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PYRANO- AND DIHYDROFURANO-ISOFLAVONES FROM *MILLETIA THONNINGII*

WILLIAM A. ASOMANING, CHARLES AMOAKO, ISAAC V. OPPONG, WILLIAM R. PHILLIPS, IVAN ADDAE-MENSAH, EMMANUEL Y. OSEI-TWUM,* REINER WAIBEL† and HANS ACHENBACH†

Department of Chemistry, University of Ghana, Legon, Ghana; *King Fahd University of Petroleum and Minerals, P.O. Box 1427, Dhahran 31261, Saudi Arabia; †Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Erlangen, Germany

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Key Word Index—*Milletia thonningii*; Leguminosae; root bark; isoflavones; *O,O*-diemthylalpinumisoflavone; 5-*O*-methyl-4′-*O*-(3-methyl-2-butenyl)alpinumisoflavone; thonninginisoflavone.

Abstract—The petrol extract of the root bark of *Milletia thonningii* afforded β-amyrin and *O,O*-dimethylalpinumisoflavone, together with the new compounds 5-*O*-methyl-4′-*O*-(3-methyl-2-butenyl)alpinumisoflavone and thonninginisoflavone. The structures were determined by spectroscopic methods and partly by chemical interconversions. The absolute configuration of thonninginisoflavone was established by chiroptical studies. The crude extract and some of the pure compounds were active in the brine shrimp lethality bioassay.

INTRODUCTION

Milletia thonningii (Schum and Thonn) Baker is a tree indigenous in West and Central Africa. The plant is used throughout the sub-region in traditional medicine as a laxative, a blood purifier, a dewormer, an analgesic and for the treatment of diarrhoea [1, 2]. The juice from the leaves is reported to be lethal to the *Bulinus* snail, the vector for schistosomiasis [2]. Chemically, however, the plant has received little attention apart from the work done on the seeds [3, 4], from which the isoflavones **1**–**5**, the coumarin **6**, thonningine-A (**7**) and -B (**8**) have been isolated. The petrol extract of the dry pulverized root bark of *M. thonningii* showed activity in the brine shrimp lethality bioassay [5] with a LD_{50} about 100 ppm. This caused our interest in its components.

RESULTS AND DISCUSSION

Chromatographic separation of the extract on silica gel using eluents with increasing polarity and further purification by TLC and recrystallization afforded β-amyrin and the isoflavones **2**, **9** and **10**.

Compound **2**, which had already been reported as a constituent of the seeds of *M. thonningii* [3, 4], was identified as *O,O*-dimethylalpinumisoflavone by comparison of its spectral properties with published data [3, 4, 6, 7] and by conversion to 4′-*O*-methylalpinumisoflavone (**3**) on treatment with BCl_3 [8].

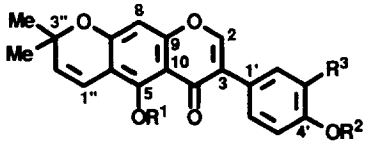
The novel isoflavone **9** was optically inactive; it had all the spectral features of a pyranoisoflavone. Comparison of the ^{1}H NMR spectra of **2** and **9** (Table 1) showed that the methoxyl singlet at δ3.82 had been replaced by the signals of a 3-methyl-2-butenyloxy group at δ1.74, 1.80 (each 3H, *d*, J = 1.0 Hz, 2 Me), 4.53 (2H, *br d*, J = 7.0 Hz, O–CH_2–CH) and 5.50 (1H, *tm*, J = 7.0 Hz, CH_2–CH =). The corresponding resonances in the ^{13}C NMR of **9** (Table 2) were observed at δ18.1, 25.8, 64.7, 119.7 and 138.1. In the mass spectrum, the molecular ion at *m/z* 418 (i.e. 54 units higher than $[M]^+$ of **2**) and a prominent fragment ion at *m/z* 69 (C_5H_9) were also in agreement with a methylbutenyl group. The ^{13}C–^{1}H correlation spectrum of **9** (Fig. 1) corroborated the proposed structure.

The novel isoflavone **10** was optically active and analysed for $C_{22}H_{20}O_5$. Comparison of the NMR data with those of **2** (Tables 1 and 2) suggested identical or analogous structures of rings A–C. However, ^{13}C–^{1}H correlation experiments (Fig. 2) established the isopropenyldihydroflurano moiety and resulted in structure **10**. The absolute configuration was determined by CD studies of the osmate ester/pyridine complex [9]; a positive Cotton effect at 480 nm indicated the *S*-configuration.

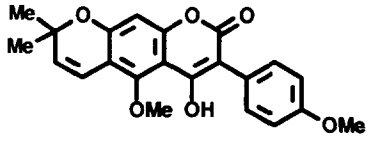
EXPERIMENTAL

General. Mps: uncorr.; IR: KBr; UV: MeOH; EIMS: 70 eV, only ions > 10% are given; ^{1}H (360 MHz) and ^{13}C NMR (90 MHz) in $CDCl_3$, δ in ppm (int. standard: TMS).

Plant material. The roots of *Milletia thonningii* (Schum and Thonn) Baker were dug up from trees on the campus of the University of Ghana in Legon (Ghana). Voucher specimens were authenticated by the Herbarium of the Department of Botany of the University of Ghana. The bark was removed, cut into small pieces and dried in the shade for three weeks.



	R^1	R^2	R^3
1	H	H	H
2	Me	Me	H
3	H	Me	H
4	H	Me	OH
5	H	$-CH_2O-$	
9	Me	$CH_2CH=C(Me)_2$	H



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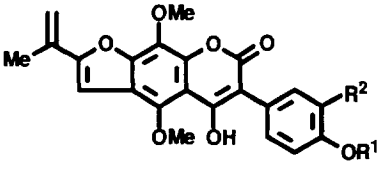
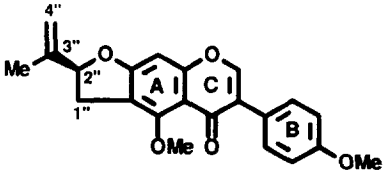


	R^1	R^2
7	$-CH_2O-$	
8	Me	H



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Extraction and isolation. Dried and pulverized root bark (2.6 kg) was extracted with petrol (60–80°) in a Soxhlet apparatus for 36 hr. After removing the solvent *in vacuo*, 117 g of a brown semi-solid residue was obtained. A portion of the crude extract (30 g) was separated by CC on silica gel into 10 fractions (F1–F10) eluting successively with petrol, petrol–EtOAc, EtOAc and finally MeOH. From F4 (2 g) β-amyrin was isolated by CC. Crystallization of F6 (7 g) afforded 5-*O*-methyl-4′-*O*-(3-methyl-2-butenyl)-alpinumisoflavone (**9**), crystallization of F7 (11 g) gave *O,O*-dimethylalpinumisoflavone (**2**), and crystallization of F8 (0.5 g) followed by prep. TLC yielded thonninginisoflavone (**10**).

β-*Amyrin.* Crystals (1.8 g), mp 194° (petrol) (ref. [10] mp 197–197.5° (EtOH)). $[\alpha]_D$ + 80° ($CHCl_3$; *c* 0.54) (ref. [10] $[\alpha]_D$ + 88.4°). All properties in agreement with an authentic sample. Brine shrimp lethality bioassay: LD_{50} > 1000 ppm.

O,O-*Dimethylalpinumisoflavone* (**2**). Granules (6.9 g), mp 138–140° (petrol) (ref. [6] mp 137–138°; ref. [3] mp 119–121°(C_6H_6)). Spectroscopic data in good agreement with published data [3, 4, 6, 7]. Brine shrimp lethality bioassay: LD_{50} 3.9 ppm.

5-O-*Methyl*-4′-O-(3-*methyl*-2-*butenyl*)*alpinumisoflavone* (**9**). Granules (4.1 g), mp 107–108° (petrol). UV λ_{max} nm (log ε): 326 (3.94), 271 (4.77), 225 (4.48). IR ν_{max} cm^{-1}: 1645, 1605, 1250, 1217, 1128. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. MS *m/z* (rel. int.): 418 $[M]^+$ (14), 351 (20), 350 (95), 336 (21), 335 (100), 332 (16), 331 (15), 322 (16), 317 (14), 307 (21), 306 (16), 60 (23).

Thonninginisoflavone (**10**). Granules (150 mg), mp 118–121° ($CHCl_3$). $[\alpha]_D$ + 63° ($CHCl_3$; *c* 0.38). UV λ_{max} nm (log ε): 294 (sh), 257 (4.73). IR ν_{max} cm^{-1}: 1641, 1626, 1467, 1246. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. MS *m/z* (rel. int.): 364 $[M]^+$ (100), 363 (20), 335 (17), 296 (26), 268 (16), 145 (18), 132 (20), 117 (17), 43 (13).

Table 1. ^{1}H NMR spectral data of compounds **2**, **9** and **10***

H	2	9	10
2	7.76 *s*	7.76 *s*	7.77 *s*
8	6.60 *d*, (0.8)	6.60 (0.8)	6.59 *s*
2′, 6′	7.46 *m*	7.45 *m*	7.46 *m*
3′, 5′	6.95 *m*	6.96 *m*	6.95 *m*
1″	6.74 *dd* (10.0, 0.8)	6.74 *dd* (10.0, 0.8)	3.11 *dd* (16.0, 7.5) 3.48 *dd* (16.0, 9.5)
2″	5.72 *d* (10.0)	5.72 *d* (10.0)	5.30 *br dd* (9.5, 7.5)
Me-3″	1.47 *s*	1.47 *s*	1.77 *br s*
Me-3″	1.47 *s*	1.47 *s*	
H_A-4″	—	—	4.95 *br s*
H_B-4″	—	—	5.10 *br s*
OMe-5	3.89 *s*	3.90 *s*	3.94
OMe-4′	3.82 *s*	—	3.82
H_2-1‴		4.53 *br d* (7.0)	
2‴		5.50 *tm* (7.0)	
Me-3‴		1.74 *br d* (1.0)	
Me-3‴		1.80 *br d* (1.0)	

*See also Refs [3, 6].
Coupling constants (J in Hz) in parentheses.

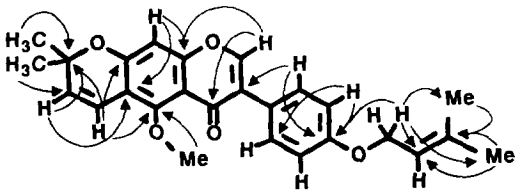

Fig. 1. Long-range couplings observed in the ^{13}C–^{1}H COSY of **9**.

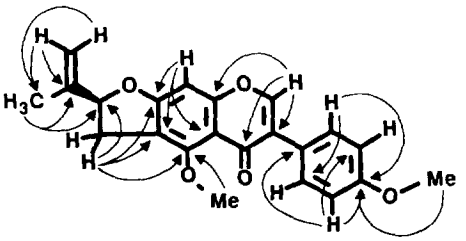

Fig. 2. Long-range couplings observed in the ^{13}C–^{1}H COSY of **10**.

Preparation and CD measurements of the osmate ester–pyridine complex of **10** [9]. A solution of **10** (0.44 mg, 1.2 μmol) in CH_2Cl_2 (65 μl) containing 25 μmol of pyridine was treated with OsO_4 (1.4 μmol in 10 μl $CHCl_3$) for about 30 min. The mixture was diluted with CH_2Cl_2 to a final vol. of 2.8 ml. The CD curve of this solution showed a positive Cotton effect at 480 nm. For comparison, the same procedure and measurement with *R*-configurated rotenone exhibited a negative Cotton effect at 460 nm.

Selective demethylation of compound **2** *with* BCl_3 [8]. A cold satd soln of BCl_3 in $CHCl_3$ was added dropwise under stirring to a cold soln of **2** (500 mg) in $CHCl_3$

Table 2. ^{13}C NMR spectral data compounds **2**, **3**, **9** and **10**

C	2*	3	9	10
2	150.3	152.5	150.3	150.2
3	125.5	123.4	125.5	125.4
4	174.9	180.0	174.9	175.1
5	155.7	156.9	155.7	156.3
6	113.2	114.5[a]	113.2	117.2
7	158.0	157.2	157.9	164.3
8	100.6	94.8	100.6	93.8
9	158.6	159.7	158.6	159.6
10	113.2	†	113.2	113.0
1′	124.2	123.0	124.1	124.2
2′	130.3	130.1	130.2	130.3
3′	113.9	114.0	114.6	113.8
4′	159.5	159.4	158.7	159.4
5′	113.9	114.0[a]	114.6	113.8
6′	130.3	130.1	130.2	130.3
1″	116.1	115.5	116.1	31.5
2″	130.7	128.2	130.6	87.6
3″	77.6	77.5	77.6	142.9
4″	—	—	—	112.8
Me-3″	28.3	28.3	28.2	15.0
Me-3″	28.3	28.3	28.2	—
OMe-5	63.0	—	62.7	61.0
OMe-4′	55.3	55.3	—	55.2
1‴	—	—	64.7	—
2‴	—	—	119.7	—
3‴	—	—	138.1	—
Me-3‴	—	—	25.8	—
Me-3‴	—	—	18.1	—

*See also ref. [4].
†Signal not recognizable.
[a]Values may be interchanged.

(20 ml) until the mixture turned brown and further addition of BCl_3 produced no change in colour. H_2O (60 ml) was added and the mixture extracted with $CHCl_3$ (3×50 ml). The solvent was removed *in vacuo* to obtain a brown residue which on crystallization yielded yellowish crystals (350 mg, 73%) of 4′-*O*-methylalpinumisoflavone (**3**). Mp 132–134° (ref. [3] mp 137–138°). UV, IR, ^{1}H NMR in agreement with published data [3, 11]. ^{13}C NMR: see Table 2.

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REFERENCES

1. Irvine, F. R. (1961) *Woody Plants of Ghana*. Oxford University Press, London.
2. Abbiw, D. K. (1990) *Useful Plants of Ghana*. Intermediate Technology Publications and The Royal Botanic Gardens, Kew.
3. Martinez Olivares, E., Lwande, W., Delle Monache, F. and Marini Bettolo, G. B. (1982) *Phytochemistry* **21**, 1763.
4. Khalid, S. A. and Waterman, P. G. (1983) *Phytochemistry* **22**, 1001.
5. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nicols, D. E. and McLaughlin, J. L. (1982) *Planta Med.* **45**, 31.
6. Chibber, S. S. and Sharma, R. P. (1979) *Indian J. Chem.* **18B**. 471.
7. Tsukayama, M., Kawamura, Y. and Tahara, H. (1992) *Heterocycles* **34**, 505.
8. Dean, F. M., Goodchild, J., Houghton, L. E., Martin, J. A., Morton, R. B., Parton, B., Price, A. W. and Somvichien, N. (1966) *Tetrahedron Letters* 4153.
9. Tahara, S., Ingham, J. L. and Mizutani, J. (1987) *Agric. Biol. Chem.* **51**, 211.
10. Buetzingham, J. E. (1982) *Dictionary of Organic Compounds*, Vol. 1, 5th Edn. Chapman & Hall, New York.
11. Jackson, B., Owen, P. J. and Scheinmann, F. (1971) *J. Chem. Soc. C* 3389.