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TWO STEROIDAL ALKALOIDS FROM *VERATRUM VIRIDE*

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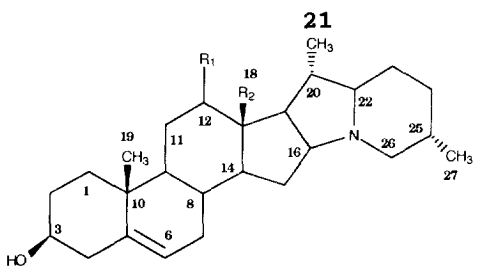
Key Word Index—*Veratrum viride*; Liliaceae; roots; rhizomes; steroidal alkaloids; rubivirine; veramivirine.

Abstract—A phytochemical study of the roots and rhizomes of *Veratrum viride* led to the isolation of the two new steroidal alkaloids, rubivirine, identified as 12β-hydroxyisorubijervine, and veramivirine, identified as 12β-hydroxyveramiline. Structural elucidation of the two compounds was aided by 2D-NMR spectral analyses.

INTRODUCTION

The roots and rhizomes of *Veratrum viride* have been extensively studied for their hypotensive [1] and cytotoxic [2] activities and in the therapy of myasthenia gravis [3]. About 25 steroidal alkaloids have been isolated from this species so far [4]. The isolation of the known alkaloids, veramarine, zygadenine, angeloylzygadenine and 15-*O*-2-methylbutyroylgermine, as well as 1α,3β-dihydroxy 5α-jervanin-12-en-11-one, a new jervanine alkaloid, has been reported [5]. Further phytochemical work on this species has led to the isolation of two new steroidal alkaloids, rubivirine (**1**) and veramivirine (**2**).



#	Compound	R_1	R_2
1	Rubivirine	β-OH	CH_2OH
3	Rubijervine	α-OH	CH_3
4	Isorubijervine	H	CH_2OH
5	Epirubijervine	β-OH	CH_3
6	Solanidine	H	CH_3

RESULTS AND DISCUSSION

The alkaloid-containing fractions were separated by a combination of flash chromatography on silica gel, preparative TLC and fractional recrystallization to give rubivirine (**1**) and veramivirine (**2**). Both compounds gave positive Dragendorff, iodoplatinate and Mayer tests, indicating their alkaloidal nature. They also gave positive Liebermann–Burchard and Salkowski tests, indicating their steroidal nature.

Rubivirine (**1**) was recrystallized from methanol to give needles. Thermospray-LC-MS analysis displayed a $[M + H]^+$ at *m/z* 430 suggesting the molecular formula $C_{27}H_{43}NO_3$. The methyl singlet which absorbed at δ 0.92 in the ^1H NMR (Table 1) is correlated with the methyl carbon at δ 19.2 and was assigned to the 19-methyl group. On the other hand, the two methyl doublets at δ 0.87 (J = 6.2 Hz) and 1.06 (J = 7.4 Hz) are correlated with the two methyl carbons at δ 18.9 and 19.4, and ascribed to the 21- and 27-methyl groups. These assignments revealed the 22,26-epiminocholestane skeleton of this alkaloid [6]. The α-axial proton H-12 resonating at δ 3.30 as a *dd* (J = 7.7 and 4.3 Hz) is correlated with a methine carbon at δ 69.0 and assigned to C-12 on the basis of HETCOR. The proton signals, which resonated at δ 3.97 (1H, *d*, J = 12.2 Hz) and 3.51 (1H, *d*, J = 12.0 Hz), and correlated with the oxygenated methylene carbon at 62.4, were assigned to a C-18 hydroxymethyl group. Confirmation of the position of the hydroxymethyl group was achieved by comparison of the ^{13}C NMR spectra (Table 2) of **1** with that reported for solanidine (**6**), which is 12,18-dideoxyrubivirine [7]. The hydroxyl group substitution at C-18 caused significant shifts at C-13 (+ 4.9), C-17 (− 5.9) as well as C-18 (+ 45.4). It also caused a significant shift at C-12 (− 2.9) as compared with rubijervine (**3**), which is 12α-hydroxysolanidine [5]. The equatorial proton H-26 absorbed at δ 2.91 as a *dd* (J = 9.1 and 2 Hz) and is geminally coupled to the axial H-26 proton which resonated

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Table 1. ^{1}H NMR spectral data of the isolated alkaloids and related compounds (300 MHz, in $CDCl_3$–CD_3OD, 1:1)

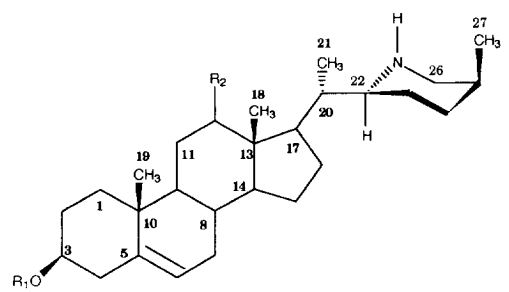
H	3	4*	1	2†
1eq	1.81 *ddd* (13.5, 3.7, 3.1)	1.81 *m*	1.66 *m*	1.82 *m*
1ax	1.09 *ddd* (13, 7.8, 3.3)	1.11 *m*	0.98 *m*	1.06 *m*
2eq	1.77 *m*	1.68 *m*	1.72 *m*	1.75 *m*
2ax	1.55 *m*	1.05 *m*	1.12 *m*	1.45 *m*
3	3.41 *dddd* (15.4, 10.5, 5.6, 5.3)	3.50 *m*	3.46 *m*	3.52 *m*
4eq	2.25 *dd* (13.5, 3.8)	2.28 *ddd* (13.5, 5.6, 1.5)	2.12 *m*	2.24 *m*
4ax	2.18 *ddd* (13.6, 8.2, 1.9)	2.22 *ddd* (13.5, 9.5, 1.4)	1.97 *m*	2.21 *m*
6	5.33 *dd* (4.1, 1.1)	5.33 *br d* (4.9)	5.33 *br s*	5.34 *br s*
7eq	1.98 *dt* (13.4, 4.9)	2.06 *dt* (13.5, 2.3)	1.92 *m*	1.95 *m*
7ax	~ 1.53 *m*	~ 1.57 *m*	~ 1.51	1.82 *m*
8	~ 1.62 *m*	~ 1.58 *m*	~ 1.60	1.56 *m*
9	1.72 *m*	~ 1.04 *m*	~ 1.01	0.97 *m*
11eq	~ 1.67	1.49 *m*	1.55 *m*	1.62 *m*
11ax	~ 0.87	1.46 *m*	0.89	1.42 *m*
12eq	3.69 *dd* (1.1, 1)	~ 1.75	—	
12ax	—	~ 1.03	3.30 *dd* (7.7, 4.3)	4.1 *dd* (8.8, 3.6)
14	1.34 *m*	1.30 *m*	1.33 *m*	1.43 *m*
15eq	1.64 *m*	1.76 *m*	~ 1.30	1.49 *m*
15ax	1.18 *m*	1.29 *m*	~ 1.25	1.41 *m*
16	2.66 *ddd* (10.5, 10, 3.2)	2.76 *ddd* (8.3, 8.2, 4.5)	1.59 *m*	1.63 *m*
17	2.27 *dd* (10.7, 10.5)	~ 1.66	1.23 *m*	1.43 *m*
18	0.88 3H *s*	3.86 *d* (12.1)	3.97 *d* (12.2)	0.71 3H *s*
	—	3.46 *d* (12.2)	3.51 *d* (12)	—
19-H3	1.01 3H *s*	1.03 3H *s*	1.04 *s*	0.98 3H *s*
20	1.61 *m*	1.89 *m*	1.82	1.89 *m*
21-H_3	0.94 3H *d* (5.9)	0.84 3H *d* (6.2)	0.87 *d* (6.2)	1.01 *d* (7.1)
22	1.59 *m*	1.61 *m*	1.75 *m*	2.78 *m*
23eq	1.63 *m*	1.63 *m*	1.62 *m*	1.71 *m*
23ax	1.17 *m*	1.56 *m*	1.53	1.34 *m*
24eq	~ 1.67 *m*	~ 1.78 *m*	~ 1.70 *m*	1.63 *m*
24ax	~ 1.60 *m*	~ 1.46 *m*	~ 1.60 *m*	1.62 *m*
25	1.69 *m*	1.84 *m*	1.81 *m*	1.91 *m*
26eq	2.91 *dd* (10.6, 3)	2.90 *dd* (9.1, 2)	2.91 *dd* (9.8, 2)	2.89 *dd* (9.1, 2)
26ax	1.46 *dd* (11.1, 3.3)	1.56 *m*	~ 1.56 *m*	1.58 *m*
27-H_3	0.86 3H *d* (6.7)	1.01 3H *d* (6.7)	1.03 *d* (6.3)	1.06 *d* (7.4)

*In $CDCl_3$.
†In $CDCl_3$, 500 MHz.

at δ 1.46. Both protons were correlated to the same methylene carbon at δ 60.4. Interestingly, the deshielded methine carbon at δ 74.4 correlated with a proton which resonated at δ 1.75 and was assigned to H-22.

Isorubijervine (**4**) and epirubijervine (**5**) are suggested to be intermediates in the biogenesis of the C-nor-D-homosteroidal *Veratrum* alkaloids [8]. The proposed pathway is acetate → mevalonate → cholesterol → solanidanes (probably isorubijervine and/or epirubijervine) → C-nor-D-homosteroidal alkaloids [9]. Rubivirine is presumably another intermediary phase in the process of *Veratrum* alkaloid biogenesis.

Veramivirine (**2**) was recrystallized from methanol to give needles. Thermospray-LC-MS analysis displayed a $[M + H]^+$ at *m/z* 416 suggesting the molecular formula $C_{27}H_{45}NO_2$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) showed a close resemblance to those of veramiline (**7**) and veramiline 3-β-D-glucopyranoside (**8**), 22,26-epiminocholestane alkaloids previously isolated from other *Veratrum* species [10–12].



#	Compound	R_1	R_2
2	Veramivirine	H	β-OH
7	Veramiline	H	H
8	Veramiline 3-glucoside	β-D-glucose	H

The two methyl singlets which resonated at δ 0.71 and 0.98 in the ^{1}H NMR spectrum were correlated with the two methyl carbons at δ 13.2 and 19.2, and were assigned to the 18- and 19-methyl groups. On the other hand, the two methyl doublets at δ 1.01 (J = 7.1 Hz) and 1.06

Table 2. ^{13}C NMR spectral data of the isolated alkaloids and related compounds (75.3 MHz, $CDCl_3$–CD_3OD, 1:1)

C	1	3	4*	2†	8‡
1	37.4	36.6	37.3	37.0	37.7
2	30.0	30.1	33.0	31.4	29.1
3	71.7	70.7	71.7	71.6	78.5
4	42.5	41.4	42.3	42.2	38.2
5	141.0	140.6	140.8	140.7	140.0
6	121.1	121.1	121.3	121.6	121.1
7	31.8	31.4	31.9	31.4	31.5
8	30.4	30.6	30.7	31.3	31.3
9	50.6	48.8	50.4	49.8	49.6
10	36.8	35.9	36.7	36.3	36.2
11	32.1	32.7	21.3	31.3	21.6
12	69.0	71.9	36.7	75.6	39.3
13	45.5	43.7	45.4	44.3	42.2
14	47.2	42.5	57.2	53.6	56.3
15	21.4	28.1	29.4	20.6	23.7
16	62.1	68.8	68.3	35.1	28.4
17	57.4	52.9	62.2	61.3	51.9
18	62.4	16.7	62.9	13.2	17.8
19	19.2	18.6	19.3	19.2	18.7
20	36.1	36.5	35.9	26.2	42.2
21	18.9	16.7	19.2	16.1	17.4
22	74.4	74.4	73.7	61.8	49.1
23	28.7	28.1	30.5	21.1	27.0
24	32.8	31.1	31.6	29.8	30.9
25	31.5	30.1	31.4	39.6	36.8
26	57.9	60.4	57.7	51.0	51.0
27	19.4	18.9	19.3	19.5	20.6

*In $CDCl_3$.
†In $CDCl_3$, 125.3 MHz.
‡In pyridine-d_5, glucose carbons are omitted [12].

(J = 7.4 Hz) were correlated with the two methyl carbons at δ 16.1 and 19.5, and are ascribed to the 21- and 27-methyl groups. These assignments revealed the 22,26-epiminocholestane skeleton of this alkaloid [6]. The α-axial proton H-12 resonating at δ 4.10 as a *dd* (J = 8.8 and 3.6 Hz) is correlated with a methine carbon at δ 75.6 and is assigned to C-12 on the basis of a HMQC-correlation spectrum. Further structural confirmation was achieved by a ^1H–^{13}C HMBC correlation experiment. The quaternary olefinic C-5, resonating at δ 140.7 showed ^3J-coupling correlations with the H-1 equatorial multiplet at δ 1.82 and the C-19 methyl singlet at δ 0.98. The latter methyl singlet exhibited ^3J-couplings with C-1 at δ 37.0 and ^2J-correlation with the quaternary C-10 at δ 36.3. Similar correlations occurred between the C-12 axial proton at δ 4.10 with C-14 and C-17 at δ 53.6 and 61.3, respectively. The C-14 at δ 53.6 displayed ^3J-correlation with the equatorial H-7 (δ 1.95), H-16 (δ 1.63), H-17 (δ 1.43) and H_3-18 (δ 0.71) proton signals. The C-21 methyl doublet signal at δ 1.01 also displayed ^3J-correlations with C-17 and C-22 at δ 61.3 and 61.8, respectively. On the other hand, the C-27 methyl doublet at δ 1.06 showed ^3J-correlations to C-24 and C-26 at δ 29.8 and 51.0, respectively. ^1H–^1H COSY, TOCSY and NOESY experiments further confirmed these assignments. Therefore, veramivirine was shown to be 12-β-hydroxyveramiline [(22*S*,25*S*)]-22,26-epiminocholest-5-ene-3β,12β-diol (**2**).

EXPERIMENTAL

General. Thermospray LC-MS were recorded on Vestec Model 201 mass spectrometer. NMR were recorded at 300 and 500 MHz for proton and 75 and 125 MHz for carbon; TMS was used as int. standard.

Plant material. Dried roots and rhizomes of *V. viride* Ait. (6.5) kg were purchased from Wilcox Drug Company, Boone, U.S.A. Identity was confirmed by comparing transverse sections of root and rhizome with those described in the lit. [13].

Extraction and isolation. The dried ethanolic extract (460 g) was dissolved in 10 l of 5% tartaric acid. The pH of the soln was adjusted to 6 and extracted with $CHCl_3$. The dried $CHCl_3$ extract (18.6 g) was flash chromatographed using 520 g silica gel (70–230 mesh), starting with cyclohexane–EtOAc–diisopropylamine (20:4:1) then with increasing proportions of EtOAc and diisopropylamine and finally isocratically with 15:8:2 (system I), collecting 100 ml frs. Frs 28–53 obtained by elution with system I were pooled and rechromatographed over silica gel. Elution with cyclohexane–EtOAc–Et_3N (10:4:1) (system II) afforded two major subfrs. Subfrs 15–16 were repeatedly chromatographed on silica gel using system II to afford rubivirine (**1**) (27 mg). Subfrs 23–26 were treated similarly to afford veramivirine (**2**) (18.5 mg).

Rubivirine (**1**). Recrystallized from MeOH as needles, mp 239–241°. $[\alpha]_D$ + 28.5 ($CHCl_3$; *c* 0.1). Themospray-LC-MS: *m/z* 430 $[M + H]^+$. IR ν^{KBr} cm^{-1}: 3460, 1450, 1370 and 1040.

Veramivirine (**2**). Recrystallized from MeOH as needles, mp 229–231°. $[\alpha]_D$ − 81 ($CHCl_3$; *c* 0.1). Thermospray LC-MS: *m/z* 416 $[M + H]^+$. IR ν^{KBr} cm^{-1}: 3500, 1460 and 1065.

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