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Studies on Constituents of *Iris* Genus Plants. III.[1] The Constituents of *Iris florentina* L. (1)

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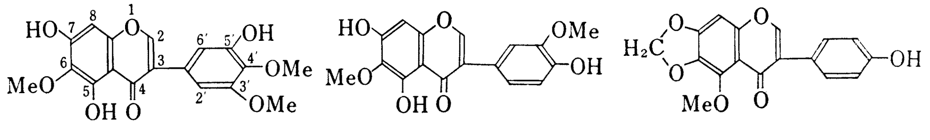
Irisflorentin, a new isoflavone has been isolated from the rhizoma of *Iris florentina* L. (Iridaceae) besides irigenin, iristectorigenin B and irisolone. The structure of irisflorentin has been determined as 5,3',4',5'-tetramethoxy-6,7-methylenedioxyisoflavone by chemical degradation and spectral means.

The presence of several isoflavones in the rhizomes of *Iris* genus (Iridaceae) has been reported.[1,3–9] From the rhizoma of *Iris florentina* L. irigenin and its glucoside iridin have been isolated.[3]

We now wish to report the structural elucidation of a new isoflavonoid to which we gave the name irisflorentin, and isolation of two known isoflavones from the rhizoma of *I. florentina* L.

The ethereal extract of the rhizoma was chromatographed on a silica gel column. The column was gradually eluted with a mixture of chloroform–methanol to give irigenin (I) and other three isoflavones (II, III and IV). II, $C_{17}H_{14}O_7$, mp 153°, and III, $C_{17}H_{12}O_6$, mp 271° were identified as iristectorigenin B and irisolone by direct comparison with authentic specimens, respectively. Compound IV (irisflorentin), mp 175°, a new isoflavone, had the composition $C_{20}H_{18}O_8$ by elemental and mass spectral analyses. The ultraviolet (UV) absorption spectrum of IV showed maxima at 266 nm (log ε 4.46), 321 nm (log ε 3.82).

The nuclear magnetic resonance (NMR) of IV (in C_6D_6) revealed the presence of four methoxyl groups at 3.53 (6H), 3.86 (3H) and 3.97 ppm (3H), a methylenedioxy at 5.21 ppm



I II III

Fig. 1.

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[1] Part I in this seies: N. Morita, M. Shimokooiyama, M. Shimizu, and M. Arisawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 730 (1972), and Part II; *Yakugaku Zasshi*, **92**, 1052 (1927).

[2] Location: *a) Gofuku 3190, Toyama; b) Aoba-yama, Sendai.*

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[8] K.W. Gopinath, A.R. Kidwai, and L. Prakash, *Tetrahedron*, **16**, 201 (1961); K.W. Gopinath, L. Prakash, and A.R. Kiawai, *Indian J. Chem.*, **1**, 187 (1963).

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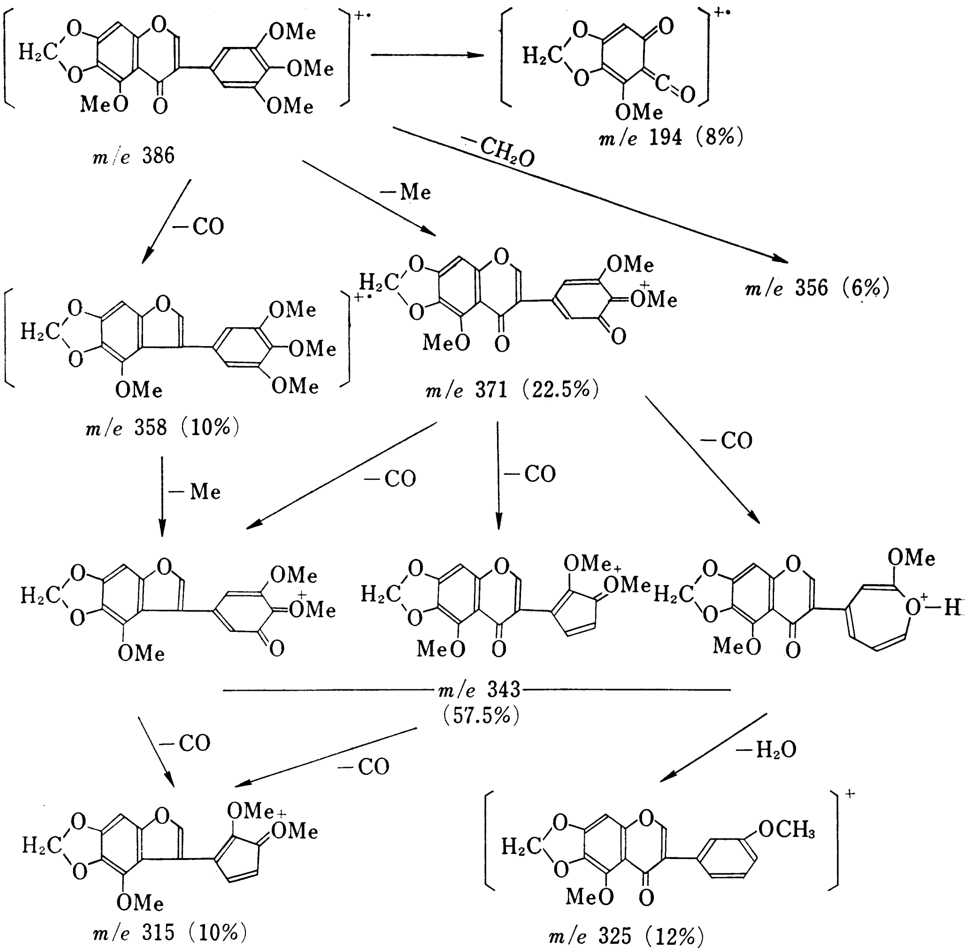

Fig. 2.

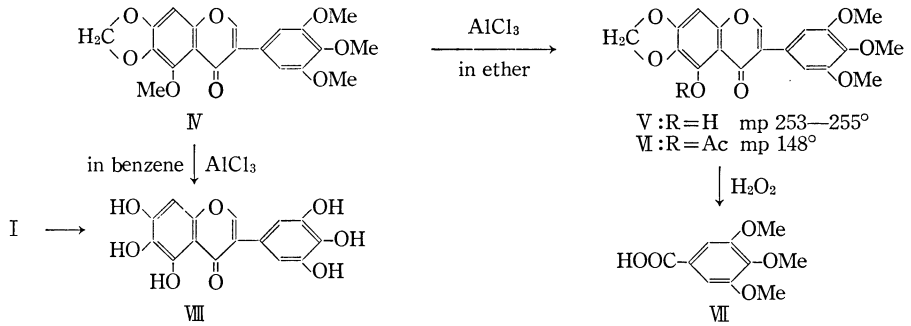

Fig. 3

and four ring protons at 6.36 (1H), 6.84 (2H) and 7.34 ppm (1H). The appearance of low-field chemical shift at 7.34 ppm was characteristic of the C-2 proton on the isoflavonoid nuclei.[10] The infrared (IR) spectrum showed absorptions at 1660 (conjugated carbonyl) and 1575 cm^{-1} (conjugated double bond) (Chart 1).

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10) T.J. Mabry, K.R. Markham, and M.B. Thomas, "*The Systemmatic Identification of flavonoids*" Springer Verlag, 1970, p. 268.

Table I. The NMR Data of IV and I-per Me. Ether

	Position	Solvent CDCl$_3$ IV	Solvent CDCl$_3$ I-per Me.	Solvent C$_6$D$_6$ IV	Solvent C$_6$D$_6$ I-per Me.
OCH$_3$	7	—	3.85(3H)	—	3.33(3H)
	3′,5′	3.89(6H)	3.88(6H)	3.53(6H)	3.59(6H)
	6	—	3.90(3H)	—	3.75(3H)
	4′	3.87(3H)		3.86(3H)	3.84(3H)
	5	4.09(3H)	3.95(6H)	3.97(3H)	3.99(3H)
$\begin{matrix}O\\O\end{matrix}\!>\!CH_2$	6,7	6.08(2H)	—	5.21(2H)	—
Aromatic H	8	6.63(1H)	6.68(1H)	6.36(1H)	6.32(1H)
	2′,6′	6.77(2H)	6.73(2H)	6.84(2H)	6.81(2H)
	2	7.81(1H)	7.81(1H)	7.34(1H)	7.48(1H)

All signals are singlet.

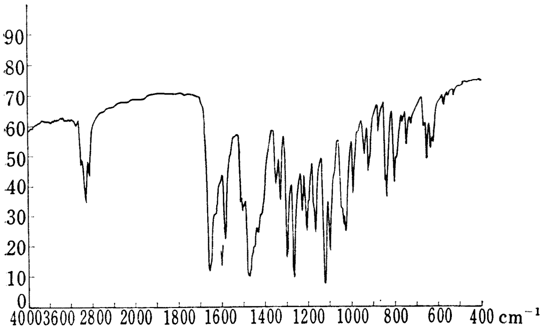

Chart 1. IR Speectrum of Irisflorentin

IV was treated with anhydrous aluminum chloride in ether, which was known as the usual procedure for demethylation of C-5 methoxyl on the chromon derivatives[11,12], to afford a monodemethylated compound (V), mp 253—255°. V gave a monoacetate (VI), mp 148° under usual acetylation.

When V was oxidized with hydrogen peroxide in 5% aqueous alkali, 3,4,5-trimethoxybenzoic acid (VII) was obtained and identified by direct comparison with an authentic samle.

The position of the substituents on IV was confirmed as follows: IV on refluxing with anhydrous aluminum chloride in benzene gave a perdemethylated compound VIII, which was identical with 5,6,7,3′,4′,5′-hexahydroxyisoflavone from irigenin as the same fashion. Consequently, irisflorentin (IV) is best reprented as 5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone.

The fragmentation pattern of irisflorentin (IV) in the mass spectrum (Fig. 2) substantiated the proposed structure.[13]

Experimental[14]

Isolation of Irigenin (I), Iristectorigenin B (II), Irisolone (III) and Irisflorentin (IV)——14.5 kg of the fresh rhizoma of *Iris florentina* L. was chopped up and extracted with MeOH. Evaporation of the solvent

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11) P. Crabbe, P.R. Leeming, and C. Djerassi, *J. Am. Chem. Soc.*, **80**, 5258 (1958).

12) E.F. King, T.J. King, and A.T. Warwick, *J. Chem. Soc.*, **1952**, 96.

13) A. Pelter, P. Stainton, A.P. Johnson, and M. Barber, *J. Heterocyclic Chem.*, 2, 256 (1965); *cf.* H. Budzikiewicz, C. Djerassi, and D.H. Williams, "*Interpretation of Mass Spectra of Organic Compounds*", Holden-Day, Inc., San Francisco, 1964, p 181.

14) Melting points were determined on a Yamato mp apparatus Model MP-21 and uncorrected. IR spectra were recorded on a Shimadzu Grating spectrophotometer IR-27G. NMR spectra were taken on a Hitachi H-60. Chemical shifts were recorded as ppm with TMS as internal standard. Mass spectra were determined with a Hitachi mass spectrometer RMU-7 at electron energy 80 eV.

yielded a viscous oily residue which was extracted with ether several times. The combined ethereal extract was concentrated and chromatographed on a silica gel column. The column was eluted with $CHCl_3$ and subsequently with a mixture of $CHCl_3$–MeOH (100: 1) to afford irisolone (III) and irisflorentin (IV). Irisolone was recrystallized from MeOH to give colorless prismes, mp 271°, no color to $FeCl_3$, green color to the Labat test for methylenedioxy group. Mg+HCl; yellow. *Anal.* Calcd. for $C_{17}H_{12}O_6$: C, 65.38; H, 3.94. Found: C, 65.53; H, 4.12. Mass Spectrum *m/e*: 312 (M^+). UV λ_{max}^{EtOH} mμ (log ε); 265 (4.55), 322 (3.88). IR ν_{max}^{KBr} cm^{-1}; 3200 (OH), 1620 (C=O), 1580 (C=C). NMR (10% solution in DMSO-d_6) δ; 3.88 (3H, singlet, OCH_3), 6.13 (2H, singlet, $\overset{O}{\underset{O}{>}}CH_2$), 6.77 (2H, doublet, J=8.5 Hz, $C_{2',6'(3',5')}$-H), 6.19 (1H, singlet, C_8-H), 7.31 (2H, doublet, J=8.5 Hz, $C_{3',5'(2',6')}$-H), 8.11 (1H, singlet, C_2-H), 9.47 (1H, singlet, OH).

III Acetate: To a solution of III was allowed to stand with acetic anhydride in pridine at room temp. for 24 hr. The reaction mixture was worked up as the usual manner. Recrystallization from MeOH gave colorless needles, mp 169—170°. NMR (10% solution in $CDCl_3$) δ: 2.28 (3H, singlet, $OCOCH_3$), 4.06 (3H, singlet, OCH_3), 6.02 (2H, singlet, $\overset{O}{\underset{O}{>}}CH_2$), 6.58 (1H, singlet, C_8-H), 7.10 (2H, doublet, J=8.5 Hz, $C_{2',6'(3',5')}$-H), 7.50 (2H, doublet, J=8.5 Hz, $C_{3',5'(2',6')}$-H), 7.51 (1H, singlet, C_2-H).

Irisflorentin (IV) was recrystallized from MeOH to give colorless needles, mp 175°, no color to $FeCl_3$, green color to the Labat test for methylenedioxy group. Mg+HCl; yellow. *Anal.* Calcd. for $C_{20}H_{18}O_8$: C, 62.18; H, 4.70. Found: C, 62.30; H, 4.87. Mass Spectrum *m/e*: 386 (M^+). P.P.C. *Rf.* 0.72 (15% AcOH). 0.88 (30% AcOH). UV λ_{max}^{EtOH} mμ (log ε): 266 (4.46), 321 (3.82). IR ν_{max}^{KBr} cm^{-1}; 1660 (C=O), 1575 (C=C). NMR data are as given in Table I.

Subsequent elution with a mixture of $CHCl_3$–MeOH (100: 1) gave irigenin (I) and iristectorigenin B. Irigenin (I) was recrystallized from MeOH to give pale yellow needles, mp 183°, greenish brown to $FeCl_3$. UV λ_{max}^{EtOH} mμ; 268, 336. P.P.C. *Rf*; 0.60 (15% AcOH, irigenin 0.60), 0.81 (30% AcOH, irigenin 0.81). Its IR spectrum was found to be superimposable with that of an authentic specimen and undepressed on admixture with an authentic sample.

I Acetate: I dissolved in acetic anhydride in the presence of a few drops of conc. H_2SO_4 was allowed to stand overnight at room temp. After the usual work-up, crystallization from MeOH gave colorless needles, mp 127—128°, undepressed on admixture with an authentic sample. NMR (10% solution $CDCl_3$) δ: 2.31 (3H, singlet, $OCOCH_3$), 2.36 (3H, singlet, $OCOCH_3$), 2.45 (3H, singlet, $OCOCH_3$), 3.84 (6H, singlet, $OCH_3 \times 2$), 3.87 (3H, singlet, OCH_3), 6.72 (1H, doublet, J=2.0 Hz, $C_{6'(2')}$-H), 6.93 (1H, doublet, J=2.0 Hz, $C_{2'(6')}$-H), 7.14 (1H, singlet, C_8-H), 7.83 (1H, singlet, C_2-H).

Iristectorigenin B (II) was recrystallized from MeOH to give pale yellow needles, mp 151°, greenish brown to $FeCl_3$. P.P.C. *Rf*; 0.49 (15% AcOH, iristectorigenin B 0.49), 0.72 (30% AcOH. iristectorigenin B 0.72). UV λ_{max}^{EtOH} mμ; 267, 292 (shoulder), 346. Its IR spectrum was found to be superimposable with that of an authentic specimen and undepressed on admixture with an authentic sample.

II Acetate: Acetate prepared from II, acetic anhydride and conc. H_2SO_4. Recrystallization from MeOH gave colorless needles, mp 160—163°, which undepressed on admixture with an authentic sample of acetyl iristectorigenin B.

Partial Demethylation of Irisflorentin (IV)——IV (200 mg) was refluxed with anhydrous aluminum chloride (2 g) in 20 ml of absolute ether. After 14 hr the reaction mixture poured into ice-water, and the yellow deposit was separated, boild with 20 ml of acetic acid and 10 ml of concd. hydrochloric acid for 5 min. Dilution with water and extraction with $CHCl_3$ yielded pale yellow crystals which were purified by passing through a column of silica gel and eluting with $CHCl_3$. Recrystallization from MeOH provided pale yellow needles of 5-demethyl-irisflorentin, mp 253—255°, greenish brown color with $FeCl_3$. *Anal.* Calcd. for $C_{19}H_{16}O_8$: C, 61.27; H, 4.33. Found: C, 61.41; H, 4.53. NMR (10% solution in DMSO-d_6) δ; 3.68 (3H, singlet, OCH_3), 3.78 (6H, singlet, $OCH_3 \times 2$), 6.12 (2H, singlet, $\overset{O}{\underset{O}{>}}CH_2$), 6.77 (1H, singlet, C_8-H), 6.83 (2H, singlet, $C_{2',6'}$-H), 6.44 (1H, singlet, C_2-H).

V Acetate (VI): Acetate was prepared from 5-demethylirisflorentin (V), acetic anhydride and conc. H_2SO_4 as described above. Recrystallization from MeOH gave colorless, mp 148°. NMR (10% solution in $CDCl_3$) δ; 2.43 (3H, singlet, $OCOCH_3$), 3.88 (9H, singlet, $OCH_3 \times 3$), 6.13 (2H, singlet, $\overset{O}{\underset{O}{>}}CH_2$), 6.67 (2H, singlet, $C_{2',6'}$-H), 6.80 (1H, singlet, C_8-H), 7.81 (1H, singlet, C_2-H).

Oxidation of V with Hydrogen Peroxide to 3,4,5-Trimethoxybenzoic Acid (VII)——To a solution of V in 5% KOH was added 3% H_2O_2 under the mechanical stirring and allowed to stand overnight at room temp. After decomposition of excess H_2O_2 with MnO_2, the reaction mixture was acidified with dilute HCl and extracted with ether. Recrystallization from MeOH provided colorless needles, mp 168—172°; undepressed on admixture with an authentic sample of 3,4,5-trimethoxybenzoic acid (VII) which was produced from 5-hydroxy-6,7,3',4',5'-pentamethoxyisoflavone.