[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND BIOCHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Chemistry of Antimycin A. IV. Studies on the Structure of Antimycin Lactone[1]

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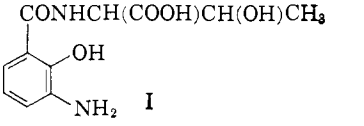
Through study of appropriate model compounds, the general structural character of the neutral product obtained by alkaline degradation of the antibiotic antimycin has been clarified.

Under mildly alkaline conditions[2] the *Streptomyces* antibiotic antimycin A (tentative molecular formula $C_{28}H_{40}N_2O_9$) is degraded to formic acid,[3] antimycic acid (I)[4] and a neutral product (II). Elucidation of the general structural nature of



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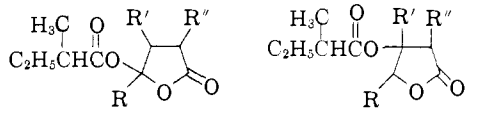
this last-named substance was the purpose of the investigations described in this contribution.

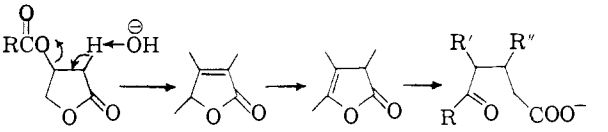
As reported previously,[2] the neutral compound is a colorless, water-insoluble, optically active liquid, which, despite many attempts, could not be crystallized. Because of the lack of a suitable criterion of purity, the ultimate analytical values were not unequivocal; however, the formula $C_{16}H_{28}O_4$ fitted the data well, and, moreover, satisfied the demands of the products obtained by further degradation as well as the proposed molecular formula for antimycin-A. The neutral product exhibited no active hydrogen, could not be reduced catalytically, and gave negative tests for phenol, alcohol, aldehyde or ketone groups. Although the substance possessed no peak in the ultraviolet region of the spectrum, it did exhibit, among others, infrared absorption bands at 5.64 μ, indicative of a γ-lactone system, and at 5.75–5.76 μ, consistent with the presence of an aliphatic ester unit. No hydroxyl absorption could be detected. Saponification of the neutral oil,[2] carried out by refluxing for two hours with 10% aqueous alkali, led *exclusively* to acidic products, a mixture which consisted principally of L(+)-2-methylbutyric acid and a keto acid, $C_{11}H_{22}O_3$.

Only two structural systems, III and IV, seemed capable of accommodating all of the evidence

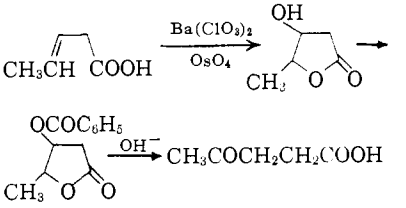


III R, R′, R″=H or alkyl IV

available at this stage. In the first case, partial hydrolysis to a γ-lactol or a hemiacetal ester would precede formation of the two main degradation acids; alternately, lactone ring-opening of IV, and β-elimination of 2-methylbutyric acid, followed by a β, γ-shift of the double bond generated, would account for the observed products. It seemed that substantiation and, possibly, differentiation of these proposals might evolve from a comparison of the properties of the antimycin lactone with those of suitable model substances.



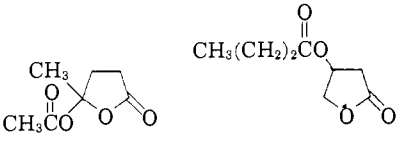
In order to confirm the hydrolysis reaction scheme postulated for the β-acyloxylactone structure, saponification of the benzoate ester of β-hydroxy-γ-valerolactone (V) was carried out.[5] The required lactone derivative was prepared by the osmium-catalyzed barium chlorate oxidation[6]



V

of Δ^3-pentenoic acid, followed by treatment of the resulting hydroxylactone with benzoyl chloride–pyridine. As anticipated, treatment of V with aqueous base under the conditions used in the antimycin lactone cleavage gave rise to benzoic acid and levulinic acid, which was characterized as the semicarbazone and as the dinitrophenylhydrazone. Similar treatment of the parent β-hydroxy-γ-lactone did not afford any detectable amount of levulinic acid, thereby indicating (*cf.* reference in footnote 5, p. 1213) that conversion to the keto acid does not proceed largely by way of this substance.

The thermal behavior of γ-acetoxy-γ-valerolactone (VI) was found to differ decidedly from that of β-butyroxy-γ-butyrolactone (VII).



VI VII

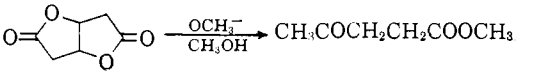
(1) From the M.S. thesis of U. C. Quarck, Department of Chemistry, University of Wisconsin, January, 1954.

(2) G. M. Tener, F. M. Bumpus, B. R. Dunshee and F. M. Strong, THIS JOURNAL, **75**, 1100 (1953).

(3) Unpublished results obtained in the Department of Biochemistry, University of Wisconsin.

(4) G. M. Tener, E. E. van Tamelen and F. M. Strong, THIS JOURNAL, **75**, 3623 (1953).

(5) After the work reported herein had been completed, R. P. Linstead, L. N. Owen and R. F. Webb published their investigations on β-eliminations of β-acyloxy esters, one example of which supports particularly well the rationalization of the antimycin lactone degaradation by base (*J. Chem. Soc.*, 1211, 1218, 1225 (1953)).



(6) J. W. E. Glattfeld and E. Rietz, THIS JOURNAL, **62**, 976 (1940).

Whereas the former substance is labile, yielding, for example, the angelica lactone at 160°,[7,8] the latter can be distilled at 173–178° without any detectable decomposition. The neutral product from antimycin is also stable at these and higher temperatures, thereby suggesting that it is a β-, rather than a γ-acyloxylactone.

As expected, the carbonyl regions in the infrared spectra of the model β- and γ-acyloxy-γ-lactones were similar to each other and to that of the antimycin lactone: the β-substituted case possessed a lactone band at 5.60 μ and an ester band at 5.75 μ; the γ-lactol ester absorbed at 5.61 and 5.75 μ. Although the carbonyl absorption by itself thus does not assist in a structural assignment, it is noteworthy that the fingerprint region of the model β-oxygenated lactone more closely resembles that of the neutral product from antimycin (Fig. 1), and to that extent may be regarded as supporting the β-formulation for the substance under investigation.[9,10]

All available evidence suggests that the individual components of the antimycin complex share the antimycic acid residue, but differ in the nature of the substitution (R, R′ and R″ in formula IV) on the actual, or potential, lactone moiety. Structural work designed to elucidate the exact nature of the branching in the various lactone entities is in progress.

Experimental[11]

β-Hydroxy-γ-valerolactone.—Sixty-four grams of Δ^3-pentenoic acid was added to a solution of 40.8 g. of barium chlorate in 150 ml. of water, and after the mixture had been stirred for 0.5 hr., 8 cc. of a 0.2% aqueous solution of osmium tetroxide was added. After stirring for 36 hr., 15 ml. more of the osmium tetroxide solution was run into the reaction mixture; and a third, 8-ml. portion of the same reagent was added after 24 hr. of subsequent stirring. After 12 additional hr. the solution was concentrated by distillation under reduced pressure such that the temperature did not rise above 50°. Extraction of the residue with absolute ethanol was followed, after removal of the excess solvent, by distillation, during which an exothermic reaction of undetermined nature occurred at about 100°. All the fractions were discarded, except that which was collected between 123° and 135° (0.4 mm.). Redistillation of this material gave 10 g. (13%) of the liquid hydroxylactone b.p. 139–141° (0.6 mm.), $n^{25}D$ 1.4632.

Anal. Calcd. for $C_5H_8O_3$: C, 51.7; H, 6.90. Found: C, 51.8; H, 7.08.

The *p*-nitrobenzoate of β-hydroxy-γ-valerolactone was prepared through the use of pyridine-*p*-nitrobenzoyl chloride. Two recrystallizations from aqueous ethanol gave pale yellow crystals, m.p. 134–135°.

Anal. Calcd. for $C_{12}H_{11}O_6N$: C, 54.3; H, 4.15. Found: C, 54.5; H, 3.72.

Treatment with pyridine–benzoyl chloride of the hydroxylactone gave the **benzoate V**, which, after three recrystallizations from aqueous ethanol, melted at 81.5–83°.

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.4; H, 5.45. Found: C, 65.5; H, 5.61.

Hydrolysis of the Benzoate of β-Hydroxy-γ-valerolactone (V).—To 9.6 ml. of 10% aqueous sodium hydroxide was added 0.49 g. of the benzoate V, and the mixture was heated under reflux for 2 hr. After this time, the reaction medium was homogeneous. On acidification of the basic solution with concentrated hydrochloric acid, 0.234 g. (87%) of benzoic acid, m.p. 120.5–121.5°, precipitated. After adjustment of the *p*H to 4.5, a solution of 0.246 g. of semicarbazide hydrochloride and 0.369 g. of sodium acetate in 3 ml. of water was added. The solid derivative which accumulated after standing in the cold for two hours was collected; the yield of crude (m.p. 178–179°) semicarbazone was 0.160 g. (42%).[12] Recrystallization from water raised the m.p. to 184.5–185°, which was not depressed on admixture with authentic levulinic acid semicarbazone, m.p. 184.5–185°.

In a separate experiment, the levulinic acid in solution was converted to the 2,4-dinitrophenylhydrazone, m.p. 206–207°. The melting point of this material was not depressed by the presence of authentic derivative, m.p. 206.5–207°.

The hydrolysis of β-hydroxy-γ-valerolactone, using the same conditions as described above, did not yield levulinic acid in such an amount that it was detectable by the semicarbazone isolation procedure utilized in the saponification experiments on the lactone benzoate.

O-Butyrate of β-Hydroxy-γ-butyrolactone.—β-Hydroxy-γ-butyrolactone was converted to the butyric acid ester by treatment with butyryl chloride and pyridine. The liquid ester boils at 106–108° (0.4–0.6 mm.), $n^{25}D$ 1.4478.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.8; H, 6.97. Found: C, 55.2; H, 6.94.

The ester was redistilled at 28 mm. pressure, b.p. 173–178°. The refractive index and the infrared spectrum of the product were identical with those of the starting material, thus indicating that the ester did not decompose under the distillation conditions.

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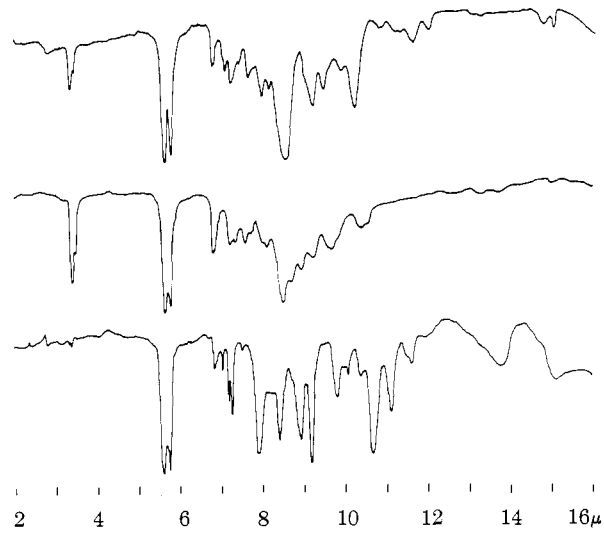

Fig. 1.—Infrared spectra: recorded on a Baird-Automatic recording spectrophotometer: top curve, β-butyroxy-γ-butyrolactone, liquid film; middle curve, antimycin lactone, liquid film; bottom curve, β-acetoxy-γ-valerolactone, 0.2 molar in $CHCl_3$ solution.

(7) J. Bredt, *Ann.*, **256**, 322 (1890).

(8) Apparently the δ-acyloxy-δ-valerolactone system is even more prone to elimination. Treatment of δ-ketohexanoic acid with acetic anhydride–acetyl chloride under conditions appropriate for the ready preparation of the lactol ester from levulinic acid (D. Vorlander and A. Knozsch, *ibid.*, **294**, 319 (1891)), followed by distillation, gave only the unsaturated lactone. Other attempts to prepare the δ-acyloxy-δ-lactone, all of which paralleled methods reported to be successful in the γ-lactone case, also failed.

(9) The β-2-methylbutyroxy-γ-lactone proposal has been incorporated into a tentative antimycin structure published elsewhere: F. Strong, "Topics in Microbial Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1958, p. 32.

(10) In a personal communication, we have been informed that Prof. H. Yonehara, University of Tokyo (Institute of Applied Microbiology) has further demonstrated the correctness of the general structure IV for the antimycin lactone through the successful periodate cleavage of the diol obtained under acidic conditions. A similar observation also has been made by Dr. R. Liu, Department of Biochemistry (unpublished results).

(11) Melting points are corrected; boiling points are uncorrected.

(12) Treatment of authentic levulinic acid under the conditions described above gave semicarbazone of comparable purity in 62% yield.