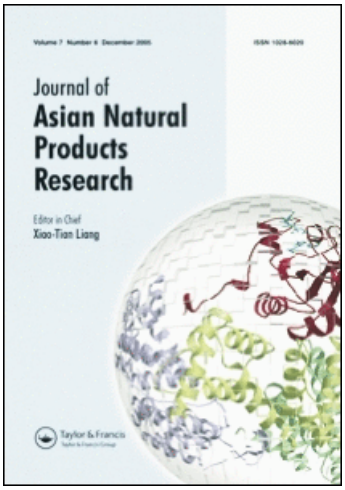

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Kang, Wen-Yi , Du, Zhi-Zhi , Yang, Xiao-Sheng and Hao, Xiao-Jiang(2005) 'Note: A new triterpene from Luculia pinciana Hook.', Journal of Asian Natural Products Research, 7: 1, 91 — 94
To link to this Article: DOI: 10.1080/10286020310001608967
URL: http://dx.doi.org/10.1080/10286020310001608967

Journal of Asian Natural Products Research, Vol. 7, No. 1, February 2005, 91–94

Note

A new triterpene from *Luculia pinciana* Hook.

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(Received 16 January 2003; revised 2 June 2003; in final form 10 June 2003)

A new triterpene named luculiaoic acid A (**1**), showing inhibitory activity of a leukaemia cell line, along with eleven known compounds, has been isolated from the ethyl acetate extract of the stems of *Luculia pinciana* Hook. All the structures were elucidated on the basis of NMR, MS, and IR methods. The activity to inhibit *Staphylococcus aureus* and *Candida albicans* of all compounds showed that ursolic acid inhibits the growth of *Staphylococcus aureus* with an MIC of 0.5 mg ml^{-1} and an MBC of 10 mg ml^{-1}, and scopletin inhibits *Candida albicans* with an MIC of 1 mg ml^{-1} and an MBC of 5 mg ml^{-1}.

Keywords: *Luculia pinciana* Hook.; Triterpene; Leukaemia cell line

1. Introduction

Luculia pinciana Hook. (Rubiaceae) is a medicinal plant widely distributed in Yunnan and Guangxi province of China [1]. As a frequently used drug for tracheitis, tuberculosis, and rheumatic diseases in traditional Chinese medicine, it can "relieve pain, dredge all the channels and vessels, promote blood circulation" [2,3]. The first investigated constituents of the plant matter were paenol [3] and volatile oil [4], and pharmacological experiments proved that the ethanol extract had antibiotic activities [5]. During phytochemical studies on this plant, by bioassay-guided fractionation, a new triterpene has been isolated from the ethyl acetate extract of the stems of *Luculia pinciana* Hook. with eleven known compounds: scopletin (**2**) [6], 2′,4′-dihydroxyacetophenone (**3**) [6], 5-methoxy-8-hydroxycoumarin (**4**) [8], daphnetin (**5**) [8], 4′-hydroxyphenyl methyl ketone (**6**) [9], 3-hydroxy-4-methyloxy-benzeic acid (**7**) [10], 5-hydroxyl-2-methyl-benzeoic acid (**8**) [11], 3-4′-hydroxyphenyl-2-propenoic acid (**9**) [11], dauricine (**10**) [6], ursolic acid (**11**) [12], and oleanolic acid (**12**) [12]. Except scopletin, the others were isolated for the first time from this genus. The activities to inhibit *Staphylococcus aureus* and *Candida albicans* of these compounds were tested. Assignments of the NMR data of the new compound were established by 2D NMR experiments, to reveal the structure of **1** as 1α,3β,24-trihydroxyurs-12-en-28-oic acid (figure 1).

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020310001608967

2. Results and discussion

Luculiaoic acid A (**1**) was obtained as a white powder. Its HR-EIMS showed a molecular peak ion at *m/z* 488.3502 which had a formula of $C_{30}H_{48}O_5$. The ^{13}C NMR and DEPT spectra showed signals for six methyls, nine methylenes, eight methines and seven quaternary carbons, including a carboxyl group, one isolated oxy-methylene group and two oxy-methine groups, which were further confirmed in the ^1H NMR spectrum. A total of 30 carbons were observed, which is characteristic of a triterpene skeleton. The urs-12-en triterpene skeleton was concluded from the chemical shifts of C-12 (δ 126.1) and C-13 (δ 138.7) [13], and was also confirmed by the protons of H-29 (δ 1.01, 3H, d, $J = \mathbf{6.6\,Hz}$), H-30 (δ 0.93, d, $J = \mathbf{5.5\,Hz}$) and H-18 (δ 2.66, 1H, d, $J = \mathbf{11.2\,Hz}$).

The IR spectrum of **1** indicated the presence of hydroxyl (ν_{max} 3430 cm^{-1}) and carboxyl (ν_{max} 1696 cm^{-1}) groups, and the EI-MS spectrum also showed characteristic peaks at *m/z* (%); 248 (100), 222 (27) and 203 (48) due to retro-Diels–Alder fission, suggesting three hydroxy groups in the A/B rings and one carboxyl group in the D/E rings of the amyrin skeleton [14]. By comparing the ^1H and ^{13}C NMR spectra of luculiaoic acid A with those of analogues reported in the literature [8,9], two secondary hydroxyl groups at δ 4.30 (1H, dd, $J = \mathbf{4.3}$, 11.6 Hz, H-3) and 5.05 (1H, brs, H-1), and a primary hydroxyl group at δ 4.40, 4.03 (each 1H, dd, $J = \mathbf{10.6}$, 10.3 Hz, H-24) were confirmed using ^1H–^1H COSY, HMQC, HMBC and NOESY experiments. In the HMBC spectrum long-range correlations were observed between H-1 and C-3, C-25, H-3 and C-24, C-23, and the ^1H–^1H COSY spectrum established the connectivities between H-1 (δ 5.05) and H-2 (δ 1.86), H-3 (δ 4.30) and H-2 (δ 1.97, 1.86), further confirming the positions of H-1, H-3 and H-24. With respect to the stereochemistry, the *J*-value of H-3 (4.3 and 11.6 Hz) indicates that the proton of C-3 is axial-H (α-H) [15,16], and NOESY correlations were observed between H-3 and H-23, H-18 and H-12, H-1 and H-24. From these data, luculiaoic acid A was elucidated as 1α,3β,24-trihydroxy-urs-12-en-28-oic acid (**1**).

3. Eexperimental

3.1 General experimental procedures

Optical rotations were measured on a HORIBA SEPA-300 high sensitive spectropolarimeter. IR spectra were recorded on a BIO-RAD FTS-135 spectrometer with KBr pellets. MS and HRMS were taken on a VG AUTO.SPCE-3000. 1D and 2D NMR experiments were

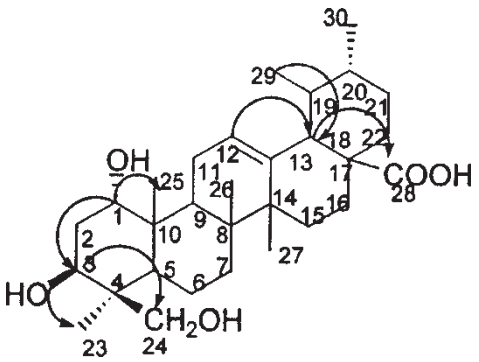

Figure 1. Structure and important HMBC correlations of **1**.

performed on a BRUKER AM-400 or DRX-500 spectrometer. Unless otherwise specified, chemical shifts were expressed in ppm with reference to the solvent signals. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (60μ; Qingdao Marine Chemical Inc., China), or Sephadex-LH-20 (25–100 μm). TLC spots were detected by spraying with 5% H_2SO_4 followed by heating.

3.2 Plant material

The stems of *Luculia pinciana* Hook. were collected in Dali, Yunnan province of China in July 2001. The plant was identified by Professor Peng Hua. A voucher specimen (No. 0358685) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, P. R. Chinese Academy of Sciences.

3.3 Extraction and isolation

Dried stems of *Luculia pinciana* Hook. (20 kg) were extracted (× 3) at room temperature with ethanol (95%). After evaporation of ethanol *in vacuo*, the concentrated extract was suspended in water and extracted successively with light petroleum, ethyl acetate and n-butanol. The ethyl acetate extract (200 g) was chromatographed on a silica gel column (1500 g, 200–300 mesh) using a gradient of (1000 ml each eluent) 0–50% methanol in chloroform (0, 2, 5, 10, 20, 50%, 1500 each). Fractions were pooled based on TLC analysis (7 combined fractions). Fraction 2 (11 g) was subjected repeatedly to column chromatography over silica gel with petrol–Me_2CO (9:1) to give **2** (901 mg), **3** (200 mg), **4** (180 mg), **5** (47 mg), **6** (23 mg), **7** (109 mg). Fraction 3 (10 g) was repeatedly chromatographed over silica gel H eluting with petrol–Me_2CO (4:1) to yield **8** (18 mg), **9** (56 mg), **10** (792 mg), **11** (871 mg), **12** (43 mg). Fraction 5 (60 g) was further separated on a silica gel H (800 g) column with methanol and chloroform (95:5 to 9:1) to give five fractions. The fourth fraction was subjected to Sephadex-LH-20 (methanol), eluting with acetone and chloroform (2:3) to give luculiaoic acid A (**1**) (87 mg).

Luculiaoic acid A (**1**); mp 260–262°C, $[\alpha]_D^{16.5} + 13.3$ (*c* 0.003, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3430, 2929, 1696, 1635, 1456, 1378, 1062, 1033. HR-EIMS *m/z*: 488.3502 calcd for

Table 1. ^1H NMR data and HMBC correlations for **1** (500 MHz δ in ppm, pyridine-d_5).

Positions	δ_H (*J in Hz*)	*NOE**	*HMBC*†
1	5.05 brs	4.03	C-3, C-25
3	4.30 dd (4.3, 11.6)	1.71	C-23, C-24
12	5.57 brs		C-18
18	2.66 d (11.2)	5.57	C-12
23	1.71 s		
24	4.40, 4.03 dd (10.6, 10.3)	5.05	
25	1.66 s		
26	1.63 s		
27	1.19 s		
29	1.01 d (6.6)	2.66	C-18
30	0.93 d (5.5)		
COOH			C-18

Signal assignments based on ^1H–^1H COSY, HMQC, HMBC and NOESY experiments.
* Numbers indicate the proton at which NOEs were observed in the NOESY spectrum, with the proton on the corresponding line.
† Numbers indicate the carbons coupled with the proton on the same row through two or three bonds (detected by the HMBC experiments).

Table 2. ^{13}C NMR data of luculiaoic acid A (125 MHz δ in ppm, pyridine-d_5).

Positions	δ_C	*Positions*	δ_C	*Positions*	δ_C
1	67.5	11	23.8	21	31.1
2	28.1	12	126.1	22	36.9
3	73.3	13	138.7	23	14.8
4	46.5	14	43.1	24	67.1
5	49.1	15	28.8	25	17.7
6	18.6	16	25.0	26	18.9
7	33.3	17	48.6	27	24.1
8	41.3	18	53.7	28	180.0
9	48.1	19	41.3	29	17.5
10	37.5	20	39.4	30	21.4

$C_{30}H_{48}O_5$ 488.3492, EI-MS *m/z* (%): 488 (M^+ 2), 470 (5), 442 (5), 288 (9), 248 (100), 222 (27), 203 (48). ^1H and ^{13}C data are given in tables 1 and 2, respectively.

3.4 Biological assay

At concentrations of 1, 10 and 100 $\mu g\,ml^{-1}$, the inhibitory rates were 11.3, 24.7 and 58.0% respectively to the HL-60 leukaemia cell line using the method of MTT. The inhibition was carried out by the National Screening Center of Pharmaceuticals, China. The activity to inhibit *Staphylococcus aureus* and *Candida albicans* of all compounds showed that **11** was inhibitory to the growth of *Staphylococcus aureus* with an MIC of 0.5 $mg\,ml^{-1}$ and an MBC of 10 $mg\,ml^{-1}$, and **2** was inhibitory to the *Candida albicans* with an MIC of 1 $mg\,ml^{-1}$ and an MBC of 5 $mg\,ml^{-1}$.

Acknowledgements

We thank the staff of the analytical group of Kunming Institute of Botany, Chinese Academy of Sciences for measuring spectra, and especially the staff of the National Screening Center of Pharmaceuticals, China.

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