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6-*C*-β-D-GLUCOPYRANOSYL-8-*C*-β-D-GALACTOPYRANOSYLAPIGENIN FROM *CERASTIUM ARVENSE*

MARIE-ALETH DUBOIS, ANNE ZOLL, KENNETH R. MARKHAM*, MARIE LOUISE BOUILLANT, GEORGETTE DELLAMONICA and JEAN CHOPIN†

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 21033 Dijon Cedex, France; *Chemistry Division, D.S.I.R., Private Bag, Petone, New Zealand; †Laboratoire de Chimie Biologique, Université de Lyon I, 69622 Villeurbanne Cedex, France.

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Key Word Index—*Cerastium arvense*; Caryophyllaceae; *C*-glycosylflavone; 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-galactopyranosylapigenin.

Abstract—The new *C*-glycosylflavone, 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-galactopyranosylapigenin, has been isolated from *Cerastium arvense* and identified on the basis of UV, MS and ^{13}C NMR spectral data and comparison with the product obtained from 6-*C*-galactosylation of vitexin.

INTRODUCTION

In continuing studies of *C*-glycosylflavones in *Cerastium arvense* L., Caryophyllaceae [1–4], we now report the isolation and identification of a new di-*C*-glycosylflavone (**1**) from the same plant.

RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous yellow solid from the water-soluble fraction of the ethanolic extract from aerial parts of *Cerastium arvense*. It showed the same UV spectrum and diagnostic shifts as apigenin [5] and the chromatographic properties of a di-*C*-glycoside, no sugar being obtained on acid hydrolysis. No apparent isomerization could be detected by TLC after acid treatment. Permethyl **1** gave the mass spectrum of a PM 6,8-di-*C*-hexosyl-apigenin: $[M]^+$ 748, $[M-15]^+$, $[M-31]^+$, $[M-163]^+$, $[M-175]^+$, $[M-189]^+$ [6]. However co-TLC with PM 6,8-di-*C*-β-D-glucopyranosyl-apigenin [7] and PM 6,8-di-*C*-β-D-galactopyranosyl-acacetin (from *C*-galactosylation of acacetin with acetobromo-α-D-galactose [8]) showed PM **1** to be different from and to migrate between the PM di-*C*-glucoside and the PM di-*C*-galactoside. From the common occurrence of glucose and galactose in natural flavone *C*-glycosides [9], this observation suggested the presence of one *C*-glucosyl and one *C*-galactosyl residue in **1**. The ^{13}C NMR spectrum is consistent with this hypothesis in that it showed between δ182.4 and 102.7 the signals of apigenin with the 10 ppm downfield shift of C-6 and C-8 expected from *C*-glycosylation, and between δ81.7 and 61.1 the signals expected for one *C*-β-D-glucopyranosyl and one *C*-β-D-galactopyranosyl residue [10]. The pattern and grouping of sugar carbon signals differs from that of vicenin-2(6,8-di-*C*-β-D-glucopyranosylapigenin) [11]. However, it is not possible by ^{13}C NMR to determine the position of linkage of each sugar residue. Therefore **1** was compared with a synthetic product previously obtained by one of us (M.L.B.) from 6-*C*-glycosylation [12] of natural vitexin (8-*C*-β-D-glucopyranosylapigenin) with acetobromo-α-D-galactose. No acid hydrolysis being used in the isolation procedure, any Wessely-Moser isomerization was precluded and the product was then assigned the structure 6-*C*-β-D-galactopyranosyl-8-*C*-β-D-glucopyranosylapigenin on the basis of its chromatographic behaviour (like diglycoside), its UV spectra showing the presence of free 5,7- and 4′-hydroxyls and the mass spectrum of its permethyl derivative (which excluded the possibility of *O*″-galactosylation [13]). Reversed-phase HPLC on a Lichosorb RP 18 column in conditions previously described for the separation of 6,8-di-*C*-glucosylapigenin and the above product [14, 15], showed the latter to be different from **1**. Similarly, the permethyl derivative was different from PM compound **1** on TLC.

It follows that **1** is 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-galactopyranosylapigenin, the first natural unsymmetrical 6,8-di-*C*-hexosylflavone to be characterized. This conclusion is also supported by HPLC of the mixture resulting from acid isomerisation of **1**, the two main peaks showing the same retention times as **1** and 6-*C*-galactosylvitexin, respectively.

EXPERIMENTAL

Plant material and isolation. See ref. [4].

6-C-β-D-Glucopyranosyl-8-C-β-D-galactopyranosylapigenin (**1**). UV λ_{max}^{MeOH} nm: 276, 304 sh, 340; + $AlCl_3$ 264 sh, 282, 306, 346, 384 sh; $AlCl_3$ + HCl 280, 304, 348, 380 sh; + NaOAc 284, 310 sh, 392; + NaOH 282, 336, 404. TLC (polyamide) R_f 0.82 (H_2O–EtOH–MeCOEt–$AcCH_2COMe$, 12:4:3:1); (cellulose) 0.27 (5% HOAc), 0.40 (15% HOAc), 0.38 (BAW, 4:1:5); (silica gel) 0.21 (EtOAc–MeOH–H_2O, 21:4:3) ^{13}C NMR (20 MHz, DMSO-d_6): 61.1–61.2 (C-6 Glc + C-6 Gal), 68.6–71.2 (C-2,4 Glc + C-2,4 Gal), 73.9–75.2 (C-1 Glc + C-1,3 Gal), 78.7–80.7 (C-3 Glc + C-5 Gal), 81.7 (C-5 Glc), 102.7–103.8 (C-3 + C-10 + C-8), 109.5

(C-6), 116.2 (C-3', C-5'), 121.3 (C-1'), 129.1 (C-2', C-6'), 153.9 (C-9), [159.1 (C-5)], 161.3–162.0 (C-7, 4'), [164.0 (C-2)], 182.4 (C-4).

Permethyl ether of **1**. EIMS 70 eV, $m/z > 300$ (rel. int.): 748 $[M]^+$ (25), 733 $[M-15]^+$ (29), 717 $[M-31]^+$ (100), 701 $[M-47]^+$ (13), 685 $[M-63]^+$ (8), 645 $[M-103]^+$ (17), 615 $[M-133]^+$ (13), 585 $[M-161]^+$ (9), 585 $[M-163]^+$ (34), 573 $[M-175]^+$ (55), 559 $[M-189]^+$ (13), 543 $[M-205]^+$ (11). TLC (silica gel) R_f 0.23 and 0.64 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1 and 5:1:4), PM 6,8-diglucosylapigenin 0.30 and 0.70, PM 6,8-digalactosylacacetin 0.14 and 0.57.

Permethyl 6,8-*di*-C-β-D-*galactopyranosylacacetin*. EIMS 70 eV, $m/z > 300$ (rel. int.): 748 $[M]^+$ (36), 733 $[M-15]^+$ (41), 717 $[M-31]^+$ (100), 703 $[M-45]^+$ (16), 685 $[M-63]^+$ (6), 645 $[M-103]^+$ (10), 643 $[M-105]^+$ (13), 585 $[M-163]^+$ (42), 573 $[M-175]^+$ (49), 559 $[M-189]^+$ (21).

C-*Galactosylation of vitexin*. To a soln of Li (0.3 g, 43 mmol) in MeOH (60 ml) were successively added vitexin (1.18 g, 2.7 mmol) and acetobromo-α-D-galactose (15.3 g, 37 mmol) with stirring at room temp. An orange ppt. appeared, which could not be dissolved in MeOH (20 ml); after 30 min, it was separated by filtration of the reaction mixture and dissolved in H_2O (30 ml). Acidification of this aq. soln led to a yellow ppt. (mainly vitexin) which was separated by filtration. 6-C-Galactosylvitexin was isolated from the aq. phase by chromatography on a polyamide column eluted with H_2O.

6-C-β-D-*Galactopyranosyl*-8-C-β-D-*glucopyranosylapigenin*. UV λ_{max}^{MeOH} nm: 272, 302 sh, 330; +$AlCl_3$ 282, 310, 359, 392 sh; +NaOAc 283, 304 sh, 386; +NaOMe 282, 331, 398. PC (Whatman No. 1): R_f 0.55 (15% HOAc), 0.35 (BAW, 4:1:5). TLC (silica gel) 0.08 (EtOAc–pyridine–H_2O–MeOH, 16:4:2:1). *Permethylether*: EIMS 70 eV, $m/z > 300$ (rel. int.): 748 $[M]^+$ (53), 733 $[M-15]^+$ (27), 717 $[M-31]^+$ (100), 703 $[M-45]^+$ (15), 687 $(M-61)^+$ (12), 643 $[M-105]^+$ (13), 585 $[M-163]^+$ (35), 573 $[M-175]^+$ (38), 559 $(M-189)^+$ (25). TLC (silica gel): R_f 0.59 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

REFERENCES

1. Dubois, M.-A. (1980) Thèse de Doctorat de 3ème cycle en Pharmacie, Université de Paris V.
2. Dubois, M.-A., Zoll, A., Bouillant, M. L. and Chopin, J. (1982) *Phytochemistry* **21**, 1141.
3. Dubois, M.-A., Zoll, A., Bouillant, M. L. and Delaveau, P. (1982) *Planta Med.* **46**, 56.
4. Dubois, M.-A., Zoll, A. and Chopin, J. (1983) *Phytochemistry* **22**, 2879.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
6. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
7. Chopin, J., Roux, B., Bouillant, M. L., Durix, A., d'Arcy, A., Mabry, T. J. and Yoshioka, H. (1969) *C. R. Acad. Sci. Ser. C* **268**, 980.
8. Chopin, J., Bouillant, M. L. and Biol, M. C. (1971) *C. R. Acad. Sci. Ser. C* **273**, 1262.
9. Chopin, J., Bouillant, M. L. and Besson, E. (1982) in *The Flavonoids, Advances in Research* (Harborne, J. B. and Mabry, T. J., eds.) p. 449. Chapman & Hall, London.
10. Markham, K. R. (1982) *Techniques of Flavonoid Identification*, p. 81. Academic Press, London.
11. Oesterdahl, B. G. (1979) *Acta Chem. Scand.* **B33**, 400.
12. Chopin, J. (1971) in *Pharmacognosy and Phytochemistry* (Wagner, H. and Hörhammer L., eds.) p. 111. Springer, Berlin.
13. Bouillant, M. L., Besset, A., Favre-Bonvin, J. and Chopin, J. (1978) *Phytochemistry* **17**, 527.
14. Lardy, C., Bouillant, M. L. and Chopin, J. (1982) Journées Internationales d'Etudes du Groupe Polyphénols, Toulouse, Résumés des communications.
15. Lardy, C. (1981) Thèse de Doctorat de 3ème cycle, Université Lyon I, N°1053.