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# C-GLYCOSYLFLAVONES IN *GNETUM GNEMON*

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**Key Word Index**—*Gnetum gnemon*; Gnetales; *C*-glycosylflavones; isovitexin; 7-*O*-glucosylisovitexin; vicenin II; swertisin; X″-*O*-glucosylswertisin; isoswertisin; swertiajaponin; isoswertiajaponin.

**Abstract**—The relationship of the Gnetales to other gymnosperms is presently ill understood. The occurrence of *C*-glycosylflavones as the only detactable flavonoids in leaf material of *Gnetum gnemon* chemically distinguishes this species from all other studied gymnosperms. Identified *C*-glycosides are: isovitexin and its 7-*O*-glucoside, vicenin II and the 7-*O*-methyl ethers of vitexin (isoswertisin), isovitexin (swertisin) and its X″-*O*-glucoside, isoorientin (swertiajaponin) and orientin (isoswertiajaponin). This is the first report of the natural occurrence of isoswertiajaponin.

## INTRODUCTION

With the exception of the Gnetales, which contain the genera *Gnetum*, *Welwitschia* and *Ephedra*, flavonoids of representatives of all groups of vascular plants have been documented [1]. The only information which has been presented concerning flavonoids of the Gnetales is that all species which have been examined lack biflavones; no positive identifications have been published [2]. The classification of the gnetalian genera is also unsettled, most authors suggesting that each genus be placed in a monotypic family. However, no one has suggested that the genera should be separated into individual classes implying that the three morphologically unique genera are related in a higher taxon.

The present paper documents the occurrence of *C*-glycosylflavones and their *O*-glucosides as the only detectable flavonoids in leaf material of *Gnetum gnemon*. The phylogenic significance of this in relation to other gymnospermous groups is discussed.

## DISCUSSION

The flavonoids identified in leaf material of *Gnetum gnemon* L. were isovitexin (**5**) and its 7-*O*-glucoside (**4**), vicenin II (**8**) and the 7-*O*- methyl-*C*-glucosylflavones, swertisin (**1**) and its X″-*O*-glucoside (**3**), isowertisin (**2**), swertiajaponin (**6**), and isoswertiajaponin (**7**). This is the first report of the natural occurrence of the latter compound. The dominant flavonoid was swertisin **1**. In addition to the above flavonoids, several trace components were partially characterized as *C*-glycosylapigenins. No other types of flavonoids were detected on heavily loaded 2-dimensional PC when viewed under UV or during the chromatographic purifications of individual compounds. This work verifies earlier observations of Sawada [3] and Swain [2] relating to the absence of biflavones in this group and indicates the absence of flavone and flavonol derivatives from this species. Future studies will reveal if this is indeed a generic and/or a class characteristic.

The only other 'gymnosperms' which have been reported to contain *C*-glycosylflavones are *Larix laricina* [4, Pinaceae] and the cycad *Dioon spinulosum* [5]. The only reported flavonoids for *L. laricina*, in addition to vitexin derivatives, were flavonol *O*-glycosides. Vitexin and orientin were observed to be very minor constituents of *D. spinulosum* with biflavone aglycones representing the dominant flavonoid component. With the exception of the Pinaceae [2, 3], all studied conifers as well as all genera of the Cycadales [5–7], have been found to contain biflavones. It is noteworthy that *Gnetum gnemon* leaf material does not contain chromatographically detectable biflavones, flavones or flavonols; considering its unique morphology as well as the present status of its chemistry, it is becoming even more evident that this genus represents a distinct group within the lower seed plants.

Table 1. UV spectra and chromatographic data for flavonoids of *Gnetum gnemon* leaf material

| Compound | Spectral maxima (nm)† | | | | | $R_f$* | | |
|---|---|---|---|---|---|---|---|---|
| | MeOH | $MeO^-$ | $AlCl_3$ | $AlCl_3$–HCl | NaOAc | TBA | HOAc | $H_2O$ |
| **1** | 337, 271 | 392, 271 | 354, 301, 278 | 349, 300, 279 | 393, 270 | 0.61 | 0.51 | 0.21 |
| **2** | 338, 266 | 393, 274 | 388, 346, 303, 272 | 383, 342, 303, 276 | 391, 270 | 0.49 | 0.22 | 0.05 |
| **3** | 333, 269 | 390, 268 | 353, 300, 275 | 345, 298, 276 | 390, 344, 268 | 0.38 | 0.77 | 0.56 |
| **4** | 337, 270 | 392, 274 | 382, 351, 299, 276 | 376, 346, 300, 279 | 396, 270 | 0.15 | 0.59 | 0.21 |
| **5** | 338, 272 | 391, 279 | 354, 301, 279, 266 | 348, 300, 280 | 394, 269 | 0.64 | 0.49 | 0.20 |
| **6** | 344, 271 | 406, 278 | 417, 335, 275 | 358, 276 | 400, 271 | 0.45 | 0.33 | 0.08 |
| **7** | 343, 268 | 408, 275 | 422, 330, 275 | 355, 298, 273 | 404, 265 | 0.27 | 0.14 | 0.02 |
| **8** | 337, 272 | 399, 332, 284 | 351, 304, 281 | 345, 303, 279 | 396, 280 | 0.12 | 0.52 | 0.20 |

* $R_f$ Rutin on same paper (TBA, 0.32; HOAc, 0.50; $H_2O$, 0.20).
† Main peaks only are given.

## EXPERIMENTAL

*Materials and methods.* Leaf material of *Gnetum gnemon* L. was collected on the campus of the University of Malaya during January, 1977. Voucher specimens are on file in the Herbarium of: The University of Malaya (B. C. Stone No. 12834) and Western Carolina University. Dried pulverized leaf material (50 g) was extracted with $Me_2CO$–$H_2O$ (1:1), the solvents removed *in vacuo*, and the residue taken up in aq. MeOH (75% MeOH) for 2D PC on Whatman 3MM paper. The maximum spotting conen was determined to be the equivalent of 1.6 g of the original plant material per chromatogram. PCs were developed according to ref. [8] in TBA and 15% HOAc. Si gel TLC (20 × 20 cm) were developed in EPWM according to ref. [9]. $R_f$ values (Table 1) were obtained by developing the chromatographically pure compounds 1D PC; rutin (Aldrich Chem. Co., R230-3) was used as an internal PC ref. UV-Visible spectra (Table 1) with 4 diagnostic reagents and PMR spectra of the TMS derivatives were also obtained according to ref. [8]. O-Glycosidic moieties were identified by GLC on 3% OV-1 (60/80 CGQ, 2 × 6 mm SS Column, 180°) by comparison to 70 monosaccharide standards. Acid reflux was carried out in 2 N HCl (2 hr) and all cochromatography was carried out 1 D PC in TBA, HOAc and $H_2O$ and EPWM (TLC).

**1** *Swertisin* (7-O-*methyl*-6-C-*glucosylapigenin*) *and* **2** *isoswertisin* (7-O-*methyl*-8-C-*glucosylapigenin*). PMR spectra agreed to those of isovitexin and vitexin, respectively [8] except for the presence of a signal corresponding to the methoxyl group (3.9 ppm). Integration of the PMR spectrum demonstrated the presence of a hexose. The presence of a *C*-glycosyl derivative was based on the plus convention of **1** and **2** after acid refluxing.

**3** *X″-O-Glucosylswertisin.* Acid hydrolysis produced glucose plus **1** and **2**. Enzyme hydrolysis (Pectinase, Sigma Chem. Co. P-4625; 16 hr, 20°) produced **1** alone.

**4** *7-O-Glucosylisovitexin.* Acid hydrolysis produced a mixture of vitexin and isovitexin. Pectinase hydrolysis produced only isovitexin.

**5** *Isovitexin.*

**6** *Swertiajaponin* (7-O-*methyl*-6-C-*glucosylisoorientin*) *and* **7** *Isoswertiajaponin* (7-O-*methyl*-8-C-*glucosylorientin*). Compound **6** formed an equilibrium mixture with **7** after acid refluxing and *vice versa*.

**8** *Vicenin II.* The absence of aglycone formation or isomerization after prolonged refluxing (4 hr, 2 N HCl) is suggestive of a 6,8-di-*C*-glycosyl compared with both glycosyl residues being identical. It is assumed that the *C*-glycosyl moieties are glucosyl based on the presence of other *C*-like compounds in the plant. Authentic vicenin II was not available for cochromatography.

Each isomer produced by acid refluxing of a parent *C*-glycosylflavone was subsequently reisomerized (2N HCl, 2 hr) to reform the pair which was detected cochromatographically. Except for vicenin II all compounds were cochromatographed with authentic compounds.

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# DI-*C*-METHYL-6,8 METHYL-3 KAEMPFEROL, NOUVEL AGLYCONE FLAVONIQUE ISOLE DE *DIDIEREA MADAGASCARIENSIS*

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**Key Word Index**—*Didierea madagascariensis*; Didiereaceae; Centrospermae; 6,8-di-*C*-methyl, 3-*O*-methyl kaempferol.

Les Didiereaceae, l'une des familles endémiques de l'île de Madagascar, regroupent onze espèces réparties en quatre genres: *Didierea*, *Alluaudia*, *Alluaudiopsis* et *Decaryia*. Ses représentants, arbres ou arbustes, se rencontrent d'ailleurs exclusivement dans la végétation xérophile du Sud et du Sud-Ouest malgache [1]. Les résultats rapportés dans ce travail présentent un double intérêt puisque, non seulement aucune analyse flavonique n'avait été conduite jusqu'à ce jour sur des représentants de cette famille, mais son chimisme polyphénolique montre d'ores et déjà une originalité certaine.

Ainsi, à partir d'un hydrolysat de l'écorce de la tige et/ou des épines de *Didierea madagascariensis*, avons-nous isolé un composé de fluorescence violette, dont les propriétés spectrales ($\lambda_{max}^{MeOH}$ 276, 335, (370)) et chromatographiques (Tableau 1) ne permettent pas l'identification à un aglycone connu. Le tracé spectral *in* MeOH suggère néanmoins qu'il s'agit d'un dérivé monosubstitué sur le phényle latéral, de type kaempférol ou apigénine. L'emploi des réactifs classiques [2], [3] permet de déceler deux —OH libres en positions 5 ($\Delta\lambda$ bande I ($AlCl_3$/HCl MeOH) 54 nm) et 4′ ($\Delta\lambda$ bande I (NaOH-