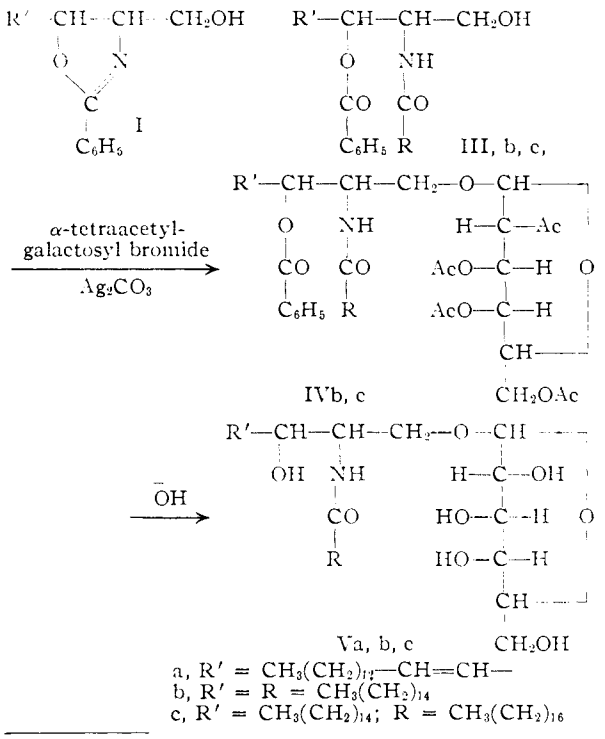
with catalytic amounts of sodium methylate[7] gave a fairly good yield of the dihydrocerebrosides (Vb, c) which were purified by crystallization from either butyl acetate or anhydrous methanol. On heating, both glycosides sintered at about 100° and turned liquid at 125–130°.[8] (Vb: found: C, 68.1; H, 11.4; N, 2.2; galactose (anthrone[9]): 23.9–24.5%; Vc: found: C, 69.1; H, 11.5; N, 2.2).

The infrared spectra of Vb and Vc (pressed in KBr) were essentially identical and showed bands at 3.0, 3.42, 3.52, 6.10, 6.46, 6.80, 7.26, 8.10, 8.56, 8.80, 8.90, 9.30, 9.44, 9.64, 11.18, 11.46, 12.72 and 13.92 μ. This is in good agreement with the spectrum of phrenosin (in KBr) published recently.[10]



(7) G. Zemplén, *Ber.*, **59**, 1254 (1926).

(8) This relatively low melting point may be due to the presence of a conglomerate of the enantiomorphs rather than a racemic compound, owing to the effect of the D-galactose moiety.

(9) F. A. Loewus, *Anal. Chem.*, **24**, 219 (1952).

(10) A. Rosenberg and E. Chargaff, *J. Biol. Chem.*, **233**, 1323 (1958).

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RECEIVED FEBRUARY 23, 1959

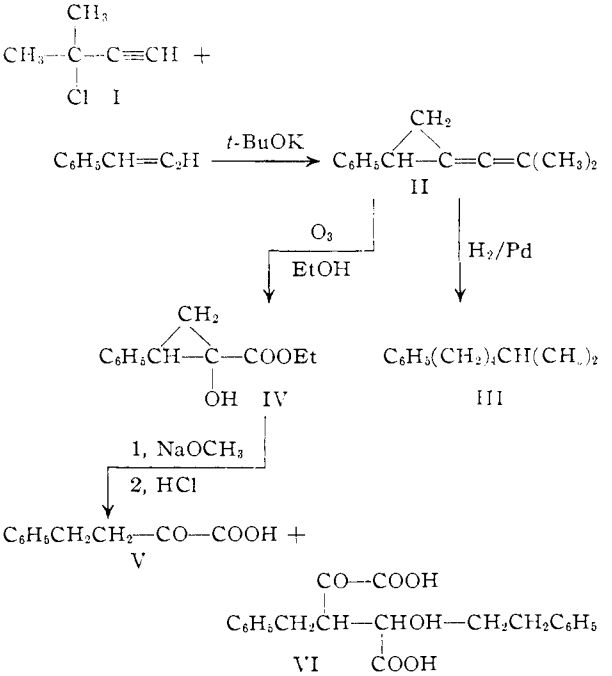
CARBENES FROM *t*-ACETYLENIC CHLORIDES. SYNTHESIS OF ALKENYLIDENECYCLOPROPANES

Sir:

Reactions of ethynyldialkyl carbinyl chlorides with nucleophilic reagents have been investigated.[1,2] Kinetics[1a,b,2] and products[1] of these reactions are rationalized by the formation of an intermediate alkenylidene carbene ($R_2C{=}C{=}C{:}$).[1] Additional evidence for the carbene intermediate has been obtained by the synthesis of alkenylidenecyclopropanes from reactions of olefins, 2-chloro-2-methyl-3-butyne (I), and base.

Addition of I to a stirred slurry of alcohol-free potassium *t*-butoxide in styrene resulted in a 48% yield of 1-(2-methylpropenylidene)-2-phenylcyclopropane (II). Found: C, 91.71; H, 8.29. The infrared spectrum of II showed the absence of an isolated or conjugated double bond, but exhibited strong absorption at 2050 cm.$^{-1}$, a position intermediate to the normal absorption of allenes and acetylenes.[3] Strong absorption at 1027 cm.$^{-1}$ suggests the presence of the cyclopropane ring.[4] The styrene chromophore was absent in the ultraviolet spectrum. The nuclear magnetic resonance spectrum of II was similar to that of styrene oxide[5] with superposition of the strong methyl absorption.

Hydrogenation of II gave 1-phenyl-5-methylhexane (III). Found: C, 88.05; H, 11.74; diacetamido derivative, m.p. 205–206°. Found: C, 70.21; H, 8.87; N, 9.49. An independent synthesis of III gave a product with identical infrared spectrum and diacetamido derivative. Ozonolysis of II in ethanol yielded acetone (65%), carbon dioxide (3%), a hydroxyester (40%), and a tarry residue. The hydroxyester (IV) is believed to be ethyl 1-hydroxy-2-phenylcyclopropanecarboxylate. Found: C, 70.19; H, 6.89. IV with sodium methoxide at room temperature for two hours gave after acidification benzylpyruvic acid (V) and its aldol condensation product (VI) (76%). Found: C, 67.56; H, 5.74. V (m.p. 36–40°) was oxidized with hydrogen peroxide to β-phenylpropionic acid. VI (m.p. 167–168° dec.) was synthesized independently[6] (mixed m.p. 167–168° dec.); IV would be expected to undergo ring opening readily to give V.[7]



(1) (a) G. F. Hennion and K. W. Nelson, THIS JOURNAL, **79**, 2142 (1957); (b) G. F. Hennion and D. E. Maloney, *ibid.*, **73**, 4735 (1951); (c) G. F. Hennion and E. G. Teach, *ibid.*, **75**, 1653 (1953).

(2) A. Burawoy and E. Spinner, *J. Chem. Soc.*, 3752 (1954).

(3) L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, pp. 48–53.

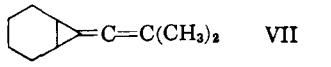
(4) *Ibid.*, pp. 27–28.

(5) J. D. Roberts, "Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 48–49.

(6) J. Bougalt, *Compt. rend.*, **155**, 477 (1912).

(7) L. Skattebøl and J. D. Roberts, THIS JOURNAL, **80**, 4085 (1958).

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]

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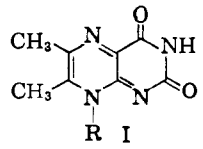
THE CONVERSION OF 6,7-DIMETHYL-8-RIBITYLLUMAZINE (6,7-DIMETHYL-8-RIBITYL-2,4[1H,3H]-PTERIDINEDIONE) TO RIBOFLAVIN BY EXTRACTS OF *ASHBYA GOSSYPII*

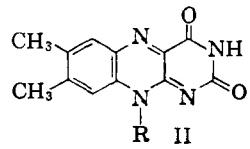
Sir:

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-rityl-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 I



R 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.

(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.

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.

(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

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