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THE ANTHRAQUINONES OF *MELANOXYLON BRAUNIA**

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Abstract—The wood and bark of *Melanoxylon braunia* Schott (Leguminosae-Ceasalpinioideae) yielded β-sitosterol, lupeol, lupenone, betulinic acid, 6,8-di-*O*-methylemodin (Ia), 6,8-di-*O*-methyl-ω-hydroxyemodin (Ib), 1,7-dihydroxy-6,8-dimethoxy-3-methylanthraquinone (IIa) and 8-methoxy-3-methyl-1,2,6,7-tetrahydroxyanthraquinone (IIIe).

INTRODUCTION

Melanoxylon braunia Schott (Leguminosae–Caesalpinioideae) is a large tree with heavy, resistant wood whose dark brown colour with even darker striping gives it great beauty. In central Brazil, it is used for railroad ties, posts and bridges.[3]

'Braúna' bark, softwood and heartwood were extracted separately with volatile solvents. A combination of chemical and chromatographic methods led to the separation of β-sitosterol, lupeol, lupenone, an orange and a red pigment from the bark extract; and of betulinic acid, β-sitosterol and two additional orange pigments from the softwood extract. One of these latter pigments is by far the most abundant extractive of the heartwood, which also contains β-sitosterol.

RESULTS

The Pigments of 'Braúna' Wood

The two orange pigments of 'braúna'-wood were identified through spectral analysis as 6,8-di-*O*-methylemodin (Ia) and 6,8-di-*O*-methyl-ω-hydroxyemodin (Ib). The identification of (Ia) was confirmed by direct comparison with an authentic sample.[4] The identification of the other pigment was confirmed through direct comparison of its diacetate with the diacetate Ic prepared by acetylation of Ia to Id, followed by benzylic bromination (Id → Ie), and substitution of the bromine by acetate. Ib, although apparently a new natural compound, has been obtained previously by methylation of questinol, a metabolite of the fungus *Penicillium frequentans* Westling.[5]

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The Pigments of 'Braúna' Bark

The two pigments isolated from the bark were also classified as anthraquinones upon inspection of their u.v., i.r. and NMR spectra. The orange pigment was recognized as containing two hydroxyl groups through the formation of a diacetate. One of the hydroxyls must be located at C-1, since it forms a chelate bridge with one of the carbonyls (ν_{max} 1642 cm^{-1}; u.v. spectral shift upon addition of $AlCl_3$). The other hydroxyl must be conjugated with the unchelated carbonyl (ν_{max} 1664 cm^{-1} weak shoulder as for other polyhydroxyanthraquinones;[6] u.v. spectral shift upon addition of NaOAc). The unperturbed carbonyls of anthraquinone give rise to a maximum at 1675 cm^{-1}.[7] Of the two positions which would allow conjugation of a hydroxyl with the unchelated carbonyl, C-2 cannot represent the correct alternative. A hydroxyl at C-2 would entail the existence of an *ortho*-dihydroxy group, which is absent (no alteration of the u.v. spectrum upon addition of H_3BO_3 + AcONa). The hydroxyl must, therefore, be located at C-7.

The formulation of this 1,7-dihydroxyanthraquinone had to be completed by allocation of two methoxyls, one methyl and three aromatic protons, structural features whose presence was indicated by the NMR spectrum. A more detailed examination of this spectrum showed that two of the aromatic protons were not only *meta*-related, but also both spin–spin coupled to the benzylic hydrogens of the C-methyl group. A CH—CCH_3=CH system fitted only into the 1-hydroxylated ring, leaving the three positions of the 7-hydroxylated ring to the two methoxy groups and the third aromatic proton. This proton should be located at C-5 *meta* to the 7-OH group, since only a slight paramagnetic shift of the corresponding singlet occurred upon passing from the NMR spectrum of the pigment to the spectrum of its acetate. The acetylation reaction can thus be formulated only as IIa → IIb. The mass spectrum favoured placement of the methoxyls as represented in IIa. The *para*-relation of one of these groups with a carbonyl would rationalize the observed facile loss of 15 a.m.u., while the *ortho*-relation of the other with a carbonyl would explain the loss of the elements of water.[8] 1,7-Dihydroxy-6,8-dimethoxy-3-methylanthraquinone (IIa), although as far as we know a new compound, has the same substitution pattern as dermoglaucin (IIc), a metabolite of the fungus *Cortinarius sanguineus* Fr.[9]

The mol. wt. of the red pigment, determined by mass spectrometry, was consistent with a methoxy-methyl-tetrahydroxyanthraquinone structure. The substitution pattern became clear when it was found that hydrolysis with sulfuric acid led to 3-methyl-1,2,6,7,8-pentahydroxyanthraquinone (IIIa) which could be acetylated to 3-methyl-1,2,6,7,8-pentaacetoxyanthraquinone (IIIb) and methylated with dimethyl sulfate to 3-methyl-1,2,6,7,8-pentamethoxyanthraquinone (IIIc). The formulae IIIa, b and c represent known compounds whose published data[10] agree with the data obtained for the derivatives of the red 'braúna' pigment. While dimethyl sulfate methylation of the original pigment also yielded IIIc, diazomethane methylation afforded a sole monohydroxyanthraquinone (later formulated IIId). This indicates the presence of only one hydroxyl at one of the two oxygenated *peri*-positions. Again, as in the previous case, the mass spectrum was consistent with the presence of a methoxyl at a *peri*-position. A fragment of high relative abundance, resulting

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through the loss of the elements of water from the molecular ion, was observed. The evidence leads to structure IIIe or IIIf. The former is preferred as the pigment is stable in alkaline solution.

Confirmation of the relative position of the hydroxyls and the methoxyl in IIIe was adduced by NMR spectrometry. The H-4 and H-5 signals are easily distinguished by their width at half height. This width is considerably larger in the case of the H-4 band, due to the benzylic coupling of the corresponding proton to the protons of the methyl group at C-3. The shifts of these signals, upon passing from the methyl ether IIIc to the acetate IIIg, can thus be followed unambiguously. As Table 1 shows, these shifts are quite different. The

TABLE 1 *O*-METHYL AND *O*-ACETYL DERIVATIVES OF IIIe AROMATIC REGION OF 60 MHz-NMR SPECTRA IN $CDCl_3$

	Peak-width at half height (ppm)		Chemical shift (τ)		Paramagnetic shift (ppm)
	IIIc	IIIg	IIIc	IIIg	Δ(IIIc-IIIg)
H-4	1·6	2 4	2 43	1 91	0 52
H-5	1·0	1 9	2 37	2 08	0 29

four hydroxyls thus are not arranged symmetrically at positions 1,2 and 7,8 and the methoxyl cannot be located at C-6. Furthermore, this paramagnetic shift is relatively small for the H-5 signal. Thus the acetylation should not have occurred at the *ortho* and *para*-positions, and the methoxyl cannot be located at C-7.

Again, although 8-methoxy-3-methyl-1,2,6,7-tetrahydroxyanthraquinone (IIIe) seems to be a new compound, anthraquinones of identical substitution pattern have been previously isolated from *Cassia obtusifolia* L.[10]

EXPERIMENTAL

For experimental techniques see Ref. 1. The direct comparison of samples included co-chromatography, mixed m p. and i r. spectra.

Isolation of 'Braúna' Constituents

Benzene extraction of the bark of M. braunia. The powdered bark (2 0 kg) was continuously extracted with hot benzene The solvent was evaporated and the residue (8 g) was chromatographed on silica (250 g), giving the following fractions with the indicated eluants: A_1 (benzene), A_2 (benzene–$CHCl_3$ 8·2), A_3 (benzene–$CHCl_3$ 1.1), A_4 (benzene–$CHCl_3$ 2 8), A_5 ($CHCl_3$), A_6 ($CHCl_3$–MeOH 95:5), A_7 ($CHCl_3$–MeOH 8 2), A_8 (MeOH) A_1, upon addition of AcOEt, gave a crystalline cmpd , m.p 81–83°, of aliphatic nature which was not further examined A_2 and A_3 gave β-sitosterol (300 mg) A_4 and A_5 gave lupenone (73 mg) A_6 gave lupeol (700 mg) A_7 was recrystallized from EtOH, yielding IIa (30 mg) A_8 gave betulinic acid (500 mg)

Ethanol extraction of the bark of M braunia The ground wood, after extraction with hot benzene (see above), was continuously extracted with EtOH. The solvent was evaporated and the residue (12 g) was washed successively with aq 20% $NaHCO_3$, 10% Na_2CO_3, and 5% NaOH The $NaHCO_3$-solution was acidified and repeatedly extracted with EtOAc The organic solution was washed with water, dried and evaporated The residue was recrystallized from MeOH, yielding IIIe (800 mg) The Na_2CO_3-solution was acidified and extracted with $CHCl_3$ The organic solution was washed with water, dried and evaporated. The residue was recrystallized from $CHCl_3$–MeOH (1 2) yielding Ia (50 mg) The NaOH solution, treated similarly, did not lead to a pure compound

Benzene extraction of the softwood of M braunia The powdered softwood (10 kg) was continuously extracted with hot benzene The solvent was evaporated and the residue (22 g) was chromatographed on

silica (500 g), giving the following fractions with the indicated eluants: B_1 (benzene), B_2 (benzene–$CHCl_3$ 7:3), B_3 (benzene–$CHCl_3$ 2·8), B_4 ($CHCl_3$), B_5 ($CHCl_3$–MeOH 95:5), B_6 ($CHCl_3$–MeOH 1·1), B_7 (MeOH) B_1 gave the same cmpd as A_1. B_2 did not give a pure cmpd. B_3 gave β-sitosterol (500 mg) B_4 and B_5 were united and rechromatographed on silica The fraction eluted with benzene–$CHCl_3$ gave β-sitosterol. The fraction eluted with $CHCl_3$ was recrystallized from $CHCl_3$–MeOH 1 2, yielding Ia (300 mg) B_5 and B_6 were united and recrystallized from $CHCl_3$–MeOH, yielding Ib (30 mg) B_6 gave betulinic acid.

Benzene extraction of the heartwood of M. braunia. The ground heartwood (12 5 kg) was continuously extracted with hot benzene The solvent was evaporated and 10 g of the residue (16 g) were chromatographed on silica (250 g), giving the following fractions with the indicated eluants: C_1 (benzene), C_2 (benzene–$CHCl_3$ 9:1), C_3 (benzene–$CHCl_3$ 7·3), C_4 (benzene–$CHCl_3$ 2·8), C_5 ($CHCl_3$), C_6 ($CHCl_3$–MeOH 95·5), C_7 (MeOH) C_1 gave the same cmpd. as A_1. C_3 gave β-sitosterol (300 mg). C_4 did not give a pure cmpd. C_5 and C_6 were united and rechromatographed on silica. The central fractions were recrystallized from $CHCl_3$–MeOH 1·2, yielding Ia (500 mg)

Properties of 'Braúna' Constituents

6,8-*Dimethoxy*-1-*hydroxy*-3-*methylanthraquinone* (Ia) Orange needles, m p 211–213° (lit.[4] m p 213–214°). Acetate (Id) m p. 204–206° Methyl ether (If) m p 230–232° (lit.[5] m p 230–232°).

6,8-*Dimethoxy*-1-*hydroxy*-3-*hydroxymethylanthraquinone* (Ib). Orange needles, m.p 237–239° (lit.[5] 238–240°)

1,7-*Dihydroxy*-6,8-*dimethoxy*-3-*methylanthraquinone* (IIa). Orange needles, m p. 238–240°. λ_{max}^{EtOH} 220, 245 sh, 284, 410 nm (ε resp. 30, 600, 11,000, 32,500, 7500); $\lambda_{max}^{EtOH+NaOH}$ 235, 312, 405, 504 (ε resp. 20,400, 33,000, 4200, 7400); $\lambda_{max}^{EtOH+AlCl_3}$ 220, 281, 414 nm (ε resp 29,500, 34,400, 6600); $\lambda_{max}^{EtOH+AcONa}$ 253, 312, 394, 500 nm (ε resp. 17,300, 25,800, 5500, 4900). ν_{max}^{KBr} 3367 (phenolic OH), 1664 w sh (unchelated CO), 1642 s (chelated CO), 1587, 1563 cm^{-1} NMR (TFA, τ)· 2 28 (s, H-5), 2 32 (broad d, *J* 2 5 Hz, H-4), 2 77 (broad d, *J* 2 5 Hz, H-2), 5 85 (s, OCH_3), 5·86 (s, OCH_3), 7 50 (s, CH_3) Found: M (high resolution mass spectrum) 314 0788. $C_{17}H_{14}O_6$ requires M 314·0791 MS: M 314 (100%), *m/e* (%), 291 (91), 297 (16), 296 (15), 281 (15), 271 (20), 253 (11), 241 (6), 225 (6), 213 (6), 200 (18), 197 (7), 172 (6), 169 (6), 148 (14), 143 (11), 128 (12), 123 (12), 115 (19), 111 (15), 109 (16)

8-*Methoxy*-3-*methyl*-1,2,6,7-*tetrahydroxyanthraquinone* (IIIe) Red crystals, m.p. 330° dec. λ_{max}^{EtOH} 225, 290, 320, 420 nm (ε resp 17,700, 34,800, 15,800, 7400) $\lambda_{max}^{EtOH+NaOH}$ 239, 340, 490 nm (ε resp. 13,300, 41,100 9100) $\lambda_{max}^{EtOH+AlCl_3}$ 229, 315, 400 sh, 487 nm (ε resp 17,700, 34,500, 4400, 10,300). $\lambda_{max}^{EtOH+AcONa}$ 295, 317, 421 nm (ε resp. 22,900, 26,600, 7400). $\lambda_{max}^{EtOH+H_3BO_3+AcONa}$ 222, 301, 421 nm (ε resp. 20,900, 39,900, 8900) ν_{max}^{KBr} 3333 (phenolic OH), 1660 w sh (unchelated CO), 1636 s (chelated CO), 1587, 1504 cm^{-1} Found: M (high resolution mass spectrum) 316 0579. $C_{16}H_{12}O_7$ requires: M 316 0583. MS· M 316 (100%), *m/e* (%), 299 (17), 298 (89), 287 (12), 286 (9), 273 (38), 270 (17), 245 (18), 242 (42), 214 (15), 199 (7), 115 (22)

Derivatives of 'Braúna' Constituents

1-*Acetoxy*-3-*acetoxymethyl*-6,8-*dimethoxyanthraquinone* (Ic). A solution of Id (70 mg) *N*-bromosuccinimide (45 mg) and benzoyl peroxide (1 mg) in CCl_4 (20 ml) was heated under reflux and illumination for 24 hr. After cooling to room temp , the solution was filtered and evaporated. The residue (40 mg) was washed repeatedly with cold water, once with hot water and extracted with $CHCl_3$. The $CHCl_3$ solution was dried, filtered and evaporated The residue was purified by silica-column chromatography, affording Ie (23 mg), ν_{max}^{KBr} 1783, 1678, 1608, 1575, 1333, 1264, 1208, 1163, 1130, 1064, 951, 853, 826, 756 cm^{-1}. Ie (23 mg) and excess anhydrous KOAc in Ac_2O (10 ml) were heated under reflux (12 hr). After cooling to room temp., the mixture was poured into ice flakes. The precipitate was collected and extracted with $CHCl_3$ which was dried and evaporated. The residue was recrystallized from MeOH, affording Ic as yellow crystals, m.p 168–170°. λ_{max}^{EtOH} 230, 241, 280, 340, 405 nm (ε resp. 17,800, 20,600, 18,000, 2800, 4100). ν_{max}^{KBr} 1770, 1751, 1681, 1667, 1608, 1570 cm^{-1}

Direct acetylation of Ib also afforded Ic.

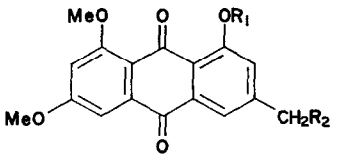
1,7-*Diacetoxy*-6,8-*dimethoxy*-3-*methylanthraquinone* (IIb) Was obtained from IIa as yellow crystals (from MeOH), m p 182–184° λ_{max}^{EtOH} 212, 265, 350 nm (ε resp 38,200, 53,700, 9200). ν_{max}^{KBr} 1783, 1681, 1610, 1587 cm^{-1}. RMN (TFA, τ): 2 22 (s, H-5), 2·28 (broad d, *J* 2 5 Hz, H-4), 2·75 (broad d, *J* 2 5 Hz, H-2), 5 83 (s, OCH_3), 5 87 (s, OCH_3), 7 52 (s, $COCH_3$), 7·77 (s, $COCH_3$).

3-*Methyl*-1,2,6,7,8-*pentahydroxyanthraquinone* (IIIa). IIIe (100 mg) in conc. H_2SO_4 (5 ml) was maintained at 100° for 90 min, and subsequently at room temp. for 24 hr. The solution was poured into ice flakes. The precipitate was separated by filtration, washed with water and crystallized from $CHCl_3$–MeOH 1:1 to red needles, m.p 326–329° dec. (lit.[10] m.p. 328–330° dec). λ_{max}^{EtOH} 235, 290, 325, 420 nm (ε resp. 9200, 23,500, 6100, 8400) ν_{max}^{KBr} 3500–3250, 1667 w sh (unchelated CO), 1629 s (chelated CO), 1587 cm^{-1}. Acetate (IIIb) m.p. 234–236° (lit.[10] m.p. 235–236°). Methyl ether (IIIc) m p. 130–132° (lit.[10] m.p. 132–133°).

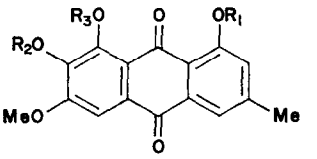
1-*Hydroxy*-3-*methyl*-2,6,7,8-*tetramethoxyanthraquinone* (IIId). IIIe was methylated with CH_2N_2 in ether, yielding IIId as yellow crystals (from EtOH), m.p. 176–178°. λ_{max}^{EtOH} 220, 280, 312, 419 nm (ε resp. 16,100, 29,500, 6900, 5200). $\lambda_{max}^{EtOH+NaOH}$ 269, 309, 515 nm (ε resp. 26,000, 7500, 4500). ν_{max}^{KBr} 1672 m (unchelated CO), 1637 s (chelated CO), 1587, 1493 cm^{-1} RMN ($CDCl_3$, τ): 2 37 (s, H-5), 2 43 (broad s, H-4)-5 95–6 02 (4 OCH_3), 7 62 (s, C-CH_3).

8-*Methoxy*-3-*methyl*-1,2,6,7-*tetraacetoxyanthraquinone* (IIIg). Was obtained from IIIe as yellow needles (from MeOH), m p. 180–182° ν_{max}^{KBr} 1795, 1692, 1600 cm^{-1}. RMN ($CDCl_3$, τ). 1 91 (broad s, H-4), 2 08 (s, H-5), 6·09 (s, OCH_3), 7·58 (s, C-CH_3), 7 62 (s, $COCH_3$), 7·66 (s, $COCH_3$)

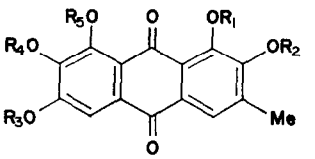
1-*Acetoxy*-3-*methyl*-2,6,7,8-*tetramethoxyanthraquinone* (IIIh) Was obtained from IIId as yellow needles (from MeOH), m.p. 159–161°. λ_{max}^{EtOH} 215, 250, 282, 348 nm (ε resp 17,000, 7300, 27,300, 7500) ν_{max}^{KBr} 1786, 1675, 1587, 1493 cm^{-1}



	R_1	R_2
Ia	H	H
b	H	OH
c	Ac	OAc
d	Ac	H
e	Ac	Br
f	Me	H



	R_1	R_2	R_3
IIa	H	H	Me
b	Ac	Ac	Me
c	H	H	H



	R_1	R_2	R_3	R_4	R_5
IIIa	H	H	H	H	H
b	Ac	Ac	Ac	Ac	Ac
c	Me	Me	Me	Me	Me
d	H	Me	Me	Me	Me
e	H	H	H	H	Me
f	Me	H	H	H	H
g	Ac	Ac	Ac	Ac	Me
h	Ac	Me	Me	Me	Me

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