

## OCCURRENCE OF XANTHONOLIGNOIDS IN GUTTIFEROUS PLANTS

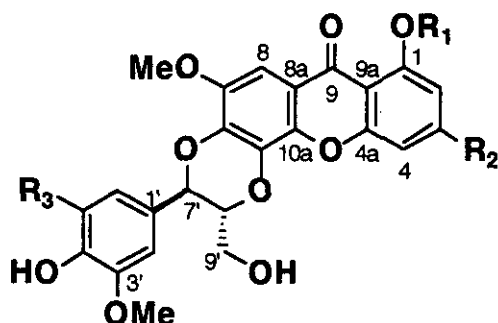
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**Abstract** — Further investigation of the chemical constituents of the Guttifereous plants (*Calophyllum inophyllum*, *C. austroindicum* and *Harungana madagascariensis*) led to isolate two new xanthonolignoids, named calophyllumins A and B, in addition to two known xanthonolignoids (cadensin C and 6-hydroxycadensin F) and four flavonoids (calophyllolic acid, quercetin, quercetin 3-*O*-rhamnoside and amentoflavone). The structures of these compounds were established by the aids of spectroscopic analysis including 2D nmr technique.

The family Guttiferae is known to rich source of xanthenes,<sup>1</sup> coumarins<sup>2</sup> and biflavonoids.<sup>3</sup> Recently various bioactivities of xanthenes (antihypoglycemic,<sup>4</sup> antiplatelet,<sup>5</sup> antioxidant activity<sup>6</sup> and so on) and coumarins (anti-HIV activity<sup>7,8</sup>) have been reported. In continuation of our studies oriented to search for biologically active substances in Guttifereous plants,<sup>9-12</sup> the chemical constituents of *Calophyllum inophyllum* L., *C. austroindicum* Kosterm ex P. F. Stevens and *Harungana madagascariensis* Lam ex Poir. were examined. We report here the isolation and characterization of two new xanthonolignoids along with six known compounds.

By chromatographic separation compounds (1-8) were isolated from the root heart of *C. inophyllum* (1, 2), the leaves of *C. inophyllum* (5-8), the wood of *C. austroindicum* (3) and the roots of *H. madagascariensis* (4), respectively.



- 1 :  $R_1 = H, R_2 = OH, R_3 = OMe$   
 2 :  $R_1 = Me, R_2 = OH, R_3 = OMe$   
 3 :  $R_1 = Me, R_2 = OH, R_3 = H$   
 4 :  $R_1 = R_2 = H, R_3 = OMe$

Compound (1), calophyllumin A, obtained as a pale yellow amorphous powder, reacted positively to  $FeCl_3$  and Gibbs tests. High-resolution (HR) FABms spectra showed  $[M-H]^-$   $m/z$  497.1084, which indicated the molecular formula of  $C_{25}H_{22}O_{11}$ . Ir absorption at  $1645\text{ cm}^{-1}$  revealed the presence of a conjugated carbonyl group. In the  $^1H$  nmr spectrum, the presence of hydroxyl groups [ $\delta$  11.04 (1H, br s) and 13.02 (1H, s)] and three methoxyl groups [ $\delta$  3.84 (3H, s), 3.76 (6H, s)] were suggested in addition to *meta*-coupled protons [ $\delta$  6.20 and 6.39 (1H each, d,  $J = 2.0$  Hz)] and an aromatic proton [ $\delta$  7.13 (1H, s)]. All carbons with hydrogen were assigned by the HMQC spectrum (Table 1). In the HMBC ( $J = 8.3$  Hz) spectrum (Figure 1), a chelated hydroxyl group caused cross peaks to three aromatic carbons ( $\delta$  98.2, 101.8 and 162.7), the former was further correlated to one of the *meta*-coupled protons at  $\delta$  6.20 in the HMQC spectrum. The aromatic carbon at  $\delta$  98.2 was correlated to the hydroxyl group at  $\delta$  11.04 in the HMBC spectrum, and another *meta*-coupled proton at  $\delta$  6.39 was correlated to quaternary carbons at  $\delta$  101.8, 157.2 and 165.2, the last of which was also correlated to the hydroxyl group at  $\delta$  11.04. These results indicated that a partial structure of 1 was shown as A in Figure 1. On the other hand, an nOe was observed between the methoxyl group at  $\delta$  3.84 and the aromatic proton at  $\delta$  7.13 which was correlated to the carbonyl carbon and four quaternary carbons ( $\delta$  112.3, 145.8, 139.8 and 141.0) through  $^2J$  or  $^3J$  in the HMBC ( $J = 8.3$  Hz) spectrum (Figure 1). Furthermore, the proton at  $\delta$  7.13 caused cross peak to the aromatic carbon at  $\delta$  132.3 through  $^4J$  in the HMBC ( $J = 1.7$  Hz) spectrum (Figure 2). These data led another plausible partial structure of B (Figure 1). The other partial structure C was determined as follows. The signals due to two aromatic protons [ $\delta$  6.76 (2H, s)] and a hydroxyl group [ $\delta$  8.61 (1H, br s)] were observed in the  $^1H$  nmr spectrum in addition to the signals of methine protons [ $\delta$  4.42 (1H, m) and 5.04

aromatic carbon at  $\delta$  132.3 through  $^4J$  in the HMBC ( $J = 1.7$  Hz) spectrum (Figure 2). These data led another plausible partial structure of **B** (Figure 1). The other partial structure (**C**) was determined as follows. The signals due to two aromatic protons [ $\delta$  6.76 (2H, s)] and a hydroxyl group [ $\delta$  8.61 (1H, br s)] were observed in the  $^1\text{H}$  nmr spectrum in addition to the signals of methine protons [ $\delta$  4.42 (1H, m) and 5.04 (1H, d,  $J = 7.8$  Hz)], methylene protons [ $\delta$  3.43 (1H, dd,  $J = 12.7, 4.4$  Hz) and 3.71 (1H, br d,  $J = 12.7$  Hz)] and a hydroxyl group [ $\delta$  5.10 (1H, t,  $J = 7.8$  Hz)] which disappeared when  $\text{D}_2\text{O}$  was added. These data as well as the  $^{13}\text{C}$  nmr and HH COSY spectral data suggested the presence of a phenylpropanoid moiety. In the HMBC ( $J = 8.3$  Hz) spectrum (Figure 1), another hydroxyl group at  $\delta$  8.61 was correlated to aromatic carbons at  $\delta$  136.3 and 148.0, the latter of which was additionally correlated to the methoxyl

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data of **1** and **3**

No.	<b>1</b> <sup>a</sup>		<b>3</b> <sup>b</sup>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ $J$ (Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ $J$ (Hz)
<b>1</b>	162.7		161.7	
<b>2</b>	98.2	6.20 (1H, d, 2.0)	95.3	6.37 (1H, d, 2.0)
<b>3</b>	165.2		163.1	
<b>4</b>	93.9	6.39 (1H, d, 2.0)	94.8	6.43 (1H, d, 2.0)
<b>5</b>	132.3		131.8	
<b>6</b>	139.8		138.2 <sup>c</sup>	
<b>7</b>	145.8		145.3	
<b>8</b>	95.9	7.13 (1H, s)	96.7	7.09 (1H, s)
<b>9</b>	178.9		172.4	
<b>4a</b>	157.2		158.7	
<b>8a</b>	112.3		115.3	
<b>9a</b>	101.8		104.9	
<b>10a</b>	141.0		139.5 <sup>c</sup>	
<b>1'</b>	125.5		126.7	
<b>2'</b>	105.7	6.76 (2H, s)	112.0	7.04 (1H, d, 1.9)
<b>3'</b>	148.0		147.6	
<b>4'</b>	136.3		147.2	
<b>5'</b>	148.0		115.0	6.82 (1H, d, 8.3)
<b>6'</b>	105.7	6.76 (2H, s)	120.7	6.89 (1H, dd, 8.3, 1.9)
<b>7'</b>	76.8	5.04 (1H, d, 7.8)	76.1	5.03 (1H, d, 7.3)
<b>8'</b>	77.7	4.42 (1H, m)	77.7	4.34 (1H, m)
<b>9'</b>	59.8	3.43 (1H, dd, 12.7, 4.4)	59.8	3.43 (1H, dd, 12.7, 4.4)
		3.71 (1H, br d, 12.7)		3.71 (1H, dd, 12.7, 2.4)
OMe-C-1			55.9	3.84 (3H, s)
OMe-C-7	55.8	3.84 (3H, s)	55.6	3.82 (3H, s)
OMe-C-3'	56.2	3.76 (6H, s)	55.7	3.78 (3H, s)
OH-C-1		13.02 (1H, s)		
OH-C-3		11.04 (1H, br s)		10.82 (1H, br s)
OH-C-4'		8.61 (1H, br s)		9.22 (1H, br s)
OH-C-9'		5.10 (1H, br t, 7.8)		5.07 (1H, br s)

Measured in  $\text{DMSO}-d_6$ . a: 500 MHz. b: 400 MHz. c: interchangeable.

proton ( $\delta$  3.76). In the  $^1\text{H}$  nmr spectrum, an nOe was observed between the methoxyl signal and the aromatic proton signal at  $\delta$  6.76 (Figure 1). Furthermore, the aromatic proton ( $\delta$  6.76) caused a cross peak

to the methine carbon at  $\delta$  76.8 which was also correlated to the methine proton at  $\delta$  5.04 in the HMQC spectrum. These results indicated that the remaining partial structure of **1** was described as C in Figure 1, which was supported by the other correlations found in the HMBC spectrum. The connective manners of the partial structures (A-C) were characterized as follows. In the HMBC ( $J = 1.7$  Hz) spectrum (Figure 2), the carbonyl carbon was correlated to the chelated hydroxyl group and the *meta*-coupled protons ( $\delta$  6.20 and 6.39) through  $^4J$ . The chemical shift of the carbonyl carbon at  $\delta$  178.9 showed a characteristic for a 1-hydroxyxanthone (a xanthone with a chelated hydroxyl group). Taking the above data and the degrees of unsaturation into consideration, **1** is a xanthonolignoid with a dioxane ring. The  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data based on the xanthone moiety closely resembled those of 1,3,5,6-tetrahydroxy-7-methoxyxanthone (caloxanthone E) previously isolated from a same plant.<sup>10</sup> The orientation of the phenylpropanoid was finally determined by the correlation between the benzylic methine proton at  $\delta$  5.04 and the aromatic carbon at  $\delta$  139.8 in the HMBC ( $J = 1.7$  Hz) spectrum (Figure 2). Thus the total structure of calophyllumin A was characterized as **1**, where H-7' and H-8' are *trans* oriented.<sup>13</sup>

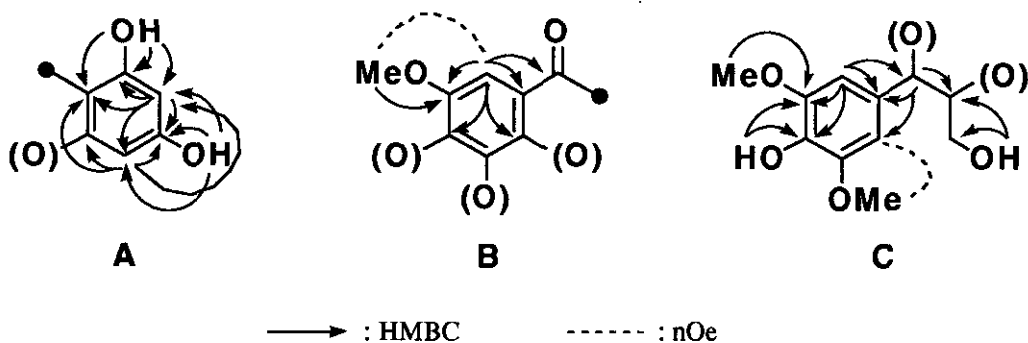


Figure 1 Partial structures (A - C), HMBC ( $J = 8.3$  Hz) spectrum and nOe experiments of **1**

Compound (**2**) was obtained as a pale yellow amorphous powder and its uv absorptions were closely similar to those of **1**. The HR-FABms showed the molecular formula of  $\text{C}_{26}\text{H}_{24}\text{O}_{11}$ . Analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data revealed that **2** had a feature of xanthonolignoid and led to a conclusion that **2** was 6-hydroxycadensin F,<sup>13</sup> the structure of which was supported by the HMBC spectrum and nOe experiments.

Compound (**3**), named calophyllumin B, obtained as a pale yellow amorphous powder, gave  $[\text{M}-\text{H}]^-$   $m/z$  481.1118 in the HR-FABms, corresponding to the molecular formula of  $\text{C}_{25}\text{H}_{22}\text{O}_{10}$ . Its uv, ir and nmr

spectral data indicated that **3** was also a xanthonolignoid. A xanthone moiety of **3** was identical to that of **2**, which was confirmed by the comparison of  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data between **2** and **3** and by the HMBC spectrum (Figure 3). The differences of **3** from **2** were due to a phenyl propanoid moiety [ $\delta$  6.82 (1H, d,  $J = 8.3$  Hz), 6.89 (1H, dd,  $J = 8.3, 1.9$  Hz), 7.04 (1H, d,  $J = 1.9$  Hz)], which showed that **3** lacked a methoxyl group at C-5' in **2**. The structure was supported by an nOe experiment and the correlations observed in the HMBC spectrum (Figure 3). Thus, the structure of calophyllumin B was concluded to be **3**.

Compounds (**4-8**) was identified as cadensin C (**4**), calophyllic acid (**5**), quercetin (**6**), amentoflavone (**7**) and quercetin 3-*O*-rhamnoside (**8**) by spectroscopic analysis including 2D nmr technique. All xanthonolignoids (**1-4**) was optically inactive as well as naturally occurring xanthonolignoids reported previously. Occurrence of xanthonolignoid is very rare in natural sources, and has been reported only in the family Guttiferae (*Cratoxylum*,<sup>13</sup> *Kielmeyera*,<sup>14</sup> *Hypericum*,<sup>15</sup> *Psorospermum*,<sup>16</sup> and *Vismia*<sup>17</sup>). We add here the occurrence of xanthonolignoids to the genus *Calophyllum* and *Harungana*.

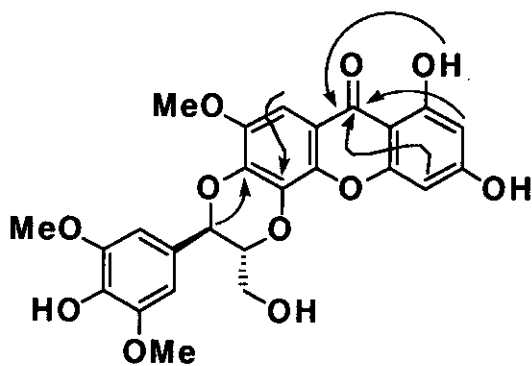


Figure 2 HMBC ( $J = 1.7$  Hz) spectrum of **1**

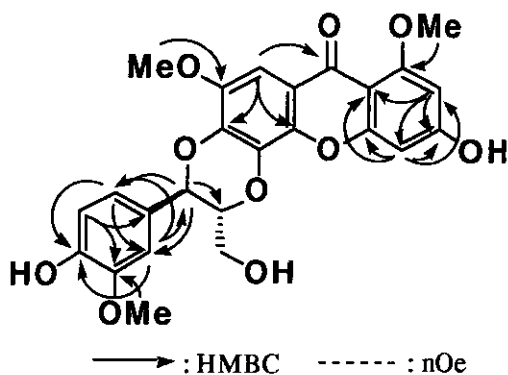


Figure 3 HMBC ( $J = 10$  Hz) spectrum and nOe experiments of **3**

## EXPERIMENTAL

**General.** The following instruments were used: Ms spectra, JEOL JMS-D300 (70 eV) instrument;  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra, JEOL JNM EX-400 or JEOL JNM-A500 (TMS as internal standard), ir spectra (on KBr pellet), JASCO IR-AI spectrophotometer; Polarimeter, JASCO DIP-370 digital polarimeter; uv (in methanol solution), Shimadzu UV-2200 spectrophotometer. The following adsorbents were used for purification:

analytical tic, Merck Kieselgel 60 F<sub>254</sub>; column chromatography, Merck Kieselgel 60, Fuji Davison Silica gel BW-300, and Pharmacia Fine Chemicals AB Sephadex LH-20.

*Plant material.* Root heart and leaves of *Calophyllum inophyllum* was collected in Okinawa, Japan, November, 1992. Wood of *C. austroindicum* was collected in India, July, 1995. Roots of *Harungana madagascariensis* was collected in Nigeria, September, 1994. Each voucher specimen is deposited in the herbarium of Gifu Pharmaceutical University.

*Extraction and isolation.* The dried and ground root heart (2200 g) of *C. inophyllum* was extracted successively with *n*-hexane, benzene, acetone and 70% MeOH. The acetone extract (45 g) was suspended into water and partitioned with benzene, EtOAc and *n*-BuOH with succession. The EtOAc soluble extract (4 g) was subjected to vacuum liquid chromatography (vlc) eluted with a benzene-acetone system to give 13 fractions. Compounds(1) (8 mg) and(2) (4 mg) were obtained from the tenth (benzene-acetone = 3 : 1) and eleventh (3 : 1) fraction, respectively.

The dried and ground leaves (600 g) of *C. inophyllum* was extracted with *n*-hexane, benzene, acetone and 70% MeOH. The acetone extract (40 g) was partitioned in similar manners mentioned above. The EtOAc soluble extract (4 g) was separated by si gel cc eluted with a benzene-acetone system to give six fractions. Compounds(5) (50 mg), (7) (30 mg) and(8) (4 mg) was obtained from the third (benzene-acetone = 5 : 1), the fifth (2 : 1) and the sixth (1 : 1) fraction, respectively. The fourth fraction (2 : 1) was subjected to vlc with the same solvent system, and the benzene-acetone (2 : 1) eluent was further purified by Sephadex LH-20 (MeOH) to give 6 (8 mg).

The dried and ground wood (1 kg) of *C. austroindicum* was extracted with benzene, acetone, and 70% MeOH under reflux. The acetone extract (25 g) was subjected to si gel cc eluted with a benzene-acetone system to give 2 (4 mg) (from benzene-acetone = 1 : 1).

The dried and ground roots (500 g) of *H. madagascariensis* were extracted successively with benzene, acetone and 70% MeOH at room temperature. The acetone extract (4 g) was subjected to vlc eluted with a benzene-acetone system. A benzene-acetone (5 : 1) eluent was purified by Sephadex LH-20 (MeOH) to give 4 (1 mg).

*Compound (1) (calophyllumin A):* A pale yellow amorphous powder;  $[\alpha]_D^{24}$ : 0° (*c* 0.05, MeOH); HR-FABms:  $[M-H]^+$  *m/z* 497.1106 (Calcd 497.1084 for C<sub>25</sub>H<sub>21</sub>O<sub>11</sub>); uv  $\lambda$  (nm): 215, 255, 283, 320, 355sh; ir  $\nu$  (cm<sup>-1</sup>): 3440, 3310, 3100, 2940, 1645, 1605, 1595; The <sup>1</sup>H and <sup>13</sup>C nmr spectral data are shown in Table 1.

*Compound (3) (calophyllumin B)*: A pale yellow amorphous powder;  $[\alpha]_D^{22}$ :  $0^\circ$  (c 0.09, MeOH); HR-FABms:  $[M-H]^+$   $m/z$  481.1118 (Calcd 481.1134 for  $C_{25}H_{21}O_{10}$ ); uv  $\lambda$  (nm): 207, 245sh, 252, 285, 311, 340sh; ir  $\nu$  ( $cm^{-1}$ ): 3375, 2920, 1640, 1605, 1595; The  $^1H$  and  $^{13}C$  nmr spectral data are shown in Table 1.

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