Improved Preparation, Chromatographic Separation and X-Ray Crystallographic Determination of the Absolute Configuration of the Enantiomers of 8-Hydroxy-2-(dipropylamino)tetralin (8-OH DPAT)

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2-Benzylamino-8-methoxytetralin (**2**), which serves as a synthetic intermediate for (+)- and (−)-8-hydroxy-2-(dipropylamino)tetralin (**1**), was resolved into the enantiomers by fractional crystallization of the di-*p*-toluoyltartrates. This procedure is more effective than that previously reported. The enantiomers of **1** were analyzed by ion-pair chromatography using *N*-benzyloxycarbonylglycyl-L-proline as a chiral counter ion added to the mobile phase (CH_2Cl_2). LiChrosorb DIOL was used as the solid phase. The separation factor obtained (1.20) enabled determination of the enantiomeric purities of (+)-**1** and (−)-**1** (> 99.8 % ee). The compound (+)-**1** · HBr crystallized in the space group $P2_1$ with unit cell dimensions a = 10.207(1), b = 13.842(4), c = 11.702(2) Å and β = 95.17(1)°. The structure was determined from X-ray diffraction data and refined by full-matrix least-squares (R = 0.041). The absolute configuration was found to be (2*R*), in agreement with a previously reported chemical correlation. The asymmetric unit contains two molecules which exhibit a small conformational difference only with respect to their *N,N*-dipropylamino moieties.

8-Hydroxy-2-(dipropylamino)tetralin (8-OH DPAT, **1**)[1] is a highly potent 5-hydroxytryptamine (5-HT)-receptor agonist[2] with pronounced selectivity for 5-HT_{1A} receptor sites[3] (for recent reviews, see Refs. 4–6). The enantiomers of **1** have been prepared from a resolved synthetic precursor (2-benzylamino-8-methoxytetralin, **2**).[1] Surprisingly, **1** turned out to be weakly stereoselective in its interaction with 5-HT receptors, (+)-**1** being only twice as potent as (−)-**1**.[1] The absolute configuration of the more potent enantiomer [(+)-**1**] has been deduced by chemical correlation with 2-(dipropylamino)tetralin to be (*R*).[7]

In this report we describe (a) an improved method for resolution of the synthetic precursor **2**, (b) a determination of the enantiomeric impurity of the enantiomers of **1** obtained by an effective ion-pair chromatographic separation achieved by addition of an optically active counter ion to the organic mobile phase,[8] and (c) an X-ray crystallographic analysis of (+)-**1** · HBr which shows that the previously reported[7] absolute configuration of (+)-**1** [i.e. (2*R*)] is correct.

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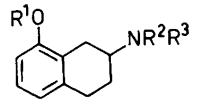

1: $R^1 = H$, $R^2 = R^3 = C_3H_7$
2: $R^1 = CH_3$, $R^2 = CH_2Ph$, $R^3 = H$

Experimental

General comments. Melting points (uncorrected) in open glass capillaries were determined on a Thomas-Hoover apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Resolution of (±)-2-benzylamino-8-methoxytetralin [(±)-**2**]. (−)-Di-*p*-toluoyl-L-tartaric acid (23.7 g, 64 mmol) was added to a hot solution of (±)-**2** (16.35 g, 61 mmol) in 165 ml of 95 % EtOH. The mixture was allowed to stand for 5 days and the crystals formed were collected. Three recrystallizations from 95 % EtOH afforded 6.65 g of the tartrate, which was converted into the hydrochloride. The yield of (2*R*)-**2** · HCl was 2.99 g (32 %). M.p. 240–241 °C; $[\alpha]_D^{20}$ +63.7° (*c* 1.0, MeOH). Lit.[7]: M.p. 239–240 °C; $[\alpha]_D^{20}$ +63.3° (*c* 1.0, MeOH).

The combined mother-liquors from the above crystallizations were concentrated and the residue was partitioned between aqueous NaOH (1 M) and ether to afford the partially resolved free base (10.45 g). The base was dissolved in hot EtOH and the solution was treated with an equimolar amount of (+)-di-*p*-toluoyl-L-tartaric acid as described above. Three recrystallizations from EtOH gave 6.85 g of the tartrate, which was converted into the hydrochloride. The yield of (2*S*)-**3** · HCl was 3.15 g (34 %). M.p. 240–241 °C; $[\alpha]_D^{20}$ −63.2 (*c* 1.0, MeOH). Lit.[7]: M.p. 240–241 °C; $[\alpha]_D^{20}$ −62.5° (*c* 1.0, MeOH).

Compounds (*S*)- and (*R*)-**2** · HCl were converted to (*S*)- and (*R*)-**1** by previously reported methods.[1,7] The enantiomers of **1** were identical (M.p., ^{1}H NMR, ^{13}C NMR, $[\alpha]_D^{20}$) to those previously reported.[1,7]

Ion-pair chromatography. The HPLC system employed an LDC Constametric III pump and the injector was a Rheodyne model 7125 with a 20 µl loop. The detector was an LDC SpectroMonitor III with a 12 µl cell monitoring at 278 nm. Two columns (length 150 mm and i.d. 3.0 mm) connected via LDV unions were used in the separation system. The solid phase was LiChrosorb DIOL (5 µm). Details on packing and testing of the columns have been described previously.[8]

The CH_2Cl_2 was dried over molecular sieves (4 Å) before use.[8] The mobile phase was prepared by mixing dry (< 30 ppm H_2O) and water-saturated CH_2Cl_2 to give a water content of about 80 ppm. The concentration of the counter ion, *N*-benzyloxycarbonylglycyl-L-proline (L-ZGP) or *N*-benzyloxycarbonylglycyl-D-proline (D-ZGP), was 2.5 mM and that of triethylamine 0.4 mM.

The mobile phase flow rate was 0.5 ml min^{-1}. The samples of (+)- or (−)-**1** were dissolved in the mobile phase before being injected.

X-Ray crystallography. General. Crystals of (+)-**1** · HBr were grown from an acetonitrile/ethanol solution, and a crystal with the dimensions 0.48×0.32×0.29 mm was used for data collection on an Enraf-Nonius CAD4F-11 diffractometer. The angular settings of 25 reflections were measured to calculate the lattice parameters. Two sets of independent reflections with $\Phi < 60°$ were collected by the $\Phi/2\Phi$ scan method using monochromated Cu$K\alpha$ radiation. Intensity measurements made on three control reflections every 2 h indicated minor decay (2 %) of the crystal. The measured intensities were re-scaled to account

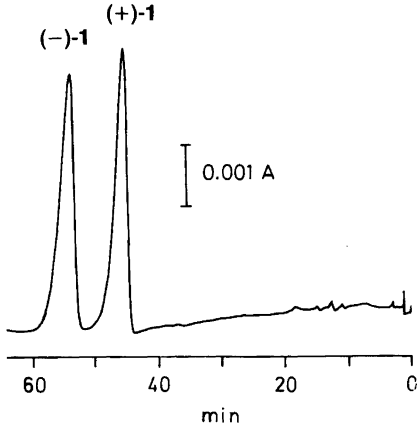

Fig. 1. Resolution of racemic 8-hydroxy-2-(dipropylamino)tetralin (**1**). Columns: two 150×3.0 mm, LiChrosorb DIOL. Mobile phase: *N*-benzyloxycarbonylglycyl-L-proline (L-ZGP) (2.50 mM) and triethylamine (0.40 mM) in dichloromethane (80 ppm water).

for this decay. A total of 5189 reflections were recorded and of these, 4449 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorentz and polarization effects but not for absorption or extinction.

Crystal data. Molecular formula $C_{16}H_{25}NO \cdot HBr$, space group P_1, unit cell a = 10.207(1), b = 13.842(4), c = 11.702(2) Å and β = 95.17(1)°, V = 1647 Å^3, Z = 4, M = 328.30, d_c = 1.324 g cm^{-3}, μ(Cu$K\alpha$) = 36.8 cm^{-1}.

Structure solution and refinement. The structure was solved by a combination of the Patterson heavy atom method and direct methods, using the program DIRDIF[9] which provided the non-hydrogen atom positions. Methyl and hydroxy hydrogen positions were determined from Fourier difference synthesis maps, and remaining hydrogen atoms were introduced at expected positions. Refinement was carried out by the full-matrix least-squares method using anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were assigned a common temperature factor (B = 5 Å^2). The hydrogen atom parameters were not refined. In order to determine the absolute configuration of (+)-**1** · HBr, anomalous dispersion factors[10] were introduced for the non-hydrogen atoms. The atomic parameters for the non-hydrogen atoms of both enantiomers were then refined. Two sets of unique reflections ($hk\pm l$, $h\text{-}k\pm l$) were used in the refinement and non-observed reflections were allowed to contribute when $F_{calc} > F_{obs}$. When the refinement was completed the residuals for the (2R)- and (2S)-enantiomers were culated to be R = 0.041 and R = 0.049 (R_w = 0.061 and R_w = 0.076), respectively. Using Hamilton's test,[11] the ratio $R_w(2S)/R_w(2R)$ is sufficiently great to reject the (2S)-enantiomer at the 0.005 significance level. Furthermore, among the 77 Bijvoet pairs for which $|F_{calc}(hkl)\text{-}F_{calc}(h\text{-}kl)| > 1.7$ for the (2R)-enantiomer, 67 of the F_{calc} differences had the same sign as the corresponding F_{obs} differences. The weighting scheme[12] used in the later part of the refinement was $w = 1/\{1+[(|F_{obs}|\text{-}18)/19]^2\}$. The form factors used were those given by Cromer and Mann.[13] All calcula-

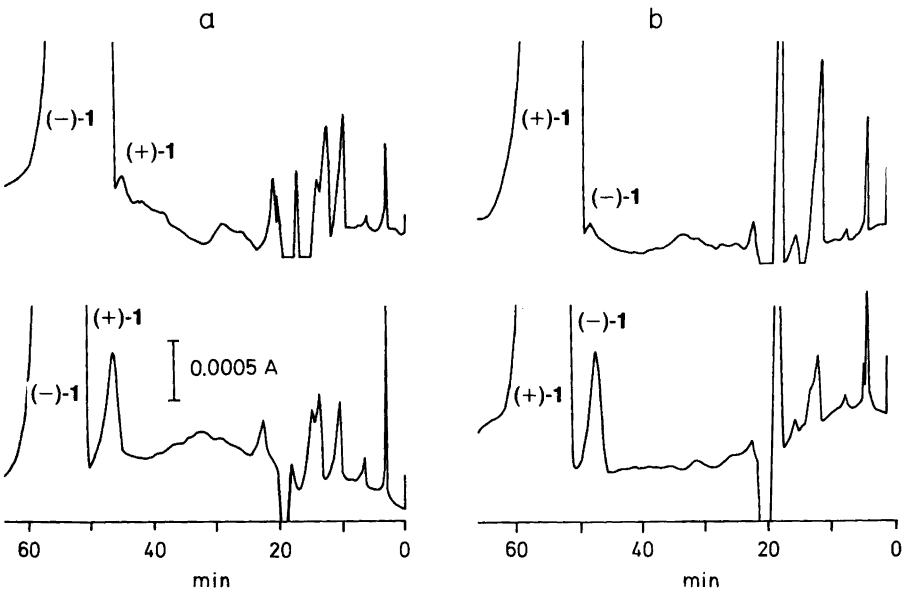

Fig. 2. Determination of the enantiomeric impurity in (−)- and (+)-8-hydroxy-2-(dipropylamino)-tetralin (**1**) [(a) and (b), respectively]. Chromatograms of samples containing an added 1 % of the respective antipode (bottom) were compared with those of the authentic samples (top). Columns: two 150×3.0 mm, LiChrosorb DIOL. Mobile phase: *N*-benzyloxycarbonylglycyl-L-proline (L-ZGP) (2.50 mM) and triethylamine (0.40 mM) in dichloromethane (80 ppm water) in (a). D-ZGP (2.50 mM) was used in (b).

Table 1. Atomic fractional coordinates and equivalent isotropic temperature factors ($x10^2$) for the non-hydrogen atoms. $U_{eq} = 1/3(U_{11} + U_{22} + U_{33} + 2 \cdot U_{13} \cdot \cos\beta)$

Atom	*x*	*y*	*z*	U_{eq}
Molecule A				
Br(1)	0.2825(1)	−0.3124(–)	0.8004(1)	6.9(1)
N(1)	0.5484(5)	−0.4107(4)	0.9231(4)	5.6(2)
O(1)	0.3953(4)	−0.7278(3)	0.7464(4)	6.8(2)
C(1)	0.4650(6)	−0.6894(4)	0.6625(6)	5.6(3)
C(2)	0.4668(6)	−0.7336(5)	0.5546(6)	6.5(3)
C(3)	0.5370(7)	−0.6897(5)	0.4743(6)	6.7(4)
C(4)	0.6064(6)	−0.6056(5)	0.4984(5)	6.0(3)
C(5)	0.6033(6)	−0.5630(4)	0.6062(5)	5.0(3)
C(6)	0.5310(5)	−0.6040(4)	0.6873(5)	4.9(3)
C(7)	0.5169(7)	−0.5591(4)	0.8028(5)	5.7(3)
C(8)	0.6047(5)	−0.4696(4)	0.8303(5)	5.3(3)
C(9)	0.6156(6)	−0.4094(5)	0.7220(5)	5.5(3)
C(10)	0.6776(6)	−0.4711(4)	0.6336(5)	5.5(3)
C(11)	0.5984(10)	−0.3014(9)	0.9462(7)	10.9(6)
C(12)	0.7287(13)	−0.3097(11)	0.9761(8)	14.5(9)
C(13)	0.7678(9)	−0.1923(6)	1.0011(7)	8.7(5)
C(14)	0.5328(8)	−0.4662(6)	1.0310(6)	7.3(4)
C(15)	0.4349(12)	−0.4343(9)	1.1009(8)	12.0(8)
C(16)	0.4214(13)	−0.4904(8)	1.2059(8)	13.2(9)
Molecule B				
Br(1)	−0.1955(1)	−0.4083(1)	0.3110(1)	5.9(1)
N(1)	0.0345(4)	−0.2704(3)	0.4331(4)	4.5(2)
O(1)	0.2191(6)	−0.2388(4)	0.0511(4)	8.3(3)
C(1)	0.1485(6)	−0.1568(5)	0.0533(5)	6.1(3)
C(2)	0.1353(7)	−0.0907(6)	−0.0371(5)	6.8(4)
C(3)	0.0594(7)	−0.0090(5)	−0.0281(5)	6.5(4)
C(4)	−0.0005(6)	0.0083(5)	0.0703(5)	6.0(3)
C(5)	0.0141(6)	−0.0554(4)	0.1621(5)	5.2(3)
C(6)	0.0886(5)	−0.1373(4)	0.1544(5)	5.0(3)
C(7)	0.1044(6)	−0.2145(4)	0.2477(5)	5.5(3)
C(8)	0.0483(5)	−0.1814(4)	0.3580(5)	4.7(3)
C(9)	−0.0807(6)	−0.1313(4)	0.3325(5)	5.2(3)
C(10)	−0.0579(6)	−0.0380(4)	0.2675(5)	5.6(3)
C(11)	−0.0467(7)	−0.2556(4)	0.5353(5)	6.1(3)
C(12)	0.0028(7)	−0.1745(5)	0.6156(5)	7.0(4)
C(13)	−0.0861(10)	−0.1721(8)	0.7152(7)	10.1(6)
C(14)	0.1662(5)	−0.3179(4)	0.4680(5)	5.8(3)
C(15)	0.1669(7)	−0.4218(5)	0.4498(6)	6.9(4)
C(16)	0.2942(7)	−0.4679(5)	0.4973(6)	7.1(4)

tions were performed on a DEC-system-10 computer using mainly the X-ray 72 program system.[14]

Results and discussion

Resolution. Previously, (±)-**2** has been resolved into the enantiomers by use of fractional crystallization of the diastereomeric tartrates.[1,7] This procedure afforded (−)-**2** in 12 % and (+)-**2** in 23 % yield. Use of di-*p*-toluoyltartaric acid instead of tartaric acid as the resolving agent improved the yield of (−)-**2** almost three-fold and that of (+)-**2** was increased by about 50 %.

Table 2. Interatomic distances (Å) and angles (°) for the non-hydrogen atoms.

Distance	A	B	Angle	A	B
C(1) – O(1)	1.371(8)	1.346(9)	C(8) – N(1) – C(11)	119.4(5)	115.2(4)
C(1) – C(2)	1.405(9)	1.396(9)	C(8) – N(1) – C(14)	113.9(5)	112.2(4)
C(1) – C(6)	1.379(8)	1.406(9)	C(11) – N(1) – C(14)	113.3(5)	112.1(4)
C(2) – C(3)	1.373(10)	1.379(10)	O(1) – C(1) – C(2)	121.6(5)	123.6(6)
C(3) – C(4)	1.379(10)	1.372(9)	O(1) – C(1) – C(6)	117.2(5)	116.9(6)
C(4) – C(5)	1.396(8)	1.388(8)	C(2) – C(1) – C(6)	121.1(6)	119.5(6)
C(5) – C(6)	1.376(8)	1.373(9)	C(1) – C(2) – C(3)	118.2(6)	119.7(6)
C(5) – C(10)	1.500(8)	1.511(9)	C(2) – C(3) – C(4)	121.6(6)	120.2(6)
C(6) – C(7)	1.506(8)	1.526(8)	C(3) – C(4) – C(5)	119.3(6)	120.9(6)
C(7) – C(8)	1.547(8)	1.529(8)	C(4) – C(5) – C(6)	120.4(5)	119.7(6)
C(8) – N(1)	1.512(8)	1.528(7)	C(4) – C(5) – C(10)	120.0(5)	120.3(5)
C(8) – C(9)	1.529(8)	1.495(8)	C(6) – C(5) – C(10)	119.6(5)	119.9(5)
C(9) – C(10)	1.523(9)	1.527(9)	C(1) – C(6(– C(5)	119.4(5)	120.0(5)
C(11) – N(1)	1.613(13)	1.529(8)	C(1) – C(6) – C(7)	117.3(5)	116.2(5)
C(11) – C(12)	1.349(16)	1.521(9)	C(5) – C(6) – C(7)	123.3(5)	123.7(5)
C(12) – C(13)	1.693(17)	1.541(12)	C(6) – C(7) – C(8)	114.6(5)	111.7(5)
C(14) – N(1)	1.499(9)	1.519(7)	N(1) – C(8) – C(7)	109.3(5)	107.4(4)
C(14) – C(15)	1.418(14)	1.455(9)	N(1) – C(8) – C(9)	111.2(5)	111.3(5)
C(15) – C(16)	1.471(16)	1.508(10)	C(7) – C(8) – C(9)	110.4(5)	111.2(5)
			C(8) – C(9) – C(10)	108.8(5)	108.7(5)
			C(5) – C(10) – C(9)	112.8(5)	112.3(5)
			N(1) – C(11) – C(12)	104.7(10)	114.2(5)
			C(11) – C(12) – C(13)	100.0(10)	107.3(6)
			N(1) – C(14) – C(15)	117.4(7)	113.8(5)
			C(14) – C(15) – C(16)	116.3(10)	112.3(5)

Ion-pair chromatographic determination of enantiomeric purity. Chromatographic systems employing *N*-benzyloxycarbonylglycyl-L-proline (L-ZGP) as chiral selector in the mobile phase have been used previously to separate enantiomers of aminoalcohols.[8,15] Use of ZGP also led to com plete resolution of racemic **1** (Fig. 1). The separation factor (α) obtained was 1.20 and the resolution factor (R_S) was 1.9. The good chromatographic resolution enables detection and determination of enantiomeric impurities even in very low concentrations. Furthermore, the use of a chiral additive (e.g. ZGP) in the mobile phase has the advantage that the accuracy of the determination of enantiomeric excess (ee) is independent of the optical purity of the counter ion as long as sufficient resolution is obtained.[15]

Chromatograms of samples of (+)- and (−)-**1** to which 1% of the opposite enantiomer had been added are shown in Fig. 2. The order of elution of the enantiomers of **1** could be regulated by using the D- or L-form of the counter ion (ZGP). Thus, the peak corresponding to the enantiomeric impurity could easily be made to elute prior to the main enantiomer, and this facilitated the determinations of the enantiomeric compositions. The % ee of the enantiomers of **1** was estimated to be greater than 99.8% (Fig. 2). Thus, the low stereoselectivity of the interaction of **1** with 5-HT_{1A} receptors is not due to the presence of enantiomeric impurities.

*X-Ray crystal structure of (+)-**1** · HBr.* The coordinates and the equivalent isotropic temperature factors for the non-hydrogen atoms are given in Table 1. Tables of structure factors, and listings of anisotropic temperature factors and hydrogen atom parameters may be obtained from one of the authors (s.s.) on request. Bond distances and angles are given in Table 2. The atom numbering and the molecular conformations of the two independent molecules A and B are shown in Fig. 3.

The tetralin moieties of molecules A and B adopt half-chair conformations with pseudo-equatorial dipropylammonium substituents. In fact, the only significant conformational differ-

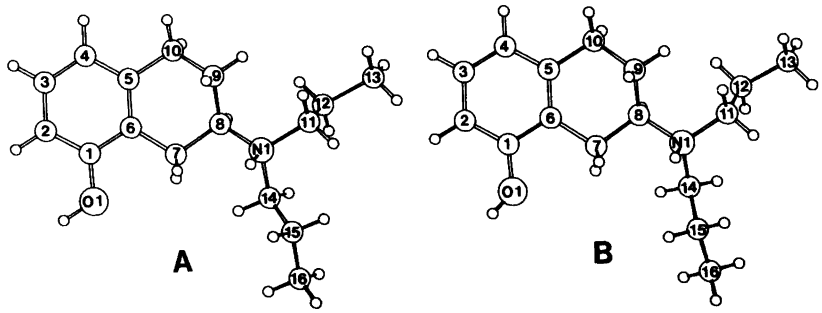

Fig. 3. Molecular conformation and atom-numbering system for the two independent molecules A and B of (2*R*)-(+)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide [(+)-**1** · HBr].

ence between A and B is found in the propyl groups, which assume slightly different conformations. The conformations of molecules A and B are very similar to a previously reported[16] molecular mechanics (MMP2)[17] conformation having a relative steric energy 0.6 kcal mol^{-1} above the lowest energy conformation identified. This particular conformation was also obtained after MMP2 minimization using the geometry of either molecule A or B as starting geometry.

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