Isolation and Identification of 1-Deoxy-1-(L-asparagino)-D-fructose Formed in the Autoclaved Culture Medium

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In the cultivation of red-colored yeast, *Sporobolomyces odoratus* AHU 3246, the growth of the yeast was stimulated when the synthetic medium was autoclaved for moderately long period. The analogous observation was also found between the autoclaved medium and the aseptically filtered medium. Such a phenomenon was generally found during the incubation of various microorganisms.¹⁾ Stimulation of initial growth of microorganisms was thought to be caused by the variation of components in the autoclaved culture solution. In connection with this heat activation effect many effective substances were identified to date, for example, the degradation products of sugars,^{2~4)} ascorbic acid⁵⁾ glucosylglycine⁶⁾ etc. The authors are interested in the formation of new nitrogen containing compounds, especially ninhydrin positive substances in the autoclaved culture medium and this paper deals with isolation and identification of these compounds.

The following medium was employed: 10% D-glucose, 0.25% L-asparagine, 0.3% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% KH_2PO_4 , 0.01% CaCl_2 and deionized water, pH 6.0.

After the medium was autoclaved, four ninhydrin positive spots appeared on the thin layer chromatogram, and two of them revealed color reaction of sugar. Fractionation of ninhydrin positive substances was performed as follows. Six liters of the autoclaved medium (at 120°C for 30 min) was concentrated and treated with cation exchange resin. The eluate with 2 N aqueous ammonia or 0.5 N trichloroacetic acid was evaporated to dryness *in vacuo* and the residue was dissolved in 70% ethanol. Insoluble material (about 12 g) was found to be L-asparagine added as a nitrogen source, and the filtrate was applied to a cellulose powder column (4.5 × 59 cm), which was eluted and fractionated with solvents of *n*-butanol-acetic acid-water (4:1:1, v/v) (solvent 1) and then 70% ethanol (solvent 2). From the fractions eluted with solvent 1, 234 mg of

crystals were isolated purely. Both the crystals thus obtained and an authentic sample of L-aspartic acid showed identical spots on TLC with solvent 1 ($R_f = 0.180$), and the same absorption bands on IR spectroscopy. Anal. Found: C, 35.62; H, 5.26; N, 10.59%. Calcd. for $\text{C}_4\text{H}_7\text{NO}_4$: C, 36.09; H, 5.30; N, 10.52%.

The column was washed with solvent 2 after the elution with 3 liters of solvent 1. From 2 liters of washings 70 mg of white powder was isolated. This substance showed both ninhydrin positive reaction and ketose positive reaction with naphthoresorcinol-phosphoric acid reagent. Therefore, this compound was estimated to be 1-deoxy-1-(L-asparagino)-D-fructose (fructose-asparagine). As it was a new compound,⁸⁾ fructose-asparagine was prepared by the method of condensation of D-glucose with L-asparagine in anhydrous methanol.⁷⁾ Both the crystals isolated and the synthetic sample showed identical spots on TLC with the following solvent system: phenol-water (4:1, v/v), $R_f = 0.47$; solvent 1, $R_f = 0.04$; pyridine-isoamyl alcohol-water (7:7:6, v/v), $R_f = 0.05$. IR spectra of both

FIG. 1. Effect of Fructose-Asparagine Added to the Medium on the Growth of *S. odoratus*.

Fructose-asparagine was added to the medium by 100 $\mu\text{g}/\text{ml}$. The yeast was cultured in 500 ml shaking flasks containing 150 ml of the media at 25°C on a reciprocal shaker. Growth of the yeast, average of 5 flasks was measured colorimetrically (O.D. at 660 nm.).

○—○, Medium without the nitrogen source autoclaved at 120°C for 15 min and L-asparagine added aseptically.

●—●, Medium without nitrogen source autoclaved, and L-asparagine and fructose-asparagine added aseptically.

samples were identical with each other. $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3500~3100 (OH), 2700~2250 ($\text{>NH}_2^{\oplus}$), 1680 (CONH_2), 1625 ($\text{COO}^{\ominus}$). *Anal.* Found for the isolated compound: C, 40.03; H, 6.29%. Found for the synthesized fructose-asparagine: C, 39.91; H, 6.59%. Calcd. for $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 39.61; H, 6.33%. $[\alpha]_{\text{D}}^{18}$ for the isolated compound -38° and for the synthesized fructose-asparagine, -40° ($c=1$, water), mp of both compounds $124\sim 125^\circ\text{C}$ (decomp.).

Another ninhydrin positive component which gave color reactions of sugar was not identified.

Effect of fructose-asparagine (100 $\mu\text{g}/\text{ml}$ medium) on the growth of the yeast is shown in Fig. 1. Fructose-asparagine affected on the initial growth of the yeast rather than its logarithmic phase.

In case of the incubation of *S. roseus* AHU 3980 more distinct additive effect was observed. For example, after 71 hr incubation the O.D. value was 0.898 for the fructose-asparagine added medium and 0.161 for the blank. L-Aspartic acid also exhibited the additive effect.

We believe that the heat activation effect will ascribe to the formation of various compounds by autoclaving the medium and though the mechanism may not be simple, the sugar-amino acid condensation product,

fructose-asparagine, isolated for the first time in this work, is one of the effective substances.

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