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Potentially Cytotoxic Triterpenoids from the Root Bark of *Siphonodon celastrineus* Griff.

Chokchai Niampoka, Rutt Suttisri, Rapepol Bavovada, Hiromitsu Takayama[1], and Norio Aimi[1]

Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand and [1]Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

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A new oleanane-triterpene, 3β-acetoxy-11α-benzoyloxy-13β-hydroxyolean-12-one (**1**), was isolated along with a known quinone-methide triterpene, pristimerin (**2**), from the root bark of *Siphonodon celastrineus* Griff., a Thai medicinal plant of the family Celastraceae. Their structures were determined based on spectroscopic analysis.

Key words: *Siphonodon celastrineus*, Celastraceae, Oleanane-triterpene, Quinone-methide triterpene

INTRODUCTION

Siphonodon celastrineus Griff. (Celastraceae) is a tree up to 25 m in height found in the northern and central parts of Thailand. Its root is used in traditional Thai medicine for the treatment of inflammation, abscess, skin diseases and as a bone tonic (Chayamarit, 1985). Recently, the ethanolic extract of its leaves was shown to be cytotoxic against the breast cancer cell line MCF-7 with an IC_{50} value of 17.1 μg/mL (Itharat *et al.*, 2004). However, no previous chemical investigations have been done on this plant. In our continuing study on the chemical constituents of celastraceous plants, we have isolated a new oleanane-triterpene, 3β-acetoxy-11α-benzoyloxy-13β-hydroxyolean-12-one (**1**), and a known quinone-methide triterpene, pristimerin (**2**), from the methanol extract of the root bark of this plant.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on a Yanagimoto micro hot-stage melting point apparatus and uncorrected. UV spectra were recorded using a Shimadzu UV-160A spectrometer in MeOH. IR spectra were obtained on a Perkin Elmer Spectrum 2000 FT-IR spectrophotometer. Optical rotation was measured on a Perkin Elmer 341 polarimeter in MeOH or $CHCl_3$. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a JEOL JNM-A500 (Alpha series) NMR spectrometer and chemical shifts are expressed as d values with TMS as an internal standard. Fast atom bombardment (FAB) mass spectra were acquired using a JEOL HX-110A mass spectrometer. Column chromatography was carried out on silica gel (Kieselgel 60, 230-400 mesh, Merck Co.) and Sephadex LH-20 (Pharmacia). TLC was performed on silica gel 60 F_{254} plates (0.25 mm, Merck Co.), and spots were detected under UV light and also by spraying with 10% H_2SO_4 solution followed by heating.

Plant materials

The root bark of *Siphonodon celastrineus* was collected in Phitsanulok, Thailand in September 2000 and identified by Prof. Thawatchai Santisuk, Royal Forest Department, Thailand. Voucher specimens (No. RB-00091) were deposited at the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Extraction and isolation

Dried and powdered root bark of *Siphonodon celastrineus* (120 g) was macerated four times with MeOH. The combined solutions were then evaporated under reduced pressure to give a MeOH extract (8.27 g), which was

Correspondence to: Rutt Suttisri, Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
Tel: 66-2-2188353, Fax: 66-2-2188354
E-mail: srutt@chula.ac.th

chromatographed on a silica gel column (0.040-0.063 mm, 400 g), eluting with *n*-hexane-EtOAc (9:1 → 1:9) to yield 7 major fractions (A-G). Recrystallization of fraction B (190 mg) in $CHCl_3$ gave compound **1** (110 mg, colorless crystals). Fraction F (1.12 g), subjected to silica gel (50 g) column chromatography, using *n*-hexane-$CHCl_3$-acetone (8:1:1) as eluent, gave 5 subfractions (subfr. F1-F5). Subfraction F2 (320 mg) was further purified on two successive silica gel columns (10 g each) eluted with *n*-hexane-acetone (9:1) and $CHCl_3$-MeOH (22:1), respectively, and, finally, on a Sephadex LH-20 column, washed down with $CHCl_3$-MeOH (1:1), to yield compound **2** (11 mg, orange-red crystals).

3β-Acetoxy-11α-benzoyloxy-13β-hydroxyolean-12-one (1)

Colorless crystals, m.p. 310°C; $[\alpha]_D$ +11° (*c* = 0.05, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 229 (4.40). IR (KBr) cm^{-1}: 3469 (OH), 2930, 1717 (C=O), 1279, 1117, 710. FAB-MS *m/z* (% rel. int.): 643 $[M+Na]^+$ (6), 621 $[M+H]^+$ (3), 603 (10), 498 (5), 481 (8), 470 (7), 439 (8), 421 (10), 154 (100), 105 (92), 77 (24). *Anal.* Calcd for $C_{39}H_{56}O_6$: C, 75.43; H, 9.10. Found: C, 75.39; H, 9.13. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, s, H-29), 0.86 (3H, s, H-30), 0.87 (3H, s, H-24), 0.90 (3H, s, H-23), 1.03 (3H, s, H-27), 1.05 (3H, s, H-25), 1.22 (3H, s, H-28), 1.49 (3H, s, H-26), 2.03 (3H, s, $OCO\underline{C}H_3$), 2.06 (1H, d, *J* = 12.6 Hz, H-9), 3.50 (1H, br s, OH-13), 4.49 (1H, dd, *J* = 11.8, 4.7 Hz, H-3), 6.44 (1H, d, *J* = 12.6 Hz, H-11), 7.45 (2H, t, *J* = 7.4 Hz, H-3', H-5'), 7.57 (1H, t, *J* = 7.4 Hz, H-4'), 8.07 (2H, d, *J* = 7.4 Hz, H-2', H-6'). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 16.4 (q, C-24, C-25), 17.7 (t, C-6), 18.9 (q, C-27), 20.9 (q, C-26), 21.4 (q, $OCO\underline{C}H_3$), 22.9 (t, C-15), 23.9 (t, C-2), 24.5 (q, C-30), 28.2 (q, C-23), 30.0 (t, C-22), 31.4 (q, C-28), 31.5 (s, C-20), 32.3 (q, C-29), 33.5 (s, C-17), 34.0 (t, C-7), 34.5 (t, C-21), 38.3 (t, C-19), 38.4 (s, C-4), 39.1 (t, C-16), 39.3 (s, C-10), 40.3 (t, C-1), 44.1 (s, C-8), 44.8 (s, C-14), 49.1 (d, C-18), 54.2 (d, C-9), 55.2 (d, C-5), 75.3 (d, C-11), 80.2 (d, C-3), 83.2 (s, C-13), 128.6 (d, C-3', C-5'), 129.7 (s, C-1'), 130.1 (d, C-2', C-6'), 133.2 (d, C-4'), 165.6 (s, C-7'), 171.2 (s, $O\underline{C}OCH_3$), 202.2 (s, C-12).

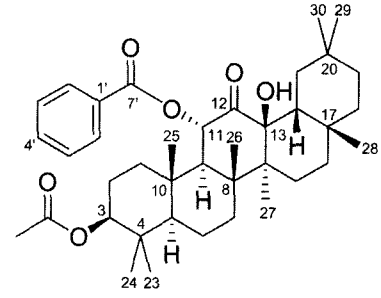
Pristimerin (2)

Orange-red crystals, m.p. 219-220°C, $[\alpha]_D$ -188° (*c* = 0.06, MeOH). UV λ_{max} (MeOH) nm (log ε): 255 (2.34), 426 (2.52). IR (KBr) cm^{-1}: 3414, 2929, 1727, 1615, 1438, 1384, 616. FAB-MS *m/z* (% rel. int.): 487 $[M+Na]^+$ (20), 465 $[M+H]^+$ (30), 464 $[M]^+$ (10), 263 (10), 202 (35), 201 (100). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.53 (3H, s, H-27), 1.10 (3H, s, H-28), 1.17 (3H, s, H-30), 1.26 (3H, s, H-26), 1.45 (3H, s, H-25), 2.20 (3H, s, H-23), 3.55 (3H, s, $COO\underline{C}H_3$), 6.34 (1H, d, *J* = 7.0 Hz, H-7), 6.53 (1H, d, *J* = 1.5 Hz, H-1), 6.96 (1H, br s, OH-3), 7.01 (1H, dd, *J* = 7.0, 1.5 Hz, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 10.2 (q, C-23), 18.3 (q, C-27), 21.6 (q, C-26), 28.6 (t, C-15), 29.6 (t, C-12), 29.9 (t, C-21), 30.5 (s, C-17), 30.9 (t, C-19), 31.6 (q, C-28), 32.7 (q, C-30), 33.6 (t, C-11), 34.8 (t, C-22), 36.4 (t, C-16), 38.3 (q, C-25), 39.4 (s, C-13), 40.4 (s, C-20), 42.9 (s, C-9), 44.3 (d, C-18), 45.0 (s, C-14), 51.6 (q, $COO\underline{C}H_3$), 117.1 (s, C-4), 118.1 (d, C-7), 119.6 (d, C-1), 127.4 (s, C-5), 134.0 (d, C-6), 146.0 (s, C-3), 164.8 (s, C-10), 170.0 (s, C-8), 178.3 (s, C-29), 178.7 (s, C-2).

RESULTS AND DISCUSSION

Column chromatography of the methanol extract of *S. celastrineus* root bark yielded compound **1** as colorless crystals and compound **2** as orange-red crystals.

Compound **1** displayed a positive FAB-MS ion peak at *m/z* 621 ($[M+H]^+$), which was in agreement with the molecular formula $C_{39}H_{56}O_6$. Its IR absorption bands at 3469 and 1717 cm^{-1} could be assigned to hydroxyl and carbonyl groups, respectively, within the molecule. In the ^{1}H-NMR spectrum of **1**, the presence of eight tertiary methyl groups [δ 0.84 (3H, s), 0.86 (3H, s), 0.87 (3H, s), 0.90 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.22 (3H, s), 1.49 (3H, s)] of an oleanane-triterpene skeleton and a three-proton singlet (δ 2.03) of an acetoxyl moiety could be observed. Two deshielded protons attaching to the oxygen-bearing methine C-3 (δ 80.2) and C-11 (δ 75.3) appeared as a doublet of doublet (1H, *J* = 11.8, 4.7 Hz) and a doublet (1H, *J* = 12.6 Hz) at δ 4.49 and 6.44, respectively. An HMBC correlation between H-3 (δ 4.49) and carbonyl



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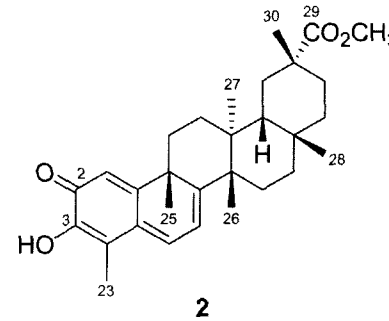

2

Fig. 1. Structure of compounds **1** and **2**

carbon signal of the acetoxyl group at δ 171.2 established the attachment of this group at C-3. Another set of proton signals at δ 8.07 (2H, d, *J* = 7.4 Hz), 7.57 (1H, t, *J* = 7.4 Hz) and 7.45 (2H, t, *J* = 7.4 Hz), together with prominent mass fragment peaks at *m/z* 105 and 77, were indicative of a benzoyl moiety within the molecule. This group is connected to the oleanane skeleton through the oxygen atom at C-11, as shown by the observed HMBC correlation between H-11 (δ 6.44) and its ester carbonyl signal at δ 165.6 (C-7'). The configurations of the acetoxyl group and the benzoyloxyl group were determined as β and α, respectively, according to the *trans* diaxial coupling constants of H-3 (*J* = 11.8 Hz) and H-11 (*J* = 12.6 Hz). ^{13}C-NMR and DEPT spectra of **1** also displayed a keto carbonyl resonance at δ 202.2 and a signal of an oxygen-bearing quaternary carbon at δ 83.2. The position of the keto carbonyl was established as at C-12, according to HMBC cross peaks between this carbonyl carbon and both H-9 (δ 2.06) and H-11 (δ 6.44), whereas the tertiary hydroxyl group could be located at C-13, which was supported by HMBC correlation between this carbon signal and Me-27 (δ 1.03). The structure of **1** is therefore quite similar to that of 3β,11α-diacetoxy-13β-hydroxyolean-12-one (Herath *et al.*, 2000), found as a constituent of the stem bark of *Gordonia ceylanica* (Theaceae), except the acetoxyl moiety at C-11 is replaced here by a benzoyloxyl group, and also to rubiprasin B (Itokawa *et al.*, 1989) isolated from the roots of *Rubia cordifolia* var. *pratensis* (Rubiaceae), except the latter compound has no substituent at C-11. The configuration of the hydroxyl group at C-13 in all of these compounds has to automatically be β in order to minimize the ring strain of the triterpene skeleton. Therefore, **1** was elucidated as 3β-acetoxy-11α-benzoyloxy-13β-hydroxyolean-12-one.

The ^{1}H-NMR spectrum of the orange-red compound **2** displayed signals for six tertiary methyl groups [δ 0.53 (3H, s), 1.10 (3H, s), 1.17 (3H, s), 1.26 (3H, s), 1.45 (3H, s), 2.20 (3H, s)] and a carbomethoxyl group (δ 3.55, 3H, s). The downfield proton resonances at δ 6.34 (1H, d, *J* = 7.0 Hz), 6.53 (1H, d, *J* = 1.5 Hz), 6.96 (1H, br s) and 7.01 (1H, dd, *J* = 7.0, 1.5 Hz), together with the UV absorption maxima of **2** at 426 and 255 nm, were indicative of the quinone-methide chromophore within the molecule. The positive FAB-MS ion peak at *m/z* 465 ([M+H]$^{+}$) and the number of its ^{13}C-NMR signals correspond to the molecular formula of **2** as $C_{30}H_{40}O_4$. The mass fragment peaks at *m/z* 201 and 202 are also characteristic of ring A/B quinone-methide triterpene with a hydroxyl group at C-3 (Brown *et al.*, 1973). The location of the ester carbonyl at C-29 was confirmed by the long-range HMBC correlation between its carbon signal (δ 178.3) and Me-30 (δ 1.17, 3H, s). Based on these spectroscopic evidences and comparison with reported NMR data (Calzada *et al.*, 1991), **2** was identified as pristimerin [(20α)-3-hydroxy-2-oxo-24-*nor*-friedela-1(10),3,5,7-tetraen-carboxylic acid-(29)-methylester]. This nortriterpene has been reported as a constituent of several celastraceous plants and has been demonstrated as possessing antitumoral (Chang *et al.*, 2003), antibacterial (Ankli *et al.*, 2000), antifeedant (Avilla *et al.*, 2000) and anti-inflammatory activities (Dirsch *et al.*, 1992; 1997). However, this is the first report of **2** in *Siphonodon* species.

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REFERENCES

Ankli, A., Heilmann, J., Heinrich, M., and Sticher, O., Cytotoxic cardenolides and antibacterial terpenoids from *Crossopetalum gaumeri*. *Phytochem.*, 54, 531-537 (2000).

Avilla, J., Teixido, A., Velazquez, C., Alvarenga, N., Ferro, E., and Canela, R., Insecticidal activity of *Maytenus* species (Celastraceae) nortriterpene quinone methides against codling moth, *Cydia pomonella* (L.) (Lepidoptera: tortricidae). *J. Agric. Food Chem.*, 48, 88-92 (2000).

Brown, P. M., Moir, M., Thonson, R. H., King, T. J., Krishnamoorthy, V., and Seshadri, T. R., Tingenone and hydroxytingenone, triterpenoid quinone methides from *Euonymus tingens*. *J. Chem. Soc., Perkin Trans.*, 1, 2721-2725 (1973).

Calzada, F., Mata, R., Lopez, R., Lineres, E., Bye, R., Barreto, V. M., and del Rio, F., Friedelanes and triterpenoid quinonemethide from *Hippocratea excelsa*. *Planta Med.*, 57, 194-195 (1991).

Chang, F. R., Hayashi, K., Chen, I. H., Liaw, C. C., Bastow, K. F., Nakanishi, Y., Nozaki, H., Cragg, G. M., Wu, Y. C., and Lee, K. H., Antitumor agents 228. Five new agarofurans, reissantins A-E, and cytotoxic principles from *Reissantia buchananii*. *J. Nat. Prod.*, 66, 1416-1420 (2003).

Chayamarit, K., Thai Medicinal Plants, Part 4, Chutima Publishing, Bangkok, pp. 330-332 (1985).

Dirsch, V., Wiemann, W., and Wagner, H., Antiinflammatory activity of triterpene quinone-methides and proanthocyanidins from the stem bark of *Heisteria pallida*. *Engl. Pharm. Pharmacol. Lett.*, 2, 184-186 (1992).

Dirsch, V. M., Kiemer, A. K., Wagner, H., and Vollmar, A. M., The triterpenoid quinonemethide pristimerin inhibits induction of inducible nitric oxide synthase in murine macrophages. *Eur. J. Pharmacol.*, 336, 211-217 (1997).

Herath, H. M. T. B., Athukoralage, P. S., and Jamie, J. F., Two

oleanane triterpenoids from *Gordonia ceylanica* and their conversions to taraxarane triterpenoids. *Phytochem.*, 54, 823-827 (2000).

Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H., and Raman, A., *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *J. Ethnopharmacol.*, 90, 33-38 (2004).

Itokawa, H., Qiao, Y.-F., Takeya, K., and Iitaka, Y., New triterpenoids from *Rubia cordifolia* var. *pratensis* (Rubiaceae). *Chem. Pharm. Bull.*, 37, 1670-1672 (1989).