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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 tubes 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°, $[\alpha]_D +9^\circ$ (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 circular dichroism (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 seichellogenin (**1**),⁴⁾ a dihydroxy-triterpene lactone (**2**), $C_{30}H_{48}O_4$, mp 205—207°, $[\alpha]_D -21^\circ$ ($CHCl_3$), 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-12 β -ol moiety has been corroborated by the proton nuclear magnetic resonance (1H -NMR) signals observed at δ 4.39 (1H, m, $W_{h/2}=12$ Hz, 12 α -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 seichellogenin (**1**) has been now defined S as based on the 1H -NMR analysis utilizing the pyridine-induced shift (Table).^{1,5)}

Methylation⁶⁾ 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-12 α -ol moiety in bivittoside A has been substantiated by the 1H -NMR signals observed at δ 4.52 (1H, d, $J=4$ Hz, 12 β -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°, $[\alpha]_D +12^\circ$

(pyr.), UV (MeOH): 256 nm ($\epsilon=10000$). 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°, $[\alpha]_D -299^\circ$ ($CHCl_3$), shows two olefinic proton signals at δ 5.85 and 6.09 (1H each, ABq, $J=9$ Hz) in the 1H -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 ($\epsilon=2500$). 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, $[\alpha]_D -5^\circ$ ($CHCl_3$), 1H -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

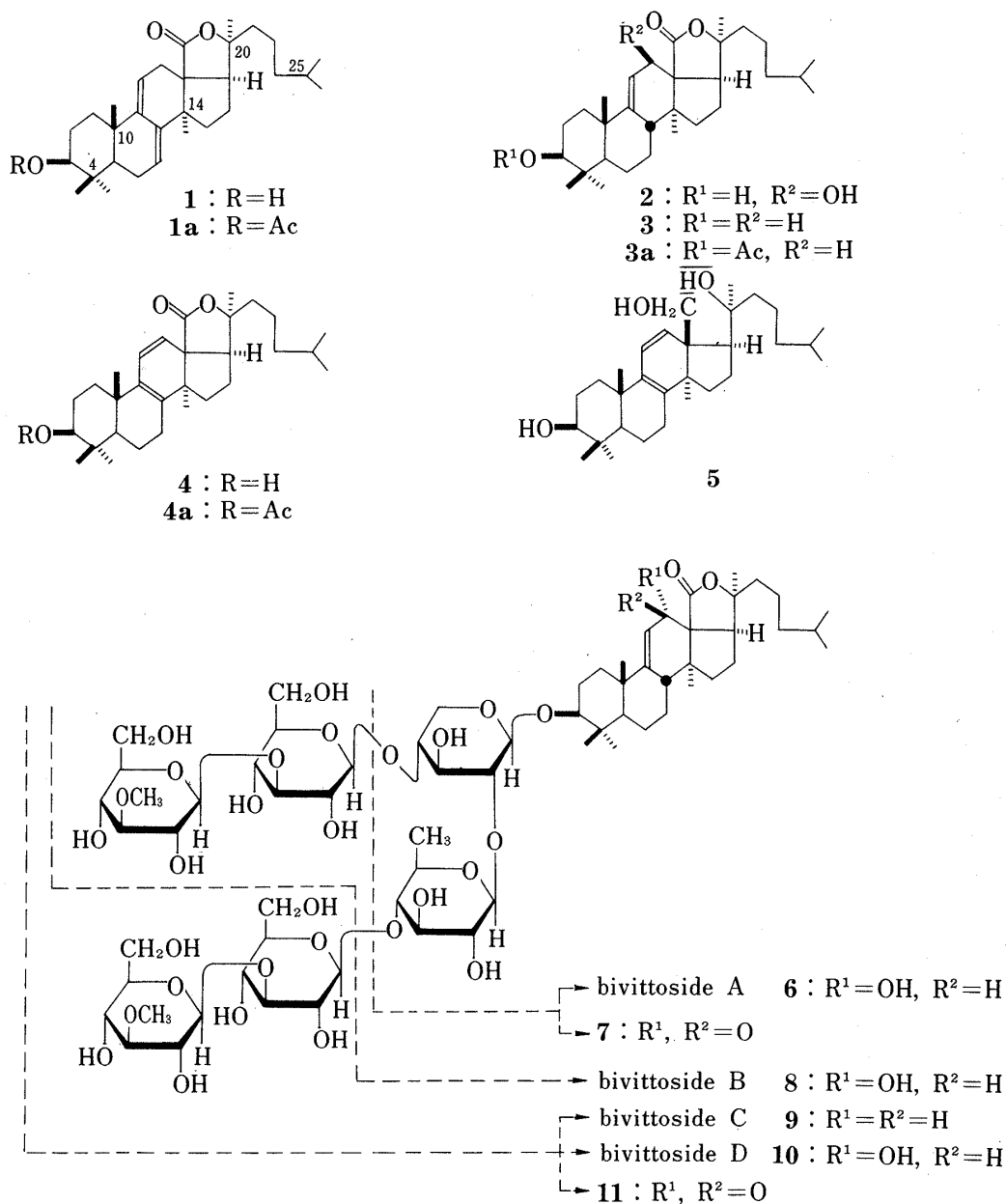


Chart 1

TABLE I. $^1\text{H-NMR}$ Data (90 MHz)

	Solvent	4-Me ₂	10-Me	14-Me	20-Me	25-Me ₂
1	$\{\text{CDCl}_3$	0.90, 1.00	1.10	1.00	1.38	0.88
	$\{d_5\text{-pyr.}$	1.11, ^{a)} 1.21	1.35	1.07 ^{a)}	1.35	0.88
2	$\{\text{CDCl}_3$	0.85, 1.00	1.19	0.91	1.58	0.88
	$\{d_5\text{-pyr.}$	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 ($\epsilon=2500$), $[\theta]_{275} -25000$ (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**), $\text{C}_{54}\text{H}_{88}\text{O}_{22} \cdot 3\text{H}_2\text{O}$, mp 270—273°, $[\alpha]_{\text{D}} +6^\circ$ (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**), $\text{C}_{67}\text{H}_{110}\text{O}_{32} \cdot 3\text{H}_2\text{O}$, mp 219—221°, $[\alpha]_{\text{D}} -7^\circ$ (pyr.) is a hexaglycoside as shown by the $^1\text{H-NMR}$ 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**), $\text{C}_{67}\text{H}_{110}\text{O}_{31} \cdot \text{H}_2\text{O}$, mp 216—218°, $[\alpha]_{\text{D}} -31^\circ$ (pyr.), furnished another sapogenol (**3**), $\text{C}_{30}\text{H}_{48}\text{O}_3$, mp 231—233°, $[\alpha]_{\text{D}} -16^\circ$ (CHCl_3), [monoacetate (**3a**), $\text{C}_{32}\text{H}_{50}\text{O}_4$, 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**), $\text{C}_{67}\text{H}_{108}\text{O}_{32} \cdot 2\text{H}_2\text{O}$, mp 218—220°, $[\alpha]_{\text{D}} -13^\circ$ (pyr.), UV (MeOH): 253 nm ($\epsilon=8300$), which was found to be identical with an oxidation product of bivittoside D (**10**) obtained by the method⁷⁾ 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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