Journal of Chromatography, 195 (1980) 292–296
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,791

Note

Gas chromatographic separation of chiral 2-hydroxy acids and 2-alkyl-substituted carboxylic acids

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(First received December 20th, 1979; revised manuscript received March 3rd, 1980)

Gas chromatographic methods are preferentially applied for the determination of the configuration of optically active compounds, especially when only small and impure samples are available. Besides the biological relevance of a certain configuration, the quantitative measurement of enantiomer composition in asymmetric syntheses has increasing importance.

Direct enantiomer resolution on chiral stationary phases has been used as the most elegant method for stereochemical assignments in the field of amino acids and amines[1–4], based on the fundamental investigations of Gil-Av and co-workers. Only recently the enantiomers of 2-hydroxy acids could be separated on special chiral stationary phases as O-trifluoroacetyl-hydroxy acid isopropyl esters[5].

Alternatively, chiral compounds may be separated as diastereomeric derivatives on achiral stationary phases. As in amino acids two functional groups are available in hydroxy acids for introducing a chiral substituent. Both techniques have been used before[6–10]. For amino acids (+)-3-methylbutan-2-ol proved to be an excellent reagent to form diastereomeric esters, which could be very well resolved as N-pentafluoropropionyl derivatives.

In this paper we report on the separation of the (+)-3-methyl-2-butyl esters of O-trifluoroacetylated (O-TFA) or O-trimethylsilylated (O-TMS) 2-hydroxy acids and of branched carboxylic acids.

EXPERIMENTAL

Materials

(+)-3-Methylbutan-2-ol was prepared, as described by Halpern and Westley[12], by esterification of L-valine with racemic 3-methylbutan-2-ol and fractional crystallization of the *p*-toluene sulphonic acid salt. After two crystallizations and alkaline hydrolysis of the ester, (+)-3-methylbutan-2-ol was obtained in 98.5% optical purity and 35% overall yield.

Esterification of 2-hydroxy acids and 2-alkyl(aryl)-carboxylic acids

Amounts of 100 μg of the acid were heated for 2 h at 80°C with 50 μl of (+)-3-methylbutan-2-ol–HCl gas (7 *N*) in a screw cap vial with a PTFE lining in the cap. Excess reagent was removed with a current of dry N_2.

0021-9673/80/0000–0000/$02.25

O-Trifluoroacetylation

To the residue of (+)-3-methyl-2-butyl esters, 200 μl of CH_2Cl_2 and 50 μl of trifluoroacetic anhydride were added and kept at room temperature for 30 min. After removal of the excess reagent with nitrogen, the residue was dissolved in 200 μl of CH_2Cl_2 and investigated by gas chromatography.

O-Trimethylsilylation

Alternatively 50 μl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA, Macherey, Nagel & Co, Düren, G.F.R.) were added to the esters and kept at room temperature for 1 h.

Gas chromatography

The preparation of glass capillaries has been described in a previous publication[13]. The SE-30 capillary was purchased from Franzen Analysentechnik (Bremen, G.F.R.). Gas chromatographic investigations were run on a Carlo Erba Model 2101 gas chromatograph with inlet split system (split ratio 30:1), hydrogen as carrier gas, and flame ionization detector.

RESULTS AND DISCUSSION

Previous investigations of diastereoisomeric 2-hydroxy acid derivatives in most cases were performed on long steel capillaries or on packed columns with peak shapes,

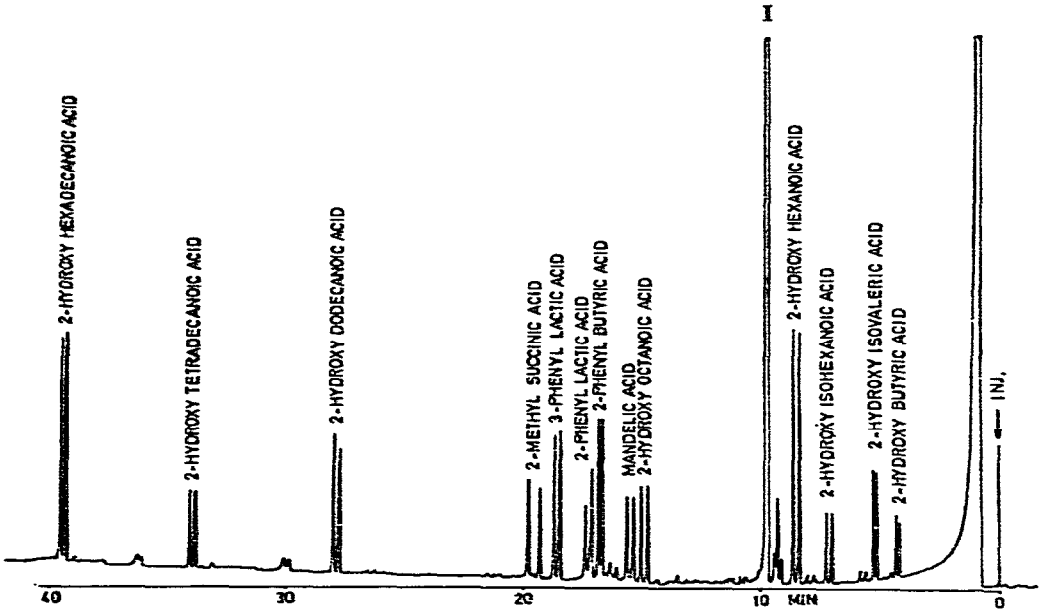

Fig. 1. Gas chromatogram of DL-2-O-TFA-carboxylic acid (+)-3-methyl-2-butyl esters and DL-2-alkyl(aryl)-carboxylic acid (+)-3-methyl-2-butyl esters on a 25-m glass capillary column (SE-30, 0.3 mm I.D.). I = N-TFA-L-valine-O-(+)-3-methyl-2-butyl ester (internal standard). Deactivation, Carbowax 20M; column temperature, 80°C; temperature programme, 3°C/min to 240°C; carrier gas, 0.7 bar hydrogen; split ratio, 1:30.

TABLE I

SEPARATION FACTORS (α) FOR 2-O-TFA- AND 2-O-TMS-CARBOXYLIC ACID (+)-3-METHYL-2-BUTYL ESTERS ON 25-m GLASS CAPILLARY A (SE-30) AND B (OV-17)

Compound	*α-Value O-TFA derivative*		*Temperature (°C)*		*α-Value O-TMS derivative*		*Temperature (°C)*	
	Column A	*Column B*	*Column A*	*Column B*	*Column A*	*Column B*	*Column A*	*Column B*
Lactic acid	1.015	1.019	100	100	1.047	1.033	100	100
2-OH-Butyric acid	1.032	1.036	100	100	1.042	1.044	100	100
2-OH-Isovaleric acid	1.029	1.032	100	100	1.034	1.039	100	100
2-OH-Isohexanoic acid	1.050	1.046	100	100	1.037	1.036	100	100
2-OH-Hexanoic acid	1.045	1.053	100	100	1.041	1.045	100	100
2-OH-Octanoic acid	1.035	1.034	130	130	1.032	1.032	130	130
2-OH-Dodecanoic acid	1.023	1.022	190	190	1.017	1.019	190	190
2-OH-Tetradecanoic acid	1.026	1.025	190	190	1.019	1.020	190	190
2-OH-Hexadecanoic acid	1.024	1.028	190	190	1.018	1.022	190	190
Malic acid	1.021	1.029	140	140	1.016	10.17	140	140
2-Phenyllactic acid	1.032	1.037	140	140	1.007	1.032	140	150
3-Phenyllactic acid	1.029	1.029	140	140	1.030	1.033	140	150
Tropaic acid	1.013	1.026	140	140	1.016	1.025	140	150
Mandelic acid	1.032	1.040	140	140	1.010	1.015	140	140
4-OH-3-Methoxymandelic acid	1.023	1.026	200	200	1.033	1.015	200	200
3-OH-4-Methoxymandelic acid	1.023	1.002	200	200	1.030	1.011	200	200
4-OH-Mandelic acid	1.011	1.033	140	140	not separated	1.019	—	200

analysis times and sensitivities not comparable with modern standards. The excellent results with amino acid derivatives[11] encouraged us to apply the same technique to 2-hydroxy and other chiral carboxylic acids. Although (+)-3-methyl-2-butyl esters of 2-hydroxy acids with free hydroxy groups are sufficiently volatile for gas chromatography, the peak shapes of acylated or silylated derivatives are better. Both types of derivative are sufficiently stable over several days. The O-TFA derivatives (Fig. 1) are more volatile and show slightly larger separation factors (α) than the O-TMS derivatives (Fig. 2). The results are summarized in Tables I and II. The order of elution of diastereoisomers was proved as far as pure enantiomers were available. In these cases (lactic acid, malic acid, mandelic acid) the L-enantiomers have the longer retention time.

The derivatives of 3-hydroxybutyric acid could not be separated. It seems to be necessary that the carboxy group, which is esterified with the chiral alcohol, is directly attached to the asymmetric center. Similar results have been obtained by Rose and co-workers[14].

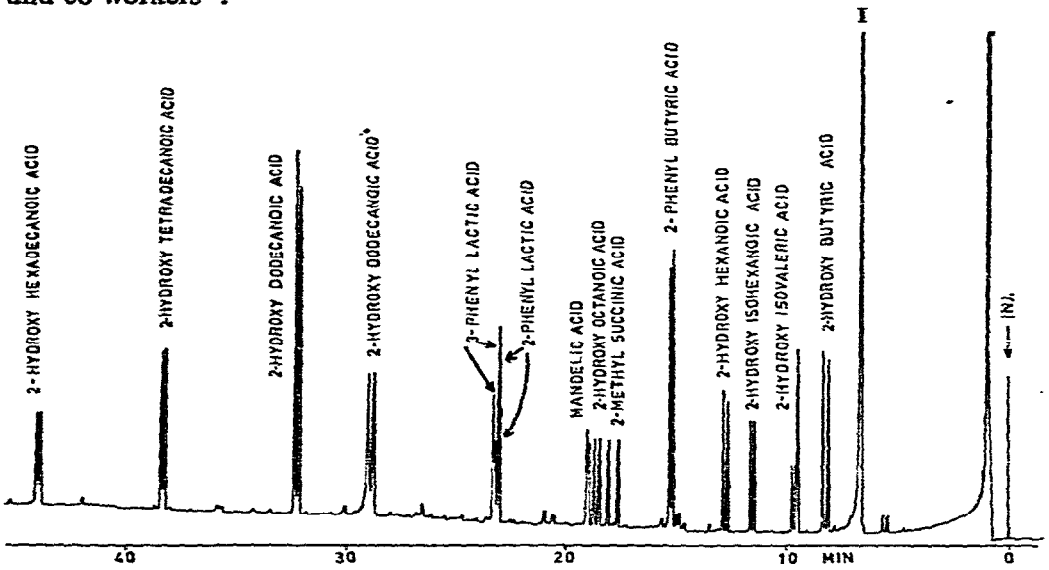

Fig. 2. Gas chromatogram of DL-2-O-TMS-carboxylic acid (+)-3-methyl-2-butyl esters. Same conditions as in Fig. 1. (* = 2-hydroxydodecanoic acid with underivatized hydroxy group).

TABLE II

SEPARATION FACTORS (α) FOR 2-ALKYL(ARYL)-CARBOXYLIC ACID (+)-3-METHYL-2-BUTYL ESTERS ON 25-m GLASS CAPILLARY A (SE-30) AND B (OV-17)

Compound	*α-Value*		*Temperature (°C)*	
	Column A	*Column B*	*Column A*	*Column B*
2-Methylsuccinic acid	1.061	1.057	140	140
2-Phenylsuccinic acid	1.032	1.022	190	190
2-Phenylbutyric acid*	1.027	1.027	100	120

* Enantiomeric 2-phenylbutyric acid amides have been separated on optically active stationary phases by Weinstein and co-workers[3].

Although all methods based on the formation of diastereoisomers are inaccurate principally because of the different reaction kinetics in the formation of the diastereomeric derivatives and because of the lack of enantiomer reagents with 100% optical purity[15], the described procedure can be recommended for the assignment of the configuration of carboxylic acids with an asymmetric centre at C-2.

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