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Six scalemic mixtures of 6-monosubstituted dihydrobenzophenanthridine alkaloids from *Chelidonium majus* and optically active structures of enantiomers

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ABSTRACT

Six pairs of previously undescribed 6-monosubstituted dihydrobenzophenanthridine alkaloids were separated as corresponding six scalemic mixtures from the aerial part of *Chelidonium majus*. The elucidation for the 2D structures of these alkaloids was achieved using regular spectroscopic and chemical methods. The assignment of scalemic-mixture nature was achieved using combined examinations of their NMR data, CD spectra, calculation of specific rotations, and chiral HPLC profiles. The identification for the relative configurations of alkaloids possessing two asymmetric carbons directly connected up by a rotatable sp^3-sp^3 carbon-carbon single bond was significantly facilitated by discussing the *erythro* and *threo* relative configurations defined by the mutuality of the orders of decreasing steric hindrances between the two sets of ligands linked to the two chiral centers. Two scalemic mixtures were assigned as (1′*R*,6*R*/1′*S*,6*S*)- and (1′*S*,6*R*/1′*R*,6*S*)-1-(dihydrochelerythrine-6-yl)ethanols, two as (1′*R*,6*R*)/(1′*S*,6*S*)- and (1′*S*,6*R*)/(1′*R*,6*S*)-1-(dihydrosanguinarine-6-yl)ethanols, one as (±)-ethyl 2-(dihydrosanguinarine-6-yl) acetate, and one as (±)-ethyl dihydrosanguinarine-6-carboxylate, respectively. The resolution of three scalemic mixtures was achieved and the absolute configurations of the three pairs of enantiomers were assigned *via* time-dependent Density Functional Theory calculations of electronic circular dichroism (ECD) data. The assignment for the absolute configurations of the other three scalemic mixtures was achieved *via* a chiral HPLC-UV/CD method plus analyzing their ECD data. The findings of this paper demonstrated that the relevant biochemical reactions concerning the construction of these 6-monosubstituted dihydrobenzophenanthridine alkaloids in the test plant are very nonselective. Scalemic mixture of (1′*R*,6*R*)/(1′*S*,6*S*)-1-(dihydrosanguinarine-6-yl)ethanol exhibited biological activity. It inhibited the growth of human MDA-MB-231 cell line at a moderate level with IC_{50} value of 5.12 μM.

1. Introduction

Chelidonium majus Linn. of the Papaveraceae family naturally grows across a sweeping region in China. It was and is widely used to treat some diseases in Chinese folk medicines (Editorial committee of Flora of China, 1999). Phytochemical investigations into this medicinal plant were reported by researchers. More than 30 isoquinoline alkaloids were isolated and characterized. According to the habitual classification of alkaloids, the reported isoquinoline alkaloids were mainly broken down into benzophenanthridines (Paulsen et al., 2015; Rosa and Vincenzo, 1992; Kadan et al., 1990, 1992), protoberberines (Táborská et al., 1994), protopines (Zhou et al., 1989), and aporphines (Shafiee and Jafarabadi, 1998), among others. In addition, triterpenoids (Guo et al., 2014), sesquiterpenoids (Guo et al., 2014), sterols (Guo et al., 2014), and caffeic acid derivatives (Hahn and Nahrstedt, 1993) were also isolated from *C. majus*. In the fields of plant taxology, chemistry, pharmacology, and pharmacognosy, alkaloids have been recognized as the most important constituents of this species. Pharmacological properties of these alkaloids, including antitumor (Capistrano et al., 2015), anti-inflammation (Vavrečková et al.,

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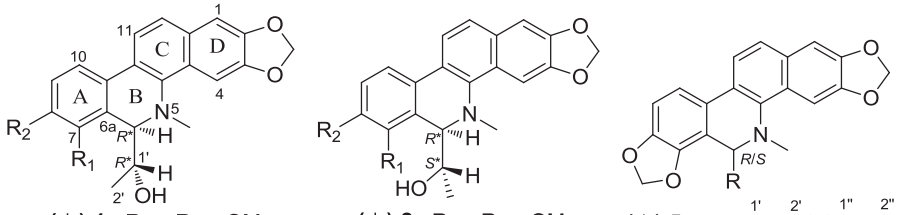
1996a; Kim et al., 2015; Lenfeld et al., 1981), antispasm (Hiller et al., 1998; Häberlein et al., 1996), antiproliferation (Vavrečková et al., 1996b), and antifungi (Meng et al., 2009), were explored and illustrated. Recently, following the isolation and identification of two previously undescribed hopane-type triterpenes (Deng et al., 2016), our group acquired six previously undescribed 6-monosubstituted dihydrobenzophenanthridine alkaloids from the ethanolic extract of the aerial part of *C. majus*. Structural elucidation found that these alkaloids shared the same core characteristic of 5-methyl-5,6-dihydrobenzo[*c*]phenanthridine substituted by oxygen-containing functional groups *via* carbon-oxygen single bond at the aromatic C-2, 3, 7, and 8 sites on the numbering system of benzo[*c*]phenanthridine (see Scheme 1). Four (**1**–**4**) of the six alkaloids also shared the same ethanol-1-yl group linked to C-6 *via* a rotatable sp^3-sp^3 carbon-carbon single bond, which led to the situation of chiral attachment of chiral group at the C-6 site, existing two directly connected chiral carbon atoms. Alkaloids **5** and **6** both bonded an achiral carbon atom on C-6, also affording structures of optical activity.

The structural features of **1**–**4** were evocative of the *erythro* and *threo* relative configurations of organic compounds. But the definition of *erythro* and *threo* relative configurations explained by the Fischer projection isn't considered to facilitate the structural identification on ^{1}H NMR coupling constants of such organic compounds. In the traditional definition, it is stipulated that two identical atoms or functional groups linked directly to the two different asymmetric carbons, respectively, are either on the same side of the sp^3-sp^3 single bond linking the two asymmetric carbons (*erythro*) or on the opposite sides (*threo*) when the molecular structures were expressed out by the Fischer projection. In order to facilitate the identification of the optically active alkaloids **1**–**4** and other similar compounds, the *erythro* and *threo* relative configurations are defined in this work in a different way, i.e., by the mutuality of the orders of decreasing steric hindrances of the two sets of ligands at the two chiral centers. As a result, this beloved definition provided a great help for the structural elucidation of **1**–**4** in this paper. In addition, as our ongoing effort to discover biologically active compounds, and according to the folk application of the title plant, alkaloids **1**–**6** were tested for the growth inhibitory activity against several human cancer cell lines using the MTT assay. This paper describes the full structures of **1**–**6** and the facility of explaining the *erythro* and *threo* relative configurations by using the steric hindrances of the two sets of ligands at the two chiral centers for the structural identification of **1**–**4**. It also reports on a preliminary bioactive screening for these alkaloids.

2. Results and discussion

The air-dried aerial part of *C. majus* was extracted with aqueous ethanol. The extract was concentrated and partitioned using petroleum ether and $CHCl_3$, respectively. The $CHCl_3$-soluble portion was subjected to procedures of isolation and purification to yield six alkaloids (**1**–**6**), all as white amorphous powder. Strong fluorescence under UV 254 nm and 365 nm on TLC plate precoated with silica gel G, positive reaction with Dragendorff's reagent, and absorption maxima at around 280–285 nm and 321–324 nm in the UV spectra were observed for all the six alkaloids. These chemical and physical natures were consistent with those of dihydrobenzophenanthridine alkaloids (Deng and Qin, 2010; Ito et al., 1990; Ng et al., 1987). The ^{1}H and ^{13}C NMR spectroscopic data of alkaloids **1**–**6** were given in Tables 1–3. All the alkaloids displayed the typical features of 6-monosubstituted dihydrobenzophenanthridine alkaloids bonding four oxygen atoms *via* carbon-oxygen single bond on aromatic C-2, 3, 7, and 8 in their ^{1}H NMR spectra by the following five specifics, (1) signals of two sets of aromatic AB spin-spin coupling system with ^3J values of 8.0–9.0 Hz assignable to CH(9) = CH(10) and CH(11) = CH(12) from two *vic*-tetrasubstituted benzene rings; (2) two isolated aromatic singlets containing one aromatic proton each in the resonance region of δ_H 6.99–8.09 assignable to CH(1) and CH(4); (3) one singlet of methyl group at δ_H 2.62–2.76 assignable to nitromethyl group linked to N-5; (4) the singlets of two aromatic methoxy groups resonated at the typical region of δ_H 3.79–4.15 and the signals of one aromatic methylenedioxy group as two one-proton virtual doublets with virtual coupling ^2J values of 0.8–1.2 Hz or one two-proton broad singlet at the typical region of δ_H 6.07–6.10 for **1** and **2**; or the signals of two aromatic methylenedioxy groups resonated as two one-proton virtual doublets with virtual coupling ^2J value of 1.2 Hz or two one-proton broad singlets and one two-proton broad singlet at the typical region of δ_H 6.04–6.13 for **3**–**5** or as two sets of two one-proton virtual doublets with virtual coupling ^2J value of 1.2 Hz for **6**; and (5) one doublet with ^3J values of 8.4–9.0 Hz (**1**–**4**) or one triplet with ^3J value of 7.6 Hz (**5**) or one singlet (**6**) containing one proton each at the aliphatic resonance region of δ_H 4.30–5.46 assignable to CH(6) bonding an electronegative heteroatom, which indicated that a methine (**1**–**4**) or a methylene (**5**) or a hydrogen-free carbon atom (**6**) were directly linked to C-6 to form the chiral carbon atoms. All of these signals were consistent with the chemical shifts, signal patterns, and area integral values of the relevant functional groups from chiral 6-monosubstituted dihydrobenzophenanthridine moieties. Based on these informations, the chiral dihydrobenzophenanthridine moieties of **1** and **2** were identified as dihydrochelerythrine-6-yl, and **3**–**6** all as dihydrosanguinarine-6-yl. For each and every alkaloid of **1**–**4**, putting off the 6-monosubstituted dihydrobenzophenanthridine core, one set of resolvable signals of ethanol-1-yl moiety was detected in the ^{1}H NMR spectra, i.e., one multiplet of oxymethine resonating at δ_H 3.67 or 3.90 and one doublet of methyl at δ_H 1.34/1.35 or δ_H 1.53/



Scheme 1. Structures of **1**-**6**.