

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

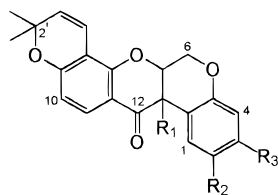
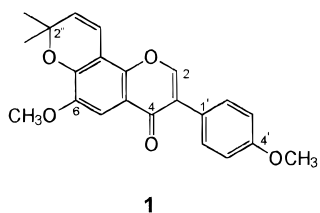
Abiy Yenesew, Jacob O. Midiwo,* and Peter G. Waterman†

Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

Received August 26, 1996[⊗]

From the seed pods of *Millettia 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-methoxycalopogonium isoflavone A). In addition, three known isoflavones (formononetin, ferrugone, jamaicin) and two rotenoids (millettone, tephrosin) were identified. All structures were determined on the basis of spectroscopic evidence.

Millettia 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-methoxycalopogonium isoflavone A (**1**), together with the known isoflavones formononetin, ferrugone, and jamaicin and the rotenoids millettone (**2**) and tephrosin (**3**). The isolation and structure elucidation of (**1**) is reported here.



2 R₁ = H; R₂, R₃ = OCH₂O

3 R₁ = OH; R₂ = R₃ = OCH₃

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 C₂₂H₂₀O₅. 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 (³*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-methoxycalopogonium isoflavone A is suggested. The ¹³C-NMR chemical shift data (Table 1) was assigned unambiguously on the basis of HC-COBI⁷ and HMBC⁶ experiments.

The seed pods of this plant also yielded three known isoflavones:^{5,8,9} 7-hydroxy-4-methoxyisoflavone (formononetin), 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,² 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

* To whom correspondence should be addressed. Phone: 254-2-440042. FAX: 254-2-446138.

† Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, Scotland, U.K.

[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1997.

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

	^1H	^{13}C	2J	3J
2	7.95s	152.7	124.4 (C-3)	124.7 (C-1'), 147.3 (C-8a), 175.7 (C-4)
3		124.4		
4		175.7		
4a		117.8		
5	7.57s	105.4	147.4 (C-6)	147.3 (C-8a), 147.6 (C-7), 175.7 (C-4)
6		147.4		
7		147.6		
8		110.4		
8a		147.3		
1'		124.7		
2'/6'	7.51d (8.8)	130.4	124.7 (C-1')	124.4 (C-3), 130.4 (C2'/6'), 159.7 (C-4')
3'/5'	6.98d (8.8)	114.1		114.1 (C-3'/5'), 124.7 (C-1')
4'		159.7		
2''		78.3		
3''	5.75d (10.0)	130.4	78.3 (C-2'')	110.4 (C-8)
4''	6.81d (10.0)	115.4	110.4 (C-8)	78.3 (C-2''), 147.3 (C-8a), 147.6 (C-7)
6-OMe	3.96s	56.5		147.4 (C-6)
4'-OMe	3.85s	55.4		159.7 (C-4')
2''-Me ₂	1.56s	28.1	78.3 (C-2'')	28.1 (Me-2''), 130.4 (C-3'')

$^1J_{\text{H-C}}$ correlations were obtained using HC-COBI; 2J and $^3J_{\text{H-C}}$ 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 (δ_{H} 7.27, δ_{C} 77.23). HMBC spectra were optimized for $J_{\text{H-C}} = 7$ Hz.

Plant Material. The seed pods of *Millettia 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 formononetin (25 mg).

6-Methoxycalopogonum Isoflavone A (1): amorphous solid from EtOAc; UV (MeOH) λ_{max} nm (log ϵ) 263 (4.38), 302 (3.98), 317 (3.99); IR ν_{max} (KBr) 1690, 1590 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 100.56 MHz), see Table 1; HREIMS (70 eV) m/z 364.1311; calcd for $\text{C}_{22}\text{H}_{20}\text{O}_5$ 364.1305; (rel. int.) 364 $[\text{M}]^+$ (37), 349 $[\text{M} - \text{Me}]^+$ (100), 264 (16).

Formononetin: needles (MeOH); mp 256–258 °C (lit. mp 255–258 °C); HREIMS m/z 268.0816, calcd for $\text{C}_{16}\text{H}_{20}\text{O}_4$ 268.0732; UV and ^1H and ^{13}C NMR identical with literature values.^{8,9}

Ferrugone and jamaicin: amorphous mixture; HREIMS m/z 408.1197; calcd for ferrugone, $\text{C}_{23}\text{H}_{20}\text{O}_7$ 408.1209; 378.1098; calcd for jamaicin 378.1103; ^1H and ^{13}C NMR were identical with those reported for the two compounds.⁵

Millettone (2): needles (MeOH); mp 179–181 °C (lit.² mp 180–181 °C); $[\alpha]_{\text{D}} -25^\circ$ (c 0.10, MeOH); UV and ^1H NMR were identical with literature values;² ^{13}C

NMR (100.56 MHz) δ 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, calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6$ 378.1098; (rel int) 378 $[\text{M}]^+$ (100), 363 $[\text{M} - \text{Me}]^+$ (83), 203 (39), 187 (63), 177 (49).

Tephrosin (3): oil; $[\alpha]_{\text{D}} -99^\circ$ (c 0.14, MeOH); UV, ^1H and ^{13}C NMR in close agreement with literature values;^{2,10} HREIMS (70 eV) m/z 410.1353, calcd for $\text{C}_{23}\text{H}_{22}\text{O}_7$ 410.1359; (rel int) 410 $[\text{M}]^+$ (8), 395 $[\text{M} - \text{Me}]^+$ (75), 207 (100), 165 (22).

Acknowledgment. One of us (A.Y.) is grateful to The African Network of Scientific and Technological Institutions (ANSTI) for the award of a scholarship and the German Academic Exchange Service (DAAD) for financial support. Mr. S. G. Mathenge is thanked for the of the plant. NMR studies were performed in the Strathclyde University NMR Laboratory.

References and Notes

- Gillet, J. B.; Polhill, R. M.; Verdcourt, B. *Flora of Tropical East Africa Leguminosae*; Whitefriars: London, 1971; pp 123–144.
- Ollis, W. D.; Rhodes, C. A.; Sutherland, I. O. *Tetrahedron* **1967**, *23*, 4741–4760.
- Dagne, E.; Mammo, W.; Bekele, A.; Odyek, O.; Byaruhanga, M. A. *Bull. Chem. Soc. Ethiopia* **1991**, *5*, 81–86.
- Yenesew, A.; Midiwo, J. O.; Waterman, P. G. *Phytochemistry* **1996**, *41*, 951–955.
- Dagne, E.; Bekele, A.; Waterman, P. G. *Phytochemistry* **1989**, *28*, 1897–1900.
- Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
- Bax, A. *J. Magn. Reson.* **1983**, *53*, 517.
- Duddeck, H.; Yenesew, A.; Dagne, E. *Bull. Chem. Soc. Ethiopia* **1987**, *1*, 36–41.
- Harborne, J. B.; Mabry, T. J. *The Flavonoids: Advances in Research*, Chapman and Hall: London, 1982; pp 19–134.
- Luyengi, L.; Lee, I.-S.; Mar, W.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **1994**, *36*, 1523–1526.

NP9605955