Pergamon

0031-9422(95)00019-4

Phytochemistry, Vol. 39, No. 4, pp. 925–927, 1995

0031-9422/95 $9.50 + 0.00

TWO IRIDOID GLUCOSIDES, 5-*O*-MENTHIAFOLOYLKICKXIOSIDE AND KICKXIN, FROM *KICKXIA* DUM. SPECIES

Nedjalka Handjieva, Liljana Tersieva,* Simeon Popov† and Ljuba Evstatieva*

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Bulgaria; *Institute of Botany, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

(*Received in revised form* 5 *December* 1994)

Key Word Index—*Kickxia elatine*; *K. spuria*; *K. commutata*; Scrophulariaceae; iridoids; 5-*O*-menthiafoloylkickxioside; kickxin; kickxioside; antirrinoside; antirride; linarioside; mussaenosidic acid.

Abstract—The iridoid compositions of *Kickxia elatine*, *K. spuria* and *K. commutata* were studied. Two new iridoid glucosides, 5-*O*-menthiafoloylkickxioside and the dimer kickxin, were isolated. Their structures were elucidated on the basis of spectral and chemical data. The structure of kickxin has been determined as an ester of mussaenosidic acid and antirrinoside between C-11 and C-6. Additionally, five known iridoid glucosides—kickxioside, antirrinoside, linarioside, antirride and mussaenosidic acid, were isolated and identified. The latter two iridoids were found for the first time in *Kickxia* species.

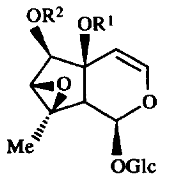
INTRODUCTION

In continuation of our investigations on iridoid glycosides in Scropulariaceae plants, we report now the iridoid composition of the three *Kickxia* species distributed in Bulgaria *K. elatine* (L.) Dum., *K. spuria* (L.) Dum. and *K. commutata* (Bernh. ex Rchb.) Fritsch. [1]. Earlier investigations showed the presence of kickxioside (**1**), antirrinoside (**2**) and linarioside (**4**) in *Kickxia* species [2–5].

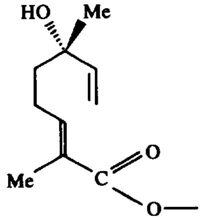
RESULTS AND DISCUSSION

Most Scrophularioideae–Antirrideae plants proved to be rich in iridoid glycosides. *Kickxia* is botanically close to the recently investigated *Linaria*. From *K. spuria*, *K. elatine* and *K. commutata*, we isolated the known iridoid glucosides kickxioside (**1**) and antirrinoside (**2**) as the main components, along with antirride (**3**), linarioside (**4**) and mussaenosidic acid (**5**), identified by spectral comparison with authentic samples [6–10]. Iridoids **3** and **5** were found for the first time in *Kickxia* species. Two new iridoids **6** and **7** were isolated from *K. elatine*. These compounds were present also in *K. spuria* and *K. commutata* although in lower concentrations.

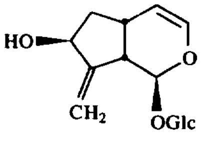
The ^{1}H and ^{13}C NMR spectra (Experimental, Table 1) of **6** showed a close resemblance to those of kickxioside (**1**) isolated from the same plant. The main significant ^{13}C NMR differences were the observed 35 carbon resonances. The 10 additional carbon and associated proton resonances were almost identical with those observed for



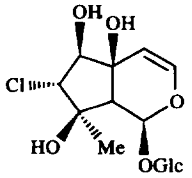
	R¹	R²
1	H	menthiafoloyl
2	H	H
6	menthiafoloyl	menthiafoloyl



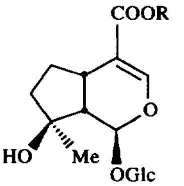
Menthiafoloyl



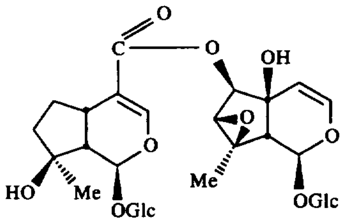
3



4



	R
5	H
8	Me



part *a* part *b*

7

---

†Author to whom correspondence should be addressed.

Table 1. ^{13}NMR spectral data for kickxioside (**1**), 5-*O*-menthiafoloylkickxioside (**6**), mussaenoside (**8**), antirrinoside (**2**) and kickxin (**7**)

					7^{e}	
C	**1**^{a}	**6**^{b}	**8**^{c}	**2**^{d}	Part *a*	Part *b*
1	99.3	92.6	95.2	94.9	94.4	93.9
3	44.3	144.6	151.9	142.9	151.8	142.1
4	106.8	103.1	113.3	107.5	112.0	106.1
5	73.8	79.7	30.3	74.5	30.8	73.6
6	77.3	76.2	29.6	77.3^{f}	29.6	78.2
7	63.5	63.8	40.4	66.0	39.7	63.3
8	63.8	64.3	80.4	64.2	79.4	63.3
9	51.5	49.3	51.4	52.7	52.4	51.2
10	16.8	16.8	23.7	17.5	23.5	16.4
11			170.6		166.9	
1′	98.6	98.6	99.1	99.4	98.8	98.6
2′	72.8	73.7	73.4	74.3	73.6	73.6
3′	76.1	76.1	76.5	77.9^{f}	76.9	76.6
4′	69.6	69.9	70.4	71.4	70.7	70.6
5′	76.5	75.2	77.1	77.9^{f}	77.4	77.2
6′	61.2	62.0	61.5	62.7	61.8	61.8
OMe			52.6			
1″	167.7	166.6				
2″	26.8	127.5				
3″	44.5	144.8				
4″	23.7	24.0				
5″	40.4	41.1				
6″	73.1	73.3				
7″	42.5	143.7				
8″	12.3	112.7				
9″	12.3	12.6				
10″	27.6	28.1				
1‴		167.3				
2‴		128.3				
3‴		144.6				
4‴		24.2				
5‴		41.1				
6‴		73.6				
7‴		143.5				
8‴		113.0				
9‴		12.5				
10‴		27.6				

^{a}Ref.[2]; CD_3OD-$CDCl_3$ (7:3); $^{b}CDCl_3$; ^{c}Ref. [9]; D_2O; ^{d}Ref. [4]; CD_3OD; $^{e}CD_3OD$; ^{f}These assignments may be reversed.

the menthiafoloyl part of kickxioside which showed the presence of a second menthiafoloyl unit. The considerable deshielding of the C-5, H-4 and H-9 signals and shielding of the C-4 and C-9 signals showed an acylation site for the second terpene unit at C-5. Thus, the structure of **6** was established as 5-*O*-menthiafoloylkickxioside.

Compound **7**, named kickxin, isolated from *K. elatine* showed 31 peaks in the ^{13}C NMR spectrum, suggesting a bisiridoid structure. In fact, one set (part *a*) of 16 signals closely corresponding to a mussaenosidic acid (**5**) moiety could be sorted out, leaving another set of 15 signals (part *b*) resembling the spectrum of antirrinoside (**2**, see Table 1). The location of the ester linkage was determined by comparison of the NMR data of **7** with those of antirrinoside (**2**) and mussaenoside (**8**) [11,12]. The upfield shift for C-6 (part *b*) and downfield shifts for C-5 and C-7 (part *b*) suggested participation of the hydroxyl group at C-6 of antirrinoside with the carboxylic group of mussaenosidic acid. The proton coupling pattern was confirmed by the H–H COSY spectrum which exhibited cross-peaks for the following couplings: part *a*—H-3/H-5, H-1/H-9, H-9/H-5, H-6/H-7, H-6/H-6, H-5/H-6 and part *b*—H-1/H-9, H-6/H-7, H-3/H-4. On the basis of these results the structure of kickxin was determined to be **7**. In addition, alkaline methanolysis of kickxin in MeOH yielded mussaenoside and antirrinoside. The native population of *K. elatine* in Rila mountain contained a higher content of kickxin and mussaenosidic acid than the population from the Black Sea coast (Tsarevo). The same observation was valid also for *K. spuria* and *K. commutata*.

These investigations confirmed antirrinoside to be characteristic for Scrophularioideae–Antirrhineae plants [5]. Kickxioside may be a marker for *Kickxia* plants but this needs to be proven by additional studies of a greater number of *Kickxia* species.

EXPERIMENTAL

^{1}H NMR: 250 MHz; ^{13}C NMR: 62.9 MHz, multiplets from DEPT; H–H COSY.

Plant material. Kickxia elatine (2 samples). *K. commutata* and *K. spuria* were collected when in flower, respectively, in Tsarevo (Black Sea coast), Rila mountain, Tsarevo and Primorsko (Black Sea coast). The plant material was identified by Dr L. Evstatieva, Inst. of Bot., Bulg. Acad. Sci. Voucher specimens SOM 281, 282, 283 and 280, respectively, are deposited in the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

Isolation of compounds. Dried aerial parts of *K. elatine* (50 g) were extracted (×2) with MeOH. After evapn of solvent *in vacuo* the residue (10.2 g) was treated with charcoal (50 g). Elution was with H_2O (1l), 5% MeOH (1l), 30% MeOH (1l), 50% MeOH (800 ml), MeOH–Me_2CO (1:1, 800 ml) and MeOH–$C_2H_4Cl_2$ (1:1, 500 ml).

The MeOH fr. (1.3 g) dissolved in H_2O was extracted with $CHCl_3$. The concd $CHCl_3$ layer (378 mg) was chromatographed on silica gel (37 g) with $CHCl_3$–MeOH–H_2O (60:15:4) to give frs 20–21 of pure **6** (56 mg) and frs 25–26 (62 mg) pure **1**. The concd water layer (790 mg) was sepd on silica gel (45 g) with consecutive elution by $CHCl_3$–MeOH–H_2O (60:22:4) and MeOH. Frs 12–15 contained pure **1** (200 mg), frs 24–25—pure **3** (99 mg), frs 28–31—pure **2** (121 mg).

The combined 30% and 50% methanolic frs from the charcoal column (1.6 g) were sepd on silica gel to give frs 13–17 of pure **1** (114 mg), frs 18–21 of pure **3** (57 mg), frs 24–26 of pure **2** (290 mg) and a polar fr. which after additional purification on a Lobar column on silica gel (7 g) gave frs containing pure **5** (19 mg). Frs 29–32 (120 mg), after additional purification on silica gel, gave pure **7** (25 mg).

Kickxioside (**1**). ^{1}H and ^{13}C NMR as reported in ref. [2].

Antirrinoside (**2**). ^{1}H and ^{13}C NMR as reported in refs [3, 4].

Antirride (**3**). ^{1}H and ^{13}C NMR as reported in ref. [5].

Linarioside (**4**). ^{1}H and ^{13}C NMR as reported in ref. [6].

Mussaenosidic acid (**5**). ^{1}H and ^{13}C NMR as reported in ref. [7]

5-O-*Menthiafoloylkickxioside* (**6**). Amorphous powder. $[\alpha]_D^{20}$ − 85.49° ($CHCl_3$; *c* 1.47). ^{1}H NMR (250 MHz, $CDCl_3$): δ6.48 (*d*, 1H, *J* = 6.5 Hz, H-3), 5.64 (*d*, 1H, *J* = 1Hz, H-1), 5.49 (*d*, 1H, *J* = 6.5 Hz, H-4), 5.13 (*d*, 1H, *J* = 2.3 Hz, H-6), 3.70 (*d*, 1H, *J* = 2.3 Hz, H-7), 2.91 (*d*, 1H, *J* = 1 Hz, H-9), 1.45 (*s*, 3H, Me-10), 4.68 (*d*, 1H, *J* = 7.6 Hz, H-1′), 3.87 (*dd*, 1H, *J* = 12 and 2.4 Hz, $H-6'_a$), 3.70 (*dd*, 1H, *J* = 12 and 5 Hz, H-6′b, 6.75 and 6.65 (each *dt*, 2 × 1H, *J* = 7.1 and 1.2 Hz, H-3″ and H-3‴), 5.87 and 5.89 (each *dd*, 2 × 1H, *J* = 17.3 and 10.7 Hz, H-7″ and H-7‴), 5.20 (*dd*, 2H, *J* = 17.3, H-8″a and H-8‴a), 5.07 (*dd*, 2H, *J* = 10.7 and 1.2 Hz, H-8″b and H-8‴b), 2.17 (*m*, 4H, 2H-4″ and 2H-4‴), 1.74 (*br s*, 6H, Me-9″ and Me-9‴), 1.58 (*m*, 4H, 2H-5″ and 2H-5‴), 1.28 (*s*, 6H, Me-10″ and Me-10‴). ^{13}C NMR (62.9 MHz, $CDCl_3$): Table 1. (Found C, 58.05; H, 7.27; $C_{35}H_{50}O_{14} \cdot 1.5\,H_2O$ requires: C, 58.24; H, 7.40%.)

Kickxin (**7**). Amorphous powder. $[\alpha]_D^{20}$ − 74.23° (MeOH; *c* 0.57). ^{1}H NMR (250 MHz, CD_3OD): part *a*: δ7.57 (*s*, 1H, H-3), 5.48 (*d*, 1H, *J* = 3.5 Hz, H-1), 4.70 (*d*, 1H, *J* = 7.8 Hz, H-1′), 3.15 (*m*, 1H, H-5), 2.3 (*m*, 1H, $H-6_a$), 2.26 (*dd*, 1H, *J* = 9.3 and 3.9 Hz, H-9), 1.68 ((*m*, 1H, $H-7_a$), 1.65 (*m*, 1H, $H-6_b$), 1.33 (*s*, 3H, Me-10), 1.28 (*m*, 1H, $H-7_b$); part *b*: δ6.41 (*d*, 1H, *J* = 6.3 Hz, H-3), 5.50 (*d*, 1H, *J* = 6.9 Hz, H-1), 5.03 (*d*, 1H, *J* = 1.7 Hz, H-6), 4.93 (*d*, 1H, *J* = 6.3 Hz, H-4), 4.68 (*d*, 1H, *J* = 7.8 Hz, H-1′), 3.53 (*d*, 1H, *J* = 1.7 Hz, H-7), 2.46 (*d*, 1H, *J* = 6.9, H-9), 1.51 (*s*, 3H, Me-10). ^{13}C NMR (62.9 MHz, CD_3OD): Table 1. (Found C, 49.06; H, 6.69; $C_{31}H_{44}O_{19} \cdot 1.5\,H_2O$ requires: C, 49.80; H, 6.34%.)

Alkaline methanolysis. Compound **7** (11 mg) was dissolved in 0.1 N methanolic NaOH (4.5 ml). After 2.5 hr, neutralization with 0.1 N HCl was carried out and active charcoal was added. The filtrate was sepd on a silica gel column to give antirrinoside and mussaenoside.

Acknowledgement—This work was supported by the National Foundation of Scientific Research of Bulgaria under contract X-45.

REFERENCES

1. Andreev, N., Anchev, M., Kozhuharov, St., Markov, M., Peev, D. and Petrova, A. (1992). In *Guide to Higher Plants in Bulgaria*, p. 36. Sofia.
2. Nicoletti, M., Serafini, M., Tomassini, L., Bianco, A. and Passacantilli, P. (1987) *Planta Med.* **53**, 295.
3. Toth, L., Kokovay, K., Bujtas, Gy. and Papay, V. (1978) *Pharmazie* **33**, 84.
4. Toth, L., Csordas, I. and Papay, V. (1978) *Acta Botany Acad. Sci. Hung.* **24**, 179.
5. Kooiman, P. (1970) *Acta Botany Neerl.* **19**, 329.
6. Bianco, A., Passacantilli, P., Polidori, G., Nicoletti, M. and Messana, I. (1983) *Gazz. Chim. Ital.* **113**, 829.
7. Chaudhuri, R. K., Afifi-Yazar, F. Ü., Sticher, O. and Winkler, T. (1980) *Tetrahedron* **36**, 2317.
8. Handjieva, N., Ilieva, E., Spassov, S. and Popov, S. (1993) *Tetrahedron* **49**, 9261.
9. König, G., Rimpler, H. and Hunkler, D. (1987) *Phytochemistry* **26**, 423.
10. Damtoft, S., Hansen, S., Jacobsen, B., Jensen, S. and Nielsen, B. (1984) *Phytochemistry* **23**, 2387.
11. Gardner, D., Narum, J., Zook, D. and Stermitz, F. (1987) *J. Nat. Prod.* **50**, 485.
12. Boros, C. and Stermitz, F. (1990) *J. Nat. Prod.* **53**, 1055.