

中华双扇蕨中一个新的贝壳杉烷型二萜

王扣^{1,2}, 李明明^{1,2}, 成晓¹, 许刚¹,
彭丽艳¹, 赵勤实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 中国科学院研究生院, 北京 100049)

摘要: 从中华双扇蕨 (*Dipteris chinensis*) 中分离得到一个新的对映贝壳杉烷型二萜和反式桂皮酸的二聚体: 16-hydroxy-17-[(Z)-p-coumaroyl]-ent-kauran-19-oic acid, 命名为 dipterinoid A (1)。同时首次从该植物中分离得到其他 13 个已知化合物。

关键词: 双扇蕨科; 中华双扇蕨; 对映贝壳杉烷型二萜; Dipterinoid A

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A New *ent*-Kaurane Diterpenoid from the Aerial of *Dipteris chinensis* (Dipteridaceae)

WANG Kou^{1,2}, LI Ming-Ming^{1,2}, CHENG Xiao¹, XU Gang¹,
PENG Li-Yan¹, ZHAO Qin-Shi^{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 Graduate University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: A new *ent*-kaurane diterpenoid, dipterinoid A (1), was isolated from the aerial parts of *Dipteris chinensis* together with thirteen known compounds. Its structure was determined as 16-hydroxy-17-[(Z)-p-coumaroyl]-*ent*-kauran-19-oic acid by extensive NMR and MS analysis. All of known compounds were isolated from this plant for the first time.

Key words: Dipteridaceae; *Dipteris chinensis*; *ent*-Kaurane-type diterpene; Dipterinoid A

Dipteris, the only living genus of the family Dipteridaceae, includes eight species which are distributed in the tropics of Asia. Three of the species have a distribution in the southwest, south China and Taiwan (Delectis Florae Reipublicae Popularis Sinicae Agenda Academiae Sinicae Edita, 2000). To the best of our knowledge, only investigation about chemical constituents of *Dipteris* plants has been reported which revealed the presence of *ent*-kaurane-type diterpenes in *D. conjugata* (Tanaka *et al.*, 1985). *Dipteris chinensis*, dis-

tributed in Yunnan, Guizhou, and Guangxi provinces of China, is used as herbal medicine to treat edema and kidney deficiency (Ching, 1980), and occasionally planted for ornament because of its large showy fan-shaped leaves. There is no phytochemical report on this plant up to now. As a part of our research work on bioactive metabolites from ferns of China (Li *et al.*, 2006; 2007), phytochemical investigation on the aerial parts of *D. chinensis* was studied, and a new *ent*-kaurane diterpenoid, dipterinoid A (1), was isolated from the

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Author for correspondence; E-mail: qinshizhao@mail.kib.ac.cn; Tel: +86-871-5223058

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作者简介: 王扣 (1983-) 女, 在读博士研究生, 主要从事蕨类植物化学与生物学的研究。

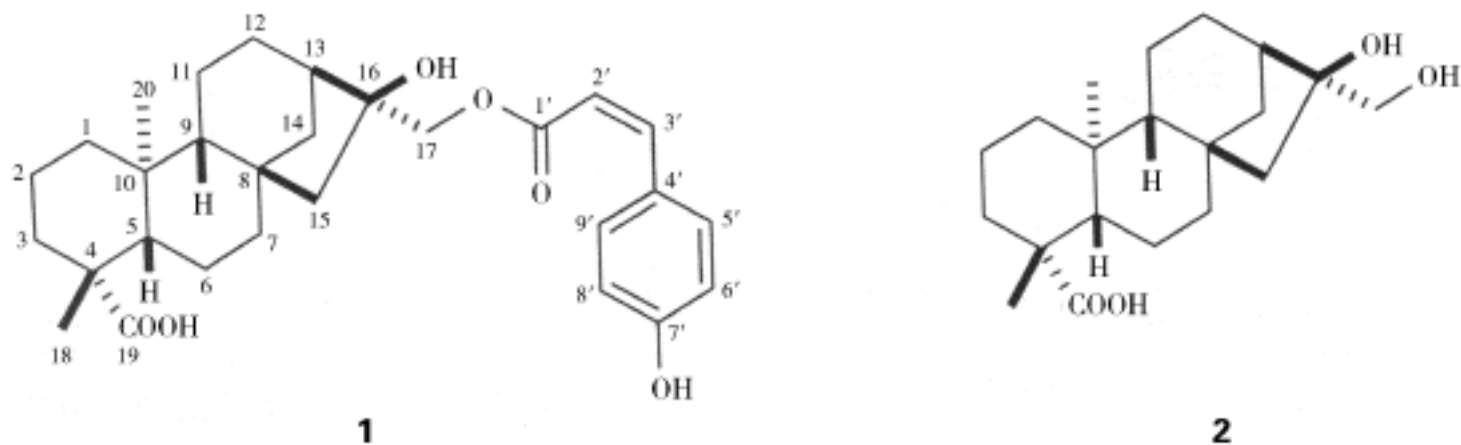


Fig. 1 The structures of compound 1 and 2

95% ethanol extract together with thirteen known compounds. Herein, the isolation and the structural elucidation of 1 was reported.

Results and Discussion

Compound 1, obtained as white amorphous powder, has the molecular formula $C_{29}H_{38}O_6$ based on the negative HR-ESI-MS ($[M-H]^-$ at m/z 481.2583; calcd for $C_{29}H_{37}O_6$, 481.2590) as well as NMR spectral data, requiring eleven degrees of unsaturation. The IR spectrum showed the absorption bands for the hydroxyl (3423 cm^{-1}) and carbonyl groups (1696 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 29 carbon signals including twenty carbon signals for a diterpenoid unit together with nine characteristic signals for a *p*-coumaroyl unit at δ_c 167.1 (s), 159.6 (s), 144.1 (d), 133.6 ($2 \times$ d), 127.4 (s), 116.8 (d) and 115.7 ($2 \times$ d). The ^1H NMR data also indicated the presence of the *p*-coumaroyl moiety at δ_H 5.81 (1H, d, $J=12.8\text{ Hz}$, H-2), 6.88 (1H, d, $J=12.8\text{ Hz}$, H-3), 7.74 (2H, d, $J=8.8\text{ Hz}$, H-5 and H-9), and 6.82 (2H, d, $J=8.8\text{ Hz}$, H-6 and H-8). This was further supported by the $^1\text{H}-^1\text{H}$ COSY (H-2 with H-3, H-5 with H-9 and H-8) and HMBC correlations (H-2 and H-3 with C-1 and C-4). In addition, the double bond was suggested as *cis*-double bond due to the coupling constant (Macias *et al.*, 1997).

The remaining diterpenoid unit was consisted of two angle methyl groups (δ_H 0.98, 1.17; δ_c 16.0, 29.2), ten methylenes (including one oxygenated carbon at δ_c 71.6 for C-17), four methines (including

δ_c 57.4, 57.1, 42.4 for C-5, C-9, C-13 respectively, and one oxygenated carbon at δ_c 78.5 for C-16), three quaternary carbons (including δ_c 44.5, 44.0, 40.4 for C-8, C-4, C-10 respectively), and one carbonyl group (δ_c 178.9). Considering the spectral characteristic discussed above, together with the fact that several *ent*-kaurane diterpenoids such as 16, 17-dihydroxy-*ent*-kauran-19-oic acid (2) (Tanaka *et al.*, 1985) and 9, 16, 17-trihydroxy-19-kauranoic acid (Richter *et al.*, 1977) isolated from *D. chinensis*, this diterpenoid unit was ascribable to be an *ent*-kaurane diterpenoid. The NMR data of 1 was very similar to those of 2 except for the nine more signals for the (*Z*)-*p*-coumaroyl unit discussed above, which indicated that 1 was an *ent*-kaurane diterpenoid with *p*-coumaroyl (Table 1). The (*Z*)-*p*-coumaroyl unit in 1 was connected to C-17, as suggested by the HMBC correlations of H-17 with C-1. In addition, the $^1\text{H}-^1\text{H}$ COSY spectrum of 1 indicated the presence of the structural fragments $-\text{CH}_2\text{CH}_2\text{CH}_2-$ (C-1-C-2-C-3), $-\text{CHCH}_2\text{CH}_2-$ (C-5-C-6-C-7), and $-\text{CHCH}_2\text{CH}_2\text{CH}-$ (C-9-C-11-C-12-C-13). Analysis of the HMBC spectrum demonstrated the correlations of H-5 (δ_H 1.07, 1H, m) with C-4, C-6, C-7, C-10, C-18, and C-19, H-9 (δ_H 1.09, 1H, m) with C-5, C-8, C-10, C-12, C-15, and Me-20, and from H-13 (δ_H 2.01, 1H, m) to C-8, C-12, and C-16. All the 2D NMR data discussed above confirmed the structure of *ent*-kaurane diterpenoid further more.

The relative stereochemistry of 1 was suggested as the same with that of 2, and the hydroxyl group at C-16 was also suggested to be β -oriented because the

Table 1 ^1H and ^{13}C NMR Data of 1 in CD_3COCD_3

No	1			2
	c	H	HMBC	c
1	41.5 t	0.85, 1.89 m	C-9, C-10, C-11, C-20	41.2 t
2	19.9 t	1.38, 1.91 m	C-3, C-4, C-10, C-18	19.8 t
3	38.8 t	1.18, 1.97 m ^a	C-5, C-15, C-18	38.6 t
4	44.0 s			43.9 s
5	57.4 d	1.07 m	C-4, C-6, C-7, C-10, C-18, C-19	57.1 d
6	22.6 t	1.81 m	C-5, C-9	22.5 t
7	42.6 t	1.44 m	C-5, C-8, C-9	42.5 t
8	44.5 s			43.9 s
9	57.1 d	1.09 m	C-5, C-8, C-10, C-12, C-15, C-20	56.7 d
10	40.4 s			40.1 s
11	19.5 t	1.55 m, 2.11 m	C-8, C-12, C-13	19.4 t
12	27.5 t	1.43 m, 1.84 m	C-14, C-16	27.6 t
13	42.4 d	2.01 m	C-8, C-12, C-16	41.6 d
14	38.8 t	1.01 m, 2.11 m ^b	C-9, C-12	38.7 t
15	53.6 t	1.45 d, $J = 14.0$ Hz 1.51 d, $J = 14.0$ Hz	C-7, C-8, C-9, C-16, C-17	53.4 t
16	78.5 s			79.1 s
17	71.6 t	3.97 d, $J = 11.2$ Hz 4.05 d, $J = 11.2$ Hz	C-13, C-15, C-16, C-1	70.4 t
18	29.2 q	1.17 s	C-3, C-4, C-5, C-19	29.4 q
19	178.9 s			180.0 s
20	16.0 q	0.98 s	C-10, C-19	16.0 q
1	167.1 s			
2	116.8 d	5.81 d, $J = 12.8$ Hz	C-1, C-3, C-4	
3	144.1 d	6.87 d, $J = 12.8$ Hz	C-1, C-4, C-5, C-9	
4	127.4 s			
5, 9	133.6 2 × d	7.74 dd, $J = 8.8, 2.0$ Hz	C-3, C-7	
6, 8	115.7 2 × d	6.82 dd, $J = 8.8, 2.0$ Hz	C-4, C-7	
7	159.6 s			

^{a, b} Assignments with the same superscript may be reversed, although those given here are preferred.

chemical shift of α -oriented C-17 CH_2OH signal (about δ_c 70.0) (Yamasaki *et al.*, 1976; Satake *et al.*, 1984) should be more downfield than that of β -oriented C-17 CH_2OH signal (about δ_c 66.0) (He *et al.*, 2005; Nishimura *et al.*, 2007; Wang *et al.*, 1995). Therefore, the structure of compound 1 was elucidated as 16 β -hydroxy-17-[(*Z*)-*p*-coumaroyl]-*ent*-kauran-19-oic acid and named as dipterinoid A (Fig. 1).

The known compounds were identified to be sandaracopimaric acid (Comte *et al.*, 1995), 16 β , 17-dihydroxy-*ent*-kauran-19-oic acid (Tanaka *et al.*, 1985), 9, 16, 17-trihydroxy-19-kauranoic acid (Richter *et al.*, 1977), (20*R*)-dammar-24-en-20-ol (Yamashita *et al.*, 1998), bergapten (Masuda *et al.*, 1998), kaempferol (Markham *et al.*, 1978), quercitrin (Hrhammer *et al.*, 1966; Zhong *et al.*, 1997), quercetin 3-robinobioside (Bresseur and Angenot, 1986), nicotiflorin (Markham *et al.*, 1978), 20-hydroxyecdysone 20, 22-

acetate (Homvisasevongs *et al.*, 2004), 4-hydroxybenzoic acid (Scott, 1972), β -sitosterol (Nes *et al.*, 1992) and daucosterol (Seo *et al.*, 1978). However, 20-hydroxyecdysone 20, 22-acetate may be an artefact because acetone was used in the course of isolation. The naturally occurring compound may be 20-hydroxyecdysone (Suksamrarn *et al.*, 2002). Their structures were determined by comparison of the spectroscopic data with those reported in the literature.

Experimental

General experimental procedures Optical rotation was measured on a Horiba SEPA-300 polarimeter. IR spectra was obtained with a Tensor 27 FT-IR spectrometer with KBr pellets. The ^1H and ^{13}C NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers in CD_3COCD_3 at room temperature (δ in ppm, J in Hz). FAB-MS was carried out on a VG Autospec-3000 spectrometer. HR-ESIMS was recorded with an API

QSTAR Pulsar i spectrometer. Silica gel F₂₅₄, Silica gel H (Qingdao Marine Chemical Ltd., China) and LiChroprep RP-18 silica gel (40-63 μm, Merck, Dramstadt, Germany) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates immersed with 15% H₂SO₄ in ethanol. Solvents were distilled prior to use.

Plant material The aerial parts of *D. chinensis* were collected from Dawei Mountain, Pingbian County, Yunnan Province, PRC, in July 2007 and were identified by professor Cheng Xiao of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation Air-dried and powdered plant materials (2.3 kg) were extracted with 95% EtOH (10 L × 3, each 3 days) at room temperature. After evaporation of the solvent in vacuo at 50 °C, the residue was dissolved in H₂O and then extracted exhaustively with EtOAc (2 L × 4). The EtOAc extract (88 g) was dissolved in 95% ethanol then subjected to column chromatography over MCI eluting with 90% ethanol. The elution was concentrated in vacuo to give a residue (71 g), which was subjected to column chromatography over silica gel (100-200 mesh) and eluted with petroleum ether-acetone (10, 9:1, 8:2, 7:3, 6:4, 1:1, 0:1) to give six fractions A-F. Fractions B-E were separated repeatedly on silica gel, and then further purified using Sephadex LH-20 with (CHCl₃-MeOH 0:1 and 1:1) and RP-18 (MeOH-H₂O gradient elution of increasing concentration). Fraction B gave (20*R*)-dammar-24-en-20-ol (65.0 mg). From fraction C, bergapten (4.5 mg), sandaracopimaric acid (5.0 mg), 4-hydroxybenzoic acid (3.0 mg), and sitosterol (2.0 g) were obtained. Fraction D afforded 1 (4.0 mg), 16, 17-dihydroxy-*ent*-kauran-19-oic acid (40.0 mg) and kaempferol (4.5 mg). Fraction E gave quercitrin (1.7 mg), 9, 16, 17-trihydroxy-19-kauranoic acid (9.2 mg) and daucosterol (200.0 mg). Fraction F (60 g) was subjected to silica gel CC and eluted with a mixture of CHCl₃-MeOH (100% CHCl₃, 9:1, 8:2, 7:3, 6:4, 100% MeOH) to afford five subfractions F1-F5. 20-Hydroxyecdysone 20, 22-acetonate (55.0 mg) was obtained from F2 by silica gel CC repeatedly and then followed by preparation HPLC. Quercetin 3-robinobioside (12.0 mg) and nicotiflorin (120 mg) were yielded from F3 by silica gel CC and Sephadex LH-20.

dipterinoid A (1, 16-hydroxy-17-[(*Z*)-*p*-coumaroyl]-*ent*-kauran-19-oic acid): white amorphous solid; $[\alpha]_D^{25.8} = -58.06$ ($c = 0.155$, MeOH); UV_{max}^{MeOH} (log ε): 311 (4.07); IR_{max}^{KBr} cm⁻¹: 3423, 2941, 2871, 2852, 1696, 1605, 1514, 1164; negative FAB-MS m/z (%): 481[M-H]⁻ (100); HR-

ESIMS m/z : 481.2583 [M-H]⁻ (calcd for C₂₉H₃₇O₆, 481.2590); NMR data see Table 1.

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