picrate (m.p. 140–142°),[3] and reduction to 5,5a,-6,7,8,9,10,10a-octahydrocyclohept[b]indole (m.p. 73–74°).[3] Conversion of I to II (calcd. for $C_{13}H_9N$: C, 87.12; H, 5.06. Found: C, 87.36; H, 4.86) was accomplished by vapor-phase (350–360°) catalytic (5% palladium–charcoal on magnesium oxide) dehydrogenation. The absorption spectra of II are: ultraviolet (alcohol solution), λ_{max} in mμ at 288, log ε 4.47, and 309, log ε 4.59 (similar to that of benz[a]azulene);[4] visible (alcohol solution), broad peak with maximum at 500 mμ, log ε 2.61; infrared in 6 to 9μ region (carbon tetrachloride solution), 6.2μ, 6.7μ, 7.1μ, 7.3μ, 7.8μ and 8.4μ. Further identification of the product as II was provided by reduction (97% of the theoretical quantity of hydrogen taken up) to I (identity by m.m.p. and infrared spectrum). Acridine has been identified (m.p., m.m.p., infrared spectrum) as a by-product of the dehydrogenation of I.

Repetition of the reaction of I with iodine and nitrobenzene afforded, in our hands, a low yield of dark red solid which was identical (infrared spectrum) with II obtained by catalytic dehydrogenation, and lesser amounts of other, unidentified substances, but no product corresponding to that described by Treibs.

II, as obtained by us, represents the first example of a heteroazulene compound of established structure.

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(4) This is in agreement with the observations of G. M. Badger, R. S. Pearce and R. Pettit (*ibid.*, 3199 (1951)) on similar systems.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF WASHINGTON A. G. ANDERSON, JR.
SEATTLE 5, WASHINGTON JAMES TAZUMA

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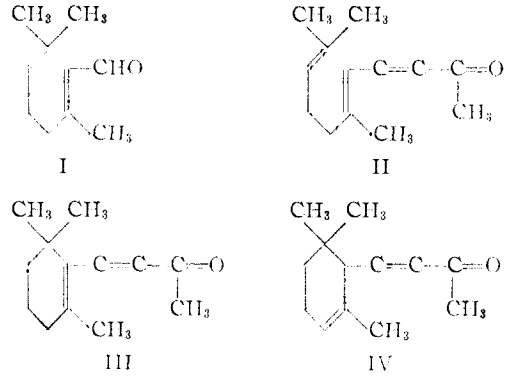
CAROTENOID PRODUCTION IN PHYCOMYCES

Sir:

Goodwin and Lijinsky[1] have reviewed hypotheses concerning carotenoid formation in plants. We have confirmed their findings that leucine with slower growth produces more β-carotene in the mold *Phycomyces blakesleeanus*[2] than asparagine with faster growth. We have tested a hypothesis that we could influence carotenoid production by providing cultures with compounds theoretically capable of providing terminal groups appropriate to specific carotenoid molecules. These include citral (I), pseudoionone (II), β-ionone (III) and α-ionone (IV). Thus I and II might be expected to form lycopene if ring closure does not occur; III to form β-carotene; IV to form α-carotene or even ϵ_1-carotene synthesized by Karrer and Eugster.[3]

Using a Wickerham (carbon-base) medium increased to 7.5% glucose, with 0.25% *dl*-leucine, we have five times demonstrated an effect of β-ionone, applied *ca.* 2 μl. per 20 ml. of medium to



72-hour cultures. Fifteen hours later, the β-carotene content of treated cultures in a typical run was 218 μg. per gram of dry mycelium, compared with 91.2 μg. per gram for control cultures. In all cases, I, II and III depressed the rate of culture development. Aerial mycelium was sparse and there were few fruiting bodies, and I and II showed no pigment production. The effect of III on β-carotene production relative to the controls is lessened progressively as the aerial mycelium develops and the cultures mature. The foregoing was demonstrated on media limited in one or more nutrients.

We therefore developed a more nearly complete medium in which growth of controls was not limited in the total time of the experiment, 60 hours. This medium contained 3 g. of Difco yeast extract and 25 g. of glucose per liter, with thiamine added at the time of inoculation, 6 mg. per liter. Twenty ml. of medium was added to petri dishes with Raschig rings or glass beads supporting a tared filter paper, *cf.* ref. 1. Cultures were inoculated and grown in the light, *ca.* 25°, for 36 hours, when aerial mycelium is thick but short (*ca.* 1/8″), with no fruiting bodies and negligible pigmentation. We then added *ca.* 5 μl. of the following to the cultures, subsequently kept in the dark: I, (Eastman Kodak Co., redistilled); II, synthesized by coupling I with acetone; III (Fritzsche Bros., Inc., Novoviol, beta, re-distilled); IV (Novoviol, alpha, extra). Since IV was demonstrated to contain some III, this will not be discussed except to remark that no α-carotene was found. Control cultures grew and developed fruiting bodies in the subsequent 12 hours. I- and II-treated cultures showed little development and no pigment production. Cultures treated with III yielded 30 μg. of β-carotene per culture after 7 hours, and 134 μg. after 23 hours. They were bright orange, contrasted with gray yellow for the controls containing 4.9 μg. after 23 hours. Similar results were obtained in a second run. Carotene formation seemingly proceeded in the presence of III at the expense of metabolites that would otherwise ensure normal culture development. Two possibilities would explain negative results with I and II, toxicity or diversion of metabolites to colorless compounds, possibly polyene in nature.

This experiment was repeated at levels of I, II and III one-tenth the previous values, and growth was more nearly normal. I and II under these conditions produced less total pigment, 75 and 88%,

(1) T. W. Goodwin and W. Lijinsky, *Biochem. J.*, **50**, 268 (1951).

(2) We thank Dr. Kenneth B. Raper, Northern Regional Research Laboratory, for providing (+) and (−) strains. The latter were used here.

(3) B. Karrer and C. H. Eugster, *Helv. Chim. Acta*, **33**, 1433, 1952 (1950).

respectively, of that for the control, *cf.* 450% for III. However, spectroscopic and chromatographic evidence indicated a small increase in the absolute amount of lycopene for I and II, compared with III and the control. The fluorescence of I- and II-treated samples was noticeably blue-green, whereas the others were characteristically green.

In summary, production of β-carotene can be markedly influenced in a short period (5 to 20 hours) and of lycopene to a minor extent by use of compounds presumably providing terminal groups in the carotenoid molecule. Results are not incompatible with a tentative scheme postulated by Garton, Goodwin and Lijinsky.[4]

(4) G. A. Garton, T. W. Goodwin and W. Lijinsky, *Biochem. J.*, **49**, 154 (1951.).

G. MACKINNEY
T. NAKAYAMA
C. D. BUSS
C. O. CHICHESTER
DIVISION OF FOOD TECHNOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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LIPOTHIAMIDE AND ITS RELATION TO A THIAMIN COENZYME REQUIRED FOR OXIDATIVE DECARBOXYLATION OF α-KETO ACIDS

Sir:

We wish to report evidence indicating that lipothiamide, a catalytic agent present in cellular extracts, is the amide of α-lipoic acid and thiamin, and suggesting that this moiety is part of a thiamin coenzyme required for oxidative decarboxylation of α-keto acids.

Natural materials contain α-lipoic acid, a catalytic factor required for pyruvate oxidation,[1] in bound condition. Acidic or basic hydrolysis degrades the bound material predominantly to α-lipoic acid, whereas enzymatic hydrolysis releases "complex" forms of α-lipoic acid.[1,2]

Our study of α-lipoic acid complexes was directed toward first obtaining an organism which required such a complex for growth. This requirement was exhibited by a mutant strain of *Escherichia coli*, developed by irradiating wild-type cells and isolating the mutant, using the penicillin technique.

Whereas the mutant did not respond to α-lipoic acid in the presence of all other known vitamins and growth factors, it did respond to the α-lipoic acid complexes present in cellular extracts, or to substances formed by incubating α-lipoic acid and thiamin with wild-type *Escherichia coli* or *Streptococcus lactis* cells.

Bioautographs of the incubation mixture revealed the presence of two active principles, possessing R_f values identical with those of two α-lipoic acid complexes present in cellular extracts. Both biosynthetic "conjugates" gave a negative thiochrome test and a positive azo test. These observations suggested that the two conjugates contain α-lipoic acid and thiamin conjugated through an amide linkage.

Chemical synthesis studies support this conclusion. Reaction mixtures obtained by heating α-lipoic acid and thiamin *in vacuo*, and by treating thiamin with the acid chloride of α-lipoic acid, produced maximum growth of the mutant at a level of 2.5×10^{-6} γ per cc. of culture medium. In a similar manner, preparations active in catalytic amounts for the mutant have been obtained by condensing α-lipoic acid or its acid chloride with 2-methyl-5-ethoxymethyl-6-aminopyrimidine, thiamin monophosphate, and thiamin pyrophosphate, respectively.

The active principles in chemical preparations of lipothiamide and its monophosphate possessed R_f values identical with the two active principles produced biosynthetically and with two of those present in cellular extracts. Furthermore, treatment of lipothiamide monophosphate or pyrophosphate with intestinal phosphatase degrades these two factors to lipothiamide, as revealed with bioautographs.

An investigation of possible coenzymatic functions of lipothiamide and its phosphorylated derivatives has revealed: (1) resting cell suspensions of the mutant will not oxidize either pyruvate or α-ketoglutarate unless one of these conjugates is added; (2) the growth requirement of the organism for lipothiamide can be completely by-passed by supplying the products (acetate, citrate and succinate) of these two blocked reactions. *All* three products, however, must be present. The induced mutation apparently destroys the capacity of the organism to conjugate α-lipoic acid and the pyrimidine moiety of thiamin, resulting in a deficiency of an α-keto acid coöxidase required for the reactions producing the three essential metabolites.

BIOCHEMICAL INSTITUTE AND
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TEXAS, AND
CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS
LESTER J. REED
BETTY G. DEBUSK

RECEIVED MAY 5, 1952

LYSINE BIOSYNTHESIS IN TORULOPSIS UTILIS[1]

Sir:

In an isotopic study of biosynthetic mechanisms in *Torulopsis utilis*, data from yeast grown on methyl- and carboxyl-labeled acetate have provided a clue to the pathway of lysine synthesis in this organism. Cultural details and other experimental procedures have already been described.[2] The lysine was isolated from the cell material by standard methods, and degraded by the following steps: decarboxylation with ninhydrin; oxidation to δ-aminovaleric acid; degradation of the latter by the Schmidt reaction[3] to carbon dioxide and 1,4-diaminobutane; oxidation of the latter to succinic acid; isolation of succinate carboxyls by the Schmidt reaction. Another sample of lysine was oxidized to glutaric acid and the glutarate carboxyls obtained by the Schmidt reaction. These procedures yielded individual activities of carbons 1, 2, 3 and 6, and the averages of carbons 4 and 5.

(1) Aided by grants from the U. S. Atomic Energy Commission, Contract No. AT(30-1)777, and by an institutional grant from the American Cancer Society.

(2) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 1726 (1952).

(3) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951); we are greatly indebted to Dr. Phares for supplying us with this method prior to publication.

(1) I. C. Gunsalus, *et al.*, *J. Biol. Chem.*, **194**, 859 (1952).

(2) L. J. Reed, *et al.*, *ibid.*, **192**, 851 (1951).