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Research Article

Alkaloids Isolated in the Roots of *Aconitum carmichaeli* Debx Growing in Vietnam

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ABSTRACT
The roots of *Aconitum Carmichaeli* Debx. is recognized as a medicinal plant by its anti-inflammatory and analgesic effects. Our phytochemical investigation of the roots led to the isolation of three alkaloids including delcosine (**1**), fuziline (**2**), and karacoline (**3**). Their structures were identified on the basis of NMR, MS, IR spectroscopic analysis and comparison with the published NMR data. Compound **1** was isolated for the first time from this plant.

Keywords: *Aconitum carmichaeli*, alkaloid, delcosine, fuziline, karacoline

INTRODUCTION

The genus *Aconitum*, belonging to the family Rannunculaceae, is widely distributed in the alpine and subalpine regions. The plants are usually perennial or biennial herbs, often with stout leafy stems, bulbs or creeping rhizomes. Leaves are mostly cauline, lobed, rarely divided and dentate. Flowers are simple or branched recemes. It comprises of over 300 species, including some ornamental and medicinal plants[1]. The tuberous roots of genus *Aconitum* are commonly applied for various diseases, such as rheumatic fever, painful joints and some endocrinal disorders. The main chemical composition of the genus *Aconitum* is alkaloids[1-3]. It has been reported that the roots of *Aconitum Carmichaeli* Debx. mainly presented including alkaloids (benzoylmesaconine, karacoline, neoline), acid amine, free sugars and organic acids[4]. In this study, we have isolated and identified structures of three alkaloids from *Aconitum Carmichaeli* Debx. growing in Ha Giang province, Vietnam.

MATERIALS AND METHODS

Plant material

The roots of *Aconitum Carmichaeli* Debx. were collected in Ha Giang province, Vietnam during 2012 and authenticated by Prof. Dr. Nguyen Van Tap and Dr. Pham Thanh Huyen from the National Institute of Medicinal Materials (NIMM). A voucher specimen has been deposited in the NIMM.

General experimental procedures

Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB Waegetechnik Rapido, Germany) and were uncorrected. The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [^{1}H (500 MHz), ^{13}C (125 MHz), and DEPT-90 and 135 MHz)] spectra were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). Chemical shifts were reported in ppm downfield from TMS with *J* in Hz. Electrospray Ionization Mass Spectra (ESI-MS) were recorded on a Varian Agilent 1100 LC-MSD mass spectrometer. Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H_2SO_4, followed by heating with a heat gun. Column chromatography was performed on silica gel (70–230 and 230–400 mesh, Merck). Organic solvents were of analytical grade.

Extraction and isolation

Powdered roots of *Aconitum carmichaeli* Debx. (2.8 kg) were extracted with 96% ethanol (6L × 3 times) at room temperature. The ethanol extracts were combined and then evaporated to dryness *in vacuo* at 40°C. This extract (300.8 g) was fractioned with n-butanol (750 mL × 3 times). The obtained n-butanol extract (130.6 g) was separated into 6 fractions (Frs. A- F) by chromatography on a silica gel column, with a gradient solvent system *n*-hexane-EtOAc (EtOAc: 0%→100%). Fractions E and D were further chromatographed over silica gel column with CH_2Cl_2-MeOH (90:10, v/v) as eluent to give compounds **1 (**16 mg) and **2** (14 mg), respectively. Finally, fraction F was subjected to a silica gel column with $CHCl_3$-MeOH (90:10, v/v) as eluent to yield compound **3** (12 mg).

Compound 1 (Delcosine): mp 203-204°; TLC [Silica gel, CH_2Cl_2-MeOH (95:5, v/v)]: R_f = 0.45; ***ESI-MS***: *m/z* 454.5 $[M+H]^+$. ***^{1}H-NMR*** *(500 MHz, CDCl$_3$)*: δ (ppm) 1.15 (3H, t, *J* = 7.0 Hz, N-CH_2CH_3), 1.48 (1H, m, H-10), 1.56 (2H,

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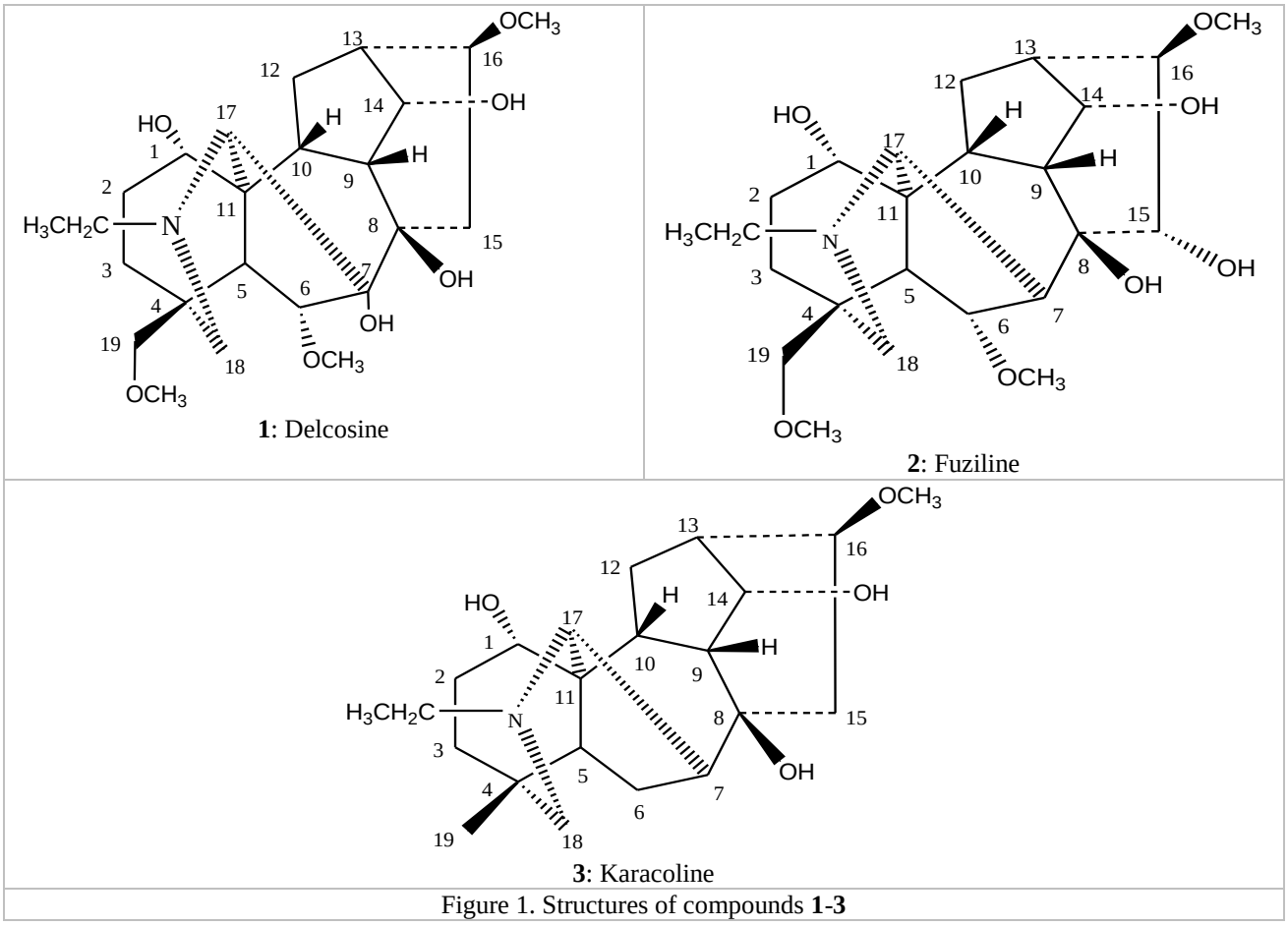

1: Delcosine

2: Fuziline

3: Karacoline

Figure 1. Structures of compounds **1-3**

t, *J* = 7.0 Hz, N-CH_2CH_3), 1.66-1.67 (1H, m, H-3a), 1.71-1.75 (2H, m, H-3b, H-12a), 1.84-1.91 (1H, m, H-2a), 2.02 (1H, t, *J* = 6.0 Hz, H-7), 2.09 (1H, d, *J* = 6.0 Hz, H-17), 2.18 (1H, m, H-12b), 2.19 (1H, d, *J* = 10.0 Hz, H-18a), 2.29 (1H, t, H-6), 2.35-2.40 (1H, m, H-14), 2.37 (1H, d, *J* = 10.0 Hz, H-18b), 2.51-2.55 (1H, m, H-2b), 2.60 -2.63 (1H, m, H-15a), 2.75 (1H, d, H-14), 3.28 (2H, t, *J* = 6.0 Hz, H-1, H-9), 3.32 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.66 (1H, d, *J* = 5.5 Hz, H-19a), 4.08 (1H, t, *J* = 4.5 Hz, H-15b), 4.18 (1H, d, *J* = 6.5 Hz, H-19b), 4.22 (1H, d, *J* = 7.0 Hz, H-8). ***^{13}C-NMR*** *(125 MHz, $CDCl_3$): δ* (ppm) 12.8 (N-CH_2CH_3), 29.2 (C-2), 29.3 (C-12), 29.7 (C-9), 37.5 (C-15), 38.1 (C-10), 40.2 (C-4), 42.6 (C-5), 44.1 (C-7), 44.8 (C-11), 48.2 (C-18), 48.4 (N-CH_2CH_3), 49.5(C-1), 52.2 (OCH_3), 56.3 (OCH_3), 56.9 (OCH_3), 57.9 (C-17), 59.1 (C-6), 63.9 (C-19), 74.1 (C-3), 75.9 (C-8), 80.1 (C-14), 81.7 (C-13), 83.0 (C-16).

Compound 2 (Fuziline): mp 214-216°; TLC: [Silica gel, CH_2Cl_2-MeOH (95:5, v/v)]: R_f = 0.52. ***ESI-MS***: *m/z* 454.5 $[M+H]^+$. ***^{1}H-NMR*** *(500 MHz,$CDCl_3$): δ* (ppm) 1.11 (3H, t, *J* = 7.0 Hz, N-CH_2CH_3), 1.51 (1H, m, H-10), 1.56 (2H, t, *J* = 7.0 Hz, N-CH_2CH_3), 1.76-1.79 (1H, m, H-3a), 1.85-1,89 (2H, m, H-3b, H-12a), 2.00-2.06 (1H, m, H-2a), 2.12 (1H, t, *J* = 6.0 Hz, H-7), 2.17 (1H, d, *J* = 6.0 Hz, H-17), 2.22-2.31 (1H, m, H-12b), 2.27 (1H, d, *J* = 10.0 Hz, H-18a), 2.33 (1H, t, H-6), 2.4-2.44 (1H, m, H-14), 2.68 (1H, d, *J* = 10.0 Hz, H-18b), 2.69-2.75 (1H, m, H-2b), 3.14-3.17 (2H, t, *J* = 6.0 Hz, H-1, H-9), 3.33 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.64 (1H, d, H-14), 3.66 (1H, d, *J* = 5.5 Hz, H-19a), 4.08 (1H, t, *J* = 4.5 Hz, H-15), 4.12 (1H, d, *J* = 6.5 Hz, H-19b), 4.25 (s, br, OH), 4.40 (1H, d, *J* = 7.0 Hz, H-2). ***^{13}C-NMR*** *(125 MHz,$CDCl_3$): δ* (ppm) 13.0 (N-CH_2**C**H_3), 29.4 ppm (C-2), 29.9 (C-12), 30.6 (C-9), 38.0 (C-10), 40.5 (C-4), 43.5 (C-5), 44.0 (C-7), 46.6 (C-11), 48.3 (C-18), 48.4 (N-CH_2CH_3), 49.2 (C-1), 56.6 (OCH_3), 57.3 (OCH_3), 57.9 (OCH_3), 59.0 (C-17), 62.5 (C-6), 72.0 (C-19), 75.5 (C-13), 78.5 (C-3), 79.0 (C-8), 80.0 (C-14), 84.3 (C-15), 90.5 (C-16).

Compound 3 (Karacoline): mp 183-184°; TLC [Silica gel, CH_2Cl_2-MeOH (95:5, v/v)]: R_f = 0.35. ***IR*** (KBr) ν_{max} (cm^{-1}): 3376 (OH), 2924, 2852 (CH), 1094 (C –O – C). ***ESI-MS***: *m/z* 378 $[M + H]^+$. ***^{1}H-NMR*** *(500 MHz, $CDCl_3$): δ* (ppm) 3.76 (1H, t, *J* =3.5, H-1), 4.22 (1H, t, *J* = 5.0, H-14), 3.39 (1H, m, H-16), 2.88 (1H, s, H-17), 0.9 (3H, s, H-18), 3.34 (3H, s, OMe-16), 1.14 (3H, t, *J* = 7,2, NCH_2CH_3). ***^{13}C-NMR*** *(125 MHz, $CDCl_3$): δ* (ppm) 25.11 (C-6), 27.51 (C-18), 29.69 (C-2), 29.69 (C-12), 31.45 (C-3), 33.11 (C-4), 39.84 (C-10), 41.96 (C-15), 44.09 (C-13), 45.22 (C-7), 46.56 (C-9), 46.61 (C-5), 48.59 (N- CH_2 CH_3), 48.59 (N-CH_2CH_3), 48.83 (C-11), 56.35 (OCH_3), 60.07 (C-19), 63.38 (C-17), 72.51 (C-1), 74.22 (C-8), 75.72 (C-14), 81.96 (C-16).

RESULTS AND DISCUSSION

A phytochemical investigation of the D, E and F fractions of the n-butanol extract led to the isolation of three compounds **1-3** (Figure 1). Their structures were identified by comparing physicochemical and spectroscopic data with the published values for delcosine (**1**)[5], fuziline (**2**)[2] and karacoline (**3**)[3]. Compound **1** was isolated as white

crystal and its positive ESI-MS showed a molecular ion peak $[M+H]^+$ at *m/z* 454.5, consistent with the molecular formula $C_{24}H_{39}NO_7$. The ^{1}H-NMR spectrum showed the presence of a methyl triplet at δ_H 1.15 (t, ^3J = 7.0 Hz, 3H, N-$CH_2C\mathbf{H_3}$), three methoxy signals at δ_H 3.32 (s, 3H, 6-OCH_3), 3.34 (s, 3H, 19-OCH_3), and 3.42 (s, 3H, 16-OCH_3), together with four oxygenated methine protons at δ_H 2.29 (t, H-6), 2.75 (d, H-14), 3.28 (t, *J* = 6.0 Hz, H-1), and 3.41 (d, *J* = 6.0 Hz, H-16). The ^{13}C-NMR spectrum indicated the presence of 24 carbon atoms, in which the upfield carbon signal at δ_C 12.8 suggested to the methyl carbon at N-CH_2CH_3 group. In addition, three methoxy carbons at δ_C 56.4 (16-OCH_3), 57.4 (6-OCH_3), and 59.1 (19-OCH_3) along with seven oxygenated carbons at δ_C 74.1, 75.9, 76.7, 77.2, 80.1, 81.7, and 83.0 were observed. Based on the above evidence and the literature data[5], compound **1** was identified as delcosine. The ^{1}H-NMR spectrum of **2** in $CDCl_3$ exhibited the presence of a N-CH_2CH_3 group like that of compound **1** due to the methyl signal at δ 1.11 (3H, t, *J* = 7.0 Hz), three methoxy groups (each 3H, s) at δ 3.33, 3.35, 3.45. Additionally, the spectrum indicated a downfield doublet at δ 3.64 a triplet at δ 4.08, attributable to typical oxygenated methine protons H-14 and H-15, respectively. The ^{13}C-NMR and DEPT spectra of **2** in $CDCl_3$ showed twenty-four signals for twenty-four carbon atoms in the molecule. The presence of a methine carbon at 84.3 ppm and the absence of the methylene signal at δ 37.5 ppm in compound **1** indicated that a secondary hydroxy group is present at C-15 in **2**. The chemical shifts of the remaining carbons are in agreement with the assigned structure **2** for fuziline[2]. The molecular formula was established as $C_{22}H_{35}NO_4$ based on a molecular ion peak at *m/z* 378 $[M + H]^+$. The IR spectrum showed typical absorption bands arising from hydroxyl (3376 cm^{-1}), -CH group (2924, 2852 cm^{-1}), and C-O-C groups (1094 cm^{-1}), respectively. In addition, the IR spectrum of which showed absorption bands of carbonyl groups in five-membered (1740 cm^{-1}) and six-membered (1665 cm^{-1}) rings, which limits the location of one of the secondary hydroxy groups to positions 6, 10, and 12. The ^{1}H-NMR spectrum of **3** showed the presence of a triplet at δ 4.16 ppm with a coupling constant of 4.5 Hz excludes positions 6 and 12 and suggests the hydroxy group to be placed in the five-membered ring at C_{10}. In addition, the ^{1}H- NMR spectrum had ten protons of multiplets at δ 7.97-7.41 ppm region. Furthermore, the proton signal of the acetyl group appears relatively in up-field at δ 1.30 ppm, which shows the presence of a hydroxy group at C_{10} and makes it possible to ascribe the tertiary hydroxy group to C_8. By comparing these data with literature[3], compound **3** was identified as karacoline

CONCLUSION

From 96% ethanol extract of the roots of *Aconitum Carmichaeli* Debx. we have isolated three alkaloids including delcosine (**1**), fuziline (**2**), and karacoline (**3**). Those structures were elucidated by extensive spectroscopic evidence and comparison with the literature data. Interestingly, compound **1** was first time isolated from this plant. Our results could be beneficial for the search of new chemical agents from Vietnamese plants.

COMPETING INTERESTS

We declare that we have no conflict of interest.

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