

Dammarane Triterpenes from the Hypocotyls and Fruits of *Ceriops tagal*

Charoen Pakhathirathien,[†] Chatchanok Karalai,^{*,†} Chanita Ponglimanont,[†] Sanan Subhadhirasakul,[§] and Kan Chantrapromma[‡]

Department of Chemistry, Faculty of Science, and Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand, and Institute of Science, Walailak University, Tasala, Nakhon Si Thammarat 80160, Thailand

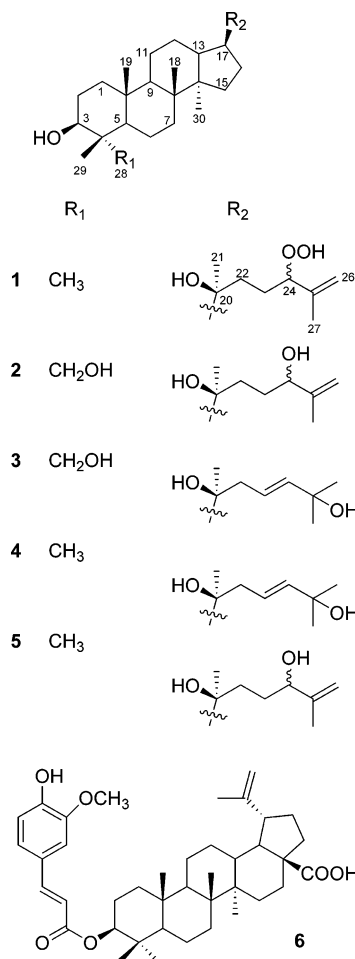
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Three new dammarane triterpenes, cereotagaloperoxide (**1**), cereotagalol A (**2**), and cereotagalol B (**3**), together with four known dammarane triterpenes, an oleanane triterpene, and 13 known lupane triterpenes were isolated from the hypocotyls and fruits of *Ceriops tagal*. The structures of **1–3** were characterized on the basis of their spectroscopic data.

Ceriops tagal (Perr.) C. B. Rob is a mangrove plant belonging to the Rhizophoraceae family. The bark of this plant has been used for the treatment of infected wounds in Thailand and for obstetric and hemorrhagic conditions in the Philippines.¹ As part of our continuing chemical studies on Thai medicinal mangrove plants, we report herein the isolation and structural elucidation of three new dammarane triterpenoids (**1–3**) along with 18 known compounds from the hypocotyls and fruits of *C. tagal*.

All compounds were obtained from *n*-hexane and dichloromethane extracts of the air-dried powdered hypocotyls and fruits of *C. tagal*, as described in the Experimental Section. Identifications of the known compounds were achieved by comparison with previously reported spectroscopic data and characterized as dammarane triterpenoids: isofouquierol (**4**),² fouquierol (**5**),² dammarenediol II,³ and ocotillol II.⁴ The stereochemistry at C-20 of all isolated dammaranes was characterized as *S* configured by comparison of the carbon chemical shifts at C-17, C-20, and C-21 with the corresponding reported dammaranes.^{3,18} The other isolates were characterized as oleanolic acid,⁵ 3 β -*E*-feruloyllupeol,⁶ 3 β -*Z*-feruloyllupeol,⁶ 3 β -*E*-feruloyl-betulin,⁷ 3 β -*E*-feruloylbetulinic acid (**6**),⁸ 3 β -*E*-caffeoylbetulinic acid,⁹ 3 β -*E*-caffeoylbetulin,⁹ 3 β -*E*-coumaroyllupeol,¹⁰ 3 β -acetylbetulinic acid,¹¹ betulin,¹² betulinic acid,¹³ 3 α -betulinic acid,¹³ betulonic acid,¹⁴ and lupeol.¹⁵ The ¹³C NMR spectroscopic data of isofouquierol and fouquierol are shown here as the first complete report (Table 2).

The ESITOFMS of **1** revealed a pseudomolecular ion peak at *m/z* 499.3754 [*M* + Na]⁺, corresponding to the molecular formula C₃₀H₅₂O₄ (calcd for C₃₀H₅₂O₄Na *m/z* 499.3763). The EIMS showed fragment peaks at *m/z* 317 [*M* – side chain]⁺, 299 [*M* – side chain – H₂O]⁺, 207 [side chain + ring D – HO]⁺, and 125 [side chain – H₂O]⁺. The IR spectrum displayed absorption bands at 3450 and 1655 cm^{–1} for hydroxyl and double-bond functionalities, respectively. In the ¹³C NMR spectrum (Table 2), compound **1** showed 30 carbon resonances, of which the signals of C-1 to C-19 and C-28 to C-30 agreed well with those of compounds **4** and **5** (Table 1). Therefore, compound **1** was suggested to be a dammarane triterpenoid derivative. Characteristic for a tetracyclic dammarane, five methyl



singlets at δ 0.78 (Me-29), 0.85 (Me-19), 0.87 (Me-30), 0.96 (Me-18), and 0.97 (Me-28) appeared in the ¹H NMR spectrum of **1**. The oxymethine proton (H-3) resonated at δ 3.20 (dd, *J* = 11.1, 5.7 Hz), showing *J* values consistent with an axial orientation.¹⁶ The ¹³C NMR spectrum and DEPT experiments revealed the side chain (C-20 to C-27) as having two methyls (δ 17.1, 24.6), three methylenes (δ 24.6, 36.5, 113.7), one oxymethine carbon (δ 89.5), and two quaternary carbons (δ 75.1, 144.1). The terminal olefinic methylene protons at δ 5.02 (2H, m) at C-26 (δ 113.7) showed the HMBC correlations with the C-27 vinyl methyl carbon (δ 17.1), the C-25 olefinic carbon (δ 144.1), and the C-24 carbon (δ 89.5). An oxymethine proton at δ 4.31 (t, *J*

* To whom correspondence should be addressed. Tel: +6674288444. Fax: +6674212918. E-mail: chatchanok.k@psu.ac.th or chatchanok_k@yahoo.com.

[†] Department of Chemistry, Prince of Songkla University.

[§] Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University.

[‡] Institute of Science, Walailak University.

Table 1. ^1H NMR Spectroscopic Data (δ ppm) of Compounds **1–3** (300 MHz, CDCl_3) (J in Hz)

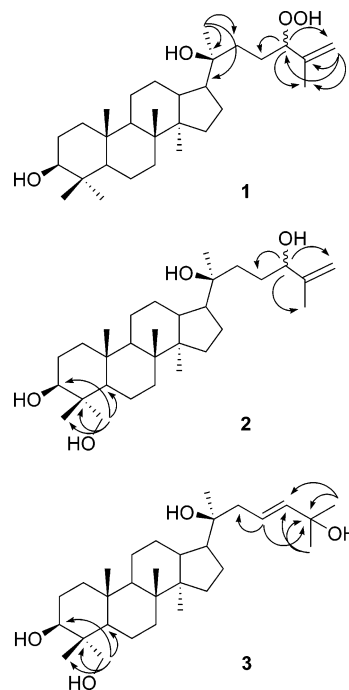
H	1	2	3
3	3.20 dd (11.1, 5.7)	3.64 dd (8.4, 7.8)	3.64 dd (8.4, 7.8)
5	0.73 m	1.36 m	1.35 m
9	1.33 m	0.88 m	0.88 m
13	1.63 m	1.62 m	1.69 m
17	1.72 m	1.75 m	1.74 m
18	0.96 s	0.96 s	0.97 s
19	0.85 s	0.89 s	0.89 s
21	1.13 s	1.15 s	1.13 s
22			2.19 m
23			5.69 m
24	4.31 t (6.3)	4.04 m	5.69 m
26	5.02 m	4.96 brs, 4.84 m	1.33 s
27	1.75 s	1.74 s	1.33 s
28	0.97 s	3.72 d (10.5), 3.42 d (10.5)	3.72 d (10.2), 3.43 d (10.2)
29	0.78 s	0.88 s	0.88 s
30	0.87 s	0.87 s	0.87 s
OOH	8.08 brs		

Table 2. ^{13}C NMR Spectroscopic Data (δ ppm) of Compounds **1–5** (75 MHz, CDCl_3)

carbon	1	2	3	4	5
1	39.0	38.7	38.7	39.0	39.0
2	27.4	27.0	27.0	27.5	27.5
3	78.8	76.6	76.6	79.0	79.0
4	38.9	42.0	42.0	39.0	39.0
5	55.8	50.6	50.6	55.9	55.9
6	18.2	18.4	18.4	18.3	18.3
7	35.2	35.0	35.0	35.2	35.2
8	40.3	40.4	40.4	40.4	40.4
9	50.6	50.4	50.4	50.6	50.6
10	37.0	37.0	37.0	37.1	37.1
11	21.5	21.5	21.5	21.5	21.5
12	24.8	24.9	24.8	24.9	24.8
13	42.3	42.3	42.4	42.4	42.4
14	50.3	50.3	50.3	50.3	50.3
15	31.1	31.2	31.1	31.1	31.2
16	27.0	27.5	27.5	27.4	27.4
17	49.6	50.1	49.9	50.1	50.1
18	15.3	15.5	15.5	15.5	15.5
19	16.1	16.5	16.6	16.2	16.2
20	75.1	75.1	75.1	75.1	75.2
21	24.6	25.4	25.8	25.7	25.3
22	36.5	36.6	43.4	43.4	36.0
23	24.6	29.3	122.4	122.4	29.7
24	89.5	76.5	142.1	142.0	75.9
25	144.1	147.6	70.8	70.8	147.6
26	113.7	110.9	30.0	29.9	110.8
27	17.1	17.8	29.9	29.9	18.0
28	27.8	71.9	71.9	28.0	28.0
29	15.3	11.3	11.3	15.4	15.4
30	16.4	16.5	16.5	16.4	16.5

= 6.3 Hz) at C-24 (δ 89.5) showed HMBC correlations with C-23 (δ 24.6), C-27 (δ 17.1), and C-26 (δ 113.7) (Figure 1). The presence of a hydroperoxy group at C-24 was supported by the molecular formula $\text{C}_{30}\text{H}_{52}\text{O}_4$, a broad downfield signal of a hydroperoxy proton at δ 8.08, and the downfield chemical shift of the oxygenated carbon C-24 at δ 89.5.¹⁷ Thus, compound **1** (cereotagaloperoxide) was determined structurally as 20(*S*)-3 β ,20-dihydroxy-24-perhydroxydammar-24-ene.

Compound **2** was obtained as an amorphous powder. The ESITOFMS showed a pseudomolecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 499.3767, indicating a molecular formula of $\text{C}_{30}\text{H}_{52}\text{O}_4$ (calcd for $\text{C}_{30}\text{H}_{52}\text{O}_4\text{Na}$ m/z 499.3763). The IR spectrum showed the same pattern as that of **1**. In the ^{13}C NMR spectrum, signals were displayed for four oxygenated carbons at δ 71.9, 75.1, 76.5, and 76.6. Comparison of ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **1** and **2** revealed

**Figure 1.** Selective HMBC correlations of **1–3**.

that the latter compound has one more hydroxymethylene group and one less methyl group on a quaternary carbon than **1**. The NMR signals for an oxymethine proton (H-24) at δ 4.04 and carbon (C-24) at δ 76.5 were shifted upfield in comparison with those of **1** (δ_{H} 4.31, δ_{C} 89.5), indicating that C-24 is connected to a hydroxyl group.^{2,18} The ^{13}C NMR spectra suggested that, except for a hydroxyl group at C-24, **1** and **2** have similar side chains; therefore, the additional hydroxymethylene group should be at the tetracyclic moiety. This was assigned at C-28 (δ 71.9) due to HMBC correlations of H₂-28 (δ 3.72, 3.42, each d, J = 10.5 Hz) with C-3 (δ 76.6), C-4 (δ 42.0), C-5 (δ 50.6), and C-29 (δ 11.3) (Figure 1). ^{13}C NMR spectroscopic data of the side chain (C-20 to C-27) of **2** agreed well with those of fouquierol (**5**) (Table 2). Thus, compound **2** (cereotagalol A) was identified as 20(*S*)-3 β ,20,24,28-tetrahydroxydammar-25-ene.

Compound **3** was obtained as a colorless gum. This compound also exhibited a pseudomolecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 499.3754 in the ESITOFMS. The ^1H and ^{13}C NMR spectra for the tetracyclic moiety of **3** (Tables 1 and 2) were similar to those of **2**, with signals for the hydroxymethylene protons (H₂-28) at δ 3.72 and 3.43 and the corresponding carbon C-28 at δ 71.9. The difference was in the side chain (C-20 to C-27). Two olefinic methine protons in **3** with the same chemical shift at δ 5.69 (m) on the sp^2 carbons at C-23 (δ 122.4) and C-24 (δ 142.1) replaced two terminal methylene protons (H₂-26) and carbons (C-25, C-26) in **2**. Three singlet methyl signals were shown at δ_{H} 1.13, 1.33, and 1.33 and δ_{C} 25.8, 30.0, and 29.9, respectively. These results were also confirmed by the HMBC correlation as follows: Me-26 (δ 1.33) and Me-27 (δ 1.33) showed correlation with C-25 (δ 70.8) and C-24 (δ 142.1), and H-23 (δ 5.69) with C-25 (δ 70.8) and C-22 (δ 43.4) (Figure 1). The NMR spectroscopic data of the side chain (C-20 to C-27) agreed well with those of isofouquierol (**4**)² and isofouquierone.¹⁸ Thus, compound **3** (cereotagalol B) was identified as 20(*S*)-3 β ,20,25,28-tetrahydroxydammar-23-ene.

Most isolates from *C. tagal* were tested for cytotoxic activity. Only 3 β -*E*-feruloylbetulinic acid (**6**) exhibited potent

cytotoxic activity for KB, BC, and NIC-H187 cell lines, with IC₅₀ values of 3.8, 3.0, and 1.8 µg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Electrothermal melting point apparatus model IA6301 and were uncorrected. Optical rotations were measured on an Autopol II automatic polarimeter. UV spectra were determined on a Shimadzu UV 160A spectrophotometer. The IR spectra were recorded on a Nicolet Magna IR 560 spectrophotometer and a Perkin-Elmer 2000 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker Avance-300 spectrometer, operating at 300 MHz for proton and 75 MHz for carbon. Chemical shifts (δ) were expressed in ppm with reference to internal TMS in CDCl₃. The EIMS was performed using a Thermofinnigan MAT 95 XL mass spectrometer. The ESITOFMS were obtained on a Micromass LCT mass spectrometer. Quick column chromatography and column chromatography were carried out on silica gel 60 F₂₅₄ and silica gel 100, respectively. Precoated plates of silica gel 60 GF₂₅₄ were used for analytical purposes.

Plant Material. The hypocotyls and fruits of *C. tagal* were collected at the Mangrove Research Station in Nakhon Si Thammarat Province, Thailand, in November 2002. The plant was identified by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand, and a voucher specimen (No. PSU 0012581) has been deposited at the same institute.

Extraction and Isolation. Dried milled hypocotyls of *C. tagal* (5.3 kg) were extracted with hexane and CH₂Cl₂, successively. Evaporation resulted in the crude extracts of hexane (32.9 g) and CH₂Cl₂ (128.6 g), respectively. A portion of the hexane extract (17.1 g) was subjected to column chromatography using gradient elution of hexane and ethyl acetate (10:0–7:3) to afford 12 fractions (A01–A12). Fraction A02 (1.43 g) was subjected to quick column chromatography using hexane and ethyl acetate mixtures with increasing polarity as eluting solvent (10:0–9:1) to afford 3β-*E*-feruloyllupeol (160 mg) after crystallization from acetone. Fraction A03 (150 mg) was subjected to quick column chromatography using hexane–ethyl acetate (9.5:0.5) as eluting solvent to afford 3β-*Z*-feruloyllupeol (64 mg). Fraction A05 (520 mg) was subjected to quick column chromatography using hexane–ethyl acetate (9:1) as eluting solvent to give 3β-acetylbetulinic acid (38 mg) after crystallization from acetone.

The gummy residue from the CH₂Cl₂ extract (25.0 g) was treated with acetone to give acetone-soluble and -insoluble fractions. The acetone-soluble fraction was concentrated to afford a gummy residue (14.3 g), which was subjected to column chromatography. The column was eluted with a gradient elution of hexane and ethyl acetate (10:0–7:3). The eluted fractions were combined on the basis of TLC to give 15 fractions (B01–B15). 3β-*E*-Feruloyllupeol (40 mg) was isolated from fraction B02 (120 mg) by crystallization from acetone. Fractions B03 (224 mg), B04 (853 mg), B06 (93 mg), B09 (194 mg), and B12 (321 mg) were successively subjected to quick column chromatography using hexane–ethyl acetate (9:1–8:2) as eluting solvent to afford lupeol (14 mg) from fraction B03, 3β-*E*-coumaroyllupeol (5 mg) and 3α-betulinic acid (15 mg) from fraction B04, betulin (7 mg) and betulinic acid (9 mg) from fraction B06, 3β-*E*-feruloylbetulinic acid (97 mg) and 3β-*E*-feruloylbetulin (64 mg) from fraction B09, and 3β-*E*-caffeoylbetulinic acid (8.1 mg) and 3β-*E*-caffeoylbetulin (7.2 mg) from fraction B12. Fractions B14 (524 mg) and B15 (347 mg) were successively subjected to quick column chromatography using hexane–acetone (8:2) as eluting solvent to afford betulinic acid (25 mg) and 3 (11 mg) from fraction B14, and compound 2 (4 mg) from fraction B15.

Dried milled fruits of *C. tagal* (574.0 g) were extracted with hexane and CH₂Cl₂, successively. Evaporation resulted in the dried hexane (6.0 g) and CH₂Cl₂ (6.1 g) extracts. The combined

dried crude hexane and CH₂Cl₂ extracts (12.1 g) was subjected to quick column chromatography using gradient elution by a mixture of hexane and ethyl acetate (10:0–7:3) to afford 10 fractions (C01–C10) on the basis of TLC analysis. Fraction C06 (443 mg) was subjected to quick column chromatography using hexane–ethyl acetate (9:1) as eluting solvent, to afford dammarenediol II (31 mg) and compound 1 (40 mg), the latter after crystallization from acetone. Fraction C07 (717 mg) was subjected to quick column chromatography using gradient elution of a hexane and acetone mixture with increasing polarity (9:1–8:2) to afford oleanolic acid (30 mg), ocotillol II (6 mg), fouquierol (12 mg), and isofouquierol (6 mg).

Bioassay. The cytotoxicity assay employed the colorimetric method.¹⁹ The reference substance, ellipticine, exhibited activities toward human oral epidermoid carcinoma (BC), human breast cancer (KB), and human small cell lung cancer (NCI-H187) cell lines in the IC₅₀ range of 0.3–0.6 µg/mL.

Cereotagaloperoxide (1): amorphous powder; mp 183–185 °C; [α]_D²⁷ +54.1 (c 0.04, MeOH); IR (KBr) ν_{max} 3450, 1655, 1460, 1375 cm⁻¹; ¹H NMR data, see Table 1; ¹³C NMR data, see Table 2; EIMS *m/z* 476 [M]⁺ (0.5), 317 (5), 299 (10), 207 (60), 125 (100); ESITOFMS *m/z* 499.3754 (calcd for C₃₀H₅₂O₄+Na, 499.3763).

Cereotagalol A (2): colorless gum; [α]_D²⁷ +52.6 (c 0.02, MeOH); IR (KBr) ν_{max} 3390, 1660, 1380 cm⁻¹; ¹H NMR data, see Table 1; ¹³C NMR data, see Table 2; ESITOFMS *m/z* 499.3767 (calcd for C₃₀H₅₂O₄+Na, 499.3763).

Cereotagalol B (3): colorless gum; [α]_D²⁷ +55.6 (c 0.02, MeOH); IR (KBr) ν_{max} 3480 cm⁻¹; ¹H NMR data, see Table 1; ¹³C NMR data, see Table 2; ESITOFMS *m/z* 499.3754 (calcd for C₃₀H₅₂O₄+Na, 499.3763).

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References and Notes

- (1) Bamroongrugs, N. *Songklanakarin J. Sci. Technol.* **1999**, *21*, 377–386.
- (2) Butruille, D.; Dominguez, X. A. *Tetrahedron Lett.* **1974**, 639–642.
- (3) Asakawa, J.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Tetrahedron* **1977**, *33*, 1935–1939.
- (4) Tanaka, R.; Masuda, K.; Matsunaga, S. *Phytochemistry* **1993**, *32*, 472–474.
- (5) Seebacher, W.; Simic, N.; Weis, R.; Saf, R.; Kunert, O. *Magn. Reson. Chem.* **2003**, *41*, 636–638.
- (6) Ponglimanont, C.; Thongdeeying, P. *Aust. J. Chem.* **2005**, *58*, 615–618.
- (7) Kuo, Y.-H.; Chang, C.-I.; Kuo, Y.-H. *Phytochemistry* **1997**, *46*, 1135–1137.
- (8) Siddiqui, B. S.; Kardar, M. N. *Phytochemistry* **2001**, *58*, 1195–1198.
- (9) Chen, B.; Duan, H.; Takaishi, Y. *Phytochemistry* **1999**, *51*, 683–687.
- (10) Kuo, Y.-H.; Chang, C.-I.; Li, S.-Y.; Chou, C.-J.; Chen, C.-F.; Kuo, Y.-H.; Lee, K.-H. *Planta Med.* **1997**, *63*, 363–365.
- (11) Evers, M.; Poujade, C.; Soler, F.; Ribeill, Y.; James, C.; Lelievre, Y.; Gueguen, J.-C.; Reisdorf, D.; Morize, I.; Pauwels, R.; Clercq, E.; Henin, Y.; Bousseau, A.; Mayaux, J.-F.; Pecq, J.-B.; Dereu, N. *J. Med. Chem.* **1996**, *39*, 1056–1068.
- (12) Tinto, W. F.; Blair, L. C.; Alli, A.; Reynolds, W. F.; McLean, S. J. *Nat. Prod.* **1992**, *55*, 395–398.
- (13) Kitajima, J.; Shindo, M.; Tanaka, Y. *Chem. Pharm. Bull.* **1990**, *38*, 714–716.
- (14) Akihisa, T.; Takamine, Y.; Yoshizumi, K.; Tokuda, H.; Kimura, Y.; Ukiya, M.; Nakahara, T.; Yokochi, T.; Ichiishi, E.; Nishino, H. *J. Nat. Prod.* **2002**, *65*, 278–282.
- (15) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517–1575.
- (16) Rouf, A. S. S.; Ozaki, Y.; Rashid, M. A.; Rui, J. *Phytochemistry* **2001**, *56*, 815–818.
- (17) Cabrera, G. M.; Seldes, A. M. *J. Nat. Prod.* **1995**, *58*, 1920–1924.
- (18) Waterman, P. G.; Ampofo, S. *Phytochemistry* **1985**, *24*, 2925–2928.
- (19) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107–1112.