Journal of Chromatography, 100 (1974) 180–184

CHROM. 7662

Note

New derivatives for the gas chromatographic resolution of amino acid enantiomers

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(Received May 28th, 1974)

In recent years, the application of gas chromatography (GC) to optical resolution has been developed in two different ways. One is the direct separation of the enantiomers on an optically active stationary phase[1–5], and the other involves the derivatization into the diastereomer followed by GC on an ordinary liquid phase. The GC resolution of the amino acid enantiomers has been investigated by several workers. Condensation with a chiral reagent yields the diastereomers, which often can be easily separated under the appropriate column conditions. For this purpose, N-trifluoroacetyl-(*S*)-(−)-prolyl (TP) chloride and its related compounds have been used as resolving agents[6–9]. In addition, Brooks *et al.*[10] recently introduced the new effective reagents drimanoyl chloride and (*R*)-(+)-*trans*-chrysanthemoyl chloride for the gas-phase analytical resolution of enantiomeric amines and alcohols. In this paper, we report the potential utility of new chiral reagents to form the diastereomers of amino acids for GC resolution on conventional columns.

EXPERIMENTAL

Gas chromatography

The apparatus used was a Shimadzu Model GC-5AIFE gas chromatograph equipped with a hydrogen flame ionization detector and a "silanized" U-shaped glass column (3 m × 3 mm I.D.). The column was packed with 1.5% SE-30, 1.5% OV-1, 1.5% OV-17 or 0.5% PEGA on Chromosorb W (100–120 mesh). Column temperatures were 160°, 180°, 170° and 155°, respectively. Both the detector and flash heater were maintained at 30° above the column temperature. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min.

Materials

Amino acids and *d*-isoketopinic acid were kindly donated by Ajinomoto (Kawasaki, Japan) and Yoshitomi Pharmaceutical Industries (Yoshitomi, Japan), respectively. *l*-Dihydroteresantalic acid and *l*-teresantalic acid were synthesized from *d*-isoketopinic acid by known procedures[11,12]. The acid chlorides were freshly prepared by treatment with thionyl chloride prior to use: *d*-isoketopinyl chloride (m.p. 128–132°), *l*-dihydroteresantalinyl chloride (m.p. 174–176°) and *l*-teresantalinyl chloride

(b.p.$_{30mm}$ 60–65°). Methyl and butyl esters of amino acids were obtained in the usual manner.

Preparation of derivatives

Procedure A. To a solution of amino acid ester (*ca.* 1 mg), dissolved in tetrahydrofuran (0.8 ml) containing pyridine (0.2 ml), was added the acid chloride (*ca.* 4 mg). The reaction product comprised the N-acyl derivative together with excess of reagent and was injected directly into the gas chromatograph.

Procedure B. The amino acid ester (*ca.* 1 mg) in acetonitrile (0.5 ml) was treated with the acid chloride (*ca.* 4 mg) in the presence of triethylamine (1 drop). The reaction product was similarly analyzed without further purification.

Separation factor

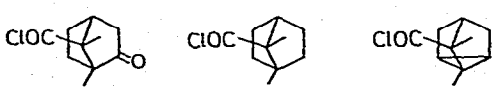
Relative retention times (RRT) were measured using *p,p'*-DDT as a reference compound. The ratio of the retention times of each enantiomeric pair (t_{R_2}/t_{R_1}) was expressed as α. The separation factor, R, was calculated from the equation

$$R = 2(t_{R_2} - t_{R_1})/(W_1 + W_2)$$

where W_1 and W_2 are the bases of triangles derived from the theoretical peaks.

RESULTS AND DISCUSSION

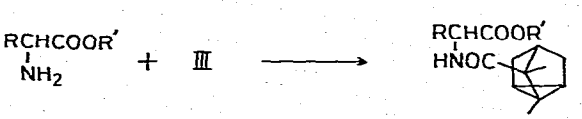
The requirement of a more rigid skeletal structure and higher volatility for the resolving agent prompted us to explore the utility of the camphor-related compounds *d*-isoketopinyl chloride (I), *l*-dihydroteresantalinyl chloride (II) and *l*-teresantalinyl chloride (III) (see Fig. 1). An initial effort was directed to testing the applicability of



I II III

Fig. 1. Structures of *d*-isoketopinyl chloride (I), *l*-dihydroteresantalinyl chloride (II) and *l*-teresantalinyl chloride (III).

three derivatization reagents employing DL-alanine as a model compound. The reaction of the amino acid ester with these reagents in the presence of a basic catalyst proceeded readily to provide the sufficiently volatile N-acyl derivative in a quantitative yield, as shown in Fig. 2. The amino acid showed a single peak of the theoretical



R' = Me or *n*-Bu

Fig. 2. Scheme of the reaction of amino acid ester and reagent I, II, or III (see text).

TABLE I

RELATIVE RETENTION TIMES AND THEIR RATIOS FOR DIASTEREOMERIC N-ACYL DL-ALANINE ESTERS*

Column	*d-Isoketopinyl*		*l-Dihydroteresantalinyl*		*l-Teresantalinyl*	
	RRT	$\alpha_{(D/L)}$	*RRT*	$\alpha_{(D/L)}$	*RRT*	$\alpha_{(D/L)}$
Methyl ester						
1.5% SE-30 (a)	0.317	1.03	0.178	1.02	0.163	1.03
	0.307		0.174		0.168	
1.5% OV-1 (b)	0.357	1.00	0.198	1.03	0.185	1.03
	0.356		0.192		0.191	
1.5% OV-17 (c)	0.360	1.00	0.130	1.02	0.125	1.05
	0.359		0.127		0.131	
0.5% PEGA (d)	0.408	1.01	0.172	1.02	0.165	1.10
	0.403		0.168		0.182	
n-Butyl ester						
1.5% SE-30 (a)	0.953	1.02	0.539	1.03	0.497	1.04
	0.934		0.523		0.517	
1.5% OV-1 (b)	0.960	1.02	0.545	1.04	0.490	1.05
	0.941		0.524		0.515	
1.5% OV-17 (c)	0.607	1.01	0.237	1.03	0.223	1.07
	0.601		0.230		0.239	
0.5% PEGA (d)	0.781	1.02	0.262	1.02	0.256	1.07
	0.765		0.256		0.274	

* *p,p'*-DDT was used as a reference compound (retention times: (a) 34.6 min; (b) 13.6 min; (c) 21.1 min; (d) 26.5 min). Column temperatures: (a) 160 °; (b) 180 °; (c) 170 °; (d) 155 °. Both the detector and flash heater were maintained at 30 ° above the column temperature.

shape on both the selective and non-selective phases, indicating the excellent GC properties of the reaction product.

The chromatographic behaviour of the N-acyl derivatives of DL-alanine methyl and butyl esters was examined with four kinds of typical liquid phases, 1.5% SE-30, 1.5% OV-1, 1.5% OV-17 and 0.5% PEGA. The retention times relative to *p,p'*-DDT and the ratio of retention values of each enantiomeric pair, α, are listed in Table I. Almost all of the pairs of diastereomers could be well distinguished from each other on either of these columns. Of the three diastereomers, the N-*l*-teresantalinyl derivative afforded the most satisfactory separation on the selective phase, in particular on the 0.5% PEGA column. The *d*-isoketopinyl derivative showed a more prolonged retention time, probably due to the presence of an oxo group. The elution order of the diastereomeric N-*l*-teresantalinyl amino acid esters was the same as that of the TP derivatives, in which the L-amino acid exhibited a larger retention value than the corresponding D-enantiomer. In contrast, both the N-*d*-isoketopinyl and N-*l*-dihydroteresantalinyl alanine esters showed the reversed elution pattern, although at present no structural reason can be suggested for this reversal.

The gas-phase analytical resolution of the representative neutral and acidic amino acid enantiomers was then undertaken on a PEGA column by forming the N-*l*-teresantalinyl derivatives. The results for twelve pairs of the enantiomers are given in Table II. The degree of separation is quantitatively expressed by the separation factor, *R*, proposed by Pattison[13,14]. In general, the methyl ester of an amino acid af-

TABLE II

SEPARATION FACTORS FOR DIASTEREOMERIC N-*l*-TERESANTALINYL DL-AMINO ACID ESTERS

Conditions: glass column (3 m × 3 mm I.D.); nitrogen flow-rate, 30 ml/min; column temperature, 155°; flash heater temperature, 185°; detector temperature, 185°.

Amino acid	*Methyl ester*			*n-Butyl ester*		
	RRT(D)*	α(L/D)	*R*	*RRT*(D)	α(L/D)	*R*
Alanine	0.164	1.10	1.24	0.256	1.07	0.75
Valine	0.191	1.05	0.63	0.329	1.03	–
Norvaline	0.242	1.08	0.70	0.401	1.05	0.65
Leucine	0.248	1.05	0.71	0.469	1.03	0.40
Isoleucine	0.250	1.03	–	0.474	1.04	–
Norleucine	0.311	1.05	0.55	0.596	1.04	0.53
Proline	0.648	1.20	1.85	1.07	1.04	–
Aspartic acid	0.922	1.05	–	2.38	1.01	–
Methionine	1.44	1.09	1.33	2.23	1.04	0.54
Ethionine	1.67	1.03	–	2.46	1.01	–
Glutamic acid	1.66	1.07	1.14	2.72	1.06	0.40
Phenylalanine	2.17	1.04	–	3.18	1.03	–

* *p,p'*-DDT was used as a reference compound (retention time, 26.5 min).

forded a more satisfactory separation than the corresponding butyl ester. No marked differences in the separation factors for normal and branched-chain amino acids were observed. Of the amino acids tested so far, the methyl ester of proline showed excellent resolution with a separation factor of 1.85. It is evident from the results that enantiomeric alanine (R = 1.24), methionine (R = 1.33), and glutamic acid (R = 1.14) were also completely separated. To the best of our knowledge, this appears to be the most successful instance of the gas-phase resolution of amino acid enantiomers with use of a chiral reagent. Diastereomeric derivatization into the N-*l*-teresantalinyl amino acid methyl ester was also effective for the optical resolution of valine, norvaline, leucine and norleucine. In these instances, the extent of overlapping of the enantiomeric peaks was less than 15% and the quantitative determination was practicable. With regard to isoleucine, aspartic acid, ethionine and phenylalanine, each set of enantiomers was still distinguishable. However, the R value was less than 0.4 and hence measurement of the peak areas was impossible because of their overlap.

It has previously been suggested that optical resolution should be achievable with compounds in which the chirality is embodied in a more rigid skeleton[10]. The potential utility of the present resolving agents may be ascribable to the rigid carbon skeleton, which is directly linked to amino acid through an amide bond. The more rigid structure of the *l*-teresantalinyl moiety, which possesses a cyclopropane ring, appears to reflect the excellent resolution of the resulting diastereomers.

It is hoped that availability of the new chiral reagents may increase the versatility of GC resolution based on diastereomeric derivatization.

ACKNOWLEDGEMENTS

The authors extend their thanks to Dr. Masahiro Torigoe, Yoshitomi Pharma-

ceutical Industries, and Mr. Hiroshi Iwase, Ajinomoto Co., for generous gifts of the samples. This work was supported in part by a Grant-in-Aid from Iatrochemical Research Foundation, Tokyo, which is gratefully acknowledged.

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