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A COUMESTONE FROM THE ROOTS OF *TEPHROSIA HAMILTONII*

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Key Word Index—*Tephrosia hamiltonii*; Leguminosae; Roots; coumestone; 2-methoxy-3,9-dihydroxy coumestone; Pongamol; Flemichapparin-B and-C.

Abstract—A new coumestone, 2-methoxy-3,9-dihydroxy coumestone, was isolated from the roots of *Tephrosia hamiltonii* together with pongamol, flemichapparin-B and -C. The structures were elucidated by spectroscopic methods.

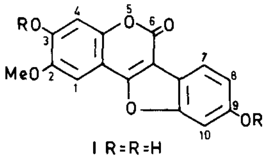
INTRODUCTION

The genus *Tephrosia* elaborates several types of flavonoid compounds [1]. The present communication reports the isolation and structural elucidation of a new coumestone (**1**) together with the known compounds pongamol (**4**), flemichapparin-B (**5**) and-C (**6**) from the roots of *T. hamiltonii.*

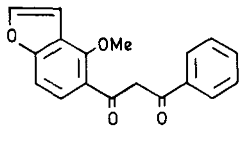
RESULTS AND DISCUSSION

Compound **1**, mp 318–320°, analysed for $C_{16}H_{10}O_6$, which was supported by $[M]^+$ at m/z 298. It formed a diacetate (**2**), mp 256°, analysed for $C_{20}H_{14}O_8$, $[M]^+$ at m/z 382 and a dimethyl ether (**3**), mp 255°, analysed for $C_{18}H_{14}O_6$ and $[M]^+$ at m/z 326. Thus the presence of two phenolic hydroxyl groups in compound **1** was indicated. In the IR spectrum of compound **1** the absorptions due to the phenolic hydroxyl (3450), δ-lactone carbonyl (1710), aromatic C–C (1620, 1600, 1500) and aromatic C–O ($1265\ \mathrm{cm}^{-1}$) were discernible. Its UV spectral data $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 246 (4.51), 304 (2.42), 350 (4.61) was strikingly similar to that of a natural coumestone, lucernol [2]. The alcoholic solution of compound **1** exhibited bright blue fluorescene, a phenomenon commonly noted in coumarins and coumestones [3]. Therefore a coumestone structure was considered for compound **1**. The UV spectrum of compound **1** showed a bathochromic shift of 25 nm ($\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 375 (4.62) in the long wavelength band in the presence of sodium acetate indicating a hydroxyl group at the 3-position of the coumestone skeleton [4]. The combination of boric acid and sodium acetate did not produce any shift in the absorption maxima indicating the absence of an ortho hydroxyl group. The mass spectral fragmentation of compound **1** with m/z 298 $[M]^+$ (90%), 283 $[M-Me]^+$ (55%), 255 $[M-Me-CO]^+$ (23%) and 227 $[M-Me-CO-CO]^+$ (5%) was in good agreement with the fragmentation of coumarins [5]. In particular, the relative intensity of the $[M-Me]^+$ ion is the characteristic feature of 6-methoxy coumarins [5]. Therefore a methoxy group may be located at the C-2 position of the coumestone. The ^{1}H NMR spectrum (90 MHz, $CDCl_3$) of its acetate (**2**) revealed the presence of one methoxyl group (δ3.95, 3H, *s*), two acetoxyl groups (2.36, 6H, *s*) and five aromatic protons. The low field doublet at δ8.05 (1H, $J = 9$ Hz) and a singlet at δ7.6 (1H) are characteristic and are assigned to H-7 and H-1, respectively [6, 7]. It may be mentioned that in 2,3,9-trioxygenated coumestones, like lucernol triacetate the H-7 appears as a low field doublet at δ8.06 and H-1 as a singlet at δ7.8 [2]. The two singlets at δ7.53 (1H) and 7.25 (1H) are assignable to H-4 and H-10, respectively. A doublet at δ7.24 (1H, $J = 9$ Hz) was assignable to H-8. Based on the above ^{1}H NMR characteristics of the acetate (**2**), the two acetoxyl groups may be located at the C-3 and C-9 positions and hence the parent compound has been assigned as 2-methoxy-3,9-dihydroxy coumestone (**1**).

In the ^{1}H NMR spectrum (90 MHz, DMSO-d_6) of compound **1** the following signals were observed: δ7.8 (1H, *d*, $J = 9$ Hz, H-7), 7.3 (1H, *s*, H-1), 7.1 (1H, *s*, H-10), 6.92 (1H, *d*, $J = 9$ Hz, H-8), 6.8 (1H, *s*, H-4), 3.97 (3H, *s*, OMe) 3.71 (1H, *br*, D_2O exchangeable, C_3-OH), 3.42 (1H, *br*, D_2O exchangeable, C_9-OH). This assignment was confirmed by a direct comparison of the dimethyl ether of compound **1** with an authentic sample of 2,3,9-trimethoxy coumestone (lucernol trimethyl ether) [2]. This is the first report of **1** as a natural product. Pongamol (**4**), flemichap-



1 R=R=H
2 R=R=Ac
3 R=R=Me



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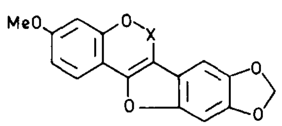

5 X = CH_2
6 X = C=O

parin-B (**5**) and- C (**6**) were also isolated and characterized by physical methods and by direct comparison with authentic samples.

EXPERIMENTAL

The air-dried roots (7 kg) of *T. hamiltonii*, collected locally were coarsely powdered and extracted successively with petrol (bp 60–80°) and $CHCl_3$ in a Soxhlet apparatus until the extracts were almost colourless. The combined petrol and $CHCl_3$ extracts on concn under red. pres. yielded a semi-solid which was mascerated with petrol. The petrol insoluble portion (20 g) was subjected to CC over silica gel (200 mesh). Fractions of 250 ml each were collected. The column was successively eluted with petrol (fractions 1–8), petrol–C_6H_6 (1:1) (fractions 9–15), petrol–C_6H_6 (1:4) (fractions 16–32), C_6H_6 (fractions 33–54), C_6H_6–EtOAc (9:1) (fractions 55–68), C_6H_6–EtOAc (4:1) (fractions 69–86), C_6H_6–EtOAc (1:1) (fractions 87–104), EtOAc (fractions 105–115), MeOH (fractions 116–125). Each fraction was monitored by TLC and similar fractions were combined. Fractions 1–8 yielded colourless oil. Fraction 9–18 yielded waxy material (0.75 g). Fractions 19–28 on rechromatography over silica gel (200 mesh) and crystallization with $CHCl_3$ gave flemichapparin-B (**5**, 0.85 g), mp 180° lit. mp 179–180° [8]. Fractions 29–54 on crystallization with C_6H_6, yielded pongamol (**4**, 7 g), mp 130°, lit. mp 128° [9]. Fractions 55–90 yielded a mixture of neutral compounds (2 g) whose separation is in progress. Fractions 91–104 yielded a light brown substance, containing coumestone (2.5 g). Fractions 105–125 gave a dark coloured resinous material (5 g).

The coumestone containing material (2.5 g) was rechromatographed over silica gel (200 mesh). Fractions of 100 ml each were collected. The column was eluted with C_6H_6 (fractions 1–6), C_6H_6–EtOAc (6:4) (fractions 7–16), EtOAc (fractions 17–30) and MeOH (fractions 31–50). Fractions 1–6 yielded small quantity and was not investigated. Fractions 7–16 on concn furnished a colourless solid, which crystallized from C_6H_6–EtOAc mixture (6:4) as colourless needles, designated as compound **1** (0.2 g), mp 318–320° [Found: C, 64.43; H, 3.34; $C_{16}H_{10}O_6$ requires: C, 64.42; H, 3.35%]. $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 246 (4.51), 304 (2.42), 350 (4.61); $\lambda_{\max}^{\mathrm{MeOH+NaOH}}$ nm: 248, 312, 372; $\lambda_{\max}^{\mathrm{MeOH+NaOAc}}$ nm: 262, 395. EIMS (probe) 70 eV, *m/z* (rel. int.): 298 $[M]^+$ (90), 283 $[M-Me]^+$ (55), 270 $[M-CO]^+$ (2), 255 $[M-Me-CO]^+$ (23), 227 $[M-Me-CO-CO]$ (5), 208 $[M-90]^+$ (60), 180 $[208-CO]^+$ (100), 165 $[180-CO]^+$ (20), 137 $[165-CO]^+$ (15), 109 $[137-CO]^+$ (2).

Diacetate (**2**). Compound **1** (50 mg) was refluxed with Ac_2O (1 ml) and pyridine (3 drops) for 30 min to yield a diacetate which was crystallized from EtOH as colourless needles, mp 256° [Found: C, 62.84; H, 3.65; $C_{20}H_{14}O_8$ requires: C, 62.83; H, 3.66%]. EIMS (probe) 70 eV, *m/z* (rel. int.): 382 $[M]^+$ (10) 340 $[M-CH_2{=}C{=}O]^+$ (35), 298 $[M-(2\times CH_2{=}C{=}O)]^+$ (100), 283 $[298-Me]^+$ (25), 255 $[283-CO]^+$ (5).

Dimethyl ether (**3**). Compound **1** (40 mg) in dry Me_2CO (25 ml) was refluxed with Me_2SO_4 (0.5 ml) and dry K_2CO_3 (200 mg) for 4 hr. The product was crystallized from $CHCl_3$ as colourless needles (40 mg) mp 255°, lit. mp 255° [2]. EIMS (probe) 70 eV, *m/z* (rel. int.): 326 (100), 311 (53), 283 (12), 255 (20), 241 (20), 190 (35), 167 (20), 162 (35), 154 (30), 100 (30), 86 (35), 73 (52), 70 (52), 59 (40), 57 (43), 42 (50).

The petrol soluble fraction was concd under red. pres. to a green semi-solid (18 g) which was subjected to CC over silica gel (200 mesh). Fractions of 250 ml each were collected. The column was successively eluted with petrol (fractions 1–4), petrol–C_6H_6 (9:2) (fractions 5–10), petrol–C_6H_6 (1:1) (fractions 11–15), C_6H_6 (fractions 16–28), C_6H_6–EtOAc (9:1) (fractions 29–38), C_6H_6–EtOAc (4:1) (fractions 39–54), C_6H_6–EtOAc (7:3) (fractions 55–69), C_6H_6–EtOAc (1:1) (fractions 70–86), EtOAc (fractions 87–98), MeOH (fractions 99–110). Fractions 1–3 yielded an oil (1.5 ml). Fractions 4–8 yielded a low melting aliphatic compound (1 g). Fractions 9–12 on concentration and crystallization with $CHCl_3$ yielded flemichapparin-B (**5**, 0.1 g). Fractions 13–28 on rechromatography over silica gel and crystallization with benzene yielded pongamol (**4**, 5 g). Fraction 29–60 yielded flemichapparin-C (**6**, 0.5 g), crystallized from benzene, mp 272°, lit. mp 272° [8], $[M]^+$ at *m/z* 310. Fractions 61–98 yielded a mixture of terpenoid compounds (1 g), whose separation is in progress. Fractions 99–110 yielded a dark resinous material (4 g).

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