# Three New Lupane-Type Triterpenes from *Diospyros maritima*

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**Three new lupane derivatives, 3-(*E*)-feruloyl-28-palmitoylbetulin (1), 3-(*Z*)-coumaroyl-28-palmitoylbetulin (2), and 3-(*Z*)-coumaroyllupeol (3) have been isolated from the stem of *Diospyros maritima*. Their structures were determined by using spectral and chemical methods.**

**Key words** *Diospyros maritima*; Ebenaceae; triterpene; 3-(*E*)-feruloyl-28-palmitoylbetulin; 3-(*Z*)-coumaroyl-28-palmitoylbetulin; 3-(*Z*)-coumaroyllupeol

Chemical studies of species of *Diospyros* (Ebenacea) grown in Taiwan include the fruits of *D. discolor* WILLD,[1] leaves of *D. kaki* THUNB,[2] barks and stems of *D. eriantha* CHAMP,[3,4] and stems of *D. morrisiana* HANCE.[5—7] The water extract of the stem of *D. maritima* BLUME (indigenous to Taiwan) has usually been used to treat rheumatic diseases locally in Taiwan.[8] Recently, we reported the isolation of some new naphthoquinones[9] and triterpenes[10] from the stem of this plant and found that the naphthoquinones exhibited strong antitumor activity.[10c] Using the same extract, we have now purified in detail and have also isolated three new triterpenes 3-(*E*)-feruloyl-28-palmitoylbetulin (**1**), 3-(*Z*)-coumaroyl-28-palmitoylbetulin (**2**), and 3-(*Z*)-coumaroyllupeol (**3**). This paper deals with the structural elucidation of these compounds.

Compound **1** was deduced to be a triterpenoid due to a positive Liebermann–Burchard test. The HR-EI-MS gave a pseudomolecular [M−ferulic acid ($C_{10}H_{10}O_4$)]$^+$ ion at *m/z* 662.5942, consistent with the molecular formula $C_{56}H_{88}O_6$. The IR spectrum showed the presence of hydroxy, ester, conjugated ester, terminal double bond, and phenyl group functionalities. The $^1$H-NMR spectrum (Table 1) of compound **1** exhibited signals characteristic of a (*E*)-feruloyl moiety, a methoxyl group [$\delta$ 3.91 (s, 3H); having nuclear Overhauser effect (NOE) correlation with a signal at $\delta$ 7.01], five singlet methyl groups, a palmitoyloxymethylene group attached to a quaternary carbon [$\delta$ 2.30 (t, 2H, $J$=7.5 Hz, H-2″), 3.81, 4.24 (d, each 1H, $J$=11.1 Hz, H-28)], an isopropenyl group [$\delta$ 1.66 (s, 3H), 4.57, 4.66 (d, each 1H, $J$=2.0 Hz)], a methine proton bearing an ester ($\delta$ 4.60, m, 1H, H-3, obscured by olefinic protons), and a typical lupene $H_\beta$-19 proton signal ($\delta$ 2.40, m, 1H). The $^{13}$C-NMR data (Table 1) of **1** also contained signals consistent with the presence of a (*E*)-feruloyl moiety.[10a] Compound **1** was considered to be a betulin (**4**) derivative with an extra palmitoyl group and an extra (*E*)-feruloyl moiety by comparison of its $^{13}$C-NMR data with those of betulin.[11] The proton detected heteronuclear multiple-bond correlation (HMBC) spectrum of **1** showed correlation between $\delta_H$ 4.60 (H-3) and $\delta_C$ 167.3 (C-9′), and $\delta_H$ 4.24 (H-28) and $\delta_C$ 174.3 (C-1″). From the above evidence, compound **1** was assigned as 3-(*E*)-feruloyl-28-palmitoylbetulin.

Compound **2** also gave a positive Liebermann–Burchard test. The HR-EI-MS gave a pseudomolecular [M−coumaric acid ($C_9H_8O_3$)]$^+$ ion at *m/z* 662.6005, consistent with the molecular formula $C_{55}H_{86}O_5$. The IR and UV data of **2** closely resembled those of **1** and the $^1$H- and $^{13}$C-NMR data

Table 1. $^1$H- and $^{13}$C-NMR Data for **1** and **2** (300 and 75 MHz in $CDCl_3$)

| No. | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 38.4 | | 38.4 | |
| 2 | 23.8 | | 23.8 | |
| 3 | 80.8 | 4.60 m | 80.9 | 4.49 dd (4.8, 10.8) |
| 4 | 38.0 | | 37.9 | |
| 5 | 55.4 | | 55.5 | |
| 6 | 18.2 | | 18.1 | |
| 7 | 34.1 | | 34.1 | |
| 8 | 40.9 | | 40.9 | |
| 9 | 50.3 | | 50.3 | |
| 10 | 37.1 | | 37.1 | |
| 11 | 20.9 | | 21.0 | |
| 12 | 25.2 | | 25.2 | |
| 13 | 37.6 | | 37.6 | |
| 14 | 42.7 | | 42.7 | |
| 15 | 27.1 | | 27.2 | |
| 16 | 29.2 | | 29.6 | |
| 17 | 46.4 | | 46.4 | |
| 18 | 48.8 | | 48.8 | |
| 19 | 47.7 | 2.40 m | 47.7 | 2.39 m |
| 20 | 150.1 | | 150.1 | |
| 21 | 28.7 | | 29.7 | |
| 22 | 34.5 | | 34.5 | |
| 23 | 28.0 | 0.86 s | 28.0 | 0.83 s |
| 24 | 16.0 | 1.02 s | 16.0 | 1.00 s |
| 25 | 16.2 | 0.85 s | 16.1 | 0.82 s |
| 26 | 16.6 | 0.90 s | 16.5 | 0.77 s |
| 27 | 14.7 | 0.96 s | 14.7 | 0.98 s |
| 28 | 62.5 | 3.81 d (11.1), 4.24 d (11.1) | 62.6 | 3.81 d (10.8), 4.24 d (10.8) |
| 29 | 109.8 | 4.57 d (2.0), 4.66 d (2.0) | 109.9 | 4.60 d (2.0), 4.67 d (2.0) |
| 30 | 19.1 | 1.66 s | 19.1 | 1.67 s |
| 1′ | 127.1 | | 127.2 | |
| 2′ | 109.2 | 7.01 d (1.6) | 130.2 | 7.61 d (8.8) |
| 3′ | 147.8 | | 115.0 | 6.76 d (8.8) |
| 4′ | 146.7 | | 156.5 | |
| 5′ | 116.3 | 6.88 d (8.4) | 115.0 | 6.76 d (8.8) |
| 6′ | 123.0 | 7.04 dd (1.6, 8.4) | 130.2 | 7.61 d (8.8) |
| 7′ | 144.3 | 7.56 d (16.0) | 143.0 | 6.80 d (12.6) |
| 8′ | 114.7 | 6.26 d (16.0) | 117.9 | 5.80 d (12.6) |
| 9′ | 167.2 | | 166.4 | |
| $-OCH_3$ | 56.0 | 3.91 s | | |
| 1″ | 174.3 | | 174.3 | |
| 2″ | 34.0 | 2.30 t (7.5) | 34.0 | 2.30 t (7.5) |
| 3″ | 25.1 | ] | 25.0 | ] |
| 4″—13″ | 29.2—29.8 | 1.20—1.30 br s | 29.1—29.7 | 1.20—1.30 br s |
| 14″ | 31.8 | ] | 31.9 | ] |
| 15″ | 22.7 | | 22.7 | |
| 16″ | 14.1 | 0.87 m | 14.1 | 0.88 m |

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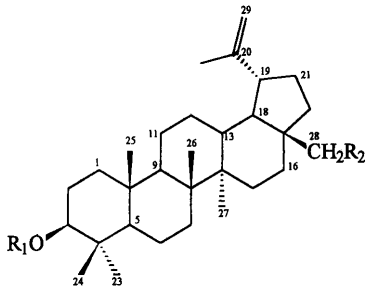
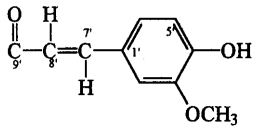
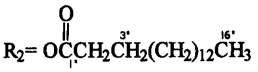
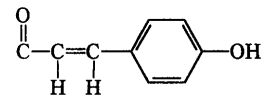
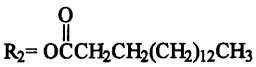
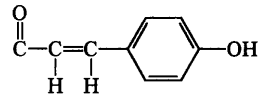


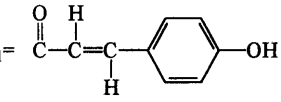
1 $R_1$=  $R_2$= 

2 $R_1$=  $R_2$= 

3 $R_1$=  $R_2$= H

4 $R_1$=H $R_2$= OH

5 $R_1$= H $R_2$= H

6 $R_1$=  $R_2$= H

(Table 1) of **2** were very similar to those of **1** except for the appearance of signals for a (*Z*)-coumaroyl group instead of a (*E*)-feruloyl group. The HMBC spectrum of **2** showed correlation between $\delta_H$ 4.49 (H-3) and $\delta_C$ 166.4 (C-9′), and $\delta_H$ 4.24 (H-28) and $\delta_C$ 174.3 (C-1″). When compound **2** was treated with 5% methanolic HCl, it gave the known compounds, 3-(*Z*)-coumaroylbetulin,[12] and methyl palmitoate.[13] Thus, the structure of compound **2** was deduced to be 3-(*Z*)-coumaroyl-28-palmitoylbetulin.

Compound **3** was also obtained in small amounts. The molecular formula , $C_{39}H_{56}O_3$, was determined through peak matching of the molecular ion at *m/z* 572.4224, observed through HR-EI-MS. EI-MS gave a [M−coumaric acid]$^+$ ion at *m/z* 408, thus compound **3** was considered to be a coumaroyl ester of lupeol (**5**). The IR spectrum showed the presence of hydroxy group, a conjugated ester, a conjugated double bond, a terminal double bond, and a phenyl group. The UV spectrum exhibited an absorption maximum at 310 nm. The $^1$H-NMR spectrum exhibited signals similar to those of 3-(*E*)-coumaroyllupeol (**6**) (isolated from the same source)[10c] except for the presence of a (*Z*)-coumaroyl moiety [$\delta$ 5.81 and 6.80 (d, each 1H, $J$=12.9 Hz)] instead of an (*E*)-coumaroyl moiety [$\delta$ 6.27, 7.57 (d, each 1H, $J$=16.0 Hz)]. From the above evidence, the structure of compound **3** was deduced as 3-(*Z*)-coumaroyllupeol.

## Experimental

**General Procedures** Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. $^1$H- and $^{13}$C-spectra were obtained on a Bruker AM-300 at 300 and 75 MHz, respectively in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. EI-MS, FAB-MS, UV, and specific rotations were taken on a JEOL JMS-HX 300, a JOEL JMS-HX 110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck, 70—230 mesh).

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

**Extraction and Isolation** The stems of *D. maritima* (16 kg) were extracted with EtOH (160 l) at 60 °C three times (10 h each time). The EtOH extract was then evaporated *in vacuo*, yielding a black residue, which was suspended in $H_2O$ (12 l), and partitioned with *n*-hexane (1 l×5). The aqueous layer was partitioned again with *n*-BuOH (1 l×4). The evaporated combined *n*-BuOH extracts (180 g) were chromatographed on silica gel (*n*-hexane–ethyl acetate and ethyl acetate–methanol step gradient) and HPLC (30% ethyl acetate and 70% *n*-hexane) repeatedly and afforded three components, 3-(*E*)-feruloyl-28-palmitoylbetulin (**1**) (8 mg), 3-(*Z*)-coumaroyl-28-palmitoylbetulin (**2**) (12 mg), and 3-(*Z*)-coumaroyllupeol (**3**) (2 mg).

3-(*E*)-Feruloyl-28-palmitoylbetulin (**1**): Amorphous solid, $[\alpha]_D^{20}$ =+25.1° ($c$=0.5, $CHCl_3$). UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 318 (4.20). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380, 1725, 1680, 1620, 1590, 1575, 960, 880. $^1$H- and $^{13}$C-NMR data see Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 662 [(M−$C_{10}H_{10}O_4$)$^+$, 3], 396 (100), 255 (47), 213 (19), 189 (18), 147 (36). HR-EI-MS Calcd for $C_{46}H_{78}O_2$: 662.6005; Found 662.5942.

3-(*Z*)-Coumaroyl-28-palmitoylbetulin (**2**): Amorphous solid, $[\alpha]_D^{20}$= +23.1° ($c$=0.3, $CHCl_3$). UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 312 (4.65). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3410, 3045, 1735, 1680, 1675, 1640, 1605, 1597, 1515, 970, 880. $^1$H- and $^{13}$C-NMR data see Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 662 [(M−$C_9H_8O_3$)$^+$, 1], 396 (2), 255 (8), 213 (12), 174 (99), 145 (52), 55 (100). HR-EI-MS *m/z* [M−$C_9H_8O_3$]$^+$ Calcd for $C_{46}H_{78}O_2$: 662.6005; Found 662.6005.

3-(*Z*)-Coumaroyllupeol (**3**): Amorphous solid, $[\alpha]_D^{20}$ =+35.2° ($c$=0.1, $CHCl_3$). UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 310 (4.56). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3360, 3045, 1715, 1684, 1660, 1610, 1595, 1510, 970, 880. $^1$H-NMR ($CDCl_3$) $\delta$: 0.77, 0.77, 0.83, 0.95, 1.01, 1.67 (s, each 3H), 2.32 (m, 1H, H-19), 4.50 (dd, 1H, $J$=10.5, 5.1 Hz, H-3), 4.55, 4.67 (br s, each 1H, H-29), 5.81, 6.80 (d, each 1H, $J$=12.9 Hz, H-8′, -7′), 6.78, 7.62 (d, each 2H, $J$=8.7 Hz). EI-MS (70 eV) *m/z* (rel. int. %): 572 (M$^+$, 17), 408 [(M−$C_9H_8O_3$)$^+$, 57], 394 (21), 365 (14), 189 (79), 147 (100). HR-EI-MS *m/z* [M]$^+$ Calcd for $C_{39}H_{56}O_3$: 572.4232; Found 572.4224.

**Partial Hydrolysis of 2 with 5% Methanolic HCl** Compound **2** (7 mg) was heated at 60 °C in 5% methanolic HCl (1.5 ml) for 4 h and the solution was then quenched with 20 ml of $H_2O$. The products were extracted and purified to yield 3-(*Z*)-coumaroyllbetulin[12] (3.0 mg) and methyl palmitoate[13] (1.5 mg).

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