6-Methoxycalpogonium Isoflavone A: A New Isoflavone from the Seed Pods of *Millettia dura*

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From the seed pods of *Milletta dura* a new isoflavone has been isolated and identified as 3-(4-methoxyphenyl)-6-methoxy-8,8-dimethyl-4H,8H-benzo[1,2-b;3,4-b’]dipyran-4-one (trivial name 6-methoxycalpogonium isoflavone A). In addition, three known isoflavones (foromonetonin, ferrugone, jamaicin) and two rotenoids (millettone, tephrosin) were identified. All structures were determined on the basis of spectroscopic evidence.

*Milletta dura* Dunn. (Leguminosae) is a shrub or a small tree indigenous to East Africa.1 Rotenoids and isoflavones have been isolated from the seeds2 and the stem and root barks3 of this plant, and recently we have reported four new isoflavones from seed pods collected in August 1994.4 A further sample of seed pods became available in March 1995, and these were studied to determine any variation in content in comparison to the earlier collection. In addition to the previously isolated compounds, this study resulted in the isolation of another new isoflavone, 6-methoxycalpogonium isoflavone A (1), together with the known isoflavones foromonetonin, ferrugone, and jamaicin and the rotenoids millettone (2) and tephrosin (3). The isolation and structure elucidation of (1) is reported here.

![Structure of 6-Methoxycalpogonium Isoflavone A](image)

Compound 1 was isolated as a colorless amorphous solid. The HRMS revealed a molecular ion peak at m/z 364.1311 corresponding to the formula C_{25}H_{30}O_{6}. The UV (302, 317 nm), 1H-NMR (δ 7.95 for H-2), and 13C-NMR (δ 152.7 for C-2) spectra of this compound were consistent with an isoflavone skeleton. The presence of a 2,2-dimethyl pyran and two methoxyl substituents were indicated from both 1H- and 13C-NMR spectra, and the base peak at m/z 349 [M − Me]+ was also consistent with the presence of the 2,2-dimethylpyran system, which forms a stable ion on the loss of one of the geminal methyls.5

A singlet aromatic proton resonating at δ 7.57 could be assigned as H-5 on the basis of the long-range (J) coupling to the carbonyl (C-4) observed in the HMBC spectrum.6 A NOESY experiment revealed a strong interaction between this proton and the methoxyl resonance at δ 3.96, thus requiring the placement of one methoxyl at C-6. The presence of AA′BB′ spin-system indicated a simple para-substituted B-ring; the second methoxyl (δ 3.85) being placed at 4′ by a NOESY correlation between the methoxyl and the δ 6.98 resonance for the 3′- and 5′ protons. Hence the 2,2-dimethylpyran substituent must be at C-7/C-8, with the anticipated oxygenation at C-7 being confirmed by the HMBC experiment (H-5 to C-7 at δ 147.6).

These data are in agreement with structure 1 for this new compound, for which the trivial name 6-methoxycalpogonium isoflavone A is suggested. The 13C-NMR chemical shift data (Table 1) was assigned unambiguously on the basis of HC-COBI7 and HMBC8 experiments.

The seed pods of this plant also yielded three known isoflavones:6,8,9 7-hydroxy-4-methoxysowflavone (foromonetonin), 3-[2,5-dimethoxy-3,4-(methylenedioxy)phenyl]-8,8-dimethyl-4H,8H-benzo[1,2-b;3,4-b’]dipyran-4-one (ferrugone) and 3-[6-methoxy-3,4-(methylenedioxy)phenyl]-8,8-dimethyl-4H,8H-benzo[1,2-b;3,4-b’]dipyran-4-one (jamaicin), and two known rotenoids:9 millettone (2) and tephrosin (3). The 13C-NMR chemical shift data for 2 do not appear to have been published. During this study they were obtained from a J-modulated spectrum and unambiguously assigned by means of HC-COBI and HMBC experiments. Chemical shift values are given in the Experimental Section.

**Experimental Section**

**General Experimental Procedures.** IR spectra (KBr) were recorded on a Mattson Genesis Series FTIR spectrophotometer, and UV spectra (MeOH) on a DU spectrophotometer. Specific rotations [α]_D were determined at the sodium-D line using a Perkin-Elmer 241 polarimeter at 21 °C. MS were recorded on a high-resolution electron impact mass spectrometer AEIMS

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Table 1. \(^{1}H\) and \(^{13}C\)-NMR Chemical Shift Data, Together with \(^2J\) and \(^3J\) Correlations, for Compound 1 (J values, in Hz, in Parentheses)

<table>
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<tr>
<th></th>
<th>(^1H)</th>
<th>(^13C)</th>
<th>(^2J)</th>
<th>(^3J)</th>
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<tr>
<td>2</td>
<td>7.95s</td>
<td>152.7</td>
<td>124.4</td>
<td>124.7</td>
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<tr>
<td>3</td>
<td>124.4</td>
<td>105.4</td>
<td>167.5</td>
<td>174.3</td>
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<td>4</td>
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<td>117.8</td>
<td>124.7</td>
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</tr>
<tr>
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<td>105.4</td>
<td>167.5</td>
<td>174.3</td>
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<tr>
<td>5</td>
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<td>124.7</td>
<td>174.3</td>
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<td>124.7</td>
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<tr>
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<td>174.3</td>
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<td>2'/6'</td>
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<td>130.4</td>
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<tr>
<td>3'/5'</td>
<td>6.98d (8.8)</td>
<td>114.1</td>
<td>114.1</td>
<td>114.1</td>
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<tr>
<td>4'</td>
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<td>130.4</td>
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<tr>
<td>6-O-Me</td>
<td>8.10d (10.0)</td>
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<tr>
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<tr>
<td>2'-O-Me</td>
<td>2.81s</td>
<td>2.81s</td>
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</tr>
</tbody>
</table>

\(^1R-H-C\) correlations were obtained using HC-COBI; \(^2J\) and \(^3J\) \(^1H\)-C correlations from HMBC.

902 double-focusing instrument (direct probe insert at 70 eV). NMR spectra were recorded on a Bruker AMX-400 spectrometer in CDCl\(_3\) as solvent (\(\delta_H 7.27, \delta_C 77.23\)). HMBC spectra were optimized for \(J_{\text{H-C}} = 7\) Hz.

Plant Material. The seed pods of Milletta dura were collected in Nairobi, March 1995. The species was identified by Mr. S. G. Mathenge of the Herbarium, Botany Department, University of Nairobi, where a voucher specimen is deposited.

Isolation of Compounds from the Seed Pods of M. dura. Dried and ground seed pods (2 kg) of M. dura were extracted with CHCl\(_3\) by cold percolation. The concentrated extract (40 g) was subjected to column chromatography on Si gel (400 g) eluting with mixtures of hexane and EtOAc of increasing polarities. In addition to the previously reported compounds,\(^4\) the fraction eluted with 5% EtOAc gave 2 (18 mg); 10% EtOAc gave a mixture of ferrugone and jamaicin (37 mg), which were not separated. Elution with 15% EtOAc gave 3 (15 mg); 20% EtOAc gave 1 (35 mg); and 25% EtOAc gave formononcitin (25 mg).

6-Methoxy-calopogonum Isolavone A (1): amorphous solid from EtOAc; UV (MeOH) \(\lambda_{\text{max}}\) nm (log e) 283 (3.83), 302 (3.95), 317 (3.99); IR \(\nu_{\text{max}}\) (KBr) 1690, 1590 cm\(^{-1}\); \(^1H\) NMR (CDCl\(_3\), 400 MHz), see Table 1; \(^13C\) NMR (CDCl\(_3\), 100.56 MHz), see Table 1; HREIMS (70 eV) m/z 364.1311; calculated for C\(_{22}\)H\(_{20}\)O\(_{5}\) 364.1305; (rel. int.) 364 [M]\(^+\) (37), 349 [M-Me]\(^+\) (100), 264 (16).

Formononcitin: needles (MeOH); mp 256–258 °C (lit. mp 255–258 °C); HREIMS m/z 268.0816, calculated for C\(_{16}\)H\(_{14}\)O\(_{4}\) 268.0732; UV and \(^1H\) and \(^13C\) NMR identical with literature values.\(^8\)\(^9\)

Ferrugone and jamaicin: amorphous mixture; HREIMS m/z 408.1197; calculated for ferrugone, C\(_{28}\)H\(_{20}\)O\(_{7}\) 408.1209; 378.1098; calculated for jamaicin 378.1103; \(^1H\) and \(^13C\) NMR identical with those reported for the two compounds.\(^5\)

Milletton (2): needles (MeOH); mp 179–181 °C (lit.\(^2\) mp 180–181 °C); [\(\alpha\)]\(_D\) -25° (c 0.10, MeOH); UV and \(^1H\) NMR were identical with those reported for the two compounds.\(^2\)

NMR (100.56 MHz) \(\delta\) 189.3 (s, C-12), 160.3 (s, C-9), 157.0 (s, C-8a), 148.6 (s, C-5), 148.0 (s, C-2), 142.6 (s, C-3), 128.7 (d, C-3'), 128.6 (d, C-11), 117.7 (d, C-10), 115.9 (d, C-4'), 112.8 (s, C-11a), 109.3 (s, C-8), 107.2 (d, C-1), 106.0 (s, C-1a), 101.3 (t, O-CH\(_2\)-O), 99.0 (d, C-4), 77.9 (s, C-2'), 72.4 (d, C-6a), 66.5 (t, C-6), 44.9 (d, C12a), 28.7 (q, 2'-Me), 28.4 (q, 2'-Me); HREIMS (70 eV) m/z 378.1097, calculated for C\(_{22}\)H\(_{20}\)O\(_{5}\); (rel. int.) 378 [M]\(^+\) (100), 363 [M-2Me]\(^+\) (83), 203 (39), 187 (63), 177 (49).

Tephrosin (3): oil; [\(\alpha\)]\(_D\) -99° (c 0.14, MeOH); UV, \(^1H\) and \(^13C\) NMR in close agreement with literature values;\(^2\) HREIMS (70 eV) m/z 410.1353, calculated for C\(_{22}\)H\(_{20}\)O\(_{7}\); (rel. int.) 410 [M]\(^+\) (8), 395 [M-Me]\(^+\) (75), 207 (100), 165 (22).

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References and Notes