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NATURAL PHOSPHONOLIPIDS

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The literature up to 1998 inclusive on the distribution and methods for isolating and identifying natural phosphonolipids is reviewed.

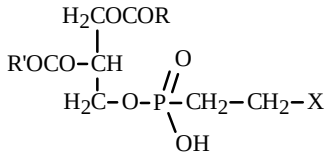
Key words: phospholipids, phosphonolipids, fatty acids.

Phosphonolipids were first found in the single-celled microbes *Tetrahynema pyriformis*, which were isolated from sheep stomach [1, 2]. Then it was discovered that this class of lipids occurs in *Protozoa* [3-11] and is widely distributed among many species of marine animals [12-28], mainly anemone, mollusks, oysters, and sponges. They were also found in sea stars [29]. However, sea stars are known to feed on gastropods and bivalve mollusks, which contain up to 20% phosphonolipids in the total phospholipids (PL) [30, 31]. It was hypothesized that phosphonolipids are not structural components of sea-star organs and tissue but exogenic in origin [29] because they are found primarily in the gastro-intestinal tract and diminish or completely disappear upon fasting [32]. Phosphonolipids were also found in fish [33], certain mammals [34-37], various bacteria strains [38], mice [39], egg yolk [40], insects [41], and other sources [42-45].

Phosphonolipids from plant sources are much less studied. In 1983, Moschidis first observed them in plants, in lipids of apricot-pit nuclei [46]. Until now, the phosphonolipid content of the large number of plants has been studied only sporadically. We found them in lipids of kenaf (*Hibiscus cannabinus*, Malvaceae) and cotton (*Gossypium hirsutum*, Malvaceae) seeds [47-49] and detected them chromatographically in lipids of calendula (*Calendula officinalis*, Asteraceae) seeds [50].

Phosphonolipids that are encountered in nature can be divided into three groups (**A-C**; R, R′ - fatty acid residues):

A. Phosphono-analogs of glycerophospholipids or diacyl-*sn*-glyceryl-3-phosphonates (**1**)



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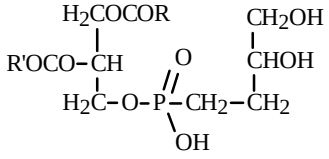
2. X = $-NH_2$ (phosphonophosphatidylethanolamines);

3. X = $-NHCH_3$ (N-methylphosphonophosphatidylethanolamines);

4. X = $-NH(CH_3)_2$ (N-dimethylphosphonophosphatidylethanolamines);

5. X = $-N^+(CH_3)_3$ (phosphonophosphatidylcholines);



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6. Phosphonophosphatidylglycerines;

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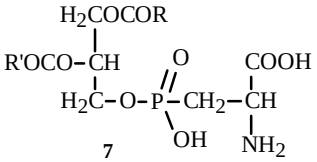

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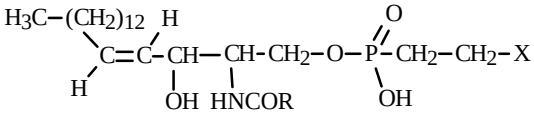
7. Phosphonophosphatidylserines.

B. N-Acylsphingosyl-1-O-(2′-aminoethyl)phosphonates or ceramide aminoethylphosphonates (CAEP)



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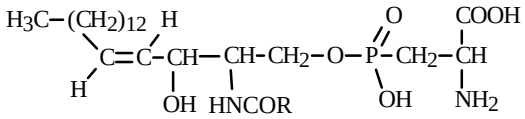
9. X = –NH_2; **10**. X = –$NH(CH_3)$; **11**. X = –$N(CH_3)_2$; **12**. X = $N^+(CH_3)_3$;



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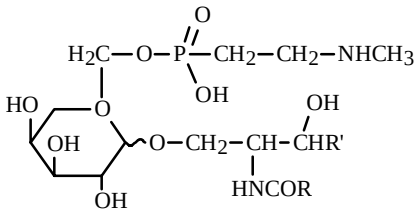
C. Phosphonosphingoglycolipids (PSGL)



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14. 1-O-[6′-O-(N-Methylaminoethylphosphonyl)galactopyranosylceramide.

Alkyl and alkenyl natural phosphonolipids have also been observed in nature. Thus, the Infusoria *Tetrahynema pyriformis* and *Paramecium tetraurelia* yielded 1-O-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)phosphonates along with diacyl type A phosphonolipids; *Entodium caudatum*, 1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)phosphonates [6].

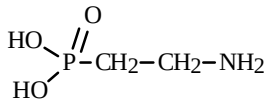
The total phosphonolipids isolated from goat spleen contained dihydro derivatives of **8** [35]. Type C phosphonolipids with five carbohydrate residues were found in several species of marine gastropod mollusks [23]. Phosphonolipids bound to protein were also found by column chromatography in homogenized lipids from marine mollusks [24]. It was established that the protein is bound to **10**. The molecular weight of the protein-phosphonolipid was 86,000. The nerve tissue of the marine anemone *Aplysia kurodai* yielded **8**, which contains the galactose ester of pyruvic acid [25, 26, 51] (Table 1).

The total lipids of biological sources are extracted mainly by the Folch [6, 13, 38, 47, 52, 53] or the Bligh—Dyer [16, 17, 23, 44, 55, 56] method. Phosphonolipids are separated from the corresponding phospholipid analogs using chromatographic methods (column, thin-layer, preparative thin-layer) and identified by the products of base and acid hydrolysis and by spectral analysis [10, 12, 20, 34, 40, 43, 46, 56, 57].

Phosphonolipids were first isolated from *Tetrahynema pyriformis* by ether—ethanol (1:1). The extract was hydrolyzed by boiling HCl with subsequent separation of the hydrolysis products on a Dowex column [1]. The physicochemical constants of one from the decomposition products were consistent with 2-aminoethylphosphonic acid.

TABLE 1. Distribution of Phosphonolipids in Nature

Source	Phosphonolipid	Reference
Simplest		
Tetrahymena pyriformis	2,5- and 1-O-alkyl-*sn*-glycero-3(2-aminoethyl)phosphonates	1, 2, 6, 8, 17,
Paramecium tetraurelia	1-O-Alkyl-*sn*-glycero-3(2-aminoethyl)phosphonates, type A compounds	6
Entodinium caudatum	1-O-Alk-1′-enyl-2-acylglycero-3(2-aminoethyl)phosphonates, **1** and **8**	6, 10, 57, 70
Marine invertebrates		
Anemones (*Anemone*)		
Anthopleura elegantissima	**8**	3, 7, 12, 13, 17, 21
Anthopleura xanthogrammica	**10, 11, 12, 13**	21, 83
Corbicula sandai	**8**	70
Teallia felina	**8**	17, 22
Metridium dianthus	**8**	12, 17, 21, 22
Metridium senile	**8**	21, 28, 68, 69
Aplysia kurodai	**14**	25, 26, 51
Oysters (*Ostrea*)	**8**	20
Sponges (*Spongia*)	**8**	14
Mollusks (*Mollusca*)	**1**, **8**, **9**, **10**, type C with five carbohydrate residues; **8**, bound to protein	10, 19, 23, 24, 30
(various species)		
Fish	**9, 10, 14**	33
Fungi		
Pythium prolatum	**9, 13**	43
Mycobacterium strains	**2**	38
Land snail (*Eobania vermiculata*)	**8, 2**	44
Insects (*Cicada oni*)	**2, 5, 8**	41
Egg yolk	**2, 5**	40
Human sperm	**5**	42
Cardiac and skeletal muscle	**2, 8**	39
Sheep and goat brain	**2, 5, 6, 7, 12**	34
Goat spleen	Dihydro-**8** (ceramide aminoethylphosphonates)	35
Rat liver	**2**	45
Human aorta	**5**	38
Apricot pits	**5** and two unidentified phosphonolipids	46
Kenaf seeds (*Hibiscus cannabinus*)	**2** and three unidentified components	47, 48
Cotton seeds (*Gossypium hirsutum*)	**2** and two unidentified components	47, 49



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The typical solvent systems used in the chemistry of lipids [47, 55] separate lipids into classes. However, they do not separate PL from the phospho-analogs. The separation of mixtures of phospholipids and phosphonolipids has been investigated [56, 58-63]. A lipid layer was separated by the Folch method [23]. Type C phosphonolipids (PSGL) containing 1-3 carbohydrate residues were isolated from the lower layer using column chromatography on silicic acid and iatrobeads. The upper layer yielded PSGL with 3-5 carbohydrate residues using first column chromatography on Sephadex QAE and then on iatrobeads. PSGL are less mobile upon chromatography than the coresponding glycosphingolipids.

In 1983, a methanol—water (2:1) mixture was used to separate synthetic mixtures of phospholipids and phosphonolipids. The PL remain at the origin; phosphonolipids have R_f = 0.8-0.9 [61]. The system was previously used to purify O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine from other lipids [62]. This solvent system was first used for TLC and PTLC of plant phosphonolipids during a study of apricot-pit lipids [46]. For kenaf and cotton seeds, it is practically impossible to observe phosphonolipids in the unpurified lipid fraction. Therefore, the total PL from seeds of these plants that were obtained according to the literature [53] and purified of carbohydrate impurities by gel filtration through G-25 molselect [64] were separated in two solvent systems: hexane—ether (7:3) (1) and acetone (2). The total phospho- and phosphonolipids, which are enriched in glycolipids and pigments, remain at the origin upon chromatography in hexane—ether. The origin band was purified of impurities using PTLC in acetone. The purified total phospho- and phosphonolipids were separated preparatively using CH_3OH—H_2O (2:1) [47].

Kenaf seeds contain 1.1% PL of the total seed mass. Of these, 1.5% are phosphonolipids [65]. Cotton seeds contain 1.6 and 1.3%, respectively [66]; apricot pits, 0.24 and 6.5% [46].

The data presented above demonstrate that the number of plants investigated for phosphonolipid content is small. This may be due to the labor-intensiveness of existing methods for isolating, purifying, and separating them and especially to the low content of this class of compounds in the total lipids or PL.

The phosphonolipid content in biological samples is calculated in various ways. The P–C bond in them is very strong and stable to prolonged treatment with HCl (6M, 110°C, 48 h) and HBr (conc., 125°C, 10 h) [67]. This property of these compounds is also used to determine phosphonates. A sample is subjected to strong acid hydrolysis. The content of phospholipid P in the hydrolysate is determined. The residue is combusted by $HClO_4$. The phosphonic P is determined [8, 17]. The phosphonolipid content is also determined from the total P content relative to the phospholipid P and from the content of **15** in the polar lipids.

Thus, the amount of phosphonolipids in various sources is calculated using various parameters. It varies over wide ranges. For example, the content of **15** in the marine anemone *Metridium* is 1.0-1.56 wt% [12, 17, 21, 22, 68, 69]. In *Anthopleura elegantissima* this quantity reaches 25% [12, 17]. According to the literature [7], the content of type B phosphonolipids in this source is 9.3% of the total lipid mass.

The content of total lipids in the bivalve mollusk *Mytilus galloprovincialis* is 1.27% of the fresh mass [30]. Of these, 61.5% are polar lipids; 11.2%, **8**. In *Entodinium caudatum*, 18% of the lipid P is found in diglyceride aminoethylphosphonate; 6%, in **8** [57, 70]. In *Tetrahymena pyriformis*, the phosphonic P in **15** is 13% of the total P [1, 17]. The ratio of PE:phosphono-PE is 13:1 [8]. Phosphonolipid P in the marine anemone *Tealia felina* is 50% of the total P [17] and 1.8% of the dry mass [22]. Various parts of oyster contain **15** in quantities from 19 to 45% of the total lipids [20]. Various strains of *Mycobacterium* contain 0.76-2.1% phosphonolipids of the total PL [38]; egg yolk, 1% phosphonolipids of total PL, 98.1% of which are phosphono-PE and 1.9%, phosphono-PC [40]. In the fungus *Pythium prolatum*, 16.5% of the lipid P occurs in the fraction of type B compounds [43]. In goat spleen, 3.43% of the total PL are phosphonolipids, 30% of which are type A lipids; 5%, type B [35].

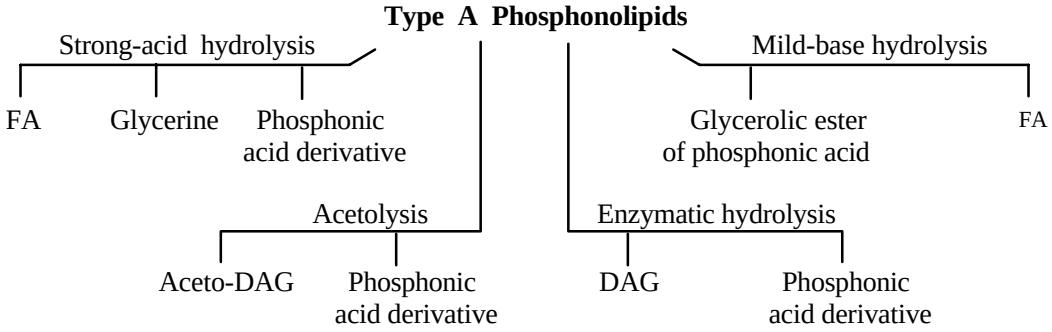
Phosphonolipids, like PL, are developed on TLC by Vaskovskii reagent [71]. There is also a specific reagent, Dittmer's reagent [72], which turns PL brown and phosphonolipids blue.

The IR spectra of phosphonolipids lack an absorption band characteristic of the P–O–C bond [73] and contain bands at 730 and 1180 cm^{-1} [7, 12, 20, 27, 34, 40, 46, 73-75].

The difference between phospho- and phosphonolipids is more evident in NMR spectra. The P–CH_2 protons usually appear at stronger field than those of the P–O–CH_2 group [76]. NMR spectra of L-α-glyceryl-N-methyl-2-aminoethylphosphonate were compared with those of synthetic L-α-glyceryl-N-methyl-2-aminoethylphosphate [77]. It was found that the methylene H atoms of the phosphate and phosphonate appear in different regions. The P–CH_2 protons give a multiplet at 2.72 ppm; the P–O–CH_2 protons, a singlet at 0.89 ppm (water was used as an internal standard).

Decomposition and identification of the resulting products of type A phosphonolipids have been studied [27, 28, 63, 79]. In principle, all methods of decomposing PL, with the exception of cleaving the polar part of the molecule, can also be used for phosphonolipids.

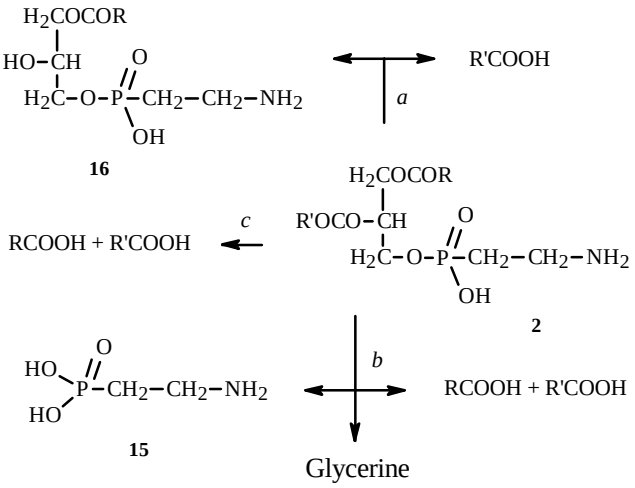
Baer [78] proposed a scheme for studing type A phsophonolipids.



Scheme 1

Enzymatic hydrolysis was performed using phospholipase C; acetolysis, a mixture of acetic anhydride and glacial acetic acid (1:4).

The structure of phosphono-PE isolated from kenaf seeds was investigated using the scheme below.



a - phosphonolipid A_2

b - 6 N HCl, 130°C, oil bath, 24 h

c - 10% KOH/CH_3OH, room temp

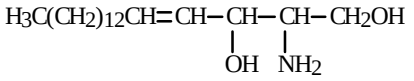
Scheme 2

Fatty acids (FA) were cleaved by mild-base hydrolysis (c). Acid hydrolysis of glycerophosphonolipids, in contrast with PL, does not give amino alcohols (ethanolamine, choline). The P–C bond is not cleaved by strong-acid hydrolysis (b). As a result, the hydrolysis products contain glycerine and **15**. Compound **15** is observed in the free state and as the glyceride or ceramide in certain species of invertebrates and single-celled organisms. It was found that **15** bound to lipid is synthesized before it accumulates in the free state [80].

TABLE 2. Composition of Sphingosine Bases of Various Parts of Oyster (*Ostrea gigas*)

Base, %	Body tissue			
	skeletal muscle	rib	skin	intestines
16:1	75.9	18.4	20.4	10.8
17:1	-	4.1	5.4	4.2
18:1	4.7	25.3	23.0	17.9
19:1	-	12.9	13.1	6.4
18:2	19.3	30.7	29.0	52.3
20:2	-	7.6	8.5	8.1

The structure of type B phosphonolipids was established using oxidative decomposition with subsequent characterization of the products [12, 13, 43, 81]. Acid-catalyzed methanolysis [10] of this type of phosphonolipids formed FA, phosphonic acid derivatives, and sphingosine bases (**17** or **18**) [11, 12, 35, 58, 78].



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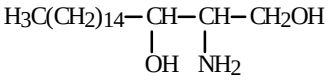

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The hydrolysis products of type B phosphonolipids contained 2-methyl- and 2-dimethyl derivatives of **15** [17, 82].

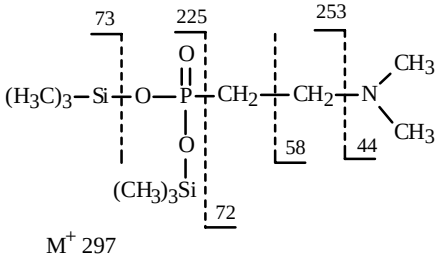
Sphingosine bases were identified by common methods [68, 83-87]. The structures of intact phosphonolipids of this type and their decomposition products are found using advanced mass spectrometric methods, GLC, and GLC-MS [11, 19, 20, 90]. Type B lipids of 15 species of marine animals were studied using mass spectrometry [19]. It was found that peaks with *m/z* 126 and 140 and 124 and 138 are characteristic of **9** and its N-methyl derivatives. The TMS derivatives of long-chain bases obtained via acid-catalyzed methanolysis of **8** from *Pinctada* mollusks were analyzed using GC [10]. Peaks corresponding to C_{18}-sphingosine (77%), C_{18}-dihydrosphingosine (16%), and unidentified compounds (7%) were observed.

The sphingosine bases that were obtained by this method from **8** that was isolated from various parts of oyster were also studied as the TMS derivatives using GC—MS [20] (Table 2).

The position of the double bonds in the sphingosine bases was confirmed by comparing their GC-MS with those of the TMS-polyols obtained from N-acetylsphingosines [88] by oxidation with OsO_4 [89].

Table 2 shows that the components of **8** from various oyster tissues differ markedly in the composition of the sphingosine bases. The principal ones in muscles are the hexadecasphingenines; in the remaining tissues, octadecasphingodienines.

Hydrolysis of type B compounds by phospholipase C produced ceramide and a water-soluble product, phosphono-N,N-dimethylethylamine, which was identified as the TMS-derivative by GC-MS by comparison with the corresponding analog phosphoryl-N,N-dimethylethylamine. It was found that the TMS-derivatives of these two compounds are separated well by GLC and give different mass spectra. The GLC retention time of the silyl derivative of N,N-dimethylphosphonoethanolamine (**19**) lies between those of phosphoryl-N,N-dimethylethanolamine (**20**) and phosphorylethanolamine.



M^+ 297

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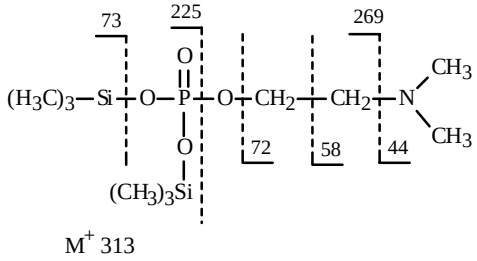

M^+ 313

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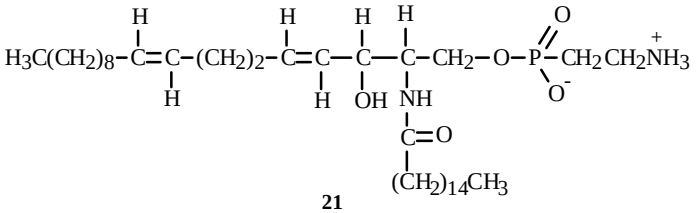
The mass spectrum contains characteristic ions with *m*/*z* 58, 72, 225, and others. The ion with *m*/*z* 314 may belong to the [M + 1] ion or, more likely, to the rearrangement ion corresponding to trimethylsilylphosphate [91].

The composition of the components of **8** that were isolated from *Metridium senile* were studied using base hydrolysis and enzymolysis by phospholipase C [68, 69]. The nonpolar part of the lipid was studied in more detail. It was shown that the principal sphingosine base in this instance is D-erythro-1,3-dihydroxy-2-amino-*trans*-4,*trans*-8-octadecadiene.

The principal molecular form of CAEP from this marine anemone was found to be **21**:



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A complex phosphonolipid (type C) was isolated in 1989 from the nerve tissue of the marine anemone *Aplysia kurodai*. It contains the galactose ester of pyruvic acid. This was the first observation of such a galactose ester as a component of animal sphingolipids. The structure of this complex phosphonolipid was established using MS, ^{1}H NMR, and a study of the permethylation reaction products [25]. Sugars were analyzed by GLC as the TMS or trimethylsilyl-N-acetyl derivatives of the methylglycosides; sphingosine bases, as the TMS derivatives. They were identified as galactosamine, glucose, fucose, and N-acetylgalactosamine. The structure 3,4-O-(1-carboxyethylidene)Galβ1-3Gal-NAc=α1→3(Fuc1→2)(2-aminoethylphosphonyl→6)Galβ1→1 ceramide was proposed. The following sphingosine composition was found: octadeca-4-sphingenin, 41%; antiisononadeca-4-sphingenin, 13%; unidentified, 46%. Pyruvic acid was identified as the 2,4-dinitrophenylhydrazone.

The partial structure of the complex type-C phosphonolipid that contains five carbohydrate residues and was isolated from mollusks [23] was proved using GLC and GC-MS of the partially deuterated acetates of the aldites and TMS derivatives of the carbohydrates and hexosaminephosphonates obtained from acid hydrolysis of the lipid. It was found that the polar part of this type-C phosphonolipid consists of methylaminoethylphosphonates bound to straight-chain and branched carbohydrate chains containing galactose, glucose, fucose, and galactosamine. The lipid contains two aminoethylphosphonates bound to galactose, galactosamine, and 3-O-methylgalactose. The ceramide part includes C_{16}- and C_{18}-sphingosine bases.

The complete FA composition of the phosphonolipids from biological sources, especially animal tissues, has been reported in the literature [20, 10, 23, 25]. The FA composition of phosphonolipids from kenaf and cotton seeds has been studied in detail (Table 3).

Table 3 shows that palmitic acid predominates in the phosphonolipids of oyster and certain species of marine anemone (*A. kurodai*, *A. elegantissima*, and *M. senile*); in fungi, oleinic and linoleic acids. In phosphono-PE of kenaf and cotton seeds, the saturated acids are dominated by 16:0; unsaturated, 18:1. The total FA in **8** of the mollusk *Pinctada martensii* [10] consist almost entirely of hydroxyacids, 2-OH-16:0 and 2-OH-18:0, where the location of the OH groups was determined by GLC-MS. The principal observed peaks had *m*/*z* 286 [M^+], 227 [M - 59], 111, 97, 90, 83, 69, 57, and 43 in addition to 314 [M^+], 255 [M - 59], 111, 97, 90, 83, 57, and 43. The peak with *m*/*z* 90 confirms that the OH group is located on C_2.

In *A. elegantissima*, 82.4% of the total FA content consists of the 16:0 acid [13]; in *M. senile*, the content is 76.5% [21, 22]. According to the literature [68, 69], the fraction of 16:0 acid in **8** of this marine anemone is 52%; 35% of the FA are branched. In PSGL with five carbohydrate residues that are obtained from mollusks, the principal FA are 18:0 and 16:0, where the 18:0 represent 75.9% [23].

In phosphonolipids of the mollusk *Mytilus galloprovincialis*, 48.4% of the FA are C_{16} acids [30].

The positional distribution of phosphono-PE FA in kenaf and cotton seeds was determined by enzymolysis by phospholipase A_2 under conditions usually used for PL [92, 93]. It was found that the overwhelming majority of saturated acids was esterified in the *sn*-1-position (64.2% in phosphono-PE of kenaf seeds; 63.3%, cotton). Unsaturated acids were esterified in the *sn*-2-position (86.2 and 82.0%, respectively). Phosphonolipids and PL form a complex with a snake-venom enzyme, which is necessary for enzymolysis. This complex is a specific substrate for phospholipase A_2. The well known rule that the *sn*-2-position of natural lipids is esterified primarily by unsaturated acids applies also to phosphonilipids of plant origin.

TABLE 3. Fatty-Acid Composition of Phosphonolipids from Certain Natural Sources

Source	Fatty acid											
	12:0	14:0	16:0	16-OH	16:1	17:0	17:1	18:0	18:1	18:2	18:3	uniden.
Compounds **8** from various oyster (*Ostrea gigas*) tissues:												
skeletal muscle			89.7	Tr.		7.0		3.3				
rib			77.0	13.2		4.8		4.7				
skin			76.2	15.1		5.0		3.2				
intestines			77.2	13.7		5.1		4.0				
Compounds **8** from the fungus *Pythium prolatum**		0.3	19.6		0.3			1.6	21.2	21.6	4.7	24.9
Type C phosphonolipids from *Aplysia kurodai*			94.2					5.8				
Type C phosphonolipids from the mollusk *Pinctada martensii***				84.8								3.5
Gastropod mollusks								79.9				20.1
Anemone:												
Anthopleura elegantisima			82.4									
Metridium senile			76.5									
Type C phosphonolipids with five carbohydrate residues from mollusks								75.9				
Compounds **2** from seeds of *H. cannabinus*:												
total	1.1	1.2	30.1		2.1	1.8	1.0	3.0	37.8	18.9	2.3	
sn-1	1.6	2.3	50.7		0.8	2.0	Tr.	7.6	25.0	10.0	Tr.	
sn-2	Tr.	0.5	13.3		2.5	Tr.	2.2	Tr.	50.8	26.8	3.9	
Compounds **2** from seeds of *G. hirsutum*:												
total	3.0	1.5	35.0		2.9			3.0	32.2	22.4	-	
sn-1	4.4	3.0	53.3		0.3			6.6	18.4	14.0	-	
sn-2	2.1	1.1	14.8		4.8			Tr.	43.9	33.0	-	

*In compound **8** from the fungus *Pythium prolatum*, 5.9% of 20:4 acid was found.
**In *Pinctada martensii*, 11.7% of 18-OH acid was found.

The positional type and composition of phosphono-PE in lipids from kenaf and cotton seeds were calculated using data for the positional distribution of FA (Table 4).

Table 4 shows that there are 50 types of phosphono-PE in kenaf seeds; 39, in cotton. The predominant molecular species in both instances are 16:0-18:1 (25.8 and 23.5%), 16:0-18:2 (13.6 and 17.8%), 18:1-18:1 (12.7 and 8.1%), and 16:0-16:0 (7.0 and 7.9%).

The composition of phosphono-PE in lipids of kenaf and cotton seeds is (%): SS, 9.3 (11.4); SU, 57.4 (55.6); US, 5.2 (6.0); and UU, 30.7 (27.0).

It is known that the principal components of *Tetrahymena pyriformis* are PC and phosphono-PE [1, 6]. The FA composition of phosphono-PE has been shown to depend on the cultivation temperature [7]. It was found that more 18:2 (6,11) and 18:3 acids are synthesized at 15°C (Table 5). Increasing the temperature (39.5°C) decreases the relative content of the 18:3 and 16:1 acids. As expected, the *sn*-2-position of the phosphonolipid is esterified mainly by unsaturated acids (92.7% at 15°C; 82.5%, 39.5°C).

TABLE 4. Position and Type of Phosphono-PE in Kenaf and Cotton Seeds

Type	Seeds, %		Type	Seeds, %	
	kenaf	cotton		kenaf	cotton
12:0-12:0	+	0.1	18:2-16:1	0.3	0.7
12:0-14:0	+	+	12:0-17:1	+	-
14:0-14:0	+	+	16:0-17:1	1.1	-
16:0-12:0	-	1.1	18:0-17:1	0.2	-
17:0-14:0	+	-	18:1-17:1	0.5	-
12:0-16:0	0.2	+	18:2-17:1	0.2	-
16:1-16:0	0.1	+	12:0-18:1	0.8	2.0
17:0-16:0	0.3	-	14:0-18:1	1.2	1.3
16:1-16:1	+	+	16:0-18:1	25.8	23.5
17:0-16:0	+	-	16:1-18:1	0.4	0.1
18:0-12:0	-	0.1	17:0-18:1	1.0	-
18:1-12:0	-	0.5	18:0-18:1	3.8	2.9
18:2-12:0	-	0.3	18:1-18:1	12.7	8.1
16:0-14:0	0.2	0.4	18:2-18:1	5.0	6.2
18:0-14:0	+	0.1	12:0-18:2	0.3	1.5
18:1-14:0	0.1	0.2	14:0-18:2	0.6	1.0
18:2-14:0	+	0.2	16:0-18:2	13.6	17.8
14:0-16:0	0.3	0.7	16:1-18:2	0.2	0.1
16:0-16:0	7.0	7.9	18:0-18:2	2.0	2.2
18:0-16:0	1.6	1.0	18:1-18:2	6.7	6.1
18:1-16:0	3.5	2.7	18:2-18:2	2.7	4.9
18:2-16:0	1.4	2.1	12:0-18:3	0.1	-
12:0-16:1	+	0.2	14:0-18:3	0.1	-
14:0-16:1	-	0.1	16:0-18:3	2.0	-
16:0-16:1	1.3	2.6	17:0-18:3	0.1	-
18:0-16:1	0.3	0.4	18:0-18:3	0.3	-
18:1-16:1	0.6	0.9	18:1-18:3	1.0	-
			18:2-18:3	0.4	-

TABLE 5. Composition and Positional Distribution of Fatty Acids in 2-Aminoethylphosphonolipids of Cells of *T. pyriformis* Grown at 15 and 39.5°C

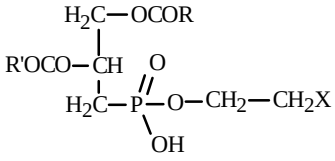
Fatty acid	39.5°C			15°C		
	tot.	*sn*-1	*sn*-2	tot.	*sn*-1	*sn*-2
14:0	5.3	26.0	2.2	4.0	20.1	1.1
16:0	6.8	39.1	3.3	4.2	23.4	1.9
16:1(9)	10.1	7.8	11.7	5.5	14.6	3.7
17:0	5.0	3.2	5.3	2.3	3.7	1.7
18:1(9)	5.0	0.8	6.8	5.7	3.3	6.7
18:2(6, 11)	7.0	0.7	7.9	22.6	3.8	28.1
18:2(9, 12)	10.0	0.8	11.4	14.9	5.9	16.3
18:3(6, 9, 12)	43.3	3.7	44.7	37.4	18.6	37.9

It was concluded that phosphonolipids in *Tetrahymena* cells may act as acceptors of unsaturated acids during thermal acclimatization.

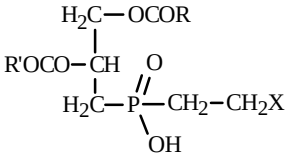
Thus, it is still premature to draw any conclusions from the scarce data on the FA composition of natural phosphonolipids. It can be noted that animal and plant tissues synthesize phosphonolipids with entirely different FA. This may be due to their specific role in membranes.

The chemical synthesis of phosphonolipids with different sets of hydrophobic and hydrophilic constituents is rather simple [94-97]. As a rule, the reaction of glycerides or ceramides with substituted phosphonic acid chlorides is used. Depending on the resulting phosphonolipid, various phosphorylating agents such as N-phthalimidoethylphosphonic acid chloride, bromoethylmetaphosphonate, (2-trimethylammoniummethyl) phosphonate, N,N-dibenzylaminoethylphosphonic acid, and others are used.

Phosphonolipids with a C–P bond between the phosphorus part and the polar lipid head (A—C types) are found in nature. However, phosphonolipids formed from 1,2-propanediol and phosphonic acid at C_3, i.e., 1,2-diacyloxypropyl-phosphonates (**22**) and phosphonic acid analogs with two P–C bonds, i.e., 1,2-diacyloxypropylphosphinates (**23**) can theoretically exist.



22



23

Phosphonolipids of type **22** and **23** have not yet been found in nature. However, they have been synthesized [98, 99] because they are interesting as inhibitors of various phospholipases and as analogs of thrombocyte-activating factors.

The biological role of phosphonolipids has not been clearly defined. Many researchers think that these lipids, like PL, are cell-membrane structural elements and point out their possible special role in membrane permeability [11, 12, 17, 26] and stabilization [100], as if protecting them from the effect of hydrolytic enzymes such as phospholipase and phosphatase. This issue was addressed more directly [101] by supposing that the presence of a P–C bond in the phosphonolipids inactivates namely phospholipase D. According to the literature [102], certain phosphonolipids inhibit phospholipase C and phosphatidylphosphohydrolases. The GI tract of the marine anemone contains many hydrolytic enzymes. It is possible in this instance that the phosphonolipids stabilize to some degree the cell membrane.

Phosphonolipids are a new class of cationic lipids and are considered to be components that facilitate movement of DNA in cells [103, 104]. Activation of phosphatidylinositol-3-kinase is known to be necessary to stimulate cell division. Phosphonolipids, like phosphatides and lyso-phosphatidic acids, were found to be selective inhibitors of this enzyme [105].

Thompson [11] studied the metabolism of phosphonolipids in *Tetrahymena* and found that phosphonolipids are synthesized differently than PL. He concluded on this basis that this class of lipids may fulfill in cells some unique function. Others [23] suggest that complex type C phosphonolipids in mollusk cells may act as receptors.

Thus, the present review shows that phosphonolipids of plant origin are being studied at a slower pace than those from animal sources. The investigations have not been systematic. Perhaps phosphonolipids are present in many plants but have remained unnoticed owing to their low content.

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