ALPHA-GLYCERYL ETHERS IN COELENTERATES

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The nonsaponifiable lipids were isolated from a number of marine coelenterates. The nonsaponifiable fraction ranged from 3.6% of the total lipids in the coral *Porites porites* to 26% in the gorgonian *Pseudoplexaura acerosa*. The α-glyceryl ether content, determined by periodate oxidation, ranged from 7% of the nonsaponifiable matter in the sea anemone *Metridium senile* to 38% in the alcyonacean *Nephtea*.

Kind and Bergmann (1) were the first to establish the presence of α-glyceryl ethers in lower marine invertebrates. They isolated batyl alcohol from the gorgonian *Plexaura flexuosa* from Bermuda.

As part of a study of the comparative biochemistry of coelenterates (2) we have analyzed a number of these organisms and two symbiotic algae which occur in sea anemones for α-glyceryl ethers.

The chemistry and biology of ether lipids have been reviewed recently (3, 4). Glyceryl ethers occur in nature uncombined, as fatty acid esters and as components of phosphatides. They have been examined for effects on growth, hematopoiesis, hemolysis, radiation sickness, and wound healing, but the significance of the results of these studies is not clear.

MATERIALS AND METHODS

The specimens analyzed for α-glyceryl ethers included the following marine organisms collected in the areas indicated in parentheses after the name of the organism. Gorgonians: *Eunicea laciniata* (Florida), *E. tourneforti* (Bimini), *Gorgonia flabellum* (Bimini), *G. ventalina* (Bermuda), *Muricea atlantica* (Bermuda), *Muriceopsis flavida* (Bimini), *Plexaura homomalla* (Bermuda), *Pseudopterogorgia americana* (Bermuda), *Ps. acerosa* (Bimini), *Pterogorgia anceps* (Bimini), *Rumphella antipathes* (Eniwetok), Zooxanthellae (symbiotic algae) from the sea anemone *Anthopleura elegantissima* (San Juan Islands, Washington), Zoochlorellae from *Anthopleura elegantissima* (San Juan Islands). Sea anemone: *Metridium senile* (San Juan Islands). Stony coral: *Porites porites* var. *furcata* (Bimini). Soft coral: *Nepthea* sp. (Eniwetok).

The zooxanthellae, the zoochlorellae and *Metridium* were obtained in April and May. All the other organisms were collected in June and July. The zooxanthellae and zoochlorellae were frozen after isolation and then placed in alcohol. The watery *Metridium* and *Nephthea* were cleaned, cut into small pieces, and dried at 65 C in an oven. The relatively dry gorgonians were dripdried in the sun and then placed in an oven at 65 C for complete drying. The dried animals, in lots of five or more specimens, were ground to a powder in a Waring Blendor.

Total lipids were extracted from the dried ground organisms in a Soxhlet extractor for 48 hr with chloroform-methanol, 1:1. The nonsaponifiable lipids were isolated by ether extraction following acetolysis (4, 5), and saponification with excess methanolic potassium hydroxide. Water was removed from the lipid fractions by distillation with benzene.

The glyceryl ether content was determined by periodate oxidation (6).

The glyceryl ethers were isolated by thin-layer chromatography of the nonsaponifiable lipids on Silica Gel G activated at 110 C for 45 min just before use; hexane: isopropyl alcohol: ammonia (250:50:1) served as developer and authentic chimyl alcohol as the glyceryl ether standard.

Thin-layer chromatography was done on 8 x 2-inch glass plates coated with 0.25 mm Silica Gel G. A thin line of nonsaponifiable lipids dissolved in benzene was applied to the plates. On the same plate a separate sample of authentic chimyl alcohol was also spotted. After the solvent had traveled ¾ the length of the plate, the plate was

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removed and dried for one minute at 100 C. The plate was put immediately into a chamber saturated with iodine vapor. As soon as the spots were visible (about one minute), the spot (line) corresponding to the glyceryl ethers, including the silicic acid to which the glyceryl ethers were absorbed, was removed carefully with a spatula and placed in a centrifuge tube. To this was added 1/10 ml of hexamethyldisilazane. The tubes were shaken and then centrifuged. The supernatant, which contained the α, β-trimethylsilyl ether derivatives of the α-glyceryl ethers, was then analyzed by gas chromatography.

The preparation of the trimethylsilyl ether derivatives had to be done rapidly while the silicic acid was still active. Freedman and Croitoru (8) have reported that hindered phenols can be converted to their trimethylsilyl ether derivatives with hexamethyldisilazane using silicic acid as a catalyst at 200 C. The glyceryl ethers and fatty alcohols can also be derivatized by this method at room temperature, but only if the fatty alcohols and glyceryl ethers are actively bound to the silicic acid.

A Microtek gas chromatograph with a hydrogen flame detector and ¼ in x 8 ft stainless steel column of 12% UCW98 (Applied Science Laboratories) on Gas Chrom Z were used in all of the gas chromatography. Chromatography of the trimethylsilyl ether derivatives of the glyceryl ethers was done at 250 C with 50 lb of helium carrier gas pressure.

A mixture of standard glyceryl ethers, C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}, was converted to the trimethylsilyl ether derivatives and gas chromatographed. A graph of the log of the retention time vs. the number of carbon atoms in the compound gives a straight line when applied to an homologous series. By extending this line in either direction it was possible to determine the retention of the glyceryl ethers not included in the standard mixture. From these data it was possible to determine which glyceryl ethers were represented by the peaks on the gas chromatograms of the glyceryl ethers from the nonsaponifiable lipids of the organisms studied. The relative areas of these peaks were determined by cutting them out of the chart paper and weighing them on an analytical balance.

The fatty alcohols were isolated by thin-layer chromatography on Silica Gel G using benzene as the solvent; 1-octadecanol was used as a control. The trimethylsilyl ethers were analyzed by gas chromatography at 230 C. A mixture of tetradecanol, octadecanol, eicosanol, and docosanol, carried through the entire procedure, was used as a standard.

RESULTS AND DISCUSSION

From the weights of the total animal, the total lipid, and the nonsaponifiable lipids, the amount of lipid in the whole animal and the amount of lipid which is nonsaponifiable were calculated. The results are given in Table 1. The relative amounts

TABLE 1. *Total lipids, nonsaponifiable lipids, and α-glyceryl ethers of coelenterates and zooxanthellae.*

Organism	Lipids in whole organism (%)	Nonsaponifiable lipids in total lipids (%)	α-Glyceryl ethers in nonsaponifiable lipids (%)
Eunicea laciniata	20	16	15
Eunicea tourneforti	5.2	13	18
Gorgonia flabellum	6.1	13	16
Gorgonia ventalina	12	6.4	7
Muricea atlantica	5.5	10	—
Muriceopsis flavida	12	5.0	16.5
Plexaura homomalla	22	9.4	21
Pseudopterogorgia acerosa	12	26	20
Pterogorgia anceps	17	17	—
Rumphella antipathes	16	—	28
Zooxanthellae from *Anthopleura elegantissima*	36	14	28
Zoochlorellae from *Anthopleura elegantissima*	34	12	6.9
Metridium senile	26	12	7.1
Porites porites	2.0	3.6	12

(moles) of the various glyceryl ethers found in the nonsaponifiable lipids of each animal examined are given in Table 2. The relative amounts of the corresponding fatty alcohols in these animals are also given. The figures were obtained by calculating the relative peak areas from the gas chromatograms. The weight ratios of the derivatives were then converted to mole ratios.

From the mole ratios of the glyceryl ethers and the total number of moles of the glyceryl ethers (obtained from periodate oxidation data) in the nonsaponifiable lipids, the amount of each glyceryl ether was approximated. From this the total weight of the glyceryl ethers was computed; the result is represented in Table 1 as the weight percent of the nonsaponifiable lipids.

In addition to the glyceryl ethers represented in Table 2, there were also trace amounts of glyceryl ethers containing 17, 19, and 20 carbon atoms in the alkyl chains in some of these animals. The saturated fatty alcohols ranged from 14 to 20 carbons in most of the animals.

In all the specimens studied, except the sea anemone, *Metridium senile*, and the zooxanthellae and the zoochlorellae from *Anthopleura elegantissima*, there was either no selachyl alcohol or the selachyl alcohol was less than three-hundredths of the batyl alcohol content, although the animals contained considerable amounts of oleyl alcohol and oleic acid.

The sea anemone *Metridium senile*, as well as the zooxanthellae and zoochlorellae from *Anthopleura elegantissima*, which contain considerable amounts of selachyl alcohol, were all collected from cold water areas, while the other specimens were taken from warm water areas.

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TABLE 2. *α-Glyceryl ethers and fatty alcohols of coelenterates and zooxanthellae.*

Organism[a]	Mole ratio of α-Glyceryl ethers R-$OCH_2CH(OH)CH_2OH$			Fatty alcohols R-OH		
	R= C_{16}	C_{18}	$C_{18:1}$	R= C_{16}	C_{18}	$C_{18:1}$
Eunicea laciniata	.062	1	.02	1.6	1	.57
Eunicea tourneforti	.34	1	.02	1.6	1	.12
Gorgonia flabellum	.89	1	0	2.1	1	.12
Gorgonia ventalina	.95	1	0	3.2	1	.49
Muricea atlantica	.93	1	.015	1.6	1	.032
Muriceopsis flavida	.22	1	0	7.8	1	.42
Plexaura homomalla	.39	1	.008	.36	1	.058
Pseudopterogorgia acerosa	.25	1	0	2.9	1	.65
Pseudopterogorgia americana	.84	1	.025	9.1	1	.64
Pterogorgia anceps	1.4	1	0	9.5	1	1.4
Rumphella antipathes	.77	1	0	3.4	1	.37
Zooxanthellae from *Anthopleura elegantissima*	6.0	1	.51	7.8	1	3.3
Zoochlorellae from *Anthopleura elegantissima*	7.3	1	.18	3.1	1	.78
Metridium senile	2.5	1	.45	5.4	1	.84
Porites porites	1.7	1	0	15.1	1	.47
Nephtea sp.	.22	1	0	.84	1	.009

[a] The gorgonians are named according to Bayer (9).

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