Fitoterapia 113 (2016) 151–157

Contents lists available at ScienceDirect

Fitoterapia

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

Diterpenoids from *Callicarpa kwangtungensis* and their NO inhibitory effects

Jing Xu [a], Shen Li [a], Xiaocong Sun [a], Jun Ma [a], Feng Liu [a], Ling Tong [b], Dongho Lee [c], Yasushi Ohizumi [d], Muhetaer Tuerhong [e,*], Yuanqiang Guo [a,**]

[a] State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300350, People's Republic of China
[b] State Key Laboratory of Core Technology in Innovative Chinese Medicine, Pharmaceutical Analysis Institute, Tasly Academy, Tianjin 300410, People's Republic of China
[c] Department of Biosystems and Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea
[d] Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan
[e] College of Chemistry and Environmental Sciences, Laboratory of Xinjiang Native Medicinal and Edible Plant Resources Chemistry, Kashgar University, Kashgar 844000, People's Republic of China

ARTICLE INFO

Article history:
Received 14 May 2016
Received in revised form 21 July 2016
Accepted 27 July 2016
Available online 1 August 2016

Chemical compounds studied in this article:
7β-Hydroxydehydroabietic acid (PubChem CID: 13370052)
7-Oxodehydroabietic acid (PubChem CID: 620189)
7α-Hydroxydehydroabietic acid (PubChem CID: 13370054)
Angustanoic acid F (PubChem CID: 10591519)

Keywords:
Callicarpa kwangtungensis
Abietane diterpenoids
NO inhibitory effects
Diterpenoids
TDDFT ECD calculations

ABSTRACT

A phytochemical investigation of the leaves of *Callicarpa kwangtungensis* led to the isolation of three new diterpenoids (**1**–**3**), callipenes A–C, and eleven known analogues (**4**–**14**). Their structures were established on the basis of extensive analysis of NMR spectroscopic data, X-ray diffraction data, and experimental and calculated electronic circular dichroism spectra. Compounds **1** and **2** are rare abietane diterpenoids possessing a peroxide bridge. All of the isolates were found to inhibit LPS-induced NO production in BV-2 cells.

1. Introduction

The genus *Callicarpa*, a member of the Verbenaceae family, contains about 190 species distributed mainly in tropical and subtropical Asia and Oceanica [1]. Some *Callicarpa* species, such as *C. macrophylla*, *C. nudiflora*, and *C. dichotoma*, have been used as folk medicines for the treatment of various medical indications since ancient times [2]. Chemical constituents reported from this genus include terpenoids, especially diterpenoids, phenylethanoids, phenylpropanoids, and flavonoids, exhibiting various biological effects, such as anti-inflammatory, hemostatic, antitubercular, antiplatelet aggregation, cytotoxic, and neurite outgrowth-promoting activities [2–18]. The species *C. kwangtungensis* Chun is a shrub distributed mainly in southern mainland China [19] and its leaves or twigs have been used as a folk medicine for the treatment of measles, migraine, and stomachache [2]. Although previous phytochemical investigation on this species were undertaken and some compounds, such as triterpenoids and phenylpropanoid glycosides, have been reported [2,5,8,20], phytochemical and pharmacological studies on *C. kwangtungensis* used as a folk medicine are insufficient. In our continuous survey on the chemical composition of folk medicines and search for natural bioactive substances [21–24], the chemical constituents of the leaves of *C. kwangtungensis*, have been investigated. This procedure led to the isolation of three new diterpenoids, callipenes

* Correspondence to: M. Tuerhong, College of Chemistry and Environmental Sciences, Kashgar University, Kashgar 844000, People's Republic of China.
** Correspondence to: Y. Guo, State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300350, People's Republic of China.
E-mail addresses: 2673134206@qq.com (M. Tuerhong), victgyq@nankai.edu.cn (Y. Guo).

http://dx.doi.org/10.1016/j.fitote.2016.07.012
0367-326X/

A–C (**1**–**3**), together with eleven known analogues (**4**–**14**). The structures of compounds **1**–**14** were elucidated on the basis of extensive 1D and 2D NMR spectroscopic data analysis, and the absolute configurations of new compounds **1**–**3** were established by X-ray diffraction and time-dependent density functional theory (TDDFT) electronic circular dichroism (ECD) calculations. Herein, details of the isolation and structural determination of three new diterpenoids as well as their NO inhibitory effects are described.

2. Experimental

2.1. General

The optical rotations were recorded on an InsMark IP120 automatic polarimeter (Shanghai InsMark Instrument Technology Co., Ltd., Shanghai, People's Republic of China). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany) with KBr disks. ECD spectra were obtained on a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, UK). 1D and 2D NMR spectra were recorded on a Bruker AV 400 instrument (Bruker Group, Fallanden, Switzerland, 400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. ESIMS spectra were acquired on a Thermo Finnigan LCQ-Advantage mass spectrometer (Finnigan Co., Ltd., San Jose, CA). HR-ESIMS were recorded by an IonSpec 7.0 T FTICR MS (IonSpec Co., Ltd., Lake Forest, CA). HPLC purifications were finished on a CXTH system, equipped with a Shodex RI-102 detector (Showa Denko Co., Ltd., Tokyo, Japan). The HPLC column used was a 20 × 250 mm i.d., 5 μm, YMC-pack ODS-AM (YMC Co., Ltd., Kyoto, Japan). X-ray crystallographic analysis was carried out on a Rigaku Saturn 944 CCD diffractometer equipped with a multilayer monochromator and Cu Kα radiation (λ = 1.54187 Å) (Rigaku Co. Ltd., Japan). The structure was solved by direct methods (SHELXL-97), expanded using Fourier techniques, and refined with full-matrix least-squares on F^2 (SHELXL-97). Silica gel (100–200 mesh) was used for column chromatography (Qingdao Haiyang Chemical Group Co., Ltd., Qingdao, People's Republic of China). Chemical reagents for isolation were of analytical grade and purchased from Tianjin Chemical Reagent Co. (Tianjin, People's Republic of China). Biological reagents were from Sigma Chemical Co. The murine microglial BV-2 cell line was from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, People's Republic of China).

2.2. Plant material

The leaves of *C. kwangtungensis* were purchased in July 2014 from Anguo Materia Medica Market, Hebei province, People's Republic of China. The botanical identification was made by one of the authors (Y. G.), and a voucher specimen (No. 20140717) was deposited at the laboratory of the Research Department of Natural Medicine, College of Pharmacy, Nankai University, People's Republic of China.

2.3. Extraction and isolation

The air-dried leaves of *C. kwangtungensis* (9.6 kg) were extracted with MeOH (3 × 81 L) under reflux. The solvent was evaporated to afford a crude extract (2.0 kg). The extract was suspended in H_2O (2 L) and partitioned with petroleum ether (6 × 2 L) and EtOAc (6 × 2 L) successively. The petroleum ether soluble part (366 g) was subjected to a silica gel column chromatography, using a gradient of acetone in petroleum ether (0–30%), to give eight fractions (F_1–F_8) based on TLC analysis. Fraction F_6 was subjected to MPLC over ODS eluting with a step gradient from 64 to 90% MeOH in H_2O to give eighteen subfractions ($F_{6\text{-}1}$–$F_{6\text{-}18}$). Compound **1** (t_R = 32 min, 11.5 mg) was isolated from the above subfraction $F_{6\text{-}4}$ by preparative HPLC (77% MeOH in H_2O), and the purification of subfraction $F_{6\text{-}5}$ with the same HPLC (77% MeOH in H_2O) afforded compounds **3** (t_R = 74 min, 8.7 mg), **12** (t_R = 51 min, 7.9 mg), and **13** (t_R = 46 min, 12.4 mg). Fraction F_4, with the same procedure as for F_6, gave its subfractions $F_{4\text{-}1}$–$F_{4\text{-}16}$. Using the above HPLC system, compound **2** (t_R = 29 min, 12.3 mg) was obtained from $F_{4\text{-}4}$ (84% MeOH in H_2O), and compound **10** (t_R = 63 min, 11.6 mg) was isolated from subfraction $F_{4\text{-}8}$ (84% MeOH in H_2O). Fraction F_3 was fractionated by the above MPLC to yield eighteen subfractions $F_{3\text{-}1}$–$F_{3\text{-}18}$. Compounds **4** (t_R = 52 min, 11.2 mg), **5** (t_R = 50 min, 12.1 mg), and **8** (t_R = 54 min, 18.0 mg) were obtained from $F_{3\text{-}12}$ (90% MeOH in H_2O). Using the same MPLC system as for the above fractions, F_7 and F_5 provided the subfractions $F_{7\text{-}1}$–$F_{7\text{-}23}$ and $F_{5\text{-}1}$–$F_{5\text{-}14}$. The subsequent purification of $F_{7\text{-}5}$ by the same HPLC system (84% MeOH in H_2O) resulted in the isolation of compounds **6** (t_R = 41 min, 13.2 mg), **7** (t_R = 36 min, 12.0 mg), and **11** (t_R = 45 min, 26.5 mg). Compounds **14** (t_R = 59 min, 13.0 mg) and **9** (t_R = 54 min, 17.2 mg) were obtained from subfraction $F_{7\text{-}3}$ (75% MeOH in H_2O) and $F_{5\text{-}6}$ (81% MeOH in H_2O), respectively, with the above HPLC system.

2.3.1. Callipene A (**1**)

Colorless crystals (MeOH); mp 179–181 °C; $[\alpha]_D^{22}$: −93 (c = 0.2, CH_2Cl_2); ECD (CH_3CN) 199 ($\Delta\varepsilon$ − 7.7), 221 ($\Delta\varepsilon$ + 1.9) nm; IR (KBr) ν_{max} cm^{-1}: 3458, 2940, 2875, 1716, 1695, 1625, 1448, 1387; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Table 1. ESIMS m/z 368 $[M + NH_4]^+$; HRESIMS m/z 368.2437 $[M + NH_4]^+$, calcd. for $C_{20}H_{34}NO_5$ 368.2437.

X-ray crystal data of callipene A (**1**): $C_{20}H_{30}O_5$, M_r = 350.44, orthorhombic, space group $P2(1)2(1)2(1)$, a = 10.0970 (2) Å, b = 13.2111 (3) Å, c = 13.5268 (3) Å, α = 90°, β = 90°, γ = 90°, V = 1804.37 (7) Å^3, T = 173(2) K, Z = 4, μ(Cu Kα) = 0.740 mm^{-1}, Dcalc = 1.290 g/cm^3, F(000) = 760, crystal dimensions 0.24 × 0.22 × 0.20 mm were used for measurements. The total number of reflections measured was 14,588, of which 3937 were unique (R(int) = 0.0294). Final R_1 = 0.0350, wR_2 = 0.0908 ($I > 2\sigma(I)$), Flack parameter = 0.01 (6). Crystallographic data of this compound have been deposited in the Cambridge Crystallographic Data Centre (CCDC 1494372).

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1**–**3** (in $CDCl_3$, δ in ppm, J in Hz).[a]

Position	**1**		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1α	1.65 m	31.4	1.65 m	33.4	1.71 m	34.0
1β	1.46 m		1.56 m		1.40 m	
2α	1.60 m	17.5	1.59 m	17.5	1.16 m	18.7
2β	1.66 m		1.67 m		1.51 m	
3α	1.69 m	37.2	1.70 m	37.0	1.83 m	36.1
3β	1.58 m		1.61 m		1.58 m	
4		46.4		46.9		46.7
5	2.25[b]	40.0	2.27[b]	38.0	2.40 dd (13.1, 3.0)	37.5
6α	1.91[b]	25.2	1.54 m	27.6	2.28 m	27.4
6β	2.09[b]		1.42 m		1.85 m	
7	5.85 br s	125.9	β 4.27 t (4.9)	74.0	β 3.53 br s	73.0
8		137.1		144.0		74.8
9		82.8		81.7		66.0
10		38.0		38.6		36.6
11a	2.13 m	26.4	2.28 m	23.8	α 1.51 m	19.2
11b	1.92 m		1.65 m		β 1.82 m	
12a	2.04 m	21.2	2.05 m	27.1	α 1.47 m	19.4
12b	1.20 m		1.42 td (4.1, 12.0)		β 1.74 m	
13		82.6		79.8		77.2
14	α 3.98 s	68.9	6.54 d (1.6)	130.6	α 1.55 m	17.8
					β 1.62 m	
15	2.24[b]	29.0	1.93 quint (6.9)	32.2	1.92 quint (7.0)	30.9
16	0.86 d (6.8)	15.5	1.01 dd (1.8, 6.9)	17.1	1.01 dd (2.0, 7.0)	17.4
17	0.88 d (6.8)	17.5	1.00 dd (1.8, 6.9)	17.4	0.99 dd (2.0, 7.0)	16.5
18		184.2		183.6		183.1
19	1.30 s	17.4	1.32 s	17.6	1.17 s	16.1
20	1.00 s	14.3	1.08 s	18.0	1.10 s	17.4
OCH_3			3.33 s	56.0	3.13 s	49.0

[a] The assignments are based on DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY, and NOESY experiments.
[b] Signals were in overlapped regions of the spectra and the multiplicities could not be discerned.