Phytochemistry, Vol. 26, No. 4, pp. 1185–1188, 1987.
Printed in Great Britain.

0031-9422/87 \$3.00 + 0.00

ACETYLATED ALLOSE-CONTAINING FLAVONOID GLUCOSIDES FROM *STACHYS ANISOCHILA*

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(*Revised received* 25 *July* 1986)

Key Word Index—*Stachys anisochila*; Labiatae; 8-hydroxyflavonoids; allose; acetylated sugars; two-dimensional NMR.

Abstract—In continuation of our chemosystematic study of *Stachys* (Labiatae) we have isolated the previously reported isoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranoside] (**1**) and 3′-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranoside] (**4**) and four new allose-containing flavonoid glycosides from *S. anisochila*. The new glycosides are hypolaetin 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranside] (**6**) as well as the three corresponding diacetyl analogues of **1**, **4** and **6**, isoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside], 3′-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside] and hypolaetin 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside]. Extensive two-dimensional NMR studies (proton-carbon correlations, COSY experiments) allowed assignment of all ^{1}H NMR sugar signals and a correction of the ^{13}C NMR signal assignments for C-2 and C-3 of the allose.

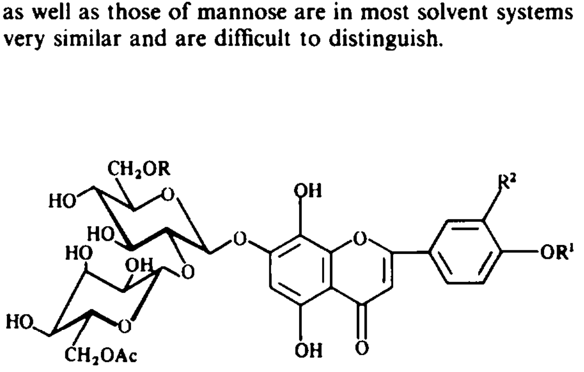
INTRODUCTION

We previously reported from *Stachys recta* (Labiatae) [1] the isolation and structure elucidation of three flavonoid glycosides (**1**, **3** and **4**), all of which contained acetylated allose. Using HPLC with modern diode array technology for a chemotaxonomical screening of 13 species of the genus *Stachys* [2] four further flavonoids were detected in *S. anisochila* in addition to **1** and **4**. Establishing a band around 300 nm in the UV, the compounds could be partially identified as 5,8-dihydroxyflavonoids with a trisubstituted A-ring [3]. Their isolation and structure elucidation are reported here. All four compounds are new acetylated allose-containing flavonoid glucosides.

RESULTS AND DISCUSSION

The flavonoid glycosides were isolated by classical methods of CC and prep. TLC and, except for **4** and **6**, all were crystallized. The UV spectra recorded with diagnostic reagents [4] resulted in grouping the compounds in three pairs (**1**/**2**, **4**/**5** and **6**/**7**, respectively). Both compounds in each pair exhibited the same UV data (Table 1), thus establishing that the members of each pair have the same aglycone skeleton. When the UV spectra obtained in $AlCl_3$ were compared for **4** and **5** as well as for **6** and **7** a further differentiation was evident. For **6** and **7** an intense maximum at 440 nm suggested for each a free *ortho*-dihydroxy group in the B-ring. Since a similar strong maximum was absent for **4** and **5**, they must be substituted at either 3′ or 4′. In the ^{1}H NMR (Table 2) one three-proton signal at δ1.88 was observed for **1**, **4** and **6**, whereas two three-proton signals were present at δ1.88 and δ2.06 for **2**, **5** and **7**. These signals correspond to aliphatic acetyl groups attached to the sugar moiety. In the region where most glycosyl proton signals occur (δ3.3–5.3) **1**, **4** and **6** (compounds with one acetyl group) were essentially identical but different from **2**, **5** and **7** (compounds with two acetyl groups); however, the latter three gave similar patterns. According to the integration and the number of glycosyl peaks all compounds appeared to be diglycosides. A signal for one methoxyl group was found at δ3.88 for **4** and **5**, thus specifying the B-ring substitution pattern postulated by the $AlCl_3$ data in the UV of these compounds. The signals in the region of δ6.0–8.5 for the aglycone moieties were in agreement with the proposed structures. Comparison of the ^{13}C NMR data (Table 3) of **4** and **5** with those of **6** and **7** resulted in the assignment of the methoxyl group to 4′: C-5′ of **4** and **5** showed an upfield shift of *ca* 3.7 ppm due to an *ortho* carbon resonance with a methoxyl group [5].

By acid hydrolysis of the compounds and subsequent HPLC analysis of the sugars [6] glucose and allose were found. On TLC and PC, the R_f values of these two sugars as well as those of mannose are in most solvent systems very similar and are difficult to distinguish.



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = H,\ R^3 = Ac$
3 $R^1 = Me,\ R^2 = R^3 = H$
4 $R^1 = Me, R^2 = OH, R^3 = H$
5 $R^1 = Me, R^2 = OH, R^3 = Ac$
6 $R^1 = R^3 = H,\ R^2 = OH$
7 $R^1 = H,\ R^2 = OH,\ R^3 = Ac$

Table 1. UV data of the isolated flavonoid glycoside pairs

	1/2	4/5	6/7
MeOH	277, 308, 323 sh	252*, 277, 298 sh, 335	252*, 276, 298 sh, 335
NaOMe	240, 270 sh, 372 decomp.	248, 313 sh, 375 decomp.	252, 392 decomp.
$AlCl_3$	276, 322, 350, 415	268 sh, 280, 310, 360, 425	276, 320, 440
$AlCl_3 + HCl$	278, 322, 342 sh, 415	262 sh, 278, 310, 327 sh, 352, 425	265 sh, 276, 312 sh, 325, 358, 420
NaOAc	275, 310, 327, 388 decomp.	252, 277, 298 sh, 332, 378 sh decomp.	262, 301 sh, 400 decomp.
$NaOAc + H_3BO_3$	277, 308, 327 sh	252, 276, 298 sh, 335	262, 300 sh, 378

*Only as shoulder in **5** and **7**.

Table 2. ^{1}H NMR data of the flavonoid glycosides (**1**, **2**, **4**–**7**)

	1	2	4	5	6	7
C-3	6.84 *s*	6.84 *s*	6.80 *s*	6.80 *s*	6.72 *s*	6.72 *s*
C-6	6.70 *s*	6.65 *s*	6.70 *s*	6.65 *s*	6.70 *s*	6.65 *s*
C-2′	7.98 *d*	7.98 *d*	7.52 *d*	7.52 *d*	7.48 *d*	7.48 *d*
	J = 8.8	*J* = 8.8	*J* = 2.2	*J* = 2.2	*J* = 2.2	*J* = 2.2
C-3′	6.95 *d*	6.95 *d*	—	—	—	—
	J = 8.8	*J* = 8.8				
C-5′	6.95 *d*	6.95 *d*	7.12 *d*	7.12 *d*	6.92 *d*	6.92 *d*
	J = 8.8	*J* = 8.8	*J* = 8.8	*J* = 8.8	*J* = 8.8	*J* = 8.8
C-6′	7.98 *d*	7.98 *d*	7.61 *dd*	7.61 *dd*	7.49 *dd*	7.48 *dd*
	J = 8.8	*J* = 8.8	*J* = 8.5/2.3	*J* = 8.5/2.3	*J* = 8.5/2.3	*J* = 8.5/2.3
G-1*	5.10 *d*	5.17 *d*	5.10 *d*	5.17 *d*	5.10 *d*	5.17 *d*
	J = 7.4	*J* = 7.4	*J* = 7.4	*J* = 7.4	*J* = 7.4	*J* = 7.4
G-2	3.60 *t*	3.64 *t*	3.60 *t*	3.64 *t*	3.60 *t*	3.64 *t*
G-3	3.53 *m*†	3.54 *t*	3.53 *m*†	3.54 *t*	3.53 *m*†	3.54 *t*
G-4	3.28 *m*‡	3.29 *t*	3.28 *m*‡	3.29 *t*	3.28 *m*‡	3.29 *t*
G-5	3.50 *m*†	3.76 *m*	3.50 *m*†	3.76 *m*	3.50 *m*†	3.76 *m*
G-6	3.53 *m*†	4.12 *dd*	3.53 *m*†	4.12 *dd*	3.53 *m*†	4.12 *dd*
	3.76 *d*	4.33 *d*	3.76 *d*	4.33 *d*	3.76 *d*	4.33 *d*
A-1*	4.94 *d*	4.92 *d*	4.94 *d*	4.92 *d*	4.93 *d*	4.92 *d*
	J = 8.0	*J* = 8.0	*J* = 8.0	*J* = 8.0	*J* = 8.0	*J* = 8.0
A-2	3.28 *m*‡	3.25 *dd*	3.28 *m*‡	3.25 *dd*	3.28 *m*‡	3.25 *dd*
A-3	3.92 *s*	3.92 *t*	3.92 *s*	3.92 *t*	3.92 *t*	3.92 *t*
A-4	3.43 §	3.42 *dd*	3.43 §	3.42 *dd*	3.43 §	3.42 *dd*
A-5	3.88 *dt*	3.85 *dt*	3.88 *dt*‖	3.85 *dt*‖	3.88 *dt*	3.85 *dt*
A-6	5.05 *d*	3.99 *d*	4.05 *d*	3.05 *d*	4.05 *d*	3.99 *d*
OCH_3	—	—	3.88 *s*	3.88 *s*	—	—
OH at C-5	12.37 *s*	12.37 *s*	12.32 *s*	12.33 *s*	12.38 *s*	12.38 *s*
CH_3 (Ac)	1.89 *s*	1.87 *s*	1.88 *s*	1.88 *s*	1.88 *s*	1.88 *s*
	—	2.06 *s*	—	2.06 *s*	—	2.06 *s*

*G, β-D-Glucoside; A, β-D-alloside.

†,‡Overlapping signals.

§Signal partially overlapped by the H_2O peak.

‖Signal partially overlapped by the OCH_3 peak.

Solvent DMSO-d_6. The spectra were run on a GN 500 spectrometer (500.10 MHz). Chemical shifts (ppm) relative to TMS as internal standard, coupling constants (*J*) in Hz.

The signal at δ82.4, corresponding to the C-2 of the glucosyl moiety, is indicative of a disaccharide in which the glucosyl group is attached to the aglycone and has a second glycosyl unit attached at C-2. The ^{13}C values for C-1 of both the glucosyl (δ99.3) and allosyl (δ102.3) units suggested pyranose rings with β-linkages: this was confirmed by ^{1}H NMR exhibiting two doublets for two anomeric protons at δ5.10 (glucosyl) and δ4.94 (allosyl) with large coupling constants of *ca* 7.4 and 8.0 Hz, respectively. Using ATP experiments (this technique distinguishes carbons with zero and two protons from those with one or three) it was possible to localize in the ^{13}C NMR the C-6 signals of both glycosyl groups. For **2**, **5** and **7** both C-6 signals are shifted downfield about 3.0 ppm from the expected values (δ*ca* 63 instead of δ60) indicating that the acetyl groups observed for **2**, **5** and **7** in

Table 3. ^{13}C NMR spectral data of the flavonoid glycosides (1, 2, 4–7)

	1	**2**	**4**	**5**	**6**	**7**
C-2	164.0	164.1	164.0	163.9	164.2	164.2
C-3	102.6	102.7	103.4	103.3	102.6	102.6
C-4	182.3	182.3	182.4	182.2	182.2	182.1
C-5	150.3	150.4	150.7	150.4	150.5	150.3
C-6	100.0	99.6	100.1	99.4	99.8	99.4
C-7	152.1	152.2	152.3	152.1	152.1	152.1
C-8	127.4	127.4	127.6	127.3	127.4	127.3
C-9	143.6	143.8	143.9	143.9	143.8	143.9
C-10	105.5	105.6	105.6	105.5	105.4	105.4
C-1′	121.1	121.2	123.1	122.7	121.5	121.4
C-2′	128.6	128.6	113.2	113.1	113.5	113.5
C-3′	115.9	115.9	146.8	146.7	145.6	145.6
C-4′	161.3	161.4	151.2	151.2	149.8	149.8
C-5′	115.9	115.9	112.1	112.1	115.8	115.8
C-6′	128.6	128.6	119.0	118.9	119.1	119.1
G-1*	99.3	99.2	99.5	99.0	99.2	99.0
G-2	82.5	82.4	82.2	81.9	82.1	81.9
G-3	75.5	75.3	75.6	75.2	75.4	75.2
G-4	69.2	69.5	69.3	69.4	69.1	69.4
G-5	77.1	73.7	77.1	73.6	77.0	73.5
G-6	60.5	63.1	60.6	63.1	60.4	63.0
A-1*	102.5	102.5	102.3	102.1	102.2	102.1
A-2	71.4	71.5	71.5	71.3	71.4	71.3
A-3	70.7	70.8	70.9	70.7	70.7	70.7
A-4	66.7	66.8	67.0	66.8	66.8	66.8
A-5	71.4	71.5	71.5	71.3	71.4	71.3
A-6	63.4	63.5	63.6	63.4	63.3	63.4
OMe	—	—	55.8	55.7	—	—
	20.4	20.5	20.4	20.3	20.3	20.3
	—	20.6	—	20.5	—	20.5
Acetyl	170.2	170.2	170.3	170.1	170.2	170.1
	—	170.3	—	170.2	—	170.2

*G, *β*-D-Glucoside, A, *β*-D-alloside.

The spectra were run on a GN 500 spectrometer (125.76 MHz). Chemical shifts (ppm) relative to TMS as internal standard. Solvent DMSO-d_6.

the ^{1}H NMR and detected also in the ^{13}C NMR (signals at δ20.3/20.5 and δ170.1/170.2) are attached at C-6 of each glycosyl moiety [5]. In **1**, **4** and **6** (one acetyl group each) the assignment of the acetyl group to the C-6 of the allose was made by comparing the ^{1}H and ^{13}C NMR data of **1**, **4** and **6** with those for **2**, **5** and **7**. The chemical shift values of all signals in the bioside moieties of **1**, **4** and **6** agree rather well with those of **2**, **5** and **7** with the exception of those for the C-5 and C-6 resonances of the glucose. Therefore, **1** is isoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranoside], **2** isoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside], **4** 3′-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranoside], **5** 3-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside], **6** hypolaetin 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-*β*-D-glucopyranoside] and **7** is hypolaetin 7-*O*-[6‴-*O*-acetyl-*β*-D-allopyranosyl-(1 → 2)-6″-*O*-acetyl-*β*-D-glucopyranoside].

The assignments of all glycosyl signals in the ^{1}H and ^{13}C NMR were based on two-dimensional NMR studies (proton–carbon correlation, COSY experiments) [7]. The second acetyl group at C-6 in the glucosyl moiety of **2**, **5** and **7** shifted downfield the ^{1}H NMR signals of the two C-6 protons as well the one of the proton at C-5, thus separating to some extent the glycosyl signals. Once the C-1, C-2 and C-6 of the glucosyl and C-1, C-4 and C-6 of the allosyl groups were assigned in the ^{13}C NMR, proton–carbon correlation experiments allowed the assignment of the corresponding ^{1}H NMR signals. Examining the cross peaks in the COSY experiment, the remaining signals in the ^{1}H NMR were assigned. The ^{1}H NMR assignments reconfirmed the ^{13}C NMR proton–carbon correlation except for the previously reported ^{13}C NMR assignments of C-2 and C-3 of the allose, which needed to be interchanged [1, 8]. In the ^{1}H NMR the signals for the protons of C-2 and C-3 of the allosyl moiety were readily assigned since the C-2 proton signal gave a broad doublet of a doublet at δ3.25 with a large coupling to the anomeric proton, while the C-3 proton appeared as a small triplet at δ3.92. In contrast, in the ^{13}C NMR the signals of the C-2 and C-3 carbons of allose have similar chemical shifts (δ70.8 for C-3 and 71.6 for C-2; moreover the latter signal is further overlapped by the C-5 signal) making these assignments more difficult thus accounting for the previous incorrect assignments.

EXPERIMENTAL

Plant material. Stachys anisochila Vis. & Pancic was collected from the State of Bosnia-Hercegovina, Yugoslavia, on the road from Sarajevo to Mostar, east of the village of Konjic, by M. Bandle and A. Lenherr, 25 May 1981. A voucher specimen (coll. no. 22494) is on deposit in the Herbarium of the Geobotanical Institute, ETH Zuerich (ZT).

Isolation of flavonoids. Freeze-dried leaves of *S. anisochila* (60 g) were exhaustively extracted with MeOH. The filtrate was concd, dissolved in H_2O and purified from lipophilic components with petrol. The freeze-dried water fraction afforded 15 g of a highly enriched flavonoid extract. This extract was chromatographed on a Polyclar (Polyclar AT, GAF corp.) column, initiated with toluene–MeOH 9:1 and gradually increased to pure MeOH in 10% increments. The flavonoids eluted in the following order: **5** (as a pure compound), **2** and **4** (as mixture), **1** and **7** (as mixture) and finally **6** (as a pure compound). The mixtures of **2** and **4** as well as of **1** and **7** were separated subsequentally on prep. silica gel TLC plates with CH_2Cl_2–MeOH–H_2O (40:10:1) as solvent system. Compounds **5** and **2** were further crystallized from a mixture of toluene–MeOH and **1** and **7** from a mixture of MeOH–H_2O; **4** was only available in a small amount and for **6** no suitable crystallization medium was found.

Sample preparation for sugar analysis. A dry sample of the flavonoid glycoside was dissolved in 0.1 N TFA and refluxed for 50 min on a steam bath. After repeated evaporation *in vacuo* of the hydrolysis soln, the residue was taken up in H_2O and the aq. soln extracted with EtOAc. After further purification by filtration over Al_2O_3 the sample was freeze-dried.

Identification of the sugars. Sugar analysis was carried out by HPLC at 80° on an Aminex HPX-87P monosaccharide column, 300 × 7.8 mm, particle size 9 μm (BIORAD che. div., California). Deionized H_2O was used as solvent (flowrate 0.2 ml/min for 0–10 min, after 0.5 ml/min). Detection by a refractive index (Altex 156 RI detector). The sample (20 μl in H_2O) was injected in a concentration of 2 mg/ml, the reference compounds in a concentration of 1 mg/ml each. Two peaks were obtained corresponding to glucose (R_t 15.1 min) and allose (R_t 25.6 min). Mannose (R_t 18.8 min) could not be detected.

Acknowledgements—This work was supported by a research grant from the Swiss National Science Foundation to Andreas Lenherr and by grants to T.J.M. from the National Science Foundation (BSR-8402017) and Robert A. Welch Foundation (F-130). The authors are indebted to Dr. B. Meier and Professor Dr. O. Sticher (Pharm. Institute ETH, Zuerich) for the earlier HPLC analysis in Stachys, to Dr. St. Sorey and Dr. B. Shoulders (Dept. of Chemistry, University of Texas at Austin) for the detailed NMR experiments and fruitful discussions, as well as to Dr. M. Gretz (Dept. of Biology, George Mason University, Fairfax, Virginia) for assistance in the sugar analysis.

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