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STRUCTURE OF HOLOTHURIN B

A PHARMACOLOGICALLY ACTIVE TRITERPENE-OLIGOGLYCOSIDE

FROM THE SEA CUCUMBER HOLOTHURIA LEUCOSPILOTA BRANDT

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Holothurin was first isolated in 1955 as a hemolytic and piscicidal principle of the sea cucumber *Holothuria leucospilota* Brandt (= *H. vagabunda* Selenka) (Japanese name: nise-kuro-namako).[1)] Afterwards, two triterpene-oligoglycosides, holothurin A and B, have been characterized from *H. leucospilota* and *H. lubrica*,[2)] and holothurin A from *Actinopyga agassizi*.[3)] Holothurin A has been presumed to be an oligoglycoside of a lanostane-type triterpene containing a sulfate, D-glucose, 3-O-Me-D-glucose, D-quinovose, and D-xylose,[3a,b)] while holothurin B to comprise the same aglycone, a sulfate, D-quinovose, and D-xylose,[2)] and to be a quinovosyl-xyloside.[2b)] Although two artifact aglycones, 22,25-epoxy-holosta-7,9(11)-diene-3β,17α-diol (1)[3a,b)] and 22,25-epoxy-12β-methoxy-holost-9(11)-ene-3β,17α-diol (2),[3c)] have been isolated, the total structure of these oligoglycosides have not yet been established. As a continuing study in a series of our works on the oligoglycoside constituents of the species of *Holothuroidea*[4)] and *Asteroidea*,[5)] we have been working on the structure determination of holothurin A and B isolated from *H. leucospilota*. This paper provides the evidence being consistent with the structure (7) for holothurin B.

Holothurin B (7), $C_{41}H_{63}O_{17}SNa$,[6)] mp 224-226°, $[\alpha]_D^{17}$ -11.0° (MeOH): UV (MeOH): transparent above 210 nm; IR (KBr, cm^{-1}): 3385 (br, OH), 1742 (γ-lactone), 1634 (C=C), 1230, 830 (sulfate),[7)] 1060 (br, C-O-C); CD (MeOH): $[\theta]_{200}$ +55400 (pos. max.), $[\theta]_{221}$ -8300 (neg. max.), was obtained in 0.8% yield from the aq. 70% EtOH ext. of the body wall of *H. leucospilota*(collected in Miya-

zaki Pref. in July). It is positive for the potassium rhodizonate reagent.[8] On hydrolysis with snail enzyme,[9] it gave two prosapogenols: DS-Pro-B (4)(major) and Pro-B (5).

DS-Pro-B (4), $C_{35}H_{54}O_{10}\cdot H_2O$, mp 291-292.5°, $[\alpha]_D^{28}$ -5.0° (MeOH); IR (KBr): 3350, 1741, 1632, 1055; CD (MeOH): $[\theta]_{202}$ +53000 (pos. max.), $[\theta]_{222}$ -9000 (neg. max.), lacks the sulfate group and furnished 1 and xylose on acid hydrolysis. The PMR spectrum of 4 (d_5-py.) shows the signals at δ1.07 (4-Me), 1.20 (25-Me_2), 1.34 (4-Me), 1.37 (10-Me), 1.69 (14-Me), 1.76 (20-Me)(all s, *tert.* Me x 7), 4.14 (t, J= 6 Hz, 22-H), 4.35 (t-like, J= 7, 3α-H), 4.84 (d, J= 7, anom. H of β-D-xylopyranoside), 4.96 (d, J= 5, 12β-H),[10] and 5.66 (d, J= 5, 11-H).[10] The CD maximum due to C=C $\pi\rightarrow\pi^*$ transition[4,11] observed in 4 and 7 in connection with $J_{11\text{-}H,12\text{-}H}$ in 4 indicates the presence of a 9(11)-en-12α-ol moiety in the lanostane-type aglycone of 4 and 7. The CMR spectrum of 4 (d_5-py.)[12] provides a further evidence supporting the 9(11)-en-12-ol structure by the signals at δ153.7 (s, C-9), 115.5 (d, C-11), 71.4 (d, C-12).[13] Presence of a tetrahydrofuran-type side chain in 4 and 7 is shown by the PMR signals together with the resembled CMR signals of C-22 and C-25 in 4 and 7 as compared with those in 1.[14] Based on the foregoing evidence, the aglycone of 4 has been formulated as 3 (with undefined stereochem. at C-20). Furthermore, the β-D-xyloside linkage in 4 has been located at 3β-OH of 3 on the basis of the glycosidation shift[15] which is observed in the CMR spectrum of 4 as compared with that of 1.[16] As for the configuration at C-20 in 3, the pyridine-induced solvent shift[17] for the 20-Me PMR signals in 1 and 1a is informative (Table 1), thus the configuration at C-20 in 1 (consequently in 3) being assigned as S. The structure of DS-Pro-B has now been expressed as 4.

Another prosapogenol Pro-B (5), $C_{35}H_{53}O_{13}SNa$ (amorph.), gave 1 and xylose on acid hydrolysis, while solvolysis with dioxane-pyridine[5,18] of 5 furnished 4. In the CMR spectrum of 5, the esterification shift[19] of C-4' is observed (Table II), thus the location of the sulfate group in 5 being suggested as 4'-OH of 4.

Solvolysis[5,18] of holothurin B (7) gave a desulfated deriv. DS-HL-B (6), $C_{41}H_{64}O_{14}\cdot 1/2H_2O$, mp 283-286°; IR (KBr): 3380, 1742, 1627, 1055; CD (MeOH): $[\theta]_{199}$ +71700 (pos. max.), $[\theta]_{220}$ -7200 (neg. max.), which, on acid hydrolysis, liberated 1 and one mole each of xylose and quinovose. The hepta-O-methyl deriv. (6a)(M^+ 878), IR (CCl_4): no OH; CD (MeOH): $[\theta]_{200}$ +63400 (pos. max.), $[\theta]_{222}$ -7400 (neg. max.), shows two anomeric proton signals at δ4.34 and 4.62 (both d, J= 7)($CDCl_3$), thus two sugars being linked with β-orientation ($^4C_1$ form). On methanolysis, 6a gave Me 2,3,4-tri-O-methyl-quinovopyranoside and Me 3,4-di-O-methyl-xylopyranoside.

On the other hand, the penta-O-methyl deriv. (7a) of holothurin B, IR ($CHCl_3$): 3440 (w),

1751, 1638, 1260, 1080, 834, shows two anomeric proton signals at δ4.47 and 4.60 (both d, J= 7). On methanolysis, 7a gave Me 2,3,4-tri-O-methyl-quinovopyranoside and Me 3-O-methyl-xylopyranoside. Based on the accumulated evidence described above, the total structure of holothurin B

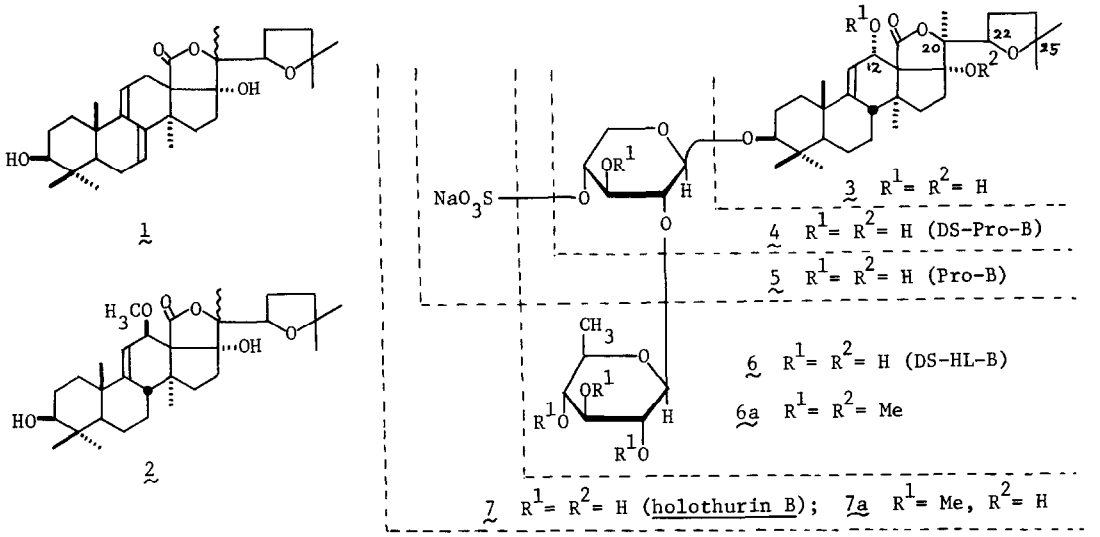

Table I (PMR at 90 MHz)

		4-Me$_2$	10-Me	14-Me	20-Me	25-Me$_2$	7-H	11-H	22-H
1	$CDCl_3$	0.90, 1.01	1.09	1.19	1.36	1.22, 1.26	5.51 (m)	5.27 (m)	4.22 (t,6)
	d_5-py.	1.08, 1.20	1.38	1.46	1.53	1.11, 1.14	5.64 (m)	5.41 (m)	4.24 (t,6)
1a	$CDCl_3$	0.89, 0.97	1.11	1.18	1.34	1.21, 1.24	5.49 (m)	5.26 (m)	4.21 (t,7)
	d_5-py.	0.91, 1.01	1.34	1.47	1.54	1.08, 1.14	5.62 (m)	5.36 (m)	4.27 (t,7)

Table II (CMR in d_5-pyridine)

	C-1'	-2'	-3'	-4'	-5'	C-1"	-2"	-3"	-4"	-5"	-6"	OMe
DS-Pro-B (4)	107.6	75.4	78.5	71.1	67.1	---	---	---	---	---	---	---
Pro-B (5)	107.1	75.2	76.1	76.1	64.5	---	---	---	---	---	---	---
DS-HL-B (6)	106.2	84.0	78.1	70.9	66.7	105.7	76.7	77.8	77.1	73.4	18.4	---
holothurin B (7)	105.7	83.4	76.6	75.1	64.0	105.2	76.0	77.4	76.6	73.4	18.5	---
Me β-D-xylo-pyranoside	106.0	74.6	78.1	70.9	67.0	---	---	---	---	---	---	56.6
Me β-D-quinovo-pyranoside	---	---	---	---	---	105.3	76.6	78.0	77.2	73.8	18.5	56.5

has been elucidated as 7, which is further supported by the CMR analyses of 4, 5, 6, and 7 (data of the carbohydrate moieties being given in Table II). The configuration at C-22 is under investigation. The pharmacological activities of holothurin B (7) will be published elsewhere by Prof. Y. Enomoto of Miyazaki University. The authors are grateful to Prof. Enomoto and his students for their kind help for collecting the sea cucumber.

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