mμ : 249, 278. *Anal.* Calcd. for $C_{18}H_{29}O_8NS_2$: C, 47.88; H, 6.47; N, 3.12. Found : C, 47.92; H, 6.44; N, 3.22. The product did not show mixed m.p. depression with the product prepared by a).

Methyl 2,3,4-Tri-O-acetyl-6-S-acetyl-6-deoxy-6-thio-β-D-glucopyranoside (XXXI)——a) A mixture of XXVIII (2 g.) and MeONa in dry MeOH (50 ml.) containing Na (1 g., 7 mole) was refluxed for 13 hr. After cooling, the solvent was removed to afford a sirup which acetylated with pyridine (20 ml.) and Ac_2O (20 ml.) at 0°. After standing at room temperature for 15 hr., the mixture was treated as described in XXIX a) to give a sirup. It was dissolved in benzene and chromatographied on silica gel (50 g.). Elution was performed using benzene, 5% ether-benzene (v/v) and ether, successively. The ether-effluent was evaporated to give a sirup which dissloved in small amount of warm ether. Petr. ether was added to give a slight turbidity and left in a refrigerator to induce crystallization. The resulting crystalline mass was collected by filtration and recrystallized from ether-petr. ether to give pure material (1 g., 40%), m.p. 94.5°, $[\alpha]_D^{20}$ −24° (c=1.05, $CHCl_3$), IR λ_{max}^{Nujol} μ : 5.9 (-SAc). *Anal.* Calcd. for $C_{15}H_{22}O_9S$: C, 47.62; H, 5.86; S, 8.47. Found : C, 47.62; H, 5.99; S. 8.57.

b) A mixture of methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucopyranoside (2 g.), prepared in a fashion similar to that used by Compton[20] and AcSK (0.7 g., 1.3 mole) in dry Me_2CO (30 ml.) was refluxed for 6 hr. After cooling, the mixture was poured into ice-H_2O, extracted with $CHCl_3$, and the $CHCl_3$-layer washed with H_2O. Moisture was removed with Na_2SO_4, filtered and the filtrate evaporated to a sirup which chromatographed as described in a). From ether-effluent crystals (1 g., 63%), m.p. 94°, $[\alpha]_D^{20}$ −28°(c=1.05,$CHCl_3$) were obtained. *Anal.* Calcd. for $C_{15}H_{22}O_9S$: C, 47.62; H, 5.86. Found : C, 47.35; H, 6.00. The product was identical with that, prepared by a), in mixed m.p. and IR.

A part of elementary analyses was carried out by the Tokyo Laboratory, Kowa Co., Ltd. to all of whom the authors' thanks are due.

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UDC 581.19 : 582.936 : 547.97 : 615.89

32. Manki Komatsu, Tsuyoshi Tomimori, and Michiko Ito : Studies on the Constituents of *Swertia japonica*. I.*[1] On the Structures of Swertisin and Isoswertisin.

(*Research Laboratory, Taisho Pharmaceutical Co., Ltd.*[*2])

Swertisin, $C_{22}H_{22}O_{10}$, m.p. 243°(decomp.), was isolated in a pure state from the whole herb of *Swertia japonica* Makino (Gentianaceae), and identified as 6-C-β-D-glucopyranosylgenkwanin.

Isoswertisin, $C_{22}H_{22}O_{10}$, m.p. 295°(decomp.), was obtained by the acid-treatment of swertisin, and formulated as 8-C-β-D-glucopyranosylgenkwanin.

At the same time, it was found that they were interconvertible into each other, reminiscent of the interrelationship between vitexin and isovitexin.

(Received June 6, 1966)

Swertia japoncia Makino (Japanese name "Senburi") is a biennial herb of the family Gentianaceae, which is widely distributed in Japan, Korea, and China.

In 1927, swertisin was first isolated from the whole herb of this plant by Nakaoki,[1] who proposed the empirical formula $C_{13}H_{10}O_6 \cdot H_2O$ which was presumed a sort of flavonoid or xanthone compound. Subsequently, Asahina, *et al.*[2] revised the formula for swertisin to $C_{23}H_{24}O_{11}$. No further investigations, however, have been made.

It has now been found that crude swertisin, obtained from this plant by Nakaoki's procedure, consisted of swertisin and a small amount of two other flavonoid compounds,

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*1 Preliminary communications were published in Tetrahedron Letters, No. 15, 1611 (1966). A part of this work was reported at the Regular Meeting of Kanto Branch, Pharmaceutical Society of Japan, in Tokyo (December, 1965).

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1) T. Nakaoki : Yakugaku Zasshi, **47**, 144 (1927).

2) Y. Asahina, J. Asano, Y. Ueno : *Ibid.*, **62**, 22 (1942).

which were isolated in pure state on polyamide chromatography, *i.e.*, compound (A) (swertisin), m.p. 243° (decomp.); compound (B), m.p. 265° (decomp.); and compound (C), m.p. 237° (decomp.).

The present paper deals with the elucidation of the structures of swertisin and its acid-converted isomer (isoswertisin), which have now been established as I and I′ respectively. The details of compound (B) and (C) will be reported later by the authors.

Compound (A) (I), identified with authentic swertisin, was obtained in pale yellow powdery crystals, m.p. 243° (decomp.), and its analytical values suggested the formula $C_{22}H_{22}O_{10}$ containing one methoxyl group. It gave a greenish brown color with ferric chloride, and the reduction tests for flavonoid were positive. The ultraviolet (UV) spectrum of swertisin, maxima at 336 and 273 mμ, is very similar to those reported for a number of flavonoids of apigenin-type. It formed a hexa-O-acetate, m.p. 155~158°, $C_{22}H_{16}O_{10}(COCH_3)_6$, on acetylation, which gave negative ferric reaction indicating the existence of six hydroxyl groups. Methylation of I with diazomethane yielded di-O-methylswertisin, m.p. 302°, $C_{21}H_{17}O_7(OCH_3)_3$, which gave no colorlation with ferric chloride, and no significant bathochromic shift of UV absorption maxima by adding aluminum chloride. The methyl ether further formed its tetra-acetate, m.p. 150~155°, or tetra-*p*-nitrobenzoate, m.p. 236°, indicating that out of the six hydroxyls in swertisin, two are phenolic and the rest alcoholic.

Table I. Ultraviolet Absorption Spectra (λ_{max} mμ (log ε))

Solvent	I	I′
EtOH	273 (4.24)	271 (4.23)
	336 (4.32)	338 (4.30)
EtOH-$AlCl_3$	281 (4.22)	278 (4.20)
	304 (4.17)	306 (4.10)
	354 (4.32)	348 (4.26)
	380 (4.23)[a]	393 (4.13)
EtOH-NaOAc	273 (4.21)	271 (4.22)
	336 (4.22)	338 (4.23)
	400 (4.01)[a]	400 (3.85)[a]
EtOH-H_3BO_3-NaOAc	273 (4.21)	271 (4.23)
	336 (4.30)	338 (4.25)

a) Shoulder

Table II. Rf Values on Paper Chromatogram

	I	I′	vitexin	Products after treatment with 10% H_2SO_4 of I	of I′	of vitexin
Solv. 1	0.59	0.21	0.25	0.59	0.59	0.42
				0.21	0.21	0.25
Solv. 2	0.78	0.69	0.60	0.78	0.78	0.71
				0.69	0.69	0.60
Solv. 3	0.61	0.51	0.45	0.61	0.61	0.61
				0.51	0.51	0.45

Alkali fission of I afforded phloroglucinol monomethylether, *p*-hydroxybenzoic acid, and *p*-hydroxyacetophenone. Boiling I with hydriodic acid in phenol gave apigenin which was further characterized as its triacetate. Oxidation of di-O-methylswertisin with nitric acid yielded *p*-anisic acid. These data show that swertisin possesses a mono-O-methylapigenin unit in the molecule. The presence of a 7-methoxyl group in

I was confirmed by comparing its UV spectrum with those in the presence of sodium acetate (no change) and of aluminum chloride (bathochromic shift of 44 mμ).[3]

These results indicated that swertisin contained a genkwanin unit, with a $C_6H_7(OH)_4$ moiety attached to the nucleus.

On the other hand, swertisin also found to be non-glycosidic because of its negative Molisch reaction and non-formation of sugar even after drastic treatment with mineral acid, and so it was suggested that swertisin might be a C-glycosyl compound. The estimation of periodate consumed for the oxidation of di-O-methylswertisin resulted in an uptake of two moles of the oxidant within five hours with the formation of one mole of formic acid. On ozonolysis of swertisin, D-glucose and D-arabinose were produced which were determined by paper and thin-layer chromatography. These results show that the C_6-moiety in swertisin is D-glucose in the form of a pyranose.

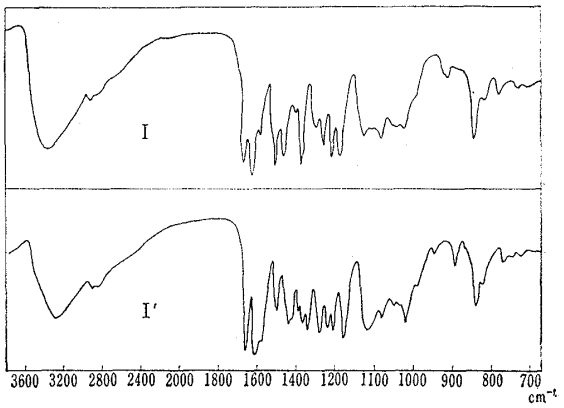

Fig. 1. Infrared Absorption Spectra of I and I' (in KBr)

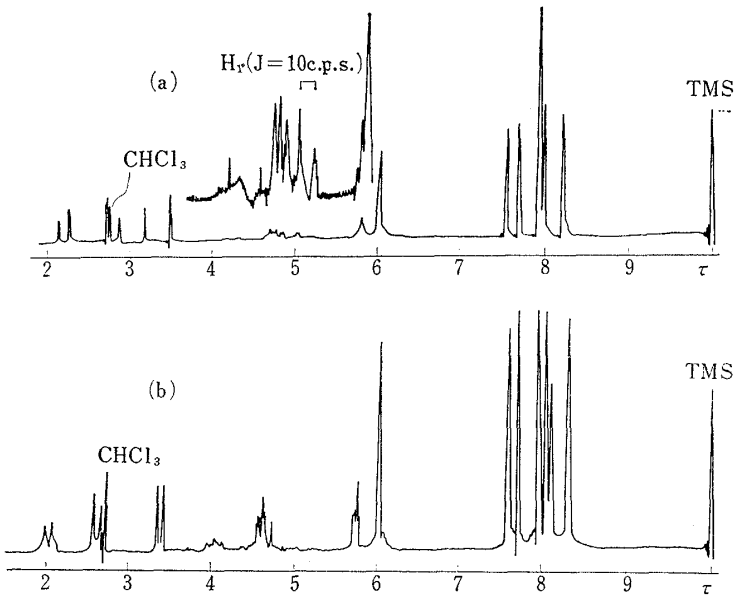

Fig. 2. Nuclear Magnetic Resonance Spectra of (a) and (b) in $CDCl_3$

a) Hexa-O-acetylswertisin, measured at 60 Mc.p.s.
b) Hexa-O-acetyliso-swertisin, measured at 100 Mc.p.s.

Further, hydrolytic decomposition of di-O-methylswertisin with aqueous barium hydroxide gave *p*-methoxyacetophenone and a degradation product, $C_{14}H_{16}O_7(OCH_3)_2$ (II), which was a fragment corresponding to A-ring and formed penta-acetate, m.p. 169~170°, $C_{14}H_{11}O_7(OCH_3)_2(COCH_3)_5$ (III). With the excess of aqueous periodic acid followed by reduction, II gave rise to 3-methyl-6-hydroxy-2,4-dimethoxyacetophenone (2,4-dinitrophenyl-hydrazone, m.p. 205°) which was identified by comparing it with a synthetic specimen starting from phloroglucinol.[4,5]

From these results, it is evident that the glucopyranosyl residue in swertisin is present in the 6-position of the genkwanin nucleus. This conclusion is also supported by the fact that swertisin gives a positive Gibbs indophenol test. Moreover, it is supported by nuclear magnetic resonance (NMR) studies that the glucopyranosyl residue must have β-configuration. As shown in Fig. 2, in the spectrum of hexa-O-acetylswertisin (34H), four proton signals display typical signal patterns for B-ring protons (AB type): $H_{2'6'}$, doublet (J=9 c.p.s.) at τ 2.25; $H_{3'5'}$, doublet (J=9 c.p.s.) at τ 2.85. Two

3) L. Jurd: "The Chemistry of Flavonoid Compounds," T. A. Geissman, Ed., 107 (1962). Pergamon Press, London.
4) K. Nakazawa, S. Matsuura: Yakugaku Zasshi, **73**, 751 (1953).
5) H. F. Birch, A. Robertson: J. Chem. Soc., **1938**, 306.

singlets at τ 3.23 (1H) and τ 3.55 (1H) could be assigned to the C_8 and C_3 proton respectively. A signal at τ 6.05 (3H) indicated the presence of one methoxyl group. A total of 18 protons is observed over the range τ 7.57~8.25, and these are attributable to the six acetyl groups. The signals over the range τ 4.0~6.0 account for the the seven protons of the glucosyl residue. One of these, a doublet centered at τ 5.10 is assigned to the $C_{1''}$ proton, the large coupling constant (J=10 c.p.s.) due to a *trans*-diaxial coupling with the $C_{2''}$ proton indicating a presence of β-configuration.

II was also proved to be identical with 3-C-β-D-glucopyranosyl-6-hydroxy-2,4-dimethoxyacetophenone by the NMR spectrum of its acetate, *i.e.*, four acetyls of glucopyranosyl group at τ 7.93 (6H), 7.96 (3H) and 8.20 (3H); two methoxyls at τ 6.15 (3H) and 6.22 (3H); Ar-O-Ac at τ 7.75 (3H); Ar-Ac at τ 7.48 (3H); benzenoid proton at τ 3.57 (1H); and the rest of seven protons at τ 4.0~6.0 indicated same signal patterns as those of β-glucopyranosyl residue in hexa-O-acetylswertisin.

Consequently, the structure of swertisin was established as 6-C-β-D-glucopyranosylgenkwanin.

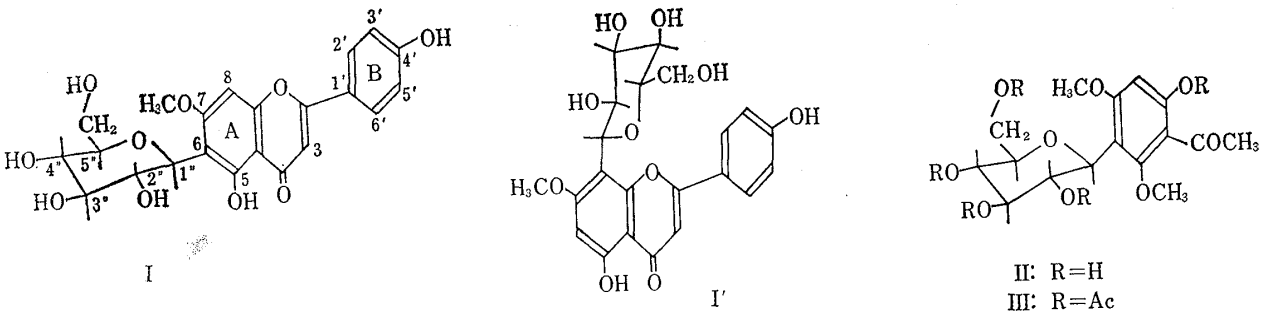

Chart 1.

In addition, treatment of swertisin under the usual hydrolytic condition yielded equilibrium mixture of which produced two spots on paper, one being identical in Rf values with the original swertisin, and the other, giving the lower Rf values, which was suggested to be the 8-C-isomer of swertisin due to a Wessely-Moser rearangement, considering the interconvertibilities between vitexin and iso-vitexin,[6,7] or orientin and homo-orientin.[8~10] The isomer, named isoswertisin, was obtained in pale yellow powdery crystals, m.p. 295° (decomp.), $C_{22}H_{22}O_{10}$. It gives a positive color reaction for flavonoid, and the UV spectrum is also very similar to that of swertisin. It gave di-O-methylisoswertisin, m.p. 290°, $C_{21}H_{17}O_7(OCH_3)_3$, which was identified as tri-O-methylvitexin, prepared from authentic vitexin, showing that isoswertisin might be mono-O-methylvitexin. The presence of a methoxyl group at 7 position in isoswertisin was confirmed by the same method as in the case of swertisin.

From these results, the structure of isoswertisin was established as 8-C-β-D-glucopyranosylgenkwanin. This conclusion is supported by the fact that swertisin gives a positive Gibbs reaction, while isoswertisin does not. The NMR spectra of hexa-O-acetyl swertisin and hexa-O-acetylisoswertisin also support the identification both of compounds as ring-isomeric C-glycosylflavones.

6) M. K. Seikel, T. A. Geissman : Arch. Biochem. Biophys., **71**, 17 (1957).
7) R. M. Horowitz, B. Gentili : Chem. Ind., **1964**, 498.
8) L. Hörhammer, H. Wagner, H. Nieschlag, G. Wildi : Arch. Pharmaz. Ber. dtsch. pharmaz. Ges., **292**, 380 (1959).
9) B. H. Koeppen : Z. Naturforsch., **19b**, 173 (1964).
10) *Idem* : Biochem. J., **97**, 444 (1965).

Experimental

All melting points were uncorrected. UV spectra were measured after Jurd[11,12] using a Hitachi Recording Spectrophotometer EPS-2U type. IR spectra were recoreded on a JASCO DS-301 spectrophotometer. NMR spectra were determined at 60 Mc. except for hexa-O-acetylisoswertisin in $CDCl_3$ solutions containing TMS as an internal standard using a JNM C-60 spectrophotometer.

Paper chromatography was carried out by the ascending method, using Toyo Filter Paper No. 50 and solvent systems of (1) 15% AcOH (solv. 1), (2) 60% AcOH (solv. 2), and (3) BuOH-AcOH-H_2O (4:1:5 by volume) (solv. 3).

Extraction and Isolation of Swertisin——The dried whole herb of *Swertia japonica* (6 kg.) was extracted 3 times with boiling MeOH and the extract concentrated to small volume. After removal of white precipitation (oleanolic acid and fatty matter), the filtrate was evaporated to dryness. The residue was dissolved in water, and then treated with ether to remove chlorophyll and swertianol. The aqueous solution was allowed to stand for a few days, saturated with ether. A mixture of flavonoids gradually deposited as pale yellow powders (25 g.) was dissolved in MeOH, and chromatographed on a column of Nylon powder (Polyamide Woelm. 2 kg.), using MeOH as an eluant. The eluate was collected in 25 ml. fractions, giving fractions 1 to 230. The faster-moving fractions (fr. 1 to 50) indicating only one spot at Rf 0.59 (solv. 1), 0.78 (solv. 2), 0.61 (solv. 3) on a paper chromatogram of the flavonoid, were combined and evaporated to dryness, giving swertisin, which was recrystallized from H_2O as pale yellow powdery crystals, m.p. 243°(decomp.), either alone or an admixture with authentic swertisin. Yield, 15 g. The IR spectrum was also found to be superimposable with that of swertisin. It gave following color reactions : $FeCl_3$(+), Mg-HCl (+), Zn-HCl (+), zircon-citric acid (−), Molisch (−), Gibbs (+), and Emerson (+). $[\alpha]_D^{20}$ −10.0 (C, 0.9, pyridine). *Anal.* Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97. Found : C, 59.62; H, 5.28.

Hexa-O-acetylswertisin——Swertisin (0.2 g.) was acetylated with Ac_2O and pyridine (3 hr. at 110°). The mixture was poured into iced water and allowed to harden. The amorphous acetate (0.3 g.) was recrystallized from $CHCl_3$-hexane. Yield : 0.1 g. of colorless prismatic needles, with a negative ferric chloride reaction, m.p. 155~158°. UV λ_{max}^{EtOH} mμ (log ε) : 260 (4.31), 308 (4.35). IR λ_{max}^{KBr} cm^{-1} 1750 ($COCH_3$); 1645 (conjugated CO); 1610, 1500, 1450 (aromatic C=C) : 1365, 1220 ($COCH_3$). *Anal.* Calcd. for $C_{34}H_{34}O_{16}$: C, 58.45; H, 4.91; OMe, 4.44. Found : C, 58.21; H, 4.70; OMe, 4.54.

Deacetylation of Hexa-O-acetylswertisin——The acetate (0.1 g.) was hydrolysed to free swertisin by suspending it in saturated $Ba(OH)_2$ solution (40 ml.). The mixture was allowed to stand overnight and acidified with dil. HCl to pH 5, giving a yellow solution, which was passed through a column of nylon powder, and then washed with H_2O. The absorbed matter was eluted with MeOH. Removal of the solvent from the eluate afforded swertisin in powdery crystals, m.p. and mixed m.p. 243°. Acetylation of this swertisin by Ac_2O-pyridine regenerated hexa-O-acetylswertisin, m.p. and mixed m.p. 155°.

Di-O-methylswertisin——A dry ethereal solution of CH_2N_2 generated from nitrosomethylurethane (20 ml.) was added to a MeOH solution of swertisin (1 g.) at 5°. The mixture was allowed to stand overnight. After removal of the solvent, the residue was washed with ether, and crystallized from MeOH, forming colorless needles (0.5 g.), m.p. 302°, with a negative ferric reaction, insoluble in cold aqueous NaOH solution. UV λ_{max}^{EtOH} mμ (log ε) : 263 (4.21), 320 (4.32). IR λ_{max}^{KBr} cm^{-1} : 3400 (OH); 1640 (conjugated CO); 1600, 1510, 1460 (aromatic C=C); 1360 (OCH_3). Rf values : 0.72 (solv. 1), 0.88 (solv. 2), 0.64 (solv. 3). *Anal.* Calcd. for $C_{24}H_{26}O_{10}$: C, 60.76; H, 5.52. Found : C, 60.87; H, 5.57.

Tetra-O-acetyl-di-O-methylswertisin——Acetylation of di-O-methylswertisin by Ac_2O-pyridine yielded the tetra-acetate which on crystallization from $CHCl_3$-hexane gave a white crystalline, m.p. 150~155°. $FeCl_3$(−). UV λ_{max}^{EtOH} mμ (log ε) : 263 (4.10), 320 (4.40). NMR (τ) : 8.25, 8.02, 8.00, 7.98 (OAc); 6.17, 6.14, 6.05 (OMe); 4.0~5.95 (multiplet, 7H); 3.45 (C_3-proton); 3.25 (C_8-proton); 3.03 (doublet, J=9 c.p.s., $C_{3'5'}$-proton); 2.25 (doublet, J=9 c.p.s., $C_{2'6'}$-proton). *Anal.* Calcd. for $C_{32}H_{34}O_{14}$: C, 59.81; H, 5.33; OMe, 14.49. Found : C, 59.38; H, 4.84; OMe, 14.70.

Di-O-methylswertisin tetra-*p*-nitrobenzoate——A mixture of di-O-methylswertisin (0.2 g.), *p*-nitrobenzoyl chloride (0.7 g.), and pyridine (2 ml.) was heated at 100° for 1 hr. and poured into 1% HCl solution. The solid was triturated with aqueous $NaHCO_3$, washed and crystallized from MeOH, giving the tetra-*p*-nitrobenzoate in colorless feathery needles, m.p. 236° with a negative ferric reaction. *Anal.* Calcd. for $C_{52}H_{38}O_{22}N_4$: N, 5.4. Found : N, 5.9.

Alkali Fission of Swertisin——A mixture of swertisin (0.2 g.) and 50% KOH solution (20 ml.) was refluxed in an atmosphere of N_2 for 2 hr. After cooling and dilution with H_2O, the reaction mixture was acidified with dil. H_2SO_4 and extracted with ether. The ether extract was fractionated by the usual method into a phenolic and an acidic fractions. The phenolic fraction was chromatographed on paper using diazotized sulfanilic acid as a spray reagent. Two spots were revealed on the paper chromatograms of the two

11) L. Jurd, R. M. Horowitz : J. Org. Chem., **22**, 1618 (1957).
12) L. Jurd : Arch. Biochem. Biophys., **63**, 376 (1956).

phenolic substances, the Rf values of which agreed with those of phloroglucinol monomethylether[13] and *p*-hydroxyacetophenone, respectively. From this fraction *p*-hydroxyacetophenone was separated on polyamide chromatography using MeOH as an eluant, identified as its 2,4-dinitrophenylhydrazone. The acidic fraction was extracted with ether and the extract was recrystallized from H_2O to prisms, m.p. 213°, which was identified as *p*-hydroxybenzoic acid by the comparison of IR spectra and mixed m.p..

Decomposition of Swertisin with Hydriodic acid——A mixture of swertisin (0.6 g.), phenol (10 ml.) and HI (12 ml. d, 1.7) was gently boiled under reflux for 8 hr. On working the reaction mixture following normal procedure, a brown solid was obtained which on crystallization from EtOH gave yellow prisms, m.p. 347~348°(decomp.). Admixture with authentic apigenin did not depress the melting point. Its acetate, prepared by the acetone-pyridine method, melted at 187°. *Anal.* Calcd. for $C_{21}H_{15}O_8$: C, 63.63; H, 4.07. Found : C, 63.36; H, 4.28.

Oxidation of Di-O-methylswertisin with HNO_3——A mixture of di-O-methylswertisin (0.1 g.), conc. HNO_3 (1.7 ml.), and H_2O (8.5 ml.) was heated under reflux for 1.5 hr., and the mixture was allowed to stand overnight. The solid that separated was filtered, recrystallized from MeOH, giving *p*-anisic acid, m.p. and mixed m.p. 182°, which was further identified by the comparison of IR spectra.

Ozonolysis of Swertisin——In the aqueous solution of swertisin (0.5 g. in 100 ml.) O_3 was bubbled for 7 hr. when the color of the solution changed to brown, gradually turning yellow. The solution was evaporated *in vacuo* and the residue was dissolved in MeOH. $Pb(OAc)_2$ and $Pb(OAc)_2 \cdot Pb(OH)_2$ solutions were added and the precipitate formed was filtered off. The excess of lead salt was removed by passing H_2S and the filtrate was concentrated. The concentrated solution was tested by paper chromatography using (a) BuOH-pyridine-H_2O (10:3:3), (b) solv. 3, and (c) phenol-H_2O (3:1), as the developing solvent systems, and aniline hydrogen phthalate as the reagent. Two sports appeared on the paper chromatogram and were identified as those of D-glucose and D-arabinose. Rf 0.37, 0.39 (a, glucose 0.37, arabinose 0.39); 0.43, 0.48 (b, glucose 0.43, arabinose 0.48); 0.36, 0.53 (c, glucose 0.36, arabinose 0.52). Thin-layer chromatography on silica gel (developer : AcOEt-iso-PuOH-H_2O=1:2:1. Reagent : anisaldehyde-sulfuric acid) afforded two spots at Rf 0.48 and 0.42 coincident with those of D-glucose and D-arabinose, respectively.

Estimation of Acid formed by the Oxidation of Di-O-methylswertisin——Di-O-methylswertisin (50.0 mg. or $1.05 \times 10^{-4}M$) was dissolved in EtOH (30 ml.) and 0.05*M*-$NaIO_4$ solution (20 ml.) was added. The mixture was allowed to stand in a dark place at 25~30°. A test solution (8.0 ml.) was added with an excess of ethylene glycol. After standing for 10 min., the solution was titrated with 0.01*N*-NaOH (f=1.197) using phenolphthalein as the indicator.

A blank test was carried out under the same condition.

HCOOH Formation

Time (hr.)	2	3.5	5	6	7	23
HCOOH (mole)	0.64	0.96	1.05	1.05	1.03	0.99

Estimation of the Consumption of $NaIO_4$ during Oxidation of Di-O-methylswertisin——To a solution of di-O-methylswertisin (50.0 g. or $1.05 \times 10^{-4}M$) in EtOH (20 ml.), 0.05*M*-$NaIO_4$ solution (20 ml.) was added and the mixture was allowed to stand in a dark place at 25°. A test solution (5 ml.) was added with saturated $NaHCO_3$ solution (10 ml.), standard 0.1*N*-Na_3AsO_3 solution (6.0 ml.), and 20% KI solution (1.5 ml.). After standing for 30 sec., the solution was titrated with 0.05*M*-I_2 solution using soluble starch solution as the indicator. A blank test was carried out under the same condition.

IO_4-Consumption

Time (hr.)	2	3	4	5	6	8	23
$NaIO_4$ (mole)	1.56	1.85	2.01	2.05	2.05	2.00	1.97

Hydrolytic Fission of Di-O-methylswertisin with $Ba(OH_2)$——Di-O-methylswertisin (1 g.) was boiled under reflux with saturated $Ba(OH)_2$ solution (150 ml.) in an atmosphere of N_2 for 3 hr. The reaction mixture was cooled, extracted with ether and the residue obtained after removal of ether was identified as *p*-methoxyacetophenone (as its 2,4-dinitrophenylhydrazone, m.p. 256°). The homogeneous alkaline liquor was acidified (pH 6.0) with dil. H_2SO_4, and evaporated *in vacuo* at 40°. The solid residue was extracted with boiling Me_2CO. Evaporation of the Me_2CO solution left a white amorphous powder (II). $FeCl_3$(+), Gibbs (−). Acetylated with Ac_2O-pyridine, it gave a penta-acetate which recrystallized from ether in colorless prisms (III), m.p. 169~170°, with a negative ferric reaction. *Anal.* Calcd. for $C_{14}H_{16}O_7(OCH_3)_2(COCH_3)_5$: C, 54.93; H, 5.67; OMe, 10.92. Found : C, 54.95; H, 5.90; OMe, 10.95.

3-Methyl-6-hydroxy-2,4-dimethoxyacetophenone——A solution of 6% aqueous HIO_4 (20 ml.) was added to II (0.4 g.) in AcOH (20 ml.), and the mixture agitated for 5 hr. at room temperature, diluted with H_2O (150 ml.), neutralized with a slight excess of $NaHCO_3$ and then kept for 18 hr. After removal of white deposit the aqueous liquors were extracted with ether. The ether extract was dried and evaporated, leaving a slight yellow product (0.2 g.), which was used for the Clemmensen reduction without further purification. A mixture of the oxidation product (0.2 g.), freshly prepared Zn-Hg (0.5 g.), AcOH (2 ml.), and conc. HCl

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(0.4 ml.) was boiled for 5 min., cooled, poured into H_2O (50 ml.) and extracted with ether. The ether extracts were washed with aqueous $NaHCO_3$, H_2O, dried and evaporated. Distillation of the residue *in vacuo* gave a pale yellow oil, b.$p_{0.2}$ 110~112°, which gave a violet coloration with $FeCl_3$. The 2,4-dinitrophenylhydrazone separated from benzene in brilliant red prisms, m.p. 205~206°, undepressed on admixture with a synthetic sample of 2,4-dinitrophenylhydrazone of 3-methyl-6-hydroxy-2,4-dimethoxyacetophenone,[4,5] which was further identified by the comparison of IR spectra.

Oxidation of Di-O-methylswertisin with $Pb(OAc)_4$——A mixture of di-O-methylswertisin (0.15 g.), $Pb(OAc)_4$, and AcOH (15 ml.) was kept at 25° for 4 days, poured into H_2O (100 ml.), and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with saturated aqueous $NaHCO_3$, dil. aqueous NaOH, and then H_2O, dried and evaporated, leaving a yellow product which gave 2,4-dinitrophenylhydrazone, orange red needles, m.p. 299~300°(decomp.), as recrystallized from AcOH. *Anal.* Calcd. for $C_{25}H_{20}O_9N_4$ (2,4-dinitrophenylhydrazone of 6-formyl-4′,5,7-trimethoxyflavone) : N, 10.60. Found : N, 10.24.

Oxidation of Di-O-methylswertisin with $NaIO_4$——0.2*M*-$NaIO_4$ (40 ml.) was added to a solution of di-O-methylswertisin (0.5 g.) in H_2O (1L.), and the mixture kept in the dark for 18 hr., filtered, and neutralized (phenolphthalein) with aqueous $Ba(OH_2)$ solution. On being evaporated to 50 ml. the filtered solution was extracted with $CHCl_3$. Evaporation of the dried extracts left a yellow solid which gave 2,4-dinitrophenylhydrazone, m.p. 299°(decomp.), undepressed on admixture with a sample prepared by the oxidation of di-O-methylswertisin with $Pb(OAc)_4$.

Isoswertisin (I′)——A mixture of swertisin (2 g.) and 10% H_2SO_4 solution (2 L.) was refluxed for 8 hr. The yellow solid (1 g.) obtained on cooling was fractionally crystallized from Me_2CO when the sparingly soluble swertisin separated first as a crystalline mass. The more soluble fraction was obtained by further concentration when yellow powder of isoswertisin separated. It was recrystallized from Me_2CO as pale yellow powdery crystals, m.p. 295°(decomp.). Yield, 0.2 g. It gave following color reactions : $FeCl_3$ (+), Mg-HCl (+), Zn-HCl (+), zircon-citric acid (−), Molisch (−), Gibbs (−), PPC (Table II). TLC on silica gel (developer : AcOEt-MeCOEt-HCO_2H-H_2O=5:3:1:1) afforded one spot at Rf 0.55 (cf. swertisin, Rf 0.4). *Anal.* Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97. Found : C, 58.84; H, 5.36.

Interconvertion of I and I′——Ten mg. each of I and I′ was respectively heated on an oil bath with 10% H_2SO_4 solution (30 ml.) for 7 hr. After cooling and dilution with H_2O, the reaction mixture was passed through a column of polyamide powder (2 g.). The column was washed with H_2O until the eluate was neutral. No sugar was detected in the passed solution. Subsequent elution of the column with MeOH afforded the mixture of flavonoid. In each case, two spots were revealed on the paper chromatograms of the two flavonoids (Table II), indicating the occurrence of I and I′ in each reaction mixture.

Hexa-O-acetylisoswertisin——Acetylation of I′ by Ac_2O-pyridine method yielded the hexa-acetate which on crystallization from $CHCl_3$-hexane gave a white crystalline having a negative ferric reaction, m.p. 134~136°. UV λ_{max}^{EtOH} mμ (log ε) : 260 (4.26), 310 (4.36). NMR spectrum of the acetate were run on a JNM-4H-100 spectrophotometer at 100 Mc.p.s. (Fig. 2). *Anal.* Calcd. for $C_{34}H_{34}O_{16}$: C, 58.45; H, 4.91. Found : C, 58.14; H, 5.08.

Di-O-methylisoswertisin (Tri-O-methylvitexin)——A dry ethereal solution of CH_2N_2 generated from nitrosomethylurethane (5 ml.) was added to a MeOH solution of I′ (0.2 g.). The mixture was maintained at 5° for 24 hr. with occasional shaking and finally at room temperature for a further 24 hr. After removal of the solvent, the residue was repeatedly methylated by the same method. The crude product was washed with ether, and crystallized from MeOH with activated charcoal, forming colorless needles, with a negative ferric reaction, m.p. 290°, which was identified as tri-O-methylvitexin, prepared from authentic vitexin, by the comparison of IR spectra and mixed m.p.. UV λ_{max}^{EtOH} mμ (log ε) : 267 (4.25), 328 (4.30). Rf values (blue fluorescence) : 0.58 (solv. 1), 0.80 (solv. 2), 0.43 (solv. 3). *Anal.* Calcd. for $C_{24}H_{26}O_{10}$: C, 60.76; H, 5.52. Found : C, 60.56; H, 5.60.

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