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NEW XANTHONES FROM THE BARKS OF *CRATOXYLUM*

SUMATRANUM SSP. *NERIIFOLIUM*

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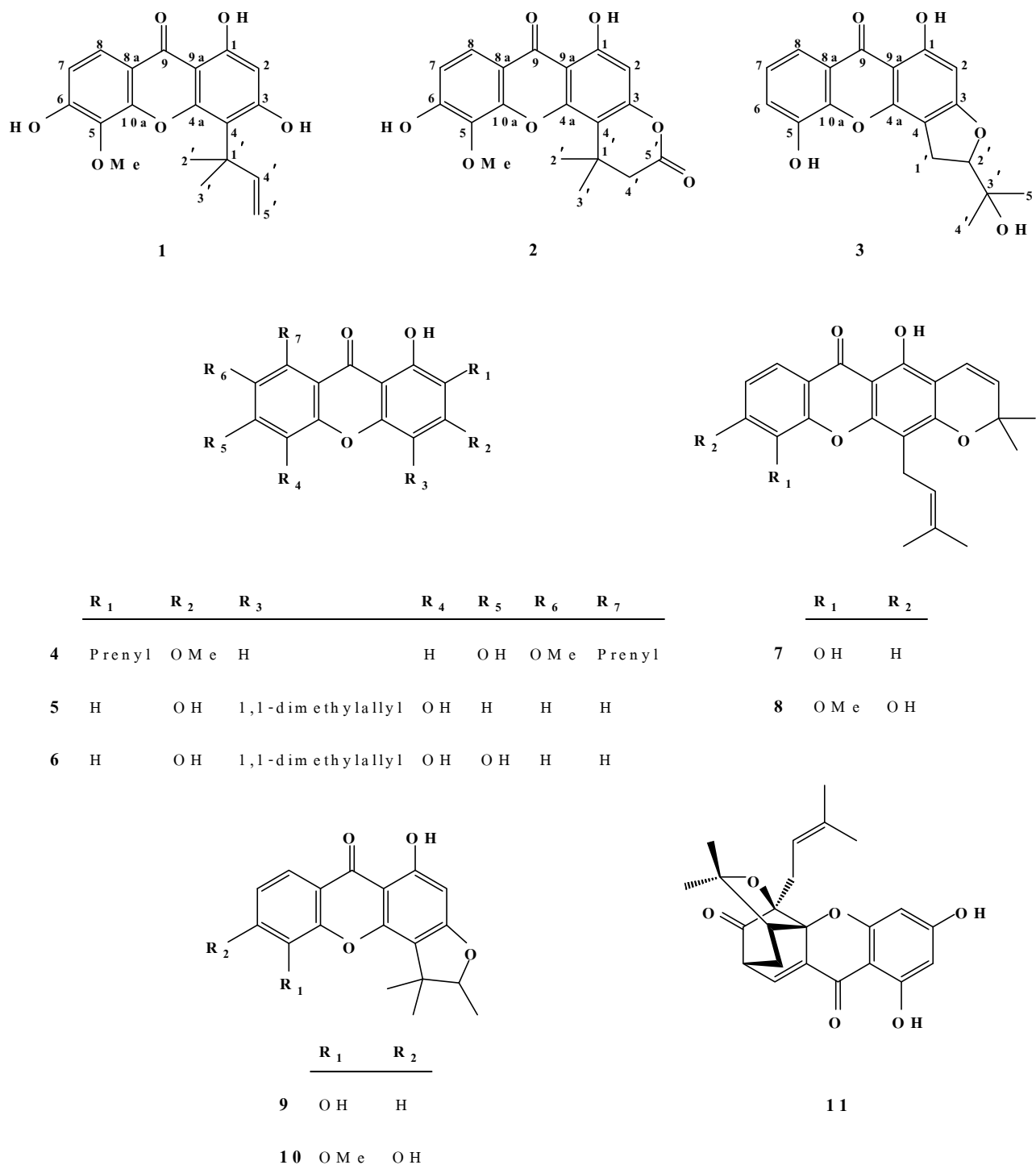
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Abstract – Three new prenylated xanthones, neriifolone A-C (**1-3**), and eight known xanthones (**4-11**) were isolated from the barks of *Cratoxylum sumatranum* ssp. *neriifolium*. All the new compounds were characterized by intensive spectroscopic methods (1D and 2D NMR, UV and IR spectroscopy and mass spectrometry).

Cratoxylum is a small genus belonging to the Clusiaceae, which is distributed in several Asian countries. Some species of this genus have been used in folk medicine as a treatment for diarrhea, flatulence¹ as well as diuretic, stomachic and tonic complaints.² Xanthone is the major chemical substance present in this genus³⁻¹¹ and some of these chemical substances showed interesting biological activities.^{4,6-8,10,11} In this paper, we describe the isolation and structural elucidation of three new xanthones (**1-3**) together with eight known xanthones (**4-11**) from the barks of *C. sumatranum* ssp. *neriifolium*.

The dichloromethane extract from the barks of *C. sumatranum* spp. *neriifolium* was separated by chromatographic techniques to afford eleven compounds: three new xanthones, neriifolone A (**1**), neriifolone B (**2**) and neriifolone C (**3**), and eight known xanthones (**4-11**). The structures of all of the new compounds were then elucidated using extensive 1D and 2D NMR spectroscopic techniques.

Figure 1. Compounds isolated from *C. sumatranum* spp. *neriifolium*

Neriifolone A (**1**) was obtained as a yellow solid. The molecular formula, C₁₉H₁₉O₆, was determined by HRMS (ESI-TOF) ([M+H]⁺ *m/z* 343.1194). The UV spectrum showed absorption bands of the xanthone skeleton⁸⁻¹¹ at 246, 256, 320 and 354 nm whereas the IR spectrum showed the stretches for the conjugated carbonyl and hydroxyl functionalities at 1647 and 3392 cm⁻¹, respectively. The ¹H NMR spectrum of **1**

(Table 1) exhibited a signal for a hydrogen bonded hydroxyl group (1-OH) at δ 13.44 and three aromatic protons at δ 7.81 (1H, *d*, J = 8.7 Hz), 7.03 (1H, *d*, J = 8.7 Hz) and 6.32 (1H, *s*) which were assigned to H-8, H-7 and H-2, respectively. Moreover, a methoxy group at δ 3.95 (3H, *s*) and a 1,1-dimethylallyl unit at δ 6.45 (1H, *dd*, J = 17.4, 10.5 Hz, H-4'), 4.96 (1H, *dd*, J = 17.4, 0.9 Hz, Ha-5') 4.89 (1H, *dd*, J = 10.5, 0.9 Hz, Hb-5') and 1.77 (6H, *s*, Me-2' and Me-3') were also observed in the ^1H NMR spectrum. The presence of a 1,1-dimethylallyl unit on C-4 was confirmed by the HMBC correlations (Figure 2) between Me-2' (δ 1.77), Me-3' (δ 1.77) and H-4' (δ 6.45) to C-4 (δ 112.8). The assignment of the methoxy group on C-5 was based on the cross peak of the methoxy protons (δ 3.95) and H-7 (δ 7.03) with C-5 (δ 136.0) in the HMBC experiment.

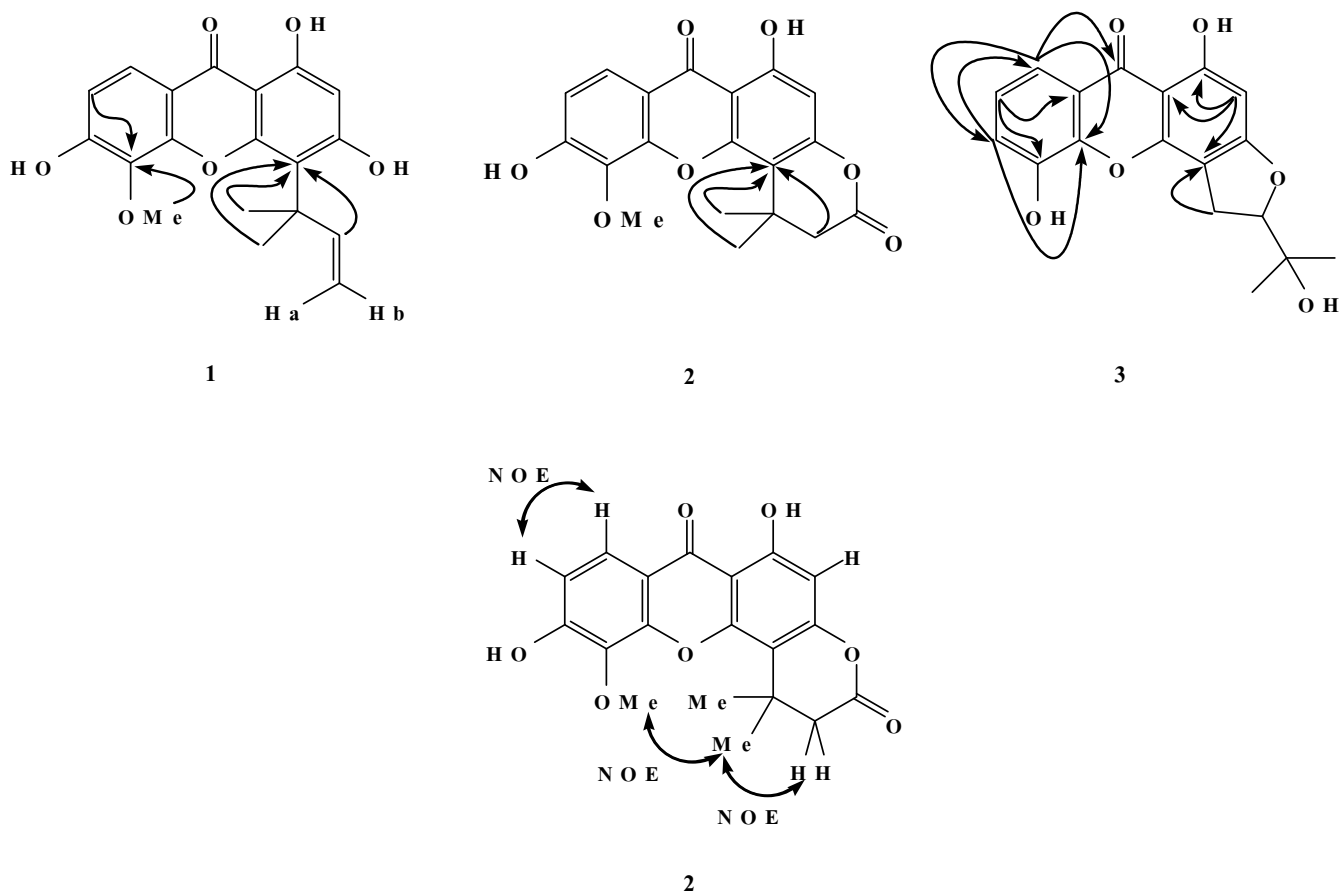


Figure 2. NOE cross peaks of **2** and HMBC correlations of **1**, **2** and **3**

Neriifolone B (**2**) was isolated as a yellow solid. The HRMS (ESI-TOF) gave a molecular ion peak at m/z 379.0789 ($[\text{M}+\text{Na}]^+$) which was consistent with the molecular formula $\text{C}_{19}\text{H}_{16}\text{O}_7$. The UV and IR spectra of **2** exhibited the same pattern as that of **1**. However, the IR spectrum of **2** also showed an additional carbonyl absorption peak at 1780 cm^{-1} . The ^1H and ^{13}C NMR spectral data of **2** (Table 1) were similar to those of **1** except for the disappearance of the 1,1-dimethylallyl unit. Compound **2** exhibited signals for the

3,3-dimethyl- δ -lactone ring which showed ^1H and ^{13}C NMR signals at δ 1.69/28.0 (Me-2' and Me-3'), 2.85/45.1 (H/C-4'), 34.8 (C-1') and 158.1 (C-3), 110.9 (C-4) and 167.0 (C-5'). Based on the HMBC correlations between Me-2' and Me-3' (δ 1.69) and H-4' (δ 2.85) to C-4 (δ 110.9), this unit was placed at C-3 and C-4 of the xanthone skeleton. The cross peak between Me-2' and Me-3' (δ 1.69) with 5-OMe (δ 4.01) in the NOE experiment also supported this placement.

Table 1. ^1H - and ^{13}C -NMR spectral data of **1**, **2** and **3** in acetone- d_6

Position	1	2	3			
	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}
1	-	162.6	-	162.8	-	162.1
2	6.32 (s)	100.1	6.41 (s)	100.2	6.19 (s)	100.0
3	-	164.8	-	158.1	-	161.6
4	-	112.8	-	110.9	-	110.6
4a	-	157.0	-	155.5	-	156.7
5	-	136.0	-	135.9	-	147.4
6	-	157.6	-	158.0	7.41 (dd , 8.1, 0.6)	120.9
7	7.03 (d , 8.7)	114.7	7.09 (d , 9.0)	115.4	7.28 (t , 8.1)	124.8
8	7.81 (d , 8.7)	121.9	7.85 (d , 9.0)	122.3	7.68 (dd , 8.1, 0.6)	116.1
8a	-	114.7	-	114.7	-	122.3
9	-	181.4	-	181.7	-	182.1
9a	-	103.8	-	106.3	-	105.1
10a	-	151.5	-	151.9	-	146.3
1'	-	42.0	-	34.8	2.03 (dd , 13.5, 2.4, $trans$ -H) 1.94 (dd , 13.5, 7.8, cis -H)	46.8
2'	1.77 (s)	30.3	1.69 (s)	28.0	5.56 (dd , 7.8, 2.4)	94.0
3'	1.77 (s)	30.3	1.69 (s)	28.0	-	109.2
4'	6.45 (dd , 17.4, 10.5)	151.2	2.85 (s)	45.1	i 1.75 (s)	32.8
5'	4.96 (dd , 17.4, 0.9, Ha) 4.89 (dd , 10.5, 0.9, Hb)	109.2	-	167.0	i 1.64 (s)	32.8
1-OH	13.44 (s)	-	13.28 (s)	-	12.98 (s)	-
5-OMe	3.95 (s)	61.9	4.01 (s)	61.9	-	-

ⁱ Interchangeable

Neriifolone C (**3**) was isolated as a yellow solid. The molecular formula was determined as $\text{C}_{18}\text{H}_{17}\text{O}_6$ by HRMS (ESI-TOF) ($[\text{M}+\text{H}]^+$ m/z 329.1018). The UV and IR spectra confirmed that **3** has a xanthone skeleton. Compound **3** exhibited the following ^1H NMR (Table 1) signals for the xanthone nucleus. A chelated hydroxyl proton was located at 12.98 ppm. A singlet aromatic proton signal at δ 6.19 which showed 2J and 3J HMBC correlations (Figure 2) with C-1 (δ 162.1), C-4 (δ 110.6) and C-9a (δ 105.1) was assigned as the isolated proton H-2. The ABM system of spectrum at δ 7.68 (dd, J = 8.1, 0.6 Hz), 7.41 (dd, J = 8.1, 0.6 Hz) and 7.28 (t, J = 8.1 Hz) and the 2J or 3J HMBC correlations of H-6 (δ 7.41) with C-8 (δ 116.1) and C-10a (δ 146.3); H-7 (δ 7.28) with C-5 (δ 147.4) and C-8a (δ 122.3) and H-8 (δ 7.68) with C-6

(δ 120.9), C-9 (δ 182.1) and C-10a (δ 146.3) were assigned as aromatic protons H-8, H-6 and H-7, respectively. Also, a characteristic signal of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran moiety at δ 1.75 (*s*, H-4'), 1.64 (*s*, H-5'), 5.56 ($J = 7.8, 2.4$ Hz, H-2'), 2.03 ($J = 13.5, 2.4$ Hz, *trans*-H) and δ 1.94 ($J = 13.5, 7.8$ Hz, *cis*-H) and the HMBC correlations of H-1' (δ 2.03 and 1.94) with C-4 (δ 110.6), confirmed that a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran moiety was fused to the xanthone nucleus at the C-3 and C-4 positions.

The remaining compounds were identified as β -mangostin (**4**),¹² pancixanthone-A (**5**),¹³ assiguxanthone-A (**6**),¹⁴ trapezifolixanthone (**7**),¹⁵ 5-*O*-methylxanthone V₁ (**8**),¹⁶ pancixanthone-B (**9**),¹³ 5-*O*-methyl-2-deprenylrheediaxanthone B (**10**),¹⁷ and (+)-4-oxa-tricyclo[4.3.1.0]decan-2-one scaffold (**11**),^{18,19} by 1D and 2D NMR techniques and compared with previous reports of these known compounds.

EXPERIMENTAL

GENERAL

Melting points were measured with using a Bibby Stuart Scientific melting point apparatus SMP3. UV spectra were measured on a UNICAM UV-310 spectrophotometer. Infrared spectra (IR) were recorded on a Perkin-Elmer 1750 FT-IR spectrophotometer. ESI-TOF mass spectra were recorded on a Micromass LCT spectrometer. NMR spectra (¹H, ¹³C, DEPT, ¹H-¹H COSY, NOESY, HMQC and HMBC) were recorded on Bruker AV300 or AV500 spectrometers. Chromatography was performed with the use of Merck prep-PLC and TLC, Merck silica gel 100 column and Sephadex LH-20 column. Optical rotation was measured in acetone solution with a sodium D line (589 nm) on an AUTOPOL[®] P-1020 (A068860638) automatic polarimeter.

PLANT MATERIAL

Barks of *C. sumatranum* ssp. *neriifolium* (Clusiaceae) were collected at Amphur Bannasan, Suratthani Province, Thailand, in February 2009. The plant was identified by Assistant Professor Dr. Maruay Mekanawakul, a specialist in the botanics, and the voucher specimen (WU 1453) is deposited at the botanic garden, Walailak University, Nakhon Si Thammarat, Thailand.

EXTRACTION AND ISOLATION

Chopped dried barks of *C. sumatranum* ssp. *neriifolium* (18.5 kg) were extracted with CH₂Cl₂ at room temperature for 3 days. After a removal of solvents, a yellow-brown viscous CH₂Cl₂ extract (366.28 g) was obtained. This extract (366.28 g) was dissolved in acetone. The acetone-soluble portion (255.28 g) was fractionated by quick column chromatography over silica gel 60H with the use of hexane, hexane-CH₂Cl₂, CH₂Cl₂, CH₂Cl₂-acetone, acetone and acetone-MeOH as eluents to afford 10 fractions (FCN1-FCN10).

Fraction FCN5 (8.01 g) was subjected to Sephadex LH-20 column chromatography with MeOH as an eluent, yielding 5 subfractions (FCN5a- FCN5e). **7** (6.4 mg) was obtained from repeated Sephadex LH-20 column chromatography with MeOH as an eluent and then by prep-TLC (CH₂Cl₂: hexane, 3:2 v/v) of subfraction FCN5e (218.7 mg). Repeated Sephadex LH20 gel filtration of fraction FCN6 (20.14 g) gave **4** (32.9 mg), **8** (1.4 mg) and **9** (110.6 mg). Fraction FCN7 (15.52 g) was separated by Sephadex LH-20 column chromatography with MeOH as an eluent, yielding **10** (40.0 mg). Fraction FCN8 (9.16 g) was purified by Sephadex LH-20 column chromatography with MeOH as an eluent and then by prep-TLC (CH₂Cl₂), affording **2** (17.5 mg). Fraction FCN8e (464.5 mg) was separated by Sephadex LH-20 column chromatography with MeOH as an eluent, yielding 6 subfractions (FCN8e1- FCN8e6). Subfraction FCN8e3 (40.4 mg) was purified by prep-PLC (100% CH₂Cl₂) to afford **1** (19.3 mg) while **5** (32.7 mg) and **3** (6.6 mg) were derived from subfraction FCN8e4 (160.0 mg) by prep-PLC (100% CH₂Cl₂). Subfraction FCN8e5 (27.0 mg) and subfraction FCN8-f (50.0 mg) were purified by prep-PLC (100% CH₂Cl₂) to afford **11** (15.0 mg) and **6** (18.9 mg), respectively.

Neriifolone A **1**: Yellow solid. mp 146–147 °C. UV $\lambda_{\max}$ (MeOH) (log ϵ): 246 (4.08), 256 (3.92), 320 (3.86), 354 (3.38) nm. IR (neat) $\nu_{\max}$: 3392 (OH), 1647 (C=O) cm⁻¹. ¹H-NMR (300 MHz, acetone-*d*₆) and ¹³C-NMR (75 MHz, acetone-*d*₆) see Table 1. TOF-MS [M+H]⁺ *m/z*: 343.1194 for C₁₉H₁₉O₆ (calcd. 343.1176).

Neriifolone B **2**: Yellow solid. mp 231–232 °C. UV $\lambda_{\max}$ (MeOH) (log ϵ): 244 (4.42), 314 (4.00), 348 (3.77) nm. IR (neat) $\nu_{\max}$: 3368 (OH), 1780 (C=O), 1651 (C=O) cm⁻¹. ¹H-NMR (300 MHz, acetone-*d*₆) and ¹³C-NMR (75 MHz, acetone-*d*₆) see Table 1. TOF-MS [M+Na]⁺ *m/z*: 379.0789 for C₁₉H₁₆O₇Na (calcd. 379.0788).

Neriifolone C **3**: Yellow solid. mp 297–298 °C. Optical rotation: $[\alpha]_D^{23.6}$ -27.42° (*c* 0.2600 %w/v in acetone). UV $\lambda_{\max}$ (MeOH) (log ϵ): 248 (4.28), 256 (4.27), 318 (4.02), 366 (3.07) nm. IR (neat) $\nu_{\max}$: 3392 (OH), 1651 (C=O) cm⁻¹. ¹H-NMR (300 MHz, acetone-*d*₆) and ¹³C-NMR (75 MHz, acetone-*d*₆) see Table 1. TOF-MS [M+H]⁺ *m/z*: 329.1018 for C₁₈H₁₇O₆ (calcd. 329.1020).

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