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A LUPANE TRITERPENE AND TWO TRITERPENE CAFFEATES FROM *RHOIPTELEA CHILIANTHA*

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Key Word Index *Rhoiptelea chiliantha*; Rhoipteleaceae; lupane triterpene; triterpene caffeate.

Abstract—A new lupane triterpene and two new triterpene caffeates were isolated from the bark of *Rhoiptelea chiliantha*, together with two known triterpene acids. Their structures were elucidated on the basis of spectral and chemical evidence.

INTRODUCTION

Rhoiptelea chiliantha Diels et Hand.-Mazz. is the only species of the family Rhoipteleaceae which is distributed in southern China and northern Vietnam. The arguments about the taxonomical relationships of this family to Betulaceae, Juglandaceae and Ulmaceae [1] led us to investigate the chemical constituents of the bark of this plant. We have previously reported on the structural elucidation of two novel triterpene–lignan esters having a dimeric structure, a known triterpene caffeate [2] and a new rearranged-ursane triterpene [3] from this source, and we report here on the isolation and structural elucidation of a new lupane triterpene (**1**) and two new triterpene caffeates (**3** and **5**), together with two known triterpenes (**2** and **4**) from the same source.

RESULTS AND DISCUSSION

The 95% ethanol extract of the air-dried bark was partitioned between diethyl ether and H_2O. The ether layer was subjected to MCI-gel CHP 20P chromatography to afford two fractions; the first fraction was chromatographed on silica gel and ODS to yield two triterpene caffeates (**3** and **5**). The second fraction was separated by silica gel chromatography and normal-phase MPLC to afford three triterpene acids (**1**, **2** and **4**). Compounds **2** and **4** were identified as 3β,28-dihydroxy-20(29)-lupen-27-oic acid [4] and 3β,27-dihydroxyolean-12-en-28-oic acid [2, 5], respectively, by comparison of their ^{1}H NMR, mass spectral and physical data with those described in the literature.

Compound **1** showed a molecular ion peak at m/z 458 in its EI-mass spectrum. The 30 carbon signals observed in the ^{13}C NMR spectrum were characterized by a DEPT experiment, which indicated that **1** was a triterpene having seven methyls, nine methylenes, eight methines and six quaternary carbons. Furthermore, the chemical shifts of three of the methine carbon signals (δ78.6, 73.1 and 74.3) suggested the presence of three hydroxyl groups. From these data, the empirical formula, $C_{30}H_{50}O_3$, was deduced. In the ^{1}H NMR spectrum, the appearance of a pair of olefinic proton signals (δ4.91 and 4.76) and an allylic methyl signal (δ1.78), which are characteristic of an isopropenyl group, and six singlet methyl signals suggested that **1** was a pentacyclic triterpene possessing a lupene, hopene or fernene skeleton. In the HMBC spectrum (Table 1), carbon signals resonating at δ78.6 (*d*), 40.4 (*s*) and 55.4 (*d*) were correlated with two singlet methyl protons (δ1.35 and 1.71), indicating that these signals were assignable to C-3, C-4 and C-5, respectively, and that a hydroxyl group was located at C-3. The locations of the remaining two hydroxyl groups were determined to be at C-6 and C-7 on the basis of the coupling (J = 4 Hz) between the oxygen-bearing methine protons (δ4.66 and 3.90) and the HMBC correlaton of the proton at δ4.66 with C-5 (δ55.4). The partial structure, which could be determined by further interpretation of the correlations found in the H-H COSY, HSQC and HMBC spectra, is delineated by heavy lines in Fig. 1. These results suggested that the structure of **1** is that of 3,6,7-trihydroxy-20(29)-lupene. The configurations of the hydroxyl groups at C-3, C-6 and C-7 were all determined to be β on the basis of the NOE interactions between H-3 and H-5 and between H-5 and both H-6 and H-7 in the NOESY spectrum. In addition, the NOE interactions shown in Fig. 2 confirmed the lupane skeleton of **1**. Consequently, **1** was characterized as 3β,6β,7β-trihydroxy-20(29)-lupene.

Compound **3** gave dark green colouration with 1% $FeCl_3$ reagent. The ^{1}H NMR data were closely related to those of **2** except for the appearance of additional signals

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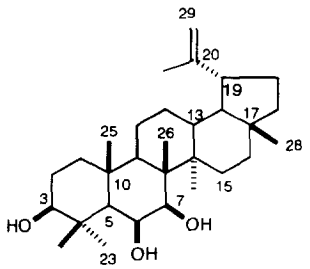
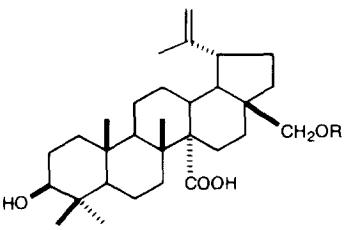
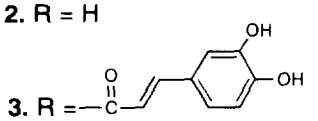


1



2. R = H



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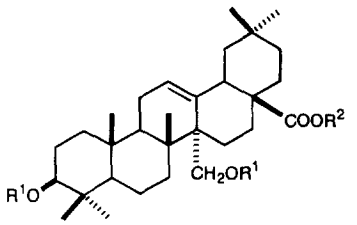
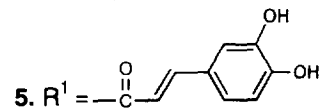


4. $R^1 = R^2 = H$

4a. $R^1 = H$, $R^2 = Me$



5. R^1 =

$R^2 = H$

Table 1. ^1H and ^{13}C NMR data of compound **1** (500 and 125 MHz, pyridine-d_5)

No.	C	H	HMBC (H to C)	No.	C	H	HMBC (H to C)
1	41.6 *t*	1.74 (*ddd*, 13, 3, 3)		16	36.5 *t*	1.50 *m*	
		1.03 (*ddd*, 13, 12, 3)	C-2, 10			1.58 *m*	
2	28.6 *t*	2.10 *m*		17	43.1 *s*		
		2.04 *m*		18	48.8 *d*	1.50 *m*	
3	78.6 *d*	3.47 (*dd*, 11, 4)	C-4, 23, 24	19	48.5 *d*	2.54 (*ddd*, 11, 11, 6)	C-13, 18, 20, 22, 29, 30
4	40.4 *s*			20	151.3 *s*		
5	55.4 *d*	0.97 (*d*, 2)	C-1, 3, 4, 6, 9, 10, 24, 25	21	30.3 *t*	2.00 *m*	
6	73.1 *d*	4.66 (*dd*, 2, 4)	C-5, 7, 8, 10			1.42 *m*	
7	74.3 *d*	3.90 (*d*, 4)	C-6, 8, 14	22	40.4 *t*	1.42 *m*	
8	46.6 *s*					1.26 *m*	
9	51.5 *d*	1.42 *m*		23	28.1 *q*	1.35 *s*	C-3, 4, 5, 24
10	37.4 *s*			24	18.24 *q*	1.71 *s*	C-3, 4, 5, 23
11	21.4 *t*	2.18 *m*		25	17.9 *q*	1.51 *s*	C-1, 5, 9, 10
		1.58 *m*		26	11.1 *q*	1.64 *s*	C-7, 8, 9, 14
12	26.1 *t*	1.90 *m*		27	15.5 *q*	1.19 *s*	C-8, 13, 14, 15
		1.26 *m*		28	18.19 *q*	0.88 *s*	C-16, 17, 18, 22
13	38.4 *d*	1.85 *m*		29	109.8 *t*	4.91 (*d*, 2)	C-19, 30
14	44.7 *s*					4.76 (*d*, 1)	C-19, 30
15	31.6 *t*	2.16 *m*		30	19.6 *q*	1.78 *s*	C-19, 20, 29
		1.55 *m*					

Assignments were based on the H–H COSY, HSQC and HMBC spectra.
The coupling constants expressed in Hz are given in parentheses.

arising from a *trans*-caffeoyl group, suggesting that **3** was a caffeoyl ester of **2**. This was confirmed by alkaline hydrolysis of **3** to give **2**. The location of the caffeoyl group was determined to be at C-28 on the basis of the downfield shifts of H_2-28 (δ4.67 and 4.51) compared with those of **2** ($\Delta\delta$ + 0.51 and + 0.76, respectively). Accordingly, the structure of **3** was established as 28-*O*-*trans*-caffeoyl-3β,28-dihydroxy-20(29)-lupen-27-oic acid.

Compound **5** also showed dark green colouration with 1% $FeCl_3$ reagent. The presence of a triterpene core in the molecule was presumed by the observation of 27 aliphatic signals, two olefinic signals and one carboxyl carbon signal in the ^{13}C NMR spectrum, the chemical shifts of which were related to those of **4**. The remaining carbon signals suggested the presence of two caffeoyl groups, which was further supported by the negative FAB–mass spectrometry (*m/z* 795 $[M - H]^-$). Methylation of **5** with diazomethane followed by methanolysis afforded **4a** [6], the methyl ester of **4** and methyl *trans*-3,4-dimethoxycinnamate. The locations of the two caffeoyl groups were determined to be at C-3 and C-27, respectively, by the downfield shifts of H-3 (δ4.52,

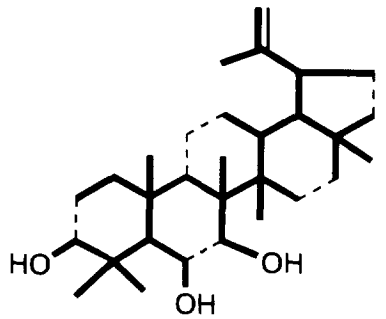


Fig. 1. Partial structures of **1** shown by heavy lines.

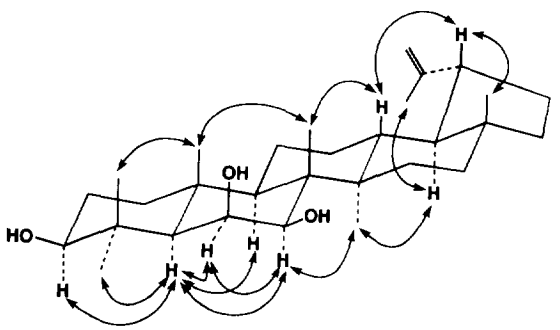


Fig. 2. NOE interactions of **1**.

$\Delta\delta$ + 1.32) and H_2-27 (δ4.43 and 4.16. $\Delta\delta$ + 0.60 and + 0.98, respectively) compared with those of **4a**. Thus, the structure of **5** was elucidated as 3,27-di-*O*-*trans*-caffeoyl-3β,27-dihydroxyolean-12-en-28-oic acid. To our knowledge, compound **5** is the first example of a triterpene di-*O*-caffeate, although di-*O*-coumaroyl triterpenes have been isolated from *Ilex asprella* [5].

Triterpene caffeates have so far been found in the plants of *Quercus* (Fagaceae) [7], *Betula* (Betulaceae) [8, 9], *Myrica* (Myricaceae) [10], *Pyracantha* (Rosaceae) [11] and *Larrea* (Zygophyllaceae) [12]. The presence of the triterpene caffeates in *R. chiliantha* may be important to the chemosystematic placement of the Rhoipteleaceae.

EXPERIMENTAL

General. Mps: uncorr.; ^{1}H (300 and 500 MHz) and ^{13}C (75 and 125 MHz) NMR: TMS as int. standard; EI-MS and FAB-MS: JEOL JMX DX-303 mass spectrometer; CC: Kieselgel 60 (70–230 mesh, Merck), MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries), Bondapak ODS (37–55 μm, Waters Associates) and Si-5 MPLC (10 μm, Kusano); TLC: precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck).

Plant material. The bark of *R. chiliantha* was collected in Huaping, Guangxi, China, in Oct., 1988. The voucher specimen was deposited in the Laboratory of Plant Chemotaxonomy, China Pharmaceutical University, Nanjing.

Extraction and sepn. The air dried ground bark (4.5 kg) was extracted with 95% EtOH. The extract (570 g) was partitioned (× 2) between Et_2O (1 l) and H_2O (1 l), then the Et_2O layer was concd and treated with MeOH. The MeOH-soluble fr. was subjected to MCI-gel CHP 20P (80–100% MeOH, then Me_2CO) to afford fr. 1 (129 g) and fr. 2 (125 g). A part of fr. 1 (77 g) was then chromatographed on silica gel ($CHCl_3$–MeOH–H_2O, 4:1:0.1), Diaion HP-20 (80–100% MeOH) and Bondapak ODS (80–100% MeOH) to afford compounds **3** (45 mg) and **5** (90 mg). Fr. 2 was chromatographed on silica gel ($CHCl_3$–MeOH–H_2O, 4:1:0.1) and MPLC Si-5 ($CHCl_3$–MeOH, 49:1) to yield compounds **1** (73.2 mg), **2** (5 mg) and **4** (123 mg).

3β,6β,7β-*Trihydroxy*-20(29)-*lupene* (**1**). Crystals ($CHCl_3$), mp 254–256°. $[\alpha]_D^{24}$ + 6 ($CHCl_3$; *c* 0.4). EI-MS *m/z*: 458 $[M]^+$, 443 $[M - Me]^+$; ^{1}H and ^{13}C NMR: Table 1. (Found: C, 76.57; H, 10.88. $C_{30}H_{50}O_3.1/2H_2O$ requires: C, 77.04; H, 10.99).

28-O-trans-*Caffeoyl*-3β,28-*dihydroxy*-20(29)-*lupen*-27-*oic acid* (**3**). A light yellow powder, $[\alpha]_D^{24}$ − 6° (MeOH, *c* 0.4). Negative FAB-MS *m/z*: 633 $[M - H]^-$, 471 $[M$ − caffeoyl$]^-$; ^{1}H NMR (500 MHz, CD_3OD): δ7.53 (1H, *d*, *J* = 16 Hz, caffeoyl-7), 7.04 (1H, *d*, *J* = 2 Hz, caffeoyl-2), 6.95 (1H, *dd*, *J* = 8, 2 Hz, caffeoyl-6), 6.79 (1H, *d*, *J* = 8 Hz, caffeoyl-5), 6.25 (1H, *d*, *J* = 16 Hz, caffeoyl-8), 4.74, 4.62 (each 1H, *d*, *J* = 2 Hz, H_2-29), 4.67, 4.51 (each 1H, *d*, *J* = 12 Hz, H_2-28), 3.14 (1H, *m*, H-3), 1.72 (3H, *s*, H_3-30), 1.03, 0.93, 0.90, 0.75 (each 3H, *s*, methyl × 4); ^{13}C NMR (75 MHz, CD_3OD): triterpene moiety—δ180.0 (C-27), 151.8 (C-20), 110.4 (C-29), 79.5 (C-3), 64.4 (C-28), 57.3, 57.0, 53.4, 50.7, 40.5, 40.2, 40.0, 46.9, 42.8, 38.7, 38.0, 36.7, 33.8, 31.7, 28.6, 28.1, 26.7, 25.3, 24.1, 22.3, 19.7, 19.5, 17.2, 17.15, 16.2; caffeoyl moiety—δ169.4 (C-9'), 149.7 (C-4'), 147.0 (C-3'), 146.9 (C-7'), 127.7 (C-1'), 123.1 (C-6'), 116.6 (C-5'), 115.9 (C-8'), 115.3 (C-2'). (Found: C, 70.58; H, 8.58. $C_{39}H_{54}O_7.3/2H_2O$ requires: C, 70.77; H, 8.68).

Alkaline hydrolysis of **3**. A soln of **3** (30 mg) in 5% aq. NaOH (2 ml) was heated at 80° for 15 min and then left at room temp. for 24 hr. After acidification with 1 N HCl, the reaction soln was extracted with Et_2O, and the Et_2O extract was sepd by silica gel CC, with $CHCl_3$–MeOH (49:1) to give **2** (3 mg).

3,27-*Di*-O-trans-*caffeoyl*-3β,27-*dihydroxyolean*-12-*en*-28-*oic acid* (**5**). A yellow powder, $[\alpha]_D^{24}$ + 88 (MeOH, *c* 0.5). Negative FAB-MS *m/z*: 795 $[M - H]^-$, 633 $[M$ − caffeoyl$]^-$; ^{1}H NMR (300 MHz, CD_3OD): δ7.52, 7.50 (each 1H, *d*, *J* = 16 Hz, caffeoyl-7',7''), 7.03, 7.02 (each 1H, *d*, *J* = 2 Hz, caffeoyl-2',2''), 6.92 (2H, *dd*, *J* = 2, 8 Hz, caffeoyl-6',6''), 6.79, 6.76 (each 1H, *d*, *J* = 8 Hz, caffeoyl-5',5''), 6.22, 6.18 (each 1H, *d*, *J* = 16 Hz, caffeoyl-8',8''), 5.61 (1H, *s*, H-12), 4.52 (1H, *m*, H-3), 4.43, 4.16 (each 1H, *d*, *J* = 13 Hz, H_2-27), 2.93 (1H, *m*, H-18), 1.01, 0.96, 0.94, 0.90, 0.85, 0.85 (each 3H, *s*, methyl × 6); ^{13}C NMR (75 MHz, CD_3OD): triterpene moiety—δ39.0 (C-1), 28.7 (C-2), 82.2 (C-3), 39.4 (C-4), 56.7 (C-5), 19.3 (C-6), 34.7 (C-7), 41.2 (C-8), 49.9 (C-9), 38.3 (C-10), 24.7 (C-11), 128.1 (C-12), 139.0 (C-13), 46.7 (C-14), 25.1 (C-15), 23.9 (C-16), 47.3 (C-17), 42.5 (C-18), 46.2 (C-19), 31.5

(C-20), 34.3 (C-21), 33.5 (C-22), 28.7 (C-23), 16.2 (C-24), 17.5 (C-25), 18.9 (C-26), 66.6 (C-27), 181.6 (C-28), 33.7 (C-29), 24.1 (C-30); caffeoyl moiety—δ169.2, 168.8 (C-9′, 9″), 149.6, 149.5 (C-4′, 4″), 147.0 × 2 (C-3′, 3″), 146.8, 146.7 (C-7′, 7″), 127.7, 127.8 (C-1′, 1″), 122.9 × 2 (C-6′, 6″), 116.5, 116.6 (C-5′, 5″), 115.6, 115.4 (C-8′, 8″), 115.1 × 2 (C-2′, 2″). (Found: C, 69.32; H. 7.73. $C_{48}H_{60}O_{10}.2H_2O$ requires: C, 69.21; H: 7.74).

Methylation and alkaline methanolysis of **5**. A soln of **5** (15 mg) in MeOH was treated with CH_2N_2 in Et_2O and then methanolized with 2.5% NaOH in H_2O–MeOH (1:1) for 2 hr. After acidification with 1 N HCl, the reaction mixt. was extracted with Et_2O, and the Et_2O extract was sepd by silica gel CC, with hexane–EtOAc (4:1), to afford methyl *trans*-3,4-dimethoxycinnamate (1.5 mg) and **4a** (3 mg) [6]: prisms, mp 217–218°, $[\alpha]_D^{24}$ + 64.0° ($CHCl_3$, *c* 0.2). EI-MS: *m/z*: 486 $[M]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ5.84 (1H, *s*, H-12), 3.83, 3.18 (each 1H, *d*, *J* = 12 Hz, H_2-27), 3.18 (1H, *m*, H-3), 2.95 (1H, *dd*, *J* = 13, 6 Hz, H-18), 0.98, 0.96, 0.90, 0.87, 0.77, 0.67 (each 3H, *s*, methyl × 6).

3β,28-*Dihydroxy*-20(29)-*lupen*-27-*oic acid* (**2**) [4]. A powder, $[\alpha]_D^{24}$ + 22 ($CHCl_3$, *c* 0.05). EI-MS: *m/z*: 472 $[M]^+$, 454 $[M - H_2O]^+$, 441 $[M - CH_2OH]^+$; ^{1}H NMR (300 MHz, pyridine-d_5): δ4.95, 4.78 (each 1H, *s*, H_2-29), 4.63, 4.23 (each 1H, *d*, *J* = 12 Hz, H_2-28), 3.41 (1H, *dd*, *J* = 7, 9 Hz, H-3), 1.78 (3H, *s*, H_3-20), 1.18, 1.15, 1.02, 0.88 (each 3H, *s*, methyl × 4). ^{1}H NMR (300 MHz, CD_3OD): δ4.70, 4.58 (each 1H, *d*, *J* = 2 Hz, H_2-29), 4.15, 3.75 (each 1H, *d*, *J* = 12 Hz, H_2-28), 3.13 (1H, *m*, H-3), 1.69 (3H, *s*, H_3-20), 0.96, 0.95, 0.88, 0.75 (each 3H, *s*, methyl × 4).

3β,27-*Dihydroxyolean*-12-*en*-28-*oic acid* (**4**) [5]. A powder, mp 220–222°, $[\alpha]_D^{24}$ + 47 (pyridine, *c* 0.2). EI-MS: *m/z*: 472 $[M]^+$, 454 $[M - H_2O]^+$, 441 $[M - CH_2OH]^+$; ^{1}H NMR (300 MHz, pyridine-d_5): δ5.89 (1H, *t*, *J* = 3 Hz, H-12), 4.10, 3.84 (each 1H, *d*, *J* = 12 Hz, H_2-27), 3.42 (1H, *m*, H-3), 3.38 (1H, *m*, H-18), 1.21 (3H, *s*, H_3-23), 1.06 (3H, *s*, H_3-25), 1.03 (3H, *s*, H_3-24), 1.02 (3H, *s*, H_3-30), 0.91 (3H, *s*, H_3-26), 0.89 (3H, *s*, H_3-29).

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