basic material. The neutralized solution was then distilled until all of the isopropyl alcohol had been removed. The distillate was assayed for the alcohol by comparison of its refractive index with that of mixtures of known composition. It was found to contain 3.1 g. (51.7 millimoles) of the alcohol. The excellent yield (88%) of alcohol demonstrates that the starting material was O-substituted. The basic fraction (22%) must have consisted, at least in part, of ammonia derived from the breakdown of cyanuric acid, although it may have contained a maximum of 12% of isopropylamine resulting from rearrangement during the prolonged heating period.

Similar results were obtained with *n*-propyl and *s*-amyl cyanurates. In these instances refluxing was continued for shorter periods of time and only traces of base were found. In a 10-g. run of *s*-amyl cyanurate, distillation of ether-extracted material gave 4.8 g. of *s*-amyl alcohol, b.p. 110–118°, $n^{20}D$ 1.4052 (lit. b.p. 119°, $n^{20}D$ 1.4053).

NORTH CHICAGO, ILLINOIS RECEIVED OCTOBER 9, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Partial Synthesis of Reichstein's Substance E[1]

BY L. H. SARETT, MAX FEURER AND KARL FOLKERS

The partial synthesis of Reichstein's Substance E, 4-pregnene-11β,17α,20β,21-tetrol-3-one, has been accomplished *via* two routes: A, from pregnane-3α,17α,20β,21-tetrol-3-one 20,21-acetonide by reduction of the 11-ketone with lithium aluminum hydride, partial oxidation by the Oppenauer method and introduction of the 4,5-double bond; B, by reduction of the 3-enol ether of cortisone acetate.

The characterization of 4-pregnene-11β,17α,20β,-21-tetrol-3-one (VI), Reichstein's Substance E, and its isolation from the adrenal glands of cattle have been reported by Reichstein[2] and Reichstein and von Euw.[3] The present paper describes the partial synthesis of this compound from pregnane-3α,17α,-20β,21-tetrol-11-one[4] and also from cortisone acetate. The former route proceeded *via* the 20,21-acetonide[4] (I) which with lithium aluminum hydride was reduced with a high degree of stereospecificity to pregnane-3α,11β,17α,20β,21-pentol 20,21-acetonide (II). The steric course of this reduction is thus the same as that of catalytic hydrogenation.[5] Extending the views of Trevoy and Brown[6] on the mechanism of reduction with lithium aluminum hydride to the present case, it is apparent that the reagent finds free entrance to the C-11 position only from the rear of the molecule. This result parallels the findings of Ott and Murray[7] who obtained estradiol-17β from estrone in high yield and those of Shoppee and Summers[8] who noted that coprostanone and cholestanone are reduced in a sterically unique sense. In contrast, cholestane-3β-ol-7-one showed no steric selectivity on reduction to the diol with lithium aluminum hydride.[9]

The partial oxidation of the pentol acetonide (II) to the desired tetrol-3-one acetonide (III) was accomplished through an Oppenauer oxidation.[10] It is interesting parenthetically that the rate of oxidation of the 11β-hydroxyl group with substances capable of liberating hypobromous acid, such as N-bromoacetamide, is greater than that of the 3α-hydroxyl (A/B *cis*). This result may be compared with the recorded observation[9] that the rate of oxidation of an hydroxyl group at the 11β-position with chromic acid is much greater than that at the 20β-position. It appears probable from these results and from those of Fieser and Rajagopalan,[11] who investigated the partial oxidation of various polyhydroxy steroids with N-bromosuccinimide that the oxidation rate of a given secondary hydroxyl group is far more dependent on the stability of the intermediate hypobromite ester (or chromic ester) than on the degree of steric hindrance of the hydroxyl group, as measured by its accessibility to acylating agents.

Hydrolysis of the acetonide (III) with warm dilute acetic acid yielded the free tetrolone (IV), from which the diacetate (V) was readily prepared. Bromination of the latter afforded an amorphous 4-bromo derivative, condensation of which with dinitrophenylhydrazine gave the dinitrophenylhydrazone[12] of Substance E diacetate, also an amorphous powder. However, chromatography of the crude pyruvic acid[12] hydrolysis product yielded a compound the properties of which agreed with those of Substance E diacetate (VII).

Substance E could also be obtained from cortisone acetate in three steps. The α,β-unsaturated carbonyl group was protected by formation of an enol ether[13] (VIII). Reduction with lithium aluminum hydride then afforded $\Delta^{3,5}$-3-ethoxypregnadiene-11β,17α,20β,21-tetrol, acid hydrolysis of which gave a hydrated pregnanetetrolone which from its physical constants and its method of preparation must be identical with the hydrate of Substance E. On acetylation this material yielded the same diacetate obtained by the first-described method.

Experimental[14]

Pregnane-3α,11β,17α,20β,21-pentol 20,21-Acetonide (II).—To a solution of 3.78 g. of pregnane-3α,17α,20β,21-tetrol-11-one 20,21-acetonide (I) in 40 cc. of absolute

(1) The substance of this paper was presented at the 118th Meeting of the American Chemical Society, September 5, 1950.

(2) Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936); **20**, 953 (1937).

(3) Reichstein and von Euw, *ibid.*, **24**, 247E (1941).

(4) Sarett, THIS JOURNAL, **71**, 1169 (1949).

(5) See, for example, Lardon and Reichstein, *Helv. Chim. Acta*, **27**, 713 (1944). The 11β-hydroxyl group is designated 11α in the cited reference, according to the convention of that period.

(6) Trevoy and Brown, THIS JOURNAL, **71**, 1675 (1949).

(7) Abstracts of American Chemical Society, 113th Meeting (1948).

(8) Shoppee and Summers, *J. Chem. Soc.*, 687 (1950).

(9) Fieser, Fieser and Chakravarti, THIS JOURNAL, **71**, 2226 (1949).

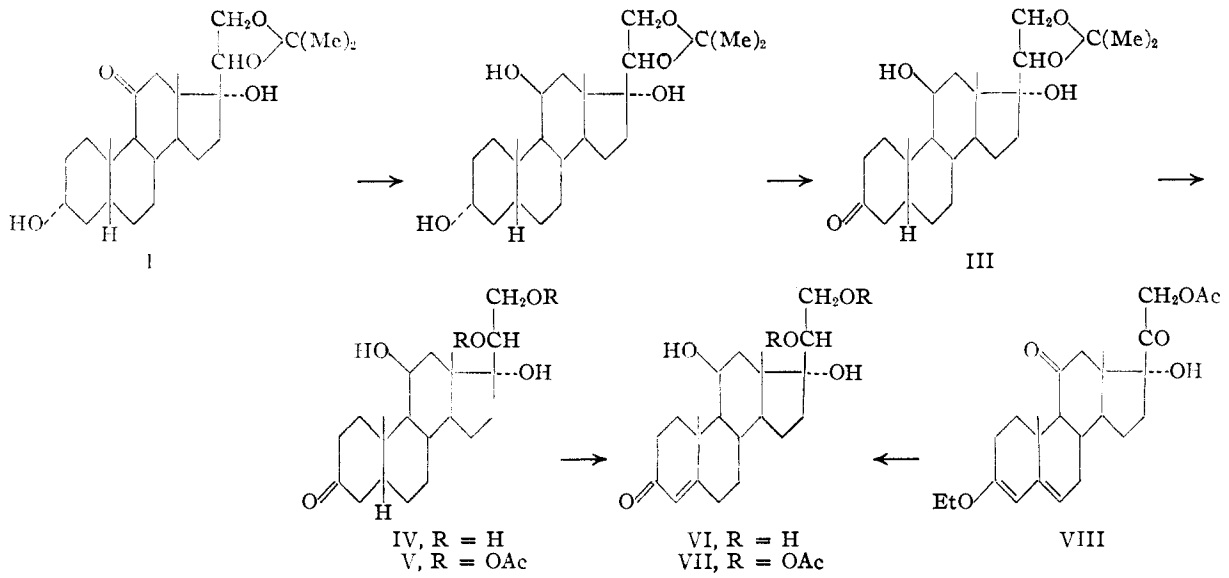
(10) *Cf.* Reich and Reichstein, *Arch. Inst. Pharmacodyn. Therap.*, **68**, 415 (1943); von Euw, Lardon and Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944).

(11) Fieser and Rajagopalan, THIS JOURNAL, **71**, 3935, 3938 (1949).

(12) The procedure of Mattox and Kendall, *ibid.*, **70**, 882 (1948); *J. Biol. Chem.*, **188**, 601 (1950).

(13) *Cf.* Serini and Koster, *Ber.*, **71**, 1766 (1938).

(14) Melting points were taken on the Kofler micro hotstage.



tetrahydrofuran, 4 g. of lithium aluminum hydride dissolved in 150 cc. of the same solvent was added slowly and with mechanical stirring. After being stirred at room temperature overnight, the mixture was treated with 50 cc. of 20% aqueous potassium hydroxide, and extracted several times with ether. Evaporation of the washed ether layer gave 3.71 g. of oily residue which after chromatographic purification on alumina (alkaline) yielded 2.05 g. of crystals, II, m.p. 179.5°; $[\alpha]^{25}D$ +35° (*c*, 0.95 in acetone). Approximately 1.5 g. of the crude reduction product was bound by the alumina even after elution with methanol. A second crystalline form of the acetonide, II, m.p. 190°, was occasionally obtained upon recrystallization. A mixture of the two forms showed a transition point at 180° followed by a sharp melting point at 190°.

Anal. Calcd. for $C_{24}H_{40}O_5$: C, 70.56; H, 9.87. Found: C, 70.54; H, 10.06.

Pregnane-3α,11β,17α,20β,21-pentol.—A solution of 150 mg. of pregnane-3α,11β,17α,20β,21-pentol 20,21-acetonide (II) in 3 cc. of 50% aqueous acetic acid was heated on the steam-bath for 20 minutes. The solution was then concentrated to a small volume *in vacuo*, dissolved in ethyl acetate and washed with sodium bicarbonate. Evaporation of the ethyl acetate solution gave the free pentol, m.p. 266–269°, after recrystallization from methanol–ethyl acetate.

Anal. Calcd. for $C_{21}H_{36}O_5$: C, 68.53; H, 9.86. Found: C, 68.51; H, 9.66.

Pregnane-11β,17α,20β,21-tetrol-3-one 20,21-Acetonide (III).—A solution of 1.5 g. of pregnane-3α,11β,17α,20β,21-pentol 20,21-acetonide (II) in 60 cc. of benzene was dried by distilling half of the solvent. After the addition of 1.0 g. of aluminum phenoxide and 2.5 g. of freshly distilled cyclohexanone, the mixture was refluxed for 16 hours. Dilution with benzene, followed by several washes with dilute potassium hydroxide and water and removal of the solvent *in vacuo* yielded 2.72 g. of an oily residue. Chromatography over 50 g. of alkaline alumina gave, as benzene eluate, 872 mg. of tetrolone acetonide III, m.p. 190°; $[\alpha]^{25}D$ +38° (*c*, 1.0 in acetone). A mixed melting point with starting material showed a depression of 20°.

Anal. Calcd. for $C_{24}H_{38}O_5$: C, 70.99; H, 9.43. Found: C, 70.58; H, 8.94.

Pregnane-11β,17α,20β,21-tetrol-3-one 20,21-Diacetate (V).—A solution of 750 mg. of pregnane-11β,17α,20β,21-tetrol-3-one 20,21-acetonide (III) in 12 cc. of 50% aqueous acetic acid was heated on the steam-bath for 20 minutes. The solvent was then removed *in vacuo*, and the residue warmed on the steam-bath for 10 minutes with a mixture of pyridine and acetic anhydride. After removal of the excess reagents *in vacuo*, the residue was taken up in ether, washed with dilute hydrochloric acid, dilute sodium bicarbonate and finally with water. Concentration of the solution to dryness gave a residue which after chromatographic purification afforded 646 mg. of the diacetate V, m.p. 182–184°; $[\alpha]^{35}D$ +95.5° (acetone).

Anal. Calcd. for $C_{25}H_{38}O_7$: C, 66.54; H, 8.50. Found: C, 66.27; H, 8.31.

4-Pregnene-11β,17α,20β,21-tetrol-3-one 20,21-Diacetate (VII) (Reichstein's Substance E Diacetate).—Pregnane-11β,17α,20β,21-tetrol-3-one 20,21-diacetate (1.38 g.) was treated with one molecular equivalent of bromine in acetic acid according to the standard procedure of Mattox and Kendall[12] to give an amorphous monobromide. This crude material was then subjected to the action of 2,4-dinitrophenylhydrazine and the amorphous dinitrophenylhydrazone (2.1 g.) cleaved with pyruvic acid under standard conditions. The crude cleavage product was reacetylated and chromatographed to give the diacetate VII, m.p. 229–231°; $[\alpha]^{24}D$ +161.3° (*c*, 0.56 in acetone). A mixed melting point with the diacetate prepared through the 3-enol ether (VIII) showed no depression.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.28. Found: C, 66.71; H, 8.05.

3-Ethoxy-3,5-pregnadiene-17α,21-diol-11,20-dione 21-Acetate (VIII).—A solution of 1 g. of 4-pregnene-17α,21-diol-3,11,20-trione 21-acetate in a mixture of 3 cc. of ethanol and 3 cc. of benzene was stirred at room temperature for 6 hours with 3 cc. of ethyl orthoformate after addition of 3 drops of absolute ethanolic hydrogen chloride (8%). After removal of the solvent, the crystalline residue was dissolved in benzene, washed with dilute potassium carbonate and water and evaporated to dryness. Recrystallization from ethanol gave the 3-enol ethyl ether VIII, m.p. 177–182°; $[\alpha]^{25}D$ +28° (dioxane).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.67; H, 7.94.

4-Pregnene-11β,17α,20β,21-tetrole-3-one (VI) (Reichstein's Substance E).—A solution of 400 mg. of 3-enol ethyl ether VIII in 45 cc. of ether was refluxed for 50 minutes with a solution of 600 mg. of lithium aluminum hydride in 10 cc. of ether. After the addition of water, the mixture was extracted with ether and the ethereal solution evaporated to dryness. The amorphous residue was refluxed for 40 minutes with a mixture of 1 cc. of methanol and 1 cc. of 1 *N* sulfuric acid and then the solvent removed *in vacuo*. Recrystallization from ethyl acetate gave the tetrolone VI as a hydrate, dec. 127–129°; $[\alpha]^{25}D$ +94° (acetone) (not corrected for water of crystallization). For analysis a sample was dried in a weighing pig at 130°.

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.19; H, 8.84. Found: C, 69.47; H, 8.84. Loss in weight on drying, calcd. for $C_{21}H_{32}O_5 \cdot H_2O$: H_2O, 4.94. Found: H_2O, 4.45.

Acetylation of a sample of this pregnenetetrolone with acetic anhydride–pyridine yielded a diacetate, m.p. 229–231°, not depressed on admixture with a sample of Substance E diacetate prepared by Method A.

Acknowledgment.—The authors wish to express their indebtedness to Mr. Richard Boos and his associates for microanalyses.

RAHWAY, N. J. RECEIVED OCTOBER 18, 1950

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. XVI. Beckmann Rearrangement of 17-Ketosteroid Oximes[1]

BY ST. KAUFMANN

The 17-oximes of dehydroisoandrosterone acetate, Δ^4-androstene-3,17-dione and estrone benzoate have been submitted to the Beckmann rearrangement. The resulting lactams (dehydroisoandrololactam acetate, testololactam and estrololactam benzoate) with a six-membered D-ring can be considered as nitrogen analogs of the D-homosteroids. The position of the NH-group has been established by selenium dehydrogenation of one of these lactams. The identification of the dehydrogenation product as 1-azachrysene proves that the NH-group is in the 17a-position.

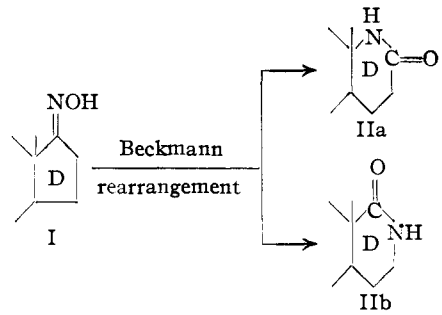
Since Westerfeld[2] described a lactone by oxidation of estrone with alkaline hydrogen peroxide, several other D-ring lactones of steroids have been reported.[3,4,5,6] Interesting physiological properties have been attributed to some of the lactones, so it seemed interesting to prepare the analogous lactams. Bachmann[7] has described a five-membered D-ring lactam of desoxyequilenin, but so far no six-membered D-ring lactams of steroids seem to have been described.

The best way for preparing this group of compounds is probably the Beckmann rearrangement of the oximes of the 17-ketosteroids (I). In fact, this rearrangement could be accomplished smoothly with the oximes of several steroids: dehydroisoandrosterone acetate, Δ^4-androsten-3,17-dione and estrone benzoate. These oximes were prepared by known methods; in the case of Δ^4-androsten-3,17-dione, however, it was necessary to protect the 3-keto group temporarily preparing its enol ether, so that only the 17-keto group was converted into the oxime group.

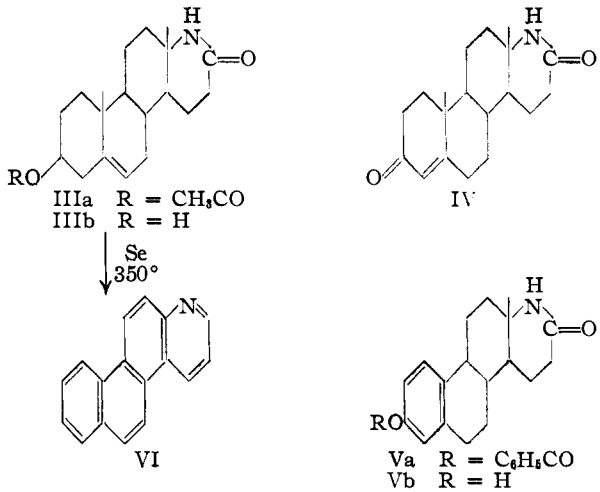
The rearrangement was carried out with *p*-acetylaminobenzenesulfonyl chloride in pyridine solution. In order to have the reaction proceed normally it was necessary to esterify the 3-hydroxy groups; no lactams could be isolated from the reaction mixture when working with the free compounds. The lactams with a free hydroxy group can be easily obtained by alkaline hydrolysis of the corresponding esters.

The lactams are very stable and high melting substances. Attempts to open the lactam ring with alkali or acids were unsuccessful. The NH-group of the lactam cannot be acetylated under ordinary conditions, but only with boiling acetic anhydride in presence of small amounts of *p*-toluenesulfonic acid.

Theoretically, the Beckmann rearrangement can proceed in two different directions to yield lactams



of the general type IIa or IIb. IIa can be considered as 17a-aza D-homosteroid and IIb as 17-aza D-homosteroid. Actually, in all cases only *one* lactam was isolated from the reaction mixture and no indications of the presence of an isomer were noted. In order to establish the position of the NH-group, the lactam obtained from dehydroisoandrosterone (IIIb) was dehydrogenated with selenium at 350°. From the acid-soluble fraction of the dehydrogenation mixture, a small amount of crystalline material was isolated, which proved to be identical with 1-azachrysene (naphtho[2,1-f]-quinoline (VI)), synthesized by Mosettig, *et al.*[8] The identity of the two substances has been established by mixed melting point,[9] ultraviolet spectrum (Fig. 1) and infrared spectrum.[10] Hence,



(1) For the preceding paper in this series see C. Djerassi and G. Rosenkranz, *Experientia*, **VI**, Feb. (1951).

(2) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).

(3) R. P. Jacobsen, *ibid.*, **171**, 61 (1947); H. Levy and R. P. Jacobsen, *ibid.*, **171**, 71 (1947).

(4) C. von Seemann and G. A. Great, THIS JOURNAL, **72**, 4073 (1950).

(5) J. W. Huffman, M. H. Lott and J. Ashmore, *ibid.*, **70**, 4268 (1948).

(6) E. B. Hershberg, E. Schwenk and E. Stahl, *Arch. Biochem.*, **19**, 300 (1948).

(7) W. E. Bachmann and F. Ramirez, THIS JOURNAL, **72**, 2525 (1950).

(8) E. Mosettig and J. Krueger, *J. Org. Chem.*, **3**, 325 (1938).

(9) I thank Dr. Mosettig for having kindly sent me a specimen of 1-azachrysene for comparison purposes.

(10) The infrared spectrum has been determined in the Sloan-Kettering Institute through the courtesy of Dr. K. Dobriner.