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ISOFLAVONOIDS FROM *MYROXYLON BALSAMUM**

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Key Word Index—*Myroxylon balsamum*; Leguminosae-Lotoideae; isoflavonoids; (±)-7-hydroxy-4′-methoxyisoflavanone; (±)-7,3′-dihydroxy-4′-methoxyisoflavanone; 2-(2′,4′-dihydroxyphenyl)-5,6-dimethoxybenzofuran.

Abstract—*Myroxylon balsamum* (Leguminosae–Lotoideae) trunk wood contains a series of biogenetically related flavonoids, including the novel (±)-7-hydroxy-4′-methoxyisoflavanone, (±)-7,3′-dihydroxy-4′-methoxyisoflavanone and 2-(2′,4′-dihydroxyphenyl)-5,6-dimethoxybenzofuran.

INTRODUCTION

The genus *Myroxylon* L.f., considered by some to be monotypic and restricted to *M. balsamum* (L.) Harms. although about 6 species have been described, comprises, according to other authors, the species *M. balsamum* and *M. peruiferum* L.f. [2]. In previous studies of *M. balsamum* trunk wood, collected in the vicinity of Rio de Janeiro, the isolation of cabreuvin (**1a**) [3] and of afrormosin (**1b**) [4] was reported. Paralleling the uncertainties of the systematics, in the present examination of *M. balsamum* wood, collected in the Rio Doce region of Espirito Santo State, and classified by Apparicio Pereira Duarte, neither of the mentioned isoflavones (**1a**, **1b**) were isolated. In substitution appeared, besides sitosterol, formononetin (**1c**) [5], 3′-hydroxyformononetin (**1d**) [6] and 3′-hydroxy-8-*O*-methylretusin (**1e**) [7], accompanied by (+)-demethylhomopterocarpin (**2**) [8], 3-hydroxy-9-methoxycoumestan (**3a**) [9], 3-hydroxy-8,9-dimethoxycoumestan (**3b**) [10], (±)-7,4′-dihydroxyflavanone (**4**) [8] and 3 novel compounds **5a**, **5b** and **6a**. The coumestan numbering system follows a recent recommendation [11].

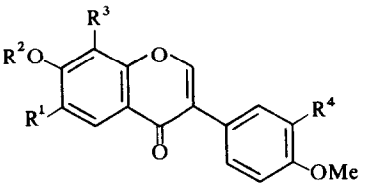
RESULTS

The known compounds were identified by comparison of mp and spectra with published data. For **1c**, **2**, **3a**, **3b** and **4**, identifications were confirmed by direct comparisons with authentic samples.

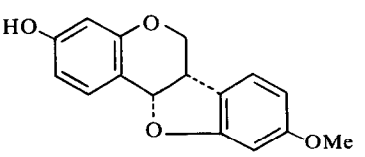
The molecular formulae of **5a** and **5b**, respectively $C_{16}H_{14}O_4$ and $C_{16}H_{14}O_5$, determined by elementary analysis and MS, were expanded to $C_{15}H_{10}O_2$.OH.OMe and $C_{15}H_9O_2(OH)_2OMe$ after comparison of the PMR spectra of the isolates with the spectra of the derived Me ethers (**5a**–**5c**, **5b**→**5d**) and acetates (**5a**→**5e**, **5b**→**5f**). These spectra also indicated the oxygenation patterns of the aromatic rings and classified the compounds as isoflavanones through the characteristic frequencies of the heterocyclic proton signals [8, 12, 13]. Both these features were confirmed by DDQ oxidation of the derived Me ethers to di-*O*-methyldaidzein [14] (**5c**→**1f**) and to cabreuvin [3] (**5d**→**1a**). The reagent was previously employed in an oxidation of this type [15]. At this point only the distribution of the OH/OMe groups among the aromatic rings remained to be considered. This was indicated by *m/e* values of the retro-Diels–Alder fragments which account for the base peaks in the MS spectra of both compounds: **5a**→$[CH_2{=}CH.C_6H_4.OMe]^+$· and **5b**→$[CH_2{=}CH.C_6H_3.OH.OMe]^+$·. In view of a positive Gibbs test [16], it is the OH and not the OMe of **1b** which is located at C-3′.

The structures **5a** and **5b** which are compatible with these data were confirmed by synthesis of their acetates via catalytic hydrogenation of the corresponding isoflavone acetates, respectively the acetate of formononetin (**1g**→**5e**) and the diacetate of 3′-hydroxyformononetin (**1h**→**5f**).

The molecular formula of **6a**, $C_{16}H_{14}O_5$, determined by high resolution MS, was expanded to $C_{14}H_6O(OH)_2(OMe)_2$ after inspection of the IR (ν_{max} 3400 cm^{-1}, no



1a $R^1 = R^3 = H$, $R^2 = Me$, $R^4 = OMe$
1b $R^1 = OMe$, $R^2 = R^3 = R^4 = H$
1c $R^1 = R^2 = R^3 = R^4 = H$
1d $R^1 = R^2 = R^3 = H$, $R^4 = OH$
1e $R^1 = R^2 = H$, $R^3 = OMe$, $R^4 = OH$
1f $R^1 = R^3 = R^4 = H$, $R^2 = Me$
1g $R^1 = R^3 = R^4 = H$, $R^2 = Ac$
1h $R^1 = R^3 = H$, $R^2 = Ac$, $R^4 = OAc$



2

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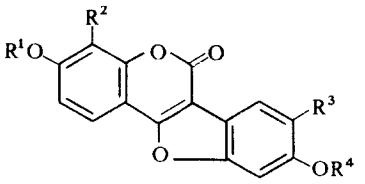
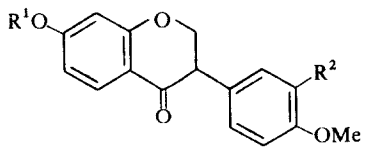
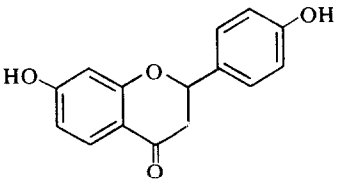


3a $R^1 = R^2 = R^3 = H$, $R^4 = Me$
3b $R^1 = R^2 = H$, $R^3 = OMe$, $R^4 = Me$
3c $R^1 = R^2 = H$, $R^3 - R^4 = OCH_2$
3d $R^1 = Me$, $R^2 = OH$, $R^3 = R^4 = H$



5a $R^1 = R^2 = H$
5b $R^1 = H$, $R^2 = OH$
5c $R^1 = Me$, $R^2 = H$
5d $R^1 = Me$, $R^2 = OMe$
5e $R^1 = Ac$, $R^2 = H$
5f $R^1 = Ac$, $R^2 = OAc$



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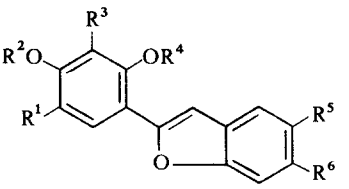

6a $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = R^6 = OMe$
6b $R^1 = R^3 = H$, $R^2 = R^4 = Me$, $R^5 - R^6 = OCH_2O$
6c $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = Me$, $R^6 = OMe$
6d $R^1 = R^5 = H$, $R^2 = R^4 = Me$, $R^3 = R^6 = OH$
6e $R^1 = R^2 = OCH_2$, $R^3 = OMe$, $R^4 = R^5 = R^6 = H$

C=O absorption) and PMR (two 3 H singlets at τ 6.08 and 6.16, 6 aromatic H) spectra. Further expansion to $C_8H_3O.C_6H_3(OH)_2(OMe)_2$ was possible through recognition in the latter spectrum of the typical proton multiplets (cf. Experimental for H-3', H-5', H-6') of a 2',4'-dioxyphenyl moiety. The C_8H_3O-unit must represent an oxa-aromatic system, possibly a benzofuran. PMR comparison with model compounds shows **6a** to be a 2-aryl derivative: H-3 τ 2.87 (**6a**), 2.92 (**6b**) [17], 2.84 (**6c**) [18], in which the protons on the benzo-moiety, represented by singlets, are *para*-related: H-4 τ 3.03 (**6a**), 3.08 (**6b**) [18]; H-7 τ 2.98 (**6a**), 3.01 (**6b**) [17].

At this point only the distribution of the OH/OMe groups among the aromatic rings remained to be considered. Both OHs cannot be situated on the benzofuran moiety, in view of the absence of a H_3BO_3–NaOAc UV shift and a positive Gibbs test [16]. The latter evidence requires one of the OHs to be placed at C-2'. Among the 3 alternative situations which are compatible with these data: 2',4'-diOH-5,6-diOMe; 5,2'-diOH-6,4'-diOMe; 6,2'-diOH-5,4'-diOMe; the first one is preferred on account of its analogy with the situation prevailing in the co-occurring isoflavonoids **1c**, **1d**, **1e**, **2**, **3a**, **3b**, **5a** and **5b** all carrying an OH at the position which biosynthetically corresponds to C-4' of the 2-arylbenzofuran **6a**. Specially significant in this respect is the distribution of the oxy-functions on the coumestan **3b**, the putative precursor of **6a**.

The transformation **3b** → **6a** would be analogous to the laboratory conversion **3c** → **6b** under alkaline methylating conditions [17]. A similar relationship may exist between **3a** and vignafuran (**6c**) [18], as well as sativol (**3d**) [19] and pterofuran (**6d**) [20]. Vignafuran and pterofuran are the sole biosynthetically analogous 2-arylbenzofurans previously isolated from higher plants. Compound **6e** was isolated from yeast [21], and should arise by an unrelated biosynthetic route.

EXPERIMENTAL

Isolation of constituents. A softwood sample (5 kg) was ground and extracted successively with C_6H_6 and EtOH. Gradual concn of the C_6H_6 soln gave two crops of crystals and a residue. The 2nd crop (1.86 g) was recrystallized from EtOH to **1c**. The residue (180 g) was chromatographed on Si gel giving in order the following fractions with the indicated eluents: A_1 (C_6H_6), A_2 (C_6H_6–$CHCl_3$, 9:1), A_3 (C_6H_6–$CHCl_3$, 1:1), A_4 (C_6H_6–$CHCl_3$, 1:4 and $CHCl_3$), A_5 ($CHCl_3$–MeOH, 19:1), A_6 (MeOH). A_1 and A_2 gave aliphatic material. A_3, washed with MeOH, gave sitosterol. A_4 was chromatographed on Si gel giving **6a**. A_5 was separated by MeOH into soluble **2** and an insoluble residue. A_6 was chromatographed on Si gel giving **5a**, **1c** and **5b**. The EtOH extract (200 g) was chromatographed on Si gel giving in order the following fractions with the indicated eluents: B_1 and B_2 ($CHCl_3$–MeOH, 19·1), B_4, B_5 and B_6 ($CHCl_3$–MeOH, 9:1). B_1 gave aliphatic material. B_2 was separated with MeOH into soluble **3a** and insoluble **5a**. B_3 was fractionally crystallized from MeOH: 1st crop **3b**. B_4 was separated by MeOH into insoluble **1c** and a mixture which was separated by Si gel chromatography into **1e**, **1c** and **5b**. B_5, washed with MeOH, gave **1d**. B_6 was chromatographed on Sephadex LH-20 giving **4**, **5b** and **1d**. Total quantities (in mg/kg) obtained: sitosterol 1, **1c** 502, **1d** 96, **1e** 60, **2** 38, **3a** 2, **3b** 3, **4** 14, **5a** 508, **5b** 412, **6a** 16.

(±)-7-Hydroxy-4'-methoxyisoflavanone (**5a**). Mp 185–188° (EtOH) [Found: C, 70.89; H, 5.35. $C_{16}H_{14}O_4$ requires: C, 71.10; H, 5.22%]. ν_{max}^{KBr} (cm^{-1}). 3260, 1662, λ_{max}^{EtOH} (nm): 282, 313 (ε resp. 11600, 6800); $\lambda_{max}^{EtOH + NaOH}$ (nm): 260, 287 inf, 341 (ε resp. 2900, 5400, 22600); $\lambda_{max}^{EtOH + NaOAc}$ (nm): 262, 287, 343 (ε resp. 6400, 5300, 20100); no $AlCl_3$-shift. Gibbs test [16]: negative PMR [$(CD_3)_2CO$, 60 MHz, τ]: 0.77 (*s*, dissapp. with D_2O, OH), 2.25 (*d*, J = 8 Hz, H-5), 2.77 (AA' pattern, H-2', H-6'), 3.12 (BB' pattern, H-3', H-5'), 3.4 (*dd*, J = 8, 3 Hz, H-6), 3.6 (*d*, J = 3 Hz, H-8), 5.35 (*d*, J = 6 Hz, 2H-2), 6.1 (*t*, J = 6 Hz, H-3), 6.24 (*s*, OMe). MS (*m/e*): 270 (25%) M, 134 (100), 119 (30), 91 (28), 81 (11). Me ether (**5c**): mp 129–130° (EtOH) [Found: C, 71.79; H, 5.93. $C_{17}H_{16}O_4$ requires: C, 71.82; H, 5.73]. ν_{max}^{KBr} (cm^{-1}). 1676, 1606. PMR ($CDCl_3$, 60 MHz, τ). 2.14 (*d*, J = 9 Hz, H-5), 2.8 (AA' pattern, H-2', H-6'), 3.13 (BB' pattern, H-3', H-5'), 3.42 (*dd*, J = 9, 2 Hz, H-6), 3.57 (*d*, J = 2 Hz, H-8), 5.39 (*d*, J = 6 Hz, H-2), 6.03–6.27 (overlapped by OMe signals, H-3), 6.19 (*s*, OMe), 6.23 (*s*, OMe). MS (*m/e*): 284 (14%) M, 150 (8), 134 (100), 119 (14). Oxidation of **5c** (72 mg) with DDQ (40 mg) in dioxane (15 ml) (reflux, 100 hr), evapn of the solvent and chromatography of the residue gave **1f** (12 mg), mp and mmp with authentic sample 162°. Acetate (**5e**): mp 149–151° (EtOH) [Found: C, 69.30; H, 4.93 $C_{18}H_{16}O_5$ requires: C, 69.22; H, 5.16%]. ν_{max}^{KBr} (cm^{-1}). 1757, 1684, 1618. PMR ($CDCl_3$, 60 MHz, τ): 2.03 (*d*,

J = 9 Hz, H-5), 2.8 (AA' pattern, H-2', H-6'), 3.13 (BB' pattern, H-3', H-5'), 3.1 (*dd*, J = 9, 3 Hz, H-6), 3.28 (*d*, J = 3 Hz, H-8), 5.35 (*d*, J = 6 Hz, H-2), 6.17 (*t*, J = 6 Hz, H-3), 6.23 (*s*, OMe), 7.7 (*s*, COMe). MS (*m/e*): 312 (8%) M, 135 (20), 134 (100), 119 (21). This acetate was obtained (a) by treatment of **5a** (54 mg) with C_5H_5N–Ac_2O (1:4) (2.5 ml) (room temp. 24 hr) and (b) by treatment of **1g** (50 mg) in EtOH (5 ml) with H_2, Pd/C (5 mg); mmp 149–151°.

(±)-*7,3'-Dihydroxy-4'-methoxyisoflavanone* (**5b**). 185–188° (EtOH) [Found: C, 66.95; H, 4.81. $C_{16}H_{14}O_5$ requires: C, 67.13; H, 4.93%]. ν_{max}^{KBr} (cm^{-1}): 3433, 3311, 1667. λ_{max}^{EtOH} (nm): 282, 315 (ε resp. 20600, 11700); $\lambda_{max}^{EtOH + NaOH}$ (nm): 300 inf., 340 (ε resp. 21700, 42300); $\lambda_{max}^{EtOH + NaOAc}$ (nm): 260, 280, 342 (ε resp. 13100, 11700, 28000); no $AlCl_3$ UV shift. Gibbs test [16]: λ_{max} (nm): 630. PMR [$(CD_3)_2CO$, 60 MHz, τ]: 0.67 (*s*, dissapp. with D_2O, OH), 2.25 (*d*, J = 8 Hz, H-5), 2.7 (*s*, dissapp. with D_2O, OH), 3.02–3.37 (*m*, H-2', H-5', H-6'), 3.42 (*dd*, J = 8, 2 Hz, H-6), 3.62 (*d*, J = 2 Hz, H-8), 5.37 (*d*, J = 6 Hz, 2H-2), 6.2 (*s*, OMe), 6.24 (*t*, J = 6 Hz, H-3). MS (*m/e*): 286 (38) M, 150 (100), 137 (96), 136 (9), 135 (77), 107 (18). DiMe ether (**5d**): mp 135–137° (EtOH) [Found: C, 68.70; H, 5.87. $C_{18}H_{18}O_5$ requires: C_1 68.78; H, 5.77%]. ν_{max}^{KBr} (cm^{-1}): 1662, 1608. PMR ($CDCl_3$, 60 MHz, τ): 2.12 (*d*, J = 9, H-5), 3.19 (*s*, H-2', H-5', H-6'), 3.42 (*dd*, J = 9, 3 Hz, H-6), 3.57 (*d*, J = 3 Hz, H-8), 5.35 (*d*, J = 6 Hz, 2H-2), 6.17 (*s*, OMe), 6.2 (*t*, J = 6 Hz, H-3). MS (*m/e*): 314 (90%) M, 165 (54), 164 (100), 149 (92), 138 (52), 122 (14), 121 (41). Oxidation of **5d** (90 mg) with DDQ (74 mg) in dry C_6H_6 (20 ml) (reflux, 78 hr), evapn of the solvent and chromatography of the residue gave **1a** (37 mg), mp and mmp with authentic sample 153–157°. Diacetate (**1h**), mp 80–83° [Found: C, 65.07; H, 5.12. $C_{20}H_{18}O_7$ requires: C, 64.86; H, 4.90%]. ν_{max}^{KBr} (cm^{-1}): 1764, 1692. PMR ($CDCl_3$, 60 MHz, τ): 2.04 (*d*, J = 7 Hz, H-5), 2.97 (*dd*, J = 7, 2 Hz, H-6), 3.03 (*d*, J = 2, H-8, H-2'), 3.2 (*d*, J = 7 Hz, H-6'), 3.25 (*dd*, J = 7, 2 Hz, H-5'), 5.35 (*d*, J = 6 Hz, H-2), 6.09 (*t*, J = 6 Hz, H-3), 6.17 (*s*, OMe), 7.7 (*s*, COMe). This diacetate was obtained (a) by treatment of **5b** (29 mg) in Ac_2O (1 ml) with TsOH (1.9 mg) (room temp., 16 hr) and (b) by treatment of **1h** (50 mg) in EtOH (5 ml) with H_2, Pd/C (5 mg); mmp 81–83°.

2-(2',4'-Dihydroxyphenyl)-5,6-dimethoxybenzofuran (**6a**). Mp 178–180°. [Found: M 286.0850. $C_{16}H_{14}O_5$ requires: M 286.0841]. ν_{max}^{KBr} (cm^{-1}): 3464, 3393, 1605. λ_{max}^{EtOH} (nm): 293 inf., 300 inf., 330, 343 (ε resp. 9100, 9700, 29200; 25200 $\lambda_{max}^{EtOH + NaOH}$ (nm): 355, 370 inf. (ε resp. 26600, 33700; no NaOAc or $AlCl_3$ UV shifts. Gibbs test [16]: λ_{max} (nm): 680. PMR ($CDCl_3$, 60 MHz, τ): 2.65 (*d*, J = 6 Hz, H-6'), 2.87 (*s*, H-3), 2.98 (*s*, H-7), 3.03 (*s*, H-4), 3.19 (*d*, J = 6 Hz, H-5'), 3.23 (*s*, H-3'), 6 (*s*, OMe), 6.05 (*s*, OMe); [$(CD_3)_2CO$, 60 MHz, τ]: 2.59 (*s*, H-3), 2.65 (*d*, J = 8 Hz, H-6'), 2.9 (*s*, H-7), 2.97 (*s*, H-4), 3.02 (*d*, J = 2 Hz, H-3'), 3.09 (*dd*, J = 8, 2 Hz, H-5'), 6.08 (*s*, OMe), 6.16 (*s*, OMe). MS (*m/e*): 286 (100%) M, 271 (35), 256 (9), 243 (6), 228 (7).

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