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Note

Optical resolution of β-blocking agents by thin-layer chromatography and high-performance liquid chromatography as diastereomeric *R*-(—)-1-(1-naphthyl)ethylureas

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The pharmacological effects of adrenergic β-blocking drugs are mainly due to the *S*-forms[1]. *S*-(−)-Propanolol, for example, is 100 times more active than the *R*-(+)-enantiomer. With the exception of stereoselective syntheses, racemates are obtained in drug synthesis. To avoid unnecessary stress on the organism caused by inactive, or in some cases toxic, forms, the resolution of racemates and the administration of pure enantiomers is an interesting approach.

Chromatographic methods have been used for analytical purposes to a great extent in recent years[2–4]. The few methods published for the HPLC resolution of racemic β-blocking agents are based on direct separation or the use of chiral derivatization reagents. A direct separation of some β-blocking agents by ion-pairing chromatography using D-camphor sulphonic acid as chiral additive to the mobile phase was described by Pettersson and Schill[5]. Hermansson and Von Bahr[6,7] used N-trifluoroacetyl-*S*-propyl chloride, *tert.*-butoxycarbonyl-L-alanine anhydride and *tert.*-butoxycarbonyl-L-leucine anhydride as chiral derivatization reagents for the HPLC resolution of *R,S*-propranolol and some other β-blockers. More recent work describes the use of *R*-(+)-1-phenylethyl isocyanate[8] and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate[9] as chiral reagents for the HPLC resolution of some β-blocking agents.

We have investigated the applicability of *R*-(−)-1-(1-naphthyl)ethyl isocyanate[10] for the resolution of β-blocking agents[11]. This reagent shows some advantages over *R*-(+)-1-phenylethyl isocyanate with the respect to resolution and sensitivity of detection. The NEIC derivatives show a high UV absorption and a strong fluorescence.

EXPERIMENTAL

Chemicals and reagents

All solvents used were of analytical grade and obtained from Merck (Darmstadt, F.R.G.). *R*-(−)-1-(1-Naphthyl)ethyl isocyanate [*R*-(−)-NEIC] was purchased from Ega Chemie (Steinheim, F.R.G.). *R,S*-Propranolol and *R,S*-alprenolol were purchased from Sigma (München, F.R.G.). *R,S*-Pindolol was obtained from Sandoz (Basel, Switzerland). *R,S*-Oxprenolol, *R,S*-bunitrolol and *R,S*-metroprolol were obtained from Hässle (Mölndal, Sweden).

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HPLC instrumentation and conditions

The HPLC system consisted of a Perkin-Elmer Series 2 liquid chromatograph equipped with a Rheodyne 7105 injector and a Perkin-Elmer LC-55 UV detector. UV detection was carried out at 290 nm. For fluorescence detection, a Parkin-Elmer spectrofluorometer MPF 44 equipped with a 20-μl Hellma flow-through cell was used. The excitation monochromator was set at 285 nm and the emission monochromator at 335 nm. The separations were carried out on a Knauer RP-18 column (20 × 0.4 cm I.D.) with methanol–water (70:30 or 60:40) as mobile phase.

TLC conditions

The solutions were applied to HPTLC silica gel plates (Merck, Darmstadt, F.R.G.) with a 200-μl Pt–Ir capillary (Antech, Bad Dürkheim, F.R.G.). Development was performed in benzene–ether–acetone (88:10:5) over a distance of 5 cm.

Derivatization procedure

To 1–50 μmol of the free bases or their salts (Fig. 1) dissolved in a mixture of dry chloroform and dimethylformamide (8:2), an equimolar amount of triethylamine and a *ca.* two-fold molar excess of *R*-(−)-NEIC were added. After a reaction time of 20 min the excess reagent was destroyed by the addition of diethylamine. After a further 15 min an aliquot of the reaction mixture was transferred to a TLC plate or to the liquid chromatograph.

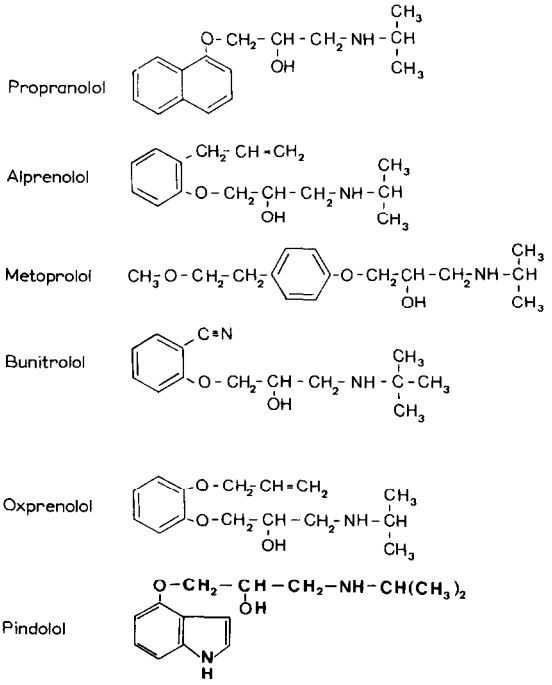


Fig. 1. Structures of the drugs investigated.

RESULTS AND DISCUSSION

Isocyanates form ureas with amines and carbamates with alcohols. This reaction principle has already been used for UV[12] and fluorescence[13,14] derivatization in HPLC and TLC. The reaction of *R*-(−)-NEIC with the investigated 1-aryloxy-3-isopropylamino-2-propanol derivatives leads uniformly to diastereomeric ureas, which can be separated by TLC or HPLC. Reaction takes place only with the amino group. The hydroxy group does not react under the conditions applied. No racemization is observed during the reaction. The addition of triethylamine allows for the direct reaction of the salts without the preliminary liberation of the free bases. To destroy the excess isocyanate, diethylamine is added after the reaction. The reaction scheme is shown in Fig. 2.

As can be seen from Fig. 3, all the derivatives investigated are well resolved on HPTLC silica gel plates. The *R*-(+) forms always have higher R_F values than the *S*-(−) isomers (Table I). HPLC separations were carried out on RP-18 columns using methanol–water as mobile phase.

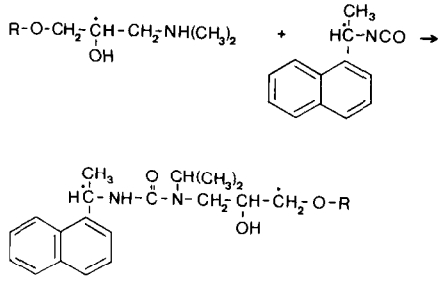


Fig. 2. Scheme of the reaction of 1-aryloxy-3-isopropylamino-2-propanols with *R*-(−)-1-(1-naphthyl)ethyl isocyanate.

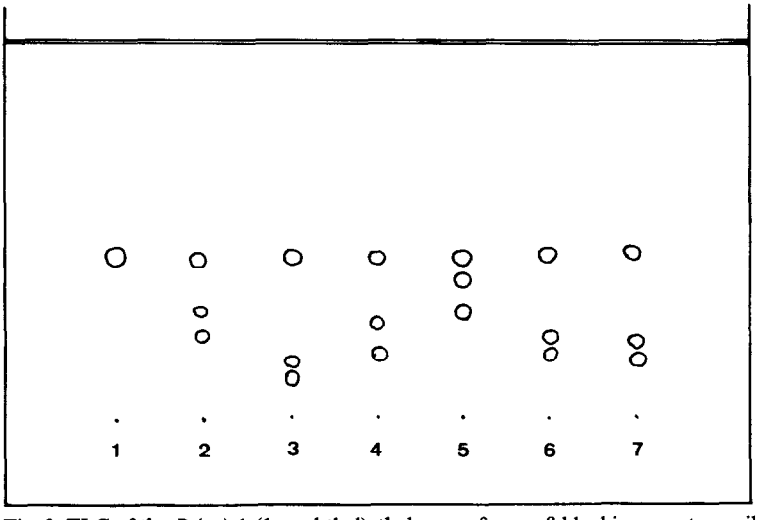


Fig. 3. TLC of the *R*-(−)-1-(1-naphthyl)ethylureas of some β-blocking agents on silica gel HPTLC plates. Solvents, benzene–ether–acetone (88:10:5). Spots: 1, blank; 2, bunitrolol; 3, metoprolol; 4, alprenolol; 5, propranolol; 6, oxprenolol; 7, pindololol.

TABLE I

R_F VALUES OF THE ENANTIOMERS OF SOME β-BLOCKING AGENTS AS THEIR NEIC DERIVATIVES

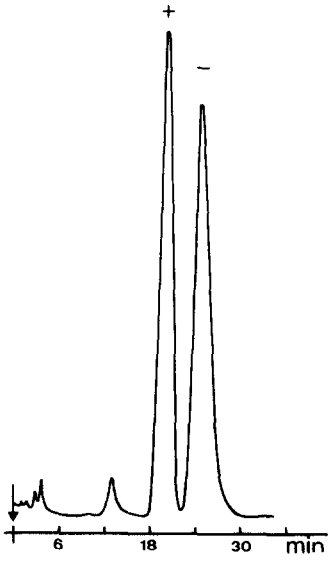
Plates, silica gel HPTLC; solvent, benzene–ether–acetone (80:10:5).

Compound	R_F	
	R	*S*
Bunitrolol	0.42	0.37
Metoprolol	0.32	0.27
Alprenolol	0.41	0.33
Propranolol	0.51	0.42
Oxprenolol	0.37	0.32
Pindolol	0.38	0.33
Blank	0.56	–

Fig. 4 shows the resolution of *R,S*-propranolol. For the determination of the elution order the pure enantiomers, resolved in a preparative scale with (+)-tolyltartaric acid, were injected after derivatization with the isocyanate.

In addition to propranolol, a series of other racemic β-blocking agents can be resolved by HPLC. The k' and α values of some derivatives are given in Table II. Fig. 5 shows the resolution of *R,S*-bunitrolol, *R,S*-metropolol and *R,S*-alprenolol.

Owing to the high molar absorptivity of the naphthylethylureas, UV absorbance is a satisfactory method of detection. Enhanced sensitivity can be obtained by



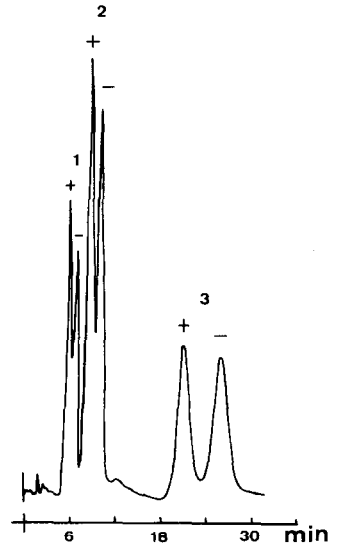


Fig. 4. Resolution of the enantiomers of propranolol as their NEIC derivatives. Column, RP-18, 25 × 0.46 cm I.D.; mobile phase, methanol–water (70:30); flow-rate, 2 ml/min; detection, UV 290 nm.

Fig. 5. Resolution of the enantiomers of bunitrolol, metoprolol and alprenolol. For conditions, see Fig. 4.

TABLE II

k' AND α VALUES OF THE ENANTIOMERS OF SOME β-BLOCKING AGENTS AS THEIR NEIC DERIVATIVES

Mobile phase, methanol–water (70:30, except for pindolol, 60:40).

	k' (R)	k' (S)	α
Bunitrolol	1.5	2.0	1.33
Metoprolol	2.5	3.2	1.3
Alprenolol	6.9	10.4	1.5
Propranolol	6.8	10.3	1.5
Oxprenolol	2.2	3.0	1.33
Pindolol	8.0	11.2	1.4

fluorescence detection. Nanogram amounts of one enantiomer derivatized with NEIC are detectable in the presence odf a 100-fold excess of the other stereoisomer. Quantitative determination of the enantiomers is also possible by this method.

As preliminary experiments have shown, the method is also applicable to sympathicomimethic drugs; *e.g.*, *R,S*-ephedrine can be resolved. Research to extend this procedure to drugs with alcoholic hydroxy groups to form the diastereomeric carbamates is also in progress.

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