were mixed in the ratio K-12:Y-53 of 100 – 1000:1. These mixtures were inoculated into a synthetic medium[4] containing 300 O.U./ml. penicillin G to give an initial cell concentration of 10^8–10^9/ml. The suspensions were incubated with shaking for four hours, when there were about 10^4–10^5 viable cells. In a number of experiments, assays of these survivors have given ratios of K-12:Y-53 ranging from 2:1 to 1:100, and representing amplifications of mutants of several hundred to several thousand-fold. No change from the initial ratio was observed when the growth factors required by Y-53 were added to the treatment medium.

New mutants have also been obtained from several *Salmonella* strains, by treatment of cells grown from irradiated inocula. Approximately half the colonies surviving the treatment failed to grow when inoculated in synthetic medium. However, only about two-thirds of these proved to be stable mutants when subcultures on nutrient agar slants were retested. No satisfactory explanation of this behavior has been found.

The mutants so far characterized require a variety of growth factors, including histidine, methionine, tryptophan, leucine, threonine, proline, phenylalanine and tyrosine.

The method would lend itself to the isolation of specific mutants by the addition of irrelevant growth factors to the treatment medium. The method should be applicable to other bacteria, but the optimal conditions will have to be worked out for each organism.

Parallel experiments with streptomycin and streptothricin[5] gave no alteration in the mutant ratio. Very few antibiotics are reputed to have the differential activity on growing cells needed for this method.

(5) Kindly provided by Merck and Co.

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RECEIVED DECEMBER 1, 1948

THE LACTONES OF 2-HYDROXYMETHYLPOLYHYDROPHENANTHRYL-1-ACETIC ACIDS

Sir:

In the course of proof of structure of steroidal 16,17-ketols prepared by the method of Stodola, *et al.*,[1] we have submitted such a ketol (now known to be a 16-keto-17(α)-hydroxysteroid) to reaction with lead tetraacetate in aqueous acetic acid to rupture the C_{16}–C_{17} bond with cleavage of steroid Ring D to produce an aldehyde group at C_{13} and an acetic acid group at C_{14}.[2] Reduction of such an aldehyde-acid to the primary alcohol stage using hydrogen and Raney nickel catalyst (or hydrogen and Adams catalyst plus ferrous ion) yields a δ-hydroxyacid which easily lactonizes to the δ-lactone.

(1) Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941).
(2) Huffman and Lott, THIS JOURNAL, in press.

By this method of synthesis we have prepared a series of 2-hydroxymethylpolyhydrophenanthryl-1-acetic acid lactones utilizing various 16-keto-17-(α)-hydroxysteroids as starting materials. Thus, from 3-methoxy-17(α)-hydroxy-16-keto-$\Delta^{1,3,5}$-estratriene[3,4] has been obtained the lactone of 7-methoxy-2-methyl-2-hydroxymethyl-1,2,3,4,9,10,11,12-octahydrophenanthryl-1-acetic acid (m. p. 176–177°).[5] *Anal.*[6] Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.85, 75.96; H, 8.03, 7.96. This lactone is easily demethylated with hydriodic acid[4] to give the free phenol (m. p. 285–287° dec.). Similarly, from 3(β),17(α)-dihydroxy-16-keto-Δ^5-androstene[7,2] has been prepared the lactone of 7-hydroxy-2,13-dimethyl-2-hydroxymethyl-1,2,3,4,-5,6,7,8,10,11,12,13 - dodecahydrophenanthryl - 1 - acetic acid (m. p. 205.5–206.5°). *Anal.* Calcd. for $C_{19}H_{28}O_3 \cdot H_2O$: C, 70.77; H, 9.38. Found: C, 70.82, 70.73; H, 9.41, 9.34. This lactone upon oxidation by the Oppenauer method furnishes the lactone of 7-keto-2,13-dimethyl-2-hydroxymethyl-1,2,3,4,5,6,7,9,10,11,12,13 - dodecahydrophenanthryl-1-acetic acid (m. p. 191–192°). *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.27, 75.22; H, 8.65, 8.75. The two isomeric lactones from 3(α),17(α)-dihydroxy-16-ketoandrostane and 3(β), 17(α)-dihydroxy-16-ketoandrostane have also been prepared; these melt at 228.5–229.5° and 201° respectively. *Anal.* Calcd. for $C_{19}H_{30}O_3$ (m. p. 228.5–229.5°): C, 74.47; H, 9.87. Found: C, 74.40, 74.32; H, 9.91, 9.84.

This series of lactones is not identical with that prepared by Westerfeld[8] and by Jacobsen[9] and co-workers. A mixed melting point determination using our 7-methoxy-2-methyl-2-hydroxymethyl-1,2,3,4,9,10,11,12-octahydrophenanthryl-1-acetic acid and the corresponding δ-lactone[10] (estrololactone methyl ether) of Dr. Jacobsen showed a depression of some thirty degrees.

(3) Huffman, *J. Biol. Chem.*, **167**, 273 (1947).
(4) Huffman, *ibid.*, **169**, 167 (1947).
(5) All melting points are uncorrected.
(6) Microanalyses performed by Dr. E. W. D. Huffman, Denver.
(7) Butenandt, Schmidt-Thomé and Weiss, *Ber.*, **72**, 417 (1939).
(8) Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).
(9) Jacobsen, *ibid.*, **171**, 61 (1947); Levy and Jacobsen, *ibid.*, **171**, 71 (1947); Jacobsen, Picha and Levy, *ibid.*, **171**, 81 (1947).
(10) Kindly supplied by Dr. R. P. Jacobsen.

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RECEIVED NOVEMBER 6, 1948

THE ION-EXCHANGE SEPARATION OF ZIRCONIUM AND HAFNIUM

Sir:

In the course of a rather cursory examination of the elution of tetra-positive ions from the cation exchange resin Dowex 50 with hydrochloric acid solutions, we have discovered a very effective method for separating zirconium from hafnium. In view of the great labor involved in preparing even reasonably pure hafnium compounds by