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BIANTHRAQUINONES AND A SPERMIDINE ALKALOID FROM *CASSIA FLORIBUNDA*

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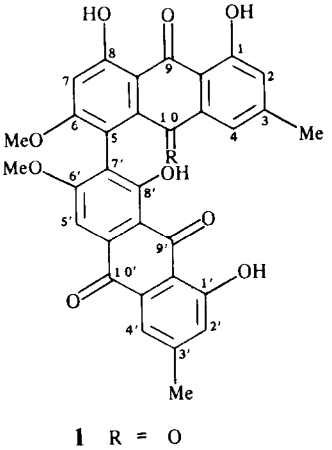
Key Word Index—*Cassia floribunda*; Leguminosae; leaves; bianthraquinones; physcion; spermidine alkaloid.

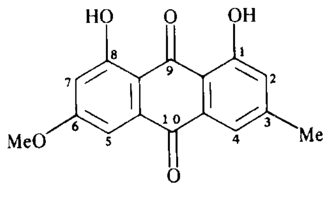
Abstract—The leaves of *Cassia floribunda* afforded N^1,N^8-dibenzoylspermidine, physcion, 5,7′-biphyscion and 5,7′-physcion-physcionanthrone whose structures were determined by MS, ^{1}H and ^{13}C NMR spectral analysis as well as by chemical methods.

INTRODUCTION

The genus *Cassia* is known to possess important medicinal properties and is a rich source of anthraquinones and flavonoids. *Cassia floribunda* Cav. (syn. *C. laevigata*) is one of the *Cassia* species growing in Ethiopia. The pods, roots and seeds of this plant have yielded the 8-mono-, and digalactosides of physcion as well as chrysophanol and emodin [1–3]. Flavonoids including ombuin and quercetin have also been reported from the roots, leaves and seeds [4–6]. Our investigation of the leaves of *C. floribunda* has resulted in the isolation and identification of two new bianthraquinones for which the names floribundone-1 (**1**) and floribundone-2 (**2**) are suggested, in addition to physcion (**3**) and N^1,N^8-dibenzoylspermidine (**4**). The spermidine alkaloid (**4**) has not been reported from natural sources previously.



1 R = O
2 R = H_2



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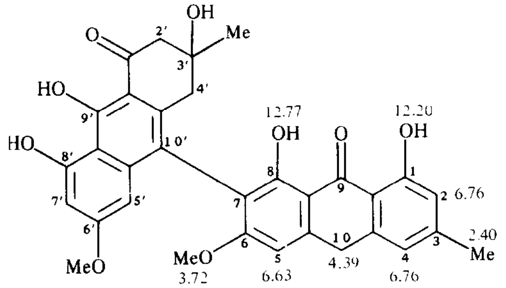
RESULTS AND DISCUSSION

The chloroform extract of the leaves of *C. floribunda* was chromatographed to afford the common anthraquinone, physcion (**3**) (see Experimental) and an orange pigment, which was shown by MS and ^{1}H NMR to be a mixture of two closely related compounds. These two compounds could not be separated, even though the mixture can be slightly resolved on oxalic acid-impregnated TLC (silica gel, benzene). This mixture was dissolved in aqueous potassium hydroxide (0.2 M), followed



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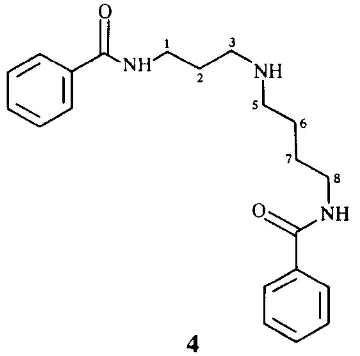

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by acidification with concentrated hydrochloric acid and extraction with chloroform to yield only one of the components of the mixture, floribundone-1, for which structure **1** is proposed. This compound shows the characteristic colour reactions of hydroxyanthraquinones. Reductive cleavage with alkaline sodium dithionite gave only physcion (TLC comparison). The UV-VIS absorption of **1** resembles that of physcion [7]. The IR spectrum of **1** showed a weak shoulder at 1665 cm^{-1} and an intense band at 1640 cm^{-1} for the non-chelated and chelated carbonyl groups. The MS showed a molecular ion at *m/z* 566, which corresponds to a dehydrodimer of physcion (*m/z* 535), which may be formed by loss of methoxy from M$^+$ and cyclization of the 8′-hydroxyl group onto C-6, and an ion at *m/z* 283 which corresponds to the physcion monomer. The ^{1}H NMR of **1** showed four distinct peaks in the chelated hydroxyl region, six aromatic proton signals, two methoxy signals and two methyl signals indicating 14 aromatic protons in the parent compound. Since anthraquinone has only eight aromatic protons, it follows that compound **1** has to be a bianthraquinone, with a molecular formula of $C_{32}H_{22}O_{10}$, which is in agreement with the value 566.1223 obtained for [M]$^+$ by HRMS. Floribundone-1 (**1**) and physcion (**3**) have similar ^{1}H NMR spectra. The chemical shifts for the protons of **1** were assigned using the data for physcion from ref. [8].

The most striking difference between the spectrum of **1** and that of physcion is the presence of two singlets at δ7.57 (1H) and 6.83 (1H) in the spectrum of **1**, whereas the corresponding signals in the spectrum of physcion appear as doublets at δ7.32 and 6.60 ($J = 2.5$ Hz). The latter signals represent the H-5 and H-7 of physcion, respectively. This is consistent with a 5-7′ coupled bianthraquinone structure, which would give rise to the appearance of two singlets for the protons at positions 5′ and 7. There is a small upfield shift for H-4 and Me-3 probably due to the twisted conformation of the dimer which would result in the shielding of these protons by the other anthraquinone moiety. The DEPT spectrum of **1** showed four methyl signals at δ22.1, 22.2, 56.5 and 56.6, and five C-H signals at δ103.6, 104.6, 121.1, 123.9 and 124.4. The intensity of the C-H signal at δ121.1 is much higher than any of the other C-H signals probably representing two C-H signals. The observation, that out of the above signals for six C-Hs, only two have chemical shifts appropriate to C-5 and C-7 [9], further supports the 5-7′ coupling of the bianthraquinone structure.

An important observation made about the second pigment **2** was its transformation to floribundone-1 (**1**) by treatment with base and subsequent acidification. This is a reaction which often brings about the conversion of anthrones to anthraquinones. It was, therefore, suspected that floribundone-2 may be a physcion-(physcion-anthrone) dimer. The HRMS of the mixture of **1** and **2** shows ions at *m/z* 552.1415 ([M]$^+$ of **2** calc. 552.1420) and fragments at *m/z* 283 and 269 arising by cleavage of the 5-7′ bond of **2**. The ^{1}H NMR spectrum showed four signals integrating for four protons in the chelated hydroxyl region, six aromatic protons, two methoxy signals and two methyl signals. Signals at δ6.53 and 7.56 for H-7 and H-5, respectively, appear as singlets supporting the 5-7′ coupling of the bianthranoid system. The ^{1}H NMR signals of **2** were assigned by comparison with those reported for anhydrophlegmacin (**5**) [10] (Table 1).

The alternative structure which would have the oxo group at C-10 and the CH_2 group at C-10′ is ruled out from the ^{1}H NMR data since in this case the protons at positions 2 and 4 would have chemical shifts greater than δ7, and those at positions 2′, 4′, 5′, and 7 would resonate below δ7. The signal of the methylene protons at C-10 slightly overlap with the methoxy signals and appear at $\delta \approx 3.9$. Anthrone hydrogen signals are usually observed at δ4.3 [10]. The shielding of *ca* 0.4 ppm observed for these hydrogens in **2** may be due to the twisted conformation of the dimer about the 5-7′ bond, which would

Table 1. ^{1}H NMR data of physcion (**3**) (100 Mhz), floribundone-1 (**1**) and floribundone-2 (**2**) (400 MHz, $CDCl_3$ TMS as int. standard).

H	**3**	**1**	**2**
1-OH	12.26 *s*	12.10 *s*	12.30 *s*
2-H	7.04 *br s*	7.04 *br d*, $J = 2.0$	6.64 *br s*
3-Me	2.45 *s*	2.35 *s*	2.25 *s*
4-H	7.57 *br s*	7.42 *br d*, $J = 2.0$	6.52 *br s*
5-H	7.32 *d*, $J = 2.5$		
6-OMe	3.92 *s*	3.82^a *s*	3.85^a *s*
7-H	6.60 *d*, $J = 2.5$	6.83 *s*	6.53 *s*
8-OH	12.05 *s*	12.05 *s*	12.05 *s*
10-H_2			3.8–3.9†
1′-OH		12.20 *s*	12.10 *s*
2′-H		7.06 *br d*, $J = 2.0$	7.08 *br d*, $J = 2.0$
3′-Me		2.45 *s*	2.45 *s*
4′-H		7.67 *br d*, $J = 2.0$	7.66 *br d*, $J = 2.0$
5′-H		7.57 *s*	7.56 *s*
6′-OMe		3.85* *s*	3.75* *s*
8′-OH		13.10 *s*	13.10 *s*

J in Hz.

*May be interchanged.

†Not distinctly observed, probably overlapped by the OMe signals.

place them in the shielding zone of the other anthraquinone moiety. Models of bianthranoids **1** and **2** show that rotation about the 5-7′ bond is hindered, which should give rise to the biphenyl type chirality. This is confirmed by the optical activity of compound **1** (See Experimental).

The very strong CD-values show clearly that they are derived from the typical CD-coupling [11] between the two practically identical achiral chromophores. At the longest wavelength such a CD-couplet can be found with maxima at 465 nm ($\Delta\varepsilon_{max} = +10.8$) and 405 nm ($\Delta\varepsilon_{max} = -3.6$). It is not conservative, but $n \rightarrow \pi^*$-Cotton effects are expected also to appear in this range. Calculations of the transition moment vectors using Streitwieser's [12] modification and parameters for such systems suggested that the first two transitions are polarized nearly along the long axis of the anthraquinone moiety. The exciton theory [11] applied to this system gives a positive CD-couplet for P-helicity (according to Cahn–Ingold–Prelog nomenclature [13, 14]). Since this is found experimentally, it is possible to assign with confidence the absolute configuration of (+)-**1** as **P**. A variety of bianthranoids have been isolated from natural sources, but these are the first 5,7′-dimers based on physcion [15, 16].

The methanol extract, when subjected to the usual acid–base extraction and subsequent crystallization (see Experimental), yielded the spermidine alkaloid **4**. HRMS of **4** gave the molecular formula $C_{21}H_{27}N_3O_2$. The IR spectrum showed absorptions at 3500, 3300 and 1666 cm^{-1} for free and hydrogen bonded N-H and amide carbonyl groups. ^{13}C NMR showed 17 signals. In the DEPT spectrum seven CH_2, four quaternary and six C-H carbon signals were observed. However, among the six C-H signals there are four showing much higher intensities compared to the others. This suggests that each of them represents two carbons, so that we can assume the compound contains 10 C-H groups. Thus, the total number of carbons is 21 as indicated by the HRMS. Two of the four signals for quaternary carbons, viz. those at δ 167.48 and 167.23, are assignable to amide carbonyls. The 400 MHz ^{1}H NMR spectrum showed signals at δ1.5–1.7 (6H, *m*, H-2, H-6, H-7), 2.1 (1H, *s*, H-4), 2.65 (2H, *t*, $J = 6.5$ Hz, H-5), 2.75 (2H, *t*, $J = 6.5$ Hz, H-3), 3.4 (2H, *m*, H-8), 3.5 (2H, *m*, H-1), 6.65 (1H, *br s*, H-NC^8), 7.3–7.8 (10H, *m*, Ar-H), 7.93 (1H, *br s*, H-NC^1) corresponding to a total of 27 protons. The proton chemical shifts for this compound were assigned with the help of a two-dimensional ^{1}H, ^{1}H-COSY experiment. The signals of the CH_2 groups at δ3.4 and 3.5 appear as multiplets due to coupling with the neighbouring amide protons and the CH_2 groups. It is interesting that the two protons on each of the amide nitrogens are nonequivalent, resonating at δ7.93 and 6.65.

Hydroxybenzoyl and hydroxycinnamoyl derivatives of spermidine have been reported as metabolites of microorganisms [17] and higher plants [18]. To our knowledge the spermidine alkaloid **4** is the first example of a simple benzoyl derivative from a natural source. Its synthesis has been reported by Bergeron *et al.* [19]. A simple cinnamoyl spermidine derivative, N^1,N^8-dicinnamoyl spermidine, has also been reported from *Maytenus chuchuuasha* [20].

EXPERIMENTAL

General. The following solvent systems were used for chromatographic separations: EtOAc–C_6H_6 (1:4) (solvent 1), petrol–EtOAc (9:1) (solvent 2), $CHCl_3$ (solvent 3), $CHCl_3$–MeOH (2:1) (solvent 4), $CHCl_3$–EtOH–Et_2NH (70:30:1) (solvent 5).

Plant material. Leaves of *C. floribunda* were collected from the experimental garden, Department of Chemistry, Addis Ababa University. Voucher specimen of the plant is deposited at the National Herbarium, Addis Ababa University.

Extraction and isolation. (A) The powdered leaves of *C. floribunda* (300 g) were soaked in HOAc (5%) and extracted with petrol (10 l, cold). The marc was then extracted with $CHCl_3$ (5 l cold). Drying and evapn of the $CHCl_3$ extract gave a gummy dark residue (14 g, 4.6%), which on TLC (solvent 3) showed 5 spots, of which the one with R_f 0.62 was the major constituent. This crude residue was chromatographed on silica gel column using solvent 3 as eluent. The first fraction gave a yellow pigment (R_f 0.92 in solvent 3, R_f 0.46 in solvent 2) which was readily identified as physcion (**3**) by direct comparison with an authentic specimen. The second fraction gave an orange powder (600 mg). This was purified by chromatography on Sephadex (LH-20) using solvent 4 and subsequently by prep. TLC (solvent 3) to yield 12 mg (0.0004%, mp > 260°) of an orange pigment as a mixture of **1** and **2**. This mixture was dissolved in aq. KOH (0.2 M) and left to stand for 30 min. Acidification with conc. HCl and extraction with $CHCl_3$ gave only **1**, mp > 260°.

(B) The dried and powdered leaves of *C. floribunda* (500 g) were extracted with petrol (3 l) and then with MeOH (7 l). The residue obtained after evaporation of the MeOH was extracted with aq. HCl (1 %) which was treated with ammonia (25%) until alkaline. Extraction of the basic soln with $CHCl_3$ followed by drying (Na_2SO_4) and evapn yielded a dark residue which was again extracted with aq HCl (1%). This acidic soln was repeatedly extracted with $CHCl_3$ until the aq. phase was almost colourless. Basification of the aq. phase with ammonia (25%) and extraction with $CHCl_3$, drying and concn gave a colourless solid (180 mg, 0.36%). TLC (silica gel, solvent 5) showed a Dragendorff-positive spot (R_f 0.42) to be the major constituent. This was dissolved in a mixture of EtOH/EtOAc and cooled to give 30 mg colourless needles of **4**, mp 133–134° (lit. 130–133° [18]).

1,1′8,8′-*Tetrahydroxy*-6,6′-*dimethoxy*-3,3′-*dimethyl*-(5,7′-*bianthracen*)-9,9′,10,10′-*tetraone, floribundone*-1 (**1**). Orange fine crystals (MeOH), mp > 260°, optical rotation $[\alpha]^{18} + 130$ (589), + 148 (578), + 244 (546), + 234 (436), + 234 (365 nm) ($CHCl_3$; *c* 0.05). UV, $\lambda_{max}^{CHCl_3}$ (log ε): 238 (4.61, sh), 257 (4.62, sh), 284 (4.75), 447 nm (4.50). CD: $\lambda_{max}(\Delta\varepsilon)$: 465 (+ 10.8), 406 (− 3.6), 296 (− 20.8), 278 (+ 31.0), 250 (− 12.6), 221 (− 24.3); IR ν_{max}^{KBr}: 1610, 1640, 1665 (sh) cm^{-1}; HRMS: 566.1223 (M^+, calc. for $C_{32}H_{22}O_{10}$: 566.1213); EIMS 70 eV, *m/z* (rel. int.): 566 [M^+] (76), 535 (100), 517 (12), 297 (14), 283 (10). ^{1}H NMR: see Table 1. ^{13}C NMR (100.4 MHz, $CDCl_3$): δ191.05, 182.58, 166.08, 164.64, 162.43, 161.14, 148.42, 148.35, 124.43, 123.87, 121.10, 113.14, 104.60, 103.64, 56.61, 56.48, 22.19, 22.13.

Mixture of floribundone-1 (**1**) *and* 1,1′,8,8′-*tetrahydroxy*-6,6′-*dimethoxy*-3,3′-*dimethyl*-(5,7′-*bianthracen*)-(10*H*)-9,9′,10′-*trione*, (**2**). HRMS: 566.1223 (M^+ for **1**; calc. for $C_{32}H_{22}O_{10}$: 566.1213); 552.1415 (M^+ for **2**; calc. for $C_{32}H_{24}O_9$: 552.1420); EIMS (70 eV) *m/z* (rel. int.): 566 (80), 552 (100), 535 (86), 517 (16), 297 (54), 283 (18), 269 (12), ^{1}H NMR of **2** see Table 1.

Reductive cleavage of **1**. To a soln of **1** (2 mg) in 2 ml aq. NaOH (5%), sodium dithionite (10 mg) was added and left for 1 hr. The soln was then acidified and extracted with $CHCl_3$, dried, and evapd to give only physcion.

N^1,N^8-*Dibenzoylspermidine* (**4**). HRMS: 353.2103 (calc. for $C_{21}H_{27}N_3O_2$: 353.2103); EIMS (70 eV) *m/z* (rel. int.): 353 (4), 219 (7), 205 (21), 191 (40), 174 (9), 134 (7), 105 (100); IR ν_{max}^{KBr}: 3500, 3300, 1666 cm^{-1}; ^{1}H NMR see text; ^{13}C NMR (100.4 MHz, $CDCl_3$): δ27.42, 27.47, 28.42, 39.78, 39.84, 48.78, 49.35, 126.80, 126.83, 128.34, 128.44, 131.11, 131.25, 134.74, 134.79, 167.23, 167.48.

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