Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 925–929
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

1-Methyl anthraquinones and their biogenetic precursors from *Stereospermum personatum*

U. Sampath Kumar[a], P. Aparna[a], R. Jagadeeshwar Rao[a], T. Prabhakar Rao[b], J. Madhusudana Rao[a,*]

[a]*Natural Products Laboratory, Division of Organic Chemistry-1, Hyderabad-500 007, India*
[b]*NMR Division, Indian Institute of Chemical Technology, Hyderabad-500 007, India*

Received 1 November 2002; received in revised form 1 November 2002

Abstract

Two novel 1(17)-methyl anthraquinones, sterequinone-A and -D, their biogenetic precursors sterequinone-B, -C, and a new naphthoquinone sterequinone-E along with a known naphthoquinone, sterekunthal-B, have been isolated from the petroleum ether extract of stem bark of *Stereospermum personatum*. Their structures were established using spectroscopic methods.

Keywords: *Stereospermum personatum*; *Bignoniaceae*; Sterequinone-A; Sterequinone-B; Sterequinone-C; Sterequinone-D; Sterequinone-E

1. Introduction

Stereospermum personatum is a well known medicinal plant that is frequently used in Ayurvedic system of medicine for different disease conditions and is one of the ingredients of the popular formulation, Dasamoola (Varier, 1994). It possesses antibacterial, antifungal, hypoglycemic and is active against p338 lymphocytic leukemic cells (Bakuni et al., 1971). The plant belongs to *Bignoniaceae* family, which is known for its antimicrobial, antiprotozoal and antiinflammatory properties (Binutu et al., 1996). Recent report on the antimalarial constituents from *S. kunthianum* prompted us to systematically isolate the constituents from the locally available plant, *S. personatum*. Previous work includes isolation of lapachol (Purushotaman and Natarajan, 1974) and specioside (De Silva et al., 1982) an iridoid glycoside from this plant. Our efforts led to the isolation of two novel anthraquinones, sterequinone-A [1], and sterequinone-D [5], their biogenetic precursors sterequinone-C [4], sterequinone-B [3] and a new naphthoquinone sterequinone-E [6] along with sterekunthal-B [2] which was recently reported from *S. kunthianum* (Onegi et al., 2002).

2. Results and discussion

Column chromatographic separation of petroleum ether extract of stem bark, led to the isolation of five new quinones namely sterequinones-A, -B, -C, -D and -E along with a known naphthoquinone, sterekunthal-B. The structures of all these compounds were established using spectroscopic methods.

Compound **1**, sterequinone-A, was isolated as yellow semisolid. The EIMS spectrum of the compound has shown a molecular ion peak at m/z 274 corresponding to the molecular formula $C_{19}H_{14}O_2$. The IR spectrum has indicated the presence of carbonyl group at 1668 cm^{-1}. The 200 MHz ^1H NMR in $CDCl_3$ and ^{13}C NMR spectrum of this compound have shown general features of substituted anthraquinone system. The ^1H NMR spectrum has shown two multiplets integrating for two protons each at δ 8.25 and 7.75 indicated a typical 1,2-disubstituted aromatic ring. A one proton singlet at δ 8.2 was attributed for H-11. An olefinic proton at δ 6.5 for H-14 was observed as an apparent triplet. The downfield shift of methylene doublet at δ 3.45 indicated its presence in between two double bonds. A singlet

* Corresponding author. Tel.: +91-40-27193166; fax: +91-40-27160512.
E-mail address: janaswamy@iict.ap.nic.in (J.M. Rao).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00128-6

integrating for 3 protons at δ 2.85 indicated the presence of an aromatic methyl group whose presence was also observed in ^{13}C NMR at δ 18.7. The doublet integrating for 3 protons at δ 2.25 revealed the presence of one more methyl group. The ^{13}C NMR spectrum of the compound in $CDCl_3$ indicated the presence of 19 carbons. The DEPT experiment revealed the presence of two methyl, a methylene, 6 methine and 8 quaternary carbons. The ^{13}C NMR values were assigned by comparison with the values of 1-methyl anthraquinone (Berger et al., 1981). The presence of a methyl group at δ 2.25 having a minute coupling constant along with the corresponding signal at δ 13.0 in its ^{13}C NMR spectrum indicated that the second methyl group is an olefinic methyl group. Based on the above data the structure of sterequinone-A was assigned as **1**.

Compound **2**, sterekunthal-B, was isolated as a yellow solid, mp 158 °C. The molecular formula $C_{20}H_{18}O_4$ could be deduced from its positive ion FABMS $(M^+ + H)$ 323. The IR spectrum has indicated the presence of three carbonyl groups at 1711 cm^{-1}, 1682 cm^{-1} and 1616 cm^{-1}. The 200 MHz ^1H NMR spectrum in $CDCl_3$ of this compound has shown identical values to that of sterekunthal-B, which was previously isolated from *S. kunthianum* (Onegi et al., 2002). This is the first occurrence of this compound from this species. The ^{13}C NMR in $CDCl_3$ has indicated the presence of 20 carbons and their assignments have been shown in Table 1.

Compound **3**, sterequinone-B was isolated as dark yellow solid with a melting point of 138 °C. The molecular formula $C_{21}H_{20}O_5$ was deduced from its positive ion FABMS $(M^+ + H)$ 353. The IR spectrum has shown three bands at 1710 cm^{-1}, 1662 cm^{-1} and 1616 cm^{-1} indicating the presence of three carbonyl groups. The 200 MHz ^1H NMR in $CDCl_3$ has displayed that this compound also possesses an aldehyde function (δ 10.22) and similar features as that of pinnatal previously isolated from *Kigelia pinnata* (Joshi et al., 1982). Two doublets at δ 8.22, 7.53 and a double doublet at δ 7.30 all integrating for single protons each have shown the presence of a 1,2,4-trisubstituted aromatic ring. The singlet at δ 3.94 is attributed for an aromatic methoxy group on the ring. It was further confirmed by the corresponding signal at δ 55.9 and downfield shift of C-4 carbon to δ 164.4 in its ^{13}C NMR spectrum. The two methyl groups were observed at similar positions as observed in pinnatal i.e., at δ 0.67 and 1.19 along with a mutiplet at δ 1.88-2.05 integrating for 4 protons. A double doublet at δ 2.93 is attributed for H-12. The position of the methoxy group was established using HMBC experiment in which C-7 has shown correlation with H-3 and H-5. The C-2 carbon has shown correlation with H-6 only. Thus the structure of 3 which was named sterequinone-B could be established as shown.

Compound **4**, sterequinone-C, was isolated as pale yellow solid mp199 °C with a molecular ion peak at *m/z* 292 in its EIMS spectrum corresponding to the molecular formula $C_{19}H_{16}O_3$. IR spectrum has shown two bands at 1669 and 1619 cm^{-1} indicating the presence of two carbonyls. The 200 MHz ^1H NMR spectrum in $CDCl_3$ has displayed similar aromatic pattern as that of compound **2** except for the absence of an aldehyde signal. Two multiplets at δ 8.25 and at 7.75 integrating for two protons were attributed for 4 aromatic protons along with an olefinic signal at δ 8.23. A double multiplet at δ 3.05 and a multiplet at δ 2.3 integrating for two protons each indicating the presence of two adjacent methylene groups. Two methyl groups were observed at δ 2.76 and at 1.65. All the above values, except aromatic signals are in close resemblance with that of kigelinol isolated from the plant *Kigelia pinnata,* which also belongs to *Bignoniaceae* family (Akunyili and Houghton, 1993). The ^{13}C NMR has indicated the presence of 19 carbons. DEPT experiment revealed the presence of five methine carbons in the aromatic region, which confirms that sterequinone-C is a deoxy analogue of kigelinol. Hence the structure of compound **4** was assigned as shown.

Compound **5**, sterequinone-D, was obtained as pale yellow syrup and has shown a molecular ion at *m/z* 304 to the corresponding molecular formula $C_{20}H_{16}O_3$. The 300 MHz ^1H NMR spectrum in $CDCl_3$ displayed similar pattern as that of compound **1** with different aromatic substitution. Two doublets at δ 8.22, 7.65 and a multiplet at 7.23 indicated that it also possesses a 1, 2, 4-trisubstituted aromatic ring. A singlet at δ 3.99 integrating for 3 protons accounts for the presence of a

Table 1
^{13}C NMR spectral data of the compounds **1–4** and **6** ** in $CDCl_3$ (75.462 MHz)

Carbon	**1**	**2**	**3**	**4**	**6**
1	185.6	193.1	193.3	185.3	183.0
2	134.8	134.7	137.2	134.9	135.6
3	127.0	127.7	109.7	126.5	109.9
4	133.8	134.3	164.4	133.9	164.3
5	133.6	135.2	123.0	133.3	123.8
6	126.5	127.8	130.1	127.1	129.6
7	133.02	135.24	128.5	132.7	127.1
8	184.3	178.7	177.9	183.5	182.2
9	136.5	139.8	139.8	153.2	154.3
10	135.2	78.2	78.4	81.2	80.8
11	133.08	142.7	141.5	119.7	121.0
12	150.7	50.5	50.3	144.5	149.6
13	128.2	84.8	84.8	131.2	133.3
14	116.3	37.5	37.5	29.2	29.3
15	38.0	25.8	25.8	41.1	42.0
16	150.3	46.8	46.8	138.6	134.0
17	140.6	56.4	56.5	149.8	96.0
18	18.7	18.7	18.7	18.4	
19	13.0	24.3	24.3	27.4	27.4
20		204.2	204.5		
$-OCH_3$			55.9		55.9

** Numbering is followed as in the latest reference (Onegi et al., 2002).

methoxy substitution on the ring. All the remaining signals were observed at similar positions as in compound 1. All the above values coupled with extra molecular weight indicated that it is a methoxy analogue of sterequinone-A and hence the structure of the compound was deduced as **5**. The presence of both the anthraquinones, sterequinone-A and sterequinone-D was also observed when the bark of the *S. personatum* was extracted with petroleum ether at room temperature.

Compound **6**, sterequinone-E, was isolated as pale yellow syrup with a molecular ion peak at m/z 308, corresponding to the molecular formula $C_{19}H_{16}O_4$ in its EI MS spectrum. The IR spectrum has shown the presence of two carbonyls at 1670 and 1631cm^{-1}. The 400 MHz ^{1}H NMR spectrum in $CDCl_3$ was similar to compound 4 but hinted a tri substituted aromatic ring like sterequinone-B along with a methoxy signal at δ 3.99. A one proton multiplet at δ 8.25, two doublets at δ 7.72 and 7.24 integrating for one proton each are assigned for three aromatic protons. Two olefinic protons observed as singlets at δ 8.23 and 8.14 are attributed for H-17 and H-11. The absence of methyl group at δ 2.76 accounts for the appearance of extra olefinic signal as a singlet at δ 8.23. The ^{13}C NMR spectrum in $CDCl_3$ has clearly indicated the signal at δ 55.9 for the aromatic methoxy group. The olefinic proton at H-11 was further supported by the presence of a methine carbon at δ 96.0 in the ^{13}C NMR spectrum. Thus, the structure of the compound **6** is confirmed as methoxy demethyl analogue of sterequinone-C (**4**). The position of the methoxy group was established by comparing the ^{13}C NMR values with that of compound **2** and on the basis of biogenetic considerations.

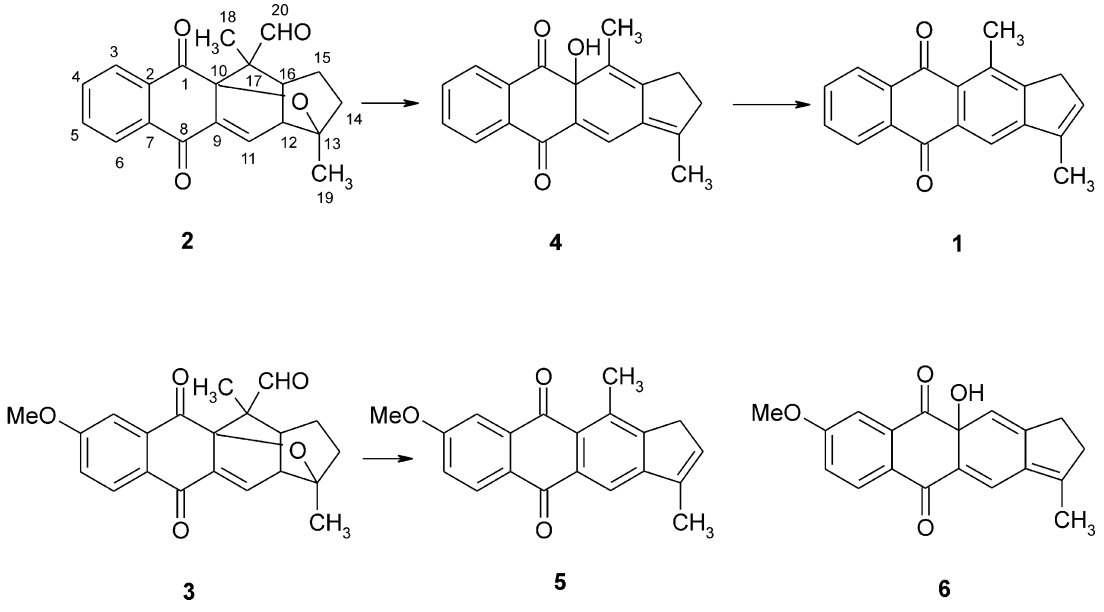
The occurrence of all these compounds is noticed in the room temperature petroleum ether extract of the stem bark indicating that none of them are artifacts.

Biogenetic consideration: (Scheme 1) Joshi et al. comment that pinnatal could have been derived from a hypothetical geranyl naphthoquinine (Joshi et al., 1982). Pinnatal is isolated from both S. *kunthianum* and *Kegelia pinnata*, thus indicating a common biogenetic pathway for these quinones. The cooccurence of both naphthaquinones and 1-methyl anthraquinones in the genus *Stereospermum* and *Kegelia* belonging to *Bignoniaceae* is unprecedented. Lapachol, which occurs in these plants (De Silva et al., 1982; Inoue et al., 1981), can be considered as a parent molecule for all the naphthoquinones and 1-methyl anthraquinones so far isolated. 1-methyl anthraquinones occur widely in nature and have been basically known to be derived by acetate malonate pathway (Yagi et al., 1978). Sterekunthal-B isolated from *S. kunthianum* together with the two quinones sterequinone-C and sterequinone-A from *S. personatum* by us completes the biogenetic sequence of these compounds. In the same manner kegelinol, isokegelinol sterequinone-D would have been synthesized from pinnatal, isopinnatal, sterequinone-B, respectively in these plants. It is interesting to note that 1-methyl group (17-methyl) is missing in sterequinone-E.

3. Experimental

3.1. General

Column chromatography was performed on silica gel (60–120 mesh). Melting points were recorded on Fisher



Scheme 1.

Johns apparatus and are uncorrected. EIMS was recorded on VG 70–70 Micromass FAB Mass was recorded on VG Auto Spec-M instrument. IR spectra were recorded on Nicolet spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on Varian 200, 400 MHz and Bruker 300 MHz spectrometer using TMS as internal standard. HMBC experiment was done on Bruker 300 MHz spectrometer.

3.2. Plant material

Stem bark of the plant was collected from the forests of Tirumala, Andhra Pradesh (India) in the month of January. It was identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Titupati. A voucher specimen of the plant is deposited in the Herbarium, Department of Botany with an accession number, 534.

3.3. Extraction and isolation

The shade dried bark of the plant (2 kg) was powdered and extracted with petroleum ether in a Soxhlet apparatus for 24 h. The solvent was evaporated under reduced pressure in a rotary flash evaporator to obtain a residue (10 g). The residue was adsorbed on silica gel and subjected to column chromatography over silica gel. The column was subjected to elution with petroleum ether first followed by mixtures containing increasing amounts of ethyl acetate. The fractions eluted at 4, 16, 18, and 20% were collected separately, concentrated and rechromatographed using silica gel (60–120 mesh) to obtain compounds **1** (18 mg), **2** (25 mg), **3** (22 mg), **4** (20 mg), **5** (8 mg) and **6** (5 mg) in pure form.

3.4. Sterequinone-A (*1*)

Pale yellow semi solid. IR (KBr) γ_{max} 2816, 1668, 1587, 1327, 1289, 1015, 714 cm^{-1}. EIMS: *m*/*z* (rel int) 274 (100) [M^+], 259 (25), 231 (17), 202 (41), 141 (19), 101 (11), 77 (14); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.25 (2H, m, H-3 and 6), 8.20 (1H, s, H-11), 7.8 (2H, m, H-4 and 5), 6.5 (1H, apparent triplet, H-14), 3.45 (2H, d, $J=0.6$ Hz, H-15), 2.85 (3H, s, H-18), 2.30 (3H, d, $J=1.2$ Hz, H-19). ^{13}C NMR: see Table 1.

3.5. Sterekunthal-B (*2*)

Pale yellow solid. mp: 158 °C; IR (KBr) γ_{max} 2985, 2829, 1711, 1662, 1615, 1589, 1282, 1182, 972, 706 cm^{-1}; FABMS: 323 ($M^+ + H$); ^{1}H NMR (200 MHz, $CDCl_3$): 8.25 (1H, dd, $J=1.0$, 8.0 Hz, H-6), 8.15 (1H, dd, $J=8.0$, 1.4 Hz, H-3), 7.7–7.85 (3H, m, H-4,5 and 11), 2.95 (1H, dd, $J=4.0$, 7.0 Hz, H-12), 2.05–2.30 (2H, m, H-15), 1.85–2.0 (3H, m, H-14, 16), 1.19 (3H, s, H-19), 0.67 (3H, s, H-18); ^{13}C NMR: see Table 1.

3.6. Sterequinone-B (*3*)

Yellow solid, mp: 138 °C; IR (KBr) γ_{max} 2917, 1710, 1662, 1616, 1591, 1298, 1157, 988, 717 cm^{-1}; FABMS: 353 ($M^+ + H$); ^{1}H NMR: (200 MHz, $CDCl_3$): 10.25 (1H, s, H-20), 8.22 (1H, d, $J=8.9$ Hz, H-6), 7.81 (1H, d, $J=6.8$ Hz, H-11), 7.53 (1H, d, $J=2.6$ Hz, H-3), 7.30 (1H, dd, $J=2.9$, 8.9 Hz, H-5), 3.94 (3H, s, Ar–OCH_3), 2.94 (1H, dd, $J=4$, 7.1 Hz, H-12), 2.28 (1H, m, H-15b), 1.88–2.05 (4H, m, H-15a, 14, 16), 1,19 (3H, s, H-19), 0.67 (3H, s, H-18). ^{13}C NMR: see Table 1.

3.7. Sterequinone-C (*4*)

Pale yellow solid mp: 199 °C; IR (KBr) γ_{max} 3487, 2825, 1669, 1618, 1585, 1328,1328, 1169, 714 cm^{-1}; EIMS: *m*/*z* (rel int): 292 (M^+, 38), 278 (100), 235 (16), 202 (18), 178 (18), 165 (10), 115 (8); ^{1}H NMR (200 MHz, $CDCl_3$): 8.25 (2H, m, H-3 and 6), 8.23 (1H, s, H-11), 7.78–7.73 (2H, m, H-4 and 5), 3.05 (2H, dm, H-15), 2.76 (3H, s, H-18), 2.28 (2H, m, H-14), 1.65 (3H, s, H-19); ^{13}C NMR: see Table 1.

3.8. Sterequinone-D (*5*)

Yellow syrup. IR (KBr) γ_{max} 2818, 1669, 1587, 1329, 1290, 717 cm^{-1}. EIMS: *m*/*z* (rel int): 304 (M^+, 100), 288 (19), 254 (12), 189 (15), 149 (54), 120 (48), 71 (40); ^{1}H NMR (300 MHz, $CDCl_3$): 8.22 (1H, d, $J=10.7$ Hz, H-6), 8.19 (1H, s, H-11), 7.65 (1H, d, $J=3$ Hz, H-3), 7.23 (1H, m, H-5), 6.45 (1H, apparent triplet, H-14), 3.99 (3H, s, Ar–OCH_3), 3.45 (2H, d, $J=0.6$ Hz, H-15), 2.85 (3H, s, H-18), 2,25 (3H, d, $J=1.2$ Hz, H-19).

3.9. Sterequinone-E(*6*)

Semi solid. IR (KBr) γ_{max} 3485, 2820, 1670, 1631, 1599, 1328, 1168, 718 cm^{-1}; EIMS: *m*/*z* (rel int): 308 (7) (M^+), 292 (100), 282 (13), 253 (16), 141 (14), 105 (18), 77 (23); ^{1}H NMR (400 MHz, $CDCl_3$): 8.25 (1H, m, H-5), 8.23 (1H, s, H-17), 8.14 (1H, s, H-11), 7.72 (1H, d, $J=2.6$ Hz, H-3), 7.24 (1H, d, $J=11.3$ Hz, H-6), 3.99 (3H, s, Ar–OCH_3), 3.05 (2H, dm, H-15), 2.30 (2H, m, H-14), 1.65 (3H, s, H-19); ^{13}C NMR: see Table.1.

Acknowledgements

The financial support from CSIR Co-ordinated program on bioactive substances from plant sources is gratefully acknowledged. We thank Drs. K. V. Raghavan, Director, IICT and J. S. Yadav, Senior Deputy Director and Head, Organic Division-1 for their invaluable suggestions. R.J.R. thanks UGC for the fellowship.

References

Akunyili, D.N., Houghton, P.N., 1993. Meroterpenoids and naphthaquinones from *Kigelia pinnata*. Phytochemistry 32, 1015–1018.

Bakuni, D.S., Dhar, M.L., Dhar, M.M., Dhawan, B.N., Gupta, B., Srimal, R.C., 1971. Screening of Indian plants for biological activity: part III. Indian. J. Exp. Biol. 9, 91–102.

Berger, Y., Berger-Deguee, M., Castonguay, A., 1981. Carbon-13 nuclear magnetic resonance studies of anthraquinones. IV—correlation of substituent effects for 1-substituted anthraquinones Organic Magnetic Resonance 15, 303–306.

Binutu, O.A., Adesogan, K.E., Okogu, J.I., 1996. Antibacterial and antifungal compounds from *Kigelia pinnata*. Planta Med. 62, 353.

Inoue, K., Inouye, H., Cheng-Chang, C., 1981. A naphthaquinone and a lignan from the wood of *Kigelia pinnata*. Phytochemistry 20, 2271–2276.

Joshi, K.C., Singh, P., Taneja, S., Cox, P.J., Howie, R.A., Thomson, R.H., 1982. New terpenoid aldehydes from *Kigelia pinnata*: crystal structure of pinnatal. Tetrahedron 38, 2703–2708.

Onegi, B., Kraft, C., Kohler, I., Freund, M., Jenett-Siems, K., Siems, K., Beyer, G., Melzig, M.F., Bienzle, U., Eich, E., 2002. Antiplasmodial activity of naphthaquinones and one anthraquinone from *Stereospermum kunthianum*. Phytochemistry 60, 39–44.

Purushotaman, K.K., Natarajan, R.K., 1974. Chemical examination of Patala (*Stereospermum tetragonum D.C*). J. Res. Indian Med. 9 (4), 107–108.

De Silva, L.B., Hearth, W.H.M.W., Jennings, R.C., Mahendran, M., Wanningama, G.P., 1982. Isolation of specioside from *Stereospermum persunatum*. Indian. J. Chem. Sect. B 21, 703–704.

Varier, P.S., 1994. Indian Medicinal Plants (Orient Longman) 5, 192.

Yagi, A., Yamanouchi, M., Nishioka, I., 1978. Biosynthetic relationship between tetrahydroanthracene and anthraquinone in *Aloe saponaria*. Phytochemistry 17, 895–897.