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STRUCTURE OF HOLOTHURIN A

A BIOLOGICALLY ACTIVE TRITERPENE-OLIGOGLYCOSIDE

FROM THE SEA CUCUMBER HOLOTHURIA LEUCOSPILOTA BRANDT

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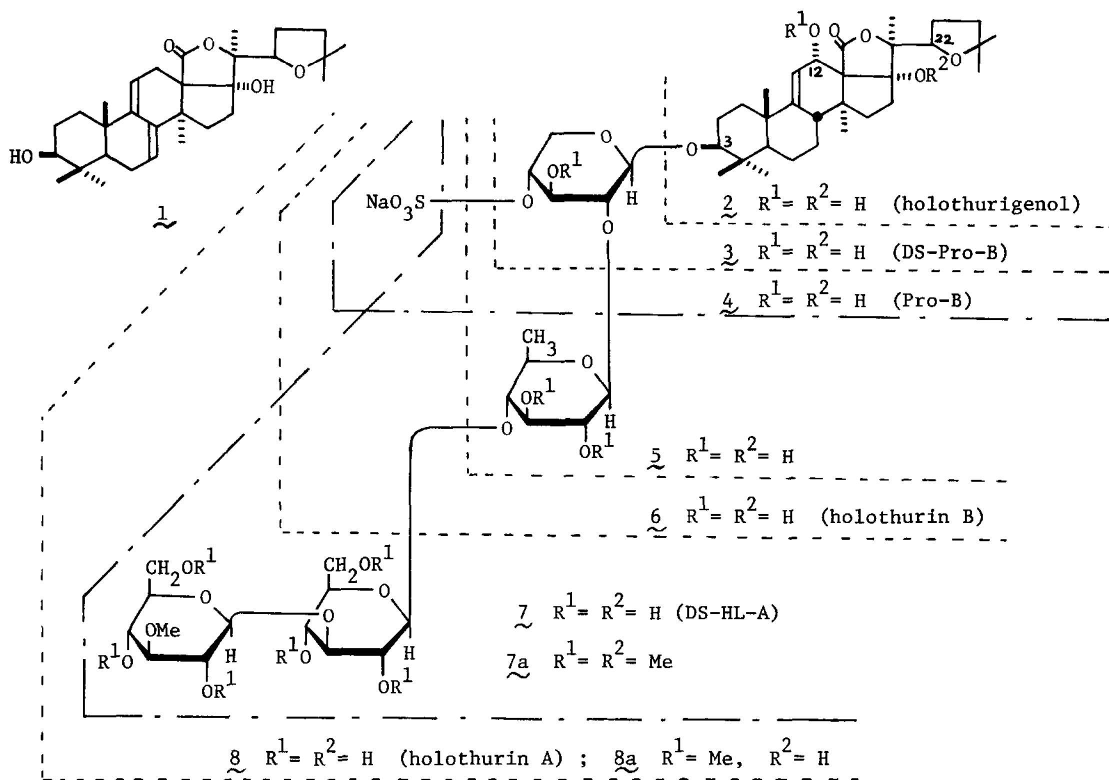
Summary: On the basis of chemical and physicochemical evidence, the structure of holothurin A, a biologically active major oligoglycoside of lanostane-type triterpene holothurigenol (2), has been elucidated to be 8.

More than a decade ago, two Japanese groups independently reported the isolation of two triterpene-oligoglycosides (named holothurin A and B) from two species of the sea cucumber: *Holothuria leucospilota* Brandt (syn. *H. vagabunda* Selenka, Japanese name "nise-kuro-namako") and *H. lubrica*.[1)] It was also suggested by Yasumoto et al.[1b)] that holothurin A from above two *Holothuria* spp. was identical with holothurin A which was previously isolated by Chanley et al.[2)] from the Bahamian sea cucumber *Actinopyga agassizi*. Of holothurin A and B, we elucidated recently the structure of holothurin B (6) which was obtained as the major oligoglycoside of the body wall of *H. leucospilota*.[3)] As a next step in a continuing study, we isolated holothurin A from the Cuvierian tubules of the same sea cucumber and determined its structure. This paper provides the evidence being consistent with the structure (8) for holothurin A.[4)]

The 70% EtOH extractive of the Cuvierian tubules of *H. leucospilota* (collected in July in Miyazaki prefecture) was subjected to solvent-partition, silica gel column chromatography, and recrystallization, thus furnishing holothurin A and B[3)] (2.1% and 0.6% respectively from the starting ext.). On acid (aq. 3N HCl) hydrolysis, holothurin A (8), $C_{54}H_{85}O_{27}SNa$,[5)] mp 228-230°, $[\alpha]_D^{18}$ -14.9° (MeOH); UV (MeOH): transparent above 210 nm; IR (KBr, cm^{-1}): 3420 (br, OH), 1745 (γ-lactone), 1640 (C=C), 1225, 827 (sulfate),[6)] 1070 (br, C-O-C); CD (MeOH): $[\theta]_{200}$ +41600 (pos.



max.), $[\theta]_{224}$ -6400 (neg. max.); potassium rhodizonate test[7]: positive, yielded one mole each of D-glucose, 3-O-methyl-D-glucose, D-quinovose, and D-xylose, in addition to an artifact aglycone (1) as from holothurin B (6).[3] In the ^{13}C-NMR spectra (Table I)[8] of holothurin A (8) and B (6), the carbon signals due to both aglycones are in good accordance with each other, suggesting that holothurin A comprises the same aglycone (holothurigenol (2)[9]) as holothurin B and the oligosaccharide moiety attached to 3β-OH of the aglycone. In addition, the chemical shifts of four anomeric carbons of holothurin A suggest the β-orientation for all of the glycosidic linkages.[10]

On hydrolysis with snail enzyme,[3] holothurin A (8) gave holothurin B (6) and its prosapogenols [DS-Pro-B (3)(major) and Pro-B (4)(trace)][3], while enzymatic hydrolysis of 8 with crude naringinase[11] furnished 4 and 6. Solvolysis of holothurin A with dioxane-pyridine[11,12] gave a desulfated product: DS-HL-A (7), mp 231-233°, $[\alpha]_D^{22}$ -27.6° (MeOH); IR (KBr): 3410 (br), 1748, 1639, 1068 (br); CD (MeOH): $[\theta]_{202}$ +59700 (pos. max.), $[\theta]_{225}$ -8600 (neg. max.), which, on acid hydrolysis, liberated 1 and an equal mole of glucose, 3-O-methyl-glucose, quinovose, and xylose.

Methylation[3] of 7 gave a dodeca-O-methyl deriv. (7a); IR (CCl_4): no OH, 1766, 1642, 1086

Table I ^{13}C-NMR Data (in d_5-pyridine, δ_C)

carbon	holothurin B (6)	holothurin A (8)*a	DS-HL-A (7)*a	carbon	holothurin A (8)*a	DS-HL-A (7)*a	Me glycopyranoside	
1	35.7(t)*b	35.5(t)	35.7(t)	1'	105.7	105.5	106.0	
2	27.4(t)*c	27.4(t)*c	27.4(t)*c	2'	83.1	83.9	74.6	Me β-D-xylo-
3	88.7(d)	88.7(d)	88.7(d)	3'	76.7	77.8	78.1	pyranoside
4	40.0(s)	40.0(s)	40.1(s)	4'	74.9	70.9	70.9	(OMe: 56.6)
5	52.6(d)	52.9(d)	53.1(d)	5'	63.8	66.4	67.0	
6	20.3(t)	20.3(t)	20.3(t)					
7	28.1(t)*c	28.2(t)*c	28.1(t)*c	1"	105.2*d	105.4*d	105.3	
8	40.9(d)	41.0(d)	41.1(d)	2"	76.0	76.0	76.6	Me β-D-quino-
9	153.6(s)	153.7(s)	153.9(s)	3"	76.7	76.3	78.0	vopyranoside
10	39.7(s)	39.7(s)	39.8(s)	4"	86.5	87.1	77.2	(OMe: 56.5)
11	115.5(d)	115.6(d)	115.6(d)	5"	71.7*c	71.7*c	73.8	
12	71.4(d)	71.7(d)*c	71.7(d)*c	6"	17.9	18.2	18.5	
13	58.7(s)	59.0(s)	59.2(s)					
14	45.9(s)	46.0(s)	46.0(s)	1"'	105.1*d	105.3*d	105.5	
15	38.4(t)	38.6(t)	38.6(t)	2"'	73.9	73.6	74.9	Me β-D-gluco-
16	27.1(t)	27.2(t)	27.1(t)	3"'	88.1	88.4	78.3	pyranoside
17	89.7(s)	89.7(s)	89.8(s)	4"'	70.8	70.9	71.4	(OMe: 56.7)
18	174.4(s)	174.3(s)	174.2(s)	5"'	77.3	77.8	78.2	
19	18.8(q)	19.0(q)	18.9(q)	6"'	62.2	62.5	62.7	
20	86.5(s)	86.5(s)	86.5(s)					
21	22.5(q)	22.5(q)	22.5(q)	1""	104.6	104.6	105.5	
22	80.6(d)	80.7(d)	80.8(d)	2""	74.9	74.9	74.6	Me 3-O-Me-β-
23	36.4(t)	36.6(t)	36.6(t)	3""	87.7	87.8	88.2	D-glucopyrano-
24	28.7(t)	28.7(t)	28.7(t)	4""	69.5	70.0	70.7	side
25	81.3(s)	81.3(s)	81.3(s)	5""	78.0	78.1	78.0	(OMe: 56.7)
26	28.7(q)*d	28.7(q)*d	28.7(q)*d	6""	61.9	62.5	62.4	
27	28.1(q)*cd	28.2(q)*cd	28.1(q)*cd	3""OMe	60.5	60.4	60.8	
28	21.2(q)	21.4(q)	21.3(q)					
29	27.4(q)*c	27.4(q)*c	27.4(q)*c					
30	16.7(q)	16.7(q)	16.8(q)					

*a Measured at 60° for 8 and at 70° for 7. *b Abbreviations given in the parentheses denote the signal patterns observed in the off-resonance experiments. *c The two-carbon intensities of respectively overlapping signals were confirmed by the hetero-decoupling without NOE method. (ref. 18). *d Assignments may be reversed in the same vertical column.

(br); CD (MeOH): $[\theta]_{202}$ +74000 (pos. max.), $[\theta]_{226}$ -7900 (neg. max.); ^{1}H-NMR ($CDCl_3$, δ_H): 4.19, 4.27, 4.54, 4.57 (1H each, all d, J= 7 Hz, four anom. H of β-glycosidic linkages), which, on methanolysis, yielded Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, Me 2,3-di-O-Me-quinovopyranoside, and Me 3,4-di-O-Me-xylopyranoside (GLC, TLC). On the other hand, a deca-O-methyl deriv. (8a); IR ($CHCl_3$): 3455 (w, OH),[13] 1754, 1640, 1238, 1090, 823, liberated, on methanolysis, Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, Me 2,3-di-O-Me-quinovopyranoside, and Me 3-O-Me-xylopyranoside (GLC, TLC).

Based on the above evidence, the structure of holothurin A has been clarified as 8, in which the C-22 configuration is currently under study in this laboratory. As given in Table I, the signals due to C-2' (xylose), C-4" (quinovose), C-3"' (glucose) in 8 and 7 show the glycosi-

dation shift[14] and the signal due to C-4' (xylose) in 8 shifts to lower field on esterification.[15] Furthermore, the values of the spin-lattice relaxation times (T_1) of the carbohydrate carbons in 7 (measured by the inversion recovery method) are also consistent with the sequence of the monosaccharide constituents in 8: *e.g.* the average NT_1 value[16] for 3-O-methyl-glucose is 0.26, glucose 0.23, quinovose 0.22, and xylose 0.18 sec. The terminal residue shows the longest NT_1 value and the most remarkable flexibility.

As for the sequence of the monosaccharide constituents in holothurin A from *A. agassizi*, Chanley et al. assumed to be quinovose, 3-O-methyl-glucose, glucose, and xylose from the terminal.[2d,e] Its sequence is inconsistent with our present conclusion. This will be the subject of our future investigation including the direct comparison of both holothurin A.

Some microbiological activities of 3, 4, 5,[3] holothurin B (6), 7, and holothurin A (8) were examined[17] and the moderate activities were observed for 6, 7, and 8 (relative activities: 7 > 6 > 8), the detail of which will be reported elsewhere.

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