

Molecular Weight of the Adrenocorticotropic Hormone

BY ELLEN BURTNER

Diffusion and sedimentation experiments have been performed with dilute solutions of the adrenocorticotropic hormone which has been prepared and described by Li, Simpson and Evans of the Institute of Experimental Biology, Berkeley, California.¹ In making up the solutions 20 mg. of the purified preparation was dissolved in 3 cc. of phosphate buffer of pH 7.0 and containing sodium chloride to give ionic strength 0.28, and dialyzed against the buffer salt system. Two sedimentation velocity experiments were performed in the standard Svedberg oil-turbine high velocity ultracentrifuge, and one diffusion experiment was carried out in a Lamm cell. The position of the peak in the former, and the spreading of the peak in the latter, at the end of successive time intervals, were observed by the Lamm scale line displacement method.

The sedimentation constant, s_{20} , was calculated by using the procedure described by Svedberg and Pedersen.² While the individual values of s_{20} calculated at half-hour intervals during each experiment vary somewhat, the two averaged values agree to within 5%. Variations between the results in individual experiments may be due to the fact that the material is light and thus travels only a short distance in each time interval, making the error in the determination of the change of position of the boundary an appreciable fraction of the total distance traveled. In the scale line displacement *vs.* distance in cell diagrams, the curves obtained were not unlike those characteristic of a single substance.

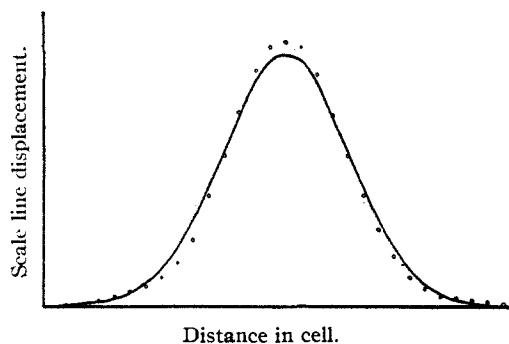


Fig. 1.

(1) C. H. Li, M. E. Simpson and H. M. Evans, *Science*, **96**, 450 (1942).

(2) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge." Oxford University Press, Oxford, 1940.

The diffusion constant was calculated from the equation $D = \sigma^2/2t$, where σ is the standard deviation of the curve obtained by plotting the scale line displacement against position in the cell. The value was then adjusted to give the diffusion constant for a hypothetical process taking place in pure water at 20°. Comparison of the experimental curve, when reduced to normal coordinates, with the Gaussian error curve, gave evidence of some inhomogeneity. In the figure the solid line represents the ideal diffusion curve of a single substance, and the circles are the experimental points.

The molecular weight was calculated by using the familiar equation $M = RTs/D(1 - V\rho)$. The partial specific volume was not determined because a sufficient amount of the hormone was not available. In the absence of information to the contrary, it was assumed to be 0.75. The frictional ratio, f/f_0 , was obtained by using the equations $f = M(1 - V\rho)/s$ and $f_0 = 6\pi\eta N(3MV)^{1/3}/(4\pi N)^{1/3}$. It gives as an approximate value 3:1 for the ratio of major to minor axis of the assumed unhydrated ellipsoidal molecule.

Experimental data, with calculations, are summarized to form Table I. For reasons given above the magnitude of the uncertainty involved in these figures is relatively high. (Probable error in molecular weight is $\pm 10\%$.)

TABLE I

MOLECULAR KINETIC DATA FOR THE ADRENOCORTICOTROPIC HORMONE (FRICTIONAL AND AXIAL RATIOS COMPUTED FOR UNHYDRATED ELLIPSOIDAL MOLECULE)

s_{20} (in S)	D_{20} (in 10^{-7} sq. cm./sec.)	M	f/f_0	a/b
2.08	10.4	20,000	1.1	3

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

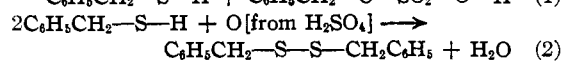
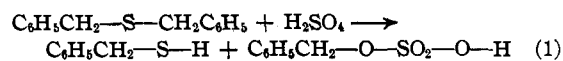
RECEIVED FEBRUARY 25, 1943

Tribenzylsulfonium Hydrogen Sulfate and Hydroxide

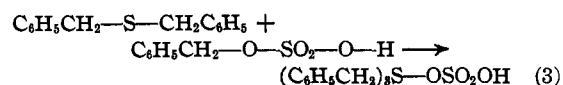
BY OTTO HAAS AND GREGG DOUGHERTY

It has been found that concentrated sulfuric acid brings about the cleavage of dibenzyl sulfide at moderate temperatures. Unlike the well known reaction of ethers and sulfuric acid, however, the products isolated are not benzyl mercaptan and benzyl hydrogen sulfate, although they are likely intermediates in the reaction. The principal product obtained is tribenzylsulfonium hydrogen

sulfate and the most probable course of the reaction is



and more complex products.



Based on this explanation of the process the yields are nearly quantitative.

The tribenzylsulfonium hydrogen sulfate like the chloride and iodide was found to be fairly stable in water but decomposed on heating in water solution in the usual way to the sulfide and benzyl alcohol. Attempts to prepare the nitrate from the sulfate by treating the latter with silver nitrate in 95% alcohol resulted unexpectedly in the formation of tribenzylsulfonium hydroxide. Apparently, the nitrate was easily hydrolyzed to the hydroxide and since this behavior is unusual in the case of sulfonium salts, the point was investigated further. Tribenzylsulfonium nitrate was prepared by the method of Renshaw and Searle¹ and it was found that it was in fact very readily converted to the hydroxide when heated with water. By a slight modification of the procedure of Renshaw and Searle the tribenzylsulfonium hydroxide was formed directly in nearly quantitative yields from tribenzylsulfonium iodide-mercuric iodide. This constitutes a convenient and very efficient preparation method for the hydroxide.

Tribenzylsulfonium Sulfate.—Three grams of dibenzyl sulfide was added to 6 g. of concentrated sulfuric acid. Little solution occurred at room temperature, but on warming to 70–80° and shaking vigorously, solution of the sulfide was complete in about ten minutes. This dissolving was accompanied by the evolution of sulfur dioxide. After the reaction mixture had cooled, it was poured into 25 cc. of cold water. This caused the precipitation of a white crystalline product, which was washed by decantation with two 25-cc. portions of water. A crude yield of 3 g. of tribenzylsulfonium sulfate, melting at 130–135°, was obtained. Crystallization from alcohol gave pure tribenzylsulfonium sulfate, m. p. 171°. (The reported melting points of this salt vary from 170 to 175°).²

Anal. Calcd. for $\text{C}_{21}\text{H}_{22}\text{O}_4\text{S}_2$: SO_4^{--} , 24. Found: (by addition of barium chloride solution to the water solution of the sulfonium sulfate), 21. This sulfonium salt forms a trinitrophenoxide (m. p., 140°) which is identical with that prepared from known tribenzylsulfonium salts

(1) Renshaw and Searle, *THIS JOURNAL*, **55**, 4951 (1933).

(2) Fichter and Sjöstedt, *Ber.*, **43**, 3428 (1910).

and picric acid. These are easily formed by mixing hot alcohol or water solutions of the reactants. Tribenzylsulfonium sulfate may be changed into the hydroxide by silver nitrate, if the reaction is carried out in 95% alcohol.

Tribenzylsulfonium Hydroxide.—Tribenzylsulfonium hydroxide was prepared in almost quantitative yields by the action of dilute aqueous silver nitrate (0.05 *N*) on an acetone solution of tribenzylsulfonium iodide-mercuric iodide. This addition complex was prepared by the general method of Smiles,³ and was obtained in the form of glistening yellow plates, m. p. 137–138°.

Anal. Calcd. for $\text{C}_{21}\text{H}_{21}\text{SI}_3\text{Hg}$: S, 3.61; I, 43.0. Found: S, 3.58; I, 42.5.

After the addition of the silver nitrate, the solution was warmed to coagulate the silver iodide, which was then removed by filtration. The excess acetone was evaporated, and on cooling the residual solution, tribenzylsulfonium hydroxide separated as a white solid. When this was recrystallized from water, the hydroxide was obtained in the form of thick, white needles, which were stable and non-hygroscopic, and which melted at 133°.

Anal. Calcd. for $\text{C}_{21}\text{H}_{22}\text{OS}$: S, 9.94. Found: S, 9.84.

This sulfonium hydroxide was also prepared from tribenzylsulfonium chloride⁴ and moist silver oxide. It may be converted into the trinitrophenoxide, sulfate, iodide (m. p. 78°), chloride, etc., by metathesis with the acids (or salts) in water solutions. On heating, the hydroxide decomposed into dibenzyl sulfide and benzyl alcohol.

Tribenzylsulfonium nitrate was prepared according to the method of Renshaw. The oily product obtained was converted into the sulfonium hydroxide by heating it with water.

(3) Smiles, *J. Chem. Soc.*, **77**, 160 (1900).

(4) Lee and Dougherty, *J. Org. Chem.*, **4**, 48 (1939).

FRICK CHEMICAL LABORATORY
PRINCETON UNIVERSITY
PRINCETON, N. J.

RECEIVED MARCH 23, 1943

Contribution to the Theory of Racemism

By C. S. HUDSON

Let the enantiomorphous forms of an optically active substance S be designated *d*-S and *l*-S. Assume that a solution containing equal quantities of *d*-S and *l*-S deposits crystals of S.

Case 1.—If these crystals are found by suitable measurements to be different from the separately and similarly prepared crystals of the single *d*-S and *l*-S components, the S crystals are a racemate, which may be designated *rac.* S. If the crystals prove to be the same as those of *d*-S and *l*-S, the racemate has not been found in the test.

Case 2.—Suppose now that the substance S is tautomeric in addition to being optically active, and assume two tautomers (S_1 and S_2) for simplicity; in this case there are two racemates possi-