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> STRUCTURES OF TOROKONINE AND GOMANDONINE, TWO NEW DITERPENE ALKALOIDS FROM ACONITUM SUBCUNEATUM NAKAI

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Chemical investigation of the roots of <u>Aconitum subcuneatum</u> Nakai resulted in the isolation of two new diterpene alkaloids torokonine (1) and gomandonine (2), along with eight known compounds, i.e. mesaconitine, jesaconitine, 14-dehydrobrowniine, neoline, 15- α hydroxyneoline, isotalatizidine, senbusine A, and virescenine. The structure and the absolute configuration of torokonine (1) were established as 7- α -hydroxyryosenamine from the spectroscopic analysis. The novel structure of gomandonine (2), having an epoxy ring, was initially deduced by the pyridine-induced solvent shift technique and then confirmed by X-ray analysis.

KEYWORDS <u>Aconitum Subcuneatum; Ranunculaseae</u>; diterpene alkaloid; torokonine; gomandonine; ¹³C-NMR; CD; solvent shift; X-ray analysis

We have determined the structure of two new diterpene alkaloids, named torokonine and gomandonine isolated from the roots of <u>Aconitum</u> <u>subcuneatum</u> Nakai collected at Mt. Gomando, Fukushima prefecture, in July 1982.

Torokonine (1), which was originally isolated from the same plants and was named Gomando-base I by Ochiai $\underline{et} \underline{al}$,¹⁾ has the following physical constants: mp 198.5-199 $^{\circ}$ C (from acetone), C₂₇H₃₁NO₅, (m/z; M⁺ Calcd 449.2202, Found 449.2234), $[\alpha]_{D}^{8}$ +71.7° (c=0.14, MeOH), IR(KBr);3400, 1720, ¹H-NMR(CD₃OD): δ 7.47-8.02(5H, aromatic H), 5.52(1H, m, C_2 -H), 5.03 and 5.00(each 1H, t, J=1.3Hz, $C_{1.7}$ -H₂), 4.53(1H, br s, C_{15} -H), 4.42(1H, d, J=2.6Hz, C_7 -H), 3.41(1H, br s, C_6 -H), 3.11 and 2.60(each 1H, d, J=12.5Hz, $C_{19}-H_2$), 2.96(1H, s, $C_{20}-H$), 1.11(3H, s, $C_{18}-H_3$). These data for torokonine, which are very similar to those of ryosenamine (3),²⁾ indicate the presence of an additional secondary hydroxy group in ryosenamine molecule. In the 1^{3} C-NMR spectrum (Table I), the appearance of a new doublet at 64.3 ppm, the absence of a triplet at 29.1 ppm (compared with ryosenamine (3)), and the downfield shift at C_6 (5.6 ppm) and at C_8 (4.8 ppm) indicate that an extra secondary hydroxy group must be present at the C7 position. Furthermore, upfield shifts of C_{14} (6.0 ppm) and of C_{15} (5.9 ppm) due to steric compression by the OH function give proof of the α -orientation of the C₇ hydroxy group. The absolute configuration of torokonine (1) was determined as follows. 1 was oxidized with active MnO₂ in CH₂Cl₂ at room temperature to yield α, β -unsaturated ketone derivative (4), mp 248-250°C(from MeOH), IR(KBr);3540, 1715, 1645, ¹H-NMR(CDCl₃) δ : 5.85, 5.10(each 1H, d, J=1Hz, C₁₇-H₂). The CD spectra of 4 [$\lambda \operatorname{dioxane}_{ext}$ nm($\Delta \epsilon$): +0.82(362), +0.82(349), +14.0(235.5)] and that of ryosenaminone (5)²) [$\lambda \operatorname{dioxane}_{ext}$ nm($\Delta \epsilon$): +0.53(363), +0.57(348), +14.30(235)] showed similar Cotton curves. Thus, the absolute configuration of 1 is identical with that of ryosenamine (3) having (-)-kaurene type. We also made it clear that an unidentified alkaloid tentatively named Toroko-base I¹) that has been isolated from <u>Aconitum subcuneatum</u> Nakai collected at Toroko(Akita Prefecture) is torokonine (1) by comparison of the physical and spectral data.



(1) R=OH, torokonine

(3) R=H , ryosenamine



(4) R=OH

(5) R=H

 Table I.
 13C
 Chemical Shifts of 1, 3, and 2

Carbon	1	3	2
1	28.9	29.2*	70.6
2	70.2	70.8	32.1
3	39.2	38.8	40.1*
4	35.4	35.9	33.8
5	51.5	54.3	52.7
6	69.7	64.1	24.1#
7	64.3	29.1*	42.6 [@]
8	48.9	44.1	44.6
9	79.6	79.3	43.9 [@]
10	49.8	50.5	51.4 "
11	36.9	37.2	25.6#
12	34.6	35.0	41.6@
13	32.7	33.6	69.1
14	36.0	42.0	39.2
15	66.6	72.5	76.5
16	153.6	155.2	65.5
17	110.5	109.6	45.0
18	29.3	29.5	26.3
19	62.2	63.7	59.6
20	73.3	74.2	68.9
C=0	165.9	166.0	
\checkmark	130.1	130.4	
	129.4	129.4	
\sim	128.8	128.6	
	133.3	133.0	42.0
N-CH3			43.9

Assignments with the same symbol are interchangeable in each column. δ (ppm) downfield from TMS in CDCl₃ 1,3 and in d₅-pyridine 2.

Gomandonine (2), [mp 248-249°C(from MeOH), $[\alpha]_D^{12}$ -42.5°(c=0.12, MeOH), $C_{21}H_{31}NO_4$], has the following spectral data: IR(KBr):3570, 3400, 3310, MS m/z(%):361(M⁺, 41), 344(M⁺-OH, 100), ¹H-NMR(d_5-pyridine+D_2O) &:5.00(1H, s, C_{15}-H), 4.37(1H, d.d, J_1=4.4HZ, J_2=8.4HZ, C_{13}-H), 4.13(1H, d.d, J_1=6.3HZ, J_2=10.9HZ, C_1-H), 3.89(1H, br s, C_{20}-H), 3.63(1H, d, J=6.3HZ, C_{17}-H_a), 2.66(1H, d, J=6.3HZ, C_{17}-H_b), 3.47(1H, br.d, d, J_1=8.2HZ, J_2=13.8HZ, C_6-\beta H), 2.25(3H, s, N-CH_3), 0.71(3H, s, CH_3). We have deduced from the above physico-chemical data that gomandonine is a C₂₀ type diterpene alkaloid having one N-CH₃ group and three secondary hydroxy groups in the molecule. But most characteristic of this new compound is the absence of the exomethylene group that always exists in the hitherto known C₂₀ diterpene alkaloids. ¹³C-NMR spectrum analysis (Table I) indicated the presence of an epoxy ring at the C₁₆-C₁₇ position. Initially, we deduced the location and the stereochemistry of the three hydroxy groups (C₁-OH, C₁₅-OH, and C₁₃-OH) utilizing

the pyridine-induced solvent shift technique in ¹H-NMR spectra.³⁾ Individual protons (C₂₀-H, C₆- β H, and C₁₅- α H) occupying positions sterically close to the hydroxy function (C₁- α OH, C₁₅- β OH, and C₁₃-OH) shift dramatically downfield (0.41 ppm, 0.77 ppm and 0.69 ppm, respectively) in d₅-pyridine relative to CDCl₃. Furthermore, one signal of the protons on C₁₇ showed a pyridine-induced solvent shift by -0.51 ppm, a fact which indicates that C₁₅- β OH and C₁₇-H_a have the 1,3-diaxial relationship, and consequently that the epoxy ring orients to the *a*-side. The structure of 2 indicated by the considerations above was confirmed by X-ray analysis. Crystals of gomandonine (2) belong to a monoclinic space group, P2₁, with the cell parameters of a=10.531(3), b=8.074(4), c=11.743(2)Å, β =112.44(2)⁰, Z=2, and Dx=1.30g/cm³. The structure was solved by the direct method MULTAN and the result was refined by a block diagonal least squares procedure to R=0.072 for 1905 unique reflections with Fo>3 σ (Fo) measured on a Rigaku AFC-5 diffractometer with CuKa radiation. The ORTEP drawing of the structure of gomandonine (2) is shown in Fig. 1.



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