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ISOFLAVONOIDS AND A PTEROCARPAN FROM *GLIRICIDIA SEPIUM*

H. M. T. B. Herath*, R. S. Dassanayake, A. M. A. Priyadarshani, Susila de Silva, G. P. Wannigama† and Joanne Jamie†

Natural Products Programme, Institute of Fundamental Studies, Kandy, Sri Lanka; Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka; † Department of Chemistry, University of Wollongong, Wollongong, NSW 2522 Australia

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Key Word Index—*Gliricidia sepium*; Leguminosae; insecticidal activity; toxicity; isoflavans; isoflavones; isovestitol; formononetin; afrormosin; pterocarpan; medicarpin.

Abstract—A new isoflavan, 7,4′-dihydroxy-3′-methoxyisoflavan has been isolated from the insecticidally active hot dichloromethane extract of the heartwood of *Gliricidia sepium*, along with the three other isoflavonoids, isovestitol, formononetin and afrormosin, a pterocarpan, medicarpin and 4-hydroxy-3-methoxycinnamaldehyde which are new to this species. © 1997 Elsevier Science Ltd

INTRODUCTION

Gliricidia sepium grows as a shade plant in the tea plantations of Sri Lanka. Previous studies on the insecticidal activity of *G. sepium* have shown that all parts of this plant have activity against Southern army worm, Cabbage looper, and Yellow woolly bear [1]. Though the main reason for planting *G. sepium* in a tea field is to cut off direct sunlight to maintain the optimum lighting conditions required by tea plants, it has been found that *G. sepium* also acts as a good diversionary host plant for *Glyptotermes dilatatus*, a tea pest responsible for causing great damage in the low country tea plantations of Sri Lanka. Olfactometer experiments showed that the attraction of the termite *G. dilatatus* towards the heartwood and the stem bark of *G. sepium*, and their organic extracts, is even significantly higher than that to some of the highly susceptible tea clones. Further feeding experiments involving different parts of the whole plant, and organic extracts of *G. sepium*, indicated that the heartwood was highly toxic and that the hexane and dichloromethane extracts were also significantly toxic to *Glyptotermes dilatatus*. These bioassay results suggested that chemical investigation of *G. sepium* would be of interest in order to isolate the semiochemicals responsible for the attraction, as well as the toxic elements causing the death of termites. Most of the previous chemical investigations of *G. sepium* have focused on the leaves and this has led to the isolation of 15 allelopathic compounds [2], including a coumarin. Two new hydrocarbons and another rare hydrocarbon have been found in the petioles of the species [3]. Two flavonoid glycosides [4, 5], an isoflavan, an isoflavene and some other flavonoid constituents have also been reported from *G. sepium* [6, 7]. This paper describes the isolation and structural elucidation of a new isoflavan, 7,4′-dihydroxy-3′-methoxyisoflavan (**1**) from the heartwood of *G. sepium* along with another isoflavan, isovestitol (**2**), two isoflavones, formononetin (**3**) and afrormosin (**4**), and a pterocarpan, medicarpin (**5**). Afrormosin (**4**) has been reported as a powerful antitumour promoting agent [8], whilst medicarpin (**5**) is known to be antifungal [9].

RESULTS AND DISCUSSION

HR-MS established the molecular formula $C_{16}H_{16}O_4$ for compound **1**. The base peak at *m/z* 150 and another prominent peak at *m/z* 123 in the mass spectrum of **1** corresponding to the basic fragment ions of the isoflavan ring system, gave evidence for an isoflavan type of structure. Signals for the aliphatic protons H-2, H-3 and H-4 in the ^1H NMR spectrum of **1** indicated the presence of an isoflavan-type C-ring (see Experimental). Decoupling studies on each of the above individual aliphatic proton signals confirmed the assignment of the proton resonances. The aromatic region of the ^1H NMR spectrum showed two sets of ABX protons. The base peak *m/z* 150 in the mass spectrum showed that the single methoxyl group of compound **1** is attached to ring B of the molecule. Irradiation of the protons of this methoxyl group

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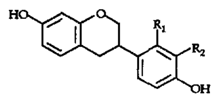
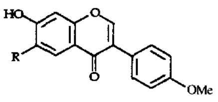
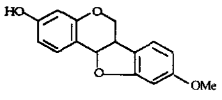
* Author to whom correspondence should be addressed.



1 R_1=H, R_2=OMe
2 R_1=OMe, R_2=H



3 R = H
4 R = OMe



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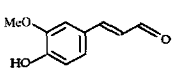

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resulted in enhancement of the signal at δ 6.27 for a *meta* coupled proton, which should thus be in ring B. Homo COSY, decoupling studies, HMQC and HMBC of **1** confirmed that the signals at δ 6.34 and 6.86 were due to the other two aromatic protons of ring B. Further, the ROESY spectrum of **1** showed that both the *meta* doublet at δ 6.27 and the double doublet at δ 6.34 are correlated with the H-4. This indicated a 1,3,4 type of substitution pattern in ring B with a methoxyl group at C-3′. The ROESY spectrum of compound **1** also indicated a correlation between the *ortho* coupled proton at δ 7.01 and H-4. Further, NOE studies of the signal at δ 7.01 showed the proximity of H-4, suggesting that the hydroxyl group of ring A should be at C-7. In addition, HMQC and HMBC of **1** confirmed that the signals at δ 7.01, 6.39 and 6.48 corresponded to the aromatic protons of ring A, H-5, H-6 and H-8, respectively. Hence the structure, 7,4′-dihydroxy-3′-methoxyisoflavan, is proposed for **1**. EIMS of **2** suggested the molecular formula $C_{16}H_{12}O_4$. The mass fragmentation pattern and the aliphatic region of the ^1H NMR of **2** were almost identical with those of **1**. This indicated a similarity in the basic structural features of **1** and **2**. ^1H, ^{13}C, Homo-COSY, HMQC and HMBC spectral data showed that the methoxyl group of **2** is attached at C-2′ whereas in **1** the methoxyl group is at C-3′. Hence the structure 7,4′-dihydroxy-2′-methoxyisoflavan is suggested for **2**. Though **2** is new to *G. sepium*, it has previously been isolated and reported as isovestitol from *Anthyllis vulneraria* and some *Tetragonolobus* species [10]. The IR, ^1H, ^{13}C, HMQC, HMBC and mass spectral data established the structure 7-hydroxy-4′-methoxyisoflavone for **3**. This is also new to the species but the spectral data and the melting points were identical with formononetin which has previously been reported [11]. Spectral data and mp gave the structure 7-hydroxy 6,4′-dimethoxyisoflavone for **4** which was first isolated from the heartwood of *Afrormosia elata* as afrormosin [12]. Spectral data confirm the structure of medicarpin as **5**. Compound **6** was identified as 4-hydroxy-3-methoxycinnamaldehyde by comparison of spectral data.

EXPERIMENTAL

Mp's were recorded on a Kofler hot-stage apparatus. Identities of compounds were established by IR, NMR and MS spectral data. The mp and co-TLC comparisons were also carried out wherever possible. ^1H and ^{13}C spectra were recorded on a Varian Unity 400 spectrometer at the Department of Chemistry, University of Wollongong, Wollongong, NSW 2522 Australia. Prep. TLC was carried out on Merck Kieselgel 60 F_{254}. Flash and medium pres. CC was carried out on Merck Kieselgel 60 (230–400 mesh ASTM).

Plant material. Gliricidia sepium was collected from Ratnapura, Sabaragamuwa province in Sri Lanka and authenticated by comparison with herbarium specimens at the National Herbarium, Royal Botanic Gardens, Peradeniya, Sri Lanka.

Extraction. Dried and crushed heartwood (7.0 kg) of *G. sepium* was extracted with hot CH_2Cl_2 in a Soxhlet extractor. The CH_2Cl_2 extract (8.0 g) was chromatographed on a medium pres. silica gel column and eluted with hexane, CH_2Cl_2 and MeOH by gradually increasing the polarity gradient. Further purification of the column frs eluted from CH_2Cl_2 to 2% MeOH–CH_2Cl_2, using flash chromatographic techniques and prep. TLC methods gave compounds **1** (17 mg), **2** (12 mg), **3** (36 mg), **4** (23 mg), **5** (24 mg) and **6** (10 mg).

7,4′-Dihydroxy-3′-methoxyisoflavan (**1**). Colourless needles, mp 149–150°; IR ν_{max} cm^{-1}: 3400, 1605, 1485, 1445, 1415, 1295, 1205, 1185, 1125, 1100, 1110; ^1H NMR (Acetone-d_6) δ 7.01 (1H, *d*, $J_{5,6} = 8.4$ Hz, H-5), 6.86 (1H, *d*, $J_{5',6'} = 8.4$ Hz, H-5′), 6.48 (1H, *d*, $J_{8,6} = 2.4$ Hz, H-8), 6.39 (1H, *dd*, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 2.4$ Hz, H-6), 6.34 (1H, *dd*, $J_{6',5'} = 8.0$ Hz, $J_{6',2'} = 2.4$ Hz, H-6′), 6.27 (1H, *d*, $J_{2',6'} = 2.4$ Hz, H-2′), 4.23 (1H, *ddd*, $J_{2eq,2ax} = 10.4$ Hz, $J_{2eq,3ax} = 3.4$ Hz, $J_{2eq,4eq} = 2.0$ Hz, H-2eq), 3.96 (1H, *dd*, $J_{2ax,2eq} = 10.4$ Hz, $J_{2ax,3ax} = 10.0$ Hz, H-2ax), 3.71 (3H, *s*, OCH_3), 3.46 (1H, *m*, H-3ax), 2.95 (1H, *dd*, $J_{4ax,4eq} = 15.6$ Hz, $J_{4ax,3ax} = 10.8$ Hz, H-4ax), 2.79 (1H, *ddd*, $J_{4eq,4ax} = 15.6$ Hz, $J_{4eq,3ax} = 5.2$ Hz, $J_{4eq,2eq} = 2.0$ Hz, H-4eq); ^{13}C NMR (Acetone-d_6) δ 160.3 (C-4′), 157.4 (C-3′), 156.6 (C-8a), 156.0 (C-7), 131.0 (C-5), 128.7 (C-6′), 120.8 (C-1′), 114.3 (C-4a), 108.7 (C-6), 105.6 (C-5′), 103.6 (C-8), 102.4 (C-2′), 70.4 (C-2), 55.3 (OCH_3), 32.6 (C-3), 31.0 (C-4); MS (70 eV) $m/z = 272$ (M^-, 20), 150 (100), 137 (31), 123 (18), 107 (17); HR-MS: $[M]^+$ found 272.1055, calcd for $C_{16}H_{16}O_4$ 272.1049.

7,4′-Dihydroxy-2′-methoxyisoflavan (**2**). Colourless needles, mp 95–97°, ^1H NMR ($CDCl_3$) δ 6.87 (1H, *d*, $J = 8.4$ Hz, H-6′), 6.80 (1H, *d*, $J = 8.2$ Hz, H-5), 6.32–6.27 (3H, *m*, H-6, H-3′ and H-5′), 6.22 (1H, *br s*, H-8), 4.20 (1H, *br d*, $J = 10.3$ Hz, H-2eq), 3.92 (1H, *m*, H-2ax), 3.66 (3H, *s*, OCH_3), 3.42 (1H, *m*, H-3ax), 2.87 (1H, *dd*, $J = 15.6$ and 15.5 Hz, H-4ax), 2.76 (1H, *dd*, $J = 15.6$ and 5.0 Hz, H-5eq); ^{13}C NMR ($CDCl_3$) δ 159.8 (C-4′), 157.0, 156.4 (C-7 and C-2′), 155.1 (C-8a), 130.5 (C-5), 128.1 (C-6′), 120.8 (C-1′), 114.0 (C-4a), 108.4 (C-6), 104.9 (C-5′), 103.2 (C-8), 102.0 (C-3′), 70.3 (C-2), 55.4 (OCH_3), 32.0 (C-3), 30.5 (C-4); MS (70 eV) $m/z = 272$ (M^+, 24), 150 (100), 137 (28), 135 (10), 124 (9).

7-Hydroxy-4′-methoxyisoflavone (**3**). Needles, mp

260° (lit [11], 261°; ^{1}H NMR (CD_3OD) δ 8.07 (1H, *d*, $J = 8.8$ Hz, H-5), 7.94 (1H, *s*, H-2), 7.44 (2H, *d*, $J = 8.7$ Hz, H-2′ and H-6′), 6.95 (2H, *d*, $J = 8.7$ Hz, H-3′ and H-5′), 6.92 (1H, *dd*, $J = 8.8$ and 2.1 Hz, H-6), 6.83 (1H, *d*, $J = 2.1$ Hz, H-8), 3.83 (3H, *s*, OCH_3); ^{13}C NMR (CD_3OD) δ 177.5 (C-4), 163.0 (C-7), 160.2 (C-4′), 158.0 (C-8a), 153.1 (C-2), 130.6 (C-2′ and C-6′), 128.0 (C-5), 125.1, 124.8 (C-3 and C-1′), 117.5 (C-4a), 115.7 (C-6), 114.3 (C-3′ and C-5′), 102.8 (C-8), 55.6 (OCH_3); MS (70 eV) $m/z = 268$ (M^+, 100), 255 (18), 134 (77), 109 (20), 91 (32).

7-*Hydroxy*-6,4′-*dimethoxyisoflavone* (**4**). Mp 228° (lit [13] 231–233°); ^{1}H NMR (DMSO) δ 8.32 (1H, *s*, H-2), 7.50 (2H, *d*, $J = 8.7$ Hz, H-2′ and H-6′), 7.42 (1H, *s*, H-5), 6.98 (2H, *d*, $J = 8.7$ Hz, H-3′ and H-5′), 6.94 (1H, *s*, H-8), 3.87 (OCH_3), 3.77 (OCH_3); ^{13}C NMR (DMSO) δ 174.2 (C-4), 158.9 (C-4′), 153.0 (C-7), 152.8 (C-2), 151.7 (C-8a), 147.0 (C-6), 130.0 (C-2 and C-6′), 124.4 (C-3), 122.6 (C-1′), 116.2 (C-4a), 113.6 (C-3′ and C-5′), 104.6 (C-5), 102.8 (C-8), 55.8 (OCH_3), 55.1 (OCH_3); MS (70 eV) $m/z = 298$ (M^+, 100), 166 (48), 149 (30), 148 (30), 132 (43), 123 (24).

3-*Hydroxy*-9-*methoxypterocarpan* (**5**). Colourless needles mp 128–129° (lit [9], 127–129°); ^{1}H NMR ($CDCl_3$) δ 7.41 (1H, *d*, $J = 8.4$ Hz, H-1), 7.15 (1H, *d*, $J = 8.9$ Hz, H-7), 6.58 (1H, *dd*, $J = 8.4$ and 2.4 Hz, H-2), 6.48 (2H, *m*, H-8 and H-10), 6.44 (1H, *d*, $J = 2.4$ Hz, H-4), 5.52 (1H, *d*, $J = 6.8$ Hz, H-11a), 4.26 (1H, *dd*, $J = 11.0$ and 5.0 Hz, H-6eq), 3.79 (3H, *s*, OCH_3), 3.64 (1H, *dd*, $J = 11.0$ and 10.9 Hz, H-6ax), 3.55 (1H, *ddd*, $J = 11$, 5.1 and 6.7 Hz, H-6a); ^{13}C NMR ($CDCl_3$) δ 161.5, 161.1 (C-10a and C-9), 157.5, 157.1 (C-3 and C-4a), 132.6 (C-1), 125.2 (C-7), 119. 5 (C-7a), 113.0 (C-1a), 102.2 (C-2), 106.8, 97.3 (C-8 and C-10), 104.1 (C-4), 79.0 (C-11a), 67.0 (C-6), 55.9 (OCH_3), 39.9 (C-6a); MS (70 eV) $m/z = 270$ (M^+, 100), 255 (37), 155 (33).

4-*Hydroxy*-3-*methoxycinnamaldehyde* (**6**). ^{1}H NMR ($CDCl_3$) δ 9.67 (1H, *d*, $J = 7.7$ Hz, H-3′), 7.43 (1H, *d*, $J = 15.8$ Hz, H-1′), 7.15 (1H, *dd*, $J = 8.2$ and 1.9 Hz, H-6), 7.09 (1H, *d*, $J = 1.9$ Hz, H-2), 6.99 (1H, *d*, $J = 8.2$ Hz, H-5), 6.62 (1H, *dd*, $J = 15.8$ and 7.7 Hz, H-2′), 6.00 (1H, *s*, OH), 3.98 (3H, *s*, OCH_3); ^{13}C NMR ($CDCl_3$) δ 194.0 (C-3′), 153.5 (C-1′), 149.4 (C-4), 147.5 (C-3), 126.9 (C-2′), 124.5 (C-6), 115.3 (C-5), 109.8 (C-2); MS (70 eV) $m/z = 178$ (M^+, 100).

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