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ISOMOLLUPENTIN-*O*-GLUCOSIDES FROM *CERASTIUM ARVENSE*

MARIE-ALETH DUBOIS, ANNE ZOLL and JEAN CHOPIN*

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 21033 Dijon Cedex, France, *Laboratoire de Chimie Biologique, Université Claude Bernard, 69622 Villeurbanne Cedex, France

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Key Word Index—*Cerastium arvense*, Caryophyllaceae, *C*-glycosylflavones, isomollupentin 7-*O*-glucoside, isomollupentin 4′-*O*-glucoside, isomollupentin 2″-*O*-glucoside

Abstract—Eight *C*-glycosylflavone *O*-glycosides including three new compounds isomollupentin 7-*O*-glucoside, isomollupentin 4′-*O*-glucoside and isomollupentin 2″-*O*-glucoside have been isolated from the leaves and flowers of *Cerastium arvense* The 27 *C*-glycosylflavones identified in this plant are tabulated

INTRODUCTION

As part of biochemical systematic investigation of *Cerastium arvense* subsp *arvense* (Caryophyllaceae) [1], several *C*-glycosylflavones have been reported mono-*C*-glycosylflavones [2], di-*C*-glycosylflavones [3, 4], 7,2″-di-*O*-glycosyl-*C*-glycosylflavones [2, 5] and two 7-*O*-glycosyl-*C*-glycosylflavones, the known saponarin and the new cerarvensin 7-*O*-glucoside [2] We now report the isolation and identification of eight *O*-glycosyl-*C*-glycosylflavones including three new isomollupentin *O*-glucosides (7-*O*-glucoside, 4′-*O*-glucoside and 2″-*O*-glucoside) All the *C*-glycosylflavones so far isolated from *C arvense* are tabulated [6]

RESULTS AND DISCUSSION

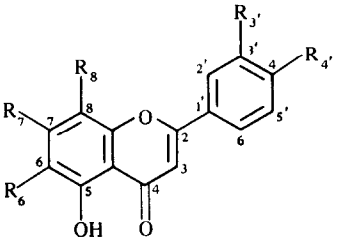
The novel compounds isomollupentin 7-*O*-glucoside (**1**), isomollupentin 4′-*O*-glucoside (**2**) and isomollupentin 2″-*O*-glucoside (**3**) as well as the known 4′-*O*-glucosylisovitexin, 4′-*O*-glucosylisoorientin, 2″-*O*-glucosylisovitexin and 2″-*O*-arabinosylisovitexin were isolated from the ethanolic extract from fresh aerial parts of *Cerastium arvense* and identified by their spectral and chromatographic properties [7, 8]

Compound **1** showed the UV spectrum and diagnostic shifts of a 7-*O*-substituted apigenin [9] and the chromatographic properties of an apigenin diglycoside Acid hydrolysis with 4 N HCl–MeOH (1 1) yielded glucose (TLC) and isomollupentin (6-*C*-arabinosylapigenin) [UV, EIMS of the permethyl (PM) ether, co-TLC with standard free and permethylated samples] accompanied by small amounts of its Wessely–Moser isomer The isomollupentin 7-*O*-glucoside structure of **1** was confirmed by the mass spectrum (EIMS) of its PM derivative which showed the characteristic fragmentation pattern of a PM 5,7-dihydroxy-6-*C*-glycosylflavone 7-*O*-glucoside [10, 11] with two homologous series of peaks corresponding to the fragmentation of PM 6-*C*-glycosylflavones, the first series related to the molecular peak $[M]^+$ *m/z* 690, $[M-15]^+$, $[M-31]^+$, $[M-119]^+$ (hM), $[M-131]^+$ (iM), $[M-145]^+$ (jM), $[M-161]^+$ (kM) (from the 6-*C*-pentosyl residue), the second series to the aglycone AH $[A]^+$ *m/z* 471, $[AH-15]^+$ (aAH), $[AH-31]^+$ (bAH), $[AH-47]^+$ (cAH), $[AH-63]^+$ (dAH), $[AH-119]^+$ (hAH), $[AH-131]^+$ (iAH), $[AH-145]^+$ (kAH) (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 The pentosyl nature of the 6-*C*-glycosyl residue is given as noted above by the difference 131 [AH – iAH] and the apigenin nature of the flavone moiety by $[iAH]^+$ *m/z* 341

Compound **2** showed the UV spectrum and diagnostic shifts of a 4′-*O*-substituted apigenin [9] and the chromatographic properties of a diglycoside Acid hydrolysis led to glucose and isomollupentin accompanied by small amounts of its Wessely–Moser isomer (identified as above) The PM derivative gave the mass spectrum (EIMS) of a PM 5,7-dihydroxy-6-*C*-glycosylapigenin 4′-*O*-glycoside [10, 11] $[M]^+$ *m/z* 690, followed by the usual $[M-15]^+$, $[M-31]^+$, $[M-47]^+$, $[M-119]^+$, $[M-131]^+$ fragments from the 6-*C*-pentosyl residue and an important aglycone ion AH $[M-218]^+$ The presence and intensity of ion h + 1 (AH) *m/z* 354, the lower intensity of the ions a (AH), b (AH), c (AH), d (AH) (*m/z* 457, 441, 425, 409, respectively) are other characteristics of a PM 4′-*O*-glycosyl-6-*C*-glycosylflavone [10, 11]. The nature of the 4′-*O*-glycosyl residue is given by the difference 218 [M – AH] corresponding to one hexose As noted above, the pentosyl nature of the *C*-glycosyl residue is given by the difference 131 [M – iM] or [AH – iAH] and the apigenin nature of the flavone moiety by $[iAH]^+$ (*m/z* 341) These data proved **2** to be isomollupentin 4′-*O*-glucoside

Compound **3** showed the UV spectrum and diagnostic shifts [9] of apigenin with free hydroxyl groups at the 5, 7 and 4′ positions and the chromatographic properties of an apigenin diglycoside Acid hydrolysis led to glucose and isomollupentin accompanied by small amounts of its Wessely–Moser isomer The position of attachment of glucose to the *C*-glycosyl residue was determined by the mass spectrum (EIMS) of the PM derivative of **3** which showed the characteristic fragmentation pattern of PM 6-*C*-pentosylapigenin 2″-*O*-glycosides [11] absence of M – 15 and M – 31 ions (showing the absence of a 2″-OMe [25]) replaced by the ions $[SO]^+$ (*m/z* 471) and $[S]^+$ (*m/z* 455) derived from the elimination of the PM 2″-*O*-glycosyl

Table 1 *C*-glycosylflavones from *Cerastium arvense*



Compound	R_3	R_4	R_6	R_7	R_8
Cerarvensin	H	OH	β-D-Xyl	OH	H
Isomollupentin	H	OH	α-L-Ara	OH	H
Isovitexin	H	OH	β-D-Glc	OH	H
Isoorientin	OH	OH	β-D-Glc	OH	H
Isoscoparin	OMe	OH	β-D-Glc	OH	H
6-*C*-xylosyl-8-*C*-arabinosylapigenin	H	OH	β-D-Xyl	OH	α-L-Ara
Schaftoside	H	OH	β-D-Glc	OH	α-L-Ara
Isocorymboside	H	OH	β-D-Gal	OH	α-L-Ara
Cerastin	H	OH	β-D-Glc	OH	β-D-Gal
Cerarvensin 7-glucoside	H	OH	β-D-Xyl	OGlc	H
Isomollupentin 7-glucoside (1)	H	OH	α-L-Ara	OGlc	H
Isovitexin 7-glucoside	H	OH	β-D-Glc	OGlc	H
Isomollupentin 4′-glucoside (2)	H	OGlc	α-L-Ara	OH	H
Isovitexin 4′-glucoside	H	OGlc	β-D-Glc	OH	H
Isoorientin 4′-glucoside	OH	OGlc	β-D-Glc	OH	H
Isomollupentin 2″-glucoside (3)	H	OH	Glc(1 → 2)Ara	OH	H
Isovitexin 2″-xyloside	H	OH	Xyl(1 → 2)Glc	OH	H
Isovitexin 2″-arabinoside	H	OH	Ara(1 → 2)Glc	OH	H
Isovitexin 2″-glucoside	H	OH	Glc(1 → 2)Glc	OH	H
Isomollupentin 7-glucoside					
2″-xyloside	H	OH	Xyl(1 → 2)Ara	OGlc	H
2″-arabinoside	H	OH	Ara(1 → 2)Ara	OGlc	H
2″-glucoside	H	OH	Glc(1 → 2)Ara	OGlc	H
Isovitexin 7-glucoside					
2″-arabinoside	H	OH	Ara(1 → 2)Glc	OGlc	H
2″-glucoside	H	OH	Glc(1 → 2)Glc	OGlc	H
2″-Feruloylisovitexin	H	OH	2-FerGlc	OH	H
2″-Feruloyl 4′-glucosyl					
isovitexin	H	OGlc	2-FerGlc	OH	H
isoorientin	OH	OGlc	2-FerGlc	OH	H

Glc, glucose, Ara, arabinose, Gal, galactose, Xyl, xylose, Fer, feruloyl

and 2″-*O*-glycosyloxy residues, respectively, and presence of an intense ion J (*m/z* 341), the molecular ion could not be found, but the chromatographic properties of the free compound (see Experimental) only agree with an isomollupentin monoglucoside structure Compound **3** is therefore isomollupentin 2″-*O*-glucoside

Isomollupentin 7,2″-di-*O*-glycosides have been previously identified in this plant [5] and in *Spergularia rubra* [12], but isomollupentin 7-*O*-glucoside, isomollupentin 4′-*O*-glucoside and isomollupentin 2″-*O*-glucoside are characterized for the first time Beside these new isomollupentin *O*-glycosides, five known 6-*C*-glycosylflavone *O*-glycosides have been isolated from *Cerastium arvense* 4′-*O*-glucosylisovitexin and 4′-*O*-glucosylisoorientin (reported from *Gentiana* sp [13, 14] and *Briza* sp [15]), 2″-*O*-glucosylisovitexin (isolated from *Oxalis acetosella* [16], *Gentiana asclepiadea* [17], *Cucumis melo* [18] and *Melandrium album* [19]), 2″-*O*-xylosylisovitexin (isolated from *Desmodium canadense* [20] and *Passiflora serratifolia* [21]) and 2″-*O*-arabinosylisovitexin isolated from *Melandrium album* [19] and *Avena sativa* [22]

All these compounds were identified by UV, acid hydrolysis, mass spectrometry (EIMS) of the PM derivatives and comparison with literature data

In addition, several feruloyl *C*-glycosylflavones have been isolated from *Cerastium arvense* Three of them were known compounds 2″-*O*-feruloylisovitexin and 2″-*O*-feruloyl-4′-*O*-glucosylisovitexin (from *Gentiana punctata* [23]), and 2″-*O*-feruloyl-4′-*O*-glucosylisoorientin (from *Gentiana burseri* [24]) The first one was identified by UV, acid and alkaline hydrolysis, EIMS of the PM derivative (= PM isovitexin) and comparison with literature data The two others were characterized by UV, acid and alkaline hydrolysis, EIMS of the PM derivatives

(= PM isovitexin and PM isoorientin, respectively), FAB-MS of the free compounds and ^{1}H NMR of the acetates Another feruloyl-4′-*O*-glucosylisovitexin and a feruloylcerastin (6-*C*-glucosyl-8-*C*-galactosyl-apigenin), both acylated on a sugar residue, were isolated in too small amount for further study

The 27 identified *C*-glycosylflavones isolated from *Cerastium arvense* during this study are given in Table 1

EXPERIMENTAL

Plant material Cerastium arvense L subsp *arvense* was collected in May 1981 on the roadside at Chamboeuf, Côte d'Or, France A voucher specimen, No 116, is deposited in the Herbarium of Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de Dijon

Extraction and isolation Fresh leaves and flowers (2 kg) were extracted with 95% EtOH (10 l) under reflux After concn under red pres the residue was taken up in hot H_2O and filtered The aq phase was then partitioned against $CHCl_3$ and Et_2O The remaining aq layer (12 g) was submitted in three portions to reversed phase HPLC on a Lichroprep RP-18 (25–40 μm) column (20 × 2 cm) Elution with a discontinuous gradient MeOH–H_2O–HOAc, 4 15 1, 6 13 1, 10 9 1 (pressure 10 bars, flow rate 10 ml/min), yielded 10 fractions, which were further separated on Lichrosorb RP-18 (10 μm) and microcrystalline cellulose columns (see ref [6] for details) Final separation of 1 was achieved by TLC (silica gel) in EtOAc–MeOH–H_2O (21 4 3) Compounds were cleaned over Sephadex LH-20 columns prior to spectral analysis Known compounds were identified by comparison of UV, chromatographic properties, and mass spectral data with those of standard compounds

Isomollupentin 7-*O*-*glucoside* (**1**) UV λ_{max}^{MeOH} nm 272, 330, + NaOAc 272, 348 sh, 394, + $AlCl_3$ 280, 300 sh, 346, 388 sh, + $AlCl_3$ + HCl 278, 302 sh, 340, 386 sh, NaOH 278, 300 sh, 348 sh, 400 TLC (polyamide) R_f 0 77 (H_2O–EtOH–MeCO Et–$AcCH_2COMe$, 12 4 3 1), (cellulose) R_f 0 22 (HOAc 5%), 0 42 (HOAc 15%), 0 48 (BAW, 4 1 5), (silica gel) R_f 0 34 (EtOAc–MeOH–H_2O, 21 4 3) Permethylether EIMS 70 eV, m/z > 295 (rel int) 690 $[M]^+$ (20), 675 $[M-15]^+$ (32), 659 $[M-31]^+$ (73), 643 $[M-47]^+$ (10), 571 $[M-119]^+$ (20), 559 $[M-131]^+$ (20), 472 $[M-218, AH]^+$ (18), 471 $[M-219, A]^+$ (47), 457 $[AH-15]^+$ (50), 441 $[AH-31]^+$ (88), 425 [AH-47]$^+$ (40), 409 $[AH-63]^+$ (59), 383 $[AH-89]^+$ (12), 355 $[AH-117]^+$ (38), 353 $[AH-119]^+$ (61), 341 $[AH-131, 1(AH)]^+$ (100), 327 $[AH-145]^+$ (76), 311 $[AH-161]^+$ (32), 297 $[AH-175]^+$ (39) TLC (silica gel) R_f 0 15 ($CHCl_3$–EtOAc–Me_2CO, 5 4 1), 0 58 ($CHCl_3$–EtOAc–Me_2CO, 5 1 4)

Isomollupentin 4′-*O*-*glucoside* (**2**) UV λ_{max}^{MeOH} nm 273, 326, + NaOAc 278, 296 sh, 390, + $AlCl_3$ 284, 300, 344, 380 sh, + $AlCl_3$ + HCl 284, 300, 344, 380 sh, NaOMe 278, 298 sh, 380 TLC (polyamide) R_f 0 90 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12 4 3 1), (cellulose) R_f 0 34 (HOAc 5%), 0 53 (HOAc 15%), 0 46 (BAW, 4 1 5), (silica gel) 0 30 (EtOAc–MeOH–H_2O, 21 4 3) Permethylether EIMS 70 eV, m/z > 295 (rel int) 690 $[M]^+$ (23), 675 $[M-15]^+$ (19), 659 $[M-31]^+$ (100), 643 $[M-47]^+$ (14), 631 $[M-59]^+$ (6), 601 $[M-89]^+$ (4), 571 $[M-119]^+$ (11), 559 $[M-131]^+$ (36), 472 $[M-218, AH]^+$ (49), 457 $[AH-15]^+$ (3), 441 $[AH-31]^+$ (17), 425 $[AH-47]^+$ (5), 409 $[AH-63]^+$ (2), 383 $[AH-89]^+$ (4), 355 $[AH-117]^+$ (14), 354 $[AH-118]^+$ (31), 353 $[AH-119]^+$ (6), 341 $[AH-131]^+$ (32), 327 $[AH-145]^+$ (29), 311 $[AH-161]^+$ (5), 297 $[AH-175]^+$ (7) TLC (silica gel) R_f 0 12 ($CHCl_3$–EtOAc–Me_2CO, 5 4 1), 0 35 ($CHCl_3$–EtOAc–Me_2CO, 5 1 4)

Isomollupentin 2″-*O*-*glucoside* (**3**) UV λ_{max}^{MeOH} nm 275, 336, + NaOAc 280 317, 332 sh, 390, + $AlCl_3$ 280, 302, 346, 384 sh, + $AlCl_3$ + HCl 280, 304, 348, 380 sh, + NaOMe 280, 328, 400 TLC (polyamide) R_f 0 73 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12 4 3 1), (cellulose) R_f 0 43 (HOAc 5%) 0 62 (HOAc 15%), 0 66 (BAW, 4 1 5), (silica gel) R_f 0 44 (EtOAc–MeOH–H_2O, 21 4 3) Permethylether EIMS 70 eV, m/z > 300 (rel int) 515 $[SO_i]^+$ (15), 501 $[SO_j]^+$ (13), 485 $[SO_k]^+$ (27), 471 $[SO]^+$ (46), 455 $[S]^+$ (100), 355 $[i]^+$ (16), 341 $[j]^+$ (58), 325 $[k]^+$ (11), 311 $[1]^+$ (7), TLC (silica gel) R_f 0 05 ($CHCl_3$–EtOAc–Me_2CO, 5 4 1), 0 34 ($CHCl_3$–EtOAc–Me_2CO, 5 1 4)

Acid hydrolysis Compound (2 mg) was heated with MeOH–4 N HCl (1 1) at 100° in a sealed tube for 1 hr 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–MeOH–H_2O (21 4 3), (cellulose) in 15% HOAc and BAW (4 1 5)

Sugars were identified by TLC (0 2 M Na_2HPO_4 impregnated silica gel plates) in Me_2CO–H_2O (9 1) against standard markers, flavones and sugars were respectively detected with bis-diazotized benzidine–Na_2CO_3 and aniline phthalate The aglycones were permethylated and cochromatographed on TLC (silica gel, $CHCl_3$–EtOAc–Me_2CO, 5 4 1) with standard PM 6-*C*-glycosylflavones

Alkaline hydrolysis The acylated glycoside (1 mg) was added to 2 N NaOH (2 ml) and the mixture left under N_2 for 2 hr at room temp, then acidified with 2 N HCl The acid was extracted with Et_2O and characterized by TLC with authentic samples The deacylated glycoside was extracted with *n*-BuOH and characterized by spectral (UV, EIMS) and chromatographic methods

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