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KNIPHOLONE: A UNIQUE ANTHRAQUINONE DERIVATIVE FROM *KNIPHOFIA FOLIOSA*

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Key Word Index—*Kniphofia foliosa*; Liliaceae; anthraquinone; chrysophanol; knipholone.

Abstract—The roots of *Kniphofia foliosa* afforded, in addition to chrysophanol, a novel anthraquinone named knipholone, whose structure was determined by spectroscopic methods as well as by degradation to known compounds.

INTRODUCTION

Several genera of the family Liliaceae viz. *Aloe*, *Asphodeline*, *Bulbine* etc. contain anthraquinones and bisanthraquinones [1–3]. The genus *Kniphofia* includes several species most of which are restricted to tropical regions. An earlier study established the presence of rhein in seeds of *K. uvaria* [4], a plant used in Zulu folk medicine in South Africa [5]. Apart from a report on the isolation of putrescine amides from leaves of *K. foliosa*, *K. flavovirens* and *K. tuckii* [6], no further studies on *Kniphofia* could be found in the literature.

The roots of *Kniphofia foliosa* Hochst., a perennial herb widely distributed in the mountainous regions of central and northern Ethiopia [7], are used in traditional Ethiopian medicine for the treatment of abdominal cramps. This prompted us to investigate the chemical constituents of sun dried roots. TLC of the crude extract revealed the presence of at least nine chloroform-soluble pigments. Separation on Sephadex LH 20 columns led to the isolation of chrysophanol (**5**) and the orange-brown main component (0.09%), which was named knipholone (**1**). Compound **1** represents a new type of anthraquinone pigment, in which the chromophore is connected to an acylphloroglucinol unit.

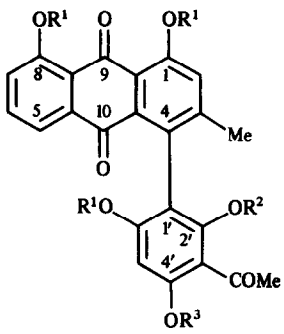
RESULTS AND DISCUSSION

Compound **1**, $C_{24}H_{18}O_8$, $[\alpha]_D^{22} + 80°$ (MeOH), has a UV/VIS spectrum (λ_{max} 224, 254, 288, 432 nm) which closely corresponds to that of **5**. The presence of a chrysophanol system is supported by the ^{1}H NMR spectrum (400 MHz, CD_3COCD_3), which shows two singlets for the chelated hydroxyl groups (δ12.00 and 12.53) and the typical ABC pattern for the protons in the 5-, 6- and 7-positions. A quartet at δ7.32 can be ascribed to H-2 (or H-4) due to the 0.7 Hz coupling caused by the adjacent methyl group (δ2.17). This leads to the conclusion that the chrysophanol nucleus is substituted by a $C_9H_9O_4$ residue either at position 4 or 2.

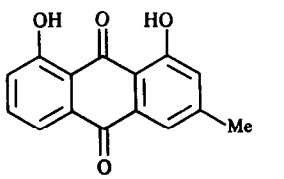
The remaining ^{1}H NMR signals at δ2.62 (*s*, Me), 3.98 (*s*, OMe), 6.24 (*s*, aromatic H), 8.95 (*s* (*br*), OH) and 14.22 (*s*, OH) and the ^{13}C NMR data (Table 1) indicate that this residue represents an acetylphloroglucinol unit etherified by a methyl group at one of the two *ortho* hydroxyl groups. In accord with this assignment **1** can be converted into tetramethyl and tetraacetyl derivatives (**3** and **4**, respectively).

Definitive proof of the principal structure was obtained by reductive cleavage of **1** with alkaline sodium dithionite [8, 9], which afforded **5** and 4,6-dihydroxy-2-methoxy-acetophenone (**6**), identified by its mp, MS and ^{1}H NMR data.

The attachment of the acetylphloroglucinol unit to the chrysophanol system follows from the proton-coupled ^{13}C NMR spectrum. It displays a doublet (J = 4 Hz) for C-10 arising from ^3J-coupling with the *peri*-proton at C-5 [10]. This proves the presence of the substituent at C-4. A doublet of doublets would have been expected for C-10 if both *peri*-protons at C-4 and C-5 were available. Except for C-4, the chemical shifts of all the other carbon atoms are in close agreement with those of **5** (Table 1). The



	R¹	R²	R³
1	H	H	Me
2	H	Me	H
3	Me	Me	Me
4	Ac	Ac	Me



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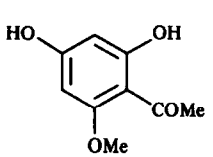

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Table 1. ^{13}C NMR spectral data of knipholone (1) and chrysophanol (5) measured at 100.4 and 22.6 MHz in CD_3COCH_3–DMSO and in $CDCl_3$, respectively (TMS as internal standard)

C	1	5	C	1	5
1	161.7 *dd* (4, 2)*	162.4	9	192.5 *s*	192.5
1a	114.7 *dd* (5, 5)†	113.7	10	181.9 *d* (4)	181.9
2	124.6 *Dqd* (161, 5, 7)	124.5	1′	104.7 *dd* (5.5, 5.5)	
3	151.6 *q* (6)	149.3	2′	163.3 *d* (5)‡	
4	128.5 *m*	121.4	3′	107.3 *m*	
4a	131.6 *s*	133.2	4′	162.4 *qd* (3, 2)§	
5	119.3 *Ddd* (165, 8, 3)	119.9	5′	91.2 *D* (*br*) (160)	
5a	134.4 *d* (8)	133.6	6′	161.9 *s*(*br*)	
6	137.4 *Dd* (162, 2.5)	136.9	$ArCH_3$	20.4 *Qd* (127, 4.5)	22.3
7	123.3 *Ddd* (163, 7, 7)‖	124.3	OCH_3	55.6 *Q* (144)	
8	161.1 *dm* (8)	162.7	$COCH_3$	32.6 *Q* (128)	
8a	115.5 *ddd* (6, 6, 6)¶	115.8	$COCH_3$	202.3 *q* (6.5)	

*Coupling constants (Hz) in parentheses.
†$D_2O \rightarrow d$.
‡$D_2O \rightarrow s$.
§Irr. at $OCH_3 \rightarrow d$.
‖$D_2O \rightarrow Dd$.
¶$D_2O \rightarrow dd$.

highfield shift of the methyl singlet in the ^1H NMR spectrum of **1** (δ2.17; **5**: 2.47) and the downfield shift of the chelated hydroxyl-proton at C-1 (δ12.52; **5**: 12.13) are in accord with the presence of a twisted acetylphloroglucinol substituent at the 4-position.

The final problem is the location of the methoxy group which could be attached either at C-4′ or C-2′ (cf. formula **1** or **2**). The correctness of formula **1** was proven by heteronuclear decoupling. Irradiation at the methoxy-proton frequency simplifies the carbon multiplet at δ162.4 into a doublet. The residual coupling $J = 2$ Hz can only be explained if the methoxy group is located at C-4′. In accord with this assignment the tetraacetate **4** exhibits two of the four acetate signals at unusually high field (δ1.75, 1.91) due to the anisotropy of the anthraquinone moiety. The chemical shift of the methoxyl signal in **1** (δ3.98) is in the normal range, whereas in the permethyl ether **3** the methoxyl groups at C-2′ and C-6′ appear at δ3.66 and 3.28, respectively.

The restricted rotation around the biaryl linkage explains the optical activity of knipholone as well as its CD curve. Compound **1** could arise biogenetically by oxidative coupling of **5** with acetylphloroglucinol or its monomethyl ether **6**. Further studies on the phenolic constituents of *K. foliosa* and related species are in progress.

EXPERIMENTAL

General. ^1H NMR: 90 and 400 MHz, TMS as the internal standard; MS: 70 eV; TLC: silica gel with EtOAc–C_6H_6 (1:4, solvent 1) and MeOH–C_6H_6 (1:9, solvent 2).

Plant material. K. foliosa tubers were collected in 1982 from Wollo province, Ethiopia, on the road from Dessie to Woreyilu, Hara Wobalo Kebele at an altitude of 2600 m. The plant was identified by Ato Zerihum Woldu, Biology Department, Addis Ababa University, and a voucher sample is kept in the National Herbarium, Addis Ababa.

Extraction and isolation of anthraquinones. The powdered roots of *K. foliosa* (1 kg) were soaked in Me_2CO at room temp and then filtered. The filtrate after solvent removal gave a reddish powder (25 g, 2.5%), which on TLC (solvent 1) showed at least nine spots, of which three with R_f 0.60, 0.86 and 0.93 were the most prominent. The crude Me_2CO extract (1.0 g) was first soaked in $CHCl_3$ (30 ml), filtered and upon removal of solvent yielded a solid substance (0.5 g) which was applied to a column packed with Sephadex LH 20 (100 g) and eluted with $CHCl_3$–MeOH (1:1). A total of 70 fractions, each 10 ml, were collected over a 24 hr period. The residue obtained after combining fractions 44–47 contained principally compound **1** (R_f 0.60) while fractions 48–52 contained mixtures of a bright red pigment (R_f 0.86) and a yellow substance (R_f 0.93). Final purification by prep. TLC (system 2) gave compound **1** (50 mg), a red pigment (8 mg) whose structure was not elucidated and a yellow substance (2 mg), which was identified as chrysophanol (**5**) by comparison with an authentic sample.

Knipholone (**1**). Orange-brown crystals (Me_2CO–petrol), mp 225° (dec.); $[\alpha]_D^{22} + 80°$ (MeOH; *c* 0.01). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 3000, 1680, 1630, 1460, 1370, 1280; UV λ_{max}^{EtOH} nm (ε): 224 (31 000), 254 (18 000), 288 (18 000), 432 (6900); bathochromic shift upon addition of one drop 5% NaOH, 232 (28 000), 250 (20 000), 330 (19 000), 500 (6000). CD (MeOH) λ_{max} 430 ($\Delta\varepsilon + 0.89$), 330 (+1.43), 293 (−0.85), 280 (+5.18), 253 (−1.43), 236 (−4.28); MS *m/z* (rel. int.): 434 $[M]^+$ (100), 419.0815 ($C_{23}H_{15}O_8$, 92), 417.1007 ($C_{24}H_{17}O_7$, 21), 321 (33), 267.0642 ($C_{16}H_{11}O_4$, 28); ^1H NMR (400 MHz, CD_3COCD_3): δ2.17 (*d*, $J = 0.7$ Hz, $ArCH_3$), 2.62 (s, $ArCOCH_3$), 3.98 (*s*, OMe), 6.24 (*s*, H-5′), 7.30 (*dd*, $J = 8$, 1.5 Hz, H-7), 7.32 (*qu*, $J = 0.7$ Hz, H-2), 7.56 (*dd*, $J = 7$, 1.5 Hz, H-5), 7.75 (*dd*, $J = 8$, 7 Hz, H-6), 8.95 (*s* (*br*), OH), 12.00 (*s*, OH), 12.53 (*s*, OH), 14.22 (*s*, OH); ^{13}C NMR: see Table 1. $C_{24}H_{18}O_8$ calc. 434.1001; found 434.1001 (MS).

Knipholone tetramethyl ether (**3**). To a soln of **1** (15 mg) in dry Me_2CO, K_2CO_3 (100 mg) and Me_2SO_4 (0.5 ml) were added and the mixture was stirred for 7 days. Filtration and evaporation of the solvent followed by purification by prep. TLC ($CHCl_3$–MeOH, 17:3) yielded **3** as a yellow oil which solidified on long

standing, mp 100–102°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1690, 1600, 1470; MS *m/z* (rel. int.): 490 $[M]^+$ (70), 475 (100), 459 (10), 295 (42), 280 (10), 230 (10). ^1H NMR (90 MHz, $CDCl_3$): δ2.13 (*s*, 3H), 2.53 (*s*, 3H), 3.28 (*s*, 3H), 3.66 (*s*, 3H), 4.02 (*s*, 3H), 6.35 (*s*, H-5'), 7.22 (*m*, 2H), 7.44 (*m*, 2H). $C_{28}H_{26}O_8$ calc. 490.1627; found 490.1620 (MS).

Knipholone tetraacetate (**4**). Acetylation of **1** (21 mg) following the usual methods gave a yellowish oil in quantitative yield which solidified on long standing, mp 98–100°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3050, 1790, 1700, 1690, 1200. MS *m/z* (rel. int.): 602 M^+ (6), 560 (45), 518 (62), 477 (27), 476 (100), 461 (14), 434 (29); ^1H NMR (90 MHz, $CDCl_3$): δ1.75 (*s*, 3H), 1.91 (*s*, 3H), 2.17 (*s*, 3H), 2.44 (*s*, 6H), 2.55 (*s*, 3H), 3.95 (*s*, OMe), 6.84 (*s*, H-5'), 7.27 (*s* (*br*), H-2), 7.33 (*dd*, J = 8, 1.5 Hz, H-7) 7.66 (*t*, J = 8 Hz, H-6), 8.00 (*dd*, J = 8, 1.5 Hz, H-5). $C_{32}H_{26}O_8$ calc. 602.1425; found 602.1428 (MS).

Reductive cleavage of compound **1**. To a soln of **1** (20 mg) in 5% NaOH (3 ml), $Na_2S_2O_4$ (30 mg) was added and the mixture was heated for 1 hr at 80°. The dark red soln was then acidified and extracted with EtOAc. After evaporation of the dried extract (Na_2SO_4), the residue was separated by filtration and recrystallized from hot EtOH to give chrysophanol (**5**) as golden yellow plates mp 188–190° (lit. [11] mp 196°). The filtrate was concd *in vacuo*, the residue dissolved in $CHCl_3$ under reflux and when allowed to stand overnight deposited crystals of 4,6-dihydroxy-2-methoxyacetophenone (**6**) mp 197–199° (lit. [12] mp 205–207°); MS *m/z* (rel. int.): 182 $[M]^+$ (32), 167.0346 ($C_8H_7O_4$, 100), 152.0099 ($C_7H_4O_4$, 11); ^1H NMR (90 MHz, CD_3COCD_3): δ2.53 (*s*, Ac), 3.86 (*s*, OMe), 5.94 (*d*, J = 2 Hz, H-5), 6.02 (*d*, J = 2 Hz, H-3). $C_9H_{10}O_4$ calc. 182.0580; found 182.0580 (MS).

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