Journal of Chromatography, 508 (1990) 333–339
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 336

Separation of carboxylic acid enantiomers by gas chromatography after rapid derivatization with (*R*)- or (*S*)-1-phenylethylamine after activation by ethyl chloroformate

ÅSA CARLSON and OLLE GYLLENHAAL*
Analytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)
(First received November 21st, 1989; revised manuscript received February 5th, 1990)

SUMMARY

The application of the chloroformate activation method for the formation of diastereometric of chiral carboxylic acids is described. This rapid procedure was extended to the gas chromatographic analysis of carboxylic acids with the chiral carbon in the 3-position with respect to the carboxylic group, mandelic and tropic acid and a carboxylic acid containing carbamate. The purities of (*R*)- and (*S*)-1-phenylethylamine chiral reagents were investigated using (*R*)-O-methylmandelic acid of high optical purity. The identities of the derivatives were studied using mass spectrometry. With the hydroxy acids not only is the expected amide formed but also the hydroxy group is converted into carbonate.

INTRODUCTION

Carboxylic acids with chiral carbons can be converted into a large number of different kinds of diastereomeric derivatives[1–3]. Amides are frequently used owing to their chemical stability and the rigidity of the amide bond. Normally the derivatization process consists of two steps, namely activation of the acid to an energy-rich intermediate and then reaction with an appropriate chiral amine. The first step frequently involves the use of noxious reagents and the second step is often time consuming. Combined with evaporation steps, such procedures can be cumbersome. In liquid chromatography, with UV or fluorescence detection, the responses of the diastereoisomers are not necessarily identical[1]. This should not be a problem when using capillary gas chromatography with flame ionization detection.

As many analytical amide-formation reactions take 0.5–1.5 h, excluding evaporation, Björkman[4] developed a method that reduced the overall derivatization time to 3 min. The method is based on the formation of a mixed anhydride with ethyl chloroformate, which is then reacted with L-leucinamide. The method was applied to the determination by liquid chromatography of the enantiomers of indoprofen[4] and ketoprofen[5] isolated from plasma. An advantage with L-leucinamide, in addition to its high enantiomeric purity[4,6], is its poor UV-absorbing properties. Papers have

0021-9673/90/$03.50

appeared using this method, sometimes with minor modifications, for the determination of anti-inflammatory drugs of the 2-arylpropionic acid type[7–12].

In this work we report the application of this rapid method to the analysis of chiral carboxylic acids by capillary gas chromatography with flame ionization detection. In the acids investigated the asymmetric carbon is in both the 2- and 3-positions with respect to the carboxylic group. The derivatization reaction has also been extended to some interesting 2-hydroxy acids such as tropic acid and mandelic acid, and to 2-(phenylaminocarbonyloxy)propionic acid.

EXPERIMENTAL

Instrumentation and chromatographic conditions

A Varian 3700 gas chromatograph equipped with a flame ionization detector was used. The injector and detector temperatures were both 300°C. The carrier gas was nitrogen with an inlet pressure of 80 kPa (120 kPa for the carbamate). The split flow-rate was 20 ml/min. The make-up gas to the detector had a flow-rate of 20 ml/min. An SE-54 fused-silica capillary column was used (25 m × 0.32 mm I.D., film thickness 0.25 μm). The chromatograms were recorded with a Hewlett-Packard 3390A integrator.

Mass spectra were recorded using the same type of column and gas chromatograph connected to a Varian-MAT 44S instrument. Helium was used as the carrier gas, the transfer line kept at 250°C and the ionization energy was 70 eV.

Reagents and chemicals

(*R*)(+)-1-Phenylethylamine (98+%) and (S)(−)-1-phenylethylamine (97–98%) were obtained from EGA-Chemie (Steinheim/Albuch, F.R.G.), and triethylamine from Eastman Kodak (Rochester, NY, U.S.A.), ethyl chloroformate (zur Synthese) from Merck (Darmstadt, F.R.G.), were redistilled. (*R/S*)-Indoprofen and (*S*(+)-naproxen were purchased from Sigma (St. Louis, MO, U.S.A.), (*R/S*)-ibuprofen from Astra (Södertälje, Sweden), (*R/S*)-ketoprofen from Bayer (Leverkusen, F.R.G.), (*R/S*)-2-phenylbutanoic acid from Fluka (Buchs, Switzerland), (*R/S*)-tropic acid from Sigma and (*R/S*)-mandelic acid from Riedel-De Haën (Seelze, Hannover, F.R.G.). (*R*)-O-Methylmandelic acid, (*R/S*)-1-indanylacetic acid, (*R/S*)-1-tetralylacetic acid and (*S*)-2-(phenylaminocarbonyloxy)propionic acid [(*S*)-PACOPA] were obtained from Jansen (Beerse, Belgium) and the metoprolol metabolite H 104/83 (4-methoxyethylphenoxymethyllactic acid) came from the Department of Organic Chemistry, Hässle (Mölndal, Sweden). Some of the structures are given in Fig. 1.

Solvents and other reagents were of analytical-reagent grade.

Derivatization

The acids were dissolved in acetonitrile and a volume (ranging from 150 to 400 μl) containing 1–3 mg of the acid was mixed with 200 μl of 50 m*M* triethylamine in acetonitrile. To the mixture were added, at intervals of 30 s, 100 μl of ethyl chloroformate (60 m*M*) in acetonitrile and 200 μl of (*S*)(−) or (*R*)(+)-1-phenylethylamine–triethylamine (0.5 *M* each) in methanol. After 2 min, 0.5 ml of hydrochloric acid (0.25 *M*) was added to stop the reaction. The derivative was then extracted with 3 ml of ethyl acetate and 2 μl were injected into the gas chromatograph.

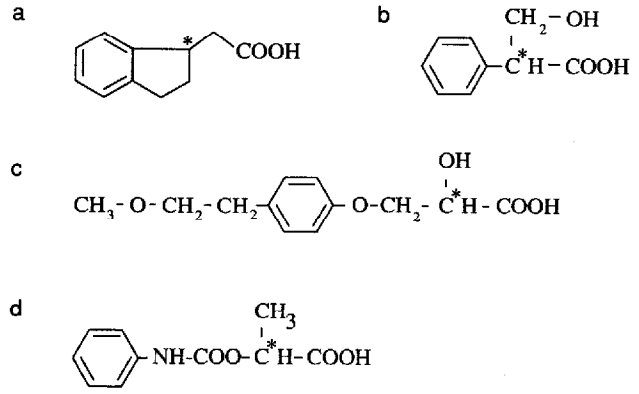

Fig. 1. Structures of (a) 1-indanylacetic acid, (b) tropic acid, (c) lactic acid metabolite of metoprolol (H 104/83) and (d) 2-phenylaminocarbonyloxy) propanoic acid (PACOPA). The asterisks indicate the asymmetric carbon atoms.

The derivatives of (*R*/*S*)-2-phenylbutanoic acid and (*R*/*S*)-mandelic acid, made for testing the possible stereoselectivity of the second step of the reaction, were prepared as above, but the reactions were stopped with 0.25 *M* hydrochloric acid 5, 10, 15, 20, 30 and 45 s and 1, 1.5, 2, 3 and 5 min after the addition of the chiral amine solution.

RESULTS AND DISCUSSION

Identification of derivatives

The amide derivative of (*R*/*S*)-1-indanylacetic acid that was formed in the reaction (Fig. 2) was identified by mass spectrometry. We obtained the expected molecular ion and fragment ions due to the indan ring. The base peak is at *m*/*z* 105 (phenethyl). In the total ion current chromatogram recorded we also observed a large peak whose mass spectrum corresponded to the ethyl carbamate of phenylethylamine.

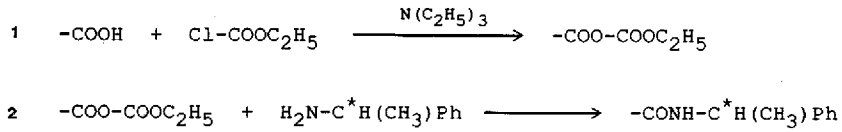

Fig. 2. Reaction scheme for the rapid formation of amide from a carboxylic acid with 1-phenylethylamine after activation with ethyl chloroformate in the presence of triethylamine. (1) Activation; (2) amidation.

The metoprolol metabolite derivative was also subjected to analysis by mass spectrometry (Fig. 3a). Based on this spectrum, the structure in Fig. 3b is proposed. Eliminated neutral fragments are carbon dioxide and ethanol (Figure 3b). This reaction to a carbonate has not been observed when using buffer (pH 8 and higher) in the derivatization of cathecolamines with methyl chloroformate followed by gas chroma-

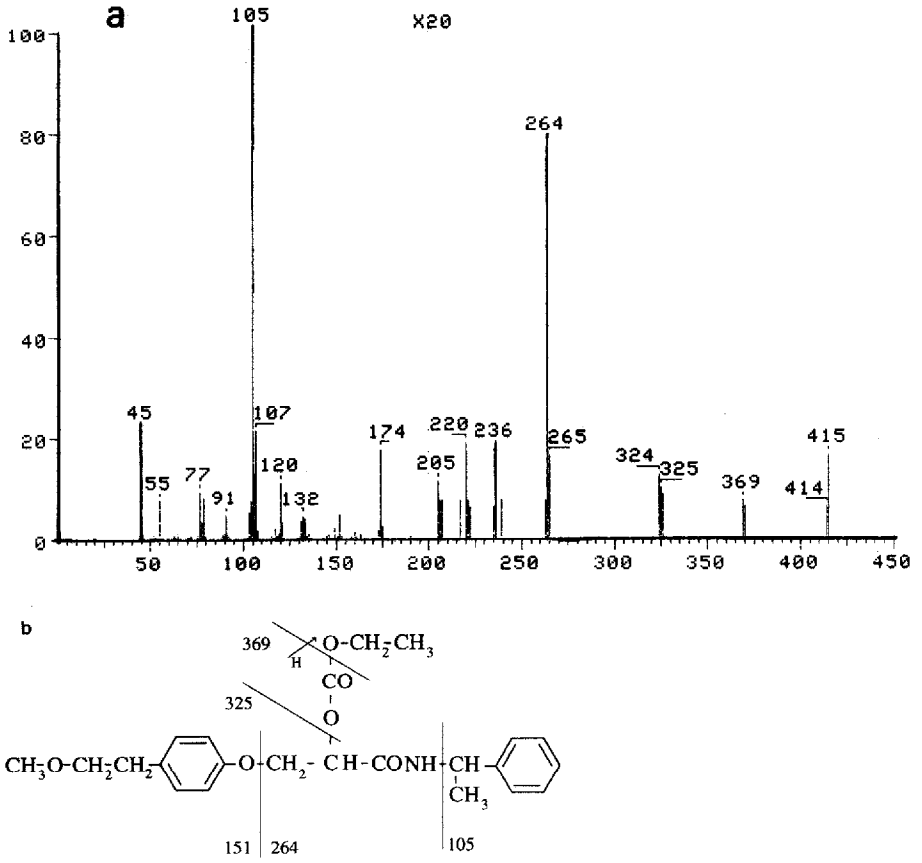

Fig. 3. (a) Mass spectrum of the carbonate/amide derivative of the metoprolol metabolite H 104/83, 1st peak. (b) Proposed structure of the carbonate/amide derivative of the metoprolol metabolite H 104/83 and some fragmentation routes. MV = 415.

tographic analysis[13]. Hence using this derivatization procedure it is not necessary to block the alcohol group by *e.g.*, trimethylsilylation. Simultaneous derivatization of hydroxy acids has been reported with isocyanates[14] but requires prolonged heating.

The mass spectra of the diastereomers of (*S*)-PACOPA gave a molecular ion at *m/z* 312 that can be cleaved to phenyl isocyanate and an alcohol[15]. The charge is predominantely retained on the isocyanate ion, which is the base peak (*m/z* 119). Some anilinium ion is observed at *m/z* 93, which can be explained by the molecular ion expelling carbon dioxide and an allenic ion at *m/z* 175 is formed[15].

Separation of (R)- and (S)-1-phenylethylamides on SE-54

The separation factors for the derivatives formed are given in Table I. For the (*S*)-naproxen and the (*R*)-O-methyl mandelic acid derivatives the second peak is the *R,R* (or *S,S*) derivative. In general, symmetrical peaks with baseline separation were observed except for indoprofen, whose peaks showed some tailing. As expected, the

TABLE I

SEPARATION OF 1-PHENYLETHYLAMINE DERIVATIVES ON AN SE-54 CAPILLARY COLUMN

Compound	*Separation factor*		*Capacity factor of peak 2 (230°C)*
	230°C	*250°C*	
1-(Indanyl)acetic acid (see Fig. 1)	1.034	1.023	4.65
1-(Tetralyl)acetic acid	1.040	1.031	7.07
2-Phenylbutanoic acid	1.075	–	1.97
Indoprofen, 2-[4-(1-oxo-2-isoindolinyl)phenyl]propionic acid	–	1.044	3.65[b]
Ibuprofen, 4-(isobutylphenyl)propionic acid	1.091[a]	1.081	3.90
Ketoprofen, 2-(3-benzoylphenyl)propionic acid	1.120[a]	1.104[a]	23.53
Naproxen, (*S*)-2-(6-methoxy-2-naphthyl)propionic acid	1.132[a]	1.113[a]	16.46
Tropic acid (see Fig. 1)	1.085	–	2.96
H 104/83 (see Fig. 1)	–	1.066	11.10[b]
Mandelic acid, 1-hydroxy-1-phenylacetic acid	1.039	–	4.44
(*R*)-O-Methylmandelic acid	1.080	–	2.13

[a] Baseline separation.
[b] 250°C.

two β-chiral acids gave lower separation factors than their α-chiral analogues (Table I). The closer the chiral centres are to each other, combined with bulky substituents, the easier is the separation[16].

The hydroxy acids investigated have lower separation factors than most of the 2-arylpropionic acids (Table I). The chromatogram in Fig. 4 shows the separation of the enantiomers of the derivatized metoprolol metabolite. This compound required a longer time to be eluted. The better separation factor of the O-methyl ether of mandel-

Fig. 4. Gas chromatogram of the mixed derivative in Fig. 3b. Column temperature, 250°C. Retention time for peak 2, 12 min.

ic acid shows that the carbonate group of the hydroxy acids reduces the difference in volatility between the diastereoisomers in the gas chromatographic system. Also for derivatized (*S*)-PACOPA the separation factor is sufficient, 1.054 (220°C), for baseline separation.

Enantiomeric purity of the 1-phenylethylamines

We chose (*R*)(+)- and (*S*)(−)-1-phenylethylamine as the chiral derivatizing agents because they have given good results in chromatographic analysis[17–22]. L-Leucinamide[4,5] is too polar to be suitable for gas chromatographic analysis.

The purity of the chiral amines is only indicated on the label, and rarely discussed in any of the references. The (*R*)-O-methylmandelic acid available, which was >99.8% pure[23] and has been used for the determination of the enantiomers of tocainide[24], was derivatized with (*R*)(+)- and (*S*)(−)-1-phenylethylamine. If we assume that the (*R*)-O-methylmandelic acid is 100% pure and we use the peak areas to measure the purity of the chiral amines, then (*R*)(+)-1-phenylethylamine was 98.34% pure [relative standard deviation (R.S.D.) = 0.03, $n = 6$] and (*S*)(−)-1-phenylethylamine 97.98% (R.S.D. = 0.10, $n = 6$). The remainder is due to the antipode, and any traces of (*S*)-O-methylmandelic acid present (<0.2%).

Enantiomeric purity of (S)-PACOPA

This compound can be used for classical resolution by recrystallization of amines and can be prepared from readily available and cheap starting materials. After derivatization with both (*R*)- and (*S*)-phenylethylamine, the chromatograms showed the presence of 80% as *S,R* and 74% as *S,S*, respectively. The discrepancy might be due to racemization under the alkaline derivatization conditions used, as the enantiomeric contamination of the amines is <2%.

Possible stereoselectivity of the second step in the derivatization reaction

The derivatization reaction of (*R/S*)-2-phenylbutyric acid was stopped with hydrochloric acid at times ranging from 5 s to 5 min after the addition of the chiral amine. The samples were then chromatographed at 210°C and the peak-area ratio was calculated. The same procedure was also performed with mandelic acid (220°C). No change in the peak-area ratios with increasing time was observed, so it is concluded that the reaction is not stereoselective to any significant extent.

ACKNOWLEDGEMENTS

We thank Magnar Ervik, Bo Lamm, Sam Larsson and Jörgen Vessman for their kind interest and valuable help in this work.

REFERENCES

1 W. Lindner, in M. Zief and L. J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, Basle, 1987, Ch. 4, pp. 91–130.
2 J. Gal, in I. W. Wainer and D. E. Drayer (Editors), *Drug Stereochemistry: Analytical Methods and Pharmacology*, Marcel Dekker, New York, Basle, 1988, Ch. 4, pp. 77–112.
3 S. Einarsson and M. Ahnoff, in W. J. Lough (Editor), *Chiral Liquid Chromatography*, Blackie, Glasgow, 1989, Ch. 4, pp. 39–80.

4 S. Björkman, *J. Chromatogr.*, 339 (1985) 339.
5 S. Björkman, *J. Chromatogr.*, 414 (1987) 465.
6 K. H. Lehr and D. Damm, presented at *ISSX, 2nd European Symposium on Foreign Compound Metabolism, Frankfurt am Main, March/April, 1987.*
7 R. T. Foster and F. Jamali, *J. Chromatogr.*, 416 (1987) 388.
8 H. Spahn, *J. Chromatogr.*, 423 (1987) 334.
9 R. Mehvar, F. Jamali and F. M. Pasutto, *J. Chromatogr.*, 425 (1988) 135.
10 H. Spahn, *J. Chromatogr.*, 427 (1988) 131.
11 D. A. Nicoll-Griffith, T. Inaba, B. K. Tang and W. Kalow, *J. Chromatogr.*, 428 (1988) 103.
12 H. Spahn, *J. Chromatogr.*, 433 (1988) 331.
13 O. Gyllenhaal, L. Johansson and J. Vessman, *J. Chromatogr.*, 190 ((1980) 347.
14 I. Benecke and W. A. König, *Angew. Chem.*, 94 (1982) 709.
15 H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, 1967, p. 500.
16 J. W. Westley, B. Halpern and B. L. Karger, *Anal. Chem.*, 40 (1968) 2046.
17 G. J. van Giessen and D. G. Kaiser, *J. Pharm. Sci.*, 64 (1975) 798.
18 G. Helmchen, H. Völter and W. Schüle, *Tetrahedron Lett.*, (1977) 1417.
19 J. K. Stoltenborg, C. V. Puglisi, F. Rubio and F. M. Vane, *J. Pharm. Sci.*, 70 (1981) 1207.
20 T. Kaneda, *J. Chromatogr.*, 366 (1986) 217.
21 A. Sioufi, D. Colussi, F. Marfil and J. P. Dubois, *J. Chromatogr.*, 414 (1987) 131.
22 B. Blessington, N. Crabb, S. Karkee and A. Northage, *J. Chromatogr.*, 469 (1989) 183.
23 S. Larsson, personal communication.
24 K. J. Hoffmann, L. Renberg and C. Bäärnhielm, *Eur. J. Drug Metab. Pharmacokinet.*, 9 (1984) 215.