STRUCTURE OF PYRIDINDOLOL, INHIBITOR OF β-GALACTOSIDASE

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Pyridindolol is a product of a streptomyces and exhibits inhibitory activity against bovine liver β-galactosidase. The structure of pyridindolol, 1-[1(*R*), 2-dihydroxyethyl]-3-hydroxymethyl-9*H*-pyrido[3,4-*b*] indole, has been established by spectroscopic analyses and an X-ray structure determination.

Pyridindolol (**I**) inhibits bovine liver β-galactosidase in acidic conditions and its production, isolation and properties were reported in a previous paper[1]. This paper is concerned with the structure determination of the new inhibitor.

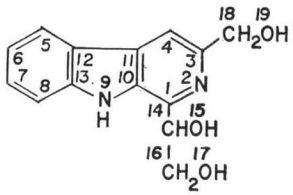
Pyridindolol was crystallized from methanol-ethyl acetate as colorless needles melting at 167~168°C. Potentiometric titration in 50 % aqueous methanol showed the presence of a weak basic function of pKa (50 % MeOH) 5.35 and the titration equivalent was 258. From elemental analysis and mass spectrometry, the molecular formula was established as $C_{14}H_{14}N_2O_3$ (MW 258). Calcd. for $C_{14}H_{14}N_2O_3$: C 65.10, H 5.46, N 10.85, O 18.59. Found: C 65.52, H 5.56, N 11.15, O 18.51. *m/e* 258.0977 (calcd. for $C_{14}H_{14}N_2O_3$ 258.1003). The UV spectrum of **I** in 95 % methanol showed absorption maxima at 216 (log ε 4.36), 238 (4.62), 292 (4.27), 345 (3.71) and 350 nm (3.71). In acidic solution these maxima were shifted to 256 (4.58), 306 (4.30) and 380 nm (3.75). The UV spectra suggest that **I** could be a β-carboline derivative[2]. Acetylation of **I** with acetic anhydride and pyridine gave a tri-O-acetyl derivative (**II**), m.p. 83~84°C, *m/e* 384.1356 (calcd. for $C_{20}H_{20}N_2O_6$ 384.1320).

The PMR spectrum of **I** measured in DMSO-d_6 solution is shown in Table 1. Methylene protons (2H) appeared at δ 3.86, coupled with an OH proton at δ 4.65~4.9 (overlapped by other methylene protons), and a methine proton at δ 5.08. The latter proton is coupled with an OH proton at δ 5.69. These coupling relations suggested the presence of a 1,2-dihydroxyethyl substituent. Other methylene protons (2H) appeared at δ 4.73 coupled with an OH proton at δ5.33, which suggested the presence of a hydroxy-

Table 1. Chemical shift and coupling constants of pyridindolol (DMSO-d_6)

Proton	Chemical shift* (multiplicity)	Coupling constant (Hz)
4−H	8.04 (s)	
5−H	8.19 (q)	$J_{5,6}=7.5$ $J_{5,7}=1.5$
6−H	7.20 (m)	$J_{6,7}=6.5$ $J_{6,8}=1.5$
7−H	7.50 (m)	$J_{7,8}=8.0$
8−H	7.68 (q)	
9−H	11.09 (s)	
14−H	5.08 (m)	$J_{14,15}=5.0$
15−H	5.69 (d)	
16,16′−H	3.56~4.1 (m)	
17−H	4.65~4.9	
18,18′−H	4.73 (d)	$J_{18,19}=5.5$
19−H	5.33 (t)	

*ppm from TMS as an internal reference (δ=0)



methyl group.

Four sequential aromatic protons assigned to C_5, C_6, C_7 and C_8 protons of a β-carboline chromophore appeared at δ 8.19, 7.20, 7.50 and 7.68 respectively, ($J_{5,6}=7.5$, $J_{5,7}=1.5$, $J_{6,7}=6.5$, $J_{6,8}=1.5$ and $J_{7,8}=8$ Hz). An NH proton at the 9 position appeared at δ 11.09 as a singlet. A nuclear OVERHAUSER effect (NOE) was observed between the NH and C_8 protons, +11 % CH{NH}. The position of the 1,2-dihydroxyethyl group can be assigned to C_1, because of the existence of a NOE between the NH and the methine proton of the dihydroxyethyl group, +9 % NH{CH} and +8 % CH{NH}. A NOE was also observed between the hydroxymethyl protons and a singlet proton appearing at δ 8.04, +18 % CH{CH_2}, which suggested that the hydroxymethyl group is at the 3 or 4 position of the β-carboline skeleton. The substitution position of the hydroxymethyl group could not be assigned unambiguously from the spectroscopic analysis, but position 3 seemed more possible from the viewpoint of biosynthesis[3,4].

To determine the absolute configuration of the 1,2-dihydroxyethyl side chain and to confirm the position of the hydroxymethyl groups, an X-ray structure determination was carried out with a single crystal of pyridindolol hydrobromide.

The hydrobromide was recrystallized from ethylacetate-methanol solution as pale yellow crystalline platelets. The lattice constants and intensity data were measured on a Rigaku four-circle X-ray diffractometer using Ni-filtered Cu$K\alpha$ radiation. The crystal data are given in Table 2. Of the 2,553 reflections with 2θ angles less than 135°, intensities of 2,223 independent reflections were measured as F_0 values greater than two times their standard deviations. For $hk1$ and $hk3$ reflections, $h\bar{k}l$ FRIEDEL reflections were also measured in order to determine the absolute configuration. The size of the crystal used for the intensity measurement was about $0.03\times0.2\times0.35$ mm. No absorption correction was applid for the intensity data.

Table 2. Crystal data

Pyridindolol hydrobromide, $C_{14}H_{14}N_2O_3\cdot HBr$
M.W.=339.2. Monoclinic, $P2_1$.
$a=17.600(9)$, $b=10.120(5)$, $c=7.692(5)$ Å,
$\beta=102.3(1)°$, $Z=4$, $D_x=1.681$ g cm^{-3}.

The structure was solved by the heavy atom method using the phase angles obtained

Fig. 1. The stereoscopic view of pyridindolol

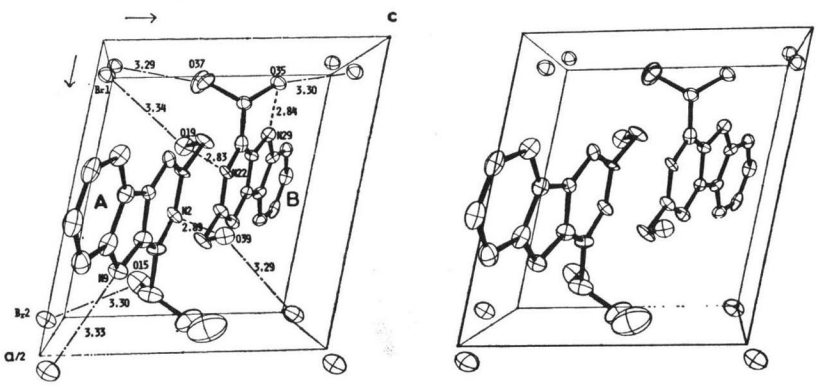


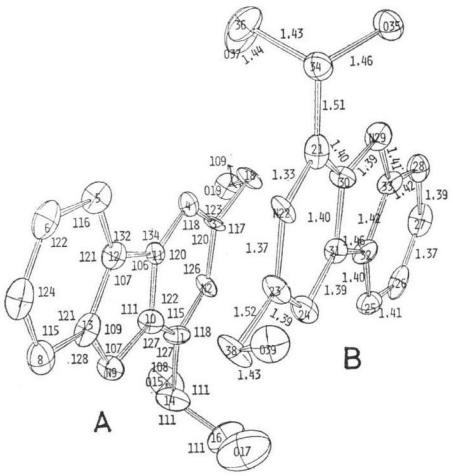
Table 3. Atomic parameters ($\times 10^4$). To represent the absolute configuration, the right-handed coordinate system should be taken. Temperature factors are of the form, $T=\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$.

	Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
	BR (1)	249 (1)	0 (0)	30 (3)	18 (1)	51 (2)	101 (3)	−2 (2)	13 (1)	8 (4)
	BR (2)	4744 (1)	105 (4)	−417 (3)	20 (1)	98 (3)	149 (5)	2 (2)	22 (2)	−8 (5)
A molecule	C (1)	3408 (12)	7169 (23)	3388 (30)	10 (7)	30 (22)	106 (42)	−1 (11)	5 (14)	9 (25)
	N (2)	2845 (11)	7856 (21)	3857 (24)	15 (6)	42 (22)	84 (35)	−3 (10)	22 (12)	3 (23)
	C (3)	2111 (12)	7422 (24)	3806 (28)	6 (7)	45 (23)	81 (38)	8 (11)	15 (13)	8 (25)
	C (4)	1907 (12)	6165 (22)	3178 (27)	16 (8)	31 (21)	59 (36)	1 (11)	12 (13)	10 (22)
	C (5)	1892 (14)	3086 (23)	1466 (30)	21 (9)	27 (22)	92 (43)	0 (12)	3 (15)	4 (26)
	C (6)	2122 (15)	1924 (25)	717 (30)	29 (10)	39 (24)	68 (40)	−4 (13)	−4 (16)	11 (25)
	C (7)	2860 (16)	1725 (27)	490 (32)	33 (11)	47 (28)	78 (43)	−1 (15)	4 (17)	−3 (29)
	C (8)	3456 (15)	2645 (28)	947 (32)	25 (10)	59 (29)	79 (43)	11 (14)	11 (16)	14 (29)
	N (9)	3679 (9)	5008 (25)	2157 (21)	15 (6)	33 (17)	105 (30)	−3 (13)	13 (10)	−16 (31)
	C (10)	3209 (13)	5890 (24)	2806 (31)	16 (8)	39 (24)	96 (42)	17 (12)	6 (15)	2 (26)
	C (11)	2449 (12)	5380 (22)	2672 (26)	12 (7)	42 (26)	51 (33)	−2 (10)	8 (12)	0 (22)
	C (12)	2469 (13)	4053 (23)	1914 (29)	17 (8)	35 (22)	69 (38)	1 (11)	4 (14)	5 (24)
	C (13)	3226 (14)	3835 (22)	1644 (29)	25 (9)	14 (22)	75 (40)	−5 (12)	−1 (15)	−4 (23)
	C (14)	4207 (15)	7822 (29)	3529 (39)	17 (9)	51 (30)	199 (60)	0 (14)	20 (18)	−13 (35)
	O (15)	4072 (12)	9190 (18)	3087 (27)	38 (8)	27 (18)	245 (44)	−13 (10)	47 (15)	6 (23)
	C (16)	4663 (18)	7656 (36)	5196 (43)	29 (13)	95 (39)	194 (67)	−7 (20)	−12 (23)	−3 (42)
	O (17)	4713 (14)	6311 (27)	5801 (35)	45 (11)	118 (31)	399 (66)	2 (16)	−16 (21)	−34 (39)
	C (18)	1558 (13)	8351 (24)	4447 (32)	14 (8)	42 (24)	123 (46)	14 (12)	27 (15)	18 (27)
	O (19)	1612 (10)	9666 (15)	3750 (22)	23 (6)	30 (19)	121 (30)	11 (8)	8 (11)	−1 (17)
B molecule	C (21)	1504 (14)	12636 (26)	6311 (31)	23 (9)	47 (26)	67 (40)	6 (13)	10 (15)	1 (27)
	N (22)	2070 (10)	11947 (21)	5832 (24)	14 (6)	47 (21)	72 (32)	2 (10)	21 (11)	0 (22)
	C (23)	2829 (13)	12349 (23)	5997 (30)	17 (8)	28 (22)	101 (41)	10 (11)	22 (15)	8 (25)
	C (24)	3056 (13)	13588 (24)	6697 (30)	16 (8)	40 (23)	98 (42)	4 (12)	18 (15)	23 (26)
	C (25)	3062 (13)	16661 (25)	8334 (31)	14 (8)	58 (28)	90 (42)	−8 (13)	2 (15)	12 (28)
	C (26)	2852 (15)	17844 (26)	9081 (30)	33 (11)	46 (27)	57 (40)	−6 (15)	−8 (16)	17 (27)
	C (27)	2116 (16)	18020 (24)	9370 (31)	33 (10)	16 (21)	89 (43)	0 (12)	5 (16)	4 (25)
	C (28)	1531 (14)	17102 (25)	8973 (29)	21 (9)	45 (24)	76 (40)	4 (13)	10 (15)	10 (25)
	N (29)	1265 (11)	14808 (21)	7678 (23)	18 (7)	41 (25)	86 (31)	9 (11)	6 (11)	−9 (23)
	C (30)	1717 (12)	13895 (24)	7000 (29)	11 (7)	37 (23)	83 (40)	0 (11)	10 (14)	−4 (25)
	C (31)	2467 (13)	14372 (22)	7176 (28)	14 (7)	22 (20)	63 (37)	7 (11)	5 (13)	0 (23)
	C (32)	2483 (13)	15695 (23)	7924 (29)	14 (8)	31 (22)	75 (40)	0 (11)	−3 (14)	−3 (25)
	C (33)	1727 (13)	15893 (22)	8234 (28)	13 (7)	20 (20)	78 (38)	0 (10)	−1 (13)	−2 (23)
	C (34)	725 (13)	12018 (26)	6004 (31)	14 (8)	51 (27)	87 (43)	5 (13)	10 (15)	6 (28)
	O (35)	303 (9)	12527 (18)	7352 (21)	17 (6)	61 (19)	99 (31)	0 (9)	19 (11)	−19 (21)
	C (36)	273 (15)	12314 (27)	4193 (33)	20 (9)	51 (27)	104 (45)	8 (14)	−12 (16)	6 (30)
	O (37)	195 (13)	13738 (21)	4001 (25)	45 (9)	63 (23)	125 (37)	7 (13)	−8 (15)	12 (24)
	C (38)	3375 (14)	11355 (27)	5384 (34)	20 (9)	62 (28)	140 (50)	9 (14)	40 (18)	2 (32)
	O (39)	3266 (9)	10126 (21)	6141 (19)	22 (6)	28 (16)	135 (28)	4 (11)	10 (10)	−4 (25)

by the two bromide ions. Some difficulties were experienced in arriving at the correct solution due to the fact that the two crystallographically independent molecules as well as the bromide ions are arranged with approximate symmetry of inversion center lying nearly at (1/4, 1, 1/2) which gives rise to the approximate space group symmetry $P2_1/a$. For several atoms, the

deviation from the center of symmetry was hardly detected. However, refinement by FOURIER and difference FOURIER syntheses and by least-squares calculations yielded the final R factor 0.091 in which the contributions of 16 hydrogen atoms were included. The absolute configuration was determined by use of the anomalous dispersion of Cu$K\alpha$ radiation by the bromine atoms. Intensities of 30 FRIEDEL pairs of reflection clearly indicated the absolute configuration as shown in Fig. 1. The final atomic parameters are given in Table 3. Figure 1 shows the stereoscopic drawing of the molecular packing viewed along the b axis. The two molecules A and B involved in an asymmetric unit, are bound together by two hydrogen bonds to form a dimer across the pseudo center of symmetry at (1/4, 1, 1/2).

Fig. 2. The bond lengths and angles.
The average values for the corresponding bonds in A and B molecules are given.



The conformations of these two molecules are very similar. They would have the antipodal structure to each other if the symmetry element relating them were the true inversion center. In the present structure however, molecules A and B have the same absolute configuration R at C(14) and C(34), respectively, which results in the great difference in the relative orientations of the two hydroxyl groups O(15) and O(35). Thus, in molecule B, the torsion angle, C(30)-C(21)-C(34)-O(35) is $-30.9°$ and the intramolecular hydrogen bond is formed between N(29)H and O(35), while in molecule A, the corresponding angle, C(10)-C(1)-C(14)-O(15) is increased to 148.8° and no hydrogen bond is formed within the molecule. The values of the bond lengths and angles averaged over the two independent molecules are shown in Fig. 2. Their standard deviations are estimated to be about 0.03 Å and 2°, respectively.

Thus, the structure of pyridindolol is determined to be 1-[1(R), 2-dihydroxyethyl]-3-hydroxymethyl-9H-pyrido[3,4-b]indol.

Experimental

Instrument: Melting points were determinated with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Carl Zeiss 0.005° photoelectric polarimeter. Potentiometric titration were determined with a Metrohm potentiograph E 436. IR spectra were taken with a Hitachi Model EPI-S2 spectrometer, and UV spectra were taken with a Hitachi Model EPS-3T spectrometer. PMR spectra were taken with a Varian HA-100 spectrometer with TMS as an internal standard. High resolution mass spectra were measured with a Hitachi RMU-7M spectrometer. X-ray diffraction intensities were measured by a Rigaku four-circle X-ray diffractometer.

Pyridindolol (**I**): Pyridindolol, prepared according to the previous paper[1], was recrystallized twice from MeOH - EtOAc (95:5) as colorless needles, mp 167~168°C.

Pyridindolol triacetate (**II**): A 13-mg sample of **I** was dissolved in 1 ml of dry pyridine under cooling with ice-water and 0.3 ml of acetic anhydride was added. This solution was

allowed to stand at room temperature overnight. One ml of cold water was added to that to give a clear solution. The reaction mixture was evaporated under reduced pressure, leaving a pale yellow oil. This material was dissolved in 0.5 ml of benzene and chromatographed on a column of silica gel (Wako gel C-200, Wako Pure Chemical Co., Ltd., Osaka) (1.1×17 cm). The column was eluted with a mixed solvent consisting of benzene, methanol, and acetic acid-(100:1:0.2). Compound II was eluted from 46 ml to 60 ml and oily material obtained after evaporation of the solvent was recrystallized from ethanol as colorless needles 12 mg, mp 83~84°C, $UV_{max}^{95\%MeOH}$ nm (log ε), 215 (4.38), 239 (4.55), 245 (4.53), 255 (4.46), 292 (4.18), 345 (3.62), 355 (3.63), $\lambda_{max}^{0.05NHCl95\%MeOH}$ nm (log ε), 235 (4.18), 261 (4.53), 311 (4.24), $IR\nu_{max}^{KBr}$: 1750 (C=O), 1635, 1575, 1500 (indole ring) 1250~1220 (acetate), PMR in d_6-DMSO, δ 2.01 (3H, s, ACO-), δ 4.16 (6H, s, 2ACO-), δ 4.45~4.83 (2H, m, -$^{16}CH_2$-), δ 5.30 (2H, s, -$^{18}CH_2$-), δ 6.41 (1H, q, -^{14}CH-).

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: C 62.49, H 5.24, N 7.29, O 24.98.
Found: C 62.35, H 5.20, N 7.25, O 24.80.

Pyridindolol hydrobromide (III): A 52-mg sample of **I** was dissolved in 0.4 ml of 4.7% hydrobromic acid, and evaporated under reduced pressure, leaving a yellow crystalline material. Recrystallization from a mixture of ethylacetate and methanol afforded 35 mg of yellow crystalline platelets of III, mp 206~214°C.

Anal. Calcd. for $C_{14}H_{14}N_2O_3 \cdot HBr$: C 49.57, H 4.45, N 8.25, O 14.14, Br. 23.55.
Found: C 49.45, H 4.10, N 8.55, O 13.85, Br. 23.00.

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