Isolation and Structural Elucidation of Bioactive Secondary Metabolites from Marine Sponges

Isolierung und Strukturaufklärung von bioaktiven Sekundärmetaboliten aus marinen Schwämmen

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Mousa S. M. AlTarabeen

aus Amman, Jordan

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Erklärung

Ich versichere, dass ich die vorliegende Dissertation mit dem Titel “Isolierung und Strukuräufklarung von bioaktiven Sekumdärmetaboliten aus marinen Schwämmen“ selbständig angefertigt habe und keine anderen als die agegebenen Quellen und Hilfsmittel verwendet habe. Die eingereichte Arbeit habe ich in dieser oder ähnlicher Form noch keinem anderen Prüfungsausschuss vorgelegt.

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Mousa AlTarabeen

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Abstract

Marine invertebrates such as cnidarians, mollusks, tunicates, and sponges represent the main source of new natural products which are currently in clinical trials or under pre-clinical evaluation. Among marine invertebrates are sponges which produce the highest and most diverse number of natural products which were and still used as drug-leads with a wide range of therapeutic properties. About 5000 species of sponges were discovered out from an estimated 8000 species, which are believed to be present in nature. Many marine sponges are still poorly investigated and so, they are representing a promising source of new secondary metabolites which have pharmacological properties and can be used in the formulation of some promising drugs. In the present study, five sponges from the Gulf of Aqaba in Jordan (**MT1**, **MT3**, **MT5**, **MT6**, and **MT8**), and one (**FB67**) from Ambon in Indonesia were investigated. Modern NMR spectroscopy (1D and 2D) and mass spectrometry (MS) techniques were involved in the structure elucidation of the isolated compounds. In this study, a total of twenty-six secondary metabolites were isolated and three of these compounds were identified as new natural products. All isolated compounds were subjected to a MTT assay against the mouse lymphoma cell line L5178Y.

1. The unknown sponge (**MT5**)

   An attempt was made to identify the unknown sponge (**MT5**) in the Naturalism Biodiversity Center in Netherland but unfortunately, it failed. Two new natural products were isolated from this sponge which are; 3-amino-1-(2-amino-4-bromophenyl) propan-1-one, as well as 7-bromoquinolin-4(1*H*)-one. In addition, four known compounds were isolated which are; bromoindole-3-carbaldehyde, caulerpin, (*Z*)-5-(4-hydroxybenzylidene)-hydantoin, and

(*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin. The last two compounds were also isolated from the sponge *Hemimycale* sp.

2. *Theonella* sp. (**MT3**) and *Diacarnus ardoukobae* (**MT1**)

More than 100 metabolites have been reported from *Theonella swinhoei* including nonribosmal polypeptides/depsipeptides (as the cytotoxic poly-theonamides and the anti-inflammatory perthamides and solomonamides), macrolides (e.g. the cytotoxic swinholide A). The investigation of this sponge afforded one natural product which is swinholide A. Reviewing the current literature revealed only a small number of hits concerning the chemistry of *Diacarnus ardoukobae*. One compound was isolated from *Diacarnus ardoukobae* which was latrunculin B.

3. *Diacarnus erythraeanus* (**MT8**)

Diacarnus erythraeanus is well known as a source of norterpene cyclic peroxides. Six known norterpene cyclic peroxide compounds were isolated from *Diacarnus erythraeanus*. Four of these compounds showed strong activities in the MTT assay against the cell line (L5178Y). These results indicated the importance of the relative configuration for the cytotoxic activity of these compounds in the MTT assay against the cell line L5178Y.

4. *Acanthostrongylophora ingens* (**FB67**)

Acanthostrongylophora ingens was previously reported to produce manzamine alkaloids. Ten compounds were isolated from *Acanthostrongylophora ingens*, one manzamine derivative was identified as a new natural product (ircinal E), in addition to seven known manzamine derivatives. Six manzamine derivatives showed strong activities in the MTT assay against the cell line L5178Y. The results of the MTT assay of these compounds indicated the importance of the *ß*-carboline moiety for the activity.

Zusammenfassung

Marine Invertebraten wie Nesseltiere, Weichtiere, Manteltiere und Schwämme repräsentieren die Hauptquelle der Naturprodukte, welche zurzeit klinischen Studien oder vorklinischen Bewertungen unterzogen werden. Unter den marinen Invertebraten produzieren Schwämme die meiste und diverseste Anzahl an Naturprodukten, welche als Leitstrukturen ein breites Spektrum an therapeutischem Potential aufweisen. 5000 Speziens von Schwämmen wurden bisher entdeckt, wobei die Anzahl der in der Natur vorhandenen Speziens auf 8000 geschätzt wird.

Viele der Meeresschwämme sind immer noch nur geringfügig erforscht und repräsentieren daher eine viel versprechende Quelle neuer Sekundärmetabolite mit pharmakologischem Potential, welche zur Formulierung einiger aussichtsreicher Arzneistoffe angewandt werden könnten. In dieser Arbeit wurden fünf Schwämme aus dem Golf von Aqaba in Jordanien (**MT1**, **MT3**, **MT5**, **MT6** und **MT8)** und einer (**FB67**) von Ambon in Indonesien, erforscht. Moderne NMR-spektroskopische (1D und 2D) und massenspektrometrische (MS) Techniken wurden zur Strukturaufklärung der isolierten Substanzen angewandt. In dieser Arbeit wurden insgesamt sechsundzwanzig Sekundärmetabolite isoliert und drei dieser Substanzen wurden als neue Naturprodukte identifiziert. Alle isolierten Substanzen wurden einem MTT-Assay gegenüber einer Mauslymphomzelllinie (L5178Y) unterzogen.

1. Unbekannter Schwamm (**MT5**)

   Es wurde ein Versuch unternommen, den unbekannten Schwamm (**MT5**) zu identifizieren, im Naturalism Biodiversity Center in den Niederlanden. Dieser war jedoch erfolglos. Zwei neue Naturprodukte wurden aus diesem Schwamm isoliert, und zwar 3-Amino-1-(2-amino-4-bromophenyl)propan-1-on, wie auch 7-Bromoquinolin-4(1*H*)-on. Zusätzlich wurden vier bekannte Substanzen isoliert, Bromoindol-3-carbaldehyd, Caulerpin,

(*Z*)-5-(4-Hydroxybenzyliden)-hydantoin und (*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin. Die beiden letzteren Substanzen wurden auch aus dem Schwamm *Hemimycale* sp. isoliert.

2. *Theonella* sp. (**MT3**) und *Diacarnus ardoukobae* (**MT1**)

Aus dem Schwamm *Theonella swinhoei* sind bereits mehr als 100 Metabolite bekannt, einschließlich nonribosmaler Polypeptide/Depsipeptide (sowie die zytotoxischen Polytheonamide und die antiinflammatorischen Perthamide und Solomonamide) und Makrolide (z.B. das zytotoxische Swinholid A). Die Erforschung dieses Schwammes erbrachte einen Naturstoff, und zwar Swinholid A. Die Recherche in aktueller Literatur zeigte nur eine geringe Anzahl an Übereinstimmungen bezüglich der Chemie von *Diacarnus ardoukobae*. Es wurde eine Substanz aus *Diacarnus ardoukobae* isoliert, namentlich das Latrunculin B.

3. *Diacarnus erythraeanus* (**MT8**)

Diacarnus erythraeanus ist als Quelle für Norterpen-Cycloperoxide bekannt. Sechs bekannte Norterpen-Cycloperoxid Substanzen wurden aus *Diacarnus erythraeanus* isoliert. Vier dieser Substanzen zeigten eine starke Aktivität im MTT-Assay gegenüber der Zelllinie L5178Y. Diese Ergebnisse verdeutlichten die Bedeutung der relativen Konfiguration für die zytotoxische Aktivität dieser Substanzen im MTT-Assay gegenüber der Zelllinie L5178Y.

4. *Acanthostrongylophora ingens* (**FB67**)

Über *Acanthostrongylophora ingens* wurde bisher berichtet, dass dieser Schwamm Manzamin-Alkaloide produziert. Zehn Substanzen wurden aus *Acanthostrongylophora ingens* isoliert, worunter ein Manzamin-Derivat als neuer Naturstoff (Ircinal E) identifiziert wurde, zusätzlich zu sieben bekannten Manzaminderivaten. Sechs der Manzamin-Derivate

zeigten eine starke Aktivität im MTT-Assay gegenüber der Zelllinie L5178Y. Die Ergebnisse der Zytotoxizität im MTT-Assay gegenüber der Zelllinie L5178Y dieser Substanzen verdeutlichte die Relevanz des ß-Carbolin Molekülfragments für die Aktivität dieser Substanzen.

Table of Contents

1. Introduction

Nature represents an endless source for bioactive molecules. These natural products have provided a considerable value to the pharmaceutical industry over the past fifty years (Mishra, and Tiwari, 2011). Moreover, the forty new drugs that were launched on the market between 2000-2010 originated from terrestrial plants, micro- and marine organisms (Newman and Cragg, 2012). Despite that the oceans and seas cover more than 70% of the earth's surface and contain more than 300,000 described species of plants and animals, the investigation of marine natural products is considerably more challenging than the investigation of natural products from terrestrial origin due to the difficulty in collection, storage, and identification of the marine organisms (Bruneton, 1995; Roberts, 2007). It was found that the adaptation mechanisms of marine organisms to this unique habitat force them to produce a wide variety of secondary metabolites (Costantino *et al*., 2004). Historically, the discovery of marine-derived natural products was initiated by the isolation of unusual nucleoside derivatives from the sponge *Tethya crypta* (Bergmann, and Feeney. 1950 and 1951). Since then, the discovery of marine natural products has been enormous (Mehbub *et al*., 2014). For instance, more than 8000 new marine natural products were recorded between 2001 and 2010 (Mehbub *et al*., 2014). To conclude, the marine environment has proven to be a very rich source of extremely potent compounds which have demonstrated significant biological activities such as antitumor, antiinflammatory, immune-modulation, antiallergic, and antiviral activities (Gerwick and Moore, 2012; Perdicaris *et al*., 2013; Mehbub *et al*., 2014).

1.1. The potential of marine natural products in drug discovery

The majority of marine natural products that have pharmacological properties and were used in the formulation of some promising drugs are produced by invertebrates such as tunicates,

mollusks, cnidarians, and sponges (Perdicaris *et al*., 2013). Examples are, **kalihinol–A** and **calyculin–A** which represent two antibiotic metabolites with a potent therapeutic value and were purified from marine sponges (Gerwick and Moore, 2012) (Figure 1.3). In addition, the anticancer agents, **PM01183**, and **aplidine** represent examples for compounds which were isolated from tunicates (Gerwick and Moore, 2012). Moreover, the anticancer agents, **SGN-75** and **bryostatin 1** represent metabolites that were isolated from mollusks and bryozoans, respectively (Gerwick and Moore, 2012). In addition, the antiflammatory agent, **simplexin E**, and the anti-HIV agent, **lobohedleolied** represent compounds which were isolated from cnidarians (Rashid *et al*., 2000; Wu *et al*., 2009).

Among the marine invertebrates, the phylum *Porifera* is considered as the most dominant source of many bioactive natural products that were used as leads to develop many pharmaceutical commercial drugs (Perdicaris *et al*., 2013). Recently, about 30% of the marine-derived drugs were discovered from this phylum (*Porifera*) (Mehbub et *al*., 2014), and were described in highly cited reviews in the last decades (Higa *et al*., 1994; Alcaraz *et al*., 2006). In conclusion, sponge-derived natural products have been shown to be a pivotal source of new leads with a wide range of therapeutic properties (Gerwick and Moore, 2012; Perdicaris *et al*., 2013). Examples of metabolites that were isolated from this phylum are **latrunculins** (Kashman *et al*., 1980; Kashman *et al*., 1985), **swinholides** (Carmely and Kashman, 1985), **manzamines** (Sakai *et al*., 1986), which have attracted the attention of both chemists and cell biologists. Moreover, **Prialt®** (ziconotide), a peptide which was isolated from a tropical marine cone snail and is used for treatment of chronic pain for example in spinal cord injury (Perdicaris *et al*., 2013). This was followed by the discovery of **Vira-A®** (vidarabine) which is an anti-HSV agent

(Perdicaris *et al*., 2013). Moreover, the nucleosides s**pongothymidine** and **spongouridine**, which were isolated from *Tethya crypta* (Bergmann, and Feeney. 1950), and their derivative **Ara-C**® (also known as 1-*β*-D-arabino furanosyl cytosine or cytrarabine) were also reported as the first marine-derived anticancer agents that are used for treatment of leukemia (Schwartsmann, 2000; Proksch *et al*., 2002). In addition, the two anticancer agents **Yondelis**® (known also as trabectedin or ET-347), and **Halaven**® also enriched natural product discovery (Gerwick and Moore, 2012) (Figure 1.1).

1.2. Marine sponges

Sponges of the phylum *Porifera* are sessile organisms and represent the simplest and oldest living group of metazoan animals. It was found that so far 5000 species of sponges were discovered out from an estimated 8000 species, which are believed to be present in nature (Perdicaris *et al*., 2013). Fossils confirmed that sponges first appeared during the Cambrian period, 700-800 million years ago (Balakrishnan *et al*., 2014). In fact, sponges are important components of all modern coral reef communities (Santos-Gandelman *et al*., 2014) and their biomass and range of ecological tolerance frequently exceed that of the coral reef building species. Moreover, they have a considerable impact on the marine environment by effectively filtering large quantities of water, modifying the reef frame work, and providing a shelter for numerous species of fishes and invertebrates (Reiswig, 1971; Glynn, 1973; Rützler, 1978). Despite their simplicity, sponges are highly diverse and well adapted organisms and they can manage to survive longer than any other metazoan (Hooper, 1997). Thus, their successful evolution and wide distribution in modern reef habitats make them highly interesting for ecological and chemical studies (Perdicaris *et al*., 2013). *Porifera* stands for "pore bearer" and these sponges are multicellular organisms that have bodies full of pores and channels allowing

water to circulate through them and they have no nervous system (Gazave *et al*., 2012). Their middle jelly-like layers have a large and varied population of cells and some types of cells in their outer layers may move into the middle layer and change their function (Paul, 1992; Sipkema *et al*., 2005; Alcaraz *et al*., 2006). Taxonomically, the phylum *Porifera* comprises four main groups of sponges based on the skeleton of these sponges, which are: Calcarea (calcarea), Hexactinellida (glass sponge), Sclerospongiae (coraline sponges), and Demospongiae (demosponges). The latter forms the largest and most diverse class and about 95% of all sponge species belong to this phylum (Gazave *et al*., 2012).

1.2.1. Marine sponges as hosts for microorganisms

Marine sponges, due to evolutionary and ecological long term changes, often inhabit diverse microbial communities (archaea, microalgae, and fungi). Many studies in the past have focused on the ecosystem of sponge associated microbial communities (Balakrishnan *et al*., 2014). It was found that, the associated microorganisms comprise up to 40% of the sponge volume and that they can contribute significantly to host metabolism (e.g. photosynthesis or nitrogen fixation) (Perdicaris *et al*., 2013). More than 30% of different microorganisms have so far been recognized in the domain of bacteria and *archaea* (Balakrishnan *et al*., 2014). Moreover, the sponge-associated microbes were found to produce a wide range of bioactive metabolites (Taylor *et al*., 2007). These microbial metabolites might have significant roles in chemical ecology of marine sponges and their homeostasis in the environment (Balakrishnan *et al*., 2014), like protecting the marine sponges against predators (Proksch *et al*., 2002) or help to fight off neighbors which are competing for space (Thakur and Müller, 2004). The symbiotic relationship between the sponge and these microorganisms could be classified into three categories, mutualism (the symbionts play an essential role in the metabolism of their host);

facultative mutualism (i.e. they have a beneficial effect on their host, but the host can survive without the symbiont); or commensalism (i.e. they are present without providing obvious beneficial effects to their host) (Balakrishnan *et al*., 2014). Thus, it has been suggested that the true producers of some biologically active secondary metabolites of the marine benthic invertebrates including sponges could be the associated microorganisms specially, the *bacteria* (Banaigs *et al*., 1993; Ireland *et al*., 1988; Balakrishnan *et al*., 2014). Some examples are, the 15 sponge-derived *actinomycetes* extracts which were obtained and tested for both antimicrobial and antifungal activities and it was found that nine among the fifteen extracts showed a promising activity against multiresistant gram-postive bacteria and/or fungi, including vancomycin-resistant *Enterococcus faecium* and multidrug-resistant *Candida albicans* (Engelhardt *et al*., 2010). In addition, a study reported that antioxidative indole derivatives which can scavenge free radicals and ROS can be produced from marine sponge-derived yeasts (Sugiyama *et al*., 2009). Moreover, ten marine bacterial strains were isolated from *Callyspongia difusa* from the Gulf of Mannar province-India and showed a promising activity against clinical bacterial pathogens (kalirajan *et al*., 2013). The former results suggested that the sponge-associated bacterial strain *Virgibacillus* sp. may contribute to the search for novel antibiotics to overcome the infections and also for the production of potential immunomodulatory agents. However, it is important to indicate that only 1% from these sponge-associated microbes can so far be cultivated in the laboratory conditions (Balakrishnan *et al*., 2014).

1.2.2. The rationale of marine sponge selection to have bioactive metabolites

Considering the enormous number and diversity of sponges and in addition to the importance of these sponges to the balance of the ecosystem of marine environment, ingenious strategies should be utilized to select the sponge or the sponge-associated microbes which are

producing bioactive compounds. Recently, a report described the distribution of new compounds from sponges based on country/geographical area of 61 countries (Mehbub *et al*., 2014). It was found that sponges from Japan had the highest number of compounds (332 compounds), followed by Indonesia (235 compounds), Korea (211 compounds), Australia (187 compounds), and then China (146 compounds). Also, the Bahamas, Mexico, Palau, Papua New Guinea, Philippines and Vanuatu are other countries that are rich in sponges and produce more than 50 new compounds as described in last ten years (Mehbub *et al*., 2014). Moreover, studies showed that the highest concentration of toxic or antioxidant sponge-metabolites are found in habitats such as coral reefs that are characterized by intense competition and feeding pressure from carnivorous fishes (Perdicaris *et al*., 2013). In addition, it was found that marine invertebrates which inhabit the Indo-Pacific regions are rich in bioactive secondary metabolites (Sabdono and Radjasa, 2008). Despite the Gulf of Aqaba is rich in oligotrophic organisms, unfortunately, Jordan is not within the potential locations for new compounds from sponges and this is due to lack of studies in this field because of the limited resources and facilities which are already available in Jordan.

1.3. Marine sponges metabolites

There are numerous examples of bioactive compounds from marine sponges in the literature such as anticancer, antioxidant, antinflammatory, antiparasitic, antiviral, antiinfective, hypocholesterolemic, immunosuppressive, and antifouling agents (Perdicaris *et al*., 2013).

1.3.1. Secondary metabolites from marine sponges as anticancer agents

Many anticancer leads were derived from marine sponges (Perdicaris *et al*., 2013). Recently, 39 apoptotic agents from marine sponges were isolated (Essack *et al*., 2011).

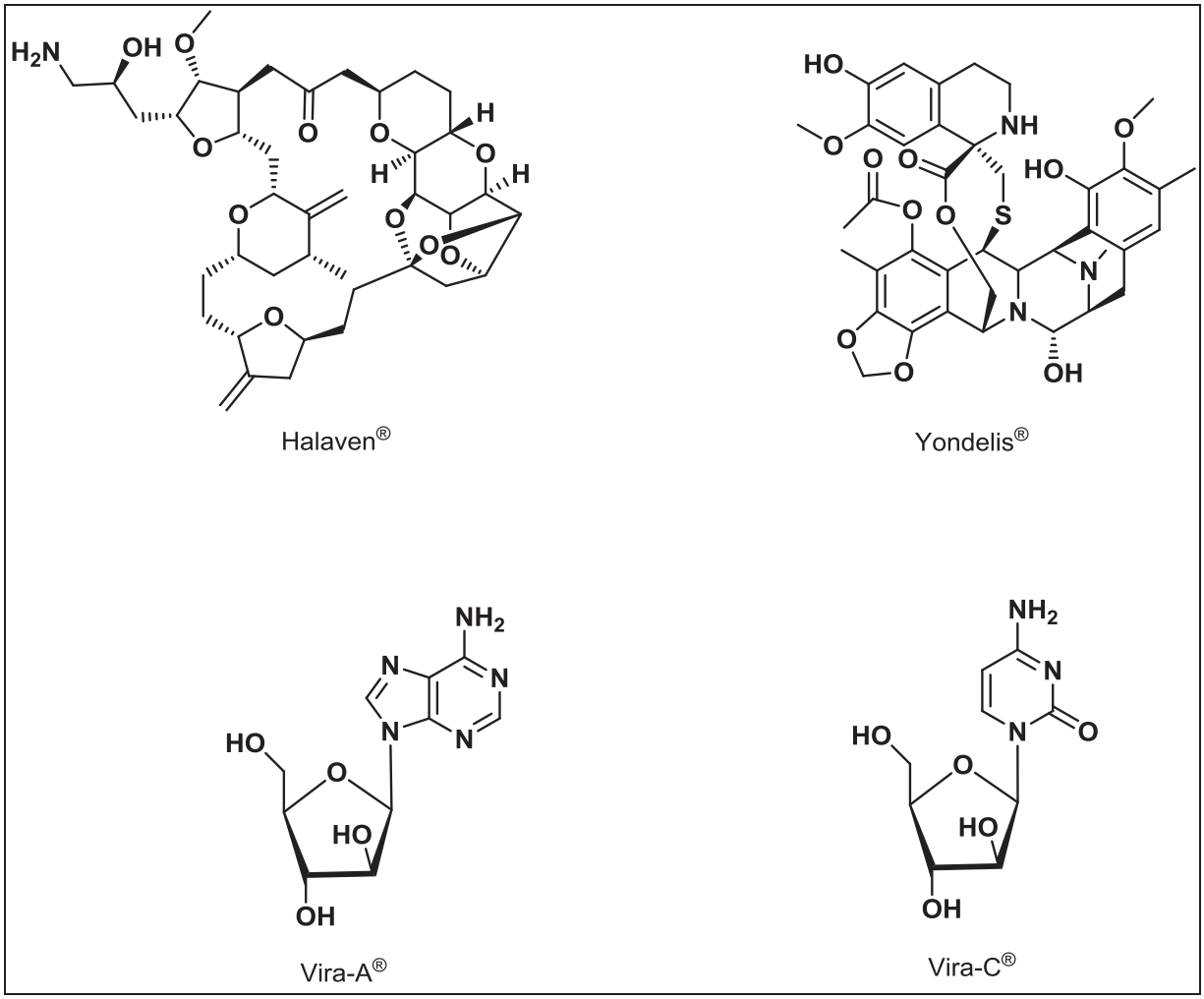


Figure 1.1: Structures of some drugs from marine invertebrates.

Moreover, many of these marine natural products are under clinical evaluation in Phases I-III clinical trials for treatment of various types of cancers (Mayer *et al*., 2010). Examples include, **discodermolide**, **hemiasterlins,** modified **halichondrin B**, **KRN-70000**, **alipkinidine**, **fascaphysins**, **isohomohalichondrin B**, **halichondrin B**, **laulimalide/ fijianolide**, **5-methoxyamphimedine** and **variolin** (Crews *et al*., 2003) (Figure 1.2). In addition, **renieramycins** which are members of the tetrahydrosio-quinoline alkaloid family were isolated from marine sponges belonging to the genus *Reniera* and possess a potent anticancer activity

(Halim *et al.,* 2011). The preclinical results revealed that **renieramycin M** induced lung cancer cells apoptosis through p53-dependent pathway and the compound may inhibit progression and metastasis of lung cancer cells (Halim e*t al.,* 2011) (Figure 1.2). In addition, **monanchocidin** which is a novel polycyclic guanidine alkaloid which was isolated from the marine sponge *Monanchora pulchra* induced cell death in human monocytic leukemia (THP-1), human cervical cancer (Hela) and mouse epidermal (JB6 C141) cells (Guzii *et al*., 2010) (Figure 1.2). Further examples of marine anticancer metabolites are the macrocyclic lactone polyether **spongistatin 1** which was isolated in 1993 from the marine sponge *Spongia* sp. (Kong *et al*., 2011) (Figure 1.2). **Spongistatin 1** was shown to inhibit mitosis, microtubule assembly, and the binding of it to tubulin thereby inducing cytotoxic cell death in numerous cancer cell lines (Bai *et al*., 1993). In 1990, a collaborative program was initiated between a natural product chemistry laboratory in University of Califormia Santa Cruz and an experimental therapeutics laboratory of Henry Ford Hospital in Detroit which succeeded in the discovery and development of many anticancer agents from different sponge extracts (Rothmeier *et al*., 2010). In this collaboration, novel *in-vitro* assays were conducted for 2036 extracts from 683 individual sponges which led to isolation of many bioactive compounds with antitumor activity (Rothmeier *et al*., 2010). Also, **smenospongine** which is an isolated sesquiterpene aminoquinone from the sponge *Smenospongia* sp. (Kondarcki and Guyot, 1987) (Figure 1.3) and the lectin which was purified from the marine sponge *Cinachyerlla apion* (CaL) were found to have cytotoxic and antiproliferative properties. Recently, studies revealed that this lectin induces cell death by apoptosis which is activated by a pro-apoptotic protein (Bax) through promoting the mitochondrial membrane permeabilization, cell cycle arrest in S-phase and acting on both dependent and/or independent of caspases pathway (Valeriote *et al*., 2012). Thus, the potential of

Cal in medical studies for treating cancers was discovered (Valeriote *et al*., 2012). Last but not the least was **heteronemin**, another anticancer sesterterpene which was isolated from the sponge *Hyrtios* sp. and it was found to have biological effects on chronic myelogenous leukemia cells. The bioassays showed that **heteronemin** affected cellular processes including cell cycle, apoptosis, mtogen-activated protein kinases (MAPKs) pathway and the nuclear factor kappa B (NF-kappa B) signaling cascade (Rabelo *et al*., 2012) (Figure 1.3).

1.3.2. Secondary metabolites from marine sponges as antioxidant and antiinflammatory agents

Many studies in last few years reported bioactive compounds from marine sponges with antioxidant, radical scavenging and antiinflammatory properties (Perdicaris *et al*., 2013). Such as, **heteronemin** (Figure 1.3) which was found to have antiinflammatory activity (Rabelo, *et al*., 2012). In addition, four marine sponges; *Smenospongia* (SP1), *Callyspongia* (SP2), *Niphates* (SP3), and *Stylissa* (SP4) were collected from the Red Sea, and a number of compounds were isolated from these sponges such as phenolic compounds, aromatic polyketides, aromatic alkaloids, and indole derivatives with strong antioxidant potential in comparison to ascorbic acid and vitamin E (Longeon *et al*., 2011; Utkina *et al*., 2009). Moreover, some aromatic polyketides isolated from marine sponge-derived fungus *Aspergillus versicolor* (Li, 2009) exhibited significantly higher antioxidant capacity than butylated hydroxytoluene (BHT).

1.3.3. Secondary metabolites from marine sponges as antiparasitic agents

Many compounds were isolated from marine sponges and showed a potent activity against some parasites like malaria (Sipkema *et al*., 2005). Examples include, those metabolites from *Diacarnus erythraeanus,* a sponge collected from the Red Sea (El Sayed *et al*., 2001; Youssef *et al*., 2001; Youssef, 2004; Lefranc *et al*., 2013), **19-hydroxypsammaplysin E** which

showed a promising antimalarial activity (Mudianta, *et al*., 2012), in addition to the **ingamine** type alkaloids, **22(*S*)-hydroxyingamine A**, and **dihydroingenamine D** which were isolated from the marine sponge *Petrosid* Ng5. **Dihydroingenamine D** and **ingamine A**, showed a strong antiplasmodial activity against chloroquine-sentive (D6) and resistant (W2) strains of *Plasmodium falciparum* (Ilias *et al*., 2012) (Figure 1.3). In addition, **tsitsikammamine C** is a bispyrroloiminoquinone alkaloid that was isolated from the marine sponge *Zyzzya* sp. and displayed a potent *in-vitro* antimalarial activity through inhibition of both ring and trophozoite stages of malaria parasite life cycle. Further antimalarial agents are, **makaluvamines** and **damirones A** which were isolated from the same sponge and displayed a potent growth inhibitory activity against *P. falciparum* (Davis *et al*., 2012) and the two ***N*-cinnamoyl-amino acids**, **iotrochamides A** and **B** which were purified from the Australian sponge *Iotochota* and were also shown to inhibit *Trypanosoma brucei* (Feng *et al*., 2012). Furthermore, four diterpenes, **8-isocyanato-15-formamidoamphilect-11(20)-ene**, **8-isothiocyanato-15-formamidoamphilect-11(20)-ene**, **8-isocyano-15-formamidoamphilect-11(20)-ene**, and **7-formamidoamphilect-11(20),15-diene** which were purified from the sponge *Stylissa cf. massa*, were proved to exhibit a strong antimalarial activity (Chanthathamrongsiri *et al*., 2012). Last but not the least are the nine bromotyrosine-derived compounds which were isolated from the Caribbean marine sponge *Verongula rigida*, were found to be active against three parasitic protozoa: *Leishmamia panamensis*, *Plasmodium falciparum* and *Trypanosoma* cruzi (Galeano *et al*., 2011).

1.3.4. Secondary metabolites from marine sponges as antiviral agents

Over 40 metabolites from marine organisms in the last few years are commercially available in the pharmacological markets and have antiviral activity (Perdicaris *et al*., 2013). As

anti-HIV agents, about 150 natural products have been isolated in the previous decade from marine organisms. Examples of those anti-HIV agents are, **avarol**, **avarone**, and **ilimaquinone** (Tziveleka *et al*., 2003) (Figure 1.3).

In addition, the nucleoside vidarabine (**Ara-A®**) which was isolated from the sponge *Tethya crypta* represents the most important antiviral lead from marine origin (Villa and Gerwick, 2010). **Vidarabine** inhibits Viral DNA polynmerase and DNA synthesis of Herpes, *vaccinica* and *vaicellazoeter* viruses (Villa and Gerwick, 2010). Furthermore, **norbatzelladine L** which was isolated from a marine sponge of the genus *Monanchora* displayed antiviral activity against *Herpes simplex* virus type (HSV-1), with 97% of inhibition in the viral adsorption phase (Kohn *et al*., 2012).

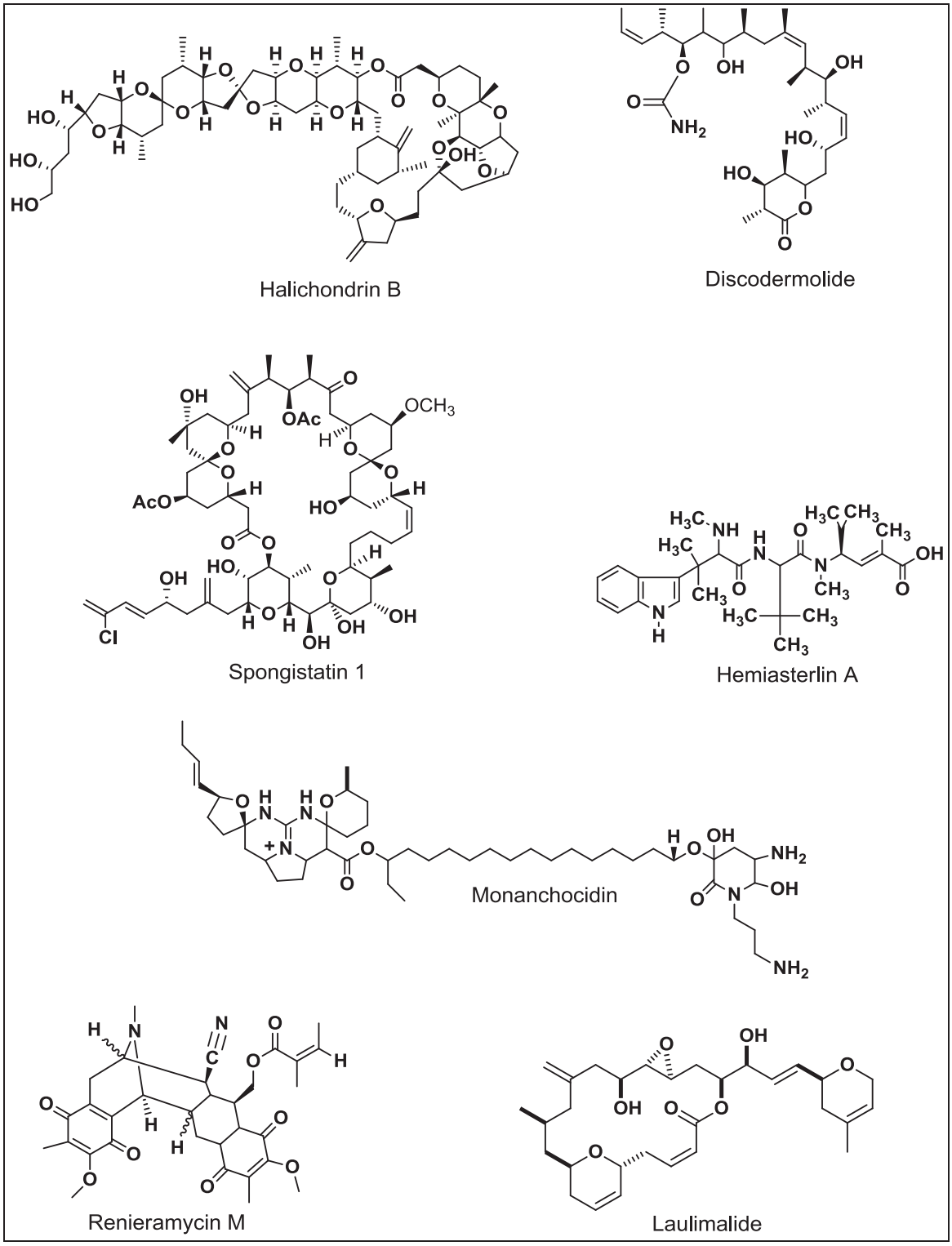


Figure 1.2: Structures of some marine natural products from sponges in preclinical and clinical trials (I, II, III).

1.3.5. Secondary metabolites from marine sponges as antiinfective agents

Marine sponges are considered as a rich source of natural products and metabolites which are used as antiinfectives with a strong inhibitory activity against fungi, and other microbes (Perdicaris *et al*., 2013). Examples are, **purpuroines A-J** which are halogenated alkaloids and were isolated from the marine sponge *lotrochota purpurea*. These **purpuroines A-J** showed inhibitory activity against diseases related to fungi and bacteria (Shen *et al*., 2012). Moreover, several novel cyclic **bis-1,3-dialkylpyridinium** alkaloids and **cyclostellettamines** which were isolated from the sponge *Halicona* sp. from Korea exhibited moderate cytotoxic and antibacterial activities against A 549 cell-line and Gram positive strains, respectively (Lee *et al*., 2012). In addition, some alkaloids which were isolated from the marine sponge *Agelas mauritiana* exhibited antifungal activity against *Cryptococcus neoformans*, and antibacterial activity against *Staphylococcus aureus* and methicillin resistant *S. aureus in-vitro* (Yang *et al*., 2012). Furthermore, the extracts which were obtained from the sponges *Cinachyrella* sp., *Halicclona* sp., and *Petromica citrine* showed antibacterial activity against 61% of coagulase-negative *Staphylococci* (CNS) strains including strains which are resistant to traditional antibiotics (Perdicaris *et al*., 2013).

1.3.6. Secondary metabolites from marine sponges as a source for hypocholesterolemic agents

In the last decade, it was reported that marine sponges could be a source of hypocholesterolemic drug leads (Perdicaris *et al*., 2013). For instance, *Spiratrella abata*-derived **lyso-PAF** analogues and **lysophosphatidylcholines** have been reported in an *in-vitro* study as successful inhibitors of cholesterol biosynthesis (Shin *et al*., 1999; Schumacher *et al*., 2010). In addition, it was reported that marine sponges are a source of hypocholesterolemic novel

lysophosphatidylchlolines and thereby stimulated an interest for compounds derived from marine sponge due to the short lifespan of the same synthetic **lysophosphatidylchlolines** *in-vivo* (Zhao *et al*., 2003).

1.3.7. Secondary metabolites from marine sponges as immunosuppressive agents

Many natural components which were isolated from marine sponges were tested for their immunosuppressive properties in recent years (Perdicaris *et al*., 2013). Two compounds were isolated from the sponge *Agelas flabellrformis* Carter (Agelasidae) and exhibited a significant immunosuppressive activity (Gunasekera *et al*., 1989). These compounds are **4a-merhyl-5a-cholest-8-en-3-ol** and **4,5-dibromo-2-pyrrolic acid** were highly active in the suppression of the response of murine splenocytes in the two-way mixed lymphocyte reaction (MLR) with little or no demonstrable cytotoxicity at low doses (Gunasekera *et al*., 1989). Moreover, an extract obtained from the marine sponge *Aurora globostellata* (Tuticorin Coast of India) which showed promising immunomodulatory activity (Chairman *et al*., 2013).

1.3.8 Secondary metabolites from marine sponges as antifouling agents

Recently, natural marine antifouling molecules attracted the interest of scientists to provide less toxic and more specific antifouling congeners (Perdicaris *et al*., 2013). Biofouling organisms such as blue mussels, bamacles, and macroalgae cause serious problems to ships hulls, cooling systems of power plants, and aquaculture materials. Various studies in last decade were directed to find the most promising alternative technologies against fouling from marine organisms and especially from marine sponges (Perdicaris *et al*., 2013). For instance, **poly 3-alkylpyridinium** salts, **saraine**, and **haminols**, which were either extracted or derived from the marine sponges *Haliclona* sp., *Reniera sarai* and the mollusk *Haminoea fusai*, or obtained by chemical synthesis showed a good antifouling activity against larvae of the barnacle

Amphibalanus amphitrite (Perdicaris *et al*., 2013). Further examples are the derivatives of agelasine which were extracted from the Indonesian marine sponge *Agelas linnaei* and inhibited the settling of larva of *Balanus improvisus* in the antifouling bioassay together with the growth of planktonic forms of biofilm forming bacteria *S. epidermidis* (Blihoghe *et al*., 2011). Thus, several sets of natural compounds which were derived from marine sponges for the development of small molecules that inhibit and/or disperse bacterial biofilms specifically through non-microbial mechanisms were discovered (Hertiani *et al*., 2010; Stowe *et al*., 2011).

1.4. Aim of the Study

Sponges are among the most dominant group of marine organisms that are responsible for large numbers of secondary metabolites which can be as leads for development of therapeutic drugs. Most researchers in the last decades focused on marine sponges because of their size and abundance. Also, it was found that the highest concentration of therapeutically important sponge-metabolites are found in habitats such as coral reefs that are characterized by intense competition and feeding pressure from carnivorous fishes as present in the Gulf of Aqaba or at Ambon in Indonesia. Thus, the aim of this study was to continue refinement of the selection, screening, dereplication, isolation and structure elucidation followed by estimation of the pharmaceutical potential of some natural products which are produced by some marine sponges. The sponges that were investigated in this study, were collected from the Gulf of Aqaba in Jordan (the unknown sponge (**MT5**), *Hemimycale* sp. (**MT6**), *Theonella* sp. (**MT3**), *Diacarnus ardoukobae* (**MT1**), and *Diacarnus erythraeanus* (**MT8**), in addition to (*Acanthostrongylophora ingens*) (**FB67**) which was collected at Ambon in Indonesia.

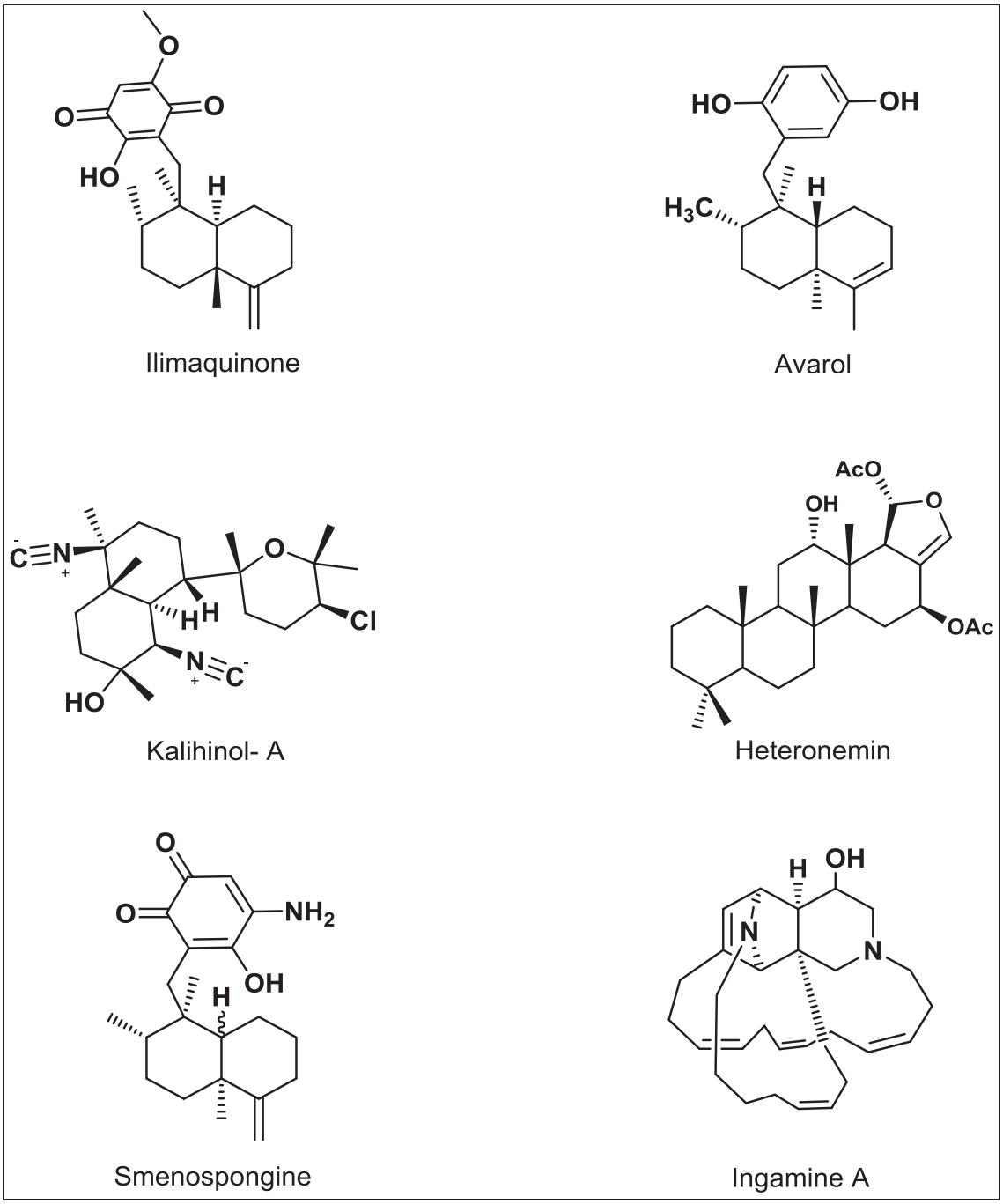


Figure 1.3. Some bioactive metabolites from sponges (showing antimalarial, antiviral, antibiotic, antibactria, or anitinflammatory activities).

1. Materials and Methods

2.1. Biological materials

The samples which were handled in the current study include sponges that were collected from the Gulf of Aqaba in Jordan and at Ambon in Indonesia. Five sponge samples were collected in front of the marine science station (MSS) from the Gulf of Aqaba in Jordan by SCUBA diving at 20 m depth in 2011, *viz*., the unknown sponge (**MT5**), *Hemimycale* sp. (**MT6**), *Theonella* sp. (**MT3**), *Diacarnus ardoukobae* (**MT1**), *Diacarnus erythraeanus* (**MT8**). In addition, *Acanthostrongylophora ingens* (**FB67**) was collected at Ambon in Indonesia by SCUBA diving at 10 m depth in 1996.

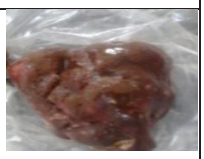
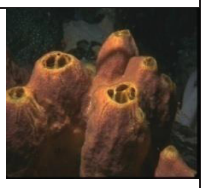
2.1.1. Sponges from the Gulf of Aqaba in Jordan

The sponge samples were transferred to Germany under dry ice and kept in a freezer at -70^{o} C in the Institute of Pharmaceutical Biology and Biotechnology in the Heinrich Heine University in Duesseldorf. Voucher samples were kept in ethanol under registration numbers **MT5**, **MT6**, **MT3**, **MT1**, and **MT8**, respectively at the National Museum of Natural History, Leiden, Netherlands. The sponges were identified by Dr. Nicole de Voogd at the same museum and an attempt was done to identify the unknown sponge (**MT5**) which unfortunately, failed (Table 2.1).

2.1.2. Sponge from Ambon in Indonesia

The sponge *Acanthostrongylophora ingens* (**FB67**) which was collected at Ambon in Indonesia was immersed in ethanol immediately after collection. Voucher specimen was kept in ethanol under registration number **FB67** at the National Museum of Natural History, Leiden, Netherlands, and was identified by Dr. Nicole de Voogd at the same museum (Table 2.1).

Table 2.1: Sponges were collected at the Gulf of Aqaba in Jordan (**1-5**), and at Ambon in Indonesia (**6**).

NO.	Code	Sponge sample	Class	Order	Family	Photo
1	**MT5**	Unknown	------	------	------	
2	**MT6**	*Hemimycale* sp.	Demospongiae	Poecilosclerida	Hymedesmiiae	
3	**MT3**	*Theonella* sp.	Demospongiae	Lithistida	Theonllidae	
4	**MT1**	*Diacarnus ardoukobae*	Demospongiae	Poecilosclerida	Podospongiidae	
5	**MT8**	*Diacarnus erythraeanus*	Demospongiae	Poecilosclerida	Podospongiidae	
6	**FB67**	*Acanthostrongyl-ophora ingens*	Demospongiae	Haploscleride	Petroiidae	

2.2. Chemicals

2.2.1. General laboratory chemicals

Solvent	Specification
n-Butanol	Merk
(*R*)-(-)-Methoxy-triflourmethylphenylacetylchloride	Aldrich
(*S*)-(-)-Methoxy- triflourmethylphenylacetylchloride	Aldrich
2,2-Diphenyl-1-picryl-hydrazyl (DPPH)	Sigma
2-Aminoethyl diphenylborionate	Fluka
Anisaldehyde (4-methoxybenzaldehyde)	Merk
Concentrated ammonia solution	Fluka
Dimethylsulfoxide	Merk Formicacid
Ninhydrin	Riedel-deHaeem
Trifluroaceticacid (TFA)	Merk

2.2.2. Chromatography

2.2.2.1. Stationary phases

Stationary phase	Specification
Pre-coated TLC plates, Silica Gel 60 F254, layer thickness 0.2 mm	Merk
Silica Gel 60, 0.04 – 0.063 mm meshsize	Merk
Pre-coated TLC plates, RP-18, F254 S, layer thickness 0.25 mm	Merk
RP-18, 0.04 – 0.0.63 mm meshsize	Merk
Sephadex LH-20, 0.25 – 0.1 mm mesh size	Merk
Diaion HP-20	Supelco

2.2.2.2. Spray reagents

The reagents were stored in amber-colored bottles and kept in the refrigerator until being used. TLC was done to monitor the identity of the fractions and the purity of the isolated compounds. It was also utilized to optimize the solvent system that would be applied for column chromatography.

2.2.2.2.1. Anisaldehyde/H_2SO_4 spray Reagent

No.	The components of the reagent	Volume (ml)	Notes
1.	Methanol	85	
2.	Glacial acetic	10	
3.	Conc. H_2SO_4	5	Added slowly
4.	Anisaldehyde	0.5	

2.2.2.2.2. Vanillin/H_2SO_4 spray reagent

No.	The components of the reagent	Amount	Notes
1.	Methanol	85 ml	
2.	Conc. H_2SO_4	15 ml	Added slowly
3.	Vanillin	1 g	

2.2.2.2.3. Flavone reagents

No.	The components of the reagent	Flavone reagent A (amount)	Flavone reagent A (amount)
1.	2-Aminoethyl diphenylborionate	1 g	-
2.	Methanol	Up to 100 ml	-
3.	Polyethylene glycol 400	-	5 ml
4.	Ethanol	-	95 ml

2.2.3. Solvents

2.2.3.1. General solvents

Dichloromethane, ethyl acetate, methanol, ethanol, *n*-hexane, acetone and acetonitrile were used. The solvents were purchased from the Institute of Chemistry, University of Duesseldorf. They were distilled before use and for spectroscopic measurements special grades of solvents.

2.2.3.2. Solvents used for the spectrometry methods

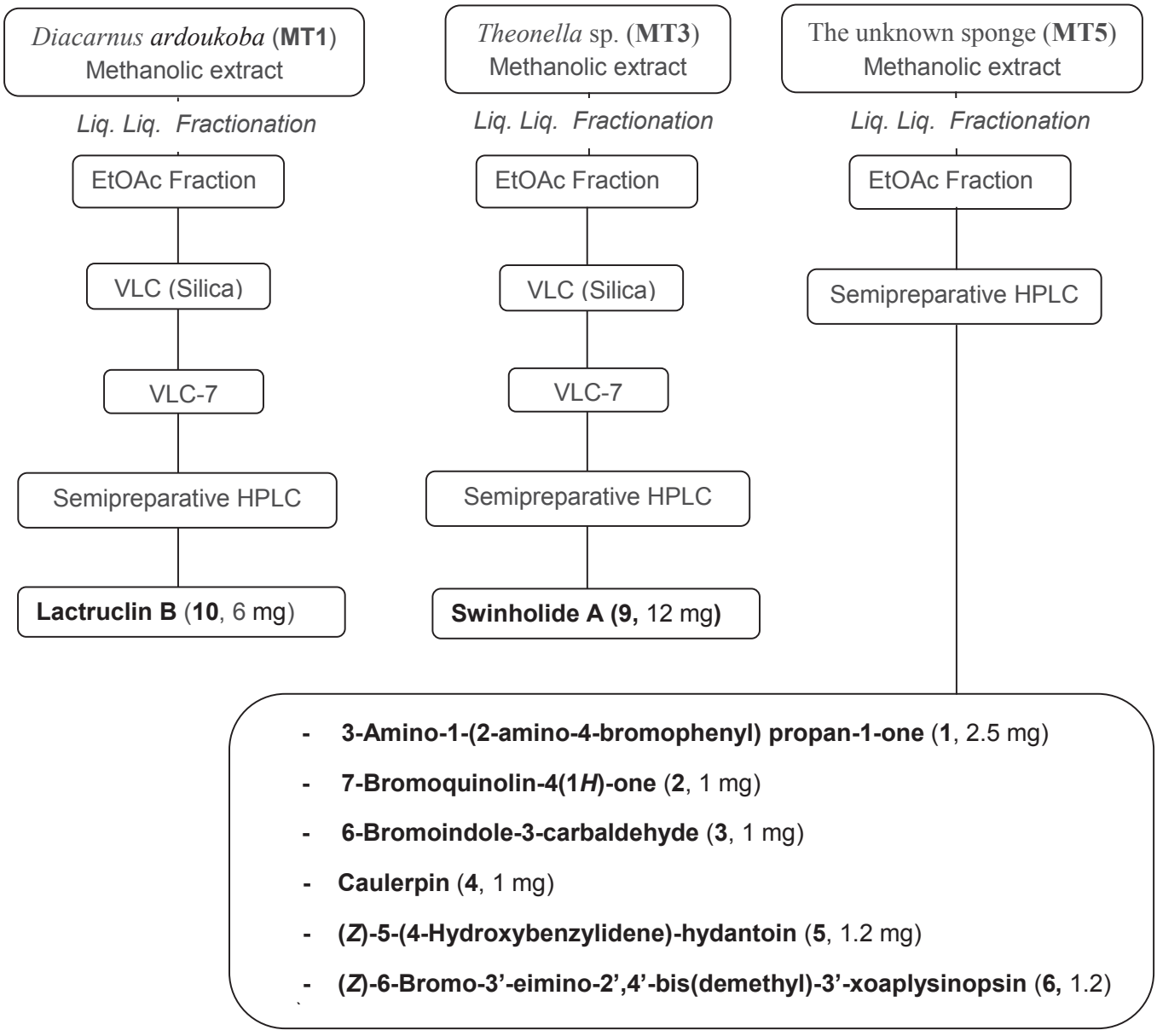
Solvent	Applied for	Specification
Methanol	HPLC	LiChroSolv HPLC grade (Merk)
Acetonitrile	HPLC	LiChroSolv HPLC grade (Merk)
Nanopurewater	HPLC	By passing distilled water through nano- and ion- exchange filter Cells (Barnstead, France).
Methanol	Optical rotation	Special grade (Sigma)
Ethanol	Optical rotation	Special grade (Sigma)
Chloroform	Optical rotation	Special grade (Sigma)
Water	Optical rotation	Special grade (Fluka)
Methanol-d_4	NMR	Uvasol, Merk
Chloroform-d	NMR	Uvasol, Merk
DMSO-d_6	NMR	Uvasol, Merk

2.3. Methods

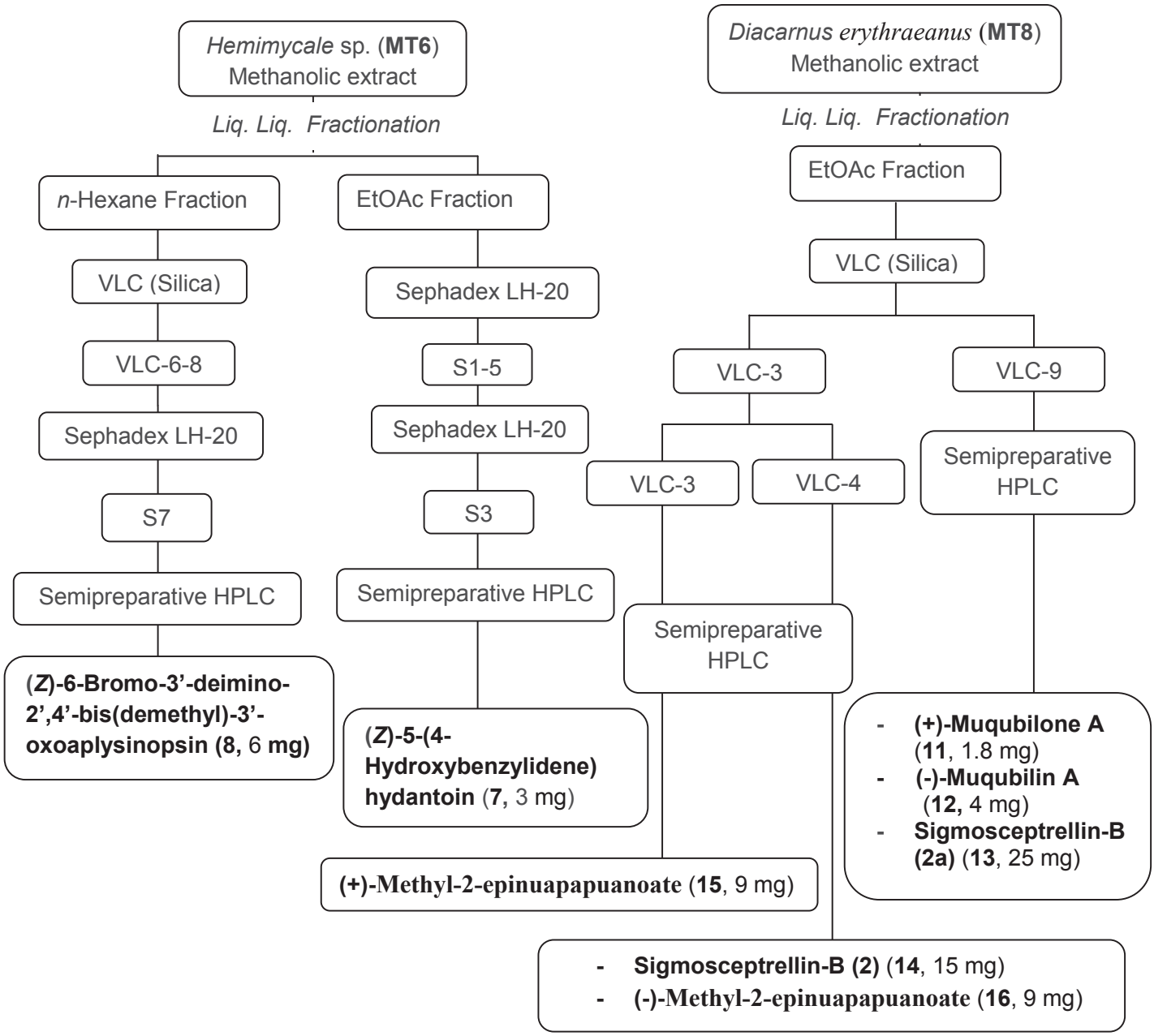
2.3.1. Isolation and purification of secondary metabolites

2.3.1.1. Isolation of secondary metabolites from the Gulf of Aqaba in Jordan

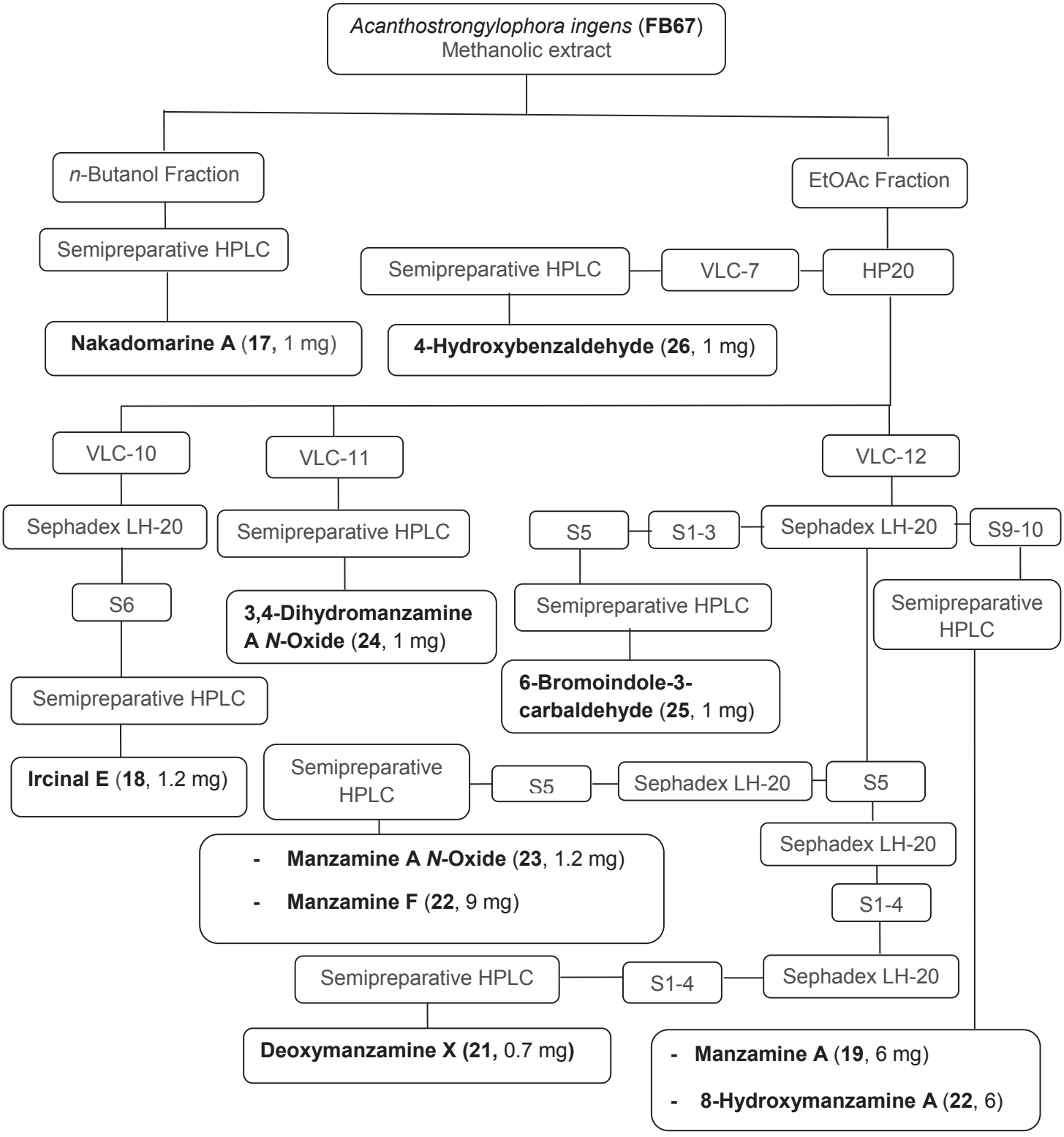
2.3.1.1.1. Isolation of secondary metabolites from *Diacarnus ardoukoba* (MT1), *Theonella* sp. (MT3), and the unknown sponge (MT5)



2.3.1.1.2. Isolation of secondary metabolites from *Hemimycale* sp. (MT6), and *Diacarnus erythraeanus* (MT8)



2.3.1.2. Isolation of secondary metabolites from *Acanthostrongylophora ingens* (FB67) at Ambon in Indonesia



2.3.2. Separation methods

2.3.2.1. Extraction and solvent-solvent partition

Extracts containing natural products contain often a mixture of components in a complex matrix. Each individual component of this mixture must be separated from other components. Therefore, the separation can be performed depending on the difference in the physical or chemical properties of the individual components. The natural products first of all are extracted from the complex matrix by organic solvents such as ethyl acetate, acetone, *n*-hexane, or methanol which are common solvents for the isolation of unknown samples. Then, this is followed by mixing two immiscible solvent, in which the compounds of the extract migrate from one phase to the other one depending on the common principle "like-dissolves-like".

2.3.2.2. Chromatographic separation method

Chromatography is a separation method where the components are distributed between two different phases, a stationary phase and a mobile phase. Depending on the physical properties of these components (e.g. size, polarity, charge, and etc.), different techniques are developed. Mostly, the stationary phase is solid, while the mobile phase could be liquid or gas depending on the physical and chemical properties of the components that are separated.

2.3.2.2.1. Thin layer chromatography (TLC)

In this technique the components are separated depending on the polarity, thus, they move at different rates along the TLC plates. This solid plate can be coated by polar stationary phase [e.g. silica gel 60 F_{254} (layer thickness 0.2 mm, E. Merk, Darmstadt, germany)] or nonpolar stationary phase [e.g. RP-18 F_{254} (layer thickness 0.25 mm, Merk, Darmstadt, germany)]. The mobile phase which is used with the polar stationary phase in this technique is depending on the polarity of the compounds *viz*., for polar compounds the solvent system

EtOAc:MeOH:H_2O with different ratios is used, for semi polar compounds the solvent system DCM:MeOH:EtOAc with different ratios is used, and for nonpolar compounds the solvent system *n*-Hexane:EtOAc with a different ratios is used. The mobile phase system MeOH:H_2O with different ratios is used for the nonpolar stationary phase and this is used only for separation of polar compounds. The separation efficiency of the compounds on TLC is checked by detection of the bands on TLC under UV lamp at 254 and 366 nm which is then followed by spraying the TLC plate with reagents like vanillin/H_2SO_4 and anisaldehyde/ H_2SO_4 followed by heating at 110^o C.

2.3.2.2.2. Vacuum liquid chromatography (VLC)

This technique is used for samples with a relatively large amount. In this method the material of the stationary phase is packed tightly in a sintered glass filter funnel with different sizes (depending on the amount of sample), and the sample is adsorbed onto a small amount of the stationary phase by using a volatile solvent. For semi and nonpolar compounds a polar stationary phase (e.g. silica gel 60) is used and a gradient elution starting from (*n*-Hexane:EtOAc) (100:0) to (0:100) and then (DMC:MeOH) (100:0) to (0:100) are used as mobile phases. For polar compounds a nonpolar stationary chromatography (e.g. RP-18) is preferred with a gradient elution starting from (H_2O:MeOH) (100:0) to (0:100) are used as mobile phases. In this technique the mobile phase flows through the stationary phase by applying a vacuum, and the column is allowed to run dry and the successive fractions were collected.

2.3.2.2.3. Other types of column chromatography

The derived fractions from the VLC column are subjected to other types of column chromatography like **size exclusion chromatography**. In this technique the stationary phase

consists of porous beads such as sephadex (LH-20) with a mobile phase MeOH, MeOH:DCM (1:1) or acetone. The compounds that have a larger molecular size are eluted first since they are excluded from the interior of the beads, while those with a smaller molecular size enter through beads and are eluted according to their ability to exist between the pores where they are trapped. It is worth to mention that in this technique the mobile phase flows through the stationary phase by the gravity force. Another type of column chromatography is **ion exclusion chromatography**, in which the separation depends on electrical charge of different compounds in different media. Therefore they are differently retained to the stationary phase which is an ion exchange resin beds (e.g. Diaion HP-20) according to their different affinity between the mobile phase and the stationary phase.

2.3.2.2.4. Preparative and semi-preparative high pressure liquid chromatography

The fractions of the compounds which already isolated from the former chromatography columns are subjected to preparative and semi-preparative HPLC techniques for more purification depending on the amount of these fractions. 20-80 mg of sample can be loaded in the preparative-HPLC column for each injection. The solvent system is pumped through the column with a flow rate 20 ml/min, while only 1-3 mg of sample can be loaded to the semi-preparative-HPLC column per each injection, and the solvent system is pumped through the column with a flow rate 5 ml/min. The mobile phase for both techniques is the same which consists of mixture of MeOH and nanopure H_2O with or without 0.01% TFA that is pumped in gradient or isocratic manner. Both instruments consist of four main parts *viz*., pump, column, detector, and recorder. The specifications of these parts in the two instruments are summarized in table 2.2.

Table 2.2 Summary of the specifications of the main parts for the preparative and semi-preparative-HPLC

The part	Specifications for Preparative HPLC	Specifications for Semi preparative HPLC
Pump	Varian, PrepStar 218	Dionex P580A LPG
Column	Varian Dynamax (250 x 4.6 mm, ID and 250 x 21.4 mm, ID), pre-packed with Microsorb 60-8 C-18, with integrated pre-column	Knauer (300 x 8 mm, ID), pre-packed with Eurosphere 100-10 C18, with integrated pre-column
Detector	Varian, PrepStar 320 UV-Vis detector	Merk Hitachi UV detector L-7400
HPLC program	Varian Star (V.6)	

2.3.2.2.5. Analytical high pressure liquid chromatography (HPLC)

Analytical HPLC instruments consist of six main parts *viz*., pump, autosampler, separation column, detector, recorder, in addition to reservoirs of mobile phases. The mobile phases which are used start from MeOH:Nanopure H_2O (10:90), and reach 100% MeOH in 35 minutes as standard gradient, or 25.5 minutes as a half-time gradient and the pH is adjusted to pH 2 by formic acid. For each injection 20 µL of sample is loaded by the autosampler and all peaks are detected at 235, 240, 280 and 340 nm by UV-VIS photodiode array detector. This instrument is used to evaluate the purity of the isolated individual compounds, in addition to identify the distribution of peaks from extracts or fractions. The specifications of the main parts for the analytical HPLC are summarized in table 2.3.

Table 2.3: Summary of the specifications of the main parts for the analytical HPLC

The part	Specifications for the analytical HPLC
Pump	Varian, Prep Star 218
Column	Kanuer (125 x4 mm, ID), pre-packed with Eurosphere 100-5 C18, with integrated pre-column
Column thermostat	STH 585
Autosampler	ASI-110T
Detector	Dionex Photodiode Array Detector UVD 340S
HPLC program	Chromeleon (V. 6.3)

2.3.3. Structure elucidation of the isolated secondary metabolites

2.3.3.1. Mass spectrometry (MS)

A mass spectrometry is a technique used to determine the molecular weight of the isolated compounds. Principally the atoms and molecules can be separated and quantified using a mass spectrometer depending on the difference in mass-to-charge ratio (*m/z*) of the ionized atoms or molecules. Therefore a mass spectrometer is a useful instrument for determination of important structural information of molecules. Basically, there are four main parts of the mass spectrometer *viz*., ionization source, mass-analyzer, detector, and the recorder. Relative intensity vs. the mass-to-charge ratio (*m/z*) is the output of a mass spectrometer.

2.3.3.2. Electrospray ionization mass spectrometry (ESI-MS)

In this type of the mass spectrometer the ions are produced by using an electrospray technique where a high voltage applied to a liquid which creates an aerosol. The three parts of the mass spectrometer are: ion source, analyzer, and detector which should be maintained under high vacuum conditions in order to maintain the travel of the ions through the

instrument without any impediment from air molecules. Once a sample is injected into the ionization source, the molecules are ionized. Then the ions are passed through the analyzer and are separated according to their mass (*m*) to charge (*z*) ratio (*m/z*). These ions flow into the detector and the signals are transmitted to the data system where the mass spectrum is recorded.

ESI technique may produce multiply charged ions, therefore, is especially suitable for producing ions from macromolecules since ESI technique after prevents the tendency of these molecules to fragment when they are ionized.

Liquid chromatography-mass spectrometry (LC-MS)

The interface of ESI-MS with LC makes an effective online LC/MS. By this method the mass spectrum of each component of complex mixtures, can be recorded individually while the compound is eluted from the column. A Finnigan LCQ-DECA mass spectrometer which is connected to a UV detector is used for carried out HPLC/ESI-MS in our institute. The gradient mobile phase consists of MeOH:nanopure H_2O (10:90) (H_2O which is adjusted to pH 2 with 0.1% HCOOH), and reaches 100% MeOH in 35 minutes (Table 2.4).

Table 2.4 Summary of the specifications of the main parts for the LC/UV/MS

The part	Specifications for the LC/UV/MS
HPLC system	Agilent 1100 series (pump, detector, and autosampler)
MS spectrometer	Finnigan LC Q-DECA
Column	Knauer, (250 × 2 mm, ID), pre-packed with Eurosphere 100-5 C18, with integrated pre-column

2.3.3.3. Electron impact mass spectrometry (EI-MS)

In this type of mass spectrometer the compound which is vaporizing in an evacuated

chamber is bombarded with electrons having 25.80 eV (2.4–7.6 kJ/mol) of energy using electron impact technique (EI). This high energy ionizes and causes extensive fragmentation of organic molecules. It is worth to mention that the energy which is required to break down the strongest single bonds in organic molecules is about 4 eV, while to ionize an organic molecules this energy is about 7-10 eV (Elsayed, 2010). The fragmentation in this method is extensive, leading to fragment ions by which the compound can be characterized. However, the recurrent obscurity of molecular ions is one of the main disadvantages of this method.

Low resolution EI-MS was measured on a Finnigan MAT 8430 mass spectrometer. Measurements were done by Dr. Peter Tommes, Institute for Inorganic and Structural Chemistry, Heinrich-Heine University, Duesseldorf.

2.3.3.4. Matrix-assisted laser desorption/ ionization (MALDI)

This technique is used in the mass spectrometry and is similar in character to electrospray ionization (ESI) technique because both of them produce ions of large molecules by soft ways in gas phase, despite that MALDI produces quit fewer multiple charged ions. MALDI is an effective technique in the analysis of bimolecules (e.g. protein, DNA, and sugars) and large organic molecules (e.g. polymers, and macromolecules). MALDI is suggested to be a three-step process *viz*., first, the sample is mixed with a suitable matrix material (e.g. 2,5-dihydroxybenzoic acid (DHB), picolinic acid (PA), and 3-hydroxy picolinic acid) and then applied to a metal plate; second, a pulsed laser irradiates the sample triggering ablation and desorption of the sample and matrix material; third, the analyte molecules ionized are being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into a mass spectrometer which is used to analyze them. MALDI was measured on a Ultaflex-Bruker mass spectrometer. Measurements were done by Dr. Peter Tommes, Institute for Inorganic and Structural Chemistry, Heinrich-

Heine University, Duesseldorf.

2.3.3.5. High resolution mass spectrometry (HRESI-MS)

In high resolution technique the ion beam is passing through two analyzers, the first one is the electrostatic analyzer, and the second one is the magnetic analyzer, respectively. By these double focusing mass spectrometer, the ion masses can be measured with accuracy of approximately 1 ppm, and by this accuracy of measurement, the atomic composition of the molecular ions can be determined.

HRESIMS was measured on Finnigan LCQ Deca and Micromass Q tof 2 mass spectrometer. Measurements were done by Dr. Peter Tommes, Institute for Inorganic and Structural Chemistry, Heinrich-Heine University, Duesseldorf.

2.3.3.6. Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei of certain atoms in a magnetic field absorb and re-emit electromagnetic radiation (energy). This energy has specific frequency that depends on the strength of magnetic field and magnetic properties of the isotope of atoms. This phenomenon only appears in nuclei possessing a property called spin (e.g. ^{1}H, ^{13}C, ^{17}O, ^{15}N, and ^{31}P). Hence, not all atoms have this property and also not all isotopes of same atom have this property (e.g. ^{2}H, ^{12}C, and ^{16}O). This technique finds applications in different fields of science. NMR spectrometry is used usually by chemists to study chemical structures by using one dimensional NMR for simple structures (e.g. ^{1}H, and ^{13}C). For more complicated structures two dimensional techniques are used (e.g. COSY, HSQC, and HMBC).

NMR spectra were recorded at 300° K on Bruker ARX-500 and on Bruker AVANCE DMX-600 NMR spectrometer by Dr. Schaper, Klaus, Institute for Inorganic and Structural Chemistry, Heinrich-Heine University, Duesseldorf. Some measurements were also performed at

the Helmholtz Centre for Infection Research, Braunschweig, by Dr. Victor Wray using an AVANCE DMX-600 NMR spectrometer and others in the Institute of Complex Systems: Strukturbiochemie, Forschungszentrum Juelich by Dr. Rudolf Hartmann using an AV600 IIIHD NMR spectrometer. All 1D and 2D spectra were obtained using the standard Bruker software. The samples were dissolved in different solvents, dependent on the solubility of these samples and the quality of NMR data. The observed chemical shift (δ) values were given in ppm and the coupling constants (*J*) in Hz.

2.3.3.7. Optical activity

Optical activity is a physical property of chiral molecules that arises from the way by which they are interact with light. Optical rotation was determined on a Perkin-Elmer-241 MC polarimeter. The substances were measured in a 0.5 mL cuvette with 0.1 dm length. The angle of rotation was measured at the wavelength at 546 and 579 nm of a mercury vapour lamp at room temperature (25° C). The specific optical rotation was calculated using the expression:

$$[\propto]_D{}^T = \frac{[\propto]_{579} \times 3.199}{4.199 - \frac{[\propto]_{579}}{[\propto]_{579}}}$$

with $[\propto]_D{}^T$ = Specific rotation at the wavelength of Sodium D-lin, 589 nm, at certain temperature T.

$[\alpha]_{579}$ and $[\alpha]_{564}$ = Optical rotation at wavelengths 579 and 564 nm, respectively, calculated using the formula:

$$[\alpha]_\lambda = \frac{100\alpha}{l \times c}$$

where, α= the measured angle of rotation in degrees,

l= the length in dmof the polarimeter tube,

c = the concentration of the substance expressed in g/100 mL.

3.3.4. Testing the biological activity

Finding the bioactive compounds from the marine sources executed only and only if assay systems have been contrived which will allow for successful biologically guided fractionation of the culture extracts.

2.3.4.1. Cytotoxicity assay

2.3.4.1.1 Microculture tetrazolium (MTT) assay

Cytotoxicity assays were carried out by Prof. Dr. W. E. G. Müller, Institute for Physiological Chemistry and Pathobiochemistry, University of Mainz, Mainz. Cytotoxicity was tested against L5178Y mouse lymphoma cells using the microculture tetrazolium (MTT) assay, and compared to that of untreated controls (Carmichael *et al*., 1987).

MTT colorimetric assay

Of the test samples, stock solutions in ethanol 96% (v/v) were prepared. Exponentially, growing cells were harvested, counted, and diluted appropriately. From the cell suspension, 50 µL containing 3750 cells were pipetted into 96-well microtiter plates. Subsequently, 50 µL of a solution of the test samples containing the appropriate concentration was added to each well. The concentration range was 3 and 10 µg/mL. The small amount of ethanol present in the wells did not affect the experiments. The test plates were incubated at 37° C with 5% CO_2 for 72 h. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at 5 mg/mL in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4) and from the solution, 20 µL was pipetted into each well. The yellow MTT penetrates the healthy living cells and in the presence of mitochondrial reductase, MTT is transformed to its blue formazan complex. After an incubation period of 3 h 45 min at 37° C in a humidified incubator with 5% CO_2, the medium was centrifuged (15 min, 20° C, 210

x g) with 200 µL DMSO, the cells were lysed to liberate the formed formazan product. After thorough mixing, the absorbance was measured at 520 nm using a scanning microtiter-well spectrophotometer.

The color intensity is correlated with the number of healthy living cells. Cell survival was calculated using the formula:

$$\text{Survival\%} = 100 \times \frac{\text{Absorbance of treated cell - Absorbance of culture cell}}{\text{Absorbance of untreated cells - Absorbance of culture cell}}$$

All experiments were carried out in triplicates and repeated three times. As controls, media with 0.1% EGAMME/DMSO were include in experiments.

Cell cultures

L5178Y mouse lymphoma cells were grown in Eagle's minimal essential medium supplement with 10% horse serum in roller tube culture. The medium contained 100 units/mL penicillin and 100 µg/ml streptomycin. The cells were maintained in a humidified atmosphere at 37° C with 5% CO_2.

2. Results

3.1. Bioscreening results

A collection of sponges from the Gulf of Aqaba in Jordan and from Ambon in Indonesia was screened for the discovery of bioactive metabolites. The methanolic extracts of these sponges were tested against the mouse lymphoma cell line (L5178Y). Among these sponges, *Diacarnus ardoukobae* (**MT1**), *Theonella* sp. (**MT3**), and *Diacarnus erythraeanus* (**MT8**) from the Gulf of Aqaba in Jordan, in addition to *Acanthostrongylophora ingens* (**FB67**) from Ambon in Indonesia exhibited promising results in this cytotoxicity assay. Based on the cytotoxic activity and the HPLC chromatograms of those extracts (Table 3.1), five sponge samples, *viz.*, *Diacarnus ardoukobae* (**MT1**), *Theonella* sp. (**MT3**), *Diacarnus erythraeanus* (**MT8**), *Hemimycale* sp. (**MT6**), and the unknown sponge (**MT5**) from the Gulf of Aqaba in Jordan, in addition to *Acanthostrongylophora ingens* (**FB67**) from Ambon in Indonesia were selected for further chemical investigation.

Table 3.1: Results of cytotoxicity assay for the methanol extracts of the sponges on the mouse lymphoma cell (L5178Y) (Conc. is 10 µg/ml).

NO.	Code	Name of the Sponge	Origin	L5178Y growth in%
1	**MT1**	***Diacarnus ardoukobae***	Gulf of Aqaba	-1.9
2	MT2	*Petrosia elephantotus*	Gulf of Aqaba	118.8
3	**MT3**	***Theonella aff. swinhoei***	Gulf of Aqaba	-1.5
4	MT4	*Amphimedon chloros*	Gulf of Aqaba	6
5	**MT5**	**Unknown sponge**	Gulf of Aqaba	14.6
6	**MT6**	***Hemimycale columella***	Gulf of Aqaba	109.8
8	**MT8**	***Diacarnus erythraeanus***	Gulf of Aqaba	1.3
9	**FB67**	***Acanthostrongylophora ingens***	Ambon	0.0

3.2. Isolation of natural products

Pure compounds have been isolated from different sponges from the Gulf of Aqaba in Jordan and from Ambon in Indonesia by using different chromatographic techniques. Moreover, several isolated compounds exhibited moderate to strong cytotoxic activity.

3.3. Isolated compounds from the Gulf of Aqaba in Jordan

The clear warm water and the abundance of marine life in the Red Sea have been attracting many scientists to investigate sponges from this area. Indeed, several biologically active compounds have been isolated from the Red Sea, like latrunculins A and B (Groweiss *et al*., 1983), subereamolline A (Shaala *et al*., 2012), and noresterterpene cylic peroxides (Lefranc *et al*., 2013). Geographically, the Gulf of Aqaba is one of the two northern branches of the Red Sea (Khalaf *et al*., 2013). Reviewing the current literature, only one study has been reported from the Gulf of Aqaba in Jordan (Fouad *et al*., 2004). In this study, two new steroidal saponins, eryloside K and L have been isolated. Thus, it was interesting to us to investigate some untouched sponges from this untouched area.

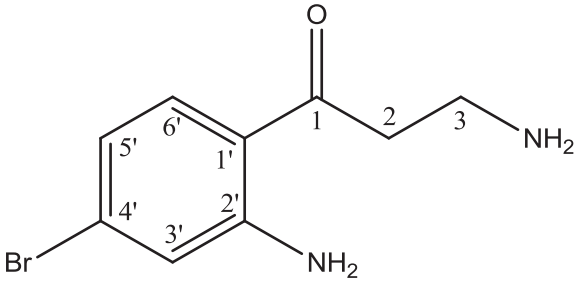
3.3.1. Metabolites from the unknown sponge (MT5)

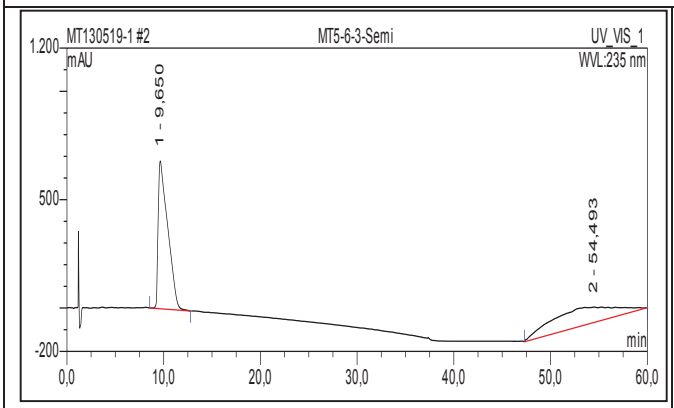
An attempt was done to identify the unknown sponge (**MT5**) in the Naturalism Biodiversity Center in Netherland but unfortunately, it failed. This sponge was collected in the front of the marine science station at 20 m depth from the Jordanian coast of the Gulf of Aqaba by SCUBA diving. The sponge (300 g) was freeze-dried and cut into small pieces, followed by exhaustive extraction with methanol. The obtained crude extract (250 mg) was further partitioned between EtOAc and H_2O. The ethyl acetate fraction was then filtered and evaporated to dryness using a rotary evaporator. The dried extract was then redissolved in methanol, and was further separated using different chromatographic techniques affording 3-amino-1-(2-amino-

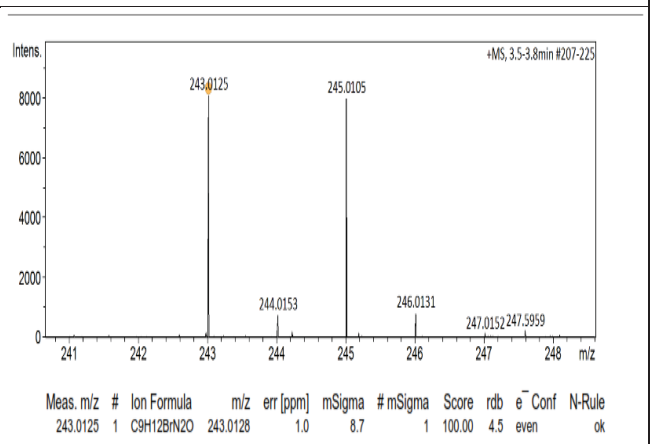
4-bromophenyl) propan-1-one (**1**, 3.5 mg), 7-bromoquinolin-4(1*H*)-one (**2**, 1 mg), 6-bromoindole-3-carbaldehyde (**3**, 1.1 mg) and caulerpin (**4**, 1 mg), (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**, 1.8 mg), (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**, 1.6 mg), respectively.

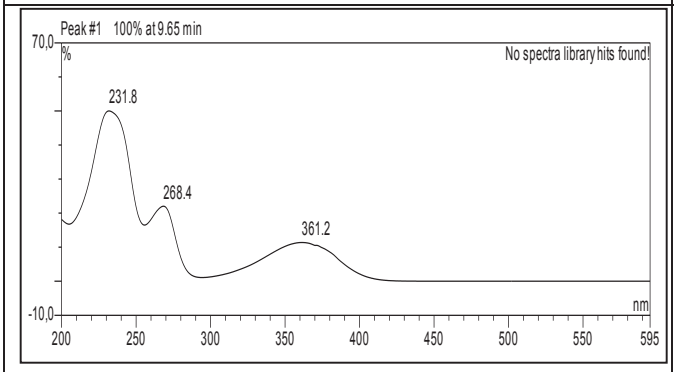
3.3.1.1. 3-Amino-1-(2-amino-4-bromophenyl) propan-1-one (1: new natural product)

3-Amino-1-(2-amino-4-bromophenyl) propan-1-one	
Synonym(s)	3-Amino-1-(2-amino-4-bromophenyl)propan-1-one
Sample code	MT5-6-3
Biological source	Unknown sponge (**MT5**)
Sample amount	3.5 mg
Physical Description	Yellow solid
Molecular Formula	$C_9H_{12}BrN_2O$
Molecular Weight	242 g/mol.
Retention time HPLC	9.65 min (standard gradient)





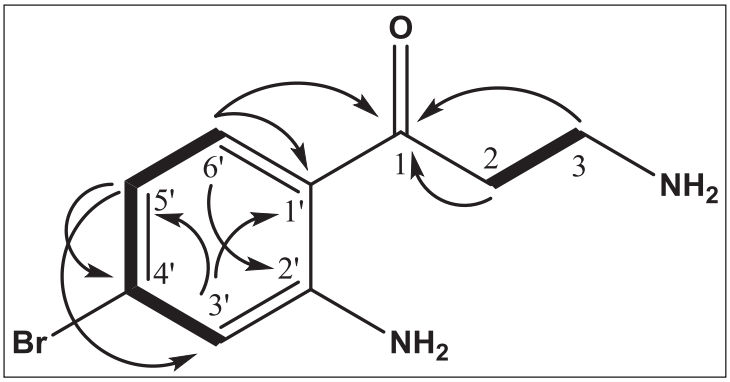




No inonization in negative ESI-MS

Results

Compound (**1**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The HRESIMS of compound (**1**) showed an isotopic cluster of $[M+H]^+$ ions in the ratio of 1:1 at *m/z* 243.0125 and 245.0105, indicating that the molecular weight is 242 g/mol. with the molecular formula $C_9H_{12}BrN_2O$ (calculated 243.0055, Δ 0.007). Compound (**1**) showed UV absorption at λ_{max} (MeOH) 231.8, 268.4, and 361.2 nm. The ^{1}H NMR spectrum (Figure 3.1) demonstrated the presence of a doublet of doublets at δ_H 6.73 ppm [dd (*J*=1.9, 8.6 Hz)] which was assigned to H-5', in addition to two doublets *viz.*, at δ_H 7.63 ppm [d (*J*=8.6 Hz)], and 6.99 ppm [d (*J*=1.9 Hz)] which were attributed to H-6', and H-3', respectively (ABX system). However, the signals of both H-2 and H-3 were overlapped by the water signal. The ^{13}C-NMR (Figure 3.2) and DEPT (Figure 3.3) spectra revealed the presence of 9 carbons including two methylene carbons at δ_C (36.7, 36.3 ppm) which were assigned to C-2 and C-3, respectively, and a deshielded signal resonating at δ_C (199.7 ppm) was attributed to C-1 (Table 3.2). The ^{1}H-^{1}H COSY spectrum (Figure 3.4) of compound (**1**) demonstrated two spin systems, the first one is CH(3')CH(5')CH(6') for the aromatic system, and the other spin system is CH_2(2)CH_3(3). Further structure elucidation was performed through a HMBC experiment. The HMBC spectrum (Figure 3.5) showed correlations from both CH_2-2, and CH_2-3 to C-1 (δ_C 199.7), from H-6' at δ_H 7.63 ppm to C-1 (δ_C 199.7), C-1' (δ_C 116.6), C-2' (δ_C 154.0), C-4'(δ_C 130.8), C-5' (δ_C 119.3), from H-3' at δ_H 6.99 ppm to C-1' (δ_C 116.6), C-4' (δ_C 130.8), and C-5' (δ_C 119.3). Further inspection of the HMBC spectrum revealed also correlations from H-5' at δ_H 6.73 ppm to C-1' (δ_C 116.6), C-3' (δ_C 120.7), and C-4' (δ_C 130.8). The assignment of the NH_2 and Br substituents to C-2' and C-4' was corroborated by the chemical shifts of their respective carbons at δ_C (154.0, and 130.8 ppm), respectively (Table 3.2).



Structure of compound (**1**).

^{1}H-^{1}H COSY

HMBC

Table 3.2: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data for compound (**1**).

Pos.	**δ_C (CD_3OD)**	**δ_H (CD_3OD)**	**HMBC**
1	199.7[a]	-	-
2	36.7	b	1
3	36.3	b	1
1’	116.6[a]	-	-
2’	154.0[a]	-	-
3’	120.7	6.99, *d* (1.9)	1’, 4’, 5’
4’	130.8[a]	-	-
5’	119.3	6.73, *dd* (1.9, 8.6)	1’, 3’, 4’
6’	132.7	7.63, *d* (8.6)	1, 1’, 2’, 4’, 5’

(a) Signals were extracted from the HMBC spectrum.

(b) Overlapped with the water signal.

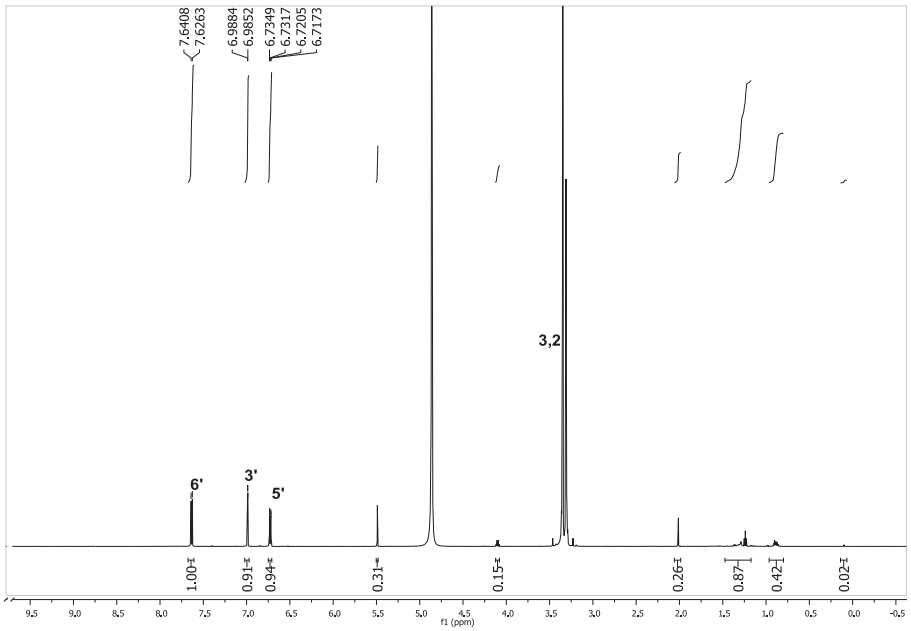


Figure 3.1: ^{1}H NMR spectrum of compound (**1**).

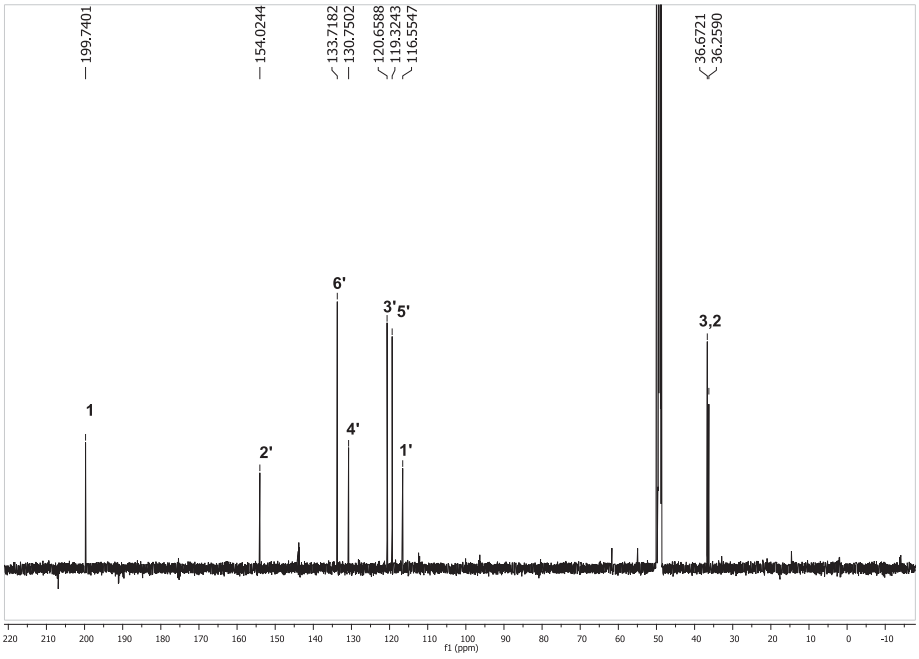


Figure 3.2: ^{13}C NMR spectrum of compound (**1**).

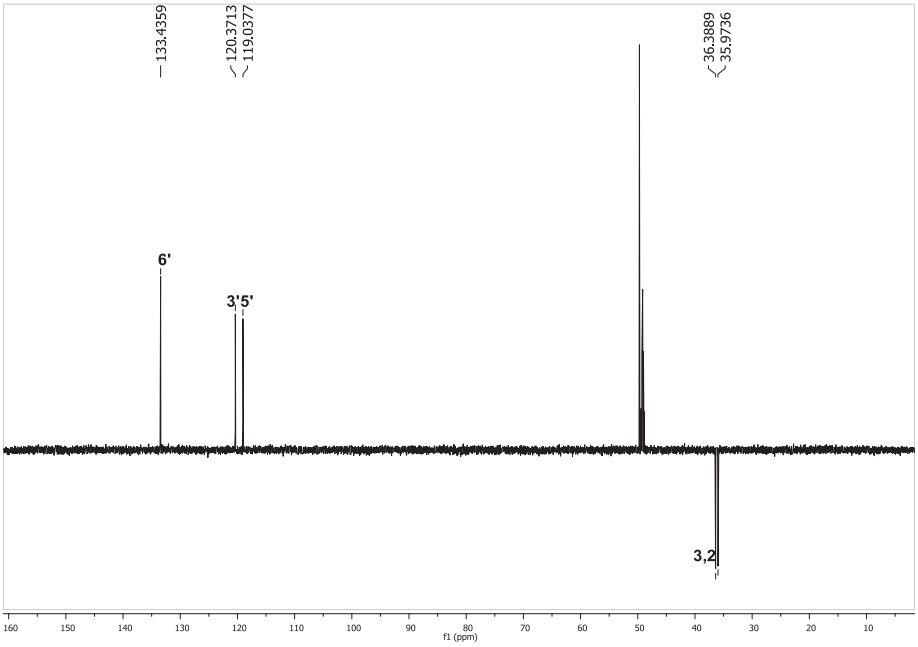


Figure 3.3: DEPT spectrum of compound (**1**).

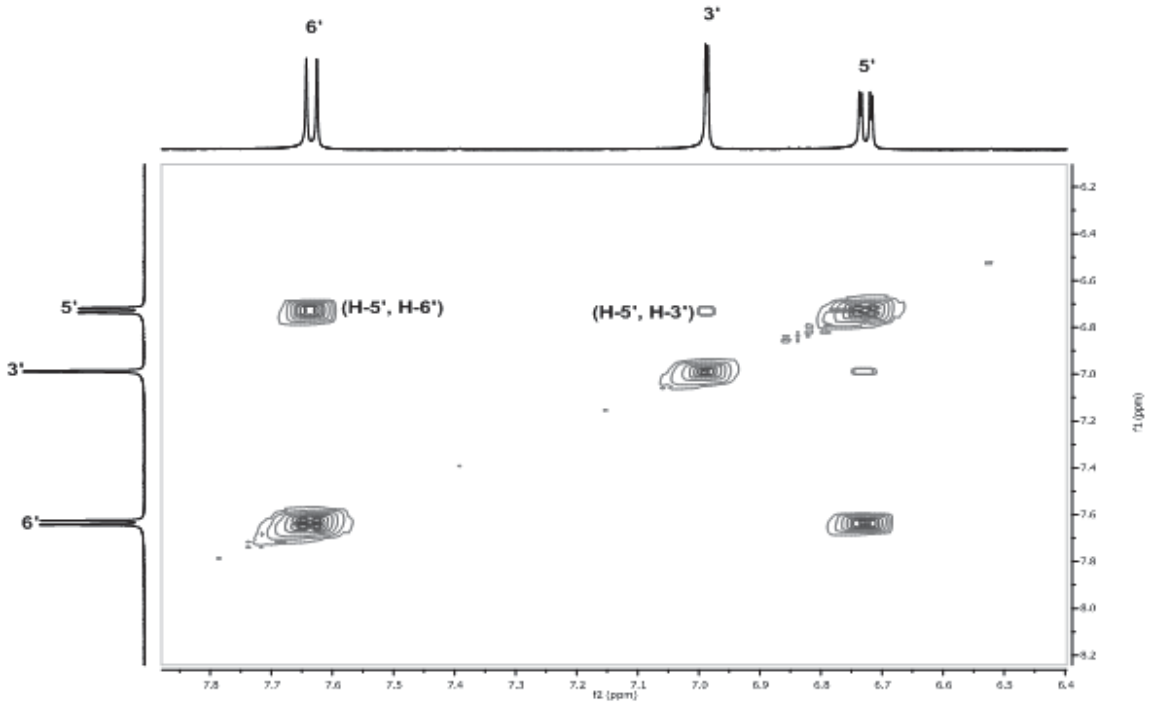


Figure 3.4: ^{1}H-^{1}H COSY spectrum of compound (**1**).

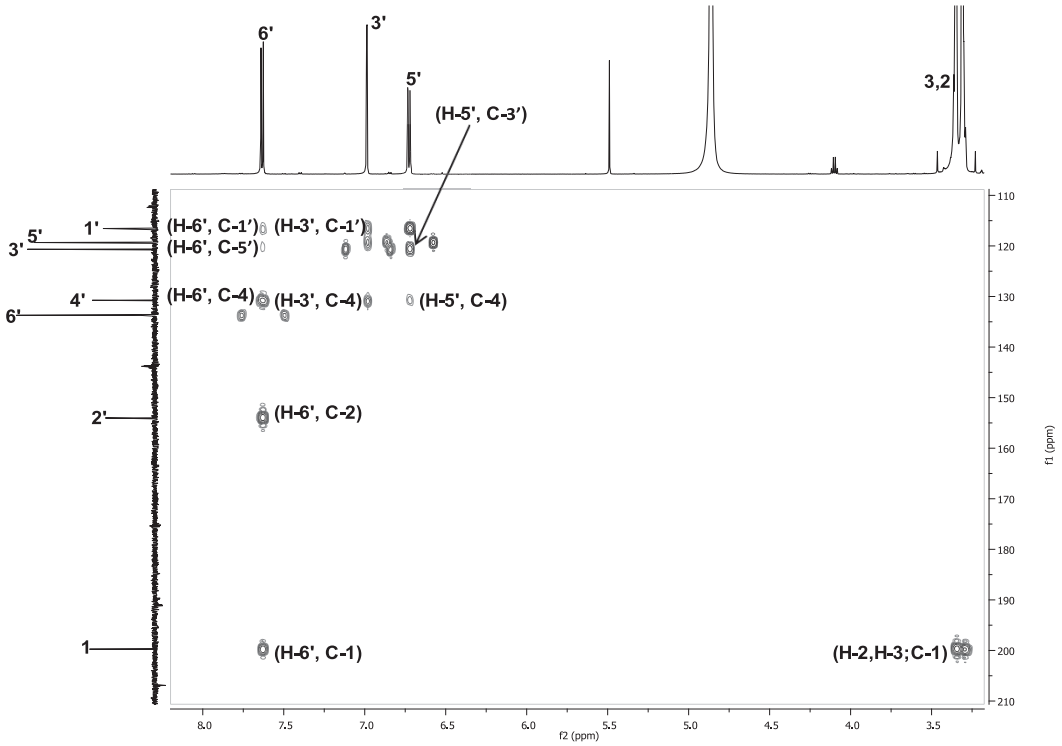
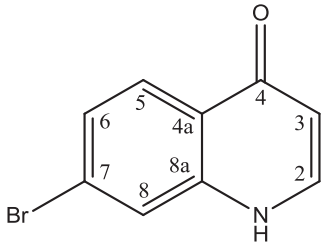
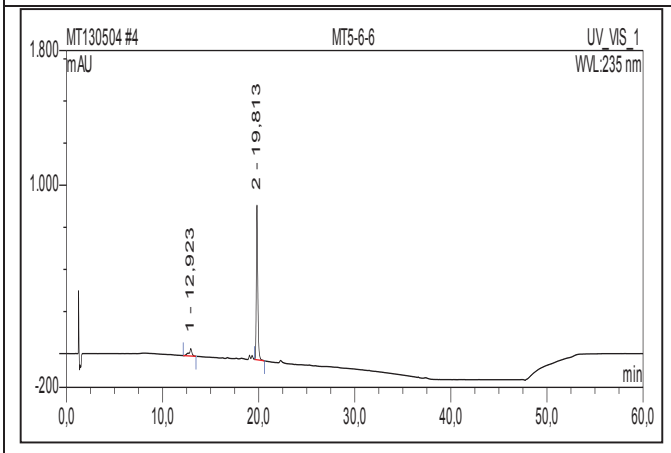


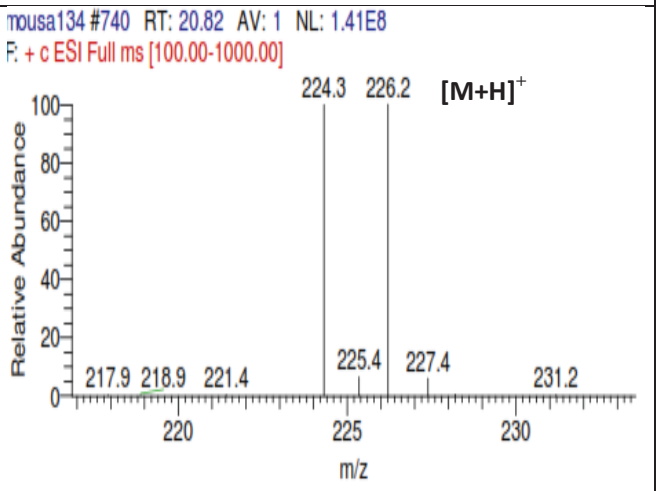
Figure 3.5: HMBC spectrum of compound (**1**).

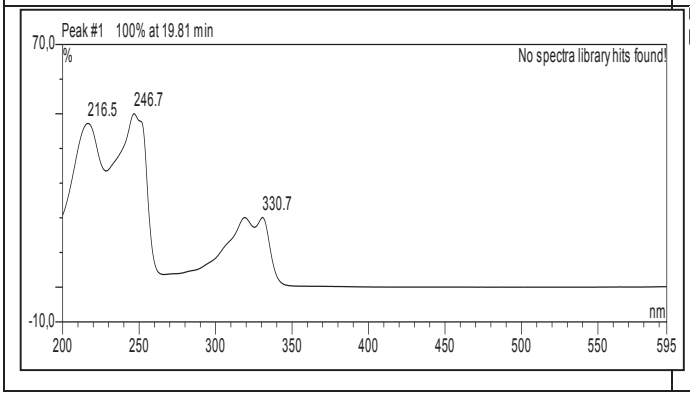
3.3.1.2. 7-Bromoquinolin-4(1*H*)-one (2: new natural product)

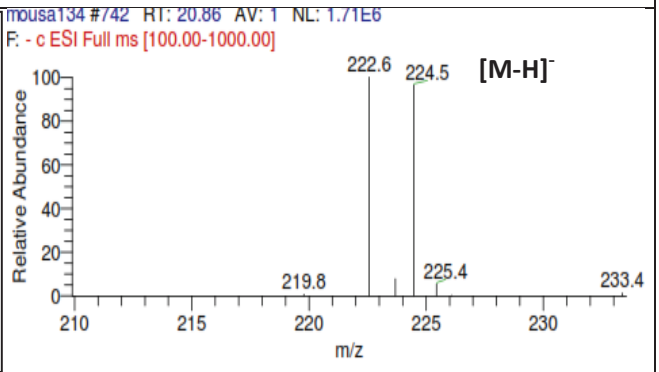
7-Bromoquinolin-4(1*H*)-one	
Synonym(s)	7-Bromoquinolin-4(1*H*)-one
Sample code	MT5-6-6
Biological source	Unknown sponge (**MT5**)
Sample amount	1 mg
Physical Description	Yellow solid
Molecular Formula	C_9H_6BrNO
Molecular Weight	223 g/mol.
Retention time HPLC	19.81 min (standard gradient)





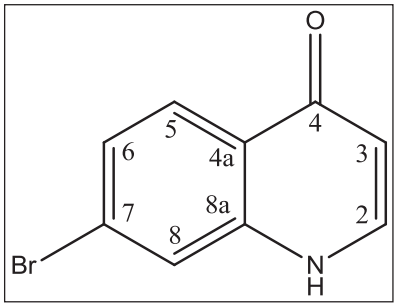






Results

Compound (**2**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The ESI mass spectrum of (**2**) revealed *pseudo*-molecular ion peaks at *m/z* 224/226.3 $[M+H]^+$ and 222.4/224.5 $[M-H]^-$ (base beak), with a ratio of (1:1), revealing a molecular weight of 223 g/mol. and containing one bromine atom. Compound (**2**) showed UV absorption at λ_{max} (MeOH) 216.5, 246.7, and 330.7 nm. The ^{1}H NMR showed the presence of one doublet of doublets at δ_H 7.55 ppm [dd (*J*=1.6, 8.7 Hz)], two doublets *viz.*, at δ_H 8.15 ppm [d (*J*=8.7 Hz)], and δ_H 7.80 ppm [d (*J*=1.6 Hz)] which were assigned to H-6, H-5, and H-8, respectively (ABX system). Moreover, two downfield doublets *viz.*, at δ_H 7.99 ppm [d (*J*=7.3 Hz)] and δ_H 6.35 ppm [d (*J*=7.3 Hz)] were attributed to the *cis*-oriented olefinic protons H-2, and H-3, respectively. The ^{1}H-^{1}H COSY spectrum (Figure 3.6) showed two spin systems, the first spin system is CH(5)CH(6)CH(8) in the aromatic ring and the second one is CH_2(2)CH_2(3). Further elucidation of (**2**) was performed through a HMBC experiment in which correlations from H-5 at δ_H 8.15 ppm to C-8a (δ_C 140.0), from H-2 at δ_H 7.99 ppm to C-4 (δ_C 177.9), and C-8a (δ_C 140.0), from H-3 at δ_H 6.35 ppm to C-2 (δ_C 123.5) were detected. Also H-3 at δ_H 6.35 ppm showed an ω-correlation to C-8a (δ_C 140.0). Further inspection of the HMBC spectrum (Figure 3.7) also revealed correlations from H-6 at δ_H 7.55 ppm to C-8 (δ_C 122.7), and from H-8 at δ_H 7.80 ppm to C-4a (δ_C 125.4). Further structural confirmation was achieved by comparison of the ^{1}H NMR, and mass spectral data of compound (**2**) with those of the same synthetic compound (Al-Awadi *et al*., 2007) (Table 3.3).



Structure of compound (**2**).

Table 3.3: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data for compound (**2**).

Pos.	δ_C (CD_3OD)[a,b]	δ_C (CD_3OD)[a]	δ_H (DMSO-d_6)[a]	δ_H (DMSO-d_6)[c]	HMBC[a]
1	-	-	-	-	-
2	123.5	7.99, *d* (7.3)	7.92, *brt* (7.15)	7.82, *d* (7.37)	4, 8a
3	108.0	6.35, *d* (7.3)	6.05, *d* (7.4)	6.04, *d* (7.37)	2, 8a
4	177.9	-	-	-	-
5	124.6	8.15, *d* (8.7)	7.99, *d* (8.6)	8.0, *d* (8.56)	8a
6	133.2	7.55, *dd* (1.6, 8.7)	7.45, *dd* (1.7, 8.6)	7.52, *d* (8.56)	-
7	129.7	-	-	-	-
8	122.7	7.80, *d* (1.6)	7.75, *d* (1.5)	7.74, *s*	4a
8a	140.0	-	-	-	-
4a	125.4	-	-	-	-

(a) Compound (**2**) (b) Signals were extracted from the HMBC spectrum (c) Al-Awadi *et al*., 2007

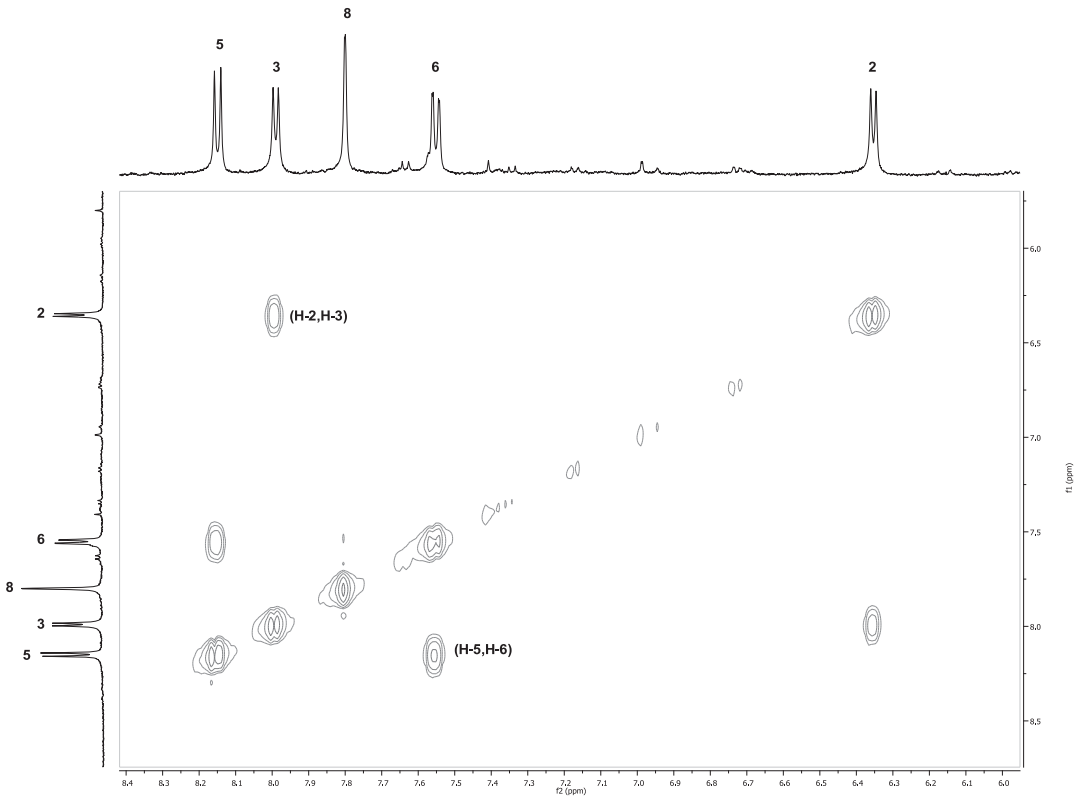


Figure 3.6: ^{1}H-^{1}H COSY spectrum of compound (**2**).

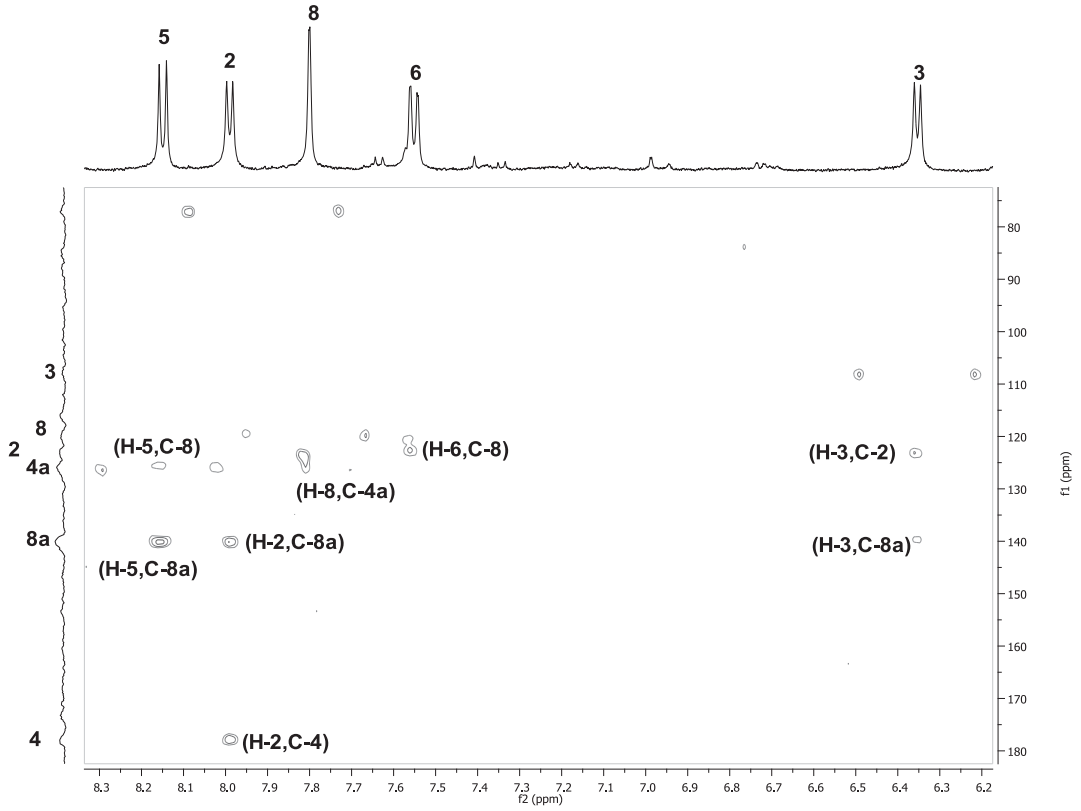
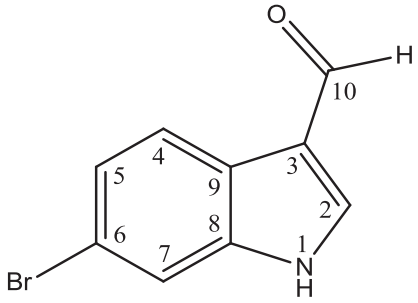
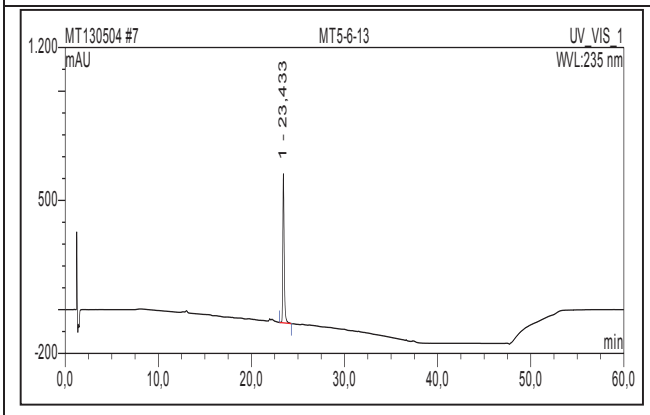


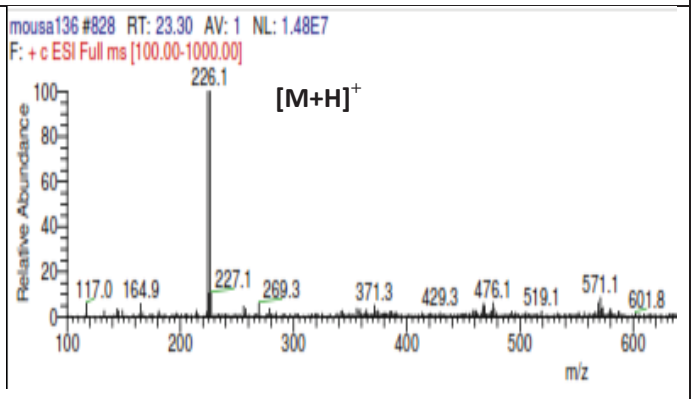
Figure 3.7: HMBC spectrum of compound (**2**).

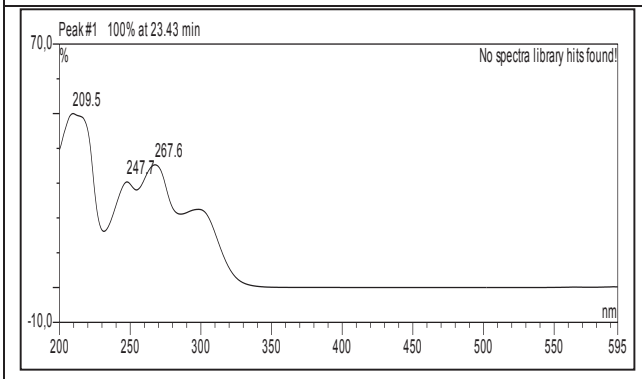
3.3.1.3. 6-Bromoindole-3-carbaldehyde: (3: known natural product)

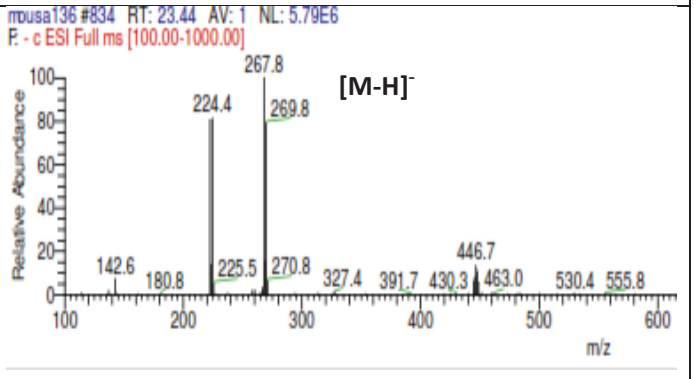
6-Bromoindole-3-carbaldehyde	
Synonym(s)	6-Bromoindole-3-carbaldehyde
Sample code	MT5-6-13
Biological source	Unknown sponge (**MT5**)
Sample amount	1.2 mg
Physical Description	yellowish solid
Molecular Formula	C_9H_6BrNO
Molecular Weight	223 g/mol.
Retention time HPLC	23.43 min (standard gradient)





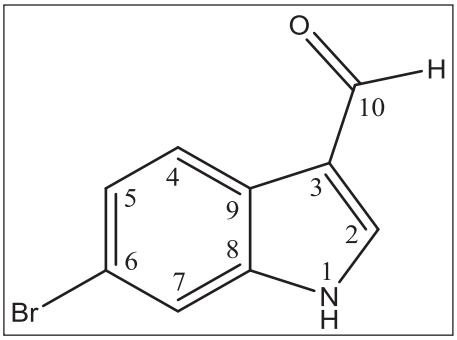






Compound (**3**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The ESI mass spectrum of compound (**3**) revealed *pseudo*-molecular ion peaks at m/z 224/226.3 $[M+H]^+$ and 222.4/224.5 $[M-H]^-$ (base beak), with a ratio of 1:1, revealing a molecular weight of 223 g/mol. and containing one bromine atom. Compound (**3**) showed UV absorption at λ_{max} (MeOH) 209.5, 2447.7, and 267.7 nm. The ^{1}H NMR spectrum for compound (**3**) showed one doublet at δ_H 8.32 ppm [d (*J*=2.4 Hz)] which was assigned to the olefinic H-2, two doublets *viz*., at δ_H 8.02 ppm [d (*J*=8.4 Hz)], and δ_H 7.71 ppm [d (*J*=1.6 Hz)] which were attributed to H-4, and H-7, and one doublet of doublets (H-5) at δ_H 7.36 ppm [d (*J*=1.6, 8.4 Hz)] was also observed, respectively (ABX system) for the aromatic ring (Table 3.4). This assumption was corroborated by the ^{1}H-^{1}H COSY spectrum (Figure 3.8) which showed two spin systems, the first spin system is CH(4)CH(5)CH(7) while the second one is CH(2)CH(10) (Table 3.4). The structure of (**3**) was finally confirmed by comparison of the ^{1}H NMR, and mass spectral data of (**3**) with those of the published literature (Olguin-Uribe *et al.*, 1997) (Table 3.4).

It worth to mention that compound (**3**) was isolated from other sponges like *Pleroma menoui* (Guella *et a*l., 1988), from other marine organisms *viz.*, *Stomozoa murrayi* tunicate (Olguin-Uribe *et al.*, 1997), *Tubastrea faulkneri* coral (Okuda *et al*., 1982), and from the marine-associated bacterium *Acinetobacter* sp. (Olguin-Uribe *et al.*, 1997).



Structure of compound (**3**).

Table 3.4: ^{1}H NMR (600 MHz) and COSY data of compound (**3**).

Pos.	**δ_H (DMSO-d_6)[a]**	**δ_H (DMSO-d_6)[c]**	**COSY[a]**
1	9.93, *s*	-	-
2	8.32, *d* (2.4)	8.20	10
3	-	-	-
4	8.02, *d* (8.4)	7.82	5
5	7.36, *dd* (1.6, 8.4)	7.44	4,7
6	-	-	-
7	7.71, *d* (1.6)	7.61	5
8	-	-	-
9	-	-	-
10	12.22, *brs*	-	2

(a) Compound (3) (b) Olguin-Uribe *et al*., 1997

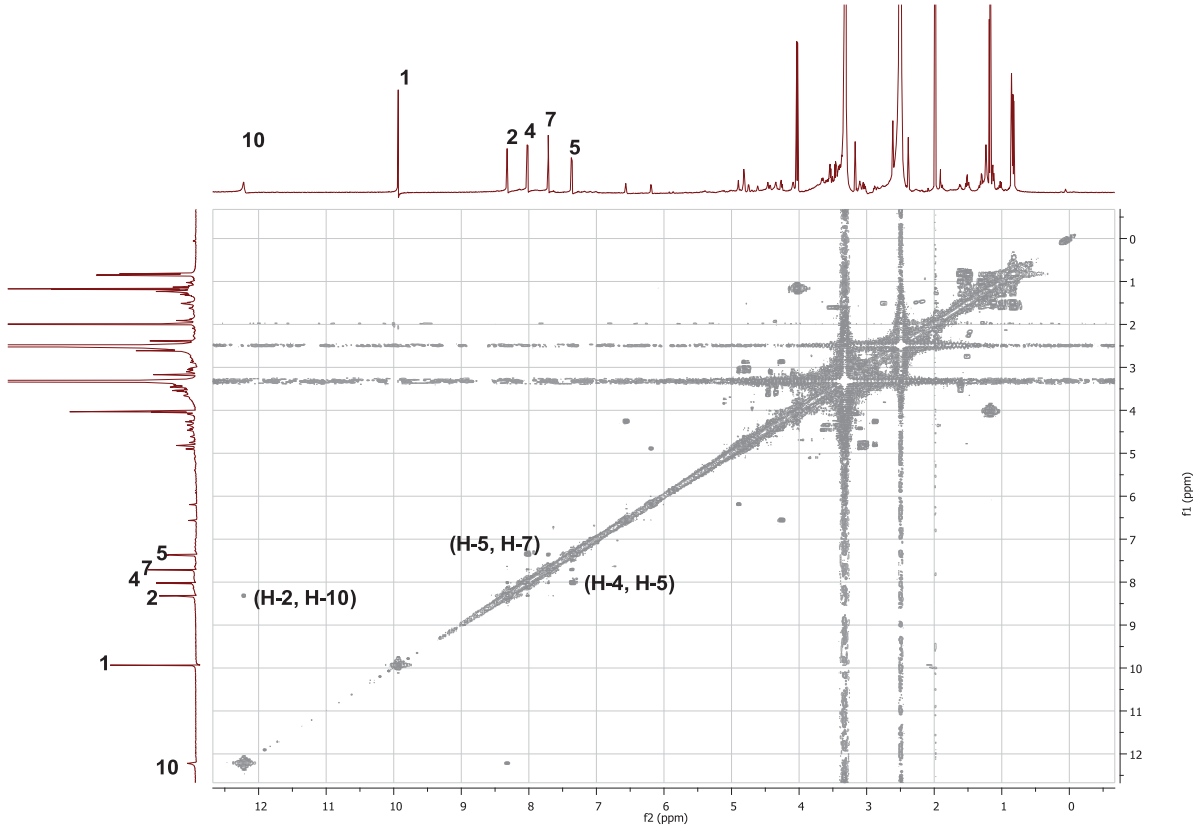
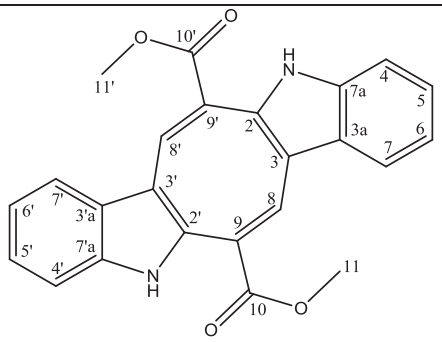
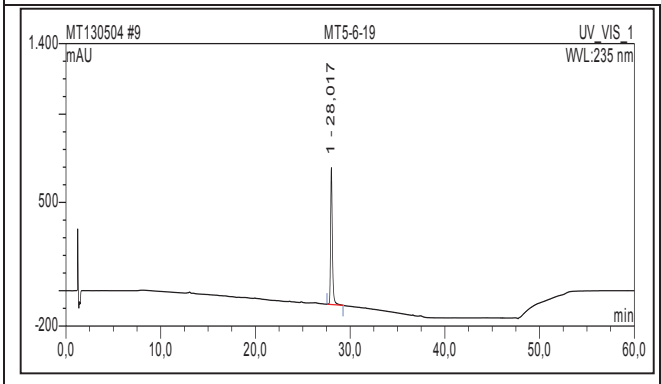


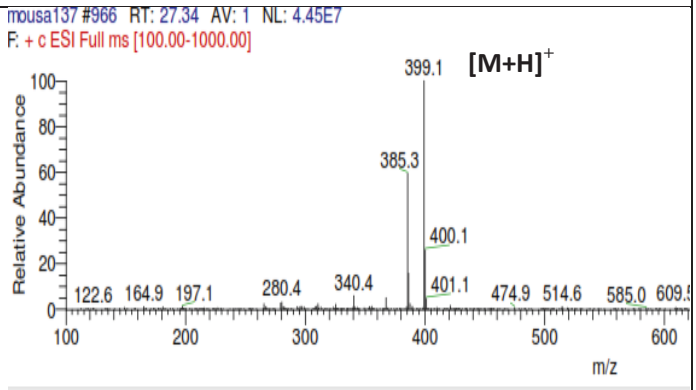
Figure 3.8: ^{1}H-^{1}H COSY spectrum of compound (**3**).

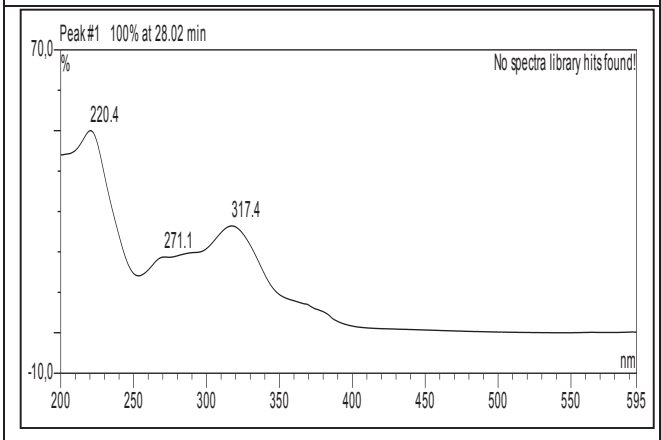
3.3.1.4. Caulerpin (4: known natural product)

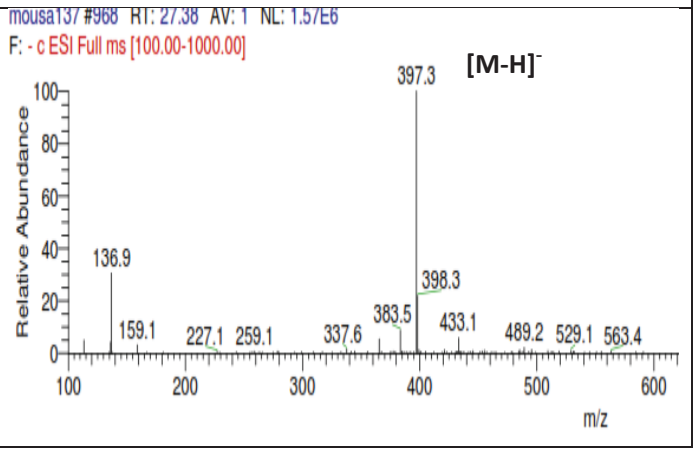
Caulerpin	
Synonym(s)	(6*E*,13*E*)-Dimethyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate
Sample code	MT5-6-19
Biological source	Unknown sponge (**MT5**)
Sample amount	1 mg
Physical Description	yellowish solid
Molecular Formula	$C_{24}H_{18}N_3O_2$
Molecular Weight	398.41 g/mol.
Retention time HPLC	28.02 min (standard gradient)





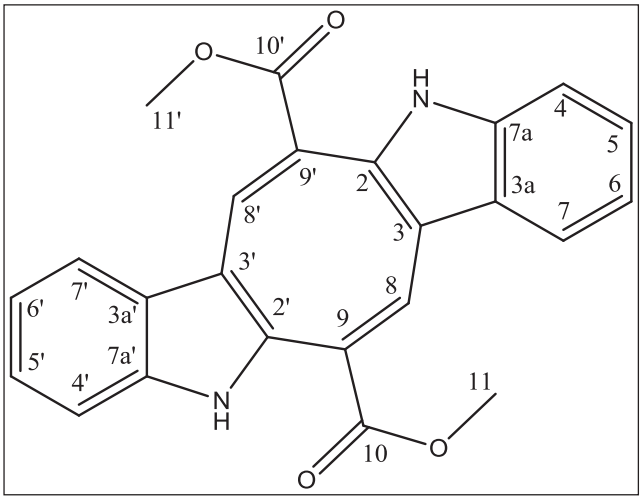






Compound (**4**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The ESI mass spectrum of compound (**4**) revealed *pseudo*-molecular ion peaks at *m/z* 399.1 $[M+H]^+$ and 397.3 $[M-H]^-$ (base peak), respectively revealing a molecular weight of 398 g/mol. Compound (**4**) showed UV absorption at λ_{max} (MeOH) 220.4, and 317.4 nm. The ^1H NMR spectrum indicated a plane of symmetry by showing two doublets *viz.*, at δ_H 7.30 ppm [2H, d (*J*=8.0 Hz)], and δ_H 7.42 ppm [2H, d (*J*=8.0 Hz)] which were assigned to H-4/H-4', and H-7/H-7', respectively, two doublets of doublets *viz.*, at δ_H 7.04 ppm [2H, dd (*J*=7.2, 8.0 Hz)], and δ_H 7.12 ppm [2H, d (*J*=7.2, 8.0 Hz)] which were attributed to H-5/H-5', and H-6/H-6', respectively, and one singlet in the down field region (H-8/H-8') at δ_H 8.16 ppm [2H, s] in the eight-membered aromatic ring. Furthermore, two methoxyl signals (OCH_3-11/OCH_3-11') at δ_H 3.85 ppm [6H, s] were also observed (Table 3.5). The ^1H-^1H COSY spectrum of (**4**) (Figure 3.6) revealed one spin system CH(4/4')CH(5/5')CH(6/6')CH(7/7'). Further elucidation of (**4**) was performed through the HMBC experiment in which correlations from OCH_3-11/OCH_3-11' at δ_H 3.85 ppm to C-10/C-10' (δ_C 168.6), from H-8/H-8' at δ_H 8.16 ppm to C-2/C-2' (δ_C 134.7), and C-10/C-10' (δ_C 168.6), from H-4/H-4' at δ_H 7.30 ppm to C-3a/C-3a' (δ_C 129.3), and C-6/C-6' (δ_C 124.1), from H-5/H-5' at δ_H 7.04 ppm to C-7/C-7' (δ_C 119.2), and C-7a/C-7a' (δ_C 140.0). Further inspection of the HMBC spectrum of (**4**) (Figure 3.9) revealed also correlations from H-6/H-6' at δ_H 7.12 ppm to C-3a/C-3a' (δ_C 129.3), and C-4/C-4' (δ_C 112.7), from H-7/H-7' at δ_H 7.42 ppm to C-5/C-5' (δ_C 121.7), and C-7a/C-7a' (δ_C 140.0). The structure of compound (**4**) was finally confirmed by comparison of ^1H NMR, ^{13}C NMR, and mass spectral data with published data of the same known compound caulerpin (Rocha *et al*., 2007) (Table 3.5). It is worth to mention that compound (**4**) is described from sponge material here for the first time while it was previously reported to be produced only

by algae (de Souza *et al.*, 2009**)**. Caulerpin belongs to a family of bisindole natural products and has an extra eight-membered ring between the two indole mioties (de Souza *et al.*, 2009**)**.



Structure of compound (**4**).

Table 3.5: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of (**4**).

Pos.	**δ_C (CD_3OD)[a,b]**	**δ_C (DMSO-d_6)[c]**	**δ_H (CD_3OD)[a]**	**δ_H (DMSO-d_6)[c]**	**HMBC[a]**
1	-	-	-	-	-
2/2'	134.7	132.69	-	-	-
3/3'	-	110.99	-	-	-
3a/3a'	129.3	126.74	-	-	-
4/4'	112.7	111.57	7.30, 2H, *d* (8.0)	7.32, 2H, *d* (8)	3a, 6
5/5'	121.7	119.90	7.04, 2H, *dd* (7.2, 8.0)	7.05, 2H, *dd* (7.2, 7.7)	7, 7a
6/6'	124.1	122.59	7.12, 2H, *dd* (7.2, 8.0)	7.14, 2H, *dd* (7.3, 7.8)	3a, 4
7/7'	119.2	117.65	7.42, 2H, *d* (8.0)	7.42, 2H, *d* (7.98)	5, 7a
7a/7a'	140.0	137.30	-	-	-
8/8'	143.9	141.44	8.16, 2H, *s*	8.16, 2H, *s*	10, 2
9/9'	127.2	125.68	-	-	-
10/10'	168.6	165.62	-	-	-
11/11'	53.0	52.09	3.85, 6H, *s*	3.77, 6H, *s*	10

(a) Compound (**4**) (b) Signals were extracted from the HMBC spectrum (c) Rocha *et al*., 2007

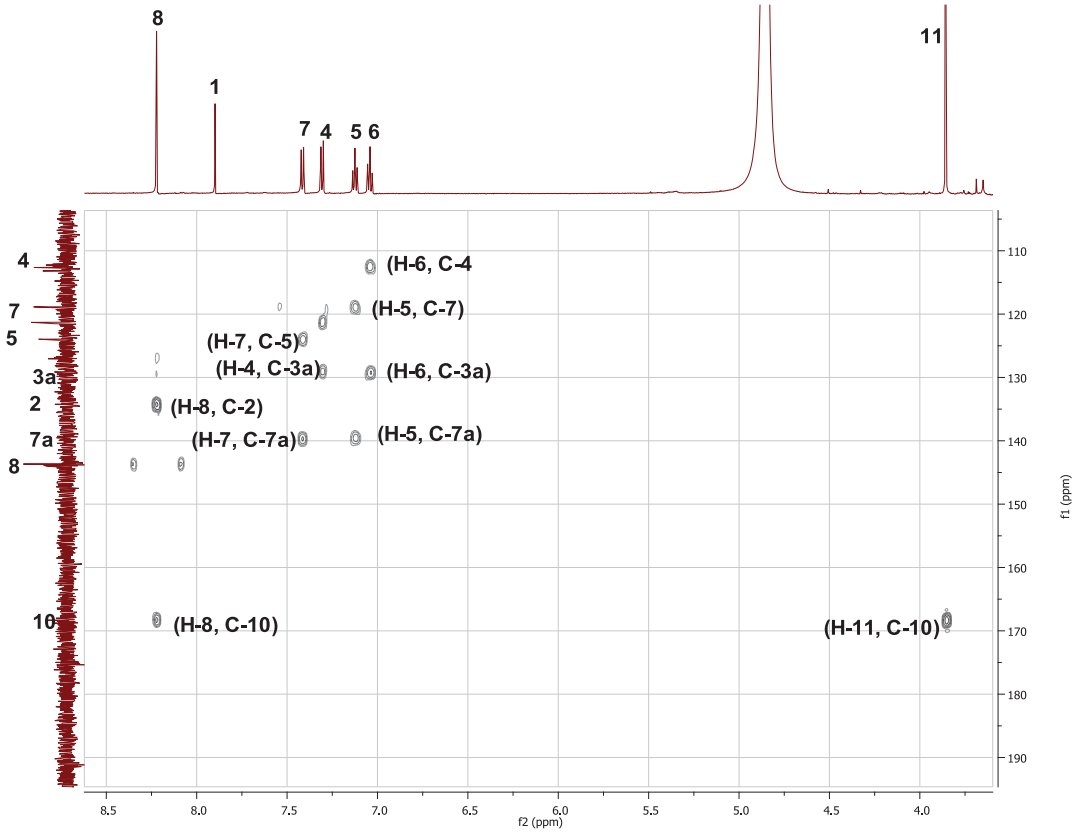
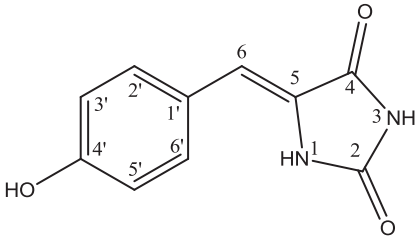
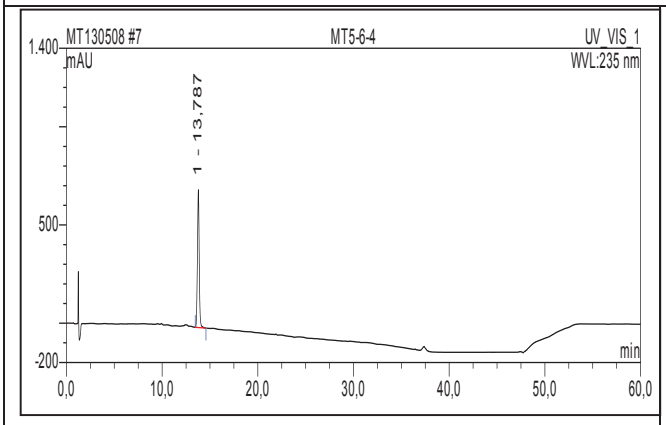


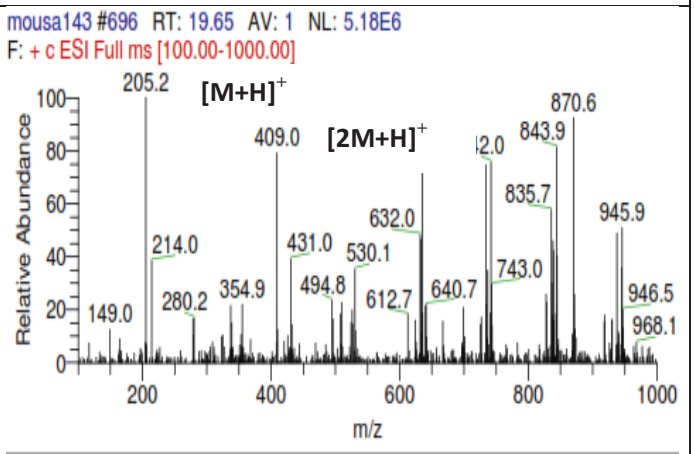
Figure 3.9: HMBC spectrum of compound (**4**).

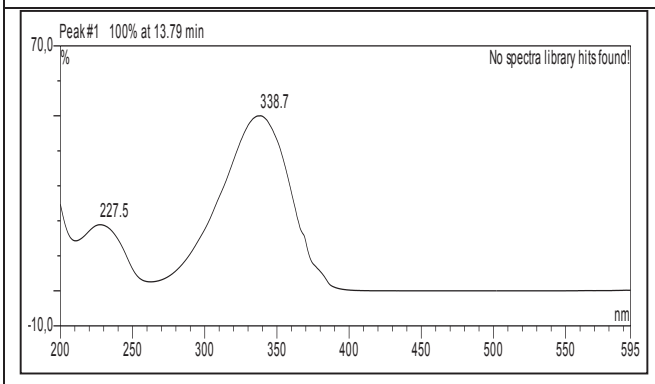
3.3.1.5. (*Z*)-5-(4-Hydroxybenzylidene)-hydantoin (PMH) (5: known natural product)

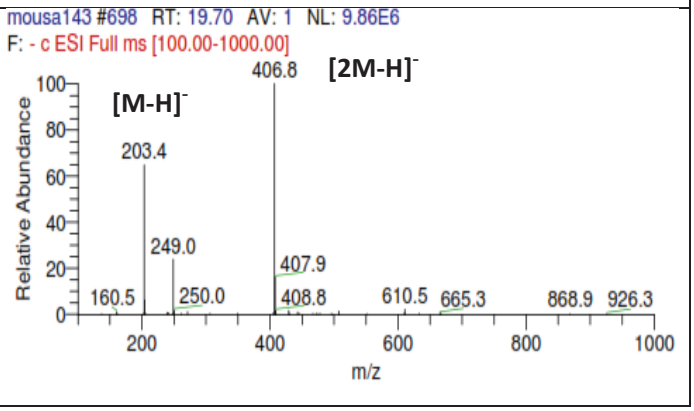
(*Z*)-5-(4-Hydroxybenzylidene)-hydantoin	
Synonym(s)	(*Z*)-5-(4-Hydroxybenzylidene)-hydantoin
Sample code	MT5-6-4
Biological source	Unknown sponge (**MT5**)
Sample amount	1.8 mg
Physical Description	yellowish solid
Molecular Formula	$C_9H_8N_2O_3$
Molecular Weight	204.18 g/mol.
Retention time HPLC	13.79 min (standard gradient)



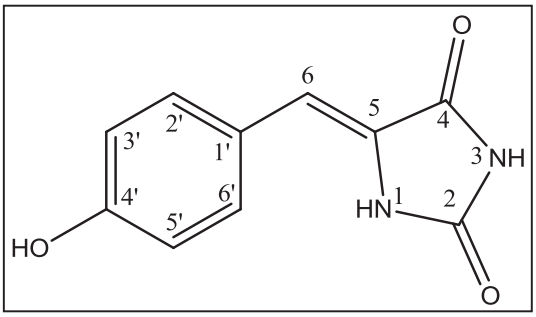








Compound (**5**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The ESI mass spectrum of compound (**5**) revealed *pseudo*-molecular ion peaks at *m/z* 205.2 $[M+H]^+$, 409.0 $[2M+H]^+$, 203.4 $[M-H]^-$, and 406.8 $[2M-H]^-$, respectively indicating a molecular weight of 204 g/mol. Compound (**5**) showed UV absorption at λ_{max} (MeOH) 226.6 nm. The ^{1}H NMR spectrum of (**5**) showed two doublets, viz., at δ_H 6.78 ppm [2H, d (*J*=7.2 Hz)], and δ_H 7.47 ppm [2H, d (*J*=7.2 Hz)] which were assigned to H-2'/H-6' and H-3'/H-5', respectively (AA'BB' system) in the aromatic ring, four signals in the down field region at δ_H 11.10 ppm (s), δ_H 10.3 ppm (s), δ_H 6.35 ppm (s), and δ_H 9.88 ppm (s) which were assigned to H-1, H-3, H-6, and OH-4', respectively (Table 3.6). This assumption was corroborated by the ^{1}H-^{1}H COSY spectrum which indicated one continuous spin system which is CH(6)CH(2')CH(3')CH(5')CH(6'). The structure of compound (**5**) was further confirmed through the HMBC spectrum (Figure 3.6). This HMBC spectrum showed correlations from H-6 at δ_H 6.35 ppm to C-4 (δ_C 165.7), C-5 (δ_C 123.9), C-2' (δ_C 132.3), and C-6' (δ_C 132.3), from H-2' at δ_H 7.47 ppm to C-6 (δ_C 110.7), C-3' (δ_C 116.4), and C-4' (δ_C 158.3), and from H-6' at δ_H 7.47 ppm to C-6 (δ_C 110.7), C-3' (δ_C 116.4), and C-4' (δ_C 158.3). Further inspection of the HMBC spectrum (Figure 3.6) also displayed correlations from H-3' at δ_H 6.78 ppm, to C-1' (δ_C 125.4), and C-2' (δ_C 132.3), and from H-5' at δ_H 6.78 ppm to C-1' (δ_C 125.4), and C-6' (δ_C 132.3). The geometrical isomerism (*E*/*Z* isomers) was possible due to the restricted rotation around the exocyclic C=C double bond of the PMHs (Khanfar *et al*., 2009). Further confirmation for the structure (**5**) with *Z*-geometry was achieved by comparison of the ^{1}H NMR, ^{13}C NMR, and mass spectral data of this compound with published data (Ha *et al*., 2011) (Table 3.6). Compound (**5**) is an imidazolidine alkaloid derivative which was isolated before from the Red Sea sponge *Hemimycale arabica* (Mudit and Elsayed, 2011).



Structure of compound (**5**).

Table 3.6: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of (**5**).

Pos.	**δ_C (DMSO-d_6)[a,b]**	**δ_C (DMSO-d_6)[c]**	**δ_H (DMSO-d_6)[a]**	**δ_H (DMSO-d_6)[c]**	**HMBC[a]**
1	-	-	11.10, *s*	11.10, *s*	-
2	155.6	156.3	-	-	-
3	-	-	10.3, *s*	10.3, *s*	-
4	165.7	166.3	-	-	-
5	123.9	126.0	-	-	-
6	110.7	110.0	6.35, *s*	6.34, *s*	4, 5, 2', 6'
1'	125.4	124.5	-	-	-
2', 6'	132.3	131.9	7.47, *d* (7.2)	7.46, *d* (8.0)	6, 3', 4'
3', 5'	116.4	116.4	6.78, *d* (7.2)	6.77, *d* (8.0)	1', 2', 6'
4'	158.3	158.7	-	-	-
OH-4'	-	-	9.88, *s*	9.84, *s*	-

(a) Compound (**5**) (b) Signals were extracted from the HMBC spectrum (c) Ha *et al*., 2011

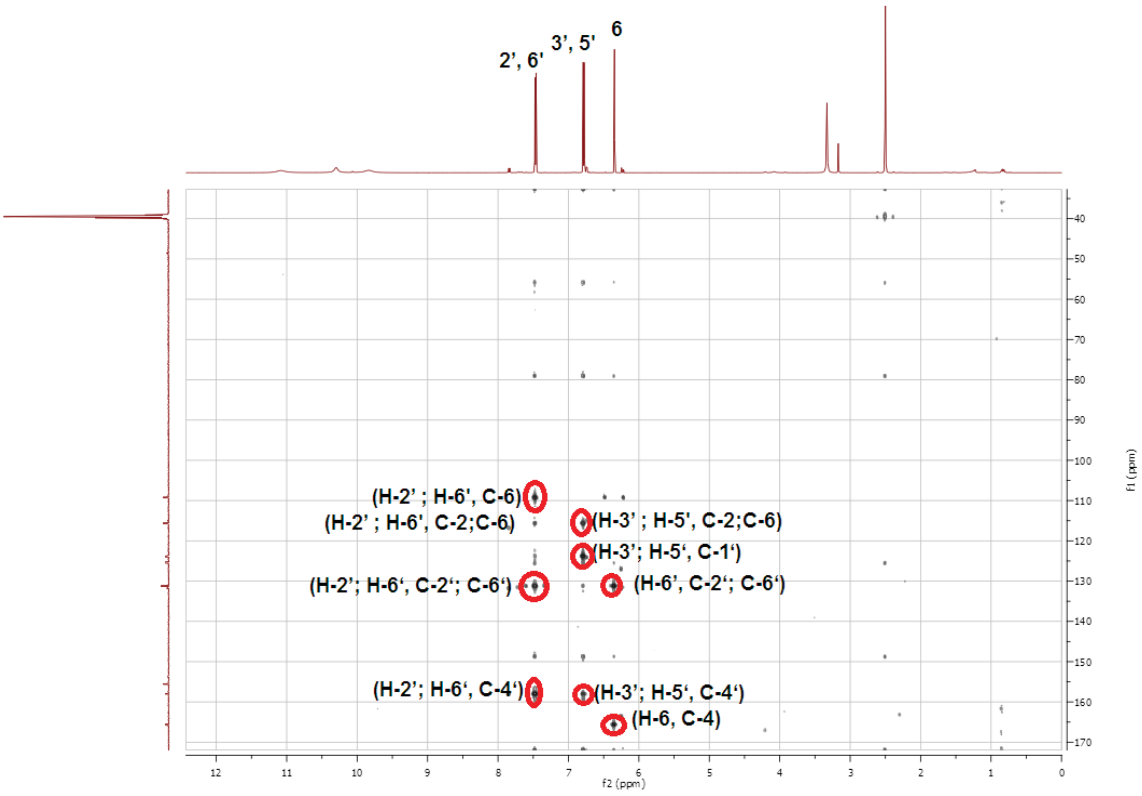
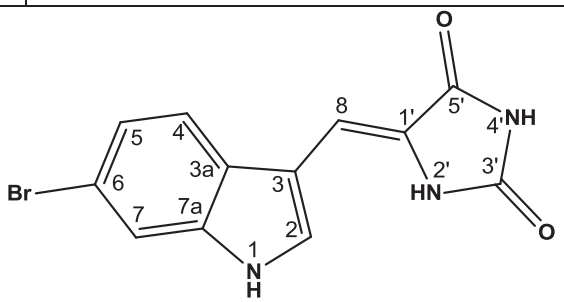
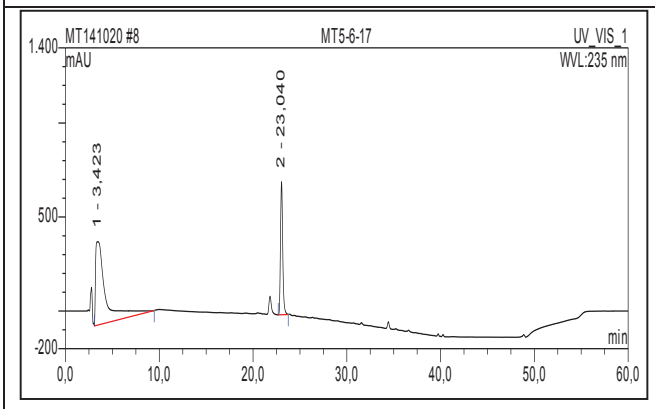


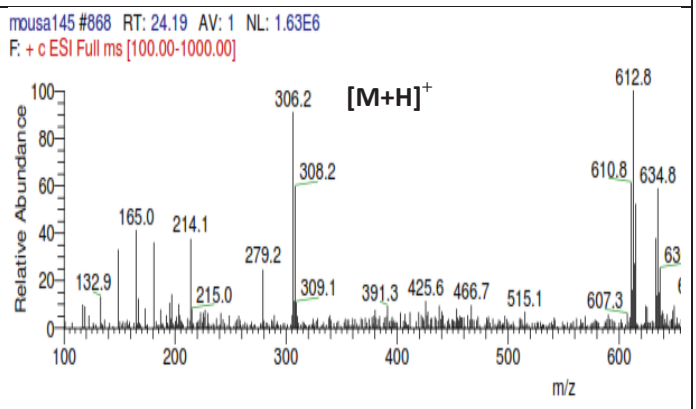
Figure 3.10: HMBC spectrum of compound (**5**).

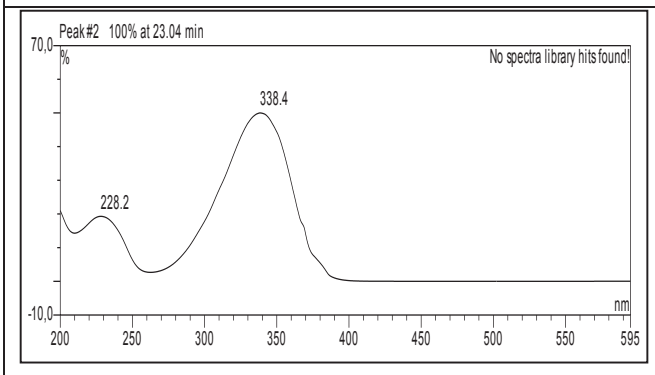
3.3.1.6. (*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (6: known natural product)

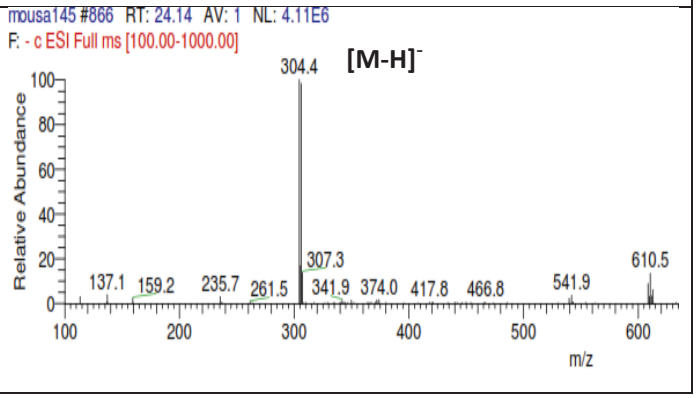
(*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin	
Synonym(s)	(*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin
Sample code	MT5-6-17
Biological source	Unknown sponge (**MT5**)
Sample amount	1.6 mg
Physical Description	yellowish solid
Molecular Formula	$C_{10}H_8BrN_3O_2$
Molecular Weight	305 g/mol.
Retention time HPLC	23.01 min (standard gradient)







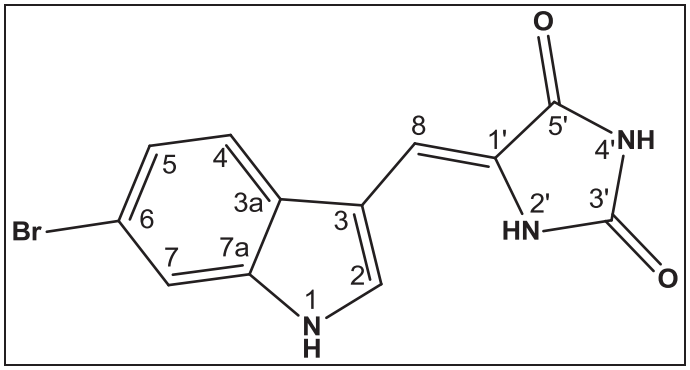




Results

Compound (**6**) was isolated as a yellowish solid from the EtOAc extract of the unknown sponge (**MT5**). The ESI mass spectrum of compound (**6**) revealed *pseudo*-molecular ion peaks at *m/z* 306.0/308.2 $[M+H]^+$, and 304.4/306.2 $[2M-H]^-$, with a ratio of (1:1) indicating a molecular weight of 305 g/mol. and containing a single bromine subtitution. Compound (**6**) showed UV absorption at λ_{max} (MeOH) 228.24, and 338.4 nm. The ^{1}HNMR spectrum of (**6**) showed three doublets *viz*., at δ_H 8.13 ppm [d (*J*=2.60 Hz)], δ_H 7.74 ppm [d (*J*=8.5 Hz)], and δ_H 7.61 ppm [d (*J*=1.5 Hz)] which were assigned to H-2, H-4, and H-7, respectively, a doublet of doublet (H-5) at δ_H 7.24 ppm [dd (*J*=8.5, 1.5 Hz)] was also revealed, and one singlet at δ_H 6.70 ppm (s) was attributed to H-8 (Table 3.7). This assumption was confirmed by the ^{1}H-^{1}H COSY experiment which showed one spin system, CH(4)CH(5)CH(7). Further elucidation of compound (**6**) was performed through a HMBC experiment. The HMBC spectrum (Figure 3.11) showed correlations from H-2' at δ_H 11.05 ppm to C-3' (δ_C 155.2), and C-1' (δ_C 124.2), from H-4' at δ_H 10.15 ppm to C-3' (δ_C 155.2), and C-1' (δ_C 124.2), from H-8 at δ_H 6.70 ppm to C-3a (δ_C 126.6), and C-2 (δ_C 127.4), and from H-2 at δ_H 8.13 ppm to C-8 (δ_C 100.9). Further inspection of HMBC spectrum revealed also correlations from H-4 at δ_H 7.74 ppm to C-3 (δ_C 108.6), C-6 (δ_C 114.9), and C-7a (δ_C 136.1), and from H-5 at δ_H 7.24 ppm to C-3a (δ_C 126.6), and C-7 (δ_C 114.4). The geometrical isomerism (*E*/*Z* isomers) of (**6**) was possible due to the restricted rotation around the exocyclic C=C double bond (Khanfar *et al*., 2009). The structure of (**6**) with *Z*-geometry was further confirmed by a comparison of ^{1}H NMR, ^{13}C NMR, and mass spectral data with those of published data of (*Z*)-6-bromo-3-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (Guella *et al*., 1988) (Table 3.7). Thus, compound (**6**) was elucidated as the known compound (*Z*)-6-bromo-3-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin.

It worth to mention that compound (**6**), which is an aplysinopsin derivative, was first isolated from the sponge *Smenspongia* [= *Polyfibrospongia*] *echina* (Dictyoceratida) of Belizeas *E*-isomer (Djura *et al*., 1980), and from scleractinain corals of the family (Dendrophyliidae) as *E*/*Z*-mixture (1:1) (Guella *et al*., 1988).



Structure of compound (**6**).

Table 3.7: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of (**6**).

Pos.	δ_C (DMSO-d_6)[a,b]	δ_C (DMSO-d_6)[c]	δ_H (DMSO-d_6)[a]	δ_H (DMSO-d_6)[c]	HMBC[a]
1	-	-	11.9, *brs*	11.98, *brs*	3, 3a, 7a
2	127.4	127.5	8.13, *d* (2.60)	8.15, *brs*	8
3	108.6	108.7	-	-	-
3a	126.6	126.3	-	-	-
4	120	120.1	7.74, *d* (8.5)	7.75, *d* (8.4)	3, 6, 7a
5	122.9	122.9	7.24, *dd* (1.5, 8.5)	7.24, *dd* (1.7, 8.4)	3a, 7
6	114.9	115.0	-	-	-
7	114.4	114.4	761, *d* (1.5)	761, *d* (1.7)	-
7a	136.1	136.6	-	-	-
8	100.9	101.0	6.70, *s*	6.71, *s*	3
1'	124.2	124.9	-	-	-
2'	-	-	11.05, *s*	11.10, *s*	3', 1'
3'	155.2	155.4	-	-	-
4'	-	-	10.15, *s*	10.20, *s*	3', 1'
5'	165.2	167.3	-	-	-

(a) Compound (**6**) (b) Signals were extracted from the HMBC spectrum (c) Guella *et al*., 1988

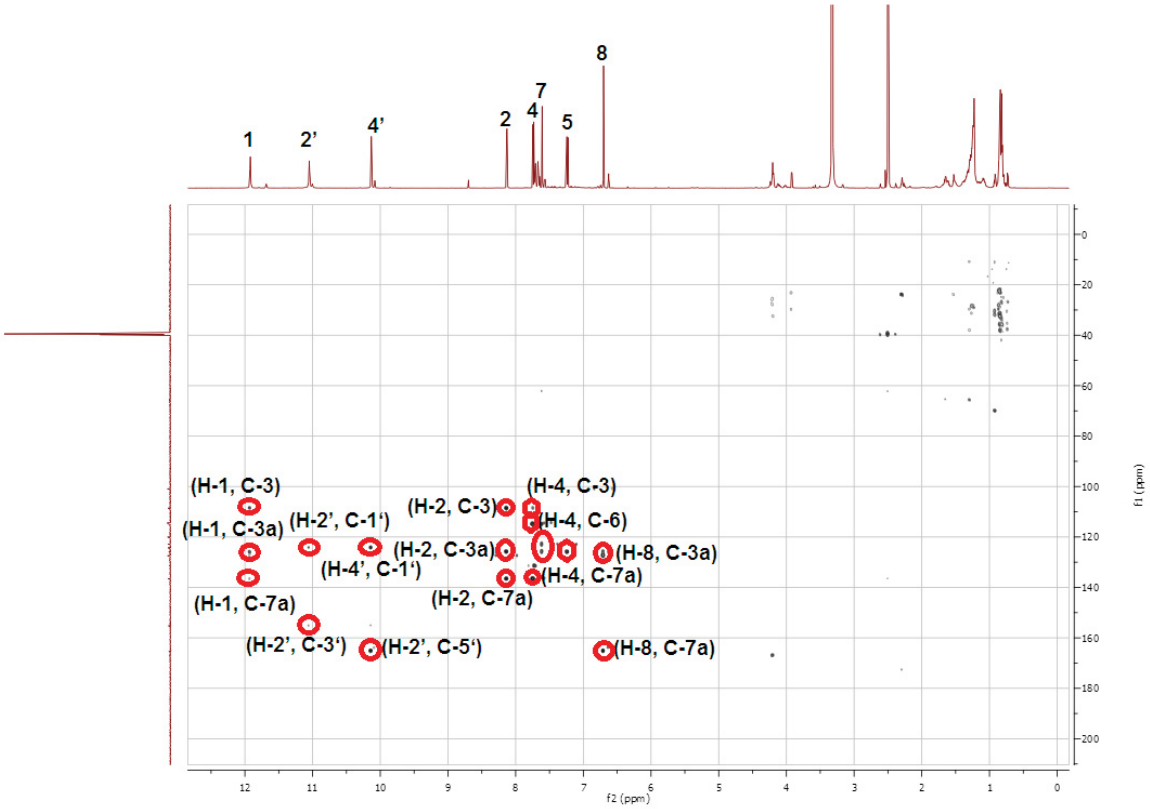


Figure 3.11: HMBC spectrum of compound (**6**).

3.3.2. Metabolites from the sponge *Hemimycale columella*:

The sponge *Hemimycale* sp. (family Mycalidae) is well known for its bioactive secondary metabolites especially the complex guanidine alkaloids (Ohtani *et al*., 1992; Louwrier *et al*., 1996). In the current study, the sponge *Hemimycale columella* was chemically and biologically investigated. This sponge was collected in the front of the marine science station at 20 m depth from the Jordanian coast of the Gulf of Aqaba by SCUBA diving. The sponge (500 g) was freeze-dried and cut into small pieces, followed by exhaustive extraction with methanol. The obtained crude extract (1.23 g) was further partitioned between 90% MeOH and *n*-hexane. The *n*-hexane fraction was then filtered and

evaporated to dryness using a rotary evaporator. The dried extract was subjected to silica VLC column chromatography using mobile phases: (*n*-hexane:EtOAc) (100:0) to (*n*-hexane:EtOAc) (0:100), then (DCM:MeOH) (100:0) to (DCM:MeOH) (0:100), to give one major fraction. This fraction was then redissolved in methanol and then further purified by different chromatographic techniques affording (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**8**, 6 mg). The 90% MeOH after partition with *n*-hexane was then evaporated to dryness using a rotary evaporator. The dried extract was redissolved in H_2O and then partition with EtOAc. This fraction was redissolved in methanol, and then was further purified by different chromatographic techniques affording (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**7**, 3 mg). The UV, NMR, and mass spectral data of compounds (**7** and **8)** were discussed in the unknown sponge (**MT5**) (see 3.3.1.5 and 3.3.1.6).

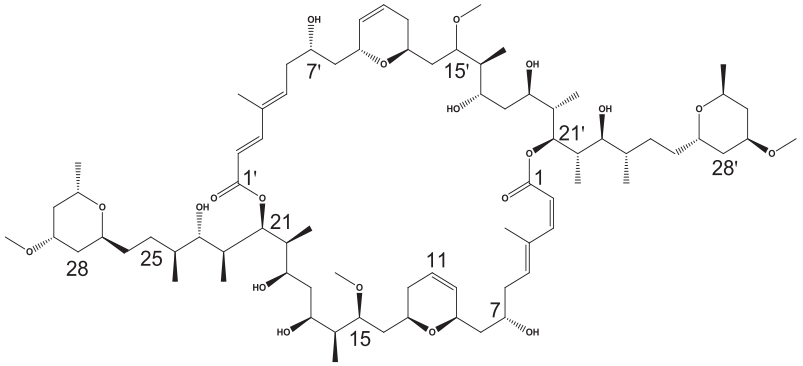
3.3.3 Metabolites from the sponge *Theonella* sp.

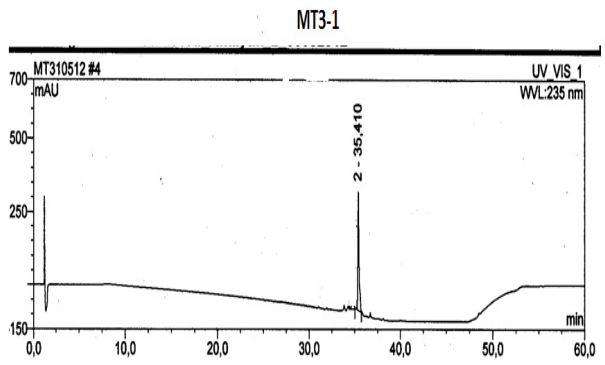
Reviewing the current literature indicated that many marine sponges of the genus *Theonella* (Lithistida, Theonellidae) have been subjected to intensive chemical investigations yielding many structurally intriguing secondary metabolites. More than 100 metabolites have been reported from *Theonella swinhoei* including nonribosmal polypeptides/depsipeptides (as the cytotoxic poly-theonamides and the anti-inflammatory perthamides and solomonamides), macrolides (e.g. the cytotoxic swinholide A), polyene derivatives (exemplified by aurantosides), and the uncommon steroids (4-methylene steroids and truncated side-chain sulfated steroids) (Sinisi *et al*., 2013). The methanolic extract of the sponge *Theonella* sp. was investigated in the present study. This sponge was collected from the same location as the former sponges by SCUBA diving. The sponge (500 g) was freeze-dried and cut into small

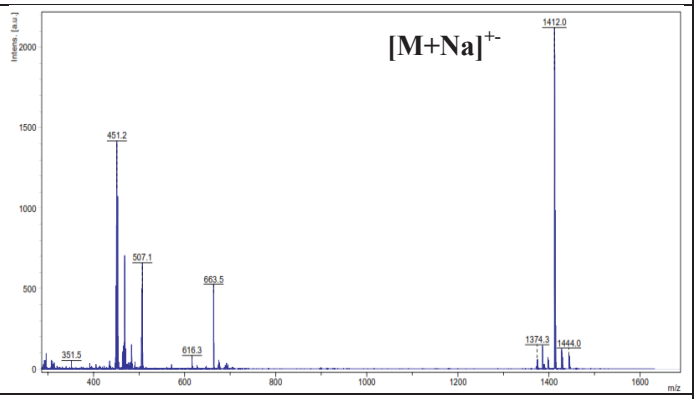
pieces, followed by exhaustive extraction with methanol. The obtained crude extract (1.22 g) was further partitioned between EtOAc and H_2O. The EtOAc fraction was then filtered and evaporated to dryness using a rotary evaporator. The dried extract was then subjected to silica VLC column chromatography using two gradient mobile phases starting from (*n*-hexane:EtOAc)(100:0) to (*n*-hexane:EtOAc) (0:100), and then (DCM:MeOH) (100:0) to (DCM:MeOH) (0:100), yielding a major subfraction. This subfraction was redissolved in methanol and then further purified by different chromatographic techniques to afford one major compound which is swinholide A (**9**, 12 mg).

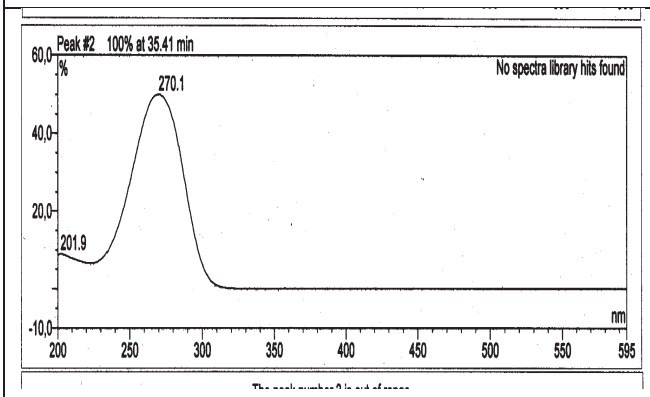
3.3.3.1. Swinholide A (9: known natural product)

Swinholide A	
Synonym(s)	Swinholide A
Sample code	MT3-1
Biological source	*Theonella* sp.
Sample amount	12 mg
Physical Description	White amorphous powder
Molecular Formula	$C_{78}H_{132}N_3O_{20}$
Molecular Weight	1390.0 g/mol.
Optical rotation$[\alpha]_D^{20}$	+10.0 (*c* 0.2, MeOH
Retention time HPLC	35.41 min (standard gradient)









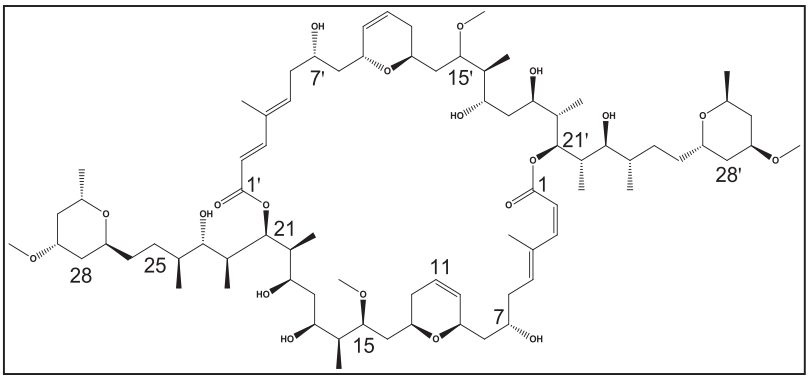
No ionization in negative ESI-MS

Results

Compound (**9**) was isolated as a white amorphous powder from the EtOAc extract of *Theonella* sp. The MALDI mass spectrum of compound (**9**) revealed a *pseudo*-molecular ion peak at *m/z*1412 $[M+Na]^+$(base peak), indicating a molecular weight of 1390.0 g/mole. Compound (**9**) showed UV absorption at λ_{max} (MeOH) 270.1 nm. The ^1H NMR spectrum exhibited a plane of symmetry by showing five olefinic signals *viz*., at δ_H 5.85 ppm [2H, d (*J*=15.7 Hz)], δ_H 7.42 ppm [2H, d (*J*=15.7 Hz)], δ_H 6.11 ppm [2H, t (*J*=7.2 Hz)]), δ_H 5.66 ppm [2H, d (*J*=10.5 Hz)], and δ_H 5.82 [2H, m] which were attributed to H-2/H-2', H-3/H-3', H-5/H-5', H-10/H-10', and H-11/H-11', respectively, five methyl signals at δ_H 0.84 pm [d (*J*=7.0 Hz)], δ_H 0.91 ppm [d (*J*=7.0 Hz)], δ_H 0.94 ppm [d (*J*=7.0Hz)], δ_H 0.84 ppm [d (*J*=6.8Hz)], and δ_H 1.19 ppm [d (*J*=6.2Hz)] which were assigned to 16/16'-Me, 20/ 20'-Me, 22/ 22'-Me, 24/24'-Me, and 31/ 31'-Me, respectively, an olefinic methyl singlet (4/ 4'-Me) was observed at δ_H 1.78 (s), and two methoxyl signals at δ_H 3.32 ppm (s), and δ_H 3.33 ppm (s) which were attributed to 15/ 15'-OMe, and 29/ 29'-OMe, respectively (Table 3.8). The ^{13}C NMR and DEPT spectra revealed signals for 39 carbon atoms *viz*., six methyl signals at δ_C (12.7, 9.2, 9.3, 10.0, 18.1, and 22.1 ppm) which were assigned to 4/4'-Me, 16/ 16'-Me, 20/ 20'-Me, 22/ 22'-Me, 24/ 24'-Me, and 31/ 31'-Me, respectively, 9 methylenes were observed at δ_C (39.1, 41.5, 32.5, 37.6, 39.4, 25.5, 30.2, 36.1, and 40.0 ppm) which were attributed to C-6/C-6', C-8/C-8', C-12/C-12', C-14/C-14', C-18/C-18', C-25/C-25', C-26/C-26', C-28/C-28', and C-30/C-30', respectively, 20 methines at δ_C (116.0, 152.5, 140.7, 66.1, 71.6,131.2, 125.1, 65.7, 78.7, 43.9, 73.8, 70.8, 30.8, 76.0, 38.3, 77.7, 34.9, 73.2, 74.6, and 68.4 ppm) which were assigned to C-2/C-2', C-3/C-3', C-5/C-5', C-7/C-7', C-9/C-9', C-10/C-10', C-11/C-11', C-13/C-13', C-15/C-15', C-16/C-16', C-17/C-17', C-19/C-19', C-20/C-20', C-21/C-21', C-22/C-22', C-23/C-23', C-24/C-24', C-27/C-27', C-29/C-29', and C-31/C-31', respectively, one quaternary carbon (C-4) at δ_C (135.5 ppm) was also observed, and

two methoxyl carbons at δ_C (57.2, and 55.6 ppm) which were attributed to 15/ 15'-OMe, and 29/29'-OMe, respectively, in addition to the deshielded signals of C-1/C-1' at δ_C (170.0 ppm) (Table 3.8). The ^1H-^1H COSY spectrum showed two spin systems, the first spin system is CH(2)CH(3), and the long second one extends from CH(5) to CH(31). Further elucidation of compound (**9**) was performed through a HMBC experiment in which correlations from H-2/H-2' at δ_H 5.85 ppm to C-1/C-1' (δ_C 170.0) and C-4/C-4' (δ_C 135.5), from H-3/H-3' at δ_H 7.42 ppm to C-1/ C-1' (δ_C 170.6), C-2/C-2' (δ_C 116.0), C-4a/C-4a' (δ_C 12.7), and C-5/C-5' (δ_C 140.7), from H-5 at δ_H 6.11 ppm to C-3/C-3' (δ_C 152.5), C-4a/C-4a' (δ_C 12.7), C-6/C-6' (δ_C 39.1), and C-7/C-7' (δ_C 66.1), and from CH_2-6 at δ_H 2.41 ppm to C-4/C-4' (δ_C 135.5), C-5/C-5' (δ_C 140.7), C-7/C-7' (δ_C 66.1), and C-8/C-8' (δ_C 41.5) were detected. Further inspection of the HMBC spectrum (Figure 3.12) also revealed correlations from H-9/H-9' at δ_H 4.48 ppm to C-10/C-10' (δ_C 131.2), from H-10/H-10' at δ_H 5.66 ppm to C-12/C-12' (δ_C 32.5), from H-19/H-19' at δ_H 3.97 ppm to C-20a/C-20a' (δ_C 9.3), and C-21/C-21' (δ_C 76.0), from H-21/H-21' at δ_H 5.46 ppm to C-1/C-1' (δ_C 170.6), C-18/C-18' (δ_C 39.4), C-19/C-19 (δ_C 70.8), C-20a/C-20a' (δ_C 9.3), and C-23/C-23' (δ_C 77.7), from H-23/H-23' at δ_H 3.11 ppm to C-25/C-25' (δ_C 25.5), and from 4/ 4'-Me at δ_H 1.78 ppm to C-3/C-3' (δ_C 152.5) (Table 3.8). Comparison of ^1H NMR, ^{13}C NMR, mass spectral data, and of the $[\alpha]_D^{20}$ of compound (**9**) with those of swinholide A (De Marino *et al*., 2011) proved that both compounds were identical (Table 3.8). It is worth to mention that swinholide A was the first 44-membered macrolide which was isolated from the Red Sea marine sponge *Theonella swinhoei* (Carmely and Kashman, 1985), and is then introduced as a product of biochemistry at symbiotic microorganisms (Andrianasolo *et al*., 2005). The structure of swinholide A was first assigned as a monomer and then revised later to be a symmetric cyclic dimer (Kobayashi *et al*.,

1989), and this was followed by determination of its absolute configuration (Kobayashi *et al*., 1990; Kitagawa *et al*., 1990; Doi *et al*., 1991).



Structure of compound (**9**)

Table 3.8: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data for (**9**).

Pos.	δ_C (CD_3OD)[a]	δ_C (CD_3OD)[b]	δ_H (CD_3OD)[a]	δ_H (CD_3OD)[b]	HMBC[a]
1/1'	170.0	170.6	-	-	-
2/2'	116.0	115.6	5.85, *d* (15.7)	5.84, *d* (15.7)	1, 4
3/3'	152.5	152.3	7.42, *d* (15.7)	7.43, *d* (15.7)	1, 2, 4a
4/4'	135.5	135.5	-	-	-
4/4'-Me	12.7	12.4	1.78, *s*	1.77, *s*	3, 4, 5
5/5'	140.7	140.5	6.11, *t* (7.2)	6.14, *t* (7.3)	3, 4a, 6
6/6'	39.1	38.8	2.41, *t* (7.2)	2.40, *t* (6.9)	4, 5, 7
7/7'	66.1	68.1	4.01, *m*	4.02, *m*	5, 8, 9
8/8'	41.5	41.0	1.76, 1.30, *m*	1.76, 1.28, *m*	6, 10
9/9'	71.6	70.5	4.48, *m*	4.47, *brd* (10.5)	8, 10
10/10'	131.2	130.9	5.66, *d* (10.5)	5.65, *dd* (1.8, 10.5)	8, 12
11/11'	125.1	124.9	5.82, *m*	5.81, *m*	9, 10
12/12'	32.5	32.2	1.94, *m*	1.94, *m*	10, 11
13/13'	65.7	65.3	3.51, *m*	3.49, *m*	11, 15
14/14'	37.6	37.2	1.77, 1.58, *m*	1.77, 1.58, *m*	13, 15
15/15'	78.7	78.2	3.75, *m*	3.76, *m*	15a, 16
15/15'-OMe	57.2	56.7	3.32, *s*	3.32, *s*	-
16/16'	40.5	43.8	1.54, *m*	1.52, *m*	14, 16a
16/16'-Me	9.2	8.8	0.84, *d* (7.0)	0.83, *d* (6.7)	16
17/17'	73.8	73.2	3.61, *m*	3.61, *m*	15, 18
18/18'	39.4	39.0	1.72, 1.63, *m*	1.74, 1.63, *m*	20
19/19'	70.8	70.1	3.97, *m*	3.97, *overlap*	17, 21
20/20'	39.8	39.4	1.90, *m*	1.94, *m*	20a
20/20'-Me	9.3	8.9	0.91, *d* (7.0)	0.91, *d* (7.0)	20
21/21'	76.0	75.6	5.46, *d* (11.4)	5.46, *d* (10.5)	19, 23
22/22'	38.3	37.9	1.97, *m*	1.98, *m*	20, 24
22/22'- Me	10.0	9.6	0.94, *d* (7.0)	0.93, *d* (6.9)	22
23/23'	77.7	77.2	3.11, *dd* (2.2, 9.4)	3.11, *dd* (1.8, 9.5)	21, 25
24/24'	34.9	34.4	1.70, *m*	1.70, *m*	24a, 25
24/24'- Me	18.1	17.7	0.84, *d* (6.8)	0.98, *d* (6.7)	24
25/25'	25.5	25.1	1.42, 1.22, *m*	1.42, 1.24, *m*	23, 27
26/26'	30.2	29.7	1.92, *m*	1.94, 1.27, *m*	24, 25
27/27'	73.2	72.7	3.98, *m*	3.99, *overlap*	25, 27
28/28'	36.1	35.8	1.86, *m*	1.87, *brd* (12.8)	26, 30
			150, *m*	152, *m*	-
29/29'	74.6	74.2	3.59, *m*	3.61, *m*	27, 29a
29/29'-O Me	55.6	55.3	3.33, *s*	3.34, *s*	29
30/30'	40.0	39.7	1.09, *m*	1.09, *dd* (10.4, 12.6)	-
			2.01, *m*	2.01, *brd* (12.6)	31
31/31'	68.4	65.7	3.88, *m*	3.74, *m*	30, 31a
31/31'- Me	22.1	21.8	1.19, *d* (6.2)	1.19, *d* (6.2)	31

(a) Compound (**9**) (b) De Marino *et al*., 2011

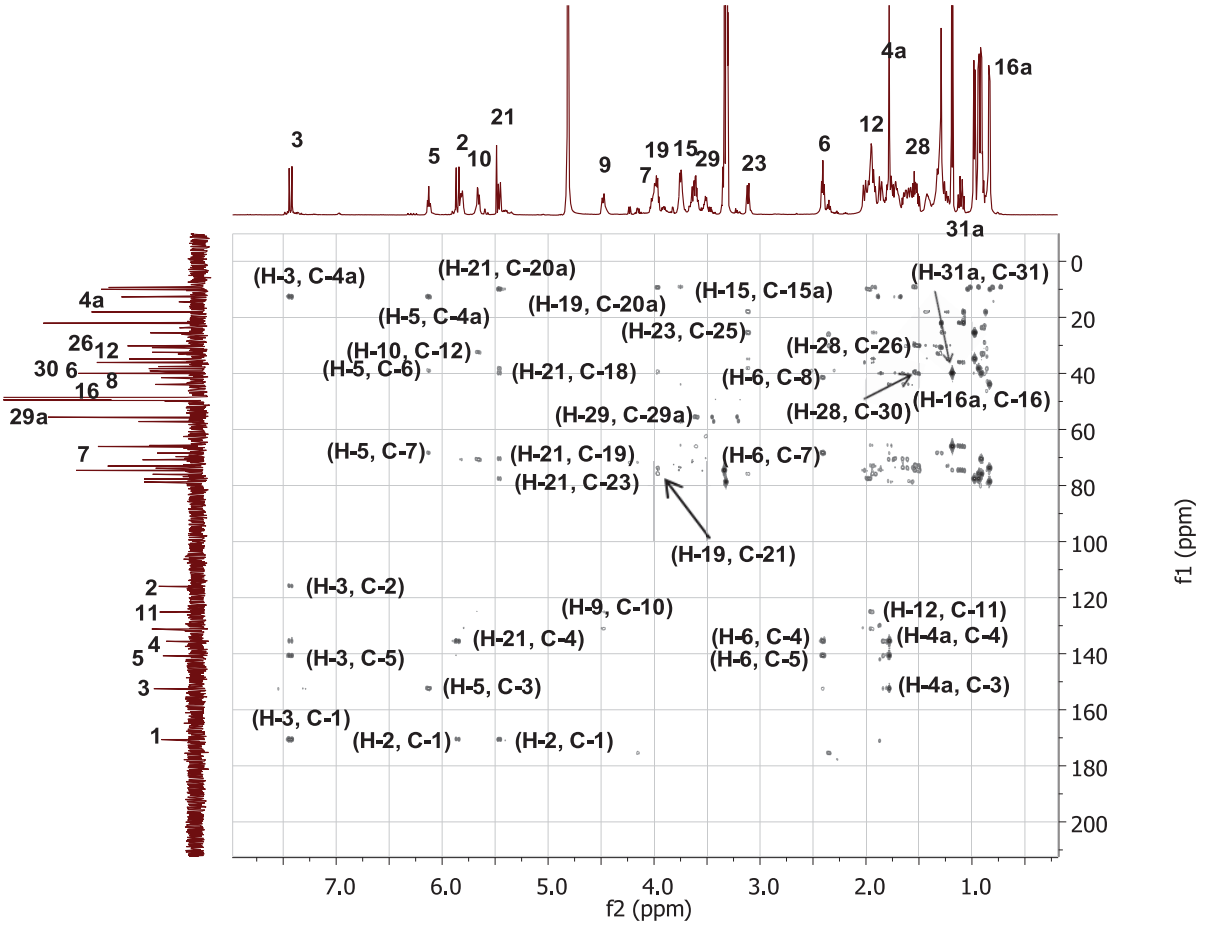


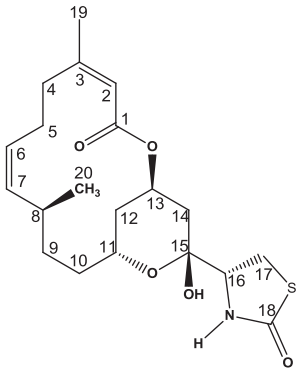
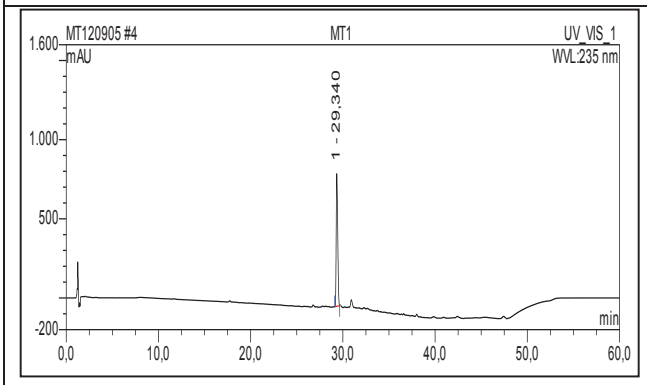
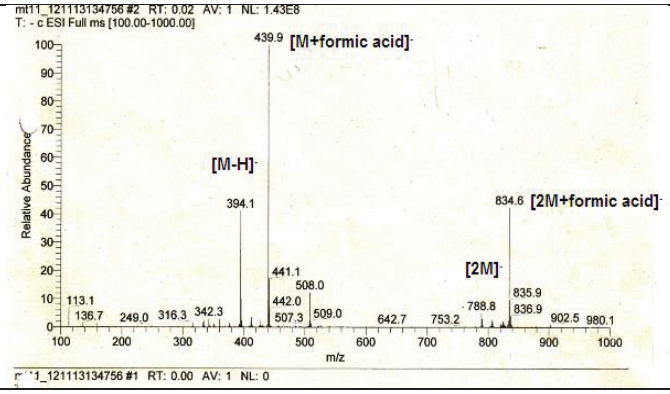
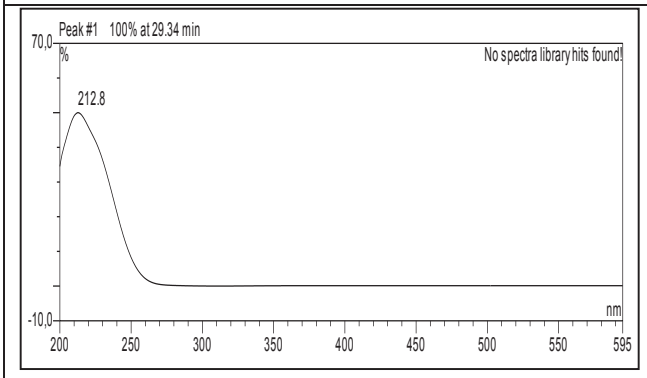
Figure 3.12: HMBC spectrum of compound (**9**)

3.3.4. Metabolites from the sponge *Diacarnus ardoukoba*:

Diacarnus ardoukoba belongs to the family (Podospongiidae). Reviewing the current literature revealed only a small number of hits concerning the chemistry of this genus. In the existing study, the methanolic extract of the sponge *Diacarnus ardoukoba* was chemically and biologically investigated. This sponge was collected in the front of the marine science station at 20 m depth from the Jordanian coast of the Gulf of Aqaba by SCUBA diving. The sponge (500 g) was freeze-dried and cut into small pieces, followed by exhaustive extraction with methanol. The obtained crude extract (1.22 g) was further partitioned between EtOAc and H_2O. The EtOAc

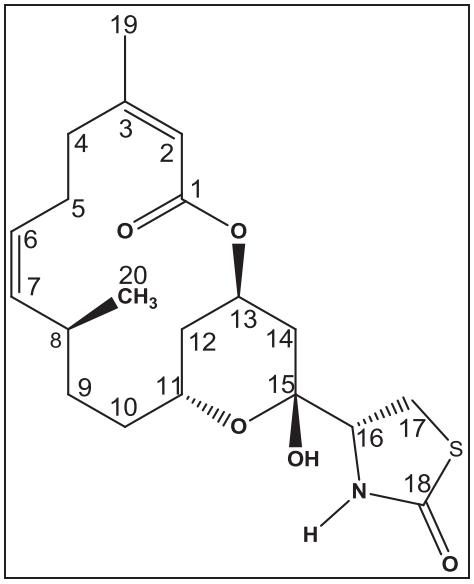
fraction was then filtered and evaporated to dryness using a rotary evaporator. The dried extract was then subjected to silica VLC column chromatography using two gradient mobile phases starting from (*n*-hexane:EtOAc) (100:0) to (*n*-hexane:EtOAc) (100:0) and then (DCM:MeOH)(100:0) to (DCM:MeOH) (100:0). One major subfraction was obtained. This subfraction was redissolved in methanol and was then further purified by different chromatographic techniques to afford one compound which is latrunculin B (**10**, 6 mg).

3.3.4.1. Latrunculin B (10: known natural product)

<table>
<tr><th colspan="2">Latrunculin B</th></tr>
<tr><td>Synonym(s)</td><td>(R)-4-((1R,4Z,8Z,10S,13R,15R)-15-Hydroxy-5,10-dimethyl-3-oxo-2,14-dioxabicyclo[11.3.1]heptadeca-4,8-dien-15-yl)thiazolidin-2-one</td></tr>
<tr><td>Sample code</td><td>MT1-1</td></tr>
<tr><td>Biological source</td><td>Diacarnus ardoukoba</td></tr>
<tr><td>Sample amount</td><td>6 mg</td></tr>
<tr><td>Physical Description</td><td>yellowish solid</td></tr>
<tr><td>Molecular Formula</td><td>$C_{20}H_{29}NO_5S$</td></tr>
<tr><td>Molecular Weight</td><td>394.51 g/mol.</td></tr>
<tr><td>Optical rotation$[\alpha]_D^{20}$</td><td>103.0 (c 0.2, MeOH)</td></tr>
<tr><td>Retention time HPLC</td><td>29.34 min (standard gradient)</td></tr>
<tr><td colspan="2">  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>  </td><td>No ionization in negative ESI-MS</td></tr>
</table>

Compound (**10**) was isolated as pale yellow oil from the EtOAc extract of *Diacarnus ardoukoba*. The ESI mass spectrum of compound (**10**) revealed *pseudo*-molecular ion peaks at *m/z* 394.1 $[M-H]^-$ (base peak), 439.9 $[M+HCOOH-H]^-$, 788.8 $[2M-H]^-$, and 834.6 $[2M+HCCOH-H]^-$, respectively, revealing a molecular might of 394 g/mol. Compound (**10**) showed UV absorption at λ_{max} (MeOH) 212.8 nm. The ^1H NMR spectrum revealed three olefinic protons, *viz*., at δ_H 5.62 ppm (s), δ_H 5.30 ppm [t (*J*=12.0 Hz)], and δ_H 5.06 ppm [t (*J*=11.0 Hz)] which were attributed to H-2, H-6, and H-7, respectively, one methyl doublet at δ_H 0.95 ppm [d (*J*=6.0 Hz)] which was assigned to CH_3-20, and one olefinic methyl at δ_H 1.94 ppm (s) which was assigned to CH_3-19 (Table 3.9). The ^{13}C NMR spectrum revealed signals for 20 carbon atoms *viz*., two methyls at δ_C (25.6, and 22.9 ppm) were attributed to C-19, and C-20, respectively, seven methylenes at δ_C (36.9, 27.8, 32.5, 32.6, 36.7, 31.7, and 29.7 ppm) were assigned to C-4,C-5, C-9, C-10, C-12, C-14, and C-17, respectively, seven methines at δ_C (119.5, 129.4, 136.7, 30.2,64.5, 70.2, and 63.7 ppm), were attributed to C-2, C-6, C-7, C-8, C-11, C-13, and C-16, respectively, two quaternary carbons were observed at δ_C (157.8, and 98.8 ppm) which were assigned to C-3, and C-15, respectively, in addition to the deshielded signals of C-1, and C-18 which were observed at δ_C (168.2, and 177.8 ppm), respectively (Table 3.9). The ^1H-^1H COSY spectrum revealed two spin systems, the first spin systems is CH_2(4)CH_2(5)CH(6)CH(7)CH(8)CH_2(9)CH_2(10)CH(11)CH_2(12)CH(13)CH_2(14), and the second one is CH(16)CH_2(17). Further elucidation of compound (**10**) was performed through a HMBC experiment. The HMBC spectrum (Figure 3.13) showed correlations from H-2 at δ_H 5.62 ppm to C-1 (δ_C 168.2), C-3 (δ_C 157.8), C-4 (δ_C 36.9), and C-19 (δ_C 25.6), from CH_3-19 at δ_H 1.94 ppm to C-2 (δ_C 119.5), C-3 (δ_C 157.8), and C-4 (δ_C 36.9), from CH_2-5 (at δ_H 2.14, and 1.78 ppm) to C-3 (δ_C 157.8), C-4 (δ_C 36.9), C-6 (δ_C 129.4) , and C-7 (δ_C 136.7), and from CH_3-20 at δ_H 0.95

ppm to C-7 (δ_C 136.7), C-8 (δ_C 30.2), and C-9 (δ_C 32.5). Further inspection of the HMBC spectrum revealed also correlations from H-11 at δ_H 4.51 ppm to C-9 (δ_C 32.5), C-10 (δ_C 32.6), C-13 (δ_C 70.2), and C-15 (δ_C 98.8), from CH_2-14 (at δ_H 2.19 ppm, and 1.78 ppm) to C-12 (δ_C 36.7), C-13 (δ_C 70.2), and C-15 (δ_C 98.8), and from H-16 at δ_H 3.8 ppm to C-15 (δ_C 98.8), C-17 (δ_C 29.7), and C-18 (δ_C 177.8). Further structure confirmation was achieved by comparison of ^{1}H NMR,^{13}C NMR, mass spectral data, in addition to the $[\alpha]_D^{20}$ of (**10**) with published data of the known compound latrunculin B (Ahmed *et al*., 2007) (Table 3.9).



Structure of compound (**10**)

Table 3.9: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data for (**10**).

Pos.	δ_C (CD_3OD)[a]	δ_C (CD_3OD)[b]	δ_H (CD_3OD)[a]	δ_H (CD_3OD)[b]	HMBC[a]
1	168.2	165.6	-	-	-
2	119.5	118	5.62, *s*	5.60, *s*	1, 3, 4, 19
3	157.8	154.7	-	-	-
4	36.9	35.8	2.04, 2.97, *m*	1.97, 3.05, *m*	2, 3, 6
5	27.8	26.9	2.14, 1.87, *m*	2.15, 2.70, *m*	7
6	129.4	127.6	5.30, *t* (12.0)	5.40, *ddd* (11.2, 11.2, 3)	7
7	136.7	135.9	5.06, *t* (11.0)	5.00, *dd* (10.8, 10.8)	-
8	30.2	28.9	2.8, *m*	2.7, *m*	9, 10, 20
9	32.5	31.2	1.09, 1.37, *m*	1.10, 1.70, *m*	7, 8, 11
10	32.6	31.2	1.56, 2H, *m*	1.70, 2H, *m*	11
11	64.5	62.6	4.51, t (10.9)	4.50, *m*	9, 10, 13
12	36.7	35.4	1.57,1.67, *m*	1.4, 1.6, *m*	-
13	70.2	68.7	5.18, *brs*	5.20, *m*	11, 15
14	31.7	31.8	1.78, *m*	2.15, *m*	12, 13, 15
			2.19, *m*	2.44, *d* (15.2)	12, 13, 15
15	98.8	97.7	-	-	-
16	63.7	61.8	3.8, *m*	4.0, *m*	14, 15, 17
17	29.7	28.7	3.1, 3.4, *m*	3.0, *m,* 3.20, d (14.4)	15, 16
18	177.8	175.3	-	-	-
19	25.6	24.1	1.94, *s*	1.96, *s*	2, 3
20	22.9	22.3	0.95, *d* (6.0)	0.90, *d* (6.0)	7, 6, 8, 9

(a) Compound (**10**) (b) Ahmed *et al*., 2007.

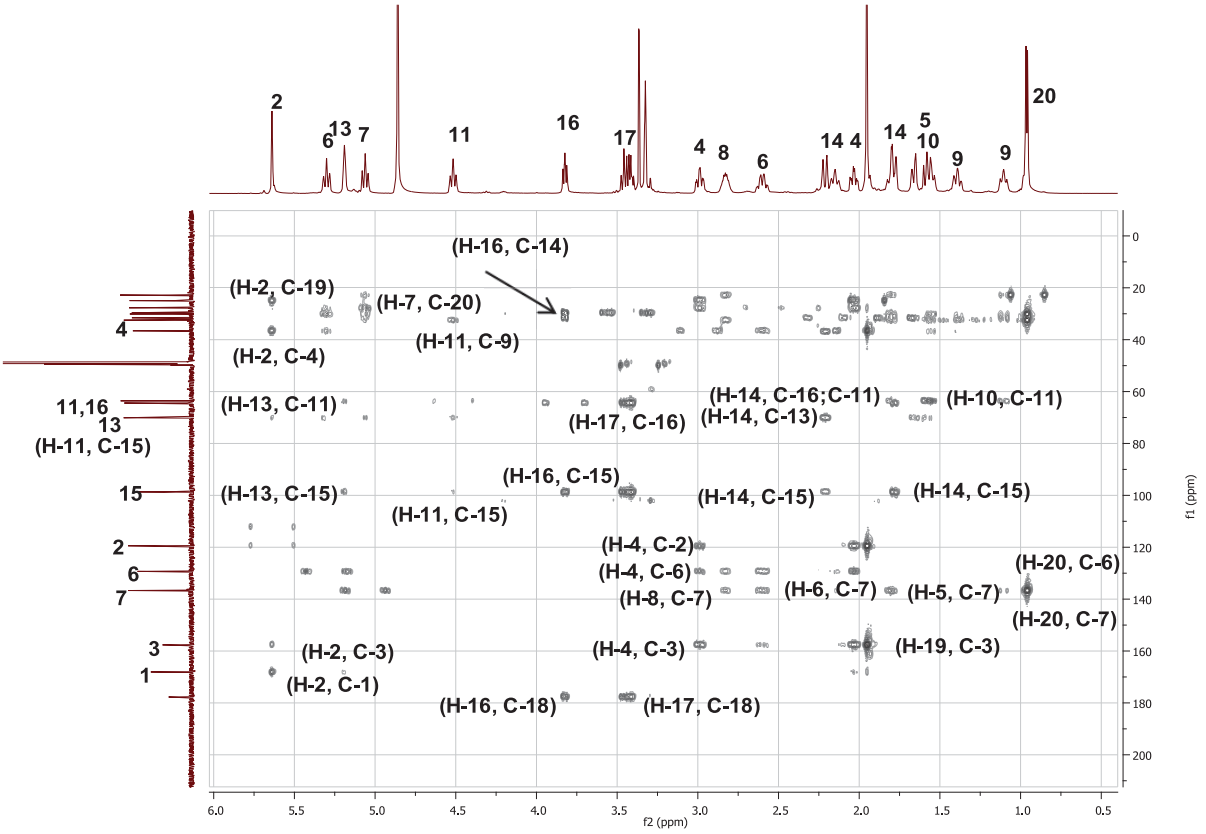


Figure 3.13: HMBC spectrum of compound (**10**).

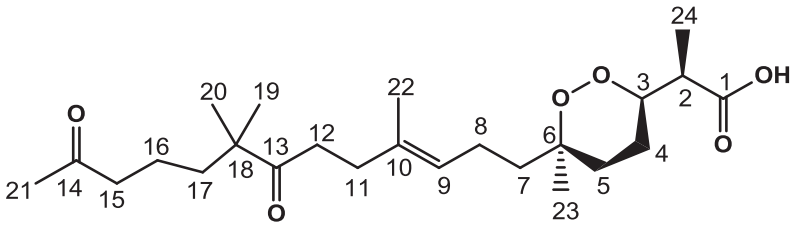
3.3.5 Metabolites from the sponge *Diacarnus erythraeanus*.

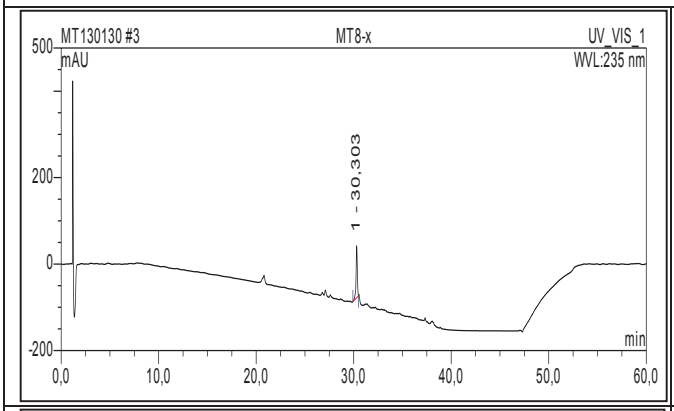
Marine sponges of the genus *Diacarnus* are well known as a source of norterpene cyclic peroxides (D´ Ambrosio *et al*., 1997; D´ Ambrosio *et al*., 1998; Sperry *et al*., 1998; El Sayed *et al*., 2001; Youssef *et al*., 2001; Youssef, 2004; Dai *et al*., 2007; Ibrahim *et al*., 2008). Norterpene cyclic peroxides possess a 1, 2-dioxane ring linked to a 2-substituted proionic acid, either free or esterified, and are characterized by an acyclic, monocyclic, or bicyclic carbon skeleton (Casteel, 1992). These metabolites which are terpene derivatives are reported also in other sponges like (Prianos) (Kashman and Rotem, 1979; Manes *et al*., 1984), and (Sigmosceptrella) (Albericci *et al*., 1979; Albericci *et al*., 1982). In the current study, the investigation of the methanolic extract of the sponge *Diacarnus erythraeanus* afforded six known norterpene cyclic peroxide

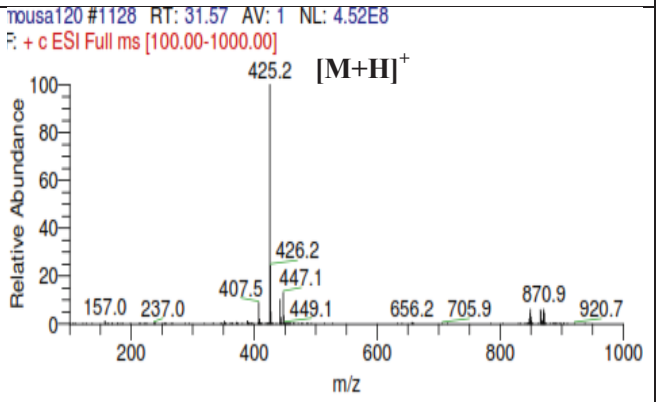
compounds. This sponge was collected in the front of the marine science station at 20 m depth from the Jordanian coast of the Gulf of Aqaba by SCUBA diving. The sponge (500 g) was freeze-dried and cut into small pieces, followed by exhaustive extraction with methanol. The obtained crude extract (2.21 g) was further partitioned between EtOAc and H_2O. The ethyl acetate fraction was then filtered and evaporated to dryness using a rotary evaporator. The dried extract was then subjected to silica VLC column chromatography using two gradient mobile phases starting from (*n*-hexane:EtOAc) (100:0) to (*n*-hexane:EtOAc) (100:0) then (DCM:MeOH) (100:0) to (DCM:MeOH) (100:0), to obtain two major fractions. Both fractions were redissolved in methanol separately, and were then further purified by different chromatographic techniques to afford (+)-muqubilone A (**11**, 1.8 mg), (-)-muqubilin A (**12**, 4 mg), sigmosceptrelin B (2a) (**13**, 15 mg), sigmosceptrelin B (2) (**14**, 25 mg), (+)-methyl-2-epinuapapuanoate (**15**, 9 mg), and (-)-methyl-2-epinuapapuanoate (**16**, 15 mg), respectively.

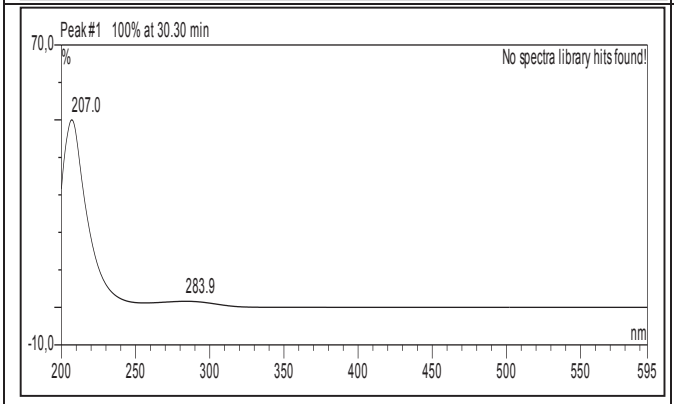
3.3.5.1. (+)-Muqubilone A (11: known natural product)

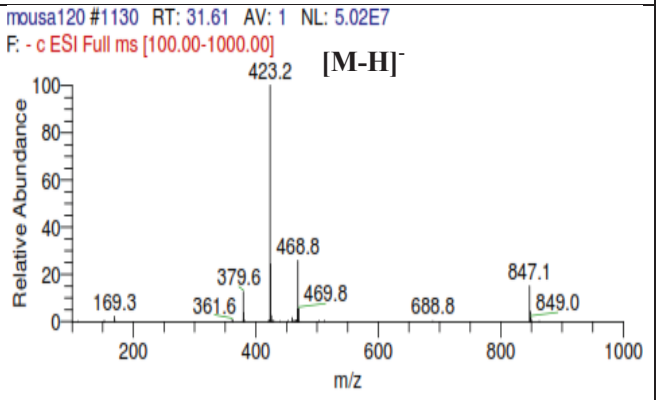
(+)-Muqubilone A	
Synonym(s)	(*R*)-2-((3*S*,6*R*)-6-Methyl-6-((*E*)-4,8,8-trimethyl-7,12-dioxotridec-3-en-1-yl)-1,2-dioxan-3-yl)propanoic acid
Sample code	MT8-x
Biological source	*Diacarnus erythraeanus*
Sample amount	1.8 mg
Physical Description	Colorless oil
Molecular Formula	$C_{24}H_{40}O_6$
Molecular Weight	424.57 g/mol.
Optical rotation $[\alpha]_D^{20}$	+52 (*c* 0.2, $CHCl_3$)
Retention time HPLC	30.30 min (standard gradient)





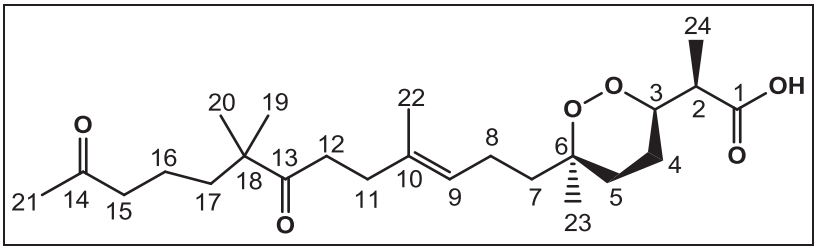






Compound (**11**) was isolated as colorless oil from the EtOAc extract of *Diacarnus erythraeanus*. The ESI mass spectrum of compound (**11**) revealed *pseudo*-molecular ion peaks at *m/z* 425.2 $[M+H]^+$ (base peak) 847.6 $[2M+H]^+$, 870.8 $[2M+Na]^+$, 423.1 $[M-H]^-$ (base peak) and 847.1 $[2M-H]^-$, indicating a molecular weight of 424 g/mol. Compound (**11**) showed UV absorption at λ_{max} (MeOH) 207.0, and 283.9 nm. The ^{1}H NMR spectrum showed one olefinic proton at δ_H 5.08 ppm [t (*J*=6.1 Hz)] which was assigned to H-9, one methyl doublet (CH_3-24) at δ_H 1.26 [d (*J*=7.2 Hz)] also was observed, one olefinic methyl was revealed at δ_H 1.58 ppm (s) which was attributed to CH_3-22, and four extra methyl signals *viz*., at δ_H 1.09 ppm (s), δ_H 1.09 ppm (s), δ_H 2.10 ppm (s), and δ_H 1.27 ppm (s) which were assigned to CH_3-19, CH_3-20, CH_3-21, and CH_3-23, respectively (Table 3.10). The indicative chemical shifts of C-3, C-4, C-5, at δ_C (81.6, 23.5, and 32.2 ppm), respectively confirmed the presence of a cyclic peroxide moiety in compound (**11**) (Capon *et al*., 1998; Ovenden and Capon, 1998). The structure of (**11**) was also corroborated by analysis of ^{1}H-^{1}H COSY spectrum (Figure 3.14) through the presence of four spin systems, the first spin system is CH_3(24)CH(2)CH(3)CH_2(4)CH_2(5), the second one is CH_2(7)CH_2(8)CH(9), the third one is CH_2(11)CH_2(12), while the fourth spin system is CH_2(15)CH_2(16)CH_2(17) (Table 3.10). The relative configuration of (**11**) was determined depending on the rule used by (Rubio *et al*., 2009). The carbon substituent at C-3 was set as equatorial since H-3 exhibited a vicinal diaxial coupling to H-4, $J_{3,4}$= 6.9 and 13.0 Hz (*J*= 3-4 Hz is expected for equatorial). The methyl group at C-6 (δ_C= 21.2 ppm) was next assigned as axial from its diagnostic ^{13}C NMR shift ($\delta_{C,eq}$= 23.5-24.0 ppm; $\delta_{C,ax}$= 20.5-20.9 ppm). The erythro/threo (*R**/*S**) configuration at C-2/C-3 was designated based on the proton chemical shift of H-20 at δ_H 1.26 ppm (standard value: $\delta_{H,erythro}$= 1.13-1.14 ppm; $\delta_{H,threo}$= 1.22-1.24 ppm). Finally, the comparison of ^{1}H NMR, ^{13}C NMR, mass spectral data, in addition to

the $[\alpha]_D^{20}$ of compound (**11**) with those of (+)-muqulibone A which had been isolated from the Red Sea derived same species (El Sayed *et al*., 2001) indicated that these two compounds are identical (Table 3.10).



Structure of compound (**11**)

Table 3.10: ^{1}H NMR (600 MHz) and COSY data of (**11**).

Pos.	δ_C $(CDCl_3)^{a,b}$	δ_H $(CDCl_3)^{c}$	δ_H $(CDCl_3)^{a}$	δ_H $(CDCl_3)^{c}$	COSY[a]
1	-	179.1	-	-	-
2	43.2	43.0	2.66, *m*	2.64, *quin* (7.5)	3, 20
3	81.6	81.2	4.13, *dd* (13.0, 6.9)	4.14, *ddd* (9.5, 7.5, 3)	2, 4
4	23.5	23.4	1.75, 2H, *m*	1.76, 2H, *m*	3, 5
5	32.2	32.0	1.64, 2H, *m*	1.65, 2H, *m*	4
6	-	80.1	-	-	-
7	39.7	39.8	1.43, 2H, *m*	1.42, 2H, *m*	8
8	22.2	21.7	1.98, 2H, *m*	1.99, 2H, *m*	7, 9
9	124.9	124.3	5.08, *t* (6.1)	5.08, *t* (7.0)	8, 22
10	-	134.5	-	-	-
11	33.8	33.5	2.17, 2H, *t* (7.6)	2.19, 2H, *t* (7.7)	12
12	36.7	35.6	2.51, 2H, *t* (8.0)	2.53, *t* (7.7)	11
13	-	215.1	-	-	-
14	-	208.7	-	-	-
15	44.3	43.9	1.45, 2H, *m*	1.46, 2H, *m*	16
16	19.4	19.0	1.42, 2H, *m*	1.42, 2H, *m*	15, 17
17	39.4	39.1	2.39, 2H, *t* (6.5)	2.40, 2H, *t* (6.9)	16, 19, 20
18	-	47.5	-	-	-
19	24.6	24.3	1.09, 3H, *s*	1.10, 3H, *s*	-
20	24.6	24.3	1.09, 3H, *s*	1.10, 3H, *s*	-
21	30.0	29.9	2.10, 3H, *s*	2.12, 3H, *s*	
22	16.0	16.1	1.58, 3H, *s*	1.60, 3H, *s*	-
23	21.2	20.6	1.27, 3H, *s*	1.28, 3H, *s*	-
24	13.5	13.3	1.24, 3H, *d* (7.2)	1.26, 3H, *d* (7.6)	2

(a) Compound (**11**) (b) signals were extracted from the HSQC spectrum (c) El Sayed *et al*., 2001

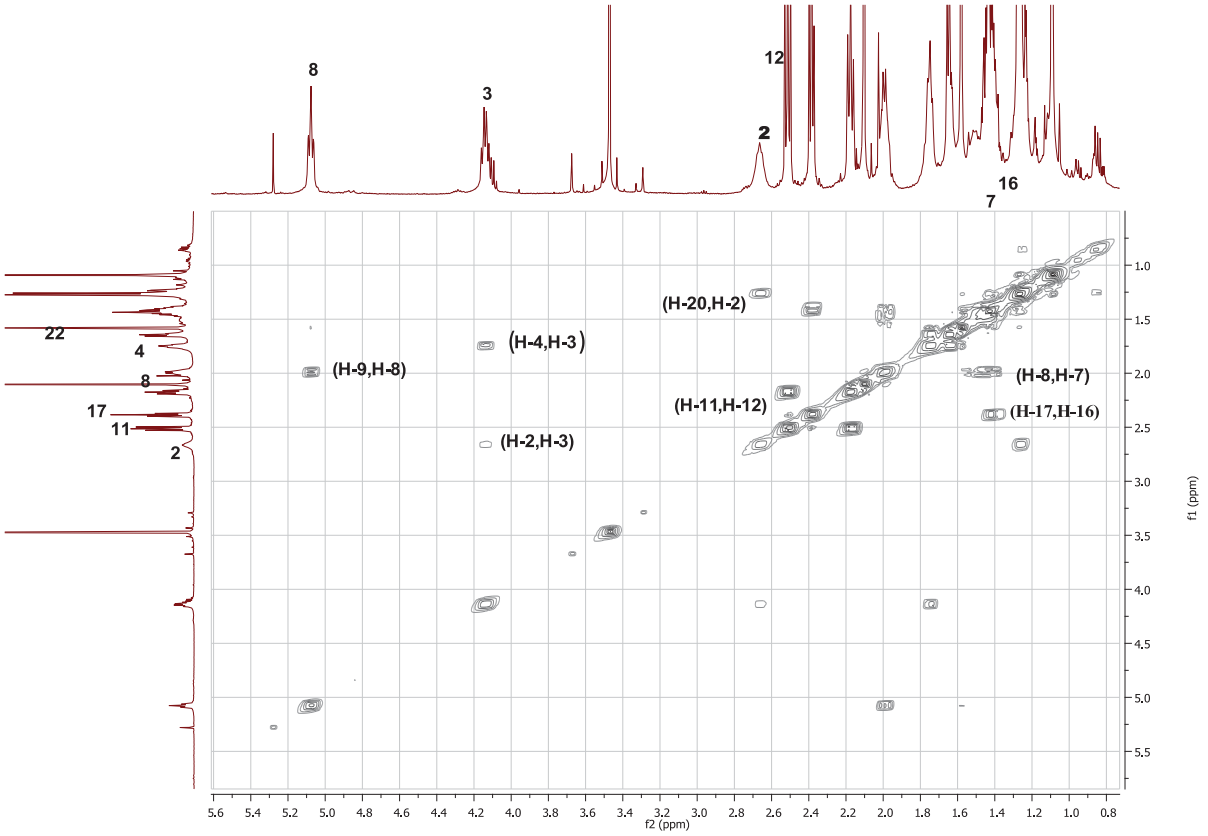
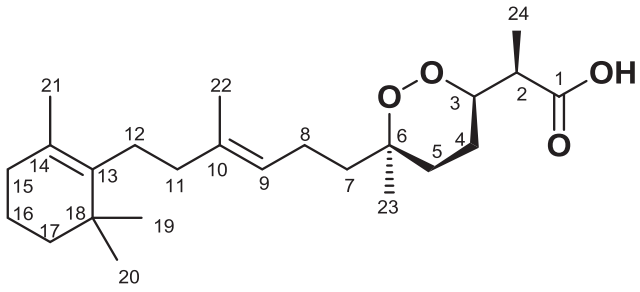
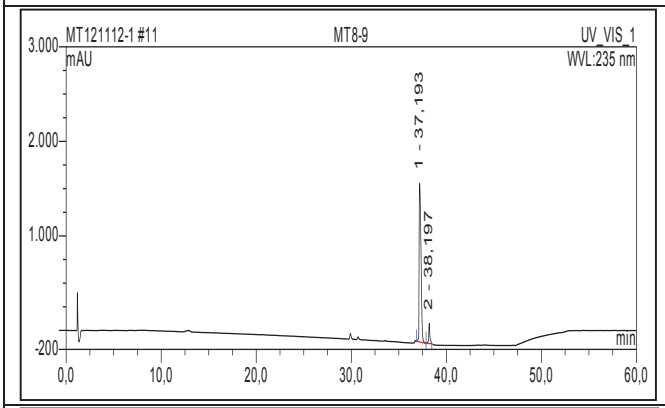


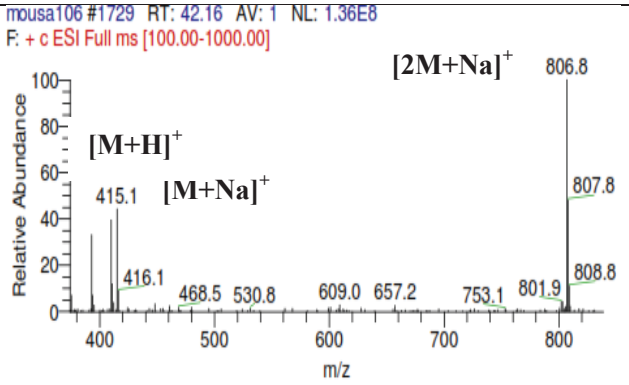
Figure 3.14: ^{1}H-^{1}H COSY spectrum of compound (**11**).

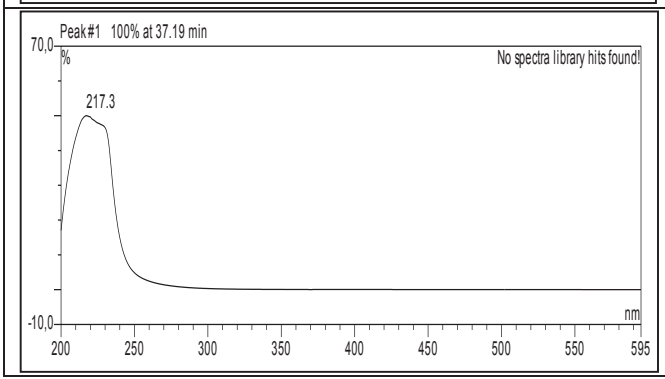
3.3.5.2. (-)-Muqubilin A (12: known natural product)

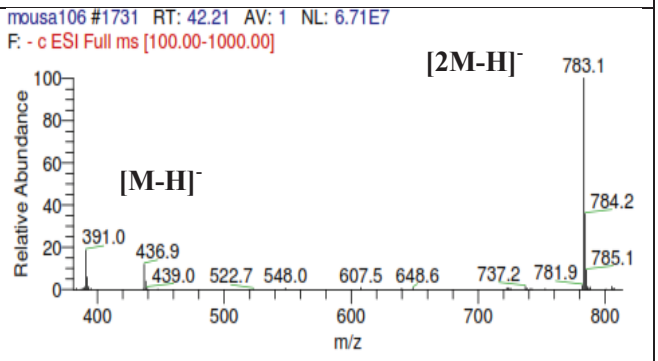
(-)-Muqubilin A	
Synonym(s)	(*R*)-2-((3*S*,6*R*)-6-Methyl-6-((*E*)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hex-3-en-1-yl)-1,2-dioxan-3-yl)propanoic acid
Sample code	MT8-9
Biological source	*Diacarnus erythraeanus*
Sample amount	4 mg
Physical Description	yellow oil
Molecular Formula	$C_{24}H_{40}O_4$
Molecular Weight	392.57 g/mol.
Optical rotation $[\alpha]_D^{20}$	-40 (*c* 0.2, $CHCl_3$)
Retention time HPLC	30.30 min (standard gradient)





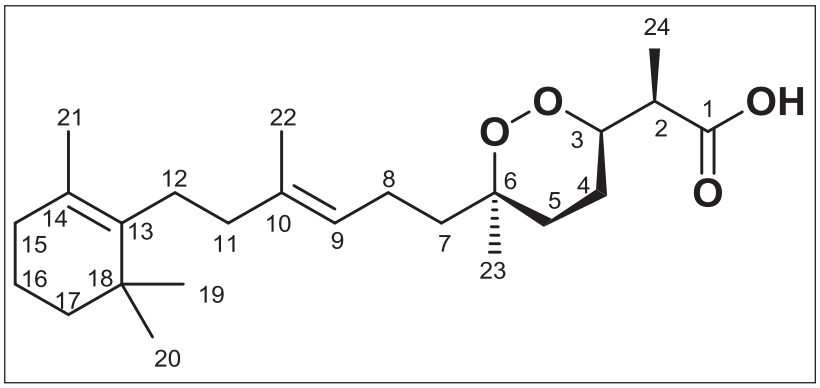






Compound (**12**) was isolated as yellowish oil from EtOAc extract of *Diacarnus erythraeanus*. The ESI mass spectrum of compound (**12**) revealed *pseudo*-molecular ion peaks at *m/z* 393 $[M+H]^+$ (base peak), 415.1 $[M+Na]^+$, 806.8 $[2M+Na]^+$, 391 $[M-H]^-$ (base peak), and 783.2 $[2M-H]^-$, indicating a molecular weight of 424 g/mol. Compound (**12**) showed UV absorption at λ_{max} (MeOH) 206.7 nm. The ^{1}H NMR spectrum revealed one olefinic proton (H-9) at δ_H 5.14 ppm [t (*J*=6.5 Hz)], one methyl doublet (CH_3-24) at δ_H 1.21 ppm [d (*J*=7.0 Hz)], two olefinic methyl signals *viz*., at δ_H 1.61 ppm (s), and δ_H 1.66 ppm (s) which were attributed to CH_3-21, CH_3-22, respectively, and three methyl signals, *viz*., at δ_H 1.00 ppm [6H, (s)], and δ_H 1.28 ppm (s) which were assigned to CH_3-19, CH_3-20, and CH_3-23, respectively (Table 3.11). The ^{13}C NMR and DEPT spectra displayed signals for twenty four carbons, *viz*., six methyl carbons were observed at δ_C (29.7, 29.7, 20.6, 16.6, 20.8, and 14.2 ppm), were assigned to C-19, C-20, C-21, C-22, C-23, and C-24, respectively, nine methylenes at δ_C (25.0, 41.3, 33.5, 23.2, 42.0, 29.6, 34.2, 41.6, and 21.1 ppm) which were attributed to C-4 , C-5, C-7 , C-8 C-11, C-12, C-15, C-16, and C-17, respectively, three methines at δ_C (44.6, and 83.2, and 125.3 ppm),were assigned to C-2, C-3, and C-9, respectively, five quaternary carbons at δ_C (81.7, 137.8, 138.7, 128.6, and 36.5 ppm), were attributed to C-6, C-10, C-13, C-14 and C-18, respectively, in addition to the deshielded signal of C-1 which was observed at δ_C (178.0 ppm) (Table 3.11). The indicative chemical shifts of C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, and C-21 at δ_C (138.7, 128.6, 34.2, 41.6, 21.1, 36.5, 29.7, 29.7, and 20.6 ppm), respectively confirmed the presence of a 2,6,6-trimethylcyclohexene moiety in compound (**12**) (Yunker and Scheuer, 1978; Tsuda *et al*., 1992; Albizati *et al*., 1987). The indicative chemical shift of C-3, C-4, C-5, at δ_C (83.2, 25.0, and 41.3 ppm), respectively further confirmed the presence of a cyclic peroxide moiety in compound (**12**) (Capon *et al*., 1998; Ovenden and Capon, 1998). The

^{1}H-^{1}H COSY spectrum showed four spin systems, the first spin system is CH_3(24)CH(2)CH(3)CH_2(4)CH_2(5), the second one is CH_2(7)CH_2(8)CH(9), the third spin system is CH_2(11)CH_2(12), and the last one is CH_2(15)CH_2(16)CH_2(17). Further elucidation of (**12**) was performed by a HMBC experiment. The HMBC spectrum (Figure 3.15) showed correlations from H-2 at δ_H 2.58 ppm to C-1 (δ_C 178.0) , and C-3 (δ_C 83.2), from CH_3-24 at δ_H 1.21 ppm to C-1 (δ_C 178.0) , and C-3 (δ_C 83.2), from CH_2-5 (at δ_H 1.52 ppm) to C-3 (δ_C 83.2), C-6 (δ_C 81.7) , C-7 (δ_C 33.5), and C-23 (δ_C 20.8), and from H-7 at (δ_H 1.70, 176 ppm) to C-6 (δ_C 81.7), C-9 (δ_C 125.3), and C-23 (δ_C 20.8). Further inspection of the HMBC spectrum also revealed correlations from CH_2-11 (at δ_H 2.02, and 2.06 ppm) to C-9 (δ_C 125.3), C-22 (δ_C 16.6), and C-13 (δ_C 138.7), from CH_2-15 (at δ_H 1.92 ppm) to C-13 (δ_C 138.7), C-17 (δ_C 21.1) and C-21 (δ_C 20.6), and from CH_2-17 (at δ_H 1.43, and 1.60 ppm) to C-13 (δ_C 138.7), C-16 (δ_C 41.6), C-18 (δ_C 36.5), C-19 (δ_C 29.7) and C-20 (δ_C 29.7). The relative configuration of (**12**) was determined depending on the rule used by (Rubio *et al*., 2009). The carbon substituent at C-3 was set as equatorial since H-3 exhibited a vicinal diaxial coupling to H-4, $J_{3,4}$= 7.5 and 12.7 Hz (J= 3-4 is expected for equatorial). The methyl group at C-6 (δ_C= 20.8 ppm) was next assigned as axial from its diagnostic ^{13}C NMR shift ($\delta_{C,eq}$= 23.5-24 ppm; $\delta_{C,ax}$= 20.5-20.9 ppm). The erythro/threo (*R**/*S**) configuration at C-2/C-3 was designated based on the proton chemical shift position of H-24, at δ_H 1.21 ppm (standard value: $\delta_{H,erythro}$= 1.13-1.14 ppm; $\delta_{H,threo}$= 1.22-1.24 ppm). Finally, structure of (**12**) was confirmed by comparison of the $[\alpha]_D^{20}$, ^{1}H NMR, ^{13}C NMR, and mass spectral data with those published for the same known compound which was isolated for the first time from *Diacarnus cf. spinopoculum* (Sperry *et al*., 1998) (Table 3.11).



Stucture of compound (**12**)

Table 3.11: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of (**12**).

Pos.	δ_C (CD_3OD)[a]	δ_C ($CDCl_3$)[b]	δ_H (CD_3OD)[a]	HMBC[a]
1	178.0	180.0	-	-
2	44.6	43.0	2.58, *brs*	1, 3
3	83.2	81.1	4.10, *dd* (12.7, 7.5)	1, 2, 5, 24
4	25.0	23.5	1.77, *m*	-
5	41.3	39.7	152, *m*	3, 6, 7, 23
6	81.7	80.3	-	-
7	33.5	32.0	1.70, 1.67, *m*	6, 9, 23
8	23.2	21.7	2.04, *m*	6
9	125.3	123.3	5.14, *t* (6.5)	7, 8, 11, 22
10	137.8	136.6	-	-
11	42.0	39.9	2.02, 2.06, *m*	9, 13, 22
12	29.6	27.9	2.02, 2.03, *m*	-
13	138.7	137.2	-	-
14	128.6	127.0	-	-
15	34.2	32.8	1.92, t (6.3)	13, 17, 21
16	41.6	39.9	1.43, 1.59, *m*	-
17	21.1	19.6	1.43, 1.60, *m*	13, 16, 18, 19, 20
18	36.5	35.0	-	-
19	29.7	28.7	1, *s*	13
20	29.7	28.7	1, *s*	13
21	20.6	19.9	1.61, *s*	-
22	16.6	16.1	1.66, *s*	-
23	20.8	20.8	1.28, *s*	-
24	14.2	13.3	1.21, *d* (7.0)	1, 3

(a) Compound (**12**). (b) Sperry *et al*., 1998.

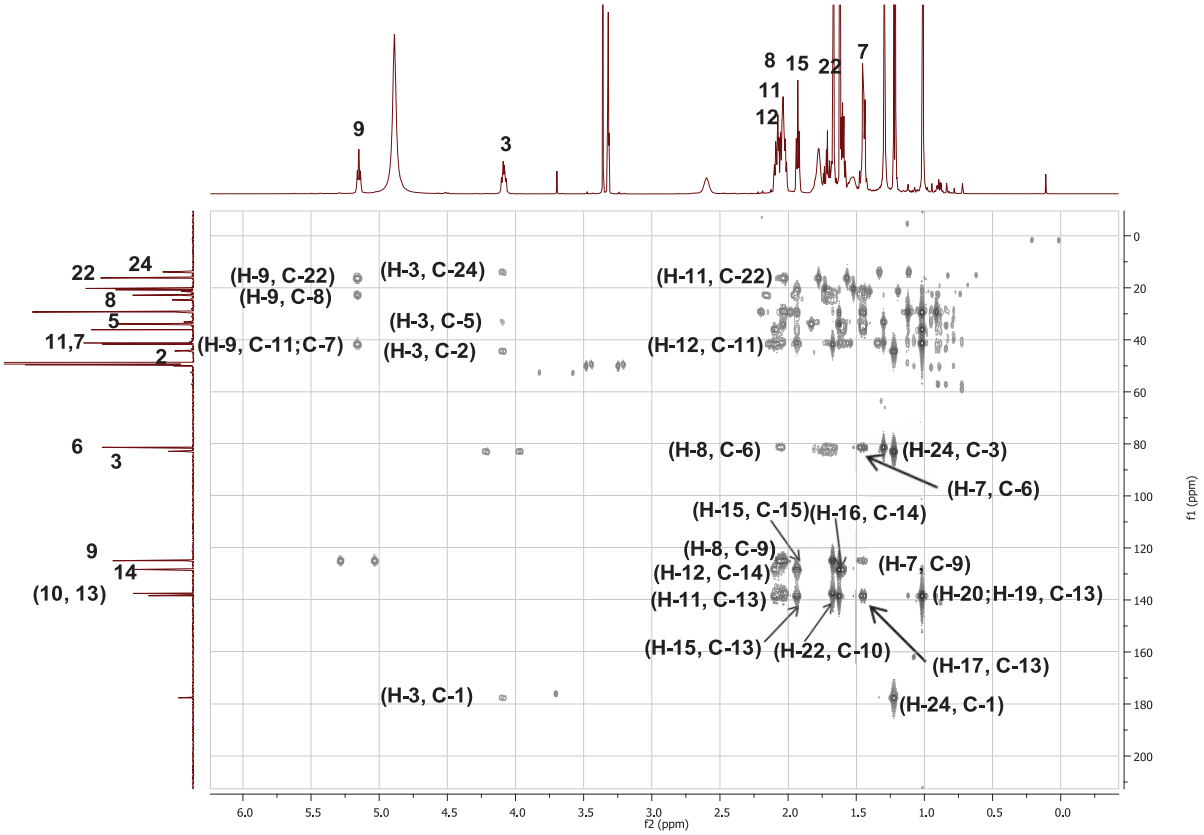
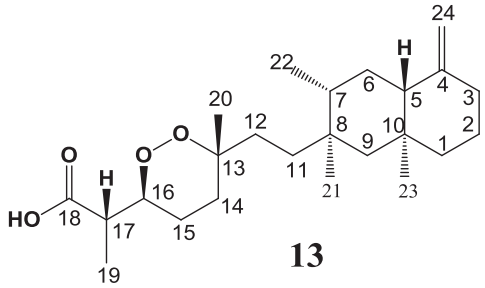
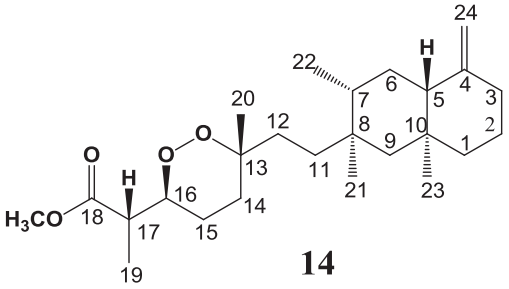


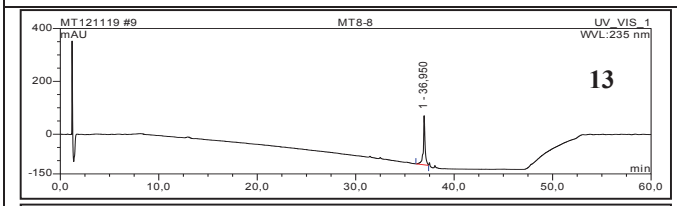
Figure 3.15: HMBC spectrum of compound (**12**).

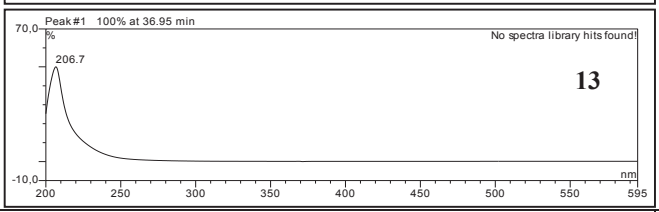
3.3.5.3. Sigmosceptrellin-B (2a) andSigmosceptrellin-B (2) (13, 14: known natural products)

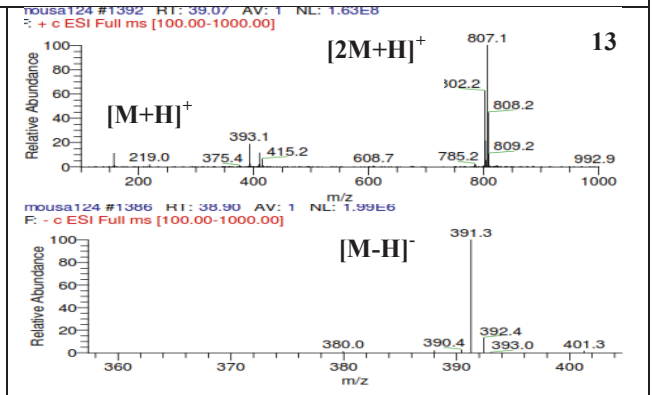
Sigmosceptrellin-B (2a) (13), Sigmosceptrellin-B (2) (14)	
Synonym(s)	Sigmosceptrellin-B (2a) (**13**), Sigmosceptrellin-B (2) (**14**)
Sample code	MT8-8 (**13**), MT8-VL4-7 (**14**)
Biological source	*Diacarnus erythraeanus*
Sample amount	**13** (25 mg), **14** (15 mg)
Physical Description	Colorless oil
Molecular Formula	**13** ($C_{24}H_{40}O_4$), **14** ($C_{25}H_{42}O_4$)
Molecular Weight	**13** (392.57 g/mol.), **14** (406.31 g/mol.)
Optical rotation$[\alpha]_D^{20}$	**13** (+50.6), **14** (+51.3) (*c* 0.2, $CHCl_3$)
Retention time HPLC	**13**(36.95 min), **14** (36.03 min) (standard gradient)

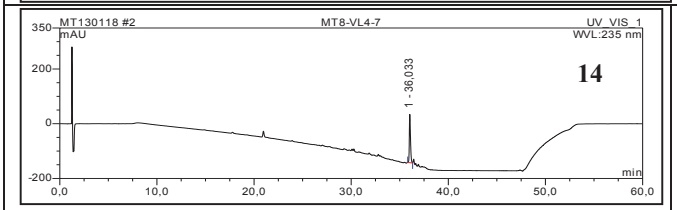


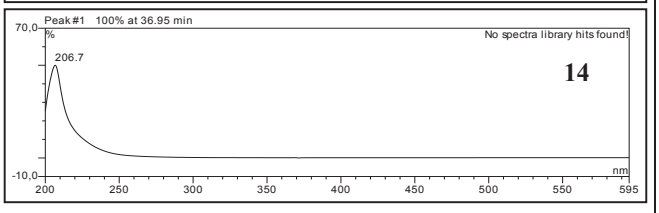


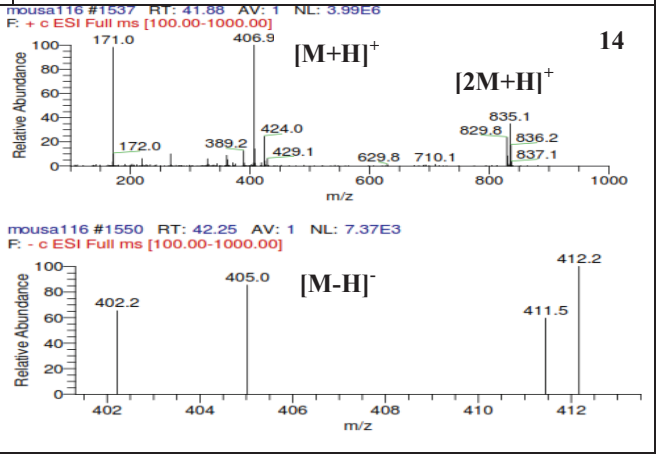






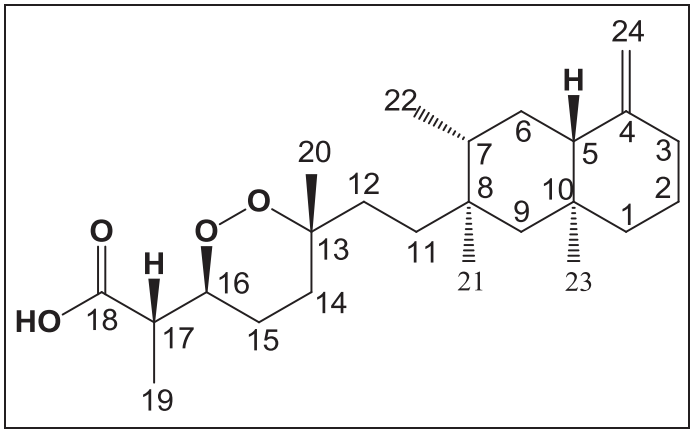




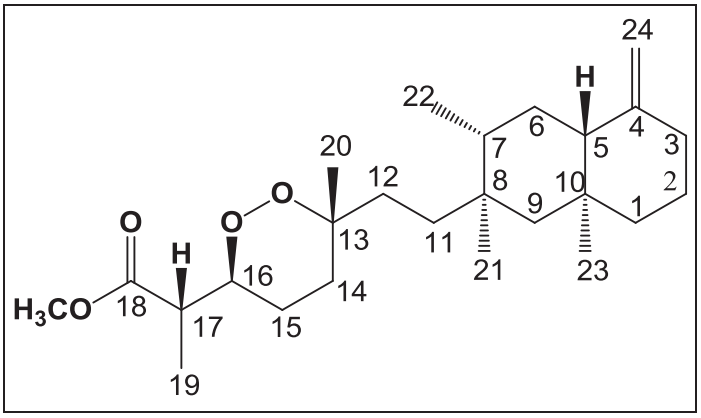


Compounds (**13** and **14**) were isolated as colorless viscous oil from the EtOAc extract of *Diacarnus erythraeanus*. The ESI mass spectrum of (**13**) revealed *pseudo*-molecular ion peaks at *m/z* 393.3 $[M+H]^+$ (base peak), 807.1 $[2M+H]^+$, and 391.3 $[M-H]^-$, respectively indicating a molecular weight of 392 g/mol., while that of (**14**) showed a *pseudo*-molecular ion peaks at *m/z* 406.9 $[M+H]^+$ (base peak), 835.1 $[2M+H]^+$, and 405.0 $[M-H]^-$, respectively revealing a molecular weight of 406 g/mol. Compounds (**13**) and (**14**) showed UV absorption at λ_{max} (MeOH) 206.7, and 206.1 nm, respectively. By comparison of the molecular weights of the two compounds, it was found that they differ by 14 amu indicating the possible presence of an extra methyl group in (**14**) in comparison to (**13**). The ^{1}H NMR spectrum of (**13**) revealed geminal-coupled olefinic protons (CH_2-24) at δ_H 4.47 ppm (brs), two methyl doublets *viz*., at δ_H 1.22 ppm [d (*J*=7.0 Hz)], and δ_H 0.76 ppm [d (*J*=6.5 Hz)] which were assigned for CH_3-19, and CH_3-22, respectively, and three methyl signals *viz*., at δ_H 1.22 ppm (s), δ_H 0.70 ppm (s), and δ_H 1.00 ppm (s) which were attributed to CH_3-20, CH_3-21, and CH_3-23, respectively. On the other side, the ^{1}H NMR spectrum of (**14**) is identical to that of (**13**) except in the presence of an extra methoxyl group at δ_H 3.66 ppm (s) instead of the hydroxyl group (Table 3.12). The structures of both compounds were confirmed by analysis of ^{1}H-^{1}H COSY spectrum (Figure 16, and 17). There are four spin systems *viz*., the first spin system is $CH_2(1)CH_2(2)CH_2(3)$, the second one is $CH(5)CH_2(6)CH(7)CH_3(22)$, the third spin system is $CH_2(11)CH_2(12)$, and the fourth one is $CH_2(14)CH_2(15)CH(16)CH(17)CH_3(19)$ (Table 3.12). The relative configuration and the structure of both compounds were confirmed also by the comparison of ^{1}H NMR, mass spectral data, in addition to the optical rotation with published data of the same known compounds Sigmosceptrellin-B (2a), and Sigmosceptrellin-B (2) which were isolated from *Sigmosceptrella laevis*, from the Vicinity of Laing Island (Paupua-New Guinea) for the first time (Albericci *et al*.,

1982) (Table 3.12). It is worth to mention that three compounds of the sigmosceptrellin type were reported from *Sigmosceptrella laevis* (Albericci *et al*. 1982) which are sigmosceptrellin-A, sigmosceptrellin-B, and sigmosceptrellin-C. These compounds have the same core structure but differ in their relative configuration.



Structure of compound (**13**)



Structure of compound (**14**)

Table 3.12: ^{1}H NMR (600 MHz) and COSY data of (**13)** and (**14)**.

Pos.	δ_H ($CDCl_3$)[a]	δ_H ($CDCl_3$)[b]	δ_H ($CDCl_3$)[c]	COSY[a,b]
1	1.44, *m*	1.44, *m*	-	2, 23
2	1.22, 1.83, *m*	1.22, 1.83, *m*	-	3, 1
3	2.26, 2.06, *m*	2.26, 2.06, *m*	-	24, 2
4	-	-	-	-
5	1.51, *m*	1.51, *m*	-	6
6	1.42, *m*	1.42, *m*	-	5, 7
7	1.38, *m*	1.38, *m*	-	22, 6
8	-	-	-	-
9	1.41, *m*	1.41, *m*	-	23
10	-	-	-	-
11	1.10-1.20, *m*	1.10-1.20, *m*	-	12, 21
12	1.30-1.43, *m*	1.30-1.43, *m*	-	11, 20
13	-	-	-	-
14	1.56, *m*	1.58, *m*	-	-
15	1.68, *m*	1.72, *m*	-	16
16	4.12, *m*	4.13, *m*	4.14, *brs*	15
17	2.64, *brs*	2.66, *brs*	2.65, *quintet* (7.0)	15, 16, 19
18	-	-	-	-
19	1.22, *d* (7.0)	1.23, *d* (7.0)	1.21, *d* (7.0)	16
20	1.22, *brs*	1.23, *brs*	1.23, *s*	12
21	0.70, *s*	0.71, *s*	0.73, s	11, 9
22	0.76, *d* (6.5)	0.77, *d* (6.4)	0.80, *d* (5.0)	7
23	1.02, *s*	1.03, *s*	1.03, *s*	1, 9
24	4.47, *brs*	4.47, *brs*	4.50, *brs*	3
25	3.66, *s*	-	3.70, *s*	-

(a) Compound (**13**). (b) Compound (**14**). (c) Albericci *et al*., 1982.

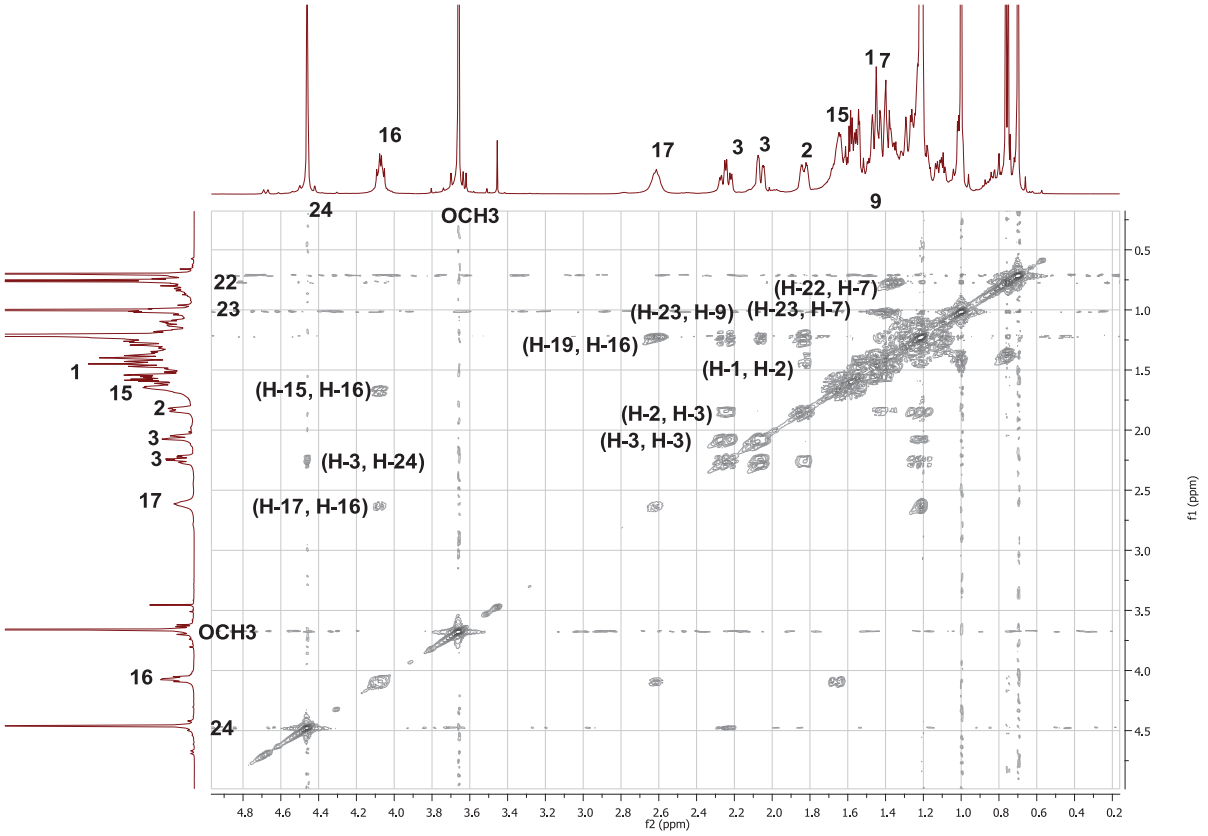


Figure 3.16: ^{1}H-^{1}H COSY spectrum of compound (**13**).

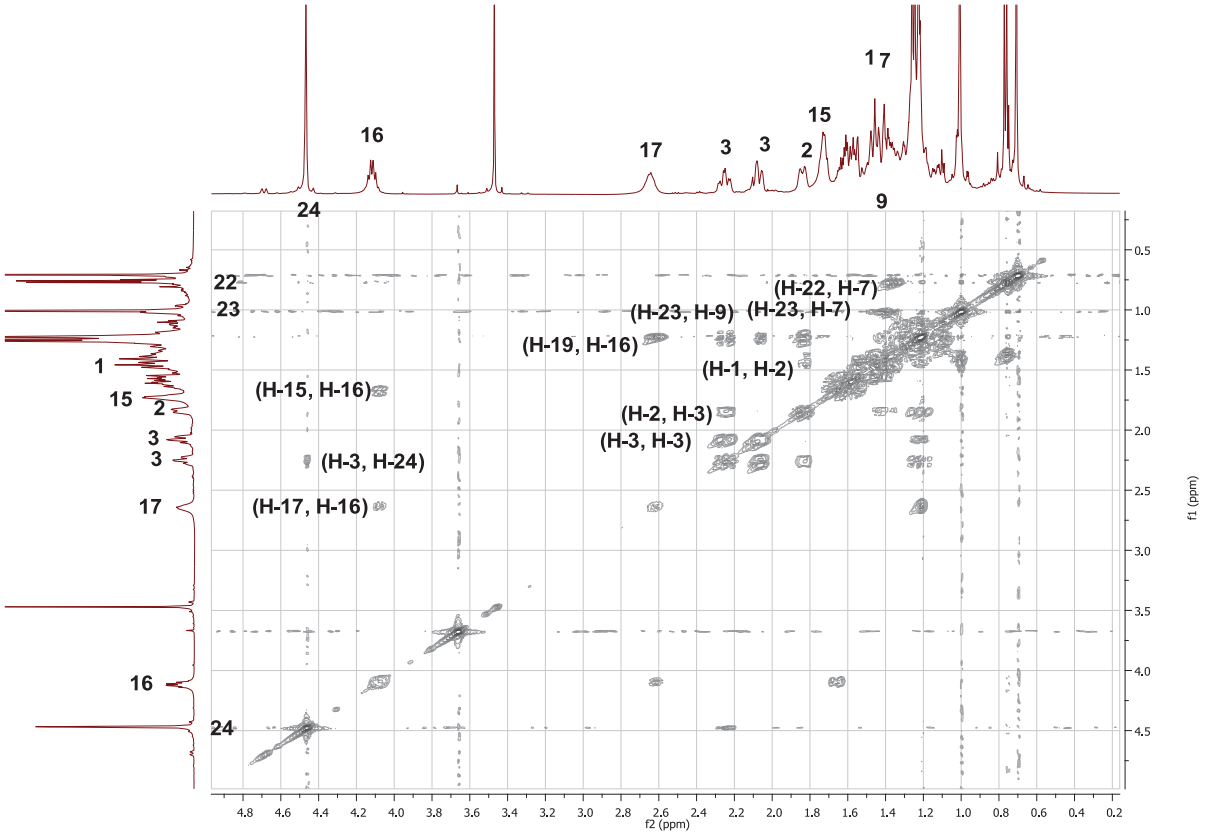
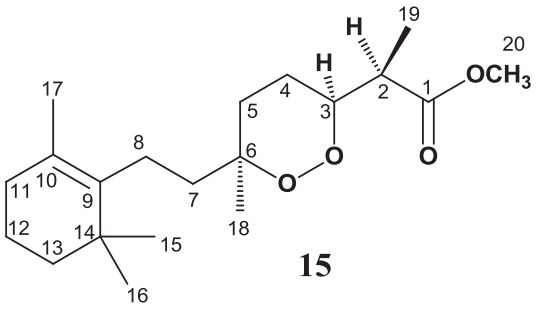
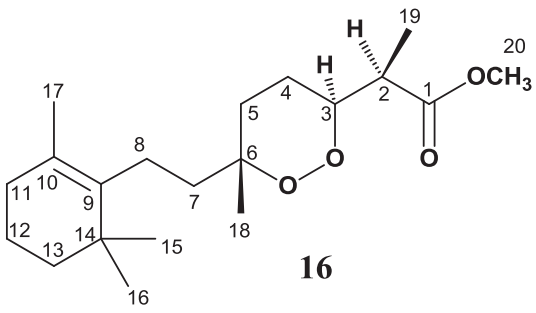


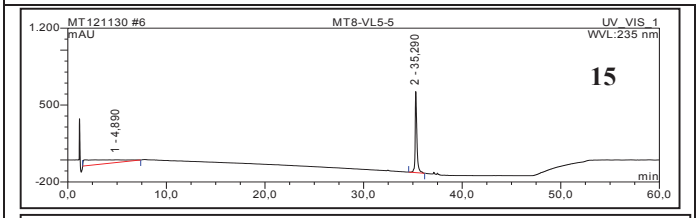
Figure 3.17: ^{1}H-^{1}H COSY spectrum of compound (**14**).

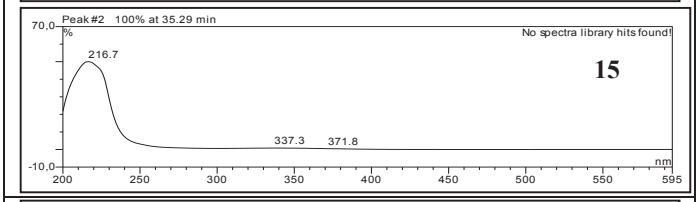
3.3.5. Methyl-2-epinuapapuanoate (15, 16: known natural products)

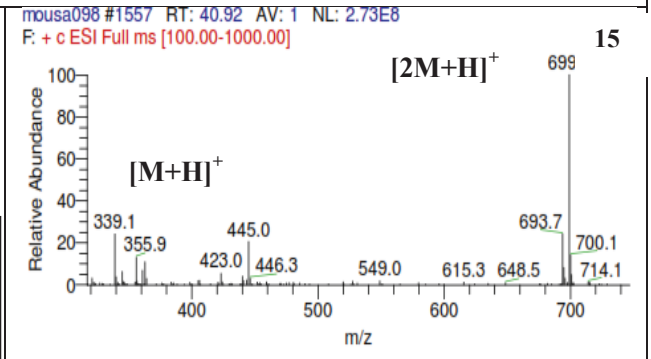
(+)-Methyl-2-epinuapapuanoate (15); (-)-Methyl-2-epinuapapuanoate (16)	
Synonym(s)	(+)-Methyl-2-epinuapapuanoate (**15**), (-)-Methyl-2-epinuapapuanoate (**16**)
Sample code	MT8-VL5-5 (**15**), MT8-VL4-2 (**16**)
Biological source	*Diacarnus erythraeanus*
Sample amount	**15** (9 mg), **16** (15 mg)
Physical Description	Colorless oil
Molecular Formula	**15** ($C_{20}H_{34}O_4$), **16** ($C_{20}H_{34}O_4$)
Molecular Weight	**15** (338.48 g/mol.), **16** (338.48 g/mol.)
Optical rotation $[\alpha]_D^{20}$	**15** (+48.8), **16** (-47.9) (*c* 0.2, $CHCl_3$)
Retention time HPLC	**15** (35.3 min), **16** (36.1 min) (standard gradient)

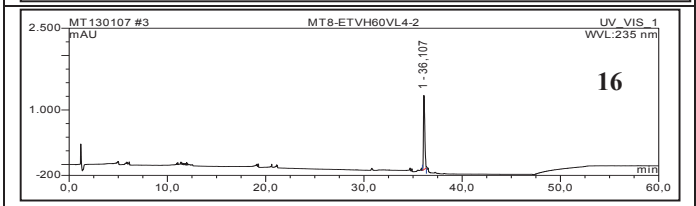


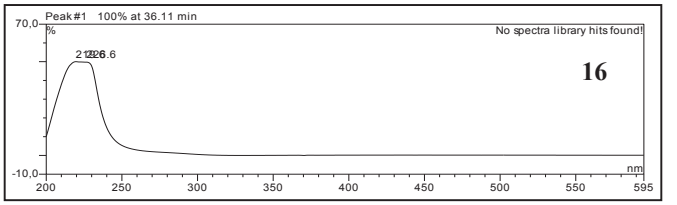


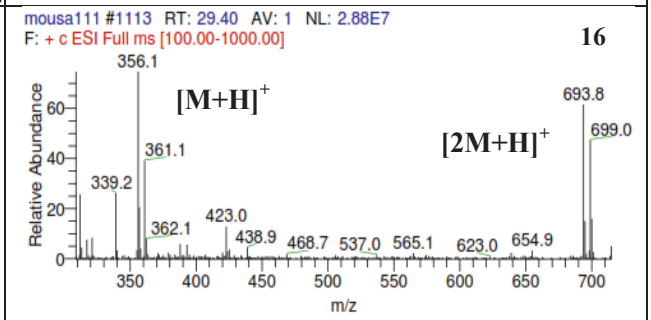








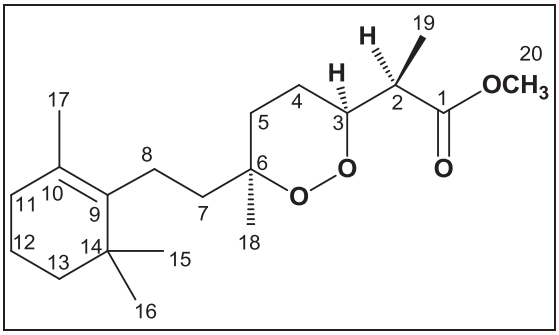




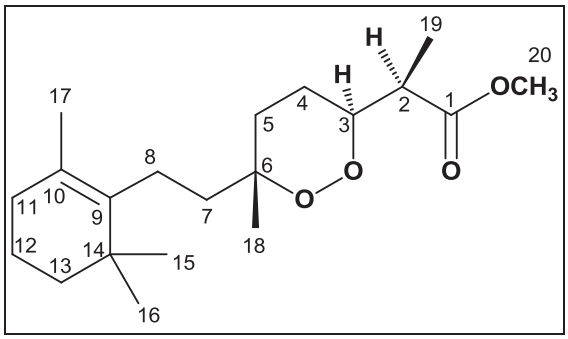
Compounds (**15** and **16**) were isolated as colorless viscous oil from the EtOAc extract of *Diacarnus erythraeanus*. The ESI mass spectrum of both compounds (**15** and **16**) revealed *pseudo*-molecular ion peaks at *m/z*339.2 $[M+H]^+$, and 699.0 $[2M+Na]^+$, indicating a molecular weight of 338 g/mol. for both compounds. Compounds (**15** and **16**) showed the same UV absorption at λ_{max} (MeOH) 2.16.7 nm. The ^1H NMR spectrum of (**15**) showed one methyl doublet at δ_H 1.11 ppm [d (*J*=7.1Hz)] which was assigned to CH_3-19, one olefinic methyl group (CH_3-17) at δ_H 1.59 ppm (s), three methyl signals at δ_H 0.97 ppm (s), δ_H 0.99 ppm (s), and δ_H 1.13 ppm (s) which were attributed to CH_3-15, CH_3-16, and CH_3-18, respectively, and one methoxyl signal at δ_H 3.67 ppm (s) which was assigned for OCH_3-20 (Table 3.13). The ^{13}C NMR and DEPT spectra of (**15**) revealed signals for 24 carbon atoms, *viz*., five methyls at δ_C (28.9, 28.7, 20.0, 23.9, and 12.0 ppm) which were attributed to C-15, C-16, C-17, C-18, and C-19, respectively. Seven methylenes were observed at δ_C (22.8, 32.8, 34.0, 22.5, 33.0, 19.8, and 41.1 ppm) which were assigned to C-4, C-5 , C-7, C-8, C-11, C-12, and C-13, respectively, four quaternary carbons at δ_C (80.3, 136.9, 127.3, and 35.0 ppm) which were assigned to C-6, C-9, C-10, and C-14, respectively, two methines at δ_C (42.9, and 81.4 ppm), were attributed to C-2, and C-3, respectively, one methoxyl carbon at δ_C (51.9 ppm), was assigned to 20-OCH_3, and the deshielded signal of C-1 was observed at δ_C (174.5 ppm) (Table 3.13). The indicative chemical shifts of C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, and C-17 at δ_C (136.9, 127.3, 33.0, 19.8, 41.1, 35.0, 28.9, 28.7, and 20.0 ppm), respectively confirmed the presence of a 2,6,6-trimethylcyclohexene moiety in compound (**15**) (Yunker and Scheuer, 1978; Albizati *et al*.,1987; Tsuda *et al*., 1992), furthermore, the indicative chemical shifts of C-3, C-4, and C-5, at δ_C (81.4, 22.8, and 32.8 ppm), respectively confirmed the presence of a cyclic peroxide moiety in compound (**15**) (Capon *et al*., 1998; Ovenden and Capon, 1998). The ^1H-^1H COSY

spectrum revealed three spin systems, the first spin system is CH_3(19)CH(2)CH(3)CH_2(4)CH_2(5), the second one is CH_2(7)CH_2(8), and the third spin system is CH_2(11)CH_2(12)CH_2(13). Further elucidation of compound (**15**) was performed by a HMBC experiment. The HMBC spectrum (Figure 3.18) showed correlations from H-2 at δ_H 2.54 ppm to C-1 (δ_C 174.5), C-3 (δ_C 81.4), C-4 (δ_C 22.8), and 19 (δ_C 12.0), from H-3 at δ_H 4.23 ppm to C-1 (δ_C 174.5), C-2 (δ_C 42.9), and C-19 at (δ_C 12.0), from CH_2-4 (at δ_H 1.63 ppm) to C-3 (δ_C 81.4), and C-6 (δ_C 80.3), and from CH_2-5 (at δ_H 1.63, and 1.75 ppm) to C-3 (δ_C 81.4), and C-6 (δ_C 80.3). Further inspection of the HMBC spectrum (Figure 3.18) also revealed correlations from CH_2-7 (at δ_H 1.91, and 1.63 ppm) to C-6 (δ_C 80.3), C-9 (δ_C 136.9), from CH_2-8 (at δ_H 2.05, and 1.91 ppm) to C-6 (δ_C 80.3), C-9 (δ_C 136.9), C-10 (δ_C 127.3), and C-14 (δ_C 35.0), from CH_2-13 (at δ_H 1.39 ppm) to C-9 (δ_C 136.9), from CH_3-15 at δ_H 0.97 ppm to C-9 (δ_C 136.9), from CH_3-16 at δ_H 0.99 ppm to C-9 (δ_C 136.9), and from CH_3-19 at δ_H 1.11 ppm to C-1 (δ_C 174.5), and C-3 (δ_C 81.4) (Table 3.13). The ^{1}H NMR data of (**16**) are identical to those of compound (**15**) except for CH_3-19 at C-1 (δ_H 1.11 ppm [d (*J*=7.14 Hz)], and δ_H 1.23 ppm [d (*J*=7.04 Hz)] for (**15**) and (**16**), respectively) and CH_3-18 for C-16 (δ_H 1.13 ppm (s), and δ_H 1.30 ppm (s) for (**15**) and (**16**), respectively) which indicated that the relative configurations at C-1 and for C-16 for the compounds (**15**), and (**16**) are different. The ^{1}H-^{1}H COSY spectrum of (**16**) revealed three spin systems, the first spin system is CH_3(19)CH(2)CH(3)CH_2(4)CH_2(5), the second one is CH_2(7)CH_2(8), and the third spin system is CH_2(11)CH_2(12)CH_2(13) (Figure 3.19). The relative configurations for (**15**) and (**16**) were determined depending on the rule used by (Rubio, *et al*., 2009). The carbon substituent at C-3 for (**15**), and (**16**) was set as equatorial since H-3 exhibited a vicinal diaxial coupling to H-4, $J_{3,4}$ in (**15**)= 8.2, and 7.3 Hz and $J_{3,4}$ in (**16**)= 8.2, and 7.9 Hz (*J*= 3-4 is expected for equatorial). The CH_3 signal at δ_C (23.9 ppm) for

C-6 of (**15**) was next assigned as equatorial while the signal for (**16**) at δ_C (20.5 ppm)* was assigned as axial depending on their diagnostic ^{13}C NMR shift ($\delta_{C,eq}$= 23.5-24.0 ppm; $\delta_{C,ax}$= 20.5-20.9 ppm). The erythro (R*/R*) configuration at C-2/C-3 was designated for compound (**15**) while it was erythro/threo (R*/S*) for compound (**16**) based on the proton chemical shift of H-19, at δ_H 1.11 ppm and 1.23 ppm for (**15**) and (**16**), respectively (Standard value: $\delta_{H,erythro}$= 1.13-1.14 ppm; $\delta_{H,threo}$= 1.22-1.24 ppm). According to this rule the relative configuration for (**15**) is 2*R*, 3*R*, 6*S* while for (**16**) is 2*R*, 3*S*, 6*R*. The structures of (**15** and **16**) were confirmed finally by comparison the ^1H NMR, ^{13}C NMR, mass spectral data, in addition to the optical rotation to the published data of the same known compound (D´Ambrosio *et al*., 1997) (Table 3.13).



Structure of compound (**15**)



Structure of compound (**16**)

Table 3.13: ^{1}H NMR (600 MHz), and ^{13}C NMR (150 MHz) data of (**15**) and (**16**).

Pos.	δ_C ($CDCl_3$)[a]	δ_C ($CDCl_3$)[c]	δ_H ($CDCl_3$)[a]	δ_H ($CDCl_3$)[b]	δ_H ($CDCl_3$)[c]
1	174.5	174.25	-	-	-
2	42.9	42.95	2.54, *m*	2.64, *brs*	2.65, *br. dq* (7.0)
3	81.4	81.30	4.23, *ddd* (8.2, 7.3, 3.0)	4.11, *ddd* (8.2, 7.9, 4.1)	4.13, *ddd* (9, 7.5, 3.3)
4	22.8	21.68	1.63, *m*	1.68, *m*	1.68, *m*
5	32.8	31.81	1.63,1.75, *m*	1.63, 1.73, *m*	1.65, *m*
6	80.3	80.35	-	-	-
7	34.0	39.0	1.91,1.63, *m*	1.69, *m*	1.68, *m*
8	22.5	21.76	2.05,191, *m*	2.0, *m*	2.02, *m*
9	136.9	136.36	-	-	-
10	127.3	127.30	-	-	-
11	33.0	32.74	1.88, *m*	1.86, *t* (6.2)	1.88, *m*
12	19.8	19.47	1.55, *m*	1.54, *m*	1.55, *m*
13	41.1	39.01	1.39, *m*	1.35, 1.44, *m*	1.41, *m*
14	35.0	35.04	-	-	-
15	28.9	28.60	0.97, *s*	0.95, *s*	0.97, *s*
16	28.7	28.57	0.99 , *s*	0.96, *s*	0.98, *s*
17	20.0	19.73	1.59, *s*	1.55, *s*	1.57, *s*
18	23.9	20.45	1.13, *s*	1.30, *s*	1.32, *s*
19	12.0	13.61	1.11, *d* (7.1)	1.23, *d* (7.1)	1.25, *d* (7.0)
20	51.9	51.74	3.67, *s*	3.67, *s*	3.67, *s*

* δ_C of C-18 for compound **16** was deduced by the comparison of the δ_H of H-18 (1.30 ppm) for (**16**) with those of (**15**) and the reference which were 1.13, and 1.32 ppm respectively.

(a) Compound (**15**). (b) Compound (**16**). (c) D´Ambrosio *et al*., 1997.

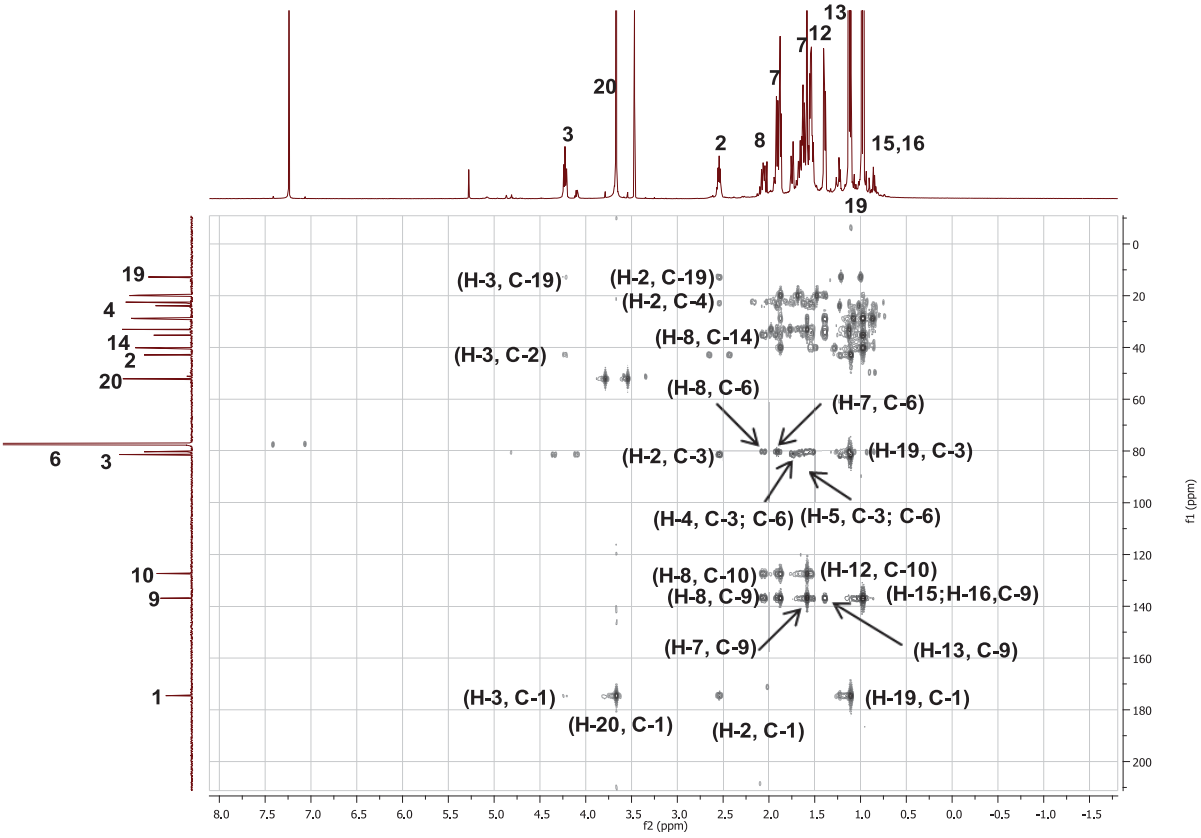


Figure 3.18: HMBC spectrum of compound **(15)**.

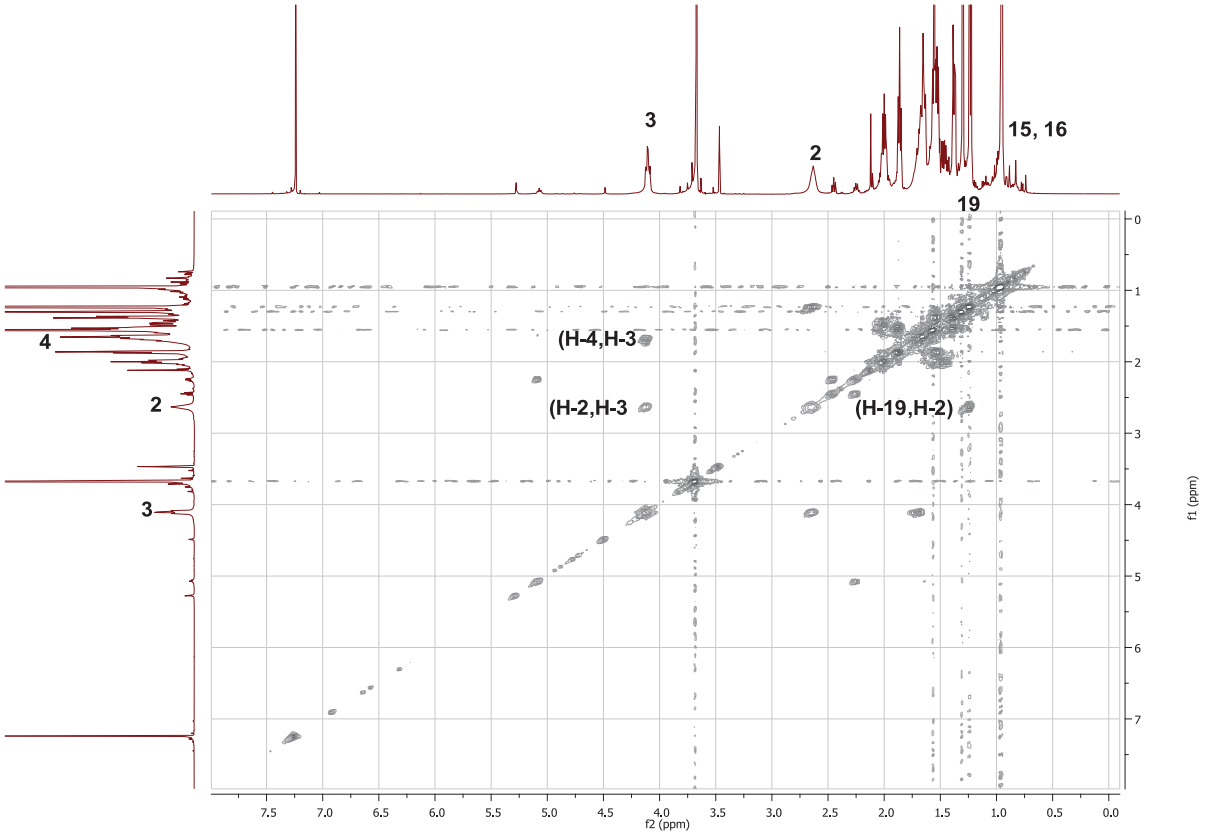


Figure 3.19: ^{1}H-^{1}H COSY spectrum of compound **(16)**.

3.3.6. Bioactivity assay results for compounds from sponges from the Gulf of Aqaba in Jordan.

The isolated compounds which were isolated from five sponges from Gulf of Aqaba in Jordan were subjected to a cytotoxicity (MTT) assay against the mouse lymphoma (L5178Y) cells. These sponges are: the unknown sponge (**MT5**), *Hemimycale* sp. (**MT6**), *Theonella* sp. (**MT3**), *Diacarnus ardoukobae* (**MT1**), and *Diacarnus erythraeanus* (**MT8**).

3.3.6.1. Bioactivity assay for compounds isolated from the unknown sponge (MT5)

Table 3.14: Cytotoxicity assay results for the compounds isolated from the unknown sponge (**MT5**)

Nr.	Compound tested	L5178Y growth in% (@ 10 μg/mL)	EC_{50}* (μg/mL)
1	3-amino-1-(2-amino-4-bromophenyl) propan-1-one	-4	>10
2	7-bromoquinolin-4(1*H*)-one	89.7	
3	6-bromo-3-carbaldehyde	75.3	
4	cuulerpin	80.4	
5	(*Z*)-5-(4-hydroxybenzylidene)-hydantoin	23	
6	(Z)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin	11	

* Data provided by Prof. W. E. G. Müller, Mainz.

The cytotoxicity assay results interestingly revealed that 3-amino-1-(2-amino-4-bromophenyl) propan-1-one (**1**), (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**) showed a moderate cytotoxic activity against the L5178Y cell line. 7-Bromoquinolin-4(1*H*)-one (**2**), 6-bromoindole-3-carbaldehyde (**3**), and caulerpin (**4**) showed a weak cytotoxic activity (Table 3.14).

3.3.6.2. Bioactivity assay for compounds isolated from *Hemimycale* sp. (MT6)

The compounds (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**7**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**8**) which were isolated from the ethyl

acetate and *n*-hexane fraction of *Hemimycale* sp. (**MT6**), respectively were subjected to cytotoxicity (MTT) assay against the mouse lymphoma (L5178Y) cells. The results revealed that (**7**), and (**8**) had a moderate cytotoxic activity against the L5178Y cell line with growth inhibition of 77%, and 89%, respectively at 10 μg/mL.

3.3.6.3. Bioactivity assay for compound isolated from *Theonella* sp. (MT3)

The isolated compound from the ethyl acetate fraction of *Theonella* sp. (**MT3**) which is swinholide A (**9**) showed a moderate cytotoxic activity against the mouse lymphoma (L5178Y) cells with growth inhibition of 87% at 10 μg/mL.

3.3.6.4 Bioactivity assay for compounds isolated from *Diacarnus ardoukobae* (MT1).

The isolated compound from the ethyl acetate fraction of *Diacarnus ardoukobae* (**MT1**) which is latrunculin B (**10**) showed a moderate cytotoxic activity against the mouse lymphoma (L5178Y) cells with a growth inhibition of 92% at 10 μg/mL.

3.3.6.5. Bioactivity assay for compounds isolated from *Diacarnus erythraeanus* (MT8)

Table 3.15: Cytotoxicity assay results for the compounds isolated from *Diacarnus erythraeanus* (**MT8**).

Nr.	Compound tested	L5178Y growth in% (@10 μg/mL)	EC_{50}* (μg/mL)	EC_{50}* (μM)
11	(+)-muqubilone A	17		
12	(-)-muqubilin A	-3.1	0.19	0.49
13	sigmosceptrellin-B (2a)	-2.4	0.5	1.2
14	sigmosceptrellin-B (2)	1.0	1.32	3.4
15	(+)-methyl-2-epinuapapuanoate	4.2	2.2	6.4
16	(-)-methyl-2-epinuapapuanoate	70.29		

* Data provided by Prof. W. E. G. Müller, Mainz.

(-)-Muqubilin A, sigmosceptrellin-B (2a) (**13**), sigmosceptrellin-B (2) (**14**), and (+)-methyl-2-epinuapapuanoate (**15**) proved to be highly effective against the L5178Y cancer cell

line. Furthermore, (+)-muqubilone A (**11**) showed a moderate cytotoxic activity against the L5178Y cancer cell line. (-)-Methyl-2-epinuapapuanoate (**16**) proved to be inactive against this cell line (Table 3.15).

3.4. Isolated compounds from sponge from Ambon in Indonesia

The current literature reported that Indonesia is within the top ten potential locations for new sponge-derived compounds (Mehbub *et al*., 2014). Recently, many new biologically active compounds have been isolated from some Indonesian sponges like, sesquibastadine (Niemann *et al*., 2012), polycarpthiamines A, and B (Pham *et al*., 2013), manadoperoxide B (Chianese *et al*., 2013), dictyoceratain-C (Arai *et al*., 2014), and variabines A, and B (Sakai *et al*., 2014).

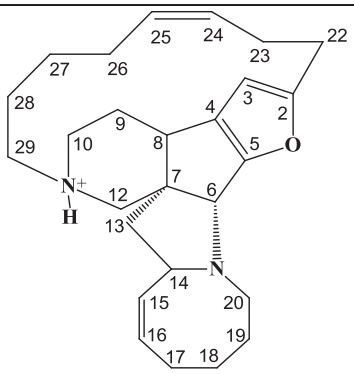
3.4.1. Isolated compounds from *Acanthostrongylophora ingens*

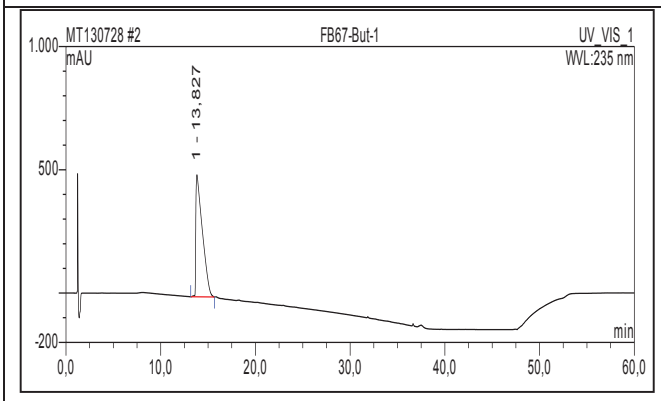
Taxonomically, *Acanthostrongylophora ingens* was previously reported to produce manzamine alkaloids similar to some other members of the family (Petrosiidae) (*Haliclona* sp. and *Petrosia* sp.) (Radwan *et al*., 2012). Manzamines are structurally interesting alkaloids that are isolated from marine sponges and are characterized by a fused tetra- or pentacyclic ring system that is attached to a *β*-carboline moiety (Radwan *et al*., 2012). Since the isolation of manzamine A (Sakai *et al*., 1986), more than 80 manzamine derivatives have been already isolated (Furusato *et al*., 2014), including nakadomarin A (Kobayashi *et al*., 1997), cantholactone and acantholactam (Wahba *et al*., 2012), zamamidine A (Yamada *et al*., 2009), zamamiphidin A (Kubota *et al*., 2013), manadomanzamine A (Rao *et al*., 2004), manzamine C (Kondo *et al*.,1992), manzamine *N*-oxide (Edrada *et al*., 1996), and the dimer kauluamine (El Sayed *et al*., 2001). Manzamine derivatives possess various biological activities, such as proteasome inhibitory (El-Desoky *et al*., 2014), intracellular proteolytic complex (FururSato *et al*., 2014), cytotoxic (Sakai *et al*., 1986), antimicrobial (Rao *et al*., 2004), antimalarial (Radwan *et al*.,

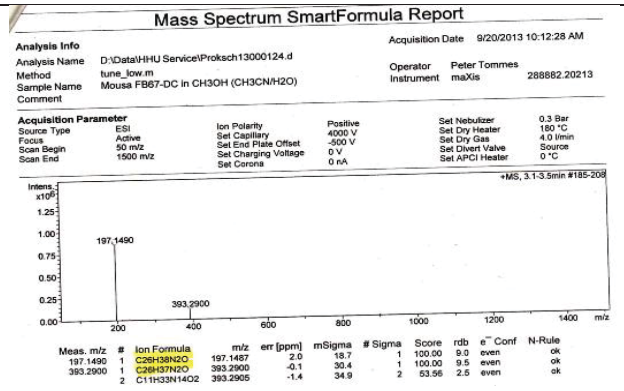
2012), antiviral, antiinflammatory (Yousaf *et al*., 2004), antiatherosclerotic (Eguchi *et al*., 2013), and insecticidal effects (Edrada *et al*., 1996). In the present study, the methanolic extract (2.25 g) which was obtained from 500 g of *Acanthostrongylophora ingens* sponge was partitioned between ethyl acetate and water. The water phase was further extracted by *n*-BuOH to give *n*-BuOH fraction while the obtained ethyl acetate fraction was then filtered and dried using a rotary evaporator and was found to be 1.65 g. The dried EtOAc fraction was then subjected to RP-20 column chromatography employing (H_2O:MeOH) (100:0) to (H_2O:MeOH) (0:100), (MeOH:acetone) (50:50), and (MeOH:acetone) (0:100) as mobile phases. Further purification was performed via Sephadex (LH-20) and semi-preparative HPLC applying a gradient elution from 5% MeOH/H2O/0.1%TFA to 100% MeOH and using a flow rate of 5 mL/min to yield 9 compounds. Yield of compounds from the ethyl acetate fraction were as follows: **18** (1 mg), **19** (5 mg), **20** (5 mg), **21** (9 mg), **22** (0.7 mg), **23** (1.1 mg), **24** (1.1 mg), **25** (1 mg), **26** (1.2 mg). The *n*-BuOH fraction was further purified by using semi-preparative HPLC applying a gradient elution from 5% MeOH/H2O/0.1%TFA to 100% MeOH and using a flow rate of 5mL/min to yield **17** (1 mg).

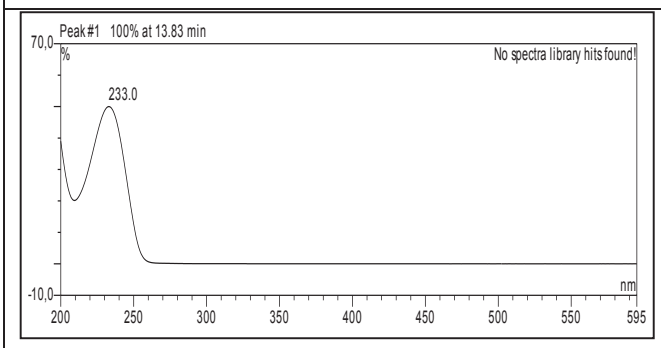
3.4.1.1. Nakadomarin A (17: known natural product)

Nakadomarin A	
Synonym(s)	Nakadomarin A
Sample code	FB67-But-1
Biological source	*Acanthostrongylophora ingens*
Sample amount	1 mg
Physical Description	Colorless amorphous solid
Molecular Formula	$C_{26}H_{36}N_2O$
Molecular Weight	392.58 g/mol.
Optical rotation$[\alpha]_D^{20}$	-4.3 (*c* 0.2, MeOH)
Retention time HPLC	13.83 min (standard gradient)







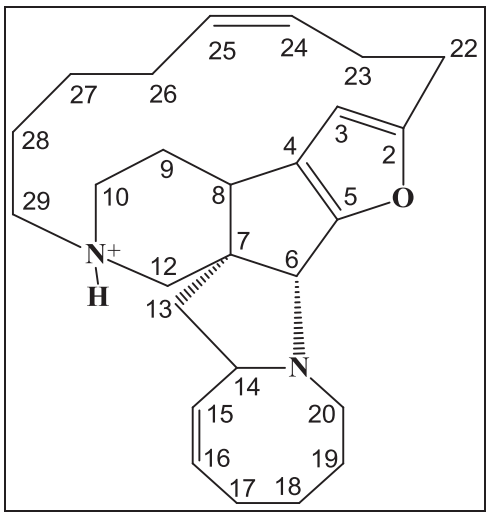


No ionization in negative ESI-MS

Results

Compound (**17**) was isolated as colorless amorphous solid from the *n*-BuOH fraction of *Acanthostrongylophora ingens*. The HRESIMS of compound (**17**) showed an ion peak at *m/z* 393.2900 $[M+H]^+$, indicating that the molecular weight of (**17**) is 392 g/mol. with a molecular formula $C_{26}H_{36}N_2O$ (calculated 393.2906, Δ 0.0006). Compound (**17**) showed UV absorption at λ_{max} (MeOH) 233.0 nm. The ^{1}H NMR spectrum revealed five olefinic protons, at δ_H 5.99 ppm (s), δ_H 5.76 ppm [dd (*J*=8.3, 10.1 Hz)], δ_H 6.13 ppm [dd (*J*=9.7,17.6 Hz)], 5.25 ppm [t (*J*=11.3 Hz)] and δ_H 5.15 ppm [dd (*J*=8.8, 10.6 Hz)] which were assigned to H-3, H-15, H-16, H-24, and H-25, respectively (Table 3.16). The ^{13}C NMR spectrum revealed signals for twenty six carbons, fourteen methylene carbons at δ_C (21.9, 44.7, 52.0, 41.4, 25.1, 26.8 , 21.7, 50.7, 29.0, 26.8, 22.2, 23.3, 20.8, and 56.5 ppm) which were assigned to C-9, C-10, C-12, C-13, C17, C-18, C-19, C-20, C-22, C-23, C-26, C-27, C-28, and C-29, respectively, eight methines at δ_C (105.3, 70.1, 40.6, 58.7 124.9, 140.0, 130.1, and 129.7 ppm) which were attributed to C-3, C-6, C-8, C-14, C-15, C-16, C-24, and C-25, respectively, and four quaternary carbons (164.6, 137.4, 148.0, and 57.5 ppm) which were assigned to C-2, C-4, C-5 and C-7, respectively (Table 3.16). The structure of (**17**) was also further elucidated by analysis of ^{1}H-^{1}H COSY spectrum (Figure 3.20). There are three spin systems, *viz*., the first spin system is CH_2(13)CH(14)CH(15)CH(16)CH_2(17)CH_2(18)CH_2(19)CH_2(20), the second one is CH(8)CH_2(9)CH_2(10)NH(11)CH_2(12), and the last one is CH_2(22)CH_2(23)CH(24)CH(25) CH_2(26)CH_2(27)CH_2(28)CH_2(29)NH(11)CH_2(12). Moreover, the COSY cross peaks CH_2(10)NH(11)CH_2(12) proved that compound (**17**) exists as a salt. Further elucidation of compound (**17**) was performed through a HMBC experiment. This HMBC spectrum (Figure 3.21) showed correlations from H-3 at δ_H 5.99 ppm to C-2 (δ_C 164.6), C-4 (δ_C 137.4), and C-5 (δ_C 148.0), from H-6 at δ_H 5.49 ppm to C-5 (δ_C 148.0), C-7 (δ_C 57.5), C-8 (δ_C 40.6), C-12 (δ_C

52.0), and C-13 (δ_C 41.4), from H-8 at δ_H 3.22 ppm to C-4 (δ_C 137.4), C-5 (δ_C 148.0), C-9 (δ_C 21.9), C-10 (δ_C 44.7), and C-13 (δ_C 41.4), from CH_2-10 (at δ_H 3.68, and 2.27 ppm) to C-7 (δ_C 57.5), C-8 (δ_C 40.6), and C-12 (δ_C 41.4), from CH_2-13 (at δ_H 2.22 ppm) to C-6 (δ_C 70.1), C-7 (δ_C 57.5), C-8 (δ_C 40.6), C-12 (δ_C 52.0), C-14 (δ_C 58.7), and C-15 (δ_C 124.9), and from H-15 at δ_H 5.76 ppm to C-13 (δ_C 41.4), C-14(δ_C 58.7), and C-17 (δ_C 25.1). Further inspection of the HMBC spectrum also revealed correlations from CH_2-17 (at δ_H 2.23 ppm) to C-15 (δ_C 124.9), and C-16 (δ_C 140.0), from CH_2-22 (at δ_H 2.96, and 2.58 ppm) to C-2 (δ_C 164.6), C-3 (δ_C 105.2), and C-24 (δ_C 130.1), and from CH_2-27 (at δ_H 1.46, and 1.59 ppm) to C-24 (δ_C 130.1), C-25 (δ_C 129.7), and C-29 (δ_C 56.5) (Table 3.16). Further structure confirmation was achieved by the comparison of ^{1}H NMR,^{13}C NMR, mass spectral data, in addition to the $[\alpha]_D^{20}$ of (**17**) with those of published data of nakadomarin A which was isolated for the first time from an Okinawan marine sponge *Amphimedon* sp. (Kobayashi *et al*., 1997) (Table 3.16).



Structure of compound (**17**)

Table 3.16: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of (**17**).

Pos.	δ_C ($CDCl_3$)[a]	δ_C (CD_3OD)[b]	δ_H ($CDCl_3$)[a]	δ_H (CD_3OD)[b]	HMBC[a]
1	-	-	-	-	-
2	164.6	164.6	-	-	-
3	105.2	105.6	5.99, *s*	5.99, *s*	2, 4, 5, 23
4	137.4	137.9	-	-	-
5	148.0	156.2	-	-	-
6	70.1	75.9	5.49, *s*	4.25, *brs*	5, 7, 8, 12, 13
7	57.5	64.2	-	-	-
8	40.6	43.2	3.22, *brs*	2.95, *dd* (2.7, 3.4)	5, 4, 10, 9, 13
9	21.9	23.1	2.41, *m*	2.04, *dddd* (2.4, 2.3, 12.1 , 14.2)	10, 7
			1.91, *m*	1.85, *ddd* (2.7, 4.5, 14.2)	
10	44.7	46.6	3.67, *d* (11.6)	2.22, *dd* (2.4, 12.1)	12, 8, 7
			2.27, *m*	2.66, *dt* (4.5, 12.1)	
11	-	-	13.17, *brs*	-	-
12	52.0	60.6	3.79, *d* (14.5)	3.13, *d* (12.2)	6, 7, 8, 10
			3.46, *d* (14.5)	2.39, *d* (12.2)	
13	41.4	43	2.22, *m*	2.17, *dd* (5, 12.7)	6, 7,8, 12, 14
				1.65, *dd* (10.9, 12.7)	-
14	58.7	60.4	4.01, *m*	4.05, *m*	-
15	124.9	129.5	5.76, *dd* (8.3, 10.1)	5.61, *dd* (8.3, 10.1)	13, 14, 17
16	140.0	136.4	6.13, *dd* (9.7, 17.6)	5.97, *dd* (10.1, 7.2)	14, 17
17	25.1	26.4	2.23, *m*	2.42, 2.19, *m*	14, 16, 15
18	26.8	28.9	1.71, 1.58, *m*	1.76, *m*, 1.51, *tt* (4.3, 13)	-
19	21.7	25.3	2.28, 1.78, *m*	1.77, *m*	29
20	50.7	51.7	3.61, *s*, 2.77, *brs*	2.93, 3.20, *m*	14, 23
21	-	-	-	-	-
22	29.0	30	2.96, *m*	2.76, *ddd* (3.0, 10, 14.6)	-
			2.58, *m*	2.77, *ddd* (2.9, 7.4, 14.6)	2, 5, 23, 24
23	26.8	29.2	2.61, 2.18, *m*	2.56, 2.22, *m*	22, 25
24	130.1	129.8	5.25, *t* (11.3)	5.28, ddd (7.8, 8.0, 10.8)	23, 26
25	129.7	132.7	5.15, *dd* (8.8, 10.6)	5.50, *dt* (10.8, 7.8)	25, 27, 23
26	22.2	27.6	1.86,1.98, *m*	1.97,1.70, *m*	24, 29
27	23.3	29.7	1.46,1.59, *m*	1.13, 0.96, *m*	25, 29
28	20.8	27.4	1.48, 1.181, *m*	1.17, 1.39, *m*	-
29	56.5	59.6	2.88, *m*	2.47, *dt* (11.9, 3.7)	27
			1.92, *m*	2.36, *dt* (11.9, 3.6)	-

(a) Compound (**17**) (b) Kobayashi *et al*., 1997

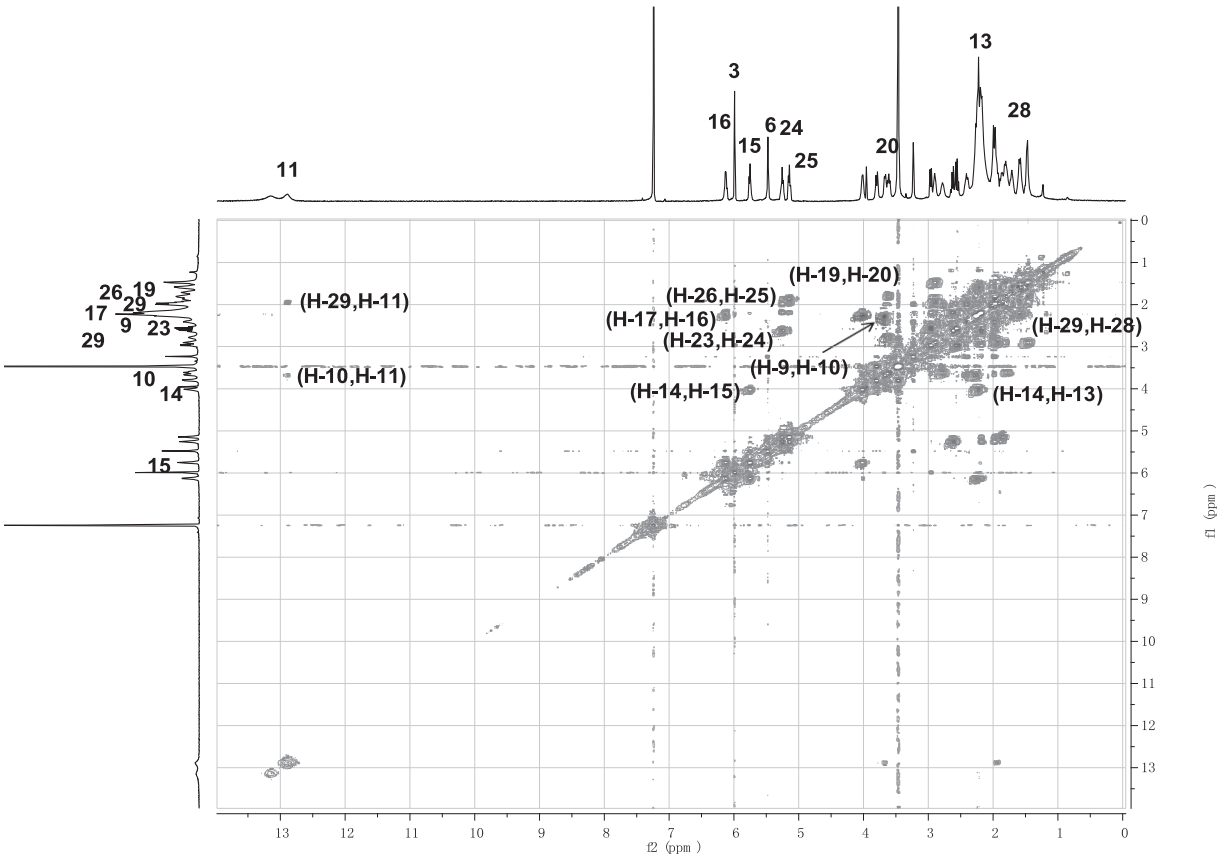


Figure 3.20: ^{1}H-^{1}H COSY spectrum for compound (**17**).

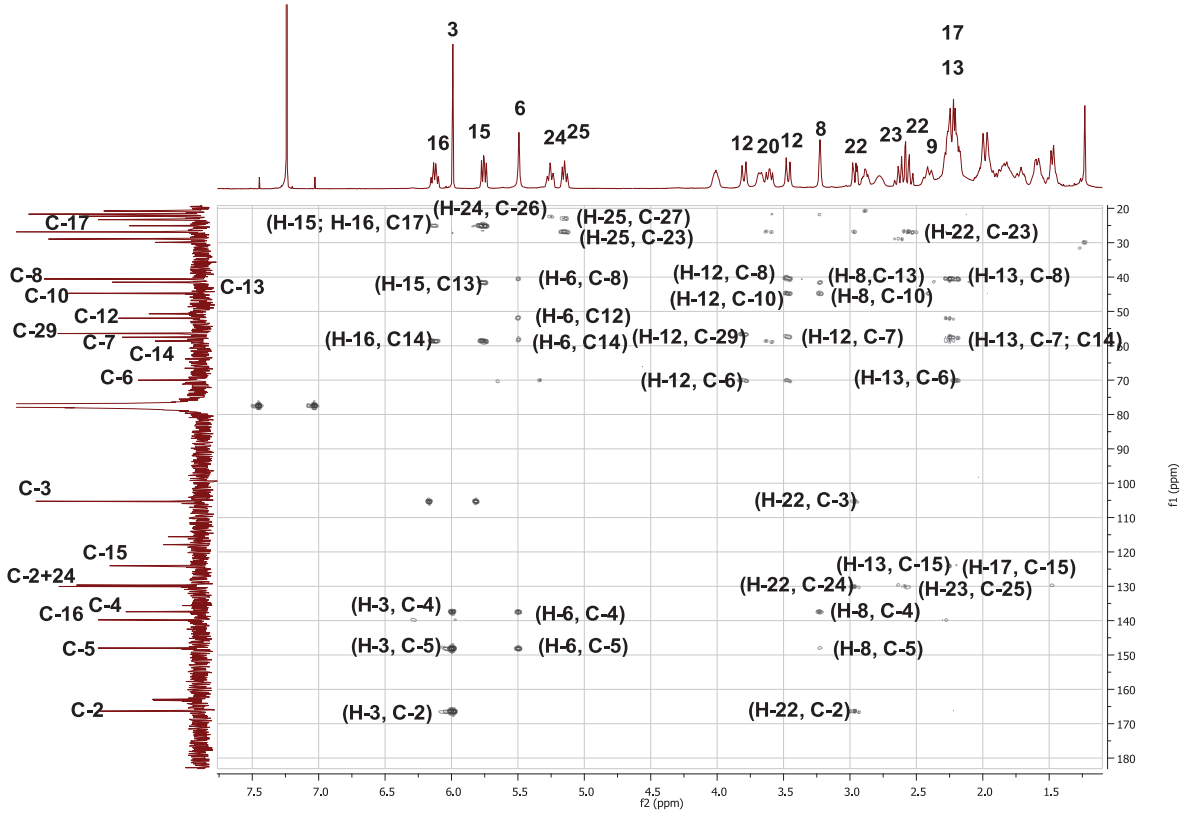
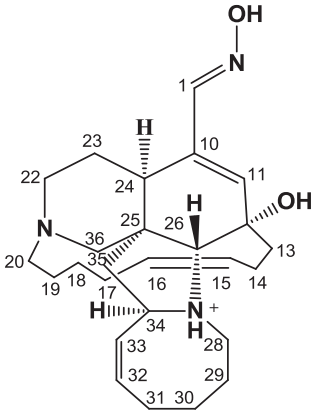
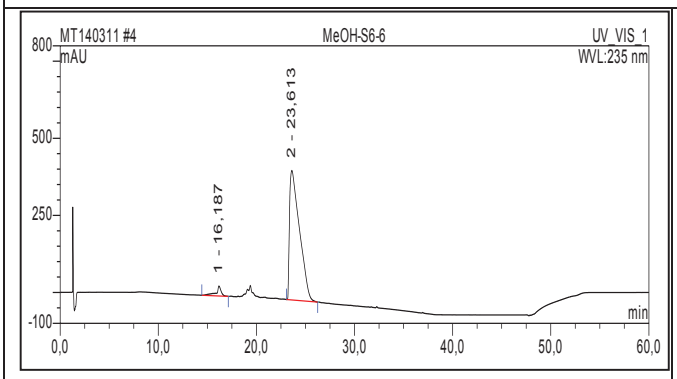


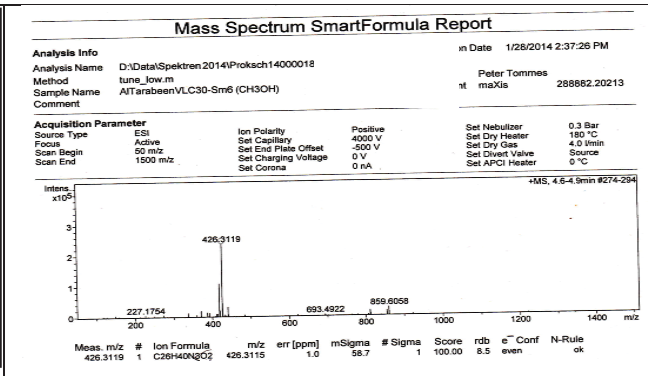
Figure 3.21: HMBC spectrum for compound (**17**).

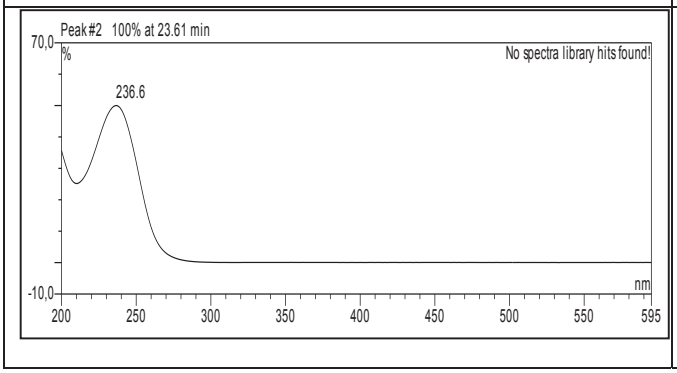
3.4.1.2. Ircinal E (18: new natural product)

Ircinal E	
Synonym(s)	Ircinal E
Sample code	MeOH-S6-6
Biological source	*Acanthostrongylophora ingens*
Sample amount	1.0 mg
Physical Description	Yellow white residue
Molecular Formula	$C_{26}H_{39}N_3O_2$
Molecular Weight	425.61 g/mol.
Optical rotation$[\alpha]_D^{20}$	+15 (*c* 0.2, $CHCl_3$)
Retention time HPLC	23.6 min (standard gradient)









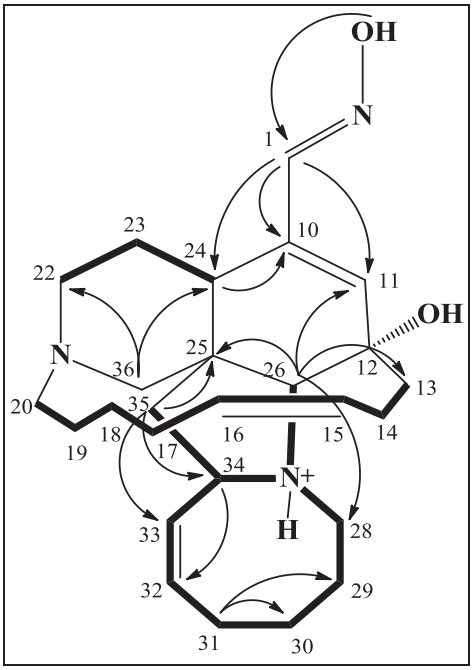
No ionization in negative ESI-MS

Results

Compound (**18**) was isolated as a yellowish white amorphous solid from the EtOAc fraction of *Acanthostrongylophora ingens*. The HRESI mass spectrum of compound (**18**) showed 426.3115 $[M+H]^+$ (base peak) indicating that the molecular weight of (**18**) is 425 g/mol. with a molecular formula of $C_{26}H_{40}N_3O_2$ (calculated 426.3120, Δ 0.0005). Compound (**18**) showed UV absorption at λ_{max} (MeOH) 236.6 nm. The ^{1}HNMR spectrum of (**18**) showed six olefinic protons at δ_H 7.77 ppm (s), 5.89 ppm (s), 5.48 ppm (m), 5.50 ppm (m), 6.26 ppm (m), and 5.39 ppm [brt (*J*=9.97)] which were assigned for H-1, H-11, H-15, H-16, H-32, and H-33, respectively (Table 3.17). In addition, the signals of three exchangeable protons at δ_H 11.12 (OH-1), 8.94 (NH-27), and 6.07 (OH-12) were observed. The analysis of the COSY spectrum (Figure 3.22) of (**18**) indicated the presence of three spin system, the first one being CH_2(22)CH_2(23)CH(24), in addition to two long spin systems within the molecule corresponding to the partial structures from CH_2-13 to CH_2-20 and from CH-26 to CH_2-35, and the COSY correlation CH_2(26)NH(27)CH_2(28) proved that compound (**18**) exists as a salt. Further elucidation of compound (**18**) was performed through a HMBC experiment (Figure 3.23) in which correlations from CH_2-36 (at δ_H 2.29, and 3.11 ppm) to C-20 (δ_C 51.8), C-26 (δ_C 75.5), and C-35 (δ_C 44.0), from CH_2-23 (at δ_H 1.22, and 1.84 ppm) to C-10 (δ_C 136.7), C-24 (δ_C 34.9), and C-25 (δ_C 45.3), from H-24 at δ_H 2.59 ppm to C-10 (δ_C 136.7), C-23 (δ_C 30.9), C-25 (δ_C 45.3), and C-26 (δ_C 75.5), and from H-26 at δ_H 3.67 ppm to C-11 (δ_C 137.5), and C-36 (δ_C 68.5) which confirmed the presence of two six-membered rings fused to a 13-membered macrocycle were observed. Moreover, the HMBC spectrum showed correlations from H-26 at δ_H 3.67 ppm to C-28 (δ_C 52.2), from H-34 at δ_H 4.63 ppm to C-25 (δ_C 45.3) and C-26 (δ_C 75.5), and from CH_2-35 (at δ_H 1.83, 2.02 ppm) to C-24 (δ_C 34.9), C-25 (δ_C 45.3), C-26 (δ_C 75.5) and C-33 (δ_C 123.8) by which the connection of the CH_2-28─CH_2-35 moiety to the previous substructure was confirmed.

Furthermore, the attachment of an oxime-function to C-10 was evidenced through HMBC correlations from OH-1 at δ_H 11.12 ppm to C-1 (δ_C 148.9), and from H-1 at δ_H 7.77 ppm to C-10 (δ_C 136.7), and C-24 (δ_C 34.9), while the correlation from OH-12 at δ_H 6.07 ppm to C-12 (δ_C 75.6) proved its position at C-12. These data were in agreement with those observed for ircinal A, except that the aldehyde group at C-10 in ircinal A was replaced by an aldoxime group in (**1**) and this confirms the difference in molecular weights between both compounds by 16 amu.

The relative configuration of (**18**) was determined by analysis of the ROESY spectrum and it was found to be identical to that of ircinal A and B (Kondo *et al*., 1992). In addition, the strong cross peak found in the ROESY spectrum (Figure 3.24) between H-1 [δ_H 7.77 (s, 1H)] and OH-1 [δ_H 11.12 ppm] confirmed the *E* configuration of the aldoxime moiety. Furthermore, the absolute configuration of ircinal E (**18**) is assumed to be identical to that of ircinal A based on the $[\alpha]_D^{20}$ values of (**18**): +15 (*c* 0.2, $CHCl_3$), which is in the positive side as observed for ircinal A, as well as based on their close biogenetic relationship. Accordingly, (**18**) was identified as a new natural product, for which the name ircinal E is proposed.



Structure of ircinal E (**18**)

^{1}H-^{1}H COSY

HMBC

Table 3.17: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), COSY and HMBC data of Ircinal E (**18**).

Pos.	δ_H (DMSO-d_6)[a]	δ_H (DMSO-d_6)	COSY	HMBC
1	148.9	7.77, *s*	-	10, 24
10	136.7	-	-	-
11	137.5	5.89, s	-	1, 10, 12, 24
12	75.6	-	-	-
13	38.5	1.50, 1.89, *m*	14	-
14	20.1	1.93, 2.26, *m*	13, 15	-
15	126.5	5.48, *m*	14, 16	-
16	133.2	5.50, *m*	15, 17	-
17	24.3	1.47, 2.56, *m*	16, 18	-
18	26.0	1.01, 1.38, *m*	17, 19	-
19	24.0	1.34, 1.72, *m*	18, 20	-
20	51.8	2.20, 2.77, *m*	19	-
21	-	-	-	-
22	48.3	1.67, 2.77, *m*	23, 22	-
23	30.9	A 1.22, *m*	24, 22	24, 25
		B 1.84, *m*	24, 22	-
24	34.9	2.59, *brs*	23	10, 23, 25, 26
25	45.3	-	-	-
26	75.5	3.67, *s*	27	11, 25, 28, 36
27	NH	8.94, *s*	26, 28, 34	-
28	52.2	3.42, 3.65, *m*	27	-
29	25.6	1.80, 1.91, *m*	28, 30	-
30	23.8	1.34, 1.80, *m*	29, 31	-
31	27.6	2.20, *m*	30, 32	29, 30, 32, 33
32	141.0	6.26, *m*	31, 33	34
33	123.8	5.39, *brt* (9.97)	32, 34	31, 34, 36,
34	56.8	4.63, *dd* (7.5, 14.0)	27, 33, 35	25, 26, 32
35	44.0	A 1.83, *m*	34	24, 25, 33, 34, 36
		B 2.02, *m*	34	25, 26, 36, 33
36	68.5	A 3.11, *d* (11.5)	36	35
		B 2.29, *d* (11.5)	36	26, 20
OH-1	-	11.12, s		1
OH-12	-	6.07, *s*		12

(a) Signals were extracted from the HSQC and HMBC spectra.

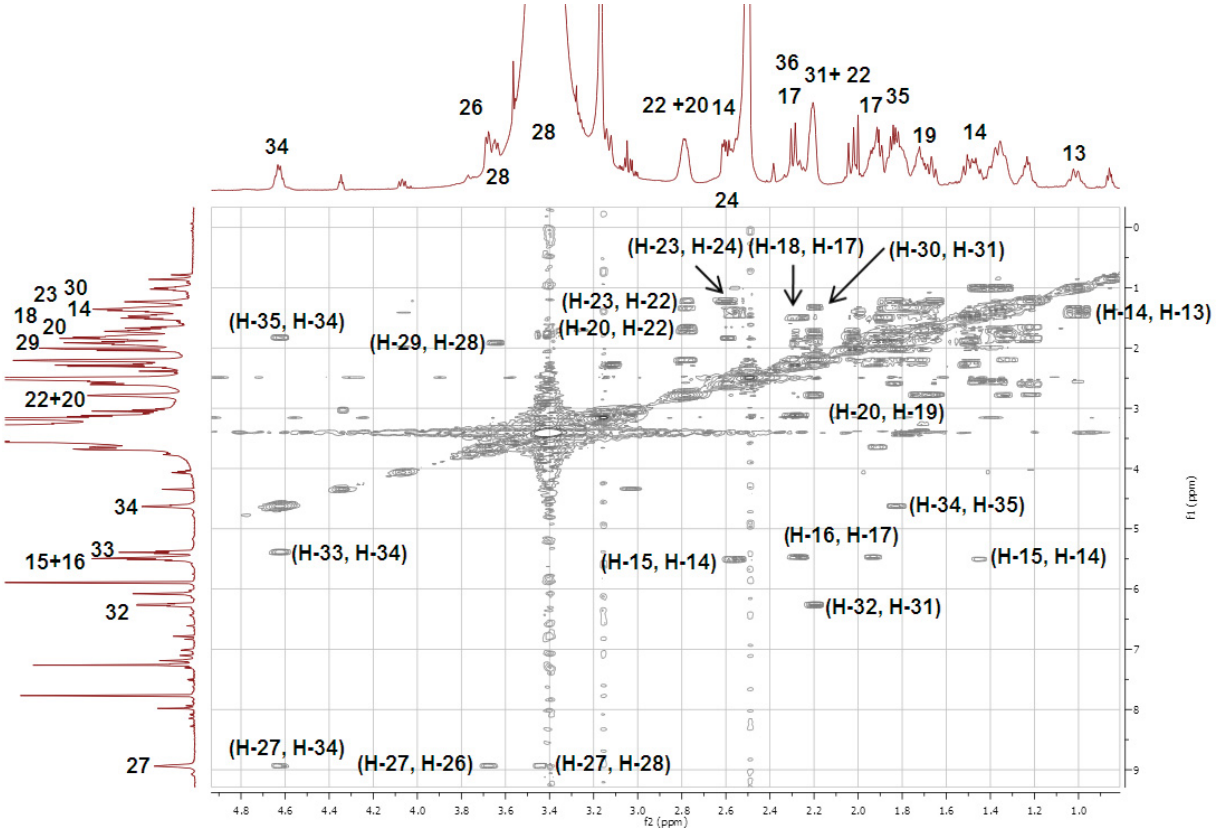


Figure 3.22: ^{1}H-^{1}H COSY spectrum for compound (**18**).

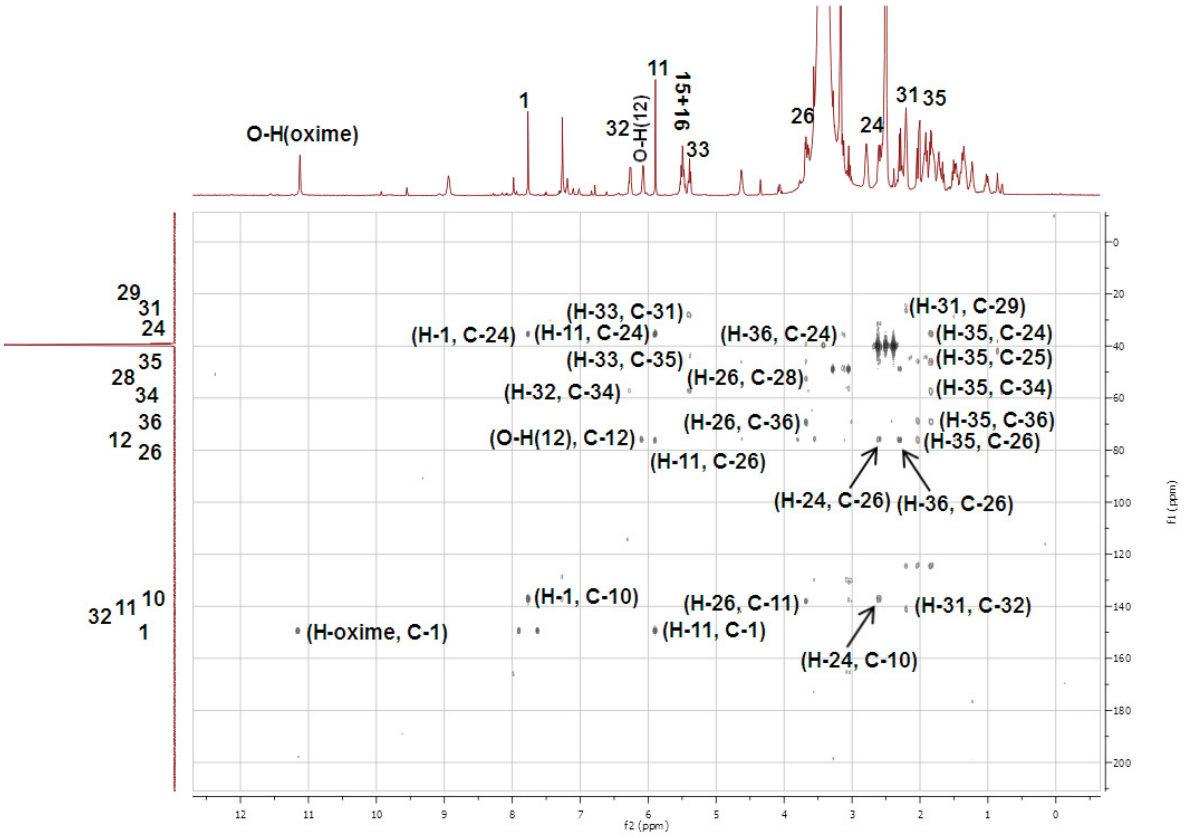


Figure 3.23: HMBC spectrum for compound (**18)**.

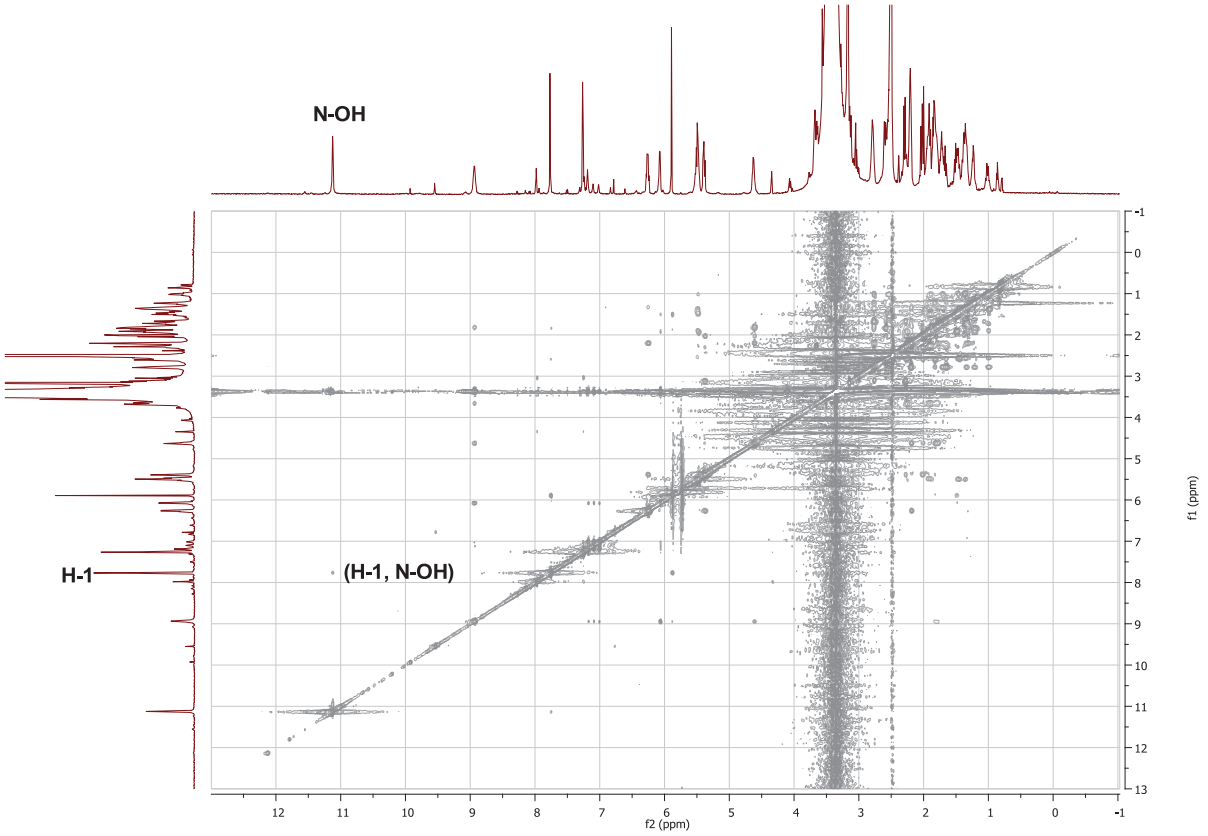
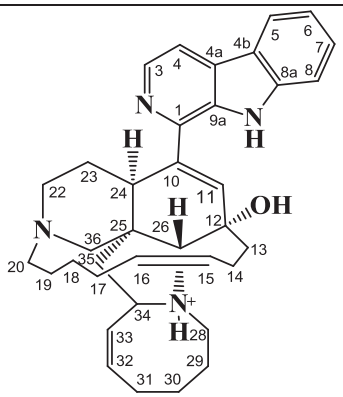
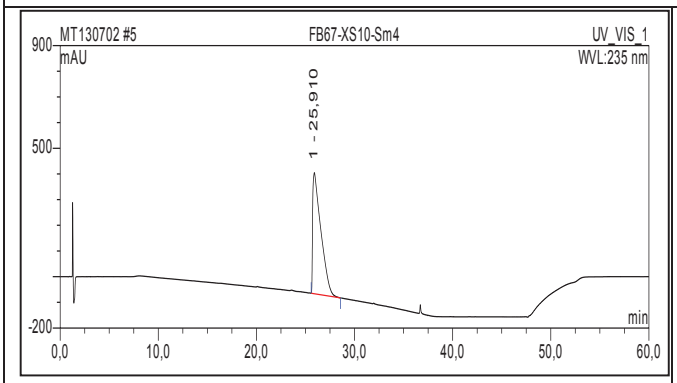


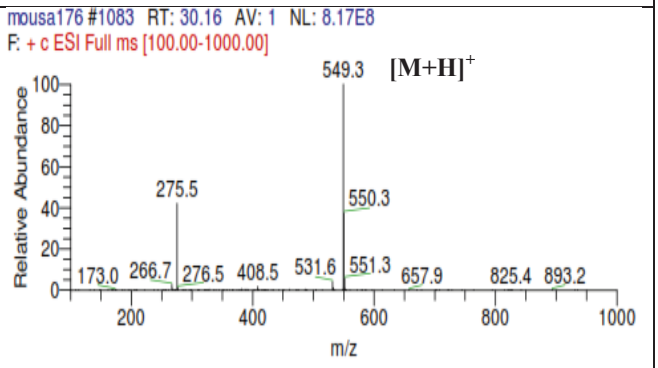
Figure 3.24: ROESY spectrum for compound (**18**).

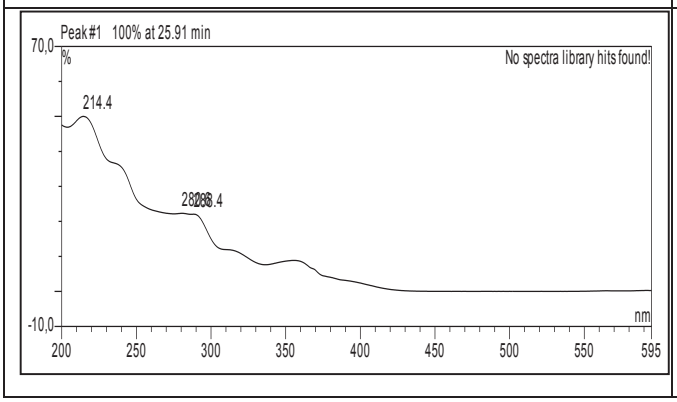
3.4.1.3. Manzmaine A (19: known natural product)

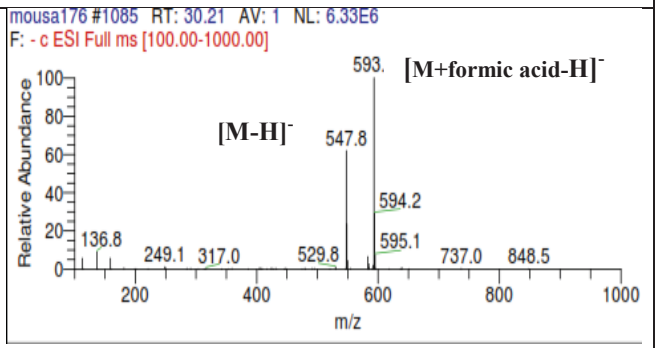
Manzamine A	
Synonym(s)	Manzamine A
Sample code	FB67-XS10-sm4
Biological source	*Acanthostrongylophora ingens*
Sample amount	5 mg
Physical Description	Yellow solid
Molecular Formula	$C_{36}H_{44}N_4O$
Molecular Weight	548.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+36.6 (*c* 0.2, $CHCl_3$)
Retention time HPLC	25.9 min (standard gradient)







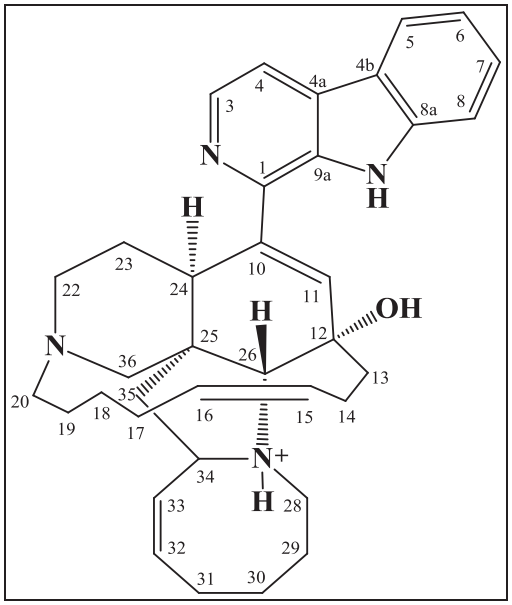




Results

Compound (**19**) was isolated as a yellowish solid from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum of (**19**) revealed *pseudo*-molecular ion peaks at *m/z* 549.3 $[M+H]^+$ (base peak), 547.8 $[M-H]^-$, and 593.1 $[M+HCOOH-H]^-$ (bas peak), indicating a molecular weight of 548 g/mol. Compound (**19**) showed UV absorption at λ_{max} (MeOH) 214.4, and 283.8 nm. The ^{1}H NMR spectrum for compound (**19**) showed six aromatic protons, four doublets at δ_H 8.37 ppm [d (*J*=5.1 Hz)], δ_H 8.07 ppm [d (*J*=5.1 Hz)], δ_H 8.26 ppm [d (*J*=7.9 Hz)], and at δ_H 7.67 ppm [d (*J*=8.0 Hz)] which were assigned to H-3, H-4, H-5, and H-8, respectively, and two triplets at δ_H 7.27 ppm [t (*J*=7.4 Hz)] and 7.58 ppm [t (*J*=7.6 Hz)] which were attributed to H-6 and H-7, respectively. Furthermore, the ^{1}H NMR spectrum showed five olefinic protons at δ_H 6.44 ppm (s), δ_H 5.61 ppm (m), δ_H 5.56 ppm (m), δ_H 6.32 ppm (m), and δ_H 5.48 ppm [t (*J*=10.1 Hz)] which were attributed to H-11, H-15, H-16, H-32, and H-33, respectively (Table 3.18). The ^{1}H-^{1}H COSY spectrum (Figure 3.25-b) showed five spin systems (Figure 3.25-a), and the COSY cross peaks CH_2(26)NH(27)CH_2(28) proved that compound (**19**) exists as a salt (Table 3.18). Further elucidation of (**19**) was performed through a HMBC experiment. This HMBC spectrum (Figure 3.26) showed correlations from H-26 at δ_H 3.83 ppm to C-11 (δ_C 135.1), C-24 (δ_C 39.1), C-25 (δ_C 46.5), C-28 (δ_C 52.9) and C-36 (δ_C 69.4), from H-34 at δ_H 4.71 ppm to C-24 (δ_C 39.1), C-25 (δ_C 47.8), C-26 (δ_C 77.8), C-32 (δ_C 141.4), and C-33 (δ_C 124.8), from CH_2-35 (at δ_H 2.18, 2.35 ppm) to C-25 (δ_C 47.8), C-26 (δ_C 77.8), C-33 (δ_C 124.8), and C-36 (δ_C 69.4), from H-11 at δ_H 6.44 ppm to C-10 (δ_C 142.8), and C-13 (δ_C 39.4), and from 12-OH at δ_H 6.04 ppm to C-12 at (δ_C 76.4) proving its position at C-12. Further inspection of the HMBC spectrum (Figure 3.26) also revealed correlation from H-3 at δ_H 8.37 ppm to C-1 (δ_C 142.5), C-4 (δ_C 114.7), and C-4a (δ_C 129.4), from H-4 at δ_H 8.07 ppm C-4b (δ_C 121.2), and C-9a (δ_C 133.5), from H-5 at δ_H 8.26 ppm to C-4a (δ_C 129.4), and C-8a (δ_C 141.1), from H-7 at δ_H 7.58

ppm to C-5 (δ_C 122.4), and C-8a (δ_C 141.1), from H-11 at δ_H 6.44 ppm to C-10 (δ_C 142.8), and C-26 (δ_C 77.8), from H-15 at δ_H 5.61 ppm to C-17 (δ_C 24.9), and from H-16 δ_H 5.56 ppm to C-14 (δ_C 21.0) (Table 3.18). The structure of (**19**) was finally confirmed by comparison of the ^{1}H NMR, ^{13}C NMR, mass spectral data, and the $[\alpha]_D^{20}$ of (**19**) with the published data for manzamine A (Zhang *et al*., 2008) (Table 3.18).



Structure of compound (**19**)

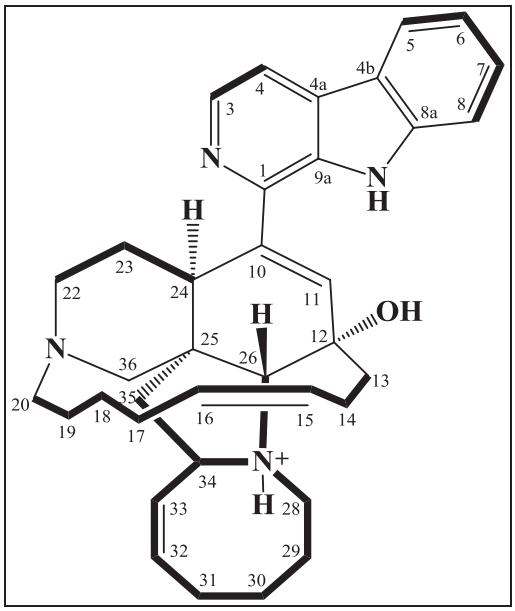


Figure3.25-a: ^{1}H-^{1}H COSY correlations for compound (**19**)

Table 3.18: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), COSY, and HMBC data of (**19**).

Pos.	δ_C (DMSO-d_6)[a,b]	δ_C (CD_3Cl)[c]	δ_H (DMSO-d_6) [a]	δ_H (CD_3Cl)[c]	COSY[a]	HMBC[a]
1	142.5	143.7	-	-	-	-
2	-	-	-	-	-	-
3	138.1	137.6	8.37, *d* (5.1)	8.34, *d* (5.2)	4	1, 4, 4a
4	114.7	113.9	8.07, *d* (5.1)	7.85, *d* (5.1)	3	9a
4a	129.4	129.5	-	-	-	-
4b	121.2	-	-	-	-	-
5	122.4	121.0	8.26, *d* (7.9)	8.08, *d* (7.9)	6	4a, 8a
6	120.3	119.3	7.27, *t* (7.4)	7.23, *t* (7.9)	5, 7	4b, 8
7	129.0	128.1	7.58, *t* (7.6)	7.52, *t* (7.9)	6, 8	4a, 5
8	112.8	112.9	7.67, *d* (8.0)	7.83, *d* (7.9)	7	6
8a	141.1	141.6	-	-	-	-
9	-	-	10.71, *s*	11.75, *brs*	-	-
9a	133.5	133.4	-	-	-	-
10	142.8	141.3	-	-	-	-
11	135.1	135.2	6.44, *s*	6.52, *s*	-	10, 13, 24, 26
12	76.4	71.3	-	-	-	-
12	(OH)	-	6.04, *s*	-	-	12
13	39.4	39.2	2.16, 2.07, *m*	1.75, 2.15, *m*	14	15, 26
14	21.0	20.8	2.05, 2.36, *m*	2.15, 2H, *m*	13, 15	15, 16
15	127.5	127.0	5.61, *m*	5.57, *m*	14, 16	17
16	133.7	132.9	5.56, *m*	5.57, *m*	15, 17	14
17	24.9	25.0	2.67, 1.50, *m*	1.60, 2.50, *m*	16, 18	-
18	26.6	26.5	1.41, 1.12, *m*	1.20, 1.45, *m*	17, 19	-
19	22.7	24.6	1.24, *m*	1.45, 1.81, *m*	18, 20	-
20	52.4	53.6	2.85, 2.24, *m*	2.38, 2.58, *m*	19	18, 22
22	49.2	49.4	2.82, 1.71, *m*	1.88, 2.93, *m*	23	-
23	31.8	33.6	1.82, 1.64, *m*	1.78, 2.95, *m*	22, 24	-
24	39.1	41.2	3.14, *brs*	2.55, *m*	23	23, 25, 26, 36
25	47.8	47.1	-	-	-	-
26	77.8	78.2	3.83, *d* (7.3)	3.72, *d* (6.0)	27	10, 13, 24, 25, 28, 36
27	-	-	8.97, *brs*	10.62, *brs*	26, 28, 34	-
28	52.9	53.5	3.75, 3.47, *m*	3.27, 4.03, *m*	27, 29	-
29	26.3	26.4	1.94, 1.84, *m*	2.00, 2.60, *m*	28, 30	-
30	24.5	24.4	1.79, 1.37, *m*	1.45, 1.95, *m*	29, 31	-
31	28.1	28.5	2.22, 2H, *m*	2.30, 2H , *m*	30, 32	30, 32 , 33
32	141.4	142.4	6.32, *m*	6.29, *m*	31, 33	34
33	124.8	123.8	5.48, *t* (10.1)	5.39, t (9.9)	32, 34	31, 34
34	57.6	57.2	4.71, *m*	4.94, *m*	35	25, 26, 32, 33
35	44.5	44.8	2.32, 2.15, *m*	1.85, 2.40, *m*	34	25, 26, 36, 33
36	69.4	70.6	3.24, *d* (11.7)	2.38, 2.88, *m*	-	24, 26
			2.40, *d* (11.6)	-	-	24, 26, 22

(a) Compound (**19**) (b) ^{13}C were extracted from the HSQC and HMBC spectra (c) Zhang *et al*., 2008

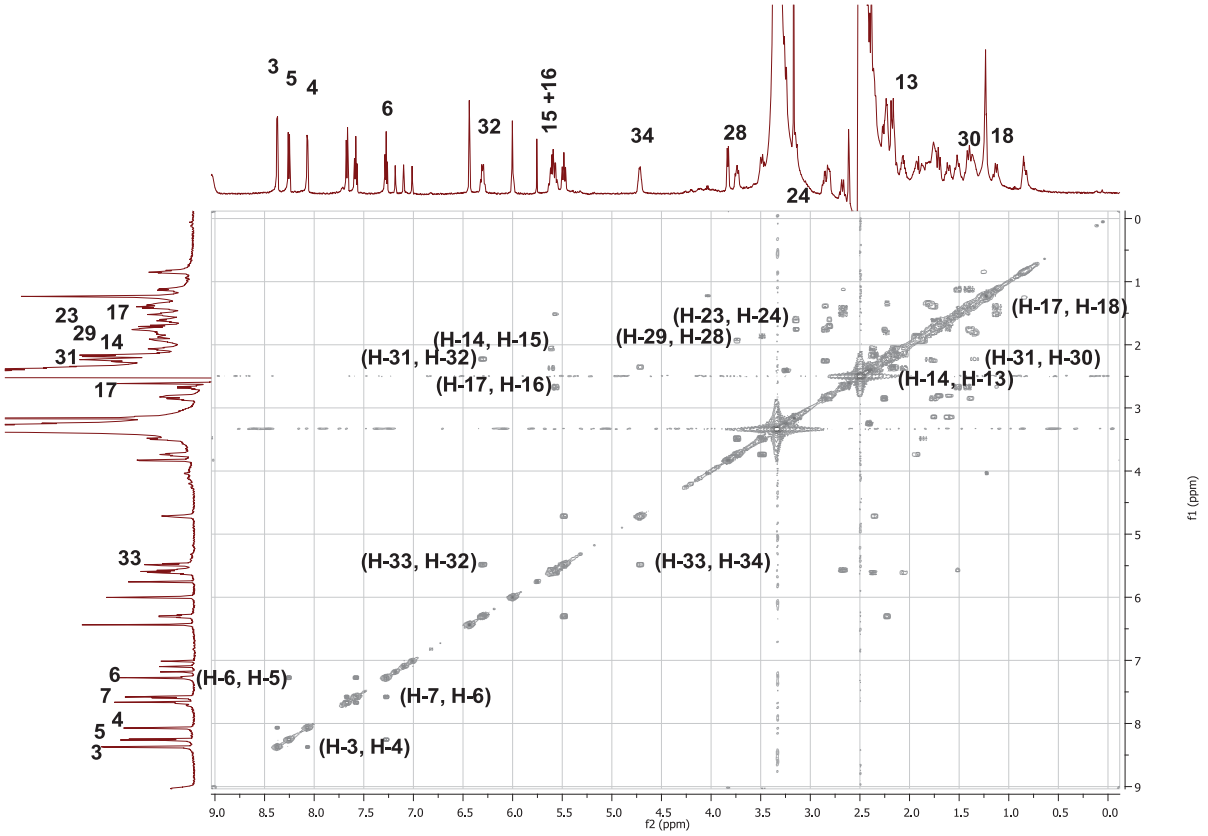


Figure 3.25-b: ^{1}H-^{1}H COSY spectrum for compound (**19**).

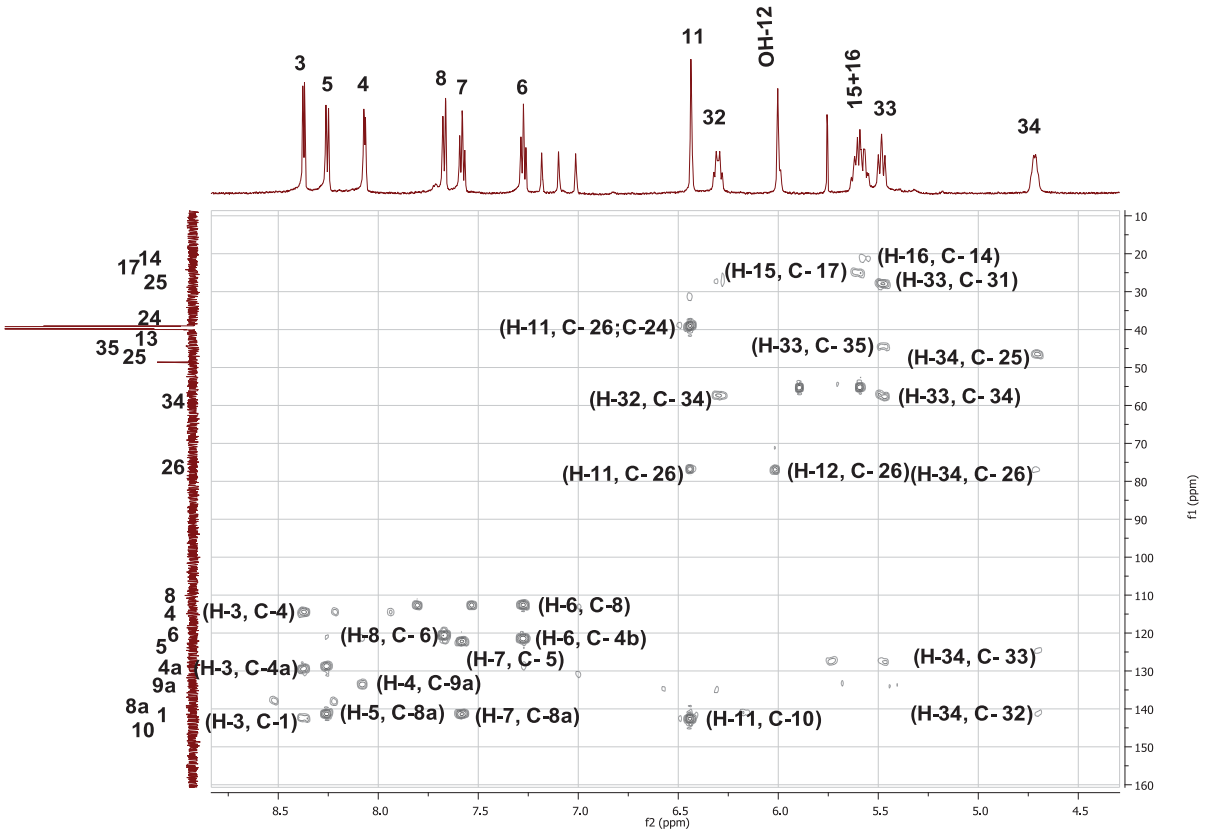
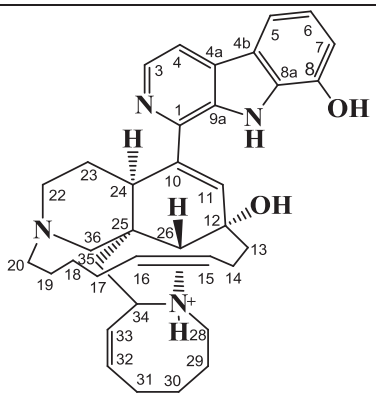
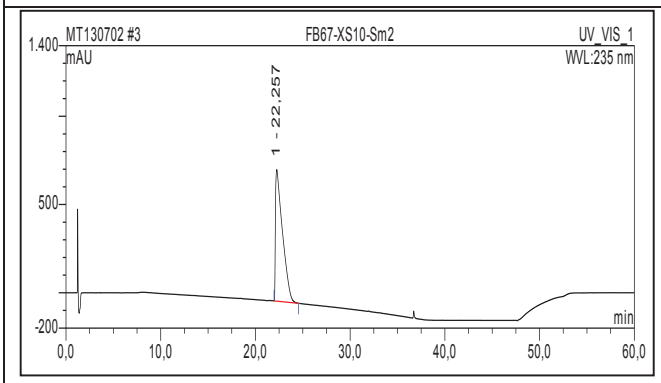


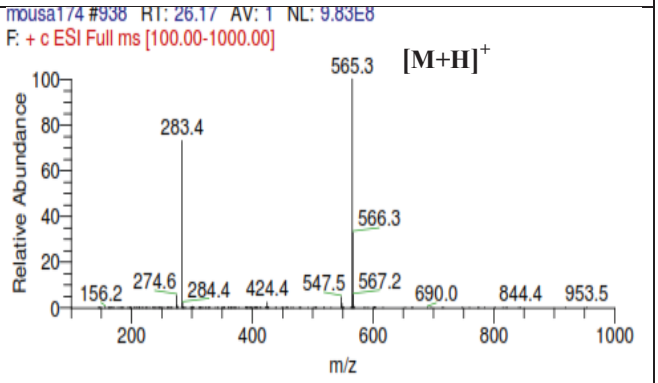
Figure 3.26: HMBC spectra for compound (**19**).

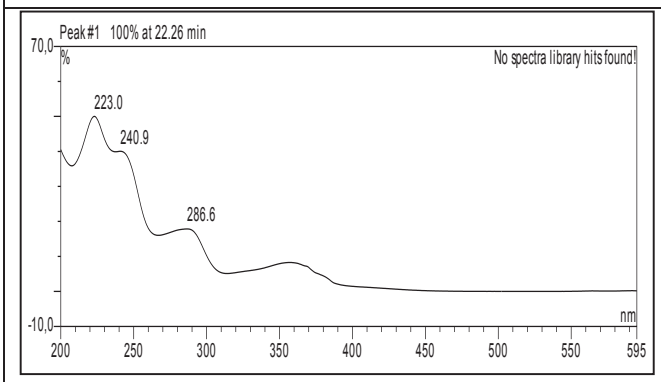
3.4.1.4. 8-Hydroxymanzmaine A (20: known natural product)

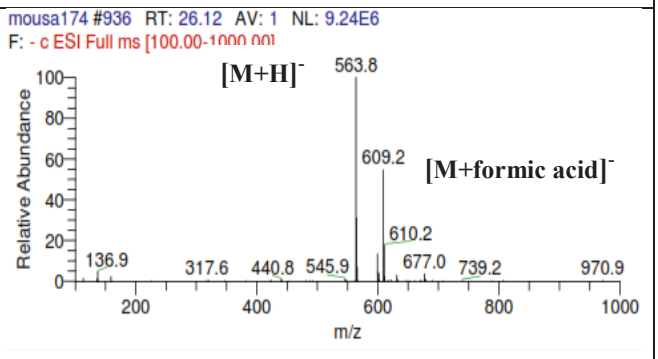
8-Hydroxymanzamine A	
Synonym(s)	8-Hydroxymanzamine A
Sample code	FB67-XS10-sm2
Biological source	*Acanthostrongylophora ingens*
Sample amount	5 mg
Physical Description	Yellowish powder
Molecular Formula	$C_{36}H_{44}N_4O_2$
Molecular Weight	564.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+34.2 (*c* 0.2, $CHCl_3$)
Retention time HPLC	22.26 min (standard gradient)







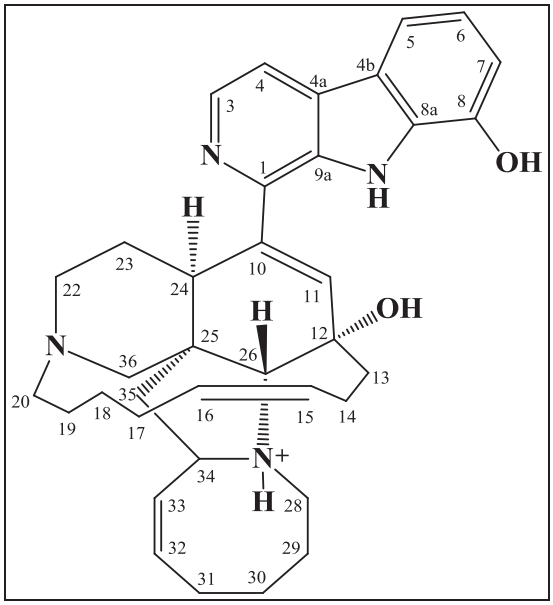




Results

Compound (**20**) was isolated as a yellowish powder from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum of (**20**) revealed *pseudo*-molecular ion peaks at *m/z* 565.3 $[M+H]^+$ (base peak), 563.8 $[M-H]^-$ (base peak), and 609.2 $[M+HCOOH-H]^-$ indicating a mass of 564 g/mol. This mass differs by 16 amu in comparison to compound (**19**) which suggests the presence an additional oxygen atom in (**20**). Compound (**20**) showed UV absorption at λ_{max} (MeOH) 223.0, 240.9, and 286.6 nm. The ^{1}H NMR spectrum for compound (**20**) showed five aromatic protons, *viz*., four doublets at δ_H 8.34 ppm [d (*J*=5.1 Hz)], δ_H 8.00 ppm [d (*J*=5.1 Hz)], δ_H 7.69 ppm [d (*J*=7.8 Hz)], and δ_H 6.99 ppm [dbr (*J*=7.7 Hz)] which were assigned to H-3, H-4, H-5, and H-6, respectively, and one triplet at δ_H 7.10 ppm [t (*J*=7.1 Hz)] and was attributed to H-7. Moreover, the ^{1}H NMR revealed five olefinic protons at δ_H 6.44 ppm (s), δ_H 5.61 ppm (m), δ_H 5.56 ppm (m), δ_H 6.30 ppm [dd (*J*=7.3, 17.7 Hz)], and 5.48 ppm [t (*J*=9.9 Hz)] which were attributed to H-11, H-15, H-16, H-32, and H-33, respectively.(Table 3.19). The ^{1}H-^{1}H COSY spectrum (Figure 3.27-b) showed five spin systems (Figure 3.27-a), and the COSY correlation CH_2(26)NH(27)CH_2(28) proved that compound (**20**) exists as a salt. Further elucidation of (**20**) was performed through a HMBC experiment. This HMBC spectrum revealed correlations from H-26 at δ_H 3.83 ppm to C-11 (δ_C 134.9), C-24 (δ_C 39.1),C-28 (δ_C 52.9), and C-36 (δ_C 69.4), from CH_2-35 (at δ_H 2.18, and 2.35 ppm) to C-24 (δ_C 39.1), C-25 (δ_C 46.9), C26 (δ_C 76.5), C-33 (δ_C 124.8), C-34 (δ_C 57.6), and C-36 (δ_C 69.4), from H-11 at δ_H 6.44 ppm to C-10 (δ_C 142.7), C-13 (δ_C 39.4) and C-26 (δ_C 76.5) , from 8-OH at δ_H 10.0 ppm to C-8 at (δ_C 144.4), from 12-OH at δ_H 6.04 ppm to C-12 at (δ_C 77.3) proved their positions at C-8, and C-12, respectively. Further inspection of the HMBC spectrum (Figure 3.28) also revealed correlations from H-3 at δ_H 8.34 ppm to C-1 (δ_C 143.5), C-4 (δ_C 115.0), and C-4a (δ_C 129.9), from H-4 at δ_H 8.00 ppm to C-4b (δ_C 124.1), and C-9a (δ_C 133.2), H-5 at δ_H 7.69 ppm to C-4a

(δ_C 129.9), and C-8a (δ_C 130.8), from H-7 at δ_H 6.99 ppm to C-5 (δ_C 112.8), and C-8a (δ_C 130.8) (Table 3.19). The structure of (**20**) was finally confirmed by comparison of the ^{1}H NMR, ^{13}C NMR , mass spectral data, in addition to the $[\alpha]^{20}_D$ of (**20**) with the published data for 8-hydroxymanzamine A (Zhang *et al*., 2008) (Table 3.19).



Structure of compound (**20**).

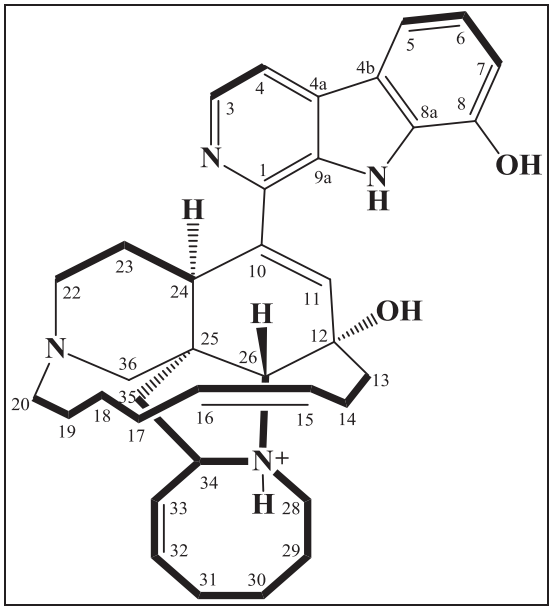


Figure3.27-a: ^{1}H-^{1}H COSY correlations for compound (**20**)

Table 3.19: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), COSY, and HMBC data of (**20**).

Pos.	δ_C (DMSO-d_6)[a,b]	δ_C (CD_3Cl)[c]	δ_H (DMSO-d_6)[a]	δ_H (CD_3Cl)[c]	COSY[a]	HMBC[a]
1	143.5	143.3	-	-	-	-
2	-	-	-	-	-	-
3	138.2	137.9	8.34, *d* (5.1)	8.33, *d* (5.1)	4	4, 4a
4	115.0	114.7	8.00, *d* (5.1)	7.83, *d* (5.1)	3	9a
4a	129.9	129.8	-	-	-	-
4b	124.1	-	-	-	-	-
5	112.8	112.6	7.69, *d* (7.8)	7.62, *d* (7.2)	6	7, 8a
6	121.2	120.7	7.10, *t* (7.1)	7.15, *t* (7.5)	5, 7	4b, 8
7	113.3	114.3	6.99, *brd* (7.7)	7.09, *dd* (0.9, 7.5)	6, 8	5, 8a
8	144.4	143.8	-	-	-	-
8	-OH	-	10.0, *s*	-	-	-
8a	130.8	130.6	-	-	-	-
9	-	-	10.71, *s*	11.5, *s*	-	-
9a	133.2	132.9	-	-	-	-
10	142.7	141.9	-	-	-	-
11	134.9	134.6	6.44, *s*	6.46, *s*	-	1, 13, 24, 26
12	77.3	71.2	-	-	-	-
12	OH	-	6.04, *s*	-	-	-
13	39.4	39.2	2.16, 2.07, *m*	1.80, 2.06, *m*	14	12
14	21.0	20.7	2.05, 2.36, *m*	2.23, 2H, *m*	13, 15	-
15	127.4	126.7	5.61, *m*	5.59, *m*	14, 16	-
16	133.6	133.0	5.56, *m*	5.59, *m*	15, 17	-
17	24.9	24.7	2.67, 1.50, *m*	1.64, 2.49, *m*	16, 18	-
18	26.6	26.5	1.12,1.41, *m*	1.23, 1.54, *m*	17, 19	-
19	24.4	24.5	1.24, *m*	123, 1.54, *m*	18, 20	-
20	52.4	53.4	2.24, 2.85, *m*	2.43, 2.62, *m*	19	-
21	-	-	-	-	-	-
22	49.1	49.2	1.71, 2.82, *m*	1.88, 2.97, *m*	23	-
23	31.8	33.4	1.64, 1.82, *m*	1.86, 2.97, *m*	22, 24	-
24	39.1	41.2	3.14, *brs*	2.53, *m*	23	10, 26
25	46.9	47.1	-	-	-	-
26	76.5	78.3	3.83, *d* (7.3)	3.77, *d* (6.9)	27	11, 13, 24, 28
27	-	-	8.79, *brs*	9.80, *brs*	-	-
28	52.9	53.7	3.47, 3.75, *m*	3.32, 4.03, *m*	27, 29	-
29	26.2	26.4	1.86, 1.94, *m*	2.30, 2.50, *m*	28, 30	-
30	23.7	24.2	1.36, 2H, *m*	1.54, 2.03, *m*	29, 31	-
31	28.1	28.4	2.22, 2H, *m*	2.35, 2H, *m*	-	-
32	141.4	142.8	6.30, *dd* (7.3, 17.7)	5.23, *s*	31, 33	-
33	124.8	123.3	5.48, *t* (9.9)	5.42, *t* (9.8)	32, 34	31
34	57.6	57.4	4.70, *dd* (8.5, 15.1)	4.98, *brq* (6.9)	35	25, 26, 32, 33
35	44.5	44.8	2.15, 2.35, *m*	1.92, 2.39, *m*	34	25, 33, 34, 36
36	69.4	70.2	2.40, *d* (11.6)	2.40, *m*	-	-
			3.24, *d* (11.7)	2.92, *m*	-	-

(a) Compound (**20**) (b) ^{13}C were extracted from the HSQC and HMBC spectra (c) Zhang *et al*., 2008.

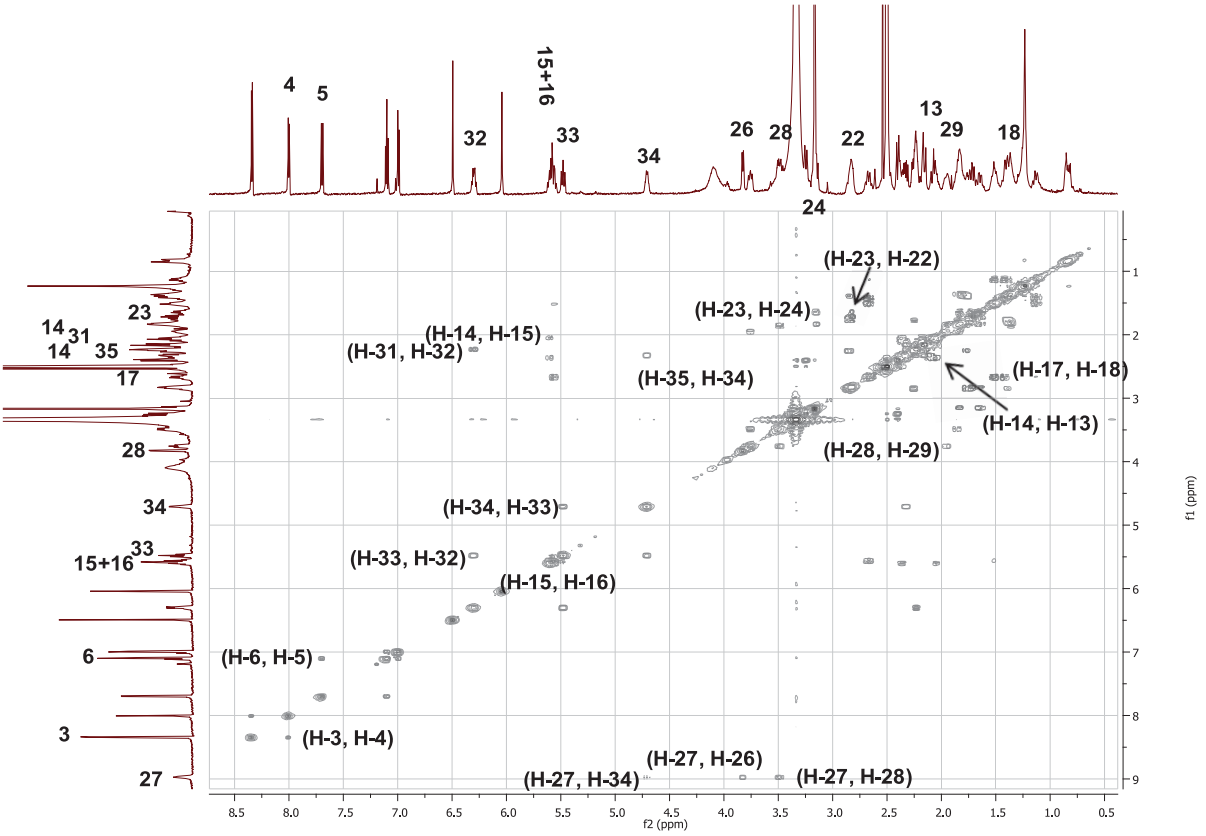


Figure 3.27-b: ^{1}H-^{1}H COSY spectrum for compound (**20**).

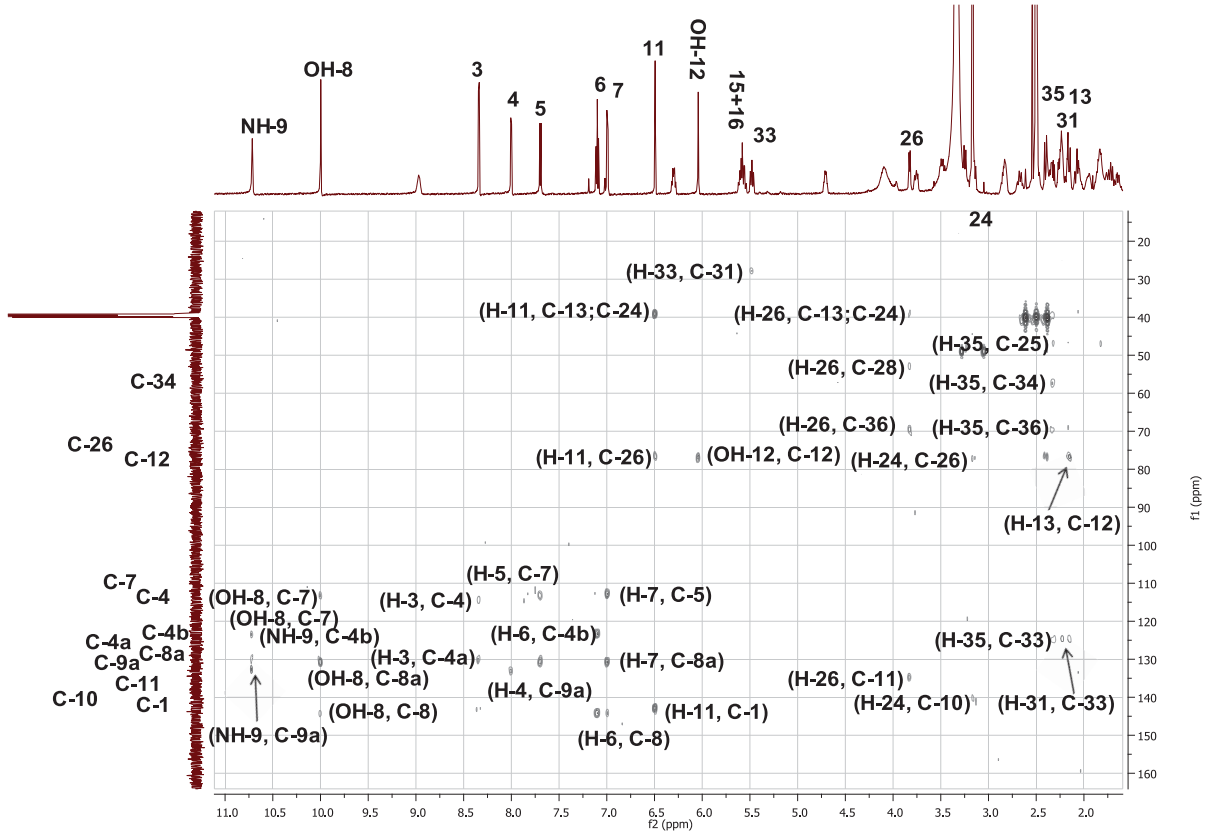
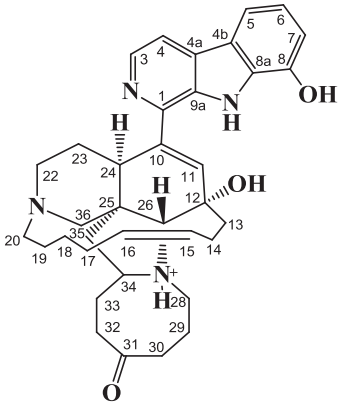
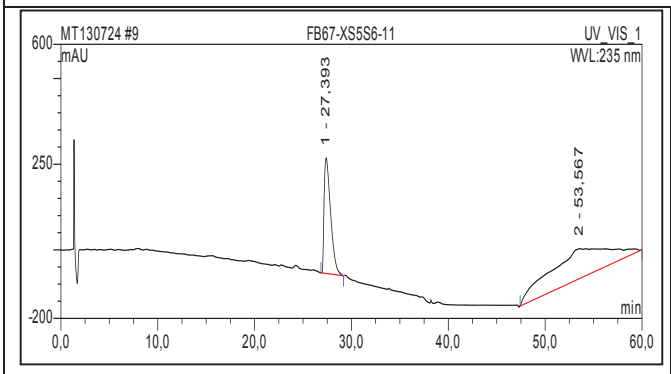


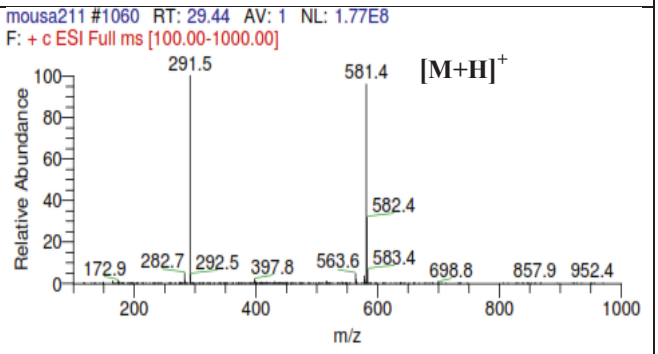
Figure 3.28: HMBC spectrum for compound (**20**).

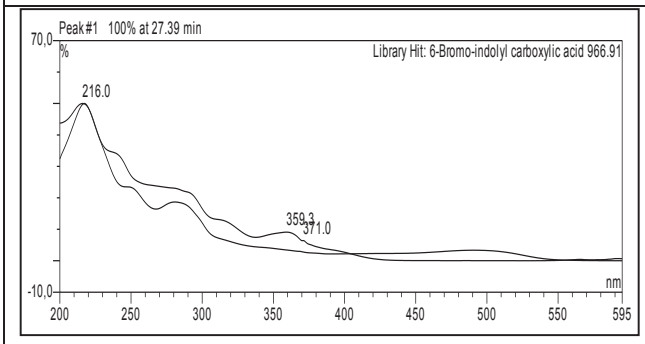
3.4.1.5. Manzamine F (21: known natural product)

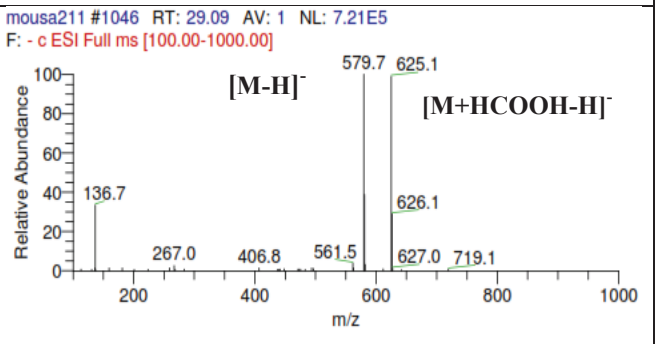
Manzamine F	
Synonym(s)	Manzamine F
Sample code	X-S5S6-11
Biological source	*Acanthostrongylophora ingens*
Sample amount	9 mg
Physical Description	yellowish powder
Molecular Formula	$C_{36}H_{44}N4O_3$
Molecular Weight	580.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+35.6 (*c* 0.2, $CHCl_3$)
Retention time HPLC	27.39 min (standard gradient)





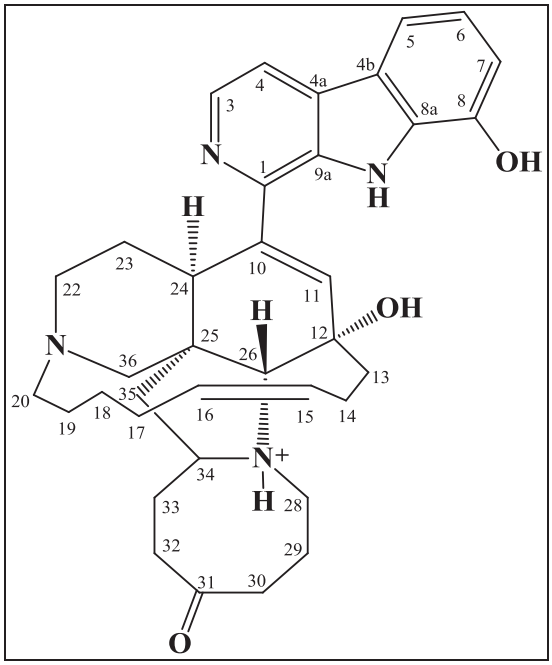






Compound (**21**) was isolated as a yellowish powder from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum of (**21**) revealed *pseudo*-molecular ion peaks at *m/z* 581.3 $[M+H]^+$, 579.7 [M-H]- , and 625.1 $[M+HCOOH-H]^-$ (base peak) indicating a molecular weight of 580 g/mol. This mass differs by 16 amu in comparison with compound (**20**) which suggests the presence of an extra oxygen atom in (**21**). Compound (**21**) showed UV absorption at λ_{max} (MeOH) 223.0, 216.0, and 359.3 nm. The ^{1}H NMR spectrum for (**21**) showed five aromatic protons, four doublets at δ_H 8.27 ppm [d (*J*=5.3 Hz)], δ_H 7.90 ppm [d (*J*=5.3 Hz)], δ_H 6.90 ppm [d (*J*=7.8 Hz)], and δ_H 7.54 ppm [d (*J*=7.6 Hz)] which were assigned to H-3, H-4, H-5, and H-7, respectively, and one triplet at δ_H 7.08 ppm [t (*J*=7.7 Hz)] which was attributed to H-7. Moreover, three protons appeared in the olefinic region at δ_H 6.40 ppm (s), δ_H 5.64 ppm [dd (*J*=8.2, 16.6 Hz)], δ_H 5.51 ppm [dd (*J*=4.9, 10.8 Hz)] which were attributed to H-11, H-15, H-16, respectively (Table 3.20). However, the other two olefinic proton signals for H-32 and H-33 were absent in the proton spectrum of (**21**) in comparison with those of (**19**), and (**20**). The ^{1}H-^{1}H COSY spectrum (Figure 3.29-b and 3.29-c) showed six spin systems (Figure 3.29-a) (Table 3.20). Further elucidation of (**21**) was performed through a HMBC experiment. This HMBC spectrum (Figure 3.30) revealed correlations from H-3 at δ_H 8.27 ppm to C-1 (δ_C 144.3), C-4 (δ_C 115.0), and C- 4a (δ_C 131.7), respectively, from H-4 at δ_H 7.90 ppm to C-3 (δ_C 138.5), C-4b (δ_C 124.1), C-9a (δ_C 134.6), from H-5 at δ_H 6.90 ppm to C-6 (δ_C 122.7), C-7 (δ_C 111.7), C-8a (δ_C 133.3), from H-6 at δ_H 7.08 ppm to C-4b (δ_C 124.1), C-5 (δ_C 114.7), and C-8 (δ_C 147.6), from H-7 at δ_H 7.54 ppm to C-5 (δ_C 114.7), C-8 (δ_C 147.6), and C-8a (δ_C 133.3), from H-11 at δ_H 6.40 ppm to C-1 (δ_C 143.3), and C-24 (δ_C 43.5), from CH_2-22 (at δ_H 2.78, and 1.81 ppm) to C-20 (δ_C 54.1), and C-24 (δ_C 43.5), from CH_2-23 (at δ_H 1.69, and 1.56 ppm) to C-24 (δ_C 43.5), and C-25 (δ_C 47.8), and C-22 (δ_C 51.0), and from H-24 at δ_H 3.15 ppm to C-23 (δ_C 34.2) , C-25 (δ_C 47.8),

and C-35 (δ_C 46.6). Further inspection of HMBC spectrum revealed also correlations from H-15 at δ_H 5.64 ppm to C-14 (δ_C 22.7), and C-17 (δ_C 27.0), from H-26 at δ_H 3.79 ppm to C-28 (δ_C 53.7) , and C-36 (δ_C 69.3), from CH_2-30 (at δ_H 2.68, and 2.26 ppm) to C-28 (δ_C 53.7), C-29 (δ_C 34.2), and C-32 (δ_C 44.6), from CH_2-32 (at δ_H 2.56, and 2.19 ppm) to C-30 (δ_C 39.7), C-33 (δ_C 25.92), and C-34 (δ_C 64.9), from CH_2-35 (at δ_H 1.90, 1.67 ppm) to C-24 (δ_C 43.5), C-25 (δ_C 47.8), C-34 (δ_C 64.9), and C-36 (δ_C 69.3), and from CH_2-36 (at δ_H 2.69, and 2.22 ppm) to C-20 (δ_C 54.1), C-22 (δ_C 51.0), C-24 (δ_C 43.5), and C-25 (δ_C 47.8) (Table 3.20). Comparison of the $[\alpha]_D^{20}$, ^{1}H NMR, ^{13}C NMR, and mass spectral data with the literature data (Ichiba *et al*., 1988) indicates that (**21**) is the known compound manzamine F.



Structure of compound (**21**).

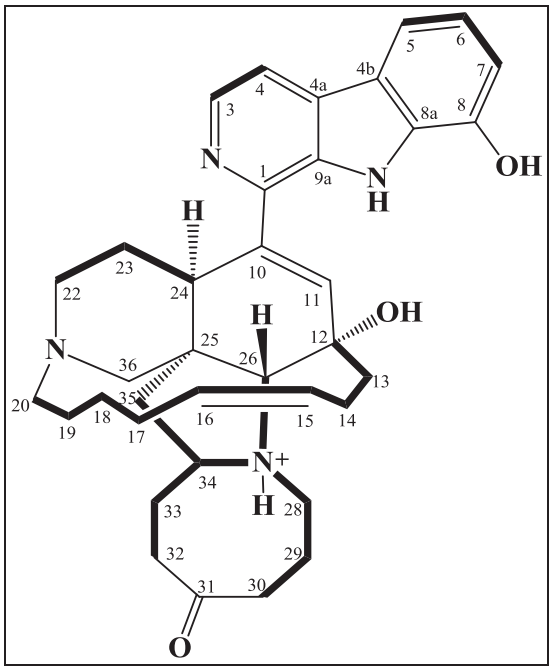


Figure 3.29-a: ^{1}H-^{1}H COSY correlations for compound (**21**)

Table 3.20: ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), COSY, and HMBC data of (**21**).

Pos.	δ_C (CD_3OD)[a,b]	δ_C (CD_3Cl)[c]	δ_H (CD_3OD)[a]	δ_H (CD_3Cl)[c]	COSY[a]	HMBC[a]
1	144.3	142.4	-	-	-	-
2	-	-	-	-	-	-
3	138.5	137.8	8.27, *d* (5.3)	8.38, *d* (5.2)	4	4, 4a
4	115.0	113.7	7.90, *d* (5.3)	7.80, *d* (5.2)	3	9a
4a	131.7	130.2	-	-	-	-
4b	124.1	122.8	-	-	-	-
5	114.7	112.0	6.90, *d* (7.8)	7.62, *d* (7.9)	6	7, 8a
6	122.7	120.9	7.08, *t* (7.7)	7.14, *m*	5, 7	4b, 8
7	111.7	113.0	7.54, *d* (7.6)	7.14, *m*	6, 8	5, 8a
8	147.6	143.4	-	-	-	-
8a	133.3	127.9	-	-	-	-
9a	134.6	133.2	-	-	-	-
10	138.7	137.3	-	-	-	-
11	141.6	140.9	6.41, *s*	6.65, *s*	-	1, 13, 24, 26
12	70.8	69.0	-	-	-	-
13	42.3	39.9	2.15, 1.78, *m*	1.87, 2.05, *m*	14	12
14	22.7	21.4	2.09, 2.30, *m*	2.1-2.3, *m*	13, 15	-
15	129.2	129.9	5.64, *dd* (8.2, 16.6)	5.63, *dd* (7.8, 10.0)	14, 16	-
16	133.2	130.1	5.51, *dd* (4.9, 10.8)	5.52, *ddd* (4.2-4.6, 10.2)	15, 17	-
17	27.0	25.5	1.72, 2.57, *m*	1.64, 2.50, *m*	16, 18	-
18	28.01	26.6	1.39, 1.40, *m*	1.30, 1.42, *m*	17, 19	-
19	26.6	25.0	1.72, 1.42, *m*	1.40, 1.75, *m*	18, 20	-
20	54.1	52.8	2.34, 2.65, *m*	2.38, 2.60, *m*	19	-
21	-	-	-	-	-	-
22	51.0	49.6	1.80, 2.78, *m*	1.90, 2.80, *m*	23	-
23	34.2	34.0	1.56, 1.69, *m*	1.56, 2.25, *m*	22, 24	-
24	43.5	42.3	3.15, *brs*	3.20, *m*	23	10, 26
25	47.8	47.3	-	-	-	-
26	81.01	81.7	3.79, *d* (7.3)	3.70, *s*	27	11, 13 , 24, 28
27	-	-	-	-	-	-
28	53.7	53.0	3.52, 2.80, *m*	2.75, 3.32, *m*	27, 29	-
29	34.2	32.7	1.94, 1.91, *m*	1.95, 2.05, *m*	28, 30	-
30	39.7	45.1	2.68, 2.26, *m*	2.22, 2.49, *m*	29, 31	-
31	216.4	216.2	-	-		-
32	44.6	38.8	2.56, 2.19, *m*	2.33, 2.62, *m*	31, 33	-
33	25.92	24.4	2.18, 1.88, *m*	1.70, 2.10, *m*	32, 34	31
34	64.9	63.6	2.91, *m*	2.98, *m*	35	25, 26, 32, 33
35	46.6	46.6	1.67, 1.90, *m*	1.57, 1.64, *m*	34	25, 33, 34, 36
36	69.3	69.0	2.22, *m*	2.30, *m*	-	-
			2.69, *m*	2.49, *m*	-	-

(a) Compound (**21**) (b) ^{13}C were extracted from the HSQC and HMBC spectra (c) Ichiba *et al*., 1988

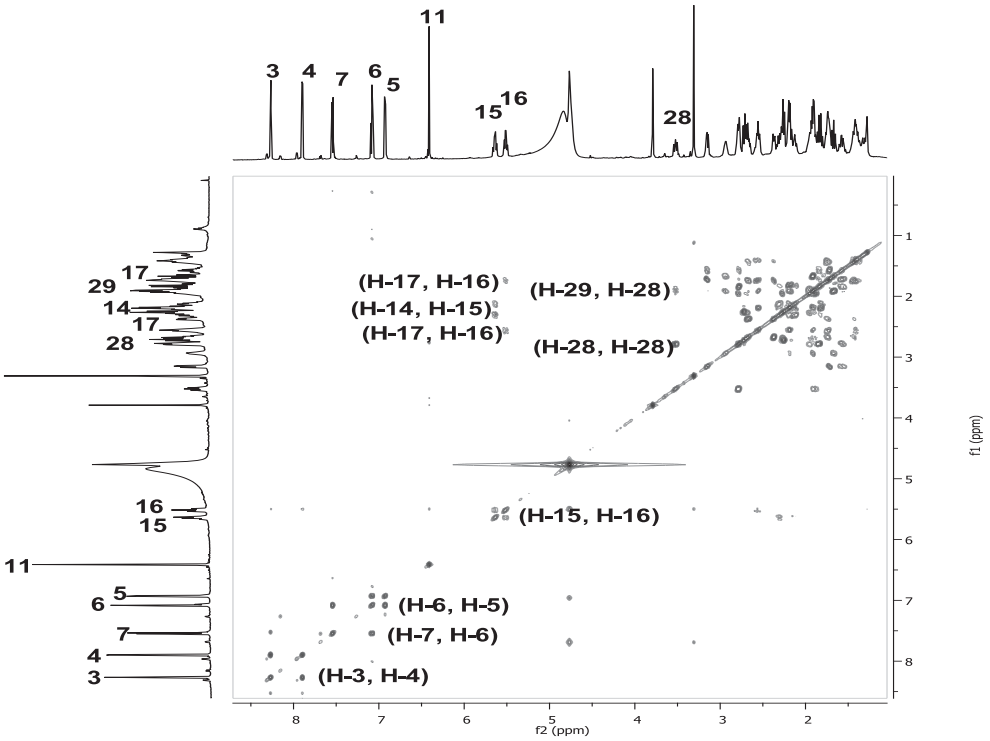


Figure 3.29-b: ^{1}H-^{1}H COSY spectrum for compound (**21**)

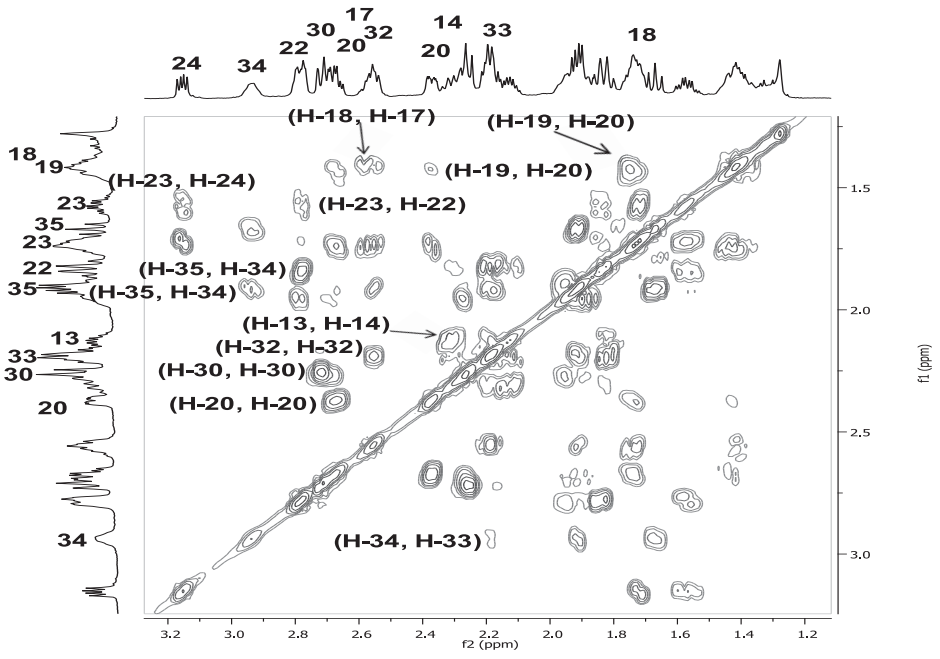


Figure 3.29-c: ^{1}H-^{1}H COSY spectrum for compound (**21**).

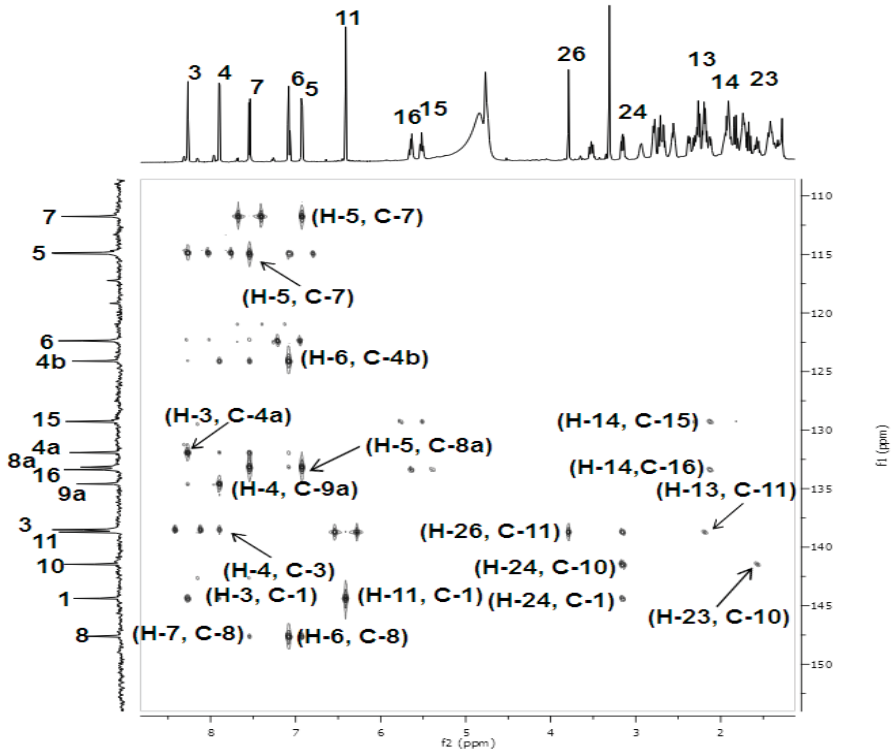


Figure 3.30-a: HMBC spectrum for compound (**21**).

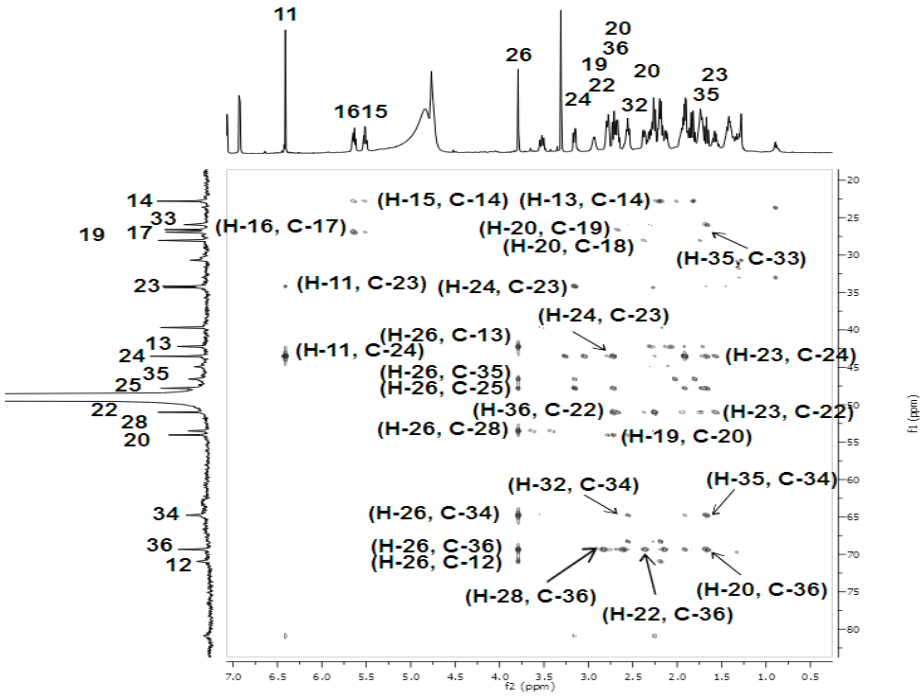
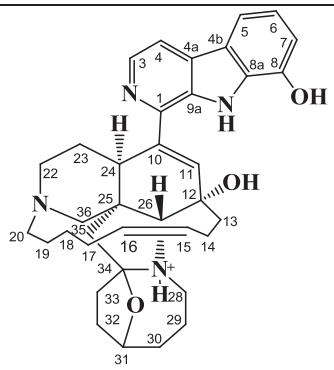
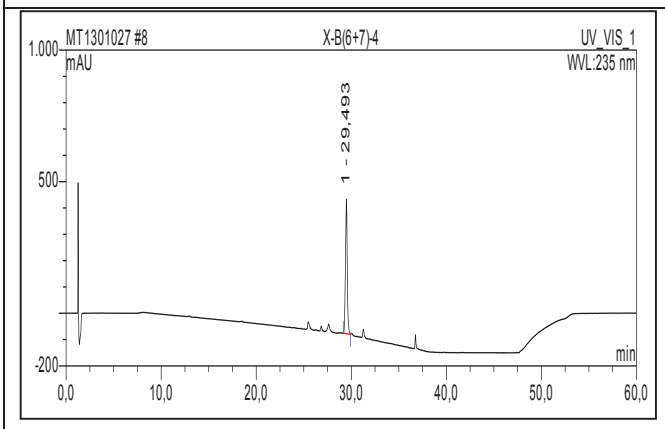


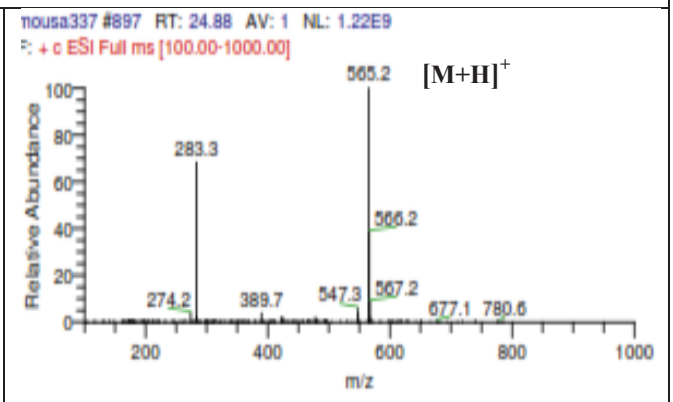
Figure 3.30-b: HMBC spectrum for compound (**21**).

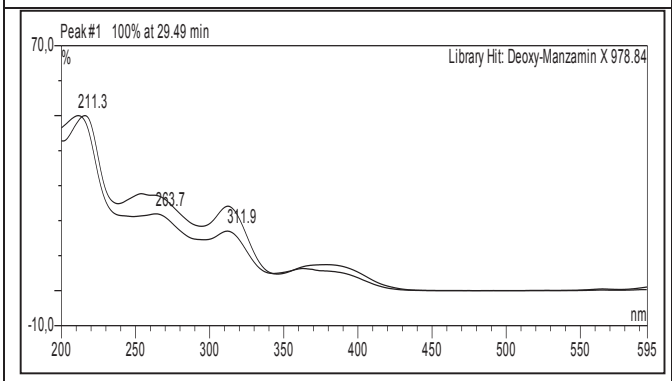
3.4.1.6. Deoxymanzamine X (22: known natural product)

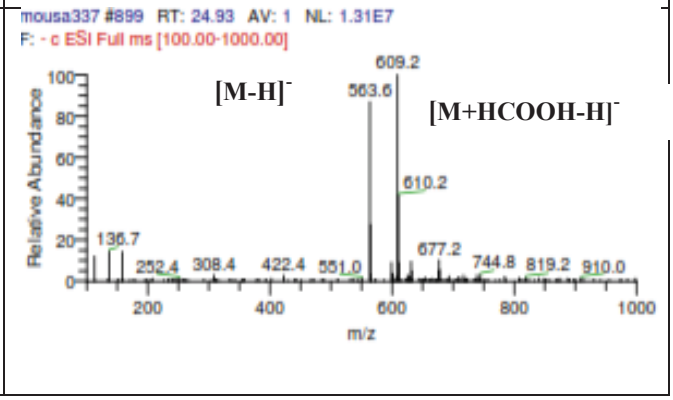
Deoxymanzamine X	
Synonym(s)	Deoxymanzamine X
Sample code	X-B(6+7)-4
Biological source	*Acanthostrongylophora ingens*
Sample amount	0.7 mg
Physical Description	Yellowish powder
Molecular Formula	$C_{36}H_{44}N_4O_2$
Molecular Weight	564.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+12.5 (*c* 0.1, $CHCl_3$)
Retention time HPLC	29.49 min (standard gradient)



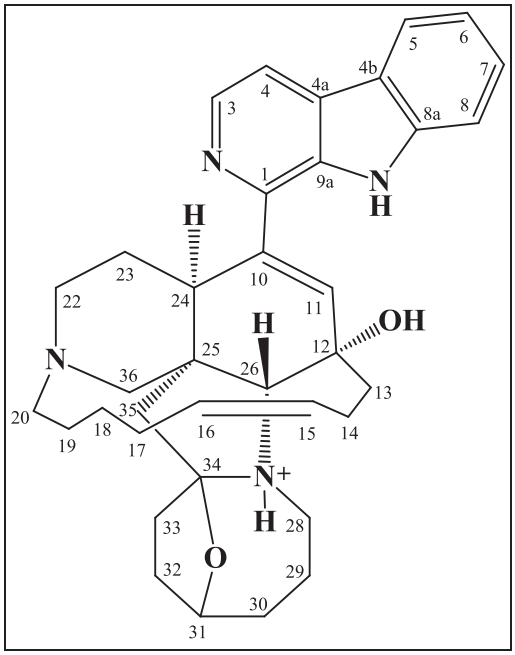








Compound (**22**) was isolated as a yellowish powder from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum showed *pseudo*-molecular ion peaks at *m/z* 565.2 $[M+H]^+$ (base peak), 563.8 $[M-H]^-$, and 609.2 $[M+HCOOH-H]^-$ (base peak) indicating a molecular weight of 564 g/mol. This mass differs by 16 amu in comparison with the mass of compound (**19**), and it is the same as the mass of compound (**20**). Compound (**22**) showed UV absorption at λ_{max} (MeOH) 212.1, 240.6, and 264.1 nm. The ^{1}H NMR spectrum for compound (**22**) showed six aromatic protons, four doublets at δ_H 8.37 ppm [d (*J*=5.1 Hz)], δ_H 8.05 ppm (brs), δ_H 8.25 ppm [d (*J*=7.9 Hz)], and δ_H 7.65 ppm [d (*J*=8.1 Hz)] which were assigned to H-3, H-4, H-5, and H-8, respectively, and two triplets at δ_H 7.27 ppm [t (*J*=7.4 Hz)], and 7.58 ppm [t (*J*=7.4 Hz)] which were attributed to H-6, and H-7, respectively. Furthermore, three protons appeared in the olefinic region at δ_H 6.40 ppm (s), δ_H 5.60 ppm (m), δ_H 5.50 ppm (m), which were assigned to H-11, H-15, H-16, respectively, while the other two olefinic proton signals for H-32 and H-33 are not present in the spectrum of (**22**) in comparison with (**19**) (Table 3.21). The proton which appeared at δ_H 11.06 ppm (s) was assigned as NH-9 by comparison with the same proton of compound (**19**). The ^{1}H-^{1}H COSY spectrum showed five spin systems (Figure 3.31). The relative configuration and the structure of (**22**) were finally confirmed by comparison of the ^{1}HNMR, mass spectral, UV data, in addition to the $[\alpha]_D^{20}$ of **22** with the published data of the same known compound (Edrada *et al*., 1996) (Table 3.21).



Structure of compound (**22**)

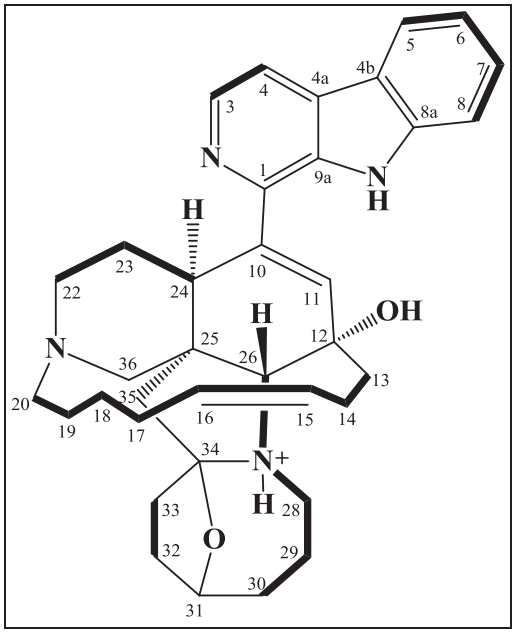


Figure 3.31: ^{1}H-^{1}H COSY correlations for compound (**22**)

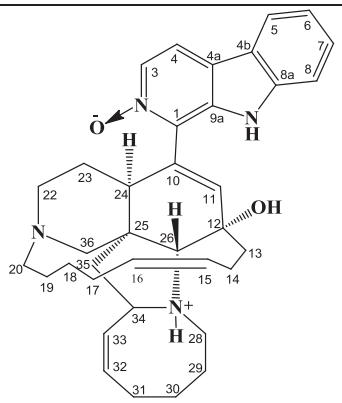
Table 3.21: ^{1}H NMR (600 MHz) data of (**22**).

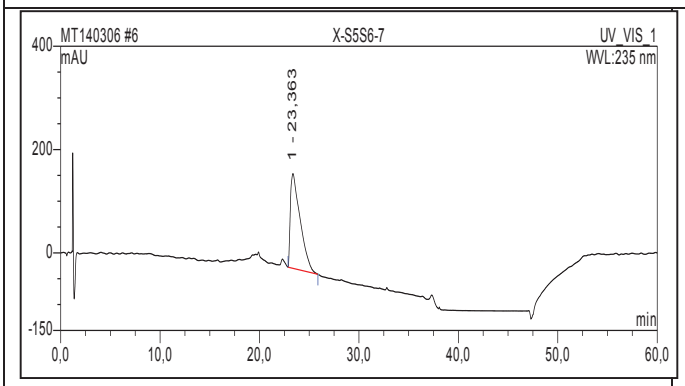
Pos.	δ_H (DMSO-d_6)[a]	δ_H (DMSO-d_6)[b]	δ_H (CD_2Cl_2)[c]
1	-	-	-
2	-	-	-
3	8.37, *d* (5.1)	8.37, *d* (5.1)	8.42, *d* (5.1)
4	8.05, *brs*	8.07, *d* (5.1)	7.87, *d* (5.2)
4a	-	-	-
4b	-	-	-
5	8.25, *d* (7.7)	8.26, *d* (7.7)	8.16, *d* (7.8)
6	7.27, *t* (7.4)	7.27, *t* (7.4)	7.33, *m*
7	7.58, *t* (7.4)	7.58, *t* (7.6)	7.57, *m*
8	7.65, *d* (8.1)	7.67, *d* (8.0)	7.57, *m*
8a	-	-	-
9	11.06, *s*	10.71, *s*	8.8, *brs*
9a	-	-	-
10	-	-	-
11	6.40, *s*	6.44, *s*	6.46, *s*
12	-	-	-
OH-12	5.17, *brs*	6.04, *s*	3.40, *brs*
13	-	2.16, 2.07, *m*	1.69, 2.10, *m*
14	-	2.05, 2.36, *m*	2.40, 2.14, *m*
15	5.60, *m*	5.61, *m*	5.68, *q* (10.0)
16	5.50, *m*	5.56, *m*	5.59, *dt* (10.8, 5.0)
17	1.86-1.76, *m*	2.67,1.50, *m*	264, 186-1.76, *m*
18	1.57-1.46, *m*	1.41,1.12, *m*	1.51, *m*
19	1.57-1.46, *m*	1.24, *m*	1.47, *m*
20	2.84-2.79, *m*	2.85, 2.24, *m*	2.54, 2.78, *m*
22	2.84-2.79, *m*	2.82, 1.71,*m*	2.01, 2.80, *m*
23	1.62-1.50, *m*	1.82, 1.64, *m*	1.58, *m*
24	2.92, *dd* (7.8, 14.6)	3.14, *brs*	3.01, *dt* (6.4, 9.3)
25	-	-	-
26	3.60, *brs*	3.83, *d* (7.3)	3.62, *s*
27	8.97, *brs*	8.97, *brs*	-
28	2.90, 3.59, *m*	3.75, 3.47, *m*	2.89, 3.63, *m*
29	1.86-1.76, *m*	1.94, 1.84, *m*	1.86-1.76, *m*
30	1.86-176, *m*	1.79, 1.37, *m*	1.66, 1.86-1.76, *m*
31	4.19, *brs*	2.22, 2H, *m*	4.54, *brd* (7.9)
32	3.27, 2.1-1.65, *m*	6.30, *m*	3.20, d (11.9), 2.05, 1.63, *m*
33	2.30-2.10, *m*	5.48, *t* (10.14)	2.25-2.16, *m*
34	-	4.70, *m*	-
35	193, 2.38, *m*	2.32, 2.15, *m*	1.94, *d* (13.9), 2.33, *d* (13.9)
36	2.22, 2H, *d* (14.5)	3.24, *d* (11.7) 2.40, *d* (11.6)	2.27, 2H, *d* (11.9)

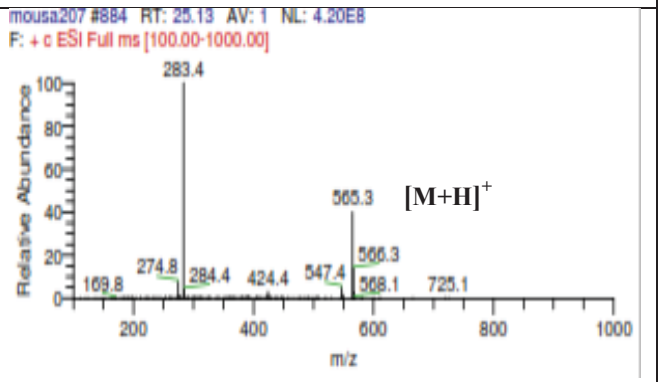
(a) Compound (**22**) (b) Compound (**19**) (c) Edrada *et al*., 1996

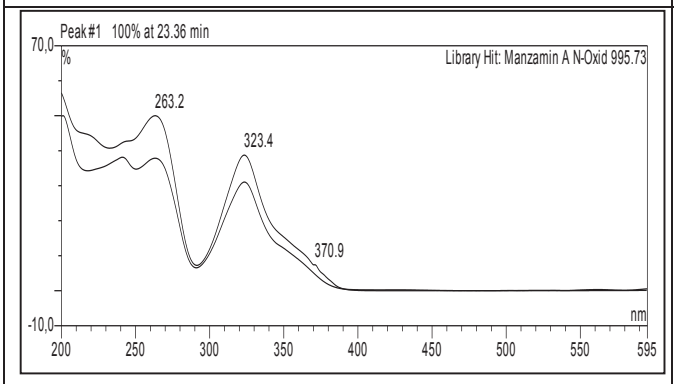
3.4.1.7. Manzamine A *N*-Oxide (23: known natural product)

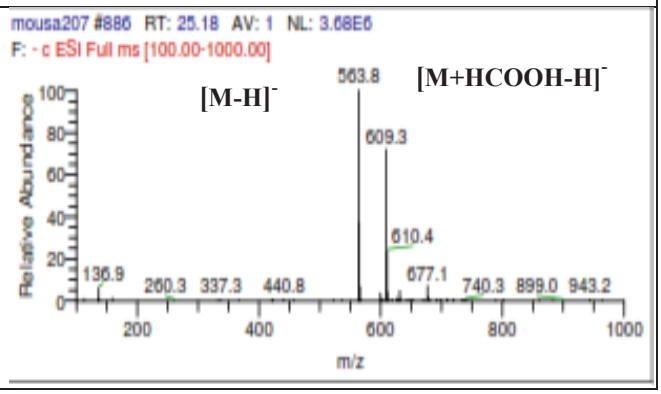
Manzamine A *N*-Oxide	
Synonym(s)	Manzamine A *N*-Oxide
Sample code	X-S5S6-7
Biological source	*Acanthostrongylophora ingens*
Sample amount	1.1 mg
Physical Description	Yellowish powder
Molecular Formula	$C_{36}H_{44}N_4O_2$
Molecular Weight	564.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+30.8 (*c* 0.2, $CHCl_3$)
Retention time HPLC	29.49 min (standard gradient)



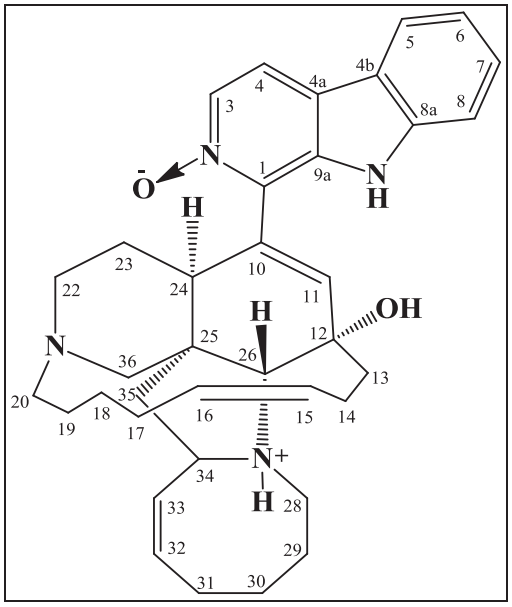








Compound (**23**) was isolated as a yellowish powder from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum revealed *pseudo*-molecular ion peaks at *m/z* 565.3 $[M+H]^+$, 563.8 $[M-H]^-$ (base peak), and 609.2 $[M+HCOOH-H]^-$ indicating a molecular weight of 564 g/mol. This mass differs by 16 amu in comparison with the mass of compound **19**, and it is same as the mass of compound (**20**), and (**22**). Compound (**23**) showed UV absorption at λ_{max} (MeOH) 263.2, 223.4, and 370.9 nm. The ^{1}H NMR spectrum for compound (**23**) showed six aromatic protons, two multiplets at δ_H 8.1 ppm [2H, m] which were assigned to H-3, and H-4, respectively, two doublets at δ_H 8.17 ppm [d (*J*=7.80 Hz)], and δ_H 7.69 ppm [d (*J*=8.2 Hz)] which were attributed to H-5, and H-8, respectively, and two triplets at δ_H 7.29 ppm [t (*J*=7.5 Hz)] and δ_H 7.49 ppm [t (*J*=8.0 Hz)] which were assigned to H-6, and H-7, respectively. Another five protons appeared in the olefinic region at δ_H 6.07 ppm (s), δ_H 5.65 ppm (m), δ_H 5.56 ppm (m), δ_H 6.28 ppm [dd (*J*=7.5, 17.7)], and δ_H 5.48 ppm [t (*J*=7.5)] which were attributed to H-11, H-15, H-16, H-32, and H-33, respectively (Table 3.22). The ^{1}H-^{1}H COSY spectrum (Figure 3.32-b) showed five spin systems (Figure 3.32-a), and the COSY cross peaks CH_2(26)NH(27)CH_2(28) proved that compound (**23**) exists as a salt. The relative configuration and the structure of (**23**) were finally confirmed by comparison of the ^{1}H NMR, mass spectral, UV data, and $[\alpha]_D^{20}$ data with the published data of the same known compound (Edrada *et al*., 1996) (Table 3.22).



Structure of compound (**23**)

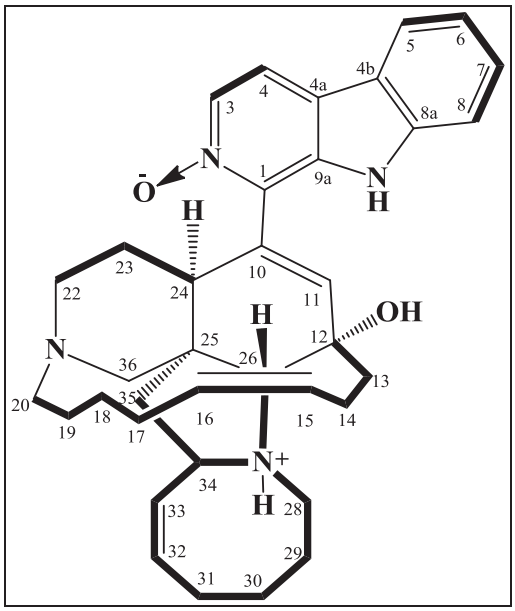


Figure 3.32-a: ^{1}H-^{1}H COSY correlations for compound (**23**)

Table 3.22: ^{1}H NMR (600 MHz) and COSY data of (**23**).

Pos.	δ_C (DMSO-d_6)[a]	δ_H (DMSO-d_6)[b]	δ_H (CD_2Cl_2)[c]	COSY
1	-	-	-	-
2	N	-	-	-
3	8.1, *m*	8.37, *d* (5.1)	8.06, *d* (6.7)	4
4	8.1, *m*	8.07, *d* (5.1)	7.77, *d* (6.7)	3
4a	-	-	-	-
4b	-	-	-	-
5	8.17, *d* (7.8)	8.26, *d* (7.9)	8.04, *d* (8.0)	6
6	7.29, *t* (7.5)	7.27, *t* (7.4)	7.32, *t* (8.0)	5, 7
7	7.49, *t* (8.0)	7.58, *t* (7.6)	7.51, *m*	6, 8
8	7.69, *d* (8.2)	7.67, *d* (8.0)	7.54, *m*	7
8a	-	-	-	-
9	10.48, *brs*	10.71, *brs*	8.8, *brs*	-
9a	-	-		-
10	-	-		-
11	6.07, *s*	6.44, *s*	6.07, *s*	-
12	-	-	-	-
OH-12	5.97, brs	6.04, *s*	3.40, *brs*	-
13	2.03, 2.15, *m*	2.07, 2.16, *m*	1.85, 2.15, *m*	14
14	2.07, 2.38, *m*	2.05, 2.36, *m*	2.10, 2.4, *m*	13, 15
15	5.65, *m*	5.61, *m*	5.72, *m*	14
16	5.56, *m*	5.56, *m*	5.60, dt (4.7, 7.9)	17
17	1.71, 2.36, *m*	2.67, 1.50, *m*	1.75, 2.55, *m*	16
18	1.38, 2H, *m*	1.41, 1.12,*m*	1.30, 1.41, *m*	-
19	1.38, 1.88, *m*	1.24, *m*	1.41, 1.81, *m*	-
20	2.36, 2.79, *m*	2.85, 2.24, *m*	2.38, 2.80, *m*	-
22	2.36, 3.00, *m*	2.82, 1.71, *m*	2.50, 2.98, *m*	-
23	1.71, 3.21, *m*	1.82, 1.64, *m*	1.75, 3.15, *m*	24
24	3.0, *brs*	3.14, *brs*	3.0, *dd* (7.3, 11.5)	23
25	-	-	-	-
26	3.78, *d* (7.2)	3.83, *d* (7.3)	3.72, *s*	-
27	9.12, *sbr*	8.97, *sbr*	-	26, 28, 34
28	3.75, 3.47, *m*	3.75, 3.47, *m*	320, 3.96, *m*	27, 29
29	1.94, *m*	1.94, *m*	1.98, 2.84, *m*	28, 30
30	1.36, *m*	1.36, *m*	1.38, 1.91, *m*	29, 31
31	2.24, 2H, *m*	2.22, 2H, *m*	2.30, 2H, *m*	30, 32
32	6.28, *dd* (7.5, 17.7)	6.30, *m*	5.95, *m*	31, 33
33	5.48, *t* (7.5)	5.48, *t* (10.1)	5.35, *m*	32, 34
34	4.71, *m*	4.70, *m*	4.29, *m*	33, 35
35	-	-	-	34
36	2.42, *d* (11.6)	2.40, *d* (11.6)	2.33, *d* (3.1)	36
	3.21, *d* (11.5)	3.24, *d* (11.7)	2.80, *d* (11.6)	

(a) Compound (**23**) (b) Compound (**19**) (c) Edrada *et al*., 1996

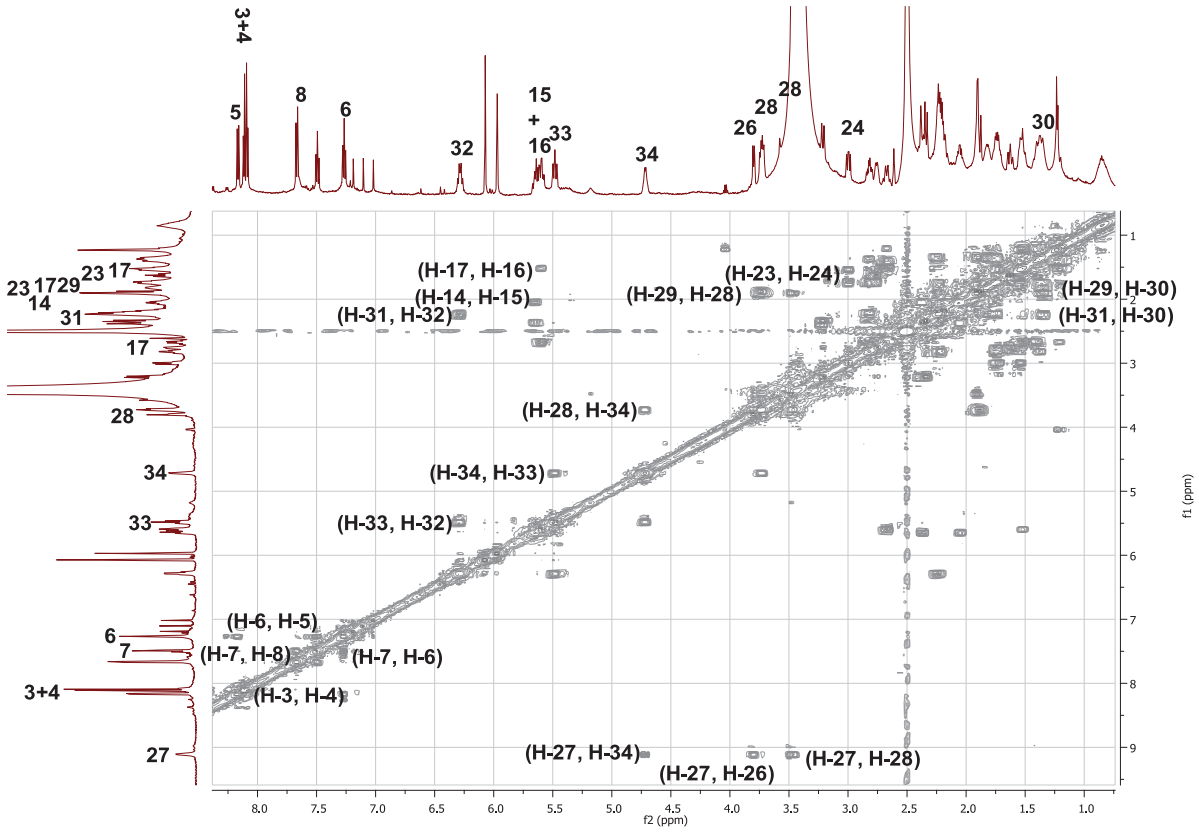
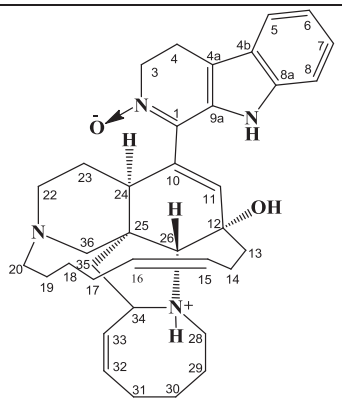
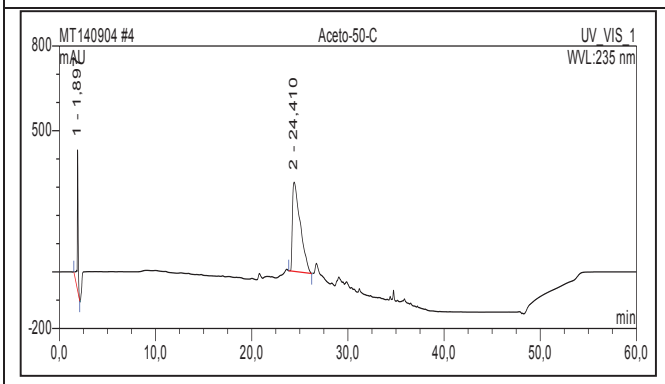


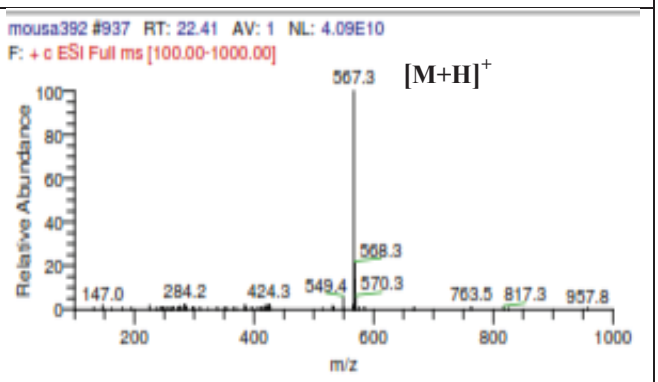
Figure3.32-b: ^{1}H-^{1}H COSY spectra for compound (**23**)**.**

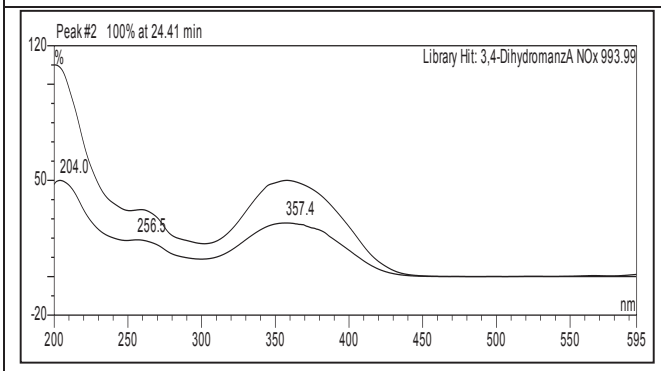
3.4.1.8. 3,4-Dihydromanzamine A *N*-Oxide (24: known natural product)

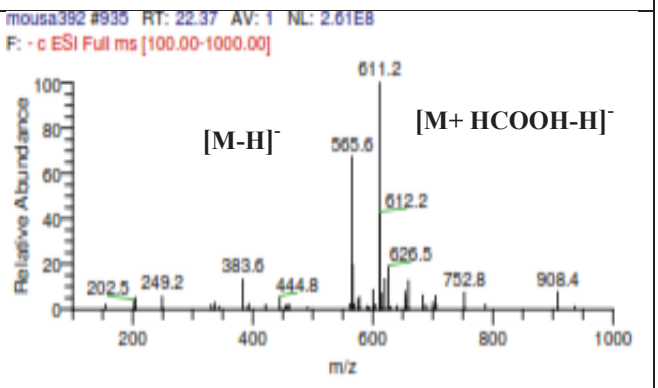
3,4-Dihydromanzamine A *N*-Oxide	
Synonym(s)	3,4-Dihydromanzamine A *N*-Oxide
Sample code	Aceto-50-C
Biological source	*Acanthostrongylophora ingens*
Sample amount	1.1 mg
Physical Description	yellowish powder
Molecular Formula	$C_{36}H_{46}N_4O_2$
Molecular Weight	566.77 g/mol.
Optical rotation$[\alpha]_D^{20}$	+30.8 (*c* 0.2, $CHCl_3$)
Retention time HPLC	24.41 min (standard gradient)



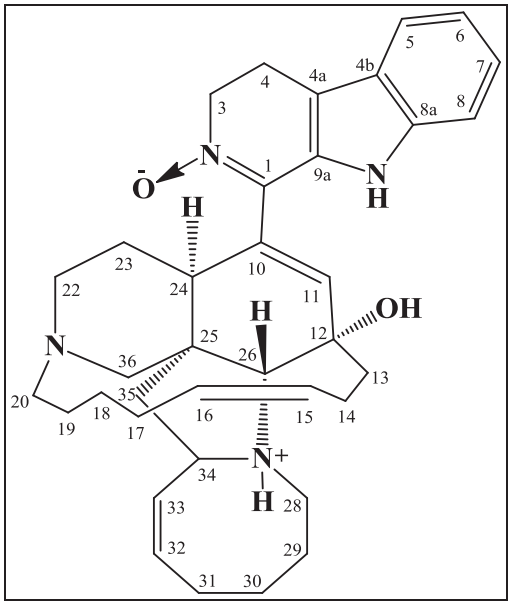








Compound (**24**) was isolated as a yellowish powder from the EtOAc fraction of *Acanthostrongylophora ingens*. The ESI mass spectrum revealed pseudo-molecular ion peaks at*m/z* 567.3 $[M+H]^+$ (base peak), 565.6 $[M-H]^-$, and 611.2 $[M+HCOOH-H]^-$ (base peak) indicating a mass of 566 g/mol. This mass differs by 2 amu in comparison with the molecular weight of compound (**23)**. Compound (**24**) showed UV absorption at λ_{max} (MeOH) 204.0, 256.5, and 357.4 nm. The ^{1}H NMR spectrum for compound (**24**) showed four aromatic protons, two doublets at δ_H 7.51 ppm [d, (*J*=8.0 Hz)], and δ_H 7.46 ppm [d (*J*=8.2 Hz)] which were assigned to H-5, and H-8, and two triplets at δ_H 7.06 ppm [t (*J*=7.5 Hz)], and 7.14 ppm [t (*J*=7.1 Hz)] which were attributed to H-6, and H-7, respectively, while the proton signals of H-3, and H-4 are missing in the ^{1}H NMR spectrum of compound (**24**) in comparison to those of (**19**), (**21**), and (**22**). Furthermore, five protons appeared in the olefinic region at δ_H 5.95 ppm (s), δ_H 5.62 ppm (m),δ_H 5.57 ppm (m), δ_H 6.27 ppm [dd (*J*=7.2,17.4 Hz)], and δ_H 5.45 ppm [t (*J*=10.0 Hz)] which were assigned to H-11, H-15, H-16, H-32, and H-33, respectively (Table 3.23). In addition, two protons appeared in the downfield region at δ_H 10.53 ppm (s), and δ_H 8.99 ppm (s) which were attributed to NH-9 and NH-27, respectively by comparison with compounds (**19**), (**20**) (Table 3.23). The last proton proved that compound (**24**) exists as a salt. The ^{1}H-^{1}H COSY spectrum showed five spin systems (Figure 3.33). Comparison of the $[\alpha]_D^{20}$, UV, ^{1}H NMR spectra (Table 3.25), and mass spectral data with the literature data (Edrada *et al*., 1996) indicates that (**24**) is the known compound 3,4-dihydromanzamine A *N*-oxide.



Structure of compound (**24**)

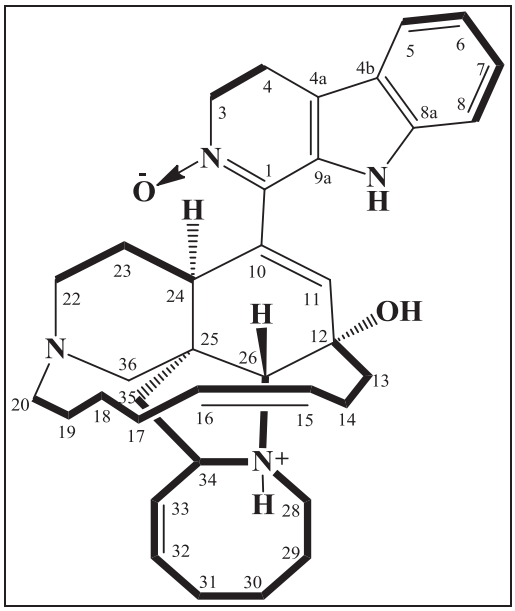


Figure 3.33: ^{1}H-^{1}H COSY correlations for compound (**24**)

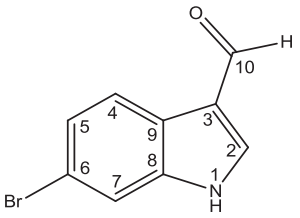
Table 3.23: ^{1}H NMR (600 MHz) data of (**24**).

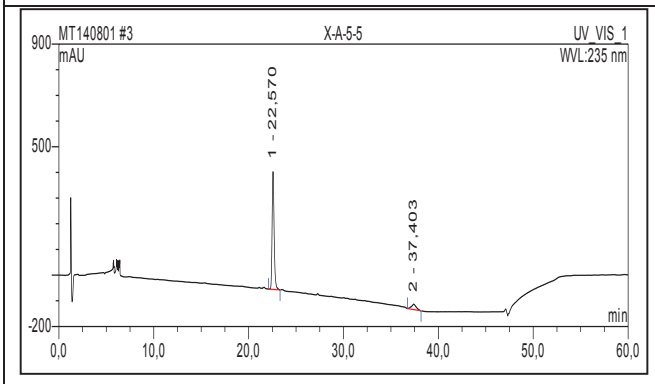
Pos.	δ_C (DMSO-d_6)[a]	δ_H (DMSO-d_6)[b]	δ_H (CD_2Cl_2)[c]
1	-	-	-
2	-	-	-
3	4.40, 4.0, *m*	8.37, *d* (5.1)	4.36, 4.21, *m*
4	3.20, *m*	8.07, *d* (5.1)	3.22, *m*
4a	-	-	-
4b	-	-	-
5	7.51, *d* (8.0)	8.26, *d* (7.9)	7.51, *d* (7.8)
6	7.06, *t* (7.5)	7.27, *t* (7.4)	7.15, *d* (8.0)
7	7.14, *t* (7.1)	7.58, *t* (7.6)	7.21, *dt* (1.1, 7.6)
8	7.46, *d* (8.2)	7.67, *d* (8.0)	7.41, *d* (8.0)
8a	-	-	-
9	10.53, *s*	10.71, *s*	8.80, *brs*
9a	-	-	-
10	-	-	-
11	5.95, *s*	6.44, *s*	5.97, *s*
12	-	-	-
OH-12	5.92, *brs*	6.04, *s*	3.40, *brs*
13	1.65, 2.01, *m*	2.16, 2.07, *m*	1.75, 2.02, *m*
14	2.39, 2.13, *m*	2.05, 2.36, *m*	2.40, 2.14, *m*
15	5.62, *m*	5.61, *m*	5.69, *m*
16	5.57, *m*	5.56, *m*	5.59, *dt* (10.8, 4.7)
17	1.88, 2.64, *m*	2.67,1.50, *m*	1.85, 2.52, *m*
18	1.35, 1.67, *m*	1.41, 1.12, *m*	1.34, 1.70, *m*
19	1.35, 1.67, *m*	1.24, *m*	1.34, 1.70, *m*
20	2.38, 2.64, *m*	2.85, 2.24, *m*	2.42, *m*, 2.63, *dt* (11.7, 4.8)
22	2.0, 2.82, *m*	2.82, 1.71, *m*	1.95, 2.79, *m*
23	1.67, 1.88, *m*	1.82, 1.64, *m*	1.60, 1.97, *m*
24	3.1, *m*	3.14, *brs*	2.98, *m*
25	-	-	-
26	3.50-3.30, *m*	3.83, *d* (7.3)	3.45, *s*
27	8.99, *brs*	8.97, *brs*	-
28	3.20, *m*	3.75, *m*, 3.47, s	3.19, *m*
29	1.50, 1.67, *m*	1.94,1.84, *m*	1.55, 1.70, *m*
30	1.36, 1.88, *m*	1.79,1.37, *m*	1.34, 1.85, *m*
31	2.31, 2.13, *m*	2.22, 2H, *m*	2.35, 2.15, *m*
32	6.27, *dd* (7.2, 17.4)	6.30, *m*	5.94, *m*
33	5.45, *t* (10.0)	5.48, *t* (10.14)	5.30, *t* (9.5)
34	4.65, *m*	4.70, *m*	4.21, *m*
35	170-1.60, 2.82, *m*	2.32, 2.15, *m*	1.65, 2.95, *m*
36	2.30, 2.82, *m*	3.24, *d* (11.7), 2.40, *d* (11.6)	2.30, *d* (2.3) 2.80, *d* (11.6)

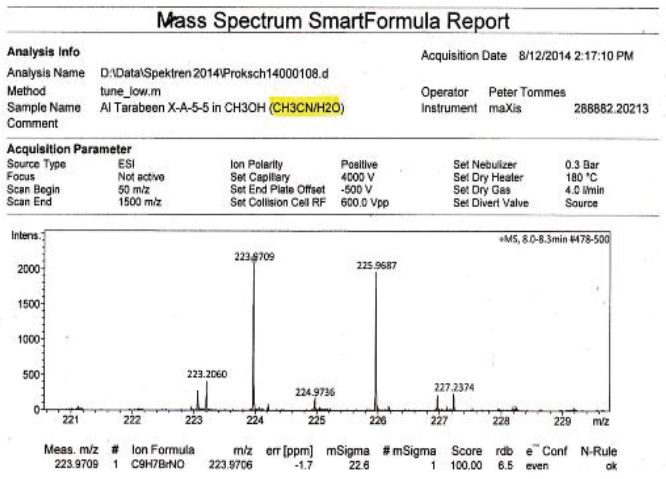
(a) Compound (**24**) (b) Compound (**19**) (c) Edrada *et al*., 1996 (**3**)

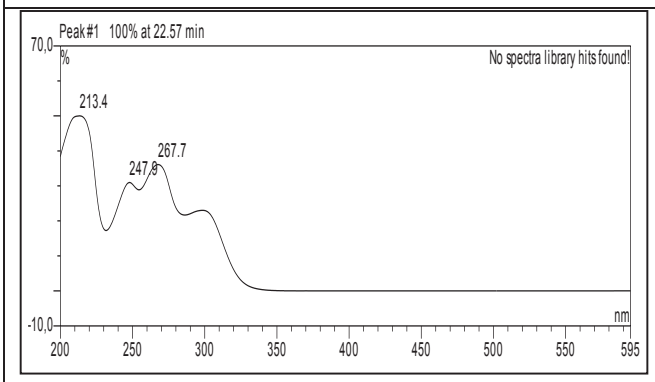
3.4.1.9. 6-Bromoindole-3-carbaldehyde: (25: known natural product)

6-Bromoindole-3-carbaldehyde	
Synonym(s)	6-Bromoindole-3-carbaldehyde
Sample code	X-A-5-5
Biological source	*Acanthostrongylophora ingens*
Sample amount	1.2 mg
Physical Description	yellowish solid
Molecular Formula	$C_{10}H_6BrNO$
Molecular Weight	223.05 g/mol.
Retention time HPLC	22.57 min (standard gradient)







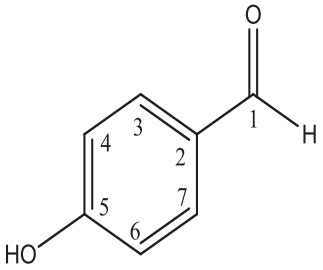


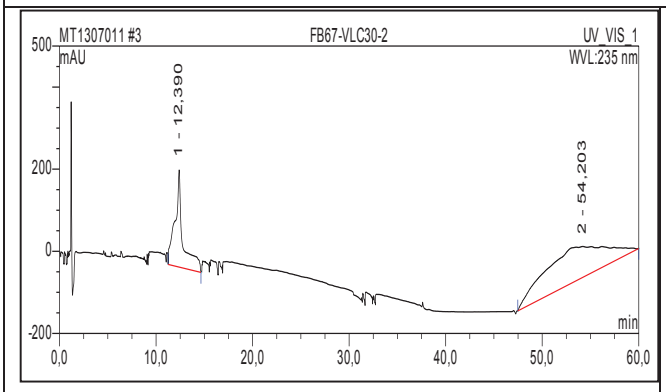
No ionization in negative ESI-MS

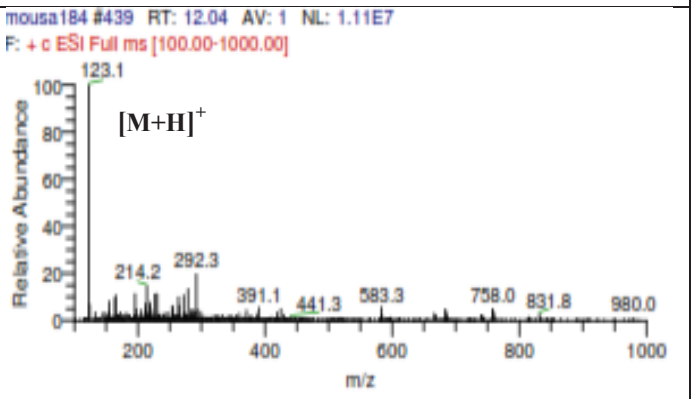
*Compound **25** was discussed before (see 3.3.1.3)

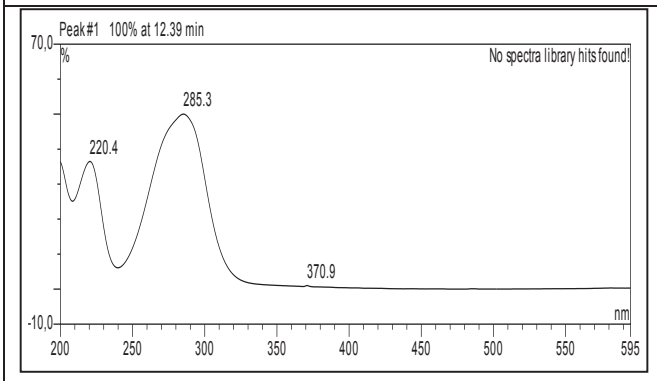
3.4.1.10. 4-Hydroxybenzaldehyde: (26: known natural product)

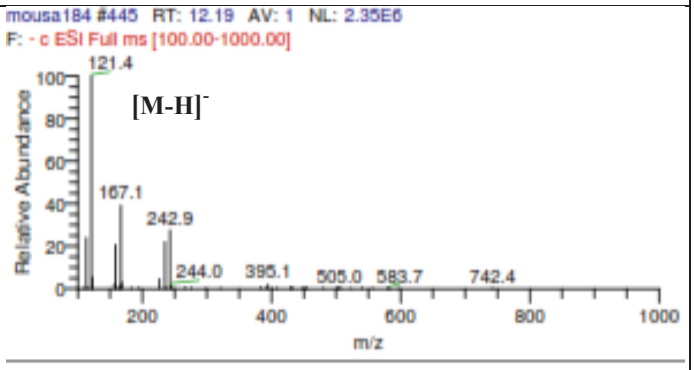
4-Hydroxybenzaldehyde	
Synonym(s)	4-Hydroxybenzaldehyde
Sample code	FB67-VLC30
Biological source	*Acanthostrongylophora ingens*
Sample amount	1.2 mg
Physical Description	yellowish solid
Molecular Formula	$C_7H_6O_2$
Molecular Weight	122.12 g/mol.
Retention time HPLC	12.39 min (standard gradient)



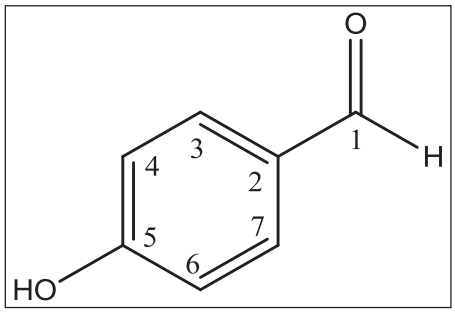








Compound (**26**) was isolated as a yellowish solid from the EtOAc extract of the *Acanthostrongylophora ingens*. The ESI mass spectrum of **26** revealed *pseudo*-molecular ion peaks at *m/z* 123.1 $[M+H]^+$ (base peak), and 121.4 $[M-H]^-$ indicating a molecular weight of 122 g/mol. Compound (**26**) showed UV absorption at λ_{max} (MeOH) 220.4, and 285.3 nm. The ^1H NMR spectrum for compound (**26**) revealed four doublets at δ_H 7.78 ppm [2H, d (*J*=8.4 Hz)], and at δ_H 6.92 ppm [2H, d (*J*=8.4 Hz)], which were assigned to H-3, H-7, H-4, and H-6, respectively (AA'BB' system) (Table 3.24). This assumption was confirmed by the ^1H-^1H COSY spectrum (Figure 3.34) which showed two spin systems, *viz*., the fist spin system is CH(3)CH(4) and the other one is CH(6)CH(7) (Table 3.24). The structure of (**26**) was finally confirmed by comparison of the ^1H NMR, and mass spectral data of (**26**) with the published data of the same known compound (Li *et al*., 1994) (Table 3.24).



Structure of compound (**26**)

Table 3.24: ^{1}H NMR (600 MHz) and COSY data of (**26**).

Pos.	**δ_H (DMSO-d_6)[a]**	**δ_H (DMSO-d_6)[b]**	**COSY[a]**
1	9.77, *s*	9.75, *s*	-
2	-	-	-
3	7.78, *d* (8.4)	7.73, *d* (8.7)	4
4	6.92, *d* (8.4)	6.87, *d* (8.7)	3, 6
5	-	-	-
6	6.92, *d* (8.4)	6.87, *d* (8.7)	4
7	7.78, *d* (8.4)	7.73, *d* (8.7)	-

(a) Compound (**26**) (b) Li *et al*., 1994

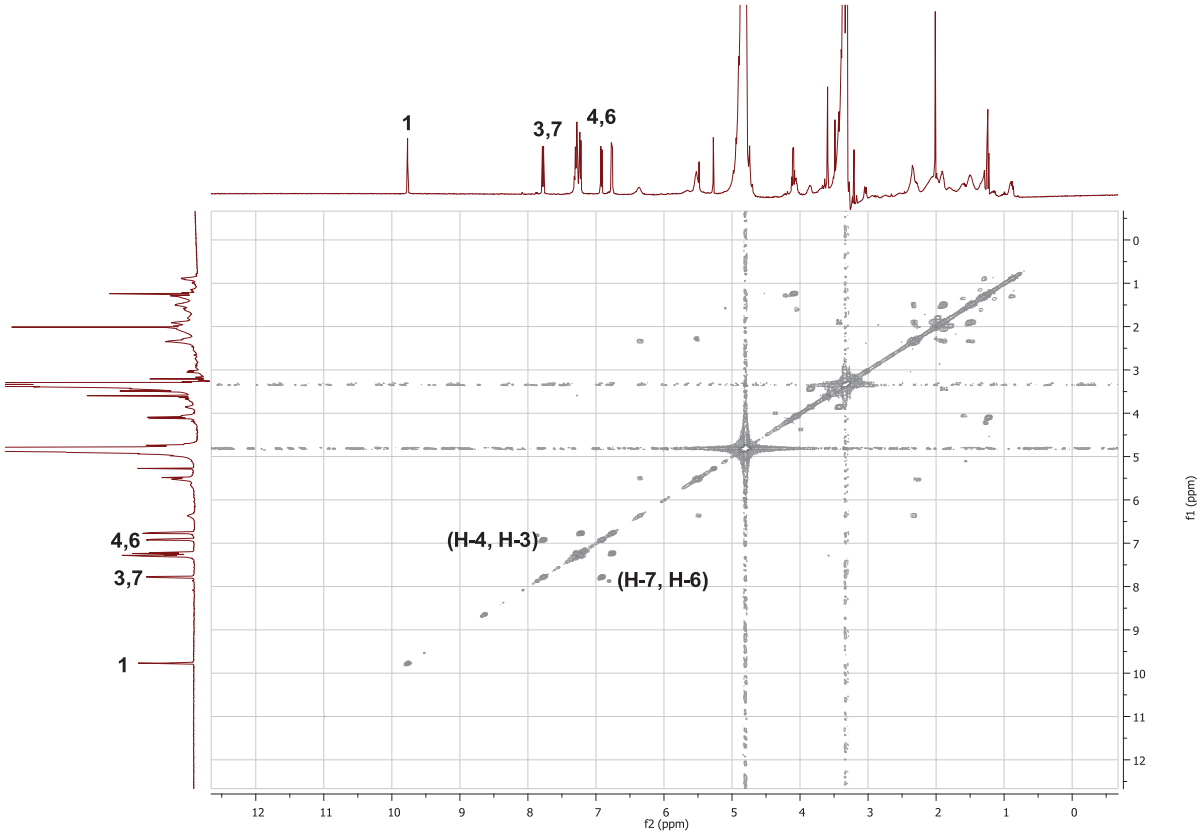


Figure 3.34: ^{1}H-^{1}H COSY spectrum for compound (**26**).

3.4.2. Bioactivity assay results for compounds from *Acanthostrongylophora ingens* from Ambon in Indonesia.

The isolated compounds from the EtOAc and *n*-ButOH of *Acanthostrongylophora ingens* sponge from Ambon in Indonesia were subjected to a cytotoxicity (MTT) assay against the mouse lymphoma (L5178Y) cells (Table 3.25).

Table 3.25: Cytotoxicity assay results for the compounds isolated from *Acanthostrongylophora ingens* sponge.

Nr.	Compounds tested	EC_{50}* (μg/mL)	EC_{50}* (μM)
17	nakadomarin A[a]		
18	ircinal E	9.2	21.66
19	manzamine A	1.8	3.29
20	18-hydroxy manzamine A	1.7	3.01
21	manzamine F	1.7	4.07
22	deoxymanzamine X	1.8	3.19
23	manzamine A *N*-Oxide	1.6	2.84
24	3.4-dihydromanzamine A *N*-Oxide	1.6	2.84
25	6-bromoindole-3-carbaldehyde[a]		
26	4-hydroxybenzaldehyde[a]		

* Data provided by Prof. W. E. G. Müller, Mainz. (a) Not active

Manzamine A (**19**), 8-hydroxymanzamine A (**20**), manzamine F (**21**), 6-deoxymanzamine X (**22**), manzamine A *N*-oxide (**23**), and 3,4dihydromanzamine A *N*-oxide (**24**) showed strong activity against the mouse lymphoma cell line (LY5178), while the new compound ircinal E (**18**) showed a moderate activity against the cell line (LY5178). However, nakadomarin A (**17**), 6-bromoindole-3-carbaldehyde (**25**), and 4-hydroxybenzaldehyde (**26**) proved to be inactive against the same cell line.

4. Discussion

4.1. Selection of marine sponges

Sponges of the phylum *Porifera* are important units of all modern coral reef communities (Santos-Gandelman *et al*., 2014). In spite of their simplicity, these sponges are highly diverse and well adapted organisms and they can manage to survive longer than any other metazoan (Hooper, 1997). The secondary metabolites produced by these sponges have a significant role in the chemical ecology of marine sponges, and their homeostasis in the marine environment (Balakrishnan *et al*., 2014). Many reviews have been published in the last decades describing the sponge-derived biochemicals (Alcaraz *et al*., 2006; Higa *et al*., 1994). In fact, sponges are important sources of many biopharmaceuticals with a wide arrange of therapeutic properties (Gerwick and Moore, 2012; Perdicaris *et al*., 2013; Mehbub *et al*., 2014). Considering not only the importance of sponges as a source of new leads, but also their importance for the balance of the ecosystem of the marine environment. Strategies should be utilized to select those sponges which are producing bioactive compounds. Recent studies showed that the highest concentration of toxic or antioxidant sponge-metabolites are found in habitats such as coral reef that characterized by intense competition and feeding pressure from carnivorous fishes (Perdicaris *et al*., 2013).

4.2. Methodologies for metabolite profiling

The main steps in natural product research involve extraction of the metabolites from the extract of samples material, followed by structure elucidation of the isolated metabolites. Secondary metabolites profiling is a challenging task because of the chemical diversity of their structures and the limitation of analytical methods (Wolfender *et al*., 2005). Therefore, screening of these metabolites in crude extracts or fractions requires validated methods with high degree of

sensitivity and selectivity, such as coupling of advanced analytical and hyphenated spectroscopic techniques to chromatographic techniques (e.g. HPLC, GC, and CE) (Figure 4.1). The power of the combining these techniques emerged from their ability to provide information about the different substructures and functional groups of the structures and it has the capability to solve difficult analytical problems (Patel *et al*., 2010) as it is applied in the present study.

4.2.1. HPLC/UV and HPLC/ESI-MS

Recently, HPLC/UV-photodiode array detection is a very useful technique to distinguish each single ingredient present in an extract. The UV spectrum is an important tool in structural elucidation of secondary metabolites; chromophores of the components absorb light in different UV regions (Cannall, 1998).

Some compounds which were isolated in this study were identified based on HPLC/UV-photodiode array detection (LC/UVDAD) *e.g*. manzamine derivatives (**21**-**23**). On the other hand, High Performance Liquid-Chroatography-Electrospray Mass Spectrometry (HPLC/ESI-MS) technique, which is a combination of HPLC with sensitive and specific MS, can be applied for the detection of a wide range of biological molecules. The major advantage of HPLC ESI-Ms comes from its ability to spontaneously detect large number of natural products in a single run (Pitt, 2009). Furthermore, closely related secondary metabolites can be easily detected by this technique

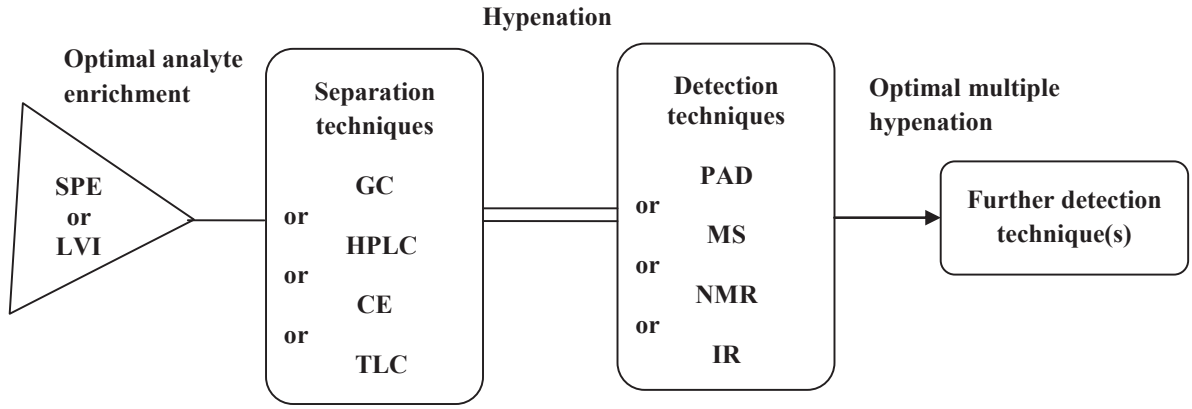


Figure 4.1: Hyphenated techniques (Patel, 2010)

4.3. Isolation of natural products

Pure secondary metabolites have been isolated from different sponge species collected by SCUBA diving from the Gulf of Aqaba in Jordan using different chromatographic techniques. These sponges are, *Diacarnus ardoukobae* (**MT1**), *Theonella* sp. (**MT3**), *Diacarnus erythraeanus* (**MT8**), *Hemimycale* sp. (**MT6**), the unknown sponge (**MT5**), in addition to *Acanthostrongylophora ingens* (**FB67**) from Ambon in Indonesia which exhibited moderate to strong cytotoxic activity.

4.4. Secondary metabolites isolated from sponges from the Gulf of Aqaba in Jordan.

4.4.1. Secondary metabolites isolated from the unknown sponge (MT5)

The methanolic extract of the unknown sponge (**MT5**) yielded one new natural product, 3-amino-1-(2-amino-4-bromophenyl) propan-1-one (**1**), in addition to 7-bromoquinolin-4(1*H*)-one (**2**) (which was previously known only as a synthetic product), together with four known compounds which are, 6-bromoindole-3-carbaldehyde (**3**), caulerpin (**4**) (which is described from sponge material here for the first time), (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**), respectively.

4.4.1.1. Bioactivity of secondary metabolites from the unknown sponge (MT5)

6-Bromoindole-3-carbaldehyde (**3**) showed 80% inhibition of barncale larvae settlement at a concentration of 10 µg/ml and showed moderate antibacterial properties (Olguin-Uribe *et al.*, 1997), while caulerpin (**4**), which was previously only known to be produced by algae, showed a variety of important biological activities which are described in the literature, e.g. its activity as a growth regulator (Xu and Su, 1996), its plant root growth stimulant properties (Raub *et al*., 1987), its antitumor activity (Ayyad, and Badria. 1994), its strong PTPIB inhibitory activity with IC_{50} value of 3.77 μM (Mao *et al*., 2006), and its antinociceptive and antiinflamatory activities *in-vivo* (de Souza *et al*. 2009). (*Z*)-5-(4-Hydroxybenzylidene)-hydantoin (**5**) showed potent *in-vitro* anti-growth and anti-invasive properties against PC-3M prostate cancer cells in a MTT, and in a spheroid disaggregation assay (Mudit *et al*., 2009; Shah *et al*., 2009). In the present study, a cytotoxicity assay revealed that 3-amino-1-(2-amino-4-bromophenyl)propan-1-one (**1**), (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**) showed a moderate cytotoxic activity against the L5178Y cell line with growth inhibition of 100% (EC_{50} > 10 μg/ml), 77%, and 89% at 10 µg/ml respectively. 7-Bromoquinolin-4(1H)-one (**2**), 6-bromoindole-3-carbaldehyde (**3**), and caulerpin (**4**) showed a weak cytotoxic activity against the same cell line (L5178Y). It is worth to mention that (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**) were separated from the *Hemimycale* sp. (**MT6**) in the current study. These compounds are (**7**), and (**8**).

4.4.1.2. Proposed biosynthesis of secondary metabolites from the unknown sponge (MT5)

Structural comparison of compounds (**1**) and (**2**) indicates that these compounds are biogenetically closely related. Their skeleton may be derived from tryptophan via the kynurenine pathway as (**1**) is a 4-bromo-derivative of the decarboxylated kynurenine and (**2**) is a 7-bromo-derivative of kynurenic acid. Furthermore, compound (**1**) shows resemblance with the 4-chloro-kynurenine residue of the recently isolated taromycin A from marine origin (Yamanaka *et al*., 2014). Interestingly, compounds (**4**-**6**) also appear to be derived from tryptophan, indicating that the metabolism of this amino acid is a prominent biochemical feature within this particular sponge (Figure 4.2).

It worth to mention that 6-bromoindole-3-carbaldehyde (**3**) was isolated from the marine-associated bacterium *Acinetobacter* sp. (Olguin-Uribe *et al.*, 1997). Thus, it could be suggested that the true producers of biologically active secondary metabolites of the marine benthic invertebrates including sponges could be the associated microorganisms (Ireland *et al*., 1988; Banaigs, 1993; Balakrishnan *et al*., 2014).

4.4.2. Secondary metabolites isolated from *Theonella* sp. (MT3)

The methanolic extract of MT3 sponge was investigated in the present study and afforded one compound which is a swinholide A (**9**). Swinholide A was the first 44-membered macrolide which was isolated from the Red Sea marine sponge *Theonella swinhoei* (Carmely and Kashman, 1985). The structure of swinholide A was first assigned as a monomer, and then was revised later to be a symmetric cyclic dimer (Kobayashi *et al*., 1989), and this was followed by determination of its absolute configuration (Kobayashi *et al*., 1990; Kitagawa *et al*., 1990; Doi *et al*., 1991). Swinholide A (**9**) showed a moderate activity against the lymphoma cell line L5178Y with growth inhibition of 87% at 10 μg/ml.

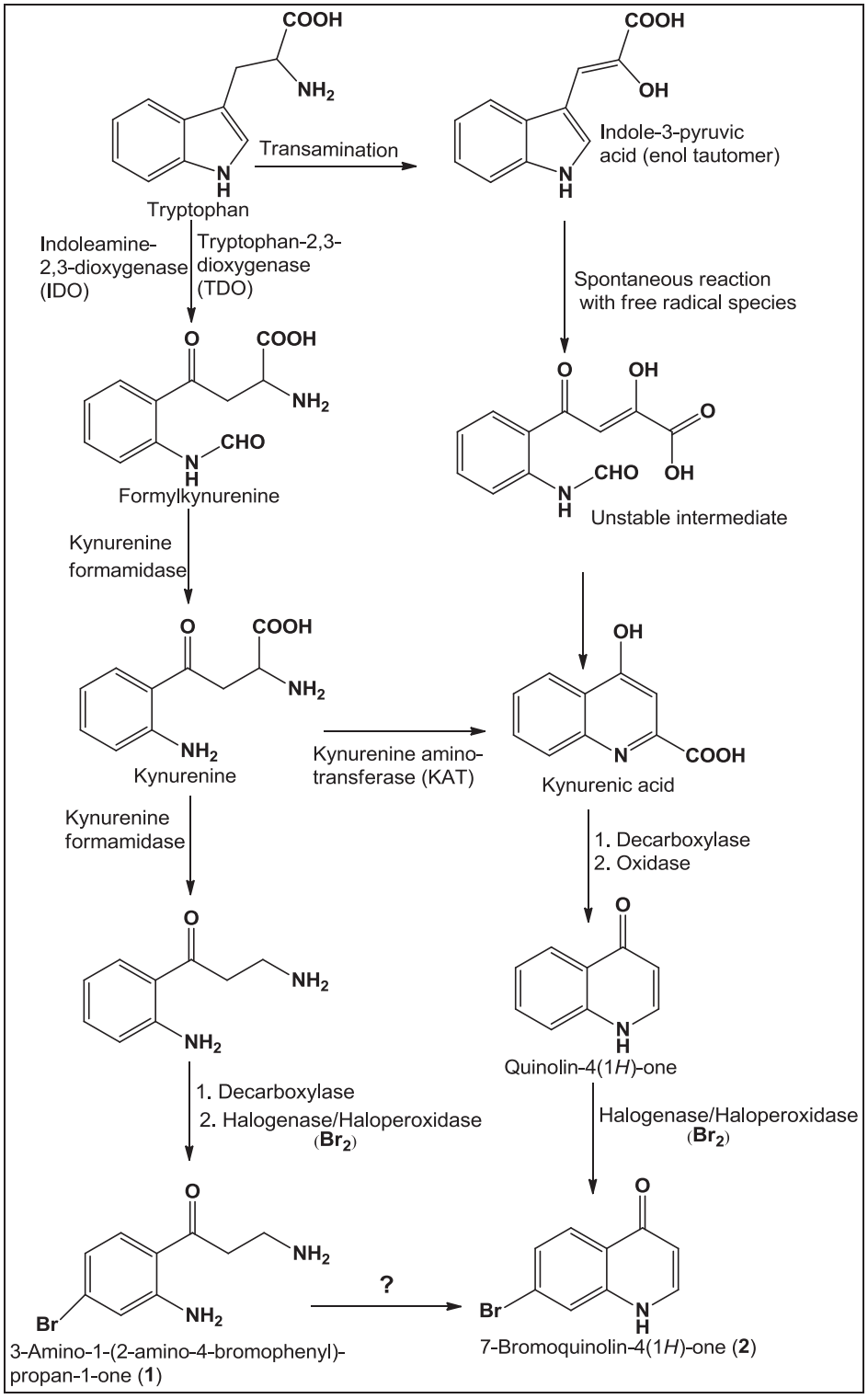


Figure 4.2: Proposed biosynthetic pathway of **1** (3-amino-1-(2-amino-4-bromophenyl) propan-1-one), and **2** (7-bromoquinolin-4(1*H*)-one).

4.4.3. Secondary metabolites isolated from *Diacarnus ardoukobae* (MT1)

In the present study, the methanolic extract of Diacarnus *ardoukoba* sponge was chemically and biologically investigated and yielded one compound which is latrunculin B (**10**). The basic structural of latrunculin B consists of a macrolide 1,3 fused to a tetrahydropyran containing a 2-thiazolidine side chain (Amagata, *et al*., 2008). Latrunculin B dispayed potent antimigratory activity in a wound healing assay and it is showed potent antimicrobial activity against *Candida albicans*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Bacillus cereus* (El Sayed *et al*., 2006). The IC_{50} values of latruncunlin B against the murine cancer cell line (HCT-116) and against the human cancer cell line (MDA-MB-435) were 1.1 and 2.8 μg/ml, respectively (Amagata *et al*., 2008). In this study, latrunculin B showed a moderate activity against the lymphoma cell line L5178Y with growth inhibition of 92% at 10 μg/ml.

4.4.4. Secondary metabolites isolated from *Diacarnus erythraeanus* (MT8)

In the current study, the investigated methanolic extract of the sponge *Diacarnus erythraeanus*, which was collected from the shore of marine science station at a depth of 20 m from the Jordanian coast of the Gulf of Aqaba by SCUBA diving, afforded six known norterpene cyclic peroxide compounds which are, (+)-muqubilone A (**11**), (-)-muqubilin A (**12**), sigmosceptrelin B (2a) (**13**), sigmosceptrelin B (2) (**14**), (+)-methyl-2-epinuapapuanoate (**15**), and (-)-methyl-2-epinuapapuanoate, respectively.

4.4.4.1. Bioactivity of norterpene cyclic peroxides

Sponges of the genus *Diacarnus*, especially *Diacarnus erythraeanus* from the Red Sea are already known to produce terpene peroxides and other related metabolites, which had also been reported to have a wide variety of biological activities (El Sayed *et al*., 2001; Youssef *et al*., 2001, Youssef *et al*., 2004; Lefranc *et al*., 2013). Challenges have been addressed in defining the chirality of their multiple stereocenters (Sperry *et al*., 1998). Norterpene cyclic

peroxides could be structurally classified into several families according to their carbon skeleton frame work, *viz*., acylic, monocyclic, and bicyclic (Capon *et al*., 1997). Interest has usually been focused on this class of compounds because they frequently possess a variety of biological activities such as antimicrobial (Capon *et al*., 1985; He *et al*., 1991), ichthyotoxicity (Albericci *et al*., 1979; Albericci *et al*., 1982), sea urchin egg cell division inhibitory (Manes *et al*., 1984), cytotoxicity (Sperry *et al*., 1998), antiviral (Tanaka *et al*., 1993), antitoxoplasmosis (El Sayed *et al*., 2001) and antimalarial activity (D´Ambrosio *et al*., 1998). In the present study the methanolic extract of *Diacarnus erythraeanus* yielded a series of terpene peroxides. Four of these terpene peroxides which are, (-)-muqubilin A (**12**), sigmosceptrellin-B (2a) (**13**), sigmosceptrellin-B (2) (**14**), and (+)-methyl-2-epinuapapuanoate (**15**) showed strong activity against the lymphoma cell line L5178Y with EC_{50} values of 0.49, 1.2, 3.4, and 6.4 μM respectively, while muqubilone A (**11**) and (-)-methyl-2-epinuapapuanoate (**16**) found to be inactive against this cell line (L5178Y) (Table 3.18).

4.4.4.2. Structure activity relationship of norterpene cyclic peroxides

According to the result of the cytotoxicity test of the isolated terpene peroxides from the sponge *Diacarnus erythraeanus* against the lymphoma cell line L5178Y (Table 3.18), it is suggested that the insertion of a cyclohexane moiety as in [sigmosceptrellin-B (2a) (**14**)] increases the activity of these compounds against the lymphoma cell line L5178Y. Furthermore, the replacement of an ester group by a hydroxyl moiety as in [sigmosceptrellin-B (2) (**13**)] increases the activity of these compounds against the lymphoma cell line L5178Y. In addition, the insertion of a prenyl unit in the central portion of the molecule makes norsesterpene cyclic peroxides more active than their norditerpene cyclic congeners (Benet *et al*., 1996; D´Ambrosio *et al*., 1997) as in (-)-muqubilin A (**12**). The oxidative opening of the

cyclohexene ring as in [(+)-muqubilone A (**11**)] decreases the cytotoxic activity against the lymphoma cell line L5178Y. Moreover, the difference of the cytotoxicity between (+)-methyl-2-epinuapapuanoate (**15**) and (-)-methyl-2-epinuapapuanoate (**16**) against this cell line (L5178Y) explored the importance of the configuration at C-3, and C-6 (Figure 4.3).

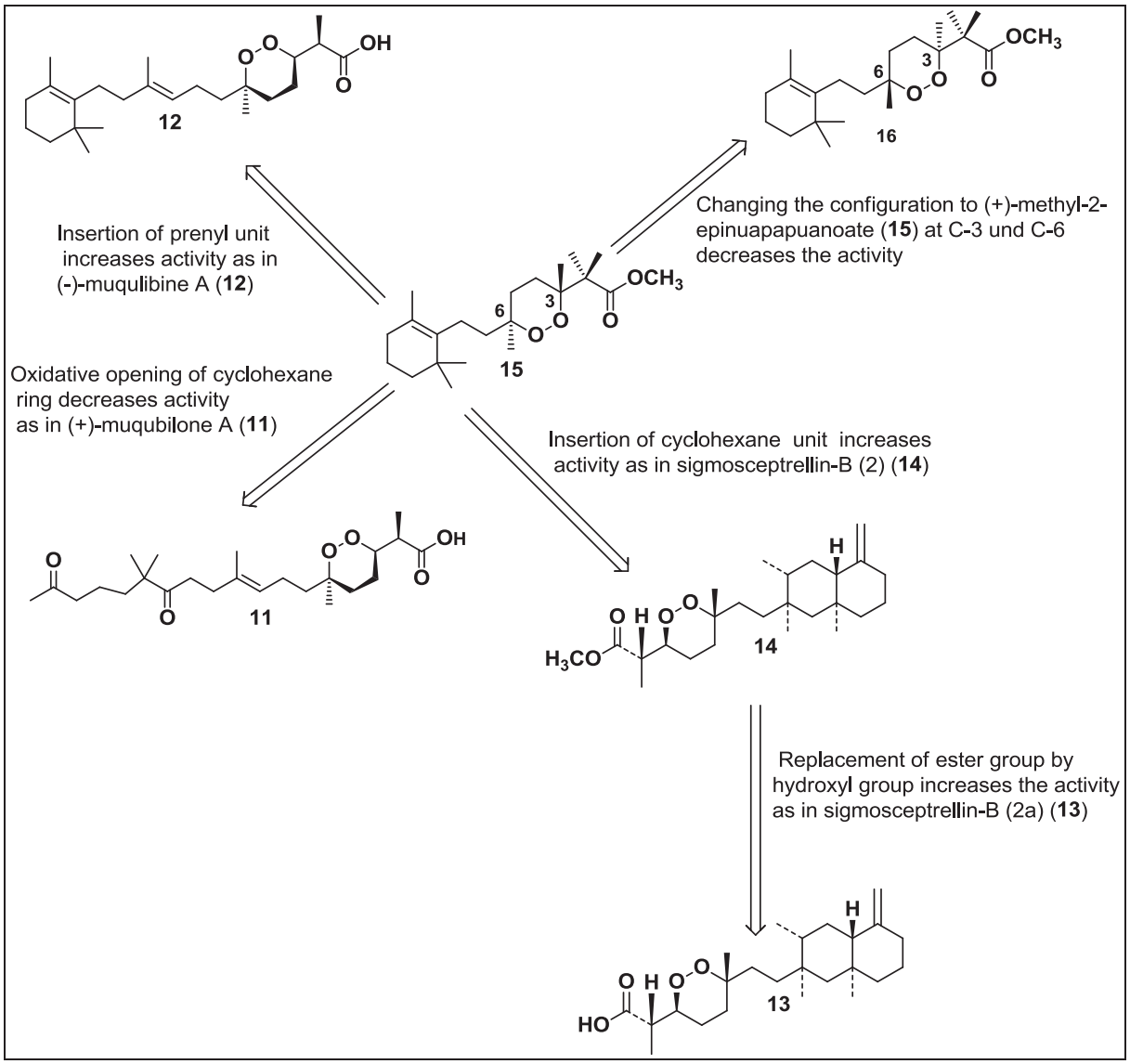


Figure 4.3: Proposed structure activity relationship of norterpene cyclic peroxides against the lymphoma cell line L5178Y.

4.4.4.3. Proposed biosynthesis of norterpene cyclic peroxides

A plausible biosynthetic pathway of norterpene cyclic peroxide is described in (Figure 4.4). (Capon *et al*., 1997) proposed that saturated cyclic peroxides could be formed by concerted oxygen addition to the (*E*, *E*)-diene followed by reduction of the resulting unsaturated peroxide intermediate (Figure 4.5).

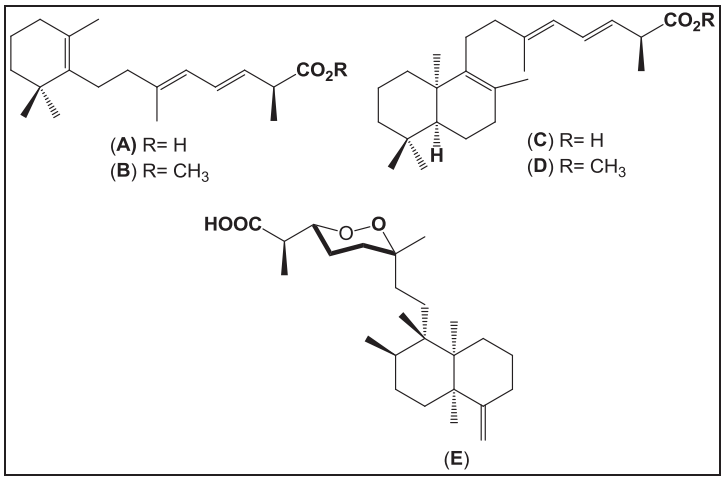


Figure 4.4: Proposed biosynthetic precursors for noterpene cylic peroxides (Capon *et al*., 1997).

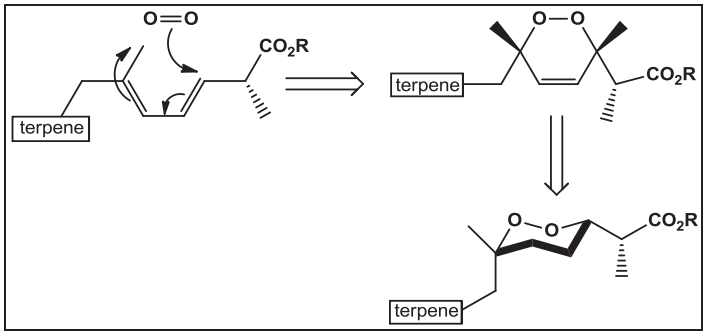


Figure 4.5: Postulated biosynthesis of noterpene cyclic peroxides by oxygen addition to noterpene diene precursors (Capon *et al*., 1997).

4.5. Secondary metabolites isolated from sponge from Ambon in Indonesia

4.5.1. Secondary metabolites from *Acanthostrongylophora ingens*

Acanthostrongylophora ingens was previously reported to contain manzamine alkaloids similar to other members of the family Petrosiidae (*Haliclona* sp. and *Petrosia* sp.) (Radwan *et al*., 2012). In the present study, investigation of the methanolic extract of this sponge yielded one new manzamine derivative in addition to nine known compounds, seven of them are manzamine derivatives. These compounds are nakadomarine A (**17**), ircinal E (**18**) (new compound), manzamine A (**19**), 8-hydroxymanzamine A (**20**), manzamine F (**21**), deoxymanzamine X (**22**), manzamine A *N*-oxide (**23**), 3,4-dihydromanzamine A *N*-Oxide (**24**), in addition to 7-bromoindole-3-carbaldehyde (**25**), and 4-hydroxybenzaldehyde (**26**).

4.5.1.1. Bioactivity of manzamines

Manzamine derivatives possess various biological activities, such as proteasomal inhibitory activity (El-Desoky *et al*., 2014), intracellular proteolytic complex (Furusato *et al*., 2014), cytotoxic (Sakai *et al*., 1986), antimicrobial (Rao *et al*., 2004), antimalarial (Radwan *et al*., 2012), antiviral, antineuroinflammatory (Yousaf *et al*., 2004), antiatherosclerotic (Eguchi *et al*., 2013), and insecticidal effects (Edrada *et al*., 1996). Compounds **19**, **20**, **21**, **22**, **23**, and **24** showed strong activity against the lymphoma cell line L5178Y with EC_{50} values of 3.29, 301, 4.07, 3.19, 2.84, and 2.84 μM, respectively, while **18** showed a moderate activity against the lymphoma cell line L5178Y. However, **17**, **25**, and **26** found to be inactive against the same cell line (L5178Y) (Table 3.28).

4.5.1.2. Structure activity relationship of manzamines

According to the result of the cytotoxic activities of the isolated manzamines from the sponge *Acanthostrongylophora ingens* against the lymphoma cell line L5178Y (Table 3.28), it is

suggested that the presence of a *β*-carboline moiety increases the cytotoxic activity against the lymphoma cell line L5178Y as in [manzamine A (**19**)], while the removal of the *β*-carboline unit decreases the activity against this cell line (L5178Y) as in [ircinal E (**18**)] and [nakadomarin A (**17**)]. The insertion of additional oxygens to manzamine A at C-8 doesn't effect on the cytotoxic activity against this cell line (L5178Y) as in [8-hydroxymanzamine A (**20**)], while the insertion of a ketone group at C-31 and the removal of the double bond between C-32 and C-33 perhaps decreases the activity against the lymphoma cell line L5178Y as in [manzamine F (**21**)] (Figure 4.6). These results suggest the importance of *β*-carboline moiety (**9**-**24**) in the enhancement of cytotoxicity of manzamines.

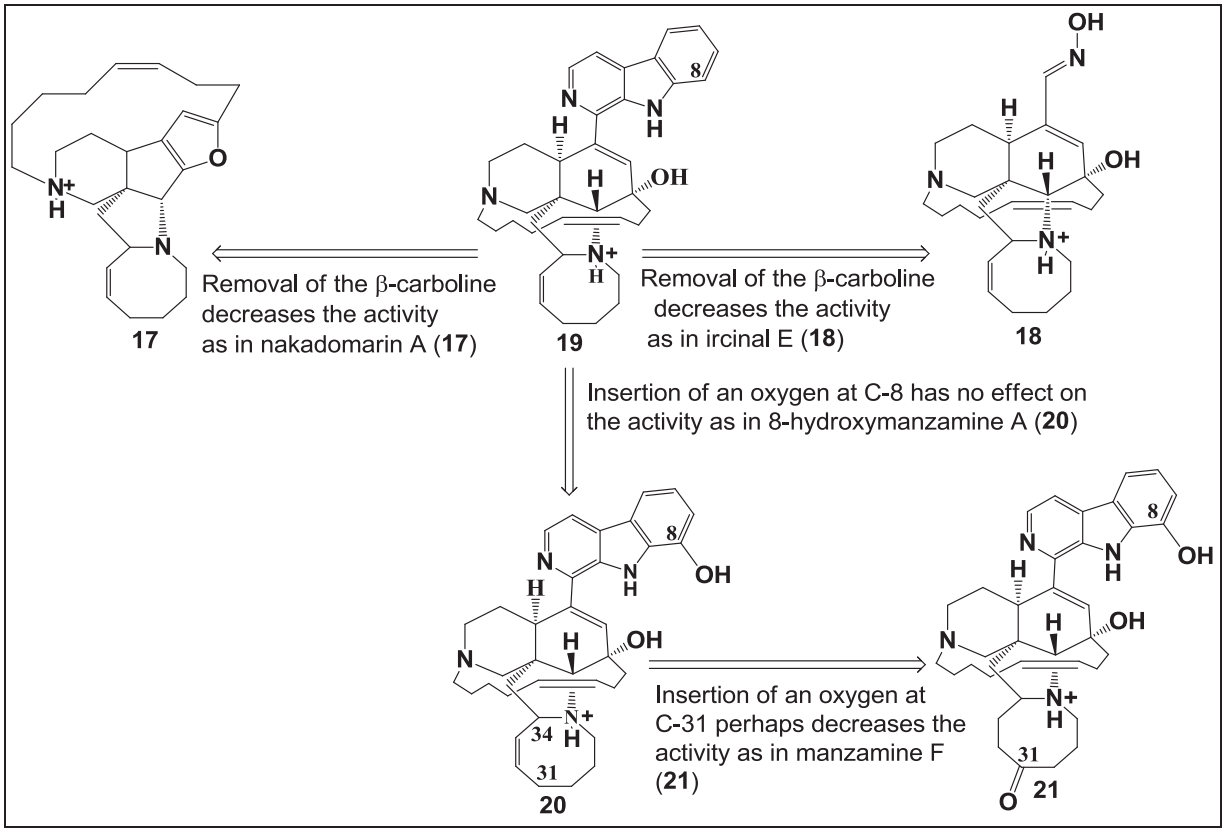


Figure 4.6: Proposed structure activity relationships of manzamines against the lymphoma cell line L5178Y.

5. Conclusion:

Sponges are among the most important groups of the marine animal and have played a vital role as sources of many biopharmaceuticals during the last decade. Habitats like coral reefs which are distinguished by intense competition and feeding pressure from carnivorous fishes are known and lead to the highest concentration of toxic or antioxidant sponge-metabolites.

As described, sponges are highly diverse and well adapted organisms and they can manage to survive longer than any other metazoan. Associated microorganisms comprise up to 40% of the sponge volume and can contribute significantly to the host metabolism (e.g. photosynthesis or nitrogen fixation). Therefore, there is a high probability to find secondary metabolites from sponges and sponge-association microorganisms.

In the present study, the sponges which were chemically investigated were collected from two locations; five sponges were collected from Gulf of Aqaba in Jordan, and one from Ambon in Indonesia. Different chromatographic techniques were applied to purify the extracts of these sponges in order to isolate their secondary metabolites. Then, the structures of the isolated metabolites were elucidated using different mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques. Finally, these metabolites were subjected to assessments for biological cytotoxic activity using MTT assay.

1. Secondary metabolites from sponges of the Gulf of Aqaba in Jordan.

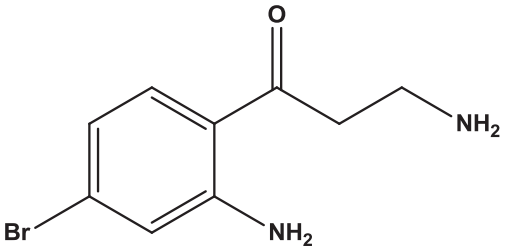
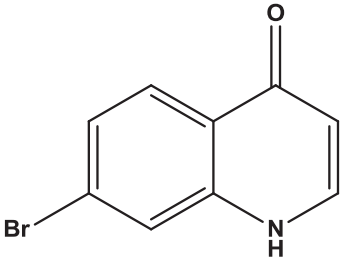
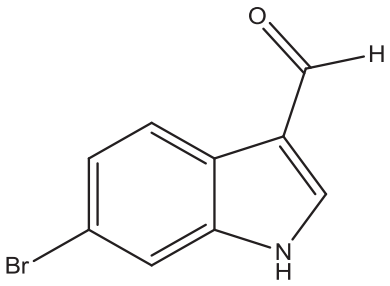
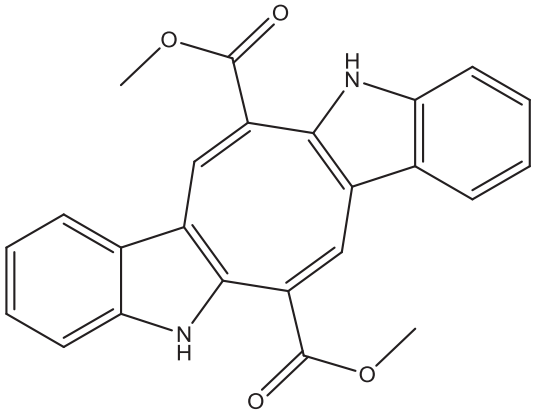
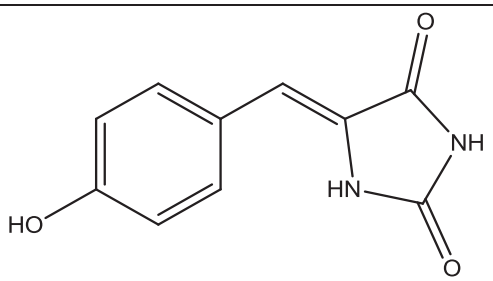
Sixteen compounds were isolated from five sponges which were collected from the Gulf of Aqaba; including two macrolide compounds which are latrunculin B (**10**) and swinholide A (**9**) which were isolated from *Diacarnus ardoukobae* (**MT1**) and *Theonella* sp. (**MT3**), respectively. Six compounds were isolated from the unknown sponge (**MT5**) including one new natural product namely, 3-amino-1-(2-amino-4-bromophenyl) propan-1-one (**1**), in addition to 7-bromoquinolin-4(1*H*)-one (**2**) (which was previously known only as a synthetic

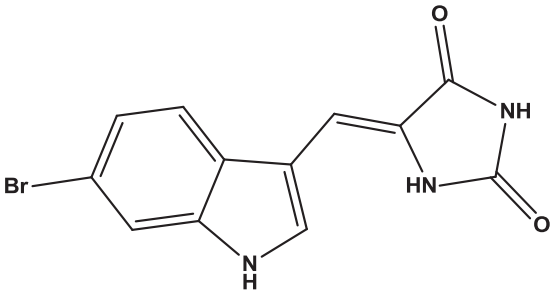
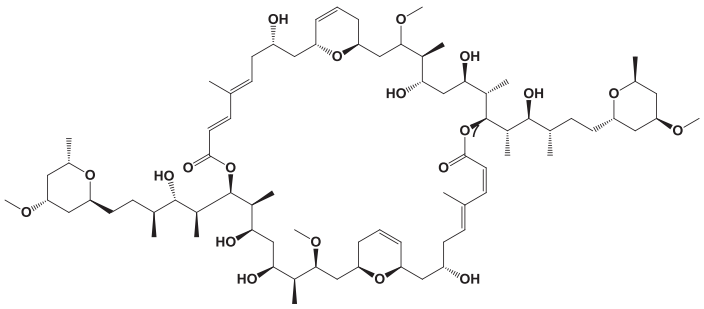
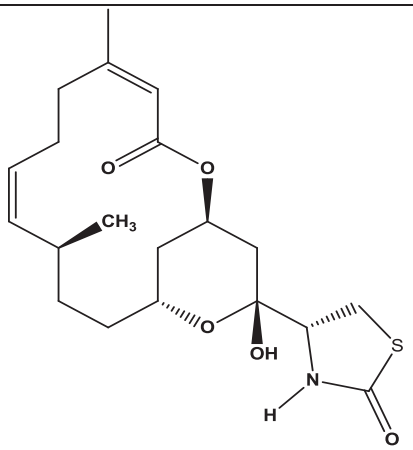
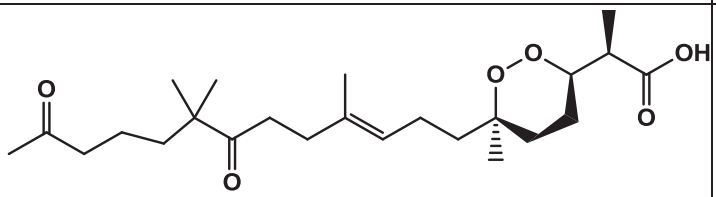
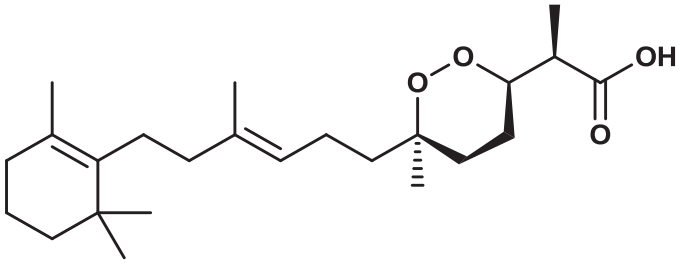
product), together with four known compounds, including 6-bromoindole-3-carbaldehyde (**3**), caulerpin (**4**) (which is described from sponge material here for the first time), (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**5**), and (*Z*)-6-bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**). The repeated isolation of the same compounds from more than one sponge in this study indicates that the sources of these compounds are perhaps sponge-associated microorganisms. Six known terpene peroxides were isolated from *Diacarnus erythraeanus*; four of them showed strong activity against the lymphoma cell line L5178Y which are, (-)-muqubilin A (**12**), sigmosceptrellin-B (2a) (**13**), sigmosceptrellin-B (2) (**14**), and (+)-methyl-2-epinuapapuanoate (**15**). (-)-Methyl-2-epinuapapuanoate (**16**) which is a stereoisomer of **15** proved to be inactive against this cell line which indicates the importance of the configuration for the activity of these compounds against this cell line (L5178Y).

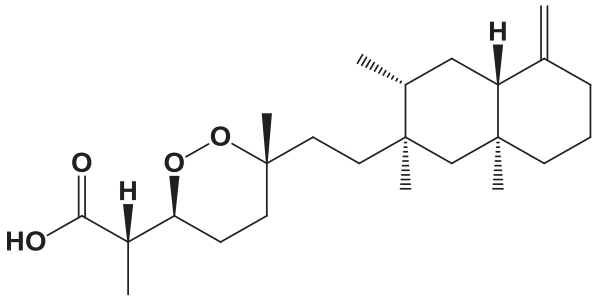
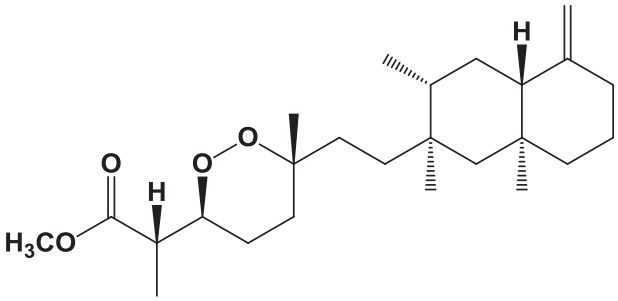
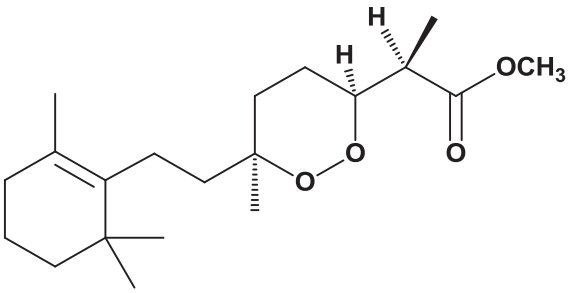
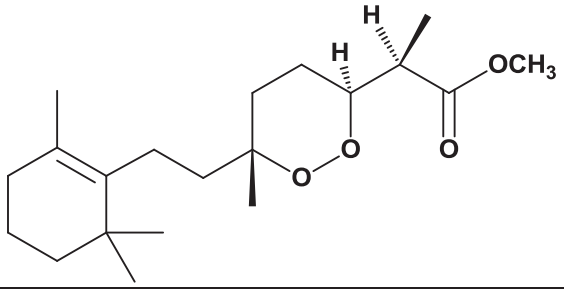
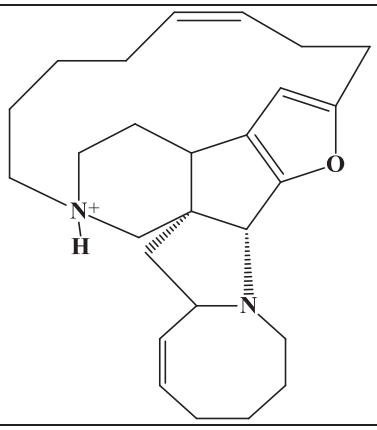
2. Secondary metabolites from sponge from Ambon in Indonesia.

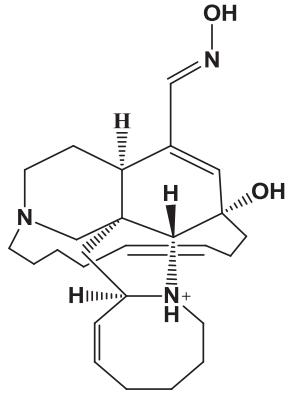
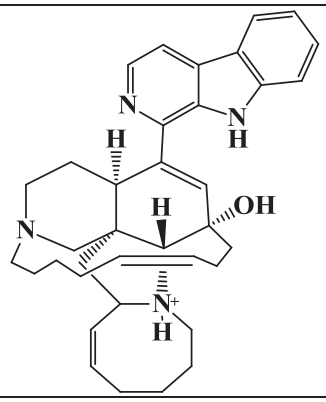
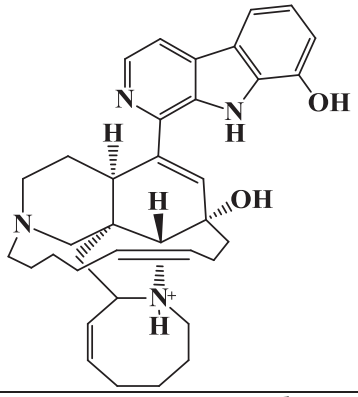
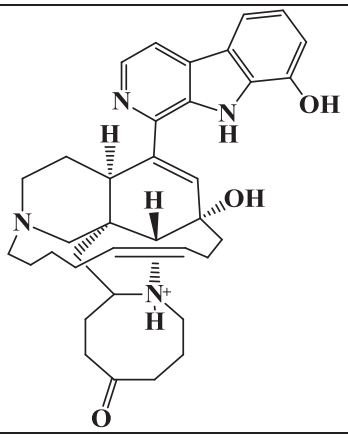
Ten compounds were isolated from the sponge *Acanthostrongylophora ingens* (**FB67**) which was collected from Ambon in Indonesia; including one new manzamine derivative named, ircinal E which showed moderate activity against the cell line L5178Y in addition to seven known manzamine compounds. Six of these compounds proved to be highly active against this cell line (L5178Y) which are, manzamine A (**19**), 8-hydroxymanzamine A (**20**), manzamine F (**21**), deoxymanzamine X (**22**), manzamine A *N*-oxide (**23**), and 3,4-dihydromanzamine A *N*-oxide (**24**). The results of MTT assay for these compounds suggest that the presence of a *β*-carboline moiety, as in **19-24**, plays an important role in the enhancement of cytotoxicity of the manzamines.

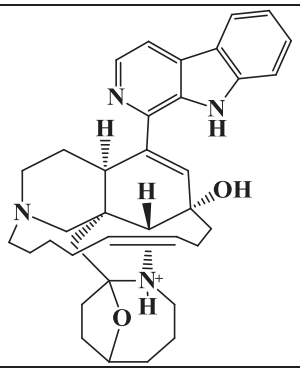
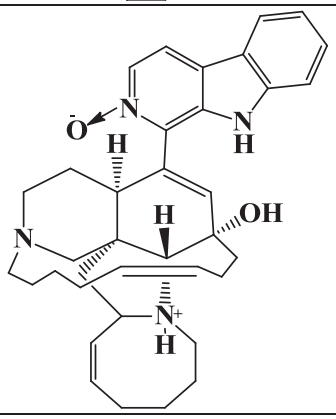
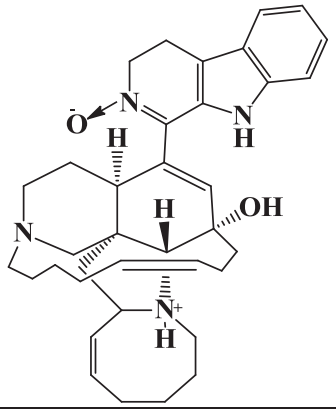
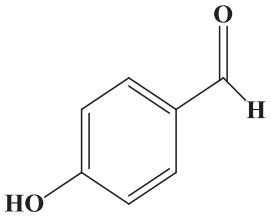
Table 5.1: Summary of the isolated compounds

Compound name	Structure	Source	Comment
3-Amino-1-(2-amino-4-bromophenyl) propan-1-one		The unknown sponge (**MT5**)	New
7-Bromoquinolin-4(1*H*)-one		The unknown sponge (**MT5**)	New as natural product
6-Bromoindole-3-carbaldehyde		The unknown sponge (**MT5**) and *Acanthostrongylophora ingens* (**FB67**)	Known
Caulerpin		The unknown sponge (**MT5**)	Known
(*Z*)-5-(4-Hydroxybenzylidene)-hydantoin		The unknown sponge (**MT5**) and *Hemimycale* sp. (**MT6**)	Known

Compound name	Structure	Source	Comment
(*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin		The unknown sponge (**MT5**) and *Hemimycale* sp. (**MT6**)	Known
Swinholide A		*Theonella* sp. (**MT3**)	Known
Latrunculin B		*Diacarnus ardoukobae* (**MT1**)	Known
(+)-Muqubilone A		*Diacarnus erythraeanus* (**MT8**)	Known
(-)-Muqubilin A		*Diacarnus erythraeanus* (**MT8**)	Known

Compound name	Structure	Source	Comment
Sigmosceptrellin-B (2a)		*Diacarnus erythraeanus* (**MT8**)	Known
Sigmosceptrellin-B (2)		*Diacarnus erythraeanus* (**MT8**)	Known
(+)-Methyl-2-epinuapapuanoate		*Diacarnus erythraeanus* (**MT8**)	Known
(-)-Methyl-2-epinuapapuanoate		*Diacarnus erythraeanus* (**MT8**)	Known
Nakadomarin A		*Acanthostrong-ylophora ingens* (**FB67**)	Known

Compound name	Structure	Source	Comment
Ircinal E		*Acanthostrong-ylophora ingens* (**FB67**)	New
Manzamine A		*Acanthostrong-ylophora ingens* (**FB67**)	Known
8-Hydroxy manzamine A		*Acanthostrong-ylophora ingens* (**FB67**)	Known
Manzamine F		*Acanthostrong-ylophora ingens* (**FB67**)	Known

Compound name	Structure	Source	Comment
Deoxymanzamine X		*Acanthostrongyl-ophora ingens* (**FB67**)	Known
Manzamine A *N*-oxide		*Acanthostrongyl-ophora ingens* (**FB67**)	Known
3,4-Dihydromanz-amine A *N*-oxide		*Acanthostrongyl-ophora ingens* (**FB67**)	Known
4-Hydroxy benzaldehyde		*Acanthostrongyl-ophora ingens* (**FB67**)	Known

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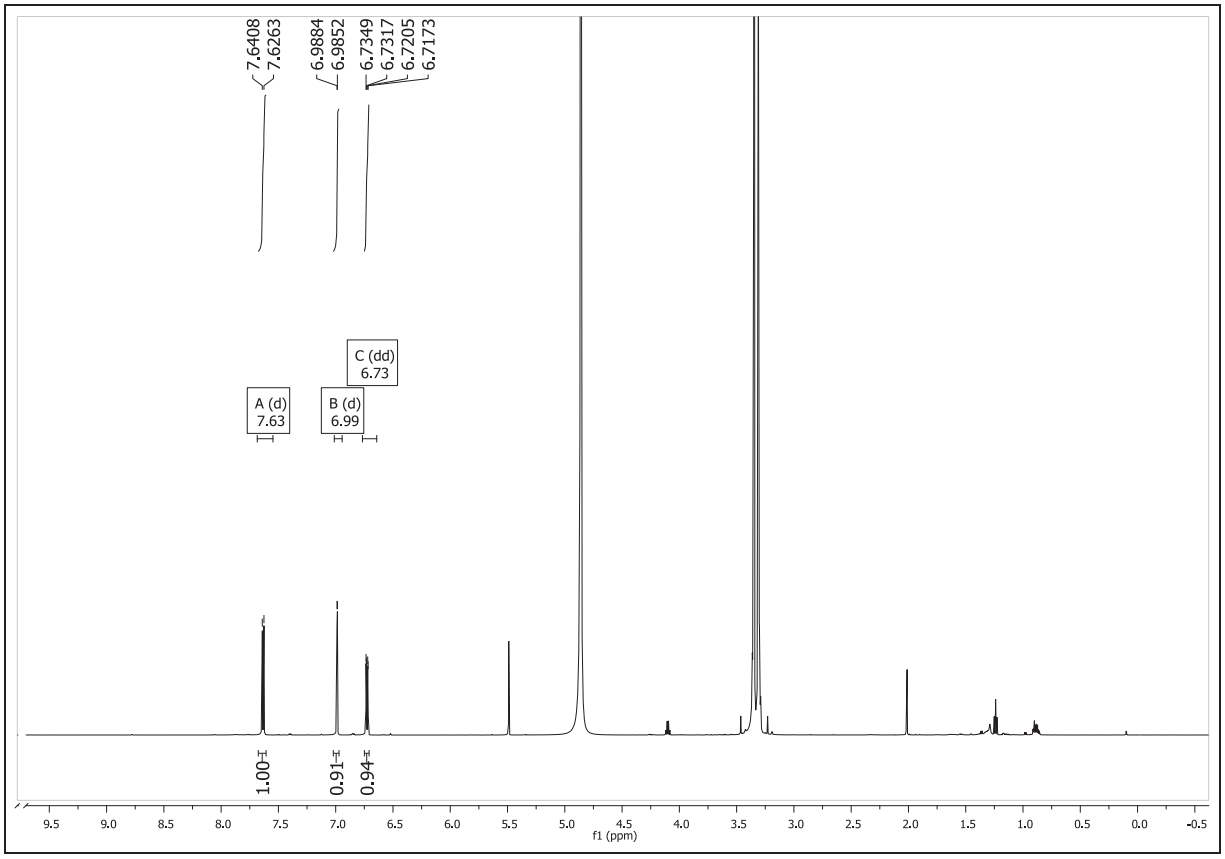
7. List of Abbreviations

$[\alpha]_D$	specific rotation at the sodium D-line
Br	broad signal
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CI	chemical ionization
COSY	correlation spectroscopy
d	doublet
DCM	dichloromethane
dd	doublets of doublet
DEPT	distortionless enhancement bypolarization transfer
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ED	effective dose
EI	electron impact ionization
ESI	electrospray ionization
et al.	*et altera* (and others)
EtOAc	ethyl acetate
eV	electron volt
g	gram
HMBC	heteronuclear multiplebond connectivity
HMQC	heteronuclear multiple quantum coherence
H_2O	water
HPLC	high performance liquid chromatography
HR-MS	high resolution mass spectrometry
Hz	herz
L	liter
LC	liquid chromatography
LC/MS	liquid chromatography-mass spectrometery
m	multiplet

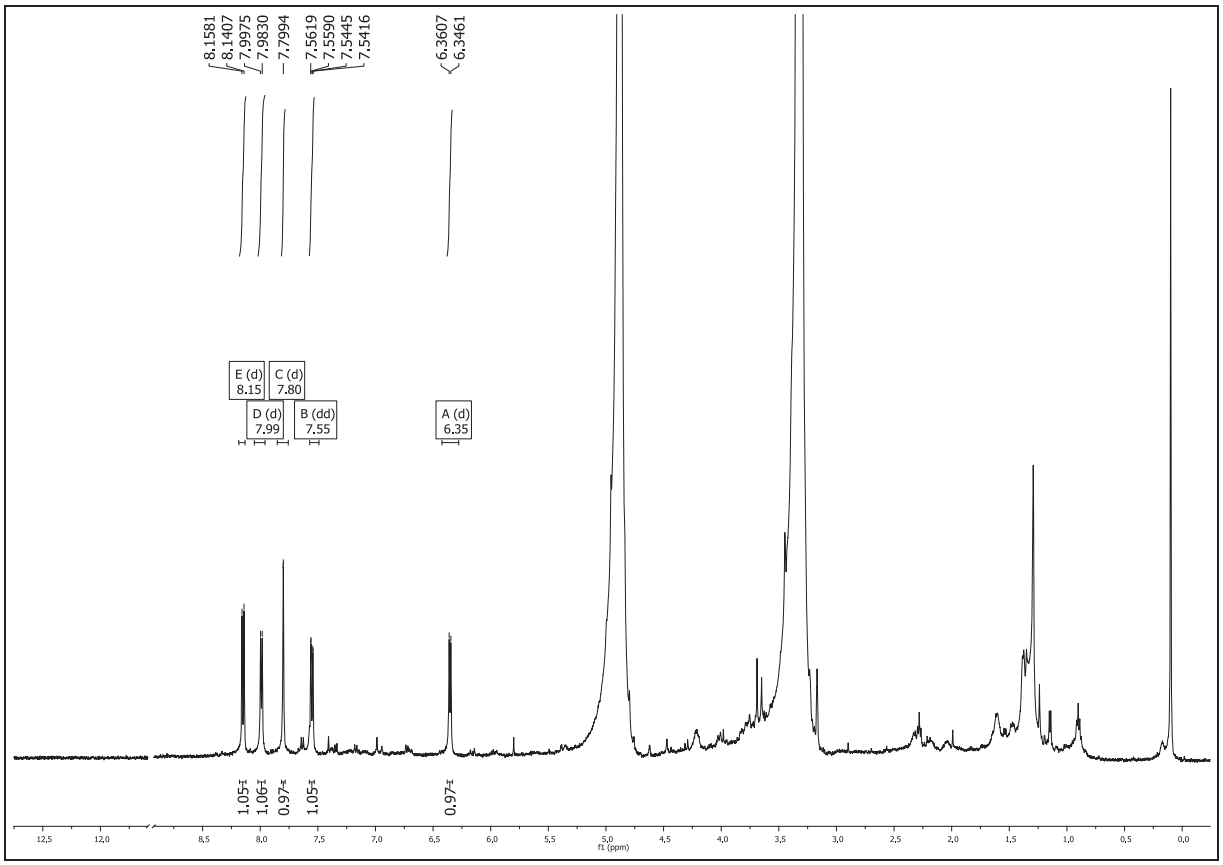
M	molar
MeOD	deuterated methanol
MeOH	methanol
mg	miligram
MHz	mega Herz
min	minute
mL	milliliter
mm	millimeter
MS	mass spectrometry
MTT	microculture tetrazolium assay
m/z	mass per charge
μg	microgram
μL	microliter
μM	micromolar
ng	nanogram
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser and exchange spectroscopy
PCR	polymerase chain reaction
ppm	parts per million
q	quartet
ROESY	rotatingframe Overhauser enhancement spectroscopy
s	singlet
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
UV	ultra-violet
VLC	vacuum liquid chromatography

8. Attachments

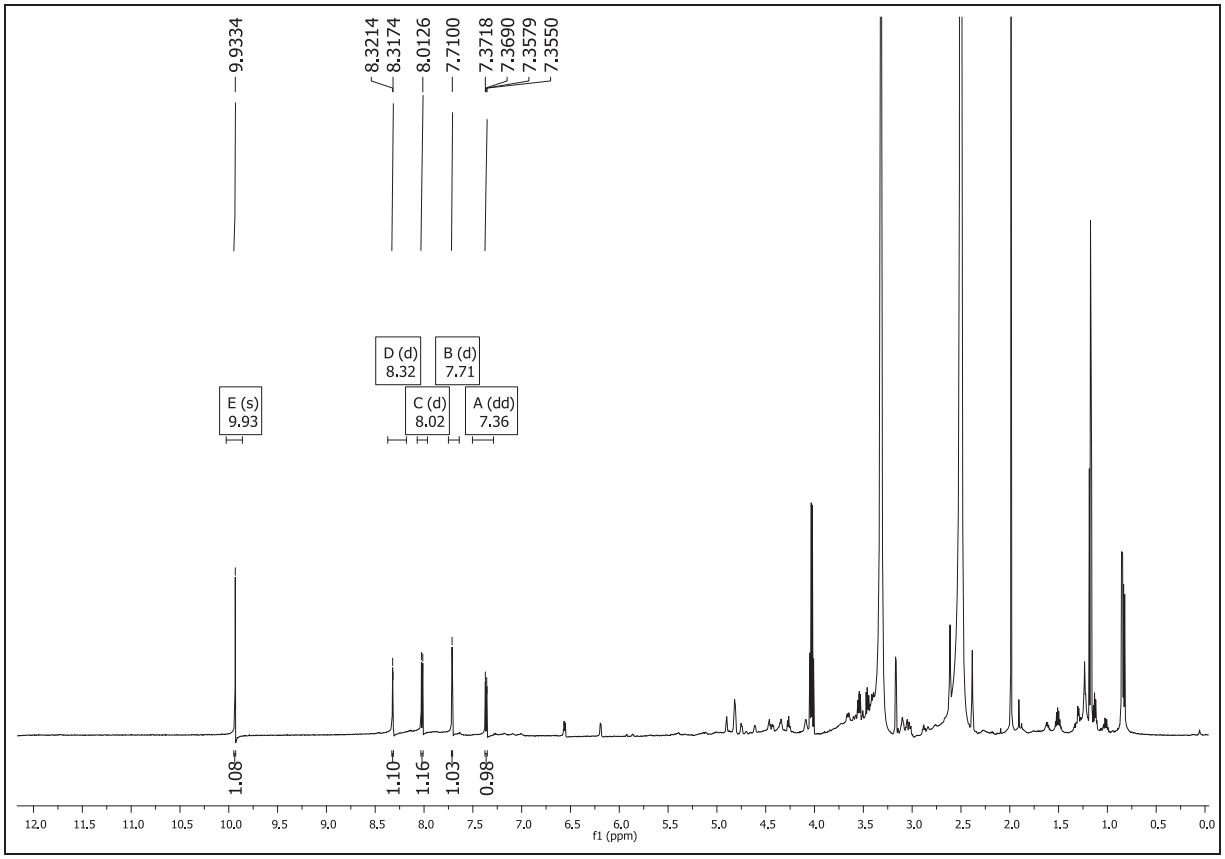
Attacment: The ^{1}H NMR spectrum of 3-Amino-1-(2-amino-4-bromophenyl)propan-1-one (**1**).



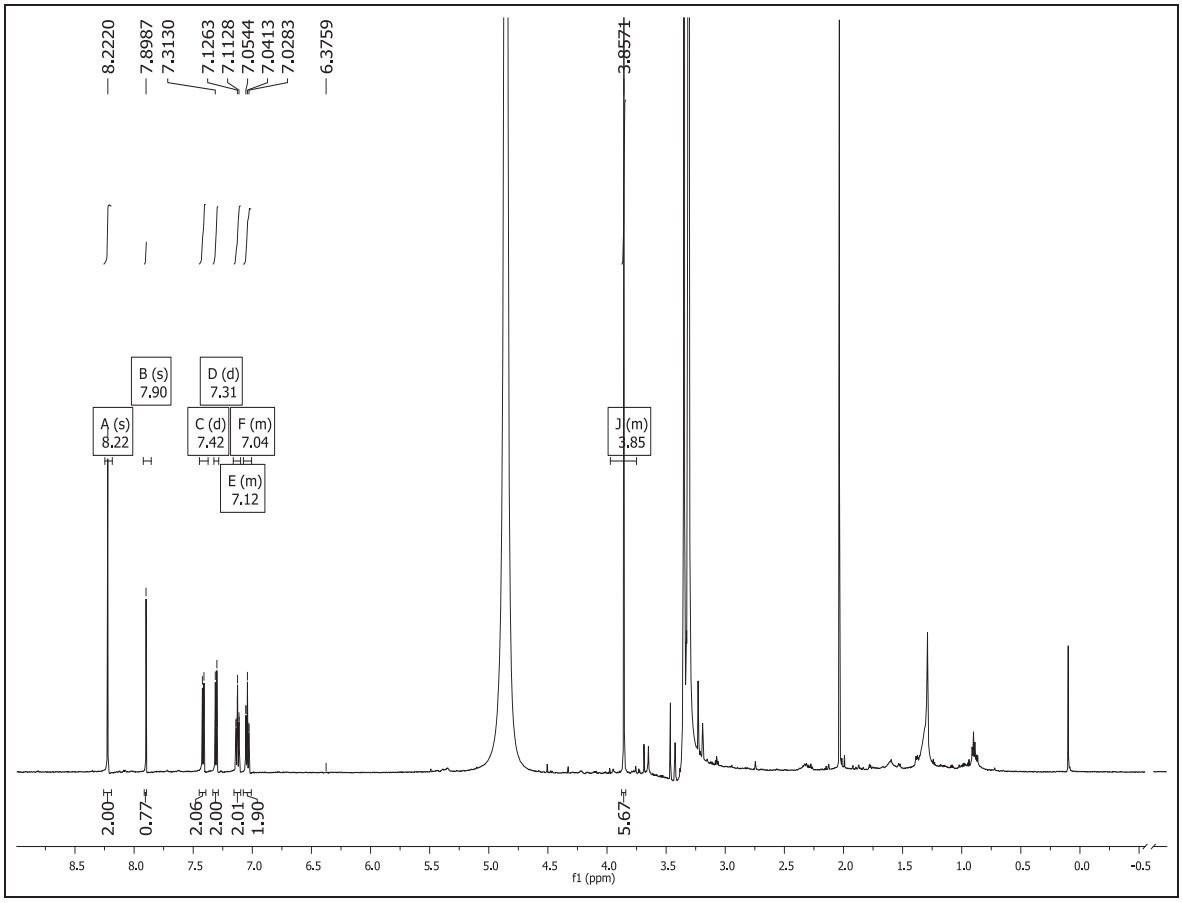
Attacment: The ^{1}H NMR spectrum of 7-Bromoquinolin-4(1*H*)-one (**2**).



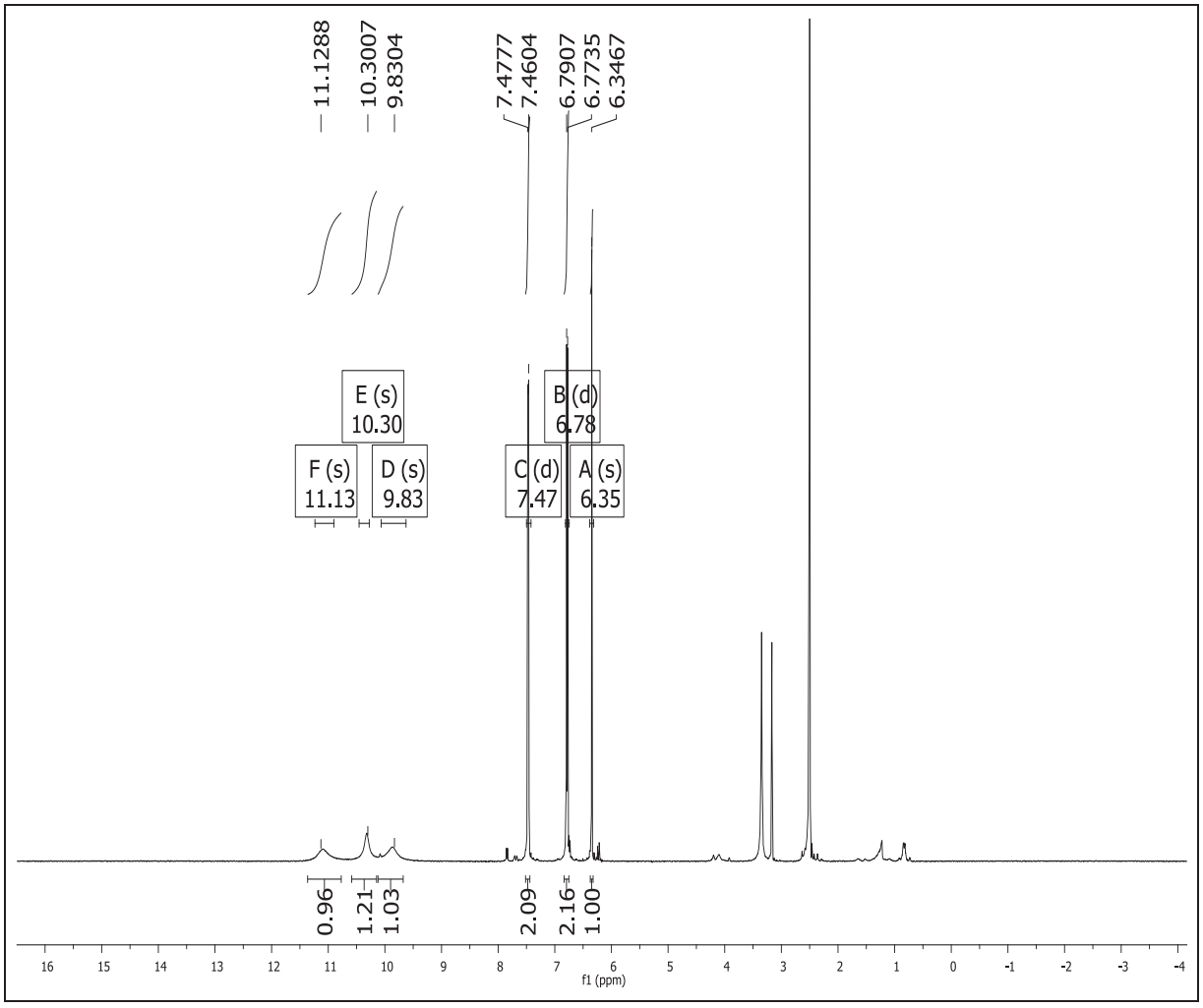
Attacment: The ^{1}H NMR spectrum of 6-Bromoindole-3-carbaldehyde (**3**).



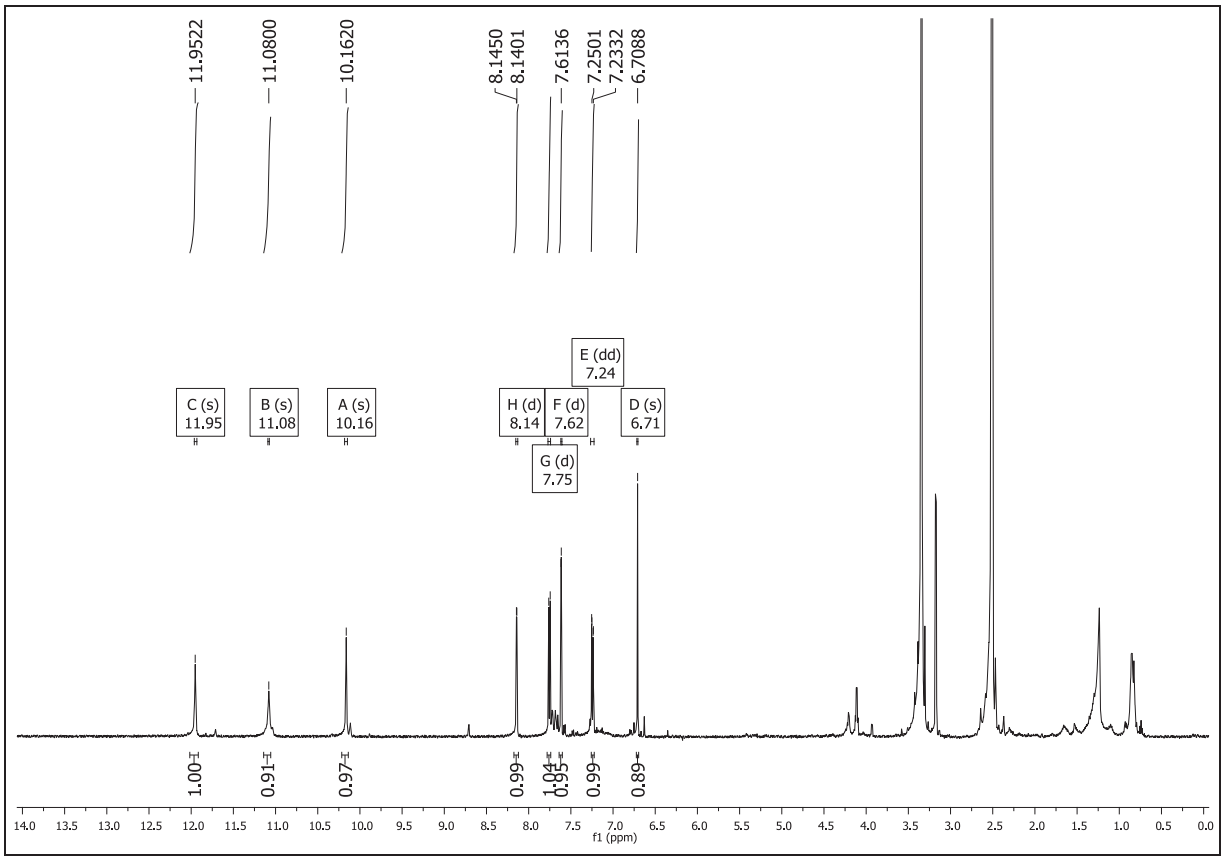
Attacment: The ^{1}H NMR spectrum of Caulerpin (**4**).



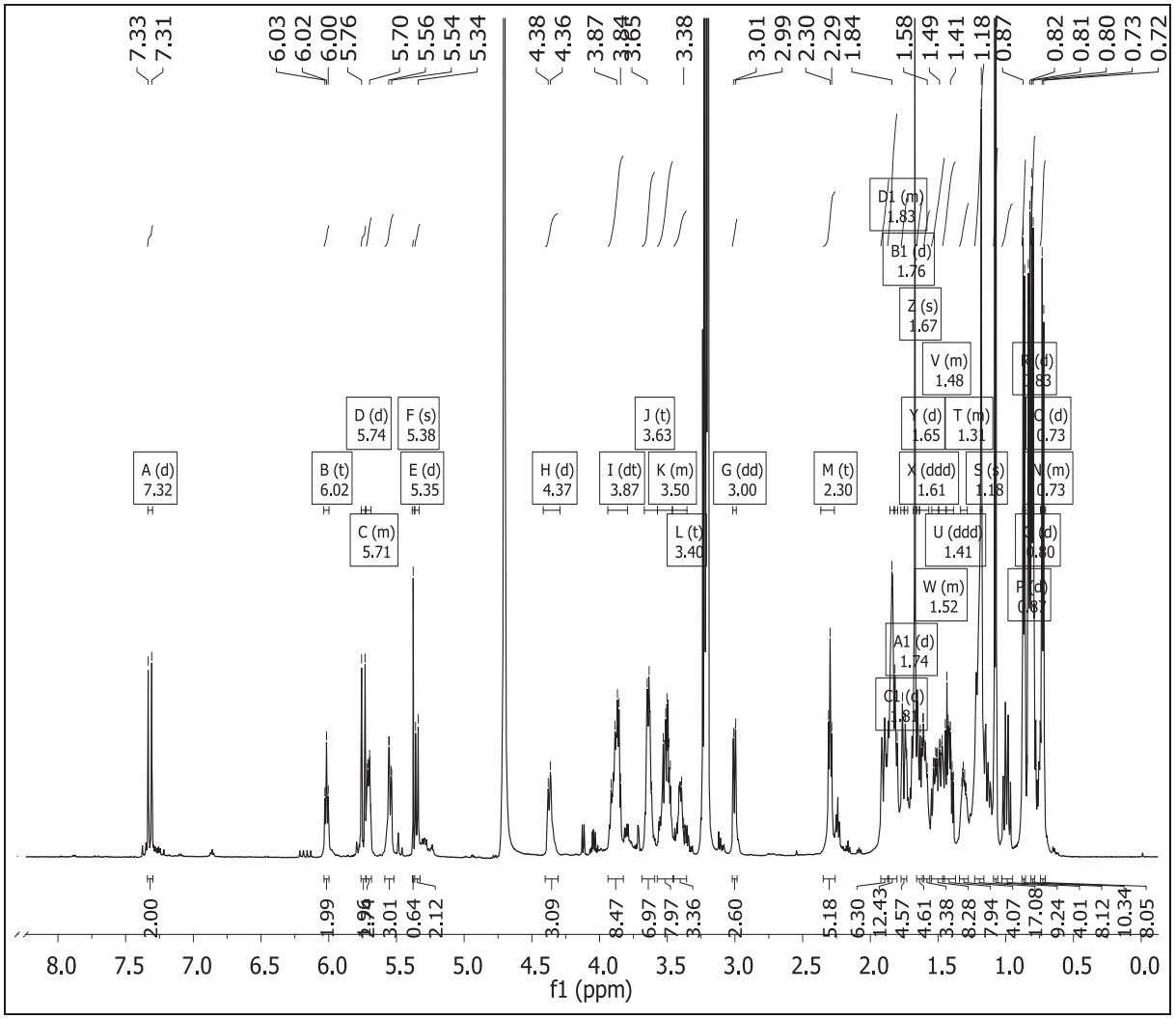
Attacment: The ^{1}H NMR spectrum of (*Z*)-5-(4-Hydroxybenzylidene)-hydantoin (**5**+**7**).



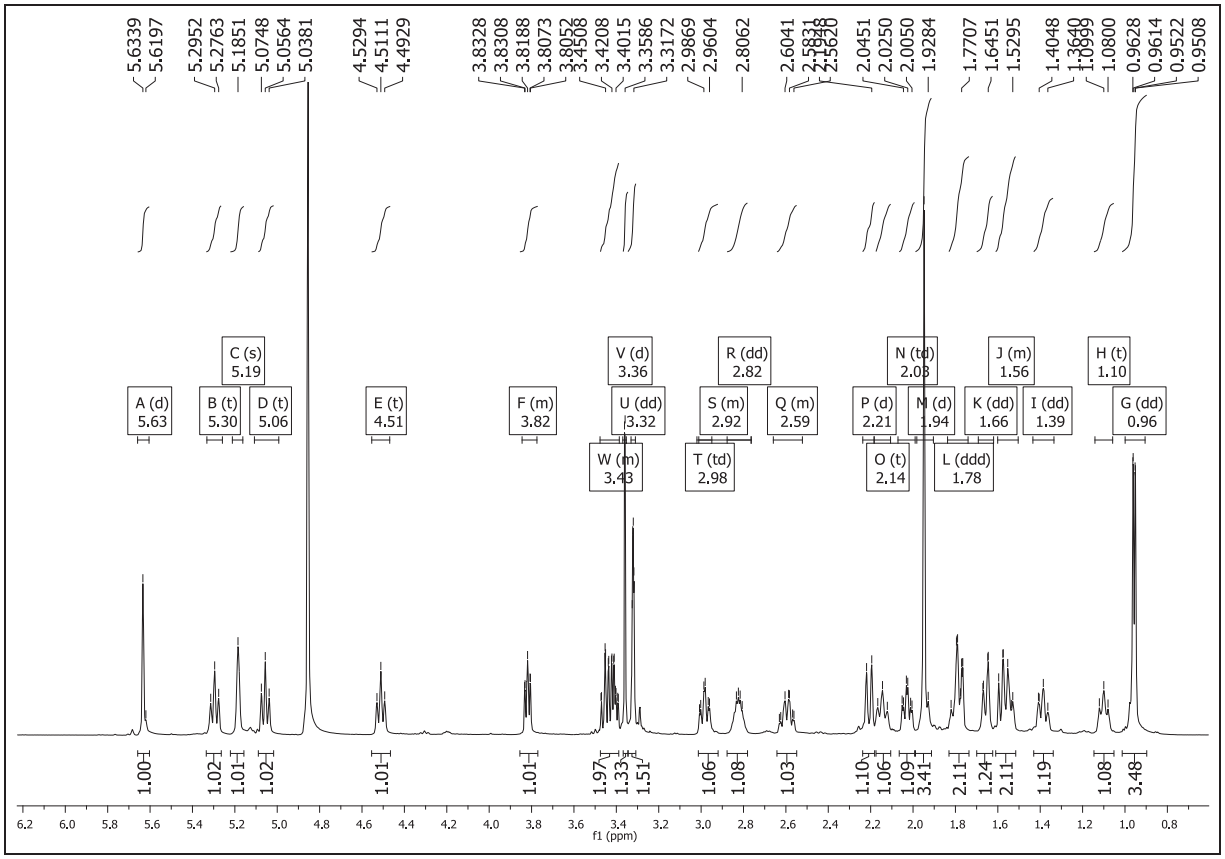
Attacment: The ^{1}H NMR spectrum of (*Z*)-6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin (**6**+**8**).



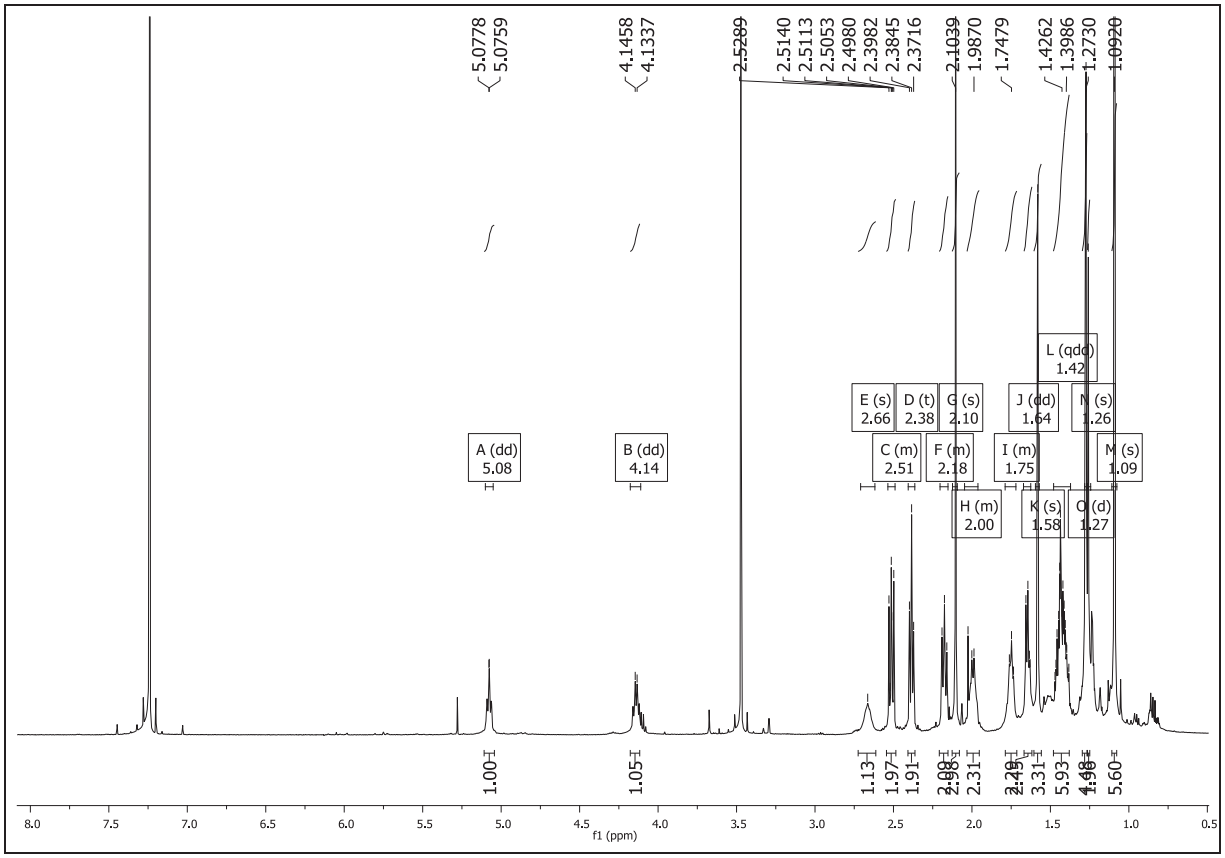
Attacment: The ^{1}H NMR spectrum of Swinholide A (**9**).



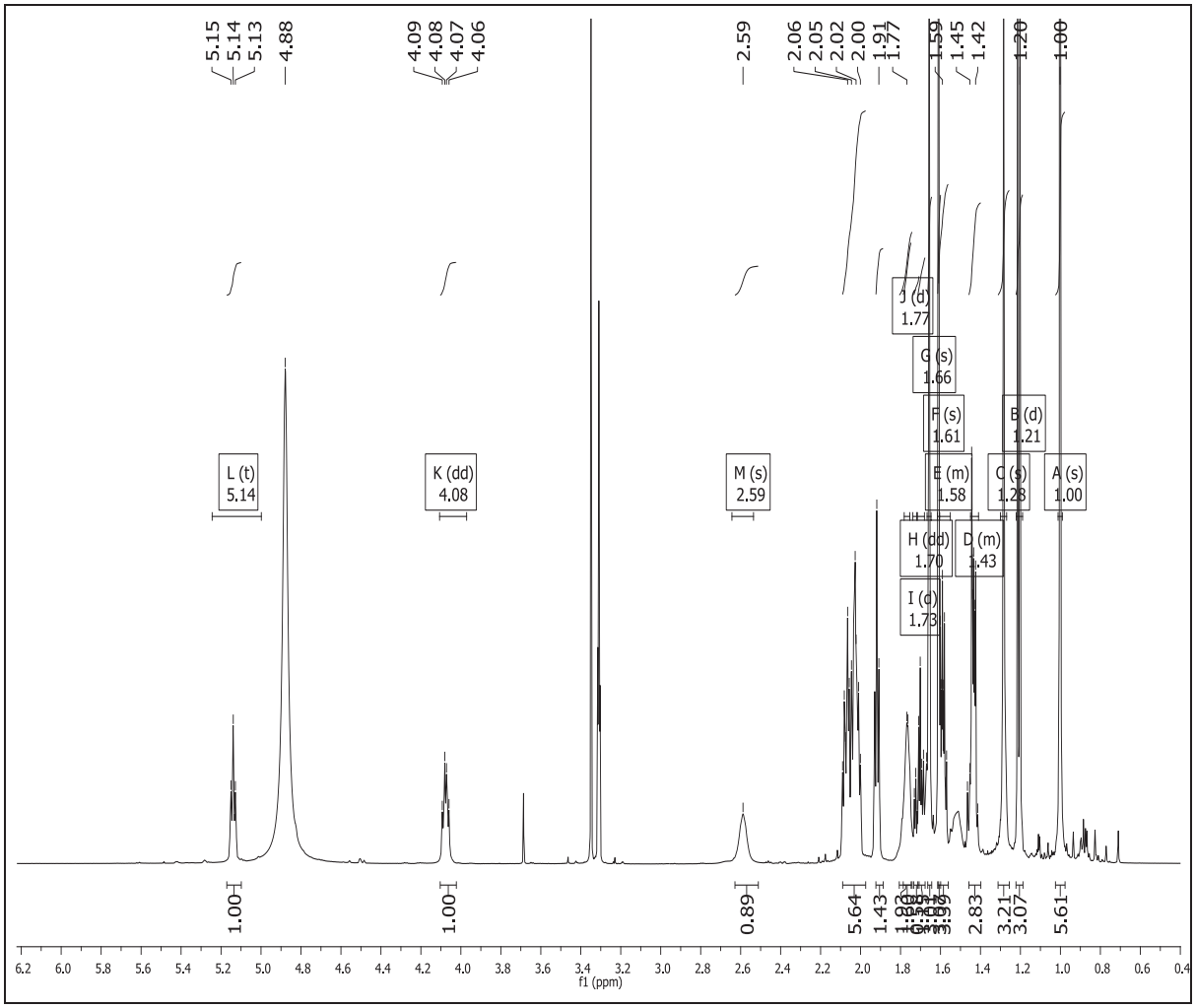
Attacment: The ^{1}H NMR spectrum of Latrunculin B (**10**).



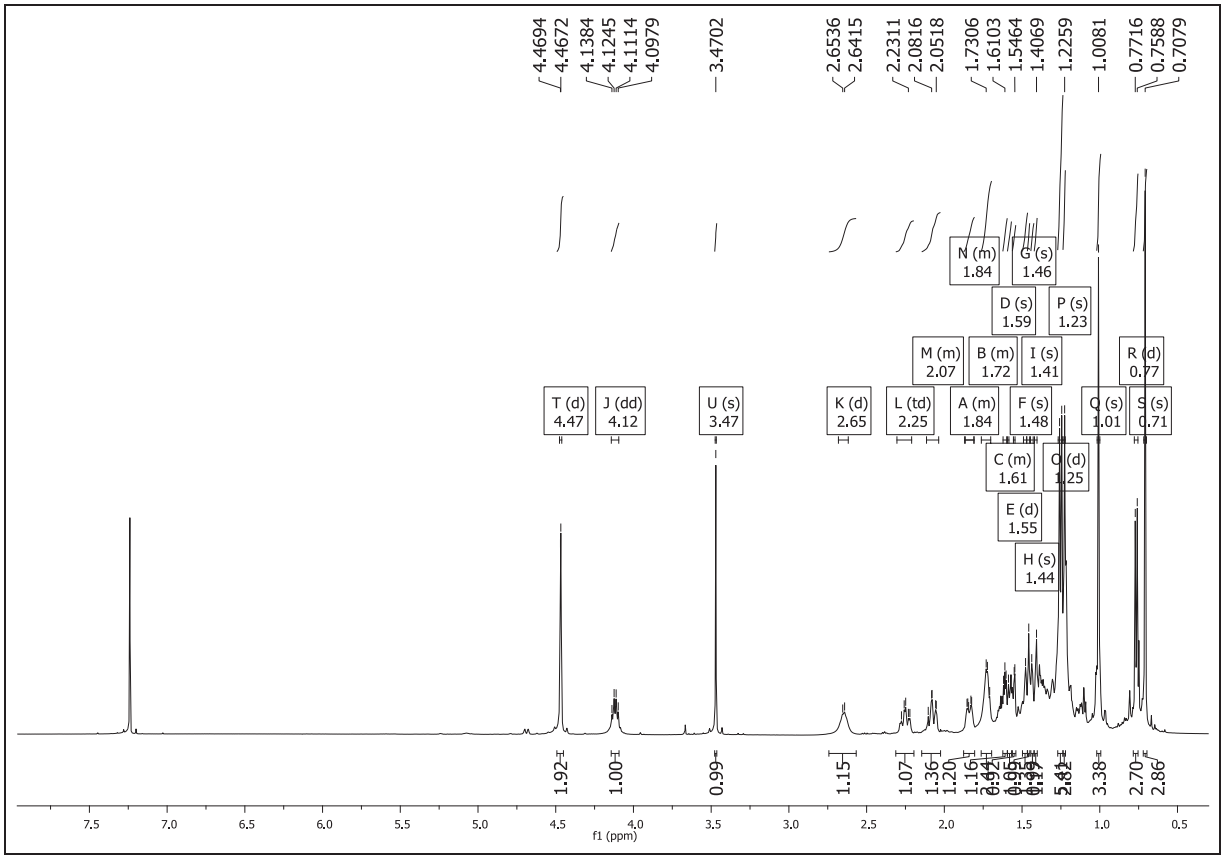
Attacment: The ^{1}H NMR spectrum of (+)-Muqubilone A (**11**).



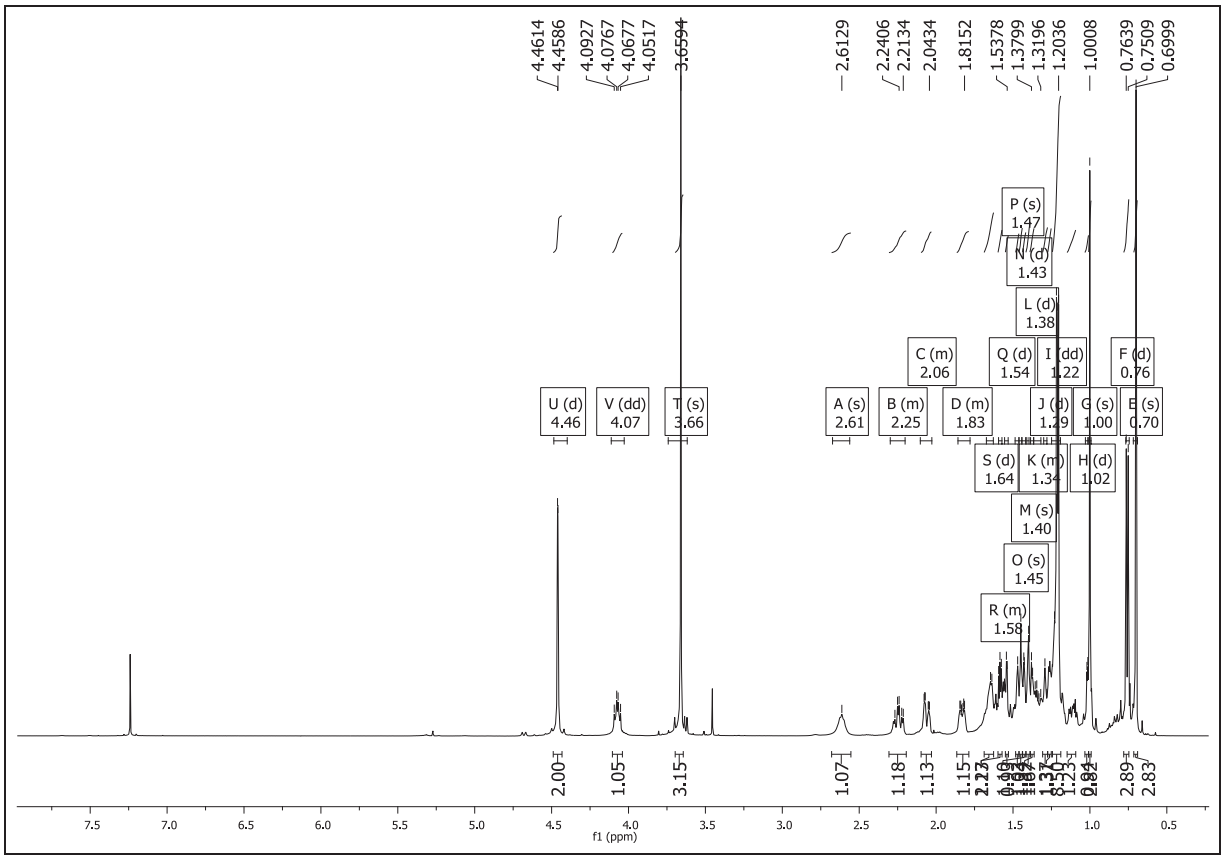
Attacment: The ^{1}H NMR spectrum of (-)-Muqubilin A (**12**).



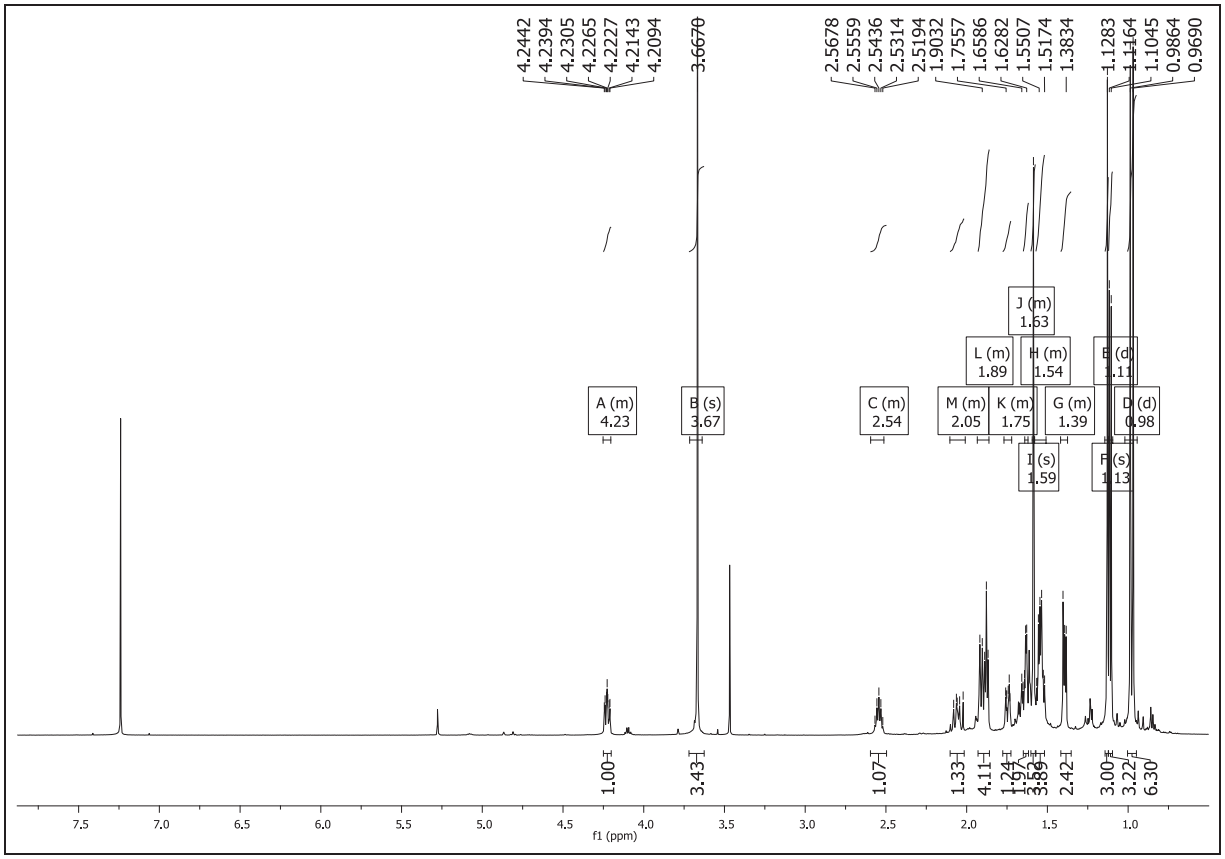
Attacment: The ^{1}H NMR spectrum of Sigmosceptrellin-B (2a) (**13**).



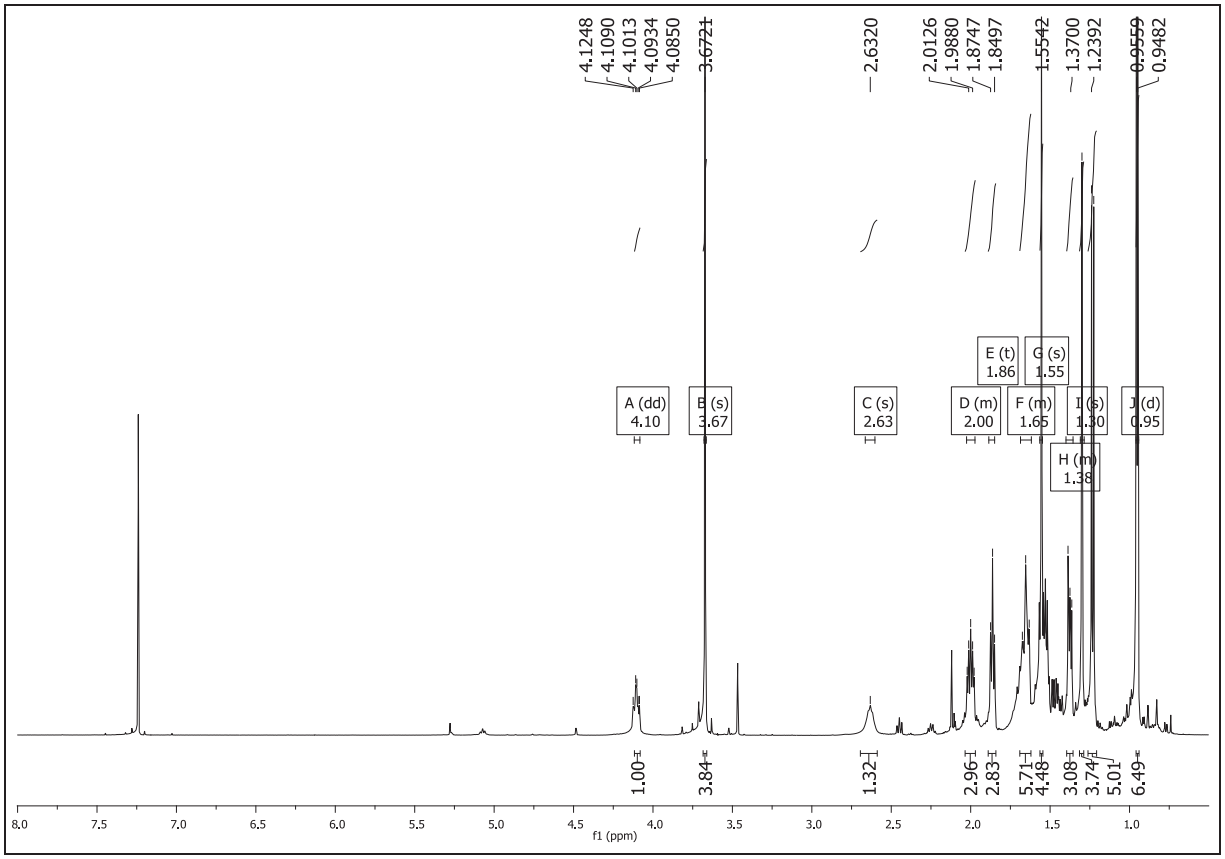
Attacment: The ^{1}H NMR spectrum of Sigmosceptrellin-B (2) (**14**).



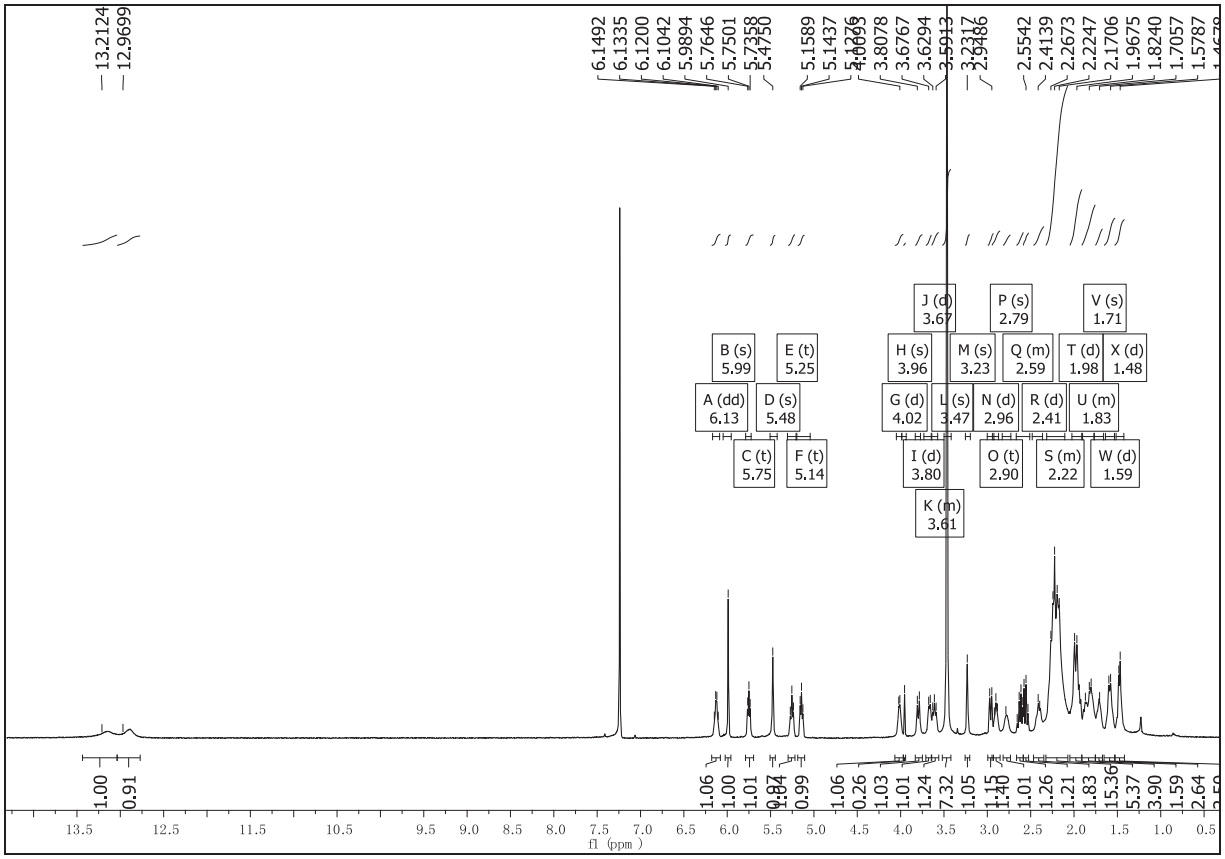
Attacment: The ^{1}H NMR spectrum of (+)-Methyl-2-epinuapapuanoate (**15**).



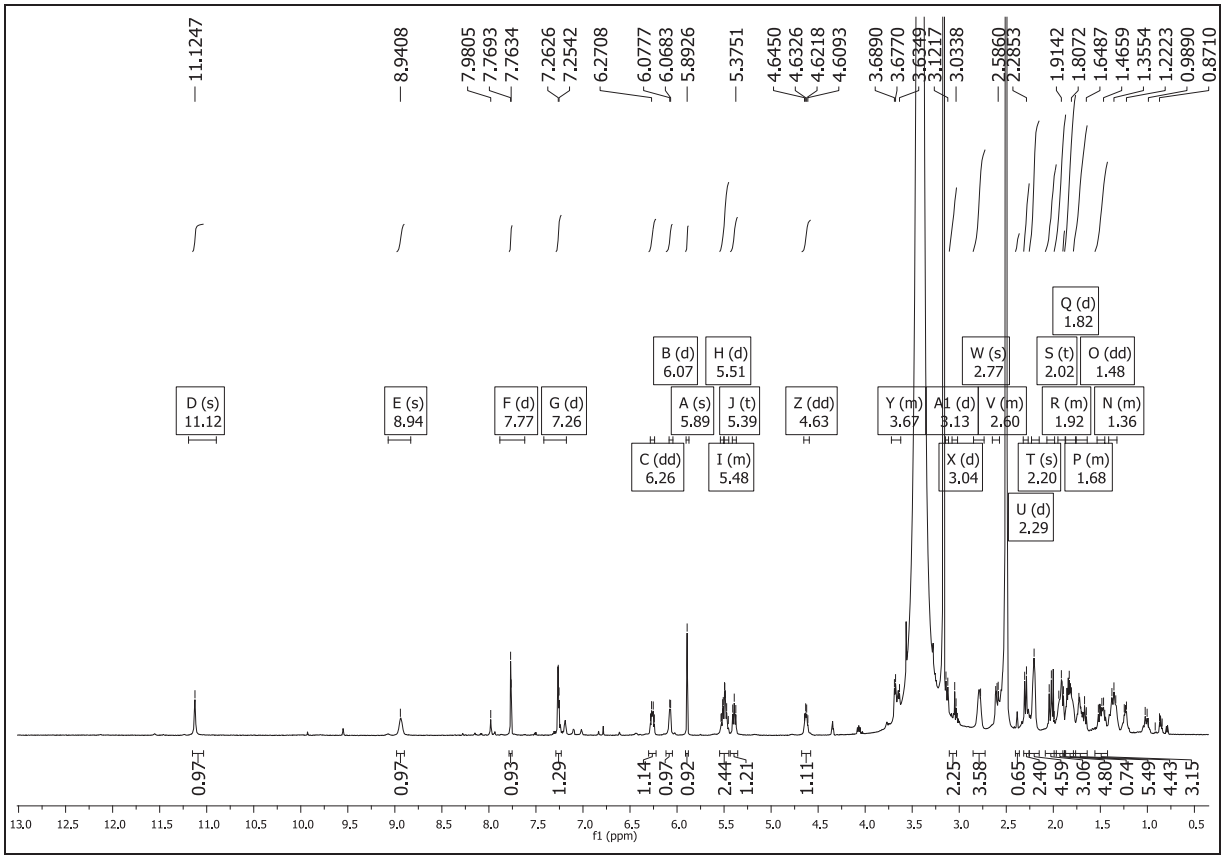
Attacment: The ^{1}H NMR spectrum of (-)-Methyl-2-epinuapapuanoate (**16**).



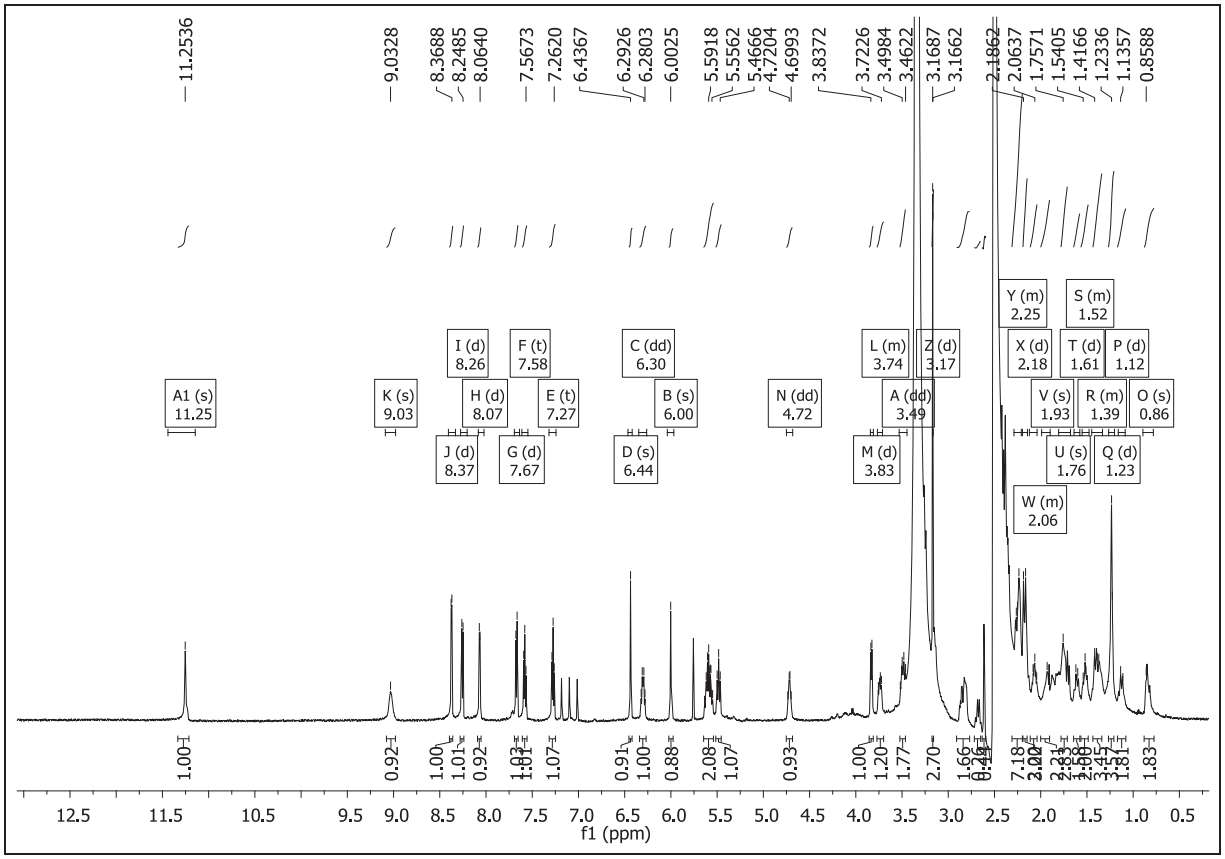
Attacment: The ^{1}H NMR spectrum of Nakadomarin A (**17**).



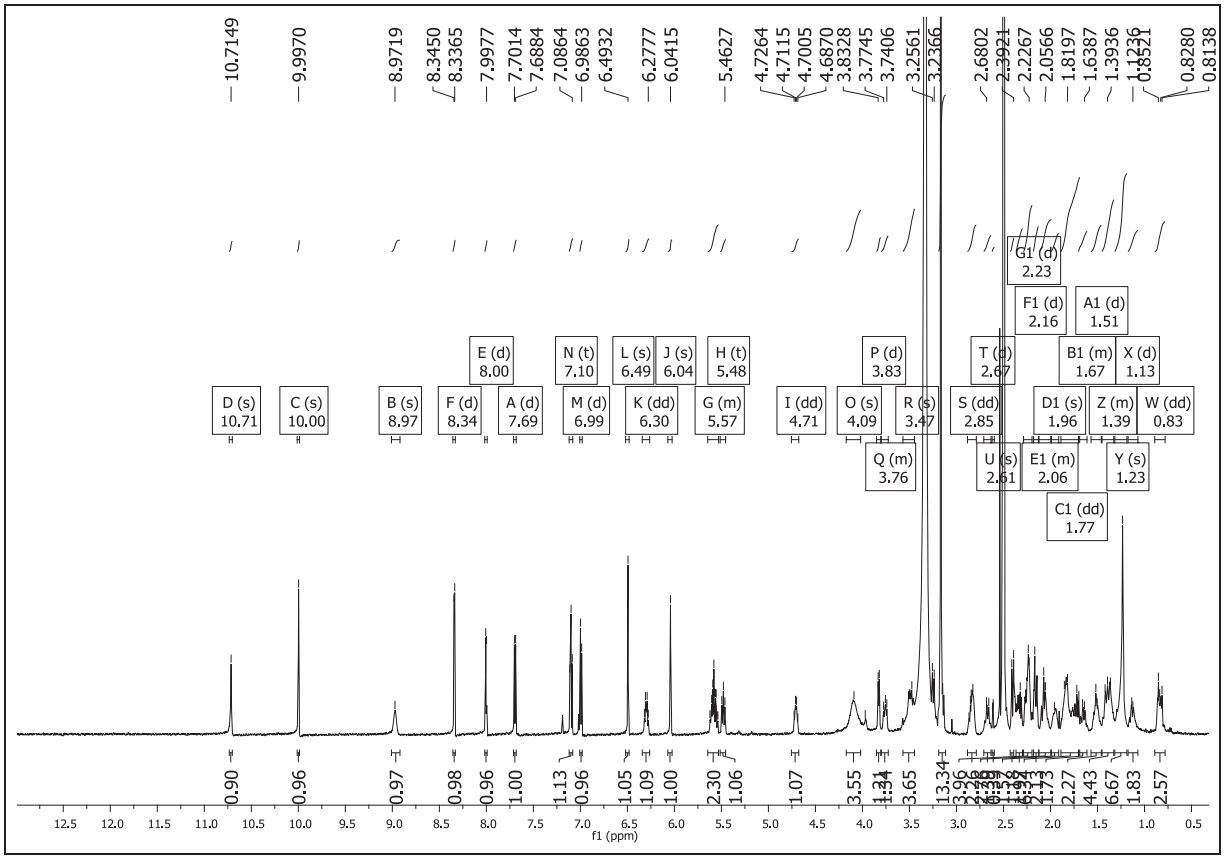
Attacment: The ^{1}H NMR spectrum of Ircinal E (**18**).



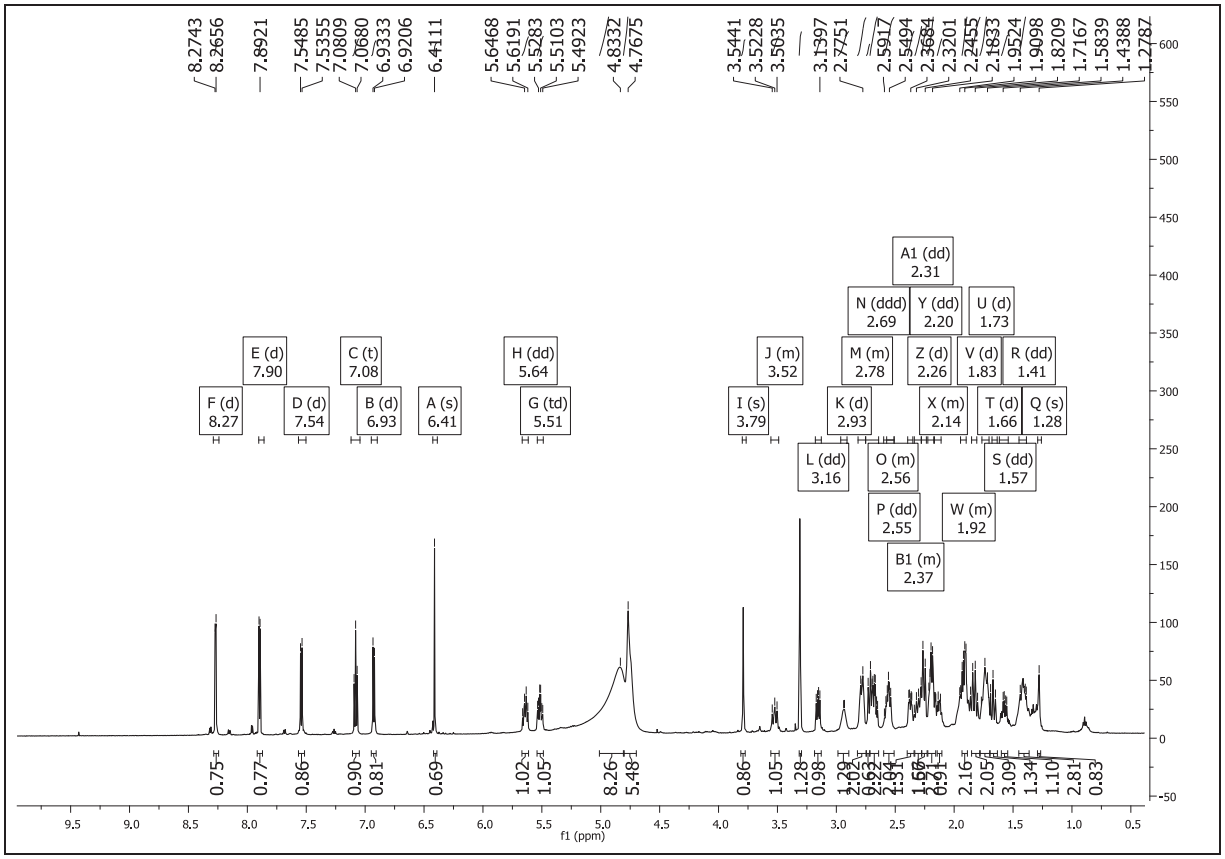
Attacment: The ^{1}H NMR spectrum of Manzamine A (**19**).



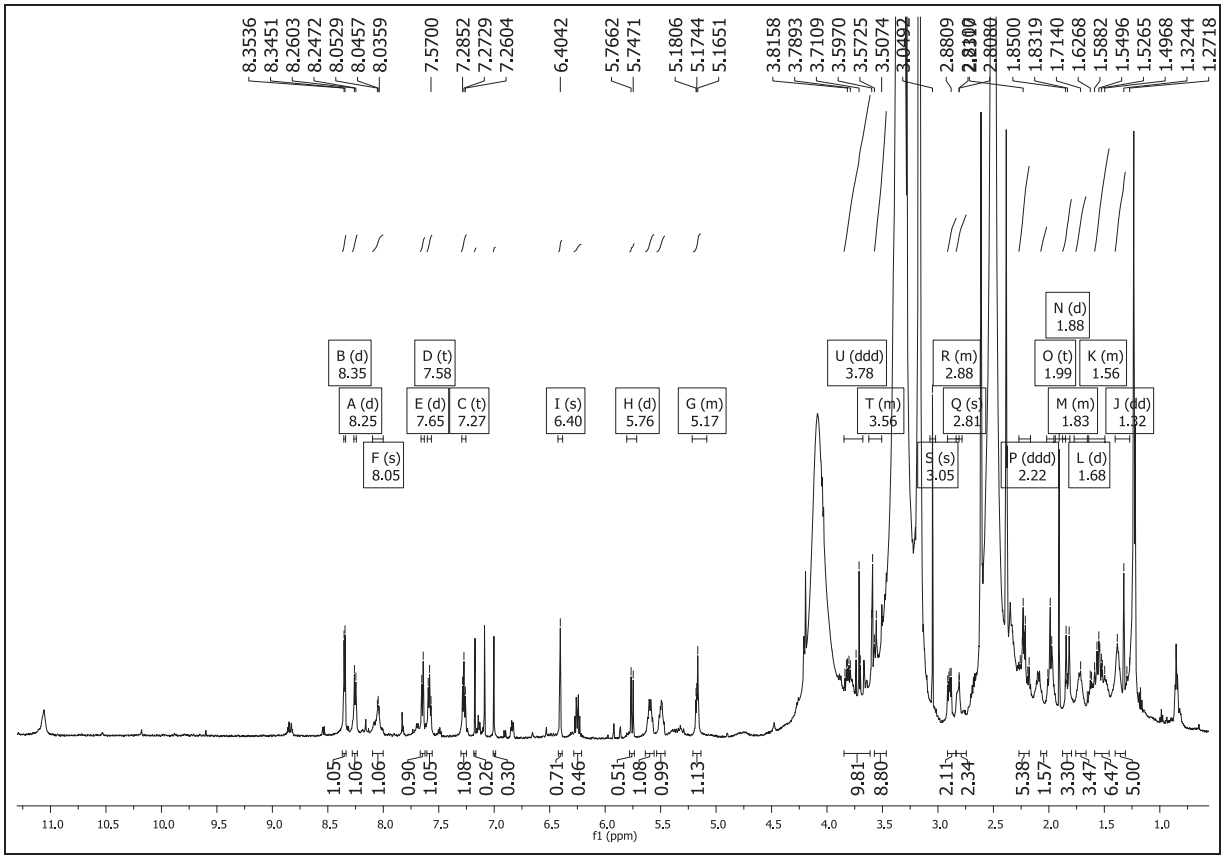
Attacment: The ^{1}H NMR spectrum of 8-Hydroxy manzamine A (**20**).



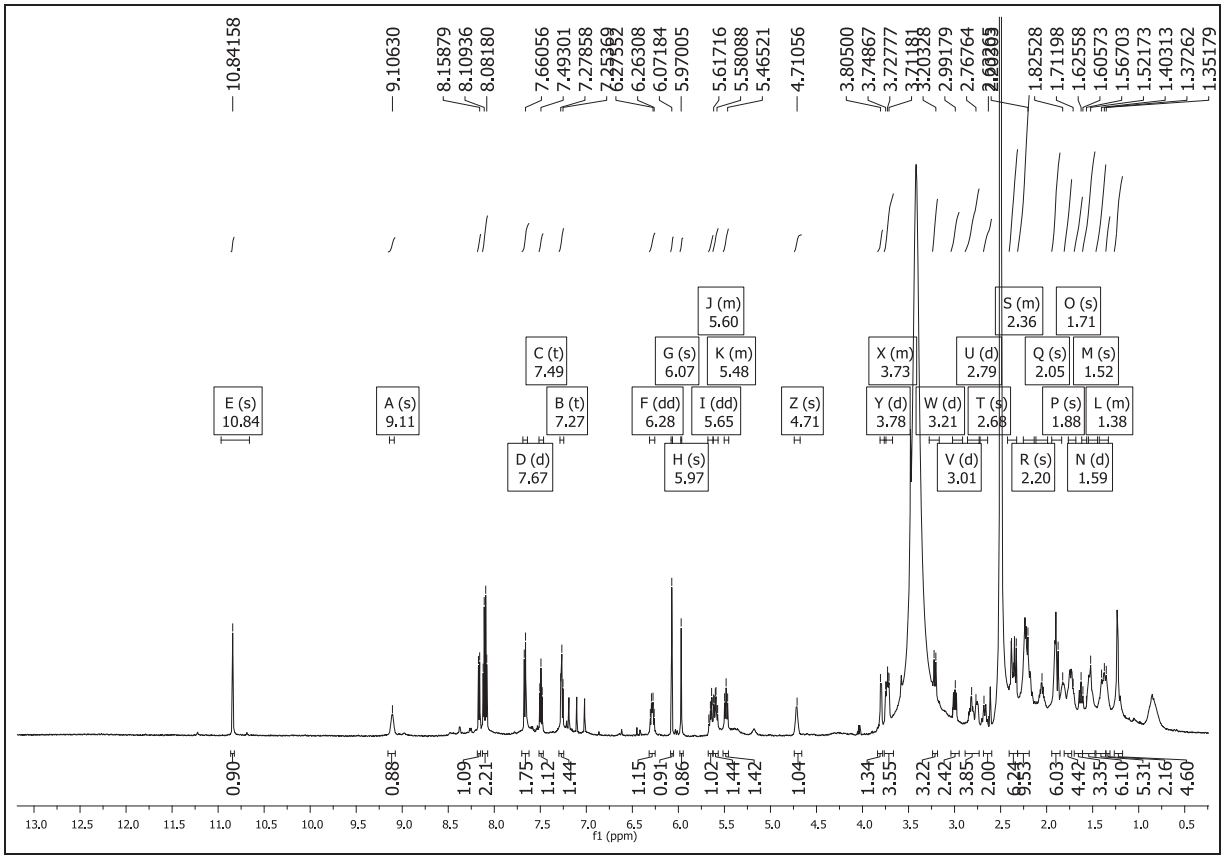
Attacment: The ^{1}H NMR spectrum of Manzamine F (**21**).



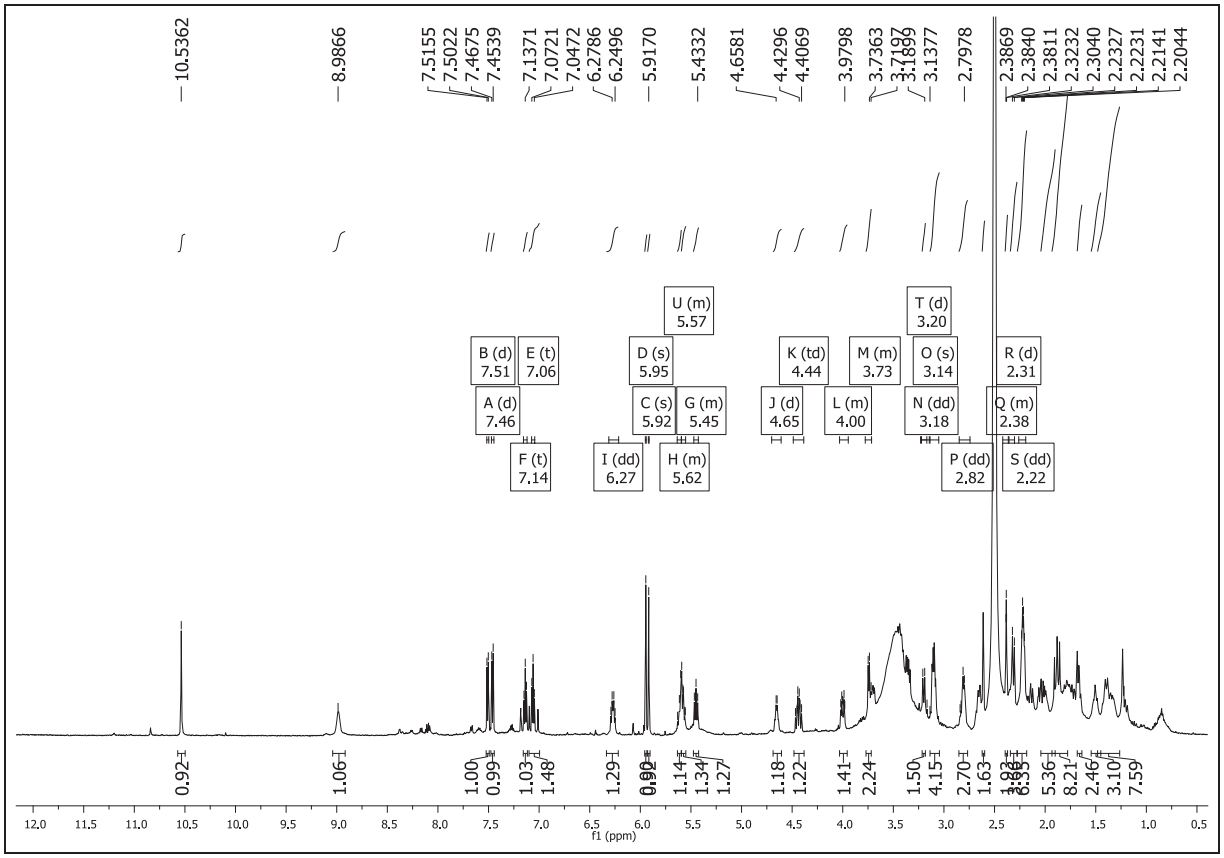
Attacment: The ^{1}H NMR spectrum of Deoxymanzamine X (**22**).



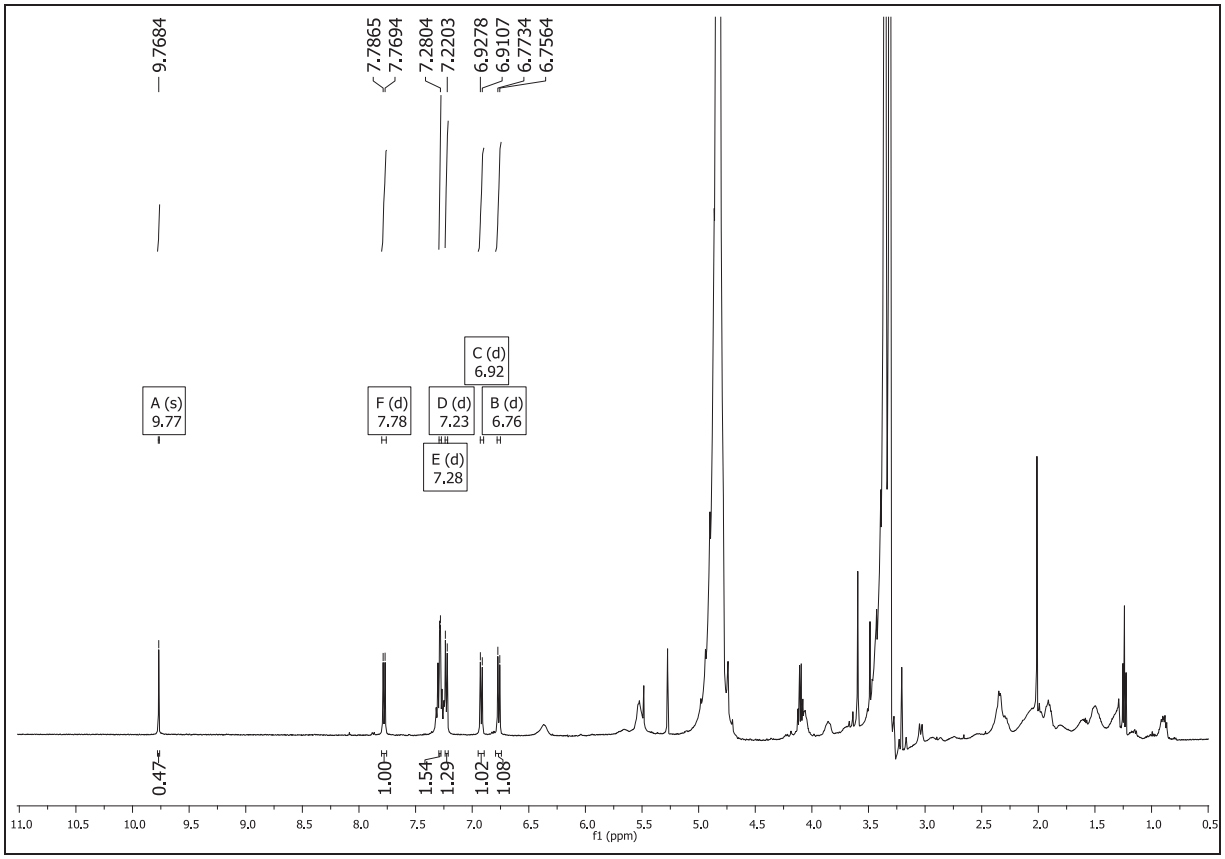
Attacment: The ^{1}H NMR spectrum of Manzamine A *N*-oxide (**23**).



Attacment: The ^{1}H NMR spectrum of 3,4-Dihydromanzamine A *N*-oxide (**24**).



Attacment: The ^{1}H NMR spectrum of 4-Hydroxybezaldehyde (**26**).



Resume

I. Personal Data

Family Name: Al-Tarabeen

Given Name : Mousa

Date of Birth: 15 Jan. 1978

Place of Birth: Amman

Nationality: Jordanian

Marital status: Married

Present Address: West Str.39-42119 Wuppertal

E-mail: moalt001@hhu.de ; mousa_e@hotmail.de

Languages (reading/writing): Arabic (native)

English (v. good)

II. Education

* M. Sc. in applied Chemistry- Jordan University of Science and Technology with (Very Good) March 2006.

* B. Sc. in applied Chemistry- Jordan University of Science and Technology with (Good) June 2001.

*General Secondary School Certificate Examination (Al-Sharif Hussein Ibn Naser School). 1996.

III.Work Experience:

1- Researcher assistant in Pharmaceutical Biology and Biotechnology Institute-HHU from 15.06.2011 to present

2- Lecture at Costal environmental department/Marine Science Colleague/Jordan University from 01.05.2010 to 01.06.2011
3- Ben Hayyan-Aqaba International Laboratories from 04.02.2008 to 01.05.2010.
4- Marine Science Station as Researcher Assistant from 01.11.2006 to 31.12.2007.
5- Research Assistant/Teaching, Department of Applied Chemistry, J.U.S.T as master student from 01.04. 2003 to 31.12. 2005.

IV. Research and Technical Experience:

***My working in organic Laboratory in Ben Hayyan-Aqaba International Laboratories includes**:

1-Analysis of Aflatoxin (B1, B2, G1, and G2) in food samples using HPLC-FLS.
2- Analysis of Aflatoxin (B1, B2, G1, and G2) in food samples using TLC.
3- Analysis of Pesticide in Foods and water using GC-MS, GC-ECD, GC-FID, and GC-NPD.
4- Analysis of Herbicides, THMs, VOCs, PAHs, and Phenol in water using GC-MS, and GC-ECD.
5- Analysis melamine using HPLC-PDA, and UPLC-MS-MS.
6- Develop method for analysis of preservative (Potassium Sorbate and Sodium Benzoate) using HPLC-PDA.
7- Analysis of Antioxidant (BHT, and BHA) in food sample using GC-FID.
8- Develop method for analysis of Oil soluble color (8 color) using HPLC-PDA
9- Analysis of Acidity in oil, cheese, and water.
10- Analysis of Peroxide in Oil.
11- Analysis of THD, TDS, and TSS in water.
12- Analysis of COD and BOD in water.
13- Analysis color in food samples using GC-FID.
14- Analysis of water soluble color in food sample using HPLC-PDA.

15- Analysis of ethanol in food sample using GC-FID.

***My work in MSS included:**

1- Collecting samples from sea include: water, sediment, and zooplankton and Phytoplankton using boat or by SCUBA Diving.

2- Analysis of Zooplankton Phytoplankton.

3-Analysis of Sea water for Nutrients, Hydrocarbon, Total carbon, chlorophyll, and dissolved oxygen.

4- Analysis of chlorinated pesticide using GC-ECD.

5-Analysis of Cartonoide, and Fucoxanthin using HPLC.

V-Training Courses:

1-Cambridge Diploma in IT (Training course from 17.05.2009 to 22.7.2009).

2-Training course on software and operation of HPLC (Agilent 1100) in Manchester- England from 22.09.2008 to 24.09.2008.

3-Training course on Trouble shooting and Maintenance of HPLC (Agilent 1100) in Manchester- England from 01.10.2008 to 03.10.2008.

4-Training course on software and operation of GC (Agilent 6890) in Manchester- England from 30.06.2008 to 04.06.2008.

5-Training Course on Analysis of Pesticide in Foods and water using GC-MS, GC-ECD, GC-FID, and GC-NPD by Expert John Cox who brought by Ben Hayyan Laboratory from 04.02.2008 to 25.02.2008 and from 14.07.2008 to 30.07.2008.

6-Training course on Analysis of THMs and VOCs in water samples using head space,

trap Technique, and Purge by Royal Scientific Society (RSS) from 22.06.2008 to 26.06.2008.

7-Training course on GC/MS and GC/MS-MS by Royal Scientific Society (RSS) from 01.06.2008 to 04.06.2008.

8-Training course on Analysis of PAHs, Phenol, and Herbicides in water samples using GC, and HPLC by Royal Scientific Society (RSS) from 9/3/2008-18/3/2008.

9-Training course on diving from 01.03.2007 to 30.03.2007 in MSS.

10- Training course in biochemistry as master student in biochemistry Laboratory in Jordan University of Science and Technology from 10.10.2004 to 01.04.2005 which contains:

-Separation of proteins using Agraose Gell Electrophoresis.

- Determine the concentration of protein usinglowery method

- Elution the concentration of the high-density lipoprotein “HDL”

- Separation of plasma from bloods using Beckman Centrifuge.

-Separation of the high-density lipoprotein using Ultracentrifuge.

- Identification of lipid composition in HDL using TLC.

11- Training course at Industrial Complex- Aqaba from 21.08.2000 to 05.10.2000 which contains:

-Water purification for drinking and Industry.

-Analysis of water (Chlorine, calcium carbonate, trace metal, PH

-Preparation and analysis of sulfuric acid.

-Preparation and analysis of phosphoric acid.

-Preparation and analysis of fertilizer.

-Preparation and analysis of aluminum florid.

VI- Publications:

1- Al Tarabeen M., Aly A. H., Perez Hemphill C. F., Rasheed M., Wray V., Proksch P. (**2015**). New Nitrogenous Compounds from a Red Sea Sponge from the Gulf of Aqaba. Zeitschrift fuer Naturforschung, C: Journal of Biosciences. **70** (3-4), 75-78.

2- Al Tarabeen M., Daletos G., Ebrahim W., Müller W. E. G., Hartmann R., Lin W., Proksch P. (**2015**). Ircinal E, a New Manzamine Derivative from the Indonesian Marine Sponge *Acanthostrongylophora ingens*. (Already accepted by Natural Product Communications Journal).