Norditerpenoid Alkaloids from the Processed Tubers of *Aconitum carmichaeli*

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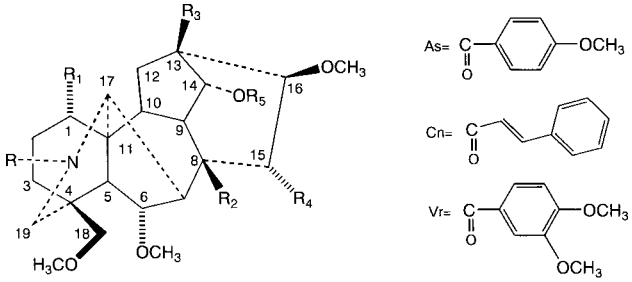
Four new and five known norditerpenoid alkaloids were isolated from the processed tubers of *Aconitum carmichaeli*. The new alkaloids are 14-*O*-cinnamoylneoline (3), 14-*O*-anisoylneoline (4) 14-*O*-veratroylneoline (5), and lipo-14-*O*-anisoylbikhaconine (8). The known alkaloids are neoline (1), 14-*O*-acetylneoline (2), foresaconitine (6), crassicauline A (7), and lipohypaconitine (9). Alkaloids 2, 6, and 7 were isolated from this plant for the first time. The structures of the new alkaloids were established by spectroscopic and chemical methods.

Key words prepared aconite; *Aconitum carmichaeli*; Ranunculaceae; norditerpenoid alkaloid

The aconite root is an indispensable and common drug in Chinese traditional medicine. The processed lateral root of *Aconitum carmichaeli* DEBEAUX (Ranunculaceae), now officially listed as "Prepared Aconite" in the *Korean Pharmacopoeia*, seventh edition, is used as an analgesic and anesthetic agent in the treatment of neuralgic and rheumatic conditions.[1] The aconite plants are known to contain a number of norditerpenoid and diterpenoid alkaloids.[2—8] During our work on diterpenoid alkaloids from *Aconitum* plants, we isolated four new norditerpenoid alkaloids (**3**—**5**, **8**) and five known ones (**1**, **2**, **6**, **7**, **9**) from the processed tubers of *A. carmichaeli*.[9] The structures were determined by detailed NMR analyses including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC) techniques. In this paper, we report on the isolation and structural elucidation of these compounds.



1 R= CH_2CH_3, R_1=R_2= OH, R_3=R_4=R_5= H

2 R= CH_2CH_3, R_1=R_2= OH, R_3=R_4= H, R_5= Ac

3 R= CH_2CH_3, R_1=R_2= OH, R_3=R_4= H, R_5= Cn

4 R= CH_2CH_3, R_1=R_2= OH, R_3=R_4= H, R_5= As

5 R= CH_2CH_3, R_1=R_2= OH, R_3=R_4= H, R_5= Vr

6 R= CH_2CH_3, R_1= OCH_3, R_2= OAc, R_3=R_4= H, R_5= As

7 R= CH_2CH_3, R_1= OCH_3, R_2= OAc, R_3= OH, R_4= H, R_5= As

8 R= CH_2CH_3, R_1= OCH_3, R_2= OLip.[a], R_3= OH, R_4= H, R_5= As

9 R= CH_3, R_1= OCH_3, R_2= OLip.[b], R_3=R_4= OH, R_5= Bz

10 R= CH_2CH_3, R_1= OCH_3, R_2=R_3= OH, R_4=R_5= H

Lip.[a]: linoleoyl, palmitoyl, stearoyl, oleoyl

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

Results and Discussion

Compound **3** was isolated as an amorphous powder. The electron impact (EI) and positive FAB-MS showed a molecular ion $[M]^+$ at *m*/*z* 567 and a quasimolecular ion $[M+H]^+$ at *m*/*z* 568, respectively. The high-resolution (HR) EI-MS showed a molecular ion peak $[M]^+$ at *m*/*z* 567.3273 corresponding to the molecular formula $C_{33}H_{45}NO_7$. The spectral data of **3** are quite similar to those of 14-*O*-acetylneoline (**2**). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with that of 14-*O*-acetylneoline (**2**)[11] clearly indicated the new alkaloid to be a neoline derivative possessing a cinnamoyl group [δ_H 6.42 (1H, d, *J*=16.2 Hz), 7.37—7.41 (3H, m), 7.51—7.54 (2H, m), 7.68 (1H, d, *J*=16.2 Hz); δ_C 130.5 (C-1′), 128.9 (C-2′, 6′), 128.2 (C-3′, 5′), 134.2 (C-4′), 145.5 (C-7′), 117.7 (C-8′), 166.1 (C-9′)] at C-14 instead of an acetoxyl group. On the basis of these data, the structure of **3** was assigned to be 14-*O*-cinnamoylneoline and confirmed by saponification of **3** to neoline (**1**), which was identical to an authentic sample by direct comparison. The only example of this type is described as 14-*O*-benzoylneoline from *A. subcuneatum* NAKAI.[12]

Compound **4** has the molecular formula $C_{32}H_{45}NO_8$, established by positive-ion HR-FAB-MS (*m*/*z* 572.3242 $[M+H]^+$; Calcd for $C_{32}H_{46}NO_8$: 572.3223) as well as ^{13}C-NMR spectral data and distortionless enhancement by polarization transfer (DEPT) experiments. Analysis of ^{1}H- and ^{13}C-NMR spectra (Table 1) indicated that its structure is quite similar to that of compound **3** except that it has an anisoyl group [δ_H 3.84 (3H, s), 6.90 (2H, d, *J*=9.0 Hz), 7.95 (2H, d, *J*=9.0 Hz); δ_C 122.5 (C-1′), 131.6 (C-2′, 6′), 113.7 (C-3′, 5′), 163.4 (C-4′), 165.8 (C=O), 55.4 (OCH_3)] at C-14 instead of a cinnamoyl group. Substitution of the C-14 hydroxyl group with an anisoyl group was also deduced by the appearance of peaks at *m*/*z* 152 [$(CH_3OC_6H_4COOH)^+$, 15.7%] and 135 [$(CH_3OC_6H_4C{\equiv}O)^+$, 100%] in EI-MS. The anisoyl moiety was positioned at C-14 of the neoline skeleton on the basis of the HMBC correlation from H-14 to anisoyl C=O. The structure of **4** was further confirmed by alkaline hydrolysis to neoline (**1**), which was identified by direct comparison with

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Table 1. ^{13}C-NMR Chemical Shifts for 14-*O*-Acetyl-, 14-*O*-Cinnamoyl-, 14-*O*-Anisoyl-, and 14-*O*-Veratroylneolines (**2**—**5**) in $CDCl_3$

Carbon no.	14-*O*-Acetyl neoline (**2**)	14-*O*-Cinnamoyl neoline (**3**)	14-*O*-Anisoyl neoline (**4**)	14-*O*-Veratroyl neoline (**5**)
1	72.0	71.9	72.0	72.0
2	29.3	29.3	29.3	29.3
3	29.8	29.6	29.8	29.7
4	38.1	38.2	38.1	38.2
5	44.4	44.4	44.4	44.4
6	83.2	83.1	83.3	83.2
7	52.7	53.1	53.0	53.3
8	74.6	74.7	74.8	74.7
9	46.1	46.1	46.0	46.0
10	43.3	43.5	43.6	43.7
11	49.7	50.8	49.8	50.0
12	29.5	29.3	29.6	29.5
13	36.5	37.2	37.5	37.6
14	77.1	76.8	76.9	76.6
15	42.6	42.5	42.5	42.6
16	81.8	82.0	81.9	81.8
17	63.4	63.4	63.4	63.4
18	80.0	80.0	80.0	79.9
19	56.9	56.9	56.9	56.9
20	48.4	48.6	48.3	48.2
21	12.9	12.7	12.9	12.7
6-OCH_3	57.9	58.0	57.9	58.0
16-OCH_3	56.1	56.2	56.1	56.2
18-OCH_3	59.1	59.2	59.1	59.2
Other	Acetyl	Cinnamoyl	Anisoyl	Veratroyl
	170.3 (CO)	130.5 (C-1′)	122.5 (C-1′)	122.6 (C-1′)
	21.2 (CH_3)	128.9 (C-2′,6′)	131.6 (C-2′,6′)	112.2 (C-2′)
		128.2 (C-3′,5′)	113.7 (C-3′,5′)	148.7 (C-3′)
		134.2 (C-4′)	163.4 (C-4′)	153.1 (C-4′)
		145.5 (C-7′)	165.8 (C-7′)	110.4 (C-5′)
		117.7 (C-8′)	55.4 (OCH_3)	123.5 (C-6′)
		166.1 (C-9′)		165.8 (C-7′)
				55.9 (OCH_3)
				56.2 (OCH_3)

an authentic sample. Thus the structure of **4** was determined to be 14-*O*-anisoylneoline.

The molecular formula of **5** was determined to be $C_{33}H_{47}NO_9$ on the basis of the quasimolecular ion peak in its HR-FAB-MS at *m/z* 602.3329 $[M+H]^+$. The ^{1}H- and ^{13}C-NMR spectra (Table 1) were also similar to those of **4** except for the absence of an anisoyl group and the presence of a veratroyl group [δ_H 3.92, 3.93 (3H each, s), 6.89 (1H, d, J=8.4 Hz), 7.58 (1H, d, J=2.1 Hz), 7.64 (1H, dd, J=2.1, 8.4 Hz); δ_C 122.6 (C-1′), 112.2 (C-2′), 148.7 (C-3′), 153.1 (C-4′), 110.4 (C-5′), 123.5 (C-6′), 165.8 (C=O), 55.9, 56.2 (2×OCH_3)]. A difference of 30 mass units between **4** and **5** supports the deduction that **5** is a veratoryl derivative of neoline. Substitution of the C-14 anisoyl group with a veratroyl group was also deduced by the appearance of peaks at *m/z* 182 $[(CH_3O)_2C_6H_3COOH^+$, 22.5%] and 165 $[(CH_3O)_2C_6H_3C{\equiv}O]^+$, 100%] in EI-MS. An HMBC correlation (H-14 and veratroyl C=O) confirmed that the veratroyl unit is attached at the C-14 position of **5**. Alkaline hydrolysis of **5** furnished neoline (**1**), that was identical in all respects to an authentic sample. Thus from the results of the foregoing spectral studies the structure of **5** was elucidated to be 14-*O*-veratroylneoline.

Compound **8** was isolated as a colorless oil. The IR spectrum showed the presence of hydroxy (3435 cm^{-1}), ester carbonyl (1719 cm^{-1}), and aromatic C=C (1638 cm^{-1}) groups. The ^{1}H-NMR spectrum closely resembled that of lipobikhaconitine,[13] with four methoxy singlets at δ 3.16, 3.27, 3.30, and 3.55, and an *N*-ethyl [δ 1.14 (3H, t, J=7.0 Hz)]. It showed three multiplets between δ 5.34—5.42, 1.28—1.80, and δ 0.89—0.92 due to long-chain ester side-chains. It exhibited signals for para-substituted aromatic protons at δ 6.92 and 8.03 (2H each, d, J=8.8 Hz) with one additional methoxy singlet signal at δ 3.87, suggesting an anisoyl group. A doublet at δ 4.88 (d, J=4.9 Hz) was assigned to an H-14β methine proton, indicating the presence of a geminal hydroxy group at C-13. The FAB-MS showed four protonated molecular ions $[M+H]^+$ of the stearoyl, oleoyl, linoleoyl, and palmitoyl ester alkaloids and a fragment ion at *m/z* 584 due to the loss of an acylium ion from the molecular ion peaks. Alkaline hydrolysis of **8** yielded bikhaconine (**10**)[14] along with a mixture of long-chain fatty acids displaying molecular ion peaks for the methyl esters of palmitic [M^+, *m/z* 270], linoleic [M^+, *m/z* 294], oleic [M^+, *m/z* 296], and stearic [M^+, *m/z* 298] acids in the GC-MS. The proportion of each acid in the acid mixture was in the ratio 48 : 10 : 37 : 5, respectively. The HMBC spectrum of **6** showed three-bond connectivity between H-14 and the anisoyl C=O, supporting the presence of an anisoyl group at C-14. Thus, in analogy to the long-chain fatty acid esters of norditerpenoid alkaloids,[6,13] a mixture C_{16} and C_{18} fatty acid esters at C-8 of 14-*O*-anisoylbikhaconine (=forestine)[15] was inferred for compound **8** and was designated as lipo-14-*O*-anisoylbikhaconine, a previously undescribed alkaloid. The known compounds, neoline (**1**),[5,6,14] 14-*O*-acetylneoline (**2**),[11] foresaconitine (**6**),[16,17] crassicauline A (**7**),[16,18] and lipohypaconitine (**9**)[6] were also isolated and identified by comparison of their physical and spectral data with the reported values. Of these isolates, three compounds (**2**, **6**, **7**) were isolated from this species for the first time. It is well-known that the processed aconite roots show variation in the alkaloid content and composition depending on the method of processing.[19,20] In particular, the contents of alkaloids in commercially available preparations show marked fluctuation.[21,22] Climatic and soil conditions, as well as the methods and date of harvesting, influence the alkaloid content and composition.[5,23,24] Nonetheless, a comparison of the results of the present study and previous ones showed some differences. It is somewhat difficult to explain the differences at this stage. Further experiments are required to clarify the reason for the variation in the results.

Experimental

General Procedures The optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in positive ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured on a Varian Gemmi 2000 instrument (300 MHz) or a Bruker AM-500 (500 MHz), and the chemical shifts were referenced to TMS. GC-MS analysis was performed as previously described[25] using a Hewlett Packard 5989B mass spectrometer equipped with a 5890 Series II$^+$ gas chromatograph. TLC was performed on silica gel $60F_{254}$ (Merck).

Plant Material The processed tubers of *A. carmichaeli* imported from Sichuan province, China, were purchased in April 2001 at Sunheung Oriental Drug Store, Seoul, Korea, and authenticated by emeritus Professor H. J. Chi of the Natural Products Research Institute, Seoul National University, as well as by Professor J. H. Park of the College of Pharmacy, Pusan National University.[26] A voucher specimen (no. KSS000403) was deposited at the

Table 2. NMR Chemical Shifts of Lipo-14-*O*-anisoylbikhaconine (**8**) in $CDCl_3$

Carbon no.	δ_H (multiplicity, *J*)	δ_C (DEPT)	^1H–^1H COSY	HMBC
1	3.01—3.06 (m)	85.41 (CH)	H-2	1-OCH_3
2	2.26—2.39 (m)	26.74 (CH_2)	H-2, H-3	
	1.95—1.99 (m)			
3	1.62—1.82 (m)	35.31 (CH_2)	H-2	H-18, H-19
4	—	39.51 (C)		H-18, H-19
5	2.11 (br d, 5.5)	49.51 (CH)	H-6	H-10, H-18
6	3.98 (d, 6.5)	83.54 (CH)	H-5, H-7, H-17	6-OCH_3, H-7, H-9 (H-17)
7	3.05 (br s)	49.69 (CH)	H-6	H-9
8	—	85.77 (C)		H-6, H-7, H-9, H-14, H-15
9	2.92 (t-like, 5.2)	45.59 (CH)	H-10, H-12	H-15 (H-7)
10	2.46—2.53 (m)	41.46 (CH)		H-9 (H-17)
11	—	50.65 (C)		H-7, H-9
12	2.79 (dd, 6.7, 12.8)	36.16 (CH_2)	H-12	13-OH
	2.44—2.49 (m)			
13	—	75.23 (C)		13-OH, H-14, H-15, H-16
14	4.88 (d, 4.9)	79.01 (CH)	H-9	H-16
15	3.04 (dd, 8.8, 15.6)	39.96 (CH_2)	H-15	
	2.41 (dd, 5.9, 15.6)			
16	3.39 (dd, 5.9, 8.8)	84.17 (CH)	H-15	13-OH, H-14, 16-OCH_3
17	2.92	62.28 (CH)		H-5 (H-10)
18	3.62 (d, 8.4)	80.74 (CH_2)	H-18	18-OCH_3
	3.16 (d, 8.4)			
19	2.48 (d, 11)	54.08 (CH_2)	H-19	H-17, H-18
	2.51 (d, 11)			
20	2.43—2.46 (m)	49.47 (CH_2)	H-20, H-21	21-CH_3
	2.55—2.63 (m)			
21-CH_3	1.10 (t, 7.1)	13.84 (CH_3)	H-20	
1-OCH_3	3.27 (s)	56.60 (CH_3)		
6-OCH_3	3.16 (s)	58.35 (CH_3)		
16-OCH_3	3.55 (s)	59.20 (CH_3)		H-16
18-OCH_3	3.30 (s)	59.48 (CH_3)		H-18
13-OH	3.83 (br s)	—		
C-14 acyl moiety				
1′	—	123.17 (C)		H-3′, H-5′
2′	8.03 (d, 8.8)	132.17 (CH)	H-3′	
3′	6.92 (d, 8.8)	114.12 (CH)	H-2′	
4′	—	164.84 (C)		H-3′, H-5′, 4′-OCH_3
5′	6.92 (d, 8.8)	114.12 (CH)	H-6′	
6′	8.03 (d, 8.8)	132.17 (CH)	H-5′	
7′	—	166.46 (C)		H-2′, H-6′, H-14
OCH_3	3.87 (s)	55.80 (CH_3)		
C-8 acyl group				
1″ (C=O)		173.04 (C)		
		173.08 (C)		
8″	2.01—2.08 (m)	27.60 (CH_2)[a,b]		
9″	5.34—5.42 (m)	130.43 (CH)[a,b,c]	H-8″, H-10″	
10″	5.34—5.42 (m)	128.46 (CH)[a,b,c]	H-9″, H-11″	
11″	2.79	26.02 (CH_2)[a]		
12″	5.34—5.42 (m)	128.27 (CH)[a]	H-11″, H-13″	
13″	5.34—5.42 (m)	130.67 (CH)[a]	H-12″, H-14″	
14″	2.01—2.08 (m)	27.60 (CH_2)[a]		
18″	0.88—0.92 (m)	14.47 (CH_3)		
		14.52 (CH_3)		
$(CH_2)_n$	1.28	22.97, 23.09, 24.62, 29.42, 29.47, 29.51, 29.59, 29.72, 29.74, 29.76, 29.86, 29.93, 30.01, 30.06, 30.10, 30.17, 31.93, 32.32, 35.20		

a) These signals are valid for linoleoyl chains only. *b*) These signals are valid for oleoyl chains only. *c*) These signals should be reversed in oleoyl chains.

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Extraction and Isolation Powdered processed tubers of *A. carmichaeli* (7.1 kg) were extracted five times with MeOH at room temperature. The MeOH extracts were combined and evaporated under reduced pressure to dryness. This extract was partitioned with 3% aqueous NH_4OH and $CHCl_3$. The $CHCl_3$ extract (30 g) was separated into 10 fractions (frs. I—X) by chromatography on a silica gel column with a gradient of MeOH in $CHCl_3$. Fraction I was separated further by chromatography on a silica gel column with EtOAc–MeOH (10 : 0.3) to afford 10 subfractions (frs. 1—10). Subfractions I-8 and I-9 were further purified by silica gel column chromatography with

cyclohexane–EtOAc–Et_2NH (10 : 1 : 0.2) to give **3** (8 mg) from fr. I-8 and **4** (10 mg) and **5** (8 mg) from fr. I-9, respectively. Each fraction underwent further chromatography on silica gel columns employing the same solvent systems to give 14-*O*-acetylneoline (**2**, 25 mg),[11] **4** (15 mg), and **5** (7 mg) from fr. I-10, neoline (**1**, 150 mg),[5,6,14] crassicauline A (**7**, 120 mg),[16,18] and **8** (25 mg) from fr. III-3, and foresaconitine (**6**, 15 mg)[16,17] and lipohypaconitine (**9**, 10 mg)[6] from fr. II-3. The known compounds were identified by comparison of their physical and spectral data with published values.

14-*O*-Cinnamoylneoline (**3**): Amorphous powder (MeOH); $[\alpha]_D^{23}$ +9.7° (c=0.35, $CHCl_3$). IR (KBr) cm^{-1} 3435 (OH), 1719 (ester CO), 1638 (aromatic C=C), 1458, 1256, 1167, 1109, 984 (*trans* C=C), 770. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.14 (3H, t, J=7.0 Hz, N-$CH_2C\underline{H}_3$), 2.21 (1H, d, J=6.9 Hz, H-5), 2.65 (1H, dd, J=4.5, 7.2 Hz, H-13), 3.28, 3.33, 3.35 (3H each, s, 3×OCH_3), 3.25, 3.63 (1H each, d, J=8.1 Hz, H-18), 3.71 (1H, t-like, H-1), 4.14 (1H, br d, J=6.3 Hz, H-6β), 5.04 (1H, t, J=4.7 Hz, H-14β), 6.42 (1H, d, J=16.2 Hz, H-8′), 7.37—7.41 (3H, m, H-3′, 4′, 5′), 7.51—7.54 (2H, m, H-2′, 6′), 7.68 (1H, d, J=16.2 Hz, H-7′). ^{13}C-NMR: see Table 1. EI-MS m/z: 567 $[M]^+$ (26.0), 550 $[M-OH]^+$ (100), 534 $[M-(OH+O)]^+$ (24.5), 518 $[M-(OH+CH_3OH)]^+$ (19.1), 436 $[M-C_6H_5CH=CHC{\equiv}O]^+$ (3.2), 418 $[M-(C_6H_5CH=CHC{\equiv}O+H_2O)]^+$ (3.9), 131 $[C_6H_5CH=CHC{\equiv}O]^+$ (44.6). Positive FAB-MS m/z: 568 $[M+H]^+$, 550 $[M-OH]^+$. Positive HR-FAB-MS m/z: 568.3273 $[M+H]^+$ (Calcd for $[C_{33}H_{45}NO_7+H]^+$: 568.3274).

14-*O*-Anisoylneoline (**4**): Amorphous powder (MeOH); $[\alpha]_D^{22}$ +22.3° (c=0.7, $CHCl_3$). IR (KBr) cm^{-1} 3430 (OH), 1719 (ester CO), 1609, 1510 (aromatic C=C), 1458, 1258, 1169, 1105, 765. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.14 (3H, t, J=7.2 Hz, N-$CH_2C\underline{H}_3$), 2.20 (1H, br d, J=6.3 Hz, H-5), 2.63 (1H, dd, J=4.8, 7.2 Hz, H-13), 3.24 (3H, s, OCH_3), 3.32 (3H each, s, 2×OCH_3), 3.84 (3H, s, OCH_3), 3.31, 3.60 (1H each, d, J=8.1 Hz, H-18), 3.72 (1H, br s, H-1), 4.12 (1H, br d, J=6.6 Hz, H-6β), 5.14 (1H, t, J=4.5 Hz, H-14β), 6.90 (2H, d, J=9.0 Hz, H-3′, 5′), 7.95 (2H, d, J=9.0 Hz, H-2′, 6′). ^{13}C-NMR: see Table 1. EI-MS m/z: 571 $[M]^+$ (2.6), 554 $[M-OH]^+$ (19.6), 538 $[M-(OH+O)]^+$ (11.3), 522 $[M-(OH+CH_3OH)]^+$ (8.8), 490 $[M-(OH+2CH_3OH)]^+$ (2.0), 152 $[CH_3OC_6H_4COOH]^+$ (15.7), 135 $[CH_3OC_6H_4C{\equiv}O]^+$ (100). Positive FAB-MS m/z: 572 $[M+H]^+$. Positive HR-FAB-MS m/z: 572.3242 $[M+H]^+$ (Calcd for $[C_{32}H_{45}NO_8+H]^+$: 572.3223).

14-*O*-Veratroylneoline (**5**): Amorphous powder (MeOH); $[\alpha]_D^{23}$ +22.1° (c=0.75, $CHCl_3$). IR (KBr) cm^{-1} 3434 (OH), 1717 (ester CO), 1603, 1518 (aromatic C=C), 1458, 1271, 1109, 764. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.14 (3H, t, J=7.0 Hz, N-$CH_2C\underline{H}_3$), 2.23 (1H, d, J=6.6 Hz, H-5), 2.63 (1H, dd, J=4.5, 7.2 Hz, H-13), 3.28, 3.33, 3.34, 3.92, 3.93 (3H each, s, 5×OCH_3), 3.28, 3.59 (1H each, d, J=8.1 Hz, H-18), 3.78 (1H, br s, H-1), 4.14 (1H, br d, J=6.0 Hz, H-6β), 5.17 (1H, t, J=4.8 Hz, H-14β), 6.89 (1H, d, J=8.4 Hz, H-5′), 7.58 (1H, d, J=2.1 Hz, H-2′), 7.64 (1H, dd, J=2.1, 8.4 Hz, H-6′). ^{13}C-NMR: see Table 1. EI-MS m/z: 584 $[M-OH]^+$ (24.5), 568 $[M-(OH+O)]^+$ (13.7), 552 $[M-(OH+CH_3OH)]^+$ (10.8), 520 $[M-(OH+2CH_3OH)]^+$ (2.5), 437 $[C_{24}H_{39}O_6N]^+$ (1.5), 420 $[C_{24}H_{39}O_6N-OH]^+$ (8.8), 182 $[(CH_3O)_2C_6H_3COOH]^+$ (22.5), 165 $[(CH_3O)_2C_6H_3C{\equiv}O]^+$ (100). Positive FAB-MS m/z 602 $[M+H]^+$. Positive HR-FAB-MS m/z: 602.3329 $[M+H]^+$ (Calcd for $[C_{33}H_{47}NO_9+H]^+$: 602.3333).

Lipo-14-*O*-anisoylbikhaconine (**8**): Colorless oil; $[\alpha]_D^{23}$ +18.7° (c=0.135, $CHCl_3$); ^{1}H- and ^{13}C-NMR : see Table 2. FAB-MS m/z: 868 (acyl=stearoyl), 866 (acyl=oleoyl), 864 (acyl=linoleoyl), 840 (acyl=palmitoyl) $[M+H]^+$, 837 $[868-CH_3O]^+$, 836 $[868-CH_3OH]^+$, 835 $[866-CH_3O]^+$, 834 $[866-CH_3OH]^+$, 833 $[864-CH_3O]^+$, 832 $[864-CH_3OH]^+$, 809 $[840-CH_3O]^+$, 808 $[840-CH_3OH]^+$, 584 $[M-lipoyl]^+$. Positive HR-FAB-MS m/z: 868.5947 $[M+H]^+$ (Calcd for $[C_{51}H_{81}NO_{10}+H]^+$: 868.5939), m/z: 866.5771 $[M+H]^+$ (Calcd for $[C_{51}H_{79}NO_{10}+H]^+$: 866.5782), m/z: 864.5630 $[M+H]^+$ (Calcd for $[C_{51}H_{77}NO_{10}+H]^+$: 864.5626), m/z: 840.5623 $[M+H]^+$ (Calcd for $[C_{49}H_{77}NO_{10}+H]^+$: 840.5626).

Alkaline Hydrolysis of 3—5 and 8 in Methanolic KOH Compounds **3**—**5** and **8** (*ca.* 5 mg each) were each dissolved in 10 ml of 5% KOH in MeOH and allowed to stand for 20 h. After removal of the MeOH by evaporating *in vacuo*, 15 ml of water was added to the mixture, and the whole was extracted 3 times with $CHCl_3$. The residue was purified using cyclohexane–EtOAc–Et_2NH (5 : 2 : 0.2) as eluent to give neoline (**1**, 3 mg) from **3**, **4**, and **5** or bikhaconine (**10**, 2 mg) from **8**. Bikhaconine (**10**): amorphous; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.07 (3H, t, J=6.9 Hz, N-$CH_2C\underline{H}_3$), 2.07 (1H, br s, H-7), 2.99 (1H, dd, J=6.9, 10.8 Hz, H-1), 3.15 (1H, br s, H-17), 3.23 (3H, s, 1-OCH_3), 3.29 (3H, s, 6-OCH_3), 3.30 (3H, s, 18-OCH_3), 3.41 (3H, s, 16-OCH_3), 3.70 (1H, d, J=8.4 Hz, H-18), 3.99 (1H, d, J=4.8 Hz, H-14); 4.10 (1H, d, J=6.9 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ: 85.6 (C-1), 26.0 (C-2), 34.9 (C-3), 39.3 (C-4), 49.9 (C-5), 82.3 (C-6), 52.4 (C-7), 72.7 (C-8), 50.4 (C-9), 42.2 (C-10), 50.1 (C-11), 36.1 (C-12), 76.7 (C-13), 80.6 (C-14), 39.8 (C-15), 84.5 (C-16), 62.5 (C-17), 79.5 (C-18), 53.9 (C-19), 49.3 (N-$\underline{C}H_2CH_3$), 13.5 (N-$CH_2\underline{C}H_3$), 56.1 (1-OCH_3), 57.3 (6-OCH_3), 57.7 (16-OCH_3), 59.1 (18-OCH_3). EI-MS m/z: 467 $[M]^+$ (4.1), 436 $[M-CH_3O]^+$ (100), 404 $[M-CH_3OH]^+$ (3.3).

Fatty Acid Analysis by GC-MS The aqueous layer from the alkaline hydrolysis of compound **8** was acidified with 3% HCl and extracted 3 times with *n*-hexane. The hexane layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to yield a fatty acid mixture. This was identified as methyl palmitate [t_R 15.6 min, 48.2%], methyl oleate [t_R 17.4 min, 37.2%], methyl stearate [t_R 17.5 min, 5.1%], and methyl linoleate [t_R 20.9 min, 9.5%] by GC-MS after methylation with CH_2N_2.

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