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Chemical Studies on the Mistletoe. V.[1] The Structure of Taxillusin, a New Flavonoid Glycoside Isolated from *Taxillus kaempferi*[2]

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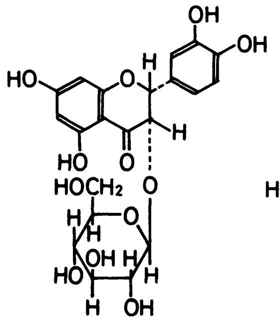
A new flavonoid glycoside, taxillusin was isolated from *Taxillus kaempferi* (DC.) Danser and (2*R*,3*R*)-taxifolin 3-*β*-D-glucopyranoside 6″-gallate was assigned to this substance from studies on the hydrolysis products and from analyses of the ^{1}H-NMR and carbon-13 NMR spectra.

Japanese mistletoe, *Taxillus kaempferi* (DC.) Danser (Matsugumi in Japanese) is a perennial shrub of *Viscaceae.* In our previous paper[1] we reported the isolation and structure of isoglucodistylin (**1**), a flavonoid constituent of this plant, which was assigned to be (2*R*,3*S*)-taxifolin 3-*β*-D-glucopyranoside. During the course of the isolation of **1** by Sephadex LH-20 column chromatography, we isolated another new flavonoid glycoside and named it taxillusin (**2**). We now wish, to report the isolation and the results which lead to the structure of taxillusin (**2**).



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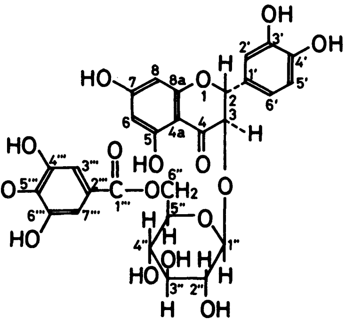

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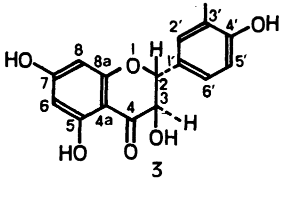
Compound **2** is hygroscopic faintly yellowish crystals, mp 198—203 °C and optically active, $[\alpha]_D$ +5.2°. Elemental analyses show that **2** has a composition of $C_{28}H_{26}O_{16}$. The UV spectrum of **2** in ethanol shows the absorption maximum at 292 nm, and this absorption maximum and its pattern were similar to those reported for a number of flavonoids of taxifolin type.[3] The IR spectrum of **2** shows absorption bands characteristic of flavonoid glycosides and indicative of the existence of a conjugated ester group at 1690 and 1250 cm^{-1} besides them.

Acetylation of **2** with acetic anhydride and pyridine gave a decaacetate of **2** as colorless fine crystals, mp 125—129 °C.

Acid hydrolysis of **2** with 5% sulfuric acid in 50% aqueous ethanol[4] gave taxifolin (**3**), gallic acid (**4**), and D-glucose as the components. Since the optical rotation value of **3** ($[\alpha]_D$ +12°) is identical with that of (+)-taxifolin,[5,6] the absolute configuration of **3** is 2*R* and 3*R*.[7] Methylation of **2** with dimethyl sulfate and potassium carbonate in acetone followed by acid hydrolysis in the same manner as described above and then air oxidation[3c] gave 3′,4′,5,7-tetra-*O*-methylquercetin (**5**),[3c,8] which was identified with the authentic sample prepared by methylation and acid hydrolysis of rutin in the same manner. This result indicates that either D-glucose or gallic acid might be linked at the 3-position of **3**.

Treatment of **2** with hydrogen chloride in methanol gave **3**, methyl gallate (**6**), methyl *α*-D-glucopyranoside (**7**), and a substance **8**. The IR spectrum of **8** shows absorption bands indicative of the existence of a conjugated ester group at 1700 and 1240 cm^{-1}. Since **8** gives **6** and **7** by further treatment with hydrogen chloride in methanol, D-glucose and gallic acid should be linked by an ester bond. The ^{1}H-NMR spectrum of a trimethylsilyl derivative of **8** in $CDCl_3$ shows a sharp singlet ascribed to the methoxyl group at *δ* 3.37 ppm, a doublet corresponding to the 1-proton of glucose at *δ* 4.62 ppm (*J*=3 Hz, axial/equatorial coupling indicative of *α*-D-glucoside), and a sharp singlet attributed to the 3′- and 7′-protons of galloyl group at *δ* 7.23 ppm. This result indicates **8** to be a gallate of methyl *α*-D-



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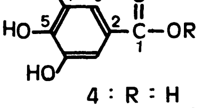
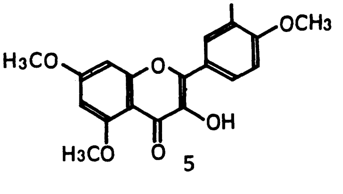


4 : R : H
6 : R : CH3



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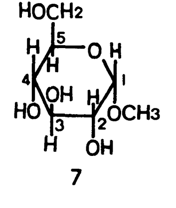

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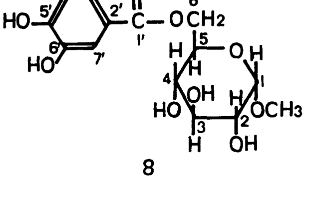

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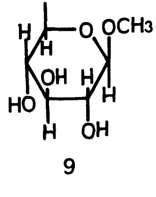

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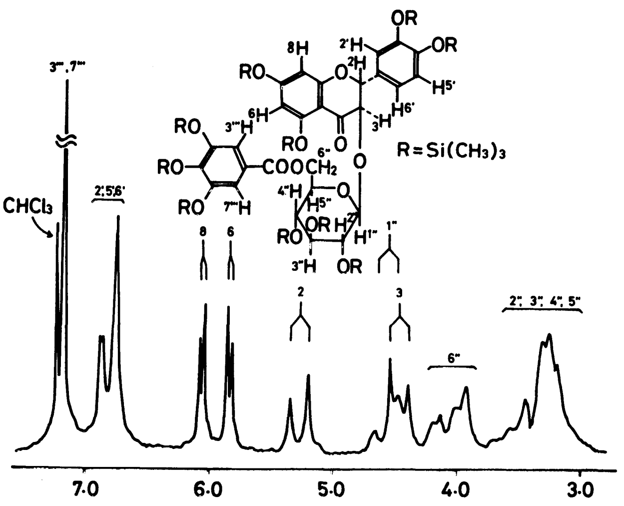

Fig. 1. The ^{1}H-NMR spectrum of trimethylsilyl derivative of taxillusin (**2**) in CDCl$_3$.

glucoside.

The position of linkage between the D-glucose and gallic acid in **8** was confirmed by comparison of the carbon-13 NMR spectra of **6**, **7**, **8**, and their trimethylsilyl derivatives as shown in Table 1. The 6-carbon of the glucose in **8** appears at a field lower by 2.7 ppm and the 5-carbon of the glucose in **8** appears at a field higher by 2.0 ppm than those of **7**. The 5- and 6-carbons of the glucose in a trimethylsilyl derivative of **8** also appear at fields higher by 2.4 ppm and lower by 1.6 ppm respectively than those of a trimethylsilyl derivative of **7**. These results indicate that the gallic acid in **8** should be linked by an ester bond at the 6-position of **7**.

Beisdes the signals characteristic of **3** as shown in Fig. 1, the ^{1}H-NMR spectrum of a trimethylsilyl derivative of **2** in CDCl$_3$ shows a sharp singlet of $3'''$- and $7'''$-protons of galloyl group at δ 7.20 ppm, signals of the protons in $2'$-, $3'$-, $4'$-, and $5'$-positions of glucose between δ 3.0 and 3.8 ppm, a broad doublet of $1''$-proton of glucose at δ 5.24 ppm (J= *ca.* 11 Hz, axial/axial coupling indicative of β-D-glucoside), and a two proton AB-type multiplet of $6''$-protons of glucose at δ 4.04 ppm (down-field shift by galloyl group). This result indicates that **2** might be (2*R*,3*R*)-taxifolin 3-β-D-glucopyranoside $6''$-gallate.

The structure of **2** is supported also by the carbon-13 NMR spectra of **1**, **2**, **3**, **6**, and methyl β-D-glucopyranoside (**9**) in DMSO-d_6 as shown in Table 1. The 1-carbon of the glucose in **2** appears at a field higher by 2.7 ppm than that of **9** and the 4-carbonyl carbon in **2** also appears at a field higher by 5.6 ppm than that of **3**. This fact is the consequence of steric compression between the C-1 of glucose and the C-4 of taxifolin. The 3- and 4-carbons of the taxifolin in **1** appear at fields higher by 1.1 ppm and lower by 0.7 ppm respectively

Table 1. Carbon-13 NMR chemical shifts[a] of **1**, **2**, **3**, **6**, **7**, **8**, **9**, and trimethylsilyl derivatives of **7** and **8**

Compound		**6**	**7**	TMS[b] of **7**	**9**	**8**	TMS of **8**	**3**	**1**	**2**
Solvent		DMSO-d_6	DMSO-d_6	CDCl$_3$	DMSO-d_6	DMSO-d_6	CDCl$_3$	DMSO-d_6	DMSO-d_6	DMSO-d_6
Taxifolin	2	—	—	—	—	—	—	82.9	80.6	80.5
	3	—	—	—	—	—	—	71.3	75.2	76.3
	4	—	—	—	—	—	—	197.5	192.6	191.9
	4a	—	—	—	—	—	—	100.2	101.0	101.0
	5	—	—	—	—	—	—	163.1	163.3	163.3
	6	—	—	—	—	—	—	95.8*	95.9*	95.9*
	7	—	—	—	—	—	—	166.5	167.0	167.0
	8	—	—	—	—	—	—	94.8*	95.0*	95.0*
	8a	—	—	—	—	—	—	162.3	161.6	161.3
	1'	—	—	—	—	—	—	127.8	126.5	126.3
	2'	—	—	—	—	—	—	115.1**	114.6**	114.4**
	3'	—	—	—	—	—	—	145.5***	145.5***	145.4***
	4'	—	—	—	—	—	—	144.7***	145.0***	145.0***
	5'	—	—	—	—	—	—	115.1**	115.4**	115.5**
	6'	—	—	—	—	—	—	119.2	118.7	118.3
Glucose	1	—	99.5	99.9	103.7	99.7	100.0	—	101.0	101.0
	2	—	72.4	74.2	73.2	71.9	74.1	—	73.4	73.5
	3	—	73.2	75.6	76.6	73.3	75.2	—	77.1	76.3
	4	—	70.2	72.3	69.9	70.5	72.9	—	69.8	69.3
	5	—	71.8	72.3	76.5	69.8	69.9	—	76.5	74.0
	6	—	60.9	62.5	60.9	63.6	64.1	—	61.0	63.2
Gallic acid	1	166.2	—	—	—	165.8	166.2	—	—	165.7
	2	119.2	—	—	—	119.2	122.6	—	—	119.4
	3	108.4	—	—	—	108.7	115.7	—	—	108.7
	4	145.4	—	—	—	145.5	148.0	—	—	145.4
	5	138.2	—	—	—	138.4	143.8	—	—	138.3
	CH$_3$	51.5	54.2	54.8	55.9	54.5	55.1	—	—	—

a) In parts per million down-field from tetramethylsilane. b) TMS: trimethylsilyl derivative. c) Asterisks indicate that assignments are not unambiguous.

than those of **2**. This fact is the consequence of the difference in the configuration at the 3-position of taxifolin between **1** and **2**. The 5- and 6-carbons of the glucose in **2** appear at fields higher by 2.5 ppm and lower by 2.3 ppm respectively than those of **9** and also appear at fields higher by 2.5 ppm and lower by 2.2 ppm respectively than those of **1**, which indicates that gallic acid is linked by an ester bond at the 6-position of D-glucose.

These ^{1}H-NMR and carbon-13 NMR spectroscopic data and all experimental results described above support the structure of taxillusin (**2**) to be (2*R*,3*R*)-taxifolin 3-*β*-D-glucopyranoside 6″-gallate.

Experimental

All melting points are uncorrected. The proton and carbon nuclear magnetic resonance spectra were measured with a JEOL JNM-PFT-60 NMR spectrometer, and the latter spectra were obtained on sampling interval of 2.0 s. Chemical shifts were obtained by δ value (ppm) from tetramethylsilane as internal standard at 15.04 MHz for carbon and 59.8 MHz for proton. The UV spectra were measured with a Hitachi EPS-3 recording spectrophotometer. The optical rotations were measured with a Union PM-101 polarimeter.

Extraction and Isolation of Taxillusin (2). The fresh leaves and twigs of the mistletoe (2 kg)[9] were extracted with ethanol (6 l) in the usual way, and the extract was condensed to a syrup under reduced pressure. The syrup was triturated with water (2 l) and chloroform (2 l), and the undissolved material which accumulated between both layers was collected and washed with water. After being dried in a desiccator over calcium chloride, the faintly yellowish powder (11.5 g) was dissolved in ethanol (50 ml), and the solution was subjected to chromatography on a column of Sephadex LH-20 (7×40 cm) eluting with ethanol.[10] The last effluent was rechromatographed with a column of Sephadex LH-20 (3.5×120 cm) in the same manner as described above. After removal of the solvent, recrystallization from aqueous ethanol gave taxillusin (**2**) as hygroscopic faintly yellowish crystals: mp 198—203 °C (decomp); yield, 9.2 g (0.46% from wet plant); $[\alpha]_D^{25}$ +5.2° (*c* 1.0, EtOH); UV (EtOH): λ_{max} 292 nm (ε 23300); IR (KBr disk): ν_{max} 3400—3200, 1690, 1653, 1620, 1610, 1550, 1531, 1516, and 1250 cm^{-1}. Found: C, 51.31; H, 4.78%. Calcd for $C_{28}H_{26}O_{16}\cdot 2H_2O$: C, 51.38; H, 4.62%.

Acetylation of 2. Acetylation of **2** with acetic anhydride and pyridine in the usual way gave a decaacetate of **2** as colorless fine crystals: mp 125—129 °C; UV (EtOH): λ_{max} 241 (ε 31900, inflexion), 263 (ε 17900), and 312 nm (ε 6490). Found: C, 55.16; H, 4.42%. Calcd for $C_{48}H_{46}O_{26}$: C, 55.49; H, 4.46%.

Acid Catalyzed Hydrolysis of 2. **2** (100 mg) was dissolved in 50% aqueous ethanol (20 ml) containing concd sulfuric acid (1 ml) and the mixture was refluxed for 3 h. After removal of ethanol by evaporation under reduced pressure, the solution was extracted with ethyl acetate. The extract was subjected to chromatography on a column of Sephadex LH-20 (2×75 cm) eluting with ethanol and gave **3** (mp 240—242 °C; yield, 32 mg; $[\alpha]_D^{25}$ +12° (*c* 0.75, EtOH)) and **6** (mp 233—237 °C (decomp); yield, 12 mg).

Hydrolyzate free from **3** and **6** was neutralized with barium carbonate and was concentrated under reduced pressure, which was subjected to thin layer chromatography for detection of the sugar component. The sugar was proved to be identical with D-glucose.

Methylation of 2. According to Fukui, Nakadome, and Ariyoshi's method,[3c] **2** was methylated with dimethyl sulfate and potassium carbonate in acetone followed by acid hydrolysis and then by air oxidation. The product was identical with authentic 3′,4′,5,7,-tetra-*O*-methylquercetin (**5**) which was prepared by methylation and acid hydrolysis of rutin in the same manner. **5**: mp 194—196 °C[3c]; UV(EtOH): λ_{max} 253 and 362 nm;[8] ^{1}H-NMR ($CDCl_3$): δ 3.92 (3H, s, CH_3O), 3.98 (9H, s, CH_3O), 6.37 (1H, d, *J*=2 Hz, H-6), 6.54 (1H, d, *J*=2 Hz, H-8), 6.99 (1H, d, *J*=9 Hz, H-5′), 7.38 (1H, broad s, 3-OH), 7.78 (1H, dd, *J*=2 and 9 Hz, H-6′), and 7.81 ppm (1H, d, *J*=2 Hz, H-2′).

Methanolysis of 2. **2** (620 mg) was dissolved in dry methanol (15 ml), and 30% methanolic hydrogen chloride (5 ml) was added to this solution. After having been allowed to stand for 12 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The residue was subjected to chromatography on a column of Sephadex LH-20 (3×120 cm) eluting with methanol in the same manner as described above and gave **3** (yield, 105 mg), **6** (mp 198—200 °C; yield, 55 mg), **7** (mp 169—170 °C; yield, 60 mg; $[\alpha]_D^{20}$ +159° (*c* 0.5, H_2O)), and **8** (colorless powder from methanol and ether; mp 120—124 °C; yield, 110 mg; $[\alpha]_D^{22}$ +37° (*c* 0.2, EtOH); IR (KBr disk): ν_{max} 3350, 1700, 1614, 1535, 1240, 1150, 1045, 878, 770, and 745 cm^{-1}; Found: C, 48.83; H, 5.23%. Calcd for $C_{14}H_{18}O_{10}$: C, 48.56; H, 5.24%).

Further treatment of **8** with methanolic hydrogen chloride in the same manner as described above gave **6** and **7**.

Acetylation of **8** with acetic anhydride and pyridine in the usual way gave a hexaacetate of **8** as colorless crystals: mp 77—79 °C. Found: C, 51.97; H, 5.01%. Calcd for $C_{26}H_{30}O_{16}$: C, 52.18; H, 5.05%.

Trimethylsilylation. Flavonoid or sugar (50 mg) was treated with anhydrous pyridine (5 ml), trimethylchlorosilane (0.5 ml), and hexamethyldisilazane (1 ml). The mixture was allowed to stand overnight at room temperature. After being evaporated to dryness under reduced pressure, the residue was dissolved in chloroform, and the undissolved materials which separated were filtered off and washed with chloroform. The filtrate and washings were evaporated to dryness under reduced pressure. No further attempt was made to purify the trimethylsilyl derivative before measurement of the NMR spectra.

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9) The mistletoe which occurred on a Japanese red pine (*Pinus densiflora*) was collected at Mt. Udo of Shizuoka City in the spring of 1977.

10) From this effluent, quercetin, (+)-taxifolin, avicularin quercitrin, hyperin, and guaijaverin were isolated besides taxillusin and isoglucodistylin.