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

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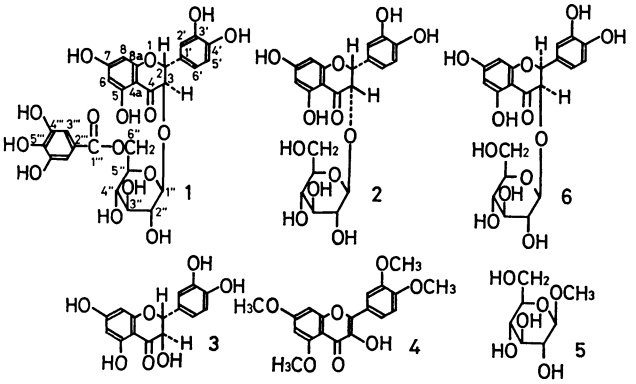
(Received March 23, 1982)

Synopsis. A new flavonoid glycoside, isoglucodistylin was isolated from *Taxillus kaempferi*, and (2*R*,3*S*)-taxifolin 3-*β*-D-glucopyranoside was assigned to this substance from studies on the hydrolysis products and on the isomerization products, and from analyses of the carbon-13 NMR spectra.

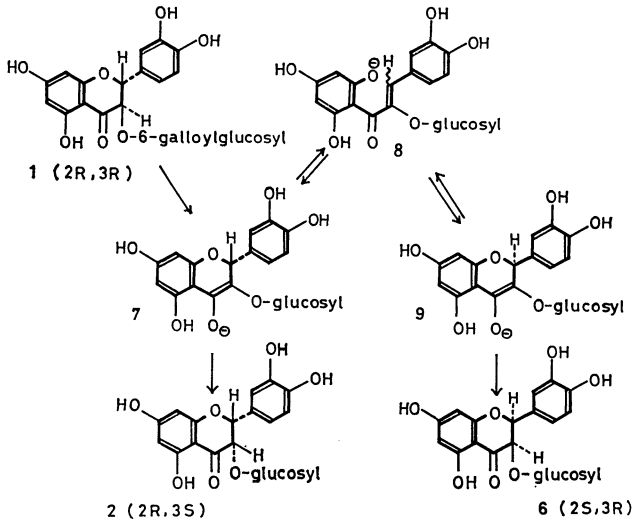
In our studies on the constituents of Japanese mistletoe, we reported the isolation and structure of taxillusin, a new flavonoid glycoside from *Taxillus kaempferi*, which was assigned to be (2*R*,3*R*)-taxifolin 3-*β*-D-glucopyranoside 6″-gallate (**1**).[2] During the course of the isolation of **1** by Sephadex LH-20 column chromatography, we found a presence of another new flavonoid glycoside and named it isoglucodistylin (**2**).



2 is amorphous white powder, mp 169—171 °C ($[\alpha]_D^{27}$ +25°), and the ultraviolet spectrum of **2** in ethanol shows the absorption maximum at 296 nm. Acid hydrolysis with hydrochloric acid gave (+)-taxifolin (**3**) ($[\alpha]_D^{27}$ +11°)[3] and glucose as the components. Methylation of **2** with dimethyl sulfate and potassium carbonate in acetone followed by acid hydrolysis and by air oxidation gave 3′,4′,5,7-tetra-*O*-methylquercetin (**4**).[4,5] This result indicates that glucose must be linked at the 3-position of taxifolin.

Taxifolin 3-glucoside (glucodistylin) had been isolated from the leaves of *Chamaecyparis obtusa* but this substance shows the ultraviolet absorption maximum at 292 nm in ethanol.[4] Both taxifolin 3-rhamnoside (astilbin) and aromadendrin 3-rhamnoside (engelitin) show their absorption maxima at 292 nm in ethanol and the configuration of the substituents at the 2- and 3-positions of both substances is *trans*.[6] While both the *cis* forms of astilbin and engelitin which have been prepared by isomerization of natural *trans* isomers show their absorption maxima at 296 nm in ethanol.[6] Therefore, glucodistylin must be a substance of *trans* form, and **2** might be 2,3-*cis*-taxifolin 3-glucoside.

The structure of **2** is supported by the carbon-13 NMR spectra of **1**, **2**, **3**, and methyl *β*-D-glucopyranoside (**5**) in DMSO-d_6 as shown in Table 1. The 3- and 4-carbons of taxifolin in **2** appear at a field



Scheme 1.

higher by 1.1 ppm and a field lower by 0.7 ppm respectively than those of **1**. This fact is the consequence of the difference in the configuration at the 3-position of taxifolin between **1** and **2**.

Acid hydrolysis of **2** gave (+)-taxifolin (**3**) of 2*R* and 3*R* configuration,[7] however, this result might be due to the isomerization at the 3-position from *cis* to *trans* form *via* enol. Therefore, the absolute configuration of **2** should be 2*R* and 3*S*.

The isomerization of **2** with aqueous sodium acetate in ethanol[6] gave a substance **6** but did not give 2,3-*trans*-taxifolin 3-*β*-glucoside. The ultraviolet spectrum of **6** in ethanol shows the absorption maximum at 295 nm. Acid hydrolysis of **6** with hydrochloric acid gave (−)-taxifolin ($[\alpha]_D^{23}$ −11°).[8] In the carbon-13 NMR spectra, the 1-carbon of glucose and the 2-carbon of taxifolin in **6** appear at fields lower by 1.8 and 0.7 ppm respectively than those of **2** as shown in Table 1. This fact is the consequence of the presence of less steric compression between the C-1 of glucose and the C-2 of taxifolin in **6**. These results indicate **6** to be (2*S*,3*R*)-taxifolin 3-*β*-D-glucopyranoside.

Hydrolysis of **1** with aqueous sodium carbonate gave **2**, **6**, and gallic acid, but did not give 2,3-*trans*-taxifolin 3-*β*-glucoside. The isomerization in the hydrolytic conditions was confirmed by high pressure liquid chromatography of recovered **1**. These results indicate that taxifolin glucosides in *cis* form might be more stable than those of *trans* in the conditions described above.

The formation of those **2** and **6** can be rationalized by the mechanistic sequence as shown in Scheme 1. During the base-catalyzed hydrolysis of the galloyl group, **1** is isomerized into **2** with inversion at the 3-position through an enolate **7**. When the C–O bond between the 1- and 2-positions of the enolate

Table 1. Carbon-13 NMR chemical shifts of **1**, **2**, **3**, **5**, and **6**, in DMSO-d_6

Compound		1	2	6	3	5
Taxifolin	2	80.5	80.6	81.3	82.9	—
	3	76.3	75.2	75.2	71.3	—
	4	191.9	192.6	192.7	197.5	—
	4a	101.0	101.0	100.9	100.2	—
	5	163.3	163.3	163.5	163.1	—
	6	95.9*	95.9*	95.9*	95.8*	—
	7	167.0	167.0	167.3	166.5	—
	8	95.0*	95.0*	95.1*	94.8*	—
	8a	161.3	161.6	161.7	162.3	—
	1′	126.3	126.5	126.4	127.8	—
	2′	114.4**	114.6**	114.8**	115.1	—
	3′	145.4***	145.5***	145.5***	145.5**	—
	4′	145.0***	145.0***	144.9***	144.7**	—
	5′	115.5**	115.4**	115.3**	115.1	—
	6′	118.3	118.7	118.6	119.2	—
Glucose	1	101.0	101.0	102.8	—	103.7
	2	73.5	73.4	73.4	—	73.2
	3	76.3	77.1	76.8	—	76.6
	4	69.3	69.8	69.9	—	69.9
	5	74.0	76.5	76.7	—	76.5
	6	63.2	61.0	61.2	—	60.9
Gallic acid	1	165.7	—	—	—	—
	2	119.4	—	—	—	—
	3	108.7	—	—	—	—
	4	145.4	—	—	—	—
	5	138.3	—	—	—	—
	CH_3	—	—	—	—	55.9

In parts per million downfield from tetramethylsilane. Asterisks indicate that assignments are not unambigous.

7 is cleaved to afford a phenolate **8**, the phenolate oxygen in **8** attacks at the 2-position from the opposite side to afford an enolate **9**, which then converted to **6** with inversion at the 3-position.

These carbon-13 NMR spectroscopic data and all experimental results described above support the structure of isoglucodistylin (**2**) to be (2*R*,3*S*)-taxifolin 3-*β*-D-glucopyranoside.

Experimental

All melting points are uncorrected. The carbon-13 NMR spectra were measured with a JEOL JNM-PFT-60 NMR spectrometer on sampling interval of 2.0 s. Chemical shifts were obtained by δ value (ppm) from TMS as internal standard at 15.04 MHz. The UV spectra were measured with a Hitachi EPS-3 recording spectrophotometer. HPLC apparatus used was JASCO TRI ROTAR high pressure liquid chromatography. Column used was 2.6 mm×250 mm stainless steal peacked with LiCrosolb RP-8. Elution system was CH_3CN–H_2O (9:1) containing 0.02 M (1 M=1 mol dm^{-3}) acetic acid.

Isolation of Isoglucodistylin (2). The fresh leaves and twigs of the mistletoe (2 kg) which occurred on *Pinus densiflora* were extracted with ethanol (6 L) and the extract was condensed to a syrup *in vacuo*. The syrup was triturated with water (2 L) and chloroform (2 L), and the undissolved material which accumulated between both layers was collected. The faintly yellowish powder (11.5 g) was subjected to chromatography on a column of Sephadex LH-20 (3.5×120 cm) eluting with ethanol. Concentrated to dryness of the fractions containing **2** and recrystallization from ethyl acetate gave **2** as colorless amorphous powder (mp 167—169 °C; yield, 100 mg (0.005% from wet plant); $[\alpha]_D^{27}$ +25° (*c* 0.3, EtOH); UV (EtOH): λ_{max} 296 nm (ε 17100)). Found: C, 51.98; H, 4.46%. Calcd for $C_{21}H_{22}O_{12}\cdot H_2O$: C, 52.07; H, 4.99%.

Acid Catalyzed Hydrolysis of 2. **2** was dissolved into 2 M hydrochloric acid and the mixture was refluxed for 2 h. Extraction with ethyl acetate and recrystallization from water gave **3** as faintly yellowish crystals (mp 240—242 °C; $[\alpha]_D^{27}$ +11° (*c* 1.0, EtOH); UV (EtOH): λ_{max} 291 nm (ε 19400)). Hydrolyzate free from **3** was subjected to TLC 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,[4] **2** was methylated with dimethyl sulfate and potassium carbonate in acetone followed by acid hydrolysis and then air oxidation. The product was identified with authentic 3′,4′,5,7-tetra-*O*-methylquercetin (**4**) which was prepared by methylation and acid hydrolysis of rutin in the same way.

Isomerization of 2 with Sodium Acetate. **2** was isomerized with ethanolic sodium acetate according to Tominaga and Yoshimura's method.[6] Separation of the isomerized mixture with a column of Sephadex LH-20 in the same way as described above gave **6** as colorless amorphous powder (mp 200—202 °C (decomp); yield, 15%; $[\alpha]_D^{27}$ +150° (*c* 0.1, EtOH); UV (EtOH): λ_{max} 295 nm (ε 18500)). Found: C, 52.16; H, 4.43%. Calcd for $C_{21}H_{22}O_{12}\cdot H_2O$: C, 52.07; H, 4.99%.

Acid Catalyzed Hydrolysis of 6. Acid hydrolysis of **6** with 2 M hydrochloric acid in the same way as described above gave (−)-taxifolin (mp 238—240 °C; $[\alpha]_D^{23}$ −11° (*c* 0.1, EtOH); UV (EtOH): λ_{max} 291 nm (ε 18800)).

Base Catalyzed Hydrolysis of 1. **1** (1.25 g) was dissolved into aqueous 0.5 M sodium carbonate (200 ml) and the mixture was stirred for 18 h at room temperature under nitrogen atmosphere. After having been acidified with hydrochloric acid, the reaction mixture was extracted with ethyl acetate. The extract was subjected to chromatography on a column of Sephadex LH-20 in the same way as described above gave **2** (75 mg), **6** (45 mg), and gallic acid (10 mg).

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