

FOUR ISOFLAVONES FROM SEED PODS OF *MILLETTIA DURA*

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Key Word Index—*Millettia dura*; Leguminosae; seed pods; isoflavones; durallone; 6-demethyldurallone; predurallone; isoerythrinin-A 4'-(3-methylbut-2-enyl) ether; NMR.

Abstract—Seed pods of *Millettia dura* yielded four novel isoflavones, which have been characterized as 3-(3,4-dimethoxyphenyl)-6-methoxy-8,8-dimethyl-4*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyrans-4-one (trivial name, durallone), 6-demethyldurallone, 3-(3,4-dimethoxyphenyl)-8-(3-methylbut-2-enyl)-6-methoxy-7-hydroxybenzopyran-4-one (trivial name, predurallone) and 3-(4-{3-methylbut-2-enyloxy})-8,8-dimethyl-4*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyrans-4-one (trivial name, isoerythrinin-A 4'-(3-methylbut-2-enyl) ether). Structures of these compounds were determined on the basis of their spectroscopic data.

INTRODUCTION

The genus *Millettia* Wight et Arn. (Leguminosae) consists of ca 100 Old World species of climbers and trees [1]. The seeds of a number of them are known for their insecticidal and piscicidal properties [1]. Previous investigations of a number of species have also shown them to be a good source of flavonoids and isoflavonoids, and of nitrogenous compounds [2].

Millettia dura Dunn. is widely distributed in east Africa where it is used as a shade and ornamental tree [3]. Previous studies on the root and stem-bark [4], and on the seeds [5], have yielded isoflavones and rotenoids. In the present paper we present the results of an investigation of seed pods and stem-bark. From the former, we have obtained four novel isoflavones, while the latter gave only known compounds.

RESULTS AND DISCUSSION

Dried and ground seed pods were extracted with chloroform. Silica gel chromatography of the concentrated extract using gradient elution from hexane to ethyl acetate yielded the four new isoflavones.

The HR-EI mass spectrum of the major compound showed a $[M]^+$ at m/z 394.1451, corresponding to the molecular formula $C_{23}H_{22}O_6$. The UV spectrum [5], and 1H (δ 7.97 for H-2) and ^{13}C (δ 151.9 for C-2) NMR spectra [6] showed this compound to be an isoflavone derivative. The 1H (Table 1) and ^{13}C (Table 2) NMR spectra further revealed the presence of a 2,2-dimethylpyran and three methoxyl substituents. The major ion at

m/z 379 $[M - Me]^+$ provided further support for the presence of a 2,2-dimethylpyran system [6].

In the 1H NMR spectrum, four aromatic protons were visible as an ABD system and a single deshielded proton (δ 7.56), the latter being typical for H-5. From the ^{13}C NMR spectrum, the occurrence of all quaternary oxygenated sp^2 carbons in the range 145–150 ppm required oxygenation to occur on adjacent carbons, viz., C-3' and C-4', and C-6 and C-7. These observations were strongly suggestive of structure 1 and this was confirmed by a study of direct and long-range heteronuclear coupling interactions using the HC-COBIdec [7] and HMBC [8] techniques, respectively. The interactions observed in the HMBC study of 1 are summarized in Fig. 1. Compound 1 has been given the trivial name durallone and is closely related to the known isoflavone, durmillone (2), which was also isolated in the present study (from the stem-bark).

A minor compound eluted from the column with 1, analysed for $C_{22}H_{20}O_6$ by HR-EI mass spectrometry and gave very similar UV and NMR spectra. The 1H and ^{13}C NMR spectra indicated the same substitution pattern as 1, but with one of the three methoxyls missing and replaced by a hydroxyl. The presence of a major ion at m/z 162 (4) strongly supported the occurrence of methoxyls at C-3' and C-4' and this was confirmed by a NOESY experiment, which revealed interactions of H-2' and H-5' with the methoxyls. The identity of this isoflavone as 3 was again confirmed by an HMBC experiment. The compound has been assigned the trivial name, 6-demethyldurallone.

The most polar of the new compounds was analysed for $C_{23}H_{24}O_6$ by HR-EI mass spectrometry. Once again, the NMR data indicated a 6,7,8,3',4'-substituted isoflavone, oxygenated at C-6, C-7, C-3' and C-4'. However, in this case, in addition to three methoxyl resonances

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Table 1. ^1H NMR chemical shift data for isoflavones isolated from *Millettia dura* (400 MHz, CDCl_3)

H	1	2	3	5	7	10	11	12
2	7.97 s	7.91 s	7.97 s	8.02 s	7.95 s	7.94 s	7.93 s	7.91 s
5	7.56 s	7.53 s	7.68 s	7.54 s	8.07 d	8.07 d	7.89 d	7.89 d
6					6.87 d	6.86 d	6.98 d	6.96 d
2'	7.25 d	7.09 d	7.22 d	7.26 d	7.49 d	7.50 d	7.18 d	7.48 d
3'					6.98 d	6.98 d		6.97 d
5'	6.91 d	6.84 d	6.92 d	6.91 d	6.98 d	6.98 d	6.93 d	6.97 d
6'	7.04 dd	6.96 dd	7.05 dd	7.06 dd	7.49 d	7.50 d	7.03 dd	7.48 d
1''				3.58 d				
2''				5.27 t				
3''	5.74 d	5.72 d	5.75 d		5.73 d	5.72 d		
4''	6.80 d	6.78 d	6.83 d		6.82 d	6.81 d		
2''/3''-Me	1.55 s	1.54 s	1.55 s	1.69 s	1.51 s	1.51 s		
				1.83 s				
1'''					5.52 t			
2'''					4.56 d			
3'''-Me					1.77 s			
					1.82 s			
OCH ₂ O		5.97 s					6.21 s	6.21 s
OH			5.77 br s	6.61 br s				
6-OMe	3.95 s	3.94 s		3.94 s				
3'-OMe	3.92 s		3.93 s	3.92 s			3.93 s	
4'-OMe	3.90 s		3.91 s	3.89 s		3.85 s	3.92 s	3.85 s

J values: H-5/H-6 8.8 Hz; H-2'/H-6' 1.7 Hz; H-5'/H-6' 8.0 Hz; H-3''/H-4'' 10.0 Hz; 1''-2'' in **5** 6.7 Hz; H-1'''/H-2''' in **7** 6.7 Hz.

Table 2. ^{13}C NMR chemical shift data for isoflavones isolated from *Millettia dura* (100 MHz, CDCl_3)

C	1	2	3	5	7	10	11	12
2	151.9	151.9	152.1	152.3	151.9	151.9	151.9	151.8
3	124.3	124.3	124.4	123.9	125.0	124.9	124.9	124.9
4	175.7	175.5	175.9	176.2	176.2	176.1	175.7	175.7
4a	117.8	117.7	118.6	117.6	118.6	118.6	120.7	120.8
5	105.2	105.3	109.2	102.3	127.0	126.9	121.4**	121.2
6	147.4	147.3*	143.3	145.3	115.4	115.4	107.5	107.5
7	147.4	147.4*	144.9	148.9*	157.5	157.5	152.4	152.4
8	110.3	110.3	109.8	115.8	109.4	109.4	134.7	134.7
8a	147.5	147.5	146.9	151.0	152.6	152.3	141.4	141.5
1'	125.1	126.1	125.1	125.2	124.3	124.5	124.5	124.1
2'	112.8	109.9	112.9	112.7	130.3	130.3	112.8	130.4
3'	148.9*	147.7*	149.0*	149.1*	114.9	114.2	149.4*	114.2
4'	149.2*	147.8*	149.3*	148.9*	159.1	159.8	149.0*	159.9
5'	111.3	108.5	111.4	111.3	114.9	114.2	111.4	114.2
6'	121.1	122.5	121.3	121.1	130.3	130.3	121.2**	130.4
1''				22.4				
2''	78.3	78.3	79.3	121.1	77.9	77.9		
3''	130.6	130.5	130.4	133.0	130.5	130.5		
4''	115.3	115.3	115.4		115.2	115.2		
2''/3''-Me	28.2	28.1	28.4	18.0	28.4	28.3		
	28.2	28.1	28.4	25.8	28.4	28.3		
1'''					65.1			
2'''					119.9			
3'''					138.5			
3'''-Me					18.4			
					26.0			
OCH ₂ O		101.3					103.5	103.5
6-OMe	56.5	56.4		56.4				
3'-OMe	56.4		56.2	56.0			56.2	
4'-OMe	56.2		56.2	56.0		55.5	56.2	55.6

*Signals in the same column with the same number of asterisks are interchangeable.

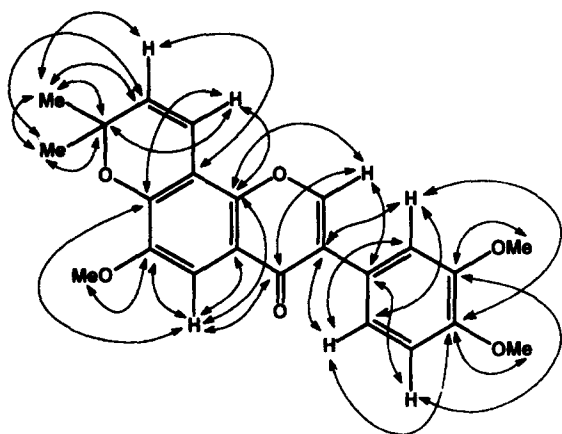
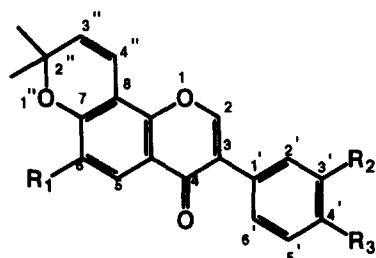


Fig. 1. HMBC correlations for compound 1.

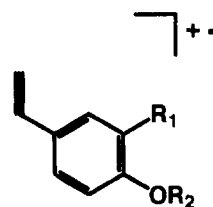
signals were observed for a 3-methylbut-2-enyl unit, rather than a 2,2-dimethylpyran. The presence of methoxyls at C-3' and C-4' was confirmed by the ion (4) in the EI-mass spectrum, leaving only the problem of whether the third methoxyl was at C-6 (5) or C-7 (6). This was

resolved by the ^{13}C NMR resonance for the methoxyls, which were all in the range 56–57 ppm. Such a shielded position for the methoxyl resonance requires the presence of an unsubstituted *ortho*-position, whereas if both *ortho*-positions are substituted it would resonate in the range 60–63 ppm [9]. Structure 5 can, therefore, be assigned. This compound appears to be the precursor of 1 and on this basis the name predurallone is assigned.

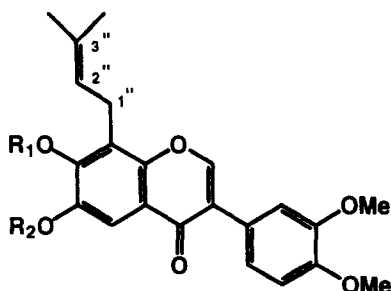
The least polar compound was analysed for $\text{C}_{25}\text{H}_{24}\text{O}_4$, with the UV and NMR spectra revealing an isoflavone with 2,2-dimethylpyran and 3-methylbut-2-enyloxy substituents, and AB and AA'BB' aromatic spin systems. This requires that the B-ring carries a single *para*-substituent, which must be the prenyloxy substituent. In the A-ring, the strongly deshielded nature of one of the AB protons places it as H-5, so that the pyran ring must be between C-7 and C-8, with the oxygen at C-7 (δ 157.5). On this basis, the isolate can be assigned structure 7, which is new. Confirmation of this was obtained from the presence of the ion 8 in the EI mass spectrum and the NOE enhancement of the H-3'/H-5' resonance on irradiation of the methylene protons of the prenyloxy



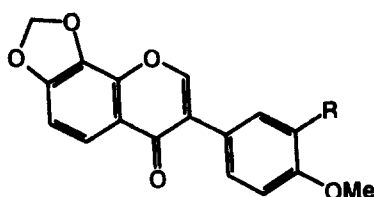
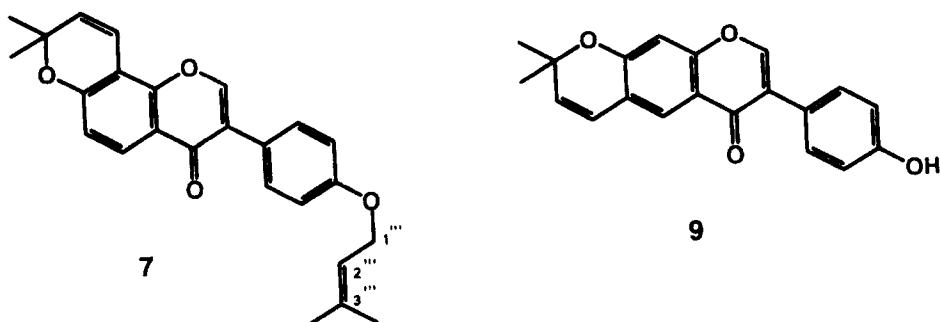
	R ₁	R ₂	R ₃
1	OMe	OMe	OMe
2	OMe	O-CH ₂ -O	
3	OH	OMe	OMe
10	H	H	OMe



	R ₁	R ₂
4	OMe	Me
8	H	CH ₂ CH=CMe ₂



	R ₁	R ₂
5	H	Me
6	Me	H



R
 11 OMe
 12 H

substituent. The known isoflavone, erythrin-A (9), isolated from *Erythrina* species [2], has the same oxygenation pattern as 7, but with the pyran ring substituent at C-6/C-7 and with a free phenol at C-4'. It seems appropriate to relate the trivial name of 7 to erythrin-A, calling it isoerythrin-A 4'-(3-methylbut-2-enyl) ether.

The stem-bark yielded durmillone (2), calopogonium isoflavone-A (10), maxamiisoflavone-D (11) and maximaisoflavone-H (12); 2 and 12 have previously been isolated from *M. dura* [4, 5], but not 10 or 11. NMR data for these compounds are included in Tables 1 and 2 for comparative purposes. It is notable that the seed pod contains an entirely unique suite of isoflavonoids in comparison with the seeds, as well as with other parts of the plant that have been examined [4, 5].

EXPERIMENTAL

Plant material. Seed pods and stem bark of *M. dura* were collected in Nairobi in August 1994. 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 seed pods. Dried and ground seed pods (1.5 kg) were extracted with CHCl_3 by cold percolation. The concd extract

(30 g) was subjected to CC on silica gel (300 g) eluting with mixts of hexane and EtOAc of sequentially increasing polarity. The fr. eluted with 10% EtOAc gave 7 (6 mg). Elution with 30% EtOAc gave a mixt. of 3 compounds which were sepd by prep. TLC on silica gel (hexane-toluene-EtOAc, 2:2:1) to give 1 (100 mg), 3 (25 mg) and 5 (23 mg).

Isolation of compounds from stem-bark. Dried and ground stem-bark (340 g) was extracted as described for seed pods. The concd extract (20 g) was fractionated in a similar way. Elution with 5% EtOAc gave 10 (250 mg), 10% EtOAc gave 12 (8 mg), 20% EtOAc gave 2 (160 mg) and 30% EtOAc gave 11 (7 mg).

Durallone (1). Needles from MeOH, mp 186–188°. Found $[\text{M}]^+$ m/z 394.1451; $\text{C}_{23}\text{H}_{22}\text{O}_6$ requires 394.1410. UV λ_{max} (MeOH) (log ϵ) nm: 253 (4.47), 332 (3.99), 345 (3.95). ^1H NMR, see Table 1. ^{13}C NMR, see Table 2. EIMS m/z (rel. int.): 394 $[\text{M}]^+$ (66), 379 $[\text{M} - \text{Me}]^+$ (39), 378 $[\text{M} - \text{Me} - \text{H}]^+$ (100).

6-Demethyldurallone (3). Amorphous solid. Found $[\text{M}]^+$ m/z 380.1233; $\text{C}_{22}\text{H}_{20}\text{O}_6$ requires 380.1294. UV λ_{max} (MeOH) (log ϵ) nm: 252 (4.45), 335 (3.97), 347 (3.94). ^1H NMR, see Table 1. ^{13}C NMR, see Table 2. EIMS m/z (rel. int.): 380 $[\text{M}]^+$ (76), 365 $[\text{M} - \text{Me}]^+$ (100), 269 (32), 162 (64), 113 (63).

Predurallone (5). Amorphous solid. Found $[\text{M}]^+$ m/z 396.1546; $\text{C}_{23}\text{H}_{24}\text{O}_6$ requires 396.1566. UV λ_{max} (MeOH)

(log ϵ) nm: 243 (4.36), 250 (4.38), 264 (4.39), 302 (4.18), 319 (4.17). ^1H NMR, see Table 1. ^{13}C NMR, see Table 2. EIMS m/z (rel. int.): 396 $[\text{M}]^+$ (66), 381 $[\text{M} - \text{Me}]^+$ (27), 363 (34), 219 (92), 193 (98), 162 (100), 113 (91).

Isoerythrin-A 4'-(3-methylbut-2-enyl) ether (7). Amorphous. Found $[\text{M}]^+$ m/z 388.1681; $\text{C}_{25}\text{H}_{24}\text{O}_4$ requires 388.1668. UV λ_{max} (MeOH) (log ϵ) nm: 263 (4.38), 302 (3.98), 317 (3.99). ^1H NMR, see Table 1. ^{13}C NMR, see Table 2. EIMS m/z (rel. int.): 388 $[\text{M}]^+$ (20), 320 (48), 305 $[\text{M} - \text{Me} - \text{C}_3\text{H}_8]^+$ (100), 187 (16).

Durmillone (2). $[\text{M}]^+$ m/z 378.1108 for $\text{C}_{22}\text{H}_{18}\text{O}_6$. *Calopogonium isoflavone A* (10). $[\text{M}]^+$ m/z 334.1198; $\text{C}_{21}\text{H}_{18}\text{O}_4$.

Maximaisoflavone D (11). $[\text{M}]^+$ m/z 326.0837; $\text{C}_{18}\text{H}_{14}\text{O}_6$.

Maximaisoflavone H (12). $[\text{M}]^+$ m/z 296.0643; $\text{C}_{17}\text{H}_{12}\text{O}_5$. Physical and spectral data are as previously reported [4–6, 10, 11]. NMR data, see Tables 1 and 2.

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