THE ROLE OF PYRIMIDINES IN THE BIOGENESIS OF PTERIDINES

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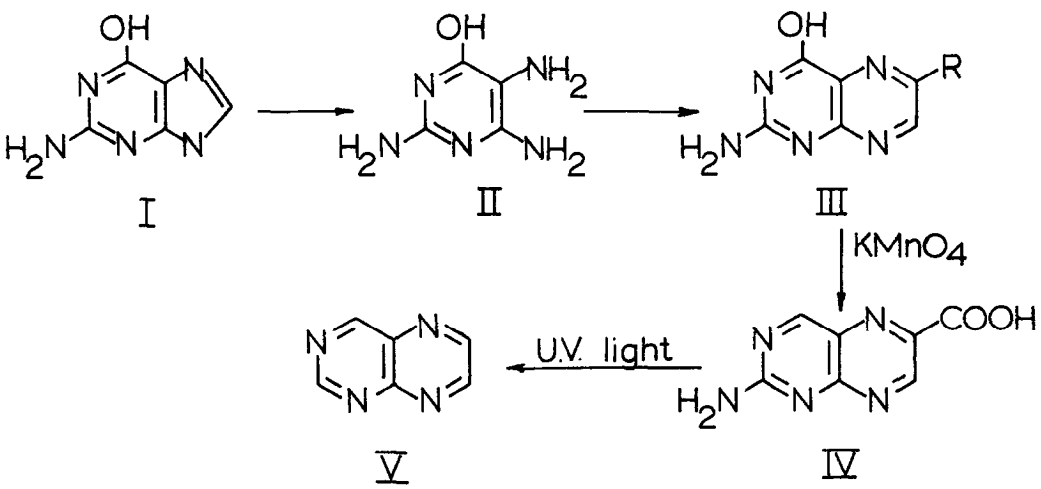
INTRODUCTION

In recent years indirect evidence, primarily from growth studies, has accumulated implicating diaminopyrimidines in the biosynthesis of pteridines. See for example the article by Stokstad, (1960). Isotopic evidence also points in this direction. It has, for example, been demonstrated that adenine-2-C^{14} but not adenine-8-C^{14} contributes its label to the pteridines of type III in scheme I below. (Vieira and Shaw, 1961). Subsequently, it was shown that guanine (I) was the more important purine



SCHEME I.

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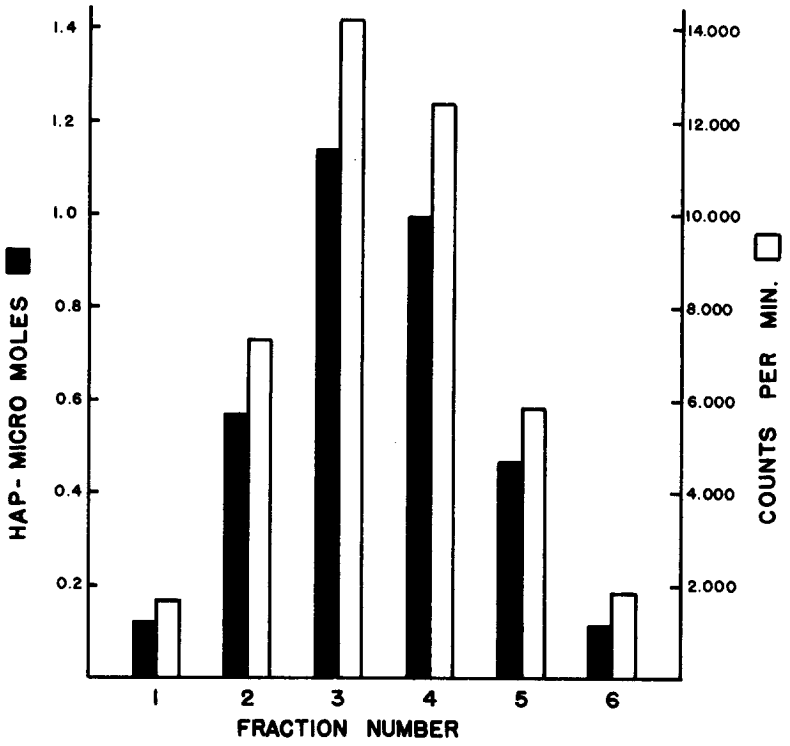
precursor in similar experiments (Baugh and Shaw, to be published). The incorporation of radioactivity from guanosine or guanylic acid into folic acid by cell free extracts of E. coli was recently reported by Reynolds and Brown (1962).

McNutt, studying riboflavin biosynthesis (McNutt, 1961) demonstrated the utilization of all the purine ring atoms except carbon-8; it has been shown that this pathway involves a pteridine intermediate (Plaut, 1960).

In this paper we present isotopic evidence both for the formation of 2,4,5-triamino-6-hydroxypyrimidine (II) from guanine (I) and its utilization in pteridine ring formation.

EXPERIMENTAL

Cultures of Corynebacterium sp. were grown in the medium of Hutchings, et al., (1948) fortified with 60 mg of II per liter labeled with carbon-14 in the five position, with a specific activity of 16,000 cpm/μmole. After five days incubation the pteridine pool was isolated by adsorption and elution from norite as described by Hutchings, et al., (1948). The mixture of pteridines so obtained was oxidized to IV and purified on Dowex 50 H^+. The U.V. spectra of this material agreed closely with that published for IV by Mowat, et al., (1948). No evidence was seen for the isomeric 7-acid. The peak fractions of IV from Dowex 50 H^+ were combined and evaporated in vacuo to dryness to remove excess HCl and at pH 6.0 was decarboxylated in U.V. light according to published methods (Vieira and Shaw, 1961). The V so obtained was purified on Dowex 50 H^+ and a single peak was found, successive fractions of which were quantitated spectrally and plated at infinite thinness for counting in a windowless, gas flow counter (Nuclear Chicago). The close agreement between radioactivity and micromoles of V through the peak may be seen in Figure I. From II, specific activity of 16,000 cpm/μmole, V was obtained with a specific activity of 13,500 cpm/μmole. This represents an 85% efficiency of conversion of II into the mixed pteridine pool.



Correlation of radioactivity and pteridine content (measured at 312 mu) of successive chromatographic fractions of 2-amino-4-hydroxypteridine derived by $KMnO_4$ oxidation of the mixed pteridine pool. Open bars radioactivity; solid bars micromoles.

FIGURE I.

Riboflavin was isolated, purified and found to be non-radioactive from this precursor (II), although riboflavin is well labeled by radioactive I. This finding indicated that the label from II was not getting into V via the known pathway through the purines. For confirmation the nucleic acids were isolated from the bacteria (Roberts, et al., 1957), hydrolyzed with H_2SO_4 (Kerr and Seraidarian, 1949), and the free bases purified on Dowex 50 H^+ (Cohn et al., 1955). The guanine and adenine so obtained were non-radioactive.

Since II has not been found in nature, experiments designed to trap this compound as a metabolite of I were carried out. Four one liter flasks containing five hundred ml of media each were fortified with 30 mg of gua-

nine-2-C-14 per flask, specific activity 86,000 cpm/μmole. After two days on the shaker 10 mg of II, 10 mg of the 5-formyl derivative of II, and 10 mg of 2-amino-4-hydroxy-6-hydroxymethyl pteridine* were added to each flask aseptically, and the incubation continued for two days, at which time the additions were repeated and incubation was continued for a total of six days. The bacteria were harvested by centrifugation and extracted with 200 ml 1% NH_4/ETOH (V/V) followed by extraction with 200 ml 1% HCl/ETOH (V/V). These extracts were taken to dryness in vacuo and the residues added to the bacterial supernatant media. The media was then reduced to 200 ml in vacuo and at pH 7.0 was passed over a 3 x 15 cm column of DEAE cellulose that had previously been equilibrated with 0.5 M phosphate buffer pH 6.0 and washed with distilled water. The column was washed until the eluate was transparent to U.V. light at 260 mμ. The eluate and washings were combined and reduced to 50 ml in vacuo, acidified to pH 2.0 and applied to a 3 x 15 cm column of Dowex 50 H^+. The column was eluted by a gradient elution using one liter each of distilled water and 3N HCl; four major peaks were obtained. The fourth peak emerged in the range of normality of HCl from 2.5-3N. This fraction was reduced to 20 ml in vacuo and reapplied to a 2 x 15 cm column of Dowex 50 H^+ and a second water - 3N HCl gradient was applied. The peak in the range 2.5-3N HCl was well separated from any other U.V. absorbing material, gave the correct spectra for II, and behaved chromatographically in the above procedure like an authentic sample. None of the 5-formyl derivative of II was recovered from the media. It is considered likely that the isolation procedures would hydrolyse 5-formyl II to II. An aliquot from the peak was plated and counted and a specific activity of 200 cpm/μmole above background was found. A fraction of this peak was refluxed for 30 minutes with an excess of 2,3-butanedione and the resulting pteridine was purified on Dowex 50 H^+. This material behaved chromatographically as an authentic sample of 2-amino-4-

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* See discussion.

hydroxy-6,7-dimethylpteridine, and gave the U.V. spectra for this compound as published by Curran and Angier, (1958). Aliquots of this material were plated and counted and the specific activity of this derivative was also found to be 200 cpm/μmole above background.

It therefore appears clear that II or a closely related derivative of II is a metabolite of I and can contribute its radioactivity with great efficiency to pteridines of type III in this organism.

DISCUSSION

These findings are in general agreement with the nutritional findings of Dewey and Kidder and co-workers who recently observed the sparing effect of II on the folic acid requirement of Tetrahymena pyriformis (Kidder and Dewey, 1961) and the ability of II to replace the Biopterin requirement of Crithidia fasciculata (Dewey, Kidder and Butler, 1959). Weygand reported that he was unable to show the incorporation of radioactivity from II-2-C^{14} into the folic acid isolated from several strains of bacteria (Weygand, et al., 1956). However, after feeding II-2-C^{14} to pierid caterpillars, he found the label in position two of xanthopterin (Weygand and Waldschmidt).

The low but definite radioactivity of II is probably a reflection of the small pool size for this pyrimidine in vivo, and may indicate that a derivative of II is the true intermediate. We have been studying the 5-formyl derivative as a possible intermediate in the ring opening reaction of I, however, since this derivative was not recovered one can only speculate as to its role in this biosynthetic pathway at this time. We are currently trying to modify our isolation procedure to permit the recovery of 5-formyl II. The 2-amino-4-hydroxy-6-hydroxymethyl pteridine was recovered radioactive. These findings will be reported in the future. It will be of interest to see if folic acid is labeled by II or its 5-formyl derivative, especially since it is known to be labeled by I. These possibilities are presently under investigation.

ACKNOWLEDGEMENT

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