Experimental[6]

The Reimer–Tiemann Reaction with 2,5-Dimethylphenol.—Ten grams of 2,5-dimethylphenol, m.p. 74.5–75.5°, was stirred, while heated under reflux with 150 ml. of chloroform, 150 g. of potassium hydroxide and 120 ml. of water for 1.5 hours. The mixture was titrated and found to be 0.16 *N* in base. The solution was then steam distilled and 240 mg. of pale yellow needles, m.p. 56.6–60.5°, was isolated from the distillate. Evaporative sublimation at 55° (0.01 mm.) yielded the analytical sample of 2-hydroxy-3,6-dimethylbenzaldehyde as pale yellow needles, m.p. 60.5–61.5° (reported 62–63° [7]). *Anal.* Calcd. for $C_9H_{10}O_2$: C, 71.98; H, 6.71; Found: C, 71.78; H, 6.73.

The Reimer–Tiemann Reaction with *p*-Hydroxybenzoic Acid.—When 13.8 g. (0.1 mole) of *p*-hydroxybenzoic acid was refluxed for one hour with 90 ml. of water, 65 g. of 85% potassium hydroxide (1.0 mole) and 16.0 g. (0.125 mole) of chloroform, there was obtained after acidification and ether extraction according to the directions of Armstrong, 14.2 g. of brown solid. The aldehyde, determined as the phenylhydrazone and 2,4-dinitrophenylhydrazone amounted to 12.5 and 11.5%, respectively. No water or ether-insoluble material was found; the remainder, 12.0 g. (87%) is considered recovered starting material (see reference 2).

The experiment was repeated, using 13.8 g. (0.1 mole) of the phenol, 81.0 g. (1.25 moles) of potassium hydroxide and 35.4 g. (0.3 mole) of chloroform, followed after a reaction period of 30 minutes with another 1.25 moles of potassium hydroxide and 0.3 mole of chloroform. Separation of the reaction product into ether-soluble and ether-insoluble material after acidification, yielded 3.77 g. (27.5%) of brown powder, molecular weight 400–425 (Rast), insoluble in water and ether, soluble in sodium bicarbonate. Calculated molecular weight of the triphenylmethane derivative is 410. The ether-soluble material, 9.21 g. (62%), was estimated to contain 16.2% aldehydic material and 51% recovered phenol by the method described above.

(6) All melting points are corrected.

(7) K. Auwers and F. Winternitz, *Ber.*, **35**, 465 (1902).

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Desthiobiotin in the Biosynthesis of Biotin

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Desthiobiotin refers to a compound derivable from biotin by Raney nickel reduction.[1] Desthiobiotin satisfies the biotin requirement of some biotin-requiring microörganisms, is inactive as a biotin source for others, and is an antimetabolite of biotin for a third group.[2] Dittmer, *et al.*,[3] have presented microbiological evidence for the conversion of desthiobiotin to biotin by growing yeast. Tatum has reported[4] evidence that desthiobiotin is the biotin derivative that accumulates during growth in the presence of pimelic acid of a biotin-requiring mutant that does not utilize desthiobiotin. Thus it is commonly accepted that desthiobiotin as well as pimelic acid is a normal precursor of biotin.

The nature of the biotin derivative that accumulates when *Aspergillus niger* is grown with aeration in the presence of pimelic acid has been investigated by Wright, *et al.*[5–7] The predominant biotin derivative is biotin L-sulfoxide.[8] Biotin itself also is converted by the mold to biotin L-sulfoxide. It was established that biotin L-sulfoxide originates enzymatically since biotin is unaffected by the medium or by an autoclaved culture of *Aspergillus niger* under conditions simulating growth of the mold.

Experiments similar to those carried out with added pimelic acid have now been completed in which *Aspergillus niger* was grown in the presence of desthiobiotin. It is demonstrated in this paper by paper chromatographic procedures that, under the conditions previously described, desthiobiotin is converted to biotin L-sulfoxide, presumably through biotin as an intermediate. This finding is clear evidence that desthiobiotin is not utilized in an anomalous manner, a point not established by previous studies, since for sulfoxide formation to occur it is *a priori* established that a thiophane ring must exist. These experiments do not necessarily prove of course that desthiobiotin is an *obligate* intermediate in the biosynthesis of biotin.

Experimental

Aspergillus niger was grown in shaker flasks on the basal medium and under conditions described previously in detail.[5] One 500-ml. lot of medium was unsupplemented and served as a control. A second flask was supplemented with 50 γ of DL-desthiobiotin and a third was supplemented with 1 mg. of DL-desthiobiotin. After growth for 5 days the mycelia were filtered off and the culture filtrates paper chromatographed (0.02-ml. culture filtrate applied 10 times, Whatman No. 1 paper, ascending technique, development time 18 hours, room temperature in butanol (40), water (50), acetic acid (10)). This solvent system readily separates a number of biotin derivatives (biocytin, R_F = 0.37, biotin L-sulfoxide, R_F = 0.46, biotin D-sulfoxide, R_F = 0.57), but biotin (R_F = 0.83) and desthiobiotin (R_F = 0.89) are not well separated. R_F values in this solvent system are not significantly influenced by salts and other extraneous materials contained in the culture filtrates studied. Areas

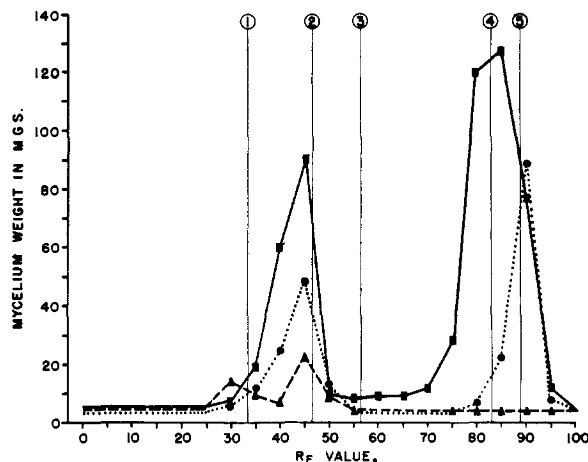

Fig. 1.—Bioautography of *Aspergillus niger* culture filtrates: —■—■—■—, medium supplemented with 1 mg. of DL-desthiobiotin/500 ml.; ..●..●..●.., medium supplemented with 50 γ of DL-desthiobiotin/500 ml.; ---▲---▲---▲---, medium unsupplemented. Vertical lines designated by circled figures represent reference R_F values for: 1, biocytin; 2, biotin L-sulfoxide; 3, biotin D-sulfoxide; 4, biotin; 5, desthiobiotin.

(1) V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *J. Biol. Chem.*, **146**, 475 (1942).

(2) V. G. Lilly and L. H. Leonian, *Science*, **99**, 205 (1944).

(3) K. Dittmer, D. B. Melville and V. du Vigneaud, *ibid.*, **99**, 203 (1944).

(4) E. L. Tatum, *J. Biol. Chem.*, **160**, 455 (1945).

(5) L. D. Wright and E. L. Cresson, THIS JOURNAL, **76**, 4156 (1954).

(6) L. D. Wright, E. L. Cresson, J. Valiant, D. E. Wolf and K. Folkers, *ibid.*, **76**, 4160 (1954).

(7) L. D. Wright, E. L. Cresson, J. Valiant, D. E. Wolf and K. Folkers, *ibid.*, **76**, 4163 (1954).

(8) This nomenclature refers to the optical rotation of the sulfoxide and not to its spacial relationship to any reference compound. Since asymmetry is present in biotin itself biotin D-sulfoxide and biotin L-sulfoxide are diamers and not enantiomorphs.

of microbiological activity were located by cutting the papers into 21 segments which were individually eluted in 25-ml. amounts of a biotin-deficient medium.[5] After elution the paper strips were removed, the flasks plugged, autoclaved and seeded with *Neurospora crassa*. The biotin requirement of this mold is satisfied equally well with biotin, desthiobiotin, biotin L-sulfoxide, biotin D-sulfoxide and biocytin. Following growth of the mold at 30° for 3–5 days the mycelia were removed, pressed, dried and weighed. By plotting mycelial weight against distance traveled the R_F may be obtained by interpolation to within 0.05 of a unit.

The results of the bioautography in butanol-water-acetic acid are summarized in Fig. 1. Additional confirmatory evidence for the existence of biotin L-sulfoxide in the desthiobiotin-supplemented culture filtrate (1 mg. of DL-desthiobiotin) was obtained by chromatography in four other solvents against a reference sample of biotin L-sulfoxide. The results obtained are summarized as

Solvent system	R_F values Culture filtrate	Biotin L-sulfoxide
Phenol (satd. with water)	0.82	0.83
n-Butyric acid (70), water (30)	.83	.80
n-Butanol (80), ethanol (10), ammonia (30)	.07	.07
Isobutyric acid (satd. with water)	.57[a]	.57

[a] Preliminary separation of microbiological activity from factors influencing migration was carried out in phenol (satd. with water) prior to chromatography at 90° to the original direction.

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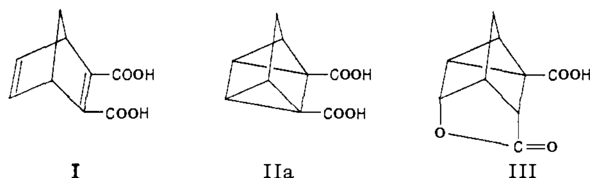
COMMUNICATIONS TO THE EDITOR

SYNTHESIS OF A NORTETRACYCLENE (TETRACYCLOHEPTANE) DERIVATIVE

Sir:

When a solution of bicyclo[2,2,1]heptadiene-2,3-dicarboxylic acid (I)[1] in absolute ether was irradiated for 8–12 hours with a General Electric AH-4 ultraviolet lamp, I was isomerized in good yield to II, m.p. 225° (dec.). *Anal.* Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.48. Found: C, 59.80; H, 4.57. II was converted to a dimethyl ester, b.p. 100–105° (0.5 mm.), $n^{25}D$ 1.5000, by diazomethane in absolute ethanol. *Anal.* Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; molecular wt., 208.2. Found: C, 63.63; H, 5.77: mol. wt. (in camphor), 208.4. II is soluble in water, ethanol, acetone and ethyl acetate, but is insoluble in less polar organic solvents. II has an infrared spectrum distinctly different from I and from the isomeric γ-lactone of 5-hydroxytricyclo[2,2,1,$0^{2,6}$]heptane-2,3-dicarboxylic acid (III),[2,3] in particular lacking carbon–carbon double bond frequencies at 6.3–6.4 μ and 14.2 μ observable in the spectrum of I and the nortricyclene frequency at 12.4 μ observable with III. In addition II and III have peaks at 3.23 and 3.21 μ, respectively, diagnostic of carbon–hydrogen bonds attached to three-membered rings.[4] Ultraviolet absorption spectral studies indicate conjugation in II.



II is isomerized to I by refluxing with palladium–charcoal catalyst in ethyl acetate. Like I, it decolorized bromine solution, but in distinction from I it reacts with both water and ethanol. These products have not yet been characterized. Unlike I, which is inert toward iodine at room temperature, II reacts quantitatively with one mole of iodine (in acetone) per mole of compound to yield a moderately stable diiodide, m.p. 170° (dec.). *Anal.* Calcd. for $C_9H_8O_4I_2$: I, 58.5. Found: I, 56.6. Both I and II are reduced rapidly by hydrogenation over palladium on charcoal to Δ^2-bicyclo[2,2,1]heptene-2,3-dicarboxylic acid.[1]

The above facts appear to be consistent with the valency tautomeric[5,6] structure tetracyclo[2,2,1,-$0^{2,6}$,$0^{3,5}$]heptane-2,3-dicarboxylic acid (IIa) for II. No compound of this ring system, which might be called "nortetracyclene," in view of its relationship to the unknown dehydroterpene tetracyclene,[7] or might be called simply "tetracycloheptane," appears to be described in the literature.

Work on this and analogous materials is continuing.

Acknowledgments.—The authors wish to acknowledge financial assistance from the Office of Naval Research. The infrared spectra were kindly determined by Mr. Jack L. Bitner and Mr. Ernest Silversmith, and analyses were performed by Galbraith Laboratories.

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(2) K. Alder and F. Brochhagen, *Chem. Ber.*, **87**, 167 (1954).
(3) A. Winston and P. Wilder, Jr., THIS JOURNAL, **76**, 3045 (1954).
(4) E. R. Lippincott, *ibid.*, **73**, 2001 (1951).
(5) J. W. Baker, "Tautomerism," George Routledge and Sons, London, 1934, pp. 201–206.
(6) A. C. Cope, A. C. Haven, Jr., F. L. Ramp and E. R. Trumbull, THIS JOURNAL, **74**, 4867 (1952).
(7) In this regard, see T. Hasselstrom and E. M. Falasco, Abstracts of Papers, 125th Meeting of the American Chemical Society, Kansas City, Mo., March, 1954, p. 39M.

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THE "CELLULOLYTIC FACTOR" ACTIVITY OF CERTAIN SHORT CHAINED FATTY ACIDS

Sir:

The presence of an unidentified nutritional factor(s) for rumen microörganisms in rumen juice