Phytochemistry, Vol. 22, No. 9, pp. 2057–2060, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00
Pergamon Press Ltd.

FLAVONE GLYCOSIDES OF *SALVIA TRILOBA*

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(*Revised received* 6 *December* 1982)

Key Word Index—*Salvia triloba*; Labiatae; 6-methoxyflavones; flavone glycosides; chemosystematics.

Abstract—From *Salvia triloba*, 13 flavonoids were isolated and identified. The 7-glucosides and 7-glucuronides of apigenin, luteolin, 6-methoxyapigenin and 6-methoxyluteolin and chrysoeriol 7-glucuronide were identified. Also present were 6,8-di-*C*-glucosylapigenin, luteolin 7-diglucoside, luteolin 7-glucuronide-3′-glucoside and 6-hydroxyluteolin 6,3′-dimethyl ether.

INTRODUCTION

Several *Salvia* species have been investigated for their flavonoid constituents. From *Salvia officinalis*, genkwanin, 6-methoxygenkwanin, 6-methoxyluteolin, 6-methoxyluteolin 7-methyl ether, hispidulin (6-methoxyapigenin), luteolin and scutellarein 5,6,7,4′-tetramethyl ether were identified [1, 2], while from *S. virgata* scutellarein 6,7,4′-trimethyl ether (salvigenin), its 5-glucoside and luteolin 7,3′,4′-trimethyl ether were isolated [3]. Kaempferol 3-mono- and 3,7-dimethyl ethers, quercetin 3,7,4′-tri- and 3,7,3′,4′-tetramethyl ethers, apigenin and genkwanin were reported from *S. glutinosa* [4]. Salvigenin was also found in *S. triloba* [5] and *S. aethiopis* [6]. In addition, the latter also contained luteolin 7,3′,4′-trimethyl ether. No flavonoid glycosides have been completely characterised in *Salvia* species [7, 8].

RESULTS AND DISCUSSION

Twelve flavonoid glycosides and one aglycone were isolated from *Salvia triloba*. Of the 12 glycosides, one was identified as the *C*-glycoside 6,8-di-*C*-β-glucosylapigenin. The remaining 11 were found to belong to five aglycones: apigenin, luteolin, chrysoeriol, 6-methoxyapigenin (hispidulin), 6-methoxyluteolin (nepetin) and 6-hydroxyluteolin 6,3′-dimethyl ether (jaceosidin), the latter in the free form. The physical properties of hispidulin, nepetin and jaceosidin are reported in Table 1 and in the Experimental.

Hispidulin, nepetin and jaceosidin have a rather limited distribution in nature, and their glycosides are of even rarer occurrence. Hispidulin and nepetin 7-glucosides and 7-glucuronides were characterized in the present study. Hispidulin 7-glucoside was first reported from *Plantago asiatica* (Plantaginaceae) [9] and the 7-glucuronide was found in *Scutellaria creticola* [10]. Nepetin 7-glucoside was first isolated from *Nepeta hindostana* [11], and its 7-glucuronide was recently identified for the first time from the leaves of *Digitalis lanata* (Scrophulariaceae) [12]. Two uncommon luteolin glycosides are also reported in the present study. The first is luteolin 7-diglucoside, which was first isolated from *Dahlia variabilis* [13], and the second, luteolin 7-glucuronide-3′-glucoside, which is reported for the first time. It was identified by chemical and UV analyses (see Experimental). Luteolin 3′-glucoside has previously been reported from *Dracocephalum thymiflorum* [14], another member of the Labiatae. The UV data of the uncommon glycosides are recorded in Table 2, and the chromatographic properties of all isolated glycosides in Table 3. Besides the above-mentioned glycosides, traces of two glycosides were also isolated and gave glucose and glucuronic acid, respectively. The aglycone of both glycosides showed the same properties as 6-hydroxyluteolin [15]. The small amounts available prevented further detailed studies.

The presence of unusual 6-hydroxylated and 6-methoxylated flavones in *Salvia triloba* agrees with the flavonoid chemistry of the Labiatae as a whole. The family is known to be rich in such compounds. Thus, baicalein (5,6,7-trihydroxyflavone) and 5,6,7,4′-tetrahydroxyflavone have been reported from *Coleus blumei* [16], *Galeopsis* sp. [17] and *Scutellaria* sp. [18]. 5,4′-Dihydroxy-6,7-dimethoxyflavone was reported in *Teucrium polium* [19], while nepetin was isolated from *Nepeta hindostana* [11] and *Rosmarinus officinalis* [20]. From *Satureia douglasii*, xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxyflavone) was isolated and identified [21]. The presence of 6,8-di-*C*-glucosylapigenin in the present study is also not surprising, since flavone *C*-glycosides have been reported in several *Vitex* species [22] from the closely related Verbenaceae.

EXPERIMENTAL

Plant material. Fresh material (*Salvia triloba* L. fil.) was collected by one of us (A.M.A.) from the north coast of Sinai, close to El-Arish. The plant was authenticated by Professor Dr. L. Boulos, NRC.

Isolation and identification of the flavonoids. The leaves and stems of the plant (1 kg) were extracted with 70% EtOH. The extract was subjected to CC (polyamide) with mixtures of H_2O and EtOH. Further fractionation was applied by elution techniques on paper. Acid hydrolysis was carried out with 2 N HCl, mild acid hydrolysis with 0.1 N HCl and enzymic hydrolysis with β-glucosidase in an acetate buffer (pH 5). Demethylation was carried out with pyridinium hydrochloride for 3 hr. The common

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Table 1. Physical properties of hispidulin, nepetin and jaceosidin

	Hispidulin (6-methoxyapigenin)	Nepetin (6-methoxyluteolin)	Jaceosidin (6-hydroxyluteolin 6,3′-dimethyl ether)
R_f values*			
BAW	83	76	79
50%	45	39	44
PhOH	92	84	92
Colour			
UV	dark brown	brown	brown
$+NH_3$	brown	yellow-brown	yellow-brown
UV data			
MeOH	274, 334	255, 272, 346	253, 272, 345
NaOMe	276, 324, 394	266, 273, 330†, 402	281, 329, 397
$AlCl_3$	284†, 310, 356, 390†	274, 303†, 333†, 420	260, 279, 300†, 365
$AlCl_3$–HCl	284†, 310, 354, 390†	260, 280, 362, 390†	268, 279, 300†, 360
NaOAc	274, 305†, 369	262, 272, 380	273, 308†, 370
NaOAc–H_3BO_3	276, 338	263, 271†, 373	274, 348
MS data			
$[M]^+$	300 (65)	316 (100)	330 (100)
$[M-1]^+$	——	315 (20)	329 (9)
$[M-15]^+$	285 (77)	301 (77)	315 (81)
$[M-18]^+$	282 (38)	298 (50)	312 (56)
$[M-43]^+$	257 (100)	273 (100)	287 (60)
$[B]^+$	——‡	137 (38)	151 (19)
$[B_2-28]^+$	——‡	109 (90)	123 (24)

*BAW, *n*-butanol–acetic acid–water (4:1:5); 50%, 50% acetic acid; PhOH, phenol–water (4:1).
†Shoulder.
‡Not clear.

glycosides were identified by standard procedures as: apigenin 7-glucoside, apigenin 7-glucuronide, luteolin 7-glucoside, luteolin 7-glucuronide and chrysoeriol 7-glucuronide. The remaining flavonoids were identified as follows.

Hispidulin. Demethylation gave scutellarein (co-chromatographed with an authentic sample), and its structure was confirmed by UV data (Table 1). The MS (Table 1) gave a $[M]^+$ at m/z 300, consistent with a tetraoxygenated flavonoid with one *O*-methyl group and a comparable peak at 285 for the expected loss of Me from the 6-methoxyl group [23].

Nepetin. Demethylation gave 6-hydroxyluteolin, which showed similar chromatographic and UV data (Table 1) to that reported in the lit. [15]. The MS (Table 1) gave a $[M]^+$ at m/z 316, consistent with a pentaoxygenated flavonoid with one *O*-methyl group, and a comparable peak at 301 for the expected loss of Me from the 6-methoxyl group [23]. The B-ring ion $[B_2]^+$ (m/z 137) indicated a free 3′,4′-oxygenation pattern.

Jaceosidin. Demethylation gave 6-hydroxyluteolin as above. The MS (Table 1) gave a $[M]^+$ at m/z 330, consistent with a pentaoxygenated flavonoid, with two *O*-methyl groups and a comparable peak at 315 for the expected loss of Me from the 6-methoxyl group. The B-ring ion $[B_2]^+$ (m/z 151) indicated a 3′,4′-oxygenation pattern with one *O*-methyl group. The UV data (Table 1) indicated a free 5-hydroxyl group ($\Delta\lambda$ with $AlCl_3$–HCl of band I = 15 nm), a free 7-hydroxyl group (the presence of a band at 329 with NaOMe) and substitution at either the 3′ or 4′ position (no shift of band I between $AlCl_3$ and $AlCl_3$–HCl or with NaOAc–H_3BO_3). The increase in intensity of band I on the addition of NaOMe indicates a free OH-4′ rather than a OH-3′.

Hispidulin and nepetin 7-glucosides and 7-glucuronides. All glycosides gave the corresponding aglycone and sugar on acid and enzymic hydrolyses. The lack of a shift with NaOAc, as well as the disappearance of the 324–330 band present in the aglycones and the appearance of a shoulder at 304 nm in NaOMe in all glycosides (Table 2), confirms that glycosylation is in position 7.

Luteolin 7-diglucoside. This glycoside gave rise to luteolin and glucose on acid hydrolysis. Mild acid and enzymic (β-glucosidase) hydrolyses both gave luteolin 7-glucoside as an intermediate. The UV data (Table 2) was identical to that of luteolin 7-glucoside.

Luteolin 7-glucuronide-3′-glucoside. This glycoside was present in small amounts and gave rise to luteolin, glucose and glucuronic acid on acid hydrolysis. Both enzymic hydrolysis with β-glucosidase and mild acid hydrolysis gave luteolin 7-glucuronide. UV data (Table 2) indicated that both positions 7 and 3′ were occupied. Thus, the lack of a shift of band II with NaOAc and the presence of a band at 305 nm with NaOMe suggested that the 7 position was occupied. The lack of a shift band I with NaOAc–H_3BO_3, or between $AlCl_3$ and $AlCl_3$–HCl indicated that either the 3′ or 4′ position was occupied. The increase in intensity of band I on the addition of NaOMe indicated a free OH-4′. The chromatographic properties are given in Table 3.

6,8-*Di*-C-β-*glucosylapigenin.* This glycoside had mp 232–235° (decomp.) (lit. 233–236° [24]). It did not change on acid hydrolysis and the UV data (Table 2) also agree with that reported in the lit. [25]. It co-chromatographed with an authentic sample from *Thymelea hirsuta* [26] and R_f values are given in Table 3.

Table 2. UV data of uncommon glycosides isolated from *Salvia triloba**

	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
Luteolin 7-glucoside	255	266	274	274	258	260
	268	280sh	295sh	298sh	266	270sh
	348	300sh	330sh	353	368	372
		392	425	388	400sh	
Luteolin 7-diglucoside	254	266	280	280	256	258
	268	280sh	300sh	300sh	272sh	276sh
	348	300sh	334	340	377	374
		404	426	391	398sh	
Luteolin 7-glucuronide-3'-glucoside	268	272	274	275	262	268
	344	305sh	300	300	390	346
		398	351	348		
			382	382		
Hispidulin 7-glucoside	274	275	283	283	273	273
	331	304sh	298	298	336	335
		388	354	350	390	
			390sh	388sh		
Hispidulin 7-glucuronide	274	275	281	281	273	273
	331	305sh	297	297	336	335
		388	353	349	390	
			390sh	388sh		
Nepetin 7-glucoside	255sh	276	275	261	262	261
	273	304sh	300	278	273sh	272sh
	345	407	330sh	298sh	370	370
			422	364		
Nepetin 7-glucuronide	255	274	274	262	260	260
	272	304sh	295sh	277	272sh	272sh
	346	410	330sh	294sh	370	370
			422	365		
6,8-Di-*C*-glucosylapigenin	274	283	265sh	262sh	283	276sh
	311sh	331	281	280	308sh	284
	333	398	306	304	334sh	323
			352	346	395	351
			390	386		414sh

sh, Shoulder.

*The UV data of the common 7-glucosides and 7-glucuronides of apigenin, luteolin and chrysoeriol are not recorded here (luteolin 7-glucoside is shown for comparison with the 7-diglucoside).

Table 3. R_f values of glycosides isolated from *Salvia triloba*

	R_f values (×100)*			
	BAW	H_2O	15%	PhoH
Apigenin 7-glucoside	66	3	14	77
Apigenin 7-glucuronide	46	13	21	37
Luteolin 7-glucoside	38	1	11	60
Luteolin 7-glucuronide	31	4	15	13
Luteolin 7-diglucoside	27	7	20	53
Luteolin 7-glucuronide-3'-glucoside	15	6	19	12
Chrysoeriol 7-glucuronide	37	3	11	41
Hispidulin 7-glucoside	50	3	23	84
Hispidulin 7-glucuronide	48	11	26	43
Nepetin 7-glucoside	41	2	14	73
Nepetin 7-glucuronide	28	9	19	24
6,8-Di-*C*-glucosylapigenin	20	16	36	44

*BAW, *n*-butanol–acetic acid–water (4:1:5); 15%, acetic acid–water (15:85); PhOH, phenol–water (4:1).

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