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C-GLYCOSYLFLAVONES FROM *GNETUM BUCHHOLZIANUM* AND *GNETUM AFRICANUM*

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Key Word Index—*Gnetum buchholzianum*; *G. africanum*; Gnetaceae; *C*-glycosylflavonoids; 2″-xylosylisoswertisin; 2″-glucosylisoswertisin; apigenin 7-neohesperidoside.

Abstract—*C*-Glycosylflavones, including two new compounds 2″-xylosylisoswertisin and 2″-glucosylisoswertisin, were isolated from the leaves of *Gnetum buchholzianum* and *G. africanum*. The occurrence of apigenin 7-neohesperidoside distinguishes the latter from the first species.

INTRODUCTION

The genus *Gnetum* contains *ca* 40 species distributed in the equatorial regions of Africa, America and Asia. The occurrence of *C*-glycosylflavones and their *O*-glucosides as the only detectable flavonoids in leaf material of *Gnetum gnemon* has been reported to distinguish this species from all other gymnosperms studied [1]. *Gnetum buchholzianum* Engl. and *Gnetum africanum* Welw. are edible plants commonly used in equatorial Africa. As part of a thorough study of these vegetables [2–4], we report the isolation and identification of *C*-glycosylflavonoids, including two new 2″-*O*-glycosides of isoswertisin, from both species, and of apigenin 7-neohesperidoside from *G. africanum*.

RESULTS AND DISCUSSION

Two novel compounds, 2″-*O*-xylosylisoswertisin (**1**) and 2″-*O*-glucosylisowertisin (**2**), as well as the known *C*-glycosylflavones vitexin (8-*C*-glucosylapigenin), 2″-*O*-xylosylvitexin, 2″-*O*-glucosylvitexin, isoswertisin (8-*C*-glucosyl-7-*O*-methylapigenin), vicenin-1 (6-*C*-xylosyl-8-*C*-glucosylapigenin), vicenin-2 (6,8-di-*C*-glucosylapigenin) and vicenin-3 (6-*C*-glucosyl-8-*C*-xylosylapigenin) were isolated from the butanol-soluble fraction of the aqueous ethanol extract of *G. buchholzianum*.

Compound **1** showed the UV spectrum and diagnostic shifts [5] of a 7-*O*-substituted apigenin and the chromatographic properties of an apigenin diglycoside. Acid hydrolysis led to xylose and isoswertisin, accompanied by small amounts of swertisin. The position of attachment of xylose to the *C*-glucosyl residue was determined by the mass spectrum of the permethyl (PM) derivative of **1** which showed the molecular peak at m/z 690 and the same fragmentation pattern as PM 2″-*O*-xylosylvitexin with prominent ions $[SO]^+$ (m/z 515) and $[j]^+$ (m/z 341) [6], and by co-chromatography of PM **1** and PM 2″-*O*-xylosylvitexin.

Compound **2** showed the same UV spectrum and diagnostic shifts as **1** and its chromatographic properties were very similar. Acid hydrolysis led to glucose and isoswertisin (+ swertisin) and the mass spectrum of PM **2** was identical with that of PM 2″-*O*-glucosylvitexin [7]: $[M]^+$ (m/z 734), $[SO]^+$ (m/z 515) and $[j]^+$ (m/z 341). The 2″-*O*-glucosylisoswertisin structure of **2** was confirmed by co-chromatography of PM **2** and PM 2″-*O*-glucosylvitexin.

Vitexin and isoswertisin were identified by UV, mass spectrometry of the PM derivatives [8] and co-chromatography with authentic samples (free compounds and PM derivatives), 2″-*O*-xylosyl and 2″-*O*-glucosylvitexin by UV, acid hydrolysis, mass spectrometry of the PM derivatives and co-chromatography of the free compounds and PM derivatives with authentic samples, vicenins-1, -2 and -3 by UV, mass spectrometry of the PM derivatives [8] and HPLC of the underivatized compounds [9].

From *G. africanum* were similarly isolated isoswertisin, 2″-*O*-xylosylisoswertisin, 2″-*O*-glucosylisoswertisin, vicenin-2, vicenin-3 and two other compounds, **3** and **4**, not found in *G. buchholzianum*. Compound **3** was identified as 2″-*O*-rhamnosylisoswertisin by UV, acid hydrolysis, mass spectrometry of the PM derivative and comparison with authentic samples (free compound and PM derivative) [10]. Compound **4** was identified as apigenin 7-neohesperidoside by UV, acid hydrolysis, mass spectrometry of the PM derivative [11] and comparison with literature data.

From these results, the occurrence of *C*-glycosylflavones appears to be a characteristic feature of the genus *Gnetum*. However, interesting differences are found in this respect between *G. gnemon* (from Malaya) and the two African species, the latter containing only 8-*C*-glucosylflavones and their 2″-*O*-glycosides whereas 6-*C*-glucosylflavones are the main components of the former. On the other hand, the absence of vitexin and 2″-*O*-glycosylvitexin in *G. africanum* is a characteristic difference between the two African species. However, the most distinctive (and perhaps interesting) feature of *G. africanum* is the occurrence of a flavone *O*-glycoside as one

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of the major flavonoids present in this species, since the absence of such compounds in *G. gnemon* has been considered as a possible characteristic of the genus *Gnetum* [1].

EXPERIMENTAL

Plant material. Gnetum buchholzianum Engl. and *Gnetum africanum* Welw. were collected on the Bateke plateau, Congo. Voucher specimens are deposited at the Marien N'Gouaby University, Brazzaville, Congo.

Extraction and isolation. Dried leaves (500 g) were extracted with 70% aq. EtOH. After concn under red. pres., the aq. residue was extracted with hexane, Et_2O, EtOAc and *n*-BuOH. The BuOH concentrate was chromatographed on a cellulose column and eluted with a gradient of H_2O–MeOH. The eluates were repeatedly fractionated by PC on Whatman 3MM paper in BAW (4:1:5) and 2 or 15% HOAc until homogeneity was reached.

2″-O-*Xylosylisoswertisin* (**1**). TLC (cellulose) R_f 0.73 (15% HOAc), 0.56 (BAW 4:1:5). UV λ_{max}^{MeOH} nm: 267, 324; + $AlCl_3$ 274, 299, 343, 386; + $AlCl_3$ + HCl 273, 299, 335, 378; + NaOH 282, 296, 389; + NaOAc 267, 296, 380. Permethyl ether: EIMS 70 eV, *m/z* (rel. int.): 690 $[M]^+$ (37), 545 $[SOj]^+$ (24), 515 $[SO]^+$ (64), 499 $[S]^+$ (12), 355 $[i]^+$ (18), 341 $[j]^+$ (100), 325 $[k]^+$ (48), 311 $[l]^+$ (16). TLC (silica gel) R_f 0.09 and 0.36 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1 and 5:1:4).

2″-O-*Glucosylisoswertisin* (**2**). TLC (cellulose) R_f 0.78 (15% HOAc), 0.72 (BAW 4:1:5). UV λ_{max}^{MeOH} nm: 268, 325; + $AlCl_3$ 276, 303, 345, 385; + $AlCl_3$ + HCl 276, 302, 339, 385; + NaOH 278, 392; + NaOAc 268, 300, 392. Permethyl ether: EIMS 70 eV, *m/z* (rel. int.): 734 $[M]^+$ (4), 545 $[SOj]$ (25), 515 $[SO]^+$ (60), 499 $[S]^+$ (19), 355 $[i]^+$ (20), 341 $[j]^+$ (100), 325 $[k]^+$ (59), 311 $[l]^+$ (60). TLC (silica gel) R_f 0.08 and 0.31 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1 and 5:1:4).

Acid hydrolysis. The samples were dissolved in MeOH–4 M HCl (1:1) and heated at 100° for 1 hr in a sealed tube. After repeated evapns of the solvent, the residue was taken up in H_2O and extracted with *n*-BuOH. The aglycones were identified in the *n*-BuOH extract by TLC (silica gel) in EtOAc–pyridine–H_2O–MeOH (16:5:2:1); (cellulose) in 15% HOAc and BAW (4:1:5), the sugars in the aq. phase by TLC on Na_2HPO_4 (0.2 M) impregnated silica gel in Me_2CO–H_2O (9:1) against standard markers. The flavones and sugars were respectively detected with bis-diazotized benzidine and aniline malonate.

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