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NEW *C*-GLYCOSYLFLAVONES FROM *CERASTIUM ARVENSE*

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Key Word Index—*Cerastium arvense*; Caryophyllaceae; *C*-glycosylflavones; cerarvensin; cerarvensin 7-*O*-glucoside; isovitexin 7,2″-di-*O*-glucoside.

Abstract—Three *C*-glycosylflavones isolated from *Cerastium arvense* have been identified as 6-*C*-xylosylapigenin (cerarvensin), its 7-*O*-glucoside and isovitexin 7,2″-di-*O*-glucoside.

INTRODUCTION

In the order Centrospermeae, the families Caryophyllaceae and Molluginaceae are not only distinguished by the presence of anthocyanins, but also of *C*-glycosylflavonoids [1]. In continuing studies of *C*-glycosylflavones in Caryophyllaceae (*Spergularia rubra* [2], *Stellaria holostea* [3] and *Cerastium arvense* [4]), we now report the isolation and identification of three new *C*-glycosylflavones from *Cerastium arvense*.

RESULTS AND DISCUSSION

Besides the known isovitexin (6-*C*-glucosylapigenin), saponarin (isovitexin 7-*O*-glucoside), vicenin-2 (6,8-di-*C*-glucosylapigenin), isocorymboside (6-*C*-galactosyl-8-*C*-arabinosylapigenin) [5] and a 6,8-di-*C*-pentosylapigenin identified with a compound isolated from *Mollugo pentaphylla* [6] and *Lespedeza cuneata* [7], compounds A–C were isolated from the water-soluble fraction of the ethanolic extract from aerial parts of *Cerastium arvense* [4].

Compound A showed the same UV spectrum and diagnostic shifts as apigenin [8] and the chromatographic properties of a *C*-glycoside, no sugar being obtained on acid hydrolysis. Permethyl compound A gave the mass spectrum of a PM 6-*C*-pentosylapigenin: $[M]^+$, 486, $[M-15]^+$, $[M-31]^+$, $[M-119]^+$, $[M-131]^+$, $[M-145]^+$ [9]. Co-TLC with PM 6-*C*-β-D-xylopyranosylapigenin [10] and PM 6-*C*-α-L-arabinopyranosylapigenin [11] showed identity with the former, confirmed by direct comparison of compound A with 6-*C*-β-D-xylopyranosylapigenin. This compound has already been found as a 2″-*O*-rhamnoside in *Phlox drummondii* [12], but not yet in the free state. The name cerarvensin is proposed for compound A. Compound B showed the UV spectrum and diagnostic shifts of a 7-*O*-substituted apigenin [8] and the chromatographic properties of a glycoside. Acid hydrolysis led to glucose and compound A accompanied by its Wessely–Moser isomer. The cerarvensin 7-*O*-glucoside structure of compound B was confirmed by the mass spectrum of its PM derivative which showed the characteristic fragmentation pattern of a PM 5,7-dihydroxy-6-*C*-glycosylflavone 7-*O*-glycoside [13] with two homologous series of peaks corresponding to the fragmentation of PM 6-*C*-glycosylflavones, the first series related to the molecular peak: 690, $[M]^+$, $[M-15]^+$, $[M-31]^+$, $[M-119]^+$ (hM), $[M-131]^+$ (iM) (from the 6-*C*-pentosyl residue) while the second series related to the aglycone: 472, $[AH]^+$, $[AH-31]^+$, $[AH-47]^+$, $[AH-63]^+$, $[AH-119]^+$ (hAH), $[AH-131]^+$ (iAH) (again from the 6-*C*-pentosyl residue). The nature of the 7-*O*-glycosyl residue is given by the difference 219 [M − A], corresponding to one hexose. As noted above, the pentosyl nature of the carbon-bound sugar is given by the difference 131 [AH − iAH], and the apigenin nature of the flavone moiety by 341 $[iAH]^+$.

Compound C showed the UV spectrum and diagnostic shifts of a 7-*O*-substituted apigenin [8] and the chromatographic properties of a glycoside. Acid hydrolysis led to glucose and isovitexin accompanied by lesser amounts of vitexin. The isovitexin 7,2″-di-*O*-glucoside structure of compound C followed from the mass spectrum of its PM derivative which showed the characteristic fragmentation pattern of a PM 5,7-dihydroxy-6-*C*-glycosylflavone 7,2″-di-*O*-glycoside [11]. The molecular peak was found at *m/z* 938 and the absence of $[M-15]^+$ and $[M-31]^+$ peaks replaced by the ions $[SO]^+$ (*m/z* 719) and $[S]^+$ (*m/z* 703) derived from the elimination of the PM 2″-*O*-glycosyl residue, respectively without and with the oxygen atom of the glycosidic bond, showed the loss of the PM 2″-*O*-glucosyl group in a first step, the hexose nature of the 2″-*O*-glycosyl residue being given by the difference [M − S], 235. Then the homologous peaks $[SO(AH)]^+$ (*m/z* 501) and $[S(AH)]^+$ (*m/z* 485), derived from $[SO]^+$ and $[S]^+$ by loss of the PM 7-*O*-glycosyl group with hydrogen transfer

showed the hexose nature of this group by the difference 218 [S − S(AH)]. Finally, the important peak [jAH]$^+$ (*m/z* 327) agreed with the apigenin nature of the flavone moiety and the difference 158 [S(AH) − jAH] with the hexose nature of the 6-*C*-glycosyl residue. Similar compounds, 2″-*O*-glucosyl- and 2″-*O*-rhamnosylisovitexin 7-*O*-galactosides [14] as well as other isovitexin 7,*x*″-di-*O*-glycosides [15] have been identified in *Melandrium album*, but isovitexin 7,2″-di-*O*-glucoside is characterized as such for the first time. It is likely to be identical with the 7-*O*-glucosyl-6-*C*-glucosylglucosylapigenin from *Melandrium album* [16], due to the more recent demonstration of genetically controlled 2″-*O*-glycosylation of isovitexin in this plant [17]. Moreover compound C may be compared with 6-*C*-arabinosylapigenin 7,2″-di-*O*-glucoside obtained from another caryophyllaceous species *Spergularia rubra* [11].

EXPERIMENTAL

Plant material. Cerastium arvense L. ssp. *arvense* was collected on the roadside at Noiron-sous-Gevrey, Côte d'Or, France (voucher specimen No. 115 is deposited in the Herbarium, Faculté de Pharmacie, Université de Dijon).

Isolation. Fresh leaves (300 g) were extracted under reflux with 95% EtOH and then percolated with hot 95% EtOH. After concn under red. pres. the residue was macerated with hot H_2O and filtered. The aq. phase was fractionated first on a polyamide column with a H_2O–MeOH gradient (0–75% MeOH), then on cellulose TLC in 5% HOAc. The bands corresponding to compounds A–C were eluted with hot MeOH and further purified by prep. cellulose TLC in BAW (4 : 1 : 5) [18]. Final purification was on a Sephadex LH 20 column with MeOH.

Compound A (*cerarvensin*). UV λ_{max}^{MeOH} nm: 276, 336; +NaOAc 280, 304sh, 340sh, 390; +$AlCl_3$ 280, 304, 350, 384sh; +$AlCl_3$ + HCl 280, 304, 350, 384sh; +NaOMe 280, 330, 396. TLC (polyamide) R_f 0.30 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12 : 4 : 3 : 1); (cellulose) 0.09 (5% HOAc), 0.27 (15% HOAc), 0.64 (BAW, 4 : 1 : 5); (Si gel) 0.63 (EtOAc–MeOH–H_2O, 63 : 12 : 9). Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 486 [M]$^+$ (19), 471 [M − 15]$^+$, [12], 455 [M − 31]$^+$ (100), 439 [M − 47]$^+$ (15), 367 [M − 119]$^+$ (17), 355 [M − 131]$^+$ (58), 341 [M − 145]$^+$ (31), 325 [M − 161]$^+$ (12), 311 [M − 175]$^+$ (12), TLC (Si gel) R_f 0.45, PM 6-*C*-β-D-xylopyranosylapigenin 0.45, PM 6-*C*-α-L-arabinopyranosylapigenin 0.23 ($CHCl_3$–EtOAc–Me_2CO, 5 : 4 : 1). Acid hydrolysis of compound A gave 6-*C*-xylosylapigenin which isomerizes to 8-*C*-xylosylapigenin.

Compound B (*O-glucosyl-7-cerarvensin*). UV λ_{max}^{MeOH} nm: 274, 334; +NaOAc 270, 394; +$AlCl_3$ 282, 304, 350, 384sh; +$AlCl_3$ + HCl 282, 304, 350, 384sh; +NaOMe 272, 310sh, 392. TLC (polyamide) R_f 0.75 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12 : 4 : 3 : 1); (cellulose) 0.19 (5% HOAc), 0.40 (15% HOAc), 0.46 (BAW, 4 : 1 : 5); (Si gel) 0.40 (EtOAc–MeOH–H_2O, 63 : 12 : 9). Acid hydrolysis gave compound A (and its 8-isomer) and glucose. Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 690 [M]$^+$, (30), 675 [M − 15]$^+$, (23), 659 [M − 31]$^+$ (55), 643 [M − 47]$^+$ (4), 571 [M − 119]$^+$ (15), 559 [M − 131]$^+$ (17), 545 [M − 145]$^+$ (6), 529 [M − 161]$^+$ (3), 472 [M − 218]$^+$, [AH]$^+$ (11), 471 [M − 219]$^+$ [A]$^+$ (24), 457 [AH − 15]$^+$ (38), 441 [AH − 31]$^+$ (100), 425 [AH − 47]$^+$ (46), 409 [AH − 63]$^+$ (80), 353 [AH − 119]$^+$ (42), 341 [AH − 131]$^+$ (56), 327 [AH − 145]$^+$ (56), 325 [AH − 147]$^+$ (21), 311 [AH − 161]$^+$ (21), 297 [AH − 175]$^+$ (21). TLC (Si gel) R_f 0.17 ($CHCl_3$–EtOAc–Me_2CO, 5 : 4 : 1), 0.60 ($CHCl_3$–EtOAc–Me_2CO, 5 : 1 : 4).

Compound C (*7,2″-di-O-glucosyl-6-C-glucosylapigenin*). UV λ_{max}^{MeOH} nm: 274, 334; +NaOAc 274, 394; +$AlCl_3$ 282, 304sh, 350, 384sh; +$AlCl_3$ + HCl 280, 302sh, 346, 380sh; +NaOMe 276, 308sh, 350sh, 390; TLC (polyamide) R_f 0.90 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12 : 4 : 3 : 1); (cellulose) 0.72 (5% HOAc), 0.80 (15% HOAc), 0.39 (BAW, 4 : 1 : 5); (Si gel) 0.19 (EtOAc–MeOH–H_2O, 63 : 12 : 9). Acid hydrolysis gave isovitexin and vitexin plus glucose. Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 938 ([M]$^+$ (12), 763 [SOi]$^+$ (4), 749 [SOj]$^+$ (7), 733 [SOk]$^+$ (12), 719 [SO]$^+$ (66), 703 [S]$^+$ (81), 545 [SOi(AH)]$^+$ (7), 531 [SOj(AH)]$^+$ (12), 515 [SOk(AH)]$^+$ (6), 501 [SO(AH)]$^+$ (31), 485 [S(AH)]$^+$ (100), 341 [iAH]$^+$ (19), 327 [jAH]$^+$ (46), 311 [kAH]$^+$ (25). TLC (Si gel) R_f 0.20 ($CHCl_3$–EtOAc–Me_2CO, 5 : 4 : 1), 0.69 ($CHCl_3$–EtOAc–Me_2CO, 5 : 1 : 4).

Acid hydrolyses. Each sample was dissolved in MeOH–4 N HCl (1 : 1) and heated at 100° for 3 hr in a sealed tube. After repeated evaporations 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 (Si gel) in EtOAc–pyridine–H_2O–MeOH (80 : 20 : 10 : 5) and EtOAc–MeOH–H_2O (63 : 12 : 9); (cellulose) in 15% HOAc and BAW (4 : 1 : 5). The sugars were extracted with pyridine from the aq. phase after neutralization and evaporation to dryness and identified by TLC on Na_2HPO_4 (0.2 M) impregnated Si gel in Me_2CO–H_2O (9 : 1) against standard markers. The flavones and sugars were respectively detected with bis-diazotized benzidine–Na_2CO_3 and aniline phthalate.

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