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GAS CHROMATOGRAPHIC RESOLUTION OF 2-HYDROXYCARBOXYLIC ACID ENANTIOMERS

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SUMMARY

Several types of derivative can be employed for gas chromatographic resolution of optical isomers of 2-hydroxycarboxylic acids, *viz.* 2-acyloxycarboxamides, 2-trimethylsiloxycarboxamides, 2-carbamoyloxycarboxylic esters, 2-carbamoyloxycarboxamides and 2-hydroxycarboxylic esters. In all cases hydrogen bonding is the main interaction which leads to chiral recognition. Each type of derivative offers specific advantages whilst exhibiting certain limitations. The choice of derivative will depend primarily upon the sample, the respective hydroxy acid and the relative amounts of isomers.

INTRODUCTION

The resolution of optical isomers of 2-hydroxycarboxylic acids is of considerable interest in biochemistry and medicine[1]. Lactic acid, for example, may occur in biological samples in both enantiomeric forms, depending upon its origin[2]. A suitable method for the analysis of the enantiomers of 2-hydroxycarboxylic acids is the conversion into diastereomeric esters with a chiral alcohol such as butanol[3,4], or else derivatization of the hydroxy group with chiral reagents such as menthyl chloroformate[5] or a chiral isocyanate[6]. However, there are several intrinsic disadvantages of the diastereomer method, notably the need for absolute optical purity of the reagent. Direct gas chromatographic (GC) resolution of the enantiomers of 2-hydroxycarboxylic acids was first reported in 1978 for the resolution of 2-pentafluoropropionoxy-N-cyclohexylcarboxamides on Chirasil–Val[7]. These derivatives exhibit much higher resolution factors than the corresponding carboxylic esters. Thus it was inferred that hydrogen-donor groups are indispensible for the high chiral recognition of Chirasil–Val or other stationary phases of the diamide type. In consequence of this generalization and the observation that certain chiral alcohols may be resolved as urethanes[8], we tested other –NH-containing derivatives for the resolution of 2-hydroxycarboxylic acid enantiomers, *viz.* the 2-carbamoyloxycarboxylic esters[9] and

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the 2-trimethylsiloxycarboxamides; the 2-carbamoyloxycarboxylic esters were later utilized extensively by other workers[10]. As the free hydroxyl group is capable of forming strong hydrogen bonds, the 2-hydroxycarboxylic esters[11] were studied, along with the 2-carbamoyloxycarboxamides[12]. We now summarize our experiences as to the specific advantages and drawbacks of each type of derivative. From this comparison we deduce that hydrogen bonding plays the most important role in enantioselective association between the stationary phase and the hydroxycarboxylic acid.

EXPERIMENTAL

Preparation of derivatives

Preparation of 2-hydroxycarboxylic methyl esters

A 1-mg amount of a mixture of eight 2-hydroxycarboxylic acids together with 0.2 ml of 5 *N* hydrochloric acid in methanol were heated in a heavy-walled conic ampoule at 110°C for 30 min. To ensure complete reaction and to neutralize the hydrochloric acid, 100 μl of ethereal diazomethane solution was added to the chilled reaction mixture. The excess of diazomethane was carefully blown off using nitrogen at room temperature.

Preparation of 2-trimethylsiloxy-N-propylcarboxamides

Aminolysis. The methyl esters, prepared as above, were heated with 0.1 ml of *n*-propylamine for 30 min to 110°C. The excess of *n*-propylamine was blown off at room temperature with a gentle stream of nitrogen.

Trimethylsilylation. N-Methyl-N-trimethylsilyltrifluoroacetamide (40 μl) was added and the mixture was heated to 60°C for 10 min. Any excess of reagent was blown off at room temperature and the product was taken up in methylene chloride.

Preparation of the 2-heptafluorobutyroxy carboxamides

The sample was aminolysed as described above, the amide was dissolved in 500 μl of methylene chloride and 50 μl heptafluorobutyric anhydride was added. The mixture was heated for 10 min at 110°C, the excess reagent and solvent was blown off at room temperature and the product was dissolved in methylene chloride.

Preparation of 2-(isopropylcarbamoyloxy)carboxylic methyl esters

A 1-mg sample was allowed to react at room temperature with 100 μl of ethereal diazomethane solution. After gas evolution ceased, the excess of diazomethane was blown off with a stream of nitrogen. Freshly distilled isopropyl isocyanate (0.1 ml) and triethylamine (0.1 ml) [triethylamine was refluxed with naphthyl isocyanate (10:1) for 1 h and then distilled] were added and the mixture was heated in a screw-capped vial to 110°C for 10 min. The excess reagent is blown off at room temperature and the product was dissolved in methylene chloride.

Preparation of 2-(isopropylcarbamoyloxy)-N-isopropylcarboxamides

A 1-mg sample was dissolved in 300 μl of methylene chloride and 100 μl of isopropyl isocyanate and the solution was heated to 110°C for 1 h. The reaction mixture was cooled to room temperature and the excess reagent was blown off. The product was taken up in methylene chloride.

Determination of reaction kinetics

Approximately 5 mg of tetradecane as internal standard were added to 5 mg of sample and the respective preparation was performed as described above. The ratio of derivative to standard was determined by gas chromatography and, where

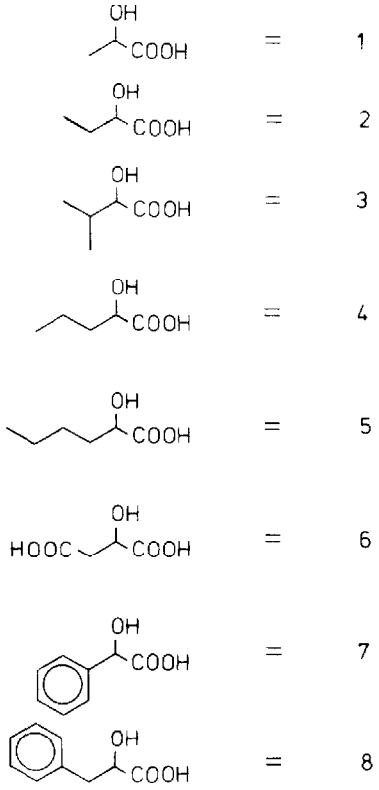
applicable, the sample was divided into five equal portions to which equal amounts of the second reagent were added. The second reaction was stopped at different times and the excess reagent blown off. The amounts of both the intermediate and the final product relative to the internal standard were determined by GC.

Gas chromatography

GC was performed on a Carlo Erba 2101 instrument with split-injector, temperature 250°C, splitting ratio 1:50, and flame ionization detector (temperature 250°C). A Duran-glass capillary, 20 m × 0.25 mm, coated with Chirasil–Val as described previously[13], was employed. For the 2-hydroxycarboxylic esters a fused silica capillary was used instead. The carrier gas was hydrogen at an inlet pressure of 40 kPa, corresponding to a mean linear carrier-gas velocity of 50 cm/sec; temperature programs were as shown in the corresponding chromatograms. Peak identities were established by mass spectrometry.

RESULTS AND DISCUSSION

For a comparison of the different derivatives the following 2-hydroxycarboxylic acids were selected: lactic acid (1), 2-hydroxybutyric acid (2), 2-hydroxyisovaleric acid (3), 2-hydroxyvaleric acid (4), 2-hydroxycaproic acid (5), malic acid (6), mandelic acid (7), and β-phenyllactic acid (8) (Scheme 1).



Scheme 1

The derivatives employed first for direct resolution of 2-hydroxy carboxylic acid enantiomers[7] were the 2-perfluoroacyloxy carboxamides. Their preparation involved a three-step derivatization (Scheme 2).

$$\text{H-OCHRCO-OH} + \text{R'OH} \xrightarrow{H^+} \text{H-OCHRCO-OR'} + H_2O$$

$$\text{H-OCHRCO-OR'} + \text{R''NH}_2 \longrightarrow \text{H-OCHRCO-NHR''} + \text{ROH}$$

$$\text{H-OCHRCO-NHR''} + (C_3F_7CO)_2O \longrightarrow C_3F_7CO\text{-OCHRCO-NHR''} + C_3F_7COOH$$

Scheme 2.

Methylation with diazomethane was not always complete, as solid samples often contained the hydroxy acids as intermolecular lactides. Nevertheless, together with the methyl esters these were converted into the corresponding amides during the second derivatization step. This reaction required high temperature to be sufficiently fast. The rate of racemization was high for mandelic acid and its analogues, since the mesomeric effect of the phenyl ring stabilizes the transient carbanion. Of L-mandelic acid 27% were inverted to the D-enantiomer under the adopted conditions. For the aliphatic hydroxy acids, inversion was in the range of only 1–2% under the same conditions. The 2-hydroxycarboxamides were finally converted into the 2-heptafluo-

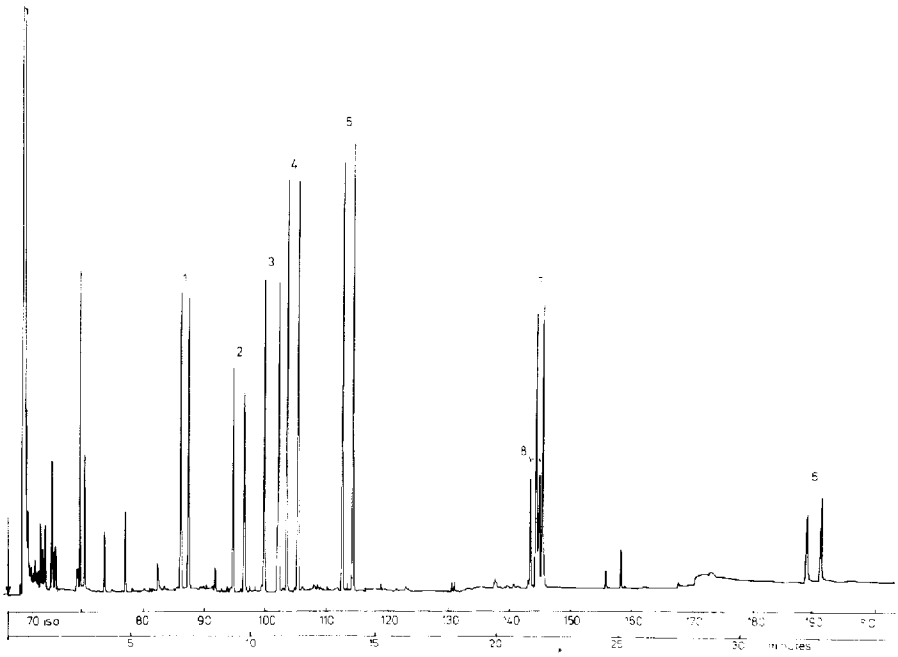


Fig. 1. Gas chromatographic separation of 2-heptafluorobutyryloxycarboxamides on a glass capillary column, 20 m × 0.25 mm, coated with Chirasil–Val. Conditions as given in the Experimental section.

robutyroxycarboxamides by heating with the corresponding anhydride. A chromatogram of the standard mixture is shown in Fig. 1. These derivatives exhibit acceptable gas chromatographic properties. Little peak-tailing is observed, and all 2-hydroxycarboxylic acids show high resolution factors. The lower homologues, the trifluoroacetoxy and the pentafluoropropionoxy derivatives, are less suitable as they are more susceptible to hydrolysis. For the same reason the GC capillary must be sufficiently well deactivated, *i.e.* the glass-wall surface should be free of basic and nucleophilic sites. These derivatives cannot be stored over several days.

Scheme 3.

For derivatization to the 2-trimethylsiloxycarboxamides the final step involved the more gentle trimethylsilylation (Scheme 3). These derivatives are preferable to the former. A chromatogram of the test mixture is shown in Fig. 2. The resolution factors are only slightly smaller owing to the higher temperature of elution; the differences in association enthalpy between isomers decrease with increasing temperature[14]. Malic acid shows a problem typical of the dicarboxylic acids, namely formation of several derivatives; owing to incomplete aminolysis three peak-pairs are found. The first two pairs represent the monoamide monoesters, with the amide group in the 1- or 4-position, respectively. The pair eluting at 197°C is the diamide. Altogether the trimethylsilyl ethers are superior to the perfluoroacyl esters. Their stability against hydrolysis is higher, and consequently they may be stored over longer times.

Since chiral alcohols can be resolved as carbamates[8], the analogous derivatives were tried for 2-hydroxycarboxylic acids. The hydroxy acids were first esterified, and then treated with isocyanate to form the corresponding carbamoyloxy derivatives (Scheme 4) but, owing to the slow reaction[14], base catalysis was necessary to achieve high yields. This is depicted in Fig. 3. Reaction with pure isopropyl isocyanate was not complete even after 3 h (B) and base-catalysis was required to achieve sufficiently fast conversion, *i.e.* in *ca.* 30 min. (A). A chromatogram of the test mixture is shown in Fig. 4. The GC properties and the simpler two-stage preparation render these derivatives quite attractive. Also, all hydroxy acids show large resolution factors including malic acid. A further advantage is the formation of only one derivative for dicarboxylic acids. In addition, racemization under the employed conditions, even for mandelic acid, is negligible. Disadvantages are formation of a number of by-products, which potentially interfere with the peaks of interest, and the need for freshly distilled isocyanate and base in order to keep the formation of unwanted by-products to a minimum.

A further extension of this general approach is the conversion of 2-hydroxy carboxylic acids into the 2-carbamoyloxy carboxamides by heating with isocyanate[12], a simple one-step derivatization with negligible racemization (Scheme 5). However, this approach has several disadvantages (Fig. 5): derivatization is slow, with *ca.* 20% yield after 1 h, the resolution factors are the smallest of all, the derivatives are relatively involatile and formation of by-products is extensive.

GC resolution of 2-hydroxy carboxylic esters is a further possibility. These

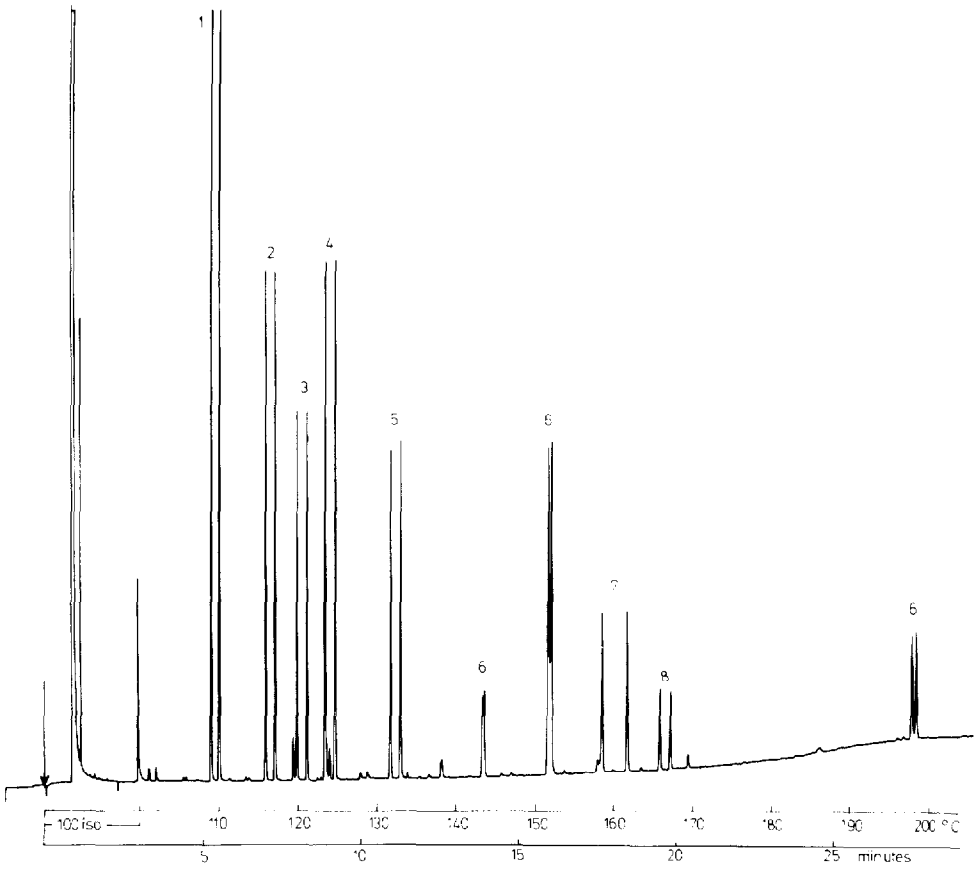


Fig. 2. Gas chromatographic separation of 2-trimethylsilyloxycarboxamides on a glass capillary column, 20 m × 0.25 mm, coated with Chirasil-Val. Conditions as given in the Experimental section.

derivatives have recently been proposed[11], but with non-deactivated glass capillaries we obtained bad peaks. Fused-silica capillaries are better suited, as shown in Fig. 6. Only β-phenyllactic acid cannot be resolved as the ester. Racemization during derivatization is negligible, the procedure is fast and complete and the derivatives are the most volatile of all; this even may cause difficulties with the lower homologues, as after derivatization they are easily lost during evaporation of reagents. Esters with different higher alcohols may sometimes be preferable[15].

The hydroxy esters have some limitations. Solutions of hydrogen chloride in alcohols are relatively involatile owing to their equilibrium with the highly solvated alkoxonium chloride. Therefore, evaporation of reagents under a stream of nitrogen requires a long time and raises the risk of losing the more volatile 2-hydroxycarboxylic esters. For esters of primary alcohols, this problem may be alleviated by "neutralization" of the residual hydrogen chloride with the corresponding diazoalkane shortly before the reagent is completely evaporated. For some samples it is preferable

$$\text{H-OCHRCO-OR'} + \text{R''NCO} \longrightarrow \text{R''NHCO-OCHRCO-OR'}$$

Scheme 4.

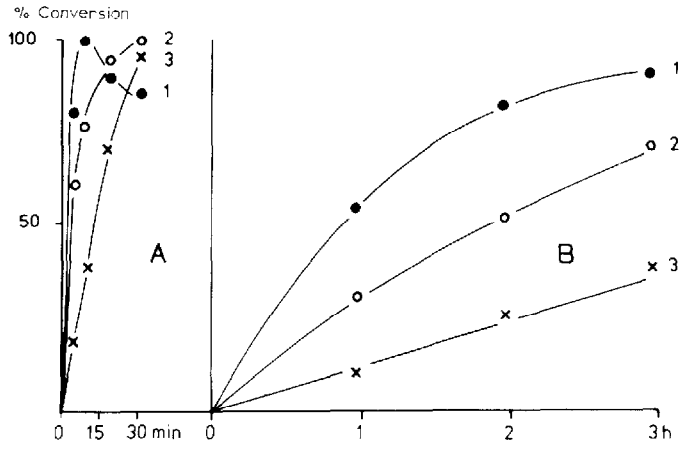


Fig. 3. Conversion of 2-hydroxycarboxylic methyl esters into 2-(isopropylcarbamoyloxy)carboxylic methyl esters by treatment with isopropyl isocyanate (50%) in triethylamine (A) or methylene chloride (B) at 110°C.

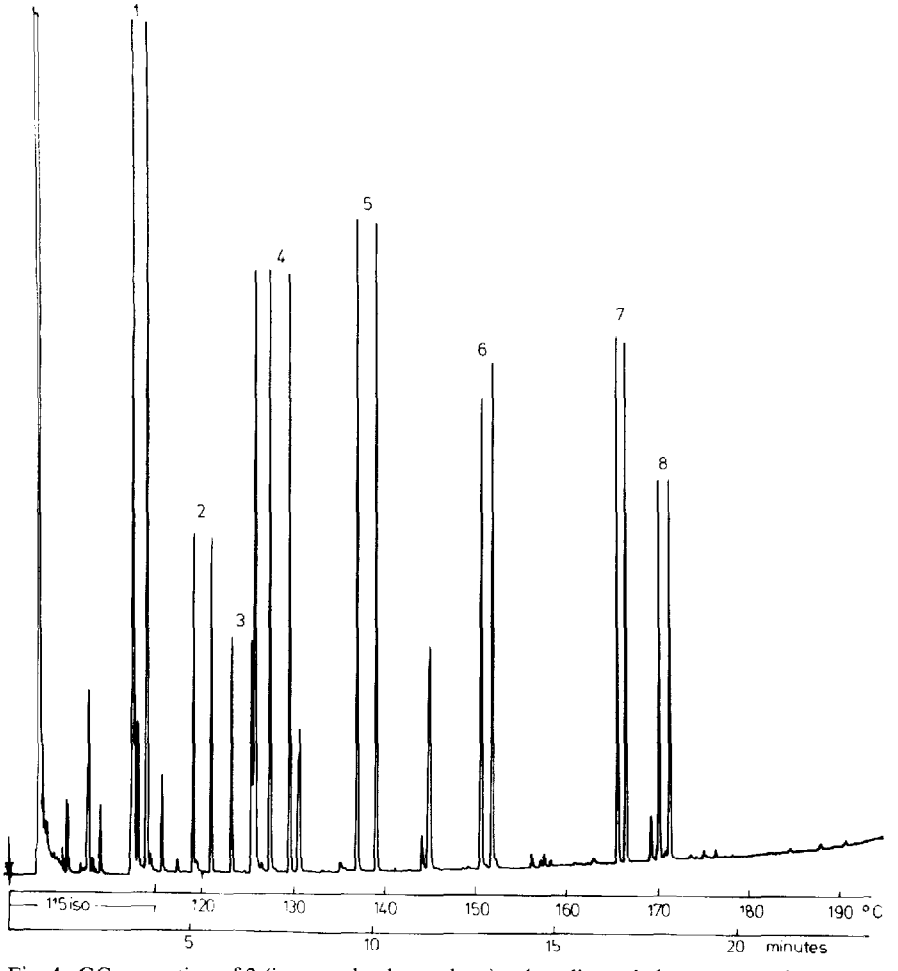


Fig. 4. GC separation of 2-(isopropylcarbamoyloxy)carboxylic methyl esters on a glass capillary column coated with Chirasil Val. Conditions as given in the Experimental section.

$$H\text{-}OCHCO\text{-}OH + 2R''NCO \longrightarrow R''NHCO\text{-}OCHRCO\text{-}NHR'' + CO_2$$

Scheme 5.

to esterify with the diazoalkane, mainly when the hydroxy acids in the sample are present only in their free forms, and not as lactides. Other possibilities for derivatization which should be investigated are pyrolytic esterification via the tetramethylammonium salts in the hot injector[16] or esterification with acetals[17].

The order of elution of the two isomers is usually D before L, only for the hydroxycarboxylic esters is the order reversed. From this fact conclusions can be drawn regarding the mechanism of chiral recognition. Apparently, a proton-donor

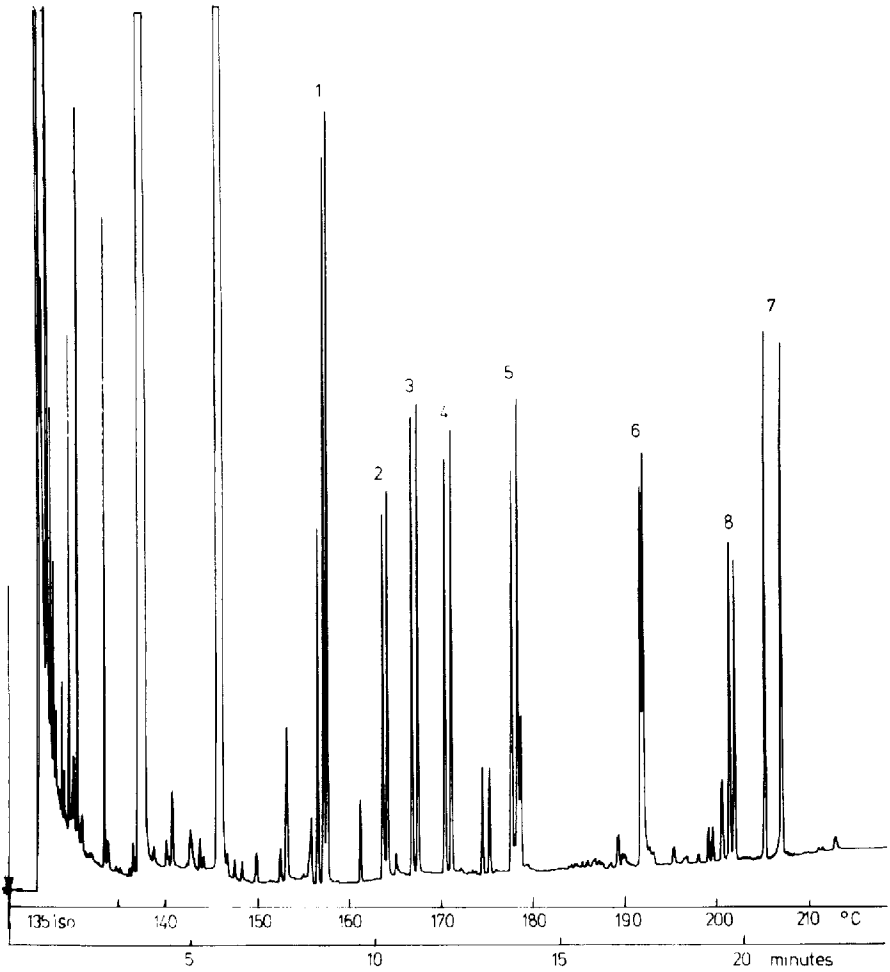


Fig. 5. GC separation of 2-(isopropylcarbamoyloxy)-N-isopropylcarboxamides on a glass capillary column, 20 m × 0.25 mm, coated with Chirasil-Val. Conditions as given in the Experimental section.

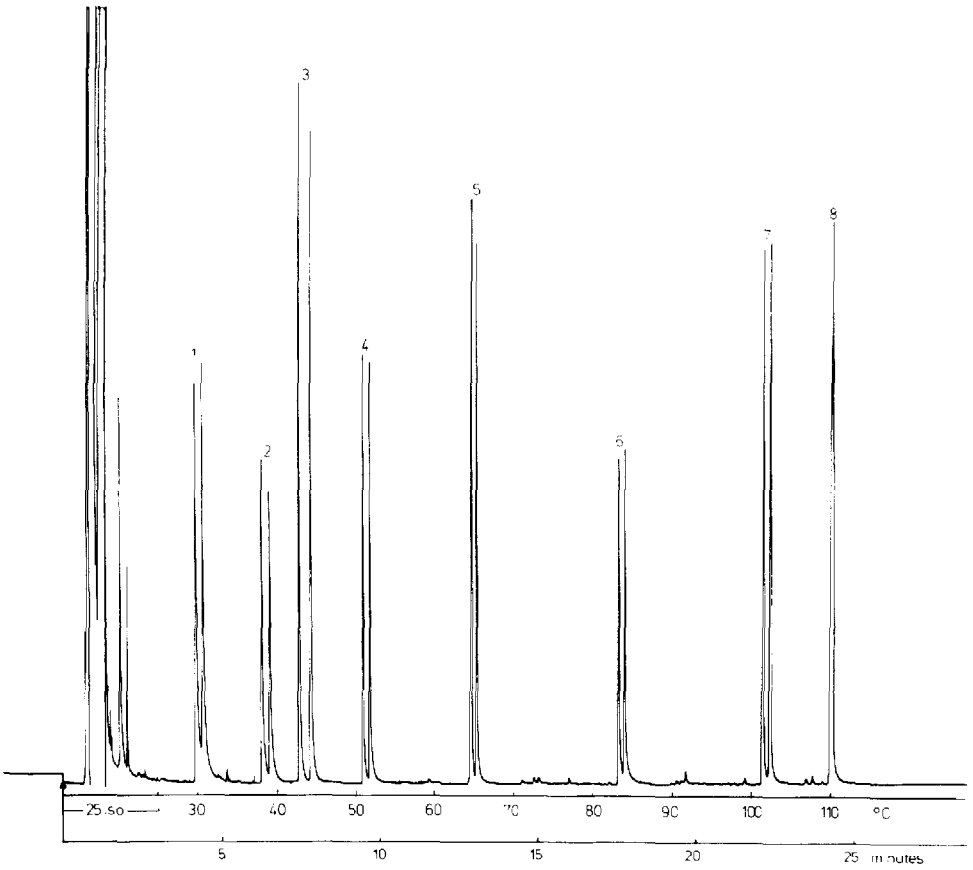
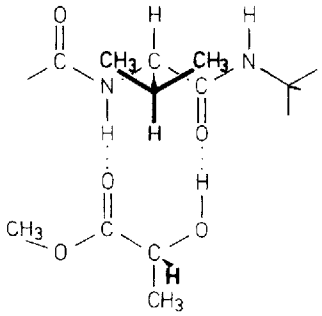


Fig. 6. GC separation of 2-hydroxycarboxylic methyl esters on a fused silica capillary column, 20 m × 0.25 mm, coated with Chirasil–Val. Conditions as given in the Experimental section.

group as the hydrogen-bonding moiety is important for diastereotropic* association with enthalpy differences between two isomers which are sufficiently large to enable resolution. The hydrogen atoms of either the amide or the carbamate moieties serve for this purpose, with, in the case of the 2-hydroxycarboxylic esters, the oxygen-bonded hydrogen.

In this context also, the 2-perfluoroacyloxycarboxylic esters should be mentioned. These can be resolved into their enantiomers on a special diastereomeric phase carrying *S*-valine-*S*-α-phenylethylamide as chiral selector[8], a chiral stationary phase later employed also by other workers[10]. These derivatives are more volatile than the 2-hydroxycarboxylic esters, and hence it is even more difficult to avoid losses during the final evaporation of the reagents; also, as the separation factors are small the derivatives need to be chromatographed at very low temperatures and with cor-

* We recommend the term "diastereotropic" to indicate that interaction of chiral selector and selectand is viewed as a transient approach and dissociation of both partners with a vectorial character of the interaction forces rather than as formation of a static, rigid diastereomeric complex.

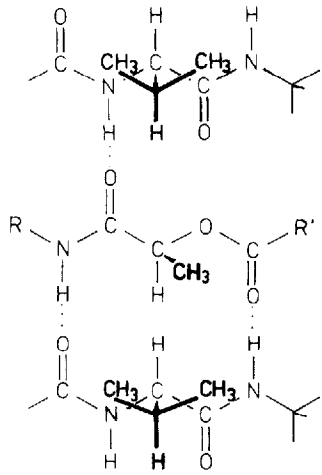


Scheme 6.

respondingly long retention times. Therefore this approach is not widely applicable.

Inspection of molecular models along with the spectral properties suggest that the conformation with lowest energy in solution or in the vapour phase involves an intramolecular C_5–hydrogen bond[17]. If this is also assumed to be the case in the non-polar silicone matrix of the stationary phase, then diastereotropic association of selector and selectand as depicted in Scheme 6, with D-lactic acid as selectand, should have the lowest association enthalpy. Exchange of hydrogen atom and methyl group at the chiral centre leads to higher steric hindrance between the methyl group of the selectand and the bulky isopropyl groups of the selector. This results in a lower relative stability for the L,L-complex. The fact that β-phenyllactic acid is not resolved into its isomers is surprising, but at present is difficult to rationalize.

For all other derivatives a trimolecular association is more likely. The most stable conformation of the selector moiety, the N-acylvaline-*tert.*-butylamide residue, is depicted in Scheme 7, with a *trans*-conformation for both amide groups and intramolecular C-5 and C-7 hydrogen bonds. Intercalation of the selectand perfluoroacyloxycarboxamide between two selectors must be assumed, and not association to one selector, as otherwise an unfavourable *cis*-conformation of the amide groups would have to be assumed.



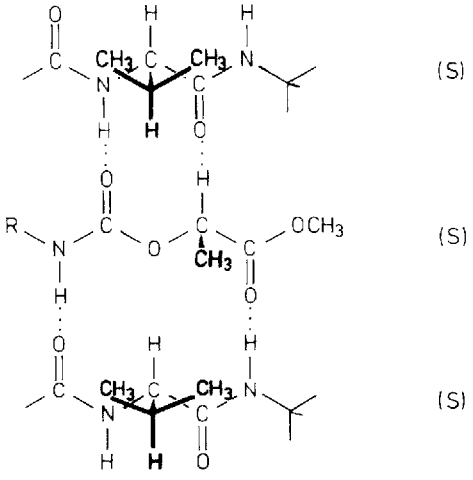
Scheme 7.

A similar association is envisaged for the 2-trimethylsiloxycarboxamides: the ester carbonyl group is absent, but the relative configuration of the small hydrogen atom and the bulky trimethylsiloxy group leaves little other choice for a well-fitting insertion of the selector. Possibly the trimethylsilylether oxygen may further stabilize the complex by forming a weak hydrogen bond to the *tert.*-butylamide hydrogen of the selector. Clearly, there are also other conceivable associations such as interaction of the selectand amide group with the *tert.*-butylamido carbonyl group of the selector. This would not contribute to chiral recognition as the two chiral centres are too far apart.

The association likely to entail highest chiral recognition of the carbamoyloxy esters is the one depicted in Scheme 8. Other possible relative arrangements do not rationalize the strong enantioselectivity as well. In the case of the carbamoyloxy amides, inspection of molecular models does not immediately disclose a clearly preferable mode of interaction. The smaller α-values probably reflect the multiple and competing possibilities of the relative arrangements of selector and selectand.

From a practical point of view the question arises which type of derivative is to be recommended for a particular analysis or sample. The selection of derivative depends mainly upon the composition of the sample, the enantiomeric ratio and the particular hydroxy acid to be determined. Normally the least time-consuming and least laborious procedure is preferred. In this respect esterification is clearly favoured. As discussed previously, the high volatility of ester derivatives may cause problems. Well deactivated capillaries are required to prevent absorption on the capillary walls; this can impair the accuracy of enantiomer ratio determinations. For β-phenyllactic acid, which is not resolved as its simple ester, conversion into the carbamoyloxy derivative or to the trimethylsiloxycarboxamide is recommended.

For dicarboxylic acids, *e.g.* malic acid, esterification or further derivatization to the carbamoyloxy derivatives is the most suitable. Tartaric acid is more problematic. Esterification with diazomethane leads also to partial methylation of one of the hydroxyl groups. Acid-catalysed esterification is therefore preferred, but the diester itself exhibits strong tailing even on well deactivated capillaries. Reaction with propyl isocyanate yields the suitable biscarbamoyloxy derivative. With *tert.*-butyl isocyanate at lower triethylamine concentrations (20%) the more volatile mono-urethane is



Scheme 8.

formed as the main product. Both derivatives exhibit sufficiently high resolution factors, but from *meso*-tartaric acid two enantiomeric monocarbomoyloxy derivatives are formed which are also resolved. A fact worth mentioning is that the enantiomer commonly denoted as D-tartaric acid elutes after the L-isomer, seemingly in contrast to the elution order of the other hydroxy acid enantiomers. With the Cahn–Prelog–Ingold denotation it becomes clear that *S*-enantiomers are retained longer than *R*-enantiomers. The *S*-configuration corresponds to the L-2-hydroxycarboxylic acids and to D-tartaric acid, possessing two *S*-chiral centres[19].

For mandelic acid and its analogues the derivatives chosen are the 2-hydroxycarboxylic esters and the 2-carbamoyloxycarboxylic esters. For aliphatic 2-hydroxycarboxylic acids, formation of the 2-trimethylsiloxycarboxamides may be considered, especially in view of their excellent GC properties and their stability. In particular, for determinations of similar amounts of two enantiomers in a complex sample, these derivatives may be useful, *e.g.* in quantitative analysis via enantiomer labelling. Perfluoroacyloxy- and carbamoyloxycarboxamides are not very suitable; the former are less stable than the trimethylsilyoxycarboxamides, while the latter exhibit relativity low resolution factors and numerous by-products are formed during derivatization. Only in some cases may they be considered, *e.g.* for shifting the peak position to avoid superposition on an otherwise co-eluting constituent.

In conclusion, we would like to emphasize that for high chiral recognition on Chirasil–Val, the presence of a hydrogen-donating functional group in the derivatives is essential. Certainly, other molecular forces such as dipole and π-electron interactions may contribute but, owing to their lower energies, they are less significant. Other appropriate relative co-ordinations of hydrogen-accepting and -donating groups in a derivative can be envisaged; 2-hydroxycarboxamides for instance also exhibit high resolution factors, but they exhibit even stronger peak tailing than hydroxy esters. Similar principles govern the resolution of the isomers of other chiral compounds, *e.g.* alcohols or sulphoxides[20].

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