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Selective biocatalytic synthesis and crystal structure of (2*R*,6*R*)-hydroxyketaminium chloride, C₁₃H₁₇Cl₂NO₂

Table 1: Data collection and handling.

Crystal:	Colorless needle
Size:	0.52 × 0.15 × 0.04 mm
Wavelength:	Mo K α radiation (0.71073 Å)
μ :	0.45 mm ⁻¹
Diffractometer, scan mode:	Xcalibur, ω
$\theta_{\max}$, completeness:	30.8°, >99%
$N(hkl)_{\text{measured}}$, $N(hkl)_{\text{unique}}$, R_{int} :	23,627, 8214, 0.056
Criterion for I_{obs} , $N(hkl)_{\text{gt}}$:	$I_{\text{obs}} > 2 \sigma(I_{\text{obs}})$, 5570
$N(\text{param})_{\text{refined}}$:	335
Programs:	Bruker [1], SHELX [2, 3], Diamond [4]

$\gamma = 73.335(4)^\circ$, $Z = 2$, $V = 711.13(5) \text{ \AA}^3$, $R_{\text{gt}}(F) = 0.0536$, $wR_{\text{ref}} = 0.0878$, $T = 290 \text{ K}$.

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A part of the hydrogen bonded polymeric title 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.

Source of material

The mutated variant I238Q/V286G/L289T/M388A of the cytochrome P450 monooxygenase CYP154E1 from *Thermobifida fusca* YX was used to catalyze the oxidation of 120 μmol (R)-ketamine hydrochloride to (2*R*,6*R*)-hydroxyketamine and -hydroxynorketamine as described by Bokel et al. [5]. The reaction mixture was incubated for 24 h at 25 °C, 250 revolutions per minute. After addition of sodium carbonate, the product mixture was extracted using ethyl acetate. A semi-preparative HPLC was used to purify the (2*R*,6*R*)-hydroxyketamine as previously described [5]. The solvents (acetonitrile/water) were removed *via* evaporation and lyophilization. Afterwards, the residue was dissolved in chloroform with hydrochloric acid to form the title compound. The solvent was removed again, and the title compound was dissolved in 2-propanol. Slow evaporation over night at room temperature gave colorless needle crystals.

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Abstract

C₁₃H₁₇Cl₂NO₂, triclinic, $P1$ (no. 1), $a = 7.1486(3) \text{ \AA}$, $b = 7.8265(3) \text{ \AA}$, $c = 13.6579(5) \text{ \AA}$, $\alpha = 81.085(3)^\circ$, $\beta = 77.487(4)^\circ$,

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Table 2: Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å²).

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{iso} */ <i>U</i> _{eq}
Cl1	0.45305 (17)	0.52741 (15)	0.19624 (10)	0.0536 (3)
Cl2	0.38590 (17)	0.73388 (14)	0.41362 (9)	0.0502 (3)
Cl3	0.54115 (16)	0.79443 (17)	0.83816 (10)	0.0527 (3)
Cl4	0.65298 (15)	1.10762 (13)	0.60081 (9)	0.0442 (3)
O1	0.4597 (5)	0.0086 (5)	0.2198 (3)	0.0531 (10)
H1O	0.418 (9)	−0.048 (8)	0.278 (5)	0.09 (2)*
O2	0.4485 (5)	0.2071 (4)	0.3715 (3)	0.0503 (9)
O3	0.5377 (6)	0.3198 (5)	0.7969 (3)	0.0589 (11)
H3O	0.578 (7)	0.287 (7)	0.744 (4)	0.053 (18)*
O4	0.5709 (4)	0.5911 (4)	0.6453 (3)	0.0468 (9)
N1	0.7089 (5)	0.3655 (4)	0.3989 (3)	0.0379 (9)
H11	0.606164	0.456825	0.386775	0.045*
H12	0.666131	0.298624	0.453246	0.045*
N2	0.3252 (5)	0.9046 (4)	0.6130 (3)	0.0323 (8)
H21	0.423604	0.934784	0.630354	0.039*
H22	0.376902	0.842764	0.559621	0.039*
C1	0.5951 (6)	0.1825 (5)	0.3067 (3)	0.0344 (10)
C2	0.7736 (6)	0.2536 (5)	0.3103 (3)	0.0319 (9)
C3	0.9361 (6)	0.0830 (5)	0.3364 (3)	0.0378 (10)
H3A	1.055368	0.116487	0.338958	0.045*
H3B	0.890821	0.027556	0.402485	0.045*
C4	0.9853 (6)	−0.0523 (6)	0.2590 (4)	0.0451 (11)
H4A	1.042621	−0.000753	0.194176	0.054*
H4B	1.083658	−0.158538	0.279374	0.054*
C5	0.8035 (7)	−0.1054 (6)	0.2481 (4)	0.0505 (13)
H5A	0.759193	−0.174117	0.309727	0.061*
H5B	0.839594	−0.181955	0.193792	0.061*
C6	0.6309 (6)	0.0572 (6)	0.2259 (4)	0.0409 (10)
H6A	0.674664	0.119639	0.160722	0.049*
C7	0.8404 (6)	0.3655 (5)	0.2135 (3)	0.0337 (9)
C8	1.0412 (7)	0.3481 (6)	0.1752 (3)	0.0447 (11)
H8A	1.134619	0.268565	0.209835	0.054*
C9	1.1064 (7)	0.4451 (7)	0.0874 (4)	0.0530 (13)
H9A	1.241835	0.427834	0.063172	0.064*
C10	0.9733 (9)	0.5658 (7)	0.0361 (4)	0.0603 (14)
H10A	1.017392	0.632335	−0.022382	0.072*
C11	0.7724 (8)	0.5886 (6)	0.0715 (4)	0.0501 (12)
H11A	0.680624	0.670667	0.036937	0.060*
C12	0.7083 (6)	0.4893 (6)	0.1584 (3)	0.0389 (10)
C13	0.8622 (7)	0.4391 (6)	0.4229 (4)	0.0482 (12)
H13A	0.969936	0.342196	0.441311	0.072*
H13B	0.804504	0.511662	0.477938	0.072*
H13C	0.911149	0.511022	0.364829	0.072*
C21	0.4192 (6)	0.6108 (5)	0.7063 (3)	0.0343 (10)
C22	0.2461 (5)	0.7826 (5)	0.6987 (3)	0.0307 (9)
C23	0.0877 (6)	0.7155 (5)	0.6653 (3)	0.0352 (10)
H23A	−0.028084	0.815040	0.658736	0.042*
H23B	0.141021	0.670888	0.599721	0.042*
C24	0.0255 (6)	0.5664 (6)	0.7408 (4)	0.0437 (11)
H24A	−0.036057	0.612871	0.805249	0.052*
H24B	−0.071611	0.526324	0.716886	0.052*
C25	0.2044 (7)	0.4081 (6)	0.7543 (4)	0.0500 (12)
H25A	0.162303	0.319156	0.805370	0.060*

Table 2: (continued)

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{iso} */ <i>U</i> _{eq}
H25B	0.255905	0.353335	0.691548	0.060*
C27	0.1694 (6)	0.8801 (5)	0.7943 (3)	0.0306 (9)
C26	0.3676 (6)	0.4632 (5)	0.7849 (4)	0.0433 (11)
H26A	0.314324	0.514211	0.849528	0.052*
C28	−0.0342 (6)	0.9715 (5)	0.8182 (3)	0.0382 (10)
H28A	−0.119239	0.967991	0.776181	0.046*
C29	−0.1098 (6)	1.0649 (6)	0.9012 (4)	0.0455 (11)
H29A	−0.244206	1.124012	0.914398	0.055*
C30	0.0120 (7)	1.0718 (6)	0.9650 (4)	0.0488 (12)
H30A	−0.039944	1.133877	1.021792	0.059*
C31	0.2115 (7)	0.9861 (6)	0.9442 (3)	0.0448 (11)
H31A	0.295375	0.990535	0.986579	0.054*
C32	0.2858 (6)	0.8937 (5)	0.8600 (3)	0.0356 (10)
C33	0.1791 (6)	1.0727 (5)	0.5820 (4)	0.0429 (11)
H33A	0.080622	1.042886	0.554803	0.064*
H33B	0.246890	1.145151	0.531613	0.064*
H33C	0.115920	1.138032	0.639500	0.064*

Experimental details

All hydrogen atoms were added using different riding models depending on the chemical surroundings [3]. The Flack parameter [−0.02(3)] was determined using 1973 quotients ([I⁺] − [I[−]])/([I⁺] + [I[−]]) using the post-refinement Parsons' quotients method [3, 6].

Comment

(2*R*,6*R*)-hydroxyketamine is one of the minor human metabolites of the anesthetic drug (*R,S*)-ketamine [7–9]. Whereas the hepatic metabolism by cytochrome P450s (CYPs) leads to a mixture of several hydroxynorketamines and hydroxyketamines, mutagenesis of the bacterial CYP154E1 allowed the selective synthesis of (2*S*,6*S*)-hydroxynorketamine from (*S*)-ketamine [10] as well as (2*R*,6*R*)-hydroxynorketamine from (*R*)-ketamine [5]. In this study, the crystal structure of one of the side products of the latter reaction is presented.

Molecular description and comparison. The asymmetric unit of the title structure contains two crystallographically independent (2*R*,6*R*)-hydroxyketaminium cations (systematic name: (1*R*,3*R*)-1-(2-chlorophenyl)-3-hydroxy-*N*-methyl-2-oxocyclohexan-1-aminium; the protonation at the methylamino group shifts the numbering scheme which includes the chiral centers) and two chloride counter anions (see the figure). In contrast to our previously published

structure of (2*R*,4*S*)-hydroxyketamine [11], the cyclohexanone ring is in a different chair conformation, which rules out an intramolecular hydrogen bond between the aminium and the hydroxy group. Instead, the conformation of the title cation is very similar to that of the cation seen in the crystal structure of (2*R*,6*R*)-hydroxynorketaminium chloride [12]. In general, all molecular geometric parameters are in the expected ranges [11, 12].

Supramolecular aspects. In the title crystal structure each cation donates three classical hydrogen bonds. In detail, each cation donates two $NH \cdots Cl$ bonds and one $OH \cdots Cl$ hydrogen bond (see the figure). Thus, the (2*R*,6*R*)-hydroxyketaminium cation exclusively donates hydrogen bonds. The fact that the carbonyl group is not involved in any classical hydrogen bond is the result of a surplus of hydrogen bond acceptors in this structure. Obviously, the charge-supported hydrogen bonds ($NH^+ \cdots Cl^-$ and $NH^+ \cdots O$) are preferred. There are two prominent hydrogen bonding motifs, which should be mentioned and classified using so-called graph set descriptors [13]. There are 12-membered rings consisting of two cation and two chloride anions (graph set descriptor: $R^2_4(12)$; see the figure). Furthermore, a simple chain motif is seen, which is formed by the NH_2 groups and the chloride anions (see the figure; graph set descriptor: $C^1_2(4)$). Consequently, a chain-type polymer is formed, which propagates along the crystallographic *b* axis (red line in the figure).

A further comparison with the aforementioned crystal structure of (2*R*,6*R*)-hydroxynorketaminium chloride [12] shows that NH_3 group instead of the H_3C-NH_2 moiety allows a further hydrogen bonding connection leading to a two-dimensional framework.

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