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Xanthones from the stem bark of *Garcinia nigrolineata*

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Abstract

Nine xanthones, nigrolineaxanthones A–I, together with nine known xanthones, were isolated from the crude methanol extract of the stem bark of *Garcinia nigrolineata*; two of which have previously been reported as synthetic xanthones. The structures were elucidated by analysis of spectroscopic data, especially using 1D and 2D NMR spectroscopic data.
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Keywords: *Garcinia nigrolineata*; Guttiferae; Trioxygenated and tetraoxygenated xanthones

1. Introduction

In our continuing phytochemical investigation of *Garcinia* plants (Guttiferae family) found in Southern Thailand, we have examined the stem bark of *Garcinia nigrolineata*. This investigation has led to the isolation and structural determination of nine new and nine known xanthones (**1**–**18**).

2. Results and discussion

The MeOH extract of the stem bark of *G. nigrolineata* was subjected to chromatographic purification to afford nine new xanthones: five trioxygenated (**1**, **3**, **4**, **6** and **8**) and four tetraoxygenated (**2**, **5**, **7** and **9**) xanthones, along with nine known xanthones: six trioxygenated ones: 1,3,5-trihydroxy-4-(3-hydroxy-3-methylbutyl)xanthone (**10**) (Gonda et al., 2000), 1,3,7-trihydroxy-2-(3-hydroxy-3-methylbutyl)xanthone (**12**) (Garcia Cortez et al., 1998), 6-deoxyjacreubin (**13**) (Owen and Scheinmann, 1974), morusignin C (**14**) (Hano et al., 1990), 1,5-dihydroxy-6′,6′-dimethylpyrano[2′,3′:3,2]xanthone (**15**) (Burkhardt et al., 1992) and tovoxanthone (**16**) (Gabriel and Gottlieb, 1972), and three tetraoxygenated ones: latisxanthone D (**11**) (Ito et al., 1997), rheediaxanthone A (**17**) (Delle Monache et al., 1981) and brasillixanthone (**18**) (Marques et al., 2000). All structures were elucidated using 1D and 2D NMR spectroscopic data. The ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra. The ^{1}H and/or ^{13}C spectral data of known xanthones were also compared with those reported in the literature.

Most of the new compounds showed four typical UV absorption bands of a xanthone chromophore at λ_{max} 230–248 (strong), 260–264 (strong), 312–320 (medium) and 370–385 (weak) nm (Ito et al., 2003) while those with a chromene unit conjugated to the xanthone nucleus, i.e. nigrolineaxanthones B (**2**), F, (**6**), G (**7**), H (**8**) and I (**9**), exhibited some bathochromic shifts of absorption bands. All compounds showed in their IR spectra hydroxyl and pyrone carbonyl stretching bands at 3328–3446 and 1627–1649 cm^{-1}, respectively. In the ^{1}H NMR spectra, a singlet at δ 12.82–13.34 indicated the presence of a hydroxyl group at C-1, chelated to the xanthone carbonyl group. A signal of a deshielded aromatic proton at δ 7.58–7.73 was attributed to H-8 due to the anisotropic effect of the carbonyl group. These assignments were confirmed by HMBC correlations (Table 3): 1-OH/C-1, C-2 and C-9a, and H-8/C-6, C-9 and C-10a.

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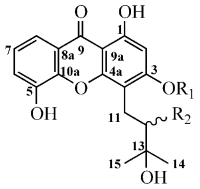

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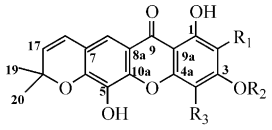
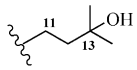
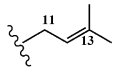
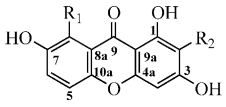
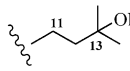
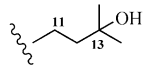


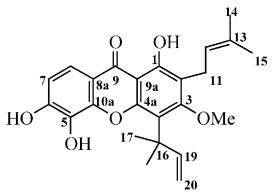
2: R_1 = H, R_2 = Me, R_3 = 

11: R_1 =  , R_2 = R_3 = H



4: R_1 =  , R_2 = H

12: R_1 = H, R_2 = 



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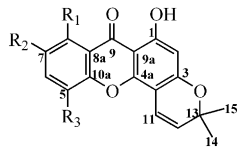

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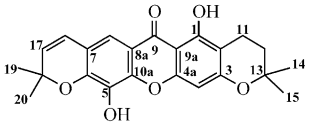

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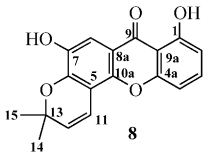

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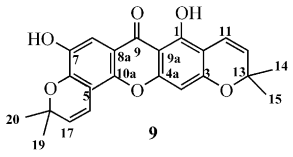

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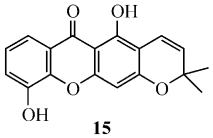

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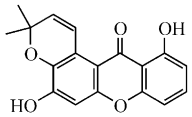

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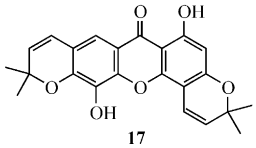

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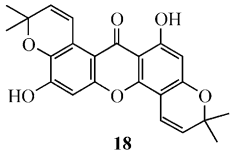

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Nigrolineaxanthone A (**1**) was isolated as a yellow solid, mp 142.8–144.6 °C. The molecular formula $C_{19}H_{20}O_6$ was deduced by HREIMS. The ^{1}H NMR spectrum (Table 1) was almost identical to the known xanthone **10**: characteristic peaks of a 1,2,3-trisubstituted benzene ring [δ 7.73 and 7.28 (1H each, *dd*, J = 8.0 and 2.0 Hz) and 7.23 (1H, *t*, J = 8.0 Hz)], a 3-hydroxy-3-methylbutyl unit [δ 2.95–2.92 (2H, *m*), 1.77–1.74 (2H, *m*) and 1.36 (6H, *s*)], a chelated hydroxy proton [δ 12.82 (1-OH, *s*)] and an aromatic-proton singlet at δ 6.39. As the lowest-field aromatic-proton (δ 7.73) of the 1,2,3-trisubstituted benzene was assigned to H-8, two other aromatic-protons at δ 7.28 and 7.23 which coupled to H-8 with coupling constants of 2 and 8 Hz, were attributed to H-6 and H-7, respectively. This was confirmed by correlation peaks between H-6 and a methine carbon (δ 115.9, C-8) and the oxygenated carbon (C-10a) as well as those between H-7 and an oxygenated (hydroxylated) aromatic carbon (δ 145.5, C-5) and a quaternary aromatic carbon (δ 120.9, C-8a). The chelated hydroxyl proton (1-OH) gave a ^3J peak with a methine aromatic carbon at (δ 94.0, C-2) which was correlated to the aromatic proton (δ 6.39, H-2) in the HMQC spectrum. Irradiation of H-2 in a NOED experiment enhanced the singlet signals of the methoxy protons (δ 3.91) and chelated hydroxy proton (δ 12.85). Consequently, the methoxyl group was assigned to be at C-3 (δ 163.6). The 3-hydroxy-3-methylbutyl unit was then located at C-4. This was confirmed by HMBC correlations of H-11 (δ 2.95–2.92)/C-3 and H-12 (δ 1.77–1.74)/C-4. Accordingly, nigrolineaxanthone A was assigned as 1,5-dihydroxy-3-methoxy-4-(3-hydroxy-3-methylbutyl)xanthone (**1**), a new monomethyl ether derivative of the known xanthone

Nigrolineaxanthone B (**2**) was isolated as yellow crystals, mp 165.0–167.2 °C. The HREIMS indicated its molecular formula as $C_{24}H_{26}O_7$. Its ^{1}H NMR spectrum (Table 1) was similar to that of **1** except for the fact that the two higher-field aromatic protons of the 1,2,3-trisubstituted benzene ring of **1** were replaced in **2** by a dimethylchromene ring [δ 6.44, 5.73 (1H each, *d*, J = 10.0 Hz) and 1.54 (6H, *s*). Irradiation of the olefinic methine proton (δ 6.44, H-16) of the dimethylchromene ring caused a NOE enhancement of the lowest-field aromatic H-8 (δ 7.45), suggesting that the dimethylchromene ring was fused in a linear fashion to the xanthone nucleus. The location of the singlet aromatic proton (δ 6.37), the methoxyl group (δ 3.91) and the 3-hydroxy-3-methylbutyl unit in the right-handed aromatic ring was established by HMBC correlations (Table 3) to be the same as **1**. Enhancements of both chelated hydroxy and methoxy proton signals after irradiation of H-2 con-

Table 1
^{1}H NMR spectral data for nigrolineaxanthones A–I (**1**–**9**)

Position	δ_H(mult., J_{Hz})								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1-OH	12.82 (*s*)	12.96 (*s*)	12.83 (*s*)	13.40 (*s*)	13.35 (*s*)	12.92 (*s*)	13.38 (*s*)	12.85 (*s*)	13.34 (*s*)
2	6.39 (*s*)	6.37 (*s*)	6.42 (*s*)	6.16 (*d*, 2.0)		6.27 (*d*, 0.5)		6.74 (*dd*, 8.5, 1.0)	
3								7.54 (*t*, 8.5)	
3-OMe	3.91 (*s*)	3.91 (*s*)	3.94 (*s*)		3.78 (*s*)				
4				6.23 (*d*, 2.0)			6.45 (*s*)	6.91 (*dd*, 8.5, 1.0)	6.33 (*s*)
5				7.11 (*d*, 8.5)		7.39 (*d*, 8.5)			
6	7.28 (*dd*, 8.0, 2.0)		7.29 (*dd*, 7.5, 1.5)	7.18 (*d*, 8.5)		7.28 (*dd*, 8.5, 3.0)			
7	7.23 (*t*, 8.0)		7.24 (*t*, 7.5)		6.97 (*d*, 9.0)				
7-OH								5.52 (brs)	5.50 (brs)
8	7.73 (*dd*, 8.0, 2.0)	7.45 (*s*)	7.73 (*dd*, 7.5, 1.5)		7.73 (*d*, 9.0)	7.60 (*d*, 3.0)	7.63 (*s*)	7.63 (*s*)	7.58 (*s*)
11	2.95–2.92 (*m*)	2.95–2.92 (*m*)	3.30 (*dd*, 15.0, 2.0) 2.83 (*dd*, 15.0, 8.0)	3.39–3.36 (*m*)	3.42 (*d*, 6.0)	6.83 (*dd*, 10.0, 0.5)	2.85 (*t*, 6.5)	6.94 (*d*, 10.0)	6.75 (*d*, 10.0)
12	1.77–1.74 (*m*)	1.78–1.74 (*m*)	3.67 (*dd*, 8.0, 2.0)	1.85–1.82 (*m*)	5.30 (*mt*, 6.0)	5.61 (*d*, 10.0)	1.87 (*t*, 6.5)	5.78 (*d*, 10.0)	5.59 (*d*, 10.0)
14	1.36 (*s*)	1.34 (*s*)	1.36 (*s*)	1.27 (*s*)	1.71 (*s*)	1.48 (*s*)	1.36 (*s*)	1.56 (*s*)	1.47 (*s*)
15	1.36 (*s*)	1.34 (*s*)	1.36 (*s*)	1.27 (*s*)	1.80 (*s*)	1.48 (*s*)	1.36 (*s*)	1.56 (*s*)	1.47 (*s*)
16		6.44 (*d*, 10.0)					6.95 (*d*, 10.0)		6.88 (*d*, 10.0)
17		5.73 (*d*, 10.0)			1.67 (*s*)		5.79 (*d*, 10.0)		5.75 (*d*, 10.0)
18					1.67 (*s*)				
19		1.54 (*s*)			6.64 (*dd*, 18.0, 10.5)		1.59 (*s*)		1.55 (*s*)
20		1.54 (*s*)			5.25 (*dd*, 18.0, 1.0) 5.05 (*dd*, 10.5, 1.0)		1.59 (*s*)		1.55 (*s*)

firmed the above assignments. Since there was no other proton signal the substituent at C-5 must be a hydroxyl group. C-5 appeared at higher field than that in **1** due to the effect of two *ortho*-oxygenated groups. Thus, nigrolineaxanthone B was assigned as 1,5-dihydroxy-3-methoxy-4-(3-hydroxy-3-methylbuty1)-6′,6′-dimethylpyrano-(2′,3′:6,7)xanthone (**2**).

Nigrolineaxanthone C (**3**) was obtained as a pale yellow solid, mp 104.5–105.8 °C. The molecular formula was determined as $C_{19}H_{20}O_7$ by HREIMS. The ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2) were similar to those of **1**, suggesting that **3** was also a 1,3,5-trioxygenated xanthone, but with the replacement of the methylene-proton signal of H-12 in **1** with an oxymethine-proton signal in **3**. The presence of an additional oxymethine carbon at δ 79.6 (C-12) supported this conclusion. Thus, the side chain was now a 2,3-dihydroxy-3-methylbutyl unit: δ 3.67 (1H, *dd, J* = 8.0 and 2.0 Hz), 3.30 (1H, *dd, J* = 15.0 and 2.0 Hz), 2.83 (1H, *dd, J* = 15.0 and 8.0 Hz) and 1.36 (6H, *s*)]. In the HMBC spectrum (Table 3), a cross peak between this oxymethine proton (H-12) with C-4 (δ 106.1) established the linkage of the 2,3-dihydroxy-3-methylbutyl unit at C-4. Irradiation of H-2 (δ 6.42, *s*) in a NOED spectrum enhanced signals of both the chelated hydroxy and methoxy protons, indicating the attachment of the methoxyl group at C-3. Thus, nigrolineaxanthone C was identified as 1,5-dihydroxy-3-methoxy-4-(2,3-dihydroxy-3-methylbutyl)xanthone (**3**).

Nigrolineaxanthone D (**4**) was isolated as a pale yellow solid, mp 196.0–197.8 °C. HREIMS established a molecular formula of $C_{18}H_{18}O_6$. The ^{1}H NMR spectrum (Table 1) showed characteristic peaks of a chelated hydroxy proton [δ 13.40 (*s*)], two *ortho*-aromatic protons [(δ 7.11 and 7.18, 1H each, *d, J* = 8.5 Hz)] and two *meta*-aromatic protons [δ 6.16 and 6.23 (1H each, *d, J* = 2.0 Hz)], in addition to typical signals of a 3-hydroxy-3-methylbutyl unit [δ 3.39–3.36 (2H, *m*), 1.85–1.82 (2H, *m*) and 1.27 (6H, *s*)]. The highly deshielded position of the methylene protons of the 3-hydroxy-3-methylbutyl unit (δ 3.37, H-11) suggested that this side chain was *peri* to a carbonyl group. This was confirmed by ^3J correlations in the HMBC spectrum (Table 3): H-11/C-7 (δ 150.9), H-11/C-8a (δ 118.8) and H-12 (δ 1.85–1.82)/C-8. Two *ortho* aromatic protons (δ 7.11 and 7.18) were attributed to H-5 and H-6, respectively, according to the HMBC correlations between H-5/C-7 and C-8a, and H-6/C-8 and C-10a (δ 151.8). Since the hydroxyl group (δ 13.40) was placed at C-1, two *meta* aromatic protons (δ 6.16 and 6.23) were then located at C-2 and C-4, respectively. A ^2J correlation between H-4 and an oxyaromatic carbon (δ 164.7, C-3) indicated the presence of a hydroxyl group at C-3. Accordingly, the structure of nigrolineaxanthone D was 1,3,7-trihydroxy-8-(3-hydroxy-3-methylbutyl)xanthone (**4**).

Nigrolineaxanthone E (**5**), isolated as a pale yellow solid, mp 102.5–103.8 °C, had the molecular formula $C_{24}H_{26}O_6$ (HRFABMS). The ^{1}H NMR spectrum (Table 1) consisted of one chelated hydroxyl signal [δ 13.35 (1H, *s*)], two *ortho*-coupled aromatic signals [δ 7.73 and 6.97 (1H each, *d, J* = 9.0 Hz)], one methoxy proton signal [δ 3.78 (3H, *s*)], characteristic signals of a 1,1-dimethylallyl group [δ 6.64 (1H, *dd, J* = 18.0 and 10.5 Hz), 5.25 (1H, *dd, J* = 18.0 and 1.0 Hz), 5.05 (1H, *dd, J* = 10.5 and 1.0 Hz) and 1.67 (6H, *s*)] and signals of a prenyl unit [δ 5.30 (1H, *mt, J* = 6.0 Hz), 3.42 (2H, *d, J* = 6.0 Hz), 1.80 and 1.71 (each 3H, *s*)]. The aromatic proton (δ 6.97) was attributed to H-7 as it coupled (*J* = 9 Hz) to the most deshielded aromatic H-8 (δ 7.73). In addition, H-7 showed correlations to C-5 (δ 130.9), C-6 (δ 149.2) and C-8a (δ 113.8) in the HMBC spectrum (Table 3). Since no other aromatic protons were observed, C-5 and C-6 substituents were hydroxyl groups. The prenyl unit was assigned to be at C-2 (δ 118.4) by the correlations between H-11 (δ 3.42) and C-1 (δ 160.0), C-2 and C-3 (δ 164.4) (Table 3). Furthermore, the enhancement of the signal of vinylic methyl protons, Me-14 (δ1.71), upon irradiation of the olefinic H-12 (δ 5.30) in a NOED spectrum, indicated that Me-14 was *cis* to H-12. The methoxyl and the 1,1-dimethylallyl groups were assigned to be at C-3 and C-4 (δ 118.6), respectively, based on the ^3J correlations (Table 3): the methoxyl protons/C-3 and the gem-dimethyl protons

Table 2
^{13}C NMR spectral data for nigrolineaxanthones A–I (**1**–**9**)

Position	δ_C								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	161.9	161.7	162.4	163.3	160.0	163.0	160.2	161.8	157.6
2	94.0	94.0	94.2	97.9	118.4	99.2	111.5	110.3	104.5
3	163.6	163.3	163.9	164.7	164.4	160.9	162.1	135.9	160.1
3-OMe	56.0	56.0	56.3		62.7				
4	108.6	108.7	106.1	93.5	118.6	100.9	94.6	106.7	94.8
4a	153.6	153.5	154.0	157.5	152.7	151.9	155.8	156.1	156.9
5	145.5	138.7	145.4	116.1	130.9	119.1	146.4	109.2	109.2
6	120.4	145.4	120.4	123.7	149.2	123.8	145.7	146.5	145.7[a]
7	123.9	118.3	124.1	150.9	112.9	152.1	109.1	146.9	141.9
8	115.9	113.1	116.1	129.8	117.8	109.3	108.7	108.7	108.7
8a	120.9	114.0	120.8	118.8	113.8	121.1	114.8	114.1	114.0
9	181.4	180.7	181.4	183.3	181.3	180.5	180.1	181.3	180.0
9a	103.5	103.2	103.5	103.7	105.6	103.5	102.8	108.5	103.2
10a	144.7	145.7	144.6	151.8	144.8	150.3	141.7	142.1	146.3[a]
11	16.4	16.6	25.7	21.3	22.9	115.0	16.0	115.4	115.6
12	41.0	41.4	79.6	42.9	122.5	127.0	41.1	129.9	127.4
13	72.7	72.2	74.4	71.4	132.2	78.3	72.7	79.5	78.1
14	29.7	29.6	24.5	29.2	25.7	28.3	29.3	28.3	28.3
15	29.7	29.6	24.5	29.2	18.0	28.3	29.3	28.3	28.3
16		121.6			42.0		115.6		115.4
17		131.0			28.7		129.7		129.9
18		78.2			28.7		79.2		79.3
19		28.5			156.8		28.3		28.2
20		28.5			104.3		28.3		28.2

[a] Interchangeable.

Table 3
Major HMBC correlations for nigrolineaxanthones A–I (**1**–**9**)

Proton	HMBC correlations								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1-OH	1, 2, 9a	1, 2, 9a	1, 2, 9a		1, 2, 9a	1, 2, 9a	1, 2, 9a	1, 2, 9a	1, 2, 9a
2	1, 3, 4, 9a	1, 3, 4, 9a	1, 3, 4, 9a	1, 2, 4, 9a		1, 3, 4, 9a		1, 4, 9a	
3								1, 4a, 9a	
3-OMe	3	3	3		3				
4				2, 3, 4a, 9a			2, 3, 4a, 9a	2, 4a, 9a	2, 3, 4a, 9a
5				7, 8a		7, 8a, 10a			
6	8, 10a		8, 10a	8, 10a		7, 10a			
7	5, 8a		5, 8a		5, 6, 8a				6, 7, 8
8	6, 9, 10a	6, 9, 10a, 16	6, 9, 10a		6, 9, 10a	6, 9, 10a	6, 9, 10a	6, 7, 9, 10a	6, 8a, 9, I0a
11	3, 4, 4a, 13	3, 4, 4a, 12	3, 4, 4a, 12, 13	7, 8, 8a, 12, 13	1, 2, 3, 12, 13	3, 4a, 13	1, 2, 3, 12, 13	6, 13	1, 2, 3, 13
12	4, 14, 15	11, 13	4, 11, 13	8, 11, 14, 15	11, 14, 15	4, 13, 14, 15	2, 11, 13, 14, 15	5, 13, 14, 15	2, 13, 14, 15
14, 15	11, 12, 14,	12, 14, 15	11, 12, 14, 15	12, 13, 14, 15	12, 13	12, 13, 14, 15	11, 12, 13, 14, 15	11, 12, 14, 15	12, 14, 15
16		6, 18					6, 18		5, 6, 10a, 18
17		7, 18			4, 16, 18, 19		7, 18		5, 18, 19, 20
18					4, 16, 17, 19				
19		17, 18, 20			4, 16, 17, 18		16, 17, 18, 20		17, 18, 20
20		17, 18, 19			16, 19		16, 17, 18, 19		17, 18, 19,

(Me-17 and Me-18, δ 1.67)/C-4. Therefore, nigrolineaxanthone E was assigned as 1,5,6-trihydroxy-3-methoxy-2-(3-methyl-2-butenyl)-4-(1,1-dimethylallyl)xanthone (**5**).

Nigrolineaxanthone F (**6**) was obtained as yellow crystals, mp 235.9–236.5 °C. The HREIMS indicated its molecular formula as $C_{18}H_{14}O_3$. The ^{1}H NMR spectrum (Table 1) showed similar signals to 6-deoxyjacareubin (**13**) (Owen and Scheinmann, 1974): a chelated hydroxy proton (δ 12.92), an aromatic proton (δ 6.27, *d*, J = 0.5 Hz) and dimethylchromene protons [δ 6.83 (1H, *dd*, J 10.0 and 0.5 Hz), 5.61 (1H, *d*, J = 10.0 Hz) and 1.48 (6H, *s*)]. These were located at the same position as found in **13** according to cross peaks in HMBC spectrum (Table 3). The typical signals of a 1,2,4-trisubstituted benzene ring [δ 7.60 (1H, *d*, J = 3.0 Hz), 7.39 (1H, *d*, J = 8.5 Hz) and 7.28 (1H, *dd*, J = 8.5 and 3.0 Hz)] were also observed in the ^{1}H NMR spectrum and were assigned to H-8, H-5 and H-6, respectively. The assignments were supported by ^3J HMBC correlations (Table 3). The ^3J correlation between H-5 and an oxyaromatic carbon (C-7, δ 152.1) established the substituent at C-7 to be a hydroxyl group. Nigrolineaxanthone F was therefore assigned as 1,7-dihydroxy-6′,6′-dimethylpyrano(2′,3′:3,4)xanthone (**6**), a new naturally occurring trioxygenated xanthone, which has previously been reported as a synthetic compound (Clarke et al., 1974).

Nigrolineaxanthone G (**7**) was obtained as yellow crystals, mp 205.8–207.2 °C. Its molecular formula $C_{23}H_{22}O_6$ was deduced by HREIMS. The ^{1}H NMR spectrum (Table 1) showed characteristic signals for a chelated hydroxyl group (δ 13.38, 1-OH), two aromatic protons (δ 7.63 and 6.45, 1H each, *s*) and typical signals of a dimethylchromene ring [δ 6.95 and 5.79 (1H each, *d*, J = 10.0 Hz) and δ 1.59 (6H, *s*)]. The spectrum further showed the presence of two methylene-proton signals as triplets [δ 2.85 and 1.87 (2H each, *t*, J = 6.5 Hz) and two methyl groups as a singlet (δ 1.36), implying the presence of a dimethylchromane ring. In the HMBC spectrum (Table 3), the chelated hydroxy proton at δ 13.38 and methylene protons (δ 2.85, H-11) of a chroman ring gave cross peaks with the same oxyaromatic carbon, (δ 160.2, C-1) while the other methylene protons (δ 1.87, H-12) correlated with a quaternary aromatic carbon, (δ 111.5, C-2). These data indicated that the dimethylchroman ring was fused to C-2 and C-3 (δ 162.1) of a xanthone nucleus with an ether linkage at C-3. The aromatic proton at δ 6.45 was then assigned to H-4 due to its cross peaks with C-2, C-3, C-9a (δ 102.8) and C-10a (δ 141.7). Irradiation of the lowest-field aromatic H-8 (δ 7.63) caused a NOE enhancement of the *cis*-olefinic proton (H-16, δ 6.95) of the dimethylchromene ring, suggesting the fusion of the dimethylchromene to C-6 (δ 145.7) and C-7 (δ 109.1) of the xanthone nucleus with an ether linkage at C-6. This was confirmed by HMBC correlations of H-16 (δ 6.95)/C-6 and H-17 (δ 5.79)/C-7). The remaining carbon signal at δ 146.4 was attributed to C-5, substituted by a hydroxyl group. Nigrolineaxanthone G was then assigned as 1,5-dihydroxy-6′,6′-dimethyldihydropyrano(2′,3′:3,2)-6″,6″-dimethylpyrano-(2″,3″:6,7)xanthone (**7**) which was previously obtained by synthesis (Delle Monache et al., 1984).

Nigrolineaxanthone H (**8**) was obtained as yellow crystals, mp 220.1–222.5 °C. The molecular formula was determined as $C_{18}H_{14}O_5$ by HREIMS. The ^{1}H NMR spectrum (Table 1) showed a sharp singlet of a chelated hydroxy proton (1-OH) at δ 12.85, a broad singlet signal

of a phenolic proton at δ 5.52, an aromatic proton (δ 7.63, *s*) and typical signals of a dimethylchromene ring [δ 6.94, 5.78 (1H each, *d*, $J=10.0$ Hz) and δ 1.56 (6H, *s*)], in addition to the presence of a 1,2,3-trisubstituted-benzene signals [δ 7.54 (1H, *t*, $J=8.5$ Hz), 6.91 and 6.78 (1H each, *dd*, $J=8.5$ and 0.5 Hz)]. In the HMBC data (Table 3), the chelated hydroxy proton (1-OH) caused a cross peak with an aromatic methine carbon at δ 110.3 which correlated to an aromatic proton of a 1,2,3-trisubstituted benzene ring at δ 6.78 in the HMQC spectrum. These results indicated that this aromatic proton was located at C-2. Two aromatic protons at δ 7.54 and 6.91 were then attributed to H-3 and H-4, respectively, according to their splitting patterns and coupling constants. In addition there were cross peaks between H-3/C-1 (δ 161.8) and C-4a (δ 156.1) and H-4/C-2 and C-9a (δ 108.5). Irradiation of the hydroxy proton at δ 5.52 in a NOE experiment enhanced the signal of the most deshielded aromatic H-8 (δ 7.63), indicating the hydroxyl group to be at C-7. The linkage of the chromene ring at C-5 (δ 109.2) and C-6 with an ether linkage at C-6 was established by HMBC data (Table 3) which showed two cross peaks: H-11 (δ 6.94)/C-6 and H-l2 (δ 5.78)/C-5. Therefore, nigrolineaxanthone H was determined as 1,7-dihydroxy-6′,6′-dimethylpyrano(2′,3′:6,5)-xanthone (**8**).

Nigrolineaxanthone I (**9**) was obtained as a yellow solid, mp 241.7–243.5 °C. The molecular formula was determined as $C_{23}H_{20}O_6$ by HREIMS. The ^{1}H NMR spectrum (Table 1) was similar to that of **8** except that **9** showed signals for two dimethylchromene units. This implied that two aromatic protons (δ 7.54 and 6.73) of a 1,2,3-trisubstituted benzene ring in **8** were replaced in **9** by a dimethylchromene ring of which the signal of *gem*-dimethyl protons resonated as a singlet at δ 1.47 and two doublet signals of two *cis*-olefinic protons (H-16 and H-17) at δ 6.75 and 5.59. In the HMBC spectrum (Table 3), cross peaks between two *cis*-olefinic protons (δ 6.75 and 5.59) and C-3 (δ 160.1) and C-2 (δ 104.5), respectively, established the location of the dimethylchromene ring at C-2 and C-3 with an ether linkage at C-3. The linkage of the other dimethylchromene ring at C-5 (δ 109.2) and C-6 (δ 145.7 or 146.3), as in **8**, was established by HMBC correlations between C-5/H-17 and C-6/H-16. The structure of nigrolineaxanthone I was therefore 1,7-dihydroxy-6′,6′-dimethylpyrano(2′,3′:-3,2)-6″,6″-dimethylpyrano(2″,3″:6,5)xanthone (**9**).

Nigrolineaxanthones A (**1**), B (**2**) and D (**4**) with 3-hydroxy-3-methylbutyl substituents showed in their EI mass spectra weak molecular ions and strong fragment ions due to loss of 73 mass units [$\cdot CH_2C(CH_3)_2(OH)$] from the molecular ion by cleavage at a benzylic bond. Similar fragmentations were found in nigrolineaxanthone C (**3**) having a 2,3-dihydroxy-3-methylbutyl moiety (loss of 89 mass units) and E (**5**) having a prenyl group (loss of 55 mass units). The remaining xanthones (**6**–**9**) with dimethylchromene units showed characteristic fragment peaks corresponding to loss of 15 mass units ($\cdot CH_3$) from the molecular ions. Interestingly, the presence of the 3-hydroxy-3-methylbutyl substituent or a dimethylchroman ring can be easily distinguished not only by the difference of 18 mass units in the molecular ion but also by the multiplicities of their methylene protons in ^{1}H NMR spectra: the methylene protons of the dimethylchroman ring appear as two triplets while those of the uncyclized chain have two sets of multiplets.

3. Experimental

3.1. General

Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. IR spectra were obtained either on a FTS 165 FT-IR spectometer or a Perkin-Elmer Spectrum GX FT-IR system. ^{1}H and ^{13}C NMR spectra were recorded on a Varian UNITY INOVA 500 MHz or a Brüker AMX 400 MHz spectrometer using deuterochloroform solutions unless otherwise stated with TMS as internal standard. UV spectra were measured with a Specord S100 spectrophotometer (Analytik Jena Ag). Optical rotations were measured in MeOH solution at the sodium D line (590 nm) 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). CC was performed on silica gel (Merck) type 100 (70–230 mesh ASTM), eluted with a gradient of $CHCl_3$–MeOH, or on reversed phase silica gel C-18 with a mixture of MeOH–H_2O as eluent. Light petroleum had bp 40–60 °C.

3.2. Plant material

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

3.3. Isolation

The crude MeOH extract (88.8 g) from 1.85 kg of material was subjected to CC on reversed phase silica gel and eluted with solvent mixtures of decreasing

polarity (1:1 MeOH–H_2O to pure MeOH) to afford 8 subfrs. Subfr. 2 (5.45 g, eluted with 1:1 MeOH-H_2O) was further purified by CC on silica gel to yield 3 subfrs. The first subfr. (215 mg, eluted with $CHCl_3$ to 1:9 MeOH–$CHCl_3$), upon flash CC on silica gel with solvent mixtures of increasing polarity ($CHCl_3$ to 1:9 MeOH–$CHCl_3$) and subsequent prep TLC with 1:4 EtOAc–hexane as a mobile phase yielded **3** (5 mg), **4** (7 mg), **10** (5 mg) and **12** (10 mg). Further separation of subfr. 3 (5.38 g, eluted with 1:1 MeOH–H_2O) on silica gel column chromatography gave 3 subfrs. The first subfr. (190 mg, eluted with $CHCl_3$ to 3:97 MeOH–$CHCl_3$) was subjected to flash CC on silica gel with solvent mixtures of increasing polarity ($CHCl_3$ to 1:10 MeOH–$CHCl_3$) and prep TLC with 1:4 EtOAc–hexane as a mobile phase to yield **1** (6 mg) and **7** (3 mg). Subfr. 4 (3.38 g, eluted with 50–60% aq. MeOH) was fractionated by silica gel CC to yield 6 subfrs. The first (17 mg, eluted with 1:99 MeOH–$CHCl_3$), third (137 mg, eluted with 3–10% MeOH in $CHCl_3$), fourth (21 mg, eluted with 10–20% MeOH in $CHCl_3$) and fifth (46 mg, eluted with 20–40% MeOH in $CHCl_3$) subfrs. were further purified by CC on reversed phase silica gel C-18 with solvent mixtures of decreasing polarity (80–90% aq. MeOH) and subsequent prep TLC with 50–70% CH_2Cl_2 in light petroleum as a mobile phase to afford **2** (3 mg), **6** (2 mg), **8** (9 mg), **11** (2 mg), **13** (4 mg), **14** (3 mg), **15** (4.5 mg) and **16** (2 mg). Subfr. 5 (38 mg, eluted with 60–70% aq. MeOH) was purified by the same method as subfr. 4 to yield **5** (8 mg), **9** (2.6 mg), **17** (2 mg) and **18** (3 mg).

3.3.1. Nigrolineaxanthone A (*1*)

Yellow solid, mp 142.8–144.6 °C; UV: λ_{max} MeOH nm: 248, 260, 318, 370; IR ν_{max} (neat) cm^{-1}: 3358 (O–H), 1645 (C=O); MS *m/z* (rel. int): 344 $[M]^+$ (3), 326 (9), 311 (8), 272 (17), 271 (100), 270 (46), 258 (7), 241 (19); HREIMS *m/z* 344.1259 [M]+ (calc. for $C_{19}H_{20}O_6$, 344.1260); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.2. Nigrolineaxanthone B (*2*)

Yellow crystals, mp 165.0–167.2 °C; UV: λ_{max} MeOH nm: 240, 274, 322, 385; IR ν_{max} (neat) cm^{-1}: 3439 (O–H), 1644 (C=O); MS *m/z* (rel. int): 408 $[M–H_2O]^+$ (6), 393 (10), 354 (24), 353 (100), 338 (9), 337 (31), 323 (10); HREIMS *m/z* 426.1673 $[M]^+$ (calc. for $C_{24}H_{26}O_7$, 426.1679); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.3. Nigrolineaxanthone C (*3*)

Pale yellow solid, mp 104.5–105.8 °C; $[\alpha]_D^{29} = -43.5$ ° ($c = 2.3\times10^{-2}$, EtOH); UV: λ_{max} MeOH nm: 244, 258, 320, 375; IR ν_{max} (neat) cm^{-1}: 3394 (O–H), 1645 (C=O); MS *m/z* (rel. int): 360 $[M]^+$ (4.8), 301 (9), 272 (20), 271 (100), 259 (16), 258 (9), 241 (22); HREIMS *m/z* 360.1217 $[M]^+$ (calc. for $C_{19}H_{20}O_7$, 360.1209); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.4. Nigrolineaxanthone D (*4*)

Pale yellow solid, mp 196.0–197.8 °C; UV: λ_{max} MeOH nm: 248, 262, 312, 380; IR ν_{max} (neat) cm^{-1}: 3328 (O–H), 1649 (C=O); MS *m/z* (rel. int): 330 $[M]^+$ (7.5), 312 (100), 297 (31), 283 (33), 270 (50), 269 (50), 257 (86); HREIMS *m/z* 330.1100 [M]+ (calc. for $C_{18}H_{18}O_6$, 330.1103); ^1H NMR ($CDCl_3$ + MeOH-d_4): Table 1; ^{13}C NMR ($CDCl_3$ + MeOH-d_4): Table 2.

3.3.5. Nigrolineaxanthone E (*5*)

Pale yellow solid, mp 102.5–103.8 °C; UV: λ_{max} MeOH nm: 240, 264, 318, 370; IR ν_{max} (neat) cm^{-1}: 3416 (O–H), 1627 (C=O); MS *m/z* (rel. int): 410 $[M]^+$ (22), 379 (12), 367 (100), 355 (88), 339 (60), 327 (21), 323 (43), 311 (46), 309 (27), 297 (27), 285 (19); HRFABMS *m/z* [M + H]+ 411.1805 (calc. for $C_{24}H_{27}O_6$, 411.1808); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.6. Nigrolineaxanthone F (*6*)

Yellow solid, mp 235.9–236.5 °C; UV: λ_{max} MeOH nm: 230, 276, 328, 385; IR ν_{max} (neat) cm^{-1}: 3446 (O–H), 1644 (C=O); MS *m/z* (rel. int): 310 $[M]^+$ (5), 295 (100); HREIMS *m/z* 310.0841 $[M]^+$ (calc. for $C_{18}H_{14}O_5$, 310.0841); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.7. Nigrolineaxanthone G (*7*)

Yellow solid, mp 205.8–207.2 °C; UV: λ_{max} MeOH nm: 240, 262, 334, 376; IR ν_{max} (neat) cm^{-1}: 3416 (O–H), 1644 (C=O); MS *m/z* (rel. int): 394 (18) $[M]^+$, 379 (29), 353 (23), 351 (36), 339 (100), 323 (32), 321 (16); HREIMS *m/z* 394.1424 $[M]^+$ (calc. for $C_{23}H_{22}O_6$, 394.1416); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.3.8. Nigrolineaxanthone H (*8*)

Yellow crystals, mp 220.1–222.5 °C; UV: λ_{max} MeOH nm: 240, 260, 338, 384; IR ν_{max} (neat) cm^{-1}: 3409 (O–H), 1642 (C=O); MS *m/z* (rel. int): 310 $[M]^+$ (20), 295 (100), 149 (15); HREIMS *m/z* 310.0848 $[M]^+$ (calc. for $C_{18}H_{14}O_5$ 310.0841); ^1H NMR: Table 1; ^{13}C NMIR: Table 2.

3.3.9. Nigrolineaxanthone 1 (*9*)

Yellow solid, mp 241.7–243.5 °C; UV: λ_{max} MeOH nm: 230, 285, 346, 385; IR ν_{max} (neat) cm^{-1}: 3424 (O–H), 1646 (C=O); MS *m/z* (rel. int): 392 $[M]^+$ (11), 377 (100), 359 (13), 347 (8); HREIMS *m/z* 392.1257 $[M]^+$ (calc. for $C_{23}H_{20}O_6$, 392.1260); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

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