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REASSIGNMENT OF THE STRUCTURE OF TUGUACONITINE FROM *ACONITUM SIBIRICUM*

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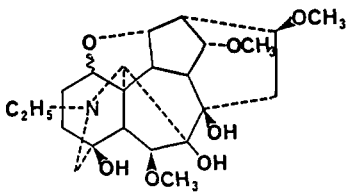
ABSTRACT.—Structure **1** previously had been assigned incorrectly to tuguaconitine, a C_{18}-diterpenoid alkaloid from *Aconitum sibiricum*. Structure **2** has been assigned to tuguaconitine on the basis of its ^{13}C-nmr spectral data and that of the 1-dehydro-oxidation product.

Tuguaconitine ($C_{23}H_{35}NO_7$) isolated from the roots of *Aconitum sibiricum* Poir (Ranunculaceae), collected at "Tae-Hwa," Kangwon-Do province, Korea, has recently been assigned structure **1** on the basis of mass, ^{1}H-nmr, and ^{13}C-nmr data (1). Subsequent investigations from our laboratories demonstrate that the correct structure for tuguaconitine is **2**.

Tuguaconitine has mp 197-199°; hrms M^+ 437.24241, $C_{23}H_{35}NO_7$ (calcd, 437.24229); ms (70 ev) *m/z* M^+ 437 (83%), 422 (100%), 406 (40%), 394 (20%), 311 (7%), 268 (32%), 222 (8%), 130 (3%), 114 (12%), and 58 (38%). The ^{1}H-nmr spectrum ($CDCl_3$) gave signals at δ 1.08 (3H, t, J=7 Hz, N-CH_2-CH_3), 3.36, 3.41, and 3.42 (each 3H, s, OCH_3).

Structure **2** was deduced from a reinterpretation of spectral data and by comparison of the ^{13}C-nmr spectra of tuguaconitine with those of monticoline (**4**) and monticamine (**5**), two C_{18}-diterpenoid alkaloids bearing a C(3)-C(4) epoxy bridge (2,3). Accurate carbon-13 measurements, involving a DEPT (distortionless enhancement by polarization transfer) experiment assisted greatly in assigning structure **2**.

The DEPT ^{13}C-nmr spectrum ($CDCl_3$) of tuguaconitine exhibited 23 signals due to 23 carbon atoms in the molecule. These have been sorted into: five signals for CH_2 carbons at 30.7: C(12), 31.5: C(2), 33.3: C(15), 49.9: (NCH_2-) and 54.2: C(19) ppm; ten signals for CH carbons, of which four are oxygenated and are represented by the down-



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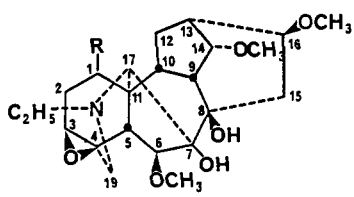
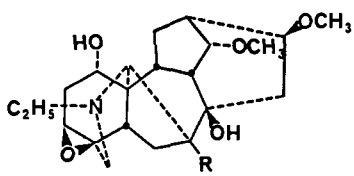


2 R=αOH tuguaconitine
3 R=O



4 R=OH monticoline
5 R=H monticamine

field signals at 77.9, 82.7, 84.2, and 90.2 ppm; four signals for quaternary carbons at 53.9, 58.6, 78.5, and 89.4 ppm. The two downfield signals at 78.5 and 89.4 ppm are attributed to the two oxygenated quaternary carbons C(8) and C(7), respectively, while the upfield signal at 53.9 ppm is assigned to the non-oxygenated quaternary C(11).

The seventh oxygen atom in the molecule forms an epoxy bridge between C(3) and C(4) as indicated by the two signals at 58.5 and 58.6 ppm for the methine at C(3) and the quaternary C(4) position, respectively. Four signals for CH_3 carbons appear at 13.9, 56.3, 57.7, and 58.8 ppm. The upfield signal at 13.9 ppm corresponds to the methyl carbon of N-CH_2CH_3, while the three downfield signals at 56.3, 57.7, and 58.8 ppm are attributed to three methoxyl carbons. Thus, the DEPT experiment distinguished the three methoxy signals from the signals at 58.5 (methine) and 58.6 (quaternary), both C(3) and C(4) carrying the epoxy group. The signal at 56.3 ppm indicates the presence of C(16)-OCH_3, since the C(16)-methoxy carbon occurs at $\sim$ 56 ppm in all C_{18}- and C_{19}-diterpenoid alkaloids, except those carrying an oxygen substituent at C(13) or C(15) (4). At the same time, the methine signal at 82.7 ppm is attributed to C(16).

The signal at 57.7 ppm is assigned to the C(14)-methoxyl carbon, and the methine signal at 84.2 ppm is attributed to C(14) as these are the characteristic positions for C(14)-OCH_3 carbons in all lycoctonine-type diterpene alkaloids with C(14)-bearing a methoxyl function.

The assignment of the remaining methoxyl signal at 58.8 ppm to C(6) relies on the presence of the methine signal at 90.2 ppm which corresponds to methoxylated C(6) in all lycoctonine alkaloids having positions 6, 7, and 8 carrying oxygen substituents (3).

Besides the epoxy and three methoxyl groups, the other three oxygen atoms in the

TABLE 1. Carbon-13 nmr Data of Tuguaconitine (**2**), **3**, Monticoline (**4**) (2,3), and Monticamine (**5**) (2,3)

Carbon	Compounds			
	2	**3**	**4**	**5**
1	77.9	203.7	77.3	77.0
2	31.5	38.3	31.8	32.3
3	58.5	58.7	58.3	57.7
4	58.6	58.6	59.5	58.7
5	43.2	43.7	45.7	46.5
6	90.2	90.3	34.2	25.9
7	89.4	89.6	86.6	45.5
8	78.5	78.6	76.9	74.4
9	48.7	50.2	46.6	45.3
10	37.9	38.2	37.1	37.2
11	53.9	53.6	54.4	53.6
12	30.7	29.7	30.5	30.6
13	42.6	41.8	42.0	42.3
14	84.2	84.2	84.6	84.6
15	33.3	33.5	36.0	42.8
16	82.7	82.8	82.5	82.6
17	67.0	66.5	65.7	64.5
18	—	—	—	—
19	54.2	54.1	53.2	57.7
N-CH_2	49.9	50.2	50.0	47.6
CH_3	13.9	14.2	14.1	13.3
'6	58.8	59.7	—	—
'14	57.7	57.8	57.6	57.6
'16	56.3	56.3	56.2	56.1

molecule occur as three hydroxyls, two of which are attached to the quaternary C(7) and C(8) (89.4 and 78.5 ppm, respectively), and the third OH group is attached to the remaining oxygenated methine carbon resonating at 77.9 ppm and assigned to C(1).

The assignment (1) of an ether linkage between C(1) and C(12) in the previously proposed structure (**1**) for tuguaconitine, and the consequent assignment of the signal at 77.9 ppm to C(1) is in error, because, if C(1) were involved in an ether linkage with C(12) or possibly with C(19), then C(1) would resonate at 84-89 ppm (5). Secondly, the signal at 58.7 ppm was assigned to C(4)-OH (structure **1**), an error because C(4) bearing a hydroxyl group usually resonates at 70-71 ppm (3).

Oxidation of tuguaconitine (**2**) using CrO_3-pyridine-H_2O gave ketone **3** as the main product. The ^{13}C-nmr spectrum of **3** exhibited the same pattern of signals as that of tuguaconitine (**2**), except for the appearance of a signal at 203.7 ppm for the CO carbon instead of a signal at 77.9 ppm, assigned to C(1) in the ^{13}C-nmr spectrum of tuguaconitine. The ^{13}C-nmr spectrum of **3**, also exhibited a signal at 38.3 ppm instead of the signal at 31.5 ppm assigned to C(2) in the ^{13}C-nmr spectrum of **2**. This downfield shift is produced by the presence of a carbonyl group at C(1).

The ir spectrum of **3** showed an absorption for a carbonyl group at 1720 cm^{-1} (CO in a six-membered ring). This result rules out the presence of a carbonyl group at C(14) in **3**, since the cyclopentanone carbonyl in such alkaloids absorbs at ~1760 cm^{-1} (6). This result also rules out the presence of a hydroxyl group at C(14) in tuguaconitine (**2**) instead of the methoxyl function.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected and were taken on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Spectra were recorded on the following instruments: ir Varian model 1420 spectrophotometer; ^{1}H-nmr on a Perkin-Elmer EM390, 90 MHz spectrometer; ^{13}C-nmr on a JEOL model FX-270 spectrometer.

ISOLATION OF TUGUACONITINE.—The voucher specimen of *A. sibiricm* is stored in the Department of Botany, College of Natural Science, Seoul National University, Korea. Professor Young Ho Chung of that department identified the plant. The dried roots (1.5 kg) of the plant were extracted with MeOH (3×5 liters, for 24 h at room temperature). The MeOH solution was concentrated to 1 liter below 40° and treated with 1 N HCl to pH 3.0. The acidic liquor was washed with 3×500 ml portions of hexane, and the aqueous layer was treated with NH_4OH to pH 11.0. This mixture was extracted with $CHCl_3$ (3×500 ml), and the $CHCl_3$ extract was dried over anhydrous Na_2SO_4; evaporation in vacuum gave 35 gm of a residue. The latter was chromatographed over a column of silica gel (70-230 mesh, Merck, 500 gm) using $CHCl_3$-Me_2CO (4:1) as an eluent, and 12 fractions were collected. Fraction 8 (2.5 gm) was rechromatographed over silica gel (100 gm) using $CHCl_3$-MeCN (1:1) as eluent. Tuguaconitine (120 mg) was obtained from the 5th fraction and crystallized from EtOAc/Et_2O mixture in colorless cubic crystals, mp 197-199°.

1-KETOTUGUACONITINE.—Tuguaconitine (**2**, 22 mg) in pyridine (0.5 ml) was added while cooling (ice bath) to Cornforth reagent prepared by adding CrO_3 (18 mg) in H_2O (0.2 ml) while cooling (ice bath) to pyridine (0.5 ml). The mixture was left in an ice bath for 2 h and then at room temperature overnight. Solvent was removed from the dark-brown mixture under reduced pressure. The residue was extracted with 3×50 ml of $CHCl_3$. The product in 20 ml of CH_2Cl_2 was chromatographed by vacuum liquid chromatography (7) over 4 g of neutral alumina (activity III) using CH_2Cl_2/MeOH mixtures. The main fraction was evaporated under vacuum to give 14 mg of colorless residue of **3**. The ir spectrum (nujol) showed absorption at 1720 cm^{-1}; ^{13}C-nmr spectrum (Table 1).

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