Benzopyran, Biphenyl, and Tetraoxygenated Xanthone Derivatives from the Twigs of *Garcinia nigrolineata*

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One new benzopyran, nigrolineabenzopyran A (1), two new biphenyls, nigrolineabiphenyls A and B (2, 3), and four new tetraoxygenated xanthones, nigrolineaxanthones T–W (4–7), were isolated from the crude methanol extract of the twigs of *Garcinia nigrolineata* along with 11 known xanthones. The xanthones isolated from the twigs as well as those from the stem bark were evaluated for antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Nigrolineaxanthone F, latissxanthone D, and brasilixanthone showed significant activity, with an equal MIC value of 2 μg/mL.

*Garcinia nigrolineata* Planch. Ex T. Anderson (Guttiferae), locally named Cha-muang, is distributed throughout Malaysia, southern Thailand, and Burma.1 Our previous investigation on its leaves2 and stem bark3 led to the isolation of many trioxygenated and tetraoxygenated xanthones. In our continuing investigation on this plant in the search for substances active against MRSA, seven new compounds, one benzopyran (1), two biphenyls (2 and 3), and four xanthones (4–7), have been identified. The antibacterial activity of xanthones isolated was investigated.

**Results and Discussion**

The MeOH extract of twigs of *G. nigrolineata* was subjected to chromatographic purification to afford seven new compounds—one benzopyran derivative, nigrolineabenzopyran A (1), two biphenyls, nigrolineabiphenyls A (2) and B (3), and four tetraoxygenated xanthones, nigrolineaxanthones T–W (4–7)—along with 11 known xanthones: dulxanthone A,4 nigrolineaxanthone A,3 1,3,5-trihydroxy-4-(3-hydroxy-3-methylbutyl)xanthone,5 forbesxanthone,6 tovophyllin A,7 6-deoxyjacareubin,8 morusignin C,9 ananixanthone,10 1,5-dihydroxy-6′,6′-dimethylypyrano[2′,3′,3:2′]xanthone,11 morusignin I,12 and rheediaxanthone A.13 Structures of all compounds were elucidated using spectroscopic data, especially 1D and 2D NMR techniques. The $^{13}$C NMR signals were assigned from DEPT, HMQC, and HMBC spectra. The $^{1}$H and/or $^{13}$C spectral data of the known xanthones compared well with those reported in the literature.

Nigrolineabenzopyran A (1) was isolated as a colorless gum (C$_{13}$H$_{14}$O$_{5}$ by HREIMS). The $^{1}$H NMR spectrum showed typical signals of a chromene ring [$\delta$ 6.62, 5.47 (1H each, d, $J = 10.0$ Hz) and 1.42 (6H, s), an aromatic proton at $\delta$ 5.97 (s), and a methoxyl group at $\delta$ 4.04 (s). The $^{3}$J HMBC correlations of H-4 ($\delta$ 6.62)/C-5 and C-8a ($\delta$ 160.6) and that of H-3 ($\delta$ 5.47)/C-4a ($\delta$ 102.8) established the fusion of the chromene ring at C-4a and C-8a with an ether linkage at C-8a. The singlet aromatic proton was attributed to H-8 according to $^{3}$J correlations with C-4a and C-6 ($\delta$ 93.5). Irradiation of H-4, in the NOEDIFF experiment, enhanced only the signal of H-3, but not H-8, confirming the assigned location for the aromatic proton. Hydroxyl groups were assigned as substituents at C-5 and C-7 on the basis of $^{13}$C chemical shift data. A correlation between the methoxy protons and a carbonyl carbon at $\delta$ 169.8 indicated the presence of the methyl ester functionality, which was then assigned to C-6, ortho to both hydroxyl...
groups. Therefore, nigrolineabenzyopyran A (1) was identified as 5,7-dihydroxy-2,2-dimethyl-6-methylcarboxylbenzopyran.

Nigrolineabiphenyl A (2) was obtained as a colorless gum. The molecular formula was established as C_{13}H_{18}O_{3} by HREIMS. The UV absorption bands were similar to those of garcibiphenyls A and B.\textsuperscript{14} The $^1$H NMR spectrum showed signals characteristic of a 1,3,4-trisubstituted benzene (δ 7.08 (1H, d, $J$ = 2.0 Hz), 6.98 (1H, dd, $J$ = 8.5 and 2.0 Hz), and 6.91 (1H, d, $J$ = 8.5 Hz)), one singlet signal of two equivalent aromatic protons (δ 6.73) belonging to a 1,3,4,5-tetrasubstituted benzene, and one singlet signal of two identical methoxyl groups (δ 3.94). In the NOEDIF experiment, irradiation of the methoxy protons affected the signal of two equivalent aromatic protons (H-2 and H-6), suggesting that two identical methoxyl groups were located at C-3 and C-5 (δ 147.2) of the tetrasubstituted benzene ring. HMBC cross-peaks between H-2 and H-6 with C-3, C-4 (δ 134.0) and C-5 (see Supporting Information) confirmed their location. The met-coupled aromatic protons of the trisubstituted benzene ring at δ 7.08 and 6.98 were assigned as H-2’ and H-6’ according to HMBC correlations of H-2’C-4’ (δ 143.0) and C-6’ (δ 119.2) and those of H-6’/C-2’ (δ 113.9) and C-4’. The remaining aromatic proton (δ 6.98) was then attributed to H-5 on the basis of the splitting pattern, values of coupling constants, and HMBC data. The position of linkage between the two aromatic rings was established by a 3/2 HMBC cross-peak of H-2’ and C-1 (δ 132.6). This was further supported by signal enhancement of H-2’ and H-6’ upon irradiation of H-2 and H-6. Since there were no other proton signals, the substituents at C-4, C-3’ (δ 144.0) and C-4’ were hydroxyl groups. Thus, nigrolineabiphenyl A (2) was assigned as 3,5-dimethoxy-(1,1′-biphenyl)-3,4,4′-triol.

Nigrolineaxanthone B (3) was isolated as a colorless gum (C_{20}H_{16}O_{3} by HREIMS). The $^1$H NMR data were similar to those of 2, but with one additional methoxyl signal at δ 3.97 (s), indicating that one of the hydroxyl groups in 2 had been replaced by a methoxyl group. This group was placed at C-3’ (δ 146.6) on the basis of its HMBC correlation with C-3’ (see Supporting Information). Signal enhancement of these methoxy protons after irradiation of H-2’ (δ 7.00) confirmed the location. The remaining HMBC (see Supporting Information) and NOEDIF data are identical to those of 2. Thus, nigrolineaxanthone B (3) was elucidated as 3,3’,5-trimethoxy-[1,1′-biphenyl]-4,4′-diol.

Nigrolineaxanthone T (4), isolated as a yellow gum, had the molecular formula C_{14}H_{14}O_{3}. Absorption bands typical of a xanthone chromophore were observed.\textsuperscript{2,3} The $^1$H NMR data were similar to those of dulxanthone A\textsuperscript{4} except for the replacement of signals for a 3-methybut-2-enyl unit in dulxanthone A with signals for a 3-hydroxy-3-methylbutyl unit (δ 2.94 (2H, m), 1.73 (2H, m), and 1.30 (6H, s)). In the HMBC spectrum (Supporting Information), 3/4 cross-peaks between H-11 (δ 2.94) and C-3 (δ 163.3) and C-4α (δ 153.8) established attachment of the 3-hydroxy-3-methylbutyl unit at C-4, the same position as the 3-methylbut-2-enyl unit in dulxanthone A. Signal enhancement of H-2 (δ 6.35, s) and H-11 of the C-4 side chain, upon irradiation of the C-3 methoxy protons, confirmed the above assignment. Thus, nigrolineaxanthone T (4) was identified as 1,5,6-trihydroxy-3-methoxy-4(3-hydroxy-3-methylbutyl)xanthone.

Nigrolineaxanthone U (5), isolated as a yellow solid, had the molecular formula C_{18}H_{18}O_{3}. The UV and IR data were similar to those of 4, indicating that 5 possessed a xanthone chromophore. The $^1$H NMR signals of rings A and B (Table 1) as well as HMBC data (Supporting Information) were almost identical to those of morusignin C\textsuperscript{9} and 1,3,5-trihydroxy-4(3-hydroxy-3-methylbutyl)xanthone,\textsuperscript{5} respectively. Therefore, the structure of nigrolineaxanthone U (5) was 1,3,5,8-tetrahydroxy-4(3-hydroxy-3-methylbutyl)xanthone.

Nigrolineaxanthone V (6), a yellow gum, gave a molecular formula of C_{24}H_{24}O_{6} by HREIMS. It exhibited $^1$H NMR signals of rings A and B (Table 1) as well as the HMBC data (Supporting Information) almost identical to those of rheediaxanthone A\textsuperscript{8} and dulxanthone A, respectively. Thus, nigrolineaxanthone V (6) was determined as 1,5-dihydroxy-3-methoxy-4(3-methylbut-2-enyl)-6′,6′-dimethylpyra[2′,3′:6,7]xanthone.

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Thus, nigrolineaxanthone U (5) and nigrolineaxanthone V (6) were determined as 1,3,5,8-tetrahydroxy-4(3-hydroxy-3-methylbutyl)xanthone.
Isolation of the New Constituents. The dried and chopped twigs (3.5 kg) were extracted with MeOH (5 L) for 5 days at room temperature three times. Filtration and subsequent evaporation of the combined MeOH extracts to dryness in vacuo afforded a dark brown residue (250 g). Purification of the crude MeOH extract was performed using two pathways. The first one started by subjecting the crude extract (76 g) to column chromatography (CC) on Sephadex LH20 eluted with MeOH to afford three fractions. Fraction 1 (2.56 g) was obtained from fraction A2. Fraction A5 (60.7 mg, eluted with 100% CHCl3) gave 6 (3.7 mg), upon silica gel CC eluted with solvent mixtures of increasing polarity (CHCl3–light petroleum, CHCl3, and 10% MeOH–CHCl3). Fraction A10 (2.38 g, eluted with 98% MeOH–CHCl3) was subjected on silica gel eluted with gradient system A to yield five subfractions. Compound 3 (3 mg) was obtained from the second subfraction (20.6 mg, eluted with 0.2–1% MeOH–CHCl3) after purification by CC with reversed-phase silica gel eluted with gradient system C. Compound 2 (4.5 mg) was afforded from the third subfraction (eluted with 2–5% MeOH–CHCl3). Fraction A12 (147.9 mg, 5% MeOH–CHCl3) was fractionated further by CC using reversed-phase silica gel with gradient system C to give five subfractions. Compound 5 (5.5 mg) was obtained from the second subfraction (6.2 mg, eluted with 60% MeOH–H2O), upon silica gel CC eluted with gradient system B. The second investigation began with dissolving the crude MeOH extract (70 g) in CHCl3. The CHCl3 solutions (36.5 g) were subjected to CC using Sephadex LH20 eluted with MeOH to give five fractions (B1–B5). Fraction B2 (2.90 g from total of 8.33 g) was further purified by silica gel CC using gradient system A to afford 1 (3.3 mg). Purification of fraction B3 (4.88 g) by silica gel CC eluted with gradient system A afforded six subfractions. The fifth subfraction (2.20 g, eluted with 40% MeOH–CHCl3) was further fractionated using CC on Sephadex LH20 eluted with 40% MeOH–CHCl3 to give 4 (10 mg).

**Nigrolineabenzopyran A (1):** colorless gum; UV (MeOH) λmax (log e) 227 (2.97), 253 (3.64), 260 (3.70), 274 (3.16), 302 (2.05); IR ( neat) νmax 3450, 1645, 1586 cm⁻¹; 1H NMR (500 MHz) 6.26 (1H, d, J = 10.0 Hz, H-4), 5.97 (1H, s, H-8), 5.47 (1H, d, J = 10.0 Hz, H-3), 4.04 (3H, s, OCH3), 1.42 (6H, s, C-2-OCH3). 13C NMR (125 MHz) 180.7 (C, C-9), 163.6 (C, C-3), 161.9 (C, C-1), 153.8 (C, C-4), 147.5 (C, C-5), 144.0 (C, C-3′), 114.6 (CH, C-7), 109.2 (C, C-4), 108.0 (C, C-8a), 102.7 (C, C-9a), 98.8 (CH-2), 71.4 (C, 13-C), 42.7 (CH3, C-12), 29.5 (CH3, C-14, C-15), 17.4 (CH2, C-11); EIMS m/z 346 [M]+ (5), 328 (25), 307 (15), 274 (25), 273 (100), 272 (36), 149 (15), 71 (20), 69 (22), 57 (41); HREIMS m/z 346.1058 (calcld for C19H20O7, 346.1053).

**Nigrolineanaphtrochalcone V (6):** yellow gum; UV (MeOH) λmax (log e) 273 (4.34), 316 (3.88), 369 (3.66); IR ( neat) νmax 3410, 1645 cm⁻¹; 1H NMR (500 MHz) (acetone-d6) 13.09 (1H, s, 1-OH), 7.47 (1H, s, H-8), 6.44 (1H, d, J = 10.0 Hz, H-16), 6.38 (1H, s, H-2), 5.72 (1H, d, J = 10.0 Hz, H-17), 5.49 (1H, s, 5-OH), 5.28 (1H, tbr, J = 7.5 Hz, H-12), 3.92 (3H, s, 3-OCH3), 3.53 (2H, s, H-17), 1.87 (3H, d, J = 10.0 Hz, H-15), 1.69 (3H, s, H-14), 1.54 (6H, s, H-19, H-20); 13C NMR (125 MHz) 130.8 (C, C-9), 136.5 (C, C-3), 161.9 (C, C-1), 153.8 (C, C-4a), 148.7 (C, C-5), 145.4 (C, C-10a), 144.7 (C, C-6), 131.9 (C, C-13), 130.9 (CH-17), 122.2 (CH, 12), 121.5 (CH, C-16), 117.6 (C, C-7), 113.4 (CH, C-8), 107.9 (C, C-4), 103.3 (C, C-9a), 103.0 (C, C-8a), 94.2 (CH, C-2), 78.7 (C, 18), 56.0 (CH3, 3-OCH3), 28.5 (CH3, C-19, C-20), 25.8 (CH3, C-14, C-17), 21.7 (CH3, C-11), 17.9 (CH3, C-15); EIMS m/z 408 [M]+ (8), 393 (17), 369 (14), 354 (19), 353 (100), 337 (23), 325 (40), 149 (42); HREIMS m/z 408.1656 (calcld for C23H22O10).

**Nigrolineanaphtrochalcone W (7):** pale yellow gum; UV (MeOH) λmax (log e) 325 (4.02), 299 (4.56), 338 (3.77), IR ( neat) νmax 3402, 1608 cm⁻¹; 1H NMR (500 MHz) (acetone-d6) 14.09 (1H, s, 1-OH), 7.99 (1H, d, J = 10.0 Hz, H-21), 6.73 (1H, dd, J = 10.0, 5.5 Hz, H-11), 6.31 (1H, d, J = 0.5 Hz, H-4), 5.77 (1H, d, J = 10.0 Hz, H-22), 5.57 (1H, d, J = 10.0 Hz, H-12), 5.27 (1H, tbr, J = 7.5 Hz, H-17), 3.57 (2H, d, J = 7.5 Hz, H-16), 1.88 (3H, s, H-20), 1.69 (3H, s, H-19), 1.49 (6H, s, H-24, H-25), 1.48 (6H, s, H-14, H-15); 1C NMR (125 MHz) 182.8 (C, C-9), 159.8 (C, C-3), 157.8 (C, C-1), 156.5 (C, C-4a), 150.9 (C, C-10a), 148.6 (C, C-16), 136.6 (C, C-7), 132.7 (C, C-12), 121.0 (CH, C-21), 120.9 (CH, C-17), 117.1 (C, C-8), 115.7 (C, C-11), 115.3 (C, C-5), 108.4 (C, C-8a), 104.3 (C, C-9a), 103.8 (C, C-2), 94.2 (CH, C-4), 77.9 (C, C-13), 76.9 (C, C-23), 28.4 (CH2, C-14, C-15), 27.4 (CH3, C-24, C-25), 25.8 (CH3, C-16), 22.6 (CH2, C-16), 18.0 (CH2, C-20); EIMS m/z 460 [M]+ (15), 445 (34), 401 (23), 178 (23), 149 (62), 83 (42), 71 (52), 69 (74), 57 (100); HREIMS m/z 460.1878 (calcld for C23H22O10).

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**Supporting Information Available:** Table of HMBC correlations for compounds 1–7 and structures of known compounds are available free of charge via the Internet at http://pubs.acs.org.

**References and Notes**

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