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PHOSPHOLIPID STUDIES OF MARINE ORGANISMS 4.[1]
(2R,21Z)-2-METHOXY-21-OCTACOSENOIC ACID, THE FIRST NATURALLY OCCURRING α-METHOXY ACID FROM A PHOSPHOLIPID

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Abstract: A novel fatty acid, (2R,21Z)-2-methoxy-21-octacosenoic acid, was shown to be present in the phospholipids of the sponge Higginsia tethyoides.

High levels of the unique C_{24-30} "demospongic" fatty acids have recently been identified in marine invertebrates. In our laboratory, the phospholipids of the sponges Aplysina fistularis and Petrosia ficiformis were shown to contain[1,2] new methyl-branched and straight chain C_{26-30} fatty acids (e.g., 1 and 2). We now wish to report the isolation and structure elucidation of a new phospholipid acid (7) from the sponge Higginsia tethyoides, which was investigated in connection with our ongoing research on membrane structure and function in lower marine organisms.

The sponge colonies were collected near Joal (ca. 100 km from Dakar, Senegal), lyophilized, extracted with cold $CHCl_3$/MeOH (1:1 v/v), and the total phospholipids separated as described earlier[2] using silicic acid column chromatography. The fatty acid methyl esters of the phospholipids were obtained by transesterification with 14% BF_3 in methanol, 2N HCl in dry methanol, or 1N NaOMe in methanol. In every case the same composition of fatty acid methyl esters was encountered.

Capillary GC and GC/MS analysis of the mixture gave a C_{30} acid (M^+ 460, three degrees of unsaturation) as the major component (24% of fatty acid mixture). Comparison of capillary GC retention times and equivalent chain values, together with mass spectral comparison of N-acyl pyrrolidide derivatives, showed it to be 3, 5,9,23-tricontatrienoic acid ($\Delta^{5,9,23}$-30:3), a previously reported[3] demospongic acid.

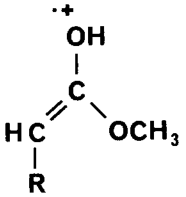
The second major (13%) demospongic acid exhibited unusual properties: on silica gel TLC, its methyl ester gave a slightly lower R_f value than that of conventional fatty acid methyl esters, implying the presence of polar functionalities. Separation from other fatty acid methyl esters was effected by silica gel column chromatography, since "normal" fatty acid methyl esters were eluted with hexane/ether (95:5 v/v) while the more polar compound was eluted in the hexane/ether (90:10 v/v) fractions. It was further purified by HPLC (column: Altex

Ultrasphere ODS; solvent: absolute methanol; flow rate: 2.0 ml/min., refractometric detector). Our compound was eluted shortly after the solvent peak, while conventional fatty acid methyl esters had a longer retention time. The high resolution mass spectrum of the new methyl ester (8) exhibited a molecular ion peak at m/z 466.43890 (42%, $C_{30}H_{58}O_3$), confirming the presence of an additional oxygen. The existence of a M^+-$COOCH_3$ fragment ion (m/z 407.42450, $C_{28}H_{55}O$) as the base peak strongly suggested an α-substitution pattern. The other diagnostic peaks were found at m/z 434.42195 (31%, $C_{29}H_{54}O_2$, M^+-MeOH) and 375.39820 (61%, $C_{27}H_{51}$, M^+-COOMe + MeOH), consistent with such an assumption. Lack of peaks at m/z 74 and 90, but presence of a fragmentation ion at 104.04862 (40%, $C_4H_8O_3$) from the characteristic McLafferty rearrangement[4] suggested a hydroxymethyl or a methoxy (6) rather than a hydroxy (5) substitution. The molecular ion peak (M^+ 505) of the N-acyl pyrrolidide[2] confirmed the $C_{30}H_{58}O_3$ empirical formula of the parent acid methyl ester which requires one degree of unsaturation in the alkyl chain.

1

2

3



4 R = H , m/z 74

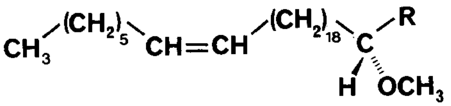
5 R = OH , m/z 90

6 R = OCH_3 or CH_2OH , m/z 104

The IR spectrum showed hydrocarbon and ester bands at 3050, 2820 and 1720 cm^{-1}, respectively. There was no prominent absorption at 980-968 cm^{-1}, indicating cis rather than trans unsaturation,[2] and no hydroxyl bands were observed. The presence of an α-methoxy group was confirmed by the NMR spectrum ($CDCl_3$), exhibiting two methoxy signals at 3.755 (methoxycarbonyl singlet, normally at 3.658 ppm, shifted because of the α-substitution) and at 3.380 ppm (δ). The NMR spectrum also showed absorbances at 0.882 (3H, t, terminal methyl) and at 5.350 (2H, m, monounsaturation) in addition to the large CH_2 absorbance at 1.253 ppm. These results indicated a straight chain structure with only one terminal methyl group and one double bond (cis orientation by IR evidence).

The hydrogenated (H_2/PtO_2, EtOH, 8 hr.) ester (10) gave a molecular ion at m/z 468 consistent with the reduction of one double bond. The prominent M^+-59 peak of the original compound at m/z 407 was shifted 2 a.m.u. to m/z 409, and the olefinic bands disappeared in the NMR spectrum. The original acid was subjected to $NaIO_4/KMnO_4$ oxidative double bond degradation in tert-butanol[5] followed by methylation. The mass spectrum of the resulting dicarboxylic acid methyl ester again gave a strong M^+-59 peak at m/z 355. Other prominent peaks were m/z 382 (M^+-32), 323 (M^+-91) and 291 (M^+-(32 + 91)) together with m/z 74 and 104, both McLafferty peaks (4,6) corresponding to an α-unsubstituted (m/z 74) and α-methoxy (m/z 104) methyl ester. The $C_{24}H_{46}O_4$ empirical formula of the dibasic ester located the double bond of the parent compound at C-21. Since the major fatty acid in the sponge is a $\Delta^{5,9,23}$-C_{30} acid (3), and biosynthetic chain elongation of fatty acids in sponges is a known process[1-3] (e.g., 21-octacosenoic acid (Δ^{21}-28:1) → 23-triacontenoic acid (Δ^{23}-30:1) → 3), unsaturation at C-21 of our acid is not surprising. The combination of MS, NMR and IR spectral data along with chromatographic and chemical properties indicated the presence of Z-2-methoxy-21-octacosenoic acid (7) as a major component of the phospholipids from the sponge *Higginsia tethyoides*. Formation of an α-methoxy group due to the extraction or esterification process is not possible, since α-hydroxy methyl esters[6,7] are obtained in the same manner.

For further structure proof, 7 was first reduced to the corresponding alcohol 9 (M^+ 438, $C_{29}H_{58}O_2$) by LAH. Its mass spectrum showed peaks at m/z 407 (loss of CH_2OH) and 385 (loss of CH_3OH and CH_2OH). The ester methoxy signal disappeared in the NMR spectrum, while the ether methoxy signal was present at 3.406 ppm together with the -CH=CH- (5.350), CH_2 (1.253) and terminal CH_3 (0.882) signals.

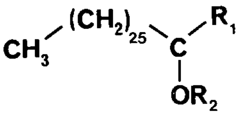


$$CH_3(CH_2)_5CH{=}CH(CH_2)_{18}\overset{R}{\underset{H\ \ OCH_3}{C}}$$

7 R = COOH

8 R = $COOCH_3$

9 R = CH_2OH



$$CH_3(CH_2)_{25}\underset{OR_2}{C}{-}R_1$$

10 R_1 = $COOCH_3$ R_2 = CH_3

11 R_1 = CH_2OH R_2 = CH_3

12 R_1 = CH_2OH R_2 = H

13 R_1 = O R_2 = CH_3

Hydrogenation (H_2/PtO_2, rt, 7 hr) of alcohol 9 yielded the saturated analog 11 with a slightly longer retention time on capillary GC. The olefinic proton signals had disappeared and the prominent M^+-CH_2OH peak at m/z 407 was shifted to m/z 409. The saturated α-methoxy alcohol 11 gave the corresponding glycol 12 by ether cleavage (47% HI in water at reflux temp., 3 hr.) as the major reaction product (no CH_3O signal in NMR). Oxidative degradation ($NaIO_4$/$KMnO_4$) of the glycol 12 followed by esterification with methanolic HCl furnished methyl heptacosanoate (13) as the final product, which was identical (MS, GC coinjection) with a commercially available authentic sample.

Circular dichroism measurements of the methyl ester 8 in methanol and in hexane demonstrated a strong negative Cotton effect at about 210 nm (Θ 19,500) and a weaker positive one at 235-240 nm (Θ 2,200). This result ensures the presence of an optically active natural product and indicates an R configuration in accordance with methyl 2-R-hydroxypalmitate, S-lactic acid ester and ether derivatives.[6,8] Naturally occurring 2-hydroxy fatty acids are known to possess the R configuration[7] and alkylation of the hydroxy group causes only slight shifts in the wavelength.[8,9] The above mentioned chemical evidence and CD spectral data define our compound as (2R,21Z)-2-methoxy-21-octacosenoic acid (7). To our knowledge this is the first example of a naturally occurring α-methoxy acid in a phospholipid.

Distribution of our compound among the individual phospholipid classes was investigated by preparative TLC separation and transesterification of each class.[2] 2-R-Methoxy-21-octacosenoic acid (7) and 5,9,23-tricontatrienoic acid (3) are major fatty acids of phosphatidyl ethanolamine and phosphatidyl serine of Higginsia tethyoides but are present only in very small amounts in phosphatidyl glycerol. Phosphatidyl choline was not present as a major class in this organism.

Other interesting membrane components of Higginsia tethyoides are currently under investigation in our laboratory.

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