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An isoflavone from *Myristica malabarica*

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Abstract

Phytochemical investigation of the heartwood of *Myristica malabarica* has led to the isolation of the new 7,4′-dimethoxy-5-hydroxyisoflavone together with two other isoflavones, biochanin A and prunetin, and a 1,3-diarylpropanol and a rare α-hydroxydihydrochalcone.

Keywords: Myristica malabarica; Myristicaceae; Heartwood; Isoflavone; Prunetin; Biochanin A; α-Hydroxydihydrochalcone; 1,3-Diarylpropanoid

1. Introduction

Previous investigations (Purushottaman, Sarada & Connolly, 1977, 1984) of *Myristica malabarica* Lam (Rampatri) (aril) revealed the presence of the long chain α,ω-diarylnonanoids, malabaricones A–D and a lignan named malabaricanol. Our phytochemical investigation of the heartwood extractives of *M. malabarica* has resulted in the isolation of a new isoflavone, along with two known isoflavones, a rare α-hydroxydihydrochalcone and a 1,3-diarylpropanol.

2. Results and discussion

The dried heartwood of *M. malabarica* was successively extracted with hot petrol and ethanol. Fractionation of the petrol extract over silica gel column afforded the new isoflavone **1**.

The UV spectrum of **1** showed maxima at 205 and 258 nm, which is typical of isoflavones. The shift (258 → 270 nm) observed on addition of $AlCl_3$ indicated the presence of a 5-hydroxyl group. Its IR spectrum contained a strong band at 1650 cm^{-1} (conjugated carbonyl). The isoflavonoid character of **1** is apparent from its ^{1}H-NMR spectrum which revealed the characteristic chemical shifts and coupling pattern δ 7.8 (*s*, 1H, H-2), 7.4 (*d*, J = 8 Hz, 2H, H-2′, H-6′), 6.9 (*d*, J = 8 Hz, 2H, H-3′, H-5′). The singlet at δ 6.3 (2H) was assigned to C_6–H and C_8–H which very often appears as singlet, although they should show as two meta coupled doublets. These data together with the presence of two phenolic methoxyl groups (δ 3.8, 6H) and one chelated hydroxyl group (D_2O exchangeable) established its structure as **1**. It was further confirmed by MS which showed along with molecular ion at m/z 298 ($C_{17}H_{14}O_5$), the RDA fragments at m/z 166, and 132. The structure **1** was finally confirmed by direct comparison (mmp, Co.IR and CoTLC) with a synthetic sample of 5-hydroxy-7,4′-dimethoxyisoflavone prepared from 5,7,4′-trimethoxyisoflavone by selective demethylation ($AlCl_3$–ether).

The ethanol extract of the heartwood gave the rare (αR) α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone **4** and (2R) 1-(2-hydroxy-4-methoxyphenyl)-3-(3,4-methylenedioxyphenyl)-propan-2-ol **5**. These were identified by analysis of their spectral data and mp described in the literature (Bezuidenhoudt, Brandt & Roux, 1981; Filho, Frota Leite & Gottlieb, 1972), and the isoflavones 5,7-dihydroxy-4′-methoxyisoflavone (biochanin A, **2**) (Wall et al., 1988) and 5,4′-dihydroxy-7-methoxyisoflavone (prunetin, **3**) (Campbell, Harper & Kemp, 1969). These were identified by analysis of their spectral data and direct comparison with authentic samples available from our laboratories.

The present work constitute the first report of the occurrence of isoflavones in the genus *Myristica*. It

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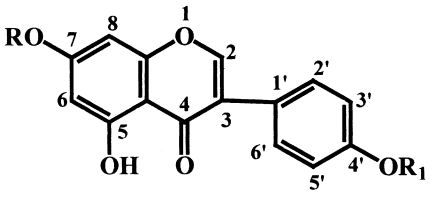
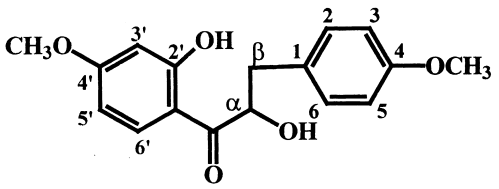
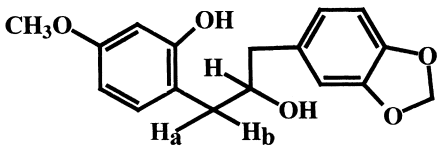
may be mentioned that isoflavonoids have been identified in the genus *Virola* (Myristicaceae) (Gottlieb, 1979; Brazfo, Pedreria, Gottlieb & Maria, 1976). α-Hydroxydihydrochalcones are rare natural products (lyonogenin from *Lyonia formosa* (Shukla, Tandon & Dhar, 1973), nubigenol from *Podocarpus nibugena* (Bhakuni, Bittner & Silva, 1973), hydroxymethoxydihydrochalcone from *Pterocarpus angolensis* (Bezuidenhoudt et al., 1981), coatine A and B from *Eysenhardtia polystachya* and *Pterocarpus* sps. (Beltrami, Bernardi, Fronza, Mellerio, Vidari & Vita-Finzs, 1982; Bezuidenhoudt, Brandt & Ferreira, 1987) and from *Xanthocercis zambesiaca* (Bezuidenhoudt, Bezuidenhoudt & Ferreira, 1988). The co-occurrence of α-hydroxydihydrochalcone along with α-methyldeoxybenzoins and isoflavones was earlier noted in *Pterocarpus* species (Leguminosae) (Bezuidenhoudt et al., 1981). The present work is another instance of this co-occurrence. The role of α-hydroxychalcones and α-hydroxydihydrochalcones in flavonoid biogenesis has previously been proposed and discussed (Bezuidenhoudt et al., 1981, 1987, 1988; Volsteedt, Rall & Roux, 1973; Roux & Ferreira, 1974).



(1) $R = R_1 = CH_3$
(2) $R = H$, $R_1 = CH_3$, **Biochanin A**
(3) $R = CH_3$, $R_1 = H$, **Prunetin**



(4)



(5)

3. Experimental

3.1. General

Plant material of *M. malabarica* LAM was obtained from Kerala, South India, through the courtesy of Dr. K.K. Purushottaman (Purushottaman et al., 1977) mps: uncorrected, UV: MeOH, IR: KBr disc. ^{1}H-NMR: 90 and 200 MHz in $CDCl_3$ with TMS as internal standard. EIMS at 70 eV (direct probe). CC: Silica gel 60–120 mesh.

3.2. Extraction and isolation

Drillings (4 kg) of the heartwood of *M. malabarica* were extracted with petrol (60–80°C) (2 × 10 l, 30 h) followed by ethanol (2 × 10 l, 30 h). Evaporation of the solvents gave a yellow gum (2 g) and a brown gum (145 g), respectively. The petrol extract was fractionated by CC over silica gel using a petrol–C_6H_6–EtOAc step gradient to afford **1**. The ethanol extract was divided into ether soluble and insoluble portions. The ether extract was fractionated by CC over silica gel using C_6H_6–EtOAc–MeOH as eluants to afford **2**, **3**, **4** and **5**.

3.3. 7,4′-Dimethoxy-5-hydroxyisoflavone *1*

Pale yellow crystals ($CHCl_3$–petrol, 1 : 2, v/v), mp 145–146°C; R_f (C_6H_6–EtOAc, 19 : 1, v/v): 0.57; ethanolic $FeCl_3$: dark brown; UV λ_{max}^{MeOH} nm: 205 and 258; + $AlCl_3$: 206, 270; IR ν_{max}^{KBr} cm^{-1}: 2900 (*br*), 1650, 1600, 1580; ^{1}H-NMR ($CDCl_3$): δ 12.8 (1H, *s*, D_2O exchangeable OH-5), 7.8 (1H, *s*, H-2), 7.4 (2H, *d*, J = 8 Hz, H-2′, 6′), 6.9 (2H, *d*, J = 8 Hz, H-3′, 5′), 6.3 (2H, *s*, H-6 and 8), 3.85 (3H, *s*, OMe), 3.8 (3H, *s*, OMe); EIMS (probe) 70 eV, *m/z* (rel. int.): 298 $[M]^+$ (100), 297 (30), 283 (1), 166 (47), 167 (5), 149 (7), 138 (98), 132 (65).

3.4. α-2′-Dihydroxy-4,4′-dimethoxydihydrochalcone *4*

Colourless needles (C_6H_6–EtOAc) mp 94°C; R_f (C_6H_6–EtOAc, 19 : 1, v/v): 0.36; $FeCl_3$: dark brown; triphenyltetrazolium: deep red; UV λ_{max}^{MeOH} nm (log ε): 214 (4.5), 274 (4.39), 316 (4.1); + $AlCl_3$: 218, 300, 360; + NaOMe: 214, 228, 274, 350; IR ν_{max}^{KBr} cm^{-1}: 3500,

2850, 1630, 1610, 1590; $[\alpha]_D^{25}$ −76.84 (MeOH, *c* 0.001015); CD $[\theta]_{222}$ +10,500, $[\theta]_{230}$ 0, $[\theta]_{232}$ −28,000, $[\theta]_{237}$ 0, $[\theta]_{246}$ −84,500, $[\theta]_{269}$ +66,000, $[\theta]_{280}$ +12,100, $[\theta]_{300}$ −55,500, $[\theta]_{317}$ −10,700, $[\theta]_{340}$ −12,000, $[\theta]_{350}$ +20,500; ^{1}H-NMR ($CDCl_3$): δ 12.2 (1H, *s*, OH-2′, D_2O exchangeable), 7.6 (1H, *d*, *J* = 10 Hz, H-6′), 7.07 (2H, *d*, *J* = 8.5 Hz, H-2 and 6), 6.8 (2H, *d*, *J* = 8.5 Hz, H-3 and 5), 6.5 (2H, *m*, H-3′ and 5′), 5.22 (1H, *m*, H-α), 3.9 (3H, *s*, OCH_3), 3.8 (3H, *s*, OCH_3), 3.54 (1H, *d*, *J* = 7.8 Hz, α-OH, D_2O exchangeable), 3.13 (1H, *dd*, *J* = 14 and 5 Hz, H-β), 2.9 (1H, *dd*, *J* = 14 and 5 Hz, H-β); EIMS (probe) 70 eV; *m*/*z* (rel. int.): 302 $[M]^+$ (5.8), 284 $(M-H_2O)^+$ (20), 181 (5), 151 (51), 121 (100).

3.5. 1-(2-Hydroxy-4-methoxyphenyl)-3-(3,4-methylenedioxyphenyl) propan-2-ol **5**

Light yellow crystals (C_6H_6–EtOAc), mp 116°C; R_f (C_6H_6–EtOAc, 19 : 1, v/v): 0.28; aq. $FeCl_3$–$K_3Fe(CN)_6$: blue; UV λ_{max}^{MeOH} nm (log ε): 206 (4.67), 280 (4.04); + $AlCl_3$: 206, 280; + NaOAc: 206, 280; + NaOMe: 210, 288; IR ν_{max}^{KBr} cm^{-1}: 3350, 3120, 2900, 1615, 1580; $[\alpha]_D^{25}$ −4.8 (MeOH, *c* 1.043); CD $[\theta]_{227}$ +8400, $[\theta]_{229}$ −9600, $[\theta]_{230}$ −3020, $[\theta]_{231}$ 0, $[\theta]_{236}$ +12,080, $[\theta]_{241}$ +23,860, $[\theta]_{250}$ +21,140, $[\theta]_{286}$ +18,120, $[\theta]_{298}$ +30,800, $[\theta]_{310}$ +30,800, $[\theta]_{335}$ +32,900; ^{1}H-NMR ($CDCl_3$): δ 8.3 (1H, *s*, 2′-OH, D_2O exchangeable), 6.91 (1H, *d*, *J* = 8 Hz, H-6′), 6.67 (3H, *m*, H-2″ 5″ 6″), 6.5 (1H, *d*, *J* = 2 Hz, H-3′), 6.42 (1H, *dd*, *J* = 8 and 2 Hz, H-5′), 5.92 (2H, *s*, –OCH_2O–), 4.1 (1H, *m*, H-2), 3.77 (3H, *s*, OMe), 2.51 (1H, *br*, 2-OH, D_2O exchangeable), 2.54–291 (4H, *m*, 2 × $ArCH_2$–); ^{13}C-NMR ($CDCl_3$); δ 159.9 (C-4′), 156.5 (C-2′), 147.5 (C-3″ or 4″), 145.8 (C-4″ or 3″), 132.0 (C-2″), 131.4 (C-6′), 122.2 (C-6″), 117.3 (C-1′), 109.5 (C-2″ or 5″), 108.5 (C-5″ or 2″), 106.1 (C-5′), 102.8 (C-3′), 100.9 (–OCH_2O–), 74.9 (C-2), 55.2 (OCH_3), 42.9 (C-1 or 3), 38.06 (C-3 or 1). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 302 $[M]^+$ (51), 284 (2), 167 (38), 165 (9), 137 (100), 135 (44), 136 (97).

3.6. Biochanin A **2**

Colourless needles (MeOH) mp 215–216°C; R_f (MeOH–$CHCl_3$, 1 : 19, v/v): 0.55; ethanolic $FeCl_3$: dark brown; UV λ_{max}^{MeOH} nm (log ε): 261 (4.5), 330 (2.7); + $AlCl_3$: 273, 310, 375; + NaOAc: 272, 327; + NaOMe: 249, 273, 327; IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1640; ^{1}H-NMR (acetone d_6): δ 13.12 (1H, *s*, OH), 9.8 (1H, *s*, D_2O exchangeable, OH-7) 8.2 (1H, *s*, H-2), 7.4 (2H, *d*, *J* = 8 Hz, H-2′, 6′), 6.84 (2H, *d*, *J* = 8 Hz, H-3′ and 5′), 6.44 (1H, *d*, *J* = 2.5 Hz, H-8), 6.32 (1H, *d*, *J* = 2.5 Hz, H-6), 3.84 (3H, *s*, 4′-OMe); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 284 $[M]^+$ (100), 283 $[M-H]^+$ (10), 142 (6), 152 (16), 132 (48), 124 (8).

3.7. Prunetin **3**

Colourless needles (MeOH) mp 238–239°C; R_f (MeOH–$CHCl_3$, 1 : 19, v/v): 0.50; Ethanolic $FeCl_3$: dark brown; UV λ_{max}^{MeOH} nm (log ε): 262 (4.54), 327 (3.6); + $AlCl_3$: 273, 309, 374; + NaOAc: 262, 330; + NaOMe: 272, 353; IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1645; ^{1}H-NMR (acetone d_6): δ 13.12 (1H, *s*, OH-5), 8.8 (1H, *s*, OH-4′, D_2O exchangeable), 8.22 (1H, *s*, H-2), 7.46 (2H, *d*, *J* = 8 Hz, H-2′ and 6′), 6.9 (2H, *d*, *J* = 8 Hz, H-3′ and 5′), 6.54 (1H, *d*, *J* = 2.5 Hz, H-8), 6.36 (1H, *d*, *J* = 2.5 Hz, H-6), 3.92 (3H, *s*, OMe). EIMS (probe) 70 eV, *m*/*z* (rel. Int.): 284 $[M]^+$ (100), 283 $[M-H]^+$ (35), 166 (47), 138 (8), 118 (50).

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