the mixture was shaken thoroughly and left at room temperature for 2 hr with occasional shaking. During this time the reaction mixture became progressively more turbid with a brown precipitate. The reaction mixture was added to 6.5 ml of cold 5 *N* hydrochloric acid, 6.5 ml of cold, distilled water, and 25 ml of diethyl ether. After shaking, the ether layer was removed and the lower layer was extracted several times with small volumes of ether. The combined extracts were washed to neutrality, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give 22 mg of a white-yellow solid.

The crude product was dissolved in chloroform and applied to an 8-g column of silicic acid. Elution with 100 ml of chloroform removed 2 mg of a mixture of unidentified products. The desired product (12 mg) was recovered by elution with 150 ml of 1% methanol in chloroform, and unreacted starting material (19 mg) was eluted with 5% methanol in chloroform. Lyophilization of an acetic acid solution of the product yielded a white powder, mp 104–105°. On thin layer chromatography in chloroform–methanol (95:5) the product gave a single spot with R_f 2.5 relative to that of N-acetyldihydrosphingosine.

Anal. Calcd for $C_{20}H_{39}NO_3$: C, 70.33; H, 11.51; N, 4.10. Found: C, 70.09; H, 10.99; N, 5.75[20] (Kjeldahl: N, 4.00).

3-Oxo-1-hydroxy-2-acetamido-4-octadecene. N-Acetylsphingosine (65.5 mg, 192 μmoles) was oxidized by chromic anhydride in benzene–pyridine as described above. Silicic acid column chromatography of the crude product (37.6 mg) gave 16.3 mg of the 3-oxo compound. The product was crystallized from pentane, mp 69–70°, $[\alpha]^{25}D$ +11.8° (*c* 11.3, ethanol), λ_{max}^{EtOH} 230 mμ (ε 14,300).

Anal. Calcd for $C_{20}H_{37}NO_3$: C, 70.75; H, 10.98; N, 4.13. Found: C, 70.47; H, 10.66; N, 4.39.

Reduction of N-Acetyl-3-oxo Bases with Sodium Borohydride. About 3 mg of sample was dissolved in 1 ml of methanol and the solution was cooled to 10–12°. One drop of 1 *N* sodium hydroxide and 0.2 ml of a solution of 20 mg of sodium borohydride in 10 ml of methanol were added. The solution was left at 10–12° for 15 min. Another 0.2 ml of sodium borohydride solution was added and the mixture was kept at 10–12° for an additional 15 min. Cold, saturated sodium chloride (1 ml) was added and the product was extracted into diethyl ether. The emulsion which resulted was broken by the addition of 1 ml of water. The aqueous layer was extracted twice more with ether, and the combined total extracts were washed with water and evaporated to dryness. Vacuum drying over calcium chloride and lyophilization from acetic acid solution gave about 3 mg of a white-yellow solid. The products were examined by thin layer chromatography, and infrared and ultraviolet spectra were recorded.

Samples of about 1 mg were reduced with excess sodium borodeuteride in methanol. After standing overnight at room temperature, excess water was added and the mixture was extracted twice with equal volumes of diethyl ether. The combined ether layers were washed once with water, dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The crude product was prepared for gas chromatography and mass spectrometry by addition of 0.1 ml of a mixture consisting of pyridine, hexamethyldisilazane, and trimethylchlorosilane in the proportions 10:2:1.[8]

Acknowledgments. This investigation was supported by Public Health Service Research Grant AM-04307 from the National Institute of Arthritis and Metabolic Diseases. The experimental studies herein reported constituted a portion of the Ph.D. Thesis of Robert C. Gaver, University of Pittsburgh, 1964. We are indebted to Mr. John Naworal and Mrs. Thelma Hamilton for assistance in recording mass spectra and in preparation of the mass spectral tables.

Communications to the Editor

5-Hydroxy-7-chlortetracycline

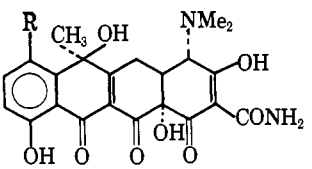
Sir:

The outstanding antibacterial properties of the tetracycline antibiotics, coupled with their remarkable clinical effectiveness, have provided the stimulus for an intensive effort to find new members of the class with superior properties. The chemical mating of oxytetracycline (IIa) and chlortetracycline (IIb) has been recognized as a desirable, but elusive, goal. We wish to report the preparation of the desired 5-hydroxy-7-chlortetracycline (IIc) and present some of its chemical and biological properties.

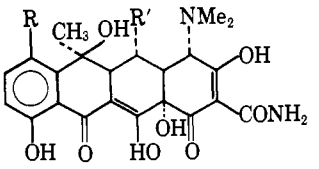
Recently the terminal stages of the biosynthesis of oxytetracycline (IIa) and tetracycline (IId) have been reported and these antibiotics were shown to arise through a common intermediate (Ia).[1] By logical extension 5a,11a-dehydrochlortetracycline (Ib) would be the biological parent of 5-hydroxy-7-chlortetracycline (IIc). Despite severe difficulties occasioned by the unusual lability of 5-hydroxy-7-chlortetracycline in neutral or alkaline solutions, we have succeeded in obtaining the antibiotic as its crystalline hydrochloride after incubation of 5a,11a-dehydrochlortetracycline hydrochloride (Ib) with a washed cell preparation of *Streptomyces rimosus*, strain BE514 (ATCC 13,224).[2]

(1) P. A. Miller, J. H. Hash, M. Lincks, and N. Bohonos, *Biochem. Biophys. Res. Commun.*, **18**, 325 (1965).

The culture was grown under conditions generally suitable for other *S. aureofaciens* and *S. rimosus* strains.



Ia, R = H
b, R = Cl



IIa, R = H; R′ = OH
b, R = Cl; R′ = H
c, R = Cl; R′ = OH
d, R = R′ = H

The cells were removed by centrifugation, washed with dilute buffer, and resuspended in one-fourth of the original volume, using pH 6.6 buffer; 500 μg/ml of substrate (Ib) was added, and, after about 6 hr, a transformation yield of approximately 200 μg/ml was obtained. The product was isolated by a process consisting of acidification, filtration, butanol extraction of the filtrate, pH gradient partition chromatography on cellulose powder at low temperatures, countercurrent distribution, and crystallization from acid–water. By working quickly and avoiding pH's above 3 and temperatures above 25° as far as possible, satisfactory isolation yields of crystalline antibiotic were obtained.

(2) The same transformation (Ib to IIc) has been observed independently by Dr. J. R. D. McCormick of these laboratories working with whole cell systems.

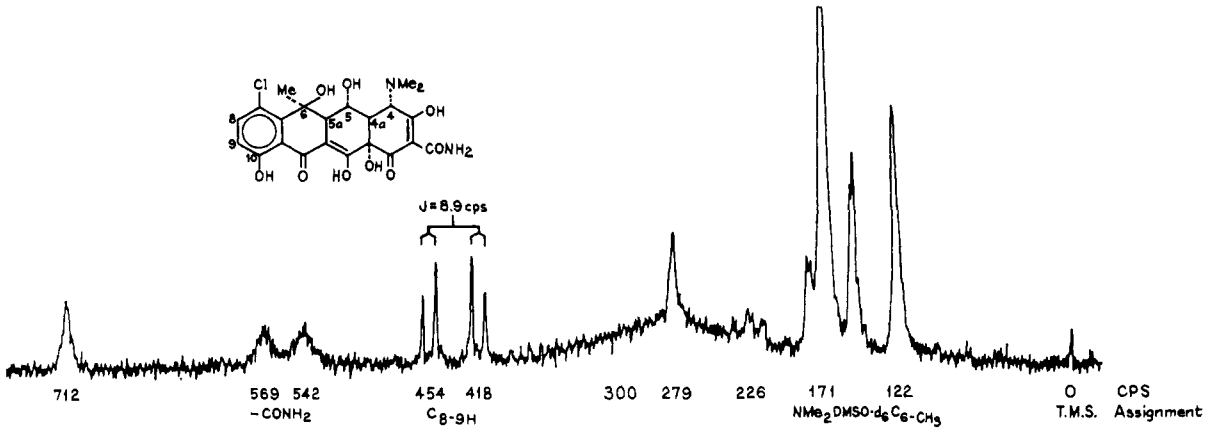


Figure 1. Nmr spectrum (60 Mc) of 5-hydroxy-7-chlortetracycline hydrochloride in dimethyl sulfoxide-d_6.

5-Hydroxy-7-chlortetracycline hydrochloride (IIc) crystallizes in small yellowish needles, $[\alpha]^{25}D$ −237° (*c* 0.880, 0.001 *N* MeOH·HCl). *Anal.* Found: C, 49.68; H, 5.12; N, 4.97; Cl, 13.05. The ultraviolet spectrum showed $\lambda_{max}^{0.01N HCl-MeOH}$ 372, 266, and 229 mμ (ε 13,800 21,120, and 20,800, respectively); and the nmr spectrum in deuterated dimethyl sulfoxide (Figure 1) is fully consistent with formulation IIc, being identical with that of oxytetracycline below τ 5 and chlortetracycline above τ 5. Hydrogenolysis using 10% palladized charcoal in 50% aqueous dimethylformamide (containing a small amount of triethylamine) yielded 70% of oxytetracycline hydrochloride, which was identified by comparison (spectra and papergram mobility in three systems) with an authentic sample. Transformation of the antibiotic to oxytetracycline leaves only the position of the chlorine atom open to question. The aromatic region of the nmr spectrum (AB pattern at τ 2.43 and 3.04) clearly shows that, as expected, the chlorine atom remained at C_7 during the biological treatment. These findings are compelling evidence that the desired microbiological reactions have occurred in the anticipated fashion.

The antibiotic is less stable than oxytetracycline, chlortetracycline, and tetracycline in neutral solutions (Table I). This factor was a serious hindrance during the isolation studies. The addition of calcium ion satisfactorily overcomes this deficiency, and, when the antibiotic is dissolved in 80% propylene glycol solution at pH 7.5–7.6 and at least 2 molar equiv of calcium chloride is added,[3] the solution retains considerable bioactivity when stored at room temperature for weeks The antibiotic possesses one-half the bioactivity of chlortetracycline when measured against *Staphylococcus aureus* C209P by a turbidimetric procedure, and it is highly active orally and subcutaneously against experimental staphylococcal infections in mice.

Table I. Stability of Various Tetracyclines

	Bioactivity remaining after 2.5 hr at 37°, %			
pH	Tetracycline	Oxytetracycline	Chlortetracycline	5-Hydroxy-7-chlortetracycline
2.0	95	96	100	96
3.0	95	97	100	81
4.0	96	93	99	84
5.0	91	90	99	76
6.0	92	94	100	57
7.0	92	96	78	25

(3) We thank Dr. L. Leeson and co-workers of the Pharmaceutical Product Development Dept. of these laboratories for preparing these solutions.

The preparation of 5-hydroxy-7-chlortetracycline provides a striking confirmation and extension of the tetracycline biosynthetic pathway presented in an earlier paper in this series.[1] Details of some of the biological studies briefly reported here will be given in a communication submitted elsewhere.

Acknowledgment. We wish to thank our colleagues in the Biochemical Research Section for performing numerous fermentations, bioassays, and large-scale isolations; the Organic Chemical Research Section for microanalyses; and J. T. Lancaster of our Central Research Division, Stamford, Conn., for nmr spectra.

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Spectroscopic Evidence for Ionic Ground States in Molecular Donor–Acceptor Complexes[1]

Sir:

It has been suggested in both experimental and theoretical contexts that charge-transfer complexes between initially neutral organic molecules that are strong donors and strong acceptors may have essentially ionic, salt-like, ground states.[2] Polarized single-crystal spectro-

(1) This work has been supported by grants from the National Science Foundation and the National Institutes of Health.

(2) (a) J. J. Weiss, *J. Chem. Soc.*, 245 (1942); *Phil. Mag.*, **8**, 1169 (1963); (b) H. Kainer, D. Bijl, and A. C. Rose-Innes, *Naturwissenschaften*, **41**, 303 (1954); D. Bijl, H. Kainer, and A. C. Rose-Innes, *J. Chem. Phys.*, **30**, 765 (1959); (c) H. Kainer and A. Uberle, *Chem. Ber.*, **88**, 1147 (1955); H. Kainer and W. Otting, *ibid.*, **88**, 1921 (1955); (d) R. Foster and T. J. Thomson, *Trans. Faraday Soc.*, **59**, 296 (1963); (e) R. Foster, *Photoelec. Spectrometry Group Bull.*, **No. 15**, 413 (1963); (f) Y. Matsunaga, *J. Chem. Phys.*, **41**, 1609 (1964); (g) P. L. Kronick, H. Scott, and M. M. Labes, *ibid.*, 40, 890 (1964) (further references are cited by these authors); (h) R. S. Mulliken, *J. Chim. Phys.*, **61**, 20 (1964); (i) H. M. McConnell, B. M. Hoffman, and R. M. Metzger, *Proc. Natl. Acad. Sci. U. S.*, **53**, 46 (1965); J. I. Krugler, C. G. Montgomery, and H. M. McConnell, *J. Chem. Phys.*, **41**, 2421 (1964).