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THE FLAVONOIDS OF *MILLETTIA FERRUGINEA* SUBSP. *FERRUGINEA* AND SUBSP. *DARASSANA* IN ETHIOPIA

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Key Word Index—*Millettia ferruginea* subsp. *darassana*; *M. ferruginea* subsp. *ferruginea*; Leguminosae; Papilionoideae; isoflavones; flavanones; chalcones; pterocarpenes; chemotaxonomy.

Abstract—Analysis of the bark and seed pods of plant material assigned to *Millettia ferruginea* subsp. *darassana* and *M. ferruginea* subsp. *ferruginea* has led to the isolation of eight isoflavones, a chalcone, a flavanone and a pterocarpene. Three of the isolated compounds appear to be novel and have been identified as 5-methoxydurmillone (5,6-dimethoxy-3′,4′-methylenedioxy-2″,2″-dimethylpyrano-[5″,6″:7,8]isoflavone), isojamaicin (3′-methoxy-4′,5′-methylenedioxy-2″,2″-dimethylpyrano[5″,6″:7,8]isoflavone) and 4′-hydroxyisolonchocarpin (4′-hydroxy-2″,2″-dimethylpyrano[5″,6″:7,8]flavanone). Patterns of flavonoid production do not appear to distinguish the two subspecies.

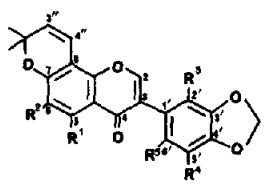
INTRODUCTION

Millettia ferruginea (Hochst.) Bak. (Amharic name Berbera) is a tree, endemic to Ethiopia [1]. Two subspecies are recognized, the typical subsp. *ferruginea* occurs in northern Ethiopia (N of 11°30′) while subsp. *darassana* (Cuf.) Gillett is restricted to the southern province of Sidamo [1]. From the area between these two clearly defined subspecies, plants show a mixture of characters attributable to both suggesting the possibility of hybridization. Previous chemical studies have been restricted to the seeds. Clark [2] reported the isolation of rotenone, deguelin and tephrosin from material collected in Addis Ababa, which is in the intermediate belt between the two clear subspecies. These rotenoids are probably responsible for the common use of the seeds as a fish poison [2]. A further study of seeds of unknown origin [3] gave two isoflavonoids, ferrugone (**1**) and durmillone (**2**).

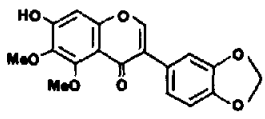
We now wish to report studies on stem bark samples attributable to both subsp. of *M. ferruginea*. A collection obtained from Sidamo Province can clearly be assigned to subsp. *darassana*. A second collection from the north (Gondar) is similarly attributable to subsp. *ferruginea* and proved to be chromatographically identical to a third sample collected in Addis Ababa.

RESULTS AND DISCUSSION

The chloroform-soluble portion of an ethanolic extract of the bark of *M. ferruginea* subsp. *darassana* was fractionated by column chromatography over silica gel and gave six flavonoids. Five of these were characterized as ferrugone (**1**), jamaicin (**3**), ichthynone (**4**), 7-hydroxy-5,6-dimethoxy-3′,4′-methylenedioxyisoflavone (**5**) and flemichapparin-B (**6**). In some cases additional ^1H and ^{13}C NMR data were obtained for the known compounds



	R^1	R^2	R^3	R^4	R^5
1	H	H	OMe	OMe	H
2	H	OMe	H	H	H
3	H	H	H	H	OMe
4	H	OMe	H	H	OMe
9	OMe	OMe	H	H	H
10	H	H	H	H	H
14	H	H	H	OMe	H



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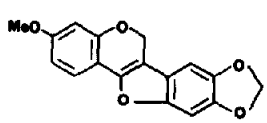

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and these are given in Tables 1 and 2 respectively. The sixth and major compound analysed for $C_{23}H_{20}O_7$ and gave the simple UV spectrum of an isoflavone. The ^1H NMR spectrum (Table 1) confirmed the isoflavone nucleus (H-2, δ7.81) and revealed the presence of 2,2-dimethylpyrano, methylenedioxy and two methoxyl substituents. Three further aromatic protons showed coupling interactions typical of an ABC system. The EIMS revealed a base peak for $[M-Me]^+$, typical of com-

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Table 1. ^{1}H NMR chemical shifts for isoflavonoids

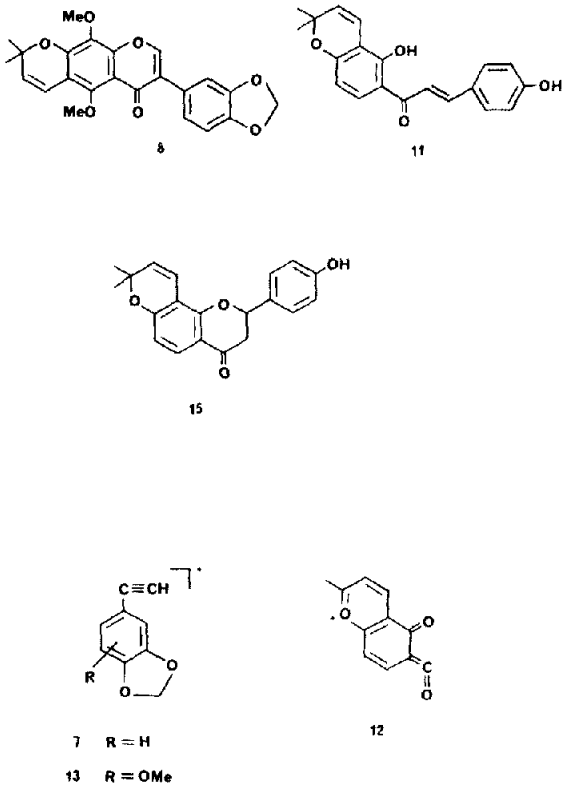
H	1	2	3	4	5	9	9*	10	14
2	7.91	7.94	7.91	7.94	7.80	7.81	7.89	7.93	7.95
5	8.05	7.55	8.04	7.56	—	—	—	8.05	8.06
6	6.86	—	6.86	—	—	—	—	6.86	6.87
8	–	–	–	–	6.79	—	—	—	—
2′	–	7.10	6.83	6.83	7.08.	7.08	7.03	7.09	6.80[a]
5′	—	6.87	6.62	6.60	6.85	6.85	6.91	6.86	–
6′	6.62	6.97	—	—	6.93	6.93	6.91	6.97	6.87[a]
3″	5.73	5.74	5.72	5.75	—	5.68	5.62	5.72	5.72
4″	6.81	6.80	6.81	6.82	—	6.76	6.71	6.81	6.80
2″-Me_2	1.50	1.55	1.50	1.57	–	1.55	1.54	1.50	1.50
O–CH_2–O	6.02	5.99	5.97	5.99	5.98	5.98	6.00	5.99	6.00
OMe	3.87	3.67	3.73	3.96	4.04	3.96	3.91	—	3.94
	3.85	—	—	3.74	3.95	3.90	—	—	—
OH	–	–	–	–	6.45	–	12.89	–	–

9* = 5-demethyl derivative of **9**.
All spectra run in $CDCl_3$, **9** at 360 MHz, **9*** at 90 MHz and the remainder at 250 MHz.
J values $_{5-6}$ = 8.8 Hz, $_{2'-6'}$ = 1.7 Hz, $_{5'-6'}$ = 8 Hz, $_{3''-4''}$ = 10 Hz.
[a]Assignments interchangeable.

Table 2. ^{13}C NMR shifts for isoflavonoids

C	1	3	4	9
2	153.4	153.7	153.5	150.2
3	122.1	121.9	129.5	125.3
4	175.7	175.6	175.3	175.0
4a	118.4	118.4	118.4	106.5
5	126.7	126.7	105.4	151.0
6	115.2	115.0	147.0	140.0
7	152.6	153.0	147.4	149.1
8	109.3	109.2	110.3	113.1
8a	157.3	157.1	154.0	153.0
1′	118.0	112.9	113.2	125.6
2′	139.2	111.2	111.2	110.0
3′	137.2	148.4	148.3	147.5
4′	136.9	141.2	141.2	147.5
5′	139.1	95.5	95.6	108.2
6′	110.5	153.0	153.0	122.5
2″	77.7	77.6	78.0	68.1
3″	115.0	115.0	115.3	114.9
4″	130.3	130.2	130.5	129.0
2″-Me_2	28.2	28.0	28.0	28.0
O–CH_2–O	101.8	101.3	101.3	100.0
OMe	60.2	56.9	56.9	62.1
	57.0	–	56.3	61.3

All spectra run in $CDCl_3$; **1**, **3**, **4** at 22.5 MHz, and **9** at 90.56 MHz.



pounds with the 2,2-dimethylpyran system and a significant fragment at *m/z* 146 (**7**) which can be assigned to ring B carrying a methylenedioxy substituent.

Thus the isoflavone must have two methoxyl and the 2,2-dimethylpyran substituents in ring A and the problem to be resolved is the placement of the methoxyl substituents. Demethylation using 48% HBr gave a product, the ^{1}H NMR spectrum of which revealed a chelated hydroxyl at δ12.89 allowing one methoxyl to be assigned to C-5 and requiring the second to be placed at C-6 or C-8. NOE experiments involving irradiation of the methoxyl resonances failed to yield significant enhancements of H-4″ which effectively eliminates the possibility of the 5,8-dimethoxy compound (**8**) and permits the assignment of structure **9**, which appears to be novel and to which we assign the trivial name of 5-methoxy-durmillone. The ^{13}C NMR spectrum of **9** (Table 2) supports the proposed structure. A comparable extraction of the seed pods of *M. ferruginea* subsp. *darassana* led to the isolation of one isoflavone, the previously reported durmillone (**2**).

The bark of the material collected in Addis Ababa and which showed a TLC profile comparable to that of authentic *M. ferruginea* subsp. *ferruginea* was defatted with petrol and then extracted with chloroform. Chromatographic separation of the petrol extract yielded trace amounts of calopogonium isoflavone B (**10**), 4-hydroxylonchocarpin (**11**) and two further flavonoids that appear to be novel. The first of these analysed for $C_{22}H_{18}O_6$ and gave the ^{1}H NMR spectrum of an isoflavone with 2,2-dimethylpyrano, methylenedioxy and methoxyl substituents (Table 1). The mass spectrum revealed fragments for ring A substituted with the pyrano substituent at *m/z* 187 (**12**) and ring B with methylenedioxy and methoxyl substituent at *m/z* 176 (**13**). The ^{1}H NMR spectrum revealed *meta* coupling between the two B-ring protons which must be placed at H-2′ and H-6′ leading to the placement of the methoxyl at C-5′. This compound must therefore have structure **14** and can be assigned the trivial name isojamaicin. The last compound analysed for $C_{20}H_{18}O_4$ with the ^{1}H NMR spectrum showing the typical ABX system for H-2 and H-3 of the flavanone nucleus. In addition, it revealed the presence of unsubstituted C-5, C-6, a simple *para* substituted B-ring and a 2,2-dimethylpyrano substituent which must be placed at C-7/C-8. These observations were sustained by the EIMS allowing formulation of structure **15** which, although previously synthesized [5], appears to be new and to which we assign the trivial name 4′-hydroxyisolonchocarpin. The chloroform extract yielded further amounts of the chalcone (**11**) and three isoflavones characterized previously in this study; **1**, **3**, and **9**.

The distribution of flavonoids isolated from the barks of the two varieties is shown in Table 3. This shows that, based on oxygenation patterns among the isoflavones isolated, no distinction can be made between the two taxa. The pterocarpene (**6**) was only found in subsp. *darassana*, where it occurred in appreciable amounts, while the chalcone **11** and the flavanone **15** were isolated only from subsp. *ferruginea*.

EXPERIMENTAL

Plant material. The bark and seed pods of *M. ferruginea* subsp. *darassana* were collected from Aleta-Wondo, Sidamo Province in December 1987. Bark of *M. ferruginea* subsp. *ferruginea* was collected in Gondar town in February 1988 and the material originating in Addis Ababa was obtained in September 1987.

Table 3. Distribution of flavonoids between the two subspecies of *Millettia ferruginea*

Flavonoid	subsp. *darassana*	subsp. *ferruginea*
Chalcone **11**	—	+
Flavanone **15**	–	+
Pterocarpene **6**	+	–
Isoflavones **1**	+	+
3	+	+
4	+	–
5	+	–
9	+	+
10	–	+
14	–	+

Voucher specimens S-108 and S-145 representing subsp. *darassana* and subsp. *ferruginea* respectively are deposited at the Herbarium of the Biology Department, Addis Ababa University.

Isolation of compounds from the bark of Millettia ferruginea *subsp.* ferruginea. The ground bark (400 g) was percolated with petrol (bp 60–80°) at room temp., dried, and then subjected to Soxhlet extraction with $CHCl_3$. The petrol extract was concd (3 g) and chromatographed over Sephadex LH20 (70 g) eluting with $CHCl_3$–MeOH (1:1). Thirty fractions (7 ml each) were collected, the first 20 of which contained only fats and were discarded. The remaining fractions were bulked and on concn yielded 10 mg of a mixture of flavonoids. These were separated by circular PTLC (silica gel, solvent, toluene–hexane–EtOAc 3:4:3) to give **10** (3 mg), **14** (1.5 mg), **11** (3 mg) and **15** (1 mg). The crude $CHCl_3$ extract (10 g) was eluted from Sephadex LH20 in the same manner and the flavonoid fraction (6 g) chromatographed over silica gel (250 g) and eluted with petrol containing increasing amounts of EtOAc. Elution with 5% EtOAc yielded **11** (120 mg); with 10% EtOAc **3** (40 mg); with 15% EtOAc a mixture of **3** and **1** was obtained and separated by PTLC over silica gel (solvent, toluene–hexane–EtOAc 3:4:3) to give **1** (45 mg) followed by a second mixture of compounds from which **9** (60 mg) was obtained by recrystallization.

Isolation of compounds from the bark of Millettia ferruginea *subsp.* darassana. The ground bark (5 kg) was percolated with EtOH (8 l) for 15 days. The extract was filtered and concd to give 149 g of solid which was fractionated between $CHCl_3$ (90 g) and MeOH (50 g). A 22 g portion of the $CHCl_3$- sol. fraction was chromatographed over silica gel (600 g). The column was washed with petrol to remove fatty material and then the flavonoids were eluted with petrol containing increasing amounts of EtOAc. Elution with 5–10% EtOAc gave only further fatty material. From 15% EtOAc a yellow oil was obtained which was pptd with MeOH and filtered; the filtrate yielded **6** (50 mg). Elution with 20% EtOAc gave a mixture of **1** and **3** (400 mg) which was not treated further. From 25% EtOAc **9** (1.5 g) was obtained. Finally, elution with 30% EtOAc resulted in a mixture which was separated by PTLC (silica gel, toluene–hexane–EtOAc 3:4:3) to give **4** (40 mg) and **5** (10 mg).

Isolation of **2** *from the seed pods of* Millettia ferruginea *subsp.* darassana. The ground pods (300 g) were percolated consecutively with petrol, $CHCl_3$ and MeOH. The $CHCl_3$-sol. material (6 g) was applied to a Sephadex LH20 column and eluted with $CHCl_3$–MeOH (1:1) to remove fats and chlorophyll. The flavonoid containing fraction was chromatographed over silica gel and eluted with petrol–EtOAc mixtures. Elution with 15% EtOAc gave **2** (30 mg).

Ferrugone (**1**). Needles from MeOH, mp 164–166° (lit. [3] 167–9°). Found: $[M]^+$ 408.1220; $C_{23}H_{20}O_7$ requires 408.1209. UV λ_{max} nm: 229, 260, 310sh, 320sh. ^{1}H NMR (see Table 1). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int): 408 $[M]^+$ (100), 393 (98), 377 (30), 363 (16), 206 (87), 187 (14).

Durmillone (**2**). Needles from MeOH, mp 179–181° (lit. [3] 182–185°). Found: $[M]^+$ 378.1080; $C_{22}H_{18}O_6$ requires 378.1103. ^{1}H NMR (see Table 1). EIMS *m/z* (rel. int.): 378 $[M]^+$ (60), 363 (100), 217 (5), 146 (9).

Jamaicin (**3**). Needles from MeOH, mp 189–192° (lit. [4] 193–194°). Found: $[M]^+$ 378.1120; $C_{22}H_{18}O_6$ requires 378.1103, UV λ_{max} nm: 228, 261, 308. ^{1}H NMR (see Table 1). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.): 378 $[M]^+$ (100), 363 (62), 347 (44), 187 (12).

Ichthynone (**4**). Needles from MeOH, mp 201–202° (lit. [6] 203–204°). Found: $[M]^+$ 408.1184; $C_{23}H_2O_7$ requires 408.1209. UV λ_{max} nm: 229, 260, 306, 330sh. ^{1}H NMR (see Table 1). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.); 408 $[M]^+$ (100), 393 (81), 377 (89), 233 (10), 176 (4), 174 (16).

7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone (**5**). Needles from Me_2CO, mp 230–231° (lit. [7] 235–237°). Found: $[M]^+$ 342.0736; $C_{18}H_{14}O_7$ requires 342.0739. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 342 $[M]^+$ (59), 327 (100), 181 (12), 146 (30).

Flemichapparin (**6**). Needles from MeOH, mp 174–179° (lit. [8] 179–180°). Found: $[M]^+$ 296.0690; $C_{17}H_{12}O_5$ requires 296.0685. UV λ_{max} nm: 230, 250, 292, 339, 358. ^1H NMR (250 MHz, $CDCl_3$) δ: 7.37 (1H, *d*, *J* = 8.2 Hz, H-1), 7.02 (1H, *s*, H-7), 6.73 (1H, *s*, H-10), 6.53 (2H, *m*, H-2, H-4), 6.00 (2H, *s*, O–CH_2–O), 5.52 (2H, *s*, H-6), 3.81 (3H, *s*, 3-OMe). ^{13}C NMR (22.5 MHz, $CDCl_3$) ppp: *s* at 160.7 (C-3), 154.8 (C-4a), 150.4 (C-10a), 147.6 (C-11a), 145.6, 144.8 (C-8, C-9), 119.0 (C-6b), 109.8 (C-11b), 106.3 (C-6a); *d* at 120.7 (C-1), 107.1 (C-2), 102.4 (C-7), 97.1 (C-4), 93.9 (C-10); *t* at 101.3 (O–CH_2–O), 65.3 (C-6); *q* at 55.3(C-3-OMe). EIMS *m/z* (rel. int.): 296 $[M]^+$ (100), 295 (61), 281 (10).

5-Methoxydurmillone (**9**). Needles from MeOH, mp 142–143° Found: $[M]^+$ 408.1223; $C_{23}H_{20}O_7$ requires 408.1209. UV λ_{max} nm (log ε): 224 (4.41), 263 (4.54), 294sh (4.15), 320sh (3.81), unchanged by addition of NaOMe. IR ν_{max} cm^{-1}: 1660, 1630, 1530, 1510, 1425, 1420, 1375, 1360, 1290, 1180, 1075. ^1H NMR (see Table 1). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.): 408 $[M]^+$ (60), 393 (100), 363 (11), 349 (12), 270 (1), 196 (11), 146 (10). *5-Hydroxydurmillone*. **9** (50 mg) was mixed with 48% HBr (23 ml) and refluxed for 3 hr. Excess HBr was removed under red. pres. and the residue chromatographed over silica gel eluting with petrol–EtOAc (9:1) to give *5-hydroxydurmillone* (6 mg), mp 205–207°. Found: $[M]^+$ 394.1044; $C_{22}H_{18}O_7$ requires 394.1052. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 394 $[M]^+$ (60), 379 (100).

Calopogonium isoflavone B. (**10**). Amorphous solid. Found: $[M]^+$ 348.1103; $C_{21}H_{16}O_5$ requires 348.0980. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 348 $[M]^+$ (99), 333 (100), 187 (45).

4-Hydroxylonchocarpin (**11**). Orange needles from MeOH, mp 189–192° (lit. [9] 201–203°). Found: $[M]^+$ 322.1210; $C_{20}H_{18}O_4$ requires 322.1205. UV λ_{max} nm: 226, 272, 305sh, 368; (+ NaOMe): 226sh, 270, 432. ^1H NMR (250 MHz, Me_2CO-d_6) δ: 14.07 (1H, *s*, 2'-OH), 8.06 (1H, *d*, *J* = 9.0 Hz, H-6'), 7.86 (1H, *d*, *J* = 15.4 Hz, H-β) 7.70 (1H, *d*, *J* = 15.4 Hz, H-α), 7.74 (2H, *d*, *J* = 8.5 Hz, H-2, H-6), 6.92 (2H, *d*, *J* = 8.5 Hz, H-3, H-5), 6.69 (1H, *d*, *J* = 10.0 Hz, H-4''), 6.36 (1H, *d*, *J* = 8.8 Hz, H-5'), 5.71 (1H, *d*, *J* = 10.0 Hz, H-3''), 1.44 (6H, *s*, 2''-Me_2). ^{13}C NMR [22.5 MHz, Me_2CO-d_6) ppm]: *s* at 193.2 (C=O), 161.7, 161.1, 160.4 (C-4, C-2', C-4'), 127.6 (C-1), 115.0 (C-1'), 108.8 (C-3'), 78/5 (C-2''); *d* at 145.5 (C-β), 132.2 (C-4''), 131.9 (C-2, C-6), 129.2 (C-6'), 118.2 (C-α), 116.8, C-3, C-5), 116.3 (C-3''), 110.0 (C-5'), *q*, *t*, 28.5 (2''-Me_2). EIMS *m/z* (rel. int.): 322 $[M]^+$ (53), 307 (67), 187 (100).

Isojamaicin (**14**). Amorphous. Found: $[M]^+$ 378.1140; $C_{22}H_{18}O_6$ requires 378.1103. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 378 $[M]^+$ (66), 363 (100), 333 (25), 206 (35), 187 (35).

4'-Hydroxyisolonchocarpin (**15**). Amorphous. Found: $[M]^+$ 328.1108; $C_{20}H_{18}O_4$ requires 328.1205. ^1H NMR (250 MHz, $CDCl_3$) δ: 7.74 (1H, *d*, *J* = 8.5 Hz, H-5), 7.36 (2H, *d*, *J* = 8.3 Hz, H-2', H-6'), 6.89 (2H, *d*, *J* = 8.8 Hz, H-3', H-5'), 6.62 (1H, *dd*, *J* = 10.0, 0.7 Hz, H-4''), 6.49 (1H, *dd*, *J* = 8.8, 0.7 Hz, H-6), 5.56 (1H, *d*, *J* = 10.3 Hz, H-3''), 5.40 (1H, *dd*, *J* = 13.1, 3.1 Hz, H-2), 3.01 (1H, *dd*, *J* = 16.8, 13.2 Hz, H-3_{ax}), 2.80 (1H, *dd*, *J* = 16.8, 3.2 Hz, H-3_{eq}), 1.47 (3H, *s*, 2''-Me)$^+$, 1.44 (3H, *s*, 2''-Me). EIMS *m/z* (rel. int.): 322 $[M]^+$ (67), 307 (87), 187 (100), 120 (37).

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