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ISOLATION AND STRUCTURE OF TWO NEW DIHYDROISOCOUMARINS FROM *KIGELIA PINNATA**

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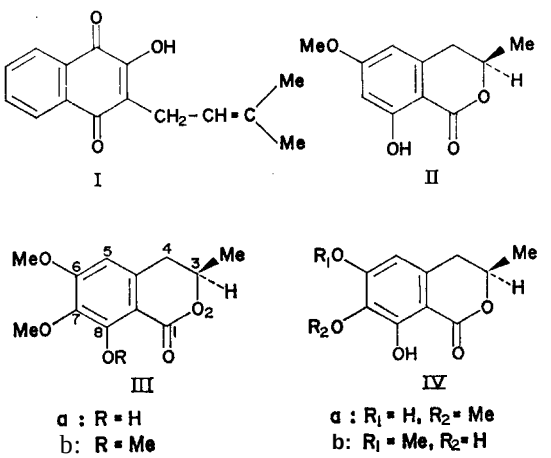
Abstract-Two new dihydroisocoumarins (IIIa and IVa) have been isolated from *Kigelia pinnata* and their structures established. Stigmasterol, β-sitosterol, lapachol and 6-methoxymellein were also identified in the roots and bark.

INTRODUCTION

Kigelia pinnata DC. (Syn. *K. africana* Benth.) (Bignoniaceae) is a small spreading tree with pendulous racemes of dull liver-coloured flowers and a long-stalked large gourd-like fruit. From its roots and bark we have isolated stigmasterol, β-sitosterol, the naphthoquinone lapachol (I), 6-methoxymellein (II) and two new phenolic compounds. We wish to present here evidence leading to structures (IIIa) and (IVa) for these two compounds.

RESULTS AND DISCUSSION

The constituents mentioned above were separated by chromatography over silica gel. The yellow compound, m.p. 140–141°, which appeared in the initial fractions, was identified as lapachol (I)' by comparison with an authentic sample. Lapachol is a known constituent of the wood of several species of the Bignoniaceae.



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The minor constituent, m.p. 76–77°, $C_{11}H_{12}O_4$, obtained in the chromatography had spectral properties in very close agreement with the reported values for (-) 8-hydroxy-6-methoxy-3-methyl-3,4-dihydroisocoumarin (II),' commonly referred to as 6-methoxy-mellein. This compound was first isolated[3] from carrots which had developed a bitter taste during storage. Subsequently this has been isolated from the cultures of the fungus *Sporormia bipartis* Cain[4] and *Sporormia affinis* Cacc., Bomm and Rouss.[2] The racemic form of (II) has been synthesized.[5] The identity of our compound was confirmed by direct comparison with authentic (—) 6-methoxymellein. The absolute configuration at C_3 is R since the circular dichroism (in MeOH) at 270 nm is negative, as in mellein.[6]

The major constituent of the plant which has m.p. 144°, [a],-79.91 °, is new and has been named kigelin. Its molecular formula $C_{12}H_{14}O_5$ was confirmed by the mass spectrum. The presence of a phenolic hydroxyl group was shown by the purple colour with $FeCl_3$ and its solubility in aqueous alkali. Its u.v., i.r. and NMR spectra showed obvious similarities with those of 6-methoxymellein (II). The NMR spectrum* of kigelin showed the presence of two methoxyls, one aromatic hydrogen, an Ar—CH_2—CH—Me unit as in 6-methoxy-

mellein and a chelated hydroxyl. The carbonyl group in kigelin appeared in the i.r. at 1665 cm-'. This was shifted to 1710 cm^{-1} in *O*-methylkigelin, obtained by methylation, indicating the presence of a chelated hydroxyl. The facile demethylation of *O*-methylkigelin with aluminium chloride in ether to give kigelin supports this conclusion.' These data indicated that kigelin is a 8-hydroxy-3-methyl-3,4-dihydroisocoumarin having two methoxyl groups in the aromatic ring. Oxidation of *O*-methylkigelin with potassium permanganate gave 3,4,5-trimethoxyphthalic acid identified as its *N*-methylimide.[8] This leads to structure (IIIb) for *O*-methylkigelin and (IIIa) for kigelin. The absolute configuration at C_3 follows from the negative CD at 271 nm.

The methylene hydrogens at C_4 of kigelin appear in $CDCl_3$ essentially as two lines centred at δ 2.87 and C_3-H as a multiplet at 8 4·7. The non-equivalence of the C_4 hydrogens is clearly shown in DMSO-d, in which they appear as the typical AB part of an ABX system. The C_3-H multiplet at δ 4·65 also shows more fine structure in DMSO-d_6.

The second new compound isolated from the plant has m.p. 148°, $[\alpha]_D$-80·78°. Its molecular formula $C_{11}H_{12}O_5$ was shown by its mass spectrum. Its u.v., i.r. and NMR spectra showed striking similarities to those of kigelin. Treatment of the compound with diazomethane for a short duration gave kigelin whereas more prolonged treatment gave *O*-methylkigelin. This leads to structure (IVa) or (IVb) for the compound. Compound (IVb) was obtained by the demethylation of Q-methylkigelin with excess boron trichloride. Boron trichloride[9] is known to cleave very readily aromatic methoxyls adjacent to a ketone group. Of the methoxyls at C_6 and C_7, the latter possessing a more electron-rich ethereal

* NMR spectra were recorded in $CDCl_3$ unless otherwise noted and δ values are given in ppm relative to TMS. Signals described are: s, singlet; d, doublet; m, multiplet.

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oxygen would be expected to cleave more readily than the former.[10,11] The demethylation product should hence be (Nb) and since it is different from the naturally occurring demethylkigelin, the latter should be (IVa). TLC examination of (IVa) and (IVb) on silica gel impregnated with 2 % sodium tetraborate[12] confirms that the latter has a catechol unit.

Kigelin and its congeners have close biogenetic and structural similarities to the ochratoxins,[13] reticulol,[14] canescin,[15] 6-hydroxyramulosin,[16] the chlorine-containing metabolites from *Sporormia affinis*[2] and actinobolin.[17]

EXPERIMENTAL

M.ps are uncorrected. Optical rotations were taken in 2–3% solutions in $CHCl_3$. U.v. spectra were determined in EtOH. Relative intensities of mass spectral peaks are given in parenthesis.

Isolation. The dried and powdered roots of *Kigeliapinnata (6* kg) were extracted with hot hexane and the extract concentrated and cooled. The solid that separated was filtered and crystallized from CH_2Cl_2–hexane to yield kigelin (IIIa) (1 g), colourless silky needles, m.p. 144°, $[\alpha]_D$ -79·91°, λ_{max} 221, 274, 308 nm (log ϵ 4.37, 4.16, 3·60), shifted to λ_{max} 230,274, 345 nm (log ϵ 4.36, 4.10, 3.72) on addition of NaOH, ν_{max} (CH_2Cl_2) 1665, 1620, 1580, 1120, 1040, 1010 cm^{-1}. NMR: δ 11·2 (1H, s, OH), 6.35 (1H, s, C_5—H), 4·7 (1H, m, C_3—H), 3.92 (3H, s, O-Me), 3.85 (3H, s, O-Me), 2.87 (2H, d, J = 9 c/s, C_4—H), 1·48 (3H, d, J = 6·5 c/s, C_3—Me). Mass spectrum: *m/e* 238 (M^+, 100), 223 (19), 220 (5), 209 (14), 205 (23), 195 (18), 191 (15), 179 (24), 177 (37), 163 (12), 151 (11). (Found: C, 60.29; H, 6.19. $C_{12}H_{14}O_5$ requires C, 60·50; H, 5·92%.) CD (in MeOH): λ_{max} 314,271, 245,226 nm ($\Delta\epsilon$ −0·562, −2·06, +1·5, -1.69).

The filtrate from kigelin was evaporated and the residue (33 g) chromatographed over silica gel in C_6H_6. The column was eluted successively with C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$ and finally $CHCl_3$–MeOH. 50 ml fractions were collected and monitored by TLC. The earlier fractions gave lapachol (I) (3 g), yellow needles (from CH_2Cl_2–hexane), m.p. 140–141°, giving a deep red colour with $FeCl_3$, $[\alpha]_D$ 0°, λ_{max} 253, 280, 330 nm (log ϵ 4·31, 4.18, 3·50), ν_{max} (CH_2Cl_2) 3410. 1655. 1605 cm^{-1}. NMR: δ 7·55–8·25 (4H, m), 7·5 (1H, OH), 5·25 (1H, t), 3·3 (2H, d), 1·77 (3H, s), 1·67 (3H, s). (Found: C, 7464; H, 5·95. Calc. for $C_{15}H_{14}O_3$: C, 74.36; H, 5·83%.) The compound was identical (mixed m.p., TLC. i.r.) with an authentic sample of lapachol.

Later fractions yielded 6-methoxymellein (II) (100 mg), colourless needles (from ether-hexane), m.p. 76–77°, $[\alpha]_D$—60·71°, λ_{max} 268, 302 nm (log • 4·00, 3·59), shifted to λ_{max} 233, 268, 335 nm (log ϵ 421, 3·80, 3·70) on addition of NaOH, ν_{max} (CH_2Cl_2) 1660, 1625. 1580 cm-'. NMR: δ 11·23 (1H, OH), 6.25 (2H, split singlets, J = 2·5 c/s), 4·62 (1H, m), 3·78 (3H, s), 2·8 (2H, d, J = 7 c/s), 1.45 (3H, d, J = 6' c/s). Mass spectrum: *m/e* 208 (M^+, 100), 193 (14), 190 (55), 179 (34), 165 (65), 164 (100), 162 (26), 147 (14), 145 (16). 136 (24), 119 (22), 91 (40), 78 (30), 69 (23), 65 (40). (Found: C, 63.99; H, 6.22. Calc. for $C_{11}H_{12}O_4$: C, 63·45; H, 5·81%.) The compound was identical (mixed m.p., TLC, i.r.) with an authentic sample of (−)-6-methoxymellein. CD (in MeOH): λ_{max} 301.270. 248. 232 nm ($\Delta\epsilon$ +0·72, −1·73, +0·72, -2.59).

Thefractions that followed gave more kigelin (1·5 g).

Later fractions gave stigmasterol (200 mg), identical (mixed m.p., TLC, i.r.) with an authentic sample.

The hexane extract of the bark (5 kg) on chromatography yielded kigelin (200 mg) and β-sitosterol, identical (mixed m.p., TLC, i.r.) with an authentic sample.

Extraction of the defatted bark with acetone and chromatography of the residue as above yielded more kigelin (0·5 g). The more polar fractions gave 6-demethylkigelin (IVa) (200 mg), pale yellow glistening plates (from $CHCl_3$–hexane), m.p. 148°, giving a purple colour with $FeCl_3$, $[\alpha]_D$—80·78°, λ_{max} 220, 275 nm (log • 1620, 1580 cm^{-1}, d to λ_{max} 246. 313 nm (log ϵ 4·28. 4·39) on addition of NaOH, ν_{max} (CH_2Cl_2) 3500. 1670. NMR: δ 11·4 (1H, s, OH), 6·77 (1H, s, OH), 6·33 (1H, s, C_5—H), 4·7 (1H, m, C_3—H), 3·95 (3H, s, O-Me), 2.88 (2H, d, J = 7·5 c/s, C_4—H), 1·5 (3H, d, J = 6·5 c/s, C_3—Me). Mass spectrum: *m/e* 224 (M^+, 100), 209 (32), 206 (48), 195 (14), 191 (32), 188 (26), 181 (12), 180 (10), 177 (12), 165 (54), 163 (58), 160 (14), 137 (25). (Found: C, 59.29; H, 5.53. $C_{11}H_{12}O_5$ requires C, 58.92; H, 5·40%.) R_f (in $CHCl_3$–5% MeOH): 0·60 (SiO_2), 0·58 (SiO_2 impregnated with 2% sodium tetraborate).

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O-*Methylkigelin* (*IIIb*) (a) ***With CH_2N_2.*** A soln of kigelin (100 mg) in MeOH was treated with excess ethereal CH_2N_2. After 48 hr, the product was chromatographed over Al_2O_3 in C_6H_6 to yield the methyl ether (80 mg), m.p. 114° (from ether-pentane), λ_{max} 218, 265, 290 (sh) nm (log ϵ 4.54, 4.09, 3·48), ν_{max} (CH_2Cl_2) 1710, 1600 cm^{-1}. NMR: δ 6·55 (1H, s, C_5—H), 4·53 (1H, m, C_3—H), 3·97 (3H, s, O-Me), 3.93 (3H, s, O-Me), 387 (3H, s, O-Me), 2.82 (2H, d, J = 7 c/s, C_4—H), 1.45 (3H, d, J = 7 c/s, C_3—Me). (Found: C, 62·28; H, 6.54. $C_{13}H_{16}O_5$ requires C, 61.89; H, 6·39%.)

(b) *With Me_2SO_4.* A soln of kigelin (1 g) in acetone (30 ml) was refluxed with anhydrous K_2CO_3 (5 g) and Me_2SO_4 (1.5 ml) for 24 hr, filtered and evaporated. Chromatography of the product over Al_2O_3 in C_6H_6 yielded 0-methylkigelin (0·7 g), identical (mixed m.p., TLC, i.r., NMR) with the above sample.

Methylation of 6-demethylkigelin (IVa). (a) *Partial methylation*: A soln of 6-demethylkigelin (100 mg) in MeOH (5 ml) was treated with ethereal CH_2N_2 (from 2 g nitrosomethylurea). After 3 hr, it was worked up to yield kigelin (IIIa) (60 mg), m.p. 144°, identical (mixed m.p., TLC, i.r.) with the natural sample.

(b) *Complete methylation.* A soln of 6-demethylkigelin (50 mg) in MeOH (2 ml) was treated with excess CH_2N_2 (from 5 g nitrosomethylurea). After 48 hr, workup yielded 0-methylkigelin (IIIb), m.p. 114°, identical (mixed m.p., TLC, i.r.) with the authentic sample.

Demethylation of O-methylkigelin (IIIb). *(a) With $AlCl_3$*: A soln of 0-methylkigelin (300 mg) in dry ether (50 ml) was refluxed with stirring with anhydrous $AlCl_3$ (1·5 g) for 1 hr, ether evaporated and dii. HCl added. Extraction with CH_2Cl_2 yielded kigelin (200 mg), identical (mixed m.p., TLC, i.r.) with the authentic sample.

(b) *With BCl_3.* A soln of 0-methylkigelin (100 mg) in CH_2Cl_2 (5 ml) was cooled to −50° and treated with BCl_3 (1 ml). After 24 hr at 25°, the soln was evaporated, treated with dil. HCl and extracted with CH_2Cl_2 to yield 7-demethylkigelin (IVb) (60 mg), heavy cubes (from CH_2Cl_2–hexane), m.p. 146–147°, giving a bluish green colour with $FeCl_3$, λ_{max} 234,278, 320nm (log ϵ 4·34, 4.12, 3·54), ν_{max} (CH_2Cl_2) 3540, 1660, 1630, 1590 cm^{-1}. NMR: δ 11.03 (1H, s, OH), 6.33 (1H, s, C_5—H), 5·53 (1H, br, OH), 4·7 (IH, m, C_3—H), 3.93 (3H, s, O-Me), 2.87 (2H, d, J = 7 c/s, C_4—H), 1.47 (3H, d, J = 6·5 c/s, C_3—Me). Mass spectrum: m/e 224 (M^+, 100), 209 (1), 206 (9), 19.5 (7), 188 (6), 178 (9), 163 (9), 160 (13), 152 (5), 135 (6). (Found: C, 59.05; H, 5.56. $C_{11}H_{12}O_5$ requires C, 58.92; H, 5·40%.) R_f (in $CHCl_3$–5% MeOH): 0·45 (SiO_2), 0·03 (SiO_2 impregnated with 2% sodium tetraborate).

$KMnO_4$ oxidation of O-methylkigelin. To a well-stirred suspension of 0-methylkigelin (0·7 g) in aq. NaOH (2N, 5 ml) was added a soln of $KMnO_4$ (3 g) in H_2O (100 ml) during 1 hr. The soln was heated at 100° for 1 hr more, cooled and excess SO_2 passed in. Continuous extraction of the acidic solution with ether for 24 hr gave a gum. This was heated at 140° *in vacuo*, treated with excess ethanolic $MeNH_2$ and the product sublimed *in vacuo* to yield 3,4,5-trimethoxy-*N*-methylphthalimide (160 mg), pale yellow needles (from MeOH), m.p. 127°, λ_{max} 244, 326 nm (log ϵ 4.63, 3·63), ν_{max} (KBr) 1740, 1680, 159.5 cm^{-1}. NMR: δ 7·1 (1H, s), 4.15 (3H, s, O-Me), 4·02 (3H, s, O-Me), 3.92 (3H, s, O-Me), 3·1 (3H, s, N-Me). (Found: C, 57.48; H, 5.42. Calc. for $C_{12}H_{13}NO_5$: C, 57.37; H, 5·22%.) The compound was identical (mixed m.p., TLC, u.v., i.r., NMR) with an authentic sample of the imide[9] prepared by $KMnO_4$ oxidation of 4,5,6-trimethoxyphthalide.[18]

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