Communications to the Editor

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Revised Structure of Lyoniatoxin, Toxin of *Lyonia ovalifolia* var. *elliptica*

The structure of lyoniatoxin, a diterpenoid from the leaves of *Lyonia ovalifolia* var. *elliptica*, has been revised to I on the basis of ^{13}C NMR evidence, providing a typical example of the use of the long-range ^{13}C-^{1}H spin decoupling technique for the structural elucidation of isoprenoid natural products.

Keywords——*Lyonia ovalifolia* var. *elliptica*; Ericaceae; diterpenoid; toxin; NMR; ^{13}C-^{1}H spin decoupling

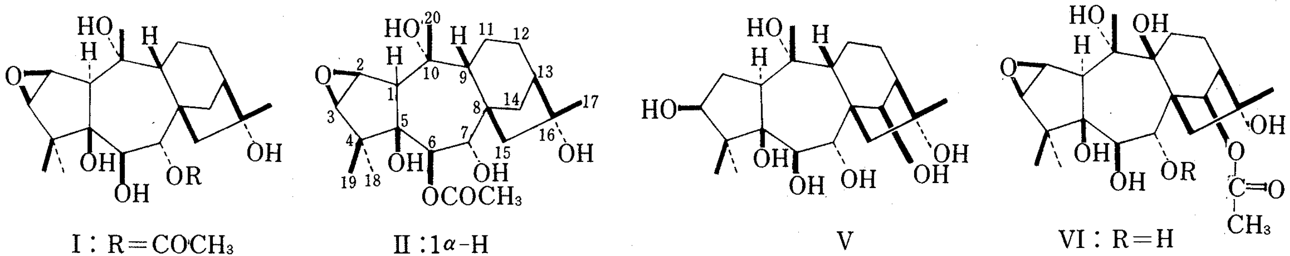
Lyoniatoxin (L) is a toxin isolated by Ikeda and Suzuki[1] in 1960 from the leaves of *Lyonia ovalifolia* Drude var. *elliptica* Handel-Mazzetti (Ericaceae). In 1970, Hikino *et al.*[2] proposed the stereostructure II for L on the basis of chemical and physical evidence. Simultaneously, Yasue *et al.*[3] reported the alternative stereostructure III for L, but their allocation of the carbon skeleton was based only on biogenetic speculation. Subsequently, Yasue *et al.*[4] performed a number of structural examinations on L and arrived at the same structure of Hikino.

Since we have recently made assignments of the carbon-13 nuclear magnetic resonance (^{13}C NMR) shifts of the ericaceous diterpenoid,[5] the ^{13}C NMR shifts of L were now assigned by correlation with those of its congeners,[5] off-resonance decoupling experiments and chemical shift considerations (Table I).

The assignments for C-6 and C-7 came into question. Comparison of the ^{13}C NMR spectra of deacetyl-L (IV) and bisdeacylasebotoxin V (deacyl-A-V, V) should reveal that a *peri*-interaction between the 7-hydroxyl and the 14-hydroxyl in the latter is absent in the former. Hence, conversion of the latter to the former may induce a greater shift of the C-7 resonance than that of C-6. In fact, the upper field signal (δ 76.2) in deacyl-L (IV) showed a significant displacement as compared with the pair corresponding to C-6 and C-7 in deacyl-A-V (V) ($\Delta\delta$ 3.0 or 4.0 ppm), while the higher field signal (δ 79.3) exhibited a lesser shift ($\Delta\delta$ 0.1 or 0.9 ppm). Thus, the C-6 and C-7 resonances in deacyl-L (IV) were assigned to the signals at lower and higher field (δ 79.3 and 76.2), respectively.

The C-6 and C-7 signals appeared at δ 78.1 and 80.5 in L. If the lower and higher field signals are assigned to C-6 and C-7, respectively, both the signals show, on passing from deacyl-L (IV) to L, lower field shifts ($\Delta\delta$ +1.2 and +1.9 ppm, respectively) which are inconsistent with the expected acetylation shifts.[5] While if the above assignments are reversed (δ 80.5 for C-7 and δ 78.1 for C-6), the former shows a downfiled shift of +4.3 ppm and the latter exhibits an upfield shift of −1.2 ppm on acetylation, in analogy with the predicted acetylation shifts.[5] Further, the changes in certain other resonances (C-4—C-8 and C-15) on going from deacyl-L (IV) to L are essentially identical with those from 14-acetyl-bisdeacylasebotoxin VII (VI) to its 7-acetate (VII) (Table I), but not in accord with those from grayanotoxin III and rhodojaponin III to their 6-acetates.[5] These data indicated that L is a 7-acetylated derivative (I).

In order to confirm the structure of L, ^{13}C-^{1}H spin couplings were determined (in $CDCl_3$–C_5D_5N). The carbinyl carbon signals at δ 77.4 and 79.6 were spin coupled to the carbinyl hydrogen signals at δ 3.61 and 5.24, respectively. These observations, together with the finding of ^{13}C-^{1}H spin coupling between the carbonyl carbon signal at δ 171.6 and the hydrogen signal at δ 5.24, revealed that the signal at δ 77.4 corresponds to the hydroxyl-bearing carbon and that at δ 79.6 is due to the acetoxyl-carrying carbon. The C-4 quaternary carbon signal may be coupled to the H-6 signal but not to the H-7 signal, while the C-8 quaternary carbon



I : R=COCH₃
IV : R=H

II : 1α-H
III : 1β-H

V

VI : R=H
VII : R=COCH₃

Chart 1

Table I. Carbon-13 Shieldings in Lyoniatoxin and Related Substances (δ in C_5D_5N)

	I[a]	IV	VII	VI
C-1	54.4	54.7	54.3	55.0
C-2	60.1	60.2	59.6	59.5
C-3	64.6	64.5	64.6	63.9
C-4	48.7	48.3	48.7	48.3
C-5	79.5	79.3	82.7	82.3
C-6	78.1	79.3	77.5	78.5
C-7	80.5	76.2	77.5	73.6
C-8	51.5	51.5	62.4	61.8
C-9	50.4	51.9	81.2	79.9
C-10	77.9	78.1	80.6	80.8
C-11	23.3	23.2	31.3	31.3
C-12	26.8	27.1	25.1	25.1
C-13	50.9	50.9	54.7	54.7
C-14	30.6	29.5	81.2	81.6
C-15	53.6	52.9	50.4	49.4
C-16	77.3	77.3	78.9	78.5
C-17	24.4	24.0	23.3	24.1
C-18	21.1	20.7	21.1	20.6
C-19	21.5	21.4	21.9	21.8
C-20	31.0	31.0	27.4	27.5

[a] δ 22.0 and 171.9 for CH_3CO-.

signal may be coupled to the H-7 and H-6 signals. Selective decoupling of the H-6 and H-7 signals, however, produced no apparent changes in the C-4 and C-8 signals. Since the C-4 and C-8 signals are coupled to a number of hydrogen signals besides the H-6 and H-7 signals, removal of only one long-range coupling would induce no apparent changes in the signals. Irradiation at δ 1—2.5, where the signals for the H-9, H-14 and H-15 signals appear, caused no change in the signal at δ 77.4 but brought about removal of the fine splittings of the signal at δ 79.6. Furthermore, irradiation at δ 2.65, where the H-1 signal occurs, resulted in decoupling of the signal at δ 77.4 but produced no change in the signal at δ 79.6. These facts clearly show that the carbon bearing the hydroxyl is C-6 while the one carrying the acetoxyl is C-7. Hence, the structure of L is revised to I.

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References and Notes

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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°