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Iridoid glucosides from *Kickxia abhaica* D.A. Sutton from Scrophulariaceae

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Abstract

Two iridoid glucosides namely; 6-acetylantirrinoside (**1**), 6′-*O*-*p*-hydroxybenzoylantirrinoside (**2**) were isolated from the aerial parts of *Kickxia abhaica*. Beside that, three known iridoid glucosides, antirrinoside (**3**), antirride (**4**) and mussaenosidic acid (**5**), one flavone glycoside (**6**) and a hexitol, D-mannitol (**7**) were isolated. The structures of the iridoid glucosides **1**–**2** were established by 1D and 2D NMR spectral data, including COSY, HMQC and HMBC experiments, as well as HRMS.

Keywords: *Kickxia abhaica*; Scrophulariaceae; Iridoid glucosides; 6-acetylantirrinoside; 6′-*O*-*p*-hydroxybenzoylantirrinoside

1. Introduction

The genus *Kickxia* is comprised of about 47 species worldwide (Mabberley, 1997). In Saudi Arabia, the genus is represented by 10 species (*Kickxia elatine*, *Kickxia aegyptiaca*, *Kickxia acerbiana*, *Kickxia collenetteana*, *Kickxia corallicola*, *Kickxia pseudoscoparia*, *Kickxia scalarum*, *Kickxia petiolata*, *Kickxia hastate* and *Kickxia abhaica*), which are distributed in different parts of the country (Chaudhary, 2001). Most of these species are distributed in the South and West regions including *K. abhaica*. Only seven *Kickxia* species world wide were chemically investigated and resulted in the isolation of mainly flavonoids and iridoid glycosides (Khan et al., 2001; Yuldashev et al., 1996; Handjieva et al., 1995; Amer, 1993; Kassem, 1992; Khan et al., 1991; Singh and Prakash, 1987; Nicoletti et al., 1987; Toth et al., 1978a,b,c; Pinar, 1973). Up to the present time nothing has been reported about the chemistry of *K. abhaica*. Therefore, the present paper reports on the isolation and characterization of the two new iridoid glucosides, 6-acetylantirrinoside (**1**), 6′-*O*-*p*-hydroxybenzoylantirrinoside (**2**) from the aerial parts of *K. abhaica*. In addition, the plant also yielded three known iridoid glucosides, antirrinoside (**3**) (Scarpati et al., 1968; Chaudhuri et al., 1980), antirride (**4**) (Handjieva et al., 1993) and mussaenosidic acid (**5**) (Damtoft et al., 1984), one flavone glycoside, hispidulin 7-neohesperidoside (**6**) (Lee et al., 1994; Park et al., 1995) and a hexitol, D-mannitol (**7**) (Khan and Aqil, 1993).

2. Results and discussion

Compound **1** was obtained as a gummy substance and its molecular formula $C_{17}H_{24}O_{11}$ was determined by HRFABMS. The 17 carbons were resolved in the ^{13}C NMR spectrum (Table 1). When compared to the spectrum of antirrhinoside (**3**), a very good correspondence could be seen for 15 of the signals, while the remaining two signals could be assigned to an acetyl moiety. Compound **1** was, therefore, a monoacetate of **3**, in agreement with the MS data. The point of attachment was evident from the ^1H NMR spectrum where the H-6 signal was seen at δ 4.86, 0.9 ppm downfield from that of **3**. The position of the acetate group at C-6 was further confirm by 2D NMR ^1H–^{13}C

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Table 1
^{1}H and ^{13}C NMR spectral data for compounds **1-2** in CD_3OD (δ values, J in parenthesis in Hz)[a]

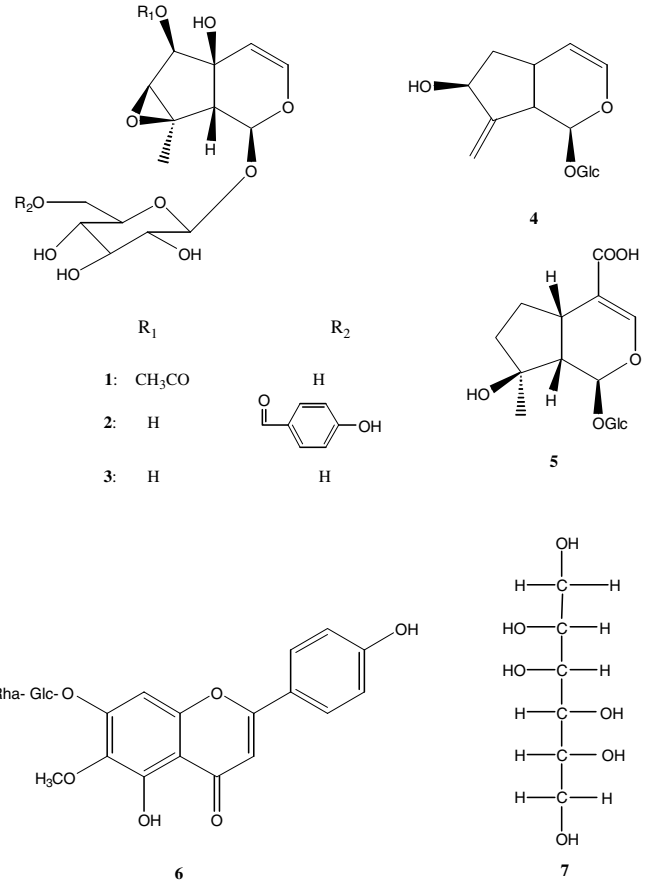
Proton	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1	5.44 *d* (6.0)	94.6	4.99 *d* (8.0)	95.6
3	6.32 *d* (6.5)	143.4	6.24 *d* (6.0)	142.8
4	4.82 *d* (6.5)	107.5	4.77 *d* (6.0)	107.7
5	–	74.7	–	74.8
6	4.86 *d* (2.0)	79.4	3.69 *d* (1.0)	78.8
7	3.39 *d* (2.0)	64.2	3.15 *br.s*	66.0
8	–	64.5	–	63.0
9	2.36 *d* (6.0)	53.3	2.22 *d* (8.0)	53.3
10	1.39 *s*	17.4	1.24 *s*	17.6
1′	4.57 *d* (8.0)	99.8	4.62 *d* (8.0)	99.9
2′	3.14 *m*	74.7	3.16 *m*	74.8
3′	3.30 *m*	77.7	3.49 *m*	75.7
4′	3.15 *m*	71.8	3.32 *m*	71.8
5′	3.30 *m*	78.6	3.33 *m*	77.7
6′a	3.53 *dd* (11.75, 6.5)	63.0	4.40 *dd* (12.0, 7.0)	64.1
6′b	3.83 *dd* (11.75, 2.5)	63.0	4.52 *dd* (12.0, 2.5)	64.1
1″	–	172.0	–	167.8
2″	2.03 *s*	20.3	–	122.2
3″/7″			7.78 *d* (9.0)	132.9
4″/6″			6.73 *d* (9.0)	116.3
5″			–	163.7

[a] Assignments made by combination of COSY, DEPT, HMQC, HMBC data and comparison with the literature.

HMBC experiments. The HMBC spectrum showed ^{3}J correlations between δ 4.82 (H-4), $\delta_{C\text{-}6}$ 79.4, $\delta_{C\text{-}9}$ 53.3, and between δ 4.86 (H-6) and $\delta_{C\text{-}1''}$ 172.0, confirming the placement of the acetate group at C-6. These findings unambiguously established the structure of **1** as 6-acetylantirrhinoside.

Compound **2**, analyzed for $C_{22}H_{26}O_{12}$ by HRFABMS, was isolated as amorphous powder and its UV spectrum exhibited absorption bands at λ_{max} 257 and 320 nm due to the presence of a conjugated system. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were diagnostic for antirrhinsoide esterified with an aromatic acid (Fauvel et al., 1995). In the ^{1}H NMR of **2** the down field shift of H-6′ protons to δ 4.40 and 4.52, ca. 0.8 ppm from the usual position strongly support that C-6′is the site of esterification. Further confirmation was made by HMBC experiment. That showed ^{3}J correlations between δ 7.78($\delta_{C3''/7''}$132.9), $\delta_{C\text{-}1''}$ 167.8 and $\delta_{C\text{-}5''}$ 163.7, and between δ 4.40, 4.52 (H-6′a, H-6′b) and $\delta_{C\text{-}1''}$ 167.8, confirming the attachment of aromatic acid moiety at C-6′.The NMR spectra of **2** were found to be similar with those reported (Dawidar et al., 1989) for 6′-*O*-cinnamoylantirrhinoside but lacking the signals for the α and β positions of cinnamoyl moiety. Based on the foregoing data, the structure of **2** was established as 6′-*O*-*p*-hydroxybenzoylantirrinoside.

During the course of isolation of the above compounds, *K. abhaica* yielded three known iridoid glucosides **3**–**5**, one flavone glycoside (**6**) and one alditol (**7**). These compounds were identified by comparison of their physical and spectroscopic data with those reported in the literatures. This is the first time that the iridoid glucosides **1**–**2** appeared in the literature and the first report of **6** from the family scrophulariaceae. In addition, compounds **4** and **5** are reported for the second time from the genus *Kickxia* (Handjieva et al., 1995).



3. Experimental

3.1. General

Mp uncorr.; UV spectra were recorded on a Hewlett–Packard HP-845 UV–Vis spectrophotometer; FTIR spectra were obtained on a Nicolet Impact 410 spectrophotometer; Specific rotation measurements were recorded on a Perkin–Elmer 242 MC polarimeter; NMR spectra were acquired in CD_3OD or DMSO on a Bruker Avance DRX-500 instrument at 500 (^{1}H) and 125 (^{13}C) MHz using the residual solvent signal as internal standard. Standard Bruker pulse programs were used for APT, DEPT, 2D NMR COSY, HMQC and HMBC spectra. HRFABMS were obtained on a Bruker Bioapex-FTMS with electrospray ionization; EIMS were measured using an E.I. Finnigan model 4600 quadrupole system or a Shimadzu QP500 GC/mass spectrometer; TLC: silica gel 60 F254 (Merck) plates; solvents: different concentration of MeOH–$CHCl_3$ and H_2O–MeOH; CC: silica gel 60/230–400 mesh (EM

Science); RP C-18 silica gel. Centrifugal preparative TLC (CPTLC; using Chromatotron®, Harrison Research Inc. model 7924): 1–4 mm silica gel P_{254} disc. The isolated compounds were visualized under short- and long-wave UV light, followed by spraying with *p*-anisaldehyde reagent.

3.2. Plant material

K. abhaica D.A. Sutton was collected in April, 2003 from Baljurashi, Saudi Arabia and identified by Dr. M. Atiqur Rahman, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 14716) was deposited at the herbarium of the College of Pharmacy, KSU.

3.3. Extraction and isolation

The air-dried aerial parts (1.0 kg) of *K. abhaica* were exhaustedly extracted with petroleum ether (12 g), followed by EtOH at room temperature to yield after evaporation 120 g. The ethanol extract was dissolved in hot methanol to afford white precipitate (7 g) identified as D-mannitol (**7**). The soluble methanol fraction was concd., diluted with water and successively extracted with $CHCl_3$ (3 × 300 ml), EtOAc (3 × 200 ml) and butanol (2 × 200 ml). The ethylacetate (2 g) and butanol (20 g) extracts were combined together and subjected to flash chromatography on silica gel (600 g) using chloroform and then increasing concentrations of MeOH (20–50%) in $CHCl_3$ to give 5 fractions; 1 (4.7 g), 2 (3.3 g), 3 (2.8 g), 4 (1.1 g), 5 (4.1 g).

Fraction 1 (4.7 g) was rechromatographed on silica gel (60 g) using 10% $CHCl_3$–MeOH to afford sub-fractions A–E. Sub-fraction A (820 mg) was separated by RP-column (30 g) using 40% H_2O–MeOH as a solvent to give two fractions a and b. Fraction a (150 mg) was purified by CPTLC (1 mm silica gel disc) using 8% MeOH–EtOAc to yelid **1** (10 mg). Fraction b (600 mg) was separated by CPTLC (4 mm silica gel disc) using 10% MeOH–EtOAc to give three fractions I–III. Fraction I (39 mg) was purified by RP-column using 40% H_2O–MeOH as a solvent to afford **2** (14 mg). Fraction C (800 mg) was subjected to CPTLC (4 mm silica gel disc) using 20% MeOH–$CHCl_3$ to give three sub-fractions i–iii. Sub-fraction ii (400 mg) was purified by CPTLC (2 mm silica gel disc) using 20% MeOH–$CHCl_3$–NH_3 to give **4** (15 mg). Portion of fraction D (250 mg) was separated by CPTLC (2 mm silica gel disc) using 20% MeOH–$CHCl_3$–acetic acid, further purification by LH-20 (40 g) using 30% MeOH–$CHCl_3$ as a solvent followed by repeated CC over silica gel using $CHCl_3$ as solvent to give **3** (28 mg).

Fraction 4 (1.1 g) was subjected to CPTLC (4 mm silica gel disc) using 25% MeOH–$CHCl_3$ to give two fractions A and B. Fraction B (0.5 g) was separated by CPTLC (2 mm silica gel disc) using 20% MeOH–$CHCl_3$–NH_3 to yielded two sub-fractions a and b. The sub-fraction a (200 mg) was purified by LH-20 (30 g) using 50% MeOH–H_2O to afford **6** (80 mg). The sub-fraction b (65 mg) was purified by RP-column (30 g) using 40% H_2O–MeOH as a solvent to give **5** (17 mg).

3.4. 6-Acetylantirrinoside (*1*)

Gum, $[\alpha]_D$ −100° (*c*; 0.04 in MeOH); UV λ_{max} (MeOH) nm (log ε): 202 (3.61), 275 (2.24); IR (film) ν_{max} cm^{-1}: 3411, 2923, 1734, 1375, 1240, 1101, 1076, 1047, 1016 and 960; ^1H and ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int. %) 241 $[M - 163]^+$ (0.25), 225 (0.58), 207 (1.8), 165 (3.3), 145 (3.5), 129 (12.4), 114 (6.5), 97 (19.9), 87 (21.9), 85 (12.1), 73 (15.8), 71 (11.7), 69 (10.1), 57 (17.3), 45 (44.7) and 43 (100); HRFABMS: 405.1393 ($[M + H]^+$); (calc. for $[C_{17}H_{24}O_{11} + H]$ 405.1397).

3.5. 6′-O-p-Hydroxybenzoylantirrinoside (*2*)

Amorphous powder, mp. 132–134 °C; $[\alpha]_D$ −51.3° (*c*; 0.07 in MeOH); UV λ_{max} (MeOH) nm (log ε): 202 (4.82), 257 (4.58), 320 (3.62); IR (film) ν_{max} cm^{-1}: 3420, 3411, 2920, 1701, 1608, 1313, 1279, 1236, 1167, 1101, 1074, 1045, 1012, 771 and 617; ^1H and ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int. %) 483 $[M + 1]^+$ (0.31), 198 (1.2), 177 (4.9), 173 (8.8), 163 (4.7), 138 (14.8), 121 (26), 93 (9.0), 73 (22.3), 69 (16), 60 (20.9), 57 (29.3), 55 (23.9), 45 (43.5), 44 (100) and 41 (37.8); HRFABMS: 483.1500 ($[M + H]^+$); (calc. for $[C_{22}H_{26}O_{12} + H]$ 483.1503).

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