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CHAPTER I

INTRODUCTION

Halogens

Iodine was first discovered in marine organisms in 1811 by Courtois (1) who found it in kelp. Fyfe (2), in 1819, reported iodine in sponges and stated that it did not exist in sponges in the same state of combination as in other organisms such as the cryptogams. Iodine is readily extracted into water from these organisms whereas it is not from sponges. Balard (3) in 1925, used the reaction between iodine and starch to demonstrate the presence of iodine in molluscs, coral, gorgonians and seaweed. In 1843, Croockewit (4) isolated from bath sponges an iodine-containing protein-like substance which resembled silk fibroin in its chemical characteristics. The iodine content, 1.08 per cent, was determined by decomposing the protein, precipitating the iodine and weighing it as palladous iodide. Vogel (5), for the first time, in 1848, declared that the iodine in sponges was in organic combination. Witting (6), in 1858, showed that the ash of algae contained iodine and chlorine and Eschle (7), in 1897, determined that certain algae contain from 0.02 to 0.03 per cent iodine.

Hundeshagen (8), in 1895, in an interesting paper on sponges claimed to have found as much as 14 per cent iodine and 1 to 2 per cent bromine and chlorine. He attributed the extraordinarily rich content of halogens to the hot climate from which the sponges came. In later work Mörner (9) stated that the variations of halogen content does not depend on climatic conditions. Ackermann and Burchard (10) have reported a low iodine content of sponges compared to Hundeshagen's figures. Both investigated Aplysina compressa. Hundeshagen reporting 9-10 per cent and Ackermann and Burchard reporting 0.43 per cent.

In 1896, two discoveries stimulated research on marine organisms containing iodine and on iodine compounds in general. Baumann (11) showed that iodine was a component of the normal sheep's thyroid and Drechsel (12) isolated a specific compound containing iodine from the gorgonian, Gorgonia cavolinii. Drechsel called this compound iodogorgoic acid. On the basis of the empirical formula he obtained, $C_4H_8NIO_2$, he suggested that it was an iodoaminobutyric acid. The protein material from the horny axial skeleton of the gorgonian, termed gorgonin (13), was the source of iodogorgoic acid. Drechsel also demonstrated, by hydrochloric acid hydrolysis, that gorgonin contained, besides iodogorgoic acid, leucine, tyrosine, lysine, lysatine and ammonia, definitely proving it to be protein in nature. He suggested that the gorgonian has a specific metabolism for incorporating iodine into the skeleton since he found very little, if any, iodine in the coenenchyma.

In 1898, Harnack (14) isolated a substance from the bath sponge, called iodospongin, similar to Drechsel's gorgonin. He showed the iodine content of the sponge to be 1.5-1.6 per cent. In 1900,

Mendel (15) repeated Drechsel's procedure for the isolation of iodogorgoic acid from Gorgonia acerosa, from the West Indies, but was unsuccessful. In 1903, Henze (16) showed that iodogorgoic acid could not be an iodoaminobutyric acid because it gave the xanthoproteic reaction. The identification of iodogorgoic acid was not completed until 1905 when Wheeler and Jamieson (17) synthesized 3,5-diiodotyrosine and showed it to be identical with the natural product. Later, in 1909, Wheeler and Mendel (18) isolated an acid from iodospongin which they showed to be identical with 3,5-diiodotyrosine. Oswald (19), in 1911, determined the 3,5-diiodotyrosine in gorgonin and iodospongin.

In 1907, Mörner (9) found bromine in gorgonians and suggested that the bromine containing acid analogous to 3,5-diiodotyrosine should also be present. This was confirmed in 1913 when Mörner (20) isolated and identified dibromo-d,l-tyrosine from the gorgonian Primnoa stengel.

In 1948, Fromagoet (21) et al. discovered monoiodotyrosine in Gorgonia verrucosa. Roche and Lafon (22, 23) after investigating the content of tyrosine and its iodinated derivatives suggested that the tyrosine content depends on the species of gorgonian, and halogenation is limited by the amount of tyrosine present, thus there are many gorgonins. Roche and Lafon (24) also reported the presence of diiodotyrosine in very small quantities in certain seaweeds.

In 1951, Roche (25) looked for and found monobromotyrosine in gorgonians. Since monoiodotyrosine is considered the precursor of diiodotyrosine in the thyroid gland, monobromotyrosine should be the precursor of the dibromotyrosine in animals.

A number of workers have investigated the halogen and nitrogen content of gorgonians. These data and the data from the present study are compared in table 1.

A number of species of gorgonians became available to the author in 1957. It was thought that a study of the iodine, bromine and nitrogen content would be of value from the standpoints of comparative biochemistry and systematic zoology. It was further thought worthwhile to see whether location or differences in physical characteristics, such as color, had anything to do with the content of halogens. The use of a new method of analysis developed by Schöniger (26) was thought to be of particular importance as it allowed a more rapid and simpler decomposition of the organic material than has been used up to this time. Iodine and bromine, if found, would be an indication of the presence of mono and dihalogenated tyrosine in the gorgonians tested and would also suggest whether or not the total tyrosine was at a high or low level.

Sulfur Containing Nitrogen Compounds

The occurrence of betaines and related nitrogen compounds is widespread in nature. Kutscher and Ackermann (31, 32, 33) have reviewed naturally occuring nitrogen containing compounds rather thoroughly in 1926, 1933 and 1936. Ackermann (34), in 1955, wrote an outstanding review on biological amines which gives, among other things, information regarding the discovery and source of these amines. Since Ackermann wrote his review, a number of sulfur containing amines have been identified in biological material. The distribution of sulfur containing amines related to taurine is summarized in table 2.

TABLE 1

COMPARISON OF HALOGEN AND NITROGEN CONTENT IN GORGONIANS

Species	Source	%I	%Br	%N	Mole ratios I/Br	I/N	Br/N	
Briareidae								
Briareum asbestinum (pallas)	Bimini	0.00	0.07	1.79	----	----	0.01	(x)
Gorgoniidae								
Eugorgia aurantiaca	Gulf of California	1.50	----	7.10	----	0.02	----	(27)
Gorgonia acerosa	Bermuda	1.94	----	15.75	----	0.01	----	(27)
(Pallas)	W. Indies	1.70	----	-----	----	----	----	(15)
(Pterogorgia acerosa ?)	Tortugas	0.79	----	-----	----	----	----	(28)
	Florida	0.90	0.66	-----	0.86	----	----	(9)
Gorgonia bicolor Val.	____	0.23	0.88	-----	0.17	----	----	(9)
Gorgonia citrina	W. Indies	0.79	----	-----	----	----	----	(9)
Esper	Tortugas	1.17	----	-----	----	----	----	(28)
(Xiphigorgia citrina ?)								
Gorgonia cavolinii v. Koch	Naples	5.49	1.98	-----	1.74	----	----	(9)
Gorgonia graminea Lamarck	----	5.58	1.31	-----	2.68	----	----	(9)
Gorgonia pinnate O F M	Sweden	0.02	----	-----	----	----	----	(9)
Gorgonia setosa Esper	Florida	0.77	----	-----	----	----	----	(9)
Gorgonia verrucosa Pallas	----	6.92	1.62	-----	2.70	----	----	(9)
Leptogorgia purpuracea Pallas	----	0.28	2.61	-----	0.07	----	----	(9)
Leptogorgia purpurea (Pallas)	----	0.02	----	-----	----	----	----	(9)
(Gorgonia pumicea Val.*								
Leptogorgia rigida	Panama	1.55	----	10.30	----	0.02	----	(27)
Verrill	Nicaragua	0.24	0.99	-----	0.15	----	----	(9)

* Old name used by the author.
(x) Gorgonian investigated in this dissertation.

TABLE 1 (Cont.)

Species	Source	%I	%Br	%N	Mole ratios I/Br	I/N	Br/N	
Leptogorgia virgulata (Lam.)	N. Carolina	1.82	----	8.67	----	0.02	----	(27)
	Florida	0.42	----	8.30	----	0.01	----	(27)
Leptogrogia sanguinolenta (Pallas) (Gorgonia petechizans Pall.)*	----	----	0.61	-----	----	----	----	(9)
Pterogorgia acerosa (Pallas)	Bermuda	0.31	0.32	5.88	0.61	0.01	0.01	(x)
	Bimini	0.27	0.16	5.82	1.06	0.01	0.01	(x)
	Bimini	0.08	0.56	12.58	0.91	0.01	0.01	(x)
Lophogorgia palma Pallas	----	0.07	2.58	-----	0.02	----	----	(9)
	----	0.08	2.30	-----	0.02	----	----	(9)
	S. Africa	0.03	----	-----	----	----	----	(9)
Pterogorgia americana (Gmelin)	Bermuda	0.36	0.50	5.20	0.46	0.01	0.02	(x)
Rhipidogorgia flabellum (Linnaeus)	Bermuda	1.88	----	13.76	----	0.02	----	(27)
	W. Indies	1.15	----	-----	----	----	----	(15)
	W. Indies	0.45	0.37	-----	0.77	----	----	(9)
	Tortugas	0.80	----	-----	----	----	----	(28)
	----	0.13	----	14.10	----	0.001	---	(29)
	Bermuda	0.32	0.33	6.78	0.61	0.01	0.01	(x)
	Bimini	0.39	0.39	5.76	0.63	0.01	0.01	(x)
	Bimini	0.79	1.01	13.11	0.50	0.01	0.01	(x)
Rhipidogorgia verriculata Ellis	W. Indies	0.62	0.75	-----	0.52	----	----	(9)
Xiphigorgia anceps (Pallas)	Florida	0.96	0.23	-----	2.62	----	----	(9)
	Tortugas	1.58	----	-----	----	----	----	(9)
Xiphigorgia citrina (Esper)	Bimini	0.82	0.64	11.92	0.81	0.01	0.01	(x)
Gorgonellidae								
Gorgonella sarmentosa Lamk	Naples	0.12	1.98	-----	0.04	----	----	(9)

* Old name used by the author.
(x) Gorgonians investigated in this dissertation.

TABLE 1 (Cont.)

Species	Source	%I	%Br	%N	Mole ratios I/Br	I/N	Br/N	
Isididae								
Isis hippuris	----	2.03	----	-----	----	----	----	(9)
Isis polyacantha Steenstr.	----	1.58	0.74	-----	1.34	----	----	(9)
Muriceidae								
Paramuricea placomus (Linnaeus)	Norway	0.25	1.18	-----	0.13	----	----	(9)
Muricea hebes	Panama	0.89	----	10.50	----	0.01	----	(27)
Muricea muricata (Pallas)	Bermuda	3.94	----	12.30	----	0.04	----	(27)
	W. Indies	0.30	----	-----	----	----	----	(9)
	W. Indies	1.02	----	-----	----	----	----	(9)
	Bermuda	1.70	1.02	13.51	1.05	0.01	0.01	(x)
Plexaurella dichotoma (Esper)	----	0.12	1.07	-----	0.07	----	----	(9)
	W. Indies	0.11	0.96	-----	0.07	----	----	(9)
	Tortugas	0.11	----	-----	----	----	----	(28)
	Bermuda	0.00	0.37	4.35	----	----	0.02	(x)
	Bermuda	0.00	0.28	2.93	----	----	0.02	(x)
Plexaurella nutans (Duchassaing and Michelotti)	Bermuda	0.11	0.22	2.91	0.32	0.01	0.01	(x)
Plexauridae								
Eunicea asperula Valenciennese	W. Indies	1.25	3.70	-----	0.22	----	----	(9)
Eunicea ehrenbergi M. A. S.	----	1.72	4.20	-----	0.26	----	----	(9)
Eunicea grandis Verrill	Bermuda	1.29	2.83	12.15	0.29	0.01	0.04	(x)
	Bermuda	1.21	3.18	11.94	0.24	0.01	0.05	(x)
	Bermuda	1.71	2.99	13.06	0.36	0.02	0.04	(x)
Eunicea laxispina Lamk	----	1.45	2.86	-----	0.32	----	----	(9)
Eunicea mammosa Lamouroux	Bimini	2.29	3.22	13.55	0.45	0.02	0.04	(x)
	Bimini	2.09	2.57	12.55	0.51	0.02	0.04	(x)
	Bimini	1.98	2.79	-----	0.45	----	----	(x)

(x) Gorgonians investigated in this dissertation.

TABLE 1 (Cont.)

Species	Source	%I	%Br	%N	Mole ratios I/Br	I/N	Br/N	
Eunicea plantaginea Lamk	California	1.03	----	-----	----	----	----	(9)
Eunicea rousseaui Milne Edwards	Bermuda	2.17	----	13.26	----	0.02	----	(27)
	Tortugas	1.62	----	-----	----	----	----	(28)
Eunicea succinea Esper	----	1.06	2.89	-----	0.26	----	----	(9)
	W. Indies	1.04	3.02	-----	0.22	----	----	(9)
Eunicea tourneforti Milne Edwards and Haime	W. Indies	1.50	----	-----	----	----	----	(9)
	Bermuda	0.89	3.44	12.18	0.16	0.01	0.05	(x)
	Bermuda	1.41	2.99	13.27	0.30	0.01	0.04	(x)
	Bermuda*	1.41	3.47	10.07	0.26	0.02	0.06	(x)
Eunicella verrucosa Pallas	----	6.78	1.70	-----	2.39	----	----	(30)
Plexaura adusta	W. Indies	1.55	----	-----	----	----	----	(9)
Plexaura antipathes Köll	W. Indies	0.88	3.50	-----	0.16	----	----	(9)
Plexaura crassa Ellis and Solander	Bermuda	2.48	----	13.27	----	0.02	----	(27)
	Tortugas	0.94	----	-----	----	----	----	(28)
	Bermuda	1.49	2.44	11.92	0.38	0.02	0.04	(x)
	Bermuda	2.16	2.44	12.78	0.56	0.02	0.03	(x)
Plexaura esperi Verrill	Bermuda	1.36	2.58	12.80	0.33	0.01	0.04	(x)
Plexaura flavida (Lamarck)	Bimini	2.60	0.63	14.17	2.56	0.02	0.01	(x)
Plexaura flexuosa Kukenthal	Bermuda	4.95	----	12.88	----	0.04	----	(27)
	Bermuda	4.63	----	11.73	----	0.04	----	(27)
	W. Indies	5.34	----	13.26	----	0.04	----	(27)
	W. Indies	0.28	----	-----	----	----	----	(15)
	Florida	1.23	3.47	-----	0.22	----	----	(9)
	Tortugas	2.63	----	-----	----	----	----	(28)
	Bermuda	1.64	3.26	12.05	0.32	0.02	0.05	(x)
	Bermuda	2.09	2.73	12.06	0.48	0.02	0.04	(x)

* A gangling form, identified as E. tourneforti by Dr. Deichmann.
(x) Gorgonians investigated in this dissertation.

TABLE 1 (Cont.)

Species	Source	%I	%Br	%N	Mole ratios I/Br	I/N	Br/N	
Plexaura homomalla Esper	----	0.84	3.65	-----	0.14	----	----	(9)
	Tortugas	1.42	----	-----	----	----	----	(28)
	Bermuda	1.32	2.95	11.62	0.28	0.01	0.05	(x)
	Bermuda	1.52	2.61	10.92	0.38	0.02	0.04	(x)
Plexaura intermedia Kölli	W. Indies	1.34	3.05	-----	0.34	----	----	(9)
Plexaura nodulifera Lamarck	----	0.74	3.02	-----	0.11	----	----	(9)
Plexaura porosa Esper	W. Indies	1.03	----	-----	----	----	----	(9)
Plexaura suffruticosa Dand	Java	0.58	----	-----	----	----	----	(9)
Primnoidae								
Primnoa lepadifera L.	Norway	0.12	3.76	-----	0.02	----	----	(9)
Primnoa verticillaris L.	----	0.05	2.95	-----	0.01	----	----	(9)

(x) Gorgonians investigated in this dissertation.

TABLE 2

DISTRIBUTION OF TAURINE AND RELATED COMPOUNDS IN NATURE

Compound	Investigator	Source
Asterubin $(CH_3)_2NC(:NH)NHCH_2CH_2SO_3H$	Ackermann (34)	Sea star
Choline sulfate $(CH_3)_3NCH_2CH_2SO_3$	Woolley and Peterson (35) Lindberg (36)	Aspergillus Red algae and lichens
Cysteic acid amide $HO_3SCH_2CH(NH_2)C:ONH_2$	Deffner and Hafter (43)	Squid
Hypotaurine $NH_2CH_2CH_2SO_2H$	Cavallini, Mondovi and DeMarco (40)	Rat urine
Isethionic acid $HOCH_2CH_2SO_3H$	Koechlin (42)	Squid
N-methyltaurine $CH_3NHCH_2CH_2SO_3H$	Lindberg (38)	Red algae
Di-N-methyltaurine $(CH_3)_2NCH_2CH_2SO_3H$	Lindberg (37)	Red algae
Taurine $NH_2CH_2CH_2SO_3H$	Lindberg (38), Koechlin (42) and many others	Red algae, Squid
Taurobetaine $(CH_3)_3NCH_2CH_2SO_3$	Ackermann and List (39) Author (this dissertation)	Sponges Gorgonian
Taurocyamine $NH_2C(:NH)NHCH_2CH_2SO_3H$	Thoai and Robin (44)	Marine worms
Thiotaurine $NH_2CH_2CH_2SO_2SH$	Cavallini, DeMarco and Mondovi (45)	Rat urine

In 1937, Woolley and Peterson (35) showed the presence of choline sulfate in Aspergillus. Lindberg (36, 37, 38), in 1955, described the occurrence of taurine, N-methyltaurine, di-N-methyltaurine and choline sulfate in lichens and red algae. Lindberg postulated that taurine might be successively methylated to taurobetaine which then might be oxidized to choline sulfate. He looked for taurobetaine but was unable to isolate it. Since then Ackermann and List (39) have reported taurobetaine in sponges from the North Sea and from the Mediteranean Sea.

Cavallini, Mondovi and DeMarco (40), in 1955 isolated and identified hypotaurine in the urine of rats which had been fed a supplement of cystine. Ouchi (41) in 1959, showed the presence of hypotaurine in five molluscs, two coelenterates (sea anemones), sponges and local salted sea urchins. Ouchi suggests that hypotaurine may be an intermediate in the conversion of cystine or cysteine to taurine.

In 1955, isethionic acid was found by Koechlin (42) in squid axoplasm. Taurine (42) and methionine (43) are also present in the axoplasm of squid. In 1959, Deffner and Hafter (43) showed the presence of cysteic acid amide and the absence of cysteic acid, cysteine and cystine in the dialysable portion of squid axoplasm.

In 1957 the author received a quantity of the gorgonian Briareum asbestinum from Bimini. During the course of investigating this gorgonian, crystals separated from the methanol extract. These crystals were identified as taurobetaine.

Lipid Materials in Gorgonians

In 1844, Silliman (46) investigated the stony corals and found that they contained from 4 to 8 percent organic matter. When pulverized

coral was treated with boiling water, jelly-like material floated to the surface. When an ether solution of this material was evaporated it yielded a yellow wax-like substance which fused below 200° F. Doree (47), in 1909, showed the presence of cholesterol in a large number of animals including sponges and sea anemones. In 1937, Montignie (48) extracted coral with alcohol and showed the presence of sterols in the unsaponifiable residue. In 1938, Bock and Wetter (49) showed the presence of pro-vitamin D in the coelenterates Alcyonium digitatum, Metridium dianthus and Urticina crassicornis. Bergmann and Lester (50, 51), during the course of an investigation of lower animals for sterols, found in 1940 that reef corals and gorgonians contain a high content of lipoid material. Madrepora cervicornis, the staghorn coral, contained 0.25 per cent of a low melting wax which melted at 50-50.5° C and was identified as cetyl palmitate. Sterols, alcohols, hydrocarbons and ketones were also thought to be present. The gorgonians also showed the presence of sterols, alcohols and hydrocarbons.

In 1942, Kind and Bergmann (52) isolated a quantity of non-steroid alcohols from the gorgonian, Plexaura flexuosa. Fractional distillation and repeated recrystallization gave an alcohol identified as octadecyl alcohol, a mixture of batyl alcohol and octadecyl alcohol, and a residue found to be batyl alcohol. Kind and Bergmann (52) also extracted an unidentified species of Xiphigorgia with acetone and obtained a wax, melting point 50-50.5° C., which was found to be cetyl palmitate by comparison with the known material and by identification of the products of hydrolysis.

In 1943, Bergmann, McLean and Lester (53) studied the sterols of the gorgonians Xiphigorgia sp. and Plexaura flexuosa. Xiphigorgia was shown to contain cholesterol and a $C_{29}H_{50}O$ sterol very similar to clinonasterol. Plexaura flexuosa yielded a sterol which had a formula of $C_{30}H_{52}O$ or $C_{31}H_{54}O$ and a melting point of 184-185° C, $[\alpha]_D$ -45. This sterol did not correspond to any known sterol and was therefore named gorgosterol.

Since 1943 no work has been done on the lipids of gorgonians, but related organisms have been investigated for their sterol, alcohol and fatty acid content. Bergmann (54), in 1949, has reviewed the work on the sterol content of various organisms. Bergmann and his associates have investigated sterols from sponges (55, 56) and sea anemones (57). They also have investigated sponges (56) and sea anemones (57, 58) for the alcohol, fatty acid and triglyceride content. These investigations have shown that palmitic acid is present in all organisms studied. Other fatty acids and alcohols identified are shown in table 3.

Since the gorgonians contain a relatively large fraction of lipid material and since not much has been done to separate and identify the individual constituents it was thought that this line of research would provide some useful information. In particular there was the possibility of it being helpful to comparative biochemists who wish to use chemical differences to clear up the confusion in the present system of classification of marine organisms, and testing Hilditch's hypothesis (59) that the more primitive the animal the more complex its mixture of fatty acids.

TABLE 3

FATTY ACIDS AND ALCOHOLS IN SPONGES AND SEA ANEMONES

Sponges (56)

Spheciospongia vesparia		*Suberites compacta*	
alcohols	acids	alcohols	acids
glycerol	palmitic	glycerol	palmitic
	stearic		α-hydroxytetracosanoic
	$\Delta^{17,20}$-hexacosadienoic		an octacosenoic
	Δ^{9}-hexacosenoic		an octacosatrienoic

Anemones (57, 58)

Bolocera tuediae		*Condylactis gigantea*	
alcohols	acids	alcohols	acids
cetyl	myristic	myristyl	myristic
stearyl	palmitoleic	cetyl	palmitic
oleyl	stearic	stearyl	stearic
11-eicosenyl	oleic	C_{20} and C_{22}	arachidic
11-docosenyl	gadoleic	(unsat.)	behenic
	cetoleic		C_{24}
	erucic		C_{26}
	C_{24}		
	C_{26}		

Material from the pentane extracts of Eunicea grandis, Muricea muricata, Briareum asbestinum, Plexaura crassa and Plexaurella nutans was used in this study.

CHAPTER II

EXPERIMENTAL

Composition of Gorgonian Skeletons

Collection and preparation of samples. During the summer of 1957 the following species of gorgonians were collected from Bermuda waters for nitrogen and halogen analysis of the horny skeletons: Eunicea grandis Verrill, Eunicea tourneforti Milne Edwards and Haime, Muricea muricata (Pallas), Plexaura flexuosa (Lamouroux), Plexaura crassa Ellis and Solander, Plexaura esperi Verrill, Plexaura homomalla (Esper), Plexaurella dichotoma (Esper), Plexaurella nutans (Duchossaing and Michelotti), Pterogorgia acerosa (Pallas), Pterogorgia americana (Gmelin) and Rhipidogorgia flabellum (Linnaeus). The gorgonians were identified with the help of Dr. Elisabeth Deichmann's "Key to the Species of Gorgonians from Shallow Water" and by comparison with preserved specimens at the Bermuda Biological Station which had previously been identified by Dr. Deichmann.

Specimens were also collected at Bimini in 1955 and 1957. These were Briareum asbestinum (Pallas), Eunicea mammosa Lamouroux, Plexaura flavida (Lamarck), Pterogorgia acerosa (Pallas), Rhipidogorgia flabellum (Linnaeus) and Xiphigorgia citrina (Esper). The specimens were identified by Dr. Elisabeth Deichmann of the Museum of Comparative Zoology of Harvard University.

The gorgonians were collected in water from five to twenty feet deep. They were cut off at the base with a sharp instrument and the whole colonies were drained of water and hung in the sun to dry.

Medium size skeletons, of each species, were chosen for analysis wherever possible. The dry skeletons were ground to a fine powder in either a small Wiley Mill or a motor driven hammermill depending on the size of the skeleton. In most cases two or three skeletons were ground as one sample. Only one branch of Plexaurella nutans was taken and the rest saved for reference because it was the only specimen available. In the case of Pterogorgia acerosa half of a skeleton was ground with the cortex on and the other half without it to see whether the analysis of skeletal nitrogen and halogens would be affected by the cortex. The cortex was not removed from Pterogorgia americana. Rhipidogorgia flabellum was taken whole, and with the cortex removed by soaking in a 2 per cent acetic acid solution on a steam bath.

Nitrogen determination. The nitrogen content of the gorgonian skeletons was obtained by the Cole-Parks (60) modification of the semi-micro Kjeldahl method. The size of the samples used in the analysis ranged from 20 to 60 milligrams. The results are summarized in table 4.

Iodine and bromine determination. The iodine and bromine contents of the powdered gorgonian skeletons were obtained by the method of Schöniger (26, 61). The Schöniger apparatus consists of an Erlenmeyer flask of 300 or 500 milliliter capacity with a standard taper joint with flared top, and a stopper is in place the platinum screen extends into the flask. The sample is weighed out on a piece of ashless paper which is rolled up and inserted in the holder. The absorbing solution for

TABLE 4

IODINE, BROMINE AND NITROGEN CONTENT OF SIXTEEN GORGONIANS

Species and origin	%I	ave	%Br	ave	%N	ave
Briareum asbestinum	0.00		0.04		1.75	
Rabbit Cay[a]	0.00	0.00	0.14		1.82	1.79
			0.02	0.07		
Eunicea grandis						
Castle Harbor[b]	1.38		2.89		12.23	
	1.20	1.29	2.77	2.83	12.07	12.15
Ferry Point[b]	1.22		3.24		11.94	
	1.20	1.21	3.12	3.18	11.93	11.94
Somerset[b]	1.66		3.01		12.05	
	1.78		2.97	2.99	13.97	13.06
	1.86	1.71				
Eunicea mammosa						
Rabbit Cay	2.25		3.23		13.69	
	2.32	2.29*	3.21	3.22*	13.41	13.55
Rabbit Cay	2.06		2.50		12.47	
	2.11	2.09*	2.64	2.57*	12.62	12.55
Rabbit Cay	1.99		2.80			
	1.96	1.98*	2.78	2.79*		
Eunicea tourneforti						
Castle Harbor	0.89		3.43		12.23	
	0.89	0.89	3.45	3.44	12.12	12.18
Somerset	1.43		3.02		13.21	
	1.38	1.41	2.96	2.99	13.33	13.27
Eunicea tourneforti**						
Ferry Point	1.47		3.43		10.00	
	1.34	1.41	3.51	3.47	10.14	10.07

[a] Bimini, B. W. I.
[b] Bermuda.
* The averages represent separate colonies.
** A gangling form, identified as E. tourneforti by Dr. Deichmann.

TABLE 4 (Cont.)

Species and origin	%I	ave	%Br	ave	%N	ave
Muricea muricata						
Castle Harbor	1.70		1.01		13.56	
	1.70	1.70	1.03	1.02	13.46	13.51
Plexaura crassa						
Castle Harbor	1.44		2.45		12.02	
	1.60		2.42	2.44	11.82	11.92
	1.60					
	1.52					
	1.45					
	1.44					
	1.43					
	1.46	1.49				
North Rock[b]	2.14		2.41		12.63	
	2.18	2.16	2.45	2.44	12.93	12.78
Plexaura esperi						
Castle Harbor	1.37		2.59		12.89	
	1.24		2.56	2.58	12.71	12.80
	1.47	1.36				
Plexaura flavida						
Rabbit Cay	2.55		0.62		13.91	
	2.64	2.60	0.64	0.63	14.40	14.17
Plexaura flexuosa*						
Ferry Point	1.70		3.23		12.04	
	1.63		3.29	3.26	12.05	12.05
	1.60					
	1.46					
	1.72					
	1.70	1.64				
Plexaura flexuosa**						
Ferry Point	2.08		2.72		11.95	
	2.09	2.09	2.73	2.73	12.18	12.06

* Skeletons from brown colored specimens only.
** Skeletons from purple colored specimens only.

TABLE 4 (Cont.)

Species and origin	%I	ave	%Br	ave	%N	ave
Plexaura homomalla						
Castle Harbor	1.32		2.94		11.67	
	1.32	1.32	2.95	2.95	11.57	11.62
Somerset	1.61		2.67		10.60	
	1.51		2.54	2.61	11.23	10.92
	1.49					
	1.48	1.52				
Plexaurella dichotoma (?)						
Castle Harbor	trace		0.34		4.33	
	0.00	trace	0.40	0.37	4.37	4.35
Somerset	0.00		0.26		3.05	
	0.00	0.00	0.30	0.28	2.80	2.93
Plexaurella nutans (?)						
North Rock	0.11		0.21			
	0.11	0.11	0.22	0.22	2.91	2.91
Pterogorgia acerosa*						
Castle Harbor	0.30		0.36		5.88	
	0.31	0.31	0.28	0.32	5.88	5.88
Pterogorgia acerosa*						
N. of N. Bimini	0.27		0.16		5.67	
	0.27	0.27	0.16	0.16	5.97	5.82
Pterogorgia acerosa						
N. of N. Bimini	0.80		0.55		12.21	
	0.80	0.80	0.57	0.56	12.95	12.58
Pterogorgia americana*						
North Rock	0.36		0.52		5.18	
	0.35	0.36	0.47	0.50	5.22	5.20
Rhipidogorgia flabellum*						
Somerset	0.33		0.31		7.09	
	0.30	0.32	0.35	0.33	6.55	6.87
Rabbit Cay	0.40		0.37		5.44	
	0.37	0.39	0.40	0.39	6.08	5.76

* Whole animal (cortex left on).

TABLE 4 (Cont.)

Species and origin	%I	ave	%Br	ave	%N	ave
Rhipidogorgia flabellum[x]						
Rabbit Cay	0.80		1.02		12.82	
	0.78	0.79	0.99	1.01	13.40	13.11
Xiphigorgia citrina						
N. Turtle Rock	0.82		0.65		11.83	
	0.82	0.82	0.62	0.64	12.00	11.92

iodine which consists of 10 milliliters of distilled water, 1 milliliter of 2 N sodium hydroxide, and 2 or 3 drops of 30 per cent hydrogen peroxide, is put in the flask and the flask is filled with oxygen. The paper is ignited and immediately inserted into the flask. After combustion of the sample has taken place, the flask is shaken to enhance absorption and allowed to stand for 30 minutes. Distilled water is added to the flared lip and as the stopper is withdrawn the joint and sides of the flask are rinsed. The flask and stopper are rinsed with a small amount of distilled water.

To obtain the amount of iodine present, samples of 10 to 20 milligrams for the 300 milliliter apparatus and 30 to 68 milligrams for the 500 milliliter apparatus were burned as described above, and the absorption solution was treated as follows. The solution was boiled for 3 to 5 minutes to get rid of excess hydrogen peroxide. Ten milliliters of a solution containing 4 milliliters of bromine and 100 grams of potassium acetate in 1000 milliliters of glacial acetic acid, were

[x] The cortex was removed with 2 per cent acetic acid on a steam bath.

added. The excess bromine was reduced with formic acid and the solution was allowed to stand for 3 minutes. After 4 milliliters of 2 N sulfuric acid and a few crystals of potassium iodide were added, the solution was titrated with 0.005 or 0.002 N sodium thiosulfate using starch as an indicator. Practice determinations were made on compounds containing known amounts of iodine and in these cases 0.02 N sodium thiosulfate was used. The results of the practice determinations are summarized in table 5 and the results of the gorgonian analyses are summarized in table 4.

To obtain the per cent bromine present, samples of 10 to 20 miligrams in the 300 milliliter apparatus and 25 to 58 milligrams in the 500 milliliter apparatus were burned as in the procedure for iodine. The absorption solution in this case was 5 milliliters of a solution containing 20 grams of sodium dihydrogen phosphate in 100 milliliters of distilled water, 20 milliliters of a solution containing 300 grams of sodium chloride in 1000 milliliters of distilled water, and 10 milliliters of sodium hypochlorite solution which was prepared by dissolving chlorine in 1.1 N sodium hydroxide till the hypochlorite concentration was 1 N. For the determination of bromine in gorgonians the concentration of bromine was small enough so that the sodium chloride solution was omitted (62). This gave a sharper end point with the starch indicator.

The absorption solution was brought to boiling, 5 milliliters of 50 per cent sodium formate was added and the solution brought to boiling again to destroy excess hypochlorite. The flask was cooled under the tap and 20 milliliters of 6 N sulfuric acid and 0.2 grams of

TABLE 5

PRACTICE DETERMINATIONS OF IODINE AND BROMINE

Compound	mgm	%I	ave	% expected
2,6-Diiodo-p-nitrophenol	4.86	65.21		
	6.41	65.54		
	9.57	65.25	65.33	64.93
p-Iodobenzoic acid	4.44	51.10		
	3.53	50.70		
	3.76	51.06*	50.95	51.17

Compound	mgm	%Br	ave	% expected
P-Bromobenzoic acid	6.45	39.33		
	5.59	39.28		
	2.57	39.11	39.24	39.75
p-Bromobenzoic acid (in the presence of organic iodide)	3.12	39.00		
	2.85	39.45		
	3.54	39.23		
	1.70	39.03		
	1.46	39.14	39.17	39.75

potassium iodide was added and the solution was made up to 100 milliliters with distilled water. The solution was then titrated with 0.004 or 0.002 N sodium thiosulfate to a starch-iodine end point. Blank determinations showed the absence of iodine and bromine in the reagents. The practice determinations on known mixtures of compounds containing iodine and bromine, using 0.02 N sodium thiosulfate are summarized in table 5. The summary of the gorgonian bromine analyses is given in table 4. As the method just described gives the total bromine and iodine present, the iodine must be subtracted to get the bromine.

* Value obtained by using the procedure for the determination of bromine.

Isolation of Taurobetaine from Briareum Asbestinum

The porous material (cortex or coenenchyma) around the skeletons of the gorgonians contains the polyps. In order to study the substances contained in the cortical material, weighed amounts of the dried material were run through an extraction procedure which removed soluble substances. A typical procedure consisted of extracting the weighed and dried cortical material in a Soxhlet extractor for 24 hours with n-pentane and, after freeing the material from pentane, extracting for 24 hours with diethyl ether and repeating the extraction with acetone and then with methyl alcohol. In this manner a rough separation was achieved, dividing the material into classes differing in polarity.

In the summer of 1955, Dr. L. S. Ciereszko, of the University of Oklahoma, collected some Briareum asbestinum near Rabbit Cay in the Bimini Islands. Briareum asbestinum has no distinct axial skeleton and was dried and ground whole. The ground material was subjected to the serial extraction procedure (see above). After the material had been extracted with methanol for 2 hours the extract began to bump because of a crystalline precipitate which had appeared in the flask. This precipitate consisted of a mixture of fine crystals and small yellowish spear point crystals. The yellow spear point crystals slowly turned dark brown when heated above 200° C and at about 300° C turned black and bubbled as if a gas were being given off. More material was extracted with methanol and after 24 hours the extract was allowed to stand for several weeks. At the end of this period several relatively large bunches of spear point crystals, amounting to a few milligrams, had appeared on the sides of the

flask. These crystals were soluble in water and slowly turned dark when heated above 200° C.

In December of 1956 additional Briareum asbestinum was obtained and 180 grams of the dry material was run through the serial extraction procedure. After one hour of methanol extraction the precipitate was filtered off from the hot extract, the extract was concentrated slightly and allowed to cool to room temperature. After standing for a day spear point crystals precipitated. These crystals were collected, washed successively with fresh methanol and anhydrous ether and then dried.

In December of 1957 more Briareum asbestinum was collected off Rabbit Cay in the Bimini Islands. The details of extracting the spear point crystals had now been worked out as follows. Dried Briareum asbestinum, 680 grams, were extracted serially with n-pentane, ether, and acetone for 24 hours each, followed by extraction for 3 hours with methanol. The methanol extract was filtered hot and allowed to stand at room temperature overnight. The spear point crystals which precipitated were washed with fresh methanol and with anhydrous ether. The dried product consisted of 1.021 grams of almost colorless crystals. The gorgonian material was extracted for 21 hours more and the hot extract was filtered from the precipitated solids and allowed to stand overnight in the refrigerator. There was another crop of spear point crystals which after washing with fresh methanol and anhydrous ether weighed 0.987 grams and appeared almost colorless. The total weight, 2.008 grams of spear point crystals, represents a yield of 0.3 per cent based on the dry weight of the whole colony.

Since slight variations in the methanol extraction procedure did not seem to change the character of the spear point crystals and since these crystals were obtained in essentially the same state of purity from different batches of Briareum asbestinum stored for different lengths of time, all recrystallized spear point crystals will be referred to as BaI. The recrystallizations were accomplished by dissolving spear point crystals in a large amount of hot methanol, cooling to room temperature and letting the solution stand in the refrigerator overnight. Recrystallization from ethanol and from an ethanol-water mixture was tried. Material of essentially the same purity was obtained from the different solvents, the crystals being colorless and the decomposition point varying from 344° to 347° C (uncorr.).

The decomposition points of BaI were all obtained on a Townson-Mercer, Model IV, melting point apparatus by raising the temperature rapidly to 300° C, then slowly to 339° C, inserting the sample, and raising the temperature 1°/minute thereafter. The BaI began to turn dark at 340° C. The decomposition point was taken as the temperature at which it turned black and bubbling took place. If the BaI were inserted at 315° C the decomposition point was 340.5° C (uncorr.) for a lot which had a decomposition point of 346° C (uncorr.) when inserted at 339° C.

BaI was soluble in water and its solution gave a neutral reaction to litmus paper. A 1 per cent solution in water showed no optical activity. Ultra violet absorption spectra were run on solutions of different concentrations of BaI in distilled water. The spectrum of the sample containing 0.033 grams/milliliter showed a very small hump

in the region of 250-280 millimicrons. When the concentration was increased to 0.1 grams/milliliter the hump in the curve did not increase in height. BaI gave no precipitate with picric acid. The silver nitrate test for halides was negative. When a few crystals were mixed with strong alkali the solution effervesced and the gas coming off turned moist litmus paper blue. After a sodium fusion, positive tests were obtained for nitrogen and sulfur.

The valence state of sulfur (63) in BaI was checked in the following manner. BaI was fused with sodium hydroxide, the fusion mixture dissolved in water and then acidified. Nothing happened to moistened lead acetate paper when held over the acidified solution indicating the absence of hydrogen sulfide. When a solution of potassium permanganate and barium chloride was held just over the acidified solution by means of a glass loop the solution was decolorized and a white precipitate of barium sulfate appeared indicating sulfur dioxide was being evolved.

A quantitative determination of nitrogen (63) was carried out on some unrecrystallized spear point crystals and a value of 8.27 per cent N was obtained. Since treatment of BaI with strong alkali had evolved a basic gas, a sample of BaI was run through the distillation part of the Kjeldahl procedure without digestion and gave a value equivalent to 8.21 per cent nitrogen. A sample of BaI was dried for several days in a desiccator and sent to the Galbraith Microanalytical Laboratories for elemental analysis, with the following results: C, 35.45 per cent; H, 8.64 per cent; N, 8.07 per cent; S, 18.90 per cent; and residue, none. The oxygen by difference, was 28.90 per cent.

The gas which was evolved from BaI upon treatment with alkali smelled like trimethylamine. To identify it, the picrate was made as follows. Saturated picric acid in water was placed in the center well of a Conway microdiffusion vessel and a small amount of BaI and 24 per cent sodium hydroxide in the space around the center well. The lid was fixed in place with stopcock grease and the vessel allowed to stand undisturbed. After 2 hours the vessel was placed in the refrigerator. A very small amount of fine yellow needles precipitated; melting point 216-222° C (uncorr.). Trimethylamine picrate (64) melts at 216° C. Picric acid has a melting point of 121.8° C and ammonium picrate decomposes at 270° C. The decomposition of a larger amount of BaI gave trimethylamine picrate; melting point 213°-216° C (corr.). Known trimethylamine picrate was made from Eastman White Label trimethylamine by bubbling the gas into a saturated solution of picric acid in water. The precipitate was recrystallized from hot water and dried; melting point 210.5-213.5° C (corr.). The trimethylamine picrate from the decomposition of synthetic taurobetaine melted at 215.5-217.5° C. (corr.).

The chloroplatinate of trimethylamine from BaI was obtained by placing chloroplatinic acid in the center well of a Conway microdiffusion vessel and treating BaI with 24 per cent sodium hydroxide in the space around the center well with the lid sealed in place with stopcock grease for several hours at 42° C. The crystals which formed in the center well were recrystallized from ethanol containing hydrochloric acid. The decomposition point of the recrystallized chloroplatinate was 199.5° C (corr.). Trimethylamine chloroplatinate was prepared from Eastman White Label trimethylamine as follows. To 1 millilliter of a water solution

containing 0.05 grams of trimethylamine and 10 per cent hydrochloric acid, was added slowly with shaking, 1 milliliter of approximately 25 per cent chloroplatinic acid (sp. gr. 2). Since no precipitate formed, 1 milliliter of water containing 0.25 grams of trimethylamine and approximately 0.25 milliliters of chloroplatinic acid (sp. gr. 2) was added. The yellow precipitate which formed was washed with 10 per cent hydrochloric acid and recrystallized from ethanol which contained some hydrochloric acid. The recrystallized trimethylamine chloroplatinate had a decomposition point of 201° C (corr.). The mixed decomposition point of the trimethylamine chloroplatinates was 202° C (corr.). The decomposition point of trimethylamine chloroplatinate is listed (65) as 242-243° C and a decomposition point of 245° C was obtained after the chloroplatinates had stood for some months.

According to James (66) trimethylamine precipitates as $H_2PtCl_6 \cdot 2N(CH_3)_3$ when it is allowed to react with chloroplatinic acid. The platinum content of this compound is 36.96 per cent. The trimethylamine chloroplatinate from Eastman White Label trimethylamine and the trimethylamine chloroplatinate from the decomposition of BaI were analyzed for platinum by igniting the samples in a furnace and weighing the remaining platinum. The results are listed in table 6.

At this point it was suspected that BaI was taurobetaine because of the elemental analysis, its physical properties and the fact that trimethylamine was evolved upon treatment with strong alkali. To show that BaI was taurobetaine the other part of the molecule had to be identified. This was done by decomposing BaI by the procedure of James (66), somewhat modified. Approximately 0.5 grams of barium hydroxide was

added to 0.2 grams of BaI in boiling water solution. When the steam was neutral to litmus, sulfuric acid was added to the alkaline solution to give a pH of 2, and the barium sulfate was filtered off. The filtrate was evaporated almost to dryness on a steam bath and was then diluted with water. Potassium carbonate was now added to pH 8.5 and the solution was evaporated to dryness on a steam bath. The residue was ground with 20 milliliters of 95 per cent ethanol and digested for 15 minutes on a steam bath. The mixture was then filtered and the filtrate set in the refrigerator to cool. After 2 hours 0.047 grams of fine silky crystals precipitated (S38).

S38 did not melt below 350° C; no gas was evolved when treated with hydrochloric acid. No precipitate was formed upon addition of barium chloride solution and it charred in a flame leaving a residue which turned litmus paper blue when dissolved in water. It was suspected that S38 was either potassium isethionate or potassium ethylene sulfonate. To distinguish between the two an equivalent weight was determined as follows. A weight of 0.02195 grams of S38 was dissolved in water and run through a column containing Dowex 50 cation exchange

TABLE 6

PLATINUM IN TRIMETHYLAMINE CHLOROPLATINATES

Source of trimethylamine	mgm of sample	mgm of Pt	% Pt	Pt expected
Eastman White Label trimethylamine	73.23	27.06	36.95	
	67.97	25.14	36.99	36.96
BaI	10.30	6.47	37.28	
	13.01	8.17	37.20	36.96

resin (50 to 100 mesh, medium porosity) in the acid form at a rate of 2 milliliters/3 minutes to convert the salt to the acid. The solution was collected till it became neutral to pH paper and was then titrated with 0.01631 N potassium hydroxide using a Gilmont Micro-buret. The equivalent weight found was 153; calculated for potassium isethionate it is 164 and for potassium ethylenesulfonate, 146.

The S-benzylthiouronium salt of S38 was prepared according to the procedure of Whitmore and Landau (67) and recrystallized from water-ethanol, melting point 142-144° C. According to Whitmore and Landau the melting point of the S-benzylthiouronium salt of ethylenesulfonic acid is 145-146° C. The melting point of the S-benzylthiourea hydrochloride used in the preparation of the thiouronium salt was 140° C. The mixed melting point of S-benzylthiourea hydrochloride and the thiouronium salt of S38 was 110°-140° C.

Since the determination of the equivalent weight of S38 did not distinguish clearly between potassium isethionate and potassium ethylenesulfonate, it was decided to prepare synthetic potassium isethionate for comparison. Lambert and Rose (68) prepared sodium-2-hydroxypropane-1-sulfonate and sodium-prop-1-ene-1-sulfonate from sodium bisulfite and propylene oxide. It was thought that potassium ethylenesulfonate and potassium isethionate could be made using potassium bisulfite and ethylene oxide. A solution containing 135 milliliters of water and 66 grams of potassium bisulfite was shaken with 22 grams of ethylene oxide. Very soon a white precipitate formed which did not disappear after 2 hours of shaking. The water was evaporated off and the residue was extracted with hot 95 per cent ethanol. The hot ethanol solution was decanted. Almost

immediately, fine white crystals precipitated. The ethanol solution was allowed to stand at room temperature overnight and the precipitate was collected and dried, melting point 195-196° C (uncorr.), after recrystallization from ethanol. After recrystallizing a second time from ethanol the melting point was 194-195° C (uncorr.) when the temperature was raised 1° C/minute. The value reported for potassium isethionate is 190° C (69). S38 did not melt below 350° C.

For comparison with the natural product obtained from Briareum, taurobetaine was synthesized from taurine and diazomethane according to the method of Kuhn and Brydowna (70). Two grams of taurine was dried and ground to a very fine powder and placed in a 500 milliliter Erlenmeyer flask with a Teflon covered stirring bar. Diazomethane was prepared by decomposing 20 grams of nitrosomethylurea in 100 milliliters of U. S. P. ether over potassium hydroxide (71). When the diazomethane was added to the taurine a fizzing sound was noticed and white fumes were given off. After about 15 minutes of stirring the yellow color disappeared and a second portion of diazomethane, equal to the first, was added. The mixture was then stirred until almost all of the color had disappeared and more diazomethane from the decomposition of 10 grams of nitrosomethylurea in 50 milliliters of ether was added. The mixture was stirred for one and one-half hours at which time the yellow color of excess diazomethane was still present.

The ether was decanted off and the residue dried. The weight of crude taurobetaine was 2.20 grams (80 per cent yield). The crude material had a decomposition point of 342° C (uncorr.) when inserted in the melting point block at 338° C. The crude taurobetaine was heated to

boiling with 300 milliliters of methanol and filtered hot. The solid which remained was heated to boiling in 30 milliliters of methanol, and water was added at the boiling point till all of the solid had dissolved except for a minute residue. The hot solution was filtered. Upon cooling colorless silky needles precipitated. The crystals were washed with a small amount of ether and sucked dry. The dry taurobetaine weighed 1.25 grams and had a decomposition point of 345° C (uncorr.) when inserted into the melting point apparatus at 338° C. The infra-red absorption spectra of the natural and synthetic taurobetaine were obtained on a Perkin-Elmer 21 spectrophotometer and were identical. The data are given in table 7.

TABLE 7

ABSORPTION MAXIMA FOR TAUROBETAINE, POTASSIUM ETHYLENE SULFONATE AND POTASSIUM ETHANESULFONATE

	$\cdot CH{:}CH_2$		$\cdot SO_3^-$		other
Potassium ethylene sulfonate	3.27 μ, 7.18 μ, 10.23 μ,	6.10 μ 7.88 μ 10.55 μ	8.20 μ, 9.50 μ,	8.40 μ	13.25 μ
Potassium ethanesulfonate	$\underline{\cdot CH_2 \cdot}$ 3.33 μ,	6.82 μ	7.95 μ, 8.35 μ, 13.25 μ	8.15 μ 9.40 μ	
Taurobetaine (natural and synthetic)	3.33 μ,	6.73 μ	8.35 μ,	9.64 μ	10.28 μ 10.90 μ 10.59 μ 12.98 μ

A small amount of synthetic taurobetaine was subjected to alkaline decomposition as described on page 29. The product corresponded to that which was obtained from natural taurobetaine. It did not melt below 360° C but turned dark slowly as the temperature was raised to that point. The S-benzylthiouronium derivative had a melting point of 139-141° C (corr.). The mixed melting point of the S-benzylthiouronium derivatives was 140-142° C (corr.). The infra-red absorption maxima of potassium ethylenesulfonate and of potassium ethanesulfonate are compared in table 7.

Since BaI was shown to be taurobetaine, which contains the N-methylammonium radical, the following experiment was done to show that the taurobetaine was not produced during extraction of Briareum asbestinum by methylation of taurine by hot methanol. A methanol extraction was run on 240 grams of Briareum asbestinum, previously subjected to the serial extraction procedure, mixed with 1.002 grams of taurine, decomposition point 327° C (uncorr.). At the end of 5 hours of extraction 0.212 grams of white fluffy material separated out, decomposition point 312° C (uncorr.). During the last 19 hours of extraction 0.781 grams of material separated out, decomposition point 312° C (uncorr.). This represents a total of 0.993 grams of taurine recovered. Some of the material from the final extraction period was recrystallized from hot aqueous methanol, decomposition point 325° C (uncorr.) when inserted at 310° C. When inserted at 322° C the decomposition point was 327° C (uncorr.).

A second way of showing the presence of taurobetaine in Briareum asbestinum was to use ethanol rather than methanol in the extraction procedure. This was done on 318 grams of Briareum asbestinum. At the

end of 10 hours 1.116 grams of the spear point crystals were obtained and extraction for 14 hours more yielded crystals weighing 0.241 grams (0.43 per cent total yield). Approximately 1.0 gram of the crude material was recrystallized from ethanol to give 0.210 grams of taurobetaine. The recrystallized material had a decomposition point of 342° C (uncorr.) when inserted at 339° C into the melting point apparatus and the infra-red absorption spectrum was identical with the spectrum of the taurobetaine obtained by the methanol extraction procedure.

Waxes from Gorgonians

The cortex from Eunicea grandis was ground in a meat grinder and extracted with pentane for 24 hours. The extract, a dark brown solution, was filtered to remove the solid material. Some of the filtered extract was run through a Magnesol column to give a colorless solution which was stored in a stoppered Erlenmeyer flask over the summer months. During this time the pentane evaporated leaving flaky colorless crystals, Eg47, of a waxy substance melting at 48-54° C (uncorr.). Eg47 was recrystallized twice from acetone, melting point 57-58° C (corr.).

Briareum asbestinum was ground in a meat grinder and extracted with pentane for 24 hours. The extract was filtered, concentrated to a thick oil, and subjected to steam distillation. The oil which did not steam distill was treated with acetone. A waxy precipitate, Ba55, was obtained which after recrystallization from hot acetone had a melting point of 50.5-51.5° C (uncorr.).

A waxy material, Pc51, which had been isolated from the pentane extract of Plexaura crassa by the formation of a urea complex

by Dr. L. S. Ciereszko, was recrystallized from acetone and showed a melting point of 55-56° C (uncorr.).

The pulverized cortex of Plexaurella nutans was extracted for 24 hours with pentane and the extract filtered. The filtrate was allowed to stand until all of the pentane had evaporated. The solid which deposited separated into a dark brown layer on the bottom and a light yellow layer on top. The yellow layer was mechanically separated, dissolved in pentane and put on a Magnesol column. A mixture of pentane and anhydrous ether was then added. The material moved very slowly and was left to run overnight. In the morning the column was dry and was eluted with anhydrous ether. Most of the color stayed on the column. The waxy material, Pn48, from the ether solution was recrystallized from acetone and had a melting point of 51-52° C (uncorr.).

The infra-red absorption spectra of cetyl palmitate and the waxes from Eunicea grandis, Plexaura crassa, Plexaurella nutans and Briareum asbestinum were run on a Perkin-Elmer 21 infra-red spectrophotometer. The spectra were typical of waxes and were identical except for a few small absorption maxima. The infra-red data are summarized in table 8 along with the melting points.

TABLE 8

THE COMPARISON OF Pn48, Ba55, Pc51 AND Eg47

WITH CETYL PALMITATE

Absorption maxima		Cetyl palmitate	Pn48	Ba55	Pc51	Eg47
3.52 μ	($\cdot CH_2 \cdot$)	p	p	p	p	p
5.75 μ	($\cdot C{:}O$)	p	p	p	p	p
6.82 μ	($\cdot CH_2 \cdot$)	p	p	p	p	p
8.47 μ	($\cdot O \cdot$)	p	p	p	p	p
13.75 μ		p	p	p	p	p
13.95 μ		p	p	p	p	p
9.45 μ *		a	a	---	p	p
9.60 μ *		p	p	p	a	a
9.70 μ *		a	a	---	p	---
Melting point		53°	51-52°	50.5-51.5°	55-56°	57-58°

* Minor absorption maxima.
p Present
a Absent

CHAPTER III

DISCUSSION AND CONCLUSIONS

Halogens and Nitrogen

Both iodine and bromine were found in all gorgonians investigated except Briareum asbestinum and Plexaurella dichotoma which contained only bromine (see table 4). Of the sixteen species examined only two, Pterogorgia acerosa (from Bimini) and Muricea muricata (from Bermuda), had a mole ratio of I/Br higher than 1.00. A low mole ratio of I/Br has been found in most gorgonians by other investigators (see table 1). Low (73) has found this to be true of the sponges.

The deviations of halogen content from species to species in the same family are not significant because the content of individual colonies of the same species vary (see E. mammosa, table 4). As a family, the Plexauridae show the least variation of halogen content. It is comparatively high.

The average I/N and Br/N mole ratios for four species of Gorgoniidae investigated were 0.01; for eight Plexauridae the average ratios were 0.016 and 0.044. The deviations from the average were not very great. Not enough individual species have been investigated yet to say anything definite but it is interesting to note that the ratios are higher for the eight Plexauridae than for the four Gorgoniidae.

The differences of content for individuals of different color, but of the same species, and for individuals collected at different places, but of the same species, were not significant.

Taurobetaine from Briareum Asbestinum

The discovery of taurobetaine by Ackermann and List (39) in sponges and independently by the author in Briareum asbestinum adds another taurine derivative to the list of those discovered in recent years. The function of taurobetaine in the gorgonian is at this time unknown. Taurine, N-methyltaurine, di-N-methyltaurine and choline sulfate are found in algae; hypotaurine, methionine, isethionic acid and cysteic acid amide are found in squid axoplasm; and thiotaurine is found in rat urine. Taurobetaine may play a role in intermediate metabolism as Lindberg (38) postulates or it may be involved in transmethylation. It could play a role in the formation of trimethylamine and trimethylamine oxide. Another possibility is that its function may be that of assisting in the maintenance of osmotic pressure. The high concentration of taurobetaine lends support to this last idea because as a highly ionized internal salt it needs no counter ion. Hoppe-Seyler and Linneweh (74) have suggested that betaines are typical excretion products and have received support for this idea from Kutscher and Ackermann (75).

The alkaline degradation of taurobetaine (see p. 30) did not give potassium isethionate as reported by James (66). The product did not melt below 350° C. Its S-benzylthiouronium salt had a melting point of 142-144° C, very close to the melting point of the S-benzylthiouronium salt of potassium ethylenesulfonate. The mixed melting point of the

S-benzylthiouronium salts of the alkaline degradation products are identical. The infra-red data (table 6) show that the degradation product is potassium ethylenesulfonate. The sulfonate maxima for potassium ethylenesulfonate correspond very well to those reported by Simon, Kriegsmann and Dutz (72) for potassium ethanesulfonate and those reported by Bellamy (76). It is probable that the maximum at 13.25μ is due to the sulfonate group as neither of the sulfonates contain other groups which normally absorb toward that end of the spectrum. The maxima for $\cdot CH{:}CH_2$ in potassium ethylenesulfonate correspond to the regions of absorption assigned to this group by Bellamy.

Lipid Materials in Gorgonians

The infra-red spectra of the waxes from Plexaurella nutans, Plexaura crassa, Briareum asbestinum and Eunicea grandis indicate ester linkages and saturation. The major maxima of the natural products and cetyl palmitate are identical. Differences in minor maxima (see table 8) show up in only the waxes from Plexaura crassa and Eunicea grandis when compared to cetyl palmitate.

The melting points of the waxes from Plexaurella nutans and Briareum asbestinum together with the infra-red information show that they are cetyl palmitate.

The melting points of the other two waxes from Plexaura crassa and Eunicea grandis are higher than that of cetyl palmitate. Because of this and the differences in the infra-red spectra compared to cetyl palmitate, it seems likely that they are higher homologs of cetyl palmitate. The wax from Plexaura crassa could be octadecyl palmitate,

melting point 55°, cetyl stearate, melting point 56.5° or stearyl stearate, melting point 62° C. The wax from Eunicea grandis might be stearyl stearate.

The finding of cetyl palmitate in Plexaurella nutans and Briareum asbestinum adds two more coelenterates to those in which cetyl palmitate is a major constituent.

CHAPTER IV

SUMMARY

Several investigators have determined the iodine, bromine and nitrogen contents of the axial skeletons of gorgonians, but no one had ever determined all three, and compared them, in the same gorgonian. The iodine, bromine and nitrogen contents of sixteen gorgonians from Bermuda and Bimini were found and compared in this study. The iodine, bromine and nitrogen per centages by dry weight of skeletons are as follows. Briareum asbestinum (Rabbit Cay), 0.00, 0.07, 1.79; Eunicea grandis, (Castle Harbor), 1.29, 2.83, 12.15; Eunicea grandis (Ferry Point), 1.21, 3.18, 11.94; Eunicea grandis (Somerset), 1.71, 2.99, 13.06; Eunicea mammosa (Rabbit Cay), 2.29, 3.22, 13.55; Eunicea mammosa (Rabbit Cay), 2.09, 2.57, 12.55; Eunicea mammosa (Rabbit Cay), 1.98, 2.70, -----; Eunicea tourneforti (Castle Harbor), 0.89, 3.44, 12.18; Eunicea tourneforti (Ferry Point; a gangling form), 1.41, 3.47, 10.07; Muricea muricata (Castle Harbor), 1.70, 1.02, 13.51; Plexaura crassa (Castle Harbor), 1.49, 2.44, 11.92; Plexaura crassa (North Rock), 2.16, 2.44, 12.78; Plexaura esperi (Castle Harbor), 1.36, 2.58, 12.80; Plexaura flavida (Rabbit Cay), 2.60, 0.63, 14.17; Plexaura flexuosa (Ferry Point; brown), 1.64, 3.26, 12.05; Plexaura flexuosa, (Ferry Point; purple), 2.09, 2.73, 12.06; Plexaura homomalla (Castle Harbor),

1.32, 2.95, 11.62; Plexaura homomalla (Somerset), 1.52, 2.61, 10.92; Plexaurella dichotoma (?) (Castle Harbor), trace, 0.37, 4.35; Plexaurella dichotoma (?) (Somerset), 0.00, 0.28, 2.93; Plexaurella nutans (?) (North Rock), 0.11, 0.22, 2.91; Pterogorgia acerosa (N. of N. Bimini; cortex left on), 0.27, 0.16, 5.82; Pterogorgia acerosa (N. of N. Bimini), 0.08, 0.56, 12.58; Pterogorgia acerosa (Castle Harbor; cortex left on), 0.31, 0.32, 5.88; Pterogorgia americana (North Rock; cortex left on), 0.36, 0.39, 5.76; Rhipidogorgia flabellum (Somerset; cortex left on), 0.32, 0.33, 6.87; Rhipidogorgia flabellum (Rabbit Cay; cortex left on), 0.39, 0.39, 5.76; Rhipidogorgia flabellum (Rabbit Cay; cortex removed with acetic acid), 0.79, 1.01, 13.11; Xiphigorgia citrina (N. Turtle Rock), 0.82, 0.64, 11.92.

The halogen and nitrogen content varied greatly from species to species and even in different specimens of the same species, a significant difference was found. The mole ratio of I/Br was low. The mole ratios of I/N and Br/N were lower in the Gorgoniidae than in the Plexauridae.

Taurobetaine was found to occur in relatively large amounts in Briareum asbestinum, a gorgonian. Taurobetaine was identified by comparison with the synthetic material and by degradation to trimethylamine and potassium ethylenesulfonate. The alkaline decomposition of taurobetaine yields a salt of ethylenesulfonic acid rather than of isethionic acid as reported previously.

The waxes isolated from Briareum asbestinum and Plexaurella nutans were identified as cetyl palmitate by comparison of their infrared spectra and melting points with those of known cetyl palmitate.

The infra-red spectra and melting points of the waxes from Eunicea grandis and Plexaura crassa indicated that they are higher homologs of cetyl palmitate.

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