retention of ^{14}C but complete loss of tritium, thereby establishing the precursor relationship of loganic acid (**1**) to gentiopicroside (**5**).[16]

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Biosynthesis of the Tetracyclines. XII.[1] Anhydrodemethylchlortetracycline from a Mutant of *Streptomyces aureofaciens*

Sir:

We recently reported the isolation of 4-aminodedimethylaminoanhydrodemethylchlortetracycline.[2] We commented at that time that no anhydrotetracycline derivative having the intact dimethylamino group had yet been observed as a product of a blocked mutant of *Streptomyces aureofaciens*. We have continued to search, however, for such a mutant, and now report a successful achievement of this goal. Anhydrodemethylchlortetracycline was successfully isolated from mutant 1E6113, a derivative of a demethylchlortetracycline-producing parental strain.[3] Mutant 1E6113 normally produces a small quantity of demethylchlortetracycline (DMCT) (about 10–25 μg/ml), but in the absorption spectrum of an acidic aqueous extract there was seen a significant amount of absorption in the 420–440 mμ region where the anhydrotetracyclines show a moderately strong maximum. Using the absorbancy in this region as a guide, we were able to isolate the substance responsible and to show that it was identical with an authentic specimen of anhydrodemethylchlortetracycline.[4] The isolation proceeded as follows: 1 l. of 1E6113 fermented mash (microbiological assay 25 μg/ml as DMCT) was acidified to pH 1.5 with perchloric acid, washed with hexane (centrifugation), and extracted with ethyl acetate (centrifugation). The extract, which by paper chromatographic examination contained essentially no demethylchlortetracycline, was concentrated to dryness and the residue, 840 mg, taken up in tetrahydrofuran (THF). The solution was dried onto 30 g of diatomaceous earth and packed onto the top of a 3 in. $\times$ 12 in. partition chromatographic column consisting of 300 g of diatomaceous earth filter aid which had been thoroughly mixed with 150 ml of 0.1 *M* ethylenediaminetetraacetic acid (EDTA) buffer at pH 8.3. The column was developed with chloroform and the eluate collected in 100-ml fractions. Spectrophotometric examination of the fractions showed anhydrotetracycline-like absorption in cuts 8 through 20. These were combined and evaporated to dryness to yield 80 mg of a partly crystalline product, identified as predominantly amphoteric anhydrodemethylchlortetracycline by paper chromatography (orange fluorescing spot at R_f 0.48 in pH 8.3 EDTA–butanol system). The compound, 80 mg, was converted to the hydrochloride by dissolving in 0.5 ml of 1-butanol plus 0.3 ml of 8 *N* hydrochloric acid. The crystalline hydrochloride, 53 mg, was shown to be pure anhydrodemethylchlortetracycline by exact correspondence of the ultraviolet and infrared spectra with those of an authentic specimen.[4] Paper chromatographic behavior, in two systems, of the isolated compound likewise was identical with that of the authentic specimen.

Although the original 1E6113 mash contained a small amount of demethylchlortetracycline, we took pains in the isolation to avoid conditions which might partly or completely dehydrate this compound to the anhydro derivative. In addition, the isolation method used was one which separated the already present anhydro derivative from the parent compound at an early point. Finally, the quantity of anhydrodemethylchlortetracycline isolated in pure form was greater than the total DMCT content of the starting mash. For these reasons we feel that there is no possibility that the anhydrodemethylchlortetracycline is an isolation artifact.

In that part of the biosynthetic pathway to the tetracyclines[5] which involves tetracyclic intermediates, blocked mutants have now been reported for each step, excepting only two. These two steps are: 4-hydroxylation of the pretetramids (a product of this hydroxylation, 4-hydroxy-6-methylpretetramid, is known[6]), and the 12a-hydroxylation of the 4-hydroxypretetramids to yield the 4-ketodedimethylaminoanhydrotetracyclines. Some experimental observations on the latter biosynthetic step will be presented in a forthcoming comunication.

(1) Previous paper in this series: J. R. D. McCormick, E. R. Jensen, N. H. Arnold, H. S. Corey, U. H. Joachim, S. Johnson, P. A. Miller, and N. O. Sjolander, *J. Am. Chem. Soc.*, **90**, 7127 (1968).
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The Reaction of Complexes of Rhodium(I) Chloride with Norbornadiene

Sir:

Norbornadiene is oligomerized by several metal catalysts[1] to dimers and trimers that are related formally to the molecules of the starting hydrocarbon by a simple cycloaddition process.[1h,2,3] The contrasting hypotheses that the new carbon–carbon bonds form simultaneously[1i,4] or sequentially[2] have both been advanced. This communication reports the reaction of

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