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Globulixanthones C, D and E: three prenylated xanthones with antimicrobial properties from the root bark of *Symphonia globulifera*☆

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Abstract

Two prenylated xanthone derivatives, named globulixanthones C and D and one bis-xanthone, designated globulixanthone E, have been isolated from the root bark of *Symphonia globulifera*. The structures of these compounds were elucidated by a detailed spectroscopic analysis. They have been shown to exhibit in vitro significant antimicrobial activity against a range of micro-organisms. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Symphonia globulifera*; Guttiferae; Globulixanthones C, D and E; Antimicrobial activity

1. Introduction

Symphonia globulifera is a large forest tree found throughout the central, south and east provinces of Cameroon where it is used as a medicinal plant, as laxative for pregnant women and a general tonic (Aubreville, 1950). Previous study of the mixture of methanol–methylene chloride (1:1) extract of the root bark of this plant has led recently to the isolation and characterization of two new xanthone derivatives with isoprenoid groups named globulixanthones A and B (Nkengfack et al., 2002). As part of our continuing investigation of new biologically active metabolites from this plant, we now report the isolation, structural elucidation and biological activities of two new xanthones, designated, globulixanthones C and D along with one new bis-xanthone, named globulixanthone E.

2. Results and discussion

Air-dried and ground root bark of *S. globulifera* was extracted at room temperature with a mixture of CH_2Cl_2–MeOH (1:1) and MeOH, successively. These extracts were concentrated to dryness under vacuum and their antimicrobial activities against a range of micro-organisms were evaluated in vitro. The MeOH–CH_2Cl_2 (1:1) extract exhibited a broad spectrum of antimicrobial activity at 150 μg/ml (MeOH extract showed weak activity at 500 μg/ml) when tested using streak-dilution technique (Mitscher, 1977). This extract was then subjected to a bioassay-directed fractionation. After successive chromatographic purifications, two new xanthones, named globulixanthones C (**1**) and D (**2**) were isolated together with a novel bis-xanthone, globulixanthone E (**3**).

☆ Part 2 in the series "*Symphonia* studies". For part 1, see Nkengfack et al., 2002.

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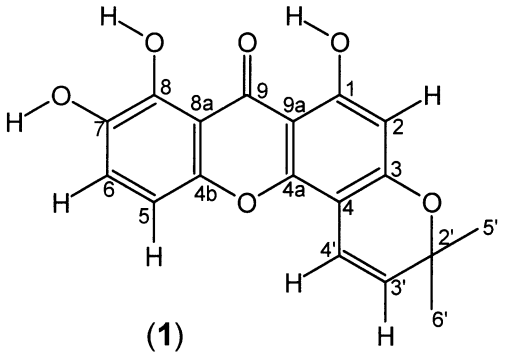

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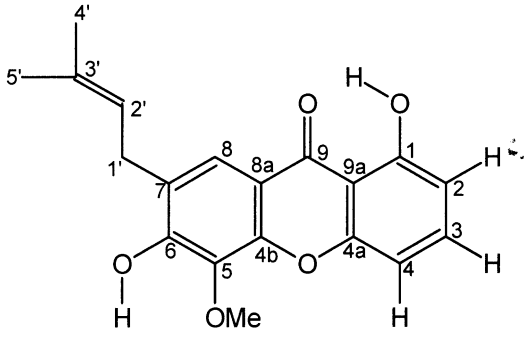

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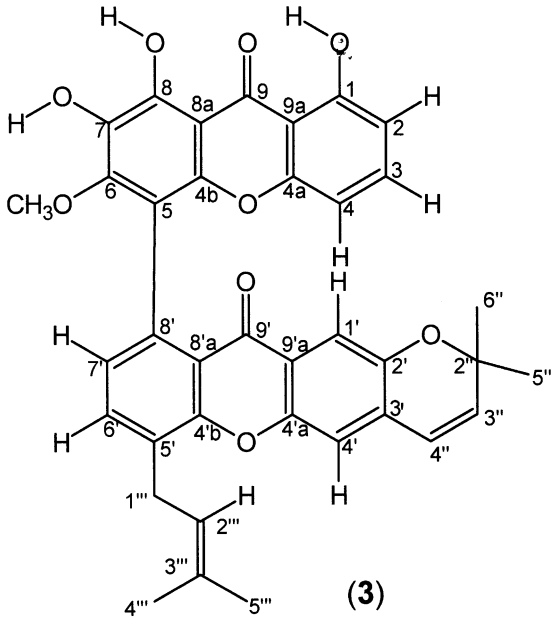
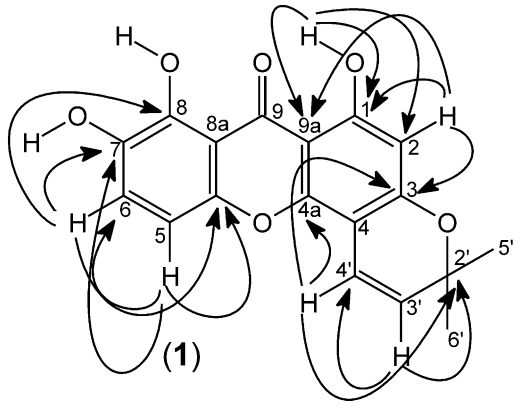


(3)

Globulixanthone C (**1**), mp 285 °C, was obtained as yellow needles, and reacted positively to $FeCl_3$ reagent. It was formulated as $C_{18}H_{14}O_6$ on the basis of its HRMS ($[M]^+$, m/z found 326.0786; calc. 326.0790). Its IR spectrum exhibited vibration bands due to hydroxyl groups (3454 cm^{-1}) and conjuguated carbonyl group (1664 cm^{-1}). The UV absorptions (204, 219, 267, 295, 337 nm) indicated (**1**) to be a xanthone derivative (Peres and Nagem, 1997; Nagem et al., 2000). The ^1H NMR of (**1**) revealed the presence of two chelated hydroxyls [δ 12.35 (1H, *s*) and 11.10 (1H, *s*)] one of which must be placed at C-1 (δ 12.35) and a free phenolic hydroxyl [δ 8.50 (1H, *brs*)], all exchangeable with D_2O. The ^1H NMR spectrum of compound (**1**) also showed signals for *ortho*-coupled aromatic protons [δ 7.26 and 6.67 (1H each, $J = 8.1$ Hz)], a single aromatic proton [δ 6.40 (1H, *s*)] and a dimethylchromene ring [δ 1.49 (6H, *s*) 6.65 and 5.85 (1H, each, *d*, $J = 10.0$ Hz)]. The presence of a 2,2-dimethylchromene moiety was further confirmed both by the ^{13}C NMR spectrum which showed characteristic signals at δ 28.0 ($C_{5'}$, $C_{6'}$), 78.7 (C-2′), 114.2 (C-4′) and 128.8 (C-3′) (Agrawal and Bansal, 1989) and by the EI-mass spectrum on which the base peak at m/z 311 $[M\text{-}15]^+$ was observed. In the HMBC spectrum (Fig. 1), cross-peaks between the chelated hydroxyl group at (δ 12.35) and C-1 (δ 160.8), C-9a (δ 102.0) and C-2 (δ 95.0) and between the single aromatic proton at δ 6.40 and C-1 (δ 160.8) C-9a (δ 102.0), C-3 (δ 156.1) and C-4 (δ 104.1) suggested that the single aromatic proton was located at C-2.

The orientation of the dimethylchromene ring was precisely determined by 2D NMR techniques (HSQC and HMBC). Again, in the HMBC spectrum, the *cis*-olefinic proton at δ 6.65 showed cross-peaks with carbon signals at C-4 (δ 104.1), C-4a (δ 156.0) and C-3 (δ 156.1), while in the NOESY spectrum (Fig. 2), no interaction between the same *cis*-olefinic proton and the chelated hydroxyl group at δ 12.35 was observed. This finding clearly indicated that the dimethylpyran ring was fused in an angular fashion to xanthone nucleus at positions C-3/C-4. On the other hand, the absence in the ^1H NMR spectrum of an aromatic signal around δ 7.40–7.60 due to H-8 *peri* to the carbonyl, indicated that the second chelated hydroxyl group most be located at the C-8 position. Thus, the free phenolic group was located at C-7 and the *ortho*-aromatic protons at positions C-5 and C-6. This was substantiated, on one hand, by the NOESY spectrum which showed strong interaction between the free phenolic group and the second chelated



(1)

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

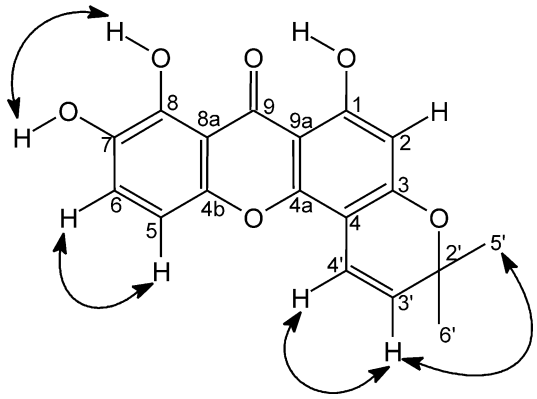


Fig. 2. Selected NOESY correlations for compound **1**.

hydroxyl group and on the other hand, by the HMBC experiment through the ^2J and ^3J correlations between the H-6 (δ 7.26) and C-7 (δ 137.3), C-8 (δ 143.5) and C-4b (δ 151.7) and between H-5 (δ 6.67) and C-7 (δ 137.3) and C-6 (δ 123.9) and C-4b (δ 151.7). From the above spectroscopic studies, the structure of globulixanthone C (**1**) can be assigned as 1,7,8-trihydroxy-2,2-dimethylpyrano[5,6:3,4] xanthone.

Compound (**2**), globulixanthone D, mp 120 °C, isolated as yellow solid, had a molecular formula $C_{19}H_{18}O_5$ on the basis of its high-resolution mass spectrum (326.1150; calc. 326.1154). The ^{13}C NMR spectrum revealed 19 carbon signals that were sorted by DEPT and Jmod experiments into two methyl and one methoxyl groups; one methylene; five sp^2 methine and ten quaternary carbons among which, one carbonyl carbon. The IR spectrum showed vibration bands at 3428 cm^{-1} due to free hydroxyl group and a chelated one at 3250 cm^{-1} and band for chelated carbonyl group at 1664 cm^{-1}. These data, together with those obtained from UV (203, 234, 247, 262, 313, 356 nm), ^{1}H NMR (two singlets, 1H each at δ 12.82 and 7.78 due to chelated OH and proton *peri* to a carbonyl moiety, respectively) and ^{13}C NMR, (Table 1) suggested compound (**2**) to be a xanthone derivative. Furthermore, in the ^{1}H NMR spectrum, an ABM spin system corresponding to a 1,2,3-trisubstituted benzene ring [δ 7.50 (1H, *t*, J = 8.5 Hz), 6.92 and 6.72, (1H each, *dd*, J = 8.4, 1.3 Hz)], phenolic hydroxyl signal [δ 6.50 (1H, *s*) exchangeable with D_2O] and signals corresponding to a 3,3-dimethylallyl group [δ 1.69 (3H, *s*), 1.79 (3H, *s*), 3.40 (2H, *d*, J = 6.8 Hz), 5.30 (1H, *t*, J = 6.8Hz)] were observed in addition to a 3H singlet at δ 4.11 due to a methoxyl group. In the HMBC spectrum, while the proton of the hydrogen bonded hydroxyl group at δ 12.82 correlated with C-1 (δ 162.0), C-9a (δ 108.5 and C-2 (δ 110.7), the double doublet at δ 6.72 showed long-range cross-peaks (^2J and ^3J) with C-1 (δ 162.0), C-4 (δ 106.8) and C-3 (δ 136.1) and the triplet at δ 7.50 gave ^3J correlations with the carbon atoms at δ 162.0 (C-1) and 156.5 (C-4a). These results clearly indicated that the aromatic ring A was 1,2,3-trisubstituted. Therefore, it remained to be established unambiguously the positions on the ring of the γ,γ-dimethylallyl, the methoxyl and the free phenolic groups. The chemical shift value of the carbon atom of the methoxyl group at δ 61.9 indicated that this substituent was linked to the C-5 aromatic carbon whose the two *ortho*-positions were occupied by an oxygen containing functional group (Iinuma et al., 1994; Kosela et al., 2000). Thus, the free phenolic hydroxyl group was attached to C-6 and the 3,3-dimethylallyl moiety to C-7. This was further confirmed by the other correlations in the HMBC and NOESY experiments as shown in Figs. 3 and 4. Therefore, the structure of (**2**) was concluded to be 1,6-dihydroxy-5-methoxy-7-(3-methylbut-2-enyl) xanthone.

Globulixanthone E (**3**) was obtained as pale yellow amorphous material and gave positive reaction to methanolic ferric chloride reagent. Its molecular formula was shown to be $C_{37}H_{30}O_9$ from the high resolution ESI-TOF mass spectrum which showed a pseudo molecular ion peak $[M+H]^+$ at m/z 619.1966 (calc. 619.1968). The IR spectrum of (**3**) disclosed vibration bands due to chelated hydroxyl (3248 cm^{-1}), chelated carbonyl (1648 cm^{-1}), free conjuguated carbonyl (1668 cm^{-1}), benzene ring moieties and other functionalities. Its UV spectrum exhibited maxima at 203, 235, 253, 293, 327 nm and was found to be similar to that of globulixanthone B (**2**). From the above data, it was inferred that compound (**3**) could be a bis-xanthone derivative bearing substituents having a total number of eleven carbon atoms. This was confirmed by the analysis of the ^{1}H NMR (Table 1) and COSY spectra which revealed the presence of an ABM spin system corresponding to a 1,2,3-trisubstituted benzene ring [δ 7.70 (1H, *t*, J = 8.2 Hz) 7.01 and 6.78 (1H each, *dd*, J = 8.2 and 1.8 Hz)], a 3,3-dimethylallyl group [δ 5.30 (1H, t, J = 6.8 Hz), 345 (2H, *d*, J = 6.8 Hz), 1.85 and 1.70 (3H each, *s*)], a pair of *ortho*-coupled aromatic protons [δ 7.32 and 6.65 (each 1H, *d*, J = 8.7Hz), a pair of *para*-coupled aromatic protons [δ 7.55 (1H, *s*) and 6.42 (1H, *d*, J = 0.9 Hz)], a dimethylchromene ring [δ 1.49 (6H, *s*) 6.68 (1H, *dd*, J = 10, 0.9 Hz), 5.79 (1H, *d*, J = 10 Hz)] and a methoxyl group [δ 4.00 (3H, *s*)], in addition to three chelated hydroxyl groups [δ 12.80 (1H, *s*), 12.30 (1H, *s*) and 11.10 (1H, *s*)] (Graham et al., 1990). The ^{13}C NMR spectrum (Table 1) analyzed with the Jmod, DEPT and HSQC techniques displayed twenty-one quaternary, ten methine, one methylene and four methyl carbons. By comparison with the ^{1}H NMR spectrum of (**2**), the ABM spin system corresponding to a 1,2,3-trisubstituted benzene ring could only be assigned to the H-2, H-3 and H-4 protons of a 1-hydroxylated xanthone ring A. It was therefore assumed that the two remaining chelated hydroxyl groups could occupy adjacent positions on xanthone ring B one of which being strongly influenced by the anisotropic effect of a carbonyl group. On the other hand, the fact that one of the

Table 1
^{1}H (300 MHz) and ^{13}C (75 MHz) assignments for globulixanthone C (**1**), globulixanthone D (**2**) and globulixanthone E (**3**)

1 (DMSO)			**2** ($CDCl_3$)			**3** (Acetone-d_6)		
Attribution	^{13}C	^{1}H [m, *J* (Hz)]	Attribution	^{13}C	^{1}H [m, *J* (Hz)]	Attribution	^{13}C	^{1}H [m, *J* (Hz)]
1	160.8	–	1	162.0	–	1	163.0	–
2	95.0	6.40 (*s*)	2	110.7	6.72 (*dd*, 8.4, 1.3)	2	112.0	6.78 (*dd*, 8.2, 1.8)
3	156.1		3	136.1	7.50 (*t*, 8.4)	3	137.0	7.70 (*t*, 8.2)
4	104.1	–	4	106.8	6.92 (*dd*, 8.4, 1.3)	4	108.3	7.01 (*dd*, 8.2, 1.8)
4a	156.0	–	4a	156.5	–	4a	158.0	–
4b	151.7	–	4b	155.0	–	4b	157.5	–
5	109.7	6.67 (*d*, 8.1)	5	133.9	–	5	124.1	–
6	123.9	7.26 (*d*, 8.1)	6	153.4	–	6	153.0	–
7	137.3	–	7	126.3	–	7	155.0	–
8	143.5	–	8	120.8	7.78 (*s*)	8	153.5	–
8a	128.8	–	8a	125.9	–	8a	104.5	–
9	184.1	–	9	180.0	–	9	181.2	–
9a	102.0	–	9a	108.5	–	9a	109.1	–
2′	78.7	–	1′	28.1	3.40 (*d*, 6.8)	1′	117.0	7.55 (*s*)
3′	128.8	5.85 (*d*, 10.0)	2′	120.8	5.30 (*t*, 6.8)	2′	158.5	–
4′	114.2	6.65 (*d*, 10.0)	3′	133.1	–	3′	107.8	–
5′	28.0	1.49 (*s*)	4′	25.8	1.79 (*s*)	4′	96.5	6.42 (*d*, 0.9)
6′	28.0	1.49 (*s*)	5′	17.8	1.69 (*s*)	4′a	154.1	–
1-OH	–	12.85 (*s*)	1-OH	–	12.82 (*s*)	4′b	154.1	–
7-OH	–	8.50 (*s*, *brs*)	5-OMe	61.9	4.11 (*s*)	5′	116.0	–
8-OH	–	11.10 (*s*)	6-OH	–	6.50 (*s*)	6′	126.0	7.32 (*d*, 8.7)
						7′	111.8	6.65 (*d*, 8.7)
						8′	111.0	–
						8′a	130.1	–
						9′	182.0	–
						9′a	124.5	–
						2″	78.0	–
						3″	129.4	5.79 (*d*, 10.0)
						4″	116.8	6.68 (*dd*, 10.0, 0.9)
						5″	28.0	1.49 (*s*)
						6″	28.0	1.49 (*s*)
						1‴	22.5	3.50 (*d*, 6.8)
						2‴	123.1	5.30 (*t*, 6.8)
						3‴	132.8	–
						4‴	18.0	1.85 (*s*)
						5‴	26.3	1.71 (*s*)
						1-OH	–	12.80 (*s*)
						6-OMe	62.0	4.00 (*s*)
						7-OH	–	11.10 (*s*)
						8-OH	–	12.30 (*s*)

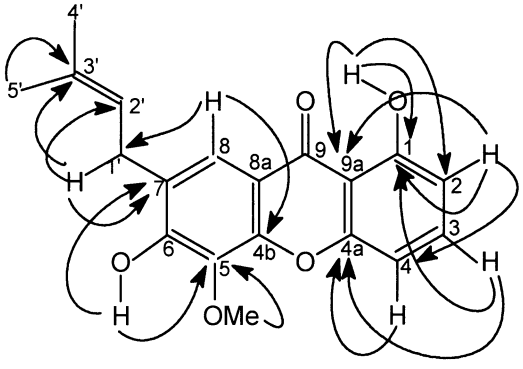


Fig. 3. Significant long-range correlations observed in ^{13}C–^{1}H HMBC for compound **2** in $CDCl_3$.

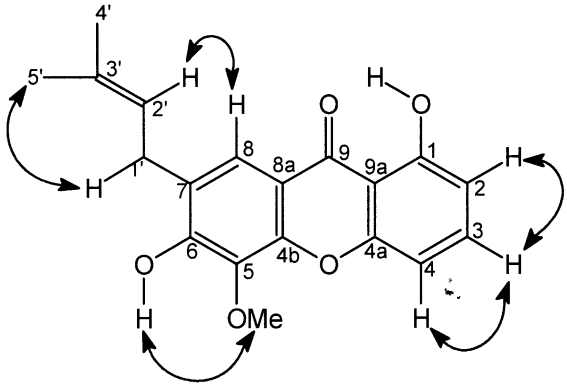


Fig. 4. Selected NOESY correlations for compound **2**.

cis-olefinic (δ 6.68) proton of the chromene ring showed a weak coupling ($J = 0.9$ Hz) with one of the *para*-coupled aromatic protons [δ 6.42 (*d*, $J = 0.9$ Hz)] suggested that the chromene ring was fused in the linear manner on the aromatic ring A′ belonging to the second xanthone unit and which bears the pair of the *para*-coupled protons. This finding was further confirmed by the chemical shift (δ 7.55) of one of the *para*-aromatic protons which must be assigned to C-1′ position, *peri* to carbonyl and by the NOESY (Fig. 5) spectrum in which a cross peak between the second *para*-aromatic proton at δ 6.42 (H-4′) and one of the *cis*-olefinic proton at δ 6.68 (H-4″) of the chromene ring was observed. Moreover, the presence in the HMBC (Fig. 6) spectrum of (**3**) of long-range correlation (^2J and ^3J) between one of the *ortho*-coupled aromatic proton at δ 7.32 (H-6′) and the methylene carbon at δ 22.5(C-1‴) and between the allyl protons at δ 3.45 and carbon atoms at 124.1 (C-5′) and 153.0 (C-6′) indicated clearly that the 3,3-dimethylallyl moiety was located on ring B′ at the positions C-5′ adjacent to one of the *ortho*-coupled aromatic protons (H-6′). This was further supported by the NOESY spectrum which showed cross-peaks between the allyl protons (δ 3.45) and the aromatic proton H-6′ at δ 7.32. It remained at this stage to establish unambiguously the position of the methoxyl group and the linkage between the two xanthone units.

This was deduced, once again, from the NOESY spectrum of (**3**) which displayed cross-peaks between the methoxyl signal at δ 4.00 and one of the aromatic *ortho*-coupled proton at δ 6.65 (H-7′) and between methoxyl signal and one of the chelated hydroxyl appearing at δ 11.10. This finding indicated that the methoxyl group was located on ring B at position C-6. Thus, the linkage between the two xanthone units was deduced to be C-5, C-8′. From the above spectroscopic studies, the structure of globulixanthone E was characterized as (**3**). Although bis-xanthones with an ether linkage (Mandal and Chatterjee, 1987) and a very few number of C-C′ linked bis-xanthones (Graham et al., 1990) have been isolated from the nature, to our knowledge, this is the first time that a C-C′ linked bis-xanthone derivative is reported from *Symphonia* genus.

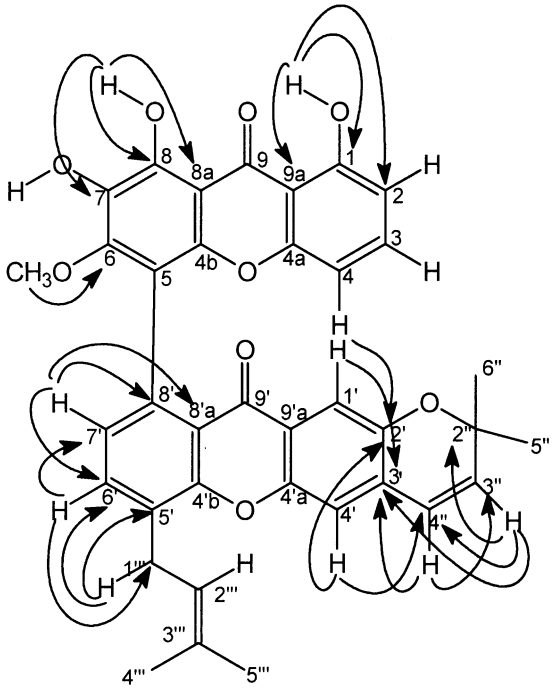


Fig. 5. Selected HMBC correlations for compound **3**.

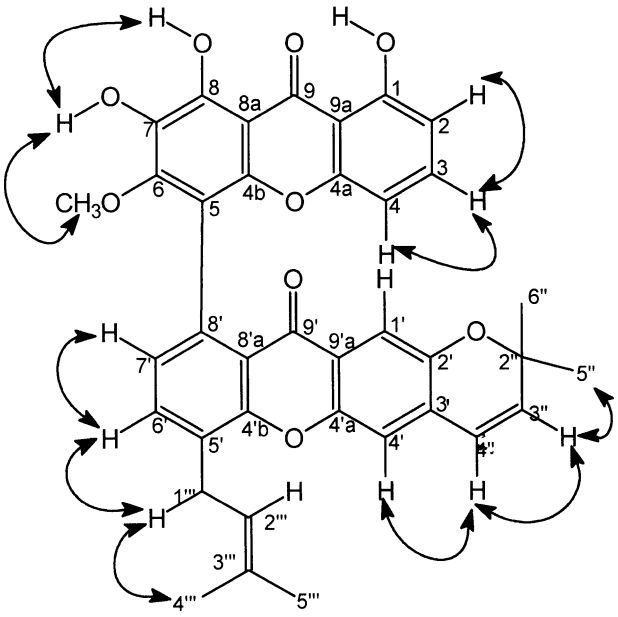


Fig. 6. Selected NOESY correlations for compound **3**.

Compounds (**1**)–(**3**) were tested for their antimicrobial potential against representative Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio anguillarium*) and Gram-negative (*Escherichia coli*) bacteria in an agar well diffusion assay. As shown in Table 2, with all the three tested Gram-positive strains (*S. aureus, B. subtilis, V. anguillarium*), the activities of the three compounds (**1**)–(**3**) were almost equivalent to or less than those

Table 2
In vitro antimicrobial activity of compounds **1**–**3** from *Symphonia globulifera*

Compound	Micro-organism tested			
	S. aureus[a]	*B. subtilis*[b]	*V. anguillarium*	*E. coli*
CH_2Cl_2-MeOH (1:1) extract	100	100	100	i$^+$
MeOH extract	500	500	500	i$^+$
Globulixanthone C	14.05	8.24	–	i$^+$
Globulixanthone D	08.0	12.5	i$^+$[a]	i$^+$
Globulixanthone E	4.51	3.12	5.56	i$^+$
Streptomycin sulfate	6.25	0.85	4.12	i$^+$

[a] Inactive.

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

demonstrated by streptomycin. But none of these compounds was active (MIC > 100) against Gram-negative bacterium, *E. coli*.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Buchi apparatus and are uncorrected. Silica gel, 230–400 Mesh (Merck) and silica gel 70–230 Mesh (Merck) were used for flash and column chromatographies, respectively, while precoated aluminium sheets silica gel 60 F_{254} (Merck) were used for TLC with the mixture of *n*-hexane-ethylacetate as eluent; spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4. IR spectra were measured on a JASCO FTIR-300E spectrometer with KBr pellets. UV spectra were recorded on a Kontron-Uvikon 932 spectrophotometer. NMR spectra were run on a Bruker instrument equipped with a 5 mm ^1H and ^{13}C probe operating at 300 and 75 MHz, respectively with TMS as internal standard.

3.2. Plant material

Root bark of *Symphonia globulifera* 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.

3.3. Extraction and isolation

Air dried powdered root bark of *S. globulifera* (6 kg) was extracted successively with a mixture of CH_2Cl_2–MeOH (1:1) and MeOH at room temperature during 24 h. Both extracts were concentrated under reduced pressure to yield brown viscous mass of CH_2Cl_2–MeOH (1:1) extract (70 g) and a sticky residue of methanol extract (120 g). These two extracts were evaluated in vitro for their antimicrobial activity and potency against a range of representative Gram-positive (*S. aureus*, *B. subtilis*, *Vibrio anguillarum*) and Gram-negative (*E. coli*) bacteria. As a result, MIC values of the CH_2Cl_2–MeOH (1:1) and MeOH extracts, when assayed against Gram-positive representative were 50 and 500 μg/ml, respectively. But, against representative a Gram-negative, *E. coli* bacterium, no activity was observed. The crude CH_2Cl_2–MeOH (1:1) extract (70 g) was then subjected to flash column chromatography over silica gel 60 (70—230 mesh, ASTM; Merck) eluting with cyclohexane–EtOAc mixture with increasing polarity. A total of 30 fractions (of ca 500 ml each were collected and combined on the basis of TLC analysis leading to three main series A–C. Fractions 1–10, eluted with a mixture of C_6H_{12}–EtOAc (17:3) gave series A (15 g); series B (20 g) was constituted of fractions 11–20 eluted with a mixture of cyclohexane–EtOAc (1:1) while series C (19 g) resulted from fractions 22–30 eluted with cyclohexane–EtOAc (1:3).

Series B was column chromatographed over silica gel packed in cyclohexane. Gradient elution was effected with cyclohexane–EtOAc mixtures. A total of 75 fractions of ca 50 ml each were collected and combined on the basis of TLC. Fractions 1–15, eluted with a mixture of cyclohexane–EtOAc (9:1), showed on TLC one spot. They were combined and evaporated to yield globulixanthone C (**1**) (200 mg). Fractions 20–31, eluted with cyclohexane–EtOAc (4:9) also contained one compound. These fractions were combined and evaporated to yield globulixanthone D (**2**) (70 mg). Series C, on repeated column chromatographic separations, eluted with the mixture of cyclohexane–EtOAc with increasing polarity, afford globulixanthone E (**3**) (50 mg).

3.4. 1,7,8-Trihydroxy-2,2-dimethylpyrano[5′,6′: 3,4]xanthone (globulixanthone C) (*1*)

Yellow needles, mp 285 °C; UV λ_{max}^{MeOH}nm (logε): 204 (4.28), 219 (3.76), 267 (3.78), 295 (4.03) and 337 (4.46); IR ν_{max}^{KBr} cm^{-1}: 3454, 3112, 2967, 1664, 1617, 1505, 1492, 1347, 1387, 1268, 1196; ^1H NMR (acetone-d_6), see Table 1, ^{13}C NMR (acetone-d_6), see Table 1; HREIMS *m*/*z* 326.0786 (calc. for $C_{18}H_{14}O_6$, 326.0790); EIMS *m*/*z* (rel. int): 326$[M]^+$ (90), 311 (100), 159 (21), 152 (18), 114 (36).

3.5. 1,6-Dihydroxy-5-methoxy-7-(3-methylbut-2-enyl)xanthone (globulixanthone D) (*2*)

Yellow crystals, mp 120 °C; UV λ_{max}^{MeOH}nm (logε) 203 (3.77), 234 (4.01), 247 (4.00), 313 (3.63) and 356 (3.57); IR ν_{max}^{KBr} cm^{-1}: 3428, 3250, 2934, 1664, 1650, 1624, 1466, 1394, 1300, 1242, 1085; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 1; HREIMS *m*/*z* 325–1150 (calc. for $C_{19}H_{18}O_5$, 326.1154); EIMS *m*/*z* (rel. int.): 326$[M]^+$(100), 283 (48), 271 (48), 234 (15), 191 (38), 179 (27), 92 (39).

3.6. Globulixanthone E (*3*)

Pale yellow amorphous powder, mp 228 °C; UV λ_{max}^{MeOH}nm (logε) 203 (4.42), 235 (4.38), 253 (3.46), 294 (4.44) and 327 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3248, 2934, 1668, 1648, 1560, 1465; 1387, 1365, 1212, 1108, ^1H NMR (acetone-d_6) see Table 1; ^{13}C NMR (acetone-d_6), see Table 1; HRESY-TOF *m*/*z* 619.1966 $[M+H]^+$ (calc. for $C_{37}H_{31}O_9$, 619.1968).

3.7. Antimicrobial assay

The extracts and purified active principles from *S. globulifera* were tested against micro-organisms, *Staphylococcus*

aureus (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Vibrio anguillarium* (ATCC 19264) and *Escherichia coli* (ATCC 8739). The qualitative antimicrobial assay employed, was the classic agar disc diffusion procedure using Mueller Hinton agar (DIFCO). Paper discs were impregnated with 20 μl of the DMSO solution of each sample (1 mg/ml) and allowed to evaporate at room temperature. Streptomycin sulfate (20 μl of a 1 mg/ml solution) was used as standard positive control. The plates with *B. subtilis* were incubated at 30 °C, while the other were incubated at 37 °C for 18 h and the diameter of the zone inhibition around each disc measured and recorded at the end of the incubation period. As the sensitivity of the disc bioassay is low, final activity was performed using a minimum inhibitory concentration (MIC) method. The MIC values were determined by the standard broth micro-dilution method in Mueller-Hinton, with an inoculum of 10^4 CFμ/ml. To ensure that the densities of the diluted cultures were within the range, serial dilution plate counts were also made for each culture. Tube inoculated with *B. subtilis* were incubated at 30 °C while tubes inoculated with others micro-organisms. The MIC values were determined after 24 h of incubation. Streptomycin sulfate was also used as positive control in the assay system.

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