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Studies on Constituents of *Iris* Genus Plants. IV. The Constituents of *Iris florentina* L. (2[1])

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Irifloside (III), $C_{23}H_{22}O_{12}\cdot H_2O$, mp 177—178°, a new isoflavone glycoside, has been isolated from the rhizoma of *Iris florentina L.* (Iridaceae) together with iriflophenone (II), irisolone (IV), irisflorentin (I), iristectorigenin B (V), irigenin (VI) and iridin (VII). The structure of irifloside (III) has been determined as 5,4′-dihydroxy-3′-methoxy-6,7-methylenedioxyisoflavone-4′-O-β-D-glucoside by chemical degradation and spectral means, and its aglycone, $C_{17}H_{12}O_7$, mp 221—223°, was named iriflogenin (VIII).

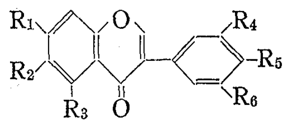
The structure of iriflophenone (II), $C_{13}H_{10}O_5$, mp 207—208°, has been determined as 2,4,6,4′-tetrahydroxybenzophenone by spectral means.

We have previously shown[1] that a new isoflavone, irisflorentin, was isolated from the ethereal extract of the rhizoma of *Iris florentina* L. and its structure was determined as 5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone.

As the continuation of this series, we now wish to report two new constituents, which have been named iriflophenone and irifloside, from the ethyl acetate extract of the rhizoma.

The ethyl acetate extract of the rhizoma was chromatographed on a silica gel column. Irisolone (IV),[1,3] irisflorentin (I),[1] iristectorigenin B (V),[1,4] and irigenin (VI)[1,5] were eluted from the column with a mixture of chloroform–methanol (100: 1). The remaining column was then eluted with a mixture of chloroform–methanol (20: 1) to give iriflophenone (II), irifloside (III) and iridin (VII).

Fig. 1



Compounds	mp (°C)	R_1	R_2	R_3	R_4	R_5	R_6
Irisflorentin (I)	175	$-O-CH_2-O-$		OMe	OMe	OMe	OMe
Irifloside (III)	177—178	$-O-CH_2-O-$		OH	OMe	O-Glu	H
Irisolone (IV)	271	$-O-CH_2-O-$		OMe	H	OH	H
Iristectorigenin B (V)	153	OH	OMe	OH	OMe	OH	H
Irigenin (VI)	185	OH	OMe	OH	OMe	OMe	OH
Iridin (VII)[5]	208	O-Glu	OMe	OH	OMe	OMe	OH
Iriflogenin (VIII)	221—223	$-O-CH_2-O-$		OH	OMe	OH	H
Iriflogenin acetate (IX)	168—170	$-O-CH_2-O-$		OAc	OMe	OAc	H

Glu.; glucose

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[1] Part III: N. Morita, M. Arisawa, Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 600 (1973).

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

[3] K.W. Gopinath, A.R. Kidwai and L. Prakash, *Tetrahedron*, **16**, 201 (1961).

[4] N. Morita, M. Shimokoriyama, M. Shimizu and M. Arisawa, *Yakugaku Zasshi*, **92**, 1052 (1972).

[5] W. Baker, *J. Chem. Soc.*, **1928**, 1022.

The combustion analysis of iriflophenone (II), mp 207—208°, showed the empirical formula $C_{13}H_{10}O_5$, which was confirmed by mass spectral measurement (M^+ 246). The ultraviolet (UV) absorption spectrum exhibited maxima at 290 nm (sh) (log ε 4.12) and 319 nm (log ε 4.27). The infrared (IR) spectrum displayed absorption bands at 3200 cm^{-1} (hydroxyl), 1635 cm^{-1} (carbonyl) and 1600 cm^{-1} (aromatic). The IR spectrum together with the positive color test for ferric chloride clearly indicated that II was a phenolic ketone.

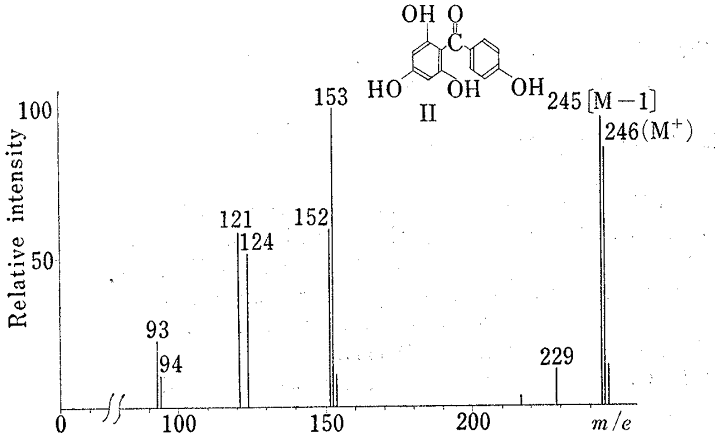

Fig. 2. Mass Spectrum of Iriflophenone (II)

II gave a trimethylsilyl derivative which was sufficiently soluble in carbon tetrachloride. The nuclear magnetic resonance (NMR) spectrum of this trimethylsilyl ether exhibited a singlet (2H) at 5.78 ppm and a pair of doublet (J=9.0 Hz, each 2H) at 6.65 and 7.55 ppm, indicating that the aromatic rings were the symmetric tetrasubstituted and *p*-substituted.

II gave a tetraacetate, mp 115—116°, on usual acetylation. The tetraacetate showed each two acetyl signals at 1.90 and 2.29 ppm. Thus the structure of II was proved to be 2,4,6,4′-tetrahydroxybenzophenone.[6] The mass spectrum (Fig. 2) of II is consistent with the assigned structure. The fragmentation pattern was given in Chart 1. Facile homolysis of the hydroxyl group giving rise to

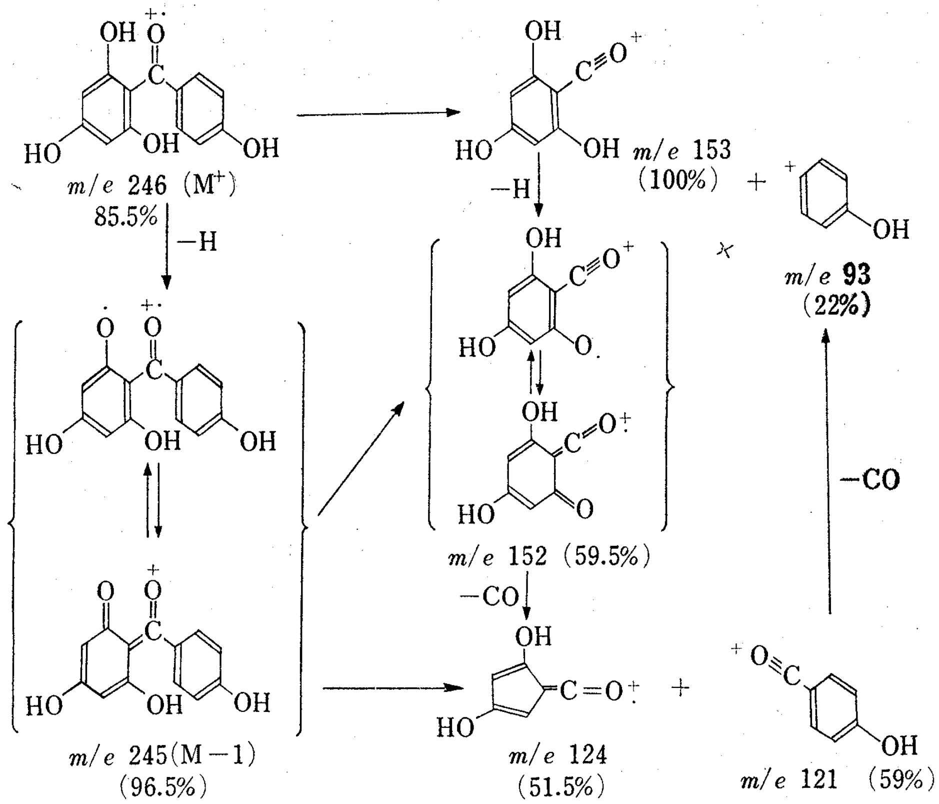

Chart 1. Fragmentation in Mass Spectrum of Iriflophenone (II)

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[6] H. Nishizawa and R. Robinson, *J. Chem. Soc.*, 121, 839 (1922).

the M-1 peak might be due to stabilization of the resulting ion radical by electron transfer. II was first isolated as a plant constituent in this time.

Irifloside (III), mp 177—178°, gave positive color test for the Gibbs reagent,[7] and analyzed for $C_{23}H_{22}O_{12}\cdot H_2O$ by combustion analysis. The UV absorption spectrum showed maxima at 251 nm (sh) (log ε 4.34), 274 nm (log ε 4.45) and 335 nm (log ε 3.65). The UV maxima changed at 249 nm (log ε 4.27) and 283 nm (log ε 4.43) by addition of aluminum chloride. The IR spectrum exhibited bands at 3400 cm^{-1} (hydroxyl), 1675 cm^{-1} (carbonyl), 1625 cm^{-1} (chelated carbonyl)[8] and 1575 cm^{-1} (aromatic). In the NMR spectrum of trimethylsilyl irifloside, a broad signal integrating six protons at 3.0—3.8 ppm and a doublet (1H, $J=6.5$ Hz) at 4.85 ppm were assigned the aliphatic protons and the anomeric proton[9] of the sugar moiety, respectively. A methoxyl group at 3.82 ppm (3H), a methylenedioxy group at 5.99 ppm (2H) and five ring protons at 6.44 (1H, assigned C_8-H[10] in flavonoids),[4,11] 6.89 and 7.14 ppm

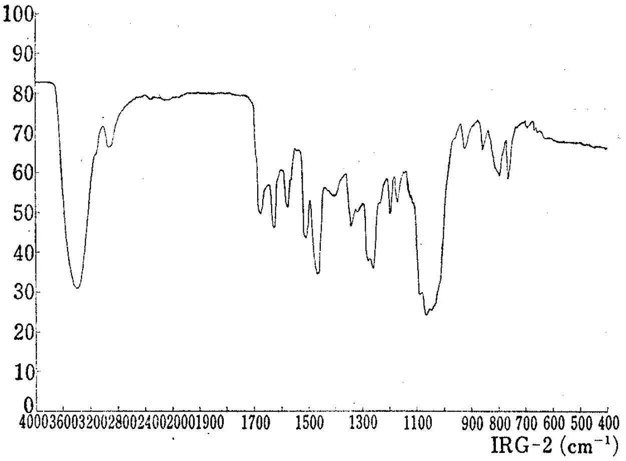


Fig. 3. IR Spectrum of Irifloside (III) (KBr)

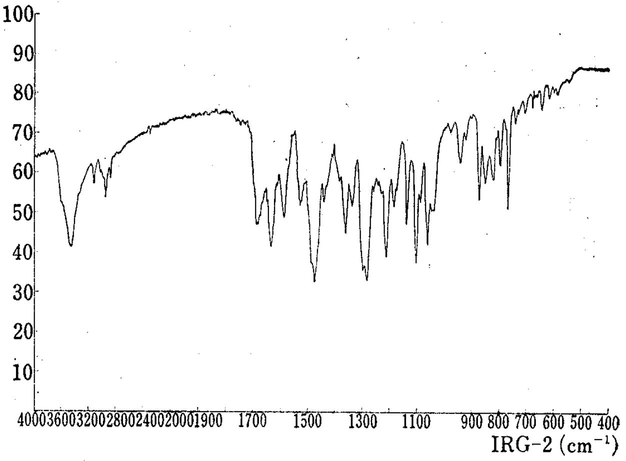
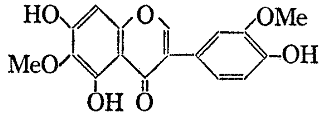
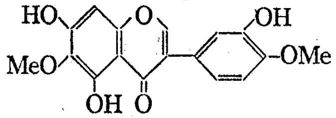


Fig. 4. IR Spectrum of Irifrogenin (VIII) (KBr)

Table I. Benzene-induced Solvent Shifts of $C_{3'}$- and $C_{4'}$-OMe

Compounds	δ (ppm)				Δ Values	
	$CDCl_3$ (a)	C_6D_6 (b)	CD_3OD (c)	CD_3OD $+C_6D_6$ (d)	$\delta_a-\delta_b$	$\delta_c-\delta_d$
 Iristectorigenin B	3.90 4.02[a]	3.17 3.62[a]	3.84 3.84[a]	3.61 3.87[a]	0.73 0.40[a]	0.23 −0.03[a]
 Iristectorigenin A[10]			3.87 3.87[a]	3.79 3.87[a]		0.08 0[a]
Iriflogenin (VIII)	3.91	3.32	3.86	3.61	0.59	0.25

[a] C_6-OMe, (CD_3OD: C_6D_6=1:1)

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[7] a) H.D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927); b) F.E. King, T.J. King and L.C. Manning, *J. Chem. Soc.*, **1957**, 563. The UV spectrum showed maxima at 628 nm.

[8] S. Baba, *Kagaku no Ryoiki, Suppl.*, **45**, 1 (1961).

[9] T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, N.Y., 1970, p. 261.

[10] The positive color reaction for Gibbs reagent together with the bathochromic shifts ($AlCl_3$), indicated the absence of a substituent at 8-position.

[11] N. Morita, M. Shimokoriyama, M. Shimizu and M. Arisawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 730 (1972).

(3H, complex two peaks) and 7.68 ppm (1H, assigned C_2-H in isoflavonoids)[9] indicated that III is an isoflavonoid. III was hydrolyzed on refluxing with 10% methanolic hydrochloric acid to give an aglycone, iriflogenin (VIII) and a sugar part. The sugar moiety was determined as glucose by direct paper chromatography and gas chromatography after preparation of its trimethylsilyl ether.

VIII, mp 221—223°, $C_{17}H_{12}O_7$, gave a diacetate (IX), mp 168—170°. When VIII was oxidized with hydrogen peroxide in an alkaline medium, vanillic acid was isolated as the acidic fragment and identified by comparison of the paper chromatographic behavior. The benzene-induced solvent shifts[12] of VIII in comparison with those of iristectorigenin B and iristectorigenin A[11] exhibited the Δ values of C_3'-OMe (Table I) and that agreed with the chemical proof. Moreover, the solvent effects of methoxyl group[13] in VIII-permethylate at the 5-position agreed with that of irisflorentin.[1]

The position of another hydroxyl group was evidenced at C-5 follows. It has been reported[14] that the 5-hydroxyl of isoflavone derivatives which has the intramolecular hydrogen bond with carbonyl group shows the bathochromic shift in the UV spectrum on addition of aluminum chloride. The UV maximum (λ_2) of VIII exhibited the shift to longer wavelength on addition of aluminum chloride.

In the fragmentation pattern of diacetate (IX), ketene elimination twice gave rise to VIII as a base peak, which further cleaved to retro-Diels-Alder ion,[15] *m/e* 148 and *m/e* 180 (Chart 2).

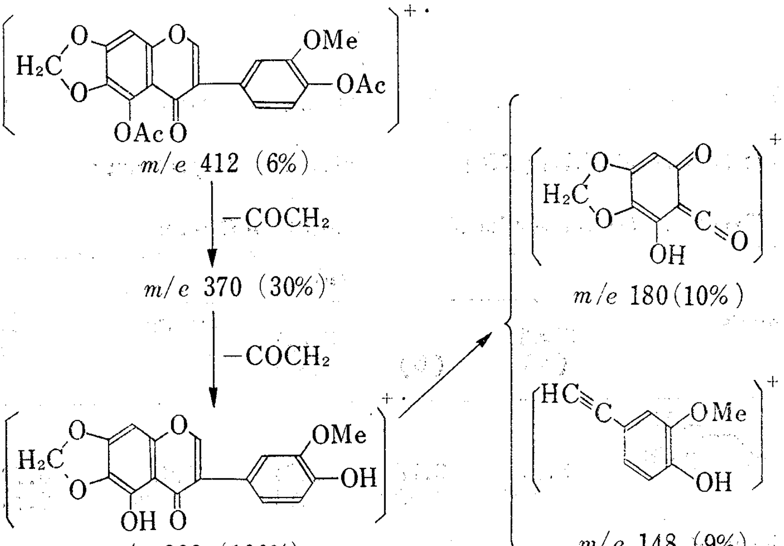

Chart 2. Fragmentation in Mass Spectrum of Iriflogenin Diacetate (IX)

From above results the structure of VIII is represented as 5,4'-dihydroxy-3'-methoxy-6,7-methylenedioxyisoflavone.

The bathochromic shifts in the UV spectrum of both III and VIII on addition of aluminum chloride and this together with the positive color test for the Gibbs reagent[7] clearly indicated the glycosyl linkage shoud be bonded at C-4'. The configuration of the sugar moiety of III

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12) R.G. Wilson, J.H. Bowie and D.H. Williams, *Tetrahedron*, 24, 1407 (1968).

13) A. Pelter and P.I. Amenechi, *J. Chem. Soc.* (*C*), 1969, 887.

14) T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, N.Y., 1970, p. 171.

15) Q.N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," John Wiley and Sons, New York, N.Y., 1971, p. 171.

was determined by means of NMR spectrum. The anomeric proton of the β-linked sugar has a diaxial coupling with the C-2$''$ proton and appears as a doublet with a coupling constant of approximately 7 Hz.[9] In the NMR spectrum of trimethylsilyl irifloside, the anomeric proton signal showed a doublet ($J=6.5$ Hz) at 4.85 ppm which can be attributed to the β-configuration.

Consequently, the structure of irifloside (III) was determined as 5,4′-dihydroxy-3′-methoxy-6,7-methylenedioxyisoflavone-4′-O-β-D-glucoside.

Experimental[16]

Isolation of Irisolone (IV), Irisflorentin (I), Iristectorigenin B (V), Irigenin (VI), Iriflophenone (II), Irifloside (III) and Iridin (VII)——14.5 kg of the fresh rhizoma of *Iris florentina* L. was chopped and extracted with MeOH. Evaporation of MeOH gave a viscous oily residue which was extracted with ether and then ethyl acetate. The combined extract with AcOEt was concentrated and chromatographed on a silica gel column. The column was eluted with a mixture of $CHCl_3$–MeOH (100: 1) to afford IV, I, V and VI, and subsequently with a mixture (20: 1) to afford II, III and VII.

Iriflophenone (II) was recrystallized from H_2O to give yellow prismes, mp 207—208° (lit. mp 210°),[6] purplish brown to $FeCl_3$. Mg+HCl; Orange, Gibbs reac.; (+). *Anal.* Calcd. for $C_{13}H_{10}O_5$: C, 63.41; H, 4.07. Found: C, 63.67; H, 4.12. Mass Spectrum *m/e*: 246 (M^+). UV λ_{max}^{EtOH} nm (log ε): 215 (sh) (4.32), 290 (sh) (4.12), 319 (4.27). IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1635 (C=O), 1600 (C=C). NMR (TMS ether of II, 10% solution in CCl_4) δ: 5.78 (2H, singlet, aromatic H×2), 6.65 (2H, doublet, $J=9.0$ Hz, aromatic H×2), 7.55 (2H, doublet, $J=9.0$ Hz, aromatic H×2).

II Tetraacetate——To a solution of II in pyridine was added acetic anhydride. After heating on a water bath for 10 hr, the reaction mixture was worked up as the usual manner. Recrystallization from MeOH gave colorless needles, mp 115—116°, no color to $FeCl_3$. NMR (10% solution in $CDCl_3$) δ: 1.90 (6H, singlet, $OCOCH_3\times2$), 2.29 (6H, singlet, $OCOCH_3\times2$), 6.99 (2H, singlet, aromatic H×2), 7.15 (2H, doublet, $J=9.0$ Hz, aromatic H×2), 7.81 (2H, doublet, $J=9.0$ Hz, aromatic H×2). Mass Spectrum *m/e*: 414 (M^+), 372, 246, 245, 153, 152, 124, 121, 94, 93.

Irifloside (III) was recrystallized from MeOH to give colorless needles, mp 177—178°, purplish brown to $FeCl_3$, green color to the Labat test[17] for the methylenedioxy group. The acid hydrolyzate reduced the Fehling reagent. Mg+HCl; (−), Gibbs reac.; (+). *Anal.* Calcd. for $C_{23}H_{22}O_{12}\cdot H_2O$: C. 54.33; H, 4.72. Found: C, 54.18; H, 4.98. UV λ_{max}^{EtOH} nm (log ε): 251 (sh) (4.34), 274 (4.45), 335 (3.65). UV $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 249 (4.27), 283 (4.43). IR ν_{max}^{KBr} cm^{-1}: 3400, 1675, 1625, 1575, 1510, 1470. NMR (TMS ether of III, 10% solution in CCl_4) δ: 3.0—3.8 (6H, broad, aliphatic H×6), 3.82 (3H, singlet, OCH_3), 4.85 (1H, doublet, $J=6.5$ Hz, anomeric H), 5.99 (2H, singlet, $\begin{smallmatrix}O\\O\end{smallmatrix}\!>\!CH_2$), 6.44 (1H, singlet, C_8-H), 6.89 and 7.14 (3H, complex two peaks, aromatic H×3), 7.68 (1H, singlet, C_2-H).

Iriflogenin (VIII) (Hydrolysis of Irifloside (III))——A solution of III in 10% HCl (MeOH) was refluxed on a water bath for 5 hr. The reaction mixture was evaporated to dryness under reduced pressure. The residue was washed with H_2O and recrystallized from MeOH to give colorless needles, mp 221—223°, purplish brown to $FeCl_3$, green color to the Labat test[17] for methylenedioxy group. Mg+HCl; (−), Gibbs reac.; (+). *Anal.* Calcd. for $C_{17}H_{12}O_7$: C, 62.20; H, 3.66. Found: C, 62.48; H, 3.55. UV λ_{max}^{EtOH} nm (log ε): 251 (sh) (4.35), 274 (4.47), 335 (3.65). UV $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 248 (4.28), 284 (4.44). NMR (3 mg of VIII in 0.5 ml of $CDCl_3$)[18] δ: 3.91 (3H, singlet, OCH_3), 6.06 (2H, singlet, $\begin{smallmatrix}O\\O\end{smallmatrix}\!>\!CH_2$), 6.46 (1H, singlet, C_8-H), 6.92—7.12 (3H, multiplet, aromatic H×3), 7.85 (1H, singlet, C_2-H). The water soluble part of the residue was evaporated to dryness *in vacuo* and allowed to stand with a few drops of TMS-imidazole over night. The TMS ether of the sugar portion was determined by gas–liquid chromatography: t_R 12.4 min (TMS ether of glucose 12.4 min, TMS ether of galactose 11.2 min; Shimazu Gas Chromatograph Model GC-1C equipped with a hydrogen flame ionaization detector, stainless steel column, 2 m long, 4 mmϕ packed with 5% SE-30 on Cerite (80—100 mesh), injector temp. 250°, column temp. 180°, detector temp. 250°, nitrogen gas flow rate 33 ml/min).

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[16] 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 Hitachi H-60 spectrometer. Chemical shifts were recorded as δ values (ppm) with TMS internal standard. Mass spectra were determined with a Hitachi mass spectrometer RMU-7 at electron energy 80 eV.

[17] M.A. Labat, *Bull. Soc. Chim.*, 5 (4), 745 (1909).

[18] Recorded on 100 Mc.

VIII Diacetate (IX)——To a solution of VIII in pyridine was added acetic anhydride. The reaction mixture was allowed to react on a water bath for 10 hr. The reaction mixture was worked up as the usual manner. Recrtstallization from MeOH gave colorless needles, mp 168—170°, no color to $FeCl_3$. NMR (3 mg of IX in 0.5 ml of $CDCl_3$)[18] δ: 2.26 (3H, singlet, $OCOCH_3$), 2.39 (3H, singlet, $OCOCH_3$), 3.79 (3H, singlet, OCH_3), 6.01 (2H, singlet, $\genfrac{}{}{0pt}{}{O}{O}\!>\!CH_2$), 6.66 (1H, singlet, C_8-H), 6.70—6.74 (3H, multiplet, aromatic H × 3), 7.66 (1H, singlet, C_2-H). Mass Spectrum *m/e*: 412 (M^+), 370, 328, 180, 148.

Oxidation of VIII with H_2O_2——To a solution of VIII in 5% KOH was added 3% H_2O_2 under the mechanical stirring and allowed to stand overnight at room temperature. After decomposition of excess H_2O_2 with MnO_2, the reaction mixture was acidified with dil. HCl and extracted with ether. The ethereal residue was examined by P.P.C.: *Rf* 0.38 (toluen: formic acid: ethyl formate = 5: 4: 1, vanillic acid 0.38, isovanillic acid 0.23. Color reaction with Diazo reagent; orange.)

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