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C-GLYCOSYLFLAVONES OF THE GNETOPSIDA

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Key Word Index—*Ephedra*; *Gnetum*; *Welwitschia*; Gnetopsida; *C*-glycosylflavones.

Abstract—*C*-Glycosylflavones have been identified in *Ephedra antisyphilitica*, *Gnetum gnemon* and *Welwitschia mirabilis*. The *C*-glycosidic moieties of apigenin and luteolin derivatives have been identified as glucose and/or xylose for these species.

INTRODUCTION

The Gnetopsida is a small group of seed plants represented by the extant genera *Ephedra*, *Gnetum* and *Welwitschia*. Except for the alkaloid constituents [1], relatively little information has been published on their chemistry. Flavonoid constituents of the group include *C*-glycosylflavones [2–6], flavonol-*O*-glycosides [6, 7] and proanthocyanidins [6, 8]. The only flavonoids to be totally characterized include vicenins 1 and 2 from *Ephedra andina* [5] and 8-hydroxykaempferol 3-*O*-glucoside from *E. equisetina* [7]. The present report, which is a continuation of a previous effort [1, 2], concerns the identification of the sugar moieties of *C*-glycosylflavones for *Gnetum gnemon*, *Ephedra antisypilitica* and *Welwitschia mirabilis* [2, 3].

RESULTS

Welwitschia mirabilis was found to accumulate 6,8-di-*C*-glucosyl derivatives of luteolin (lucenin 2) and chrysoeriol (luteolin-3′-*O*-Me). The previously reported [3] 6-*C*-glycosylchrysoeriol was not available for further characterization. *Ephedra antisyphilitica* accumulates 6,8-di-*C*-glucosyl derivatives of apigenin (vicenin 2) and luteolin (lucenin 2) as well as their 6-*C*-xylosyl-8-*C*-glucosyl (vicenin 1, lucenin 1), and 6-*C*-glucosyl-8-*C*-xylosyl (vicenin 3, lucenin 3) derivatives. The presence of 6-*C*-glucosyl-7-*O*-methylapigenin (swertisin) was verified for *Gnetum gnemon*. Acid refluxates (1 hr, 2 N HCl) of a portion of the whole-plant extract verified the presence of proanthocyanidins for species of *Ephedra* [6, 8]; however, the presence of flavonols [6] could not be verified for *Welwitschia mirabilis*.

EXPERIMENTAL

Location of voucher specimens, work-up of flavonoids and UV–visible spectra have been presented elsewhere for *G. gnemon* [2] and *E. antisyphilitica* [3]. *W. mirabilis* (300 g dry wt) was a gift from Mr. Frank von Blottnitz, Namib Research Institute, Gobabeb, Walvis Bay, Namibia. The dry plant material was extracted with CH_2Cl_2 (Soxhlet) and subsequently with Me_2CO–H_2O (1:1). The latter was concentrated *in vacuo*, taken up in MeOH–H_2O for CC (Whatman Cellulose Powder CF-11) and developed with increasing concentration of MeOH in H_2O. The first flavonoid band, was separated into two bands by 1-D PC (TBA; 3 × overdevelopment, [9]). Permethylation and MS procedures were previously described [10]. R_f values were determined by 1-D PC using rutin as an int. standard. The R_f values × 100 for rutin were: TBA, 47; 15% HOAc, 55; H_2O, 24; phenol-saturated H_2O, 51; 2% HOAc, 31; 30% HOAc, 68; and 3% NaCl, 21. The R_f values presented below are in the same sequence.

E. antisyphilitica bands: lucenins 1, 2, 3: PC $R_f \times 100$: 11, 41, 12, 45, 17, 56, 10. PC chromatographic area 1 when permethylated produced two bands on Si TLC after three successive developments ($CHCl_3$–EtOAc–Me_2CO; 5:4:1), The upper band I-PM-2 showed the MS of a PM 6-*C*-xylosyl-8-*C*-hexosylluteolin (M^+ 734, M-119 > M-131 > M-145, M-131 > M-175). The lower band I-PM-1 showed superimposition of the MS of a PM 6,8-di-*C*-hexosylluteolin (M^+ 778) and of a PM 6-*C*-hexosyl-8-*C*-pentosylluteolin (M^+ 734) in the ratio 2:1. This band cochromatographed with PM 6,8-di-*C*-glucosyl-luteolin. Therefore PC chromatographic area 1 contains lucenins 1, 2, and 3. Vicenins 1, 2, 3: PC $R_f \times 100$: 26, 51, 21, 66, 31, 67, 19. PC chromatographic area 2 similarly led to two permethyl derivatives. The MS of II-PM-2 indicated a PM 6-*C*-xylosyl-8-*C*-hexosylapigenin (M^+ 704, M-119 > M-131 > M-145, M-131 > M-175). The MS of II-PM-1 showed superimposition of a PM 6,8-di-*C*-hexosylapigenin (M^+ 748) with a PM 6-*C*-hexosyl-8-*C*-pentosylapigenin (M^+ 704) in the ratio of 3:1. This band cochromatographed with PM 6,8-di-*C*-glucosylapigenin. Therefore PC chromatographic area 2 contains vicenins 1, 2, and 3.

W. mirabilis bands: lucenin 2: PC $R_f \times 100$: 08, 42, 14, 41, 17, 60, 14. UV nm: MeOH, 345, 273; MeO^-, 407, 345 (sh), 282; $AlCl_3$, 418, 279; $AlCl_3$/HCl, 380 (sh), 354, 290 (sh), 278; NaOAc, 406, 282 (sh), 281. Compound 1 was separated from compound 2 by a triple overdevelopment on PC in TBA [9]. The PM ether produced M^+ 778 and the fragmentation pattern of a PM 6,8-di-*C*-hexosylluteolin and cochromato-

✠ Deceased 4 August 1980.

graphed with PM 6,8-di-*C*-glucosylluteolin. The free compound 1 cochromatographed with 6,8-di-*C*-glucosylluteolin.

6,8-Di-*C*-glucosylchrysoeriol: PC $R_f \times 100$: 12, 49, 14, 68, 17, 66, 13. UV nm: MeOH, 340, 286 (sh), 275; MeO^-, 407, 337 (sh); $AlCl_3$, 388 (sh), 361, 282; $AlCl_3$/HCl, 385 (sh), 356, 282; NaOAc, 397, 321, 283. The PM ether produced M^+ 778 and the fragmentation pattern of a 6,8-di-*C*-hexosylluteolin and cochromatographed with PM 6,8-di-*C*-glucosylluteolin. The free compound cochromatographed with 6,8-di-*C*-glucosylchrysoeriol.

G. gnemon. Swertisin: UV, NMR, and R_f values for this compound have been published [2]. The previous identification was verified by cochromatography with authentic swertisin using conditions where *C*-glucosides separate from *C*-galactosides (activated Si gel TLC: EPWM).

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CONFIRMATION OF STRUCTURE OF THE FLAVONOL GLUCOSIDE TAMBULETIN

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Key Word Index—*Zanthoxylum alatum*; Rutaceae; tambuletin; 3,5,3′-trihydroxy-7,4′-dimethoxy-8-*O*-β-D-glucopyranosylflavone; structure determination.

Abstract—The structure of tambuletin, present in seeds of *Xanthoxylum* species has been confirmed as the 8-glucoside of gossypetin 7,4′-dimethyl ether.

INTRODUCTION

Zanthoxylum acanthopodium and *Z. alatum* (Rutaceae) are well-known Indian indigenous plants [1]. Bose and Bose [2] isolated two flavonols—tambulin and tambulol— from *Z. acanthopodium* and assigned the structure of 5,7-dihydroxy-3,8,3′-trimethoxy or 5,7-dihydroxy-6,8,4′-trimethoxy flavone for tambulin. Later Balakrishna and Seshadri [3] isolated tambuletin (in the place of tambulin and tambulol) from the same plant and characterized it as 8-*O*-methylherbacetin. They also considered, on synthetic grounds, that tambulin was 7,8,4′-tri-*O*-methylherbacetin. Subsequently, Harborne *et al.* [4]. showed that tambuletin isolated by Balakrishna and Seshadri [3] was a glycoside with 3,5,3′-trihydroxy-7,4′-dimethoxy-8-*O*-glucosyl or (less likely) 3,5,3′-trihydroxy-8,4′-dimethoxy-7-*O*-glucosylflavone structure; they also suggested that tambulin could be