Isoflavene, Isoflavan, and Flavonoid Constituents of *Gliricidia sepium*

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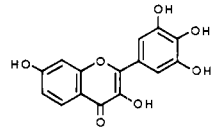
The extracts of *Gliricidia sepium* heartwood have yielded two new isoflavenes, viz. 2',3',7-trihydroxy-4'-methoxyisoflav-3-ene (sepiol) and 3',7-dihydroxy-2',4'-dimethoxyisoflav-3-ene (2'-*O*-methylsepiol), a new phenolic isoflavan, robinetin, and 7,3',4'-trihydroxyflavanone.

The heartwood of the Panamanian tree *Gliricidia sepium* (Leguminosae) is moderately resistant to attack by marine organisms (Southwell and Bultman, 1971). Its constituents have not previously been investigated, although robinetin **1** was isolated (Subramoni and Rangaswami, 1973) from a related Indian species, *Gliricidia maculata*.

After removal of robinetin and other unidentified acidic flavonoids by extraction with sodium bicarbonate and carbonate solutions, the sodium borate soluble fraction of ether extracts of the heartwood of *Gliricidia sepium* yields a new, colorless phenol, $C_{16}H_{14}O_5$, called sepiol and identified as an isoflav-3-ene **2a** (Jurd, 1976). In addition, the sodium borate and carbonate insoluble fraction of the ether extract yields two new minor isoflavanoid constituents, viz. $C_{17}H_{16}O_5$ and $C_{17}H_{18}O_5$, and a third phenol, $C_{15}H_{12}O_5$, identified as the known 7,3',4'-trihydroxyflavanone.



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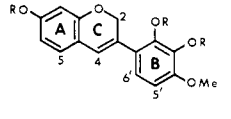

2a, R = H **2c**, R = Me
2b, R = COMe **2d**, R = Et

EXPERIMENTAL SECTION

All melting points are uncorrected. NMR spectra, unless otherwise stated, were determined in $CDCl_3$ with a tetramethylsilane internal standard on a modified Varian HA-100 instrument.

Extraction of *Gliricidia sepium* Heartwood. Milled heartwood (3 kg) was extracted continuously with boiling Skellysolve F (3 days) and with ether (3 days). The ether solution was evaporated and the residue was extracted with warm benzene (2 × 500 mL). Evaporation of the benzene solution gave a gum which was redissolved in ether (800 mL) and successively washed with saturated aqueous $NaHCO_3$ (3 × 200 mL) and $Na_2B_4O_7$ (7 × 200 mL). The residual ether solution, as described below, was chromatographed to give the minor phenolic components.

The aqueous borax solution was acidified and extracted with ether. Evaporation of the extract gave a gum which crystallized from wet methanol (20 mL). Recrystallized from aqueous methanol, sepiol **2a** separated as slightly brown, glistening prisms, mp 209–210 °C (2.30 g), which rapidly reduce ammoniacal silver nitrate and give an intense blue color changing to an emerald green with methanolic ferric chloride: λ_{max} (EtOH) 323 (4.44), 220 (4.57) nm (log ϵ); *m/e* (%) 286 (100), 285 (43), 271 (36), 270 (12), 269 (15), 153 (10), 152 (11), 147 (12), 135 (22), 134 (12). Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.1; H, 4.93. Found: C, 67.1; H, 5.06.

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Warmed with acetic anhydride (0.5 mL) and pyridine (0.1 mL) sepiol (0.3 g) formed the triacetate **2b**, which crystallized from methanol as colorless prisms: mp 143 °C (0.32 g); NMR δ 2.27 (3 H, s), 2.29 (3 H, s), 2.31 (3 H, s), 3.87 (3 H, s), 4.94 (2 H, d, *J* = 1 Hz), 6.46–6.70 (2 H, m), 6.60 (1 H, br s), 6.88 (1 H, d, *J* = 9 Hz), 7.02 (1 H, d, *J* = 9 Hz), 7.19 (1 H, d, *J* = 9 Hz). Anal. Calcd for $C_{22}H_{20}O_8$: C, 64.1; H, 4.89. Found: C, 64.0; H, 4.83.

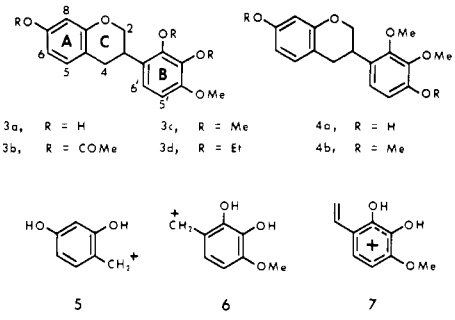
Tri-*O*-methylsepiol (2c). A mixture of sepiol (0.40 g), dimethyl sulfate (2.0 mL), potassium carbonate (4.0 g), and acetone (20 mL) was heated under reflux for 1 h. The product obtained on adding water crystallized from methanol to give tri-*O*-methylsepiol **2c** as colorless needles: mp 102 °C (0.41 g); *m/e* (%) 328 (100), 327 (27), 313 (39), 297 (15), 161 (12), 156.5 (16); NMR δ 3.80 (3 H, s), 3.85 (3 H, s), 3.88 (3 H, s), 3.91 (3 H, s), 5.02 (2 H, d, *J* = 1 Hz), 6.42 (1 H, br s), 6.40–6.58 (2 H, m), 6.66 (1 H, d, *J* = 8 Hz), 6.98 (1 H, d, *J* = 8 Hz), 7.01 (1 H, d, *J* = 8 Hz). Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.5; H, 6.14. Found: C, 69.5; H, 6.25.

2c (0.1 g) was hydrogenated in tetrahydrofuran (20 mL) with 5% Pd/C catalyst. Tri-*O*-methyldihydrosepiol **3c** crystallized from methanol as colorless needles: mp 76–77 °C (0.08 g); λ_{max} (EtOH) 288 (3.60), 280 (3.70) nm (log ϵ); *m/e* (%) 330 (37), 194 (100), 182 (40), 181 (26), 179 (44), 149 (36); NMR δ 2.88 (1H, br s), 2.96 (1 H, s), 3.34–3.80 (1 H, m), 3.78 (3 H, s), 3.86 (3 H, s), 3.90 (3 H, s), 3.92 (3 H, s), 4.00 (1 H, d of d, *J* = 10, 10 Hz), 4.31 (1 H, d of d, *J* = 10, 3 Hz), 6.44 (1 H, d, *J* = 2 Hz), 6.48 (1 H, d of d, *J* = 8, 2 Hz), 6.64 (1 H, d, *J* = 8 Hz), 6.81 (1 H, d, *J* = 8 Hz), 6.97 (1 H, d, *J* = 8 Hz). Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.1; H, 6.71. Found: C, 69.2; H, 6.72.

Tri-*O*-ethylsepiol (2d). Sepiol (0.5 g), treated with diethyl sulfate (2.0 mL), potassium carbonate, and acetone as described above, formed tri-*O*-ethylsepiol **2d**, which crystallizes from acetone–methanol as colorless needles: mp 69–70 °C (0.51 g). Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.3; H, 7.08. Found: C, 71.5; H, 7.01.

Catalytic hydrogenation of tri-*O*-ethylsepiol (0.20 g) in tetrahydrofuran (30 mL) with Pd/C catalyst gave tri-*O*-ethyldihydrosepiol **3d**, colorless brittle prisms from methanol: mp 78–79 °C (0.17 g); NMR δ 1.26–1.52 (9 H, m), 2.86 (1 H, br s), 2.95 (1 H, br s), 3.42–3.74 (1 H, m), 3.84 (3 H, s), 3.87–4.38 (8 H, m), 6.40–7.05 (5 H, m); NMR (C_6D_6) δ 1.00–1.40 (9 H, m), 2.82 (1 H, br s), 2.90 (1 H, br s), 3.39 (3 H, s), 3.66 (2 H, q, *J* = 7 Hz), 3.70 (1 H, m), 3.88–4.08 (6 H, m), 6.33–6.97 (5 H, m). Anal. Calcd for $C_{22}H_{28}O_5$: C, 70.9; H, 7.58. Found: C, 71.1, H, 7.50.

Dihydrosepiol 3a. Catalytic hydrogenation of sepiol (0.2 g) in tetrahydrofuran (15 mL) gave dihydrosepiol **3a**, which crystallized from benzene as colorless prisms: mp 172–173 °C (0.18 g): *m/e* (%) 288 (80), 167 (13), 166 (100), 165 (12), 154 (55), 153 (43), 135 (21), 133 (21), 123 (76); NMR δ 2.90–3.00 (2 H, m), 3.40–3.70 (1 H, m), 2.86 (3 H, s), 4.08 (1 H, d of d, *J* = 10, 10 Hz), 4.35 (1 H, d of d, *J* = 10, 3 Hz), 4.63 (1 H (OH), s), 5.42 (1 H (OH), s), 5.48 (1 H, (OH), s), 6.30–6.48 (3 H, m), 6.61 (1 H, d, *J* = 9 Hz), 6.93 (1 H, d, *J* = 9 Hz). Anal. Calcd for $C_{16}H_{16}O_5$: C, 66.7;



3a, R = H
3b, R = COMe
3c, R = Me
3d, R = Et
4a, R = H
4b, R = Me

5 6 7

H, 5.59. Found: C, 66.5; H, 5.58.

Acetylation of **3a** with acetic anhydride and pyridine gave dihydrosepiol triacetate **3b**, colorless needles from methanol: mp 172–173 °C; NMR δ 2.29 (3 H, s), 2.31 (3 H, s), 2.33 (3 H, s), 2.89 (1 H, br s), 2.98 (1 H, br s), 3.20–3.44 (1 H, m), 3.84 (3 H, s), 3.97 (1 H, d of d, J = 10, 10 Hz), 4.29 (1 H, d of d, J = 10, 3 Hz), 6.56–6.68 (2 H, m), 6.85 (1 H, d, J = 9 Hz), 7.04 (1 H, d, J = 9 Hz), 7.06 (1 H, d, J = 9 Hz). Anal. Calcd for $C_{22}H_{22}O_8$: C, 63.8; H, 5.35. Found: C, 63.8; H, 5.44.

3-(2,3,4-Trimethoxyphenyl)-7-methoxycoumarin (9c). (Tri-*O*-methylsepiol (0.2 g) was added to a suspension of chromium trioxide (0.2 g) in pyridine (40 mL) at 50 °C. After 30 min TLC showed oxidation was complete. Dichloromethane (50 mL) was added and the filtered solution was washed with water and aqueous HCl and evaporated. The residue crystallized from methanol to give 3-(2,3,4-trimethoxyphenyl)-7-methoxycoumarin (**9c**) as colorless needles: mp 141–142 °C (0.09 g); NMR δ 3.89 (3 H, s), 3.90 (3 H, s), 3.91 (3 H, s), 3.93 (3 H, s), 6.72 (1 H, d, J = 8.0 Hz), 6.80–6.90 (2 H, m), 7.11 (1 H, d, J = 8.0 Hz), 7.39 (1 H, d, J = 8.0 Hz), 7.68 (1 H, s). Anal. Calcd for $C_{19}H_{18}O_6$: C, 66.7; H, 5.30. Found: C, 66.6; H, 5.23.

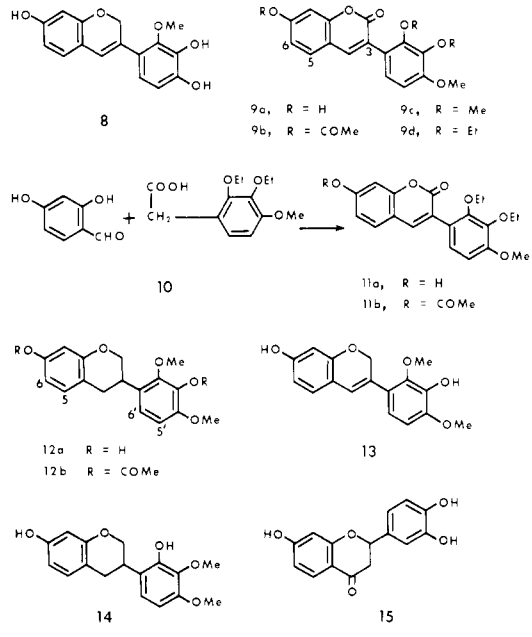
Oxidation of Sepiol Triacetate. Sepiol triacetate (0.8 g) was added to a suspension of chromium trioxide (1.0 g) in pyridine (15 mL) at 50 °C. After 1 h dichloromethane (125 mL) was added and the filtered solution washed with water and 10% HCl (100 mL). The solution was evaporated and the residue crystallized from methanol to give 3-(2,3-diacetoxy-4-methoxyphenyl)-7-acetoxycoumarin (**9b**) as colorless plates: mp 188–189 °C (0.53 g); NMR δ 2.19 (3 H, s), 2.31 (3 H, s), 2.36 (3 H, s), 3.89 (3 H, s), 6.93 (1 H, d, J = 9.0 Hz), 7.06 (1 H, d of d, J = 9.0, 2.0 Hz), 7.15 (1 H, d, J = 2.0 Hz), 7.33 (1 H, d, J = 9.0 Hz), 7.49 (1 H, d, J = 9.0 Hz), 7.67 (1 H, s). Anal. Calcd for $C_{22}H_{18}O_9$: C, 62.0; H, 4.26. Found: C, 62.3; H, 4.43.

A solution of the above triacetate (0.30 g) in acetone (5 mL) and methanol (10.0 mL) was hydrolyzed by warming for 5 min with 10% aqueous NaOH (5.0 mL) containing a little sodium dithionite. Water was added and the solution was acidified and concentrated until crystallization of the product began. Recrystallized from acetone–methanol, 3-(2,3-dihydroxy-4-methoxyphenyl)-7-hydroxycoumarin (**9a**) separated as slightly yellow needles: mp 262–263 °C (0.20 g). The same product was obtained by hydrolysis of the triacetate in ethanolic sulfuric acid. Anal. Calcd for $C_{16}H_{12}O_6$: C, 64.0; H, 4.03. Found: C, 63.7; H, 4.22.

The above phenolic coumarin (0.2 g) was ethylated by warming with diethyl sulfate and potassium carbonate in acetone for 2 h. The product obtained on adding water crystallized from methanol to give 3-(2,3-diethoxy-4-methoxyphenyl)-7-ethoxycoumarin (**9d**) as colorless, soft needles: mp 93–94 °C (0.19 g); NMR δ 1.18–1.60 (9 H, m), 3.91 (3 H, s), 4.14 (6 H, q, J = 7.0 Hz), 6.73 (1 H, d, J = 9.0 Hz), 6.78–6.92 (2 H, m), 7.15 (1 H, d, J = 9.0 Hz), 7.40 (1 H, d, J = 9.0 Hz), 7.74 (1 H, s). Anal. Calcd for $C_{22}H_{24}O_6$: C, 68.7; H, 6.29. Found: C, 69.0; H, 6.33.

Synthesis of 3-(2,3-Diethoxy-4-methoxyphenyl)-7-ethoxycoumarin. A mixture of 2,3-diethoxy-4-methoxyacetophenone (7.14 g), sulfur (0.96 g), and morpholine (2.61 g) was heated under reflux for 6 h. The oily product which separated on addition of ice–water was collected and hydrolyzed by warming with 80% ethanol (40 mL) and KOH (10 g) for 18 h. The solution was concentrated, diluted with water, and extracted with ether. Evaporation of the ether gave 2,3-diethoxy-4-methoxyphenylacetic acid as an oil. Without further purification this was heated under reflux with 2,4-dihydroxybenzaldehyde (4.0 g), potassium acetate (6.0 g), and acetic anhydride (25.0 mL) for 4 h. The product which separated on addition of water crystallized from methanol to give 3-(2,3-diethoxy-4-methoxyphenyl)-7-acetoxycoumarin (**11b**) as colorless needles: mp 128 °C (3.65 g); NMR δ 1.22 (3 H, t, J = 7.0 Hz), 1.41 (3 H, t, J = 7.0 Hz), 2.36 (3 H, s), 3.89 (3 H, s), 4.13 (4 H, q, J = 7.0 Hz), 6.71 (1 H, d, J = 9.0 Hz), 7.00–7.16 (2 H, m), 7.14 (1 H, d, J = 9.0 Hz), 7.49 (1 H, d, J = 9.0 Hz), 7.77 (1 H, s). Anal. Calcd for $C_{22}H_{22}O_7$: C, 66.3; H, 5.57. Found: C, 66.2; H, 5.59.

A solution of the above acetate (1.0 g) in boiling methanol (30 mL) was treated with 10% aqueous KOH (2.0 mL) for 5 min. The solution was diluted and acidified. The solid product crystallized from methanol to give 3-(2,3-diethoxy-4-methoxyphenyl)-7-hydroxycoumarin (**11a**) as colorless needles: mp 180–181 °C (0.75 g). Anal. Calcd. for $C_{20}H_{20}O_6$: C, 67.4; H, 5.66. Found: C, 67.7; H, 5.74.



8 9a, R = H 9b, R = COMe 9c, R = Me 9d, R = Et
10 11a, R = H 11b, R = COMe
12a R = H 12b R = COMe 13 14 15

The above phenol (0.30 g) was heated under reflux with diethyl sulfate (2.0 mL), potassium carbonate (10 g), and acetone (50 mL) for 2 h. Water was added and, after hydrolysis of excess diethyl sulfate, the product was collected. Recrystallized from methanol 3-(2,3-diethoxy-4-methoxyphenyl)-7-ethoxycoumarin (**9d**) was obtained as colorless needles: mp and mmp with the product from tri-*O*-ethylsepiol 93–94 °C (0.25 g); NMR and R_f values were identical with the sepiol product. Anal. Calcd for $C_{22}H_{24}O_6$: C, 68.7; H, 6.29. Found: C, 68.7; H, 6.40.

Minor Phenolic Constituents of *Gliricidia sepium*. After removal of the carbonate and borate soluble constituents of the benzene-soluble fraction of the ether extract, the residue was preparatively chromatographed on silica gel (benzene), LH-20 (chloroform–methanol), and again on silica gel (chloroform). Column elution gave

successively the three phenols subsequently identified as 2′-*O*-methylsepiol **13**, the phenolic isoflavan **14** (or **3a**), and 7,3′,4′-trihydroxyflavanone. 2′-*O*-Methylsepiol **13** was obtained as an oil, which formed a diacetate on warming with acetic anhydride and pyridine. The diacetate crystallized from methanol as colorless needles: mp 104–105 °C; *m/e* (%) 384 (80), 342 (73), 300 (100), 285 (48), 283 (47), 147 (24); NMR δ 2.29 (3 H, s), 2.37 (3 H, s), 3.77 (3 H, s), 3.86 (3 H, s), 5.03 (2 H, d, *J* = 1.0 Hz), 6.61 (1 H, br s), 6.60–6.65 (2 H, m), 6.74 (1 H, d, *J* = 9.0 Hz), 7.04 (1 H, d, *J* = 9.0 Hz), 7.15 (1 H, d, *J* = 9.0 Hz). Anal. Calcd for $C_{21}H_{20}O_7$: M^+ 384.1210. Found: M^+ 384.1202.

Hydrogenation of the diacetate in the presence of palladium/charcoal yielded the isoflavan as an oil: NMR δ 2.26 (3 H, s), 2.35 (3 H, s), 2.91 (1 H, br s), 2.99 (1 H, br s), 3.52 (1 H, m), 3.81 (3 H, s), 3.82 (3 H, s), 4.00 (1 H, d of d, *J* = 10.0, 10.0 Hz), 4.32 (1 H, d of d, *J* = 10.0, 3.0 Hz), 6.60–6.63 (2 H, m), 6.70 (1 H, d, *J* = 9.0 Hz), 6.95 (1 H, d, *J* = 9.0 Hz), 7.06 (1 H, d, *J* = 9.0 Hz).

The phenolic isoflavan **14** (or **3a**) crystallized from chloroform as slightly yellow needles: mp 152–153 °C; [α] −5.3° (acetone); *m/e* (%) 302 (57), 180 (100), 168 (45), 167 (45), 135 (14), 133 (20), 123 (12); NMR (acetone-d_6) δ 2.91 (1 H, br s), 2.98 (1 H, br s), 3.41 (1 H, m), 3.81 (3 H, s), 3.83 (3 H, s), 4.00 (1 H, d of d, *J* = 10.0, 10.0 Hz), 4.28 (1 H, d of d, *J* = 10.0, 3.0 Hz), 6.29 (1 H, d, *J* = 2 Hz), 6.36 (1 H, d of d, *J* = 9.0, 2.0 Hz), 6.50 (1 H, d, *J* = 9.0 Hz), 6.84 (1 H, d, *J* = 9.0 Hz), 6.91 (1 H, d, *J* = 9.0 Hz), 7.87 (1 H (OH), s), 8.03 (1 H (OH), s). Anal. Calcd for $C_{17}H_{18}O_5$: M^+ 302.1155. Found: 302.1153.

With acetic anhydride and pyridine **14** formed a diacetate (oil): NMR δ 2.31 (3 H, s), 2.38 (3 H, s), 2.90 (1 H, br s), 2.98 (1 H, br s), 3.26 (1 H, m), 3.86 (3 H, s), 3.87 (3 H, s), 3.97 (1 H, d of d, *J* = 10.0, 10.0 Hz), 4.29 (1 H, d of d, *J* = 10.0, 3.0 Hz), 6.56–6.68 (2 H, m), 6.80 (1 H, d, *J* = 9.0 Hz), 6.87 (1 H, d, *J* = 9.0 Hz), 7.05 (1 H, d, *J* = 9.0 Hz).

7,3′,4′-Trihydroxyflavanone **15** crystallized from ether–benzene as yellow needles: mp 222 °C (lit. mp 224–226 °C; Shinoda, 1929); NMR (acetone-d_6) δ 2.68 (1 H, d of d, *J* = 17, 4 Hz) 3.03 (1 H, d of d, *J* = 17, 12 Hz), 3.82 (1 H, br s), 5.40 (1 H, d of d, *J* = 12, 4 Hz), 6.43 (1 H, d, *J* = 2 Hz), 6.67 (1 H, d of d, *J* = 9, 2 Hz), 6.87 (2 H, br s), 7.05 (1 H, d, *J* = 2 Hz), 7.74 (1 H, d, *J* = 9 Hz), 8.43 (2 H, br s). Anal. Calcd for $C_{15}H_{12}O_5$: M^+ 272.0686. Found: 272.0690.

The compound formed a triacetate: mp 126 °C (lit. mp 123–125 °C); NMR δ 2.31 (9 H, s), 2.87 (1 H, d of d, *J* = 18, 6 Hz), 3.10 (1 H, d of d, *J* = 18, 11 Hz), 5.50 (1 H, d of d, *J* = 11, 6 Hz), 6.73–6.89 (2 H, m), 7.20–7.43 (3 H, m), 7.96 (1 H, d, *J* = 10 Hz).

RESULTS AND DISCUSSION

In accord with structure **2a** sepiol contains one methoxyl and three phenolic hydroxyl groups and is optically inactive. The NMR spectrum of its triacetate indicates the presence of five aromatic protons, three of which show only ortho (or ortho,para) coupling, and of a methylene proton at δ 4.94 which is allylically coupled (*J* = 1 Hz) to a methine proton at δ 6.60. The chemical shift of the methylene group suggests an isoflav-3-ene structure for sepiol, since in isomeric isoflav-2-enes the methylene group at C_4 appears upfield at about δ 3.50 (Anirudhan et al., 1966). An isoflavene structure was further confirmed by the mass spectra of sepiol and its tri-*O*-methyl derivative **2c** which show prominent ions at $M^+ - 1$ due to the facile formation of the corresponding isoflavylium cations.

The NMR spectrum of dihydrosepiol (**3a**), $C_{16}H_{16}O_5$, formed by catalytic hydrogenation of sepiol, reveals the characteristic splitting pattern previously observed (Kurosawa et al., 1968; Ferreira et al., 1974) for C ring protons of isoflavans. Furthermore, the spectrum of tri-*O*-methyldihydrosepiol (**3c**) clearly indicates that the four methoxyl groups are substituted at positions 7, 2′, 3′, and 4′ on the isoflavan nucleus. Thus, the aromatic protons at C_5' and C_6' appear as ortho-coupled doublets at δ 6.64 and 6.81, the C_5 proton as an ortho,para-coupled doublet at δ 6.97, the C_6 proton as an ortho,meta-coupled doublet at δ 6.48, and the C_8 proton as a meta,para-coupled doublet at δ 6.44. The C_4 methylene group of the C ring appears as two broad 1 H singlets at δ 4.00 and 4.31, and C_3 methine proton as a multiplet at δ 3.60. The NMR, UV, and mass spectra of tri-*O*-methyldihydrosepiol closely agree in all respects with those reported (Pelter and Amenechi, 1969) for an optically active di-*O*-methyl derivative **4b** (mp 65–67 °C) of laxifloran **4a**, a dihydroxyisoflavan detected (but isolated and characterized only as its methyl derivative) in *Lonchocarpus laxiflorus*. Tri-*O*-methyldihydrosepiol, therefore, is a higher melting racemate (mp 76–77 °C) of di-*O*-methyllaxifloran and has structure **3c**. Tri-*O*-methylsepiol is the corresponding isoflav-3-ene **2c**.

The mass spectrum of dihydrosepiol has prominent ions at *m/e* 123, 153, and 166 due to the formation of the ions, **5**, **6**, and **7**, respectively. These data show that in sepiol and dihydrosepiol ring A carries a single hydroxyl group and ring B the methoxyl and two hydroxyl groups. It remains, therefore, only to establish the location of the methoxyl relative to the two hydroxyl groups in the B ring. Sepiol reduced silver nitrate and its λ_{max} in alcohol (323 nm) undergoes a bathochromic shift of 15 nm on addition of boric acid–sodium acetate, indicating (Jurd, 1962) that the hydroxyl groups are ortho and that the methoxyl is located at the 4′ position as in **2a**, or at the 2′ position as in the possible alternative structure **8**. Pelter and Amenechi (1969) observed that signals from methoxyl groups ortho to hydrogen in fully methylated compounds move upfield >0.3 ppm on changing solvent from $CDCl_3$ to C_6D_6. In accord with this, only two of the four methoxyl signals of tri-*O*-methyldihydrosepiol **3c** show shifts >0.3 ppm on changing solvent from $CDCl_3$ (δ 3.78, 3.86, 3.90, and 3.92) to C_6D_6 (δ 3.39, 3.41, 3.67, and 3.75). Application of the procedure to the tri-*O*-ethyl derivatives of sepiol and dihydrosepiol unambiguously locates the methoxyl at the 4′ position as in **2d**. With tri-*O*-ethylsepiol **2d** the methoxyl signal appears at δ 3.87 in $CDCl_3$ and the three methylene signals of the ethoxyl groups at δ 4.00, 4.07, and 4.13. In C_6D_6 the methoxyl and one of the methylene signals shift upfield by 0.43 and 0.38 ppm, respectively, the remaining two methylene signals shifting less than 0.08 ppm. Similarly, with tri-*O*-ethyldihydrosepiol **3d** the methoxyl (δ 3.84 $CDCl_3$; δ 3.89 C_6D_6) and one methylene group (δ 4.01, $CDCl_3$; δ 3.66 C_6D_6) shift, and two methylene groups are unaffected (δ 4.01 in both solvents). These observations exclude structure **8** for sepiol. Solvent shifts of *O*-ethyl derivatives have also been used recently by Merlini and his associates (Arnone et al., 1975) with red sandalwood pigments, and should prove to be generally useful modification of the Pelter procedure for locating methoxyl groups in other phenolic natural products.

The isoflav-3-ene structure of sepiol was chemically confirmed by allylic oxidation of its tri-*O*-methyl derivative to yield a fluorescent (UV light) compound, $C_{19}H_{18}O_6$, identified by its spectral properties as a 3-phenylcoumarin (**9c**). A similar oxidation of sepiol triacetate gave **9b** which was hydrolyzed to the phenolic coumarin **9a** and subsequently ethylated to yield **9d**. The structure of **9d** and,

therefore, of sepiol, was established by its synthesis from 2,3-diethoxy-4-methoxyacetophenone. This was converted via the Willgerodt reaction (Brown, 1975) to the phenylacetic acid **10**, which was condensed with 2,4-dihydroxybenzaldehyde in acetic anhydride and potassium acetate (Donnelly and Kavanagh, 1974) to yield the 3-phenylcoumarin (**11b**). Hydrolysis of this monoacetate gave **11a** which was then ethylated to yield 3-(2,3-diethoxy-4-methoxyphenyl)-7-ethoxycoumarin (**9d**), identical in all respects with the product obtained from sepiol.

The minor phenolic constituent, $C_{17}H_{16}O_5$, of *Gliricidia sepium* does not reduce ammoniacal silver nitrate. It contains two methoxyl groups and forms a diacetate whose NMR spectrum shows the presence of a methylene group at δ 5.03 allylically coupled to a methine proton at δ 6.61. The aromatic region of the spectrum is closely similar to that of sepiol triacetate in all respects. This phenol, therefore, is a 7-hydroxyisoflavene derived from sepiol by methylation of one of the two hydroxyl groups. On catalytic hydrogenation the diacetate yields a diacetoxyisoflavan (oil), in which three aromatic protons appear as ortho-coupled doublets at δ 6.70, 6.95, and 7.06 and two aromatic protons as a 2 H multiplet at δ 6.60–6.63. These chemical shifts agree precisely with those reported (Donnelly et al., 1973) for the protons at positions 5′, 6′, 5, 6, and 8, respectively, in mucronulatol diacetate (**12b**). On this basis the *Gliricidia* phenol is the 2′-*O*-methyl derivative **13** of sepiol.

The phenol, $C_{17}H_{18}O_5$, isolated in a very small amount from the heartwood, is optically active, and contains two methoxyl and two hydroxyl groups. Both the mass spectrum, which has prominent ions at 123, 167, and 180, and the NMR spectrum of the phenol establish that it is a 7-hydroxyisoflavan with two methoxyls and a hydroxyl group located at positions 2′, 3′, and 4′ of the B ring. The melting point and rotation of the phenol and the NMR spectrum of its diacetate differ, however, from those reported (Kurosawa et al., 1968; Donnelly et al., 1973) for mucronulatol **12a**. This new phenol, therefore, is either the isomeric isoflavan **14** or laxifloran **3a**, which as previously noted has been isolated and described only in the form of its dimethyl derivative **3b**. Because of a lack of material, an unequivocal decision between these two possible structures **14** and **3a** for the *Gliricidia* phenol cannot be made at this time.

The third minor constituent of the heartwood, $C_{15}H_{12}O_5$, reduces silver nitrate and gives a flavanone color reaction. On the basis of its physical and spectral properties, it is clearly identical with the known (Shinoda, 1929) 7,3′,4′-trihydroxyflavanone (**15**).

Prior to the isolation of sepiol and 2′-*O*-methylsepiol only one other isoflavene has been detected in plants (Brink et al., 1974). However, since they are highly reactive intermediates, they may be expected to play a central role in the biosynthesis of isoflavans and other types of isoflavanoids, such as the 3-phenylcoumarins (Donnelly and Kavanagh, 1974).

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New Natural Products from Marine Borer Resistant Woods. A Review.

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The examination of the heartwood extractives of three tropical woods (*Dalbergia retusa*, *Tabebuia guayacan*, and *Cordia alliodora*), specifically regarding their established natural resistance to marine borer attack, has resulted in the characterization of several new natural products. These new compounds include several structural types: cinnamylphenols, isoflavones, naphthaquinones, dibenzoxanthene, oxadibenzoxanthone, and geranylhydroquinone. Several of the new compounds or their derivatives have been successfully synthesized.

Long-term marine exposure tests of 115 tropical woods (Southwell and Bultman, 1971) have established several woods to be naturally resistant to attack by a variety of marine boring organisms. While some of these resistant woods have been examined chemically, no attempt has previously been made to determine the constituents responsible for resistance to natural marine borers. Biologically active compounds obtained from these recognized resistant species may serve as models for the development

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