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FLAVONOIDS OF *MILLETTIA DURA*

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Abstract- The root bark, stem bark and seeds of *Millettia dura* afforded 11 flavonoids four of which are new to the genus. The pattern of flavonoid distribution in *M. dura* is compared with that in *M. ferruginea* in order to draw chemotaxonomic conclusions.

INTRODUCTION

The genus *Millettia* is represented by about 200 species spread out in the forested parts of tropical and subtropical Africa, Asia and Australasia [1]. The seeds of these species are used as insecticides, piscicides [2] and in folk medicine [3]. Some 100 *Millettia* species are believed to occur in Africa [4]. Only a few of these species have been subjected to phytochemical studies i.e. seeds [5, 6, 7] and bark [8, 9] of *M. ferruginea*, seeds of *M. dura* [10] and *M. thonningii* [11, 12] and leaves of *M. zechiana* [13].

M. dura is a shrub or small tree growing up to 13m high and is cultivated in East Africa as a shade or ornamental tree [1]. Previous phytochemical study on the seeds of this plant by Ollis *et al* [10] resulted in the isolation of three isoflavones (durlettone, durmillone and mildurone) and five rotenoids (rotenone, tephrosin, dehydrodeguelin, milletone and milletosin).

It has been pointed out that *M. dura* is taxonomically "much confused with the Ethiopian *M. ferruginea*" [1]. Since we have previously investigated the constituents of the different parts of *M. ferruginea*, we decided to undertake a comparative phytochemical study of the stem, root bark and seeds of these two species. The results presented below show *M. dura* to be chemically quite different from *M. ferruginea*.

RESULTS AND DISCUSSION

The stem and root bark of *M. dura* was successively extracted with petrol and CH_2Cl_2 by percolation at room temperature. CC of the crude ext. led to the isolation of the ubiquitous triterpene lupeol, three isoflavones and two chalcones.

Compound **1** showed in its ^1H NMR spectrum a sharp singlet at δ7.91 (H-2) indicating the presence of an isoflavone nucleus. The occurrence of a 4'-oxygenated B-ring was deduced from the 2H doublets at δ6.96 (H-3' and H-5') and 7.46 (H-2' and H-6'). In addition, two *ortho*-coupled doublets appeared at δ6.94 and 7.90 which are attributed to H-6 and H-5, respectively. This allowed the placement of the methylenedioxy group at C-7 and 8 and the OCH_3 at C-4'. The MS showed a molecular ion at *m/z* 296 and RDA fragment ions at *m/z* 164 and 132 supporting the attachment of the OCH_3 and methylenedioxy groups to the B and A rings, respectively. Furthermore, the ^{13}C NMR spectrum showed the presence of one primary, one secondary, seven tertiary and eight quaternary carbon atoms. The above evidence confirms that compound **1** is maximaisoflavone H, a compound previously isolated from *Tephrosia maxima* [14, 15]. However, this is the first finding of **1** in the genus *Millettia*. C-8 oxygenated isoflavones are very rare in the genus *Millettia* and the only known

examples are thonningine A and B from *M. thonningii* [12] and aurmillone from *M. auriculata* [16].

Compound **2** displayed in its MS a molecular ion at *m/z* 350 and an RDA fragment ion at *m/z* 146 consistent with a methylenedioxy substituted B-ring. The presence of 21 carbons was evident from the ^{13}C NMR spectrum which showed two primary, two secondary, eight tertiary and nine quaternary carbon resonances. The presence of an *O*-prenyl group was derived from both ^{13}C and ^{1}H NMR spectra. The appearance of two sets of ABX patterns in the ^{1}H NMR spectrum corresponding to the protons on the A and B rings allowed the placement of the *O*-prenyl group on C-7. Compound **2** was thus identified as maximaisoflavone B, a compound which was earlier reported from *Tephrosia maxima* [14]. However, this is the first report of **2** from the genus *Millettia*.

Compound **3** showed in its MS a molecular ion peak at *m/z* 326 and the ^{13}C NMR spectrum showed 18 carbon resonances corresponding to two primary, one secondary, six tertiary and nine quaternary carbon atoms. The ^{1}H NMR spectrum displayed 3H singlets at δ3.73 and 3.92 (2 x OCH_3) and a 2H singlet at δ5.96 (OCH_2O). The aromatic region showed singlets at δ6.63, 6.83 and 7.88 attributable to H-3', H-6' and H-2, respectively, and an ABX pattern at δ6.85 (*d*), 6.98 (*dd*) and 8.18 (*d*) corresponding to H-8, H-6 and H-5, respectively. Consequently compound **3** was identified as 7,2'-dimethoxy-4',5'- methylenedioxyisoflavone.

Compound **4** displayed in its HRMS a molecular ion at *m/z* 338 consistent with the molecular formula $C_{21}H_{22}O_4$. The ^{13}C NMR spectrum showed 21 carbon resonances corresponding to three primary, one secondary, nine tertiary and eight quaternary carbon atoms. The presence of a C-prenyl, an OCH_3 and a chelated OH group was evident from the ^{1}H NMR spectrum. In addition, the aromatic region showed the presence of a *trans* double bond, a *para* substituted phenyl ring and a 4,5,6-trisubstituted phenacyl ring. The placement of the OCH_3 at C-4' was confirmed by a decoupling experiment. Thus, irradiating the OCH_3 resonance at δ3.88 resulted in a 10% enhancement of the H-5' resonance at δ6.47. The above spectroscopic data allowed the identification of compound **4** as 4-hydroxyderricin.

Compound **5** showed in its ^{1}H NMR spectrum a 6H singlet at δ1.55 and 1H doublets at δ5.64 and 6.76 suggesting the presence of a dimethylpyran ring. The remaining proton resonances closely resemble those of 4-hydroxyderricin (**4**). The ^{1}H NMR, ^{13}C NMR, and MS data allowed the establishment of compound **5** as 4-hydroxylonchocarpin.

Compound **5** was earlier reported from the bark of *M. ferruginea* subspecies *ferruginea* [8].

The seeds of *M. dura* were first defatted with petrol and extracted with $CHCl_3$. CC of the $CHCl_3$ ext. afforded two isoflavones (durmillone and durlettone) and four rotenoids (tephrosin, milletone, deguelin and 12-hydroxymilletone). Except for deguelin, all of these compounds were previously reported from the seeds of *M. dura* [10]. Table 1 shows the distribution of flavonoids isolated from the seeds of *M. dura* and *M. ferruginea*. As can be seen from Table 1, the same type of rotenoids occur in both species except for milletone and 12-hydroxymilletone and *M. ferruginea* appears to be richer in isoflavonoids. A more striking difference between the two species is however observed in the flavonoid distribution in the stem and root bark.

Compounds **1-4** have not previously been encountered in the genus *Millettia* and none of the compounds isolated from *M. dura* are C-5 oxygenated. On the other hand the rare C-8 oxygenated compound (**1**) is elaborated by *M. dura*. Previous studies on *M. ferruginea* [7,8] have revealed the occurrence of three isoflavones with oxygenation at C-5 but no isoflavone with C-8 oxygenation. The presence of compound **1** in *M. dura* and the occurrence of the 8-oxygenated isoflavones, 5-methoxydurmillone, 7-hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone and pre-5-methoxydurmillone, in *M. ferruginea* may serve to clearly delineate the two species.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR: at 90, 300 and 400 MHz. ^{13}C NMR: at 22.5 MHz.

Plant material. Stem bark, root bark and seeds of *M. dura* (Dunn) were obtained from Makerere

Table 1. Distribution of flavonoids between *M. dura* and *M. ferruginea* seeds and bark.

Flavonoid	*M. dura*		*M. ferruginea*	
	seeds	bark	seeds	bark
Rotenone	+ [10]	-	+ [5, 7]	-
Tephrosin	+	-	+ [5, 7]	-
Deguelin	+	-	+ [5]	-
Ferrugone	-	-	+ [6, 7]	+ [8]
Durmillone	+	-	+ [6, 7]	-
12a-Hydroxyrotenone	-	-	+ [7]	-
Calopogonium isoflavone A	-	-	+ [7]	-
Calopogonium isoflavone B	-	-	-	+# [8]
Barbigerone	-	-	+ [7]	-
Milletone	+	-	-	-
12-Hydroxymilletone	+	-	-	-
Durlettone	+	-	-	-
Preferrugone	-	-	+* [7]	-
Predurmillone	-	-	+* [7]	-
Prebarbigerone	-	-	+# [7]	-
Nordurlettone	-	-	+* [9]	-
4-Hydroxyderricin	-	+	-	-
4-Hydroxylonchocarpin	-	-	-	+# [8]
4'-Hydroxyisolonchocarpin	-	-	-	+# [8]
Maximaisoflavone H	-	+	-	-
Maximaisoflavone B	-	+	-	-
7,2'-Dimethoxy-4',5'-methylene-dioxyisoflavone	-	+	-	-
5-Methoxydurmillone	-	-	-	+ [8]
Jamaicin	-	-	-	+ [8]
Isojamaicin	-	-	-	+# [8]
Ichthynone	-	-	-	+* [8]
7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone	-	-	-	+* [8]
Flemichapparin B	-	-	-	+* [8]
4'-*O*-Geranylisoliquiritigenin	-	-	-	+* [9]
7-*O*-Geranylformononetin	-	-	-	+* [9]

* found from subspecies *darassana*

found from subspecies *ferruginea*

University campus, a voucher specimen under the cipher K287 is deposited in the Herbarium of the Botany Department, Makerere University.

Isolation of compounds from the seeds of M. dura. 234 g of the powdered seeds of *M. dura* were first soaked in petrol for 24 hrs and the marc was subsequently extracted with hot $CHCl_3$. Removal of the $CHCl_3$ yielded 10 g of crude ext. which was applied on silica gel CC and eluted with petrol and petrol:EtOAc mixtures of increasing polarities. Fraction 17 afforded 12-hydroxymilletone and milletone. Fraction 18 gave durlettone. Fractions 24-26 were rechromatographed to afford deguelin. Fractions 27-28 gave tephrosin after rechromatography. Fractions 29-34 were combined and rechromatographed to afford durmillone.

Isolation of compounds from stem bark. Dried 160 g of the stem bark was soaked in petrol at room temp. for 8 days, filtration and removal of the solvent yielded 2 g of crude ext. The marc was further extd. by percolation with CH_2Cl_2 for 4 days to yield 4 g of crude ext. From the CH_2Cl_2 ext. 18 mg of **4** pptd out. The petrol ext. when applied on silica gel CC and eluted with petrol and increasing polarities of EtOAc yielded 13 mg of lupeol, 15 mg of **1** and 7mg of **4**. The CH_2Cl_2 ext. was likewise applied on CC and eluted with petrol and petrol:EtOAc mixtures of increasing polarities to yield 68 mg of lupeol, 4 mg of **2**, 16 mg of **1**, 4 mg of **5**, 14 mg of **4** and 7 mg of **3**. All of the above described compounds were also obtained from the root bark following similar procedure.

Maximaisoflavone H (**1**). Mp 191-193° (lit. [14] 190-191°); IRυ_{max} cm^{-1}: 3120, 2980, 1655, 1510, 1460, 1400, 1270, 1020; ^1H NMR (90 MHz, $CDCl_3$): δ3.84 (3H, *s*, OCH_3), 6.20 (2H, *s*, OCH_2O), 6.94 (1H, *d*, J = 9 Hz, H-6), 6.96 (2H, *d*, J = 9 Hz, H-3', H-5'), 7.46 (2H, *d*, J = 9 Hz, H-2', H-6'), 7.90 (1H, *d*, J = 9 Hz, H-5), 7.91 (1H, *s*, H-2); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ175.0 (C=O), 159.9 (C-4'), 152.2 (C-7), 151.5 (C-2), 147.8 (C-9), 134.4 (C-8), 130.2 (C-2', C-6'), 124.4 (C-3), 123.8 (C-1'), 121.0 (C-4a, C-5), 114.1 (C-3', C-5'), 107.1 (C-6), 103.2 (OCH_2O), 55.4 OCH_3; MS *m/z* (rel. int.): 296 $[M]^+$ (49), 281 (6), 165 (10), 164 (100), 146 (31), 132 (28), 126 (13), 117 (10), 106 (21), 89 (20).

Maximaisoflavone B (**2**). Mp 119-121° (lit. [14] 126-128°); IRυ_{max} cm^{-1}: 3050, 2980, 1660, 1530, 1470, 1275; ^1H NMR (90 MHz, $CDCl_3$): δ1.76 (3H, *s*, CH_3), 1.84 (3H, *s*, CH_3), 4.60 (2H, *d*, J = 7, H-2"), 5.48 (1H, *t*, J = 7, H-3"), 6.76-7.12 (5H, H-2', H-5', H-6', H-6, H-8), 7.88 (1H, *s*, H-2), 8.16 (1H, *d*, J = 9 Hz, H-5); 176.2 (C=O), 163.6 (C-7), 158.1 (C-8a), 152.1 (C-2), 147.9 (C-3', C-4'), 138.9 (C-4"), 127.9 (C-5), 126.1 (C-1'), 125.3 (C-3), 122.6 (C-2"), 119.2 (C-3"), 119.1 (C-4a), 115.1 (C-6), 109.9 (C-6'), 108.4 (C-5'), 101.4 (OCH_2O), 101.2 (C-8), 65.8 (C-2"), 25.7 (C-4"), 18.3 (C-5"); MS *m/z* (rel. int.): 350 $[M]^+$ (2), 282 (12), 268 (13), 151 (6), 146 (9), 139 (4), 132 (2), 118 (3), 107 (3), 89 (6), 69 (100).

7,2'-Dimethoxy-4',5'-methylenedioxyisoflavone (**3**). Mp 215-217° (lit. [17] 210-212°); IRυ_{max} cm^{-1}: 1660, 1520, 1460, 1330, 1280, 1260, 1200, 1045; ^1H NMR (300 MHz, $CDCl_3$): δ3.73 (3H, *s*, OCH_3), 3.92 (3H, *s*, OCH_3), 5.96 (2H, *s*, OCH_2O), 6.63 (1H, *s*, H-3'), 6.83 (1H, *s*, H-6'), 6.85 (1H, *d*, J = 2.1 Hz, H-8), 6.98 (1H, *dd*, J = 9, 2.1 Hz, H-6), 7.88 (1H, *s*, H-2), 8.18 (1H, *d*, J = 9 Hz, H-5); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ177.0 (C=O), 164.2 (C-7), 158.1 (C-8a), 154.1 (C-2), 153.3 (C-2'), 148.6 (C-4'), 141.6 (C-5'), 128.0 (C-5), 122.7 (C-3), 119.0 (C-4a), 114.2 (C-6), 113.6 (C-1'), 111.4 (C-6'),

101.4 (OCH_2O), 100.6 (C-8), 96.1 (C-3'), 57.3 (OCH_3), 55.7 (OCH_3); MS *m/z* (rel. int.): 326 $[M]^+$ (91), 308 (8), 295 (100), 281 (8), 253 (8), 182 (6), 176 (13), 175 (16), 151 (34), 148 (27), 147 (35).

4-Hydroxyderricin (**4**). Amorphous (lit. [18, 19] mp 146-147°); IRν_{max} cm^{-1}: 3450, 1640, 1600, 1590, 1520, 1460, 1400, 1380, 1300, 1260, 1130, 1090; ^1H NMR (400 MHz, $CDCl_3$): δ1.66 (3H, *s*, CH_3), 1.77 (3H, *s*, CH_3), 3.36 (2H, *d*, H-1"), 3.88 (3H, *s*, OCH_3), 5.20 (1H, *m*, H-2"), 6.47 (1H, *d*, *J* = 9 Hz, H-5'), 6.88 (2H, *br*, H-3, H-5), 7.42 (1H, *d*, *J* = 15.5 Hz, H-α), 7.50 (2H, *d*, *J* = 8 Hz, H-2, H-6), 7.76 (1H, *d*, *J* = 9 Hz, H-6'), 7.79 (1H, *d*, *J* = 15.5 Hz, H-ß), 13.5 (1H, *s*, 2'-OH); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ192.7 (C=O), 163.6 (C-4'), 163.3 (C-2'), 158.5 (C-4), 144.1 (C-ß), 131.6 (C-3"), 130.5 (C-2, C-6), 129.2 (C-6'), 128.0 (C-1), 122.5 (C-2"), 118.8 (C-α), 118.2 (C-1'), 116.3 (C-3, C-5), 115.1 (C-3'), 102.4 (C-5'), 55.9 (OCH_3), 25.7 (4"-CH_3), 22.0 (C-1"), 17.8 (5"-CH_3); HRMS *m/z* (rel. int): 338.1516 $[M]^+$ (76) (calc. for $C_{21}H_{22}O_4$: 338.1512), 323 (7) 296 (23), 295 (100), 284 (21), 283 (39), 218 (9), 203 (17), 190 (12), 175 (17), 164 (15), 163 (100), 147 (28), 120 (35), 119 (35), 115 (18), 107 (21), 105 (25).

4-Hydroxylonchocarpin (**5**). Amorphous (lit. [18] mp 203-205°); IRν_{max} cm^{-1}: 3250, 1645, 1625, 1600, 1560, 1525, 1500, 1380, 1300, 1245, 1220, 1175, 1130; ^1H NMR (90 MHz, $CDCl_3$): δ1.55 (6H, *s*, 2xCH_3), 5.64 (1H, *d*, *J* = 9.7 Hz, H-3"), 6.40 (1H, *d*, *J* = 9 Hz, H-5'), 6.76 (1H, *d*, *J* = 9.7 Hz, H-4"), 6.90 (2H, *d*, *J* = 9 Hz, H-3, H-5), 7.48 (1H, *d*, *J* = 16.2 Hz, H-α), 7.59 (2H, *d*, *J* = 9 Hz, H-2, H-6), 7.66 (1H, *d*, *J* = 9 Hz, H-6'), 7.88 (1H, *d*, *J* = 16.2 Hz, H-ß), 13.68 (1H, *s*, 2'-OH); MS *m/z* (rel. int.): 322 $[M]^+$ (9.5), 307 (23), 203 (4), 188 (12), 187 (100), 174 (4), 163 (5), 159 (5), 147 (7), 131 (8), 120 (10).

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