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ISOFLAVONOIDS OF *MILDBRAEDEODENDRON EXCELSA*

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Key Word Index—*Mildbraedeodendron excelsa*; Leguminosae; isoflavonoids; *Cordyla africana*.

Abstract—The heartwood of *Mildbraedeodendron excelsa* has yielded five isoflavones and an (±)-isoflavanone. The structure of 7,4′-dihydroxy-6-methoxyisoflavone (glycetein) was confirmed by synthesis.

INTRODUCTION

The genus *Mildbraedeodendron* (Leguminosae) is monotypic [1]. *M. excelsa* was first reported by Harms in 1911, and listed by Hutchinson [2] in the sub-family Lotoideae and tribe Swartzieae; however Engler and Melchior [3] place this taxon in the subfamily Caesalpinoideae. Only three genera in this tribe have been analysed phytochemically. *Swartzia madagascariensis* [4] and *S. leiocalycina* [5] have yielded fourteen pterocarpinoids whilst *Aldina heterophylla* [6] has (−)-maackiain as the major component and minor amounts of (±)-maackiain and demethylhomopterocarpin. The sample of the third tribe studied *Cordyla africana* [7] was shown to have nine isoflavones.

The position of Swartzieae is still disputed but the phytochemical evidence reported for *Swartzia*, *Aldina* and *Cordyla* species and now herein for *M. excelsa* would subscribe to their being placed in the Lotoideae.

RESULTS AND DISCUSSION

Five known isoflavones (7,4′-dihydroxy-6-methoxy-(**1**); 6-hydroxy-7,2′-dimethoxy-4′,5′-methylenedioxy-(**2**); 6,7,2′,4′,5′-pentamethoxy-(**3**); 6,7,2′-trimethoxy-4′,5′-methylenedioxy (**4**); 6,7,3′-trimethoxy-4′,5′-methylenedioxy-(**5**) and (±)-6,7-dimethoxy-3′,4′-methylenedioxyisoflavanone(**6**) were isolated from *M. excelsa* heartwood. 6-Demethylmilldurone (**2**) was the major component. The assignment of structures to the isoflavonoids **2**–**6** was based on UV, IR, NMR and mass analyses.

Due to insufficient material, a detailed spectral analysis was not feasible of the isoflavone (**1**). This isoflavone was reported to occur as its glucoside in soya beans (Phaseoleae) [9] but a sample was unavailable and the aglycone had not been synthesised. To confirm the structure of the isolate, 7,4′-dihydroxy-6-methoxyisoflavone was prepared. 4-Benzyloxy-2-hydroxy-5-methoxyacetophenone, obtained from resacetophenone by benzylation, persulphate oxidation and subsequent partial methylation, was condensed with *p*-benzyloxybenzaldehyde. The 2′-hydroxyl group of the chalcone was benzylated prior to Tl(III) acetate treatment. Debenzylation afforded 1-(2,4-dihydroxy-5-methoxyphenyl)-2-(4-hydroxyphenyl)-3,3-dimethoxypropan-1-one, and treatment of this intermediate with acid gave the expected isoflavone (**1**). Attempts to prepare the isoflavone (**1**) by a shorter route, i.e. by the action of Tl(III) nitrate reagent on the 2′-hydroxychalcone were unsuccessful.

The NMR spectrum of the isoflavanone (**6**) exhibited a degenerate ABC resonance pattern for the 2′- 5′- and 6′-protons. A singlet which integrates for three protons occurs at 6·79δ [7b]. Similarly situated protons in tetrahydrodurmillone [8], otobain [10], safrole, 3,4-methylenedioxybenzyl cyanide and 3,4-methylenedioxyphenylacetyl chloride also give sharp singlets. In order to alter the $\Delta\nu/J$ through specific solvent effect,

an initial study on 3,4-methylenedioxyphenylacetic acid was undertaken. The spectra were run in C_6D_6 and C_5D_5N. $\delta(CDCl_3)$ 6·2 (*s*, C-2; C-5; C-6); $\delta(C_6D_6)$ 6·2 (*bs*, C-6), 6·65 (*bs*, C-2; C-5); $\delta(C_5D_5N)$ 7·12 (*bs*, C-6), 6·9 (*bs*, C-2; C-5). The ortho coupling is not apparent but the 2- and 5- proton signals are at a lower frequency. The spectrum of the isoflavanone (**6**) in C_6D_6 showed a similar pattern, the 2'- 5'- protons gave a signal at δ6·65 a shift due to the collision complex of the solvent and the carbonyl group. The calculated spectra (NMREN) gave similar patterns.

A comparative examination of the isolates from the heartwoods of *M. excelsa* and *Cordyla africana* [7] showed the presence in the latter wood of the isoflavanoids (**2–6**) and in addition 5,6,7,8-tetramethoxy-3',4'-methylenedioxy- and 6,7,3',4'-tetramethoxy-isoflavones. Milldurone was the major component in the extract of *C. africana*. The basis [1] for the creation of *Mildbraedeodendron* and its distinction from *Cordyla* might be worthy of reanalysis.

EXPERIMENTAL

Unless otherwise stated mps were determined on a Kofler hot stage apparatus. IR spectra were measured for KBr discs, UV spectra were determined in MeOH and 60MHz, NMR spectra for solns in $CDCl_3$ (tetramethylsilane as internal reference). Only significant bands in IR and NMR spectra are quoted. MS were obtained with an AEI MS 902 (direct inlet) instrument. Optical rotations were measured on a Perkin–Elmer moder 141 Polarimeter. Merck Kiselgel HF_{254} and $PF_{254+366}$ were used for TLC and PLC respectively. During isolation processes, the appropriate combination of fractions were determined by TLC. TLC plates were examined with UV illumination and spraying with chlorosulphonic acid in HOAc.

Isolation of the constituents of Mildbraedeodendron excelsa. Heartwood shavings (1·6 kg) were extracted with *n*-hexane (40h) and subsequently with hot Me_2CO (35h). The *n*-hexane extract (5·5 g) was fractionated by column chromatography (Si gel; 500 g). Elution with $CHCl_3$–Me_2CO (98:1; 19:1; 9:1) gave a series of fractions which were subsequently purified by PLC (eluent: $CHCl_3$–MeOH/9:1). 6,7-Dimethoxy-3',4'-methylenedioxyisoflavanone (**6**) [7a] was crystallized from MeOH or C_6H_6 as cubes (90 mg) mp 201–202°; $[\alpha]_D^{21}$ 0° (Found: C, 66·0; H, 4·9. Calc. for $C_{18}H_{16}O_6$ C, 65·9; H, 4·9%); MS (rel int) M^+ 328 (32) 180(96) 165 (20) 148 (100) 147 (27) 137 (14); NMR: δ 7·36 (1H, *s*, C-5), 3·84 (3H, *s*, C-2', C-5', C-6'), 6·53 (1H, *s*, C-8), 6·0 (2H, *s*, $O.CH_2.O$), 4·66 (2H, α, $J_{2,3}$ 7 Hz, (C-2), 3·95 (1H, *t*, C-3), 3·98, 3·93 (6H, 2Xs, OMe). 6,7,3'-Trimethoxy-4',5'-methylenedioxyisoflavone (**5**) (21 mg), identical (mp, IR, UV, NMR) with an authentic sample [7a]. 6,7,2'-Trimethoxy-4',5'-methylenedioxyisoflavone (**4**) (88 mg) was crystallized from $CHCl_3$–MeOH in needles mp 233–234°. MS (rel int) M^+ 356 (74) 326 (24) 325 (100) 309 (12) 181 (28)176 (12) 175 (18) 162 (19). Identical (IR, UV, NMR) with milldurone [8]. 6,7,2',4', 5'-Pentamethoxyisoflavone (**3**) (65 mg) in needles from MeOH, mp 169–170° (lit. [7a] mp 171–172°). MS (rel int) M^+ 372 (100) 357 (19) 343 (10) 341 (51) 329 (14) 181 (12). λ_{max} (nm) (log ϵ) 302 (4·56) 257 (4·75). NMR: δ 8·05 (1H, *s*, C-2), 7·7 (1H, *s*, C-5), 7·06 (1H, *s*, C-8), 6·95 (1H, *s*, C-6'), 6·71 (1H, *s*, C-3'), 4·02, 3·97, 3·90, 3·82 (12H, 4Xs, OMe). The Et_2O soluble fraction (13·2 g) of the Me_2CO extract yielded, besides additional amounts of isoflavones (**3–6**), 6-hydroxy-7,2'-dimethoxy-4',5'-methylenedioxyisoflavone (**2**) (925 mg) in needles from MeOH, mp 251–252° (lit. [7b] mp 252–253°). Acetylation (Ac_2O-C_5H_5N) afforded a *monoacetate* as needles from MeOH, mp 215–217° (Found: C, 63·0; H, 4·3. $C_{20}H_{16}O_8$ requires C, 62·5; H, 4·2%); NMR: δ 8·03 (1H, *s*, C-2), 6·03 (2H, *s*, $O.CH_2.O$), 4·0, 3·8 (6H, *s*, OMe), 2·4 (3H, *s*, OCOMe). ν_{CO} 1760 cm^{-1}, 1635 cm^{-1}. A slow moving oil which solidified on addition of MeOH yielded 7,4'-dihydroxy-6-methoxyisoflavone (**1**) (57 mg), which was crystallized from MeOH in needles mp 311–312° (lit. [9] mp 311–313°). The product was identical with an authentic sample synthesized below.

Synthesis of 7,4'-dihydroxy-6-methoxyisoflavone. NaOH (40%, 5 ml) was added to a soln of 4-benzyloxy-2-hydroxy-5-methoxyacetophenone (1·2 g) and 4-benzyloxybenzaldehyde (0·938 g) in EtOH (25 ml). The mixture was heated (30 min) under reflux and retained at 21° for 8 days, then diluted with H_2O (200 ml) and acidified to pH3. Extraction with $CHCl_3$ gave an oil which crystallized from MeOH–$CHCl_3$ (1:1) to afford 4,4'-bisbenzyloxy-2'-hydroxy-5'-methoxychalcone (611 mg) as needles, mp 147–148° (found: C, 77·6; H, 5·6. $C_{30}H_{26}O_5$ requires C, 77·3; H, 5·6%); ν_{max} 3450, 1635 cm^{-1}; NMR: δ 13·5 (1H, *s*, OH, exchanges D_2O) 7·49 (10H, *s*, Ph) 7·15 (1H, *s*, C-6'), 6·61 (1H, *s*, C-3'), 7·69 (2H, *d*, $J_{2,3}$ 8·5 Hz, C-2, C-6), 7·09 (2H, *d*, C-3, C-5), 7·23 (1H, *d*, $J_{\alpha\beta}$ 16·5 Hz, C-α), 7·71 (1H, *d*, C-β). 5·21, 5·15 (4H, 2X5, $OC\underline{H}_2Ph$), 3·94 (3H, *s*, OMe). Benzylation with K_2CO_3–NaI–DMF and benzyl chloride afforded 2,4,4'-*tribenzyloxy-5-methoxychalcone* which was crystallized from MeOH in cubes mp 87–88° (Found: C, 79·6; H, 5·6 $C_{37}H_{32}O_5$ requires C, 79·9; H, 5·8%); ν_{max} 1650, 1610 cm^{-1}. NMR: δ 5·27, 5·14, 5·03 (6H, *s*, $OC\underline{H}_2Ph$). Tl(III) acetate (750 mg) was added to the preceding chalcone (425 mg) in MeOH (25 ml) and the soln was heated (4 days) under reflux. Purification of the only product by PLC gave 1-(2,4-bisbenzyloxy-5-methoxyphenyl)-2-(4-benzyloxyphenyl)-3,3 dimethoxypropan-1-one (30 mg) as an oil (Found: C, 72·1; H, 5·9. $C_{32}H_{32}O_4$ requires C, 72·6; H, 6·0%). ν_{max} 1660, 1610 cm^{-1}. NMR: δ 7·44 (15H, *s*, Ph), 7·18 (1H, *s*, C-6'), 6·97 (1H, *s*, C-3'), 5·51 (1H, *d*, *J* 10.2Hz, C-2), 5·34 (1H, *d*, C-3), 5·12 (6H, *m*, $O.C\underline{H}_2.Ph$), 3·91 (3H, *s*, OMe), 3·45, 3·12 (6H, 2Xs, OMe). Palladised charcoal (10%; 50 mg) was added to the foregoing acetal (30 mg) in EtOAc (10 ml). The mixture was stirred in an atmosphere of H_2 until uptake ceased; removal of the catalyst and evaporation of the solvent yielded 1-(*2,4-dihydroxy-5-methoxyphenyl*)-2-(*4-hydroxyphenyl*)-3,3-*dimethoxypropan-1-one* (17 mg). NMR: δ 12·65 (1H, *s*, C-2' OH), 7·3 (2H, *d*, *J* 8·4 Hz C-2'', C-6''), 6·85 (2H, *d*, C-3'', C-5''), 7·29 (1H, *s*, C-6'), 6·54 (1H, *s*, C-3'), 5·09 (1H, *d*, *J* 17 Hz, C-2) 4·69 (1H, *d*, C-3), 4·27, 4·16 (2H, 2Xs, $O\underline{H}$), 3·84 (3H, *s*, OMe), 3·27, 3·3 (6H, 2Xs, OMe). Concentrated HCl (0·5 ml; 10%) was added to the preceding acetal (17 mg) in EtOH and the soln was warmed (30 min) on a water-bath. Addition of H_2O and subsequent extraction gave 7,4'-dihydroxy-6-methoxyisoflavone which crystallized from MeOH in needles (7 mg), mp 313° (Found: C, 65·4; H, 4·4. Calc. for $C_{16}H_{12}O_5$ C, 64·9; H, 5·0%). The diacetate crystallized from MeOH in needles, mp and mmp (with diacetate of the natural product) 212–214°. NMR: δ 8·1 (1H, *s*, C-2), 7·88 (1H, *s*, 5-H), 7·72 (2H, *d*, $J_{2,3}$ 8·5 Hz, C-2', C-6'), 7·28 (2H, *d*, C-3', C-5'), 7·36 (1H, *s*, C-8), 4·01 (3H, *s*, OMe), 2·41, 2·36 (6H, *s*, OMe).

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