J. Natn. Sci. Coun. Sri Lanka 1983 **11** (2): 185 – 190

A Comparative Study of the Glycoside Fractions of some Holothurians Found in Sri Lankan Waters

M. MAHENDRAN*, T. W. ABRAHAM, S.R. KRISHNARAJAH
Department of Chemistry, University of Colombo, Colombo, Sri Lanka.

AND

PADMINI ELANGANAYAGAM
Department of Zoology, Batticaloa University College, Vantharumoolai, Chenkaladi, Sri Lanka

(Date of receipt: 03 May 1983)

(Date of acceptance: 23 November 1983)

Abstract: Glycoside fractions from ten holothurians were isolated and subjected to a comparative examination. All ten species contained holothurin A. Except *Holothuria edulis* and *Havelockia versicolor* all other species were found to contain holothurin B. The acid hydrolysis of glycoside fractions of all species examined yielded mainly two genins (22, 25 - epoxy - 7, 9 (11) holostadien-3-17-diol and its deoxvanalogue) and four sugars namely glucose, xylose. 3-0-methyl glucose and quinovose.

1. Introduction

The toxicity of sea-cucumbers has been known for many years.[5] The active compounds, the greatest amount of which occurs in the Cuvierian glands[17] of the animal, have been given the general name holothurins.[11] These holothurins are triterpene glycosides and it has been ascertained that these glycosides possess diverse physiological activity.[2,9,10]

Extracts of sea-cucumbers possess antitumoural activity.[10] They show neurotoxic,[9] cytotoxic,[7] antifungal[14] and antiviral[13] effects. They also affect blood,[6] stimulate activity of RNA-polymerases in rat liver nuclei,[4] inhibit seed respiration,[8] as well as the incorporation of radioactive nucleosides and amino acids in rat marrow cells.[2]

This present study was carried out in order to compare the glycoside fractions of some of the holothurians found in Sri Lankan waters with the glycoside fractions of the holothurians found in the Pacific waters.[3]

2. Materials and Methods

2.1 Isolation of Glycoside Fractions:

Dried samples were ground and first extracted with CH_2Cl_2 in order to remove fat. Extraction was carried out in a soxhlet apparatus. This was followed by extractions with methanol. The methanol solution was evaporated to dryness (in

* Present address - Department of Chemistry, Mc Master University, Hamilton, Ontario, Canada

vacuo). Water was added to the dry residue and this was extracted with butanol with subsequent evaporation of the extract under vacuum until a slight turbidity appeared. Cholesterol was added to the solution while vigorously agitating (70-80 mg of cholesterol per 1 g of dry ethanol extract residue). The mixture was held at 40-50°C for 15 min and left to stand for 24 hrs. The resultant precipitate was separated by centrifugation and washed with ether. In order to dissociate the complex, the residue was dissolved in pyridine (3 ml of pyridine per 0.1 g of residue) and after being left to stand for 5-6 hrs the solution was diluted with ether (4-5, v/v). The glycoside residue which separated was washed with ether.

2.2 Thin-layer Chromatography of glycoside fractions

The glycosides were analysed on silica gel plates (Merck, Kieselgel 60 F 254) using the following solvent systems;

(1) Butanol-Ethanol, 5:1, v/v, saturated with water.

(2) Chloroform-Methanol-water, 75:25:1, v/v
Spray reagent-Vanillin (lg) in concentrated sulphuric acid (100ml)[15]

2.3 Glycoside hydrolysis

Hydrolysis of the glycoside was carried out in 2N HCl (1 ml per 40 mg of saponin). After filtration, the residue (aglycones) was extracted into ether and the ether evaporated.

2.4 Examination of Aglycones

The aglycones were analysed by TLC on silica gel plates (Merck, Kieselgel 60 F 254) using benzene-ethyl acetate; 10:1 (v/v) as the solvent system.

2.5 Analysis of sugars

The sugars were analysed by Paper Chromatography (Whatman, No. 1) using the following solvent systems.

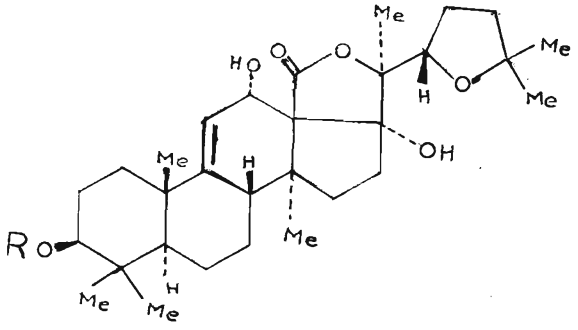
(1) Butanol: Acetic Acid : Water mixture, 4 : 1 : 2.
Spray reagent - Diphenyl amine, Aniline & Phosphoric acid in Ethanol.[15]

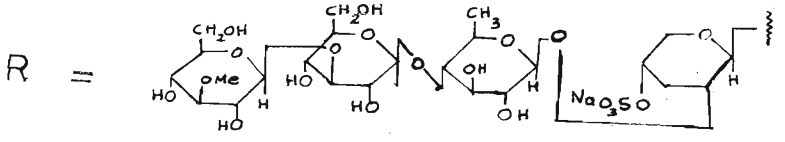
(2) Phenol-Water mixture, 100: 40 (v/v)
Spray reagent - Phthallic anhydride/aniline in Butanol.[15]

3. Results and Discussion

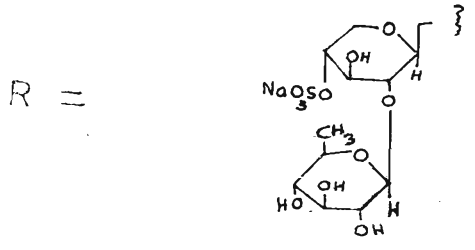
The results obtained for ten species of sea cucumbers are given in Table I.

All ten species studied contain predominantly the glycosides, holothurins A and B, except *Holothuria edulis* and *Havelockia versicolor* which lacked the latter





HOLOTHURIN A



HOLOTHURIN B

Figure 1. Structures of Holothurins A and B

Table 1. Glycoside Composition and Hydrolysis Products of the Glycoside Fraction from the Family Holothuriidae

	Species	Location	% Saponin Gravimetry	Holothurins●				Arlycones○			Products of Hydrolysis			
				Y*	A	B	K*	I	II	P*	Quinovose	Sugars 3 - 0 - Me - Glu	Kylose	Glucose
1.	Holothuria atra	Mandativu	1.06	-	+	+	+	+	+	-	+	+	+	+
2.	Holothuria leucospilota	Mandativu	0.91	-	+	+	+	+	+	-	+	+	+	+
3.	Holothuria nobilis	Trincomalee	0.28	-	+	+	+	+	+	-	+	+	+	+
4.	Holothuria scabra	Mandativu	0.30	-	+	+	+	+	+	-	+	+	+	+
5.	Holothuria edulis	Mannar	0.70	-	+	-		+	+	-	+	+	+	+
6.	Holothuria spinifera	Poonakari	1.03	-	+	+	+	+	+	-	+	+	+	+
7.	Stichopus chloronatus	Trincomalee	0.74	-	+	+	+	+	+	+	+	+	+	+
8.	Stichopus variegatus	Mandativu	1.08	-	+	+	-	+	+	-	+	+	+	+
9.	Bohadschia marmorata	Trincomalee	0.55	-	+	+	+	+	+	-	+	+	+	+
10.	Havelochia versicolor	Mandativu	2.14	+	+	-	+	+	+	-	+	+	+	+

*Unidentified

● Refer Figure 1

○ Refer Figure 2

holothurin. In addition, all ten species, except *Holothuria edulis* and *Stichopus variegatus*, contained an unidentified holothurin (denoted as X). The species *Havelockia versicolor* was unique in that it contained a holothurin (denoted as Y) which was found to be absent in the other nine species.

It is known that, with acid hydrolysis holothurin A and B from 22, 25-epoxy-7, 9(11), holostadien-3-17-diol (figure 2.1) as the main component, in lesser quantities its deoxyanalouge (figure 2.11) and four sugars namely glucose, xylose, 3-0 methyl glucose and quinovose.

The acid hydrolysis of the glycosides (of all ten Sri Lankan species) also yielded the same genins (figures 2.1 and 2.11) and the four sugars.

The results indicate that the glycosides present in the species found in Sri Lankan waters do not differ much from those found in the Pacific waters. Elyakov *et.al.*[3] have reported that *Holothuria nobilis* (Viti Levu Island, Fiji) contained only holothurin A and genin I whereas our studies indicate the presence of both holothurins A & B and genins I & II. They also report the absence of holothurin A in the species *Holothuria scabra* (Efate Island, New Hebrides) whereas the same species studied by us contains holothurin A. The species *Holothuria edulis* was found to lack holothurin

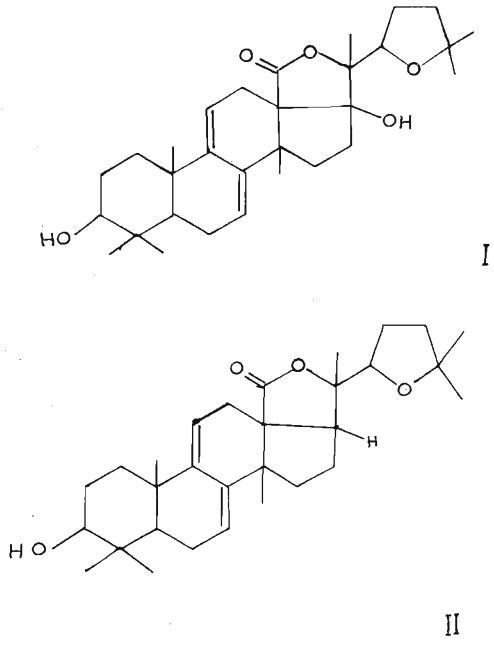


Figure 2. Structures of Aglycones

whereas Elyakov *et al.* have reported its presence in *H. edulis* collected at Maraki Islands and Gilbert Islands.

The species of *Bohadschia marmorata* studied by us contains both holothurins A & B and both genins I & II unlike the results obtained by Elyakov *et.al* which indicates the absence of any of them. Our studies on *Stichopus chloronatus* and *Stichopus variegatus* gave completely different results to that obtained by Elyakov *et al.* Both species were found to contain both holothurins A & B and also contained the two genins I & II.

Acknowledgements

The authors wish to thank the Natural Resources, Energy and Science Authority of Sri Lanka for financial support, Prof. G. B. Elyakov for the authentic samples of holothurin A, B, the genins and the sugars and Prof. A. L. Gunatilake for the authentic sample of 22, 25- oxidoholothriogenin.

References

1. CHANLEY, J. D., MAZZETTI T. & SOBOTKA H. (1966). *Tetrahedron letts.* **22**, 1857-1884.
2. ELYAKOV, G. B. & OVODOV, YU. S. (1972). *Khim.Prir. Soedin.* **6**, 697 (in Russian).
3. ELYAKOV, G. B., STONIK , V. A., LEVINA, E. V., SLANKA, V. P., KUZENTSOVA, T. A. & LENIN, V. S. (1973).*Comp. Biochem. Physiol.* Vol. 44 B. p. 325-336.
4. HIAI, S., OURA, H., TSUKADA, K. & HIRAI, Y. (1971). *Chem. Pharm. Bull.* **19**, 1656.
5. HYMAN, L. H. (1955). *"The Invertebrates-Echinodermata"* McGraw - Hill, N. Y.
6. JAKOWSKA, S., NIGRELLI, R. F., MURRAY, P. M. & VELTRI, A. M. (1958). *Analyt. Res.* **132**, 459.
7. LASLEY, B. J. & NIGRELLI, R. F. (1970). *Toxicon* **8**, 301.
8. MAYEVSKY, A. & MARCHAIM, U. (1972) *Pl. Cell. Physiol.***13**, 927.
9. NIGRELLI, R. F. (1952). *Zoologica* **37**, 89-90.
10. NIGRELLI, R. F. & ZAHL, P. (1952). *Proc. Soc. Exp. Biol. Med.* **81**, 379-380.
11. NIGRELLI, R. F. & JAKOWSKA, S., (1960). *Ann N.Y. Acad. Sci.* **90**, 884.
12. PETTIT, G. R. *et.al. J. Pharm. Sci.* **65**, 1558.
13. RAO, G. S., SINSHEIMER, J. E. & COCHRAN, K. W. (1974), *J. Pharm. Sci.* **63**, 471.
14. SCHIMADA, S. (1969). *Science* **163**, 1462.
15. STAHL, E. (1969). *Thin layer Chromatography*, Springer-vedag, Berlin-Heidelberg-New York.
16. THRON, C. D., DURANT, R. C. & FRIESS, S. L. (1964). *Toxic. Appl. Pharmac.* 6: 182.
17. YAMANOUCHI, T. (1955). *Publ. Seto. Marine Biol. Lab.* **4**, (Part 2) 183.