

Communication

A Novel Triterpene from *Centella asiatica*

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Abstract: A novel triterpene, 2 α ,3 β ,20,23-tetrahydroxyurs-28-oic acid (**1**), was isolated from the aerial part of *Centella asiatica*. Its structure was elucidated by spectroscopic methods, including 2D-NMR spectra. It displayed no activity against Hela and A549 cell lines at concentrations of 10 and 30 μ g/mL, respectively.

Keywords: *Centella asiatica*; triterpene; 2 α ,3 β ,20,23-tetrahydroxyurs-28-oic acid.

Introduction

The perennial subshrub *Centella asiatica* (L.) Urban (Umbelliferae/Apiaceae family, commonly known as Gutu kola, Asiatic pennywort, Indian pennywort or Spadeleaf) has been widely cultivated as a vegetable or spice in China, Southeast Asia, India, Sri Lanka, Africa and Oceania. It has been used in Southeast Asia for the treatment of a wide variety of afflictions such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration and diarrhea [1]. It was also used

in Europe for treatment of wounds and ulcers. Earlier work on this plant has led to the isolation of more than 70 constituents, such as triterpenoid saponins [2-4], polyacetylenes [5], flavones [6], sterols and lipids [7]. A systematic study of the chemical constituents and antitumor activities of *C. asiatica* led us to isolate a new urs-type triterpene compound **1**, together with ten known compounds, namely asiatic acid, madecassic acid, indocentoic acid, bayogenin, kaempferol, quercetin, euscaphic acid, terminolic acid, 3 β -6 β -23-tri-hydroxyolean-12-en-28-oic acid, and 3 β -6 β -23-trihydroxyurs-12-en-28-oic acid. This paper deals with the structural elucidation of the new triterpene **1**.

Results and Discussion

Compound **1** was a white powder, $[\alpha]_D^{25} +26.6$ (c 0.1, MeOH). Its HRFABMS showed a $[M-H_2O]^+$ peak at m/z 489.7028, corresponding to the molecular formula $C_{30}H_{50}O_6$ (calcd. 489.7033). Its IR spectrum showed absorption bands at 3433 and 1722 cm^{-1} , ascribable to hydroxyl and carboxyl functions, respectively. ^{13}C - and DEPT 135 $^\circ$ NMR spectra showed six signals for Me carbons, ten methylenes, seven methines, and six quaternary carbons, together with a carboxyl group. A total of 30 carbon resonances were observed, which confirmed its triterpenic nature. The following NMR data suggested the structural features of urs-28-oic acid for compound **1**: a methyl doublet (δ 1.03, d, $J = 6.9$ Hz, Me-29), and the carbonyl carbon resonance at δ 180.1 (C-28). The spectrum also showed signals at δ 3.68 and 3.34 ($J = 9.4$ Hz) ascribable to the 2 β - and 3 α -protons on carbons bearing a hydroxyl function, respectively. An AB doublet, δ 3.50 ($J = 11.0$ Hz) and 3.26 ($J = 11.0$ Hz), indicated the presence of a $-CH_2OH$ function. The chemical shifts of C-4 and Me-24 led to placement of the $-CH_2OH$ at the C-23 position. The A and B ring proton and carbon signals matched those reported for asiatic acid (2 α ,3 β ,23-trihydroxyurs-12-en-28-oic acid) [8], but the 1H -NMR spectrum of **1**, compared with that of asiatic acid, lacked a methyl doublet (Me-30) and contained a signal corresponding to a methyl singlet at δ 1.32 in the 1H -NMR, as well as a quaternary hydroxylated carbon (δ 86.2) in the ^{13}C -NMR spectrum. The carbon signals of the E ring were in agreement with those reported for 3 β -*O*-(β -D-xylopyranosyl-(1-3)- α -L-arabinopyranosyl)-2 α ,20 β ,23-trihydroxyurs-12-en-28-*O*-[β -D-glucopyranosyl-(1-6)- β -D-glucopyranosyl] ester [9]. The 1H - and ^{13}C -NMR spectra were completely assigned by detailed 2D-NMR experiments (Table 1), which showed the HMBC correlations between H-30 and C-19, C-20, C-21, H-29 and C-18, C-19, C-20, H-3 and C-2, C-4, C-23, C-24. NOESY correlation of H-2 and H-25, H-3 and H-23 further corroborated the above conclusions. In summary, compound **1** was identified as 2 α ,3 β ,20,23-tetrahydroxyurs-28-oic acid (Figure 1).

Figure 1. Structure of Compound **1**.

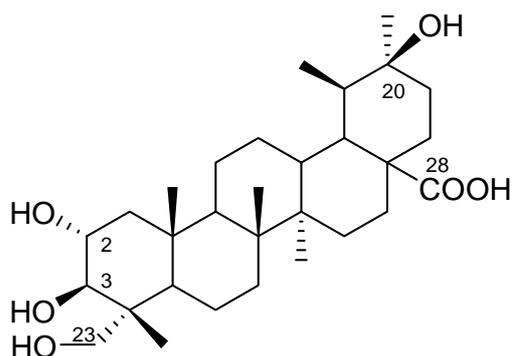


Table 1. ^1H -NMR (300 MHz), and ^{13}C -NMR (75 MHz) data of **1** (CD_3OD , TMS, δ ppm).

Carbon No.	δ_{H}	δ_{C}
1		48.3 (t)
2	3.68 (1H, m)	70.0 (d)
3	3.34 (1H, d, $J = 9.4$ Hz)	78.2 (d)
4		44.2 (s)
5		48.5 (d)
6		19.1 (t)
7		34.6 (t)
8		41.7 (s)
9		51.9 (d)
10		39.3 (s)
11		22.4 (t)
12		28.6 (t)
13		44.5 (d)
14		42.4 (s)
15		28.1 (t)
16		33.2 (t)
17		49.7 (s)
18		49.5 (d)
19		43.5 (d)
20		86.2 (s)
21		28.5 (t)
22		26.4 (t)
23	3.50 (1H, d, $J = 11.0$ Hz) 3.26 (1H, d, $J = 11.0$ Hz)	66.3 (t)
24	0.67 (3H, s)	13.8 (q)
25	0.95 (3H, s)	18.5 (q)
26	0.96 (3H, s)	16.3 (q)
27	0.98 (3H, s)	14.7 (q)
28		180.1 (s)
29	1.03 (3H, d, $J = 6.9$ Hz)	19.0 (q)
30	1.32 (3H, s)	24.4 (q)

Biological Activity

The EtOH extract of *C. asiatica* and the individual compounds were screened for anti-cancer activity against Hela and A549 cell lines. The MTT method was used to determine cytotoxic activity. No activity was observed at concentrations of 10 and 30 $\mu\text{g}/\text{mL}$, respectively.

Experimental

General

NMR spectra were run on a Bruker AVANCE 300 instrument using TMS as internal standard. MS data was obtained on a JEOL JMS D-300 instrument. Column chromatography was performed on silica-gel (Qingdao Haiyang Chemical Co., Ltd), and Toyopearl HW-40 (Tosoh). The HPLC instrument was a JASCO Gulliver Series equipped with a PU-1580 (pump), RI-1530 and UV-1575 (detectors). Semi-Preparative HPLC was performed using a YMC-Pack ODS-A, SH-343-5 column. IR spectra were recorded on a Nicolet 380 FT-IR spectrophotometer (Thermo Electron Corporation). Optical rotation was measured with a MC 241 digital polarimeter (Perkin-Elmer).

Plant material and product isolation

Aerial parts of *C. asiatica* were collected in September 2003, in Hebei province, P.R. China. A voucher specimen, identified by Dr. Wen-Yuan Gao, was deposited under registration No. TJU-03928 at the herbarium of the Department of Natural Products and Traditional Chinese Medicine, Tianjin University. The plant material (3 kg) was refluxed three times with 95% EtOH. The extract was concentrated under reduced pressure to give a residue (700 g) which was partitioned between ethyl acetate and H₂O. The EtOAc extract (160 g) was chromatographed on a silica gel column with an eluent of increasing polarity and eluates of similar composition, according to TLC analysis, were pooled to yield 19 fractions. Fraction 16 (7.7 g, $R_f = 0.5$, eluted with 9:1 CHCl₃-MeOH) was chromatographed on Toyopearl HW-40, and then further purified by reverse phase HPLC (8:2 MeOH-H₂O) and GPC (MeOH) to give compound **1** (6 mg).

References

1. Jiang Su New Medical College; *Dictionary of Chinese Materia Medica*; ShangHai Scientific and Technical Publishing House: Shanghai, P.R. China, **1977**; p. 1874.
2. Jiang, Z. Y.; Zhang, X. M.; Zhou, J.; Chen, J. J. New triterpenoid glycosides from *Centella asiatica*. *Helv. Chim. Acta* **2005**, *88*, 297-303.
3. Matsuda, H.; Morikawa, T.; Ueda, H.; Yoshikawa, M. Masayuki. Medicinal foodstuffs. XXVI. Inhibitors of aldose reductase and new triterpene and its oligoglycoside, centellasapogenol A and centellasaponin A, from *Centella asiatica* (Gotu Kola). *Heterocycles* **2001**, *55*, 1499-1504.
4. Kuroda, M.; Mimaki, Y.; Harada, H.; Sakagami, H.; Sashida, Y. Five new triterpene glycosides from *Centella asiatica*. *Nat. Med.* **2001**, *55*, 134-138.
5. Schulte, K.E.; Ruecker, G.; Abdul Bary, E. Constituents of medical plants. XXVII. Polyacetylenes from *Hydrocotyle asiatica*. *Arch. Pharm.* **1973**, *306*, 197-209.
6. Prum, N.; Illel, B.; Raynaud, J. Flavonoid glycosides from *Centella asiatica* L. (Umbelliferae). *Pharmazie* **1983**, *38*, 423.
7. Kapoor, R.; Ali, M.; Mir, S. R. Phytochemical investigation of *Centella asiatica* aerial parts. *Oriental J. Chem.* **2003**, *19*, 485-486.

8. Kojima, H.; Ogura, H. Triterpenoids from *Prunella vulgaris*. *Phytochemistry* **1986**, *25*, 729-733.
9. Giuseppina, C.; Aurora, B.; Cosimo, P.; Fabio, V.; Nunziatina, D. T. Triterpene Saponins from *Tupidanthus calyptratus*. *J. Nat. Prod.* **2001**, *64*, 750-753.

Sample availability: Available from the corresponding author.

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