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Structures of Four New Triterpenoidal Oligoglycosides, Bivittoside A, B, C, and D, from the Sea Cucumber *Bohadschia bivittata* Mitsukuri

On the basis of chemical and physicochemical evidence, the structures of four triterpenoidal oligoglycosides, bivittoside A, B, C, and D from the sea cucumber *Bohadschia bivittata* Mitsukuri, have been elucidated as **6**, **8**, **9**, and **10**, respectively. A new homoannular dienic sapogenol was isolated as the acetate and the highly strained structure (**4a**) has been elucidated.

Keywords——sea cucumber; *Bohadschia bivittata*; lanostane-type triterpenoid; oligoglycoside; bivittoside; strained homoannular diene; *Turbo cornutus* glycosidase

During the course of systematic studies on the biologically active constituents of echinoderm,[1] we have recently isolated four new triterpenoidal oligoglycosides, named bivittoside A (**6**), B (**8**), C (**9**), and D (**10**), from the sea cucumber *Bohadschia bivittata* Mitsukuri collected in Okinawa Prefecture in July. This communication deals with the evidence being consistent with the proposed structures.[2]

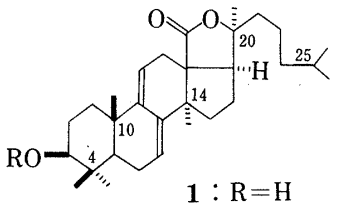
The MeOH extract of the Cuvierian tubles of *B. bivittata* afforded bivittoside A, B, C, and D, after solvent-fractionation and chromatographic separation, in 2, 2, 2, and 8% yields (respectively from the MeOH ext.).

Bivittoside A (**6**), $C_{41}H_{66}O_{12}\cdot H_2O$,[3] mp 267—268°, $[\alpha]_D$ +9° (pyr.), UV (MeOH): transparent above 210 nm, shows the infrared(IR) absorption bands [3400 (br), 1070 (br) cm^{-1}] characteristic to glycoside and the band due to γ-lactone (1750 cm^{-1}). The circulardichroism (CD) spectrum (MeOH) of bivittoside A demonstrates chirality of the γ-lactone moiety by a negative maximum: $[\theta]_{222}$ −7800. On acidic hydrolysis, bivittoside A furnished the dienic artifact sapogenol seychellogenin (**1**),[4] a dihydroxy-triterpene lactone (**2**), $C_{30}H_{48}O_4$, mp 205—207°, $[\alpha]_D$ −21° ($CHCl_3$), IR (KBr): 3350 (br), 1753 (br) cm^{-1}, and one mole each of D-xylose and D-quinovose, and another minor sapogenol (*vide post*). The structure of **2** having the 9(11)-en-12β-ol moiety has been corroborated by the proton nuclear magnetic resonance(^{1}H-NMR) signals observed at δ 4.39 (1H, m, $W_h/_2$=12 Hz, 12α-H) and δ 5.12 (1H, br.s, $W_h/_2$=6 Hz, 11-H) and the CD spectrum (MeOH): $[\theta]_{204}$ +32000 (pos. max.) [9(11)-ene],[5b] and also by the ready conversion of **2** giving **1** on acidic treatment. The unknown configuration at C-20 of seychellogenin (**1**) has been now defined S as based on the ^{1}H-NMR analysis utilizing the pyridine-induced shift (Table).[1,5]

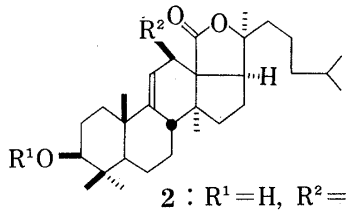
Methylation[6] of bivittoside A gave the fully methylated hexa-O-methyl derivative (**6a**) [two β-anomeric proton signals at δ 4.33 and 4.62 (1H both, d, J=7 Hz)], which, on methanolysis, liberated methyl pyranosides of 2,3,4-tri-O-methylquinovose and 3,4-di-O-methylxylose. Presence of the 9(11)-en-12α-ol moiety in bivittoside A has been substantiated by the ^{1}H-NMR signals observed at δ 4.52 (1H, d, J=4 Hz, 12β-H) and δ 5.70 (1H, d, J=4 Hz, 11-H)[5b] and the CD spectrum (MeOH): $[\theta]_{212}$ +8100! and by oxidation with CrO_3-pyridine-*n*-BuOH-aq.H_2SO_4[7] providing the 12-keto derivative (**7**), $C_{41}H_{64}O_{12}\cdot 2H_2O$, mp 263—264°, $[\alpha]_D$ +12°

(pyr.), UV (MeOH): 256 nm (ε=10000). The latter conversion simultaneously shows that the oligosaccharide moiety in bivittoside A attaches to 3β-OH of the sapogenol. Based on the above-mentioned evidence, the structure of bivittoside A has been elucidated as **6**. The C-12 configuration of **6** has been inverted during the acidic hydrolysis to furnish **2**.

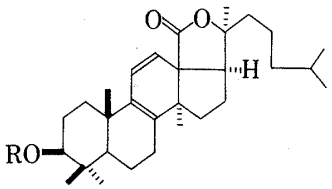
Isolation of the above-mentioned minor sapogenol was effected after acetylation. The monoacetate (**4a**), $C_{32}H_{48}O_4$, mp 172—173°, $[\alpha]_D$ −299° ($CHCl_3$), shows two olefinic proton signals at δ 5.85 and 6.09 (1H each, ABq, J=9 Hz) in the ^{1}H-NMR spectrum. It gave seychellogenin acetate (**1a**) on acidic treatment, thus presence of the 8,11-diene moiety being suggested. The UV spectrum of the acetate shows the homoannular diene absorption maximum with unusual large red-shift: λ_{max} 302 nm (ε=2500). The similar characteristic has been observed in the CD spectrum (MeOH): $[\theta]_{302}$ −89000 (neg. max.) (homoannular diene $\pi\rightarrow\pi^*$) and $[\theta]_{239}$ +40000 (pos. max.) (γ-lactone). However, $LiAlH_4$ reduction of the acetate gave a triol (**5**), $C_{30}H_{50}O_3$, amorphous, $[\alpha]_D$ −5° ($CHCl_3$), ^{1}H-NMR (δ): 6.04 (2H, s, 11-H, 12-H), 3.56, 3.86 (1H each, ABq, J=11 Hz, 18-H_2). The UV and CD spectra of the



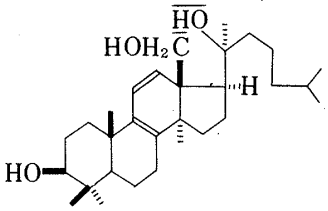
1 : R=H
1a : R=Ac



2 : R^1=H, R^2=OH
3 : R^1=R^2=H
3a : R^1=Ac, R^2=H



4 : R=H
4a : R=Ac



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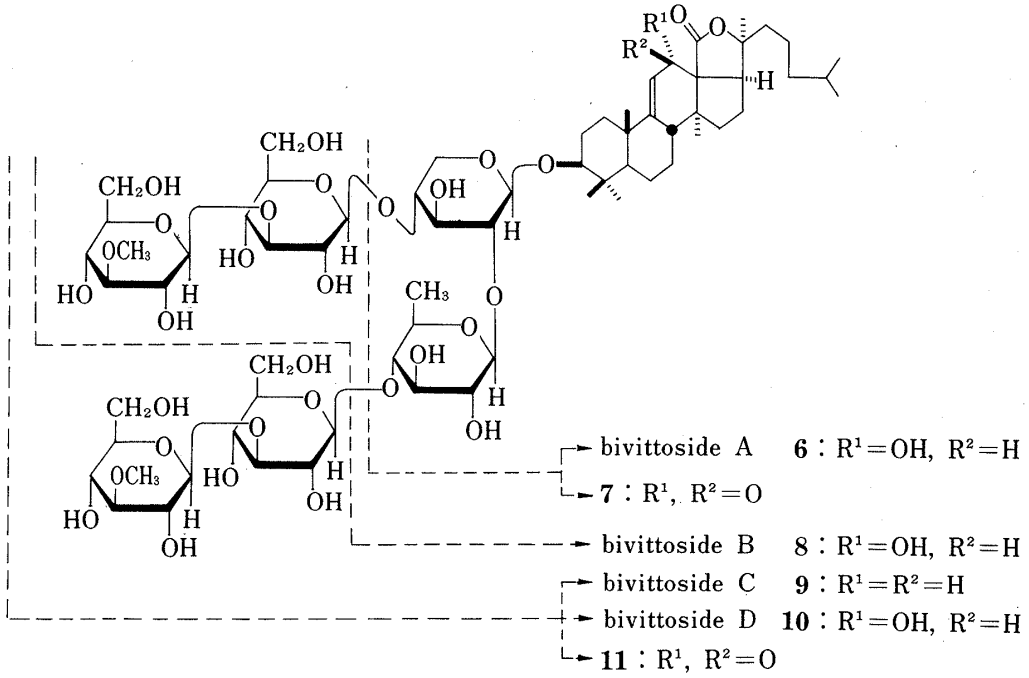

bivittoside A **6** : R^1=OH, R^2=H
7 : R^1, R^2=O
bivittoside B **8** : R^1=OH, R^2=H
bivittoside C **9** : R^1=R^2=H
bivittoside D **10** : R^1=OH, R^2=H
11 : R^1, R^2=O

Chart 1

Table I. ^{1}H-NMR Data (90 MHz)

	Solvent	4-Me$_2$	10-Me	14-Me	20-Me	25-Me$_2$
1	$CDCl_3$	0.90, 1.00	1.10	1.00	1.38	0.88
	d_5-pyr.	1.11,[a] 1.21	1.35	1.07[a]	1.35	0.88
2	$CDCl_3$	0.85, 1.00	1.19	0.91	1.58	0.88
	d_5-pyr.	1.03,[a] 1.20	1.34	0.98[a]	1.89	0.88

a) The assignments are interexchangeable.

triol show the homoannular diene maximum at the normal wave length: λ_{max} 275 nm (ε=2500), $[\theta]_{275}$ −25000 (neg. max.), thus the structure **4a** being evidenced. It has been assumed that the sapogenol (**4**) with the 8,11-diene moiety may be an intermediary compound in the process providing seychellogenin (**1**) from bivittoside A (**6**). The reason for the unusual large red-shift of the UV absorption maximum of **4a** is yet unclear, although the severe strain on the homoannular diene chromophore may be ascribable as one of the reasons.

Enzymic hydrolysis of bivittoside B (**8**), $C_{54}H_{88}O_{22}\cdot 3H_2O$, mp 270—273°, $[\alpha]_D$ +6° (pyr.), with crude hesperidinase afforded bivittoside A (**6**).[8] On methanolysis, the dodeca-O-methyl derivative (**8a**) [four β-anomeric proton signals at δ 4.32 (d, J=8 Hz), 4.43 (d, J=7 Hz), 4.70 (d, J=8 Hz), and 4.72 (d, J=8 Hz)] liberated methyl pyranosides of 2,3,4,6-tetra-O-methylglucose, 2,4,6-tri-O-methylglucose, 2,3,4-tri-O-methylquinovose, and 3-O-methylxylose, thus the structure **8** being substantiated.

Bivittoside D (**10**), $C_{67}H_{110}O_{32}\cdot 3H_2O$, mp 219—221°, $[\alpha]_D$ −7° (pyr.) is a hexaglycoside as shown by the ^{1}H-NMR spectrum of the octadeca-O-methyl derivative (**10a**) which shows six β-anomeric proton signals at δ 4.11 (1H, d, J=7 Hz), 4.31 (1H, d, J=8 Hz), 4.55 (1H, d, J=7 Hz), and 4.89 (3H, d, J=7 Hz). Enzymic hydrolysis of **10** with mixed glycosidase from *Turbo cornutus* afforded bivittoside B (**8**), while methanolysis of the fully methylated derivative gave methyl pyranosides of 2,3,4,6-tetra-O-methylglucose, 2,4,6-tri-O-methylglucose, 2,3-di-O-methylquinovose, and 3-O-methylxylose. Therefore, the structure **10** has been proposed to bivittoside D.

On acidic hydrolysis, bivittoside C (**9**), $C_{67}H_{110}O_{31}\cdot H_2O$, mp 216—218°, $[\alpha]_D$ −31° (pyr.), furnished another sapogenol (**3**), $C_{30}H_{48}O_3$, mp 231—233°, $[\alpha]_D$ −16° ($CHCl_3$), [monoacetate (**3a**), $C_{32}H_{50}O_4$, mp 225—226°]. The structure of bivittoside C (**9**) has been elucidated as based on the following evidence. Thus, acetylation followed by oxidation with *t*-butyl chromate and subsequent deacetylation of bivittoside C gave the 9(11)-en-12-one derivative (**11**), $C_{67}H_{108}O_{32}\cdot 2H_2O$, mp 218—220°, $[\alpha]_D$ −13° (pyr.), UV (MeOH): 253 nm (ε=8300), which was found to be identical with an oxidation product of bivittoside D (**10**) obtained by the method[7] as described above for oxidation of **6** giving **7**.

The antifungal activities of these bivittosides will be reported elsewhere.

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References and Notes

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