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A new isoflavanone from *Iresine herbstii*

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ABSTRACT

A new isoflavanone 2′,2,5-trimethoxy-6,7-methylenedioxyisoflavanone was isolated from the aerial parts of *Iresine herbstii*, together with isoflavone tlatlancuayin (2′,5-dimethoxy-6,7-methylenedioxyisoflavone). The structure was identified using spectroscopic analysis. This is the first description of a methoxy group occurrence at position 2 of the isoflavanone skeleton. Both isolated compounds were tested for α-glucosidase inhibitory activity, but showed only a low effect compared to hyperoside.

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1. Introduction

Iresine herbstii Hook. (Amaranthaceae) is a perennial herb with variegated leaves of purplish or deep purple to green. It is native to tropical South America and has now spread over the entire world. It is used in traditional medicine of Brasil topically, to treat eczema and for healing wounds [1,2]. As a decoction made from the leaves, it has an antipyretic effect [1]. In China, it is traditionally used in the treatment of anemia [3], and in Thailand it is used as a tonic [4]. It was used in Peruvian Andes for ritual treatments, often in combination with other plants (e.g. *Trichocereus*) [1].

Previous pharmacological studies have shown the effect of *I. herbstii* on the central nervous system [5]. Its extract interacts with central serotonin and dopamine receptors [6]. The ethanolic extract also inhibits lipid peroxidation [7].

Previous phytochemical studies of *I. herbstii* have revealed the presence of 3′,4′,7-trihydroxy-6-methoxyflavone [7], *N*-feruloyltyramine [8], glycinebetaine and trigonelline [9], simple and acylated betacyanins — amaranthine, isoamaranthine, iresinine I, isoiresinine I (= iresinin II), iresinin III and iresinin IV [10,11].

Herein, we report the isolation and structural determination of a new isoflavanone 2′,2,5-trimethoxy-6,7-methylenedioxyisoflavanone, together with the closely related isoflavone tlatlancuayin (2′,5-dimethoxy-6,7-methylenedioxyisoflavone), which was described earlier by our group [12]. To the best of our knowledge, the newly isolated 2′,2,5-trimethoxy-6,7-methylenedioxyisoflavanone was not found in any natural sources to date. Furthermore, this is the first report of an isoflavanone with methoxy group at position 2 of the benzopyrane ring. The α-glucosidase inhibitory activity of isolated compounds was also measured.

2. Experimental

2.1. General

IR spectra were obtained on a FTIR Nicolet Impact 410 spectrometer (Nicolet, CZ) using a KBr method. Melting points were measured by a melting point apparatus Büchi 535 (Büchi Labortechnik, CH) and are uncorrected. Column chromatography was performed by silica gel L40/100 and

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Abbreviations: ACN, acetonitrile; α-PNPG, *p*-nitrophenyl-α-D-glucopyranoside; PNP, *p*-nitrophenol.

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L40/60 (Lachema, CZ), respectively. ESI-MS spectra were recorded using a HP 1100 LC-MSD Trap VL (Hewlett–Packard, USA) combined with electrospray ionisation (nebulizer pressure 50 psi, dry gas 10 L/min, dry temp. 350 °C). HPLC analysis was performed with an Agilent 1100 HPLC system equipped with DAD UV/Vis (Agilent Technologies, USA): column ABZ + Plus, 150 × 4.6 mm, 3 μm particle size; mobile phase (gradient elution): 10% ACN and 90% HCOOH 0.2% in 0 min to 100% ACN for 36 min, flow rate 1 mL/min, temp. 30 °C). Analytical grade solvents were purchased from Sigma (Sigma, CZ). NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C). The spectra were measured in DMSO-d_6 at 303 K. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signals of the solvent [2.50 ppm (^{1}H) and 39.50 ppm (^{13}C)] and are reported relative to tetramethylsilane (TMS). The 2D NMR experiments were used to assign the individual ^{1}H and ^{13}C resonances. The gradient in NOESY [13] was applied during a mixing time of 800 ms, and the spectra were processed using the STATES-TPPI protocol [14]. The gradient-selected HSQC experiment [15] was adjusted for $^{1}J_{H,C} = 145$ Hz and ^{1}H–^{13}C GSQMBC [16] and gs-HMBC [17] experiments for $^{n}J_{H,C} = 7.5$ Hz and $^{n}J_{H,C} = 3.5$ Hz. For other parameters, please see our most recent work [18–20]. Absorbance value for the determination of α-glucosidase inhibition was measured on a Microplate Reader Synergy HT (Bio-Tek, USA).

2.2. Plant material

The plant material was collected in May 2002 from the greenhouse of the Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, and identified by Prof. Václav Suchý. A voucher specimen (IH20020502) was deposited in the herbarium of the Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno.

2.3. Extraction and isolation

The lyophilized and powdered aerial sections of *I. herbstii* (1028 g) were extracted in a Soxhlet extractor with 96% ethanol. The obtained extract was then concentrated at a reduced pressure at 40 °C (210 g) and subsequently partitioned between chloroform and water. The chloroform fraction was evaporated to until dry under the reduced pressure, dissolved, and partitioned again between petrolether and 90% methanol (*v/v*). The methanolic fraction was again concentrated under a reduced pressure (26.2 g) and separated using column chromatography on silica gel L60/100 with mobile phase benzene: ethylacetate 8:2 (v/v) and subsequently re-chromatographed with benzene:ethylacetate 97:3 (v/v). Compounds **1** (0.059 g) and **2** (1.729 g) were obtained after crystallization from the corresponding fractions.

2.3.1. 2′,2,5-Trimethoxy-6,7-methylenedioxyisoflavanone

2′,2,5-Trimethoxy-6,7-methylenedioxyisoflavanone (**1**); m.p. 142–145 °C; IR ν_{max} (KBr) 2938, 1670, 1474, 1248, 1103, 1062, and 933 cm^{-1}; UV λ_{max} 214, 242, 282, and 338 nm; MS-ESI *m/z* 327.2 (100), 381.2 (10.0) and 359.2 (3.3). For ^{1}H and ^{13}C NMR data see Table 1.

Table 1
^{1}H and ^{13}C NMR chemical shifts [a] for compounds **1** and **2** in DMSO-d_6 at 303 K.

1			2		
Atom	δ_H	δ_C	Long-range correlation with H-atom [b]	δ_H	δ_C
2	5.33	103.05	H-3, H-9	8.08	151.96
3	3.98	52.92	H-2 [c], H-3′ [c], H-6′	–	122.63
4	–	188.16	H-2, H-3, H-8	–	173.40
4a	–	109.13	H-3 [c], H-8	–	113.11
5	–	142.09	H-8, H-10	–	140.33
6	–	132.89	H-8, H-11	–	135.89
7	–	154.24	H-8, H-11	–	152.49
8	6.50	93.92		6.98	93.60
8a	–	155.34	H-2, H-8	–	153.98
9	3.38	55.71	H-2	–	–
10	3.88	60.05		3.88	60.75
11	6.07, 6.09	102.06		6.17	102.54
1′	–	123.68	H-2, H-3, H-3′, H-4′, H-5′	–	121.20
2′	–	156.76	H-3, H-3′, H-4′, H-5′, H-6′, H-7′	–	157.39
3′	7.04	111.33	H-4′, H-6′	7.06	111.18
4′	7.28	128.77	H-5′, H-6′	7.36	129.43
5′	6.87	120.44	H-3′, H-4′	6.98	119.96
6′	6.97	128.96	H-3, H-3′, H-4′, H-5′	7.19	131.51
7′	3.78	55.59		3.71	55.45

[a] Referenced to the residual DMSO-d_5 (^{1}H, δ 2.50 ppm) and DMSO-d_6 (^{13}C, δ 39.50 ppm).
[b] Adjusted for $^{n}J_{H,C} = 7.5$ Hz.
[c] Observed in the experiment adjusted for $^{n}J_{H,C} = 3.5$ Hz.

2.3.2. 2′,5-Dimethoxy-6,7-methylenedioxyisoflavone (tlatlancuayin)

2′,5-Dimethoxy-6,7-methylenedioxyisoflavone (**2**); m.p. 147–149 °C; IR ν_{max} (KBr) 2908, 1649, 1468, 1256, 1103, 1048, and 929 cm^{-1}; UV λ_{max} 218, 246, and 320 nm; MS-ESI *m/z* 327.2 (100) and 349.1 (22.5). For ^{1}H and ^{13}C NMR data see Table 1.

2.4. Inhibition of α-glucosidase

The inhibition of α-glucosidase was assayed on a microplate reader according to the standard method, [21] with slight modification: 180 μL of 0.1 M phosphate buffer (pH 7.0), 20 μL of the enzyme solution (0.2 U/mL α-glucosidase in 0.1 M phosphate buffer) and 10 μL of the compound solution (MeOH, 50 μM) were mixed. After an incubation time (15 min, 37 °C) the reaction was initiated by adding 20 μL of the substrate solution (2.5 mM α-PNPG in 0.1 M phosphate buffer). After an additional 15 min at 37 °C, the reaction was stopped by adding 0.2 M Na_2CO_3 (80 μL). The amount of PNP released was quantified at 405 nm. One set of mixtures prepared with an equivalent volume of MeOH, instead of tested samples was used as a blank. Another set of mixtures prepared with an equivalent volume of phosphate buffer instead of enzyme was used as a control. The inhibitory rates (%) were calculated according to the formula $[1 - (Abs_{test} - Abs_{control}) / Abs_{blank}] \times 100\%$. Hyperoside was used as the reference compound. All samples were tested in triplicates.

3. Results and discussion

Compound **1** was obtained as white to pale yellow needles with m.p. 142–145 °C. The molecular weight of compound **1**

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