ELSEVIER

Journal of Chromatography A, 773 (1997) 239–247

JOURNAL OF CHROMATOGRAPHY A

Reaction of fatty acid amides and ethanolamides with trimethylsulfonium hydroxide

Klaus Vosmann[a], Erika Klein[b], Nikolaus Weber[b,*]

[a]*Institut für Chemie und Physik der Fette, BAGKF, Piusallee 76, D-48147 Münster, Germany*

[b]*Institut für Biochemie und Technologie der Fette, H.P. Kaufmann-Institut, BAGKF, Piusallee 68, D-48147 Münster, Germany*

Received 12 November 1996; revised 31 January 1997; accepted 7 February 1997

Abstract

The preparation of fatty acid methyl esters by transesterification of acyl lipids using trimethylsulfonium hydroxide (TMSH) as a base catalyst is a convenient GC derivatization procedure of growing interest. It has now been found that lipids containing amido groups, e.g. fatty acid amides and fatty acid ethanolamides, are partially N- and/or O-methylated by the pyrolytic reaction with TMSH occurring in the injector of the gas chromatograph. The various reaction products, i.e. N-methyl and N,N-dimethyl fatty acid amides as well as N-methyl, O-methyl and N,O-dimethyl fatty acid ethanolamides, were analyzed by GC and GC–MS. Our results show that derivatization with TMSH is not generally recommended for the derivatization of lipid mixtures containing fatty acid amides or ethanolamides. However, some of the methylation products as well as the sequence of reactions which finally lead to N,N-dimethyl fatty acid amides and N,O-dimethyl fatty acid ethanolamides, respectively, can be of diagnostic value for the structural analysis of the various fatty acid amides by GC–MS. © 1997 Elsevier Science B.V.

Keywords: Derivatization, GC; Fatty acid amides; Amides; Trimethylsulfonium hydroxide; Fatty acid ethanolamides

1. Introduction

Trimethylsulfonium hydroxide (TMSH) is a powerful reagent for the base-catalyzed transmethylation of acyl lipids, such as triacylglycerols, for the preparation of fatty acid methyl esters [1–4]. Under pyrolytic conditions in the hot injection port of a gas chromatograph, fatty acid methyl esters were formed from free fatty acids by the reaction with TMSH as well [5]. In a previous paper, we have described the pyrolytic O-methylation of hydroxy groups of lipids in the presence of TMSH [6]. N-Methylation of amido and amino groups of lipids by the pyrolytic reaction with TMSH has not been studied, so far. However, on-column alkylation with tetraalkylammonium [7–10] and trialkylsulfonium salts or hydroxides [8–10], respectively, has been recommended for GC derivatization of various acidic chemicals including carboxylic acids, phenols and sulfonamides.

Fatty acid amides and ethanolamides are well known in industrial applications, such as the control of viscosity and foam properties of detergent and cosmetic formulations [11–14]. In nature, these classes of compounds have been detected in total lipids of microorganisms, plants and animals. For example, a great variety of fatty acid amides and their N-alkyl derivatives have been isolated from

*Corresponding author.

PII S0021-9673(97)00138-6

microorganisms [15,16] and plants [17–19]. Very recently, both fatty acid amides [20] and ethanolamides [21–24] have been found to be physiological signalling molecules in human and animal brain. GC separation of fatty acid amides has been studied occasionally [16,25–27], whereas extensive thermal dehydration of fatty acid ethanolamides has been observed under GC conditions [22]. Recently, we have described side reactions occurring with lipids containing hydroxy groups during on-column derivatization with TMSH [6]. Here we report the pyrolytic reaction of TMSH with both fatty acid amides and ethanolamides leading to N- and/or O-methylated derivatives.

2. Experimental

2.1. Materials

Fatty acid methyl esters, acyl chlorides and acyl anhydrides were purchased from E. Merck (Darmstadt, Germany) and Sigma-Aldrich (Deisenhofen, Germany). TMSH reagent (0.2 *M* TMSH in methanol) was a product of Macherey–Nagel (Düren, Germany). Monomethyl ammonium chloride and an aqueous solution of dimethyl amine were obtained from Serva (Heidelberg, Germany). Ethanolamine, N-methyl- and O-methylethanolamine, technical grade stearamide and erucamide were products of Sigma–Aldrich.

2.2. Preparation of standards

Fatty acid amides as well as N-methyl and N,N-dimethyl fatty acid amides were prepared by the reaction of fatty acid anhydrides or acyl chlorides with gaseous ammonia, methylamine or dimethylamine. Fatty acid ethanolamides and the corresponding N-methyl and O-methyl derivatives were prepared by the reaction of fatty acid methyl esters with ethanolamine, N-methyl- and O-methylethanolamines, respectively, following the procedure of Feairheller et al. [14]. N,O-Dimethylethanolamides of fatty acids were prepared from the ethanolamides by the reaction with methyl bromide in the presence of KOH. Synthetic fatty acid amides and ethanolamides as well as their N- and O-methyl derivatives were purified by thin-layer chromatography on 0.3 mm silica gel H (E. Merck) layers using dichloromethane–methanol (95:5, v/v) or dichloromethane–methanol–acetic acid (95:5:2, v/v/v) as solvent systems.

2.3. Derivatization for GC

Under standard conditions, 60 μl TMSH reagent (0.2 *M* TMSH in methanol) were added to 0.8 mg lipid containing primary or secondary amide groups (molar ratio around 1:4). The mixture, 1 μl, was immediately injected into a gas chromatograph.

2.4. GC conditions

GC of fatty acid amides and ethanolamides was carried out using a Hewlett-Packard (Böblingen, Germany) HP-5890 Series II gas chromatograph fitted with a flame ionization detection (FID) system. N-Methyl derivatives of fatty acid amides were separated on a 0.15 μm FFAP-CB fused-silica capillary column (Macherey–Nagel), 25 m×0.25 mm I.D., using nitrogen as the carrier gas. The N-methyl derivatives of fatty acid amides, except erucic acid amide, were separated initially at 200°C for 5 min, followed by linear programming from 200 to 240°C at 10°C per min. The final temperature was kept constant for 10 min. Erucic acid amide was separated from its N-methylated derivatives isothermally at 240°C. The split ratio was 1:10, the injector and flame-ionization detector temperature 270°C. N-Methyl, O-methyl, and N,O-dimethyl as well as 2-oxazoline derivatives of fatty acid ethanolamides were separated on a 0.15 μm Silar 5CP fused-silica capillary column (Macherey–Nagel), 25 m×0.25 mm I.D., using the temperature program described above.

2.5. GC–MS analysis

GC–MS of N-methyl derivatives of fatty acid amides, N-methyl, O-methyl and N,O-dimethyl derivatives of fatty acid ethanolamides was performed in the electron ionization (EI) mode (70 eV) on a Hewlett-Packard instrument Model 5890 Series II/5989 A. The GC instrument was equipped with a 0.23 μm Permabond OV-1 fused-silica capillary

column (Macherey–Nagel) 25 m×0.32 mm I.D. The carrier gas was He at a flow-rate of 1.0 ml/min. The column temperature was initially kept at 180°C for 5 min and then programmed from 180° to 240°C at 10°C/min; the final temperature was held for 25 min. Other operating conditions were split/splitless injector in splitless mode (temperature 250°C), interface temperature 280°C and ion-source temperature 200°C.

2.6. Structural analysis

GC peaks were assigned by comparison of their retention times with those of synthetic standards. Structural assignments were made for fatty acid amide standards, their N-methyl and N,N-dimethyl derivatives which were prepared by established procedures. The structure of fatty acid ethanolamides, their N-methyl, O-methyl, and N,O-dimethyl derivatives was determined in a similar manner. Peaks of N-methyl and N,N-dimethyl derivatives of fatty acid amides as well as N-methyl, O-methyl, and N,O-dimethyl derivatives of fatty acid ethanolamides obtained by the pyrolytic reaction with TMSH were assigned a structure if ions corresponding to all the important mass fragments were present and no other mass ions of high intensity were detected.

3. Results

Single compounds or mixtures of lipids containing amido groups, such as fatty acid amides and ethanolamides, were reacted with TMSH under pyrolytic conditions in the injection port of GC [5]. The formation of N- and O-methylated derivatives was studied by GC and GC–MS analyses and the results were summarized in Fig. 1.

3.1. Fatty acid amides

Fig. 2 shows the gas chromatographic separation of the reaction products of technical stearamide, i.e. a mixture of palmitic and stearic acid amides, with TMSH. The formation of both N-methyl and N,N-dimethyl derivatives was confirmed by GC and MS comparison with synthetic standards. It is obvious from Fig. 1 that a total of around 70% of the technical stearamide was converted to the two N-methylated products with strong preference for the N-monomethyl compounds (around 60%).

The GC separation of the products of the reaction of oleic acid amide with TMSH is shown in Fig. 3. The ratio of non-reacted amide to N-methyl plus N,N-dimethyl derivatives is slightly reduced as compared to that given above for the technical stearamide. This is due to the different molar ratios of amide to TMSH reagent used. Studies concerning the optimal molar ratio between amide substrates and TMSH reagent revealed that the ratio of pyrolytic conversion of fatty acid amides to the corresponding N-methylated products in the presence of TMSH depended on the molar excess of the methylating reagent (Fig. 4). Extensive conversion was reached already at a molar ratio of about 1:4 to 1:6. Under these conditions, a total of around 60% of oleic acid amide was converted to the above reaction products, mainly to the N-monomethyl derivative. A 24-fold excess of TMSH increased yield of N-methyl deriva-

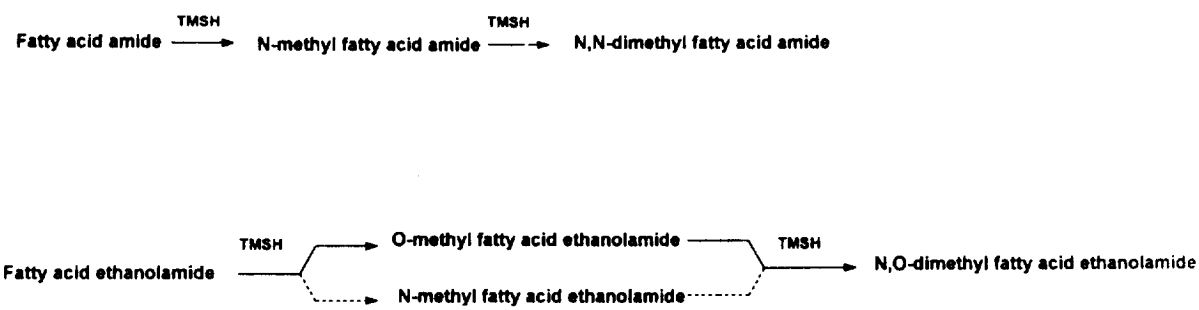

Fig. 1. Reaction scheme of the formation of N-methylated derivatives of fatty acid amides as well as N- and O-methylated derivatives of fatty acid ethanolamides by the pyrolytic reaction of trimethylsulfonium hydroxide.

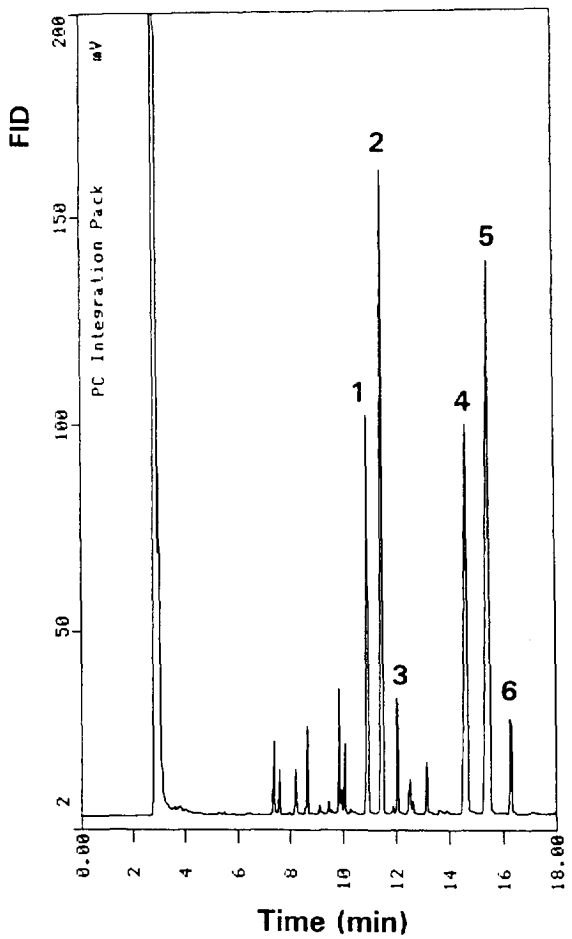

Fig. 2. Gas chromatogram of the reaction products of technical stearamide (stearic acid amide) with TMSH: (1) palmitic acid amide, not reacted; (2) N-methylpalmitic acid amide; (3) N,N-dimethylpalmitic acid amide; (4) stearic acid amide, not reacted; (5) N-methylstearic acid amide; (6) N,N-dimethylstearic acid amide.

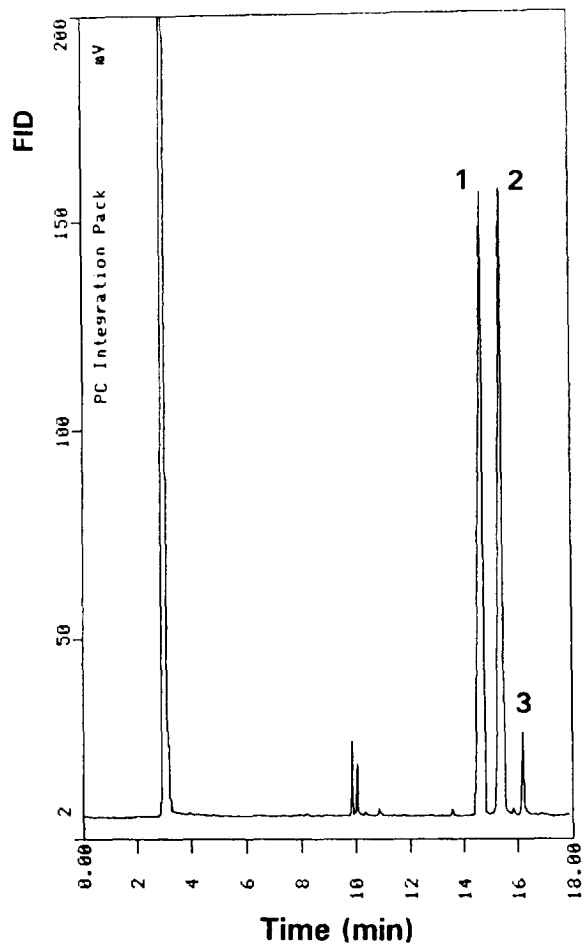

Fig. 3. Gas chromatogram of the reaction products of oleic acid amide with TMSH: (1) oleic acid amide, not reacted; (2) N-methyloleic acid amide; (3) N,N-dimethyloleic acid amide.

tives by a small extent only (Fig. 4). Similar results were obtained for other fatty acid amides such as lauramide, myristamide, erucamide and linolenamide. In contrast, monoalkyl amides with long N-alkyl chains, such as N-octadecyl oleic acid amide, as well as alkyl cyanides were not N-methylated by TMSH reagent (data not shown).

The mass spectra of the various fatty acid amides as well as their N-methyl and N,N-dimethyl derivatives show the expected molecular ions. It is evident from our results that molecular ions of the N-methyl and N,N-dimethyl derivatives are of about 1.5–3.5 fold higher intensity than the non-reacted fatty acid amides. In addition to the characteristic fragments at m/z 59 (McLafferty rearrangement ion) and m/z 72 (γ-cleavage peak) in the low mass region of the original fatty acid amides, similar fragments were recorded at m/z 73 and 86 for the N-methyl as well as at m/z 87 and 100 for the N,N-dimethyl derivatives. These results are in excellent agreement with the data given in the literature [20,25].

3.2. Fatty acid ethanolamides

Fig. 5 shows the gas chromatographic separation of the 2-oxazoline derivative formed by thermal

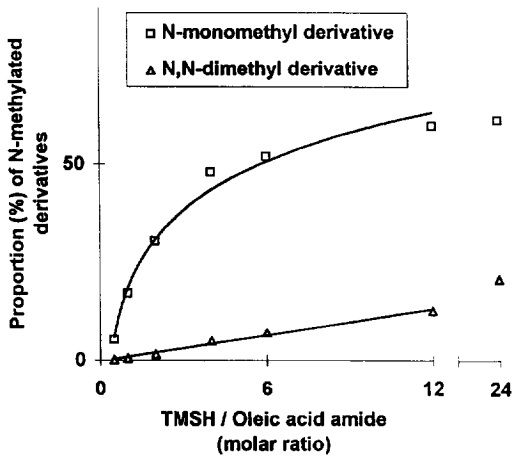

Fig. 4. Formation of N-methyl and N,N-dimethyl oleic acid amide in relation to the molar ratio of TMSH (all values are mean of three determinations).

dehydration of palmitic acid ethanolamide [22] as well as the reaction products of this ethanolamide with TMSH. The formation of O-methyl and N,O-dimethyl palmitic acid ethanolamides as well as small proportions of the corresponding N-methyl derivative was confirmed by GC comparison with synthetic standards and by MS (cf. Table 1).

The GC separation of the reaction products of arachidonic acid ethanolamide (anandamide) with TMSH is demonstrated in Fig. 6. It is evident that a number of minor reaction products are formed in addition to the main products, i.e. 2-oxazoline derivative as well as O-methyl and N,O-dimethyl arachidonic acid ethanolamides. The small peaks appearing between 17 and 19 min all demonstrate the prominent fragments of the 2-oxazoline derivative at *m/z* 329, 218, 178, 98 and 85 suggesting structural similarities with this thermal dehydration product. In a similar manner, the peaks between 20 and 23 min show prominent fragments at *m/z* 361, 276, 236, 178, 131, 117, 91, and 76 suggesting structural similarities with the O-methyl and N,O-dimethyl derivatives of arachidonic acid ethanolamide. It is of interest that significant proportions of the minor reaction products containing N- and/or O-methyl groups are formed only by the reaction of arachidonic acid ethanolamide with TMSH, but not of other saturated or unsaturated ethanolamides, such as palmitic or linolenic acid ethanolamides. Moreover,

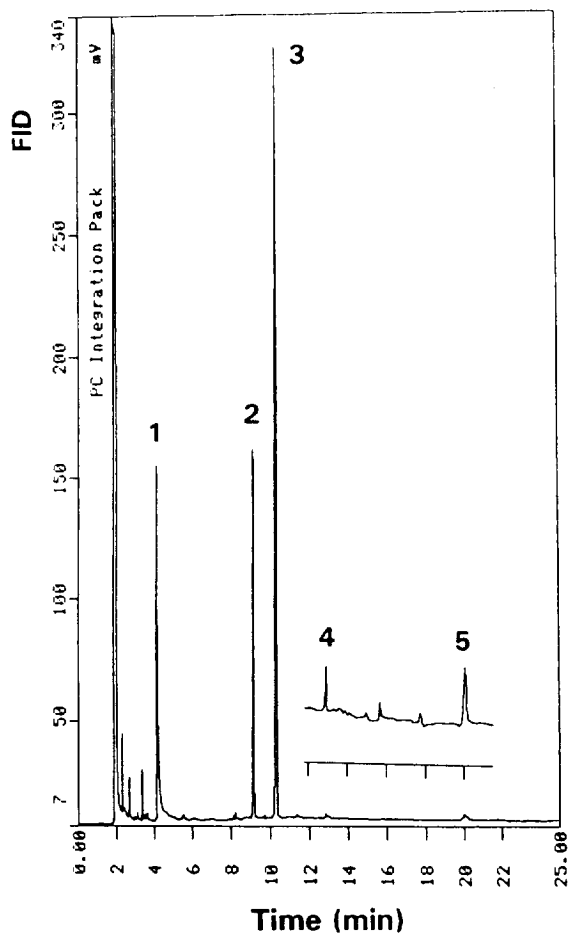

Fig. 5. Gas chromatogram of the reaction products of palmitic acid ethanolamide with TMSH: (1) 2-oxazoline derivative of palmitic acid; (2) O-methylpalmitic acid ethanolamide; (3) N,O-dimethylpalmitic acid ethanolamide; (4) N-methylpalmitic acid ethanolamide; (5) palmitic acid ethanolamide, not reacted; insert: FID response×1/5.

it was observed that after GC injection in the presence of TMSH fatty acid ethanolamides were almost completely transformed to 2-oxazoline derivatives as well as O-methyl and N,O-dimethyl derivatives, whereas only traces, if any, of N-methyl ethanolamides and unreacted compounds were detected (cf. Fig. 5).

Thermal dehydration of fatty acid ethanolamides, e.g. anandamide, led to the corresponding 2-oxazoline, *m/z* 329 $[M]^+$, upon electron ionization [22]. Similar dehydration reactions leading to 2-oxazoline derivatives were found for all other fatty

Table 1
EI mass spectral data of various derivatives of fatty acid ethanolamides

Derivatives of fatty acid ethanolamides	Important mass fragments, *m/z* (rel. %)[a]					
2-Oxazoline derivatives of						
Palmitoyl ethanolamide	299 (4.3)	256 (34.3)	239 (5.8)		116 (20.7)	103 (100)
	$[M]^+$	$[M-C_2H_3O]^+$	$[M-C_2H_6NO]^+$		$C_5H_{10}NO_2$	$C_4H_9NO_2$
Stearoyl ethanolamide	327 (3.4)	284 (29.3)	267 (3.5)		116 (21.3)	103 (100)
	$[M]^+$	$[M-C_2H_3O]^+$	$[M-C_2H_6NO]^+$		$C_5H_{10}NO_2$	$C_4H_9NO_2$
Oleoyl ethanolamide[b]	325 (15.7)	282 (4.4)	264 (3.9)		116 (51.1)	103 (54.4)
	$[M]^+$	$[M-C_2H_3O]^+$	$[M-C_2H_7NO]^+$		$C_5H_{10}NO_2$	$C_4H_9NO_2$
Linoleoyl ethanolamide[b]	323 (5.0)				116 (16.4)	103 (26.4)
	$[M]^+$				$C_5H_{10}NO_2$	$C_4H_9NO_2$
Linolenoyl ethanolamide[b]	321 (4.3)				116 (20.0)	103 (22.9)
	$[M]^+$				$C_5H_{10}NO_2$	$C_4H_9NO_2$
Arachidonoyl ethanolamide	329 (3.6)				98 (37.6)	85 (100)
	$[M]^+$				C_5H_8NO	C_4H_7NO
N-Methyl ethanolamides						
N-Methylpalmitoyl ethanolamide	313 (4.6)	270 (9.2)	239 (1.6)		130 (16.1)	117 (100)
	$[M]^+$	$[M-C_2H_3O]^+$	$[M-C_3H_8NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
N-Methyloleoyl ethanolamide[c]	339 (32.5)	296 (2.9)			130 (50.7)	117 (55.9)
	$[M]^+$	$[M-C_2H_3O]^+$			$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methyl ethanolamides						
O-Methylpalmitoyl ethanolamide	313 (6.1)	281 (9.9)	239 (13.5)		130 (21.4)	117 (100)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_8NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methylstearoyl ethanolamide	341 (3.9)	309 (9.4)	267 (7.0)		130 (19.5)	117 (100)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_8NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methyloleoyl ethanolamide[d]	339 (20.7)	307 (1.5)	265 (3.5)		130 (37.1)	117 (36.6)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_8NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methyllinoleoyl ethanolamide[d]	337 (7.5)	305 (1.4)	262 (1.5)		130 (8.9)	117 (14.3)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_9NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methyllinolenoyl ethanolamide[d]	335 (5.3)	303 (1.6)	260 (2.2)		130 (9.9)	117 (13.1)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_9NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
O-Methylarachidonoyl ethanolamide[d]	361 (2.9)	329 (1.4)	286 (2.1)		130 (19.2)	117 (62.0)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_3H_9NO]^+$		$C_6H_{12}NO_2$	$C_5H_{11}NO_2$
N,O-Dimethyl ethanolamides						
N,O-Dimethylpalmitoyl ethanolamide[e]	327 (11.1)	295 (9.9)	280 (5.4)	239 (3.1)	144 (11.8)	131 (84.4)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_2H_7O]^+$	$[M-C_4H_{10}NO]^+$	$C_7H_{14}NO_2$	$C_6H_{13}NO_2$
N,O-Dimethylstearoyl ethanolamide[e]	355 (6.9)	323 (8.9)	308 (10.5)	267 (8.3)	144 (15.6)	131 (61.9)
	$[M]^+$	$[M-CH_4O]^+$	$[M-C_2H_7O]^+$	$[M-C_4H_{10}NO]^+$	$C_7H_{14}NO_2$	$C_6H_{13}NO_2$
N,O-Dimethyloleoyl ethanolamide[f]	353 (30.7)		308 (6.1)	264 (2.8)	144 (36.7)	131 (43.0)
	$[M]^+$		$[M-C_2H_5O]^+$	$[M-C_4H_{11}NO]^+$	$C_7H_{14}NO_2$	$C_6H_{13}NO_2$
N,O-Dimethyllinoleoyl ethanolamide[f]	351 (18.9)		306 (4.2)	262 (4.9)	144 (10.1)	131 (17.1)
	$[M]^+$		$[M-C_2H_5O]^+$	$[M-C_4H_{11}NO]^+$	$C_7H_{14}NO_2$	$C_6H_{13}NO_2$
N,O-Dimethyllinoleoyl ethanolamide[f]	349 (16.4)		303 (8.6)	260 (6.9)	144 (11.9)	131 (15.4)
	$[M]^+$		$[M-C_2H_6O]^+$	$[M-C_4H_{11}NO]^+$	$C_7H_{14}NO_2$	$C_6H_{13}NO_2$
N,O-Dimethylarachidonoyl ethanolamide[f]	375 (7.4)		329 (5.4)		144 (11.1)	131 (76.9)
	$[M]^+$		$[M-C_2H_6O]^+$		$C_7H_{14}NO_2$	$C_6H_{13}NO_2$

[a] Intensities relative to base peak.
[b] Base peak: *m/z* 62 ($[H_3N-CH_2-CH_2-OH]^+$).
[c] Base peak: *m/z* 76 ($[CH_3-NH_2-CH_2-CH_2-OH]^+$).
[d] Base peak: *m/z* 76 ($[H_3N-CH_2-CH_2-OH-CH_2]^+$).
[e] Base peak: *m/z* 57.
[f] Base peak: *m/z* 90 ($[CH_3-NH_2-CH_2-CH_2-OH-CH_2]^+$).

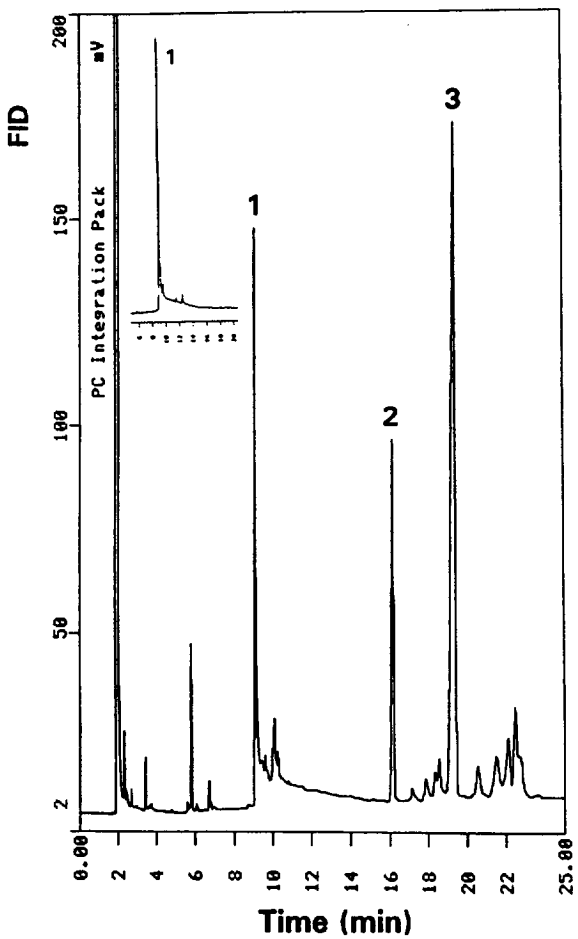

Fig. 6. Gas chromatogram of the reaction products of anandamide (arachidonic acid ethanolamide) with TMSH: (1) 2-oxazoline derivative of arachidonic acid; (2) O-methylarachidonic acid ethanolamide; (3) N,O-dimethylarachidonic acid ethanolamide; insert: 2-oxazoline derivative of arachidonic acid ethanolamide formed by thermal dehydration under GC conditions without TMSH treatment.

acid ethanolamides studied. Furthermore, GC–MS analysis revealed a distinct fragmentation pattern in the low-mass range, e.g. the ions at m/z 85 (McLafferty rearrangement ion) and m/z 98 (product of γ-cleavage). With the exception of anandamide, an ion at m/z 112 was formed due to γ-cleavage which cannot be formed from the anandamide molecule because of the Δ^5-double bond [23]. In addition, the mass spectra showed fragmentations with sequential loss of m/z 14 and 26 suggesting gradual cleavage of CH_2 and CH=CH groups. These results are consistent with the literature [22,23].

Table 1 gives the mass spectral data of 2-oxazoline derivatives of various fatty acid ethanolamides, N-methyl, O-methyl and N,O-dimethyl derivatives of fatty acid ethanolamides. The spectra of all compounds showed the expected molecular ions. The occurrence of the McLafferty rearrangement ion and an additional ion in the low mass region, which had been formed by γ-cleavage, was characteristic of all compounds studied.

Ethanolamides and N-methylethanolamides undergo two important fragmentation reactions. The cleavage of m/z 43 is caused by a β-cleavage at the N–C bond with rearrangement of two hydrogen atoms, whereas the losses of m/z 60 (or 61) and m/z 74, respectively, result from the cleavage of the amide bond [28,29]. Similar fragmentation has been observed for O-methyl ethanolamides (loss of m/z 74 or 75) as well as N,O-dimethyl ethanolamides (loss of m/z 88 or 89). Another typical fragmentation reaction of O-methyl ethanolamides is the loss of methanol (m/z 32). This reaction occurs preferentially with saturated N,O-dimethyl ethanolamides, while the mass spectra of unsaturated N,O-dimethyl ethanolamides showed non-characteristic fragments with m/z 45 (C_2H_5O) or 46 (C_2H_6O).

4. Discussion

Fatty acid amides, e.g. 'cocoamide' and erucamide, as well as fatty acid ethanolamides, e.g. stearic acid ethanolamide, are ingredients of various technical formulations [11–14]. Recently, the biological importance of fatty acid amides and ethanolamides was confirmed by the identification of various novel biological signalling molecules, such as oleic and erucic acid amides, arachidonic acid ethanolamide and several other fatty acid ethanolamides, which were detected as physiologically active compounds in total lipids of human and animal brain [20,22–24]. Fatty acid ethanolamides such as palmitic acid ethanolamide, were found in plants as well [26]. Moreover, a number of fatty acid amides, e.g. all (Z)-9,12,15-octadecatrienoic acid amide (linolenamide), were found to be inhibitors of microbial phospholipase A_2 [15]. With this back-

ground, GC–MS was employed for the structural analysis of N-methyl and N,N-dimethyl derivatives of fatty acid amides as well as N-methyl, O-methyl and N,O-dimethyl derivatives of fatty acid ethanolamides formed by the pyrolytic reaction with TMSH.

4.1. Fatty acid amides

All fatty acid amides studied showed the characteristic fragmentation pattern as reported in the literature [16,27]. In addition, fatty acid amides were converted to N-methyl and N,N-dimethyl fatty acid amides in the injector of the gas chromatograph at temperatures of 250–270°C by the pyrolytical reaction with TMSH [5], whereas both fatty acid amides and ethanolamides do not form methyl derivatives when reacted with TMSH at 70°C for several hours (data not shown). Typical fragments of pyrolytic reaction products have been identified by GC–MS as described above. The appearance of N-methyl and N,N-dimethyl derivatives clearly demonstrates the presence of an NH_2 group in the original compounds (Figs. 2 and 3). Because of the rather low rate of formation of N-methyl and, in particular, N,N-dimethyl derivatives from long-chain alkylamines, e.g. octadecylamine (data not shown), and the relatively high rate of conversion of the various fatty acid amides into both N-methyl and N,N-dimethyl derivatives it may be possible to distinguish between amino and amido groups of lipid mixtures. Furthermore, it is of interest for structural analysis that long-chain N-monoalkyl amides, such as N-octadecyloleic acid amide, are not methylated at all by the pyrolytic reaction with TMSH (data not shown). This effect demonstrates the higher sterical hindrance by the long-chain alkyl moiety as compared to the methyl group. Finally, alkyl cyanides are not methylated by the reaction with TMSH, either.

4.2. Fatty acid ethanolamides

Substantial proportions of O-methyl derivatives were formed from fatty acid ethanolamides in the pyrolytic reaction with TMSH and it is obvious that methylation of the primary hydroxy group is the preferred reaction (Figs. 5 and 6). The O-methyl derivative is then utilized for the methylation of the secondary amide group leading to N,O-dimethyl fatty acid ethanolamides (Fig. 1). A less important sequence of reactions may lead first to the N-methyl derivative which is finally O-methylated to the N,O-dimethyl derivative (Fig. 1).

In contrast to fatty acid amides, the ethanolamides tend to undergo thermal dehydration. Under GC conditions high proportions of the corresponding 2-oxazoline derivatives are formed which have been utilized for MS analysis of fatty acid ethanolamides [22,23]. The pyrolytic reaction of fatty acid ethanolamides with TMSH mainly leads to the O-methyl and N,O-dimethyl derivatives, both of which can give additional information in GC–MS analyses (Table 1). Moreover, important information on the structure of fatty acid ethanolamides may be available from the sequence of reactions leading finally to N,O-dimethyl derivatives (Fig. 1).

In conclusion, we have found that fatty acid amides and fatty acid ethanolamides are partially N- and/or O-methylated by the pyrolytic reaction with TMSH, and, therefore, this method is not generally recommended for the derivatization of lipid mixtures containing various fatty acid amides. However, the methylation products as well as the sequence of methylation reactions which finally leads to N,N-dimethyl fatty acid amides and N,O-dimethyl fatty acid ethanolamides, respectively, can be of diagnostic value for GC–MS analyses of fatty acid amides and ethanolamides.

Acknowledgments

We thank Dr. A. Nehrings, Macherey-Nagel Co., for a generous gift of TMSH.

References

[1] W.J. Butte, J. Chromatogr. 261 (1983) 142–145.
[2] E. Schulte, K. Weber, Fat Sci. Technol. 91 (1989) 181–183.
[3] E. Schulte, in K.D. Mukherjee and N. Weber (Editors), Analysis of Lipids, CRC Press, Boca Raton, FL, 1993, pp. 139–148.
[4] W.W. Christie, in W.W. Christie (Editor), Advances in Lipid Methodology-2, Oily Press, Dundee, 1993, pp. 69–111.
[5] W. Butte, J. Eilers, M. Kirsch, Anal. Lett. 15A (1982) 841–850.

[6] K. Vosmann, E. Schulte, E. Klein, N. Weber, Lipids 31 (1996) 349–352.
[7] I. Brondz, I. Olsen, J. Chromatogr. 598 (1992) 309–312.
[8] M. Amijee, R.J. Wells, J. Chromatogr. A 662 (1994) 123–137.
[9] M. Amijee, J. Cheung, R.J. Wells, J. Chromatogr. A 738 (1996) 43–55.
[10] M. Amijee, J. Cheung, R.J. Wells, J. Chromatogr. A 738 (1996) 57–72.
[11] M.W. Formo, in D. Swern (Editor), Bailey's Industrial Oil and Fat Products, Vol. 2, Wiley-Interscience, New York, 4th ed., 1982, pp. 343-405.
[12] R.A. Reck, J. Am. Oil Chem. Soc. 62 (1985) 355–365.
[13] R.A. Reck, in R.W. Johnson and E. Fritz (Editors), Fatty Acids in Industry, Marcel Dekker, New York, Basel, 1989, pp. 177–199.
[14] S.H. Feairheller, R.G. Bistline Jr., A. Bilyk, R.L. Dudley, M.F. Kozempel, M.J. Haas, J. Am. Oil Chem. Soc. 71 (1994) 863–866.
[15] M.K. Jain, F. Ghomashchi, B.-Z. Yu, T. Bayburt, D. Murphy, D. Houck, J. Brownell, J.C. Reid, J.E. Solowiej, S.-M. Wong, U. Mocek, R. Jarrell, M. Sasser, M.H. Gelb, J. Med. Chem. 35 (1992) 3584–3586.
[16] T. Kaneshiro, R.F. Vesonder, R.E. Peterson, D. Weisleder, M.O. Bagby, J. Am. Oil Chem. Soc. 71 (1994) 491–494.
[17] F.A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer, J. Chem. Soc. (1956) 4163–4169.
[18] F. Bohlmann, J. Ziesche, H. Robinson, R.M. King, Phytochemistry 19 (1980) 1535–1537.
[19] R. Martin, H. Becker, Phytochemistry 24 (1985) 2295–2300.
[20] B.F. Cravatt, O. Prospero-Garcia, G. Siuzdak, N.B. Gilula, S.J. Henriksen, D.L. Boger, R.A. Lerner, Science 268 (1995) 1506–1509.
[21] N.R. Bachur, K. Masek, K.L. Melmon, S. Undenfriend, J. Biol. Chem. 240 (1965) 1019–1024.
[22] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Science 258 (1992) 1946–1949.
[23] L. Hanus, A. Gopher, S. Almog, R. Mechoulam, J. Med. Chem. 36 (1993) 3032–3034.
[24] P.C. Schmid, R.J. Krebsbach, S.R. Perry, T.M. Dettmer, J.F. Maasson, H.H.O. Schmid, FEBS Lett. 375 (1995) 117–120.
[25] B.A. Andersson, W.H. Heimermann, R.T. Holman, Lipids 9 (1974) 443–449.
[26] D.A. Brengartner, J. Am. Oil Chem. Soc. 63 (1986) 1340–1343.
[27] C.N. Wang, L.D. Metcalfe, J. Am. Oil Chem. Soc. 61 (1984) 581–583.
[28] F.W. McLafferty, Interpretation of Mass Spectra, 2nd ed., W.A. Benjamin, Reading, 1973, pp. 146–147 and 163–166.
[29] G. Spiteller, Massenspektrometrische Strukturanalyse organischer Verbindungen, Verlag Chemie, Weinheim, 1966, pp. 137–139.