Phytochemistry 150 (2018) 85–92

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Alkaloids from *Corydalis decumbens* suppress neuronal excitability in primary cultures of mouse neocortical neurons

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ARTICLE INFO

Article history:
Received 4 December 2017
Received in revised form
5 February 2018
Accepted 11 March 2018

Keywords:
Corydalis decumbens
Chemical correlation
Alkaloids
Neuron excitability

ABSTRACT

Eight previously undescribed alkaloids, named corydemine, dihydrocorydemine, corydedine, 8,13-dioxo-14-hydroxytetrahydropalmatine, egenine-α-*N*-oxide, egenine-β-*N*-oxide, 7′-*O*-ethylegenine-α-*N*-oxide, and 7′-*O*-ethylegenine-β-*N*-oxide, together with three known ones, muramine, L-tetrahydropalmatine, and (+)-egenine, were isolated from the bulbs of *Corydalis decumbens*. Their structures were elucidated by comprehensive spectroscopic analysis and chemical correlation. The isolated compounds were tested for their ability to modulate neuronal excitability in primary cultured neocortical neurons. Four of the compounds, corydemine, dihydrocorydemine, muramine, and L-tetrahydropalmatine, inhibited neuronal excitability with IC_{50} values of 3.6, 16.7, 13.5 and 14.0 μM, respectively.

1. Introduction

Corydalis decumbens (Thunb.) Pers., a member of the family Papaveraceae (recorded as "Xiatianwu" in the Chinese Pharmacopoeia), is a commonly used traditional Chinese medicine. The bulbs of *C. decumbens* have been used clinically to treat post-stroke hemiplegia, headache, rheumatic arthritis, and traumatic injuries (China Pharmacopoeia Committee, 2015). Chemical and pharmacological studies have demonstrated that alkaloids are the main active chemical constituents of *C. decumbens* (Wang et al., 2006). Several classes of alkaloids such as the protopines (protopine), protoberberines (berberine, tetrahydropalmatine), phthalideisoquinolines (bicuculline, decumbenine C), aporphines (bulbocapnine, isocorydine), benzylisoquinolines (*epi*-coryximine, decumbenine B) have been isolated from *C. decumbens* (Liao et al., 1995; Joshi et al., 1990; Zhang et al., 1995; Liao et al., 2014). These alkaloids have displayed a range of biological activities, especially several that affect the central nervous system. For example, bicuculline, a specific $GABA_A$ receptor antagonist, modulates neuroplasticity (learning and memory) through an activity-dependent signaling pathway (He and Bausch, 2014) as well as dose-dependently induced tonic-chronic seizure in mice and rats (Meldrum and Nilsson, 1976; Rubio et al., 2010). Tetrahydropalmatine, a marketed medicine worldwide, has been reported to possess analgesic, sedative, and hypnotic effects through modulating dopamine receptor activity (Zhu, 1991; Chu et al., 2008). Recent studies have also demonstrated that tetrahydropalmatine interacts with Ca^{2+} (Huang et al., 1999) and acid-sensing (Liu et al., 2015) ion channels, possibly contributing to its effect on the central nervous system. Protopine was reported to display neuroprotective effects in the rat focal cerebral ischemic injury model (Xiao et al., 2007) as well as possess anti-depression (Xu et al., 2006), anti-inflammatory (Saeed et al., 1997) and antithrombotic effects (Ko et al., 1989; Shiomoto et al., 1990).

Neuronal excitability can influence neuronal circuit activity and resultant behaviors. Disruption of neuronal excitability can lead to

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https://doi.org/10.1016/j.phytochem.2018.03.006
0031-9422/

seizure, sedation (Navidhamidi et al., 2017), allodynia/hyperalgesia (Woolf, 2011) and mental diseases (Levitt et al., 2004). As a model system for studying neuronal excitability, murine primary cultured neurons form synaptic networks and display spontaneous synchronized Ca^{2+} oscillations (SCOs) (Pacico and Mingorance-Le Meur, 2014). These SCOs occur simultaneously with action potential generation and are controlled by the balance of excitatory and inhibitory neuronal inputs; therefore, they are intimately involved in neuronal excitability phenomena (Dravid and Murray, 2004; George et al., 2009; Cao et al., 2015). In addition, aberrant Ca^{2+} signals can regulate neuronal death, development (Cao et al., 2014a), and plasticity (He and Bausch, 2014).

In an effort to discover new neuroactive compounds, we systematically investigated the chemical constituents of the ethanolic extract of bulbs of *C. decumbens* and evaluated their ability to modulate neuronal excitability. Eight previously undescribed alkaloids (**1**–**8**) together with three known ones (**9**–**11**), were isolated from these bulbs, fully characterized by detailed spectroscopic analysis as well as chemical correlations, and four were found to inhibit neuronal excitability in primary cultured neocortical neurons.

2. Results and discussion

The dried bulbs of *C. decumbens* were extracted with 95% EtOH and the extract was partitioned between 3% tartaric acid-H_2O and ethyl acetate. The aqueous acidic solution was basified and then extracted with $CHCl_3$ to yield an alkaloid-enriched fraction. This fraction was subjected to silica gel column chromatography eluting with a gradient of MeOH in $CHCl_3$ to provide six fractions (A–F). Further purification of alkaloids from fractions A–F was achieved using a combination of silica gel, RP-18, Sephadex LH-20 column chromatography (CC) and semi-preparative HPLC, and yielded eight previously undescribed alkaloids as well as three known compounds. Elucidation of their structures was performed by analysis of NMR spectra (^1H, ^{13}C, COSY, NOESY, HSQC, and HMBC) and mass spectrometry (HRESIMS). The eight previously undescribed alkaloids (Fig. 1) were named corydemine (**1**), dihydrocorydemine (**2**), corydedine (**3**), 8,13-dioxo-14-hydroxytetrahydropalmatine (**4**), egenine-*α*-*N*-oxide (**5**), egenine-*β*-*N*-oxide (**6**), 7′-*O*-ethylegenine-*α*-*N*-oxide (**7**), and 7′-*O*-ethylegenine-*β*-*N*-oxide (**8**). Compounds **1** and **2** possess a unique nine-membered dibenzo [*c*,*f*]azepine ring which is in contrast to the ten-membered ring system common in protopine alkaloids (Guinaudeau and Shamma, 1982). Compound **3** is the first naturally occurring pseudobenzylisoquinoline alkaloid with a benzyl substituent at C-4. Compound **4** is a highly oxygenated protoberberine alkaloid. Compounds **5**–**8** represent the first examples of phthalideisoquinoline hemiacetal *N*-oxide alkaloids.

Compound **1** was obtained as a yellowish powder. Its molecular formula was determined as $C_{20}H_{19}NO_6$ based on the HRESIMS ion at *m/z* 370.1294 $[M + H]^+$, indicating 12 degrees of unsaturation. Analysis of the ^{13}C NMR spectrum revealed all 20 carbon resonances which could be assigned to a ketone carbonyl (δ_C 199.0), an amide carbonyl (δ_C 158.6), 14 aromatic carbons, and 4 aliphatic carbons. The absorptions at 3396, 1713, 1644 cm^{-1} in the IR spectrum indicated the presence of NH or OH functionalities as well as confirmed the presence of two carbonyl groups. The ^1H NMR spectrum (Table 1) possessed resonances for two singlet aromatic protons at δ_H 7.40 (1H, s) and 7.14 (1H, s), a pair of *ortho*-coupled aromatic protons at δ_H 7.26 (1H, d, $J = 9.0$ Hz) and 6.42 (1H, d, $J = 9.0$ Hz), and two additional aromatic proton resonances at 7.65 (1H, d, $J = 5.5$ Hz) and δ_H 8.45 (1H, d, $J = 5.5$ Hz). The latter deshielded aromatic proton was attached to a carbon resonating at δ_C 140.3 in the HSQC spectrum, and suggested that the double bond was substituted with an *N*-atom. In addition, characteristic resonances at δ_H 4.05 (3H, s), 3.96 (3H, s), 3.95 (3H, s), and 3.92 (3H, s) were assigned to four methoxy groups. These NMR resonances suggested that compound **1** was structurally related to muramine (**9**) (Guinaudeau and Shamma, 1982). HMBC correlations (Fig. 2) between the methoxy methyl groups and their subtending carbons [2-OCH_3 (δ_H 3.96 to δ_C 151.3), 3-OCH_3 (δ_H 4.05 to δ_C 153.5), 9-OCH_3 (δ_H 3.95 to δ_C 136.7), 10-OCH_3 (δ_H 3.92 to δ_C 159.4) defined their attachment to aromatic rings A and C. HMBC correlation between H-6 (δ_H 8.45) and the amide carbonyl carbon (δ_C 158.6) placed the amide moiety at C-8, whereas an HMBC correlation between H-12 (δ_H 7.26) and the ketone carbonyl carbon (δ_C 199.0) confirmed that one carbon atom was lost in **1** compared to muramine (Guinaudeau and Shamma, 1982). HMBC correlations were also observed from H-4 (δ_H 7.14) to C-5 (δ_C 121.4) and from H-5 (δ_H 7.65) to C-4 (δ_C 105.1), C-4*α* (δ_C 134.2), and C-13*α* (δ_C 122.6), supporting the structural assignment of **1** (Fig. 2). The structure of **1** was elucidated as shown and named corydemine.

Compound **2** was isolated as a yellowish powder with a molecular formula of $C_{20}H_{21}NO_6$, as determined by HRESIMS of the $[M + H]^+$ ion at *m/z* 372.1446. The resonances observed in ^{13}C NMR spectrum of **2** were similar to those of **1**. The major difference came from the chemical shifts of C-5 and C-6. The two carbon resonances at δ_C 140.3 and 121.4 in compound **1** were replaced by two methylene carbon resonances at δ_C 47.0 and 25.6 in **2**. Corresponding differences were also observed in the ^1H NMR spectrum as the two aromatic proton resonances at δ_H 8.45 and 7.65 in **1** were replaced by the methylene resonances at δ_H 3.90 and 2.82 in **2**. This deduction was supported by the observation of HMBC correlations from H-4 (δ_H 6.74) to C-5 (δ_C 25.6), and from H-5 (δ_H 2.82) to C-4 (δ_C 110.9), C-4*α* (δ_C 131.3), and C-13*α* (δ_C 119.5). Thus, the structure of **2** was characterized as shown and named dihydrocorydemine.

Compound **3** was separated as a yellowish powder with the molecular formula $C_{20}H_{15}NO_6$ established by HRESIMS (*m/z* 366.0978 $[M + H]^+$). Analysis of the ^1H NMR spectrum (Table 2) indicated resonances for four singlet aromatic protons at δ_H 9.15, 7.96, δ_H 7.83, and 7.53, two coupled aromatic protons at δ_H 7.25 (1H, d, $J = 8.0$ Hz) and 6.75 (1H, d, $J = 8.0$ Hz), two methylenedioxy groups at δ_H 6.33 (2H, s) and 5.98 (2H, s), a methylene group at δ_H 4.69 (2H, s), and a methyl singlet at δ_H 4.32 (3H, s) which was assigned to a deshielded *N*-methyl group. The significant downfield chemical shift of the two aromatic protons at δ_H 9.15 and 7.96 indicated the presence of an electron-withdrawing group. The ^{13}C NMR spectrum showed all 20 carbon resonances including those for a carboxylic acid group (δ_C 176.1) and relatively shielded methylene group (δ_C 28.7). The key HMBC correlations (Fig. 3) from the *N*-methyl protons (δ_H 4.32) to C-1, from H-8 (δ_H 7.53) to C-1, and from H-5 (δ_H 7.83) to C-4 confirmed the existence of an isoquinoline unit. Together these data indicated that compound **3** possessed a benzylisoquinoline skeleton (Gözler et al., 1983). The substitution pattern of the benzyl moiety was revealed by HMBC correlations (Fig. 3) between H-5′ (δ_H 7.25) and C-7′ (δ_C 176.1) and C-3′ (δ_C 149.0). This placed the carboxylic acid group at C-6′ and a methylenedioxy group bridging between C-2′ and C-3′. The HMBC correlations between H-9 (δ_H 4.69) and C-4 (δ_C 136.9), and between H-3 (δ_H 7.96) and C-9 (δ_C 28.7) demonstrated that the benzyl moiety was connected to C-4. The downfield shift of C-7′ (δ_C 176.1) and the *N*-methyl group (δ_H 4.32) suggested that compound **3** existed as a zwitterion (Chang et al., 2015). Based on this evidence, the structure of **3** was established as a substituted 4-benzylisoquinoline and named corydedine.

Compound **4**, obtained as a colorless powder, gave an $[M + Na]^+$ peak at *m/z* 422.1215 by HRESIMS corresponding to a molecular formula $C_{21}H_{21}NO_7$. The IR spectrum displayed absorptions for NH or OH (3395 cm^{-1}) and two carbonyl groups (1700 and 1646 cm^{-1}).