# Studies on the Viscous Substance Dripping from the Leaves of Shiia-trees

## Part 3

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The plant secrete, viscous substance dripped from Shiia-trees (*Shiia* and *Lithocarpus*), with microscopic observation, the amount of secrete with periodical determination and with environmental conditions and age of tree, and the predominate components of secrete, glucose, fructose, sucrose, and inositol, identified with paper chromatography were published on the previous paper.[1)]

The chemical components which were identified with paper chromatography were confirmed with gas chromatography. These compounds were decided as fructose, $\alpha$-glucose, $\beta$-glucose, myo-inositol and sucrose.

## EXPERIMENTAL AND RESULTS

### Paper chromatography

There are various kinds of component in leaf is easily considered. Then, the predominate component which was classified as one saccharide and was related closely to viscous substance dripping from leaf of Shiia-tree was investigated in this experiment.

The reference experiment which was one preliminary experiment for detect the difference depending upon proceeding time was carried on in a periodic interval.

Each group of one handred leaves was cut out from the branch during May to September in a periodic interval. The record of sampling was shown in Table 1.

Table 1.
Record of sampling

| Date | 1st May | 16th June | 31st July | 15th September |
|---|---|---|---|---|
| Weight (g) of one handred leaves | 27.1 | 33.7 | 36.8 | 29.9 |

These one hundred leaves were extracted instantly with 300 ml of 90% ethyl alcohol which contained 2.5 g of calcium carbonate for 30 min in boiling water bath. And this ethyl alcohol extraction was repeated again to protect a extraction loss which became a viscous material. Then, ethyl alcohol was evaporated until the alcoholic solution was condensed to about 200 ml under reduced pressure.

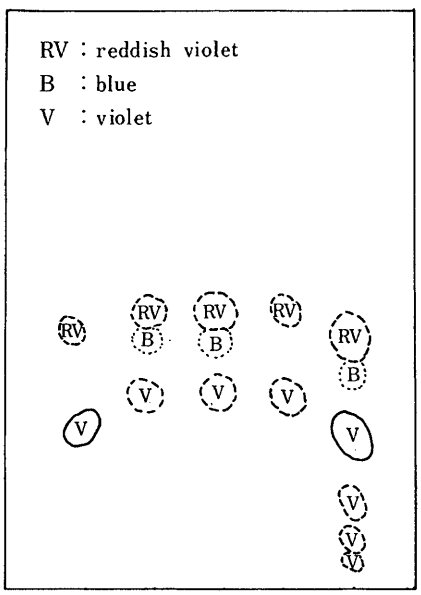
The procedure was an application of Partridge's method.[2)]

The condensed solution was spotted many times to concentrate on the same starting point. It was developed in the phase which was prepared with n-butyl alcohol:acetic acid: water (4:1:5, v/v) ascending 18 cm high. And this ascending was repeated three times. The indicator which was composed with 0.2% naphtoresorsin alcoholic solution: concentrated phosphoric acid (10:1, v/v) was sprayed to detect the compounds.

The characteristics that the colour of background, aldose, and ketose showed pale reddish pink, blue, and violet respectively made to be easy to detect them. And the colour appeared of spot after development and spraying was stable especially for a long time.

The result was shown in Fig. 1.

The detected compounds by the above described treatment were mono-saccharides and they were same to these compounds detected by the treatment which was published on the previous paper[1)].




1 : 1st May 2 : 16th June 3 : 31st July
4 : 15th September s : sample

Fig. 1.

**Gas chromatography**

The leaf that the plant secrete deposited on the external surface was washed with a neutral detergent solution. 1 g of the leaf that the midrib was taken out was cut to very small pieces. They and very small amount of sodium carbonate were added into 50 ml of 90% ethyl alcohol which was prepared to extract the components of leaf.

After the first ethyl alcohol extraction was carried on for 12 min in boiling water bath, the first extracted leaf was again extracted with 30 ml of 90% ethyl alcohol for 10 min. Then, they were filtered again. After the extraction and filtration procedures were repeated again. This fraction was called the third ethyl alcohol extraction fraction. The leaf extracted was used to detect a starch in tissue with iodine using Sachs' method.[3)]

Then, the filtrate was evaporated to about 2 ml. The protein in filtrate was excepted with 3 to 5 ml of saturated solution which was prepared with lead acetate and ammonium oxalate. The filtrate was diluted with water to 100 ml of the solution which was called protein free solution. Then, the pH of 20 ml of the protein free solution was adjusted to 7.5–7.8 with N/10 barium hydroxide. The solution was filtered with two layers of hard filter papers (No.5C).[4)] Its filtrate was passed through the column (6×1, cm) packed with Dowex 50H (60–80 mesh).

And 20 ml of the passed solution was evaporated to about 2 ml and dried up using test tube (15/25) under reduced pressure.

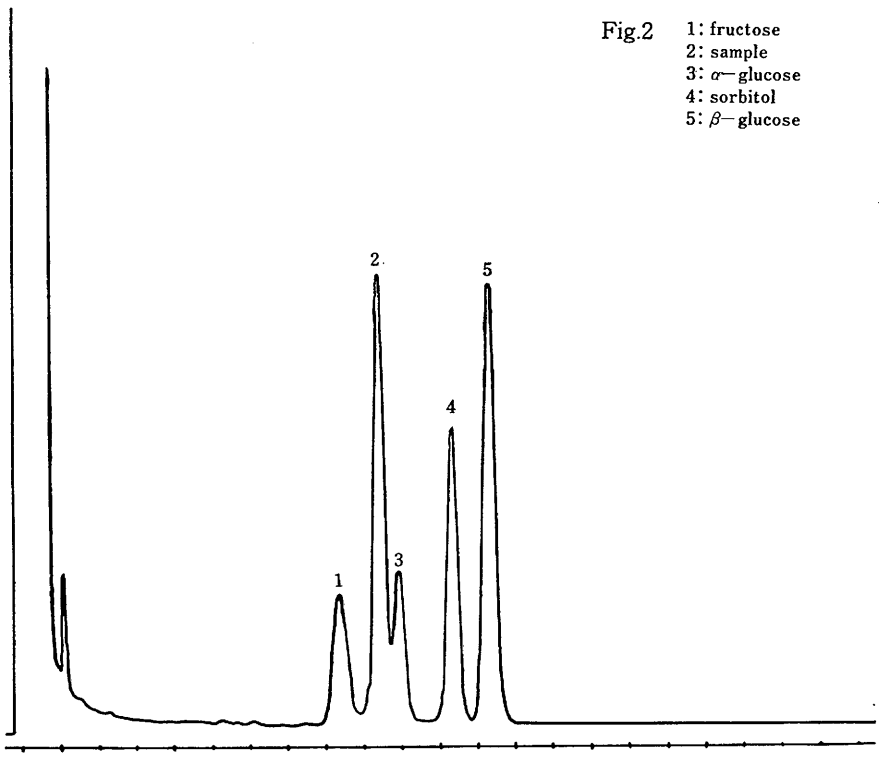



Fig.2 1: fructose
2: sample
3: α–glucose
4: sorbitol
5: β–glucose

And 1 ml of pyridine, 0.1 ml of trimetlylchlorosilane (TMCS), and 0.2 ml of hexamethyldisilazane (HMDS) were added into the test tube to produce TMCS-compound. 2$\mu$l of TMCS-compound was analyzed with gas chromatography.

The result obtained was shown in Fig. 2. And the result of the authentic compounds which was as one kind of comparative substance was shown in Fig. 2.

Running experimental condition of gas chromatography was as follows;

| | |
|---|---|
| Column: | SE−30 (5 %) 1.5 m glass column |
| Temperature: | 160−280°C, 4°C/min |
| Column: | 160−250°C, 2°C/min, programming |
| Detector: | 300°C (FID) |
| Inject: | 300°C |
| Gas: | $N_2$; 60 ml/min, $H_2$; 0.7 kg/cm$^2$, Air; 1 kg/cm$^2$ |
| Sensitivity: | $10^3$ |
| Range: | 1/32 |
| Chart speed: | 5 mm/min (one division; 2 min) |
| Sample: | 2$\mu$l |
| Apparatus: | SHIMAZU G.C. 4BM PFE |

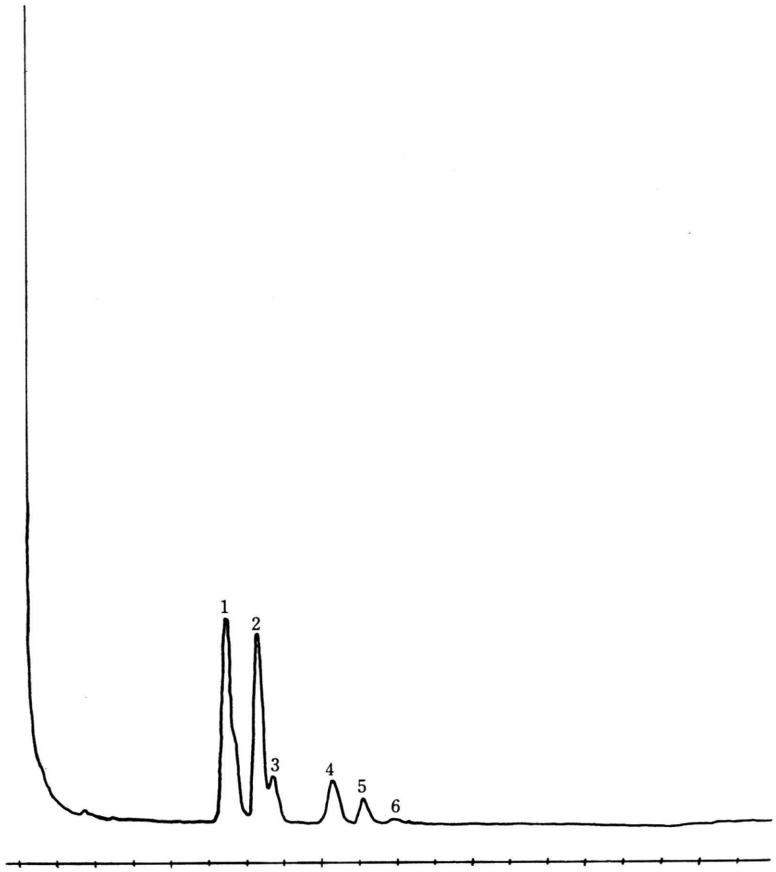
The six peaks could be detected gas chromatographically in comparison with the authentic compounds. The result was shown in Fig. 3.

The first peak which appeared at early time had an undetected little shoulder. But, it was assigned to fructose. And the third one, the fourth one, the fifth one and the sixth one were assigned to $\alpha$-glucose, sorbitol, $\beta$-glucose and inositol respectively. However, the second peak was not assigned.

The predominate components of leaf in early summer was analyzed with gas chromatography. But, the amount of secrete at that time was a very small amount which could not almost be collected. The photograph of the state of leaf at that time was shown in Fig. 4. Then, the secrete might be periodic in year was induced under the consideration that this phenomenon had to be recognized chemically again. Also, the components of leaf itself had to be detected in the same analytical procedure as a reference.

The predominate components of secrete deposited on external surface of leaf were analyzed chemically and gas chromatographically.

0.1 g of the secrete which was taken out from the surface was dissolved in 30 ml of 90 % ethyl alcohol. And its solution was evaporated to 2 ml under reduced pressure. And the protein in the condensed solution was excepted with 3 to 5 ml of saturated solution of lead acetate and ammonium oxalate. After the solution was diluted to 100 ml with water, 20 ml of the solution was adjusted at pH 7.5−7.8 with N/10 barium hydroxide using pH meter. Then, the adjusted solution was filtered with two layers of hard filter paper (No.5C)[4]. After the filtrate was evaporated to about 2 ml under reduced pressure, it was dried up in




1: fructose
2: unknown
3: α—D—glucose
4: sorbitol
5: β—glucose
6: inositol

Fig. 3.

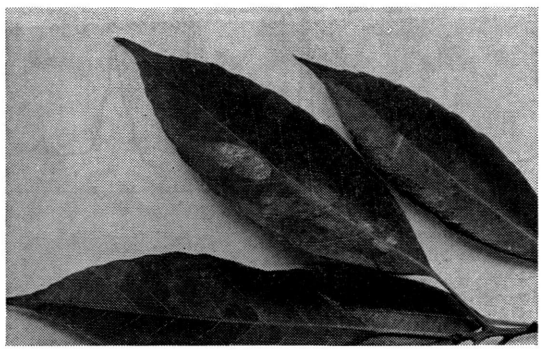


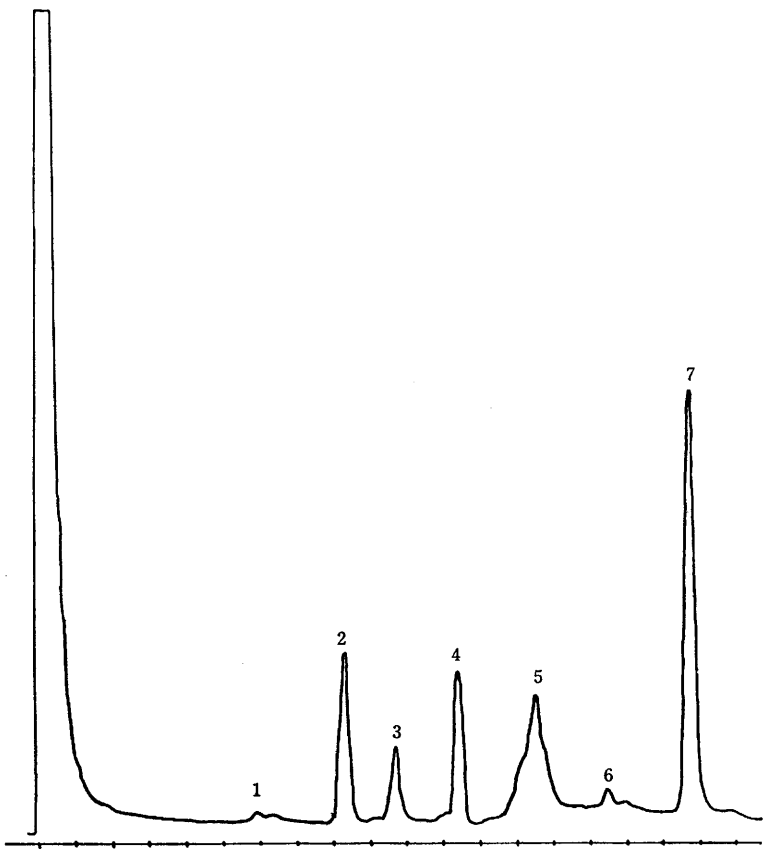
Fig.4

test tube (15/25). And 1 ml of pyridine, 0.1 ml of trimethylchlorosilane (TMCS), and 0.2 ml of hexamethyldisilazane (HMDS) were added into the test tube. 2$\mu$l of TMCS-compound was analyzed with gas chromatography.

The result obtained was shown in Fig. 5.

The detected compounds were five carbohydrates in appeared seven peaks. And these peaks, the first peak to the fifth peak were identified to sucrose, $\alpha$-glucose, D-fructose, $\beta$-glucose and inositol respectively. Especially, inositol was decided to myo-inositol in comparison with a authentic sample.

These stereochemical structure requires other several experiments, so the absolute conformation might be decided in future.




1 : unknown
2 : fructose
3 : $\alpha$—glucose
4 : $\beta$—glucose
5 : inositol
6 : unknown
7 : sucrose

Fig. 5.

**Starch**

This analytical procedure was partially performed by the method of Sachs.[5)]

1 g of leaf was extracted with 90 % ethyl alcohol three times. The liquid phase, extract, was consumed to determine mono- and oligosaccharide, like glucose, sucrose etc., but the solid phase, many small pieces, was consumed to determine polysaccharide, like starch etc. The solid phase, many small pieces, was added into 10 ml of 20 % perchloric acid. They were crushed and ground to syrupy material in porcelain motor for 5 min. After they were left on a desk for 1 hour, the syrupy material was taken into 50 ml flask washing the inside of motor with 20 % perchloric acid. And its solution was filled up to 50 ml exactly with 20 % perchloric acid. And it was centrifugated for 10 min at 4000 r.p.m. 5 ml of the supernatant solution was diluted to 25 ml with 20 % perchloric acid. And 0.2 ml of N/100 iodine-potassium iodate solution was added into it. The colour of sample solution became a very slight violet blue. The absorbance was determined at 600 nm in comparison with the starch-iodine standard curve.

The starch solution that 50 ml of soluble starch dissolved in 50 ml of 20 % perchloric acid was used as standard starch solution. After each 1 ml, 2 ml, and 5 ml, of the standard starch solution was taken into 25 ml volumetric flask respectively, 0.2 ml of N/100 iodine-potassium iodate solution added into each starch solution. And each solution was diluted to 25 ml exactly with 20 % perchloric acid. The absorbances of standard starch solution and sample solution were summarized in Table 1.

Table 1.
Absorbances of standard starch solution and sample solution at 600 nm

| Starch solution (content, mg) | 1 | 2 | 5 | |
|---|---|---|---|---|
| Sample solution (conversion, mg) | | | | 1.6 |
| Absorbance | 0.066 | 0.127 | 0.273 | 0.205 |

The estimation of saccharide on gas chromatogram was made approximately. This calculation was seemed to be valuable for qualitative one only.

Fructose: Weight;

area (0.188 $cm^2$) x coefficient (1.688) ÷ factor (2.00)

= weight (0.0555 mg)

Percentage;

weight (0.0555 mg) ÷ constant (1.5) x 100 = 3.70 (%)

Glucose = $\alpha$-glucose + $\beta$-glucose:
weight;
area (0.231 + 0.80 $cm^2$) x coefficient (1.994) ÷ factor (2.00)
= weight (0.0578 mg)
Percentage;
weight (0.0578 mg) x constant (1.5) x 100 = 3.85 (%)

Sucrose: Weight;
area (0.506 $cm^2$) x coefficient (0.333) ÷ factor (2.00)
= weight (0.7601 mg)
Percentage;
weight (0.7601 mg) x constant (1.5) x 100 = 50.68 (%)

Coefficient 1.688, 1.994, or 0.333 was decided by these data of concentration of sample, retention time and area of peak when the authentic sample, fructose, glucose, or sucrose, was analyzed under the constant condition carried out unchangeably in this experiment.

Factor 1.5 was decided by the datum of performed condition, 6 ml of sample, in comparison with the datum of the standard condition, 4 ml of sample.

The sum of percentage determined was 58.23, so the rest percentage might include inositol and raffinose which was possible to detect with paper chromatography. Also, other material or impure material might be included.

## ACKNOWLEDGEMENT

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## SUMMARY

The predominate components of Shiia-tree sampled in a periodic interval, lst May, 16th June, 31st July, 15th September, were investigated to detect the proceeding of metabolism with paper chromatography.

Fructose and sucrose were identified in four samples. However, glucose was detected only in the sample on 16th June and 31st July.

The predominate components of leaf sampled on 17th July were determined with gas chromatography as TMCS-compounds. Fructose, $\alpha$-glucose, sorbitol, $\beta$-glucose, and inositol were identified in comparison with the authentic compounds.

Also, the predominate compounds of secrete sampled at the beginning March were determined with gas chromatography like the analysis of components of leaf as TMCS-compounds.

And sucrose, $\alpha$-glucose, D-fructose, $\beta$-glucose and myo-inositol were identified according to the authentic compounds. Then, starch of leaf sampled on 17th July was determined approximate qualitatively with the absorbane of iodine starch reacted solution at 600 nm. The approximate amount of starch to leaf was about 1.6 mg per g in weight.

## REFERENCES

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2) Partridge, S.M.: Biochem. J. 42, 238 (1948).
3) Sachs. J.: Ex. Nu. Botany 382(1959)〔in Japanese〕
4) analytical grade, TOYO ROSHI CO. LTD.

〔内容抄録〕 椎の木の葉から落下する粘稠性物質に関する研究（第3報）

高橋敬三　堀津圭佑　南雲葉子　三吉淑子

椎の木の葉から滴下する植物分泌物，粘稠性物質の顕微鏡観察，分泌物質量の期間的測定，環境条件と樹令とその量，ペーパークロマトグラフで同定された葡萄糖，果糖，庶糖とイノシトールの分泌物の主要成分を本報に報告した。

ペーパークロマトグラフで同定された化学的成分はガスクロマトグラフで確認された。それらの化合物は，$\alpha$-葡萄糖，$\beta$-葡萄糖，マイオイノシトールと庶糖と決定した。