[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY AND THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XXX. COMPONENT ACIDS OF LIPIDS OF SPONGES. I.[1]

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In previous communications of this series, (1) attention has been called to the fact that the greatest diversity of sterols is to be found among the lowest forms of animal life. It appeared of interest to ascertain whether an analogous variation is also shown by other groups of typical constituents of primitive invertebrates. The comprehensive comparative studies of Hilditch on the component acids of animal fats have led this investigator to conclude that "the fats of the simplest and most primitive organisms are usually made up of very complex mixtures of fatty acids whilst, as biological development has proceeded, the chief component acids have become fewer in number" (2). Assuming the validity of this generalization, one should expect to find among the most primitive invertebrates, such as sponges, fatty acids of a diversity substantially greater than that encountered among animals of higher organization.

At present there is actually very little known about the fatty acids of invertebrates in general, and about those of lower, marine species in particular. Significant data are available only for certain species of mollusks, arthropods, and annelids, but not for any members representing the lower phyla. Thus, the literature contains only the most fragmentary references to the fatty acids of sponges. As late as 1903, Cotte (3) disputed the presence of typical fatty acids in the common Mediterranean sponge, *Suberites domuncula*. A few years later, however, Henze (4) described the presence in the same species of a mixture of acids in which oleic acid was one of the components. In addition he obtained an acid, $C_{12}H_{24}O_2$, m.p. 110°, which he tentatively regarded as a branched-chain isomer of lauric acid. On the basis of our present knowledge such a possibility is contraindicated by the high melting point of the acid. The presence of butyric and oleic acid, and the absence of palmitic acid in *Reniera simulans* (3) as well as the occurence of phosphatides in *S. domuncula* (5) have been reported. In the most recent publication on this subject, *Clarke* and *Mazur* (6) have described the presence of significant amounts of free fatty acids in the lipids of freshwater and marine sponges.

The present investigation deals with the fatty acids of *Spheciospongia vesparia* and *Suberites compacta*. Both sponges belong to the order of HADROMERINA, the former to the family of *Choanitidae*, and the latter to that of *Suberitidae*. The significant differences in the nature of the sterols present in species of the two respective families have been discussed in a previous communication (7). These

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sponges had been selected because they may readily be secured in quantity, and because preliminary investigations had indicated that they contain fatty acids of an unexpectedly high molecular weight.

Spheciospongia vesparia

The starting material was a dark oil which had been obtained several years ago by an acetone extraction of rapidly air-dried sponges. It was anticipated that the more unsaturated components of this oil had undergone some decomposition during the drying of the sponges and the subsequent storage of the oil. The fatty acids obtained from this oil were at once converted into the methyl esters which were separated by distillation into three fractions. (Table IV). The high boiling points of the last two fractions and their saponification equivalents indicated the presence of substantial quantities of esters of acids higher than C_{20}.

The acids obtained from the lowest-boiling ester fraction (L, Table IV) were separated into saturated and unsaturated acids through their lead salts, and their respective methyl esters were fractionally distilled. (Tables V and VI). From certain of the saturated fractions there was eventually obtained pure palmitic and stearic acid.

The iodine value, 103, and the saponifiaction equivalent, 358, of the second ester fraction (M, Table IV) contraindicated the presence of significant quantities of material with more than two double bonds. This fraction was subdivided by distillation into twelve portions (Table VII). Hydrogenation followed by saponification of one of the fractions (M 9) afforded pure tetracosanoic acid. Another fraction (M 11) yielded a mixture of C_{26}-acids in which the presence of a hexacosadienoic acid was ascertained by bromination and isolation of a nicely crystalline tetrabromohexacosanoic acid.

The highest-boiling ester fraction (H, Table IV) contained some unsaponifiable material. Its separation was effected by saponification of the mixture and conversion of the acids to their lead salts (Table VIII). In contrast to the unsaponifiable matter, the lead salts were only sparingly soluble in hot ethanol. The acids obtained from the insoluble lead salts gave neutralization equivalents which indicated that they were of the order of C_{26} and C_{28}. They were separated into a solid and a liquid fraction by crystallization from acetone. Both fractions were converted into the methyl esters, which were then fractionally distilled (Table IX), and similar fractions were combined and redistilled. Eventually there was obtained a 12-g. fraction of an acid mixture consisting practically exclusively of hexacosenoic and hexacosadienoic acids. A new hexacosadienoic acid, m.p. 61.4–61.8°, was obtained from this fraction by a series of recrystallizations. Upon catalytic hydrogenation it gave hexacosanoic acid, and upon bromination a crystalline tetrabromide, m.p. 98.5–99°. The absorption spectrum of the acid, and its failure to react with maleic anhydride proved the absence of conjugated double bonds.

Because of lack of material the oxidative degradation of the pure acid was not attempted. Instead, an impure sample containing some hexacosenoic acid was

oxidized in acetone with potassium permanganate. The principal oxidation products were two monocarboxylic acids, caproic and heptadecanoic acid, and two dicarboxylic acids, pentadecamethylene-1,15-dicarboxylic acid and azelaic acid. In addition a number of secondary oxidation products were obtained, such as oxalic, succinic, and suberic acid and other dicarboxylic acids of lower molecular weight. These data suggest that the caproic acid and the pentamethylene-1,15-dicarboxylic acid have been derived from a $\Delta^{17,20}$-hexacosadienoic acid (I), and that this acid is identical with the one of m.p. 61.4–61.8° which has been described above. They also indicate that the heptadecanoic acid and azelaic

$$\underset{\text{I}}{CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_{15}COOH} \qquad \underset{\text{II}}{CH_3(CH_2)_{15}CH{=}CH(CH_2)_7COOH}$$

acid are products of the oxidative fission of a Δ^9-hexacosenoic acid (II) which was present in the original acid mixture. The isolation of this acid in a pure state has not yet been accomplished.

TABLE I

THE COMPONENT ACIDS OF THE LIPIDS FROM *Spheciospongia vesparia*

SATURATED ACIDS			UNSATURATED ACIDS		
Acid	Wt.-%	Mole-%	Acid	Wt.-%	Mole-%
Myristic	1.7	2.6	Tetradecenoic	0.4	0.6
Palmitic	9.1	12.1	Hexadecenoic	3.1	4.1
Stearic	0.7	0.8	Octadecenoic	15.9	18.4
			C_{20}	6.5	7.1
			C_{22}	4.4	4.4
			C_{24} (>−2H)	4.5	4.2
			C_{26}	38.8	33.6
			C_{28}	14.9	12.1

Fractional distillation of the highest-boiling ester fraction (6, Table IX) gave material which upon saponification afforded mixtures of acids consisting chiefly of the order C_{28}. Lack of material prevented the isolation of a pure component.

The over-all composition of the mixture of acids from *Spheciospongia vesparia* which gave distillable methyl esters is shown in Table I. It was estimated on the basis of yields, iodine values, and saponification equivalents, following the procedures recommended by Hilditch (8), and the methods of calculation suggested by Charnley (9), Longnecker (10), and Rapson, *et al.* (11).

Suberites compacta

The starting material was an acetone extract of sponges which had been freshly collected in Long Island Sound. Unexpected difficulties were encountered during the isolation of the crude fatty acids. A certain fraction of the acids showed a pronounced tendency to form a gel in the aqueous phase, thereby impeding a smooth separation of water and ether layers.

The acids of normal behavior were divided into numerous fractions by separa-

tion of their lead salts, fractional distillation of their methyl esters (Tables X–XII), and recrystallizations. The over-all composition of the acid mixture is shown in Table II. It was calculated by methods analogous to those mentioned above.

Palmitic acid was the only one of the lower acids isolated in a pure state. From the highest-boiling ester fractions there was eventually obtained a material

TABLE II

THE COMPONENT ACIDS OF THE LIPIDS FROM *Suberites compacta*[3]

SATURATED ACIDS			UNSATURATED ACIDS		
Acids	Wt.-%	Mole-%	Acid	Wt.-%	Mole-%
Myristic	0.3	0.5	Tetradecenoic	0.2	0.3
Palmitic	6.6	8.8	Hexadecenoic	3.1	4.1
Stearic	1.0	1.2	C_{18} (−2.3H)	11.1	13.3
Arachidic	1.1	1.2	C_{20} (−2.3H)	7.8	8.6
Behenic	0.6	0.6	C_{22} (−6.3H)	17.8	18.1
			C_{24} (−7.3H)	11.6	10.9
			C_{26} (−2.9H)	9.4	8.1
			C_{26} (−xH)	12.2	10.7
			C_{28} (−4.5H)	17.0	13.7

TABLE III

COMPARISON OF THE HYDROXYACID FROM *Suberites* WITH α-HYDROXYTETRACOSANOIC ACID

PROPERTY	SUBERITES ACID	α-HYDROXYTETRACOSANOIC ACID
Melting point	98.6–99.2°	99.5–100.5° (12), 99° (13)
Rotation	$[\alpha]_D^{22}$ +3.02° (pyridine)	$[\alpha]_D^{11}$ +3.7° (pyridine) (12) $[\alpha]_D^{22}$ −3.13 (anethole) (13)
Neutralization equivalent	384	384.6
Analysis	C, 74.67; H, 13.06	C, 74.97; H, 12.58.

(7, Table XII) consisting practically exclusively of methyl esters of C_{28}-acids. Catalytic hydrogenation of a sample of this fraction gave a quantitative yield of methyl octacosanoate, m.p. 67.2–67.5°. Repeated recrystallization of the acid mixture obtained from this fraction afforded a crystalline, new octacosenoic acid, m.p. 57.3–57.8°. Upon bromination it afforded a crystalline dibromide, m.p. 64–64.5°. When the C_{28}-acid mixture was brominated there was obtained a crystalline hexabromoöctacosanoic acid, m.p. 114.5–115.5°. Its formation proved the presence of an octacosatrienoic acid in the mixture.

The acids forming the gelatinous fraction of the original mixture were con-

[3] Exclusive of α-hydroxyacids.

verted into their magnesium salts. From the salts which were least soluble in ethanol, there was obtained an optically active acid, $C_{24}H_{28}O_3$, in form of cauliflower-like, hard crystals, which are characteristic of α-hydroxyacids. In Table III the properties of this acid are compared with those of α-hydroxytetracosanoic acid. Their great similarity suggests the identity of the two compounds.

DISCUSSION OF THE RESULTS

The data presented in Tables I and II show that each of the two sponges under investigation contains 20 or more different fatty acids. They prove that in sponges the diversity of sterols is paralleled by that of fatty acids, and they lend support to Hilditch's tenet that the more primitive the animal the more complex the mixture of fatty acids derived from it.

As has been pointed out before various factors have contributed to destroy most of the fatty acids of *Spheciospongia vesparia* with more than two double bonds. Consequently, the picture given by Table I is somewhat distorted. Nevertheless, the composition of the acid mixtures of the two sponges is strikingly similar, particularly in the most significant detail, *i.e.* the predominance of acids of the order C_{26} and C_{28}. Where they differ is at those points which one would expect to be most strongly affected by the loss of unsaturated material. Preliminary studies on the fatty acids of other sponges of the order HADROMERINA have furnished similar data.

At present sponges may be regarded as one of the best sources for unsaturated fatty acids of higher molecular weight. With but two apparent exceptions, brain cerebrosides and mussel phosphatides, no animal source has so far been reported to contain more than traces of normal, unsaturated fatty acids of orders higher than C_{24}. The presence in brain cerebrosides of small amounts of unsaturated C_{26}- and C_{28}-acids has been convincingly demonstrated by Klenk (14), and Lovern (15) has found that 20% of mussel phosphatides is represented by a mixture of unsaturated C_{28}-acids and acids which are difficult to esterify. They have not been further investigated. Shibic acid (hexacosapentaenoic acid) and thynnic acid (hexacosahexaenoic acid) have been reported by Ueno and Yonese (16) to occur in traces in tunny liver-oil, and Smith and Brown (17) have found acids higher than C_{24} in menhaden body fats. Saturated acids of the order C_{26}–C_{32} are of course common constituents of many waxes from plants and animals. No evidence has so far been obtained for the presence of any significant quantities of such acids in sponges.

As far as the authors are aware the $\Delta^{17,20}$-hexacosadienoic acid from *Spheciospongia*, and the octacosenoic acid from *Suberites* have not been previously found in nature. The possibility exists that the Δ^{9}-hexacosenoic acid, when obtained in a pure state, will prove to be identical with the hexacosenoic acid of m.p. 45°, first isolated by Klenk and Schumann (14) from brain cerebrosides. It is of interest to note in this connection that the α-hydroxytetracosanoic acid from *Suberites* has also been found to be a moiety of brain cerebrosides (18).

It is probable that at least a part of the higher fatty acids were present in the acetone-benzene soluble lipids of the sponges as phosphatides or in cerebroside-

like combinations rather than as triglycerides. In this preliminary study, carried out with limited material, no effort was made to differentiate between triglycerides or phospholipids. The unpleasant amine-like odor given off during the saponification of the original extract gave evidence of the presence of phospholipids.

In 1948, Hilditch (19) pointed out that but for two known exceptions all monoethenoid acids from plant and animal sources bear a structural relationship to oleic acid in that they contain the heptamethylene group $(CH_2)_7$ on one or the other sides of the double bond. In acids originating from plants this fragment occurs exclusively to the left of the double bond with the latter moving progressively further away from the carboxyl group as the chain length increases. In animal fats no such strict relationship exists, but more often than not the unsaturation is found at the ninth carbon atom. It is of interest to note therefore that the Δ^9-hexacosenoic acid from *Spheciospongia* is so constituted.

Much less is known about straight chain, diethenoid fatty acids. As far as the authors are aware, the only unconjugated representatives which have so far been found in nature are linoleic acid, $\Delta^{11,14}$-eicosadienoic acid, $\Delta^{11,14}$-docosadienoic acid (20), and $\Delta^{18,16}$-docosadienoic acid (21). Of these the most reliable information is available for the first and the last, and they both contain the group $CH_3(CH_2)_4CH{=}CHCH_2CH{=}$. The same terminal group is present in the $\Delta^{17,20}$-hexacosadienoic acid obtained from *Spheciospongia*.

EXPERIMENTAL

All melting points are corrected.

Spheciospongia vesparia

To one liter of 10% alcoholic potassium hydroxide was added 300 ml. of the oil obtained by the acetone extraction of air-dried sponges. After the solution had been heated for several hours in an atmosphere of nitrogen about one-half of the alcohol was removed under reduced pressure. Water (500 ml.) was added and another 250 ml. of solvent distilled off. The solution was made up to one liter by addition of water and extracted four times with 250-ml. portions of ether. During each extraction a blackish, semi-solid interphase was formed, which was withdrawn, washed with water and ether, and discarded. The combined ether-extracts gave 116 g. ofunsaponifiable material (22). The black soap solution was acidified with sulfuric acid. The brown curdy acids were coalesced to a semi-solid mass by alternate warming and cooling and the aqeous layer was decanted. The latter was extracted twice with ether and reserved for the determination of glycerol. The acids were dissolved in ether, and the solution was washed free of mineral acid and evaporated to dryness. Water was removed from the residue by co-distillation with benzene. The final residue weighed 113 g. Several other lots of oil were similarly saponified.

The crude acids (182 g.) were converted into the methyl esters by refluxing with methanol containing 1% of conc'd sulfuric acid (800 ml.). The neutral methyl esters (176 g.) were rapidly distilled from a 300-ml. Claisen flask with a built-in Vigreux column. The results are shown in Table IV.

Fraction L. Saponification of this yellow oil gave 25.5 g. of an acid mixture. It was separated into three fractions by way of the lead salts; L1, solid acids, (5.2 g); L2, liquid acids, (15.5 g.), and L3, acids whose lead salts were of intermediate solubility, (4.1 g.).

Fraction L1. The acids were converted into their methyl esters (5.2 g.) which were then fractionated. The distillation was carried out through a column 35 cm. long and 6 mm.

wide containing a removable Pyrex spiral. Two thermometers were attached to the tube, one with the bulb near the top and the other at the bottom. The column was encased in a Pyrex tube of 30 mm. diameter surrounded by a Nichrome wire heating unit. This was in turn surrounded by a Pyrex tube of about 45 mm. diameter. The efficiency of the column had been established by the separation of a 1 g. mixture of cetyl palmitate and stearate. The results of the fractionation of L1 are shown in Table V.

Palmitic acid. A sample (0.67 g.) of fraction 3 (Table V) was saponified and the resulting acid was recrystallized twice from acetone (0.38 g.); m.p. 62.5–62.9°. It did not give a depression of the m.p. when mixed with authentic palmitic acid.

Anal. Calc'd for $C_{16}H_{32}O_2$: N. E., 256.4; Found: N. E., 256.

TABLE IV

PRELIMINARY FRACTIONATION OF THE METHYL ESTERS OF ACIDS FROM *Spheciospongia*

FRACTION	B.P.,[a] °C.[4]	WT. G.	S. E.[5]	I. V.[6]
L	135–165	27.7	282	56.3
M	165–215	35.2	358	103
H	216–228	37.1	460	112
	285–300	15.0	469	114

[a] Pressure: 1.5–2.0 mm.

TABLE V

FRACTIONATION OF THE METHYL ESTERS OF THE SOLID ACIDS FROM L

FRACTION	B.P., °C.[4]	WT., G.	S. E.[5]	n_D^{45}
1	125	0.87	250	1.4292
2	126–131	.90	267	1.4310
3	132–135	1.73	—	1.4318
4	135–138	0.65	281	1.4343
5	139–145	.82	293	1.4390
Res.		.2		

[a] Pressure: 1.3 mm.

Stearic acid. The acid obtained from fraction 5 (Table V) was recrystallized six times from acetone, 60 mg; m.p. 68.9–69.4°.

Anal. Calc'd for $C_{18}H_{36}O_2$: N. E., 284.5; Found: N. E., 284.

Fraction L2. The acids were converted to the methyl esters (15.1 g. which were then fractionated in the still described above. The results are shown in Table VI.

Fraction L3. Recrystallization of this fraction from acetone at 5° gave 0.9 g. of a solid fraction, (N. E. 259; Iodine Value, 7.4), and 3.1 g. of a liquid fraction, (N. E., 278.5; Iodine Value, 76.3). Fraction L3 therefore appeared to consist principally of palmitic and oleic acids.

Fraction M. The high saponification equivalent and low iodine values of this fraction suggested the possibility that it was contaminated with unsaponifiable material. Saponification of the mixture, however, yielded only minute traces of material insoluble in alkali.

[4] Temperature measured at the head of the column.

[5] Saponification equivalent.

[6] The iodine values were determined according to Yasuda's modification of the Rosenmund-Kuhnhenn method (32).

The acids were then reconverted into the methyl esters which were fractionally distilled as described above. The results are shown in Table VII.

Fraction M6. Catalytic hydrogenation of this fraction, followed by saponification and recrystallization of the acids gave a product (175 mg.) of m.p. 75–75.5°; N. E., 344. Its properties were analogous to those of a mixture containing 88 mole-% docosanoic acid and 12 mole-% tetracosanoic acid.

TABLE VI

FRACTIONATION OF THE METHYL ESTERS OF THE LIQUID ACIDS FROM L

FRACTION	B.P.,[a] °C.[4]	WT. G.	S. E.[5]	I. V.[6]	n_D^{25}
1	117	0.15	—	—	1.4550
2	119–123	5.50	262	31.9	1.4423
3	124–127	3.00	274	61.4	1.4480
4	135–139	3.22	291	80.2	1.4513
5	140–145	2.40	300	83.5	1.4533
Res.		0.82			

[a] Pressure: 0.9 mm.

TABLE VII

FRACTIONATION OF M

FRACTION	B.P.,[a] °C.[4]	WT., G.	S. E.[5]	I. V.[6]
M 1	131–138	1.72	282	81.3
M 2	140–144	3.12	295	84.4
M 3	145–147	1.69	299	78.8
M 4	155–159	5.07	322	93.8
M 5	159–163	2.97	341	83.1
M 6	166–170	1.68	353	80.2
M 7	173–179	0.49	359	72.3
M 8	180–185	1.35	369	83.4
M 9	185–192	2.25	380	80.4
M 10	199–203	2.95	398	107.6
M 11	203–205	6.25	411	108
M 12	200–195	0.87	413	112
Res.	Tar	2.53		

[a] Pressure: 0.1–0.2 mm.

Fraction M9. (*Tetracosanoic acid*). Catalytic hydrogenation of this fraction, followed by saponification and two recrystallizations of the resulting acid from acetone gave tetracosanoic acid, m.p. 84–84.4°. The melting point with authentic tetracosanoic acid was 83.9–84.2°.

Anal. Calc'd for $C_{24}H_{48}O_2$: N. E., 368.6. Found: N. E., 368.

Fraction M11. (*Tetrabromohexacosanoic acid*). A sample (330 mg.) of the acid mixture obtained from this fraction was dissolved in ether (10 ml.). At 0° bromine was added dropwise until absorption no longer took place. An equal volume of petroleum ether was added and the mixture kept at 5° for two days. The precipitate (175 mg.) was recrystallized five times from petroleum ether (30–60°); microcrystalline powder, m.p. 120–121°.

Anal. Calc'd for $C_{26}H_{46}Br_4O_2$: C, 43.8; H, 6.8; Br, 44.9.

Found: C, 44.3; H, 6.8; Br, 44.1.

Fraction H. The reddish-brown fraction was saponified in an atmosphere of nitrogen,

and the mixture of acids was at once dissolved in 500 ml. of 95% ethanol. To the boiling solution was added 500 ml. of a hot solution of 45 g. of lead acetate and 2 g. of acetic acid in 95% ethanol. Most of the lead salts thus formed were insoluble in the boiling ethanol. They melted around 50°, and formed a liquid layer on the bottom of the flask. The more soluble material was removed by decanting the supernatant liquid and by repeated extraction of the residue with hot ethanol.

The combined alcoholic solutions were concentrated to 750 ml. and cooled. A small amount of a yellow granular precipitate was obtained. It was recrystallized from 400 ml. of ethanol. The mother liquors were combined and concentrated in *vacuo* to a very small volume. The lead salts from the three fractions were converted into the free acids which are listed in Table VIII.

TABLE VIII

ACIDS FROM LEAD-SALT SEPARATION OF FRACTION H

FRACTION	WT. G.	N. E.	I. V.[6]	PROPERTIES OF Pb-SALTS
H 1	0.84	407	89.1	Recrystallizeable from EtOH
H 2	15.11	600	—	Soluble in EtOH
H 3	33.0	401	101	Insoluble in hot EtOH

TABLE IX

FRACTIONATION OF THE METHYL ESTERS OF THE LIQUID ACIDS OF H 3

FRACTION	B.P.,[a] °C.	WT., G.	n_D^{25}	I. V.[6]
1	200	0.37	1.4590	
2	200–205	4.72	1.4610	
3	207–211	4.13	1.4616	
4	212–222	5.62	1.4623	107
5	223–231	3.94	1.4650	
6	232–237	2.06	1.4790	
Res.	Tar	3.89		

[a] Pressure: 0.3 mm.

Fraction H1. Several recrystallizations of this fraction from acetone, methanol, and petroleum ether gave a colorless powder (0.4 g); m.p. 52–54.5°; N. E. 403; I. V. 80. The properties of this material were those of a mixture of C_{26}- and C_{28}-acids.

Fraction H2. This fraction was converted into the methyl esters which were then fractionally distilled at 1 mm. and below. The largest fraction (7.1 g.) was optically active, $[\alpha]_D$ −4.4°; S. E. 692; I. V. 145. The rotation and high S. E. value were due to the presence of unsaponificable material. The latter was isolated by a chromatographic separation of part of this fraction. It consisted of a mixture of cliona- and porifera-sterol (22). Another sample of this fraction (570 mg.) was hydrogenated. It yielded a material (195 mg.) of m.p. 62.8–63°, which gave no depression of the melting point when mixed with an authentic sample of methyl hexacosanoate of m.p. 63°. It may be concluded therefore that this fraction consisted essentially of a mixture of sterols and methyl esters of C_{26}-acids.

Fraction H3. The neutralization equivalent of this fraction indicated that it consisted principally of acids of the order C_{26} and C_{28}. The fraction was dissolved in acetone (150 ml.) and the solution cooled overnight. There was obtained a colorless, crystalline solid (7.2 g.), m.p. 46–55°; N. E. 399; I. V. 104. Evaporation of the mother liquor gave a mixture of liquid acids (25 g.). Both fractions were subjected to a series of fractional distillations of

their methyl esters and recrystallizations which eventually led to the isolation of C_{26}-acids. Since the methods involved were essentially the same in both cases, only the separation of the liquid acid mixture will be discussed.

This fraction was converted into the methyl esters (24.7 g.) which were then fractionated in the still described above. The results are shown in Table IX.

Hexacosadienoic acid. Fractions 2–5 (Table IX) were combined and refractionated. Several of the new fractions appeared to consist exclusively of methyl esters of hexacosenoic and hexacosadienoic acids. These fractions were combined (12.7 g.); S. E. 408; I. V. 106, and converted into the acids (12 g.). These were dissolved in anhydrous ether (50 ml.), and the solution cooled to −5°. The crystalline material (0.91 g.), m.p. 58–59.5°, was recrystallized from acetone until a constant melting point was reached (0.5 g.). The acid crystallized in glistening, waxy plates, m.p. 61.4–61.8°, setting point 61.0°. It showed no absorption in the ultraviolet between 220 and 270 mμ. Several unsuccessful attempts were made to shift the double bonds of this acid into conjugation according to the method of Hilditch, *et al.* (21, 23).

Anal. Calc'd for $C_{26}H_{48}O_2$: C, 79.54; H, 12.32; N. E., 392.6; I. V., 129.5.
Found: C, 79.75; H, 12.36; N. E., 394, 392; I. V., 130, 129.

Tetrabromohexacosanoic acid. The bromination of the above acid was carried out according to the procedure of Kass, *et al.* (24) with slight modifications. To the acid (50 mg.) in a mixture of equal parts of ether and petroleum ether (10 ml.) was added dry bromine dropwise and with vigorous stirring. Throughout the addition the temperature was kept at 0°. Addition was continued until comparison with a blank showed the bromine to be in excess by about two drops, and until the color did not change during 20 minutes. A few drops of β-amylene were then added to remove the bromine excess. After standing overnight at 5° the solution had deposited some crystalline material (51 mg.); m.p. 92–98°. This was recrystallized twice from a mixture of ether and petroleum ether, b.p. 30–60°, and eight times from petroleum ether. The tetrabromide was microcrystalline; m.p. 98.5–99°.

Anal. Calc'd for $C_{26}H_{48}Br_4O_2$: Br, 44.88; N. E., 712.2.
Found: Br, 44.67; N. E., 709, 712.

Hexacosanoic acid. A sample of the dienoic acid (132 mg.) in a mixture of equal parts of ether and ethanol (60 ml.) was hydrogenated at 40 p.s.i. with a platinum catalyst. Concentration of the filtrate gave a crystalline material (125 mg.), m.p. 84–86°. After two recrystallizations from acetone the acid was obtained in form of glistening, waxy plates, m.p. 86.8–87.5°, setting at 86°. When mixed with an authentic sample of hexacosanoic acid, m.p. 87.4–87.6°, the melting point was 87–87.5°.

Anal. Calc'd for $C_{26}H_{52}O_2$: N. E., 396.7. Found: N. E., 396.

Oxidation of the mixture of hexacosenoic and hexacosadienoic acids. The acids obtained from the mother liquors of the crystallization of the dienoic acid, described above, were oxidized according to the method of Brown and Farmer (25). In one example, 5% potassium permanganate (300 ml.) was added during one day to the acids (2.5 g.) and sodium carbonate (0.7 g.) in water (600 ml.). The mixture was left at room temperature with occasional shaking for two days. It was then decolorized with sodium bisulfite and the manganese dioxide removed. The latter was washed repeatedly with hot water, and the washings were combined with the filtrate.

The basic solution was concentrated (500 ml.), acidified with hydrochloric acid, and steam-distilled. The distillate was collected in 500-ml. portions. With the second portion a white solid began coming over. The distillation was continued for two days until this material had ceased to distil. A total of 16 liters of distillate was collected. Each was filtered separately to secure the white solid, Fraction A. All filtrates were combined; Fraction B. The material remaining in the distilling flask was Fraction D.

Heptadecanoic acid (margaric acid). Fraction A was dissolved in ether, and the solution was dried over sodium sulfate, treated with Norit, and evaporated to dryness (550 mg.). After one recrystallization from acetone (10 ml.) this yellowish, waxy material gave small, glistening plates (500 mg.), m.p. 56.5–57.5°. After one recrystallization each from acetone

and methanol, the acid melted at 59–59.2°, setting at 58.4°. The melting point did not change during subsequent repeated recrystallizations from acetone, methanol, and petroleum ether. The mixture melting point with authentic margaric acid was 59.2–60°.

Anal. Calc'd for $C_{17}H_{34}O_2$: C, 75.55; H, 12.59; N. E., 270.44.

Found: C, 75.75; H, 12.27; N. E., 269.3, 269.8.

Caproic acid. Fraction B was made basic and concentrated to 500 ml. It was acidified and extracted with ether three times. Washing, drying, and evaporation of the combined ether extracts gave a yellow oil (350 mg.) with the characteristic odor of valeric or caproic acid. The oil was distilled from a semimicrodistillation apparatus at 65 mm. The fraction (240 mg.) boiling between 135–140° was a colorless oil; n_D^{20} 1.4166. Reported for caproic acid, n_D^{20} 1.4170.

A sample of the acid (50 mg.) was converted to the *p*-bromphenacyl ester by the standard procedure. After four recrystallizations from 75% ethanol the derivative (78 mg.) was obtained in form of colorless, small plates, m.p. 71–72°. It did not give a depression of the melting point when mixed with authentic *p*-bromophenacyl caproate of m.p. 71.6–72°.

Anal. Calc'd for $C_{14}H_{17}BrO_3$: Br, 25.61. Found: Br, 25.46.

Fraction D. The aqueous liquid (400 ml.) remaining in the distilling flask contained some yellow, scummy material. It was extracted four times with 75-ml. portions of ether. The combined ether extracts were washed twice with cold water, dried over sodium sulfate, treated with Norit, and evaporated to dryness. The residue (1.5 g.), Fraction E, was a waxy material interspersed with a powdery solid.

Succinic acid. Digestion of Fraction E with benzene afforded a small amount of insoluble material which was recrystallized from a mixture of ether and benzene (40 mg.); m.p. 183–184°. It gave no depression of the melting point when mixed with authentic succinic acid.

Anal. Calc'd for $C_4H_6O_4$: N. E., 59.05. Found: N. E., 58.2, 59.0.

The benzene solution from which the succinic acid had been removed was evaporated to dryness and the residue was extracted four times with hot water. An oily material, floating on the surface of the aqueous extracts, was conveniently removed by filtration through a layer of cotton. The low-melting wax, remaining on the cotton, was combined with the remainder of the water-insoluble residue, Fraction E1. The combined aqueous filtrates were kept at 5° for 12 hours when the precipitate, Fraction E2, was collected (267 mg.), m.p. 98–110°.

Azelaic acid. A solution of Fraction E2 in hot water (100 ml.) was made basic with ammonia and then boiled for a few minutes with aqueous calcium chloride. A curdy precipitate formed which was collected after one day and washed thoroughly with water. It was then treated in a separatory-funnel with dilute hydrochloric acid (25 ml.), and the mixture was extracted five times with 25-ml. portions of ether. The combined extracts were washed with cold water, dried over sodium sulfate, and concentrated to a small volume (50 ml.) An equal volume of petroleum ether was added, and the mixture was kept at 5° for six hours. The powdery solid (20 mg.), m.p. 96–104°, was recrystallized four times from an acetone-petroleum ether mixture and finally from water; small platelets (71 mg.), m.p. 106–107°.

Anal. Calc'd for $C_9H_{16}O_4$: N. E., 94.1. Found: N. E., 93.5.

Suberic acid. The filtrate from the calcium azelate was acidified with hydrochloric acid and extracted five times with 25-ml. portions of ether. The ether extracts were combined, washed free of mineral acid, dried over sodium sulfate, and concentrated to a small volume (10 ml.). Addition of petroleum ether (20 ml.) gave a white powder (83 mg.), m.p. 108–117°. After six recrystallizations from an acetone-petroleum ether mixture the suberic acid was obtained in form of small, colorless needles (41 mg.), m.p. 140–142°.

Anal. Calc'd for $C_8H_{14}O_4$: N. E., 87.1. Found: N. E., 86.8, 87.3.

Pentadecamethylene-1,15-dicarboxylic acid. Systematic fractional crystallization of Frac-

tion E1 eventually afforded this acid as a microcrystalline material (80 mg.), m.p. 114–115°. Reported, m.p. 118° (26), 116° (27).

Anal. Calc'd for $C_{17}H_{32}O_2$: C, 68.00; H, 10.67; N. E., 150.2.
Found: C, 67.99; H, 10.61; N. E., 150.2, 150.3.

In another experiment the methyl esters of the original C_{26}-acids were oxidized according to the procedures recommended by Haworth (28) and Hilditch (29). The mixture of half-esters of dicarboxylic acids thus obtained was saponified, and the resulting acids were separated. The yield of the pentadecamethylene dicarboxylic acid was somewhat higher than that obtained in the process described above.

C_{28}-acids. Highest-boiling methyl ester fractions, such as fraction 6, (Table IX), were combined and fractionally distilled at 0.3–0.5 mm. The fractions with saponification equivalents of 425–427 were combined and converted to the acids. Repeated recrystallization of these acids gave products of indefinite m.p.'s only, such as 62–79°, I. V. 113. Hydrogenation of the mixture also led to a mixture, m.p. 79–85°.

Anal. Calc'd for $C_{26}H_{52}O_2$: N. E., 398; for $C_{28}H_{56}O_2$: N. E., 424.
Found: N. E., 417.

These results indicate that this acid mixture contained a preponderance of C_{28}-acids.

Glycerol. A portion of the acidic solution from which the original fatty acids had been extracted was made basic with sodium hydroxide and evaporated almost to dryness on a steam-bath. The residue was mixed with anhydrous sodium sulfate and the resulting cake ground to a fine powder. This was throughly extracted with acetone (Soxhlet). The oil (800 mg.) remaining after evaporation of the acetone was twice distilled from a semi-micro distillation apparatus. In each case a black residue was left in the pot. The distillate boiled at 120° at 10 mm.; n_D^{25} 1.4726; reported for glycerol, n_D^{25} 1.4730.

A sample of the glycerol was benzoylated with benzoyl chloride in pyridine, and the benzoate was recrystallized five times from ethanol, m.p. 75.2–76°. When mixed with authentic glycerol tribenzoate of m.p. 76°, it melted at 75.5–76°.

Suberites compacta

Isolation of the lipid. The sponges, freshly collected near Block Island, Long Island Sound, were passed through a meat grinder into two 20-l. bottles. Sufficient acetone was added to each flask to double the volume therein, and the mixture was allowed to stand for two weeks. The supernatant liquid was then withdrawn, and the solids were shaken twice with acetone and finally collected. They were charged in approximately 800-g. batches into a large Soxhlet apparatus (1) and extracted for one day with acetone. The insoluble material, after drying, weighed 3.49 kg.

All liquid extracts were combined, and most of the acetone was distilled under somewhat reduced pressure in the presence of nitrogen. The oil was removed from the remaining liquid by repeated extraction with ether. The combined ether extracts (4 l.) were concentrated to about 200 ml. The remainder of the ether and the water were removed by co-distillation with benzene. The benzene was then evaporated under reduced pressure in an atmosphere of nitrogen.

The lipid was a dark viscous mass (115 g.) which was practically solid at 0°; S. E. 588; I. V. 83.

Isolation of the fatty acids. The lipid was saponified with 1 *N* alcoholic potassium hydroxide (500 ml.) in an atmosphere of nitrogen. Most of the ethanol was then removed at reduced pressure while the total volume was kept almost constant by the occasional addition of water. The solution was then made strongly acid with sulfuric acid and extracted four times with 100-ml. portions of ether. The combined ether layers were washed with water. All aqueous layers were reserved for the isolation of glycerol.

The ether solution was extracted six times with 100-ml. portions of 5% sodium carbonate and three times with water. Evaporation of the ether gave 33 g. of unsaponifiable material (30).

All soap solutions were combined and acidified with sulfuric acid. The jelly-like mass of acids which had separated seemed only partially soluble in ether. When shaken with this solvent, an emulsion was formed which did not separate into layers even after long standing. Centrifugation of the mixture brought about a partial separation of the layers. It was found to be more advantageous at this stage to add a small amount of ethanol to the mixture to facilitate a normal separation of the layers. The ether layer was washed with water containing some ethanol. The use of water alone emulsifies the mixture. Evaporation of the ether extracts gave a dark green, wax-like material.

Lead salt separation of the acids. The fatty acids were dissolved in boiling ethanol (500 ml.), and to this solution was added a hot solution of lead acetate (50 g.) and acetic acid (5 ml.) in ethanol (500 ml.) A large portion of the lead salts precipitated in form of a heavy oil which solidified around 60°.

The supernatant liquid was decanted and cooled to 15°. The precipitate which had formed was recrystallized from ethanol (3.6 g.), m.p. 115–120°. It was lead-free, insoluble in sodium carbonate solution, and hence unsaponifiable material.

TABLE X

FRACTIONATION OF THE METHYL ESTERS OF FRACTION A OF THE *Suberites* ACIDS

FRACTION	B.P.,[a] °C.[4]	WT. G.	S. E.[5]	I. V.[6]
1	123–128	0.95	264	60.0
2	129–134	1.35	274	78.8
3	135–139	4.15	293	90.3
4	140–146	2.03	339	171
5	147–154	4.12	346	220
6	155–160	2.46	356	255
7	161–168	3.76	370	275
Res.		5.16	402	

[a] Pressure: 0.3 mm.

The alcoholic solution containing the soluble lead salts was concentrated under reduced pressure to about 50 ml. Water and hydrochloric acid were then added, and the mixture was extracted with ether containing a small amount of alcohol. The ether extract was washed several times with a 5% sodium carbonate and water. Upon evaporation it gave another quantity (5.5 g.) of unsaponifiable material. The carbonate extracts were acidified and the acids were taken up in ether. Atempts to wash the ether extracts with water once more led to emulsions. A separation was eventually effected by adoption of the following procedure.

The emulsion was centrifuged until a clear separation into layers had been achieved. The ether layer was decanted, and the gelatinous, aqueous phase was shaken with ether and centrifuged again. The process was repeated until the ether layer remained practically colorless. The combined ether layers were then shaken with water, and the resulting emulsion was centrifuged and treated as before. This process was repeated until a shaking of the combined ether layers no longer formed significant emulsions. They were then dried and evaporated to dryness. The residue, representing the "liquid" acids, Fraction A, weighed 25.1 g.

The insoluble lead salts were decomposed with hydrochloric acid and the acids taken up in ether. Here also emulsions were formed, which were separated as described above. Evaporation of the ether extract gave 20.2 g. of "solid" acids, Fraction B.

The gelatinous, aqueous fractions from the liquid and solid acid separations were combined, and the water was removed in *vacuo*. There remained 11.45 g. of a gummy liquid containing some solid (Fraction C).

Fraction A. The acids were converted to the methyl esters, of which 24.1 g. was fractionally distilled in the apparatus described above. The results are shown in Table X. The range of saponification equivalents indicated the presence of esters of acids from C_{14} to C_{28}.

Fraction B. The acids were converted into their methyl esters which were then distilled. The results are shown in Table XI. The bulk of the distillate consisted of esters of acids of the order C_{26}–C_{28}. Fraction 2 was refractionated at 1 mm. One of the fractions upon saponification gave pure palmitic acid, m.p. 62.5–62.9°; N. E., 256.

α-Hydroxytetracosanoic acid. Fraction 4 (Table XI) contained some solid material, suspected of being a derivative of a hydroxy acid. The entire fraction was therefore saponi-

TABLE XI

FRACTIONATION OF THE METHYL ESTERS OF FRACTION B OF THE *Suberites* ACIDS

FRACTION	B. P.,[a] °C.[4]	WT., G.	S. E.[5]	I. V.[6]	PRESSURE
1	110–114	0.41	265	2.8	
2	115–119	2.52	281	20.2	0.2 mm.
3	138–144	2.56	323	66.6	
4	145–215	12.92	411	99.6	0.05 mm.
Res.		1.72	432	86.5	

TABLE XII

FRACTIONATION OF THE METHYL ESTERS OF ACIDS FROM FRACTION 4 (Table XI)

FRACTION	B.P.,[a] °C.[4]	WT., G.	S. E.[5]	I. V.[6]
1	159–160	0.17	332	51.3
2	172–179	0.53	350	59.0
3	184–193	0.80	376	67.2
4	194–199	0.96	400	84.8
5	200–204	3.29	413	98.6
6	205–209	1.98	428	123
7	210–213	3.48	434	133
Res.		0.36	464	74.6

[a] Pressure: 0.03–0.04 mm.

fied and the acids converted into their magnesium salts. Decomposition of the ethanol-insoluble magnesium salt with hydrochloric acid afforded an acid which after one recrystallization melted at 88–93° (260 mg.).

Solutions of this acid in ether, benzene, chloroform, and petroleum ether gelled when cooled slowly. The acid was best recrystallized by rapid cooling of a solution in acetone. After several recrystallizations from this solvent the melting point remained constant at 98.6–99.2°; $[\alpha]_D$ 25° +3.02° (61.4 mg. in 3.09 ml. of pyridine, α +0.06°).

Anal. see Table III.

Octacosenoic acid. The soluble magnesium salts (see above) were converted into the acids, the methyl esters (11.6 g.) of which were then fractionally distilled. The results are shown in Table XII. The acid mixture obtained from a portion of fraction 7 was divided, by crystallization from acetone at −5°, into a solid (160 mg.) and a liquid fraction (900 mg.). After six recrystallizations of the solid fraction the melting point remained constant at 57.3–57.8°, setting at 56.4°. It crystallized in the form of fine, waxy needles.

Anal. Calc'd for $C_{28}H_{54}O_2$: C, 79.56; 12.88; N. E., 422.7; I. V. 60.1.

| Found: C, 79.26; H, 13.05; N. E., 424; I. V., 60.5, 61.4.

Dibromoöctacosanoic acid. A sample (35 mg.) of the above acid was brominated in petroleum ether (5 ml.). The crude bromide (40 mg.) was recrystallized from petroleum ether, m.p. 64–64.5°.

Anal. Calc'd for $C_{28}H_{54}O_2$: Br, 27.43; N. E., 582.5.

Found: Br, 27.74; N. E., 584.

Methyl octacosanoate. A sample (450 mg.) of fraction 7 (Table XII) was hydrogenated at 40 p.s.i. in a mixture of equal parts of ether and ethanol (50 ml.) with a platinum black catalyst. After filtration and concentration the saturated ester was obtained (396 mg.), m.p. 66.8–67.4°. After recrystallization from acetone it gave large, colorless plates, m.p. 67.2–67.5°. Reported for methyl octacosanoate, m.p. 67.5° (31).

Anal. Calc'd for $C_{29}H_{58}O_2$: S. E., 438.7. Found: S. E., 439.

Octacosanoic acid. Saponification of the ester described above gave octacosanoic acid in form of large, glistening plates, m.p. 90.1–90.4°. Reported 90.3–90.9° (31).

Anal. Calc'd for $C_{28}H_{56}O_2$: N. E., 424.7. Found: N. E., 424.

Hexabromoöctacosanoic acid. The liquid fraction, obtained during the isolation of the octacosenoic acid, was dissolved in ethanol. To the solution was added dropwise a hot solution of barium hydroxide in ethanol until no more precipitation could be observed. The barium salts were collected, washed throughly with ethanol, and then dissolved in ether. The ether solution was washed three times with dilute hydrochloric acid and three times with water, dried over sodium sulfate, and concentrated to a small volume (10 ml.).

The solution was cooled to 0°, and bromine was added in fine drops until decolorization no longer took place. After five hours standing at 5°, the precipitate was recrystallized five times from ether until a constant melting point was reached. The product was a fine, white powder, m.p. 114.5–115.3°.

Anal. Calc'd for $C_{28}H_{50}Br_6O_2$: Br, 53.58. Found: Br, 53.29.

Fraction C. This fraction was dissolved in equal parts of ether and acetone. Some insoluble, inorganic material was removed and the solution was cooled to 0°. The precipitate (2.9 g.) was collected and the filtrate evaporated to dryness. There remained a brown oil of disagreeable, fishy odor, which was not further investigated.

The precipitated acids were converted into their magnesium salts by treatment with a hot, 5% solution of magnesium acetate in 95% ethanol. The salts were washed repeatedly with hot ethanol, and they were then decomposed in a mixture of ether and dilute hydrochloric acid. The acids obtained from the ether extract (2.3 g.) were repeatedly recrystallized from acetone, (440 mg.), m.p. 91–95.5°. The product was once more purified over the magnesium salt and then recrystallized nine times from acetone. The final product crystallized in form of cauliflower-like globules which are regarded as characteristic of higher hydroxy acids (14), m.p. 98.6–99.2°. A mixture of this acid with the hydroxytetracosanoic acid described above did not give a depressed melting point.

Anal. Calc'd for $C_{24}H_{48}O_3$: N. E., 384.6. Found: N. E., 385.

Glycerol. Glycerol was isolated from the acidic solution from which the original acids had been extracted by means of the procedure described under *Spheciospongia vesparia*. It was converted to the tribenzoate, m.p. 75.5–76°.

SUMMARY

The component acid mixtures of the lipids from the sponges, *Spheciospongia vesparia* and *Suberites compacta* have been investigated. Their most characteristic features are their great complexity and the preponderance of C_{26}- and C_{28}-acids.

Palmitic acid and stearic acid have been isolated from the acid mixture obtained from *Spheciospongia*. The presence in this mixture of unsaturated C_{24}-acids has been demonstrated by their conversion to tetracosanoic acid.

A new dienoic acid has been isolated from the mixture of C_{26}-acids. It was

identified by its conversion to a tetrabromide and to hexacosanoic acid. Oxidative fission has given results indicating that this acid is $\Delta^{17,20}$-hexacosadienoic acid. The presence of Δ^{9}-hexacosenoic acid has also been demonstrated.

Palmitic acid and α-hydroxytetracosanoic acid have been isolated from the acid mixture obtained from *Suberites*. A new octacosenoic acid has been obtained from the C_{28}-acid fraction of this mixture. It was identified by its conversion to a dibromide and to octacosanoic acid. The presence in the mixture of an octacosatrienoic acid was demonstrated by the isolation of a hexabromoöctacosanoic acid.

Glycerol has been isolated from the lipids of both sponges.

The significance of these observations has been discussed.

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