a rate as to keep the pH in the range 6.5–6.8. Evaporating the resulting solution to dryness, washing with 5–10 ml. of water to remove sodium chloride, and recrystallizing twice from benzene gave 3 g. (32%) of IV melting at 109–110° (lit.[2] 110°).

Anal. Calcd. for C_2H_4ClNO: C, 25.6; H, 4.3; N, 15.0; Avail. Cl, 75.9. Found: C, 25.7; H, 4.3; N, 15.6; Avail. Cl, 75.0.

Chlorination in the *p*H range 7–8.5 did not produce any material containing available chlorine. Chlorination at a *p*H of 5 caused a yellow oil to separate. The oil was destroyed by the addition of excess sodium hydroxide solution and was not studied further.

Chlorination of barbituric acid with pH control. Barbituric acid (2.6 g., 0.02 mol.) suspended in water (200 ml.) was treated with chlorine (4 g.) over a 1-hr. period while either *N* sodium hydroxide or sodium bicarbonate was added at such a rate as to keep the *p*H in the range 5.9–6.1. The resulting solution was evaporated *in vacuo* to about 5 ml. and filtered to remove a small amount of solid. The solid (1 g., m.p. 149–151°) was recrystallized from water to give white, crystalline α,α-dichloroacetylurea (X) (0.6 g.) melting at 155–156°. The lit.[12] reports X to melt at 151°.

Anal. Calcd. for $C_3H_4Cl_2N_2O_2$: C, 21.0; H, 2.6; N, 16.4; M.W. 171. Found: C, 21.1; H, 2.3; N, 16.4; M.W. 175.

The infrared spectrum was identical with that of an authentic sample of X.

Evaporation of the original aqueous filtrate gave a mixture of sodium chloride and some tacky white solid. The mixture contained 10–11% available chlorine but attempts to isolate a discrete compound containing available chlorine by extraction with a variety of solvents were unsuccessful.

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Stereospecific Reduction of Prednisone by Alfalfa Seedlings

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The ability to transform steroids in submerged culture is very widespread in the world of microorganisms. Bacteria, yeast, actinomycetes, filamentous fungi, and protozoa have been reported to perform various types of reactions without apparent taxonomic limitations.[1]

It became of interest to us to study the activities of some higher plants in this regard. We have now found that alfalfa seedlings (*Medicago sativa*) grown in shake culture will bring about the reduction of the 20-keto group in prednisone to the 20α-hydroxyl group, yielding thereby 1,4-pregnadiene-17α,20α,21-triol-3,11-dione. The product was characterized by conversion to its 20,21-diacetate, which was compared and found identical with an authentic sample.[2]

The conversion does not occur unless the seedlings are growing. Attempted transformation with seedlings killed by autoclaving with 15-lb. steam at 121° for 15 minutes effected no change in the steroidal substrate. In an experiment in which the alfalfa seedlings were disrupted in a Waring Blendor prior to the addition of prednisone, extraction and chromatography revealed what appeared to be only a trace of product; the starting material appeared essentially undiminished.

Reduction of the 20-carbonyl group to the corresponding 20α-hydroxyl group is found infrequently outside mammalian systems. Only two such conversions have been reported with microorganisms, one incidental to a more complex Wagner-Meerwein rearrangement with an unidentified yeast,[3] employing 16α,17α-oxidoprogesterone as substrate, and the other with the yeast *Rhodotorula longissima*,[2] wherein reduction at 20- was the only identified transformation with Reichstein's Compound S, cortisone and prednisone as substrates.

EXPERIMENTAL

Two-gram quantities of alfalfa seed (*Medicago sativa*) were surface sterilized by immersing for 30 min. in 100 ml. of a 1% solution of sodium hypochlorite. The sodium hypochlorite solution was decanted and the seeds were rinsed 3 times with 100-ml. aliquots of sterile water. After the final rinse, the seeds were resuspended in 100 ml. of sterile tap water in 300-ml. Erlenmeyer flasks and placed on a reciprocal shaker operating at one hundred 1.5-in. strokes per min. Four 75-watt frosted bulbs were used as a constant light source. Temperature was maintained at 28°.

After 96 hr., when the seedlings generally had attained a length of 10 to 20 mm., 10 mg. of prednisone dissolved in 1 ml. of dimethylformamide was added to each flask. Directly before the addition of the steroid, a sample of the water in the flasks was taken and checked microscopically or added to sterile broth as a sterility control. Ninety-six hr. after addition of the steroid the aqueous contents of the flasks were decanted and extracted with chloroform in order to recover the steroid.

By paper chromatography in the Shull system[4] it was estimated that at the 10-mg. level all but a very small amount of the starting material was converted to the product.

The combined extracts of a series of flasks from the 96-hr. run were chromatographed over 10 g. of Florisil and eluted with methylene chloride and methylene chloride–methanol mixtures. Unreacted prednisone was eluted with 1% methanol-in-methylene chloride. A series of semisolid fractions from 3% and 4% methanol-in-methylene chloride were free of prednisone as shown by paper chromatography in Shull's system[4] and consisted predominantly of a single, ultraviolet-absorbing, TPTZ-negative product with a migration rate the same as 1,4-pregnadiene-17α,20α,21-triol-3,11-dione. These fractions were pooled and acetylated with 1 ml. of acetic anhydride in 1 ml. of pyridine overnight. Water precipitation afforded 20 mg. of 1,4-pregnadiene-17α,20α,21-triol-3,11-dione 20,21-diacetate, m.p. 245–249°, the infrared spectrum of which was identical with that of an authentic sample.[2]

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When 8 mg. of starting material were added, the transformation appeared to go to completion in the 96-hr. period. Chromatographic analysis of a 17-day aliquot indicated that the transformation at the 10-mg. level had gone to completion and that further changes had not occurred.

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Synthesis of 1,4-Pregnadiene-17α,20α,21-triol-3,11-dione

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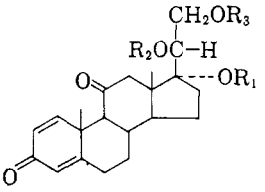
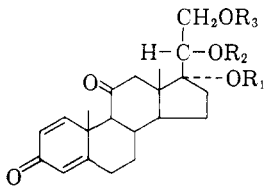
In the metabolism of adrenocortical hormones reduction of the C_{20}-ketone to a 20α- and (or) 20β-ol is frequently encountered.[1-5] After administration of prednisone or prednisolone to humans, the 20-dihydro derivatives (Δ^1-Reichstein's Substance U and E) appear to be important metabolites.[6-8] In addition A-ring reduced compounds may be produced.[8]

We have been interested in the synthesis of these 17,20,21-triols because of their metabolic significance and because of their glycogen deposition properties.[9,10] Like Szpilfogel and co-workers we also had found the *20β-ols* to have glucocorticord activity. For this reason we undertook the synthesis of a *20α-ol* such as 1,4-pregnadiene-17α,20α,21-triol-3,11-dione, which was suggested to be one of the urinary metabolites isolated after administration of prednisone to humans.[5]

Selective reduction of prednisone with sodium borohydride[9,11] yielded Δ^1-Reichstein's U 21-monoacetate (Ia). The general method of Fukushima *et al.*[12] was used to invert the configuration at C_{20} from β to α as follows: mesylation gave the 20-mesylate (Ib); acid-catalyzed acetylation formed the 17,21-diacetate-20-mesylate (Ic); internal displacement of the 20-mesylate by the 17-acetate function *with inversion at* C_{20} yielded a 20,-21-diacetate in the 20α-series (IIa); alkaline hydrolysis of the acetate groups afforded 1,4-pregnadiene-17α,20α,21-triol-3,11-dione (IIb).



Ia. R_1 = R_2 = H, R_3 = CH_3CO
b. R_1 = H, R_2 = CH_3SO_2, R_3 = CH_3CO
c. R_1 = R_3 = CH_3CO, R_2 = CH_3SO_2



IIa. R_1 = H, R_2 = R_3 = CH_3CO
b. R_1 = R_2 = R_3 = H

An attempt to apply this method to the preparation of the 11β-hydroxy analog of IIb failed due to acetylation of the 11-hydroxyl group during the acid-catalyzed C_{17}-acetylation stage. We were not able to remove this 11-acetate even with sodium methoxide.

In contrast to the reported[9] glucocorticoid activity of the corresponding 20β-ol, compound IIb was inactive in the liver glycogen test.[13]

EXPERIMENTAL[14]

1,4-Pregnadiene-17α,20β,21-triol-3,11-dione-20-mesylate-21-acetate (Ib). One gram of 1,4-pregnadiene-17α,20β,21-triol-3,11-dione-21-acetate (Ia)[9] was dissolved in 3.0 ml. of pyridine and cooled in an ice bath. To this solution was added 1.5 ml. of methanesulfonyl chloride. After standing at room temperature for 2 hr. the reaction mixture was poured into water. The resultant solid was filtered and recrystallized from methanol to give 920 mg. of the desired 20-mesylate (Ib), m.p. 173–178° (dec.).

Anal. Calcd. for $C_{24}H_{32}O_8S$: C, 59.99; H, 6.71. Found: C, 60.52; H, 6.61.

1,4-Pregnadiene-17α,20β,21-triol-3,11-dione-20-mesylate-17,21-diacetate (Ic). To 920 mg. of the mesylate (Ib) was added 16 ml. of acetic acid, 8 ml. of acetic anhydride, and 160 mg. of *p*-toluenesulfonic acid. After standing at room temperature for 3 days the mixture was poured into ice and water which contained 1.0 ml. of pyridine. It was then extracted 4 times with ethyl acetate, washed with 5% hydrochloric acid, water, and saturated sodium bicarbonate solution. The extract was dried and evaporaged under reduced pressure to yield 1.06 g. of an oil (Ic) suitable for the next step.

1,4-Pregnadiene-17α,20α,21-triol-3,11-dione-20,21-diacetate (IIa). To 1.0 g. of Ic above, 150 ml. of 96% acetic acid, and 7.5 ml. of acetic anhydride was added 15 g. of potassium acetate. This reaction mixture was heated under reflux for 3 hr. and then poured into ice water containing 1 ml. of pyridine. This was extracted thrice with ethyl ace-

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