New Lipo Norditerpenoid Alkaloids from Root Tubers of Aconitum ferox

Jampani Bhogi Hanuman, and Alfred Katz

J. Nat. Prod., **1994**, 57 (1), 105-115• DOI:
10.1021/np50103a015 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 105-115, January 1994

NEW LIPO NORDITERPENOID ALKALOIDS FROM ROOT TUBERS OF *ACONITUM FEROX*[1]

JAMPANI BHOGI HANUMAN and ALFRED KATZ*

Natural Products Research Laboratory, Oberwilerstrasse 9, CH-4054, Basel, Switzerland

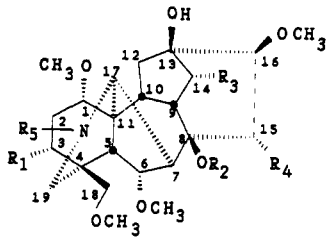
ABSTRACT.—Four new lipoalkaloids, **1–4**, were isolated from basic extracts of unprocessed and Ayurvedic processed root tubers of *Aconitum ferox*. Spectral investigation showed each to be a mixture of C-8 linoleoyl, palmitoyl, stearoyl esters of norditerpenoid alkaloids, namely of veratroylpseudaconine [**6**], anisoylyunaconine [**7**], benzoylindaconine [**8**] and veratroylbikhaconine [**9**].

In the course of our comparative investigation of Ayurvedic processed and unprocessed root tubers of the Indian drug *Aconitum ferox* (Ranunculaceae) [for taxonomy see (2)], we isolated, besides the known C-8-acetyl alkaloids pseudaconitine, yunaconitine, indaconitine, and bikhaconitine [**10–13**] (3), four new norditerpenoid alkaloids [**1–4**], as mixtures esterified with long-chain fatty acids affixed to C-8. Separation of **1–4** from the parent alkaloids **10–13** and their purification were achieved by multiple preparative tlc. Mixed esters of lipoalkaloids have been isolated previously by Kitagawa and coworkers (4), who found in the processed aconite tubers "chuanwu" (representing the Chinese species *Aconitum carmichaeli* Debx.), lipoaconitine [**14**], lipodeoxyaconitine [**15**], lipomesaconitine [**16**], and lipohypaconitine [**17**]. On methanolysis, each of these alkaloids yielded a mixture of methyl-linoleate, -palmitate, -oleate, -stearate, and -linolenate besides methyl benzoate and the norditerpenoid alkaloidal moieties (4). Data corroborated by ^{1}H, ^{13}C, HETCOR, ^{1}H,^{1}H-COSY nmr, ms and gc-ms elucidated each of our new isolates **1–4** as a mixture of C-8 esters of saturated and unsaturated fatty acids of veratroylpseudaconine [**6**] (2,5), anisoylyunaconine [**7**] (2,5), benzoylindaconine [**8**] [= ludaconitine (2,5)] and veratroylbikhaconine [**9**] (2,5). In an earlier communication (2) we discussed the nmr spectra of **6** and **9**, and we report here complete ^{1}H-nmr spectra of **7** and **8**. For comparison we prepared **7** and **8** by acid hydrolysis (6) of yunaconitine [**11**], and indaconitine [**12**], respectively. The alkaloids **1–3** were obtained from drug supplied by Elan Pharmaceuticals, and alkaloid **4** from drug supplied by Zandu Pharmaceutical Works Ltd.

RESULTS AND DISCUSSION

The ir spectra of isolates **1–4** showed hydroxyl, ester, and aromatic bands. In compounds **1** and **4** the 765 cm^{-1} band indicated a 1,3,4-trisubstituted, in compound **2** the 848 cm^{-1} band a 1,4-disubstituted, and in compound **3** the 712 cm^{-1} band a mono-substituted aromatic nucleus. The uv spectra also showed the characteristic absorptions for an aromatic nucleus. The ^{1}H-nmr spectra of isolates **1–4** resembled the reported spectra of pseudaconitine [**10**] and bikhaconitine [**13**] (2), but showed two additional multiplets between δ 5.20–5.48 and 1.25–1.80 and a distorted triplet at δ 0.85–0.92 (Table 1) due to the lipo ester side-chains. Isolates **1** and **4** exhibited signals for two ortho- protons and one meta- proton of a 1,3,4-trisubstituted aromatic system and two sharp singlets of aromatic methoxy signals. This suggested a veratroyl group. Isolate **2** displayed two doublets in the down-field region of the spectrum, each integrating for two protons and one aromatic methoxy singlet, thus suggesting an

[1]Part 13 on *Aconitum*. For Part 12 see Hanuman and Katz (1).



	R_1	R_2	R_3	R_4	R_5		R_1	R_2	R_3	R_4	R_5
1	OH	Lip.[a]	OVr	H	Et	**10**	OH	Ac	OVr	H	Et
2	OH	Lip.[a]	OAs	H	Et	**11**	OH	Ac	OAs	H	Et
3	OH	Lip.[a]	OBz	H	Et	**12**	OH	Ac	OBz	H	Et
4	H	Lip.[a]	OVr	H	Et	**13**	H	Ac	OVr	H	Et
5	OH	Lip.[b]	OBz	H	Et	**14**	OH	Lip.[c]	OBz	OH	Et
6	OH	H	OVr	H	Et	**15**	H	Lip.[c]	OBz	OH	Et
7	OH	H	OAs	H	Et	**16**	OH	Lip.[c]	OBz	OH	Me
8	OH	H	OBz	H	Et	**17**	H	Lip.[c]	OBz	OH	Me
9	H	H	OVr	H	Et						

[a]Lip.: linoleoyl, palmitoyl and stearoyl
[b]Lip.: linoleoyl.
[c]Lip.: linoleoyl, palmitoyl, oleoyl, stearoyl and linolenoyl.

anisoyl group. Compound **3** showed a three-proton multiplet and a two-proton multiplet in the aromatic region indicating the presence of a benzoyl ester moiety.

In isolates **1**, **2**, and **3**, a doublet of doublets at δ 3.78 is attributed to the C-3 proton coupled with the neighboring C-2 methylene protons. In isolate **4** this signal is missing, and a multiplet is observed at δ 1.55–1.69. Moreover, the ^{13}C-nmr chemical shifts of C-3 in **1–3** appeared at δ 71.70 and the corresponding shift of **4** at δ 34.96 (Table 2) suggesting that **4** is a C-3 deoxy-, while **1–3** are C-3 hydroxy-norditerpenoid alkaloids. The DEPT nmr spectrum of **4** displayed 19 upfield methylene carbon signals while **1–3** showed 18 such signals. Twelve of these methylenes are attributed to the non-diterpenoid moiety of the molecule. The ^{13}C-nmr spectra of compounds **1–4** displayed, besides the signals of the aromatic moiety, four methine carbons in the downfield region. These observations as well as the connectivities found in the ^{1}H-^{1}H-COSY nmr spectrum (Table 3) indicated the presence of unsaturated and saturated side-chains. Accordingly, the mass spectra of isolates **1–4** each showed three molecular ion peaks of the stearoyl-, linoleoyl-, and palmitoyl-ester alkaloids and three fragments due to loss of 32 units [OH+CH_3] from the M+1$^+$ peaks. The base peaks of compounds **1**, **3**, and **4** are due to loss of the lipoyl fragments leading to the basic alkaloidal moieties of veratroylpseudaconine [**6**], benzoylindaconine [**8**], and veratroylbikhaconine [**9**]. In isolate **2** the M+1$^+$ peak of the palmitoyl ester is the base peak, with the peak of anisoylyunaconine [**7**] measuring only 14%. Further, solvolysis (7) of compounds **1–4** yielded alkaloids **6–9**, respectively, along with mixtures of long-chain fatty acids that displayed, by gc, three peaks having the same retention times as linoleic, stearic and palmitic acid. Gc-ms of the fatty acid mixtures showed molecular ion peaks for palmitic acid (M$^+$ 256), linoleic acid (M$^+$ 280), and stearic acid (M$^+$ 284). The proportion of palmitic acid in the acid mixture amounts to 73.1–82.4%, of linoleic acid to 10.2–19.6%, and of stearic acid to 6.7–10.8%.

The ^{13}C-nmr spectra of the alkaloidal moiety are in accordance with the spectra of 8-*O*-linoleoyl-benzoylaconine [**5**] (4), which we prepared according to the procedure of Kitagawa *et al.* (4) and for which we are reporting the complete nmr data. The assignments of the ^{13}C-nmr signals of the linoleoyl, palmitoyl and stearoyl side-chains

TABLE 1. ^{1}H-Nmr Data of Compounds **1–9**.

Proton	Compound								
	1	**2**	**3**	**4**	**5**[a]	**6**	**7**[b]	**8**[c]	**9**
H-1	3.05–3.15, m	3.07–3.13, m	3.07–3.14, m	2.99–3.07, br t	3.08–3.14, m	3.12, dd (6.0, 8.0)	3.12, dd (6.1, 8.8)	3.13, dd (6.2, 8.6)	3.04, br t
H-2	1.90–2.03, m 2.27–2.35, m	1.88–2.03, m 2.29–2.51, m	1.94–2.04, m 2.25–2.41, m	1.90–2.03, m 2.26, br d (9.9)	1.93–2.13, m 2.31–2.43, m	2.03–2.17, m 2.25–2.45, m	1.95–2.10, m 2.27–2.40, m	2.05–2.23, m 2.25–2.40, m	1.90–2.08, m 2.19–2.33, m
H-3	3.78, dd (4.2, 8.2)	3.78, dd (4.4, 8.6)	3.78, dd (4.0, 8.0)	1.55–1.69, m —	3.76–3.81, m —	3.64–3.78, m —	3.76, dd —	3.73, m	1.55–1.72, m 1.55–1.72, m
H-5	2.06–2.17, m	1.93–2.20, m	2.07–2.17, m	2.06–2.12, m	2.07–2.15, m	2.03–2.13, m	1.98–2.08, m	2.05–2.15, m	2.04–2.12, m
H-6	4.02, d (6.5)	4.02, d (6.1)	4.01, d (6.7)	3.94, m	4.02, d (6.7)	4.07, d (6.7)	4.08, d (6.8)	4.08, d (6.6)	4.02, d (6.6)
H-7	3.03, br s	3.03, br s	3.04, br s	3.03, br s	3.08–3.14, m	2.03–2.13, m	—	—	2.03–2.17, m
H-9	2.89–2.94, m	2.85–2.94, m	2.85–2.93, m	2.90, br s	2.84–2.94, m	2.51–2.57, m	2.46–2.58, m	2.49–2.63, m	2.47–2.61, m
H-10	2.06–2.15, m	2.10–2.25, m	2.05–2.15, m	2.06–2.12, m	2.07–2.15, m	2.03–2.13, m	1.98–2.08, m	2.05–2.23, m	2.04–2.12, m
H-12	2.06–2.15, m 2.50–2.65, m	2.10–2.25, m 2.55–2.70, m	2.05–2.15, m 2.55–2.68, m	1.99–2.12, m 2.73–2.81, m	2.07–2.15, m 2.68–2.72, m	2.03–2.13, m 2.31–2.57, m	1.98–2.08, m 2.50–2.60, m	2.05–2.23, m 2.45–2.60, m	2.00–2.09, m 2.49–2.69, m
H-14	4.86, d (5)	4.85, d (5)	4.89, d (5)	4.85, d (5)	4.86, d (4.9)	5.12, d (5)	5.14, d (5)	5.17, d (4.7)	5.15, d (5)
H-15	2.42–2.48, m 3.03, br t (4)	2.29–2.51, m 2.99–3.07, m	2.36–2.44, m 3.00–3.15, m	2.35–2.50, m 2.99–3.20, m	4.44, dd, (5.3, 2.8)	2.22–2.42, m 2.51–2.68, m	2.15–2.30, m 2.48–2.66, m	2.23–2.41, m 2.49–2.68, m	2.19–2.33, m 2.50–2.69, m
H-16	3.37–3.40, m	3.40, br d (8)	3.41, dd (6, 8)	3.35–3.42, m	3.34, d (5.4)	3.31–3.42, m	3.31–3.38, m	3.31-3.40, m	3.26–3.39, m
H-17	2.85, br s	2.85, br s	2.85, br s	2.90, m	2.80–2.94, m	3.00, br s	3.02, br s	3.03, br s	3.00–3.09, m
H-18	3.48, d (9) 3.62, d (9)	3.49, d (8.9) 3.62, d (8.9)	3.48, d (8.9) 3.62, d (8.9)	3.12, d 3.61, d (8.4)	3.44, d (8.8) 3.61, d (8.8)	3.64–3.78, m —	3.71, br s —	3.71, br s —	3.26–3.39, m 3.66, d (8.4)
H-19	2.35–2.45, m 2.89–2.94, m	2.32–2.51, m 2.85–2.94, m	2.34–2.44, m 2.85–2.93, m	2.35–2.50, m 2.99–3.20, m	2.31–2.37, m 2.77–2.94, m	2.42, d (12) 2.94 d (12)	2.43, d (11) 2.95, d (11)	2.44, d (11) 2.95 d (11)	2.19–2.33, m 2.41–2.69, m
H-20	2.45–2.60, m 2.45–2.60, m	2.48–2.60, m 2.48–2.60, m	2.48–2.62, m 2.48–2.62, m	2.36–2.59, m 2.36–2.59, m	2.37–2.43, m 2.68–2.85, m	2.39–2.57, m 2.39–2.57, m	2.48–2.67, m 2.48–2.67, m	2.43–2.68, m 2.43–2.68, m	2.41–2.69, m 2.41–2.69, m
21-Me	1.09, t (7)	1.09, t (7.1)	1.10, t (7)	1.08, t (7.1)	1.09, t (7)	1.12, t (7.0)	1.11, t (7.1)	1.12, t (6.9)	1.09, t (7.1)
1-OMe	3.25, s	3.25, s	3.25, s	3.25, s	3.26, s	3.27, s	3.31, s	3.31, s	3.27, s
6-OMe	3.15, s	3.15, s	3.15, s	3.14, s	3.16, s	3.25, s	3.25, s	3.26, s	3.26, s
16-OMe	3.54, s	3.55, s	3.56, s	3.52, s	3.76, s	3.42, s	3.40, s	3.41, s	3.39, s
18-OMe	3.30, s	3.25, s	3.29, s	3.28, s	3.30, s	3.31, s	3.28, s	3.28, s	3.30, s
3-OH		2.39, s		—		2.06, s	2.25, s	2.23, s	—
13-OH	3.89, s	3.89, s	3.91, s	3.82, s	3.96, s	3.86, s	3.91, s	3.89, s	3.86, s
15-OH	—	—	—	—	4.49, d (2.8)	—	—	—	—
8-OH	—	—	—	—	—	2.27, s			2.25, s

TABLE 1. Continued.

Proton	Compound								
	1	2	3	4	5[a]	6	7[b]	8[c]	9
aromatic									
2′-H	7.61, d (1.6)	8.00, d (8.8)	8.04–8.08, m	7.62,d (2)	8.00–8.05, m	7.59, d (1.7)	7.98, d (8)	8.02–8.05, m	7.59, d (1.7)
3′-H	—	6.91, d (8.8)		—	7.41–7.49, m	—	6.93, d (8)	7.41–7.49, m	—
4′-H	—	—	7.40–7.56, m	—	7.53–7.62, m	—	—	7.54–7.63, m	—
5′-H	6.79, d (8.3)	6.91, d (8.8)		6.88, d (8)	7.41–7.49, m	6.90, d (8.4)	6.93, d (8)	7.41–7.49, m	6.90, d (8.4)
6′-H	7.70, d (1.6, 8.3)	8.00, d (8.8)	8.04–8.08, m	7.70, d (2, 8)	8.00–8.05, m	7.67, dd (1.7, 8.4)	7.98, d (8)	8.02–8.05, m	7.69, dd (1.7, 8.4)
3′-OMe	3.93, s	—	—	3.90, s	—	3.93, s	—	—	3.93, s
4′-OMe	3.96, s	3.85, s	—	3.92, s	—	3.94, s	3.86, s	—	3.94, s
lipo side chain									
OCO									
CH_2[d]	1.65–1.80, m 0.97–1.13, m	1.65–1.77, m 0.97–1.13, m	1.63–1.78, m 1.01–1.16, m	1.69–1.77, m 0.89–1.01, m	1.71–1.90, m 0.96–1.04, m				
$(CH_2)_n$[d,e]	1.25–1.42, m	1.25–1.42, m	1.26–1.38, m	1.25–1.35, m	1.22–1.35, m				
CH_2	1.90–2.03, m	1.93–2.10, m	1.91–2.04, m	1.99–2.12, m	1.93–2.12, m				
CH	5.30–5.45, m	5.31–5.47, m	5.32–5.45, m	5.25–5.45, m	5.32–5.37, m				
CH	5.25–5.36, m	5.26–5.47, m	5.27–5.42, m	5.20–5.40, m	5.31–5.41, m				
CH_2	2.73–2.79, m	2.74–2.80, m	2.74–2.80, m	2.73–2.81, m	2.72–2.84, m				
CH	5.25–5.36, m	5.26–5.41, m	5.27–5.42, m	5.20–5.40, m	5.32–5.37, m				
CH	5.30–5.45, m	5.31–5.47, m	5.32–5.48, m	5.25–5.45, m	5.31–5.41, m				
CH_2	1.90–2.03, m	1.93–2.10, m	1.91–2.04, m	1.99–2.12, m	1.93–2.12, m				
$(CH_2)_2$[d]	1.25–1.42, m	1.25–1.42, m	1.26–1.38, m	1.25–1.35, m	1.25–1.35, m				
CH_2[d]	1.25–1.42, m 1.65–1.80, m	1.25–1.42, m 1.65–1.77, m	1.26–1.38, m 1.63–1.78, m	1.25–1.35, m 1.69–1.77, m	1.25–1.35, m 1.71–1.90, m				
CH_3[d]	0.85–0.90, m	0.88, m	0.85–0.92, m	0.85–0.89, m	0.85–0.92, m				

[a]Chemical shifts of prepared compound.
[b]Chemical shifts of hydrolyzed product of yunaconitine.
[c]Chemical shifts of hydrolyzed product of indaconitine.
[d]These signals are also valid for palmitoyl and stearoyl chains.
[e]n=5 for stearoyl and linoleoyl chains, *n*=3 for palmitoyl chains.

TABLE 2. ^{13}C-Nmr Chemical Shifts of Compounds **1–9**.[a]

Carbon	Compound									Carbon	Compound				
	1	**2**	**3**	**4**	**5**[b]	**6**[b]	**7**[b,c]	**8**[b,d]	**9**[b]		**1**	**2**	**3**	**4**	**5**
C-1	82.33	82.31	82.29	85.04	82.44	82.59[e]	82.69	82.67	85.50	C-1″	172.68	172.68	172.66	172.63	175.16
C-2	33.51	33.51	33.51	26.36	33.59	33.65	33.75	33.74	26.18	C-2″[f]	34.80	34.84	34.77	34.80	34.80
C-3	71.65	71.70	71.67	34.96	71.55	71.97	72.07	72.04	35.05	C-3″[f]	24.24	24.26	24.15	24.23	24.18
C-4	43.19	43.19	43.20	39.15	43.15	43.29	43.39	43.40	39.36	C-4″[e,f]	29.11	29.12	29.07	29.11	29.01
C-5	47.07	47.10	47.11	49.23	46.54	47.91	48.05	48.10	49.82	C-5″[e]	29.11	29.12	29.07	29.11	29.01
C-6	83.27	83.27	83.28	83.17	83.54	82.49[e]	83.56	82.56	82.61	C-6″[e,f]	29.36	29.36	29.37	29.35	29.36
C-7	48.74	48.73	48.74	49.23	44.77	53.47	53.41	53.41	53.68	C-7″[e,f]	29.69	29.69	29.71	29.63	28.88
C-8	85.35	85.39	85.40	85.36	91.77	73.83	73.87	73.88	73.77	C-8″[g]	27.23	27.23	27.24	27.23	27.22
C-9	44.83	44.83	44.79	45.24	44.35	47.91	48.17	48.10	48.41	C-9″[g]	130.02	130.02	130.03	130.04	130.00
C-10	40.95	40.95	40.95	41.09	41.03	42.00	42.14	42.19	42.36	C-10″[g]	128.10	128.09	128.12	128.07	128.16
C-11	50.38	50.39	50.40	50.30	50.14	50.27	50.35	50.36	50.32	C-11″[g]	25.65	25.65	25.65	25.64	25.67
C-12	35.10	35.19	35.18	35.73	35.80	35.83	36.08	36.06	36.46	C-12″[g]	127.90	127.89	127.90	127.91	127.90
C-13	74.80	74.75	74.75	74.91	74.09	75.88	76.06	76.09	76.15	C-13″[g]	130.26	130.28	130.27	130.27	130.31
C-14	78.65	78.61	78.90	78.69	79.01	79.83	80.02	80.32	80.11	C-14″[g]	27.23	27.23	27.24	27.23	27.22
C-15	40.03	39.89	39.87	39.75	79.01	42.35	42.25	42.12	42.14	C-15″[e,f]	29.11	29.12	29.07	29.11	28.01
C-16	83.83	83.68	83.61	83.99	90.09	83.23	83.54	83.23	83.49	C-16″[f]	31.93	31.93	31.93	31.93	31.56
C-17	61.60	61.59	61.60	61.89	61.27	61.84	61.88	61.89	62.26	C-17″[f]	22.70	22.60	22.60	22.70	22.60
C-18	77.00	77.04	77.04	80.36	76.75	77.42	77.53	77.51	80.66	C-18″[f]	14.10	14.11	14.10	14.10	14.08
C-19	47.53	47.57	47.59	53.70	47.06	47.45	47.53	47.54	53.77						
C-20	48.74	48.67	48.74	49.07	48.91	48.93	49.00	49.01	49.28						
C-21	13.31	13.30	13.31	13.45	13.34	13.52	13.59	13.56	13.67						
C-1′	56.01	55.83	55.84	56.01	55.91	55.86	56.06	56.06	56.07						
C-6′	58.00	57.98	57.99	58.00	58.18	57.55	57.56	57.58	57.59						
C-16′	58.90	58.88	58.88	58.84	60.98	58.40	58.38	58.36	58.40						
C-18′	59.14	59.14	59.14	59.09	59.11	59.17	59.23	59.23	59.22						

TABLE 2. Continued.

Carbon	Compound									Carbon	Compound				
	1	2	3	4	5[b]	6[b]	7[b,c]	8[b,d]	9[b]		1	2	3	4	5
aromatic															
O=C	165.91	165.98	166.23	166.00	166.04	166.35	166.53	166.83	166.53						
C-1	122.73	122.67	129.77	122.88	129.82	122.40	122.40	130.12	122.60						
C-2	110.38	131.79	129.77	110.37	129.74	110.41	131.84	129.77	110.48						
C-3	148.76	113.78	128.54	148.75	128.66	148.70	113.87	128.57	148.77						
C-4	153.14	163.53	133.12	153.09	133.28	153.17	163.82	133.18	153.20						
C-5	112.04	113.78	128.54	112.10	128.66	112.17	113.87	128.57	112.28						
C-6	123.79	131.79	128.77	123.80	129.74	123.75	131.84	129.77	123.80						
C-3′	55.85	—	—	56.01	—	56.30	—	—	56.01						
C-4′	55.85	55.42	—	55.84	—	56.01	55.48	—	55.94						

[a]The ^{13}C-nmr shift multiplicities were determined by DEPT.

[b]Our own data on known compound.

[c,d]Chemical shifts of hydrolyzed product of yunaconitine and indaconitine respectively.

[e]Interchangeable values.

[f]These signals are valid for linoleoyl, palmitoyl and stearoyl chains.

[g]These signals are valid for linoleoyl chains only. For C-4 to C-12 of palmitoyl and C-4 to C-14 of stearoyl chains assignments cannot be made on the basis of our data.

TABLE 3. ^{1}H-^{1}H COSY Nmr Data for Compounds **1–9**.

Proton	Compound									H/H Connectivities
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
H-1	3.10	3.12	3.10	3.03	3.12	3.12	3.12	3.13	3.04	H_a-2/H_b-2
H_a-2	1.96	1.96	1.99	1.95	1.98	2.08	2.00	2.10	2.00	H_b-2/H-1 $H_{a,b}$-3
H_b-2	2.30	2.32	2.33	2.26	2.37	2.35	2.30	2.35	2.26	H_a-2/H-1 $H_{a,b}$-3
$H_{a,b}$-3	3.80	3.80	3.78	1.62	3.78	3.71	3.75	3.73	1.63	H_a-2/H_b-2
H-5	2.10	2.12	2.12	2.09	2.12	2.08	2.05	2.10	2.08	H-6
H-6	4.02	4.00	4.01	3.94	4.02	4.07	4.08	4.08	4.02	H-5, H-7
H-7	3.03	3.03	3.04	3.03	2.90	—	—	—	—	H-6
H-9	2.90	2.89	2.89	2.90	2.90	2.54	2.52	2.56	2.54	H-10/H-14
H-10	2.10	2.15	2.10	2.09	2.10	2.08	2.05	2.10	2.08	H-9/H_a/H_b-12
H_a-12	2.10	2.15	2.10	2.05	2.15	2.08	2.05	2.10	2.08	H_b-12
H_b-12	2.55	2.62	2.60	2.77	2.70	2.46	2.52	2.57	2.60	H_a-12/H-10
H-14	4.86	4.85	4.89	4.85	4.86	5.12	5.14	5.17	5.15	H-9
H_a-15	2.45	2.38	2.40	2.42	4.44	2.32	2.25	2.32	2.26	H_b-15/H-16
H_b-15	3.03	3.05	3.05	3.09	—	2.59	2.57	2.58	2.59	H_a-15/H-16
H-16	3.40	3.38	3.41	3.38	3.34	3.36	3.34	3.35	3.32	H_a-15/H_b-15
H-17	2.85[a]	2.85[a]	2.85[a]	2.90[a]	3.11[a]	3.00[a]	3.02[a]	3.03[a]	3.04[a]	H-6_a
H_a-18	3.48	3.49	3.48	3.12	3.44	—	—	—	3.32	H_b-18
H_b-18	3.62	3.62	3.62	3.61	3.62	—	—	—	3.66	H_a-18
H_a-19	2.35	2.38	2.35	2.42	2.35	2.42	2.43	2.44	2.26	H_b-19
H_b-19	2.90	2.89	2.89	3.09	2.90	2.94	2.95	2.95	2.55	H_a-19
$H_{a,b}$-20	2.52	2.54	2.55	2.50	2.40 2.75	2.50	2.57	2.55	2.55	$H_{b,a}$-20, Me-21
Me-21	1.09	1.09	1.10	1.08	1.09	1.11	1.11	1.12	1.09	$H_{a,b}$-20

TABLE 3. Continued.

Proton	Compound									H/H Connectivities
	1	2	3	4	5	6	7	8	9	
aromatic										
H-2′	7.61	8.00	8.06	7.62	8.02	7.59	7.98	8.03	7.59	H-6′/H-3′/H-4′
H-3′	—	6.91	7.44	—	7.45	—	6.93	7.45	—	H-2′/H-4′/H-5′
H-4′	—	—	7.48	—	7.57	—	—	7.58	—	H-2′/H-3′/H-5′/H-6′
H-5′	6.79	6.91	7.56	6.88	7.45	6.90	6.93	7.45	6.90	H-3′/H-4′/H-6′
H-6′	7.70	8.00	8.06	7.70	8.02	7.67	7.98	8.03	7.69	H-2′/H-4′/H-5′
lipo side chain										
H_a-2″	1.05	1.05	1.08	1.05	1.00					H-3″
H_b-2″	1.72	1.71	1.70	1.73	1.80					H-3″
H-3″	1.33	1.33	1.32	1.30	1.28					H-2″
H-4″-H-x″[b,c]	1.33	1.33	1.32	1.30	1.28					
H-8″	2.00	2.01	1.97	2.05	2.02					H-9″
H-9″	5.37	5.39	5.40	5.35	5.36					H-8″/H-10″
H-10″	5.30	5.36	5.34	5.30	5.34					H-9″/H-11″
H-11″	2.76	2.77	2.77	2.77	2.78					H-10″/H-12″
H-12″	5.30	5.36	5.34	5.30	5.34					H-11″/H-13″
H-13″	5.37	5.39	5.40	5.35	5.36					H-12″/H-14″
H-14″	2.00	2.01	1.97	2.05	2.02					H-13″/H-15″,H-16″
H-15″,16″	1.33	1.33	1.32	1.30	1.30					H-14″/H_b-17″
H_a-17″	1.33	1.33	1.32	1.30	1.30					H_b-17″
H_b-17″	1.72	1.71	1.70	1.73	1.80					H_a-17″
H-18″	0.87	0.88	0.88	0.87	0.88					H_a-17″

[a]Long-range coupling.
[b]x=7 for stearoyl- and linoleoyl groups, x=5 for palmitoyl group. For palmitoyl group the following numbering of H has to be changed accordingly.
[c]Connectivities for H-4″ to H-X″ cannot be given.

were based on literature values given for esters of these acids (8). However, each of the lipo acid mixtures of compounds **1–4** showed a signal at 31.53 ppm, which we could not assign. We presume that this is caused by an additional unsaturated fatty acid present in a minor amount which we have not identified. The ^{1}H- and ^{13}C-nmr spectra of compounds **6–9** obtained by solvolysis were in good agreement with the reported spectra of the same alkaloids isolated from aconite tubers (2,5). We also prepared **7** and **8** by acid hydrolysis (6) of yunaconitine [**11**] and indaconitine [**12**], respectively. Tlc of **7** prepared from **2** and **11** showed an identical R_f, as did **8** prepared from **3** and **12**. We wish to mention that the ^{1}H-nmr spectrum of the lipo acid mixtures showed a quartet at δ 2.90–3.00, which was not observed in the alkaloids **1–4**, and which we cannot explain yet. Presumably it is due to a by-product formed during the hydrolysis.

The reported data identify the new isolates as lipopseudaconitine [**1**], lipoyunaconitine [**2**], lipoindaconitine [**3**], and lipobikhaconitine [**4**] and give their complete ^{1}H- and ^{13}C-nmr chemical shift data (Tables 1 and 2). They are mixed C-8 linoleoyl, palmitoyl, and stearoyl esters of the norditerpenoid alkaloids **6–9**. However, we cannot exclude the possibility that the isolates **1–4** contain minor amounts of C-8 substitution by other fatty acids.

The complete ^{1}H-nmr data of the previously known compounds **6** and **7** (2,5) are reported for the first time. Some ^{13}C-nmr assignments of the literature (5) had to be adapted in accordance with our earlier publication (2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded in $CDCl_3$ on a Bruker 200 spectrometer (200 MHz), with chemical shifts (δ) expressed downfield to TMS, and nmr data given in Tables 1–4; gc-ms was performed on a Varian 3400-Saturn using a DB-5 capillary column (30×0.25 mm) and the retention times (R_t) are given in min:sec; mass spectra were recorded on a VG 70-SE mass spectrometer at 70 eV by eims or fabms. The matrix for fabms was thioglycerine; ir spectra were taken on a Perkin-Elmer 281 in KBr pellets; uv spectra were obtained on a Perkin-Elmer Lambda 5 instrument in $CHCl_3$. Optical rotations were recorded in $CHCl_3$ at 25°. Cc was conducted with Merck Kieselgel 60 and neutral Al_2O_3 activity IVa, and tlc on Merck Si gel and neutral Al_2O_3 plates (0.25 mm). For compound yields % refers to powdered tubers; C_6H_{12}=cyclohexane.

PLANT MATERIAL.—Two batches (A and B) of Ayurvedic processed and unprocessed root tubers, labeled *Aconitum ferox*, were extracted. Batch A was supplied by Elan Pharmaceuticals, Bombay, India. Batch B was received from Zandu Pharmaceuticals, Bombay, India. Voucher root specimens are deposited in the senior author's herbarium.

EXTRACTION AND ISOLATION.—From unprocessed powdered root tubers (400 g) of batch A the purified basic extract (3.2 g) was prepared as described earlier (2). It was column chromatographed over Si gel (150 g) with a polarity gradient solvent system starting with C_6H_{12}-$CHCl_3$-Et_2NH (7.5:2:0.5) and 250 ml fractions were collected. Fraction 1 (775 mg) was rechromatographed on Al_2O_3 (25 g) and 250-ml fractions were collected. Fraction 9 [C_6H_{12}-EtOAc-EtOH (7.5:2:0.5)] yielded 126 mg of an alkaloidal mixture that was separated on prep. tlc plates (Al_2O_3; 20 cm×20 cm, 0.25 mm) to give crude alkaloids **1–3** on multiple development with the same solvent system. Final purification was carried out with this same tlc system. Alkaloids **1–3** were also isolated from processed tubers of batch A, while processed and unprocessed tubers of batch B, extracted and purified in the same way, yielded isolate **4**. For yields see Table 5.

Lipopseudaconitine [**1**].—Colorless oil, $[\alpha]_D$ +12.4° (c=0.5); uv λ max (log ε) 220.7 (3.90), 250.4 (4.21) nm; ir ν max 3532 (br), 1723, 1603, 1516, 1460, 1272, 1223, 1179, 1098, 1026, 983 and 765 cm^{-1}; fabms *m/z* 914 {[M+1]$^+$, R=stearoyl (38%)}, 910 {[M+1]$^+$, R=linoleoyl (58%)}, 886 {[M+1]$^+$, R=palmitoyl (56%)}, 882 (28%), 878 (30%), 854 {[M−OH+CH_3]$^+$ (33%)}, 630 {[M−lipoyl]$^+$ (100%)}.

Lipoyunaconitine [**2**].—Colorless oil, $[\alpha]_D$ +12.0° (c=0.5); uv λ max (log ε) 222.2 (3.95), 244.7 (4.29) nm; ir ν max 3527 (br), 1722 (ester), 1608, 1513, 1460, 1282, 1257, 1171, 1695, 1032, 985, 848, 771 and 695 cm^{-1}; fabms *m/z* 884 {[M+1]$^+$, R=stearoyl (34%)}, 880 {[M+1]$^+$, R= linoleoyl (37%)}, 856 {[M+1]$^+$, R=palmitoyl (100%)}, 848 (4%), 852 (3%), 824 (7%), 600 {[M−lipoyl]$^+$ (14%)}.

Lipoindaconitine [**3**].—Colorless oil, $[\alpha]_D$ +1.1° (c=0.5); uv λ max (log ε) 208.7 (3.86), 240.9 (4.01) nm; ir ν max 3518 (br), 1724 (ester), 1603, 1540, 1512, 1452, 1280, 1182, 1098, 1029, 985 and 712; fabms

TABLE 4. ^{1}H-^{13}C HETCOR Nmr Observations for Compound **1**.

Carbon	δC	δH	Carbon	δC	δH
1	82.20	3.10	OCO	172.70	—
2	33.50	—	CH	34.80	—
3	71.90	3.80	CH_2	24.00	1.25
5	47.00	2.12	CH_2	29.00	1.33
6	83.20	4.00	CH_2	29.00	1.33
7	48.70	3.05	CH_2	29.40	1.33
9	45.00	2.90	CH_2	29.70	1.33
10	41.00	2.12	CH_2	27.20	2.00
12	35.00	2.10	CH	130.00	5.37
		2.54	CH	128.00	5.30
14	79.00	4.90	CH_2	25.70	2.75
15	40.00	2.45	CH	128.00	5.30
		3.05	CH	130.00	5.37
16	84.00	3.40	CH_2	27.00	2.00
17	61.60	2.85	CH_2	29.80	1.33
18	77.00	3.50	CH_2	32.00	1.33
		3.65	CH_2	22.70	1.33
19	47.50	2.38			1.72
		2.89	CH_3	14.00	0.90
20	48.70	2.55			
21	13.31	1.10			
OMe-1	56.00	3.25			
OMe-6	58.00	3.15			
OMe-16	59.00	3.50			
OMe-18	59.00	3.30			
aromatic					
2′	110.50	7.60			
5′	112.00	6.90			
6′	124.00	7.70			
OMe-3′	56.00	3.95			
OMe-4′	56.00	3.95			

m/z 854 {$[M+1]^+$, R=stearoyl (40%)}, 850 {$[M+1]^+$, R=linoleoyl (86%)}, 826 {$[M+1]^+$, R=palmitoyl (59%)}, 822 (26%), 818 (48%), 794 {$[M-OH+CH_3]^+$ (33%), 570 {$[M-$lipoyl$]^+$ (100%)}.

Lipobikhaconitine [**4**].—Colorless oil, $[\alpha]_D$ +2.0° (*c*=0.4); uv λ max (log ε) 264.2 (4.08), 291.3 (3.79) nm; ir ν max 3542 (br), 1721, 1602, 1515, 1465, 1346, 1271, 1223, 1178, 1092, 1026, 987 and 765 cm^{-1}; fabms *m/z* 898 {$[M+1]^+$, R=stearoyl (17%)}, 894 {$[M+1]^+$, R=linoleoyl (37%)}, 870 {$[M+1]^+$, R=palmitoyl (43%)}, 866 (11%), 862 (14%), 838 {$[M-OH+CH_3]^+$ (14%)}, 614 {$[M-$lipoyl$]^+$ (100%)}.

Solvolysis of isolate **1**: 50 mg of **1** dissolved in 25 ml of dioxane-H_2O (1:1) was refluxed at 120° for 18 h. The reaction mixture was concentrated *in vacuo*, adjusted to pH 1 with 0.1 N HCl and extracted 3 times with 25 ml of Et_2O. The aqueous layer was adjusted to pH 9 with 25% NH_4OH and extracted 3 times with 25 ml $CHCl_3$. Yield at pH 9: 35 mg of compound **6**, which showed in tlc and co-tlc the same R_f as **6** isolated earlier (2,5). Yield at pH 1: 15 mg mixture of lipo acids.

Solvolysis of isolates **2**, **3**, and **4**: 8 mg each of **2**, **3**, and **4** were solvolysed analogously to **1**, to give **7**, **8**, and **9** respectively.

Yields at pH 9: **7** (4 mg) showed in tlc and co-tlc the same R_f as **7** prepared from yunaconitine [**11**]; **8** (3 mg) showed in tlc and co-tlc the same R_f as **8** prepared from indaconitine [**12**]; **9** (4 mg) showed in tlc and co-tlc the same R_f as **9** isolated earlier (2,5).

Yield at pH 1: 3.5 mg, 3.5 mg and 2.0 mg lipo acid mixtures from isolates **2**, **3**, and **4** respectively.

Gc of lipo acid mixtures: From Isolate **1**: Palmitic acid, R_t 17:10, 81.7%; linoleic acid, R_t 18:56, 10.2%; stearic acid, R_t 19:08, 8.1%. From isolate **2**: Palmitic acid, R_t 17:08, 82.4%; linoleic acid, R_t 18:56, 10.9%;

TABLE 5. Yields of Extracts and Alkaloids.

Root tubers	Batch A				Batch B			
	Unprocessed 400 g		Processed 475 g		Unprocessed 492 g		Processed 517 g	
	g	%	g	%	g	%	g	%
Crude extracts ...	5.67	1.42	3.88	0.81	9.50	1.98	6.59	1.27
Purified	3.20	0.80	1.39	0.29	5.30	1.07	2.60	0.37
Pentane	2.32	0.74	2.48	0.52	3.46	0.86	3.14	0.60
	mg	%	mg	%	mg	%	mg	%
Isolate **1**	50	0.012	30	0.006				
Isolate **2**	24	0.006	10	0.002				
Isolate **3**	22	0.005	8	0.0017				
Isolate **4**					38	0.007	20	0.004

stearic acid, R_t 19:06, 6.7%. From isolate **3**: Palmitic acid, R_t 17:10, 73.9%; linoleic acid, R_t 18:56, 15.1%; stearic acid, R_t 19.06, 10.8%. From isolate **4**: Palmitic acid, R_t 17:12, 73.1%; linoleic acid, R_t 18:58, 19.0%; stearic acid, R_t 19:08, 7.7%. Reference mixture: Palmitic acid, R_t 17:03; linoleic acid, R_t 18:08, stearic acid, R_t 19:00.

Solvolysis of yunaconitine [**11**].—Compound **11** (10 mg) dissolved in 0.1 N H_2SO_4 (2.5 ml) was heated in a sealed tube at 140° for 24 h, adjusted to pH 9 with 25% NH_4OH, and extracted 3 times with 25 ml of $CHCl_3$. Yield: 7 mg of **7**.

Solvolysis of indaconitine [**12**].—Compound **12** (10 mg) was solvolyzed as described for **11**. Yield: 7 mg of **8**.

ACKNOWLEDGMENTS

We are grateful to Prof. Dr. H.H.A. Linde, Pharmaceutical Institute of the University of Basel, Switzerland, for providing the facilities to record spectra and specific rotations at the Institute; to Dr. Vera Bernhardt, of the same Institute, for gc-ms recordings; to Mr. Ch. Quiquerez, ms Laboratory, Sandoz A.G., Basel, Switzerland, for the measurement of mass spectra; to Dr. K.M. Parikh and Dr. S.S. Mahajani, Zandu Pharmaceutical Works Ltd., Bombay, India, for their generous supply of aconite tubers and to Mr. D.V.P. Raju, Elan Pharmaceuticals, Bombay, India, for providing root tubers from the Bombay market.

LITERATURE CITED

1. J.B. Hanuman and A. Katz, *Nat. Prod. Lett.*, **3**, 227 (1993).
2. J.B. Hanuman and A. Katz, *J. Nat. Prod.*, **56**, 801 (1993).
3. S.W. Pelletier and B.S. Joshi, in: "Alkaloids: Chemical and Biological Perspectives." Ed. by S.W. Pelletier, John Wiley and Sons, New York, 1984, Vol. 2, pp. 288, 395, 433, and 460.
4. I. Kitagawa, M. Yoshikawa, Z.L. Chen, and K. Kobayashi, *Chem. Pharm. Bull.*, **30**, 758 (1982).
5. K.S. Khetwal, B.S. Joshi, H.K. Desai, and S.W. Pelletier, *Heterocycles*, **34**, 441 (1992).
6. K.K. Purushothaman and S. Chandrasekharan, *Phytochemistry*, **13**, 1975 (1974).
7. H. Hikino, C. Konno, H. Takata, Y. Yamada, C. Yamada, Y. Ohizumi, K. Sugio, and H. Fujimura, *J. Pharmacodyn.*, **3**, 514 (1980).
8. J.G. Batchelor, R.J. Cushley, and J.H. Prestegard, *J. Org. Chem.*, **39**, 1698 (1974).

Received 1 July 1993