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PHYTOCHEMISTRY

Isoflavones and coumarins from *Milletia thonningii*

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Abstract

From the root wood and the pods of *Milletia thonningii* six isoflavones and a novel coumarin were isolated. They are alpiniumisoflavone and the 5-*O*-methyl, *O*,*O*-dimethyl, 3′-hydroxy-4′-*O*-methyl and the 5-*O*-methyl-4′-*O*-(3-methyl-2-butenyl) derivatives, together with robustone and the new coumarin, thonningine-C. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Milletia thonningii*; Leguminosae; Isoflavones; Coumarins; 5-*O*-methylalpinumisoflavone; Thonningine-C

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1. Introduction

Milletia thonningii (Schurn and Tonn) Baker is a tree indigenous in West and Central Africa. In traditional medicine preparations of the plant are used against various diseases (Irvine, 1961; Abbiw, 1990). A more recent report (Abbiw, 1990), that the juice from the leaves can kill the *Bilinus* snail, the vector for schistosomiasis, has heightened our interest in this plant. In earlier studies from the seeds three coumarins, thonningine A (**1**), thonningine B (**2**) and robustic acid (**3**), as well as the isoflavones **4**–**8** have been isolated (Martinez Olivares, Lwande, Delle Monache, & Marini Bettolo, 1982; Khalid & Waterman, 1983). As previously reported, the petrol extract of the root bark afforded the isoflavones **7**–**10** together with β-amyrin (Asomaning, Amoako, Oppong, Phillips, Addae-Mensah, Osei-Twum, Waibel, & Achenbach, 1995). In continuation of our work on the constituents of *M. thonningii* we have now investigated the root wood and the mature pods of the plant.

2. Results and discussion

Chromatographic separation of the petrol extract of the root wood of *M. thonningii* resulted in the isolation of the isoflavones **4**, **7**–**9** and **11**. Compounds **4** and **7**–**9** were identified by comparison with authentic samples (Asomaning et al., 1995), whilst **11** was determined by comparison of its spectral properties with literature data (Tsukayama, Kawamura, & Tahara, 1992). This latter compound has only been reported as a synthetic product, but hitherto has not been isolated from a natural source. Separation of the petrol extract of the pods of the plant by chromatography on silica gel afforded the known isoflavones **5** and **7**, whereas chromatographic separation of the $CHCl_3$ extract of the pods yielded the new coumarin (**12**), being named thonningine-C.

Compound **12** exhibited ^1H and ^{13}C NMR data very similar to those reported for thonningine-A (**1**) and thonningine-B (**2**), respectively (Khalid & Waterman, 1983) (Tables 1 and 2), suggesting the compound might be 5-hydroxy-4-9-dimethoxy-6-(3,4-dimethoxyphenyl)-2-(1-methylethenyl)-7H-furano[3,2-g] chromen-7-one(=3′-methoxythonningine-B) with a fully substituted ring A. However, HMQC and HMBC experiments gave evidence for a hydrogen sub-stituted ring A but due to the crowding of quat-ernary carbons we did not succeed to derive the complete structure from the heteronuclear correlation experiments run with **12**. Therefore the compound was hydrogenated to yield a mixture of **13** (main product) and **14** which was separ-

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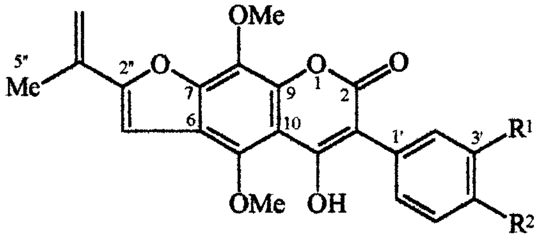
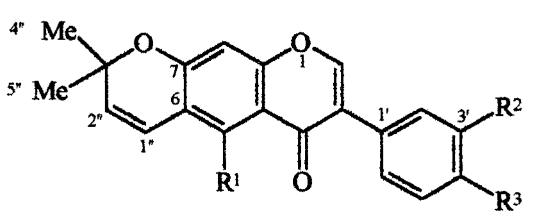
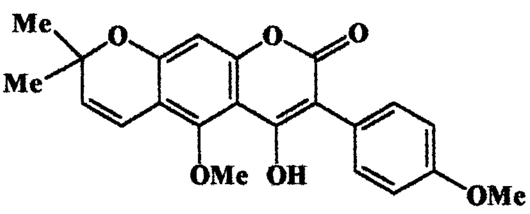


1 $R^1 + R^2 = OCH_2O$

2 $R^1 = H$, $R^2 = OMe$



	R^1	R^2	R^3
4	OH	H	OH
5	OH	OH	OMe
6	OH	H	OMe
7	OMe	H	OMe
8	OH	OCH_2O	
9	OMe	H	$OCH_2CH{=}C(CH_3)_2$
11	OMe	H	OH



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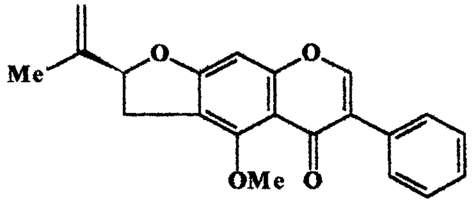

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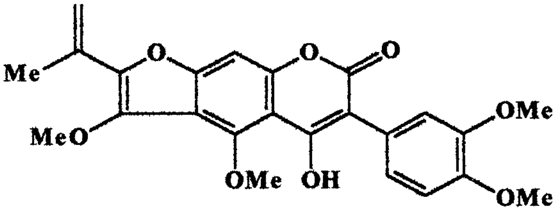

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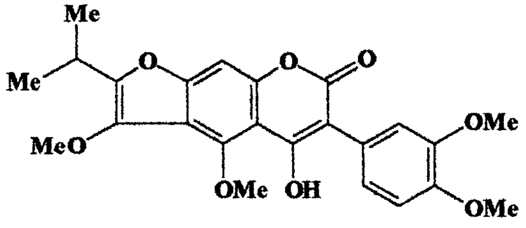

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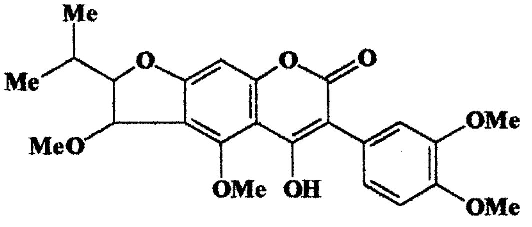
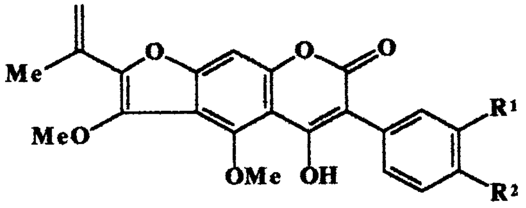


14

ated by HPLC. HMQC and HMBC experiments with **14** (Fig. 1) confirmed its structure and thereby the structure of **12**. Further corroboration came from NOE measurements performed with **12** and **14** respectively (Fig. 2).

The close similarity of NMR data of the coumarins **1** and **2** with those of **12** and the occurrence of these compounds in the same plant, suggest a reexamination of the constitutions of thonningine-A (**1**) and thonningine-B (**2**) to definitely exclude structures like **15** and **16.** Unfortunately the compounds were not available anymore.



15 $R^1 + R^2 = OCH_2O$

16 $R^1 = H$, $R^2 = OMe$

Table 1
^{1}H NMR data of compounds **1** and **12**–**14** (δ in CDCl$_3$, J (Hz))

H	1[a]		12		13		14	
	δ	Multiplicity; J	δ	Multiplicity; J	δ	Multiplicity; J	δ	Multiplicity; J
8	7.23	s	7.24	s	7.23	s	6.65	s
2′	[b]	[b]	7.11	d; 2	7.11	d; 2	7.06	d; 2
5′	[b]	[b]	6.97	d; 8	6.97	d; 8	6.95	d; 8
6′	[b]	[b]	7.15	dd; 8,2	7.15	dd; 8, 2	7.10	dd; 8, 2
1″	–	–	–	–	–	–	5.21	d; 5
2″	–	–	–	–	–	–	4.03	dd; 10.5, 5
3″	–	–	–	–	3.26	sep;7.5	2.30	m
4″	5.79	br s	5.78	br s	1.37	d; 7.5	1.14	d; 7
	5.25	q; 1	5.25	dq; 1.5, 1.5				
5″	2.24	d; 1	2.24	3H; br s	1,37	d; 7.5	1.22	d; 7
4-OH	10.43	s	10.43	s	10.50	s	10.00	s
5-OMe	4.26	s	4.26	s	4.25	s	4.29	s
3′-OMe	[b]	[b]	3.91[c]	s	3.91[c]	s	3.90[c]	s
4′-OMe	[b]	[b]	3.92[c]	s	3.92[c]	s	3.91[c]	s
1″-OMe	3.94	s	3.94	s	3.89	s	3.20	s

[a] Values taken from Khalid & Waterman, 1983 and arranged to correlate with the data of **12**.

[b] 7.01–7.09 (3H; m) and 6.00 (2H; s).

[c] Values might be interchanged within a column.

Table 2
^{13}C NMR shifts of compounds **1** and **12** (δ in CDCl$_3$)

C	12	1[a]	14[b]
2	162.4	160.3	n.d.
3	105.1	104.9	103.5
4	160.7	160.7	161
5	148.3	148.2	154.5
6	113.7	113.6	110.5
7	151.0	151.0	164.5
8	97.3	97.2	95
9	154.4	160.2	156
10	104.0	103.9	100.5
1′	123.6	124.5	123.5
2′	114.0	108.0	114
3′	148.6	147.1	148.5
4′	148.7	147.3	148.5
5′	111.0	111.1	111
6′	123.3	124.3	123.5
1″	137.5	137.4	75.5
2″	145.8	145.7	94.5
3″	132.0	132.0	27
4″	115.4	115.3	19
5″	19.3	19.2	19.5
5-OMe	65.4	65.2	61.5
3′-OMe	55.9	[c]	56
4′-OMe	55.9	[c]	56
1″-OMe	62.8	62.6	54

[a] Values taken from Khalid & Waterman, 1983 and arranged to correlate with the data of **12**.

[b] Values taken from HMQC and HMBC spectra with an inaccuracy of ca. 0.5 ppm.

[c] 100.9 (-OCH$_2$O-).

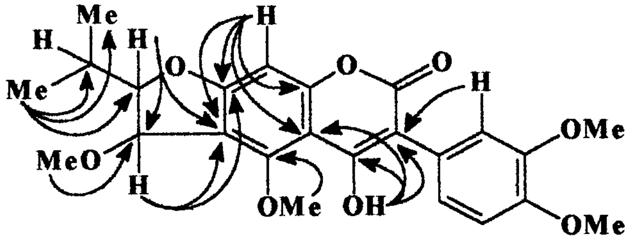


Fig. 1. Important long-range C–H correlations observed in the HMBC of compound **14**.

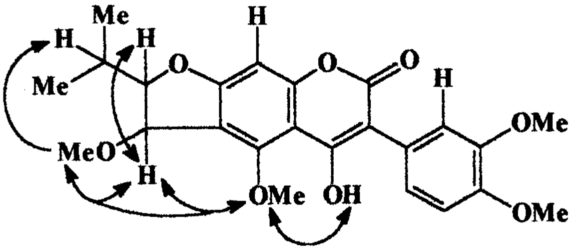


Fig. 2. Important NOEs observed in compound **14**.

3. Experimental

3.1. General

Mps uncorr. TLC was performed on precoated plates (Silica gel 60 F$_{254}$, Merck) using petrol–Me$_2$CO

(1:1); detection by UV and anisaldehyde reagent (Stahl & Kaltenbach, 1961). CC on silica gel. HPLC on Eurospher RP-18, 7 μm (Knauer). IR: KBr; UV: MeOH. ^{1}H and ^{13}C in $CDCl_3$ at 360 and 90 MHz, respectively; int. standard: TMS for ^{1}H solvent for ^{13}C. EIMS at 70 eV with direct inlet; unless key ions only ions ≥10% and *m/z* >100 are given.

3.2. Plant material

The roots of *M. thonningii* (Schum and Thonn) Baker were dug from a tree on the campus of the University of Ghana and the bark removed. The wood was prepared for extraction in a manner as reported in an earlier paper (Asomaning et al., 1995). Mature, green pods, harvested from the same tree were opened and all seeds removed. The empty pods were cut into small pieces and dried in the shade for three weeks. For both the root wood and the pods, voucher specimen were authenticated by the Ghana National Herbarium, Department of Botany, University of Ghana.

3.3. Extraction and isolation

The pulverized root wood (1.8 kg) was extracted with petrol (60–80°C) in a Soxlet apparatus for 48 h. The solvent was removed in vacuo to obtain a brown solid (16 g). CC of 15 g of this solid on silica gel eluting with petrol, petrol with increasing amounts of EtOAc, EtOAc and finally MeOH afforded six fractions (F1–F6). Fraction F1 yielded *O*,*O*-dimethylalpinumisoflavone (**7**) (629 mg), F3 alpinumisoflavone (**4**) (64 mg), F4 5-*O*-methyl-4′-*O*-(3-methyl-2-butenyl)-alpinumisoflavone (**9**) (1.65 mg) and F5 robustone (**8**) (10.5 mg). Fraction F6 deposited a solid from which 5-*O*-methylalpinumisoflavone (**11**) (36 g) was isolated by repeated CC on silica gel. The pods (1.4 kg) were soaked in cold petrol (60–80°) five times each for 24 h. The extracts were combined and the solvent removed in vacuo to yield a dark brown syrup (5.5 g). The dried defatted plant material was further extracted with cold $CHCl_3$ (5 × 24 h) to yield a brown solid (6.2 g). CC of the petrol extract (4.2 g) as described for the root wood afforded four fractions (P1–P4). From P2 crystallized *O*,*O*-di-methylalpinumisoflavone (**7**) (36 mg). Fraction P3 after purification by CC on silica gel gave 3′-hydroxy-4′-*O*-methylalpinumisoflavone (**5**) (46 mg). CC of the $CHCl_3$ extract on silica gel using petrol with increasing amounts of $CHCl_3$ as the eluant gave three fractions (C1–C3). From fraction C1 3′-hydroxy-4′-*O*-methylalpinumisoflavone (*5*) (85 mg) was isolated, and from C3 after purification by CC thonningine-C (**12**) (25 mg).

3.4. 3′-Hydroxy-4′-O-methylalpinumisoflavone (5)

Yellowish crystals from petrol, mp 151–153° (Ref. (Martinez Olivares et al., 1982) mp 155–156°). TLC: R_f 0.54, anisaldehyde: blue. ^{13}C NMR: δ 180.8 (C-4), 159.5 (C-7), 157.2 (C-9), 157.0 (C-5), 152.6 (C-2), 146.8 (C-4′), 145.7 (C-3′) 128.1 (C-2″), 124.0 (C-1′), 123.4 (C-3), 121.0 (C-6′), 115.5 (C-1″), 115.1 (C-2′) 110.7 (C-5′) 106.1 (C-6), 105.6 (C-10), 94.8 (C-8), 76.5 (C-3″), 28.3 (C-4″, C-5″), 56.0 (4′-OMe). Other spectroscopic properties in agreement with published data (Martinez Olivares et al., 1982).

3.5. 5-O-methylalpinumisoflavone (11)

White granules from petrol, mp 199–200° (Ref. (Tsukayama et al., 1992) mp 134–135° from MeOH–H_2O), TLC: R_f 0.49, anisaldehyde: yellow. IR ν_{max} cm^{-1}: 1650, 1640. UV λ_{max} nm (log ε): 282 (4.70), 225 (4.22). ^{13}C NMR: δ 175.7 (C-4), 158.8 (C-9), 158.2 (C-7), 156.3 (C4′), 155.7 (C-6), 150.7 (C-2), 130.8 (C-2″), 130.5 (C-2′, C-6′), 125.9 (C-3), 123.5 (C-1′), 116,1 (C-1″), 115.7 (C-3′, C-5′), 113.4 (C-6 or C-10), 113,1 (C-6 or C-10), 100.7 (C-8), 77.8 (C-3″), 62.9 (*O*–Me), 28.3 (C-4″, C-5″). EIMS *m/z* (rel. int. %): 350.1155 $[M]^+$ (63) (calcd. for $C_{21}H_{18}O_5$:350.1154), 336 (20), 335 (100), 317 (16), 307 (24), 306 (20), 168 (22), 118 (12). ^{1}H NMR in agreement with published data (Tsukayama et al., 1992).

3.6. Thonningine-C (12)

Yellow granules from petrol, mp 199–200°. TLC: R_f 0.49, anisaldehyde: yellow, IR ν_{max} cm^{-1}: 3500, 2950, 1710, 1630, 1580, 1460, 1070, 1040, UV λ_{max} (log ε): 218 (3.24), 281 (4.23), 292 (4.42), 308 (sh), 350 (4.26). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int. %): 438.1315 $(m)^+$ (60) (calcd for $C_{24}H_{22}O_8$: 438. 1315), 260 (100), 245 (66), 190 (17).

3.7. Hydrogenation of thonningine-C (12)

Compound **12** (5 mg) dissolved in MeOH was hydrogenated over Pd/C (5%) for 4 h (room temp/1 bar). After filtration and removal of the solvent, the residue was separated by HPLC using MeOH–H_2O 4:1 as the eluent to yield 3″,4″,-dihydrothonningine-C (**13**) (3 mg) and 1″,2″,3″,4″-tetrahydrothonningine-C (**14**) (0.8 mg).

3.8. 3″,4″-Dihydrothonningine-C (13)

Colorless oil. TLC: R_f 0.50, anisaldehyde: pale yellow. ^{1}H NMR: Table 1. EIMS *m/z* (rel. int. %): 440 $(M)^+$ (56), 425 (14), 263 (29), 262 (98), 247 (100), 233

(40), 213 (40), 205 (30), 193 (17), 191 (15), 178 (30), 165 (15).

3.9. 1″,2″,3″,4″-Tetrahydrothonningine-C (**14**)

Colorless oil. TLC: R_f 0.43, anisaldehyde: pale yellow. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. EIMS m/z (rel. int. %): 442 $(M)^+$ (60), 410 (22), 367 (21), 339 (33), 265 (22), 233 (42), 232 (18), 203 (22), 191 (23), 178 (100), 163 (22).

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