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COMPOSITION OF LIPIDS, FATTY ACIDS, AND ALDEHYDES OF THE

HYDROID "CROSS" MEDUSA Conionemus vertens

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The composition of the lipids, fatty acids, and aldehydes of the hydroid "cross" medusa C. vertens has been investigated. It has been shown that the free sterols are the main components of the phospholipids of the medusa and 78% of them consists of cholesterol. Phosphatidylethanolamine is the main phospholipid of the medusa and 74.4% of it is represented by the plasmalogen form. The amount of phosphatidylcholine is 1.7 times less than that of phosphatidylethanolamine and it contains no plasmalogen form. The 22:4ω6 and 22:5ω3 acids are the main polyenic acids of the medusa. More than 55% of its fatty aldehydes consists of saturated molecular species, the main ones being the 18:0 and 20:1 aldehydes.

The Coelenterata are widely distributed in nature and form an important component of marine ecosystems. The lipids of the Coelenterata have been little studied, however; in the main, information relates to representatives of the class of Anthozoa — actinians and reef-forming corals. There is considerably less information on the composition of the lipids of representatives of other classes of Coelenterata — Hydrozoa, Scyphozoa, and Cubozoa [1]. A paper by É. Ya. Kostetskii [2] has recently appeared which is devoted in part to a comparative analysis of the phospholipids of hydroid and scyphoid Coelenterata. Unfortunately, the author gives quantitative information of the composition of the phospholipids for only five out of the 31 species studied.

In the present paper we describe the results of an investigation of the lipids of the hydroid "cross" medusa Conionemus vertens, which is a characteristic poisonous representative of the hydroid Coelenterata [3].

The micro-TLC of the lipids of C. vertens in a system for neutral lipids showed that the medusa was rich in sterols and contained only a small amount of triacylglycerols. A similar composition of these lipids is known for several representatives of the Coelenterata. Thus, the neutral lipids of the siphonophore Physalia physalis contained 26% of triacylglycerols and 42% of cholesterol [4], while in the medusa Cyanea capillata the triacylglycerols amounted to 14% and the sterols to 47% of all the neutral lipids [1]. Judging from the size and intensities of the spots on a chromatogram, in C. vertens the relative amount of triacylglycerols was even lower. The neutral lipids of C. vertens also contained sterol esters, alkyl glyceryl ether, and free fatty acids. The GLC of the free sterols of the medusa showed that they contained 78% of cholesterol.

Below we give the composition of the phospholipids of C. vertens (% on the lipid phosphorus):

PC	23.3 ± 1.0*	PI	1.7 ± 0.9
PE	31.9 ± 0.8	DPG	2.5 ± 0.7
CAEP	21.2 ± 1.4	X_1	2.1 ± 0.5
PS	16.8 ± 3.4	X_2	0.6 ± 0.5

The micro-TLC of the medusa lipids in systems for polar lipids and the results of the qualitative and quantitative determination of the phospholipids showed that the main polar lipids of the "cross" medusa were phosphatidylethanolamine (PE), phosphatidylcholine (PC), and ceramide aminoethylphosphonate (CAEP). These lipids are the main ones for representatives of a large number of species of Coelenterata [1, 2, 5, 6]. However, their ratio in the lipids of C. vertens differed appreciably from their ratio in the lipids of other species of Coelenterata. Thus, in the lipids of animals of this type the amount of PCs is generally one-and-a-half times as great as that of PE [1, 2, 5-9]. In C. vertens the opposite ratio was observed (1.7 times more PEs than PCs). Appreciable amounts of CAEP are found among the lipids of Coelenterata [1, 2, 5]. The amount of CAEP in the C. vertens lipids, although it was close to the mean value derived by É. Ya. Kostetskii and Yu. A. Shchipunov for Coelenterata [5], was almost twice the amount in the lipids of a number of hydroids [2]. The amount of PS in the lipids of C. vertens also considerably exceeded its level in the majority of other hydroid and scyphoid Coelenterata [2]. Only Mason [7] has found such a high level in the lipids of the actinian Metridium senile. The absence of phosphatidic acid in the lipids of C. vertens must be pointed out, while it was detected in all the species investigated by É. Ya. Kostetskii [2]. Earlier, no phosphatidic acid had been found in M. senile [7] or in the scyphoid medusa Pelagia noctiluca [6]. The latter did not contain either PS or PI, either, although it had a fairly high level of DPG (9.1%).

The reaction micro-TLC of the lipids of C. vertens showed that 74.4% of the phosphatidylethanolamine consisted of the plasmalogen form, while the PC contained no plasmalogen. Rouser et al. [9] have shown previously that the PC and PE of the actinian Anthopleura elegantissima contains alkenylacyl forms [9], and V. M. Dembitskii and V. E. Vaskovskii showed that 73% of the phosphatidylethanolamine of the actinian M. senile fimbriatum is plasmalogenic [10].

Nakhel et al. [6] detected a high level of phospholipids with alkyl bonds in the medusa P. noctiluca: 62% of the phosphatidylcholine and 55% of the phosphatidylethanolamine of the medusa were represented by alkylacyl forms [6]. In order to determine the presence of alkylacyl forms in the phospholipids of C. vertens, a lipid extract was treated successively with acid and with sodium methanolate. No lysophospholipids were detected among the reaction products. This means that the phospholipids of C. vertens contain practically no forms with alkyl bonds, although they were detected in the neutral lipids.

The lipids of C. vertens included one which was obviously a cerebroside. This followed from its chromatographic behavior on micro-TLC and its specific reaction on detection by the reagent for glycolipids. Cerebrosides have been found previously in small amounts in the lipids of Metridium senile fimbriatum, Anthopleura sp., Aurelia aurita [11], and M. senile [7].

As analysis of the fatty acids by the GLC method showed, the lipids of C. vertens contained substantial amounts of the 18:0 and 18:1 acids (Table 1). The main polyenic acids of the cross medusa were the 22:4ω6 and 22:5ω3 long-chain fatty acids. These acids have been described previously as the main ones for only two species of hydroid medusae Nemopsis bachei and Maeotias inexpectata [1], while the siphonophore P. physalis — a peculiar symbiont of polypoid and medusoid individuals belonging to the same class of Hydrozoa — had the usual composition of fatty acids with eicosapentaenoic, docosahexaenoic, and arachidonic acids as the main polyenic components that is characteristic for the majority of marine organisms, including scyphoid medusae [1, 4]. These acids were also present in appreciable amounts in C. vertens. The acid X_1 was probably one of the iso-16:1 acids. These acids have been detected previously in marine animals, including Coelenterata [1, 4, 12, 13].

The information on the composition of the fatty aldehydes of C. vertens is the first not only for the class of Hydrozoa but also for the whole type of Coelenterata. Characteristic for the fatty aldehydes of C. vertens is a high content of saturated representatives (more than 55%) and the presence of branched aldehydes with 17-21 carbon atoms in the chain.

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*Mean value and standard deviation, n = 3.

TABLE 1. Composition of the Fatty Acids and Aldehydes of the Total Lipids of C. vertens*

Chain length, number and position of double bonds	Fatty acid	Fatty aldehyde	Chain length, number and position of double bonds	Fatty acid	Fatty aldehyde
	%			%	
12:0	0,2	—	18:1ω6	—	0,8
14:0	0,6	0,1	18:2ω6	1,2	—
14:1	—	0,1	i19:0	0,3	—
i15:0	0,2	0,2	ai19:0	0,3	—
ai15:0	0,1	—	19:0	0,8	0 8
15:0	0,2	0,2	X₂	—	1,2
15:1	0,2	0,1	20:0	3,6	1,1
i16:0	0,2	0,2	20:1ω9+ω7	2,2	25,6
16:0	8,5	10,3	20:2	0,3	0.6
16:1	2,4	1,0	ai21:0	-	2.5
X₁	1,9	—	20:4ω6	7,0	—
i17:0	0,1	0,7	20:4ω3	0,3	—
ai17:0	0,2	0,6	20:5ω3	9,1	—
17:0	0,5	2,0	22:1	0,9	—
17:1ω8	0,7	—	22:4ω6	18,8	—
17:1ω6	2,3	—	22:5ω3	11,6	—
i18:0	0,2	1,0	22:6ω3	4,0	—
18:0	10,8	39,1	Unidentified	0,9	0,7
18:1ω9+ω7	9,4	10,5			

*Components in amounts of ≥0.1% are given.

The 18:0 and 20:1 aldehydes of the cross medusa are the main ones, making up about 65% of all the aldehydes of the animal. The limited nature of literature information on the composition of the fatty aldehydes of marine invertebrates [14-16] does not permit the recognition of any regular differences whatever in the composition of the aldehydes of C. vertens. However, a feature common for the composition of the fatty aldehydes of C. vertens and of species investigated earlier may be noted — the high degree of their saturation.

Thus, it is possible to state that the composition of the lipids of C. vertens has a number of peculiar features: a ratio of PCs, PEs, CAEP, and PSs different from that in all the other hydroids investigated, an unusually high content of the 22:4ω6 and 22:5ω3 acids, and a high degree of saturation of the fatty aldehydes.

EXPERIMENTAL

The medusae were collected in August, 1987, in Amur Bay (Peter the Great Bay, Sea of Japan). The lipids were extracted by the method of Bligh and Dyer [17] and were separated on plates for micro-TLC with a layer of silica gel strongly fixed by a silicic acid sol [18]. The polar lipids were chromatographed in the systems chloroform—methanol—28% ammonia—benzene (65:30:6:10) (first direction) and chloroform—methanol—acetone—acetic acid—water—benzene (70:30:5:4:1:10) (second direction) [19]. Reaction micro-TLC was carried out as described previously [20]. The neutral lipids were separated in the hexane—diethyl ether—acetic acid (85:15:1) system. For the nonspecific detection of the lipids we used a 10% solution of sulfuric acid in methanol or ethanol followed by heating. The lipids were identified by comparison with authentic samples (the lipids of egg yolk were taken as a sample) and with the aid of specific reagents: for phospholipids [21], for phosphorus-containing components [22]; for amino-containing lipids (0.2% ninhydrin in acetone); for choline-containing lipids [23]; and for glycolipids [24]. Lipid phosphorus was determined by the method of Vas'kovskii et al. [21]. TLC of the products of acid and alkaline hydrolysis was conducted as described previously [25] using the systems described above for polar lipids and the lower phase of the chloroform—diethyl ether—acetone—28% ammonia (90:30:15:15) system for the detection of alkylmonoglycerides.

The fatty acid methyl esters, dimethyl acetals, and free sterols were analyzed by GLC on a Shimadzu GC-9A chromatograph with a Chromatopak C-R3A data-processing stage. Quartz capillary columns, 25 m, with FFAP (177°C — for fatty acid methyl esters and dimethyl acetals) and with OV-101 (240°C — for fatty acid methyl esters; 280°C — for sterols). The carrier gas was helium and the flow split 1:60.

The free sterols were isolated by preparative TLC in the system for neutral lipids. Cholesterol was identified by comparison with an authentic sample (Serva, FRG). Fatty acid methyl esters and dimethyl acetals were obtained by a known method [25]. They were purified by preparative TLC in benzene [26]. The fatty acids and aldehydes were identified by comparison with standard palmitic and stearic acids and by using the "carbon number" method [27, 28]. For the identification of the fatty aldehydes we used, additionally, their catalytic hydrogenation, which was carried out in 2-3 ml of methanol—benzene (1:1) in the presence of 5 mg of PtO_2 with stirring for 1 h.

SUMMARY

1. The composition of the lipids, fatty acids, and aldehydes of the hydroid medusa Conionemus vertens has been investigated. It has been shown that the free sterols of C. vertens are the main components of its neutral lipids and these contain 78% of cholesterol. PE is the main phospholipid of the medusa and contains 74.4% of the plasmalogen form. The amount of PC is 1.7 times less than the amount of PE and it does not contain the plasmalogen form.

2. The main polyenic acids of C. vertens are the 22:4ω6 and 22:5ω3 acids.

3. More than 55% of the fatty aldehydes of the medusa is represented by their saturated molecular forms. The 18:0 and 20:1 aldehydes are the main ones, making up about 65% of all the aldehydes of the animal.

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FLAVONOIDS OF Thermopsis alterniflora.

CROTONOYLTHERMOPSOSIDE AND CROTONOYLCOSMOSIIN — NEW ACYLATED
FLAVONE GLYCOSIDES

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From the epigeal part of Thermopsis alterniflora Rgl.et Schmalh. (Fabaceae),
in addition to formononetin, ononin, cynaroside, and rothindin, two new
acylated flavone glycosides have been isolated and, on the basis of chemi-
cal transformations and spectral characteristics, their structures have
been established as 4',5,7-trihydroxy-3'-methoxyflavone 7-O-(6''-O-croto-
noyl-β-D-glucopyranoside) and 4',5,7-trihydroxyflavone 7-O-(6''-O-crotonoyl-
β-D-glucopyranoside).

Thermopsis alterniflora Rgl. et Schmalh. (family Fabaceae) is a medicinal plant and is
used for obtaining cytisine [1]. The flavonoids chrysoeriol, thermopsoside, genistin,
and teralin have been obtained from this plant previously [2, 3].

In an investigation of the flavonoids of the epigeal part of T. alterniflora, in addi-
tion to chrysoeriol, thermopsoside, and genistin we have isolated another six compounds
(I-VI).

Compounds (I-III) were identified on the basis of IR, UV, PMR, and mass spectra and
by comparison with authentic samples.as, respectively, formononetin [4], ononin [4], and
cynaroside [5].

Compound (IV), according to its spectral parameters, was an isoflavone glycoside.
On acid hydrolysis, glycoside (IV) was split with the formation of an aglycon, identified
as pseudobaptigenin (7-hydroxy-3',4'-methylenedioxyisoflavone), and D-glucose. A study
of the mass and PMR spectra of the tetraacetate of (IV) and a comparison of its physico-
chemical properties with those given in the literature showed that the substance isolated
was a monoglycoside identical with rothindin. In the present paper we consider the struc-
ture of the two new acylated flavone glycosides (V) and (VI).

The absorption maxima at 254, 259.5, and 351 nm observed in the UV spectrum of com-
pound (V) showed that it was a flavone derivative [7]. This was confirmed by the forma-
tion of chrysoeriol (4',5,7-trihydroxy-3'-methoxyflavone (VII) [2]) on the acid hydrolysis
of glycoside (V). In addition to chrysoeriol the hydrolysate was found to contain D-
glucose and crotonic (trans-β-methylacrylic) acid.

In the IR spectrum of compound (V), in addition to the absorption bands of the func-
tional groups characteristic of the flavonoid class, there was an absorption band at 1719
cm⁻¹ showing the presence of an ester function in the molecule. Treatment of glycoside
(V) with a 0.5% solution of potassium hydroxide led to the formation of thermopsoside
(chrysoeriol 7-O-β-D-glucopyranoside (VIII) [3]) and crotonic acid.

When compound (V) was acetylated with acetic anhydride in pyridine, the pentaacetyl
derivative (IX) was formed. The mass spectrum of the latter had the peak of the molecular