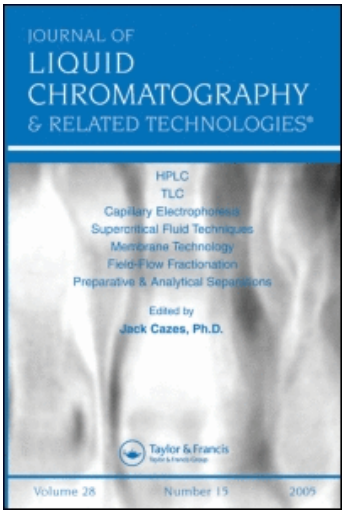

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CHIRAL SEPARATION OF SEVERAL THIOBARBITURATES ON A CELLULOSE TRIS(4-METHYLBENZOATE) PHASE UNDER REVERSED-PHASE CONDITIONS

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ABSTRACT

The chiral separation of several thiobarbiturates on a tris(4-methylbenzoate) phase (Chiralcel OJ-R, 15 × 0.46 cm and Chiralcel OJ-R 15 × 0.20 cm) under reversed-phase conditions is described. The separation properties of a normal sized and a newly developed narrow-bore column were compared. The influence of buffer and organic modifiers on enantioselectvity was investigated. The three mobile phases used were water/acetonitrile (70:30, v/v), water/acetonitrile/methanol (50:25:25, v/v), and water/acetonitrile/

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methanol (55:15:30, v/v). Of seven thiobarbiturates investigated, five have been resolved. The values of α (1.11–1.85) and R_s (0.71–5.75) have been reported.

INTRODUCTION

Thiobarbiturates are the drugs commonly used as sedative, hypnotic, and anticonvulsant agents (1). There are differences in the pharmacological activities of the thiobarbiturates enantiomers (1). The quantitative differences in the activities of the enantiomers of thiopental (2,3) and thiohexital have been reported (4). The chirality of these compounds is based on the presence of an asymmetric C-atom at position 5 of the pyrimidine ring or/and in the side chain situated at C-5 (Fig. 1).

Various chiral stationary phases, i.e., cyclodextrins, proteins, and polysaccharides, have been used for the enantiomeric resolution of barbiturates (1). However, the cellulose-based phases were found to exhibit good enantioselectivity for barbiturates.The racemic barbiturates were resolved on microcrystalline cellulose triacetate (5–9) and on cellulose tris(4-benzoate) (Chiracel OJ) (10,11). It was also shown that enantiomeric resolution of barbiturates is possible under reversed-phase conditions using a Chiracel OJ-R column (12,13).

Compared to normal-phase OJ columns, handling of the Chiracel OJ-R columns is more convenient with regard to the choice of a variety of mobile phases.This study deals with the enantiomeric separation of thiobarbiturates under reversed-phase conditions, comparing a 15 × 0.46 cm Chiracel OJ-R column with a newly developed 15 × 0.2 cm narrow-bore column of the same type.

EXPERIMENTAL

Chemicals and Materials

All reagents were of analytical grade. Methanol and acetonitrile (gradient grade) were obtained from Merck (Darmstadt, Germany). Compounds 1–3 were from Abbott (North Chicago, IL, USA). Compound 4 was obtained by treatment of diethyl ethylmalonate with *N*-ethylthiourea, and compounds 5–7 were obtained by methylation of 2-thiophenobarbital (4).

High Performance Liquid Chromatography (HPLC) Conditions

HPLC was performed using a HP 1090 system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode array ultraviolet detector. The samples were

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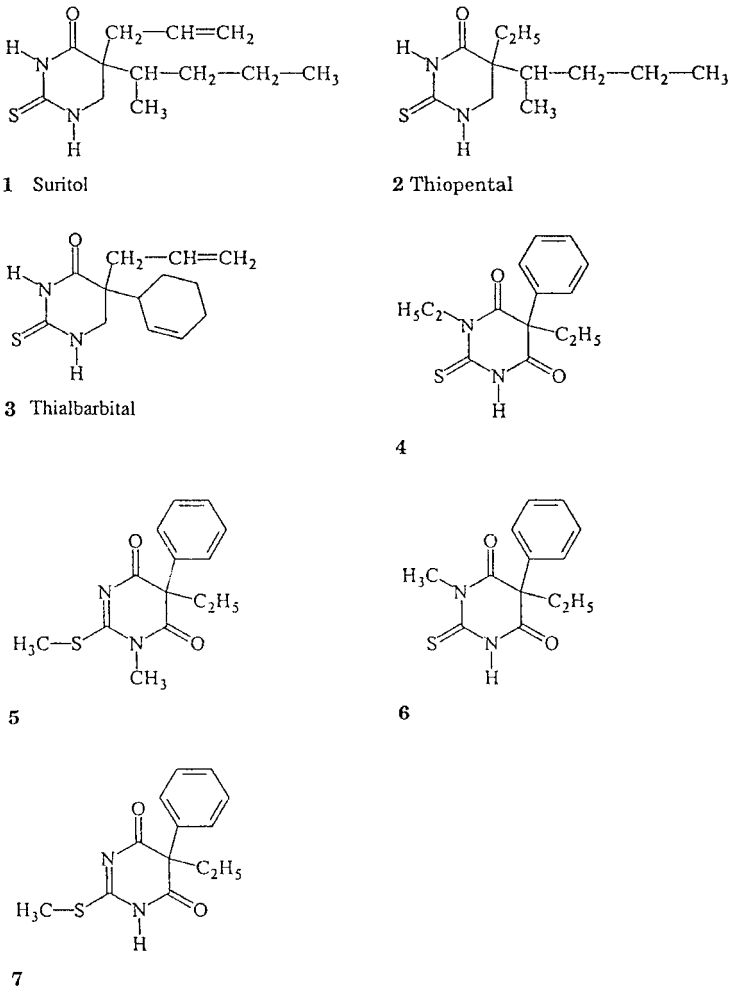

Figure 1. Structures of the compounds investigated.

injected by an autosampler, and the amount injected was 8 μL. The detection was performed at 235 nm. The OJ-R 15 $\times$ 0.2 cm and OJ-R 15 $\times$ 0.46 cm chiral stationary-phase columns were obtained from Daicel Co. (Tokyo, Japan).

The mobile phases used were different mixtures of methanol, acetonitrile, and water. The flow rate of the mobile phases were 0.2 and 0.5 mL/min for the OJ-R 15 $\times$ 0.2 cm and OJ-R 15 $\times$ 0.46 cm columns, respectively. All separations were carried out at room temperature.

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RESULTS AND DISCUSSION

The chromatographic parameters, capacity factor (k'), separation factor (α), and resolution factor (R_s) for the resolved enantiomers of thiobarbiturates under the reversed-phases mode are presented in Table 1. The typical chromatograms of some of the resolved enantiomers are given in Figures 2a–2e. The comparative studies on the chiral separation of thiobarbiturates were carried out using 15 × 0.46 cm and a 15 × 0.2 cm narrow-bore Chiracel OJ-R columns. Both columns exhibited comparable enantioselectvity; however, the narrow-bore column showed several advantages, as the separations were faster with significantly sharper peak shapes and with less solvent consumption.

While the normal-sized column was operated at 0.5 mL/min, a flow rate of 0.2 mL/min was chosen for the narrow-bore column to reducing backpressure and increase the lifetime of the column. However, since the pressure limit recommended by the supplier was not reached at this flow rate, a slight increase in flow rate would be still possible.

Table 1. Separation Data for Thiobarbiturates on a OJ-R 15 × 0.2 cm Column (Flow = 0.2 mL/min) and a OJ-R 15 × 0.46 cm Column (Flow = 0.5 mL/min)

	OJ-R (15 × 0.2 cm)				OJ-R (15 × 0.46 cm)			
Compounds	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
	Mobile phase: water/acetonitrile (70:30, v/v)							
1	8.27	9.40	1.14	0.96	9.76	10.97	1.21	0.99
2	7.55	8.48	1.12	0.77	8.51	9.48	1.11	0.97
3	8.93	11.04	1.24	1.41	10.04	12.37	1.23	1.95
4	n.e.[a]				n.e.			
5	7.84	13.12	1.67	4.06	9.05	14.44	1.60	4.76
6	n.e.				n.e.			
7	6.55	—	1.00	0.00	14.56	—	1.00	0.00
	Mobile phase: water/acetonitrile/methanol (50:25:25, v/v)							
1	3.63	4.13	1.14	0.71	3.72	4.22	1.13	0.90
2	3.68	4.24	1.15	0.74	3.93	4.51	1.15	0.90
3	4.39	5.68	1.29	1.22	4.38	5.65	1.29	1.67
4	23.08	42.21	1.83	5.75	n.e.			
5	4.43	8.18	1.85	3.10	4.70	8.68	1.85	4.01
6	44.33	—	1.00	0.00	n.e.			
7	4.78	—	1.00	0.00	10.11	—	1.00	0.00
	Mobile phase: water/acetonitrile/methanol (55:15:30, v/v)							
2	1.46	2.00	1.37	0.98				

[a]n.e., not eluted within 120 min.

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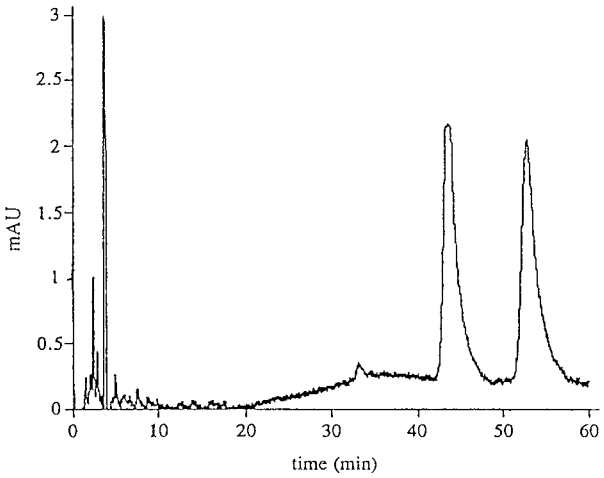

Figure 2a. Separation of compound 3 on the 15 × 0.46 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile (70:30, v/v) with flow rate 0.5 mL/min.

Different mobile phase compositions, such as water or buffer/acetonitrile mixtures, and ternary mixtures consisting of water or buffer, acetonitrile, and methanol were investigated. Extremely high retention times were observed with mobile phases containing only 20% acetonitrile. As is typical for reversed phases,

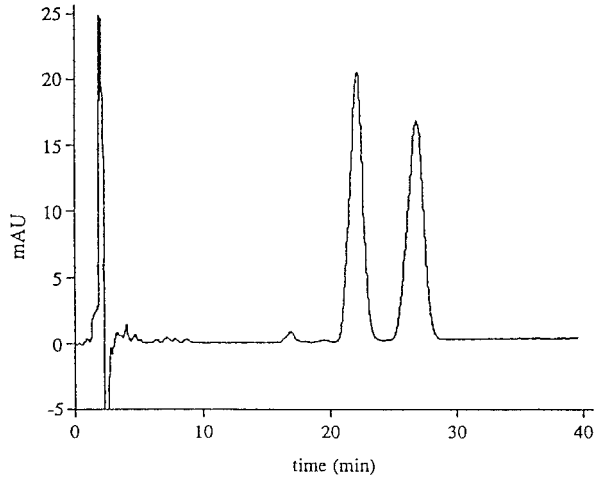

Figure 2b. Separation of compound 3 on the 15 × 0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile (70:30, v/v) with flow rate 0.2 mL/min.

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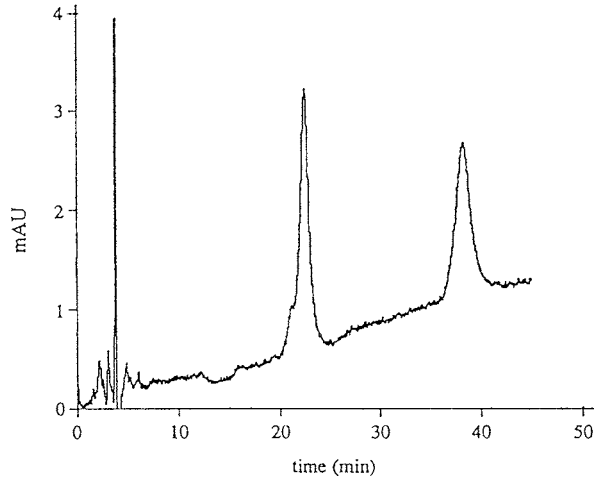

Figure 2c. Separation of compound 5 on the 15 × 0.46 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (50:25:25, v/v) with flow rate 0.50 mL/min.

a decrease in retention time was observed with increasing acetonitrile content. Therefore, the acetonitrile content was systematically varied. Figure 3 shows the influence of acetonitrile percentage on resolution of thiobarbiturates. It is clear from Figure 3 that any slight increase in the acetonitrile content resulted in the decrease

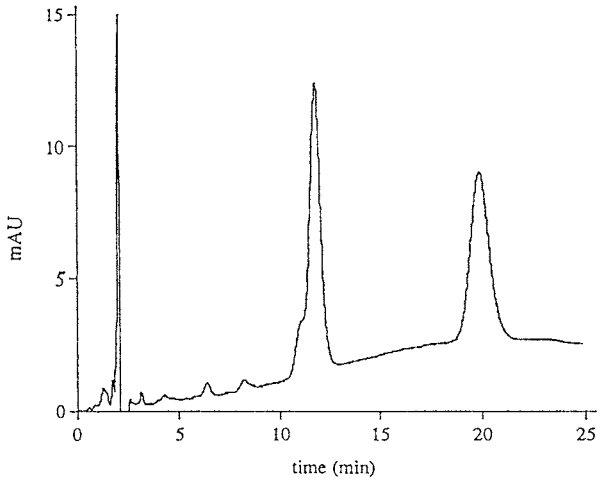

Figure 2d. Separation of compound 5 on the 15 × 0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (50:25:25, v/v) with flow rate 0.20 mL/min.

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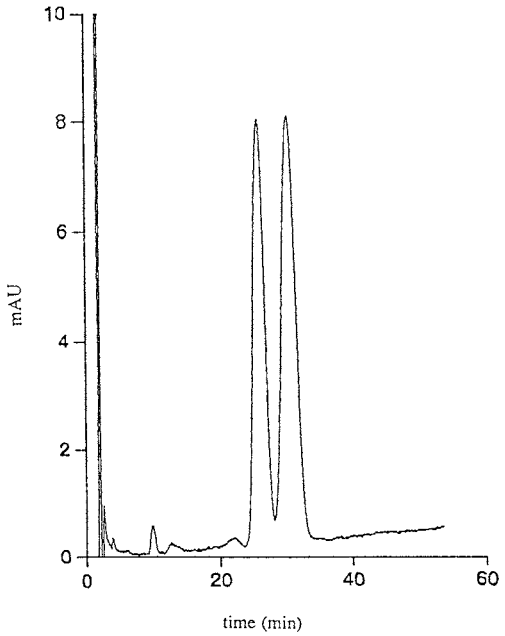

Figure 2e. Separation of compound 2 on the 15 × 0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (55:15:30, v/v) with flow rate 0.20 mL/min.

of resolution factor. The substitution of water by a perchlorate buffer resulted in a slight increase in retention, coupled with more pronounced band broadening. Ishikawa and Shibata (15) reported an increase in retention and improvement of resolution of acidic compounds with increasing pH.

The influence of pH was investigated over a range of 7 to 2 using a perchlorate buffer; however, no significant change in retention and resolution was observed. This might be due to the fact that the thiobarbiturates are rather weak acids (pK_a about 8–9), except compound 5 which is neutral. Therefore, in further experiments, no buffer was used. Ternary mobile phases containing water/methanol/acetonitrile showed the best results.

Table 1 shows that compound 4 possesses an extremely high retention time. By changing to water/acetonitrile/methanol (40:40:20) as a mobile phase, baseline resolution was still obtained with a reasonable separation time. Surprisingly, compound 6, which differs from 4 only in the substituent on the nitrogen, was not resolved, despite the fact it was also strongly retained. Compound 7 was rather weakly retained and was not resolved in any of the mobile phases investigated. This may be due to the poor hydrogen bonding at the sulfur atom that is affected by the steric effect of the methyl group attached to it.

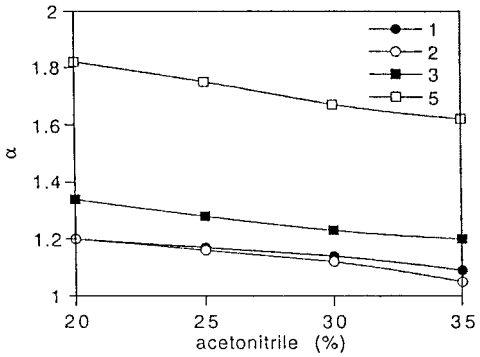

Figure 3. Influence of actonitrile content on selectivity. Column: Chiracel OJ-R 15 × 0.2 cm (flow = 0.2 mL/min); mobile phase: water with different amounts of acetonitrile.

The same is the case with compound 5. The baseline resolution for compound 2 can be achieved by using water/acetonitrile/methanol (55:15:30) as a mobile phase.

The chiral recognition mechanism on cellulose-based phases involves: a) the inclusion of aromatic moieties or other bulky substituents of the analytes into cavities of the chiral selectors; and b) attractive interactions such as dipole-dipole, π-π, and hydrogen bonding (15–19). In the case of thiobarbiturates, hydrogen bonding and dipole-dipole stacking between the imide group of the analytes and the ester groups of the selector might be assumed to be the main interactions responsible for chiral recognition.

Since the compounds containing an aromatic moiety, such as compound 4, showed significantly higher retention and better resolution compared to compounds with aliphatic side chains, inclusion into the cavities paired with π-π interactions between the phenyl group on C-5 and the benzoyl group of the chiral selector might be taken into account in these particular cases. The same might apply also for compound 3, which contains an alicyclic ring, except with weaker π-π interactions, as reflected in a slight decrease in resolution. These facts are in good agreement with observations made for non-sulfur-containing barbiturates (12,13).

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