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Separation of some enantiomers and diastereomers of propranolol derivatives by high-performance liquid chromatography

C. Facklam*, A. Modler

Max-Planck-Gesellschaft, Arbeitsgruppe "Asymmetrische Katalyse" an der Universität Rostock (in the "Institut für Organische Katalyseforschung an der Universität Rostock eV), Buchbinderstrasse 5–6, 18055 Rostock, Germany

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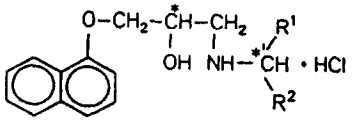
Abstract

The separation of some propranolol analogues **I** into their enantiomers and diastereomers respectively, by HPLC succeeded with cellulose tris(3,5-dimethylphenylcarbamate) as a chiral stationary phase and hexane–alcohol and aqueous $NaClO_4$–acetonitrile as mobile phases. Retention times and α values depend on the substituents R^1 and R^2. With non-aromatic substituents R^1 or R^2 the *R*-enantiomers are eluted first whereas with aromatic substituents the *S*-enantiomers, surprisingly, have shorter retention times.



I

enantiomers: $R^1 = R^2$
diastereomers: $R^1 \neq R^2$

1. Introduction

Owing to their cardiovascular properties, β-blockers play an important role in the treatment of heart diseases. As the chirality of drugs is generally an important issue from pharmacological, toxicological and regulatory points of view, the development of methods in order to determine their optical purity is important. Enantiomeric separations of propranolol derivatives by liquid chromatography may be performed after derivatization with optically pure reagents [1,2]. A different approach, however, is the chromatographic separation of enantiomers by means of chiral stationary phases (CSPs). Lee *et al.* [3] separated the enantiomers of β-blockers using various CSPs, *e.g.*, cellulose tris(3,5-dimethylphenylcarbamate) CSP with hexane–alcohol eluents. Cellulose tris(3,5-dimethylphenylcarbamate) CSP is also suitable for reversed-phase chromatography [4]. CSPs of the α_1-acid glycoprotein type have also been utilized [5,6]. Very efficient enantiomeric resolutions of β-blockers with short retention times have been achieved by means of supercritical fluid chromatography (SFC) [7,8]. Capillary electrophoresis is also useful [9,10]. Compounds of type **I** having

* Corresponding author.

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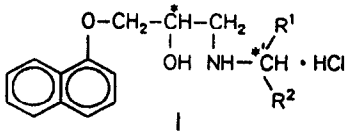


I

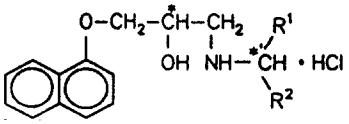
a second chiral centre ($R^1 \neq R^2$) have been investigated by Krause *et al.* [11]. We were interested in separating compounds of type I into their various stereoisomers by HPLC using Chiralcel OD-H and Chiralcel OD-R in order to analyse the products of such a synthesis.

2. Experimental

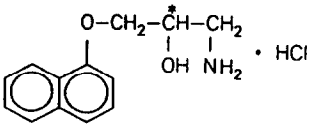
Chromatography was performed with a Model 1090 Series II liquid chromatograph equipped with a diode-array detector (Hewlett-Packard) and a Chiralyser (IBZ Messtechnik, Hannover, Germany). Separations were carried out on a Chiralcel OD-H analytical column (250 × 4.6 mm I.D.); except for resolutions at different temperatures (see Fig. 1), (150 × 4.6 mm I.D.) and a Chiralcel OD-R analytical column (250 × 4.6 mm I.D.) (Baker Chemikalien, Gross Gerau, Germany). The detection wavelengths were set at 230 and 270 nm. Chromatograms were measured at 27°C.

Acetonitrile, hexane and 2-propanol of HPLC grade were purchased from Ferak (Berlin, Germany), ethanol from Laborchemie Apolda (Apolda, Germany) and sodium perchlorate from Merck (Darmstadt, Germany). The compounds were dissolved in hexane–2-propanol at a concentration of 1 mg/ml.

Table 1
Enantiomeric separation on Chiralcel OD-H



Compound	R^1	R^2	k'_1	k'_2	α	Eluent[a]
1	H	H	2.00	5.15	1.36	1[b]
2	CH_3	CH_3	1.30	1.94	1.49	2[b]
3	C_2H_5	C_2H_5	0.52	1.32	2.54	4[b]
4	C_6H_5	C_6H_5	2.17	2.46	1.13	3[c]



Compound	R^1	k'_1	k'_2	α	Eluent
5		0.92	1.43	1.55	3[b]
6		1.05	1.39	1.32	3[b]

[a] Eluents: 1 = *n*-hexane–ethanol–2-propanol (90:2:10); 2 = *n*-hexane–ethanol–2-propanol (90:10:10); 3 = *n*-hexane–ethanol–2-propanol (90:20:10); 4 = *n*-hexane–ethanol–2-propanol (90:30:10, v/v/v).
[b] Flow-rate = 1.5 ml/min.
[c] Flow-rate = 0.5 ml/min.

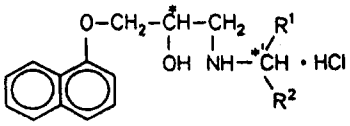
3. Results and discussion

Enantiomeric separation of propranolol analogues **1–6** (see Table 1) was performed using hexane–alcohol mobile phases. In some instances small amounts of unidentified by-products were resolved from the main products with hexane–ethanol–2-propanol eluents, but not with hexane–2-propanol eluents. Table 1 gives the k' and α values and chromatographic conditions. The retention times were dependent on the steric requirements of substituents R^1 and R^2. Depending on the size of R^1 and R^2 the alcohol content of the eluent had to be varied in order to keep the retention times low. Chiralcel OD-H shows excellent enantioselectivity for samples of **1–3** and also for **5** and **6**. Although the α value of **4** ($R^1 = R^2 = C_6H_5$) turned out to be smaller, baseline resolution of the enantiomers of **4** was achieved with eluent 3 and a flow-rate of 0.5 ml/min, but not with eluent 4 and a flow-rate of 1.5 ml/min (see Table 1). Overall, the effectiveness of separation appears to be largely dependent on the nature (aliphatic *vs.* aromatic) and size of the substituents R^1 and R^2. Although the chiral cavities of the CSP are distinguished by a high affinity for aromatic groups [12], which is in agreement with our result that compound **4** is more retained than compounds with aliphatic substituents R, the chiral recognition mechanism of the CSP–substrate interaction does not necessarily give rise to a better separation of the aromatically substituted enantiomers.

With non-aromatic substituents R^1 or R^2 the *R*-enantiomers are eluted first whereas with aromatic substituents the *S*-enantiomers [no ORD spectra of the propranolol analogues have been recorded to prove the absolute configuration, but all our substances with the *S*-configuration were prepared in the same way [11] and shown −-rotation in methanol], surprisingly, have shorter retention times. The capacity factors of the diastereomers **7** and **8** (Table 2) are sufficiently different to allow easy resolution of the four isomers of **7**; three of the four isomers of **8**, which were available to us, were likewise separated. In both instances diastereomers with the *R,R*-configuration were eluted first. Increased flow-rates and higher temperatures up to 40°C cause a decrease in retention times but do not alter the effectiveness of the separations (Fig. 1).

The enantiomeric resolution of all propranolol derivatives can be performed likewise using reversed-phase eluents with Chiralcel OD-R and

Table 2
Diastereomeric separation on Chiralcel OD-H



Compound	R^1	R^2	Configuration (**')	k'	Eluent[a]
7a	CH_3	C_6H_5	*R,R'*	2.95	1
7b	CH_3	C_6H_5	*R,S'*	4.26	1
7c	CH_3	C_6H_5	*S,S'*	4.93	1
7d	CH_3	C_6H_5	*S,R'*	6.90	1
8a	CH_3	C_6H_{11}	*R,R'*	1.10	2
8b	CH_3	C_6H_{11}	*S,S'*	2.15	2
8c	CH_3	C_6H_{11}	*S,R'*	4.71	2

[a] Eluents: 1 = *n*-hexane–ethanol–2-propanol (90:3:10); 2 = *n*-hexane–ethanol–2-propanol (90:10:10 v/v/v). Flow-rate, 1.0 ml/min.

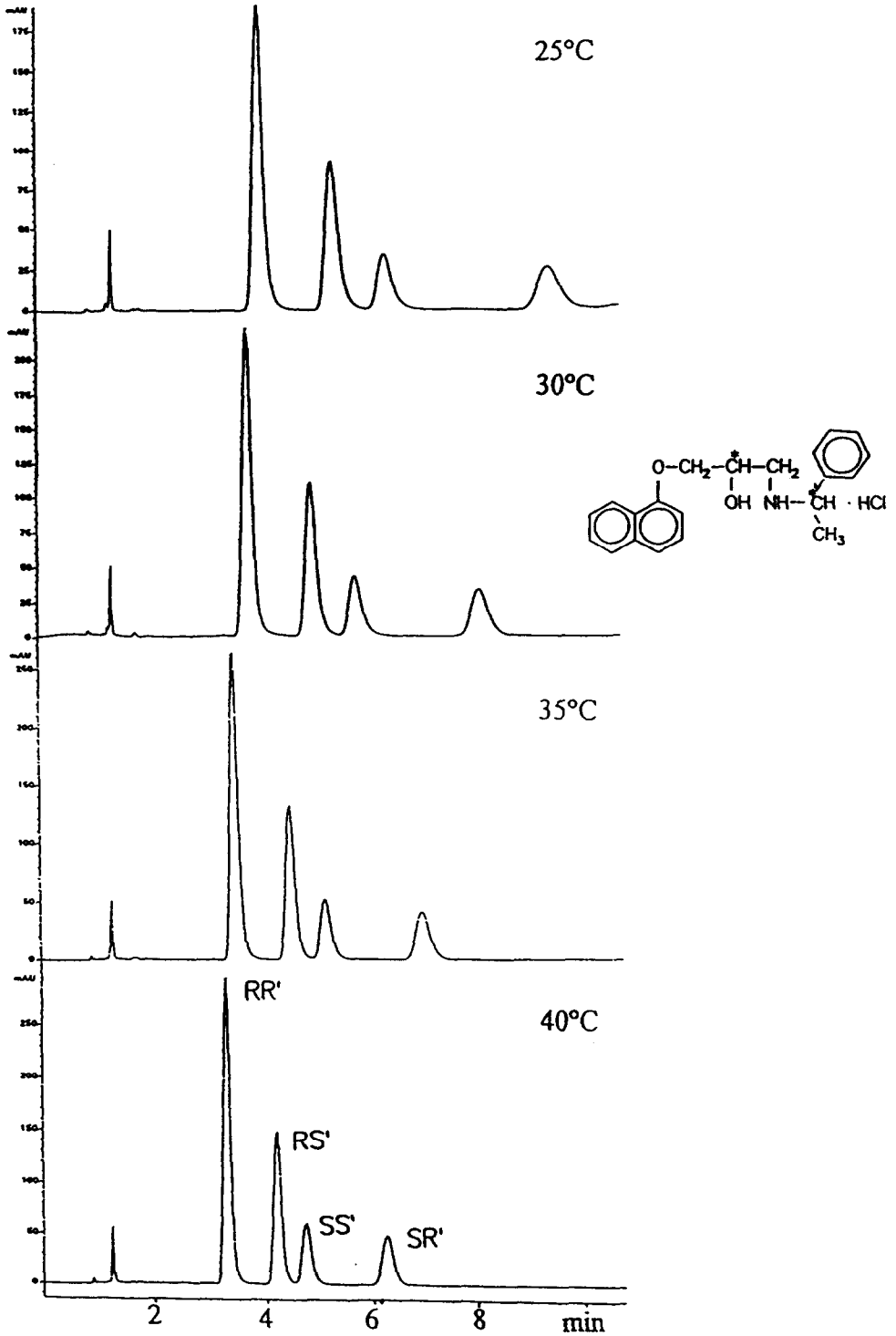
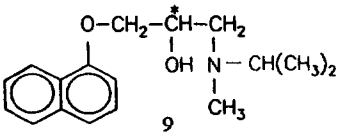


Fig. 1. Isomeric separation of **7a–d** on Chiralcel OD-H. Eluent, *n*-hexane–ethanol–2-propanol (90:3:10, v/v/v); flow-rate, 2.0 ml/min.

aqueous 1 *M* $NaClO_4$–acetonitrile and even using reversed-phase conditions again only compound **4** shows the exception from the rule that *R*-enantiomers are eluted first. Capacity and separation factors are given in Tables 3 and 4. We were able to separate the diastereomers of **7** and **8** with Chiralcel OD-R and $NaClO_4$–acetonitrile with slightly greater difficulty, the isomers of **7** being separated only after a relatively long retention time (up to 40 min) and the separation of the isomers of **8** remaining incomplete. The influence of pH and buffer was not investigated. Advances might be achieved by further optimizing the mobile phases as shown, for example, by Ishikawa and Shibata [4].

Both normal- and reversed-phase conditions were applied to separate the isomers of **9–11**.

We believe interactive forces due to stereospecific hydrogen bond formation between the CSPs and the chiral substrates to be responsible



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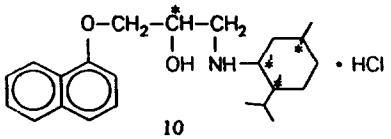

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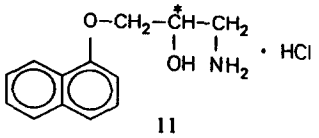

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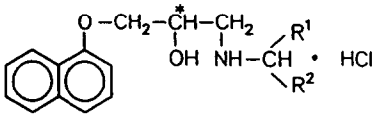
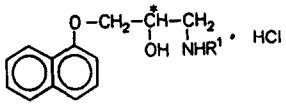
Table 3
Enantiomeric separation on Chiralcel OD-R



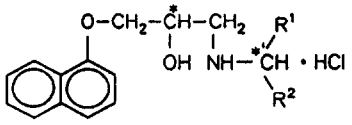
Compound	R^1	R^2	k'_1	k'_2	α	Eluent[a]
1	H	H	5.20	6.00	1.15	1[b]
2	CH_3	CH_3	9.60	13.50	1.41	1[b]
3	C_2H_5	C_2H_5	6.80	9.60	1.40	1[c]
4	C_6H_5	C_6H_5	3.35	4.18	1.24	2[c]



Compound	R^1	k'_1	k'_2	α	Eluent
5		1.08	1.84	1.70	3[c]

[a] Eluents: 1 = 1 *M* $NaClO_4$–acetonitrile (60:40); 2 = 1 *M* $NaClO_4$–acetonitrile (10:90); 3 = 1 *M* $NaClO_4$–acetonitrile (40:60, v/v).
[b] Flow-rate, 0.5 ml/min.
[c] Flow-rate, 1.0 ml/min.

Table 4
Diastereomeric separation on Chiralcel OD-R



Compound	R^1	R^2	Configuration (**')	k'	Eluent[a]
7a	CH_3	C_6H_5	*R,R'*	9.50	1
7b	CH_3	C_6H_5	*R,S'*	11.60	1
7c	CH_3	C_6H_5	*S,R'*	12.70	1
7d	CH_3	C_6H_5	*S,S'*	14.70	1
8a	CH_3	C_6H_{11}	*R,R'*	4.60	2[b]
8b	CH_3	C_6H_{11}	*S,R'*	5.80	2[b]
8c	CH_3	C_6H_{11}	*S,S'*	6.60	2[b]

[a] Eluents: 1 = 1 *M* $NaClO_4$–acetonitrile (60:40); 2 = 1 *M* $NaClO_4$–acetonitrile (50:50, v/v). Flow-rate, 1.0 ml/min.
[b] Temperature, 29°C.

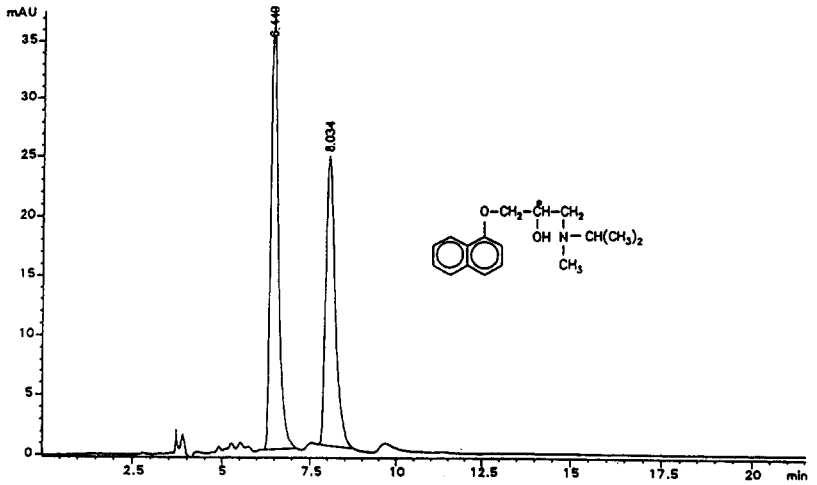


Fig. 2. Enantiomeric separation of racemate **9** on Chiralcel OD-H. Eluent, *n*-hexane–ethanol–2-propanol (90:10:10, v/v/v); flow-rate, 1.0 ml/min.

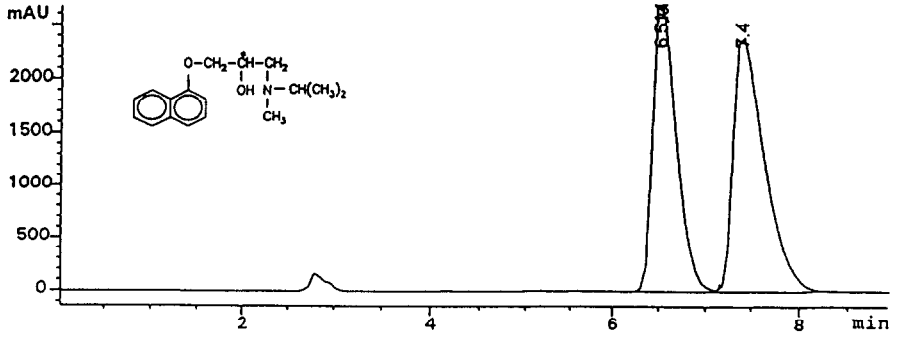


Fig. 3. Separation of racemate **9** on Chiralcel OD-R. Eluent, 1 *M* $NaClO_4$–acetonitrile (50:50, v/v); flow-rate, 1.0 ml/min.

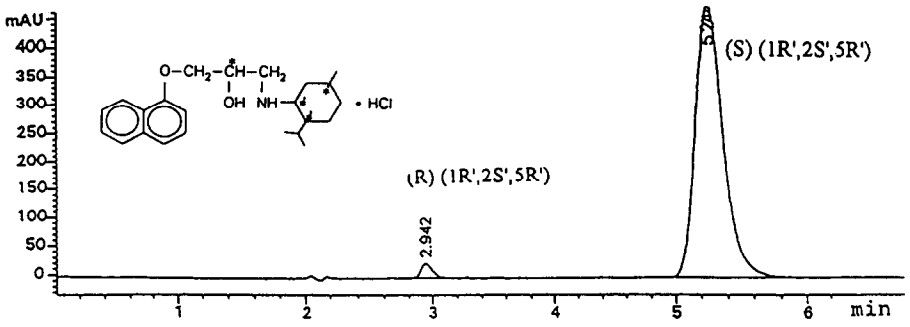


Fig. 4. Separation of **10** [excess of (*S*)(1*R'*,2*S'*,5*R'*)-compound] on Chiralcel OD-H. Eluent, *n*-hexane–ethanol–2-propanol (90:30:10, v/v/v); flow-rate, 1.5 ml/min.

for differences in the retention times of the enantiomers [13]. Siret *et al.* [8] described the chiral separation of β-blockers on a ChyRoSine-A CSP by SFC. However, the resolution of derivatives with tertiary amino groups was shown to be ineffective. Our compound **9**, lacking an NH proton, was readily separated into its enantiomers by using Chiralcel OD (Figs. 2 and 3), which may be explained by the availability of the OH group in **9** for hydrogen-bond interactions. The influence of the NH proton on the resolution is small. Compounds **2** land **9** shows similar α values. Derivative **10**, bearing a (1*R*,2*S*,5*R*)-menthyl substituent, was also separated (Figs. 4 and 5). Compound **11** contains a primary amino group; its isomers were separated under reversed-phase conditions (Fig. 6). Hexane–alcohol eluents proved to be ineffective, but on addition of formic acid **11** was straightforwardly separated with the *R*-isomer being eluted first (Fig. 7).

4. Conclusions

Some enantiomers and diastereomers of propranolol derivatives **I** with different substituents on the nitrogen were separated by HPLC on cellulose tris(3,5-dimethylphenylcarbamate) as chiral stationary phase. Excellent chiral separations of enantiomeric propranolol analogues were obtained with $NaClO_4$–acetonitrile eluents. Baseline separation was achieved for enantiomeric and diastereomeric propranolol analogues (with amine alkyl and amine aryl substituents) using hexane–alcohol eluents. To resolve compound **11** the addition of formic acid was necessary. The presence of an amine proton is not

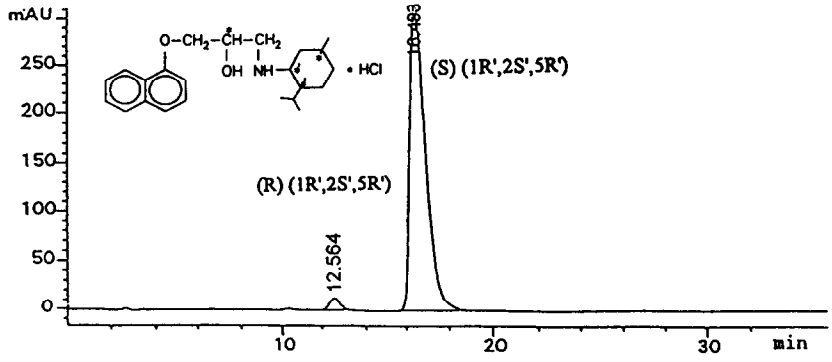


Fig. 5. Separation of **10** [excess of (*S*)(1*R'*,2*S'*,5*R'*)-compound] on Chiralcel OD-R. Eluent, 1 *M* $NaClO_4$–acetonitrile (40:60, v/v); flow-rate, 1.0 ml/min.

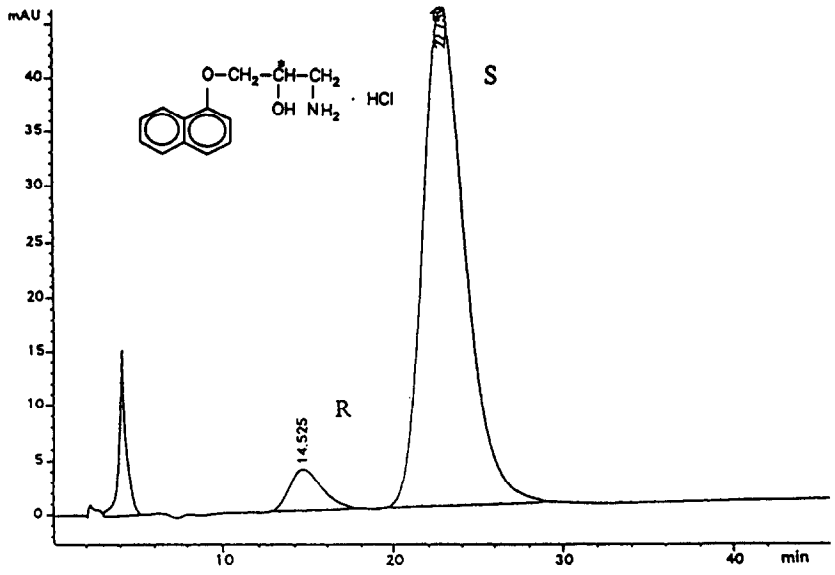


Fig. 6. Enantiomeric separation of **11** (enantiomeric excess of *S*-enantiomers) on Chiralcel OD-R. Eluent, 1 *M* $NaClO_4$–acetonitrile (70:30, v/v); flow-rate, 1.0 ml/min.

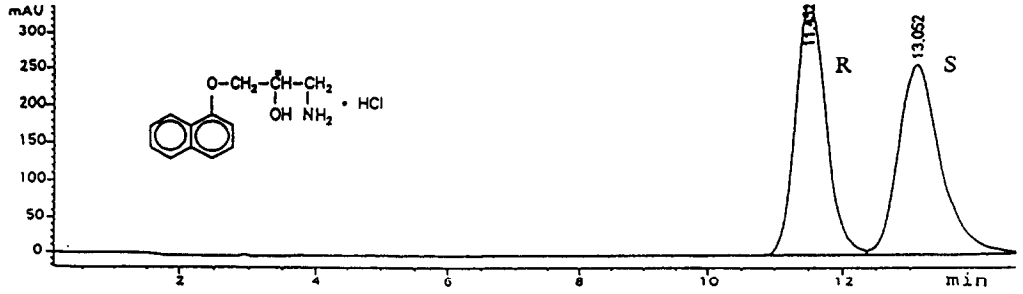


Fig. 7. Enantiomeric separation of racemate **11** on Chiralcel OD-H. Eluent, hexane–2-propanol–formic acid (80:20:1, v/v/v); flow-rate, 1.5 ml/min.

necessary to achieve chiral recognition. In this class of 1,2-amino alcohols, the *R*-enantiomers are eluted first with both normal- and reversed-phase systems if one or two amine substituents R are aliphatic. With two aromatic substituents the *S*-enantiomer has the shorter retention time.

5. Acknowledgements

We acknowledge the gift of all propranolol analogues from Professor H.-W. Krause and Mrs. U. Schmidt. We thank Mrs. H. Baudisch for the mass spectra of the compounds after the isolation of chromatographically resolved substances. Professor G. Oehme, Dr. R. Selke and Dr. H. Mennenga are thanked for helpful discussions.

6. References

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