

Short Communication

Neothyoside B, a Triterpenoid Diglycoside from the Pacific Sea Cucumber *Neothyone gibbosa*

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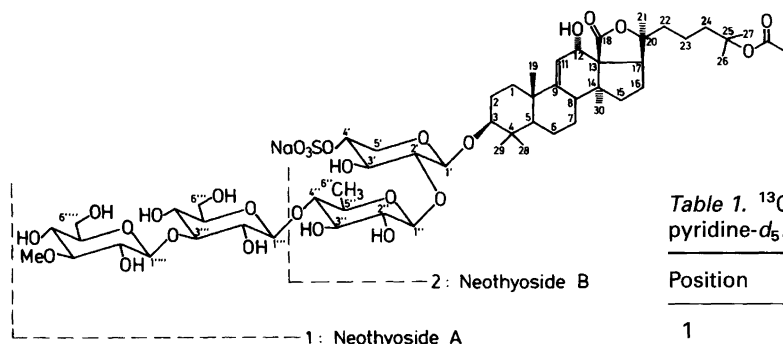
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The dendrochirotid holothurian *Neothyone gibbosa* Deichman has previously been found to contain the tetraglycoside neothyoside A, 3-*O*-[3-*O*-methyl- β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-quinovopyranosyl-(1 \rightarrow 2)-4-*O*-sodiumsulfonato- β -D-xylopyranosyl]-12 α -hydroxy-25-acetoxylolost-9(11)en-3 β -ol (1).¹

During attempts to reisolate this compound, in order to proceed with biological evaluation of the activity, the

(1.67 ppm), while the remaining occupied tertiary positions. The signals at 1.50 and 1.52 ppm were proved to be singlets by a decoupling experiment.

According to ¹³C NMR spectral analysis (Table 1) the structure of the aglycone is identical with the one present in neothyoside A. All the assigned values deviate less than 0.6 ppm from the ones published,¹ except for the signal assigned to C-12, which deviates by 2.5 ppm. This assignment was confirmed by the COSY ¹H-¹H



material in hand showed slightly different properties from that of neothyoside A. Closer inspection of the sample revealed that a different product had been secured. From the FAB negative mass spectrum obtained in glycerol matrix a pseudo-molecular ion appeared at *m/z* 887 corresponding to C₄₃H₆₇O₁₇S. A positive FAB mass spectrum showed a pseudo-molecular ion at *m/z* 911 corresponding to C₄₃H₆₇NaO₁₇S. In the ¹H NMR spectrum the signals of nine methyl groups appeared at δ 1.15, 1.29, 1.31, 1.41, 1.50, 1.52, 1.62, 1.67 and 2.01. Only one methyl group occupied a secondary position

Table 1. ¹³C NMR spectral assignments of neothyoside B in pyridine-*d*₅.

| Position | δ_c | Position | δ_c | Position | δ_c |
|----------|------------|----------|------------|----------|-------------------|
| 1 | 36.4 | 16 | 29.9 | Me(Ac) | 22.0 |
| 2 | 27.0 | 17 | 47.0 | CO(Ac) | 170.1 |
| 3 | 88.8 | 18 | 177.2 | 1' | 105.8 |
| 4 | 40.0 | 19 | 18.6 | 2' | 83.3 |
| 5 | 52.8 | 20 | 84.6 | 3' | 76.7 ^a |
| 6 | 21.2 | 21 | 22.2 | 4' | 75.6 ^a |
| 7 | 28.8 | 22 | 39.6 | 5' | 64.1 |
| 8 | 40.1 | 23 | 19.0 | 1'' | 105.2 |
| 9 | 153.0 | 24 | 41.2 | 2'' | 76.0 ^a |
| 10 | 39.6 | 25 | 81.9 | 3'' | 77.8 ^a |
| 11 | 116.1 | 26 | 26.0 | 4'' | 76.9 ^a |
| 12 | 68.2 | 27 | 26.3 | 5'' | 73.4 |
| 13 | 64.2 | 28 | 28.1 | 6'' | 18.6 |
| 14 | 46.6 | 29 | 16.7 | | |
| 15 | 37.2 | 30 | 22.5 | | |

^a Signals may be interchanged. Multiplicities were confirmed by an APT experiment.

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correlation, where connectivity between a doublet at 5.72 ppm (H-11, $J=4.4$ Hz) and a doublet at 4.58 ppm (H-12, $J=4.8$ Hz) was observed. The signal for C-10 is expected to coincide with the signal assigned to C-22. It is concluded that the aglycone part of neothyoside B is 12 α -hydroxy-25-acetoxyholost-9(11)en-3 β -ol.

The structure of the sugar moiety was established by comparison of ^{13}C NMR data with those reported for Holothurin B₁.² All values deviate less than 0.5 ppm from the positions reported. The most probable candidate for the signal originating from carbon 6'' was that already assigned to C-19 in the aglycone. In accordance with the literature,^{2,3} this signal includes both C-19 and C-6''. The presence of the methyl group in quinovose was established by COSY ^1H - ^1H correlation. In the 2D-spectrum a coupling between a methyl doublet at 1.67 ppm ($J=5.7$ Hz) and a sugar proton at 3.75 ppm (multiplet) was observed. These data define the sugar moiety attached at 3-*O* as [β -D-quinovopyranosyl-(1 \rightarrow 2)-4-*O*-sodiosulfonato- β -D-xylopyranosyl].

Based on these results we conclude that neothyoside B is 3-*O*-[β -D-quinovopyranosyl-(1 \rightarrow 2)-4-*O*-sodiosulfonato- β -D-xylopyranosyl]-12 α -hydroxy-25-acetoxyholost-9(11)en-3 β -ol (**2**). Neothyoside B differs from neothyoside A only in the structure of its sugar moiety. The holothurins often display the 12 α -hydroxy-9(11)-ene fragment in the aglycone and either a disaccharide or a tetrasaccharide moiety with the sulfate group attached to C-4 of the xylose residue.⁴ For example two compounds from the sea cucumber *Holothuria leucospilota* Brandt, holothurin A and B differ only in the absence of two monosaccharides in the sugar moiety.^{5,6} These findings are compatible with a biogenesis where two monosaccharides or a disaccharide are added to the aglycone followed, in certain cases, by the addition of similar units to the sugar moiety.

Neothyoside B exhibits inhibition (9.5 mm) of the pathogenic yeast *Candida albicans* in filter paper disc assay at 1 mg/disc.

Experimental

The ^1H and ^{13}C NMR spectra were recorded on a Varian UNITY 400 spectrometer, operating at 400 MHz for protons and at 100.6 MHz for carbons, respectively. All spectra were recorded in pyridine-*d*₅ which was also used as an internal standard in the ^{13}C NMR spectroscopy; in ^1H NMR spectroscopy Me₄Si was used as an internal standard. Mass spectra were obtained on a JEOL JMS-HX/HX110A spectrometer using the direct inlet system. The ethanol used was of commercial quality and distilled. Methanol (99.9%) was obtained from Baker as was the acetone (99.5%). Chloroform (99.8%) was purchased from VWR Brand and butanol (99.4%) from Monterrey Industry.

Isolation and purification of neothyoside B. Sea cucumbers were collected May 19, 1987 from Esperitu Santo Island,

north of La Paz, B.C.S. (México). Identification was performed by B. M. Sergio Keer Garcia according to Brusca. A voucher specimen is retained at the Pharmacognosy Laboratory of the Agronomy Department at *Universidad Autónoma de Baja California Sur (México)*.

The wet body wall of freshly collected *Neothyone gibbosa* (4.5 kg) was cut into small pieces and subjected to exhaustive soxhlet extraction with distilled EtOH (95%). Concentration under reduced pressure gave the crude ethanolic extract (40 g), which after dissolution in water (400 ml) was repeatedly extracted with butanol (18 \times 60 ml). The combined butanol phases left 18.98 g after evaporation of the solvent at reduced pressure. Repeated washing with hot methanol left a crude mixture of glycosides (5.2 g) and 12.25 g of methanol soluble material. The latter material was washed with acetone (3 \times 70 ml) and CHCl₃-MeOH-H₂O (6:4:1) (3 \times 70 ml) leaving 5.95 g of residual material which was subjected to flash column chromatography on Silica Gel 230-400 mesh (109 g) with successive elution with acetone, CHCl₃-MeOH-H₂O (7:3:0.5), CHCl₃-MeOH-H₂O (6:4:1), MeOH and water. Of 10 fractions secured, fraction 3 (263 mg) which eluted with approx. solvent composition CHCl₃-MeOH-H₂O (7.0:3.0:0.5), was subjected to rechromatography on the same material successively eluted with CHCl₃, CHCl₃-EtOH (9:2), (9:3), (9:5), (9:6), (1:1), and finally EtOH. Of the 13 fractions resulting from the latter experiment the fraction which eluted with solvent CHCl₃-EtOH (ca. 1:1), was treated with charcoal (40 mg) in refluxing MeOH (10 min) and the filtered solution was taken to dryness under reduced pressure to leave neothyoside B (18.7 mg) as a yellowish-white solid. UV [MeOH (log ϵ): 258 (3.29), CD (c 0.05, MeOH), λ_{ext} ($\Delta\epsilon$) 228, (+0.15), 233 (-0.42), 255 (+0.75), 328 (-0.18).

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