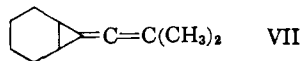


Reaction of I with potassium *t*-butoxide slurried in cyclohexene gave a 29% yield of allene (strong absorption at 2005 and 1995 cm^{-1}). After distillation and chromatography on Florisil the product was still slightly impure. Found: C, 87.80; H, 11.32. By analogy with II structure VII would be assigned to the allene.



Experiments with olefin mixtures are in progress to determine the reactivity of the intermediate as compared with CBr_2 ,⁸ CCl_2 ,⁹ and CHCOOEt .¹⁰

(8) P. S. Skell and A. Y. Garner, *THIS JOURNAL*, **78**, 5430 (1956).

(9) W. von E. Doering and W. A. Henderson, Jr., *ibid.*, **80**, 5274 (1958).

(10) P. S. Skell and R. M. Etter, *Chem. and Ind.*, 624 (1958).

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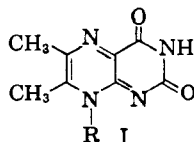
RECEIVED FEBRUARY 9, 1957

THE CONVERSION OF 6,7-DIMETHYL-8-RIBITYLLUMAZINE (6,7-DIMETHYL-8-RIBITYL-2,4[1H,3H]PTERIDINEDIONE) TO RIBOFLAVIN BY EXTRACTS OF *ASHBYA GOSSYPII*

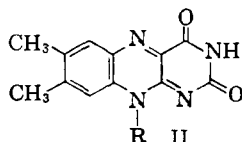
Sir:

In previous studies it was shown that the addition of formate- C^{14} or other known labeled precursors of riboflavin¹ to intact cells of *Ashbya gossypii* led to a specific radioactivity in isolated 6,7-dimethyl-8-ribityllumazine (6,7-dimethyl-8-ribityl-2,4[1H,3H]pteridinedione) (I) higher than that of riboflavin (II) in the early time periods of incubation.² These findings suggest that I is not a biological degradation product of II, but is a probable intermediate on the pathway of biosynthesis of the vitamin.

The demonstration of the biological conversion of I to II would provide more direct evidence that this substance is on the path of biogenesis of riboflavin from simpler precursor compounds. Attempts to obtain riboflavin synthesis from I with washed intact cell suspensions of *A. gossypii* were unsuccessful. This is due presumably to the inability of the compound to penetrate the cells. That a permeability barrier may indeed be responsible for the lack of success in these experiments is suggested by the observation that incubation of washed cells of *A. gossypii* with added riboflavin-2- C^{14} for 24 hours under aerobic conditions did not lead to incorporation of radioactivity into intracellular II.³



R = Ribityl



However, addition of I to a reaction mixture containing cell-free extracts of *A. gossypii* in the presence of pyruvate, adenosine triphosphate, and a

(1) G. W. E. Plaut, *J. Biol. Chem.*, **208**, 513 (1954).

(2) G. F. Maley and G. W. E. Plaut, *Federation Proc.*, **17**, 268 (1958); *J. Biol. Chem.*, **234**, 641 (1959).

(3) G. F. Maley and G. W. E. Plaut, unpublished observations.

crude coenzyme preparation led to an enhanced formation of II. In addition, it was observed (Table I) that the specific radioactivity of II formed *de novo* by the extract from *A. gossypii* is approximately the same as that of added 6,7-dimethyl-8-ribityllumazine-2- C^{14} , indicating that the added I is converted directly to II, and does not merely stimulate the transformation of another compound in the crude extract to the vitamin.

TABLE I

THE ENZYMIC INCORPORATION OF 6,7-DIMETHYL-8-RIBITYLLUMAZINE-2- C^{14} INTO RIBOFLAVIN

All reaction vessels contained 40 μmoles of sodium pyruvate, 40 μmoles of ATP, 10 mg. of crude coenzymes (Armour), particles and supernatant from centrifuged *A. gossypii* sonicate, volume adjusted to 3.1 ml. with buffer mixture at pH 6.9. Incubated in air in Warburg flasks with shaking for 14.5 hours at 30°. Riboflavin was separated from 6,7-dimethyl-8-ribityllumazine and purified by column and paper chromatography to constant specific radioactivity.² Riboflavin was estimated by measurement of the light absorption in 0.1N NaOH at 450 m μ .

Compound added or isolated	Control	Experimental
6,7-Dimethyl-8-ribityllumazine, added, μmoles	None	1.97
Riboflavin, initial, μmoles	0.474	0.489
14.5 hours, μmoles	0.513	0.613
Increase, μmoles	0.039	0.124
Total radioactivity, c.p.m.	...	7590
formed, c.p.m./ μmole	...	61000
6,7-Dimethyl-8-ribityllumazine, added, c.p.m./ μmole	...	67200

The conversion of I to II can be visualized to occur by way of the addition of two two-carbon compounds, *e.g.*, acetyl-CoA, or possibly a four-carbon fragment (*e.g.*, acetoin or diacetyl) to the methyl groups of the lumazine derivative. Such a mechanism would be consistent with the pattern of labeling of the aromatic ring of riboflavin observed in experiments with intact cells of *A. gossypii*.⁴

In view of its structural relationship to the pterins it is possible that I could be a precursor of other pterins, *e.g.*, folic acid, as well as of the flavins.

(4) G. W. E. Plaut, *J. Biol. Chem.*, **211**, 111 (1954).

(5) Fellow of the American Heart Association.

(6) Senior Research Fellow U.S.P.H.S. (SF261). These studies were aided by fund from the Williams Waterman Fund and the National Institutes of Health (H3891).

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GLADYS F. MALEY⁵
G. W. E. PLAUT⁶

RECEIVED FEBRUARY 21, 1959

NEW REACTION OF RECOIL HYDROGEN ATOMS WITH ALKENES¹

Sir:

Studies of the reaction of recoil tritium atoms, such as produced by the $\text{He}^3(\text{n,p})\text{T}$ process, with gaseous alkanes, have shown that "hot" hydrogen atoms can react efficiently by a simple displacement to form HT and the labeled form of the

(1) Work supported by United States Atomic Energy Commission.