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TRITERPENES FROM *DIOSPYROS MARITIMA**

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Key Word Index—*Diospyros maritima*; Ebenaceae; triterpenes; 3-(*E*)-feruloylbetulin; 28-acetyl-3-(*E*)-coumaroylbetulin.

Abstract—Two new lupane derivatives, 3-(*E*)-feruloylbetulin and 28-acetyl-3-(*E*)-coumaroylbetulin, have been isolated from the stem of *Diospyros maritima*. Their structures were determined using spectral and chemical methods. © 1997 Published by Elsevier Science Ltd

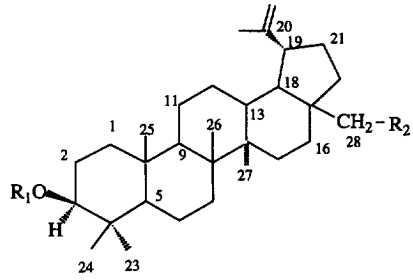
INTRODUCTION

Thirteen species of *Diospyros* are grown in Taiwan, from these, several workers have described the chemical constituents of some species including fruits of *D. discolor* Willd [1], leaves of *D. kaki* Thunb [2], barks and stems of *D. eriantha* [3, 4], and stems of *D. morrisiana* Hance [5–7]. These contain triterpenes, lignans, steroids, benzoquinones, and naphthoquinones. The stems of *D. maritima* Blume are usually used in the treatment of rheumatic diseases in the traditional regimen of Taiwan.

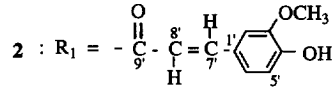
RESULTS AND DISCUSSION

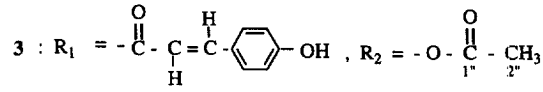
Five triterpenes were extracted from the ethanol extract of stems of *D. maritima*. Three of the triterpenes were readily identified as taraxerol [8], erythrodiol [9], and betulin (**1**) [10]. The remaining two were the new betulin derivatives, 3-(*E*)-feruloylbetulin (**2**) and 28-acetyl-3-(*E*)-coumaroylbetulin (**3**).

Compound **2** was deduced to have the molecular formula $C_{40}H_{58}O_5$, on the basis of its HRMS, and was considered to be a triterpenoid due to a positive Liebermann-Burchard test. Analysis of the IR spectrum of **2** suggested that it contained a hydroxy group (3360 cm^{-1}), a conjugated ester (1685 cm^{-1}), a conjugated double bond (1620 and 960 cm^{-1}), a terminal double bond (3050, 1660, and 880 cm^{-1}), and a phenyl group (1590, 1580, and 1500 cm^{-1}). The ^1H NMR spectrum (Table 1) exhibited signals for five singlet methyl groups, a hydroxymethyl group attached to a quaternary carbon [δ 3.31 and 3.78 (each 1H, *d*, $J = 10.7$ Hz)], an isopropenyl group [δ 1.67 (3H, *br s*), 4.57 and 4.67 (each 1H, *d*, $J = 2.0$ Hz)], an (*E*)-feruloyl moiety [δ 3.91 (3H, *s*), 5.82 (1H, *s*, -OH, disappeared on D_2O exchange), 6.26 and 7.56 (each 1H, *d*, $J = 16.0$ Hz)], a methine proton bearing an ester (δ 4.61, *m*, obscured by olefinic proton), and a typical lupenol H_β-19 proton signal (δ 2.37, *m*). By comparison of the ^1H NMR data with those of betulin (**1**), compound **2** was assigned as a betulin derivative



1 : $R_1 = H$, $R_2 = OH$





* Dedicated to Professor Yu-Shia Cheng on the occasion of her 65th birthday.

† Author to whom correspondence should be addressed.

Table 1. ^{13}C and ^{1}H NMR data for compound **2**

	δ_C	δ_H		δ_C	δ_H
1	38.4 *t*		21	29.7 *t*	
2	23.7 *t*		22	34.2 *t*	
3	80.8 *d*	4.61 *m*	23	28.0 *q*	0.86 *s*
4	38.1 *s*		24	16.0 *q*	1.01 *s*
5	55.4 *d*		25	16.2 *q*	0.85 *s*
6	18.2 *t*		26	16.6 *q*	0.87 *s*
7	34.0 *t*		27	14.7 *q*	0.97 *s*
8	40.9 *s*		28	60.7 *t*	3.31 *d* (10.7), 3.78 *d* (10.7)
9	50.3 *d*		29	109.7 *t*	4.57 *d* (2.0), 4.67 *d* (2.0)
10	37.1 *s*		30	19.1 *q*	1.67 *s*
11	20.9 *t*		1′	127.1 *s*	
12	25.2 *t*		2′	109.2 *d*	7.01 *d* (1.6)
13	37.3 *d*		3′	146.7 *s*	
14	42.7 *s*		4′	147.8 *s*	
15	27.0 *t*		5′	116.2 *d*	6.88 *d* (8.2)
16	29.2 *t*		6′	123.0 *d*	7.04 *dd* (8.2, 1.6)
17	47.8 *s*		7′	144.3 *d*	7.56 *d* (16.0)
18	48.7 *d*		8′	114.6 *d*	6.26 *d* (16.0)
19	47.8 *d*	2.37 *m*	9′	167.1 *s*	
20	150.5 *s*		OMe	56.0 *q*	3.91 *s*

[a] Numbers in parentheses are coupling constant.

with an extra (*E*)-feruloyl moiety. Hydrolysis of compound **2** with 5% aqueous KOH solution yielded two products, betulin (**1**) and ferulic acid [11]. Therefore the structure of **2** agreed with the assigned structure, 3-(*E*)-feruloylbetulin. The ^{13}C NMR data (Table 1) of **2** also confirmed the structure.

Compound **3** was also deduced to be a triterpenoid due to a positive Liebermann–Burchard test and was assigned the molecular formula $C_{41}H_{58}O_5$ on the basis of its HRMS. The IR spectrum of **3** showed bands attributable to a hydroxy group (3360 cm^{-1}), an alkyl acetate (1730 cm^{-1}), a conjugated ester (1690 cm^{-1}), a terminal double bond (3040, 1660, and 870 cm^{-1}), a conjugated double bond (1620 and 960 cm^{-1}), and a phenyl group (1595, 1590 and 1500 cm^{-1}). The ^{1}H NMR spectrum (Table 1) exhibited signals for five singlet methyl groups, an acetoxymethyl group attached to a quaternary carbon [δ 2.03 (3H, *s*), 3.82 and 4.24 (each 1H, *d*, J = 10.6 Hz)], an isopropenyl group, a (*E*)-coumaroyl moiety [δ 6.26 and 7.57 (each 1H, *d*, J = 16.0 Hz), 5.40 (1H, *s*, -OH, disappeared on D_2O exchange), 6.82 and 7.41 (each 2H, *d*, J = 8.6 Hz)], a methine proton bearing an ester (δ 4.56, *m*, obscured by olefinic proton), and a typical lupenol H_β-19 proton signal. Compound **3** was considered as a betulin derivative with an extra acetyl group and an extra (*E*)-coumaroyl moiety by comparison of its ^{1}H NMR data with those of betulin. When compound **3** was treated in 5% methanolic HCl it gave a known compound, 3-(*E*)-coumaroylbetulin [12]. From the above evidence, compound **3** can be assigned as 28-acetyl-3-(*E*)-coumaroylbetulin. The ^{13}C NMR data (Table 2) of **3** gave the further proof of this structure. The HMBC spectrum of **3** showed that δ 4.56 (H-3) and δ 167.2 (C-9′), and δ_H 4.24 (H-28) and δ_C 171.8 (C-1″) are correlated.

EXPERIMENTAL

General. Mps: uncorr; CC: silica gel.

Plant material. Stems of *D. maritima* Blume were collected in Lin-Ko in 1993. The plant material was identified by Mr Gun Muh-Tsuen, formerly a technician of the Department of Botany of the National Taiwan University, and a voucher specimen has been deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

Extraction and isolation. The heartwoods of *D. maritima* (16 kg) were extracted with EtOH at 60° (160 l × 3, 10 hr ea. time). To the EtOH extract was added H_2O to 12 l, and this phase was then partitioned (× 5) with 1 l of hexane. The aq. layer was partitioned (× 4) again with 1 l of BuOH. The combined BuOH extracts (180 g) were chromatographed on silica gel and HPLC repeatedly. Five compounds, taraxerol (56 mg), erythrodiol (5 mg), betulin (**1**) (45 mg), 3-(*E*)-feruloylbetulin (**2**) (8 mg), and 28-acetyl-3-(*E*)-coumaroylbetulin (**3**) (9 mg) were isolated.

3-(*E*)-*Feruloylbetulin* (**2**). Mp 152–154°; $[\alpha]_D^{28}$ +16.2 ($CHCl_3$, *c* 0.4); UV λ_{max} (log ε): 325 (4.50), 297 (4.41), 234 (4.53), 215 (4.63) nm; IR ν_{max}^{KBr}: 3360, 3050, 1685, 1660, 1620, 1590, 1580, 1500, 1380, 1365, 1260, 1162, 960, 880 cm^{-1}; ^{1}H and ^{13}C NMR: Table 1; EIMS (70 eV) *m/z* (rel. int.): 618 $[M]^+$ (14), 194 (50), 177 (100),

Table 2. ^{13}C and ^{1}H NMR data for compound **3**

	δ_C	δ_H		δ_C	δ_H
1	38.4 *t*		22	35.5 *t*	
2	23.8 *t*		23	28.0 *q*	0.86 *s*
3	80.8 *d*	4.56 *m*	24	16.0 *q*	1.01 *s*
4	38.0 *s*		25	16.2 *q*	0.85 *s*
5	55.4 *d*		26	16.7 *q*	0.89 *s*
6	18.2 *t*		27	14.7 *q*	0.95 *s*
7	34.1 *t*		28	62.9 *t*	3.82 *d* (10.6), 4.24 *d* (10.6)
8	40.9 *s*		29	109.9 *t*	4.57 *d* (1.6), 4.66 *d* (1.6)
9	50.3 *d*		30	19.1 *q*	1.67 *s*
10	37.1 *s*		1′	127.5 *s*	
11	20.8 *t*		2′	129.9 *d*	7.41 *d* (8.6)
12	25.2 *t*		3′	115.8 *d*	6.82 *d* (8.6)
13	37.6 *d*		4′	157.4 *s*	
14	42.7 *s*		5′	115.8 *d*	6.82 *d* (8.6)
15	27.1 *t*		6′	129.9 *d*	7.41 *d* (8.6)
16	29.6 *t*		7′	143.8 *d*	7.57 *d* (16.0)
17	46.3 *s*		8′	116.4 *d*	6.26 *d* (16.0)
18	48.8 *d*		9′	167.2 *s*	
19	47.7 *d*	2.41 *m*	1″	171.8 *s*	
20	150.1 *s*		2″	21.1 *q*	2.03 *s*
21	29.7 *t*				

[a] Numbers in parentheses are coupling constants.

145 (20); HRMS *m/z*: 618.4288, $C_{40}H_{58}O_5$ requires 618.4286.

28-*Acetyl*-3-(*E*)-*coumaroylbetulin* (**3**). Mp 147–149°; $[\alpha]_D^{23}$ +7.7 ($CHCl_3$, *c* 0.4); UV λ_{max} (log ε): 253 (3.32), 303 (4.30), 312 (4.40) nm; IR ν_{max}^{KBr}: 3360, 3040, 1730, 1690, 1660, 1620, 1595, 1590, 1500, 1380, 1360, 1261, 1172, 960, 870 cm^{-1}; ^{1}H and ^{13}C NMR: Table 2; EIMS (70 eV) *m/z* (rel. int.): 630 $[M]^+$ (2), 466 [M-coumaric acid, 60],$^+$ 451 (3), 423 (18), 393 (9), 164 (22), 147 (100); HRMS *m/z*: 630.4279, $C_{41}H_{58}O_5$ requires 630.4286.

Hydrolysis of **2** *with aqueous KOH.* Compound **2** (6 mg) was added to 5% aq. KOH (3 ml) and the reaction mixt. was heated at *ca* 60° for 3 hr. H_2O (10 ml) was added and the reaction mixt. was extracted with Et_2O. The ether extract was purified and yielded betulin (4 mg). The aq. layer was acidified with 3% HCl and then subsequently extracted with CH_2Cl_2 affording ferulic acid (1 mg).

Partial hydrolysis of **3** *with 5% methanolic HCl.* Compound **3** (7 mg) was heated at 60° in 5% methanolic HCl (1.5 ml) and, after 4 hr., the reaction was quenched with 20 ml of H_2O. The product was extracted and purified to yield 3-(*E*)-coumaroylbetulin (3.8 mg) [12].

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