

pyrans. Good yields of 2,6-dialkoxy-3-(1-alkoxy-alkyl)tetrahydropyrans are obtained.

The latter compounds are readily converted by known methods to 2-alkylideneglutaraldehydes and 2-alkyl-1,5-pentanediois. Since many 2-alkoxy-3,4-dihydro-2*H*-pyrans are obtainable,<sup>2</sup> the reactions described here make available a wide variety of 2-alkylideneglutaraldehydes and their derivatives.

## EXPERIMENTAL

**2,6-Diethoxy-3-(1-ethoxy-2-methylpropyl)tetrahydropyran.** Isobutyraldehyde diethyl acetal, 219 g. (1.5 moles), was combined with 3 ml. of boron trifluoride etherate and cooled to 5°. Over a 45-min. period, 2-ethoxy-3,4-dihydro-2*H*-pyran, 128 g. (1 mole), was added with stirring and cooling to maintain the temperature at 5–10°. Stirring was continued for 30 min. without cooling while the temperature rose to 18°. The catalyst was neutralized by adding a solution of 20 g. of potassium carbonate in 30 ml. of water, and the organic phase was separated and distilled. After recovery of 73 g. of isobutyraldehyde diethyl acetal, 174 g. (63.5%) of 2,6-diethoxy-3-(1-ethoxy-2-methylpropyl)tetrahydropyran, b.p. 86–89° (ca. 1 mm.),  $n_D^{20}$  1.4400, was obtained.

*Anal.* Calcd. for  $C_{15}H_{26}O_4$ : C, 65.66; H, 11.02. Found: C, 65.54; H, 10.98.

Treatment of the product with 2,4-dinitrophenylhydrazine reagent gave the bis(2,4-dinitrophenylhydrazone) of 2-isobutylideneglutaraldehyde, m.p. 217–219°.

*Anal.* Calcd. for  $C_{21}H_{22}N_8O_8$ : C, 49.03; H, 4.31. Found: C, 49.06; H, 4.52.

**2,6-Diethoxy-3-(1-ethoxypropyl)tetrahydropyran.** Addition of 2-ethoxy-3,4-dihydro-2*H*-pyran, 250 g. (2 moles), to propionaldehyde diethyl acetal, 290 g. (2.2 moles), containing 2 ml. of boron trifluoride etherate, as described above, gave 405 g. (78%) of 2,6-diethoxy-3-(1-ethoxypropyl)tetrahydropyran, b.p. 79–82° (ca. 1 mm.),  $n_D^{20}$  1.4378.

*Anal.* Calcd. for  $C_{14}H_{26}O_4$ : C, 64.58; H, 10.84. Found: C, 64.39; H, 10.96.

**2,6-Diethoxy-3-(1-ethoxybutyl)tetrahydropyran.** This compound (b.p. 93–95° at ca. 1 mm.,  $n_D^{20}$  1.4389) was obtained in 64% yield from butyraldehyde diethyl acetal as described above.

*Anal.* Calcd. for  $C_{15}H_{30}O_4$ : C, 65.66; H, 11.02. Found: C, 65.48; H, 11.12.

**2-Butyl-1,5-pentanediol.** A mixture of 500 ml. of *p*-dioxane, 88.5 ml. of water, 17.5 ml. of concentrated hydrochloric acid, and 250 g. (0.92 mole) of 2,6-diethoxy-3-(1-ethoxybutyl)tetrahydropyran was stirred at 45° until it became homogeneous, and then stirred for an additional 4 hr.<sup>3</sup> The mixture was then neutralized by the addition of solid sodium bicarbonate and filtered. The filtrate was subjected to hydrogenation over Raney nickel at 150° and 100 atm. The catalyst was removed by filtration, and the filtrate was distilled to obtain 107.5 g. (73%) of 2-butyl-1,5-pentanediol, b.p. 105–109° (ca. 1 mm.),  $n_D^{20}$  1.4603.

*Anal.* Calcd. for  $C_9H_{20}O_2$ : C, 67.45; H, 12.57. Found: C, 67.28; H, 12.32.

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(2) See, for example, R. I. Longley, Jr., and W. S. Emerson, *J. Am. Chem. Soc.*, **72**, 3079 (1950).

(3) R. I. Longley, Jr., W. S. Emerson, and T. C. Shafer, *J. Am. Chem. Soc.*, **74**, 2012 (1952).

## Chlorination of 1,3-Diacetylurea and of Barbituric Acid

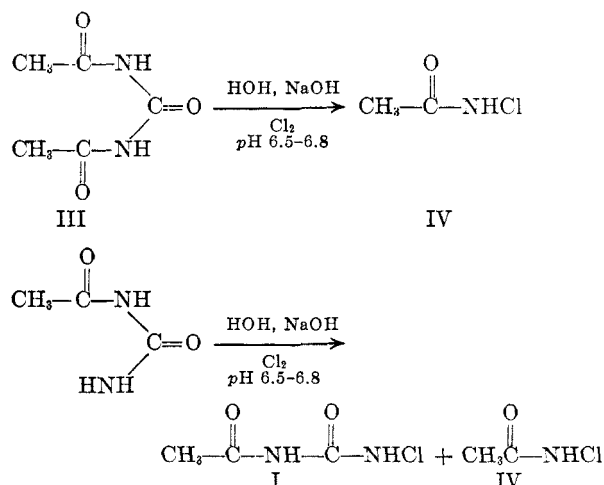
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1-Acetyl-3-chlorourea (I) is readily made by the chlorination of acetylurea in aqueous solution.<sup>1</sup> The compound decomposes with considerable vigor at 155–156°. It was thought that 1,3-diacetyl-1,3-dichlorourea (II) might be reasonably stable by virtue of the fact that the chlorine would be bound to imidic nitrogen atoms.

Attempts were made to prepare II by the chlorination of 1,3-diacetylurea (III) in an aqueous phase over a variety of *pH* ranges. Saturation of an aqueous solution of III with chlorine but without *pH* control gave recovered III as the only identifiable product. When III was chlorinated in such a fashion that the *pH* was maintained in the range 7–9 during the reaction, no product containing available chlorine was detected. The available chlorine containing product resulting from chlorination at *pH* 6–7 was shown to be *N*-chloroacetamide (IV).<sup>2–6</sup> Maintaining the *pH* at about 5 resulted in the formation of a yellow oil which was immediately deliberately destroyed by the addition of excess sodium hydroxide solution. The yellow oil may have been the explosive nitrogen trichloride or the oily, yellow *N,N*-dichloroacetamide.<sup>4</sup>

Saturation with chlorine, of an aqueous solution of acetylurea without *pH* control resulted in the formation of minute droplets of yellow oil which exploded upon being subjected to mechanical



(1) F. D. Chattaway and D. F. S. Wunsch, *J. Chem. Soc.*, **95**, 129 (1909).

(2) A. W. Hoffmann, *Ber.*, **15**, 410 (1882).

(3) C. Mauguin, *Annales de Chimie*, [8] **22**, 305 (1911).

(4) E. Boismenu, *Annales de Chimie*, [9] **9**, 161 (1918).

(5) K. J. P. Orton and A. E. Bradfield, *J. Chem. Soc.*, 993 (1927).

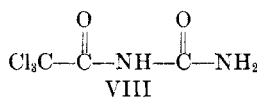
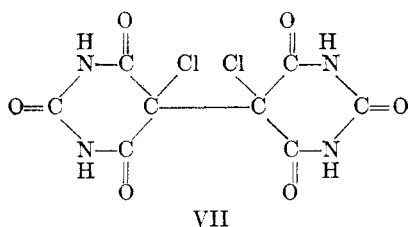
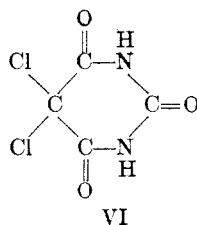
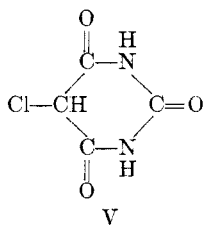
(6) G. H. Coleman, R. L. Peterson, and G. F. Goheen, *J. Am. Chem. Soc.*, **58**, 1874 (1936).

shock. Chlorinating an aqueous solution of acetylurea in the pH range 6.5–6.8 gave a mixture from which I and IV were isolated.

The chlorination of III gave IV in a relatively clean-cut manner while the chlorination of monoacetylurea, under similar conditions, gave a mixture of I and IV. Thus, one might conclude that chlorination of III does not necessarily pass through a monoureide as one might expect from the report<sup>7</sup> of the ease of hydrolysis of 1,3-diacetylureas to the monoureide and fatty acid salt.

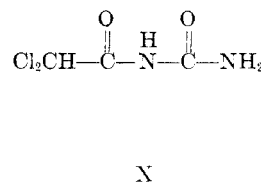
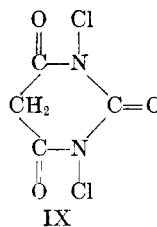
It is possible that as sodium hydroxide solution (to control pH) is dropped into the aqueous solution or suspension into which chlorine is being passed simultaneously, a localized, low concentration of sodium hypochlorite forms. This dilute sodium hypochlorite may cleave III directly to give IV. Free hypochlorous acid formed should leave III unaffected. Under similar circumstances, monoacetylurea could be cleaved by sodium hypochlorite to give the observed IV while hypochlorous acid formed could give I.

The literature reveals that the aqueous phase chlorination of barbituric acid results in the formation of 5-chlorobarbituric acid (V),<sup>8,9</sup> 5,5-dichlorobarbituric acid (VI),<sup>8-11</sup> 5,5'-dichlorohydurilic acid (VII)<sup>9</sup> and trichloroacetylurea (VIII).<sup>9</sup> Since there was no reported attempt to control pH in these aqueous chlorinations, it is reasonable to assume that they were carried out under quite acidic conditions.



We carried out a series of aqueous phase chlorinations of barbituric acid under conditions of controlled pH (the over-all span studied was pH

5.5–8.5) in an attempt to prepare *N,N'*-dichloro-barbituric acid (IX). However, the only material



isolated from the reaction was  $\alpha,\alpha$ -dichloroacetylurea (X), which, though known,<sup>12</sup> is a heretofore unreported product of the chlorination of barbituric acid.

#### EXPERIMENTAL<sup>13,14</sup>

*1-Acetyl-3-chlorourea* (I).<sup>1</sup> Acetylurea was chlorinated in water to give a white solid, m.p. 156–157°, accompanied by a sharp "pop".

*Anal.* Calcd. for  $C_3H_5ClN_2O_2$ : Avail. Cl, 52. Found: Avail. Cl, 51.7.

*Overchlorination of acetylurea.* A solution of acetylurea (0.3 g.) in water (20 ml.) at room temperature was saturated with chlorine. After standing 1 hr. a trace of solid and minute oily, yellow droplets were observed. Upon being touched with a stirring rod the oil exploded. It is believed to have been nitrogen trichloride.

*Chlorination of acetylurea with pH control.* Chlorine (4 g.) was added over a 30-min. period to a chilled (5–10°), stirred solution of acetylurea (2.0 g, 0.02 mole) in water (70 ml) while *N* sodium hydroxide solution was added at such a rate as to maintain the pH of the mixture in the range 6.6–6.8. The pH was measured by having the electrodes of a Beckman, model H-2, pH meter immersed in the solution during the chlorination. Evaporation, *in vacuo*, gave 2.5 g. of solid. Extraction of the solid with three 5 ml. and one 10 ml. portions of hot benzene gave, after removal of the benzene, 0.08 g. of white solid. Two further recrystallizations from benzene left 0.02 g. of material, m.p. 107–108°. A mixture with *N*-chloroacetamide (IV) (m.p. 109–110°) melted at 107–108.5°.

*Anal.* Calcd. for  $C_2H_4ClNO$ : Avail. Cl, 75.9. Found: Avail. Cl, 72.6.

The residue from the benzene extraction was extracted with 100 ml. of chloroform. Removal of the chloroform left 0.07 g. of material, m.p. 148–149°, containing 42% available chlorine and having properties encountered in other samples of impure I.

*Attempted preparation of 1,3-diacetyl-1,3-dichlorourea* (II). A suspension of III (3.5 g.) in water (60 ml.) was saturated with chlorine over a period of 1 hr. The solution was extracted with four 50-ml. portions of chloroform. Removal of the chloroform gave 2.1 g. (60%) of recovered III, m.p. 153–155°. The mixture m.p. with an authentic sample was 154–156°. No available-chlorine-containing material was observed.

*Chlorination of diacetylurea with pH control.* Chlorine (15 g., 0.21 mol.) was passed, over a 30–40 min. period, into a suspension of III (14.4 g., 0.1 mole) in 125 ml. of water while 3 *N* sodium hydroxide solution was added at such

(12) I. A. Pearl and W. H. Dehn, *J. Am. Chem. Soc.*, **61**, 13 (1939).

(13) All melting points are uncorrected.

(14) Elemental and infrared analyses by the Diamond Alkali Co. Research Analytical Laboratory. Available chlorine determinations are by sodium thiosulfate titration. The percent available chlorine is taken as twice the weight percent of chlorine attached to nitrogen.

(7) R. W. Stoughton, *J. Org. Chem.*, **2**, 514 (1938).

(8) H. Biltz and T. Hamburger, *Ber.*, **49**, 642 (1916).

(9) W. Bock, *Ber.*, **56**, 1224 (1923).

(10) R. Behrend, *Ann.*, **236**, 64 (1886).

(11) A. K. Macbeth, T. H. Numan, and D. Traill, *J. Chem. Soc.*, 1251 (1926).

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.<sup>2</sup> 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 pH range 7–8.5 did not produce any material containing available chlorine. Chlorination at a pH 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 pH 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  $\alpha, \alpha$ -dichloroacetylurea (X) (0.6 g.) melting at 155–156°. The lit.<sup>12</sup> 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.<sup>1</sup>

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 20 $\alpha$ -hydroxyl group, yielding thereby 1,4-pregnadiene-17 $\alpha, 20\alpha, 21$ -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.<sup>2</sup>

(1) E. Vischer and A. Wettstein, *Advances in Enzymol.*, **XX**, 237 (1958); S. C. Beesch and Fred W. Tanner, Jr., *Ind. Eng. Chem.*, **50**, 1341 (1958); A. Wettstein, *Experientia*, **XI**, No. 12, 465 (1955).

(2) F. Carvajal, O. F. Vitale, M. J. Gentles, H. L. Herzog, and E. B. Hershberg, *J. Org. Chem.*, **24**, 695 (1959).

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 20 $\alpha$ -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,<sup>3</sup> employing 16 $\alpha, 17\alpha$ -oxidoprogesterone as substrate, and the other with the yeast *Rhodotorula longissima*,<sup>2</sup> 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<sup>4</sup> 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<sup>4</sup> and consisted predominantly of a single, ultraviolet-absorbing, TPTZ-negative product with a migration rate the same as 1,4-pregnadiene-17 $\alpha, 20\alpha, 21$ -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-17 $\alpha, 20\alpha, 21$ -triol-3,11-dione 20,21-diacetate, m.p. 245–249°, the infrared spectrum of which was identical with that of an authentic sample.<sup>2</sup>

(3) B. Camerino and A. Vercellone, *Gazz. chim. ital.*, **86**, 260 (1956).

(4) G. M. Shull, Abstracts of Papers, 126th Meeting of the American Chemical Society, September 1954, New York, p. 9A.