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The Constituents of *Iris florentina* L. (3).[1] Structure of Irisxanthone, a New C-Glycosylxanthone

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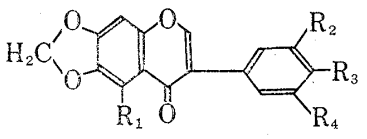
Irisxanthone (IV), $C_{20}H_{20}O_{11}\cdot H_2O$, decomposing over 208° without melting, a new C-glycosylxanthone, has been isolated from the rhizoma of *Iris florentina* L. (Iridaceae) along with mangiferin. The structure of irisxanthone (IV) has been determined as 2-C-β-D-glucopyranosyl-5-methoxy-1,3,6-trihydroxyxanthone by chemical and spectral studies.

In the precending papers[1,3] we presented isolation of two new isoflavonoids, irisflorentin (I) and irifloside (II), from the rhizoma of *Iris florentina* L. and their identification as 5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone and 5,4′-dihydroxy-3′-methoxy-6,7-methylene dioxyisoflavone-4′-β-D-glucoside, respectively.



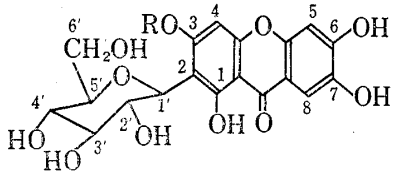
	R_1	R_2	R_3	R_4
irisflorentin (I):	OMe	OMe	OMe	OMe
irifloside (II):	OH	OMe	O·Gl.	H

Gl.; glucose

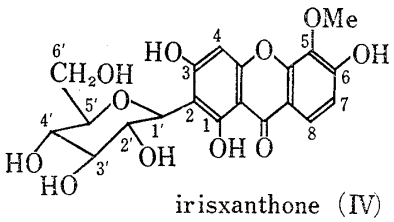
Chart 1

In the continuous investigation on more polar constituents a new C-glycoside of polyoxygenated xanthone was isolated from the ethyl acetate extract of the rhizoma.

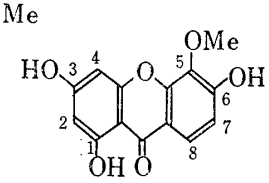
The ethyl acetate extract of the rhizoma was chromatographed on a silica gel column. After removal of several isoflavonoids by elution with a mixture of chloroform–methanol (20: 1), the column was eluted with a mixture of chloroform–methanol (10: 1) to afford a new xanthone C-glycoside, which has been named irisxanthone.



mangiferin (III) : R=H
homomangiferin (V) : R=Me



irisxanthone (IV)



1, 3, 6-trihydroxy-5-methoxyxanthone (VI)

Chart 2

1) Studies on Constituents of *Iris* Genus Plants (Part V). Part IV: M. Arisawa, N. Morita, Y. Kondo, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2323 (1973).
2) Location: *a*) *Gofuku 3190, Toyama*; *b*) *Aobayama, Sendai*.
3) Part III: N. Morita, M. Arisawa, Y. Kondo, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 600 (1973).

From the subsequent elution with a mixture of chloroform–methanol (5: 1) there was isolated mangiferin (III) which was identical with an authentic sample of mangiferin by physical and spectral data comparison.

The separation of both xanthone C-glycosides was accomplished most readily by running on the preparative thin-layer chromatography.

Irisxanthone (IV) was crystallized from methanol to give yellow needles, decomposing over 208° without melting. Microanalysis of IV established its molecular formula as $C_{20}H_{20}O_{11}\cdot H_2O$. IV was resistant to hydrolysis with hydrochloric acid, but on ferric chloride oxidation arabinose and glucose were proved.[4]

The ultraviolet (UV) absorption spectrum of IV showed maxima at 245 nm (log ε 4.75), 277 nm (log ε 4.31), 323 nm (log ε 4.39) and 360 nm (log ε 4.37) which were comparable to those given by 1,3,6-trihydroxy-5-methoxyxanthone.[5]

In the nuclear magnetic resonance (NMR) spectrum of trimethylsilyl (TMS) ether of IV, a broad signal integrating six protons at 3.1—3.8 ppm and a doublet (1H, J=9.0 Hz) at 4.71 ppm were assigned to the aliphatic protons and the anomeric proton[6] of the sugar moiety, respectively.

IV gave a crystalline heptaacetate, mp 112—114°, under usual acetylation. The NMR spectrum of the heptaacetate showed a methoxyl signal at 3.99 ppm as well as seven separate acetyl signals at 1.79, 2.00, 2.02, 2.04, 2.35, 2.46 and 2.51 ppm. The one at 1.79 ppm is assigned to the acetoxyl at C-2″ of C-β-D-glucopyranosyl compounds.[6] The aromatic protons of IV appeared as *ortho* split doublets centered at 7.07 and 7.85 ppm and a singlet at 6.58 ppm which were assignable to C-7, C-8 and C-4 protons of xanthone nucleus,[7] respectively (Table I). These observations unequivocally indicated that the sugar moiety is linked at the C-2 position of xanthone nucleus.

Table I. NMR Spectral Data of 1,3,5,6- and 1,3,6,7-Oxygenated Xanthone (in DMSO-d_6, δ (ppm))

Compounds	Assingnment				
	2-H	4-H	5-H	7-H	8-H
Irisxanthone (IV)	—	6.58	—	7.07 (d, J=9)	7.85 (d, J=9)
		(6.48)[a]		(6.83)[a] (d, J=9)	(7.70)[a] (d, J=9)
Xanthone C (VI)[5]	6.21 (d, J=3)	6.51 (d, J=3)	—	6.90 (d, J=9.6)	7.55 (d, J=9.6)
Mangiferin (III)	—	6.37	6.85	—	7.39
Homomangiferin (V)	—	6.64	6.93	—	7.48

a) in CD_3OD solvent

The position of the only methoxyl group was determined as following manner. The UV absorption maxima of IV unchanged on addition of boric acid-sodium acetate, while those in the presence of aluminum chloride or sodium acetate indicated the characteristic bathochromic shift[8] (see Experimental). Additionally, the C-4 proton signal of IV showed closely resembled downfield shift as mangiferin on conversion of these to acetates (Table II).

4) B.H. Koeppen and D.G. Roux, *Biochem. J.*, **97**, 444 (1965).
5) R.K. Chaudhuri and S. Ghosal, *Phytochem.*, **10**, 2425 (1971); S. Ghosal, R.K. Chaudhuri, and A. Nath, *J. Pharm. Sci.*, **62**, 137 (1973).
6) W.E. Hillis and D.H.S. Horn, *Aust. J. Chem.*, **18**, 531 (1965).
7) L.J. Haynes and D.R. Taylor, *J. Chem. Soc.* (*C*), **1966**, 1685.
8) *a*) T.R. Govindachari, B.R. Pai, P.S. Subramanian, U.R. Rao, and N. Muthukmaraswany, *Tetrahedron*, **23**, 243 (1967); *b*) K.R. Markham, *Tetrahedron*, **21**, 3687 (1965); *c*) O.R. Gottlieb, M.T. Magalhaes, M.O. de Silva Pereira, A.A.L. Mesquita, D. de B. Correa, and G.G. de Oliveira, *Tetrahedron*, **24**, 1601 (1968).

Table II. NMR Spectral Data of the Ring Protons on Xanthone Derivatives (in DMSO-d_6, δ (ppm))

	4-H	5-H	7-H	8-H
Mangiferin octaacetate (A)	7.54	7.71		8.01
Mangiferin (B)	6.37	6.85		7.39
Δ(A−B)	1.17	0.86		0.62
Homomangiferin heptaacetate (C)	7.32	7.74		8.15
Homomangiferin (D)	6.64	6.93		7.48
Δ(C−D)	0.68	0.81		0.67
Irisxanthone heptaacetate (E)	7.74		7.36	7.98
Irisxanthone (F)	6.58		7.07	7.85
Δ(E−F)	1.16		0.29	0.13
			$3'$ $5'$-H	$2'$ $6'$-H
Cosmosiin hexaacetate (G)			7.34	8.13
Cosmosiin (H)			6.98	7.99
Δ(G−H)			0.36	0.14

The UV spectral and NMR data indicated that the methoxyl group of IV locate at either C-5 or C-6 position and the absence of *ortho* diphenol grouping.

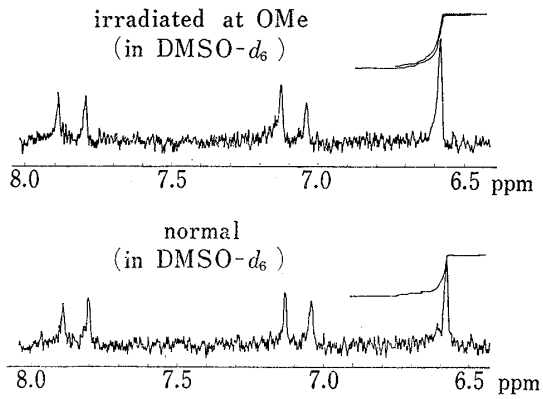


Fig. 1. NMR of Irisxanthone (IV)

Application of the nuclear Overhauser effect (NOE) to the aromatic systems can be used to determine the position of substituent groups.[9] In the NOE studies of IV, saturation of the methoxyl protons caused a 32% area enhancement in the C_4-H (Fig. 1). Similarly the NOE was observed for the methoxyl signal when the C-4 proton was saturated. No NOE was observed for the other aromatic protons on irradiating the methoxyl protons. The distance between the C-4 proton and a proton of methoxyl group was 2.4 Å as measured from Dreiding model, and at the distance the NOE of approximately 40% can be expected.[10]

From these NMR experiments, the methoxyl group in irisxanthone (IV) must be located at C-5. Consequently, the structure of irisxanthone was represented by 2-C-β-D-glucopyranosyl-5-methoxy-1,3,6-trihydroxyxanthone. Irisxanthone is the first example of 1,3,5,6-tetraoxygenated xanthone C_2-β-D-glucoside in nature.

Experimental[11]

Isolation of Irisxanthone (IV) and Mangiferin (III)——The fresh rhizoma of *Iris florentina* L. were continuously extracted with MeOH. Removal of MeOH gave a viscous oily residue which was extracted with ether and then with ethyl acetate. i) The ethyl acetate was chromatographed on silica gel column. The column was gradually eluted with a mixture of $CHCl_3$–MeOH (100: 1 to 20: 1). This eluent included several

9) *cf.* G.E. Bachers and T. Schaefer, *Chem. Revs.*, **71**, 617 (1971) and references cited therein.
10) R.E. Bell and J.K. Saunders, *Can. J. Chem.*, **48**, 1114 (1970).
11) All melting points are uncorrected and were taken on a Yanagimoto micro melting point apparatus. IR and UV spectra were recorded on a Shimazu Grating Spectrophotometer IR-27G, and on a Hitachi Spectrophotometer, Model 124, respectively. NMR spectra were obtained on a Japan Electron Optics Lab., JNM Ps-100. Chemical shifts were recorded as δ values (ppm) with TMS internal standard.

isoflavonoids.[1] The remaining column was eluted with a mixture of $CHCl_3$–MeOH (10: 1) to afford IV, and then with a mixture of $CHCl_3$–MeOH (5: 1) to afford III. ii) The ethyl acetate extract was separated on silica gel preparative thin-layer chromatography (TLC) using a mixture of $CHCl_3$–MeOH (3: 1) as a solvent. The separated bands were visualized under UV light. The bands of *Rf* 0.2 (TLC) were collected and extracted with MeOH. Evapolation of MeOH gave a crude IV.

Irisxanthone (IV)——Irisxanthone (IV) was recrystallized from MeOH to give yellow needles, which began to blacken at about 208° and gradually decomposed without melting. Greenish brown to $FeCl_3$. Mg+HCl; Orange red, Gibbs reac.; (+), Molish reac.; (+). Orange fluorescence under UV light. PPC *Rf*; 0.68 (15% AcOH). *Anal.* Calcd. for $C_{20}H_{20}O_{11}\cdot H_2O$: C, 52.86; H, 4.85. Found: C, 53.01; H, 5.12. UV λ_{max}^{EtOH} nm (log ε): 245 (4.75), 277 (4.31), 323 (4.39), 360 (4.37). UV $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 238 (4.63), 250 (4.68), 262 (4.57), 338 (4.57), 380 (4.23). UV $\lambda_{max}^{EtOH+AcONa}$ nm (log ε): 244 (4.73), 263 (sh), (4.45), 275 (sh), (4.39), 368 (4.52). UV $\lambda_{max}^{EtOH+H_3BO_3-AcONa}$ nm (log ε): 245 (4.75), 277 (4.31), 323 (4.39), 360 (4.37). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1645 (C=O), 1615 (C=C), 1575 (C=C). NMR (10% solution in CD_3OD)[12] δ: 3.94 (3H, singlet, OMe), 6.48 (1H, singlet, aromatic H), 6.83 (1H, doublet, J=9.0 Hz, aromatic H), 7.70 (1H, doublet, J=9.0 Hz, aromatic H). NMR (TMS ether of IV, 10% solution in CCl_4)[12] δ: 3.1—3.8 (6H, broad, aliphatic H), 3.93 (3H, singlet, OMe), 4.71 (1H, doublet, J=9.0 Hz, anomeric H), 6.30 (1H, singlet, aromatic H), 6.77 (1H, doublet, J=9.0 Hz, aromatic H), 7.82 (1H, doublet, J=9.0 Hz, aromatic H).

Mangiferin (III)——Mangiferin (III) was recrystallized from MeOH to give yellow needles, mp 268—270° (decomp.) with forgoing blackening at about 250°. Greenish brown to $FeCl_3$. Mg+HCl; Orange red, Gibbs reac.; (+), Molish reac.; (+). Orange fluorescence under UV light. PPC *Rf*: 0.45 (15% AcOH, mangiferin 0.45). Mixed melting point determination and comparison of IR spectra indicated its identity with an authentic mangiferin.

Irisxanthone Heptaacetate——To a solution of IV in pyridine was added acetic anhydride. The reaction mixture was allowed to react on water bath for 10 hr. The reaction mixture was worked up as the usual manner. Recrystallization from MeOH gave colorless micro needles, mp 112—114°, no color to $FeCl_3$.

NMR (in $CDCl_3$) δ: 1.79 (3H, singlet, aliphatic OAc), 2.00 (3H, singlet, aliphatic OAc), 2.02 (3H, singlet, aliphatic OAc), 2.04 (3H, singlet, aliphatic OAc), 2.35 (3H, singlet, aromatic OAc), 2.46 (3H, singlet, aromatic OAc), 2.51 (3H, singlet, aromatic OAc), 3.8 (1H, multiplet, AcO–C<u>H</u>), 3.99 (3H, singlet, OMe), 4.44 (1H, multiplet, AcO–C<u>H</u>), 4.82 (1H, doublet, J=9.0 Hz, anomeric H), 5.2 (2H, multiplet, AcO–CH_2), 5.7 (1H, multiplet, AcO–C<u>H</u>), 7.01 (1H, doublet, J=9.0 Hz, aromatic H), 7.28 (1H, singlet, aromatic H), 7.91 (1H, doublet, J=9.0 Hz, aromatic H). *Anal.* Calcd. for $C_{34}H_{34}O_{18}\cdot H_2O$: C, 54.55; H, 4.81. Found: C, 54.34; H, 5.03.

Ferric Chloride Oxidation of IV——IV (30 mg) was refluxed with aqueous solution of $FeCl_3$ (0.1 g in 5 ml H_2O) for 6 hr. After being cooled the reaction mixture was filtered and the filtrate was passed through the column of Amberlite IR-120 (H^+ form) and IR-4B (OH^- form) and evaporated *in vacuo* to a syrup, which was examined by PPC. *Rf*: 0.18 (brown), 0.21 (reddish brown) (*n*-butanol: acetic acid: water=4: 1: 2, glucose 0.18 (brown), arabinose 0.21 (reddish brown)); 0.38 (brown), 0.52 (reddish brown) (75% phenol, glucose 0.38 (brown), arabinose 0.52 (reddish brown)). Color reaction with 0.1N aniline hydrogen phthalate.

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12) NMR spectra were obtained on a Hitachi H-60 spectrometer.