THE MECHANISM OF RIBOSE FORMATION IN ESCHERICHIA COLI*

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Metabolic pathways by which ribose may be formed have been studied in this laboratory (1, 2). There is now ample proof of the existence in many organisms of enzyme systems able to form ribose-5-phosphate from glucose-6-phosphate with intermediate stages of 6-phosphogluconate and ribulose-5-phosphate. Recent studies on the metabolism of ribulose-5-phosphate have shown that it may be split by a transketolase into triose phosphate, and an active 2-carbon moiety that reappears at the hexose or heptulose level (3–6). This enzyme has been demonstrated in yeast, rat liver, and plant tissue, and in two cases the synthesis in vitro of ribulose-5-phosphate has been achieved by the action of this enzyme in the presence of glyceraldehyde-3-phosphate and either D-erythrulose or hydroxypyruvate. Although the true biological donor of the ketol fragment is not yet known, a synthetic action of the transketolase must be considered as a possible source of ribulose.

The hypothesis that ribose formation represents a synthesis from smaller molecules was the basis for the demonstrations of enzymatic synthesis of pentose in vitro by aldolases of animal and plant origin from triose phosphate and glycolaldehyde. However, the product of these syntheses was xyloketose-1-phosphate (7), and a direct synthesis of the ribose configuration by an aldolase is not likely.

Two studies of the incorporation in vivo of small molecules into the ribose of rat liver ribose nucleic acid have been made. The carboxyl carbon atom of glycine has been shown by Low to contribute to the carbon of the pentose (8), but the fact that the specific activity of the isolated ribose was less than that of the mixed polynucleotides suggests that the incorporation of glycine carboxyl into ribose is via a less direct route than its incorporation into the 4 position of the purine bases. The incorporation of doubly

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labeled acetate into ribose was also studied by Low, who showed that its
2 carbons were not incorporated as a unit (9).

A study in vivo by Bernstein is pertinent to the question of the pathway
of ribose synthesis (10). After administration of carboxyl-labeled acetate
to the chick, he showed that the C₃ and C₄ atoms of the glucose isolated
from glycogen were equally labeled. However, the C₆ of ribose derived
from nucleic acid contained a greater amount of radioactivity than the C₂
atom. This finding is inconsistent with the hypothesis that the 5-carbon
chain of ribose arises directly from glucose after loss of C₁.

Escherichia coli has been shown to contain enzymes of the phospho-
gluconate pathway; it has been estimated that, under conditions of aerobic
growth in a synthetic medium where glucose is the sole carbon source, from
16 to 38 per cent of the metabolized glucose was degraded via this pathway
(11). However, under conditions of bacteriophage infection when no rib-
ose nucleic acid is synthesized, the amount of glucose degraded by this
pathway is reduced, supporting the hypothesis that in E. coli ribose is
formed directly from glucose by loss of its C₁-carbon atom. Glucose-1-C¹⁴
was used to provide more conclusive evidence for the origin of ribose in
this organism.

Methods

Growth of Bacteria—E. coli, strain B, was grown in a well aerated syn-
thetic medium (12) supplemented with 1 mg. per cc. of glucose-1-C¹⁴ as
the sole carbon source. After an initial lag period, growth was exponential
with a division time of 55 minutes. The cells were harvested when a
break in the growth rate indicated that the glucose was exhausted. The
initial titer in these experiments was 5.6 × 10⁷, and the final titer 9 × 10⁸.
To a suspension of washed cells, trichloroacetic acid was added to give a
concentration of 5 per cent. The resulting precipitate was washed with 5
per cent trichloroacetic acid, alcohol, and ether, and dried. The ribose
nucleotides were obtained from this dried material by the procedure of
Schmidt and Thannhauser (13). The desoxyribose nucleic acid could be
obtained from the acid-insoluble fraction by the extraction procedure of
Ogur and Rosen (14). These procedures showed that the dry material was
12 to 15 per cent ribose nucleic acid, and 3 to 4 per cent desoxyribose nu-
cleic acid. Yields for the individual steps in a typical experiment are
shown in Table I.

Purification of Ribose Nucleotides—The silver salts of the nucleotides
were precipitated from the ribose nucleic acid fraction of the Schmidt-
Thannhauser procedure after adjustment to pH 7.5. This precipitate was
washed and decomposed with H₂S. Analysis of the resulting solution
showed a molar ratio of total phosphorus (15) to orcinol-reactive ribose
(16) of 1.4.
Preparation of Furfural—The nucleotide solution was made 9 N with 
$\text{H}_2\text{SO}_4$, and steam-distilled with maintenance of approximately constant 
volume. Under these conditions theoretical recovery of furfural may be 
expected from purine-bound ribose, but experiments showed that very 
little furfural was obtained from the pyrimidine nucleotides unless the 
volume of the mixture was permitted to decrease slowly by one-third to 
one-half, until slight darkening occurred and acid fumes began to appear. 
After the first 200 cc. of distillate were collected, this concentration was 
routinely made. In this way, 75 to 80 per cent of the theoretical yield 
of furfural from uridylic acid and 60 to 65 per cent from cytidylic acid 
may be obtained.

Conversion of Furfural to Derivative for Radioactivity Determination—The 
method of Low (8) involves an oxidation of furfural to furoic acid by use 
of ammoniacal silver, with subsequent purification of the derivative by

<table>
<thead>
<tr>
<th>Table I</th>
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<tbody>
<tr>
<td><strong>Furfural 2,4-Dinitrophenylhydrazone Obtained As Derivative from Cells of E. coli</strong></td>
</tr>
<tr>
<td>No. of cells</td>
</tr>
<tr>
<td>Dry weight, acid ppt., mg</td>
</tr>
<tr>
<td>Ribose nucleic acid nucleotides, total P, $\mu$M</td>
</tr>
<tr>
<td>&quot; &quot; &quot; ribose (orcinol-reactive), $\mu$M</td>
</tr>
<tr>
<td>&quot; &quot; &quot; in acid ppt., % (calculated from P = 9.52%)</td>
</tr>
<tr>
<td>Furfural, $\mu$M</td>
</tr>
<tr>
<td>&quot; 2,4-dinitrophenylhydrazone, red form, $\mu$M</td>
</tr>
<tr>
<td>&quot; yellow form, $\mu$M</td>
</tr>
</tbody>
</table>

sublimation. We experienced difficulty in the use of the published method, 
and modified the oxidation conditions. It was found necessary to use a 
large excess of the freshly prepared ammoniacal silver solution (approximately 150 $\times$ theoretical) and a heating period of at least 30 minutes at 
80°. The furoic acid solution obtained after removal of the silver did not 
have the absorption spectrum of pure furoic acid. Some purification was 
possible by ether extraction of the furoic acid from the crude solution, but 
the best preparations still showed ultraviolet absorption at low wave-
lengths in addition to the 2450 A maximum. Difficulties undoubtedly 
arose from the scale of the operations. The available amounts of pentose 
nucleic acid were approximately one-fifth those used by Low, and final puri-
fication by sublimation was impractical. Although some of our results 
were obtained by isolation of furoic acid, the following preferred procedure 
was developed.

Preparation and Isolation of 2,4 Dinitrophenylhydrazone of Furfural—

The 2,4-dinitrophenylhydrazone of furfural has been shown by Bredereck 
(17) to occur in two forms, thought to be the cis and trans isomers. Ab-
sorption spectra of specimens prepared by us according to the procedures of Bredereck are shown in Fig. 1. Derivatives obtained by the chromatographic procedure to be described below also have these absorption spectra. The 2,4-dinitrophenylhydrazone derivative was obtained from the furfural-containing distillate by addition of a hot solution of 5 times the theoretically required amount of 2,4-dinitrophenylhydrazine in 10 cc. of 0.1 N HCl. The mixture was warmed on a hot-plate until it became cloudy, and then cooled overnight. The precipitate was collected in a sintered glass crucible; on a weight basis the yield of this crude derivative was quanti-

![Graph](http://www.jbc.org/)

Fig. 1. Absorption spectra of the isomeric furfural dinitrophenylhydrazones

...ative. Purification was accomplished by column chromatography of a CHCl₃-C₆H₆ (1:1) solution on alumina. Commercial CHCl₃ had to be freed of alcohol, and used before formation of phosgene occurred; commercial benzene was redistilled. The alumina used was that marketed by Merck and Company, Inc., for chromatographic work; it was washed with 0.2 M NaHCO₃, H₂O, 0.1 N HCl, H₂O and methanol, air-dried, and finally activated at 150°C. Freshly activated alumina held the hydrazone derivative too firmly, requiring excessive development of the column; the absorbancy of the alumina was decreased by exposure to the atmospheric humidity. An arbitrary test was used to estimate absorbancy. 2 gm. of alumina in a 44 × 7 mm. column should permit recovery in 8 cc. of eluate of 0.25 mm. of furfural 2,4-dinitrophenylhydrazone (red form) placed on the column in 0.5 cc. of the solvent mixture. Alumina meeting this test was stored in a tightly closed bottle.
Development of the crude furfural 2,4-dinitrophenylhydrazone on an alumina column (33 × 85 mm.) by the CHCl₃-C₆H₆ mixture revealed four components. The fastest moving component on the column was the "yellow" or lower melting form of the derivative; the "red" form moved more slowly, and on an alumina column of the proper degree of absorbancy and sufficient length the two were sharply separated. A third component moved on the column very slowly when the developing solvent was alcohol-free; after removal of the first two components, this could be accelerated by addition of as little as 1 per cent ethanol to the developing solvent. This component was always small in amount and was not identified. In addition to the components described, some material was strongly absorbed at the top of the column, and could not be eluted with organic solvents. It could be recovered by extrusion of the absorbent, evaporation of the organic solvents, and extraction with 0.2 M NaHCO₃. This behavior is typical of the acidic hydrazones (e.g. levulinic acid 2,4-dinitrophenylhydrazone). However, the material was not homogeneous, as shown by its separation into several bands by development on the column with aqueous-ethanol mixtures. It was not further investigated.

Identity of the two furfural 2,4-dinitrophenylhydrazone fractions was checked by examination of their ultraviolet absorption in ethanol solution. The red form of the derivative was always in excess; when prepared as described above, it constituted 85 to 90 per cent of the total.

Radioactivity Determinations—A dioxane solution of furfural 2,4-dinitrophenylhydrazone was spread directly on aluminum planchets and counted with a windowless gas flow counter flushed with a helium-isobutane mixture. Counts ranging from 3 to 30 times the background were made over periods long enough to reduce the counting error to 5 per cent. The density of material on the planchets did not exceed 0.15 mg. per sq. cm., and no corrections were made for self-absorption. The D-glucose-1-C¹⁴, obtained from Dr. Isbell of the National Bureau of Standards, was similarly

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Glucose-1-C¹⁴ added to growth medium</th>
<th>Derivative prepared</th>
<th>Derivative Ribose activity</th>
<th>Glucose activity</th>
<th>c.p.m. per μM</th>
<th>c.p.m. per μM</th>
<th>per cent</th>
</tr>
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<tbody>
<tr>
<td>X</td>
<td>2028</td>
<td>2,4-Dinitrophenylhydrazone</td>
<td>572</td>
<td>31.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>2018</td>
<td>&quot;</td>
<td>547</td>
<td>27.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>1270</td>
<td>Furoic acid</td>
<td>248</td>
<td>19.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>420</td>
<td>&quot;</td>
<td>86</td>
<td>20.5</td>
<td></td>
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</table>
plated; that used in Experiments X and XI was diluted in dioxane, while in Experiments VII and VIII the glucose and the furoic acid were plated as aqueous solutions.

Radioactivity values obtained in four experiments are shown in Table II. The furoic acid derivatives were 20 per cent as active per mole as the initial glucose; the more reliable values obtained for the 2,4-dinitrophenylhydrazone derivatives average 29 per cent.

DISCUSSION

In evaluating the above results, the following postulates were made. If the ribose of ribose nucleic acid is formed by a C₃ + C₂ condensation, if the C₃ fragment is derived from glucose by the Embden-Meyerhof pathway, and if it is assumed that glyceraldehyde-3-phosphate equilibrates with dihydroxyacetone phosphate by the well known isomerase reaction, the C¹⁴ content of the triose phosphate should be 50 per cent of the labeled glucose-1-C¹⁴. The C₂ fragment would probably be equally radioactive, since all known reactions which generate a C₂ fragment from triose phosphate involve the conservation of the carbon atom derived from the C₁ of glucose, e.g. the conservation of the methyl carbon of pyruvate in acetate and acetaldehyde. However, it is not known whether the C₂ fragment used in the condensation is derived similarly, but if it is, the ribose will contain 100 per cent of the activity of the glucose.

On the other hand, if the ribose is formed directly from glucose-1-C¹⁴ via the phosphogluconate pathway, the C₁ is lost and the ribose should be unlabeled. However, if ribulose-5-phosphate, derived from the phosphogluconate pathway, equilibrated with triose phosphate derived from the Embden-Meyerhof pathway, isotope could be incorporated into ribulose and ribose in this way.

In view of these considerations, the low isotope content of the ribose (20 to 30 per cent) indicates that most of this pentose was formed via a pathway in which the C₁ of glucose was lost. However, a part apparently was derived from a C₃ + C₂ condensation, but it is not evident at present whether this involved the equilibration of ribulose phosphate with triose phosphate or net synthesis of the ribulose phosphate by such a condensation. The estimation of the utilization of the phosphogluconate pathway (16 to 38 per cent of all glucose metabolized) by E. coli under conditions comparable to those of these experiments indicates that the amount of ribulose-5-phosphate generated by this pathway is more than that needed for ribose formation (11).

SUMMARY

1. A new method is described for isolation of a pure derivative of the ribose moiety of small quantities of ribose nucleic acid.
2. This method has been employed in studying the radioactivity resident in ribose synthesized by *Escherichia coli* with D-glucose-1-C\(^{14}\) as the sole carbon source.

3. The results are consistent with the hypothesis that in this organism the major pathway of ribose synthesis from glucose involves a splitting off of the C\(_1\) atom of the hexose. However, this does not appear to be the only mechanism utilized.

**BIBLIOGRAPHY**

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