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.

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(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.

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.

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(1) Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941).

(2) Huffman and Lott, THIS JOURNAL, in press.

(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 existing methods, we feel that this procedure will prove very valuable to those interested in obtaining hafnium compounds free of zirconium.

Although the conditions which give satisfactory separation were first worked out using microgram amounts of material and the radioactive tracer technique, the run described below, involving milligrams of material, illustrates the applicability of the method to the production of significant amounts of pure hafnium and zirconium.

Thirty-five milligrams of zirconium oxide and 15 mg. of hafnium oxide were dissolved in sulfuric and hydrofluoric acids, hafnium and zirconium tracer added, and the mixture fumed to dryness. The residue was taken up in concentrated hydrochloric acid and the hydroxides precipitated with ammonium hydroxide and washed. The hydroxides were again dissolved in hydrochloric acid and the oxychlorides crystallized by evaporation. One cubic centimeter of 250 to 500 mesh Dowex 50 spheres, in the ammonium form, was suspended in 30 cc. of 2 *M* perchloric acid and the oxychlorides added a few milligrams at a time over a period of fifteen minutes, the mixture being continually agitated by bubbling air through it. Under these conditions, *i. e.*, dilute solution of zirconium and hafnium in 2 *M* perchloric acid, the zirconium and hafnium are not appreciably polymerized[1] and about 80% of each went on the resin. The slurry of resin was placed on the top of an ion-exchange column 1 sq. cm. in area and 30 cm. long which had been packed with the same resin and washed with 6 *M* hydrochloric acid to convert it to the acid form. On elution with 6 *M* hydrochloric acid the curve shown in Fig. 1 was obtained. The outline of the curve was obtained by counting the tracers (65-day Zr^{95} and 46-day Hf^{181}) and the dotted portions by optical spectrographic analysis.

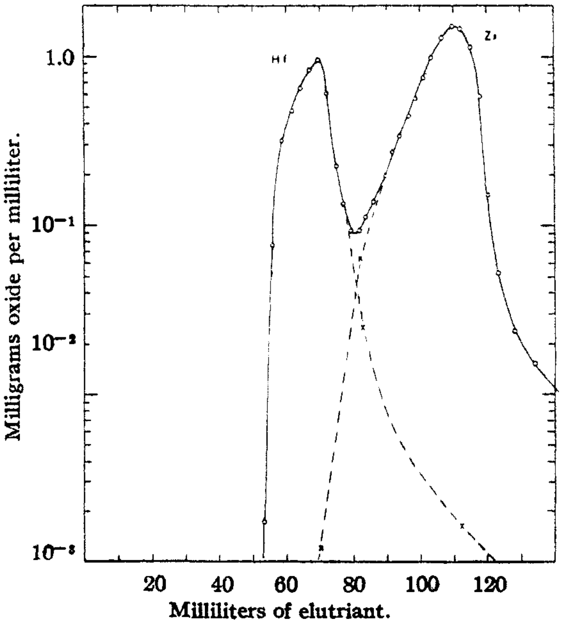

Fig. 1.—Elution of zirconium and hafnium with 6.0 *M* HCl.

Thus it can be seen that ~66% of the starting hafnium oxide, *i. e.*, 10 mg., is obtained containing ~0.1% zirconium oxide by weight. The column used in these experiments was relatively small and thus gram amounts of material should be easily handled on columns of only moderate size.

This work was done under the auspices of the Atomic Energy Commission.

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[1] R, E. Connick and W. H. McVey, private communication.

THE ISOLATION OF METHYL ARACHIDONATE BY CHROMATOGRAPHIC ADSORPTION. THE OCCURRENCE OF EICOSAPENTENOIC ACID IN ADRENAL LIPIDS

Sir:

The purest specimens of methyl arachidonate (MA) hitherto isolated have been made by debromination of methyl octabromoarachidate[1–5] which is best obtained by brominating the methyl esters of the fatty acids of beef adrenal phosphatides. The arachidonate thus prepared is likely to be contaminated with geometric and other isomers, in line with our experience with linoleic and linolenic acids.[6] Methyl arachidonate prepared solely by physical procedures,[3,4] has not been obtained more than 90–95% pure.

We have isolated methyl arachidonate of high purity by chromatographic adsorption. The arachidonate in the methyl esters of adrenal phosphatides was first concentrated by low temperature crystallization to a product of iodine number 247 containing 65 per cent. methyl arachidonate, methyl oleate and other unsaturated esters. This mixture was separated by passage over Harshaw alumina (Al-2) by the flowing chromatogram technique, the solvents being petroleum ether containing 1.5% of ethyl ether, and ethyl ether. Operations were carried out in an atmosphere of nitrogen gas. Separations and compositions of fractions were followed roughly from the refractive indices.

To illustrate the efficacy of separation by the adsorption technique, the first main fractionation was carried out by passing 98.8 g. of the concentrate over 2000 g. of alumina with 8 l. of petroleum ether–ethyl ether to give the following fractions (iod. no. in parentheses): 0.8 g. (135); 1.3 g. (152); 8.9 g. (216); 42.2 g. (246); 10.8 g. (252); 8.0 g. (265); 8.6 g. (291); 11.0 g. (313). By selected refractionations we finally obtained 8.6 g. of ester of iodine number 319.2 and the three small

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[1] Brown, *J. Biol. Chem.*, **80**, 455 (1928).
[2] Ault and Brown, *ibid.*, **107**, 615 (1934).
[3] Shinowara and Brown, *ibid.*, **134**, 331 (1940).
[4] Mowry, Brode and Brown, *ibid.*, **142**, 671 (1942).
[5] White, Orians and Brown, *J. Am. Oil Chem. Soc.*, in press.
[6] Matthews, Brode and Brown, THIS JOURNAL, **63**, 1064 (1941).