

Isolation and Characterization of 5-Hydroxy-4', 7- Dimethoxyisoflavone from the Root Bark of *Millettia Mannii* (Leguminoceae)**

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ABSTRACT

The phytochemical studies on the root bark of *Millettia Mannii*, a species belonging to the family leguminoceae and sub-family papillionaceae, led to the isolation and determination of the structure of 5-hydroxy-4',7-dimethoxyisoflavone (6). Though previously synthesized, characterized and pre-screened (SEKIZAKI and YOKOSAWA, 1988), compound (6) has been isolated for the first time from a natural source.

Keywords: Leguminoceae; Papillionaceae; Root bark; *Millettia mannii*, 5-hydroxy-4', 7-dimethoxyisoflavone

RÉSUMÉ

Les études phytochimiques sur les écorces des racines de *Millettia mannii*, une espèce de la grandefamille des légumineuses (sous-famille des papillonacées), ont permis l'isolement et la détermination de la structure de 5-hydroxy-4', 7-diméthoxyiso-flavone (6). Bien que précédemment synthétisé, caractérisé et pré-testé, le composé (6) a été isolé pour la première fois d'une source naturelle.

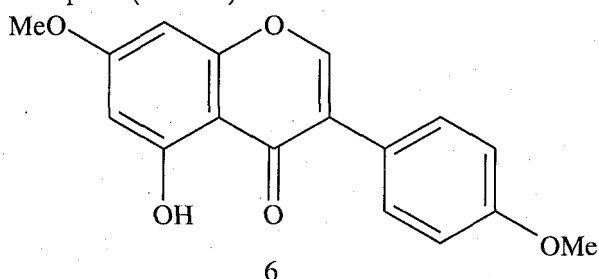
Mots clés: Léguminocées; Papillionacées; écorces de racines; *Millettia mannii*, 5-hydroxyl-4', 7-diméthoxyisoflavone

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INTRODUCTION

The genus *Millettia* has about 200 species worldwide, with about 41% found in tropical Africa alone (GILLET *et al.*, 1971). Interest on the genus *Millettia* resulted from their constant use in Africa as a source of insecticides and molluscides, vermifuges and as substances for stupefying fish. The number of *Millettia* species in Cameroon is about 21, a good number of which have been shown already to possess biologically active constituents (SINGHAL *et al.*, 1982 and DEWICK, 1993). We report on the isolation and structural determination of 5-hydroxy-4',7-dimethoxyisoflavone (**6**), which was first synthesized by SEKIZAKI and YOKOSAWA, (1988) and also shown in their study to have a significant attracting activity to *Aphanomyces euteiches* zoospore (Table 1).



EXPERIMENTAL

The melting point was determined on a Yanagimoto micro melting point apparatus and is uncorrected. The proton nuclear magnetic resonance (¹H NMR) spectrum was recorded at 300 MHz in CDCl₃ with TMS as internal standard, using the Bruker AC 300 spectrometer. The mass spectrum was determined at 70 eV using an apparatus of type R10-10.

The root bark of *Millettia mannii* was collected from the edges of the sub-equatorial forest around Yaounde in February 1995. Voucher material documenting the collection was authenticated by the Director of the National Herbarium, Yaounde, Cameroon, where a specimen has been deposited. The dried, ground root bark gave 2.1 kg of coarse powder which was successively and exhaustively extracted with MeOH and MeOH/CH₂Cl₂ (1:1). Concentration of both filtrates at reduced pressure yielded 205 g and 90 g respectively. On the basis of their tlc, both extracts were combined to give a total of 295 g of crude extract which was chromatographed on a silica gel column packed in hexane. Elution was then effected with a cyclohexane-CH₂Cl₂ gradient. On elution with

Table 1: Attracting Activity of Isoflavonoids to zoospores of *Aphanomyces euteiches* (AE-F3)^a

Comp No.	Degree of attracting activity ^b and Concentration (M)			10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹
	R ¹	R ²	R ³					
1	H	H	H	+++	++	+	-	-
2	Me	H	H	+++ +	+++ +	++	+	-
3	H	Me	H	+	-	-	-	-
4	H	H	Me	+++	++	+	-	-
5	Me	Me	H	-	-	-	-	-
6	Me	H	Me	+++	++	+	-	-
7	H	Me	Me	-	-	-	-	-
8	Me	Me	Me	-	-	-	-	-

^a AE-F3 is *Aphanomyces euteiches* isolate fraction 3, isolated from pea root rot soils

^b, -, no effect; +, attractive effect vanishes within 10 min.; ++, attractive effect persists for 30 to 60 min; ++++, attractive effect persists for more than 60 min.

hexane/CH₂Cl₂ (65:35), a series of identical fractions were obtained and assembled from which (6) crystallized out. Recrystallization in MeOH yielded 9 mg of light yellow crystals:

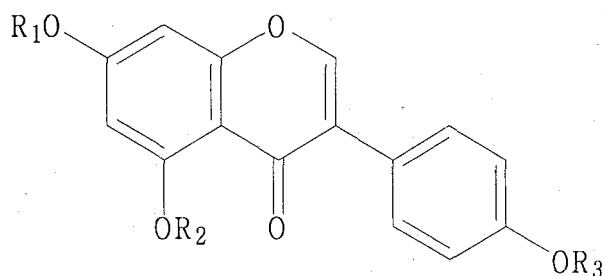
5-hydroxy-4',7-dimethoxyisoflavone (6) crystals, mp 139-140 °C (EtOH). EI-MS, *m/z* (rel.int.): 198 ([M]⁺ (100), 283(10), 166(22), 138(12), 132(20), 117(12), 95(22), 89(24). HRMS *m/z* [M]⁺ 298 (C₁₇H₁₄O₅)).

RESULTS AND DISCUSSION

The dried and ground root bark of *Millettia mannii* was successively extracted with MeOH and MeOH/CH₂Cl₂ (1:1). Silica gel chromatography of the combined extracts using gradient elution from cyclohexane to methylene chloride yielded 5-hydroxy-4',7-dimethoxyisoflavone (6), which was obtained as slightly yellow crystals (mp 139-140 °C (EtOH) (SEKIZAKI and YOKOSAWA, 1988).

The EI mass spectrum of (6) showed a prominent [M]⁺ at *m/z* 298 as the base peak. The molecular formula C₁₇H₁₄O₅ was determined from high resolution EI data. The peak at *m/z* 283 (10%) resulted from the loss of a CH₃ radical and those at *m/z* 166 (22%) and 132 (20%) arise from the RDA-fragmentation of the isoflavonoid molecular ion.

The ¹H NMR spectrum was fascinatingly simple, showing a signal for the characteristic H-2 proton of isoflavonoids (DODDRELL *et al*, 1982) at δ 7.84 ppm (1H, s); a chelated hydroxyl proton (offset) at δ 12.84 ppm, exchangeable with D₂O; two signals at δ 3.82 (3H, s) and 3.85 (3H, s) indicating two groups of methoxyl protons on rings B and C respectively; doublet signals at δ 6.95 ppm



(2H, dd) and 7.44 (2H, dd) indicating the AA'BB' signals for the four protons of the para-substituted ring C; signals at δ 6.36 ppm (1H, d) and δ 6.39 ppm (1H, d) indicating the two *meta* protons of ring B.

These data make it possible to conclude that this compound, isolated from a natural source for the first time, has the same structure as compound (6) synthesized and pre-screened by SEKIZAKI and YOKOSAWA, (1988). It has been reported that naturally occurring genistein (1), prunetin (2) and biochamin A (4) have estrogenic properties (SEKIZAKI and YOKOSAWA, 1988). Since the synthetic 5-hydroxy-4',7-dimethylisoflavone (6) exhibited significant activity to *Aphanomyces euteiches* zoospores (Table 1), the isolation and characterisation of natural compound (6) is strong evidence that this group of isoflavonoids, possess both estrogenic and attracting activities.

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Table 2: ¹H NMR data for 5-hydroxy-4', 7- dimethoxyisoflavone.

Proton	• (ppm)
H-2	7.84 (s)
H-6	6.36 (d)
H-8	6.39 (d)
H-2'	7.44 (dd)
H-3'	6.95 (dd)
H-5'	6.95 (dd)
H-6'	7.44 (dd)
4'-OMe	3.85 (s)
5-OH	12.84 (s)
7-OMe	3.82 (s)

REFERENCES

- Dewick, P.M., (1993) in *The Flavonoids: Advances in Research since 1986*. Harborne, J.B., Ed., New York: Chapman and Hall. p. 117
- Doddrell, D.M; Pegg, B.T. and Bendall, M.R., (1982), "Nuclear Spin Relaxation in Liquids and Gases", *J. Magn. Reson.*, **48**, 323
- Gillet, J.; Polhill, M. R. and Verdcourt, B., *Flora of Tropical East Africa*. 1971. London: Crown Agents for Overseas Governments and Administration. Parts 3 and 4.
- Sekizaki, H., and Yokosawa, R., (1988), "Studies on Zoospore-Attracting Activity. I. Synthesis of Isoflavones and Their Attracting Activity to *Aphanomyces euteiches* Zoospore", *Chem. Pharm. Bull.*, **36**(12), 4876-4880.
- Singhal, A.K.; Sharma, R.P.; Baruah, J.N.; Govindan, S.V. and Herz, W., (1982), "Rotenoids from roots of *Millettia pachycarpa*", *Phytochemistry*, **21**, 949-951.

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