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Antiplasmodial activity of naphthoquinones and one anthraquinone from *Stereospermum kunthianum*☆

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Abstract

A lipophilic extract of the root bark of *Stereospermum kunthianum* revealed antiplasmodial activity in vitro. Bioassay-guided fractionation led to the isolation of four novel naphthoquinones (sterekunthals A and B, pyranokunthones A and B) and one novel anthraquinone (anthrakunthone) together with the known naphthoquinone pinnatal. The structures of the novel compounds were determined by comprehensive analyses of their 1D and 2D NMR data. The antiplasmodial activities and toxicity against the endothelial cell line ECV-304 of the isolated compounds have been assessed. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Stereospermum kunthianum*; Bignoniaceae; Naphthoquinones; Anthraquinone; Antiplasmodial activity

1. Introduction

Malaria still is one of the biggest health problems in Tropical Africa. It is estimated that two to three million people die from this parasitic disease every year (WHO, 1997). The situation is even worsened due to a spreading resistance of *Plasmodium falciparum* to standard antimalarials as chloroquine (Peters, 1998). In Africa, the use of indigenous plants still plays an important role in malaria treatment (Gessler et al., 1994; Benoit-Vical et al., 1998). These plants might be an interesting source for the detection of novel antiplasmodial compounds.

The Bignoniaceae (Trumpet Creeper Family) are known for their antimicrobial, antiprotozoal, and anti-inflammatory properties (Binutu et al., 1996). They are mostly tropical trees or shrubs comprising about 100 genera with 800 species. The root bark of *Stereospermum kunthianum* Cham. is a valued remedy of certain tribes in Uganda to treat fever. This prompted us to evaluate the in vitro antiplasmodial activity of this species. We report here the isolation and structure elucidation of four new naphthoquinones (sterekunthal A [**2**], sterekunthal B [**3**], pyranokunthone A [**5**], pyranokunthone B [**6**]) and one novel anthraquinone (anthrakunthone [**4**]) together with pinnatal, a naphthoquinone previously isolated from *Kigelia pinnata* DC. (Joshi et al., 1982). IC_{50} values of all compounds have been determined against two different strains of *P. falciparum*. The cytotoxicity was also assessed, taking into account that naphthoquinones as well as anthraquinones have been reported to exhibit strong anti-proliferative properties on mammalian cells (Rao and Kingston, 1982).

2. Results and discussion

Extracts of different polarity of the root bark of *S. kunthianum* were tested for their in vitro activity against

☆ Part 6 in the series "Herbal remedies traditionally used against malaria", for part 5 see Kraft et al. (2001).

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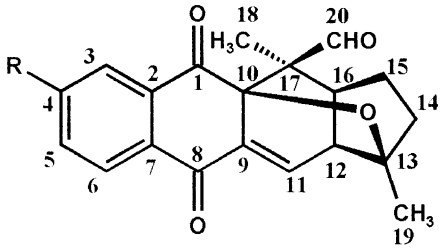

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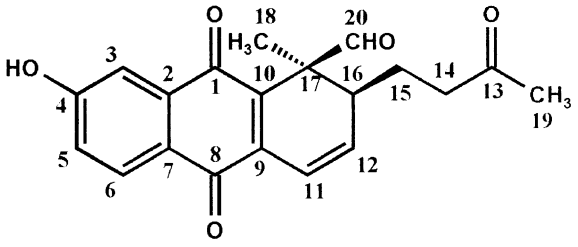

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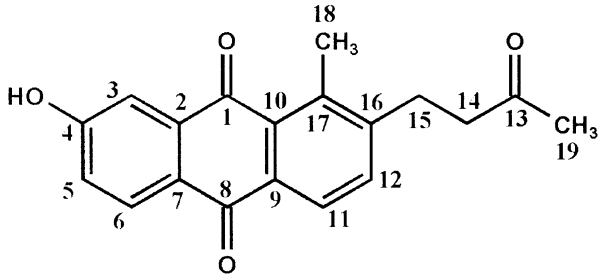

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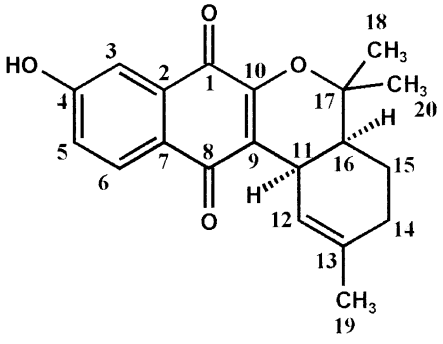

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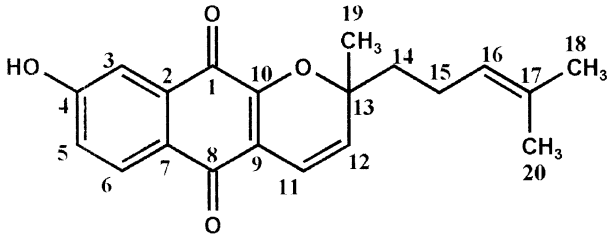

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P. falciparum. A lipophilic extract (petrol ether–EtOAc 1:1) displayed good antiplasmodial activity (Table 3). Bioassay-guided fractionation of this extract led to the isolation of a number of naphthoquinones and one anthraquinone.

The NMR data of the main constituent (**1**) were quite similar to those reported for isopinnatal from *K. pinnata* (Akuniyli and Houghton, 1993), nevertheless the position of the hydroxy group could be unambiguously deduced from the HMBC spectrum, revealing **1** to be the isomeric pinnatal (Joshi et al., 1982). Compound **3** gave a molecular ion peak of *m/z* 322 in the EIMS spectrum, corresponding to a molecular formula of $C_{20}H_{18}O_4$ which is a difference of 16 units compared to **1**. The ^{1}H NMR spectrum (Table 1) displayed signals for a 1,2-disubstituted instead of a 1,2,4-tri-substituted aromatic ring, whereas the remaining signals were similar to those of **1**, suggesting **3** to be its 4-deoxy derivative (sterekunthal B).

Compound **2** showed again a molecular ion peak of *m/z* 338, having an identical molecular composition as **1** ($C_{20}H_{18}O_5$). In addition, the same prominent fragments at *m/z* 252 and 239 (base peak) could be observed, which could be explained by loss of three or all four carbons of the side-chain and the aldehyde function at C-17, respectively. The ^{1}H NMR (Table 1) spectral data revealed the presence of an aldehyde moiety and the same aromatic system as in **1**. In addition, it displayed two olefinic signals at δ 6.95 (*d*, 9.5) and 6.44 (*dd*, 6.0, 9.5), suggesting a *cis*-configurated double bond with one vicinal proton. Of the two methyl groups, one was shifted downfield, thus hinting to a methylketone substructure,

Table 1
^{1}H NMR spectral data of compounds **2–6** in $CDCl_3$

Position	2	3	4	5	6
3	7.49 *d* (2.5)[a]	8.15 *dd* (8.0, 1.5)[b]	7.58 *d* (2.5)	7.40 *d* (2.5)	7.52 *d* (2.5)
4	–	7.76 *td* (8.0, 1.5)[c]	–	–	–
5	7.16 *dd* (8.5, 2.5)	7.82 *td* (8.0, 1.0)[c]	7.16 *dd* (8.5, 2.5)	7.04 *dd* (2.5, 8.0)	7.14 *dd* (2.5, 8.5)
6	7.99 *d* (8.5)	8.28 *dd* (1.0, 8.0)[b]	8.11 *d* (8.5)	7.89 *d* (8.0)	8.01 *d* (8.5)
11	6.95 *d* (9.5)	7.88 *d* (7.0)	8.11 *d* (8.0)	3.45 *brs*	6.68 *d* (10.0)
12	6.44 *dd* (6.0, 9.5)	2.96 *dd* (4.0, 7.0)	7.52 *d* (8.0)	6.03 *d* (2.0)	5.67 *d* (10.0)
14	2.49 *m*	1.77 *dd* (2.0, 11.0)	2.77 *t* (7.5)	1.92 *m*	1.71 *m*
		1.85–2.05 *m*		1.22 *m*	1.92 *m*
15	1.85 *m*	2.28 *ddd* (6.0, 8.0, 14.0)	3.06 *t* (7.5)	1.89 *m*	1.92 *m*
		1.85–2.05 *m*		1.22 *m*	2.10 *m*
16	2.49 *m*	1.85–2.05 *m*	–	1.70 *m*	5.07 *t* (7.0)
18	1.29 *s*	0.68 *s*	2.72 *s*	1.48 *s*	1.63 *s*[d]
19	2.13 *s*	1.19 *s*	2.20 *s*	1.60 *s*	1.50 *s*
20	9.95 *s*	10.27 *s*	–	1.25 *s*	1.55 *s*[d]

[a] Values in parentheses are J = Hz.
[b] Interchangeable.
[c] Interchangeable.
[d] Interchangeable.

Table 2
^{13}C NMR spectral data and HMBC correlations for compounds **2** and **4–5** in $CDCl_3$

	2		4		5	
C	C	HMBC	C	HMBC	C	HMBC
1	185	3	187	3	181	3
2	135	6	138	6	134	3, 6
3	113	5	113	–	113	5
4	163	6	162	6	161	3, 5, 6
5	122	3	122	–	122	3
6	130	–	130	–	128	–
7	125	3, 5	127	–	127	3, 5, 6
8	182	6, 11	183	6, 11	185	6
9	139	12	134	3, 5, 12	124	16
10	140	11, 16, 18	133	11, 18	n.d.	–
11	120	16	127	–	32	12
12	138	15, 16	135	15	121	19
13	208	14, 19	207	14, 15, 19	137	11, 19
14	42	15, 19	44	15, 19	30	19
15	24	–	29	–	21	–
16	44	11, 12, 14, 18	148	11, 14, 15, 18	41	11, 12, 18, 20
17	49	12, 18, 20	141	12, 15, 18	81	18, 20
18	18	16, 20	18	–	26	20
19	31	–	31	14	24	12
20	204	18	–	–	25	20

which was supported by the signal at δ 208 in the ^{13}C NMR (Table 2). The methyl protons showed long range correlations to the carbonyl and to a methylene group at δ 42, the corresponding protons at δ 2.49 were coupled to another methylene group at δ 1.85. These methylene groups displayed correlations to C-16 and C-12, respectively, in the HMBC spectrum (Table 2), thus establishing the structure of **2** (sterekunthal A) as given. The stereochemistry is assumed to be identical with **1**.

Compound **4** showed a molecular ion peak at m/z 308 in the EIMS spectrum, its molecular formula could be determined as $C_{19}H_{16}O_4$ by HRMS. The ^{1}H NMR spectrum revealed that it possessed the same substituted aromatic ring system as the other isolated quinones but no aldehyde moiety. The two methyl groups were both shifted downfield. One showed again HMBC correlations to a carbonyl group at δ 207 and to a methylene group at δ 44, leading to the same linear substructure as in **3**. The other methyl group coupled long-range to three quarternary carbon atoms at δ 133, 141, and 148, respectively. In addition, two *o*-coupled aromatic protons at δ 8.11 (*d*, 8.0) and 7.52 (*d*, 8.0) could be observed. As H-15 (δ 3.06, *t*, 7.5) which was notably downfield-shifted, displayed HMBC correlations to one of these aromatic carbons (δ 135) as well as to two other aromatic signals (C-16, C-17), the structure of **4**, which we named anthrakunthone, could be established as shown.

Compound **5** displayed a molecular ion peak at m/z 324 in the EIMS spectrum, corresponding to a molecular formula of $C_{20}H_{20}O_4$. The ^{1}H NMR spectrum of **5** again hinted to the same aromatic ring system but on the other hand differed notably from those of the previous substances, showing three instead of two methyl groups. Two of them (δ 1.48 and 1.25) had to be attached to the same carbon atom as could be deduced from the HMBC spectrum. The shift of this carbon in the ^{13}C NMR spectrum (δ 81) hinted to an oxygen substitution. The third methyl group (δ 1.60) turned out to be olefinic. It showed long range correlations to two olefinic carbons at δ 121 and 137, as well as to a methylene group at δ 30. In the ^{1}H–^{1}H-COSY spectrum, this methylene group was coupled to another methylene group, whereas the olefinic proton at δ 6.03 coupled to

Table 3
In vitro activity of extracts and quinones obtained from *Stereospermum kunthianum* against *Plasmodium falciparum* and ECV-304 cell line

Extract/compound	Mean IC_{50} values (μg/ml)±S.D.[a]		
	poW	Dd2	ECV-304
Lipophilic extract	7.0	16.8	–
Pinnatal (**1**)	5.6±0.6	3.6±0.9	2.2±0.3
Sterekunthal A (**2**)	1.3±0.1	0.4±0.1	0.9±0.02
Sterekunthal B (**3**)	23.3±4.2	15.2±1.7	16.0±1.0
Anthrakunthone (**4**)	14.7±0.25	14.7±5.3	7.9±0.5
Pyranokunthone A (**5**)	11.7±4.0	>25.0	>200.0
Pyranokunthone B (**6**)	8.9±1.2	7.8±1.3	88.2±4.6
Chloroquine×2 H_3PO_4	0.008±0.002	0.092±0.01	30.0±1.5

[a] Values are given as a mean of 3 experiments±standard deviation.

the methine proton at δ 3.45, which was coupled to another methine proton at δ 1.70. The corresponding carbon atom at δ 41 displayed long-range correlations to the two geminal methyl groups. From these data, the structure of **5** (pyranokunthone A) could be established. A similar tetracyclic naphthoquinone had been synthesized by Bock et al. (1986). In this compound the protons at C-11 and C-16 were *trans*-oriented, showing a coupling constant of 11.0 Hz. As H-11 of **5** displayed only small coupling constants, we assume that in our substance H-11 and H-16 are *cis*-oriented.

The EIMS spectrum of **6** showed again a molecular ion peak at *m*/*z* 324, but a different fragmentation pattern compared to **5**. A base peak at *m*/*z* 241, corresponding to the loss of C_6H_{11} could be observed. The ^1H NMR spectrum showed, in addition to the substituted aromatic ring system, two olefinic methyl groups (δ 1.55 and 1.63) and one olefinic proton at δ 5.07, coupled to a proton of a methylene group at δ 2.10. This was further coupled to another methylene group. This coupling sequence led to a substructure with a molecular composition of C_6H_{11} which was already postulated from the EIMS spectrum. Additionally, one methyl group at δ 1.50 and two olefinic protons belonging to a *cis*-double bond could be observed. Thus, the structure of **6** (pyranokunthone B) could be deduced as shown.

All isolated quinones showed different degrees of activity against two strains of *P. falciparum* (Table 3), with sterekunthal A (**2**) being the most effective one [IC_{50} values: 1.3 μg/ml (PoW); 0.4 μg/ml (Dd2)]. Obviously, the 4-hydroxy group is one structural feature relevant for the antiplasmodial activity of these compounds, as sterekunthal B (**3**) is distinctly less active as pinnatal (**1**). The IC_{50} values are comparable to those of related naphthoquinones isolated from *Kigelia pinnata* DC. (Weiss et al., 2000), another famous African bignoniaceous species. On the other hand, compounds **1–4** also exhibited marked toxicity against endothelial ECV-304 cells (Table 3). Thus, their antiplasmodial effect seems to be due to general cytotoxicity. Compounds **5** and **6** which are only moderately active against *P. falciparum* are much less toxic, displaying selectivity indices (ratio of cytotoxicity over antiplasmodial activity) of about 10.

In conclusion, our results support the ethnobotanical use of *S. kunthianum* but also show that some of its constituents may have adverse side effects on mammalian cells, too.

3. Experimental

3.1. General

For fractionation silica gel 60 (70–230 mesh) was utilised. Preparative high performance liquid chromatography (HPLC) was performed on a Knauer instrument equipped with Eurochrom 2000 on a Nucleosil P 300 C-18 (10 μm) reversed-phase column. EIMS were recorded on a Finnigan MAT CH7A (70 eV); FAB MS and HR–EIMS on a Finnigan MAT 711 (80 eV). ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, HMQC and HMBC spectra were obtained on a Bruker AVANCE DPX 400 MHz or a Bruker DRX 500 MHz spectrometer (TMS as int. standard; $CDCl_3$ as solvent).

3.2. Plant material

Root bark of *S. kunthianum* was collected near Pacego (Nebbi District, Uganda) in the dry season. A voucher specimen is deposited at the Makerere University Herbarium (001 BO).

3.3. Extraction and isolation

Ground root bark (690 g) of *S. kunthianum* was extracted with 3×2 l petrol–EtOAc 1:1 each at room temperature. The solvent was evaporated under reduced pressure at 40 °C and the residue (3.2 g) subjected to CC over silica gel. It was eluted with increasingly polar mixtures of cyclohexane–EtOAc and MeOH to afford 6 fractions. Fraction 3 which eluted with 30% EtOAc in cyclohexane was further fractionated by preparative

reversed-phase HPLC (MeOH–H_2O 45:55 to 80:20 in 80 min, flow rate = 5.0 ml/min) to afford **3** (29 mg), **5** (17.5 mg) and **6** (40 mg). Fraction 5 which eluted with 30% cyclohexane in EtOAc was again purified by prep. reversed-phase HPLC (MeOH–H_2O 35:65 to 70:30 in 60 min, flow rate = 5.0 ml/min) and yielded **1** (100 mg), **2** (43 mg), and **4** (40 mg).

3.4. Pinnatal (*1*)

Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, *s*, H-18), 1.18 (3H, *s*, H-19), 1.73 (1H, *m*, H-14a), 1.84–2.04 (3H, *m*, H-14b, H-15a, H-16), 2.26 (1H, *ddd*, $J = 6$, 8, 14 Hz, H-15b), 2.94 (1H, *dd*, $J = 4$, 7 Hz, H-12), 7.28 (1H, *dd*, $J = 2.5$, 8.5 Hz, H-5), 7.54 (1H, *d*, $J = 2.5$ Hz, H-3), 7.83 (1H, *d*, $J = 7$ Hz, H-11), 8.22 (1H, *d*, $J = 8.5$ Hz, H-6), 10.26 (1H, *s*, H-20) ; ^{13}C NMR (125 MHz, acetone-d_6): δ 19.1 (*q*, C-18), 24.5 (*q*, C-19), 26.4 (*t*, C-15), 38.3 (*t*, C-14), 47.6 (*d*, C-16), 50.8 (*d*, C-12), 57.0 (*s*, C-17), 79.2 (*s*, C-10), 85.5 (*s*, C-13), 113.2 (*d*, C-3), 124.0 (*d*, C-5), 128.6 (*s*, C-7), 131.3 (*d*, C-6), 138.2 (*s*, C-2), 140.8 (*s*, C-9), 142.6 (*d*, C-11), 164.0 (*s*, C-4), 178.0 (*s*, C-8), 194.1 (*s*, C-1), 206 (*d*, C-20); EIMS (80 eV): *m*/*z* (rel. int.) 338 $[M]^+$ (5), 310 (28), 295 (23), 277 (46), 252 (93), 239 (100).

3.4.1. Sterekunthal A (*2*)

Yellow oil. $[\alpha]_D^{20}$ −10° ($CHCl_3$, *c* 0.44); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m*/*z* (rel. int.) 338 $[M]^+$ (4), 320 (13), 310 (11), 295 (11), 277 (80), 252 (93), 239 (100); HREIMS: *m*/*z* 338.1152 (calc. for $C_{20}H_{18}O_5$ 338.1154).

3.4.2. Sterekunthal B (*3*)

Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; EIMS (80 eV): *m*/*z* (rel. int.): 322 $[M]^+$ (10), 294 (15), 279 (16), 242 (38), 236 (32), 225 (100), 165 (18), 81 (35); HREIMS: *m*/*z* 322.1204 (calc. for $C_{20}H_{18}O_4$ 322.1205).

3.4.3. Anthrakunthone (*4*)

Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m*/*z* (rel. int.) 308 $[M]^+$ (24), 293 (62), 290 (17); (+)-FABMS: *m*/*z* 309 $[M+H]^+$; HREIMS: *m*/*z* 308.1099 (calc. for $C_{19}H_{16}O_4$ 308.1049).

3.4.4. Pyranokunthone A (*5*)

Yellow solid. $[\alpha]_D^{20}$ −38° ($CHCl_3$, *c* 0.04); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; EIMS (80 eV): *m*/*z* (rel. int.) 324 $[M]^+$ (100), 309 (47), 281 (22), 121 (15); HREIMS: *m*/*z* 324.1362 (calc. for $C_{19}H_{16}O_4$ 324.1352).

3.4.5. Pyranokunthone B (*6*)

Orange oil. $[\alpha]_D^{20}$ 0° ($CHCl_3$, *c* 0.2); ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1; EIMS (80 eV): *m*/*z* (rel. int.) 324 $[M]^+$ (49), 309 (18), 305 (34), 281 (12), 241 (100), 121 (24); HREIMS: *m*/*z* 324.1362 (calc. for $C_{19}H_{16}O_4$ 324.1343).

3.5. In vitro antiplasmodial activity

The bioassay was performed as described earlier (Kraft et al., 2000). The chloroquine-sensitive strain poW (IC_{50} for chloroquine×2 H_3PO_4 = 0.015 μM) and the chloroquine-resistant clone Dd2 (IC_{50} for chloroquine×2 H_3PO_4 = 0.14 μM) of *Plasmodium falciparum* were maintained in continuous culture as described by Trager and Jensen (1976). The antiplasmodial assay was carried out according to the method of Desjardins et al. (1979) and activities were determined by a ^{3}H-hypoxanthine-incorporation assay.

3.6. In vitro cytotoxicity assay

The cytotoxicity of the substances was estimated by a proliferation assay using the MTT-assay (Mosmann, 1983). Test substances were dissolved in acetone and diluted with medium to the desired concentrations. Human endothelial cells (ECV-304) were cultivated in Eagle Medium 199 supplemented with 10% fetal calf serum in 96-well plates in an atmosphere of 5% CO_2 at 37 °C in a humidified environment. Endothelial cells were seeded at a density of approximately 1000 cells per well. After 24 h they were supplemented with 100 μl test substance in medium and cultivated for further 4 days. The cell viability was measured by the MTT-assay using DMSO to dissolve the formed purple formazan. The absorbance was quantified at 580 nm with an ELISA plate reader. Data are presented as the mean of 3 independent experiments with 8 parallel samples for each concentration. The IC_{50} values were calculated by linear regression.

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