Enzyme-mediated synthesis and crystal structure of (2R,4S)-hydroxyketamine, $C_{13}H_{16}ClNO_2$

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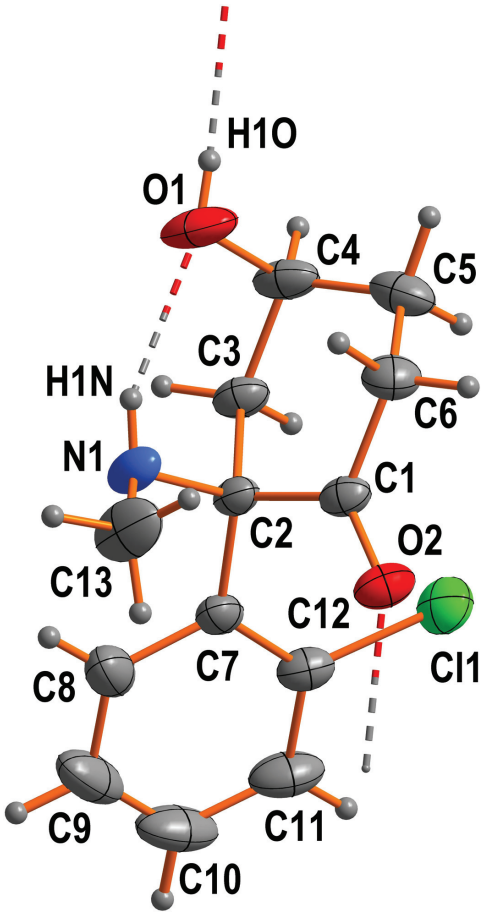

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Abstract

$C_{13}H_{16}ClNO_2$, monoclinic, $P2_1$ (no. 4), $a = 7.4945(7)$ Å, $b = 7.2336(6)$ Å, $c = 11.5401(10)$ Å, $\beta = 92.555(2)°$, $V = 624.99(10)$ Å^3, $Z = 2$, $R_{gt}(F) = 0.0415$, $wR_{ref}(F^2) = 0.0947$, $T = 291$ K.

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The molecular structure is shown in the figure. Table 1 contains crystallographic data and Table 2 contains the list of the atoms including atomic coordinates and displacement parameters.

Table 1: Data collection and handling.

Crystal:	Colorless block
Size:	0.90 × 0.80 × 0.56 mm
Wavelength:	Mo $K\alpha$ radiation (0.71073 Å)
μ:	0.30 mm^{-1}
Diffractometer, scan mode:	Bruker APEX-II, φ and ω
θ_{max}, completeness:	30.0°, >99%
$N(hkl)_{measured}$, $N(hkl)_{unique}$, R_{int}:	6118, 3527, 0.087
Criterion for I_{obs}, $N(hkl)_{gt}$:	$I_{obs} > 2\ \sigma(I_{obs})$, 3225
$N(param)_{refined}$:	164
Programs:	Bruker [1], SHELX [2, 3], Diamond [4]

Source of material

An amount of 360 µmol (98.7 mg) of (*R*)-ketamine hydrochloride was converted to the title compound by an *Escherichia coli*-based whole-cell biocatalyst expressing the double mutant I238G/M388A of the cytochrome P450 monooxygenase CYP154E1 from *Thermobifida fusca* YX and two redox partner proteins as recently described by Bokel et al. [5]. The reaction was carried out in a total volume of 180 mL with an educt concentration of 2 mM. After incubation at 25 °C, 250 revolutions per minute for 20 h, the product mixture was extracted using ethyl acetate after addition of sodium carbonate. The title compound (2*R*,4*S*)-hydroxyketamine {systematic name: (2*R*,4*S*)-2-(2-chlorophenyl)-4-hydroxy-2-methylamino)-cyclohexanone} was purified by flash chromatography on silica as previously described [5]. The solvent was evaporated and the residue was dissolved in methanol. Crystals were obtained by slow evaporation overnight from methanol.

Table 2: Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2).

Atom	*x*	*y*	*z*	U_{iso}*/U_{eq}
Cl1	0.05378(9)	0.25991(10)	0.07451(5)	0.05572(19)
O1	0.1888(4)	−0.3498(3)	0.3458(2)	0.0702(7)
H1O	0.150(5)	−0.448(6)	0.350(3)	0.069(11)*
O2	0.0475(2)	0.2845(2)	0.34835(15)	0.0480(4)
N1	0.3678(3)	0.0021(3)	0.3870(2)	0.0507(5)
H1N	0.341(4)	−0.102(5)	0.403(3)	0.044(8)*
C1	0.0783(3)	0.1234(3)	0.32893(17)	0.0354(4)
C2	0.2580(3)	0.0585(3)	0.28374(18)	0.0342(4)
C3	0.2383(3)	−0.1139(3)	0.2045(2)	0.0411(5)
H12	0.198160	−0.073719	0.127520	0.049*
H13	0.355195	−0.169700	0.198221	0.049*
C4	0.1107(4)	−0.2606(3)	0.2448(2)	0.0503(6)
H44	0.092957	−0.352762	0.183122	0.060*
C5	−0.0678(4)	−0.1767(4)	0.2699(3)	0.0561(7)
H5A	−0.146834	−0.272738	0.296116	0.067*
H5B	−0.121082	−0.124178	0.199197	0.067*
C6	−0.0492(4)	−0.0262(4)	0.3625(2)	0.0480(5)
H6A	−0.165444	0.027927	0.374096	0.058*
H6B	−0.006709	−0.081142	0.435259	0.058*
C7	0.3512(3)	0.2111(3)	0.2179(2)	0.0374(4)
C8	0.5293(3)	0.2550(5)	0.2475(3)	0.0586(6)
H8	0.587639	0.193452	0.308931	0.070*
C9	0.6209(5)	0.3883(5)	0.1871(5)	0.0807(12)
H9	0.738859	0.415501	0.209126	0.097*
C10	0.5397(5)	0.4799(5)	0.0958(4)	0.0785(12)
H10	0.602036	0.568930	0.055869	0.094*
C11	0.3633(5)	0.4393(4)	0.0628(3)	0.0612(8)
H11	0.306591	0.500376	0.000529	0.073*
C12	0.2731(3)	0.3065(3)	0.1242(2)	0.0414(5)
C13	0.3658(5)	0.1213(5)	0.4897(3)	0.0738(10)
H13A	0.388078	0.246823	0.467645	0.111*
H13B	0.456806	0.081628	0.545421	0.111*
H13C	0.251167	0.113289	0.523331	0.111*

Experimental details

Coordinates of hydrogen atoms attached to nitrogen and oxygen were refined without constraints or restraints. The carbon-bound hydrogen atoms were placed using a riding model (AFIX 13/23/43/137) implemented in the SHELXL system using the standard parameters for the constrained U_{iso}(H) values [3]. The absolute structure determination succeeded as the derived Flack parameter is found to be near zero [0.01(5) from 1306 selected quotients] using Parsons's method [6]. The classical calculation of the Flack parameter showed a slightly worse result 0.02(8) using all reflections [3, 6].

Comment

The anesthetic and antidepressant ketamine (2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone) is mainly metabolized by cytochrome P450s (CYPs) in the liver leading to, *inter alia*, several hydroxynorketamines and hydroxyketamines [7–11].

In order to synthesize some of these metabolites, which are considered to be the pharmacologically active compounds in treatment of depression [10–13], protein engineering of the cytochrome P450 CYP154E1 from the thermotolerant soil bacterium *Thermobifida fusca* YX was applied as described previously [5]. CYP154E1 was chosen as an appropriate candidate because it has been found to possess high substrate promiscuity and substrate-dependent high regioselectivity [5, 14–16]. Mutagenesis and screening among a number of CYP154E1 mutants revealed the double mutant I238G/M388A that enabled the regio- and stereoselective hydroxylation at position 4 of (*R*)-ketamine as well as of norketamine (not shown). To the best of our knowledge, the only hydroxylated at position 4 metabolites of ketamine reported so far are stereoisomers of 4-hydroxynorketamine.

Description and comparison. There is one hydroxyketamine molecule in the asymmetric unit of the title structure (*cf.* the Figure). As the compound crystallizes in the non-centrosymmetric space group $P2_1$ it is quite clear that the investigated crystal contains (2*R*,4*S*)-hydroxyketamine only. All bond lengths and angles derived from our diffraction experiment are in the expected ranges [17, 18]. The title molecule possesses a conformational flexibility by a rotation about the C2—C7 and C2—N1 single bonds, respectively. It is evident that these potential rotations will not take place independently from one another. Furthermore, the intramolecular NH$\cdots$O hydrogen bond [N1$\cdots$O1 = 2.907(3); see the Figure] reduces the possibilities. Thus it is of interest to compare the conformation of the title structure with examples found in the literature. In the title structure C2—N1 and the chlorophenyl moiety are almost coplanar (rms deviation of fitted atoms N1, C2, C7—C12 = 0.045 Å). For the parent compound ketamine, structural reports for the racemic single crystal [17] as well as for both enantiomeric forms [18] are described. For all these ketamine structures the chlorophenyl moiety is almost coplanar with the aforementioned central C—N single bond. A CSD database survey [19] yielded structures of all four possible stereoisomers of the closely related 4-hydroxynorketamine [20]. In accordance with our results, the mean plane of the chlorophenyl group is almost coplanar with the C—N bond in the structures of the (2*S*,4*S*)- and (2*R*,4*R*)-stereoisomers. However, the stereoisomers (2*R*,4*S*)- and (2*S*,4*R*)-hydroxynorketamine were crystallized as hydrochlorides showing a rotation about C5—C7 as well as the other chair conformation of the cyclohexanone ring: the amino and hydroxy group are found in equatorial positions. Thus, an intramolecular hydrogen bond is not possible. In this context it should not be kept unmentioned that during our database survey we identified a ketamine hydrochloride, which shows the same rotation of the mean plane of the chlorophenyl moiety (rotation about C5—C7) and

the other chair conformation, too [21]. Protonation at N1 and formation of a salt with some more hydrogen bonds seems to seriously affect the general conformation. This crucial aspect should be investigated in the future.

Supramolecular aspects. Adjacent molecules are connected *via* intermolecular OH···O hydrogen bonds [O1···O2′ = 2.850(3) Å; ′ = x, $-1+y$, z; see the Figure] to form a chain structure along the crystallographic b axis. Similar to other simple molecules containg almost rigid moieties [22], the packing scheme is a compromise between the conformational possibilities and hydrogen bonding interactions.

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