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ISOFURANONAPHTHOQUINONES AND PHENOLIC AND KNIPHOLONE DERIVATIVES FROM THE ROOTS OF *BULBINE CAPITATA*

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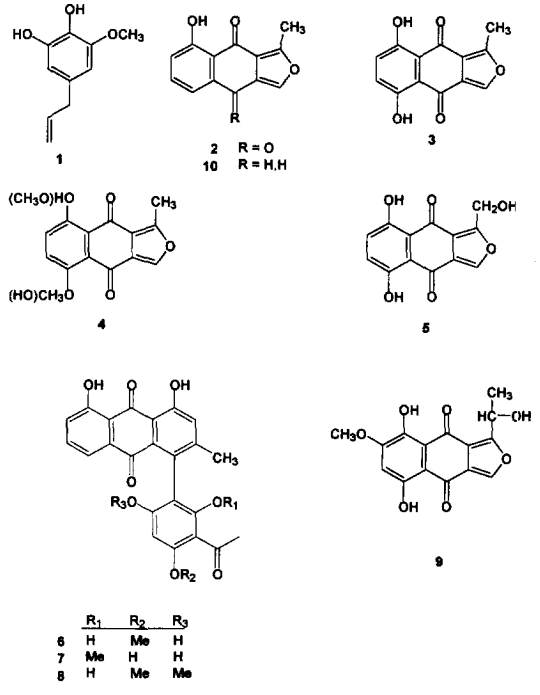
Key Word Index—*Bulbine capitata*; Asphodelaceae; roots; isofuranonaphthoquinones; naphtho[2,3-c]furan; knipholone-6′-methyl ether; 2-hydroxy-3-methoxy-5-(2-propenyl)phenol.

Abstract—The roots of *Bulbine capitata* yielded five new compounds, 2-hydroxy-3-methoxy-5-(2-propenyl) phenol, 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione, 5-hydroxy-8-methoxy- or 8-hydroxy-5-methoxy-1-methylnaphtho[2,3-c]furan-4,9-dione, 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-c]furan-4,9-dione and knipholone-6′-methyl ether in addition to the known compounds 8-hydroxy-1-methylnaphtho[2,3-c] furan-4,9-dione, chrysophanol, 10,10′-chrysophanol bianthrone, knipholone and isoknipholone. The structures were determined on the basis of spectroscopic data. © 1997 Elsevier Science Ltd

INTRODUCTION

The distribution of the genus *Bulbine* is centred in Southern Africa [1] where the occurrence of 41 species is recorded [2]. *B. capitata* V. Poelln (syn. *B. stenophylla* Verdoorn) is one of six *Bulbine* species occurring in Botswana. It also occurs in other parts of Southern Africa [2, 3]. *Bulbine* species are used in traditional medicine for the treatment of various ailments that probably arise from bacterial and fungal infections [4]. The milk decoction of the roots of *B. capitata* are used for the treatment of body rash and sexually transmitted diseases in Botswana. Only a few taxa of *Bulbine* have been investigated for secondary metabolites. *B. frutescens* and *B. abyssinica* are reported to contain knipholone, knipholone anthrone, islandicin, aloe-emodin and chrysophanol [5]. The last two pigments were also found in *B. annua* and *B. asphodeloides* [6]. Knipholone has also been reported from *B. latifolia* and *B. frutescens* [7]. To our knowledge, there is no previous phytochemical work on *B. capitata*. In this paper, we describe the isolation, from the roots, of 10 compounds, five of which are new.



RESULTS AND DISCUSSION

Flash column chromatography of the residue from the CH_2Cl_2–MeOH extract of the roots of *B. capitata* followed by gel permeation chromatography using Sephadex LH-20 and/or preparative TLC for the various fractions led to the isolation of 10 compounds. Chrysophanol and 10,10′-chrysophanol bianthrone were readily identified from their ^1H NMR spectra and by direct comparison with authentic materials available in our laboratory.

Compound **1** was assigned the molecular formula

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$C_{10}H_{12}O_3$ (HRMS, $[M]^+ = m/z$ 180.0783). It had UV-VIS absorption bands at λ_{max} 237 and 240 nm. The IR spectrum showed absorptions at 3600, 3415, 3000, 2850 and 895 cm^{-1}. The ^1H NMR spectrum of compound **1** showed signals for a pair of *meta* coupled protons at δ 6.31 and 6.47, a methoxy at δ 3.89, a two proton doublet at δ 3.29 (2H), a two proton multiplet at δ 5.08 (2H) and a one proton multiplet at δ 5.95. The ^{13}C NMR spectrum showed 10 signals among which those at δ 143.8, 130.6 and 146.8 suggested an aromatic ring with three adjacent oxygenated carbons. The signal at δ 56.2 was assigned to an aromatic methoxy in which at least one of the *ortho* positions had no substituent. Decoupling of the signal at δ 5.95 in the ^1H NMR spectrum resulted in the collapse of the doublet at δ 3.29 to a singlet and decoupling of the doublet at δ 3.29 simplified the multiplet at δ 5.95 to a double doublet (J = 10.04 and 16.94 Hz). The DEPT spectrum showed two signals attributable to methylene carbons resonating at δ 40.2 and 115.8 and a methine carbon signal at δ 137.6. The above data together with the analysis of the ^1H-^1H COSY spectrum established the presence of $CH_2 = CH\text{-}CH_2$- group. The location of the methoxy group was established by a NOE experiment which resulted in the enhancement of the Ar-H signal at δ 6.31 upon irradiation of the methoxy resonance. On the basis of these data, compound **1** was shown to be 2-hydroxy-3-methoxy-5-(2-propenyl)phenol.

Compound **2** was assigned the molecular formula $C_{13}H_8O_4$ (HRMS, $[M]^+ = m/z$ 228.0433). It had UV-VIS absorption bands at λ_{max} 252, 304 and 382 nm. The ^1H NMR and IR data obtained for compound **2** were identical to those reported for 8-hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione isolated from *Aloe ferox* along with 8-hydroxy-1-methylnaphtho[2,3-c]furan-9(4H)-one (**10**) by Koyama *et al.* [8]. These workers were able to establish structure **10** by ^{13}C-^1H long range correlation. They also reported the formation of **2** by oxidation of **10**.

Compound **3** was assigned the molecular formula $C_{13}H_8O_5$ (HRMS, $[M]^+ = m/z$ 244.0361) UV-VIS absorptions were observed at 222, 239, 301, 343 and 444 nm. In the IR spectrum an absorption due to a chelated carbonyl appeared at 1637 cm^{-1}. The ^1H NMR spectrum of compound **3** (Table 1) showed two singlets at δ 8.07 (1H) and 2.78 (3H) consistent with an isofuranonaphthoquinone structure similar to that of **2**. The three proton ABC system in **2** was no longer observed in the ^1H NMR spectrum of **3**, instead there was a two proton AB system centred at δ 7.25 (J = 9.6 Hz). There were also two D_2O exchangeable signals at δ 12.97 and 12.82 which were assigned to *peri* hydroxyls at positions 5 and 8. The above data showed compound **3** to be 5,8-dihydroxy-1-methylnaphtho-[2,3-c]furan-4,9-dione.

Compound **4** was assigned the molecular formula $C_{14}H_{10}O_5$ (HRMS, $[M]^+ = m/z$ 258.0538). UV-VIS absorptions were observed at 204, 239, 282, 334, 340 and 435 nm. Absorptions at 1678 and 1636 cm^{-1} in its IR spectrum were due to unchelated and chelated carbonyls, respectively. The ^1H NMR spectrum of compound **4** exhibited singlets from a chelated hydroxyl (δ 13.21), an aromatic proton (δ 7.98), a methoxy (δ 3.98) and a methyl (δ 2.76) and two *ortho* coupled aromatic proton signals at δ 7.36 (*d*, J = 9.0 Hz) and 7.27 (*d*, J = 9.0 Hz). The ^1H NMR spectrum was similar to that of **3** with one hydroxyl group signal in **3** replaced by a methoxy in **4**. Hence, **4** was a monomethyl ether of **3**. A NOE experiment showed no measurable signal enhancement due to any proton on irradiation of the methoxy signal. These data, however, are not adequate to distinguish compound **4** from the two alternative structures where the positions of the OH and OMe are interchanged.

Compound **5**, molecular formula $C_{13}H_8O_6$ (HRMS, $[M]^+ = m/z$ 260.0313), gave rise to UV-VIS absorptions at 240, 302 and 457 nm. The IR spectrum showed absorptions at 3415 and 1618 cm^{-1} due to a free hydroxyl and chelated carbonyl groups, respectively. The ^1H NMR spectrum consisted of signals of two chelated hydroxyls at δ 12.81 and 12.60, and singlets at δ 8.14 (1H), 7.30 (2H) and 4.98 (2H). The ^1H NMR spectrum of **5** was, therefore similar to that of **3**, except that the methyl signal in **3** was replaced by a singlet at δ 4.98 (2H) suggesting a -CH_2OH group in **5**. A DEPT experiment showed a methylene carbon signal resonating at δ 54.5. The signals at δ 184.6 and 184.1

Table 1. ^1H NMR spectral data of isofuranonaphthoquinones **2**–**5** (300 MHz, $CDCl_3$) with J (Hz) values, given in parentheses

H	2	3	4	5
3	8.05	8.07	7.98	8.14
5	7.80 *dd* (1.2, 8.0)	—	—	—
6	7.67 *t* (8.0)	7.25	7.27* *d* (9.0)	7.30
7	7.27 *dd* (1.2, 8.0)	7.25	7.36* *d* (9.0)	7.30
1-Me	2.78	2.78	2.76	—
1-CH_2OH	—	—	—	4.98
OMe	—	—	3.98	—
5,8-OH	12.86	12.97 12.82	13.21	12.81 12.60

* May be interchanged.

in the ^{13}C NMR spectrum confirmed the presence of two chelated carbonyl groups. The spectroscopic data discussed above led to the identification of compound **5** as 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-c] furan-4,9-dione.

There are only a few examples of natural isofuranonaphthoquinones. The first compound of this type was nectriafurone (**9**) isolated in 1983 from cultures of the fungus *Nectria haematococca* [9]. In higher plants, isofuranonaphthoquinones have been isolated from *Ventilago* species (Rhamnaceae) [10–12] and from *Aloe ferox* [8]. This is the first report of isofuranonaphthoquinones from *Bulbine* species. These isofuranonaphthoquinones are typically characterized by lacking oxygenation at positions 6 and 7.

Compounds **6** and **7** displayed similar ^1H NMR, UV-VIS, IR and mass spectra except for the marked difference in the chemical shift values of the OMe group which appeared at δ 3.90 in compound **6** as opposed to δ 3.29 in compound **7**. The ^1H NMR data reported in the literature for knipholone [13] and isoknipholone [14] were in complete agreement to those generated from compounds **6** and **7**, respectively. Direct TLC comparison with an authentic sample further confirmed compound **6** as knipholone. Isoknipholone (**7**) was previously reported from *Kniphofia foliosa* [14]. This is the first report of this compound from the genus *Bulbine*.

Compound **8**, molecular formula $C_{25}H_{20}O_8$ (HRMS, $[M]^+ = m/z$ 448.1166), gave rise to UV-VIS absorptions at 255, 289 and 436 nm suggesting a *peri* hydroxyl anthraquinone moiety. IR absorptions at 1680 and 1618 cm^{-1} were due to unchelated and chelated carbonyls, respectively. Three signals in the ^1H NMR spectrum for chelated phenolic hydroxyl groups at δ 13.92, 12.61 and 12.08 together with one aromatic hydrogen singlet at δ 6.13 were characteristic of knipholone type structure. In addition, one aromatic proton singlet at δ 7.26, a 3H ABC system aromatic protons resonating at δ 7.61 (*dd*, $J = 1.9, 7.7$ Hz), 7.56 (*t*, $J = 7.7$ Hz) and 7.21 (*dd*, $J = 1.9, 7.7$ Hz), two methoxy signals at δ 4.00 and 3.77, acetyl and aromatic methyls at δ 2.67 and 2.10, respectively, were observed. These data fit the general structure of knipholone (**6**). The difference between the two is the presence of one extra methoxy signal at δ 3.77 in **8**. The molecular mass of compound **8** is 14 amu higher than knipholone. Hence, compound **8** is a monomethyl ether of knipholone (**6**). There are two possible sites, C-2′ or C-6′, for the extra methoxy group. The observed ^{13}C NMR signals for these groups at δ 55.8 and 55.5 require the placement of the extra methoxy on C-6′. When the signals at δ 4.00 and 3.77 were irradiated, NOEs of 4.4 and 7.1%, respectively, were observed at δ 6.13 (H-5′) supporting the placement of the methoxy groups on C-6′ and C-4′. The above spectroscopic data established compound **8** as the hitherto unreported knipholone-6′-methyl ether. The

Table 2. ^{13}C NMR spectral data of compounds **6–8** (75.5 MHz)

C	**6*** [13]	**6**†	**7**†	**8**‡
1	161.7 *dd* (4, 2)§	161.3	159.8	162.4
2	124.6 *Dqd* (161, 5, 7)	124.5	125.9	125.3
3	151.6 *q* (6)	151.1	151.9	151.5
4	128.5 *m*	128.2	126.5	128.4
5	119.3 *Ddd* (165, 8, 3)	119.1	120.1	119.8
6	137.4 *Dd* (162, 2, 5)	137.3	137.3	136.8
7	123.3 *Ddd* (163, 7, 7)¶	123.5	124.2	123.5
8	161.1 *dm* (8)	160.1	159.4	161.8
9	192.5 *s*	191.9	192.7	193.0
10	181.9 *d* (4)	181.9	183.0	182.6
4a	131.6 *s*	131.3	132.3	131.4
10a	134.4 *d* (8)	134.4	134.3	134.7
8a	115.5 *ddd* (6, 6, 6)‖	115.4	115.5	115.7
9a	114.7 (*dd* (5, 5)**	114.6	115.0	115.0
1′	104.7 *dd* (5.5, 5.5)	104.4	103.4	106.2
2′	163.3 *d* (5)††	163.0	165.5	163.1
3′	107.3 *m*	107.1	110.0	108.9
4′	162.4 *qd* (3, 2)‡‡	162.1	163.0	162.6
5′	91.2 D (*br*) (160)	91.0	100.3	86.2
6′	161.9 *s* (*br*)	161.6	162.0	162.5
Ar-COMe	202.3 *q* (6.5)	202.4	203.5	203.6
Ar-OMe	55.6 *Q* (144)	55.6	60.7	55.5, 55.8
Ar-COMe	32.6 *Q* (128)	32.8	31.6	33.4
Ar-Me	20.4 *Qd* (127, 4.5)	20.3	21.4	21.1

* Measured $(CD_3)_2CO$ + DMSO-d_6, (100.4 MHz).† DMSO-d_6.

§ Coupling constants (Hz) in parentheses.

¶ $D_2O \rightarrow Dd$, ‖ $D_2O \rightarrow dd$, ** $D_2O \rightarrow d$, †† $D_2O \rightarrow s$, ‡‡ Irr. at $OCH_3 \rightarrow d$

^{13}C spectral assignments (Table 2) for compounds **6–8** were made by comparing measured values with those reported [13] for compound **6**.

Except for one report [15] that describes the occurrence of knipholone in *Senna didymobotrya* (Fabaceae), knipholone and related compounds have been confined to few genera of higher plants namely, *Bulbine*, *Bulbinella* and *Kniphofia* [5]. The isolation of compounds **6–8** from *B. capitata* is consistent with the above claim.

EXPERIMENTAL

General. Mps: uncorr.; ^1H NMR: 300 MHz; ^{13}C NMR: 75.5 MHz; $CDCl_3$ or DMSO-d_6 using the solvent peak as int. ref. El/ClMS: direct inlet; IR: KBr discs. UV-VIS: $CHCl_3$ soln. Prep. TLC: 0.50 or 0.75 mm thick layer silica gel; Flash CC: silica gel (particle size 40–63 μm) impregnated with 5% aq. oxalic acid.

Plant material. Bulbine capitata was purchased from a vendor at the Gaborone Station, Botswana. More plant material was collected from Molopolele 55 km north of Gaborone, Botswana, with the help of the same vendor who revealed her usual collection site. Identification of the plant was made by Dr van B-E. Wyk of the Rand Afrikaans University, Republic of South Africa. Simultaneous identification of the same plant as *B. capitata* was also made by Dr L. Turton and confirmed by G. A. Poe of the Royal Botanic Gardens at Kew, U.K. A specimen is deposited at the University of Botswana herbarium under the code BA204.

Extraction and isolation. Dried and powdered roots of *B. capitata* (200 g) were successively extracted with 2 l CH_2Cl_2–MeOH (1:1) overnight and 1.5 l MeOH (15 min). The residue (12 g) obtained by red. pres. distillation (at $T \leqslant 40°$) of the combined extracts was subjected to silica gel flash CC and eluted as follows: $CHCl_3$ (frs 1–7), $CHCl_3$-EtOAc (1:1) (frs 8 and 9) and EtOAc–MeOH (9:1) (frs 10 and 11). Fr. 2 was filtered through a short flash silica gel column using as solvents petrol, petrol–EtOAc (20:1) and petrol–EtOAc (9:1). The petrol–EtOAc (20:1) eluted frs on prep. TLC (hexane–EtOAc 9:1) afforded 12 mg of chrysophanol. Frs 3 and 4 were combined and submitted to prep. TLC (hexane–EtOAC 9:1). This gave 3 bands. The top band gave chrysophanol and the bottom band gave 4.9 mg of 10,10′-chrysophanol bianthrone. The middle band upon prep. TLC (C_6H_6–$CHCl_3$ 9:1) yielded compound **2** (3.4 mg) and compound **3** (4.7 mg). Frs 5 and 6 from the flash column of the crude extract were pooled (2.8 g) and subjected to silica gel flash CC with elution with petrol-$CHCl_3$ (2:1) (frs 1–5), $CHCl_3$–EtOAc (2:1) (fr. 6) and EtOAc (fr. 7). Frs 3 and 4 were combined, loaded on a Sephadex LH-20 column and eluted with $CHCl_3$–MeOH (2:1) to yield 4 frs Fr. 4 from the column was partitioned into $CHCl_3$-soluble and -insoluble parts. The insoluble portion turned out to be pure compound **5** (18.4 mg). The $CHCl_3$-soluble part on prep. TLC (C_6H_6–2% HCl in MeOH 4:1) gave compound **1** (9.1 mg). Fr. 2 from the Sephadex LH-20 column on prep. TLC (C_6H_6–2% HCl in MeOH 4:1) gave 20 mg knipholone (**6**) and 19.9 mg knipholone-6′-methyl ether (**8**). Fr. 4 from the flash silica gel column on Sephadex LH-20 ($CHCl_3$–MeOH 2:1) gave four frs. Repeated purification of the second and third frs. on prep. TLC, developed by either $CHCl_3$–EtOAc (9:1) or C_6H_6–2% HCl in MeOH (4:1) resulted in the isolation of 20.1 mg knipholone (**6**) and 13.1 mg isoknipholone (**7**). The last fr. was purified on prep. TLC to afford 2 mg of **4**.

2-Hydroxy-3-methoxy-5-(2-propenyl)phenol (**1**). Light yellow oil; found $[M]^+$: 180.0783 $C_{10}H_{12}O_3$, requires 180.0785; UV-VIS $\lambda^{CHCl_3}_{max}$ nm (log ε): 237 (1.21), 240 (1.50); Rν^{KBr}_{max} cm^{-1}: 3600, 3415, 3000, 2850, 2926, 1517, 1458, 1266, 1110, 895, 605; EIMS (probe) 70 eV, *m/z* (rel. int.): 180 $[M]^+$ (100), 91 (24); ^1H NMR (300 MHz, $CDCl_3$): δ 6.47 (1H, *br s*, H-6), 6.31 (1H, *br s*, H-4), 5.95 (1H, *m*, CH_2 = C<u>H</u>-), 5.08 (2H, *m*, <u>CH_2</u> = CH-), 3.89 (3H, *s*, OMe), 3.29 (2H, *d*, -<u>CH_2</u>-); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 146.8 (*s*, C-3), 143.8 (*s*, C-1), 137.6 (*d*, -<u>C</u>H = CH_2), 132.0 (*s*, C-5), 130.6 (*s*, C-2), 115.8 (*t*, -<u>CH_2</u>-), 108.8 (*d*, C-6), 103.4 (*d*, C-4), 56.2 (*q*, OMe), 40.2 (*t*, = CH-<u>CH_2</u>-).

8-Hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**2**). Yellow solid, mp 188–90° (lit. 180–183° [8]). Found $[M]^+$: 228.0433 $C_{13}H_8O_4$, requires 228.0422; UV-VIS $\lambda^{CHCl_3}_{max}$ nm (log ε): 252 (1.88), 304 (1.49), 382 (1.82); IR ν^{KBr}_{max} cm^{-1}: 3414, 2923, 1682, 1648, 1593, 1565, 1456, 1308, 1253, 1141, 1112, 1011, 884, 858, 745, 703, 599; EIMS (probe) 70 eV, *m/z* (rel. int.): 228 $[M]^+$ (100), 199 (43), 115 (21); ^1H NMR: Table 1.

5,8-Dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**3**). Orange solid, mp 176–177°. Found $[M]^+$: 244.0361 $C_{13}H_8O_5$, requires 244.0371: UV-VIS $\lambda^{CHCl_3}_{max}$ nm (log ε): 222 (1.67), 239 (2.11), 301 (1.57), 343 (1.47), 444 (1.92); IR ν^{KBr}_{max} cm^{-1}: 3414, 2361, 1637, 1560, 1508, 1458, 1351, 1243, 1144, 868, 774, 592; EIMS (probe) 70 eV, *m/z* (rel. int.): 244 $[M]^+$ (100), 165 (8), 111 (14), 57 (26); ^1H NMR: Table 1.

5-Hydroxy-8-methoxy or 8-hydroxy-5-methoxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**4**). Yellow solid, mp 194–196°. Found $[M]^+$: 258.0538 $C_{14}H_{10}O_5$, requires 258.0527; UV-VIS $\lambda^{CHCl_3}_{max}$ nm (log ε): 204 (4.3), 239 (4.1), 282 (3.6), 334 (3.4), 340 (3.4), 435 (3.8); IR ν^{KBr}_{max} cm^{-1}: 3415, 2361, 1678, 1636, 1560, 1458, 1262, 1171; CIMS (CH_4 probe) *m/z* (rel. int.): 259 $[M]^+$ (100), 258 (63), 229 (17), 119 (22), 83 (32), 73 (41); ^1H NMR: Table 1.

5,8-Dihydroxy-1-hydroxymethylnaphtho[2,3-c]furan-4,9-dione (**5**). Shiny brick red solid, mp 139–140°. Found $[M]^+$: 260.0313 $C_{13}H_8O_6$, requires 260.0320; UV-VIS $\lambda^{CHCl_3}_{max}$ nm (log ε): 240 (2.96), 302 (2.37), 457 (2.73); IR ν^{KBr}_{max} cm^{-1}: 3415, 2360, 1618, 1560, 1457, 1346, 1246, 1196, 1121, 1001, 845; EIMS (probe) 70 eV, *m/z* (rel. int.) 260 $[M]^+$ (100), 214 (38), 52 (22); ^1H NMR: Table 1.; ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 54.5 (*t*, -CH_2OH), 114.3[a] (*s*, C-4a), 114.4[a] (*s*, C-8a), 115.9 (*s*, C-3a), 122.0 (*s*, C-9a) 129.0[b]

(*d*, C-6), 129.2[b] (*d*, C-7), 146.6 (*d*, C-1), 156.9 (*s*, C-5), 156.9 (*s*, C-8), 161.9 (*s*, C-3), 184.1[c] (*s*, C-4), 184.6[c] (*s*, C-9) (Values with the same superscript may be interchanged). Assignments were made by comparison with reported data for ventilone-C [10].

Knipholone (**6**). Reddish pigment, mp 218–220° (dec.) (lit. 237° [7] and 225° [13]); $[\alpha]_D^{24}+53.6°$ ($CHCl_3$, *c* 0.3); UV-VIS $\lambda_{max}^{CHCl_3}$ nm (log ε): 238 (2.05), 257 (2.04), 289 (2.08), 332 (1.57), 434 (1.71); IR ν_{max}^{KBr} cm^{-1}: 3414, 2361, 1660, 1618, 1458, 1363, 1276, 1211, 1114, 803, 669, 607; EIMS (probe) 70 eV, *m/z* (rel. int.) 434 $[M]^+$ (100), 419 (78), 32 (33), 267 (44), 125 (35); ^1H NMR (300 MHz, DMSO-d_6) δ 14.10 (1H, *s*, *peri* OH), 12.45 (1H, *s*, *peri* OH), 11.95 (1H, *s*, *ortho* OH), 7.73 (1H, *t*, *J* = 8.0 Hz, H-6), 7.47 (1H, *dd*, *J* = 8.0, 1.1 Hz, H-5), 7.36 (1H, *s*, H-2), 7.32 (1H, *dd*, *J* = 8.0, 1.1 Hz, H-7), 6.17 (1H, *s*, H-5′), 3.90 (3H, *s*, OMe), 2.59 (3H, *s*, Ar-COMe), 2.06 (3H, *s*, Ar-Me); ^{13}C NMR: Table 2.

Isoknipholone (**7**). Orange solid, mp 225–230° (dec.) lit. 252°, dec [14]); $[\alpha]_D^{24}+33.3°$ ($CHCl_3$, *c* 0.1); UV-VIS $\lambda_{max}^{CHCl_3}$ nm (log ε): 2.04 (4.3), 239 (4.1), 254 (4.1), 283 (3.9), 326 (3.4), 436 (3.6); IR ν_{max}^{KBr} cm^{-1}: 3415, 1654, 1618, 1508, 1459, 1364, 1276, 1084, 617; EIMS (probe) 70 eV, *m/z* (rel. int.): 434 $[M]^+$ (100), 419 (28), 279 (19), 267 (87); ^1H NMR (300 MHz, DMSO-d_6) δ 13.22 (1H, *s*, *peri* OH), 12.85 (1H, *s*, *peri* OH), 11.98 (1H, *s*, *ortho* OH), 7.76 (1H, *t*, *J* = 8.0 Hz, H-6), 7.52 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-5), 7.39 (1H, *s*, H-2), 7.35 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-7), 6.22 (1H, *s*, H-5′), 3.29 (3H, *s*, OMe), 251 (3H, *s*, Ar-COMe), 2.10 (3H, *s*, Ar-Me); ^{13}C NMR: Table 2.

Knipholone-6′-methyl ether (**8**). Red solid, mp 255–260° (dec.); $[\alpha]_D^{24}+130°$ ($CHCl_3$, *c* 0.2). Found $[M]^+$: 448.1166 $C_{25}H_{20}O_8$ requires 448.1156; UV-VIS $\lambda_{max}^{CHCl_3}$ nm (log ε): 255 (2.45), 289 (2.48), 436 (2.09); IR ν_{max}^{KBr} cm^{-1}: 3410, 2923, 1680, 1618, 1458, 1278, 1211, 1127; EIMS (probe) 70 eV, *m/z* (rel. int.): 448 $[M]^+$ (100), 417 (46), 309 (35), 283 (61), 255 (41); ^1H NMR (300 MHz, $CDCl_3$): δ 13.92 (1H, *s*, *peri* OH), 12.61 (1H, *s*, *peri* OH), 12.08 (1H, *s*, *ortho* OH), 7.61 (1H, *dd*, *J* = 1.9, 7.7 Hz, H-5), 7.56 (1H, *t*, *J* = 7.7 Hz, H-6), 7.26 (1H, *s*, H-2), 7.21 (1H, *dd*, *J* = 1.9, 7.7 Hz, H-7), 6.13 (1H, *s*, 5′-H), 4.00 (3H, *s*, OMe), 3.77 (3H, *s*, OMe), 2.67 (3H, *s*, Ar-COMe), 2.10 (3H, *s*, Ar-Me); ^{13}C NMR: Table 2.

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REFERENCES

1. Dahlgren, R. M. T., Cliford, H. T. and Yeo, P. F., *The Families of the Monocotlydons*. Springer, Berlin, 1985, p. 179.
2. Reid, C., in *Plants of Southern Africa: Names and Distribution*, ed. T. H. Arnold and B. C. de Wett. National Botanical Institute, Pretoria, 1993, p. 133.
3. Turton, L. M. and Kalake, E., *A List of the Flowering Plants of Botswana In the Herbaria at the National Museum, Sebele and University of Botswana*. The Botswana Society and The National Museum, Monuments and Art Gallery, Gaborone, 1994, p. 46.
4. Watt, J. M. and Breyer-Brandwijk, M. G., *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed. Livingstone, Edinburgh, 1962, p. 695.
5. van Wyk, B-E., Yenesew, A. and Dagne, E., *Biochemical Systematics and Ecology*, 1995, **23**, 277.
6. van Rheede van Oudtshoorn, M. C. B., *Planta Medica*, 1963, **11**, 332.
7. van Staden, L. F. and Drewes, S. E., *Phytochemistry*, 1993, **35**, 685.
8. Koyama, J., Ogura, T. and Tagahara, K., *Phytochemistry*, 1994, **37**, 1147.
9. Parisot, D., Devys, M., Ferezou, J-P. and Barbier, M., *Phytochemistry*, 1983, **12**, 1301.
10. Hanumaiah, T., Rao, G. S. R., Rao, C. P., Rao, K. V. J., Cowe, H. J., Cox, R. J., Howie, R. A., Marshall, D. S. and Thomson, R. H., *Tetrahedron*, 1985, **41**, 635.
11. Jammula, S. R., Peppala, S. B., Telikepalli, H., Rao, K. V. J. and Thomson, R. H., *Phytochemistry*, 1990, **30**, 2427.
12. Ali, S., Read, R. W. and Sotheeswaran, S., *Phytochemistry*, 1994, **35**, 1029.
13. Dagne, E. and Steglich, W., *Phytochemistry*, 1984, **23**, 1729.
14. Yenesew, A., Dagne, E., Muller, M. and Steglich, W., *Phytochemistry*, 1994, **37**, 525.
15. Alemayehu, G., Hailu, A. and Abegaz, B. M., *Phytochemistry*, 1996, **42**, 1423.