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SYMPHONIN: A NEW PRENYLATED PYRANOXANTHONE WITH ANTIMICROBIAL ACTIVITY FROM THE SEEDS OF *SYMPHONIA GLOBULIFERA* (GUTTIFERAE)

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ABSTRACT. A new prenylated pyranoxanthone, symphonin (**1**), 2-(3,3-dimethylallyl)-1,5-dihydroxy-6,7-dimethoxy-2",2"-dimethylpyrano (5",6":3,4) xanthone, the known compounds guttiferone A, oleanolic acid and sitosterol were isolated from the methanol extract of the seeds of *Symphonia globulifera* (Guttiferae). The structural elucidation of the new compound was based mainly on spectroscopic analyses. The new xanthone showed antimicrobial activity against *Staphylococcus aureus*, *Streptococcus feacalis*, *Klebsiella pneumonia* and *Escherichia coli*.

KEY WORDS: *Symphonia globulifera*, Seeds, Xanthone, Antimicrobial activity

INTRODUCTION

The species *Symphonia globulifera* Linn. f. (syn. *S. gabonensis* Pierre) is a tree found mainly in lowland rainforests of tropical regions. In Cameroon, the inhabitants of the Central, South and Eastern provinces use the plant as a laxative for pregnant women and as a general tonic [1]. This species is a rich source of xanthones [2-5] and benzophenones [6-8]. There is a growing interest in constituents of the species because a number of secondary metabolites from it have been found to possess cytotoxic, antioxidant, antimicrobial, antimalarial and HIV-1 protease inhibitory activities [7-12]. As part of our research into members of the Guttiferae, we have investigated the seeds of *S. globulifera* and have isolated and characterized a new xanthone with antimicrobial activity.

RESULTS AND DISCUSSION

Air-dried and ground seeds of *Symphonia globulifera* were extracted at room temperature with methanol and the extract concentrated to dryness under vacuum. The residue showed weak activity at 300 µg/mL when tested against *Staphylococcus aureus, Streptococcus feacalis, Klebsiella pneumonia and Escherichia col*i in the agar dilution-streak assay [13]. This residue, on repeated column chromatographic separation over silica gel, afforded pure compounds, including the new xanthone derivative (**1**) and the known compounds, guttiferone A (**2**), oleanolic acid (**3**) and sitosterol, which were identified by comparison with reported spectroscopic data [7, 14].

Compound (**1**), m.p. 213-214 °C, was obtained as a yellow solid and reacted positively with $FeCl_3$, suggesting the presence of phenolic hydroxyl group (s). The molecular formula $C_{25}H_{26}O_7$, with 13 degrees of unsaturation, was deduced from EIMS and 1D NMR data (Table 1). The IR spectrum of **1** exhibited strong absorption due to phenolic hydroxyl(s) (3290 cm^{-1}), a hydrogen-

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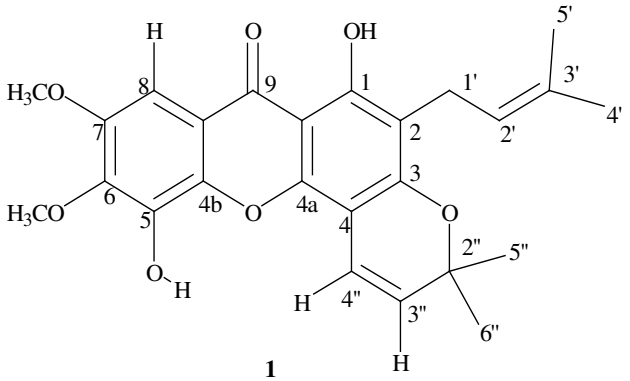
bonded carbonyl (1662 cm^{-1}) and aromatic rings (1590 cm^{-1}). The UV spectrum of **1** showed the specific absorption [λ_{max} (237, 253, 285 and 346 nm)] of a xanthone nucleus [15]. This was confirmed in the ^{1}H (two singlets, 1H each at δ_H 13.20 and 7.20 due to a chelated OH and a proton *peri* to a carbonyl function, respectively) and ^{13}C NMR spectra (Table 1) [2, 11, 12]. The ^{13}C NMR spectrum revealed 25 carbon signals which were sorted by DEPT and HMQC experiments into four methyls, two methoxyls, four methines and 15 quaternary carbons including a carbonyl function (δ_C 180.2). The ^{1}H NMR spectrum of **1** exhibited the characteristic spin system of a 3,3-dimethylallyl moiety [δ_H 1.80 (3H, s); 1.60 (3H, s); 3.30 (2H, d, J = 7.2 Hz) and 5.20 (1H, m)], together with signals for two methoxyl groups [δ_H 3.95 (3H, s) and 4.05 (3H, *s*)] and for the expected chelated hydroxyl group [δ_H 13.20 (1H, *s*)]. Another hydroxyl proton appeared as a singlet, exchangeable with D_2O, at δ_H 5.80. The presence of proton signals at δ_H 1.40 (6H, s, 2 x Me), 5.50 and 6.80 (1H each, d, J = 10.0 Hz) suggested the presence of a 2,2-dimethyl-2H-pyrano ring in **1**. This was further supported by the set of signals at δ_C 28.2, 78.0, 126.8 and 115.5 in the ^{13}C NMR spectrum [16]. In the HMBC spectrum (Figure 1, Table 1) the single aromatic proton at δ_H 7.20 gave cross-peaks with the carbonyl (δ_C 180.2) and two quaternary aromatic carbons bearing oxygens [δ_C 140.9 (C-7) and 149.2 (C-6)], consistent with its location at C-8, *peri* to the carbonyl group. In the same experiment, the methylene proton of the 3,3-dimethylallyl group at δ 3.34 (d, J = 7.2 Hz) showed cross-peaks with three quaternary carbons C-1 (δ_C 160.0), C-2 (δ_C 111.7) and C-3' (δ_C 131.5) while the protons of the AB system of the 2,2-dimethyl-2H-pyrano ring (δ_H 6.80 and 5.50) were correlated to C-3 and C-2''and and to C-4 and C-2", respectively. The chelated proton (δ_H 13.20) had correlations to C-1, C-2 and C-9a. These results indicated that the 3,3-dimethylallyl group was attached to C-2 while the chromene moiety was attached to C-3 and C-4 of ring A of the xanthone nucleus. The remaining substituents, two methoxyls and the free OH, were placed on ring B. The free OH (δ_H 5.80) had cross-peaks with C-6 (δ_C 140.9) whereas the two methoxyl groups at δ_H 3.95 and 4.04 correlated with C-6 (δ_C 140.9) and C-7 (δ_C 149.2), respectively. In addition, the C-7 methoxy had a NOE with H-8. These observations clearly demonstrated that the two methoxyls were located at C-6 and C-7 and the free OH at C-5. Thus the structure of the new xanthone, which we have named symphonin, was characterized as **1,** 2-(3,3-dimethylallyl)-1,5-dihydroxy-6,7-dimethoxy-2'',2"-dimethylpyrano (5",6":3,4) xanthone.

In addition to **1**, three known compounds were also isolated and identified as the guttiferone A (**2**), oleanolic acid (**3**) and sitosterol [7, 14].



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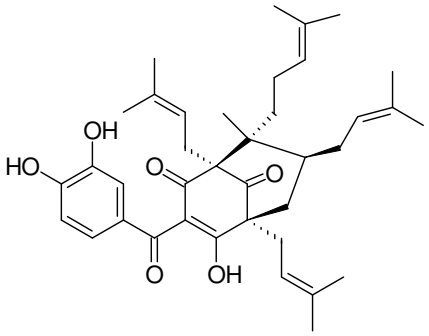

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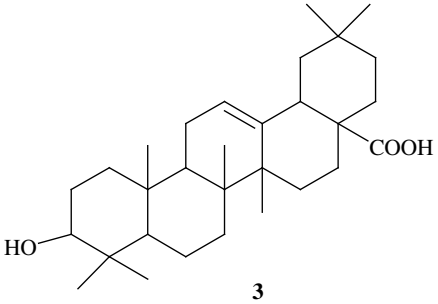

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Table 1. ^{1}H (400.1 MHz) and ^{13}C (100.6 MHz) NMR chemical shift assignments for symphonin **1**.

N°	^{13}C ($CDCl_3$) (m)	^{1}H ($CDCl_3$) (m) J (Hz)	HMBC correlations
1	160.0 (s)	-	
2	111.7 (s)	-	
3	158.3 (s)	-	
4	100.9 (s)	-	
4a	150.0 (s)	-	
4b	137.9 (s)	-	
5	140.9 (s)	-	
6	140.9 (s)	-	
7	149.2 (s)	-	
8	96.9 (d)	7.20 (s)	C-6, C-7, C-9
8a	116.0 (s)	-	
9	180.2 (s)	-	
9a	103.0 (s)	-	
1’	21.8 (s)	3.34 (d, 7.2)	C-1, C-2, C-3, C-2’, C-3’
2’	122.1 (s)	5.20 (m)	
3’	131.5 (s)	-	
4’	25.8 (q)	1.60 (s)	C-2’, C-3’, C-5’
5’	18.2 (q)	1.80 (s)	C-2’, C-3’, C-4’
2’’	78.0 (s)	-	
3’’	126.8 (d)	5.50 (d, 10)	C-2”, C-4
4’’	115.5 (d)	6.80 (d, 10)	C-3, C-2’’
5’’, 6’’	28.2 (q)	1.40 (s)	C-2’’, C-3’’
6-OMe	61.4 (q)	4.04 (s)	C-6
7-OMe	56.2 (q)	3.96 (s)	C-7
1-OH	-	13.20 (s)	C-1, C-2, C-9a
5-OH	-	5.80 (s)	C-6

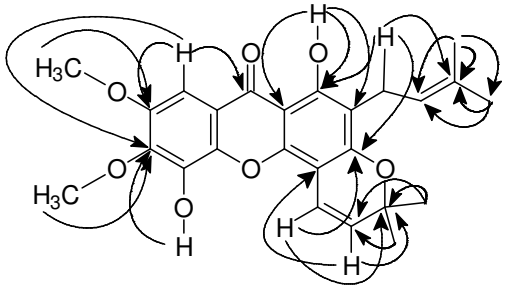

Figure 1. Significant long-range correlations observed in ^{13}C-^{1}H HMBC for compound **1**.

Compound **1** was tested *in vitro* for its antimicrobial activity against the micro-organisms, *Staphylococcus aureus, Streptococcus feacalis, Klebsiella pneumonia and Escherichia coli* using the agar diffusion-streak method [13]. As shown in Table 2, with all the four tested strains (*Staphylococcus aureus*, *Streptococcus feacalis*, *Klebsiella pneumonia* and *Escherichia coli*) the activity of compound **1** was almost equivalent to or less than that demonstrated by gentamicin.

Table 2. *In vitro* antimicrobial activity of **1** from *Symphonia globulifera*.

Compound	Micro-organism tested			
	Staphylococcus aureus	*Streptococcus feacalis*	*Klebsiella pneumonia*	*Escherichia coli*
MeOH extract	300	300	300	300
1	3.12	4.20	25.90	inactive
Gentamicin	0.60	4.52	8.00	0.90

The data are represented as minimum inhibitory concentration (MIC in μg/mL) that prevented growth of the micro-organism.

EXPERIMENTAL

General. Melting points were determined on a Buchi melting point apparatus B-545. UV spectra were obtained on a Shimadzu-265 spectrophotometer. IR spectra were recorded on a Perkin-Elmer B4FT-IR spectrometer with KBr pellets. NMR spectra were run on a Bruker spectrometer equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400.1 and 100.6 MHz, respectively, with TMS as internal standard. The chemical shifts (δ) are reported in ppm with the solvent signal, (δ_H 7.25 and δ_C 77.0 for $CDCl_3$) as reference, while coupling constants (J) are given in Hertz.

Plant material. The seeds of *Symphonia globulifera* were collected in January 2002 at Fundong in the North-West province of Cameroon. The plant was identified by Dr G. Achoundong of the National Herbarium Yaoundé, Cameroon where a voucher specimen (N° 50787 NHC) has been deposited.

Extraction, isolation and characterization. The air-dried powdered seeds of *S. globulifera* (0.5 kg) were extracted with MeOH (2 L x 3) at room temperature and the extract concentrated to dryness to obtain a viscous residue (155 g). This residue was then subjected to flash chromatography with silica gel (230-400 mesh) as stationary phase eluting with a hexane/EtOAc system as mobile phase. 70 fractions of 300 mL each were collected and grouped on the basis of

TLC analysis to yield three main series labelled A, B, and C. Fraction A contained only oils. Fraction B was concentrated to dryness and the residue subjected to column chromatography over silica gel (70-230 mesh) eluting with a gradient of increasing polarity with hexane-EtOAc to afford 30 mg of sitosterol and 100 mg of oleanolic acid (**3**). Chromatography of the fraction C led to more oleanolic acid (150 mg) and a mixture which was further subjected to repeated column chromatography to give 18 mg of symphonin (**1**) and 22 mg of guttiferone A (**2**).

Known compounds were identified by comparison of their spectral data with those reported in the literature [7, 14].

Symphonin (**1**). Yellow powder, m.p. 213-214 °C; ^{13}C (100.6 MHz, $CDCl_3$) and ^{1}H (400.13 Hz, $CDCl_3$) NMR: see Table 1; EIMS: *m/z* (rel. int.): 438 $[M]^+$ (44), 423 [M-15] (100), 383(50), 373 (31), 369 (37), 227 (74), 195 (74).

Antimicrobial assay [13]. Extract and purified active principles (1 mg/mL) were tested against the micro-organisms, *Staphylococcus aureus*, *Streptococcus feacalis*, *Klebsiella pneumonia* and *Escherichia coli*. The four strains of bacteria were cultivated in Mueller Hinton agar medium at 37 °C. After one day, their growth was assessed visually. The lowest concentration of the tested compounds in which no visible growth occurred was defined as the minimum inhibitory concentration.

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