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NEW $\Delta^{5,9}$ FATTY ACIDS IN THE PHOSPHOLIPIDS OF THE SEA ANEMONE *STOICHACTIS HELIANTHUS*

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ABSTRACT.—The diunsaturated fatty acids 5,9-heneicosadienoic acid, 5,9-docosadienoic acid and 5,9-tricosadienoic acid, the brominated fatty acids 6-bromo-5,9-heneicosadienoic acid and 6-bromo-5,9-docosadienoic acid, and the polyunsaturated fatty acids 5,9,13-eicosatrienoic acid and 5,9,13-docosatrienoic acid, were identified for the first time in nature in the phospholipids (mainly phosphatidylethanolamine and phosphatidylserine) of the anemone *Stoichactis helianthus*. Structural elucidation was accomplished by means of mass spectrometry and chemical transformations such as pyrrolidide and dimethyldisulfide derivatization. Other $\Delta^{5,9}$ fatty acids such as 5,9-hexadecadienoic acid, 5,9-octadecadienoic acid, and 5,9-eicosadienoic acid were also identified in the anemone. Our results indicate that $\Delta^{5,9}$ phospholipid fatty acids are not unique to sponges, as previously recognized, but can be found in other marine invertebrates such as an anemone. 24-Methylated sterols predominated in the sterol composition of the anemone investigated.

One interesting type of marine fatty acid diunsaturation has been the $\Delta^{5,9}$ pattern (1), which has been found almost exclusively in marine sponges and is practically unknown in other marine organisms. Examples of typical $\Delta^{5,9}$ fatty acids include 5,9-hexacosadienoic acid and 5,9,23-triacontatrienoic acid, which have been reported in the phospholipids of numerous sponges (1). All of these fatty acids have been reported to possess cis stereochemistry, with the exception of a series of iso/anteiso fatty acids of between 21 and 23 carbons which were reported from the sponge *Plakortis halichondroides* and shown to have 5*E*,9*E*- stereochemistry (2). These $\Delta^{5,9}$ fatty acids normally predominate in phosphatidylethanolamine, and show no preference for the C-1 or C-2 glycerol positions. For example, the 1,2-di-(5*Z*,9*Z*)-5,9-hexacosadienoyl-*sn*-glycero-3-phosphoethanolamine was isolated from the sponges *Microciona prolifera* and *Parasperella psila* and characterized by fabms and ms/ms (3), while the sponge *Aplysina fistularis* was found to contain 1,2-di-5,9,23-triacontatrienoyl-*sn*-glycero-3-phosphoethanolamine (4).

The more unusual 6-brominated $\Delta^{5,9}$ fatty acids with chain lengths between 24 and 28 carbons have also been reported in sponges (5–9). Some examples include (5*E*,9*Z*)-6-bromo-5,9-hexacosadienoic acid and (5*E*,9*Z*)-6-bromo-24-methyl-5,9-pentacosadienoic acid (8). It is significant that all of these fatty acids were found principally in phosphatidylethanolamine and that the bromine atom was always found at the vinylic C-6 position of the 5,9-diene function of the demospongic acid, regardless of carbon chain length or methyl branching.

The biogenesis of $\Delta^{5,9}$ fatty acids has been studied in the sponge *Microciona prolifera* (10). The conclusion from radiolabeling experiments targeting the 5,9-hexacosadienoic acid suggested that the order of double-bond introduction follows a random order; that is, either the Δ^5 double bond or the Δ^9 double bond can be introduced first in the acyl chain and then the associated Δ^9 or Δ^5 double bonds are introduced. This is in contrast with mammalian fatty-acid biochemistry where the first double bond is normally introduced at the Δ^9 position (10). As to the origin of the bromine in the 6-bromo-$\Delta^{5,9}$ fatty acids, radiolabeling experiments targeting the (5*E*,9*Z*)-6-bromo-5,9-hexacosadienoic acid have shown that the bromine is introduced at the final stages of the biosynthesis, that is, once the $\Delta^{5,9}$ diunsaturation has been introduced (6).

Is the $\Delta^{5,9}$ diunsaturation unique for sponges? This is the question we decided to

answer in this paper where we present, for the first time, information on a series of novel $\Delta^{5,9}$ fatty acids from a marine organism different from a sponge, namely, from the sea anemone *Stoichactis helianthus* Ellis, order Actiniaria, family Stoichactidae.

RESULTS AND DISCUSSION

The phospholipids from *S. helianthus* were isolated and separated as indicated in the Experimental. The main phospholipids were identified as phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylcholine (PC). Transesterification of the total and individual phospholipids with 1.0 N HCl/MeOH allowed the characterization of the principal fatty acids as methyl esters by gc-ms. Table 1 shows the fatty acid distribution in the phospholipids. The principal fatty acids in the anemone were characterized as hexadecanoic (16:0), 5,8,11,14,17-eicosapentaenoic (20:5 *n*-3), and 7,10,13,16,19-docosapentaenoic (22:5 *n*-3). In fact, polyunsaturated fatty acids accounted for 34% of the total fatty acid composition of the anemone. The polyunsaturated fatty acid 6,9,12,15-octadecatrienoic acid (18:4 *n*-3) was found almost exclusively in phosphatidylethanolamine (PE), while 8,11,14,17-eicosatetraenoic acid (20:4 *n*-3) preferred phosphatidylserine (PS). This type of specificity has not been observed before in marine phospholipids.

Other identified fatty acids and aldehydes deserve special mention. For example, both stereoisomers of 7-methyl-6-hexadecenoic acid were found in the anemone, but they were only identified in phosphatidylethanolamine (PE). This is the first time that this acid has been associated with a specific phospholipid (11). On the other hand, 2-hydroxyhexadecanoic acid was found mainly in the phosphatidylserine (PS), a fact not previously recognized for marine 2-hydroxy fatty acids. Four aldehydes of between 16 and 19 carbons, essentially arising from 1-*O*-alkenyl phospholipids or plasmalogens and particularly predominant in phosphatidylethanolamine (PE), were also identified in the anemone.

Of particular interest was the finding of 12 fatty acids with the $\Delta^{5,9}$ diunsaturation pattern accounting for 15% of the total fatty acid components of the anemone. Nine of these acids have not been identified before in nature. The new $\Delta^{5,9}$ fatty acids can be classified into four different families: (1) normal-chain $\Delta^{5,9}$ diunsaturated fatty acids of between 16 and 23 carbons, (2) 6-bromo-$\Delta^{5,9}$ fatty acids of between 20 and 22 carbons, (3) triunsaturated $\Delta^{5,9,13}$ fatty acids of between 20 and 22 carbons with the unprecedented ethylene-interruption between the double bonds, and (4) 6-bromo-$\Delta^{5,9,x}$ triunsaturated fatty acids of between 22 and 24 carbons. The unprecedented normal-chain $\Delta^{5,9}$ fatty acids were characterized as 5,9-heneicosadienoic acid [**1**], 5,9-docosadienoic acid [**2**], and 5,9-tricosadienoic acid [**3**] by mass spectrometry. The methyl ester derivatives of these acids were initially identified by a base peak at *m/z* 81, characteristic for $\Delta^{5,9}$ fatty acid methyl esters (9). The double-bond positions were rigorously confirmed by two independent methods. The method of choice was to synthesize the corresponding pyrrolidides (12). The eims of the corresponding pyrrolidide derivatives displayed a strong peak at *m/z* 180, due to allylic cleavage between C-7 and C-8, and this confirmed the double-bond arrangements. The second approach was dimethyl disulfide derivatization to the corresponding cyclic thiophenes (13). For example, dimethyl disulfide derivatization of methyl 5,9-docosadienoate afforded 2-(4-methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthiotridecan-1-yl)tetrahydrothiophene as the only product. This compound had a $[M]^+$ at *m/z* 476. Cleavage between C-5 and C-6 afforded the predominant fragments at *m/z* 161 (23) $[C_7H_{13}SO_2]^+$ and *m/z* 315 (22) $[C_{18}H_{35}S_2]^+$. The *m/z* 161 fragment either readily loses a mole of CH_3OH to give a fragment at *m/z* 129 (26) $[C_6H_9SO]^+$, or a mole of CH_3SH to give a fragment at *m/z* 113 (26) $[C_6H_9O_2]^+$. On the other hand, cleavage between C-9 and C-10 afforded the fragments at *m/z* 229 (15)

TABLE 1. Fatty Acids and Aldehydes from Total Lipids of *S. helianthus* and Its Major Phospholipids.

Compound	Abundance (wt %)			
	Total Lipids[a]	PE	PC	PS
Aldehydes[b]				
hexadecanal (16:0)	1.3	5.3	0.1	5.7
heptadecanal (17:0)	0.2	0.7	—	—
octadecanal (18:0)	4.3	15.7	2.3	2.5
nonadecanal (19:0)	0.2	4.0	—	—
Fatty Acids				
tetradecanoic (14:0)	1.3	1.8	3.9	3.1
pentadecanoic (15:0)	0.2	—	0.3	0.5
5,9-hexadecadienoic (16:2)	2.1	—	—	2.7
9-hexadecenoic (16:1)	3.7	2.2	4.0	6.3
hexadecanoic (16:0)	15.3	5.1	24.8	11.2
7-methyl-6-hexadecenoic (17:1)[c]	2.7	3.3	—	—
heptadecenoic (17:1)	1.1	3.5	0.1	—
heptadecanoic (17:0)	0.6	0.8	3.4	0.8
2-hydroxyhexadecanoic (*h*-16:0)	2.7	—	1.9	2.5
6,9,12,15-octadecatetraenoic (18:4)	3.1	5.5	—	—
9,12,15-octadecatrienoic (18:3)	1.1	—	—	—
5,9-octadecadienoic (18:2)	4.1	3.0	3.2	4.0
9,12-octadecadienoic (18:2)	1.1	1.6	—	2.1
9-octadecenoic (18:1)	2.1	10.0	18.2	12.1
11-octadecenoic (18:1)	1.6	3.0	10.4	6.9
13-octadecenoic (18:1)	0.3	1.8	7.6	5.5
octadecanoic (18:0)	6.2	3.1	5.4	5.8
nonadecanoic (19:0)	0.3	—	0.3	0.5
5,8,11,14-eicosatetraenoic (20:4)	2.3	1.9	—	1.2
5,8,11,14,17-eicosapentaenoic (20:5)	10.6	10.0	3.8	7.7
5,9,13-eicosatrienoic (20:3)[d] **[6]**	2.3	0.4	—	0.5
8,11,14,17-eicosatetraenoic (20:4)	1.5	—	—	2.0
5,9-eicosadienoic (20:2)	2.3	2.8	1.2	1.6
11-eicosenoic (20:1)	0.6	0.4	1.9	1.0
13-eicosenoic (20:1)	1.9	0.6	0.6	2.0
eicosanoic (20:0)	0.8	—	0.3	0.5
5,9-heneicosadienoic (21:2)[d] **[1]**	0.6	—	—	—
10,13,16,19-docosatetraenoic (22:4)	3.1	—	2.0	4.7
7,10,13,16-docosatetraenoic (22:4)	3.9	1.9	1.4	—
7,10,13,16,19-docosapentaenoic (22:5)	5.4	2.7	2.9	6.0
5,9,13-docosatrienoic (22:3)[d] **[7]**	0.3	—	—	—
5,9-docosadienoic (22:2)[d] **[2]**	2.1	1.6	—	—
13-docosenoic (22:1)	1.1	3.2	—	—
15-docosenoic (22:1)	1.1	3.0	—	—
5,9-tricosadienoic (23:2)[d] **[3]**	0.2	—	—	—
tetracosanoic (24:0)	0.6	—	—	—
6-bromo-5,9-heneicosadienoic (21:2)[d] **[4]**	0.4	0.3	—	0.3
6-bromo-5,9-docosadienoic (22:2)[d] **[5]**	0.8	0.7	—	0.3

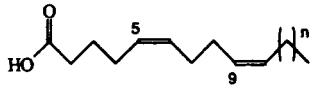
[a]These are the total phospholipids. Other minor components, identified in trace amounts, were 2-methylhexadecanoic acid, 17-tetracosenoic acid, 5,9,x-tetracosatrienoic acid, 6-bromo-5,9,x-docosatrienoic acid, and 6-bromo-5,9,x-tetracosatrienoic acid.

[b]Originated from plasmalogens and were characterized as dimethyl acetals.

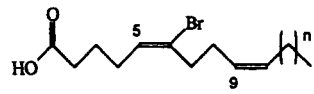
[c]Both (*E*) and (*Z*) isomers were observed in the total phospholipids.

[d]Occurrence of these acids in nature is novel.

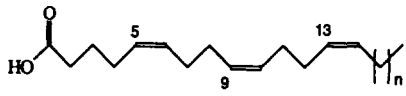
$[C_{14}H_{29}S]^+$ and *m/z* 247 (27) $[C_{11}H_{19}S_2O_2]^+$, but the latter ion also readily loses a mole of CH_3SH resulting in an abundant fragment at *m/z* 199 (88) $[C_{10}H_{15}SO_2]^+$ followed by the loss of a mole of CH_3OH resulting in the *m/z* 167 (64) $[C_9H_{11}SO]^+$ fragment. These



1 $n=10$
2 $n=11$
3 $n=12$



4 $n=10$
5 $n=11$



6 $n=5$
7 $n=7$

predominant fragmentations, therefore, confirm the double-bond positions between C-5 and C-6 and between C-9 and C-10. The other normal-chain $\Delta^{5,9}$ fatty acids were characterized in a similar way. The cis double-bond stereochemistry in these $\Delta^{5,9}$ fatty acids was confirmed by ^{13}C-nmr spectroscopy. The two methylenic carbons adjacent to cis double bonds resonate at a higher field (ca. 26–27 ppm) than those bonded to trans double bonds, which resonate at 31–34 ppm (14). In our fatty acid methyl ester mixture no absorbance between 31–34 ppm was observed with the exception of the C-2 carbons at 34 ppm and the ω-3 carbons at 31.9 ppm, but instead many absorptions in the 25–29 ppm region were detected. Therefore, most of the fatty acids in the mixture have the cis stereochemistry.

The new brominated fatty acids 6-bromo-5,9-heneicosadienoic acid [**4**] and 6-bromo-5,9-docosadienoic acid [**5**] were also identified in trace amounts in *S. helianthus,* in particular in PE and PS. This type of 6-bromo-$\Delta^{5,9}$ diunsaturation has only been identified before in sponges. Methyl esters **4** and **5** presented spectral characteristics very similar to those acids previously reported and this permitted their rapid characterization (5–9). In fact, **4** and **5** are the shorter-chain analogues of the 6-bromo-$\Delta^{5,9}$ fatty acids previously recognized in sponges. For example, methyl 6-bromo-5,9-heneicosadienoate presented an Equivalent Chain Length (ECL) value of 23.10 while methyl 6-bromo-5,9-docosadienoate had an ECL value of 24.11. The gc-ms of the brominated fatty acid methyl esters showed strong mass spectral peaks at *m/z* 74 (due to the McLafferty rearrangement typical of fatty acid methyl esters) and $[M-79]^+$ peaks as molecular ions, indicating facile loss of bromine under electron impact. Other fragmentations of interest were observed either at *m/z* 303 or *m/z* 317 $[M-Br-CH_3OH]^+$, *m/z* 81, and at *m/z* 194 $[C_{14}H_{26}]^+$ or *m/z* 208 $[C_{15}H_{28}]^+$. The gc-ms of the corresponding brominated fatty acid pyrrolidides was critical for identifying the bromine substituent and the double-bond positions. The *N*-6-bromoheneicosa-5,9-dienoylpyrrolidide afforded a strong peak at *m/z* 374 due to the loss of bromine, while a similar peak was observed at *m/z* 388 for *N*-6-bromodocosa-5,9-dienoylpyrrolidide. However, common to all of these pyrrolidides was a base peak at *m/z* 113 resulting from the McLafferty rearrangement, an intense peak at *m/z* 180 $[C_{11}H_{18}NO]^+$ corresponding to a double allylic fragmentation between C-6 and C-9 with the loss of bromine, and a strong doublet of equal intensity at *m/z* 258 and *m/z* 260 due to the same fragmentation with the bromine substituent intact (5–9). Catalytic hydrogenation (PtO_2) of the brominated methyl esters yielded the corresponding saturated fatty acids and this excluded the possibility of any methyl branching. For example, methyl 6-bromo-5,9-heneicosadienoate afforded methyl heneicosanoate and methyl 6-bromo-5,9-docosadienoate afforded methyl docosanoate, as confirmed by gc co-injection with authentic samples obtained from Sigma. These results indicated that

S. helianthus had the C_{21}–C_{22} analogues of the unusual sponge 6-bromo-$\Delta^{5,9}$ acids. The almost trace amounts of these compounds precluded us from unequivocally assigning the double-bond stereochemistry, but from gc retention times and mass spectral data we tentatively assign it as 5*E*,9*Z*.

Two unprecedented ethylene-interrupted triunsaturated fatty acids, namely, 5,9,13-eicosatrienoic acid [**6**] and 5,9,13-docosatrienoic acid [**7**], were also characterized in *S. helianthus*. Again, mass spectrometry was the key to the characterization of these acids. For example, methyl 5,9,13-eicosatrienoate displayed a $[M]^+$ at *m/z* 320 indicating a methyl eicosatrienoate. However, the mass spectrum of the methyl ester displayed a base peak at *m/z* 67 (100) and the characteristic fragment of $\Delta^{5,9}$ fatty acid methyl esters at *m/z* 81 (99), in contrast to the normal mass spectrum of methylene-interrupted methyl trienoates, where the base peak normally occurs at *m/z* 79. Synthesis of the corresponding *N*-5,9,13-eicosatrienoylpyrrolidine was used for locating the double-bond positions. The mass spectrum of this derivative displayed a $[M]^+$ at *m/z* 359 and a base peak at *m/z* 113 $[C_6H_{11}NO]^+$. Prominent fragments were identified at *m/z* 180 (17), resulting from allylic cleavage between C-7 and C-8, and at *m/z* 234 (5) $[C_{15}H_{24}NO]^+$, only possible from a second allylic cleavage between C-11 and C-12. This indirectly determined the double-bond positions to be at $\Delta^{5,9,13}$. The Δ^{13} double-bond position was also confirmed from a 12 mass unit difference between fragments at *m/z* 260 (C-13) and *m/z* 248 (C-12) in the ms of *N*-5,9,13-eicosatrienoylpyrrolidine (11). The 5,9,13-docosatrienoic acid **7** was identified in a similar fashion. Catalytic hydrogenation (PtO_2) of these methyl trienoates afforded only methyl eicosanoate and methyl docosanoate, thus excluding the possibility of methyl branching. The cis double-bond stereochemistry in these $\Delta^{5,9,13}$ fatty acids was also confirmed by ^{13}C-nmr spectroscopy as previously discussed.

Among components present in trace amounts, almost at gc baseline level, we were able to identify two 6-brominated triunsaturated fatty acids, namely, 6-bromo-5,9,x-docosatrienoic acid and 6-bromo-5,9,x-tetracosatrienoic acid. For example, the ms of methyl 6-bromo-5,9,x-docosatrienoate displayed fragmentations at *m/z* 347 $[M-Br]^+$, *m/z* 315 $[M-Br-CH_3OH]^+$, *m/z* 273 $[M-Br-C_3H_6O_2]^+$, and at *m/z* 205 $[C_{15}H_{25}]^+$. The corresponding *N*-6-bromodocosa-5,9,x-trienoylpyrrolidine afforded a strong peak at *m/z* 386 due to the loss of bromine. A similar peak was observed for *N*-6-bromotetracosa-5,9,x-trienoylpyrrolidine at *m/z* 414. These $[M-Br]^+$ ions implied the presence of three double bonds in the acyl chain. Common to all of these pyrrolidides was a base peak at *m/z* 113 due to McLafferty rearrangement, a less prominent peak at *m/z* 180, which corresponded to a double allylic fragmentation between C-6 and C-9 with the loss of bromine, and a strong doublet of equal intensity at *m/z* 258 and *m/z* 260 due to the same fragmentation with the bromine substituent intact. Unfortunately, due to the extremely small amounts of these acids, the third double bond in the chain could not be unequivocally determined from the corresponding pyrrolidide. On biosynthetic grounds it is very likely that the methyl 6-bromo-docosatrienoate is methyl 6-bromo-5,9,13-docosatrienoate. In all marine organisms where these 6-bromo-$\Delta^{5,9}$ fatty acids have been previously identified, the corresponding non-brominated $\Delta^{5,9}$ fatty acids have also been present (5–9).

We also studied the sterols from *S. helianthus* with the help of hplc, 300-MHz ^{1}H-nmr spectroscopy, ms, and comparison with authentic samples. The following sterols were isolated and characterized: 24-methylenecholesterol (11.4%), 24-methylcholesta-5,22-dien-3β-ol (13%), cholesterol (42.4%), and 24-methylcholesterol (28%). It was striking that 24-methylated sterols were the most abundant sterols in the mixture and that no 24-ethylated sterols, such as 24-ethylcholesterol, were observed.

The results presented in this work are not only important from the point of view of

new structures, but also from a biosynthetic and comparative biochemistry standpoint. Our results indicate that the $\Delta^{5,9}$-diunsaturation can be found in marine invertebrates other than sponges, a fact not before recognized in the marine environment. Moreover, *S. helianthus* seems to biosynthesize $\Delta^{5,9}$ fatty acids of between 16 and 23 carbons, in contrast to the greater chain lengths of between 24 and 30 carbons normally predominant in sponges.

The $\Delta^{5,9,13}$ fatty acids reported in this work represent a new class of polyunsaturated fatty acids with the unusual ethylene-interruption, in contrast with the usual cis-methylene-interruption found in nature. A question easily arises as to the biogenesis of these unusual fatty acids, in particular if the Δ^5 double bond is introduced before the Δ^9 double bond or vice versa. What is the biosynthetic origin for these fatty acids? Possible biosynthetic routes are shown below for both the 5,9,13-eicosatrienoic acid and 5,9,13-docosatrienoic acid. The 5,9,13-eicosatrienoic acid could arise from chain-elongation of 9-hexadecenoic acid to 13-eicosenoic acid followed by alternative Δ^5 and Δ^9 desaturations. On the other hand, the 5,9,13-docosatrienoic acid could originate from chain elongation of 9-octadecenoic acid to 13-docosenoic acid followed by either Δ^5 and Δ^9 or Δ^9 and Δ^5 desaturation.

16:0$\rightarrow\Delta^9$-16:1$\rightarrow\Delta^{13}$-20:1$\rightarrow\Delta^{5,13}$- and $\Delta^{9,13}$-20:2$\rightarrow\Delta^{5,9,13}$-20:3
18:0$\rightarrow\Delta^9$-18:1$\rightarrow\Delta^{13}$-22:1$\rightarrow\Delta^{5,13}$- and $\Delta^{9,13}$-22:2$\rightarrow\Delta^{5,9,13}$-22:3

The 6-bromo-$\Delta^{5,9}$ fatty acids presented in this work result from a type of substitution only previously identified in the phospholipids of sponges. As to their origin we can only speculate, but it is likely that they originated from the action of a marine haloperoxidase (15) on the analogous non-brominated fatty acids which were also found in this anemone. In fact, the biosynthesis of 6-bromo-5,9-hexacosadienoic acid was previously investigated by Djerassi *et al.* (6) utilizing radiolabeled precursors, and they concluded that bromination was the terminal step in the biosynthesis of these unusual acids. Therefore, it is possible that the biosynthesis of the brominated acids **4** and **5** follows the biosynthetic routes shown below.

15:0$\rightarrow$21:0$\rightarrow\Delta^5$- and Δ^9-21:1$\rightarrow\Delta^{5,9}$-21:2$\rightarrow$6-Br-$\Delta^{5,9}$-21:2
16:0$\rightarrow$22:0$\rightarrow\Delta^5$- and Δ^9-22:1$\rightarrow\Delta^{5,9}$-22:2$\rightarrow$6-Br-$\Delta^{5,9}$-22:2

Although partially identified in trace amounts, the 6-bromo-5,9,x-docosatrienoic acid and the longer-chain 6-bromo-5,9,x-tetracosatrienoic acid represent a unique class of 6-bromotrienoic fatty acids. Their identification in *S. helianthus* is important since it indicates that bromination in $\Delta^{5,9}$ fatty acids can still take place and be specific for the C-6 position even when three double bonds are either present or to be introduced in the acyl chain. In conclusion, other marine invertebrates will have to be analyzed in order to expand our present knowledge of $\Delta^{5,9}$ fatty acids in the marine environment.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Fatty acid methyl esters were analyzed by gc-ms using a 5972A MS ChemStation (Hewlett-Packard, Palo Alto, CA) equipped with a 30 m×0.25 mm special performance capillary column (HP-5MS) crosslinked with 5% PhMe silicone. The temperature program was as follows: 130° for 2 min, then increased at 3°/min to 270° and maintained for 40 min. The carrier gas was He at a pressure of 10 psi. ^{1}H- and ^{13}C-nmr spectra were recorded on a GE 300 MHz spectrometer.

ANIMAL MATERIAL.—*S. helianthus* was collected in June 1993, near Cayo Enrique, La Parguera, Puerto Rico, at a depth of 60 cm. It was frozen immediately after collection. The anemone was freeze-dried or lyophilized before analysis. A voucher specimen of the anemone is available at the Department of Marine Sciences, University of Puerto Rico, Mayagüez Campus, Puerto Rico.

EXTRACTION AND ISOLATION.—The anemone (50–60 g, dry wt) was carefully cleaned of all debris and cut into small pieces. Extraction with 2×250 ml of $CHCl_3$-MeOH (1:1) yielded the total lipids (ca. 5 g).

The neutral lipids, glycolipids and 30 mg of phospholipids were separated by cc on Si gel (60–200 mesh) using the procedure of Privett *et al.* (16). The phospholipid classes were fractionated by prep. tlc using Si gel 60 and $CHCl_3$-MeOH-NH_4OH (65:35:5) as solvent. Ninhydrin was used to specifically identify phosphatidylethanolamine (PE) and phosphatidylserine (PS), while Dragendorff reagent was used to visualize phosphatidylcholine (PC). The separated phospholipids were scraped off the plate and individually esterified with methanolic HCl.

PREPARATION AND ISOLATION OF FATTY ACID DERIVATIVES.—The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipids with methanolic HCl (17) followed by cc purification eluting with *n*-hexane-Et_2O (9:1). The double-bond positions of the monoenoic and dienoic fatty acids were determined by preparing the corresponding dimethyldisulfide derivatives by dissolving the esters (2 mg) in dimethyldisulfide (0.2 ml) and adding a solution (0.05 ml) of I_2 in Et_2O (60 mg/ml), heating the solution at 50° for 24 h, followed by the standard work-up (18). *N*-Acylpyrrolidide derivatives were prepared by direct treatment of the methyl esters with pyrrolidine-HOAc (10:1) in a capped vial for 93 h at 100° followed by ethereal extraction from the acidified solution and purification by prep. tlc. Hydrogenations were carried out in 10 ml of MeOH and catalytic amounts of PtO_2. Spectral data for the key fatty acids in this discussion follow.

Spectral data for all fatty acid methyl esters from S. helianthus.—^{1}H nmr ($CDCl_3$, 300 MHz) δ 0.85 (3H, t, CH_3CH_2-), 1.23 (m, -CH_2-), 1.59 (2H, m, -CH=CH-CH_2-CH_2-), 2.05 (2H, m, -CH_2-CH=CH-), 2.28 (2H, t, -CH_2-CO_2CH_3), 2.82 (2H, m, -CH=CH-CH_2-CH=CH-), 3.64 (3H, s, CH_3O), 5.35 (m, CH=CH); ^{13}C nmr ($CDCl_3$, 75 MHz), 14.04 (q, W-1), 22.63 (t, W-2), 24.60–24.95 (t, C-3), 26.47–27.32 (t, CH_2-CH=CH), 29.09–29.95 (t, -CH_2-), 31.87 (t, W-3), 34.04 (t, C-2), 51.36–63.78 (q, CH_3O), 126.94–131.92 (d, CH=CH), 174.04–174.32 (s, C-1).

Methyl 5,9,13-eicosatrienoate.—Eims (70 eV) *m/z* $[M]^+$ 320 (0.6), $[M-31]^+$ 289 (0.4), 288 (0.4), 247 (1.6), 235 (5), 221 (7), 219 (5), 215 (2), 205 (11), 163 (13), 147 (11), 141 (14), 135 (23), 133 (14), 131 (10), 121 (35), 109 (38), 107 (22), 95 (37), 93 (43), 91 (42), 85 (10), 81 (99), 79 (79), 77 (25), 67 (100).

N-*Eicosa-5,9,13-trienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M]^+$ 359 (2), 302 (0.2), 288 (0.3), 274 (0.3), 260 (0.3), 248 (0.4), $[C_{15}H_{24}NO]^+$ 234 (5), 220 (0.6), 208 (0.4), 206 (0.5), 194 (0.7), $[C_{11}H_{18}NO]^+$ 180 (17), 166 (1.2), 154 (0.7), 140 (1.4), 127 (3), 126 (13), 113 (100), 105 (1.3), 98 (13), 95 (1.3), 93 (2), 91 (3.2), 85 (9.4), 81 (3), 79 (5), 72 (10), 70 (12), 55 (15).

Methyl 5,9,13-docosatrienoate.—Eims (70 eV) *m/z* $[M]^+$ 348 (1.2), $[M-31]^+$ 317 (1.5), 291 (1.6), 289 (3), 263 (5), 249 (6), 235 (3.5), 219 (7), 205 (7.5), 201 (3.5), 191 (4.5), 189 (4.5), 187 (5.6), 177 (6.2), 173 (8), 155 (12), 149 (16), 145 (14), 135 (27), 131 (15), 123 (16), 121 (38), 109 (38), 95 (66), 93 (60), 91 (63), 81 (87), 74 (23), 69 (44), 67 (100).

N-*Docosa-5,9,13-trienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M]^+$ 387 (1.4), 385 (1.2), $[C_{15}H_{24}NO]^+$ 234 (5), 208 (2), 207 (0.9), 194 (2.5), 182 (2), $[C_{11}H_{18}NO]^+$ 180 (16.6), 168 (2), 154 (3.4), 153 (1), 140 (3.7), 126 (24.5), 113 (100), 98 (19), 93 (5), 91 (8), 85 (9), 81 (6), 79 (10), 72 (20), 70 (24), 55 (21).

Methyl 6-bromo-5,9-heneicosadienoate.—Eims (70 eV) *m/z* $[M-Br]^+$ 335 (19), 315 (5.4), $[M-Br-CH_3OH]^+$ 303 (10), 285 (7), 261 (9), 207 (5), $[C_{14}H_{26}]^+$ 194 (19), 180 (11.4), 165 (20), 161 (16), 159 (16), 149 (25.7), 147 (18), 145 (15.4), 141 (24), 139 (20), 135 (26.5), 123 (16), 121 (25), 119 (26), 109 (40), 105 (30), 103 (32.5), 97 (42.6), 95 (45.8), 91 (58), 81 (74), 74 (42), 67 (71), 57 (53), 55 (100).

N-*6-Bromoheneicosa-5,9-dienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M-Br]^+$ 374 (12.6), 260 (5.4), $[C_{11}H_{17}NOBr]^+$ 258 (5.5), 194 (1.3), $[C_{11}H_{18}NO]^+$ 180 (5.2), 126 (20), $[C_6H_{11}NO]^+$ 113 (100), 98 (18), 85 (11).

Methyl 6-bromo-5,9-docosadienoate.—Eims (70 eV) *m/z* $[M-Br]^+$ 349 (37), $[M-Br-CH_3OH]^+$ 317 (20), 299 (10), 275 (12.4), 233 (6.7), 221 (6), $[C_{15}H_{28}]^+$ 208 (32), 180 (10), 163 (14), 161 (18), 159 (17), 149 (25.6), 147 (16), 145 (12.5), 141 (33.6), 139 (35), 135 (26.6), 129 (4.7), 123 (14), 121 (23), 119 (19.4), 109 (42.4), 105 (19.2), 103 (11), 97 (48), 95 (41), 91 (31), 81 (72), 74 (34.5), 67 (65), 57 (52), 55 (100).

N-*6-Bromodocosa-5,9-dienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M-Br]^+$ 388 (32), 260 (11.8), $[C_{11}H_{17}NOBr]^+$ 258 (11.2), 208 (0.5), $[C_{11}H_{18}NO]^+$ 180 (6.5), 126 (10.4), $[C_6H_{11}NO]^+$ 113 (100), 98 (14), 85 (9), 83 (4), 81 (4).

Methyl 5,9-heneicosadienoate.—Eims (70 eV) *m/z* $[M]^+$ 336 (6), $[M-CH_3OH]^+$ 304 (3), 287 (3), 262 (4), 235 (2), 221 (3), 208 (2), 194 (5), 185 (4), 181 (5), 166 (4), 164 (6), 150 (17), 141 (36), 136 (17), 131 (6), 123 (11), 121 (13), 109 (64), 105 (8), 99 (19), 97 (25), 95 (32), 93 (17), 87 (14), 81 (100), 74 (27), 67 (66), 57 (26), 55 (59).

N-*5,9-Heneicosadienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M]^+$ 375 (2), 233 (2), 180 (21), 140 (2), 126 (17), 113 (100), 98 (13), 85 (9), 78 (4), 72 (10), 55 (15).

2-(4-Methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthiododecan-1-yl)tetrahydrothiophene.—Eims (70 eV) *m/z* $[M-CH_3SH]^+$ 414 (8), $[C_{17}H_{33}S_2]^+$ 301 (7), $[C_{16}H_{29}S]^+$ 253 (44), $[C_{11}H_{19}S_2O_2]^+$ 247 (10), $[C_{13}H_{27}S]^+$ 215 (10), $[C_{10}H_{15}SO_2]^+$ 199 (41), $[C_9H_{11}SO]^+$ 167 (48), $[C_7H_{13}SO_2]^+$ 161 (13), $[C_6H_9SO]^+$ 129 (22), $[C_6H_9O_2]^+$ 113 (25), $[C_5H_5O]^+$ 81 (54), 79 (55), 74 (44), 69 (54), 67 (71), 55 (100).

Methyl 5,9-docosadienoate.—Eims (70 eV) *m/z* $[M]^+$ 350 (8), $[M-CH_3OH]^+$ 318 (4), 301 (4), 276 (5), 262 (1), 249 (2), 235 (4), 221 (1), 208 (5), 194 (2), 181 (6), 164 (7), 163 (6), 154 (6), 150 (19), 141 (37), 140 (18), 136 (19), 135 (14), 123 (12), 121 (13), 109 (62), 107 (12), 99 (18), 97 (24), 95 (30), 93 (17), 87 (9), 81 (100), 74 (21), 67 (67), 57 (24), 55 (59).

N-*5,9-Docosadienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M]^+$ 389 (2), 234 (1), 180 (23), 140 (1), 126 (16), 113 (100), 98 (13), 85 (7), 79 (4), 72 (10), 55 (14).

2-(4-Methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthiotridecan-1-yl)tetrahydrothiophene.—Eims (70 eV) *m/z* $[M]^+$ 476 (4), $[M-CH_3S]^+$ 429 (3), $[C_{18}H_{35}S_2]^+$ 315 (22), $[C_{17}H_{31}S]^+$ 267 (64), $[C_{11}H_{19}S_2O_2]^+$ 247 (27), $[C_{14}H_{29}S]^+$ 229 (15), $[C_{10}H_{15}SO_2]^+$ 199 (88), $[C_9H_{11}SO]^+$ 167 (64), $[C_7H_{13}SO_2]^+$ 161 (23), $[C_6H_9SO]^+$ 129 (26), $[C_6H_9O_2]^+$ 113 (26), $[C_5H_5O]^+$ 81 (69), 79 (56), 74 (36), 69 (52), 67 (70), 55 (100).

Methyl 5,9-tricosadienoate.—Eims (70 eV) *m/z* $[M]^+$ 364 (6), $[M-CH_3OH]^+$ 332 (6), 315 (3), 290 (3), 276 (1), 249 (3), 235 (2), 222 (4), 207 (3), 194 (3), 181 (5), 164 (7), 163 (7), 154 (6), 150 (18), 141 (36), 140 (15), 136 (17), 135 (18), 123 (12), 121 (18), 109 (61), 107 (18), 99 (17), 97 (29), 95 (40), 93 (29), 87 (16), 81 (100), 74 (34), 67 (71), 57 (41), 55 (68).

N-*5,9-Tricosadienoylpyrrolidine.*—Eims (70 eV) *m/z* $[M]^+$ 403 (2), 207 (2), 180 (16), 140 (2), 126 (23), 113 (100), 105 (4), 98 (14), 85 (9), 79 (7), 72 (13), 70 (20), 55 (20).

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