# THONNINGINE-A AND THONNINGINE-B: TWO 3-PHENYLCOUMARINS FROM THE SEEDS OF MILLETTIA THONNINGII

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Key Word Index—Millettia thonningii; Leguminosae; isoflavones; 3-phenylcoumarins; robustic acid; thonningine-A; thonningine-B.

**Abstract**—The seeds of *Millettia thonningii* have yielded four isoflavones and three 4-hydroxy-3-phenylcoumarins, of which two are novel. On the basis of spectral analyses the novel compounds have been identified as 4-hydroxy-5,8-dimethoxy-3-(3',4'-methylenedioxy)phenyl-2"-isopropenylfurano (4",5": 6,7)coumarin (thonningine-A) and 4-hydroxy-5,8-dimethoxy-3-(4'-methoxy)phenyl-2"-isopropenylfurano(4", 5": 6,7)coumarin (thonningine-B).

## INTRODUCTION

Millettis thonningii (Schum. et Thonn.) Bak. is a deciduous tree found in moist situations in savanna areas of west Africa [1]. Previous investigations of the seeds have yielded the amino acid canavanine [2], the isoflavones 1-4 and the 3-phenylcoumarin robustic acid (5) [3]. Two flavonols are also recorded as present [4] but this is yet to be confirmed.

In this paper we report the results of an examination of mature seeds of M. thonningii collected in Ghana. From this material, which was unfortunately received unvouchered, seven flavonoids have been characterized; isoflavones 1–3, 5, another isoflavone, robustone (6) and the novel 3-phenylcoumarins thonningine-A (7) and thonningine-B (8). The authenticity of the material has subsequently been confirmed by comparison with a second sample of seeds collected in the Shai Hills, Ghana, and vouchered by living material.



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### RESULTS AND DISCUSSION

Concentration of an ethyl acetate extract of defatted seeds gave a precipitate (MT-1). CC of the supernatant over Si gel gave three bands. Recrystallization of the material of the first band gave MT-2 and mother liquors containing a mixture of MT-2 and MT-2A. Preparative TLC of the second band gave MT-3 and MT-4 and similar treatment of the third band gave MT-5 and a small amount of another compound (MT-5A) still contaminated with MT-5.

Compounds MT-3 and MT-4 were characterized as alpinum isoflavone (1) and its dimethyl ether (2) by interconversion and comparison of spectral and physical data with those published [3]. Compound MT-2 analysed for C<sub>21</sub>H<sub>16</sub>O<sub>6</sub> and exhibited UV and IR spectra consistent with a 5-hydroxyisoflavone [5]. The  $^{1}HNMR$  spectrum showed signals for rings A and C of an isoflavone substituted as in 1. The five remaining protons occurred as a 2H singlet at  $\delta 6.00$  for a methylenedioxy substituent and a 3H multiplet in the aromatic region for an AA'B system; collectively indicating a piperonyl B-ring (confirmed by EIMS, m/z 146). These data are compatible with the known isoflavone robustone (6), previously recorded from Derris robusta (Roxb. ex DC.) Benth. [6]. The possibility of the alternate 7,8pyrano compound was precluded by preparation of the acetate of 6, the <sup>1</sup>H NMR spectrum of which exhibited the anticipated shifts for H-4" and H-3" peri to the acetyl [7]. The minor component MT-2A could only be obtained in a mixture with 6 and its identification as 4'-methylalpinum isoflavone (3) is based on comparison of spectra of the mixtures with those of pure 6. Spectral data for 3 complied closely with that recently published [3], placement of the methoxyl at C-4' being confirmed by EIMS (m/z 132).

Compound MT-1 gave spectral data typical of a 3phenylcoumarin [6, 8] and which agreed closely with that reported for robustic acid (5) [3, 6]. The previously unreported <sup>13</sup>C NMR spectrum showed the C-5 methoxyl signal to be deshielded at  $\delta 64.6$ , thus requiring C-6 to be substituted and confirming the linear position of the pyran ring [9].

Compound MT-5 was obtained only in an amorphous form. It analysed for  $C_{23}H_{18}O_8$  and gave UV and IR spectra similar to those of 5. The <sup>1</sup>H NMR spectrum revealed the presence of two methoxyl groups ( $\delta$ 3.94 and 4.26) and the characteristic OH-4 resonance at  $\delta$  10.43. A 2H singlet at  $\delta 6.00$  for a methylenedioxy substituent, together with three aromatic protons in a pattern identical to that seen in 6, indicated a piperonyl B-ring and this was confirmed by the ion m/z 162 (9) in the EIMS. The remaining six protons were observed as a sharp singlet (1H) at  $\delta$  7.23, two broad singlets (1H each) at  $\delta$  5.25 and 5.79, the former showing long range coupling to a broad singlet (3H) at  $\delta$  2.24. This pattern agrees closely with that reported for isopropenylfuran ring systems in the coumarins hortiolone [10], and arnocoumarin and arnottiacoumarin [11].

On the basis of these data MT-5 must be a 4-hydroxy-3-(3',4'-methylenedioxy)phenylcoumarin substituted ring-A with two methoxyl groups and an isopropenylfuran moiety. This was confirmed by the EIMS which showed, in addition to 9, a base peak at m/z 260, attributable to 10 or an isomer thereof. The positions of substituents on ring-A were established from the <sup>13</sup>C NMR spectrum. Resonances for C-2--C-4 and C-4a were in close agreement with those observed for 6 and those for the B-ring with reported values for 3',4'-substituted Brings in isoflavones [12]. In comparison with 6 the signals for C-7 and C-8a had undergone shielding of between  $\delta 6$ 











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and 10; this being caused by oxygenation at C-8. The C-8 signal was observed at  $\delta$ 132.0, strongly shielded by the effects of two ortho oxygens. Resonance values for C-7, C-8 and C-8a showed close agreement with those observed for the comparable positions in xanthotoxin (11) [13]. Signals at  $\delta$ 160.7 and 118.6 can be assigned to C-5 and C-6, respectively. The alternative 5,6,7-oxygenation pattern can be ruled out as it would require the  $\delta$  160.7 resonance to be assigned to C-8a; this is well in excess of recorded values for this position in coumarins [13, 14]. For the isopropenylfuran system a triplet at  $\delta 115.3$  and a singlet at  $\delta$  137.4, together with a quartet at  $\delta$  19.2, conform to published data [15] for isopropenyl substituents of rotenoids. The two remaining signals, a singlet at  $\delta$ 151.0 and a doublet at  $\delta$ 97.2, are compatible with anticipated values for C-2" and C-3" of a 2"-substituted furan [13, 15].

On the basis of the above information MT-5 can be characterized 4-hydroxy-5,8-dimethoxy-3-(3',4'as methylenedioxy)phenyl-2"-isopropenylfuran-(4",5": 6,7)coumarin (7). This compound, which appears to be novel, has been given the trivial name thonningine-A.

The supernatant liquors, from which 7 was obtained, contained a mixture of 7 and a second compound, MT-5A. Comparison of the EIMS of 7 and the mixture indicated that the minor component was  $C_{23}H_{20}O_7$  and that on RDA cleavage it gave ions 10 and 12, suggesting that it differed from 7 only in B-ring substitution. This was confirmed by the <sup>1</sup>H NMR spectrum of the mixture which was identical to that of 7 except for the presence of an additional methoxyl resonance ( $\delta$  3.84) and an AA'BB' system centred at  $\delta$  6.90 and 7.53. The <sup>13</sup>C NMR spectrum of the mixture also showed resonances compatible with the presence of a *para*-methoxylated B-ring (cf. 2). In light of the established occurrence of 7 the minor component of the mixture can clearly be characterized as the corresponding 4'-methoxyl compound, 8, which is assigned the trivial name thonningine-B.

Within Millettia there is now some information on the flavonoids of eight species [3, 4]. It is interesting to note that in only two of these species, M. thonningii and M. auriculata Bak. ex Brand. does the 5-hydroxy group of the flavonoid appear to be retained. A similar division between species producing 5-hydroxyflavonoids and species where that substituent has been lost is noted in Tephrosia [16]. The relationship between M. thonningii and M. auriculata is extended to the occurrence of 8oxygenation, at present unknown from other Millettia species.

#### EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in EtOH and IR spectra as KCl discs. NMR spectra were run in CDCl<sub>3</sub> using TMS as int. standard. EIMS were determined at 70 eV using probe insertion.

Plant material. The major sample of Millettia thonningii seeds was supplied by Dr. L. E. Fellows, Royal Botanic Gardens, Kew, from a collection made in Ghana. A second sample of seeds, collected in the Shai Hills of Ghana, was supplied by Dr. M. D. Swaine, University of Aberdeen, Plants from the seeds collected by Dr. Swaine are growing in the greenhouses of the Botany Department, University of Aberdeen. The authenticity of the seed samples has been confirmed by Dr. R. M. Polhill, Royal Botanic Gardens Kew

Extraction and isolation of compounds. The ground seeds (270 g) were defatted with petrol (bp 40-60°) and then extracted with EtOAc. On concn the EtOAc extract gave a yellow oil (23 g) which, left overnight, yielded MT-1 (392 mg). The supernatant was subjected to CC over Si gel. Elution with petrol (bp 60–80°) followed by petrol containing increasing amounts of EtOAc gave: from 15% EtOAc, MT-2 and MT-2A as a mixture (340 mg); from 20% EtOAc, MT-3 and MT-4 as a mixture (3 g); from 30% EtOAc, MT-5 and MT-5A as a mixture (38 mg). Each mixture was subjected to prep. TLC on Si gel (solvent: CHCl<sub>3</sub>–MeOH, 99:1). This procedure yielded pure MT-2 ( $R_f$  0.76; 163 mg), MT-3 ( $R_f$  0.10; 210 mg), MT-4 ( $R_f$  0.27; 1.12 g) and MT-5 ( $R_f$  0.54; 22 mg).

*Robustic acid* (5). MT-1 gave needles from Me<sub>2</sub>CO, mp 206-210° (lit. [17] 208-210°). Found: M<sup>+</sup> 380.1261; C<sub>22</sub>H<sub>20</sub>O<sub>6</sub> requires 380.1260. UV, IR, <sup>1</sup>H NMR, EIMS in agreement with lit. [3]. <sup>13</sup>C NMR (25.1 MHz):  $\delta$ 28.1 (q, Me<sub>2</sub>-2″), 55.4 (q, OMe-4'), 64.6 (q, OMe-5), 77.8 (s, C-2″), 101.9 (d, C-8), 102.2, 104.2 (2 × s, C-3 and C-4a), 111.0 (s, C-6), 113.9 (d, C-3′, C-5′), 115.4 (d, C-3″), 123.8 (s, C-1′), 131.8 (d, C-4″), 132.2 (d, C-2′, C-6′), 154.0, 154.3 (2 × s, C-5 and C-8a), 157.6, 159.4 (2 × s, C-4′ and C-7), 160.6 (s, C-2), 162.9 (s, C-4). Synthesis of 4′-methyl ether and 4′-acetyl robustic acid gave material with physical and spectral characteristics in close agreement with published data [3].

*Robustone* (6). MT-2 gave needles from EtOH, mp 170–172° (lit. [6] 170–172°). Found: M<sup>+</sup> 364.0998; C<sub>21</sub>H<sub>16</sub>O<sub>6</sub> requires 364.0947. UV  $\lambda_{max}$  nm: 228, 281, 350, IR  $\nu_{max}$  cm<sup>-1</sup>: 1660. <sup>1</sup>H NMR (90 MH2): δ1.47 (6H, s, Me<sub>2</sub>-2″), 5.63, 6.72 (2H, ABq, J = 10 Hz, H-3″, H-4″), 6.00 (2H, s, OMeO-3′, OMeO-4′), 6.33 (1H, s, H-8), 6.95 (3H, m, H-2′, H-5′ and H-6′), 7.81 (1H, s, H-2), 13.19 (1H, s, replaceable by D<sub>2</sub>O, OH-5). EIMS m/z (rel. int.): 364 [M]<sup>+</sup> (56), 349 [M – Me]<sup>+</sup> (100), 203 (3), 146 (6). Acetylation of **6** (40 mg) followed by normal work-up gave robustone acetate (32 mg), mp 175–180°. Found: M<sup>+</sup> 406.1044; C<sub>23</sub>H<sub>18</sub>O<sub>7</sub> requires 406.1052. IR  $\nu_{max}$  cm<sup>-1</sup>: 1760, 1640, 1610. <sup>1</sup>H NMR (90 MHz): δ2.43 (3H, s, OAc-5).

4'-Methylalpinumisoflavone (3). MT-2A was obtained only in a mixture with 6. After elimination of signals attributable to  $6^{1}$ H NMR and EIMS spectra showed close agreement with those published for 3 and allied compounds [3].

Alpinum isoflavone (1). MT-3 gave pale yellow needles from  $Me_2CO$ -hexane, mp 210-212° (lit. [3] 210-212°). Found: M<sup>+</sup> 336.0960;  $C_{20}H_{16}O_5$  requires 336.0998. UV, IR, <sup>1</sup>H NMR, EIMS in close agreement with published data [3, 17]. Acetylation of 1 gave two products characterized as the 4'-acetate (mp 207° from CHCl<sub>3</sub>) and the 5,4'-diacetate (mp 210° from CHCl<sub>3</sub>). Methylation of 1 with CH<sub>2</sub>N<sub>2</sub> gave a product identical in all respects to 2.

Dimethylalpinum isoflavone (2). MT-4 gave needles from CHCl<sub>3</sub>-petrol (bp 40-60°), mp 120-121° (lit. [3] 119-121°). Found: M<sup>+</sup> 364.1255;  $C_{22}H_{20}O_5$  requires 364.1311. UV, IR, <sup>1</sup>H NMR, EIMS in close agreement with published data [3, 18]. <sup>13</sup>C NMR (25.1 MHz):  $\delta$  28.5 (q, Me<sub>2</sub>-2″), 55.5 (q, OMe-4′), 63.0 (q, OMe-5), 77.9 (s, C-2″), 101.0 (d, C-8), 113.6 (s, C-4a, C-6), 114.2 (d, C-3′, C-5′), 116.5 (d, C-3″), 124.7, 126.0 (2 × s, C-3 and C-1′), 130.7 (d, C-2′, C-6′), 131.1 (d, C-4″), 150.8 (d, C-2), 156.3 (s, C-8a), 158.5, 159.2 (2 × s, C-5 and C-4′), 160.0 (s, C-7), 175.5 (s, C-4).

*Thonningine-A* (7). MT-5 gave an amorphous solid from  $Me_2CO$ ,  $mp 205-208^{\circ}$ . Found:  $M^+ 422.0990$ ;  $C_{23}H_{18}O_8$  requires 422.1002. UV  $\lambda_{max}$  nm: 218, 270sh, 280, 291, 305sh, 350. IR  $\nu_{max}$  cm<sup>-1</sup>: 3250, 1710, 1640. <sup>1</sup>H NMR (90 MHz):  $\delta 2.24$  (3H, d, J = 1 Hz, Me-1'''), 3.94, 4.26 (2 × 3H, 2 × s, OMe-5, OMe-8), 5.25 (1H, q, J = 1 Hz, H-2'''), 5.79 (1H, br s, H-2'''), 6.00 (2H, s, OMeO-3', OMeO-4'), 7.01-7.09 (3H, m, H-2', H-5', H-6'), 7.23 (1H, s, H-3''), 10.43 (1H, s, replaceable by D<sub>2</sub>O, OH-4). <sup>13</sup>C NMR (90.56 MHz):  $\delta 19.2$  (q, Me-1'''), 62.6, 65.2 (2 × q, OMe-5 and OMe-8), 97.2 (d, C-3''), 100.9 (t, OMeO-3', OMeO-4'), 103.9, 104.9 (2 × s, C-3 and C-4a), 108.0 (d, C-2'), 111.1 (d, C-5'), 113.6 (s, C-6), 115.3

(t, C-2"), 124.3 (d, C-6'), 124.5 (s, C-1'), 132.0 (s, C-8), 137.4 (s, C-1"), 145.7, 147.1, 147.3, 148.2 ( $4 \times s$ , C-7, C-8a, C-3' and C-4'), 151.0 (s, C-2"), 160.2, 160.3, 160.7 ( $3 \times s$ , C-2, C-4, C-5). EIMS *m/z* (rel. int.): 422 [M]<sup>+</sup> (81), 260 [M - C<sub>9</sub>H<sub>6</sub>O<sub>3</sub>]<sup>+</sup> (100), 245 (38), 162 (3).

Thonningine-B (8). MT-5A was obtained only as a mixture with 7. Found: M<sup>+</sup> 408.1170;  $C_{23}H_{20}O_7$  requires 408.1209. UV and IR spectra were not separable from those of 7. <sup>1</sup>H NMR (90 MHz): signals at  $\delta$ 2.24, 3.94, 4.26, 5.25, 5.79 as 7; 3.84 (*s*, OMe-4'), 6.90, 7.53 (AA'BB', H-3', H-5' and H-2', H-6'), 10.39 (*s*, replaceable by D<sub>2</sub>O, OH-4). <sup>13</sup>C NMR (90.56 MHz): signals at  $\delta$ 19.2, 62.6, 65.2, 97.2, 103.9, 104.9, 113.6, 115.3, 124.5, 132.0, 137.4, 145.7, 147.1, 160.2, 160.3, 160.7 as 7; 55.2 (*q*, OMe-4'), 113.6 (*d*, C-3', C-5'), 131.7 (*d*, C-2', C-6'), 154.4 (*s*, C-4'). EIMS *m*/*z* (rel. int.): 408 [M]<sup>+</sup> (10), 260 (100), 148 (2).

Acknowledgements—We extend our thanks to Dr. L. E. Fellows, Jodrell Laboratory, Royal Botanic Gardens, Kew, for the original supply of plant material and Dr. M. D. Swaine, Department of Biological Sciences, University of Aberdeen, for a second sample of authenticated seeds. Dr. P. Bladon, Department of Chemistry, University of Strathclyde, and Dr. I. Sadler, Department of Chemistry, University of Edinburgh, supplied, respectively, 25.1 MHz and 90.56 MHz <sup>13</sup>C NMR spectra.

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