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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; $^{13}$C-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), [$C_{27}H_{31}NO_4$, mp 213 - 215°C, $[\alpha]_D^{12}$ +96.8°(c=0.20, MeOH)], named after Mt. Ryosen, showed the following spectral data; [IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450 (OH), 1710(C=O); UV $\lambda_{max}^{EtOH}$ nm (log ε): 230(4.10), 273.5(2.97), 281(2.88); $^1$H-NMR ($CDCl_3$) $\delta_{ppm}^{270MHz}$ : 7.43 - 8.03(5H, arom. H), 5.54(1H, m, $C_2$-H), 5.00, 4.97(each 1H, s, $=CH_2$), 4.12(1H, s, $C_{15}$-H), 3.33(1H, br s, $C_6$-H), 3.31(1H, br s, $C_{20}$-H), 3.04 (1H, d, J=13 Hz, $C_{19}$-Ha), 2.62(1H, d, J=13 Hz, $C_{19}$-Hb), 1.06(3H, s, $C_{18}$-$H_3$); 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 $C_{20}$ type diterpenoid alkaloid bearing a benzoyloxy group and a $C_{15}$- OH group. From the molecular formular, another OH group was thought to exist elsewhere. The deshielded chemical shift values of $C_{19}$-Ha and $C_{20}$-H are explained on the basis of the anisotropic effect by an axial α-oxygen atom at the $C_2$-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 $Ac_2O$ - pyridine gave mono acetate (IV), [$C_{29}H_{35}NO_5$,

mp 184.5 - 185°C, MS m/z(%): 475($M^+$, 26), 458($M^+$- OH, 100), 415($M^+$- AcOH, 38)], whose $^1$H-NMR spectrum shows the singlet signal of $C_{15}$-H shifted downfield to δ5.50. 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).

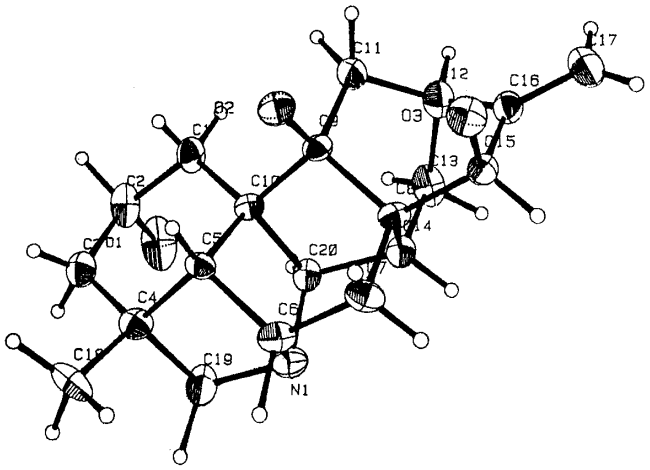
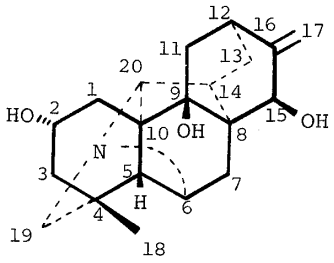



Fig. 1. An ORTEP Drawing of the Structure of (II)




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° (c=0.38, MeOH), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420(OH)].

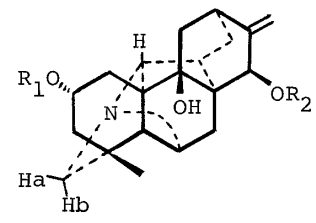
The structure of (II) was determined by X-ray analysis. Crystals of ryosenaminol (II), belong to a monoclinic space group $P2_1$, with cell constants of $a$= 8.990(1), $b$=11.313(2), $c$= 7.924(1)Å and β= 97.58(1)°.
A total of 1590 unique and significant reflections (Fo > 3σ(Fo)) were measured on a 4-circle diffractometer using CuKα radiation (λ=1.54 Å). The structure was solved by MULTAN[2] and refinement by the block diagonal least squares method converged at R = 0.071. The ORTEP drawing[2] 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; $^1$H-NMR ($CDCl_3$) $\delta_{ppm}^{100MHz}$ : 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 $C_2$-position.

The CD data of (VI) are as follows; [$\lambda_{ext}^{dioxane}$ nm($\Delta\varepsilon$): 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 acetylhypognavinone (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 $CH_2Cl_2$ and a catalytic amount of diisopropylethylamine. Spectral data of (VIII) are as follows; [high resolution MS m/z $M^+$: Found 567.2658, Calcd for $C_{35}H_{37}NO_6$ 567.2622; CD $\lambda_{ext}^{MeOH}$ nm($\Delta\varepsilon$): 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-deoxyhypognavine.



(V) hypognavine

(I) $R_1$=Bz, $R_2$=H (II) $R_1$=$R_2$=H
(IV) $R_1$=Bz, $R_2$=Ac
(VIII) $R_1$=Bz, $R_2$=-CO-C$_6$H$_4$-OCH$_3$

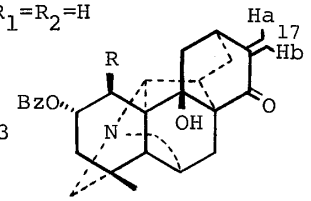
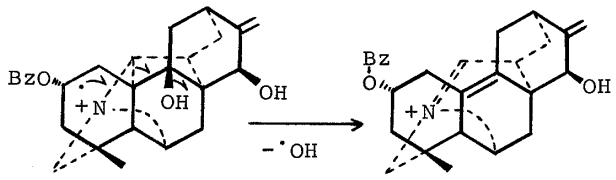


Chart 1

(VI) R=H, (VII) R=OAc



(I)

Chart 2

Table 1. $^{13}$C-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 $CDCl_3$.

b) Assignments bearing * may be interchanged.

Ibukinamine (III), [$C_{23}H_{35}NO_7$, mp 243 - 246°C, $[\alpha]_D^{19}$ +71.7°(c=0.12, MeOH)], named after *Aconitum ibukiense*, has following spectral data; [IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3530, 3360(OH), 1440, 1400, 1110, 1090; $^1$H-NMR ($d_5$-pyridine) $\delta_{ppm}^{100MHz}$: 1.08(3H, t, J=7 Hz, N-$CH_2\underline{CH_3}$), 3.14, 3.32(each 3H, s, -O$\underline{CH_3}$), 3.90(1H, d, J=4 Hz, $C_1$-H), 4.38(1H, t, J=5 Hz, $C_{14}$-H), 4.84(1H, s, $C_6$-H), 6.08(2H, s -like, olefine H), 5.70, 7.30(each 1H, -OH); MS m/z(%): 437($M^+$, 96), 422($M^+$ - $CH_3$, 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 Fo > 3σ(Fo) within the range $3° \leqq 2\theta \leqq 70°$ were measured on the diffractometer using MoKα radiation (λ=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 lycoctonine 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.

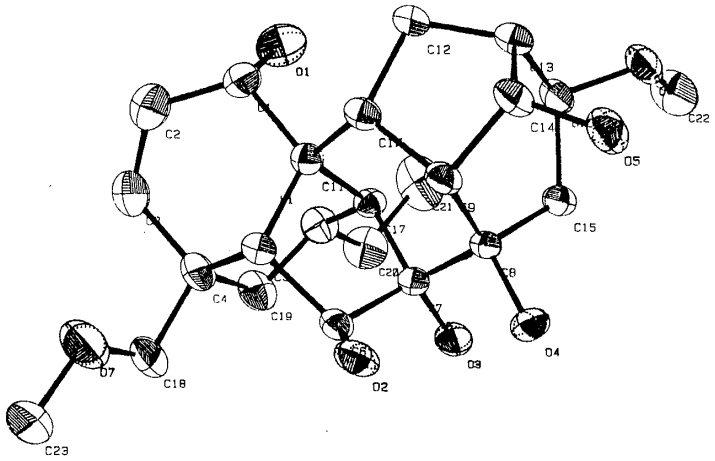
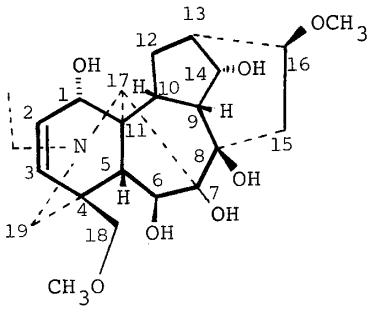



Fig. 2. An ORTEP Drawing of the Structure of (III)




(III) ibukinamine
(IX) 2,3-dihydroderivative: delphinifoline

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