Phytochemistry, Vol. 29, No. 8, pp. 2679–2682, 1990.
Printed in Great Britain.

0031 9422/90 $3.00+0.00

C-PRENYLATED ISOFLAVONES FROM *MILLETTIA FERRUGINEA*

ERMIAS DAGNE and AMHA BEKELE

Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

(*Received* 31 *October* 1989)

Key Word Index—*Millettia ferruginea* subsp. *ferruginea* and subsp. *darassana*; Leguminosae; seeds; root bark; *C*-prenylated isoflavones; rotenoids.

Abstract—Immature seeds of *M. ferruginea* subsp. *ferruginea* and subsp. *darassana* yielded three rotenoids (rotenone, tephrosin and 12a-hydroxyrotenone), four pyrano-isoflavones (ferrugone, durmillone, calopogonium isoflavone-A and barbigerone) and three novel *C*-prenylated isoflavones named preferrugone, predurmillone and prebarbigerone. The root bark of the latter subspecies also afforded the novel *C*-prenylated isoflavone pre-5-methoxydurmillone.

INTRODUCTION

The genus *Millettia*, represented by over 200 species, is distributed in tropical Africa, Asia and Australasia [1]. The seeds and other parts of *Millettia* species are commonly employed to catch fish [2], as insecticides [3] and in folk medicines [4]. The seeds of the common endemic tree, *M. ferruginea* (Hochst.) Bak. are also used in Ethiopia as a fish poison. Two subspecies namely *M. ferruginea* subsp. *ferruginea* and subsp. *darassana* (Cuf.) Gillet are recognized to occur in Ethiopia [1]. Two previous chemical studies of the seeds revealed the presence of three rotenoids namely rotenone, deguelin and tephrosin [5] and two isoflavones, ferrugone and durmillone [6]. We also recently reported the isolation and characterization of eight isoflavones, a chalcone and a pterocarpene from the stem bark of the two subspecies [7]. We now report the results of investigations on the immature seeds of the two subspecies and the root bark of subsp. *darassana*.

The studies on the seeds have resulted in the isolation of three novel C-prenylated isoflavones, preferrugone (**1**), predurmillone (**2**) and prebarbigerone (**3**). In addition the root bark yielded the new compound pre-5-methoxydurmillone (**4**). These novel substances may be considered as precursors of the respective pyranoisoflavones, i.e. ferrugone (**5**), durmillone (**6**), barbigerone (**7**) and 5-methoxydurmillone (**8**). It should be noted that prebarbigerone could be considered as a methyl ether derivative of **3a**, a more likely precursor of **7** though not detected in this study. Tahara *et al.* [8] have recently provided further biosynthetic proof for the conversion of 8-prenylated isoflavones to pyrano and other related derivatives.

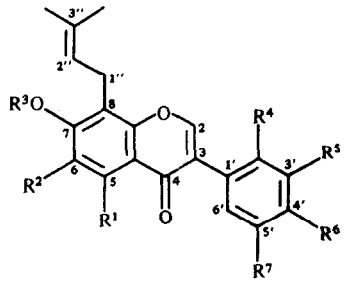
RESULTS AND DISCUSSION

An ethanol extract of the immature seeds of *M. ferruginea* subsp. *darassana*, when subjected to column chromatography on silica gel using increasing proportions of ethyl acetate in petrol led to the isolation of seven isoflavones and two rotenoids. Four of the isoflavones were readily identified as the known isoflavones ferrugone (**5**), durmillone (**6**), barbigerone (**7**) and calopogoniumisoflavone-A (**9**). Compounds **7** and **9** are reported here for the first time from the genus *Millettia*. Two of the remaining isoflavones were characterized based on evidence presented below as the novel compounds preferrugone (**1**) and predumillone (**2**). The two rotenoids were identified as 12a-hydroxyrotenone and tephrosin by comparison (co-TLC, ^{1}H NMR) with authentic samples.

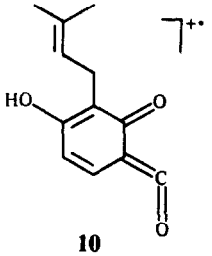
A comparable extraction of the root bark of *M. ferruginea* subsp. *darassana* resulted in the isolation of the novel compound pre-5-methoxydurmillone (**4**), a likely precursor of the isoflavone **8**, which we recently reported in the stem bark of both subspecies [7]. The chloroform extract of the immature seeds of *M. ferruginea* subsp. ferruginea, after chromatographic separations, yielded in addition to ferrugone (**5**), durmillone (**6**), rotenone, barbigerone (**7**) and calopogoniumisoflavone-A (**9**) together with the novel compound prebarbigerone (**3**).

High resolution mass spectrometry of compound **1** revealed the molecular formula $C_{23}H_{22}O_7$. The presence in its ^{1}H NMR spectrum of a singlet at δ7.88 and two *ortho*-coupled doublets at δ7.87 and 6.81 (J=8.7 Hz) indicated an isoflavone nucleus lacking substituents at C-5 and C-6 positions. A one proton triplet at δ5.18, a two proton doublet at δ3.51 and two methyl signals at δ1.75 and 1.60 clearly suggested the presence of a dimethylallyl group. The ^{1}H NMR further revealed the presence of two methoxy groups and a methylenedioxy substituent. The placement of the dimethylallyl moiety on ring A and the methoxy and the methylenedioxy groups on ring B was possible from the appearance in the mass spectrum of fragments with *m/z* 204 (**10**) and 206 (**11**). That the hydroxyl and dimethylallyl groups are located on adjacent carbons was deduced from the ease with which **1** was cyclized with 98% formic acid to yield the chroman **12**. Final proof for the proposed structure was obtained by cyclodehydrogenation of compound **1** to ferrugone (**5**) using DDQ. The structure of preferrugone is therefore 7-hydroxy-2′,5′-dimethoxy-3′,4′-methylenedioxy-8-(3,3-dimethylallyl)isoflavone.

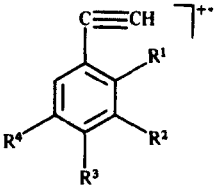
The isoflavone **2** had the molecular formula $C_{22}H_{20}O_6$. The appearance of H-5 as a singlet indicated



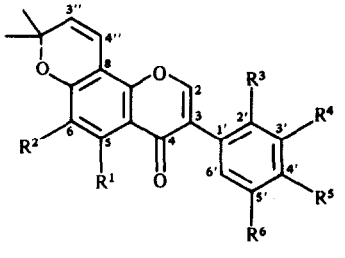
	R¹	R²	R³	R⁴	R⁵	R⁶	R⁷
1	H	H	H	OMe	—OCH_2O—		OMe
2	H	OMe	H	H	—OCH_2O—		H
3	H	H	Me	OMe	H	OMe	OMe
3a	H	H	H	OMe	H	OMe	OMe
4	OMe	OMe	H	H	—OCH_2O—		H



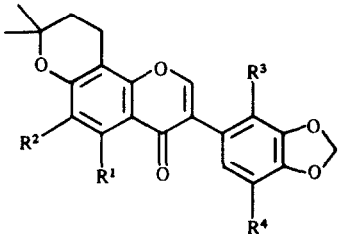
10



	R¹	R²	R³	R⁴
11	OMe	—OCH_2O—		OMe
13	H	—OCH_2O—		H
16	OMe	H	OMe	OMe



	R¹	R²	R³	R⁴	R⁵	R⁶
5	H	H	OMe	—OCH_2O—		OMe
6	H	OMe	H	—OCH_2O—		H
7	H	H	OMe	H	OMe	OMe
8	OMe	OMe	H	—OCH_2O—		H
9	H	H	H	H	OMe	H



	R¹	R²	R³	R⁴
12	H	H	OMe	OMe
14	H	OMe	H	H
15	OMe	OMe	H	H

an otherwise fully substituted ring A. The presence of a dimethylallyl, a methoxy, a methylenedioxy and a hydroxy substituent was clearly deduced from the ^{1}H NMR spectrum. The ^{1}H NMR spectrum further allows assignment of a 3′,4′-disubstituted ring B based on the ABC pattern of three aromatic protons (Table 1), a proposition supported by the presence in the mass spectrum of fragments at *m/z* 146 (**13**). Treatment of compound **2** with 98% formic acid resulted in the cyclized product **14** which supports the placement of the methoxy and hydroxy substituents at C-6 and C-7 positions, respectively, and the dimethylallyl group at C-8, thus allowing the assignment of structure **2**, viz. 7-hydroxy-6-methoxy-3′,4′-methylenedioxy-8-(3,3-dimethylallyl)isoflavone, for this compound.

Compound **3** which analysed for $C_{24}H_{26}O_6$ displayed in its ^{1}H NMR the presence of four methoxy groups and a dimethylallyl substituent, two *ortho*-coupled doublets at δ8.15 and 7.00 assignable to H-5 and H-6, respectively, and two *para* protons appearing at δ6.50 and 6.95. The presence in the mass spectrum of the RDA fragment **16** with *m/z* 192 coupled with the ^{1}H NMR spectral data allow the placement of the three methoxy groups at the 2′,4′ and 5′ positions, consistent with structure **3** for this new natural product. Confirmation of the structure of **3** as 7,2′,4′,5′-tetramethoxy-8-(3,3-dimethylallyl)isoflavone, was further obtained from the ^{13}C NMR spectrum (Table 2).

The final novel compound **4** analysed for $C_{23}H_{22}O_7$ and was established to be an isoflavone from its ^{1}H NMR, which exhibited a singlet at δ7.84 in addition to signals for two methoxy, one dimethylallyl and one methylenedioxy substituents. The ABC pattern with two doublets at δ6.83 (J = 8 Hz) and 7.06 (J = 1.8 Hz) and a double doublet at δ6.92 (J = 8, 1.8 Hz), coupled with the presence

Table 1. ^{1}H NMR spectral data of isoflavones (90 MHz, $CDCl_3$)

H	1	2	3	4	12	14	15
2	7.88	7.95	8.02	7.84	7.80	7.80	7.81
5	7.87	7.53	8.15	—	8.01	7.50	—
6	6.81	—	7.00	—	6.85	—	—
2′	—	7.10	—	7.06	—	7.10	7.07
3′	—	—	6.5	—	—	—	—
5′	—	6.85	—	6.83	—	6.83	6.83
6′	6.40	6.97	6.95	6.92	6.50	6.93	6.93
1″	3.51	3.51	3.58	3.48	—	—	—
2″	5.18	5.25	5.20	5.22	—	—	—
3″	—	—	—	—	1.86	1.87	1.87
4″	—	—	—	—	2.88	2.82	2.83
Me	1.75	1.80	1.85	1.80	1.45	1.45	1.45
	1.60	1.68	1.70	1.68	1.45	1.45	1.45
OCH_2O	5.90	6.00	—	5.95	6.00	5.98	5.95
OMe	3.75	4.00	4.00	4.00	3.85	3.91	3.91
	3.70	—	3.95	3.89	3.80	—	3.81
			3.85				
			3.80				
OH		6.35					

J (Hz): J_{5-6} = 8.8; $J_{2'-6'}$ = 1.8; $J_{5'-6'}$ = 8.1; $J_{1''-2''}$ = 7.6 (**1**), 7.3 (**2**, **4**), 7.5 (**3**), 6.4 (**12**), 7.0 (**14**), 6.6 (**15**).

Table 2. ^{13}C NMR spectral data for isoflavones (22.6 MHz, $CDCl_3$)

C	3	4	6	7	9
2	154.4	150.1	151.6	153.8	151.6
3	121.3	124.7	126.2	121.8	125.8
4	176.1	174.6	175.2	175.5	175.2
4a	118.1	111.4	117.8	118.9	118.5
5	125.3	151.5	106.2	126.8	126.6
6	109.9	137.9	147.4[a]	115.1	113.9
7	155.3	149.4	147.7[a]	152.5	152.3
8	113.6	112.9	110.3	110.0	109.1
8a	161.1	152.1	—*	157.3	157.2
1′	119.2	125.8	125.5	113.4	124.7
2′	152.5	107.7	109.8	152.5	130.0
3′	99.9	147.2	147.7[a]	100.0	114.9
4′	150.4	147.2	147.4[a]	150.5	147.6
5′	143.4	109.7	108.3	143.9	114.9
6′	116.9	122.1	122.4	117.0	130.0
1″	22.3	22.1	—	—	—
2″	121.8	121.4	78.1	77.6	78.5
3″	132.2	131.7	115.2	115.1	113.9
4″	—	—	130.3	130.1	126.6
Me	29.6	25.2	28.1	28.1	28.1
	25.5	17.4	28.1	28.1	28.1
	57.5	—	—	—	—
OMe	57.2	61.4	56.5	57.1	55
	56.6	61.1	—	57.1	—
	56.3	—	—	56.5	—
$-OCH_2O-$	—	100.6	101.8	—	—

[a]Assignments interchangeable.
*Signal not observed.

in the mass spectrum of the RDA fragment **13** with *m/z* 146 allows placement of the methylenedioxy group on ring B. This assignment was unequivocally confirmed by transformation of **4** to **8** by means of cyclodehydrogenation with DDQ and also to the corresponding cyclic derivative **15** by treatment with 98% formic acid. Thus, compound **4** is 7-hydroxy-5,6-dimethoxy-3′,4′-methylenedioxy-8-(3,3-dimethylallyl)isoflavone.

EXPERIMENTAL

General. Mps: uncorr. Analytical TLC sepns were carried out on Merck pre-coated silica gel plates (F-254; layer thickness, 0.25 mm) using *n*-hexane–toluene–EtOAc (4:3:3). ^1H and ^{13}C NMR were measured at 90 and 22.6 MHz, respectively. UV spectra were recorded in MeOH solns.

Plant material. Root bark of *M. ferruginea* subsp. *darassana* was collected in December 1987 and the seeds in August 1988 from Aleta-Wondo, Sidamo Province. Seeds of *M. ferruginea* subsp. *ferruginea* were collected from the Blue Nile Gorge on the Gojam province side at an altitude of 2300 m in July 1988. Voucher specimens S-108 and Sebsebe 2381 representing subsp. *darassana* and subsp. *ferruginea*, respectively, are deposited at the National Herbarium, Addis Ababa University.

Isolation of compounds from seeds of M. ferruginea *subsp.* darassana. Ground seeds (1 kg) were percolated with EtOH. The extract was filtered and concd to give a dark yellowish syrup (22 g), which when applied on VLC and eluted successively with petrol, $CHCl_3$ and EtOAc yielded 5, 10 and 4 g, respectively, of crude frs. The petrol fr. contained fatty substances and was not examined further.

The $CHCl_3$ fr. was subjected to CC over silica gel and eluted with increasing polarities of petrol and EtOAc. Elution with 15% EtOAc yielded **9** (400 mg) and **5** (370 mg); with 20% EtOAc **6** (320 mg); with 30% EtOAc **2** (10 mg); with 35% EtOAc 12-hydroxyrotenone (20 mg) and tephrosin (50 mg); with 40% EtOAc **7** (210 mg). The EtOAc fr. was likewise subjected to CC. Elution with 20% EtOAc gave 15 mg of an unidentified compound while with 30% EtOAc **1** (20 mg) was obtained.

Isolation of compounds from seeds of M. ferruginea *subsp.* ferruginea. Ground seeds (800 g) were extracted with $CHCl_3$ under reflux (5 hr). The yellowish syrup (20 g) obtained after removal of solvent was chromatographed over silica gel (400 g) and eluted as above but combined frs were further purified by Sephadex LH-20 CC (MeOH–$CHCl_3$, 1:1). Elution with 15% EtOAc gave **9** (410 mg) and **5** (520 mg); with 20% EtOAc rotenone (820 mg) and **6** (200 mg); with 30% EtOAc **7** (100 mg); with 40% EtOAc **3** (30 mg).

Isolation of compounds from root bark of M. ferruginea *subsp.* darassana. Powdered root bark (1 kg) was percolated with EtOH at room temp. and concd to give 81 g of a yellowish syrup, 5 g of which was chromatographed over silica gel and eluted as above. Elution with 30% EtOAc afforded **4** (100 mg).

Preferrugone (**1**). Needles from petrol–$CHCl_3$, mp 160–162°. Found: $[M]^+$ *m/z* 410.1367; $C_{23}H_{22}O_7$ requires 410.1366. UV λ_{max} nm (log ε): 244 (4.03), 250 (4.04), 256 (4.01), 261 (3.72), 310 (3.60). IR ν_{max} cm^{-1}: 3250, 1650, 1620, 1530, 1460, 1310. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 410 $[M]^+$ (100), 380 (11), 379 (28), 350 (17), 349 (10), 335 (36), 323 (22), 208 (52), 207 (27), 206 (15), 205 (33), 204 (6), 203 (23), 190 (13), 187 (12), 149 (50), 137 (70).

Cyclization of **1** *to chroman* **12**. Compound **1** (6 mg) was mixed with 98% HCO_2H (1 ml) [9] and conc. H_2SO_4 (2 drops) and stirred at room temp. for 24 hr. The reaction mixt. was poured into H_2O, extracted with $CHCl_3$, concd *in vacuo* and purified by CC over silica gel to yield the chroman **12** (3 mg). Oil. ^1H NMR (see Table 1). MS *m/z* (rel. int.): 410 $[M]^+$ (23), 379 (3), 340 (4), 285 (5), 256 (10), 208 (80), 207 (21), 186 (10), 148 (28), 109 (57), 92 (93), 82 (60), 53 (100).

Cyclodehydrogenation [10] *of* **1** *to ferrugone* (**5**). A soln of **1** (6 mg) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (3 mg) in dry benzene (2 ml) was refluxed for 9 hr. The reaction mixt was filtered and concd. The residue after CC over silica gel (petrol–EtOAc, 4:1) yielded ferrugone, identical with an authentic sample (co-TLC, ^1H NMR).

Predurmillone (**2**). Needles from petrol–$CHCl_3$, mp 195–197°. Found: $[M]^+$ *m/z* 380.1260; $C_{22}H_{20}O_6$ requires 380.1260. UV λ_{max} nm (log ε): 244 (4.35), 249 (4.36), 256 (4.37), 262 (4.33), 300 (4.17), 321 (4.16). IR ν_{max} cm^{-1}: 3450, 2950, 1660, 1630, 1520, 1500, 1460, 1310, 1260, 1060, 960, 880, 840. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.): 380 $[M]^+$ (100), 365 (10), 326 (17), 325 (84), 324 (45), 175 (10), 146 (24).

Cyclization of **2** *to chroman* **14**. Compound **2** (4 mg) was treated as above with HCO_2H to yield **14** (2 mg). Oil. ^1H NMR (see Table 1). MS *m/z* (rel. int.): 380 $[M]^+$ (50), 325 (25).

Prebarbigerone (**3**). Needles from petrol–$CHCl_3$, mp 140–143°. Found: $[M]^+$ *m/z* 410.1728; $C_{24}H_{26}O_6$ requires 410.1729. UV λ_{max} nm (log ε): 294 (4.18), 242 (4.34). IR ν_{max} cm^{-1}: 2950, 1665, 1640, 1540, 1490, 1460, 1300, 1240, 1190, 1100, 1080, 840. ^1H NMR (see Table 1). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.): 410 $[M]^+$ (41), 379 (16), 342 (5), 217 (4), 191 (6), 72 (100).

Pre-5-methoxydurmillone (**4**). Oil, found: $[M]^+$ *m/z* 410.1346; $C_{23}H_{22}O_7$ requires 410.1364. UV λ_{max} nm (log ε): 294 (3.77), 262 (3.94), 256 (3.93), 250 (3.90). IR ν_{max} cm^{-1}: 3450, 2950, 1640, 1620, 1485, 1420, 1240, 1020. ^1H NMR (see Table 1). ^{13}C NMR (see

Table 2). EIMS *m/z* (rel. int.); 410 $[M]^+$ (67), 396 (26), 395 (83), 341 (24), 339 (100), 311 (25), 166 (31), 165 (33), 149 (45), 146 (14).

Cyclization of **4** *to chroman* **15**. Compound **4** (10 mg) was treated as above to yield **15** (8 mg). Oil. ^{1}H NMR (see Table 1). MS *m/z* (rel. int.): 410 $[M]^+$ (29), 396 (17), 395 (59), 339 (55), 311 (13), 190 (13), 149 (100), 133 (15), 111 (14).

Cyclodehydrogenation of **4** *to 5-methoxydurmillone* (**8**). Treatment of **4** (10 mg) as above with DDQ yielded 5-methoxydurmillone, identical (^{1}H NMR, co-TLC) with the authentic natural product.

Barbigerone (**7**). Needles from MeOH, mp 155–157° (lit. [11] 153–154°). Found: $[M]^+$ *m/z* 394.1420; $C_{23}H_{22}O_6$ requires 394.1416. IR ν_{max} cm^{-1}: 1660, 1600, 1540, 1480, 1420, 1300, 1230, 1185, 1050. ^{1}H NMR ($CDCl_3$) 7.95 (1H, *s*, H-2), 8.05 (1H, *d*, J = 8.3 Hz, H-5), 6.84 (1H, *d*, J = 8.3 Hz, H-6), 6.55 (1H, *s*, H-3′), 6.90 (1H, *s*, H-6′), 5.65 (1H, *d*, J = 10.8 Hz, H-3″), 6.8 (1H, *d*, J = 10.8 Hz, H-4″), 3.90, 3.80, 3.75 (3 × OMe), 1.5 (2″-Me_2). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.); 394 $[M]^+$ (100), 380 (20), 379 (77), 366 (37), 349 (19), 203 (10), 189 (29), 168 (23).

Calopogonium isoflavone A (**9**). Needles from MeOH, mp 138–141°. Found: $[M]^+$ *m/z* 334.1187; $C_{21}H_{18}O_4$ requires 334.1204. IR ν_{max} cm^{-1}: 1645, 1590, 1450, 1400, 1280, 1110, 1040. ^{1}H NMR ($CDCl_3$) 7.95 (1H, *s*, H-2), 8.10 (1H, *d*, J = 8.5 Hz, H-5), 6.90 (1H, *d*, J = 8.5 Hz, H-6), 7.65 (2H, *d*, J = 8.5 Hz, H-2′, H-6′), 7.00 (2H, *d*, J = 8.5 Hz, H-3′, H-5′), 5.65 (1H, J = 10.5, H-3″), 6.85 (1H, *d*, J = 10.5, H-4″), 3.85 (4′-OMe), 1.55 (2″-Me_2). ^{13}C NMR (see Table 2). EIMS *m/z* (rel. int.): 334 $[M]^+$ (32), 319 (100), 187 (21).

Acknowledgements—This work was supported by a research grant from SAREC Sweden. The authors thank Prof R. W. Rickards (Australian National University, Australia) for MS analysis.

REFERENCES

1. Thulin, M. (1983) *Opera Bot.* **68**, 71.
2. Teesdale, C. (1954) *East African Med. J.* **31**, 351.
3. Singhal, A. K., Sharma, R. P., Baruah, J. N., Govindan, S. V. and Herz, W. (1982) *Phytochemistry* **21**, 949.
4. Kokwaro, J. O. (1976) *Medicinal Plants of East Africa*. East Afr. Literature Bureau, Nairobi, Kenya.
5. Clark, E. P. (1943) *J. Am. Chem. Soc.* **65**, 27.
6. Highet, R. J. and Highet, P. F. (1967) *J. Org. Chem.* **32**, 1055.
7. Dagne, E., Bekele, A. and Waterman, P. G. (1989) *Phytochemistry* **28**, 1897.
8. Tahara, S., Ingham, J. L. and Mizutani, J. (1989) *Phytochemistry* **28**, 2079.
9. Mizuo, M., Tamura, K., Tanaka, T. and Iinuma, M. (1988) *Phytochemistry* **27**, 2975.
10. Miyase, T., Ueno, A., Noro, T. and Fukushima, S. (1980) *Chem. Pharm. Bull.* **28**, 1172.
11. Vilain, C. (1980) *Phytochemistry* **19**, 1988.