N-Fructosyl anthranilic acid* as a possible intermediate in the synthesis of indole by *Sacccharomyces***

Terminal steps in the biosynthesis of tryptophan involve the compounds anthranilic acid and indole as direct precursors of the amino acid. The condensation of indole and serine to yield tryptophan has been well studied[1,2]. While the synthesis of indole from anthranilic acid has received considerable interest recently, a mechanism for this transformation has yet to be proposed. It is known that in *E. coli* the two carbon atoms incorporated into the pyrrole nucleus may arise from carbon atoms one and two of either glucose or ribose[3]. That the nitrogen of anthranilic acid is retained in this conversion[4], and the carboxyl carbon lost as CO_2[5], had previously been determined. Closure of the pyrrole ring onto the benzene portion is mediated apparently as a direct result of the decarboxylation reaction. This is suggested by the fact that the new ring is formed only through the carbon atom of anthranilic acid which previously held the carboxyl group. The third carbon of the anthranilic acid is not involved[6].

An auxotroph of *Saccharomyces*, designated *tr-1*,[7] can have its tryptophan requirement met by either indole or tryptophan. A second auxotroph of this yeast, *tr-2*, will grow with tryptophan, indole or anthranilic acid. The high specificity for these particular compounds unmodified structurally has been investigated and reported elsewhere[8].

Clone *tr-1*, which is blocked at a step between anthranilic acid and indole, was found to accumulate in its growth medium an intermediate which fluoresced when observed on filter paper under a Mineralight model V41 ultraviolet lamp. Ascending chromatography of the growth medium using the solvent systems of YANOFSKY[3], MASON AND BERG[9], or HAYAISHI[10] revealed two fluorescent areas. The faster-moving spot was able to feed *tr-2*, had an ultraviolet absorption spectrum identical with anthranilic acid, and did not separate from anthranilic acid when co-chromatographed with an authentic sample of this substance.

The slower-moving fluorescent material had an R_F value approximately one-half that of the faster-moving spot, and was also capable of feeding *tr-2*. That this material was similar in structure to anthranilic acid was suggested by similarity in ultraviolet absorption spectra and fluorescence under ultraviolet light. Chromatographed, concentrated medium in which *tr-1* had grown was used for studying the unknown derivative. Material cut and extracted from the filter paper in the area of the slower-moving spot gave positive tests for diazotizeable amines[11], keto-sugars[12], and reducing sugars. Reactions for pentoses[13], desoxy-sugars[14], hydroxy, and dihydroxy aromatic acids[15] were negative, and phosphate determinations[16] were inconclusive. After acid hydrolysis, the material gave a positive test for fructose[17]. Chromatograms of the hydrolyzed material when sprayed with a reagent specific for keto-sugars[18] gave a positive reaction at an R_F identical with that of free fructose.

Clones with the *tr-1* gene apparently secrete both anthranilic acid and a fructose derivative of anthranilic acid into the growth medium. The position of the fructose on the anthranilic acid molecule is suggested from a variety of evidence. The β-glucosidic esters of anthranilic acid are insoluble in ether and are readily destroyed in acid solution[19]. The ability of both anthranilic acid and the fructose conjugate to be extracted with ether at an acid pH was utilized to separate these compounds from free fructose. This suggests that fructose is not connected in the derivative through the carboxyl but through the amino group. N-Fructosyl anthranilic acid was synthesized[20,21] and material that co-chromatographed and possessed the same R_F value and ultraviolet absorption spectrum as the secreted intermediate was obtained. The synthesized compound also fed *tr-2*.

Speculation concerning the role of the derivative in the metabolism of the yeast offers two possibilities. Conjugation of the anthranilic acid with fructose could be a detoxification mechanism. When supplied with anthranilic acid and glucose or fructose, non-proliferating cells failed to yield the derivative. In fact, free anthranilic acid was synthesized by resting cells of *tr-1* when they were supplied with glucose and ammonium chloride. The N-fructosyl derivative was found only in growing cultures. This would suggest the alternate possibility that the derivative is in fact an intermediate in the biosynthesis of indole in which carbon atoms one and two of the fructose would supply the two carbon atoms of the pyrrole ring.

The mechanism for closing the ring would involve a decarboxylation of the anthranilic acid unit. The completed ring would be formed between carbon one of the anthranilic acid nucleus and the second carbon of fructose, with a loss of one molecule of water. N-Fructosyl-anthranilic acid would accumulate in the growth medium if the genetically blocked reaction involved the decarboxylation mechanism.

* N-(2-Carboxyphenyl)-1-amino-fructose-6-phosphate.

** These investigations were supported in part by the Biological and Medical Research Fund of the State of Washington, and the National Institutes of Health, Public Health Service, Grant No. E-328.

In the normal biosynthetic scheme the derivative would probably occur phosphorylated as proposed for intermediates in histidine auxotrophs of *Neurospora*[22]. Absence of phosphate in the derivative formed by *tr-1* could be due to phosphorolytic action by phosphatases or hydrolysis during the isolation procedure.

A mechanism of indole synthesis which incorporates the N-fructosyl-acid, and evidence available from the literature, is proposed as follows. N-(2-Carboxyphenyl)-1-amino-fructose-6-phosphate could arise from a direct condensation of anthranilic acid and fructose-phosphate. Glucose-phosphate could supply the carbohydrate unit with the enol or keto form resulting from an Amadori-type rearrangement[23] of the glucose unit. Decarboxylation and dehydration results in ring closure to form indole tetrose-phosphate. Elimination of tetrose-phosphate would yield free indole.

Further work is in progress to test the proposed scheme and more precisely define the indole-synthesizing reactions in *Saccharomyces*.

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Received October 15th, 1956

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Isolation and properties of ribosenucleic acid from tobacco leaves*

There is a considerable amount of work on the isolation and analysis of nucleic acids from animal and bacterial sources. The information on the nucleic acids of plant tissues is meagre. Pentose nucleic acid was isolated from wheat embryo by OSBORNE AND CAMPBELL[1] and LUSENA[2] and from barley roots by TAKASUGI[3]. Nucleoprotein was isolated from normal tobacco leaves by PIRIE[4] and EGGMAN, SINGER AND WILDMAN[5]. HOLDEN AND PIRIE[6] prepared nucleic acid from tobacco leaf nucleoprotein by three different procedures: (1) by adding an equal volume of 0.1 *M* NaCl at pH 8 and boiling the mixture, (2) by exposing nucleoprotein solution to 0.5 *N* NaOH for 40 minutes at 0° C and (3) by adding 50 g per liter trichloroacetic acid and keeping at room temperature for 8–12 hours. These procedures might yield degraded products. THOMAS AND SHERRATT[7] estimated the purine and pyrimidine composition of nucleic acids extracted from

* This investigation was supported by grants from the Rockefeller Foundation and the U.S. Public Health Service.