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BIANTHRAQUINONES FROM *SENNA DIDYMOBOTRYA*

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Key Word Index—*Senna didymobotrya*; Leguminosae; bianthraquinones; knipholone; emodin; chrysophanol; physcion; 10-hydroxy-10-(physcion-7′-yl)-chrysophanol anthrone; 5,10-dihydroxy-2-methyl-9-(physcion-7′-yl)-1,4-anthraquinone.

Abstract—Emodin, chrysophanol, physcion, knipholone and two new bianthraquinones, 10-hydroxy-10-(physcion-7′-yl)-chrysophanol anthrone and 5,10-dihydroxy-2-methyl-9-(physcion-7′-yl)-1,4-anthraquinone, are reported from the pods of *Senna didymobotrya*. Knipholone is isolated from the genus *Senna* for the first time. The compounds are identified on the basis of their colour reactions, comparison with authentic samples and spectroscopic data.

INTRODUCTION

Senna didymobotrya Fresen is one of the 18 *Senna* species growing in Ethiopia [1]. The plant is known for its value in traditional medicine [2]. Previous investigations have resulted in the isolation of anthraquinones and flavonoid glycosides from the leaves [3, 4]. Our report, which constitutes the first report on the pods of this taxon, presents the isolation and structural elucidation of two new bianthraquinones and four known anthraquinones.

RESULTS AND DISCUSSION

A chloroform extract of the pods of *S. didymobotrya* was subjected to repeated silica gel column and preparative thin-layer chromatography to yield chrysophanol, physcion, emodin, knipholone (**4**) and the bianthraquinones **1** and **3**. The common anthraquinones, chrysophanol, physcion and emodin, were identified from their ^{1}H NMR spectra and by TLC comparison with authentic samples.

Compound **4** is a reddish pigment which turned deep red on a TLC plate upon spraying with 5% methanolic KOH. Its ^{1}H NMR spectrum in $CDCl_3$, which showed three signals for chelated hydroxyl groups at δ 14.9, 12.59 and 11.98 ppm, aromatic methyl and carbonyl methyl signals at δ 2.15 and 2.65, respectively, and aromatic proton signals at δ 6.14 (H-5′), 7.30 (H2, H-7), 7.56 (H-6) and 7.65 (H-5), was very similar to that reported in the literature for knipholone [5]. This was confirmed by TLC comparison with authentic sample of knipholone kindly donated by Dr E. Dagne [5].

Compound **1**, an orange pigment, does not show measurable optical activity. The UV spectrum showed bands at 224, 271, 303, 384 and 468 mm, suggesting a quinonoid chromophore. The IR spectrum showed absorptions at 3428, 1667 and 1616 cm^{-1}, indicating the presence of hydroxyl, non-chelated and chelated carbonyl groups, respectively. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of two aromatic methyls, a methoxyl four chelated hydroxyl groups and eight aromatic protons, which can be accommodated on a bianthraquinone skeleton. The ^{1}H NMR spectrum of **1** resembles that of **2** previously isolated by our group from *S. longiracemosa* [6] (Table 1). A close comparison of the spectra, however, revealed that the two spectra have important differences. The ^{1}H NMR spectrum of **2** showed *ortho*-coupled protons resonating at δ 8.64 and 7.98 that could be assigned to H-5′ and H-6′, while the spectrum of **1** showed a singlet at δ 7.40 assignable to H-5′, and a methoxyl signal at 3.80. Since H-5′ resonates as a sharp singlet, it was assumed that the methoxyl group must be at position 6′. This led to the proposal that **1** might be a bianthraquinone based on chrysophanol and physcion.

The two *meta*-coupled protons at δ 7.01 (H-2) and 6.80 (H-4), together with an ABC system at δ 6.95 (H-5), 7.55 (H-6) and 7.12 (H-7) are assignable to the protons of the chrysophanol moiety. The signals attributed to H-4 (δ 6.80) and H-5 (δ 6.95) are relatively upfield, indicating the anthrone nature of the chrysophanol skeleton. Since there are no signals that can be assigned to the anthrone protons at C-10, it was

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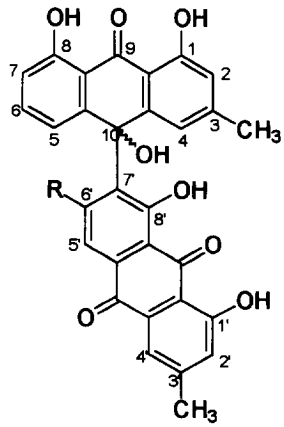
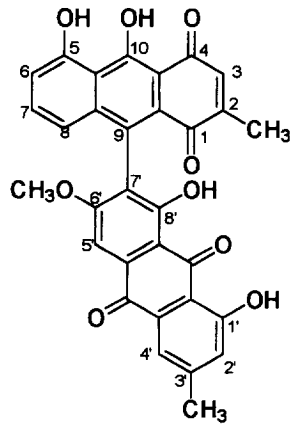


1 R = OCH_3
2 R = H



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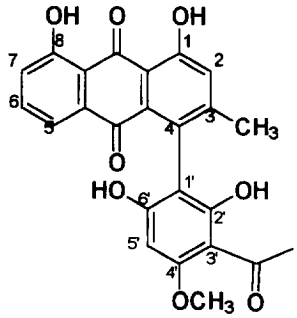

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assumed that the coupling is at this position of the chrysophanol moiety.

The signals at δ 7.21, 7.64, and 7.40 (see Table 1) are assigned to H-2, H-4 and H-5, respectively, of the physcion moiety. The fact that there is no signal attributable to H-7 and that the signal of H-5 appears as a sharp singlet led to the conclusion that the chrysophanol anthrone is coupled to position 7 of the physcion skeleton. The mass spectrum HR at 538.1278 (calculated for $C_{31}H_{22}O_9$ = 538.1262) gave a molecular formula of $C_{31}H_{22}O_9$, which is in agreement with the proposed structure **1**.

Table 1. ^{1}H NMR spectral data for compounds **1** (300 MHz, acetone-d_6) and **2** (400 MHz, $CDCl_3$ [6]), TMS as internal standard (J in Hz)

H	1	2
1-OH*	12.30 *s*	11.75 *s*
2	7.01 *br s*	6.76 *d* (J = 2.0)
3-Me	2.30 *s*	2.20 *s*
4	6.80 *br s*	6.58 *d* (J = 2.0)
5	6.95 *d* (J = 8.0)	6.78 *dd* (J = 7.8, 2.0)
6	7.55 *t* (J = 8.0)	7.38 *t* (J = 7.8)
7	7.12 *d* (J = 8.0)	6.94 *dd* (J = 7.8, 2.0)
8-OH*	12.32 *s*	12.15 *s*
1′-OH*	12.38 *s*	12.35 *s*
2′	7.21 *br s*	7.02 *d* (J = 2.0)
3′-Me	2.50 *s*	2.40 *s*
4′	7.64 *br s*	7.60 *d* (J = 2.0)
5′	7.40 *s*	8.64 *d* (J = 7.8)
6′ (OMe)	3.80 *s*	7.98 *d* (J = 7.8)
8′-OH*	12.40 *s*	12.45 *s*

*Signals in the same column may be interchangable.

Compound **3** is a brown pigment, which turned pink on TLC when sprayed with 5% methanolic KOH. The UV spectrum showed absorption maxima at 239, 300, 446, 500 and 580 nm, suggesting a quinonoid chromophore. The IR spectrum showed bands for an hydroxyl, free and chelated carbonyl groups at 3432, 1680 and 1626 cm^{-1}, respectively. The ^{1}H NMR spectrum (see Experimental) revealed six aromatic protons, two methyl groups, a methoxyl and four chelated hydroxyl groups, suggesting a bianthraquinone skeleton. The two hydroxyl groups resonating at δ 12.05 and 12.25, a methoxyl signal at δ 3.84 and three aromatic proton signals at δ 7.62 (H-5′), 7.70 (H-4′) and 7.15 (H-2′) belong to a physcion moiety.

The other moiety contains chelated and doubly chelated hydroxyl groups resonating at δ 10.6 and 17.1, respectively, three aromatic protons with an ABC splitting pattern, an aromatic methyl group and one quinonoid-H indicating a 1,4-anthraquinone structure. This is supported by the UV spectrum, which showed a

bathochromic shift of its long wavelength absorption maxima.

The signals at δ 7.12, 7.50, 6.80, 6.95 and 2.10 are assigned to H-6, H-7, H-8, Q-H and the methyl group of the 1,4-anthraquinone moiety, respectively. The signal attributable to 9-H of the 1,4-anthraquinone moiety, which would be relatively downfield (*ca* δ 8), is absent, establishing that the physcion moiety is coupled to position 9 of the 1,4-anthraquinone moiety. Besides, the absence of a signal attributable to H-7′ and the appearance of the H-5′ signal as a sharp singlet suggested that the 1,4-anthraquinone is coupled to position 7 of the physcion skeleton. These conclusions are supported by the HR mass spectrum 536.1127 (calculated for $C_{31}H_{20}O_9$ = 536.1106), which gave a molecular formula of $C_{31}H_{20}O_9$ consistent with structure **3**.

EXPERIMENTAL

General. Mps: uncorr. FT-IR: KBr discs. ^{1}H NMR at 300 MHz. ^{1}H chemical shifts are referenced to the residual $CHCl_3$ (δ 7.26) signal. HRMS were measured by Dr P. R. Boshoff, Mass Spectrometry Unit, P.O. Box 652, Cape Town 8000, South Africa. Analyt. TLC: silica gel (Merck, Kieselgel 60_{254} 0.25 mm); flash CC: silica gel (Merck 9385 Kieselgel 60, particle size 0.040–0.063 mm, impregnated with 5% aq. oxalic acid). Prep. TLC: silica gel (Merck 7748, Kieselgel 60 $Pf_{254+366}$, 1 mm). Spots were visualized with 5% methanolic KOH.

Plant material. The pods of *S. didymobotrya* were collected from the Addis Ababa University garden on 14 November 1994 and identified by Dr Sebsebe Demissew, the National Herbarium, Biology Department, Addis Ababa University (Voucher Nos. AH-2 and AH-3).

Extraction and isolation. The dried and ground pods (1.5 kg) were soaked in 5% HOAc (5 l) for 24 hr and dried. The acid-treated material was then defatted with cold petrol (30 l) and the marc was extracted with $CHCl_3$ (30 l). The $CHCl_3$ extract, upon evapn of solvent, gave a dark residue (21.4 g, 1.4%) which on TLC (silica gel, $CHCl_3$) showed 4 coloured spots with R_fs of 0.57, 0.43, 0.34 and 0.17. The dark residue (10 g) was pre-adsorbed on silica gel (30 g), applied to an oxalic acid washed silica gel packed column and eluted with $CHCl_3$. Seven frs (250 ml each) were collected. The 1st fr. gave chrysophanol and physcion. Frs 2–6, by TLC monitoring, were combined, and sepd by prep. TLC (silica gel, $CHCl_3$) to yield pure **1** and **3**. The 7th fr. was applied to Sephadex LH-20 and eluted with $CHCl_3$–MeOH (2:1) to yield 2 frs. The 1st fr. contained mainly chlorophyll and was discarded. The 2nd fr. was sepd by prep. TLC (silica gel; petrol–EtOAc, 4:1) to yield emodin and knipholone.

10-*Hydroxy*-10-(*physcion*-7′-*yl*)-*chrysophanol*-9-*anthrone* (**1**). Mp 182–184°; ^{1}H-NMR: see Table 1; MS *m/z* (rel. int. %): 538 $[M]^+$ (48), 520 $[M - H_2O]^+$ (100), 505 $[M - H_2O - CH_3]$ (63), 284 [M-physcion]$^+$ (6), 255 $[M + H\text{-chrysophanol}]^+$ (40); UV–Vis λ_{max}^{MeOH} nm (log ε): 225 (3.90), 271 (4.10), 303 (3.90), 384 (3.70), 468 (3.50); IR ν_{max}^{KBr} cm^{-1}: 3428, 1667, 1616.

5,10-*Dihydroxy*-2-*methyl*-9-(*physcion*-7′-*yl*)-1,4-*anthraquinone* (**3**). Mp 150–152° (dec.); ^{1}H NMR (300 MHz, $CDCl_3$): 17.10 *s* (10-OH), 12.25 *s* (8′-OH), 12.05 *s* (1′-OH), 10.60 *s* (5-OH), 6.95 *s* (3-H), 6.98 *d* (8-H, J = 8.0 Hz), 7.50 *t* (7-H, J = 8.0 Hz), 7.12 *d* (6-H, J = 8.0 Hz), 7.15 *br s* (2′-H), 7.70 *br s* (4′-H), 7.62 *s* (5′-H), 3.84 *s* (6′-OMe), 2.45 *s* (3′-Me), 2.10 *br s* (2-Me); HRMS *m/z*: 536. 1127 $[M]^+$ (calc. 536, 1106); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 239 (3.95), 288 (3.72), 300 (3.66), 446 (3.64), 500 (3.03), 536 (3.01), 580 (2.75); IR ν_{max}^{KBr} cm^{-1}: 3452, 1680, 1626.

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