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Studies on Constituents of Genus *Iris*. VIII.[1] The Constituents of *Iris unguicularis* Poir. (2)[2]

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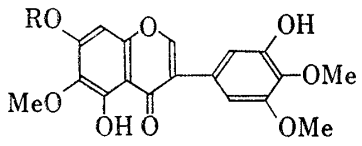
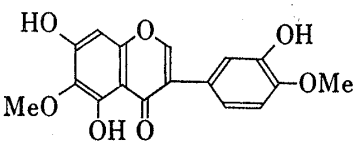
Kanzakiflavone-2 (I), a new flavone has been isolated from the ethereal extract and three known compounds, iridin (VI), mangiferin (VII) and isomangiferin (VIII) were isolated from the *n*-butanolic extract of *Iris unguicularis* Poir. respectively. The structure of kanzakiflavone-2 (I) has been determined as 5,4′-dihydroxy-6,7-methylenedioxyflavone by chemical and spectral means.

We have previously shown[1] that a new flavone, kanzakiflavone-1, was isolated from the ethereal extract of the rhizomes of *Iris unguicularis* Poir. We now wish to report the structural elucidation of a new flavone to which we gave the name kanzakiflavone-2 (I) from the ethereal extract and isolation of three known flavonoids from the *n*-butanolic extract of the rhizomes of the same plant.

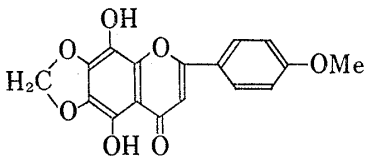
The ethereal extract of the rhizomes was chromatographed on a silica gel column. Irigenin (II),[1] iristectorigenin A (III)[1] and kanzakiflavone-1 (IV)[1] were eluted from the column with a chloroform. The remaining column was then eluted with ethyl acetate to give kanzakiflavone-2 (I).



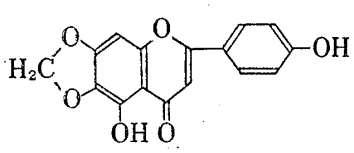
II : R=H
VI : R=glucosyl



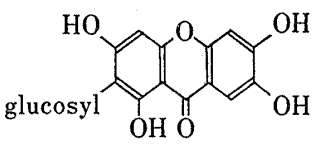
III



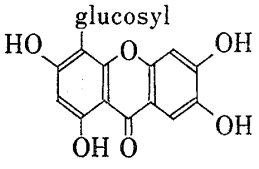
IV



I



VII



VIII

Chart 1

The combustion analysis of I, mp over 310°, showed the empirical formula $C_{16}H_{10}O_6$, which was confirmed by mass spectral measurement (M^+ 298). The ultraviolet (UV) absorption spectrum of I showed maxima at 284, 336 nm (log ε 4.14, 4.33). The UV maxima changed at 289 (sh.), 301, 354 nm (log ε 4.14, 4.19, 4.33) by addition of aluminum chloride. The infrared (IR) spectrum displayed absorption bands at 3280 cm^{-1} (hydroxyl), 1680 cm^{-1} (carbonyl),

1) M. Arisawa and N. Morita, *Chem. Pharm. Bull.* (Tokyo), **24**, 815 (1976).
2) The 38th and 41th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, June, 1973 and November, 1975.
3) *Location; 3190, Gofuku, Toyama, 930, Japan.*

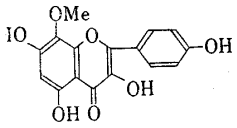
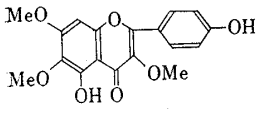
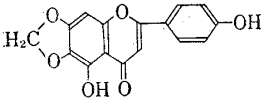
1620 cm^{-1} (chelated carbonyl) and 1610 cm^{-1} (aromatic). These spectral data together with the positive color test for flavonoid clearly indicated that I has a 5-hydroxyflavone nucleus.

In the proton nuclear magnetic resonance (PMR) spectrum of trimethylsilyl-I, a pair of 2H doublets (J=9.0 Hz) centered at 7.67 and 6.83 ppm assignable to the proton located at 2′, 6′ and 3′,5′-position, and two 1H singlets at 6.52 and 6.30 ppm assignable to the proton located at 8 and 3-position on aromatic ring, respectively. 2H singlet at 6.00 ppm was attributed methylenedioxy group.

I gave a diacetate (V), mp 237—238°, on usual acetylation. The diacetate showed two acetyl signals at 2.07 and 2.15 ppm in the PMR spectrum.

In the fragmentation pattern of diacetate (V), ketone elimination twice gave rise to I (m/e 298), which further cleaved to retro-Diels-Alder ion, m/e 270, m/e 180 and m/e 118.

Table I. Chemical Shifts of C_6 and C_8-H on Trimethylsilyl Flavonoids (in CCl_4, δ(ppm))

Flavonoids	C_6-H	C_8-H
	6.12	—
	—	6.47
	—	6.52
kanzakiflavone-2 (IV)		

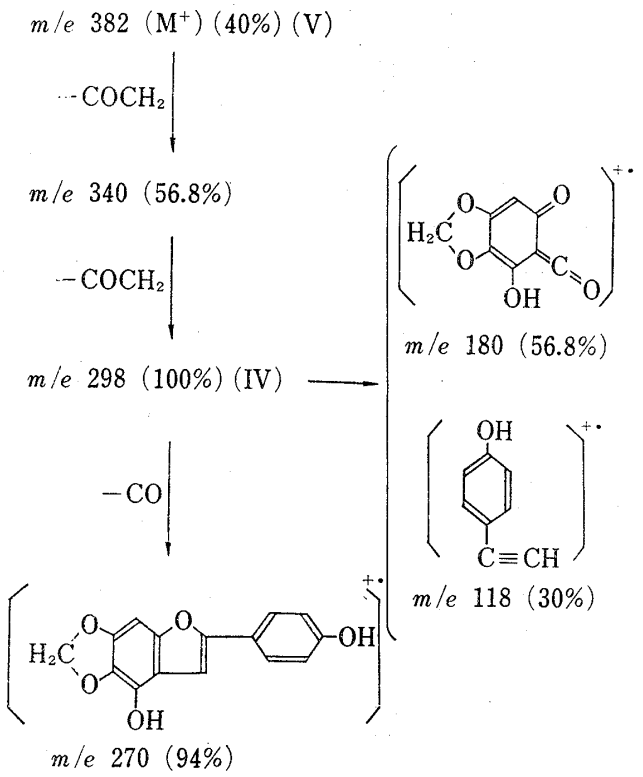


Chart 2. Fragmentation in Mass Spectrum of Kanzakiflavone-2 Diacetate (V)

From above result the structure of kanzakiflavone-2 (I) is represented as 5,4′-dihydroxy-6,7-methylenedioxyflavone.

A part of the n-butanolic extract was chromatographed on a silica gel column. The column was eluted with a mixture of chloroform–methanol to afford iridin (VI). The other part of the extract was chromatographed on a polyamide column. The column was eluted with aqueous methanol to afford mangiferin (VII) and VIII.

VIII was decomposed over 240° without melting. Microanalysis of VIII established its molecular formula as $C_{19}H_{18}O_{11}\cdot H_2O$. VIII was resistant to hydrolysis with hydrochloric acid, but ferric chloride oxidation arabinose and glucose were proved. The UV absorption spectrum of VIII showed maxima at 240, 258, 315, and 371 nm which were comparable to those given by mangiferin (VII).

In the PMR spectrum of VIII taken hexadeuterodimethyl sulfoxide (DMSO-d_6), a broad signal integrating six protons at 3.0—4.2 ppm and a doublet (1H, J=9.0 Hz) at 4.74 ppm were assigned to the aliphatic protons and the anomeric proton of sugar moiety, respectively. The aromatic protons of VIII appeared as three singlets at 6.23, 6.84 and 7.37 ppm which were assignable to C-2, C-5 and C-8 proton of xanthone nucleus, respectively (see Table II.).

Table II. Chemical Shifts of the Aromatic Proton on Xanthones

	C_2-H	C_4-H	C_5-H	C_7-H	C_8-H
Xanthone C[a]	6.21 (d, J=3)	6.51 (d, J=3)	—	6.90 (d, J=9)	7.55 (d, J=9)
Mangiferin	—	6.37	6.85	—	7.39
VIII	6.23	—	6.84	—	7.37

a) R.K. Chaudhuri and S. Ghosal, *Phytochem.*, **10**, 2425 (1971); S. Ghsal, R.K. Chaudhuri, and A. Nath, *J. Pharm. Sci.*, **62**, 137 (1973)

These data unequivocally indicated that the sugar moiety is linked at the C-2 position of xanthone nucleus and therefore VIII is isomangiferin.[4]

Experimental[5]

Extraction and Separation——The fresh rhizomes of *Iris unguicularis* Poir. were chopped up and extracted with MeOH. The methanolic extract was extracted with ether and then with ethyl acetate. The insoluble part was further extracted with *n*-butanol. The combined ethereal extract was concentrated and chromatographed on silica gel column. The column was eluted with chloroform to afford II, III and IV. The remaining column was then eluted with ethyl acetate to give kanzakiflavone-2 (I). A part of *n*-butanolic extract was chromatographed on a silica gel column. The column was eluted with a mixture of chloroform–methanol (20: 1) to afford iridin (VI). The other part of the extract was chromatographed on a polyamide column. The column was eluted with an aqueous methanol to afford mangiferin (VII) and isomangiferin (VIII).

Kanzakiflavone-2 (I)——Recrystallization from a mixture of pyridine–methanol gave pale yellow microneedles, mp over 310°, greenish brown to $FeCl_3$, yellow to Mg–HCl, orange yellow to Zn–HCl, green color to Labat reaction for methylenedioxy group, dark blue to Gibbs reaction, dark brown under UV light. PPC *Rf*: 0.27(30% AcOH), 0.71(60% AcOH), 0.89(*n*-BuOH–AcOH–H_2O (4:1:2)). *Anal.* Calcd. for $C_{16}H_{10}O_6$: C, 64.41; H, 3.52. Found: C, 64.60; H, 3.77. Mass Spectrum *m/e*: 298 (M^+), 270, 240, 212, 184, 180, 118. UV λ_{max}^{EtOH} nm (log ε): 284 (4.14), 336 (4.33). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm (log ε): 289 (sh.) (4.14), 301 (4.19), 354 (4.33). UV $\lambda_{max}^{EtOH\text{-}AcONa}$ nm (log ε): 283 (4.10), 337 (4.29). UV $\lambda_{max}^{EtOH\text{-}AcONa\text{-}H_3BO_3}$ nm (log ε): 281 (4.14), 334 (4.31). IR ν_{max}^{KBr} cm^{-1}: 3280, 1680, 1620, 1610. PMR (TMS ether of I, 10% solution in CCl_4) δ(ppm): 0.30 (9H, singlet, Me_3Si), 0.33 (9H, singlet, Me_3Si), 6.00 (2H, singlet, $\begin{smallmatrix}-O\\-O\end{smallmatrix}\!>\!CH_2$), 6.30 (1H, singlet, C_3–H), 6.52 (1H, singlet, C_8–H), 6.83 (2H, doublet, J=9.0 Hz, $C_{3',5'}$–H), 7.67 (2H, doublet, J=9.0 Hz, $C_{2',6'}$–H).

I Diacetate (V)——To a solution of III in pyridine was added acetic anhydride. After heating on a water bath for 1 hr, the reaction mixture worked up as usual manner. Recrystallization from MeOH gave colorless needles, mp 237—238°, no color to $FeCl_3$. *Anal.* Calcd. for $C_{20}H_{14}O_8$: C, 62.81; H, 3.69. Found: C, 63.00; H, 3.91. Mass Spectrum *m/e*: 382 (M^+), 340, 298, 270, 240, 212, 184, 180, 118. PMR (10% solution in C_5D_5–DMSO-d_6) δ (ppm): 2.07 (3H, singlet, $OCOCH_3$), 2.15 (3H, singlet, $OCOCH_3$), 6.04 (2H, singlet, $\begin{smallmatrix}-O\\-O\end{smallmatrix}\!>\!CH_2$, 6.58 (1H, singlet, C_3–H), 7.00 (1H, singlet, C_8–H), 7.08 (2H, doublet, J=9.0 Hz, $C_{3',5'}$–H), 7.80 (2H, doublet, J=9.0 Hz, $C_{2',6'}$–H).

Isomangiferin (VIII)——Recrystallization from a mixture of pyridine–methanol gave pale yellow microneedles, which began to blacken at about 240° and gradually decomposed without melting, greenish brown to $FeCl_3$, orange red to Mg–HCl, positive to Molish reaction, negative to Gibbs reaction, resistant to hydrolysis with HCl and orange fluorescence under UV light. PPC *Rf*: 0.29 (15% AcOH), 0.45 (30% AcOH), 0.53 (60% AcOH), 0.43 (*n*–BuOH–AcOH–H_2O (4: 1: 2)). *Anal.* Calcd. for $C_{19}H_{18}O_{11}\cdot H_2O$: C, 58.14; H, 5.14. Found: C, 58.40; H, 5.33. Mass Spectrum *m/e*: 422, 404, 387, 369, 352, 351, 327, 314, 301, 298, 285, 274. UV λ_{max}^{EtOH} nm: 240, 258, 315, 371. IR ν_{max}^{KBr} cm^{-1}: 3250, 1690, 1615, 1570, 1485. PMR (10% solution in DMSO-d_6) δ (ppm): 3.0—4.2 (6H, broad, aliphatic H×6), 4.74 (1H, doublet, J=9.0 Hz, anomeric H), 6.23 (1H, singlet,

4) N. Aritomi and T. Kawasaki, Abstracts of Papers, 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, 1969, p. 344.

5) All melting points are uncorrected and were taken on a Yanagimoto micro melting point apparatus. IR and UV spectra were recorded on a Japan Spectroscopic Co., Spectrophotometer, Model IR-E and on a Hitachi Spectrophotometer, Model 124, respectively. Mass and PMR spectra were obtained on a Japan Electron Optics Lab., JMS-OISG-2 and JNMC-60H, respectively. Chemical shifts were recorded as δ values (ppm) with tetramethylsilane (TMS) internal standard.

C_2–H), 6.84 (1H, singlet, C_5–H), 7.37 (1H, singlet, C_8–H), 13.81 (1H, singlet, C_1–OH). Glucose and arabinose were proved on ferric chloride oxidation. Acetate was prepared from VIII, acetic anhydride and pyridine. Recrystallization from MeOH gave colorless needles, mp 201—203°.

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