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ISOLATION AND IDENTIFICATION OF FOUR NORDITERPENOID ALKALOIDS FROM PROCESSED AND UNPROCESSED ROOT TUBERS OF *ACONITUM FEROX*[1]

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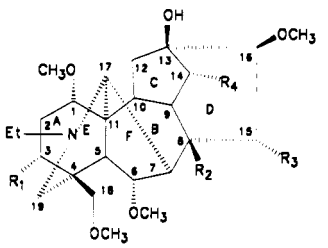
ABSTRACT.—From Ayurvedic processed and unprocessed root tubers of *Aconitum ferox* (Ranunculaceae) of commerce four norditerpenoid alkaloids, bikhaconitine [**1**], pseudaconitine [**2**], veratroylbikhaconine [**3**], and veratroylpseudaconine [**4**], were isolated. ^{1}H, ^{13}C, H,H-COSY, CH-HETCOR, and homonuclear decoupling nmr studies of **3** and **4** led to the assignment of all proton signals of compounds **1**–**4**, as well as of aconitine [**5**] and benzoylaconine [**6**]. The yield of alkaloids of the Ayurvedic processed tubers amounts to about 40% of the unprocessed tubers.

The root tubers of *Aconitum ferox* Wall (Ranunculaceae) are used for various medical indications in India, either as such or after processing by an Ayurvedic method (2–4). The therapeutic indications are remarkably varied (2), including fever, neuralgia, and rheumatism. The provenance of commercial *A. ferox* tubers is not clear. They are mixtures of various Himalayan aconite species (2,3) including *A. ferox*.

We investigated the changes in the alkaloidal components brought about by the Ayurvedic method of processing in cow urine. The tubers were supplied by Zandu Pharmaceutical Works Ltd., Bombay, India. They were labeled "Aconitum ferox." We based the macroscopic and microscopic examination of the material on the work of A. Goris (5) who used the taxonomy of P. Brühl (6). As far as the material could be identified, the cross sections showed a stellate cambium circle with numerous rays typical for the subspecies *laciniatum* P. Br. of *A. ferox*, which later was renamed *Aconitum laciniatum* Stapf by O. Stapf (7).

From the less polar fractions of the processed and unprocessed tubers we isolated bikhaconitine [**1**], pseudaconitine [**2**], 14-veratroylbikhaconine [**3**], and 14-veratroylpseudaconine [**4**]. Compounds **1**, **2**, and **4** were isolated earlier from tubers of



	R_1	R_2	R_3	R_4
1	H	OAc	H	OVr
2	OH	OAc	H	OVr
3	H	OH	H	OVr
4	OH	OH	H	OVr
5	OH	OAc	OH	OBz
6	OH	OH	OH	OBz

[1]Communication No. 11 about *Aconitum*. For communication no. 10, see Katz (1).

A. ferox (8–12). The isolation of **3** from *Aconitum balfourii* Stapf was reported by K.S. Khetwal *et al.* (13) while this paper was being written. We identified the four compounds on the basis of uv, ir, ms, and ^{1}H,^{1}H-COSY, homonuclear spin-decoupling, ^{13}C, and HETCOR, and by comparison with the earlier reported ^{13}C spectra (12–15). The nmr data are given in Tables 1–3, and the characteristic and noteworthy features of the spectra are discussed. For comparison we have also included the ^{1}H spectra of aconitine [**5**] (16) and benzoylaconine [**6**].

TABLE 1. ^{1}H Chemical Shifts of Compounds **1–6**.

Proton	Compound					
	1	**2**	**3**	**4**	**5**[a]	**6**[b]
H-1	2.96–3.08 m	3.08–3.18 m	3.04 brt	3.12 dd (8,6)	3.10–3.13 m	3.13 brt
H-2	1.90–2.11 m	1.95–2.11 m	1.90–2.08 m	2.03–2.17 m	1.97–2.16 m	1.81–1.95 m
	2.18–2.34 m	2.25–2.45 m	2.19–2.33 m	2.25–2.45 m	2.31–2.43 m	2.25–2.45 m
H-3	1.64–1.73 m	3.76 dd (9,5)	1.55–1.72 m	3.64–3.78 m	3.75–3.80 m	3.83 m
	1.64–1.73 m	—	1.55–1.72 m	—	—	—
H-5	2.08–2.11 m	2.05–2.16 m	2.04–2.12 m	2.03–2.13 m	2.06–2.16 m	2.00–2.20 m
H-6	3.96 m	4.02 d (6.4)	4.02 d (6.6)	4.07 d (6.7)	4.04 d (6.4)	4.07 d (6.8)
H-7	2.40–2.57 m	2.90–2.08 m	2.03–2.17 m	2.45–2.51 m	2.83 m	2.95 s
H-9	2.85–2.98 m	2.87–2.91 m	2.47–2.61 m	2.51–2.57 m	2.88–2.91 m	2.45–2.60 m
H-10	2.00–2.11 m	2.05–2.15 m	2.04–2.12 m	2.03–2.13 m	2.10–2.16 m	2.07–2.20 m
H-12	2.00–2.08 m	2.05–2.15 m	2.00–2.09 m	2.03–2.13 m	2.10–2.16 m	2.07–2.20 m
	2.71–2.82 m	2.55–2.69 m	2.49–2.69 m	2.31–2.57 m	2.71–2.78 m	2.51–2.66 m
H-14	4.87 d (5.2)	4.87 d (5)	5.15 d (4.8)	5.12 d (5)	4.87 d (5.2)	5.00 d (5)
H-15	2.40–2.57 m	2.40–2.55 m	2.19–2.33 m	2.22–2.42 m	4.47 dd (6,3)	4.52 m
	2.96–3.08 m	3.06 m	2.50–2.69 m	2.51–2.68 m	—	—
H-16	3.38 dd (8,5)	3.36–3.43 m	3.26–3.39 m	3.31–3.42 m	3.34 d (5.4)	3.22–3.29 m
H-17	3.00 brs	3.00 brs	3.00–3.09 m	3.00 brs	3.10–3.13 m	3.25 s
H-18	3.17 m	3.51 m	3.26–3.39 m	3.64–3.78 m	3.50 d (8.9)	3.45–3.53 m
	3.61 d (8.4)	3.64 d (8.9)	3.66 d (8.4)	3.64–3.78 m	3.63 d (8.9)	3.61 d (8.8)
H-19	2.40–2.57 m	2.25–2.45 m	2.19–2.33 m	2.42 d (12)	2.31–2.39 m	2.43 d (11)
	2.96–3.08 m	2.89 m	2.41–2.69 m	2.94 d (12)	2.83–2.91 m	2.86 d (11)
H-20	2.48–2.71 m	2.36–2.60 m	2.41–2.69 m	2.39–2.57 m	2.31–2.43 m	2.28–2.50 m
					2.66–2.84 m	2.51–2.73 m
21-Me	1.09 t (7)	1.10 t (7.1)	1.09 t (7.1)	1.12 t (7)	1.09 t (6.9)	1.09 t (7)
1-OMe	3.26 s	3.26 s	3.27 s	3.27 s	3.16 s	3.22 s
6-OMe	3.15 s	3.16 s	3.26 s	3.25 s	3.26 s	3.29 s
16-OMe	3.52 s	3.53 s	3.39 s	3.42 s	3.30 s	3.29 s
18-OMe	3.28 s	3.30 s	3.30 s	3.31 s	3.75 s	3.70 s
3′- OMe	3.91 s	3.92 s	3.93 s	3.93 s	—	—
4′- OMe	3.94 s	3.94 s	3.94 s	3.94 s	—	—
8-OAc	1.31 s	1.33 s	—	—	1.39 s	—
3-OH	—	2.09 s	—	2.06 s	2.73 s	1.91 s
8-OH	—	—	2.25 s	2.27 s	—	2.48 s
13-OH	3.82 s	3.86 s	3.86 s	3.86 s	3.96 s	3.83 s
15-OH	—	—	—	—	4.39 d (3)	4.31 s
Aromatic						
2′- H	7.61 d (1.8)	7.62 d (1.2)	7.59 d (1.7)	7.59 d (1.7)	8.00–8.04 d	7.98–8.02 m
3′- H	—	—	—	—	7.41–7.49 m	7.36–7.44 m
4′- H	—	—	—	—	7.54–7.63 m	7.51–7.59 m
5′- H	6.89 d (8.4)	6.90 d (8.0)	6.90 d (8.4)	6.90 d (8.4)	7.41–7.49 m	7.36–7.44 m
6′- H	7.71 dd	7.70 dd	7.69 dd	7.67 dd	8.00–8.06 m	7.98–8.02 m
	(1.8,8.4)	(1.2,8)	(1.7,8.4)	(1.7,8.4)		

[a]New chemical shift values.
[b]Known compound with chemical shift assignments reported for the first time.

RESULTS AND DISCUSSION

The molecular ion peak *m/z* $[M]^+$ 631 and the 34 signals exhibited by the ^{13}C spectrum of **3** suggested the molecular formula $C_{34}H_{49}NO_{10}$ of a norditerpenoid ester alkaloid. The DEPT spectrum revealed six MeO, seven methylene, nine methine, three unsubstituted, and four quaternary carbons, one of which (δ 166.5) is due to an ester carbonyl. The ir spectrum showed OH (3482 cm^{-1}, br), ester carbonyl (1715 cm^{-1}), aromatic nucleus (1603, 1517, 1456 cm^{-1}), symmetrical and asymmetrical stretchings of =C-O-C (1271, 1024 cm^{-1}), and 1,3,4-trisubstituted aromatic nucleus (766 cm^{-1}). Signals uv absorptions at λ max 260.0 nm (log ε 4.09) and 289.8 nm (log ε 3.83) confirmed the aromatic moiety.

In the ^1H spectrum (Table 1) of compound **3** the A_2B_2X system of H-1, H_2-2, and H_2-3 was documented by a triplet of H-1 and two multiplets of H_2-2 and one multiplet of H_2-3, whereas the ABXY system of **4** showed a doublet of doublet of H-1 and a multiplet of H_2-2 as well of H-3.

The H-6 of both compounds **3** and **4** showed coupling with the H-5, while there was no coupling with H-7 due to the dihedral angle (ca. 110°) of H_6/H_7. The HETCOR spectrum (Table 5) of **4** showed that the ^{13}C signal at δ 47.91 (s) was due to C-5 as well as to C-7.

The assignments of the ring C protons, i.e., H-14, H-12, H-10, and H-9, were based on irradiation studies (Table 2). In the ^1H-COSY (Table 3) spectrum long range coupling due to W configuration between H-14/H-16 was observed.

The ABX system of H_2-15/H-16 in compound **4** was noted. Irradiation of H-16 produced two doublets of H_2-15. Irradiation of H_B-15 (δ 2.45) changed the multiplet of H-16 to a doublet of 4.0 Hz, while the irradiation of H_A-15 (δ 2.28) changed the

TABLE 2. Spin-decoupling Data of Compounds **3** and **4**.

3				**4**			
Irradiated Position	ppm	Responding Position	ppm	Irradiated Position	ppm	Responding Position	ppm
H-1	3.06	H_a-2	2.05	H-1	3.12	H_a-2	2.06
		H_b-2	2.25			H_b-2	2.25
H_a-2.....	2.05	H_b-2	2.55	H_a-2	2.04	H_b-2	2.30
		H-1	3.08			H-1	3.12
		H_2-3	1.63			H-3	3.67
H_b-2	2.32	H_a-2	2.05	H_b-2	2.47	H_a-2	2.08
		H-1	3.08			H-1	3.12
		H_2-3	1.63			H-3	3.67
H_2-3	1.67	H_a-2	2.05	H-3	3.67	H_a-2	2.08
		H_b-2	2.25			H_b-2	2.30
H-5	2.05	H-6	4.02	H-5	2.08	H-6	4.07
H-6	4.03	H-5	2.08	H-6	4.07	H-5	2.00
H-9	2.56	H-14	5.15	H-9	2.55	H-14	5.10
						H-10	2.10
				H-10	2.04	H-9	2.57
H_a-12.....	2.05	H_b-12	2.55	H_a-12	2.47	H_b-12	2.08
H-14	5.15	H-9	2.54	H-14	5.12	H-9	2.55
H-16	3.32	H_a-15	2.25	H-16	3.32	H_a-15	2.30
		H_b-15	2.55			H_b-15	2.60
H_a-18.....	3.66	H_b-18	3.32				
H_a-19.....	2.25	H_b-19	2.55	H_a-19	2.94	H_b-19	2.40
H_2-20	2.56	H_3-21	1.09	H_2-20	2.55	H_3-21	1.09
H_3-21	1.10	H_2-20	2.55	H_3-21	1.09	H_2-20	2.53

multiplet of H-16 to a doublet of 8.0 Hz. Hence, the signal δ 2.51–2.68 was assigned to H_α- 15, and the signal at δ 2.22–2.42 to H_β-15. The situation in **3** was complex as the H-16 signal was merged with signals of MeO- and one H-18. Accordingly, irradiation of H-16 not only changed the multiplicity pattern of H_2-15 but also changed the doublet of the second H-18 to a singlet.

In compounds **3**, **4**, and **6**, H-16 and 13-OH assumed W conformation and exhibited long range coupling. In these compounds the aromatic ester carbonyl was bonded by 8-OH (Figure 1a). In the parent 8-acetyl derivatives **1**, **2**, and **5** there is no such bonding. The acetoxy group forces the aromatic ester group in a position close to 13-OH favoring the formation of a hydrogen bond with the aromatic ester carbonyl (Figure 1b), whereby it looses the W conformation with H-16. Consequently there is no long range coupling between 13-OH and H-16.

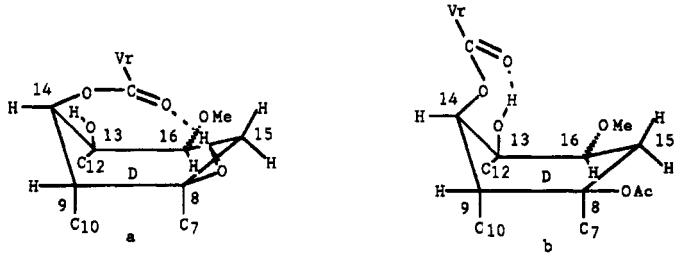


FIGURE 1. Partial structures of compounds **3** and **4** (a), **1** and **2** (b).

In **4**, H_2-19 exhibited a doublet each at δ 2.42 (H_A of AB) and δ 2.94 (H_B of AB). H_A changed to a broad singlet when H_B was irradiated, while the broad singlet of H_B resulting from the irradiation of H_A exhibited a fine splitting (J=2 Hz) due to long range coupling with H-5 and/or H-17, both of which showed W-shaped bonding connections with H_B-19. This also discloses that H_A-19 (δ 2.42) is the axial and H_B-19 (δ 2.94) is the equatorial proton.

In the ^{13}C-nmr spectra of **3** and **4**, several assignments given by K.S. Khetwal *et al.* (13) were interchanged (Table 4). Based on HETCOR spectra of the compounds, we interchanged the assignments to C-1, C-6, and C-16 in compounds **3** and **4**, and by analogy also in compound **1**. Also based on HETCOR the signals of C-7 and C-9 in **3** and **4** were reversed. By analogy to aconitine [**5**], for which the assignments were made by Joshi *et al.* (19) on the basis of HETCOR, we interchanged the assignments of C-2 and C-12, as well as of C-19 and C-20 in compounds **2** and **4**. We found the shift of C-2 in compound **2** at δ 26.18. Comparing the ^{13}C-nmr shifts given in literature for C-13 in the parent 8-acetyl compounds, yunaconitine (18), indaconitine (18), aconitine (17,19), crassicauline A (18), forestine (19), and pseudaconitine (13), to the corresponding 8-deacetyl compounds (13,18,20), downfield shifts of 1.0±0.3 ppm were observed. By analogy we assigned in compound **3** the signals at 73.77 ppm to C-8 and at 76.15 to C-13, thus having a downfield shift of 1.22 ppm in the C-13 shifts of compounds **1** to **3**.

This is the first report of complete ^{1}H-nmr spectra of compounds **1–4** and **6**.

In order to verify that compounds **3** and **4** are not artifacts due to the extraction procedure, we also prepared a $CHCl_3$ extract without addition of alkali. Tlc comparison of the neutral and the alkaline extracts showed that compounds **3** and **4** are present in

TABLE 3. ^{1}H-^{1}H COSY for **1–6**.

Proton	Compound						H/H Connectivities
	1	**2**	**3**	**4**	**5**	**6**	
H-1	3.02	3.10	3.04	3.12	3.11	3.13	H_a-2/H_b-2
H_a-2	2.00	2.03	2.00	2.08	2.06	1.90	H_b-2/H-1
							$H_{a,b}$-3
H_b-2	2.26	2.35	2.26	2.35	2.37	2.35	H_a-2/H-1
							$H_{a,b}$-3
$H_{a,b}$-3	1.68	3.76	1.63	3.71	3.77	3.83	H_a-2/H_b-2
H-5	2.09	2.10	2.08	2.08	2.11	2.10	H-6
H-6	3.96	4.02	4.02	4.07	4.04	4.07	H-5/H-7
H-7	—	—	—	—	2.83	2.95	H-6
H-9	2.91	2.89	2.54	2.54	2.89	2.52	H-10/H-14
H-10	2.05	2.10	2.08	2.08	2.13	2.13	H-9/H_a/H_b-12
H_a-12	2.05	2.10	2.08	2.08	2.13	2.13	H_b-12
H_b-12	2.76	2.62	2.60	2.46	2.74	2.58	H_a-12/H-10
H-14	4.87	4.87	5.15	5.12	4.87	5.00	H-9
H_a-15	2.48	2.47	2.26	2.32	4.47	4.52	H_b-15/H-16
H_b-15	3.02	3.06	2.59	2.59	—	—	H_a-15/H-16
H-16	3.38	3.39	3.32[a]	3.36[a]	3.34	3.25[a]	H_a-15/H_b-15
							OH-13[a]
H-17	3.00[a]	3.00[a]	3.04[a]	3.00[a]	3.12[a]	3.25[a]	H-6[a]
H_a-18	3.17	3.51	3.32	—	3.50	3.49	H_b-18
H_b-18	3.61	3.64	3.66	—	3.63	3.61	H_a-18
H_a-19	2.48	2.35	2.26	2.42	2.35	2.43	H_b-19
H_b-19	3.02	2.89	2.55	2.94	2.87	2.86	H_a-19
$H_{a,b}$-20	2.59	2.48	2.55	2.50	2.37	2.39	$H_{b,a}$-20, Me-21
					2.75	2.62	
Me-21	1.09	1.10	1.09	1.12	1.09	1.09	$H_{a,b}$-20
Aromatic							
H-2′	7.61	7.62	7.59	7.59	8.02	8.00	H-6′/H-3′/H-4′
H-3′	—	—	—	—	7.45	7.40	H-2′/H-4′/H-5′
H-4′	—	—	—	—	7.58	7.55	H-2′/H-3′/H-5′/H-6′
H-5′	6.89	6.90	6.90	6.90	7.45	7.40	H-3′/H-4′/H-6′
H-6′	7.71	7.69	7.69	7.67	8.03	8.00	H-2′/H-4′/H-5′

[a]Long range coupling.

the extracts prepared without addition of alkali, both from processed and from unprocessed tubers. Hence both Ayurvedic processed and unprocessed tubers of *A. ferox* contained the alkaloids **1–4**.

The processing of the tubers with cow urine reduced the alkaloidal components of the extract by about 60%.

14-Veratroylbikhaconine [**3**], prepared by acid hydrolysis of bikhaconitine [**1**] (12), showed the same R_f value in tlc and co-tlc as isolated **3**. It is interesting to note that boiling H_2O hydrolyzes the 8-acetyl group of aconitine [**5**] (16), which has an OH at C-15 neighboring C-8, to yield 14-benzoylaconine [**6**], while bikhaconitine [**1**], which has no 15-OH, is not hydrolyzed under these conditions.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Kofler hot-stage and are uncorrected. Nmr spectra were recorded on a Bruker 200 spectrometer in $CDCl_3$; δ are reported in ppm downfield relative to TMS as internal standard, *J* in Hz. Ms was recorded on a VG 70-SE mass spectrometer at 70 eV by eims and fabms. The matrix used for fabms was thioglycerin. Ir spectra were recorded on a Perkin-Elmer 281. Cc was done on Merck Kieselgel 60, and tlc on Merck tlc plates Si gel 0.25 mm and neutral Al_2O_3 0.25

TABLE 4. ^{13}C Chemical Shifts of Compounds **1–4**.

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	84.95[b]	82.33[a]	85.50[b]	82.59[a]
C-2	26.24	33.65[b]	26.18	33.65[b]
C-3	34.74	71.62	35.05	71.97
C-4	39.15	43.22	39.36	43.29
C-5	49.10	48.69	49.82	47.91
C-6	83.06[b]	83.16[a]	82.61[b]	82.49[a]
C-7	49.10	47.33	53.68	53.47[b]
C-8	85.53	85.55	73.77[b]	73.83[b]
C-9	45.11	44.67	48.41[b]	47.91
C-10	41.01	40.87	42.36	42.00
C-11	50.24	50.28	50.32	50.27
C-12	35.70	35.19[b]	36.46	35.83[b]
C-13	74.93	74.83	76.15[b]	75.88
C-14	78.64	78.61	80.11	79.83
C-15	39.52	39.76	42.14	42.35
C-16	83.89[b]	83.77[b]	83.49[b]	83.23[b]
C-17	62.01	61.70	62.26	61.84
C-18	80.36	77.03	80.66	77.42
C-19	53.77	47.46[b]	53.77	47.45[b]
C-20	49.21	48.82[b]	49.28	48.93[b]
C-21	13.41	13.36	13.67	13.52
C-1′	56.05	56.05	56.07	55.86
C-6′	57.86	57.81	57.59	57.55
C-16′	58.78	58.84	58.40	58.40
C-18′	59.13	59.16	59.22	59.17
O=C	169.87	169.87	—	—
CH_3	21.69	21.68	—	—
Aromatic				
O=C	166.08	166.00	166.53	166.35
C-1	122.83	122.72	122.60	122.40
C-2	110.34	110.46	110.48	110.41
C-3	148.70	148.74	148.77	148.70
C-4	153.05	153.11	153.20	153.17
C-5	112.04	112.03	112.28	112.17
C-6	123.75	123.75	123.80	123.75
C-3′	56.27	55.88	56.01	56.30
C-4′	55.86	55.88	55.94	56.01

[a]These values are interchangeable.
[b]New assignments.

mm. The reported R_f values were determined on Alox plates with the solvent system C_6H_{12}-EtOAc-EtOH (75:20:5). Yield refers to percent of powdered tubers.

PLANT MATERIAL.—Ayurvedic processed and unprocessed root tubers of *A. ferox*, both belonging to the same source of supply, were provided by Zandu Pharmaceutical Works Ltd., Bombay, India. Voucher specimens of the tubers are deposited in the author's herbarium.

AYURVEDIC PROCESSING OF THE TUBERS.—According to Zandu Pharmaceutical Works Ltd., Bombay, India, the tubers (100 g) were broken into pieces, soaked in cow urine (500 ml), and kept in sunlight for 3 days. Once a day the cow urine was changed. The pieces of tuber were washed with H_2O and dried.

EXTRACTION AND ISOLATION.—To the powdered tubers (100 g) of *A. ferox*, both unprocessed and Ayurvedic processed, 25% NH_4OH solution was added until the mixture was at pH 9 (checked with reagent

TABLE 5. HETCOR of **3** and **4**.

Position	Compound			
	3		**4**	
	δ_C	δ_H	δ_C	δ_H
1	85.50	3.00	82.50	3.10
2	26.00	2.00	33.90	—
		—		2.10
3	35.00	1.60	72.00	3.70
5	50.00	2.05	48.00	2.10
6	82.60	4.00	82.50	4.07
7	54.00	2.05	53.60	2.00
9	48.50	2.55	48.0	2.55
10	42.20	2.05	42.50	2.05
12	36.50	2.05	36.00	2.45
		2.62		—
14	80.00	5.15	80.00	5.10
15	42.00	2.25	42.50	2.30
		2.60		2.60
16	83.50	3.30	83.50	3.35
17	62.30	3.00	62.00	3.00
18	80.70	3.30	77.50	3.70
		3.65		—
19	54.00	—	47.50	2.45
		2.55		2.90
20	49.30	2.55	49.00	2.50
21	13.70	1.10	13.90	1.10
1-OMe	56.10	3.25	56.00	3.25
6-OMe	57.60	3.25	57.50	3.25
16-OMe	58.20	3.40	58.50	3.45
18-OMe	59.50	3.30	59.40	3.35
3′-OMe	56.00	3.90	56.50	3.90
4′-OMe	56.00	3.90	56.50	3.90

paper Alkalit Merck). The mixture was extracted 3 times with 400 ml of $CHCl_3$ at room temperature. The $CHCl_3$ extracts were evaporated to dryness in vacuo. The dark brown extract was dissolved in EtOH (3 ml/1 g of extract), acidified with 1 N HCl to pH 1, and extracted with pentane. The aqueous layer was brought to pH 9 by addition of 25% aqueous NH_4OH solution and extracted 3 times with $CHCl_3$. The extract was subjected to cc on Si gel (40 g of Si gel/1 g of extract). Twenty fractions of 150 ml were eluted with a polarity gradient solvent system starting with C_6H_{12}-$CHCl_3$-Et_2NH (7:2:0.5). From fractions 6 and 7 {C_6H_{12}-$CHCl_3$-Et_2NH (7:2:1)] compounds **1** and **3** were isolated after rechromatographing on a Si gel column followed by purification of the isolates by preparative tlc on Alox plates. Compounds **2** and **4** were eluted with C_6H_{12}-$CHCl_3$-Et_2NH (5:4:1) (fractions 8 and 9) in impure state. Further purification by preparative tlc on Alox plates with C_6H_{12}-EtOAc-EtOH (8:1:1) gave pure **2** and **4**.

Subsequently the powdered tubers were extracted in the same way first with $CHCl_3$ at pH 12, then air-dried at room temperature and subsequently extracted with $CHCl_3$-MeOH (25:75) at pH 9. Purification of these extracts was effected as described above. The yields are summarized in Table 6.

Bikhaconitine [**1**].—Colorless, fluffy crystals: mp 102–104° [lit. (12) 118°] (Et_2O/pentane); R_f 0.69, identical with an authentic sample of **1**; ir ν max (KBr) 3485 (br), 2933, 1717, 1601, 1514, 1464, 1347, 1273, 1224, 1177, 1093, 1022, 765; ^1H and ^{13}C nmr see Tables 1–3.

Pseudaconitine [**2**].—Colorless crystals: mp 205–207° (Me_2CO/hexane) [lit. (12) 208°]; R_f 0.42, identical with an authentic sample of **2**; ir ν max (KBr) 3510 (br), 2937, 1700, 1600, 1520, 1463, 1348, 1270, 1229, 1180, 1093, 1016, 983, 765; ^1H and ^{13}C nmr see Tables 1–3.

TABLE 6. Yields of Extracts and Alkaloids from Root Tubers of *Aconitum ferox*.

Extract	Unprocessed (492 g)				Processed (517 g)			
	pH 9		pH 12		pH 9		pH 12	
	g	%	g	%	g	%	g	%
Crude $CHCl_3$ extract	5.4	1.1	1.5	0.35	3.5	0.68	2.3	0.44
Purified	2.4	0.49	1.2	0.24	1.9	0.23	0.35	0.07
Pentane	3.0	0.62	0.28	0.06	2.3	0.44	0.42	0.08
Crude $CHCl_3$/MeOH extract	2.6	0.53			0.79	0.15		
Purified	1.7	0.34			0.35	0.07		
Pentane	0.89	0.18			0.42	0.42		
	pH 9 and pH 12				pH 9 and pH 12			
	mg		%		mg		%	
Compound **1**	745		0.15		125		0.024	
Compound **2**	39		0.008		82		0.016	
Compound **3**	46		0.009		5		0.001	
Compound **4**	10		0.002		5		0.001	

14-O-veratroyl-bikhaconine [**3**].—Amorphous [lit. (13) 90–92°]; R_f 0.65; $[\alpha]^{25}D$ +55.2 (c=0.51, $CHCl_3$); uv λ max ($CHCl_3$) (log ε) 260.0 (4.09), 289.8 (3.83) nm; ir ν max (KBr) 3482 (br), 2930, 1715, 1603, 1517, 1456, 1418, 1345, 1294, 1271, 1223, 1178, 1092, 1024, 766 cm^{-1}; fabms *m/z* (%) $[M+H]^+$ 632 (54); eims *m/z* (%) $[M]^+$ 631 (16), 614 (36), 600 (80), 584 (10), 602 (3); ^1H and ^{13}C nmr see Tables 1–3.

14-O-veratroyl-pseudaconine [**4**].—Colorless crystals, mp 210–211° (Me_2CO/hexane) [lit. (12) 212°]; R_f 0.36; $[\alpha]^{25}D$ +36.5° (c=0.5, EtOH); uv λ ($CHCl_3$) (log ε) 260.2 (4.21), 288.9 (3.96) nm; ir λ max (KBr) 3414 (br), 2932, 1705, 1604, 1516, 1465, 1422, 1301, 1273, 1227, 1177, 1105, 1029, 982, 765 cm^{-1}; ^1H and ^{13}C nmr see Tables 1–3.

PARTIAL HYDROLYSES OF BIKHACONITINE [**1**].—Compound **1** (7 mg), dissolved in 1.8 ml 0.1 N H_2SO_4, was heated in a sealed tube at 140° for 24 h. After adjustment to pH 9 with 25% NH_4OH, **3** was extracted 3 times with 20 ml of $CHCl_3$. Yield: 6 mg of **3**, which showed in tlc and co-tlc the same R_f as isolated **3**.

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