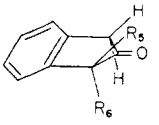
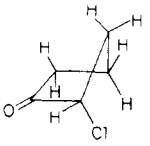
Our intermediate values check closely with some 1-halo-2-indanones[8] which should have the same halo-carbonyl geometry (III) since the five membered ring has four carbon atoms which must be in one plane and a fifth, the carbonyl group, which can maintain favorable bisections with the α-substituents by remaining in the plane.



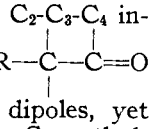
III



IV

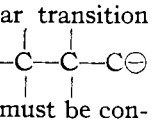
It follows that if the α-halocyclopentanones were planar, the shift for α-bromine should be about 14 cm.$^{-1}$ and α-chlorine 18–19 cm.$^{-1}$. In actual fact the shift for α-bromocyclopentanone, as measured by Corey[5c] and confirmed by us is only 8 cm.$^{-1}$ and for α-chlorocyclopentanone[5c] the shift is only 13 cm.$^{-1}$, within the axial range.

The species most likely predominant in solution (IV) involves puckering of the C_3 atom about 0.7 Å. which develops a favorable staggered C_2-C_3-C_4 interaction, reduces the repulsion of



by increasing the distance between dipoles, yet maintains the favorable bisection of the C_5 methylene group by the carbonyl substituent.

As a consequence it can be inferred that the anion of *trans*-2-chlorocyclopentol[2] must be quite puckered in the transition state for epoxide formation, unexpectedly favoring a coplanar transition state, since the repulsive forces in



with a full negative charge on oxygen must be considerable even though the geometry is not quite the same as in α-chlorocyclopentanone.

The framework outlined previously[2] and herein will accommodate recent comments on the puckered cyclopentane ring,[9,10] while LeFevre's recent polarizability data[11] are also in general argeement.

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(9) H. Kwart and W. G. Vosburgh, THIS JOURNAL, **76**, 5400 (1954).
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THE HALIDE METABOLISM OF *STREPTOMYCES AUREOFACIENS* MUTANTS. THE BIOSYNTHESIS OF 7-CHLORO-, 7-CHLORO^{36}- AND 7-BROMOTETRACYCLINE AND TETRACYCLINE

Sir:

We wish to report recent studies of the halide metabolism of two classes of experimental mutants of *Streptomyces aureofaciens* Duggar. These studies are concerned with the utilization of inorganic chloride and bromide by the mutants of the two classes to form 7-chlorotetracycline[1] and 7-bromotetracyline[2] and with the behavior of the mutants in the absence of halide.

Class I, represented by Mutant BC-41, contains those mutants whose rate of utilization of chloride for 7-chlorotetracycline (CTC) is independent of chloride concentration over the wide range of chloride concentrations from 0.02 to 10 stoichiometric equivalents of the total tetracyclines potential of the system. The total tetracyclines potential is the sum of the millimoles per liter of CTC, 7-bromotetracycline (BTC), and tetracycline (TC)[3] present at the end of the fermentation cycle. Class II, represented by Mutants S-580 and S-1055, contains those mutants whose rate of utilization of chloride for CTC depends on chloride concentration over the above range.

Chlorination by BC-41 to yield CTC is slightly inhibited by bromide; chlorination by S-580 and by S-1055 is substantially inhibited by bromide. Within limits, these inhibitions are reversible by excess chloride and competitive in nature. Halogenation in all three mutants is substantially inhibited by thiocyanate. The thiocyanate inhibition of BC-41 is not reversible by excess chloride; within limits, the thiocyanate inhibitions of S-580 and S-1055 are reversible by excess chloride. The response of all three mutants to the repression of halogenation, either through inhibition by bromide or by thiocyanate or through halide denial, is the biosynthesis of the unsubstituted product, TC.

In the absence of chloride, BC-41 utilizes bromide for BTC at a rate independent of bromide concentration over the above range; the over-all rate of bromide utilization for BTC is 0.3 times the rate of chloride utilization. Utilization by S-580 and by S-1055 of bromide for BTC could not be detected.

The total tetracyclines potential of each of the three mutants is independent of halide concentration over the 0.02 to 10 equivalents range; that is, the effect of halide variation is solely a change in the identity of product.

In the case of BC-41, the magnitude of the chlorination rate and its independence of chloride concentration allow complete utilization of chloride for CTC for chloride concentrations up to 93% that equivalent to the total tetracyclines potential of the system. Lesser initial chloride concentrations result in chloride exhaustion. Similarly, BC-41 completely utilizes bromide for BTC when bromide concentrations do not exceed 25% of that equivalent to the total tetracyclines potential of the system. S-1055 approaches complete utilization of chloride for CTC when chloride concentrations do not exceed 25% of the equivalent concentration. In the case of S-580, chloride is never completely utilized for CTC, and chloride exhaustion is not observed.

At high excess chloride concentrations, under the particular uniform fermentation conditions of these

(1) The trademark of the American Cyanamid Company for 7-chlorotetracycline is Aureomycin.
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(3) The trademarks of the American Cyanamid Company and of Charles Pfizer and Co. for tetracycline are Achromycin and Tetracyn, respectively.

experiments, CTC represents a maximum of 93%, 70% and 80% of the total terminal tetracyclines in the cases of BC-41, S-580, and S-1055, respectively. No appreciable quantities of chlorinated organic materials other than CTC were detected in fermented mashes to which excess chloride[36] originally had been added.

Mutants S-580 and S-1055 were isolated from cultures of BC-41 and possess, respectively, 65–70% and 80–85% of the total tetracyclines potential of BC-41 under the uniform fermentation conditions used for this study. All are descendents through a series of mutation treatments of the original *S. aureofaciens* A-377 isolate of Duggar. *S. aureofaciens* A-377 and most of its progeny studied are members of Class I. Both Class I and Class II contain mutants differing substantially in their morphology and physiology.

Biosynthetic TC has been isolated and found identical in its properties with TC produced by the catalytic hydrogenation of CTC.[4] BTC produced by a BC-41 fermentation has been separated by partition chromatography (butanol-chloroform moving over stationary Celite-aqueous HCl) from the TC concurrently produced and has been isolated as the crystalline hydrochloride. Small, variable quantities of CTC were present in the BTC preparations, arising from the efficient utilization by BC-41 of traces of chloride for CTC even in the presence of large excesses of bromide. A variety of counter-current extraction, partition chromatographic, and paper strip chromatographic systems useful in separating CTC, TC, oxytetracycline[5] and their quatrimycin isomers[2] failed to separate mixtures of varying ratios of CTC and BTC. The properties of BTC·HCl, corrected for

TABLE I

ULTRAVIOLET SPECTRA IN 0.1 *N* H_2SO_4

BTC·HCl		CTC·HCl	
λ max.	ε max. × 10^{-3}	λ max.	ε max. × 10^{-3}
368 mμ	9.15	368 mμ	10.7
260	18.9	265	18.3
228	17.2	228	17.6

TABLE II

HALF LIVES IN ACID AND ALKALI

Conditions	BTC	CTC	TC
*p*H 10.0 carbonate buffer, 22°	10.9 min.	18.6	>600
0.2 *N* H_2SO_4 (aq.), 100°	18.8	8.2	<2

the CTC·HCl present, are: $[\alpha]D$ (0.5% in 0.03 *N* aq. HCl): BTC·HCl, −205°; CTC·HCl, −235°. Solubility in water (25°): BTC·HCl, 1.36%; CTC·HCl, 1.40%. Solubility in dry *n*-butanol (25°): CTC·HCl, 0.013%; BTC·HCl, 0.038%. M.p.: BTC·HCl, browns at 218°, dec. at 235°; CTC·HCl, dec. at 210°: Response on a weight basis of BTC·HCl in terms of CTC·HCl to analytical procedures: turbidimetric (*S. aureus*), 95%; turbidimetric (*E. coli*), 90%; fluorometric,[6] 26.0%; Hiscox,[7] 45.0%; spectrophotometric (368 mμ, 0.1 *N* H_2SO_4), 77.0%. Catalytic hydrogenation (palladium on carbon, 1.1 atmosphere of hydrogen) of BTC has yielded TC.

A BC-41 fermentation containing no chloride except chloride[36] in concentration less than 93% that stoichiometrically equivalent to the total tetracyclines potential of the system has given an essentially quantitative conversion of chloride[36] to 7-Cl^{36}-TC; partition chromatographic separation of this material from the TC also produced and crystallization has given an over-all yield of 53% from chloride[36] to pure, crystalline 7-Cl^{36}-TC·HCl possessing essentially the same molar radioactivity as the HCl^{36} available from the Atomic Energy Commission.

(4) J. H. Booth, *et al.*, THIS JOURNAL, **75**, 4621 (1953); L. H. Conover, *et al.*, *ibid.*, **75**, 4622 (1953).

(5) The trademark of Charles Pfizer and Company for oxytetracycline is Terramycin.

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RECEIVED DECEMBER 28, 1955

PREPARATION OF BORON MONOXIDE AT HIGH TEMPERATURES

Sir:

In a series of attempts to produce elemental boron of very high purity for use in a study of certain borides,[1] considerable difficulty was experienced in removing the last traces of impurity. This was found to be true particularly in the case of boron produced by the direct reduction of boron trioxide. It has been suggested that this residual impurity may be oxygen combined with the boron as a suboxide.[2,3,4] Since the literature concerning the nature of the suboxide is limited, a study was made of its preparation and properties.

Boron monoxide has been prepared from $H_4B_2O_4$ by dehydration[5,6,7] and at high temperatures by Zintl[8] who prepared it by heating a mixture of boron and ZrO_2 at 1800° in vacuum. But he and others[9] have stated that it cannot be produced by heating a mixture of boron and B_2O_3 due to the higher vapor pressure of B_2O_3 compared to that of the monoxide. We have found that boron monoxide of high purity can be obtained by this method at temperatures as low as 1050°.

Our procedure was to heat the B–B_2O_3 mixtures in a tantalum crucible, covered with a perforated tantalum lid, and suspended in an all-glass vacuum system. The heating chamber was an air cooled Pyrex cylinder provided with a reëntrant water cooled cold finger which served as a condenser. Heat was generated in the tantalum crucible by means of a high frequency induction unit.

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