COMMUNICATIONS TO THE EDITOR

SYNTHESIS OF COMPOUNDS IN THE THIOCTIC ACID SERIES

Sir:

The name "thioctic acid" was proposed for 5,8-dithioöctanoic acid, a biologically active compound,[1] and it was suggested that the number of the carbon atom to which the secondary sulfur is attached might be used to designate various compounds in this series. It has now become evident that the biological activity of 5-thioctic acid is much less than that of 6-thioctic acid (6,8-dithiooctanoic acid) which has been synthesized by a new method. The synthesis of thioctic acid from γ-(2-tetrahydrofuryl)-butyric acid was described.[2] Although this synthesis would be expected to yield primarily 5,8-dithioöctanoic acid, the possibility of producing other isomers by rearrangement was pointed out.[2] We have improved this synthesis and have isolated three isomeric compounds.

The expected DL-5-thioctic acid, m.p. 58° (calcd. for $C_8H_{14}O_2S_2$: neut. equiv., 206; C, 46.6; H, 6.8; S, 31.1. Found: neut. equiv., 207; C, 46.2; H, 7.0; S, 31.5) was isolated in 22% over-all yield from our original synthesis starting with γ-(2-tetrahydrofuryl)-butyric acid. A second isomer, m.p. 81–86° (found: mol. wt., 200 (Rast camphor); C, 46.7; H, 7.0; S, 31.6) was isolated in 1% yield. This product was the only compound isolated (7% yield) when 4-hydroxy-8-bromoöctanoic acid or its lactone was treated with hydrobromic acid and thiourea followed by alkaline hydrolysis and iodine oxidation. This isomer is presumed to be 4-thioctic acid.

The third isomer was formed in approximately 5% over-all yield as measured by bioassay and was obtained in pure form only after oxidation to the sulfoxide and conversion to the crystalline S-benzylthiuronium salt, m.p. 143–144°.[2] This pure salt was converted to the acid, reduced with sodium borohydride to the dithiol and reoxidized with iodine to the intramolecular disulfide. This third isomer has been shown to be DL-6-thioctic acid, since by X-ray diffraction studies the S-benzylthiuronium salt of the sulfoxide was identical with the corresponding salt of the sulfoxide prepared from a sample of DL-6-thioctic acid obtained by the following synthesis: ethyl adipyl chloride was condensed with ethylene in the presence of aluminum chloride to yield on distillation ethyl Δ^7,6-ketooctenoate, b.p. 116–118° at 1.5 mm. (I). Thioacetic acid was added to I and the mixture was then reduced with sodium borohydride in methanol and hydrolyzed to give DL-8-thiol-6-hydroxyoctanoic acid (II), b.p. 164° at 0.05 mm. II was converted to crude DL-6,8-dithioloctanoic acid (dihydro-6-thioctic acid) (III) by treatment with excess thiourea in refluxing 50% hydriodic acid followed by alkaline hydrolysis. Crude III was oxidized in dilute chloroform solution to the intramolecular disulfide, DL-6-thioctic acid (IV) with iodine in potassium iodide solution. The crude IV was purified by vacuum distillation, b.p. 160–165° at 0.1 mm., and recrystallization from cyclohexane, m.p. 60–61°; found: neut. equiv. 202; C, 45.08; H, 7.08; S, 30.84. The biological activities of these three thioctic acids are compared in Table I.

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **74**, 1868 (1952).
(2) M. W. Bullock, *et al.*, *ibid.*, **74**, 1868 (1952).

TABLE I

BIOLOGICAL ACTIVITIES OF COMPOUNDS IN THE THIOCTIC ACID SERIES

Acid	Relative potency for *Streptococcus faecalis*[3]	Mμg per ml. of medium for half-maximum growth *Tetrahymena geleii*[4]	*Corynebacterium*[5]	Relative pyruvate oxidation factor activity[6]
DL-6-thioctic	100	0.376	0.5	100[a]
DL-5-thioctic	0.08	98	610	0.3
DL-4-thioctic	0.04	575	770	0.1

[a] In the test system employed 10 millimicrograms of DL-6-thioctic acid gave an oxygen uptake of 225, 290 and 259 microliters per hour on different days.

(3) F. P. Day, *et al.*, *Bacteriological Proceedings*, Soc. of Am. Bacteriologists, p. 136 (1951).
(4) By G. W. Kidder, Amherst College, Amherst, Mass.
(5) E. L. R. Stokstad, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **74**, 571 (1950).
(6) I. C. Gunsalus, M. I. Dolin and L. Struglia, *J. Biol. Chem.*, **194**, 849 (1952).

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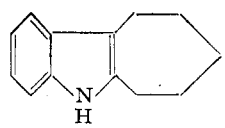
1-AZABENZ[b]AZULENE

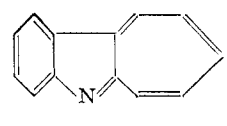
Sir:

Treibs[1] recently described 1-azabenz[b]azulene (II), prepared by reaction of I with iodine and nitrobenzene, as a blue solid which was unstable to heat and light. The basic character of the substance and analysis (Cl, N) of its unstable hydrochloride constituted the only evidence for the structure of the product. No data identifying the starting material were given.

Prior to the publication of Treibs' results we had obtained II as a dark red solid (m.p. 140–141°) stable to heat and light and basic in character (soluble in aqueous acid).



I



II

Our starting material (I) was prepared by the method of Rogers and Corson[2] and identified by its m.p. (140–141°),[3] preparation of a dark red

(1) v. W. Treibs, *Ann.*, **576**, 110 (1952).
(2) C. U. Rogers and B. B. Corson, THIS JOURNAL, **69**, 2910 (1947).
(3) N. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, 2583 (1928).

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.

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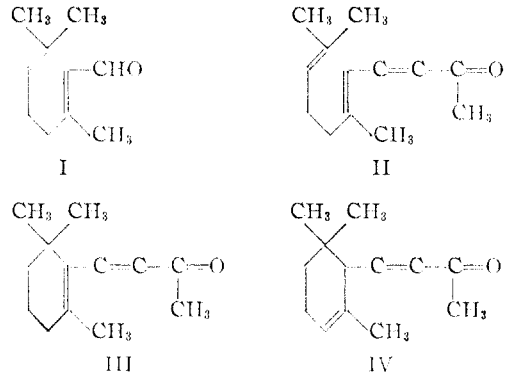
CAROTENOID PRODUCTION IN PHYCOMYCES

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).