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CORRECTION OF THE SPECTROSCOPIC DATA OF HOKBUSINE A: CONFIRMATION OF THE C-8 METHOXYL GROUP

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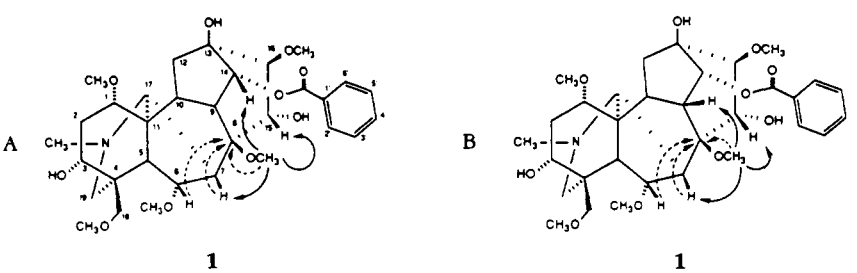
We recently isolated a C_{19}-diterpenic alkaloid **1** from the tubers of *Aconitum carmichaeli* Debx. (Ranunculaceae) used in the Chinese traditional medicines, "Fu Zi" and "Chuan Wu" (1). This structure, however, was previously assigned to hokbusine A, also reported from *A. carmichaeli* (2) and *Aconitum napellus* (3); yet significant differences existed in the reported chemical shifts and multiplicities of the ^{13}C-nmr signals. Because evidence for the original structure assignment did not rule out the C-13 methoxy,C-8 hydroxy alternative, it was conceivable that hokbusine A may have this isomeric structure. Comparison with an authentic sample revealed that hokbusine A and **1** are the same compound, thus requiring a correction of the ^{13}C-nmr data for hokbusine A (Table 1).

In order to assign structure **1**, it was thus necessary to confirm the location of the C-8 methoxyl group. The ^{13}C-nmr spectrum of **1** revealed a quartet (DEPT) at δ 50.23 (CD_3OD, δ 49.95 in $CDCl_3$) that correlated with the up-field methoxyl singlet (δ 3.15 in $CDCl_3$) in the HETCOR spectrum. This high-field methoxy methyl signal was not reported in the ^{13}C-resonances of hokbusine A (CD_3OD); the C-8 methoxyl carbon was assigned δ 59.0 (^{1}H nmr δ 3.13). Molecular models reveal that a C-8 methoxyl group would be located in a restricted molecular pocket with the methyl group placed in the shielding cone of the aromatic ring of the 14α-benzoate ester, thus accounting for the high-field frequencies of both the ^{1}H and ^{13}C resonances of this methoxy methyl group. In addition, the ^{13}C-T_1 relaxation time of this methoxy methyl resonance was relatively rapid (Table 1), supporting the suggestion of significant steric hindrance resulting in a slower τ_c.

Saturation of the δ 3.15 methoxy methyl resonance resulted in enhancements of the H-7, H-14, H-15, and the 2′,6′-*o*-protons of the benzoate group in the ^{1}H spectrum. In addition, an enhancement was also observed at 2.59, but this signal could not be unambiguously assigned to H-9 because three signals, H-9, H-19, and H-19′, all overlap at this region of the spectrum. A heteronuclear nOe to the singlet, δ 82.05, assigned to C-8 was also observed



1 **1**

FIGURE 1. Coupling interactions used to identify C-8 methoxyl group. Solid lines represent homonuclear (^{1}H-^{1}H) interactions; dashed lines represent heteronuclear (^{1}-^{13}C) interactions. (A) Dipolar couplings (nOe's); (B) long-range scalar couplings.

TABLE 1. ^{13}C-nmr Chemical Shifts (93.93 kG, 100 MHz).[a]

Carbon	Reported for **1**[b] CD_3OD	**1**[c] CD_3OD	**1** $CDCl_3$ (T_1, s)
C-1	83.9 d	83.22 d	82.34 d
C-2	35.8 t	30.80 t	33.10 t
C-3	69.9 d	70.20 d	71.06 d
C-4	44.7 s	44.90 s	42.39 s
C-5	46.7 d	45.3 d[d]	44.95 d
C-6	84.7 d	84.34 d	82.82 d
C-7	43.1 d	43.61 d	41.86 d
C-8	83.7 s	83.96 s	82.05 s
C-9	49.9 d	46.45 d	44.95 d
C-10	42.4 d	42.27 d	41.16 d
C-11	51.3 s	51.77 s	50.50 s
C-12	38.2 t	38.11 t	35.92 t
C-13	74.2 s	76.37 s	76.58 s
C-14	80.9 d	81.07 d	79.24 d
C-15	77.6 d	77.56 d	77.34 d
C-16	95.3 d	95.66 d	93.11 d
C-17	63.2 d	65.0 d[d]	63.20 d
C-18	76.1 t	78.12 t	76.99 t
C-19	50.3 t	51.2 t[d]	50.13 t
NCH_3	42.7 q	42.58 q	42.39 q
1-OMe	56.3 q	56.17 q	56.18 q (1.01)
6-OMe	59.0 q	59.24 q	58.62 q (1.20)
8-OMe	59.0 q	50.23 q	49.95 q (0.40)
16-OMe	62.2 q	62.55 q	62.25 q (0.80)
18-OMe	59.0 q	59.24 q	59.13 q (1.20)
COC_6H_5	167.8 s	167.99 s	166.25 s
C-1′	131.4 s	131.65 s	130.04 s
C-2′,6′	130.6 d	130.93 d	129.69 d
C-3′,5′	129.2 d	129.56 d	128.37 d
C-4′	133.9 d	134.28 d	132.93 d

[a]Multiplicities were assigned with DEPT and APT experiments. Signals for C-13, C-15, and C-18, overlapped by $CDCl_3$ solvent peak in the broad band proton decoupled spectrum, were revealed in the DEPT experiment. Assignments were confirmed using HETCOR and long-range HETCOR (4–6).

[b]From Hikino *et al.* (2).

[c]For direct comparison with hokbusine A, the ^{13}C-nmr spectrum of **1** was run in CD_3OD. Due to severe line broadening of some signals, CD_3OD was not a particularly suitable solvent, and $CDCl_3$ was subsequently used.

[d]Resonance was not observed at 24° due to severe broadening signal. Chemical shift is reported for the spectrum recorded at 45°.

upon saturation of the ^{1}H 3.15 singlet. Confirmation of the assignment of the δ 82.05 singlet to C-8 was obtained via heteronuclear nOe's (7) observed upon saturation of the H-6 (4.05, d, J = 6 Hz) and H-7 (2.80, br s) resonances (Figure 1A).

The locations of the other four methoxyl groups were confirmed by three-bond heteronuclear couplings (long-range HETCOR) between methoxy methyl protons and the corresponding carbinol carbons. The structural conclusions drawn from the heteronuclear nOe's were confirmed by selective INEPT (8) and long-range homonuclear COSY experiments (Figure 1B).

The original misinterpretation of the ^{13}C-nmr spectrum of **1** may have been due to severe line broadening of the signals of carbons C-1, C-2, C-5, C-17, and C-19, which existed in the ^{13}C-nmr spectrum of **1** in CD_3OD at 24°, suggesting relatively slow conforma-

tional changes in the "left-hand" portion of the molecule on the nmr time scale. Thus, the C-2 triplet in **1** appeared at 30.80 in CD_3OD but was reported to be δ 35.8. At 45°, these resonances sharpened considerably.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected, nmr spectra (Varian XL-400, 93.93 kG: ^1H, 400 MHz, ^{13}C, 100 MHz) $CDCl_3$ unless otherwise stated, residual $CHCl_3$ signal (δ=7.25 in ^1H-nmr spectra, δ=77.00 in ^{13}C-nmr spectra) as internal standard, T_1's measured by inversion-recovery, ir (Perkin-Elmer 577) KBr; ms (Finnegan MAT 8200).

EXTRACTION AND ISOLATION.—"Fu-Zi" (7.5 kg, dry wt) from the Jian-You region of Si Chuan Province, China (voucher specimen deposited in the herbarium of the Second Military Medical College, Shanghai), was mechanically pulverized, moistened with 5% Na_2CO_3 solution, and periodically shaken for 3 days with C_6H_6. The C_6H_6 layer was recovered by filtration and the extraction repeated two more times. The combined C_6H_6 extracts were thoroughly extracted with 1% aqueous HCl, and the aqueous layer subsequently adjusted to pH 13 with NaOH. Crystalline material precipitated and was removed by filtration (hypaconitine, aconitine, and mesaconitine). The aqueous filtrate was extracted with $CHCl_3$, and the $CHCl_3$ layer subsequently was washed with H_2O, dried with Na_2SO_4, and the $CHCl_3$ evaporated under reduced pressure. The residue (16 g) was chromatographed on basic alumina (400 g), eluting first with petroleum ether (bp 35–60°), then with Et_2O. The Et_2O was evaporated under reduced pressure to give a residue (1.92 g) that was subsequently triturated with Et_2O and the Et_2O-soluble material obtained by filtration. The Et_2O was removed from the filtrate under reduced pressure and chromatographed on basic alumina (200 g), eluting with Et_2O to yield **1** (50 mg), along with karakoline and beiwutine.

HOKBUSINE A [**1**].—Mp 205–207°; $[\alpha]^{25}D$ −16.8° (*c*=0.500, EtOH); hrms *m/z*: Found 603.3304 $[M]^+$ ($C_{32}H_{45}NO_{10}$ requires 603.3044); eims *m/z* (% rel. int.) $[M]^+$ 603 (2.9), 572 (100), 540 (3.2), 105 (70); ir ν cm^{-1} 3400 (OH), 1710, 1280 (ester), 1110 (methyl ether); uv λ max (EtOH) nm 230; ^1H nmr δ 1.98 (1H, m, H-2_A), 2.05–2.20 (4H, overlapped m, H-5, H-10, H-12_A, H-12_B), 2.35 (1H, m, H-2_B), 2.52 (3H, br s, H-Me), 2.57 (2H, br m, H-19_A, H-19_B), 2.59 (1H, br d, H-9), 2.80 (1H, br s, H-7), 2.93 (1H, br d, H-17), 3.15 (3H, s, 8-OMe), 3.19 (1H, br t, *J*=6 Hz, H-1), 3.29 (3H, s, 6-OMe), 3.31 (6H, s, 1-OMe, 18-OMe), 3.31 (1H, overlapped, H-16), 3.54 (1H, d, *J*=9 Hz, H-18_A), 3.60 (1H, d, *J*=9 Hz, H-18_B), 3.75 (3H, s, 16-OMe), 3.88 (1H, m, H-3), 4.05 (1H, d, *J*=6 Hz, H-6), 4.56 (1H, d, *J*=5.9 Hz, H-15), 4.83 (1H, d, *J*=5.1 Hz, H-14), 7.44 (2H, t, *J*=8 Hz, H-3′, H-5′), 7.53 (1H, t, *J*=8 Hz, H-4′), 8.02 (2H, d, *J*= Hz, H-2′, H-6′); in addition, two hydroxyl protons, 3.05 (1H, br, OH), 3.7 (1H, br, OH), were observed. The third hydroxyl proton was overlapped by the methoxyl signals. For ^{13}C nmr see Table 1.

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