*Phytochemistry*, Vol. 24, No. 2, pp. 369–371, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00


# FLAVONOIDS FROM THE STEM BARK OF *MILLETTIA HEMSLEYANA*

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(*Received* 8 *June* 1984)

**Key Word Index**—*Millettia hemsleyana*; Leguminosae; Papilionoidae; chalcones; milletenone; dihydrochalcones; dihydromilletenone methyl ether; dihydroisomilletenone methyl ether; pongaflavone; lanceolatin-B; 3',4'-methylenedioxy-7-methoxyflavone.

**Abstract**—The stem bark of *Millettia hemsleyana* has yielded six simple flavonoids of which three are novel. Dihydromilletenone methyl ether and dihydroisomilletenone methyl ether represent the two keto–enol tautomers of the known β-hydroxychalcone milletenone, trapped by methylation and reduction, and the third new compound, 3',4'-methylenedioxy-7-methoxyflavone, is the cyclized form of a demethylated milletenone. All compounds were identified on the basis of detailed spectral analysis.

## INTRODUCTION

Previous examination of a number of species of *Millettia* have shown them to be a rich source of flavonoids and isoflavonoids typical of the Tephrosieae tribe of the Papilionoidae [1]. In this paper we report the results of an examination of the stem bark of *Millettia hemsleyana* Prain, a medium–large tree found in the rain forests of south Thailand and peninsular Malaya [2].

## RESULTS AND DISCUSSION

Six flavonoids were isolated from a petrol extract of the stem bark by column chromatography over silica gel followed, in some cases, by preparative circular TLC. Two of the isolated compounds were characterized as the flavones pongaflavone, previously recorded from *Pongamia pinnata* [3], and lanceolatin-B, known from several species [1] including *Millettia ovalifolia* [4]. A third flavone analysed for $C_{17}H_{12}O_5$. The $^1$H NMR spectrum revealed the presence of methylenedioxy and methoxy substituents, a singlet at δ7.08 for H-3 and two series of three coupled aromatic protons showing ABB' patterns. The EI-mass spectrum gave ions at *m/z* 146 and 134 typical of a methylenedioxy substituted B-ring [5], thus requiring the flavone to be assigned structure **1**.

The three remaining compounds were all chalcones, oxygenated on the β-carbon. One was identified as the known compound milletenone (**2**), previously reported from *Millettia ovalifolia* [4]. The $^1$H NMR spectrum of **2** was in close agreement with that previously recorded by Khan and Zaman [4] showing that the greater part of **2** existed in the tautomer depicted.

The second, a dihydrochalcone, analysed for $C_{19}H_{20}O_6$ and was identified as **3** on the basis of the following data. The $^1$H NMR spectrum showed aromatic coupling patterns identical to **2** but differed in the presence of an ABX system centred at δ3.22, 3.47 and 4.70 and a singlet (3H) at δ3.18, typical of a methoxy substituent on the aliphatic β-carbon. The EIMS base peak at *m/z* 165 could be attributed to both ions **4** and **5**.

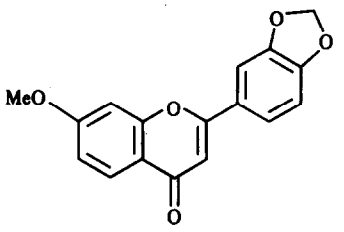
The final compound also analysed for $C_{19}H_{20}O_6$ and exhibited $^1$H NMR signals indicating an aliphatic methoxyl and ABX system as in **3**. However, while coupling patterns among the aromatic protons were identical to those of **3** there were considerable differences in chemical shift value. In **3** the most deshielded aromatic proton, that *peri* to the carbonyl, appeared as a sharp doublet showing *ortho* coupling. In this compound there were two deshielded protons, one showing both *ortho* and *meta* coupling and the other only *meta* coupling, thereby indicating that both positions *peri* to the carbonyl were unsubstituted. This suggested structure **6**, with placement of the carbonyl next to the piperonyl ring, which was confirmed by the EI-mass spectrum which gave ions **7** and, as base peak, **8**. Compounds **3** and **6** obviously represent the two keto–enol tautomers of the dihydrochalcone derived from milletenone, trapped by formation of the corresponding methyl ethers. As ion **4** is the base peak in the EI-mass spectrum of milletenone, it would seem that **2** exists predominantly in the same keto form as **3**. By contrast, ion **7** occurs at a relative intensity of *ca* 40%. On this basis, **3** has been assigned the trivial name dihydromilletenone methyl ether whilst **6** has been named dihydroisomilletenone methyl ether.

## EXPERIMENTAL

Mps uncorr.; UV: MeOH; IR: KCl disc; $^1$H NMR: 250 MHz with TMS as internal standard, solvent as stated; EIMS: 70 eV at 130–160°, direct probe. Petrol refers to bp 40–60° fraction.

*Plant material.* Bark was collected at Kuala Lompat in the Krau Game Reserve, West Malaysia, in 1982 and a voucher has been deposited at the Forest Research Institute, Kepong, Malaysia.

*Isolation and purification of flavonoids.* Ground stem bark (410 g) was extracted with petrol. The conc. extract was subjected to CC over silica gel and gave, on elution with petrol containing increasing amounts of EtOAc: (a) with 10% EtOAc, pongaflavone (331 mg); (b) with 15% EtOAc, a mixture which was separated by CC over silica gel, eluting with $C_6H_6$–EtOAc (49:1), to give **2** (218 mg); (c) with 25% EtOAc, a mixture which was separated by circular prep. TLC over silica gel (solvent:



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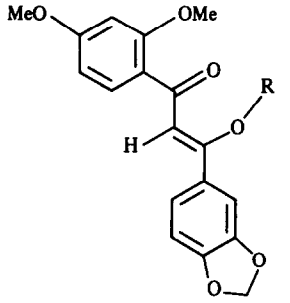

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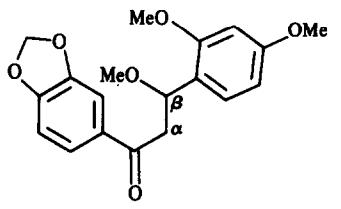

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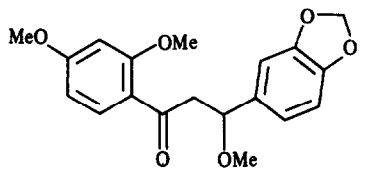

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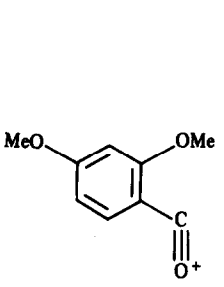

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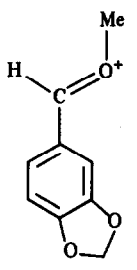

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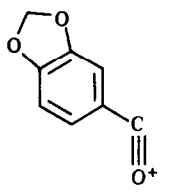

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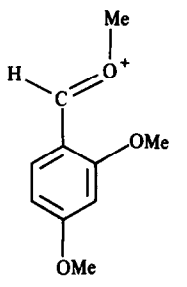

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$C_6H_6$–EtOAc, 49:1) to give **6** (17 mg) followed by **3** (39 mg); further elution with 25% EtOAc gave lanceolatin-B (51 mg); (d) with 40% EtOAc, **1** (51 mg).

*Identification of flavonoids. Pongaflavone.* Needles from petrol–EtOAc, mp 146° (lit. [3] 149°). Found: $[M]^+$ 334.1200; $C_{21}H_{18}O_4$ requires: 334.1205. UV, IR, $^1H$ NMR in agreement with published data [3].

*Milletenone* (**2**). Yellow needles from petrol–EtOAc, mp 142° (lit. [4] 138°). Found: $[M]^+$ 328.0960; $C_{18}H_{16}O_6$ requires: 328.0947. UV, IR, $^1H$ NMR ($C_5D_5N$) in agreement with literature [4]. EIMS *m/z* (rel. int.): 328 $[M]^+$ (81), 310 (16), 297 (73), 165 $[C_9H_9O_3]^+$ (100), 149 (40), 138 (59), 122 (8).

*Dihydromilletenone methyl ether* (**3**). Oil. Found: $[M]^+$ 344.1250; $C_{19}H_{20}O_6$ requires: 344.1260. UV $\lambda_{max}$ nm: 227, 265, 290. IR $\nu_{max}$ $cm^{-1}$: 1660, 1605, 1495, 1240, 1220, 1020. $^1H$ NMR ($CDCl_3$): $\delta$3.18 (3H, *s*, $\beta$-OMe), 3.22 (1H, *dd*, $J$ = 16.9, 4.9 Hz, $\alpha$-$H_{eq}$), 3.47 (1H, *dd*, $J$ = 16.9, 8.0 Hz, $\alpha$-$H_{ax}$), 3.84, 3.85 (2 × 3H, 2 × *s*, 4′-OMe, 6′-OMe), 4.73 (1H, *dd*, $J$ = 8.0, 4.9 Hz, $\beta$-$H_{ax}$), 5.95 (2H, *s*, O–$CH_2$–O), 6.43 (1H, *d*, $J$ = 2 Hz, H-5′), 6.51 (1H, *dd*, $J$ = 8, 2 Hz, H-3′), 6.76 (1H, *d*, $J$ = 8 Hz, H-5), 6.82 (1H, *dd*, $J$ = 8, 2 Hz, H-6), 6.88 (1H, *d*, $J$ = 2 Hz, H-2), 7.78 (1H, *d*, $J$ = 8 Hz, H-2′). EIMS *m/z* (rel. int.): 344 $[M]^+$ (32), 329 (31), 262 (19), 165 $[C_9H_9O_3]^+$ (100), 160 (22), 150 (20), 149 (36).

*Dihydroisomilletenone methyl ether* (**6**). Yellow needles from $Et_2O$, mp 92–94°. Found: $[M]^+$ 344.1252; $C_{19}H_{20}O_6$ requires: 344.1260. UV $\lambda_{max}$ nm: 230, 275, 309. IR $\nu_{max}$ $cm^{-1}$: 1670, 1610, 1500, 1460, 1280, 1095, 1020, 930, 820. $^1H$ NMR ($CDCl_3$): $\delta$3.10 (1H, *dd*, $J$ = 15.9, 3.2 Hz, $\alpha$-$H_{eq}$), 3.24 (3H, *s*, $\beta$-OMe), 3.29 (1H, *dd*, $J$ = 15.9, 9.2 Hz, $\alpha$-$H_{ax}$), 3.81, 3.82 (2 × 3H, 2 × *s*, 2-OMe, 4-OMe), 5.14 (1H, *dd*, $J$ = 9.2, 3.2 Hz, $\beta$-$H_{ax}$), 6.04 (2H, *s*, O–$CH_2$–O), 6.47 (1H, *d*, $J$ = 2 Hz, H-3), 6.53 (1H, *dd*, $J$ = 8, 2 Hz, H-5), 6.84 (1H, *d*, $J$ = 8 Hz, H-3′), 7.33 (1H, *d*, $J$ = 8 Hz, H-6), 7.49 (1H, *d*, $J$ = 2 Hz, H-6′), 7.59 (1H, *dd*, $J$ = 8, 2 Hz, H-2′). EIMS *m/z* (rel. int.): 344 $[M]^+$ (7), 281 (28), 181 $[C_{10}H_{13}O_3]^+$ (100), 149 (19), 121 (5).

*Lanceolatin-B.* Needles from petrol–$C_6H_6$, mp 144° (lit. [4] 135–142°). Found: $[M]^+$ 262.0622; $C_{17}H_{10}O_3$ requires: 262.0630. UV, IR, $^1H$ NMR, EIMS in close agreement with published data [4, 6].

*3′,4′-Methylenedioxy-7-methoxyflavone* (**1**). Plates from $Et_2O$, mp 208–210°. Found: $[M]^+$ 296.0687; $C_{17}H_{12}O_5$ requires: 296.0685. UV $\lambda_{max}$ nm: 314, 334. IR $\nu_{max}$ $cm^{-1}$: 1660, 1620, 1505, 1420. $^1H$ NMR ($C_5D_5N$): $\delta$3.80 (3H, *s*, 7-OMe), 6.08 (2H, *s*, O–$CH_2$–O), 6.99 (1H, *d*, $J$ = 8 Hz, H-5′), 7.05 (1H, *dd*, $J$ = 9, 2 Hz, H-6), 7.08 (1H, *s*, H-3), 7.15 (1H, *d*, $J$ = 2 Hz, H-8), 7.54 (1H, *dd*, $J$ = 8, 2 Hz, H-6′), 7.60 (1H, *d*, $J$ = 2 Hz, H-2′), 8.35 (1H, *d*, $J$ = 9 Hz, H-5). EIMS *m/z* (rel. int.): 296 $[M]^+$ (100), 268 (12), 253 (18), 150 (5), 146 (61), 134 (15).

*Acknowledgement*—Dr. G. Davison, Department of Zoology, University of Kebangsaan, Malaysia, is thanked for arranging the supply of plant material.

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*Phytochemistry*, Vol. 24, No. 2, pp. 371–372, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00


# FLAVONOL GLYCOSIDES OF *EUPHORBIA RETUSA* AND *E. SANCTAE-CATHARINAE*

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(*Revised received* 6 *August* 1984)

**Key Word Index**—*Euphorbia retusa*; *E. sanctae-catharinae*; Euphorbiaceae; flavonol glycosides; kaempferol and quercetin 3-glucuronide-7-glucosides.

**Abstract**—The flavonoid glycosides of *Euphorbia retusa* and *E. sanctae-catharinae* are reported. Besides a number of common flavonol glycosides, kaempferol and quercetin 3-glucuronide-7-glucosides are reported for the first time.

*Euphorbia* is represented in Egypt by some 35 species [1]. A number of these species have been studied previously, thus quercetin 3-rhamnoside has been isolated from *Euphorbia hypericifolia* [2] and *E. geniculata* [3]. Quercetin 3-galactoside and 3-arabinoside were identified in *E. paralias* [4], while rhamnetin 3-galactoside was detected in *E. hypericifolia* [2] and *E. prostrata* [3]. *E. granulata* proved to contain apigenin 7-glucoside [5], which was also found in *E. prostrata* [3]. Four other *Euphorbia* species have also been investigated. Thus from *E. esula* kaempferol 3-glucuronide was isolated [6] and quercetin 3-galactoside-2″-gallate was identified in both *E. verrucosa* [7] and *E. sequieriana* [8], while its isomer quercetin 3-galactoside-6″-gallate was detected in *E. platiphyllos* [7].

In the present report, two more *Euphorbia* species were investigated, namely *E. retusa* Forssk. (= *E. kahirensis* Raeusch.) and *E. sanctae-catharinae* A. Fayed. The latter is a newly reported species [9]. Both plants proved to contain kaempferol and quercetin glycosides with their 3-glucuronides forming the major glycosides in *E. retusa* and quercetin 3-rhamnoside forming the major glycoside in *E. sanctae-catharinae*. The results are outlined in Table 1. Kaempferol and quercetin 3-glucuronide-7-glucosides are reported here for the first time.

## EXPERIMENTAL

*Material.* A fresh sample of *Euphorbia sanctae-catharinae* A. Fayed was collected from Mt. Catherine, Sinai. A sample of *E. retusa* Forssk. was collected from Wadi Firan, Sinai. Both were

Table 1. The flavonoids of *Euphorbia retusa* and *E. sanctae-catharinae*

| Flavonol glycosides | *E. retusa** | *E. sanctae-catharinae** |
|---|---|---|
| Kaempferol 3-glucuronide | − | +++ |
| Kaempferol 3-glucuronide-7-glucoside | − | ++ |
| Quercetin 3-glucoside | + | − |
| Quercetin 3-glucuronide | ++ | +++ |
| Quercetin 3-rhamnoside | +++ | − |
| Quercetin 3-rutinoside | + | − |
| Quercetin 3-glucuronide-7-glucoside | + | ++ |

* +++ = major, ++ = strong, + = weak, − = absent.