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Note

Separation of enantiomers of derivatised or underivatised propranolol by means of high-performance liquid chromatography

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During investigations leading towards the development of stereoselective radioimmunoassays for the enantiomers of propranolol, we wished to study the immunogenecity of drug–protein conjugates in which the nitrogen atom of N-desisopropylpropranolol was coupled to a protein. The separation of enantiomers of N-desisopropylpropranolol has not been described, although a number of methods for the separation of the optical antipodes of propranolol have been reported. Most of these methods are based on the derivatisation of propranolol with chiral derivatising agents [1–8]. Other methods [8–12] have made use of chiral chromatographic columns, although it was found necessary to convert propranolol to suitable achiral derivatives in order to promote good chromatographic separation of enantiomers [9–12]. On the other hand, Petterson and Schill [11] separated the underivatised enantiomers of propranolol by means of a high-performance liquid chromatography (HPLC) system which employed *d*-10-camphorsulfonate in the mobile phase as a chiral counter ion.

In the present study, we were faced with the task of separating the enantiomers either of N-desisopropylpropranolol itself or of some useful derivative. We attempted to separate the optical antipodes of propranolol and two secondary aminoester derivatives which were needed in the preparation of chiral haptens for the development of stereoselective radioimmunassays. This paper describes a simple HPLC system which achieved baseline resolution of the β-aminoester derivative by means of a recycle device attached to the HPLC pump.

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EXPERIMENTAL

Materials and apparatus

The chromatography was performed with a Waters Model 590 HPLC pump equipped with a solvent select valve with recycling capability (a device to recycle the eluate through the HPLC column as required). The detector was a Model 480 Lambda Max variable ultraviolet (UV) detector (Waters Scientific, Mississauga, Canada) operating at 280 nm. The injector was Rheodyne Model 7126 with 2-ml loop. The separation was carried out on a Zorbax cyano column, 25 cm × 9.5 mm I.D. (Dupont).

Melting points were determined in open glass capillaries with a Gallenlamp melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Beckman infrared spectrophotometer, Model Acculab 4. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained at 60 MHz on a T-60-NMR spectrometer with deuterated chloroform as the solvent and tetramethylsilane (TMS) as the internal standards. Chemical shifts are expressed in δ units (parts per million, ppm) relative to TMS. Low-resolution electron-impact mass spectra were recorded on a VG micromass MM 16F instrument, 70 eV. Optical rotations were determined with a Perkin-Elmer 241 MC polarimeter.

d-10-Camphorsulfonic acid (99%), *tert*-butylamine (98%), triethylamine (99+% Gold Label), diethylamine (98%), *n*-butylamine (99%), nonylamine (98%) and methylacrylate were purchased from Aldrich (Montreal, Canada). All solvents used for the preparations of mobile phase were of HPLC grade and were supplied by Fisher Scientific (Edmonton, Canada).

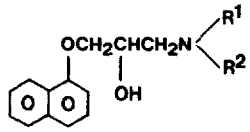
Method

N-Desisopropylpropranolol was synthesised by a published method [12].

dl-1-(2-Methoxycarbonylethyl)amino-3-(1-naphthoxy)-2-propanol (compound A).

Methylacrylate (0.172 g, 2.0 mmol) was added all at once to a solution of N-desisopropylpropranolol (0.434 g, 2.0 mmol) in 50 ml of methanol and stirred at room temperature for 4 h [13]. The solvent was evaporated under reduced pressure and the oily residue so obtained was purified by column chromatography (silica gel, solvent, chloroform). Recrystallization from dichloromethane yielded 0.303 g (50%) of yellow crystals, m.p. 50°C. ^{1}H NMR (C^2HCl_3): γ 2.6 (m, 7H), 3.65 (s, 4H), 4.2 (m, 3H), 7.45 (m, 7H). IR (KBr): 3300, 3090, 1740 cm^{-1}, MS: *m/z* 303 (M^+., 9%), 271 (15), 230 (29), 144 (33), 116 (100), 115 (18) and 84 (47). Found: C, 67.15; H, 6.85; N, 4.63. Calculated for $C_{17}H_{21}NO_4$ requires C, 67.30; H, 6.97; N, 4.61.

Compound A was separated by HPLC into its *d*- and *l*-enantiomers with the aid of solvent selector unit attached to the HPLC pump (see Fig. 2). The mobile phase was dichloromethane–hexane–acetonitrile (79:20:1), containing 5 m*M* *d*-10-camphorsulfonic acid and 2.5 m*M* *tert.*-butylamine. Degassing of the mobile phase was carried out by Millipore filtration (Millipore, Bedford, MA, U.S.A.). The column was equilibrated with the mobile phase for about 6 h so as to achieve constant retention times. The racemic compound A (60 μg) was introduced as



Propranolol: R^1 = H, R^2 = -$CH(CH_3)_2$
Hapten A: R^1 = H, R^2 = -$CH_2CH_2COOCH_3$
Hapten B: R^1 = -$CH(CH_3)_2$, R^2 = -$CH_2CH_2COOCH_3$

Fig. 1. Structures of the secondary amino derivative (A) and the sterically hindered tertiary amino derivative (B) of propranolol.

free base dissolved in mobile phase (50 μl). Baseline separation of enantiomers was achieved by setting the solvent selector unit to recycle the eluate through the column three times. Appropriate fractions of HPLC eluate were then collected. The enantiomers of hapten A were recovered from the mobile phase after disruption of the ion pair with 0.1 *M* sodium hydroxide and subsequently salting out the free base by addition of sodium chloride. The free bases of the enantiomers were then converted to amorphous hydrochloride salts by addition of a cold solution of hydrogen chloride gas in diethyl ether. Elemental analysis of each enantiomer agreed closely with the calculated value. Calculated for *dl*-1-(2-methoxycarbonylethyl)amino-3-(1-naphthoxy)-2-propanol hydrochloride, $C_{17}H_{21}NO_4HCl$: C, 60.09; H, 6.53; N, 4.12. Found: *d*-A-HCl: C, 60.15; H, 6.40; N, 4.15; *l*-A-HCl: C, 60.01; H, 6.47; N, 4.18. Specific rotation: *d*-A-HCl: +16.0° (*c* 0.1, ethanol); *l*-A-HCl: −16.05° (*c* 0.1, ethanol). Compound B (Fig. 1) was prepared similarly by the reaction of propranolol with methyl acrylate. The enantiomers of B did not separate, even after several passes through the HPLC column.

Calculations

The capacity factor (k) was calculated from the expression $k=(V_1-V_0)/V_0$ where V_1 was the retention (volume, time or distance) of the sample and V_0 was the retention of the void volume. The separation factor (α) was calculated from the expression $\alpha=k_2/k_1=(V_2-V_0)/(V_1-V_0)$ where V_2 and V_1 are the retention volumes of the two enantiomers.

RESULTS AND DISCUSSION

Samples of optically pure propranolol and the propranolol hapten A (Fig. 1) were conveniently isolated by means of the HPLC system described above which employed a polar bonded cyanopropyl silica stationary phase and a relatively low polarity mobile phase containing *d*-10-camphorsulfonic acid as a chiral counter-ion and *tert.*-butylamine as a competing base. There was no resolution of enantiomers in the absence of a competing base, or when a non-polar (μBondapak C_{18}) stationary phase was employed. Using the HPLC system described above, however, the enantiomers of underivatised propranolol showed baseline separa-

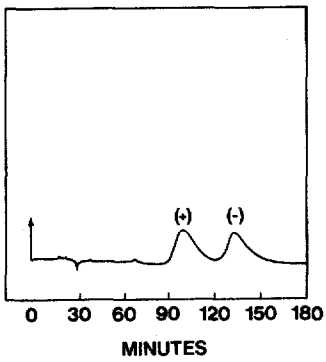


Fig. 2. Separation of propranolol in a single pass.

tion (Fig. 2), although the *dl*-hapten A was only partially resolved after a single pass through the cyanopropyl silica HPLC column ($\alpha = 1.06$). Attempts were made to improve the separation of the enantiomers of the hapten A by modification of the relative proportions of the constituents of the mobile phase. Changes in the concentrations of acetonitrile, hexane, *d*-10-camphorsulfonic acid or *tert.*-butylamine tended to alter retention times (capacity factors) of both enantiomers, without significant effect on the separation factor. The problem was solved (Fig. 3) by recycling the eluate three to four times through the column by means of the convenient recycling device attached to the HPLC pump. The separation factor increased in a linear manner with the number of passes through the HPLC system (Fig. 4). Fig. 2 shows that the HPLC system gave good peak symmetry which is important if the recycling technique is to be successful. Important in this regard was the selection of *tert.*-butylamine as the competing base. A variety of other competing bases gave comparable separation factors (Table I), but none matched the peak symmetry achieved by *tert.*-butylamine. After collection of eluate fractions and isolation of enantiomers, specific rotation measurements revealed that the *d*-enantiomer eluted before the *l*-antipode.

It would appear that the mechanism of separation involved a process in which the protonated enantiomers of propranolol or hapten A competed with *tert.*-butylamine for interaction with *d*-10-camphorsulfonate. It is likely that there was dynamic ion exchange of the enantiomers of A with competing base from *tert.*-butylamine/*d*-10-camphorsulfonate ion pairs immobilized on the stationary phase.

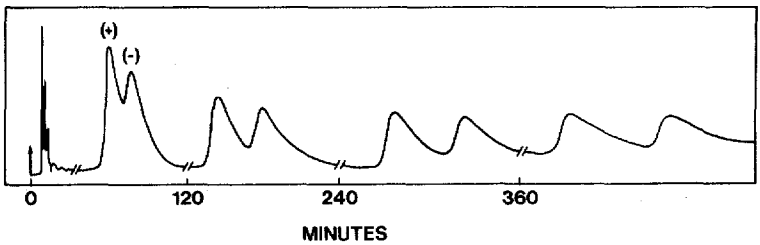


Fig. 3. Chromatogram showing the separation of *dl*-A by recycling the eluate four times through the HPLC column.

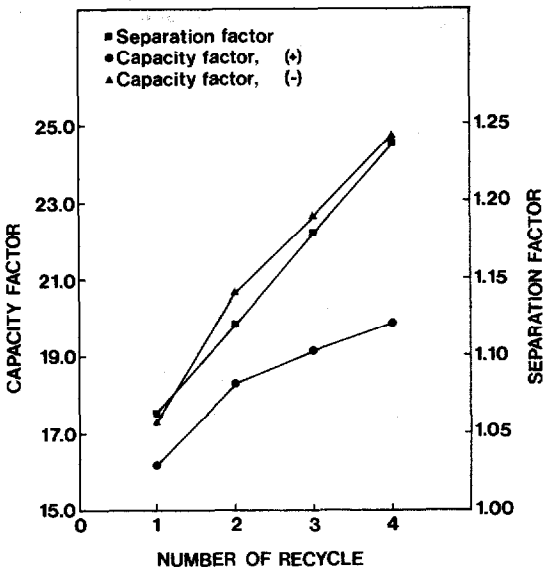


Fig. 4. Effects of number of recycle passes through the HPLC column on the capacity factor (k) and the separation factor (α).

The propranolol or hapten A/*d*-10-camphorsulfonate formed are essentially diastereomers, having different degrees of solvation. Fulfillment of the Dalgliesh three-point rule [14] for separation of enantiomers can be attributed to (i) hydrophobic interaction between the ring systems of protonated propranolol or hapten A and *d*-10-camphorsulfonate, (ii) to electrostatic interaction between their charged centers and (iii) to hydrogen bonding between the hydroxy group of propranolol or hapten A and the oxo group of *d*-10-camphorsulfonate. The latter interaction would be critically dependent on the configuration of the chiral centre and, thus, would be a crucial factor in the separation of enantiomers.

It is noteworthy that hapten B (Fig. 1) was not resolved by the present system. Unlike propranolol or hapten A which are secondary amines, the amino group of

TABLE I

EFFECT OF DIFFERENT COMPETING BASE IN THE MOBILE PHASE

Mobile phase: 5 m*M* *d*-10-camphorsulfonic acid-2.50 m*M* competing base in dichloromethane–hexane–acetonitrile (79:20:1).

Competing base	Capacity factor (k)		Separation factor (α)
	(+)	(−)	
tert.-Butylamine	16.24	17.28	1.064
Triethylamine	23.62	25.03	1.060
Diethylamine	13.24	13.60	1.030
n-Butylamine	12.58	13.22	1.050
N-Nonylamine	96.30	101.60	1.055

hapten B is substituted with three bulky groups which would likely hinder ionic interaction between protonated B and *d*-10-camphorsulfonate.

According to Petterson and Schill [11], enantiomeric separation of propranolol can be achieved using a Nucleosil cyano column and a mobile phase containing *d*-10-camphorsulfonic acid but no competing base. We found that the latter system, however, gave poor peak symmetry and erratic chromatographic behaviour. By contrast, the present HPLC system gives good peak symmetry (Fig. 2) in the separation of the enantiomers of propranolol. Furthermore, our system does not require the elimination of water from the solvents prior to eluent preparation and stable operating conditions were obtained after 6 h of recirculation of the mobile phase through the column. These observations suggest that our system differs in mechanism of retention from that proposed by Petterson and Schill [11].

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REFERENCES

1 B. Silber, N.H.G. Holford and S. Riegelman, J. Pharm. Sci., 71 (1982) 699.
2 C. Von Bahr, J. Hermansson and K. Tawara, Br. J. Clin. Pharmacol., 14 (1982) 79.
3 C. Von Bahr, J. Hermansson and M.J. Lind, Pharmacol. Exp. Ther., 222 (1982) 458.
4 J. Hermansson and C. Von Bahr, J. Chromatogr., 221 (1980) 109.
5 B. Silber and S. Riegelman, J. Pharmacol. Exp. Ther., 215 (1980) 643.
6 J.A. Thompson, J.L. Holtzman, M. Tsuru, C.L. Lerman and J.L. Holtzman, J. Chromatogr., 238 (1982) 470.
7 A.J. Sedman and J. Gal, J. Chromatogr., 278 (1983) 199.
8 Y. Okamoto, M. Kawashima, R. Aburarani, K. Hatada, T. Nishiyama and M. Masuda, Chem. Lett., (1986) 1237.
9 W.H. Pirkle, J.M. Finn, J.L. Schreiner and B.C. Hamper, J. Am. Chem. Soc., 103 (1981) 3964.
10 I.W. Wainer, T.D. Doyle, K.H. Donn and J.R. Powell, J. Chromatogr., 306 (1984) 405.
11 C. Pettersson and G. Schill, J. Chromatogr., 204 (1981) 179.
12 T. Walle and T.W. Gaffney, J. Pharmacol. Exp. Ther., 182 (1972) 83.
13 J.W. Hubbard, K.K. Midha and J.K. Cooper, J. Pharm. Sci., 67 (1978) 1563.
14 C.E. Dalgliesh, J. Chem. Soc., (1952) 3940.