Phytochemistry, Vol 23, No 10, pp 2343–2345, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

THREE FLAVONOID GLYCOSIDES CONTAINING ACETYLATED ALLOSE FROM *STACHYS RECTA*

ANDREAS LENHERR*, MOHAMMED F LAHLOUB†‡ and OTTO STICHER†

*Geobotanisches Institut and †Pharmazeutisches Institut, Eidgenossische Technische Hochschule (ETH), CH-8092 Zurich, Switzerland

(*Received* 9 *February* 1984)

Key Word Index—*Stachys recta*, Labiatae, flavone glycosides, 4′-*O*-methylisoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside), isoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside), 3′-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside)

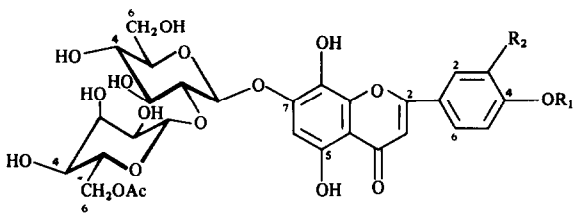
Abstract—By means of ^{13}C and ^{1}H NMR spectroscopy three flavone glycosides, obtained from *Stachys recta*, were identified as 7-*O*-(2‴-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranosides) of 4′-*O*-methylisoscutellarein, isoscutellarein and 3′-hydroxy-4′-*O*-methylisoscutellarein The latter two compounds are isolated for the first time Only mannose and glucose have been reported previously as sugar components of flavonoids of the genus *Stachys*

INTRODUCTION

Stachys recta (Labiatae) represents the widest distributed species of a polymorphic species group (about 10 species), with a main centre of diversity in the Balkan peninsula As part of a biosystematic and chemotaxonomic study of this group [1], three flavone glycosides from *Stachys recta* were isolated as reference compounds for the chemotaxonomical investigations In the present communication the isolation and structure elucidation of these flavonoids is described Glycosides **2** and **3** are new compounds

RESULTS

Glycosides **1**, **2** and **3** are the main components of a methanolic extract of lyophilized leaves from *Stachys recta* After preliminary TLC experiments glycoside **1** was separated by CC from glycosides 2 and 3 using dichloromethane–methanol–water (80 20 2) Glycosides **2** and **3** were isolated by subsequently CC with ethyl acetate–propanol–water (4 2 7, upper phase) All three substances were purified by semipreparative HPLC, using varying mixtures of methanol–water as mobile phase After lyophilization ^{1}H and ^{13}C NMR data as well as UV spectra with diagnostic reagents [2] were recorded



1 R_1 = Me, R_2 = H
2 R_1 = R_2 = H
3 R_1 = Me, R_2 = OH

The structure of the aglycone moiety of all three glycosides was established by UV data (Table 1) All three compounds exhibit band II at about 280 nm, which is typical for flavonoids with a 5,7,8 hydroxylated A-ring Band II of glycoside **3** shows two absorption maxima (255 and 278 nm), a characteristic of 3′,4′-oxygenated flavones The 4′-oxygenated equivalents, glycosides **1** and **2**, have only one maximum for band II The addition of sodium methoxide to all three compounds produces a bathochromic shift of more than 40 nm in band I However, only for glycoside **2** there is no decrease in intensity, which is diagnostic for the presence of a free 4′-hydroxyl group For glycoside **1** and **3** (methoxylated in 4′ position) the intensity of band I decreases Thus the aglycone of **1** is 4′-*O*-methylisoscutellarein, that of **2** is isoscutellarein and that of **3** is 3′-hydroxy-4′-*O*-methylisoscutellarein These structures were confirmed by the ^{1}H and ^{13}C NMR data (Tables 2 and 3) The chemical shift values of the three compounds differ only in position C-1′ to C-6′, the other values being identical

According to TLC (after acid hydrolysis) and the ^{1}H and ^{13}C NMR data the sugar components of all three flavonoids are glucose and allose Glucose, allose as well as mannose have very similar R_f values on TLC in most solvent systems Using pyridine–ethyl acetate–acetic acid–water (36 36 7 21) as solvent system and cellulose as support, two spots on TLC were observed Mannose was excluded by ^{1}H NMR data Altona and Haasnoot [3] assign to the coupling constant of the proton at C-1 of β-D-mannose values of *ca* 1 2 Hz For those of β-D-glucose and β-D-allose they give values of 7 8 Hz and 8 4 Hz respectively For all three flavonoids isolated in this work values of 7 4 Hz (= G-1, cf Table 2) and 8 0 Hz (= A-1) were obtained

The presence of an aliphatic acetyl group in all three

‡Present address Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

Table 1 UV data of compounds 1–3

	1	2	3
MeOH*	279, 306, 326 sh	277, 307 5, 322 sh	255, 278, 298, 335
$AlCl_3$	282, 322, 347, 427	282, 323, 343	264 sh, 282, 314, 360
NaOMe	311	243, 376	243, 270 sh, 318, 380
	decomp	decomp	decomp

*Identical values were obtained in MeOH–NaOAc as in pure MeOH

Table 2 ^{1}H NMR spectral data of compounds 1–3

	1	2	3
C-3	6 91 *s*	6 81 *s*	6 78 *s*
C-6	6 71 *s*	6 70 *s*	6 70 *s*
C-2′	8 10 *d*	7 98 *d*	7 50 *d*
	J = 8 8	*J* = 8 8	*J* = 2 2
C-3′	7 14 *d*	6 95 *d*	—
	J = 8 8	*J* = 8 8	
C-5′	7 14 *d*	6 95 *d*	7 12 *d*
	J = 8 8	*J* = 8 8	*J* = 8 7
C-6′	8 10 *d*	7 98 *d*	7 61 *dd*
	J = 8 8	*J* = 8 8	*J* = 8 5/2 3
G-1*	5 08 *d*	5 05 *d*	5 08 *d*
	J = 7 4	*J* = 7 3	*J* = 7 4
G-2	4 98 *s* (*br*)	5 00 *s* (*br*)	4 94 *s* (*br*)
G-3			
G-4	4 12–3 28	4 10–3 16	4 10–3 26
G-5			
G-6	5 45–4 69	5 50–4 60	5 42–4 62
A-1*	4 94 *d*	4 93 *d*	4 94 *d*
	J = 8 0	*J* = 8 0	*J* = 8 0
A-2			
A-3	4 12–3 28	4 10–3 16	4 10–3 26
A-4			
A-5			
A-6	5 45–4 69	5 50–4 60	5 42–4 62
OMe	3 88 *s*	—	3 89 *s*
OH at C-5	12 32 *s*	12 34 *s*	12 32 *s* (*br*)
Me (Ac)	1 89 *s*	1 88 *s*	1 88 *s*

*G, β-D-Glucoside, A, β-D-alloside Solvent DMSO-d_6 The spectra were run on a Bruker Spectrospin WM 300 spectrometer (300 13 MHz) Chemical shifts (ppm) relative to TMS as internal standard, coupling constants (*J*) in Hz

Table 3 ^{13}C NMR spectral data of compounds 1–3

	1	2	3
C-2	163 7	164 0	164 0
C-3	103 4	102 5	103 4
C-4	182 4	182 3	182 4
C-5	150 6	150 4	150 7
C-6	100 2	100 0	100 1
C-7	152 2	152 1	152 3
C-8	127 6	127 4	127 6
C-9	143 8	143 7	143 9
C-10	105 6	105 5	105 6
C-1′	122 8	121 1	123 1
C-2′	128 4	128 6	113 2
C-3′	114 5	115 9	146 8
C-4′	162 4	161 4	151 2
C-5′	114 5	115 9	112 1
C-6′	128 4	128 6	119 0
G-1*	99 6	99 4	99 5
G-2	82 5	82 6	82 2
G-3	75 6	75 5	75 6
G-4	69 3	69 2	69 3
G-5	77 2	77 1	77 1
G-6	60 5	60 5	60 6
A-1*	102 6	102 5	102 3
A-2	70 8	70 7	70 9
A-3	71 6	71 5	71 5
A-4	66 9	66 8	67 0
A-5	71 6	71 5	71 5
A-6	63 6	63 5	63 6
OMe	55 5	—	55 8
Acetyl	20 5	20 4	20 4
	170 4	170 2	170 3

*G, β-D-Glucoside, A, β-D-alloside The spectra were run on a Bruker Spectrospin WM 300 spectrometer (75 47 MHz), chemical shifts (ppm) relative to TMS as internal standard Solvent DMSO-d_6

molecules is evident from the signals at 20 5 ppm (Me) and 170 3 ppm (C=O) in the ^{13}C NMR spectrum This acetyl group must be localized at the sugar moiety According to Markham and Chari [4], acetylation at OH–C–6 of a sugar is evidenced by the downfield shift of 1 8–3 ppm in the C-6 signal and an upfield shift of about 3 4 ppm in the C-5 signal This was observed for the allose in all three compounds

DISCUSSION

Flavonoid **1** was identified therefore as 4′-*O*-methylisoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside), which was earlier isolated by Chari *et al* [5] from *Veronica filiformis* Smith The ^{13}C NMR data of flavonoid **1** agrees well with those reported by Chari *et al* [5] Flavonoid **2**, isoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside), and flavonoid **3**, 3-hydroxy-4′-*O*-methylisoscutellarein 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside) were isolated for the first time

Until now flavonoid glycosides from the genus *Stachys*

have been isolated only by Russian scientists from some Caucasian species [6–10] All compounds reported contain a scutellarein- or isoscutellarein skeleton with 2-mannosylglucose as the sugar moiety Allose as sugar moiety has never been reported In recent publications [11–13] they described also some flavonoids with an acetyl group at the sugar moiety, however the position of the acetyl group was not determined

EXPERIMENTAL

Plant material Leaves of *Stachys recta* L were collected on 13 August, 1980 in Switzerland by R Lang and A Lenherr at Ruggplangge in the Walenstadterberg, Kt St Gallen, at an altitude of 1300 m Voucher specimens of the whole plants (coll no 80/1642) are deposited in the Herbarium of the Geobotanical Institute, ETH Zurich (ZT)

Isolation and identification The lyophilized leaves were extracted in MeOH The filtrate was concd, dissolved in water and purified from lipophilic components with petrol In TLC experiments with CH_2Cl_2–MeOH–H_2O (80 20 2) on silica gel **1** has R_f 0 63, **2** 0 33, and **3** 0 41, with EtOAc–*n*-PrOH–H_2O (4 2 7, upper phase) **1** has R_f 0 58, **2** 0 65 and **3** 0 51 Both solvent systems are subsequently used for the separation of the three glycosides by CC on silica gel The purification of the three glycosides was carried out by semipreparative HPLC on a Knauer C_{18} reversed phase column (25 cm × 16 mm i d) The spectral data of the purified compounds are given in Tables 1–3 The sugar components are supplementary identified by TLC on cellulose after acid hydrolysis with pyridine–EtOAc–HOAc–H_2O (36 36 7 21) as solvent system (glucose R_f 0 60, allose R_f 0 63 and mannose R_f 0 66) Detection by a solution of aniline-phthalate in H_2O-saturated BuOH

REFERENCES

1 Lenherr, A (1983) Doctoral thesis, ETH Zürich, No 7453
2 Mabry, T J , Markham, K. R and Thomas, M B (1970) in *The Systematic Identification of Flavonoids* Springer, Berlin
3 Altona, C and Haasnoot, C A G (1980) *Org Magn Reson* **13**, 417
4 Markham, K R and Chari, V M (1982) in *Recent Advances in Flavonoid Research, 1975–1980* (Harborne, J B and Mabry, T J , eds) Chapman & Hall, London
5 Chari, V M , Grayer-Barkmeijer, R J , Harborne, J B and Oesterdahl, B G (1981) *Phytochemistry* **20**, 1977
6 Sheremet, I P and Komissarenko, N F (1971) *Chem Nat Compds* **7**, 563, 700, 829
7 Sheremet, I P and Komissarenko, N F (1972) *Chem Nat Compds* **8**, 616
8 Komissarenko, N F , Sheremet, I P , Derkach, A I and Pakaln, D A (1976) *Chem Nat Compds* **12**, 88
9 Komissarenko, N F , Derkach, A I , Sheremet, I P , Kovalev, I P , Gordienko, V G and Pakaln, D A (1978) *Chem Nat Compds* **14**, 445
10 Derkach, A I , Komissarenko, N F , Gordienko, V G , Sheremet, I P , Kovalev, I P and Pakaln, D A (1980) *Chem Nat Compds* **16**, 128
11 Kostyuchenko, O I , Komissarenko, N F , Zinchenko, T V and Derkach, A I (1981) *Khim Prir Soedin* **15**, 389, cf *Chem Abstr* **95**, 165591 t
12 Kostyuchenko, O I , Komissarenko, N F , Kovalev, I P , Derkach, A I and Gordienko, V G (1982) *Chem Nat Compds* **18**, 170
13 Kostyuchenko, O I , Komissarenko, N F , Zinchenko, T V , Derkach, A I and Gordienko, V G (1982) *Chem. Nat Compds* **18**, 235