

rated. The residue was crystallized from methanol, m. p. 179–180°, yield 1.5 g.

*Anal.* Calcd. for  $C_{23}H_{34}O_4$ : C, 73.7; H, 9.2. Found: C, 73.8; H, 9.1.

The above material, 500 mg., was hydrolyzed by boiling with 250 mg. of sodium hydroxide in 50 cc. of ethanol. The neutral fraction melted at 223–225° after crystallization from aqueous methanol.

*Anal.* Calcd. for  $C_{21}H_{32}O_3$ : C, 75.8; H, 9.7. Found: C, 75.5; H, 9.6.

The hydrolysis product could be reacylated with hot acetic anhydride to give the original acetate melting at 179–180°.

**Treatment of  $\Delta^{16}$ -allo-Pregnenol-3( $\beta$ )-one-20 Acetate with Hydrogen Peroxide.**—A mixture of 5 g. of  $\Delta^{16}$ -allo-pregnenol-3( $\beta$ )-one-20 acetate, 250 cc. of acetic acid, and 50 cc. of 30% hydrogen peroxide was heated to 70° for five hours. The reaction mixture was worked up as described for pregnenolone acetate. The neutral material melted at 185–186° after crystallization from methanol, yield 2.5 g.

*Anal.* Calcd. for  $C_{23}H_{34}O_4$ : C, 73.7; H, 9.2. Found: C, 73.8; H, 9.0.

Hydrolysis of this material with alcoholic sodium hydroxide gave a product which melted at 181–182° after crystallization from methanol. This substance gave a large depression in melting point when mixed with the acetate, m. p. 185–186°.

*Anal.* Calcd. for  $C_{21}H_{32}O_3$ : C, 75.8; H, 9.7. Found: C, 75.5; H, 9.6.

Reacylation with hot acetic anhydride gave the original acetate melting at 185–186°.

SCHOOL OF CHEMISTRY AND PHYSICS  
THE PENNSYLVANIA STATE COLLEGE  
STATE COLLEGE, PENNA.

RECEIVED APRIL 1, 1941

### Crystalline Avidin

BY DERROL PENNINGTON, ESMOND E. SNELL AND ROBERT E. EAKIN

Using essentially the procedures described in a previous communication for obtaining concentrated material,<sup>1</sup> we have obtained the biotin-inactivating protein, avidin, in crystalline form. Highly concentrated material obtained by this means was dissolved (50 mg. per cc.) in one-half saturated ammonium sulfate solution, at room temperature, and saturated ammonium sulfate solution was added to incipient precipitation. After centrifuging out this first turbidity, the clear solution was placed in a refrigerator. Within an hour, the avidin precipitated in the form of fine needle-like crystals. When care was taken that the crystallization proceeded more slowly, larger plates (Fig. 1) were obtained. Both forms were equally active. Such material was recryst-

(1) Eakin, Snell and Williams, *J. Biol. Chem.*, **140**, 535–543 (1941).



Fig. 1.—Avidin crystals magnified 1000 times.

tallized repeatedly by the same method and retained high activity. For assay purposes, salt-free avidin was obtained by dissolving the crystallized material in dilute salt solution, and dialyzing the solution against distilled water until the avidin was completely precipitated.

The crystallization procedure was somewhat destructive to the activity of the avidin. The potency of three times recrystallized material was approximately 4000 units per gram, while the most active amorphous material previously obtained had a potency of approximately 7000 units per gram. Crystals which stood in the refrigerator in contact with the mother liquor lost approximately three-fourths of their activity in three weeks. Dry crystals which stood for three months at summer temperature lost no activity.

Analyses on two independent batches of crystallized avidin gave the following results:

	C	H	N	S	Residue
Sample 1	43.72	7.60	12.10	1.32	2.09
Sample 2	44.26	7.28	12.83	...	0.75

Both samples gave a positive Molisch test for carbohydrate, and the analyses indicate that the substance may be a protein with a large carbohydrate moiety. Further study of the substance will await its production on a large scale.

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF TEXAS  
AUSTIN, TEXAS

RECEIVED OCTOBER 11, 1941

### An Improved Method for the Preparation of Benzenediazonium Salts

BY WILLIAM SMITH AND CHAS. E. WARING

The usual method of preparing diazo salts is that given by Hantzsch and Jochem.<sup>1</sup> Essen-

(1) Hantzsch and Jochem, *Ber.*, **34**, 3337 (1901).