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THREE NEW ALKALOIDS, RYOSENAMINE,
RYOSENAMINOL, AND IBUKINAMINE FROM *ACONITUM IBUKIENSE* NAKAI

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Three new alkaloids, ryosenamine (I), ryosenaminol (II) and ibukinamine (III), were isolated from *Aconitum ibukiense* Nakai (ibuki torikabuto). The structure of ryosenamine (I) was elucidated as 1-deoxyhypognavine by spectroscopical study and determined by its correlation with ryosenaminol (II), the structure of the latter being confirmed by X-ray analysis. The structure of ibukinamine (III) was also determined by X-ray analysis.

KEYWORDS — diterpenoid alkaloid; *Aconitum ibukiense* Nakai; Ranunculaceae; ryosenamine; ryosenaminol; ibukinamine; X-ray analysis; ¹³C-NMR; CD; absolute configuration

Three new diterpenoid alkaloids, ryosenamine (I), ryosenaminol (II), and ibukinamine (III) were isolated from *Aconitum ibukiense* Nakai, which was collected at Mt. Ryosen, Shiga prefecture, Japan, in July 1981.

Ryosenamine (I), [C₂₇H₃₁NO₄, mp 213 - 215°C, [α]_D¹² +96.8° (c=0.20, MeOH)], named after Mt. Ryosen, showed the following spectral data; [IR ν_{max}^{KBr} cm⁻¹: 3450 (OH), 1710 (C=O); UV λ_{max}^{EtOH} nm (log ε): 230(4.10), 273.5(2.97), 281(2.88); ¹H-NMR (CDCl₃) δ_{ppm}^{270MHz}: 7.43 - 8.03(5H, arom. H), 5.54(1H, m, C₂-H), 5.00, 4.97(each 1H, s, =CH₂), 4.12(1H, s, C₁₅-H), 3.33(1H, br s, C₆-H), 3.31(1H, br s, C₂₀-H), 3.04(1H, d, J=13 Hz, C₁₉-Ha), 2.62(1H, d, J=13 Hz, C₁₉-Hb), 1.06(3H, s, C₁₈-H₃); MS m/z(%): 433(M⁺, 20), 416(M⁺-OH, 100), 312(M⁺-OBz, 21)].

We have deduced from the above physico-chemical data that ryosenamine (I) is a C₂₀ type diterpenoid alkaloid bearing a benzoyloxy group and a C₁₅-OH group. From the molecular formula, another OH group was thought to exist elsewhere. The deshielded chemical shift values of C₁₉-Ha and C₂₀-H are explained on the basis of the anisotropic effect by an axial α-oxygen atom at the C₂-position.¹⁾ The base peak at m/z 416 (M⁺-OH) in the MS strongly indicates that (I) has a hydroxy group at the carbon where the lone pair of the nitrogen atom participates, as shown in chart 2.

Acetylation of (I) with Ac₂O - pyridine gave mono acetate (IV), [C₂₉H₃₅NO₅,

mp 184.5 - 185°C, MS $m/z(\%)$: 475(M^+ , 26), 458($M^+ - OH$, 100), 415($M^+ - AcOH$, 38)], whose 1H -NMR spectrum shows the singlet signal of C_{15} -H shifted downfield to δ 5.50. On the other hand, the mass spectrum of (IV) still shows the ($M^+ - OH$) fragment as the base peak at m/z 458 and shows the existence of a tertiary OH group which easily cleaves, so that the OH group was assigned tentatively at the C_9 -position (Chart 2).

Fig. 1. An ORTEP Drawing of the Structure of (II)

ryosenaminol (II)

Comparison of the ^{13}C -NMR spectrum of ryosenamine (I) with that of hypognavine (V) shows that ryosenamine has no hydroxy group at C_1 , because the triplet C_1 signal of (I) appears ca. 39 ppm higher than the corresponding carbon signal of hypognavine (V). Further, both C_9 singlet signals appear at about 80 ppm, therefore a tertiary OH group was thought to exist at the C_9 -position in ryosenamine the same as in hypognavine. This structure of (I) was confirmed in connection with the structure of ryosenaminol (II), whose structure was established by X-ray analysis (*vide infra*).

Ryosenaminol (II), which crystallized in colorless prisms from MeOH, showed the following data; $[C_{20}H_{27}NO_3]$, mp 287 - 290°C(dec.), $[\alpha]_D^{29} +66.8^\circ$ ($c=0.38$, MeOH), IR $\nu_{\max}^{KBr}$ cm^{-1} : 3420(OH).

The structure of (II) was determined by X-ray analysis. Crystals of ryosenaminol (II), belong to a monoclinic space group $P2_1$, with cell constants of $a=8.990(1)$, $b=11.313(2)$, $c=7.924(1)\text{\AA}$ and $\beta=97.58(1)^\circ$. A total of 1590 unique and significant reflections ($F_o > 3\sigma(F_o)$) were measured on a 4-circle diffractometer using $CuK\alpha$ radiation ($\lambda=1.54\text{\AA}$). The structure was solved by MULTAN²⁾ and refinement by the block diagonal least squares method converged at $R=0.071$. The ORTEP drawing²⁾ of the structure of ryosenaminol (II) is shown in Fig. 1.

The hydrolysis of (I) gave rise to ryosenaminol (II). Oxidation of (I) with pyridinium dichromate gave (VI); $[C_{27}H_{29}NO_4]$, mp 275 - 278°C(dec.); IR $\nu_{\max}^{KBr}$ cm^{-1} : 1680, 1630; 1H -NMR ($CDCl_3$) δ_{ppm}^{100MHz} : 5.15(1H, s, C_{17} -Ha), 5.88(1H, s, C_{17} -Hb); MS $m/z(\%)$: 431(M^+ , 6)]. The deshielded chemical shift value of C_{17} -Hb is due to the anisotropic effect of the newly formed $C_{15}=O$ group and therefore (VI) is an α,β -

unsaturated ketone, 15-dehydroryosenamine. The formation of (VI) gave proof that the benzoyl group of (I) was attached to the C₂-position.

The CD data of (VI) are as follows; [$\lambda_{\text{ext}}^{\text{dioxane}}$ nm($\Delta\epsilon$): 235(+14.30), 335 sh(+0.41), 348(+0.57), 355.5(+0.47), 363(+0.53)]. This CD curve was very similar to that of acetylhyppognavinone (VII),³⁾ whose absolute configuration had already been revealed to be the (-)-kaurene type.

Further, in order to apply the method of Harada *et al.*,⁴⁾ 15-O-anisoylryosenamine (VIII), mp 205.5 - 208.5°C, was derived from (I) with anisoylchloride in CH₂Cl₂ and a catalytic amount of diisopropylethylamine. Spectral data of (VIII) are as follows; [high resolution MS m/z M⁺: Found 567.2658, Calcd for C₃₅H₃₇NO₆ 567.2622; CD $\lambda_{\text{ext}}^{\text{MeOH}}$ nm($\Delta\epsilon$): 256(+3.43)]. This CD spectrum shows that the two long axes of double bond and anisoyl chromophores constitute a positive exciton chirality, right handed screwness. This method also gave the (-)-kaurene type absolute configuration. The new diterpenoid alkaloid ryosenamine (I) corresponds to 1-deoxyhyppognavine.

Table 1. ¹³C-NMR Spectra of (I) and (V)

Carbon	(I)	(V)
1	29.2*	68.1
2	70.8	73.2
3	38.8	33.0
4	35.9	35.8
5	54.3	50.6
6	64.1	64.1
7	29.1*	29.0
8	44.1	44.3
9	79.3	80.3
10	50.5	54.9
11	37.2	39.2
12	35.0	34.8
13	33.6	33.5
14	42.0	42.4
15	72.5	72.4
16	155.2	154.6
17	109.6	110.0
18	29.5	29.3
19	63.7	63.5
20	74.2	71.8

a) Chemical shifts in ppm downfield from TMS; solvent CDCl₃.

b) Assignments bearing * may be interchanged.

Ibukinamine (III), [C₂₃H₃₅NO₇, mp 243 - 246°C, [α]_D¹⁹ +71.7° (c=0.12, MeOH)], named after *Aconitum ibukiense*, has following spectral data; [IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3530, 3360(OH), 1440, 1400, 1110, 1090; ¹H-NMR (d₅-pyridine) $\delta_{\text{ppm}}^{100\text{MHz}}$: 1.08(3H, t, J=7 Hz, N-CH₂CH₃), 3.14, 3.32(each 3H, s, -OCH₃), 3.90(1H, d, J=4 Hz, C₁-H), 4.38(1H, t, J=5 Hz, C₁₄-H), 4.84(1H, s, C₆-H), 6.08(2H, s-like, olefine H), 5.70, 7.30(each 1H, -OH); MS m/z(%): 437(M⁺, 96), 422(M⁺ - CH₃, 100), 406(M⁺ - OMe, 44)].

Because of the small amount of sample, the X-ray analysis of (III) was imme-

diately carried out. Ibukinamine crystallized in the orthorhombic space group $P2_12_12_1$ with $a=15.926(3)$, $b=16.088(2)$, $c=8.350(1)$ Å and $z=4$. Intensity data of 3812 unique reflections with $F_o > 3\sigma(F_o)$ within the range $3^\circ \leq 2\theta \leq 70^\circ$ were measured on the diffractometer using MoK α radiation ($\lambda=0.71$ Å). The structure was solved by the direct method MULTAN and refined anisotropically (isotropically for hydrogens) by the least squares method to $R = 0.075$. The elucidated structure of ibukinamine belongs to the lycotinine type C_{19} diterpenoid alkaloid and has a double bond between C_2 and C_3 . Recently Aiyar *et al.*⁵⁾ reported the isolation and the structure determination of delphinifoline (IX), whose structure corresponds to 2,3-dihydroibukinamine.

Fig. 2. An ORTEP Drawing of the Structure of (III)

(III) ibukinamine

(IX) 2,3-dihydroderivative:
delphinifoline

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