

Novel Cytotoxic, Polyprenylated Heptacyclic Xanthonoids from Indonesian *Garcinia gaudichaudii* (Guttiferae)

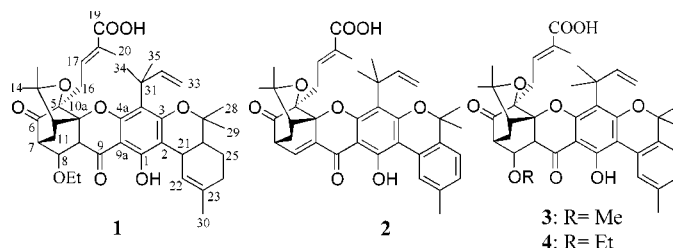
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ABSTRACT



The structures of novel gaudichaudiic acids F–I (1–4), isolated from the bark of Indonesian *Garcinia gaudichaudii*, have been elucidated by detailed spectral analysis. Gaudichaudiic acid I (4) is probably derived from 1 as a result of allylic oxidation at C-24 and C-21, followed by aromatization.

Garcinia gaudichaudii is found mainly in Indo-China, the Malay Peninsula, and Borneo. The leaf juice of the plant has been used as a native medicine to rub on cuts and minor wounds.¹ The Malaysian species *G. gaudichaudii* is a prolific producer of polyprenylated xanthonoids and was previously reported to contain 15 novel cytotoxic compounds; gaudichaudiones A–H, gaudichaudiic acids A–E, including the known morellic acid, and forbesione.² The isolation and detailed characterization of 4 novel gaudichaudiic acids F–I (1–4) from the barks of Indonesian *G. gaudichaudii* have provided a substantially different profile of caged prenylated xanthonoids. Gaudichaudiic acids F–I, which are cytotoxic,

form a unique class of heptacyclic xanthonoids, and three of them contain an unusual toluene-fused dimethylpyran ring (Figure 1).

The dichloromethane-soluble fraction of the dried ground bark of *G. gaudichaudii* was fractionated on a silica gel column, affording two major fractions. Fraction 1 yielded 63 mg (1×10^{-4} %) of gaudichaudiic acid F (1). Further extensive purification on fraction 2 by size-exclusion and silica gel chromatographic methods afforded 15 mg of G (2), 15 mg of H (3), and 12 mg of I (4).

Gaudichaudiic acid F (1), $[\alpha]_D^{31.2} -261.2^\circ$ (*c* 0.17, MeOH), isolated as a yellow oil, was found to have a molecular formula of $C_{40}H_{50}O_9$ by HR-EIMS $[M]^+ 674.3449$, calcd 674.3455. The IR spectrum showed absorption bands at 3447 (OH), 1743 (unconjugated carbonyl), 1682 (ortho-hydroxyl chelated α,β -carbonyl), and 1628 (acid) cm^{-1} . The UV spectrum indicated a modified xanthonoid absorption band [λ_{max} nm (log ϵ), 211 (3.68), 282 (3.20), 305 (3.41),

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(2) Cao, S. G.; Sng, H. L.; Wu, X. H.; Sim, K. Y.; Tan, B. H. K.; Pereira, J. T.; Goh, S. H. *Tetrahedron* **1998**, *54*, 10915–10924.

Table 1. NMR Data for Gaudichaudiic Acids **1**, **2**, **3**, and **4**^a

no.	gaudichaudiic acid F (1)		gaudichaudiic acid G (2)		gaudichaudiic acid H (3)		gaudichaudiic acid I (4)	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	12.88 s (OH)	161.4	14.36 s (OH)	161.0	13.44 s (OH)	160.0	13.45 s (OH)	160.0
2		106.0		105.5		105.6		105.5
3		162.0		163.5		163.0		163.0
4		114.7		114.4		115.9		115.9
4a		154.5		158.6		156.6		156.6
5		86.3		83.9		86.3		86.2
6		208.5		203.5		208.2		208.2
7	2.80 t (4.5)	44.6	3.54 t (5.2)	47.5	2.87 t (4.6)	44.3	2.85 t (4.5)	44.5
8	4.49 dd (4.5, 1.2)	72.4	7.63 d (7.1)	133.0	4.42 br d (4.6)	74.3	4.53 br d (4.5)	72.7
8a	3.12 br s	48.0		136.4	3.20 br s	47.9	3.23 br s	48.6
9		194.1		179.9		194.8		194.9
9a		101.9		101.8		102.9		102.9
10a		87.8		91.3		88.2		88.3
11	A: 1.97m B: 1.40 m	19.9	A: 2.36 dd (8.1, 5.2) B: 1.39 dd (8.1, 5.2)	26.5	A: 1.98 dd (14.5, 6.1) B: 1.44 dd (14.5, 8.4)	19.9	A: 1.96 dd (14.8, 6.1) B: 1.44 dd (14.7, 8.5)	20.0
12	2.55 d (9.5)	43.7	2.59 d (9.1)	49.4	2.58 d (8.4)	43.8	2.57 d (8.5)	43.7
13		81.6		83.7		81.6		81.7
14	1.11 s	27.4	1.26 s	28.3	1.13 s	27.3	1.13 s	27.3
15	1.39 s	30.6	1.73 s	31.6	1.38 s	30.3	1.37 s	30.4
16	A: 3.02 dd (15.3, 6.1) B: 2.78 dd (15.3, 8.3)	29.0	A: 2.85 dd (14.9, 3.9) B: 2.70 dd (14.9, 4.8)	29.2	A: 3.07 dd (15.5, 6.5) B: 2.83 dd (13.8, 6.5)	28.6	A: 3.07 dd (15.5, 5.5) B: 2.84 dd (15.5, 5.5)	28.7
17	7.20 dd, (8.3, 6.1)	138.6	6.39 dd (9.9, 4.8)	135.7	7.20 t (7.7)	138.4	7.09 t (7.7)	138.6
18		128.5		129.1		127.9		127.9
19		172.3		169.7		172.0		171.5
20	1.84 s	12.3	1.44 s	11.7	1.87 s	12.3	1.87 s	12.3
21	3.54 dd (9.8, 1.5)	30.9		126.3		126.3		126.3
22	6.26 d (1.5)	121.9	8.30 br s	127.3	8.28 br s	126.9	8.28 br s	126.9
23		134.2		137.2		137.1		137.1
24	1.93 m (2H)	30.5	7.09 br s	127.8	7.09 br s	128.0	7.09 br s	128.4
25	A: 1.35 m B: 1.92 m	20.7	7.09 br s	121.5	7.09 br s	121.7	7.09 br s	121.7
26	1.74 ddd (9.8, 6.4, 3.1)	39.0		135.4		135.2		135.2
27		81.6		79.9		80.0		80.0
28	1.27 s (3H)	25.1	1.68 s	26.6	1.71 s	26.6	1.71 s	26.6
29	1.41 s (3H)	25.4	1.54 s	26.9	1.55 s	26.9	1.57 s	26.9
30	1.67 s	23.6	2.39 s	21.6	2.39 s	21.6	2.39 s	21.6
31		41.3		41.5		41.2		41.2
32	6.11 dd (17.5, 10.6)	150.3	6.22 dd (16.2, 8.3)	150.7	6.22 dd (17.6, 10.3)	150.6	6.21 dd (17.4, 10.6)	150.6
33	E: 4.77 d (10.6) Z: 4.80 d (17.5)	107.8	E: 4.80 d (8.3) Z: 4.77 d (16.2)	107.1	E: 4.87 d (10.3) Z: 4.96 d (17.6)	108.1	E: 4.86 d (10.6) Z: 4.95 d (17.4)	108.1
34	1.54 s	29.4	1.71 s	30.8	1.66 s	31.2	1.65 s	31.3
35	1.57 s	30.6	1.68 s	29.7	1.58 s	28.2	1.60 s	28.2
36	A: 3.44 dq (20.0, 7.0) B: 3.46 dq (20.0, 7.0)	63.9			3.33 s	55.9	A: 3.60 q (6.9) B: 3.48 q (6.9)	64.0
37	1.12 t (7.0)	14.9					1.14 t (6.9)	30.9

^a Recorded in CDCl₃ at 500 MHz (¹H NMR) and 125 MHz (¹³C NMR); chemical shift (δ) and coupling constants are in parentheses (*J*/Hz) for single protons unless otherwise stated.

349 (2.80)]. The ¹H NMR spectrum of **1** revealed the presence of one chelated hydroxyl group [δ_{H} 12.88 (1H, s, 1-OH)], eight methyl groups [δ_{H} 1.11, 1.27, 1.39, 1.41, 1.54, 1.57, 1.67, 1.84 (each 3H)], and five olefinic protons [δ_{H} 4.77 (1H, d), 4.80 (1H, d), 6.11 (1H, dd), 6.26 (1H, d), 7.20 (1H, dd)] (Table 1). All protonated carbons were assigned by the HMQC spectrum. From the HMBC correlations, five coupled systems including isoprenyl-derived groups were observed. The first system was a 1-methylcyclohexene ring

fused with dimethylpyran ring via C-21 [δ_{C} 30.9] and C-26 [δ_{C} 39.0]. This was deduced from the correlations of H-22 [δ_{H} 6.26] with C-21 [δ_{C} 30.9] and C-26 [δ_{C} 39.0]. In addition, the dimethylpyran group was also connected to a phloroglucinol-type ring which bears an isoprenyl group at C-4 [δ_{C} 114.7]. Further, the presence of a 3-carboxylbut-2-enyl-substituted tricyclo-4-oxa[4.3.1.0^{3,7}]decan-2-one system² was established as part of the xanthonoid. The attachment of the α,β -unsaturated acid was assigned to C-5 on the basis of

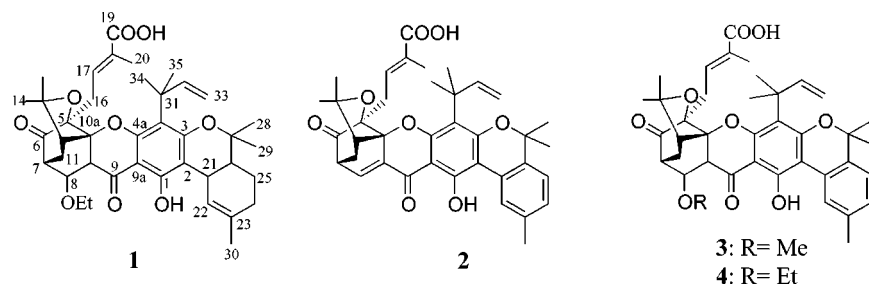


Figure 1. Structures of gaudichaudiic acids F (**1**), G (**2**), H (**3**), and I (**4**).

the correlations of H₂-16 [δ_{H} 2.78, 3.02] and H-17 [δ_{H} 7.20] with C-5 [δ_{C} 86.3]. Noteworthy is the effect of the deshielding core on H-17. The correlations between methylene protons H₂-11 [δ_{H} 1.40] with C-8 [δ_{C} 72.4], and the methine proton H-12 [δ_{H} 2.55] to C-5 [δ_{C} 86.3], C-10a [δ_{C} 87.8], and C-11 [δ_{C} 19.9], clearly supported the establishment of a tricyclo-4-oxa[4.3.1.0^{3,7}]decan-2-one system. The position of ethoxyl group was established at C-8, according to the HMBC correlations between H-36 [δ_{H} 3.44, 3.46] and C-8 [δ_{C} 72.4] and that, H-36, to C-37 [δ_{C} 14.9]. The 3-carboxylbut-2-enyl group was determined to be in the *E*-configuration from the ROESY cross-peaks between H-20 [δ_{H} 1.84] and H₂-16 [δ_{H} 2.78, 3.02] (Figure 2).

Gaudichaudiic acid G (**2**), [α]_D³¹ −248.9° (*c* 0.37, MeOH), isolated as a yellow oil, was found to have a molecular formula of C₃₈H₄₀O₈ by HR-ESIMS [*M* + Na]⁺ 647.2607, calcd 647.2621. The IR spectrum showed absorption bands at 3433 (OH), 1738 (unconjugated carbonyl), 1689 (ortho-hydroxyl chelated α,β -carbonyl), and 1631 (acid) cm^{−1}. The UV spectrum indicated modified xanthonoid absorptions [λ_{max} 224 nm (log ϵ 3.90), 290 (3.73), 350 (3.45), 363 (3.48)]. The ¹H NMR spectrum of **2** revealed the presence of one chelated hydroxyl group [δ_{H} 14.36 (s, 1-OH)], eight methyl groups [δ_{H} 1.26, 1.44, 1.54, 1.68, 1.68, 1.71, 1.73, 2.39 (s, 3H each)], four olefinic protons [δ_{H} 4.77 (1H, d), 4.80 (1H, d), 6.22 (1H, dd), 6.39 (1H, dd)], and three aromatic protons [δ_{H} 7.09 (2H, br s), 8.30 (1H, br s)]. The chemical shift of H-17 of **2**, in contrast to **1**, seemed to be counterbalanced due to the shielding by the $\Delta^{8,8a}$ -olefinic bond. All protonated carbons were assigned from the HMQC spectrum. There were five coupled systems involving isoprenyl groups and the connectivity of the systems was established by HMBC. The HMBC correlations of H-22 [δ_{H} 8.30] to C-2 [δ_{C} 105.5] and the ROESY correlations of H-25 [δ_{H} 7.09] to H-28 [δ_{H} 1.68] and H-29 [δ_{H} 1.54] showed that a novel toluene group is fused onto a dimethylpyran ring at the C-21 [δ_{C} 126.3] and C-26 [δ_{C} 135.4] positions. In addition, the latter ring was also attached by another aromatic ring at C-3 [δ_{C} 163.5] and C-2 [δ_{C} 105.5], which bears an isoprenyl group at C-4 [δ_{C} 114.4]. The typical low-field singlet at δ_{H} 14.36 was assigned to the chelated hydroxyl group at C-1 [δ_{C} 161.0], which was *peri* to the ketonic carbon C-9 [δ_{C} 179.9] and *para* to the isoprenyl group at C-4. Like **1**, the structure of the aromatic ring was substituted by a phloroglucinol, which became xanthonoid by fusion onto the

oxygenated C-4a [δ_{C} 158.6] and quaternary C-9a [δ_{C} 101.8]. The HMBC correlations of H-12 [δ_{H} 2.59] to C-7 [δ_{C} 47.5], C-10a [δ_{C} 91.3], C-11 [δ_{C} 26.5], C-13 [δ_{C} 83.7], C-15 [δ_{C}

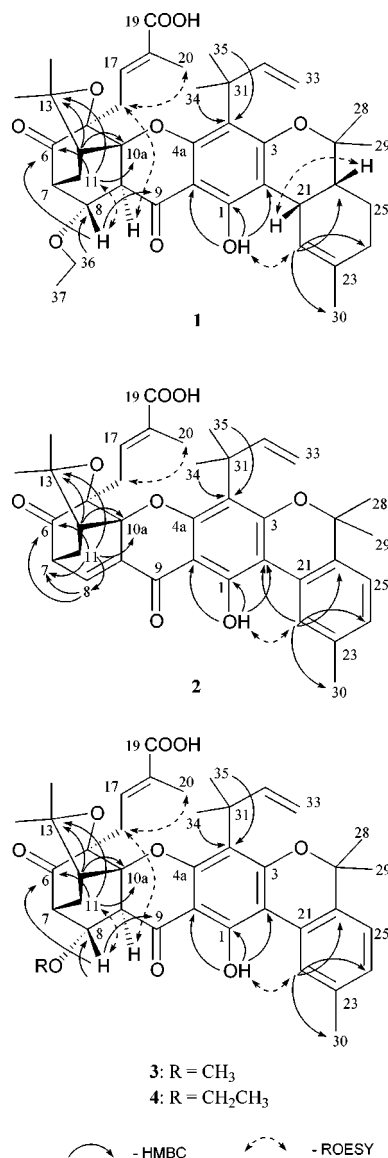


Figure 2. Selected HMBC and ROESY correlations.

31.6] and H-11 [δ_{H} 1.39, 2.36] to C-6 [δ_{C} 203.5], C-7 [δ_{C} 47.5], C-8 [δ_{C} 136.4], C-10a [δ_{C} 91.3], C-13 [δ_{C} 83.7] showed the presence of a tricyclo-4-oxa[4.3.1.0^{3,7}]decan-2-one as part of the xanthonoid system, similar to **1**. Since a ROESY cross-peak was observed between H-20 and H-16, the orientation of the 3-carboxylbut-2-enyl group at C-5 was in the *E*-configuration. The occurrence of a toluene group as part of the dimethylpyran ring via C-21 and C-26 in the xanthonoid is novel. It is presumed that the extra aromatic ring of **2–4** was derived from **1** as a result of aromatization via allylic oxidation at C-21 and C-24.

Gaudichaudiic acid H (**3**), [α]^{31.2}_D -25.13° (*c* 0.41, MeOH), isolated as a yellow oil, was found to have a molecular formula of C₃₉H₄₄O₉ by HR-ESIMS [$\text{M} + \text{Na}$]⁺ 679.2890, calcd 679.2883. The IR (3444, 1744, 1685, and 1631 cm⁻¹) and UV spectra [212 (4.56), 2.79 (4.57), 317 (4.06), and 3.64 (3.52)] were suggestive of a xanthonoid nucleus. The ¹H NMR spectrum of **3** was almost identical to that of **2**, except for an additional methoxy group. Analogous to **2**, **3** contained five coupled systems involving the tricyclo-4-oxa[4.3.1.0^{3,7}]decan-2-one system bearing a 3-carboxylbut-2-enyl group and a phloroglucinol-like ring which was flanked by a 2,3-dihydropyran-4-one group and a toluene-fused dimethylpyran ring. The connectivity of the systems was established by HMBC and detailed comparison with the spectroscopic data of **2**. The position of methoxy group was determined at C-8 because H₃₋₃₆ (methoxyl singlet) showed a HMBC correlation with C-8 [δ_{C} 74.3] and ROESY cross-peaks with H-7 [δ_{H} 2.87], H-8 [δ_{H} 4.42], and H-8a [δ_{H} 3.20]. The orientation of the 3-carboxylbut-2-enyl group was found to be the *E*-configuration since ROESY

cross-peaks are observed between H-20 [δ_{H} 1.87] and H₂₋₁₆ [δ_{H} 2.83, 3.07].

Gaudichaudiic acid I (**4**), [α]^{31.2}_D $+17.2^{\circ}$ (*c* 0.32, MeOH), isolated as a yellow oil, was found to have a molecular formula of C₄₀H₄₆O₉ by HR-FABMS [$\text{M} - 1$]⁺ 669.3087, calcd 669.3063. The IR and UV spectra, similar to those of **3**, suggested a xanthonoid nucleus. The ¹H NMR spectrum of **4** was almost identical to that of **3**, except for the presence of a β -ethoxyl group instead of a methoxy. The xanthonoid structure of **4** was established by HMBC and a detailed comparison of spectroscopic data with those of **3** and **2**. On the basis of the ROESY cross-peaks, the 3-carboxylbut-2-enyl group was also found to be the *E*-configuration, identical to **3**. Although **1**, **3**, and **4** can be possibly obtained by EtOH or MeOH addition to unsaturated $\Delta^{8,8a}$ precursors, e.g., morellic acid,² they were confirmed to be not artefacts by extraction of the bark using alcohol-free dichloromethane or chloroform solvents.

Gaudichaudiic acids **1**, **2**, **3**, and **4** showed cytotoxicity to the P388 cell line at IC₅₀ values of 4.6, 3.4, 2.0, and 1.7 $\mu\text{g}/\text{mL}$, respectively. **2**, **3**, and **4** were also cytotoxic to the P388/DOX cell line at IC₅₀ values of 3.4, 2.2, and 1.4 $\mu\text{g}/\text{mL}$, respectively. Finally, **2**, **3**, and **4** exhibited cytotoxicity at IC₅₀ values of 3.8, 3.0, and 3.0 $\mu\text{g}/\text{mL}$ to the Messa cell line, respectively.

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