The authors wish to express their indebtedness to Dr. R. M. Bock for his counsel.

The Sedimentation Behavior of Bovine Serum Albumin in Acid Solutions[1]

BY P. BRO,[2] S. J. SINGER AND J. M. STURTEVANT

RECEIVED APRIL 22, 1955

An extension of the calorimetric studies reported by Gutfreund and Sturtevant[3] has revealed that at low pH bovine serum albumin (BSA) and bovine serum mercaptalbumin (BSMA) may undergo an exothermic reaction in addition to the previously reported endothermic reaction. In an effort to find the cause of the exothermic reaction, the calorimetric work was complemented by sedimentation studies of BSA and BSMA in 0.1 M ionic strength HCl–NaCl solutions.

In agreement with published results, we have found that aggregates are formed[4,5] at low pH and that the sedimentation constants of the unaggregated[6] and aggregated species decrease with pH. The nature of the aggregation phenomenon is under investigation and will not be discussed further here. A detailed study has been made,
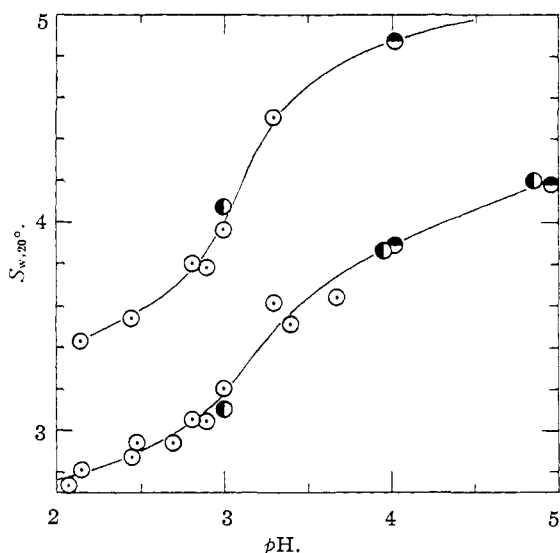

Fig. 1.—Sedimentation curves of BSA and aggregate; 0.1 M ionic strength HCl–NaCl solutions: ⊙, Armour and Pentex bovine serum albumin; ◑, bovine serum mercaptalbumin: ◓, B.S.A. after exposure to pH 3.1.

however, of the sedimentation constants as a function of pH in solutions approximately 1% in protein (Fig. 1). Several features may be noted. In the range of pH 3.8 to 2.8 the sedimentation constants show an inflection; at higher pH, the slopes of the sedimentation curves do not appear to decrease to zero near the isoelectric point; the changes in the sedimentation constants are reversible; and the behavior of BSA and BSMA are indistinguishable.

There are at least three factors which might be involved in the decrease in sedimentation constants: a decrease in molecular weight; the presence of electric potentials during sedimentation of charged particles; and configurational changes in the molecule which produce an increased frictional coefficient. The first of these is unlikely since the unaggregated and aggregated species behave in a parallel manner. The contribution of the second factor may be evaluated, as shown by Pedersen.[7] At pH 3.0 the charge correction amounts to less than 1% of the isoelectric sedimentation constant. The difference between the isoelectric sedimentation constant (corrected for the charge effects at various pH levels) and the observed values at lower pH, together with the pronounced inflection in the curve of Fig. 1, therefore provide additional evidence for the occurrence of a reversible configurational change in the BSA molecule in acid solution.[8,15] It is interesting that the aggregate appears to undergo a similar change.

In order to investigate the nature and magnitude of this configurational change, sedimentation constants extrapolated to infinite dilution should properly be determined. If the observed and isoelectric sedimentation constants in 1% solutions are compared, the magnitudes of the average changes so calculated will most likely be too large. Assuming that the molecule "swells" isotropically in acid solution, and using the model of the hydrodynamically equivalent sphere for the BSA molecule, in conjunction with Stokes' law, we find[16]

(7) T. Svedberg and K. O. Pedersen, "Die Ultracentrifuge," Steinkopff Verlag, Dresden and Leipzig, 1940, p. 22. The net charge values employed by Tanford[8] were used to calculate the charge corrections to the isoelectric sedimentation constant. The sedimentation constants of the non-protein ions were estimated from the ionic diffusion coefficients and the partial specific volume of sodium chloride. The ionic diffusion coefficients were calculated by means of the Nernst equation and the limiting ionic conductances,[9] to which the appropriate activity correction was applied. The partial specific volume of NaCl[10] was apportioned between the sodium and chloride ions on the basis of the hydrated molal volumes obtained by Sutra.[11] The partial specific volume and the diffusion coefficient of BSA are given by Edsall.[12] It was assumed that the electrolyte could be considered to be entirely NaCl. The equivalent conductance of a 0.1 M NaCl solution[13] was used to calculate the conductivity of the protein solution. Experimental mobility data[14] were employed for BSA.

(8) C. Tanford, paper presented at the meeting of the Electrochemical Society in New York, April, 1953.

(9) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd Ed., Reinhold Publ. Corp., New York, N. Y., 1950, p. 172.

(10) H. S. Harned and B. B. Owen, ref. 9, p. 253.

(11) G. Sutra, J. chim. phys., 43, 189 (1946).

(12) J. T. Edsall in H. Neurath and K. Bailey, "The Proteins," Academic Press, New York, N. Y., 1953, p. 636.

(13) H. S. Harned and B. B. Owen, ref. 9, p. 537.

(14) S. J. Singer and D. H. Campbell, THIS JOURNAL, 77, 3504 (1955).

(15) J. T. Yang and J. F. Foster, ibid., 76, 1588 (1954).

(16) The partial specific volume of the protein is assumed to change very little with pH.

(1) This research was aided by grants from the National Science Foundation (No. G 179) and the United States Public Health Service (No. RG 3996 C).

(2) General Electric Company Fellow, 1954–1955.

(3) H. Gutfreund and J. M. Sturtevant, THIS JOURNAL, 75, 5447 (1953).

(4) M. E. Reichmann and P. A. Charlwood, Can. J. Chem., 32, 1092 (1954).

(5) H. A. Saroff, G. I. Loeb and H. A. Scheraga, THIS JOURNAL, 77, 2908 (1955). We are indebted to Dr. Scheraga for communicating his results to us in advance of publication.

(6) T. Svedberg and B. Sjögren, THIS JOURNAL, 52, 2855 (1930).

that at pH 3.0, for example, the radius of the equivalent sphere is not more than 28% greater than its value at the isoelectric point. The estimated changes are smaller than those calculated by Tanford[8] from his titration data; he found a radial "swelling" of approximately 50% at pH 3.0.

Although unequivocal correlations cannot be made as yet between the calorimetric and sedimentation behavior of BSA in acid solutions, the endothermic reaction of BSA has been tentatively associated with the reversible configurational changes. The correlation between the exothermic reaction and the aggregation phenomenon is under investigation.

CONTRIBUTION No. 1297
STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

The Conversion of Androstane-3,17-dione to Androsterone[1]

BY CARL DJERASSI, A. J. MANSON AND MARVIN GORMAN

RECEIVED MAY 19, 1955

In spite of the fact that androsterone (II) was the first male sex hormone to be isolated and possesses a relatively simple structure, no short synthesis has been available for this steroid.[2] This in turn has prevented extensive clinical experiments with this substance, which many endocrinologists feel merits more attention. In connection with Raney nickel desulfurization experiments under way in this Laboratory, we have made certain ancillary observations which indicate that a facile synthesis of androsterone should be possible and these are being reported in the present note.

The only complicating feature of any androsterone synthesis is the formation of a 3α-alcohol of the 5α-series, which usually requires conditions (e.g., platinum-catalyzed hydrogenation in an acid medium)[3] which also would reduce the 17-keto group unless protected.[2] It now has been observed that while androstane-3,17-dione (I) is reduced to a mixture of diols when refluxed with fresh W-2 Raney nickel[4] in ethanol solution, the 17-keto group is not affected by non-pyrophoric (old) Raney nickel catalyst. This same non-pyrophoric catalyst, however, was quite effective in reducing a saturated 3-keto function as demonstrated by the conversion of cholestan-3-one to a 2:1 mixture of cholestan-3α-ol and cholestan-3β-ol. A noteworthy feature of this reduction is the relatively high proportion of 3α-epimer formed, which suggested that a one-step conversion of androstane-3,17-dione (I), a commercial intermediate in the manufacture of estrone, to androsterone (II) should be feasible.
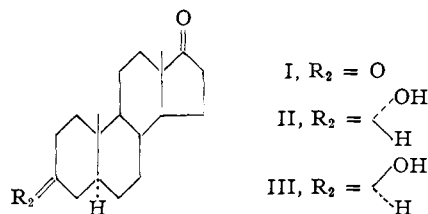
Indeed, when androstane-3,17-dione (I) was re-

(1) We are indebted to the American Cancer Society on recommendation of the Committee on Growth of the National Research Council for a research grant.

(2) For the most recent synthesis and leading references see J. Iriarte, G. Rosenkranz and F. Sondheimer, J. Org. Chem., 20, 542 (1955).

(3) Cf. L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, 1949, 3rd ed., p. 99.

(4) "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 181.

fluxed with non-pyrophoric Raney nickel catalyst, i.e., W-2 catalyst which had been stored for a period of one to three months, nearly 50% of androsterone (II) was formed, together with 34% of the epimeric epiandrosterone (III). The two isomers could, of course, be separated quantitatively by means of digitonin, but chromatographic separation also proved feasible. No attempt was made to determine the optimum conditions for the synthesis of androsterone, but it was noted that "hydrogen-free" Raney nickel catalyst[5] was equally effective. In view of the fact that cholestan-3-one is not reducible[6] by fresh W-2 Raney nickel in benzene or acetone solution and yields chiefly cholestan-3β-ol when heated in ethanol solution, it appears that the formation of large amounts of the 3α-epimer with non-pyrophoric or hydrogen-free catalyst in ethanol must proceed by a different mechanism. A possible explanation is that the latter reduction involves a hydrogen transfer from ethanol to the ketone, in spite of the fact that this does not appear to be operative[7] in Raney nickel desulfurizations.

I, $R_2 = O$

II, $R_2 = \left\langle{\begin{matrix}OH\\H\end{matrix}}\right.$

III, $R_2 = \left\langle{\begin{matrix}OH\\H\end{matrix}}\right.$

Experimental[8]

Reduction of Cholestan-3-one with Non-pyrophoric W-2 Raney Nickel.—A solution of 600 mg. of cholestan-3-one in 75 cc. of absolute ethanol was refluxed with ca. 6 g. of W-2 Raney nickel[4] (1 month old) for 5 hours. After filtering and evaporating to dryness, 588 mg. of colorless solid was obtained, which by infrared spectroscopy (no infrared carbonyl band) was shown to consist of a mixture of the epimeric cholestan-3-ols. Chromatography on 24 g. of alumina and elution with benzene–ether (4:1) yielded 390 mg. of cholestan-3α-ol; after recrystallization from ethyl acetate, it exhibited m.p. 185–186°, $[\alpha]^{20}D +26°$ (CHCl₃), $\lambda_{max}^{CHCl_3}$ 10.0 μ; acetate, m.p. 94–96°, $[\alpha]^{20}D +42°$ (EtOH). Further elution with the same solvent furnished 200 mg. of cholestan-3β-ol, m.p. 140–141°, $\lambda_{max}^{CHCl_3}$ 9.68 μ. Identity was established in each case by mixture melting point and infrared comparison with authentic samples.

When the reaction was repeated with fresh (one day old) W-2 catalyst, 83% of cholestan-3β-ol was formed.

Reduction of Androstane-3,17-dione with Non-pyrophoric W-2 Raney Nickel.—A solution of 500 mg. of androstane-3,17-dione was refluxed in 50 cc. of absolute ethanol with 5.0 g. of W-2 Raney nickel (identical results were obtained with catalyst of 1 and 3 months age) and the total reduction product passed through 18 g. of alumina. The bulk was eluted with benzene–ether (9:1) while 57 mg., representing a diol mixture ($\lambda_{max}^{CHCl_3}$ 2.8–3.0, 9.62 and 10.0 μ) was eluted with ether–methanol (9:1). The pooled benzene–ether eluates consisted of a mixture of androsterone (II) and epiandrosterone (III) as demonstrated by infrared examination ($\lambda_{max}^{CHCl_3}$ 5.76 (17-ketone), 9.62 (epiandrosterone) and 10.0 μ (androsterone)). Separation was achieved by means of digitonin,[9] the material being dissolved in 60 cc. of 90% ethanol and added to a solution of 2.28 g. of digitonin in 140 cc. of 90% ethanol. After 2 hours, the precipitate was filtered, washed with ethanol and ether and the

(5) H. Hauptmann and B. Wladislaw, THIS JOURNAL, 72, 707 (1950).

(6) C. Djerassi, M. Gorman and J. A. Henry, ibid., 77, 4647 (1955).

(7) W. A. Bonner, ibid., 74, 1033 (1952); cf. M. L. Wolfrom and J. V. Karabinos, ibid., 66, 909 (1944).

(8) All melting points were determined on the Kofler block.