Riboflavin synthetase has been shown to catalyze the formation of 1 molecule of riboflavin from 2 molecules of 6,7-dimethyl-8-ribityllumazine (Fig. 1) (1, 2). The reaction involves the donation of 4 carbon atoms from the methyl groups and the carbon 6 and carbon 7 portion of one molecule of the lumazine to a second molecule of lumazine to form riboflavin.

2(6,7-Dimethyl-8-ribityllumazine) → riboflavin + X

In this reaction, an additional compound (X) should be formed in amounts equal to that of riboflavin.

It has been shown (2) that all of the atoms of the pyrimidine portion, nitrogen atom 8, and the ribityl moiety of 6,7-dimethyl-8-ribityllumazine are retained in the unknown compound. Furthermore, during isolation by column chromatography, the unknown compound was cleaved into ribitylamine and a compound retaining all of the atoms of the pyrimidine ring of the substrate. It was shown that the latter is either alloxan or closely related chemically to alloxan. Thus, the evidence suggested that Compound X is chemically unstable and is probably a derivative of 6-ribitylaminouracil.

In this report, the isolation of the second product of the riboflavin synthetase reaction and its characterization as 4-((1'-ribitylamino)-5-amino-2,6-dihydroxypyrimidine (6-ribitylamino-5-aminouracil) is described.

EXPERIMENTAL PROCEDURE

Materials

6,7-Dimethyl-8-ribityllumazine-2-14C was synthesized according to published procedures (2). 8-Ribityllumazine-2-14C was prepared from 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine-2-14C. Riboflavin-2-14C was obtained from 6,7-dimethyl-8-ribityllumazine and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine by enzymic conversion. Riboflavin synthetase was purified from bakers' yeast by a modification of a procedure previously described (2). Glyoxal was purified by distillation of 30% technical grade glyoxal (Eastman Organic Chemical) under reduced pressure. Acid alumina AG-4 (chromatographic aluminum oxide, 200 to 400 mesh) and the cation exchange resin, Dowex AG-50W-H+ (200 to 400 mesh) were from Bio-Rad Laboratories; Lloyd's reagent was from Hartmann-Leddon Company, Philadelphia; and riboflavin (U.S.P. grade) was from Merck and Company, Inc.

Methods

Paper Chromatography and Thin Layer Chromatography—Plates for thin layer chromatography were prepared with Machery Nagel Cellulose 300 G (Brinkmann Instruments, Inc.). Materials were chromatographed on Whatman No. 3MM paper and developed by the ascending method for 16 to 18 hours at ambient temperatures. The following solvent systems were used in both techniques: System I, 1-butanol-ethanol-water (500:175:360); System II, 1-butanol-acetic acid-water (200:30:75); System III, isobutyric acid-NH4OH-H2O (100:60:16); System IV, isopropyl ether-formic acid (9:6); System V, 1-propanol-NH4OH-H2O (6:3:1); System VI, 3% NH4Cl. Assays—Purified enzyme preparations from bakers' yeast (specific activity, 90 to 450 mmoles of riboflavin formed per mg of protein per hour at 37°) were used in these studies. The general method of incubation and methods for the determination of riboflavin, 6,7-dimethyl-8-ribityllumazine, and of radioactivity have been described previously (2). The specific composition of the incubation mixtures used in each experiment is described at the appropriate place in the communication.

Synthesis of Lumazine Derivatives and 1-ribitylamino-5-amino-2,6-dihydroxypyrimidine

8-(1'-D-Ribityl)lumazine—4-(1'-D-Ribityl)lumazine-5-amino-2,6-dihydroxypyrimidine was prepared by reduction of 500 mg (1.7 mmoles) of 4-(1'-ribityl)lumazine-5-nitroso-2,6-dihydroxypyrimidine in 10 ml of water with 1.0 g of sodium hydrosulfite. To the resulting solution were added 1.5 g of sodium thiosulfate. The resulting solution was heated for 40 minutes at 82° with swirling until solution was complete. Spectrophotometric analysis of an aliquot of the sample indicated a yield of 323 mg of 8-(1'-ribityl)lumazine (1.1 mmoles, 62%). The reaction mixture was a different mixture (3) when commercial 30% glyoxal (Eastman Kodak, technical
applied to a column of 70 g of acid alumina (prepared in a 2-cm diameter column, washed with water until the ultraviolet absorbance of the washings was negligible), and the lumazine was eluted with water. The green-fluorescent eluate was evaporated to dryness in a vacuum and taken up in 20 ml of water. The lumazine was extracted from the aqueous phase with ten 20-ml portions of benzyl alcohol (saturated with water) and with three 20-ml portions of pure benzyl alcohol, permitting 95% extraction of the lumazine. The combined benzyl alcohol layers were filtered, an equal volume of ether added, and the lumazine transferred back into water. The aqueous layer was evaporated, and the residual yellow oil was crystallized from 80% ethanol, yielding 285 mg of a pale yellow solid. A solution of 40 mg of the material in 2 ml of water was placed on a column of Dowex 50-X12 (H+ form) (1 X 18 cm), and the lumazine was eluted with water. The residues, after evaporation of the aqueous eluates, were recrystallized several times from water.

8-Methylumazine, 75% yield, m.p. above 290°.

\[ \text{C}_9\text{H}_{10}\text{O}_3\text{N}_4 \]

Calculated: C 47.2%, H 3.4%, O 18.0%, N 31.5%

Found: C 47.0%, H 3.6%, O 18.0%, N 30.9%

Absorption spectrum: in 0.1 N \(\text{H}_2\text{SO}_4\), maxima at 392 \(\mu\) (\(\epsilon = 9,900 \text{ m}^{-1} \text{ cm}^{-1}\)), 256 \(\mu\) (15,500), and minimum at 295 \(\mu\) (440); in 0.1 N \(\text{NaOH}\), maxima at 300 to 120 \(\mu\) (\(\epsilon = 605 \text{ m}^{-1} \text{ cm}^{-1}\)), 305 \(\mu\) (9,000), 281 \(\mu\) (13,400), 231 \(\mu\) (21,200), and minimum at 353 \(\mu\) (7,200), 207 \(\mu\) (7,500), and 253 \(\mu\) (6,200).

8-(\(\beta\)-Hydroxyethyl)umazine, 59% yield, m.p. 221–226° (with decomposition).

\[ \text{C}_9\text{H}_{10}\text{O}_2\text{N}_4 \]

Calculated: C 46.1%, H 4.0%, O 23.9%, N 26.9%

Found: C 46.1%, H 4.0%, O 23.9%, N 26.9%

Absorption spectrum: in 0.1 N \(\text{H}_2\text{SO}_4\), maxima at 397 \(\mu\) (\(\epsilon = 9,400 \text{ m}^{-1} \text{ cm}^{-1}\)), 256 \(\mu\) (15,400) and minimum at 298 \(\mu\) (450); in 0.1 N \(\text{NaOH}\), maxima at 300 to 120 \(\mu\) (\(\epsilon = 605 \text{ m}^{-1} \text{ cm}^{-1}\)), 305 \(\mu\) (9,000), 281 \(\mu\) (13,400), 231 \(\mu\) (21,200), and minimum at 353 \(\mu\) (7,800), 205 \(\mu\) (7,500), and 253 \(\mu\) (6,200).

All lumazine samples were tested for purity by thin layer chromatography with Solvent Systems I, II, III, and VI for development.

The compounds described above have absorption spectra very similar to those exhibited by lumazine derivatives substituted in positions 6, 7, and 8 (8, 9).4 However, the latter substances show a major peak of absorbance with maxima at 404 \(\mu\) to 415 \(\mu\) in acid solution (8), while the corresponding maximal absorbances of the present lumazines, which are unsubstituted at positions 6 and 7, occur at 362 \(\mu\) to 379 \(\mu\). 8-Ribityllumazine and 8-β-hydroxyethylumazine in acid solution show identical molar absorbance at 397 \(\mu\). The former is determined spectrophotometrically under these conditions in the present study.

Preparation of Solid 4-((\(\beta\)-Ribitylamino)-\(\alpha\)-amino-2,6-dihydroxypyrimidine—4-Ribitylamin-5-nitroso-2,6-dihydroxypyrimidine (112 mg) was dissolved in 2.5 ml of hot water. To the heated solution were added 213 mg of sodium hydrosulfite. Heating was continued until all of the red color had disappeared. The solution was cooled in ice, and 1 ml of concentrated HCl was added. The precipitate formed was removed by filtration, and the filtrate was evaporated to dryness in a vacuum and taken up in 20 ml of water. The ultraviolet absorption of the washings was negligible, and the substance was crystalized from 65% ethanol; m.p. 229-231° with sintering at 218°.

\[ \text{C}_9\text{H}_{10}\text{O}_2\text{N}_4 \cdot 4\text{H}_2\text{O} \]

Calculated: C 43.0%, H 4.9%, O 33.8%, N 18.3%

Found: C 42.9%, H 4.9%, O 33.4%, N 18.7%

The compound showed the following spectral characteristics: in 0.1 N \(\text{H}_2\text{SO}_4\), maxima at 397 \(\mu\) (\(\epsilon = 9,500 \text{ m}^{-1} \text{ cm}^{-1}\)), 956 \(\mu\) (15,900), and minima at 298 \(\mu\) (470); in 0.1 N \(\text{NaOH}\), maxima at 310 \(\mu\) (\(\epsilon = 8,200 \text{ m}^{-1} \text{ cm}^{-1}\)), 280 \(\mu\) (13,100), 235 \(\mu\) (21,200), and minima at 293 \(\mu\) (6,500), 258 \(\mu\) (7,400).

8-Methylumazine and 8-(\(\beta\)-Hydroxyethyl)umazine—The diamino pyrimidines were prepared from the corresponding 5-nitrosopyrimidines (4–7)5 and condensed with glyoxal bisulfite in the manner described. The reaction mixtures were passed over columns prepared from 80 to 90 g of acid alumina and eluted with water. The residues, after evaporation of the aqueous eluates, were recrystallized several times from water.

\[ \text{C}_9\text{H}_{10}\text{O}_2\text{N}_4 \]

Calculated: C 38.7%, H 5.4%, O 25.8%, N 30.1%

Found: C 38.3%, H 5.4%, O 25.2%, N 30.0%

Under our conditions, glyoxal reacted with the diaminouracil derivatives shown to form the corresponding 8-substituted lumazines as the major products. Bis-dihydropurinyl compounds have been obtained in good yields by the condensation of glyoxal and certain diamino pyrimidines derivatives by Fidler and Wood (10). The latter have absorption spectra which are distinctly different from those of the pteridines (10), and are not present in our final products.
the filtrate was treated with two 5-ml portions of carbon disulfide. The aqueous phase was stored in a refrigerator overnight, and the precipitate formed was removed by filtration. The solution was chilled in an ice bath and was saturated with gaseous HCl. The sodium chloride precipitate was removed by filtration. The clear filtrate was brought to room temperature, and 12 ml of ethanol were added dropwise with stirring. The resulting suspension was stored in the refrigerator overnight, filtered, and the residue washed with about 1 ml of ethanol. The white residue was dried in a vacuum at room temperature.

4-Ribitylamino-5-amino-2,6-dihydroxypyrimidine HCl, 50% yield.

C₉H₁₄O₄N₄.HCl

Calculated: C 34.6%, H 5.5%, O 30.7%, N 17.9%, Cl 11.3%

Found: C 34.4%, H 5.4%, O 30.6%, N 17.4%, Cl 11.3%

Absorption spectrum in 0.1 N HCl, maximum at 268 mp (ε = 24,500 cm⁻¹ cm⁻¹).

RESULTS

Isolation and Characterization of 4-Ribitylamino-5-aminol2,6-dihydroxypyrimidine—6,7-Dimethyl-8-ribityllumazine-2-¹⁴C and enzyme were incubated under the conditions described in Fig. 2. The reaction was terminated by the addition of 0.5 ml of 15% trichloroacetic acid to each milliliter of incubation mixture. The protein precipitate was removed by centrifugation, and the riboflavin content of the supernatant liquid was determined spectrophotometrically. The solution was saturated with benzyl alcohol, and the precipitate was removed by centrifugation. All of the riboflavin and a portion of the 6,7-dimethyl-8-ribityllumazine, but none of the 4-ribitylamino-5-aminol2, 6-dihydroxypyrimidine, were extracted into benzyl alcohol by treatment of the aqueous phase with five 4-ml portions of the organic solvent. The benzyl alcohol dissolved in the aqueous layer was removed by treatment with four 4-ml portions of chloroform.

The aqueous solution was then applied to a column of Dowex 50-X4 (0.7 × 12 cm) equilibrated with 0.05 M HCl. The column was washed with 0.05 M HCl. An unknown component appeared at the front of the elution pattern, apparently a chemical decomposition product of 6,7-dimethyl-8-ribityllumazine, since it was formed either when the enzyme was present (Fig. 2A) or absent (Fig. 2B). 6,7-Dimethyl-8-ribityllumazine followed the unknown component; 0.1 M HCl was then applied to the column. Only incubation mixtures containing enzyme led to the appearance of a radioactive compound with maximal absorbance at 268 mp in the 0.1 M HCl effluent (compare Fig. 2A and B). This substance had an absorption spectrum identical with that of 4-ribitylamino-5-aminol2, 6-dihydroxypyrimidine (and of 4-(β-hydroxyethyl)amino-5-aminol2,6-dihydroxypyrimidine); it has been shown in separate experiments that the authentic substance emerges from the column in identical effluent fractions.

The methods described above were then used to estimate the stoichiometry of product formation in the riboflavin synthetase reaction. As can be seen in Table I, 4-ribitylamino-5-aminol2, 6-dihydroxypyrimidine was isolated in a yield corresponding to 79% of the amount of riboflavin formed; and the molar specific radioactivities of the two substances were in excellent agreement. 4-Ribitylamino-5-aminol2, 6-dihydroxypyrimidine was identified further by condensing it with glyoxal to form 8-ribityllumazine (Fig. 1). The latter retained the same specific radioactivity as the 4-ribitylamino-5-aminol2, 6-dihydroxypyrimidine from which it was derived, even after extensive purification (Table I, Steps A to E).

Trapping of 4-Ribitylamino-5-aminol2, 6-dihydroxypyrimidine by Glyoxal during Enzymic Incubation—6,7-Dimethyl-8-ribityllumazine was incubated with enzyme in the presence of redistilled glyoxal® for 30 minutes at 37°. The reaction mixture (Table II) was chilled, and 0.5 ml of 15% trichloroacetic acid was added.

In separate experiments under appropriate conditions, condensation of the enzymically formed product with 2,3-butane-dione or with ethyl pyruvate yielded, respectively, 6,7-dimethyl-8-ribityllumazine (11) and 6-methyl-7-hydroxy-8-ribityllumazine (12).

Under certain conditions, glyoxal can react nonenzymically with 6,7-dimethyl-8-ribityllumazine to form a green-fluorescent compound with an absorption maximum at 412 mp. This reaction can be eliminated by using the lowest concentration of glyoxal possible for efficient condensation and by processing the reaction mixture rapidly.

![Fig. 2](http://www.jbc.org/)

**Fig. 2. Separation of products of riboflavin synthetase reaction by chromatography on Dowex 50-X4. A, enzyme present (40 mg of protein; specific activity, 90) in 6.0 ml of incubation mixture. B, enzyme absent. Total volume of reaction mixture was 3.0 ml. The incubation mixtures in both cases contained per ml: 27 Mmoles of potassium phosphate at pH 7.0, 20 Mmoles of sodium sulfite at pH 7.0, and 0.07 Mmole of 6,7-dimethyl-8-ribityllumazine 2-¹⁴C. Incubations in the presence and in the absence of enzyme were performed at 37° for 18 minutes. Subsequent treatments are described in the text. All effluents from the columns were collected in 5-ml fractions. ---, radioactivity; - - - - , absorbance at 268 mp; · · · · , absorbance at 407 mp.)
Table I
Enzymic conversion of 6,7-dimethyl-8-ribityllumazine-2-14C to riboflavin and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount*</th>
<th>Total radioactivity</th>
<th>Specific radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,7-Dimethyl-8-ribityllumazine (added)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin (formed)</td>
<td>1.88</td>
<td>212,000</td>
<td>112,000</td>
</tr>
<tr>
<td>4-Ribitylamino-5-amino-2,6-dihydroxypyrimidine (recovered)</td>
<td>1.49</td>
<td>167,800</td>
<td>110,000</td>
</tr>
<tr>
<td>8-Ribityllumazine (recovered)</td>
<td>1.14</td>
<td>167,800</td>
<td>138,000</td>
</tr>
<tr>
<td>Purification of 8-ribityllumazine†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step A</td>
<td>1.07</td>
<td>126,000</td>
<td>117,000</td>
</tr>
<tr>
<td>Step B</td>
<td>0.96</td>
<td>108,600</td>
<td>113,000</td>
</tr>
<tr>
<td>Steps C + D</td>
<td>0.723</td>
<td>82,000</td>
<td>113,000</td>
</tr>
<tr>
<td>Step E</td>
<td></td>
<td></td>
<td>116,000</td>
</tr>
</tbody>
</table>

* The amounts of all compounds were determined spectrophotometrically under the following conditions: riboflavin at 450 nm (ε = 12,200 M⁻¹ cm⁻¹), 6,7-dimethyl-8-ribityllumazine at 407 nm (ε = 10,300); 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine at 308 nm (ε = 25,000); 8-ribityllumazine at 390 nm (ε = 9,600).
† To the combined fractions of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine, 6,7-dimethyl-8-ribityllumazine was added 0.7 ml (0.5 mmole) of glyoxal. The reaction mixture was brought to about pH 3.0 by adding 330 mg of NaHCO₃, and heated at 90° for 1 hour.
‡ The solution containing 8-ribityllumazine was evaporated to dryness. The residue was taken up in water, made up to 80% dryness. The residue was taken up in water, made up to 80% dryness.

Table II
Condensation of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine with glyoxal in riboflavin synthetase reaction

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount of reaction component*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,7-Dimethyl-8-ribityllumazine (consumed)</td>
<td>0.37</td>
</tr>
<tr>
<td>8-Ribityllumazine (formed)</td>
<td>0.19</td>
</tr>
<tr>
<td>Riboflavin (formed)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Determined spectrophotometrically with the use of the molar absorbances cited in Footnote* of Table I.

was added. Denatured protein was removed by centrifugation, and 0.3 ml of 0.05 M HCl was added to the supernatant solution. This liquid (1.70 ml) was divided into three 0.50-ml aliquots. 8-Ribityllumazine-2-14C, 6,7-dimethyl-8-ribityllumazine-2-14C, and riboflavin 2-14C were added to the three aliquots, respectively. Each aliquot was applied to a column of Dowex 50-X4 (1 × 14 cm) previously washed with 0.05 M HCl. The columns were eluted with 0.05 M HCl, and fractions of approximately 10-ml volume were collected.

The substances emerged from the columns in the order 8-ribityllumazine, 6,7-dimethyl-8-ribityllumazine, and riboflavin, in distinctly separate peaks. The separation was complete, since radioactivity from added labeled compounds appeared only in the column effluent fractions corresponding to the substance added. It was particularly significant that the 8-ribityllumazine isolated from the sample containing added 6,7-dimethyl-8-ribityllumazine-2-14C was not radioactive and hence not contaminated by 6,7-dimethyl-8-ribityllumazine. The isolated 8-ribityllumazine was further characterized by comparison with the authentic substance; mobilities in paper chromatographic Systems I, II, and III, and absorption spectra were identical.

The formation of 8-ribityllumazine and riboflavin was measured in the appropriate column effluents by spectrophotometric analysis (Table II).

Discussion
Consideration of the stoichiometry of the enzymic conversion of 6,7-dimethyl-8-ribityllumazine to riboflavin and inspection of the chemical nature of the substances involved in the riboflavin synthetase reaction made it appear that a 4-ribitylamino-2,6-dihydroxypyrimidine derivative should be the second product of this reaction (see structures in Fig. 1). In the previous report, evidence was presented which supported the formation of such a second product by riboflavin synthetase (2), but its exact chemical nature remained to be established. Indications that 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine could be this product were obtained by the detection of 2,10-dihydro-4,6,8-trihydroxy-10-ribityl-2-oxopyrimido[5,4-g]pteridine in certain chromatographic fractions during an attempt to isolate the unknown compound from an incubation mixture. The pyrimidopteridine may also have been present in incubation mixtures containing 6,7-dimethyl-8-ribityllumazine and a crude extract of Exserohiium ashbyii, since it was reported by Asai, Masuda, and Kuwada (13) that this mixture showed a fluorescent spot on a system of paper chromatography with the same mobility as 2,10-dihydro-4,6,8-trihydroxy-10-ribityl-2-oxopyrimido[5,4-g]pteridine. This compound is readily formed by self-condensation of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (14), and by reaction of this diaminopyrimidine with alloxaan (15). However, such evidence cannot be considered conclusive since in the case of Asai, Masuda, and Kuwada (13) other products, e.g., 6-methyl-7-hydroxy-8-ribityllumazine, were also present in incubation mixtures containing the crude cell extract, but absent when a purified enzyme was used (2). These authors (16) had reported previously that they had been unable to find 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine in Exserohiium ashbyii. In addition, our previous observation that Compound X could be decomposed easily during the process of isolation to form a highly reactive substance such as alloxaan (10).
made it possible that 2,10-dihydro-2,6,8-trihydroxy-10-ribityl-2-oxopyrimido[5,1-g]-pteridine could have been formed by the fortuitous combination of products related to but different from 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine. Therefore, to demonstrate the existence of the dianimopyrimidine compound, it became necessary to isolate the compound, or a characteristic derivative, in amounts concomitant with the stoichiometry of the over-all reaction. This condition has been fulfilled in the present study.

The separation of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine from the other substances of the reaction mixture became possible with the finding that these vic-diaminopyrimidine derivatives are relatively stable in solutions of dilute mineral acids. By following the procedures developed (Table I), 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine was isolated from an incubation mixture in a yield (79%) compatible with the appearance of riboflavin. The 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine formed did not arise from decomposition of 6,7-dimethyl-8-ribityllumazine, since none of the dianimopyrimidine appeared in the absence of enzyme (Fig. 2B). The dianimopyrimidine isolated was characterized further by condensing it with glyoxal, forming 8-ribityllumazine (Fig. 1) in 77% yield. The properties of the latter were identical with those of the authentic substance. Furthermore, the specific molar radioactivities of the 6,7-dimethyl-8-ribityllumazine-2-14C, riboflavin, and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine isolated were identical (Table I).

It is unlikely that 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine is formed from yet another substance during the process of isolation, since it has been shown that the substance can be made to react with glyoxal to form 8-ribityllumazine under the mild conditions prevailing during enzymic incubation. Under these circumstances, formation of 8-ribityllumazine and of riboflavin occurred in nearly equivalent amounts (Table II). Control experiments with a heat-denatured enzyme or omitting glyoxal showed no formation of 8-ribityllumazine.

The results of the present study indicate that the riboflavin synthetase reaction can be described by the balanced equation

\[
2(6,7\text{-Dimethyl}-8\text{-ribityllumazine}) \to \text{riboflavin} + 4\text{-ribitylamino-5-amino-2,6-dihydroxypyrimidine}
\]

characteristic absorption at 268 nm, and that treatment of this percolate with Dowex 50 led to the formation of significant amounts of alloxan.

**SUMMARY**

The second product arising from the conversion of 6,7-dimethyl-8-(1'-n-ribityl)llumazine to riboflavin by riboflavin synthetase has been shown to be 4-(1'-n-ribitylamino)-5-amino-2,6-dihydroxypyrimidine. This pyrimidine was isolated from the incubation mixture in high yields by column chromatography on the cation exchange resin, Dowex 50-X4. The ultraviolet absorption spectrum of this compound was identical with that of the authentic substance and of the analogue, 4-(β-hydroxyethyl)-5-amino-2,6-dihydroxypyrimidine. Its identity was established further by its condensation with glyoxal, ethyl pyruvate, or 2,3-butanedione, which yielded 8-(1'-n-ribityl)llumazine, 6-methyl-7-hydroxy-8-(1'-d-ribityl)llumazine, and 6,7-dimethyl-8-(1'-ribityl)llumazine, respectively. Incubation of 6,7-dimethyl-8-ribityllumazine with the enzyme in the presence of glyoxal led to the formation of equal amounts of riboflavin and 8-ribitylumazine.

The preparation and properties of 8-(1'-n-ribityl)llumazine, 8-methyllumazine, 8-(β-hydroxyethyl)llumazine, and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine have been described.

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4-(1'-d-Ribitylamino)-5-amino-2,6-dihydroxypyrimidine, the Second Product of the Riboflavin Synthetase Reaction

H. Wacker, R. A. Harvey, C. H. Winestock and G. W. E. Plaut