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A PYRANO-ISOFLAVONE FROM SEEDS OF *MILLETIA THONNINGII**

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Key Word Index—*Milletia thonningii*; Tephrosieae; Leguminosae; seed; isoflavones; 3′, 5-dihydroxy-4′-methoxy-2″, 2″-dimethylpyrano-(5″, 6″; 6, 7)-isoflavone; robustic acid.

Abstract—Extraction of the seeds of *Milletia thonningii* gave alpinumisoflavone, its monomethyl and dimethyl derivatives, robustic acid and a new pyrano-isoflavone. The structure of the latter was established by chemical transformation and spectroscopic means.

INTRODUCTION

During a systematic investigation on flavonoids and rotenoids of Tephrosieae (Leguminosae) we examined many species of the closely related genera *Lonchocarpus* and *Derris* [1, 2] and subsequently of the genus *Tephrosia* [3, 4]. We here report the results obtained with the seeds of *Milletia thonningii*, a decidous tree growing in the savanna of west tropical Africa [5].

Chemical studies on eight *Milletia* spp. have shown the presence of isoflavones and rotenoids, mainly in *M. auriculata, M. dura, M. ferruginea, M. pachycarpa* and *M. taiwaniana* [6–8], and flavones and flavanone-chalcones from *M. ovalifolia* and *M. stuhlmanii* [6, 9]. No flavonoids or rotenoids were detected by us in the seeds of *M. grandis* [2].

RESULTS AND DISCUSSION

The chloroform-soluble portion of the methanol extract of the seeds of *M. thonningii* on CC and prep. TLC gave four isoflavones and one 3-aryl-4-hydroxycoumarin.

Spectral data of the major component suggested that it was an isoflavone with a *para*-substituted B ring, and a chelated hydroxyl and a dimethylpyran in the A ring (see Experimental). Methylation with diazomethane gave a dimethyl- and a monomethyl derivative, the latter containing the chelated hydroxyl. Spectral data and mps were in close agreement with those previously reported [10] for alpinumisoflavone, **1**, a constituent of *Laburnum alpinum*, and its dimethyl (**2**) and monomethyl derivative (**3**).

Both the second major and the minor components of *M. thonningii*, were also found to be isoflavones and shown to be identical by direct comparison with dimethylalpinumisoflavone (**2**) and 4′-methyl-alpinumisoflavone (**3**), respectively. This is the first report of **3** as a natural product, while dimethyl-alpinumisoflavone was recently isolated from the seeds of *Derris robusta* [11].

The fourth compound was a new isoflavone (**5**) with the same substitution pattern in the A ring as alpinumisoflavone, but with a methoxy group in the B ring. This was indicated by the ^{1}H NMR (see Experimental) and mass spectra (M^+ 366), where the appropriate shift of the fragment ion (*m/z* 149) arising from the B ring was observed [10]. The linear fusion of the dimethylpyran ring, suggested by a slow change of the maximum in the UV spectrum on addition of aluminium chloride, was corroborated by the downfield shift ($\Delta\delta + 0.17$) of the H-4″ signal in deuteropyridine [12]. Confirmation of this was obtained by the preparation of the diacetylderivative (**6**), in which the acetylation of the 5-hydroxyl caused the expected [13] diamagnetic shift ($\Delta\delta - 0.20$) of the H-4″ signal in the NMR spectrum. The substitution in the B ring cannot be inferred from the complicated pattern in the aromatic region of the ^{1}H NMR spectrum ($CDCl_3$) of **5**, but in that of **6** the three aromatic protons appeared as *ortho, ortho–meta* and *meta* coupled, respectively. This is possible only for 2′–4′, 2′–5′ or 3′–4′ oxygenation patterns, but the first two were ruled out because the mass spectrum fragmentation lacked the characteristic ions of 2′-substituted isoflavones [14]. Finally, a 3′-hydroxy-4′-methoxy distribution of the substituents was preferred because of the higher paramagnetic shifts on acetylation of H-2′ and H-6′ (*ortho* and *para* to the

*Part 12 in the series "Tephrosieae". For Part 11 see ref. [2].

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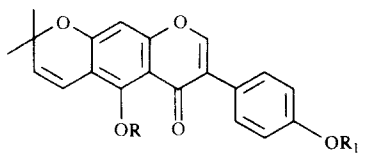
hydroxyl, respectively) with respect to that of H-5'. The chemical shift in deuteropyridine of H-2' (in **5**) is in agreement with this assumption.

The last compound isolated from *M. thonningii* gave UV (λ_{max} 254, 337 nm) and IR spectra (ν_{max} 1705 cm^{-1}) compatible with a 3-aryl-4-hydroxycoumarin. The ^1H NMR spectrum gave signals corresponding to a dimethylpyran, two methoxyls, an isolated aromatic proton and a *para*-disubstituted aromatic ring. The presence of a free hydroxyl suggested by the IR spectrum was confirmed by the preparation of a monoacetyl- and a monomethyl derivative. These data and mass fragmentation [15], as well as the mps of the compound and its derivatives, allowed its identification as robustic acid **7**, previously reported [15, 16] from *Derris robusta*. It should be noted that dimethylalpinumisoflavone (**2**) is the isoflavone equivalent to robustic acid (**7**) and that these two classes of natural product frequently occur together.

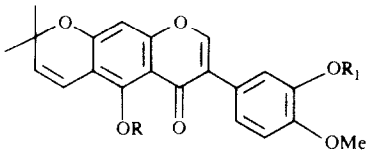
EXPERIMENTAL

Plant material. Seeds of *M. thonningii* (Schumach-Thonn.) Bak. from west Africa were supplied by the Royal Botanic Gardens, Kew, and authenticated by the Professor A. Polhill Herbarium.

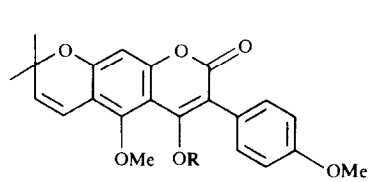
Extraction and fractionation. Seeds (29 g) were powdered and continously extracted with hot MeOH and the extract evapd. The residue was dissolved in $CHCl_3$–H_2O and the organic layer separated and evapd (4 g). The crude oily residue was roughly separated on Si gel into five fractions: F_1 (C_6H_6; 1.9 g triacylglycerols), F_2, F_3, F_4 (C_6H_6–EtOAc, 97:3; 90 mg, 250 mg and 1.2 g, respectively) and F_5 (C_6H_5–EtOAc, 9:1; 300 mg, not examined further). Extended CC of F_2 gave 4'-methylalpinumisoflavone (40 mg). Crystallization (C_6H_6) of F_3 afforded 3',5 dihydroxy-4'-methoxy-2'',2''-dimethylpyrano-(5'', 6''; 6, 7)-isoflavone (125 mg). Extended CC of F_4 gave alpinumisoflavone (745 mg) and a mixture of two spots, which were separated by prep. TLC ($CHCl_3$, 3 developments) yielding robustic acid (96 mg) and dimethylalpinumisoflavone (143 mg).



	R	R_1
1	H	H
2	Me	Me
3	H	Me
4	Ac	Me



	R	R_1
5	H	H
6	Ac	Ac



	R
7	H
8	Ac
9	Me

Alpinum isoflavone (**1**). Mp 210–212° (Me_2CO–hexane) (lit. [10] mp 213–214°). UV λ_{max}^{MeOH} 284 (4.77) nm; λ_{max}^{NaOAc} 284 nm; $\lambda_{max}^{AlCl_3}$ 299 nm after 30 min. IR $\nu_{max}^{CHCl_3}$ 3570, 3300, 1650 cm^{-1}. ^1H NMR (60 MHz; $CDCl_3$–CD_3OD, 9:1): δ 13.20 (0.5 H, *s*), 7.72 (1H, *s*, H-2), 7.25 (2H, *d*, J = 8.5 Hz, H-2', H-6'), 6.80 (2H, *d*, J = 8.5 Hz, H-3', H-5'), 6.60 (1H, *d*, J = 10 Hz, H-4''), 6.23 (1H, *s*, H-8), 5.53 (1H, *d*, J = 10 Hz, H-3''), 1.40 (6H, *s*). Methylation (CH_2N_2) gave a monomethyl derivative, (**3**) and a dimethyl derivative (**2**) identical (mmp, TLC and ^1H NMR) to the natural products.

Dimethylalpinumisoflavone (**2**). Mp 119–121° (C_6H_6) (lit. [10, 11], mp 119–120°). ^1H NMR (60 MHz, $CDCl_3$): δ 7.60 (1H, *s*, H-2), 7.30 (2H, *d*, J = 8.5 Hz, H-2', H-6'), 6.78 (2H, *d*, J = 8.5 Hz, H-3', H-5'), 6.63 (1H, *d*, J = 10 Hz, H-4''), 6.40 (1H, *s*, H-8), 5.53 (1H, *d*, J = 10 Hz, H-3''), 3.80 (3H, *s*), 3.73 (3H, *s*), 1.40 (6H, *s*).

4'-Methylalpinumisoflavone (**3**). Mp and mmp 137–138° (C_6H_6) (lit. [10] mp 136–137°). ^1H NMR (60 MHz, $CDCl_3$): δ 13.20 (1H, *s*), 7.77 (1H, *s*, H-2), 7.40 (2H, *d*, J = 8.5 Hz, H-2', H-6'), 6.92 (2H, *d*, J = 8.5 Hz, H-3', H-5'), 6.67 (1H, *d*, J = 10 Hz, H-4''), 6.27 (1H, *s*, H-8), 5.56 (1H, *d*, J = 10 Hz, H-3''), 3.80 (3H, *s*), 1.43 (6H, *s*). OMe, $\Delta\delta$ ($CDCl_3$–C_6D_6), +0.5. H-4'', $\Delta\delta$ (C_5D_5N–$CDCl_3$), +0.16. Acetylation (Ac_2O–NaOAc) gave 5-acetyl-4'-methylalpinumisoflavone (**4**), mp 180–181° (C_7H_{16}) (lit. [10] mp 179–181°). ^1H NMR (60 MHz, $CDCl_3$): δ 7.70 (1H, *s*), 7.33 (2H, *d*, J = 8.5 Hz), 6.87 (2H, *d*, J = 8.5 Hz), 6.63 (1H, *s*, H-8), 6.43 (1H, *d*, J = 10 Hz, H-4''), 5.7 (1H, *d*, J = 10 Hz, H-3''), 3.78 (3H, *s*), 2.40 (3H, *s*), 1.46 (6H, *s*). $\Delta\delta$ (**4**–**3**) H-4'', −0.24; H-3'', +0.14; H-8, +0.38.

3', 5-Dihydroxy-4'-methoxy-2'', 2''-dimethylpyrano-(5'', 6''; 6, 7)-isoflavone (**5**). Mp 155–156° (C_6H_6), yellow plates. UV λ_{max}^{MeOH} 285 (4.62) nm; λ_{max}^{NaOAc} 284 nm; $\lambda_{max}^{AlCl_3}$ 303 nm after 20 min. IR $\nu_{max}^{CHCl_3}$ 3550, 1660, 1630 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 13.0 (1H, *s*), 7.70 (1H, *s*, H-2), 7.03–6.85 (3H, *m*; H-2', H-5', H-6'), 6.63 (1H, *d*, J = 10 Hz, H-4''), 6.22 (1H, *s*, H-8), 5.75 (1H, *br s*, OH, exchangeable with D_2O), 5.53 (1H, *d*, J = 10 Hz, H-3''), 3.80 (3H, *s*), 1.42 (6H, *s*): (C_5D_5N) 7.55 (1H, *d*, J = 2 Hz, H-2'), 6.80 (1H, *d*, J = 10 Hz, H-4''). H-4'', $\Delta\delta$ (C_5D_5N–$CDCl_3$) +0.17. EI-MS (probe) 70 eV, *m/z* (rel. int.): 366 $[M]^+$ (40), 365 (9), 351 $[M-15]^+$ (100), 336 $[351-15]^+$ (26), 247 (18), 233 (4), 204 (3), 203 (RDA of $[M-15]^+$) (5), 175.5 $[M-15]^{2+}$ (27), 168 (7), 154 (18), 149(B ring) (4), 140 (3), 135 (6), 105 (4). Acetylation (Ac_2O–NaOAc) gave 3', 5-diacetoxy-4'-methoxy-2'', 2''-dimethyl-pyrano-(5'', 6''; 6, 7)-isoflavone (**6**), mp 206–208° (CH_2Cl_2–C_7H_{16}), white needles. ^1H NMR (60 MHz, $CDCl_3$): δ 7.70 (1H, *s*, H-2), 7.27 (1H, *dd*, J = 2 and 8.5 Hz, H-6'), 7.13 (1H, *d*, J = 2 Hz, H-2'), 6.91 (1H, *d*, J = 8.5 Hz, H-5'), 6.63 (1H, *s*, H-8), 6.43 (1H, *d*, J = 10 Hz, H-4''), 5.70 (1H, *d*, J = 10 Hz, H-3''), 3.77 (3H, *s*), 2.40 (3H, *s*), 2.27 (3H, *s*), 1.43 (6H, *s*). $\Delta\delta$ (**6**–**5**) H_α, −0.20; H_β, +0.17; H-8, +0.41.

Robustic acid (**7**). Mp 202–205° (C_6H_6) (lit. [15], mp 208–210°; lit. [16], mp 208–209°). UV λ_{max}^{MeOH} 233, 256, 338 nm; λ_{max}^{NaOAc} and λ_{max}^{NaOMe} 248, 277, 330 nm. IR $\nu_{max}^{CHCl_3}$ 3520, 3280, 1705, 1630, 1610 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 7.42 (2H, *d*, J = 8.5 Hz, H-2', H-6'), 6.90 (2H, *d*, J = 8.5 Hz, H-3', H-5'), 6.57 (1H, *s*, H-8), 6.45 (1H, *d*, J = 10 Hz, H-4''), 5.70 (1H, *d*, J = 10 Hz, H-3''), 3.96 (3H, *s*), 3.77 (3H, *s*), 1.44 (6H, *s*). EI-MS (probe) 70 eV, *m/z* (rel. int.): 380 $[M]^+$ (100), 365 $[M-15]^+$ (92), 337 (18), 321 (8), 307 (6), 233 (30), 232 (15), 217 (67), 203 (14), 189 (4), 175 (11), 161 (15), 148 (21), 135 (8), 133 (4). Acetylation (Ac_2O–pyridine) gave 4'-acetylrobustic

acid (**8**), mp 194–196° (CH_2Cl_2–C_7H_{16}) (lit. [16] 198–199°). ^1H NMR (60 MHz, $CDCl_3$): δ 7.35 (2H, *d*, J = 8 Hz, H-2′, H-6′), 6.90 (2H, *d*, J = 8 Hz, H-3′, H-5′), 6.60 (1H, *s*, H-8), 6.53 (1H, *d*, J = 10 Hz, H-4″), 5.70 (1H, *d*, J = 10 Hz, H-3″), 3.80 (3H, *s*), 3.76 (3H, *s*), 2.13 (3H, *s*), 1.44 (6H, *s*). Methylation of **7** with CH_2N_2 gave the corresponding methyl ether (robustic acid methyl ether) **9**, mp 194–195° (EtOH) (lit. [15], mp 198–200°; lit. [16], mp 195–196°). ^1H NMR (60 MHz, $CDCl_3$): δ 7.36 (2H, *d*, J = 8.5 Hz, H-2′, H-6′) 6.93 (2H, *d*, J = 8.5 Hz, H-3′, H-5′), 6.57 (1H, *d*, J = 10 Hz, H-4″), 6.53 (1H, *s*, H-8), 5.67 (1H, *d*, J = 10 Hz, H-3″), 3.83 (3H, *s*), 3.80 (3H, *s*), 3.53 (3H, *s*), 1.46 (6H, *s*).

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