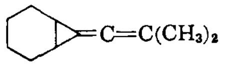
Reaction of I with potassium *t*-butoxide slurried in cyclohexene gave a 29% yield of allene (strong absorption at 2005 and 1995 cm.$^{-1}$). After distillation and chromatography on Florisil the product was still slightly impure. Found: C, 87.80; H, 11.32. By analogy with II structure VII would be assigned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


VII

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]

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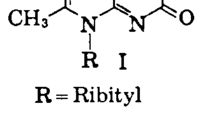
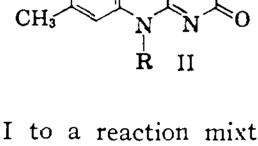
THE CONVERSION OF 6,7-DIMETHYL-8-RIBITYLLUMAZINE (6,7-DIMETHYL-8-RIBITYL-2,4[1H,3H]-PTERIDINEDIONE) TO RIBOFLAVIN BY EXTRACTS OF *ASHBYA GOSSYPII*

Sir:

In previous studies it was shown that the addition of formate-C^{14} or other known labeled precursors of riboflavin[1] 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]


I


II

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 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 ENZYMATIC 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 *p*H 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 0.1*N* NaOH at 450 mμ.

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.

---

[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.

[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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RECEIVED FEBRUARY 21, 1959

NEW REACTION OF RECOIL HYDROGEN ATOMS WITH ALKENES[1]

Sir:

Studies of the reaction of recoil tritium atoms, such as produced by the $He^3(n,p)T$ 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

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[1] Work supported by United States Atomic Energy Commission,

TABLE I

RELATIVE YIELDS OF RADIOACTIVE PRODUCTS (NORMALIZED TO 100 FOR ACTIVITY IN PARENT HYDROCARBON)

Molecules produced by mechanism described are boldface. Hydrocarbons are represented by appropriate symbols: thus /\/ butane; /‾\ *cis*-butene, etc.

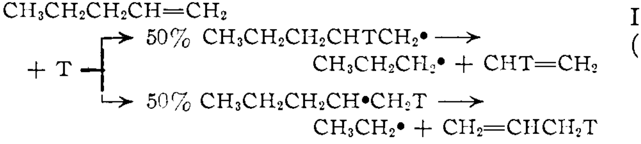
Tritiated products / Hydrocarbon	H_2	CH_4	/	⫽	/\	/⑊	/\/	>—	/\⫽	/⑊/	/‾\	>=	/\/\	/\/⑊	/\/\ + /\=/
/\⫽	143	13	3	**41**	0	**41**	31	0	100	7	4	0	0	0	0
/⑊/	171	11	1	4	0	**60**	46	0	12	100	13	0	0	0	0
/‾\	191	15	1	5	0	**69**	58	0	14	13	100	0	0	0	0
>=	116	9	0	0	0	**21**	0	10	0	0	0	100	0	0	0
/\/⑊	178	9	6	**33**	0	**42**	0	0	4	0	0	0	24	100	9
/\⫽\ + /\=/	184	13	2	0	0	**23**	0	0	**22**	2	0	0	30	6	100
/\/	235	15	15	5	6	1	100	0	0	0	0	0	0	0	0
/\⫽ + He(1:50)	500	14	0	**50**	0	**75**	44	0	100[a]	10	8	0	8	0	0
/\⫽ + O_2 (50:1)	160	5	0	**49**	0	**46**	0	0	100	4	4	0	0	0	0

[a] Yield of butene-1 activity in this run reduced by order of magnitude compared to run containing no moderator.

alkane.[2,3,4] Only small amounts of labeled degradation products, apparently formed by displacement of alkyl groups instead of hydrogen atoms, are formed.

The present work on the reaction of recoil tritium with alkenes indicates the existence of an additional reaction mechanism. As with alkanes (see Table I, butane) about 60–80% of the tritium is found in HT and the labeled parent molecules. However, appreciable yields (10–20%) of labeled degradation products are also found. Table I shows that the yield pattern of these unsaturated fragments varies in a very specific manner with the alkene reagent.

The following mechanism for this "fragmentation" reaction is postulated. A tritium atom, having some residual recoil energy, adds on to the double bond of the alkene, forming a "hot" radical. This radical then decomposes by cleaving a carbon–carbon bond without rearrangement, to form a smaller radical and an olefin. Because the tritium atom carries excess energy, it will attack the double bond indiscriminantly and form approximately equal amounts of both possible "hot" radicals.



$$\begin{array}{l} CH_3CH_2CH_2CH{=}CH_2 \\ + T \begin{cases} \rightarrow 50\%\ CH_3CH_2CH_2CHTCH_2\bullet \longrightarrow CH_3CH_2CH_2\bullet + CHT{=}CH_2 \\ \rightarrow 50\%\ CH_3CH_2CH_2CH\bullet CH_2T \longrightarrow CH_3CH_2\bullet + CH_2{=}CHCH_2T \end{cases} \end{array}$$

For example, the tritium atom will react with pentene-1 as shown, to give similar amounts of tritiated propylene and ethylene. From pentene-2, however, the same reaction will yield butene-1 and propylene.

This mechanism is in accord with results on the decomposition of deuterated butyl radicals at 500°.[5] Since addition of a hydrogen atom to a double bond is exothermic, the reaction probably could proceed at thermal energies. However, in the presence of an excess of helium as a moderator, the yields of the labeled fragments, as well as the yield of the parent molecule, are reduced greatly. On the other hand, these products are unaffected by the presence of a small amount of oxygen as scavenger. Therefore, this reaction to form labeled fragments apparently also takes place before the recoil tritium reaches thermal energies. Thus, while this is not a "hot-atom" mechanism occurring *only* at very high kinetic energies, it may be termed "epithermal" to denote that it competes effectively only at energies above the thermal range.

This mechanism accounts for the results so far at hand, some of which are shown in Table I. Work to demonstrate the further predictions of this model is continuing.

The authors wish to thank Professor W. Doering for his interest and criticism.

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RECEIVED FEBRUARY 11, 1959

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[2] M. El-Sayed and R. Wolfgang, THIS JOURNAL, **79**, 3286 (1957).

[3] M. El-Sayed, P. Estrup and R. Wolfgang, *J. Phys. Chem.*, **62**, 1356 (1958).

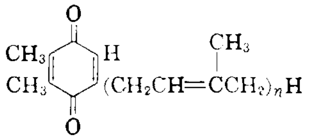
[4] D. Urch and R. Wolfgang, unpublished work.

[5] J. McNesby, D. Drew and A. Gordon, *J. Chem. Phys.*, **24**, 1260 (1956).

COENZYME Q. VIII. STRUCTURE STUDIES ON A PLANT QUINONE

Sir:

We have isolated from alfalfa a quinone having coenzyme Q-like activity[1]; structural data support I, 2,3-dimethyl-5-[3′-methyl-2′-butenyl-oktakis-(3′-methyl-2′-butenylene)]-benzoquinone.



I. $n = 9$
II. $n = 3$

Crane and Lester[2,3] isolated from alfalfa a quinone (Q-254), m.p. 42–43°, which appeared to be a trimethylbenzoquinone with a fourth substituent of nine mono-unsaturated isoprenoid units. Kofler reported[4] a substituted benzoquinone, m.p. 48–49°, from alfalfa.

The coenzyme Q-like activity[2,3] of Q-254 and studies[5–8] on "unidentified factors" in alfalfa in

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[1] Kindly tested by Dr. F. L. Crane, University of Texas, Austin.

[2] F. L. Crane and R. L. Lester, *Plant Physiol.*, **33**, (Suppl.) VII (1958).

[3] F. L. Crane, *ibid.*, in press.

[4] M. Kofler, "Jubilee Volume, Emil Barell," F. Hoffmann-LaRoche and Co., Ltd., Basel, 1946.

[5] M. G. Vavick, A. Werty and A. R. Remmerer, *Poultry Sci.*, **32**, 433 (1953).

[6] H. M. Scott, H. Fisher and J. M. Snyder, *ibid.*, **32**, 555 (1953).

[7] C. H. Hill, R. L. Borchers, C. W. Ackerson and F. E. Mussehl, *ibid.*, **32**, 775 (1953).

[8] B. March, J. Biely and S. P. Touchburn, *ibid.*, **34**, 968 (1955).