

metabolite, 4-aminodimethylaminoanhydrodemethylchlorotetracycline⁷ (**2**). This mutant of a 6-demethylchlorotetracycline (DMCT) producing parental strain was observed to accumulate very little antibiotic (less than 2 $\mu\text{g}/\text{ml}$ as DMCT). In mixed fermentations of 1E1407 with several other point-blocked mutants of *S. aureofaciens*, significant quantities of tetracycline antibiotics were produced by cosynthesis,⁸ suggesting that 1E1407 was also point blocked in the biosynthetic pathway to the tetracyclines. The nature of the antibiotic accumulated in each instance was indicative of the relative locations of the blocks in 1E1407 and its cosynthesizing partner, as we have observed that a cosynthetic response is usually due to transfer of a partially finished tetracycline molecule from a donor to an acceptor cell. Thus a positive result usually has been observed only in transfer of an intermediate from the mutant having the later block to the one having the earlier block.⁹ The positive results in these experiments led to testing a killed preparation of 1E1407 mash by addition to living cultures of other mutants, and now evidence of accumulation of a stable precursor (or precursors) by 1E1407 was found. In this situation, antibiotic is produced only when the precursor is a stable substance and occupies a place in the biosynthetic chain which is later than the point at which the test culture is blocked.

Absorption spectra of an acidic aqueous extract of 1E1407 fermented mash suggested the presence of an anhydrotetracycline-like substance, and this, together with the biological conversion data mentioned above, strongly suggested that the active precursor might be **2**.

Isolation of the precursor was accomplished by an adaptation of the method of Miller, *et al.*,⁵ in which ethyl acetate extraction of the perchloric acid acidified

(7) This compound has been previously described in terms of its chromatographic behavior and some chemical and biochemical properties.⁵

(8) J. R. D. McCormick, U. Hirsch, N. O. Sjolander, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 5006 (1960).

(9) CF-I, a transferable hydrogenation cofactor, is the one exception to this. See P. A. Miller, N. O. Sjolander, S. Nalesnyk, N. Arnold, S. Johnson, A. P. Doerschuk, and J. R. D. McCormick, *ibid.*, **82**, 5002 (1960).

whole mash was followed by simple partition of the crude material between chloroform and 0.1 *N* hydrochloric acid. The aqueous phase from the partition was evaporated to dryness to yield a partly crystalline crude product which was about 60% pure. Recrystallization was accomplished by dissolving the crude product in ten parts of 2 *N* hydrochloric acid in methoxyethanol and precipitating with toluene to give the pure product in good yield; absorption spectrum, λ_{max} $m\mu$ (ϵ): 424 (8600), 329 (3520), 314 sh (3720), 302 sh (5470), 269 (53,400), 223 (35,000); R_f 0.39 in butanol-0.1 *M* EDTA, pH 4.9, and 0.13 in butanol-0.1 *M* EDTA, pH 6.0. *Anal.* Found for $\text{C}_{19}\text{H}_{15}\text{N}_2\text{O}_7\text{Cl}_2 \cdot \text{H}_2\text{O}$: C, 48.45; H, 3.50; N, 5.85; H_2O , 4.06.

Biological conversion of **2** to DMCT was demonstrated in the usual way² utilizing *S. aureofaciens* mutant V828. A 34% conversion was found based on microbiological assay; the product, DMCT, was identified by paper chromatographic comparison with authentic material in two chromatographic systems. The pure substance, **2**, was shown by direct spectrophotometric and paper chromatographic comparison to be identical with the principal component in the partially purified material¹⁰ reported by Miller, *et al.*⁵

This isolation of an anhydrotetracycline derivative from a mutant of *S. aureofaciens* affirms the earlier presumed role of the anhydrotetracyclines as intermediates in the biosynthetic pathway to the tetracyclines and reinforces the conclusions of Miller, *et al.*,⁵ that the anhydrotetracyclines themselves arise by way of N-methylation of their amino analogs.¹¹

(10) A comparison sample of this material was kindly supplied by Dr. L. A. Mitscher of these laboratories.

(11) The earlier conclusion of one of us (J. R. D. M.) that N-methylation preceded reduction at C-4 has since been found to have been based on an isolation artifact at a key point. (See J. R. D. McCormick in "Antibiotics, Vol. 2, Biogenesis," D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin-Heidelberg, 1967.)

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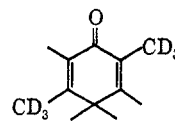
Additions and Corrections

Divinyl Sulfide: Copolymerization and Spectra [*J. Am. Chem. Soc.*, **81**, 2672 (1959)]. By CHARLES E. SCOTT and CHARLES C. PRICE, Chemistry Departments, University of Notre Dame, Notre Dame, Indiana, and University of Pennsylvania, Philadelphia, Pennsylvania.

In Table IV, change the values for ϵ_{max} for divinyl sulfide from 41,800 and 38,000 to 8350 and 7600.

Intermediates in the Photochemical Rearrangements of Bicyclo[3.1.0]hexenones [*J. Am. Chem. Soc.*, **89**, 1874 (1967)]. By HAROLD HART and DAVID W. SWATTON, Department of Chemistry, Michigan State University, East Lansing, Michigan 48823.

On page 1876, formula **11** should be



Acylation of Cyclooctatetraene Dianion and the Chemistry of Its Products [*J. Am. Chem. Soc.*, **89**, 5868 (1967)]. By THOMAS S. CANTRELL and HAROLD SHECHTER, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210.