Marine Natural Products. XVIII.[1] Four Lanostane-Type Triterpene Oligoglycosides, Bivittosides A, B, C, and D, from the Okinawan Sea Cucumber *Bohadschia bivittata* MITSUKURI

Isao KITAGAWA,*[,a] Motomasa KOBAYASHI,[a] Manabu HORI,[a] and Yoshimasa KYOGOKU[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1-6, Yamada-oka, Suita, Osaka 565, Japan and Institute for Protein Research, Osaka University,[b] 3-2, Yamada-oka, Suita, Osaka 565, Japan. *Received July 18, 1988*

A full account of the structure elucidation of four lanostane-type triterpene oligoglycosides, bivittosides A (6), B (8), C (9), and D (10), is presented. Bivittosides were isolated from body-walls and Cuvierian tubules of the Okinawan sea cucumber *Bohadschia bivittata* MITSUKURI, and among these four oligoglycosides, bivittoside D (10) showed significant antifungal activities. Acidic hydrolysis of bivittosides A (6), B (8), and D (10) provided three artifact sapogenols, among which a homoannular-dienic sapogenol named preseychellogenin (4), exhibiting characteristically red-shifted ultraviolet and circular dichroism spectra, was identified as the monoacetate (4a).

Keywords sea cucumber; *Bohadschia bivittata*; bivittoside; lanostane-type triterpene oligoglycoside; homoannular-diene strained; oligoglycoside ^{13}C-NMR; oligoglycoside T_1; *Turbo cornutus* glycosidase; deoxybivittogenin; preseychellogenin

In search of new biologically active substances from marine organisms,[2,3a)] we have been investigating antifungal constituents in sea cucumber of echinoderm.[3)] In a continuing study, we isolated four triterpenoidal oligoglycosides named bivittosides A (**6**), B (**8**), C (**9**), and D (**10**) from the sea cucumber *Bohadschia bivittata* MITSUKURI (Holothuriidae, futasuji-namako in Japanese), which was collected in the Okinawan coral reefs, and elucidated their structures. In the following, we present the full details of our studies on these oligoglycosides.[4)]

The body walls and Cuvierian tubules of the sea cucumber collected in July in Okinawa Prefecture were separately extracted and the extracts were subjected to separation and purification procedures as shown in Chart 1. Thin-layer chromatography (TLC) of the 1-butanol phase from both organs revealed the presence of large amounts of bivittosides (Fig. 1). After chromatographic purification, bivittosides A (**6**), B (**8**), C (**9**), and D (**10**) were obtained in 2, 2, 2, and 8% yields from the 1-butanol extracts of the Cuvierian tubules and in 3, 3, 3, and 6% yields from the 1-butanol extract of the body walls.

Bivittoside A (**6**) was obtained as colorless needles of mp 267—268 °C. It lacked an ultraviolet (UV) absorption maximum at above 210 nm, while it showed an absorption band due to a γ-lactone moiety (1750 cm^{-1}) and strong broad absorptions (3400, 1070 cm^{-1}) reminiscent of glycosidic structure in its infrared (IR) spectrum. The circular dichroism (CD) spectrum of **6** showed a negative maximum ($[\theta]_{222}-7800$) due to the n→π^* transition of its γ-lactone moiety.

Bivittoside A (**6**) is a diglycoside containing one mol each of xylose and quinovose. Acidic hydrolysis of **6** provided an artifact sapogenol, seychellogenin (**1**),[5)] together with two other sapogenols: **2** (named isobivittogenin) and **4** (named preseychellogenin, in minor quantity). Isobivittogenin (**2**) was readily converted to seychellogenin (**1**) by further acidic treatment. The CD spectrum of **2** showed a positive maximum ($[\theta]_{204}+32000$)[6)] due to the $\pi\rightarrow\pi^*$ transition of the 9(11) double bond, whereas the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** showed signals ascribable to 12α-H (geminal to 12β-OH) at δ4.39 (m, $W_{h/2}=12$ Hz) and olefinic 11-H at δ5.12 (brs, $W_{h/2}=$

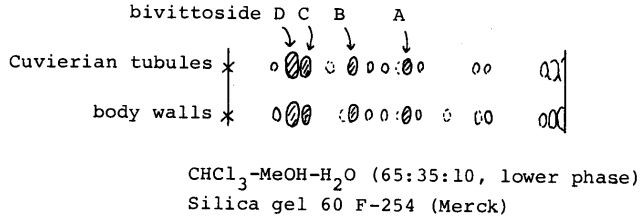


Fig. 1. TLC Diagram of the 1-BuOH Phase

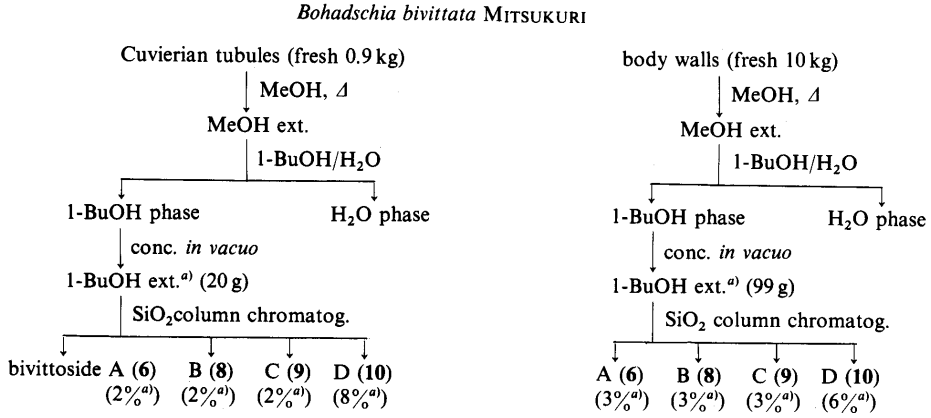
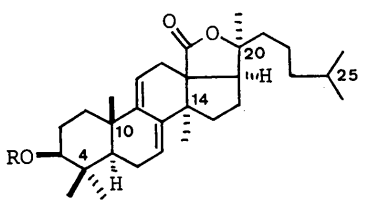
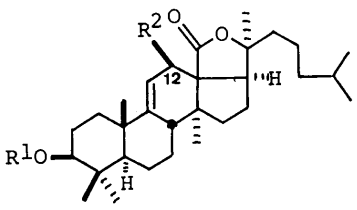


Chart 1. Isolation Procedures for Bivittosides

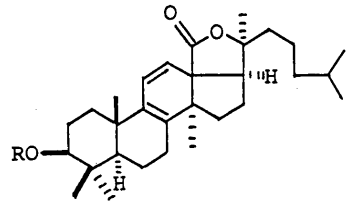
a) The yields were based on the 1-BuOH extract.



1 : R= H (seychellogenin)
1a : R= Ac



2 : R^1= H, R^2= OH (isobivittogenin)
2a : R^1= Ac, R^2= OAc
3 : R^1= R^2= H (deoxybivittogenin)
3a : R^1= Ac, R^2= H



4 : R= H (preseychellogenin)
4a : R= Ac

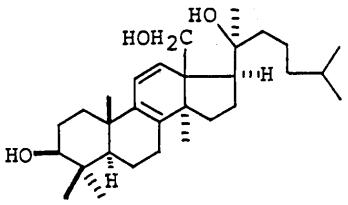


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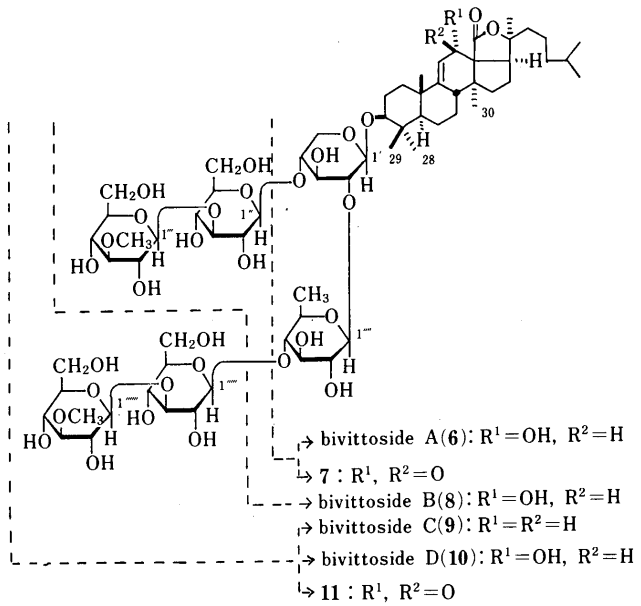
Chart 2



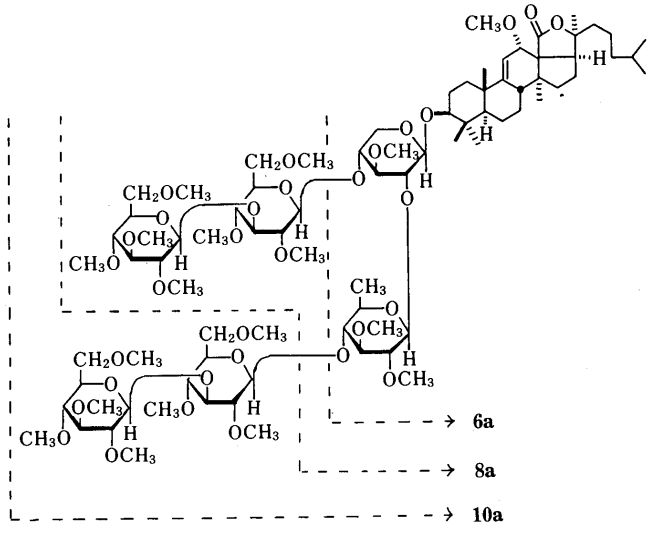


Chart 3

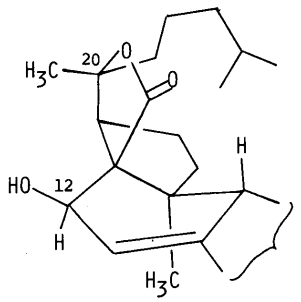


Fig. 2

6 Hz).[7] Thus, the presence of a 9(11)-en-12β-ol moiety in **2** was indicated. In regard to the unknown 20-CH_3 configuration in seychellogenin (**1**),[5] pyridine-induced shifts observed in the ^{1}H-NMR spectrum of **2** proved it to be *S*. Thus, as depicted in Fig. 2, the 20-CH_3 residue in **2** is located in the vicinity of the 12β-OH, which may result in the lower-field shift of the 20-CH_3 signal, when the spectrum was taken in pyridine-d_5, as compared with the spectrum measured in $CDCl_3$.[4,7,8]

Preseychellogenin (**4**) was purified as the monoacetate (**4a**). The ^{1}H-NMR spectrum of **4a** showed an AB quartet signal (δ 5.85, 6.09; J=9 Hz) due to two olefinic protons. Acidic treatment of **4a** afforded seychellogenin acetate (**1a**), thus supporting the 8,11-homoannular diene structure in **4a**, and **4** was suggested to be an intermediary sapogenol from the genuine sapogenol to seychellogenin (**1**). The UV spectrum of **4a** showed a homoannular diene absorption maximum at 302 nm (ε=2500) which was unusually red-shifted. The abnormality was also observed in the CD spectrum of **4a**. It showed a positive maximum ($[\theta]_{239}$+ 40000) ascribable to the γ-lactone moiety [the sign was reversed from bivittoside A (**6**)] and also showed a red-shifted negative maximum ($[\theta]_{302}$−89000) due to the homoannular diene chromophore. Dreiding model examination of **4a** has led us to presume that these unusual spectral properties may be due to a skeletal strain partly caused by the presence of the γ-lactone moiety. In order to

verify this presumption, **4a** was converted to a triol (**5**) by lithium aluminum hydride reduction. The ^{1}H-NMR spectrum of **5** showed an AB quartet signal (δ 3.56, 3.86; J= 11 Hz) due to the 18-CH_2 and a two-proton singlet at δ 6.04 due to the olefinic protons at C-11 and C-12. The UV and CD spectra of **5** showed maxima at ordinary wavelength [λ_{max} 275 nm (ε=2500) and $[\theta]_{275}$ −25000 (neg. max.)] due to the homoannular-diene chromophore. Thus, the red-shift in the UV spectrum of **4a** was ascribable to a distortion of the homoannular diene chromophore presumably caused by the γ-lactone ring formation.

The ^{1}H-NMR spectrum of bivittoside A (**6**) showed an olefinic proton signal at δ 5.70 (d, J=4 Hz) due to the 11-H and a doublet (J=4 Hz) at δ 4.52 due to the 12β-H geminal to the 12α-OH. Therefore, the genuine sapogenol of **6** was proved to have a 9(11)-en-12α-ol moiety.[6] Furthermore, mild oxidation of bivittoside A (**6**) with chromium trioxide-pyridine in 1-butanol-aqueous sulfuric acid[7,9] furnished a 9(11)-en-12-one derivative (**7**), thus proving that the carbohydrate chain in **6** is attached to the 3-OH of the sapogenol.

In order to elucidate the sequence of the carbohydrate moiety, bivittoside A (**6**) was subjected to complete methylation to afford the hexa-*O*-methyl derivative (**6a**). The ^{1}H-NMR spectrum of **6a** showed two one-proton doublets (δ 4.33, 4.62; J=7 Hz each) ascribable to β-anomeric protons. Methanolysis of **6a** liberated methyl 2,3,4-tri-*O*-methylquinovopyranoside and methyl 3,4-di-*O*-methylxylopyranoside. Consequently, the structure of bivittoside A (**6**) has been elucidated as shown.

Bivittoside B (**8**) is a tetraglycoside of mp 270—273 °C containing one mol each of xylose, quinovose, glucose, and 3-*O*-methylglucose. Acidic hydrolysis of bivittoside B (**8**) yielded seychellogenin (**1**) and isobivittogenin (**2**) as in the case of bivittoside A (**6**), whereas enzymatic hydrolysis of **8** with crude hesperidinase afforded **6** in a quantitative yield. The ^{1}H-NMR spectrum of the dodeca-*O*-methyl derivative (**8a**), obtained by complete methylation of bivittoside B (**8**), showed four one-proton doublets (δ 4.32, J=8 Hz; δ 4.43, J=7 Hz; δ 4.70, J=8 Hz; δ 4.72, J=8 Hz) due to β-anomeric protons. Methanolysis of **8a** liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,4,6-tri-*O*-methylglucopyranoside, methyl 2,3,4-tri-*O*-methylquinovopyranoside, and methyl 3-*O*-methylxylopyranoside. Based on the above-mentioned evidence, the structure of bivittoside B has been determined as **8**.

Bivittoside D (**10**) is a hexaglycoside of mp 219—221 °C, containing one mol each of xylose and quinovose and two mol each of glucose and 3-*O*-methylglucose. On acidic hydrolysis, it yielded seychellogenin (**1**) and isobivittogenin (**2**) as in the case of bivittosides A (**6**) and B (**8**). Enzymatic hydrolysis of bivittoside D (**10**), using a glycosidase mixture from the wreath shell *Turbo cornutus*, furnished bivittoside B (**8**) quantitatively. The ^{1}H-NMR spectrum of the octadeca-*O*-methyl derivative (**10a**), obtained by complete methylation of bivittoside D (**10**), showed the presence of six β-oriented anomeric protons in **10a** (δ 4.11, 1H d, J= 7 Hz; δ 4.31, 1H d, J=8 Hz; δ 4.55, 1H d, J=7 Hz; δ 4.89, 3H d, J=7 Hz). Methanolysis of **10a** provided methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,4,6-tri-*O*-methylglucopyranoside, methyl 2,3-di-*O*-methylquinovopyranoside, and methyl 3-*O*-methylxylopyranoside in 2:2:1:1 ratio. Based on the combined evidence, bivittoside D was concluded to have the structure **10**.

Bivittoside C (**9**) is also a hexaglycoside of mp 216—218 °C having one mol each of xylose and quinovose and two mol each of glucose and 3-*O*-methylglucose, like bivittoside D (**10**). Acidic hydrolysis of **9** followed by acetylation afforded deoxybivittogenin acetate (**3a**). The mass spectrum (MS) of **3a** gave a molecular ion peak at m/z 498. The ^{1}H-NMR spectrum of **3a** showed signals due to the 3β-acetoxyl group (δ 2.03, 3H s; δ 4.47, 1H m) and an olefinic proton at C-11 (δ 5.18, 1H m), whereas the CD spectrum of **3a** showed the presence of a γ-lactone moiety ($[\theta]_{221}$ −23000 (neg. max.)) and a double bond at 9(11) ($[\theta]_{208}$ +19000!). Thus, deoxybivittogenin (**3**) was presumed to be a 12-deoxy derivative of isobivittogenin (**2**). Detailed comparison of the carbon-13 (^{13}C)-NMR spectra of bivittosides C (**9**) and D (**10**) led us to presume that bivittoside C (**9**) was a 12-deoxy derivative of bivittoside D (**10**) (Table I). In order to verify this presumption, the following conversion was carried out. Thus, the hydroxyl functions of bivittoside C (**9**) were first protected by acetylation and the acetate was subjected to allylic oxidation with *tert*-butyl chromate and subsequent deacetylation to afford a 9(11)-en-12-one derivative (**11**). On the other hand, mild oxidation of bivittoside D (**10**) with chromium trioxide–pyridine in 1-butanol–aqueous sulfuric acid[7,9] afforded an enone derivative which was identical with the above enone hexaglycoside (**11**). Thus, the struc-

TABLE Ia. ^{13}C-NMR Data for Aglycones of Bivittosides A (**6**), B (**8**), C (**9**), and D (**10**) (25 or 50 MHz, Pyridine-d_5, δ_C)[a,b]

Carbon	Bivittoside A (**6**)	Bivittoside B (**8**)	Bivittoside C (**9**)	Bivittoside D (**10**)
1	36.5 (t)	36.6 (t)	36.0 (t)	36.5 (t)
2	27.2 (t)[d]	27.1 (t)[d]	27.0 (t)[d]	27.1 (t)[d]
3	88.9 (d)	88.7 (d)	88.8 (d)	88.6 (d)
4	39.7 (s)	39.9 (s)	39.7 (s)	39.7 (s)
5	53.0 (d)	53.2 (d)	53.4 (d)	53.1 (d)
6	21.3 (t)[c]	21.3 (t)[c]	21.4 (t)[c]	21.3 (t)[c]
7	28.9 (t)[d]	28.9 (t)[d]	29.8 (t)[d]	28.9 (t)[d]
8	40.1 (d)	40.2 (d)	40.3 (d)	40.2 (d)
9	153.1 (s)	153.3 (s)	151.6 (s)	153.3 (s)
10	39.5 (s)	39.7 (s)	39.5 (s)	39.5 (s)
11	116.1 (d)	116.1 (d)	111.3 (d)	116.0 (d)
12	68.2 (d)	68.3 (d)	33.6 (t)	68.5 (d)
13	64.1 (s)	64.1 (s)	58.2 (s)	64.2 (s)
14	46.6 (s)	46.7 (s)	47.5 (s)	46.6 (s)
15	24.2 (t)	24.2 (t)	24.2 (t)	24.2 (t)
16	39.5 (t)	39.5 (t)	39.5 (t)	39.5 (t)
17	47.0 (d)	47.0 (d)	51.8 (d)	47.2 (d)
18	177.2 (s)	177.2 (s)	177.2 (s)	176.9 (s)
19	22.1 (q)[e]	22.0 (q)[e]	22.3 (q)[e]	21.9 (q)[e]
20	84.7 (s)	84.6 (s)	83.8 (s)	84.6 (s)
21	26.4 (q)	26.5 (q)	27.0 (q)	26.4 (q)
22	37.1 (t)	37.2 (t)	36.8 (t)	37.1 (t)
23	22.3 (t)[c]	22.2 (t)[c]	22.3 (t)[c]	22.2 (t)[c]
24	39.5 (t)	39.5 (t)	39.5 (t)	39.5 (t)
25	28.2 (d)	28.2 (d)	28.3 (d)	28.2 (d)
26	22.7 (q)[e]	22.6 (q)[e]	22.6 (q)[e]	22.6 (q)[e]
27	22.7 (q)[e]	22.6 (q)[e]	22.6 (q)[e]	22.6 (q)[e]
28	28.0 (q)	28.0 (q)	27.9 (q)	27.9 (q)
29	16.8 (q)	16.7 (q)	16.7 (q)	16.7 (q)
30	22.7 (q)[e]	22.6 (q)[e]	19.9 (q)	22.6 (q)[e]

a) Measured at 60 °C. *b*) Abbreviations given in parentheses denote signals observed in the off-resonance experiments. *c*—*e*) Assignments may be interchangeable in the same column.

ture of bivittoside C (**9**) has been determined as shown.

Finally, the detailed ^{13}C-NMR spectral examination of bivittosides A (**6**), B (**8**), C (**9**), and D (**10**) supported their structures and the carbon signals were assigned as given in Table I.

Table Ib. ^{13}C-NMR Data for Sugar Moieties of Bivittosides A (**6**), B (**8**), C (**9**), and D (**10**) (25 or 50 MHz, Pyridine-d_5, δ_C)[a,b]

Carbon	Bivittoside A (**6**)	Bivittoside B (**8**)	Bivittoside C (**9**)	Bivittoside D (**10**)
1′	105.7 (d)	105.3 (d)	104.5 (d)	104.5 (d)
2′	84.0 (d)	83.4 (d)	83.2 (d)	83.3 (d)
3′	78.1 (d)	75.7 (d)	76.3 (d)	76.2 (d)
4′	70.8 (d)	77.6 (d)	78.1 (d)	78.0 (d)
5′	66.7 (t)	64.0 (t)	64.1 (t)	64.2 (t)
1″		102.8 (d)	102.9 (d)	102.8 (d)
2″		73.1 (d)	73.7 (d)	73.6 (d)
3″		88.1 (d)	88.3 (d)	88.3 (d)
4″		69.8 (d)	70.0 (d)	69.9 (d)
5″		78.1 (d)	78.1 (d)	78.1 (d)
6″		62.1 (t)	62.5 (t)	62.4 (t)
1‴		106.0 (d)	105.5 (d)	105.4 (d)
2‴		75.1 (d)	74.9 (d)	74.9 (d)
3‴		87.8 (d)	87.7 (d)	87.6 (d)
4‴		70.6 (d)	70.9 (d)	70.8 (d)
5‴		78.2 (d)	78.1 (d)	78.0 (d)
6‴		62.3 (t)	62.5 (t)	62.4 (t)
3‴-OMe		60.7 (q)	60.4 (q)	60.4 (q)
1⁗	106.2 (d)	105.6 (d)	105.0 (d)	105.4 (d)
2⁗	76.7 (d)	76.6 (d)	75.8 (d)	75.8 (d)
3⁗	77.8 (d)	77.4 (d)	73.0 (d)	73.0 (d)
4⁗	77.1 (d)	77.0 (d)	86.9 (d)	87.0 (d)
5⁗	73.4 (d)	73.4 (d)	71.7 (d)	71.6 (d)
6⁗	18.7 (q)	18.6 (q)	18.2 (q)	18.1 (q)
1⁗′			105.5 (d)	105.4 (d)
2⁗′			73.7 (d)	73.6 (d)
3⁗′			88.3 (d)	88.2 (d)
4⁗′			70.0 (d)	69.9 (d)
5⁗′			78.1 (d)	78.1 (d)
6⁗′			62.5 (t)	62.4 (t)
1⁗″			105.5 (d)	105.4 (d)
2⁗″			74.9 (d)	74.7 (d)
3⁗″			87.7 (d)	87.6 (d)
4⁗″			70.9 (d)	70.8 (d)
5⁗″			78.1 (d)	78.0 (d)
6⁗″			62.5 (t)	62.4 (t)
3⁗″-OMe			60.4 (q)	60.4 (q)

a) Measured at 60 °C. *b*) Abbreviations given in parentheses denote signals observed in the off-resonance experiments.

Table II. Minimum Growth Inhibitory Concentrations (μg/ml)

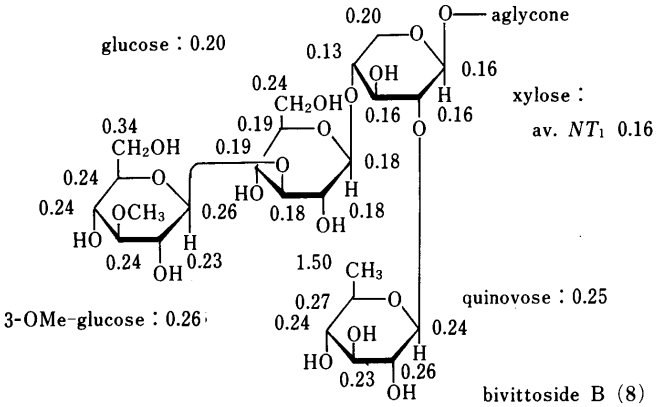
	Bivittoside				11	12
	A (**6**)	B (**8**)	C (**9**)	D (**10**)		
Aspergillus niger	>100	12.5	>100	6.25	25	1.56
Aspergillus oryzae	6.25	50	>100	3.12	25	1.56
Penicillium citrinum	>100	50	>100	6.25	>100	3.12
Penicillium chrysogenum	6.25	12.5	>100	1.56	12.5	1.56
Mucor spinescens	>100	>100	>100	>100	>100	100
Cladosporium herbarum	>100	>100	>100	50	50	100
Rhodotorula rubra	>100	>100	>100	6.25	25	3.12
Trichophyton mentagrophytes	>100	>100	>100	6.25	25	6.25
Trichophyton rubrum	25	12.5	>100	3.12	6.25	3.12
Candida albicans	>100	>100	>100	12.5	>100	50
Candida utilis	>100	50	>100	6.25	12.5	3.12

Some triterpene oligoglycosides hitherto isolated from sea cucumber in our laboratory exhibited significant antifungal activities.[3a] We have examined the present bivittosides and two derivatives for antifungal activities, and the results are shown in Table II.

Among the four bivittosides, bivittoside D (**10**) was found to exhibit significant antifungal activities. It is noteworthy that the 12-keto derivative (**11**) retained only weak antifungal activity and bivittoside C (**9**), which lacked the 12α-OH function of bivittoside D (**10**), had lost the activities completely. Thus, the presence of the 12α-OH function in bivittosides seemed to be essential for exhibiting antifungal activities.

It is also interesting that the desulfated derivative (**12**) of echinoside A,[7] which was previously isolated from the sea cucumber *Actinopyga echinites*, showed significant antifungal activities while bivittoside B (**8**), having a 12α-OH function like bivittoside D (**10**), did not show any antifungal activity. Both desulfated echinoside A (**12**) and bivittoside B (**8**) are tetraglycosides with the same four monosaccharide constituents (*i.e.* xylose, quinovose, glucose, and 3-*O*-methylglucose) but differing in their sequences (linear in **12** but branched in **8**). Although **8** lacked the 17α-OH function as compared with **12**, the difference in these carbohydrate sequences of **12** and **8** seems to be critical. In the other words, the linear sequence of the carbohydrate chain (as in **12**) may play an important role in the antifungal activities.

Furthermore, in order to compare the physicochemical



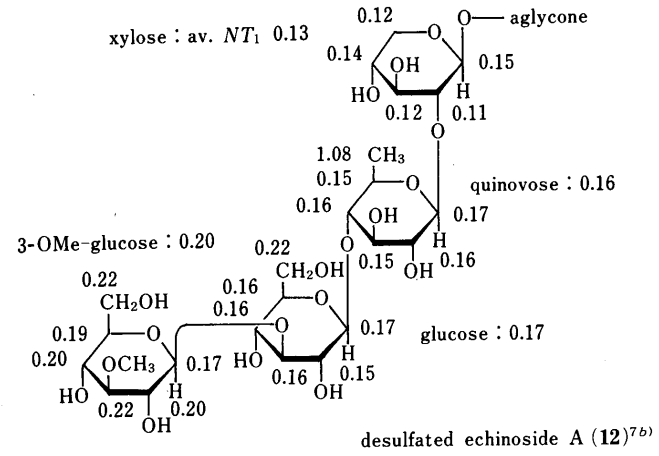


Fig. 3. NT_1 Values (s) for **8** and **12**

properties of the carbohydrate moieties of desulfated echinoside A (**12**) and bivittoside B (**8**), the spin-lattice relaxation times (T_1) of carbons in the carbohydrate moieties of **12** and **8** were examined.[7b,10,11] The T_1 values observed for individual carbons were as shown in Fig. 3. The average NT_1 values ($NT_1 = T_1 \times$ number of proton(s) attached to the carbon) for individual monosaccharide units in bivittoside B (**8**) were 0.16 s (xylose), 0.25 s (quinovose), 0.20 s (glucose), and 0.26 s (3-*O*-methylglucose), respectively.[12] These values are diverse when compared with the average NT_1 values for monosaccharide units in desulfated echinoside A (**12**),[7b] where the values increased in order from xylose to terminal 3-*O*-methylglucose.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our preceding paper.[7a]

Isolation of Bivittosides A (6), B (8), C (9), and D (10) As shown in Chart 1, the Cuvierian tubules (0.9 kg) of the sea cucumber *Bohadschia bivittata* MITSUKURI (collected in July in Okinawa Prefecture) were extracted with MeOH (4 l each) under reflux 3 times for 3 h each. The MeOH extract (39 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water (1 l each) mixture to give the 1-butanol-soluble portion (20 g). The 1-butanol-soluble portion (19 g) was then purified repeatedly by column chromatography [Silica gel 60, 60—230 mesh (Merck), 600 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1 (lower phase)→7 : 3 : 1 (lower phase)] to give bivittoside A (**6**) (0.9 g), bivittoside B (**8**) (0.8 g), bivittoside C (**9**) (0.8 g), and bivittoside D (**10**) (3.1 g). The fresh body walls (10 kg) of the same sea cucumber were extracted with MeOH (20 l each) under reflux 3 times for 4 h each. The MeOH extract (150 g) thus obtained was partitioned into a 1-butanol–water (4 l each) mixture to give the 1-butanol-soluble portion (99 g). The 1-butanol extract (20 g) was subjected to silica gel column chromatography as above to give bivittoside A (**6**) (0.8 g), bivittoside B (**8**) (0.7 g), bivittoside C (**9**) (0.8 g), and bivittoside D (**10**) (1.5 g).

Bivittoside A (**6**), colorless needles, mp 267—268 °C (MeOH), $[\alpha]_D^{15}$ +9° (c = 0.8, pyridine). *Anal.* Calcd for $C_{41}H_{66}O_{12} \cdot H_2O$: C, 64.04; H, 8.91. Found: C, 63.83; H, 8.88. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1750, 1070 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 1.5 \times 10^{-2}$, MeOH): $[\theta]_{256}$ 0, $[\theta]_{222}$ −7800 (neg. max.), $[\theta]_{215}$ 0, $[\theta]_{212}$ +8000! ^{1}H-NMR (90 MHz, pyridine-d_5, δ): 0.88 (6H, d, J = 6 Hz), 1.16, 1.27, 1.31, 1.39, 1.57 (each 3H, s), 4.52 (1H, d, J = 4 Hz), 4.74 (1H, d, J = 6 Hz), 5.11 (1H, d, J = 6 Hz), 5.70 (1H, d, J = 4 Hz). ^{13}C-NMR (25 MHz, pyridine-d_5, δ_C): Table I. Bivittoside B (**8**), colorless needles, mp 270—273 °C ($CHCl_3$–MeOH–H_2O), $[\alpha]_D^{20}$ +6° (c = 0.7, pyridine). *Anal.* Calcd for $C_{54}H_{88}O_{22} \cdot 3H_2O$: C, 56.82; H, 8.29. Found: C, 57.04; H, 8.01. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1750, 1062 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 6.3 \times 10^{-2}$, MeOH): $[\theta]_{249}$ 0, $[\theta]_{221}$ −7800 (neg. max.), $[\theta]_{212}$, 0, $[\theta]_{209}$ +14000! ^{13}C-NMR (25 MHz, pyridine-d_5, δ_C): Table I. Bivittoside C (**9**), colorless needles, mp 216—218 °C ($CHCl_3$–MeOH–H_2O), $[\alpha]_D^{28}$ −31° (c = 1.3, pyridine). *Anal.* Calcd for $C_{67}H_{110}O_{31} \cdot 2H_2O$: C, 55.59, H, 7.94. Found: C, 55.77; H, 8.00. IR ν_{max}^{KBr} cm^{-1}: 3380 (br), 1754, 1070 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 2.9 \times 10^{-2}$, MeOH): $[\theta]_{253}$ 0, $[\theta]_{221}$ −17000 (neg. max.), $[\theta]_{212}$ 0, $[\theta]_{204}$ +34000! ^{13}C-NMR (25 MHz, pyridine-d_5, δ_C): Table I. Bivittoside D (**10**), colorless needles, mp 219—221 °C ($CHCl_3$–MeOH–H_2O), $[\alpha]_D^{28}$ −7° (c = 0.9, pyridine). *Anal.* Calcd for $C_{67}H_{110}O_{32} \cdot 3H_2O$: C, 54.31, H, 7.89. Found: C, 54.11; H, 7.73. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1750, 1060 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 5.9 \times 10^{-2}$, MeOH): $[\theta]_{246}$ 0, $[\theta]_{220}$ −8000 (neg. max.), $[\theta]_{213}$ 0, $[\theta]_{208}$ +18000! ^{13}C-NMR (25 MHz, pyridine-d_5, δ_C): Table I.

Carbohydrate Compositions of Bivittosides A (6), B (8), C (9), and D (10) A solution of bivittoside A (**6**), B (**8**), C (**9**), or D (**10**) (10 mg each) in anhydrous 2.5 N AcCl–MeOH (1.5 ml) was heated under reflux for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. Evaporation of the filtrate under reduced pressure to dryness yielded a residue, which was dissolved in pyridine (0.1 ml) and treated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (0.25 ml) at room temp. (25 °C) for 5 min. The reaction mixture was subjected to gas liquid chromatographic (GLC) analysis (5% SE-52 on Chromosorb WAW DMCS 80—100 mesh, 3 mm × 2 m; column temp. 150 °C; N_2 flow rate 30 ml/min) to obtain the following results. Bivittoside A (**6**): xylose (t_R 12 min 14 s), quinovose (t_R 15 min 14 s); B (**8**): xylose, quinovose, 3-*O*-methylglucose (t_R 18 min 02 s), glucose (t_R 37 min 37 s); C (**9**): xylose, quinovose, 3-*O*-methylglucose × 2, glucose × 2; D (**10**): xylose, quinovose, 3-*O*-methylglucose × 2, glucose × 2. All constituent monosaccharides were deduced to be D-form, since these sugars hitherto obtained from sea cucumber saponins were all D as proved by their $[\alpha]_D$ values.[3]

Acidic Hydrolysis of Bivittoside A (6) 1) A mixture of bivittoside A (**6**) (100 mg) and aq. 2 N HCl (10 ml) was heated for 1 h under reflux on a water-bath. After dilution with water, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with aq. sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (30 mg), which was purified by column chromatography (SiO_2 5 g, benzene–acetone = 4 : 1) to furnish seychellogenin (**1**)[5] (17 mg) and isobivittogenin (**2**) (3 mg). Seychellogenin (**1**), colorless needles, mp 232—236 °C (MeOH), $[\alpha]_D^{23}$ −9.5° (c = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{46}O_3 \cdot 1/2H_2O$: C, 77.70; H, 10.22. Found: C, 77.72; H, 10.20. IR ν_{max}^{KBr} cm^{-1}: 3300 (br), 1762. UV λ_{max}^{MeOH} nm: 238 (sh, ε = 17000), 245 (19000), 253 (sh, 13500). CD ($c = 2.1 \times 10^{-2}$, MeOH): $[\theta]_{254}$ 0, $[\theta]_{247}$ −38000 (neg. max.), $[\theta]_{236}$ 0, $[\theta]_{225}$ +41000 (pos. max.), $[\theta]_{202}$ 0. ^{1}H-NMR (90 MHz, δ): (in $CDCl_3$) 0.88 (6H, s, 25-$(CH_3)_2$), 0.90 (3H, s, 4-CH_3), 1.00 (6H, s, 14-CH_3), 1.10 (3H, s, 10-CH_3), 1.38 (3H, s, 20-CH_3); (in pyridine-d_5) 0.88 (6H, s, 25-$(CH_3)_2$), 1.11 (3H, s, 4-CH_3), 1.07 (3H, s, 14-CH_3), 1.21 (3H, s, 4-CH_3), 1.35 (6H, s, 10,20-CH_3). MS m/z (%): 454 (M^+, 58), 421 ($M^+ - CH_3 - H_2O$, 54), 367 (M^+ − ring A, 87). Isobivittogenin (**2**), colorless needles, mp 205—207 °C (MeOH), $[\alpha]_D^{18}$ −21° (c = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 75.95; H, 10.16. IR ν_{max}^{KBr} cm^{-1}: 3350 (br), 1753. UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 2 \times 10^{-2}$, MeOH): $[\theta]_{254}$ 0, $[\theta]_{224}$ −19000 (neg. max.), $[\theta]_{214}$ 0, $[\theta]_{204}$ +32000 (pos. max.), $[\theta]_{200}$ +25000! ^{1}H-NMR (90 MHz, δ): (in $CDCl_3$) 0.85 (3H, s, 4-CH_3), 0.88 (6H, s, 25-$(CH_3)_2$), 0.91 (3H, s, 14-CH_3), 1.00 (3H, s, 4-CH_3), 1.19 (3H, s, 10-CH_3), 1.58 (3H, s, 20-CH_3); (in pyridine-d_5) 0.88 (6H, s, 25-$(CH_3)_2$), 0.98 (3H, s, 14-CH_3), 1.03, 1.20 (both 3H, s, 4-$(CH_3)_2$), 1.34 (3H, s, 10-CH_3), 1.89 (3H, s, 20-CH_3). MS m/z (%): 472 (M^+, 3), 454 ($M^+ - H_2O$, 13), 395 ($M^+ - H_2O - CO_2 - CH_3$, 100).

2) A sapogenol mixture (66 mg), obtained by acidic hydrolysis of bivittoside A (**6**) (150 mg) through a procedure as described above, was treated with pyridine (2 ml) and Ac_2O (0.5 ml) at 40 °C for 15 h. The reaction mixture was poured into ice-water and whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave an acetylated product (70 mg), which was purified by column chromatography to furnish seychellogenin acetate (**1a**) (25 mg), isobivittogenin acetate (**2a**) (5 mg), and preseychellogenin acetate (**4a**) (10 mg). **1a**, colorless needles, mp 215—217 °C (EtOH–acetone), $[\alpha]_D^{20}$ +20° (c = 0.7, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{48}O_4$: C, 77.38; H, 9.74. Found: C, 77.15; H, 9.91. IR ν_{max}^{KBr} cm^{-1}: 1775, 1730. UV λ_{max}^{MeOH} nm: 238 (sh, ε = 12800), 245 (14000), 253 (sh, 10000). ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (6H, d, J = 7 Hz), 0.90, 0.97, 1.01, 1.13, 1.39 (each 3H, s), 2.05 (3H, s), 4.52 (1H, t-like), 5.22 (1H, m), 5.59 (1H, m). MS m/z (%): 496 (M^+, 36), 437 ($M^+ - CO_2 - CH_3$, 38), 421 ($M^+ - CH_3 - AcOH$, 51), 43 (100). **2a**, colorless needles, mp 228—229 °C (hexane–acetone), $[\alpha]_D^{15}$ −26° (c = 0.4, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1754, 1740. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (6H, d, J = 6 Hz), 0.87, 1.00, 1.22, 1.25, 1.36 (each 3H, s), 2.02, 2.06 (both 3H, s), 4.50 (1H, t-like), 5.02 (1H, br s, $W_{h/2}$ = 6 Hz), 5.63 (1H, br s, $W_{h/2}$ = 6 Hz). MS m/z (%): 496 ($M^+ - AcOH$, 18), 437 (100). CI-MS m/z (%): 557 ($M^+ + 1$, 3), 497 (72), 437 (100). **4a**, colorless needles, mp 172—173 °C (EtOH–acetone), $[\alpha]_D^{15}$ −299° (c = 0.9, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{48}O_4$: C, 77.38; H, 9.74. Found: C, 77.12; H, 9.89. IR ν_{max}^{KBr} cm^{-1}: 1750, 1723, 1625. UV λ_{max}^{MeOH} nm: 302 (ε = 2500). CD ($c = 2.5 \times 10^{-2}$, MeOH): $[\theta]_{352}$ 0, $[\theta]_{302}$ −89000 (neg. max.), $[\theta]_{253}$ 0, $[\theta]_{239}$ +40000 (pos. max.), $[\theta]_{225}$ 0, $[\theta]_{215}$ −30000 (neg. max.), $[\theta]_{204}$ 0, $[\theta]_{200}$ +10000! ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.87 (6H, d, J = 6 Hz), 0.90 (6H, s), 1.06, 1.09, 1.42 (each 3H, s), 2.03 (3H, s), 4.49 (1H, t-like), 5.85, 6.09 (2H, ABq, J = 9 Hz). MS m/z (%): 496 (M^+, 12), 437 ($M^+ - CO_2 - CH_3$, 100), 377 (437 − AcOH, 27).

Acidic Treatment of Isobivittogenin (2) A mixture of isobivittogenin (**2**) (5 mg) and 1 N AcCl–MeOH (1 ml) was heated under reflux for 30 min. The reaction mixture was examined by TLC (*n*-hexane–AcOEt = 4 : 1) and seychellogenin (**1**) (Rf = 0.40) was identified as the sole product.

Acidic Treatment of 4a A mixture of **4a** (5 mg) in 1 N AcCl–MeOH (1 ml) was heated under reflux for 15 min. The reaction mixture was examined by TLC (*n*-hexane–AcOEt = 8 : 1) to identify seychellogenin acetate (**1a**) (Rf = 0.50) and seychellogenin (**1**) (Rf = 0.20) as the products.

Acidic Hydrolysis of Bivittosides B (8) and D (10) Bivittoside B (**8**) (20 mg) or D (**10**) (30 mg) was hydrolyzed with aq. 2 N HCl as described for acidic hydrolysis of bivittoside A (**6**), and seychellogenin (**1**) (3 mg) thus

obtained was shown to be identical with an authentic sample by TLC comparison and mixed melting-point determination.

$LiAlH_4$ Reduction of 4a A solution of **4a** (32 mg) in tetrahydrofuran (THF) (2 ml) was added dropwise into a suspension of $LiAlH_4$ (50 mg)–THF (3 ml) and the whole mixture was heated under reflux for 1 h. The reaction mixture was treated successively with AcOEt, water, and aq. 2 N H_2SO_4 and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a reduction product (30 mg), which was purified by column chromatography (SiO_2 3 g, *n*-hexane–AcOEt = 3 : 1) to furnish a triol (**5**) (15 mg). **5**, amorphous, $[\alpha]_D^{24}$ −5° (c = 0.4, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3350 (br), 1600 (br). UV λ_{max}^{MeOH} nm: 275 (ε = 2500). CD (c = 8.1×10^{-2}, MeOH): $[\theta]_{312}$ 0, $[\theta]_{275}$ −25000 (neg. max.), $[\theta]_{229}$ 0, $[\theta]_{206}$ +5000! ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (6H, d, J = 7 Hz), 0.81, 0.91, 0.98, 1.02, 1.43 (each 3H, s), 3.24 (1H, m), 3.56, 3.86 (2H, ABq, J = 11 Hz), 6.04 (2H, s). MS m/z (%): 458 (M^+, 1), 440 ($M^+ - H_2O$, 17), 422 ($M^+ - 2H_2O$, 24). High-MS Found: 458.376, 443.354, 440.362. Calcd for $C_{30}H_{50}O_3$ = 458.376, $C_{29}H_{47}O_3$ = 443.352, $C_{30}H_{48}O_2$ = 440.365.

Oxidation of Bivittoside A (6) with CrO_3–Pyridine–1-BuOH–Aq. H_2SO_4 1) Preparation of reagent A[9]: A solution of chromium trioxide (500 mg) in pyridine (30 mg) was poured into 1-butanol saturated with water (250 ml) and the whole solution was left standing for 1 d. The solvent was then removed below 45 °C under reduced pressure and the residue was treated with water (100 ml). The resulting precipitates were collected by filtration and washed successively with water and $CHCl_3$ and dried in a desiccator under reduced pressure to yield reagent A.

2) A solution of bivittoside A (**6**) (200 mg) in dioxane (10 ml)–water (10 ml) was treated with aq. 2 N H_2SO_4 (20 ml) and reagent A (600 mg) and the whole mixture was stirred at room temp. (20 °C) for 18 h. The reaction mixture was diluted with water and the whole was extracted with 1-butanol. The 1-butanol phase was taken and neutralized with aq. 2 N NaOH and washed with water. Removal of the solvent from the 1-butanol phase under reduced pressure gave a product (250 mg), which was purified by column chromatography [SiO_2 25 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1 (lower phase)] to afford **7** (50 mg) and **6** (125 mg, recovered). **7**, colorless needles, mp 263—264 °C (MeOH), $[\alpha]_D^{20}$ +12° (c = 1.1, pyridine). *Anal.* Calcd for $C_{41}H_{64}O_{12} \cdot 2H_2O$: C, 62.73; H, 8.73. Found: C, 62.61; H, 8.46. IR ν_{max}^{KBr} cm^{-1}: 3400, 1751 (br), 1591, 1070. UV λ_{max}^{MeOH} nm: 256 (ε = 10000). CD (c = 1.7×10^{-2}, MeOH): $[\theta]_{373}$ 0, $[\theta]_{328}$ −13000 (neg. max.), $[\theta]_{283}$ 0, $[\theta]_{254}$ +41000 (pos. max.), $[\theta]_{226}$ 0, $[\theta]_{214}$ −13000 (neg. max.), $[\theta]_{207}$ 0, $[\theta]_{206}$ +8700! ^{1}H-NMR (90 MHz, pyridine-d_5, δ): 0.88 (6H, d, J = 6 Hz), 0.92, 1.19, 1.33, 1.47, 1.57 (each 3H, s), 4.74 (1H, d, J = *ca.* 6 Hz), 5.14 (1H, d, J = *ca.* 6 Hz), 5.89 (1H, br s).

Methylation of Bivittoside A (6) Followed by Methanolysis 1) A solution of bivittoside A (**6**) (98 mg) in dimethyl sulfoxide (DMSO) (15 ml) was treated with a dimsyl carbanion solution (8.4 ml) [prepared from NaH (2 g), which was washed with dry *n*-hexane before use, and DMSO (35 ml) by stirring at 70 °C for 1 h under an N_2 atmosphere] and the whole mixture was stirred at room temp. (25 °C) for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (3.7 ml) under ice-cooling and stirred at room temp. for a further 1 h in the dark. The reaction mixture was then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with water, then dried over $MgSO_4$. Removal of the solvent from the AcOEt extract under reduced pressure gave a product (120 mg), which was purified by column chromatography (SiO_2 10 g, benzene–acetone = 20 : 1) to furnish the hexa-*O*-methyl derivative (**6a**) (109 mg). **6a**, colorless needles, mp 87—88 °C ($CHCl_3$), $[\alpha]_D^{18}$ +11° (c = 1.6, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{78}O_{12}$: C, 67.60; H, 9.41. Found: C, 67.29; H, 9.45. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1749. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 4.33 (1H, d, J = 7 Hz), 4.62 (1H, d, J = 7 Hz), 5.59 (1H, d, J = 4 Hz).

2) A solution of **6a** (5 mg) in 2.5 N AcCl–MeOH (1 ml) was heated under reflux for 1 h and neutralized with Ag_2CO_3. The whole mixture was filtered and the filtrate was subjected to GLC analysis [1) 15% polyethylene glycol succinate (PEGS) on Chromosorb WAW (80—100 mesh), 3 mm × 1 m; column temp. 150 °C; N_2 flow rate 30 ml/min, and 2) 15% neopenthyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temp. 170 °C; N_2 flow rate 30 ml/min] and TLC (*n*-hexane–acetone = 2 : 1). The following methyl glycosides were identified: methyl 2,3,4-tri-*O*-methylquinovopyranoside [I] [1) PEGS t_R = 1 min 21 s, 2 min 02 s; 2) NPGS t_R = 2 min 51 s, 3 min 54 s; Rf = 0.75, 0.65] and methyl 3,4-di-*O*-methylxylopyranoside [II] [1) PEGS t_R = 8 min 35 s, 10 min 54 s; 2) NPGS t_R = 9 min 58 s, 11 min 45 s; Rf = 0.25, 0.20].

Methylation of Bivittoside B (8) Followed by Methanolysis 1) A solution of bivittoside B (**8**) (100 mg) in DMSO (10 ml) was treated with a dimsyl carbanion solution (8 ml) and the whole mixture was stirred at room temp. (25 °C) for 1 h under an N_2 atmosphere. The reaction mixture was then treated with CH_3I (4 ml) under ice-cooling and stirred at room temp. in the dark for 1.5 h. Work-up of the reaction mixture as described above gave an AcOEt extract (135 mg), which was purified by column chromatography (SiO_2 20 g, *n*-hexane–AcOEt = 2 : 1) to furnish the dodeca-*O*-methyl derivative (**8a**) (105 mg). **8a**, amorphous, $[\alpha]_D^{18}$ 0° ($CHCl_3$). *Anal.* Calcd for $C_{65}H_{110}O_{22}$: C, 62.78; H, 8.92. Found: C, 62.66; H, 9.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 4.32 (1H, d, J = 8 Hz), 4.43 (1H, d, J = 7 Hz), 4.70 (1H, d, J = 8 Hz), 4.72 (1H, d, J = 8 Hz), 5.59 (1H, d, J = 4 Hz).

2) A solution of **8a** (10 mg) in 2.5 N AcCl–MeOH (1.5 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was examined by GLC [2) 15% NPGS on Chromosorb WAW (80—100 mesh) 3 mm × 2 m; column temp. 170 °C; N_2 flow rate 35 ml/min, and 3) 5% butane-1,4-diol succinate (BDS) on Uniport B (80—100 mesh), 3 mm × 2 m, column temp. 150 and 175 °C, N_2 flow rate 33 ml/min] and TLC (benzene–acetone = 2 : 1) to identify [I] [2) NPGS t_R = 2 min 24 s, 3 min 24 s; 3) BDS (150 °C), t_R = 2 min 22 s, 3 min 23 s; Rf = 0.80], methyl 2,3,4,6-tetra-*O*-methylglucopyranoside [III] [2) NPGS t_R = 7 min 12 s, 9 min 48 s; 3) BDS (150 °C), t_R = 8 min 08 s, 11 min 59 s; Rf = 0.75), methyl 2,4,6-tri-*O*-methylglucopyranoside [IV] [2) NPGS t_R = 19 min 18 s, 28 min 48 s; 3) BDS (175 °C), t_R = 10 min 24 s, 15 min 48 s; Rf = 0.20, 0.35], and methyl 3-*O*-methylxylopyranoside [V] [2) NPGS t_R = 19 min 18 s, 28 min 48 s; 3) BDS (175 °C), t_R = 12 min 22 s, 17 min 36 s; Rf = 0.15, 0.10].

Enzymatic Hydrolysis of Bivittoside B (8) with Crude Hesperidinase A suspension of bivittoside B (**8**) (300 mg) in an AcONa–AcOH buffer solution (pH 5.0, 60 ml) was treated with crude hesperidinase (600 mg, Lot No. 680930 provided by Tanabe Pharma. Co.) and the whole mixture was stirred at 38 °C for 5 d. The reaction mixture was then treated with 1-butanol (10 ml), heated at 50 °C for 5 min and filtered. The filtrate was extracted with 1-butanol and the 1-butanol extract was washed with water and concentrated under reduced pressure to give a product (350 mg). Purification of the product by column chromatography [SiO_2 80 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1 (lower phase)] furnished bivittoside A (**6**) (30 mg) and **8** (180 mg, recovered). **6** was shown to be identical with an authentic sample by melting-point determination and $[\alpha]_D$, IR, and ^{13}C-NMR comparisons.

Enzymatic Hydrolysis of Bivittoside D (10) with a Glycosidase Mixture from *Turbo cornutus* A suspension of bivittoside D (**10**) (500 mg) in an AcONa–AcOH buffer solution (pH 5.0, 150 ml) was treated with a glycosidase mixture from *Turbo cornutus* (800 mg, Seikagaku Kogyo Co., Ltd.) and the whole mixture was stirred at 37 °C for 3 d. The reaction mixture was treated with 1-butanol (10 ml), heated at 50 °C for 5 min and filtered. The filtrate was then extracted with 1-butanol and the solvent was evaporated off under reduced pressure to give a product (800 mg). Purification of the product by column chromatography [SiO_2 30 g, $CHCl_3$–MeOH–H_2O = 7 : 3 : 1 (lower phase)] furnished bivittoside B (**8**) (250 mg) and **10** (180 mg, recovered). **8** was shown to be identical with an authentic sample by mixed melting-point determination and $[\alpha]_D$, TLC, IR, and ^{13}C-NMR comparisons.

Methylation of Bivittoside D (10) Followed by Methanolysis 1) A solution of bivittoside D (**10**) (100 mg) in DMSO (15 ml) was treated with a dimsyl carbanion solution (10 ml) and the whole solution was stirred at room temp. (25 °C) for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (3.5 ml) under ice-cooling and then stirred at room temp. in the dark for a further 2 h. Work-up of the reaction mixture as described for methylation of bivittoside A (**6**) gave a product (130 mg), which was purified by column chromatography to furnish the octadeca-*O*-methyl derivative (**10a**) (80 mg). **10a**, amorphous, $[\alpha]_D^{20}$ −3° (c = 1.1, $CHCl_3$). *Anal.* Calcd for $C_{83}H_{142}O_{32}$: C, 60.34; H, 8.66. Found: C, 60.16; H, 8.91. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1756. ^{1}H-NMR (90 MHz, benzene-d_6, δ): 4.11 (1H, d, J = 7 Hz), 4.31 (1H, d, J = 8 Hz), 4.55 (1H, d, J = 7 Hz), 4.89 (3H, d, J = 7 Hz), 5.51 (1H, m).

2) A solution of **10a** (7 mg) in 2 N AcCl–MeOH (1.2 ml) was heated under reflux for 1.5 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered and the filtrate was analyzed by GLC [2) 15% NPGS on Chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temp. 180 °C, N_2 flow rate 38 ml/min and 3) 5% BDS on Uniport B (80—100 mesh), 3 mm × 2 m; column temp. 175 °C; N_2 flow rate 30 ml/min] and TLC (benzene–acetone = 2 : 1) to identify [III] [2) NPGS t_R = 3 min 15 s, 4 min 22 s; 3) BDS t_R = 7 min 42 s, 10 min 24 s; Rf = 0.75], methyl 2,3-di-*O*-methylquinovopyranoside [VI] [2) NPGS t_R = 3 min 15 s, 4 min 03 s; 3) BDS t_R = 7 min 10 s, 9 min 02 s; Rf = 0.65, 0.45], [IV] [2) NPGS t_R = 9 min 08 s, 15 min 52 s; Rf = 0.20, 0.35], and [V] [2) NPGS t_R = 9 min 45 s, 14 min

52 s; Rf=0.15, 0.10].

Acidic Hydrolysis of Bivittoside C (9) A mixture of bivittoside C (**9**) (100 mg) in aq. 2 N HCl (3 ml) was heated under reflux for 2 h. After dilution with water, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed successively with aq. sat. $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure gave a product (30 mg), which was purified by column chromatography (SiO_2 5 g, *n*-hexane–acetone=4:1) to furnish deoxybivittogenin (**3**) (24 mg). **3**, colorless needles, mp 231—233 °C (MeOH), $[\alpha]_D^{23}$ −16° (c=1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280 (br), 1750. CD (c=8.5×10^{-2}, MeOH): $[\theta]_{262}$ 0, $[\theta]_{224}$ −24000 (neg. max.), $[\theta]_{212}$ 0, $[\theta]_{208}$ +23000! ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.87 (6H, d, J=7 Hz), 0.84, 0.87, 0.91, 1.17, 1.38 (each 3H, s). High-MS Found: 456.359; 441.336; 423.325. Calcd for $C_{30}H_{48}O_3$=456.361; $C_{29}H_{45}O_3$=441.336; $C_{29}H_{43}O_2$=423.326.

Acetylation of Deoxybivittogenin (3) Giving the Monoacetate (3a) A solution of **3** (23 mg) in pyridine (1 ml) and Ac_2O (0.3 ml) was left standing at room temp. (25 °C) for 15 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product (24 mg), which was purified by recrystallization from EtOH–acetone to furnish the monoacetate (**3a**) (15 mg). **3a**, colorless needles, mp 225—226.5 °C (EtOH–acetone), $[\alpha]_D^{20}$ −3° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 76.88; H, 9.92. IR ν_{max}^{KBr} cm^{-1}: 1750, 1725. CD ($c=1.0\times10^{-1}$, MeOH): $[\theta]_{260}$ 0, $[\theta]_{221}$ −23000 (neg. max.), $[\theta]_{211}$ 0, $[\theta]_{208}$ +19000! ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (6H, d, J=7 Hz), 0.84, 0.86, 0.91, 1.18, 1.37 (each 3H, s),2.03 (3H, s), 4.47 (1H, m), 5.18 (1H, br s). MS m/z (%): 498 (M^+, 36), 483 (M^+-CH_3, 11), 438 (M^+−AcOH, 20), 423 (M^+−AcOH−CH_3, 100).

Acetylation of Bivittoside C (9) Followed by Oxidation with *tert*-Butyl Chromate and Deacetylation A solution of bivittoside C (**9**) (500 mg) in pyridine (10 ml) and Ac_2O (5 ml) was left standing at room temp. (26 °C) for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product (600 mg). The product (300 mg) was dissolved in CCl_4 (5 ml) and the solution was treated with a (*tert*-BuO)$_2CrO_2$ solution [(*tert*-BuO)$_2CrO_2$–AcOH–Ac_2O=5:2:1] (5 ml) and stirred at 80 °C for 3 h. After cooling to 0 °C, the reaction mixture was treated with an aqueous solution (6 ml) of $(COOH)_2$ (600 mg). The reaction mixture was allowed to cool to room temperature, then further $(COOH)_2$ (400 mg) was added, and the whole was stirred for 1 h, poured into ice-water and extracted with AcOEt. The AcOEt extract was washed successively with water, aq. sat. $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure gave a product (300 mg), which was treated with 0.1 M K_2CO_3 in aq. 85% MeOH (20 ml) and stirred at room temp. (28 °C) for 40 min. After neutralization with aq. dil. HCl, the reaction mixture was extracted with 1-butanol and the 1-butanol extract was washed with water. Removal of the solvent from the 1-butanol extract under reduced pressure gave a product (150 mg), which was purified by column chromatography [SiO_2 10 g, $CHCl_3$–MeOH–H_2O=7:3:1 (lower phase)] to furnish **11** (100 mg). **11**, colorless needles, mp 218.5—220 °C (MeOH), $[\alpha]_D^{26}$ −12.5° (c=1.1, pyridine). *Anal.* Calcd for $C_{67}H_{108}O_{32}\cdot2H_2O$: C, 55.06; H, 7.72. Found: C, 54.94; H, 7.68. IR ν_{max}^{KBr} cm^{-1}: 3390 (br), 1750, 1673 (br). UV λ_{max}^{MeOH} nm: 253 (ε=8100). CD ($c=1.2\times10^{-2}$, MeOH): $[\theta]_{360}$ 0, $[\theta]_{327}$ −16000 (neg. max.), $[\theta]_{285}$ 0, $[\theta]_{253}$ +53000 (pos. max.), $[\theta]_{221}$ 0, $[\theta]_{210}$ −8000 (neg. max.), $[\theta]_{206}$ 0, $[\theta]_{203}$ +11000! ^{13}C-NMR (50 MHz, pyridine-d_5, δ_C): 194.1 (s), 175.1 (s), 171.5 (s), 118.3 (d), 105.5 (2C, d), 105.1 (d), 104.7 (d), 102.9 (d).

Oxidation of Bivittoside D (10) with CrO_3–Pyridine–1-BuOH–Aq. H_2SO_4 A solution of bivittoside D (**10**) (1 g) in dioxane (120 ml)–water (120 ml) was treated with aq. 2 N H_2SO_4 (240 ml) and reagent A (*vide supra*) (3.14 g). The whole mixture was stirred at room temp. (22 °C) for 6 h and diluted with water. The reaction mixture was then extracted with 1-butanol and the 1-butanol extract was washed with aq. dil. NaOH and water. Removal of the solvent from the 1-butanol extract under reduced pressure gave a product (1.36 g), which was purified by column chromatography [SiO_2 60 g, $CHCl_3$–MeOH–H_2O=7:3:1 (lower phase)] to furnish **11** (800 mg). **11** thus obtained here was shown to be identical with the product (**11**) obtained from bivittoside C (**9**) by mixed melting-point determination and $[\alpha]_D$, IR, UV, CD, and ^{13}C-NMR comparisons.

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References and Notes

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