through the stem by the procedure described previously [1]. After 2 days of incubation in the greenhouse, the young shoot was harvested and analysed.

Extraction and isolation of a new limonoid. Limonoids were extracted from 15 seedlings (82 g) by procedures previously described [1]. The total extract was placed on the top of a silica gel column (1.6 × 18 cm). The column was eluted, stepwise, by increasing concns of EtOAc in hexane; 500 ml each of 35, 40, 45, 50 and 55% EtOAc. Fractions containing the compound of interest were still contaminated with nomilin. Therefore, the combined fractions were further purified by a preparative TLC plate, which was developed with solvent system b (see below).

Analysis of limonoids. The isolate obtained above was analysed by TLC, and ^{1}H NMR and ^{13}C NMR spectra. NMR spectra were obtained with a JEOLFT spectrometer, JNM-GX 270 WB. Labelled metabolites obtained from [^{14}C]acetate feeding were analysed with a Berthold Automatic TLC-linear Analyzer LB 2832. TLC plates were developed with solvent systems: (a) EtOAc–cyclohexane (3:2); (b) CH_2Cl_2–MeOH (97:3) and (c) EtOAc–CH_2Cl_2 (2:3). Limonoids were revealed by spraying plates with Ehrlich's reagent followed by HCl gas exposure [7].

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A BIANTHRAQUINONE AND A TRITERPENOID FROM THE SEEDS OF *CASSIA HIRSUTA*

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Key Word Index—*Cassia hirsuta*; Leguminosae; seeds; 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone); pentacyclic triterpernoid sapogenol; isohopane series; 3β,16β,22-trihydroxyisohopane.

Abstract—From the seeds of *Cassia hirsuta* a new bianthraquinone, 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone) and a triterpenoid 3β,16β,22-trihydroxyisohopane have been isolated.

INTRODUCTION

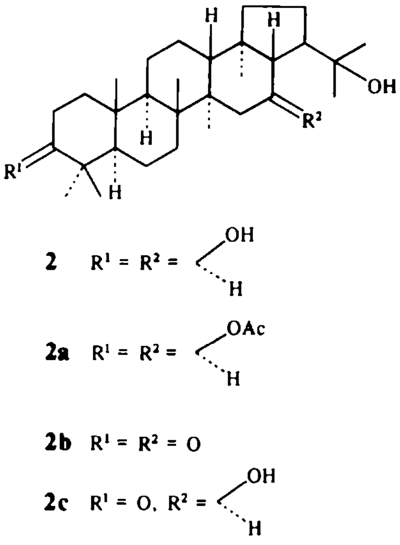
Species of *Cassia* are rich sources of flavonoids [1], anthraquinones [2] and polysaccharides [3] and the plant possesses important medicinal properties [4].

RESULTS AND DISCUSSION

A yellow crystalline pigment (**1**), $C_{32}H_{22}O_{12}$, ($[M]^+$ at m/z 598), mp > 300° (dec.) and a triterpenoid (**2**), $C_{30}H_{52}O_3$, ($[M - H_2O]^+$ at m/z 442), mp 211° were isolated from the ethyl acetate extract of the defatted seeds of *Cassia hirsuta*.

Compound **1** contained two methyl groups, two methoxyl groups and gave a pink colour with methanolic magnesium acetate [5] for the presence of a chelated (α-hydroxy) hydroxyl group. The solubility of the compound in 5% aq. sodium bicarbonate and a peak at 3440 cm^{-1} in its IR spectrum showed the presence of at least one β-hydroxyl group. A medium band at 2910 and 2890 cm^{-1} indicated the presence of a methoxyl and a methyl group, respectively. Sharp and strong bands at 1630 and 1668 cm^{-1} suggested the presence of chelated and non-chelated carbonyl groups. The λ_{max}^{EtOH} at 260, 390 and 440 nm indicated that **1** was a polyhydroxy anthraquinone [6, 7].

On acetylation, a hexaacetyl derivative, $C_{44}H_{34}O_{18}$ (**1a**), mp 213°, was obtained. The IR spectrum of **1a** showed a phenolic acetate band at 1770 cm^{-1} and a non-chelated carbonyl at 1668 cm^{-1}. Thus, out of twelve oxygen atoms in compound **1**, four occur as quinone carbonyl groups, which are both chelated (1630 cm^{-1}) and non-chelated (1668 cm^{-1}) as revealed from a com-

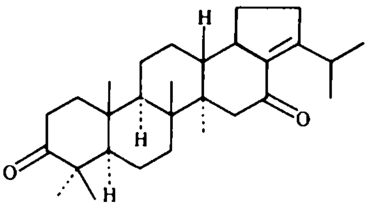


2 $R^1 = R^2 =$

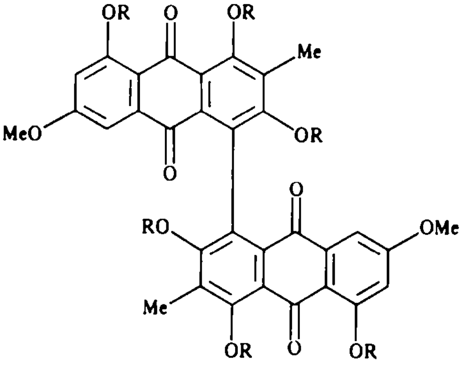
2a $R^1 = R^2 =$

2b $R^1 = R^2 = O$

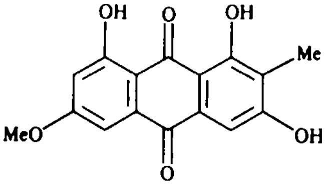
2c $R^1 = O$, $R^2 =$



2d



1 R = H
1a R = Ac



1b

parison of the IR spectra of **1** and **1a**. This confirmed the presence of a bianthraquinonoid chromophore in **1**. The mass spectrum in addition to the molecular ion peak at *m/z* 598 had strong peaks at [M − 17], [M − 18], [M − 75] and [M − 103] exhibiting the loss of water and multiple loss of carbon monoxide typical of hydroxyl anthraquinone [8]. The high molecular weight of **1** ($[M]^+$ at *m/z* 598) also supported the bianthraquinonoid nature of **1**.

Reductive cleavage of **1** with lithium aluminium hydride or with alkaline sodium dithionite [9] gave **1b**, $C_{16}H_{12}O_6$, mp 250°, as the only product. Compound **1b** was identified as 1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone by colour reactions [10, 11], UV, IR, NMR, MS and co-chromatography with a natural sample [12]. This suggests that **1** is the dehydro dimer of **1b**, $C_{16}H_{12}O_6$.

The ^1H NMR spectrum of **1a** showed signals at $\delta 2.10$ (*s*, 6H, 2 × C–Me), 3.89 (*s*, 6H, 2 × OMe), 2.45 (*s*, 6H, 2 × Ac), 2.40 (*s*, 6H, 2 × Ac), 2.34 (*s*, 6H, 2 × Ac), 7.65 (*d*, 2H, *J* = 2.5 Hz, H-5, H-5′) and 6.84 (*d*, 2H, *J* = 2.5 Hz, H-7, H-7′). Comparison with the NMR spectrum of the acetate of **1b** [signals at $\delta 2.10$ (*s*, 3H, C–Me), 3.89 (*s*, 3H, OMe), 7.89 (*s*, 1H, H-4), 7.67 (*d*, 1H, *J* = 2.5 Hz, H-5), 6.85 (*d*, 1H, *J* = 2.5 Hz, H-7), 3.89 (*s*, 3H), 2.43 (*s*, 3H), 2.39 (*s*, 3H) (each due to Ac)] confirms that **1** is a symmetrical dehydro dimer of **1b**.

In the ^1H NMR spectrum of **1a** a singlet at $\delta 7.89$ (assigned to H-4) is absent, showing a C-4, C-4′ inter anthraquinone linkage. Thus the compound is 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone).

Compound **2**, $C_{30}H_{52}O_3$ ($[M - H_2O]^+$ at *m/z* 442), mp 211°, gave all the colour tests for a triterpenoid and its IR spectrum showed a peak at 3240 cm^{-1} for hydroxyl groups. Acetylation gave a monohydroxy diacetate (**2a**), $C_{34}H_{56}O_5$, ($[M]^+$ at *m/z* 544), mp 225°. The IR spectrum of **2a** showed peaks at 3600 (tertiary-OH), 1735, 1755 and 1240–1250 cm^{-1} (acetoxy). The ^1H NMR spectrum (300 MHz, $CDCl_3$) of **2a** showed signals at $\delta 0.85$ (3H, *s*, 18α-Me); 1.05 (3H, *s*, 4β-Me); 1.1 (3H, *s*, 4α-Me); 1.16 (3H, *s*, 10β-Me) and 1.19 (6H, *s*, 8β- and 14α-Me) for six tertiary methyl groups [13, 14]. Two methyl groups in a hydroxyisopropyl side chain (Me–C–(OH)–Me) appeared at $\delta 1.50$ (3H, *s*, C-29) and 1.52 (3H, *s*, C-30). The signals at $\delta 2.06$ (3H, *s*) and 2.08 (3H, *s*) were due to the two acetoxyl methyl groups (2 × OAc). The singlet at $\delta 2.92$ (1H) which disappeared with D_2O was due to the tertiary hydroxyl groups. The 3-axial (α) proton appeared as a doublet at $\delta 4.2$ (1H, $J = 7.5$ Hz). A weak multiplet at $\delta 5.55$ (1H) for the 16β (axial) proton, as is usually observed in 3β- and 16β-acetoxy triterpenes [15].

The mass spectrum of **2a** showed a molecular ion peak at m/z 544 together with peaks at m/z 526 $[M - H_2O]^+$, 466 $[M - H_2O - HOAc]^+$, 426 $[M - HOAc - 58]^+$, 424 $[M - 2 \times HOAc]^+$, 249, 59.

The oxidation of **2** with CrO_3–pyridine [16] gave a monohydroxy diketone (**2b**), mp 232°, and a dihydroxy monoketone (**2c**), $C_{30}H_{50}O_3$, mp 228°. The diketone **2b** readily isomerized to the α,β-unsaturated ketone **2d** by refluxing with EtOH-HCl (5%); $C_{30}H_{46}O_2$, mp 234°. The easy isomerization indicated the presence of two other hydroxyl groups at C-16 (secondary) and C-22 (tertiary), respectively. Easy acetylation indicated the two secondary hydroxyl groups of **2** appeared to be equitorial (β). On considering all the above facts compound **2** may be represented at $3\beta,16\beta$,22-trihydroisohopane. This is also confirmed by mp, mmp and co-chromatography with an authentic sample obtained from the flowers of *Mollugo hirta* [17].

EXPERIMENTAL

Dried and crushed seeds were extracted with refluxing petrol and then with refluxing EtOAc and concd. The concd extract contained five spots (silica gel TLC) which were separated by a silica gel column using different solvents. The EtOAc fraction gave a single spot on TLC (silica gel, C_6H_6-EtOAc, 4:1), yellow compound (**1**) crystallized from EtOAc–petrol (1:3) mp > 300°. (Calc.: C, 64.21; H, 3.68. Found: C, 64.20; H, 3.70%). UV $\lambda_{\max}^{\text{EtOH}}$ nm: 240, 290, 430. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3340, 2960, 2800, 1675, 1630, 1150. The acetate was prepared with Ac_2O–pyridine mp 213°. UV $\lambda_{\max}^{\text{EtOH}}$ nm: 260, 290, 440. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1770, 1663, 1668, 1452, 1348.

Vacuum dried product **1** (100 mg) was dissolved in aq. 1 N Na_2CO_3 (200 ml) and a soln of sodium dithionite (20 mg) in H_2O (100 ml) was added through a filter paper. After gentle shaking for 5 min a vol. of 1 N HCl equivalent to the Na_2CO_3 present was cautiously added. The yellow solid (**1b**) was precipitated and crystallized from HOAc, mp 250°.

The C_6H_6–MeOH (4:1) eluate of the silica gel column gave a single spot on TLC (silica gel, EtOAc) of compound **2**, crystallized with $CHCl_3$-MeOH, mp 211°. (Calc.: C, 78.21; H, 11.38. Found: C, 78.01%; H, 11.16%.) The acetate was prepared with Ac_2O–pyridine, mp 225°. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3600, 1755, 1735, 1240, 1250. ^1H NMR (300 MHz, $CDCl_3$): $\delta 0.85$ (3H, *s*, Me), 1.05 (3H, *s*, Me), 1.10 (3H, *s*, Me), 1.16 (3H, *s*, Me), 1.19 (6H, *s*, 2 × Me), 1.50 (3H, *s*, H-29), 1.52 (3H, *s*, H-30), 2.06 (3H, *s*) and 2.08 (3H, *s*), both due to acetoxy methyl groups (2 × OAc), 4.2 (1H, *d*, $J = 7.5$ Hz), 5.55 (*s*, 1H).

Oxidation of compound **2**. A cold soln of **2** in dry pyridine (5 ml) was added to a slurry of CrO_3–pyridine complex at 0° with stirring and left overnight at room tmep. The reaction mixture was poured into ice and the recovered product crystallized from petrol–$CHCl_3$, mp 232°.

α,β-Unsaturated ketone **2d** *from* **2b**. Compound **2b** was dissolved in dry EtOH (4 ml) and dry EtOH-HCl (5%, 4 ml) was added. The soln was refluxed for 3 hr. The reaction mixture was poured into ice and the recovered product crystallized from MeOH, mp 234°.

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