[Chem. Pharm. Bull. 29(7)1942—1950(1981)]

Marine Natural Products. VII.[1] Bioactive Triterpene-Oligoglycosides from the Sea Cucumber *Holothuria leucospilota* Brandt (1). Structure of Holothurin B

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(Received February 16, 1981)

Two lanostane-type triterpene oligoglycosides designated as holothurin A and holothurin B were isolated from the sea cucumber *Holothuria leucospilota* Brandt (=*H. vagabnda* Selenka). The chemical structure of holothurin B, which is contained mainly in the body walls, has been established as 3-O-(2′-O-β-D-quinovopyranosyl-β-D-xylopyranosyl)-holothurigenol 4′-O-sodium sulfate (**6**), on the basis of chemical, physicochemical, and biochemical evidence. The genuine aglycone of holothurin B was designated as holothurigenol (**2**) and the C-20 and C-22 configurations of **2** and the artifact aglycone 22,25-oxidoholothurinogenin (**1**) have been elucidated as S.

Keywords——sea cucumber; *Holothuria leucospilota*; lanostane-type oligoglycosides holothurin A; holothurin B; holothurigenol; ^{1}H NMR; ^{13}C NMR; CD; snail enzyme

The sea cucumber *Holothuria leucospilota* Brandt (=*H. vagabunda* Selenka, Japanese name: nisekuro-namako) inhabits the Pacific and Indian Oceans and is known in China by the name of "yuzu haishen" as a remedy for convulsion caused by paralysis, cerebral concussion, and spinal injury.[2]

In 1942, Yamanouchi first isolated a hemolytic and piscicidal oligoglycoside from the sea cucumber and named it holothurin.[3] Afterwards, Matsuno and Iba[4] and Hashimoto *et al.*[5] found that holothurin was also produced by another kind of sea cucumber *H. lubrica* and it comprised two triterpene-oligoglycosides designated as holothurin A and holothurin B. On the other hand, Nigrelli reported in 1952 the isolation of a piscicidal and antitumor principle named "holothurin" from the Bahamean sea cucumber *Actinopyga agassizi*.[6] Later on, "holothurin" was renamed "holothurin A" and the chemical structure was investigated by Chanley *et al.*[7] In their report in 1967, Hashimoto *et al.* pointed out the probable identity of "holothurin A" from *A. agassizi* with holothurin A from *H. leucospilota*.[5,8]

In connection with the pharmacological investigation of holothurin B from *H. leucospilota* undertaken by Enomoto *et al.*,[9] we initiated structure studies of holothurin A and holothurin B isolated from *H. leucospilota*. This paper deals in detail with the structure elucidation of holothurin B (**6**).[10]

The body walls and the Cuvierian tubules separated from the sea cucumber *H. leucospilota*, which was collected at the Nichinan Coast of Miyazaki Prefecture in July, were each extracted with 70% ethanol and the extracts were both treated as shown in Chart 1. Holothurin B was isolated in 14.6% yield from the *n*-butanol soluble portion from the body walls and in 3.7% yield from the same fraction from the Cuvierian tubules, while holothurin A was obtained in 12.8% yield from the *n*-butanol soluble portion from the Cuvierian tubules.

Holothurin B (**6**), $C_{41}H_{63}NaO_{17}S$, was crystallized from methanol as colorless needles of mp 224—226 °C. It shows no absorption maximum in its ultraviolet (UV) spectrum. The infrared (IR) spectrum of holothurin B shows a γ-lactone absorption band (1742 cm^{-1}) together with strong absorption bands characteristic of glycosidic structure [3385 (br), 1060 (br) cm^{-1}].

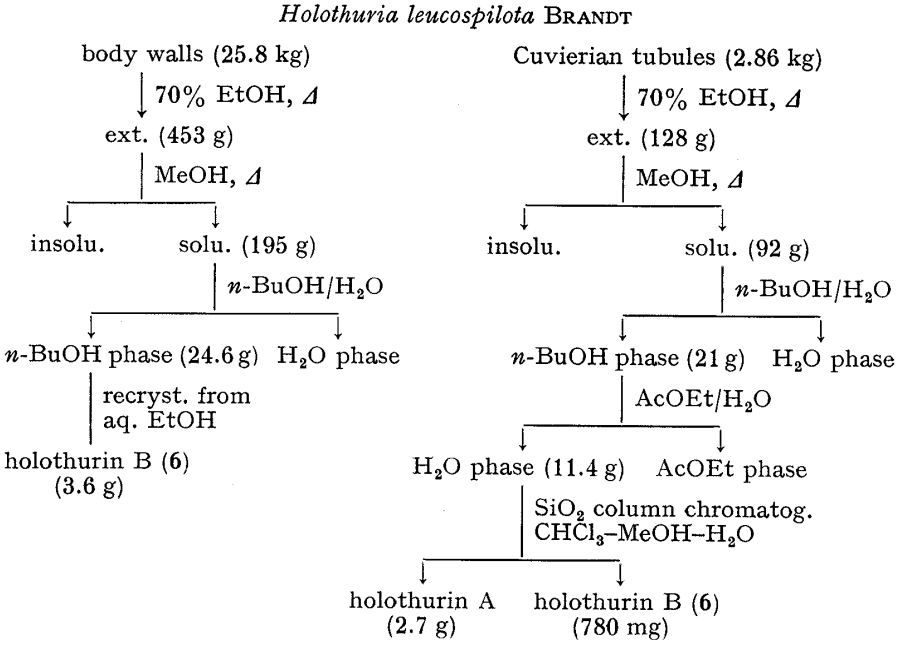

Chart 1

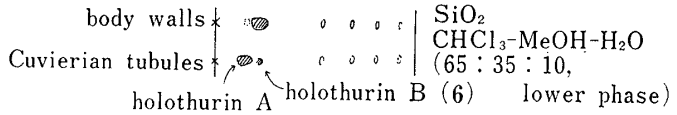

Fig. 1. TLC Diagram of the *n*-BuOH Phase

In the circular dichroism (CD) spectrum holothurin B exhibits a negative maximum of $[\theta]_{221}$ −8300 due to a γ-lactone moiety and a positive maximum of $[\theta]_{200}$ +55400 ascribable to an olefin moiety.[1a,b,e11] The presence of a sulfate group in holothurin B has been suggested on the basis of the positive potassium rhodizonate test[1c,12] and the IR absorption bands at 1230 and 830 cm^{-1}.[13]

On acidic hydrolysis with aq. 3 N hydrochloric acid, holothurin B liberated 22,25-oxidoholothurinogenin (**1**, without definition of the configurations at C-20 and C-22[7a,c]) and equimolar amounts of xylose and quinovose. The aglycone was an artifact as judged by the heteroannular diene absorption maxima observed in its UV spectrum. On enzymic hydrolysis using snail enzyme prepared according to the method of Okano *et al.*,[14] holothurin B yielded two prosapogenols designated as DS–Pro–B (**3**) and Pro–B (**4**).

DS–Pro–B (**3**), $C_{35}H_{54}O_{10}\cdot H_2O$, was crystallized from methanol as colorless needles of mp 291—292.5 °C. The IR and CD spectra of **3** indicate retention of the γ-lactone moiety [1741 cm^{-1}, $[\theta]_{222}$ −9000 (neg. max.)] of parent holothurin B, while **3** lacks the sulfate group as judged by the negative potassium rhodizonate test and the IR spectrum. Acidic hydrolysis of **3** gave the same artifact aglycone 22,25-oxidoholothurinogenin (**1**) as was obtained from holothurin B together with xylose.

In the proton nuclear magnetic resonance (^1H NMR) spectrum of **3** [in pentadeutero(d_5-)-pyridine], signals due to seven tertiary methyl groups are observed at δ 1.07 (3H, s, 4-CH_3), 1.20 (6H, s, 25-$CH_3\times2$), 1.34 (3H, s, 4-CH_3), 1.37 (3H, s, 10-CH_3), 1.69 (3H, s, 14-CH_3), and 1.76 (3H, s, 20-CH_3) together with signals at δ 4.14 (1H, t, J=6 Hz, 22-H) and δ 4.35 (1H, t-like, J=*ca.* 7 Hz, 3-H) which are assignable to two protons attached to carbons bearing an ether linkage. The ^1H NMR spectrum also shows signals due to two neighboring protons of one carbinyl methine (δ 4.96, d, J=5 Hz, 12-H) and one olefin (δ 5.66, d, J=5 Hz, 11-H)[15]

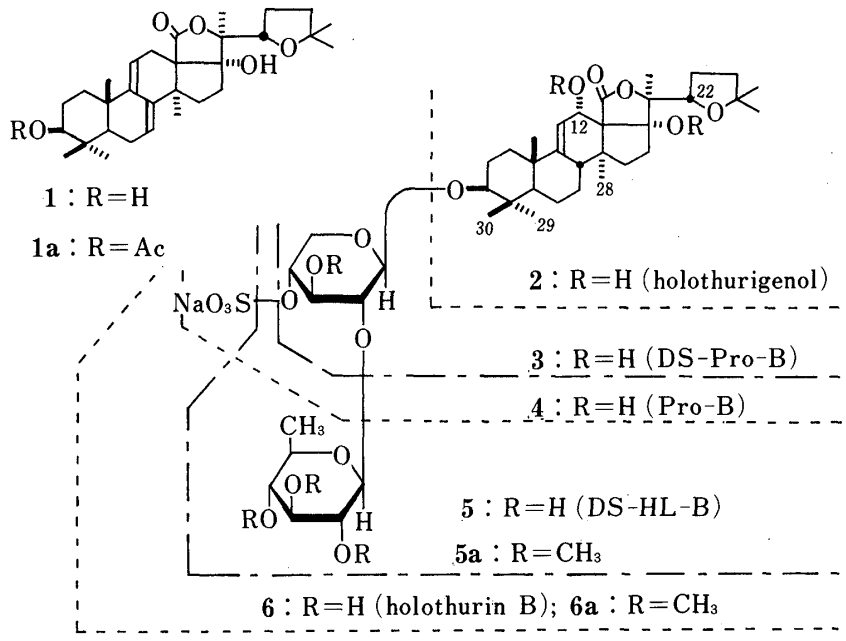

Chart 2

and signals at δ 4.84 (d, J=7 Hz, β-orientation in $^4C_1$) assignable to one anomeric proton. In addition to a negative maximum due to the γ-lactone moiety mentioned above, the CD spectrum of **3** exhibits a positive maximum of $[\theta]_{202}$ +53000 due to a $\pi-\pi^*$ transition of the olefin at C-9(11).[1a,b,e,11]

Based on the above-mentioned evidence together with examination with a Dreiding model and consideration of the coupling constant (J=5 Hz) of 11-H and 12-H (Fig. 2), the presence of a 9(11)-en-12α-ol moiety in **3** has been assumed. This assumption was further substantiated by the carbon nuclear magnetic resonance (^{13}C NMR) spectrum of **3** (Table I), which shows signals due to C-9, C-11, and C-12 at δ 153.7 (s), 115.5 (d), and 71.4 (d), respectively. The presence of a tetrahydrofuran residue in holothurin B (**6**) and DS–Pro–B (**3**), as in **1**, was indicated by the ^{13}C NMR and ^{1}H NMR data: signals of C-22 and C-25 (Table I) and 25-CH_3 (Table II, *vide supra* for **3**). The location of the β-xyloside moiety in **3** has been assigned as 3β-OH of the aglycone (**2**, holothurigenol)[16] on the basis of the glycosidation shift[17] observed in the ^{13}C NMR data for C-3 of **1** and **3** (Table I).

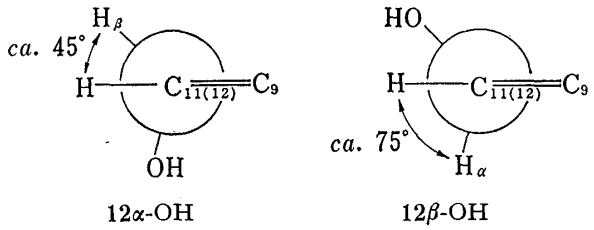

Fig. 2

In order to elucidate the configurations at C-20 and C-22 of holothurigenol (**2**), 22,25-oxidoholothurinogenin (**1**), which was obtained by acidic hydrolysis of holothurin B (**6**) and DS–Pro–B (**3**) and retained the same tetrahydrofuran residue as **2**, was subjected to the following investigation.

Comparative examination of the ^{1}H NMR spectra of **1** and its 3-O-monoacetate (**1a**) taken in deuterochloroform and d_5-pyridine (Table II) has revealed that the signals due to 14-CH_3 and 20-CH_3 of **1** and **1a** suffer a pyridine-induced solvent shift,[18] thus corroborating the 20S configuration of **1** and **2**.

Lithium aluminum hydride reduction of **1** followed by acetylation furnished a tetraol-diacetate (**7**), which, on lead tetraacetate oxidation, was decomposed to afford a 17-ketone (**8**) and a highly volatile methyl ketone. The methyl ketone was proved to be 2,2-dimethyl-5S-

acetyl-tetrahydrofuran (**9**) by the following conversion. Thus, 5,5-dimethyl-S-tetrahydrofurfuryl alcohol (**11**), which was prepared from D-mannitol (**10**),[19] was subjected to Jones oxidation and the resulting carboxylic acid (**12**) was treated with methyl lithium. The methyl ketone (**9**) thus obtained was found to be identical with the above-mentioned volatile methyl ketone (including the optical rotations). Consequently, the structure of holothurigenol (**2**), the genuine aglycone of holothurin B (**6**), has been clarified. The results also define the C-22 configuration of the artifact aglycone 22,25-oxidoholothurinogenin (**1**) as S.

TABLE I. ^{13}C-NMR Data (in d_5-pyridine, δc)

Carbon	Holothurin B (6)	DS–HL–B (5)	Pro–B (4)	DS–Pro–B (3)	22,25-Oxido-holothurinogenin (1)	
1	35.7(t)[a]	35.5(t)	35.6(t)	35.5(t)	35.9(t)	
2	27.4(t)[b]	27.4(t)[b]	27.4(t)[b]	27.4(t)[b]	28.5(t)	
3	88.7(d)	88.8(d)	88.5(d)	88.5(d)	78.1(d)	
4	40.0(s)	40.1(s)	39.9(s)	40.0(s)	39.4(s)	
5	52.6(d)	52.8(d)	52.6(d)	52.7(d)	50.1(d)	
6	20.3(t)	20.3(t)	20.3(t)	20.3(t)	25.4(t)	
7	28.1(t)[b]	28.1(t)	28.1(t)	28.1(t)	119.9(d)	
8	40.9(d)	40.9(d)	40.8(d)	40.9(d)	142.1(s)	
9	153.6(s)	153.7(s)	153.6(s)	153.7(s)	147.9(s)	
10	39.7(s)	39.7(s)	39.7(s)	39.7(s)	38.1(s)	
11	115.5(d)	115.5(d)	115.5(d)	115.5(d)	112.7(d)	
12	71.4(d)	71.4(d)	71.4(d)	71.4(d)	38.4(t)	
13	58.7(s)	58.7(s)	58.7(s)	58.7(s)	57.8(s)	
14	45.9(s)	45.9(s)	45.9(s)	45.9(s)	48.7(s)	
15	27.1(t)	27.1(t)	27.1(t)	27.1(t)	28.8(t)	
16	38.4(t)	38.4(t)	38.4(t)	38.4(t)	34.2(t)	
17	89.7(s)	89.7(s)	89.7(s)	89.7(s)	85.8(s)	
18	174.4(s)	174.4(s)	174.4(s)	174.4(s)	175.6(s)	
19	18.8(q)	18.8(q)	18.9(q)	18.8(q)	18.1(q)	
20	86.5(s)	86.5(s)	86.6(s)	86.5(s)	85.5(s)	
21	22.5(q)	22.6(q)	22.5(q)	22.5(q)	23.5(q)	
22	80.6(d)	80.6(d)	80.6(d)	80.6(d)	81.0(d)	
23	36.4(t)	36.8(t)	36.4(t)	36.4(t)	36.6(t)	
24	28.7(t)[b]	28.7(t)[b]	28.7(t)[b]	28.7(t)[b]	28.9(t)	
25	81.3(s)	81.3(s)	81.3(s)	81.3(s)	81.3(s)	
26	28.7(q)[b,c]	28.7(q)[b,c]	28.7(q)[b,c]	28.7(q)[b,c]	28.7(q)[c]	
27	28.1(q)[b,c]	28.2(q)[c]	28.2(q)[c]	28.3(q)[c]	27.8(q)[c]	
28	21.2(q)	21.5(q)	21.1(q)	21.1(q)	23.1(q)	
29	27.4(q)[b]	27.4(q)[b]	27.4(q)[b]	27.4(q)[b]	27.5(q)	
30	16.7(q)	16.8(q)	16.9(q)	17.0(q)	16.7(q)	
1′	105.7(d)	106.2(d)	107.1(d)	107.6(d)	106.0	Me-β-D-xylo-pyranoside
2′	83.4(d)	84.0(d)	75.2(d)	75.4(d)	74.6	
3′	76.6(d)	78.1(d)	76.1(d)[b]	78.5(d)	78.1	
4′	75.1(d)	70.9(d)	76.1(d)[b]	71.1(d)	70.9	
5′	64.0(t)	66.7(t)	64.5(t)	67.1(d)	67.0	(OMe: 56.6)
1″	105.2(d)	105.7(d)			105.3	Me-β-D-quinovo-pyranoside
2″	76.0(d)	76.7(d)			76.6	
3″	77.4(d)	77.8(d)			78.0	
4″	76.6(d)	77.1(d)			77.2	
5″	73.4(d)	73.4(d)			73.8	(OMe: 56.5)
6″	18.5(q)	18.4(q)			18.5	

a) Abbreviations given in parentheses denote signal patterns observed in the off-resonance experiments.
b) The two-carbon intensities of overlapping signals were confirmed by the hetero-decoupling without NOE method*.
c) Assignments may be interchangeable in a given column.
* R. Freeman, K.G.R. Pachler, and G.N. LaMar, *J. Chem. Phys.*, **55**, 4586 (1971).

Table II. ^{1}H NMR Data for **1** and **1a** (90 MHz, δ)

		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)

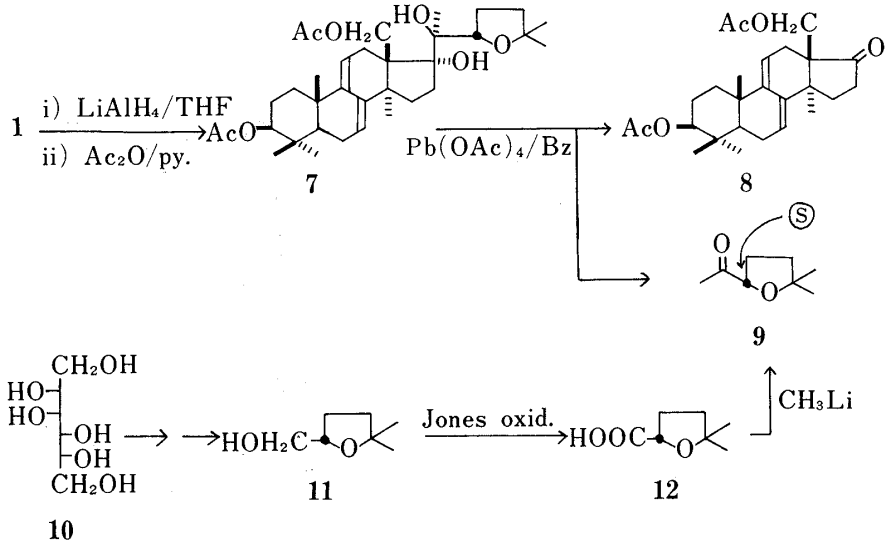

Chart 3

Another prosapogenol, Pro–B (**4**), amorphous, $C_{35}H_{53}NaO_{13}S$, retains the sulfate group as shown by its IR absorption bands at 1240 (br) and 828 cm^{-1} and the positive potassium rhodizonate test. Pro–B (**4**) is a sulfate of DS–Pro–B (**3**), since it was readily converted to **3** by solvolysis with dioxane and pyridine.[1c,d,20] In the ^{13}C NMR spectrum of **4** (Table I), signals of aglycone carbons are observed at chemical shifts nearly identical with those of **3**, while signals due to the xylose moiety are observed at somewhat shifted positions. The signal of C-4′ of **4** is observed at δ 76.1 (d), which is shifted lower due to sulfation[21] as compared with the signal of C-4′ of **3** (δ 71.1); thus, the location of the sulfate group in **4** has been shown to be C-4′.

Finally, the structure of holothurin B (**6**) was elucidated as described below. Solvolysis of holothurin B (**6**) with dioxane and pyridine furnished a desulfated derivative DS–HL–B (**5**), $C_{41}H_{64}O_{14}\cdot 1/2H_2O$, mp 283—286 °C. On methylation with methyl iodide and dimsyl carbanion, **5** was converted to a hepta-O-methyl derivative (**5a**). Signals due to two β-anomeric protons of **5a** are observed as doublets (J=7 Hz) at δ 4.34 and 4.62. Methanolysis of **5a** liberated methyl 2,3,4-tri-O-methylquinovopyranoside and methyl 3,4-di-O-methylxylopyranoside. On the other hand, the hexa-O-methyl derivative (**6a**), prepared from holothurin B (**6**) by methylation, yielded methyl 2,3,4-tri-O-methylquinovopyranoside and methyl 3-O-methylxylopyranoside. Consequently, the location of terminal quinovose at 2′-OH and the sulfate group at 4′-OH of the xylose moiety has become unequivocal. Furthermore, the glycosidation shift[17] observed in the C-2′ signals of **5** and **6** and the esterification shift[21] observed in the C-4′ signals of **4** and **6** confirm the assignment.

The chemical structure of holothurin B has now been formulated as 3-O-(2′-O-β-D-quinovopyranosyl-β-D-xylopyranosyl)-holothurigenol 4′-O-sodium sulfate (**6**).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous papers[1] unless otherwise specified. Signal multiplicities for ^1H and ^{13}C NMR data: s=singlet, d=doublet, t=triplet, br.s=broad singlet, m=multiplet, and q=quartet. Coupling constants (J values) are given in Hz.

Isolation of Holothurin A and B (6)——As shown in Chart 1, sea cucumbers (*Holothuria leucospilota*) collected at the Nithinan Coast in July 1976 were dissected to obtain the body walls and the Cuvierian tubules. The body walls (25.8 kg) were chopped finely with a knife and extracted under reflux with 70% EtOH four times (once with 16 l, three times with 8 l each). The extract (453 g), obtained after removal of the solvent under reduced pressure, was treated with boiling MeOH three times (5 l each). The MeOH soluble portions were taken and the solvent was evaporated off under reduced pressure to give a residue (195 g), which was partitioned into a *n*-BuOH–H_2O (1: 1) mixture (5.8 l). The *n*-BuOH phase was separated and removal of the solvent under reduced pressure afforded an oligoglycoside-containing residue (24.6 g). Repeated crystallization of the residue from 90% EtOH furnished holothurin B (**6**) (3.6 g, 14.6% from the *n*-BuOH phase). The mashed Cuvierian tubules (2.86 kg) were extracted under reflux with 70% EtOH five times (1.5 l each). The extract (128 g), obtained as described above, was treated with boiling MeOH three times (2 l each) and removal of the solvent under reduced pressure from the combined MeOH soluble portion gave a residue (92 g) which was partitioned into a *n*-BuOH–H_2O (1: 1) mixture (3 l). Concentration of the *n*-BuOH phase gave 21 g of residue which was again partitioned into an AcOEt–H_2O (2: 1) mixture (3 l). Concentration of the water phase under reduced pressure gave an oligoglycoside mixture which was chromatographed on a silica gel column (SiO_2, 1.1 kg) with $CHCl_3$–MeOH–H_2O (7: 3: 1, lower phase) to furnish holothurin B (**6**) (780 mg, 3.7% from the *n*-BuOH phase). Subsequent development with $CHCl_3$–MeOH–H_2O (65: 35: 10, lower phase) furnished holothurin A (2.7 g, 12.8% from the *n*-BuOH phase).

Holothurin B (**6**), mp 224—226°C (colorless needles from 90% EtOH), $[\alpha]_D^{17}$ −11.0° (c=0.3, MeOH). *Anal.* Calcd for $C_{41}H_{63}NaO_{17}S$: C, 55.77; H, 7.19; S, 3.63; Na, 2.61. Found: C, 55.58; H, 7.15; S, 3.80; Na, 2.76. IR ν_{max}^{KBr} cm^{-1}: 3385 (br), 1742, 1634 (w), 1060 (br), 1230, 830 (sulfate). UV (EtOH): transparent abave 210 nm. CD ($c=6.04\times10^{-4}$, MeOH, 21°C): $[\theta]_{246}$ 0, $[\theta]_{221}$ −8300 (neg. max.), $[\theta]_{214}$ 0, $[\theta]_{200}$ +55400 (pos. max.). ^{13}C NMR: Table I. Holothurin B gave a yellow spot at Rf 0.56 on the paper in the potassium rhodizonate test.[1c,12]

Acidic Hydrolysis of Holothurin B (6)——A mixture of holothurin B (**6**, 180 mg) in aq. 3 N HCl (12 ml) was heated in a boiling water-bath for 3 h. After cooling, the reaction mixture was centrifuged to separate a precipitate (105 mg), which was purified by column chromatography (SiO_2 10 g, benzene–acetone=50: 1) to furnish the aglycone, which was identical with authentic 22,25-oxidoholothurinogenin (**1**)[4] as judged by mixed mp determination (mp 301°C from AcOEt) and TLC and IR (KBr) comparisons. ^1H and ^{13}C NMR data are given in Tables II and I. The supernatant was neutralized with Dowex-1×2 (OH^-) and concentrated under reduced pressure to give a residue which was shown by PPC, TLC, and GLC (after trimethylsilylation) to contain quinovose and xylose. i) PPC (Toyo Filter Paper No. 50, double development for 20 h each with isopropanol-*n*-BuOH–H_2O=7: 1: 2; detection with aniline hydrogen phthalate): quinovose (Rf=0.72, xylose (0.62), ii) TLC (cellulose Avicel SF; development with phenol-H_2O=5: 2; detection as above): quinovose (Rf=0.49), xylose (0.34), iii) GLC (column: 2% SE-52 on Chromosorb WAWDMCS 80—100 mesh; 3 mm×2 m, temp. 130°C; N_2 30 ml/min): xylose (10′18″, 13′32″) and quinovose (12′14″, 16′21″) in a ratio of 1: 1.

Hydrolysis of Holothurin B (6) with Snail Enzyme——Snail enzyme (202 mg) prepared from 88 snails (in total, 300 mg of the enzyme was obtained),[14] which were collected at Settsu-kyo in Osaka Prefecture, was added to a solution of holothurin B (**6**, 300 mg) in AcOH–AcONa buffer (pH 5.2, 200 ml) and the whole was incubated with gentle stirring at 40°C for 14 days. After treatment with *n*-BuOH (10 ml), the whole mixture was heated in a boiling water-bath for 15 min, treated with Celite 535 (20 g) with stirring and then filtered. The filtrate was extracted with *n*-BuOH three times (50 ml each) and the combined *n*-BuOH phase was evaporated to dryness under reduced pressure. The residue (84 mg) was subjected to preparative TLC (Camag D-5) to furnish DS-Pro-B (**3**, 32 mg) and Pro-B (**4**, 17 mg).

DS–Pro–B (**3**), mp 291—292.5°C (MeOH), $[\alpha]_D^{28}$ −5.0° (c=0.3, MeOH). *Anal.* Calcd for $C_{35}H_{54}O_{10}\cdot H_2O$: C, 64.41; H, 8.59. Found: C, 64.12; H, 8.83. IR ν_{max}^{KBr} cm^{-1}: 3350 (br), 1741, 1632 (w), 1055 (br). CD ($c=1.66\times10^{-3}$, MeOH, 25°C): $[\theta]_{248}$ 0, $[\theta]_{222}$ −9000 (neg. max.), $[\theta]_{214}$ 0, $[\theta]_{202}$ +53000 (pos. max.). ^1H NMR (d_5-pyridine, δ): 1.07 (3H), 1.20 (6H), 1.34 (3H), 1.69 (3H), 1.76 (3H) (all s, *tert.* $CH_3\times7$), 4.14 (1H, t, J=6, 22-H), 4.35 (1H, t-like, 3-H), 4.84 (1H, d, J=7, 1′-H), 4.96 (1H, d, J=5, 12-H), 5.66 (1H, d, J=5, 11-H). ^{13}C NMR: Table I. DS–Pro–B (**3**) was negative in the potassium rhodizonate test.[1c,12]

Pro–B (**4**), amorphous, $[\alpha]_D^{28}$ −7.5° (c=0.2, MeOH). *Anal.* Calcd for $C_{35}H_{53}NaO_{13}S$: C, 57.05; H, 7.25; S, 4.35; Na, 3.12. Found: C, 56.80; H, 7.46; S, 4.63; Na, 3.02. IR ν_{max}^{KBr} cm^{-1}: 3380 (br), 1738, 1633 (w), 1055 (br); 1240, 828 (sulfate). CD ($c=4.99\times10^{-4}$, MeOH, 27°C): $[\theta]_{250}$ 0, $[\theta]_{223}$ −11200 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{202}$ +51000 (pos. max.). ^{13}C NMR: Table I. Pro–B (**4**) was positive (giving a yellow spot) to the potassium rhodizonate test.[1c,12]

Acidic Hydrolysis of DS-Pro-B (3) and Pro-B (4)——A mixture of DS–Pro–B (**3**) or Pro–B (**4**) (10 mg

each) in aq. 3 N HCl (1 ml) was heated in a boiling water-bath for 2 h. After dilution with water (2 ml), the reaction mixture was filtered. The product obtained by filtration after each reaction was found to be identical with **1** by TLC (*Rf* 0.62 for benzene–acetone=3:1, *Rf*=0.41 for *n*-hexane–acetone=2:1). Each filtrate was neutralized with Ag_2CO_3 and filtered. The residue obtained by removal of water from the filtrate under reduced pressure was examined by PPC and TLC and identified as xylose, as described above for the acidic hydrolysate of holothurin B (**6**).

Acetylation of 22,25-Oxidoholothurinogenin (1)——A solution of **1** (100 mg) in Ac_2O (4 ml)–dry pyridine (4 ml) was left to stand at room temperature for 18 h, then poured into ice-water (50 ml). The whole mixture was filtered to give the 3-O-monoacetate (**1a**, 110 mg).[7c] **1a**, mp 285—286°C (from AcOEt), $[\alpha]_D^{25}$ +8.0° (c=0.3, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3530, 1775, 1728, 1241. ^1H NMR ($CDCl_3$, δ): 2.04 (3H, s, OAc), 2.38 (1H, s, OH, disappeared on D_2O addition), and others as given in Table II.

Degradation of 1 *via* the Tetraol-diacetate (7) giving the 17-Ketone (8) and the Methyl Ketone (9)——A solution of **1** (190 mg) in dry THF (15 ml) was treated with a suspension of $LiAlH_4$ (360 mg) in dry THF (10 ml) and the whole was heated under reflux for 4 h. After cooling, the reaction mixture was treated successively with ether saturated with water (100 ml) and 2 N H_2SO_4 (30 ml), and the whole was extracted with ether five times (30 ml each). After neutralization with aq. sat. $NaHCO_3$, the ether extract was worked up in the usual manner. The product was acetylated with Ac_2O (5 ml) and dry pyridine (6 ml) overnight at room temperature. The reaction mixture was poured into ice-water (25 ml) and extracted with benzene three times (20 ml each). After being washed once with dil. HCl (10 ml), the benzene solution was worked up in the usual manner and the product was purified by column chromatography (SiO_2 10 g, benzene) to furnish the tetraol-diacetate (**7**, 118 mg).[7c] **7**, mp 212—213°C (petr. ether), $[\alpha]_D^{20}$ +41° (c=0.3, MeOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 3400, 1735 (sh), 1715, 1620 (w), 1245. UV λ_{max}^{MeOH} nm (ε): 237 (11000), 244 (13000), 253 (9000). ^1H NMR ($CDCl_3$, δ): 0.88, 0.93, 0.99, 1.22, 1.26, 1.31, 1.36 (all 3H, s, *tert.* $CH_3\times7$), 2.01, 2.03 (both 3H, s, OAc×2), 3.86, 4.27 (both 1H, ABq, J=13, CH_2OAc), 4.21 (1H, t, J=7, 22-H), 4.51 (1H, t-like, 3-H), 5.37 (2H, m, 7-H, 11-H). $Pb(OAc)_4$ (600 mg) was added to a solution of **7** (278 mg) in dry benzene (2 ml) and the whole mixture was stirred at room temperature for 4 h then filtered. The filtrate was distilled on an oil bath at 100°C to afford a distillate and a residue. The residue was purified by preparative TLC (Camag D-5, benzene–AcOEt=9:1) to furnish the 17-ketone (**8**, 116 mg). **8**, mp 164—165°C (colorless needles from petr. ether), $[\alpha]_D^{20}$ +98° (c=0.2, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 72.90; H, 8.68. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1742, 1725 (sh). UV λ_{max}^{MeOH} nm (ε): 236 (14000, sh), 242 (15000), 250 (9000, sh). ^1H NMR ($CDCl_3$, δ): 0.90 (6H, s, 4-$CH_3\times2$), 0.98 (3H, s, 10-CH_3), 1.06 (3H, s, 14-CH_3), 2.03, 2.08 (both 3H, s, OAc×2), 3.70, 4.30 (both 1H, ABq, J=12, CH_2OAc), 4.53 (1H, t-like, 3-H), 5.46, 5.59 (both 1H, m, 7-H, 11-H).

The distillate was purified by preparative GLC (column: 15% PEGS, 3 mm×2 m, temp. 65°C, He 1 kg/cm², t_R 27′) to furnish the methyl ketone (**9**), colorless oil, $[\alpha]_D^{19}$ −8° (CCl_4), MS *m/z* (%): 99 (47, M^+-43), 43 (100), which was identical with authentic 2,2-dimethyl-5S-acetyl-tetrahydrofuran (**9**) on GLC (column: HB-2000, 45 m Golay Column, temp. 100°C, N_2 45 ml/min, t_R 5′39″).

Synthesis of 2,2-Dimethyl-5S-acetyl-tetrahydrofuran (9) from 5,5-Dimethyl-S-tetrahydrofurfuryl Alcohol (11) *via* Carboxylic Acid (12)——An ice-cooled stirred solution of **11** (1.5 g), $[\alpha]_D^{20}$ −12.9° (EtOH) (ref.[19] −13.6° at 18°C), in acetone (30 ml) was treated with Jones reagent (20 ml) and the reaction mixture was stirred at 0°C for 20 min then poured into ice-water (200 ml). The whole mixture was extracted with $CHCl_3$ five times (50 ml each) and the combined $CHCl_3$ extract was worked up in the usual manner. Purification of the product by column chromatography (SiO_2 80 g, $CHCl_3$–MeOH=20:1) furnished the carboxylic acid (**12**, 980 mg). **12**, colorless liquid,[22] $[\alpha]_D^{22}$ −18° (c=0.4, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1778, 1733. ^1H NMR ($CDCl_3$, δ): 1.28, 1.36 (both 3H, s, gem. $CH_3\times2$), 1.79 (2H, t, J=7, 4-H_2), 1.99—2.56 (2H, 3-H_2), 4.50 (1H, t, J=7, 2-H), 7.00 (1H, br.s, COOH, disappeared on D_2O addition). MS *m/z* (%): 129 (7, M^+-15), 99 (100).

A solution of **12** (150 mg) in dry ether (5 ml) was treated with 2 ml of MeLi (1.84 M/ether) and the whole mixture was stirred under a nitrogen atmosphere at 18°C for 30 min then shaken with ice-water (2 ml) in a separatory funnel. The ether phase was taken and the aqueous phase was extracted with ether five times (3 ml each). The combined ether extract was washed with water, dried over $MgSO_4$, and distilled on an oil bath (temp. 50°C). The distillate was then purified by preparative GLC (column: 15% PEGS, 3 mm×3 m, temp. 90°C, He 1 kg/cm², t_R 12′24″) to furnish **9** (12.8 mg), colorless liquid,[22] $[\alpha]_D^{18}$ −12° (c=0.1, CCl_4), ^1H NMR (CCl_4, δ): 1.22, 1.26 (both 3H, s, 5-$CH_3\times2$), 2.11 (3H, s, $COCH_3$), 4.16 (1H, t, J=7, 2-H), MS *m/z* (%): 99 (25, M^+-43), 43 (100).

Solvolysis of Pro-B (4) giving DS-Pro-B (3)——A solution of **4** (50 mg) in dioxane (3 ml) and pyridine (3 ml) was heated under reflux for 30 min. After cooling, the solution was diluted with MeOH (2 ml) and filtered. Removal of the solvent from the filtrate under reduced pressure yielded a residue, which was crystallized from MeOH to furnish **3** (20 mg), mp 301°C (AcOEt). This product was identical with an authentic sample as judged by mixed mp determination and IR spectroscopy.

Solvolysis of Holothurin B (6) giving DS-HL-B (5)——A solution of **6** (235 mg) in dioxane (5 ml) and pyridine (5 ml) was heated under reflux for 40 min. After dilution with MeOH (5 ml), the whole mixture was filtered. The residue, obtained by removal of the solvent from the filtrate under reduced pressure, was purified by column chromatography (SiO_2 30 g, $CHCl_3$–MeOH–H_2O=15:2:1, lower phase) to furnish **5**

(147 mg), mp 283—286°C (colorless needles from MeOH), $[\alpha]_D^{18}$ −13.2° (c=0.3, MeOH). *Anal.* Calcd for $C_{41}H_{64}O_{14}\cdot 1/2H_2O$: C, 62.36; H, 8.24. Found: C, 62.10; H, 8.10. IR ν_{max}^{KBr} cm^{-1}: 3380 (br), 1742, 1627 (w), 1055 (br). UV (MeOH): transparent above 210 nm. CD ($c=4.88\times10^{-4}$, MeOH, 21°C): $[\theta]_{242}$ 0, $[\theta]_{220}$ −7200 (neg. max.), $[\theta]_{215}$ 0, $[\theta]_{199}$ +71700 (pos. max.). ^{13}C NMR: Table I. Potassium rhodizonate test: negative.

Methylation of DS-HL-B (5) followed by Methanolysis——A solution of **5** (78 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (6 ml) (prepared from 1 g of NaH and 17.5 ml of DMSO) and the whole mixture was stirred at 18°C under a nitrogen atmosphere for 1 h CH_3I (2.6 ml) was added under ice-cooling, and the whole was stirred in the dark at 18°C under a nitrogen atmosphere for 2 h. The reaction mixture was poured into ice-water (50 ml) and extracted with AcOEt five times (20 ml each). The combined AcOEt extract was washed with aq. sat. $Na_2S_2O_3$ and worked up in the usual manner. Purification of the product by column chromatography (SiO_2 10 g, *n*-hexane–acetone=15: 1) furnished the hepta-O-methyl derivative (**5a**, 74 mg), amorphous,[23] $[\alpha]_D^{27}$ −3° (c=0.14, MeOH), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1768, 1640 (w), 1087 (br), CD ($c=1.56\times10^{-3}$, MeOH, 18°C): $[\theta]_{252}$ 0, $[\theta]_{222}$ −7400 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{200}$ +63400 (pos. max.). ^{1}H NMR ($CDCl_3$, δ): 4.34 (1H, d, J=7), 4.62 (1H, d, J=7) (anomeric H×2).

A solution of **5a** (5 mg) in 2.5 N HCl–MeOH (1 ml) was heated under reflux for 1 h and neutralized with Ag_2CO_3. After filtration, removal of the solvent with a stream of nitrogen gave a residue which was analyzed by GLC (column: 15% NPGS, 3 mm×2 m, temp. 170°C, N_2 25 ml/min) and TLC (*n*-hexane–acetone=2: 1). It contained methyl 2,3,4-tri-O-methylquinovopyranoside (t_R 3′21″, 4′17″, *Rf* 0.63, 0.51) and methyl 3,4-di-O-methylxylopyranoside (t_R 9′50″, 11′24″; *Rf* 0.15).

Methylation of Holothurin B (6) followed by Methanolysis——A solution of **6** (400 mg) in DMSO (5 ml) was treated with a dimsyl carbanion solution (26.6 ml) (2 g NaH/35 ml DMSO) and the whole mixture was stirred under a nitrogen atmosphere at 16°C for 1 h. CH_3I (12 ml) was added under ice-cooling, and the whole was stirred at 16°C in the dark under a nitrogen atmosphere for a further 2 h. The reaction mixture was poured into ice-water (300 ml) and extracted with *n*-BuOH five times (50 ml each). After washing with aq. sat. $Na_2S_2O_3$, removal of the solvent from the *n*-BuOH extract under reduced pressure gave a residue which was purified by preparative TLC (Camag D-5, $CHCl_3$–MeOH–H_2O=7: 3: 1, lower phase, *Rf*=0.6) to furnish the hexa-O-methyl derivative (**6a**, 166 mg), amorphous,[23] $[\alpha]_D^{25}$ −5.0° (c=0.3, MeOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (w),[24] 1751, 1638 (w), 1080 (br); 1260, 834 (sulfate). CD ($c=7.24\times10^{-3}$, MeOH, 18°C): $[\theta]_{256}$ 0, $[\theta]_{224}$ −7000 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{201}$ +61000 (pos. max.). ^{1}H NMR ($CDCl_3$, δ): 4.47 (1H, d, J=7), 4.60 (1H, d, J=7) (anomeric H×2).

A solution of **6a** (5 mg) in 2.5 N HCl–MeOH (1 ml) was heated under reflux for 30 min and neutralized with Ag_2CO_3. Removal of the solvent from the filtrate with a stream of nitrogen gave a product which was shown to comprise methyl 2,3,4-tri-O-methylquinovopyranoside (t_R 3′40″, 4′54″; *Rf* 0.51, 0.54) and methyl 3-O-methylxylopyranoside (t_R 19′15″, 28′11″; *Rf* 0.18, 0.20) by GLC (column: 15% NPGS, 3 mm×2 m, temp. 150°C, N_2 30 ml/min) and TLC (benzene–acetone=2: 1).

Acknowledgement The authors are grateful to Prof. Y. Enomoto of Miyazaki University and his co-workers for their kind help in collecting sea cucumbers. They are also indebted to Dr. Y. Naya of Suntory Institute for Bioorganic Research for preparative GLC and to Dainippon Pharm. Co. for elemental analyses.

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