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

Part 2

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The several compounds of dripped viscous substance from leaves of Shiia-trees (*Shiia* and *Lithocarpus*) were detected or identified with paper chromatography. The identified and reported[1] compounds were fructose, glucose, sucrose, and raffinose.

And, on this paper, it is reported that the secreted substances were composed with fructose, glucose, sucrose, raffinose and inositol according to paper chromatography and that they were different from secrete of insect. Also, the results from some several observations about secrete in tissue of leaf were shown photographically.

EXPERIMETAL AND RESULTS

Tree

These trees are classified to *Shiia cuspidata Makino* and *Lithocarpus glabra Nakai.*

The selected three test trees, No.1, No.2, and No.3, which were grown in the authors' campus were 50 cm, 110 cm, 215 cm of round length at 140 cm high from ground. They which were selected as sample tree were represented as relative different tree in age.

Microscopic observation

(a) External surface of leaf

The passing of secrete from cell was observed under microscopic condition, so the microscopic view were photographied as shown in Fig.1 to Fig.4. Each sample leaf was treated with the method of Sachs[2] that was applied mainly to observe suger derivative in this case.

After 1 g of round part punched from leaf except vein was heated in boiling water bath for 3 to 5 min, it was extracted with 100 ml of ethyl alcohol in boiling water bath for 35 min. Then, after its extracted leaf was dried on silica gel in desiccator, the dried leaf was immersed in iodine-chloral hydrate solution that iodine was saturated in 2 ml of water contained 5 g of chloral hydrate.

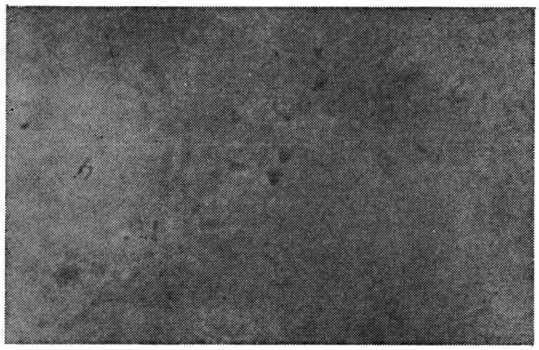


Fig. 1

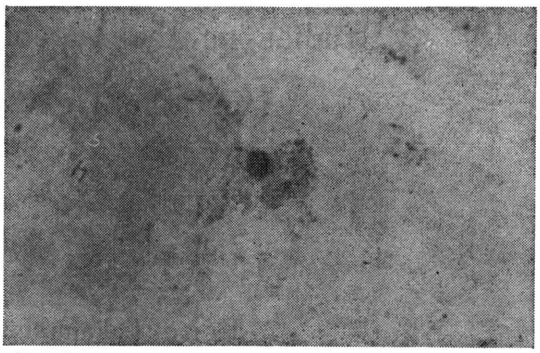


Fig. 2

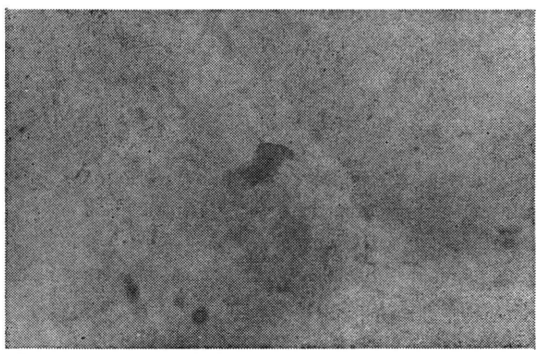


Fig. 3

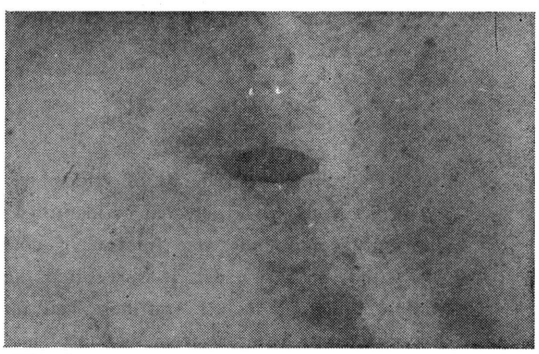


Fig. 4

(b) **Thin section of leaf**

After these sample leaves were cut out from the branch, the observation and the photographing about each one of many thin sections prepared with a microtome were carried out to research the distribution of secrete in tissue rapidly and carefully as possible. Because this kind of experiment had to be performed before the unexpective changes that the secrete moved to other site or converted to other compounds occured in unstable cell.

The first preparate was treated at -20°C using dry ice, the thin section was prepared at about 10μ using a microtome. This thin section was treated with very small amount of the above described iodine-chloral hydrate solution. And instantly, the microscopic observation was recorded photographically.

These photographs were shown in Fig.5 to Fig.8.

Secrete

The amounts of secrete dripped on each glass plate (30×30, cm^2) against their leaves of these three trees, No.1, No.2, and No.3, were estimated for this experimental period, from 9th May to 24th June, which was the best sampling time in a year to collect much secrete. Each glass plate was figured with square section (5×5, cm^2) and the number of drop of secrete dripped on ten predominate sections was numerated. And each glass plate was exchanged by fresh one at three setting times, 9:00 a.m., 0:00 p.m. and 4:00 p.m.

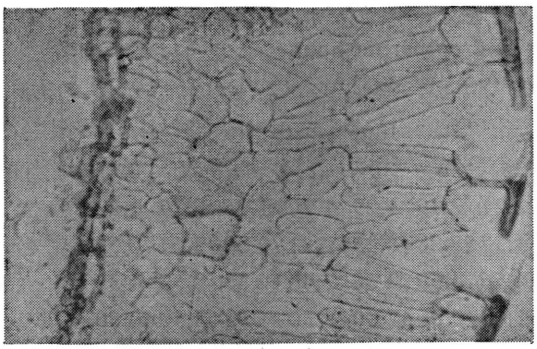


Fig. 5

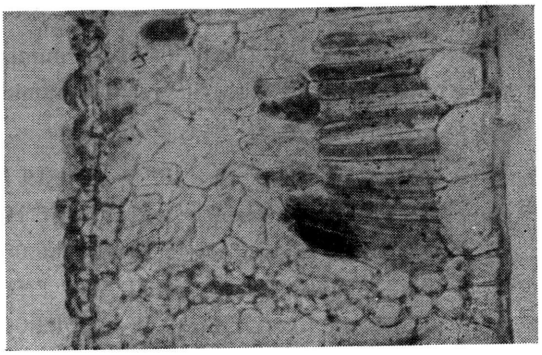


Fig. 6

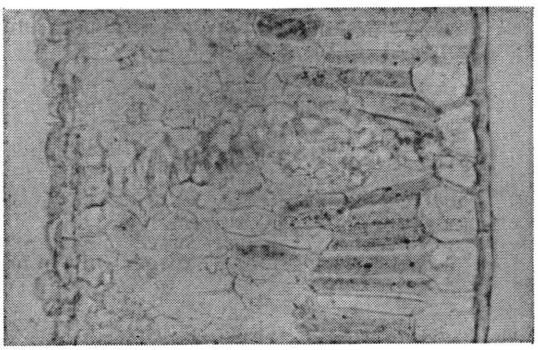


Fig. 7

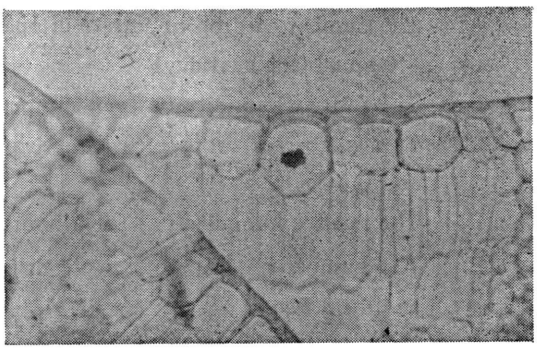


Fig. 8

The estimations of amounts of secrete were carried on a day or a hour against some environmental conditions which were determined a day or a hour. However, the determination time of environmental condition was partially different from sampling time. The temperature, the relative humidity, and the amount of transpiration were determined at 9:00 a.m., 0:00 p.m. and 3:00 p.m.

These results are summarized in Table 1 to Table 3 respectively.

Table 1.
The relation between the number of drop of secrete and the relative humidity a day

Date	May							June						
	10	11	12	13	17	18	19	25	26	1	2	3	7	24
A	9.44	12.83	12.29	6.01	3.57	5.30	6.82	2.84	2.92	2.06	1.96	2.66	1.10	2.12
B	45	68	68	74	45	56	73	57	57	56	62	72	71	70

A: Average number of drop of secrete dripping on 5x5 cm^2 a day, between 4:00 p.m. and 4:00 p.m. (the next day).

B: Relative humidity(%) a day, between 3:00 p.m. and 3:00 p.m. (the next day).

Table 2.

The ratios of the number of drop per set hour
to the relative humidity (%) per set hour

Date	May									June				
	10	11	12	13	17	18	19	25	26	1	2	3	7	24
A	8.73	11.75	11.14	6.01	3.17	4.74	6.19	2.21	2.39	1.29	1.20	1.95	0.79	1.72
B	57.00	53.00	72.42	68.71	45.14	58.00	66.00	64.57	55.28	48.28	59.71	70.00	72.57	76.57
C	9.63	14.60	14.66		4.50	5.80	8.56	4.56	4.50	3.36	3.40	4.83	2.50	4.30
D	32.66	55.00	50.50	75.00	30.50	48.00	63.00	51.00	49.00	42.00	48.50	66.00	63.50	60.50
E	12.32	16.07	15.37		4.57	7.32	8.20	4.22	3.97	4.37	4.10	4.07	1.37	4.92
F	47.33	58.50	49.00	71.50	34.00	42.50	64.00	43.00	47.50	45.00	46.00	58.50	62.00	57.50

A: The number of drop of secrete dripped on 5x5 cm per 17 hours, between 4:00 p.m. and 4:00 p.m. (the next day).

B: The relative humidity (%) per 18 hours, between 3:00 p.m. and 9:00 a.m. (the next day).

C: The number of drops of secrete dripped on 5x5 cm^2 per 3 hours, between 9:00 a.m. and 0:00 p.m.

D: The relative humidity (%) per 3 hours, between 9:00 a.m. and 0:00 p.m.

E: The number of drops of secrete dripped on 5x5 cm^2 per 4 hours, between 0:00 p.m. and 4:00 p.m.

F: The relative humidity (%) per 3 hours, between 0:00 p.m. and 3:00 p.m.

Table 3.

The relation between the number of drop of
secrete and the amount of transpiration

Date	May							June						
	10	11	12	13	17	18	19	25	26	1	2	3	7	24
A	74.6	102.7	98.3	102.2	28.6	40.4	56.7	22.8	23.4	16.5	15.7	21.4	8.8	20.6
B	54	35	49	24	83	42	18	49	51	62	29	27	35	44

A: The number of drop of secrete dripped on 5x5 cm^2 a day, between 4:00 p.m. and 4:00 p.m. (the next day).

B: The amount of transpiration (unit, 0.1mm) a day, between 3:00 p.m. and 3:00 p.m. (the next day).

From these results obtained, the most amount of secrete might be secreted from a intermediate aged tree, No.2, between these three trees since the biological activity of the tree might be at the highest level at least in comparison with other two trees. The amount of secrete might be influenced by sunlight time, temperature, relative humidity, and amount of transpiration.

The obtained results proved partially that a growth, an assimilation, or a dessimilation of plant were in a certain basic biological periodicity. The secreted amounts all the year over were assumed to be of periodic type depending upon a large environmental condition like season. A few increase or decrease of secrete might be changed by daily weather condition. But, such a determination requires many kinds of ovservations and experiments.

A secreted amount were related to relative humidity closely as shown in Table 1 and Table 2 was possible to understand easily, and there was one relation between it and amount of transpiration. These relations were reasonable in point of biological equilibrium.

Also, animal excreted substance which attached on the leaf might be not included in this plant secrete were condisered under this observation. However, the size of *Acarina* which was a representative animal, was in a range of 400μ, so a separation of animal substance and plant substance requires various microscopic, physical or chemical experimental procedures in planning.

Paper chromatography

(a)

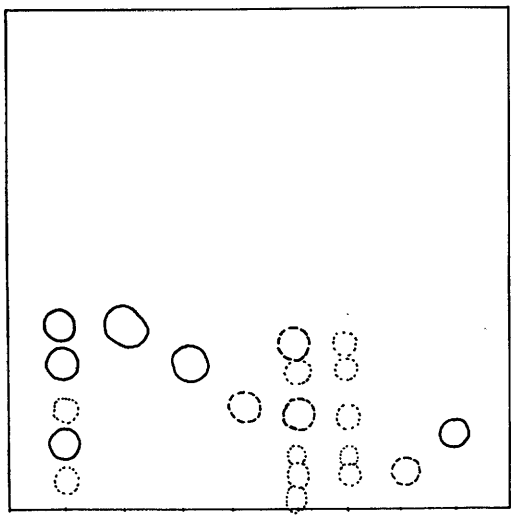
The method of Hough[3] was applied to this procedure. After about 1.5 g of the deposited secrete on the external surface of leaf was taken out, it dissolved in a small amount of water.

Then, about 50 ml of acetone was added into the aqueous solution until the precipitate appeared. After the acetone was discarded, the precipitate was dried on silica gel in desiccator. One part, 10 mg, of dried white amorphous material which was kept in desiccator was dissolved in a small amount of water. After its aqueous solution was spotted on a paper, the paper was developed with n-butyl alcohol:acetic acid: water (4:1:5,v/v), and it was dried. Then, the indicator that concentrated ammonium hydroxide solution was added dropwise into 5% silver nitrate solution until the precipitate disappeared was sprayed. And the paper was heated at 100–110°C for 5 to 10 min.

The result was shown in Fig. 9.

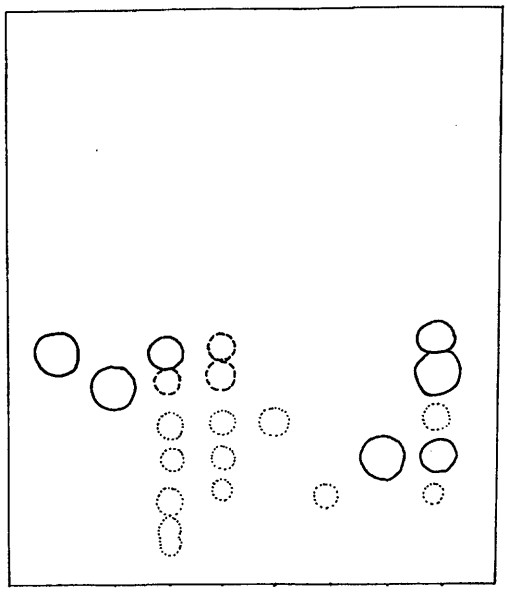
(b)

The detection of Trevelyan[4] was applied to this experiment. The sample solution was prepared as the description of (a). The paper was developed in the phase which was prepared with n-butyl alcohol : acetic acid : water (4:1:5, v/v) and was dried.



a : fructose b : glucose c : sucrose d : raffinose
e : inositol f : mixture(a,b,c,d,e) s : sample

Fig. 9



a : fructose b : glucose c : sucrose d : raffinose
e : inositol f : mixture(a,b,c,d,e) s : sample

Fig. 10

On the other hand, 20 ml of acetone was added into 0.1 ml saturated silver nitrate solution. The produced precipitate was dissolved with addition of one or two drops of water. The paper which was dried in air after development was immersed in that silver nitrate solution as possible as rapid and it was dried again. Then, 0.5 N sodium hydroxide alcoholic (80%) solution was sprayed and the paper was allowed to stand on a desk, so the distinct clear spots appeared on the paper. And the paper was immersed in 1 N sodium thiosulfate solution instantly and was washed completely with much water.

And it was dried again in air. The result was shown in Fig. 10.

Then, in this experiment, the deposited material on the surface of leaf was taken out from the leaf, and it was taken into acetone rapidly as possible. So, the hydrolysis of sucrose did not occur in this short time of chemical procedure was thought.

SUMMARY

The appearance of the plant secrete, viscous substance dripped from leaves of Shiia-tree, was periodically in year. It's maximum period was at the beginning of May and the amount of secrete decreased gradually at the end of June.

The amount of secrete was related closely to relative humidity and was more from intermediate aged tree than from young or old tree.

The state of secrete in plant tissue was observed microscopically using a microtome after it was fixed by Sach's method.

The predominate components of secrete were detected with paper chromatography. Then, glucose, fructose, sucrose and inositol which were classified as carbon compound were identified respectively.

Also, after the deposited material on the surface of leaf was taken out as sample, it was taken into acetone rapidly as possible for protect some changes.

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3) Hough. L.: Nature 165, 400 (1950)
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〔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2報）

高橋敬三　堀津圭佑　三吉淑子　南雲葉子

椎の木の葉から滴下する粘稠性物質の数種の化合物は，ペーパークロマトグラフにより検出，同定された。同定され，報告された化合物は，果糖，葡萄糖，庶糖，ラフイノーズであった。本報では，ペーパークロマトグラフによった果糖，葡萄糖，庶糖，ラフイノースとイノシトールが分泌物質を構成し，それらは昆虫の分泌物とは異る点を報告した。また，葉の組織中の分泌物についてのいくつかの観察結果を写真で示した。