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Original Article

Cathayanalactone G and other constituents from leaves and twigs of *Callicarpa cathayana*

Shuang Gong, Bin Cheng, Tiantian Sun, Xiaoli Li *, Xinxin Liang, Yuan Wang, Xiaochang Dai, Weilie Xiao *

Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, Yunnan Research & Development Center for Natural Products, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China

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ABSTRACT

Objective: To study the chemical constituents from the leaves and twigs of *Callicarpa cathayana*.
Methods: The chemical constituents were isolated and purified by column chromatography on silica gel, MCI gel CHP 20P/P120, Sephadex LH-20, and HPLC. The structures of the compounds were determined by HR-ESI-MS, 1D and 2D NMR data.
Results: A total of 24 compounds were isolated from the 85% methanol extract of leaves and twigs of *C. cathayana*. They were identified as cathayanalactone G (**1**), a new diterpene, and 23 known compounds as patagonic acid (**2**), (-)-16-hydroxycledroda-3,13-dien-16,15-olide-18-oic acid (**3**), 15-methoxypatagonic acid (**4**), oleanolic acid (**5**), ursolic acid (**6**), siaresinolic acid (**7**), pomolic acid (**8**), α-amyrin (**9**), tormentic acid (**10**), lupeol (**11**), 5,7-dihydroxy-3,4′-dimethoxyflavone (**12**), 5,4′-dihydroxy-3,7,3′-dimethoxyflavone (**13**), 5-hydroxy-3,6,7,4′- tetramethoxyflavone (**14**), salvigenin (**15**), kaemferol (**16**), astragalin (**17**), pinoresinol 4-*O*-*β*-*D*-glucopyranoside (**18**), paulownin (**19**), *β*-sitosterol (**20**), *β*-sitosterol *β*-*D*-glucopyranoside (**21**), 5-hydroxy-coumarin (**22**), isocopoletin (**23**), and 4-hydroxycinnamic acid (**24**).
Conclusion: Compound **1** is a new labdane diterpene. Compounds **10**, **13**, **16** and **17** are isolated from the genus *Callicarpa* for the first time. Compounds **7**, **8**, **9**, **12**, **14**, **23** and **24** are reported from *C. cathayana* for the first time.

1. Introduction

The genus *Callicarapa* H. T. Chang contains about 190 species which grows in tropical and subtropical areas in Asia and Oceania. There are 46 species in China, mainly locate in southern China (Chen and Gilbert, 1994b). *C. cathayana* is distributed in the southern area of the Yangtze River (Chen and Gilbert, 1994a). The genus *Callicarpa*, as a medicinal plant, is widely used in China for the treatment of hemorrhage, decreasing swelling, and relieving pain. *C. cathayana*, as an endemic species to China, is also used for the same disease (Tu et al., 2013). Previous phytochemical investigations reported the existence of diverse components in *C. cathayana*, including clerodane diterpenoids (Wang et al., 2019), triterpenoids (Zhou et al., 2005), and flavonoids (Zhou et al., 2005), etc.

One new labdane diterpene, cathayanalactone G (**1**) and 23 known compounds (**2**–**24**) were isolated from the ethyl acetate soluble part of *C. cathayana*. Compounds **10**, **13**, **16**, and **17** were isolated from the genus *Callicarpa* for the first time. Compounds **7**, **8**, **9**, **12**, **14**, **23** and **24** were reported from *C. cathayana* for the first time.

2. Materials and methods

2.1. General

The Bruker AV-400 and AV-III-600 HD spectrometer were used in the NMR experiments (Bruker Corporation, Switzerland). UV spectra were acquired on a Shimadzu UV2401PC spectrophotometer (Shimadzu Corporation, Japan). IR spectra were recorded on a Thermo NICOLET iS10 spectrophotometer with KBr pellets (Thermo Fisher Scientific, USA). Optical rotations were obtained on a JASCO P-1020 polarimeter (Jasco Corporation, Japan). HR-ESI-MS were recorded on an Agilent 1100 HPLC/TOF spectrophotometer (Agilent Technologies, Santa Clara, USA). Semi-preparative HPLC was performed on an Agilent 1260 Liquid Chromatograph System (Agilent Technologies Inc., Waldbronn, Germany) with a Zorbax SB-C_{18} semi-preparative column (9.4 mm × 250 mm, Agilent Technologies Inc., Santa Clara, USA) and Zorbax SB-C_{18} analytical column (4.6 mm × 250 mm, Agilent

* Corresponding authors.
E-mail addresses: lixiaoli@ynu.edu.cn (X.I. Li), xiaoweilie@ynu.edu.cn (W.I. Xiao).

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Technologies Inc., Santa Clara, USA). Silica gel (80–100 mesh, 200–300 mesh, Qingdao Haiyang Chem. Co., Ltd., China), MCI gel CHP 20P/P120 (Mitsubishi Chemical Corporation, Japan), Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) were used for column chromatography. TLC analysis was used for monitoring the fractions (GF_{254} plates, Qingdao Marine Chemical Factory, Qingdao, China). All solvents (Kunming Teng Branch Technology Co., Ltd., China) used for isolation were of analytical grade. 5% H_2SO_4 in anhydrous ethanol was used as chromogenic reagent for TLC analysis, followed by heating.

2.2. Plant materials

In this study, the leaves and twigs of *C. cathayana* were collected in September 2017 in Honghe, Yunnan Province, China. The specimen was identified by Dr Xiao Chen, Kunming Institute of Botany, Chinese Academy of Sciences. A key specimen (YNU 20170045) was deposited at School of Chemical Science and Technology, Yunnan University.

2.3. Extraction and isolation

The dry powder of *C. cathayana* (7 kg) was extracted at room temperature with 85% MeOH (20L × 3 times). The extract was concentrated on a rotary evaporator to obtain the crude extract (450 g). The crude extract was suspended in H_2O and extracted with ethyl acetate (EA). The EA extract (107 g) was subjected to silica gel (80–100 mesh) column using $CHCl_3$-Acetone (50:1–0:1, volume percentage). Each fraction was evaporated to dryness to give six fractions.

Fraction 2 was passed through silica gel (200–300 mesh) column (PE-EA) to give 10 fractions. Fraction 2–1 was separated by silica gel (200–300 mesh) column (PE-Acetone) to give **20** (64.9 mg) and **21** (27.8 mg). Fraction 2–6 was subjected to silica gel (200–300 mesh) column (CH_2Cl_2-MeOH) to give **11** (11.4 mg). Fraction 2–8 was passed through silica gel (200–300 mesh) column (PE-EA) to give two fractions. Fraction 2–8-1 was separated by Sephadex LH-20 (MeOH) and silica gel (200–300 mesh) column (PE-Acetone) to give **5** (43.7 mg), **10** (10.9 mg), and **15** (11.0 mg). Fraction 2–8-2 was fractioned on silica gel (200–300 mesh) column (CH_2Cl_2-MeOH) to give **6** (212.2 mg) and **17** (5.1 mg).

Fraction 3 was passed through MCI column (MeOH-H_2O: 50%–100%) to give nine fractions. Fraction 3–3 was separated by Sephadex LH-20 (MeOH) to give four fractions. Compound **16** (32.2 mg) was isolated from fraction 3–3-4. Fraction 3–3-2 was fractioned on silica gel (200–300 mesh) column (PE-Acetone) to give **12** (8.9 mg) and **14** (20.0 mg), and then by HPLC (Zorbax SB-C_{18}: 9.4 mm × 250 mm, 28%–75% CH_3CN-H_2O) to afford **13** (27.1 mg, t_R 5.3 min) and **18** (12.1 mg, t_R 10.8 min). Fraction 3–6 was passed through silica gel (200–300 mesh) column (PE-EA) to give seven fractions. Fraction 3–6-5 was separated by silica gel (200–300 mesh) column (PE-acetone) to give **7** (11.9 mg) and **8** (6.9 mg), and then by HPLC (Zorbax SB-C_{18}: 9.4 mm × 250 mm, 70%CH_3CN-H_2O) to give **9** (3.3 mg, t_R 9.4 min). Fraction 3–6-6 was fractioned on silica gel (200–300 mesh) column (PE-acetone) to give **1** (2.4 mg) and **2** (5.4 mg). Fraction 3–7 was passed through silica gel (200–300 mesh) column (PE-acetone) to give five fractions. Fraction 3–7-2 was separated by HPLC (Zorbax SB-C_{18}: 9.4 mm × 250 mm, 60% CH_3CN-H_2O) to give **3** (95.0 mg, t_R 6.2 min) and **4** (18.4 mg, t_R 8.2 min). Compound **19** (7.3 mg) was isolated from fraction 3–7-3 by Sephadex LH-20 (MeOH). Fraction 3–8 was fractioned on Sephadex LH- 20 (MeOH) and then by silica gel (200–300 mesh) column (PE-acetone) to give **22** (13.0 mg), **23** (39.9 mg), and **24** (2.8 mg).

3. Results

One new labdane diterpene, cathayanalactone G (**1**) and 23 known compounds (**2**–**24**) were isolated from the ethyl acetate soluble part of *C. cathayana*. The structures of isolated compounds were identified by extensive spectroscopic analysis, including NMR and HR-ESI-MS, and compared with the literature data. The 23 known compounds were identified as patagonic acid (**2**) (Huang & Liu, 2004), (−)-16-hydroxycledroda-3,13-dien-16,15-olide-18-oic acid (**3**) (Gao et al., 2013), 15-methoxypatagonic acid (**4**) (Costa et al., 1999), oleanolic acid (**5**) (Zhu et al., 2020), ursolic acid (**6**) (Kuang et al., 2019), siaresinolic acid (**7**) (Wang & Fang, 2012), pomolic acid (**8**) (D'Abrosca et al., 2006), α-amyrin (**9**) (Liu et al., 2010), tormentic acid (**10**) (Rocha et al., 2007), lupeol (**11**) (Fotie et al., 2006), 5,7-dihydroxy-3,4′-dimethoxyflavone (**12**) (Wei et al., 2013), 5,4′-dihydroxy-3,7,3′-dimethoxyflavone (**13**) (Al-Dabbas et al., 2006), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**) (Hòrie et al., 1998), salvigenin (**15**) (Jassbi et al., 2002), kaemferol (**16**) (Ding et al., 2013), astragalin (**17**) (Jayasinghe et al., 2004), pinoresinol 4-*O*-*β*-*D*-glucopyranoside (**18**) (Jia & Li, 1996), paulownin (**19**) (Angle et al., 2008), *β*-sitosterol (**20**) (Zou et al., 2020), *β*-sitosterol *β*-*D*-glucopyranoside (**21**) (Isaev et al., 2007), 5-hydroxy-coumarin (**22**) (Takaishi et al., 2008), isocopoletin (**23**) (Jerezano et al., 2011), 4-hydroxycinnamic acid (**24**) (Xie et al., 2016) (Fig. 1).

Cathayanalactone G (**1**): white powder; [α]26 D: +7.09 (*c* 0.07, MeOH); UV λ_{max} (log ε): 197 (6.24) nm; IR ν_{max} (cm^{-1}): 3429, 2924, 1750, 1460, 1386, 1076; ^1H and ^{13}C NMR data: see Table 1; HR-ESI-MS (*m*/*z*): 343.2248 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3$, 343.2244). The IR spectrum showed a hydroxyl absorption at 3429 cm^{-1}, a double band absorption at 2924 cm^{-1} and an ester group absorption at 1750 cm^{-1}. The ^{13}C NMR data (Table 1) showed 20 carbon signals, corresponding to the HR-ESI-MS data. The signals from ^1H and ^{13}C NMR of Table 1 showed α, *β*- unsaturated lactone ring (δ_C 175.7, 146.1, 133.5, 70.7, and δ_H 7.27, 4.73). The signals, δ_C 32.6, 29.6, 20.8, 14.2, and δ_H 1.07, 0.88, 0.79, 0.76, showed four methyl groups. Combining with other NMR data, the structure of compound **1** was proposed as labdane diterpene. Comparison of the ^1H and ^{13}CNMR data with known compound 8*β*,19-dihydroxy-*ent*-labd-13-en-16,15-olide, indicated that the signals of basic skeleton and α, *β* -unsaturated lactone ring were similar and compound **1** didn't show the signal of hydroxymethyl but a signal of methyl group (δ_C 32.6). The chemical shift value of C-4 was shifted from δ_C 39.2 in 8*β*,19-dihydroxy-*ent*-labd-13-en-16,15-olide to δ_C 32.8 in **1** (Chen et al., 2007). Apart from the orientation of CH_3-18 and CH_3-19, the absolute configuration of other parts of the compound could be confirmed by the comparison. The deduction was confirmed through the HMBC experiment (Table 1, Fig. 2) showing the correlations of H-19 (δ_H 0.79) with C-3 (δ_C 41.9), C-4 (δ_C 32.8), C-5 (δ_C 56.0) and the ROESY (Table 1, Fig. 3) showing the correlations of H-19 (δ_H 0.79) with H-1α (δ_H 1.63), H-6 α (δ_H 1.38), H-17 (δ_H 1.07), suggesting α-orientation of CH_3-19. Thus, the orientation of CH_3-18 was *β*-orientation. The HMBC experiment (Table 1, Fig. 2) displayed the correlations of H- 17 (δ_H 1.07) with C-7 (δ_C 41.8), C-8 (δ_C 72.3), C-9 (δ_C 58.9) and the ROESY (Table 1, Fig. 3) displayed the correlations H- 17 (δ_H 1.07) with H-6α (δ_H 1.38), H-7α (δ_H 1.66), H-19 (δ_H 0.79), H-20 (δ_H 0.88), suggesting α-orientation of CH_3-17 and *β*-orientation of hydroxyl. Moreover, combining with the HMBC experiment, the ^1H–^1H COSY displayed the correlations of H-1*β* (δ_H 0.80) with H-2α (δ_H 1.54), H-2α (δ_H 1.54) with H-3α (δ_H 1.29), H-1*β* (δ_H 0.80) with H-3α (δ_H 1.29), and the correlations of H-5 (δ_H 0.81) with H-6*β* (δ_H 1.51), H-6α (δ_H 1.38) with H-7α (δ_H 1.66), suggesting the basic structure of the labdane diterpene. The correlations of H-9 (δ_H 0.75) with H-11 (δ_H 1.59), H-11 (δ_H 1.59) with

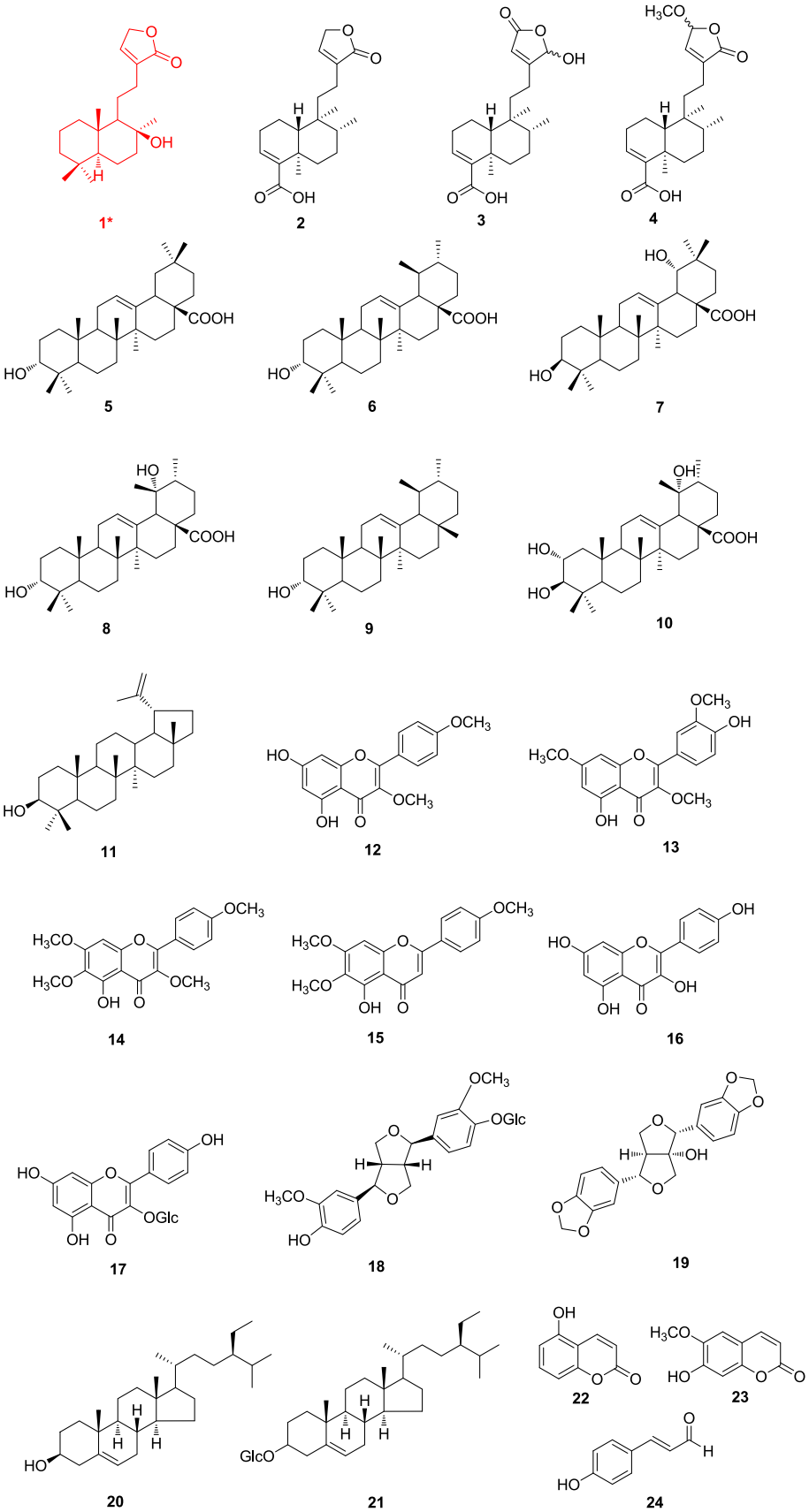

Fig. 1. Structures of compounds isolated (**1**–**24**).

Table 1
NMR data of compound **1** (600/150 MHz, methanol d_4).

No.	δ_C	δ_H (mult, J = Hz)	HMBC	COSY
1	39.3	1.63 (1H, dt, J = 12.42, 4.80 Hz, αH) 0.80 (1H, m, βH)	H-20	H-2β
2	17.9	1.54 (1H, dt, J = 13.98, 3.54 Hz, βH) 1.32 (1H, m, αH)		H-1β, 3β
3	41.9	1.29 (1H, m, αH) 1.10 (1H, dd, J = 13.80, 3.90 Hz, βH)	H-18, 19	H-2α H-2β
4	32.8		H-18, 19	
5	56.0	0.81 (1H, m)	H-18, 19, 20	
6	18.1	1.51 (1H, dd, J = 12.00, 3.69 Hz, βH) 1.38 (1H, m, αH)		H-5
7	41.8	1.66 (1H, dt, J = 13.98, 3.12 Hz, αH) 1.37 (1H, m, βH)	H-17	H-6β
8	72.3		H-17	
9	58.9	0.75 (1H, m)	H-17, 20	
10	38.8			
11	23.1	1.59 (1H, dd, J = 10.53, 5.31 Hz) 1.44 (1H, m)		H-9
12	28.3	2.21, 2.17 (each 1H, m)		H-11α, 11β
13	133.5		H-15	
14	146.2	7.27 (1H, d, J = 1.62 Hz)	H-15	H-15
15	70.7	4.73 (2H, t, J = 1.62 Hz)		
16	175.7			
17	29.6	1.07 (3H, s)		
18	20.8	0.76 (3H, s)		
19	32.6	0.79 (3H, s)		
20	14.2	0.88 (3H, s)		
OH		4.50 (1H, s)		

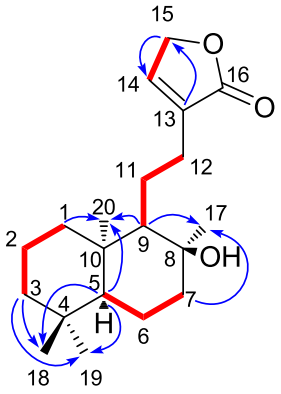

Fig. 2. Key HMBC ( ) and ^{1}H–^{1}H COSY ( ) correlations of compound **1**.

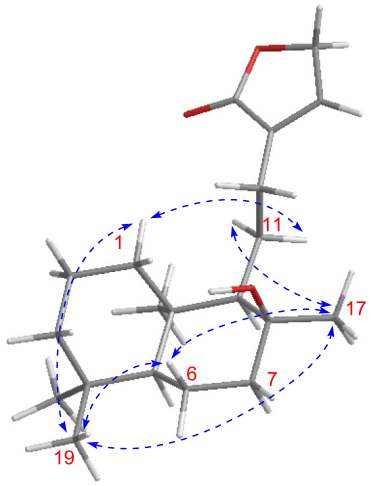

Fig. 3. Selected ROSEY correlations of compound **1**.

H-12 (δ_H 2.17). H-14 (δ_H 7.27) with H-15 (δ_H 4.73) confirmed the rest part of the structure. Therefore, the structure of cathayanalactone G was established as 8β-hydroxy-ent-labd-13-en-16,15-olide as shown in Fig. 1.

4. Discussion

The present study reported the identification of 24 compounds. There are a new labdane diterpenoid cathayanalactone G (**1**) and three known diterpenoids (**2**–**4**), seven triterpenoids (**5**–**11**), six flavonoids (**12**–**17**), two ligans (**18**, **19**), two sterols (**20**, **21**), two cumarins (**22**, **23**), and a benzene derivative (**24**). In this study, four compounds (**10**, **13**, **16** and **17**) were isolated for the first time from the genus *Callicarpa* and eight compounds (**7**, **8**, **9**, **12**, **14**, **23** and **24**) were reported from *C. cathayana* for the first time. According to the former research about the genus *Callicarpa*, the isolated diterpenes shows great anti-inflammatory activity. Further study about the anti-inflammatory activity of *C. cathayana* needs to be done. In summary, the present study enriches the chemical diversity and provided support for further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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