COMMUNICATIONS TO THE EDITOR

TETRACYCLINE[1]

Sir:

During catalytic reduction studies on chlorotetracycline (Aureomycin) using a platinum catalyst it was noted that the mixture of compounds obtained showed a low chlorine content. Since dechlorination was occurring under these conditions, chlorotetracycline was then subjected to catalytic reductions more favorable to selective removal of an aromatic halogen.

Chlorotetracycline was found to be reductively dehalogenated at room temperature and atmospheric pressure in the presence of 10% palladium on charcoal catalyst and one mole of triethylamine. Slightly over one mole of hydrogen was absorbed in 15–20 minutes using about 100 mg. of chlorotetracycline/cc. in methyl cellosolve. Some heat is produced during this rapid hydrogenolysis and the uptake of hydrogen practically stops after one mole is absorbed.

After the catalyst is removed the filtrate is poured into 5 volumes of water and the free base of tetracycline crystallizes. This product which occurs as a trihydrate can be recrystallized from methanol and water. The anhydrous form can be obtained by drying at 60° *in vacuo* for 8 hours. Either form begins to swell at 165–170° melting with decomposition at 170–173°.

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot 3H_2O$: C, 53.0; H, 6.0; N, 5.6; H_2O, 10.8. Found: C, 52.9; H, 6.2; N, 5.5; H_2O, 10.9.

Tetracycline base dissolved in *n*-butanol by adding hydrochloric acid crystallizes from this solution as a hydrochloride; m.p., darkens gradually and melts with gas at about 214°; $[\alpha]^{25}D$ − 257.9° (0.5% in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot HCl$: C, 55.0; H, 5.2; N, 5.8; Cl, 7.4. Found: C, 54.9; H, 5.3; N, 5.8; Cl, 7.3.

The ultraviolet absorption spectrum in 0.1 *N* hydrochloric acid shows maxima at 220 mμ (ϵ, 13,000), 268 mμ (ϵ, 18,040), and 355 mμ (ϵ, 13,320).

Treatment of tetracycline with hydrochloric acid gives anhydrotetracycline completely identical with that prepared from chlorotetracycline by treatment with hydriodic acid.[2] This would indicate that no structural changes other than removal of the halogen took place during the reduction.

Tetracycline is a potent antibiotic having an antibiotic spectrum very similar to chlorotetracycline. The former compound exhibits increased stability in neutral or alkaline solution.

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(1) The use of this name as a generic term is discussed in This Journal, **74**, 4976 (1952).

(2) C. W. Waller, *et al.*, *ibid.*, **74**, 4981 (1952).

IDENTIFICATION OF AN ANTIBIOTIC POLYACETYLENE FROM *CLITOCYBE DIATRETA* AS A SUBERAMIC ACID ENE-DIYNE*

Sir:

The presence of two antibiotic polyacetylenes in culture liquids of the Basidiomycete *Clitocybe diatreta* was reported recently.[1] For one of these, which was obtained crystalline, the tentative formula $C_{17}H_{12}N_2O_6$ was suggested. Analysis of a fresh sample, however, indicates the formula $C_8H_5NO_3$ rather than that above. The compound can be purified by recrystallization from methanol. It does not melt, but explodes at 198° (uncor.). Found: C, 59.01; H, 3.15; O, 29.34; N, 8.42; mol. wt. (ebullioscopic), 159 (neut. eq., 170, from the previous analysis). Calcd. for $C_8H_8NO_3$ (163.13): C, 58.90; H, 3.09; O, 29.42; N, 8.59; mol. wt., 163. The new formula was further supported by analysis of the catalytic reduction product, the values for which agreed with the formula $C_8H_{15}NO_3$. This compound is a colorless crystalline solid, m.p. 144–145° (uncor). Found: C, 55.51; H, 8.77; N, 8.05. Calcd. for $C_8H_{15}NO_3$ (173.21): C, 55.48; H, 8.73; N, 8.09.

The above data, taken in conjunction with the ultraviolet absorption spectrum (Fig. 1) point to an octadioic acid monoamide containing an ene-diyne grouping, as the probable structure of the polyacetylene. Thus, the ultraviolet absorption maxima are close to those exhibited by the lachnophyllum esters,[2,3,4] compounds of known structure containing an ene-diyne system conjugated to an (esterified) carboxyl group. The remaining CH_2NO indicated by the analysis is most readily accounted for as an amide group.

The reduction product of the antibiotic compound was identified as suberamic acid, the product to be expected on reduction of a polyacetylene of the proposed structure. To establish identity it was necessary to prepare an authentic sample of suberamic acid, since the only literature reference found to the compound is in a paper by Étaix,[5] who reports a melting point of 125–127°. Monomethyl suberate, obtained by the method of Hunsdiecker and Hunsdiecker[6] for the partial esterification of some dibasic acids, was converted to the amide by the method used by Jeffery and Vogel[7] for the preparation of some -amic acids (not including suberamic). The amide melted at 144–145° (uncor.) and gave no depression with the reduction product of the polyacetylene. Found: C, 55.57; H, 8.80; N, 8.02. Calcd. for suberamic acid, $C_8H_{15}NO_3$ (173.21): C, 55.48, H, 8.73; N, 8.09. Further proof of identity of the reduction product

(1) M. Anchel, This Journal, **74**, 1588 (1952).

(2) N. A. Sörensen and K. Stavholt, *Acta. Chem. Scand.*, **4**, 1575 (1950).

(3) T. Bruun, C. M. Haug and N. A. Sörensen, *ibid.*, **4**, 850 (1950).

(4) W. W. Wiljams, V. S. Smirnov and V. P. Goljmov, *J. Gen. Chem. (U.S.S.R.)*, **5**, 1195 (1935).

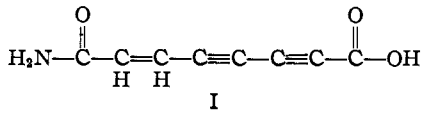
(5) L. Étaix, *Ann. chim. phys.*, [7] **9**, 356 (1896).

(6) H. Hunsdiecker and C. Hunsdiecker, *Ber.*, **75**, 291 (1942).

(7) G. H. Jeffery and A. I. Vogel, *J. Chem. Soc.*, 1101 (1934).

with suberamic acid was obtained by alkaline hydrolysis (of the former) to suberic acid, identified by mixed melting point.

Accordingly, the polyacetylene must have the structure I or II.



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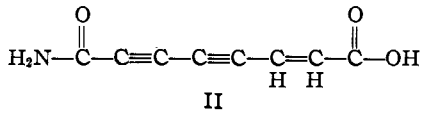

II

Choice between these two formulations could probably best be made on the basis of synthetic model compounds or of authentic samples of the compounds themselves.

Acknowledgments.—This investigation was supported in part by a research grant (E-226) from the National Microbiological Institute of the National Institutes of Health, Public Health Service. The author is indebted to Dr. Julian Wolff for the preparation of Figure 1.

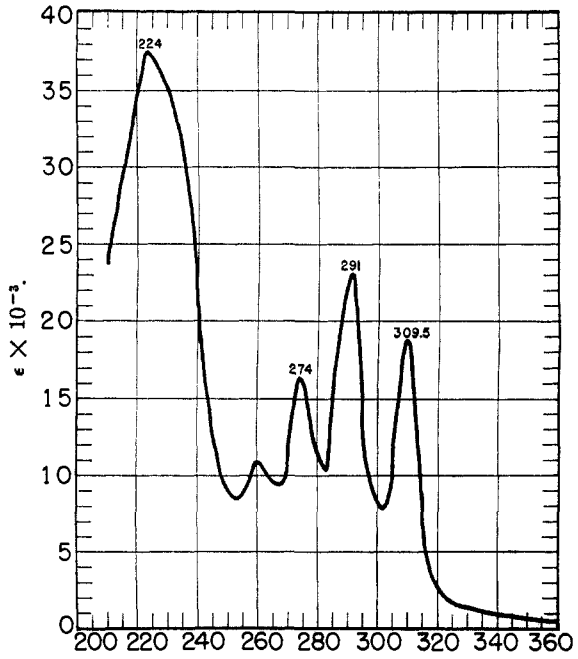

Fig. 1.—Ultraviolet absorption spectrum of the suberamic acid ene-diyne, 0.0005% in 95% ethanol. The readings were made on a Beckmann DU spectrophotometer.

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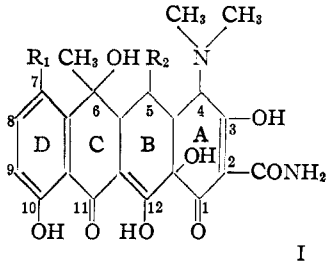
TERRAMYCIN. XI. TETRACYCLINE

Sir:

In a previous communication,[1] it has been indicated that the structure I, designated tetracycline, is common to the broad spectrum antibiotics, Terramycin (II) and Aureomycin (III).[2] At this time, we wish to report the preparation and antibiotic activity of tetracycline, I (4-dimethylamino - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,-12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo-2-naphthacenecarboxamide).[2a]



I, R_1 = H, R_2 = H
II, R_1 = *H*, R_2 = OH
III, R_1 = Cl, R_2 = H

I

Treatment of a dioxane–methanol solution of chlorotetracycline with hydrogen in the presence of palladized carbon resulted in the ready hydrogenolysis of the aromatic halogen atom to give the hydrochloride of tetracycline. The latter was converted to the crystalline base, I; m.p. 170–175° dec., $[\alpha]^{25}D$ − 239° (*c* 1% in methanol), pK_a 8.3, 10.2 (50% aqueous dimethylformamide). *Anal.* Calcd. for $C_{22}H_{24}N_2O_8$: C, 59.45; H, 5.44; N, 6.31; mol. wt., 444. Found: C, 59.35; H, 5.41; N, 6.15; equiv. wt. (titration), 227.

On treatment with methanolic hydrogen chloride, tetracycline was readily converted to the previously reported deschloroanhydroaureomycin.[3]

The ultraviolet absorption spectrum of I exhibits maxima at 268 mμ, log ε 4.27, and 363 mμ, log ε 4.14, in 0.01 *M* methanolic hydrogen chloride, and at 246 mμ, log ε 4.24, and 372 mμ, log ε 4.20, in 0.01 *M* methanolic sodium hydroxide.

The ultraviolet spectra of tetracycline in acidic and basic solution are nearly identical with the corresponding spectra[4] for oxytetracycline and provide further confirmation of the structure assigned to tetracycline. The spectra of tetracycline and chlorotetracycline[5] in acid solution are very similar. A slight hypsochromic shift of the long wave length tetracycline peak is attributed to the removal of the aromatic chlorine.[6] In contrast to chlorotetracycline,[7] tetracycline is quite stable in alkaline solution, and its spectrum in this medium is very similar to that of oxytetracycline. This observation demonstrates the profound influence of the aromatic chlorine on the stability of the C ring in chlorotetracycline[8] and is in agreement with

(1) C. R. Stephens, L. H. Conover, F. A. Hochstein, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **74**, 4976 (1952).

(2) Terramycin is the registered trade name of Chas. Pfizer and Company for the antibiotic whose generic name is oxytetracycline. Aureomycin is the registered trade name of Lederle Laboratories for the antibiotic whose generic name is chlorotetracycline.

(2a) This name and numbering system follows suggestions kindly made by the Editors of *Chemical Abstracts*.

(3) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).

(4) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, *ibid.*, **73**, 4211 (1951).

(5) R. Broschard, A. Dornbush, S. Gordon, B. Hutchings, A. Kohler, G. Krupka, S. Kushner, D. Lefemine and C. Pidacks, *Science*, **109**, 199 (1949).

(6) The ultraviolet spectra of oxytetracycline and chlorotetracycline are discussed in reference 1.

(7) *Cf.* M. S. Bryer, E. B. Schoenbach, C. A. Chandler, E. A. Bliss and P. H. Long, *J. Am. Med. Assoc.*, **138**, 117 (1948).

(8) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).