Flavones in Green Tea

Part II. Identification of Isovitexin and Saponarin

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In the previous paper,¹⁾ the author reported that nineteen flavones were newly found in green tea infusion. One of them, pigment 11, was identical with vitexin and pigment 1 seemed to be isovitexin (saponaretin) on comparison of its UV spectrum and *R_f* values with those of authentic preparation.

As pigment 1 could not be crystallized by repeated crystallization procedures, it was finally freeze-dried. The melting point (212~

214°C) remained undepressed on admixture with an authentic sample of isovitexin* (measured by a micro hot stage). The IR spectrum was superimposable. (Fig. 1).

The occurrence of saponarin (vitexin-7-glucoside) was not indicated in the previous paper because it could not be obtained from 2 kg of green tea. However a large-scale isolation procedure made it possible to prepare a relatively large amount of saponarin from

FIG. 1. IR Spectra of Authentic Isovitexin (A) and the One Isolated from Green Tea (B), (KBr disc).

1) Y. Sakamoto, *Agr. Biol. Chem.*, **31**, 1029 (1967).

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TABLE I. PAPER CHROMATOGRAPHIC DATA OF SAPONARIN ISOLATED FROM GREEN TEA

	Solvents			No reagent		Color reagents				FeCl ₃
	I	II	III	visible	uv.	AlCl ₃		Na ₂ CO ₃		
						vis.	uv.	vis.	uv.	
A	0.54	0.38	0.65	pale-Y	RBr	p-Y	GrY	Y	LY	Br
B	0.55	0.38	0.65	pale-Y	RBr	p-Y	GrY	Y	LY	Br

A: Saponarin isolated from green tea.

B: Authentic saponarin

Solvents I: *n*-BuOH:AcOH:H₂O=4:1:2 Vol. %

II: 2% AcOH

III: 75% aqueous phenol

FIG. 2. UV Spectra of Isolated Saponarin (13 mg/liter) (in EtOH)

20 kg of green tea.

The isolation method of pigments from green tea infusion was described in the previous paper.¹⁾ By means of nylon powder and then silica gel-water saturated *n*-butanol column chromatographies, the fractions containing saponarin only were separated from the other pigments. Crude saponarin was obtained as a brownish yellow sandy powder. Recrystallization from a large volume of boiling ethanol gave about 150 mg of pure saponarin.

The isolated saponarin was a pale yellow substance and melted at 229°C (with a micro hot stage). When it was dried at about 135°C (xylol bath) for 2.5 hr over phosphorus per

FIG. 3. IR Spectra of Authentic Saponarin (A) and the One Isolated from Green Tea (B), (KBr disc).

toxide *in vacuo*, 24.89 mg of the sample lost 1.07 mg of the weight which corresponds to 1.5 moles of H_2O (4.25%).

Anal. Found*: C, 50.86; H, 5.62. Calcd. for $C_{27}H_{32}O_{16} \cdot 1\frac{1}{2}H_2O$ ($H_2O=4.22\%$): C, 50.70; H, 5.48%. Moreover, it was identical with authentic saponarin** on comparison of their *Rf* values, UV and IR spectra, mp, and color reactions. *Rf* values of saponarin are shown in Table I. The sample showed absorption maxima at 272 $m\mu$ ($\log \epsilon=4.38$) and 335 $m\mu$ ($\log \epsilon=4.44$), and the absorption spectral data on aluminum chloride complex-, sodium acetate-, and sodium ethylate shift coincident with those of the authentic preparation as shown in Fig. 2.***

The IR spectra of the sample and authentic saponarin were also completely superimposable as shown in Fig. 3.*

In green tea infusion, the content of saponarin was generally so small (150 mg/20 kg) that the spot was supposed not to be detected on the paper chromatogram. However, further paper chromatographic studies revealed that the saponarin content varied depending on the source of green tea, for example, varieties, the places of production and so forth. Therefore, the spot of saponarin was detected on the two dimensional paper chromatograms if the samples which had relatively high contents of it.

* Elementary analysis was carried out by the Lab. of Elem. Anal., Shizuoka Univ. of Pharm.

** The authentic saponarin was given by Dr. N. Morita, Prof. of Toyama Univ., to whom the author wishes to express his thanks.

*** UV spectra were examined with a Beckman DB spectrophotometer with a potentiometric recorder.

* IR spectra were recorded with an Infrared spectrophotometer, IR-G, Japan Spectroscopic Co.