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STUDIES ON THE CONSTITUENTS OF TWO *IRIS* SPECIES

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ABSTRACT: *A new isoflavonoid glycoside, 5-hydroxy, 8,5'-dimethoxy-5-O-isoflavone glycoside, together with irisflorentin, irisolone, irisolone-4'-diglucoside, iristectorin A and irisxanthone were isolated and characterized from the rhizomes of Iris germanica L. var. alba. A flavone-C-glycoside, swertisin, xanthone glycosides mangiferin and isomangiferin were isolated from the leaves of Iris germanica L. var. alba. Two isoflavones irisolidone and irigenin together with an isoflavone glycoside iridin were isolated from the rhizomes of Iris pseudacorus L.*

INTRODUCTION

Both *Iris germanica* L. var. alba and *Iris pseudacorus* L. are cultivated in Egypt as ornamental plants.

The presence of several isoflavones and C-glycosyl xanthones in the *Iris* genus (Iridaceae) has been reported[1-3]. Previously we reported the isolation of irigenin, irisolidone and iridin from the rhizomes of *Iris germanica* L. var. alba[4], as well as the isolation of a new isoflavonoid aglycone, irisgermanin, together with the isoflavonoid glycosides iridin, irisolidone 7-O-glycoside and 5,3'-dihydroxy,4', 5-dimethoxy-isoflavone-7-O-glycoside[5].

EXPERIMENTAL

All melting points are uncorrected. The UV spectra were measured in MeOH, ^{1}H-NMR spectra were measured in DMSO and CD_3OD and TMS as internal standard. SiO_2 refers to silica gel (Merck) for CC and silica gel G and Whatman 3MM for TLC and PPC, respectively. The plant materials were obtained from rhizomes and leaves of *Iris germanica* L. var. alba and rhizomes of *Iris pseudacorus* L. from flowering plants (during March-May 1986) cultivated in the Experimental Station of Medicinal Plants of the Faculty of Pharmacy, Assiut University.

Extraction and isolation: The air-dried rhizomes (3kg) and leaves (1kg) of *I. germanica* L. var. alba and (1kg) of the air-dried rhizome of *I. pseudacorus* L. were extracted with 70% methanol and evaporated under vacuum. Each residue was successively extracted with ether, ethyl acetate and n-butanol.

Each extract was separately dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The ether fraction of the rhizome of *I. germanica* L. var. alba (20 g) was chromatographed over SiO_2 column and eluted with $CHCl_3$ and subsequently with a mixture of $CHCl_3$-MeOH gradient up to 20% MeOH to afford irisflorentin and irisolone.

The ethyl acetate soluble fraction of the rhizome extract (30 g) was chromatographed over SiO_2 column and eluted with a mixture of $CHCl_3$-MeOH gradient up to 40% MeOH to afford (1) and irisxanthone. The n-butanol fraction of the rhizome extract (25 g) was chromatographed on a SiO_2 column and eluted with $CHCl_3$-MeOH mixtures ending with pure methanol to afford iristectorin A and irisolone-4'-diglucoside.

The ethyl acetate and n-butanol fraction (40 g) of the leaf of *I germanica* L. var. alba was passed through SiO_2 column and eluted with $CHCl_3$-MeOH mixtures with increasing polarity to elute irigenin and then iridin, the other polar eluates were concentrated under reduced pressure (12 g) and rechromatographed on

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cellulose column, that was eluted with $CHCl_3$-MeOH mixtures gradient up to pure methanol to afford swertisin, mangiferin and isomangiferin.

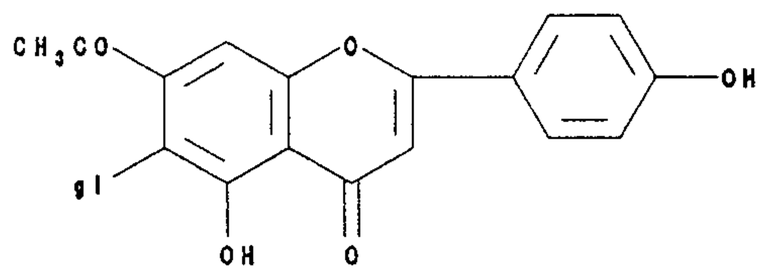
The ethyl acetate fraction of the rhizome extract of *I. pseudacorus* L. (15 g) was chromatographed on a SiO_2 column and eluted with $CHCl_3$-MeOH mixture gradient up to 30% methanol to afford irisolidone, irigenin and iridin.

Compound 1: yellow needles from methanol-chloroform (2:1), mp. 220-221°C, TLC R_f 0.51, $CHCl_3$-MeOH (3:1).
UV λ_{max} (MeOH) (nm): 272, 330 sh, UV λ_{max} (MeOH+$AlCl_3$) (nm): 272, 338 sh, UV λ_{max} (MeOH+NaOAc) (nm): 272, 334 sh.
^{1}H-NMR (DMSO-d_6) δ: 3.6 (3H,S,OCH_3), 3.7 (3H,S,OCH_3), 6.8 (2H,d,H-3',H-5'), 6.9 (1H,S,H-6), 7.3 (2H,d,H-2',H-6'), 8.2 (1H,S,H-2) and 12.7 (1H,S, exchangeable, OH-5). The sugar protons appeared as a broad signal at δ 3.1 (6H) and δ 8.4 (1H,d, J=6.5 Hz, anomeric protons).

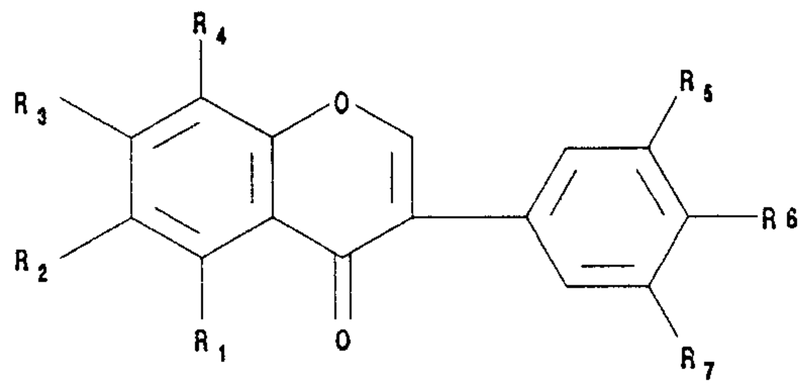
Acid hydrolysis of (1): A solution of 20 mg of (1) in methanol and 10% H_2SO_4 was refluxed over a boiling water bath for 2h. The aglycone was extracted with ether and then recrystallized from methanol. PC R_f 0.32 (15% acetic acid).
UV λ_{max} (MeOH) (nm): 268, 330 sh, UV λ_{max} (MeOH+$AlCl_3$) (nm): 270, 345, UV λ_{max} (MeOH+NaOAc) (nm): 268, 338 sh.
M/Z: M^+ 314 (100%), 299 (35%), 296 (60%), 271 (60%), 157 (10%), 139 (14%), 133 (12%), 132 (12%).

After removal of the aglycone, the mother liquor was treated as usual and chromatographed with standard markers on Whatman 3MM using BAW(4:1:2), detected with aniline hydrogen phthalate and identified as glucose.

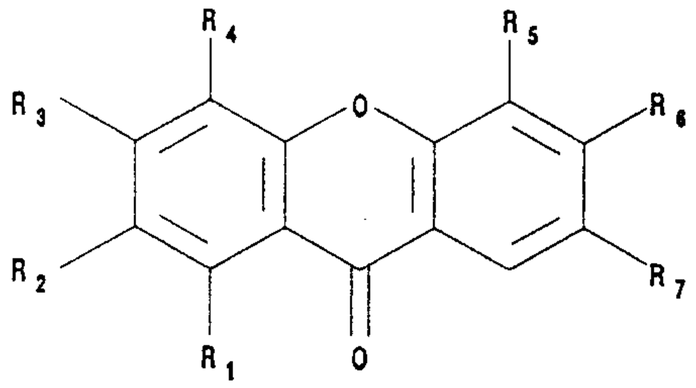
Identification of the known compounds: irisflorentin, irisolone, irisolone-4'-diglucoside, iristectorin A, swertisin, mangiferin, isomangiferin, irisolidone, irigenin and iridin was achieved by using UV, ^{1}H-NMR and MS and confirmed by TLC with authentic samples.



Swertisin m.p. 200° (dec)



	m.p.(°C)	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Irisflorentin	156	OCH_3	O-CH_2-O-		H	OCH_3	OCH_3	OCH_3
Irisolone	246	OCH_3	O-CH_2-O-		H	H	OH	H
Compound (1)	220-221	OH	H	O-gl	OCH_3	H	OCH_3	H
Irisolone-4' diglucoside	-	OCH_3	O-CH_2-O-		H	H	O-digl	H
Iristectorin A	202-204	OH	OCH_3	O-gl	H	OH	OCH_3	H
Irisolidone	184-185	OH	OCH_3	OH	H	H	OCH_3	H
Irigenin	180-182	OH	OCH_3	OH	H	OH	OCH_3	OCH_3
Iridin	207-208	OH	OCH_3	O-gl	H	OH	OCH_3	OCH_3



	m.p.(°C)	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Irisxanthone	200(dec.)	OH	gl.	OH	H	OCH_3	OH	H
Mangiferin	270(dec.)	OH	gl.	OH	H	H	OH	OH
Isomangiferin-	-	OH	H	OH	gl.	H	OH	OH

RESULTS AND DISCUSSION

Compound 1, m.p. 220-221°C, exhibited UV absorption at λ_{max} 272 and 330 nm indicating to be an isoflavone. The presence of a hydroxy group at C-5 but not C-7 was indicated by the application of diagnostic shift reagents[6]. The ^{1}H-NMR spectrum (90 MHz, DMSO) established its nature as an isoflavone. A sharp singlet at δ 8.2 was assigned to a C-2 proton, a pair of doublets (J= 8.5 Hz), each integrating for 2H, A_2B_2 pattern at δ 6.8 and δ 7.3 assigned to C-3', C-5' and C-2', C-6' protons respectively and a singlet at δ 6.9 (1H) due to C-6 proton. Two methoxy groups appeared as two singlets at δ 3.6 and 3.7 (3H, each). A characteristic signal for the 5-hydroxy proton at δ 12.7 disappeared on deuterium exchange as well as a characteristic distorted signal at δ 4.8 for one anomeric glucosyl proton (ruling out 3-glycosylation and suggesting 7-glycosylation)[7] and a broad signal at δ 3.1 (6H) for the other aliphatic sugar protons.
Acid hydrolysis of 1 gave an aglycone with a M.Wt 314, formula $C_{17}H_{14}O_6$ and glucose. The UV of the aglycone exhibited absorption maxima at 268 and 330 nm, and the bathochromic shift appeared after addition of NaOAc indicated that the sugar moiety is linked at C-7[6]. So that B ring is monosubstituted with one of the two methoxy groups which is linked at C-4'; this was supported by the peak at m/z 132 arising from retro-Diels-Alder cleavage of the heterocyclic ring[8].

Compound 1: is therefore a 7-O-glucoside with OH at C-5, OCH_3 at C-4' and the other OCH_3 in ring A which may be either at C-6 or C-8. The first possibility would correspond to irisolidone-7-O-glucoside, previously isolated[5] and should be excluded on the basis of a wide R_f discrepancy and the different physical properties. Thus the structure of 1 was identified as 5-hydroxy, 8,4' dimethoxyisoflavone-7-O-glucoside.

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دراسة محتـــويات نوعين من أنـواع الايـــــرس

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نبات الايرس الالمانى (السوسن) الذى يحمل الأسم العلمى أيرس جيرمانيكا لينيه الباونبات الايرس بسوداكورس لينيه هما من نباتات الزينة الدائمة الخضرة ويتبعا العائلة السوسنية. ونظرا لما ذكر فى بعض المراجع عن الأهمية الطبية للعائلة السوسنية وإستعمالها فى علاج بعض الأمراض لذا فقد تمت الدراسة الكيميائية لهذين النباتين.

وقد تم فصل خمسة مركبات فلافونيدية من ريزومات الايرس جرمانيكا الصنف الابيض وهم ايرسلفلورنين ، ايرسولون ، ايرسولون-٤'-ثنائى جليكوزيد ، ايرستيكتورين أ بالاضافة الى ٥-هيدروكسى ، ٨ و ٥'-ميثوكسى ، ٧-أ-ايزوفلافون جليكوزيد وهو مركب جديد يفصل لأول مرة بينما تم فصل فلافونيد جليكوزيد سويرتزين وزانون جليكوزيدات مانجفرين وايزومانجفرين من أوراق النبات.

أما ريزومات الايرس بسوداكورس فقد تم فصل ايزوفلافونيد ايربجينين وايرسوليدون وجليكوزيد ايريدين.

وقد تم التعرف على هذه المركبات عن طريق التحاليل الطيفية المختلفة ودراسة خواصها الكروماتوجرافية ودرجة انصهارها ومقارنتها بمواد أصيلة.

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