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J. Nat. Prod., **1991**, 54 (2), 525-531• DOI:
10.1021/np50074a027 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 2, pp. 525-531, Mar-Apr 1991

TWO NEW DITERPENOID ALKALOIDS FROM *ACONITUM PALMATUM*

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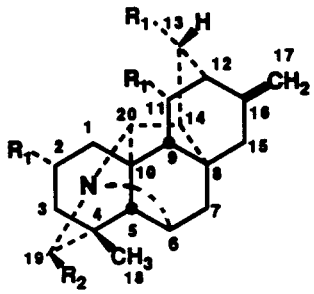
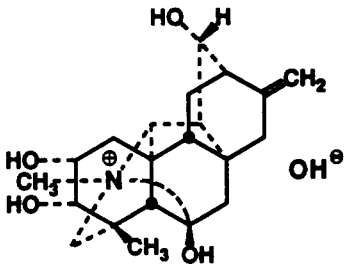
ABSTRACT.—Two new diterpenoid alkaloids, vakhmatine [**1**] and vakhmadine [**2**], have been isolated from *Aconitum palmatum*, together with the known alkaloids atisine and hetisine. The structures of **1** and **2** were established by 2D nmr and nOe studies of their acetyl derivatives **4** and **8**.

The roots of *Aconitum palmatum* Don. (Ranunculaceae) have been used as a folk medicine ("Vakhma") in India for many years. Singh and Singh reported in 1965, the isolation of five diterpenoid alkaloids: vakognavine, palmasine, vakatisine, vakatisinine, and vakatidine (1). In 1971, we established the structure of vakognavine by a single-crystal X-ray analysis of vakognavine hydriodide (2). This was the first example reported of an *N*, 19-seco diterpenoid alkaloid, an interesting compound for biogenetic speculation (2). Several reports of early studies were related to vakognavine and vakatisine (3–5), but the structures of the remaining alkaloids have not been elucidated.

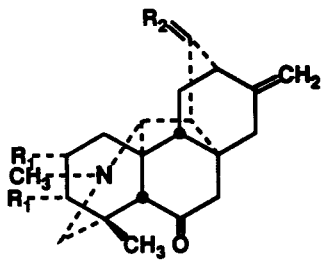
Our recent examination of the alkaloidal constituents of *A. palmatum* disclosed four new and four known diterpenoid alkaloids (6). Except for vakognavine, none of these alkaloids can be assigned to any one of the earlier reported alkaloids on the basis of the limited data provided in the early studies. We now wish to report the isolation and structural elucidation from the same plant of two new diterpenoid alkaloids, designated as vakhmatine [**1**] and vakhmadine [**2**], as well as the known alkaloids, atisine and hetisine.



1 $R_1=R_2=OH$ (vakhmatine)
3 $R_1=OH$, $R_2=H$
4 $R_1=R_2=OAc$
5 $R_1=OAc$, $R_2=OH$
6 $R_1=OAc$, $R_2=H$



2 (vakhmadine)



7 $R_1=OH$, $R_2=\alpha$–OH, β–H
8 $R_1=OAc$, $R_2=\alpha$–OAc, β–H
9 $R_1=OAc$, $R_2=O$

Because there is substantial confusion in the literature in the manner in which the configuration of the C-13 hydroxyl in hetisine derivatives is represented [both α and β] (7–12), the convention used in this paper is defined as follows: the boat containing both C-11 and C-13 and formed by carbon atoms 8, 9, 11, 12, 13, and 14 is selected as the reference ring. The hydroxyls at C-11 and C-13 are then both α. This representation of the configuration for the ψ-axial C-13 hydroxyl as α is opposite to that shown in a series of our earlier papers (6, 9–14).

RESULTS AND DISCUSSION

The EtOH extract of the roots of *A. palmatum* was fractionated by a routine gradient pH extraction. The pH-14 aqueous solution, after extraction with CH_2Cl_2, was continuously extracted with $CHCl_3$ for 4 days to afford an H_2O-soluble base fraction. Further separation of this fraction yielded the H_2O-soluble alkaloids, vakhmatine [**1**] and vakhmadine [**2**].

Vakhmatine [**1**], $C_{20}H_{27}NO_4$, $[M]^+$ 345, $[\alpha]^{24}D$ +12.6° (c = 0.2, MeOH), has an mp 170.5–174.5° (MeOH). The molecular formula was derived from the high resolution mass spectrum of its tetraacetyl derivative **4** given below. Four oxygens in the molecule indicated the presence of four hydroxyl groups because of the absence of any carbonyl absorption in the ir and ^{13}C-nmr spectra. Comparison of the ^{13}C-nmr spectra (Table 1) of **1** and the known alkaloid hetisine [**3**] suggested that this diterpenoid alkaloid was closely related to hetisine because both compounds showed similar δ_C values for C-7–C-17. The existence of a doublet at δ_C 95.5, but absence of a triplet usually at δ_C 65–70 characteristic of the 19-CH_2 signal of C_{20}-diterpenoid alkaloids, strongly suggested that one of hydroxyl groups was situated at the C-19 position.

Acetylation of **1** with Ac_2O/pyridine in the presence of *p*-dimethylaminopyridine (DMAP) at room temperature afforded a mixture that was chromatographed to give tetra- and triacetyl derivatives **4** and **5**. Compound **4**, $C_{28}H_{35}NO_8$, $[M]^+$ 513.2364 (calcd 513.2363), contained four acetyl groups (δ_H 2.04, 2.12, 3H each; 2.08, 6H), an *N*-Me group (δ_H 0.92), and an exocyclic methylene group (δ_H 4.81, 4.98). Examination of the 2D-COSY spectrum allowed three acetoxyl groups assigned to the C-2α, C-11α, and C-13α positions, and their geminal protons appeared at δ_H 5.20 (br m, $W_{1/2}$ = 12.0 Hz), 5.14 (d, J = 9.1 Hz) and 5.11 (dd, J_1 = 9.7, J_2 = 2.7 Hz), respectively. The site of the remaining acetoxyl group at C-19 was established by a down-field singlet at δ_H 5.73 and a doublet at δ_C 92.6. Comparison of the δ_C values of **4** and triacetylhetisine [**6**] (12) revealed an α effect of 19-OAc on C-19 causing a down-field shift of 29.0, a β effect on C-4 of 6.6, and a γ-effect on C-18 of −8.0 ppm, as well as a steric effect giving rise to up-field shifts on C-6 and C-20 of 2.0 and 3.0 ppm, respectively. The C-19 acetoxyl group was determined to have a β orientation, simply because in the nOe-difference experiment (Figure 1) the irradiation of the H-19 caused a strong enhancement of the H-20 (δ_H 3.65, s) and vice versa. Distinguishing between two sets of vicinal protons H-11–H-9 and H-13–H-14, and the respective carbons, C-11–C-9 and C-13–C-14 (δ_C 75.3, 52.7 and 72.7, 50.0), is not straightforward because two sets of signals have very close chemical shifts and coupling patterns. Here, unequivocal assignments of these two sets of protons and carbons began first with the location of H-14 by its nOe enhancement to H-20 (Figure 1) followed by correlation of the protons with the carbons through a 2D-HETCOR (^1J) spectrum. H-14 exhibits a very weak coupling with H-20 observed in the 2D-COSY spectrum owing to their dihedral angle of nearly 90°. Compound **5**, $C_{26}H_{33}NO_7$, $[M]^+$ 471, showed spectral features similar to those of **4** with the exception of H-19, which appeared at δ_H 4.63 as a singlet. Based on the above chemical and spectral evidence, structure **1** is assigned to vakhmatine.

TABLE 1. The ^{13}C-nmr Assignments of Compounds **1–6**, **8** and **9**.[a]

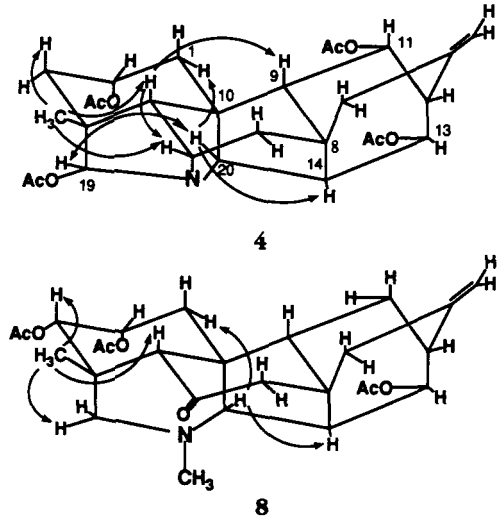
Carbon	Compound							
	1	**3**	**4**	**5**	**6**	**2**	**8**	**9**
C-1	35.1	35.1	29.4	29.8	29.6	30.0	33.7	34.6
C-2	62.9	67.8	69.3	69.8	70.1	69.3	67.9	67.2
C-3	38.5	39.9	35.7	35.4	36.5	73.5	75.8	75.6
C-4	42.4	37.7	43.2	42.5	36.6	40.6	41.3	41.2
C-5	60.6	62.6	61.2	61.4	61.1	58.9	57.8	58.1[b]
C-6	61.6	65.5	62.2	60.7	64.2	105.0	202.4	201.5
C-7	36.8	37.3	35.2	35.5	36.1	40.1	51.2	50.2
C-8	45.3	44.6	44.3	44.8	44.1	41.5	41.4	41.7
C-9	56.8	56.7	52.7	52.9	53.1	45.3	49.3	49.6
C-10	51.5	52.0	50.2	50.4	50.4	45.2	43.9	44.2
C-11	76.9	77.0	75.3	75.7	75.7	21.4	23.5	22.8
C-12	52.4	52.3	44.7	44.8	44.9	41.5	39.7	52.6
C-13	73.0	73.2	72.7	73.3	73.2	67.8	74.6	211.4
C-14	53.2	53.4	50.0	49.9	50.2	48.1	51.3	63.0[b]
C-15	34.5	34.6	33.6	33.9	34.0	31.8	35.2	34.4
C-16	148.2	148.3	142.7	143.0	143.3	148.1	146.6	141.7
C-17	107.6	107.6	110.2	110.2	110.0	107.2	107.0	110.8
C-18	27.5	30.2	21.8	22.2	29.8	25.3	25.5	25.4
C-19	95.5	64.1	92.6	91.4	63.6	66.7	57.2	56.1
C-20	66.1	69.1	65.7	65.0	68.7	73.2	65.7	70.4
N-Me	—	—	—	—	—	36.3	43.2	43.2
O-C=O	—	—	169.8	169.7	170.1	—	170.2	170.1
CH$_3$	—	—	21.6	21.7	21.9	—	21.3	21.1
O-C=O	—	—	170.1	169.7	170.2	—	169.4	169.5
CH$_3$	—	—	21.3	21.4	21.6	—	20.7	20.6
O-C=O	—	—	170.3	170.5	170.6	—	169.8	—
CH$_3$	—	—	20.9	21.0	21.1	—	20.9	—
O-C=O	—	—	170.7	—	—	—	—	—
CH$_3$	—	—	20.7					

[a]Chemical shifts in ppm downfield from TMS in CD_3OD for **1**, **3**; in $CDCl_3$ for **4**, **5**, **6**, **8**, **9**, and in D_2O for **2**; multiplicities determined by DEPT sequence except **5** and **6** (7).

[b]The assignments in Sun *et al.* (8) should be interchanged.

Vakhmadine [**2**], $C_{21}H_{30}NO_4 + OH^-$, $[\alpha]^{24}D$ −37.8° (*c* = 0.1, MeOH), mp 263.0–273.0° (EtOH), was isolated in the form of a quaternary base. The absence of any carbonyl group in the ir (Nujol) and ^{13}C-nmr (D_2O) spectra demonstrated that the C-6 keto group was masked in both crystalline form and solution although its hrms spectrum gave *m/z* 359.2091 (calcd 359.2097) as a molecular ion peak, indicative of a free base **7** ($C_{21}H_{29}NO_4$) with a carbonyl group at C-6. The existence of such a quaternary-*N* center in the molecule was apparent by its deshielding effects on *N*-Me (δ_H 2.58), H_2-19 (δ_H 2.97, 4.05, 1H each, d, J = 11.7 Hz) and H-20 (δ_H 4.22, s) compared with the values of free hetidine-type alkaloids, and also by the presence of a C-6 singlet at δ_C 105.0 and a γ effect of 6-OH causing an up-field shift on *N*-Me (δ_C 36.3 ppm).

Acetylation of **2** with Ac_2O/pyridine in the presence of DMAP at 60°, followed by chromatographic purification, gave as a major product a triacetyl derivative **8**, $C_{27}H_{35}NO_7$, $[M]^+$ 485, mp 261.0–262.0° (Me_2CO). The presence of a keto group (δ_C 202.4, ir 1695 cm^{-1}), as well as three acetyl groups (δ_C 170.2, 169.4, 169.8) indicates that the masked C-6 keto group in **2** was restored by the acetylation. This deriva-



4

8

FIGURE 1. NOe difference experiments of **4** and **8** (300 MHz, $CDCl_3$).

tive showed structural features of hetidine-like alkaloids, i.e., the existence of an *N*-Me (δ_H 2.22, δ_C 43.2), an exocyclic methylene (δ_H 4.68, 4.84; δ_C 146.6, 107.0), and the C-6 ketone mentioned above. The 2D COSY spectrum exhibited a strong coupling of two down-field vicinal protons at δ_H 4.63 (d, $J = 4.6$ Hz) and 5.44 (ddd, $J_1 = J_2 = 4.6$, $J_3 = 2.1$ Hz), which were reasonably placed at ring A for such a skeleton. The doublet, which showed an nOe enhancement to the 4-Me (δ_H 1.48) (Figure 1), was assigned to the H-3β, and consequently the other one at δ_H 5.44 should be the H-2β according to its coupling pattern. Thus, two out of three acetyl groups were placed at the C-2α and C-3α positions. The existence of a down-field signal at δ_H 4.97 (dt, $J_1 = 9.6$, $J_2 = 1.8$ Hz) allowed the third acetyl group to be placed at the C-13α position because this signal was strongly coupled with the vicinal H-14 at δ_H 2.43 (dd, $J_1 = 9.6$, $J_2 = 2.5$ Hz), as shown in the 2D COSY spectrum, while the latter was characterized by its nOe enhancement to the H-20 (δ_H 2.80, s) (Figure 1). The placement of three acetyl groups was also supported by comparison of the δ_C values of **8** and diacetylhetidine [**9**]. All signals of the two compounds were the same except for C-12, C-13, C-14, C-16, C-17, and C-20. The differences were due to the effect of the change from the C-13 keto group in **9** to the 13α-OAc group in **8**. The early literature reported the assignment of δ_C value of 62.9 to C-5 (and δ_C 58.2 to C-14) for **9** (8), which differs from the C-5 value of **8** by 5.1 ppm. Here, the assignment of δ_C 57.8 for C-5 was based on its correlation with the H-5 at δ_H 1.68 (s) as shown in the 2D HETCOR (^1J) spectrum, while the latter was located from its nOe relationship with the 4-Me (Figure 1). Thus, the assignments for C-5 and C-14 in the original paper (15) should be interchanged. According to the above reasoning, the triacetyl derivative of vakhmadine has structure **8**, and structure **2** is assigned to vakhmadine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken on a Thomas-Kofler hot stage equipped with a microscope and a polarizer and are corrected. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Ir spectra were taken on a Perkin-Elmer model 1420 spectrophotometer. ^1H, ^{13}C, nOe difference, and 2D nmr spectra were recorded in the specified solvents on Bruker AM-250, Bruker WM-300, GNΩ300 and JEOL FX-270 instruments. Mass spectra were determined on a Finnegan Quadrupole 4023 instrument at an ionizing voltage of 70 eV. For chromatographic separation on a

Chromatotron (16), rotors were coated with a 1-mm-thick layer of Al_2O_3 (EM Art. 1085) or Si gel PF 254 + 365 (EM Art. 7741); for vacuum liquid chromatography (vlc) (17), Al_2O_3 (EM Art. 1085) was employed.

EXTRACTION AND GRADIENT pH FRACTIONATION OF THE CRUDE ALKALOIDS.—*A. palmatum* was purchased from United Chemical and Allied Products of Calcutta, India. The plant was collected at 12,000–13,000 feet in the Himalayas in January 1986, and was compared against a voucher specimen in the Botanica Survey of India, Calcutta, where a voucher specimen is deposited. The sun-dried powdered root (1773 g) was percolated at room temperature with 70% EtOH (16.0 liters) for 6 days, and the percolate was evaporated in vacuo to give 1188.5 g of residue, equivalent to 759.2 g of the dried extract.

The concentrate was divided into five roughly equal portions, and each portion was diluted with H_2O (ca. 300 ml) and 1.5% H_2SO_4 solution (ca. 150 ml). The acidic solution (pH 4) was extracted with hexane (5 × 700 ml) and then filtered to remove some non-alkaloid precipitate. The aqueous layer was extracted with CH_2Cl_2 (5 × 500 ml), and the dried CH_2Cl_2 extract was designated as the pH-4 fraction. The acidic layer was basified in an ice-H_2O bath with 20% NaOH solution to pH 7 and then with 10% $NaHCO_3$ to pH 8.2. The basic solution was extracted with CH_2Cl_2 (5 × 600 ml), and the dried CH_2Cl_2 extract was designated as the pH-8.2 fraction. A precipitate, which did not dissolve in CH_2Cl_2, was collected and designated as the pH-8.2 precipitate. The aqueous layer was treated with 20% of NaOH solution in an ice-H_2O bath to pH 10 and extracted with CH_2Cl_2 (5 × 700 ml), and the dried CH_2Cl_2 extract was designated as the pH-10 fraction. The basic solution was further basified with an excess of 20% NaOH solution in an ice-H_2O bath to pH 14 and extracted with CH_2Cl_2 (5 × 800 ml), and the dried CH_2Cl_2 extract was designated as the pH-14 fraction. The aqueous layer was treated with 200 g of NaCl (ca. 20% solution) and then continuously extracted with $CHCl_3$ (500 ml) in a liquid-liquid extractor for 4 days; the dried $CHCl_3$ extract was designated as the water-soluble-base fraction. This process was repeated for the other four portions and the corresponding fractions were combined, producing six fractions: pH-4 fraction (17.94 g), pH-8.2 fraction (11.77 g), pH-8.2 precipitate (3.34 g), pH-10 fraction (12.94 g), pH-14 fraction (14.93 g) and water-soluble-base fraction (8.64 g).

ISOLATION OF ATISINE.—A part of the pH-10 fraction (6.980 g) was adsorbed on Al_2O_3 (24 g) and eluted on an Al_2O_3 vlc column (100 g, ø 4.5 × 7.0 cm) with hexane, hexane-Et_2O (1:1), CH_2Cl_2-MeOH (5:1), and $CHCl_3$-MeOH (2:1), yielding five fractions. Fraction 1 (4.350 g) contained a mixture of atisine and isoatisine, and fractions 2 and 3 (0.470 g and 0.570 g) contained crude atisine. Elution of the crude atisine (146 mg) on an Al_2O_3 Chromatotron plate with Et_2O-hexane (1:3) (80 ml), Et_2O-hexane (1:2) (60 ml), Et_2O-hexane (1:1) (120 ml), and Et_2O (40 ml) gave amorphous atisine (0.115 g), identical with an authentic sample by the co-tlc behavior and ir, ^{1}H-nmr, and ^{13}C-nmr spectra.

ISOLATION OF HETISINE, VAKHMATINE [**1**], AND VAKHMADINE [**2**].—The water-soluble-base fraction (8.640 g) was adsorbed on Al_2O_3 (24 g) and eluted on an Al_2O_3 vlc column (100 g, ø 4.5 × 7.0 cm) with Et_2O and $CHCl_3$ with increasing percentages of MeOH to give 8 fractions.

Fraction 4 (1.0 g) contained crude hetisine and was crystallized from Me_2CO to produce crystalline hetisine (225 mg, mp 251.5–254.5°), which was identical with an authentic sample in the tlc behavior, mp, ir, ^{1}H-nmr, and ^{13}C-nmr spectra.

A mixture of fractions 6 and 7 (a total of 1.32 g) adsorbed on Al_2O_3 (5 g) was chromatographed on an Al_2O_3 vlc column (30 g, ø 4.5 × 2.5 cm) by elution with $CHCl_3$ with increasing percentages of MeOH and washing with 95% EtOH-concentrated NH_4OH (3:1), generating six fractions. Fractions 3 and 5 (0.299 g and 0.231 g) contained a mixture of vakhmatine and vakhmadine. Fraction 4 (0.369 g) containing mostly vakhmatine was crystallized from MeOH to produce 66 mg of vakhmatine. The mother liquor was combined with fraction 5, and the mixture (0.542 g) was divided into three parts. Each part was separated on a Si gel Chromatotron plate by elution with 95% EtOH-concentrated NH_4OH (20:1) 120 ml, 95% EtOH-concentrated NH_4OH (3:1) 120 ml to give pure vakhmatine and vakhmadine. A total of 337 mg of vakhmatine and 110 mg of vakhmadine was obtained from the three parts. Fraction 3 was separated in a similar manner to give 65 mg of vakhmatine and 183 mg of vakhmadine.

Vakhmatine [**1**]: $C_{20}H_{27}NO_4$; mp 170.5–174.5° (MeOH); $[\alpha]^{29}D$ +12.6° (c = 0.2, MeOH); ms *m/z* (% rel. int.) $[M]^+$ 345 (4.6), 327 (7.4), 309 (14.0), 281 (13.8), 222 (7.5), 173 (13.6), 144 (14.2), 128 (18.4), 115 (17.4), 105 (27.3), 91 (47.1), 77 (30.6), 55 (45.2), 43 (67.5), 41 (100.0); ir (Nujol) ν max 3550 (OH), 3320 (br, OHs), 3060, 1650, 875 (C=CH_2), 1080, 1035, 1205, 950 cm^{-1}; ^{1}H nmr (300 MHz, CD_3OD) δ 1.04 (3H, s, 4-Me), 1.55 (1H, dd, J_1 = 15.2, J_2 = 4.8 Hz, H-3β), 1.91 (1H, dd, J_1 = 9.0, J_2 = 2.1 Hz, H-9), 1.99, 2.25 (1H each, br d, J = 17.7 Hz, H_2-15), 2.12 (1H, dd, J_1 = 9.3, J_2 = 1.8 Hz, H-14), 2.35 (1H, d, J = 2.6 Hz, H-12), 3.00 (1H, br d, J = 15.3 Hz, H-1α), 3.38 (1H, br s, H-6), 4.02 (1H, br m, H-2β), 4.11 (1H, dt, J_1 = 9.3, J_2 = 2.3 Hz, H-13β), 4.18 (1H, s, H-19), 4.22 (1H, d, J = 9.1 Hz, H-11β), 4.67, 4.84 (1H each, br s, 2H-17); ^{13}C nmr see Table 1.

Vakhmadine [**2**]: $C_{21}H_{30}NO_4 + OH^-$; mp 263.0–273.0° (EtOH); $[\alpha]^{24}D$ −37.8° (c = 0.1;

MeOH), ms *m/z* (% rel. int.) $[M]^+$ 359.2091 (3.9), (calcd 359.2097 for $C_{21}H_{29}NO_4$; loss of H_2O from $C_{21}H_{30}NO_4 + OH^-$), 342 (8.0), 44 (100.0); ir (Nujol) ν max 3340 (br, OH), 3060, 1650, 870 ($C=CH_2$), 1110, 1090, 1075, 1050, 1020, 990 cm^{-1}; ^1H nmr (250 MHz, D_2O) δ 1.40 (3H, s, 4-Me), 2.58 (3H, s, *N*-Me), 2.97, 4.05 (1H each, d, $J = 11.7$ Hz, H_2-19), 3.33 (1H, d, $J = 4.3$ Hz, H-3β), 3.93 (1H, d, $J = 11.0$ Hz, H-13β), 3.97 (1H, br m, H-2β), 4.22 (1H, s, H-20), 4.59, 4.73 (1H, each, s, 2H-17); ^{13}C nmr see Table 1.

ACETYLATION OF VAKHMATINE [**1**].—Vakhmatine [**1**] (200 mg) was treated with Ac_2O (1 ml) in pyridine (3 ml) in the presence of a catalytic amount of DMAP at room temperature for 3 days, followed by routine workup, to afford a mixture that was separated on a Si gel Chromatotron plate by elution with $CHCl_3$-hexane (1:1) (140 ml), $CHCl_3$-hexane (3:1) (80 ml), $CHCl_3$-MeOH (80:1) (80 ml), $CHCl_3$-MeOH (10:1) (40 ml), and $CHCl_3$-MeOH (5:1) (40 ml) to give four fractions. Fractions 2 and 3 (a total of 153 mg) were mixtures; fraction 1 gave a pure amorphous **4** and fraction 4 yielded a pure amorphous **5**. Compound **4**: $C_{28}H_{35}NO_8$; ms *m/z* (% rel. int.) $[M]^+$ 513.2364 (3.3) (calcd 513.2363), 471 (5.0), 454 (1.4), 411 (7.7), 43 (100.0); ir (Nujol) ν max 1740, 1730, 1250, 1230 (OAc), 1650, 970 ($C=CH_2$), 1080, 1030 cm^{-1}; ^1H nmr (300 Hz, $CDCl_3$) δ 0.92 (3H, s, 4-Me), 1.51 (1H, dd, $J_1 = 15.6$, $J_2 = 4.6$ Hz, H-3β), 1.64 (1H, s, H-5), 1.68 (1H, dd, $J_1 = 13.7$, $J_2 = 2.6$ Hz, H-7β), 1.69 (1H, dd, $J_1 = 15.2$, $J_2 = 5.0$ Hz, H-1β), 1.84 (1H, dd, $J_1 = 13.7$, $J_2 = 3.1$ Hz, H-7α), 2.04, 2.12 (3H each, s, 2 × OAc), 2.08 (6H, s, 2 × OAc), 2.09, 2.34 (1H each, br d, $J = 18.1$ Hz, H_2-15), 2.16 (1H, br d, $J = 15.6$ Hz, H-3α), 2.22 (1H, dd, $J_1 = 9.6$, $J_2 = 2.2$ Hz, H-9), 2.39 (1H, d, $J = 9.2$ Hz, H-14), 2.62 (1H, d, $J = 2.6$ Hz, H-12), 2.86 (1H, d, $J = 15.1$ Hz, H-1α), 3.52 (1H, br s, H-6), 3.65 (1H, s, H-20), 4.81, 4.98 (1H each, br s, H_2-17), 5.11 (1H, dd, $J_1 = 9.7$, $J_2 = 2.7$ Hz, H-13β), 5.14 (1H, d, $J = 9.1$ Hz, H-11β), 5.20 (1H, br m, $W_{1/2} = 12.0$ Hz, H-2β), 5.73 (1H, s, H-19); ^{13}C nmr see Table 1; nOe difference see Figure 1. Compound **5**: $C_{26}H_{33}NO_7$; ms *m/z* (% rel. int.) $[M]^+$ 471 (1.0), 412 (1.0), 411 (3.9), 351 (0.9), 309 (1.9), 43 (100.0); ir (Nujol) ν max ca. 3160 (weak, OH), 1740, 1730, 1250, 1220 (OAc), 1660, 970 ($C=CH_2$), 1040, 1020 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ 1.02 (3H, s, 4-Me), 2.03 (6H, s, 2 × OAc), 2.04 (3H, s, OAc), 2.57 (1H, d, $J = 2.6$ Hz, H-12), 2.83 (1H, d, $J = 15.3$ Hz, H-1α), 3.54 (1H, s, H-20), 3.60 (1H, br s, H-6), 4.63 (1H, s, H-19), 4.80, 4.98 (1H each, br s, H_2-17), 5.10 (1H, d, $J = 8.6$ Hz, H-11β), 5.13 (1H, d, $J = 9.0$ Hz, H-13β), 5.15 (1H, br m, $W_{1/2} = 12.0$ Hz, H-2β); ^{13}C nmr see Table 1.

ACETYLATION OF VAKHMADINE [**2**].—Vakhmadine (80 mg) was heated with Ac_2O (1 ml) in pyridine (3 ml) in the presence of a catalytic amount of DMAP at 60° for 5 h, placed at room temperature for 17 h, and worked up to yield a major product (105 mg), which was purified on an Al_2O_3 Chromatotron plate by elution with Et_2O-hexane (4:1) (50 ml), Et_2O-MeOH (40:1) (80 ml), Et_2O-MeOH (20:1) (80 ml), Et_2O-MeOH (10:1) (80 ml), and Et_2O-MeOH (5:1) (60 ml). The resulting pure product **8** (67 mg) in Me_2CO gave crystals with mp 261–262°. Compound **8**: $C_{27}H_{35}NO_7$; ms *m/z* (% rel. int.) $[M]^+$ 485 (0.6), 442 (0.5), 426 (31.1), 43 (100.0); ir (Nujol) ν max 1740, 1730, 1250, 1220, 1210 (OAc), 1695 (C=O), 1660, 880 ($C=CH_2$), 1100, 1030, 930 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 1.48 (3H, s, 4-Me), 1.63 (1H, m, H-11β), 1.67 (1H, d, $J = 15.3$ Hz), 1.68 (1H, s, H-5), 1.76 (1H, br d, $J = 10.7$ Hz, H-9), 2.00, 2.07, 2.08 (3H each, s, 3 × OAc), 2.11 (1H, d, $J = 12.0$ Hz, H-11α), 2.12 (1H, d, $J = 18.6$ Hz, H-7β), 2.22, 2.35 (1H each, d, H_2-15), 2.22 (3H, s, *N*-Me), 2.24 (1H, m, H-12), 2.28 (1H, dd, $J_1 = 15.1$ Hz, $J_2 = 2.1$ Hz, H-1α), 2.37 (1H, d, $J = 12.0$ Hz, H-19β), 2.43 (1H, dd, $J_1 = 9.6$ Hz, $J_2 = 2.5$ Hz, H-14), 2.68 (1H, d, $J = 18.6$ Hz, H-7α), 2.80 (1H, s, H-20), 2.99 (1H, d, $J = 12.0$ Hz, H-19α), 4.63 (1H, d, $J = 4.6$ Hz, H-3β), 4.68 (1H, d, $J = 1.3$ Hz), 4.84 (1H, d, $J = 1.1$ Hz, H_2-17), 4.97 (1H, dt, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz, H-13β), 5.44 (1H, ddd, $J_1 = J_2 = 4.6$ Hz, $J_3 = 2.1$ Hz, H-2β); ^{13}C nmr see Table 1; nOe difference see Figure 1.

ACKNOWLEDGMENTS

We thank Mr. Yiping Sun at Purdue University for the hrms spectra and Dr. Balawant S. Joshi for reviewing the manuscript.

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Received 13 September 1990