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C-GLYCOSYLFLAVONES FROM ROOTS OF *GLYCINE MAX*

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Key Word Index—*Glycine max* var. Amsoy 71; Leguminosae; soya bean; *C*-glycosylflavones; di-*C*-hexosylgenkwanin.

Abstract—Chemical investigation of the roots of *Glycine max* yielded six *C*-glycosylflavones identified on the basis of spectral data as carlinoside, isocarlinoside, vitexin, vitexin 2″-*O*-rhamnoside, isoschaftoside and a new compound 6,8-di-*C*-hexosylgenkwanin.

INTRODUCTION

Glycine max is an economically important legume which has already been investigated from many viewpoints such as nitrogen metabolism [1], its symbiotic relationship with *Rhizobium* [2], and phytoalexin production [3]. Our aim is to analyse within the rhizosphere both quantitatively and qualitatively the phenolic root exudates in order to determine their influence on soil micro-organisms [4]. Identification of the phenolic exudates was achieved by comparison with natural compounds occurring in root material. From fresh roots, a number of phenolic compounds were isolated, mostly isoflavonoids and flavonoids. Previous investigations have established the presence of isoflavonoids [5–7]; so, the present work deals with the structural elucidation of six of more than ten *C*-glycosylflavones isolated.

RESULTS AND DISCUSSION

Compounds **1** and **2** were shown to be luteolin derivatives from UV spectral data [8], which gave evidence for four free hydroxyl groups in the 5, 7, 3′ and 4′ positions. In both cases MS of the PM derivatives detected a molecular peak at *m/z* 734 and a fragmentation pattern typical for dissymetric di-*C*-glycosylluteolins [9]: PM **1** showed the MS of a PM 6-*C*-pentosyl-8-*C*-hexosylluteolin ($[M]^+$ 734, $[M-119]^+$ and $[M-131]^+$ giving rise to peaks greater than those of $[M-163]^+$ and $[M-175]^+$). The pentosyl group could be identified as arabinosyl ($[M-131]^+ > [M-119]^+ > [M-145]^+$); moreover, PM **1** showed the same R_f as PM isocarlinoside (PM 6-*C*-α-L-arabinosyl-8-*C*-β-D-glucopyranosylluteolin). Isocarlinoside was first identified from the leaves of *Lespedeza capitata* [10] and is recorded here for the second time.

The MS pattern of PM **2** agreed with an 6-*C*-hexosyl-8-*C*-pentosylluteolin ($[M]^+$ 734, $[M-175]^+ > [M-131]^+$) [9]; cochromatography of **2** and carlinoside and their PM derivatives showed them to be identical [11]. These data suggested that **2** is 6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranosylluteolin.

Compound **3** was identified as vitexin by MS and cochromatography of the permethylated compound. Compound **4** exhibited the same UV spectrum and diagnostic shifts as **3**, and gave vitexin, isovitexin and rhamnose on acid hydrolysis; rhamnose was identified by GC of its TMS derivative. Finally, permethylation of **4** and TLC gave one main product with the same R_f and MS as PM vitexin 2″-*O*-rhamnoside: $[M]^+$ 704 [48%], M–PM rhamnose 515 [100%], PM apigenin-CH=$^+$OH 341 [91%] [12, 13].

From cochromatography of the free compound and PM derivative, **5** was found to be identical with isoschaftoside (6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin). This identification was supported by MS of PM **5** which showed the same fragmentation pattern as PM **1** but with a molecular ion *m/z* 704.

Compound **6** was not changed by acid hydrolysis; UV spectral data agreed with an apigenin aglycone with a methoxyl group at the 7-position ($\Delta\lambda_{II}$ NaOAc–MeOH = 0). MS of PM **6** exhibited a molecular peak $[M]^+$ 748 typical for a PM 6, 8-di-*C*-hexosylapigenin with significant fragmentation $[M-31]^+$ (100%), $[M-175]^+$ (42%) and $[M-163]^+$ (34%). Cochromatography of PM **6** showed it to be different from PM vicenin 2 and synthetic PM 6,8-di-*C*-galactosylacacetin and as **6** could not be either di-*C*-glucosyl, or di-*C*-galactosylgenkwanin, it must be a dissymetric di-*C*-galactosylglucosylgenkwanin. Compound **6** and its PM derivative have been compared chromatographically with a sample isolated from *Cerastium arvense* [Dubois, M. A., personal communication], for which the structure 6-*C*-glucosyl-8-*C*-galactosylapigenin has been proposed; both PM derivatives appeared identical. However we cannot consider this comparison as exact proof of the structure of **6** since the work of Dubois has not yet been published but it is the first report of genkwanin 6, 8-di-*C*-hexoside in plants. Unfortunately, **6** was isolated in such small quantity that it was impossible to run ^{13}C NMR and to perform all the chromatographic tests for exactly locating the sugar moieties.

From a physiological point of view, *C*-glycosylflavones were not detected in *in vitro* culture either in root material or in the culture medium, even of 30-day-old seedlings. In contrast, adult root material collected in the field from 80 day old plants contained a significant amount of *C*-glycosylflavones (about 0.5% dry weight) which was

excreted into the soil as roots were dying. From a phytochemical point of view, the great diversity of *C*-glycosylflavones in soyabean is of note and similar complexity has been reported in other legumes: e.g. in *Coronilla* [14], *Lupinus* [15] and *Rhynchosia* [16].

EXPERIMENTAL

Plant material. Glycine max L. var. Amsoy 71 was collected in August 1978 in a field of the INRA station near Dijon, France.

Isolation procedure. 900 g air dried root material was extracted with hot EtOH–H_2O (1:1) and the concd extract taken up in boiling H_2O. The H_2O extract was extracted with EtOAc and subsequently *n*-BuOH. Compound **5** precipitated from the EtOAc extract (250 mg). The BuOH extract was separated on sephadex LH 20 by elution with MeOH into three large fractions from which were isolated, by prep. PC Whatman n° and DC 6 polyamide TLC, five compounds in very small quantities (0.5–3 mg). Purity of these compounds was monitored on TLC (cellulose and polyamide). Solvents: PC and cellulose TLC, 15% HOAc; DC 6 polyamide TLC, C_6H_6–MeCOEt–MeOH (4:3:3) and H_2O–EtOAc–MeOH–HOAc–dioxan (16:2:2:1:1); TLC silica gel, $CHCl_3$–EtOAc–Me_2CO (5:4:1 and 5:1:4).

Acid isomerisation. The pure compounds were treated with 2 N HCl under reflux at 100° for 2 hr. Hydrolysates were extracted with *n*-BuOH and products isolated by prep. TLC. The comparisons with authentic samples were performed according to the classical procedures (UV and R_f).

Sugar identification. Sugars were identified by GC after silylation with C_5H_5N and BSTFA + 1% HMDS, on Gas Chrom Q, 80–100 mesh, with 5% SE 52 [17].

Permethylation and MS. Preparation of PM derivatives was achieved as previously described [18, 19].

Compound **1** (*isocarlinoside*). TLC cellulose R_f 0.23 (15% HOAc), 0.22 (BAW, 4:1:5); polyamide R_f 0.58 (H_2O–*n*-BuOH–Me_2CO–dioxan, 13:3:2:1). UV λ_{max}^{MeOH} nm: 258, 269, 349; + NaOAc 280, 400; + NaOAc + H_3BO_3 266, 372; + $AlCl_3$ + HCl 262 sh, 277, 298, 354, 384; + NaOH 278, 334, 404. MS of PM ether, 70 eV, *m/z* (rel. int.): 734 $[M]^+$ (21), 719 $[M-15]^+$ (25), 703 $[M-31]^+$ (100), 615 $[M-119]^+$ (24), 603 $[M-131]^+$ (35), 589 $[M-145]^+$ (11), 571 $[M-163]^+$ (6), 559 $[M-175]^+$ (11). PM isocarlinoside, TLC (silica gel) $CHCl_3$–EtOAc–Me_2CO, 5:4:1: (R_f 0.16); 5:1:4: (R_f 0.60).

Compound **2** (*carlinoside*). TLC cellulose R_f 0.33 (15% HOAc), 0.25 (BAW, 4:1:5); polyamide R_f 0.55 (H_2O–*n*-BuOH–Me_2CO–dioxan, 13:3:2:1). UV λ_{max}^{MeOH} nm: 259, 270, 349; + NaOAc 282, 402; + NaOAc + H_3BO_3 268, 378; + $AlCl_3$ + HCl 264 sh, 280, 360, 384; + NaOH 278, 334, 404. MS of PM ether, 70 eV, *m/z* (rel. int.): 734 $[M]^+$ (12), 719 $[M-15]^+$ (26), 703 $[M-31]^+$ (100), 631 $[M-103]^+$ (10), 603 $[M-131]^+$ (8), 571 $[M-163]^+$ (27), 559 $[M-175]^+$ (40), 545 $[M-189]^+$ (7). PM carlinoside, TLC (silica gel) $CHCl_3$–EtOAc–Me_2CO, 5:4:1 (R_f 0.19); 5:1:4 (R_f 0.72).

Compound **3** (*vitexin*). TLC cellulose R_f 0.23 (15% HOAc), 0.43 (BAW, 4:1:5); polyamide R_f 0.27 (H_2O–*n*-BuOH–Me_2CO–dioxan, 13:3:2:1). UV λ_{max}^{MeOH} nm: 271, 335; + NaOAc 279, 395; + NaOAc + H_3BO_3 272, 339; + $AlCl_3$ + HCl 275, 298, 337, 382; + NaOH 280, 326, 400. MS of PM ether, 70 eV, *m/z* (rel. int.): 530 $[M]^+$ (84), 397 $[M-133]^+$ (2), 369 $[M-161]^+$ (10), 355 $[M-175]^+$ (100), 341 $[M-189]^+$ (21), 340 $[M-190]^+$ (17), 325 $[M-205]^+$ (10). PM vitexin, TLC (silica gel) $CHCl_3$–EtOAc–Me_2CO, 5:4:1: (R_f 0.16).

Compound **4** (*vitexin 2″-O-rhamnoside*). TLC cellulose R_f 0.64 (15% HOAc); polyamide R_f 0.56 (H_2O–*n*-BuOH–Me_2CO–dioxan, 13:3:2:1). UV λ_{max}^{MeOH} nm: 269, 330; + NaOAc 280, 388; + NaOAc + H_3BO_3 271, 340; + $AlCl_3$ + HCl 278, 303, 345, 380; + NaOH 281, 334, 396. MS of PM ether, 70 eV, *m/z* (rel. int.), fragments named according to ref. [12]: 704 $[M]^+$ (48), 545 $[SO_j]$ (24), 544 $[SO_{j-1}]$ (38), 515 [SO] (100), 499 S (13), 485 [S − 14] (5), 467 [S − 32] (6), 397 [f] (3), 355 [i] (11), 341 [j] (91), 325 [k] (37), 312 [1 + 1] (21), 311 [1] (19). PM vitexin 2″-*O*-rhamnoside, TLC (silica gel) $CHCl_3$–EtOAc–Me_2CO, 5:1:4: (R_f 0.37); 5:4:1: (R_f 0.08).

Compound **5** (*isoschaftoside*). TLC (cellulose) R_f 0.34 (15% HOAc), 0.35 (BAW, 4:1:5); polyamide R_f 0.62 (H_2O–*n*-BuOH–Me_2CO–dioxan 13:3:2:1). UV λ_{max}^{MeOH} nm: 272, 332; + NaOAc 281, 390; + NaOAc + H_3BO_3 274, 320, 340; + $AlCl_3$ + HCl 280, 305, 345, 382; + NaOH 281, 400. MS of PM ether, 70 eV, *m/z* (rel. int.): 704 $[M]^+$ (10), 689 $[M-15]^+$ (21), 673 $[M-31]^+$ (100), 585 $[M-119]^+$ (27), 573 $[M-131]^+$ (41), 559 $[M-145]^+$ (15), 541 $[M-163]^+$ (4), 529 $[M-175]^+$ (13). PM isoschaftoside, TLC (silica gel) $CHCl_3$–EtOAc–Me_2CO, 5:1:4 (R_f 0.67); 5:4:1 (R_f 0.18).

Compound **6** (6,8-*di*-C-*hexosylgenkwanin*). TLC (cellulose) R_f 0.41 (15% HOAc); polyamide R_f 0.67 (H_2O–*n*-BuOH–Me_2CO–dioxan, 13:3:2:1). UV λ_{max}^{MeOH} nm: 274, 334; + NaOAc 273, 390; + NaOAc + H_3BO_3 274, 320, 340; + $AlCl_3$ + HCl 280, 304, 346, 384; + NaOH 282, 334, 400. MS of PM ether, 70 eV, *m/z* (rel. int.): 748 $[M]^+$ (17), 733 $[M-15]^+$ (37) 717 $[M-31]^+$ (100), 701 $[M-47]^+$ (10), 685 $[M-63]^+$ (6), 645 $[M-103]^+$ (10), 615 $[M-133]^+$ (8), 587 $[M-161]^+$ (10), 585 $[M-163]^+$ (34), 573 $[M-175]^+$ (42). PM 6,8-di-*C*-hexosylgenkwanin, TLC (silica gel) $CHCl_3$–EtOAc–Me_2Co, 5:1:4 (R_f 0.75) (PM vicenin 2, 0.79), (PM 6,8-di-*C*-galactosyl acacetin 0.71); 5:4:1 (R_f 0.25) (PM vicenin 2, 0.35), (PM 6,8-di-*C*-galactosyl acacetin 0.17).

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