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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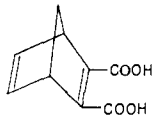
# COMMUNICATIONS TO THE EDITOR

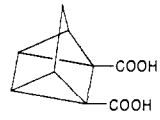
## SYNTHESIS OF A NORTETRACYCLENE (TETRACYCLOHEPTANE) DERIVATIVE

*Sir:*

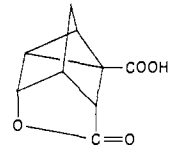
When a solution of bicyclo[2,2,1]heptadiene-2,3-dicarboxylic acid (I)[1] in absolute ether was irradiated for 8–12 hours with a General Electric AH-4 ultraviolet lamp, I was isomerized in good yield to II, m.p. 225° (dec.). *Anal.* Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.48. Found: C, 59.80; H, 4.57. 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.




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 Δ²-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,-$O^{2,6}$,$O^{3,5}$]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.

(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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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

which stimulates the rate of cellulose digestion *in vitro* has been reported from this laboratory.[1] We now wish to report that a volatile fraction from acidified rumen juice and various straight and branched chain fatty acids have cellulolytic factor activity for rumen microörganisms *in vitro*.

The addition of centrifuged rumen juice to a medium composed of mineral salts, purified wood cellulose, biotin, *p*-aminobenzoic acid and glucose inoculated with rumen microörganisms obtained by supercentrifugation of strained rumen juice increased cellulose digestion from 19% as observed in the unsupplemented flask to 58% during a 30-hour fermentation (see Table I). When the distillates obtained by ordinary distillation from rumen juice were added to the medium, the distillates from the acidified rumen juice (*p*H 2.0 to 2.5) with $H_3PO_4$ were active whereas distillates from juice made alkaline (*p*H 10 to 11) with NaOH prior to distillation were inactive. When the material remaining in the distillation flasks was neutralized and added to the fermentation flasks, the alkaline residue was active while there was little activity left in the acid residue.

TABLE I

| Additions to basal medium | Expts. | Av. cellulose digested, % pct. |
|---|---|---|
| None | 13 | 19.2 |
| Centrifuged rumen juice (CR J) | 13 | 58.2 |
| Dist. from *p*H 2–2.5 CR J | 6 | 53.3 |
| Res. from acid dist. CR J | 3 | 16.3 |
| Dist. from *p*H 10–11 CR J | 4 | 18.2 |
| Res. from alk. dist. CR J | 4 | 61.1 |
| *n*-Valeric acid[a] | 8 | 56.3 |
| Iso-valeric acid | 1 | 38.6 |
| Iso-butyric acid | 1 | 38.7 |
| Caproic acid | 3 | 49.5 |
| Valine | 8 | 38.5 |
| Proline | 3 | 36.3 |
| Valine plus proline | 1 | 55.7 |

[a] Acids and amino acids added at level of 0.004 to 0.01%.

The behavior of the active material to distillation suggests that this substance(s) is a steam volatile fatty acid. The addition of short-chained volatile fatty acids to the fermentation medium increased cellulose digestion. Caproic and *n*-valeric acids were the most active although iso-valeric and iso-butyric acids were found to have some stimulatory effect. Acetic, propionic and butyric acids, the normal end products of cellulose digestion by rumen microörganisms, were inactive as were samples of $C_7$ to $C_{10}$ straight chain fatty acids tested.

The occurrence of five-carbon acids in animal fat has been reported recently.[2] Valeric acid as well as branched chain five-carbon acids have been isolated from rumen juice[3] as rumen microörganisms form these acids from amino acids and proteins.[4]

Besides the volatile component obtained from rumen juice which would appear to be one or more of the volatile fatty acids tested above, there appears to be additional non-volatile cellulolytic factor(s) which are responsible for the factor activity found in certain feeds and yeast or yeast by-products. This factor may be related to the amino acid content of these feedstuffs. Preliminary evidence now available indicates that α-amino homologs of the volatile fatty acids used in this study are also active.

The activity of these fatty acids or their amino acid precursors is not restricted entirely to cellulose digestion. The conversion of urea nitrogen into trichloroacetic acid precipitable protein is also increased.

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RECEIVED AUGUST 31, 1954

(1) O. G. Bentley, R. R. Johnson, S. Vanecko and C. H. Hunt, *J. An. Sci.*, **13**, 581 (1954).

(2) R. P. Hansen and A. G. McInnes, *Nature*, **173**, 1093 (1954).

(3) E. F. Annison, *Biochem. J.*, **57**, 400 (1954).

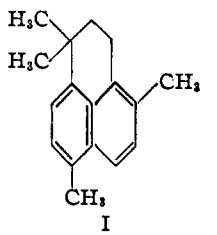
(4) K. El-Shazly, *ibid.*, **51**, 640 (1952).

## THE STRUCTURE OF CATIVIC ACID

*Sir:*

"Cativo," an oleoresinous exudate of *Prioria capaifera*, Griseb., was found by Kalman[1] to consist of an easily esterifiable, unsaturated acid, $C_{20}H_{34}O_2$, and a neutral fraction which was assumed to be the corresponding ester, cativyl cativate. We wish to report now the complete structure of this diterpenic acid.

Cativic acid, m.p. 80–82°,[2] $[\alpha]^{26}D$ −6.54° (EtOH) [*Anal.* Calcd. for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18; neut. equiv., 303. Found: C, 78.22; H, 10.92; 306] was dehydrogenated with palladium-charcoal to **1,2,5,6-tetramethylnaphthalene,** m.p. 114.5–115°; **picrate,** m.p. 151–153°; **trinitrobenzolate,** m.p. 180–181°.[3] These constants as well as the ultraviolet and infrared spectra are closely comparable to those of an authentic sample of this hydrocarbon and its reported derivatives. Dehydrogenation experiments with selenium at 325° led to a $C_{17}H_{20}$ hydrocarbon as major product, identical in all respects to that obtained by a similar dehydrogenation of agathic acid[4] and, by way of infrared spectra comparison, to **1,1,4,7-tetramethylphenalan** (I), recently synthesized by Büchi and Pappas[5]: m.p. 39–40.5°; **picrate,** m.p. 136–138°; **styphnate,** m.p. 153–155°.

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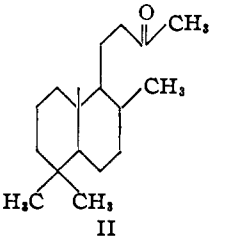


II

Cativic acid, on catalytic hydrogenation, absorbed one mole equivalent of hydrogen and, after Fischer esterification, yielded methyl dihydrocativate, m.p. 43–44°, $[\alpha]^{27}D$ +23.4°. This ester was

(1) N. V. Kalman, THIS JOURNAL, **60**, 1423 (1938).

(2) Kalman's acid was non-crystalline due to isomeric inhomogeneity. The isomerization of cativic acid will be discussed in a future paper.

(3) E. Lederer, *et al.*, *Helv. Chim. Acta*, **29**, 1354 (1946), also have isolated this anomalous product from the Pd-C dehydrogenation of ambreinolide.

(4) L. Ruzicka and J. R. Hosking, *ibid.*, **13**, 1402 (1930).

(5) G. Büchi and J. J. Pappas, THIS JOURNAL, **76**, 2963 (1954).