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鹅不食草中具有抗菌活性的三萜类成分*

梁恒兴[1,2], 宝福凯[3], 董晓萍[2], 吕 青[1], 程永现[1]**
(1 中国科学院昆明植物研究所植物化学与西部植物资源持续利用国家重点实验室, 云南 昆明 650204;
2 成都中医药大学药学院, 四川 成都 610075; 3 昆明医学院微生物与免疫学系, 云南 昆明 650031)

摘要: 从鹅不食草 (*Centipeda minima*) 全草的乙醇提取物中分离得到 3 个乌苏烷型三萜, 其中一个新化合物用波谱学方法鉴定为 ursane-20 (30)-en-3β, 16β, 21α-triol (**1**), 二个已知化合物的结构分别为 taraxasterol acetate (**2**), taraxasterol (**3**)。抗菌试验表明化合物 **2** 和 **3** 具有较强的抗菌活性。

关键词: 鹅不食草; 菊科; 三萜; 抗菌活性
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Antibacterial Triterpenes from Centipeda minima (Compositae)

LIANG Heng-Xing[1,2], BAO Fu-Kai[3], DONG Xiao-Ping[2],
LU Qing[1], CHENG Yong-Xian[1]*
(1 *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650204, China; 2 *College of Pharmacy, Chengdu University of Traditional Chinese Medicine*, Chengdu 610075, China;
3 *Department of Microbiology and Immunology, Kunming Medical College*, Kunming 650031, China)

Abstract: A new triterpene, ursane-20 (30)-en-3β, 16β, 21α-triol (**1**), together with two known compounds, taraxasterol acetate (**2**) and taraxasterol (**3**), was isolated from the whole plants of *Centipeda minima*. Their structures were identified by spectroscopic analysis. Antibacterial properties of compounds **2** and **3** were evaluated against eight disease-associated microorganisms by the agar dilution method, Both of them displayed potential antibacterial activities.

Key words: *Centipeda minima*; Compositae; Triterpenes; Antibacterial activity

Centipeda minima (L.) is a Compositae plant distributing over south Asia and Oceania (Shi and Fu, 1983). It has been using as an important medicinal herb for the treatment of cold, nasal allergy, diarrhea, malaria, and asthma in China (Jiangsu New Medical College, 1992). Previous studies revealed that flavonoids, sesquiterpenes are the main bioactive constituents (Wu *et al*, 1985, 1991; Iwakami *et al*, 1992; Taylor and Towers, 1998). During our search for new antimicrobial agents from traditional Chinese medicine, *C. minima* was investigated. From the ethanol extracts of the whole plants, three usane-type triterpenes, including a new naturally occurring compound, were purified and structurally characterized. In this paper, we describe the isolation and structural identification of the new compound, and antibacterial properties of compounds **2** and **3**.

Compound **1** was obtained as a colorless crystal. The HREIMS at m/z 481.3665 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3Na$ 481.3657) established the molecular formula of **1** as $C_{30}H_{50}O_3$. Besides a double bond, the ^{13}C NMR and DEPT spectra of **1** (Table 1) is characteristic of an ursane-type triterpene. The molecular of **1** is 32 Da larger than that of **3**, indicating the presence of two

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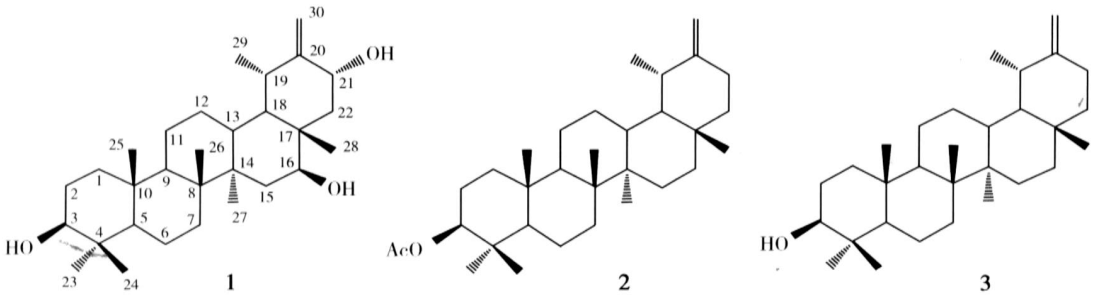
** Author for correspondence; E-mail: yxcheng@mail.kib.ac.cn; Tel/Fax. +86-871-5223048
Received date: 2007-02-02, Accepted date: 2007-04-16
作者简介: 梁恒兴 (1978-) 男, 博士, 主要从事中药化学研究。

more hydroxyl groups in **1**, which are attributed to C-16 at δ 76.1 and C-21 at δ 70.4, respectively, by the following HMBC interactions: H-15 (δ 1.97) with C-14 (δ 42.6), C-16 and C-27 (δ 16.6), H-16 (δ 3.83) with C-22 (δ 47.1) and C-28 (δ 13.0), H-28 (δ 1.19) with C-17 (δ 40.5), C-22 and C-16, H-22 (δ 3.08, 2.01) with C-16, C-17, C-18 (δ 47.8), C-28, C-20 (δ 157.7) and C-21 (δ 70.4), H-21 (δ 4.82) with C-22, C-17, C-19 (δ 38.7), C-20 and C-30 (δ 111.7). The stereochemistry of C_{16}-OH and C_{21}-OH was determined to be β, α, respectively, on the basis of the following NOESY interactions: H-28 with H-21 (δ 4.82) and H-22β (δ 3.08), H-16 with H-22α (δ 2.01) and H-29 (δ 1.55). The β configuration of C_3-OH was determined by comparison of the chemical shift of C-3 (δ 78.1) with those reported data, and further supported by the observation of HMBC correlation of H-3 (δ 3.47) with H-24 (δ 0.89). Therefore, the structure of **1** was assigned as 20 (30)-taraxasten-3β, 16β, 21α-triol. It was noted that the ester forms of **1** have been previously isolated from *Arnica lonchophylla* (Schmidt *et al*, 2004). During the structural elucidation of 20 (30)-taraxastene-3β, 16β, 21α-triol 3-laurate, myristate, -palmitate, and -stearate, Schmidt and coworker speculated these esters having a same unit by MS fragment at *m/z* 458, and thus named this unit as arnitriol A (Schmidt *et al*, 2004). However, **1**, as a natural product, was not previously isolated.

Two known triterpenes were identified as taraxasterol acetate (**2**) (Reynolds *et al*, 1986), and taraxasterol (**3**) (Reynolds *et al*, 1986), respectively, by comparison of their spectroscopic data with literature values. They were isolated from *C. minima* for the first time.

Bioassay revealed that compounds **2** and **3** exhibited antimicrobial effects against some bacteria investigated (Table 2). Compound **2** was found to be most effective against *Salmonella typhimurium* and *S. paratyphi*-A with the MIC of 6.25 μg/mL comparable to that of cefradine and gentamycin with the MIC of 7.5 μg/mL and 3.25 μg/mL, respectively. Compound **3** could inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *S. typhimurium* with the MIC value of 50 μg/mL. However, the other microorganisms were not sensitive to these two compounds even at the concentration of 100 μg/mL.



1 2 3

Experimental

General Experimental Procedures Melting points was obtained on an XRC-1 micromelting apparatus. Optical rotations was determined on a JASCO-20C digital polarimeter. UV spectra was recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra was obtained with a Bruker Tensor 27 FT-IR spectrophotometer with KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AM-400 spectrometer with TMS as an internal reference. 2D NMR spectra were measured with a DRX-500 spectrometer. EIMS (70 eV) were recorded on a VG Auto Spec-3000 spectrometer. ESIMS and HRESIMS were carried our with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh and 10-40 μm) for column chromatography and GF254 for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Sephadex LH-20 was obtained from Amersham Pharmacia Biotech, Sweden. RP-18 silica gel (40-63 μm) used for open column chromatography was purchased from Daiso Co., Japan. Diaion HP20 and MCI gel CHP 20P (75-150 μm) were obtained from Mitsubishikasei, Tokyo, Japan. Fractions were monitored by TLC and spots were visualized after spraying with 10% H_2SO_4 in ethanol or anisaldehyde reagent followed by heating.

Plant Material The whole plants of *C. minima* were purchased from Yunnan Corporation of Materia Medica, Yunnan province, People's Republic of China, and identified by Mr. H. Y. Sun at Yunnan Corporation of Materia Medica. A voucher specimen (CHYX0159) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Table 1　^{1}H and ^{13}C NMR data for compound **1** in C_5D_5N (δ in ppm, J in Hz) [a]

No.	^{1}H	^{13}C	No.	^{1}H	^{13}C
1	0.97 (m)	39.5	16	3.83 (dd, 11.4, 4.0)	76.1
2	1.88 (m)	28.3	17		40.5
3	3.47 (br t, 8.0)	78.1	18	1.59 (m) [b]	47.8
4	0.80 (m)	39.2	19	2.41 (m)	38.7
5		55.9	20		157.7
6[a]	1.56 (m) [b]	18.7	21	4.82 (dd, 8.8, 4.4)	70.4
6[b]	1.40 (m) [c]		22[a]	3.08 (dd, 13.6, 8.8)	47.1
7	1.43 (m) [c]	34.5	22[b]	2.01 (dd, 13.6, 4.4)	
8		41.3	23	1.22 (s)	28.6
9	1.37 (m)	50.3	24	0.89 (s)	16.6
10		36.9	25	1.05 (s)	16.1
11	1.58 (m) [b]	21.7	26	1.05 (s)	16.4
12	1.77 (m)	26.6	27	0.89 (s)	16.6
13	1.72 (m)	39.1	28	1.19 (s)	13.0
14		42.6	29	1.55 (d, 6.8) [b]	28.1
15	1.97 (m)	37.3	30	7.56 (br s), 7.19 (br s)	111.7

a: The spectra were obtained at 400 MHz for ^{1}H and 100 MHz for ^{13}C. b, c: Signals with the same superscripts are overlapped.

Table 2　Antibacterial activities of compounds **2** and **3** (MIC values, μg/mL)

Pathogen	**2**	**3**	cefradine	gentamycin
Reference strains				
S. aureus CMCC26001	> 100	50	15	7.5
E. coli CMCC44103	> 100	50	7.5	7.5
S. typhimurium CMCC80087	6.25	50	7.5	7.5
S. flexneri CMCC51335	> 100	> 100	3.25	3.25
Clinically isolated strains				
S. epidermidis	> 100	> 100	3.25	7.5
B. subtilis	> 100	> 100	3.25	3.25
S. paratyphi-A	6.25	> 100	3.25	3.25
S. paratyphi-B	> 100	> 100	3.25	3.25

Extraction and Isolation　Dried and powdered whole plant materials of *C. minima* (10 kg) were extracted with 95% EtOH under reflux for three times, the extracts were evaporated and suspended into water followed by successive partition with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc extracts (170 g) were subjected to column chromatography (CC) over silica gel (200-300 mesh) and eluted with $CHCl_2$-MeOH (8∶1) to give fractions 1-4. Fraction 2 (30 g) was chromatographed on Diaion HP20 (95% EtOH) to decolor, the eluents were then subjected to CC on silica gel (200-300 mesh), eluting with $CHCl_3$-MeOH (1∶0-5∶1) to give fractions 2.1-2.8. Fraction 2.2 (10 g) was passed through MCI gel CHP 20P (MeOH-H_2O, 9∶1) to decolor, and then subjected to Sephadex LH-20 (MeOH), RP-18 (MeOH-H_2O 1∶1→1∶0) and repeated vacuum liquid chromatography (VLC) to yield **1** (11 mg). The petroleum ether extracts (197 g) were subjected to CC over silica gel (200-300 mesh) and eluted with petroleum ether-EtOAc (3∶1) to give fractions A-E. Fraction A (35 g) was subjected to repeated CC on silica gel, eluting with petroleum ether-EtOAc (1∶0-0∶1) to afford a colorless crystal **2** (570 mg). Fraction C (26 g) was repeatedly chromatographed on silica gel (200-300 mesh) with petroleum ether-Me_2CO (50∶1-10∶1) as eluent to afford six fractions C1-C6. Fraction C2 (4 g) was passed through MCI gel CHP 20P (MeOH-H_2O, 9∶1) to decolor, followed by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 6∶4) and repeated VLC to yield **3** (36 mg).

Compound 1: colorless crystal; mp: 239-240 ℃; $[\alpha]_D^{21}$ +65.0 (*c* 0.1, $CHCl_3$/MeOH 2∶1); UV $\lambda_{max}^{CHCl_3}$ (log ε) nm: 248 (2.51); IR ν_{max}^{KBr} cm^{-1}: 1639, 1444, 1388; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS *m/z* 458 $[M]^+$ (4), 440 (4), 422 (3), 299 (54), 207 (55), 189 (100), 175 (30), 161 (32), 149 (42), 135 (88), 121 (98), 107 (98), 95 (93), 81 (78), 69 (76), 55 (81); HRESIMS *m/z* 481.3665 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3Na$ 481.3657).

Bioassay　Antibacterial activity was tested by agar dilution method (Baker *et al*, 1994). The bacterial strains employed were *Staphylococcus aureus* CMCC26001 (CMCC, National Center for Medical Culture Collections, Beijing, China), *Escherichia coli* CMCC44103, *Salmonella typhimurium* CM-

CC80087, and *Shigella flexneri* CMCC51335, and the following clinically isolated strains: *Staphylococcus epidermidis*, *Bacillus subtilis*, *Salmonella paratyphi*-A, *Salmonella paratyphi*-B. The bacterial strains were removed from storage kept in -70 ℃ refrigerator and streaked onto tryptone soy agar (TSA, Oxoid) plates, and then incubated for 18 to 24 hour at 37 ℃. The inoculum was prepared by culturing each isolated bacterial colony in brain heart infusion broth (Oxoid) at 37 ℃ to a turbidity equivalent to McFarland 0.5 standard (1.0×10^8 CFU/mL) and subsequently diluting the organism to 1.0×10^6 CFU/mL for susceptibility testing. For agar dilution tests, compounds (2 mg each) were first dissolved in 0.2 mL DMSO, serial dilutions of test compounds (ten serial two-fold dilutions per compound) were prepared as described by CLSI (Clinical and Laboratory Standards Institute, Wayne, PA, USA) (formerly NCCLS, National Committee for Clinical Laboratory Standards) (Clinical and Laboratory Standards Institute, 2006). Nine mililiters of molten (48 ℃) Mueller-Hinton agar was added to each milliliter of diluted compound, mixed completely, and put into plates. With a Steers replicator, an organism density of 10^4 CFU/spot was inoculated onto the appropriate plate with various concentrations of test compounds (range of final concentrations: 0.195 - 100 μg/mL). The plates were incubated overnight in ambient air at 37 ℃ for 24 hours. The minimum inhibition concentration was taken as the lowest concentration that inhibited visible growth after incubation at 37 ℃ for 24 h. Cefradine and gentamycin were used as reference standards in order to control the sensitivity of the test strains. Plates containing only MHA and MHA and 1 % DMSO in medium served as negative and solvent controls. Tests were performed in triplicate and repeated once.

References:

Baker CN, Banerjee SN, Tenover FC, 1994. Evolution of Alamar colorimetric MIC method for antimicrobial susceptibility testing of Gram-negative bacteria [J]. *J Clinic Microbiol*, **32**: 1261—1267

Clinical and Laboratory Standards Institut, 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically [M]. Approved Standards, 7th Ed. (M7-A7). Wayne: Clinical and Laboratory Standards Institute

Iwakami S, Wu JB, Ebizuka Y *et al*. 1992. Platelet activating factor (PAF) antagonists contained in medicinal plants: Lignans and sesquiterpenes [J]. *Chem Pharm Bull*, **40**: 1196—1198

Jiangsu New Medical College, 1992. Zhongyao Dacidian (Dictionary of Traditional Chinese Medicine) [M]. Shanghai Science and Technology Publishing House: Shanghai, People's Republic of China, 2400

Reynolds WF, McLean S, Poplawski J, 1986. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivatives by 2D NMR: An investigation of potential utility of ^{1}H chemical shifts in structural investigations of complex natural products [J]. *Tetrahedron*, **42**: 3419—3428

Schmidt TJ, Raison J, Willuhn G, 2004. New triterpene esters from flowerheads of *Arnica lonchophylla* [J]. *Planta Med*, **70**: 967—977

Shi Z, Fu GX, 1983. Flora of China [M]. Beijing: Science Press, 132—133

Taylor RSL, Towers GHN, 1998. Antibacterial constituents of the Nepalese medicinal herb, *Centipeda minima* [J]. *Phytochemistry*, **47**: 631—634

Wu JB, Chun TT, Ebizuka Y *et al*. 1985. Biologically active constituents of *Centipeda minima*: Isolation of a new plenolin ester and the anti-allergy activity of sesquiterpene lactones [J]. *Chem Pharm Bull*, **33**: 4091—4094

Wu JB, Chun TT, Ebizuka Y *et al*. 1991. Biologically active constituents of Centipeda minima: Sesquiterpenes of potential anti-allergy activity [J]. *Chem Pharm Bull*, **39**: 3272—3275