Antibacterial Xanthones from the Leaves of *Garcinia nigrolineata*

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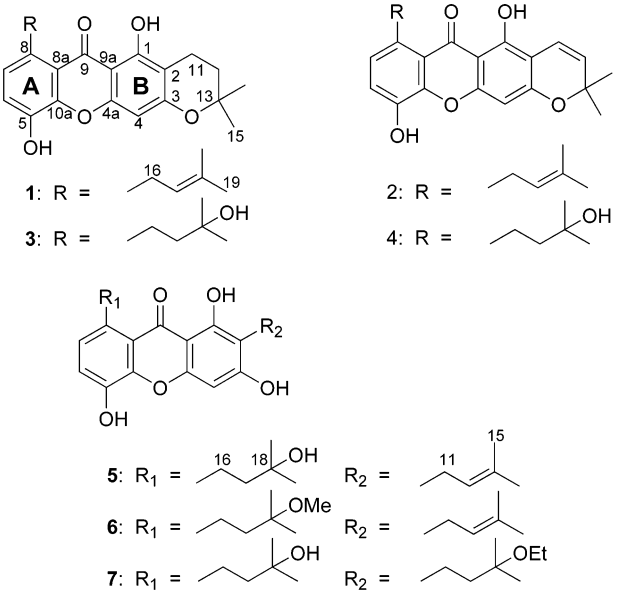
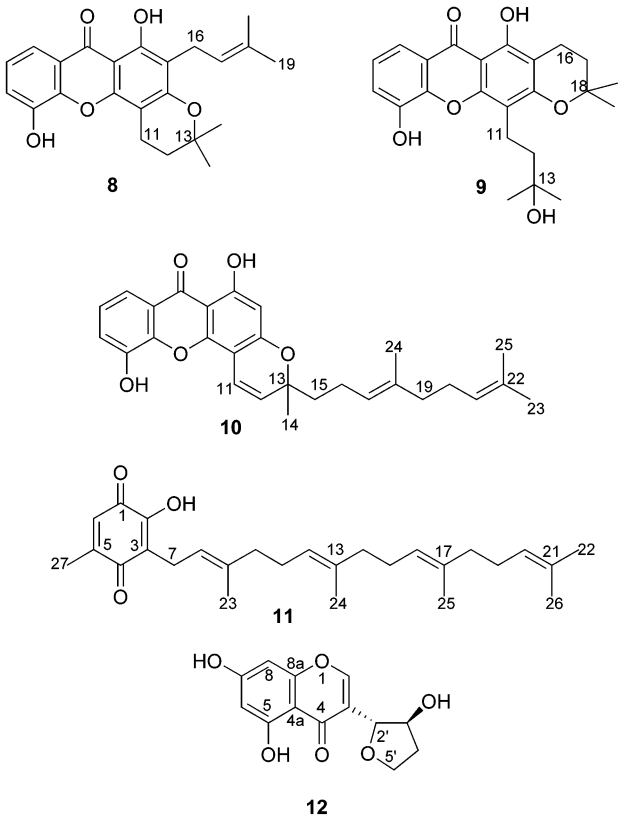
Ten new 1,3,5-trioxygenated xanthones, nigrolineaxanthones J−S (**1**−**10**), one new quinone derivative, nigrolineaquinone A (**11**), and one new isoflavone-like compound, nigrolineaisoflavone A (**12**), were isolated from the leaves of *Garcinia nigrolineata* along with four known xanthones and friedelin. Among the xanthones, only nigrolineaxanthone N (**5**) showed significant antibacterial activity against methicillin-resistant *Staphylococcus aureus*.

Garcinia nigrolineata Planch. Ex T. Anderson (Guttiferae) is distributed throughout Malaysia, southern Thailand, and Burma[1] and is locally named Cha-muang. We recently isolated and characterized several xanthones from its stem bark.[2] Continuing investigation on this plant has resulted in the isolation of 12 new and five known compounds from the leaves.

Results and Discussion

The MeOH extract of the leaves of *G. nigrolineata* was subjected to chromatographic purification to afford 12 new compounds, which included 10 1,3,5-trioxygenated xanthones, nigrolineaxanthones J−S (**1**−**10**), a benzoquinone derivative, nigrolineabenzoquinone A (**11**), and an isoflavone-like compound, nigrolineaisoflavone A (**12**). Friedelin and four known xanthones, 8-desoxygartanin,[3] ananixanthone,[4] 1,5-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)-xanthone,[5] and 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone,[6] were also isolated. All structures 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.





All of the xanthones showed typical UV and IR absorption bands. They were all 1,3,5-trioxygenated xanthones with hydroxyl groups at C-1 and C-5. The substituent at C-3 was either a hydroxyl group or an oxygen atom of a chromene or chromane ring. The C-1 hydroxyl group was evident by a deshielded, H-bonded hydroxy proton signal at δ 13.00−13.65 in the ^{1}H NMR spectra.

Nigrolineaxanthone J (**1**) showed a molecular formula of $C_{23}H_{24}O_5$ (HREIMS). The ^{1}H NMR spectrum (Table 1)

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Table 1. ^{1}H NMR Spectral Data (δ) of Nigrolineaxanthones J−R (**1**−**9**)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]	**8**	**9**
1-OH	13.60 (s)	13.56 (s)	13.60 (s)	13.44 (s)	13.65 (s)	13.77 (s)	13.43 (s)	12.86 (s)	13.00 (s)
4	6.30 (s)	6.31 (s)	6.32 (s)	6.33 (d, 0.5)	6.56 (s)	6.38 (s)	6.41 (s)		
5-OH	5.87 (brs)	5.69 (brs)						5.66 (brs)	
6	7.19 (d, 8.0)	7.20 (d, 8.0)	7.20 (d, 8.5)	7.22 (d, 8.5)	7.25 (d, 8.0)	7.20 (d, 8.0)	7.19 (d, 8.0)	7.31 (dd, 8.0, 1.5)	7.25 (dd, 8.0, 1.5)
7	7.02 (d, 8.0)	7.04 (d, 8.0)	7.00 (d, 8.5)	7.02 (d, 8.5)	7.04 (d, 8.0)	7.01 (d, 8.0)	6.98 (d, 8.0)	7.26 (t, 8.0)	7.20 (t, 8.0)
8								7.79 (dd, 8.0, 1.5)	7.72 (dd, 8.0, 1.5)
11	2.73 (t, 10.0)	6.75 (d, 10.0)	2.72 (t, 6.5)	6.74 (dd, 10.0, 0.5)	3.36 (d, 7.0)	3.47 (d, 7.5)	2.77 (t, 6.5)	2.90 (t, 7.0)	3.00 (m)
12	1.85 (t, 10.0)	5.60 (d, 10.0)	1.85 (t, 6.5)	5.61 (d, 10.0)	5.29 (mt, 7.0)	5.31 (mt, 7.5)	1.80 (t, 6.5)	1.91 (t, 7.0)	1.79 (m)
14	1.38 (s)	1.48 (s)	1.38 (s)	1.48 (s)	1.65 (s)	1.78 (s)	1.23 (s)	1.41 (s)	1.32 (s)
15	1.38 (s)	1.48 (s)	1.38 (s)	1.48 (s)	1.79 (s)	1.86 (s)	1.23 (s)	1.41 (s)	1.32 (s)
16	3.98 (d, 7.5)	3.98 (d, 7.0)	3.28 (m)	3.27 (m)	3.31 (m)	3.22 (m)	3.27 (m)	3.36 (d, 7.5)	2.79 (t, 7.5)
17	5.36 (mt, 7.5)	5.36 (mt, 7.0)	1.76 (m)	1.77 (m)	1.76 (m)	1.75 (m)	1.76 (m)	5.27 (mt, 7.5)	1.77 (t, 7.5)
18-OMe						3.29 (s)			
19	1.74 (s)	1.74 (s)	1.31 (s)	1.32 (s)	1.30 (s)	1.29 (s)	1.31 (s)	1.68 (s)	1.30 (s)
20	1.74 (s)	1.74 (s)	1.31 (s)	1.32 (s)	1.30 (s)	1.29 (s)	1.31 (s)	1.82 (s)	1.30 (s)

[a] Protons of the ethoxyl group appeared at δ 3.58 (q, 7.0) and 1.31 (t, 7.0).

showed the presence of two hydroxyl groups, two *ortho*-coupled aromatic protons, and one singlet aromatic proton. Other signals could be attributed to a prenyl group and a dimethylchromane ring. The appearance of the methylene protons (H_2-16) of the prenyl group at δ 3.98 together with ^3J cross-peaks in the HMBC spectrum (see Supporting Information) with an aromatic methine carbon (C-7, δ 124.9) and a quaternary aromatic carbon (C-8a, δ 118.4) suggested that this side chain was at C-8 (δ 135.4), a *peri* position to a carbonyl group. The correlation between one of the *ortho*-coupled aromatic protons (H-7, δ 7.02) and C-7 in the HMQC spectrum established the attachment of this proton at C-7. Thus, the other *ortho*-coupled aromatic proton at δ 7.19 was attributed to H-6. H-7 also gave ^3J cross-peaks with C-16 (δ 33.1) of the prenyl side chain and a hydroxylated aromatic carbon (C-5, δ 142.5) in the HMBC spectrum. Enhancement of the H-7 signal upon irradiation of H-16 of the prenyl group together with HMBC data confirmed these assignments. In the HMBC spectrum, the chelated hydroxy proton (1-OH, δ 13.60) and the methylene protons (H_2-12, δ 1.85) of the chromane ring gave cross-peaks with the same quaternary aromatic carbon (C-2, δ 104.4), indicating that the dimethylchromane ring was fused to C-2 and C-3 of the xanthone nucleus with an ether linkage at C-3. The remaining aromatic proton at δ 6.30 was then assigned to H-4 due to its cross-peaks with C-2, C-3, C-4a, and C-9a. Therefore, nigrolineaxanthone J (**1**) was assigned as 1,5-dihydroxy-8-(3-methylbut-2-enyl)-6′,6′-dimethyldihydropyrano(2′,3′:3,2)xanthone.

The molecular formula of nigrolineaxanthone K (**2**) ($C_{23}H_{22}O_5$) indicated that **2** contained two hydrogen atoms less than **1**. The ^{1}H NMR data (Table 1) were almost identical with those of **1** except for replacement of two triplet signals of the dimethylchromane in **1** by signals of two *cis*-olefinic protons of a dimethylchromene (δ 6.75 and 5.60). The correlations of H-11/C-1 (δ 158.2), H-11/C-3 (δ 160.4), and H-12/C-2 (δ 104.9) in the HMBC spectrum indicated that the dimethylchromene ring was located at the same location as the chroman ring in **1**. A small coupling constant (J = 0.5 Hz) between the more deshielded chromene hydrogen (H-11) and the aromatic proton (H-4, δ 6.31) by a ^5J extended W pathway confirmed the location of the dimethylchromene ring. Nigrolineaxanthone K (**2**) was thus assigned as 1,5-dihydroxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:3,2)xanthone.

Nigrolineaxanthone L (**3**) ($C_{23}H_{26}O_6$) also showed ^{1}H NMR (Table 1) and HMBC data (see Supporting Information) similar to those of **1**. Multiplet signals of two methylene groups at δ 3.28 (H_2-16) and 1.76 (H_2-17) replaced signals of the olefinic proton and methylene protons of the C-8 prenyl group in **1**. These data suggested that **3** contained a 3-hydroxy-3-methylbutyl moiety instead of a prenyl group. The highly deshielded position of H-16 of the 3-hydroxy-3-methylbutyl unit together with the HMBC data of H-16/C-7 (δ 125.7) and C-8a (δ 118.3) confirmed the attachment of this side chain at C-8. Therefore, nigrolineaxanthone L (**3**) was assigned as 1,5-dihydroxy-8-(3-hydroxy-3-methylbutyl)-6′,6′-dimethyldihydropyrano-(2′,3′:3,2)xanthone, a hydrated derivative of **1**.

Nigrolineaxanthone M (**4**) ($C_{23}H_{24}O_6$) by HREIMS showed ^{1}H NMR signals of ring A and B (Table 1) as well as the HMBC data almost identical with those of **3** and **2**, respectively. Thus, nigrolineaxanthone M (**4**) was determined as 1,5-dihydroxy-8-(3-hydroxy-3-methylbutyl)-6′,6′-dimethylpyrano(2′,3′:3,2)xanthone.

Nigrolineaxanthone N (**5**) ($C_{23}H_{26}O_6$) showed ^{1}H and ^{13}C NMR and HMBC data of ring A similar to those of **3** and **4**, with a 3-hydroxy-3-methylbutyl group at C-8. In addition, the ^{1}H NMR spectrum showed signals for a prenyl group and an aromatic proton signal at δ 6.56 (s). In the HMBC spectrum, the methylene protons (H_2-11) of the prenyl group gave cross-peaks with a quaternary aromatic carbon at δ 111.1 (C-2) and two oxygenated carbons at δ 161.5 (C-1) and 163.8 (C-3), indicating that the prenyl unit was located at C-2. The NOEDIFF data revealed that the methyl signal at δ 1.65 was *cis* to an olefinic proton (H-12). Thus, nigrolineaxanthone N (**5**) was determined as 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-8-(3-hydroxy-3-methylbutyl)xanthone.

Nigrolineaxanthone O (**6**) ($C_{24}H_{28}O_6$) showed ^{1}H NMR and HMBC data almost identical with those of **5** except for an additional methoxyl signal at δ 3.29. The ^{13}C NMR spectrum showed the same number of quaternary, methine, and methylene carbons as found in **5** except for one more methyl carbon signal, at δ 49.2. The methoxy protons (HMBC) gave a cross-peak with a quaternary carbon (C-18, δ 74.9), indicating attachment of the methoxyl group

at C-18. Thus, the side chain at C-8 in **6** was a 3-methoxy-3-methylbutyl unit, and nigrolineaxanthone O (**6**) was assigned as 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-8-(3-methoxy-3-methylbutyl)xanthone, a methyl ether of **5**.

Nigrolineaxanthone P (**7**) ($C_{25}H_{32}O_7$) showed ^{1}H NMR and HMBC data of ring A identical with those of **3**, **4**, and **5**. Proton signals and the correlations in the HMBC spectrum of ring B were also similar to **5** except that signals of the C-2 prenyl group in **5** and **6** were replaced by signals of a 3-ethoxy-3-methylbutyl unit. The location of an ethoxyl group at C-13 (δ 76.3) was confirmed by a ^3J cross-peak between the methylene protons of the ethoxyl group with C-13 of the C-2 side chain. Thus, nigrolineaxanthone P (**7**) was determined as 1,3,5-trihydroxy-2-(3-ethoxy-3-methylbutyl)-8-(3-hydroxy-3-methylbutyl)xanthone.

Nigrolineaxanthone Q (**8**) ($C_{23}H_{24}O_5$) showed a ^{1}H NMR spectrum similar to that of ananixanthone. However, doublets belonging to the olefinic protons, H-11 and H-12, of the dimethylchromene residue in ananixanthone were replaced by two triplets of the methylene protons of the dimethylchromane unit in **8**. In the HMBC spectrum, H-11 (δ 2.90) of the chromane ring and H_2-16 (δ 3.36) of the prenyl group gave cross-peaks with the same oxyaromatic carbon (C-3, δ 159.4), while H-12 (δ 1.91) correlated with a quaternary aromatic carbon (C-4, δ 99.0). Thus, nigrolineaxanthone Q (**8**) was assigned as 1,5-dihydroxy-2-(3-methylbut-2-enyl)-6′,6′-dimethyldihydropyrano(2′,3′:3,4)-xanthone, a dihydro derivative of ananixanthone.

Nigrolineaxanthone R (**9**) ($C_{23}H_{26}O_6$) showed ^{1}H NMR and HMBC data of ring A almost identical with those of **8**. In addition, the ^{1}H NMR spectrum showed characteristic signals of a dimethylchromane ring, a 3-hydroxy-3-methylbutyl unit, and a chelated hydroxy proton. The location of the dimethylchromane ring, the 3-hydroxy-3-methylbutyl group, and the chelated hydroxyl group on ring B was established by the following HMBC data. The chelated hydroxyl proton, at C-1 (δ 157.7), showed cross-peaks with C-2 (δ 111.1) and C-9a (δ 102.9). The dimethylchromane ring was fused in a linear fashion at C-2 through an oxygen at C-3 (δ 160.5) according to the HMBC correlations between H_2-16 (δ 2.79) and C-1 and C-3 as well as a correlation between H_2-17 (δ 1.77) and C-2. The remaining 3-hydroxy-3-methylbutyl unit was attached at C-4 (δ 107.9) by the correlations of H_2-11 (δ 3.00)/C-3, H_2-11/C-4a (δ 152.6), and H_2-12 (δ 1.79)/C-4. Therefore, nigrolineaxanthone R (**9**) was assigned as 1,5-dihydroxy-4-(3-hydroxy-3-methylbutyl)-6′,6′-dimethyldihydropyrano(2′,3′:3,2)xanthone.

The molecular formula of nigrolineaxanthone S (**10**) ($C_{28}H_{30}O_5$) was determined by its molecular ion at *m/z* 447 $[M + H]^+$ in the HRFABMS spectrum. The ^{1}H NMR signals and HMBC data of ring A were similar to those of **8**. Furthermore, the ^{1}H NMR signals of ring B indicated a chelated hydroxyl group, an aromatic proton, characteristic signals of a methylchromene ring, and proton signals of a 4,8-dimethylnona-3,7-dienyl group. The fusion of the methylchromene ring at C-3 (δ 161.4) and C-4 (δ 100.8) with an ether linkage at C-3 was established by correlations of H-11/C-3 and H-12/C-4. The small coupling (J = 0.5 Hz) between the more deshielded chromene hydrogen and the aromatic proton, by the ^5J extended W pathway, suggested that the aromatic proton was at C-2 (δ 99.6), *ortho* to the chromene oxygen. The ^2J cross-peaks between H-2 and C-1 (δ 163.4) and C-3 and a ^3J cross-peak with C-4 confirmed this assigment. The structure of the dienyl moiety was also established using HMBC correlations. Cross-peaks between the methylene protons, H_2-15 with C-12 (δ 126.6) and C-13 (δ 80.9), linked C-15 (δ 41.6) of the dienyl moiety to C-13 of the methylchromene ring. The *E*-configuration of the C-17/C-18 double bond in the 4,8-dimethylnona-3,7-dienyl side chain was deduced from the NOEDIFF spectra. The presence of the dienyl unit was further confirmed by a base peak at *m/z* 295 in the EIMS spectrum due to loss of a $C_{11}H_{19}$ unit from the molecular ion. Thus, nigrolineaxanthone S (**10**) with $[\alpha]^{29}_D$ +58.8 was assigned as 1,5-dihydroxy-6′-methyl-6′-(4,8-dimethylnona-3,7-dienyl)pyrano-(2′,3′:3,4)xanthone.

Nigrolineaquinone A (**11**) ($C_{27}H_{38}O_3$) exhibited IR absorption bands for hydroxyl and carbonyl groups. The UV absorption band at 264 nm suggested the presence of a *p*-benzoquinone moiety.[9] The carbon signals at δ 183.4 and 187.4 were attributed to *p*-benzoquinone carbonyl carbons. The ^{1}H NMR spectrum showed a quinonoid methyl signal at δ 2.12, a quinonoid proton signal at δ 6.59, and an enolic hydroxyl signal at δ 6.94. Signals of four olefinic methine protons, seven methylene groups, and five methyl groups suggested that a substituent of the *p*-quinone unit consisted of four isoprene units. Most of the methyl signals did not show an NOE enhancement to any signal of olefinic protons except for Me-22 (δ 1.71), which enhanced a signal of the olefinic proton, H-20 (δ 5.14). This indicated that the configuration of all the internal double bonds was *E* and the methyl group at 1.71, not the methyl group at δ 1.63, was *cis* to H-20. The chemical shifts of all carbons were assigned by comparing these data with those of (2*E*,6*E*)-farnesol[7] together with the HMBC data. This C_{20} substituent was placed at C-3 according to HMBC correlations between the methylene protons, H_2-7 (δ 3.19) of the side chain, and the quinonoid carbons. The quinonoid proton (H-6, δ 6.59) showed cross-peaks with C-4, a hydroxylated quinonoid carbon (C-2), and a methyl carbon (C-27, δ 16.6), while the methyl protons (Me-27, δ 2.12) showed cross-peaks with C-4 and a quinonoid-methine carbon (C-6, δ 128.4). Thus, nigrolineaquinone A had the structure **11**. This was supported by the FABMS, which showed a base peak at *m/z* 153 $[C_8H_8O_3 + H]^+$ together with a fragment ion of the trienyl side chain ($C_{19}H_{31}$) at *m/z* 259.

Nigrolineaisoflavone A (**12**) ($C_{13}H_{12}O_6$) showed UV absorption bands at 229, 251, 258, and 295 nm. The IR spectrum indicated absorption bands for hydroxyl and carbonyl groups. The ^{1}H NMR spectrum showed signals of two methine protons and two sets of nonequivalent methylene protons. The HMBC correlations of H-2′/C-5′ (δ 68.3), H-3′/C-5′, H_a-5′/C-2′ (δ 83.0), and H_a-5′/C-3′ (δ 76.8) together with chemical shift values of C-2′ and C-5′ established a tetrahydrofuran unit with an ether linkage between C-2′ and C-5′ and a hydroxyl group at C-3′. Signals of a chelated hydroxyl group, one free hydroxyl group, two *meta*-coupled aromatic protons, and an olefinic proton were also present in the ^{1}H NMR spectrum. The olefinic proton (δ 7.85, H-2) was allylically coupled with H-2′ and showed correlations with a carbonyl carbon (C-4) and two oxycarbons, C-8a and C-2′, of the 3-hydroxytetrahydrofuran unit. These data together with the chemical shift values of H-2 suggested that **12** possessed an isoflavone-like structure linked to 3-hydroxytetrahydrofuran at C-3, instead of a phenyl ring. The hydroxyl group (δ 12.62, 5-OH) was placed at C-5 because it formed an intramolecular hydrogen bond with the carbonyl group. The *meta*-coupled doublets at δ 6.39 and 6.27 were then attributed to H-6 and H-8, respectively. The ^2J correlations between H-6 and H-8 and an oxyaromatic carbon (δ 166.2, C-7) established the substituent at C-7 to be a hydroxyl group. Irradiation of H-2′ enhanced the signal of H-2, but not the signal of H-3′. These results

Table 2. ^{13}C NMR Spectral Data (δ) of Nigrolineaxanthones J−R (**1**−**9**)

position	C-type	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]	**8**	**9**
1	C	161.0	158.2	161.0	158.2	161.5	161.0	160.5	158.1	157.7
2	C	104.4	104.9	104.5	104.9	111.1	109.1	112.1	112.1	111.1
3	C	161.3	160.4	161.5	160.6	163.8	162.2	163.3	159.4	160.5
4	C								99.0	107.9
	CH	94.1	94.2	94.2	94.2	93.5	93.4	94.0		
4a	C	154.0	155.5	154.0	155.6	155.6	154.3	154.5	151.9	152.6
5	C	142.5	142.5	142.7	142.7	145.0	142.5	142.7	144.1	145.4
6	CH	119.2	119.4	119.4	119.6	120.6	119.5	119.3	119.6	120.1
7	CH	124.9	125.2	125.7	126.1	126.5	126.0	125.7	123.8	123.5
8	C	135.4	135.5	136.7	136.8	136.6	137.1	136.6		
	CH								117.1	115.8
8a	C	118.4	118.4	118.3	118.4	119.4	118.5	118.3	121.3	121.0
9	C=O	182.9	182.9	182.7	182.7	183.7	182.7	182.6	180.7	181.3
9a	C	103.3	104.2	103.3	104.2	104.0	104.0	103.5	103.0	102.9
10a	C	145.3	145.1	145.5	145.3	147.1	145.2	145.4	144.3	145.0
11	CH		115.5		115.4					
	CH_2	16.1		16.1		21.9	21.4	15.3	16.5	16.9
12	CH		127.5		127.6	123.3	121.2			
	CH_2	31.8		31.7				41.5	31.5	41.6
13	C	76.4	78.3	76.5	78.4	131.4	136.0	76.3	76.1	71.5
14	CH_3	26.8	28.4	26.7	28.4	25.8	25.9	24.5	26.8	29.1
15	CH_3	26.8	28.4	26.7	28.4	17.8	17.9	24.5	26.8	29.1
16	CH_2	33.1	33.0	29.8	29.8	30.7	29.8	29.7	21.4	16.6
17	CH	123.1	122.9						122.1	
	CH_2			45.9	45.9	46.9	41.4	45.9		41.2
18	C	132.6	132.7	70.7	70.7	70.4	74.9	70.8	131.5	71.3
18-OMe	CH_3						49.2			
19	CH_3	25.9	25.9	29.3	29.3	29.5	29.1	29.3	25.8	29.2
20	CH_3	18.0	18.0	29.3	29.3	29.5	25.1	29.3	17.9	29.2

[a] Carbons of the ethoxyl group appeared at δ 57.5 and 15.5.

indicated that H-2′ and H-3′ were *trans*. The mass spectrum showed a fragment ion typical of an isoflavone at *m*/*z* 153 caused by retro Diels−Alder fragmentation of the chromone ring and protonation; the base peak at *m*/*z* 207 could arise by loss of a C_3H_5O fragment from the molecular ion. Therefore, nigrolineaisoflavone A had the new isoflavone-like structure **12** with $[\alpha]^{29}_D$ −62.5. The biogensis of **12** is unclear.

All of the xanthones were examined for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Nigrolineaxanthone N (**5**) showed the best activity with a minimum inhibitory concentration (MIC) of 4 μg/mL, while standard vancomycin had a MIC value of 2 μg/mL. 8-Desoxygartanin and ananixanthone inhibited MRSA at 16 and 32 μg/mL, respectively. The other xanthones were inactive.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. IR spectra were obtained on a FTS165 FT-IR spectometer or a Perkin-Elmer Spectrum GX FT-IR system. ^1H and ^{13}C NMR spectra were recorded on a Varian UNITY INOVA 500 MHz or Bruker AMX 400 MHz spectrometer using $CDCl_3$ solutions unless ortherwise stated, with TMS as internal standard. UV spectra were measured with a Specord S100 spectrophotometer (Analytik Jena Ag). Optical rotations were measured in MeOH at the sodium D line on an AUTOPOL II automatic polarimeter. EI and HREI mass spectra were measured on a Kratos MS 25 RFA spectrometer at 70 eV. ESI and HRESI spectra were measured on a Finnegan MAT 900XL spectrometer. FAB and HRFAB spectra were measured on a VG ZAB 2SEQ spectrometer. TLC and precoated TLC were performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (70−230 mesh ASTM) eluted either with gradient system A ($CHCl_3$−MeOH) or B (20% $CHCl_3$−light petroleum, $CHCl_3$−EtOAc, and EtOAc) or on reversed-phase silica gel C_{18} with gradient system C (MeOH−H_2O), unless otherwise stated. Light petroleum had a bp of 40−60 °C.

Plant Material. The leaves of *G. nigrolineata* were collected at the Ton Nga Chang Wildlife Sanctuary, Hat Yai, Songkhla, Thailand, in June 2000. The plant was identified by Prakart Sawangchote, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, where a voucher specimen (SN184700) has been deposited.

Isolation. The dried and ground leaves (5 kg) were extracted with MeOH (5 L) for 5 days at room temperature three times. Evaporation of the combined methanolic extracts to dryness in vacuo afforded a dark green residue (300 g). The crude extract (300 g) was subjected to column chromatography eluted with a gradient system (light petroleum−$CHCl_3$−MeOH) to afford 14 fractions. Fraction 3 (310 mg, eluted with 25% $CHCl_3$−light petroleum) was further purified on column chromatography eluted with gradient system B to yield **11** (5 mg). Further separation of fraction 7 (109.6 mg, eluted with 30% $CHCl_3$−light petroleum) on column chromatography eluted with gradient system B, followed by preparative TLC with 40% $CHCl_3$−light petroleum, afforded **1** (6.8 mg) and **2** (5.5 mg). Fraction 9 (2.88 g, eluted with 60−90% $CHCl_3$−light petroleum) was subjected to column chromatography on silica gel with solvent mixtures of increasing polarity (70% $CHCl_3$−hexane to 50% MeOH−$CHCl_3$) to afford 8-desoxygartanin[3] (23.4 mg), ananixanthone[4] (9.8 mg), 1,5-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone[5] (40.0 mg), and 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone[6] (5.1 mg). Fraction 11 (568.5 mg, eluted with 60−90% $CHCl_3$−light petroleum) was purified by silica gel column chromatography with solvent mixtures of increasing polarity (30% $CHCl_3$−light petroleum, $CHCl_3$−MeOH to pure MeOH) to yield **8** (3.6 mg) and **10** (5.2 mg). Fraction 12 (4.70 g, eluted with 90% $CHCl_3$−light petroleum−10% MeOH−$CHCl_3$) was further subjected to column chromatography on reversed-phase silica gel C_{18} eluted with gradient system C to afford **3** (5.0 mg) and **4** (2.8 mg). Fraction 13 (33.16 g, eluted with 10−30% MeOH−$CHCl_3$) was purified by column chromatography eluted with gradient system A to afford **5** (30.8 mg), **6** (8.5 mg), **7** (12.5 mg), **9** (5.2 mg), and **12** (19.7 mg).

Nigrolineaxanthone J (1): pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 321 (3.93), 376 (3.31); IR (neat) ν_{max} 3365, 1646 cm^{-1}; ^1H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 380 $[M]^+$ (22), 338 (19), 337 (100), 324 (10), 282

(16), 281 (72), 269 (20); HREIMS *m*/*z* 380.1623 (calcd for $C_{23}H_{24}O_5$, 380.1624).

Nigrolineaxanthone K (2): yellow gum; UV (MeOH) λ_{max} (log ϵ) 295 (3.77), 322 (3.58), 380 (3.02); IR (neat) ν_{max} 3394, 1650 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 378 $[M]^+$ (38), 363 (79), 335 (36), 307 (15); HREIMS *m*/*z* 378.1471 (calcd for $C_{23}H_{22}O_5$, 378.1467).

Nigrolineaxanthone L (3): yellow gum; UV (MeOH) λ_{max} (log ϵ) 296 (3.15), 319 (3.33), 373 (2.73); IR (neat) ν_{max} 3416, 1646 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 398 $[M]^+$ (4), 380 (15), 351 (8), 338 (22), 337 (100), 324 (21), 283 (26), 281 (63), 269 (26); HREIMS *m*/*z* 398.1722 (calcd for $C_{23}H_{26}O_6$, 398.1729).

Nigrolineaxanthone M (4): yellow solid, mp 161−162 °C; UV (MeOH) λ_{max} (log ϵ) 295 (3.94), 316 (3.80), 376 (3.24); IR (neat) ν_{max} 3424, 1646 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 396 $[M]^+$ (5), 378 (12), 364 (21), 363 (100), 345 (25), 335 (63), 308 (23), 307 (45); HREIMS *m*/*z* 396.1577 (calcd for $C_{23}H_{24}O_6$, 396.1573).

Nigrolineaxanthone N (5): yellow solid, mp 199−200 °C; UV (MeOH) λ_{max} (log ϵ) 220 (4.53), 245 (4.48), 264 (4.28), 273 (4.24), 317 (4.19), 369 (3.66); IR (neat) ν_{max} 3409, 1643 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6), Table 1; ^{13}C NMR (125 MHz, acetone-d_6), Table 2; EIMS *m*/*z* 398 $[M]^+$ (3), 381 (15), 380 (46), 337 (54), 324 (83), 309 (100), 307 (27), 283 (42), 281 (55), 269 (21); HREIMS *m*/*z* 398.1733 (calcd for $C_{23}H_{26}O_6$, 398.1729).

Nigrolineaxanthone O (6): pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 220 (3.98), 245 (4.05), 264 (3.85), 273 (3.80), 319 (3.78), 369 (3.32); IR (neat) ν_{max} 3421, 1646 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 380 $[M - MeOH]^+$ (51), 363 (13), 353 (19), 337 (80), 324 (100), 309 (83), 307 (43), 295 (18), 283 (45), 281 (81), 269 (47); HREIMS *m*/*z* 412.1887 (calcd for $C_{24}H_{28}O_6$, 412.1886).

Nigrolineaxanthone P (7): pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 220 (5.34), 243 (5.31), 273 (5.05), 315 (5.04); IR (neat) ν_{max} 3374, 1646 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 444 $[M]^+$ (5), 398 (14), 380 (47), 339 (84), 337 (78), 325 (66), 324 (83), 309 (84), 307 (33), 284 (38), 283 (100), 281 (55), 269 (40); HREIMS *m*/*z* 444.2149 (calcd for $C_{25}H_{32}O_7$, 444.2148).

Nigrolineaxanthone Q (8): yellow gum; UV (MeOH) λ_{max} (log ϵ) 320 (3.89), 374 (3.22); IR (neat) ν_{max} 3424, 1642 cm^{-1}; ^{1}H NMR (500 MHz), Table 1; ^{13}C NMR (125 MHz), Table 2; EIMS *m*/*z* 380 $[M]^+$ (18), 337 (37), 325 (100), 309 (53), 281 (45), 269 (95); HREIMS *m*/*z* 380.1631 (calcd for $C_{23}H_{24}O_5$, 380.1624).

Nigrolineaxanthone R (9): yellow gum; UV (MeOH) λ_{max} (log ϵ) 218 (4.22), 242 (4.19), 258 (4.09), 319 (3.88); IR (neat) ν_{max} 3380, 1643 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD), Table 1; ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD), Table 2; EIMS *m*/*z* 398 $[M]^+$ (5), 380 (8), 343 (19), 325 (58), 324 (19), 309 (19), 269 (100), 268 (32); HREIMS *m*/*z* 398.1727 (calcd for $C_{23}H_{26}O_6$, 398.1729).

Nigrolineaxanthone S (10): yellow gum; $[\alpha]^{29}_D$ +58.8 (*c* 0.017); UV (MeOH) λ_{max} (log ϵ) 294 (3.67), 310 (3.77), 329 (3.78), 376 (3.26); IR (neat) ν_{max} 3418, 1647 cm^{-1}; ^{1}H NMR (500 MHz) δ 13.00 (1H, s, 1-OH), 7.79 (1H, dd, J = 8.0, 1.5 Hz, H-8), 7.34 (1H, dd, J = 8.0, 1.5 Hz, H-6), 7.27 (1H, t, J = 8.0 Hz, H-7), 6.83 (1H, dd, J = 10.0, 0.5 Hz, H-11), 6.30 (1H, d, J = 0.5 Hz, H-2), 5.67 (1H, brs, 5-OH), 5.61 (1H, d, J = 10.0 Hz, H-12), 5.11 (1H, mt, J = 7.5 Hz, H-17), 5.08 (1H, mt, J = 7.5 Hz, H-21), 2.16−2.11 (2H, m, H-16), 2.08−2.00 (2H, m, H-20), 1.98−1.94 (2H, m, H-19), 1.86−1.78 (1H, m, H-15), 1.73−1.68 (1H, m, H-15), 1.67 (3H, d, J = 1.0 Hz, H-23), 1.59 (3H, s, H-25), 1.58 (3H, s, H-24), 1.47 (3H, s, H-14); ^{13}C NMR (125 MHz) δ 180.7 (C, C-9), 163.4 (C, C-1), 161.4 (C, C-3), 150.9 (C, C-4a), 144.3 (C, C-5), 144.1 (C, C-10a), 135.8 (C, C-18), 131.4 (C, C-22), 126.6 (CH, C-12), 124.2 (CH, C-7, 21), 123.4 (CH, C-17), 121.2 (C, C-8a), 120.4 (CH, C-6), 117.1 (CH, C-8), 115.0 (CH, C-11), 103.5 (C, C-9a), 100.8 (C, C-4), 99.6 (CH, C-2), 80.9 (C, C-13), 41.6 (CH_2, C-15), 39.6 (CH_2, C-19), 27.1 (CH_3, C-14), 26.6 (CH_2, C-20), 25.7 (CH_3, C-23), 22.5 (CH_2, C-16), 17.7 (CH_3, C-25), 16.0 (CH_3, C-24); FABMS *m*/*z* 447 $[MH]^+$ (37), 295 (100), 257 (6); HREIMS *m*/*z* 446.2096 (calcd for $C_{28}H_{30}O_5$, 446.2093).

Nigrolineaquinone A (11): orange-red gum; UV (MeOH) λ_{max} (log ϵ) 264 (3.87); IR (neat) ν_{max} 3380, 2915 cm^{-1}; ^{1}H NMR (500 MHz) δ 6.94 (1H, s, 2-OH), 6.59 (1H, q, J = 1.5 Hz, H-6), 5.17 (1H, mt, J = 7.5 Hz, H-8), 5.14 (1H, mt, J = 7.5 Hz, H-16, H-20), 5.11 (1H, mt, J = 7.5 Hz, H-12), 3.19 (2H, d, J = 7.5 Hz, H-7), 2.12 (3H, d, J = 1.5 Hz, H-27), 2.09 (6H, mt, J = 7.5 Hz, H-11, H-15, H-19), 2.00 (6H, mt, J = 7.5 Hz, H-10, H-14, H-18), 1.78 (3H, s, H-23), 1.71 (3H, d, J = 1.0 Hz, H-22), 1.63 (3H, s, H-26), 1.62 (3H, s, H-24 or H-25), 1.61 (3H, s, H-25 or H-24); ^{13}C NMR (125 MHz) δ 187.4 (C, C-4), 183.4 (C, C-1), 150.6 (C, C-2), 149.1 (C, C-5), 137.2 (C, C-9), 135.1 (C, C-13), 134.9 (C, C-17), 131.3 (C, C-21), 128.4 (CH, C-6), 124.4 (CH, C-20), 124.3 (CH, C-16), 124.1 (CH, C-12), 120.6 (C, C-3), 119.6 (CH, C-8), 39.7 (CH_2, C-10, C-14, C-18), 26.8 (CH_2, C-19), 26.7 (CH_2, C-15), 26.5 (CH_2, C-11), 25.7 (CH_3, C-22), 22.1 (CH_2, C-7), 17.7 (CH_3, C-26), 16.6 (CH_3, C-27), 16.2 (CH_3, C-24 or C-25), 16.0 (CH_3, C-23, C-25 or C-24); FABMS *m*/*z* 411 $[MH]^+$ (75), 259 (26), 221 (26), 219 (24), 207 (27), 205 (41), 193 (50), 191 (36), 177 (35), 153 (100), 137 (84), 127 (100), 123 (70), 109 (93), 97 (100), 85 (100); HREIMS *m*/*z* 410.2796 (calcd for $C_{27}H_{38}O_3$, 410.2821).

Nigrolineaisoflavone A (12): pale yellow crystals, mp 186−187 °C; $[\alpha]^{29}_D$ −62.5 (*c* 0.016); UV (MeOH) λ_{max} (log ϵ) 229 (3.77), 251 (3.89), 258 (3.92), 295 (3.47); IR (neat) ν_{max} 3367, 1657 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 12.62 (1H, s, 5-OH), 7.95 (1H, d, J = 2.0 Hz, H-2), 6.39 (1H, d, J = 2.0 Hz, H-6), 6.27 (1H, d, J = 2.0 Hz, H-8), 4.76 (1H, dd, J = 2.5, 2.0 Hz, H-2′), 4.43 (1H, brs, 7-OH), 4.36 (1H, td, J = 5.5, 2.5 Hz, H-3′), 4.12 (1H, dt, J = 8.0, 3.0 Hz, H-5′β), 4.02 (1H, ddd, J = 8.5, 8.0, 6.5 Hz, H-5′α), 2.14−2.05 (1H, m, H-4′β), 1.91−1.86 (1H, m, H-4′α); ^{13}C NMR (125 MHz, acetone-d_6) δ 183.0 (C, C-4), 166.2 (C, C-7), 164.2 (C, C-5), 160.1 (C, C-8a), 155.1 (CH, C-2), 124.1 (C, C-3), 106.1 (C, C-4a), 100.4 (CH, C-6), 95.0 (CH, C-8), 83.0 (CH, C-2′), 76.8 (CH, C-3′), 68.3 (CH_2, C-5′), 34.4 (CH_2, C-4′); EIMS *m*/*z* 246 $[M - H_2O]^+$ (14), 223 (38), 218 (22), 207 (100), 205 (29), 153 (20); HRESMS *m*/*z* 265.0704 $[M + H]^+$ (calcd for $C_{13}H_{13}O_6$, 265.0712).

Antibacterial Activity Testing. MICs were determined by the agar microdilution method.[10] The test substances were dissolved in DMSO (Merck, Germany). Serial 2-fold dilutions of the test substances were mixed with melted Mueller-Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 128 to 0.03 μg/mL. MRSA isolated from a clinical specimen, Songklanakarin Hospital, was used as test strain. Inoculum suspensions (10 μL) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin was used as positive control drug. Growth controls were performed on agar containing DMSO.

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Supporting Information Available: Table of HMBC correlations for compounds **1**−**12** is available free of charge via the Internet at http://pubs.acs.org.

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