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Anthraquinones from the stem bark of *Stereospermum zenkeri* with antimicrobial activity

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Abstract

Two anthraquinones, zenkequinones A and B were isolated from the stem bark of *Stereospermum zenkeri* together with known sterequinone-F, *p*-coumaric acid, sitosterol-3-O-β-D-glucopyranoside and 3β-hydroxyolean-12-en-28-O-β-D-glucopyranoside. Their structures were established by spectroscopic methods. The antimicrobial activity of the isolated compounds was evaluated against six multiresistant strains of pathogens. Zenkequinone B showed the best antibacterial activity (MIC 9.50 μg/ml) against gram-negative *Pseudomonas aeruginosa*.

Keywords: *Stereospermum zenkeri*; Bignoniaceae; Zenkequinones A and B; Antimicrobial

1. Introduction

Stereospermum zenkeri K. Schum. ex De Wild (Bignoniaceae) is a medium size plant, native to Africa which grows to a height of about 10 m (Burtt et al., 1984). In Cameroon, its bark is used in traditional medicine against bronchitis; its roots and leaves are used to cure fever and microbial infections. To the best of our knowledge, no phytochemical study has been carried out on this species. Several species from Bignoniaceae family are well known for their antimicrobial, antiprotozoal and anti-inflammatory properties (Binutu and Lajubutu, 1994; Binutu et al., 1996). Previous studies on plants of *Stereospermum* genus have led to the isolation of several bioactive compounds such as anthraquinones and lignans (Kumar et al., 2003, 2005; Onegi et al., 2002; Ghogomu et al., 1985; Kanchanapoom et al., 2006). As part of our continuing search for biologically active compounds from Cameroonian medicinal plants, we have examined the stem bark of *S. zenkeri* and report here the isolation and structural elucidation of two new anthraquinones, zenkequinone A (**1**) and B (**2**) as well as the antimicrobial activity of the new and known isolated compounds.

2. Results and discussion

Air-dried and ground stem bark of *S. zenkeri* was extracted successively at room temperature with *n*-hexane,

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EtOAc and MeOH. These extracts were concentrated to dryness under vacuum. The EtOAc extract residue was subjected to repeated column chromatographic separations to yield zenkequinone A (**1**) and B (**2**), sterequinone F (**3**), *p*-coumaric acid (**4**), sitosterol-3-O-β-D-glucopyranoside and 3β-hydroxyolean-12-en-28-O-β-D-glucopyranoside (**5**).

Compound **1**, zenkequinone A, was obtained as a yellow semisolid, reacting positively with Borntrager's test characteristic of anthraquinone derivatives. Its molecular formula, $C_{19}H_{16}O_3$, was deduced from the high resolution electro spray ionisation-time of flight (HR-ESITOF+) mass spectrometry which showed the pseudo molecular ion $[M+H]^+$ at *m*/*z* 293.3012, corresponding to 12 double-bond equivalents. The IR spectrum displayed absorptions at 1667 and 1619 cm^{-1} due to carbonyl groups. Its UV spectrum exhibited maxima at 204, 260 and 335 nm. The ^1H NMR spectrum (Table 1) revealed two sets of overlapping multiplets integrating for two protons each at δ_H 8.20–8.25 and 7.72–7.81, indicating a typical 1,2-disubstituted aromatic ring; a singlet at δ_H 8.20 attributed to H-11. Two multiplets at δ_H 2.90, 3.08 with a total integral of 2 protons, and a multiplet at 2.30 (2H) indicated the presence of two adjacent methylene groups (H-3 and H-2) and two singlets at δ_H 2.67 (3H) and 1.64 (3H) indicated two methyl groups. The presence of two adjacent methylene groups was confirmed by the 2D COSY ^1H NMR spectra. The above NMR values are similar to those of sterequinone C (**1a**) isolated from *Stereospermum personatum* (Kumar et al., 2003). The ^{13}C NMR spectrum (Table 1) revealed 19 carbon signals which were sorted by Jmod and HSQC techniques into two methyl, five methine, two methylene and 10 quaternary carbons including two carbonyl groups at δ_C 184.9 and 183.2, and a carbinol at δ_C 80.9. The differences between compound **1** and sterequinone C (**1a**) were clearly observed in the HMBC spectrum of **1** (Fig. 1), where all the methylene protons correlated with the quaternary carbon at δ_C 80.9 (C-1). The HMBC spectrum of compound **1** also showed correlations between the protons of the methylene group at δ_H 2.90 and 3.08, and C-4 (δ_C 138.2), C-2 (δ_C 40.7), C-1 (δ_C 80.9), C-11a (δ_C 152.9); and the protons of the second methylene group at δ_H 2.30 (H-2) also correlated with C-3 (δ_C 28.8), C-1 (δ_C 80.9), C-11a (δ_C 152.9), and C-12 (δ_C 149.5). Furthermore the singlet at δ_H 8.20 (1H, H-11) showed correlations with

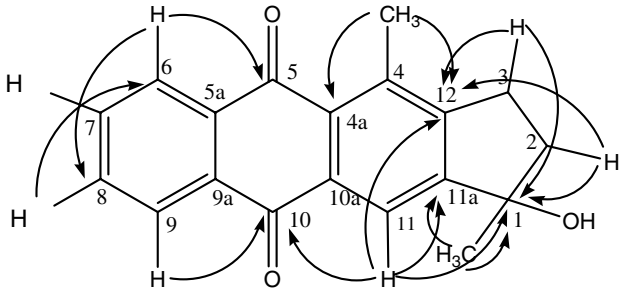

Fig. 1. Selected HMBC correlations for compound **1**.

Table 1
^1H and ^{13}C NMR chemical shift assignments for compounds **1** and **2** in $CDCl_3$

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	80.9	–	43.2	3.38 (1H, *d*, *J* = 19.0 Hz) 3.57 (1H, *d*, *J* = 19.0 Hz)
2	40.7	2.30 (2H, *m*)	68.6	–
3	28.8	2.90 (1H, *m*) and 3.08 (1H, *m*)	33.9	1.90 (2H, *m*)
4	138.2	–	27.6	2.93 (1H, *m*) and 3.24 (1H, *m*)
4a	130.8	–	144.2	–
5	184.9	–	134.4	7.53 (1H, *d*, *J* = 8.1 Hz)
5a	133.6	–	–	
6	126. 8	8.20–8.25 (1H, *m*)	125.3	8.15 (1H, *d*, *J* = 8.1 Hz)
6a	–	–	131.7	–
7	132.9	7.72–7.81 (1H, *m*)	183.5	–
7a	–	–	135.0	–
8	132.3	7.72–7.81 (1H, *m*)	126.5	8.17–8.22 (1H, *m*)
9	126. 2	8.20–8.25 (1H, *m*)	134.0	7.70–7.77 (1H, *m*)
9a	133.5	–	–	–
10	183.2	–	133.4	7.70–7.77 (1H, *m*)
10a	134.5	–	–	–
11	119.4	8.20 (1H, *s*)	127.1	8.17–8.22 (1H, *m*)
11a	152.9	–	131.5	–
12	149.5	–	185.7	–
12a	–	–	132.6	–
13	–	–	138.4	–
1-CH_3	27.1	1.64 (3H, *s*)	–	–
2-CH_3	–	–	30.3	1.50 (3H, *s*)
4-CH_3	18.1	2.67 (3H, *s*)	–	–

C-1 (δ_C 80.9), C-4a (δ_C 130.8) and C-12 (δ_C 149.5). All these findings clearly indicated that the hydroxyl group was linked to C-1. This was confirmed by the correlations observed between the protons of C-1 methyl group at δ_H 1.64 and C-1 (δ_C 80.9), C-11a (δ_C 152.9) and the C-4 methyl protons at δ_H 2.67 and C-4a, C-12 and C-4. The above data and other correlations shown in Fig. 1 led to structure **1** for zenkequinone **A**.

Compound **2**, zenkequinone B, was isolated as a yellow powder, m.p. 202.6–203.0 °C. Its molecular formula, $C_{19}H_{16}O_3$, could be deduced from its HR-ESITOF+ mass spectrum which showed a pseudo molecular ion $[M+Na]^+$ at m/z 315.2981 corresponding to 12 double-bond equivalents. The IR spectrum displayed absorption bands at 1660 and 1651 cm^{-1} due to carbonyl groups. Its UV spectrum exhibited maxima at 207, 256 and 338 nm. Compound **2** gave a positive Borntrager's test characteristic of anthraquinone derivatives.

The ^{13}C NMR spectrum (Table 1) revealed 19 carbon signals that were sorted by DEPT and Jmod experiments into one methyl and three methylene groups, six sp^2 methine and nine quaternary carbons including two carbonyls at δ_C 185.7 and 183.5, and a carbinol at δ_C 68.6. The ^1H NMR spectrum (Table 1) of compound **2** revealed two sets of overlapping multiplets integrating for two protons each at δ_H 8.17–8.22 and 7.70–7.77, indicating a typical 1,2-disubstituted aromatic ring. In addition, it displayed two doublets at δ_H 8.15 (1H, *d*, $J = 8.1$ Hz) and 7.53 (1H, *d*, $J = 8.1$ Hz), suggesting the presence of two ortho-coupled aromatic protons (H-6 and H-5); two multiplets at δ_H 2.93 and 3.24 with a total integral of 2 protons, and a multiplet at δ_H 1.90 (2H) indicated the presence of two adjacent methylene groups (H-3 and H-4). Two doublets at δ_H 3.57 (1H, *d*, $J = 19.0$ Hz) and 3.38 (1H, *d*, $J = 19.0$ Hz) corresponded to two geminal methylene protons (H-1), whose downfield shift could be due to its proximity both to carbonyl and to hydroxyl groups; and one singlet at δ_H 1.50 (3H) accounted for the single methyl group of compound **2**. All these data suggested that the aromatic moiety of compound **2** was similar to that of known strerequinone F (**3**) and so the last unsaturation in **2** was due to an additional cycle. Thus, compound **2** might be a cyclic derivative of compound (**3**). Further confirmation was obtained from HMBC and NOESY spectra. In fact, in the HMBC spectrum of **2** (Fig. 2), methylene protons at δ_H 3.57 (H-1) and 3.38 (H-1′) showed cross-peaks with C-12a (δ_C 132.6), C-2 (δ_C 68.6), C-13 (δ_C 138.4), C-3 (δ_C 33.9), C-4a (δ_C 144.2); and the methyl singlet at δ_H 1.50 (CH_3) correlated with C-1 (δ_C 43.2), C-2 (δ_C 68.6) and C-3 (δ_C 33.9). In the same spectrum, the methylene protons at δ_H 1.90 (*m*) were correlated with C-2, C-3, C-4, C-4a and those at 2.93 (*m*) and 3.24 (*m*) with C-2, C-4a, C-5 (δ_C 134.4), and C-13. All these findings clearly indicate that the compound **2** is presumably a product of the angular cylisation of sterequinone F (**3**). The above data and other correlations shown in Fig. 2 led to the structure **2** for zenkequinone B.

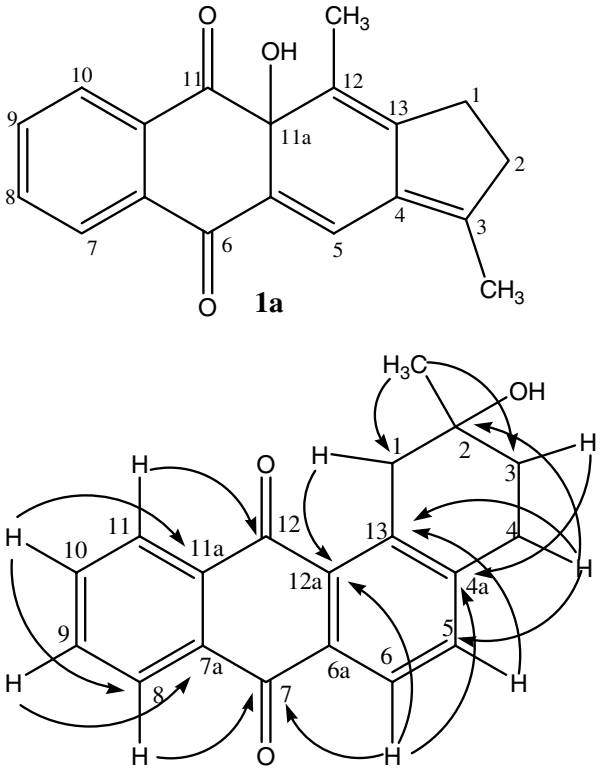

Fig. 2. Selected HMBC correlation for compound **2**.

In addition to **1** and **2**, four known compounds were isolated and characterized as sterequinone-F (**3**), *p*-coumaric acid (**4**), sitosterol 3-O-β-D-glucopyranoside and 3β-hydroxyolean-12-en-28-O-β-D-glucopyranoside (**5**) (Kumar et al., 2005; Ralph et al., 1994; Clifford et al., 2006; Srivastava and Jain, 1989).

Compounds **1**–**4** were tested *in vitro* for their antimicrobial activity. The MIC and MBC obtained with these molecules indicated that they possessed antimicrobial properties (see Table 2). Zenkequinone B (**2**) demonstrating the best activity (MIC 9.50 μg/ml) against gram-negative *Pseudomonas aeruginosa*. The antimicrobial activities of quinones may be linked to their properties to complex irreversibly with nucleophilic amino acids in proteins often leading to inactivation of the protein and loss of biological function (Cowan, 1999).

3. Experimental

3.1. General methods

Melting points were determined on Buchi melting point apparatus B-545. Ultra-violet (UV) spectra were measured on a CARY 100 Bio UV–visible spectrometer. IR spectra were obtained with a Nicolet 5-sxc-FTIR spectrometer. NMR spectra were recorded 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.

Table 2
Minimum inhibitory concentration and minimum bactericidal concentration of compounds **1**–**4** and ampicillin in μg/ml

Microbial strains	**1**		**2**		**3**		**4**		Ampicillin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	37.50	75.00	18.75	37.5	nt	–	nt	–	0.40	0.80
Pseudomonas aeruginosa	18.75	37.50	9.50	18.75	nt	–	nt	–	0.80	1.60
Proteus vulgaris	37.50	75.00	37.50	37.5	nt	–	75.00	75.00	0.40	0.80
Staphylococcus aureus	nt	–	nt	–	nt	–	37.50	75.00	0.80	1.60
Bacillus subtilis	37.50	75.00	37.50	75.50	37.50	75.00	18.75	37.50	1.60	3.20
Bacillus megaterium	37.50	75.00	18.75	37.50	18.5	37.50	18.75	37.50	1.60	3.20

nt: not tested; –: not determined

Silica gel 230–400 mesh (Merck) and silica gel 70–230 mesh (Merck) were used for flash and column chromatography, while precoated aluminium silica gel 60 F254 sheets were used for TLC with a mixture of cyclohexane and EtOAc as eluents; spots were visualised under UV lamps (254 and 365 nm) or by spraying MeOH–H_2SO_4 reagent followed by the heating of the plate at 105 °C during 10 min.

3.2. Plant material

The stem bark of *S. zenkeri* was collected in October 2005 at Mont Eloumdem (Yaoundé) in the centre province of Cameroon. The plant was identified by Mr. Nana Victor, botanist at the National Herbarium of Cameroon where a voucher specimen (No. 1022/SRFK) has been deposited.

3.3. Extraction and isolation

The air-dried powdered stem bark of *S. zenkeri* (2.8 kg) was extracted successively with *n*-hexane, EtOAc and MeOH at room temperature. The extracts were concentrated to dryness to obtain a viscous residue of 12.0, 42.0 and 194.0 g from hexane, EtOAc and MeOH extracts, respectively. Forty grams of the EtOAc extract residue was subjected to flash column chromatography over silica gel (230–400 mesh) as stationary phase eluted with hexane–EtOAc mixtures of increasing polarity. Fifty-nine fractions of 400 ml each were collected and combined on the basis of TLC analysis to yield three main fractions labelled S1 (11 g), S2 (12 g) and S3 (14 g). Fraction S1 (11 g) was essentially an oil.

Fraction S2 (12 g) was subjected to column chromatography over silica gel (70–230 mesh) eluting with a cyclohexane–EtOAc gradient of increasing polarity and resulted in the collection of 90 fractions of 150 ml each, which were combined on the basis of TLC analysis. Sub-fractions S_2A (17–23), eluted with a mixture of cyclohexane–EtOAc (9:1), afforded sterequinone F (250 mg). Sub-fractions S_2B (24–35), eluted with a mixture of cyclohexane–EtOAc (85:15), afforded zenkequinone A (53 mg).

Fraction S3 (14 g) was subjected to column chromatography over silica gel (70–230 mesh) eluting with cyclohexane–EtOAc (80:20–50:50). The subfractions eluted at 20% and 40% cyclohexane–EtOAc mixtures were concentrated and rechromatographed using silica gel to obtain zenkequinoneB (23 mg), *p*-coumaric acid (400 mg), sitosterol-3-O-β-D-glucopyranoside (72 mg) and 3β-hydroxy-olean-12-en-28-O-β-D-glucopyranoside (15 mg).

3.4. Zenkequinone A (*1*)

Yellow semisolid, $[\alpha]_D^{25} + 10.4$ (acetone; $c = 0.067$). UV: λ_{max}^{MeOH} nm (log ε) 204 (4.12), 260 (4.38) and 335 (3.76). ^1H (300 MHz, $CDCl_3$) NMR and ^{13}C (75 MHz, $CDCl_3$) NMR: see Table 1. EIMS: *m/z* (rel. int.): 292 (M^+, 30), 277 (21), 274 (100), 246 (11), 235 (16). +HR ESI-TOF-MS *m/z*: 293.3012 $[M+H]^+$ (Calcld. 293.3324 for $C_{19}H_{17}O_3$).

3.5. Zenkequinone B (*2*)

Yellow powder, m.p. 202.6–203 °C $[\alpha]_D^{25} - 236$ (acetone; $c = 0.050$); UV: λ_{max}^{MeOH} nm (log ε) 207 (4.12), 256 (4.16) and 338 (4.76). ^1H (300 MHz, $CDCl_3$) NMR and ^{13}C (75 MHz, $CDCl_3$) NMR: see Table 1. EIMS: *m/z* (rel. int.): 292 (M^+, 23), 277 (100), 105 (49), 91 (21). +HR ESI-TOF-MS *m/z* 315.2981 $[M+Na]^+$ (Calcld. 315.3177 for $C_{19}H_{16}O_3Na$).

3.6. Antimicrobial assay

Seven bacterial species, including Gram positive (*S. aureus*, *B. subtilis*, *B. megaterium*) and Gram negative (*E. coli*, *P. aeruginosa*, *P. vulgaris*) were used. These strains were clinical isolates from patients obtained from 'Centre Pasteur du Cameroun' and presenting multiresistance to commonly used antibiotics. They were maintained on agar slant at 4 °C in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) where the antimicrobial tests were performed. The strains were incubated at 37 °C for 24 h on nutrient agar (NA), prior to any screening. The Mueller Hinton Agar (MHA) and the nutrient broth (NB) were used for the antimicrobial assays.

For the screening, a method described by Berghe and Vlietinck (1991) was used. The minimum inhibitory concentration (MIC), considered as the lowest concentration of the sample which inhibits the visible growth of microbe, was determined by the microbroth dilution method (Car-

bonnelle et al., 1987) in Mueller Hinton Broth supplemented with 5% glucose and 0.05% phenol red.

For minimum bactericidal concentration (MBC) determination, the content of each tested tube showing no decoloration was used to inoculate by flooding fresh antimicrobial free MHA. This was further incubated for 18–24 h at 37 °C, after which the lowest dilution that yield no growth was considered as the MBC (Mims et al., 1993).

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