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Hypotensive Principles of *Diospyros kaki* Leaves[1]

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The extract of a traditional Oriental medicine "kaki-yō," *Diospyros kaki* leaves, was fractionated, monitoring for hypotensive activity against urethane-anesthetized rats; astragalin and isoquercitrin were obtained as the active principles.

Keywords——*Diospyros kaki* leaves; Ebenaceae; astragalin; isoquercitrin; hypotensive activity

The crude drug "kaki-yō", prepared from the leaves of *Diospyros kaki* THUNBERG (Ebenaceae), has been utilized as a hypotensive drug in traditional medicine in Japan.

In 1956, Yamashita[3] examined the clinical utility of "kaki-yō" and noted that it exhibited hemostatic, diuretic, laxative and hypotensive effects with no appreciable side actions. Active principles were simultaneously investigated, and it was found that part of the activity was elicited by vitamin C, which is contained in large quantities in the leaves, and is known clinically to have hypotensive activity. However, the presence of other hypotensive principles was also suggested. Tannin,[4] carotin, cryptoxanthin, rutin, quercetin,[3] and astragalin[5] have been isolated from the crude drug "kaki-yō" in addition to vitamin C,[6] but none of them were thought to be hypotensive principles. In order to confirm the hypotensive activity of the crude drug, a methanol extract was subjected to examination for this pharmacological activity using urethane-anesthetized rats, and hypotensive action was indeed found. The present work was therefore initiated to identify the active principle.

The methanol extract of the crude drug was fractionated as indicated in Chart 1, monitoring the hypotensive activity. The extract was partitioned with water and ether, and the water layer was extracted with ethyl acetate. The acetate-soluble portion, which was physiologically active, was chromatographed twice on silica gel to afford an active fraction which exhibited two spots on thin-layer chromatography (TLC), one giving dark green coloration and the other dark brown coloration in the ferric chloride reaction. Since separation of the two substances could not be achieved efficiently by means of silica gel chromatography, it was accomplished by Sephadex LH-20 chromatography.

One active principle was found to be positive to the magnesium–hydrochloric acid reaction and the zinc–hydrochloric acid reaction and to exhibit maxima in its ultraviolet (UV) spectrum at 265, 295 (shoulder) and 348 nm, indicating that it was a flavonoid. On hydrolysis with dil. sulfuric acid it afforded kaempferol and glucose, and on acetylation it gave the heptaacetate, showing that it was a kaempferol monoglucoside. The displacements of the UV maxima on addition of sodium hydroxide, aluminum chloride, aluminum chloride–hydrochloric acid, sodium acetate, and sodium acetate–boric acid, when compared with known data, indicated it to be a kaempferol 3-glucoside. In the ^{1}H nuclear magnetic resonance (NMR) spectrum of the heptaacetate, the coupling constant of the doublet attributed to the

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1) Part XVII in the series on the validity of the Oriental medicines.
2) Location: *Aoba-yama, Sendai 980, Japan.*
3) H. Yamashita, *Fukuoka Igaku Zasshi*, 47, 824 (1956).
4) S.H. Hsu, H.L. Chang, and Y.W. Tien, *J. Chem. Eng. China*, 7, 26 (1940) [*Chem. Abstr.*, **36**, 2438 (1942)].
5) T. Nakaoki and N. Morita, *Yakugaku Zasshi*, **80**, 1298 (1960).
6) C.G. Vinson and F.B. Cross, *Science*, **96**, 430 (1942).

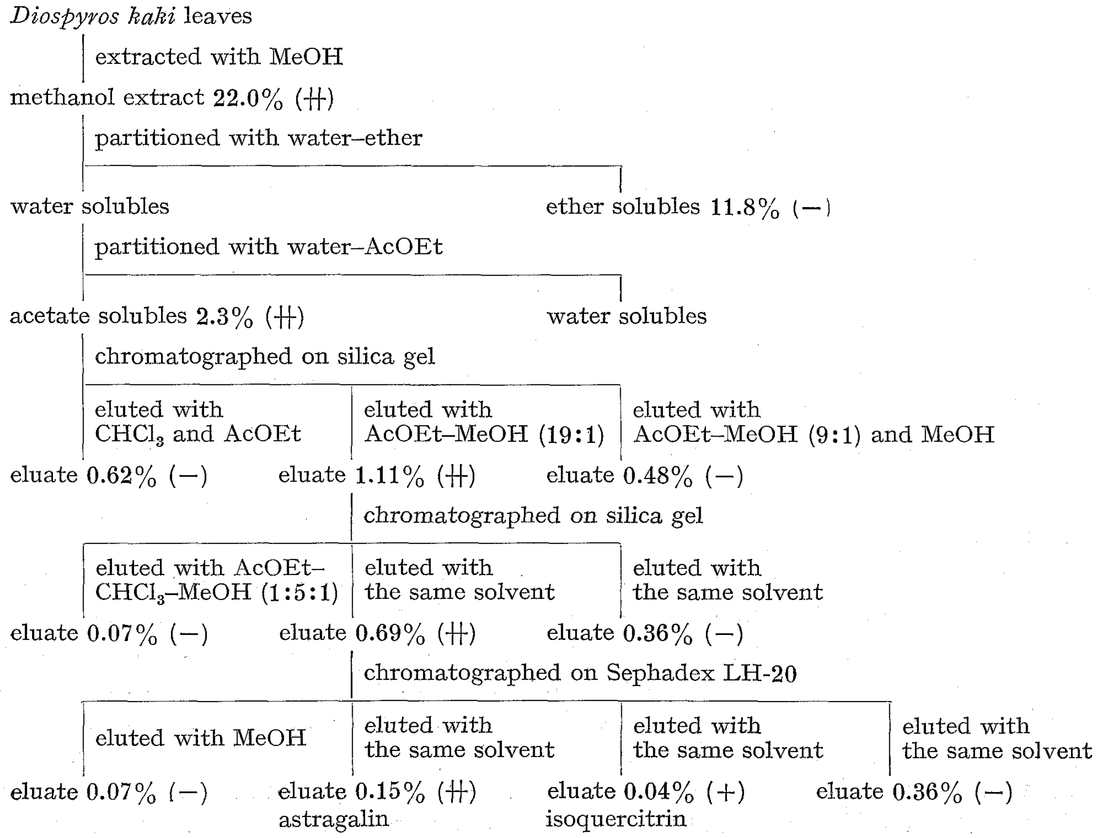

Chart 1. Flow Diagram of the Isolation of Astragalin and Isoquercitrin from *Diospyros kaki* Leaves[a]

[a] Yields (%) were calculated on the basis of the plant material, and scores in parentheses indicate the relative hypotensive activities of the fractions.

anomeric hydrogen was found to be 8 Hz, showing that glucose was present in a β-glucoside linkage. On the basis of the above evidence, the first principle was concluded to be kaempferol 3-β-glucoside. Its identity with astragalin was confirmed by direct comparison. The astragalin obtained was found by bioassay to show a hypotensive activity (2.0 mg/kg *i.v.*, 41 ± 18 mmHg fall, $n=4$).

Another active principle was also found to be positive to the magnesium–hydrochloric acid reaction and the zinc-hydrochloric acid reaction, and to display UV maxima at 256, 295 (shoulder) and 355 nm, again suggesting flavonoid nature. On hydrolysis with dil. sulfuric acid, quercetin and glucose were obtained, and acetylation yielded the octaacetate. Consequently, it was considered to be a quercetin monoglucoside. As in the case of astragalin, the shifts of the UV maxima on addition of various chelators as well as the coupling constant of the anomeric hydrogen signal in the ^{1}H NMR spectrum of the octaacetate demonstrated that this second principle was quercetin 3-β-glucoside. The melting point of a specimen crystallized from methanol remained at 181—185°, showing depression on admixture with an authentic sample of isoquercitrin, mp 237—239°. Subsequent crystallization of the specimen from ethanol–water (1: 1) raised the melting point to 235—236°, and no depression was seen on admixture with the authentic sample. Other data were also identical with those of the authentic sample. The isoquercitrin obtained was found by bioassay to exhibit hypotensive activity (2.0 mg/kg *i.v.*, 10 ± 3 mmHg fall, $n=3$).

Although astragalin has already been isolated from the same plant material,[5] this is the first time that isoquercitrin has been isolated from this genus, *Diospyros*.

As mentioned above, laxative and diuretic actions, in addition to the hypotensive action, have been reported as clinical effects of the crude drug "kaki-yō".[3] It is also known that glycosides of kaempferol and quercetin mediate the laxative and diuretic actions.[7] Therefore, the effects of the crude drug may be rationalized at least in part by the presence of the principles, astragalin and isoquercitrin, found in the present work in addition to the above compounds.

Experimental[8]

Isolation of Astragalin and Isoquercitrin from *Diospyros kaki* Leaves——The dried leaves of *Diospyros kaki* were extracted 3 times with cold MeOH for 1 day (each extraction). The combined MeOH solution was concentrated under reduced pressure to yield an extract, which was fractionated as shown in Chart 1.

In the last Sephadex LH-20 chromatography, elution with MeOH gave astragalin as pale yellow needles (from MeOH), mp 163—165°. UV $\lambda_{\max}^{\text{MeOH}}$ nm: 265, 295 (sh), 348; UV $\lambda_{\max}^{\text{MeOH-NaOH}}$ nm: 275, 324, 398; UV $\lambda_{\max}^{\text{MeOH-AlCl}_3}$ nm: 274, 304, 347, 396; UV $\lambda_{\max}^{\text{MeOH-AlCl}_3\text{,HCl}}$ nm: 274, 304, 345, 396; UV $\lambda_{\max}^{\text{MeOH-AcONa}}$ nm: 272, 303, 364; UV $\lambda_{\max}^{\text{MeOH-AcONa,H}_3\text{BO}_3}$ nm: 266, 298 (sh), 350. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3300, 1650, 1599. ^{1}H NMR (CD_3OD) δ: 6.23 (1H, d, $J=2$), 6.43 (1H, d, $J=2$), 6.92 (2H, d, $J=9$), 8.08 (2H, d, $J=9$); ^{1}H NMR (pyridine-d_5) δ: 6.73 (2H, s), 7.23 (2H, d, $J=9$), 8.50 (2H, d, $J=9$). Identity with an authentic sample was confirmed by comparison of the infrared (IR) spectra.

Successive elution with MeOH in the last Sephadex LH-20 chromatography afforded isoquercitrin as pale yellow needles (from MeOH and then from 50% aq. EtOH), mp 235—236°. UV $\lambda_{\max}^{\text{MeOH}}$ nm: 256, 295 (sh), 355; UV $\lambda_{\max}^{\text{MeOH-NaOH}}$ nm: 272, 322 (sh), 407; UV $\lambda_{\max}^{\text{MeOH-AlCl}_3}$ nm: 275, 304 (sh), 334 (sh), 431; UV $\lambda_{\max}^{\text{MeOH-AlCl}_3\text{,HCl}}$ nm: 268, 299 (sh), 355 (sh), 401; UV $\lambda_{\max}^{\text{MeOH-AcONa}}$ nm: 273, 318 (sh), 380; UV $\lambda_{\max}^{\text{MeOH-AcONa,H}_3\text{BO}_3}$ nm: 262, 379. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3250, 1644, 1593. ^{1}H NMR (CD_3OD) δ: 6.18 (1H, d, $J=2$), 6.36 (1H, d, $J=2$), 6.88 (1H, d, $J=8$), 7.58 (1H, dd $J=8$, 2), 7.74 (1H, d, $J=2$); ^{13}C NMR (CD_3OD) δ: 62.6, 71.2, 75.8, 78.1, 78.3, 94.8, 99.9, 104.5, 105.7, 116.0, 117.6, 123.1, 123.2, 135.7, 145.9, 147.7, 149.9, 158.4, 163.0, 166.0, 179.5. Identity with an authentic sample was confirmed by mixed melting point and comparison of the UV and IR spectra.

Acetylation of Astragalin——Astragalin was acetylated with Ac_2O–pyridine and crystallized from $CHCl_3$–MeOH to give astragalin heptaacetate as colorless needles, mp 204—207°. *Anal.* Calcd. for $C_{35}H_{34}O_{18}$: C, 56.60; H, 4.62. Found: C, 56.10; H, 4.42. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1740, 1620. ^{1}H NMR ($CDCl_3$) δ: 1.93 (3H, s), 2.00 (3H, s), 2.02 (3H, s), 2.12 (3H, s), 2.84 (6H, s), 2.96 (3H, s), 3.55 (1H, m), 3.95 (2H, m), 5.03 (1H, dd, $J=9$, 8), 5.13 (1H, dd, $J=8$, 8), 5.29 (1H, dd, $J=9$, 8), 5.54 (1H, d, $J=8$), 6.82 (1H, d, $J=2$), 7.20 (2H, d, $J=9$), 7.27 (1H, d, $J=2$), 8.03 (2H, d, $J=9$).

Acid Hydrolysis of Astragalin——Astragalin was hydrolyzed with 2.5% H_2SO_4 to give a yellow precipitate and the mother liquor.

The precipitate was crystallized from MeOH to give kaempferol as yellow needles, mp 260—264°. UV $\lambda_{\max}^{\text{MeOH}}$ nm: 266, 318 (sh), 365. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3275, 1657, 1610, 1570. ^{1}H NMR (CH_3OH) δ: 6.02 (1H, d, $J=2$), 6.21 (1H, d, $J=2$), 6.73 (2H, d, $J=9$), 7.90 (2H, d, $J=9$). Identification with kaempferol was performed by mixed melting point and comparison of the UV, IR, and ^{1}H NMR spectra.

The mother liquor was passed through Amberlite IRA-400 (OH^- type) and the eluate concentrated to give a pale brown syrup. Identification with glucose was performed by avicel TLC (*Rf* 0.43; pyridine–AcOEt–AcOH–H_2O (5:5:1:3)), and by gas–liquid chromatography (GLC) of the trimethylsilyl derivative.

Acetylation of Isoquercitrin——Isoquercitrin was acetylated with Ac_2O–pyridine to give isoquercitrin octaacetate as a colorless powder. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1774, 1753, 1629. ^{1}H NMR ($CDCl_3$) δ: 1.92 (3H, s), 2.00 (3H, s), 2.02 (3H, s), 2.12 (3H, s), 2.34 (3H, s), 2.36 (6H, s), 2.43 (3H, s), 3.60 (1H, m), 3.98 (2H, m), 5.20 (3H, m), 5.66 (1H, d, $J=8$), 6.81 (1H, d, $J=2$), 7.29 (1H, d, $J=2$), 7.31 (1H, d, $J=9$), 7.92 (1H, d, $J=2$), 7.96 (1H, dd, $J=9$, 2).

Acid Hydrolysis of Isoquercitrin——Isoquercitrin was hydrolyzed with 2.5% H_2SO_4 to yield a yellow precipitate and the mother liquor. The precipitate was crystallized from MeOH to give quercetin as yellow needles, mp 303—307°. UV $\lambda_{\max}^{\text{MeOH}}$ nm: 257, 371. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3300, 1660, 1601. Identification with quercetin was performed by mixed melting point and comparison of the UV and IR spectra.

The mother liquor was passed through Amberlite IRA-400 (OH^- type) and the eluate concentrated to give a pale brown syrup. Identification with glucose was performed as described above.

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8) Melting points are uncorrected. NMR spectra were determined at 100 MHz for ^{1}H and at 25 MHz for ^{13}C. The ^{13}C NMR spectrum was measured as described previously.[9] Chemical shifts (δ) are given in ppm from internal tetramethylsilane (TMS) and the coupling constants (J) in Hz. Abbreviations: s=singlet, d=doublet, m=multiplet, dd=doublet of doublets.

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Blood Pressure Measurement——The measurement was performed described as previously.[10] The blood pressures of the rats employed were 80—110 mmHg.

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Elucidation of the Structure of a New Lignan Glucoside from *Olea europaea* by Carbon-13 Nuclear Magnetic Resonance Spectroscopy

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The structure of a new lignan glucoside isolated from the stem of *Olea europaea* Linn. (Oleaceae) was elucidated as (+)-1-acetoxypinoresinol-4′-β-D-glucoside [(1*S*,2*R*,5*R*,6*S*)-1-acetoxy-2-(3′-methoxy-4′-hydroxyphenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane 4′-β-D-glucopyranoside] by analysis of the carbon-13 nuclear magnetic resonance and circular dichroism spectra.

Keywords——*Olea europaea* L.; Oleaceae; new lignan glucoside; (+)-1-acetoxypinoresinol-4′-β-D-glucoside; ^{13}C-NMR spectra; MS spectra; CD curves

During an examination of Oleaceae we have investigated the stem constituents of *Olea europaea* Linn., the fruits of which are a rich source of olive oil.

We report here the isolation of a new lignan glucoside (**1**) [(+)-1-acetoxypinoresinol-4′-β-D-glucoside], related to (+)-pinoresinol-β-D-glucoside[2] (**8**), and assign the structure based on carbon-13 nuclear magnetic resonance (^{13}C–NMR) and circular dichroism (CD) data.

The extraction was carried out as described in "Experimental".

Compound **1** was isolated from the chloroform extract as colorless needles, $C_{28}H_{34}O_{13}\cdot 1/2H_2O$, mp 183.5—185°, $[\alpha]_D^{22}$ +7.9° (ethanol).

Infrared (IR) absorption of **1** at 1735 cm^{-1} suggested the presence of one alcoholic acetoxyl, which was confirmed by the appearance of a signal at δ 1.67 in the proton nuclear magnetic resonance (PMR) spectrum.

Deacetylation of **1** with ammonia in methanol gave compound **3**, $C_{26}H_{32}O_{12}\cdot 1.5H_2O$, $[\alpha]_D^{23}$ −1.1° (ethanol) as an amorphous powder.

Acetylation of **1** with acetic anhydride–pyridine gave compound **7** as a colorless syrup, $[\alpha]_D^{20}$ −6.3° (ethanol), mass spectrum (MS) *m/e*, 788 [M$^+$].

The PMR spectrum of **7** showed the presence of five alcoholic acetoxyls (δ 1.67, 2.03, 2.10), one phenolic acetoxyl (δ 2.33), two aromatic methoxyls (δ 3.83, 3.87) and six aromatic protons (δ 6.73—7.27).

The ultraviolet (UV) spectrum of **1** showed absorption maxima at 231 and 279.5 nm. The bathochromic shift of the absorption maximum with sodium ethoxide was very similar to that of **8**, suggesting a pinoresinol-type lignan glucoside structure.

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[1] Location: *a*) *Ishikari-Tobetsu, Hokkaido, 061-02, Japan*; *b*) *Yamashiro-cho, Tokushima, 770, Japan.*

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