Structure–Analgesic Activity Relationship Studies on the C_{18}- and C_{19}-Diterpenoid Alkaloids

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For evaluation of C_{18}- and C_{19}-diterpenoid alkaloids as analgesics, three C_{19}-diterpenoid alkaloids were isolated from the roots of *Aconitum hemsleyanum* var. *circinatum* and *A. transsecutum*; and twenty-five semisynthetic C_{18}- or C_{19}-diterpenoid alkaloids were prepared from lappaconitine, crassicauline A or yunaconitine. In a mice acetic acid-induced abdominal constriction assay, four crassicauline A analogs and three yunaconitine analogs exhibited good analgesic activities with 77.8—94.1% inhibition range in 0.1—10 mg/kg subcutaneous (s.c.) dose range at the point of 20 min after drug administration. Among them, 8-*O*-deacetyl-8-*O*-ethylcrassicauline A (ED_{50}=0.0972 mg/kg) and 8-*O*-ethylyunaconitine (ED_{50}=0.0591 mg/kg) were the most potent analgesics relative to the reference drugs lappaconitine (ED_{50}=3.50 mg/kg) and crassicauline A (ED_{50}=0.0480 mg/kg). Analgesic activity data of these C_{18}- and C_{19}-diterpenoid alkaloids indicate that a tertiary amine in ring A, an acetoxyl or an ethoxyl group at C-8, an aromatic ester at C-14, and the saturation state of the ring D are important structural features necessary to the analgesic activity of the C_{19}-diterpenoid alkaloids.

Key words diterpenoid alkaloid; lappaconitine; crassicauline A; yunaconitine; analgesic activity

Aconitum and *Delphinium* plants have been medicinally used for centuries.[1,2] The traditional Chinese medicine "Cao-Wu," the tubers of *Aconitum* species, has been extensively employed for the clinical treatment of pains, rheumatics and neurological disorders.[3,4] The pharmacological effects of *Aconitum* plants are attributed to their characteristic diterpenoid alkaloids, a group of complex natural products displaying a lot of interesting chemistry[5,6] and biological activities.[1] The analgesic activities of C_{18}- and C_{19}-diterpenoid alkaloids have been extensively investigated since 1981, among which 3-acetylaconitine,[7] lappaconitine,[8,9] and crassicauline A (bulleyaconitine A)[10] have been reported to exhibit marked analgesic activities and have been developed to be analgesic drugs clinically used for the treatment of various pains in China.[12—16] As compared with the known analgesics, such as morphine, methadone, *etc.*, all these three alkaloids induced neither morphine-like tolerance nor physical dependence.

Although there are a few reports[11,13,17—21] on the structure–analgesic activity relationship studies on the C_{18}- and C_{19}-diterpenoid alkaloids, further specific information on the structure features necessary for the analgesic activity of these compounds is still needed. In this paper, twenty-five semisynthetic derivatives were prepared from lappaconitine, crassicauline A, and yunaconitine. Analgesic activities of these semisynthetic alkaloids, as well as three naturally occurring alkaloids, were evaluated in a mice acetic acid-induced abdominal constriction assay. We describe herein the preparation and structure–analgesic activity relationship (SAR) studies for the C_{18}- and C_{19}-diterpenoid alkaloids.

Results and Discussion

Chemistry To further investigate the analgesic activities of C_{18}- and C_{19}-diterpenoid alkaloids, five lappaconitine derivatives (see structures in Fig. 2), seventeen crassicauline A derivatives (see structures in Fig. 3), and six yunaconitine analogs (see structures in Fig. 4) were synthesized from their parent compounds or isolated from *Aconitum* plants. *N*-Deethyllappaconitine (**1**), *N*-deethyllappaconitine imine (**4**) and *N*-deethyl-5″-bromolappaconitine imine (**5**) were prepared by treatment of lappaconitine with *N*-bromosuccinimide (NBS)-HOAc by the similar procedure previously developed by us.[22,23] *N*-Deethyl-*N*,8,9-triacetyllappaconitine (**2**) and *N*-deethyl-*N*-acetyllappaconitine (**3**) were synthesized by acetylation of compound **1** with acetic anhydride-pyridine.

8-*O*-Deacetyl-8-*O*-ethylcrassicauline A (**9**) and 8-*O*-deacetyl-8-*O*-isopentylcrassicauline A (**10**) were prepared from crassicauline A based on the procedure reported in the

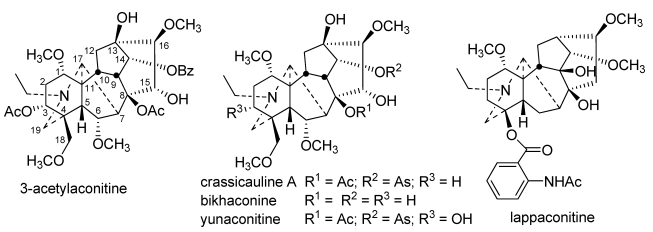

Fig. 1. C_{18}- and C_{19}-Diterpenoid Alkaloids that Exhibit Analgesic Activities

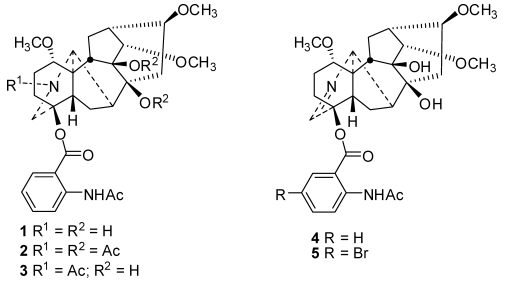

Fig. 2. Lappaconitine Analogues

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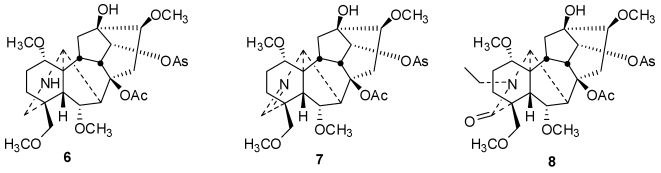

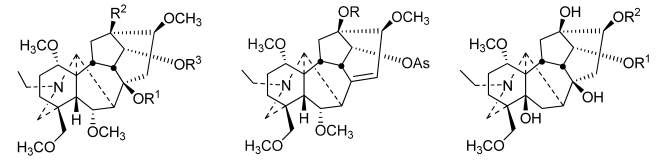

	R^1	R^2	R^3
9	Et	OH	As
10	$(CH_2)_2CH(CH_3)_2$	OH	As
11	Bz	OH	As
12	Bz	OH	Bz
13	H	OH	Ac
14	H	OH	Boc
15	Boc	OH	As
16	Boc	Boc	As
17	Et	OH	Et
18	H	OH	Et

19 R = H
20 R = Boc

21 R^1 = As R^2 = H
22 R^1 = H R^2 = As

As = OC-C_6H_4-OCH_3(*P*)

Fig. 3. Crassicauline A Analogues

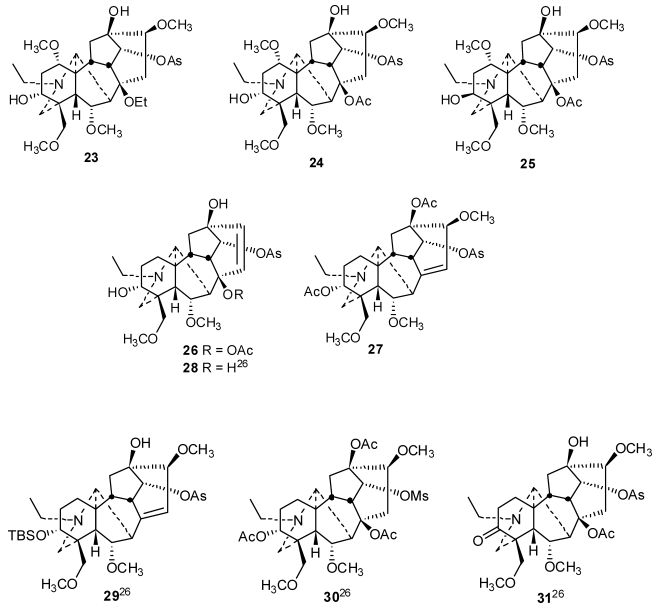

Fig. 4. Yunaconitine Analogues

literature.[24] 8-*O*-Deacetyl-8-*O*-benzoylcrassicauline A (**11**), 8-*O*-deacetyl-8-*O*-*t*-butoxycarbonyloxycrassicauline A (**15**) and 8-*O*-deacetyl-8-*O*,13-di-*t*-butoxycarbonylcrassicauline A (**16**) were synthesized by selective hydrolysis of crassicauline A with dioxane–H_2O (2 : 1) according to Murayama's method[4] followed by acetylation with benzoyl chloride (BzCl) or di-*tert*-butyl dicarbonate [$(Boc)_2O$]. Three acetylated bikhaconine derivatives, 8,14-dibenzoylbikhaconine (**12**), 14-acetylbikhaconine (**13**) and 14-*t*-butoxycarbonylbikhaconine (**14**), were produced by acetylation of bikhaconine (derived from crassicauline A) with BzCl, Ac_2O, and $(Boc)_2O$, respectively. Two ethylated bikhaconine derivatives, 8,14-*O*-diethylbikhaconine (**17**) and 14-*O*-ethylbikhaconine (**18**), were obtained by ethylation of hydroxyl in the bikhaconine with ethyl bromide. *N*-Deethylcrassicauline A (**6**), *N*-deethylcrassicauline A imine (**7**) and crassicauline A lactam (**8**) were achieved by reaction of crassicauline A with *N*-bromosuccinimide (NBS)-glacial acetic acid based on the methods developed by us.[22,23] Pyrocrassicauline A (**19**) and 13-*t*-butoxycarbonyl-pyrocrassicauline A (**20**) were prepared by selective hydrolysis of crassicauline A with dioxane–H_2O (2 : 1) according to Murayama's method[4] followed by refluxing with $(Boc)_2O$ in 41% and 35% yields, respectively. 1,16-Didemethoxy-$\Delta^{15,16}$-yunaconitine (**26**), 1-demethoxy-3,13-diacetyl-14-methane sulfonyl-pyrobikhaconine (**27**) were readily prepared according to the method reported in the literature.[25] 1-Demethoxyyunaconitine (**24**, 70%) and 3-epi-1-demethoxyyunaconitine (**25**, 18%) were obtained by reduction of 1-demethoxy-3-dehydroyunaconitine (**31**, derived from yunaconitine,[26,27]) with sodium borohydride. Crassicauline A analogues, hemsleyanisine (**21**) and isohemsleyanisine (**22**), as well as yunaconitine analogue 8-*O*-ethylyunaconitine (**23**), were isolated from the roots of *Aconitum hemsleyanum* var. *circinatum* and *A. transsecutum* according to the procedures described in the literature.[27,28]

Analgesic Activities Pharmacological studies were carried out to assess the *in vivo* analgesic activities of the above-mentioned C_{18}- and C_{19}-diterpenoid alkaloids. According to the one-dose preliminary assay data, the compounds that showed marked inhibition of writhing movement (>50%) induced by acetic acid were selected for their ED_{50} value evaluation. Four most potent analgesics (**6**, **9**, **23**, **28**[26]) were selected for acute toxic examination, based on *in vivo* analgesic activity data. All these pharmacological data and toxic examination data were summarized in Table 1. In a mice model 0.7% acetic acid-induced abdominal constriction assay,[29] a 10 mg/kg or less subcutaneous (s.c.) dose of *N*-deethylcrassicauline A (**6**), *N*-deethylcrassicauline imine (**7**), 8-*O*-deacetyl-8-*O*-ethylcrassicauline A (**9**), hemsleyanisine (**21**), 8-*O*-ethylyunaconitine (**23**), 1-demethoxyyunaconitine (**24**), and 1,16-didemethoxy-8-*O*-deacetyl-$\Delta^{15,16}$-yunaconitine (**28**) exhibited good analgesic activities (78—94% inhibition range) at the point of 20 min after drug administration (see data in Table 1). Among them, compounds **6**, **9**, **23**, and **28** (ED_{50}=0.0591—2.58 mg/kg) exhibited superior analgesic activity compared to the reference drug lappaconitine (ED_{50}=3.50 mg/kg); compounds **9** and **23** (ED_{50}=0.0591, 0.0972 mg/kg) exhibited comparable analgesic activity to the reference drug crassicauline A (ED_{50}=0.0480 mg/kg). The other alkaloids under investigation showed moderate to inactive analgesic activities in the acetic acid-induced abdominal constriction assay.

The structure–activity relationship (SAR) data acquired for lappaconitine derivatives show that the analogues derived from the modification on ring A of lappaconitine, including *N*-deethyl derivative (**1**), the amides (**2**, **3**), and the imines (**4**, **5**), exhibit significantly decreased analgesic activities (0—48% inhibition range at a 10 mg/kg s.c. dose) relative to the parent compound lappaconitine (ED_{50}=3.50 mg/kg).

The *in vivo* data acquired for crassicauline A analogues (**9**—**22**) show that analgesic activities can be manipulated by varying the electronic and steric properties of substituents attached to C-8. For example, compound **9** possessing an

Table 1. Antinociceptive Effects of Compounds on Acetic Acid-Induced Writhing Test and Preliminary Acute Toxicity in Mice

Compound	Dose (mg/kg)	Inhibition (%)	ED_{50} (mg/kg)	Toxic dose (mg/kg)	Mortality[a] (died/tested)
6	0.8	90.0	0.411	4	5/5
7	10	84.2	6.42	—	—
9	0.2	86.4	0.0972	1	2/5
21	6	77.8	3.42	—	—
23	0.1	84.0	0.0591	0.6	3/5
24	10	94.1	7.98	—	—
28	4	94.1	2.58	26	5/5
1	10	48.0	—	—	—
2	10	0.0	—	—	—
3	10	10.0	—	—	—
4	10	20.0	—	—	—
5	10	18.2	—	—	—
8	10	25.0	—	—	—
10	10	15.0	—	—	—
11	10	36.4	—	—	—
12	10	16.7	—	—	—
13	10	11.1	—	—	—
14	10	34.6	—	—	—
15	10	20.0	—	—	—
16	10	21.7	—	—	—
17	10	23.1	—	—	—
18	10	19.2	—	—	—
19	10	25.0	—	—	—
20	10	15.0	—	—	—
22	10	11.1	—	—	—
25	10	16.7	—	—	—
26	10	41.2	—	—	—
27	10	22.2	—	—	—
Lappaconitine	—	—	3.50	11.7	—
Crassicauline A	—	—	0.0480	0.92	—

The acetic acid induced writhing data is displayed as percent reduction. *a*) Dead mice were counted at 1 h after injection of test samples.

ethoxyl group at C-8 instead of an acetoxyl group can retain potential analgesic activity (ED_{50}=0.0972 mg/kg), while 8,14-diethoxyl analogue **17** is inactive. In contrast, replacement of the acetoxy group at C-8 of crassicauline A with a bulky alkoxyl (isopenthoxyl) group (**10**) or bulky ester such as Boc (**15**, **16**) or BzO (**11**, **12**) abolishes the analgesic activity. It is also interesting to note that 14-acetylbikhaconine (**13**), 14-*t*-butoxycarbonyl-bikhaconine (**14**), and 14-*O*-ethyl-bikhaconine (**18**), possessing a hydroxyl group at C-8 instead of an acetoxyl group in crassicauline A and a substitute at C-14 different from crassicauline A, show very weak activity. Introduction of a double bond at C-8/C-15 *via* elimination of C-8 acetoxyl group (**19**, **20**) dramatically diminishes the activities. The point of attachment of a *p*-methoxy-benzoyloxy (OAs) substituent is a determinant of analgesic activity because the 14-OAs compound (**21**), structurally similar to parent compound crassicauline A, shows good analgesic activity (ED_{50}=3.42 mg/kg). In contrast, the 16-OAs regioisomer (**22**) is inactive. On the other hand, ring A modified derivatives of crassicauline A, *N*-deethyl derivative (**6**), imine (**7**) and lactam (**8**), exhibit less activity than their parent compound crassicauline A even though analogues **6** and **7** still maintain good analgesic activity (ED_{50}=0.411, 6.42 mg/kg).

The mice acetic acid-induced abdominal constriction assay data show that yunaconitine subgroup (**23**—**28**) exhibits a wide range (low-to-good) of analgesic activities (see data in Table 1). In this subgroup, 8-*O*-ethylyunaconitine (**23**) is an equipotent analgesics (ED_{50}=0.0591 mg/kg) relative to crassicauline A (ED_{50}=0.0480 mg/kg), indicating that the replacement of C-8 acetoxyl group with ethoxyl group does not affect the analgesic activities. Similar to crassicauline A derivatives, $\Delta^{8(15)}$ derivative (**27**) is inactive. Similarly, $\Delta^{15(16)}$ derivative (**26**) possesses less activity (41.2% inhibition at a 10 mg/kg s.c. dose) compared to its parent compound (**24**, 94.1% inhibition at a 10 mg/kg s.c. dose). Stereochemistry at C-3 is important for the activity since 3α-hydroxyl analogue **24** exhibits good activity (ED_{50}=7.98 mg/kg), while its isomer, 3β-hydroxyl analogue **25**, is inactive.

In conclusion, the above-mentioned structure–activity studies show that an *N*-ethyl substituted tertiary amine in ring A, an acetoxyl or ethoxyl group at C-8, an aromatic ester (OBz or OAs) at C-14, and the saturation state of the ring D are necessary for the manifestation of important analgesic activity of C_{18}- and C_{19}-diterpenoid alkaloids. Also, 3α or 5-hydroxyl group is helpful for the analgesic activity. The structure–activity data established in this study constructs the critical pharmacophore of C_{18}- and C_{19}-diterpenoid alkaloids as analgesics, which could be beneficial in searching for the potential analgesics that is equal or more active, but with lower toxicity, than currently clinical used C_{18}- and C_{19}-alkaloid-type analgesics.

Experimental

General IR spectra were recorded on a Nicolet 200 SXV spectrometer; mass spectra were obtained with a Finnigan LCQ and Micromass Auto Spec Ultima-Tof spectrometer; ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker AC-E 200 or a Varian INOVA-400/54 spectrometer in $CDCl_3$, with tetramethylsilane (TMS) as internal standard; Silica GF254 and gel H (10—40 μm, Qingdao Sea Chemical Factory, China) were used for TLC and CC. Only key signals for all products in the ^{1}H-NMR spectra were reported. The starting materials crassicauline A, yunaconitine, and lappaconitine were purchased from Kunming Institute of Botany at the Chinese Academy of Sciences and Lanzhou Pharmaceutical Company in China.

***N*-Deethyllappaconitine (1)** To a solution of lappaconitine (100 mg, 0.17 mmol) in glacial acetic acid (5 ml) was added NBS (910 mg, 0.51 mmol), and the subsequent reaction solution was allowed to stand at room temperature for 1.5 h. After a general work-up, column chromatography (silica gel, 3 g, $CHCl_3$–MeOH=94 : 6) of the crude residue gave title compound **1** (92 mg, 95%): ^{1}H-NMR (200 MHz) δ: 2.22 (3 H, s, NHCO$\underline{CH_3}$), 3.30, 3.30, 3.40 (each 3H, s, OCH_3×3), 7.02 (1H, m, H-5″), 7.50 (1H, m, H-4″), 7.90 (1H, d, *J*=8.0 Hz, H-3″), 8.66 (1H, d, *J*=8.4 Hz, H-6″), 11.03 (1H, br s, N$\underline{H}$COCH$_3$). ^{13}C-NMR (50 MHz) δ: 82.5 (C-1), 23.7 (C-2), 29.6 (C-3), 83.5 (C-4), 52.3 (C-5), 26.2 (C-6), 44.2 (C-7), 75.9 (C-8), 77.3 (C-9), 36.8 (C-10), 52.7 (C-11), 24.4 (C-12), 49.2 (C-13), 90.0 (C-14), 44.0 (C-15), 82.3 (C-16), 57.0 (C-17), 50.8 (C-19), 55.8 (C-1′), 56.0 (C-14′), 57.8 (C-16′), 169.0, 25.2 (NH$\underline{C}$O$\underline{C}$H$_3$), 167.2 ($\underline{C}$OO), 115.1 (C-1″), 141.5 (C-2″), 120.1 (C-3″), 134.3 (C-4″), 122.3 (C-5″), 130.9 (C-6″). HR-ESI-MS *m*/*z*: 557.2847 $[M+H]^+$ (Calcd for $C_{30}H_{41}N_2O_8$: 557.2857).

***N*-Deethyl-*N*,8,9-triacetyllappaconitine (2) and *N*-Deethyl-*N*-acetyllappaconitine (3)** The acetylated C_{18}-diterpenoid alkaloids **2** and **3** were synthesized by acetylation of **1** following a general procedure. **2**: 89% yield. ^{1}H-NMR (200 MHz) δ: 2.03, 2.13, 2.17, 2.21 (each 3H, OCO$\underline{CH_3}$×4), 3.23, 3.33, 3.41 (each 3H, s, OCH_3×3), 4.78 (1H, ABq, *J*=14.5 Hz, H-19), 4.90 (1H, *J*=4.9 Hz, H-14β), 7.03 (1H, m, H-5″), 7.54 (1H, m, H-4″), 7.84 (1H, d, *J*=8.4 Hz, H-3″), 8.66 (1H, d, *J*=8.4 Hz, H-6″), 10.97 (1H, br s, N$\underline{H}$Ac). ^{13}C-NMR (50 MHz) δ: 81.0 (C-1), 23.3 (C-2), 31.1 (C-3), 88.0 (C-4), 46.4 (C-5), 26.2 (C-6), 45.9 (C-7), 82.7 (C-8), 83. 9 (C-9), 38.1 (C-10), 50.6 (C-11), 25.2 (C-12), 48.7 (C-13), 82.1 (C-14), 40.1 (C-15), 80.6 (C-16), 57.8 (C-17), 46.6 (C-19), 170.2 (C-21), 25.4 (C-22), 56.5 (C-1′), 55.7 (C-14′), 57.5 (C-16′), 169.2, 169.0, 22.4, 22.3 (OAc×2), 169.2, 23.4 (NH$\underline{C}$O$\underline{C}$H$_3$), 167.0 ($\underline{C}$OO), 115.1 (C-1″), 141.9 (C-2″), 120.4 (C-3″), 134.7 (C-4″), 122.3 (C-5″), 130.6 (C-6″). HR-ESI-MS *m*/*z*: 705.2967 $[M+Na]^+$ (Calcd for $C_{36}H_{46}N_2O_{11}Na$: 705.2994). **3**: 86% yield. ^{1}H-NMR (200 MHz) δ: 2.17, 2.22 (each 3H, s, OCO$\underline{CH_3}$×2), 3.22, 3.31, 3.34 (each 3H, s, OCH_3×3), 7.01 (1H, t, *J*=7.8 Hz, H-5″), 7.49 (1H, t, *J*=8.0 Hz, H-4″), 7.89 (1H, d, *J*=8.4

Hz, H-3″), 8.66 (1H, d, *J*=8.4 Hz, H-6″), 11.03 (1H, br s, NHAc). ^{13}C-NMR (50 MHz) *δ*: 82.8 (C-1), 24.4 (C-2), 31.1 (C-3), 82.5 (C-4), 53.4 (C-5), 26.2 (C-6), 49.7 (C-7), 75.0 (C-8), 77.8 (C-9), 36.6 (C-10), 50.9 (C-11), 25.2 (C-12), 53.7 (C-13), 89.7 (C-14), 44.5 (C-15), 80.9 (C-16), 58.6 (C-17), 47.0 (C-19), 169.2 (C-21), 22.3 (C-22), 56.2 (C-1′), 55.7 (C-14′), 57.9 (C-16′), 169.1, 22.5 (NHCOCH$_3$), 167.0 (COO), 115.0 (C-1″), 141.7 (C-2″), 120.2 (C-3″), 134.5 (C-4″), 122.2 (C-5″), 130.8 (C-6″). HR-ESI-MS *m/z*: 621.2775 [M+Na]$^+$ (Calcd for $C_{32}H_{42}N_2O_9Na$: 621.2783).

***N*-Deethyllappaconitine Imine (4)** The imine **4** was obtained in 73% yield by the same synthetic procedure as that of **1**. ^{1}H-NMR (200 MHz) *δ*: 2.18 (3H, s, OCOCH$_3$), 3.22, 3.31, 3.41 (each 3H, s, OCH$_3$×3), 7.03 (1H, m, H-5″), 7.52 (1H, m, H-4″), 7.96 (1H, d, *J*=8.0 Hz, H-3″), 8.68 (1H, d, *J*=8.4 Hz, H-6″), 11.0 (1H, br s, NHAc). ^{13}C-NMR (50 MHz) *δ*: 82.3 (C-1), 23.2 (C-2), 27.0 (C-3), 88.6 (C-4), 48.1 (C-5), 26.9 (C-6), 41.3 (C-7), 75.4 (C-8), 88.0 (C-9), 36.8 (C-10), 53.8 (C-11), 26.9 (C-12), 52.4 (C-13), 89.7 (C-14), 44.2 (C-15), 81.3 (C-16), 62.1 (C-17), 159.2 (C-19), 56.2 (C-1′), 56.4 (C-14′), 57.9 (C-16′), 169.0, 25.5 (NHCOCH$_3$), 167.0 (COO), 115.1 (C-1″), 141.8 (C-2″), 120.2 (C-3″), 134.7 (C-4″), 122.3 (C-5″), 130.8 (C-6″). HR-ESI-MS *m/z*: 577.2532 [M+Na]$^+$ (Calcd for $C_{30}H_{38}N_2O_8Na$: 577.2520).

***N*-Deethyl-5″-bromolappaconitine Imine (5)** A solution of lappaconitine (100 mg, 0.17 mmol) and NBS (246 mg, 1.36 mmol) in glacial acetic acid (5 ml) was allowed to stand at room temperature overnight. After a general work-up, column chromatography (silica gel, 3 g, petroleum ether–acetone=2 : 1) of the crude residue afforded **5** (94 mg, 87%). ^{1}H-NMR (200 MHz) *δ*: 2.21 (3H, s, NHAc), 3.22, 3.31, 3.34 (each 3H, s, OCH$_3$×3), 7.67 (1H, dd, *J*=10.0, 2.4 Hz, H-4″), 8.04 (1H, d, *J*=2.4 Hz, H-6″), 8.62 (1H, d, *J*=10.0 Hz, H-3″), 10.93 (1H, br s, NHAc). ^{13}C-NMR (50 MHz) *δ*: 82.3 (C-1), 22.3 (C-2), 29.6 (C-3), 89.3 (C-4), 52.3 (C-5), 26.8 (C-6), 41.2 (C-7), 75.3 (C-8), 76.5 (C-9), 36.8 (C-10), 53.9 (C-11), 23.2 (C-12), 48.1 (C-13), 89.7 (C-14), 44.2 (C-15), 82.6 (C-16), 62.1 (C-17), 158.8 (C-19), 56.5 (C-1′), 56.2 (C-14′), 57.9 (C-16′), 169.0, 25.2 (NHCOCH$_3$), 165.8 (COO), 114.0 (C-1″), 140.8 (C-2″), 121.9 (C-3″), 137.4 (C-4″), 116.6 (C-5″), 133.1 (C-6″). HR-ESI-MS *m/z*: 655.1637 [M+Na]$^+$ (Calcd for $C_{30}H_{37}N_2O_8BrNa$: 655.3631).

***N*-Deethylcrassicauline A (6)** To a solution of crassicauline A (100 mg, 0.15 mmol) in 5 ml of glacial acetic acid was added NBS (80 mg, 0.45 mmol). The reaction mixture was allowed to stand at room temperature overnight and basified to ph 10 using 25% NH_4OH. The subsequent mixture was extracted with chloroform. The combined extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo* to afford compound **6** (83 mg, 90%). ^{1}H-NMR (200 MHz) *δ*: 1.34 (3H, s, OCOCH$_3$), 3.16, 3.22, 3.28, 3.53, 3.84 (each 3H, s, OCH$_3$×5), 3.98 (1H, d, *J*=5.4 Hz, H-6*β*), 4.88 (1H, d, *J*=5.0 Hz, H-14*β*), 6.88, 7.97 (each 2H, AA′BB′ system, *J*=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 83.1 (C-1), 23.4 (C-2), 35.2 (C-3), 39.0 (C-4), 44.1 (C-5), 82.5 (C-6), 53.4 (C-7), 85.5 (C-8), 40.2 (C-9), 43.6 (C-10), 50.3 (C-11), 29.0 (C-12), 74.5 (C-13), 78.7 (C-14), 39.7 (C-15), 83.1 (C-16), 55.3 (C-17), 79.9 (C-18), 49.3 (C-19), 57.5 (C-1′), 58.6 (C-6′), 57.7 (C-16′), 59.0 (C-18′), 169.5, 21.4 (OAc), 165.7 (COO), 122.3 (C-1″), 131.5 (C-2″, 6″), 113.6 (C-3″, 5″), 163.3 (C-4″), 55.3 (4″-OCH$_3$). HR-ESI-MS *m/z*: 616.3088 [M+H]$^+$ (Calcd for $C_{33}H_{46}NO_{10}$: 616.3116).

***N*-Deethylcrassicauline A Imine (7)** To a solution of crassicauline A (1 g, 1.55 mmol) in 25 ml of glacial acetic acid was added NBS (2.2 g, 12.44 mmol), and the reaction was allowed to stand at room temperature overnight. The reaction mixture was basified to pH 10 using 25% NH_4OH, and the subsequent mixture was extracted with chloroform. The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo* to afford the crude product. Purification of the product by silica gel (25 g) column chromatography using petroleum ether–acetone (5 : 2, with 1% diethylamine) as eluant furnished **7** (693 mg, 73%): ^{1}H-NMR (200 MHz) *δ*: 1.27 (3H, s, OCOCH$_3$), 3.04, 3.15, 3.27, 3.51 (each 3H, s, OCH$_3$×4), 4.87 (1H, d, *J*=4.7 Hz, H-14*β*), 6.87, 7.96 (each 2H, AA′BB′ system, *J*=8.6 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 83.5 (C-1), 22.5 (C-2), 35.8 (C-3), 46.5 (C-4), 53.9 (C-5), 82.0 (C-6), 42.8 (C-7), 84.2 (C-8), 40.1 (C-9), 45.7 (C-10), 51.5 (C-11), 27.8 (C-12), 74.6 (C-13), 78.6 (C-14), 38.6 (C-15), 82.1 (C-16), 61.2 (C-17), 77.8 (C-18), 165.8 (C-19), 55.9 (C-1′), 58.7 (C-6′), 57.1 (C-16′), 59.0 (C-18′), 169.5, 21.4 (OAc), 165.8 (COO), 122.4 (C-1″), 131.6 (C-2″, 6″), 113.7 (C-3″, 5″), 163.4 (C-4″), 55.3 (4″-OCH$_3$). HR-ESI-MS *m/z*: 614.2945 [M+H]$^+$ (Calcd for $C_{33}H_{44}NO_{10}$: 614.2960).

Crassicauline A Lactam (8) To a solution of crassicauline **A** (1 g, 1.55 mmol) in 25 ml of glacial acetic acid was added NBS (2.2 g, 12.44 mmol), and the reaction solution was allowed to stand at room temperature overnight. After a general work-up, purification of the crude residue over column chromatography (silica gel, 25 g, petroleum ether–acetone=5 : 2, with 1% diethylamine) furnished **8** (50 mg, 4.9%): ^{1}H-NMR (200 MHz) *δ*: 1.05 (3H, t, *J*=7.1 Hz, NCH$_2$CH$_3$), 1.30 (3H, s, OCOCH$_3$), 3.12, 3.28, 3.37, 3.53 (each 3H, s, OCH$_3$×4), 4.77 (1H, br s, H-14*β*), 6.89, 7.97 (each 2H, AA′BB′ system, *J*=8.2 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 84.9 (C-1), 25.5 (C-2), 31.0 (C-3), 41.0 (C-4), 50.7 (C-5), 81.4 (C-6), 50.6 (C-7), 84.0 (C-8), 42.5 (C-9), 45.7 (C-10), 51.3 (C-11), 33.6 (C-12), 75.6 (C-13), 78.5 (C-14), 40.1 (C-15), 83.4 (C-16), 60.1 (C-17), 77.8 (C-18), 124.0 (C-19), 56.1 (C-1′), 58.7 (C-6′), 57.1 (C-16′), 59.5 (C-18′), 169.7, 21.4 (OAc), 168.0 (COO), 122.3 (C-1″), 131.6 (C-2″, 6″), 113.7 (C-3″, 5″), 163.4 (C-4″), 55.4 (4″-OCH$_3$). HR-ESI-MS *m/z*: 658.3230 [M+H]$^+$ (Calcd for $C_{35}H_{48}NO_{11}$: 658.3227).

8-*O*-Deacetyl-8-*O*-ethylcrassicauline A (9) A solution of crassicauline A (100 mg, 0.17 mmol) in ethanol (5 ml) was kept refluxing for 3 d, and then the ethanol was removed under reduced pressure. The residue obtained was purified over silica gel column chromatography employing chloroform–methanol (97 : 3) as eluent to give the title compound **9** (84 mg, 89%). ^{1}H-NMR (200 MHz) *δ*: 0.57 (3H, t, *J*=6.8 Hz, NCH$_2$CH$_3$), 1.07 (3H, t, *J*=7.0 Hz, OCH$_2$CH$_3$), 3.24, 3.24, 3.28, 3.32 (each 3H, s, OCH$_3$×4), 3.85 (3H, s, Ar-OCH$_3$), 3.99 (1H, d, *J*=3.7 Hz, H-6*β*), 4.82 (1H, d, *J*=5.1 Hz, H-14*β*), 6.88, 8.01 (each 2H, AA′BB′ system, *J*=8.7 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 85.3 (C-1), 26.3 (C-2), 36.4 (C-3), 38.9 (C-4), 48.9 (C-5), 82.8 (C-6), 48.9 (C-7), 78.0 (C-8), 41.3 (C-9), 46.3 (C-10), 50.4 (C-11), 29.6 (C-12), 75.3 (C-13), 79.0 (C-14), 37.2 (C-15), 84.0 (C-16), 61.3 (C-17), 80.0 (C-18), 53.7 (C-19), 49.1 (C-21), 13.4 (C-22), 56.2 (C-1′), 58.6 (C-6′), 58.6 (C-16′), 58.9 (C-18′), 166.2 (COO), 123.3 (C-1″), 131.6 (C-2″, 6″), 113.3 (C-3″, 5″), 163.0 (C-4″), 55.7 (4″-OCH$_3$), 55.7 (OCH$_2$), 15.2 (OCH$_2$CH$_3$). HR-ESI-MS *m/z*: 630.3635 [M+H]$^+$ (Calcd for $C_{35}H_{52}NO_9$: 630.3642).

8-Deacetoxy-8-isopentoxycrassicauline A (10) and Pyrocrassicauline A (19) A solution of crassicauline A (300 mg, 0.45 mmol) in isopentyl alcohol (50 ml) was heated at 80 °C for 3 d. After a general work-up, the crude residue was subjected to column chromatography (silica gel, 10 g, petroleum ether–acetone=11 : 2) to give C-8 isopentoxylated analogue **10** (90 mg, 30%) and pyrocrassicauline A **19** (126 mg, 41%). **10**: ^{1}H-NMR (200 MHz) *δ*: 1.09 (3H, t, *J*=7.2 Hz, NCH$_2$CH$_3$), 3.26, 3.26, 3.29, 3.54 (each 3H, s, OCH$_3$×4), 3.85 (3H, s, 4″-OCH$_3$), 4.00 (1H, d, *J*=6.4 Hz, H-6*β*), 4.80 (1H, d, *J*=4.9 Hz, H-14*β*), 6.90, 8.02 (each 2H, AA′BB′ system, *J*=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 85.3 (C-1), 26.3 (C-2), 37.4 (C-3), 39.0 (C-4), 48.2 (C-5), 82.9 (C-6), 49.1 (C-7), 78.0 (C-8), 41.4 (C-9), 46.3 (C-10), 50.6 (C-11), 36.3 (C-12), 75.2 (C-13), 79.1 (C-14), 39.0 (C-15), 84.0 (C-16), 61.3 (C-17), 80.0 (C-18), 53.7 (C-19), 48.9 (C-21), 13.4 (C-22), 56.3 (C-1′), 58.7 (C-6′), 58.7 (C-16′), 58.9 (C-18′), 166.1 (COO), 123.3 (C-1″), 131.8 (C-2″, 6″), 113.3 (C-3″, 5″), 163.1 (C-4″), 55.3 (4″-OCH$_3$), 61.3, 30.2, 24.6, 22.6, 22.3 (OCH$_2$CH$_2$CH(CH$_3$)$_2$). HR-ESI-MS *m/z* 672.4109 [M+H]$^+$ (Calcd for $C_{38}H_{58}NO_9$, 672.4106). **19**: ^{1}H-NMR (200 MHz) *δ*: 1.07 (3H, t, *J*=7.1 Hz, NCH$_2$CH$_3$), 3.24, 3.28, 3.30, 3.38 (each 3H, s, OCH$_3$×4), 3.85 (3H, s, 4″-OCH$_3$), 4.20 (1H, d, *J*=6.6 Hz, H-6*β*), 4.94 (1H, d, *J*=2.8 Hz, H-14*β*), 5.54 (1H, d, *J*=6.2 Hz, H-15), 6.88, 8.00 (each 2H, AA′BB′ system, *J*=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 86.0 (C-1), 25.1 (C-2), 38.4 (C-3), 39.8 (C-4), 48.5 (C-5), 83.5 (C-6), 50.9 (C-7), 146.9 (C-8), 46.6 (C-9), 48.4 (C-10), 51.6 (C-11), 35.3 (C-12), 77.6 (C-13), 78.3 (C-14), 116.0 (C-15), 83.6 (C-16), 59.2 (C-17), 80.3 (C-18), 54.0 (C-19), 49.8 (C-21), 13.5 (C-22), 56.3 (C-1′), 58.1 (C-6′), 57.1 (C-16′), 59.2 (C-18′), 167.8 (COO), 122.8 (C-1″), 131.9 (C-2″, 6″), 113.4 (C-3″,5″), 163.2 (C-4″), 55.3 (4″-OCH$_3$). HR-ESI-MS *m/z*: 584.3221 [M+H]$^+$ (Calcd for $C_{33}H_{46}NO_8$: 584.3218).

8-Deacetoxyl-8-benzoyloxycrassicauline A (11) A solution of crassicauline A (1 g, 1.55 mmol) in dioxane–H_2O (1 : 1) (50 ml) was refluxed overnight. After a general work-up, residue **A** (913 mg, 98%) was obtained. To a solution of residue **A** (100 mg, 0.16 mmol) in 5 ml of pyridine was added 4-dimethylaminopyridine (DMAP) (100 mg) and 4.24 mmol of benzoyl chloride, and the resulting reaction mixture was kept refluxing overnight and cooled to room temperature. After a general work-up, the crude residue was subjected to column chromatography (silica gel, 3 g, petroleum ether–acetone=7 : 1, with 1% of diethylamine) to furnish **11** (50 mg, 43%). ^{1}H-NMR (200 MHz) *δ*: 1.12 (3H, t, *J*=6.9 Hz, NCH$_2$CH$_3$), 3.23, 3.24, 3.27, 3.29 (each 3H, s, OCH$_3$×4), 3.83 (3H, s, 4″-OCH$_3$), 4.05 (1H, d, *J*=6.7 Hz, H-6*β*), 5.68 (1H, d, *J*=4.8 Hz, H-14*β*), 5.54 (1H, d, *J*=6.2 Hz, H-15), 6.89—8.07 (8H, m, Ar-H). ^{13}C-NMR (50 MHz) *δ*: 85.0 (C-1), 22.6 (C-2), 35.7 (C-3), 39.2 (C-4), 53.1 (C-5), 79.2 (C-6), 53.1 (C-7), 84.1 (C-8), 42.9 (C-9), 46.8 (C-10), 49.5 (C-11), 29.6 (C-12), 73.6 (C-13), 77.1 (C-14), 42.4 (C-15), 82.6 (C-16), 61.8 (C-17), 80.5 (C-18), 54.0 (C-19), 46.8 (C-21), 13.5 (C-22), 56.0 (C-1′), 57.8 (C-6′), 57.5 (C-16′), 59.1 (C-18′), 165.6 (COO), 122.4 (C-1″), 132.0 (C-2″, 6″), 113.7 (C-3″, 5″), 163.4

(C-4″), 55.3 (OCH_3-4″), 165.6 (COO), 122.4 (C-1‴), 129.8 (C-2‴, 6‴), 128.1 (C-3‴, 5‴), 132.6 (C-4‴). HR-ESI-MS *m/z*: 706.3557 $[M+H]^+$ (Calcd for $C_{40}H_{52}NO_{10}$: 706.3586).

8,14-Dibenzoylbikhaconine (12) Crassicauline A (500 mg, 0.75 mmol) was added to a solution of NaOH in methanol (20 ml), the subsequent reaction solution was heated at 50 °C for 30 min. The methanol was removed to give a residue, to which were added DMAP (300 mg), pyridine (10 ml), and benzoyl chloride (20 of drops, 8.48 mmol). The subsequent reaction mixture was allowed to stand at room temperature overnight. After a general work-up, the crude residue was purified over column chromatography (silica gel, 10 g, petroleum ether–acetone=3 : 2) to yield **12** (47 mg, 13%). ^{1}H-NMR (200 MHz) δ: 1.12 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 3.24, 3.25, 3.28, 3.30 (each 3H, s, $OCH_3\times4$), 4.06 (1H, d, J=6.7 Hz, H-6β), 5.73 (1H, d, J=4.9 Hz, H-14β), 7.26—8.12 (10H, m, Ar-H). ^{13}C-NMR (50 MHz) δ: 85.1 (C-1), 26.0 (C-2), 35.8 (C-3), 39.3 (C-4), 49.6 (C-5), 79.2 (C-6), 53.3 (C-7), 84.3 (C-8), 43.0 (C-9), 46.8 (C-10), 50.4 (C-11), 35.0 (C-12), 73.6 (C-13), 77.4 (C-14), 42.4 (C-15), 82.6 (C-16), 61.8 (C-17), 80.6 (C-18), 54.0 (C-19), 49.1 (C-21), 13.6 (C-22), 56.0 (C-1′), 57.8 (C-6′), 57.5 (C-16′), 59.1 (C-18′), 166.0, 165.6 (COO×2), 130.2, 130.7 [(C-1″)×2)], 129.8, 129.9 [(C-2″)×2)], 128.2, 128.5 [(C-3″, 5″)×2)], 132.6, 133.0 [(C-4″)×2)]. HR-ESI-MS *m/z*: 676.3457 $[M+H]^+$ (Calcd for $C_{39}H_{50}NO_9$: 676.3480).

14-Acetylbikhaconine (13) and 14-*t*-butoxycarbonylbikhaconine (14) Bikhaconine analogue **13** (92 mg) was obtained in 86% yield by acetylation of bikhaconine (100 mg, 0.21 mmol) with acetic anhydride (1 ml) in 5 ml of pyridine. Similarly, bikhaconine analogue **14** (38 mg) was prepared in 32% yield by treatment of bikhaconine (100 mg, 0.21 mmol) with $(Boc)_2O$ (93 mg, 0.42 mmol) and DMAP (100 mg) in 10 ml of pyridine. **13**: ^{1}H-NMR (200 MHz) δ: 1.08 (3H, t, J=7.1 Hz, $NCH_2\underline{CH_3}$), 2.08 (3H, s, $OCO\underline{CH_3}$), 3.23, 3.30, 3.30, 3.36 (each 3H, s, $OCH_3\times4$), 4.02 (1H, d, J=6.8 Hz, H-6β), 4.91 (1H, d, J=4.9 Hz, H-14β). ^{13}C-NMR (50 MHz) δ: 85.5 (C-1), 25.9 (C-2), 37.3 (C-3), 39.3 (C-4), 50.2 (C-5), 82.7 (C-6), 53.2 (C-7), 85.0 (C-8), 42.4 (C-9), 48.9 (C-10), 50.3 (C-11), 35.0 (C-12), 73.5 (C-13), 81.2 (C-14), 41.5 (C-15), 83.6 (C-16), 62.1 (C-17), 80.6 (C-18), 53.8 (C-19), 49.4 (C-21), 13.6 (C-22), 56.2 (C-1′), 58.6 (C-6′), 57.4 (C-16′), 59.2 (C-18′), 171.8, 21.2 (OAc). HR-ESI-MS *m/z*: 510.3042 $[M+H]^+$ (Calcd for $C_{27}H_{44}NO_8$: 510.3061). **14**: ^{1}H-NMR (200 MHz) δ: 1.08 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 1.47 (9H, s, $(\underline{CH_3})_3$C-OCO), 3.23, 3.29, 3.30, 3.34 (each 3H, s, $OCH_3\times4$), 4.03 (1H, d, J=6.4 Hz, H-6β), 4.70 (1H, d, J=4.6 Hz, H-14β). ^{13}C-NMR (50 MHz) δ: 85.6 (C-1), 29.6 (C-2), 37.5 (C-3), 37.6 (C-4), 50.1 (C-5), 82.3 (C-6), 52.8 (C-7), 82.4 (C-8), 42.2 (C-9), 49.7 (C-10), 49.2 (C-11), 35.1 (C-12), 73.1 (C-13), 82.3 (C-14), 41.0 (C-15), 83.8 (C-16), 62.1 (C-17), 80.6 (C-18), 53.6 (C-19), 49.2 (C-21), 13.6 (C-22), 56.2 (C-1′), 58.5 (C-6′), 57.3 (C-14′), 59.1 (C-16′), 154.0 s, 76.8 s, 27.6 q [$(\underline{C}H_3)_3\underline{C}$–O$\underline{C}$O]. HR-ESI-MS *m/z*: 568.3478 $[M+H]^+$ (Calcd for $C_{30}H_{50}NO_9$: 568.3480).

8-*O*-Deacetyl-8-*O*-*t*-butoxycarbonylcrassicauline A (15) and 8-*O*-Deacetyl-8-*O*,13-di-*t*-butoxy-carbonylcrassicauline A (16) To a solution of residue **A** (100 mg, 0.33 mmol) in dichloromethane (10 ml) were added DMAP (200 mg) and $(Boc)_2O$ (177 mg, 0.81 mmol), and the reaction was allowed to stand at room temperature overnight. After a general work-up, the crude residue was purified by column chromatography (silica gel, 8 g, petroleum ether–acetone=9 : 1) to yield **15** (90 mg, 34%) and **16** (118 mg, 51%). **15**: ^{1}H-NMR (200 MHz) δ: 1.07 (3H, t, J=6.9 Hz, $NCH_2\underline{CH_3}$), 1.11 (9H, s, $(\underline{CH_3})_3$C–OCO), 3.17, 3.24, 3.27, 3.49 (each 3H, s, $OCH_3\times4$), 3.84 (3H, s, 4″-OCH_3), 4.00 (1H, d, J=6.8 Hz, H-6β), 4.89 (1H, d, J=5.0 Hz, H-14β), 6.89, 8.01 (each 2H, AA′BB′ system, J=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) δ: 84.9 (C-1), 26.3 (C-2), 36.0 (C-3), 39.1 (C-4), 48.9 (C-5), 82.8 (C-6), 49.7 (C-7), 85.3 (C-8), 41.2 (C-9), 45.4 (C-10), 50.1 (C-11), 34.7 (C-12), 75.2 (C-13), 78.5 (C-14), 39.2 (C-15), 83.8 (C-16), 61.9 (C-17), 80.5 (C-18), 53.8 (C-19), 49.1 (C-21), 13.4 (C-22), 57.5 (C-1′), 58.8 (C-6′), 57.5 (C-16′), 59.1 (C-18′), 166.2 (COO), 123.2 (C-1″), 131.9 (C-2″, 6″), 113.5 (C-3″, 5″), 163.2 (C-4″), 55.3 (4″-OCH_3), 151.8 s, 80.6 s, 27.4 q [$(\underline{C}H_3)_3\underline{C}$–O$\underline{C}$O]. HR-ESI-MS *m/z*: 702.3816 $[M+H]^+$ (Calcd for $C_{38}H_{56}NO_{11}$: 702.3848). **16**: ^{1}H-NMR (200 MHz) δ: 1.05 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 1.06, 1.46 (each 9H, s, $(\underline{CH_3})_3$C–OCO×2), 3.16, 3.23, 3.26, 3.42 (each 3H, s, $OCH_3\times4$), 3.83 (3H, s, 4″-OCH_3), 3.98 (1H, d, J=6.6 Hz, H-6β), 5.08 (1H, d, J=5.2 Hz, H-14β), 6.88, 8.02 (each 2H, AA′BB′ system, J=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) δ: 84.8 (C-1), 26.2 (C-2), 35.6 (C-3), 39.1 (C-4), 48.8 (C-5), 80.2 (C-6), 49.4 (C-7), 85.2 (C-8), 41.4 (C-9), 43.9 (C-10), 50.2 (C-11), 34.7 (C-12), 76.6 (C-13), 76.5 (C-14), 39.4 (C-15), 82.8 (C-16), 61.3 (C-17), 80.5 (C-18), 53.9 (C-19), 48.9 (C-21), 13.5 (C-22), 55.8 (C-1′), 58.1 (C-6′), 57.5 (C-16′), 59.1 (C-18′), 165.9 (COO), 123.2 (C-1″), 132.1 (C-2″, 6″), 113.4 (C-3″, 5″), 163.1 (C-4″), 55.3 (4″-OCH_3), 152.4 s, 151.7 s, 82.5 s, 81.7 s, 27.7 q, 27.3 q [$(\underline{C}H_3)_3\underline{C}$–O$\underline{C}$O×2]. HR-ESI-MS *m/z*: 802.4340 $[M+H]^+$ (Calcd for $C_{43}H_{64}NO_{13}$: 802.4372).

8,14-*O*-Diethylbikhaconine (17) and 14-*O*-Ethylbikhaconine (18) To a solution of bikhaconine (200 mg, 0.42 mmol) in THF (10 ml) was added NaH (200 mg). The resultant mixture was refluxed for 2 h before adding ethyl bromide (5 drops). The subsequent reaction mixture was kept refluxing for an additional 4 h, and cooled to room temperature. After a general work-up, the crude product was purified by column chromatography (silica gel, 8 g, petroleum ether–acetone=16 : 10, with 1% diethylamine) to give ethylated analogues **17** (66 mg, 30%) and **18** (52 mg, 25%). **17**: ^{1}H-NMR (400 MHz) δ: 1.07 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 1.13, 1.17 (each 3H, t, J=7.0 Hz, $OCH_2\underline{CH_3}\times2$), 3.25, 3.29, 3.30, 3.32 (each 3H, s, $OCH_3\times4$), 4.07 (1H, d, J=6.6 Hz, H-6β). ^{13}C-NMR (50 MHz) δ: 86.1 (C-1), 25.9 (C-2), 36.2 (C-3), 39.3 (C-4), 50.0 (C-5), 82.2 (C-6), 52.1 (C-7), 82.5 (C-8), 42.2 (C-9), 49.4 (C-10), 50.0 (C-11), 35.2 (C-12), 73.4 (C-13), 80.0 (C-14), 41.4 (C-15), 84.0 (C-16), 62.47 (C-17), 80.7 (C-18), 53.7 (C-19), 49.3 (C-21), 13.6 (C-22), 56.1 (C-1′), 57.2 (C-6′), 57.0 (C-18′), 65.7, 58.6, 16.1, 15.5 ($OCH_2\underline{CH_3}\times2$). HR-ESI-MS *m/z*: 524.3607 $[M+H]^+$ (Calcd for $C_{29}H_{50}NO_7$: 524.3582). **18**: ^{1}H-NMR (200 MHz) δ: 1.05 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 1.14 (3H, t, J=7.0 Hz, $OCH_2\underline{CH_3}$), 3.20, 3.27, 3.28, 3.36 (each 3H, s, $OCH_3\times4$), 4.04 (1H, d, J=6.8 Hz, H-6β). ^{13}C-NMR (50 MHz) δ: 85.4 (C-1), 26.2 (C-2), 37.3 (C-3), 39.1 (C-4), 49.9 (C-5), 82.9 (C-6), 52.3 (C-7), 76.3 (C-8), 41.6 (C-9), 49.4 (C-10), 50.0 (C-11), 34.9 (C-12), 73.9 (C-13), 82.4 (C-14), 42.4 (C-15), 85.3 (C-16), 62.1 (C-17), 80.7 (C-18), 53.7 (C-19), 49.1 (C-21), 13.5 (C-22), 56.1 (C-1′), 57.8 (C-6′), 57.3 (C-16′), 59.1 (C-18′), 66.2, 15.8 ($O\underline{CH_2}\underline{CH_3}$). HR-ESI-MS *m/z*: 496.3289 $[M+H]^+$ (Calcd for $C_{27}H_{46}NO_7$: 496.3269).

13-*t*-Butoxycarbonyl-pyrocrassicauline A (20) To a solution of the residue **A** (200 mg, 0.32 mmol) in 5 ml of pyridine were added $(Boc)_2O$ (300 mg, 1.83 mmol) and DMAP (200 mg, 0.32 mmol), and the resultant reaction solution was kept refluxing overnight. After a general work-up, the residue obtained was subjected to column chromatography (silica gel, 8 g, petroleum–acetone=11 : 1) to produce acetylated product **20** (76 mg, 35%): ^{1}H-NMR (200 MHz) δ: 1.05 (3H, t, J=6.9 Hz, $NCH_2\underline{CH_3}$), 1.46 (9H, s, $(\underline{CH_3})_3$C–OCO), 3.21, 3.21, 3.28, 3.49 (each 3H, s, $OCH_3\times4$), 3.82 (3H, s, 4″-OCH_3), 4.29 (1H, d, J=6.6 Hz, H-6β), 5.24 (1H, d, J=2.5 Hz, H-14β), 5.54 (1H, d, J=6.6 Hz, H-15), 6.87, 8.07 (each 2H, AA′BB′ system, J=8.8 Hz, Ar-H). ^{13}C-NMR (50 MHz) δ: 85.9 (C-1), 25.1 (C-2), 37.2 (C-3), 39.8 (C-4), 48.1 (C-5), 80.0 (C-6), 50.9 (C-7), 145.2 (C-8), 44.8 (C-9), 48.0 (C-10), 51.7 (C-11), 35.3 (C-12), 81.9 (C-13), 77.4 (C-14), 116.6 (C-15), 83.5 (C-16), 74.7 (C-17), 80.3 (C-18), 54.2 (C-19), 49.6 (C-21), 13.5 (C-22), 56.0 (C-1′), 58.1 (C-6′), 57.6 (C-16′), 59.1 (C-18′), 166.4 (COO), 123.2 (C-1″), 132.3 (C-2″, 6″), 113.3 (C-3″, 5″), 163.2 (C-4″), 55.3 (4″-OCH_3), 152.6 s, 85.4 s, 27.7 q [$(\underline{CH_3})_3\underline{C}$–O$\underline{C}$O]. HR-ESI-MS *m/z*: 684.3722 $[M+H]^+$ (Calcd for $C_{38}H_{54}NO_{10}$: 684.3742).

1-Demethoxyyunaconitine (24) To a solution of 1-demethoxy-3-dehydroyunnaconitine (**31**)[26] (2.812 g, 4.485 mmol) in methanol (90 ml) was added $NaBH_4$ (544 mg, 14.3 mmol), and the reaction was allowed to proceed with stirring at room temperature for 1 h. After removal of methanol, the residue was diluted with water (5 ml), and the subsequent suspension was extracted with chloroform (80 ml×3). The combined extracts were dried over anhydrous sodium sulfate, and the organic solvent was removed under reduced pressure. The residue obtained was purified over silica gel (75 g) column chromatography eluting with petroleum ether–acetone (5 : 1) to give compound **24** (1.961 g, 70%): ^{1}H-NMR (400 MHz) δ: 1.17 (1H, dd, J=14.0, 6.0 Hz, H-1β), 1.87—1.92 (1H, m, H-1α), 1.70—1.73 (1H, m, H-2β), 1.64—1.70 (1H, m, H-2α), 3.73 (1H, dd, J=12.0, 5.2 Hz, H-3β), 1.99 (1H, d, J=6.4 Hz, H-5), 4.00 (1H, d, J=6.8 Hz, H-6β), 2.97 (1H, s, H-7), 2.84 (1H, dd, J=7.2, 5.6 Hz, H-9), 2.11 (1H, dd, J=14.0, 12.8 Hz, H-10), 1.55 (1H, dd, J=14.4, 5.6 Hz, H-12), 1.87—1.92 (1H, m, H-12), 4.88 (1H, d, J=5.2 Hz, H-14β), 2.40—2.43 (1H, m, H-15), 3.02 (1H, dd, J=15.6, 8.8 Hz, H-15), 3.32 (1H, m, H-16), 2.54 (1H, s, H-17), 3.59, 3.79 (each 1H, ABq, J=9.2 Hz, H_2-18), 2.25, 2.95 (each 1H, ABq, J=11.6 Hz, H-19), 1.92—1.94, 2.45—2.50 (each 1H, m, H_2-21), 1.10 (3H, t, J=7.2 Hz, $NCH_2\underline{CH_3}$), 3.15 (3H, s, OCH_3-6), 3.54 (3H, s, OCH_3-16), 3.30 (3H, s, OCH_3-18), 1.34 (3H, s, OAc-8), 7.99, 6.92 (each 2H, AA′BB′ system, J=12.0 Hz, H-2″, 6″, H_2-3″, 5″), 3.86 (3H, s, OCH_3-4″). ^{13}C-NMR (100 MHz) δ: 28.9 (C-1), 28.9 (C-2), 73.8 (C-3), 43.0 (C-4), 48.6 (C-5), 83.1 (C-6), 48.3 (C-7), 85.9 (C-8), 44.0 (C-9), 40.4 (C-10), 45.8 (C-11), 36.9 (C-12), 74.5 (C-13), 78.5 (C-14), 40.0 (C-15), 83.7 (C-16), 63.8 (C-17), 76.4 (C-18), 50.8 (C-19), 48.6 (C-21), 13.2 (C-22), 57.4 (C-6′), 58.7 (C-16′), 58.9 (C-18′), 169.6, 21.4 ($OCO\underline{CH_3}$-8), 165.6 (COO), 122.3 (C-1″), 131.4 (C-2″, 6″), 113.6 (C-3″, 5″), 163.3 (C-4″), 55.2 (OCH_3-4″). HR-ESI-MS *m/z*: 630.3297 $[M+H]^+$ (Calcd for $C_{34}H_{48}NO_{10}$: 630.3273).

3-Epi-1-demethoxyyunaconitine (25) To a solution of 1-demethoxy-3-dehydroyunaconitine (**31**,[26] 2.812 g, 4.485 mmol) in methanol (90 ml) was

added gradually $NaBH_4$ (544 mg, 14.3 mmol), and the reaction mixture was kept stirring at room temperature for 1 h. Removal of solvent gave a residue, which was diluted with water (50 ml). The resulting suspension was extracted with chloroform (80 ml×3), the combined extracts were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue obtained was subjected to silica gel (75 g) column chromatography eluting with petroleum ether–acetone (5 : 1) to give the title compound **25** (525 mg, 18%): ^{1}H-NMR (400 MHz) δ: 1.64—1.68 (1H, m, H-1), 1.41—1.48 (1H, m, H-2α), 2.43—2.51 (1H, m, H-2β), 3.76 (1H, t, J=2.4 Hz, H-3α), 2.69 (1H, d, J=6.8 Hz, H-5), 4.08 (1H, d, J=12.0 Hz, H-6β), 2.95 (1H, s, H-7), 2.85 (1H, dd, J=6.8, 5.6 Hz, H-9), 2.11 (1H, dd, J=13.0, 12.4 Hz, H-10), 1.56 (1H, dd, J=14.0, 4.8 Hz, H-12), 1.99—2.05 (1H, m, H-12), 4.89 (1H, d, J=4.2 Hz, H-14β), 2.37—2.42 (1H, m, H-15), 3.02 (1H, dd, J=15.2, 8.8 Hz, H-15), 3.30 (1H, hidden, H-16), 2.53 (1H, s, H-17), 3.18, 4.03 (each 1H, ABq, J=8.4 Hz, H_2-18), 2.23 (1H, ABq, J=11.2 Hz, H-19), 2.47—2.53 (2H, m, H_2-21), 1.09 (3H, t, J=7.2 Hz, $\underline{N}CH_2\underline{C}H_3$), 3.17 (3H, s, OCH_3-6), 3.54 (3H, s, OCH_3-16), 3.32 (3H, s, OCH_3-18), 1.36 (3H, s, $OCO\underline{C}H_3$-8), 7.99, 6.92 (each 2H, AA′BB′ system, J=12.0 Hz, H-2″, 6″, H-3″, 5″), 3.86 (3H, s, OCH_3-4″). ^{13}C-NMR (100 MHz) δ: 25.8 (C-1), 27.7 (C-2), 78.1 (C-3), 42.3 (C-4), 42.6 (C-5), 83.3 (C-6), 48.2 (C-7), 85.7 (C-8), 44.2 (C-9), 40.5 (C-10), 45.9 (C-11), 36.8 (C-12), 74.7 (C-13), 78.6 (C-14), 40.1 (C-15), 83.9 (C-16), 64.3 (C-17), 81.2 (C-18), 50.8 (C-19), 48.7 (C-21), 13.2 (C-22), 57.5 (C-6′), 58.7 (C-16′), 58.9 (C-18′), 169.5, 21.5 (OAc-8), 165.7 (COO), 122.5 (C-1″), 131.5 (C-2″, 6″), 113.6 (C-3″, 5″), 163.3 (OCH_3-4″), 55.3 (OCH_3-4″). HR-ESI-MS *m/z*: 630.3271 $[M+H]^+$ (Calcd for $C_{34}H_{48}NO_{10}$: 630.3273).

1,16-Didemethoxy-$\Delta^{15,16}$-yunaconitine (26) To a solution of compound **29**[26] (70 mg, 0.10 mmol) in MeOH (3 ml) was added *p*-TsOH (31 mg) and acetic acid. The reaction was allowed to proceed with stirring at room temperature for 4 h. Basification of the reaction mixture with 10% NaOH to pH 12, the subsequent mixture was extracted with chloroform (10 ml×3), the combined extracts was dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The residue obtained was purified by column chromatography over silica gel (2.0 g) eluting with petroleum ether–acetone (8 : 2) to afford compound **26** (37 mg, 64%). ^{1}H-NMR (200 MHz) δ: 1.04 (3H, t, J=7.0 Hz, $NCH_2\underline{C}H_3$), 1.39 (3H, s, $OCO\underline{C}H_3$-8), 3.13, 3.29, 3.83 (each 3H, s, OCH_3×3), 4.14 (1H, d, J=6.6 Hz, H-6β), 4.91 (1H, d, J=3.4 Hz, H-14β), 5.98 (1H, dd, J=10.0, 1.4 Hz, H-15), 6.54 (1H, dd, J=10.0, 1.4 Hz, H-16), 6.87, 7.91 (each 2H, AA′BB′ system, d, J=8.8 Hz, H-3″, 5″, and H-2″, 6″). ^{13}C-NMR (50 MHz) δ: 28.8 (C-1), 29.3 (C-2), 74.7 (C-3), 43.2 (C-4), 48.9 (C-5), 82.5 (C-6), 44.7 (C-7), 83.7 (C-8), 43.5 (C-9), 41.8 (C-10), 45.6 (C-11), 39.1 (C-12), 75.8 (C-13), 77.8 (C-14), 126.3 (C-15), 136.0 (C-16), 65.2 (C-17), 77.3 (C-18), 47.2 (C-19), 48.9 (C-21), 13.3 (C-22), 57.1 (C-6′), 59.1 (C-18′), 169.5, 21.7 ($O\underline{C}O\underline{C}H_3$), 166.4 (COO), 122.3 (C-1″), 131.6 (C-2″, 6″), 113.6 (C-3″, 5″), 163.4 (OCH_3-4″), 55.3 (OCH_3-4″). HR-ESI-MS *m/z*: 598 $[M+H]^+$ (100). HR-ESI-MS *m/z*: 598.3001 $[M+H]^+$ (Calcd for $C_{33}H_{44}NO_9$: 598.3011).

1-Demethoxy-3,13-diacetoxy-14-methanesulfonyl-pyrobikhaconine (27) Compound **30**[26] (2.6 g, 3.95 mmol) was placed in 500 ml of round flask and heated under vacuum (0.5 mmHg) to give a residue, which was chromatographed over silica gel H (55 g) using cyclohexane–acetone (10 : 1) as eluent to yield the title compound **27** (1.4 g, 60%). ^{1}H-NMR (400 MHz) δ: 1.11 (3H, t, J=7.2 Hz, $NCH_2\underline{C}H_3$), 2.05, 2.10 (each 3H, s, $OCO\underline{C}H_3$×2), 3.15 (3H, s, $OSO_2\underline{C}H_3$), 3.24, 3.32, 3.37 (each 3H, s, OMe×3), 4.88 (1H, dd, J=12.4, 5.2 Hz, H-3α), 4.94 (1H, d, J=3.6 Hz, H-14*)*, 5.54 (1H, d, J=6.4 Hz, H-15). HR-ESI-MS *m/z*: 598.2664 $[M+H]^+$ (Calcd for $C_{29}H_{44}NO_{10}$: 598.2680). Its structure was also confirmed by comparison with the authenticated sample in TLC plate using different solvent systems (petroleum ether–acetone=2 : 1, $CHCl_3$–MeOH=98 : 2, $CHCl_3$–acetone=9 : 1).

Analgesic Activity Assay in a Mice Model Acetic Acid-Induced Writhing Test: Material and Methods. Preparation of Test Samples for Bioassay All test samples were given subcutaneously to test animals in 0.1 ml/10 g body weight after dissolving in DMSO or 0.1 M HCl followed by diluting to certain concentration with distilled water. The animals of control group received the same experimental handling as those of the testing groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle.

Animals Female Kunming strain mice (20±2 g) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences. All animals were kept under a controlled light/dark cycle and temperature (20±2 °C), with free access to food and water. The animals were left for 2 d for acclimatization to animal room conditions. A minimum of six animals were used in each group. Experiments were carried out under principles of Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China on November 14, 1988 and followed the guidelines of the Animal Care and Use Committee of Shanghai Institute of Materia Medica.

Acetic Acid Writhing Test The writhing test in mice was performed according to Koster's method (1959).[29] Mice were injected intraperitoneally with 0.1 ml/10 g body weight of 0.7% acetic acid in normal saline 20 min after s.c. administration of the test samples or the vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) occurring between 5 and 15 min after the acetic acid injection was recorded. The data represent average of the total number of writhes observed. The analgesic activity was expressed as percentage change from writhing controls.

Acute Toxicity Five female mice for each group subcutaneously injected with different doses of compounds were observed for 1 h and kept alive for 24 h; the morbidity or mortality, if happens, was recorded for each group at the end of observation period. Four of the most analgesics (**6**, **9**, **23**, **28**) were selected for acute toxic examination, based on *in vivo* analgesic action data. The mice showed toxic symptom such as eructation, shiver and salivary in 20 min after administrating the test samples at a dose of 10 times of their ED_{50} value. The mortality was 5/5, 2/5, 3/5, 5/5, respectively, at 1 h after injection of the compounds.

For calculating the ED_{50} values, a linear regression was made based on at least four dosages for each compound (with the high efficacy over 80%, and low efficacy less than 20%), without providing the 95% confidence limits.

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