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

Steroids. XLIII.[1] A Ten Step Conversion of Progesterone to Cortisone. The Differential Reduction of Pregnane-3,20-diones with Sodium Borohydride[2]

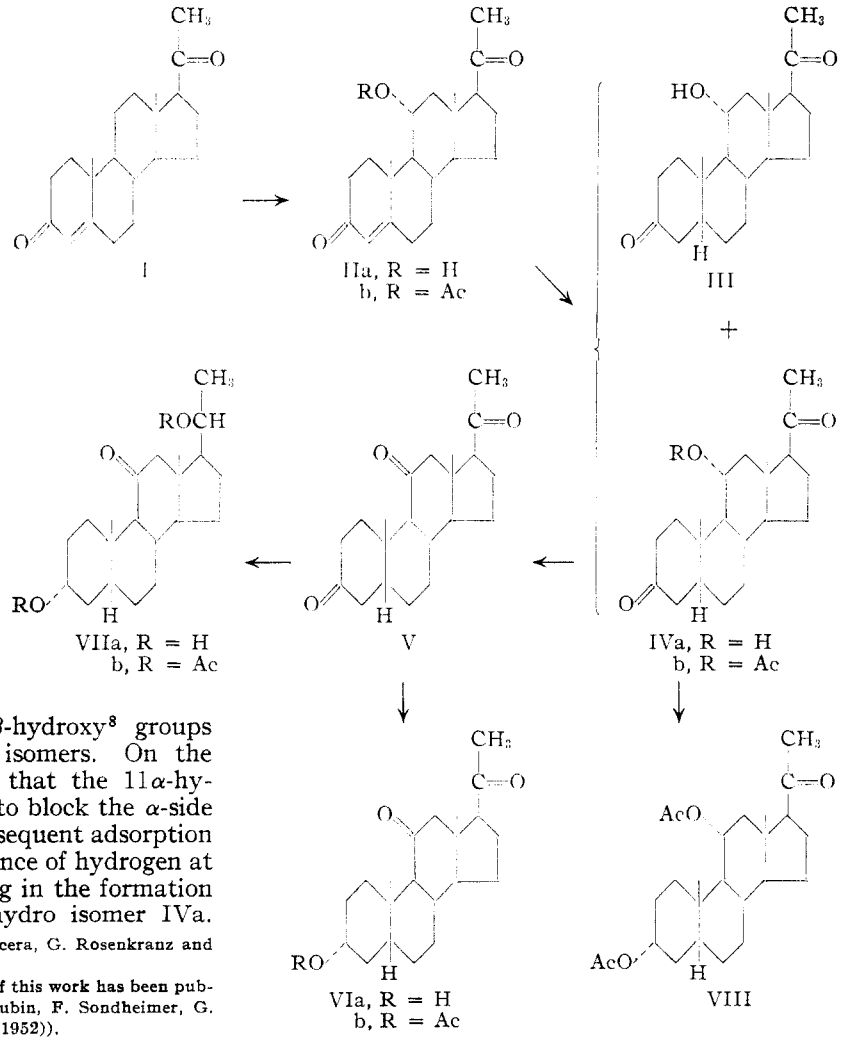
BY O. MANCERA, HOWARD J. RINGOLD, CARL DJERASSI,[3] G. ROSENKRANZ AND FRANZ SONDHEIMER

RECEIVED NOVEMBER 3, 1952

The hydrogenation of 11α-hydroxyprogesterone (IIa) has been shown to yield mainly pregnan-11α-ol-3,20-dione (IVa), which was oxidized to pregnane-3,11,20-trione (V). The differential reduction at C-3 of pregnane-3,20-dione (IX) and of allopregnane-3,20-dione (XI) could be brought about smoothly by means of sodium borohydride, and V was similarly reduced by this reagent to pregnan-3α-ol-11,20-dione (VIa), a cortisone intermediate. Since 11α-hydroxyprogesterone can be obtained in one step by microbiological oxidation of progesterone, the presently described experiments complete a ten step synthesis of cortisone from progesterone. This differential reduction of pregnane-3,20-diones has provided the basis for a new route to Δ^4-pregnene-17α,21-diol-3,20-dione (Reichstein's substance S), in which the crucial step is the reduction of pregnan-17α-ol-3,20-dione (XV) to pregnane-3α,17α-diol-20-one (XVI).

The discovery[2,4] that the microbiological oxidation of progesterone (I) effects 11-oxygenation, with the formation of 11α-hydroxyprogesterone(IIa),[5] has lent considerable interest to this latter compound as a possible source of cortisone. We have now been able to convert IIa by a simple three step synthetic sequence to pregnane-11,20-dione-3α-ol (VIa), a compound which has already been transformed, in six steps, to cortisone.[6] This combined microbiological–chemical route to cortisone presents the attractive features of starting with readily available materials, and of proceeding in relatively few steps (ten from progesterone or fourteen from "diosgenin"), all in good yield.

The first step consisted in the catalytic hydrogenation with a palladium catalyst of the Δ^4-double bond of 11α-hydroxyprogesterone (IIa). It is known that this type of hydrogenation of Δ^4-3-ketosteroids bearing 11-keto[7] and 11β-hydroxy[8] groups leads chiefly to the 5α (allo) isomers. On the other hand it was anticipated that the 11α-hydroxy group in IIa would tend to block the α-side of the steroid molecule, with consequent adsorption on the catalyst surface and entrance of hydrogen at the less hindered β-side, resulting in the formation of the desired 5β (normal) dihydro isomer IVa. This indeed proved to be the case, and with a palladized charcoal catalyst in ethanol solution



only 33% of the insoluble and readily isolatable 5α-isomer III[5] was obtained; under comparable conditions 11-ketoprogesterone had given[8] 68% of the corresponding allo derivative. The "normal" isomer, pregnan-11α-ol-3,20-dione (IVa), formed as the major product, proved to be a low-melting compound, difficult to isolate in the pure state, but could be characterized by its more tractable acetate IVb. The hydrogenation was run

(1) Paper XLII, F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **75**, 1282 (1953).

(2) A preliminary announcement of part of this work has been published (O. Mancera, A. Zaffaroni, B. A. Rubin, F. Sondheimer, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 3711 (1952)).

(3) Department of Chemistry, Wayne University, Detroit, Michigan.

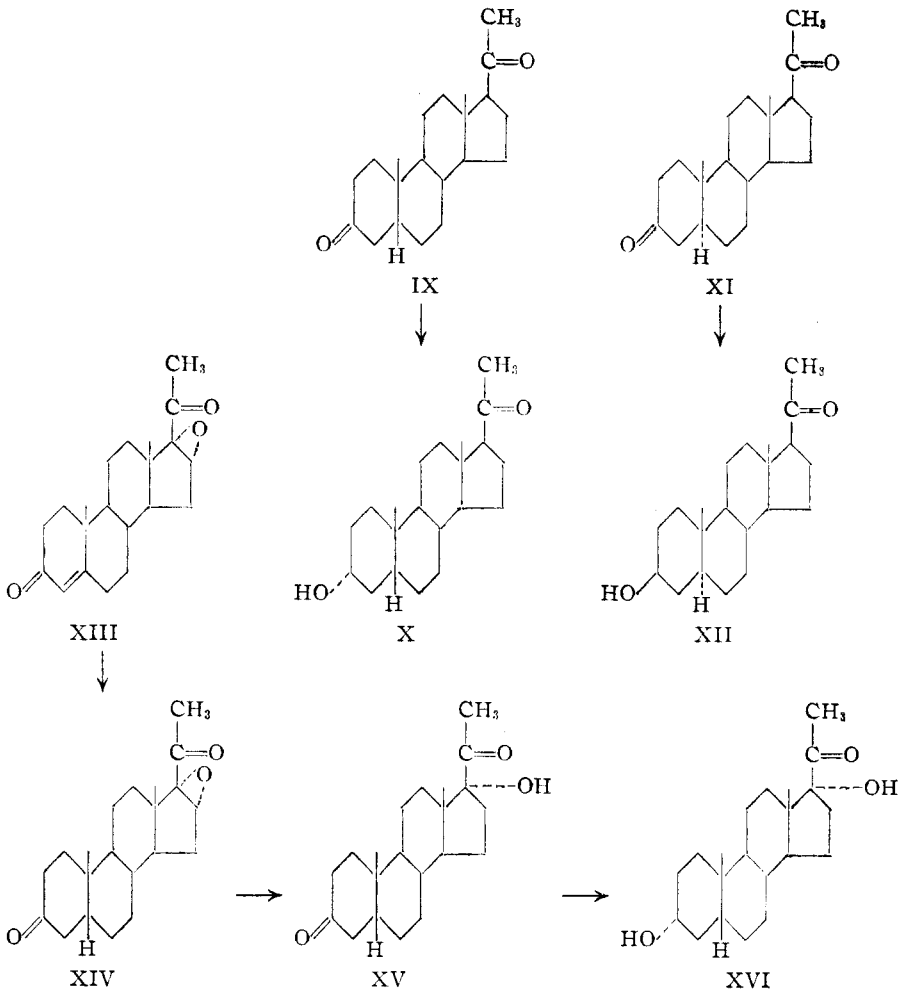
(4) (a) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952); (b) J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952).

(5) O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **17**, 1066 (1952).

(6) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **74**, 483 (1952).

(7) C. Djerassi, G. Rosenkranz, J. Pataki and S. Kaufmann, *J. Biol. Chem.*, **194**, 115 (1952).

(8) J. Pataki, G. Rosenkranz and C. Djerassi, *ibid.*, **195**, 751 (1952), and references cited therein.



under a variety of conditions, and the isomer ratio was most easily determined by the amount of the allo-derivative III which crystallized directly. It had been found in the case of ring C unsubstituted Δ^4-3-ketones that the presence of alkali[9] favored hydrogenation to the 5β-derivatives, and the present reduction was therefore run in the presence of potassium hydroxide. A very marked effect was indeed found; with a comparatively small quantity (5:1 ratio of steroid to potassium hydroxide) the amount of allo isomer III which could be isolated was *ca.* 20%, while with larger quantities only quite small amounts (*ca.* 5%) separated. The use of piperidine, while not as effective as potassium hydroxide, also caused a decrease in allo formation. The substitution of dioxane for ethanol as solvent was found to have no marked effect on the isomer ratio. The 11-acetate IIb was also hydrogenated (in the presence of potassium hydroxide), as the more bulky 11α-acetoxy group might be expected to favor formation of the 5β derivative even more than the hydroxy group. However no appreciable variation in the isomer ratio seemed to occur.

The next step, the oxidation of pregnan-11α-ol-3,20-dione (IVa) with chromium trioxide to the known[10] pregnane-3,11,20-trione (V) proceeded smoothly. In practice it was found advantageous to oxidize the crude hydrogenation mixture, after removal of the allo isomer III, in view of the losses inherent in the purification of IVa. In this way, when the hydrogenation was performed under conditions most favorable to "normal" formation, an over-all yield of 58% was realized in the two steps.

The third step, the differential reduction of the 3-keto group to yield the important cortisone intermediate[6] pregnan-3α-ol-11,20-dione (VIa) was carried out in 82% yield (53–62% conversion) by means of sodium borohydride in pyridine solution. Sodium borohydride was employed, as we had previously found that the 20-keto group both in pregnane-3,20-dione (IX) and in allopregnane-3,20-dione (XI) was reduced by this reagent much more sluggishly than the 3-keto group, and smooth preferential reductions to pregnan-3α-ol-20-one (X) and allopregnan-3β-ol-20-one (XII),[11] respectively, could be accomplished.[12] It was found rather surprisingly, that although these last mentioned reductions were carried out in alcohol

(9) P. L. Julian, "Recent Advances in Hormone Research," Vol. VI, Academic Press, Inc., New York, N. Y., 1951; R. Yashin, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 4654 (1951).

(10) *Inter al.* J. von Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944); C. Meystre and A. Wettstein, *ibid.*, **30**, 1262 (1947).

(11) The configuration of the 3-ols produced (3α in the normal series and 3β in the allo series) was to be expected from the work of C. W. Shoppee and G. H. R. Summers (*J. Chem. Soc.*, 687 (1950)) and of W. G. Dauben, R. A. Micheli and J. F. Eastham (THIS JOURNAL, **74**, 3852 (1952)). Small amounts of carbinols with the opposite configurations were undoubtedly formed, but were not isolated. It is worth noting that this type of differential reduction at C-3 of pregnane-3,20-dione (IX) and of allopregnane-3,20-dione (XI) has previously been carried out through catalytic hydrogenation over a platinum catalyst in neutral or acid solution (R. E. Marker, O. Kamm and E. L. Wittle, *ibid.*, **59**, 1841 (1937); A. Butenandt and G. Muller, *Ber.*, **71**, 191 (1938); G. Fleischer, B. Whitman and E. Schwenk, THIS JOURNAL, **60**, 79 (1938); R. E. Marker and E. J. Lawson, *ibid.*, **61**, 588 (1939)). We have found that this method is not as satisfactory in detail as the one described in this paper.

(12) E. Elisberg, H. Vanderhaeghe and T. F. Gallagher (*ibid.*, **74**, 2814 (1952)) have recently described the differential reduction at C-3 of etiocholane-3,17-dione and androstane-3,17-dione by means of sodium borohydride (slightly more than one equivalent). We would like to thank Dr. J. T. Gallagher for informing us of these experiments prior to publication. In their paper these authors also reported upon their observation that 20-ketosteroids are reduced only with difficulty with sodium borohydride. It may be noted that in our experiments the use of a considerable excess of the reagent was found to be satisfactory, an obvious advantage when working on a small scale, and when the purity of the hydride is unknown.

solution, when the reduction of pregnane-3,11,20-trione (V) was performed in this solvent only very little VIa was produced, the chief product being the known[13] pregnane-3α-20β-diol-11-one (VIIa) arising from attack at C-20 as well as at C-3.

A similar differential sodium borohydride reduction of the above described pregnan-11α-ol-3,20-dione acetate (IVb) in pyridine solution, followed by acetylation, readily yielded pregnane-3α,11α-diol-20-one diacetate (VIII), identical with a sample obtained from the 20-ethylene ketal[14] of VIa through reduction at C-11 with lithium and alcohol in liquid ammonia, followed by cleavage at C-20 and acetylation.[1]

The discovery of this very facile preferential reduction of pregnane-3,20-diones opened up the way for a new route to Δ^4-pregnene-17α,21-diol-3,20-dione (Reichstein's substance S), a substance of considerable importance at the present time (*cf.* [4b,15]). The starting point for this route was 16α,17α-oxidoprogesterone (XIII), which is very easily obtainable from "diosgenin" by side-chain degradation to $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate, followed by epoxidation to 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one[16] and Oppenauer oxidation.[17] Catalytic hydrogenation of the Δ^4-double bond in XIII over a palladium–charcoal catalyst in the presence of potassium hydroxide[9] gave nearly exclusively the 5β (normal derivative, 16α,17α-oxidopregnane-3,20-dione (XIV). The latter was converted by means of hydrogen bromide in acetic acid to the bromohydrin, which was debrominated through hydrogenation over a palladium–calcium carbonate catalyst (the Kendall[18] modification of the Julian[16,19] 17α-hydroxy introduction) to the known[20] pregnan-17α-ol-3,20-dione (XV). When this compound was now reduced with sodium borohydride in pyridine solution, again only the C-3 carbonyl function was attacked and pregnane-3α,17α-diol-20-one (XVI)[20] was produced in 65–70% yield.[21] The latter was then converted to "substance S" essentially by the published method.[22]

Experimental[23]

Hydrogenation of 11α-Hydroxyprogesterone (IIa).—11α-Hydroxyprogesterone (IIa) (1.0 g.)[24] in 50 cc. of dioxane together with 0.2 g. of potassium hydroxide dissolved in 3 cc. of methanol was hydrogenated at atmospheric pressure (580 mm.) and room temperature over 0.1 g. of a 10% palladium–charcoal catalyst (American Platinum Works, Newark, N. J.). After 3 hours one mole of hydrogen had been absorbed, and gas uptake had ceased. The catalyst was removed by filtration, the solution was neutralized with acetic acid, concentrated *in vacuo*, diluted with water and the product extracted with ether. Trituration of the residue with ether yielded 0.18 g. (18%) of allopregnan-11α-ol-3,20-dione (III) with m.p. 185–192°, raised to 194–196° on one crystallization (reported[6] m.p. 193–195°). Identity with an authentic specimen was established through mixture melting point determination and infrared comparison. The mother liquors were crystallized from ether–pentane to yield 0.67 g. (67%) of crude pregnan-11α-ol-3,20-dione (IVa) with m.p. 90–105°, no absorption in the 220–300 mμ region. The analytical sample was obtained by crystallization from chloroform–hexane, which involved considerable losses. It had m.p. 116–118°, $[\alpha]_D$ +91°, $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free hydroxyl band.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.97; H, 9.92.

The crude solid "normal" compound IVa (0.25 g., m.p. 90–105°) was acetylated with acetic anhydride and pyridine on the steam-bath. The 11-acetate IVb was crystallized from acetone–hexane; 0.19 g. with m.p. 139–143° was obtained, raised on further crystallization to 148–149°, $[\alpha]^{20}D$ + 65°, $\lambda_{max}^{CHCl_3}$ 1736, 1720 and 1700 cm.$^{-1}$, no free hydroxyl band.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.19; H, 9.34.

The remaining crude normal compound IVa (0.42 g., m.p. 90–105°) dissolved in 10 cc. of acetic acid was oxidized with 0.11 g. of chromium trioxide in 0.5 cc. of water and 5 cc. of acetic acid for 2 hours at room temperature. Crystallization of the product from acetone–hexane gave 0.21 g. of pregnane-3,11,20-trione (V) with m.p. 145–149°. Further purification yielded material with m.p. 158–160°, $[\alpha]^{20}D$ +128°, $\lambda_{max}^{CHCl_3}$ 1702 cm.$^{-1}$, no free hydroxyl band. An authentic specimen[10] was found to exhibit m.p. 160–162°, $[\alpha]^{20}D$ +126°, and identity was established through mixture melting point determination and infrared comparison.

When the above described hydrogenation of IIa was carried out in ethanol solution, but otherwise under identical conditions, the reaction was complete in 15–30 minutes and the isomer ratio seemed to be unchanged. An 18–20% yield of the allo isomer III with m.p. 186–193° was isolated; in one experiment the total mother liquors were acetylated to give a 65% over-all yield of the acetate IVb with m.p. 140–144°, while in another the total mother liquors were oxidized with chromium trioxide to furnish a 40% over-all yield of the triketone V with m.p. 146–150°.

When the hydrogenation was performed in ethanol solution with piperidine (0.1 cc. for 0.6 g. of IIa) instead of potassium hydroxide, a 27% yield of the allo compound III with m.p. 188–195° was isolated. On acetylation of the mother liquors an over-all yield of 53% of the acetate IVb with m.p. 145–147° was obtained.

When the hydrogenation was carried out in ethanol solution without base, a 33% yield of the allo isomer III was obtained. Acetylation of the mother liquors furnished the acetate IVb, m.p. 140–144°, in 47% yield.

Hydrogenation of 11α-Acetoxyprogesterone (IIb).—11α-Acetoxyprogesterone (IIb) (0.79 g. with m.p. 169–172°, prepared from 0.71 g. of IIa) in 50 cc. of dioxane together with 0.15 g. of potassium hydroxide in 3 cc. of methanol was hydrogenated over 0.1 g. of a 10% palladium–charcoal catalyst. Crystallization of the product from acetone–hexane gave 0.47 g. (59%) of the "normal" acetate IVb with m.p. 138–143°, raised on further crystallization to 146–148°, $[\alpha]^{20}D$ +63°. Identity was established with the above described product by mixture melting point determination and infrared comparison.

Pregnane-3,11,20-trione (V). Preparative Procedure.—11α-Hydroxyprogesterone (IIa) (1.0 g.) in 40 cc. of ethanol

(13) L. H. Sarett, THIS JOURNAL, **70**, 1690 (1948).

(14) G. Rosenkranz, J. Pataki and C. Djerassi, *J. Org. Chem.*, **17**, 290 (1952).

(15) D. R. Colingworth, M. P. Brunner and W. J. Haines, THIS JOURNAL, **74**, 2381 (1952).

(16) P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, *ibid.*, **72**, 5145 (1950).

(17) P. L. Julian, E. W. Meyer and I. Ryden, *ibid.*, **72**, 367 (1950).

(18) F. B. Colton, W. R. Nes, D. A. van Dorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

(19) P. L. Julian, E. W. Meyer, W. J. Karpel and I. Ryden, THIS JOURNAL, **71**, 3574 (1949).

(20) T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 184 (1951).

(21) This preferential reduction could alternatively be carried out by hydrogenation over a Raney nickel catalyst in ethanol or ethyl acetate solution. However, this procedure resulted in a mixture of the 3α- and the 3β-ols; although this mixture of epimers could be used for the further transformation to "substance S," it proved troublesome to separate it from the accompanying impurities formed in the reduction.

(22) B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 189 (1951).

(23) Melting points are uncorrected. Unless noted otherwise, rotations were determined in chloroform solution. We are grateful to Srta. Paquita Revaque for these measurements as well as for the infrared spectra, which were determined on a Perkin–Elmer model 12C spectrometer with sodium chloride prism. Thanks are due to Srta. Amparo Barba and staff for microanalyses.

(24) We are indebted to Dr. A. Zaffaroni of our Biochemistry Department for providing a very generous supply of this material, obtained through the microbiological oxidation of progesterone.[2]

containing 0.6 g. of potassium hydroxide was hydrogenated over 0.1 g. of a 10% palladium–calcium carbonate catalyst as above. Trituration of the product with ether gave only 0.04 g. (4%) of the allo compound III with m.p. 184–191°. The mother liquors (0.98 g.) were oxidized for 2 hours at room temperature with 0.23 g. of chromium trioxide in acetic acid (20 cc.) and water (1 cc.). The neutral oily product (0.91 g.) was purified chromatographically to give 0.58 g. (58% over-all) of the trione V with m.p. 150–153°, $[\alpha]^{20}D$ +131°, which was of sufficient purity for the next step. Recrystallization raised the m.p. to 158–160°.

Pregnan-3α-ol-20-one (X).—A solution containing pregnan-3,20-dione (IX) (3.0 g.), and sodium borohydride (0.3 g.) in ethanol (250 cc.) was allowed to stand at room temperature for 1 hour. The excess reagent was destroyed by the addition of a little acetic acid, the solution was concentrated *in vacuo*, diluted with water, and extracted thoroughly with ether. Crystallization from acetone–hexane yielded 1.21 g. of pregnan-3α-ol-20-one (X) with m.p. 144–146°, while chromatography of the mother liquors furnished another 0.68 g. with m.p. 145–146° (total yield 63%). On further crystallization the substance had m.p. 147–148°, $[\alpha]^{20}D$ + 108°, λ_{max}^{mull} 1700 cm.$^{-1}$ and free hydroxyl band (reported[25] m.p. 148–149°, $[\alpha]^{19}D$ +114° (EtOH)).

The acetate (pyridine–acetic anhydride, 1 hour, steam-bath) was crystallized from pentane, and exhibited m.p. 97–98°, $\lambda_{max}^{CHCl_3}$ 1728 and 1700 cm.$^{-1}$ (reported[25] m.p. 99°).

Allopregnan-3β-ol-20-one (XII).—The reaction was run with allopregnane-3,20-dione (XI) (3.0 g.) and sodium borohydride (0.3 g.) in ethanol (250 cc.) in the manner described above for the normal compound. After dilution with water the product was collected by filtration and crystallized from ethyl acetate–hexane. The allopregnan-3β-ol-20-one (XII) weighed 2.30 g. (76%), and had m.p. 195–196°, $[\alpha]^{20}D$ + 94°, $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free hydroxyl band. An authentic specimen showed m.p. 194–196°, $[\alpha]^{20}D$ +95°, and identity was established through mixture melting point determination and by infrared comparison.

The acetate was crystallized from ethyl acetate–hexane, and had m.p. 144–145°, $[\alpha]^{20}D$ + 83°, $\lambda_{max}^{CHCl_3}$ 1728 and 1700 cm.$^{-1}$. An authentic specimen had m.p. 145–146°, $[\alpha]^{20}D$ +85°, and identity was established in the usual manner.

Pregnan-3α-ol-11,20-dione (VIa).—A solution containing 0.70 g. of pregnane-3,11,20-trione (V) and 0.07 g. of sodium borohydride in 5 cc. of pyridine was allowed to stand at room temperature for 7 hours. It was then poured into water, 5 drops of acetic acid were added (this resulted in the decomposition of the excess hydride, despite the presence of pyridine, as evidenced by the vigorous effervescence observed), and the product was extracted with chloroform. Chromatographic purification over 50 g. of ethyl acetate-washed alumina yielded two fractions. The one eluted with benzene on crystallization from acetone–hexane gave 0.25 g. (36%) of recovered starting material with m.p. 150–153°; the other eluted with benzene–ether (6:4 and 4:6) on crystallization from the same solvent pair furnished 0.37 g. (82% yield, 53% conversion) of the 3α-ol VIa with m.p. 160–165°, $[\alpha]^{20}D$ +109°. Further crystallization yielded material with m.p. 169–171°, $[\alpha]^{20}D$ +105°, $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free hydroxyl band. An authentic sample[26] had m.p. 168–171°, $[\alpha]^{20}D$ +103°, and identity was established in the usual way.

The acetate VIb was crystallized from acetone–hexane and had m.p. 134–135°, $[\alpha]^{20}D$ +135°, $\lambda_{max}^{CHCl_3}$ 1736, 1718 and 1700 cm.$^{-1}$. It was identified with an authentic specimen[26] with m.p. 134–135°, $[\alpha]^{20}D$ +138°.

It was found that the addition of a small amount of water to the reduction mixture increased the rate of reaction. Thus, when the above described experiment was carried out in the presence of 6 drops of water, a 62% conversion was achieved after 7 hours reaction time, and unchanged material was again recovered.

Pregnane-3α,20β-diol-11-one (VIIa).—The reduction of pregnane-3,11,20-trione (V) (0.60 g.) was carried out with sodium borohydride (0.06 g.) in ethanol (35 cc.) at room temperature for 1 hour. A little acetic acid (*ca.* 10 drops) was added, the solution was concentrated and diluted with water. The precipitate was collected and crystallized from acetone–hexane. The pregnane-3α,20β-diol-11-one (VIIa) weighed 0.37 g. (61%) and had m.p. 225–229°. Further crystallization yielded the analytical sample as long felted needles with m.p. 231–233°, $[\alpha]^{20}D$ +29°, λ_{max}^{mull} 1700 cm.$^{-1}$ and free hydroxyl band (reported[13] m.p. 236–238°).

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.51; H, 10.00.

The diacetate VIIb (acetic anhydride–pyridine, 1 hour, steam-bath) after crystallization from acetone–hexane showed m.p. 157–159°, $[\alpha]^{20}D$ +79° (acetone), λ_{max} 1728 and 1700 cm.$^{-1}$ (reported[13] m.p. 160.5–161°, $[\alpha]^{25}D$ +81° (acetone)).

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.96; H, 9.45.

Pregnane-3α,11α-diol-20-one Diacetate (VIII).—Pregnan-11α-ol-3,20-dione acetate (IVb) (0.55 g.) was reduced with sodium borohydride (0.06 g.) in 5 cc. of pyridine containing 5 drops of water for 7 hours at room temperature. Water was added, then 5 drops of acetic acid, and the product was extracted with ether. The total residue, after removal of solvent, was directly acetylated (acetic anhydride–pyridine, 1 hour, steam-bath), and the solid product obtained by the addition of water was crystallized from acetone–hexane. The 3,11-diacetate VIII weighed 0.39 g. (63%) and had m.p. 138–142°. The analytical sample showed m.p. 141–143°, $[\alpha]^{20}D$ +61°, $\lambda_{max}^{CHCl_3}$ 1720 and 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 72.09; H, 9.42.

16α,17α-Oxidopregnane-3,20-dione (XIV).—16α,17α-Oxidoprogesterone (XIII)[17] (500 g.) in 4.5 l. of dioxane (previously distilled over Raney nickel) together with 10 g. of potassium hydroxide dissolved in 500 cc. of methanol was hydrogenated for 24 hours over 50 g. of a prereduced 5% palladium–charcoal catalyst at 30 lb. pressure at room temperature. The catalyst was filtered off, washed well with hot dioxane, and the filtrate poured into ice-water (40 l.). The precipitate was collected and dried. It weighed 485 g. (96%) and consisted of crude oxidopregnane-3,20-dione (XIV) with m.p. 163–168°, $[\alpha]^{20}D$ + 81°, no appreciable absorption at 240 mμ; it was of sufficient purity to be used in the next step. The analytical sample, obtained by crystallization from acetone, had m.p. 168–170°, $[\alpha]^{20}D$ +79°, $\lambda_{max}^{CS_2}$ 1714 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.19; H, 9.38.

Pregnan-17α-ol-3,20-dione (XV).—The crude 16α,17α-oxidopregnane-3,20-dione (XIV) (500 g., m.p. 163–168°) was dissolved in 3 l. of glacial acetic acid, and the solution was ice cooled until the acid started to crystallize. A 32% solution of hydrogen bromide in acetic acid (1 l.) was added with stirring and continued cooling at such a rate that the temperature did not exceed 18°. After being allowed to stand without cooling for 15 minutes, the solution was poured into 50 l. of iced distilled water with stirring. The crude bromohydrin was collected and air dried between filter paper.

Anal. Calcd. for $C_{21}H_{31}O_3Br$: Br, 19.42. Found: Br, 20.86.

The bromohydrin in 15 l. of 96% ethanol (previously distilled from Raney nickel) was hydrogenated over 1.5 kg. of a prereduced 1.5% palladium–calcium carbonate catalyst for 24 hours at 30 lb. pressure and room temperature. The catalyst was filtered off, washed with hot alcohol, the filtrate was evaporated to a small volume and poured into ice-water. The solid was collected and crystallized once from acetone. The pregnan-17α-ol-3,20-dione (XV) weighed 345 g. (69% based on XIV) and had m.p. 208–211°, $[\alpha]^{20}D$ +53° (EtOH). The analytical sample was obtained by further crystallization from acetone and exhibited m.p. 210–212°, $[\alpha]^{20}D$ +53° (EtOH), $\lambda_{max}^{CHCl_3}$ 1710 cm.$^{-1}$ and free hydroxyl band (reported[20] m.p. 215–217°, $[\alpha]^{25}D$ +53.9° (EtOH)).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.69; H, 9.44.

Pregnane-3α,17α-diol-20-one (XVI) by Sodium Borohydride Reduction of XV.—A solution of 500 g. of pregnan-17α-ol-3,20-dione (XV) and 50 g. of sodium borohydride in 6 l. of pyridine and 250 cc. of distilled water was allowed

(25) A. Butenandt and G. Muller, *Ber.*, **71**, 191 (1938).

(26) Kindly supplied by Dr. M. Tishler of Merck and Co., Inc.

to stand at room temperature for 24 hours. It was then poured into 45 l. of ice-water and acetic acid was slowly added to decompose excess reagent until no further effervescence was observed. The precipitate was collected and washed well with dilute hydrochloric acid, water, sodium bicarbonate solution and water. The dried product was crystallized from ethyl acetate to yield 325 g. (65%) of pregnane-3α,17α-diol-20-one (XVI) with m.p. 206–211°, $[\alpha]^{20}D$ +56° (EtOH), which was suitable for transformation[22] to "substance S." On further crystallization pure XVI was obtained with m.p. 212–214°, $[\alpha]^{20}D$ +59° (EtOH), $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free hydroxyl band, identified by direct comparison with an authentic specimen (reported[20] m.p. 213–214°, $[\alpha]^{25}D$ +63° (EtOH)). The mother liquors, through chromatography, could be made to yield another 25 g. of XVI with m.p. 205–210° (total yield 70%). It was however more economical to reoxidize them with N-bromoacetamide in pyridine solution (*cf.* footnote 20), when *ca.* 15% of the original diketone XV were recovered.

When the amount of water in the above described experiment was decreased, the rate of reduction was considerably reduced.

MEXICO CITY 17, D. F.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE SCIENCE RESEARCH INSTITUTE, OREGON STATE COLLEGE]

β-Aletheine[1] and Pantetheine[2]

BY TSOO E. KING, CHARLES J. STEWART AND VERNON H. CHELDELIN

RECEIVED SEPTEMBER 15, 1952

β-Aletheine (N-(β-alanyl)-2-aminoethyl mercaptan) was prepared by condensation of carbobenzoxy-β-alanyl chloride with β-aminoethyl mercaptan, reduction and isolation of the crystalline oxalate. β-Aletheine was then condensed with pantoyl lactone to produce pantetheine of high purity in good yields. β-Aletheine could not replace β-alanine for growth of yeast in a pantothenic acid-free medium, nor was it inhibitory. The pantetheine made by the present method possessed about 20,000 LBF units per mg. when assayed by *L. helveticus*, and upon enzyme digestion yielded at least 95% of the theoretical amount of pantothenic acid.

Pantetheine (*Lactobacillus bulgaricus* factor, LBF) has been synthesized previously by Snell, *et al.*,[3] by condensing methyl pantothenate with 2-aminoethyl mercaptan. It also has been shown[4] that LBF can be converted to coenzyme A (CoA) in a liver enzyme system in two hours in about 25% yield. Since the isolation of CoA is tedious, its amounts of pantetheine by the above method resulted in variable yields of material of inconsistent purity. For this reason a method for the synthesis of β-aletheine[1] was developed. This compound in turn condensed with pantolactone to give LBF in a smooth reaction and with good yields. The reactions carried out are summarized in Fig. 1. The

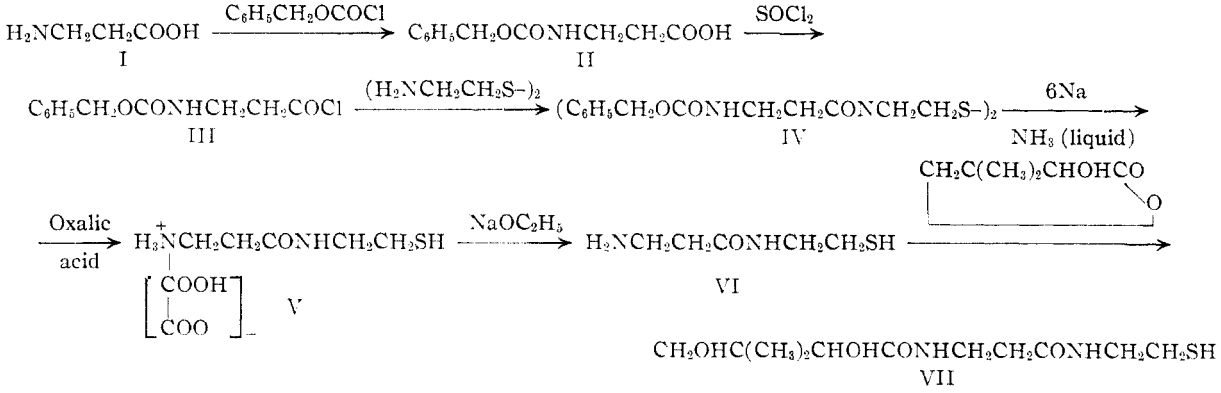


Fig. 1.—Reaction scheme for the synthesis of pantetheine.

preparation through enzymatic synthesis seems feasible. Thus a liberal quantity of pantetheine is needed. However, attempts to prepare large general scheme is somewhat similar to that reported by Baddiley and Thain[5] during the period that the present work was underway. Where comparable, the present work is in agreement with that of the English workers. In addition, the simplified procedure employed herein for preparing carbobenzoxy-β-aletheine, and the device of isolating β-aletheine through its oxalate salt, provide an easy route to this important intermediate.

Experimental

Carbobenzoxy-β-alanine (II).—This compound was synthesized according to Sifferd and du Vigneaud[6] with the exception that pure β-alanine was the starting material. The product was obtained in 90% yield with a melting point of 103–105° (Sifferd and du Vigneaud reported 102–104°

(1) The name β-aletheine is proposed for N-(β-alanyl)-2-aminoethyl mercaptan and β-alethine for its disulfide form, in conformance with the nomenclature of pantetheine and pantethine, respectively (*cf.* ref. 3).

(2) Paper No. 13 of a series on pantothenic acid studies. Reported in part before The Northwest Regional Meeting of the American Chemical Society, June, 1952. A portion of this work was submitted by C. J. S. for the M.S. degree, June, 1952, Oregon State College. This study has been supported by The Nutrition Foundation, Inc.; The Eli Lilly Laboratories, Inc.; The Abbott Laboratories, Inc.; and the Office of Naval Research, Contract NR 123-058. Published with the approval of the Monographs Publications Committee, Oregon State College, Research paper No. 217, School of Science, Department of Chemistry.

(3) E. E. Snell, G. M. Brown, V. J. Peters, J. A. Craig, E. L. Wittle, J. A. Moore, V. M. McGlohon and O. D. Bird, THIS JOURNAL, **72**, 5349 (1950).

(4) T. E. King and F. M. Strong, *J. Biol. Chem.*, **189**, 325 (1951).

(5) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 800 (1952).

(6) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).