イエシロアリの分泌物について：職蟻の分泌物中の糖類とアミ酸の構成(林学科)

ON THE SECRETION OF TERMITES,

Coptotermes formosanus SHIRAKI

The components of sugars and amino acids in the secretion of workers

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I. INTRODUCTION

There are many kinds of termites, but most of them are termites, *Coptotermes formosanus* SHIRAKI (4,5), in Okinawa.

Termites like to eat wood, so it appear that all source of their food is supplied only wood. Termites are similar to such as honeybees and ants from the viewpoint of making a collective living, but termites differ from them for the source of food.

It is thinkable that termites are social insects with the class system of queen, vice-queen, king, vice-king, nymph, soldier and worker. They are living in one nest.

They are sharing partial charge of their duties. The queen is the absolute ruler and her main duty is to increase the clan.

The vice-queen and the vice-king take charge of succession in case of trouble occurred on the queen and king. Nymph is unripe. Soldiers defend their clan against the enemy and also they watch over workers. It is thinkable that the workers acquire the food and water for themselves, and support all other termites not to speak of the queen.

Now, if the soldiers and workers, who have lived under such community life up the present, are kept in a different surroundings.

What type of made of life would they take?

At first, it is thinkable that they work only to obtain food for themselves. Otherwise, it is thinkable that they still belong to their old nest and behave in the same way that they have done up to the present. The soldiers and workers were transfered without impetus to the fresh wood meal of Ryukyu matsu by a mere accident.

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Sci. Bull. Coll. Agr. Univ. Ryukyus, 19 : 481~488 (1972)

After then, it had been observed that the secretion were released from the mouth and anus of termites at a certain place. It has been studing about termites by MOORE (7) for the point of Pheromone. But there are no literatures for the secretion which has been obtained by the author at this time. As soldiers eat the secretion, it is thinkable that the secretion has relation with other termites not speak of the queen. For the constitution of the secretion were investigated.

II EXPERIMENT

II-1 The secretion:

The secretion of termites (soldiers 30, workers 300) were gathered in August (5g) and January (2g) respectively.

II-2 Separation of the secretion:

Add 6N-HCl to the milled secretion and then heat 48 hrs in the boiling water. Filtrates the hydrolysis solution with glass-filter in an ordinary manual, and passes the filtrates through in Amberlite IR-120 (R-H Type) (1ml/min). Only cations of co-exist-salt are adsorbed. Anions of inorganic, organic acid ions and sugars were all flowed out. Then, wash the column with a little water.

Most of amino acids were eluted first than inorganic cations by passing 2N-NH_4OH through the column. Concentrate the effluent while removing ammonium hydroxide. Most of amino acids were adsorbed and other co-exist cations were flowered out by passing the concentrates through Amberlite IRA-410 (R-H Type) column. After the distilled water washing elute the adsorbed amino acids by passing 2N-HCl to the column. Concentrate the effluent (amino acids effluent) at reduced pressure (50°C). Keep them for sugars and amino acids samples.

II-3 Quantitave of sugars (Somogyi-nelson method):

II-3-1 Adjustment and measurment of the sample:

a) Put 0.5 ml of the sugars sample which were obtained from II-2 into a test tube and add 7.5 ml of H_2O, 1 ml of $Ba(OH)_2$ solution. Mix well with tapping the test tube by fingertips.

Fathermore add 1ml of $ZnSO_4$ solution. Shake hard the test tube.

b) Mix well after adding 1 ml of Copper reagent.

c) As soon as stands the test tube accurately for 10 minutes in boiling water, cool it for 1-2 minutes with water.

d) After adding 1 ml of Nelson reagent with mixing, stands het test

tube for 5 minutes. Dilute to 25 ml with distilled water. Measure the color photometrically at 530 mμ. The measurment must be done with handling zero adjustment using brank.

(Used Hitachi Parkin Elmer UV. vis Spectrophotometer)

(1) 5% $ZnSO_4$ solution

(2) 0.3N$Ba(OH)_2$ solution:

Dissolve 45 g $Ba(OH)_2 \cdot 8H_2O$ in 1 *l* boiled and cooled distilled water. Filtrate the $Ba(OH)_2$ solution if it become turbid. It has no importance for consistency of $ZnSO_4$ solution and $Ba(OH)_2$ solution at this experiments. But it must be neutral or slight alkalinity both equivalent mixing solution.

(3) Dissolved anhydrous sodium carbonate 24g and 12g of potassium sodium tartrate into about 250 ml of distilled water, and add 40 ml of 10% $CuSO_4$ solution with mixing. Furthermore, add 16g of sodiumbicarbonate. In another beaker dissolved 180g anhydrous Na_2SO_4 with 500 ml of warmed distilled water. This solution added into the previous solution, and make up to 1 *l* with distilled water at room temperature. Filtrate the precipitate after stands this reagent for several days.

(4) Nelson-reagent:

Dissolved 50g of ammonium molybdate into 900 ml of distilled water and put 42 ml of conc. H_2SO_4. Furthermore, 6g of pure $Na_2HAsO_4 \cdot 7H_2O$ which was dissolved with 50 ml of distilled water into the above solution after mixing well. Stands this solution 24-48 hrs at 38°C. Now, it ready for use. Keep this reagent in a brown bottle. This reagent has golden and transparent. If it has a tinge of green, put a few drops of bromine and mix well.

After the organic matter were completly decomposed, remove bromine in draft.

II-4 Chromatography of sugars :

One dimentional paper for sugars sample from II-2, Chromatography were done as follows. Used a glass container with a cover for a development container (6 cm×45 cm). Development were done by rising method at a room temperature. n-BuOH, pyridine, and H_2O were adopted for a development solvent. (n-BuOH : py. : H_2O= 3 : 2 : 1.5) Each solvents except H_2O were used after redistillation. Eight mixtures of guarantied sugar samples (D-glucose, D-galactose, D-mannose, D-xylose, L-rhamnose, lactose and D-glucronic acid) were developed. The silver nitrate in NH_4OH solution were spraied to the standard sugar samples and unknown sample from II-2. After heating the paper at 105°C for 5-10 minutes and it was compared and detected.

II-5 Quantitative of amino acids :

After amino acids unknown samples from Ⅱ-2 were developed in development solvent (AcOH : Pr : H_2O : n-buOH= 4 : 1 : 1 : 2), separate the part of pink colored and the part of yellowish colored respectively. Stand each separate samples for 20 minutes in boiling water. Afterwards, put 1 ml of ninhidrin solution and a little quantity of pyridin, cooled immediately. Dilute these extraction with a mixture of n-propanol and H_2O (1 : 1) to a fixed volume.

Colorimetried at 570 mμ for amino acids which was colored pink, and at 440 mμ for tyron group which was colored yellowish.

Ninhidrin reagent:

Dissolved 5g of ninhidrin reagent and 0.5g of $SnCl_2 \cdot 2H_2O$ with 500 ml of methylcellosolve. In this solution add 250 ml of 2N-CH_3COOH.

II-6 Two dimentional chromatography of amino acids :

Two dimentional paper chromatography for the amino acids samples, Ⅱ-2, were done as follows. Used a glass container with a cover (20×25×28cm) for a develpment container, and were done in rising method at room temperature. Adoped n-BuOH : Py : H_2O (1 : 1 : 1) for a development solvent. Adoped phenol : H_2O : conc NH_3 (160g : 40 : 1) which is contained a little quatitity of KCN for two dimentional solvent. Distillate phenol by adding 0.1% of aluminium and 0.05% $NaHCO_3$. Use n-buOH after redistillation. The authentic amino acids were sent from Kyushu university. Fifteen mixture of amino acids (L-cystine, L-tyrosine, L-asparatic acid, L-leucine, L-lysine, DL-metionine, L-pheylalanine, L-histidine · HCl · H_2O, Alanine, Glycine, DL-tryptophan, L-glutamic acid, L-arginine·HCl, L-serine, and L-proline) were development and unknown samples were compared and detected.

II-7 The orientation and aggregation test for Y-type

The experiments were done by using reformed SAMESHIMA'S Y-type (11) tester. Shown as photo.1, Y-type inducement tester was made by insect a test tube(1.5 x 16 cm) into a polyvinyl cup (6 × 10 cm) and fixed by water proof adhesives. The test tube on the both sides were connected to the vaccum pump and passed air through into the tube for 10 minutes. Afterwards, insert filter paper which contained a fixed samples and equivalent quantity of water into the test tube on the both sides, and were put 5 soldiers, 50 workers into polyvinyl cup and were observed, action of termites every 10 minutes, and

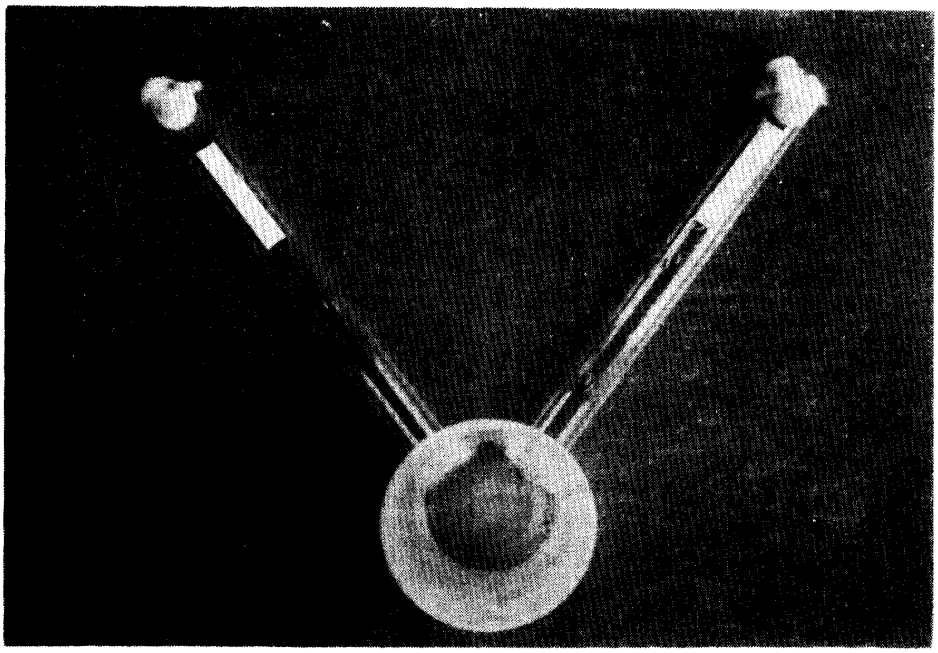

Photo. 1. The orientation and aggregation test apparatus

were checked number of termites which completely attacked the filter paper. Same experiment as above were done by replace left sample with right sample.

III. RESULT AND CONSIDERATION

II-1 Sugars

The secretion which was obtained in August and January were composed of about 55% sugars. It was identified by paper chromatography that sugars fraction gave D-glucronic acid (Rf 0.75), ? (Rf 0.32), D-galactose (Rt 0.40), D-glucose (Rf 0.45), D-xylose (Rf 0.55), L-rhamnose (Rf 0.64), ? (Rf 0.79), (1:1:2:2:2:1:1).

There are some literatures (6,12) for wood sugars, but there are no appearance of D-glucronic acid and L-rhamnose in the secretion. So it appears that L-rhamnose and D-glucronic acid made after termite adsorbed wood meal.

III-2 Amino acids

The secration which obtained in August and January were composed of 20% crude protein respectively. There are differs from that qualified the nest of termites by NAKASHIMA (8). It is indistinct that the secration gave more large quantity of crude protein than the crude protein quantity (about 10%) in Ryukyu mastu. ERGENE (1) insist that bacteria which lived on *Kalo-terms flavicollus* fix free nitrogen. As shown Fig. 1, it was identified by two

dimentional paper chromatography that amino acids gave asparatic acid, glutamic acid, alanin, tyrosine, D, E, G, (1 : 1 : 2 : 2 : 1 : 1 : 1). This is a literature for amino acids in wood by FUKUDA (2). Amino acids which

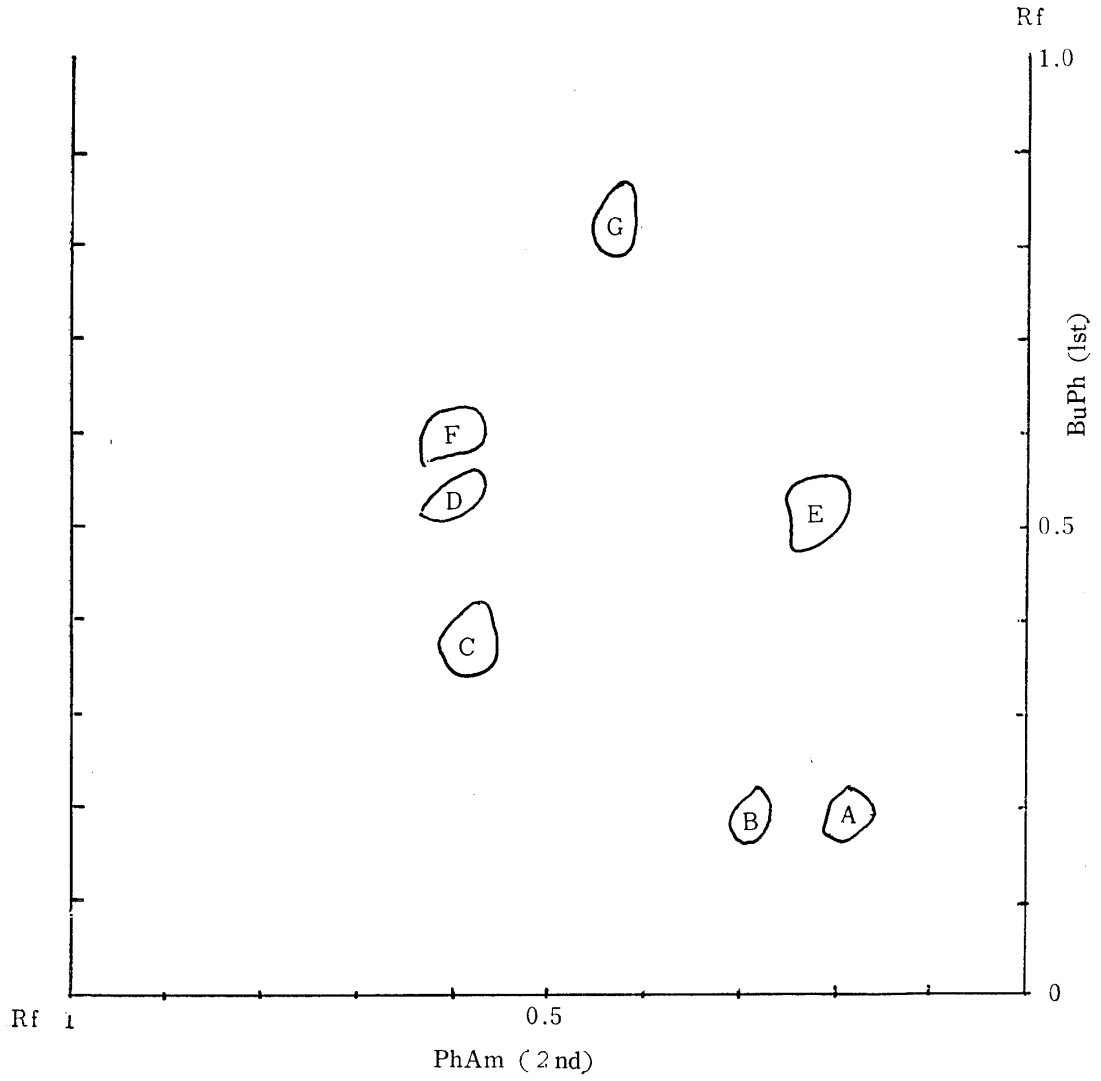

Fig. 1. Diagaram showing two-dimensional paper chromatography of secretion from termites

A. L-asparatic acid; B. L-glutamic acid; C. alanine; F. L-tyrosine; D. E. G. undetermined spots

obtained from the secretion were included in wood, but some amino acids in wood were disappeared by taking into termite. It is not clear if the disappeared amino acids are needed for worker. About free amino acids in worker are investigated by FUJII et al. (3) and others. Most of amino acids except

L-tyrosine, alanin were not identified with the secretion.

III-3 The orientation and aggregation test for termites:

As there are some literatures (9,10) for food of termites, the orientation and aggregation test for termites were studied on the secretion.

On the test for termites, it was shown that termites attacked completly hydrolysis rather than the secration.

In the hydrolysis, amino acids shown powerfully to induce termites.

Furthermore, in the amino acids, especially mixture of asparatic acid and tyrosine shown powerfully to induce termites.

IV. SUMMARY

The Secreion from the mouth and anus of termites have investigated. On the hydrolysis, the yield of sugars and amino acids were about 55%, and 20% respectively. It was identified by paper chromatogrphy that sugars fraction gave D-glucronic acid, (Rf 0.32) D-galactose, D-glucose, D-xylose, L-rhamnsoe, (Rf 0.79), (1 : 1 · 2 · 2 : 2 : 1 · 1 : 1) and amino acids fraction gave asparatic acid, L-glutamic acid, alanine, L-tyrocine, D,E,G, (1 : 1 : 2 : 2 : 1 : 1 : 1),

Besides, on the orientation and aggregation test of termites, it was shown that termites attacked amino acids fraction. In the amino acids, especially, mixture of L-asparatic acid and L-tyrosine shown powerfully to inducet ermites.

Acknowledgment——In succession of this study, the author wishs to thank Professor Tamio KONDO, Assistant professor Masasi SUMIMOTO, and Assistant Isako SAEKI, Faculty of Agriculture, Kyushu University for their consistently guidance.

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摘　　要

イエシロアリの口腔および肛門から排出された分泌物について検討した。酸加水分解によって，分泌物は約55%の糖と20%のアミノ酸で構成されていた。ペーパークロマトグラフイにより糖の構成は D-gluculonic acid, ? (Rf 0.32), D-galactose, D-glucose, D-xylose ,L-rhamnose, ? (Rf 0.79) がスポット面積比で1：1：2：2：1：1であり，アミノ酸の構成は Asparatic acid,L-glutamic acid, Alanine, L-tyrosine, D, E, G がスポット面積比で1：1：2：2：1：1：1であることを示した。

一方嗜好性テストで糖部よりはアミノ酸部に嗜好性を示し，アミノ酸でも，とくに Asparatic acid とL-tyrosine の混合物に強い嗜好性を示した。