OCCURRENCE OF XANTHONOLIGNOIDS IN GUTTIFEREOUS PLANTS

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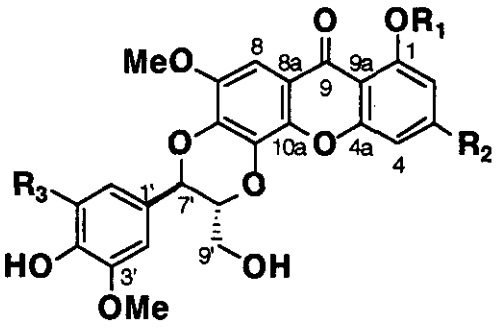
Abstract — Further investigation of the chemical constituents of the Guttifereous plants (*Calophyllum inophyllum*, *C. austroindicum* and *Harungana madagascariensis*) led to isolate two new xanthonolignoids, named calophyllumins A and B, in addition to two known xanthonolignoids (cadensin C and 6-hydroxycadensin F) and four flavonoids (calophyllic 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 xanthones,[1] coumarins[2] and biflavonoids.[3] Recently various bioactivities of xanthones (antihypoglycemic,[4] antiplatelet,[5] antioxidant activity[6] and so on) and coumarins (anti-HIV activity[7,8]) have been reported. In continuation of our studies oriented to search for biologically active substances in Guttifereous plants,[9-12] 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 cm^{-1} revealed the presence of a conjugated carbonyl group. In the ^1H nmr spectrum, the presence of hydroxyl groups [δ 11.04 (1H, br s) and 13.02 (1H, s)] and three methoxyl groups [δ 3.84 (3H, s), 3.76 (6H, s)] were suggested in addition to *meta*-coupled protons [δ 6.20 and 6.39 (1H each, d, J = 2.0 Hz)] and an aromatic proton [δ 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 (δ 98.2, 101.8 and 162.7), the former was further correlated to one of the *meta*-coupled protons at δ 6.20 in the HMQC spectrum. The aromatic carbon at δ 98.2 was correlated to the hydroxyl group at δ 11.04 in the HMBC spectrum, and another *meta*-coupled proton at δ 6.39 was correlated to quaternary carbons at δ 101.8, 157.2 and 165.2, the last of which was also correlated to the hydroxyl group at δ 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 δ 3.84 and the aromatic proton at δ 7.13 which was correlated to the carbonyl carbon and four quarternary carbons (δ 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 δ 7.13 caused cross peak to the aromatic carbon at δ 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 [δ 6.76 (2H, s)] and a hydroxyl group [δ 8.61 (1H, br s)] were observed in the ^1H nmr spectrum in addition to the signals of methine protons [δ 4.42 (1H, m) and 5.04

aromatic carbon at δ 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 [δ 6.76 (2H, s)] and a hydroxyl group [δ 8.61 (1H, br s)] were observed in the ^{1}H nmr spectrum in addition to the signals of methine protons [δ 4.42 (1H, m) and 5.04 (1H, d, J = 7.8 Hz)], methylene protons [δ 3.43 (1H, dd, J = 12.7, 4.4 Hz) and 3.71 (1H, br d, J = 12.7 Hz)] and a hydroxyl group [δ 5.10 (1H, t, J = 7.8 Hz)] which disappeared when D_2O was added. These data as well as the ^{13}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 δ 8.61 was correlated to aromatic carbons at δ 136.3 and 148.0, the latter of which was additionaly corrrelated to the methoxyl

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

	1[a]		**3**[b]	
No.	δ_C	δ_H J (Hz)	δ_C	δ_H J (Hz)
1	162.7		161.7	
2	98.2	6.20 (1H, d, 2.0)	95.3	6.37 (1H, d, 2.0)
3	165.2		163.1	
4	93.9	6.39 (1H, d, 2.0)	94.8	6.43 (1H, d, 2.0)
5	132.3		131.8	
6	139.8		138.2[c]	
7	145.8		145.3	
8	95.9	7.13 (1H, s)	96.7	7.09 (1H, s)
9	178.9		172.4	
4a	157.2		158.7	
8a	112.3		115.3	
9a	101.8		104.9	
10a	141.0		139.5[c]	
1'	125.5		126.7	
2'	105.7	6.76 (2H, s)	112.0	7.04 (1H, d, 1.9)
3'	148.0		147.6	
4'	136.3		147.2	
5'	148.0		115.0	6.82 (1H, d, 8.3)
6'	105.7	6.76 (2H, s)	120.7	6.89 (1H, dd, 8.3, 1.9)
7'	76.8	5.04 (1H, d, 7.8)	76.1	5.03 (1H, d, 7.3)
8'	77.7	4.42 (1H, m)	77.7	4.34 (1H, m)
9'	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 DMSO-d_6. a: 500 MHz. b: 400 MHz. c: interchangeable.

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

to the methine carbon at δ 76.8 which was also correlated to the methine proton at δ 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 (δ 6.20 and 6.39) through ^4J. The chemical shift of the carbonyl carbon at δ 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}H and ^{13}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.[10] The orientation of the phenylpropanoid was finally determined by the correlation between the benzylic methine proton at δ 5.04 and the aromatic carbon at δ 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.[13]

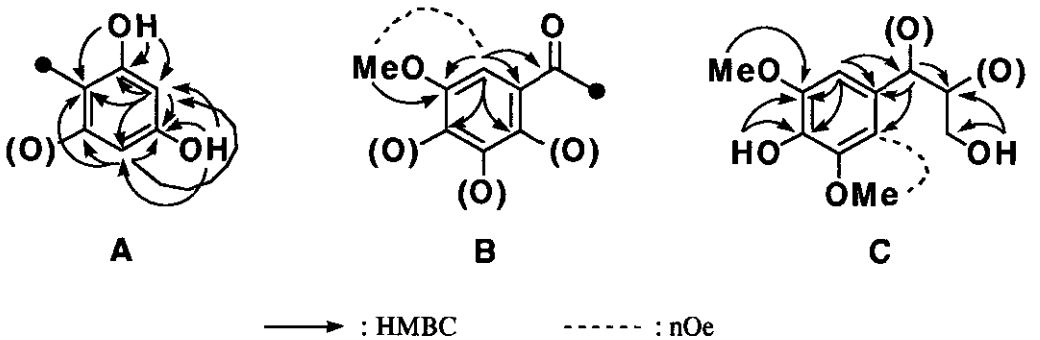


Figure 1 Partial structures (**A** - **C**), HMBC (J = 8.3 Hz) spectrum and nOe experiments of **1**

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 $C_{26}H_{24}O_{11}$. Analysis of the ^{1}H and ^{13}C nmr spectral data revealed that **2** had a feature of xanthonolignoid and led to a conclusion that **2** was 6-hydroxycadensin F,[13] 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 $[M-H]^-$ *m/z* 481.1118 in the HR-FABms, corresponding to the molecular formula of $C_{25}H_{22}O_{10}$. 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}H and ^{13}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 [δ 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 occuring xanthonolignoids reported previously. Occurrence of xanthonolignoid is very rare in natural sources, and has been reported only in the family Guttiferae (*Cratoxylum*,[13] *Kielmeyera*,[14] *Hypericum*,[15] *Psorospermum*,[16] and *Vismia*[17]). We add here the occurrence of xanthonolignoids to the genus *Calophyllum* and *Harungana*.

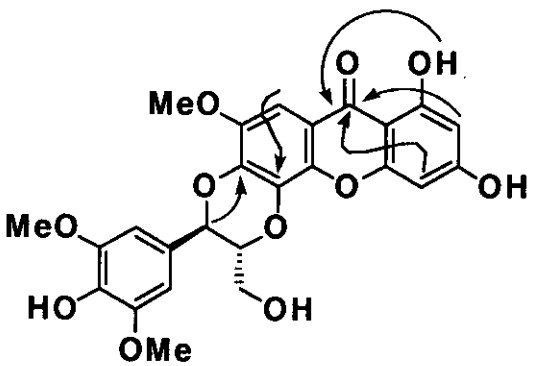


Figure 2 HMBC (J = 1.7 Hz) spectrum of **1**

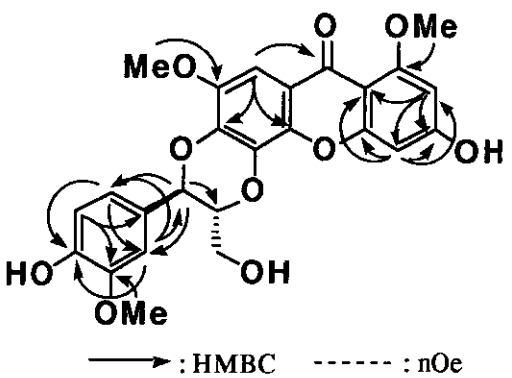


Figure 3 HMBC (J = 10 Hz) spectrum and nOe experiments of **3**

EXPERIMENTAL

General. The following instruments were used: Ms spectra, JEOL JMS-D300 (70 eV) instrument; ^{1}H and ^{13}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 tlc, Merck Kieselgel 60 F_{254}; 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 forth 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 $C_{25}H_{21}O_{11}$); uv λ (nm): 215, 255, 283, 320, 355sh; ir ν (cm^{-1}): 3440, 3310, 3100, 2940, 1645, 1605, 1595; The ^1H and ^{13}C nmr spectral data are shown in Table 1.

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

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