Mass spectrometry-guided isolation of two new benzoquinoline alkaloids from Macleaya cordata

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1. Introduction

Macleaya cordata (Wild.) R. Br. belongs to genus Macleaya of the Papaveraceae family and has been used for over a thousand year as a traditional folk medicine (Zeng et al. 2013). Isoquinoline alkaloids, including benzophenanthridine, dihydro-benzophenanthridine, protopine and protoberberine, are its major biological activities components (Kosina et al. 2010; Feng et al. 2012). These alkaloids have several biological activities including antibacterial (Cheng et al. 2014), anti-inflammatory, antimicrobial (Li et al. 2015), anti-tumour, anti-HIV
and animal growth promotion (Qing et al. 2014). A set of products (e.g. BoLuoHui ZhuSheYe®, BoLuoHui ZhongYangDing®) has been developed successfully in China due to those bioactive alkaloids. Meanwhile, sanguinarine and chelerythrine, two main alkaloids isolated from this plant medicine, have been used for promoting animal growth as a popular natural feed additive (e.g. Sangrovit®) (Stiborova et al. 2008; Zdarilova et al. 2008). In addition to these well-known chemical components, a series of alkaloids, which may have potential biological activities, is still unknown and requires further isolated and identified.

To the best of our knowledge, traditional phytochemistry isolation provides a comprehensive chemical foundation in the discovery of new drug candidates from plant medicines. However, this method is time consuming and low efficiency. Therefore, bioactivity-guided isolation is applied for rapid excavating the chemical components which respond to the relevant biological activities (Kang et al. 2013). In this study, a liquid chromatography–mass spectrometry (LC–MS)-guided isolation technology is also applied for rapid uncovering the alkaloids which have not been reported from M. cordata. Finally, two new benzoquinoline alkaloids, named 2,3-methylenedioxy-7,10-dimethyl-7,8,9,10-tetrahydro-benzoquinoline (1) and 2,3-methylenedioxy-7,10-dimethyl-8-carboxyl-benzoquinoline (2) (Figure 1), were rapidly isolated under the guiding of mass spectrometry, and then identified on the basis of numerous spectroscopic data.

2. Result and discussion

2.1. Structure elucidation of compound 1

Compound 1 was isolated as reddish-brown powder. It showed positive reaction to Dragendorff reagent on thin-layer chromatography (TLC). High resolution-Q-TOF-MS of 1 exhibited quasi-molecular ion ([M + H⁺]) at m/z 256.1331, corresponding to the molecular formula C₁₆H₁₇NO₂. The ¹H NMR spectra (see Figure S1) of 1 displayed, in the aromatic region, two one-proton signals at δ_H 7.41 (H-1) and 7.06 (H-4), one pair of AB-type ortho-coupling quartet at δ_H 7.32 (d, J = 8.4 Hz, H-5) and 7.14 (d, J = 8.4 Hz, H-6). The ¹³C and DEPT NMR spectra (see Figure S1) of the compound showed six aromatic quaternary carbons (δ_C 148.8, 148.2, 143.8, 131.5, 130.7, 126.3), four aromatic tertiary carbons (δ_C 126.3, 123.3, 106.2, 101.1), two secondary carbons (δ_C 49.8, 24.7) and one tertiary carbon (δ_C 31.5). All these signals indicated the skeleton of compound 1 was tetrahydro-benzoquinoline.

The remaining data in the ¹H, ¹³C, DEPT and HMQC NMR spectra (see Figure S1), indicated signals of a methylenedioxy group at δ_H 5.97 (2H, s)/ δ_C 102.8, a N-methyl group at δ_H 2.82 (3H, s)/ δ_C 43.9, a methyl group at δ_H 1.30 (3H, s)/ δ_C 23.5. In the HMBC spectra (see Figure S1), the methylenedioxy group (δ_H 5.97) showed clear correlations with C-2 (δ_C
148.2) and C-3(δ_c 148.8), indicating the direct linkage of the methylenedioxy with C-2, C-3. The methyl and N-methyl group were located at para-position in the non-aromatic ring by multiple signals at δ_h 2.16 (H-8), 1.67 (H-8') and δ_h 3.35 (H-9), 3.28 (H-9'). Due to correlations of -CH_3 to C-7, C-8, C-6a and N-CH_3 to C-9, C-10a in HMBC signals, the methyl and N-methyl group can be assigned at C-7 and position-10, respectively (Figure 1). Other significant HMBC correlations for the structural elucidation of compound 1 are given in Figure S1. Finally, the structure of compound 1 was determined and identified as 2,3-methylenedioxy-7,10-dimethyl-7,8,9,10-tetrahydro-benzoquinoline.

2.2. Structure elucidation of compound 2

Compound 2 was obtained as yellowish-brown powder. It also showed positive reaction to Dragendorff reagent on TLC. High resolution-Q-TOF-MS of 2 gave the quasi-molecular ion peak at m/z 296.0908 ([M + H^+]), showed its molecular formula C_{17}H_{14}N_4O_4. The ^1H NMR spectra (see Figure S2) of 2 displayed, in the aromatic ring, three one-proton signals at δ_H 9.22 (H-9), 8.25 (H-1) and 7.57 (H-4), one pair of AB-type ortho-coupling quartet at δ_H 8.13 (d, J = 8.8 Hz, H-5) and 8.19 (d, J = 8.8 Hz, H-6). The ^13C and DEPT NMR spectra (see Figure S2) of the compound showed eight aromatic quaternary carbons (δ_c 154.2, 152.9, 150.8, 140.3, 136.6, 136.6, 131.4, 120.7), five aromatic tertiary carbons (δ_c 149.3, 132.4, 121.7, 107.6, 106.9). All these signals indicated the skeleton of compound 2 was benzoquinoline.

The ^1H, ^13C, DEPT and HMQC NMR spectra (see Figure S2) of alkaloid 2 demonstrated signals of a methylenedioxy group at δ_H 6.30 (2H, s)/δ_c 104.8, a N-methyl group at δ_H 4.84 (3H, s)/δ_c 52.6, a methyl group at δ_H 3.16 (3H, s)/δ_c 18.0. The neutral loss of a CO_2 molecular and formed the fragment ion at m/z 252.1008 (Δppm-0.79) was appeared in the tandem mass spectrometry (MS/MS) of 2 (see Figure S3), which indicated the signal of a carboxy group. Meanwhile, the –COOH group was further confirmed by the ^13C NMR data (δ_c 169.6). In the HMBC spectra (see Figure S2), –OCH_2O– (δ_H 6.30) group showed clear correlations with C-2 (δ_c 152.9) and C-3(δ_c 150.8), indicating the direct linkage of the methylenedioxy with C-2, C-3. Due to correlations of CH_3 to C-7, C-8, C-6a and N-CH_3 to C-9, C-10a in HMBC signals, the methyl and N-methyl group can be assigned at C-7 and position-10, respectively (Figure 1). The chemical shift of C-9 (δ_c 149.3) and H-9 (δ_h 9.22) were moved to low field compared with other aromatic tertiary carbons and protons, which indicated C-9 and H-9 were influenced by electron withdrawing group. Meanwhile, in the HMBC spectra, the –COOH (δ_c 169.6) group showed clear correlation with H-9 (δ_h 9.22). Therefore, the carboxy group can be assigned at C-8. Other significant HMBC correlations for the structural elucidation of compound 2 are given in Figure S2. Finally, the structure of compound 2 was determined and identified as 2,3-methylenedioxy-7,10-dimethyl-8-carboxyl-benzoquinoline.

3. Experimental

3.1. General experimental procedure

Ultraviolet (UV) spectra were taken on a Hitachi UV-3310 spectrophotometer. IR spectra were obtained with a Nicolet Impact 410 infrared spectrometer with KBr disc technique. 1D and 2D NMR spectra were acquired using a Bruker ACF-400 spectrometer (the ^1H NMR spectra at 400 MHz and ^13C NMR spectra at 100 MHz). The mass spectra were determined by an Agilent 1290 HPLC system coupled with a 6530 Q-TOF/MS accurate-mass spectrometry. Agilent preparative HPLC system including a binary pump, an injector and a UV detector was used.
for isolation the target compounds. The reversed-phase Xcharge C18 (10 μm, 20 × 250 mm i.d., Da Lian, RP China) was employed as chromatographic column. Silica gel (200–300 mesh) used for column chromatography (CC) and silica GF$_{254}$ for TLC was supplied by Qingdao Marine Chemical Factory, Qingdao, RP China.

3.2. Plant material

The *M. cordata* fruits were collected from Jinzhai country (Anhui province, RP China) in August, 2014, and were authenticated by Prof. Jian-Guo Zeng (Hunan University of Chinese Medicine). A voucher specimen (AHJZ20140824) was deposited in the herbarium of the School of Pharmacy, Hunan University of Chinese Medicine, Hunan, RP China.

3.3. Detection, extraction and isolation

The structures of different types of alkaloid in *M. cordata* fruits have been speculated and elucidated in detail by their mass spectrometry (Qing et al. 2014; Qing, Cheng, et al. 2015; Qing, Liu, et al. 2015). However, two compounds were different from the well-known alkaloids according to their fragmentation pathways (see Figure S3). Therefore, the two compounds were regarded as new type of alkaloid in *M. cordata*. In order to determine the structure and investigation of the potential biological activities, the two alkaloids were isolated by the preparative HPLC system under the guiding of mass spectrometry.

The air-dried *M. cordata* fruits (50 kg) were extracted three times (3 × 1 h) under conditions of reflux with 95% ethanol, and the ratio of solid and liquid is 1:20. After evaporation of the ethanol in vacuum, a viscous residue was obtained which was suspended in 450 L (3 × 150 L) hydrochloric acid solution (PH3), and the acid water layer was obtained after standing for 24 h. Then, NH$_3$·H$_2$O was added for adjusting the PH to 10 and the alkaline water layer and undissolved residue (650 g) were isolated after standing for 24 h.

The undissolved residue was subjected to silica gel CC (200–300 mesh, 10 kg) eluted with CH$_2$Cl$_2$-MeOH (97:3) to provide five fractions according to TLC detection on silica gel plates. Fraction 4 (56.4 g) was separated by CC on silica gel (200–300 mesh, 1.0 kg) eluted with CH$_2$Cl$_2$-MeOH (95:5) to obtain six fractions. Fraction 5 (2.4 g) was isolated by preparative RP-HPLC, eluted with MeOH and 0.1% formic acid aqueous solution (60:40, v/v), to give compound 1 (76 mg, 20 ml/min, $t_R = 6.2$ min) and fraction 3 (42 mg). Compound 2 (12.6 mg, 10 ml/min, $t_R = 9.9$ min) was finally purified from the fraction 3 by the HPLC system, eluted with MeOH and 0.1% formic acid aqueous solution (50:50, v/v). The target alkaloids were always monitored and detected by mass spectrometry in the process of exaction and isolation, and relevant chromatograms linked to isolation procedure were showed in Figure S4.

3.4. Characterisation data

Compound 1: reddish-brown powder, UV (MeOH) $\lambda_{\text{max}}$ 224, 259 nm; IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$ 2979, 2863, 2742, 1608, 1500, 1247, 927; ESIMS: $m/z$: 256.1331 [M+H]$^+$, 241.1092 [M-CH$_3$]$^+$, 226.0858 [M-CH$_3$-CH$_3$]$^+$. $^1$H NMR (400 MHz CD$_3$OD) $\delta_H$: 7.41 (1H, s, H-1), 7.32 (1H, d, $J = 8.4$, H-5), 7.14 (1H, d, $J = 8.4$, H-6), 7.06 (1H, s, H-4), 5.97 (2H, s, –oCH$_2$–), 5.97 (2H, s, –OCH$_2$O–), 3.35 (1H, m, H-7), 2.82 (3H, s, N-CH$_3$), 2.16 (1H, m, H-8), 1.67 (1H, m, H-8'), 1.30 (3H, s, 7-CH$_3$); $^{13}$C NMR (100 MHz CD$_3$OD) $\delta_C$: 148.8 (C-3), 148.2 (C-2), 143.8 (C-10a), 131.5 (C-4a), 130.7 (C-6a),...
126.3 (C-6), 126.3 (C-10b), 123.3 (C-5), 106.2 (C-4), 102.8 (–OCH₂O–), 101.1 (C-1), 49.8 (C-9), 43.9 (N–CH₃), 31.5 (C-7), 24.7 (C-8), 23.5 (7-CH₃).

Compound 2: yellowish-brown powder, UV (MeOH) λ max 209, 240, 306 nm; IR (KBr) ν max cm⁻¹ 2985, 2874, 2719, 1751, 1613, 1467, 1264, 920; ESIIMS: m/z: 296.0908 [M+H]+, 281.0666 [M-CH₃]+, 252.1008 [M-CO₂]+, 224.1041 [M-CO₂-CO]+, 194.0952 [M-CO₂-CO-CH₂O]+.

1H NMR (400 MHz CD₃OD) δ H: 9.22 (1H, s, H-9), 8.25 (1H, s, H-1), 8.19 (1H, d, J = 8.8, H-6), 8.13 (1H, d, J = 8.8, H-5), 7.57 (1H, s, H-4), 6.30 (2H, s, –OCH₂O–), 4.84 (3H, s, N-CH₃), 3.16 (3H, s, 7-CH₃); 13C NMR (100 MHz CD₃OD) δ C: 169.6 (–COOH), 154.2 (C-7), 152.9 (C-2), 150.8 (C-3), 149.3 (C-9), 140.3 (C-10a), 136.6 (C-8), 136.6 (C-4a), 132.4 (C-5), 131.4 (C-6a), 121.7 (C-6), 120.7 (C-10b), 107.6 (C-4), 106.9 (C-1), 104.8 (–OCH₂O–), 52.6 (N-CH₃), 18.0 (7-CH₃).

Supplementary material
Supplementary material relating to this article is available online, alongside Figures S1–S4.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
Cheng P, Wang B, Liu XB, Liu W, Kang W, Zhou J, Zeng JG. 2014. Facile synthesis of tetrahydroprotoberberine and protoberberine alkaloids from protopines and study on their antibacterial activities. Nat Prod Res. 28:413–419.

Feng F, Ye FZ, Li CL, Liu WY, Xie N. 2012. Two new benzophenanthridine isoquinoline alkaloids from Macleaya cordata. Chin J Nat Med. 10:0378–0382.

Kang YJ, Yi YL, Zhang C, Wu SQ, Shi CB, Wang GX. 2013. Bioassay-guided isolation and identification of active compounds from Macleaya microcarpa (Maxim) Fedde against fish pathogenic bacteria. Aquacult Res. 44:1221–1228.

Kosina P, Gregorova J, Gruz J, Vacek J, Kolar M, Vogel M, Roos W, Naumann K, Simanek V, Ulrichova J. 2010. Phytochemical and antimicrobial characterization of Macleaya cordata herb. Fitoterapia 81:1006–1012.

Li CM, Yang XY, Zhong YR, Yu JP. 2015. Chemical composition, antioxidant and antimicrobial activity of the essential oil from the leaves of Macleaya cordata (Willd) R. Br. Nat Prod Res. 29:1–5.

Qing ZX, Cheng P, Liu XB, Liu YS, Zeng JG. 2015. Systematic identification of alkaloids in Macleaya microcarpa fruits by liquid chromatography tandem mass spectrometry combined with the isoquinoline alkaloids biosynthetic pathway. J Pharm Biomed Anal. 103:26–34.

Qing ZX, Cheng P, Liu XB, Liu YS, Zeng JG, Wang W. 2014. Structural speculation and identification of alkaloids in Macleaya cordata fruits by high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry combined with a screening procedure. Rapid Commun Mass Spectrom. 28:1033–1044.

Qing ZX, Liu XB, Wu HM, Cheng P, Liu YS, Zeng JG. 2015. An improved separation method for classification of Macleaya cordata from different geographical origins. Anal Methods. 7:1866–1871.

Stiborova M, Vostalova J, Zdarilova A, Ulrichova J, Hudecek J, Tschirner K, Simanek V. 2008. Macleaya cordata extract and sangrovit genotoxicity assessment in vivo. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 152:35–39.
Zdarilova A, Vrublova E, Vostalova J, Klejduš B, Stejskal D, Proskova J, Kosina P, Svobodova A, Vecera R, Hrbac J, et al. 2008. Natural feed additive of *Macleaya cordata*: safety assessment in rats a 90-day feeding experiment. Food Chem Toxicol. 46:3721–3726.

Zeng JG, Liu YS, Liu W, Liu XB, Liu FQ, Huang P, Zhu PC, Chen JJ, Shi MM, Guo F, et al. 2013. Integration of transcriptome, proteome and metabolism data reveals the alkaloids biosynthesis in *Macleaya cordata* and *Macleaya microcarpa*. PLoS ONE 8:e53409.