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THE STRUCTURES OF AMENTOFLAVONE GLYCOSIDES ISOLATED FROM *PSILOTUM NUDUM*

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Key Word Index—*Psilotum nudum*; Psilotaceae; amentoflavone *O*-glucosides, vicenin-2.

Abstract—On the basis of new spectroscopic evidence, structures are proposed for three amentoflavone glycosides and an apigenin di-*C*-glycoside previously isolated from *Psilotum nudum*. The major glycoside is identified as the 7,4′,4‴-tri-*O*-β-D-glucopyranoside, minor glycosides as the 4′,4‴-di-*O*-β-D-glucopyranoside and 7,4‴-di-*O*-β-D-glucopyranoside, and the apigenin di-*C*-glycoside as vicenin-2. The amentoflavone glucosides are all new natural products.

INTRODUCTION

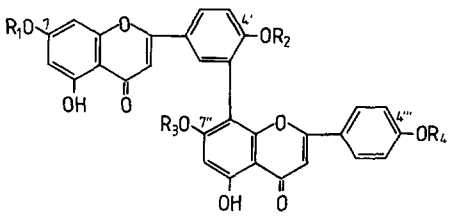
Biflavone glycosides are exceedingly rare in nature and amentoflavone glycosides have been found only once, in the primitive vascular plants belonging to the family Psilotaceae [1]. The one reported detection and isolation of these glycosides (from *Psilotum* and *Tmesipteris*) appeared in 1978 [2] and in that communication it was established that these glycosides are accompanied not only with trace quantities of the 7-*O*-glucoside, 7-*O*-rhamnoglucoside and 7-*O*-rhamnoglucoside-4′-glucoside of apigenin, but also with an apigenin 6,8-di-*C*-glycoside and massive amounts of amentoflavone itself. The structures of the biflavone glycosides were not defined beyond the fact that they are all amentoflavone *O*-glucosides, and the *C*-glycoside was tentatively identified as vicenin-2. In the present communication additional evidence is presented for the *P. nudum* compounds which defines their structures more precisely.

RESULTS AND DISCUSSION

Amentoflavone glucoside, Pn-V

Pn-V, the major biflavone glycoside in *P. nudum* gave on acid hydrolysis amentoflavone (**1**) and glucose as the only products [2]. The ratio of glucose to amentoflavone was shown to be 3:1 by integration of the ^{1}H NMR spectrum using a WEFT sequence (see Experimental). Permethylation of Pn-V followed by acid hydrolysis liberated only 2,3,4,6-tetra-*O*-methylglucose suggesting that each of the three glucoses is in the pyranose form and is glycosidically attached to a separate amentoflavone hydroxyl group. This was confirmed by ^{13}C NMR spectroscopy, which revealed sugar carbon signals at 100/100.8, 77.1, 76.4, 73.2, 69.5 and 60.6 ppm typical of simple mono-*O*-β-glucopyranosides [3].

The distribution of the three glucose units on the amentoflavone nucleus was defined initially by absorption spectroscopy (see Table 1). Pn-V gave a spectrum in methanol with band II at 270 nm and band I at 317 nm. Addition of sodium methoxide caused band II to shift to 284 nm with a marked increase in intensity and band I to shift to 364 nm with a decrease in intensity. Such behaviour is typical of amentoflavones derivatized at the 4′- and 4‴-hydroxyls [4, 5]. Addition of sodium acetate caused a doubling and partial bathochromic shift of band II indicating the presence of a free 7- or 7″-hydroxyl group [5]. On this basis Pn-V can possess either of two structures, amentoflavone 7,4′,4‴-tri-*O*-glucoside (**2**) or amentoflavone 4′,7″,4‴-tri-*O*-glucoside (**3**). Comparison of the absorption spectra of Pn-V with those of the methyl ether equivalents of **2** and **3**, namely sciadopitysin (amentoflavone 7,4′,4‴-tri-*O*-methyl ether, **4**) and kayaflavone (amentoflavone 4′,7″,4‴-tri-*O*-methyl ether, **5**), strongly supports structure **2** for Pn-V. Thus, whereas the spectra of kayaflavone differ markedly from those of Pn-V, those of sciadopitysin are superimposable when allow-



		R^1	R^2	R^3	R^4
1	Amentoflavone	H	H	H	H
2	Pn-V	glc	glc	H	glc
3		H	glc	glc	glc
4	Sciadopitysin	Me	Me	H	Me
5	Kayaflavone	H	Me	Me	Me
6	Sotetsuflavone	H	H	Me	H
7	Sequoiaflavone	Me	H	H	H
8	Isoginkgetin	H	Me	H	Me
9	Pn-IV	H	glc	H	glc
10	Podocarpusflavone-A	H	H	H	Me
11	Podocarpusflavone-B	Me	H	H	Me
12	Bilobetin	H	Me	H	H
13	Ginkgetin	Me	Me	H	H
14	Pn-II	glc	H	H	glc

Table 1. Absorption spectra of amentoflavone glucosides and known methyl ether standards

Amentoflavone derivative	Substitution pattern*	Absorption spectra (nm)†		
		MeOH	NaOMe	NaOAc
Pn-V (2)	7,4′,4‴	270, 317	284↑, 364↓	272, 280, 310 sh, 333
Sciadopitysin (4)	7,4′,4‴	270, 325	291↑, 370↓	270, 281, 313 sh, 340
Kayaflavone (5)‡	4′,7″,4‴	270, 326	279↑, 295 sh, 372↓	276, 303 sh, 332
Pn-V derived monomethyl ether (and sotetsuflavone, 6)	7″	270, 334	273, 303 sh, 394↑	272, 300 sh, 376
Sequoiaflavone (7)§	7	269, 336	280↑, 295 sh, 385↑	268, 288, 346
Pn-IV (9)	4′,4‴	271, 320	281↑, 295 sh, 366↓	277, 300 sh, 344
Isoginkgetin (8)	4′,4‴	270, 326	277↑, 300 sh, 368↓	278, 300 sh, 355
Pn-II (14)	7,4‴	268, 283 sh, 312 sh, 330 sh	276, 292 sh, 388	267, 285, 312 sh, 333 sh
Podocarpusflavone-A (10)	4‴	269, 289, 315 sh, 333	276↑, 292sh, 311sh, 392	276, 290 sh, 310 sh, 363 sh
Bilobetin (12)	4′	270, 331	277↑, 292 sh, 387	277, 298 sh, 360
Ginkgetin (13)	7,4′	270, 330	282↑, 325 sh, 392	272, 282 sh, 343
Podocarpusflavone-B (11)	7,4‴	270, 283 sh, 328	277, 295 sh, 388	270, 283, 313 sh, 332 sh
Pn-II derived dimethyl ether‖	4′,7″	267, 330	270, 300 sh, 380	271, 300 sh, 353
Pn-VI	7	271, 332	280, 392	272, 286, 340–360 br

*Methylation or glucosylation

†Symbols used: ↑, marked increase in intensity; ↓, marked decrease in intensity (relative to MeOH spectrum).

‡Spectral data from ref. [5].

§Sample from Dr. H. Geiger.

‖Some amentoflavone contamination.

ance is made for the small hypsochromic effect of glycosylation [6].

Confirmation of structure **2** for Pn-V was obtained by examination of the monomethyl ether derived by methylation with diazomethane. Acid hydrolysis of this yielded an amentoflavone monomethyl ether ($[M]^+$ 552) which gave a set of absorption spectra (see Table 1) identical to those of authentic sotetsuflavone (amentoflavone 7″-*O*-methyl ether, **6**) but different from those of sequoiaflavone (amentoflavone 7-*O*-methyl ether, **7**). Further, this methyl ether cochromatographed with sotetsuflavone (but not with sequoiaflavone) as also did its acetylated product with the equivalent acetate of sotetsuflavone. On the basis of the above data, the structure of the major amentoflavone glycoside in *P. nudum* is defined as amentoflavone 7,4′,4‴-tri-*O*-β-D-glucopyranoside (**2**).

Several other minor amentoflavone glucosides are also found in *P. nudum* [2] and these all appear to be structurally related to Pn-V. Controlled acid hydrolysis of Pn-V produced a mixture of unchanged Pn-V with Pn-IV, Pn-II and 'Pn-VI' all of which are thus seen as structural precursors of Pn-V with free 7″-hydroxyl groups.

Amentoflavone glucoside, Pn-IV

Pn-IV possesses R_f values (relative to Pn-V) consistent with a diglucoside formulation and integration of its ^{1}H NMR spectrum confirms the presence of only two glucose units. As with Pn-V, the shifts observed in the absorption spectrum on addition of sodium methoxide indicate that both the 4′- and 4‴-hydroxyl groups are substituted. The sizeable (6 nm) bathochromic shift of band II in sodium acetate confirms that it is the 7-hydroxyl group that has been liberated in the conversion of Pn-V to Pn-IV [e.g. compare spectra of sciadopitysin (**4**) and isoginkgetin (**8**) in Table 1]. The spectra of Pn-IV approximate closely to those of isoginkgetin as would be expected for an amentoflavone 4′,4‴-di-*O*-glucoside and accordingly Pn-IV is assigned structure **9**.

Amentoflavone glucoside, Pn-II

Pn-II also possesses R_f values consistent with a diglucoside and integration of its ^{1}H NMR spectrum confirms that it is a diglucoside. In the absorption spectra of Pn-II (Table 1) the bathochromic shift of band I to 388 nm on the addition of sodium methoxide (with only a slight decrease in intensity compared with Pn-V) suggests that a glucose has been lost from either the 4′- or the 4‴-position in the production of Pn-II from Pn-V. The presence of a free hydroxyl at the 4′-position is favoured by the fact that the very distinctive absorption spectra of Pn-II (e.g. the pronounced doubling of the band II absorption) approximate closely to those of podocarpusflavone-A (**10**) and -B (**11**), both of which contain free 4′-hydroxyls and substituted 4‴-hydroxyls, but not to those of bilobetin (**12**) and ginkgetin (**13**), which contain free 4‴-hydroxyls and substituted 4′-hydroxyls. The spectrum of Pn-II, in contrast to that of amentoflavone 4‴-methyl ether (**10**), is unaffected by sodium acetate thus confirming that the 7-*O*-glucosyl function present in Pn-V still remains intact. Absorption data therefore suggest that Pn-II is amento-

flavone 7,4‴-di-*O*-glucoside (**14**) and this is also supported by the near equivalence of the spectra of Pn-II and podocarpusflavone-B (**11**, see Table 1).

Methylation of Pn-II with diazomethane followed by acid hydrolysis gave an amentoflavone dimethyl ether ([M]$^+$ 566). This had absorption spectra (Table 1) consistent with the presence of a 4′- or 4‴-hydroxyl (slight decrease in band I intensity in sodium methoxide) and a 7- or 7″-hydroxyl (band II + 5 nm in sodium acetate). It did not cochromatograph on TLC with amentoflavone 7″,4‴-dimethyl ether. In the mass spectrum of this dimethyl ether the molecular ion loses 46 mu to produce a cyclic ion (**16**, see Scheme 1) [4]. Loss of 46 mu (i.e. MeOMe) in this way requires that the dimethyl ether be a 4′,7″-dimethyl ether (**15**). Under the same conditions, compounds **6**, **13** and amentoflavone 7″,4‴-dimethyl ether produced this same cyclic ion with the loss of only 32 mu (i.e. MeOH). The presence of retro-Diels–Alder fragments at *m/z* 153 (A_1 + H) and at 121 (B_2) is also consistent with this formulation and not with the alternative 7,4‴-dimethyl ether (e.g. B_2 is also present in the spectra of **6** and **13** but not amentoflavone 7″,4‴-dimethyl ether in which B_2 has *m/z* 135). It follows that Pn-II is amentoflavone, 7,4‴-di-*O*-β-D-glucopyranoside (**14**).

Amentoflavone glucoside, Pn-VI

Pn-VI appears on a 2D-paper chromatogram close to the aglycone, amentoflavone, and has R_f values which require it to be a monoglucoside. The 'Pn-VI' produced by partial hydrolysis of Pn-V appeared as a rather large diffuse spot and was probably a mixture of several monoglucosides. This may also be true for the small amounts of Pn-VI isolated from *P. nudum*, since the absorption spectra vary somewhat from isolation to isolation (cf. data in Table 1 with that in ref. [2]). The predominant glucoside in the present study appears to be the 7-*O*-glucoside since the absorption data match better with those of amentoflavone 7-*O*-methyl ether (sequoiaflavone, **7**) than with those of amentoflavone-4′-methyl ether (**12**) or 4‴-methyl ether (**10**), in general form.

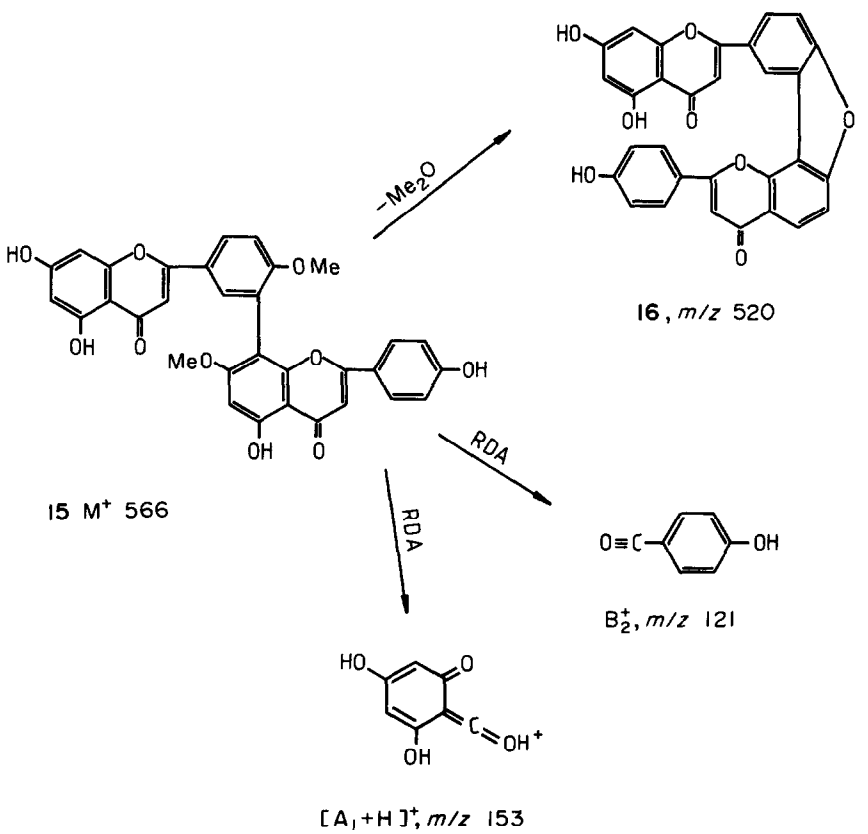
Flavone C-glycoside, Pn-VIII

The previous tentative identification of Pn-VIII as vicenin-2 [2] has now been confirmed. Not only was Pn-VIII chromatographically identical to authentic vicenin-2 under a wide range of chromatographic conditions but also its permethyl ether cochromatographed with the permethyl ether of authentic vicenin-2 in a range of solvents on cellulose and silica gel TLC.

EXPERIMENTAL

Plant material and extraction. As described in ref. [1].

NMR spectroscopy. All spectra were measured at 30° on a Varian FT80A, using DMSO-d_6 as solvent for ^{13}C NMR and D_2O for ^{1}H NMR For the proton spectra a WEFT (water elimination Fourier transform) sequence [7] was used to elim-



Scheme 1. Key electron-impact mass spectral fragmentations of the dimethyl ether from Pn-II.

inate the effect of traces of H_2O on the integrated values for glucose levels.

Mass spectroscopy. EIMS were measured on a Kratos MS-30.

Absorption spectra. Absorption spectra were measured with and without shift reagents in accordance with procedures previously outlined [6] For data see Table 1.

Permethylation method. Standard method using DMF solvent, NaH and MeI as described in ref. [6].

Methylation with CH_2N_2 The amentoflavone glucoside in MeOH (but dissolved in H_2O first if necessary) was treated at 0° with excess ethereal CH_2N_2, repeatedly, over a period of 2 hr. The product, after removal of solvent, was dissolved in 3 M HCl and heated at 100° for 45 min. The methylated aglycone recovered by evapn was then compared by TLC against standards. Purification for MS work was achieved by TLC on silica gel in BEAA (C_6H_6–EtOAc–HOAc, 10:3:2)

Partial hydrolysis of Pn-V. Pn-V in 1 M TFA was heated at 100° for 30 min and the products were recovered by rotary evaporation. Product analysis by 2D-TLC revealed a pattern of UV-absorbing spots similar to that previously reported for *P. nudum* biflavone glycosides [2] Cochromatography of each of the products with authentic *P nudum* glycosides on cellulose TLC in TBA (*t*-BuOH–HOAc–H_2O, 3:1.1), HOAc (15% HOAc), and BEW (*n*-BuOH–EtOH–H_2O, 4:1:2.2) revealed the presence of Pn-V, Pn-IV, Pn-II and possibly Pn-VI (see Discussion) in the hydrolysis products.

Pn-V data. R_f (PC) 0.2 (TBA), 0 81 (HOAc), $[\alpha]_D^{20}$ (H_2O; 0.4 g/100 ml) −9.3°; ^{1}H NMR ratio of aromatic to sugar protons, 1:1.85 (≡ 3 15 sugars), ^{13}C NMR (measurement conditions maximized for measurement of sugar carbons), clear signals observed at 132.5/131.7/131.0 (C-2‴,6‴), 127.9 (C-2′,6′), 116 3 (C-3‴, 5‴, 5′), 104 0, 103.4, 100.8/100.0 (C-6, 6″, glc C-1), 94.6 (C-8), 77 1/76 4 (glc C-3, 5), 73 2 (glc C-2), 69.5 (glc C-4), 60.6 (glc C-6).

Methyl ether (**6**) *from Pn-V* R_f values (amentoflavone, 7″-methyl ether, and 7″,4‴-methyl ether standards)· BEAA (silica gel) 0.41 (0.19, 0.41, 0.56); BPF (C_6H_6–pyridine–HCO_2H, 36:9:5) silica gel 0.24 (0 07, 0 24, 0 37), *n*-BuOH saturated with 0 88 NH_4OH plus MeOH to homogenize (silica gel) 0.26 (0.23, 0.26, —); 50% HOAc (cellulose) 0.79 (0 73, 0 79, —); MeOH–HOAc–H_2O, 18.1.1 (polyamide) 0.19 (0 07, 0.19, 0 33). **6** was distinguishable from **7** on silica gel in C_6H_6–dioxan–H_2O, 90.25·4 (repetitive runs) but not in BPF, BEAA or BPEFD [5] MS (major ions common also to the spectrum of sotetsuflavone): *m/z* 552 $[M]^+$*, 534, 521, 520, 121 $[B_2]^+$. *Methyl ether* (**6**) *acetate* prepared by heating **6** with pyridine–Ac_2O. TLC analysis (silica gel, C_6H_6–dioxan–H_2O, 90:25.4 and C_6H_6–Me_2CO, 2:1) revealed two blue fluorescing acetates (UV) which cochromatographed with the two blue fluorescing acetates produced from sotetsuflavone under the same conditions 2,3,4,6-*Tetramethylglucose* was produced from Pn-V by permethylation (see above) followed by hydrolysis of the product (1 M HCl, 45 min, 100°). After dilution with H_2O and $CHCl_3$ extraction, the aq. product was cochromatographed with authentic 2,3,4,6-tetramethyl-, 2,3,4-trimethyl- and 2,4,6-trimethyl-glucose on paper in BAW and BBPW (*n*-BuOH–C_6H_6–pyridine–H_2O, 5:1.3:3). Subsequent spraying with aniline hydrogen phthalate [6] revealed that the sole product from Pn-V was chromatographically equivalent to 2,3,4,6-tetramethylglucose

Pn-IV data. R_f (PC) 0 60 (TBA), 0.76 (HOAc); ^{1}H NMR proton ratios (see Pn-V); 1:1.4.

Pn-II data. R_f (PC) 0.48 (TBA), 0.53 (HOAc); ^{1}H NMR proton ratios (see Pn-V), 1:1.35 *Methyl ether* (**15**) *from Pn-II:* R_f (BEAA, silica gel; also amentoflavone, 7″-methyl ether and 7″,4‴-methyl ether standards) 0.46 (0 17, 0.3, 0.48), MS (major ions) *m/z* (rel int.): 566 $[M]^+$ (100)†, 534 (25), 520 (20), 153 (35), 121 (100).

Pn-VI data. R_f (PC) 0.75 (TBA), 0.30 (HOAc).

Pn-VIII data. Pn-VII cochromatographed with authentic vicenin-2 (from *Vitex lucens* and *Conocephalum conicum*) in the following systems: PC: R_f 0.28 (TBA), 0.50 (HOAc); TLC-cellulose: 0.21 (BAW); TLC-silica gel· 0.13 (EtOAc–pyridine–H_2O–MeOH, 16:4:2:1). The permethyl ether derivative of Pn-VIII cochromatographed with authentic PM-vicenin-2 in the following: TLC-silica gel: R_f 0 35 ($MeCOMe$–$CHCl_3$, 1·4), 0 70 ($CHCl_3$–EtOAc–MeOH, 5:4:1); TLC-cellulose· 0.63 (15% HOAc).

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*A lower intensity ion evident at *m/z* 566 was also present in the spectra of sotetsuflavone and sequoiaflavone.

†As in the spectra of **13** and amentoflavone-7″,4‴-dimethyl ether, a lower intensity ion at *m/z* 580 was also evident. This ion disappeared when the amount of sample used was reduced.