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Studies on Medicinal Resources. XXXII.[1] The Components of Rhizome of *Iris tectorum* MAXIMOWICZ (Iridaceae)

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Besides tectroridin, two components, one of which is novel, have now been isolated from *n*-butanol extract of the rhizome of *Iris tectorum* MAXIMOWICZ. The one (I), mp 210—214°, colorless microneedles, was idetntified as androsin (acetovanillon-β-D-glucoside). The other (II$_A$) $C_{23}H_{24}O_{12}$, mp 212—214°, was assumed to be 5,7,3′-trihydroxy-6,4′-dimethoxyisoflavone-7-β-D-glucoside. We proposed the names iristectorin A and iristectorigenin A for the glycoside and its aglycone, respectively.

Iris tectorum MAXIMOWICZ (Iridaceae) is a decorative and a medicinal plant. Its purplish blue or white flower opens in early summer. Though the rhizome of the plant named "Enbikon" has locally been used as an emetic or purgative drug for a long time, any other components have not been investigated but tectroridin.[3]

From the water-soluble part of the roots of this plant, four new components I, II$_A$, II$_B$ and IV have now been isolated. The present paper deals with the result of experiments carried out on the elucidation of the chemical structures of I, II$_A$ and III in Fig. 1. The fresh rhizomes were extracted with methanol and the methanolic extract was extracted with ether and then with ethyl acetate. The insoluble part was further extracted with *n*-butanol. Thin-Layer Chromatogram(TLC) of the *n*-butanolic extract is shown in Fig. 1, and five crystalline compounds which were provisinonally named I, II$_A$, II$_B$, III and IV were isolated by column chromatography. The first fraction gave I as cololress needles, mp 210—214°, and showed ultraviolet(UV) absorption maximum at 224, 268 and 302 mμ. Its infrared (IR) spectrum indicated the presence of hydroxyl group, carbonyl group and double bond in its molecule. I corresponded to the molecular formula of $C_{15}H_{20}O_8$. Hydrolysis of I with 10% sulfuric acid afforded an aglycone, mp 107—110°, in 48.3% yield, which corresponded to the molecular formula of $C_9H_{10}O_3$. The sugar portion was treated as usual and the sugar was identified as glucose by paper partition chromatography(PPC) and its osazone, mp 205°. The nuclear magnetic resonance (NMR) spectrum of trimethylsilyl (TMS) ether of I (in CCl_4), showed a complex multiplet for 2H centered at δ 7.54 ppm, and a doublet(J=9.0 cps) for 1H centered at 7.06 ppm which were attributable to protons on aromatic ring, respectively. Broad doublet (J=7.0 cps) (1H) centered at 5.02 ppm assignable to the anomeric proton of the glucoside linkage and the signals (6H) at 3.45—3.90 ppm to the other aliphatic protons on

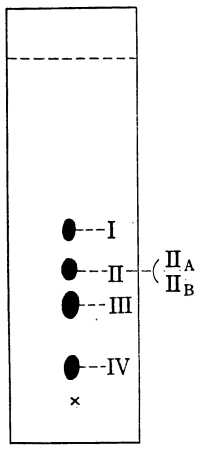


Fig. 1.

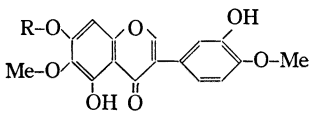
plate; Kieselgel G, solvent; $CHCl_3$:MeOH=5:1, coloring; H_2SO_4.

1) Part XXXI: N. Morita, M. Shimizu and S. Uchida, *Yakugaku Zasshi*, **88**, 1311 (1968).
2) Location: *a*) *Gohuku 3190, Toyama*; *b*) *Hongo 7-3-1, Bunkyo-ku, Tokyo.*
3) B. Shibata, *Yakugaku Zasshi*, **47**, 280 (1927); Y. Asahina, B. Shibata and Z. Ogawa, *Yakugaku Zasshi*, **48**, 1087 (1928).

glucose portion, respectively. Two signlets (3H each) at 3.94 and 2.51 ppm were attributed to a methoxy group and an acetoxy group, respectively. The aglycone of I showed a positive reaction to ferric chloride solution, absorption maximum of its UV spectrum at 228, 277 and 305 mμ, and the presence of hydroxyl group and carbonyl group in its IR spectrum. The NMR spectrum of the aglycone of I (in $CDCl_3$) showed a complex multiplet for 2H about 7.5 ppm, and a doublet (J=9.0 cps) for 1H centered at 6.96 ppm on aromatic ring, respectively. Two singlets (3H each) 3.93 and 2.53 ppm were attributed to a methoxy group and an acetoxy group, respectively.

Based on these facts mentioned above, the aglycone of I is concluded to be acetovanillon (4-hydroxy-3-methoxyacetophenon, apocynin).[4] The identification was established by direct comparison with the authentic sample of acetovanillon. Consequently, I is acetovanillon-β-D-glucoside, *i.e.*, androsin, mp 218—220°.[5]

The second fraction, colorless needles (II), gave two spots (II_A and II_B for the lower and upper spots) on PPC (5% acetic acid). II_A formed colorless silky needles, mp 212—214°, and showed absorption maximum of its UV spectrum at 268 (log ε=4.31) and 340 (log ε=3.10) mμ. Its IR spectrum indicated the presence of hydroxyl group, carbonyl group and double bond in its molecule. II_A corresponded to the molecular formula of $C_{23}H_{24}O_{12}$. Hydrolysis of II_A with 10% sulfuric acid afforded an aglycone, mp 231°, in 65.2% yield, which corresponded to the molecular formula of $C_{17}H_{14}O_7$. The sugar portion was treated by usual manner, and glucose was detected by PPC and its osazone, mp 205°. In these aspects mentioned above, II_A was expected as an isoflavone glucoside. The NMR spectrum of TMS ether of II_A (in CCl_4) showed two 1H singlets at 7.72 and 6.62 ppm, assignanle to the protons located at 2 and 8-position, and complex two peaks for 3H about at 7.14 and 6.81 ppm, assignable to 2′,5′ and 6′-position in isoflavone nucleus, respectively. 1H complex multiplet near 5.0 ppm, assignable to the anomeric proton of the glucoside linkage and the signals (6H) at 3.5—4.0 ppm to the other aliphatic protons on glucose portion, respectively. Two 3H singlets at 3.75 and 3.85 ppm were attributed to two methoxy groups. The aglycone of II_A showed absorption maximum of its UV spectrum at 268 and 340 mμ, and its IR spectrum indicated the presence of hydroxyl group and carbonyl group in its molecule. The NMR spectrum of TMS ether of the aglycone (in CCl_4) showed two 1H singlets at 7.71 and 6.46 ppm assignable to the proton located at 2 and 8-position, and complex two peaks for 3H at 7.15 and 6.82 ppm assignable to 2′,5′ and 6′-position in isoflavone nucleus, respectively. Two 3H singlets at 3.73 and 3.84 ppm were attributed to two methoxy groups. Oxidation of II_A with hydrogen peroxide afforded isovanillic acid, and by alkaline decompostion of the aglycone, iretol as phenol portion and isovanillyl acetic acid as acid portion were obtained. Terefore the aglycone has the structure of 5,7,3′-trihydroxy-6,4′-dimethoxyisoflavone, and II_A is monoglucoside of that.



R	name
glucose:	iristectorin A (II_A)
H :	iristectorigenin A (aglycone of II_A)

The proton signal at 8-position is NMR spectrum of TMS ether of isoflavones which contain the common 5,7-dihydroxy substitution pattern, usually appears in the range 6.3—6.5 ppm, while the signal for the 8-position is shifted downfield to the range 6.5—6.9 ppm when a sugar is attached to the oxygen at 7-position.[6]

Glucose commonly forms a β-linkage in flavonoid glycosides and the anomeric proton of the β-linked sugar has a diaxial coupling with the C_2 proton. Thus the anomeric proton

4) H. Finnemore, B. Sc, *J. Chem. Soc.*, **93**, 1513, 1520 (1908).
5) C.W. Moore, *J. Chem. Soc.*, **95**, 734 (1909).
6) T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids" Springer–Verlab, 1970, p. 261.

usually appears as a doublet with a coupling constant of about 7 cps. In flavonoid 7-O-glucosides, however, the anomeric proton does not appear as a sharp doublet but instead gives a complex multiplet.[7]

Based on these facts mentioned above, II_A is concluded to be 5,7,3′-trihydroxy-6,4′-dimethoxyisoflavone-η-β-D-glucoside. We propose the names iristectorin A and iristectorigenin A for II_A (glycoside) and its aglycone, respectively. III formed colorless needles, mp 256°, which were identified as tectoridin by direct comparison with an authentic sample. The structures of II_B and IV will soon be reported.

Experimental[8]

Extraction and Isolation——5 kg of the fresh rhizome was cut and extracted with MeOH, and the methanolic extract was extracted with ether and then with ethyl acetate. The insoluble part was further extracted with *n*-butanol, and the butanolic solution was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the mixture of glycosides were obtained as yellowish powder about 0.1% yield. The glycosides were separated by silicagel chromatography employing $CHCl_3$–MeOH mixture as the solvent system. The each eluted fraction recrystallized from MeOH, afforded I, II, III and IV, yields being 90, 360, 800, and 500 mg, when the solvent system are 24:1, 19:1, 14:1 and 9:1, respectively. II gave two spots, II_A and II_B on PPC (5% AcOH). When II was treated with MeOH, first II_A rystallized as colorless needles and when the filtrate of II_A was treated with activated charcoal and then concentrated, II_B crystallized as colorless needles.

Properties of Androsin (I)——Colorless microneedles, mp 210—214°. The dilute acid hydrolyzate reduced the Fehling reagent. PPC *Rf*; 0.96 (15% AcOH). UV λ_{max}^{EtOH} mμ (log ε); 224 (4.24), 268 (4.10), 302 (3.85). IR ν_{max}^{KBr} cm^{-1}; 3200—3600 (OH), 1660 (C=O), 1600 (C=C). *Anal.* Calcd. for $C_{15}H_{20}O_8$: C, 51.80; H, 5.84. Found: C, 52.01; H, 6.03. NMR (TMS ether of I, 10% solution in CCl_4) δ; 0.14 (9H, singlet, O-Si(CH_3)$_3$), 0.17 (27H, singlet, O-Si(CH_3)$_3\times3$), 2.51 (3H, singlet, CO–CH_3), 3.45—3.90 (6H, broad, aliphatic H in glucose$\times6$), 3.94 (3H, singlet, O-CH_3), 5.02 (1H, broad doublet, anomeric H in glusose), 7.06 (1H, doublet, J=9.0 cps, aromatic H), 7.54 (2H, complex multiplet, aromatic H$\times2$).

Acetovanillon (Hydrolysis of Androsin (I))——A solution of 70 mg of I in 10% H_2SO_4 was warmed on a water bath for 2 hr. The precipitated aglycone was collected, and recrystallized from benzene. Almost colorless prismatic needles, mp 114—115°, yielding 33.8 mg. PPC *Rf*; 0.775 (15% AcOH). UV λ_{max}^{EtOH} mμ (log ε); 229 (4.08), 277 (3.92), 305 (3.86). IR ν_{max}^{KBr} cm^{-1}; 3300—3400 (OH), 1670 (C=O), 1610 (C=C). *Anal.* Calcd. for $C_9H_{10}O_3$: C, 65.03; H, 6.07. Found: C, 65.12; H, 6.06. NMR (10% solution in $CDCl_3$), 2.35 (3H, singlet, CO–CH_3), 3.93 (3H, singlet, O-CH_3), 6.96 (1H, doublet, J=9.0 cps, aromatic H), 7.55 (2H, multiplet, aromatic H$\times2$). Its IR spectrum was found to be superimposable with that of the authentic specimen. After removal of the aglycone, the mother liquor was treated as usual. PPC *Rf*; 0.31 (*n*-butanol: AcOH: H_2O=4:1:2, glucose 0.31), 0.38 (*n*-butanol: pyridine: H_2O=6:4:3, glucose 0.38). Color reaction with 0.1N aniline hydrogen phthalate; reddish brown.

Properties of Iristectorin A(II_A)——Colorless needles, mp 212—214°, greenish brown to $FeCl_3$ and negative to reduction test for flavonoids. The dilute acid hydrolyzate reduced the Fehling reagent. TLC *Rf*; 0.52 ($CHCl_3$: MeOH=5:1). PPC *Rf*; 0.48 (5% AcOH), 0.74 (15% AcOH). UV λ_{max}^{EtOH} mμ (log ε); 268 (4.31), 340 (3.10). UV $\lambda_{max}^{EtOH+AlCl_3}$ mμ (log ε); 280 (4.31), 390 (3.14). UV $\lambda_{max}^{EtOH+AcONa}$ mμ (log ε); 268 (4.26), 340 (3.00). UV $\lambda_{max}^{EtOH+EtONa}$ mμ (log ε); 275 (4.17), UV $\lambda_{max}^{EtOH+H_3BO_3}$ mμ (log ε); 268 (4.26), 340 (3.07). IR ν_{max}^{KBr} cm^{-1}; 3300—3500 (OH), 1660 (C=O), 1620 (C=C). *Anal.* Calcd. for $C_{23}H_{24}O_{12}$: C, 56.08; H, 4.92. Found: C, 56.31; H, 5.08. NMR (TMS ether of II_A, 10% solution in CCl_4) δ; 0.02 (9H, singlet, O-Si(CH_3)$_3$), 0.17 (27H, singlet, O-Si(CH_3)$_3\times3$), 0.23 (9H, singlet, O-Si(CH_3)$_3$), 0.28 (9H, singlet, O-Si(CH_3)$_3$), 3.35—3.95 (12H, O-$CH_3\times2$ (3.75 and 3.85), aliphatic H in glucose$\times6$), 5.0 (1H, complex multiplet, anomeric H in glucose), 6.62 (1H, singlet, C_8-H), 6.81 and 7.14 (3H, complex two peaks, aromatic H$\times3$), 7.72 (1H, singlet, C_2-H).

Iristectorigenin A(Hydrolysis of Iristectorin A(II_A))——A solution of 100 mg of II_A in 10% H_2SO_4 was warmed on water bath for 2 hr. The aglycone that extracted with ether, was recrystallized from MeOH, yielding 65.2 mg. Colorless needles, mp 231°, greenish brown to $FeCl_3$ and negative to reduction test for flavonoids. PPC *Rf*; 0.515 (15% AcOH), 0.74 (30% AcOH). UV λ_{max}^{EtOH} mμ (log ε); 268 (4.33), 340 (3.41). UV $\lambda_{max}^{EtOH+AlCl_3}$ mμ (log ε); 278 (4.30), 380 (3.23). UV $\lambda_{max}^{EtOH+AcONa}$ mμ (log ε); 270 (4.28), 340 (3.60). UV $\lambda_{max}^{EtOH+H_3BO_3}$ mμ (log ε); 268 (4.31), 340 (3.45). IR ν_{max}^{KBr} cm^{-1}; 3300—3500 (OH), 1665 (C=O), 1625 (C=C). *Anal.* Calcd. for $C_{17}H_{14}O_7$: C, 61.80; H, 4.27. Found: C, 62.03; H, 4.49. NMR (TMS ether of iristectro-

7) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids" Springer-Verlab, 1970, p. 268.

8) All melting points were uncorrected. NMR spectrum; tetramethylsilan as an internal standard.

rigenin A, 10% solution in CCl_4) δ; 0.21 (9H, singlet, O-Si(CH_3)$_3$), 0.26 (18H, singlet, O-Si(CH_3)$_3$×2), 3.73 (3H, singlet, O-CH_3), 3.84 (3H, singlet, O-CH_3), 6.46 (1H, singlet, C_8-H), 6.82 and 7.15 (3H, complex two peakes, C_2'. C_5' and C_6'-H), 7.71 (1H, singlet, C_2-H). After removal of the aglycone, the mother liquor was treated as usual. PPC *Rf*; 0.31 (4:1:2, glucose 0.31), 0.38 (6:4:3, glucose 0.38). Color reaction with 0.1 N aniline hydrogen phthalate; reddish brown. The osazone was formed as yellow needles, mp 205°, undepressed on admixture with glucoosazone, mp 207°.

Acetylation of the aglycone with Ac_2O and H_2SO_4 in the usual manner gave its acetate as colorless needles, mp 206—208°, no color to $FeCl_3$. NMR (10% solution in $CDCl_3$) δ; 2.32 (3H, singlet, CO-CH_3), 2.37 (3H, singlet, CO-CH_3), 2.46 (3H, singlet, CO-CH_3), 3.85 (6H, singlet, O-CH_3×2), 6.97—7.15 (3H, C_2'. C_5' and C_6'-H), 7.18 (1H, singlet, C_8-H), 7.87 (1H, singlet, C_2-H).

Oxidation of II_A with H_2O_2——To a solution of II_A in 5% KOH was added 3% H_2O_2 and the mixture was allow to stand over night. After decomposition of excess H_2O_2 with MnO_2, the reaction mixture was acidified with dilute hydrochloric acid and extracted with ethyl acetate. The aqueous solution was submitted to PPC. PPC *Rf*; 0.63 (15% AcOH, isovanillic acid 0.63), 0.23 (toluen: formic acid: ethyl formate=5:4:1, isovanillic acid 0.23). Color reaction with Diazoreagent; orange red (isovanillic acid, orange red).

Alkali Decomposition of Iristectorigenin A——A mixture of 1 mg of iristectorigenin A, 1 g of KOH and 0.5 ml of H_2O, was boiled for 3 min. After cooling, the mixture was acidified with 5% H_2SO_4, and extracted with ether several times. The combined ethereal extract was washed with saturated $NaHCO_3$ solution several times. The alkali solution was acidified with 5% H_2SO_4 and extracted with ether.

Phenolic Portion: Color reaction with Diazo-reagent; orange red (iretol, orange red). PPC *Rf*; 0.615 (5% AcOH, iretol 0.615), 0.76 (15% AcOH, iretol 0.76).

Acid Portion: Color reaction with Diazo-reagent; orange (isovanillyl acetic acid, orange). PPC *Rf*; 0.77 (5% AcOH, isovanillyl acetic acid 0.77), 0.82 (15% AcOH, isovanillyl acetic acid 0.82).

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