Studies on the Constituents of Japanese Mistletoes from Different Host Trees, and Their Antimicrobial and Hypotensive Properties

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The chemical constituents of Japanese mistletoes, *Taxillus yadoriki* DANSER, *Taxillus kaempferi* DANSER, and *Korthalsella japonica* ENGLER, epiphyting to different host trees were compared, and the antimicrobial and hypotensive properties of some isolated flavonoids were examined. Two known flavonoid glycosides, hyperin and quercitrin, were isolated from *Taxillus yadoriki* DANSER, together with fatty acids, phytosterol, and phytosterol-glucoside. There was remarkable variation of contents of quercitrin among the plants on different host trees. From *Taxillus kaempferi* DANSER, fatty acids, phytosterol, phytosterol-glucoside, quercetin, avicularin, and taxillusin were isolated, and quercitrin and hyperin were also identified. There was no remarkable variation of compositions of flavonoid glycosides among the plants on different host trees. A known flavone glycoside, chrysoeriol-4′-*O*-glucoside, was isolated from *Korthalsella japonica* ENGLER, together with fatty acids, phytosterol, oleanolic acid, and phytosterol-glucoside. Chrysoeriol-4′-*O*-glucoside is contained in this plant irrespective of the host trees.

Keywords mistletoe; Loranthaceae; flavonoid; antimicrobial activity; hypotensive effect; hyperin; quercitrin; avicularin; taxillusin; chrysoeriol-4′-*O*-glucoside

Japanese mistletoes (Loranthaceae) are classified into four genera: *Hyphear* DANSER, *Taxillus* VAN TIEGH, *Viscum* LINN., and *Korthalsella* VAN TIEGH.[1] The chemical constituents of *Viscum album* LINN. var. *coloratum* OHWI (yadorigi in Japanese) have been reported by several authors.[2,3] Ohta and Yagishita[2] reported the isolation and the structure determination of three new flavonoids, flavoyadorinin-A (rhamnazin-3-*O*-glucoside), flavoyadorinin-B (7,3′-di-*O*-methylluteolin-4′-*O*-glucoside) and homo-flavoyadorinin-B (7,3′-di-*O*-methylluteolin-4′-*O*-glucoapioside) from the leaves of this plant epiphyting to *Pyrus communis* LINN. Sakurai and Okumura[3] reported the isolation and identification of two new flavonoid glycosides, taxillusin ((2*R*, 3*R*)-taxifolin 3-*O*-glucoside 6″-gallate) and isoglucodistylin ((2*R*, 3*R*)-taxifolin 3-*O*-glucoside), and various known flavonoid constituents, quercetin, (+)-taxifolin, avicularin (quercetin-3-*O*-arabinofuranoside), quercitrin (quercetin-3-*O*-rhamnoside), hyperin (quercetin-3-*O*-galactoside), and guaijaverin (quercetin-3-*O*-arabinopyranoside) from *Taxillus kaempferi* DANSER (matsugumi in Japanese) epiphyting to *Pinus densiflora* SIEB. *et* ZUCC. But the chemical constituents of the other Japanese mistletoes have not been reported. In our previous investigation on the constituents of *Hyphear tanakae* HOSOKAWA (hozakiyadorigi in Japanese),[4] we reported on the isolation and the structural elucidation of a new triterpene fatty acid ester, a mixture of lup-20(29)-ene-7β,15α-diol-3β-palmitate, stearate, arachidate, behenate and lignocerate, and four know flavonoid glycosides, rhamnocitrin-3-*O*-rhamnoside, afzelin (kaempferol-3-*O*-rhamnoside), rhamnetin-3-*O*-rhamnoside, and quercitrin. The present paper describes the isolation and the structural elucidation of the constituents in the leaves and twigs of three Japanese mistletoes, *Taxillus yadoriki* DANSER (ohbayadorigi in Japanese), *Taxillus kaempferi* DANSER, and *Korthalsella japonica* ENGLER (hinokibayadorigi in Japanese), compares the constituents of these plants epiphyting to different host trees, and presents the antimicrobial and hypotensive properties of some isolated flavonoids.

The methanolic extract of *Taxillus yadoriki* DANSER epiphyting to *Quercus glauca* THUNB. was partitioned between *n*-hexane and water. The aqueous layer was further extracted with chloroform and *n*-butanol. Each extract was concentrated and subjected to silica gel column chromatography. We isolated fatty acids (I), phytosterol (II), and phytosterol-glucoside (III) from the hexane extract, and a known flavonoid glycoside, compound IV from the butanol extract. On the basis of the results of hydrolysis, the bathochromic shifts with diagnostic reagents (EtONa, $AlCl_3$, $AlCl_3$/HCl, and AcONa) in the ultraviolet (UV) spectra, and the infrared (IR), the proton nuclear magnetic resonance (^{1}H-NMR), and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data,[5–7] compound IV was identified as hyperin. The methanolic extract of *Taxillus yadoriki* DANSER epiphyting to *Catanopsis cuspidata* var. *sieboldii* NAKAI was concentrated and subjected to silica gel column chromatography. We isolated two known flavonoid glycoside, compound IV and compound V. On the basis of the results of hydrolysis, melting point determination, and the IR data comparisons, compound V was identified as quercitrin. A comparison by thin layer chromatography (TLC) of the constituents from *Taxillus yadoriki* DANSER epiphyting to different host trees, *Acer palmatum* THUNB. var. *matsumurae* MAKINO, *Rhododendron keampferi* PLANCH., *Castanopsis cuspidata* var. *sieboldii* NAKAI, *Quercus glauca* THUNB., *Quercus serrata* THUNB., *Prunus yedoensis* MATSUM., and *Camellia japonica* LINN., showed

TABLE I. Contents of Flavonoids of *Taxillus yadoriki* DANSER (mg/g)

Host species	Compound	IV	V
Acer palmatum THUNB. var. *matsumurae* MAKINO (Aceraceae)		1.57	6.06
Rhododendron kaempferi PLANCH. (Ericaceae)		2.47	0.03
Castanopsis cuspidata var. *sieboldii* NAKAI (Fagaceae)		1.21	5.12
Quercus glauca THUNB. (Fagaceae)		1.61	0.02
Quercus serrata THUNB. (Fagaceae)		1.60	0.02
Prunus yedoensis MATSUM. (Rosaceae)		1.92	0.02
Camellia japonica LINN. (Theaceae)		2.41	0.10

TABLE II. Comparative Study on the Composition of Fatty Acids

	$CH_3(CH_2)_nCOOH$												
$n=$	14	15	16	17	18	19	20	21	22	23	24	25	26
	Area %												
Hyphear tanakae HOSOKAWA	+	−	+	−	5	+	52	3	34	+	3	−	−
Taxillus yadoriki DANSER	−	−	−	−	4	−	3	−	8	−	67	−	12
Taxillus kaempferi DANSER	4	+	8	+	15	+	14	3	24	3	26	−	−
Korthalsella japonica ENGLER	+	27	+	12	+	12	3	9	5	+	8	−	8

+: not more than 3%. −: not detected.

that compounds II—IV are contained in this plant irrespective of the host trees. The contents of flavonoids IV and V were analyzed by high-performance liquid chromatography (HPLC). There was remarkable variation of contents of compound V among the plant on different host trees (Table I).

The methanolic extract of *Taxillus kaempferi* DANSER epiphyting to *Pinus densiflora* SIEB. *et* ZUCC. was partitioned between *n*-hexane and water. The aqueous layer was further extracted with chloroform and *n*-butanol. The hexane and chloroform extracts were each concentrated and subjected to silica gel column chromatography. We isolated fatty acids (VI) and phytosterol (VII) from the hexane extract, and phytosterol-glucoside (VIII) from the chloroform extract. The butanol extract was chromatographed on silica gel and Sephadex LH-20 columns to give known flavonoids, quercetin (IX), avicularin (X) and taxillusin (XI), and the presence of compounds IV and V was proved by HPLC. A HPLC comparison of the constituents from *Taxillus kaempferi* DANSER epiphyting to *Pinus densiflora* SIEB. *et* ZUCC., *Pinus thunbergii* PARLAT., and *Abies firma* SIEB. *et* ZUCC. showed that there was no remarkable variation of the compositions of compounds IV, V, X, and XI.

The methanolic extracts of *Korthalsella japonica* ENGLER epiphyting to *Eurya japonica* THUNB. and *Ligustrum ovalifolium* HASSK. were partitioned between chloroform and water. The aqueous layer was further extracted with *n*-butanol. We isolated fatty acids (XII), phytosterol (XIII), oleanolic acid (XIV) and phytosterol-glucoside (XV) from the chloroform extract, and a known flavonoid glycoside, compound XVI, from the butanol extract. On the basis of the results of hydrolysis, the bathochromic shifts with diagnostic reagents in the UV spectra, the IR, ^{1}H-NMR, and ^{13}C-NMR spectral data, and a molecular ion peak at *m/z* 462 and prominent peaks at *m/z* 301, 153, and 148 in the electron impact-mass (EI-MS) spectrum, compound XVI was identified as chrysoeriol-4′-*O*-glucoside (5,7-dihydroxy-3′-methoxyflavone-4′-*O*-glucoside). A comparison of the constituents from *Korthalsella japonica* ENGLER epiphyting to different host trees, *Cinnamomum japonicum* SIEBOLD, *ex* NAKAI, *Ligustrum japonicum* THUNB., *Ligustrum ovalifolium* HASSK., *Camellia japonica* LINN. var *hortensis* MAKINO, *Camellia sasanqua* THUNB., and *Eurya japonica* THUNB., showed that compounds XIII, XIV, and XV (by TLC) and compound XVI (by HPLC) are contained in this plant irrespective of the host trees. *Korthalsella japonica* ENGLER characteristically contained a large quantity of fatty acids of odd carbon numbers, compared with the other species (Table II).

TABLE III. The Antimicrobial Activity of Flavonoids

Compound	Organisms 1	2	3	4
	MIC (μg/ml)			
Quercetin	400	>400	>400	>400
Quercitrin	>400	>400	>400	400
Hyperin	>400	>400	>400	6.25
Avicularin	>400	>400	>400	>400
Taxillusin	>400	>400	>400	12.5

Organisms: 1, *Staphylococcus aureus* 209P PPD; 2, *Escherichia coli* NIHJ JC2; 3, *Pseudomonas aeruginosa* ATCC 27853; 4, *Klebsiella pneumoniae* ATCC 10031.

TABLE IV. Hypotensive Effects of Flavonoids in Normal Rats

Compound	Dose	Maximal decrease of blood pressure (mmHg) Rat 1	Rat 2	Rat 3
Quercitrin	10 mg/kg	−55	−10	0
Rhamnetin-3-*O*-rhamnoside	10 mg/kg	−85	−100	−35
Rhamnocitrin-3-*O*-rhamnoside	10 mg/kg	−70	−80	−35
Homo-flavoyadorinin-B	10 mg/kg	−60	−25	0
Acetylcholine	0.01 μg/kg	−70	−45	−45
	0.5 μg/kg	−85	−90	−70

Taxillus kaempferi DANSER and *Taxillus yadoriki* DANSER, classified as the same genus taxonomically, were found to contain common flavonoids, but no common aglycones of flavonoids were found among the other three genera. On the basis of these chemotaxonomical results, we consider that aglycones of flavonoid glycosides are of value as taxonomic markers.[8]

The Chinese medicine "Sohkisei", derived from *Viscum album* LINN. var. *coloratum* OHWI and *Taxillus yadoriki* DANSER, is prescribed as an anodyne or tonic. *Taxillus kaempferi* DANSER has been prescribed as a hypotensive or diabetic folk medicine (named Matsunomidori) in Japan.[9] Consequently, we examined the antimicrobial and hyproperties of some isolated flavonoids.[10]

A study was made of the antimicrobial activity of five flavonoid compounds obtained from *Taxillus yadoriki* DANSER and *Taxillus kaempferi* DANSER. Hyperin and taxillusin were found to have antimicrobial activity toward *Klebsiella pneumoniae* (Table III). Four flavonoids obtained from *Taxillus yadoriki* DANSER, *Hyphear tanakae* HOSOKAWA, and *Viscum album* LINN. var. *coloratum* OHWI were tested for their hypotensive property using normal rats. Intravenous administration of these compounds to anesthetized rats produced a temporary hypotensive response (Table IV). In view of these anti-

microbial and hypotensive properties, we presume flavonoids play some part in the action of the folk medicine.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were obtained with a JASCO IR-810 spectrometer. UV spectra were recorded on a Hitachi 220A spectrophotometer. Gas liquid chromatography (GLC) was run on a Shimadzu GC-15A with a flame ionization detector, using glass columns (2 m × 3 mm i.d.) packed with 5% Unisole 400 on Uniport S (60—80 mesh) or with 2% SE-30 on Uniport HP (60—80 mesh). MS were recorded on a JEOL DX300 mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken at 400 and 100 MHz with a Bruker AM 400 spectrometer, and chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet). TLC was carried out on precoated Kieselgel 60F_{254} plates (Merck). Spots were detected on the basis of UV absorbance (254, 365 nm) and by spraying 10% H_2SO_4 or anisaldehyde reagent followed by heating. Column chromatography was carried out with Wakogel C-200 (Wako Pure Chemical Ind. Ltd.), Diaion HP-20 (Mitsubishi Chemical Ind. Ltd.), and Sephadex LH-20 (Pharmacia Fine Chemicals). The HPLC apparatus used was a JASCO TRI-ROTAR SR2 or TWINCLE (column, TSK gel ODS-80TM (4.6 mm i.d. × 150 mm); mobile phase, pH 4.0, 5 mM phosphate buffer–MeOH (3 : 2); detection UV 335 nm).

Extraction and Isolation Dried leaves and twigs (7.4 kg) of *Taxillus yadoriki* DANSER epiphyting to *Quercus glauca* THUNB., collected at Usami, Shizuoka Prefecture, in June 1987, were extracted three times with methanol (*ca.* 36 l). The methanolic extract was evaporated to dryness (3.67 kg) and then partitioned between *n*-hexane and water to give the hexane extract (282 g). The aqueous layer was further extracted with $CHCl_3$ to give the chloroform extract (10 g) and with *n*-BuOH to give the butanol extract (176 g), successively. The hexane extract (282 g) was subjected to silica gel column chromatography using the solvent systems hexane–EtOAc (4 : 1, and 7 : 3), EtOAc, and EtOAc–MeOH (1 : 1), successively. Each fraction was further purified by silica gel column chromatography eluting with $CHCl_3$–EtOAc (9 : 1), and $CHCl_3$–MeOH (9 : 1). These procedures gave 583 mg of I, 48 mg of II, and 70 mg of III. The butanol extract was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH (4 : 1, 7 : 3, and 1 : 1). Each fraction was further purified by silica gel column chromatography. These procedures gave 1.50 g of IV. Dried leaves and twigs (100 g) of *Taxillus yadoriki* DANSER epiphyting to *Catanopsis cuspidata* var. *sieboldii* NAKAI, collected at Ito, Shizuoka Prefecture, in Jan. 1988, were extracted twice with methanol (2 l). The methanolic extract was subjected to silica gel column chromatography with $CHCl_3$–MeOH (4 : 1 and 7 : 3), and $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer). These procedures gave 37 mg of IV, and 96 mg of V. Dried leaves and twigs (5 g) of *Taxillus yadoriki* DANSER epiphyting to *Acer palmatum* THUNB. var. *matsumurae* MAKINO, *Rhododendron kaempferi* PLANCH., *Castanopsis cuspidata* var. *sieboldii* NAKAI, *Quercus glauca* THUNB., *Quercus serrata* THUNB., and *Prunus yedoensis* MATSUM., collected at Ito, Shizuoka Prefecture in Jan. 1988, and *Camellia japonica* LINN., collected at Izaku, Kagoshima Prefecture in Oct. 1988, were extracted with methanol (100 ml). Each methanolic extract was analyzed by TLC and HPLC.

Dried leaves and twigs (9.4 kg) of *Taxillus kaempferi* DANSER epiphyting to *Pinus densiflora* SIEB. *et* ZUCC., collected at Aida, Okayama Prefecture, in June 1986, were extracted three times with methanol (*ca.* 36 l). The methanolic extract was evaporated to dryness (1.57 kg) and then partitioned between *n*-hexane and water to give the hexane extract (272 g). The aqueous layer was further extracted with $CHCl_3$ to give the chloroform extract (27 g) and with *n*-BuOH to give the butanol extract (374 g), successively. The hexane extract (81 g) was subjected to silica gel column chromatography eluting with hexane–EtOAc (4 : 1), EtOAc, and EtOAc–MeOH (1 : 1). The chloroform extract (27 g) was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH (9 : 1). Each fraction was further purified by silica gel column chromatography. These procedures gave 114 mg of VI, 210 mg of VII, and 28 mg of VIII. The butanol extract (139 g) was subjected to Diaion HP-20 column chromatography eluting with H_2O–MeOH (3 : 2, and 2 : 3), and MeOH. Each fraction was further purified by silica gel column chromatography eluting with $CHCl_3$–MeOH (9 : 1, 4 : 1, 7 : 3, and 0 : 100), $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer), and $CHCl_3$–MeOH (1 : 1). These procedures gave 195 mg of IX, 133 mg of X, 1.93 g of XI. Dried leaves and twigs (20 g) of *Taxillus kaempferi* DANSER epiphyting to *Pinus thunbergii* PARLAT., and *Abies firma* SIEB. *et* ZUCC, collected at Matsuyama, Ehime Prefecture, in Aug. 1988, were extracted twice with methanol (200 ml). Each methanolic extract was analyzed by TLC and HPLC.

Dried leaves and twigs (249 g) of *Korthalsella japonica* ENGLER epiphyting to *Eurya japonica* THUNB., collected at Kagoshima, Kagoshima Prefecture, in Aug. 1986, were extracted four times with methanol (3 l). The methanolic extract was evaporated to dryness (71 g) and then partitioned between $CHCl_3$ and water to give the chloroform extract (16 g). The aqueous layer was further extracted with *n*-BuOH to give the butanol extract (18 g). The chloroform extract (16 g) was subjected to silica gel column chromatography using the solvent system hexane–EtOAc (4 : 1, and 7 : 3), EtOAc, and EtOAc–MeOH (1 : 1), successively. Each fraction was further purified by silica gel column chromatography eluting with $CHCl_3$–EtOAc (9 : 1), and $CHCl_3$–MeOH (9 : 1). These procedures gave 115 mg of XII, 32 mg of XIII, 514 mg of XIV, and 53 mg of XV. Dried leaves and twigs (506 g) of *Korthalsella japonica* ENGLER epiphyting to *Ligustrum ovalifolium* HASSK., collected at Kozushima, Tokyo in June 1987, were extracted four times with methanol (3 l). The methanolic extract was evaporated to dryness (96 g) and then partitioned between $CHCl_3$ and water. The aqueous layer was further extracted with *n*-BuOH to give the butanol extract (19 g). The butanol extract was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH (4 : 1, 3 : 2), and MeOH. Each fraction was further purified by chromatography on silica gel and Sephadex LH-20 eluting with MeOH. These procedures gave 9.9 mg of XVI. Dried leaves and twigs (5 g) of *Korthalsella japonica* ENGLER epiphyting to *Ligustrum japonicum* THUNB., and *Eurya japonica* THUNB., collected at Kagoshima, Kagoshima Prefecture, in Aug. 1986, *Cinnamomum japonicum* SIEBOLD, *ex* NAKAI, *Ligustrum ovalifolium* HASSK., and *Camellia japonica* LINN. var. *hortensis* MAKINO, collected at Kozushima, Tokyo, in June 1987, and *Camellia sasanqua* THUNB., collected at Fukuoka, Fukuoka Prefecture, in Oct. 1988, were extracted with methanol (100 ml). Each methanolic extract was analyzed by TLC and HPLC.

Hyperin (IV) Yellowish powder, mp 235—237 °C. IR ν_{max}^{KBr} cm^{-1}: 3325, 1655, 1607, 1203. UV λ_{max}^{EtOH} nm (log ε): 363 (4.27), 256 (4.37); $\lambda_{max}^{EtOH+EtONa}$ nm: 414, 271; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 434, 272; $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 410, 363 sh, 267; $\lambda_{max}^{EtOH+AcONa}$ nm: 377, 268. ^{1}H-NMR (DMSO-d_6) δ: 5.38 (1H, d, J=7.6 Hz, anomeric proton), 6.21 (1H, s), 6.41 (1H, s), 6.82 (1H, d, J=8.4 Hz), 7.54 (1H, s), 7.68 (1H, d, J=8.4 Hz). Acid hydrolysis: galactose (GLC).

Quercitrin (V) Yellowish crystals, mp 175—178 °C. IR ν_{max}^{KBr} cm^{-1}: 3340, 1658, 1604, 1201. Acid hydrolysis: rhamnose (TLC).

Avicularin (X) Yellowish powder, mp 202—205 °C. IR ν_{max}^{KBr} cm^{-1}: 3310, 1641, 1608, 1199. UV λ_{max}^{EtOH} nm (log ε): 358 (4.21), 256 (4.32); $\lambda_{max}^{EtOH+EtONa}$ nm: 407, 271; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 438, 274; $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 402, 361 sh, 269; $\lambda_{max}^{EtOH+AcONa}$ nm: 371, 268. ^{1}H-NMR (DMSO-d_6) δ: 5.59 (1H, s, anomeric proton), 6.21 (1H, d, J=1.3 Hz), 6.41 (1H, d, J=1.3 Hz), 6.86 (1H, d, J=8.4 Hz), 7.49 (1H, d, J=1.7 Hz), 7.56 (1H, dd, J=1.7, 8.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 60.6 (t), 76.9 (d), 82.0 (d), 85.8 (d), 93.5 (d), 98.6 (d), 103.9 (s), 107.8 (d), 115.5 (d), 115.5 (d), 120.9 (s), 121.6 (d), 133.3 (s), 145.0 (s), 148.4 (s), 156.3 (s), 156.8 (s), 161.1 (s), 164.2 (s), 177.6 (s). Acid hydrolysis: arabinose (TLC).

Taxillusin (XI) Pale yellowish powder, mp 190—192 °C. IR ν_{max}^{KBr} cm^{-1}: 3405, 1687, 1650, 1618, 1246, 1092. UV λ_{max}^{EtOH} nm (log ε): 339 sh, 289 (4.38). CD (c=1.66 × 10^{-4}, MeOH) $[\theta]^{25}$ (nm): 13500 (325), −31700 (293). ^{1}H-NMR (DMSO-d_6) δ: 4.62 (1H, d, J=5.8 Hz), 5.53 (1H, d, J=5.8 Hz), 5.84 (1H, d, J=1.7 Hz), 5.90 (1H, d, J=1.7 Hz), 6.59 (1H, dd, J=1.7, 8.2 Hz), 6.65 (1H, d, J=8.2 Hz), 6.76 (1H, d, J=1.7 Hz), 6.99 (2H, s). ^{13}C-NMR (DMSO-d_6) δ: 63.2 (t), 69.3 (d), 73.3 (d), 74.0 (d), , 76.1 (d), 76.3 (d), 80.4 (d), 94.9 (d), 95.8 (d), 101.0 (s), 101.4 (d), 108.6 (d), 108.6 (d), 114.3 (d), 115.4 (d), 118.2 (d), 119.4 (s), 126.3 (s), 138.3 (s), 145.1 (s), 145.4 (s), 145.4 (s), 145.5 (s), 161.4 (s), 163.4 (s), 165.7 (s), 167.1 (s), 191.8 (s).

Chrysoeriol-4′-*O*-glucoside (XVI) Yellowish powder, mp 269—271 °C. EI-MS m/z: 462 (M^+), 301, 153, 148. IR ν_{max}^{KBr} nm^{-1}: 3465, 1652, 1603, 1258. UV λ_{max}^{EtOH} nm (log ε): 332 (4.29), 269 (4.29); $\lambda_{max}^{EtOH+EtONa}$ nm: 374, 307 sh, 277; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 386 sh, 348, 279; $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 383 sh, 343, 280; $\lambda_{max}^{EtOH+AcONa}$ nm: 349 sh, 310 sh, 276. UV λ_{max}^{EtOH} nm (after hydrolysis): 345, 267, 249; $\lambda_{max}^{EtOH+EtONa}$ nm: 409. ^{1}H-NMR (DMSO-d_6) δ: 3.90 (3H, s, OCH_3), 5.31 (1H, br s, anomeric proton, tentative), 6.21 (1H, d, J=2.1 Hz), 6.55 (1H, d, J=2.1 Hz), 6.99 (1H, s), 7.25 (1H, d, J=8.6 Hz), 7.61 (1H, d, J=2.1 Hz), 7.65 (1H, dd, J=2.1, 8.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 55.9 (q), 60.5 (t), 69.5 (d), 73.0 (d), 76.7 (d), 77.0 (d), 94.0 (d), 98.8 (d), 99.5 (d), 103.7 (s), 104.0 (d), 110.2 (d), 115.0 (d), 119.6 (d), 124.0 (s), 149.1 (s), 149.7 (s), 157.3 (s), 161.3 (s), 163.0 (s), 164.2 (s), 181.7 (s). Acid hydrolysis: glucose (GLC).

Determination of Minimum Inhibitory Concentration (MIC) Sub-

cultures of *Staphylococcus aureus* 209P PPD, *Escherichia coli* NIHJ JC2, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 10031, in Meuller–Hinton broth (37 °C, 24 h) were used in this study. The MIC of quercetin, quercitrin, hyperin, avicularin, and taxillusin were determined by the agar plate dilution method.[11] The samples were dissolved in 100% dimethyl sulfoxide, and serial twofold dilutions with H_2O of the solution were added to sensitivity test agar (Eiken) to give final concentrations ranging from 400 to 0.025 ppm. An adjusted inoculum (10^6 CFU) was applied to each agar plate. The MIC was the lowest concentration that prevented visible growth on inoculated plates incubated at 37 °C for 20 h.

Hypotensive Activity The test was carried out on male Jcl-SD rats (Nippon Clea) weighing *ca.* 300 g, anesthetized with phenobarbital 120 mg/kg. The carotid artery was exposed and cannulated, and the blood pressure was recorded *via* a pressure transducer. Acetylcholine was used as a positive control of hypotensive effect.

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