Phytochemistry, Vol. 20, No. 8, pp. 1977–1979, 1981.
Printed in Great Britain.

0031–9422/81/081977–03 $02.00/0
Pergamon Press Ltd.

AN ACYLATED ALLOSE-CONTAINING 8-HYDROXYFLAVONE GLYCOSIDE FROM *VERONICA FILIFORMIS*

V. MOHAN CHARI, RENÉE J. GRAYER-BARKMEIJER,* JEFFREY B. HARBORNE* and BENGT-GÖRAN ÖSTERDAHL†‡

Institut für Pharmazeutische Biologie, Karlstr. 29, 8 München 2, West Germany; *Plant Science Laboratories, The University, Reading, RG6 2AS, U.K.; †Institute of Chemistry, Organic Chemistry Department, University of Uppsala, Uppsala, Sweden

(*Received* 19 *February* 1981)

Key Word Index—*Veronica filiformis*; Scrophulariaceae; flavone glycosides; isoscutellarein 4′-methyl ether; 7-*O*-β-(6‴-*O*-acetyl-2″-*O*-β-allosylglucoside); ^{13}C NMR.

Abstract—A novel flavone glycoside has been obtained from the whole plant of *Veronica filiformis* and identified by means of ^{13}C NMR spectroscopy as isoscutellarein 4′-methyl ether 7-*O*-β-(6‴-*O*-acetyl-2″-*O*-allosylglucoside). The related isoscutellarein glycoside is also present. This is the first report of 2-allosylglucose as a disaccharide unit of flavonoids. ^{13}C NMR data on some A-ring trioxygenated flavonoids are also presented.

INTRODUCTION

A chemosystematic study of the genus *Veronica* has revealed *inter alia* a range of flavone glycosides, based mainly on apigenin, luteolin, scutellarein and 6-hydroxyluteolin, in these plants [1, 2]. One group of five species in the section *Alsinebe*, subsection *Agrestis*, which includes *V. filiformis*, is distinguished from other *Veronica* species by the presence of 8-hydroxyflavone glycosides as the major leaf constituents [2]. Two of these glycosides have now been isolated and characterized. The present results indicate that these two glycosides are also distinctive in carrying acyl substitution and in containing a new disaccharide moiety.

RESULTS AND DISCUSSION

Extraction of the whole plant of *Veronica filiformis* gave two major glycosides, **1** and **2**, which were only clearly separable after paper chromatography in $CHCl_3$–HOAc–H_2O (2:1:1, lower layer). Glycoside **1** gave 4′-*O*-methylscutellarein (5,6,7-trihydroxy-4′-methoxyflavone), glucose and allose on acid hydrolysis. The latter hexose has R_f values very similar to those of glucose in most solvent systems but is clearly separated from it in phenol–water. The colour properties of **1** and, in particular, the ^{13}C NMR data (see below) showed that it was not a scutellarein but an isoscutellarein derivative. Acid hydrolysis had clearly caused the well known Wessely–Moser rearrangement to take place so that the original 5,7,8-trihydroxyflavone was converted to the corresponding 5,6,7-trihydroxy isomer. Similar behaviour has been recorded in the acid hydrolysis of other 8-hydroxyapigenin or 8-hydroxyluteolin glycosides [3, 4].

The complete structure of **1** was then established by its ^{13}C NMR spectrum (Table 1). The absence of an aromatic methine carbon signal in the range 90.0–96.0 ppm

Table 1. Chemical shift data for A-ring trioxygenated flavonoid 7-*O*-β-glycosides

	1	3	4
C-2	163.4	163.8	147.4
C-3	103.3	102.2	135.9
C-4	182.2	181.8	176.3
C-5	150.5	146.5	150.3
C-6	100.0	130.4	97.9
C-7	152.0	150.9	151.5
C-8	127.6	94.3	126.8
C-9	143.7	148.7	143.5
C-10	105.5	105.7	104.7
C-1′	122.7	121.1	122.1
C-2′	128.4	127.9	115.3
C-3′	114.5	115.6	145.1
C-4′	162.2	160.7	147.9
C-5′	114.5	115.6	115.6
C-6′	128.4	127.9	120.3
G-1*	99.2	101.2	101.5
G-2	82.5	72.9	73.3
G-3	75.5	75.8	75.7
G-4	69.2	69.7	69.8
G-5	77.2	77.0	77.3
G-6	60.6	60.6	60.7
		Methyl-β-D-allopyranoside	
A-1*	102.5	101.6	
A-2	70.7	70.6	
A-3	71.5	71.3	
A-4	66.9	67.8	
A-5	71.5	74.3	
A-6	63.6	61.7	
OMe	55.6	55.8	
Acetyl	20.5, 170.1		

*G = β-D-Glucoside, A = β-D-alloside. Solvent DMSO-d_6, 80°. The spectra were run on a Jeol FX-100 NMR spectrometer.

‡Present address: Food Laboratory, The National Food Administration, Box 622, S-751 26 Uppsala, Sweden.

indicates that C-8 is substituted [5]. As can be seen from the Table 1, the signal for the unsubstituted C-8 in scutellarein 7-*O*-β-glucoside (**3**) is at 94.3 ppm. The ready differentiation between a 5,6,7- and a 5,7,8-trioxygenation pattern on the basis of the chemical shift of the A-ring methine resonance has been previously indicated [5]. The presence of an acetyl group in the molecule is clearly evident from the signals at 20.5 ppm (Me) and 170.1 ppm (C=O). This was also apparent when treatment of **1** with a crude esterase preparation gave a new glycoside with lower R_f values. Comparison of the signals of the glucose moiety in **1** with the corresponding ones of **3** and **4** clearly show that 2″-hydroxyl is glycosidated by a second sugar and that the acetyl group must be situated on the second hexose moiety. The chemical shift values of the allose unit in **1** compared well with those of methyl-β-D-allopyranoside except for C-5 and C-6 indicating that the site of acetylation must be C-6‴ of the allose moiety. With the exception of the C-5‴ and C-6‴ resonances, the chemical shift values for the bioside moiety are very similar to those of chrysoeriol 7-*O*-(2″-*O*-β-D-allopyranosyl-β-D-glucospyranoside) isolated from *Sideritis grandiflora* [6]. These data thus show that **1** is 4′-*O*-methylisoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside).

The second glycoside **2** was very similar to **1** in all its properties, except that it gave scutellarein instead of the 4′-*O*-methyl ether on acid hydrolysis. Insufficient material was available for detailed spectral analysis, but the close similarity in R_f and the co-occurrence with **1** suggests that it is probably the related isoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside).

The chemical shift data for related A-ring trioxygenated flavonoid aglycones **5**–**10** are presented in Table 2. The resonances for the unsubstituted C-8 are all below 94.0 ppm except in the case where C-5 is methylated as in **9**. Substitution at C-2′, as in wightin (**10**), leads to an approximate 6.0 ppm downfield shift of the C-3 signal, compared with other flavones without C-2′ substitution. A similar observation has been made in the case of 2′-methoxyflavone [7].

Table 2. ^{13}C chemical shift data for some A-ring trioxygenated flavonoids*

	5	6	7	8	9	10
C-2	147.1	163.1	163.0	163.9	161.1	162.6
C-3	135.5	102.9	102.8	102.9	107.3	109.0
C-4	176.1	181.5	181.8	182.2	177.1	182.1
C-5	151.8†	152.0	152.4†	152.5†	152.6	156.6
C-6	130.9	132.0	131.2	131.9	140.4	96.0
C-7	157.2	158.1	157.0	158.5	157.7	158.5
C-8	93.7	91.8	94.0	91.3	96.3	128.4
C-9	151.4†	151.7	152.1†	152.1†	154.5	149.0
C-10	103.5	104.9	103.9	105.1	108.7	103.9
C-11	122.1	122.5	122.7	121.5	121.4	125.4
C-2′	115.2	127.5	127.9	110.1	111.2	146.2
C-3′	145.1	114.0	114.3	150.8	149.3	151.0
C-4′	147.8	161.9	162.0	148.0	151.9	119.2
C-5′	115.7	114.0	114.3	115.8	112.8	120.1
C-6′	120.1	127.5	127.9	120.4	119.6	124.5

*The spectra were run on a Jeol FX-100 NMR spectrometer, in DMO-d_6, except in the case of **6**, which was dissolved in $CDCl_3$.

†Assignments bearing the same superscripts in any one spectrum may be reversed.

Allose is a rare natural sugar and it has only recently been identified in association with flavonoids, following the isolation of kaempferol 3-*O*-β-alloside from the fern *Osmunda asiatica* [8]. This is then the second report of allose in a flavonoid derivative and the first report of 2-*O*-β-allopyranosylglucopyranose as the disaccharide component of a naturally occurring flavonoid bioside. Iridoid allosides have been isolated from *Mentzelia* [9] and the disaccharide derivative 2,3-di-*O*-acetyl-4-*O*-β-xylopyranosylallopyranose has been reported as the sugar moiety in the iridoid from *Viburnum opulus* [10]. Other reports of naturally occurring allosides are those of the 6′-*O*-cinnamate, 6-*O*-benzoate and the 6-*O*-β-phenylpropionate of 2-hydroxy-4-hydroxymethylphenyl-β-D-allopyranoside in the leaves of *Protea rubropilosa* Beard [11] as well as 1-(2,4-dimethoxy-6-hydroxy)-phenylbut-2-*O*-β-D-allopyranosyl-1-one from the fern *Arachinoides standishii* Ohwi [12].

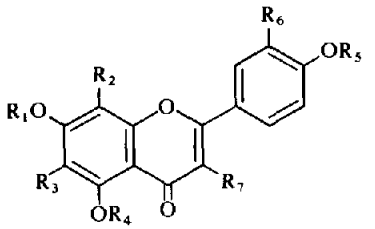
EXPERIMENTAL

Plant material. Plants of *Veronica filiformis* Sm. were collected in Switzerland by members of the Plant Systematics Laboratory of the University of Leiden and in the U.K. by one of us (R.J.G.-B.). Both samples gave identical 2-D chromatographic profiles for flavonoids and were combined. Voucher specimens are deposited in the herbarium at Leiden under the numbers LEP 16072 and LEP 21668, respectively.

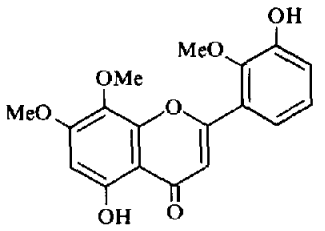
Isolation and identification of flavones **1** *and* **2**. The conc. 70% EtOH extract of the whole plant was purified by prep. PC in BAW and 15% HOAc, the major glycosidic fraction being then separated into **1** and **2** (R_fs 0.47 and 0.16) by prep. PC in CAW (for solvent key see ref. [13]). The two compounds were finally purified by PC in H_2O. Both were unstable in soln and on keeping, oxidized rapidly to black polymers, so that all operations in soln were carried out as quickly as possible. Glycoside **1** has R_fs 0.50 in BAW, 0.42 in 15% HOAc, 0.12 in H_2O and 0.88 in PhOH and appears on paper as dark absorbing in UV or UV + NH_3 and as bright yellow in daylight. The spectral λ_{max} in MeOH are: 281, 303 and 330 (shoulder); + $AlCl_3$ 321, 346, 430 nm; + NaOAc or NaOAc + H_3BO_3 no shifts; + NaOH, decomp. MS of permethyl ether: (AEI-MS-30, 70 eV, direct inlet) *m/z* (rel. int.) M^+ 778 (< 10), 515 (< 10), 391 (25), 328 (100), 313 (69), 299 (31), 243 (25), 219 (13), 187 (75), 173 (25), 167 (13), 157 (13), 155 (31), 101 (81). MS of peracetate: (AEI-MS-30, 70 eV, direct inlet) *m/z* (rel. int.) M^+ 1002 (< 10), 619 (< 10), 343 (< 10), 342 (< 10), 332 (14), 331 (90), 301 (10), 300 (60), 299 (< 10), 289, 284, 271, 229, 211, 187, 170 (all < 10), 169 (100), 145, 139 (< 10), 127 (26), 115 (10), 109 (< 10).

On hydrolysis with crude esterase (in an anthocyanase preparation), **1** gave a second glycoside, **1a**, with similar properties but with R_fs of 0.34 in BAW and 0.25 in 15% HOAc. On acid hydrolysis **1** gave scutallarein 4′-methyl ether, glucose and allose, all three components being identified by direct spectral and chromatographic comparisons with authentic markers. Allose has similar R_fs to glucose in BAW, BEW and BBPW, but separates clearly in PhOH (allose R_f 0.44, glucose 0.34).

Glycoside **2** has R_fs 0.42 in BAW, 0.39 in 15% HOAc, 0.10 in H_2O and 0.85 in PhOH. Spectral λ_{max} in MeOH: 280, 311, 330; + $AlCl_3$ 326, 349, 424 nm; no shifts with NaOAc or NaOAc + H_3BO_3; rapid decomp. with NaOH.



(1) R_1 = 6-*O*-Acetyl-β-D-allo-(1 → 2)-β-D-glucosyl, $R_3 = R_4 = R_6 = R_7 = H$, $R_2 = OH$, $R_5 = Me$
(3) R_1 = β-D-Glucosyl, $R_2 = R_4 = R_5 = R_6 = R_7 = H$, $R_3 = OH$
(4) R_1 = β-D-Glucosyl, $R_2 = R_6 = R_7 = OH$, $R_3 = R_4 = R_5 = H$
(5) $R_1 = R_2 = R_4 = R_5 = H$, $R_6 = R_7 = OH$, $R_3 = OMe$
(6) $R_1 = R_5 = Me$, $R_2 = R_4 = R_6 = R_7 = H$, $R_3 = OMe$
(7) $R_1 = R_2 = R_4 = R_6 = R_7 = H$, $R_3 = OMe$, $R_5 = Me$
(8) $R_1 = R_2 = R_4 = R_5 = R_7 = H$, $R_3 = R_6 = OMe$
(9) $R_2 = R_7 = H$, $R_1 = R_4 = R_5 = Me$, $R_3 = R_6 = OMe$



(10)

Acknowledgements—We thank Prof. T. R. Govindachari (Madras, India) for a sample of wightin and Dr. E. Nilsson (Uppsala, Sweden) for a sample of scutellarein 7-*O*-β-glucoside.

REFERENCES

1. Grayer-Barkmeijer, R. J. (1978) *Biochem. System. Ecol.* **6**, 131.
2. Grayer-Barkmeijer, R. J. (1979) Doctoral thesis, University of Leiden.
3. Harborne, J. B. (1969) *Phytochemistry* **8**, 2071.
4. Markham, K. R. and Porter, L. J. (1979) *Phytochemistry* **18**, 611.
5. Markham, K. R. and Chari, V. M. (1981) in *Recent Advances in Flavonoid Research*, 1975–1980 (Harborne, J. B. and Mabry, T. J., eds.) in press.
6. Rabanal, R. A., Valverde, S., Martin-Lomas, M., Rodriguez, B. and Chari, V. M. (1981) *Phytochemistry* (to be submitted).
7. Joseph-Nathan, P., Mares, J. and Hernandez, M. C. (1974) *J. Mag. Reson.* **16**, 447.
8. Okuyama, T., Hosoyama, K., Kiraga, Y., Kurono, G. and Takemoto, T. (1978) *Chem. Pharm. Bull.* **26**, 3071.
9. Jensen, S. R., Mikkelsen, C. B. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 71.
10. Bock, K., Jensen, S. R., Nielsen, B. J. and Norn, V. (1978) *Phytochemistry* **17**, 753.
11. Perold, G. W., Beylis, P. and Howard, A. S. (1973) *J. Chem. Soc. Perkin Trans.* **1**, 643.
12. Tanaka, N., Machashi, H., Saito, S., Murakami, T., Saiki, Y., Chen, C.-M. and Iitaka, Y. (1980) *Chem. Pharm. Bull.* **28**, 3070.
13. Harborne, J. B. (1973) *Phytochemical Methods*. Chapman & Hall, London.