ing the two extractions were done in the presence of dark red light.

The results suggest that, discounting separation-induced exchange,[6] complete exchange occurred in less than seven seconds.

It is interesting to note (see Table I) that the precipitate formed upon addition of pyridine to the reaction mixture contained two moles of iodine per mole of stannic iodide.

(6) R. J. Prestwood and A. C. Wahl, THIS JOURNAL, **71**, 3137 (1949).

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11-Oxygenated Steroids. IX. The Elimination of the 11α-Hydroxyl Group *via* the Tosylate

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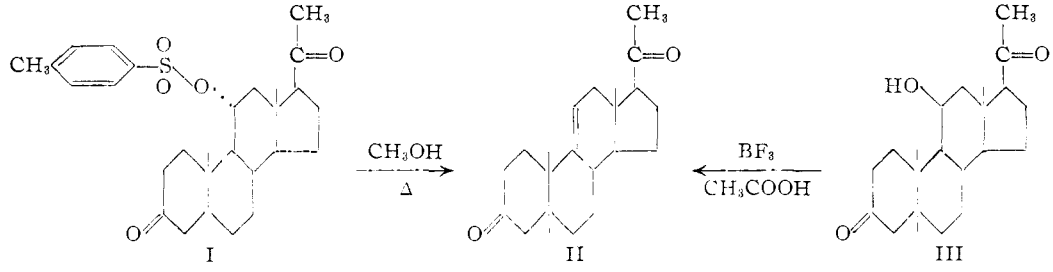
The recent communication of Fried and Sabo[1] describing, in part, the detosylation of Δ^4-pregnen-11α,17α,21-triol-3,20-dione 11-tosylate 21-acetate by sodium acetate in acetic acid prompts us to report our experiences with the detosylation of pregnan-11α-ol-3,20-dione tosylate (I). The tosylate (I), prepared in the usual way, was detosylated by refluxing in methanol solution.[2] It was possible to isolate *p*-toluenesulfonic acid in about 80% yield from the reaction, indicating very substantial or complete elimination or displacement of tosylate ion from I. Chromatographic separation of the products permitted us to separate $\Delta^{9(11)}$-pregnen-3,20-dione (II) in 22% yield.



The structure of II was established by an independent preparation. Boron trifluoride catalyzed dehydration[3] of pregnan-11β-ol-3,20-dione[4] (III) afforded II, identical in all respects with that obtained from the detosylation.

Experimental[5]

Pregnan-11α-ol-3,20-dione Tosylate (I).—To a solution of 13.5 g. of pregnan-11α-ol-3,20-dione[6] in 200 ml. of dry pyridine was added 13.5 g. of *p*-toluenesulfonyl chloride. The reaction mixture was held at room temperature for 18 hours and was then poured onto 1 l. of ice-water. Crystallization occurred after several hours and the resulting precipitate was removed by filtration, dried and crystallized from methylene chloride–hexane. There resulted 10.1 g. (51%) of I, m.p. 156–157°, $[\alpha]^{25}D$ +77.1° (1% in chloroform).

Anal. Calcd. for $C_{26}H_{34}O_5S$: S, 6.58. Found: S, 6.27.

The aqueous pyridine filtrate from the initial precipitation was extracted with methylene chloride, the methylene chloride solution was washed free of pyridine with dilute sulfuric acid and water and dried over magnesium sulfate. The dried solution was concentrated and hexane was added. On further concentration of the hexane solution an oil precipitated which was then brought back into solution with ether. Cooling and seeding of the resulting solution afforded a crystalline precipitate which was separated by filtration; yield 4.1 g., m.p. 106–110° dec. This product was identical with starting material.

Elimination of the 11α-Tosyl Group; $\Delta^{9(11)}$-Pregnen-3,20-dione (II).—A solution of 10 g. of I in 1 l. of C.P. methanol was refluxed for five hours. The reaction mixture was then concentrated *in vacuo*. The residual oil was taken up in methylene chloride, cooled and the insolubles were removed by filtration. There was isolated 2.76 g. of a strongly acidic, water-soluble crystalline substance, m.p. 103–105° (m.p. of *p*-toluenesulfonic acid 106–107°). The recovery of *p*-toluenesulfonic acid indicated that elimination or displacement had occurred to the extent of at least 78%.

The methylene chloride filtrate was concentrated to a small volume, hexane was added and the solution was chromatographed on activated alumina (Merck, chromatographic grade, 300 g.) prepared with hexane. Following preliminary elution with hexane and 10% ether–hexane a total of 12 fractions of 20% ether–hexane was collected with the following pattern of m.p.

Fraction no.	M.p., °C.
20–26 (150-ml. fractions)	*ca.* 135–141
27 (500-ml. fractions)	137–141
28 (500-ml. fractions)	132–143
29 (500-ml. fractions)	126–132
30 (500-ml. fractions)	125–137
31 (500-ml. fractions)	105–125

All succeeding fractions were oily. The column was stripped with ether, methylene chloride and methanol in that order. The material balance for the reaction mixture was

p-Toluenesulfonic acid	2.74 g.
Fractions 20–28	0.86 g.
Fractions 29–31	0.59 g.
Oils (all other fractions)	5.01 g.
	9.20 g.

Recrystallization of combined fractions no. 20–28 from acetone–water afforded II, m.p. 149–150°, $[\alpha]^{25}D$ +96.6° (acetone). The product could also be recrystallized from methylene chloride–hexane. The infrared spectrum of the combined material from fractions no. 29–31 was identical with that from fractions no. 20–28.

Boron Trifluoride Catalyzed Dehydration of Pregnan-11β-ol-3,20-dione (III).—To a solution of 0.5 g. of III in 50 ml. of glacial acetic acid was added 7.5 ml. of boron trifluoride etherate (47% solution—Matheson, Coleman and Bell). After standing 70 hours at room temperature the reaction mixture was diluted with methylene chloride and washed neutral with ice-water. The methylene chloride solution was dried, concentrated to a small volume and hexane was added to induce crystallization. There resulted as a first

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953).

(2) H. R. Nace, *ibid.*, **74**, 5937 (1952).

(3) H. Heymann and L. Fieser, *ibid.*, **73**, 5252 (1951).

(4) E. P. Oliveto, T. Clayton and E. B. Hershberg, *ibid.*, **75**, 486 (1953).

(5) All melting points are corrected. Analyses and optical data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories.

(6) E. P. Oliveto, H. L. Herzog and E. B. Hershberg, THIS JOURNAL, **75**, 1505 (1953).

crop 0.23 g. of II, m.p. 148–150°, and an additional 0.095 g., m.p. 144–147° was isolated from the mother liquor by further concentration. Recrystallization from methylene chloride–hexane raised the m.p. to 149–150°, $[\alpha]^{25}D$ +94.3° (acetone).

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.34; H, 9.32.

The sample of II prepared from III had an infrared spectrum identical with that of II isolated from the detosylation procedure.

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A Method for Determining the Solubility Characteristics of Components in Protein Mixtures[1]

BY E. L. HESS AND DORIS S. YASNOFF

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A rational approach to the chemical separation of a mixture of proteins requires some knowledge of the effect of *p*H and ionic strength upon the solubility of the components. In many fractionation studies the available quantity of starting material severely limits the investigation of these parameters. This report outlines a method for obtaining such information. As little as 0.1 g. of a crude mixture is sufficient for a fairly complete study of ionic strength and *p*H effects. The simplicity of the method and the economy of material recommends its use, particularly in work with tissue extracts. The earlier work of Kober,[2] Morse,[3] and Wannow[4] indicated the possible use of the method in the fractionation of protein mixtures.

The method consists of diluting a protein mixture to a concentration appropriate to optical density measurements in an ultraviolet spectrophotometer and reading the scale deflection given by the mixture.

A series of readings are made at various *p*H values with the concentration and ionic strength held constant. When *p*H and concentration are held constant the ionic strength may be varied. At those *p*H and ionic strength conditions where precipitation occurs, the optical density resulting from turbidity is superposed upon the intrinsic absorption from the soluble portion of the system. The precipitate, in our experience, remains suspended at the low concentrations employed. The turbidity is an inverse function of the solubility as can be seen in Fig. 1. The solubility curve in Fig. 1 was obtained by centrifuging the turbid solutions and reading the optical density (λ 280 mμ) of the clear supernatants in a Model DU Beckman spectrophotometer. For protein systems, where the solubility relationships are such that precipitation does not occur, the optical density remains constant over the *p*H range normally of interest. Although most of our studies have been carried out at 260 and 280 mμ, it would seem preferable to make the turbidity measurements at 250 mμ. At this lower wave length most proteins are close to the minimum of their characteristic ultraviolet absorption curve. Because the scattered radiation is an inverse function of the fourth power of the wave length the measured turbidity will be greater at the lower wave length. When precipitation does not occur within the *p*H and ionic strength deemed suitable for separation, the effect of salts such as ammonium sulfate or miscible solvents with low dielectric properties, *e.g.*, ethanol, can be explored. The use of thermospacers on the spectrophotometer allowing temperature control of the solution is recommended when organic solvents are employed.

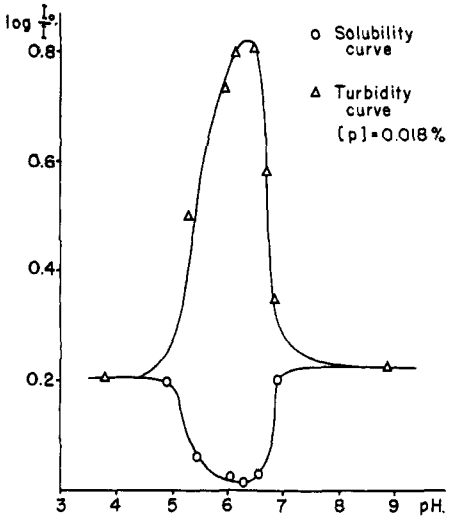

Fig. 1.—Comparison of solubility and turbidity measurements as a function of *p*H: fraction 6.2P, μ = 0.0; λ 280 mμ.

The application of the method is illustrated in Figs. 2 and 3. The turbidity curves of several fractions from bovine palatine tonsils are shown in Fig. 2. The ionic strength effect can be seen in Fig. 3. The electrophoretic patterns of the corresponding fractions are given in Fig. 4. In this instance the turbidity curves are much more revealing than are the electrophoretic patterns. For example, compare 3.0S curve in Fig. 2 and the electrophoresis pattern in Fig. 4 C.

The various fractions listed in Fig. 2 are related as outlined below. Fraction 5.1P, the crude starting material in this report, is the equivalent of Ppt.B in the fractionation scheme of a preceding publication.[5] It is of interest that although 5.1P shows a fairly symmetrical pattern at *p*H 8.6, μ = 0.10, veronal buffer in the Tiselius apparatus this fraction is shown to contain at least four separate components. 5.1P is separated at *p*H 3.0 and μ = 0.10 into a precipitate (3.0P) amounting to 70% of 5.1P. The supernatant (3.0S) constitutes the remaining 30% of 5.1P. 3.0S is separated at *p*H 6.2 and μ = 0.0 into a precipitate (6.2P) amounting to 80% of 3.0S while the supernatant (6.2S) constitutes the remaining 20% of the 3.0S fraction.

The choice of *p*H 6.2 and an ionic strength of zero for the separation of 3.0S was made from inspection of the two 3.0S curves shown in Fig. 3. Additional

(1) *This report represents work done under contract with the* U. S. Atomic Energy Commission. Project No. 6 to Contract AT (11-1)89 with Northwestern University.

(2) P. A. Kober, *J. Ind. Eng. Chem.*, **10**, 556 (1918).

(3) J. F. Morse, *Analyst*, **62**, 11 (1937).

(4) H. A. Wannow, *Koll.-Z.*, **87**, 311 (1939).

(5) E. L. Hess, W. Ayala and A. Herranen, THIS JOURNAL, **74**, 5419 (1952).