Desorption chemical ionization mass spectrometry of 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholines and analogues[1]

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Abstract Ammonia desorption chemical ionization of ether-linked phospholipids of the type 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factors) and a series of analogues revealed a systematic fragmentation pattern that is characteristic for these compounds. The predominant ions included the protonated molecular ion and a series of fragments derived from the molecular ion having the following nominal mass losses: MH-14, MH-42, MH-59, and MH-183. Deuterated ammonia was used to elucidate the nature of several fragments. In addition, desorption chemical ionization was used to quantitate 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine at the nanogram/sample level. **—Thomas, M. J., M. Samuel, R. L. Wykle, J. R. Surles, and C. Piantadosi.** Desorption chemical ionization mass spectrometry of 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholines and analogues. *J. Lipid Res.* 1986. **27:** 172–176.

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The identification and quantitation of 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholines (1-*O*-alkyl-2-acetyl-GPCs; platelet-activating factor; PAF), and related compounds are important to understanding the physiologic role of these molecules. 1-*O*-Alkyl-2-acetyl-GPCs containing various alkyl chains and several of their analogues elicit pronounced biological responses in several cell types at sub-nanomolar concentrations. For example, platelet aggregation (1) and degranulation (2) can be induced in rabbit platelets at 10^{-11} to 10^{-10} M. Several cell types, including polymorphonuclear neutrophils, produce 1-*O*-alkyl-2-acetyl-GPC when appropriately stimulated (3). A more extensive discussion of the biology and biochemistry of PAF can be found in references (4, 5). Because these compounds are present in very low concentrations (6), they are difficult to detect chemically in cell preparations. However, recent mass spectrometric analyses of glycerophosphocholines demonstrate that this sensitive technique can be used to identify underivatized phosphocholines (7–18). No detailed comparative mass spectral study of 1-*O*-alkyl-2-acetyl-GPCs and analogues by this technique has been reported; this report presents preliminary results using the desorption chemical ionization (D/CI) technique to analyze the structure of and to quantitate 1-*O*-alkyl-2-acetyl-GPC. The relationship of this work to other studies is also discussed.

MATERIALS AND METHODS

The 1-*O*-hexadecyl-2-*O*-methoxy-*sn*-glycero-3-phosphocholine was obtained commercially from R. Berchtold, Biochemisches Labor, Bern, Switzerland. 1,2-Dioctadecanoyl-*sn*-glycero-3-phosphocholine was purchased from Serdary Research Laboratories, London, Ontario, Canada. All other compounds were prepared by published procedures (19–21). Lipid phosphorus was determined by the method of Rouser, Siakotos, and Fleischer (22). Deuterated ammonia was from Merck and Co., St. Louis, MO. Solvents were of the highest commercial grade and were checked by mass spectrometry before use. The mass spectra were obtained on a Ribermag R10-10C quadrupole mass analyzer from R. D. S. Nermag/Delsi equipped with a desorption probe for the CI source. The samples were dissolved in methanol and 50–500 ng was applied to the D/CI filament. To quantitate the MS response, solutions

Abbreviations: D/CI, desorption chemical ionization; GPC, glycero-3-phosphocholine; PAF, platelet-activating factor; MS, mass spectrometry.

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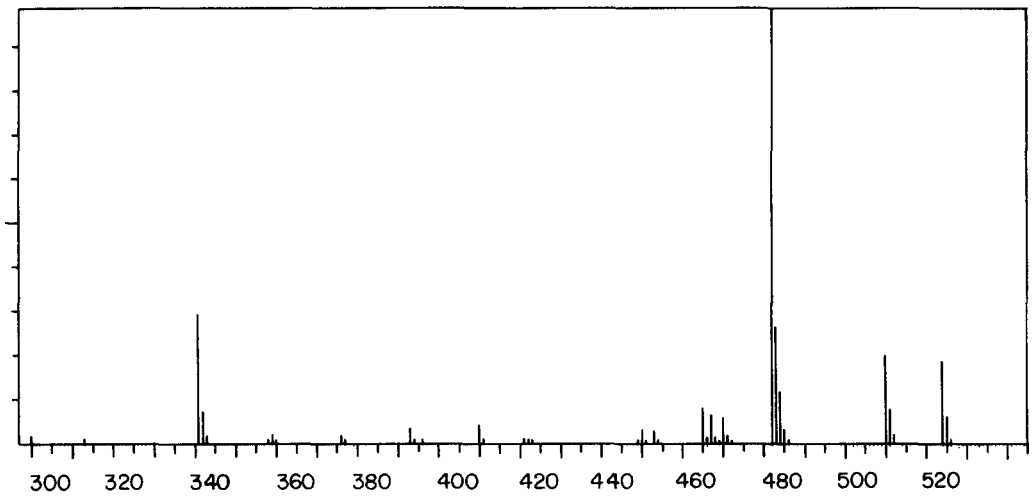

Fig. 1. Ammonia D/CI spectrum of 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine.

containing 50 ng of internal standard 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine and varying amounts of PAF were applied to the D/CI filament and allowed to dry. The probe was inserted into the source and then heated by passing 450 milliamperes through the filament. Source conditions were as follows: source temperature, 100°C; electron energy, 70 ev; source filament current, 0.2 milliamperes; trap current, 8 μamperes; reagent gas, ammonia at 1.4 $\times$ 10^{-1} torr.

RESULTS AND DISCUSSION

Fig. 1 shows an ammonia D/CI spectrum of a typical ether phospholipid 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine, 1, from 300 to 550 m/z. Major ion fragments observed for this compound (MH-14, -42, -59, and -183) are typical of the PAF molecules. **Table 1** lists the masses of characteristic ion fragments for ether phospholipids of the general structure depicted in **Fig. 2.**

TABLE 1. Principal ions in ammonia D/CI spectra of ether phospholipids[a]

R_1	R_2	R_3	$(MH)^+$	$(MH-14)^+$	$(MH-42)^+$	$(MH-59)^+$	$(MH-183)^+$
16:0[b]	OAc	H	524 (20)	510 (21)	482 (98)	465 (9)	341 (100)
16:0	OH	H	482 (33)	468 (74)		423 (100)	
16:1	OAc	H	522 (42)	508 (16)	480 (94)	463 (16)	339 (100)
16:0	Et	Et	522 (34)	508 (6)	480 (39)	463 (100)	339 (7)
16:0	sec-But	H	522 (36)	508 (20)	480 (100)	463 (80)	339 (8)
16:0	n-But	H	522 (28)	508 (15)	480 (44)	463 (100)	339 (8)
16:0	Me	Me	494 (11)	480 (9)	452 (12)	435 (100)	311 (9)
16:0	OMe	H	496 (32)	482 (5)	454 (3)	437 (100)	313 (3)
16:0	18:2-acyl	H	744 (11)	730 (6)	702 (28)		561 (100)
15:0	OAc	H	510 (20)	496 (3)	468 (26)	451 (10)	327 (100)
15:0	20:4-acyl	H	754 (12)	740 (25)	712 (29)	695 (8)	571 (100)
17:0	OAc	H	538 (73)	524 (9)	496 (66)	478 (17)	355 (100)
17:0	20:4-acyl	H	782 (36)	768 (22)	740 (37)	723 (7)	599 (100)
18:0	OAc	H	552 (24)	538 (18)	510 (100)	493 (5)	369 (51)
18:1	OAc	H	550 (45)	536 (10)	508 (100)	491 (6)	367 (100)
18:2	OAc	H	548 (36)	534 (17)	506 (81)	489 (13)	365 (100)
18:0	OEt	H	538 (25)	524 (28)	496 (2)	479 (100)	355 (9)
19:0	OAc	H	566 (11)	552 (8)	524 (27)	507 (4)	383 (100)

[a]Relative intensities are enclosed in parentheses.

[b]Abbreviations: nn:n, alkyl chain length:no of double bonds; OAc, O_2CCH_3; Et, CH_2CH_3; sec-But, $CH(CH_3)CH_2CH_3$; n-But, $(CH_2)_3CH_3$; Me, CH_3; 18:2-acyl, 9,12-octadecadienoyl; 20:4-acyl, 5,8,11,14-eicosatetraenoyl.

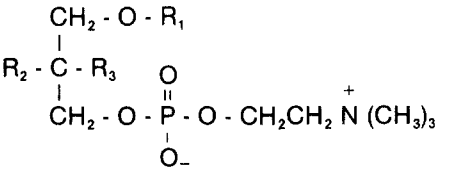

Fig. 2. General structure of the ether phospholipids examined in this work. See Table 1 for the identity of the substituents.

These fragments are arranged in order of decreasing mass. The protonated molecular ion MH^+ was always observed. More intense ions were found at $(M-42)^+$ and $(MH-183)^+$. Ions at $(MH-14)^+$ and $(MH-59)^+$ are generally the same intensity as MH^+. Structures consistent with these losses are shown in **Fig. 3.** No other substantial losses are consistently observed.

To provide further evidence supporting the structures proposed in Fig. 3, we obtained spectra of 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine using deuterated ammonia. The results are summarized in **Table 2.** Ions that show mass increases of 1 amu were MH^+ and $(MH-59)^+$. Fragment $(MH-59)^+$ can be derived by the loss of $N(CH_3)_3$, possibly involving the structure shown in Fig. 3. Loss of 183 amu from MH^+ may result from cleavage of the glycerol C3-0 bond yielding an ion (7, 10, 15–17) or it could be formed by a McLafferty type rearrangement in molecules with R^3 = H giving a neutral fragment that is subsequently protonated (11, 13). Cleavage of the C3–0 bond has been reported when some form of heating was used to vaporize the sample (7, 8, 10, 11, 13, 16, 18), but it is not commonly found in fast atom bombardment studies (7, 11, 12, 15). With ND_3, ions of m/z 369 and m/z 370 were obtained, suggesting that the two fragmentation paths yield two different ions of m/z 369 in the NH_3 spectrum (cf. Fig. 3). Ayanoglu et al. (11) reported that these dual paths operated simultaneously in only 1,2-diacetyl-*sn*-glycero-3-phosphocholine of the several compounds they studied. Fragment $(MH-42)^+$ shows an increase of 4 mass units with ND_3, consistent with displacement of $N(CH_3)_3$ by ammonia. Both Crawford and Plattner (13) and Ayanoglu et al. (11) have demonstrated this displacement in diacyl phosphocholines. This particular fragment disappears when ammonia is replaced with methane. The ion corresponding to $(MH-14)^+$ showed an increase of 2 amu with ND_3, indicating replacement of CH_3 with H (D). Loss of CH_3 and replacement by H appears to be characteristic of our method.

Confirmation of the various losses was obtained from analogues of 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine containing modified polar head groups: 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycerophosphohomocholine, 2, in

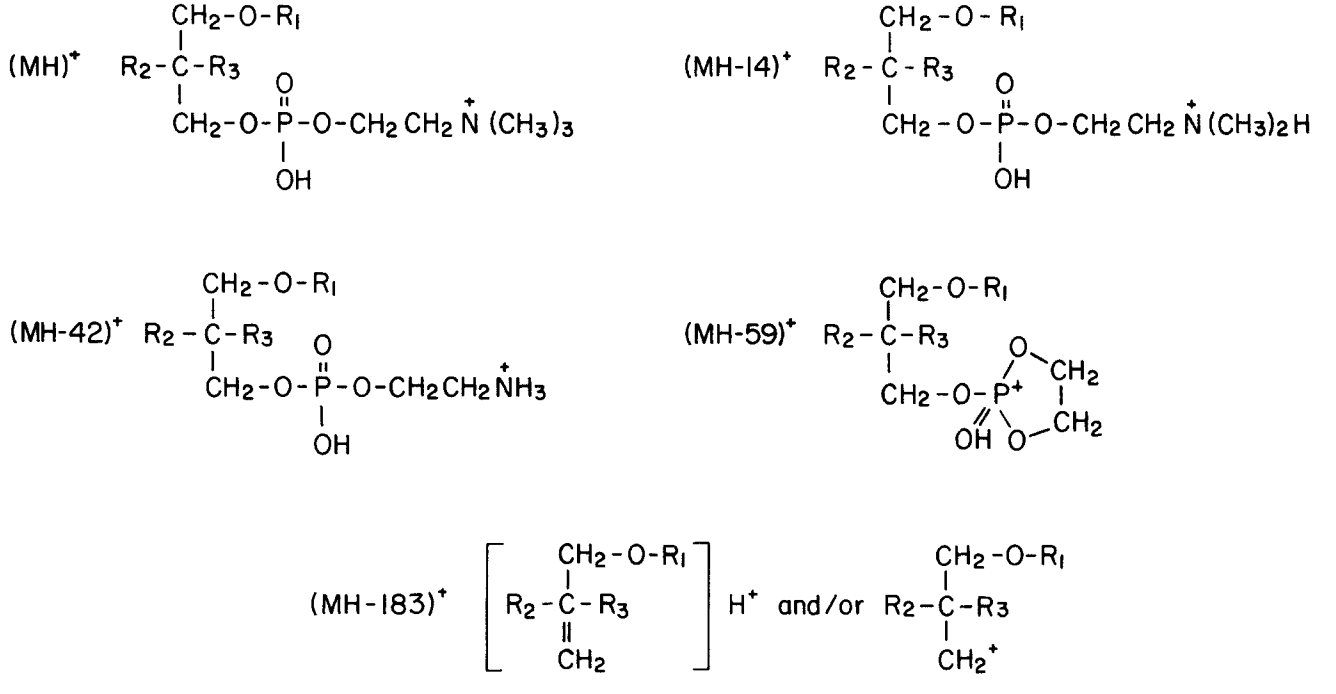

Fig. 3. Proposed structures for the ion fragments from the ether phospholipids.

TABLE 2. Comparison of ion fragments from 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine using either NH_3 or ND_3 as the CI gas[a]

NH_3		ND_3	
(m/z)	Assignment	(m/z)	Assignment
552	MH^+ (26)	553	MD^+ (46)
538	$(MH_2\text{-}CH_3)^+$ (18)	540	$(MD_2\text{-}CH_3)^+$ (23)
510	$(MH\text{-}N(CH_3)_3 + NH_3)^+$ (100)	514	$(MD\text{-}N(CH_3)_3 + ND_3)^+$ (100)
493	$(MH\text{-}N(CH_3)_3)^+$ (5)	494	$(MD\text{-}N(CH_3)_3)^+$ (11)
369	$(MH\text{-}HPO_4\ (CH_2)_2N(CH_3)_3)^+$ and $(MH\text{-}H_2PO_4\ (CH_2)_2N(CH_3)_3 + H)^+$ (61)	369	$(MD\text{-}DPO_4\ (CH_2)_2N(CH_3)_3)^+$ (72)
		370	$(MD\text{-}DHPO_4\ (CH_2)N(CH_3)_3 + D)^+$ (44)

[a]Relative intensities are given in parentheses.

TABLE 3. Comparison of major fragments from three ether phospholipid analogues with different polar head groups[a]

1[b]		2		3	
524	MH^+ (20)	538	MH^+ (60)	566	MH^+ (32)
510	$MH^+\text{-}CH_3 + H$ (21)	524	$MH^+\text{-}CH_3 + H$ (44)	538	$MH^+\text{-}CH_2CH_2$ (70)
482	$MH^+\text{-}N(CH_3)_3 + NH_3$ (98)	496	$MH^+\text{-}N(CH_3)_3 + NH_3$ (40)	482	$MH^+\text{-}N(CH_2CH_3)_3 + NH_3$ (100)
465	$MH^+\text{-}N(CH_3)_3$ (9)	479	$MH^+\text{-}N(CH_3)_3$ (100)	465	$MH^+\text{-}N(CH_2CH_3)_3$ (26)
341	$MH^+\text{-}HO_4P(CH_2)_2N(CH_3)_3$ (100)	341	$MH\text{-}HO_4P(CH_2)_3N(CH_3)_3$ (53)	341	$MH\text{-}HO_4P(CH_2)_2N(CH_2CH_3)_3$ (24)

[a]Relative intensities are enclosed in parentheses.
[b]1, 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine; 2, 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycerophosphohomocholine 3, 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-(1-phospho-2-N,N,N,-triethylaminoethanol).

which the alkyl chain between phosphate and amine has three methylenes instead of two, and 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-(1-phospho-2-N,N,N-triethylaminoethanol), 3, in which $N(CH_3)_3$ is replaced by $N(CH_2CH_3)_3$. With 2, all of the mass losses are the same as 1, except that involving breaking the glycerol 3C-O bond. Compound 3, shows a loss of $N(CH_2CH_3)$, replacement of $N(CH_2CH_3)_3$ by NH_3, and a loss of ethylene rather than CH_3. All of the ether lipids show an ion of m/z 341. **Table 3** summarizes the pertinent fragment data and the anticipated losses.

We did not observe several fragments that arose from cleavage between the glycerol oxygen and phosphorus reported by Ayanoglu et al. (11) and others (7, 16–18). We attribute the differences between the results of Ayanoglu et al. (11) and those in this report to the rate of filament heating. In our hands, rapid heating of the D/CI filament gives less fragmentation and an enrichment in high mass ions.

Platelet-activating factor is found at low concentration in most cells. For example, there are approximately 1.7 ng of PAF/10^6 human polymorphonuclear leukocytes and approximately 100 pg of PAF/10^6 rabbit leukocytes (6). We have used selective ion monitoring to acquire the areas of MH^+ (524 m/z) from PAF 1 and of the internal standard MH^+ (790 m/z). A plot of the ratio of ion intensity of 524 m/z to that of 790 m/z (I_{524}/I_{790}) versus nanograms of 1 is shown in **Fig. 4.** A linear relationship was obtained down to 1 ng of 1 with a signal-to-noise ratio of greater than 6.

In summary, D/CI mass spectra of 1-*O*-alkyl-2-acetyl-

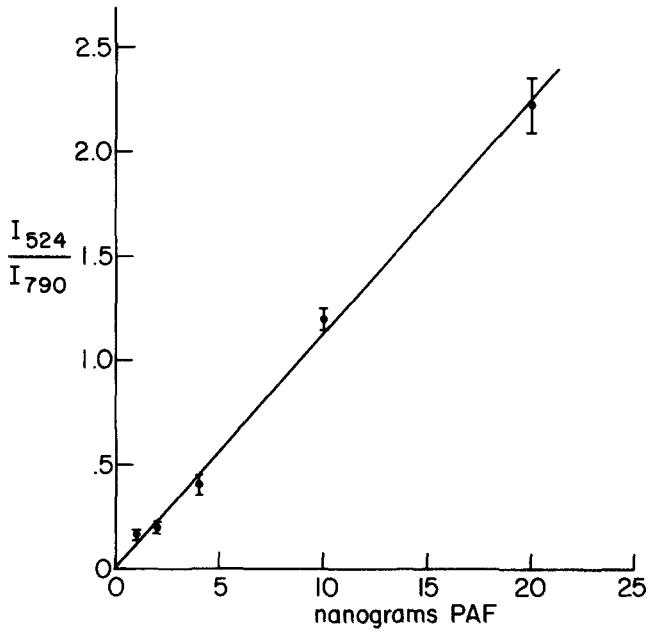

Fig. 4. Plot of the ratio of ion intensities at 524 m/z (I_{524}) and 790 m/z (I_{790}) for samples containing 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine and 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine. See Materials and Methods for details.

GPCs and analogues display a consistent fragmentation pattern that is useful in elucidating 1-*O*-alkyl-2-acetyl-GPCs of unknown structure. In addition, the technique has sufficient sensitivity to allow detection of masses that approach physiologic levels.

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