FATTY ALDEHYDES OF THE PHOSPHATIDYLETHANOLAMINES OF MARINE INVERTEBRATES

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UDC 577.115

The compositions of the fatty aldehydes from the phosphatidylethanolamines of 26 species of marine invertebrates belonging to nine classes of six types have been investigated. It has been shown that for the aldehydes of all the animals investigated a high degree of saturation and the presence of substantial amounts of branched aldehydes and aldehydes with odd numbers of carbon atoms in the chain of the molecule are characteristic. The amounts of the main – 16:0, 18:0, 18:1, and 20:1 – aldehydes changes appreciably according to the systematic position occupied by the animal; the nature of their distribution can serve to a certain extent as a chemotaxonomic marker. The bryozoan Bugula neritina differs from all the other animals studied by an unusually high content of the 17:0 aldehyde and the almost complete absence of unsaturated aldehydes.

In recent years, interest in lipids with an ether bond has increased considerably. This is connected with the detection of an antitumoral effect of a number of alkyl-containing lipids [1] and the identification of the "platelet activation factor" (PAF) as an alkylacetyl analogue of phosphatidylcholine [2-4]. It is known that the biological activity of these compounds depends to a considerable degree on the structure of the alkyl radicals – the maximum activity is exhibited by compounds with hexadecyl and octadecyl radicals [5, 6].

One of the groups of animal organisms richest in lipids with an ether bond, particularly plasmalogens, is the marine invertebrates [7]. The distribution of plasmalogens over the classes of phospholipids and their relative amounts in representatives of different systematic groups of these animals have been characterized in detail [8-10]. However, the composition of fatty aldehydes has scarcely been studied – there have been only a few fragmentary communications [11-13]. To fill this gap in information on the lipids with ether bonds of marine invertebrates it was decided to investigate the composition of the aldehydes of the phosphatidylethanolamines (PEs) – the plasmogen-containing lipids – of those species of invertebrates that, by their massiveness and availability, can be regarded as a raw material for obtaining biologically active lipids with ether bonds.

We investigated 26 species of marine invertebrates belonging to nine classes of six types. For the investigation we usually took the total soft tissues of the animals. For two large mollusks analysis was carried out separately for the most important organs. A total of more than 30 fatty aldehydes was identified. However, the concentration of many of them rarely exceeded a fractional percentage. Therefore, Table 1 gives information only for the main fatty aldehydes of the animals that are significant from the raw-materials point of view.

It can be seen from Table 1 that marine invertebrates are richest in the 18:0 aldehyde. There was not one species in which the relative amount of this aldehyde was less than 18%, while in the holothurians and some starfish it reached 70% and above. The three aldehydes next in importance were (in decreasing order) the 20:1, 16:0, and 18:1 types – for all of them there are sources containing from 20 to 40-50% of some of these aldehydes. The amount of the 17:0 aldehyde on the whole did not exceed 5-7%, but in the aldehydes of the PEs of the bryozoan Bugula neritina its proportion amounted to 22.6%.

More than 20 aldehydes, information on which has not been given in the table, can be divided arbitrarily into two groups. Characteristic for the first of them – 15:0, i17:0,

Institute of Marine Biology, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 621-625, September-October, 1989. Original article submitted December 7, 1988; revision submitted March 6, 1989.

0009-3130/89/2505-0525$12.50

TABLE 1. Main Aldehydes of the PEs of Marine Invertebrates, % on the Total of All the Aldehydes

Species	16:0	17:0	i18:0	18:0	Σ18:1†	Σ20:1†
Phylum Coelenterata **Class** Anthozoa						
Metridium senile[1]	38,8	5.6	0.9	29,4	11,6	5,5
Cnidopus japonica[1]	15.9	5.2	1.2	43,8	12,8	13,1
Anthopleura sp.[1]	17,0	4,7	1,4	29,4	10,0	21,1
Phylum Sipuncula						
Phascolosoma japonicum[1]	1,3	3,1	3,3	40,2	3,7	32,5
Phylum Arthropoda **Class** Crustacea						
Hemigrapsus sanguineus[2]	19,6	4 8	4,8	45,7	11,7	3,2
Pagurus brachiomastus[2]	29,8	5,5	2,7	29,9	19,7	4,7
Phylum Mollusca **Class** Gastropoda						
Nucella heyseana[3]	17,7	4,1	5,9	26,6	8,1	20,6
Class Bivalvia						
Mytilus edulis	4,0	4,4	5.2	47,8	5,9	15,7
Callista brevisiphonata[3]	5,0	2,3	7,5	30,1	4,8	32,0
Spisula sachalinensis[3]	4,0	1,2	5,1	23,5	2.7	52,5
Peronidia venulosa	9,9	3,9	11,5	36,4	3,5	18.2
Modiolus difficilis, **gills**	5,5	3,7	8,8	47,9	3,3	14,4
Crenomytilus grayanus, **gills**	5,1	3,4	8.1	48,5	4,1	14,6
gonads	4,3	7.0	8.6	49.4	2,3	8,5
Patinopecten jessoensis, **gills**	4,4	1 7	8,7	18,5	2,0	33,1
gonads	5,4	1,4	9,5	20,6	3,6	43,4
muscle	2,4	1,4	9,3	26,7	3,5	47,0
Phylum Echinodermata **Class** Holothurioidea						
Cucumaria japonica[4]	2,8	2,2	—	77.9	1,5	2.2
Cucumaria fraudatrix[4]	3,2	2,4	—	67,6	3,0	—
Stichopus japonicus[4]	0,3	1,0	0,9	75,9	0,1	2,5
Class Asteroidea						
Patiria pectinifera[5]	2,0	2.7	0,6	70,8	0,1	11,0
Lysastrosoma anthosticta[5]	2,7	4,0	3,2	56,5	1,1	17,2
Aphelasterias japonica[5]	3,7	4,6	5,5	45,3	1,5	7,1
Asterias amurensis[5]	4,0	5,3	5.9	61,9	0,4	5,2
Evasterias retifera t.[5]	3.9	4,9	6,0	45,5	3,3	7,5
Class Echinoidea						
Strongylocentrotus nudus[6]	9,8	6,9	3,5	47,5	7,5	8,8
S. intermedius[4]	6 8	4,3	1,6	62,0	5,3	6,1
Scaphechinus mirabilis[6]	1,7	2,3	0,7	48,6	6,6	30,7
Phylum Bryozoa **Class** Gymnolaemata						
Bugula neritina[1]	21,8	22,6	11,5	22,6	1,1	0,4

†Sum of the isomers.
[1]Whole animals; [2]muscle tissues; [3]foot; [4]musculocutaneous sac; [5]liver and gonads; [6]gonads.

ai17:0, xMe17:0,* ai18:0, i19:0; ai19:0, i20:0, 20:0, 19:0, 20:2,† 21:1, 22:0, and 22:1 is the fact that while they were present in low amount or even absent in a number of species, in individual animals the amounts of these aldehydes would reach 5% and more. Thus, 15.1% of the 22:1 aldehyde was found in the PEs of Evasterias retifera tabulata, 7% of the 22:0 aldehyde in the PEs of Aphelasterias japonica, and 8.3% of the i20:0 aldehyde in the PEs of the gills of Patinopecten jessoensis. In addition, the PEs of the whole series of animals contained fairly substantial amounts of the xMe17:0 aldehyde. In the aldehydes of the PEs of Crenomytilus grayanus (gonads), Strongylocentrotus nudus, and P. jessoensis (gills) the amount of this aldehyde reached 4, 5, and 7.1%, respectively, and in the starfish Aphelasterias japonica as much as 12.2%. The amounts of aldehydes of the second group (i14:0, 14:0, xMe14:0, i15:0, ai15:0, xMe15:0, i16:0, ai16:0, xMe16:0, 16:1, xMe17:1, ai20:0, and 21:0) did not exceed 1% in any of the species investigated. The xMe16:1 aldehyde was detected only in PEs of the gills of P. jessoensis, but in an amount of 3.2%.

*Aldehydes branched in mid-chain; xMe designates presence of methyl groups with an unknown number of branch points; 17.0 is number of carbon atoms and number of double bonds in main branch of the chain.
†Total isomeric 20:2 aldehydes.

In order to determine whether, in the isolation of the plasmalogens, there is any sense in preparatively separating the animals into organs, an investigation was made of the composition of the aldehydes of the PEs of the most important organs of two mollusks. As can be seen from Table 1, there were no substantial and regularly expressed differences in the amounts of the fatty aldehydes of the PEs of the organs investigated.

Thus, as can be seen from the results presented above, for the aldehydes of the PEs of all the species of marine invertebrates investigated, without exception, a high degree of saturation and the presence of substantial amounts of branched aldehydes and of aldehydes with odd numbers of carbon atoms in the chain of the molecule were characteristics.

The amounts of the 16:1, 18:0, 18:1, and 20:1 aldehydes in the PEs of marine invertebrates change substantially according to the systematic position occupied by the animal. For the PEs of Anthozoa the presence of all four of these aldehydes in appreciable amounts is characteristic, and for the PEs of Mollusca and Phascolosoma japonicum – the 18:0, 16:0, and 18:1 aldehydes. Echinodermata form a clearly defined group of organisms with the prevalence in their PEs of the 18:0 aldehyde, which reaches the highest concentration in representatives of the class Holothurioidea. The only representative of the class Bryozoa investigated – B. neritina – differs from all the other animals studied by an unusually high content of the 17:0 aldehyde and the almost complete absence of unsaturated aldehydes.

Unfortunately, the limited nature of literature information on the composition of the aldehydes of marine invertebrates does not permit a sufficient comparison of them with the results described in the present paper. Earlier, Joh and Hata [13] investigated the composition of the aldehydes of four species of mollusks and found that for Haliotis discus hannai and Turbo cornutus, which feed on algae, the main ones were the 18:0, 16:0, and 16:1 aldehydes while for the filter-feeders Spisula sachalinensis and Placopecten magellanicus they were the 18:0, 20:1, and 16:0 aldehydes. These results differ somewhat for those obtained for Sea of Japan species (thus, the concentrations of the 16:0 and 16:1 aldehydes in the PEs of the latter do not exceed 17.7 and 1%, respectively), but the 20:1 and 18:0 aldehydes are likewise the main ones for the S. sachalinensis that we investigated as also for the mollusks as a whole. V. M. Dembitskii has determined the composition of the aldehydes of the PEs and phosphatidylcholine of five species of Ophiuroidea [11] and of the starfish Henricia sp. [12]. He found that in the PCs of the Ophiuroidea the aldehydes were represented mainly by saturated and monoenic compounds (16:0, 18:0, 18:1, and 20:1), and in the PEs by monoenic compounds (16:1, 18:1, 20:1, and 22:1). In the lipids of the Henricia sp., in addition to the 16:0 compounds, the main aldehydes were monoenic – 18:1, 20:1, and 21:1. These results are in sharp contrast with those obtained for echinoderms in the present work. Additional investigations are necessary to determine the reasons for such differences in the composition of the aldehydes of White Sea and Sea of Japan echinoderms.

The investigation performed has shown that marine invertebrates can serve as a convenient source for the preparative isolation of phospholipids, especially those with an 18:0 alkyl group, and also with 20:1, 16:0, 18:1, and 17:0 groups. It has also been found that the distribution of these aldehydes in the PEs of marine invertebrates may serve to a certain extent as a chemotaxonomic marker for these animals.

EXPERIMENTAL

The animals were gathered in August-September, 1987 and 1988, in Starka Strait, Peter the Great Bay, Sea of Japan. The lipids were extracted by the method of Bligh and Dyer [14]. For TLC we used plates with a layer of silica gel firmly fixed by a silicic acid sol [15]. The DMA and the fatty acid methyl esters were analyzed by GLC on a Shimadzu GC-9A chromatograph with a flame-ionization detector and a Chromatopac C-R3A data-processing station (Japan). Capillary quartz columns, 25 m, with FFAP and Silar-5CP (180°C). The carrier gas was helium (40 ml/min) and the flow splitter gave a ratio of 1:60.

Isolation of the PEs. The PEs were isolated by preparative TLC on plates with a double amount of silica gel in the chloroform–methanol–28% ammonia–benzene (60:20:4:2) system after preliminary chromatography in acetone. The PE zone was transferred to a microcolumn and the PEs were eluted with 10 ml of ethanol. The eluate was evaporated in a rotary evaporator, and the residue was dissolved in 1 ml of benzene. The aldehydes were analyzed in the form of dimethyl acetals (DMAs).

Preparation of the DMAs. The DMA were obtained by the method of Carreau and Dubacq [16] and were purified by TLC in o-xylene or toluene [17]. The DMA zone was collected in a micro-

column and was eluted with 5 ml of chloroform. The chloroform was evaporated off and the DMAs were dissolved in a minimum amount of hexane.

The DMAs were identified from their relative retention times with the aid of the "carbon number" method [18, 19]. The DMAs were separated according to their degree of unsaturation by TLC in benzene on plates impregnated with silver nitrate.

The catalytic hydrogenation of the unsaturated DMAs was effected by passing hydrogen through 2 ml of a solution of the DMAs in methanol–benzene (1:1) in the presence of 2 mg of PtO_2 for 1 h.

The conversion of the DMAs into fatty acid methyl esters was carried out mainly as described in [20], in the following way. The DMAs obtained from the polar lipids of the starfish Asterias amurensis as described above were dissolved in 1 ml of 90% acetic acid, and the mixture was treated with 0.1 ml of 1 N hydrogen chloride in methanol and was heated in a sealed tube in a drying chest at 90°C for 1 h. After the reaction, the mixture was cooled and was treated with 2 ml of water, and the free aldehydes formed were extracted with 3-ml portions of hexane. The hexane was evaporated off, the residue was dissolved in 0.5 ml of 1 N sodium methanolate and, after the addition of 0.5 ml of a saturated methanolic solution of silver nitrate and 1.5 ml of methanol (a brown precipitate deposited), the mixture was stirred periodically for one and a half hours at room temperature. After this, 2 ml of water was added to it together with a few drops of concentrated hydrochloric acid to pH 3. The free fatty acids formed were extracted with three 3-ml portions of hexane. The hexane was evaporated off and the free fatty acids were dissolved in 1 ml of benzene and were methylated with 3 ml of a 1 N solution of hydrogen chloride in methanol as described previously [16].

The author expresses his graditute to N. V. Naumenko (DVGU [Far Eastern State University], Vladivostok) for supplying the procedure for converting the dimethyl acetals into fatty acid methyl esters and to V. E. Vas'kovskii for participation in a discussion of the results.

SUMMARY

1. The composition of the fatty aldehydes of the phosphatidylethanolamines of 26 species of marine invertebrates belonging to nine classes of six types has been investigated. It has been shown that for the aldehydes of all the animals investigated a high degree of saturation and the presence of substantial amounts of branched aldehydes and of aldehydes with odd numbers of carbon atoms in the molecular chain are characteristics.

2. The level of the main – 16:0, 18:0, 18:1, and 20:1 – aldehydes varies considerably according to the systematic position occupied by the animal, and the nature of the distribution may to a certain extent serve as a chemotaxonomic marker.

3. The bryozoan Bugula neritina differs from all the other animals studied by an unusually high content of the 17:0 aldehyde and the almost complete absence of unsaturated aldehydes.

LITERATURE CITED

1. H. K. Mangold and F. Paltauf, Ether Lipids: Biochemical and Biomedical Aspects, Academic Press, New York (1983), p. 277.
2. C. A. Demopoulos, R. N. Pinckard, and D. J. Hanahan, J. Biol. Chem., 254, No. 19, 9355 (1979).
3. M. Chignard, J. P. Le Couedic, M. Tence, B. B. Vargaftig, and J. Benveniste, Nature (London), 279, No. 5716, 799 (1979).
4. M. L. Blank, F. Snyder, L. W. Byers, B. Brooks, and E. E. Muirhead, Biochem. Biophys. Res. Commun., 90, No. 4, 1194 (1979).
5. K. Yu. Gordeev, G. A. Serebrennikova, and R. P. Evstigneeva, Bioorg. Khim., 10, No. 12, 1589 (1984).
6. F. Snyder, Medical Res. Rev., 5, No. 1, 107 (1985).
7. S. Chapelle, Comp. Biochem. Physiol., 88B, No. 1, 1 (1987).
8. V. M. Dembitskii and V. E. Vas'kovskii, Biol. Morya, No. 5, 68 (1976).
9. V. M. Dembitskii, Biol. Morya, No. 5, 86 (1979).
10. V. M. Dembitskii, Zh. Evol. Biokhim. Fiziol., 21, No. 1, 70 (1985).
11. V. M. Dembitskii, Khim. Prir. Soedin., No. 5, 547 (1986).
12. V. M. Dembitskii, Bioorg. Khim., 13, No. 3, 409 (1987).
13. Y. G. Joh and M. Hata, Han'guk Susan Hakhoe Chi, 12, No. 3, 181 (1979) [Chem. Abstr., 93, Ref. 92070u (1980)].

14. E. G. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, No. 8, 911 (1959).
15. B. T. Belen'kii, E. S. Gankina, L. S. Litvinova, I. I. Efimov, V. E. Vas'kovskii, S. V. Khotimchenko, and V. P. Dikarev, Bioorg. Khim., 10, No. 2, 244 (1984).
16. J. P. Carreau and J. P. Dubacq, J. Chromatogr., 151, No. 3, 384 (1978).
17. G. R. Jamieson, J. Chromatogr. Sci., 13, 10, 491 (1975).
18. J. Flanzy, M. Boudon, C. Leger, and J. Pihet, J. Chromatogr. Sci., 14, No. 1, 17 (1976).
19. F. T. Gillan, J. Chromatogr. Sci., 21, No. 7, 293 (1983).
20. G. V. Marinetti, Lipid Chromatographic Analysis, Marcel Dekker, New York, Vol. 3 (1976), p. 901.

GC–MS ANALYSIS OF HYDROXY AND EPOXY ACIDS FROM SEED OIL OF Hippophaë rhamnoides

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UDC 543.51+547.915

By the GC–MS method using a packed column, in the mixture of TMS derivatives of hydroxy acid methyl esters obtained from the seed oil of the sea buckthorn, in ten chromatographic peaks (CPs) 13 monohydroxy compounds have been characterized by their mass spectra, the main ones being derivatives of dimorphecolic and coriolic acids. Structures are proposed for four dihydroxy acids of the C_{17}–C_{20} series. The mixture of methyl esters of di- and tetra-TMS derivatives obtained from the mixture of epoxy acids from the same source has been analyzed by a similar method. In seven CPs, 11 compounds, reflecting the presence of nine epoxy acids in the initial mixture, have been characterized by their mass spectra. The main component of the mixture was 15,16-epoxyoctadeca-9,12-dienoic acid.

Having made use of the results of high-resolution mass spectrometry, metastable defocusing spectra, and known laws of the fragmentation of the TMS derivatives of oxidized fatty acids under electron impact (EI) [1], we predicted the presence of 20 hydroxy acids with chain lengths of C_{13}–C_{23} [2] and 14 C_{16}–C_{18} epoxy acids [3] in certain fractions of the seed oil of Zeravshan sea buckthorn. In the present work we have attempted to check the compositions of these fractions by chromato-mass spectrometry (GC–MS) using a packed chromatographic column.

The GC–MS analysis of hydroxy and dihydroxy acids has been performed previously in the study of the composition of a number of fractions of apple cutin [4], the products of the autooxidation of soybean oil [5], and other biological materials [6], but the authors of the papers referred to mentioned as the main disadvantage of the method used the incompleteness of the separation of the components.

Figure 1a shows a chromatogram of a fraction of TMS derivatives of the methyl esters of the hydroxy acids (I-XVII in Table 1) of sea buckthorn seed oil. Table 1 includes information on the mass numbers of the molecular and characteristic fragmentary ions and on the structures of the individual components of the mixture. The mass-spectral results are based on an analysis of the mass spectra recorded at the moment of reaching the apex of the corresponding chromatographic peak. On the basis of the mass spectra of ten chromatographic peaks (CPs) (Fig. 1) 17 compounds [13 derivatives of monohydroxy acids (I-XIII) and 4 derivatives of dihydroxy acids (XIV-XVII)] were detected, the presence of only 8 of them having been predicted in [2]: (I), (IV), (VI), (IX), (X), (XI), (XII), and (XVI), among which the main

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 626-634, September-October, 1989. Original article submitted December 14, 1988; revision submitted February 16, 1989.

0009-3130/89/2505-0529$12.50