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Quantitative gas chromatographic analysis of mandelic acid enantiomers

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SUMMARY

A procedure was developed for the quantitative gas chromatographic (GC) analysis of mixtures of mandelic acid enantiomers. Past methods for the GC resolution of α-hydroxy acid enantiomers on optically active stationary phases are systematically reviewed, with special attention to mandelic acid, which is particularly prone to racemization during derivatization. Modification of an earlier procedure involving the conversions: mandelic acid → mandelyl chloride → N-propylmandelamide → O-trimethylsilyl-N-propylmandelamide finally permitted the preparation of O-trimethylsilyl-N-propylmandelamide on a macro scale with negligible racemization and allowed the precise GC analyses of mandelic acid enantiomers on a stainless-steel capillary column coated with N-docasanoyl-L-valine *tert.*-butylamide.

INTRODUCTION

A recent need to evaluate the precise enantiomeric composition of mixtures of *R* and *S*-mandelic acid has prompted us to explore the options for the gas chromatographic (GC) resolution of α-hydroxy acid enantiomers. The GC resolution of enantiomers has been accomplished by either one of two general techniques: (1) conversion of the enantiomers into volatile diastereomer derivatives by reaction with an appropriate optically active reagent, followed by separation on conventional GC phases[1,2] and (2) conversion of the enantiomers into a suitable volatile derivative, followed by separation on an optically active stationary phase. Both methods were originally developed primarily for the resolution of amino acids, and Gil-Av and co-workers[3,4] were the first to describe the use of optically active stationary phases for such applications. These high-molucular-weight phases, which included N-trifluoroacetyl (TFA)-L-isoleucine lauryl ester[3], N-TFA-L-valyl-L-valine cyclohexyl ester[4] and other L-L-dipeptide ester derivatives[5], as well as N-lauroyl-L-valine *tert.*-butylamide[6] and N-docosanoyl-L- valine *tert.*-butylamide[7], were capable of cleanly resolving racemic amino acids as their N-TFA ester derivatives, and offered a number of practical advantages[8] over previous resolutions of amino acids as diastereomeric ester derivatives using conventional GC phases[1,2]. An evaluation of several of these

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procedures for the precise enantiomeric analysis of a number of known mixtures of leucine enantiomers was subsequently undertaken[9], with the finding that such methods allowed for comparable accuracy (0.03–0.7%) and precision (0.03–0.06% standard deviation) in replicate analyses. The above optically active phases had the drawback, however, of being limited to relatively low operating temperatures, thus jeopardizing their application to higher-molecular-weight amino acids[8,10]. This difficulty was overcome in 1977 with the development of "Chirasil-Val", an optically active polysiloxane phase made by coupling a copolymer of dimethylsiloxane and carboxy-alkylmethylsiloxane with L-VALINE *tert.*-butylamide[10]. Since that time this versatile GC phase has been used for the resolution not only of amino acids, but also of the enantiomers of a wide variety of additional classes of organic compounds[11–13].

The GC resolution of α-hydroxy acid enantiomers has not received the attention hitherto devoted to amino acids. In a study which included the resolution of certain amino acids, amines and amino alcohols, lactic acid was first resolved as its O-pentafluoropropionyl N-cyclohexylamide derivative using glass capillary columns coated with Chirasil-Val[14]. In a later study utilizing Chirasil-Val the resolution of eighteen different α-hydroxy acids as five different alkyl ester derivatives was described[15]. The peak tailing due to the free hydroxyl groups was overcome by the use of fused-silica capillary columns and the separation factor, α, for the 3-pentyl esters was found satisfactory. Frank *et al.*[16] studied the resolution of eight different α-hydroxy acids as a number of different derivatives using Chirasil-Val on glass or fused-silica capillary columns. The derivatives included methyl esters, O-heptafluorobutyryl N-propylamides, O-trimethylsilyl N-propylamides, O-isopropylcarbamoyl methyl esters and O-isopropylcarbamoyl N-isopropylamides. Generally speaking, baseline resolution of each enantiomer pair was achieved, but it was found that mandelic acid was racemized to the extent of 54% in some of the derivitization procedures. The authors speculate broadly on the nature of the intermolecular interactions between various groups on the chiral derivative and the Chirasil-Val phase. Later, Wang *et al.*[17] applied one of the above derivatives, the O-trimethylsilyl N-propylamides to the baseline separation and resolution of a mixture of nine α-hydroxy acids of increasing molecular weight ranging from that of lactic to that of *m*-hydroxymandelic acid on Chirasil-Val. Using the readily racemized mandelic acid as a probe, they also worked out the details for a three-step derivatization procedure that was accompanied with a minimum of racemization. Finally, the most recent application of Chirasil-Val to the separation of mandelic acid enantiomers has been that of Koppenhoefer and Allmendinger[18], who converted mandelic acid into a number of derivatives, each of which was analyzed independently for the extent of racemization during preparation. These included methyl, isopropyl and 3-pentyl esters, N-isopropylamide, N-isopropylurethane methyl and isopropyl esters and N-isopropylurethane N-isopropylamide. The extent of racemization at each step of the derivatization was measured by GC on Chirasil-Val and it was found that only the simple esterification of mandelic acid with an alcohol (*e.g.* 3-pentanol) was free (<0.1%) of significant racemization in these multi-step derivatization procedures.

Since 1980 numerous chiral stationary phases other than Chirasil-Val have been employed in generally successful attempts to resolve α-hydroxy acid enantiomers. These phases include optically active Cu(II) complexes of salicylaldehyde Schiff bases[19] and 1,3,5-triazine derivatives of L-valine di- and tripeptide isopro-

pyl esters[20], each of which has been used to resolve the lower alkyl and/or cycloalkyl esters of lactic acid. König *et al.*[21] used O-benzyloxycarbonyl derivatives of *S*-mandelic acid *tert.*-butylamide and *S*-α-phenylethylamide as optically active phases on glass capillary columns to resolve six different α-hydroxy acids as their O-TFA isopropyl esters. They later extended the study[22] to include a number of additional chiral phases derived from *R*- and *S*-mandelic acid as well. A year later König *et al.*[23,24] employed *S*-α-phenylethylamide or *tert.*-butylamide derivatives of other optically active α-hydroxy or O-benzyloxycarbonyl-α-hydroxy acids as stationary phases to resolve ten different hydroxy acids, again as their O-TFA isopropyl esters. At the same time these investigators introduced three new optically active polymeric phases, hexanoyl-*S*-valine-OV-225 and XE-60–*S*-valine–*S(or R)*-α-phenylethylamide, made by chemically modifying the conventional polysiloxane phases OV-225 and XE-60. These phases proved capable of resolving amino acids, amines, amino alcohols and carbohydrates, as well as α-hydroxy acids. König *et al*[25] later extended the use of the XE-60–*S*-valine–*S*-α-phenylethylamide phase to the resolution of some fourteen different α-hydroxy acid enantiomers as their methyl or isopropyl ester isopropylurethane derivatives, formed by reaction of their hydroxyl groups with isopropyl isocyanate. The latter reagent was subsequently proposed[26,27] as a one-step "universal" reagent to form derivatives for the GC resolution of optically active alcohols, amines, amino and N-methylamino acids, as well as α- and β-hydroxy carboxylic acids. Derivatizations were reported to proceed without racemization. The same XE-60–*S*-valine phase was later reported[28] effective in resolving a number of derivatives formed by the action of phosgene on optically active diols, amino alcohols, N-methylamino acids and α-hydroxycarboxylic acid, the latter as 1,3-dioxolan-2,4-diones. Again, the derivatizations were reported to proceed without racemization.

As opposed to the above optically active stationary phases for resolving α-hydroxy acids and other enantiomers directly, the earlier diastereomer GC resolution technique, originally pioneered for resolving amino acids, has received only scant application to hydroxy acids. König and Benecke[29] partially or completely resolved the O-TFA and O-trimethylsilyl (TMS) derivatives of seventeen different α-hydroxy acids as their (+)-3-methyl-2-butyl ester diastereomers on conventional Carbowax 20M, SE-30 and OV-17 phases and later Slessor *et al.*[30] resolved several higher alcohols as well as methyl 9-hydroxy-2-decenoate as their diastereomeric S(+)-lactyl esters on a methylsilicone DB-1 phase.

Previous resolutions of α-hydroxy acids have thus generally employed the optically active stationary phases indicated above, loaded onto glass or fused-silica capillary columns. Stainless steel columns have not been utilized, nor have the earlier optically active GC phases such as N-docosanoyl-*S*-valine *tert.*-butylamide (or the enantiomeric *R*-valine phase[31]), so successfully utilized for amino acids. In fact, it has been stated[22] that the N-acyl-*S*-valine *tert.*-butylamides, while excellent for amino acids, "have no enantioselectivity for α-hydroxy acid derivatives". In addition, while the above studies frequently extol the use of optically active phases for quantitative enantiomer analyses, they generally report only the separation factors achieved with the phase in question, or display actual GC traces showing the separations and the (usually) baseline resolution of the α-hydroxy acid derivatives involved. Thus an actual quantitative GC analysis, checking on accuracy and reproducibility, of mixtures of an α-hydroxy acid of known enantionmeric composition, similar to that

accomplished for leucine enantiomer mixtures[9], has not previously been undertaken. With the above in mind, the objectives of our present work were to develop a method for the accurate GC analysis of mixtures of *R*- and *S*-mandelic acid enantiomers using, if possible, our previously employed[31] stainless steel capillary columns coated with N-docosanoyl-*S* (or *R*)-valine *tert.*-butylamide phases.

EXPERIMENTAL

Gas chromatography

The GC column employed was a 44 m × 0.1 mm I.D. open tubular stainless-steel capillary column, wall-coated with N-docosanoyl-*S*-valine-*tert.*-butylamide (75 mg). This was installed in a Hewlett-Packard Model 5700A gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard Model 18740B capillary inlet system and attached to a Hewlett-Packard Model 3380A digital electronic integrator-recorder. Each product to be analyzed was dissolved in dichloromethane at the molar concentrations indicated in the experiments described below, and 1 μl of each solution was injected onto the column at the temperatures (isothermal) and carrier gas (nitrogen) pressures specified below.

Methyl R-mandelate

R(−)-Mandelic acid (Aldrich, 99+%; 948.3 mg) was esterified by heating with methanol (25 ml) and concentrated sulfuric acid (0.7 ml). The product, 974.7 mg (94.1%) of clear oil, crystallized on standing, m.p. 55.5–56.5°C; $[\alpha]_D^{25}$ − 182° (*c*, 0.9; C_6H_6). Literature: m.p. 55.5°C[32]; $[\alpha]_D^{25}$ − 173° (*c*, 15; C_6H_6)[33].

Propylaminolysis of methyl R-mandelate

The above methyl *R*-mandelate (103.0 mg; 0.620 mmol) was dissolved in propylamine (Aldrich, 99+%; 1.0 ml; 12.2. mmol). The solution was placed in a 1-dm polarimeter tube and its rotation at 25°C was observed at the time intervals shown in Table I. After 2785 min the reaction mixture was stripped of volatiles at 65°C using a rotary evaporator ("vacuum evaporated") and the residue was "chased" twice by dissolving in dichloromethane (3 ml) and vacuum evaporating. This yielded 113.1 mg (94.2%) of syrupy N-propyl-*R*(−)-mandelamide.

The constancy of the observed rotation between 1275 and 2785 min indicates that, once formed, the N-propyl-*R*(−)-mandelamide product was optically stable at 25°C in propylamine solution. The relative consistency of the first-order rate constants calculated for six observed rotations between 115 and 425 min indicates further that the above propylaminolysis is a simple pseudo first order reaction.

The optical stability of methyl R-mandelate in triethylamine

A solution of 30 mg methyl *R*-mandelate [having $[\alpha]_D^{25}$ − 171.8° (*c*, 1.77; C_6H_6)] in 1.00 ml triethylamine (Aldrich) had an observed rotation of −2.315°/dm at 25°C. The rotation remained essentially constant on standing and after 22.75 h was −2.298°. In another experiment a solution of 81.1 mg of the above ester in 3.00 ml triethylamine was heated on the steam bath for 45 min. After cooling to 25°C the solution had an observed rotation of −2.265°/dm.

TABLE I

PROPYLAMINOLYSIS OF METHYL *R*-MANDELATE

Time (min)	α°/dm	$k(c)\ min^{-1}\ (\times 10^3)^c$
0	−8.00[a]	
10	−7.33	
15	−6.98	
115	−1.89	16.4
155	−1.41	15.8
175	−1.23	16.0
245	−0.94	15.8
365	−0.82	15.0
425	−0.80	15.5
1275	−0.79	
1805	−0.79	
2785	−0.79[b]	
Mean ± S.D.		15.8 ± 0.5

[a] By extrapolation.
[b] Corresponds to $[\alpha]_D^{25} = -6.6^\circ$ (*c*, 12.0; propylamine) for N-propyl-*R*(−)-mandelamide.
[c] Calculated by $k = (2.3/t) \times \log([\alpha_0 - \alpha_\infty]/[\alpha_0 - \alpha_t]$, where t = time (min).

O-Trifluoroacetyl-N-propyl-R-mandelamide (R-I)

The above N-propyl-*R*-mandelamide (113.1 mg) was dissolved in dichloromethane (1 ml) and treated with trifluoroacetic anhydride (TFAA; 1.0 ml). After 30 min the volatiles were removed at 60°C by vacuum evaporation, chasing twice with dichloromethane (3 ml). An amount of 171.1 mg (101%) of crude, crystalline *R*-I was obtained. This was recrystallized by dissolving in acetone (0.5 ml), adding hexane (3 ml) and chilling. The purified *R*-I (62 mg) had m.p. 124.5–125°C and showed one sharp peak at 12.81 min under the GC conditions: 0.01 *M*, T = 140°C, P = 0.45 p.s.i. Pure *R*-I product proved unstable, liquefying on standing for several days. GC of this liquid failed to show the previous 12.81 min peak. GC of the original crude *R*-I showed the presence of a small shoulder at a longer retention time on the main peak, suggesting that crude *R*-I contained a small, undetermined amount of the *S*-enantiomer.

O-Trifluoroacetyl-N-propyl-RS-mandelamide (RS-I)

A mixture of methyl *RS*-mandelate (167.5 mg; 1.01 mmol) and propylamine (1.5 ml; 18.3 mmol) was allowed to stand for 96 h, then was processed as above to yield 202.4 mg (103.9%) of crude crystalline N-propyl-*RS*-mandelamide. An amount of 107.1 mg of this was dissolved in dichloromethane (0.5 ml), treated with TFAA (0.5 ml) and after 30 min was processed as above to yield 154.9 mg (96.6%) of solid *RS*-I. This was recrystallized as above to yield 84.4 mg of fine needles, m.p. 105–105.5°C. Under the GC conditions used above for the *R*-enantiomer, *RS*-I was incompletely resolved and variations in the GC parameters were accordingly investigated. For GC conditions of: 0.005 *M*, P = 0.2 p.s.i., T = 140, 145 and 150°C approximately baseline resolution was achieved with retention times [*R*:*S* (min)], respectively, of 28.70:31.42, 24.26:26.10 and 20.53:21.97. The quantitative GC analyses [*R*:*S* (%)]

under these conditions were: 140°C (53.3:46.7), 145°C (50.3:49.7) and 150°C (48.6:51.4).

O-Trimethylsilyl-N-propyl-RS-mandelamide (RS-II)

A portion of the above crude N-propyl-*RS*-mandelamide (95.3 mg; 0.494 mmol) was dissolved in pyridine (2.5 ml) and treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA; Aldrich; 970 mg; 3.77 mmol). The mixture was heated on the steam bath for 30 min then vacuum evaporated, and the residue was chased twice with benzene (4 ml) and thrice with dichloromethane (4 ml). An amount of 139.6 mg (106%) of thick oily *RS*-II was obtained which was analyzed using GC conditions of: 0.001 *M*, P = 0.35 p.s.i., *T* = 150°C. These led to baseline resolution, with *R*-II eluting at 18.77 and *S*-II at 21.52 min and an enantiomer analysis of *R:S*(%):50:50.

N-Propyl-*RS*-mandelamide has been previously resolved as its O-heptafluorobutyryl[16] and O-trimethylsilyl[16,17] derivatives on a Chirasil-Val glass capillary column, but no quantitative data were given.

O-Trimethylsilyl-N-propyl-R-mandelamide (R-II)

Since the above propylaminolysis of methyl *R*-mandelate appeared to be attended by an undetermined amount of racemization, an alternative preparation of N-propyl-*R*-mandelamide was employed. Finely powdered *R*-mandelic acid (50.0 mg) and thionyl chloride (0.5 ml) were placed in a test tube and sonicated for 15 min at 25°C. Additional thionyl chloride (0.5 ml) was added, sonication was continued for 15 min, and the mixture was transferred to a small flask with the aid of dichloromethane (2 ml). The volatiles were vacuum evaporated at 25°C and the residue was chased at 25°C with dichloromethane (4 ml), benzene (4 ml) and thrice with additional dichloromethane (4 ml), yielding 47.4 mg (84.5%) of oily mandelyl chloride. This was dissolved in dichloromethane (2 ml) and treated with propylamine (1 ml). After 5 min the solution was vacuum evaporated at 25°C and chased thrice with dichloromethane (4 ml), to yield 70.8 mg (112%) of N-propyl-*R*-mandelamide, a sweet-smelling, thick oil. This was dissolved in triethylamine (Aldrich; 1.0 ml), treated with BSTFA (0.5 ml) and heated on the steam bath for 30 min. The amber solution was vacuum evaporated at 50°C, chasing twice with benzene (4 ml) and four times with dichloromethane (4 ml). An amount of 94.0 mg (108%) of *R*-II was obtained, an amber oil which was analyzed immediately using GC conditions: *ca.* 0.01 *M*, *T* = 150°C, *P* = 0.35 p.s.i. A sharp peak at 18.79 min was noted, along with a very small (<0.12%) peak at slightly longer retention time, indicating that insignificant racemization occurred during the preparation. The next day similar GC results were obtained on the original *ca.* 0.01 *M* dichloromethane solution. The neat *R*-II product, however, appeared unstable. The original oil lost 18% of its weight on standing 24 h and the 18.79 min GC peaked had vanished. Similar instability was noted for an oily *RS*-II product prepared as above form *RS*-mandelic acid.

Quantitative analyses of known mixtures of R- and S-mandelic acid enantiomers as their O-trimethylsilyl-N-propylamides (R-II and S-II)

Mixture 1. A mixture (28.5 mg) containing 73.0% *R*(−)- and 27.0% *S*(+)-mandelic acid was converted to 49.7 mg (100%) of a mixture of R and *S*-II exactly as described above for *R*-II. Half of the product was dissolved in dichloromethane for

immediate analysis using the GC conditions employed above for *R*-II. The *R*-II eluted at 18.68 and the *S*-II at 21.46 min, resulting in: %*R*: 72.2, 72.3; %*S*: 27.8, 27.7. Two and six days later, respectively, the same dichloromethane solution showed: %*R*: 73.7, 72.3; %*S*: 26.3, 27.7; overall average: %*R*: 72.6; %*S*: 27.4 ± 0.7. After 3 weeks the same solution showed: %*R*: 70.2; %*S*: 29.8. By contrast, the original oily product showed a 7% weight loss on standing for 2 days and its GC showed a new peak at 15.41 min, with the original 18.68 and 21.46 min peaks absent. Thus *R*-II and *S*-II appear reasonably stable in dichloromethane solution at 25°C, but decompose rapidly when undissolved.

Mixture 2. A mixture (25.5 mg) containing 39.6% *R*(−)- and 60.4% *S*(+)-mandelic acid was converted to 44.5 mg (114%) of a mixture of *R*-II and *S*-II exactly as above and the product was dissolved (0.01 *M*) in dichloromethane. Immediate GC analyses under the above conditions showed: %*R*: 39.1, 39.2; %*S*: 60.9, 60.8. Replicate analyses were conducted on the same dichloromethane solution after 1, 4 and 5 days with comparable results. The overall average of six analyses was: %*R*: 38.8; %*S*: 61.2 ± 0.4.

RESULTS AND DISCUSSION

Since mandelic acid is so readily racemized, our initial goal was to develop a derivatization method proceeding with minimum racemization. While simple esterification of mandelic acid proceeds with negligible inversion (<0.1%)[15,18], subsequent conversions of the ester to the N-isopropylamide (with isopropylamine in dichloromethane, 80°C) and then to the N-isopropylurethane (with isopropylisocyanate in dichloromethane, 100°C) were reported to involve up to 4–14% inversion[18], and the direct formation of N-isopropylmandelamide N-isopropylurethane was accompanied by a host of byproducts[18] as well. The three-step conversion of mandelic acid to its O-heptafluorobutyryl N-propylamide has been reported to proceed with up to 27% inversion[16]. Thus the most promising procedure appeared to be the three-step conversion of Wang *et al.*[17] of mandelic acid to O-trimethylsilyl-N-propylmandelamide (II) using (1) thionyl chloride (25°C), (2) propylamine (25°C) and (3) BSTFA–pyridine (80°C), during which racemization was reported as low as 2%. Variations of this procedure were accordingly explored.

The conversion of methyl *R*-mandelate into N-propyl-*R*-mandelamide was accomplished by dissolving the ester in propylamine at 25°C. The propylaminolysis was complete in *ca.* 7 h (Table I), proceeding with pseudo first order kinetics. The resulting N-propyl-*R*-mandelamide was optically stable once formed (Table I), but there apeared to be a small amount of racemization of the starting ester by action of the propylamine prior to or during its conversion to the amide, as indicated by GC analysis after subsequent conversion of the amide to its O-TFA derivative (*R*-I). Applying the same reaction sequence to methyl *RS*-mandelate gave O-trifluoroacetyl N-propyl-*RS*-mandelamide (*RS*-I), which could be reasonably but not optimally resolved under our GC conditions. Conversion of the above amide to its trimethylsilyl ether using BSTFA, however, gave O-trimethylsilyl-N-propyl-*RS*-mandelamide (*RS*-II), which could be quantitatively resolved.

When the Wang *et al.*[17] conversion of *RS*-mandelic acid to *RS*-II was scaled up from the original μg level to the 50-mg level, GC analysis of the *RS*-II product showed

baseline resolution and reasonable quantitative agreement: %*R*: 49.4; %*S*: 50.6. When the same conversion was applied on this scale to *R*-mandelic acid, however, the anticipated *R*-II product proved to contain 25% of the *S*-II enantiomer. In another experiment where N-propyl-*R*-mandelamide, prepared as above by the propylaminolysis of methyl *R*-mandelate, was converted to its O-trimethylsilyl ether using BSTFA in pyridine at 80°C, the *R*-II product contained 15.4% *S*-II.

Since methyl *R*-mandelate was found to be optically stable in triethylamine solution, we thought that substituting triethylamine for pyridine in the final trimethylsilation step of the Wang *et al.* conversion[17] might lead to less overall racemization. This proved to be the case since when *R*-II was prepared in this way it contained $<0.12\%$ of the *S*-II enantiomer.

Finally, the latter procedure was applied to the quantitative analyses of two known mixtures of mandelic acid enantiomers, the first having *R*: 73.0; *S*: 27.0% and the second having *R*: 39.6; *S*: 60.4%. Replicate analyses of the II product from the first mixture showed *R*: 72.6; *S*: 27.4 ± 0.7% and from the second mixture *R*: 38.8; *S*: 61.2 ± 0.4%. Since the first analysis missed the known composition by only 0.4% and the second by 0.8%, we conclude that the above procedure enables reasonably accurate and reproducible GC analyses of mandelic acid enantiomer mixtures using N-docosanoyl-L–valine *tert*.butylamide phase on a stainless-steel capillary column.

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