in Table I were calculated using the data corresponding to those conditions under which this effect should be at a minimum, and even when the maximum possible error is assumed, the values for the energy of activation in the presence and in the absence of a catalyst are 7500 and 4300 cal./mole, respectively. For the ammonolysis of pilocarpine, ethyl benzoate, and 2-chlorobenzothiazole, data on the concentrations used, the reproducibility of results, etc., may be found in earlier publications.[1,3,4] Too much dependence may not be placed upon the results for ethyl benzoate because data on the energy of activation in the absence of a catalyst are not available.

Additional experimental studies on the rate and order of these reactions under conditions in which the concentration of the ester or halide and, especially, the concentration of catalyst are varied more widely will be necessary before definite conclusions may be drawn as to the nature of the effect involved. Nevertheless, although the absolute values of the energies of activation may be questionable in some cases, it is significant that for all ammonolytic reactions in liquid ammonia for which data are now available, the addition of electrolytes appears consistently to increase the energy of activation.

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TEXAS
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RECEIVED JULY 21, 1941

Sterols. CXXXII. Sapogenins. LIV. The Action of Hydrogen Peroxide on the Pseudosapogenin Acetates and on the Pregnenolones

BY RUSSELL E. MARKER, ELDON M. JONES AND EMERSON L. WITTBECKER

Hydrogen peroxide in acetic acid reacts upon pseudosarsasapogenin[1] to give a neutral compound of the formula $C_{27}H_{44}O_5$. Under similar conditions sarsasapogenin acetate[1] gives pregnanetriol-3(β),16,20, resulting from rupture of the side-chain between C_{20} and C_{22}. We have treated pseudosarsasapogenin diacetate in acetic acid with hydrogen peroxide and have obtained Δ^{16}-pregnenol-3(β)-one-20 acetate, which was produced by splitting of the side-chain between C_{20} and C_{22}. Likewise, pseudotigogenin diacetate gave Δ^{16}-*allo*-pregnenol-3(β)-one-20 acetate. The reaction of hydrogen peroxide in acetic acid upon Δ^{16}-pregnenol-3(β)-one-20 acetate and Δ^{16}-*allo*-pregnenol-3(β)-one-20 acetate gave a good yield of an unidentified crystalline product. These experiments show that the pregnene compounds are not formed as a direct oxidation product of the pseudosapogenins, but that an intermediate is first formed which requires hydrolysis to give the pregnene derivative.

We wish to thank Parke, Davis and Company for their generous help.

Experimental Part

Treatment of Pseudosarsasapogenin Diacetate with Hydrogen Peroxide.—Pseudosarsasapogenin, 3 g., was converted to the acetate by refluxing with acetic anhydride. The residue from the evaporation of the excess anhydride was dissolved in 200 cc. of acetic acid, and 30 cc. of 30% hydrogen peroxide was added. The mixture was heated to 70° for five hours, then concentrated by evaporation *in vacuo* and poured into water. The mixture was extracted with ether and the extract was washed with water. The ether was evaporated and the residue was hydrolyzed with boiling methanolic potassium hydroxide. The neutral fraction melted at 180–183° after crystallization from aqueous methanol.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 7.97; H, 10.2. Found: C, 79.7; H, 10.2.

With boiling acetic anhydride this substance gave an acetate which melted at 140–141° after crystallization from aqueous methanol. It gave no depression in melting point when mixed with an authentic sample of the acetate of Δ^{16}-pregnenol-3(β)-one-20.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.0; H, 9.6. Found: C, 77.0; H, 9.4.

Treatment of Pseudotigogenin Diacetate with Hydrogen Peroxide.—A mixture of 10 g. of pseudotigogenin diacetate, 50 cc. of 30% hydrogen peroxide, and 500 cc. of acetic acid was heated to 70° for six hours. Water was added and the mixture was extracted with ether. The extract was washed with water and the ether was evaporated. The residue was hydrolyzed with hot methanolic potassium hydroxide, poured into water, and extracted with ether. The extract was washed with water and the ether was evaporated. The residue was refluxed with excess acetic anhydride and the excess solvent was evaporated *in vacuo*. The residue was crystallized from methanol and the first fraction, which appeared to be a mixture, was discarded. The second fraction melted at 159–162° and did not depress the melting point of an authentic sample of the acetate of Δ^{16}-*allo*-pregnenol-3(β)-one-20.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.0; H, 9.6. Found: C, 77.1; H, 9.7.

Treatment of Δ^{16}-Pregnenol-3(β)-one-20 Acetate with Hydrogen Peroxide.—A mixture of 5 g. of Δ^{16}-pregnenol-3(β)-one-20 acetate, 250 cc. of acetic acid, and 50 cc. of 30% hydrogen peroxide was heated to 70° for five hours. The solvent was partially evaporated *in vacuo* and the remainder was dissolved in ether. The solution was washed with water and dilute sodium carbonate solution. Acidification of the alkaline wash gave no acids. The ether solution was washed with water and the ether was evapo-

(1) Marker, Jones and Krueger, THIS JOURNAL, **62**, 2532 (1940).

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 reacetylated with hot acetic anhydride to give the original acetate melting at 179–180°.

Treatment of Δ^{16}-*allo*-Pregnenol-3(β)-one-20 Acetate with Hydrogen Peroxide.—A mixture of 5 g. of Δ^{16}-*allo*-pregnenol-3(β)-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.

Reacetylation 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,[1] 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 recrystallized 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.

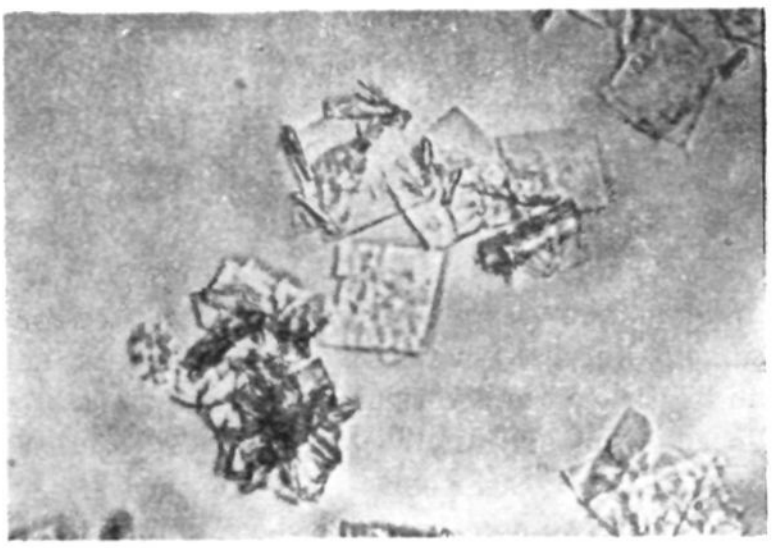

Fig. 1.—Avidin crystals magnified 1000 times.

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.[1] Essen-

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

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