Phytochemistry, 1977, Vol. 16, pp. 2041–2043. Pergamon Press. Printed in England.

C-GLYCOSYLFLAVONES FROM *AVENA SATIVA*

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(*Received* 10 *June* 1977)

Key Word Index—*Avena sativa*; Gramineae; *C*-glycosylflavones; isoswertisin 2″-rhamnoside; vitexin 2″-rhamnoside; isovitexin 2″-arabinoside; isoorientin 2″-arabinoside; isoscoparine; 6.8-di-*C*-glucosylapigenin.

Abstract—*Avena sativa* leaves, stems and inflorescences contain a range of new *C*-glycosylflavone 2″-*O*-glycosides, including vitexin and isoswertisin 2″-rhamnosides, isovitexin and isoorientin 2″-arabinosides. The structure of 'vitexin 4′-rhamnoside' from *Crataegus oxyacantha* is revised in vitexin 2″-rhamnoside.

INTRODUCTION

In previous papers on the localization of flavonoids in plastids, Weissenböck *et al.* [1–3] reported the isolation, from etioplasts and chloroplasts of *Avena sativa* (cv Gelbhafer), of four *C*-glycosylflavonoids F1, F2, F3 and F5 which were considered, on the basis of UV and acid hydrolysis data, as genkwanin 8-*C*-(rhamnosyl, glucosyl) glycoside, apigenin 6-*C*-arabinosyl-glucoside, apigenin 8-*C*-glucosylglucoside and isovitexin (apigenin 6-*C*-glucoside) respectively. However some of these assignments had to be revised from MS data and we now report on the structures of F1, F2, F3 and other *C*-glycosylflavones isolated from *Avena* leaves, stems and inflorescences during a study of the flavonoid pattern changes during development of the oat plant [4].

RESULTS AND DISCUSSION

F1, F2 and F3 were permethylated and the permethylation products separated by TLC [5]. F1 led to one main product, PM F1, which gave the characteristic MS [6] of a PM *O*″-deoxyhexosyl 8-*C*-hexosylapigenin, F3 to one product showing the same R_f and MS as PM F1, and F2 to two products, PM F21 and PM F22. The former gave the characteristic MS [6] of a PM *O*″-pentosyl-6-*C*-hexosylapigenin in which absence of M-15 and M-31 peaks allowed the assignment of the pentose residue to the 2″ position [7]. PM F22 showed the MS of a *C*-methyl derivative of PM F21, an artifact arising from permethylation [5].

The presence of rhamnose in PM F1 and PM F3 was proved by methanolysis and GLC of the resulting methyl ethers. Confirmation of the existence of only one mole of rhamnose in F1 itself came from methanolysis of F1, followed by trifluoroacetylation and quantitative GLC of the products. Identity of PM F1 and PM F3 showed the 7-substituent in F1 to be OMe, in agreement with the previous hypothesis and with the presence of a three protons singlet (δ 4.05) in the NMR spectrum of the acetate of F1.

Identification of the *C*-hexosyl group in F1 and F2 as a *C*-glucosyl one came from co-chromatography of F1 and F2 acid hydrolysis products with authentic vitexin and isovitexin in conditions where *C*-galactosyl and *C*-glucosylapigenins can be differentiated [8]. From these and previous data [1], F1 and F3 appeared as isoswertisin (7-*O*-methylvitexin) and vitexin *O*″-rhamnosides and F2 as isovitexin 2″-arabinoside.

The 2″-rhamnoside position in F1 and F3 and the 2″- arabinoside position in F2 were in agreement with absence of the high field 2″-OAc signals [9] in the NMR spectra of their acetates, with chromatographic and MS identity of PM F1 and PM F3 with the permethyl derivative of synthetic 8-*C*-neohesperidosyl-acacetin [7] and with chromatographic and MS identity of the hexamethyl vitexins resulting from acid hydrolysis of PM F1, PM F3 and PM vitexin 2″-xyloside [10].

On the other hand, comparison of F3 with an authentic sample of 'vitexin 4′-rhamnoside' from *Crataegus oxyacantha* [11] showed identity of R_f on PC and TLC, mp and mmp, UV spectra, diagnostic shifts and IR spectra for the free compounds, of R_f on TLC and MS for the PM derivatives. Consequently the vitexin 4′-rhamnoside structure of the compound from *Crataegus oxyacantha* must be revised to vitexin 2″-rhamnoside. This conclusion was in agreement with the recent assignment of a vitexin 2″-rhamnoside structure to the vitexin rhamnoside isolated from *Crataegus monogyna* [12].

F3 and F2 structures correspond with those of the *C*-glycoflavones A1 and A2 isolated by Harborne and Hall [13] from *Avena sativa* (cv Blenda) and which had provisionally been formulated as apigenin 8-*C*-rhamnosylglucoside and apigenin 8-*C*-arabinosyl-hexoside respectively, in the ignorance of saponaretin (isovitexin) structure at that time. F1 may be identical with an isoswertisin O″-rhamnoside recently isolated from *Avena sativa* (cv Shokan 1) [14].

When oat plants were grown under field conditions, two new *C*-glycosylflavones F_a and F_e, appeared in primary leaves. The main one, F_a, was hydrolysed

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by acid into F_e and arabinose, F_e being identified with isoorientin (6-*C*-β-D-glucopyranosyluteolin) by direct comparison. The isoorientin 2″-arabinoside structure for F_a was assigned on the basis of the absence of M-15 and M-31 peaks in the MS of its PM derivative [7]. The only isoorientin *O*″-arabinoside of definite structure previously known is isoorientin 6″-arabinoside from *Swertia perennis* in which the arabinose position was deduced from ^{13}C-NMR spectra [15].

In stems, another new *C*-glycosylapigenin, AD, was found in addition to the preceding ones. It could be identified by MS of its PM derivative [5] as a 6,8-di-*C*-hexosylapigenin and, by co-chromatography of free and permethylated compounds, as 6,8-di-*C*-glucosylapigenin (vicenin-2).

Inflorescences showed a very complex flavonoid pattern, in which additional *C*-glycosylflavones could be isolated with difficulty and in very small amounts. One of them, F_f, unhydrolyzable, was identified as isoscoparin (6-*C*-β-D-glucopyranosylchrysoeriol) [16] by direct comparison. Two others, F_c and F_d, isoorientin *O*″-glycosides from their UV spectra and acid hydrolysis products, although chromatographically homogeneous, were shown to be heterogeneous by TLC of the permethylation mixture, two main bands being obtained from each of them. The four bands led to the same MS as PM isoorientin 2″-arabinoside, except that the molecular peak could not be observed. Thus F_c and F_d are mixtures of isoorientin 2″-diosides since the molecular peak could always be found in the MS of PM 6-*C*-glucosyl-O″-monosides. This would be in agreement with the difficulties encountered in their separation.

An interesting observation about the *C*-glycosylflavonoid pattern found in *Avena sativa*: both 6- and 8-*C*-glucosylapigenins are present as 2″-glycosides, but the bound sugar is different, suggesting some specificity of the *O*-glycosylating enzyme for 6- or 8-C-glucosyl apigenin, unlike the glucosylflavonoid pattern found in *Fortunella margarita* where 6- and 8-*C*-neohesperidosyl acacetin occur with 7-*O*-neohesperidosyl acacetin [17]. Brederode and Nigtevecht [18] have demonstrated the existence, in the petals of *Melandrium album*, of two different UDP-glucose : isovitexin glucosyltransferases, one catalysing the transfer of glucose to the 7-hydroxyl group of isovitexin, the other catalysing the transfer of glucose to the carbon–carbon bound glucose of isovitexin, but vitexin was not tested as a substrate.

EXPERIMENTAL

For plant material, isolation procedures, chromatographic properties, acid hydrolysis, UV spectra, see [19], permethylation and purification of PM derivatives see [5]. NMR and MS were recorded on Varian HA-100 and AEI MS 902 instruments.

Compound F1 (isowertisin 2″-rhamnoside). Acetate (Ac_2O + Py), NMR ($CDCl_3$) $\delta^{10^{-6}}_{TMS}$ 4.05 (*s*, OMe), 2.1–1.9. (*m*, aliph. OAc); permethyl ether (PM F1), MS (*m/e*): 704 (M^+, 41%), 690 (M-14, 4%), 675 (M-29, 3%), 544 (M-160, 44%), 515 (M-189, 100%), 501 (10%), 499 (15%), 485 (5%), 467 (7%), 355 (10%), 341 (100%), 325 (33%), 311 (25%), 209, 193, 189, 152, 143, 135, 115, 101, 88.

Hexamethylvitexin from PM F1. Hydrolysis of PM F1 in 4N HCl–MeOH (1:1) 2 hr at 100°, evapn, extraction with $CHCl_3$ and PLC (Si gel H) in $CHCl_3$–EtOAc–Me_2CO (4:1:4); MS (*m/e*) 516 (M^+, 100%), 502 (M-14, 3%), 487 (M-29, 4%), 485 (M-31, 3%), 397 (4%), 368 (25%), 355 (31%), 341 (37%), 325 (17%), 311 (12%), 209 (23%), 193 (11%), 135, 115, 111, 101, 95, 88.

Compound F2 (isovitexin 2″-arabinoside). Acetate (Ac_2O + Py), NMR, ($CDCl_3$) $\delta^{10^{-6}}_{TMS}$ 7.95 (*d*, *J* = 9 Hz) H-2′, 6′; 7.51 (*s*) H-8; 7.31 (*d*, *J* = 9 Hz) H-3′, 5′; 6.65 (*s*) H-3; 5.5–3.0 (*m*) sugar protons; 4.87 (*d*, *J* = 10 Hz) H-1″; 2.45, 2.41, 2.33 (*s*) arom. OAc; 2. 1–1. 94 (*m*) aliph. OAc; permethyl ethers, PM F21, MS (*m/e*): 690 (M^+, 8%), 529 (3%), 515 (M-175, 45%), 499 (100%), 467 (9%), 397 (6%), 381 (5%), 355 (9%), 341 (58%), 325 (14%), 311 (7%); PM F22, (*C*-methyl ether), MS (*m/e*): 704 ($M^{+\cdot}$, 8%), 529 (M-175, 41%), 513 (100%), 355 (50%), 339 (10%), 325 (7%).

Compound F3 (vitexin 2″-rhamnoside). Mp 208–210°; acetate (Ac_2O + Py), NMR ($CDCl_3$) $\cdot\delta^{10^{-6}}_{TMS}$ 7.91 (*d*, *J* = 9 Hz) H-2′, 6′; 7.38 (*d*, *J* = 9 Hz) H-3′, 5′; 6.67 (*s*) H-3; 6.61 (*s*) H-6; 5.5–3.0 (*m*) sugar protons; 2.45, 2.39, 2.34 (*s*) arom. OAc; 2.1–1.8 (*m*) aliph. OAc; 0.76 (*m*) rhamnose Me; permethyl ether (PM F3), MS (*m/e*) 704 (M^+ 57%), 690 (M-14, 1%), 675 (M-29, 3%), 544 (M-160, 41%), 515 (M-189, 100%), 499 (16%), 485 (5%), 467 (6%), 355 (9%), 341 (98%), 325 (30%), 311 (25%), 209, 193, 189, 152, 143, 135, 101, 88.

Hexamethylvitexin from PM F3. MS (*m/e*) 516 (M^+, 100%), 502 (M-14, 3%), 487 (M-29, 6%), 485 (M-31, 4%), 397 (7%), 367 (10%), 355 (40%), 341 (54%), 325 (29%), 311 (19%), 209 (42%), 193 (20%), 152, 135, 115, 111, 101, 95, 88.

Hexamethylvitexin from PM vitexin 2″-xyloside. MS (*m/e*) 516 (M^+, 100%), 502 (M-14, 13%), 487 (M-29, 4%), 485 (M-31, 3%), 397 (3%), 367 (6%), 355 (20%), 341 (29%), 325 (15%), 311 (10%), 209 (15%), 193 (7%), 152, 135, 115, 111, 101, 88.

Vitexin rhamnoside (from Crataegus oxyacantha). Permethyl ether MS(*m/e*) 704 (M^+, 37%), 690 (M-14, 3%), 675 (M-29, 3%), 544 (M-160, 34%), 515 (M-189, 92%), 499 (12%), 485 (4%), 467 (6%), 355 (8%), 341 (100%), 325 (26%), 311 (25%), 209, 193, 189, 143, 135, 115, 101, 88.

Sugar identification in PM F1 and PM F3. Methanolysis by heating 8 hr in 0.5 N anh. MeOH–HCl at 80°, evapn to dryness N_2; GLC on 3% Carbowax 6000 (temp. progression 90–200°, 2°/min): permethylrhamnose (two peaks) from both.

Sugar determination in F1. Methanolysis of F1 (0.6 mg) and mesoinositol (0.05 mg) as internal standard, evapn to dryness N_2; 10 min heating at 150° with TFA anhydride–CH_2Cl_2; GLC on 5% OV 210 (temp. progression 110–210°, 2°/min): TFA methylrhamnoside (two peaks)/TFA inositol = 3.

Compound F_a (isoorientin 2″-arabinoside). Permethyl ether, MS (*m/e*) 720 (M^+, 4%), 589 (2%), 575 (3%), 559 (5%) 545 (M-175, 38%), 529 (M-191, 100%), 497 (10%), 411 (6%), 385 (14%), 371 (73%), 355 (25%), 341 (16%).

Compound AD (6,8-di-C-glucosylapigenin). Permethyl ether, MS (*m/e*) 748 (M^+, 26%), 733 (M-15, 30%), 717 (M-31, 100%), 703 (M-45, 6%), 701 (M-47, 10%), 685 (M-63, 63%), 645 (M-103, 15%), 615 (M-133, 7%), 585 (M-163, 33%), 573 (M-175, 48%), 559 (M-189, 9%), 543 (M-205, 10%), 541 (M-207, 10%).

Acknowledgements—This work was supported by CNRS at the University of Lyon, by the Alexander von Humboldt-Stiftung (G.P.) and the Deutsche Forschungsgemeinschaft (G.W.). We gratefully acknowledge the help of J. Favre-Bonvin for MS, of Dr. Jaggy (Firma Schwabe, Karlsruhe) for a sample of vitexin rhamnoside from *Crataegus oxyacantha* and of Dr. Nikolov (Sofia) for the text of his communication in Munich.

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Phytochemistry, 1977, Vol. 16, pp. 2043–2044. Pergamon Press. Printed in England.

SUR LA PRESENCE D'AMENTOFLAVONE CHEZ *TMESIPTERIS TANNENSIS*

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(*Received* 15 *July* 1977)

Key Word Index—*Tmesipteris tannensis*; Psilotales; Pteridophyta; amentoflavone; 3′-8″ biapigenin; chemosystematics; phylogeny.

L'analyse flavonique d'un échantillon fraîchement récolté de *Tmesipteris tannensis* a permis de mettre en évidence diverses flavones dont l'une—composé majeur—présente un spectre UV-visible, dans le MeOH, de type flavone monosubstituée sur le phényle latéral (λ_{max} en nm: 270, (291), 334). Chromatographié dans divers systèmes (Tableaux 1 et 2), ce composé révèle un comportement analogue à celui d'une biflavone, apigényl C—C apigénine. Les données de la SM, pic moléculaire nettement apparent M^+ à *m/e* 538 (40%) ($C_{30}H_{18}O_{10}$), confirment les donnés précédentes: dimère de l'apigénine mode de liaison des monomères de type C—C. L'interprétation du spectre de RMN (12 H) (Tableau 3) permet tout d'abord de retenir un mode de liaison entre le noyau B d'un monomère et le noyau A de l'autre; en effet, —au niveau des protons du noyau A, nous retrouvons les H-6 et H-8 d'un des monomères (A_1) (doublets centrés respectivement à 6,20 et 6,45 ppm et présentant chacun une constante de couplage de type *méta*) et un seul proton pour l'autre monomère (A_2) dont le δ (6,37 *s*) l'identifie à un H-6″ (H-6″, δ 6,40 ppm selon [1], H-8 δ 6,68 selon [2]). C'est donc sur ce monomère et au niveau du C 8″ que se situe un des points de liaison de la biflavone;—au niveau du noyau B, le second monomère (B_2) nous montre évidemment un double système AB, correspondant aux protons 2‴-6‴ (δ 7,59 J = 9 Hz) et 3‴-5‴ (δ 6,70 J = 9 Hz), au premier monomère (B_1), nous attribuons le multiplet centré à 7,99 ppm (J = 9 Hz) aux protons en 2′ et 6′ et le doublet centré à 7,12 ppm (J = 9 Hz) au proton 5′ [2]: le second point de liaison de la biflavone se situe donc au niveau du carbone en 3′. L'ensemble de ces données et les valeurs chromatographiques nous permettent donc d'identifier le composé à l'amentoflavone.

La diagnose flavonique de *Tmesipteris* se révèle donc identique à celle de *Psilotum triquetrum* [3]: absence de proanthocyane, de flavonol et de flavone, présence de biflavone. Ce résultat que nous n'avions pu obtenir lors d'une précédente analyse effectuée sur un gramme

Tableau 1. R_f (× 100) des flavones

Solvants	Système I 1	Système I 2	Système I 3	Système II 4
Apigénine	50	90	90	90
Vitexine	0–10	0–10	62	59
Amentoflavone (*Psilotum*)	10	28	90	92
Composé *Tmesipteris*	10	27	90	92

Système I: TLC gel de Si G Merck, solvant 1: C_6H_6–Py–(NH_4)OH (80:20:une goutte); solvant 2: C_6H_6–Dioxane–HOAc (90:25:4); solvant 3: AcOEt–Py–H_2O (80:12:10) + *ca* 5 cm^3 MeOH; système II: Papier Whatman 1, solvant B.A.W. *n*-BuOH–HOAc–H_2O (4:1:5) (phase supérieure).

Tableau 2. R_f (× 100) des biflavones

Solvants	Données expérimentales 1	Données expérimentales 2	Données de la littérature [12] 1	Données de la littérature [12] 2
Amentoflavone (*Psilotum*)	17	40	17	40
Composé *Tmesipteris*	17	40	—	—
Sotetsuflavone	—	—	37	52
Hinokiflavone	—	—	32	47
Cupressuflavone	15	37	16	36

Système: TLC gel de Si; solvant 1: C_6H_6–HCO_2H–Py (36:5:9); solvant 2: C_6H_6–Py–HCO_2H–Dioxane (5:1:2:2).