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NEW STEROIDAL ALKALOIDS FROM RHIZOMES OF *VERATRUM ALBUM*

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ABSTRACT.—The EtOH extract of the rhizomes of *Veratrum album* has afforded two new alkaloids, (+)-jervinone [**1**] and (+)-verabenzoamine [**2**]. The structures **1** and **2** were determined by spectral analysis (^{1}H and ^{13}C nmr, DEPT, 2D COSY, and NOESY).

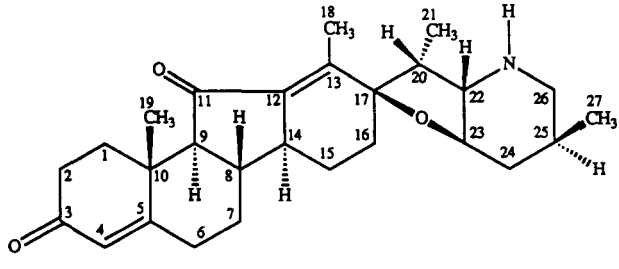
Veratrum species have been noted for their pharmacological activity for well over 300 years. Until about 1950 the extracts of *Veratrum* species were mainly used as insecticides. The alkaloids from *Veratrum* have also found use in the treatment of hypertension (1,2). Since 1930 *Veratrum* species have been studied chemically, yielding over 100 alkaloids, some of which have been pharmacologically evaluated (3–6). We have recently restudied *Veratrum album* L. (Liliaceae) and obtained several new alkaloids (7). We report here the isolation and structure elucidation of two new alkaloids, the jerveratrum-type (+)-jervinone [**1**] and cevane-type (+)-verabenzoamine [**2**].

RESULTS AND DISCUSSION

(+)-Jervinone [**1**], $C_{27}H_{37}NO_3$ (hreims *m/z* 423.2823, calcd 423.2773) possessed a mol wt 2 amu less than that of jervine [**3**] (8). Furthermore, the spectral characteristics (ms, ^{1}H and ^{13}C nmr) showed a distinct resemblance to those of **3**. Compound **1** possessed a uv spectrum with an absorption maximum at 246 nm, characteristic of an α,β-unsaturated cyclopentenone. The ir spectrum displayed intense bands at 3605 (N-H), 1723 (α,β-unsaturated cyclopentenone), 1707 (α,β-unsaturated cyclohexenone), 1620 (C=C), and 1020 (C-O) cm^{-1}.

The ^{1}H-nmr spectrum of **1** manifested signals representing two secondary methyl groups (Me-27 and Me-21) at δ 0.96 (J = 6.6 Hz) and 1.01 (J = 7.5 Hz), respectively. A three-proton singlet at δ 1.15 was assigned to the Me-19 protons. Another three-proton doublet resonating at δ 2.16 (J = 1.9 Hz) was ascribed to the C-18 allylic methyl protons, while the multiplet at δ 3.41 clearly arose from H-23α geminal to etheric oxygen. A triplet at δ 2.78 (J = 9.9 Hz) was ascribed to H-22. A singlet at δ 5.75 was assigned to the C-4 vinylic proton.

The connectivity and coupling information was directly obtainable from the COSY 45° spectrum. The H-21 doublet at δ 1.01 showed cross peaks with the C-20 methine proton at δ 2.51, while another doublet for the Me-27 protons at δ 0.96 showed COSY coupling with the C-25 methine proton at δ 1.62. Homoallylic coupling between the



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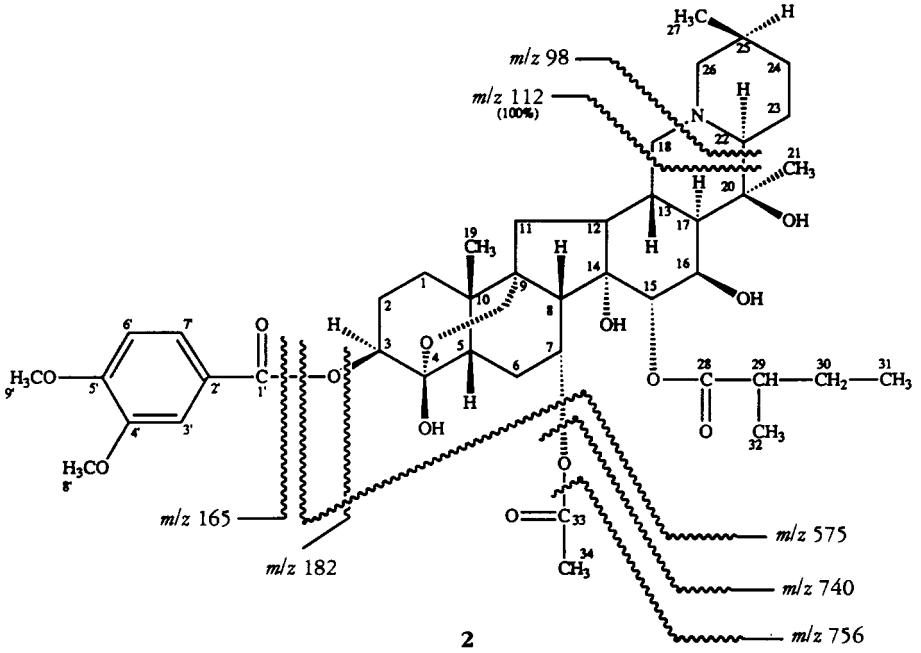

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Me-18 protons (δ 2.16) and the C-14 methine proton (δ 2.00) was also observed. The C-23 methine proton showed COSY interactions with the protons at δ 1.20, 2.22, and 2.78. The former two signals (δ 1.20 and 2.22) were geminally coupled with each other and were therefore assigned to the C-24 methylenic protons, while the latter signal (δ 2.78) was ascribed to the C-22 methine proton since it also showed vicinal coupling with the C-20 methine proton (δ 2.51).

The ^{13}C-nmr spectrum (75 MHz, $CDCl_3$) of (+)-jervinone [**1**] showed 27 carbon resonances. The multiplicity of the carbons was determined by DEPT and GASPE (9) experiments, which showed the presence of four methyl, eight methylene, and eight methine signals. The broad-band-decoupled spectrum showed all 27 carbons. The C-3 and C-11 carbonyl carbons appeared at δ 199.5 and 206.3, respectively, while the olefinic C-12 and C-13 carbons appeared at δ 136.6 and 146.2, respectively. The peaks at δ 75.30 and 65.90 were due to C-23 and C-22, respectively. The ^{13}C-nmr chemical shifts showed distinct similarity with those of jervine [**3**] (3) and are presented in Table 1.

The carbon-hydrogen heteronuclear shift correlation spectrum (HETCOSY), optimized for one-bond coupling, further confirmed various carbon and proton assignments. The C-4 resonated at δ 125.6 and showed a cross peak with the proton signal at δ 5.75 (H-4). ^{13}C-^{1}H cross peaks between C-22 (δ 65.90)/H-22 (δ 2.78) C-23 (δ

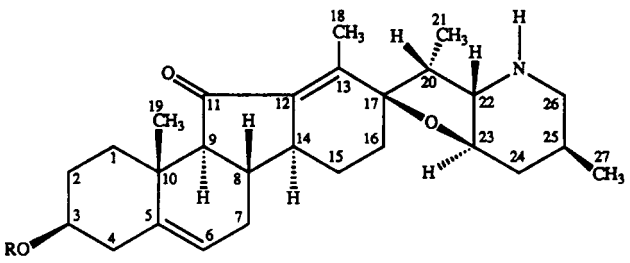

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TABLE 1. ^{13}C-nmr Spectral Data for Compounds **1–4**[a].

Carbon	Compound			
	1	**3**	**2**	**4**
C-1	35.27	36.8	31.93	32.40
C-2	33.61	31.1	26.8	26.50
C-3	199.56	71.6	75.02	75.47
C-4	125.69	41.4	105.7	105.22
C-5	168.14	142.3	46.5	45.91
C-6	32.71	120.9	29.3	28.55
C-7	30.65	38.9	66.93	66.56
C-8	41.47	37.9	46.3	47.84
C-9	64.32	62.5	93.2	92.87
C-10	[b]	37.0	46.9	46.20
C-11	206.33	206.8	33.08	33.65
C-12	136.67	137.1	45.12	47.21
C-13	146.24	145.8	[b]	33.63
C-14	43.80	44.8	81.09	81.13
C-15	23.90	24.3	68.93	69.65
C-16	38.41	30.7	69.92	69.23
C-17	85.73	85.5	47.97	45.34
C-18	12.28	12.10	61.49	61.25
C-19	16.96	18.40	17.00	19.11
C-20	39.74	40.3	72.85	72.91
C-21	11.12	10.8	19.06	19.84
C-22	65.91	66.5	70.30	69.56
C-23	75.35	76.4	18.08	18.32
C-24	29.90	30.90	29.71	28.83
C-25	30.28	31.5	27.06	27.26
C-26	53.73	54.6	61.49	61.25
C-27	18.65	18.80	17.40	17.02
C-28	—	—	176.0	175.69
C-29	—	—	41.27	41.12
C-30	—	—	32.56	26.76
C-31	—	—	11.64	11.59
C-32	—	—	22.70	16.80
C-33	—	—	161.0	176.32
C-34	—	—	18.08	74.83
C-35	—	—	—	33.23
C-36	—	—	—	7.73
C-37	—	—	—	25.67
C-1′	—	—	166.6	—
C-2′	—	—	122.9	—
C-3′	—	—	110.2	—
C-4′	—	—	153.2	—
C-5′	—	—	148.7	—
C-6′	—	—	123.73	—
C-7′	—	—	112.4	—
OMe	—	—	56.02	—
OMe	—	—	56.03	—

[a]Recorded at 100 MHz in $CDCl_3$.
[b]Missing.

75.30)/H-23 (δ 3.41), C-14 (δ 43.80)/H-14 (δ 1.95), and C-25 (δ 30.28)/H-25 (δ 1.85) were also observed in the HETCOSY spectrum. The methyl carbons resonating at δ 11.12 (C-21), 12.28 (C-18), 16.96 (C-19), and 18.65 (C-27) exhibited cross peaks with their corresponding protons at δ 1.01, 2.16, 1.15, and 0.96, respectively.

The NOESY spectrum of **1** was recorded to establish the relative stereochemistry at various asymmetric centers. NOe interaction between the C-4 methine (δ 5.75) and C-6β methylenic (δ 2.40) protons was consistent with their allylic disposition. Absence of nOe interactions between the C-22 and C-23 protons indicated the trans diaxial disposition of the two protons. Similarly, the H-19β methyl protons showed nOe with various protons present on the β face of the molecule. The ms closely resembled that of **3** with the base peak at *m/z* 110 (8). These studies led to structure **1** for the new alkaloid, named (+)-jervinone.

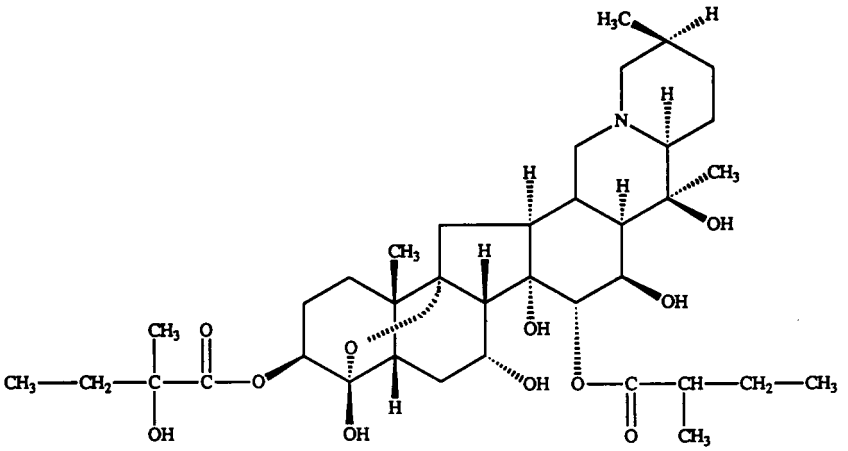
The second compound, (+)-verabenzoamine [**2**], $C_{43}H_{61}NO_{13}$, had the cevane-type structure **2** as established by detailed ^{13}C-nmr and mass spectroscopic studies. It was also isolated from the so-called "acidic fraction" of *V. album* (see Experimental). The uv spectrum of **2** afforded strong absorptions at 218, 261, and 291 nm, characteristic of the benzoate chromophore. The ir spectrum included strong absorptions at 3400 (O-H), 1725 (C=O), 1720 (C=O), 1705 (Ar-C=O), and 1596 (C=C) cm^{-1}.

The ^{1}H-nmr spectrum of **2** was particularly informative, although complex. A three-proton broad triplet at δ 0.88 (J = 7.4 Hz) was assigned to the Me-31 protons of the O^{15}-butyryl group. This signal showed vicinal coupling with the C-30 α and β diastereotopic methylenic protons, which resonated as multiplets at δ 1.40 and 1.70. The ^{1}H-nmr spectrum also showed two three-proton singlets at δ 1.00 and 1.14 for the C-19 and C-21 tertiary methyl protons, while two three-proton doublets at δ 1.08 and 1.13 were due to C-27 and C-32 (of O^{15}-butyryl) secondary methyl protons. A singlet at δ 2.01 was assigned to the acetyl methyl protons. The presence of four downfield methine signals at δ 5.34 (d, $J_{15a,16e}$ = 3.5 Hz), 5.18 (bd, J = 3.7 Hz), 4.62 (m), and 4.34 (dd, $J_{16,15}$ = 3.5 Hz, $J_{16e,17}$ = 1.7 Hz) in the ^{1}H-nmr spectrum could be assigned to the protons of oxygen-bearing carbons at C-15, C-3, C-7, and C-16. Two three-proton singlets at δ 3.91 and 3.93 were due to the MeO proton substituted on the phenyl ring of the benzoyl substituent. Other protons of the phenyl ring appeared at δ 7.63 (dd, J = 8.4 Hz, J = 2.0 Hz), 6.86 (d, J = 8.4 Hz), and 7.54 (d, J = 2.0 Hz).

In the 2D COSY-45° spectrum of **2**, cross peaks appeared between the C-15 (δ 5.34) and C-16 (δ 4.34) methine protons. The splitting of H-16 as a double doublet indicated the presence of another coupling, possibly with H-17. This is consistent with an arrangement -CH(O)-CH(O)-CH- in ring D of the steroidal skeleton. The multiplet at δ 4.62 (H-7) showed COSY interactions with two different protons at δ 2.70 and 2.33. The signal at δ 2.70 was a clear doublet (J = 4.8 Hz) and therefore was assigned to H-8 flanked with quaternary carbons (C-9 and C-13) α to it. The multiplet at δ 2.33, assigned to a C-6 methylene proton, showed COSY interaction with its geminal partner which appeared at δ 1.80. These interactions represented a substructure, CH_2-CH(O)-CH, as a part of ring B. The chemical shifts convincingly correlate with the ^{1}H-nmr chemical shifts of other closely related germine-type alkaloids (10–12) such as 15-(2-methylbutyryl)-germine and 3-(2-hydroxy-2-methylbutyryl)-15-(2-methylbutyryl)-germine [**4**] (13).

The ^{13}C-nmr spectrum of compound **2** was particularly informative and showed 42 carbon resonances. The gated spin-echo experiments helped to distinguish between Me or CH_2 and CH or quaternary carbon atoms. The ^{13}C chemical shift assignments (in ppm) for various carbons are presented in Table 1 and compared with the structurally related compound **4** (13).

The fabms of compound **2** showed a peak at *m/z* 756 which resulted from the loss of an acetyl group from the molecular ion. The hreims showed the highest mass peak at *m/z* 740.3978, corresponding to the formula $C_{41}H_{58}NO_{11}$ (calcd 740.3978) resulting from the probable loss of an acetate moiety from the molecular ion. The base peak at *m/z* 112 was due to the dimethyl piperidine fragment resulting from the cleavage of ring E



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at C-18/C-13 and C-17/C-20 bonds (14). Other major peaks were at *m/z* 575, 182, and 165. The key mass fragmentations are shown in structure **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Polartronic D instrument. Mp's were taken on a Buchi 535 instrument and are uncorrected. Uv spectra were recorded with Hitachi U-3200 and ir spectra on a JASCO A-302 spectrophotometer. ^{1}H nmr and ^{13}C nmr were determined in $CDCl_3$ solutions, with TMS as internal standard, and the signals were recorded at δ values, on a Bruker 300 MHz instrument with an Aspect 3000 computer. Ms were taken using Varian MAT 112S (low resolution) and Jeol JMS HX 100 (high resolution and fab) instruments. Tlc was accomplished on Riedel-DeHaen Si gel coated plates, and the spots were visualized in 254 nm uv light.

PLANT MATERIAL.—Rhizomes of *V. album* were collected from Trabzön, in the northern part of Anatolia, Turkey, in June 1989, and the plant material was identified by Prof. Bilge Sener. A voucher specimen was deposited in the herbarium of the faculty of pharmacy, Gazi University, Ankara.

EXTRACTION AND PURIFICATION.—The fresh rhizomes (50 kg) of *V. album* were ground in a grinder, and the resulting mash was transferred into EtOH (200 liters), shaken, and filtered. The filtrate was concentrated under vacuum to 200 g. The resulting EtOH extract was dissolved in 1 liter of 10% HOAc (pH 3.5) and again filtered. The filtrate was extracted with three 1-liter portions of petroleum ether (40–60°) to remove the chlorophyll part. The acidic mixture was re-extracted with three 1-liter portions of $CHCl_3$, and the combined $CHCl_3$ layers were concentrated under vacuum to a crude gum, 7.0 g. The separation was carried out on a column packed with Si gel (70–230 mesh, Merck) by increasing the polarities. Elution with $CHCl_3$-MeOH (97:3) gave a fraction which was then concentrated to give 200 mg of a yellowish brown material. Further purification with preparative tlc on Si gel (Riedel-DeHaen) precoated plates with $CHCl_3$-petroleum ether-diethylamine (2:7:1) afforded compound **1** as a white amorphous powder. Using $CHCl_3$-petroleum ether-diethylamine (3:7:1) as the eluent, compound **2** was obtained as a light yellow amorphous powder.

(+)-Jervinone [**1**].—Amorphous powder (55 mg): mp 246–248°; $[\alpha]^{25}$D +38.5 (c = 1.00, $CHCl_3$); uv λ max (MeOH) nm 246, ν max ($CHCl_3$) 3605, 1723, 1707, 1620, 1020 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ (ppm) 0.96 (d, J = 6.6 Hz, 3H, Me-27), 1.01 (d, J = 7.5 Hz, 3H, Me-21), 1.15 (s, 3H, Me-19), 2.16 (d, J = 1.9 Hz, 3H, Me-18), 2.78 (t, J = 9.9 Hz, H-22), 3.41 (m, H-23), 5.75 (s, H-4); ^{13}C nmr see Table 1; hrms found 423.2823 (calcd 423.2773 for $C_{27}H_{37}NO_3$); eims *m/z* [M]$^+$ 423 (36%), 408 (12), 394 (32), 312 (10), 125 (67), 113 (53), 110 (100).

(+)-Verabenzoamine [**2**].—Amorphous powder (8.2 mg): mp 181–182°, $[\alpha]^{25}$D +16.6° (c = 1.00, $CHCl_3$); uv λ max (MeOH) nm 218, 261, 291 nm; ν max ($CHCl_3$) 3400, 1725, 1720, 1705, 1596 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ ppm 0.88 (bd, J = 7.4 Hz, H-31), 1.00 (s, 3H, Me-19), 1.08 (d, J = 6.7 Hz, 3H, Me-27), 1.13 (d, H_3-32), 1.14 (s, H_3-21), 1.40 (m, H-30), 2.01 (s, H_3-34), 3.91 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 4.34 (dd, J = 3.5 Hz, J = 1.7 Hz, H-16), 4.62 (m, H-7), 5.18 (bd, J = 3.7 Hz, H-3), 5.34 (d, J = 3.5 Hz, H-15), 6.86 (d, J = 8.4 Hz, H-6′), 7.54 (d, J = 2.0 Hz, H-3′),

7.63 (dd, $J=8.4$ Hz, $J=2.0$ Hz, H-7'); fabms *m/z* 756; hrms found *m/z* 740.3978 $[M-CH_3COO]^+$ (calcd 740.3978 for $C_{41}H_{58}NO_{11}$); eims *m/z* 740 (20%), 638 (0.5), 575 (10), 182 (21), 165 (22), 112 (100), 98 (21); ^{13}C nmr see Table 1.

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