Chemo-Physiology of the Corn Smut Fungus, Ustilago Zeae (Beckm.) Unger

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Preface

A large quantity of the fungous galls caused by Ustilago zeae, the corn smut fungus, was collected by the senior author in the experimental field of the College of Dairy Agriculture, Nopporo, Hokkaido and he was engaged in studying chiefly the sugar components of the gall. He happened to discover a new polysaccharide in young fungous galls.

In 1966, he pursued his investigation, as a research student, at the Department of Agricultural Chemistry, Tohoku University, Sendai, under the direction of Dr. Kazuo Matsuda to elucidate the chemical structure of this unknown polysaccharide. Acid hydrolysis, isolation of the hydrolysisates by carbon-celite column chromatography as well as by filter-paper partition chromatography, and the acetate of each compo- have shown that the polysaccharide in question is a new glucan composed of a main chain of 1-6 bond, supposedly having a small amount of side chain of 1-3 bond, and this polysaccharide was named, "zeagallan" ("zea" comes from genus Zea, "gall" coming from the word, gall and "an" coming from a glucan).

A question was naturally aroused, how zeagallan was derived, or originated from, in other words, whether it is only as a metabolice product of the fungus, or the host tissue is mainly responsible for it.

In order to give a clue to this question the present investigation was undertaken. The fungus was artificially cultured for the purpose of searching for such a polysaccharide as zeagallan in the metabolic products of the fungus.

I. Experiments and the Effects

1. Isolation of the Corn Smut Fungus

A small fungous gall at a very early stage was sterilized in the solution of 0.1 percent mercuric chloride for three minutes, washed with sterilized water, and bits of the inner tissue of gall were scratched with a forceps, transplanted into the nutrient agar slant in test tubes, or plated in petri dishes, held at 30 C., Within two to three days, whitish colonies appeared. Under a microscope, abundant sporidia or conidia in short thread shape were observed.

2. Shake Culture of the Fungus

The growth of the fungus was found to be generally slow on agar media as Sartoris had pointed out, but quite rapid in shake culture. The following two kinds of nutrient media were selected in this experiment. Good results were obtained from these media.

a) Carrot-corn decoction

200 g of carrot root pieces, and 200 g of young corn stem and leaf pieces were put in 500 ml of water, smashed with a mixer, boiled for fifteen minutes, and filtered. The filtrate supplied with 5% glucose was used as a nutrient medium for shake culture.

b) The synthetic medium postulated by Haskins

The formula is as follows:

Dihydrogen potassium phosphate	0.1 %
Magnesium sulphate crystals	0.05 %
Ferrous sulphate crystals	0.001 %
Urea	0.06 %
Yeast extract	0.06 %
Glucose	5.0 %
Water	1000 ml

Cultures were grown in 100 ml amounts in 500 ml cotton-plugged Sakaguchi's fermentation flasks. and shaken at 30 C. The inoculation of the flask was made by pipetting, under aseptic conditions, aliquots of suspensions of the fungus in cultural solution: that is, the flasks plugged with cotton were autoclaved, and three ml of four days culture of the fungus were carefully inoculated in each flask with a sterilized long pipette in a germ-free box, then the flasks being fixed in a shaker held at 30 C. After six days the milky, turbid cultural solution was centrifuged to make all the conidia wifh mucilaginous substances precipitate. To the supernatant was added an equal quantity of methyl alcohol. Very soon, mucilaginous masses began to float in the solution. The day after, mucilaginous masses ware collected by a small tea strainer, dehydrated and kept for futher investigation. The remaining solution was filtered by filter paper, and to the filtrate was added a double volume of methyl alcohol. After two to three days cloudy precipitates sank at the bottom of the vessel. The clear

supernatant was decanted, and all the precipitates were collected by centrifugation, dehydrated with methyl alcohol, aceton, and ether, respectively. 0.5 g of slightly tinged powdery masses was obtained from 4000 ml of shake culture Haskins' medium for six days. These powdery masses were refluxed with 90% ethyl alcohol for one hour. The residue was tested with the Molisch's reagent, and the results were positive. This residue was tentatively termed, ustilago polysaccharide, and was supplied for acid hydrolysis.

3. Acid Hydrolyses of Ustilago Polysaccharide

In order to compare the chemical composition of ustilago polysaccharide with that of zeagallan, hydrolyses of both substances were carried out in parallel with dilute sulphuric acid at the atmospheric pressure and at a higher pressure. To obtain paper partition chromatograms the hydrolysates were spotted and irrigated with the solvent, pyridine : BuOH : wate (4 : 6 : 3), and sprayed with aniline hydrogen phthalate, or first sprayed with 1 % $AgNO_3$ in 80 % aceton solution, then followed with 1 % NaOH in 80 % ethyl alcohol solution. For the detection of ketones resorcinol reagent[4] was used.

a) Hydrolyses at the atmospheric pressure

0.1 g of ustilago polysaccharide and zeagallan, respectively, in each 10 ml of 0.2N and 2N sulphuric acid was heated in boiling water for six hours, neutralized with barium carbonate, filtered, then spotted on Toyo filter paper No. 51, irrigated with the solvent abovementioned, and sprayed with the pyridine-n-butanol mixture, or the silver nitrate-sodium hydroxide solution. A paper partition chromatogram is shown in fig. 1.

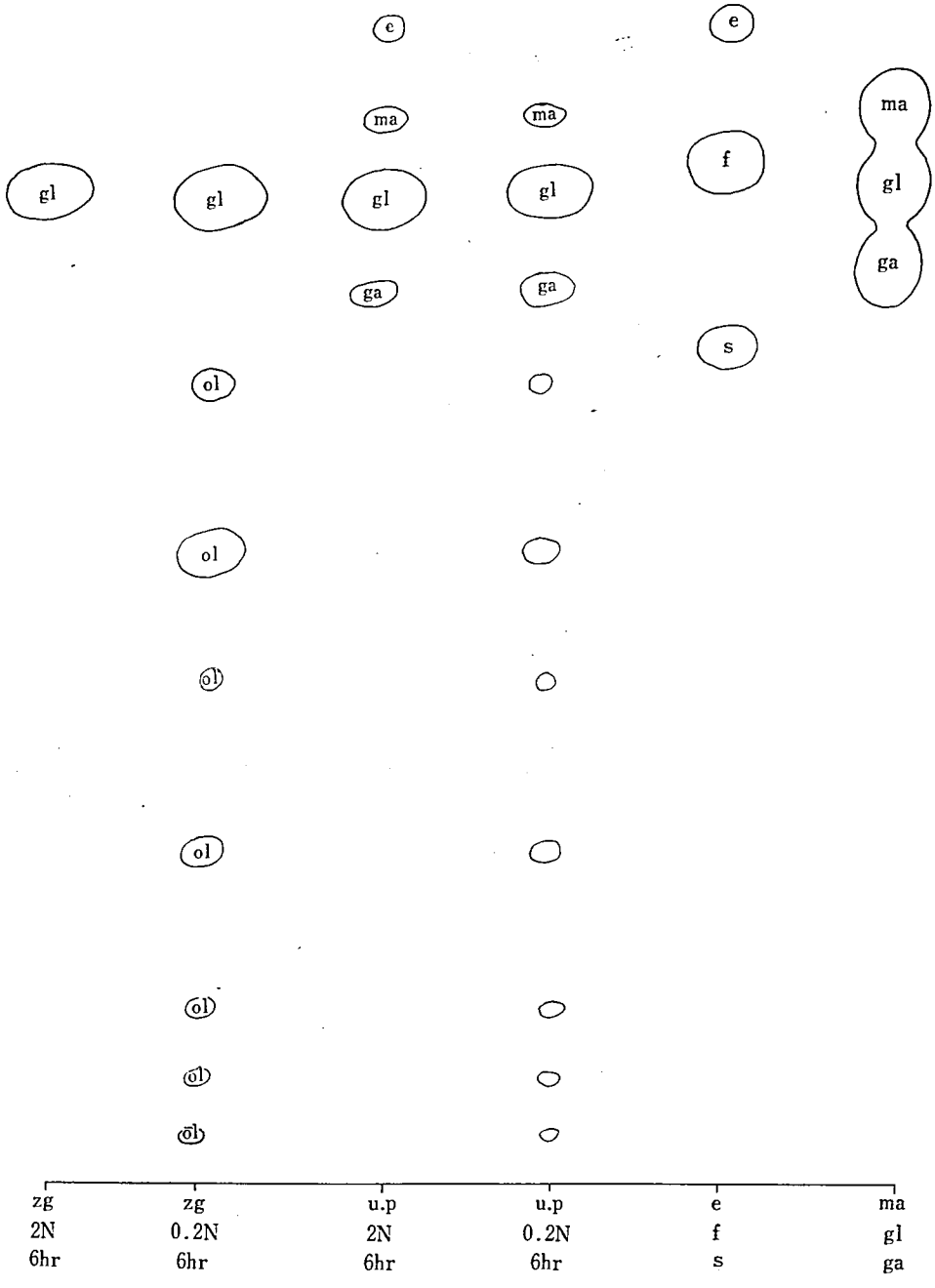
It is seen from the figure that ustilago polysaccharide seems to contain galactose, glucose, mannose and erythritol, while zeagallan is composed of glucose. It is noteworthy, however, that of partial hydrolysates of both plysaccharides, the distribution of oligosaccharides in various grades resembles closely with each other. The above may be suggested that the fungus plays a role in the biosynthesis of zeagallan within the gall of the host plant.

b) Hydrolyses at a higher pressure

0.1 g of ustilago polysaccharide and zaagallan in each 10 ml of 0.05N, 0.1N, and 0.2N sulphuric acid, respectively, was hydrolysed at 120 C. for one hour, neutralized, filtered, spotted, on Toyo filter paper No. 51, and paper partition chromatograms were obtained.

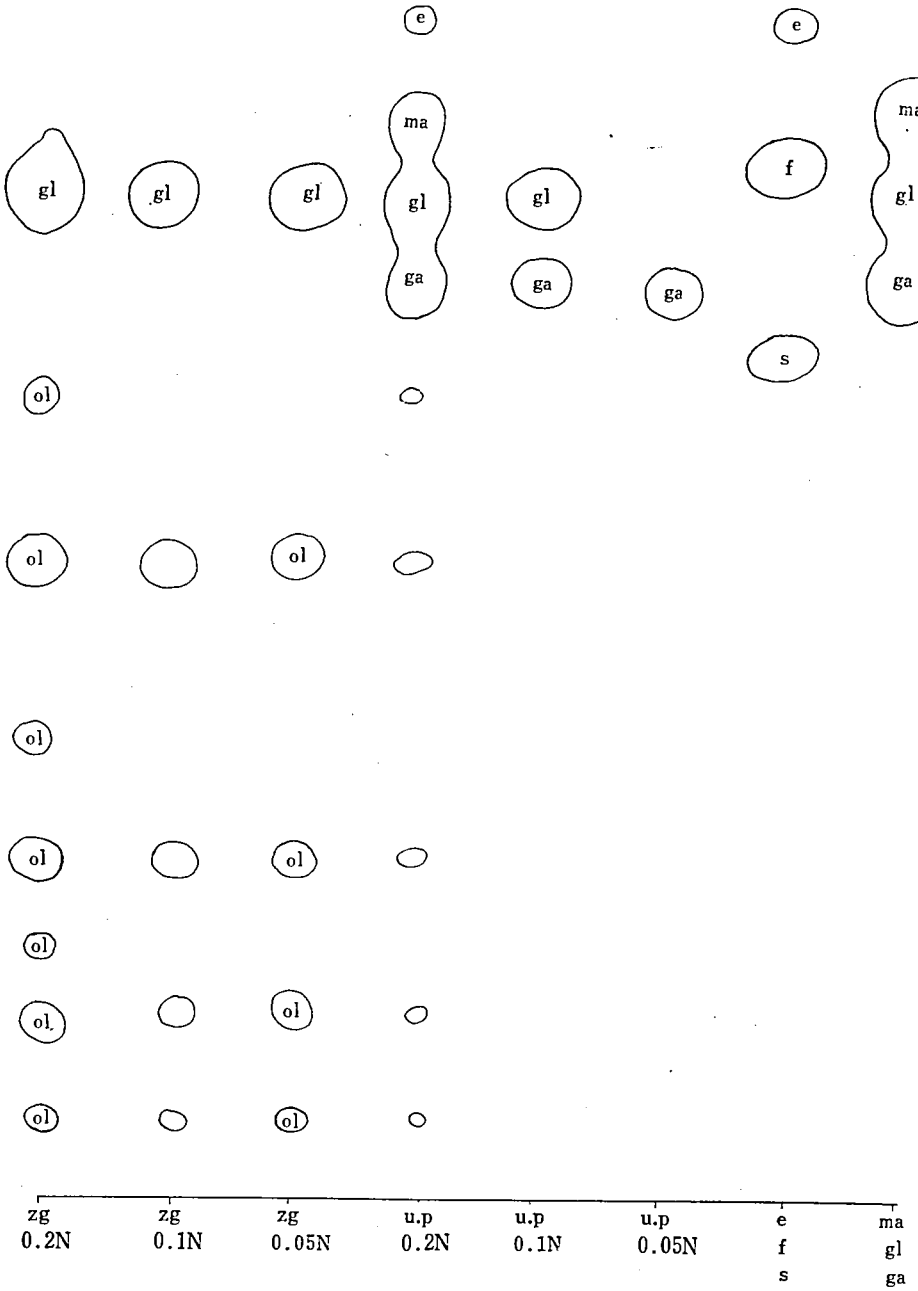
As shown in fig. 2, the distribution of the hydrolasate is different in both polysaccharides, but the distribution of the oligosaccharides resembles with each other. It is noticeable that galactose appeared as a hydrolysate of ustilago polysaccharide with 0.05N sulphuric acid, while galactose and glucose appeared with 0.1N sulphuric acid, and finally galactose, glucose, mannose, and erythritol with 0.2N sulphuric acid at 120 C. for one hour.

Fig, 1 Paper partition chromatogram of ustilago polysaccharide and zeagallan hydrolysed in boiling water



Z. g, zeagallan; U. P, ustilago polysaccharide; e, erythritol; f, fructose; s, saccharose; ma, mannose; gl, glucose; ga, galactose

Fig. 2 Paper partition chromatogram of ustilago polysaccharide and zeagallan hydrolysed at 120 C. for one hour,



Z. g, zeagallan; U. P, ustilago polysaccharide; e, erythritol; f, fructose; s, saccharose; ma, mannose; gl, glucose; ga, galactose

The above-mentioned results indicate that, although ustilago polysaccharide and zeagallan are of different kinds, the fungus may play a roll in the biosynthesis of zeagallan in the gall of the host plant, since the distribution of the oligosaccharides of hydrolysates shows a close resemblance between the two.

II. Discussion

Since the time of O. Brefeld,[5] much work[6] has been done on the artificial culture of Ustilago zeae (Beckm.) Unger (Ustilago maydis), but little has been known of the metabolic products of the fungus.

Perhaps, the Canadian workers represented by R. H. Haskins[3] have done the most extensive contribution to the knowledge of the metabolites of the corn smut fungus. Hskins[3] was the first to discover that Ustilago zeae is a remarkable producer of an antibiotic, the chemical structure of which is clarified by R. U. Lemieux[7] et al, and is termed, ustilagic acid. In a series of entitled, "Biochemistry of the Ustilaginales" they have reported the fats, the sterols, the alkaloids as metabolites of the fungus. Further, they[8] hav found a water-soluble compound identified as D-mannopyranosyl-l-meso-erythritol in aerobic submerged culture of Ustilago sp. PRL 627, and that the extracellular oil produced at the same time by shis fungus contains D-mannose, meso-erythritol, acetic acid, and a number of saturated and unsaturated fatty acids. In addition, itaconic acid and dianthrone have been also proved to be present in the oily metabolites. However, no polysaccharide as described in the present paper has been reported yet. Our ustilago polysaccharide seems to be closely related to D-mannopyranosyl-l-meso-erythritol elucidated by Haskins[8] et al.

The data mentinoied above, although not conclusive because of the inaccuracy of paper chromatographic identification, still throw some light on the biosynthesis of zeagallan in the gall of the corn plant, suggesting that the causal fungus may play a role at least.

III. Summary

1. In order to give a clue to the biosynthesis of zeagallan, a new polysaccharide found in the gall of the corn plant, the present investigation was undertaken.
2. The causal fungus, Ustilago zeae (Beckm.) Unger (Ustilago maydis) was artificially cultured in a shaker held at 30 C., and a polysaccharide was obtained as a metabolite of the fungus.
3. This Polysaccharide, tentatively name ustilago polysaccharide was hydrolysed with dilute sulphuric acid, and the hydrolysates were compared with those of zeagallan.
4. It was found that ustilago polysaccharide and zeagallan are different in their components, but there is aclose resemblance between the two, particularly, in the

distribution of the oligosaccharides in the paper chromatogram.

5. Erom these results it is likely that the causal fungus may play a role in the biosynthesis of zeagallan within the gall of the host plant.

Further studies on purification, isolation and acetates of the hydrolysates are in progress.

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