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Tomentosones A and B, Hexacyclic Phloroglucinol Derivatives from the Thai Shrub *Rhodomyrtus tomentosa*

Asadhawut Hiranrat,[†] Wilawan Mahabusarakam,*[,†,‡] Anthony R. Carroll,[§,∥] Sandra Duffy,[∥] and Vicky M. Avery[∥]

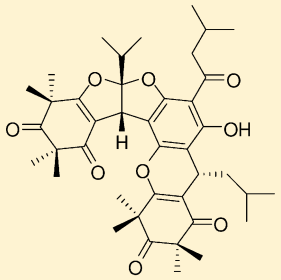
[†]Department of Chemistry and [‡]Natural Products Research Center, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[§]Environmental Futures Centre, Griffith University, Gold Coast, QLD 4222, Australia,

[∥]Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia

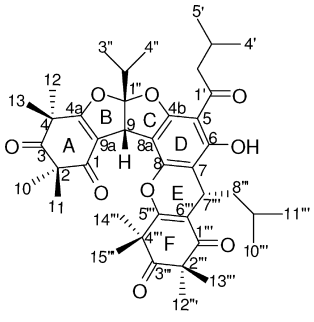
Supporting Information

ABSTRACT: Two phloroglucinols named tomentosones A and B (**1** and **2**) that each possess a novel hexacyclic ring system were isolated from the CH_2Cl_2 extract of *Rhodomyrtus tomentosa* leaves. Their structures were elucidated from analyses of 2D NMR spectroscopic data. Tomentosone A inhibited the growth of chloroquine-resistant and -sensitive strains of the malaria parasite *Plasmodium falciparum*, with IC_{50} values of 1.49 μM and 1.0 μM, respectively, while tomentosone B was significantly less active.

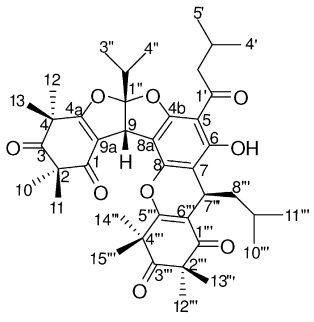


Tomentosone A

Rhodomyrtus tomentosa (Aiton) Hassk. is an evergreen shrub belonging to the family Myrtaceae and is a native of and widely distributed throughout Southeast Asia including Thailand.[1] Several biological activities have been reported for extracts of this plant, and some have exhibited antibacterial[2,3] and antihepatitis properties.[4] Furthermore, it is used in formulations of skin-whitening, antiaging, and skin-beautifying products.[5] Previous work on the antibacterial activities showed that an ethanolic extract demonstrated good activity against several types of Gram-positive bacteria.[3,6−8] We have previously reported the structures of four new acylphloroglucinols, rhodomyrtosones A−D, and six known compounds that were isolated from an acetone extract of the leaves.[9] Further investigation on a CH_2Cl_2 extract resulted in the isolation of two phloroglucinols named tomentosones A and B (**1** and **2**), each containing six continuous rings.



1



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Purification of the CH_2Cl_2 extract of *R. tomentosa* by first redissolving the extract in MeOH and removing insoluble material, followed by repeated silica gel column chromatography of the MeOH soluble material, eluting a gradient from hexane to acetone, yielded tomentosone A (22.2 mg, 0.001%) and tomentosone B (15.4 mg, 0.0007%).

Tomentosone A (**1**) was obtained as a yellowish gum with $[\alpha]^{25}_{D}$ −9.3 (*c* = 0.92, $CHCl_3$). The UV spectrum exhibited maximum absorption bands at 246, 260, 304, and 347 nm. The IR spectrum showed the absorption of a hydroxyl (3135 cm^{-1}), conjugated carbonyl (1656 cm^{-1}) and nonconjugated carbonyl (1720 cm^{-1}) groups. A molecular ion peak at *m*/*z* 688.3610 in the HREI-MS spectrum corresponded to the molecular formula of $C_{41}H_{52}O_9$. The ^{1}H NMR spectrum (Table 1) contained resonances for eight tertiary methyl groups at δ_H 1.47 (H_3-12), 1.43 (H_3-10), 1.36 (H_3-13), 1.24 (H_3-11), 1.42 (H_3-13‴), 1.36 (H_3-12‴), 1.74 (H_3-15‴), and 1.67 (H_3-14‴), six secondary methyl groups at δ_H 1.03 (H_3-4′), 1.01 (H_3-5′), 1.11 (H_3-3″), 1.09 (H_3-4″), 0.75 (H_3-10‴) and 0.83 (H_3-11‴), two methylene groups at δ_H 2.99 and 2.89 (H_2-2′) and 1.40 (H_2-8‴), five methine protons at δ_H 4.74 (*s*, H-9), δ_H 4.28 (*t*, H-7‴), δ_H 2.37 (*hept*, H-2″), δ_H 2.23 (H-3′) and 1.40 (H-9‴), and one hydrogen-bonded hydroxyl proton at δ_H 13.62 (6-OH). Correlations observed in a COSY spectrum allowed isopropyl, isobutyl and isopentyl moieties to be assigned in **1**. The resonances of five carbonyl carbons, 10 sp^2 hybridized quaternary carbons, five sp^3 hybridized quaternary carbons,

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Table 1. NMR Data for Tomentosone A (1) and B (2) in $CDCl_3$[a]

	1		2	
no.	δ_C (mult)[b]	δ_H (J, Hz)	δ_C (mult)[b]	δ_H (J, Hz)
1	192.0 s		192.1 s	
2	56.1 s		55.7 s	
3	212.1 s		212.0 s	
4	45.5 s		45.4 s	
4a	176.0 s		176.9 s	
4b	158.4 s		158.7 s	
5	104.0 s		103.9 s	
6	162.5 s		162.8 s	
7	108.7 s		107.9 s	
8	152.2 s		152.1 s	
8a	104.9 s		103.8 s	
9	45.9 d	4.74 (s)	46.3 d	4.81 (s)
9a	113.0 s		113.2 s	
10	23.8 q	1.43 (s)	24.8 q	1.36 (s)
11	25.8 q	1.24 (s)	24.1 q	1.24 (s)
12	24.5 q	1.47 (s)	24.2 q	1.49 (s)
13	23.8 q	1.36 (s)	24.0 q	1.41 (s)
1′	204.6 s		204.7 s	
2′	51.9 t	2.99 (dd; 15.0, 6.9)	52.0 t	3.12 (dd; 14.7, 6.6)
		2.89 (dd; 15.0, 6.9)		2.73 (dd; 14.7, 6.6)
3′	25.6 d	2.23 (m; 6.9)	26.0 d	2.18 (m; 6.6)
4′	22.8 q	1.03 (d; 6.9)	22.5 q	0.99 (d; 6.6)
5′	22.7 q	1.01 (d; 6.9)	22.9 q	1.03 (d; 6.6)
1″	128.8 s		128.9 s	
2″	34.9 d	2.37 (hept; 6.9)	34.9 d	2.35 (hept; 6.9)
3″	15.8 q	1.11 (d; 6.9)	15.7 q	1.11 (d; 6.9)
4″	15.8 q	1.09 (d; 6.9)	15.8 q	1.08 (d; 6.9)
1‴	197.5 s		198.3 s	
2‴	56.3 s		55.3 s	
3‴	212.5 s		212.6 s	
4‴	47.6 s		47.9 s	
5‴	167.7 s		165.9 s	
6‴	113.9 s		113.6 s	
7‴	25.3 d	4.28 (t; 5.4)	25.4 d	4.32 (dd; 5.7, 4.2)
8‴	45.4 t	1.40 (obscure)	45.3 t	1.51 (m)
9‴	25.1 d	1.40 (obscure)	25.1 d	1.40 (obscure)
10‴	23.2 q	0.83 (d; 6.6)	23.3 q	0.85 (d; 6.3)
11‴	23.6 q	0.75 (d; 6.6)	23.4 q	0.69 (d; 6.3)
12‴	22.0 q	1.36 (s)	24.3 q	1.37 (s)
13‴	24.7 q	1.42 (s)	24.2 q	1.39 (s)
14‴	25.3 q	1.67 (s)	25.8 q	1.81 (s)
15‴	23.2 q	1.74 (s)	21.9 q	1.42 (s)
6-OH		13.62 (s)		13.64 (s)

[a] ^{1}H NMR at 300 MHz referenced to residual $CHCl_3$ solvent (δ_H 7.26) and ^{13}C NMR at 75 MHz referenced to $CDCl_3$ (δ_C 77.0). [b] Multiplicity obtained from the DEPT experiment.

five methine carbons, two methylene carbons, and 14 methyl carbons were deduced from ^{13}C, DEPT, and HMQC NMR data (Table 1). Correlations observed from the eight methyl singlets in a HMBC spectrum (Figure 1) allowed two 1,1,3,3-tetramethyl β triketone moieties to be assigned in **1**. The isopentyl group was attached to one of these β-triketones since HMBC correlations were observed from H-7‴ to C-6 (δ_C 162.5), C-8 (δ_C 152.9), C-1‴ (δ_C 197.5) and C-5‴ (δ_C 167.7). The presence of a phloroglucinol moiety was deduced from correlations between H-7‴ and C-6 (δ_C 162.5), C-8 (δ_C 152.9), H-9 to C-4a, C-8 and C-8a′ and 6-OH to C-5, C-6 and C-7. Additional HMBC correlations from the methine proton H-9 to C-4a, C-9a and C-2″ as well as that of the methyl protons of the isopropyl group to C-1″ (δ_C 128.8) indicated that the second β-triketone was attached to the phloroglucinol moiety via a bisfuran fused-ring bearing the isopropyl group as for rhodomyrtosone A.[9] HMBC correlations from the isobutyl protons H-2′ and H-3′ to the only remaining unassigned carbonyl carbon C-1′ indicated that an isovaleryl group was present in the molecule. Since the phenolic proton 6-OH resonated significantly downfield it must show a strong intramolecular hydrogen bond, and this suggested that the isovaleryl group was attached to the carbon *ortho* to it at C-5. Finally, an additional ring between C-8 and C-5‴ via an oxygen bridge was required to fulfill the double bond equivalence defined by the molecular formula assigned to **1**.

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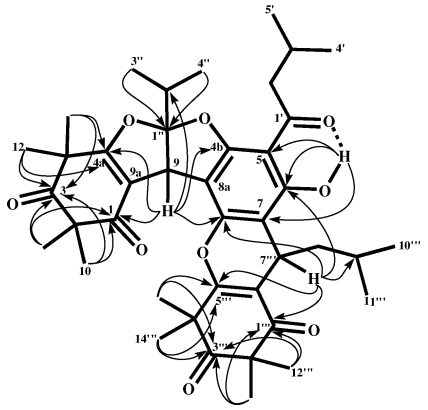

Figure 1. Major HMBC correlations observed for tomentosone A (**1**) and B (**2**).

Tomentosone B (**2**) was obtained as a pale yellow solid with mp 198−199 °C and $[\alpha]^{25}_{D}$ −10.7 (*c* = 0.68, $CHCl_3$). Its molecular formula of $C_{41}H_{52}O_9$ (M^+ 688.3610) obtained from HREIMS and other spectroscopic data (UV, IR, MS, ^1H NMR (Table 1) and ^{13}C NMR (Table 1)) were very similar to those of **1** with the only significant difference being the methyl proton signals (H_3-14‴/H_3-15‴, δ_H 1.81/1.42 for **2**; δ_H 1.67/1.74 for **1**) and nonequivalent methylene protons (H_2-2′, δ_H 3.12/2.73 for **2**; δ_H 2.99/2.89 for **1**) in the ^1H NMR spectrum. A similar combination of HMBC correlations in **2** to those observed for **1** (Table S1, Supporting Information) suggested **2** was a diastereomer of **1**.

Since both compounds possess three stereogenic centers, **1** and **2** could be diasteriomeric at C-9, C-1″, and/or C-7‴. The relative configurations of the three stereogenic centers in **1** and **2** were determined from analysis of correlations observed in ROESY spectra acquired for both compounds. ROESY correlations were observed between H-9 and H-2″, 3″-CH_2, and 4″-CH_2 in both compounds indicating that the bisfurano group bears a *cis* ring junction, and thus, **1** and **2** must be epimeric at C-7‴. Correlations were also observed from H-9 in **1** and **2** to the methyl protons, 15‴-CH_3, that lie on the β face in both compounds. Correlations between 15‴-CH_3 and the other β methyl, 13‴-CH_3, in ring F were also observed in the ROESY spectra in both compounds. A ROESY correlation was observed between 13‴-CH_3 and H-7‴ indicating that H-7‴ also occupied the β face in **1**. This assignment was corroborated by a series of ROESY correlations from substituents occupying the α face of ring E and F (14‴-CH_3, 12‴-CH_3 correlated to the methyl protons 10‴-CH_3 and 11‴-CH_3). In comparison, ROESY correlations were observed between the α methyl protons, 12‴-CH_3 and 14‴-CH_3 to H-7‴ in **2** indicating that H-7‴ was α, while the β methyl protons 13‴-CH_3 correlated to 10‴-CH_3 and 11‴-CH_3 confirming the β configuration of the isobutyl side chain attached to C-7‴ in **2**. Tomentosone A therefore possesses 1″ S*, 9 R*, 7‴ S* and tomentosone B possesses 1″*S**,9*R**,7‴*R** relative configuration.

Tomentosone A and B possess a ring system new to science. The closest related structures are myrtucommulones D, G, and I isolated from either *Myrtus communis* or *Corymbia scabrida*.[10,11] The myrtucommulones also possess a phloroglucinol attached to two alkyl-substituted β-triketone moieties but differ from **1** and **2** because one of the β-triketones forms a pyran through cyclization of a phenolic hydroxyl in the phloroglucinol onto one of the ketone carbons, whereas the phloroglucinol phenol and an enol hydroxyl from the β-triketone in **1** and **2** form a bisfuran through cyclization onto the carbon β to the bridging methine between the phloroglucinol and the β-triketone.

Tomentosone A and B were tested for their ability to inhibit the growth of chloroquine-sensitive (3D7) and -resistant (Dd2) strains of the malaria parasite, *Plasmodium falciparum*.[12] Tomentosone A (**1**) inhibited the growth of chloroquine resistant and sensitive strains of the malaria parasite *Plasmodium falciparum*, with IC_{50} values of 1.49 ± 0.45 μM (n = 2) and 1.0 μM (n = 1), respectively, while tomentosone B (**2**) was significantly less active against both strains reaching only 75% and 45% inhibition at the highest dose (40 μM) tested, respectively. This result suggests that the orientation of the side chain attached at C-7‴ is important for antiplasmodial activity with the β orientation of the isobutyl side chain being associated with higher antiplasmodial activity. Both compounds were also tested for cytotoxicity toward human embryonic kidney cells (HEK) and neither compound showed any toxicity up to the highest dose tested (40 μM). This suggested that tomentosone A could be a candidate for further development.

EXPERIMENTAL SECTION

Plant Material. *R. tomentosa* leaf samples were collected from Singha Nakorn District, Songkhla Province, in the southern part of Thailand during February 2007. The voucher specimen (A. Hiranrat 001) was identified by J. Wai and has been deposited in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

Extraction and Fractionation. The air-dried ground leaves of *R. tomentosa* (2.1 kg) were successively extracted at room temperature with CH_2Cl_2, Me_2CO, and MeOH (3 × 6 L, each 3 days). The CH_2Cl_2 extract (37.8 g) was fractionated by dissolving in MeOH to produce soluble (27.9 g) and insoluble (9.9 g) fractions. The soluble fraction (20.8 g) was separated by CC on silica gel and eluted with a Me_2CO−hexane gradient solvent system. The eluted fractions were combined into fractions A−O on the basis of their TLC characteristics. Fraction B (1.33 g) was subjected to CC on silica gel eluting with Me_2CO−hexane (2:98) to give nine fractions. Fraction B7 (60.7 mg) was then purified by CC on silica gel using Me_2CO−hexane (2:98) as an eluent to produce **1** (22.2 mg) as a yellowish gum. Fraction G (1.42 g) was subjected to CC on silica gel and eluted with gradient solvents of Me_2CO−hexane (5:95 to 10:90) to give fractions G1−G10. Compound **2** (15.4 mg) was obtained as a yellowish solid by crystallization from fraction G2 (55.2 mg) using Me_2CO−hexane (1:5) as a solvent.

Tomentosone A (1): yellowish gum; $[\alpha]^{25}_{D}$ −9.3 (*c* = 0.92, $CHCl_3$); IR (neat) cm^{-1} 3135, 2975, 2945, 1720, 1656, 1617, 1500, 1452, 1303, 1190; UV λ_{max} ($CHCl_3$) nm (log ε) 246 (4.28), 260 (4.36), 304 (4.26), 347sh (3.68); ^1H and ^{13}C NMR ($CDCl_3$) data, see Table 1: HR-EI-MS *m*/*z* 688.3610 $[M]^+$ (calcd for $C_{41}H_{52}O_9$ 688.3611); EI-MS *m*/*z* 688 $[M]^+$, 632, 630, 617, 561, 477, 385, 247, 177.

Tomentosone B (2): pale yellow solid; mp 198−199 °C (from Me_2CO/hexane); $[\alpha]^{25}_{D}$ −10.7 (*c* = 0.68, $CHCl_3$); IR (neat) cm^{-1} 3128, 2969, 2935, 1718, 1650, 1617, 1502, 1452, 1303; UV λ_{max} ($CHCl_3$) nm (log ε) 246 (4.23), 262 (4.28), 306 (4.21), 350sh (3.72); ^1H and ^{13}C NMR ($CDCl_3$) data, see Table 1; HR-EI-MS *m*/*z* 688.3610 $[M]^+$ (calcd for $C_{41}H_{52}O_9$ 688.3611); EI-MS *m*/*z* 688 $[M]^+$, 632, 630, 617, 561, 477, 385, 247, 177.

ASSOCIATED CONTENT

Supporting Information

Detailed description of general experimental procedures and 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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AUTHOR INFORMATION

Corresponding Author

*E-mail: wilawan.m@psu.ac.th.

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REFERENCES

(1) Winotai, A.; Wright, T.; Goolsby, A. J. *Fla. Entomol.* **2005**, *88*, 104−105.

(2) Dachriyanus, S.; Sargent, M. V.; Skelton, B. W.; Soediro, I.; Sutisna, M.; White, A. H.; Yulinah, E. *Aust. J. Chem.* **2002**, *55*, 229−232.

(3) Saising, J.; Hiranrat, A.; Mahabusarakam, W.; Ongsakul, M.; Voravuthikunchai, S. P. *J. Health Sci.* **2008**, *54*, 589−595.

(4) Ruqiang, H.; Yonglu, C. Abstracts of Papers. *232nd ACS National Meeting*, San Francisco, CA, Sep 10−14, 2006; American Chemical Society: Washington, DC, 2006; AGFD-113.

(5) Miyake, Y.; Nojima, J. Japan Patent JP 2006199678, 2006; *Chem. Abstr.* **2006**, *145*, 173623.

(6) Voravuthikunchai, S. P.; Limsuwan, S.; Chusri, S. *Natural Products II: Recent Progress in Medicinal Plants*; Govil, J. N., Singh, V. K., Siddqui, N. T., Eds.; Studium Press: Houston, 2007; pp 41−101.

(7) Limsuwan, S.; Voravuthikunchai, S. P. *FEMS Immunol. Med. Microbiol.* **2008**, *53*, 429−436.

(8) Limsuwan, S.; Trip, E. N.; Kouwen, T. R.; Piersma, S.; Hiranrat, A.; Mahabusarakam, W.; Voravuthikunchai, S. P.; Van Dijl, J. M.; Kayser, O. *Phytomedicine* **2009**, *16*, 645−651.

(9) Hiranrat, A.; Mahabusarakam, W. *Tetrahedron* **2008**, *64*, 11193−11197.

(10) Sheen, F.; Ahmad, M.; Khan, S. N.; Hussain, S. S.; Anjum, S.; Tashkhodjaev, B.; Turgunov, K.; Sultankhodzhaev.; Choudhary, M. I.; Rahman, A. *Eur. J. Org. Chem.* **2006**, 2371−2377.

(11) Carroll, A. R.; Lamb, J.; Moni, R.; Guymer, G. P.; Forster, P. I.; Quinn, R. J. *J. Nat. Prod.* **2008**, *71*, 1564−1568.

(12) Descriptions of bioassay methods can be found in: Carroll, A. R.; Duffy, S.; Avery, V. M. *J. Nat. Prod.* **2009**, *72*, 764−768.