Structure–Analgesic Activity Relationship Studies on the C18- and C19-Diterpenoid Alkaloids

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For evaluation of C18- and C19-diterpenoid alkaloids as analgesics, three C18-diterpenoid alkaloids were isolated from the roots of Aconitum hemsleyanum var. cinnatum and A. transsectum; and twenty-five semi-synthetic C18- or C19-diterpenoid alkaloids were prepared from lappaconitine, crassicauline A or yunaconitine. In 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 (ED50 = 0.0972 mg/kg) and 8-O-ethylunaconitine (ED50 = 0.0591 mg/kg) were the most potent analogs relative to the reference drugs lappaconitine (ED50 = 3.50 mg/kg) and crassicauline A (ED50 = 0.0480 mg/kg). Analgesic activity data of these C18- and C19-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 C19-diterpenoid alkaloids.

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

Aconitum and Delphinium plants have been medicinally used for centuries. 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. 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 and biological activities. The analgesic activities of C18- and C19-diterpenoid alkaloids have been extensively investigated since 1981, among which 3-acetylaconitine, lappaconitine, and crassicauline A (bulleyaconitine A) 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. 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 on the structure–analgesic activity relationship studies on the C18- and C19-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 semi-synthetic 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 C18- and C19-diterpenoid alkaloids.

Results and Discussion

Chemistry To further investigate the analgesic activities of C18- and C19-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-Deethyllapponaconitine (1), N-deethyllapponaconitine imine (4) and N-deethyl-5'-bromolapponaconitine imine (5) were prepared by treatment of lappaconitine with N-bromosuccinimide (NBS)-HOAc by the similar procedure previously developed by us. N-Deethyl-N,8,9,10-tetraacylcrassicauline (2) and N-deethyl-N-acetyllapponaconitine (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
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-bromo succinimide (NBS)-glacial acetic acid based on the methods developed by us.22,23) Pyrocrassicauline A (19) and 13-t-butoxy carbonyl-pyrocrassicauline A (20) were prepared by selective hydrolysis of crassicauline A with dioxane–H2O (2 : 1) according to Murayama’s method4) followed by refluxing with (Boc)2O in 41% and 35% yields, respectively. 1,16-Didemethoxy-4,15,16-yunaconitine (26), 1-demethoxy-3,13-diacyl-14-methane sulfonyl-pyrobikhaconine (27) were readily prepared according to the method reported in the literature.24) 1-Demethoxyyunaconitine (24, 70%) and 3-epi-1-demethoxyyunaconitine (25, 18%) were obtained by reduction of 1-demethoxy-3-dehydroyunaconitine (31, derived from yunaconitine26,27)) with sodium borohydride. Crassicauline A analogues, hemsleyanisine (21) and isohemsleyanisine (22), as well as yunaconitine analogue 8-O-ethyl yunaconitine (23), were isolated from the roots of Aconitum hemsleyanum var. circinatum and A. transectum according to the procedures described in the literature.25,26)

**Analgesic Activities**  Pharmacological studies were carried out to assess the in vivo analgesic activities of the above-mentioned C15- and C19-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 ED50 value evaluation. Four most potent analgesics (6, 9, 23, 28) 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 A imine (7), 8-O-deacetyl-8-O-ethylcrassicauline A (9), hemsleyanisine (21), 8-O-ethyllyunaconitine (23), 1-dimethoxylyunaconitine (24), and 1,16-didemethoxy-8-O-deacetyl-4,15,16-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 (ED50=0.0591—2.58 mg/kg) exhibited superior analgesic activity compared to the reference drug lappaconitine (ED50=3.50 mg/kg); compounds 9 and 23 (ED50=0.0591, 0.0972 mg/kg) exhibited comparable analgesic activity to the reference drug crassicauline A (ED50=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 (ED50=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

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Inhibition (%)</th>
<th>ED_{50} (mg/kg)</th>
<th>Toxic dose (mg/kg)</th>
<th>Mortality(a)</th>
</tr>
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<td>Crassicauline A</td>
<td>0.8</td>
<td>90.0</td>
<td>0.411</td>
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<tr>
<td>7</td>
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<tr>
<td>9</td>
<td>0.2</td>
<td>86.4</td>
<td>0.0972</td>
<td>1</td>
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</tr>
<tr>
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<td>6</td>
<td>77.8</td>
<td>3.42</td>
<td>—</td>
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<tr>
<td>23</td>
<td>0.1</td>
<td>84.0</td>
<td>0.0591</td>
<td>0.6</td>
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<tr>
<td>24</td>
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<tr>
<td>28</td>
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<tr>
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<td>10</td>
<td>22.2</td>
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<tr>
<td>Lappaconitine</td>
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<td>11.7</td>
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<tr>
<td>Crassicauline A</td>
<td>—</td>
<td>—</td>
<td>0.0480</td>
<td>0.92</td>
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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 acetyl 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 (isopentoxyl) 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 acetyl group in crassicauline A and a substitute at C-3 is important for the activity since 3α-ester 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 acetyl 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}^{19} and C_{19}^{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}^{19} and C_{19}^{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}^{19} and C_{19}^{19}-alka-loid-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; 1H- and 13C-NMR spectra were acquired on a Bruker AC-E 200 or a Varian INOV A-400/54 spectrometer in CDCl_3, with tetramethylsilane (TMS) as internal standard; Silica GF254 and gel H (10—40 mm, Qingdao Sea Chemical Factory, China) were used for TLC and CC. Only key signals for all products in the 1H-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-Deethyl-lappaconitine (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, 3g, CHCl_3–MeOH=94:6) of the crude residue gave title compound 1 (92 mg, 95%): 1H-NMR (200 MHz) δ: 2.22 (3 H, s, NHOCH_3), 3.30, 3.40 (each 3H, s, OCH_3), 7.02 (1H, m, H-5, H-6, H-7), 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, NHCOCH_3). 13C-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-18), 55.8 (C-19), 56.7 (C-216), 169.0, 25.2 (NHCOCH_3), 167.2 (COO), 115.1 (C-1'), 141.5 (C-2'), 120.1 (C-3'), 134.3 (C-4'), 122.3 (C-5'), 130.9 (C-6').

N-Deethyl-3-ethyl-lappaconitine (2) * Synthesized by acetylation of 1 with Ac_2O in glacial acetic acid via a general procedure. Details can be found in Table 1. In this subgroup, 8-O-ethylunaconitine (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 acetoxy group with ethoxyl group does not affect the analgesic activities. Similar to crassicauline A derivatives, Δ^{10(15)} derivative (27) is inactive. Similarly, Δ^{10(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). Stereochmistry at C-3 is important for the activity since 3α-acetoxy analogue 24 exhibits good activity (ED_{50}=7.98 mg/kg), while its isomer, 3β-acetoxy analogue 25, is inactive.

The 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).
Overnight and basified to pH 10 using 25% NH₄OH. The subsequent mixture was extracted with chloroform. The combined extracts were dried (Na₂SO₄), concentrated in vacuo, and chromatographed (silica gel, 25 g, petroleum ether–acetone, 9:1) of the crude residue afforded 3: 0.57 (3H, s, OCH₃). HR-ESI-MS m/z: 577.2532 [M⁺Na⁺] (Calculated for C₃₅H₅₂NO₉NaO₂Br: 577.2560).

N-Dehydroxy-l-bromolappaconitine Imine (6) A solution of lappaconitine (100 mg, 0.15 mmol) in 5 ml of glacial acetic acid was added NBS (20 mg, 0.15 mmol) in 5 ml of glacial acetic acid was added. The reaction mixture was allowed to stand at room temperature overnight and then the crude residue was column chromatography (silica gel, 10 g, petroleum ether–acetone: 11:2) to give C₈-isopentoxylated analog 10 (90 mg, 30%) and pyrocarnosine A (126 mg, 41%).

8-Deacetoxy-8-isopentoxycrassicauline A (10) and Pyrocrassicauline A (19) A solution of crassicauline A (100 mg, 0.15 mmol) in isopentyl alcohol (150 ml) was heated at 80 °C for 3 d. After a general work-up, the crude residue was column chromatography (silica gel, 10 g, petroleum ether–acetone: 11:2) to give C₈-isopentoxylated analog 10 (90 mg, 30%) and pyrocarnosine A (126 mg, 41%).

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55.3 (OCH3-4), 47.6 (OCH3-5), 20.7, 68.7 (H-2), 36.2 (C-3), 39.3 (C-4), 42.4 (C-5), 48.9 (C-10), 50.3 (C-11), 35.0 (C-12), 73.5 (C-13), 81.2 (C-14), 141.5 (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), 56.0 (C-1), 57.8 (C-6), 57.5 (C-16)*, 166.0, 165.6 (COO), 132.0, 130.7 [C(1′)-2′]*, 128.2, 128.8 (C-3′, 5′)*, 132.6, 133.0 [C(4′)-2]*. HS-ESI-MS m/z: 676.3457 [M+H]+ (Caled for C51H45NO13).

14-Acetyl-bikacidaline (14) and 14-butyrocarbonylbikacidaline (14) Bikacidaline analogue 14 (92 mg) was obtained in 86% yield of acetylcylohexylacetate (14) in 5 ml of pyridine. Similarly, bikacidaline analogue 14 (38 mg) was prepared in 32% yield by treatment of bikacidaline (100 mg, 0.21 mmol) with (Boc)2O (93 mg, 0.42 mmol) and DMAP (100 mg) in 10 ml of pyridine.

NMR (200 MHz) 4.15 (C-15), 83.6 (C-16), 62.1 (C-17), 80.6 (C-18), 53.7 (C-19), 49.3 (C-21), 13.5 (C-22), 56.1 (C-1), 57.6 (C-6), 57.3 (C-16)*, 166.0, 165.6 (COO), 132.0, 130.7 [C(1′)-2′]*, 128.2, 128.5 (C-3′, 5′)*, 132.6, 133.0 [C(4′)-2]*. HS-ESI-MS m/z: 676.3457 [M+H]+ (Caled for C51H45NO13).

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added gradually NaBH₄ (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 ex-
tracted with chloroform (80 ml×3), the combined extracts were dried over
anhydrous sodium sulfate, and the solvents was removed under reduced pres-
sure. The residue obtained was subjected to silica gel (75 g) column
chromatography eluting with petroleum ether–acetone (3:1) to give the title
compound 25 (525 mg, 18%). H-NMR (400 MHz) δ: 1.64–1.68 (1H, m,
H-1), 1.41–1.48 (1H, m, H-2a), 2.43–2.51 (1H, m, H-2b), 3.76 (1H, t,
J=2.4 Hz, H-3m), 2.69 (1H, d, J=6.8 Hz, H-5), 4.08 (1H, d, J=12.0 Hz, H-
6b), 2.95 (1H, s, H-7), 2.85 (1H, d, J=6.8, 5.6 Hz, H-9), 2.11 (1H, d,
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-14b), 2.37–2.42 (2H, 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=12, 6.4 Hz, H-15). HR-ESI-MS
m/z: [M+H]+ 598.2664 (Calcd for C₃₄H₄₈NO₁₀: 598.3019).

1.6-Dimethyl-3,13-diaceoxy-14-methanesulfonyl-pyrolhakaconine (26)
To a solution of compound 29δ (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, and the subsequent mixture was extracted with chloroform (10 ml
30×3), the combined extracts were dried over anhydrous Na₂SO₄, and the solvent
was removed in vacuo. The residue obtained was puriﬁed by column chromatog-
raphy over silica gel (2.0 g) eluting with petroleum ether–acetone (8:2) to afford
compound 26 (37 mg, 64%). H-NMR (200 MHz) δ: 1.04 (3H, t,
J=7.0 Hz, NCH₂CH₃), 1.39 (3H, s, OCOME₃), 3.13, 3.29, 3.83 (each 3H,
s, OCH₃)δ: 1.44 (1H, d, J=6.6 Hz, H-6b), 4.91 (1H, d, J=3.4 Hz, H-14b),
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=11.6, 9.3 Hz, H-3′, 5′, and H-2′, 6′).13C-NMR (50 MHz) δ: 28.8 (C-1), 29.3 (C-2), 74.7 (C-3), 43.2 (C-4), 48.9
(C-5), 82.6 (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), 126.3 (C-13), 136.0 (C-16), 65.2 (C-17), 77.3 (C-18), 47.2 (C-19), 48.9 (C-21), 133.3 (C-22), 57.1 (C-26), 59.1
(C-18′), 169.5, 21.7 (OCOCH₃), 166.4 (COO), 122.3 (C-1′), 131.5 (C-2′, 6′),
113.6 (C-3′, 5′), 163.3 (OCOCH₃), 55.3 (OCOCH₄). HR-ESI-MS m/z:
630.3271 [M+H]+ (Calcd for C₃₃H₄₄NO₉: 630.3273).

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