

AN ANTHRONE, AN ANTHRAQUINONE AND TWO OXANTHRONES  
FROM *KNIPHOFIA FOLIOSA*

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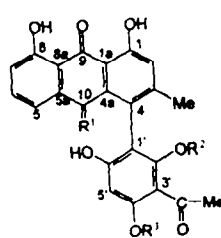
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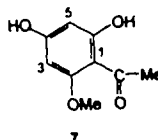
**Key Word Index**—*Kniphofia foliosa*; Asphodelaceae; stem; anthrone; anthraquinone; oxanthrones; isoknipholone anthrone; isoknipholone; foliosone; isofoliosone.**Abstract**—The compounds isoknipholone, isoknipholone anthrone, foliosone and isofoliosone were isolated from the stem of *Kniphofia foliosa* and their structures determined by spectral analyses. In addition, the known compounds, aloesaponol III, aloesaponol III-8-methyl ether and 4,6-dihydroxy-2-methoxyacetophenone were isolated and identified.

## INTRODUCTION

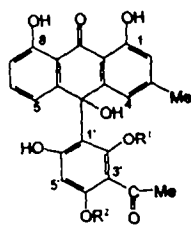
The genus *Kniphofia* Moench, comprising nearly 67 species and now placed in the new family Asphodelaceae [1], is mainly confined to the continent of Africa [2]. We report here the isolation and characterization of four novel compounds from the stem of *K. foliosa* Hochst, namely isoknipholone anthrone (1), the anthraquinone isoknipholone (2), and two oxanthrones, isofoliosone (3) and foliosone (4). Previous investigation of this plant has resulted in the isolation of several anthraquinones including the unique pigment knipholone (5), in which an acetylphloroglucinol methyl ether unit is attached to a chrysophanol moiety [3]. Knipholone anthrone (6), the most likely precursor of 5, has also recently been reported from the same plant [4].



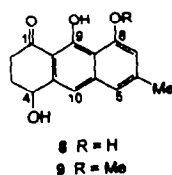
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
1	2H	Me	H
2	O	Me	H
3	O	H	Me
4	2H	H	Me



7



	R <sup>1</sup>	R <sup>2</sup>
3	Me	H
4	H	Me



8 R = H  
9 R = Me

## RESULTS AND DISCUSSION

The ethyl acetate extract of the stem of the perennial herb *K. foliosa* when concentrated yielded copious amounts (ca 1%) of the recently reported anthrone 6 [4]. Flash chromatography of the mother liquor using oxalic acid-impregnated silica gel resulted in the isolation of seven minor compounds, of which four turned out to be new natural products.

*Isoknipholone anthrone (1)*

HRMS analysis of this compound showed a molecular ion peak at  $m/z$  420.1217 corresponding to the molecular formula  $C_{24}H_{20}O_7$ . In addition, the fragment ion at  $m/z$  239 ( $C_{15}H_{11}O_3$ ) resulting from the cleavage of the internuclear bond, indicated the presence of a chrysophanol anthrone moiety coupled to an acetyl phloroglucinol methyl ether unit [4]. The presence of an anthrone group was supported by the UV-VIS ( $\lambda_{max}$  239, 264, 287, 353 nm),  $^1H$  NMR ( $\delta$ 4.01,  $d$ ,  $J = 23$  Hz, 3.84,  $d$ ,  $J = 23$  Hz for C-10 methylene) and  $^{13}C$  NMR ( $\delta$ 32.4 for C-10) data. In addition, the  $^1H$  NMR (Table 1) spectrum showed signals of two chelated hydroxyl groups, a methyl group and methylene groups as well as an isolated aromatic proton and three ABC aromatic protons indicating the chrysophanol anthrone part of the molecule to be substituted at C-2 or C-4.

The substituent was confirmed to be acetylphloroglucinol methyl ether from the  $^1H$  NMR spectrum, which showed signals assignable to an isolated aromatic proton, a chelated hydroxyl, methoxyl and acetyl groups. In a NOE experiment, irradiation of the signal at  $\delta$ 3.84 (H-10) resulted in enhancement (3%) of the signal for H-5 ( $\delta$ 6.85,  $br$   $d$ ) while the broad singlet at  $\delta$ 6.96 (H-2) was

Table 1.  $^1\text{H}$  NMR (400 MHz) spectral data for compounds 1–6 ( $\delta$  in ppm,  $\text{CDCl}_3$ )

H	1	2	3	4	5	6
2	6.96 <i>br s</i>	7.31 <i>br s</i>	6.66 <i>br s</i>	6.66 <i>br s</i>	7.32 <i>br s</i>	6.88 <i>br s</i>
4	--	--	6.90 <i>br s</i>	6.86 <i>br s</i>	--	--
5	6.85 <i>br s</i>	7.63 <i>br d</i>	7.08 <i>br d</i>	7.05 <i>br d</i>	7.56 <i>br d</i>	6.40 <i>br d</i>
6	7.41 <i>t</i>	7.64 <i>t</i>	7.47 <i>t</i>	7.41 <i>t</i>	7.75 <i>t</i>	7.49 <i>t</i>
7	6.76 <i>br d</i>	7.28 <i>br d</i>	6.85 <i>br d</i>	6.84 <i>br d</i>	7.30 <i>br d</i>	6.67 <i>br d</i>
10	4.10 <i>d</i>	--	--	--	--	4.07 <i>br s</i>
	3.84 <i>d</i>	--	--	--	--	--
5'	6.40 <i>s</i>	6.34 <i>s</i>	6.31 <i>s</i>	6.03 <i>s</i>	6.24 <i>s</i>	6.30 <i>s</i>
Ar-Me	2.20 <i>s</i>	2.19 <i>s</i>	2.29 <i>s</i>	2.29 <i>s</i>	2.17 <i>s</i>	2.12 <i>s</i>
COMe	2.63 <i>s</i>	2.65 <i>s</i>	2.24 <i>s</i>	2.46 <i>s</i>	2.62 <i>s</i>	2.63 <i>s</i>
OMe	3.35 <i>s</i>	3.33 <i>s</i>	2.64 <i>s</i>	3.89 <i>s</i>	3.98 <i>s</i>	4.00 <i>s</i>
OH	13.46 <i>s</i>	13.47 <i>s</i>	12.31 <i>s</i>	14.10 <i>s</i>	14.22 <i>s</i>	14.32 <i>s</i>
	12.60 <i>s</i>	12.61 <i>s</i>	12.25 <i>s</i>	12.34 <i>s</i>	12.53 <i>s</i>	12.35 <i>s</i>
	12.17 <i>s</i>	12.00 <i>s</i>	11.96 <i>s</i>	12.27 <i>s</i>	12.00 <i>s</i>	12.25 <i>s</i>
	--	--	11.19 <i>s</i>	11.17 <i>s</i>	--	--
	--	--	4.80 <i>br s</i>	4.13 <i>br s</i>	--	--

$$J_{5,6}, J_{6,7} = 8 \text{ Hz}, J_{10,gem} = 23 \text{ Hz}.$$

unaffected, clearly indicating that the acetylphloroglucinol methyl ether unit is attached to C-4. The chemical shift value of the methoxyl group is up field in the  $^1\text{H}$  NMR ( $\delta$ 3.35) and down field in the  $^{13}\text{C}$  NMR ( $\delta$ 61.7) relative to the resonances,  $\delta$ 4.00 ( $^1\text{H}$  NMR),  $\delta$ 56.6 ( $^{13}\text{C}$  NMR) of the same moiety in compound 6. These shifts in the methoxyl resonance appear to have been caused by the anisotropy of the chrysophanol anthrone part of the molecule. Interestingly, irradiation of the methoxyl signal at  $\delta$ 3.35 showed positive NOEs for the resonances at  $\delta$ 4.01 (1%, H-10), 2.63 (1%, COMe) and 2.20 (0.6%, 3-Me). These observations indicate placement of the OMe at C-2'. The foregoing data allow therefore assignment of structure 1 to this anthrone.

#### Isoknipholone (2)

The UV-VIS spectrum indicated this compound to be an anthraquinone derivative. HRMS analysis established the molecular formula  $\text{C}_{24}\text{H}_{18}\text{O}_8$  indicating the attachment of an acetylphloroglucinol unit to a chrysophanol moiety [3]. The  $^1\text{H}$  NMR spectrum (Table 1) of compound 2 is similar to that of 5 except for the marked difference in the chemical shift value of the OMe group which resonates at  $\delta$ 3.33 in compound 2 as opposed to  $\delta$ 3.95 in 5. The position of the OMe group was determined to be at C-2' by a NOE experiment in which irradiation of the OMe protons showed signal enhancement for COMe ( $\delta$ 2.65) and Me-3 ( $\delta$ 2.19) protons. Thus compound 2 is an isomer of knipholone (5), for which we suggest the trivial name isoknipholone (2). Compound 2 was also obtained by treatment of isoknipholone anthrone (1) with methanolic KOH.

#### Isofoliosone (3)

This compound,  $\text{C}_{24}\text{H}_{20}\text{O}_8$  (HRMS), yielded upon reductive cleavage chrysophanol and 4,6-dihydroxy-2-

methoxyacetophenone (7). In the aromatic region of the  $^1\text{H}$  NMR spectrum (Table 1), resonances for three adjacent aromatic protons with ABC pattern and two *meta* coupled protons were observed, clearly indicating that the acetylphloroglucinol methyl ether unit is attached to C-10. The  $^{13}\text{C}$  NMR spectrum showed a quaternary carbon resonance at  $\delta$ 78.7 for C-10 indicating oxygenation at this position and hence the presence of an oxanthrone moiety. An interesting feature of the  $^1\text{H}$  NMR spectrum is the appearance of the OMe signal at a relatively up field chemical shift value of  $\delta$ 2.64. This assignment for the OMe was confirmed by a  $^1\text{H}$ - $^{13}\text{C}$  COSY experiment which showed connectivity between the signals at  $\delta$ 2.64 ( $^1\text{H}$  NMR) and 63.2 ( $^{13}\text{C}$  NMR). Furthermore, a NOESY experiment (Fig. 1) showed, among others, correlation between the OMe and H-4 as well as H-5' and 6'-OH, allowing the placement of the OMe group at C-2'. The relative up field shift of the OMe resonance in the  $^1\text{H}$  NMR spectrum and the down-field shift in the  $^{13}\text{C}$  NMR spectrum is most likely due to the anisotropy of the oxchrysophanol anthrone moiety. The above data are in accord with structure 3 for this compound for which we suggest the trivial name isofoliosone.

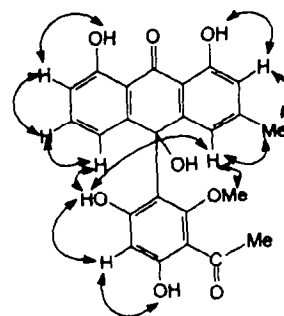


Fig. 1. NOESY correlations of 3; arrows show NOE relationship.

*Foliosone* (4)

The HRMS ( $C_{24}H_{20}O_8$ ) and UV indicated this compound to be isomeric with isofoliosone (3). As in compound 3, the  $^1H$  NMR spectrum showed signals for three adjacent aromatic protons with ABC pattern and two *meta* coupled protons, indicating that the acetylphloroglucinol group is attached to C-10. Contrary to the situation in compound 3, the methoxyl protons in compound 4 appeared at  $\delta$ 3.89, suggestive of placement of this group at C-4' [3]. This was confirmed by a NOESY experiment which showed a positive NOE between the OMe ( $\delta$ 3.89) and H-5' ( $\delta$ 6.03) protons. Interestingly, the CD spectra (Fig. 2) of compounds 3 and 4 showed opposite Cotton effects, indicating a difference in configuration at C-10.

## Other compounds

In this study 4,6-dihydroxy-2-methoxyacetophenone (7), aloesaponol III (8) and aloesaponol III-8-methyl ether (9) were also isolated from the stem of *K. foliosa*. These compounds are reported here for the first time from the genus *Kniphofia*. The phloroglucinol derivative 7, has earlier been reported as a natural product [5]. Compound 8 was reported to occur in the roots of *Aloe* [6], while 9 was reported earlier only as a derivative of 8 [7]. To our knowledge this is the first report of the occurrence of compound 9 in nature.

This study reveals the versatile reactive nature of 4,6-dihydroxy-2-methoxyacetophenone (7), which is capable of coupling with a precursor of chrysophanol to yield a variety of anthrones, anthraquinones and oxanthrones. The coupling products encountered so far from *K. foliosa* involve attachment of either C-4 or C-10 of the chrysophanol side with C-3 or C-5 of 7. The coupling between C-4 of the chrysophanol side and C-5 of 7 results in the formation of the major secondary metabolite of this plant i.e. knipholone anthrone (6). Although it is not possible at this stage to be conclusive about the nature of the precursor for the chrysophanol side, it may be reasonable to postulate the involvement of a preanthraquinone such as 8 in the biogenesis of compounds 1–6.

## EXPERIMENTAL

**Plant material.** The stem of *K. foliosa* was collected in February 1993, from the Science Faculty campus, Addis Ababa University, Ethiopia. The plant was identified by Dr Sebsebe Demissew of the National Herbarium, Ethiopia, where a voucher specimen under the cipher ED-S459 was deposited.

**Extraction and isolation.** The powdered stem (1.5 kg) was extracted with EtOAc by percolation at room temp. The extract was concd and upon standing afforded a yellow precipitate. Recrystallization of the precipitate from  $Me_2CO$  afforded 6 (15 g) [4]. The mother liquor was subjected to flash CC on oxalic acid-impregnated silica gel and eluted with mixts of petrol and EtOAc with increasing polarities. A total of 15 frs each containing ca 250 ml was collected. PTLC sepn of fr. 9 (petrol-EtOAc; 9:1), over silica gel ( $CH_2Cl_2$ , multiple development) afforded 3 (45 mg), and 4 (15 mg). Fr. 10 (8.5:1.5) was rechromatographed on Sephadex LH 20 ( $CHCl_3$ -MeOH, 1:1) to give 1 (12 mg) and 7 (40 mg). Rechromatography (flash) on oxalic acid impregnated-silica gel of frs 11–15 (4:1) gave 2 (5 mg) and that of frs 15–17 (3:1) gave 5 (50 mg) [3], 8 (10 mg) and 9 (5 mg).

**Isoknipholone anthrone (1).** Amorphous;  $[\alpha]_D +126^\circ$  ( $Me_2CO$ ;  $c$  0.002); found  $[M]^+$  420.1217  $C_{24}H_{20}O_7$ , requires 420.1213; UV  $\lambda_{max}^{MeOH}$  nm: 220, 238, 264, 287, 353; IR  $\nu_{max}^{KBr}$   $cm^{-1}$ : 3490, 3200, 2924, 2852, 1616, 1464, 1418, 1363, 1260;  $^{13}C$  NMR (25.5 MHz,  $CDCl_3$ ):  $s$  at  $\delta$ 204.9 (C-OMe), 195.1 (C-9), 167.3, 164.6, 164.0, 162.5, 161.9 (C-1, C-8, C-2', C-4', C-6'), 150.3 (C-3), 143.8, 143.4 (C-4a, C-5a), 122.8 (C-4), 116.7, 116.0 (C-1a, C-8a), 111.7, 111.3 (C-1', C-3');  $d$  at 137.5 (C-6), 120.3 (C-2), 118.8, 116.6 (C-5, C-7), 101.7 (C-5');  $t$  at 32.4 (C-10);  $q$  at 61.7 (OMe), 33.0 (C-OMe), 22.4 (Ar-Me); EIMS  $m/z$  (rel. int.): 420  $[M]^+$  (100), 405  $[M - Me]^+$  (44), 239 ( $C_{15}H_{11}O_3$ , 42), 187 (22);  $^1H$  NMR: Table 1.

**Conversion of isoknipholone anthrone (1) to isoknipholone (2).** A soln of 1 (15 mg) in 5% methanolic KOH was stirred overnight. The resulting red soln was acidified, diluted with  $H_2O$  and extracted with EtOAc. Purification of the extract by PTLC (silica gel,  $CHCl_3$ ) afforded 2

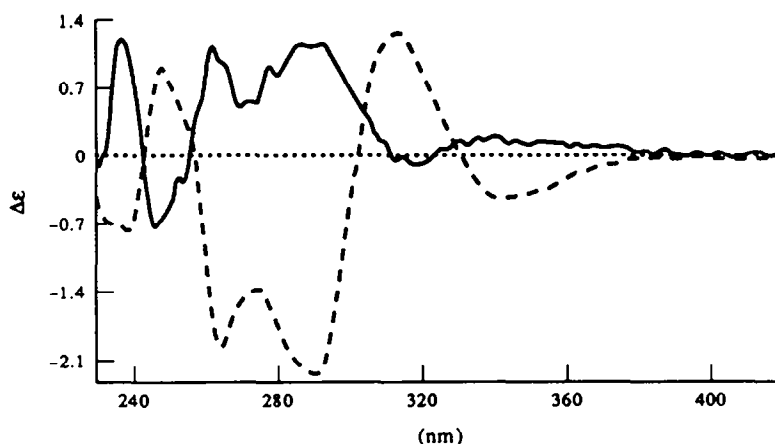


Fig. 2. Circular dichroism spectra (run in MeCN) of foliosone (—) and isofoliosone (---).

(5 mg), which was identical (UV, co-TLC) to isoknipholone isolated from the same plant.

*Isoknipholone* (2). Orange crystals; mp 252° (dec);  $[\alpha]_D -6^\circ$  (Me<sub>2</sub>CO; *c* 0.001). Found  $[M]^+$ : 434.1031 C<sub>24</sub>H<sub>18</sub>O<sub>8</sub>, requires 434.1001; UV  $\lambda_{max}^{MeOH}$  nm: 228, 254, 286, 315, 430; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3406, 3181, 2926, 2853, 1623, 1459, 1365, 1275; EIMS *m/z* (rel. int.): 434  $[M]^+$  (100), 419 (65), 403 (10), 267 (83); <sup>1</sup>H NMR: Table 1.

*Isofoliosone* (3). Amorphous;  $[\alpha]_D +25^\circ$  (Me<sub>2</sub>CO; *c* 0.01). Found  $[M]^+$ : 436.1112 C<sub>24</sub>H<sub>20</sub>O<sub>8</sub>, requires 436.1163; UV  $\lambda_{max}^{MeOH}$  nm: 222, 270, 288, 387; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3490, 3216, 2948, 1620, 1485, 1449, 1355, 1271, 1268; <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>): *s* at  $\delta$  202.6 (COMe), 191.1 (C-9), 163.9, 163.5, 162.0, 161.8, 161.1 (C-1, C-8, C-2', C-4', C-6'), 149.8 (C-3), 148.1, 147.9 (C-4a, C-5a), 115.4, 112.9 (C-1a, C-8a), 110.8, 110.1 (C-1', C-3'), 78.7 (C-10, observed when spectrum was recorded in Me<sub>2</sub>CO-*d*<sub>6</sub>); *d* at 137.4 (C-6), 121.1, 119.9, 118.1, 117.8 (C-2, C-4, C-5, C-7), 101.4 (C-5'); *q* at 63.2 (OMe), 29.0 (COMe), 22.4 (Ar-Me). EIMS *m/z* (rel. int.): 436  $[M]^+$  (10), 418  $[M-H_2O]^+$  (100), 403 (43), 387 (47), 254  $[C_{15}H_{10}O_4]^+$  (7); <sup>1</sup>H NMR: Table 1.

*Reductive cleavage of isofoliosone* (3). Compound 3 was cleaved using Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and methanolic KOH as described in ref. [3] to give chrysophanol and 7 (co-TLC).

*Foliosone* (4). Pale yellow crystals (Me<sub>2</sub>CO); mp 238° (dec.);  $[\alpha]_D -22^\circ$  (Me<sub>2</sub>CO; *c* 0.01). Found  $[M]^+$ : 436.1196 C<sub>24</sub>H<sub>20</sub>O<sub>8</sub>, requires 436.1163; UV  $\lambda_{max}^{MeOH}$  nm: 222, 271, 280, 384; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3352, 3117, 2923, 2852, 1610, 1485, 1450, 1366, 1274, 1268; EIMS *m/z* (rel. int.): 436  $[M]^+$  (10), 418  $[M-H_2O]^+$  (100), 403 (43), 387 (47), 254  $[C_{15}H_{10}O_4]^+$  (7); <sup>1</sup>H NMR: Table 1.

*4,6-Dihydroxy-2-methoxyacetophenone* (7). Crystals (Me<sub>2</sub>CO) mp 195–198° (lit. [3] 197–199°); <sup>1</sup>H NMR (400 MHz, Me<sub>2</sub>CO-*d*<sub>6</sub>):  $\delta$  13.90 (1H, *s*, OH), 6.02 (1H, *d*, *J* = 2 Hz, H-3 or H-5), 5.59 (1H, *d*, *J* = 2 Hz, H-5 or H-3), 3.92 (3H, *s*, OMe), 2.52 (3H, *s*, COMe); <sup>13</sup>C NMR (22.5 MHz, Me<sub>2</sub>CO-*d*<sub>6</sub>):  $\delta$  203.4 (COMe), 168.3, 165.7,

164.8 (C-2, C-4, C-6), 105.6 (C-1), 96.8, 91.9 (C-3, C-5); 56.1 (OMe), 32.8 (COMe); EIMS *m/z* (rel. int.): 182  $[M]^+$  (37), 167  $[M-Me]^+$  (100), 152 (8), 129 (17).

*Aloesaponol III* (8). Amorphous (lit. [7] mp 198–200°, dec.). Found  $[M]^+$ : 258.0892 C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> requires 258.0894; UV, IR, <sup>1</sup>H NMR data as in ref. [7]; EIMS *m/z* (rel. int.): 258  $[M]^+$  (100), 240  $[M-H_2O]^+$  (65), 225 (36), 212 (25), 202 (34).

*Aloesaponol III-8-methyl ether* (9). Amorphous (lit. [7] mp 173–175°). Found  $[M]^+$ : 272.1025 C<sub>16</sub>H<sub>16</sub>O<sub>4</sub>, requires 272.1051; UV and IR data as in ref. [7]; <sup>1</sup>H NMR (90 MHz, CDCl<sub>3</sub>):  $\delta$  2.68 (1H, *m*, H-2), 3.04 (1H, *m*, H-2), 2.26 (2H, *m*, H-3), 4.92 (1H, *dd*, 5, 4 Hz, H-4), 7.15 (1H, *d*, *J* = 2 Hz, H-5 or H-7), 6.68 (1H, *d*, *J* = 2 Hz, H-7 or H-5), 2.43 (3H, *s*, Ar-Me), 4.01 (3H, *s*, OMe) 15.15 (OH); EIMS *m/z* (rel. int.): 272  $[M]^+$  (100), 254  $[M-H_2O]^+$  (12).

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