Two New Lupane-Type Triterpenes from *Diospyros maritima*

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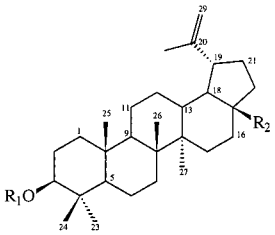
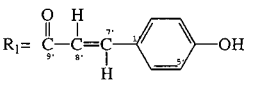
Two new lupane derivatives, 3-(*E*)-coumaroylbetulinaldehyde (**1**) and 3-(*E*)-coumaroyl-28-palmitoylbetulin (**2**), have been isolated from the stems of *Diospyros maritima*. Their structures were determined by using spectral and chemical methods.

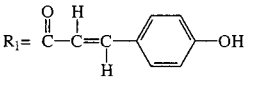
Of 13 species of *Diospyros* (Ebenacea) growing in Taiwan, several have been studied for their chemical constituents, resulting in the isolation and structure elucidation of various triterpenes, lignans, steroids, benzoquinones, and naphthoquinone. Species investigated include fruits of *D. discolor* 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 stems of *D. maritima* Blume have been used in the treatment of rheumatic diseases in the traditional regimen of Taiwan.[8] We have previously reported in the isolation of some new naphthoquinones[9] and triterpenes,[10,11] and found that some naphthoquinones exhibited strong antitumor activity from this plant.[12] In our continuing work on this plant, we have isolated and elucidated two new triterpenes, 3-(*E*)-coumaroylbetulinaldehyde (**1**) and 3-(*E*)-coumaroyl-28-palmitoylbetulin (**2**), from the stem part.

3-(*E*)-Coumaroylbetulinaldehyde (**1**) was deduced to be a triterpenoid through a positive Liebermann−Burchard test and a molecular formula of $C_{39}H_{54}O_4$ on the basis of its HREIMS. Analysis of the IR spectrum of **1** suggested that it contained a hydroxy group (3360 cm^{-1}), an aldehyde group (1715 cm^{-1}), a conjugated ester (1684 cm^{-1}), a conjugated double bond (1610 and 970 cm^{-1}), a terminal double bond (3045, 1660, and 880 cm^{-1}), and a phenyl group (1595, 1585, and 1510 cm^{-1}). The UV spectrum exhibited an absorption maximum at 312 nm. The ^1H NMR spectrum exhibited five singlet methyl groups, an aldehyde group [δ 9.66 (1H, s)], an isopropenyl group [δ 1.68 (3H, s), 4.61, and 4.73 (1H, d, J = 2.0 Hz)], a (*E*)-coumaroyl moiety [δ 6.27 and 7.57 (1H each, d, J = 16.7 Hz), 5.26 (1H, s, −OH, disappeared on D_2O exchange), 6.81, and 7.41 (2H each, d, J = 8.8 Hz)], a methine proton in proximity to an ester group (δ 4.57, m, obscured by olefinic proton, H-3), and a typical lupene Hβ-19 proton. Compound **1** was considered as a betulinaldehyde derivative with an extra (*E*)-coumaroyl moiety by comparison of its ^{13}C NMR data with those of betulinaldehyde (**3**).[13] The HMBC spectrum of **1** showed a long-range correlation between δ_H 4.57(H-3) and δ_C 167.2(C-9′). The ^{13}C NMR data of **1** also confirmed the structure.

Compound **2** was also a triterpenoid, based on a positive Liebermann−Burchard test. It contains hydroxy, ester, a conjugated ester, a conjugated double bond, a terminal double bond, and a phenyl function as discerned by the IR absorption bands at 3400, 3040, 1730, 1680, 1670, 1640, 960, and 877 cm^{-1}. The UV spectrum exhibited an absorption maximum at 309 nm. In the ^1H NMR spectrum, compound **2** exhibited signals that are characteristic of a (*E*)-coumaroyl moiety. The HREIMS of **2** gave a pseudomolecular [M − coumaric acid $(C_9H_8O_3)$]$^+$ ion at m/z 662.5977, consistent with the molecular formula of $C_{55}H_{86}O_5$. The ^{13}C NMR data of **2** also contained resonances consistent with the presence of (*E*)-coumaroyl moiety. The ^1H NMR spectrum exhibited five singlet methyl groups, a palmitoyloxymethylene group attached to a quaternary carbon [δ 2.32 (2H, t, J = 7.5 Hz, H-2″), 3.81, and 4.27 (1H each, d, J = 10.8 Hz, H-28)], an isopropenyl group [δ 1.70 (3H, s), 4.57, and 4.66 (1H each, br s)], a methine proton neighboring an ester group (δ 4.55, 1H, m, obscured by olefinic proton, H-3), and a typical lupene Hβ-19 proton. Compound **2** was considered a betulin derivative with a palmitoyl group and a (*E*)-coumaroyl moiety by comparison of its ^{13}C NMR data with those of betulin (**4**).[14] The HMBC spectrum of **2** showed long-range correlation between δ_H 4.55 (H-3) and δ_C 167.3 (C-9′), and δ_H 4.24 (H-28) and δ_C 174.6 (C-1″). The ^{13}C NMR data of **2** gave the further proof of the structure. Upon heating in 5% methanolic HCl, **2** gave the known 3-(*E*)-coumaroylbetulin[15] and methyl palmitate.[16] From the above data, compound **2** was identified as 3-(*E*)-coumaroyl-28-palmitoylbetulin.



1 R_1=  R_2= CHO

2 R_1=  R_2= $CH_2O\overset{O}{\overset{\|}{C}}CH_2CH_2(CH_2)_{10}CH_2CH_2CH_3$

3 R_1= H R_2= CHO

4 R_1= H R_2= CH_2OH

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin−Elmer 781 spectrophotometer. ^1H and ^{13}C NMR spectra were performed on Bruker AM-300 at 300 and 75 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard.

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EIMS, FABMS, UV, and specific rotations were taken on a JEOL JMS−HX 300, a JEOL JMS−HX 110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on Si gel (Merck 3374, 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. A voucher specimen has been deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

Extraction and Isolation. Dried pieces of stems of *D. maritima* (16 kg) were extracted three times with EtOH (160 L) at 60 °C (10 h for each time). The EtOH extract was evaporated in vacuo, yielding a black residue, which was suspended in H_2O (12 L), and then partitioned (5 ×) with 1 L of *n*-hexane. The aqueous layer was partitioned again with (4 × 1 L) *n*-BuOH. The combined *n*-BuOH extract (180 g) was chromatographed on Si gel using *n*-hexane and EtOAc of increasing polarity as eluent and further purified by HPLC, eluting with EtOAc−*n*-hexane (3:7). Two components, 3-(*E*)-coumaroylbetulinaldehyde (**1**) (10 mg) and 3-(*E*)-coumaroyl-28-palmitoylbetulin (**2**) (15 mg), were obtained in pure state.

3-(*E*)-Coumaroylbetulinaldehyde (1): amorphous solid; $[\alpha]^{20}_D$ +20.2° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 312 (4.60) nm; IR (dry film) ν_{max} 3360, 3045, 1715, 1684, 1660, 1610, 1595, 1585, 1510, 970, 880 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.57 (1H, m, H-3), 2.85 (1H, m, H-19), 0.88 (3H, s, H-23), 0.83 (3H, s, H-24), 0.85 (3H, s, H-25), 0.90 (3H, s, H-26), 0.96 (3H, s, H-27), 9.66 (3H, s, H-28), 4.61 (1H, d, J = 2.0 Hz, H-29), 4.73 (1H, d, J = 2.0 Hz, H-29), 1.68 (3H, s, H-30), 7.41 (2H, d, J = 8.8 Hz, H-2′, 6′), 6.81 (2H, d, J = 8.8 Hz, H-3′, 5′), 7.57 (1H, d, J = 16.7 Hz, H-7′), 6.27 (1H, d, J = 16.7 Hz, H-8′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.0 (t, C-1), 23.8 (t, C-2), 80.8 (d, C-3), 38.7 (s, C-4), 55.4 (d, C-5), 18.2 (t, C-6), 34.3 (t, C-7), 40.8 (s, C-8), 50.4 (d, C-9), 37.1 (s, C-10), 20.8 (t, C-11), 25.5 (t, C-12), 38.4 (d, C-13), 42.6 (s, C-14), 29.2 (t, C-15), 28.8 (t, C-16), 59.3 (s, C-17), 48.0 (d, C-18), 47.5 (d, C-19), 149.7 (s, C-20), 29.8 (t, C-21), 33.2 (t, C-22), 28.0 (q, C-23), 15.9 (q, C-24), 16.2 (q, C-25), 16.6 (q, C-26), 14.2 (q, C-27), 206.8 (d, C-28), 110.2 (t, C-29), 19.0 (q, C-30), 127.4 (s, C-1′), 129.9 (d, C-2′), 115.8 (d, C-3′), 157.5 (s, C-4′), 115.8 (d, C-5′), 129.9 (d, C-6′), 143.9 (d, C-7′), 116.4 (d, C-8′), 167.2 (s, C-9′); EIMS (70 eV) *m/z* 586 $[M]^+$ (21) 558 (8), 422 (26), 394 (14), 189 (39), 147 (100); HREIMS *m/z* 586.4047 (calcd for $C_{39}H_{54}O_4$, 586.4024).

3-(*E*)-Coumaroyl-28-palmitoylbetulin (2): amorphous solid; $[\alpha]^{20}_D$ +32.1° (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 309 (4.70) nm; IR (dry film) ν_{max} 3400, 3040, 1730, 1680, 1670, 1640, 1600, 1595, 1507, 960, 877 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.55 (1H, m, H-3), 2.39 (1H, m, H-19), 0.86 (3H, s, H-23), 1.00 (3H, s, H-24), 0.85 (3H, s, H-25), 0.88 (3H, s, H-26), 0.95 (3H, s, H-27), 3.81 (1H, d, J = 10.8 Hz, H-28), 4.27 (1H, d, J = 10.8 Hz, H-28), 4.57 (1H, d, J = 2.0 Hz, H-29), 4.66 (1H, d, J = 2.0 Hz, H-29), 1.70 (3H, s, H-30), 7.41 (2H, d, J = 8.8 Hz, H-2′, 6′), 6.81 (2H, d, J = 8.8 Hz, H-3′, 5′), 7.58 (1H, d, J = 16.0 Hz, H-7′), 6.27 (1H, d, J = 16.0 Hz, H-8′), 2.32 (2H, t, J = 7.5 Hz, H-2″), 1.20−1.30 (26H, br s, H-3″-15″), 0.87 (3H, m, H-16″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.4 (t, C-1), 23.8 (t, C-2), 80.8 (d, C-3), 38.0 (s, C-4), 55.4 (d, C-5), 18.1 (t, C-6), 34.1 (t, C-7), 40.9 (s, C-8), 50.3 (d, C-9), 37.1 (s, C-10), 21.0 (t, C-11), 25.2 (t, C-12), 37.6 (d, C-13), 42.7 (s, C-14), 27.1 (t, C-15), 29.6 (t, C-16), 46.4 (s, C-17), 48.8 (d, C-18), 47.7 (d, C-19), 150.1 (s, C-20), 29.7 (t, C-21), 34.5 (t, C-22), 28.0 (q, C-23), 16.0 (q, C-24), 16.2 (q, C-25), 16.7 (q, C-26), 14.7 (q, C-27), 63.0 (t, C-28), 109.9 (t, C-29), 19.1 (q, C-30), 127.2 (s, C-1′), 130.2 (d, C-2′), 115.9 (d, C-3′), 157.8 (s, C-4′), 115.9 (d, C-5′), 130.2 (d, C-6′), 144.0 (d, C-7′), 116.2 (d, C-8′), 167.3 (s, C-9′), 174.6 (s, C-1″), 33.9 (t, C-2″), 25.1 (t, C-3″), 29.1−29.7 (t, C-4″-13″), 31.9 (t, C-14″), 22.7 (t, C-15″), 14.1 (q, C-16″); EIMS (70 eV) *m/z* 662 $[M - C_9H_8O_3]^+$ (38), 619 (15), 424 (24), 203 (76), 189 (100), 147 (40); HREIMS *m/z* 662.5977 $[M - C_9H_8O_3]^+$ (calcd for $C_{46}H_{78}O_2$: 662.6005).

Partial Hydrolysis of 2 with 5% Methanolic HCl. Compound **2** (8 mg) was heated at 60 ° C in 5% methanolic HCl (1.5 mL) for 4 h. The reaction was quenched with 20 mL of H_2O; the products were extracted with 10 mL of EtOAc and purified by HPLC, eluting with EtOAc−*n*-hexane (2.5:7.5), to yield 3-(*E*)-coumaroyllbetulin (3.0 mg)[15] and methyl palmitate (1.5 mg).[16]

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References and Notes

(1) Lin, S. R. *J. Taiwan Pharm. Assoc.* **1978**, *30*, 132−135.
(2) Chou, C. J. *Ann. Rep. Nat. Res. Inst. Chin. Med.* **1984**, 117−114.
(3) Chen, C. C.; Yu, H. J.; Huang, Y. L. *Chin. Pharm. J.* **1992**, *44*, 229−233.
(4) Chen, C. C.; Yu, H. J.; Ou, J. C.; Pan, T. M. *J. Chin. Chem. Soc.* **1994**, *41*, 195−198.
(5) Lee, T. J.; Shih, T. S.; Lin, Y. M.; Chen, F. C. *Formosan Sci.* **1984**, *38*, 147−151.
(6) Chen, H. C.; Lin, Y. M.; Shih, T. S.; Chen, F. C. *Formosan Sci.* **1987**,*41*, 46−52.
(7) Chen, H. K.; Chen, K. J.; Lin Y. S.; Chen, F. C. *Formosan Sci.* **1991**, *44*, 63−71.
(8) Kan, W. S. *Pharmaceutical Botany*, National Research Institute of Chinese Medicine: Taipei, 1997; p 440.
(9) Kuo, Y. H.; Chang, C. I.; Kuo, Y. H. *J. Chin. Chem. Soc.* **1996**, *43*, 511−514.
(10) Kuo, Y. H.; Chang, C. I.; Kuo, Y. H. *Phytochemistry* **1997**, *46*, 1135−1137.
(11) Kuo, Y. H.; Chang, C. I.; Kuo, Y. H. *Chem. Pharm. Bull.* **1997**, *45*, 1221−1222.
(12) Kuo, Y. H.; Chang, C. I.; Kuo, Y. H. *Planta Med.* **1997**, *63*, 363−365.
(13) Monaco, P.; Previtera, L. *J. Nat. Prod.* **1984**, *47*, 463−468.
(14) Tinto, W. F.; Blair, L. C.; Ali, A.; Reynolds, W. F.; McLean S. *J. Nat. Prod.* **1992**, *55*, 395−398.
(15) Rashid, M. A.; Gray, A. I.; Waterman, P. G. *J. Nat. Prod.* **1992**, *55*, 851−859.
(16) Kuo, Y. H.; Yeh, M. H. *J. Chin Chem. Soc.* **1997**, *44*, 379−383.

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