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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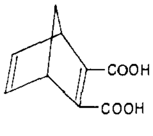
COMMUNICATIONS TO THE EDITOR

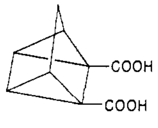
SYNTHESIS OF A NORTETRACYCLENE (TETRACYCLOHEPTANE) DERIVATIVE

Sir:

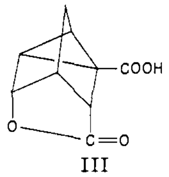
When a solution of bicyclo[2,2,1]heptadiene-2,3-dicarboxylic acid (I)[1] in absolute ether was irradiated for 8–12 hours with a General Electric AH-4 ultraviolet lamp, I was isomerized in good yield to II, m.p. 225° (dec.). *Anal.* Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.48. Found: C, 59.80; H, 4.57. II was converted to a dimethyl ester, b.p. 100–105° (0.5 mm.), $n^{25}\mathrm{D}$ 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.



I



IIa



III

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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(1) O. Diels and K. Alder, *Ann.*, **490**, 236 (1931).

(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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ROBERT L. SNELL

RECEIVED AUGUST 9, 1954

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