Comp. Biochem. Physiol., 1973, *Vol.* 44B, *pp.* 325 *to* 336. *Pergamon Press. Printed in Great Britain*

GLYCOSIDES OF MARINE INVERTEBRATES—I. A COMPARATIVE STUDY OF THE GLYCOSIDE FRACTIONS OF PACIFIC SEA CUCUMBERS

G. B. ELYAKOV, V. A. STONIK, E. V. LEVINA, V. P. SLANKE, T. A. KUZNETSOVA and V. S. LEVIN

Institute of Biologically Active Substances, Far East Science Centre, Academy of Sciences of the U.S.S.R., Vladivostok-22, U.S.S.R.

(*Received* 23 *May* 1972)

Abstract—1. Glycoside fractions from thirty-four sea cucumber species were isolated by means of precipitation with cholesterol and subjected to a comparative examination.

2. Previously unknown triterpene glycosides were detected in the genera *Bohadschia*, *Stichopus*, *Thelenota*, *Cucumaria* and *Afrocucumis*.

3. Glycoside fraction hydrolysis products were investigated.

4. A relationship was established between the systematic position of the animal and its glycoside content. The families Holothuriidae, Stichopodidae and Cucumariidae have different sets of glycosides; cholesterol-precipitated glycosides were not detected in Synaptidae.

INTRODUCTION

TRITERPENE glycosides are known to be vital for numerous plants with a broad range of biological activity. Nigrelli *et al.* (1955) and Yamanouchi (1955) were the first to discover that marine animals, sea cucumbers in particular, may also be a source of triterpene glycosides. At present, there are data in the literature on the isolation of several triterpene glycosides from certain holothurians (Chanley *et al.*, 1959; Yasumoto *et al.*, 1967; Elyakov *et al.*, 1969; Elyakov *et al.*, 1971).

On the other hand, no data are available on comparative chemical studies of triterpene glycoside distribution in Holothurioidea. Hence, the following points still remain obscure: (1) the degree of variety of triterpene glycosides in sea cucumbers and (2) the presence or absence of a relationship between the systematic position of the animals and their glycoside content.

In this work, we have tried to provide an answer to the above problems.

MATERIALS AND METHODS

Sea cucumber sources

Most of the species were collected in the islands of the West Pacific during the summer months of 1971; three species, viz. *Stichopus japonicus*, *Cucumaria japonica* and *C. fraudatrix*, were collected in Posiet Bay, Sea of Japan, during the winter months of the same year.

Isolation of glycoside fractions

To isolate glycosides, we used a complex-forming reaction with cholesterol. Unlike the usual procedure (Chanley *et al.*, 1959), in which the reaction is conducted in aqueous ethanol, we used water-saturated butanol as the reaction medium in which cholesterol and sea cucumber glycosides proved to be readily soluble. This made it possible to avoid any significant dilution of the reaction mixture and promoted fuller precipitation of triterpene glycosides.

The ground-up animals (0·1–2 kg) were covered with 90% ethanol and kept for several weeks. The ethanol solutions were evaporated dry *in vacuo*. The dry residue was extracted with water-saturated butanol with subsequent evaporation of the extract under vacuum until a slight turbidity was seen. Cholesterol was added to the solution under intense agitation (70–80 mg of cholesterol per 1 g of dry ethanol extract residue). The mixture was kept at 40–50°C for 15 min and left for 24 hr. The resultant precipitate was separated by centrifuging and washed off with ether. To dissociate the complex, the residue was dissolved in pyridine (3 ml of pyridine/0·1 g of residue), and after 5–6 hr the solution was diluted with ether (4–5 v/v). The glycoside residue was separated and washed with ether.

To desalinate the glycoside fractions, they were subjected to gel-chromatography on Sephadex G-15 columns and eluted with water. The fraction compositions were analysed with the aid of thin-layer chromatography (TLC) (H_2SO_4 was detected). Fractions similar in composition were taken together and evaporated *in vacuo*.

Thin-layer chromatography. Glycosides were analysed on plates (90 × 120 cm) with a fixed KSK silica gel layer in the following systems: chloroform–methanol–water, 60 : 30 : 4, v/v (A) and butanol–ethanol, 5 : 1, v/v, saturated with water (B). Aglycones were chromatographed in benzene–ethyl acetate = 2 : 1 (v/v) and aglycone acetates in benzene–ethyl acetate = 10 : 1 (v/v).

A glycoside fraction of *Holothuria leucospilota* (= *H. vagabunda*) was used as a reference substance; it contained a mixture of holothurins A and B (Matsuno *et al.*, 1966; Yasumoto *et al.*, 1967). Clearer chromatograms were obtained with (A).

Glycoside hydrolysis and examination of hydrolysis products. Fifty mg of the glycoside fraction were heated for 2·5 hr with 7 ml of 10% HCl at 90–100°C. The mixture was then cooled, and the precipitated aglycone residue separated and washed with water.

To isolate the individual aglycones, the aglycone mixture was chromatographed on a KSK silica gel column (150–200 mesh) with benzene–ethyl acetate elution (gradually increasing the ethyl acetate content from 10 : 1 to 2 : 1, v/v).

The filtrate was neutralized with Dowex-HCO_3^-. Monosaccharides were washed off the resin with aqueous methanol, and the solution was evaporated to dryness. The carbohydrate mixture was analysed by means of paper chromatography on Whatman No. 1 filter paper in phenol–water = 100 : 40 (v/v) and, at the same time, as aldononitrile peracetates by means of gas–liquid chromatography (GLC), using the method of Easterwood & Byron (1969), on a Zvet-3 instrument (chromosorb W, 8% butanediol succinate).

RESULTS

The results obtained for thirty-four sea cucumber species are shown in Tables 1–3.

As is apparent from Table 1, all the species belonging to the genera *Holothuria* and *Actinopyga* contain, as main glycosides, holothurin A or holothurin B (or very similar holothurins) or their mixtures.

To confirm this conclusion, we studied the hydrolysis products of the glycoside fractions.

TABLE 1—GLYCOSIDE COMPOSITION AND HYDROLYSIS PRODUCTS OF THE GLYCOSIDE FRACTION FROM FAMILY HOLOTHURIIDAE

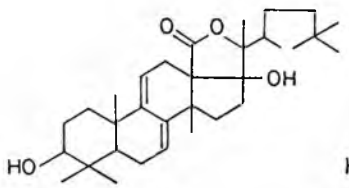
Species	Locality	Yield* (%)	Holothurins			Products of hydrolysis				
			A	B	C	Genin	Glu†	3-O-Me-glu	Xyl	Quin
Holothuria atra	New Guinea	0·5	–	+++	–	I, II	–	–	+++	+++
H. atra	Nauru Island	0·6	+++	+++	–	I, II	++	++	+++	+++
H. atra	Efate Island, New Hebrides	0·8	+++	+++	–	I, II	++	++	+++	+++
H. atra	New Caledonia	2·0	++	+++	–	I, II	+	+	+++	+++
H. atra	Upolu Island, Samoa	2·1	+++	+++	–	I, II	+	+	+++	+++
H. atra	Funafuti Island, Ellice Islands	0·6	+++	+++	–	I, II	++	++	+++	+++
H. atra	Marakei Island, Gilbert Islands	2·5	+++	++	–	I, II	+++	+++	+++	+++
H. arenicola	Lord Howe Island	2·7	+++	–	–	I	+++	+++	+++	+++
H. cinerascens	Funafuti Island, Ellice Islands	0·1	+++	–	–	I, II	+++	+++	+++	+++
H. coluber	Efate Island, New Hebrides	0·9	+	+++	–	I, II	–	–	+++	+++
H. difficilis	Lord Howe Island	0·85	+++‡	–	–	I	+++	+++	+++	+++
H. edulis	Marakei Island, Gilbert Islands	4·0	+++	+++	–	I, II	+	+	+++	+++
H. fuscocinerea	Lord Howe Island	2·5	++	+++	–	I, II	+	+	+++	+++
H. gracilis	New Caledonia	2·0	++	+++	–	I, II	+	+	+++	+++
H. hilla	Upolu Island, Samoa	2·3	+++	+++	–	I, II	++	++	+++	+++
H. impatiens	Upolu Island, Samoa	0·2	+++‡	–	–	I	+++	+++	+++	+++
H. leucospilota	Efate Island, New Hebrides	1·1	+	+++	–	I, II	+	+	+++	+++
H. nobilis	Viti Levu Island, Fiji	2·0	+++	–	–	I	+++	+++	+++	+++
H. pervicax	New Caledonia	1·2	+	+++	–	I, II	+	+	+++	+++
H. pulla	Funafuti Island, Ellice Islands	5·0	++	+++	–	I, II	+	+	+++	+++

[Continued

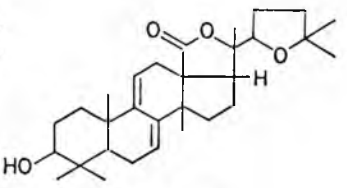
Species	Locality	Yield (%)	Holothurins			Products of hydrolysis				
			A	B	C	Genin	Glu†	3-O-Me-glu	Xyl	Quin
H. scabra	Efate Island, New Hebrides	2·2	–	+ + +	–	I, II	–	–	+ + +	+ + +
Holothuria sp.	New Guinea	1·0	+ +	+ + +	–	I, II	+	+	+ + +	+ + +
Actinopyga mauritiana	New Guinea	0·2	+ + +	+ + +	–	I, II	+ +	+ +	+ + +	+ + +
A. mauritiana	Nauru Island	0·5	+ + +	+ + +	–	I, II	+ +	+ +	+ + +	+ + +
A. mauritiana	Marakei Island, Gilbert Islands	0·2	+ +	+ + +	–	I, II	+	+	+ + +	+ + +
A. miliaris	Upolu Island, Samoa	0·8	+ +	+ + +	–	I, II	+	+	+ + +	+ + +
A. echinites	New Caledonia	0·7	+ + +	+ + +	–	I, II	+ +	+ +	+ + +	+ + +
A. lecanora	New Caledonia	1·5	+ +	+ + +	–	I, II	+	+	+ + +	+ + +
Actinopyga sp.	Butaritari Island, Gilbert Islands	0·6	+	+ + +	–	I, II	+	+	+ + +	+ + +
Bohads. argus	New Guinea	1·0	–	–	+	III	+ + +	+ + +	+ + +	+ + +
B. argus	Efate Island, New Hebrides	5·0	–	–	+	III	+ + +	+ + +	+ + +	+ + +
B. argus	New Caledonia	2·3	–	–	+	III	+ + +	+ + +	+ + +	+ + +
B. graeffei	New Guinea	1·5	+ +	+ + +	–	I§	+	+	+ + +	+ + +
B. marmorata	Marakei Island Gilbert Islands	20·0‖	–	–	+	III	+ + +	+ + +	+ + +	+ + +
Bohadschia sp.	Efate Island, New Hebrides	0·5	–	–	+	III	+ + +	+ + +	+ + +	+ + +

* Yield of the glycoside fraction per weight of the dry residue of the ethanolic extract.
† Glu = glucose; 3-O-Me-glu = 3-O-methylglucose; Xyl = xylose; Quin = quinovose.
‡ Glucoside is comparable to holothurin A, but with a different polarity.
§ Besides I some unidentified genins were detected.
‖ For glycosides from Cuvier's gland the yield of glycoside from the body wall is equal to 3 per cent.

It is known (Chanley *et al.*, 1966; Matsuno, 1966; Yasumoto *et al.*, 1967) that with acid hydrolysis holothurins A and B form 22,25-epoxy-7,9(II),holostadien-3-17-diol (I) as the main component (nomenclature of Habermehl *et al.*, 1971), and in lesser quantities its deoxyanalogue (II).



I



II

At the same time, the monosaccharide composition in A and B differed. Holothurin A contained glucose, xylose, quinovose and 3-O-methyl glucose, while holothurin B only contained quinovose and xylose (Matsuno, 1966; Yasumoto, 1967).

It has been found that in all the cases studied the Holothuriidae representatives contain glycosides that form holothurinogenins with acid hydrolysis (I and II). Substances I and II were identified with TLC in the presence of authentic samples.

Moreover, I and II were isolated in a chemically pure form by sampling from several species (*Holothuria atra*, *H. pervicax*, *H. cinerascens* and *Actinopyga mauritiana*). They were identified by comparing the spectral data (i.r., u.v. and mass) with those of the authentic samples.

The glycoside fractions which, according to TLC, contained mainly holothurin B, with acid hydrolysis, gave only xylose and quinovose; the fractions containing holothurin A gave a mixture of four monosaccharides (xylose, quinovose, 3-O-methyl glucose and glucose). Gas chromatograms for aldononitrile peracetate mixtures obtained from the hydrolysis products of certain sea cucumbers are shown below.

Of the four *Bohadschia* species studied, only *B. graeffei* contained a set of glycosides typical for *Holothuria* and *Actinopyga*. The main component in this set was holothurin B, whereas the rest contained a different main glycoside. With acid hydrolysis, this substance, which we called holothurin C, forms a mixture of quinovose, xylose, 3-O-methyl glucose and glucose. However, holothurinogenins (I–II) were absent among the aglycones obtained. According to the i.r.-spectrum, the principal aglycone contained a five-membered lactone (1760 cm^{-1}), and a haeme-dimethyl group (1380 cm^{-1}); the u.v.-spectrum showed the presence of 7(9),II-diene, max 237, 244 and 252 nm. The molecular weight of the aglycone was 454, the same as for seishellogenin previously isolated by Roller *et al.* (1969) from *B. koellikeri*.

Yet, even though the i.r.-spectra for (III) and the seishellogenin authentic sample are very similar, they do not coincide in the hydroxyl group absorption range.

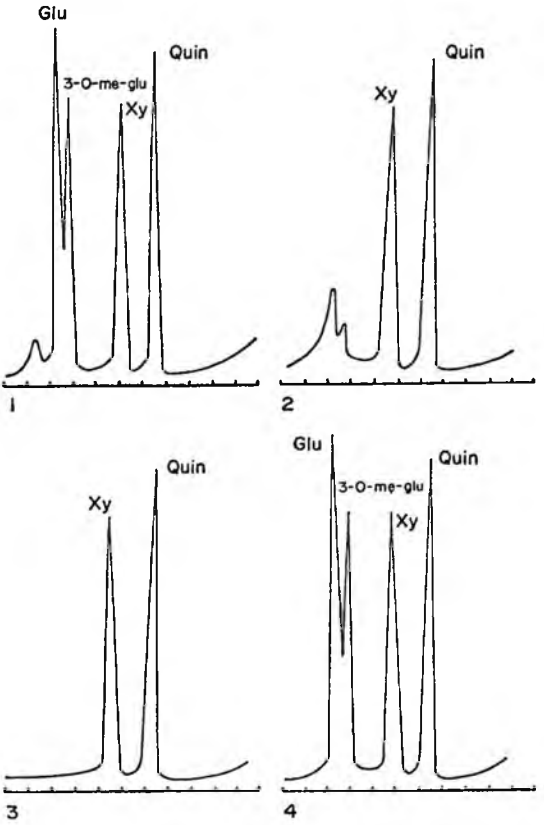
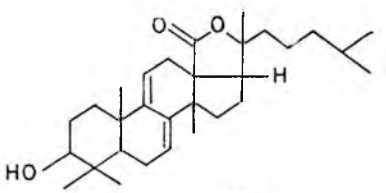


FIG. 1. Gas–liquid chromatograms for aldononitrile peracetates obtained from hydrolysis products of glycoside fractions. (Zvet-3 instrument, chromosorb W, 8% butanediol succinate). (1) Fraction of *H. arenicola* (holothurin A). (2) Fraction of *A. mauritiana* (holothurins A + B). (3) Fraction of *H. scabra* (holothurin B). (4) Mixture = glucose–3-O-methyl glucose–xylose–quinovose (1 : 1 : 1 : 1).

Probably, the aglycone we isolated is isomerous to seishellogenin and, like the latter, may be represented by the formula:



III

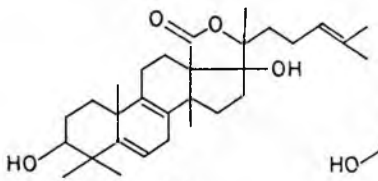
Triterpene glycosides contained in the four Stichopodidae species examined differ sharply from holothurins A, B and C (see Table 2).

TABLE 2—GLYCOSIDE COMPOSITION AND HYDROLYSIS PRODUCTS OF THE GLYCOSIDE FRACTION FROM FAMILY STICHOPODIDAE

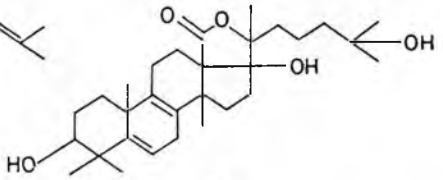
Species	Locality	Yield* (%)	Stichoposides		Other glycosides	Products of hydrolysis				
			A, C	Other		Genin	Glu†	3-O-Me-glu	Xyl	Quin
Stichopus chloronotus	Viti Levu Island, Fiji	2·3	–	+	–	VI, VII	+ + +	+ + +	+ + +	+
S. chloronotus	Butaritari Island, Gilbert Islands	4·0	–	+	–	VI, VII	+ + +	+ + +	+ + +	+
S. chloronotus	Efate Island, New Hebrides	2·0	–	+	–	VI, VII	+ + +	+ + +	+ + +	+
S. variegatus	Viti Levu Island, Fiji	0·3	–	+	–	VI, VII	+ + +	+ + +	+ + +	+
Thelenota ananas	New Guinea	2·4	–	+	–	VI, VII	+ + +	+ + +	+ + +	+
S. japonicus	Possjet Bay, Japan Sea	1	+	–	–	IV, V	+ + +	+ +	+ + +	+

* and † See Table 1.

Stichopus japonicus contains mainly two known glycosides, i.e. stichoposides A and C (Elyakov *et al.*, 1968). With acid hydrolysis of the glycoside fraction, stichopogenins (IV, V) form along with xylose, quinovose, glucose and 3-O-methyl glucose (Elyakov *et al.*, 1969). These stichopogenins do not contain a heteroannular diene chromophore typical of genins I-III.



IV



V

Three other representatives of this family contain a set of three to four main glycosides. These glycosides form glucose, xylose, 3-O-methyl glucose and a slight amount of quinovose.

With acid hydrolysis, they produce, in the main, two aglycones featured by the absence of chromophore in the heteroannular diene.

According to the i.r.-spectra, the first aglycone (VI) has two types of esters, viz. an acetate (1735 cm^{-1}) and a five-membered lactone (1760 cm^{-1}). The aglycone molecular weight was 514 (mass spectrometry).

The other aglycone (VII) has a molecular weight of 472 and lacks an acetate group; however, it retains the five-membered lactone. The aglycone structures will be discussed in a separate communication.

The investigation results for five species belonging to the families Cucumariidae and Synaptidae are shown in Table 3.

Glycoside fractions isolated from three Cucumariidae species contained a series of new glycosides differing from the previously described cucumarioside C (Elyakov *et al.*, 1970). Figure 2 shows chromatograms for the glycoside fractions of the cucumariosides studied. All three Cucumariidae species contained glycosides forming different aglycones at hydrolysis. The main aglycone *Cucumaria fraudatrix* (VIII) does not contain a heteroannular diene (in the u.v. spectrum, no absorption was observed above 210 nm) and it has a molecular weight equal to 512. *C. japonica* (IX, X) aglycones likewise do not contain the said diene, and their molecular weights are 468 and 486, respectively.

The *Afrocucumis africana* (XI) aglycone differs from the Cucumariidae aglycones in spectral characteristics.

Using the above procedure with cholesterol, we did not succeed in isolating triterpene glycoside fractions from the two Synaptidae representatives.

To elucidate the influence of animal habitat conditions on the glycoside composition, we examined in some cases several samples of one species; the samples were collected from various regions. It turned out (see Tables 1–3) that the

TABLE 3—GLYCOSIDE COMPOSITION AND HYDROLYSIS PRODUCTS OF THE GLYCOSIDE FRACTION FROM FAMILIES CUCUMARIIDAE AND SYNAPTIDAE

Family, species	Locality	Yield* (%)	Cucumariosidae			Other glycosides	Products of hydrolysis				
			I gr.	II gr.	III gr.		Genin	Glu†	3-O-Me-glu	Xyl	Quin
Cucumariidae											
Cucumaria fraudatrix	Possjet Bay, Japan Sea	3·0	+	–	–	–	VIII	+ + +	+	+ + +	+ + +
C. japonica	Possjet Bay, Japan Sea	0·2	–	+	–	–	IX, X	+ + +	+ + +	+ + +	+ + +
Afrocucumis africana	Butaritari Island, Gilbert Islands	2·5	–	–	+	–	XI	+ + +	+ +	+ +	+ +
Synaptidae											
Synapta maculata	New Guinea	—	–	–	–	–	–	–	–	–	–
S. maculata	Efate Island, New Hebrides	—	–	–	–	–	–	–	–	–	–
Euapta godeffroyi	Funafuti Island, Ellice Islands	–	–	–	–	–	–	–	–	–	–

* and † See Table 1.

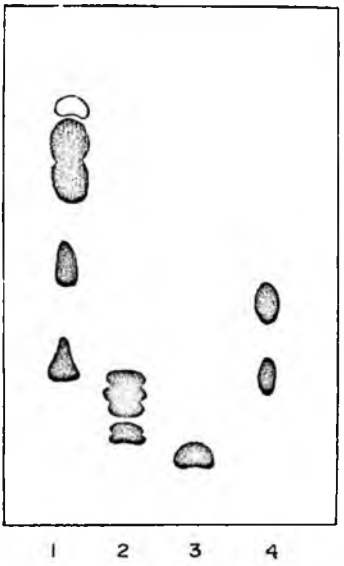


FIG. 2. Chromatograms for some glycoside fractions of Cucumariidae. (System A). (1) *C. fraudatrix*; (2) *C. japonica*; (3) *A. africana*; (4) *H. atra* (for comparison).

glycoside fraction content and glycoside ratio in the fraction depend to a considerable extent on the habitat conditions. Nevertheless, the whole glycoside set, characteristic of a given species, is mainly constant.

DISCUSSION

The works of several authors have shown sea cucumbers to contain triterpene glycosides, holothurins A and B (Chanley *et al.*, 1955; Matsuno, 1966; Yasumoto, 1967), stichoposides A and C (Elyakov, 1968) and cucumarioside C (Elyakov, 1971). We have now established that, apart from the above-mentioned substances, Holothurioidae contain a whole series of unknown triterpene glycosides.

An analysis of the results cited in Tables 1–3 indicates an obvious relationship between the systematic position of the animal and its glycoside content. In some cases, the glycoside composition is in good agreement with the accepted holothurian system (Baranova, 1971; Clark & Rowe, 1971), while in others obscurities arise.

Depending on their triterpene glycoside content, all the Holothuriidae, Stichopodidae, Cucumariidae and Synaptidae studied may be divided into the following groups:

A. Holothuriidae: sea cucumbers containing glycosides that with acid hydrolysis give holothuriogenins with a conjugated diene system of the genin (I-III) type.

B. Stichopodidae: sea cucumbers containing glycosides that with acid hydrolysis give genins lacking a conjugated diene group.

C. Cucumariidae: sea cucumbers giving various glycosides that sharply differ from the glycosides of the first two groups.

D. Synaptidae: sea cucumbers lacking cholesterol precipitated triterpene glycosides.

Within group A, embracing representatives of the Holothuridae family, definite differences were observed between the genera *Holothuria* and *Actinopyga*, on the one hand, and *Bohadschia* on the other. Thus, *Holothuria* and *Actinopyga* build up their glycosides on the basis of aglycones, which, with acid hydrolysis, convert into holothurinogenins (I-II), whereas *Bohadschia* frequently represents other glycosides based on proseishellogenin and affined substances.

Differences were likewise observed within Stichopodidae. Thus, *Thelenota ananas*, *Stichopus chloronotus* and *S. variegatus* have a rather similar set of glycosides and *S. japonicus* differs from them in this respect. This makes it possible to assume that the distinctions between *S. japonicus* and other representatives of the family are greater than is usually presumed.

The same applies to *Cucumaria japonica* and *C. fraudatrix*, which also contain different triterpene glycoside fractions.

It is noteworthy that the differences in the carbohydrate content of glycoside molecules contained in various sea cucumbers are ostensibly of a less basic character, since all the species examined use mainly the same sugars to produce glycosides, viz. glucose, xylose, quinovose and 3-O-methyl glucose.

Acknowledgements—The authors extend their profound gratitude to Professor Y. Chanley for the holothurinogenin (I, II) samples and to Professor C. Djerassi for the seishellogenin (III) sample. They also thank J. C. Shapiro for translating the paper from the Russian.

REFERENCES

Baranova Z. I. (1971) Echinoderms from the Possjet Bay of the Sea of Japan. In *Fauna and Flora of the Possjet Bay of the Sea of Japan*, pp. 242–264. Nauka, Leningrad. (In Russian.)

Chanley J. D., Ledean R., Wax J., Nigrelli R. F. & Sobotka H. (1969) Holothurin—I. The isolation, properties and sugar components of holothurin A. *J. Am. Chem. Soc.* **81**, 5180–5183.

Chanley J. D., Mazzetti T. & Sobotka H. (1966) The holothurinogenins. *Tetrahedron Letts* **22**, 1857–1884.

Clark A. M. & Rowe T. W. E. (1971) *Monograph of Shallow-water Indo-West Pacific Echinoderms*, 1–238. London.

Easterwood V. M. & Huff B. J. L. (1969) Carbohydrate analysis gas chromatography of acetylated aldonitriles. *Svensk Papperstidning* **72**, 768–772.

Elyakov G. B., Kuznetsova T. A., Dzizenko A. K. & Elkin Y. N. (1969) A chemical investigation of the trepang (*Stichopus japonicus* Selenka): the structure of triterpenoid aglycones obtained from trepang glycosides. *Tetrahedron Letts* **15**, 1151–1154.

Elyakov G. B., Kuznetsova T. A. & Vaskovsky V. E. (1968) A chemical investigation of the trepang (*Stichopus japonicus* Selenka). *Khim. Prirod. Soedin., Acad. Nauk Uz. SSR*, **4**, 253–254. (In Russian.)

Elyakov G. B. & Peretolchin N. V. (1970) Cucumarioside C—new triterpenoid glycosides from holothurian *Cucumaria fraudatrix*. *Khim. Prirod. Soedin., Acad. Nauk Uz. SSR*, **5**, 637. (In Russian.)

HABERMEHL G. & VOLKWEIN G. (1971) Aglycones of the toxins from the Cuvierian organs of *Holothuria forskali* and a new nomenclature for the aglycones from *Holothurioideae*. *Toxicon* **9**, 319–326.

MATSUNO T. & IBA J. (1966) On the saponins of the sea-cucumber. *Yakugaku Zasshi*, **86**, 637–638.

NIGRELLI R. F., CHANLEY J. D., KOHN S. K. & SOBOTKA H. (1955) The chemical nature of holothurin, a toxic principle from the sea-cucumber (*Echinodermata*; *Holothurioideae*). *Zoologica* (*N. Y.*) **40**, 47–48.

ROLLER P., DJERRASSI C., CLOETENS R. & TURCH B. (1969) Chemical studies of marine invertebrates—V. Isolation of three new holothurinogenins and their chemical correlation with lanosterol. *J. Am. Chem. Soc.* **91**, 4918–4920.

YAMANOUCHI T. (1955) On the poisonous substance contained in holothurians. *Publ. Seto Mar. Biol. Lab.* **4**, 184–202.

YASUMOTO T., NAKAMURA K. & HASHIMOTO Y. (1967) A new saponin, holothurin B, isolated from sea-cucumber, *Holothuria vagabunda* and *Holothuria lubrica*. *Agr. biol. Chem.* **31**, 7–10.

Key Word Index—Glycosides; triterpenoid glycosides; sea cucumber; *Holothurioidea*; hydrolysis of glycosides; *Holothuriidae*; *Stichopodidae*; *Cucumariidae*; *Synaptidae*.