Globulixanthones A and B, Two New Cytotoxic Xanthones with Isoprenoid Groups from the Root Bark of *Symphonia globulifera*

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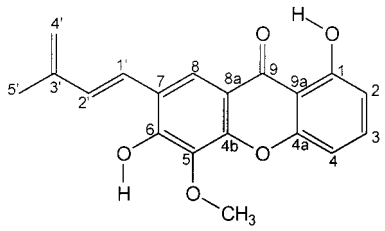
Bioassay-guided fractionation of a root bark extract of S*ymphonia globulifera* has yielded, in addition to stigmasterol, two new xanthones with isoprenoid units, named globulixanthones A (**1**) and B (**2**). The structures of these compounds have been elucidated by spectroscopic means. They possess significant cytotoxicity in vitro against the KB cell line.

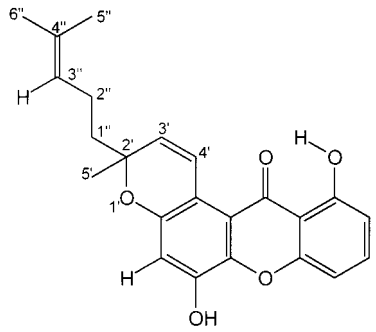
The genus Symphonia (Guttiferae), which is encountered mainly in lowland rainforests of the tropical world, has been extensively investigated from phytochemical and biological points of view. Prenylated xanthones[1−4] and prenylated benzophenones[5,6] have been isolated from African, South American, and Asian species. Some of these exhibit a wide range of biological and pharmacological activities, for example, cytotoxic,[7,8] antimicrobial,[7,8] antioxidant,[7,8] antimalarial,[7,8] and HIV-1 protease inhibitory activity.[5,6] The stem bark, heart wood, and root bark of S*ymphonia globulifera* L. f., a plant used in Cameroonian folk medicine as a laxative for pregnant women and a general tonic,[9] were previously reported to contain xanthones[1−4] and benzophenones.[5,6] In our search for biologically active compounds from Cameroonian plants, we reexamined the root bark of *Symphonia globulifera* and now report the isolation, structural determination, and biological activity of two new oxygenated xanthones with isoprenoid units named globulixanthones A (**1**) and B (**2**).

Dried and powdered root bark of S*ymphonia globulifera* was extracted at room temperature with a mixture of CH_2Cl_2−MeOH (1:1) then MeOH. The CH_2Cl_2−MeOH (1:1) extract, which exhibited 50% growth inhibition of KB cells at 10 μg/mL, was concentrated to dryness to give a sticky residue. Bioassay-directed fractionation of this residue by silica gel column chromatography monitored by cytotoxicity toward a cell culture derived from a human epidermoid carcinoma of the nasopharynx (KB cell line) afforded active fractions, from which were isolated compounds **1** and **2** along with the known stigmasterol.

Globulixanthone A (**1**), isolated as yellow powder, mp 240−242 °C, gave a positive reaction with ferric chloride reagent, indicating its phenolic nature. Its high-resolution EI-mass spectrum showed the molecular ion $[M]^+$ at *m*/*z* 324.0996, in agreement with the empirical formula $C_{19}H_{16}O_5$ (requires *m*/*z* 324.0998) corresponding to 12 degrees of unsaturation. The broad-band decoupled ^{13}C NMR spectrum of **1** showed 19 carbon signals. The analysis of this spectrum with the aid of APT, DEPT, and HMQC techniques unequivocally indicated the presence of two methyl groups, one sp^2 methylene carbon, and six sp^2 methines. Thus, there were 10 quaternary carbons, all sp^2. The IR spectrum disclosed strong vibration bands for chelated hydroxyl (3294 cm^{-1}), conjugated carbonyl (1664 cm^{-1}), and



(**1**)



(**2**)

an aromatic ring. IR, ^{13}C NMR, and UV spectral data indicated **1** to be a xanthone derivative.[10−12] The ^1H NMR and COSY spectra provided signals of most of the functional groups, including two hydroxyl groups [δ 12.67 (1H, s, chelated OH) and δ 10.30 (1H, brs)], one methoxyl group [δ 3.89 (3H, s)], and a 3-methylbuta-1,3-dienyl moiety [δ 7.11 (1H, d, J = 16.3 Hz), 6.76 (1H, d, J = 16.3 Hz), 5.29 (1H, d, J = 2.2 Hz), 5.16 (1H, d, J = 2.2 Hz), and 2.07 (3H, s)]. The ^1H NMR spectrum also exhibited evidence for an ABC spin system corresponding to a 1,2,3-trisubstituted benzene ring [δ 7.72 (1H, t, J = 8.4 Hz) 7.07 and 6.81 (1H each, dd, J = 8.4 and 1.9 Hz)] and one single aromatic proton at δ 7.83. This proton, which gave cross-peaks in the HMBC spectrum (Table 1) with the carbonyl carbon (δ 181.5), two quaternary carbons (δ 127.6 and 116.6), and two other quaternary aromatic carbons bearing an O-function (δ 150.9 and 141.7), was assigned to H-8 *peri* to the carbonyl group. Also, in the HMBC spectrum, the proton of the hydrogen-bonded hydroxyl group at δ 12.67 was correlated with C-1 (δ 161.0) and C-9a (δ 107.9), while a double doublet at δ 6.81 (H-2) showed a cross-peak with C-1 (δ 161.1) and C-4 (δ 107.3); and the triplet at δ 7.72 (H-3) with C-1 (δ 161.0), C-4 (δ 107.3), and C-4a (δ 155.7)

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Table 1. ^{1}H NMR Data for Globulixanthones A (**1**) and B (**2**)

	globulixanthone A (**1**)				globulixanthone B (**2**)			
position	^{1}H[a]			HMBC[c]	^{1}H[b]			HMBC[c]
2	6.81	dd	(8.4, 1.9)	1, 4, 9a	6.71	dd	(8.3, 1.9)	1, 9a
3	7.72	t	(8.4)	1, 4, 4a	7.48	t	(8.3)	1, 2, 4a
4	7.07	dd	(8.4, 1.9)	2, 4a, 9a	6.83	dd	(8.3, 1.9)	2, 3
6					6.84	s		4b, 5, 7, 8
8	7.83	s		1′, 4b, 6, 8a				
1′	6.76	d	(16.3)	6, 8				
2′	7.11	d	(16.3)	4′, 5′				
3′					5.76	d	(10.0)	1″, 2′, 4′, 5′
4′	5.29	d	(2.2)	2′, 3′, 5′	8.03	d	(10.0)	2′, 8, 8a
	5.16	d	(2.2)	2′, 3′, 5′				
5′	2.07	s		2′, 3′, 4′	1.44	s		1″, 2′, 3′
1″					1.81	dt	(11.6, 6.5)	2″, 2′, 3′, 5′
					1.78	dt	(11.6, 6.5)	2″, 2′, 3′, 5′
2″					2.11	m		1″, 2′, 3″, 4″
3″					5.08	t	(6.8)	1″, 2″, 4″
5″					1.65	s		3″, 4″, 6″
6″					1.58	s		3″, 4″, 5″
1-OH	12.67	s		1, 2, 9a	13.08	s		1, 2
5-OH					6.30	s		4b, 5
5-OMe	3.89	s		5				
6-OH	10.30	s						

[a] Recorded in DMSO at 300 MHz. [b] Recorded in $CDCl_3$ at 300 MHz. [c] Carbon that correlated with the proton resonance.

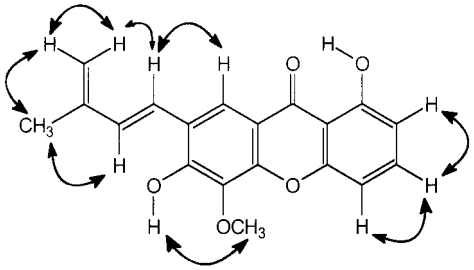

Figure 1. NOESY correlations for compound **1**.

and the double doublet at δ 7.07 (H-4) with C-2 (δ 110.1) and C-4a (δ 155.7). These results clearly indicated that the aromatic ring A, to which is attributed the ABC spin system, was 1,2,3-trisubstituted with the chelated hydroxyl group located at the C-1 position. Thus, the second hydroxyl group, the methoxyl substituent, and the 3-methylbuta-1,3-dienyl moiety were located on ring B. The positioning of these substituents on this ring was deduced as follows. Since it is well known that the chemical shift of the methoxyl groups in the aromatic ring are of diagnostic value in deciding whether the methoxyl group is attached to the di-*ortho* (more hindered methoxyl group, at ca. δ 60−61) O-substituted carbon or to a carbon bearing one or no *ortho* O-substituent[13−16] (less hindered, at ca. δ 55−56), consideration of the methoxyl group chemical shift at δ 60.8 of compound **1** leads to the conclusion that this substituent was located at position C-5 on ring B. Thus, the second hydroxyl group was attached to C-6 and the 3-methylbuta-1,3-dienyl moiety to C-7. This was further confirmed by the NOESY spectrum (Figure 1), which showed no cross-peak between the methoxyl signal and the olefinic proton H-2′ but a cross-peak between H-8 and the olefinic proton H-1′ and another cross-peak between the methoxyl signal at δ 3.89 and the second hydroxyl proton at δ 10.30. From the above spectroscopic studies, compound **1** was characterized as 1,6-dihydroxy-5-methoxy-7-[3-methylbuta-1,3-dienyl]xanthone.

Compound **2**, globulixanthone B, isolated as a sticky yellow oil, $[\alpha]_D^{20}$ +9.5° (*c* 0.07, MeOH), reacted positively to $FeCl_3$ reagent and had a molecular formula $C_{23}H_{22}O_5$ on the basis of its HREIMS (378.1465; calcd 378.1467). Its IR and UV spectra showed absorption bands characteristic of a hydroxylated xanthone. In the ^{1}H NMR spectrum (Table 1) of **2**, a chelated hydroxyl group at δ 13.08, a one-proton singlet exchangeable with D_2O due to a free hydroxyl group at δ 6.30, and a singlet aromatic proton at δ 6.84 were observed. The ^{1}H NMR spectrum of **2** also showed the presence of an ABC spin system at δ 7.48 (t, J = 8.3 Hz), 6.83 (dd, J = 8.3, 1.9 Hz), and 6.71 (dd, J = 8.3, 1.9 Hz) assignable to a 1,2,3-trisubstituted benzene ring and signals due to a methyl chromene ring at δ 1.44 (3H, s), 5.76, and 8.03 (each 1H, d, J = 10 Hz) and a 4-methylpent-3-enyl moiety at δ 5.08 (1H, t, J = 6.8 Hz), 2.11 (2H, m), 1.81 (dt, J = 11.6, 6.5 Hz), 1.78 (dt, J = 11.6, 6.5 Hz), 1.65 (3H, s), and 1.58 (3H, s). Analysis of its HMQC and HMBC spectra allowed the unambiguous assignments of all proton and carbon signals (Tables 1 and 2), which also clarified the position of the substituents. Several HMBC couplings (Table 1), including correlations of the chelated

Table 2. ^{13}C NMR Assignments for Compounds **1** and **2**

carbon	**1**[a]	**2**[b]
1	161.0 s	161.9 s
2	110.1 d	110.1 d
3	137.1 d	135.8 d
4	107.3 d	106.3 d
4a	155.7 s	155.7 s
4b	150.9 s	151.4 s
5	145.9 s	153.4 s
6	141.7 s	102.4 d
7	116.6 s	137.1 s
8	111.4 d	109.1 s
8a	127.6 s	119.5 s
9	181.5 s	183.7 s
9a	107.9 s	108.6 s
1′	121.7 d	
2′	133.6 d	79.6 s
3′	138.7 s	131.7 d
4′	118.9 t	123.6 d
5′	18.3 q	25.7 q
1″		40.4 t
2″		22.8 t
3″		121.2 d
4″		132.2 s
5″		25.6 q
6″		17.7 q
5-OCH_3	60.8 q	

[a] Recorded in DMSO at 75 MHz. [b] Recorded in $CDCl_3$ at 75 MHz.

hydroxyl group at δ 13.08 with C-1 (δ 161.9), C-2 (δ 110.1), and C-9a (δ 108.6), the double doublet at δ 6.71 (H-2) with C-1 (δ 161.9) and C-9a (δ 108.6), the triplet at δ 7.48 (H-3) with C-1 (δ 161.9), C-2 (δ 110.1), and C-4a (δ 155.7), and the double doublet at δ 6.83 (H-4) with C-3 (δ 135.8) and C-2 (δ 110.1), revealed that, as in the case of compound **1**, the aromatic ring A in compound **2** was also 1,2,3-trisubstituted with the chelated hydroxyl group in position C-1. The absence of the signal (δ 7.75−7.80) due to the aromatic proton (H-8) located at a *peri* position to the carbonyl group suggested that the methyl chromene ring was fused in a angular form at C-8 through an oxygen atom at C-7. This was confirmed, on one hand, by the chemical shift of one *cis*-olefinic proton of the chromene ring which appeared in the lower field (δ 8.03) caused by the anisotropic effect of the carbonyl group and, on the other hand, by the HMBC spectrum in which the same olefinic proton showed cross-peaks with C-8 (δ 109.1) and C-8a (δ 119.5). Furthermore, the cross-peaks observed in the HMBC spectrum between the single aromatic proton at δ 6.84 with C-7 (δ 137.1), C-8 (δ 109.1), C-5 (δ 153.4), and C-4b (δ 151.4) and between the free hydroxyl proton at δ 6.30 with C-5 (δ 153.4) and C-4b (δ 151.4) indicated that this proton and the second hydroxyl group were located on ring B at positions C-6 and C-5, respectively. By a process of elimination, the remaining 4-methyl-3-pentenyl moiety could be located on the chromene ring at position 2. This was further confirmed by the HMBC spectrum, in which cross-peaks between the olefinic proton at δ 5.76 with C-2′ (δ 79.6) and C-1″ (δ 40.4) were observed. Therefore, the structure of globulixanthone B (**2**) was assigned as 1,5-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)pyrano(5′,6′:7,8)xanthone.

Compounds **1** and **2** were evaluated in vitro for their cytotoxic activity against human epidermoid carcinoma of the nasopharynx (KB) cells. As a result, IC_{50} (μg/mL) values of these compounds were 2.15 for **1** and 1.78 for **2**, respectively.

Experimental Section

General Experimental Procedures. All melting points were determined on a Buchi apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer B4FT-IR spectrometer and UV on a Kontron-Uvikon 932 spectrometer. EIMS were obtained on a Nermag Sidar V3.0 and with LKB 9000S mass spectrometers and the HRMS with a V. G. analytical ZAB-H mass spectrometer. ^{1}H (300.13 MHz) and ^{13}C (75.45 MHz) NMR spectra were performed on an AC300 Bruker instrument, with TMS as internal standard.

Plant Material. Root bark of S*ymphonia globulifera* L. f. was collected in March 2001, at Yaounde, Cameroon. A voucher specimen (2235/SRFK) documenting the collection is on deposit at the National Herbarium Yaounde, Cameroon.

Extraction and Isolation. Air-dried powdered root bark of S*ymphonia globulifera* (6 kg) was extracted twice during 48 h (at each time) at room temperature with a mixture of CH_2Cl_2−MeOH (1:1) (8 L) and MeOH (8 L). The solvents were removed under reduced pressure to yield the CH_2Cl_2−MeOH (1:1) residue (70 g) and the MeOH residue (120 g), respectively. Both residues were evaluated in vitro for their cytotoxic activity against the KB cell line, and IC_{50} values of 10 and 300 μg/mL were obtained for the first and the second residues, respectively. Since the CH_2Cl_2−MeOH (1:1) residue showed strong cytotoxic activity compared to the methanol residue, it was first subjected to flash chromatography over a short column (5 × 50 cm) containing silica gel (230−400 mesh, 40−63 μm, ASTM Merck). Thirty fractions (ca. 500 mL each) were collected. The three most active ones (300 mg, IC_{50} 5.4 μg/mL) were combined and further subjected to column chromatography over silica gel (70−230 mesh, 63−200 μm, ASTM Merck) packed in cyclohexane and eluted with C_6H_{12}−EtOAc mixtures. In all, 50 fractions (of ca. 250 mL each) were collected and combined on the basis of TLC analysis, leading to three main series (A−C). Fractions 1−30, eluted with a mixture of C_6H_{12}−EtOAc (17:3), gave series A, from which stigmasterol (**3**) (150 mg) crystallized. Fractions 32−42, eluted with a mixture of C_6H_{12}−EtOAc (4:1), gave series B, which was further subjected to repeated column chromatography over silica gel eluted with a mixture of C_6H_{12}−EtOAc (17:3) to yield globulixanthone A (**1**) (100 mg). Series C, resulting from the combination of fractions 43−50 eluted with C_6H_{12}−EtOAc (3:1), was subjected to preparative TLC over silica gel eluted with a mixture of toluene−acetone (7:3) to afford globulixanthone B (**2**) (20 mg).

Globulixanthone A (1): isolated as yellow powder (C_6H_{12}−EtOAc), mp 240−242 °C; UV (MeOH) λ_{max} (log ϵ) 226 (4.21), 233 (4.32), 273 (4.42), 289 (4.46), 381(3.66) nm; IR (KBr) ν_{max} 3294, 2940, 1664, 1657, 1605, 1578, 1466, 1262, 1242, 1083, 940, 807, 769 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz), see Table 1; ^{13}C NMR (DMSO, 75 MHz), see Table 2; EIMS *m/z* 324 $[M]^+$ (100), 309 (20), 294 (71), 278 (18), 270 (11), 265 (21), 202 (12), 92 (10); HREIMS *m/z* 324.0996 (calcd for $C_{19}H_{16}O_5$, 324.0998).

Globulixanthone B (2): yellow sticky oil; $[\alpha]_D^{20}$ +9.5° (*c*, 0.07 MeOH); UV (EtOH) λ_{max} (log ϵ) 211 (2.55), 244 (2.58), 265 (2.42), 318 (2.30), 383 (1.86); IR (Nujol) 3426, 3276, 1648, 1618, 1569, 1516, 1474, 1320, 1240, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR (75 MHz), see Table 2; EIMS *m/z* 378 $[M]^+$ (100), 363 (37), 335 (42), 324 (26), 295 (27), 286 (11), 271 (35), 243 (21); 203 (16), 92 (14); HREIMS *m/z* 378.1465 (calcd for $C_{23}H_{22}O_5$ 378.1467).

Stigmasterol was identified by comparison of its TLC, R_f, and ^{1}H and ^{13}C NMR data with authentic sample.

Cytotoxicity Assay. Cytotoxicity of the crude extracts, fractions, and the purified compounds against human epidermoid carcinoma (KB) of the nasopharynx cancer cells line was evaluated using the protocol described in the literature by Likhitwitaywuid et al.[17]

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