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13-*O*-ACETYLVAKHMATINE, A NEW DITERPENOID ALKALOID FROM THE SEEDS OF *CONSOLIDA AMBIGUA*

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ABSTRACT.—Vakhmatine [**1**] and the new diterpenoid alkaloid 13-*O*-acetylvakhmatine [**3**] have been isolated from the polar alkaloidal fractions of the seeds of *Consolida ambigua*, together with ajacine, delcosine, gigactonine, and takaosamine. Structure **3** has been established on the basis of spectroscopic data and chemical correlation with **1**.

The aerial parts and the seeds of *Consolida ambigua* L.P.W. Ball and V.H. Heywood (syn. *Delphinium ajacis* L.) (Ranunculaceae) (1) are rich in alkaloids, with most of the these being norditerpenoids (2,3). Thirty-three norditerpenoid alkaloids (19 carbons) of the lycoctonine-type have been isolated from the seeds and various parts of this plant. These are 14-acetylbrowniine, 14-acetyldelcosine, 14-acetyldelectine, ajacine, ajacusine, ajadelphine, ajadelphinine, ajadine, ajadinine, ajanine, ambiguine, anthranoyllycoctonine, browniine, 14-deacetylajadine, 14-deacetylambiguine, delajacine, delajacirine, delajadine, delcosine, delectine, delphatine, delpheline, delphisine, delsoline, deltaline, deltatsine, gigactonine, lycoctonine, 18-methoxygadesine, methyllycaconitine, 19-oxoanthranoyllycoctonine, 19-oxodelphatine, and takaosamine (4–11). The two diterpenoid alkaloids (20 carbons) isolated from the seeds are ajaconine and dihydroajaconine (6). Ajabicine, isolated from the leaves, is an interesting diterpenoid alkaloid of twenty carbon atoms, but possessing the bicyclo[3,2,1]octane ring system, found in all the norditerpenoid alkaloids (12).

In this paper we report the isolation and structure determination of vakhmatine [**1**] and a new diterpenoid alkaloid, 13-*O*-acetylvakhmatine [**3**], from the seeds of *C. ambigua*.

RESULTS AND DISCUSSION

After defatting, the dried and crushed seeds of *C. ambigua* were extracted with 70% EtOH and the crude alkaloidal fraction was obtained as described in the Experimental. On chromatographic separation by vlc (13) and by centrifugally accelerated, radial tlc (Chromatotron) (14,15) this fraction afforded ajacine. The polar vlc fraction was chromatographed by high-performance centrifugal partition chromatography (hpcpc) (16,17) to give delcosine, takaosamine, and gigactonine. The most polar fractions afforded an amorphous new alkaloid in 0.0046% yield, and the known alkaloid vakhmatine [**1**] reported previously from *Aconitum palmatum* Don (18). The molecular formula of the new alkaloid, $C_{22}H_{29}NO_5$, was established by hrms which showed a $[M+H]^+$ at *m/z* 388.2141; calcd for $C_{22}H_{30}NO_5$, $[M+H]^+$, 388.2124. The preliminary ^{13}C-nmr spectrum of the alkaloid indicated the presence of 22 carbon signals at 172.8, 144.8, 108.7, 90.9, 76.6, 75.8, 66.1, 65.0, 61.6, 60.2, 55.1, 50.2, 49.9, 48.5, 44.2, 42.0, 40.7, 35.6, 33.7, 32.8, 22.7, and 21.1 ppm. The DEPT spectrum showed five quaternary, ten methine, five methylene, and two methyl carbons (Table 1). The ^1H-nmr spectrum showed the presence of a tertiary methyl group at δ 1.00 (3H, s) and an acetate

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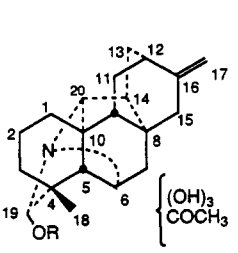
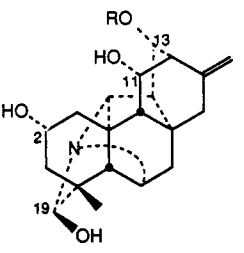
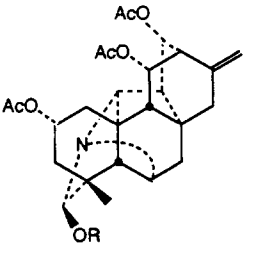


2 Partial structure
R=H or $COCH_3$



1 Vakhmatine (R=H)
3 13-Acetylvakhmatine ($R=\underset{21}{C}O\underset{22}{C}H_3$)



4 R=Ac
5 R=H

methyl at δ 2.22 (3H, s). The downfield part of the spectrum showed the presence of an exocyclic methylene group at δ 4.70 and δ 4.87 (each 1H, s). These inferences were supported by the characteristic carbon resonances at δ 22.7 (*tert.*-CH_3), δ 172.8 ($OCOCH_3$), δ 21.1 ($OCOCH_3$), δ 144.8 (s), and δ 108.7 (t) (-C=CH_2). The presence of an acetate accounts for two carbons, and the alkaloid should therefore be an acetate of an alkaloid possessing twenty carbon atoms. Biogenetic considerations and the absence of methoxy, *N*-methyl, or *N*-ethyl groups suggested that this should be a diterpenoid and not a norditerpenoid alkaloid. Of the various skeletons which are known for diterpenoid alkaloids (19), the new alkaloid isolated appeared to bear the hetisane skeleton, having one *O*-acetate and three hydroxyl groups.

The heteronuclear COSY (HETCOR) nmr spectrum was helpful in the assignment of many of the carbons and protons of the molecule. The COSY spectrum (Table 1) was employed in confirming the connectivities of some of the protons. In the ^{13}C-nmr spectrum, no triplet was observed for the C-19 *N*-CH_2 carbon which normally appears in hetisanes in the range 60.8–63.0 ppm. This methylene therefore bears an oxygen function. The doublet signal at δ 90.9 which is close to the carbinolamine carbon signals of septentriosine (δ 95.3) (20), spiramine D (δ 91.5) (21), and *N*-deethyldehydrolucidusculine (δ 87.8) (22), confirmed this assignment to C-19. In the ^{1}H-nmr spectrum, H-19 appeared as a singlet at δ 4.71. A partial structure **2** could thus be derived for the new alkaloid.

The sp^2 singlet carbon resonance appearing at δ 144.8 for C-16 indicated clearly that C-15 does not bear a hydroxyl group. In the case of alkaloids not bearing a hydroxyl at C-15, C-16 is observed at δ 144–147; e.g., hetisine: δ 146.6 (23), Guan Fu Base Z: δ 144.6 (24,25), and spirasine XI: δ 147.2 (26, S. Sakai, personal communication, July 1986). When a hydroxyl group is present at C-15, the signal for C-16 appears about 151–158 ppm; e.g., sanyonamine: δ 158.1 (27), isohypognavine: δ 150.4 (28), and nominine: δ 156.8 (29). As there was no sp^3 singlet carbon signal downfield of 60.0 ppm, no quaternary carbon bearing an oxygen function was present in the molecule. We have already assigned the carbon signals at δ 172.8 and δ 144.8 to the acetate carbonyl and C-16, respectively. The remaining three sp^3 singlets in the nmr spectrum of **2** appearing at δ 42.0, 44.2, and 50.2 must be assigned to C-4, C-8, and C-10, respectively. In all the hetisane-type alkaloids, C-10 appears in the range 46.5–55.5 ppm, depending on the location of the oxygen substituent. The signal at δ 50.2 was therefore assigned to C-10. The signals at δ 42.0 and 44.2 have been assigned to C-4 and C-8, respectively, on the basis of selective INEPT (SINEPT) nmr data (see below). In the SINEPT experiment, saturation of the C-18 methyl protons at δ 1.00 showed a polarization transfer to C-4 (δ 42.0), two bonds removed and also to C-3 (δ 40.7), C-5 (δ 61.6) and C-19 (δ 90.9), all three bonds away. In separate experiments, saturation of H-13 (δ 5.00), H-6 (δ 3.55),

TABLE 1. ^{13}C- and ^{1}H-Nmr Chemical Shift Assignments of 13-*O*-Acetylvakhmatine [**3**].[a]

Carbon	δ	Proton	δ	Mult. *J* (Hz)	COSY correlation
C-1	32.8 t	H-1_β	1.82	dd, $J_{1\beta,1\alpha}$=15.0 $J_{1\beta,2\beta}$=4.0	H-1_α
		H-1_α	2.66	br d, $J_{1\alpha,1\beta}$=15.0	H-1_β
C-2	66.1 d	H-2_β	4.18	br s, $W_{1/2}$=8.0	H-3_β, H-3_α
C-3	40.7 t	H-3_β	1.55	dd, $J_{3\beta,3\alpha}$=7.8 $J_{3\beta,2\beta}$=2.1	H-2_β
		H-3_α	1.98	br d, $J_{3\beta,3\alpha}$=7.8	H-2_β
C-4	42.0 s	—	—		
C-5	61.6 d	H-5	1.45	s	
C-6	60.2 d	H-6	3.55	br s, $W_{1/2}$=4.0	H-7_β, H-7_α
C-7	35.6 t	H-7_β	1.56	m	H-9_β
		H-7_α	1.71	dd, $J_{7\alpha,7\beta}$=14.0 $J_{7\alpha,6}$=2.7	
C-8	44.2 s	—	—		
C-9	55.1 d	H-9	1.91	d, J=9.0	H-7_β
C-10	50.2 s	—	—		
C-11	75.8 d	H-11_β	4.23	d, J=9.0	H-9_β
C-12	48.5 d	H-12	2.42	d, $J_{12,13\beta}$=2.5 $J_{12,11\beta}$=<1	H-13_β
C-13	76.6 d	H-13_β	5.00	br d, $J_{13\beta,14}$=9.0	H-12, H-14
C-14	49.9 d	H-14	2.38	d, $J_{14,13\beta}$=9.0	H-13_β
C-15	33.7 t	H-15_β	2.18	AB, J_{gem}=18.0	H-17[b]
		H-15_α	2.03	AB, J_{gem}=18.0	H-17[b]
C-16	144.8 s	—	—		
C-17	108.7 t	H-17_a	4.86	s	H-15_α,[b] H-15_β[b]
		H-17_b	4.70	s	H-15_β,[b] H-15_α[b]
C-18	22.7 q	H-18	1.00	s	
C-19	90.9 d	H-19	4.71	s	
C-20	65.0 d	H-20	3.28	s	
C-21	172.8 s	—	—		
C-22	21.1 q	CH_3-22	2.22	s	

[a]These assignments were made on the basis of DEPT and HETCOR spectra.
[b]Long-range coupling.

and H-20 (δ 3.28), showed polarization transfer to C-8 (δ 44.2), three bonds removed. This assignment for C-8 was confirmed in SINEPT experiments by saturation of H-7 (δ 1.56, 1.71), H-15 (δ 2.03, 2.18), and H-14 (δ 2.38) (Table 2).

An oxygen function should be present in ring A of the alkaloid, because there is no methylene carbon triplet upfield of δ 32.8. If ring A does not bear an oxygen function, one should observe a methylene signal at 19–20 ppm (30,31). An oxygen function in the form of a hydroxyl, an acetate or an ether group, is therefore present in ring A. The quaternary carbon signal for C-10 appearing at 50.2 ppm is in the normal range, not having the β-effect of an oxygen function located at C-1; e.g., C-10 appears at δ 54.9 in hypognavine (32) and δ 56.8 in vacognavine (33) bearing a C-1 oxygen function. The quaternary carbon signal at δ 42.0 assigned to C-4, indicated the absence of an oxygen function (OH or OAc) at C-3, which would have contributed to a β-effect, bringing it downfield by 6–8 ppm (31). One of the oxygen functions (OH or OAc) should therefore be placed at C-2. In the ^{1}H-nmr spectrum, H-2 appeared as a broad singlet at δ 4.18 and this showed a correlation with δ 66.1 in the HETCOR spectrum. The H-2 proton showed a correlation with H-3_α (δ 1.98) and H-3_β (δ 1.55) in the COSY spectrum. In a selective INEPT experiment, saturaton of the H-2 signal at δ 4.18 showed polarization

TABLE 2. Nmr Data of 13-*O*-Acetylvakhmatine [**3**] from Selective INEPT Experiments.

Irradiation of proton (δ)	Assignment	Enhanced carbon signal(s) (δ)
5.00	H-13_β	172.8 (C-21), 75.8 (C-11), 48.5 (C-12), 44.2 (C-8)
4.70	H-17_b	48.5 (C-12), 33.7 (C-15)
4.23	H-11	144.8 (H-16), 76.6 (C-13), 50.2 (C-10), 48.5 (C-12)
4.18	H-2	50.2 (C-10), 32.8 (C-1)
3.55	H-6	44.2 (C-8)
3.28	H-20	76.6 (C-13), 44.2 (C-8)
2.66	H-1_α	66.1 (C-2), 65.0 (C-20), 61.6 (C-5), 50.2 (C-10)
2.42	H-12	144.8 (C-16), 108.7 (C-17), 76.6 (C-13), 75.8 (C-11), 49.8 (C-14), 33.7 (C-15)
2.38	H-14	76.6 (C-13), 50.2 (C-10), 44.2 (C-8), 33.7 (C-15)
2.18	H-15_β	144.8 (C-16), 44.2 (C-8)
2.03	H-15_α	144.8 (C-16), 44.2 (C-8), 35.6 (C-7)
1.91	H-9	75.8 (C-11), 49.8 (C-14), 48.5 (C-12), 44.2 (C-8), 35.6 (C-7)
1.71	H-7_α	44.2 (H-8)
1.56	H-7_β	44.2 (H-8)
1.00	CH_3-18	90.9 (C-19), 61.6 (C-5), 42.0 (C-4), 40.7 (C-3)

transfer to C-1 and C-10, two and three bonds removed, respectively. Of the ten sp^3 doublet signals present in the molecule, C-2 and C-19 (bearing oxygen functions) and C-5, C-6, C-9, C-12, C-14, and C-20 account for eight methine carbons. We were thus left with the problem of locating two oxygen functions on the remaining methylenes at C-7, C-11, or C-13, accounting for a total of ten methines. When an OH group is present at C-7, the quaternary carbon C-8 appears at 48.5 ppm as in sadosine (34). As discussed earlier, C-8 appeared at δ 44.2. The presence of a hydroxyl at C-7 was thus discounted. Two of the remaining oxygen functions were thus located at C-11 and C-13. The new alkaloid is therefore a monoacetyl derivative of vakhmatine [**1**]. The location of the acetoxyl group was decided by selective INEPT nmr experiments.

Alkaline hydrolysis of the new alkaloid afforded vakhmatine [**1**], identical with an authentic sample (18). Acetylation of the new alkaloid afforded 2,11,13-tri-*O*-acetylvakhmatine [**4**] and 2,11,13,19-*O*-tetraacetylvakhmatine [**5**] (18), identified by comparison of the spectral and physical data with those of authentic samples.

These correlations with vakhmatine [**1**] did not fix the location of the acetoxyl group in the new alkaloid. However, in the ^{1}H-nmr spectrum, the proton attached to the acetoxyl group-bearing carbon should be the most downfield signal among the protons adjacent to hydroxyl and acetoxyl groups in the molecule. This signal appeared as a doublet at δ 5.00. In a selective INEPT experiment (Table 2), when the signal at δ 3.28 (H-20; ^{13}C δ 65.0) was selectively pulsed, the carbon signal at δ 76.6 (^{1}H, δ 5.00) was enhanced, which could only be assigned to H-13 three bonds away. This proton also showed a COSY correlation with H-12 and H-14 (Table 1). When H-11 (δ 4.23) was selectively pulsed, the responses observed were for the two methines at δ 76.6 (C-13) and δ 48.5 (C-12) separated by three and two bonds, respectively, besides the quaternary carbons at δ 50.2 (C-10) and δ 144.8 (C-16), both three bonds away. These results unambiguously ascertained that the acetoxyl group in the new alkaloid is on C-13, thereby confirming the structure **3** for the new alkaloid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a Thomas-Kofler hot stage instrument equipped with a microscope and polarizer. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Ir spectra were taken on a Perkin-Elmer model 1420 spectrophotometer. ^{1}H- and ^{13}C- and 2D

nmr spectra were recorded in $CDCl_3$ on Bruker AC-250 and Bruker AC-300 instruments. For chromatographic separation on a Chromatotron, rotors coated with 1-mm thick Al_2O_3, 60 PF 254+366 (Type E, EM 1104) or Si gel 60 G PF 254 (EM 7749) were used; for vlc, Al_2O_3, 60 H Basic (EM 1085) or Si gel 60H (EM 7736) was used. Hpcpc was carried out on a Sanki 1000 series, model LLB-M No. 3126 equipped with a hplc pump model SS1 222C. All the known alkaloids reported in this work were identified by comparing their tlc, ^{1}H- and ^{13}C-nmr data with those of authentic samples.

PLANT MATERIAL.—The seeds of *C. ambigua* were identified and supplied by the horticulture department (grounds) of the University of Georgia.

EXTRACTION AND ISOLATION.—Ground seeds of *C. ambigua* (1 kg) were defatted with hexane (3×3 liters) and extracted at room temperature with 70% EtOH (7×3 liters). The EtOH extract was evaporated to a syrupy mass *in vacuo* and treated with $CHCl_3$ (500 ml) and extracted with 2% H_2SO_4 (300 ml×5). The ice-cooled, acidic layer was basified with Na_2CO_3 to pH 10 and extracted with $CHCl_3$ (250 ml×4). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated *in vacuo* to afford a crude alkaloidal fraction (9.76 g).

Ajacine, delcosine, takaosamine, and gigactonine.—The crude alkaloid mixture (9.76 g) was chromatographed on a vlc column of Al_2O_3 and gradient eluted with hexane, $CHCl_3$, and MeOH. Fractions 36–62 (400 mg) eluted with hexane-$CHCl_3$ (80:20) were purified twice on an Al_2O_3 rotor to afford ajacine (140 mg; 0.014%). The vlc fraction 99 eluted with $CHCl_3$-MeOH (9:1) afforded a mixture (1.34 g). This mixture was partitioned on the hpcpc using the bi-phasic solvent system C_6H_6-$CHCl_3$-MeOH-H_2O (5:5:7:2). The upper layer was used as the stationary phase and the lower layer as the mobile phase. The crude mixture of alkaloids (1 g) was dissolved in 4 ml each of the upper and the lower layers and loaded on the hpcpc instrument operated in the descending mode. Fractions (12 ml each) were collected on an automatic fraction collector every 4 min at a flow rate of 3 ml/min, and a rotor speed of 1300 rpm. We collected 103 fractions. Fraction 14 gave a colorless compound (100 mg; 0.013%) identified as delcosine. Fractions 21–23 gave takaosamine (87 mg; 0.004%). Fractions 15–16 (284 mg) were resubmitted to hpcpc under similar conditions to give gigactonine (19 mg; 0.0026%).

13-O-Acetlvakhmatine [**3**], *takaosamine, and vakhmatine* [**1**].—The vlc fractions 100–103 eluted with $CHCl_3$-MeOH (90:10) and MeOH (100%) were combined on the basis of the tlc profile to give 1.05 g of polar alkaloids. Part of this (0.91 g) was partitioned by hpcpc with the same solvent system used earlier, the upper layer being the stationary one and the lower layer the mobile phase. We collected 140 fractions (6 ml each) were collected on a fraction collector at the rate of 2 ml/min. The rotor speed was kept at 1000 rpm. Fractions 56–58 afforded 13-*O*-acetylvakhmatine (46 mg) as an amorphous compound. Fractions 59–73 (151 mg) were separated on a Si gel rotor with a gradient of $CHCl_3$-MeOH-NH_4OH. Fractions 21–29 (59 mg) eluted with $CHCl_3$-MeOH (1:1) were further purified on an Al_2O_3 rotor to afford takaosamine (5 mg) and 13-*O*-acetylvakhmatine (10 mg) [**3**]. Fractions 30–45 eluted with $CHCl_3$-EtOH (1:1) and EtOH-NH_4OH (99:1) afforded vakhmatine (**1**; 45 mg).

13-O-Acetylvakhmatine [**3**].—$[\alpha]_D$ −20° (c=3.11, $CHCl_3$); hrms m/z 388.2141 $[M+H]^+$ (calcd for $C_{22}H_{30}NO_5$ $[M+H]^+$, 388.2124); ir (nujol) ν max 3450, 1720, 1455, 1375, 1250, 1030, 960, 880 cm^{-1}; for ^{1}H- and ^{13}C-nmr assignments, see Table 1.

HYDROLYSIS OF 13-*O*-ACETYLVAKHMATINE [**3**] TO VAKHMATINE [**1**].—A solution of alkaloid **3** in 5% methanolic KOH (5 ml) was refluxed on a steam bath for 16 h. The mixture was basified with 5% methanolic H_2SO_4 and the solvent removed *in vacuo*. The residue was extracted with $CHCl_3$ and *n*-BuOH to give vakhmatine [**3**]; fabms m/z $[M+H]^+$ 346; calcd for $C_{20}H_{27}NO_4$, $[M+H]^+$ 346.

2,11,13,19-TETRAACETYLVAKHMATINE [**4**] AND 2,11,13-TRI-*O*-ACETYLVAKHMATINE [**5**].—The alkaloid **1** (10 mg) was dissolved in MeCOCl (2 ml) and kept at room temperature for 6 days. Acetyl chloride was removed *in vacuo* and the product was chromatographed on a short Al_2O_3 column eluting with hexane and Et_2O. Fractions (8–10 ml) were collected. Fractions 5–10 eluted with hexane-Et_2O (70:30) afforded 2,11,13,19-tetraacetylvakhmatine [**4**] (4 mg) and fraction 13 eluted with Et_2O gave 2,11,13-triacetylvakhmatine [**5**] (2.5 mg).

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