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Enantiomeric separation of racemic α-substituted α-methylbutyric acid derivatives on microcrystalline cellulose triacetate

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ABSTRACT

High-performance liquid chromatography on microcrystalline cellulose triacetate was examined for the enantiomeric separation of five racemic α-substituted α-methylbutyric acid derivatives. For four pairs the retention and the separation factor increased with increasing water content in methanol. The retention of the fifth derivative increased slowly, but separation did not occur. The concentration of the second-eluted enantiomer increased and the detection improved when a step gradient was used. Small amounts of the second-eluted enantiomer in the first could be determined.

INTRODUCTION

Pyrazoloxyisobutyric acids are potential hypolipaemic drugs with various pharmacological properties, such as inhibition of blood platelet aggregation. Their synthesis was accomplished by means of the Link reaction of 1-substituted 3-hydroxypyrazoles with chloroform and acetone [1,2]. The same method allowed acetone to be replaced with methyl ethyl ketone. α-Substituted α-methylbutyric acids with an asymmetric centre at the α-carbon were obtained. The object of this work was to determine whether the resolution of the enantiomers would bring about a differentiation of the pharmacological effects among the enantiomers.

Microcrystalline cellulose triacetate (CTA) is a useful stationary phase for enantiomeric resolution by high-performance liquid chromatography (HPLC). Since the introduction of CTA by Hesse and Hagel [3–5], investigations have shown that enantiomers belonging to different classes of organic compounds can be separated on this phase [6–11]. Free acids have not been separated on cellulose derivatives, except for the recently reported work of Okamoto *et al.* [12]. We have prepared the methyl esters of the α-substituted α-methylbutyric acids for separation on CTA.

EXPERIMENTAL

The synthesized substances are shown in Fig. 1. Separation of the enantiomers was carried out either by fractional recrystallization of the cholesterol esters or by separation of the methyl esters by HPLC on CTA.

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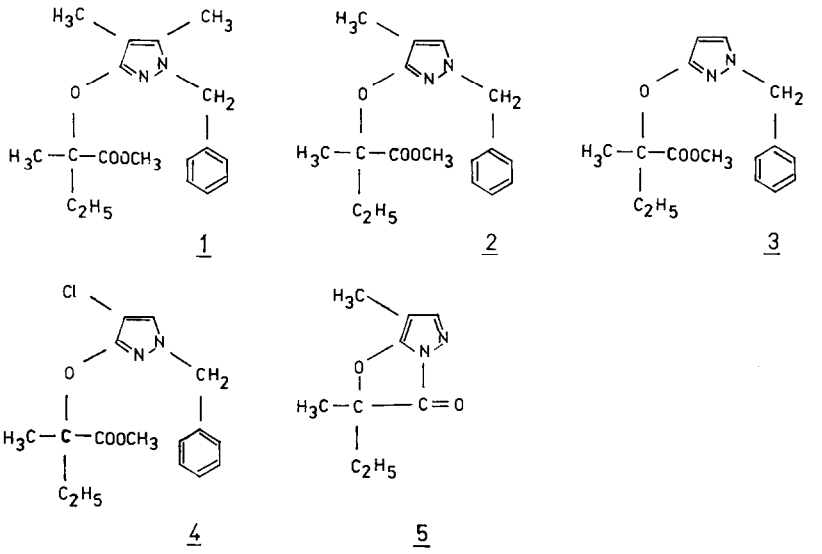

Fig. 1. Structures of the compounds studied.

Microcrystalline CTA (15–20 μm) was purchased from E. Merck (Darmstadt, F.R.G.). Methanol and ethanol of analytical-reagent grade were supplied by VEB Laborchemie (Apolda, G.D.R.). Water was deionized and distilled twice.

Stainless-steel tubes (250 × 4.6 mm I.D.) were slurry-packed with CTA. CTA was swollen in boiling ethanol for 30 min, cooled, decanted, suspended again and treated in an ultrasonic bath. During packing the flow-rate was *ca.* 3 ml/min.

TABLE I

EFFECT OF MOBILE PHASE COMPOSITION ON THE CAPACITY FACTORS OF THE ENANTIOMERS

Column A; flow-rate, 0.5 ml/min; temperature, 23 ± 1°C.

Mobile phase	Capacity factors of derivatives[a]									
	1		**2**		**3**		**4**		**5**	
	k'_a	k'_b	k'_a	k'_b	k'_a	k'_b	k'_a	k'_b	k'_a	k'_b
Ethanol–water (96:4, v/v)	0.52		1.33	2.83	1.89	6.03	3.68	6.18	1.18	2.44
Methanol–water (90:10, v/v)	1.11		0.45	0.66	0.73	1.47	1.52	1.94	0.74	1.00
Methanol–water (85:15, v/v)	–		0.90	1.66	0.94	2.26	2.26	3.11	0.92	1.31
Methanol–water (80:20, v/v)	2.94		1.76	3.85	1.50	4.24	4.51	6.74	1.03	1.59
Methanol–water (75:25, v/v)	3.11		2.62	6.43	–	–	–	–	–	–

[a] a indicates the first eluting enantiomer, b the second one.

Two columns were used. Column A was well packed. The unretained peak of 1,3,5-tri-*tert.*-butylbenzene had a plate height of 45 μm at a flow-rate of 0.1 ml/min. In column B the plate height was only 110 μm under the same conditions.

A Merck–Hitachi L6200 "intelligent" pump (E. Merck), a Rheodyne (Berkeley, CA, U.S.A.), Model 7120 sample injector with a 20-μl loop an LCD 2563 UV–VIS detector (Laboratorní Přístroje, Prague, Czechoslovakia), and a K201 recorder (Carl Zeiss, Jena, G.D.R.) were used for gradient elution chromatography.

Preparative and thermostated chromatography was performed using a Hewlett-Packard (Waldbronn, F.R.G.) Model 1081B pump, the same injector and detector as above and an Endim 621.02 recorder (Messapparatewerk Schlotheim, Schlotheim, G.D.R.).

RESULTS AND DISCUSSION

The influence of the solvents on the retention and selectivity of the compounds investigated is summarized in Table I and Fig. 2. Both the retention and selectivity improved with increase in the water content in methanol, except for compound **1**, where only an increase in retention was observed.

The temperature influenced the retention and selectivity as shown in Fig. 3. Working at room temperature or thermostating at low temperature is recommended.

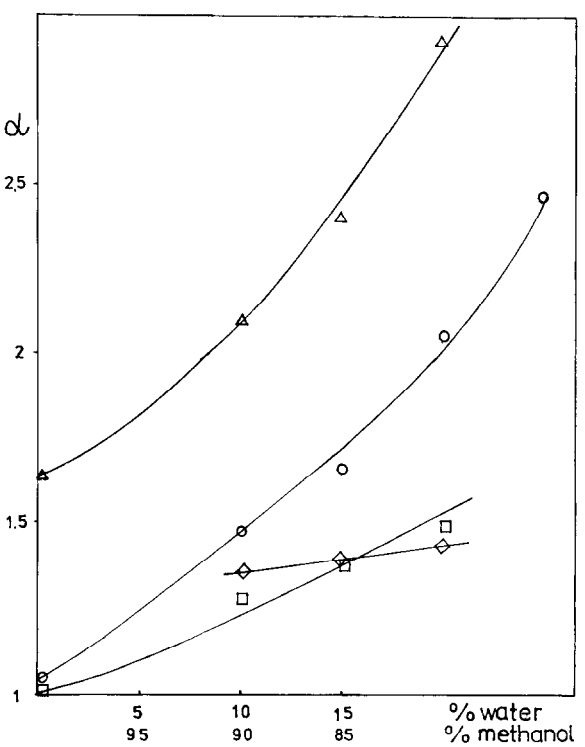

Fig. 2. Dependence of the separation factor of derivatives [(○) **2**; (△) **3**; (□) **4**; (◇) **5**] on the water content in methanol. Column A; temperature, 23 ± 1°C.

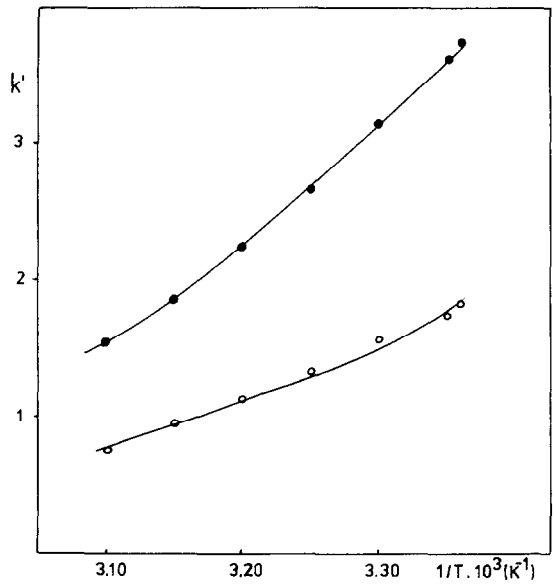

Fig. 3. Dependence of the k'_a [●, (+)-enantiomer] and k'_b [○, (−)-enantiomer] of derivative **2** on the temperature. Column A; mobile phase, methanol–water (80:20, v/v); flow-rate, 0.5 ml/min.

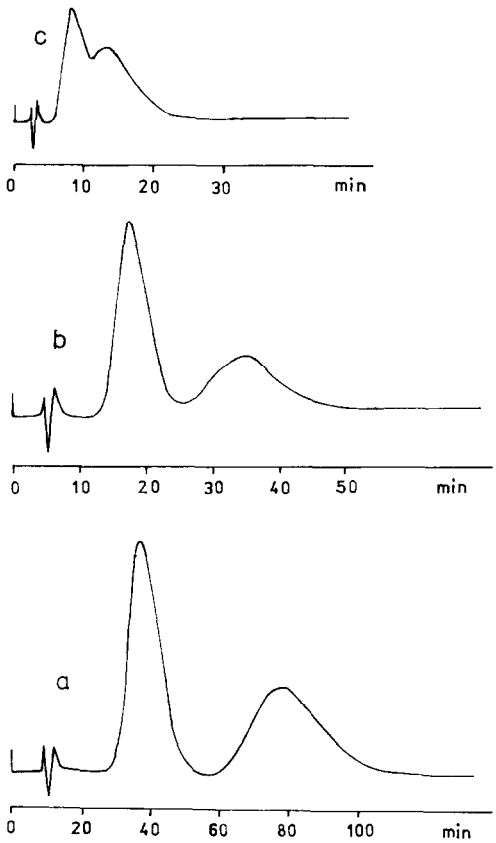

Fig. 4. Dependence of the resolution for derivative **2** on the mobile phase flow-rate. Column B; temperature, 25°C; mobile phase, methanol–water (80:20, v/v). Flow-rate: (a) 0.1; (b) 0.2; (c) 0.5 ml/min.

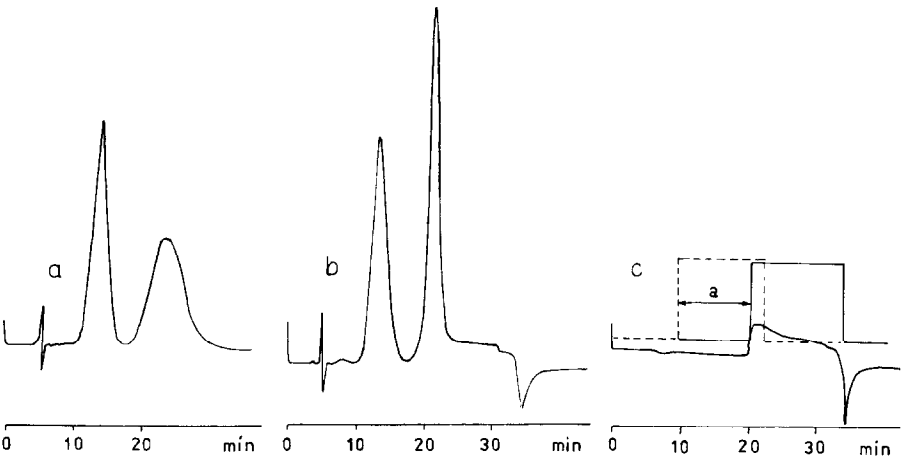

Fig. 5. Separation of enantiomers of racemic derivative **2**. Column A; temperature, 23°C; flow-rate, 0.5 ml/min. (a) Mobile phase, methanol–water (80:20, v/v); (b) step gradient, methanol–water (80:20) for 11 min, methanol for 11 min, methanol–water (80:20) to the end; (c) absorbance of the "empty" step gradient; a = interval between gradient generation and the end of the column.

The flow-rate drastically influenced the retention, as shown in Fig. 4. This shows that the slow mass transfer of solutes between CTA and the mobile phase requires adequate flow-rates. Obviously, the resolution also depends on the quality of the packing. The chromatographic conditions for Fig. 5a were the same as those for Fig. 4c. In column A in Fig. 5 the unretained peak of 1,3,5-tri-*tert.*-butylbenzene had

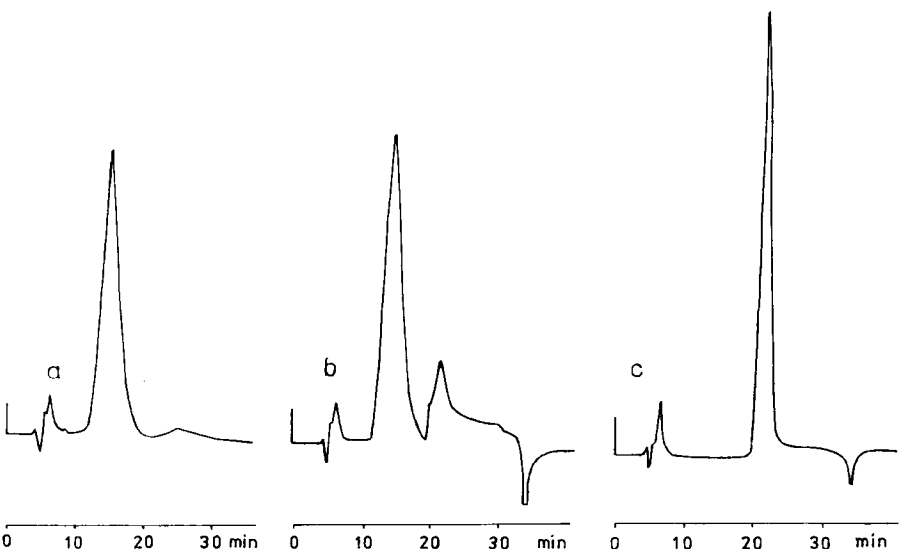

Fig. 6. Enantiomers of derivative **2**, separated by fractional recrystallization of the cholesterol esters. (a) (+)-Enantiomer, mobile phase methanol–water (80:20, v/v); (b) (+)-enantiomer, step gradient as in Fig. 5; (c) (−)-enantiomer, step gradient as in Fig. 5.

a plate height of 45 μm at a flow-rate of 0.1 ml/min, but in column B in Fig. 4 the plate height was only 110 μm under the same conditions.

Figs. 5 and 6 show how the peak shape is influenced by using a step gradient. The relatively broad and low second peak became narrow and high, and determination of the (−)- in the (+)-enantiomer became possible.

CONCLUSIONS

A step gradient may be useful both for determining the optical purity of the first-eluted enantiomer and for preparative separation when the retention and the separation factor are high. Hence solvents and time can be saved.

REFERENCES

1 H. Dorn and R. Ozegowski, *East Ger. Pat.*, DD 197 820, 1977; *Ger. Offen*, DE 2 809 183, 1984.
2 H. Dorn and R. Ozegowski, *East Ger. Pat.*, DD 242 041, 1987.
3 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277.
4 G. Hesse and R. Hagel, *Chromatographia*, 9 (1976) 62.
5 G. Hesse and R. Hagel, *Justus Liebigs Ann. Chem.*, (1976) 996.
6 K. R. Lindner and A. Mannschreck, *J. Chromatogr.*, 193 (1980) 308.
7 H. Koller, K.-H. Rimböck and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89; and references cited therein.
8 A. Mannschreck, H. Koller and R. Wernicke, *Kontakte (Darmstadt)*, 1 (1985) 40.
9 G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341.
10 E. Francotte, R. M. Wolf, D. Lohmann and R. Mueller, *J. Chromatogr.*, 347 (1985) 25.
11 T. Shibata, J. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313; and reference cited therein.
12 Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 448 (1988) 454.