Phytochemistry, 1979, Vol. 18, pp. 657–658. ©Pergamon Press Ltd. Printed in England. 0031-9422/79/0401-0657 $02.00/0

GENETICALLY CONTROLLED 2″-*O*-GLYCOSYLATION OF ISOVITEXIN IN THE PETALS OF *MELANDRIUM ALBUM*

ELISABETH BESSON†, ANDRÉ BESSET†, MARIE LOUISE BOUILLANT†, JEAN CHOPIN†, JAN VAN BREDERODE‡ and GERRIT VAN NIGTEVECHT‡

†Laboratoire de Chimie Biologique, Université de Lyon I, 69621 Villeurbanne, France;
‡Rijksuniversiteit Utrecht, Vakgroep populatie en evolutie biologie, Padualaan 8, Utrecht 2506, The Netherlands

(*Received* 30 *September* 1978)

Key Word Index—*Melandrium album*; Caryophyllaceae; isovitexin 2″-*O*-arabinoside; isovitexin 2″-*O*-rhamnoside; isovitexin 2″-*O*-glucoside; permethylisovitexin 3″-*O*-rhamnoside; MS fragmentation.

Abstract—The genetically controlled *O*-glycosylation of the 6-*C*-glucosyl residue of isovitexin in the petals of *Melandrium album* has been shown to take place in the 2″-position, by MS of the corresponding permethylated isovitexin glycosides.

In the petals of *Melandrium album*, the glycosylation of isovitexin (6-C-glucosylapigenin) is governed by a series of genes. Two of them respectively transfer glucose and xylose to the 7-hydroxyl group of isovitexin [1, 2]. Three others respectively control the transfer of arabinose, rhamnose and glucose to the 6-*C*-glucose of isovitexin [3, 4]. In this paper, this transfer is shown to take place in the 2″-position of the 6-*C*-glucose of isovitexin from the characteristic fragmentation pattern observed in the MS of the permethyl ethers of the corresponding isovitexin glycosides.

Indeed Bouillant *et al.* [5] have recently reported that permethylated 2″-*O*-glycosides of 6-*C*-glycosylflavones can easily be distinguished from their 4″ or 6″ isomers by the absence, in the MS of the former, of the M − 15 and M − 31 peaks characteristic of PM 6-*C*-glycosylflavones, respectively replaced by M − *O*-glycosyl and M − *O*-glycosyloxy peaks. Moreover *C*-rungiosylation of acacetin could be realized in the meantime [6], allowing the preparation of the permethyl 6-*C*-rungiosyl acacetin **1** required for completion of the work.

The main fragments observed in the MS of **1** (5,7,4′,2″,4″,6″,2‴,3‴,4‴-nonamethylisovitexin 3″-*O*-rhamnoside) are compared in Table 1 with those of PM 6-*C*-neohesperidosylacacetin **2** (5,7,4′,3″,4″,6″,2‴,3‴,4‴-nonamethylisovitexin 2″-*O*-rhamnoside), PM 6-*C*-rutinosylacacetin **3** (5,7,4′,2″,3″,4″,2‴,3‴,4‴-nonamethylisovitexin 6″-*O*-rhamnoside) and PM 6-*C*-cellobiosylacacetin **4** (5,7,4′,2″,3″,6″,2‴,3‴,4‴,6‴-decamethylisovitexin 4″-*O*-glucoside). As expected, the MS of **1** showed M − 15 and M − 31 peaks of the same importance as those observed in the MS of **3** and **4** when compared with the respective molecular peaks.

Every possible glycosylation position of the 6-*C*-

Table 1. MS data for PM 6-C-diholosylflavones **1**–**7**: *m/e* values (relative intensities, %) of the main fragments (≥5%)

Compound	1	2	3	4	5	6	7
M^+	704 (46)	704 (13)	704 (19)	734 (22)	690 (8)	704 (5)	734 (6)
M-15	689 (7)	—	689 (10)	719 (6)	—	—	—
M-31	673 (33)	—	673 (22)	703 (18)	—	—	—
SO_i^*	559 (12)	559 (6)	559 (15)	—	—	559 (5)	559 (6)
SO_j	545 (23)	545 (13)	545 (12)	—	545 (22)	545 (14)	545 (5)
SO_k	529 (16)	—	529 (8)	—	—	—	—
SO	515 (25)	515 (59)	515 (13)	—	515 (66)	515 (53)	515 (45)
SO-2	513 (28)		513 (23)	—	—	—	—
S + 2	501 (21)	—	501 (10)	—	—	—	—
S	499 (74)	499 (100)	499 (14)	499 (18)	499 (93)	499 (100)	499 (100)
S-14	485 (23)	—	485 (21)	—	—	—	—
SO-32	483 (42)	—	—	—	—	—	—
S-32	467 (60)	467 (6)	467 (8)	467 (8)	467 (7)	467 (6)	467 (8)
g	427 (9)	—	427 (13)	427 (5)	—	—	—
g − 2	425 (28)	—	425 (5)	425 (5)	—	—	—
f	397 (11)	397 (7)	397 (11)	—	397 (15)	397 (8)	397 (8)
n + 2	371 (11)	—	371 (15)	371 (19)	—	—	—
n	369 (42)	—	369 (32)	369 (24)	—	—	—
h	367 (28)	—	367 (15)	367 (8)	—	—	—
i	355 (69)	355 (7)	355 (100)	355 (100)	355 (35)	355 (10)	355 (11)
j	341 (100)	341 (61)	341 (26)	341 (25)	341 (100)	341 (75)	341 (35)
k	325 (63)	325 (13)	325 (15)	325 (10)	325 (25)	325 (20)	325 (13)
l	311 (39)	311 (8)	311 (13)	311 (7)	311 (25)	311 (10)	311 (6)

* See ref. [5] for peak nomenclature.

glucopyranose of isovitexin having now been studied, the following conclusion can be drawn: *in the MS of a PM* 6-C-*glucopyranosyl flavone* O''-*glycoside, the absence of a significant M* − 31 *peak* (*i.e. higher than* 50% *of the molecular peak*) *is characteristic of a* 2''-O-*glycoside.* On the other hand, the MS of **1**, **3** and **4** showed that 3''-*O*-glycosides can be easily distinguished from their 4'' or 6'' isomers by the importance of the peak *S* (M − *O*-glycosyloxy) and the peak j being the base peak. When the isovitexin *O*''-arabinoside, *O*''-rhamnoside and *O*''-glucoside were extracted from the petals of the appropriate genotypes of *Melandrium album* and permethylated, the MS of their permethylethers **5**, **6**, **7**, the main fragments of which are listed in Table 1, showed the characteristic fragmentation pattern of PM isovitexin 2''-*O*-glycosides.

Thus *Melandrium album* affords a further example of 2''-*O*-glycosylation of a *C*-glycosylflavone and it may be worth mentioning that, in the present state of our knowledge, this position of glycosylation appears to be more highly favoured in flavone *C*-glycosides than in flavone *O*-glycosides.

EXPERIMENTAL

Plant material. From *Melandrium* seed of the appropriate genotype, plants were raised with one single flavonoid glycoside in the petals. The various genotypes were obtained by inbreeding and selection. The plants were grown in the open in the experimental garden of the Department of Population and Evolutionary Biology, University of Utrecht. From each genotype 10 g petals were collected and stored in 70% MeOH, 1% HCl, at 4°.

Isolation of isovitexin O''-*glycosides.* The MeOH extract of petals was filtered, neutralized with 4 N NaOH, evapd to dryness and taken up in water. The aq. soln was extracted with *n*-BuOH. PC and TLC of the BuOH extract showed in each case only one main component. The main component was identified as an isovitexin *O*''-glycoside by UV spectrometry after purification by PC in 15% HOAc. The spectral data in MeOH and the diagnostic shifts with NaOAc + H_3BO_3, $AlCl_3$, $AlCl_3$ + HCl and NaOH were in each case the same as those given by apigenin [8]. Then the BuOH extract was evapd to dryness, the residue was permethylated in the usual way [7] and the main product was separated from the resulting mixture by PTLC on Si gel H in $CHCl_3$–EtOAc–Me_2CO (5:1:4) and (5:4:1). MS were recorded on an AEI MS 902 spectrograph (70 eV) by J. Favre-Bonvin in the Centre de Spectrométrie de Masse de l'Université de Lyon I. Temps. (sample and source in the same order) varied between 150 and 190°.

REFERENCES

1. van Brederode, J. and van Nigtevecht, G. (1972) *Genen Phaenen* **15**, 3.
2. van Brederode, J. and Nigtevecht, G. (1972) *Mol. Gen. Genet.* **118**, 247.
3. van Nigtevecht, G. and van Brederode, J. (1972) *Genen Phaenen* **15**, 9.
4. van Brederode, J. and van Nigtevecht, G. (1975) *Biochem. Genet.* **11**, 65.
5. Bouillant, M. L., Besset, A., Favre-Bonvin, J. and Chopin, J. (1978) *Phytochemistry* **17**, 527.
6. Besset, A. unpublished results.
7. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, New York.