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Note

Enantiomer separation of chiral barbiturates and of α-lipoic acid by capillary gas chromatography with modified cyclodextrins as chiral stationary phases

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Knowledge of the difference in activity of stereoisomeric drugs has initiated the development of new methods for enantiomer separation and stereochemical analysis[1]. Sensitive and accurate procedures for the determination of the enantiomeric composition of chiral drugs are not only important in cases where one of the enantiomers has toxic properties (*e.g.*, DOPA, penicillamine) but may also be utilized to study pharmacokinetics and the enantioselective metabolism of a chiral drug.

In previous investigations we have demonstrated the gas chromatographic enantiomer separation of pharmaceuticals of the amino alcohol type[2–4] (β-blockers, adrenergic drugs), some barbiturates[2], panthenol[5], penicillamine[6] and some others[1] using chiral polysiloxanes, *e.g.*, XE-60–L-valine–(*R*)-α-phenylethylamide, as stationary phases. More recently we have shown that hydrophobic derivatives of cyclodextrins can also be applied for the separation of pharmaceuticals (succinimides[7], drugs of the amine[8] and amino alcohol[9] type).

In this work, improved resolutions of racemic barbiturates and the first separation of the enantiomers of α-lipoic acid are demonstrated.

EXPERIMENTAL

The preparation of hexakis(2,3,6-tri-O-pentyl)-α-cyclodextrin[10] (Lipodex A) and of heptakis(3-O-acetyl-2,6-di-O-pentyl)-β-cyclodextrin[8] (Lipodex D) has been described previously (Pyrex glass capillary columns containing Lipodex cyclodextrin derivatives are available from Macherey, Nagel & Co., Düren, F.R.G.). Pyrex glass capillaries were coated according to the static procedure[11] using a Silanox interlayer[2].

Carlo Erba Model 2101 gas chromatographs with split injection and flame ionization detection were used for gas chromatographic investigations.

The optically active barbiturates were prepared by the separation of racemic

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intermediates according to the procedure described by Knabe *et al.*[12]. Racemic α-lipoic acid and both enantiomers were kindly supplied by B. Büchele (Kirchberg, F.R.G.).

RESULTS AND DISCUSSION

About 40% of the pharmaceuticals which are obtained synthetically are chiral but only about 10% of them are applied as pure enantiomers, 90% being used in the racemic form, in spite of the fact that in many instances the enantiomers show distinct differences in their pharmacological effects. With barbiturates, it was proved by Knabe *et al.*[12] that the narcotic effects of the enantiomers of N-alkylated barbiturates differ markedly. In some instances one of the enantiomers even displays convulsive properties. The enantiomers also show different pharmacokinetic properties. The enantiomeric purity of barbiturates has been determined by the isotope dilution method or, more efficiently, by NMR spectroscopy using chiral lanthanide shift reagents. The precision of these methods for small amounts of enantiomeric impurities is far from satisfactory.

We have found that alkylated or selectively alkylated and acylated derivatives of cyclodextrins are highly enantioselective chiral stationary phases and can be used to separate a wide variety of compounds by capillary gas chromatography[13]. These separations are independent of hydrogen-bonding interactions and are therefore possible also with substrates of medium or low polarity (saturated and unsaturated

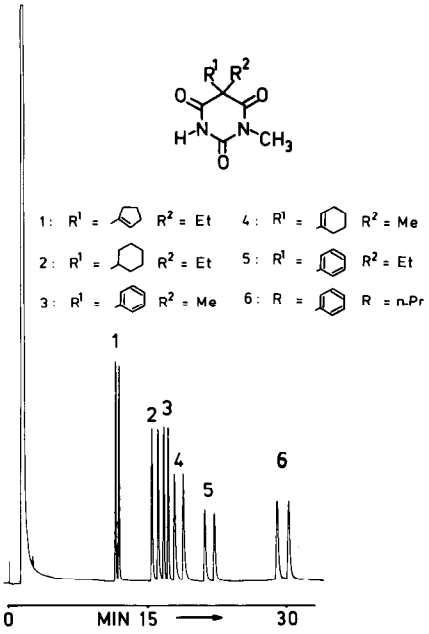

Fig. 1. Enantiomer separation of some N-alkylated barbiturates; (*R*)-enantiomers are eluted before (*S*)-enantiomers. Pyrex glass capillary column (36 m) with hexakis(2,3,6-tri-O-hexyl)-α-cyclodextrin; column temperature 180°C; carrier gas, hydrogen at 1 bar. Et = Ethyl; Me = methyl; Pr = propyl.

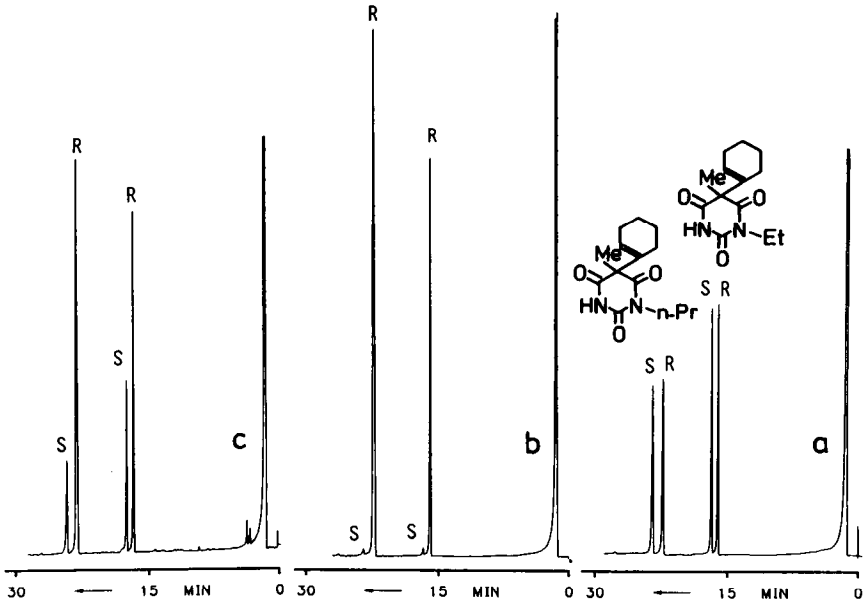

Fig. 2. (a) Enantiomer separation of N-alkyl-*nor*-hexobarbitals, (b) investigation of enantiomeric purity of (*R*)-enantiomers and (c) assignment of the order of elution. Column temperature, 195°C; for other details see Fig. 1.

hydrocarbons, alkyl halides, spiroacetals, lactones, ketones, etc.) that cannot be separated on columns with chiral polysiloxanes[14]. The enantiomers of N-alkylated barbiturates can be resolved on peralkylated cyclodextrins (*e.g.*, perpentylated α-cyclo-

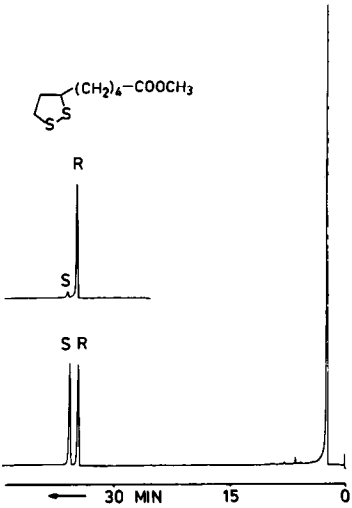

Fig. 3. Enantiomer separation of α-lipoic acid methyl ester (esterification with diazomethane) and determination of enantiomeric purity of the (*R*)-enantiomer. Pyrex glass capillary column (40 m) with Lipodex D; column temperature, 180°C; carrier gas, hydrogen at 1 bar.

dextrin, Lipodex A or perhexylated α-cyclodextrin) at column temperatures between 180 and 220°C, as shown in Figs. 1 and 2. Even minute contributions of enantiomeric impurities can be detected. In Fig. 2b the ratio of (*R*)- to (*S*)-enantiomers is 97.99:2.01 for the N-ethyl derivative and 98.64:1.36 for the N-*n*-propyl derivative. It can be assumed that enantiomeric contributions of less than 1% could be quantified in this instance. The smallest precisely detectable contribution may be even smaller if the order of elution of the enantiomers is reversed. The detection limit greatly depends, however, on the separation factor (α-value) and may be as low as 0.1% in favourable instances. In all separations the (*R*)-enantiomers are eluted prior to the (*S*)-enantiomers. Compounds substituted at the chiral centre by two alkyl groups differing by only one CH_2 group were separated incompletely or not at all.

Some chiral barbiturates have also been separated by liquid chromatography. Thus hexobarbital was resolved on triacetylcellulose by Koller *et al.*[15] and the same compound together with other barbiturates by Yang *et al.*[16] on a Pirkle-type phase.

α-Lipoic acid (Fig. 3) is involved in the oxidative decarboxylation of pyruvic acid to activated acetaldehyde, which is bound to α-lipoic acid and transferred to coenzyme A to form acetyl coenzyme A. It is also claimed to be an efficient detoxicant and is administered as its racemate (thioctacid) in the treatment of liver diseases. Although natural (+)-α-lipoic acid has the (*R*)-configuration, it is not known if the unnatural (*S*)-enantiomer has equivalent activity. As shown in Fig. 3, the enantiomers can be easily separated on heptakis(3-O-acetyl-2,6-di-O-pentyl)-β-cyclodextrin (Lipodex D), and it would be interesting to study the relationship between the stereochemistry and pharmacological activity of this compound.

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REFERENCES

1 W. A. König, in I. W. Wainer and D. E. Drayer (Editors), *Drug Stereochemistry*, Marcel Dekker, New York, Basle, 1988, p. 113.
2 W. A. König and K. Ernst, *J. Chromatogr.*, 280 (1983) 135.
3 W. A. König, K. Ernst and J. Vessman, *J. Chromatogr.*, 294 (1984) 423.
4 W. A. König, O. Gyllenhaal and J. Vessman, *J. Chromatogr.*, 356 (1986) 354.
5 W. A. König and U. Sturm, *J. Chromatogr.*, 328 (1985) 357.
6 W. A. König, E. Steinbach and K. Ernst, *J. Chromatogr.*, 301 (1984) 129.
7 W. A. König, S. Lutz, C. Colberg, N. Schmidt, G. Wenz, E. von der Bey, A. Mosandl, C. Günther and A. Kustermann, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 621.
8 W. A. König, S. Lutz, G. Wenz and E. von der Bey, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 506.
9 W. A. König, *Carbohydr. Res.*, 192 (1989) 51.
10 W. A. König, S. Lutz and G. Wenz, *Angew. Chem.*, 100 (1988) 989; *Angew. Chem., Int. Ed. Engl.*, 27 (1988) 979.
11 J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.
12 J. Knabe, W. Rummel, H. P. Büch and N. Franz, *Arzneim.-Forsch./Drug Res.*, 28 (1978) 1048.
13 W. A. König, *Nachr. Chem. Tech. Lab.*, 37 (1989) 471.
14 W. A. König, *The Practice of Enantiomer Separation by Capillary Gas Chromatography*, Hüthig, Heidelberg, 1987.
15 H. Koller, K. H. Rimbock and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 80.
16 Z. U. Yang, S. A. Barkan, C. Brunner, J. D. Weber, T. D. Doyle and I. W. Wainer, *J. Chromatogr.*, 324 (1985) 444.