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GAS CHROMATOGRAPHIC DETERMINATION OF OPTICAL ISOMERS OF SOME CARBOXYLIC ACIDS AND AMINES WITH OPTICALLY ACTIVE STATIONARY PHASES

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SUMMARY

We recently developed some novel, optically active stationary phases (containing two asymmetric carbon atoms attached to both the nitrogen and carbon atoms of the amide group) for the gas chromatographic separation of carboxylic acid and amine enantiomers. We now report analytical methods for the determination of the optical isomers of some chiral carboxylic acids and amines with these phases. We found that (1) all four optical isomers of chrysanthemic acid [(+)-*cis*, (−)-*cis*, (+)-*trans* and (−)-*trans*] can be determined as the *tert.*-butylamide derivatives with N-(1*R*, 3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine, and (2) *R*- and *S*-isomers of 1-phenyl-2-(4-tolyl)ethylamine can be determined as the N-pentafluoropropionyl derivatives with N-lauroyl-(*S*)-proline)-(*S*)-1-(α-naphthyl)ethylamide.

INTRODUCTION

Gas chromatography is an important and convenient method for the determination of optical isomers. Generally the optical purity of enantiomeric compounds can be determined by forming derivatives with optically active reagents and analysing the resulting mixture of diasteroisomers with conventional achiral stationary phases[1]. However, in this method a standard reagent of known optical purity is required. If the optical purity of the derivative-forming reagent is less than 100%, a considerable error might be introduced in the analysis.

A more direct approach to the determination of the optical purity of a mixture of enantiomers is chromatography on optically active stationary phases, without prior conversion into diastereoisomers. This method has received considerable attention in recent years.

Many chiral phases have proved to be excellent for the separation of amino acid enantiomers since the first success by Gil-Av *et al.*[2], but they often showed lower separation factors for carboxylic acid and amine enantiomers. Weinstein *et al.*[3] reported that it sufficed for a chiral stationary phase to contain an amide group and an asymmetric carbon atom, attached to the nitrogen atom [RCONHCH(CH_3)R'], in order to show selectivity in its interaction with the enantiomers of amides such as N-

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acyl amines and α-substituted carboxylic acid amides, and that the best efficiency is obtained when R′ is aromatic, particularly α-naphthyl as in N-lauroyl-(*S*)-1-(α-naphthyl)ethylamine. We prepared a *s*-triazine derivative of tripeptide ester[4] as a chiral stationary phase and accomplished the direct separation of various α-alkyl phenylacetic acid and aryl alkylamine enantiomers[5,6]. However, unfortunately, the chromatographic properties of these phases were insufficient to determine the optical purity of some chiral carboxylic acids and amines such as chrysanthemic acid and 1-phenyl-2-(4-tolyl)ethylamine.

Recently we have developed some novel amide stationary phases that contain two asymmetric carbon atoms attached to the nitrogen and carbon atoms of the amide group, respectively, and found that these phases have very excellent chromatographic properties[7,8]. In this paper, we report a method for determining the optical purity of chrysanthemic acid and 1-phenyl-2-(4-tolyl)ethylamine by gas chromatography with these novel amide stationary phases.

EXPERIMENTAL

Reagents

The optically active stationary phases, N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine[7] and N-lauroyl-(*S*)-proline (*S*)-1-(α-naphthyl)ethylamide[8], were synthesized as described previously. Optical isomers of chrysanthemic acid and 1-phenyl-2-(4-tolyl)ethylamine were prepared in our laboratory as reported by Murano[9,10]. All other reagents and solvents were of analytical- or laboratory-reagent grade.

Gas chromatography

The experiments were carried out with a Shimadzu GC-7A gas chromatograph equipped with a flame-ionization detector. The glass capillary columns were coated with an 8–10% solution of each stationary phase in chloroform.

Analytical methods

Chrysanthemic acid. To a solution of 25 mg of chrysanthemic acid in 1 ml of dry *n*-hexane, 0.25 ml of oxalyl chloride was slowly added with stirring, kept at room temperature for 30 min, then evaporated *in vacuo* at 50°C so as the remove the excess of oxalyl chloride. The residue was dissolved in a solution of *tert.*-butylamine (70 mg) in 2 ml of dry toluene. The mixture was kept at room temperature for 10 min, and acidified with 5 ml of 1 *N* hydrochloric acid. After stirring, the organic phase was dried over anhydrous sodium suiphate. A 2-μl volume of this solution was injected for gas chromatographic analysis.

The chromatographic conditions used were as follows: column, 40 m × 0.25 mm I.D. glass capillary coated with N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl) ethylamine; column temperature, 110°C; injector and detector temperature, 200°C; attenuation, 10 × 2; carrier gas, helium at a flow-rate of 1.0 ml/min; and splitting ratio, 1/80.

Each peak area was measured by a conventional trianguration method with approximate tangents on the chromatogram. The ratios of optical isomers were obtained from the ratios of the peak area of each isomer and the total peak area of four isomers.

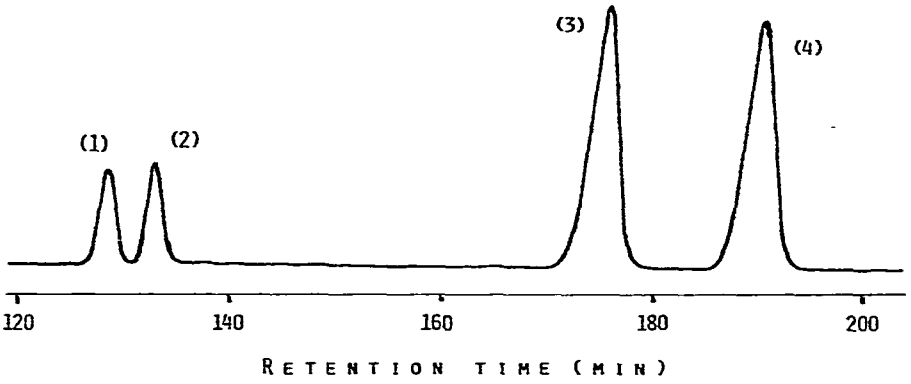

Fig. 1. Gas chromatogram of chrysanthemic acid *tert.*-butylamide. GC conditions as in Table I. (1) (−)-*cis*-isomer; (2) (+)-*cis*-isomer; (3) (+)-*trans*-isomer; (4) (−)-*trans*-isomer.

1-Phenyl-2-(4-tolyl)ethylamine. A mixture of 100 mg of 1-phenyl-2-(4-tolyl)ethylamine and 3 ml of a solution of 0.12 ml of pentafluoropropionyl anhydride in toluene containing 10% of ethyl acetate was kept at room temperature for 5 min, then 5 ml of water were added to decompose the excess of pentafluoropropionyl anhydride. The organic phase was dried over anhydrous sodium sulphate and 0.4 μl of this solution was injected for gas chromatographic analysis. The chromatographic conditions used were as follows: column, 30 m × 0.25 mm I.D. glass capillary coated with N-lauroyl-(*S*)-proline (*S*)-1-(α-naphthyl)ethylamide; column temperature, 160°C; injector and detector temperature, 220°C; attenuation, 10 × 8; carrier gas, helium at a flow-rate of 0.85 ml/min; and splitting ratio, 1/90.

Each peak area was measured by using a digital integrator (Shimadzu C-R1A). The ratios of optical isomers were obtained from the ratios of the peak area of each isomer and the total peak area of two isomers.

RESULTS AND DISCUSSION

Chrysanthemic acid, which is a very important constituent of various insecticidal pyrethroids, has four isomeric forms: (+)-*trans*, (−)-*trans*, (+)-*cis* and (−)-*cis*.

TABLE I

COMPARISON OF EXPECTED AND FOUND RATIOS OF OPTICAL ISOMERS OF CHRYSANTHEMIC ACID

Column: 40 m × 0.25 mm I.D. glass capillary coated with N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine. Column temperature: 110°C. Carrier gas (helium) flow-rate: 1.0 ml/min.

Sample No.	*Expected (%)*				*Found (%)*			
	(+)-cis	*(−)-cis*	*(+)-trans*	*(−)-trans*	*(+)-cis*	*(−)-cis*	*(+)-trans*	*(−)-trans*
1	20.1	0.2	78.6	1.1	19.9	0.2	78.8	1.1
2	17.9	2.1	70.7	9.3	17.9	1.9	71.1	9.1
3	16.5	3.4	65.5	14.6	16.5	3.2	65.8	14.5
4	14.7	5.0	59.1	21.2	15.1	4.6	59.5	20.8

TABLE II

ANALYSES OF TECHNICAL GRADE-SAMPLES OF OPTICALLY ACTIVE CHRYSANTHEMIC ACID

GC conditions as in Table I.

Sample No.	*This method (%)*				*Diastereoisomer method*[9] *(%)*			
	(±)-cis	*(−)-cis*	*(+)-trans*	*(−)-trans*	*(+)-cis*	*(−)-cis*	*(+)-trans*	*(−)-trans*
1	19.3	1.0	75.8	3.9	19.9	1.0	75.1	4.0
2	20.6	0.4	77.2	1.8	20.2	0.3	77.6	1.9
3	19.3	0.5	77.8	2.4	18.8	0.5	78.2	2.5
4	18.3	0.5	78.6	2.6	19.4	0.5	77.5	2.6
5	18.3	0.4	79.3	2.0	18.7	0.4	78.9	2.0

As is well known, optical isomers of pyrethroid esters have different toxicities for insects, depending on the configuration of the chrysanthemoyl group in the molecule, so it is important to establish the ratio of optical isomers.

Hitherto four isomers were analysed in the form of esters with (+)-2-octanol[9] or in the form of amides with (+)-α-methylbenzylamine[11]. However, as the optical purities of these derivative-forming reagents are usually less than 100%, it is necessary to correct the values obtained according to the optical purity of the chiral reagents and therefore the direct separation with optically active stationary phase was very desirable. Although we have already accomplished[12] the direct gas chromatographic separation of four isomers of chrysanthemic acid in the form of cyclohexyl-, 1,1,3,3-tetramethylbutyl-, 1-adamantyl- and α-dimethylbenzylamides on OA-300

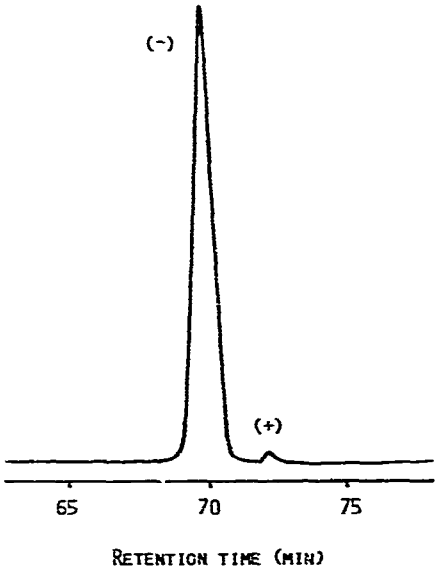

Fig. 2. Gas chromatogram of N-pentafluoropropionyl-1-phenyl-2-(4-tolyl)ethylamine showing the separation of 1% of (+)-isomer and 99% of (−)-isomer. GC conditions as in Table III.

TABLE III

COMPARISON OF EXPECTED AND FOUND RATIOS OF OPTICAL ISOMERS OF 1-PHENYL-2-(4-TOLYL)ETHYLAMINE

Column: 30 m × 0.25 mm I.D. Glass capillary coated with N-lauroyl-(*S*)-proline (*S*)-1-(α-naphthyl)ethylamide. Column temperature: 160°C. Carrier gas (helium) flow-rate: 0.85 ml/min.

Sample No.	*Expected (%)*		*Found (%)*	
	(+)-	*(−)-*	*(+)-*	*(−)-*
1	87.4	12.6	87.1	12.9
2	74.8	25.2	74.7	25.3
3	59.8	40.2	60.3	39.7
4	39.8	60.2	39.4	60.6
5	24.9	75.1	24.9	75.1
6	11.1	88.9	11.4	88.6
7*	50.0	50.0	50.0	50.0

* Racemic sample.

{N,N′-[2,4-(6-ethoxy-1,3,5-triazine)diyl]bis(L-valyl-L-valyl-L-valine isopropyl ester)}[4] as the optically active stationary phase, the separation factors were too low to determine the optical isomers accurately.

All four isomers were completely separated in the form of *tert.*-butylamide with N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine as a novel optically active stationary phase. A typical chromatogram is shown in Fig. 1. The order of elution was (−)-*cis*, (+)-*cis*, (+)-*trans*, (−)-*trans*. The ratios of the optical isomers were easily calculated by measuring peak areas. To verify that no racemization took place during the formation and analysis of the mixed amides, the expected ratios of four isomers are compared with the ratios found by analysis in Table I. Table II gives the results of analyses of some technical-grade samples. Each sample was analyzed in duplicate, and the mean value was shown in Table II. Errors were found from duplicate analyses to be ±0.3%. The precision of this methods expressed as the coefficient of variation was 0.49% (6 measurements) for the

TABLE IV

ANALYSES OF TECHNICAL-GRADE SAMPLES OF OPTICALLY ACTIVE 1-PHENYL-2-(4-TOLYL)ETHYLAMINE

GC conditions as in Table III.

Sample No.	*This method (%)*		*Diastereoisomer method*[10] *(%)*	
	(+)-	*(−)-*	*(+)-*	*(−)-*
1	98.3	1.7	98.6	1.4
2	95.0	5.0	95.7	4.3
3	95.2	4.8	95.8	4.2
4	0.1	99.9	0.2	99.8
5	17.8	82.2	17.2	82.8

(+)-*trans*-isomer of sample No. 5 in Table II. The optical isomer ratios obtained by this method were in agreement with the values obtained for the diastereoisomeric ester using (+)-2-octanol as the derivative-forming chiral reagent[9].

We have already reported[13] the gas chromatographic determination of optical isomers of 1-phenyl-2-(4-tolyl)-ethylamine, which is widely used to resolve asymmetric acids, with OA-300 as the chiral stationary phase. However, the separation (separation factor $\alpha = 1.025$, resolution $R = 1.28$) was insufficient for accurate determination. In particular, when the content of the (+)-isomer is very low, the small peak of the (+)-isomer is overlapped by the tail of the large peak of the (−)-isomer in the chromatogram and an error is often introduced. A novel optically active stationary phase, N-lauroyl-(*S*)-proline (*S*)-1-(α-naphthyl)ethylamide, has improved the separation ($\alpha = 1.038$, $R = 1.76$), as shown in Fig. 2. As both isomers were completely separated, even a small amount of the (+)-isomer can be easily determined. The results of analyses of some mixtures of (+)- and (−)-isomers are shown in Table III; the analytical values are in good agreement with the calculated values.

Table IV gives the results of the analysis of some technical-grade samples. Each sample was analyzed in duplicate, and the mean value was shown in Table IV. Errors were found from duplicate analyses to be ±0.2%. The precision of this method expressed by coefficient of variation was 0.04% (8 measurements) for the (+)-isomer of sample No. 1 in Table IV. The optical isomer ratio determined by this method was in good agreement with the value obtained in the form of diastereoisomeric amide using (*S*)-proline as the chiral reagent[10].

We have also developed analytical methods for the determination of optical isomers of some other chiral carboxylic acids, such as 3-(2,2-dichlorovinyl)cyclopropanecarboxylic acid, and chiral amines, such as 1-(α-naphthyl)ethylamine, using these novel amide phases. Details will be reported elsewhere.

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