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Contributions to the Study of Marine Products. XL. Waxes and Triglycerides of Sea Anemones[1]

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Certain lipid fractions from three sea anemones have been investigated. The warm-water anemone *Condylactis gigantea* has been shown to contain substantial quantities of solid lipids. These were found to consist of a mixture of myristyl myristate and myristyl palmitate and symmetrical palmityldimyristin. Two cold-water anemones *Bolocera tuediae* and *Actinostola collosa* have been shown to contain substantial quantities of liquid lipids. These were found to consist mainly of esters of unsaturated alcohols and acids of the order C_{20} and C_{22} and triglycerides of acids of the same order. Two new alcohols, 11-eicosenol and 11-docosenol, have been isolated. Cholesterol has been shown to be the principal sterol of the cold-water anemones.

It has been shown in previous papers of this series[4,5] that the lipids of sponges contain an unusual variety of sterols and that their component acids are also of an extraordinary diversity. The latter observation lends support to Hilditch's tenet[6] that the more primitive the animal the more complex its mixture for fatty acids. It should be borne in mind, however, that the sponges constitute an exceptional group of animals. They are recognized as a very early and probably polyphyletic branch off the main stem of evolution, and hence are ancestrally unrelated to animals of other phyla. Interesting as they may be *per se*, chemical data derived from sponges should be used with reservations in attempts at tracing the biochemical history of certain groups of compounds during the course of evolution. It was hoped to secure somewhat more pertinent data from a comparative study of the lipids of *Coelenterata*, a phylum of animals less primitive than the sponges but more representative of the main stream of evolution.

According to a concept of biochemical evolution in the direction of the use of fewer component acids, and the almost exclusive use of cholesterol[5] coelenterates should be expected to yield fatty acids and sterols in a diversity significantly greater than those encountered in higher animals. Data presented in previous communications to this series[7] do indeed point to a multiplicity of sterols in this phylum. Our knowledge of the fatty acids of coelenterates, however, is too fragmentary to permit even a tentative generalization.[8] Palmitic acid has been found to be the most conspicuous if not the most widely distributed fatty acid in stony corals (*Madreporaria*),[9] gorgonias,[10] and colonial anemones.[7] It appears to occur largely in form of cetyl palmitate, a compound which with its homologs is one of the most characteristic and most readily recognizable chemical features of this phylum.

The present communication deals with studies on lipids of two of the solitary sea anemones (*Anthozoa*). It is primarily concerned with the elucidation of the degree of diversity of the fatty acids, and their distribution among the waxes and the less conspicuous triglycerides. To ascertain relations, if any, between the fatty acid composition and mean temperature of their environment, anemones were selected which are representative of two different temperature zones. *Condylactis gigantea* was chosen as a representative species from the warm waters of the Bermudas, and *Bolocera tuediae* and *Actinostola callosa* as typical denizens of the colder waters of the Gulf of Maine.

Condylactis gigantea

It has previously been shown that the lipids of this animal comprise one-third of its dry weight, and that the unsaponifiable fraction consists mainly of myristyl alcohol together with some of its *homologs* and a sterol.[7] In this work attempts to separate the waxes and triglycerides resulted in the isolation of a crystalline material of uniform appearance amounting to nearly half of the total lipids. Both

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(4) Bergmann and Swift, *J. Org. Chem.*, **16**, 1206 (1951).

(5) Bergmann, *J. Marine Research (Sears Foundation)*, **8**, 137 (1949).

(6) Hilditch, *The Chemical Constitution of Natural Fats*, 2nd Edition, New York, 1949, p. 9.

(7) Bergmann, Feeney, and Swift, *J. Org. Chem.*, **16**, 1337 (1951).

(8) Haurowitz, *Z. physiol. Chem.*, **122**, 45 (1922).

(9) Lester and Bergmann, *J. Org. Chem.*, **6**, 120 (1941).

(10) Kind and Bergmann, *J. Org. Chem.*, **7**, 424 (1942).

the saponification value and the infrared spectrum[11] of the solid fraction showed it to be a mixture of waxes and triglycerides. Their sharp separation was accomplished by chromatography on a silica column. The wax was obtained in a yield approximately twice that of the triglyceride. Its composition expressed in *molar*-per cent, was as follows: myristyl myristate 63; myristyl palmitate 24, and combined cetyl myristate and cetyl palmitate 13.

Saponification of the triglyceride afforded myristic acid and palmitic acid in a molar ratio of two to one. The melting point of a purified sample of triglyceride, 56.5°, was higher than that reported for the unsymmetrical palmityldimyristin, 54°,[13,14] although not as high as that of the symmetrical isomer, 58.5°.[15] X-ray measurements of the crystal spacings, performed by Professor Malkin,[16] gave photographs identical with those obtained from a synthetic symmetrical palmityldimyristin.[15]

The non-crystalline fraction of the total lipids was saponified, and the unsaponifiable material was fractionated under reduced pressure. In addition to the components reported previously,[7] it contained a small amount of higher, unsaturated alcohols, probably identical with the eicosenol and docosenol to be discussed below, and some batyl alcohol (Table I). The saturated acids (27%) consisted mainly of myristic acid (66%) and palmitic acid (25%) and appeared to have mainly originated from incomplete removal of the solid waxes and triglycerides. Among the unsaturated acids the C_{14}-fraction was no longer the most prominent, but the C_{14}- and C_{16}-acids still constituted more than half of the total.

Bolocera tuediae and *Actinostola callosa*

These large anemones which are quite common on the fishing grounds of the Gulf of Maine are as rich in lipids as the Bermuda species. The lipids are viscous oils at temperatures above zero degrees and contain about twenty per cent of unsaponifiable material. The latter, also a viscous oil, could readily be fractionated under reduced pressure. The nonvolatile fraction consisted of cholesterol and some batyl alcohol. Two-thirds of the distillate represented a single fraction which upon further purification afforded a crystalline alcohol m.p. 33°. It was shown to be 11-docosenol[17] by its formation of dibromide, hydrogenation to eicosanol, and oxidation to undecanoic acid and undecanedioic acid. The second largest fraction of the unsaponifiable matter, m.p. 21–22°, was shown to be 11-eicosenol by its hydrogenation to eicosanol and oxidation to nonanoic acid and undecanedioic acid. The lower-boiling fractions afforded octadecanol, some octadecanol, hexadecanol, and a small amount of myristyl alcohol. (Table I)

TABLE I

COMPARISON OF THE UNSAPONIFIABLE FRACTIONS AND COMPONENT ACIDS FROM *Condylactis* AND *Bolocera*[20]

	Unsaponifiable Matter				Acids			
	Condylactis		*Bolocera*		*Condylactis*		*Bolocera*	
C	%	Alcohol	%	Alcohol	%	Acid	%	Acid
C_{14}	71	Myristyl	.5		51	Myristic	13	Myristic
C_{16}	14	Cetyl	10.5	Cetyl	31	Palmitic	13	Palmitoleic
C_{18}	3	Stearyl	7	Stearyl, oleyl	5	Stearic	9	Stearic, oleic
C_{20}	5	Unsaturated	27	11-Eicosenyl	4	Arachidic	20	Gadoleic
C_{22}			47	11-Docosenyl	3	Behenic	21	Cetoleic, erucic
C_{24}					1		22	
C_{26}					.1		1	

The composition of the fatty acid mixture from *Bolocera* was ascertained through two different separations. In order to obtain reasonably accurate figures on chain length distribution, and to avoid polymerization of unsaturated material, a freshly prepared acid mixture (iodine value 124) was immediately hydrogenated, and esterified by diazomethane. Table I presents the average results of the fractionation of several ester mixtures.

(11) The spectra of cetyl palmitate and trimyristin differ mainly in the region between 7.0 and 9.0 microns. The wax exhibits sharp bands at 7.10 and 7.20, the C—CH_3 stretching band at 7.26, a stronger band at 7.40, a broad band at 7.93 and the primary C—O stretching band at 8.52 microns.[12] The triglyceride reveals a band of increased intensity at 7.10 but none at 7.20. The 7.26 band is more pronounced, the one at 7.40 weaker and the broad band shifted to 8.07. The C—O stretching band is broader and shifted to 8.62 due to the additional secondary C—O linkage.[12]

(12) Zeiss and Tsutsui, *J. Am. Chem. Soc.*, **75**, 897 (1953).

(13) Jackson and Lutton, *J. Am. Chem. Soc.*, **71**, 1976 (1949).

(14) Carter and Malkin, *J. Chem. Soc.*, 577 (1939).

(15) Malkin and Meara, *J. Chem. Soc.*, 103 (1939).

(16) The authors are greatly indebted to Professor T. Malkin, Department of Organic Chemistry, The University of Bristol, England, and to Dr. E. S. Lutton, The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio, for their most generous assistance and expert counsel.

(17) The alcohol appears to be the *cis*-isomer, for its infrared spectrum lacks the strong band at 10.34 microns associated with *trans*-orientation.[18,19]

(18) Rao and Daubert, *J. Am. Chem. Soc.*, **70**, 1102 (1948).

(19) Treumann and Wall, *Anal. Chem.*, **21**, 1161 (1949).

(20) Only those alcohols and acids are cited which have been identified.

In another separation a larger amount of crude and somewhat oxidized acids was separated into a series of fractions through distillation of the methyl esters. The fractions then were subdivided by further distillations in efforts to isolate and identify individual components. During the process much of the higher unsaturated acids underwent decomposition. Separation of the saturated acids afforded myristic, palmitic, stearic, and arachidic acid. Only a few monounsaturated acids were isolated in a reasonable state of uniformity, and their identity was shown by the results of their hydrogenation and oxidative degradation. They were oleic acid, 9-eicosenoic acid (gadoleic acid), 11-docosenoic acid (cetoleic acid), and 13-docosenoic acid (erucic acid).

Quite similar results were obtained from fractions of the unsaponifiable matter and the acid mixture from *Actinostola callosa*. This anemone also contained cholesterol as its principal sterol.

DISCUSSION OF THE RESULTS

In the course of a histological study of sea anemones (*Heliactics bellis*), Arndt[21] was so startled at the abundance of oil droplets in the tissues, that at first he suspected it to be the result of some fatty degeneration. Eventually, however, he convinced himself of the normalcy of this phenomenon which he named "*steatosis physiologicus*," *i.e.*, a non-pathological fatty infiltration. The observations recorded in the present and previous communications leave no doubt about the normalcy of a very high lipid content in sea anemones. The accumulation of lipids most probably serves as a food storage mechanism, for the carbohydrate content was found to be quite low in the few coelenterates which have been studied. Thus no carbohydrate was detected in *Velella*,[8] whose lipid content is twenty-seven per cent of the dry weight.

The original expectation of obtaining from anemones component acid mixtures of considerable complexity was not altogether realized. The acid mixture from *Bolocera* is complex but not much more so than that from many other marine animals, and the mixture from *Condylactis* may even be regarded as rather simple in composition. The over-all composition of the acetone-benzene-soluble lipids, however, is remarkable and provides the first insight into the curious relationship between waxes and triglycerides in coelenterates. In *Condylactis* the two groups are present in approximately equal amounts. The wax differs from that of other coelenterates such as corals, gorgonias, and colonial anemones, in that it consists principally of myristyl myristate rather than the ubiquitous cetyl palmitate. The abundant occurrence of this ester has not previously been noted in animals.

Even more remarkable is the presence of large amounts of a saturated triglyceride, 2-palmityldimyristin. The comparative ease with which it is obtained from the lipid mixture is paralleled only by seed fats. Hilditch[22] has recently drawn attention to the regularity of triglycerides which are derived from two different acids. The acid occurring in the lesser amount is usually attached to the central position of glycerol. In simple glycerides there is a tendency to assume symmetrical configuration when one of the component acids is present in a large excess over the other. In *Condylactis* lipids these regularities are observed; myristic acid outweighs palmitic acid almost three to one and the palmityldymyristin is symmetrical.

In the wax fraction the most conspicuous alcohol, myristyl alcohol, is esterified by myristic acid and palmitic acid in proportions of roughly two to three to one. This is nearly the same proportion in which the same two acids are represented in the solid triglyceride. It remains to be seen whether these similarities in proportions are merely coincidental or of some hidden significance. Also of interest is the extraordinary similarity between the crystals spacings of the total solid lipid and its components the wax and triglyceride. The results of the measurements for which the authors are greatly indebted to Dr. Lutton and Professor Malkin[16] are shown in Table II. The great similarities probably account for the considerable difficulties encountered in separating the mixture by simple recrystallizations.

The solid, rather high melting, wax-triglyceride mixture constitutes about fifteen per cent of the dry weight of the warm water anemone, *Condylactis*, where it is distributed, with the remainder of the lipids in droplets throughout the tissues. The occurrence of similar high-melting material in similar quantities in anemones inhabiting the cold northern waters would *a priori* seem quite unlikely. It is of great interest therefore that in *Bolocera* the curious wax-triglyceride relationship is retained, but also that both groups have changed their composition in the direction of greater chain length coupled with greater unsaturation and accompanied by a lowering of the freezing point. Although no waxes and triglycerides, liquid at room temperature, have yet been isolated, their composition may be estimated from the structure and proportions of the products of their hydrolysis.

In the unsaponifiable fraction from *Bolocera* and *Actinostola* myristyl alcohol, the conspicuous constituent of *Condylactis* is but a minor, hardly discernible component, and proportionally its place is occupied by 11-docosenol. Similarly cetyl alcohol, next in quantity in *Condylactis*, is present in *Bolocera* in rather small amounts, and is largely replaced by 11-eicosenol. In *Condylactis* the most conspicuous acids are saturated and of the order C_{14}

(21) Arndt, *Zool. Jahrb.* (*Zool. Abt.*), **34**, 27 (1913).

(22) Hilditch, *Ann. Rev. Biochem.*, **22**, 125 (1953).

TABLE II

CRYSTAL SPACING OF THE SOLID LIPID, WAX, AND TRIGLYCERIDE FROM *Condylactis*

Sample	M.P., (°C) Min.	Max.	Long Spacing	Main Short Spacings		
Solid Lipids	37.7	40.7	38.6	4.31W	4.14VS	3.77S
Waxes	40.3	41.0	38.5	4.30W	4.15VS	3.77S
Triglyceride	36	56	38.6	4.31	4.12	3.81
Synthetic MPM	38	58.5	38.5	4.31	4.13	3.81

and C_{16}, and the unsaturated acids of the order C_{20} and C_{22} are only poorly represented. In *Bolocera* the situation is nearly reversed and the higher acids constitute one-half of the total mixture. (See Table I).

In the original *Bolocera* lipids the C_{20} and C_{22} unsaturated alcohols must occur to a large extent as the esters of C_{20}- and C_{22}-unsaturated acids. Convincing evidence for this rests on the fact that hydrogenation of a low temperature fraction afforded material consisting essentially of docosanyl docosanoate. Here again the similarity to a seed lipid is rather striking. Thus Hilditch[23] and associates have shown that the oil from the seeds of *Simmondsia californica* (Boxwood Family) contains mainly the 11-eicosenoic esters of 13-docosenol and 11-eicosenol. Similar eicosenyl eicosenates and eicosenyl eicosidienolates constitute about 9% of sperm whale oil.[24] In *Bolocera* lipids the proportions of the C_{20}–C_{22}-alcohols and C_{20}–C_{22}-acids demand that a substantial amount of the latter is also present in the triglyceride fraction. Here then the docoso- and eicoso-enates and polyenates take the place of the palmityldimyristin in *Condylactis*.

When the 11-docosenol and 11-eicosenol had first been isolated from *Bolocera* in this laboratory in 1951, they were either unknown compounds or their natural occurrence had not been reported. Rather recently, however, the occurrence of the two alcohols in fish has been reported from Japan. 11-Docosenol, also called lotella-alcohol, m.p. 31.2–31.8°, was found to constitute as much as 18 per cent of the liver-oils from *Lotella phycis* and *Laemonemia morosum*.[25] Substantial quantities of 11-eicosenol were also found in the ovary oil of one of the toxic blowfish (*Tetraodons*).[26] It is also of interest to note in this connection that Collin, *et al.*,[27] have isolated an unsaturated alcohol from zooplankton oil and have tentatively identified it as eicosenol. The data, however, reported for the hydrogenated material and its derivatives upon re-examination favor C_{22} rather than C_{20} and suggest that the original material was a mixture of docosenol and eicosenol in proportions of four to one.

While the possibility is not excluded that the differences in lipid composition between the two anemones are caused by dietary fats, they more likely represent an adaptation of the species to the mean temperature of its environment. The influence of temperature on the composition of fat is, of course, well known, but it has probably never before been observed so strikingly. In 1909, Dorée[28] in his classical paper on the distribution of sterols in animals reported the occurrence of cholesterol in two sea anemones, *Tealia crassicornis* and *Actina equina*. In more recent years other sea anemones have yielded a variety of sterols but not cholesterol, a fact which threw some doubt on the accuracy of Dorées original observation. The discovery of cholesterol in *Bolocera* and *Actinostola*, however, once again show that it may also occur as the principal sterol of actinians.

EXPERIMENTAL

Condylactis gigantea[29]

The lipids were extracted as previously described,[7] but the solid material, which separates upon cooling of the acetone extract, was kept apart. A typical extraction afforded 330 g. of dried, extracted anemones, 64 g. of crystalline material, and 85 g. of oily lipids, corresponding to a lipid content of 31.1%.

Solid lipids. The solid fraction, m.p. 38–40°, was decolorized with Norit in acetone, then dissolved in pentane, and the solution was passed through a column of magnesium oxide. The solid obtained from the effluent was recrystallized from acetone; m.p. 39.5–41°; n_D^{45} 1.4433; S.E., 386.

A 10-g. sample dissolved in *n*-pentane (300 ml.) was chromatographed on 100 g. of activated silica gel in a tube of 4.0-cm. diameter. Elution with four 1000-ml. volumes of pentane containing 5, 10, 20, and 20% of chloroform gave 2.22, 3.04, 1.21, and 0.23 g. of wax respectively. Subsequent elution with 450 ml. of chloroform and two 575-ml. volumes of chloroform containing 21% of ethyl acetate gave 0.71, 2.29, and 0.22 g. of triglyceride respectively.

Wax. The combined wax fractions were recrystallized from acetone (100 ml.); m.p. 40.9–42.2°; n_D^{50} 1.4402; S.E., 450, 452. A sample was saponified, and the unsaponifiable fraction and the acids were separated in the conventional manner. The unsaponifiable matter (0.685 g.) was slowly distilled *in vacuo* at a pressure below 1 mm. and a bath temperature of 100–110°. The first three fractions (0.56

(23) Green, Hilditch, and Stainsby, *J. Chem. Soc.*, 1750 (1936).

(24) Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 87.

(25) Komori and Agawa, *J. Chem. Soc. (Japan), Pure Chem. Sect.*, **74**, 1053 (1953); **75**, 1951 (1954).

(26) Umezawa, *J. Pharm. Soc. (Japan)*, **74**, 682 (1954).

(27) Collin, Drummond, Hilditch, and Gunther, *J. Exptl. Biol.*, 11, 198 (1931).

(28) Dorée, *Biochem. J.*, **4**, 72 (1909).

(29) The authors are indebted to the personnel of the Bermuda Biological Station for Research for its valuable help in obtaining these animals.

TABLE III

FRACTIONATION OF THE METHYL ESTERS OF THE HYDROGENATED *Condylactis* ACIDS

Fract.	Temp.	Weight, g.	S.E.	n_D^{80}[31]	M.p., °C.
1–3	85–89	2.74	241	1.4133	
4	95	.31	243	1.4161	
5–11	95–104	3.99	269	1.4172–1.4175	29.2–29.4
12–13	109–114	.58		1.4186–1.4201	
14–19	118–125	1.46	299	1.4212–1.4216	38.2–39.3
20–21	128–130	.43		1.4221–1.4233	
22–30	130–138	1.58	326	1.4230–1.4243	44.2–45.5
31–32	141–143	.20		1.4249–1.4251	
33–43	144–154	1.11	353	1.4263–1.4265	51.8–53.2
44–46	158–161	.49		1.4270–1.4278	
47–50	161–165	.33	381	1.4280–1.4284	56.8–57.5
51	178	.11	441	1.4328	63–65
52–57	183–192	.52		1.4346–1.4546	
Tarry residue		1.08			

g., 81.7%) consisted of myristyl alcohol, m.p. 38.3°; phenylurethan, m.p. 71°. The residue (86 mg., 12.5%), after several recrystallizations from acetone, afforded cetyl alcohol, m.p. 73°.

The acids were converted by diazomethane to the methyl esters (1.683 g.) which were fractionated through a spinning band column at pressures below 0.1 mm. The first seven fractions, 1.08 g., S.E., 241–245, n_D^{45} 1.4271–1.4273, m.p. 54.5°, when saponified afforded myristic acid. The eighth fraction was a mixture, and the following three fractions, 0.347 g., S.E., 270–272; n_D^{45} 1.4311–1.4313; m.p. 28–29°, gave palmitic acid, m.p. 62.3–63.1°, when hydrolyzed. Assuming the still residue, 0.04 g., and the hold-up loss, 0.18 g., to have been methyl palmitate, the observed molecular ratio of myristic to palmitic acid was 2:1.

Triglyceride. The combined triglycerides were treated with Norit in ether, recrystallized four times from acetone, and once each from hexane and ethyl acetate. One sample was shaken with hydrogen and a platinum catalyst then chromatographed twice over silica gel and recrystallized a few more times from acetone. The triglyceride crystallized in rosettes of fine, hard needles, m.p. 56–56.5°. The γ-form was obtained by warming the triglyceride to 60° in a capillary tube and freezing it immediately at −80°, m.p. 36°.

The triglyceride (1.325 g.) was saponified, the acids isolated in the usual manner and esterified with diazomethane. The esters (1.27 g.) were fractionally distilled at pressure below 1 mm. The first nine fractions (0.77 g.), S.E. 245, consisted of methyl myristate; saponification gave myristic acid, m.p. 54–54.5°; n_D^{60} 1.4310.

The tenth fraction (0.102 g.) was a mixture, but the following three fractions (0.197 g.); S.E., 289–273, n_D^{45} 1.4312–1.4314; m.p. 28–29°, consisted of methyl palmitate; saponification gave palmitic acid, m.p. 62.5–63°. Careful rinsing of the distilling flask and column gave methyl palmitate (0.153 g.), m.p. 28–29; n_D^{45} 1.4314. The molecular ratio of the two acids was 333:172, or approximately 2:1.

Oily lipids. The oily material remaining after the removal of the solids was divided by a series of low-temperature crystallizations into fractions representing −10°, −25°, −45°, and −65°, and the residue. Each fraction was separated over a magnesium oxide column into free acids and neutral lipids; the average free acid content was about 8%. The neutral fats were saponified. The content of unsaponifiable matter fluctuated between 21-34%. The neutralization equivalents of the acids increased from 268–303, and the iodine values[30] from 54.6–181, corresponding to −1.1H to −4.3H.

Since no very significant separation was obtained by this method all acids were recombined and separated by recrystallization from acetone at −25° into saturated acids (26%), Iodine Value, 0, and unsaturated acids (74%), I.V., 131.[28] The solid acids were methylated with diazomethane and the methyl esters (14.51 g.) were fractionally distilled through a spinning band column at below 0.1 mm. pressure and a reflux ratio of 1:48. A total of twenty fractions was collected.

Fractions 1–7 (8.9 g., 61.4%), n_D^{45} 1.4273–1.4274 consisted of methyl myristate. Fraction 8 (0.55 g.) was a mixture and fractions 9–13 (3.19 g., 22.2%) were methyl palmitate, m.p. 28.6–29.8, n_D^{45} 1.4314. Fraction 14 was another mixture; it was followed by fractions 15–17 (0.21 g., 0.14%) consisting of methyl stearate, m.p. 38.1–39.4°, n_D^{45} 1.4344–1.4346. The remaining fractions (0.59 g.) contained mixtures of methyl arachidate and methyl behenate, m.p. 42–44°, S.E., 340, n_D^{80} 1.4272–1.4310.

The liquid acids were hydrogenated with a platinum catalyst under moderate pressure in ethyl acetate. The solid reaction product was recrystallized from acetone at −65°, and the acids were methylated and distilled as described above. A 15.55 g. sample of the esters gave 57 fractions. (See Table III.)

Saponification of fractions 1–3 gave myristic acid, m. p. 52.5–53.5°, n_D^{70} 1.4272; of 5–11, palmitic acid, m. p. 62.4–62.8°; n_D^{70} 1.4309; of 14–19, stearic acid, 69.2–70.2; n_D^{70} 1.4336; of 22–30, arachidic acid, m. p. 73.8–75.4°; of 33–43, behenic acid, m. p. 80.1–80.3°, and of 47–50, lignoceric acid, m. p. 83.1–83.8°.

The unsaponifiable fractions from the oily *Condylactis* lipids were combined and fractionally distilled. As previously reported,[7] it consisted mainly of myristyl alcohol, smaller quantities of cetyl and stearyl alcohol, and sterol. A chromatographic separation of the higher-boiling fractions gave a small quantity of liquid, unsaturated alcohols, which upon catalytic hydrogenation afforded a solid mixture, m.p. 67–68°. It is probable that the unsaturated alcohols were mixtures of eicosenol and docosenol, both of which are present in large quantities in the anemones to be discussed below. A summary of the composition of the unsaponifiable fraction of *Condylactis* is given in Table I.

(30) The iodine values were determined according to Yasuda's method, *J. Biol. Chem.*, **94**, 401 (1931).

(31) The refractive indices for methyl esters at 80° have recently been reported by Krewson, *J. Am. Chem. Soc.*, **73**, 1365 (1951); *viz.* myristate, 1.4131; palmitate, 1.4173; stearate, 1.4213; arachidate, 1.4238; behenate, 1.4262; lignocerate, 1.4283; cerotate, 1.4301; and montanate, 1.4320.

Bolocera tuediae and *Actinostola callosa*

The large anemones[32] which had been preserved in a 5% solution of formalin in sea water were extracted according to the method previously described. In the case of *Bolocera*, the dried, extracted anemones weighed 620 g., and the acetone-benzene-soluble lipids, 271 g.; the lipid content of the dry anemone was therefore 30.3%. *Actinostola callosa*, another large anemone contained 28% of lipid material. The lipids were viscous oils, and their solutions in acetone did not deposit crystalline material at temperatures above 5°. A fraction obtained by low temperature crystallization will be discussed below.

Unsaponifiable matter. The lipids were saponified, and the unsaponifiable matter and the acids were isolated in the conventional manner.[7] The yield of unsaponifiable matter was 23.5% and 16.8% for *Bolocera* and *Actinostola*, respectively.

The unsaponifiable from *Bolocera* (63.5 g.) was subjected to a flash vacuum distillation under nitrogen at about 1 mm. The distillate weighed 53.6 g. (84.4%) and the residue 9.0 g. (14.1%). *Cholesterol.* The residue from the distillation of either the *Bolocera* or *Actinostola* unsaponifiable was refluxed with acetic anhydride. The acetate separating upon cooling was decolorized with Norit and recrystallized from methanol-ether, m.p. 114–115°; $[\alpha]_D^{25°}$ −44.3 ($CHCl_3$). It was converted to cholesteryl acetate dibromide, m.p. 112°; cholesterol, m.p. 145°; cholesterol dibromide, m.p. 117° and cholesteryl benzoate, m.p. 145°; 179°. Neither of the derivatives gave a depression of the m.p. when mixed with authentic material.

Fractional Distillation. In another case the unsaponifiable matter, 10.3 g., was divided into nearly fifty fractions by distillation through a spinning band column; see Table IV. The data given in the table were derived from *Actinostola* fractions, but those from *Bolocera* were quite similar.

Hexadecanol (Cetyl alcohol). The fractions 2–6 (Table IV), or their equivalents from other distillations were recrystallized from pentane, m.p. 48.5–49.3°; *3,5-dinitrobenzoate*, m.p. 71.5–72.2°; no depression of the m.p. when mixed with authentic material.

Anal. Calc'd for $C_{27}H_{42}N_2O_6$: C, 66.09; H, 8.63. Found: C, 66.39; H, 8.66.

Catalytic hydrogenation gave eicosanol, m.p. 64–65°; reported, m.p. 65.5°.

The eicosenol was oxidized with potassium permanganate according to the directions given below in connection with docosenol. A 1.093 g. sample gave 0.564 g. of dicarboxylic acid and 0.358 g. of monocarboxylic acid. The former was recrystallized successively from water, benzene, and ether; m.p. 106–107°; N.E. 106.9. Undecanedioic acid, m.p. 110°; N.E., 107.5.

Anal. Calc'd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.64; H, 9.19.

The volatile, monocarboxylic acid (nonanoic acid) was distilled and converted to the benzimidazole derivative.[33] It was recrystallized four times from hexane; m.p. 135°; 2-octylbenzimidazole, m.p. 139.5–140.5°.

Anal. Calc'd for $C_{15}H_{22}N_2$: C, 78.21; H, 9.63. Found: C, 77.87; H, 9.29.

11-Docosenol. This was always the main fraction of all distillations of the unsaponifiable from *Bolocera* and *Actinostola*. Fractions such as 28–48 (Table IV) were recrystallized several times from pentane; m.p. 31.5–33.5°, I.V., 83.0. Its large, radiating crystals set the alcohol apart from the more granular microcrystals of hexa- and octa-decanol.

Dibromide. A slight excess of a 5% solution of bromine in glacial acetic acid was added to a solution of docosenol in the same solvent. A curdy precipitate formed which was washed with cold pentane and recrystallized from pentane; fibrous sheaves of needles, m.p. 32–33.5°.

Anal. Calc'd for $C_{22}H_{44}Br_2O$: C, 54.54; H, 9.17. Found: C, 54.31; H, 9.47.

Phenylurethan. Equal weights of the docosenol and freshly distilled phenyl isocyanate were heated to 100° for 30 minutes. The excess reagent was removed *in vacuo* and the docosenyl phenylurethan was recrystallized from pentane, m.p. 45.8–46.8°.

Anal. Calc'd for $C_{29}H_{49}NO_2$: C, 78.50; H, 11.13. Found: C, 78.69; H, 11.25.

Docosanol. Docosenol, 0.655 g., in ethyl acetate was hydrogenated with a platinum black catalyst; up-take at STP, 51 ml.; calc'd for $C_{22}H_{44}O$, 49.5 ml.; m.p. 70–71.5°; docosanol 70.6°.[34] The *phenylurethan* was prepared as described above; m.p. 87°; docosanyl phenylurethan, m.p. 86–86.5°.[35]

Oxidation of 11-docosenol. (*a*). Powdered potassium permanganate (8 g.) was added over a period of 90 minutes to a solution of docosenol (2.09 g.) in purified acetone[36] (25 ml.). The mixture was refluxed for 16 hours and the solvent was removed *in vacuo*. The brown residue was

TABLE IV

FRACTIONAL DISTILLATION OF 10.3 g. OF UNSAPONIFIABLE MATTER

Fract.	B.p./1 mm.	Weight, g.	n_D	t, °C.	Principal component
1	80–100	0.053	1.4289	70.5	
2–6	100–108	.914	1.4317	70.5	Hexadecanol
7	108–110	.271	...		
8–13	110–118	.556	1.4328	70.5	Octadecanol
14	118–124	.091	...		
15–26	124–130	2.601	1.4512	45.5	11-Eicosenol
27	130–134	0.105	1.4515	45.5	
28–48	134–140	4.770	1.4527	45.5	11-Docosenol

Octadecanol (Stearyl alcohol). The fractions 8–13 (Table IV), or their equivalents from other distillations when crystallized from acetone afforded octadecanol, m.p. 56–57°; *3,5-dinitrobenzoate*, m.p. 77.5°; no depression of the m.p. when mixed with authentic material.

The mother liquor of a larger C_{18}-fraction gave an oily material which upon hydrogenation gave octadecanol. It appeared to consist mainly of 9-octodecanol (oleyl alcohol).

11-Eicosenol. The fractions 15–26 (Table IV) were combined and recrystallized twice from pentane at −10°; m.p. 21–22°; I.V., 87. The *3,5-dinitrobenzoate* was prepared in the usual manner, m.p. 39.5–40°.

(32) The animals were collected by Dr. W. D. Hartman of Yale University in the Gulf of Maine during Cruise No. 28 of Albatross III, September 1949.

(33) Pool, Harwood, and Ralston, *J. Am. Chem. Soc.*, **59**, 178 (1937).

(34) Levene and Taylor, *J. Biol. Chem.*, **59**, 905 (1924).

(35) Willstätter and Mayer, *Ber.*, **41**, 1478 (1908).

(36) Armstrong and Hilditch, *J. Soc. Chem. Ind.* (*London*), **44**, 43T (1925).

suspended in water and treated with excess sulfur dioxide. The organic matter then was extracted with ether, and the extracts were washed successively with concentrated sodium chloride solution and a 20% sodium carbonate solution. The alkaline extracts were washed with ether, acidified with sulfuric acid, and subjected to steam-distillation until all volatile material had been removed. The volatile acids were extracted with ether from the distillate to which sulfuric acid had been added. The acids (1.155 g.) were distilled from a semi-micro vacuum still with a jacketed spiral column. The bulk of the material came over at 90–95° at 0.7 mm; m.p. 26°, N.E., 185.7; undecanoic acid, m.p. 28.5°; N.E., 186.3.

The non-volatile acids separated in flocculent needles from the solvent in the distilling flask. They were extracted with ether, and the crude product (1.325 g.) was decolorized with Norit and recrystallized from methanol and pentane; m.p. 95°. The material appeared to be a mixture of an ω-hydroxy acid and a dicarboxylic acid. To obtain a uniform product it was oxidized with chromic anhydride in glacial acetic acid at room temperature. Water was added after 20 minutes, and the acid was collected by filtration and thrice recrystallized from water; m.p. 109°; N.E. 107; undecanedioic acid, m.p. 109–110°; N.E., 107.5.

(*b*). Docosenol (2.035 g.) in anhydrous pyridine (25 ml.) was treated with an excess of benzoyl chloride. The crude, noncrystalline, benzoate was isolated in the customary manner and without further purification was oxidized with potassium permanganate as described above. The volatile acid contained some benzoic acid. This was removed by passing a solution of the mixture in a few ml. of pentane through a short Norit-Celite column. The aliphatic acid (0.94 g.), m.p. 20°, was converted to the 2-benzimidazole derivative,[33] which was recrystallized from ethanol-water; m.p. 113.5°; no depression of the m.p. when mixed with authentic 2-decylbenzimidazole, m.p. 114–114.5°.

The non-volatile material showed no tendency to crystallize. It was saponified, the benzoic acid was removed by steam-distillation and the 11-hydroxyundecanoic acid was extracted with ether and recrystallized from ether and water; fine needles, m.p. 68–69°; reported m.p. 70°.

Docosanyl docosanate. A part of the original acetone-benzene-soluble lipid from *Actinostola* was dissolved in ten parts of acetone and the solution cooled to −10°. The solid material which formed was separated from the mother liquor by centrifugation in the cold and the process was repeated three more times. The semi-crystalline material liquefied between 0 to 5°. It was converted by catalytic hydrogenation, followed by recrystallization from acetone, into a colorless, crystalline solid, m.p. 70–72°; S.E., 92; docosanyl docosanate,[37] m.p. 75°; S.E., 86.5.

Anal. Calc'd for $C_{44}H_{88}O_2$: C, 81.4; H, 13.6. Found: C, 81.2; H, 13.9.

Saponification gave an acid, m.p. 75°, N.E., 334.2; docosanoic acid (behenic acid), m.p. 79.9°; N.E., 340.6. The N.E. and the m.p.[38] indicate that the isolated acid consists of about 80% docosanoic and 20% eicosanoic acid.

The unsaponifiable fraction was recrystallized a few times from acetone; m.p. 67–68°; docosanol, m.p. 70.6°; eicosanol, m.p. 65.5°.

Component acids. In one experiment the acids, I.V., 124, were hydrogenated catalytically, the saturated acids were converted into the methyl esters, and the latter were divided into 40–50 fractions by distillation through a spinning band column at pressures below 1 mm. The results of several fractionations have been averaged and the data thus obtained are given in Table I.

Otherwise the crude acids were esterified with methanol by Fischer's method and the methyl esters first were divided by vacuum distillation into three fractions which in turn were subdivided into numerous small fractions by distillation through a spinning band column.

C_{14}-fraction. Fractions of S.E. 250–255, I.V., 60–85 were combined, dissolved in ten parts of acetone, and the solution was cooled to −27°. The crystalline material was collected on a cooled funnel, —it melted at room temperature—and then quickly was distilled through a short path vacuum still; S.E., 245; I.V., 4.5; n_D^{30} 1.4336. It was saponified and the acid was recrystallized from acetone; m.p. 51.5–52°; N.E., 230, n_D^{70} 1.4279, myristic acid, m.p. 54.5°; n_D^{70} 1.4273; N.E., 228.4.

C_{16}-fraction. Fractions of S.E., 280; I.V., 89–100; by low temperature crystallization followed by distillation and recrystallization gave methyl palmitate, m.p. 29.8–30.2°; S.E., 270–272; n_D^{45} 1.4319; reported m.p. 30.6°; n_D^{45} 1.4313.

C_{18}-fraction. Crystallization of a fraction, S.E., 307, I.V., 97 from acetone at −25° gave methyl stearate, m.p. 36.2° S.E., 302; reported m.p. 38.7; S.E., 298.5. Saponification gave stearic acid, m.p. 68.2–68.8°; N.E., 287.

Another fraction, S.E., 303; I.V., 87.1, n_D^{25} 1.4500 consisted essentially of methyl oleate. Upon catalytic hydrogenation followed by saponification stearic acid, m.p. 68.5°; N.E., 288 was obtained.

Oxidation of the ester, and hydrolysis of the oxidation product with potassium permanganate was carried out as described above. There were obtained a monocarboxylic acid, n_D^{20}, 1.3220; N.E., 157.4; nonanoic acid, n_D^{20} 1.4322; N.E., 158.2; and a dicarboxylic acid, m.p. 106–107°; N.E., 93.6; nonanedioic acid, m.p. 107°; N.E., 94.1.

C_{20}-fraction. A certain fraction, S.E., 325, I.V., 77.3, appeared to consist entirely of an eicosenoic methyl ester; S.E., 324.5; I.V., 78.2. Upon hydrogenation, followed by saponification it gave eicosanoic acid m.p. 73.5–74.2°; N.E., 311; reported m.p. 75.35°; N.E., 312.5.

Oxidation of the ester by the method described above gave nonanedioic acid, m.p. 105.8–106.8°; N.E., 93.8; reported, m.p. 107°; N.E., 94.1; and undecanoic acid, m.p. 27–27.5°; N.E., 185; reported, m.p. 29.3°; N.E., 186.3. The monounsaturated acid was converted to the benzimidazole derivative, m.p. 114.2–114.8°; 2-*n*-decylbenzimidazole, m.p. 114–114.5°. The unsaturated acid was therefore 9-eicosenoic acid (gadoleic acid).

C_{22}-fraction. A fraction, S.E., 344; I.V., 81, was dissolved in 10 parts of acetone and the solution was cooled to −32°. The precipitate was collected and crystallized from acetone. There were obtained but a few mg. of crystals, m.p. 34–36°. The combined mother liquors were cooled to −55°, and the gelatinous precipitate was filtered at the same temperature. The process was repeated at −65°.

A part of the precipitated ester was saponified and the acids were hydrogenated catalytically and recrystallized from pentane, m.p. 77.1–77.8°; N.E., 336.4. The N.E. and m.p.[38] indicate that the acid was 90% pure docosanoic acid (behenic acid).

The unsaturated ester was oxidized with potassium permanganate as described above. The first fraction of the steam-volatile acids was nonanoic acid, N.E., 151, n_D^{30} 1.4279. The second fraction was purified by a short-path distillation, m.p. 27–28°; n_D^{40} 1.4316, N.E., 183; undecanoic acid, m.p. 29.3°; n_D^{40} 1.4319, N.E., 186.3.

Isolation of a pure sample of the dicarboxylic acid met with the difficulties not infrequently encountered.[23] The acid showed the N.E. (106) of the expected undecanedioic acid, (108.2) but its m.p. remained eight degrees below the reported m.p. 111°. It required paper chromatographic separation to obtain an acid of m.p. 109–110°; N.E., 107.8. The impurity responsible for the depression of the m.p. was tentatively identified as nonanedioic acid, m.p. 120–130°; N.E., 99.

(37) Brigl and Fuchs, *Z. physiol. Chem.*, 119, 280 (1922).

(38) Markley, *Fatty Acids*, Interscience Publishers, Inc., New York, N. Y., 1947, p. 121.

The formation of both undecanoic acid and undecanedioic acid proves that the unsaturated fraction had consisted mainly of the methyl ester of 11-docosenoic acid (cetoleic acid).

Solutions remaining from the S.E. determinations of the C_{22}-ester fractions upon standing for several days deposited a nicely crystalline material; from acetone, m.p. 32.5–33°; N.E., 340; erucic acid, m.p. 33.5°; N.E., 338.6. It was converted by catalytic hydrogenation to docosanoic acid, m.p. 78.8–79.8°; N.E., 342.

A sample of the unsaturated acid was rearranged to the *trans*-isomer, brassidic acid, by the nitrous acid method of Dorée and Pepper.[39] The rearranged product was recrystallized from ethanol and from acetone at −25°; m.p. 57–58°; N.E., 342; brassidic acid, m.p. 60°; N.E., 338.6.

Oxidation of the erucic acid from *Bolocera* with potassium permanganate (see above) gave nonanoic acid, N.E., 155.6 and tridecanedioic acid (brassylic acid), m.p. 108.5–110°; N.E., 120.8; reported, m.p. 112°; N.E., 122.2.

Batyl alcohol. Several of the highest-boiling, liquid ester fractions deposited some crystalline material upon long standing. It was collected, washed with cold pentane and recrystallized successively from acetone-hexane, ethyl acetate, and methanol; shiny plates, m.p. when mixed with authentic batyl alcohol, 69–70°; $[\alpha]^{25}_{D}$ +3.8°. The infrared spectra of 10% solutions of the two samples in chloroform were found to be alike. Because of the relatively high solubility of batyl alcohol in aqueous solvent the alcohol frequently is found in the acid phase and eventually among the high-boiling ester fractions.

(39) Dorée and Pepper, *J. Chem. Soc.*, 477 (1942).

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