Chinese Herbal Medicines 11 (2019) 364–368

Contents lists available at ScienceDirect

Chinese Herbal Medicines

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

Original Article

A phytochemical study on *Dichocarpum auriculatum*, an endangered medicinal plant peculiar to China

Pei Li, Shuang-shuang Liu, Qing Liu, Jie Shen, Chun-nian He*, Pei-gen Xiao

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China

ARTICLE INFO

Article history:
Received 12 March 2019
Revised 18 April 2019
Accepted 20 May 2019
Available online 23 September 2019

Keywords:
benzylisoquinoline alkaloids
chemical constituents
cyanides
Dichocarpum auriculatum (Franch.) W. T. Wang et Hsiao
megastigmane glycosides

ABSTRACT

Objective: *Dichocarpum auriculatum*, an endangered perennial herb, is endemic to China and has been used as folk medicines for the treatment of cough, hepatitis, scrofula, and epilepsy. However, there is no phytochemical report on this herbal so far. For the resource development and protective importance of this endangered medicinal plant, a phytochemical study was undertaken.
Methods: The chemical constituents were purified by silica gel column, Sephadex LH-20 column, and semi-preparative reversed phase HPLC. NMR and MS spectra were used for structural identification.
Results: Thirteen compounds were isolated from *D. auriculatum*. Their structures were characterized as jatrorrhizine (**1**), berberine (**2**), steponine (**3**), magnoflorine (**4**), coclauril (**5**), menisdaurin (**6**), menisdaurilide (**7**), aquilegiolide (**8**), (6*R*, 9*S*)-3-oxo-α-ionol-β-*D*-glucopyranoside (**9**), blumenol C glucoside (**10**), palmitic acid (**11**), dibutylphthalate (**12**), and auriculatum A (**13**).
Conclusion: Compound **13** is a new diester terephthalate derivative. All the compounds are obtained from the genus *Dichocarpum* for the first time, and compounds **9** and **10** have potential chemotaxonomic significance to the genus *Dichocarpum*.

1. Introduction

Dichocarpum auriculatum (Franch.) W. T. Wang et Hsiao, a perennial herb in the family Ranunculaceae, is mainly growing in shady wet places on slopes or growing near rocks in forests of Sichuan, Yunnan, Fujian, and Hubei Provinces of China. (Flora of China, 2001). This specie is a widely used traditional folk herb in southern China for clearing away heat and toxin, relieving cough, and eliminating phlegm. Its roots commonly called Muzhucao or Shanhuanglian have been used for the treatment of epilepsy in Junlian County, Sichuan Province (State Administration of Editorial Board of Chinese Materia Medica, 1999). There are no phytochemical reports on *D. auriculatum* and the chemical constituents of the genus *Dichocarpum* were hardly investigated so far. In previous studies, only five compounds, including one alkanol [(10*S*)-nonacosan-10-ol] and four triterpenoids (kaiyangense A, daucoster, calcoside D, and Anhuienoside B) were isolated from *Dichocarpum kaiyangense* S. Z. He, sp. nov. (Wu, 2011). To investigate the constituents of *D. auriculatum*, 13 compounds were isolated from ethyl acetate and acetone soluble parts of methanol extract of *D. auriculatum*. Their structures were characterized as jatrorrhizine (**1**), berberine (**2**), steponine (**3**), magnoflorine (**4**), coclauril (**5**), menisdaurin (**6**), menisdaurilide (**7**), aquilegiolide (**8**), (6*R*, 9*S*)−3-oxo-α-ionol-β-*D*-glucopyranoside (**9**), blumenol C glucoside (**10**), palmitic acid (**11**), dibutylphthalate (**12**), and auriculatum A (**13**) based on the physicochemical properties and NMR spectral analysis. Compounds **1**–**12** were reported for the first time in the genus *Dichocarpum*. Compound **13** is a new diester terephthalate derivative. In this paper, we report the isolation and structure elucidation of these compounds.

2. Materials and methods

2.1. General experimental procedures

Solvent used in this study was analytical grade (Beijing Chemical Works, Beijing, China) and chromatographic grade (Honeywell B&J). Analytical TLC was performed on the silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, China). Column chromatography (CC) was performed on silica gel (200–300 mesh and 300–400 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 gel (25–100 μm, Amersham Pharmacia Biotechnology AB, Uppsala, Sweden) as well as MCI CHP-20P resin (Japan Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on an YMC Pack ODS-A (YMC, Kyoto, Japan) column (250 × 10 mm). NMR spectra data was determined on a

* Corresponding author.
E-mail address: cnhe@implad.ac.cn (C.-n. He).

https://doi.org/10.1016/j.chmed.2019.05.009
1674-6384/

Bruker DRX-500 spectrometer (Bruker Corp., Karlsruhe, Germany) using TMS as internal standard.

2.2. Plant materials

Samples of *D. auriculatum* used in this study were collected in Junlian County, Sichuan Province, China, in May 2017, and identified by Prof. Pei-gen Xiao from the Institute of Medicinal Plant Development and Chinese Academy of Medical Sciences, Beijing, China. A voucher specimen (QY2017Dic001) was deposited in the Pharmaphylogeny Center at the Institute of Medicinal Plant Development, Beijing, China.

2.3. Extraction and isolation

Dry plant materials of *D. auriculatum* (2.0 kg) were pulverized and extracted by iterative macerations with acetone (10 L × 24 h, each time) at room temperature for five times. The acetone extract were concentrated under reduced pressure to yield 16.8 g crude extract, which was then subjected to column chromatography (CC) (MCI GEL, H_2O-MeOH, 10:0, 9:1, 7:3, 5:5, 3:7, and 0:10, volume ratio) to afford sixteen pooled fractions (BW1–BW16) according to the TLC analysis. Fraction BW4 (2.5 g) was loaded onto CC over silica gel using $CHCl_3$-MeOH (95:5–60:40, volume ratio) as eluent to obtain fractions BT1–BT5. The separation of BT2 with the Sephadex LH-20 (MeOH-H_2O, 1:1, volume ratio) CC produced twelve portions M1–M12, followed by further purification of M7 with the Sephadex LH-20 (MeOH) CC to yield compound **1** (20 mg) and compound **13** (7 mg). Likewise, compound **2** (15 mg) was separated as yellow needle crystals from Fr. BW6 on Sephadex LH-20 CC with MeOH as eluants. Fr. BW7 (2.0 g) was chromatographed on silica gel using a gradient of $CHCl_3$ in acetone (90:10–75:25) to give fractions W1–W9. Fr. W7 was purified by semi-preparative HPLC with 7% MeOH-H_2O mixtures and 2.5 mL/min flow rate to afford compound **5** (5 mg) and **7** (8 mg). The Fr. BW12 soluble in methanol was separated by column chromatography over Sephadex LH-20 with elution by methanol to produce fractions C1–C4. Fr. C3 and C4 were purified several times by semi-preparative HPLC with 60% MeOH-0.1% FA-H_2O and 3 mL/min flow rate to produce compound **9** (4 mg) and **10** (3.5 mg), respectively.

The residue after iterative macerations with acetone were immersed in mixture solvent (MeOH-H_2O, 2:3, 10 L × 24 h, each time) at room temparature for six times to provide a total extract of 840.6 g, which was then suspended in water and extracted successively with ethyl acetate and *n*-butanol. The ethyl acetate extract (7.8 g) mixed with MCI GEL were chromatographed over MCI GEL CC using H_2O-MeOH (9:1, 7:3, 5:5, 2:8, and 0:10, volume ratio) for elution, which yielded 16 fractions YS1–YS16. The obtained fractions were analyzed by TLC using $CHCl_3$-MeOH (8:2, volume ratio). Purification of Fr. YS-6 (842 mg) by column chromatography over Sephadex LH-20 CC using a CH_3OH-H_2O gradient resulted in isolation of compound **12** (3.5 mg). Fr. YS-8 was loaded on to CC over silica gel ($CHCl_3$-MeOH, 9:1–7:3, volume ratio) and subsequently separated by semi-preparative HPLC using MeOH-H_2O (3:7, volume ratio) as eluents with 3 mL/min flow rate to obtain compound **8** (10 mg) and compound **6** (40 mg). Fr. YS-13 was subjected to Sephadex LH-20 CC and eluted with MeOH to provide compound **11** (5.5 mg). The *n*-butanol (45.8 g) gave compound **4** (9.2 mg) and compound **3** (780 mg) after CC (MCI GEL, MeOH-H_2O, 30:70–100:0, volume ratio) and purification by semi-preparative HPLC at 3 mL/min flow rate.

3. Results

The structures of compounds **1**–**13** were identified based on their NMR and MS spectral data and by comparison with those reported in literatures. All the structures of compounds were shown in Fig. 1. This is the first chemical study on *D. auriculatum* and all the compounds are obtained from the genus *Dichocarpum* for the first time.

Compound **1** (jatrorrhizine): yellow powder. ESI-MS *m/z*: 338 $[M]^+$ $C_{20}H_{20}NO_4$; ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 9.87 (s, 1H, H-8), 9.43 (s, 1H, 3-OH), 8.83 (s, 1H, H-13), 8.20 (d, J = 9.2 Hz, 1H, H-12), 8.06 (d, J = 9.1 Hz, 1H, H-11), 7.56 (s, 1H, H-4), 7.06 (s, 1H, H-1), 4.96–4.90 (m, 2H, H-6), 4.09 (s, 3H, –OCH_3), 4.07 (s, 3H, –OCH_3), 3.90 (s, 3H, –OCH_3), 3.24–3.16 (m, 2H, H-5); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 151.10 (C-9), 150.67 (C-2), 146.84 (C-3), 145.86 (C-8), 143.99 (C-10), 138.27 (C-13a), 133.61 (C-12a), 132.03 (C-1a), 129.15 (C-4a), 127.14 (C-12), 124.08 (C-11), 121.81 (C-13b), 120.08 (C-13), 119.61 (C-8a), 112.67 (C-4), 111.86 (C-1), 62.37 (–OCH_3), 57.52 (–OCH_3), 56.36 (–OCH_3), 55.98 (C-6), 26.43 (C-5). It was identified as jatrorrhizine by comparing with reported spectral data of the literature (Jung, Yoon, Bae, Min & Choi, 2008).

Compound **2** (berberine): yellow needle crystal. ESI-MS *m/z*: 336 $[M]^+$ $C_{20}H_{18}NO_4$; ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 9.90 (s, 1H, H-8), 8.94 (s, 1H, H-13), 8.21 (d, J = 13.5 Hz, 1H, H-11), 8.00 (d, J = 9.1 Hz, 1H, H-12), 7.80 (s, 1H, H-1), 7.10 (s, 1H, H-4), 6.18 (s, 2H, O-CH_2-O), 4.93 (t, J = 6.3 Hz, 2H, H-6), 4.09 (s, 3H, –OCH_3), 4.07 (s, 3H, –OCH_3), 3.23 (t, J = 6.3 Hz, 2H, H-5); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 150.89 (C-3), 150.31 (C-10), 148.17 (C-2), 145.97 (C-9), 144.10 (C-8), 137.97 (C-13a), 133.42 (C-12a), 131.19 (C-4a), 127.20 (C-11), 124.01 (C-12), 121.88 (C-8a), 120.93 (C-13), 120.67 (C-1a), 108.93 (C-4), 105.90 (C-1), 102.58 (O-CH_2-O), 62.39 (C-9, –OCH_3), 57.51 (C-10), 55.65 (C-6), 26.79 (C-5). It was identified as berberine by comparing with reported spectral data of the literature (Yong et al., 2006).

Compound **3** (steponine): white powder. ESI-MS *m/z*: 342 $[M]^+$ $C_{20}H_{24}NO_4$; ^{1}H NMR (600 MHz, CD_3OD, TMS) δ: 6.95 (d, J = 8.4 Hz, 1H, H-11), 6.86 (s, 1H, H-4), 6.71 (t, J = 4.1 Hz, 2H, H-1, H-12), 4.83 (d, J = 16.1 Hz, 1H, H-8a), 4.69 (dd, J = 10.1, 5.8 Hz, 1H, H-8b), 4.65–4.61 (m, 1H, H-13a), 3.86 (d, J = 4.0 Hz, 3H, –OCH_3), 3.85 (s, 3H, –OCH_3), 3.26 (s, 3H, *N*-CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ: 148.52 (C-2), 146.08 (C-3), 145.89 (C-10), 143.06 (C-9), 124.00 (C-12a), 121.58 (C-1a), 118.84 (C-4), 118.32 (C-12), 112.89 (C-8a), 112.86 (C-11), 111.61 (C-1), 65.65 (C-13a), 59.43 (C-8), 55.20 (–OCH_3), 55.05 (–OCH_3), 52.20 (C-6), 49.45 (*N*-CH_3), 33.34 (C-13), 22.71 (C-5). It was identified as steponine by comparing with reported spectral data of the literature (Tanahashi, Su, Nagakura, & Nayeshiro, 2000; Wang, Liao, Lei, & Chen, 2013).

Compound **4** (magnoflorine): brown powder. ESI-MS *m/z*: 342 $[M]^+$ $C_{20}H_{24}NO_4$; ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 6.85 (d, J = 8.0 Hz, 1H, H-9), 6.78 (s, 1H, H-3), 6.71 (d, J = 7.9 Hz, 1H, H-8), 4.43 (d, J = 12.9 Hz, 1H, H-6a), 3.78 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.62 (td, J = 12.6, 4.9 Hz, 1H, H-5), 3.33 (s, 3H, -*N*CH_3), 3.25 (dd, J = 12.7, 2.8 Hz, 1H, H-7), 3.19 (ddd, J = 18.0, 12.6, 5.6 Hz, 1H, H-4), 2.90 (s, 3H, *N*-CH_3), 2.93 (d, J = 4.2 Hz, 1H, H-4), 2.65 (t, J = 13.2 Hz, 1H, H-7); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 150.49 (C-2, C-10), 149.81 (C-11, C-1), 126.02 (C-7a), 121.39 (C-11a, C-11b), 121.01 (C-11c),117.57 (C-8), 117.26 (C-3a),111.13 (C-9), 110.14 (C-3), 68.88 (C-6a), 63.25 (C-5), 56.31 (–OCH_3), 56.04 (–OCH_3), 53.32 (*N*-CH_3), 43.17 (*N*-CH_3), 30.45 (C-7), 23.62 (C-4). It was identified as magnoflorine by comparing with reported spectral data of the literature (Chen, Du, Shen, & Yang, 2009; Tanahashi et al., 2000).

Compound **5** (coclauril): white needle crystal. ESI-MS *m/z*: 151 $[M]^+$ $C_8H_9NO_2$; ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 6.42 (dd, J = 10.0, 2.2 Hz, 1H, H-2), 6.23 (dd, J = 10.0, 1.5 Hz, 1H, H-3), 5.56 (s, 1H, H-α), 4.40 – 4.35 (m, 1H, H-4), 4.23 (ddd, J = 12.8, 3.9, 1.8 Hz, 1H, H-6), 2.27 – 2.20 (m, 1H, H-5), 1.43 (dt, J = 12.8, 11.0 Hz, 1H, H-5); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 162.19 (C-1), 144.88 (C-3), 122.80 (C-2), 117.89 (C≡N), 90.76 (C-α), 67.00 (C-6), 66.25 (C-4), 42.82 (C-5). It was identified as coclauril by comparing with

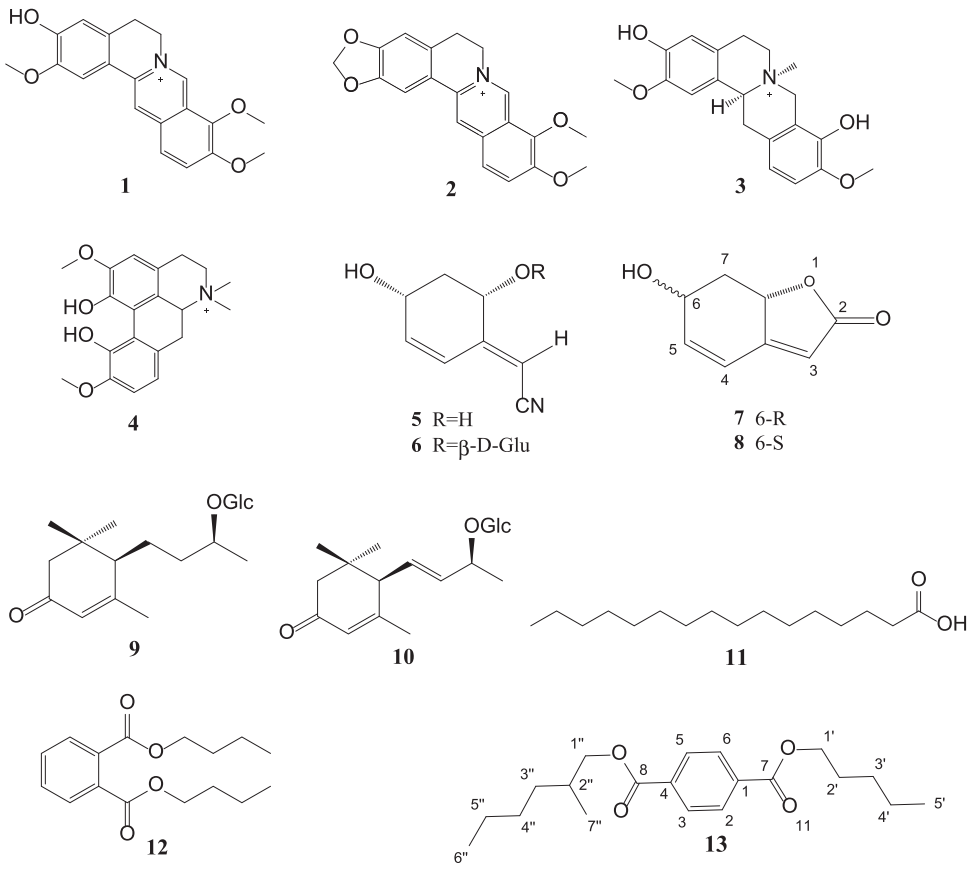

Fig. 1. Structures of compounds **1**–**13** isolated from *D. auriculatum*.

reported spectral data of the literature (Yogo, Ishiguro, Murata, & Furukawa, 1990).

Compound **6** (menisdaurin): white needle crystal. ESI-MS *m/z*: 314 $[M+H]^+$ $C_{14}H_{19}NO_7$; ^1H NMR (600 MHz, DMSO–d_6, TMS) δ: 6.22 (dd, J = 10.1, 1.5 Hz, 1H,H-2), 6.14 (dd, J = 9.9, 2.7 Hz, 1H, H-3), 5.63 (s, 1H, H-7), 4.74 (ddd, J = 10.5, 3.7, 1.8 Hz, 1H, H-6), 4.41 (d, J = 7.3 Hz, 1H, H-1′), 3.69 (d, J = 10.1 Hz, 1H, H-6′ b), 3.21–3.11 (m, 3H, H-2′, H-3′, H-5′), 3.05–2.98 (m, 1H, H-4′), 2.27 (dt, J = 12.2, 4.5 Hz, 1H, H-5), 1.65 (ddd, J = 12.3, 10.6, 8.3 Hz, 1H, H-5); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 156.35 (C-1), 142.00 (C-3), 126.56 (C-2), 117.57 (C-α), 99.77 (C-1′), 95.26 (C-7), 77.40 (C-3′), 77.21 (C-5′), 73.18 (C-6), 70.76 (C-2′), 70.74 (C-4′), 64.75 (C-4), 61.95 (C-6′), 36.57 (C-5). It was identified as menisdaurin by comparing with reported spectral data of the literature (Li, Zhang, & Yu, 2005; Yogo et al., 1990).

Compound **7** (menisdaurilide): white powder. ESI-MS *m/z*: 151 $[M-H]^-$ $C_8H_8O_3$; ^1H NMR (600 MHz, DMSO–d_6, TMS) δ: 6.60 (dd, J = 9.9, 2.5 Hz, 1H, H-2), 6.31 (d, J = 9.9 Hz, 1H, H-3), 5.94 (s, 1H, H-α), 5.55 (d, J = 6.3 Hz, 1H, 6-OH), 5.06 (ddd, J = 13.2, 4.9, 1.7 Hz, 1H, H-6), 4.48 (dd, J = 15.3, 5.7 Hz, 1H, H-4), 2.69 (dt, J = 10.5, 5.1 Hz, 1H, H-5), 1.42 (dt, J = 13.3, 10.4 Hz, 1H, H-5); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 173.48 (C-2), 164.91 (C-3a), 146.53 (C-5), 119.28 (C-4), 110.52 (C-3), 78.29 (C-7a), 65.72 (C-6). It was identified as menisdaurilide by comparing with reported spectral data of the literature (Yogo et al., 1990).

Compound **8** (aquilegiolide): white needle crystal. ESI-MS *m/z*: 151 $[M-H]^-$ $C_8H_8O_3$; ^1H NMR (600 MHz, CD_3OD, TMS) δ: 6.66 (d, J = 9.7 Hz, 1H, H-2), 6.35 (dt, J = 11.4, 5.8 Hz, 1H, H-3), 5.86 (d, J = 5.8 Hz, 1H, H-α), 5.30 (ddd, J = 12.7, 5.1, 1.5 Hz, 1H, H-6), 4.58–4.52 (m, 1H, H-4), 2.52 (dd, J = 12.6, 5.1 Hz, 1H, H-5), 1.77 (td, J = 12.7, 4.2 Hz, 1H, H-5); ^{13}C NMR (151 MHz, CD_3OD) δ: 174.47 (C-2), 164.31 (C-3a), 138.47 (C-5), 120.80 (C-4), 111.21 (C-3), 76.86 (C-7a), 63.61 (C-6). It was identified as aquilegiolide by comparing with reported spectral data of the literature (Guerriero & Pietra, 1984; Yogo et al., 1990).

Compound **9** (6*R*, 9*S*)−3-oxo-α-ionol-β-*D*-glucopyranoside**):** white powder. ESI-MS *m/z*: 395 $[M+Na]^+$ $C_{19}H_{32}O_7$; ^1H NMR (600 MHz, DMSO–d_6, TMS) δ: 5.81 (s, 1H, H-4), 5.74 (dd, J = 15.2, 9.5 Hz, 1H, H-7), 5.51 (dd, J = 15.3, 6.7 Hz, 1H, H-8), 4.36 (p, J = 6.5 Hz, 1H, H-9), 4.10 (d, J = 7.8 Hz, 1H, H-1′), 3.64 (ddd, J = 11.6, 6.1, 1.8 Hz, 1H, H-6′), 3.41 (dt, J = 11.7, 5.9 Hz, 1H, H-6′), 3.08–2.99 (m, 5H, H-2′,3′,4′,5′), 2.62 (d, J = 9.5 Hz, 1H, H-6), 2.39 (d, J = 16.5 Hz, 1H, H-2a), 1.97 (d, J = 1.2 Hz, 1H, H-2b), 1.88 (d, J = 0.9 Hz, 3H, H-13), 1.19 (d, J = 6.5 Hz, 3H, H-10), 0.95 (s, 3H, H-12), 0.90 (s, 3H, H-11); ^{13}C NMR (151 MHz, DMSO–d_6) δ: 197.35 (C-3), 161.30 (C-5), 134.43 (C-8), 128.26 (C-7), 124.27 (C-4), 99.30 (C-1′), 76.50 (C-5′), 76.35 (C-3′), 72.63 (C-2′), 71.55 (C-9), 69.35 (C-4′), 60.41 (C-6′), 54.06 (C-6), 46.54 (C-2), 35.07 (C-1), 26.69 (C-11), 26.09 (C-12), 22.30 (C-13), 21.38 (C-10). It was identified as (6*R*, 9*S*)−3-oxo-α-ionol-β-D-glucopyranoside by comparing with reported spectral data of the literature (Mpondo, Garcia, Chulia, & Mariotte, 1989; Pabst, 1992).

Compound **10** (blumenol C glucoside): white powder. ESI-MS *m/z*:393 $[M+Na]^+$ $C_{19}H_{30}O_7$; ^1H NMR (600 MHz, DMSO–d_6, TMS) δ: 5.72 (s, 1H, H-4), 4.97 (d, J = 5.1 Hz, 1H, H-1′), 3.41 (dt, J = 11.7, 5.9 Hz, 1H, H-9), 3.13–2.87 (m, 4H, H-2′, 3′, 4′, 5′), 2.38 (d, J = 17.2 Hz, 1H, H-2b), 1.97 (s, 3H, CH_3-13), 1.89–1.84 (m, 2H, H-6, H-7a), 1.74–1.65 (m, 1H, H-2a), 1.59–1.47 (m, 3H, H-7b, H-8a, H-8b), 1.15 (d, J = 6.3 Hz, 3H, CH_3-10), 1.00 (s, 3H, CH_3-12), 0.94 (s, 3H, CH_3-11). ^{13}C NMR (151 MHz, DMSO–d_6) δ: 197.43 (C-3), 165.29 (C-5), 123.54 (C-4), 101.82 (C-1′), 76.16 (C-3′), 76.12 (C-5′), 74.22 (C-9), 72.93 (C-2′), 69.46 (C-4′), 60.50 (C-6′), 49.72 (C-6), 46.33 (C-2), 35.38 (C-8), 35.05 (C-1), 27.85 (C-12), 26.14 (C-11), 24.01 (C-7), 23.52 (C-13), 20.93 (C-10). It was identified as blumenol C

glucoside by comparing with reported spectral data of the literature (Takeda et al., 1997).

Compound **11** (palmitic acid): colorless oil. ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 11.98 (s, 1H), 2.18 (t, J = 7.4 Hz, 2H), 1.51–1.43 (m, 2H), 1.23 (s, 24H), 0.85 (t, J = 7.0 Hz, 3H). It was identified as palmitic acid by comparing with reported spectral data of the literature (Qin, Yu, & Huang, 2005).

Compound **12** (dibutylphthalate): colorless oil. ^{1}H NMR (600 MHz, DMSO–d_6, TMS) δ: 7.75–7.71 (m, 2H, H-2″, H-5″), 7.69–7.66 (m, 2H, H-3″, H-6″), 4.23 (t, J = 6.6 Hz, 4H, H-3, H-3′), 1.64 (dt, J = 14.3, 6.6 Hz, 4H, H-4, H-4′), 1.41–1.34 (m, 4H, H-5, H-5′), 0.91 (t, J = 7.4 Hz, 6H, H-6, H-6′); ^{13}C NMR (151 MHz, CD_3OD) δ: 167.91 (C-1, C-1′), 132.18 (C-3″, C-4″), 130.96 (C-1″, C-6″), 128.47 (C-2″, C-5″), 65.25 (C-3, C-3′), 30.32 (C-4, C-4′), 18.87 (C-5, C-5′), 12.66 (C-6, C-6′). It was identified as dibutylphthalate by comparing with reported spectral data of the literature (Argay et al., 1997).

Compound **13** (auriculatum A) [1-pentyl-4-(2″-methyl-*n*-hexyl)-terephthalate] was obtained as a pale-pink oil. ESI-MS *m/z*: 335.2217 $[M+H]^+$; The ^{1}H NMR together with the ^{13}C NMR spectroscopic data supported a molecular formula of $C_{20}H_{30}O_4$, implying 6° of unsaturation. Its UV spectrum exhibited absorptions at λ_{max} 197.01, 223.22 and 275.38 nm. The ^{1}H NMR spectrum (Table 1) revealed the presences of one dual-substituted aromatic ring and four aromatic protons [δ_H 7.67 (2H, dd, H-2 and H-6) and 7.72 (2H, dd, H-3 and H-5)]. The ^{13}C NMR data combined with an DEPT 135° experiment revealed six aromatic carbon atoms and two carbonyl carbon atoms resonance, and indicated a symmetrical dual-substituted aromatic ring.

Attachment of substituent groups was further determined by HSQC and HMBC spectra. The HMBC correlation from H-2 (δ_H 7.67) and H-6 (7.67) to C-7 (δ C167.46), as well as H-3 (7.72) and H-5 (7.72) to C-8 (167.44) suggested the presence of a terephthalate skeleton in **13**. Combined a series of aliphatic protons showed that the structure of compound **13** was similar with 1-isobutyl-4-(2′-ethyl-*n*-hexyl)-terephthalate (Ma, Zhang, Su, Zeng, & Li, 2004). The HMBC H-1′ (4.22) / C-7 (167.46), C-2′ (30.47) and of H-1″ (4.13) / C-8 (167.44), C-2″ (38.53) proved the correlations of C-7—C-1′, C-8—C-2′, and H-7″ (0.86) / C-2″ (38.53), H-7″ (0.86) / C-8 (167.44) proved the correlation of C-7″-C-2″ (Fig. 2). These assignments combined with other observed HMBC signals and the comparison with literature established the planar structure of **13**. ^{1}H–^{1}H COZY experiment was used for the confirmation of structure. It is speculated that compound **13** is a racemic mixture for there is no obvious CD signals. However, the racemic mixture was not split due to its low content in this study, and the relative and absolute configuration of C-2″ was not determined. This challenge requires further efforts.

Table 1
^{1}H (600 MHz) and ^{13}C (151 MHz) NMR data of compound **13** in DMSO–d_6 (δ, ppm; J, Hz).

No.	δ_C, type	δ_H (J in Hz)	HMBC (H-C)
1	132.17, CH	–	–
2	132.09, CH	7.67 dd (6.6, 3.0)	C-1, 3, 7, 4, 6
3	129.14, CH	7.72 dd (6.6, 2.4)	C-1, 2, 4, 5, 8
4	132.16, CH	–	–
5	129.14, CH	7.72 dd (6.6, 2.4)	C-1, 6, 3, 4, 8
6	132.02, CH	7.67 dd (6.6, 3.0)	C-1, 5, 7, 2, 4
7	167.46, C	–	–
8	167.44, C	–	–
1′	67.85, CH_2	4.22 t (6.6)	C-2′, 7
2′	30.47, CH_2	1.63 m	C-1′, 7
3′	23.70, CH_2	1.29 m	C-2′
4′	19.13, CH_2	1.38 m	C-2′
5′	14.39, CH_3	0.85 m	C-3′, 5′
1″	65.50, CH_2	4.13 m	C-2″, 8, 3″
2″	38.53, CH	1.63 m	C-1″, 3″, 7″
3″	30.25, CH_2	1.25 m	C-2″
4″	28.83, CH_2	1.32 m	C-3″, 5″
5″	22.88, CH_2	1.30 m	C-6″, 4″
6″	14.04, CH_3	0.91 m	C-5″, 4″
7″	11.28, CH_3	0.86 m	C-2″

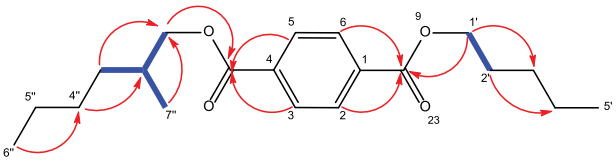

Fig. 2. Key HMBC (arrows) correlations and ^{1}H–^{1}H COZY (bold lines) of compound **13**.

The chemical structure of compound **13** is similar with some conventional plasticizers (Charles, Dennis, Thomas, & William, 1997). Considering the possible contamination of foreign impurities during separation and purification process, a UPLC-UV method was developed to detect the presence of compound **13** in original plant samples in this paper. Chromatographic detection was performed on a YMC-Pack ODS-A column (3 μm, 4.6 mm × 150 mm). A mobile phase system of A (MeOH) and B (water, contains 0.1% formic acid) was applied under the following gradient system: 0–10 min, 70%–30% B; 10–15 min, 30%–5% B; 15–18 min, 5%–5% B; 18–22 min, 70%–70% B. The flow rate was 0.3 mL/min. The temperature of column and sample pan temperature was 35 °C and 10 °C respectively. The injection volume was 2 μL. Results showed that the compound **13** was a nature product from *D. auriculatum* (Fig. 3).

It is reported that dibutylphthalate (compound **12**) is a widely used plasticizer (Kavlock et al., 2002) and have been previously isolated from many plants (Chi, Deng, & Wang, 2010; Sun & He, 2016; Wang et al., 2016), which provide evidence to indicate that compound **12** is also a nature-based product from *D. auriculatum*.

4. Discussion

The genus *Dichocarpum*, which belongs to the subfamily Thalictroideae based on molecular phylogenetic studies (Cossard et al., 2016) and comprises approximately 19 species in the world, is endemic to eastern Asia, and was separated from the genus *Isopyrum* by one of the authors Pei-gen Xiao and his colleague according to the morphological characteristics in 1964 (Hsiao & Wang, 1964). About seven species in this genus have long been traditionally used as folk medicines for the treatment of some common diseases in several provinces of China, such as bruise, indigestion, rheumatism, and so on (Li, 2010). In recent years, scholars used plastid chloroplast and nuclear DNA sequences and found that the placement of *Dichocarpum* as sister to *Isopyrum* and *Enemion* (Cossard et al., 2016; Xiang et al., 2017). However, there is no chemical evidence to support the classification of this genus because there is little phytochemical study on the genus *Dichocarpum* recently.

This current study reported 13 compounds from *D. auriculatum* for the first time, including a new diester terephthalate derivative (**13**), four benzylisoquinoline alkaloids (**1**–**4**), two cyanides (**5**–**6**), two lactones (**7**–**8**), and two megastigmane glycosides (**9**–**10**). All compounds are firstly isolated from the genus *Dichocarpum*. Among them, two protoberberine alkaloids (**1**–**2**) was previously reported in *Isopyrum thalictroides* L (Košťálová, Hrochová, Uhrín, & Tomko, 1987) and *Thalictrum henanense* (Yan & Paul, 1993). Magnoflorine **(4)** as aporphine alkaloid was reported in subfamily *Thalictroideae* (Peng, Chen, Liu, Wang, & Xiao, 2006; Xiao, 1980). Cyanogenic glycoside **6** and corresponding aglycon **5** were previously isolated from the genus *Thalictrum* (Khamidullina, Gromova, Lutsky, & Owen, 2006). Compounds **7** and **8** can be produced after hydrolysis and condensation of compounds **5** and **6** and readily re-

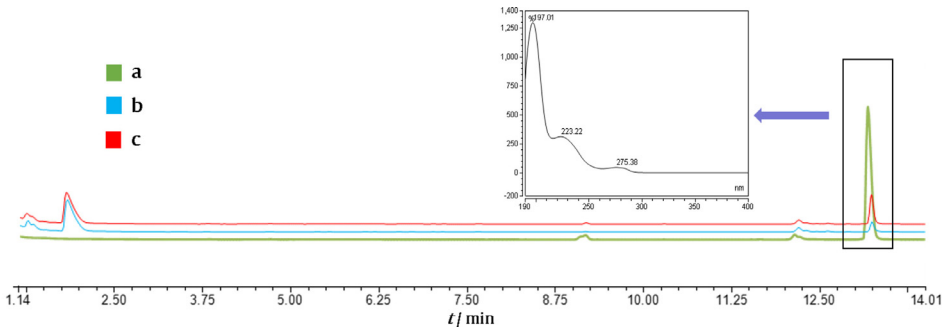

Fig. 3. UPLC-UV chromatogram of compound **13** and *D. auriculatum* (Superimposed chromatogram from bottom to top were compound 13 (a), *D. auriculatum* (b), and *D. auriculatum* + compound 13 (c), respectively. Retention time of three chromatographic peaks shown in black box was consistent and they have the same spectrum shown in small diagram).

lease HCN which is toxic to the organism (Nahrstedt, 1985). It was found that the content of compounds **7** and **8** was much higher than that of compounds **5** and **6** in *D. auriculatum* during our phytochemical isolation produce. Compounds **9** and **10** are monocyclofarnesane sesquiterpenes of megastigmane glycosides of sesquiterpenes, and were firstly reported from subfamily Thalictroideae. The results showed that compounds **9** and **10** in this paper have potential chemotaxonomic significance to the genus *Dichocarpum*, and further work needs to be done on the distribution of these compounds in the genus *Dichocarpum*.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the CAMS Innovation Fund for Medical Sciences (CIFMS) ID: 2016-I2M-1012 and Major Scientific and Technological Special Project for “Significant New Drugs Creation” (No. 2018ZX09711001-008007).

References

Argay, G., Kalman, A., Kapor, A., Ribar, B., Petrovic, S., & Gorunovic, M. (1997). Crystal structure of a mixture of lupeol-acetate tautomers isolated from *Hieracium plumulosum* A. Kerner. *Asteraceae. Journal of Molecular Structure, 435*(435), 169–179.

Charles, A. S., Dennis, R. P., Thomas, F. P., & William, J. A. (1997). The environmental fate of phthalate esters: A literature review. *Chemosphere, 35*(4), 667–749.

Chen, J. H., Du, Z. Z., Shen, Y. M., & Yang, Y. P. (2009). Aporphine alkaloids from *Clematis parviloba* and their antifungal activity. *Archives of Pharmacal Reaearch, 32*(1), 3–5.

Chi, F., Deng, J., & Wang, Y. (2010). Chemical constituents from *Lagotis brevituba*. *China Journal of Chinese Materia Medica, 35*(7), 869.

Cossard, G., Sannier, J., Sauquet, H., Damerval, C., Craene, L. R., Jabbour, F., et al. (2016). Subfamilial and tribal relationships of Ranunculaceae: Evidence from eight molecular markers. *Plant Systematics and Evolution, 302*(4), 419–431.

Fu, D. Z., & Orbélia, R. R. (2001). *Dichocarpum*. In W. T. Wang, P. K. Hsiao, Z. Y. Wu, & H. R. Peter (Eds.), *Flora of China* (vol. 6, pp. 302–305). China: Science Press, Beijing.

Guerriero, A., & Pietra, F. (1984). Butenolide atypical of the Ranunculaceae: Aquilegiolide from *Aquilega atrata* (var. *atroviolacea*). *Phytochemistry, 23*(10), 2394–2396.

Hsiao, P. K., & Wang, W. T. (1964). A new genus of Ranunculaceae—*Dichocarpum*, W. T. Wang et Hsiao. *Acta Phytotax. Sinca, 9*, 315–333.

Jung, H. A., Yoon, N. Y., Bae, H. J., Min, B. S., & Choi, J. S. (2008). Inhibitory activities of the alkaloids from *Coptidis rhizoma* against aldose reductase. *Archives of Pharmacal Research, 31*(11), 1405–1412.

Kavlock, R., Boekelheide, K., Chapin, R., Cunningham, M., Faustman, E., Foster, P., et al. (2002). NTP center for the evaluation of risks to human reproduction: Phthalates expert panel report on the reproductive and developmental toxicity of di-*n*-butyl phthalate. *Reproductive Toxicology, 16*(5), 489–527.

Khamidullina, E. A., Gromova, A. S., Lutsky, V. I., & Owen, N. L. (2006). Natural products from medicinal plants: Non-alkaloidal natural constituents of the *Thalictrum* species. *Natural Product Reports, 23*(1), 117–129.

Košťálová, D., Hrochová, V., Uhrín, D., & Tomko, J. (1987). Isoquinoline alkaloids of *Isopyrum thalictroides* L. *Chemistry, 42*(6), 841–843.

Li, Y. C. (2010). *Studies on taxonomy of* Dichocarpum *in China*. Guiyang University of Chinese Medicine.

Li, Y., Zhang, D. M., & Yu, S. S. (2005). A new stilbene from *Cercis chinensis* Bunge. *Journal of Integrative Plant Biology, 47*(8), 4.

Ma, X. Q., Zhang, G. W., Su, J. Y., Zeng, L. M., & Li, H. (2004). Studies on the secondary metabolites of the soft coral *sarcophyton* sp. *Journal of Instrumental Analysis, 23*(2), 5–8.

Mpondo, M. E., Garcia, J., Chulia, A. J., & Mariotte, A. M. (1989). A new C13 glycoside from *Gentiana pneumonanthe*. *Planta Medica, 55* 406-406.

Nahrstedt, A. (1985). Cyanogenic compounds as protecting agents for organisms. *Plant Systematics and Evolution, 150*, 35–47.

Pabst, A. (1992). Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. *Phytochemistry, 31*(31), 1649–1652.

Peng, Y., Chen, S. B., Liu, Y., Wang, L. W., & Xiao, P. G. (2006). Pharmaphylogenetic study on *Isopyroideae* (Ranunculaceae). *China Journal of Chinese Materia Medica, 31*(14), 1210–1213.

Qin, M. J., Yu, Y. B., & Huang, W. Z. (2005). Studies on chemical constituents of *Pseudostellaria heterophylla* cultivated in jiangsu. *Research and Practice of Chinese Medicines, 23*(6), 15–18.

State Administration of Editorial Board of Chinese Materia Medica (1999). In *Chinese materia medica: 7* (pp. 232–233). Shanghai: Shanghai Science and Technology Press.

Sun, X. L., & He, F. (2016). Study on the chemical constituents in ethyl acetate extract of burdock roots. *China Pharmacist, 19*(11), 2017–2018.

Takeda, Y., Zhang, H., Masuda, T., Honda, G., Otsuka, H., Sezik, E., et al. (1997). Megastigmane glucosides from *Stachys byzantina*. *Phytochemistry, 44*(7), 1335–1337.

Tanahashi, T., Su, Y., Nagakura, N., & Nayeshiro, H. (2000). Quaternary isoquinoline alkaloids from *Stephania cepharantha*. *Chemical & Pharmaceutical Bulletin, 48*(3), 370–373.

Wang, J. Z., Liao, J., Lei, Y., & Chen, X. R. (2013). Study on the alkaloid constituents of *Cyclea racemosa*. *West China Journal of Pharmaceutical Sciences, 28*(1), 006–009.

Wang, X. T., Ran, X. K., Xu, Y. B., Khin, W. A., Xue, F. Q., & Dou, D. Q. (2016). Phytochemical study on *Smallanthus sonchifolius* leaves. *Modern Chinese Medicine, 18*(9), 1111–1113.

Wu, M. (2011). *Study on the classification of chemical fingerprints of* Dichocarpum *in China and the chemical constituents of* D. kaiyangense. Guiyang University of Chinese Medicine.

Xiang, K. L., Zhao, L., Erst, A. S., Yu, S. X., Jabbour, F., & Wang, W. (2017). A molecular phylogeny of *Dichocarpum* (Ranunculaceae): Implications for eastern Asian biogeography. *Molecular Phylogenetics & Evolution, 107*, 594–604.

Xiao, P. K. (1980). Preliminary study of the correlation between phylogeny, chemical constituents and pharmaceutical aspects in the taxa of Chinese Ranunculaceae. *Acta Phytotax. Sinca, 18*(2), 142–153.

Yan, Y. N., & Paul, L. S. (1993). Studies on alkaloids of *Thalictrum honanense* W. T. Wang et S. H. Wang. *China journal of Chinese Materia Medica, 18*(18), 615–639.

Yogo, M., Ishiguro, S., Murata, H., & Furukawa, H. (1990). Coclauril, a nonglucosidic 2-cyclohexen-1-ylideneacetonitrile, from *Cocculus lauriforius* DC. *Chemical & Pharmaceutical Bulletin, 38*(1), 225–226.

Yong, D. M., Min, C. Y., Lee, K. H., Kim, K. R., Sang, U. C., & Kang, R. L. (2006). Protoberberine alkaloids and their reversal activity of P-gp expressed multidrug resistance (MDR) from the rhizome of *Coptis japonica* Makino. *Archives of Pharmacal Research, 29*, 757.