Journal of Chromatography, 508 (1990) 349–356
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 326

Pentafluorophenyldiazoalkanes as novel derivatization reagents for the determination of sensitive carboxylic acids by gas chromatography–negative-ion mass spectrometry

UTE HOFMANN, SABINE HOLZER and CLAUS O. MEESE*
Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstrasse 112, D-7000 Stuttgart 50 (F.R.G.)
(First received October 12th, 1989; revised manuscript received February 2nd, 1990)

SUMMARY

A convenient access to analytically useful pentafluorobenzyl esters of sensitive carboxylic acids is described. The novel derivatization procedure takes advantage of the chemoselectivity of diazo compounds towards acids and utilizes pentafluorophenyldiazoalkanes as derivatization reagents. The superiority of the diazo reagent over pentafluorobenzyl bromide is demonstrated with the efficient derivatization of sensitive carboxylic acids which decompose extensively when subjected to the routine procedure. The α-methylpentafluorobenzyl (MPFB) esters of mono- and dicarboxylic acids thus formed exhibit excellent gas chromatographic properties. The scope and limitation of the use of diastereomeric MPFB esters is also discussed.

INTRODUCTION

Pentafluorophenyl derivatives of various functional groups are widely used in analytical gas chromatography (GC)[1–3], owing to their excellent GC properties and electron-capturing capabilities. Further, it has been demonstrated that the selectivity and sensitivity of the analytical methods may be greatly improved by use of a mass spectrometric detector which registers selected negative ions generated in the ion source by chemical ionization and electron capture[4–7].

Carboxylic acids (**1**), and in particular eicosanoids[8,9], are readily derivatized to their corresponding 2,3,4,5,6-pentafluorobenzyl ester derivatives **4a** by use of 2,3,4,5,6-pentafluorobenzylbromide (**2a**, Fig. 1). After ionization, the ester **4a** is subjected to a unique tailor-made fragmentation (Fig. 2) to yield the neutral 2,3,4,5,6-pentafluorobenzyl radical (M = 181) and the carboxylate anion $[M-181]^-$, the latter being recorded by means of negative-ion chemical ionization mass spectrometry (NICI-MS) at the femtomole to attomole level. So far the required carboxylic ester derivatives **4** have been prepared exclusively from the analytes **1** and bromide **2a** in the presence of a non-nucleophilic base (*e.g.*, N,N-diisopropyl-

0021-9673/90/$03.50

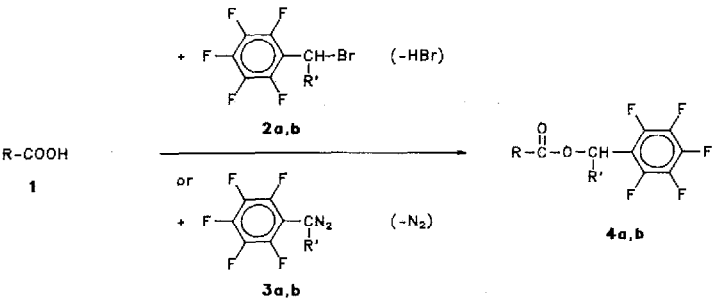

Fig. 1. Derivatization of carboxylic acids (**1**) with pentafluorobenzylbromides **2a** and **2b** and pentafluorophenyldiazoalkanes **3a** and **3b**. Series **a**, R′ = H; series **b**, R′ = CH_3.

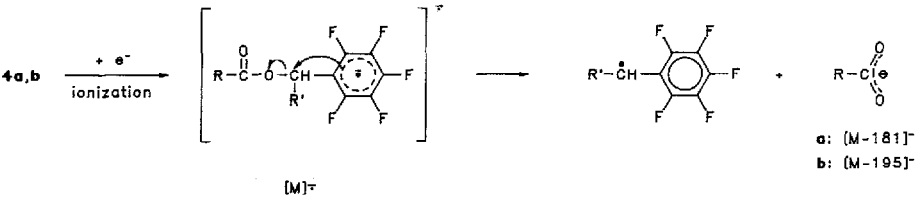

Fig. 2. Mass spectrometric (NICI-MS) fragmentation of esters **4a** and **4b**. Series **a**, R′ = H; series **b**, R′ = CH_3.

ethylamine, "Hünig's base"). This reaction, however, seems to be critical with respect to the selectivity of the ester formation and recovery of the product. In general, large amounts of unwanted side-products are formed and excess or decomposed reagent has to be removed which otherwise would cause elevated chemical noise levels[10,11].

In order to circumvent these problems volatile and more selective reagents for analytical pentafluorobenzyl ester formation from **1** are required. In this paper, an efficient solution to the problem of selective esterification of sensitive and/or nucleophilic acids and dicarboxylic acids is presented. The method consists of the smooth and convenient conversion of a carboxylic acid function into 2,3,4,5,6-pentafluorobenzyl esters (PFB esters, **4a**) or 1-(pentafluorophenyl)ethyl esters (α-methyl-2,3,4,5,6-pentafluorobenzyl esters, MPFB esters, **4b**) using the new reagents (pentafluorophenyl)diazomethane (**3a**) or 1-(pentafluorophenyl)diazoethane (**3b**)[12], respectively.

EXPERIMENTAL

Chemicals

The following acids and reagents were obtained from Fluka (Neu-Ulm, F.R.G.) and Aldrich (Steinheim, F.R.G.) and used as received: erucic acid (*cis*-13-docosaenoic acid), behenic acid (docosanoic acid), suberic acid (octanedioic acid), thiodiglycolic acid, (±)-2-phenylpropionic acid, 2,3,4,5,6-pentafluorobenzylbromide (**2a**), (±)-1-(pentafluorophenyl)ethanol and bis(trimethylsilyl)trifluoroacetamide (BSTFA). Thiodiglycolic acid sulphoxide (dicarboxymethyl sulphoxide)[13], dithiodiglycolic acid

(dicarboxymethyl disulphide)[13], (±)-3-(carboxymethylthio)lactic acid[13] and (±)-[13,17,17,18,18,19,19-$^2H_7$]-2,3,4,5,20-pentanor-19-carboxy-15-dehydro-13,14-dihydroprostaglandin E_1 (d_7-PGE-MM)[14] were prepared as described.

The homogeneity of the reagents and derivatives was checked by thin-layer chromatography (TLC) on 0.2 mm precoated silica gel plates (silica gel 60 F_{254}) (E. Merck, Darmstadt, F.R.G.) using the mobile phase ethylacetate–*n*-hexane (1:5, v/v) or/and ^{1}HNMR spectroscopy (80 MHz, δ values in ppm referred to internal TMS). The melting points reported are uncorrected.

(±)-1-(Pentafluorophenyl)ethyl bromide (**2b**)[15] was synthesized from (±)-1-(pentafluorophenyl)ethanol and PBr_3 in the presence of pyridine at −20°C and isolated in 65% yield after vacuum distillation as a colourless liquid, b.p. 73–76°C (17 mmHg). TLC: R_F 0.81. ^{1}H NMR ($CDCl_3$): 2.12 (d, J = 7.1 Hz, 3H, CH_3), 5.41 (q, J = 7.1 Hz, 1H, CHBr). Analytically pure pentafluorophenyldiazoalkanes **3a** and **3b** were prepared as described previously[12]. In the frozen state (−80°C) both compounds are stable for at least 5 years. Although no hazard has been observed, undiluted diazoalkane reagents should generally be handled with care[16].

Synthesis of reference compounds

2,3,4,5,6-Pentafluorobenzyl docosanoate. A mixture of docosanoic acid (190 mg, 0.56 mmol), 2,3,4,5,6-pentafluorobenzyl bromide (**2a**, 180 mg, 0.69 mmol) and N,N-diisopropylethylamine (150 mg, 1.2 mmol) in 2 ml of dry dimethylformamide (DMF) was stirred at 40°C for 16 h. After diluting with ethyl acetate (50 ml), the mixture was washed with 0.1 *M* HCl, water, half-saturated aqueous $NaHCO_3$ and water (3 × 5 ml each). The dried (Na_2SO_4) solution was evaporated under vacuum and the residue was dissolved in a minimum volume of ethyl acetate–*n*-hexane (1:5, v/v) and purified by column chromatography on silica gel 60 (20 g) (E. Merck) using the same solvent mixture as the mobile phase. Collection of the appropriate fractions, evaporation and drying under vacuum gave pure, crystalline ester in 97% yield (282 mg). An analytical sample was recrystallized from *n*-hexane, m.p. 68.5°C. TLC: R_F 0.77. Calculated for $C_{29}H_{45}F_5O_2$ (520.6), C 66.90, H 8.71; found, C 66.79, H 8.22%.

Related esters. In a similar manner, the following esters were prepared from the corresponding carboxylic acids and bromides **2a** and **2b**.

2,3,4,5,6-Pentafluorobenzyl *cis*-13-docosaenoate was obtained as a colourless oil (96% yield) which slowly crystallized on treatment with *n*-hexane (−40°C), m.p. 21–23°C. TLC: R_F 0.69. Calculated for $C_{29}H_{43}F_5O_2$ (518.6), C 67.16, H 8.36; found, C 67.02, H 8.32%. ^{1}H NMR ($CDCl_3$): 0.88 (bt, $J \approx 6$ Hz, 3H, CH_3), 1.26 (bs, 30 H), C2.02 (m, 4H, H-12/15), 2.33 (t, $J \approx 7$ Hz, 2H, H-2), 5.19 (t, J = 1.5 Hz, 2H, OCH_2), 5.36 (t, J = 4.6 Hz, 2H, H-13/14).

1-(Pentafluorophenyl)ethyl docosanoate was isolated in 75% yield after warming the reaction mixture at 40°C for 96 h; m.p. 55.5°C (from methanol). TLC: R_F 0.86. Calculated for $C_{30}H_{47}F_5O_2$ (534.7), C 67.39, H 8.86; found, C 67.32, H 9.11%. ^{1}H NMR ($CDCl_3$): 0.88 (bt, J = 4.9 Hz, 3H, H-22), 1.25 (bs, 38 H), 1.64 (d, J = 6.8 Hz, 3H, $OCHCH_3$), 2.32 (t, J = 7.3 Hz, 2H, H-2), 6.10 (q, J = 6.8 Hz, 1H, OCH).

2,3,4,5,6-Pentafluorobenzyl [13,14-$^2H_2$]docosanoate. Tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst) (60 mg) was dissolved in 10 ml of ethyl acetate under an atmosphere of molecular deuterium gas (>99.7% ^{2}H). To the rapidly stirred homogeneous mixture was added a solution of 2,3,4,5,6-pentafluoro-

benzyl *cis*-13-docosaenoate (100 mg, 0.19 mmol) in 2 ml of ethyl acetate. After further stirring at room temperature for 14 h, excess, *n*-hexane was added and the mixture was filtered through silica. The crude product obtained after evaporation was recrystallized from hot methanol to give 82 mg (81%) of the deuterated ester, m.p. 68°C. Calculated for $C_{29}H_{43}{}^2H_2F_5O_2$(522.6), C 66.65, $^1H+{}^2H$ 9.06; found, C 66.66 $^1H+{}^2H$ 9.24%. MS [positive-ion, electron impact, direct inlet mode (PI-EI-DIP), 20 eV]: 522 (52%), 341 (100%), 323 (82%), 305 (17%), 241 (8%). High-resolution MS ($M^{+\bullet}$): calculated, 522.3465; found, 522.3462.

*1-(Pentafluorophenyl)ethyl [13,14-$^2H_2$]docosanoate.*The crude 1-(pentafluorophenyl)ethyl *cis*-13-docosaenoate (MW 532.6), which was obtained in 69% yield from the reaction of erucic acid with **2b**, was used without further purification in the subsequent deuteration step. After purification as described above, crystalline material was obtained, m.p. 55°C (from methanol), yield 61%. Calculated for $C_{30}H_{45}{}^2H_2F_5O_2$ (536.7), C 67.14, $^1H+{}^2H$ 9.20; found, C 67.34, $^1H+{}^2H$ 9.02%. ^1H NMR ($CDCl_3$): 0.88 (bt, $J \approx 6$ Hz, 3H, H-22), 1.25 (bs, 36 H), 1.64(d, $J = 6.8$ Hz, 3H, $OCHCH_3$), 2.32 (t, $J = 7.5$ Hz, 2H, H-2), 6.11 (q, $J = 6.8$ Hz, 1H, OCH).

Instrumentation

Gas chromatography–mass spectrometry (GC–MS) was performed with a Hewlett-Packard 5985 quadrupole mass spectrometer operating in the negative-ion chemical ionization (NICI) mode (130 eV, reactant gas methane) coupled with an HP 5840 A gas chromatograph (carrier gas helium with an inlet pressure of 50 kPa). An SE-52 fused-silica capillary column was used (25 m × 0.32 mm I.D., 0.25 μm film thickness) (Chromatographie Service, Langerwehe, F.R.G.), initial temperature 110°C held for 1 min and then increased at a rate of 30°C/min to 320°C. For GC–electron capture detection (ECD) a Hewlett-Packard 5890 gas chromatograph was used, with nitrogen as carrier gas (50 kPa) and make-up gas (55 ml/min) and a detector temperature of 320°C.

Positive-ion and high-resolution mass spectra were obtained in the direct inlet mode (DIP, EI, 20 and 70 eV) on a MAT 711 instrument.

Standard derivatization procedures

PFB esters[8]. The samples were reacted with 30 μl of a 35% (v/v) solution of PFB bromide (**2a**) in acetonitrile and 10 μl of N,N-diisopropylethylamine for 20 min at room temperature. The solvent and most of the excess of reagent were removed by evaporation under a stream of nitrogen. For GC–MS measurements the remaining residue was dissolved in dodecane whereas an additional purification step was included for studies in the GC–ECD configuration. Thus, water (200 μl) was added to the samples and the derivatives were extracted with *n*-hexane (2 × 500 μl). The combined extracts were applied to a Sep-Pak silica cartridge which had been preconditioned with *n*-hexane (5 ml). After washing with *n*-hexane (2 ml) followed by *n*-hexane–diethyl ether (10:1, v/v) (1 ml) and discarding the washings the esters were eluted with 8 ml of the latter solvent mixture.

MPFB esters[12]. A 50–60-fold excess of a 0.3 *M* solution of **3b** in benzene was added to the sample and the mixture was left at room temperature for 16 h. Excess of diazoalkane was either removed by evaporation or destroyed by addition of acetic acid (10 μl). Water (200 μl) was added and the esters were isolated by extraction with

n-hexane. After evaporation of the solvent, the samples were reconstituted in dodecane for measurement by GC–MS or GC–ECD.

Time course of derivatizations

A mixture of docosanoic acid (200 nmol) and 1-(pentafluorophenyl)ethyl [13,14$^2H_2$]docosanoate (200 nmol) was treated at both 22 and 40°C with 50 μl of a 0.3 *M* solution of **3b** in benzene. Aliquots (5 μl) were taken after 15 min, 30 min, 1 h, 2 h, 4 h and 16 h and the reaction was quenched by the addition of acetic acid. The samples were worked up and analysed by GC–MS as described.

Derivatization of d_7-PGE-MM

Analytical amounts (range 30 pmol–20 nmol) of d_7-PGE-MM were converted into the bismethoxime by treatment with 1.5 ml of a solution of O-methylhydroxylamine hydrochloride (100 mg/ml) in 1.5 *M* acetate (buffer pH 5) for 30 min at room temperature. The mixture was extracted with diethyl ether (3 × 2 ml) and, after evaporation of the combined extracts, the samples were derivatized with **2a** or **3b** as described above. Prior to injection the dried samples were dissolved in BSTFA.

Response of PFB and MPFB esters

A mixture of 10 nmol of the carboxylic acid and 10 nmol of suberic acid as internal standard were derivatized to the PFB or MPFB esters as described above. The samples were dissolved in 1 ml of dodecane.

The samples with (±)-3-(carboxymethylthio)lactic acid esters were dissolved in 100 μl of BSTFA and diluted 1:10 with dodecane.

(±)-d_7-PGE-MM (10 nmol) was converted into its methoxime as described above. Hexadecanedicarboxylic acid (10 nmol) as internal standard was then added, the sample divided into two equal parts and transformed into the corresponding PFB or MPFB esters. The samples were dissolved in 100 μl of BSTFA and diluted with dodecane to a final concentration of 10 pmol/μl.

RESULTS AND DISCUSSION

Whereas diazomethane is widely accepted as a reagent for derivatization of carboxylic acids, other diazoalkanes such as phenyldiazomethane[17] or (trideutero-)-diazoethane[18] have been used far less frequently for this purpose. However, none of these reagents offers any advantages with respect to GC–ECD or GC–NICI-MS techniques. Further, previous attempts to prepare electrophoric esters via derivatization with trifluorodiazoethane were unsuccessful because this reagent failed to react with carboxylic acids[19]. Although the novel diazo compounds **3a** and **3b** contain the electronegative pentafluorophenyl residue, their reactivity towards organic acids is still sufficient to give the corresponding esters[12].

In order to determine the optimum conditions for derivatization with the diazoalkanes **3a** and **3b**, the time course of derivatization was investigated for the reaction with docosanoic acid at two different temperatures. This long-chain fatty acid is expected to react very slowly and additionally should represent a good model for eicosanoids. For reaction with **3b** esterification is nearly complete after 16 h (Fig. 3). As expected, **3a** reacts much more slowly[12] and is therefore less convenient for routine derivatizations as it requires reaction times of several days (data not shown).

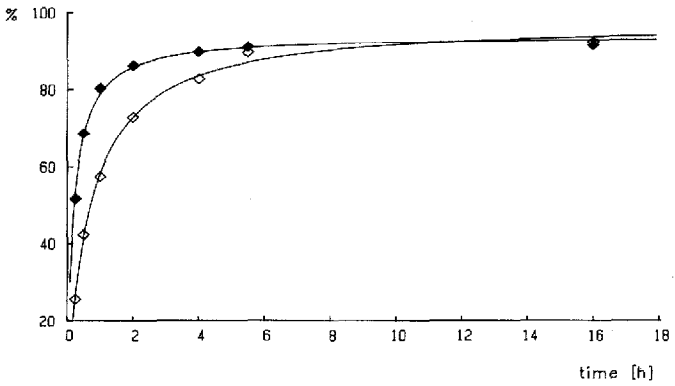

Fig. 3. Time course of derivatization of docosanoic acid with **3b**. ◇ = 25°C (average of two determinations); ◆ = 40°C.

Because the absolute responses of pure PFB and MPFB esters are identical (±5%) for carboxylic acids such as docosanoic or suberic acid (data not shown), the latter acid was employed as an internal standard to compare the two derivatization procedures. Several sensitive dicarboxylic acids which show partial decomposition when derivatized to the PFB ester in the usual manner were then converted to the PFB and MPFB esters using either **2a** or **3b**. The response of the main fragments was determined in relation to the corresponding suberic acid esters (Table I). In all cases the relative response of the MPFB ester, which essentially reflects a higher derivatization reaction yield, is better than that of the PFB ester. Thus, for sensitive carboxylic acids,

TABLE I

RELATIVE RESPONSES OF THE MAIN FRAGMENTS ($[M-181]^-$ OR $[M-195]^-$) OF THE PENTAFLUOROBENZYL (PFB) AND α-METHYLPENTAFLUOROBENZYL (MPFB) ESTERS OF DIFFERENT DICARBOXYLIC ACIDS IN RELATION TO THE RESPONSES OF THE SUBERIC ACID ESTERS (SET AT 100%)

Esters were prepared as described under Experimental.

Compound	*Relative area*		*Relative response, MPFB ester/PFB ester*
	PFB ester	*MPFB ester*	
Suberic acid	100.0	100.0	1.0
Thiodiglycolic acid	19.0	142.0	7.5
Thiodiglycolic acid sulphoxide	–[a]	0.5[b]	–
Dithiodiglycolic acid	11.8	16.7	1.4
(±)-3-(Carboxymethylthio)-lactic acid	4.5	53.0	11.8
(±)-d_7-PGE-MM[c]	35.9	173.0	4.8

[a] Not detectable.
[b] Main fragment $[M-1]^-$.
[c] Bismethoxime trimethylsilyl ether derivative; internal standard, hexadecanedicarboxylic acid.

3b seems to be an excellent alternative to the bromide **2a**, in particular as no additional purification step is necessary.

Fig. 4 shows the chromatogram of a mixture of bis-MPFB esters of aliphatic saturated dicarboxylic acids (C_6–C_{16}). The peak shape and separation of the bis-MPFB esters are identical with those of the bis-PFB esters. Although the derivatization reagent **3b** introduced two centres of chirality into the newly formed dicarboxylates, neither a separation of the diastereomers nor any peak broadening was observed in capillary GC. For chiral carboxylic acids, however, where the carboxy group is directly attached to the asymmetric centre, a separation of the diastereomers can be achieved (Table II).

The reagents **3b** may further be useful for a rapid check of the separability of chiral acids. If a separation of the diastereomeric esters could be achieved, then a conventional esterification of these acids with enantiomerically pure 2-(pentafluorophenyl)ethanol[20] should allow for the GC determination of their optical purity.

As observed for PFB esters, MPFB esters of the dicarboxylic acids studied exhibit a unique mass spectrometric fragmentation pattern and, with the exception of the corresponding derivative of thiodiglycolic acid sulphoxide (Table I), up to 90% of the total ion current accounts for the $[M-195]^-$ fragment and the natural abundance isotope masses. Because of the simple derivatization procedure described above and the excellent GC and MS properties of MPFB esters, a promising application of **3b** is

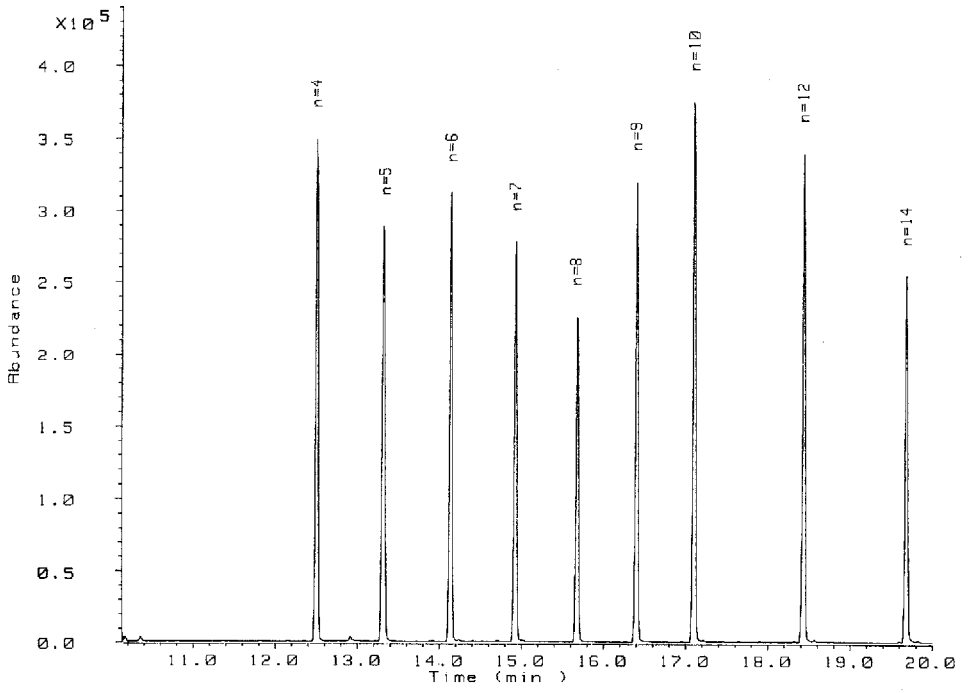

Fig. 4. $HOOC(CH_2)_nCOOH$ dicarboxylic acid mixture derivatized with **3b**. GC conditions: 25 m × 0.32 mm I.D. SE-52 fused-silica capillary column, temperature programmed at 10°C/min from 110 to 320°C.

TABLE II

SEPARATION FACTORS, α, FOR THE MPFB ESTERS OF SOME CHIRAL CARBOXYLIC ACIDS

Compound	α	*Temperature (°C)*[a]
(±)-2-Phenylpropionic acid	1.193	150
(+)-/(−)-Mandelic acid[b]	1.305	150
	1.246	170
(+)-/(−)-Mandelic acid *tert.*-butyl dimethylsilyl ether[b]	1.035	170
(±)-3-(Carboxymethylthio)-lactic acid	1.193	210

[a] Isothermal, GC conditions as indicated under Experimental.

[b] Equal amounts of both individual enantiomers of mandelic acid show identical responses of their diastereomeric esters.

expected in the trace analysis of organic acids. Preliminary results on the analysis of some of the sulphur-containing acids listed in Table I, in conjunction with stable isotope-labelled analogues as internal standards, indicate their successful identification and quantification in biological fluids in the lower femtomole range[21].

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (Grant Me 792) and the Robert-Bosch Foundation (Stuttgart, F.R.G.).

REFERENCES

1 E. D. Pellizzari, *J. Chromatogr.*, 98 (1974) 323.
2 K. Blau and G. S. King, *Handbook of Derivatives for Chromatography*, Heyden, London, 1977.
3 D. R. Knapp, *Handbook of Analytical Derivatization Reactions*, Wiley, New York, 1979.
4 D. F. Hunt and F. W. Crow, *Anal. Chem.*, 50 (1978) 1781.
5 H. Brandenberger, *Recent Dev. Mass Spectrom. Biochem. Med.*, 2 (1979) 227.
6 R. C. Dougherty, *Anal. Chem.*, 53 (1981) 625 A.
7 H. Budzikiewicz, *Mass Spectrom. Rev.*, 2 (1983) 515.
8 R. J. Strife and R. C. Murphy, *J. Chromatogr.*, 305 (1984) 3.
9 H. Schweer, H. W. Seyberth, C. O. Meese and O. Fürst, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 143.
10 O. Gyllenhaal, *J. Chromatogr.*, 153 (1978) 517.
11 M. I. Daneshvar and J. B. Brooks, *J. Chromatogr.*, 433 (1988) 248.
12 C. O. Meese, *Liebigs Ann. Chem.*, (1985) 1711.
13 C. O. Meese, D. Specht and U. Hofmann, *Arch. Pharm. (Weinheim)*, in press (1990).
14 C. O. Meese, S. Holzer and U. Hofmann, in preparation.
15 T. D. Petrova, T. I. Savchenko, T. F. Ardynkova and G. G. Yakobson, *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*, 3 (1970) 119; *C.A.*, 74 (1971) 53393q.
16 M. Regitz, *Diazoalkane*, Georg Thieme, Stuttgart, 1977.
17 H. P. Klemm, U. Hintze and G. Gercken, *J. Chromatogr.*, 75 (1973) 19.
18 C. O. Meese, *J. Labelled Compd. Radiopharm.*, 20 (1983) 1047.
19 C. O. Meese, *Synthesis*, (1984) 1041.
20 C. O. Meese, *Liebigs Ann. Chem.*, (1986) 2004.
21 U. Hofmann, M. Eichelbaum, S. Seefried and C. O. Meese, in preparation.