Gas chromatographic separation of diastereoisomeric and enantiomeric forms of some fluorinated amino acids on glass capillary columns

V. Vlasáková*, V. Tolman and K. Živný

*Institute of Nuclear Biology and Radiochemistry, Czech Academy of Sciences, Vídeňská 1083,
142 20 Prague 4 (Czech Republic)*

ABSTRACT

The monofluorinated analogues of 2-aminocarboxylic acids up to C_7 were efficiently separated into the diastereomers on glass capillary columns coated with achiral phases BP-1, BP-10 and OV-330. In addition, some difluoro and trifluoro analogues were also measured. Chiral resolution was achieved on capillary well-coated open tubular fused-silica columns coated with chiral phases XE-60–L-Val–L-(1-phenylethyl)amide, Chirasil–L-Val and Behenoyl–L-Val–*tert.*-butylamide. The separation factors and the Kovats indices of the fluorinated amino acids were determined and compared. The *erythro* racemates display a higher degree of resolution than the *threo* ones. The order of elution was found to be the L- after the D-solute on all L-phases.

INTRODUCTION

Gas chromatographic methods using chiral stationary phases have been used for efficient separations of a number of racemic substances. A large body of work has been reported since the first studies describing a direct resolution of amino acid enantiomers on chiral phases by Gil-Av and co-workers [1–5]. Bayer and co-workers [6–10] have introduced polymeric siloxane phases of low volatility and high thermal stability, such as Chirasil–L-Val, formed by coupling of the L-Val–*tert.*-butylamide moiety to a copolymer of dimethylsiloxane and carboxyalkylmethylsiloxane units. Analogously, König and co-workers [11–14] have prepared a chiral phase by connecting L-Val–L-(1-phenylethyl)amide to a methylcyanoethyl-polysiloxane

XE-60 and obtained a highly heat-resistant polymeric phase with high enantioselectivity that was used to resolve amines, amino alcohols, amino acids, hydroxy acids, carbohydrates and other substances. Also, some monoamine phases, such as N-lauroyl-D-[1-(1′-naphthyl)ethyl]amine, are conveniently used for the resolution of 2-halogenocarboxylic acids and other compounds [15].

There is a rapidly increasing demand from many scientific branches for analytical methods capable of a rapid and efficient enantiomeric separation of diverse compounds. These methods are indispensable for the resolution of drugs whose individual enantiomers exhibit different pharmacological properties and have a considerable clinical importance. A wide group of chiral compounds with biological and/or pharmaceutical importance is constituted by many fluorinated analogues of amino acids. The GC study of some of them is the subject of this paper.

The analysed fluoro derivatives of amino acids are divided into three groups. The first group is

---

* Corresponding author.

formed by five linear-chain amino acids with one fluorine atom at C-3, which form a homologous series: 3-fluoroalanine, 2-amino-3-fluorobutyric acid, 3-fluoronorvaline, 3-fluoronorleucine and 2-amino-3-fluoroheptanoic acid. The second group consists of three amino acids containing a terminal trifluoromethyl: 3,3,3-trifluoroalanine, 4,4,4-trifluorovaline and 5,5,5-trifluoroleucine. In addition, three other amino acids were measured separately: 3-fluorovaline, 5-fluoronorleucine and 5,5-difluoronorleucine.

EXPERIMENTAL

Chemicals

The standards of fluorinated amino acids were received from the authors who described their syntheses: 3-fluoroalanine, 2-amino-3-fluorobutyric acid, 3-fluoronorvaline, 3-fluoronorleucine, 2-amino-3-fluoroheptanoic acid and 3-fluorovaline [16] were kindly provided by H. Gershon, 5-fluoronorleucine [17] and 5,5-difluoronorleucine [18] by M. Hudlický, 3,3,3-trifluoroalanine [19] by A. Uskert, 4,4,4-trifluorovaline [20,21] and 5,5,5-trifluoroleucine [21] by H.M. Walborsky. Methanol and other organic solvents were of analytical grade and were supplied by Lachema (Brno, Czech Republic).

Gas chromatographic analysis

The fluorinated amino acids were converted into the N-trifluoroacetylated methyl esters according to Darbre and Islam [22]. A Varian Model 3700 (Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector was used. All achiral measurements were performed on glass capillary columns (12 m × 0.32 mm) coated with phases BP-1 (phase I), $d_f = 0.25$ μm; BP-10 (phase II), $d_f = 0.25$ μm; and OV-330 (phase III), $d_f = 0.20$ μm. Chiral separations were carried out on capillary wall-coated open tubular fused-silica columns coated with XE-60–L-Val–L-(1-phenylethyl)-amide (phase IV), 50 m × 0.25 mm, $d_f = 0.12$ μm; Chirasil-L-Val (phase V), 25 m × 0.25 mm, $d_f = 0.12$ μm; and with Behenoyl–L-Val–tert.-butylamide (phase VI), 25 m × 0.25 mm, $d_f =$

0.20 μm. Phases I and II were manufactured by SGE, Sydney, Australia, phases III and VI by Serva, Heidelberg, Germany, and phases IV and V by Chrompack, Middelburg, Netherlands. Nitrogen was used as carrier gas. Sample volumes of 1 μl were injected onto the columns using the splitting technique; the split ratio was 1:100. The retention times t_R' are corrected for the dead column volume. Separation factors are defined as $\alpha = t_{R_2}'/t_{R_1}'$, where R_1 refers to the first peak and R_2 to the second one. The Kovats indices I were calculated for the separation temperatures indicated; for the temperatures, the optimal values were chosen, taking into account the measured temperature dependence of separation factors, the time of analysis and the peak width. Chromatograms were quantitatively evaluated using a Varian integrator CDS 111.

RESULTS AND DISCUSSION

GC separation of diastereoisomers

The presence of two chiral centres in the 2-amino-3-fluoro carboxylic acids (with the exception of 3-fluoroalanine and 3-fluorovaline) gives rise to two racemic diastereoisomers. Separation of the diastereoisomers was performed on glass capillary columns coated with achiral phases of different polarity, i.e. phases I–III. The highest values of the separation factors α were found on phase II at 110°C ($\alpha = 1.22$–1.24), lower on III at 150°C ($\alpha = 1.16$–1.18) and the lowest on phase I at 90°C ($\alpha = 1.07$–1.10).

The elution sequence of the erythro and threo forms was deduced partly from analogy with 4-fluoroglutamic acid diastereoisomers [23], partly from their chromatographic behaviour. The erythro forms of all compounds within this study exhibited shorter retention times than the corresponding threo forms on all phases. The same sequence of diastereoisomers was described, for example, by Rose et al. [24] in the separation of diastereoisomeric esters and by Abalain et al. [25] for the diastereomers of alkane-2,3-diols. The separation mechanism was deduced from the conformation immobility along the C–C bond bearing the asymmetric centres. In addition, the threo isomers of monofluorinated

aliphatic amino acids present a greater spatial bulk than the *erythro* forms.

The retention characteristics of the individual diastereoisomers, *i.e.* the retention times and Kováts indices (I), along with the values of the separation factors on phases I–III are summarized in Table I. The logarithms of retention times of both the *erythro* and *threo* forms in the homologous series were plotted against the number of carbon atoms, yielding two parallel lines that confirmed the linear relationship between these two quantities. This relationship was found on all three phases (Fig. 1).

A comparison of the separation factor values in the homologous series of 2-amino-3-fluoro carboxylic acids reveals that these values are nearly identical for all members of the series on each one of the phases used. On the other hand, comparison of the separation factors of 3-fluoronorleucine and 5-fluoronorleucine under the same chromatographic conditions shows the value of the latter to be much lower than that of the former (Table I). This is in accordance with the concept of Karger and co-workers [24,26] concerning the effect of the distance between the two chiral centres on the separation of a dia-

TABLE I

GC SEPARATION OF FLUORINATED AMINO ACIDS ON ACHIRAL PHASES

Compound	Isomer	Phase I BP-1 (90°C)			Phase II BP-10 (110°C)			Phase III OV-330 (150°C)		
		t'_R	α	I_{90}	t'_R	α	I_{110}	t'_R	α	I_{150}
3-Fluoroalanine	–	1.01	–	922	1.56	–	1187	3.63	–	1398
2-Amino-3-fluoro-butyric acid	*erythro*	1.36		966	1.57		1187	2.83		1344
			1.07			1.24			1.17	
	threo	1.46		977	1.94		1224	3.31		1378
3-Fluoronorvaline	*erythro*	2.41		1054	2.46		1267	3.78		1408
			1.07			1.22			1.16	
	threo	2.59		1064	3.01		1300	4.40		1441
3-Fluoronorleucine	*erythro*	4.27		1140	4.00		1351	5.22		1477
			1.10			1.24			1.17	
	threo	4.68		1152	4.95		1388	6.13		1511
2-Amino-3-fluoro-heptanoic acid	*erythro*	7.98		1236	6.92		1444	7.80		1563
			1.10			1.24			1.18	
	threo	8.79		1251	8.57		1481	9.18		1598
3-Fluorovaline	–	1.95	–	1021	2.00	–	1229	2.64	–	1330
5-Fluoronorleucine	*erythro*	6.50			8.52			12.97		
			1.07	[a]		1.11	[a]		1.07	[a]
	threo	6.97			9.45			13.92		
5,5-Difluoro-norleucine	–	5.54	–	[a]	8.50	–	[a]	12.79	–	[a]
3,3,3-Trifluoro-alanine	–	0.27	–	840	0.45	–	966	0.50	–	974
4,4,4-Trifluoro-valine	–	1.34	–	979	1.92	–	1224	2.10	–	1282
5,5,5-Trifluoro-leucine	–	2.77	–	1042	4.70	–	1378	4.88	–	1463

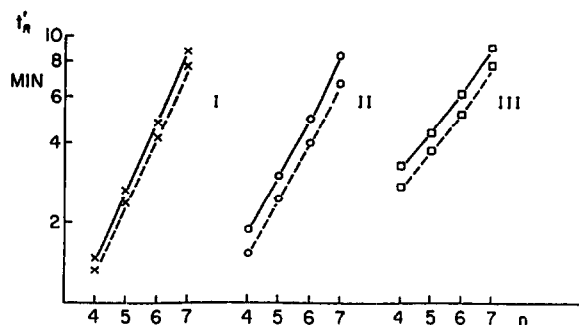[a] For 5-fluoronorleucine and 5,5-difluoronorleucine the I values were not estimated.

Fig. 1. Homologous 2-amino-3-fluoro carboxylic acids: logarithms of retention times (t_R') on achiral phases I–III plotted against the number of carbon atoms (n). Solid lines = *threo* forms; dashed lines = *erythro* forms.

stereoisomeric pair. According to the proposed separation mechanism the distance between the chiral centres affects the conformational mobility of groups along the C–C bond; the lower the distance the higher the immobility of the groups and the higher the chromatographic difference between the diastereoisomers. The constant distance between the chiral centres in a homologous series is reflected in the constant values of separation factors, whereas the larger distance in 5-fluoronorleucine lowers the separation factor (1.11 as compared with 1.24 for 3-fluoronorleucine on phase II).

TABLE II

GC RESOLUTION OF THE HOMOLOGOUS SERIES OF 2-AMINO-3-FLUORO CARBOXYLIC ACIDS ON CHIRAL PHASES

Compound	Isomer	Phase IV (140°C) XE-60–S-Val–S-PEA			Phase V (100°C) Chirasil–L-Val			Phase VI (110°C) Behenoyl–L-Val		
		t_R'	α	I_{140}	t_R'	α	I_{100}	t_R'	α	I_{110}
3-Fluoroalanine	D	2.90		1412	1.80		1146	1.20		1010
			1.03			1.00			1.13	
	L	3.00		1419	1.80		1146	1.35		1027
2-Amino-3-fluoro-butyric acid	erythro-D	2.35		1368	1.80		1146	1.15		1004
			1.04			1.08			1.22	
	erythro-L	2.45		1377	1.94		1161	1.40		1032
	threo-D	2.87		1410	2.55		1199	1.62		1051
			1.06			1.00			1.06	
	threo-L	3.05		1422	2.55		1199	1.71		1060
3-Fluoronorvaline	erythro-D	3.23		1434	2.92		1225	2.01		1084
			1.04			1.07			1.15	
	erythro-L	3.37		1443	3.11		1234	2.31		1104
	threo-D	4.09		1483	4.21		1284	3.00		1142
			1.04			1.00			1.05	
	threo-L	4.25		1491	4.21		1284	3.15		1149
3-Fluoronorleucine	erythro-D	4.72		1513	4.92		1312	3.49		1164
			1.05			1.08			1.17	
	erythro-L	4.95		1523	5.29		1322	4.08		1187
	threo-D	6.00		1562	6.99		1368	5.25		1222
			1.04			1.01			1.06	
	threo-L	6.25		1571	7.09		1378	5.55		1230
2-Amino-3-fluoro-heptanoic acid	erythro-D	7.35		1605	8.80		1405	6.56		1254
			1.05			1.08			1.16	
	erythro-L	7.70		1614	9.47		1417	7.60		1275
	threo-D	9.40		1656	12.58		1463	9.95		1314
			1.04			1.02			1.06	
	threo-L	9.80		1664	12.78		1466	10.55		1322

The effect of length of the aliphatic chain is perceptible in the separation only by slightly higher α values of 3-fluoronorleucine and 2-amino-3-fluoroheptanoic acid, this being found only on the non-polar phase I (Table I).

GC resolution of enantiomers

Separation of enantiomers of the fluorinated amino acids under study was investigated on three types of chiral phases, IV–VI. The values of separation factors were determined and the Kováts indices calculated (Tables II and III). The complete separation of all measured enantiomeric pairs was observed on phase IV under isothermal conditions at 140°C (Fig. 2) and on phase VI at 110°C. Phase V failed to resolve 3-fluoroalanine and the threo isomers of 2-amino-3-fluorobutyric acid and 3-fluoronorvaline at 100°C. However, the resolution of all erythro enantiomeric pairs was complete also on this phase (Fig. 3).

We also investigated the effect of various chiral phases on the resolution of racemic erythro and threo forms of the 2-amino-3-fluoro carboxylic acids. The results, summarized in Table II, clearly show larger α values for enantiomers of the erythro forms than for those of the threo forms. Separation factors were nearly identical for all members of the homologous series with each type of chiral phase.

Optical isomers of the trifluoro derivatives of alanine, valine and leucine were separated on all three phases, IV–VI. In all cases the α values increased from 3,3,3-trifluoroalanine to 5,5,5-tri-

TABLE III

GC RESOLUTION OF MISCELLANEOUS FLUORINATED AMINO ACIDS ON CHIRAL PHASES

Compound	Isomer	Phase IV (140°C) XE-60–S-Val–S-PEA			Phase V (100°C) Chirasil–L-Val			Phase VI (110°C) Behenoyl–L-Val		
		t'_R	α	I_{140}	t'_R	α	I_{100}	t'_R	α	I_{110}
3,3,3-Trifluoro-alanine	D	0.72		1123	0.76		1002	0.36		837
			1.04			1.07			1.03	
	L	0.75		1131	0.81		1017	0.37		841
4,4,4-Trifluoro-valine	D	2.38		1371	2.80		1215	1.47		1039
			1.08			1.09			1.11	
	L	2.56		1386	3.04		1232	1.63		1054
5,5,5-Trifluoro-leucine	D	6.11		1566	8.48		1398	3.75		1174
			1.10			1.14			1.21	
	L	6.70		1585	9.66		1420	4.54		1201
3-Fluorovaline	D	2.30		1364	1.55		1047	1.55		1047
			1.03			1.05			1.05	
	L	2.38		1371	1.63		1054	1.63		1054
5-Fluoronorleucine	erythro-D	11.03			11.51			7.44		
			1.09	^{a}		1.16	^{a}		1.13	^{a}
	erythro-L	12.01			13.40			8.37		
	threo-D	12.00			13.38			9.61		
			1.08	^{a}		1.08	^{a}		1.12	^{a}
	threo-L	12.93			14.50			10.81		
5,5-Difluoro-norleucine	D	10.84			12.01			7.06		
			1.09	^{a}		1.13	^{a}		1.30	^{a}
	L	11.86			13.57			9.19		

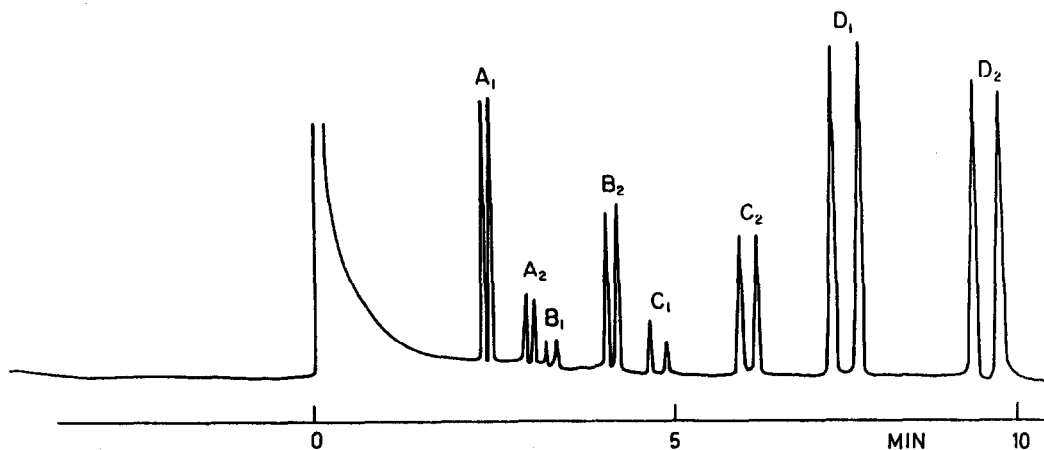^{a} Not estimated (see footnote in Table I).

Fig. 2. Homologous 2-amino-3-fluoro carboxylic acids: GC separation of the four enantiomers on chiral phase IV at 140°C. (A) 2-Amino-3-fluorobutyric acid; (B) 3-fluoronorvaline; (C) 3-fluoronorleucine; (D) 2-amino-3-fluoroheptanoic acid. The *erythro* forms are indexed by 1, the *threo* forms by 2. In each pair of peaks the left one represents the D-antipode.
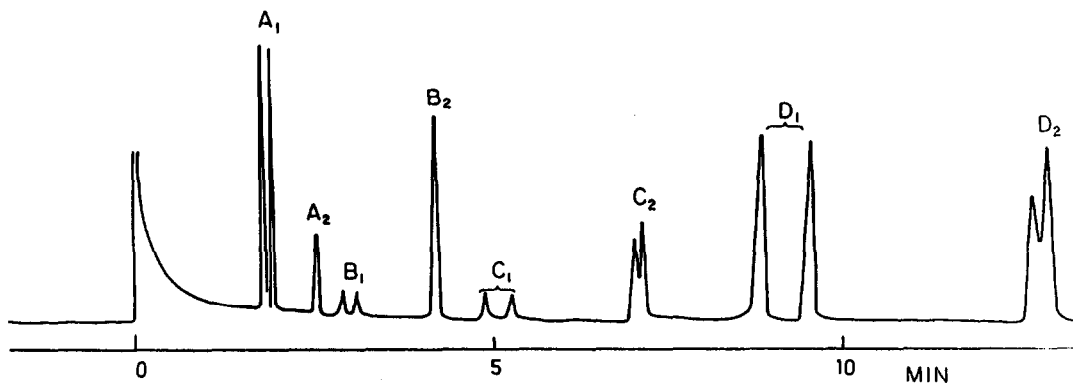

Fig. 3. Homologous 2-amino-3-fluoro carboxylic acids: GC separation of the four enantiomers on chiral phase V at 100°C. Legend as at Fig. 2; A_2 and B_2 are unresolved *threo*-D,L-2-amino-3-fluorobutyric acid and *threo*-D,L-3-fluoronorvaline, respectively.

fluoroleucine. Table III summarizes the retention characteristics and the α values.

The sequence of eluted enantiomers on all chiral phases was derived from analogy with the same types of aliphatic non-fluorinated 2-amino acids of known steric relevance and by comparison with isolated optical isomers of 4-fluoroglutamic acid [23]. On all L-phases the D-isomers were eluted first. The same sequence was observed for the esters of 3-fluoroalanine on phase V by Wagner *et al.* [27].

The linear relationship between the logarithms of retention times of the individual enantiomers and the number of carbon atoms in both the *erythro* and *threo* series was documented (Fig.
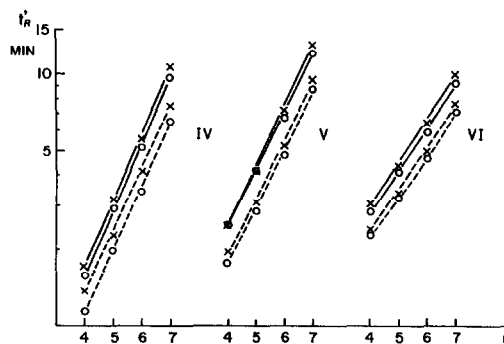

Fig. 4. The four individual enantiomers of homologous 2-amino-3-fluoro carboxylic acids: logarithms of retention times on chiral phases IV–VI plotted against the number of carbon atoms. Solid lines = *threo* forms; dashed lines = *erythro* forms; $\times$ = L; $\bigcirc$ = D.

4). This relationship was confirmed on all three phases, IV–VI.

REFERENCES

1 E. Gil-Av, B. Feibush and R. Charles-Sigler, *Tetrahedron Lett.*, (1966) 1009.
2 E. Gil-Av, B. Feibush and R. Charles-Sigler, in A.B. Littlewood (Editor), *Gas Chromatography*, Institute of Petroleum, London, 1967, p. 227.
3 S. Nakaparksin, P. Birrell, E. Gil-Av and J. Oró, *J. Chromatogr. Sci.*, 8 (1970) 177.
4 B. Feibush and E. Gil-Av, *Tetrahedron*, 26 (1970) 1361.
5 R. Charles, U. Beitler, B. Feibush and E. Gil-Av, *J. Chromatogr.*, 112 (1975) 121.
6 W. Parr, J. Pleterski, C. Yang and E. Bayer, *J. Chromatogr. Sci.*, 9 (1971) 141.
7 W.A. König, W. Parr, H.A. Lichtenstein, E. Bayer and J. Oró, *J. Chromatogr. Sci.*, 8 (1970) 183.
8 H. Frank, G.J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
9 H. Frank, G.J. Nicholson and E. Bayer, *Angew. Chem.*, 90 (1978) 396.
10 H. Frank, G.J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
11 W.A. König, S. Sievers and I. Benecke, in R.A. Kaiser (Editor), *Proceedings of the IVth Symposium on Capillary Chromatography, Hindelang, 1981*, Institut für Chromatographie, Bad Dürkheim and Hüthig, Heidelberg, 1981, p. 703.
12 W.A. König, I. Benecke and S. Sievers, *J. Chromatogr.*, 217 (1981) 71.
13 W.A. König, I. Benecke and S. Sievers, *J. Chromatogr.*, 238 (1982) 427.
14 W.A. König, W. Francke and I. Benecke, *J. Chromatogr.*, 239 (1982) 227.
15 K. Watabe and E. Gil-Av, *J. Chromatogr.*, 318 (1985) 235.
16 H. Gershon, M.W. McNeil and E.D. Bergmann, *J. Med. Chem.*, 16 (1973) 1407.
17 M. Hudlický and B. Kakáč, *Coll. Czech. Chem. Commun.*, 31 (1966) 1101.
18 M. Hudlický, *Coll. Czech. Chem. Commun.*, 32 (1967) 453.
19 A. Uskert, A. Néder and E. Kasztreiner, *Magyar Kémiai Folyóirat*, 79 (1973) 333; *Chem. Abstr.*, 79 (1973) 79147r.
20 D.F. Loncrini and H.M. Walborsky, *J. Med. Chem.*, 7 (1964) 369.
21 H.M. Walborsky, M. Baum and D.F. Loncrini, *J. Am. Chem. Soc.*, 77 (1955) 3637.
22 A. Darbre and A. Islam, *Biochem. J.*, 106 (1968) 923.
23 V. Tolman, V. Vlasáková and K. Živný, *J. Chromatogr.*, 315 (1984) 421.
24 H.C. Rose, R.L. Stern and B.L. Karger, *Anal. Chem.*, 38 [3] (1966) 469.
25 J.H. Abalain, D. Picart and F. Berthou, *J. Chromatogr.*, 274 (1983) 305.
26 B.L. Karger, R.L. Stern, W. Keane, B. Halpern and J.W. Westley, *Anal. Chem.*, 39 (1967) 229.
27 J. Wagner, E. Wolf, B. Heinzelmann and C. Gaget, *J. Chromatogr.*, 392 (1987) 211.