Journal of Natural Products
Vol. 53, No. 4, pp. 936-939, Jul-Aug 1990

TWO NEW DITERPENE ALKALOIDS, 10-HYDROXYNEOLINE AND 14-*O*-ACETYL-10-HYDROXYNEOLINE, FROM *ACONITUM FUKUTOMEI*

HIROMITSU TAKAYAMA, MASAKI YOKOTA, NORIO AIMI, SHIN-ICHIRO SAKAI*

Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Chiba 260, Japan

SHEN-TEH LU, and IH-SHENG CHEN

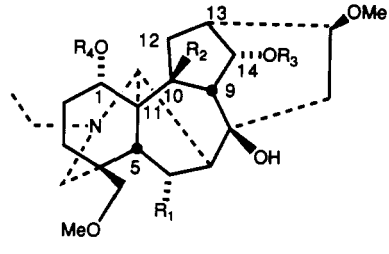
Kaohsiung Medical College, No. 100, Shih-Chuan 1st Road, Kaohsiung, Taiwan, Republic of China

ABSTRACT.—The structures of two new C_{19}-diterpene alkaloids, 10-hydroxyneoline [**1**] and 14-*O*-acetyl-10-hydroxyneoline [**2**], isolated from *Aconitum fukutomei* native to Mt. Neng-Gao in Taiwan, were determined by spectroscopic analysis and chemical reactions.

Chemical investigation of the roots of *Aconitum fukutomei* Hay. (Ranunculaceae), collected at Mt. Neng-Gao, Dai-Zhong Province in Taiwan, resulted in the isolation of two new diterpene alkaloids **1** and **2** along with seven known compounds, neoline [**3**] (1) (11% based on the crude base), 14-acetylneoline (2) (0.7%), 15-α-hydroxyneoline (3,4) (0.3%), senbusine A (5) (0.2%), isotalatizidine (6) [**5**] (0.03%), mesaconitine (1) (0.07%), and lassiocarpine (7) (0.3%). Here we describe the structural elucidation of the alkaloids **1** and **2**.

RESULTS AND DISCUSSION

The new alkaloid **1** (16.5% based on the crude base) was obtained as colorless prisms, mp 75–79° (from C_6H_6), $[\alpha]^{26}D$ +35.8° (c = 0.41, $CHCl_3$), whose high resolution mass spectrum showed the $[M]^+$ at m/z 453.2728, corresponding to the formula $C_{24}H_{39}NO_7$. The ^{1}H-nmr spectrum showed the characteristic signals of C_{19}-diterpenoid alkaloids due to N-CH_2CH_3 (δ 1.13, t, J = 7.2 Hz), OMe × 3 (δ 3.33, 3.34, and 3.35), H-6 (δ 4.14, d, J = 6.4 Hz), and H-14 (δ 4.66, dd, $J_1 = J_2$ = 5.2 Hz). The mass spectral fragmentation pattern of **1** ($[M]^+$ 13%, $[M-OH]^+$ 100%) strongly indicated the presence of a hydroxy group at the C-1 position (8,9). Treatment of **1** with Ac_2O in pyridine at room temperature afforded the diacetate **4**. In the ^{1}H-nmr spectrum of **4**, two characteristic signals were observed at δ 5.37 (dd, J_1 = 6.6, J_2 = 10.2 Hz), and δ 5.26 (dd, $J_1 = J_2$ = 5.2 Hz), which appeared at δ 4.66 and δ 4.02, respectively, in the spectrum of **1**. This indicates that two secondary hydroxy groups exist at the C-1 and C-14 positions in **1**. The ^{13}C-nmr spectrum of **1** resembled that of neoline [**3**] (10) except for a few changes (Table 1). The appearance of an extra singlet at δ 82.6



1 R_1=OMe, R_2=OH, R_3=R_4=H
2 R_1=OMe, R_2=OH, R_3=Ac, R_4=H
3 R_1=OMe, R_2=R_3=R_4=H
4 R_1=OMe, R_2=OH, R_3=R_4=Ac
5 R_1=R_2=R_3=R_4=H
6 R_1=R_3=R_4=H, R_2=OH

TABLE 1. ^{13}C-nmr Chemical Shifts[a] and Assignments for Neoline [**3**], 10-Hydroxyneoline [**1**], 14-*O*-Acetyl-10-hydroxyneoline [**2**], Isotalatizidine [**5**], and 10-Hydroxyisotalatizidine [**6**].

Carbon	Compound				
	3	**1**	**2**	**5**	**6**
C-1	72.3	69.4	69.5	72.2	69.2
C-2	29.5[b]	30.9	30.9[b]	28.7[b]	26.6
C-3	30.9[b]	29.4	29.4[b]	29.7[b]	30.8
C-4	38.2	37.9	37.8	37.2	36.9
C-5	44.9	41.0	40.8	41.6	40.5
C-6	83.3	82.7	82.9	24.9	25.0
C-7	52.3	51.8	52.4	45.1	44.7
C-8	74.3	72.6	72.9	74.3	73.4
C-9	48.3	57.8	55.7	46.6	56.1
C-10	44.3	82.6	81.8	40.1	82.3
C-11	49.6	54.2	54.5	48.6	53.3
C-12	29.8[b]	40.1	40.5	26.7	39.1
C-13	40.7	41.0	37.2	43.9	37.5
C-14	75.9	74.5	77.5	75.7	74.3
C-15	42.7	43.7	43.6	42.4	43.4
C-16	82.3	81.2	81.1	82.0	81.3
C-17	63.6	64.2	63.8	64.0	64.8
C-18	80.3	80.2	80.2	79.0	78.9
C-19	57.2	57.0	57.0	56.5	56.6
N-CH_2	48.2	48.4	48.4	48.5	48.5
CH_3	13.0	13.0	13.0	13.1	13.0
6-OMe	57.8	58.0	58.0	—	—
16-OMe	56.3	56.3	56.1	56.3	56.3
18-OMe	59.1	59.2	59.2	59.4	59.4
OCOMe	—	—	170.5	—	—
$OCOCH_3$	—	—	21.3	—	—

[a]Chemical shifts in δ downfield from TMS. Solvent $CDCl_3$.
[b]Values in the same column may be interchanged.

in the spectrum of **1**, as well as other data described above, afforded the evidence for the presence of an additional tertiary hydroxy group in **1** compared with neoline [**3**]. The signals due to C-9, C-11, and C-12 of **1** were observed downfield 9.5, 4.6, and 10.3 ppm, respectively, lower than the corresponding signals of neoline [**3**], whereas those of C-5 and C-13 were observed upfield 3.1 and 3.3 ppm, respectively, higher than the corresponding signals of neoline [**3**]. These phenomena can be interpreted by the introduction of a hydroxy group at the C-10 position in **3**. The chemical shifts at C-6, C-8, C-14, and C-16 in **1** are very close to those of **3**, so that the presence of the tertiary hydroxy group at the C-7, C-9, or C-13 position can be excluded. Furthermore, the ^{13}C-nmr spectral relationship between isotalatizidine [**5**] and 10-hydroxyisotalatizidine [**6**], whose structure was determined by X-ray analysis (11), was well compatible with that of the new alkaloid **1** and neoline [**3**] (Table 1). In order to confirm this assignment, the 2D ^{1}H-^{13}C COSY and COLOC (correlation spectroscopy via long-range coupling spectrum) (12) spectra were measured. A resonance at δ 86.2 gave the characteristic cross peaks with H-1 (δ 4.02, br s), H-17 (δ 2.53 br s), H-9 (δ 2.06, d, J = 5.2 Hz), and H-12 (δ 2.30, d, J = 15.1 Hz). These results established the placement of the extra tertiary hydroxy group at the C-10 position. Therefore, the structure of the new alkaloid **1** was concluded to be 10-hydroxyneoline. All the attempts at the selective deoxygenation of the tertiary hydroxy group (13,14) at the C-10 position in **1** in order to correlate with neoline [**3**] were unsuccessful.

The second new alkaloid **2** (0.3% based on the crude base) was isolated as an amorphous powder, $[\alpha]^{23}D$ +37.1° (c = 0.52, $CHCl_3$). The high resolution mass spectrum of **2** showed the molecular ion m/z 495.2863, corresponding to the formula $C_{26}H_{41}NO_8$. This is 42 amu (MeCO) higher than the corresponding peak in **1**. The intensive peak at 1740 cm^{-1} in the ir spectrum of **2** showed the presence of an ester group. From the fragmentation pattern in the mass spectrum [m/z $[M]^+$ 495 (9%), $[M-OH]^+$ 478 (100%)] and the downfield shift of H-14 (δ 5.29, t, J = 4.9 Hz) in the ^{1}H-nmr spectrum, we assigned the structure of **2** as 14-*O*-acetyl-10-hydroxyneoline. ^{13}C-nmr spectral data strongly supported this conclusion. Thus, the signals at C-9 and C-13, β to C-14 (77.5 ppm), were observed upfield 2.0 and 3.8 ppm, respectively, higher than the corresponding signals of 10-hydroxyneoline [**1**] by the introduction of an acetyl group on the C-14 hydroxy group. Finally, the structure of **2** was proved by the regioselective acetylation (2) of **1**. Compound **1** was treated with trifluoroacetic acid in HOAc at 80–90° for 6 h to give the 14-acetyl derivative in 62% yield, which was identical with natural **2** in all respects (co-tlc, ir, ms, and ^{1}H-nmr spectra).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Yamato MP-21 apparatus and are uncorrected. Ir spectra were measured with a Hitachi 260 spectrometer. ^{1}H-nmr spectra were recorded on JEOL GX-270 (270 MHz) and JEOL GSX-500 (500 MHz) spectrometers with TMS as an internal standard in $CDCl_3$. ^{13}C-nmr spectra were measured with JEOL GX-270 (67.8 MHz) and JEOL GSX-500 (125 MHz) spectrometers in $CDCl_3$. Mass spectra were taken with Hitachi RMU-6E and RMU-7M spectrometers. Tlc was performed on Merck precoated Si gel 60F-254 and aluminium oxide 60F-254 plates. Cc utilized Merck Si gel 60 (70–230 and 230–400 mesh) and Merck Al_2O_3 (activity II–III).

EXTRACTION AND SEPARATION OF ALKALOIDAL FRACTION.—The plant material was collected and identified by the authors (S.L. and I.C.); a voucher specimen is stored in Kaohsiung Medical College. The dried powdered roots (1.16 kg) of the plant were extracted with 3% aqueous HCl solution (3 × 4 liters) for 2 weeks. The combined extracts were basified with solid Na_2CO_3 at 0° and extracted with 5% MeOH/$CHCl_3$ three times. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure to give the crude base (12.813 g) (1.1% based on dry roots). The portion of the alkaloidal fraction (6.09 g) was roughly separated with Al_2O_3 (220 g) cc and purified with Si gel cc and/or preparative tlc as described below.

ISOLATION OF 10-HYDROXYNEOLINE [**1**].—The 10% and 20% MeOH/EtOAc eluent from the first Al_2O_3 chromatography column was subjected to the Si gel cc, and from the 10% MeOH/$CHCl_3$ fraction colorless prisms of 10-hydroxyneoline [**1**] (1004 mg, 16.5% based on the crude base) were obtained: mp 75–79° (from C_6H_6); $[\alpha]^{26}D$ +35.8° (c = 0.41, $CHCl_3$); hrms calcd for $C_{24}H_{39}NO_7$ m/z $[M]^+$ 453.2728, found m/z $[M]^+$ 453.2728; ir (KBr) 3380 and 1100 cm^{-1}; ^{1}H-nmr (500 MHz) δ 7.51 (1H, br d, J = 8.8 Hz, 1α-OH), 4.66 (1H, dd, $J_1 = J_2$ = 5.2 Hz, H-14β), 4.14 (1H, d, J = 6.4 Hz, H-6β), 4.02 (1H, br s, H-1β), 3.68 and 3.26 (1H each, d, J = 8.0 Hz, H_2-18), 3.35, 3.34, and 3.33 (3H each, s, OMe), 3.01 (1H, br s, OH), 2.72 and 2.32 (each 1H, d, J = 10.5 Hz, H_2-19), 2.53 (1H, br s, H-17), 2.42 (1H, d, J = 6.1 Hz, H-5), 2.35 (1H, d, J_1 = 9.1, J_2 = 15.7 Hz, H-15), 2.30 (1H, d, J = 15.1 Hz, H-12), 2.10 (1H, dd, J_1 = 6.1, J_2 = 15.7 Hz, H-15), 2.06 (1H, d, J = 5.2 Hz, H-9), 2.02 (1H, br s, H-7), 1.13 (3H, t, J = 7.2 Hz, N-CH_2CH_3); eims m/z (%) $[M]^+$ 453 (16), $[M-OH]^+$ 436 (100); ^{13}C nmr (125 MHz) see Table 1.

ACETYLATION OF 10-HYDROXYNEOLINE.—A mixture of **1** (51 mg), Ac_2O (0.4 ml), and dry pyridine (0.6 ml) was stirred at room temperature for 25 h. After removal of the solvent, 5% $NaHCO_3$ solution was added to the residue, which was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give a residue, which was purified by preparative tlc (developed with 5% MeOH/$CHCl_3$) to yield 50 mg (83%) of the diacetyl derivative **4** as an amorphous powder: ir ($CHCl_3$) 3560, 1740, 1725, 1230, 1095 cm^{-1}; ^{1}H nmr (270 MHz) δ 5.37 (1H, dd, J_1 = 10.2, J_2 = 6.6 Hz, H-1β), 5.26 (1H, dd, $J_1 = J_2$ = 5.2 Hz, H-14β), 4.11 (1H, d, J = 6.6 Hz, H-6β), 3.34, 3.30, and 3.23 (3H each, s, OMe), 2.06 and 2.04 (3H each, s, OAc), 1.11 (3H, t, J = 7.2 Hz, N-CH_2CH_3); eims m/z (%) $[M]^+$ 537 (0.3), $[M-OAc]^+$ 478 (100).

ISOLATION OF 14-*O*-ACETYL-10-HYDROXYNEOLINE [**2**].—The EtOAc eluent from the first Al_2O_3 cc was purified with Si gel cc using 10% MeOH/$CHCl_3$ as an eluent followed by Si gel cc (5% MeOH/

$CHCl_3$ saturated with aqueous NH_3) to give colorless, amorphous **2** (20 mg, 0.3% based on the crude base): $[\alpha]^{23}D$ +37.1° (c = 0.52, $CHCl_3$); hrms calcd for $C_{26}H_{41}NO_8$, *m/z* $[M]^+$ 495.2896, found *m/z* 495.2863; ir ($CHCl_3$) 3590, 1740, 1220, 1100 cm^{-1}; ^1H nmr (270 MHz) δ 5.29 (1H, dd, $J_1 = J_2 = 4.9$ Hz, H-14β), 4.10 (1H, d, J = 6.6 Hz, H-6β), 4.06 (1H, br s, H-1β), 3.35, 3.33, and 3.25 (3H each, s, OMe), 2.07 (3H, s, OAc), 1.13 (3H, t, J = 7.3 Hz, N-CH_2CH_3); eims *m/z* (%) $[M]^+$ 495 (9), $[M - OH]^+$ 478 (100); ^{13}C nmr (67.8 MHz) see Table 1.

PREPARATION OF 14-*O*-ACETYL-10-HYDROXYNEOLINE [**2**] FROM **1**.—To a solution of **1** (100 mg) in glacial HOAc (2 ml), trifluoroacetic acid (0.3 ml) was added, and the mixture was stirred at 80–90° for 6 h. The reaction mixture was concentrated under reduced pressure, and aqueous NH_3 solution was added to the residue. The whole was extracted with $CHCl_3$ three times, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was subjected to flash cc using 5% MeOH/$CHCl_3$ as an eluent to yield 68 mg (62%) of **2**, which was identical with natural **2** by the comparison of tlc (5% MeOH/$CHCl_3$ saturated with NH_4OH), ir ($CHCl_3$), eims, and ^1H nmr (270 MHz) spectra.

ACKNOWLEDGMENTS

We thank Mrs. H. Seki, Miss R. Hara, and Mr. T. Kuramochi in the Analytical Center of Chiba University for measurements of spectral data.

LITERATURE CITED

1. S.W. Pelletier and Z. Djarmati, *J. Am. Chem. Soc.*, **98**, 2626 (1976).
2. H. Takayama, A. Tokita, M. Ito, S. Sakai, F. Kurosaki, and T. Okamoto, *Yakugaku Zasshi*, **102**, 245 (1982).
3. H. Takayama, S. Hasegawa, S. Sakai, J. Haginiwa, and T. Okamoto, *Chem. Pharm. Bull.*, **29**, 3078 (1981).
4. S.W. Pelletier, N.V. Mody, K.I. Varughese, and C. Szu-Ying, *Heterocycles*, **18**, 47 (1982).
5. C. Konno, M. Shirasaka, and H. Hikino, *J. Nat. Prod.*, **45**, 128 (1982).
6. S.W. Pelletier, L.H. Keith, and P.C. Parthasarathy, *J. Am. Chem. Soc.*, **89**, 4146 (1967).
7. H. Takayama, J. Sun, N. Aimi, S. Sakai, S. Lu, and I. Chen, *Tetrahedron Lett.*, **30**, 3441 (1989).
8. O.E. Edwards, in: "The Alkaloids." Ed. by J.E. Saxton, The Chemical Society, London, 1971, Vol. 1, p. 369.
9. S.W. Pelletier and S.W. Page, in: "The Alkaloids." Ed. by J.E. Saxton, The Chemical Society, London, 1973, Vol. 3, p. 235.
10. H. Bando, K. Wada, T. Amiya, Y. Fujimoto, and K. Kobayashi, *Heterocycles*, **27**, 2167 (1988).
11. H. Takayama, Y. Hitotsuyanagi, K. Yamaguchi, N. Aimi, and S. Sakai, *Chem. Pharm. Bull.*, **37**, 548 (1989).
12. H. Kessler, C. Griesinger, J. Zarbock, and H.R. Looshi, *J. Magn. Reson.*, **57**, 331 (1984).
13. S.W. Pelletier, J.A. Glinski, B.S. Joshi, and S.-Y. Chen, *Heterocycles*, **20**, 1347 (1983).
14. P. Kulanthaivel and S.W. Pelletier, *Tetrahedron Lett.*, **28**, 3883 (1987).

Received 9 February 1990