Journal of Natural Products
Vol. 52, No. 2, pp. 248-251, Mar-Apr 1989

NEOTHYOSIDE A, PROPOSED STRUCTURE OF A TRITERPENOID TETRAGLYCOSIDE FROM THE PACIFIC SEA CUCUMBER, *NEOTHYONE GIBBOSA*[1]

ROSALBA ENCARNACION,* GABRIEL CARRASCO, MARICELA ESPINOZA,

Departamento de Biologia Marina, Universidad Autonoma de Baja California Sur, A.P. 219-B, La Paz, Baja California Sur 23080, Mexico

UFFE ANTHONI, PER H. NIELSEN, and CARSTEN CHRISTOPHERSEN*

Marine Chemistry Section, The H.C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

ABSTRACT.—Neothyoside A, a triterpenoid tetraglycoside, was isolated from the Pacific sea cucumber *Neothyone gibbosa*. The structure was proposed based on spectroscopic studies. The sugar portion was identical to the one encountered in echinoside A, and the aglycone formally derived from the one of bivittoside A by an unprecedented acetoxy substitution at C-25.

Sea cucumbers (Holothuroidea) are conspicuous animals. Nevertheless, they seem to have only very few predators, a fact believed to reflect the pronounced biological activity of triterpenoid saponins present in these echinoderms.

We wish to report the isolation and structure elucidation of the main holothurin in the dendrochirotid holothurian *Neothyone gibbosa* Deichmann (1). The genus was established in Cucumariidae by Deichmann in 1941 (1) and later assigned to the subfamily Sclerodactylinae Panning (2). A revised classification of Dendrochirota raises Sclerodactylinae to family level juxtaposed with Cucumariidae (3).

N. gibbosa is a small to medium-sized cucumber with a white to purple body. It occurs throughout the Gulf of California and has been reported as far south as Peru (4).

RESULTS AND DISCUSSION

The sea cucumbers were collected off Cerralvo Island near La Paz, Mexico, and the body wall was extracted with EtOH. The purified extract yielded pure neothyoside A, which was homogeneous in several hplc systems. The sample was subjected to fabms to give a pseudomolecular ion at *m/z* 1271 corresponding to $[M + Na]^+$. To substantiate the sodium content the sample was treated with LiCl giving rise to the expected pseudomolecular ion $[M + Li]^+$ at *m/z* 1255. These results indicated the molecular weight to be 1248 corresponding to the composition $C_{56}H_{89}O_{27}SNa$.

The structure of the sugar moiety was established by comparison of ^{13}C-nmr data as identical to that reported for echinoside A (5,6). The multiplicity of all ^{13}C-nmr signals (pyridine-d_5) was inferred from DEPT experiments. Except for the signal at 68.3 ppm assigned C-4$''''$, which deviates 1.0 ppm from the published value (5), and the signal from C-2$''''$, which was not resolved, all 24 values deviate less than 0.6 ppm from the reported positions. This agreement is actually better than the match between two independent sets of data for echinoside A (5,6). The chemical shift for carbon 2$''''$ is expected near 75.0 ppm [lit. (5) 75.0 ppm, lit. (6) 74.7 ppm]. Allowing for a deviation of ± 3 ppm, compared to echinoside A, the only candidates for the carbon 2$''''$ signal are those already assigned to carbons 3′, 4′, 2″, 3″, 5″, 2‴, and 5‴. Although the intensity of the carbon resonances is not usually directly related to the number of atoms resonating, it is noteworthy that the signal at 75.0 ppm (C-4′) appears with a relative intensity of 9.6 while the remaining signals in this group have average relative intensities about 5. Ac-

[1]This paper is dedicated to the memory of Alfonso Miranda Lory.

cordingly we assigned the signal at 75.0 ppm to the unresolved resonances originating from C-4′ and C-2′′′′ (Table 1). It is concluded that the sugar moiety of neothyoside A is the 3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiosulfonato-β-D-xylopyranosyl radical.

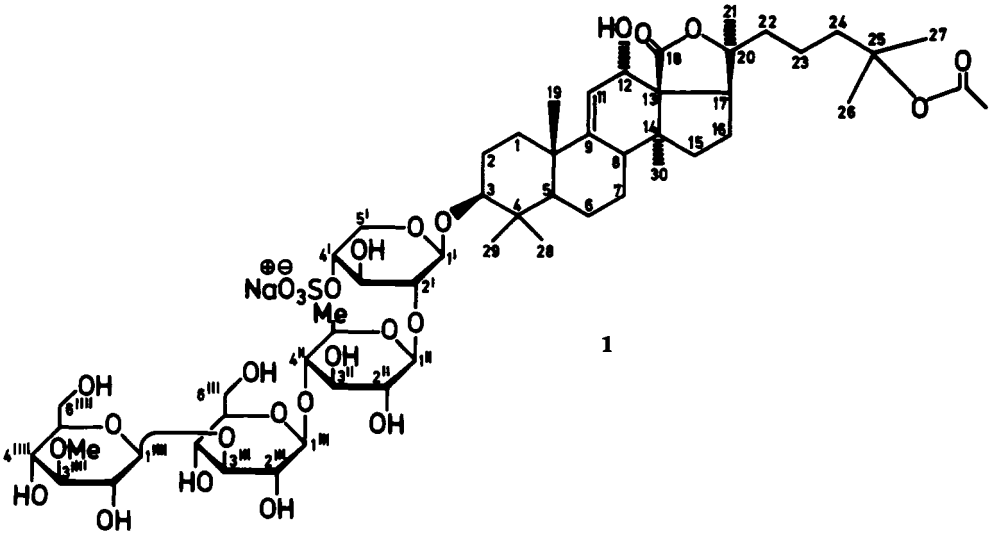
TABLE 1. Assignment of ^{13}C-nmr Data of Neothyoside A [**1**] in Pyridine-d_5.[a]

C	δ ppm	C	δ ppm
1	36.5	29	16.8
2	27.1	30	22.6
3	88.9	Me(Ac)	22.1
4	40.1	CO(Ac)	170.4
5	52.9	1′	105.8
6	21.3	2′	83.3
7	28.9	3′	76.3
8	40.2	4′	75.0
9	153.1	5′	64.3
10	39.7	1″	105.4
11	116.2	2″	75.4
12	70.7	3″	76.0
13	64.3	4″	86.7
14	46.7	5″	72.0
15	37.3	6″	18.1
16	30.0	1‴	105.3
17	47.1	2‴	74.1
18	177.4	3‴	87.9
19	18.1	4‴	69.6
20	84.8	5‴	77.7
21	22.4	6‴	61.9
22	39.7	1′′′′	104.8
23	19.1	2′′′′	75.0
24	41.2	3′′′′	87.8
25	82.1	4′′′′	68.3
26	26.1	5′′′′	78.2
27	26.2	6′′′′	62.2
28	28.3	MeO-3′′′′	60.9

[a]Close-lying signals with the same multiplicity may be interchanged.

According to the ^{13}C-nmr analysis the structure of the aglycone resembles echinoside A (6) except for the absence of the 17α hydroxy function and the introduction of an acetoxy group in position 25. Actually, with the sole exception of carbon atoms at α or in β and γ position to the affected centers C-17 and C-25 and the methyl group at C-10 (C-19), all values deviate less than 0.8 ppm from the values reported for echinoside A (5,6). The signal from C-13 was not resolved. Most likely it coincides with the C-5′ signal at δ 64.3 (7). The signals assigned to carbon atoms at the deoxygenated center (C-17) or the oxygenated center (C-25) and those of the β and γ substituents lie close to the predicted values (8). The presence of the acetoxy function was further established by the ^{1}H-nmr spectrum (chemical shift for Me, 2.02 ppm) and the occurrence of two bands in the C=O stretching region in the ir (1735 and 1752 cm^{-1}, KBr) as opposed to the single maximum in echinoside A reported in the range 1730–1738 cm^{-1} (5,6). Both ir and ^{1}H-nmr spectra are in accordance with the proposed structure of neothyoside A, but in neither case is the information sufficiently specific to allow a positive identification of structural elements.

Based on the above results we conclude the neothyoside A is 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiosulfonato-β-D-xylopyranosyl]-12α-hydroxy-25-acetoxyholost-9(11)en-3β-ol [**1**]. The name is based on holostane [(20*S*)-hydroxy-5-α-lanostane-18-carboxylic acid (18→20) lactone] following a suggestion by Habermehl and Volkwein (9).



1

This saponin encompasses elements encountered in various other holothurins. The tetrasaccharide part of the molecule is identical with the one encountered in echinoside A from *Actinopyga echinites*, in holothurin A from several holothurians, and in 24-dehydroechinoside A from *Actinopyga agassizi* (10). The triterpenoid aglycone resembles the one present in bivittosides A, B and D from *Bohadschia bivittata* (11) except for the oxygenation of C-25. The latter structural element appears to be unprecedented in naturally occurring holothurins but is recognized in an artifact produced by acid hydrolysis of 24-dehydroechinoside A (10). In our opinion, the isolation procedure used here excludes the possibility that neothyoside A might, in fact, be formed during extraction and purification.

Previously complete saponin structures have been published for only one dendrochirotid sea cucumber, *Cucumaria fraudatrix* (Cucumariidae). Cucumarioside G_1, a tetrasaccharide saponin from this species, differs from neothyoside A in the sugar moiety (terminal sugar 3-*O*-methyl-D-xylose). Moreover, the aglycone has endocyclic C-7 (8) and exocyclic C-24 (25) double bonds and a 16β-acetoxy group but lacks the C-9 (11) double bond and the 12- and 25-hydroxyl functions (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The fab mass spectra were recorded on a Kratos MS 50 RF instrument on samples prepared in a glycerol matrix. ^{13}C-nmr (pyridine-d_5) spectra originate from a Bruker AM 500 instrument operating at 125.76 MHz. Optical rotations were measured on a Perkin-Elmer 141 instrument.

MATERIALS.—The sea cucumbers were collected April 18, 1985, in El Faro off Cerralvo Island (109°33'W, 24°13'N) east of La Paz, Baja California Sur, Mexico. The sample was identified by Sergio Keer Garcia according to Brusca (4). A voucher specimen is retained at the Pharmacognosy Laboratory of the Marine Biological Department of Universidad Autonoma de Baja California Sur, Mexico.

EtOH 95% was commercial quality distilled over $KMnO_4$, MeOH was 99.8% and *n*-BuOH 99.7%, and all other solvents were twice distilled.

EXTRACTION AND PURIFICATION.—Small pieces of the body wall (2 kg) of freshly collected *N. gibbosa* were extracted at room temperature with distilled EtOH (95%). Evaporation under reduced pressure gave the crude EtOH extract (26.63 g). Extraction with boiling MeOH (3 × 250 ml) yielded after evaporation in vacuo a residue (10.35 g) that was partitioned between *n*-BuOH and H_2O (1:1) (300 ml). The *n*-BuOH phase after evaporation at reduced pressure left 1.3 g, which after repeated washings with hot MeOH yielded 230 mg (1.2×10^{-2}% of wet wt) neothyoside A [**1**] as a white solid: mp 204–206° (dec); elemental analysis calcd for $C_{56}H_{89}O_{27}SNa$, C 53.85%, H 7.13%, S 2.56%, found C 50.88%, H 7.07%, S 2.40%; $[\alpha]_{589}$ −5.0°, $[\alpha]_{578}$ −5.0°, $[\alpha]_{546}$ −5.0°, $[\alpha]_{436}$ −10.0°, $[\alpha]_{365}$ −13.8° [c = 0.8, H_2O-EtOH (1:1)].

ACKNOWLEDGMENTS

We are indebted to Dr. K. Bock for access to the 500-MHz Bruker nmr instrument and to Dr. J. Møller for the fabms data. The authors thank Miguel Betancourt for technical assistance. Thanks are also due to Secretaria de Educacion Publica-Direccion General de Investigacion Cientifica y Superacion Academica (SEP-DGICSA, C 86-01-0208) for financial support.

LITERATURE CITED

1. E. Deichmann, *Allan Hancock Pac. Exped.*, **8**, 61 (1941).
2. A. Panning, *Zool. Jahrb., Abt. Syst. Oekol. Geogr. Tiere*, **78**, 404 (1949).
3. D.L. Pawson and H.B. Fell, *Breviora*, **214**, 1 (1965).
4. R.C. Brusca, "A Handbook to the Common Intertidal Invertebrates of the Gulf of California," The University of Arizona Press, Tucson, 1973, p. 343.
5. S. Bhatnagar, B. Dudovet, A. Ahond, C. Poupat, O. Thoison, A. Clastres, D. Laurent, and P. Potier, *Bull. Soc. Chim. Fr.*, 124 (1985).
6. I. Kitagawa, M. Kobayashi, T. Inamoto, M. Fuchida, and Y. Kyogoku, *Chem. Pharm. Bull.*, **33**, 5214 (1985).
7. A. Clastres, A. Ahond, C. Poupat, P. Potier, and A. Intès, *Experientia*, **34**, 973 (1978).
8. J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972, p. 139.
9. G. Habermehl and G. Volkwein, *Toxicon*, **9**, 319 (1971).
10. D.J. Burnell and J.W. Apsimon, in: "Marine Natural Products. Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, 1983, Vol. 5, p. 287.
11. I. Kitagawa, M. Kobayashi, M. Hori, and Y. Kyogoku, *Chem. Pharm. Bull.*, **29**, 282 (1981).
12. S.S. Afiyatullov, L.Ya. Tishchenko, V.A. Stonik, A.I. Kalinovskii, and G.B. Elyakov, *Khim. Prir. Soedin.*, 1985, 244; *Chem. Nat. Prod.*, **21**, 228 (1985).

Received 23 June 1988