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196. **Masakazu Aritomi** : Terniflorin, a New Flavonoid Compound in Flowers of *Clematis terniflora* A. P. de CANDOLLE var. *robusta* TAMURA.

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Clematis terniflora A. P. de CANDOLLE var. *robusta* TAMURA (Japanese name : " Senninsô ") is a perennial, climbing herb of the family Ranunculaceae, which is distributed throughout Japan, and being known as one of the poisonous plants in Japan.

As described in detail in the experimental part, a new flavonoid compound, which would be named terniflorin hereafter has now been isolated from the flowers of this plant, and with the description and discussion of the result of experiments carried out on its structure elucidation is concerned the present paper.

Terniflorin was obtained as pale yellow needles, m.p. 266~267° (decomp.), and analyzed for $C_{30}H_{26}O_{12}\cdot H_2O$. It had no methoxyl group, and gave hexa-O-acetate, m.p. 174~175°, analyzed for $C_{30}H_{20}O_6(AcO)_6\cdot H_2O$, indicative of the presence of six free hydroxyl groups in its molecule.

Exploratory alkali fission with 50% potassium hydroxide of terniflorin gave three products, which were proved by paper chromatography to be identical with *p*-coumaric acid, *p*-hydroxybenzoic acid, and phloroglucinol, respectively.

Mild alkaline hydrolysis with 1% potassium hydroxide of terniflorin gave one mole each of *p*-coumaric acid and a flavone glycoside, m.p. 232~233° (decomp.), which was shown to be identical with cosmosiin (apigenin-7-β-D-glucoside)[1] by mixed fusion and comparison of infrared absorption.

Furthermore, drastic hydrolysis with conc. hydrochloric acid of terniflorin gave *p*-coumaric acid, apigenin, and D-glucose.

These facts indicate that terniflorin might well be structurally formulated as one of the *p*-coumarates of cosmosiin.

In harmony with the foregoing structure, the infrared spectrum of terniflorin (Fig. 1) indicates two sharp absorption bands at 1655 cm^{-1} (C=O in the γ-pyrone ring)[2,3] and at 1686 cm^{-1} (C=O, ester), respectively,

To locate the position of ester linkage in the molecule of terniflorin, use was made of ultraviolet spectrophotometry after the procedure of Jurd.[4,5]

In a medium of sodium ethylate, terniflorin showed three peaks at 270, 317, and 398 mμ. A peak at 398 mμ might well be attributable to the longer wave length absorption maximum at 321 mμ of terniflorin shifted by the addition of this reagent. This is indicative of the fact that the hydroxyl group at the position of 4′ in the molecule of terniflorin is unsubstituted.

The ultraviolet absorption curve of terniflorin was significantly altered by the addition of aluminum chloride, exhibiting four peaks at 281, 301, 325, and 380 mμ, characteristic of apigenin derivatives with a free 5-hydroxyl group in their molecules.

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2) H. L. Hergert, E. F. Kurth : J. Am. Chem. Soc., **75**, 1622 (1953).

3) G. E. Inglett : J. Org. Chem., **23**, 93 (1958).

4) L. Jurd, R. M. Horowitz : *Ibid.*, **22**, 1618 (1957).

5) L. Jurd : " Spectral Properties of Flavonoid Compounds " in " The Chemistry of Flavonoid Compounds," Ed. by T. A. Geissman, 108 (1962), Pergamon Press, Oxford.

Now it follows that terniflorin might well be represented as the structure of apigenin-7-(*p*-coumaroyl)-*β*-D-glucoside.

It is of interest that the occurrence of aroylated flavonoid compounds in plants has been reported so far by several groups of workers. Tiliroside present in the flowers of *Tilia argentea* Desf., *T. cordata* Mitt., and *T. platyphyllos* Scop.,[6,7] in the fruits of *Rosa canina* Linnaeus,[6~8] and in the leaves of *Platanus occidentalis* Linnaeus[9] was shown

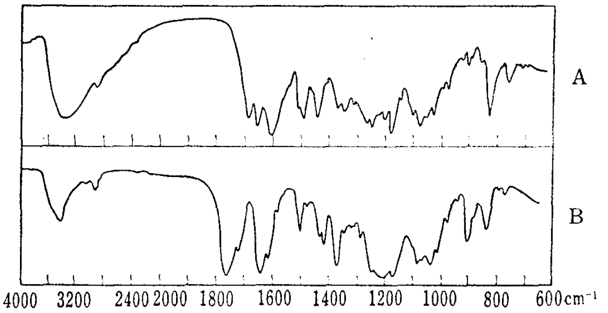

Fig. 1. Infrared Spectra of Terniflorin and its Acetate

A : Terniflorin
B : Terniflorin acetate

Table I. Ultraviolet Photometric Data of Terniflorin and Related Compounds (UV λ mμ (log ε))

	EtOH		EtOH-EtONa		EtOH-AlCl$_3$	
	max.	min.	max.	min.	max.	min.
Terniflorin	270(4.32)	279(4.26)	270(4.22)	278(4.17)	281(4.35)	284(4.34)
	321(4.53)		317(4.36)	353(4.17)	301(4.44)	309(4.39)
			398(4.38)		325(4.45)	370(4.09)
					380(4.10)	
Cosmosiin	269(4.26)	290(4.04)	264(4.14)	307(3.74)	278(4.21)	289(4.12)
	341(4.36)		398(4.48)		299(4.15)	310(3.88)
					341(4.33)	367(4.10)
					385(4.15)	
Methyl *p*-coumarate	313(4.24)		312(3.86)	316(3.85)	313(4.24)	
			368(4.43)			
Apigenin	270(4.22)	284(4.04)	277(4.30)	295(3.70)	277(4.16)	288(4.09)
	341(4.28)		330(4.08)	348(4.01)	302(4.14)	313(3.84)
			400(4.49)		344(4.24)	370(4.07)
					382(4.08)	
Acacetin	270(4.31)	288(4.14)	278(4.49)		282(4.20)	288(4.19)
	311(4.17)[a]		295(4.30)[a]	326(3.82)	303(4.25)	313(3.94)
	333(4.24)		374(4.10)		339(4.28)	369(4.00)
					383(4.03)	
4′,5-Di-O-methylapigenin	266(4.27)	282(4.10)	275(4.51)	324(3.93)	266(4.27)	276(4.05)
	329(4.26)		359(4.10)		329(4.26)	
4′,7-Di-O-methylapigenin	270(4.24)	285(4.07)	292(4.44)		280(4.22)	289(4.18)
	331(4.32)				303(4.24)	312(3.94)
					339(4.31)	370(4.04)
					380(4.06)	
Tilianin[b]	270(4.26)	287(4.08)	292(4.39)	345(3.85)	279(4.24)	289(4.19)
	331(4.33)		367(3.87)		300(4.23)	310(3.97)
					338(4.32)	366(4.04)
					382(4.07)	

a) Inflection

b) The sample was furnished through the courtesy of Prof. Dr. Naokata Morita, Faculty of Pharmaceutical Sciences, University of Toyama, to whom the writer is deeply indebted.

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6) L. Hörhammer, L. Stich, H. Wagner : Naturwiss., **46**, 358 (1959).

7) *Idem* : Arch. Pharm., **294**, 687 (1961).

8) D. Öiseth, A. Nordal : Pharm. Acta Helv., **32**, 109 (1957) (C. A., **51**, 12430 (1957)).

9) A. Stambouli, R. Paris : Ann. Pharm. France, **19**, 732 (1961) (C. A., **57**, 5023 (1962)).

to be of the structure of kaempferol-7-*p*-coumaroyl-3-β-D-glucoside, and petunoside isolated from various Petunia species to be of the structure of kaempferol-3-β-feruloylsophoroside.[10)]

Terniflorin may be enumerated as the third aroylated flavonoid glycoside in nature.

Experimental

Paper chromatography: The flavonoid compounds were run in the solvent systems of (1) BuOH-AcOH-H_2O (4:1:5 by volume) (solv. 1), (2) 60% AcOH (solv. 2), and (3) 15% AcOH (solv. 3). Spray reagent used was 5% Na_2CO_3 solution. The component sugar was run in the solvent system of BuOH-pyridine-H_2O (3:2:1 by volume) by double ascending method, and detected by spraying with 0.1% solution of *p*-anisidine·HCl in MeOH. The phenolic compounds were run in the solvent systems of (1) H_2O saturated with Et_2O, and (2) BuOH saturated with aqueous NH_4OH, and detected by spraying with diazotized sulfanilic acid.

UV spectra: UV-spectra were measured by using a self-recording spectrophotometer (Hitachi EPS 2 Type). A $10^{-4}M$ solution of each compound in EtOH was prepared and used as a stock solution. For determining the spectra, the stock solution was diluted as follows to give a concentration of $2\times10^{-5}M$. Spectra in EtOH: Five ml. of the stock solution was diluted to 25 ml. with EtOH. Spectra in EtOH-EtONa: To 5 ml. of the stock solution was added 5 ml. of $10^{-3}M$ solution of EtONa in EtOH, and the resulting solution was diluted to 25 ml. with EtOH. After 5 min., the solution was subjected to spectrophotometry. Spectra in EtOH-$AlCl_3$: To 5 ml. of the stock solution was added 5 ml. of $10^{-3}M$ solution of $AlCl_3$ in EtOH, and the resulting solution was diluted to 25 ml. with EtOH.

Isolation of Terniflorin——The air-dried flowers of *Clematis terniflora* A.P. de CANDOLLE var. *robusta* TAMURA were extracted twice with boiling MeOH. After removal of MeOH, the residue was digested with boiling H_2O, and the resulting aqueous solution was successively extracted with benzene, Et_2O, and then with AcOEt (20 times). The combined AcOEt extracts were dissolved in MeOH, treated with a saturated solution of $(AcO)_2Pb$ in MeOH, and the precipitate was filtered off. The filtrate was concentrated to a small volume, and allowed to stand in an ice-box for a week or more. Terniflorin gradually separated as a crystalline mass, which was collected, washed repeatedly with MeOH, and recrystallized from dioxane-H_2O (1:1). Yield, 300 mg. from 4 kg. of the dried flowers.

Terniflorin——Terniflorin crystallized as pale yellow needles, m.p. 266~267°(decomp.). It is sparingly soluble in H_2O and usual organic solvents except dil. dioxane and pyridine, and soluble in conc. HCl and caustic alkalis with formation of yellow solutions. It gave a yellow color with Mg-HCl, a reddish violet color with Zn-HCl, and a faint blue color with $FeCl_3$. It had Rf values 0.80 (solv. 1), 0.80 (solv. 2), and 0.00 (solv. 3) with tailing. IR $\nu_{C=O}$ cm^{-1}: 1655 (C=O in the γ-pyrone ring), 1686 (*p*-coumarate). *Anal.* Calcd. for $C_{30}H_{26}O_{12}\cdot H_2O$: C, 60.40; H, 4.80; H_2O, 3.0. Found: C, 60.49; H, 4.99; H_2O, 3.2.

Terniflorin Acetate——1) A mixture of terniflorin, Ac_2O, and anhydrous AcONa was refluxed for 3 hr. After being cooled, the reaction mixture was poured into H_2O, and the resulting precipitate was collected, washed with H_2O, and recrystallized from MeOH to colorless needles, m.p. 172~174°, which was raised to 174~175° by drying *in vacuo* over P_2O_5 at 100°. It gave an orange color with Mg-HCl, an orange color with Zn-HCl, and no coloration with $FeCl_3$. UV λ_{max}^{EtOH} mμ (log ε): 259 (4.55), 294 (4.67). IR $\nu_{C=O}$ cm^{-1}: 1640 (C=O in the γ-pyrone ring), 1716 (*p*-coumarate), 1762 (acetate). *Anal.* Calcd. for $C_{42}H_{38}O_{18}\cdot H_2O$: C, 59.43; H, 4.72. Found: C, 59.62; H, 4.66.

2) A mixture of terniflorin, Ac_2O, and pyridine was set aside at room temp. overnight. Next day, the reaction mixture was poured into H_2O, and the precipitate was collected, washed with H_2O, and recrystallized from MeOH, giving colorless needles, m.p. 171~175°. After drying as above, it melted sharply at 174~175°. Admixture with the foregoing acetate did not depress the melting point.

Alkali Fission of Terniflorin——Two mg. of terniflorin was refluxed with 0.5 ml. of 50% KOH solution for 15 min. The reaction mixture was diluted with H_2O, acidified with dil. HCl, and extracted with Et_2O. The Et_2O extract was subjected to paper chromatography using *p*-coumaric acid, *p*-hydroxybenzoic acid, and phloroglucinol as checks. Three spots were revealed on the paper chromatograms of three phenolic compounds, the Rf values of which agreed with those of authentic specimens.

Alkaline Hydrolysis——A solution of 126 mg. of terniflorin in 100 ml. of 1% KOH solution was allowed to stand at room temperature overnight. Next day, the reaction mixture was acidified with dil. HCl, and crude cosmosiin deposited as a gelatinous mass was collected, washed with H_2O, and dried. Yield, 92 mg.

The filtrate was extracted with Et_2O, and the Et_2O extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crystalline residue was recrystallized from H_2O to almost

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10) L. Birkofer, C. Kaiser: Z. Naturforsch., **17b**, 279 (1962) (C.A., **57**, 7216 (1962)).

colorless needles, m.p. 213~214°(decomp.), either alone or on admixture with *p*-coumaric acid. Yield, 33 mg.

Cosmosiin——Crude cosmosiin was recrystallized from pyridine-H_2O to yellow needles, m.p. 232~233° (decomp.), either alone or on admixture with authentic cosmosiin. It gave an orange color with Mg-HCl, an orange color with Zn-HCl, and a reddish brown color with $FeCl_3$. Co-chromatography on paper and comparison of the UV and IR absorptions established the identity of the compound and cosmosiin. IR: $\nu_{C=O}$ 1657 cm^{-1}. *Anal.* Calcd. for $C_{21}H_{20}O_{10}\cdot H_2O$: C, 56.00; H, 4.89. Found: C, 56.04; H, 4.97.

Cosmosiin was boiled with conc. HCl on a water bath for 15 min., and the reaction mixture was diluted with H_2O. Next day, the aglucon was collected, washed with H_2O, and subjected to paper chromatography. The Rf values were found to be indistinguishable from those of apigenin.

The aglucon was acetylated with Ac_2O and pyridine in the usual manner, and the acetate was recrystallized from MeOH to colorless needles, m.p. 181~182°, either alone or on admixture with authentic tri-O-acetylapigenin. *Anal.* Calcd. for $C_{21}H_{16}O_8$: C, 63.63; H, 4.07. Found: C, 63.62; H, 4.11.

The mother liquor was neutralized by passing through a column of Amberlite IR-45 (OH type), concentrated to a small volume *in vacuo*, and subjected to paper chromatography. One spot was revealed on the paper chromatogram of a sugar, the running distance of which was found to be the same as that of D-glucose.

Acidic Hydrolysis of Terniflorin——A mixture of ca. 10 mg. of terniflorin and 1 ml. of conc. HCl was boiled on a water bath for 15 min. After being cooled, the reaction mixture was diluted with H_2O, and allowed to stand overnight. Next day, the aglucon was centrifuged, washed with H_2O, and chromatographed on paper. Rf values of the spots revealed on the paper chromatograms were found to be indistinguishable from those of apigenin.

The supernatant was extracted several times with Et_2O, and the combined Et_2O extracts were subjected to paper chromatography. Rf values and colors of the spots revealed on the paper chromatograms were found to be indistinguishable from those of *p*-coumaric acid.

The mother liqour was neutralized as above, concentrated to a small volume, and subjected to paper chromatography. The running distance of the compound on paper was found to be indistinguishable from that of D-glucose.

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Summary

The presence of a new flavonoid glycoside, terniflorin, $C_{30}H_{26}O_{12}\cdot H_2O$, m.p. 266~267° (decomp.), is demonstrated in flowers of *Clematis terniflora* A. P. de CANDOLLE var. *robusta* TAMURA (Ranunculaceae).

On basis of composition, hydrolysis, and ultraviolet and infrared spectra, terniflorin is identified as apigenin-7-(*p*-coumaroyl)-*β*-D-glucoside.

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