amylase formation of the lysate was considerably inhibited while that of the vegetative cells not affected.

The authors are indebted to Mr. S. Naono

of Osaka University and to Mr. H. Yamada of Kyoto University, for kindly supplying the ribonuclease and glucose-6-phosphate used in this work.

[Bull. Agr. Chem. Soc. Japan, Vol. 22, No. 3, p. 174~176, 1958]

Shikimic Acid in the Tobacco Leaf

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Received January 29, 1958

Shikimic acid in flue-cured tobacco leaf was identified and determined by paper chromatography. The presence of shikimic acid in the flue-cured leaf was confirmed, although the content was very small, 0.02 per cent of dry matter.

In a previous paper,¹⁾ the author has reported that chlorogenic acid in tobacco leaves is newly formed in considerably great quantities during flue-curing and it seems reasonable to conclude that the aromatic ring, caffeic acid, is synthesized by a route through sedoheptulose, that is, shikimic acid. Recently, Geisman has reported²⁾ that caffeic acid in the tobacco leaves fed in the seedling stage with labeled precursor, phenylalanine-2-C¹⁴, derive directly and specifically its whole molecule from phenylalanine, and it is concluded that in higher plants such as in microorganisms, aromatic rings are made by two pathways one through shikimic acid and the other through head to tail linkage of acetic units.

The author has attempted to identify and determine shikimic acid, that seemed to be a normal intermediate in the route of aromatic

ring formation in the tobacco leaf. In a recent survey of organic acids in fruits,³⁻⁵⁾ grass,⁶⁾ and other plant tissues,⁷⁻¹⁰⁾ shikimic acid has been found in a considerable amount. However, in the tobacco leaf shikimic acid has not been found up to the present. In this paper, shikimic acid in the tobacco leaf was identified and determined by paper chromatography¹¹⁾ and the spectrophotometric method described by Yoshida and Hasegawa.¹⁰⁾

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2) T. A. Geisman and T. Swain, *Chem. & Ind.*, **1957** 984.

EXPERIMENTAL

Preparation of Extracts. One hundred grams of moisture-free material of Japanese flue-cured tobacco, produced in Kagoshima district, 1956 crop (Rapper, 2 grade), which had been ground in a mill fine enough to pass a 1-mm sieve, were boiled for one hour under a reflux condenser with 1500 ml of 80 per cent methanol. The mixture was filtered, and the residual tobacco was boiled again for one hour with 1000 ml of 80 per cent methanol. The mixture was subsequently filtered and washed with 80 per cent methanol. The combined filtrate and washings were concentrated under reduced pressure, and then 1000 ml of water was added to the residue and filtered. The filtrate was first passed through a column of Dowex 50X4 resin in the hydrogen form. The collected effluent was passed through a column of Amberlite IR4B resin in the hydroxyl form. In order to recover the acids from the resin a column of IR4B was eluted with 1N aqueous ammonia. The emergent solution below pH 10 was collected, and then treated with Dowex 50X4 resin in the hydrogen form. The solution containing organic acid was concentrated under reduced pressure and transferred quantitatively to a 25 ml volumetric flask and made to volume with water.

Qualitative Separation and Identification. The methanolic extracts were investigated by paper chromatography using the following two solvents: (I) methylethylketone-cineole-formic acid-water (50:50:20:16),¹¹⁾ (II) amylalcohol-5N formic acid (1:1).¹²⁾ Shikimic acid was observed in both solvents with R_F values, 0.15 and 0.20 respectively, and gave a red color on spraying periodate and anilin reagents described by Yoshida and Hasegawa.¹⁰⁾ Moreover, shikimic acid gave a greenish yellow color on spraying with periodate followed by sodium nitroprusside and piperazine described by Cartwright and Roberts.¹³⁾ Identical R_F values (alone and mixed) and the same colors were given for the acid spot detected and an authentic shikimic acid in the above two solvent systems and reagents.

Quinic acid in the tobacco extracts was separated and observed with R_F values in both solvents, 0.09 and 0.08 respectively.

Quantitative Assay. A spectrophotometric determination of shikimic acid¹⁰⁾ was applied to the aqueous

eluate of paper chromatogram (I). One hundred and forty microliters of the methanolic extracts (25 ml) were applied to Whatman No. 1 paper sheets. The organic rich phase of solvent (I), described above, was added to the trough and chromatograms were run descending-wise. After about eighteen hours, the strip was removed and dried in a forced-draft hot oven at 70° for one hour. The areas, detected by test-spots on both sides of paper sheets, were cut out and eluted with water. The eluate was transferred quantitatively to a 10 ml volumetric flask and made to volume with water. An aliquot of 2 ml of the eluate was used for the color reaction, based on the reaction of the periodate oxidation of shikimic acid with anilin to yield a Schiff base, and the other aliquot was used for a blank solution without aniline for zeroing the spectrophotometer.

Among the reagents, described by Yoshida and Hasegawa, ethylene glycol solution was modified as follows: thirty milliliters ethylene glycol is dissolved in 100 ml distilled water to obtain a final clear solution for measurement.

Optical density measurements were made in a Beckman B spectrophotometer at 510 m μ using a blank solution described above.

The recovery of shikimic acid from paper chromatogram resulted in 88 per cent.

RESULTS AND DISCUSSION

The color reactions of shikimic acid contained in the tobacco leaf were identified, although both colors were pale. A greenish yellow color based on the reaction of the periodate oxidation product of shikimic acid with sodium nitroprussid and piperazine was specific for quinic, shikimic and dehydroshikimic acids.^{3,4)} On the other hand, a red color on the reaction of the periodate oxidation product of shikimic acid with aniline was specific for shikimic acid, while other organic acids did not react under these conditions. Neither sugars nor amino acids gave inhibitory effects, except polyhydroxy compounds such as carbohydrates or polyols.¹⁰⁾ These observations give aid to identification. Moreover, the R_F values (alone and mixed) were identical with authentic shikimic acid by the two solvent systems. Therefore, the presence of

12) M. L. Buch, R. Montgomery and W. L. Porter, *Anal. Chem.*, **24**, 489 (1952).

13) R.A. Cartwright and E.A.H. Roberts, *Chem. & Ind.*, **1955**, 230.

shikimic acid in flue-cured tobacco leaf was confirmed, although the content was very small. Shikimic acid content found in the flue-cured tobacco leaf was 0.02 per cent to dry matter by spectrophotometric determination.

The results of this investigation may lead to some support of the presence of a direct oxidative pathway in the tobacco leaf and the fact that chlorogenic acid is formed during flue-curing through sedoheptulose as reported previously,¹⁾ and is also in accordance with Geisman's report²⁾ that in the tobacco plant there is a possibility of aromatic ring

formation through shikimic acid.

Quinic acid, which formed chlorogenic acid with caffeic acid, is present in the tobacco leaf, and the determination of quinic acid is now in progress.

ACKNOWLEDGMENT

The author wishes to thank Mrs. Taeko Taguchi for her assistance in carrying out the determination of shikimic acid, and the author is very grateful to Mr. S. Yoshida, of the Botanical Institute, University of Tokyo, for his criticism.

[Bull. Agr. Chem. Soc. Japan, Vol. 22, No. 3, p. 176~185, 1958]

Taxonomical Study of Glutamic Acid Accumulating Bacteria, *Micrococcus glutamicus* nov. sp.

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Received December 19, 1957.

Taxonomical studies of a new group of strains of glutamic acid accumulating bacteria isolated in our laboratory were carried out. It was found that these strains belong to Genus *Micrococcus* because they are gram-positive, none-sporulating, and catalase possessing cocci. They are also pale yellowish, aerobic, and capable to reduce nitrate. Moreover, they neither utilize ammonium salt as a sole source of nitrogen in Hucker's medium nor liquefy gelatin. From these observations, it seems that they are related to *M. aurantiacus* and *M. epidermidis*. However, our strains are different from them in the manner of acid production in milk and lactose media, and also in pigmentation, serological reactions and glutamic acid accumulation.

The name of *Micrococcus glutamicus* nov. sp. was, therefore proposed to these strains.

The phenomenon of cell elongation and characteristics of the pigments of these strains was also investigated.

A new group of strains of *Micrococcus* which accumulates a large quantity of glutamic acid in culture medium was isolated by one of the authors.¹⁾ This article is concerned with the

identification of these strains to belong to a new species of genus *Micrococcus* which we named *M. glutamicus* nov. sp.

EXPERIMENTAL METHODS

Diagnostic tests were carried out according to

1) Shukuo Kinoshita, Shigezo Uda and Masakazu Shimonō:
Presented at the meeting of the Agricultural Chemical Society of Japan. April 9-11, 1957. Tokyo.