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

STEROIDS. XIV.[1] STUDIES IN THE 3,6-DIHYDROXYPREGNANE SERIES (PART I)

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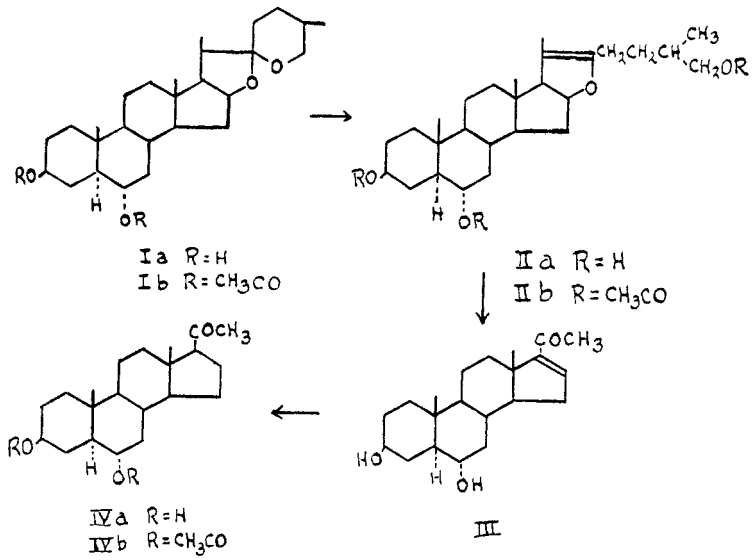
As part of an extensive biological investigation of various representatives of the pregnane series, it was necessary to prepare certain 3,6-dihydroxypregnanes. Three obvious approaches presented themselves for the synthesis of these compounds: (a) degradation of hyodesoxycholic acid (3α,6α-dihydroxycholanic acid), (b) degradation of 22-isoallospirostane-3β,6α-diol (chlorogenin)[2] (Ia), and (c) direct introduction of a 6-hydroxyl group into pregnane derivatives. In view of the unavailability of hyodesoxycholic acid in Mexico, the present report is limited to the last two approaches.

The degradation of 22-isoallospirostane-3β,6α-diol (Ia) is essentially a repetition of Marker's work (2, 3) and is reported briefly because of inadequate characterization of intermediates and lack of rotation data in this particular sequence of reactions. Conversion of I to Δ^{20}-allofurostene-3β,6α,26-triol (IIa) (ψ-chlorogenin) proceeded in the reported manner (2). Oxidation of the oily triacetate (IIb) followed by saponification afforded the previously unknown Δ^{16}-allo-



pregnene - 3β,6α - diol - 20 - one (III) and catalytic hydrogenation led to allopregnane-3β,6α-diol-20-one (IVa), further characterized by the previously undescribed diacetate (IVb). The difficulty in crystallizing some of these sub-

[1] For paper XIII, see ref. 9.

[2] For nomenclature of the steroidal sapogenins, see ref. 1.

stances has already been commented upon (3) and may account for the fact that III and IVb were not isolated earlier (3).

With respect to the direct introduction of the 6-hydroxyl group, it appeared attractive to proceed *via* a 3,5,6-triol, where partial dehydration would result in the formation of Δ^4-3,6-dihydroxy derivatives which were of especial interest for physiological testing, since they possess a double bond in the same position as in all biologically active corticosteroids. Ehrenstein (4, 5) investigated the perbenzoic acid oxidation and subsequent acetolysis of the resulting oxides in the case of Δ^5-pregnen-3β-ol-20-one (Va) and Δ^5-pregnen-3β,21-diol-20-one 21-acetate (Vb), which led to the required 3,5,6-triols and their 3,6-diacetates. In the present instance, the synthesis of the 3β,6β-diacetoxy-5α-hydroxy derivatives VIa and VIb could be considerably simplified by employing the performic acid hydroxylation procedure recently reported (6) in the cholesterol series, and which does not require any isolation of intermediates. Dehydration of the 5-hydroxyl group was accomplished in high yield by short treatment at 0° with thionyl chloride and led to the unknown Δ^4-3β,6β-diacetoxypregnenes VIIa and VIIb. It is interesting to note that these derivatives gave no color with tetranitromethane, and that the double bond was resistant to oxidation with monoperphthalic acid and lead tetraacetate. Thus, when Δ^4-pregnene-3β,6β-diol-20-one 3,6-diacetate (VIIa) was heated in acetic acid solution with one mole of lead tetraacetate, the corresponding 21-acetoxy derivative VIIb could be isolated readily.

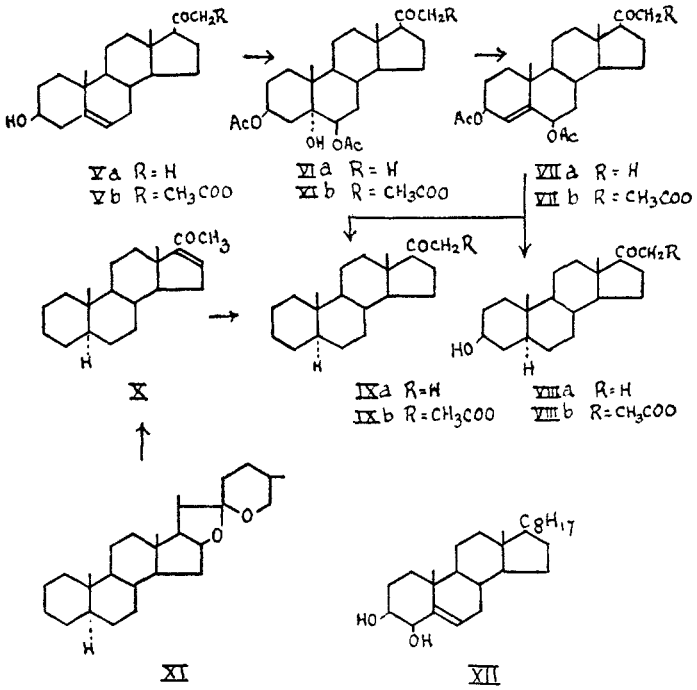
The catalytic hydrogenation of a Δ^4-3,6-dihydroxy steroid appears to have been studied only once. Rosenheim and Starling (7) observed that catalytic hydrogenation of Δ^4-cholestene-3β,6β-diol 3,6-diacetate[3] yielded in addition to the saturated diol, cholestane and cholestan-3β-ol. Similar products were isolated in the reduction of Δ^5-cholestene-3β,4β-diol (XII). We have now investigated the catalytic hydrogenation (palladium-on-charcoal or platinum oxide) of the two unsaturated alcohols VIIa and VIIb. Nearly three moles of hydrogen were absorbed in each instance and none of the saturated diol was isolated. Instead there was obtained *ca.* 30% of the known, saturated 3β-hydroxyallopregnane derivatives VIIIa and VIIIb and up to 60% of the hydrogenolysis product IX. In the case of Δ^4-pregnene-3β,6β-diol-20-one 3,6-diacetate (VIIa), allopregnane-20-one (IXa) was isolated and identified by comparison with a specimen obtained by degradation of 22-isoallospirostane (XI) (desoxytigogenin)[4] *via* Δ^{16}-allopregnen-20-one (X). The product obtained in the hydrogenation of Δ^4-pregnene-3β,6β,21-triol-20-one 3,6,21-triacetate (VIIb) possessed an elementary analysis consistent with that of allopregnan-21-ol-20-one 21-acetate (IXb) and its structure was confirmed by synthesis through a lead tetraacetate oxidation of allo-

[3] The substance was at that time considered to be Δ^5-cholestene-3β,4α-diol, but the correct structure has since been established [Butenant and Hausmann, *Ber.*, **70**, 1154 (1937) and Petrow, Rosenheim, and Starling, *J. Chem. Soc.*, 677 (1938)].

[4] Desoxytigogenin is known (8), but its degradation does not seem to have been carried out. The present specimen of desoxytigogenin was prepared by a new method (9).

pregnan-20-one (IXa) which proceeded in 65% yield. This appears to be the highest yield recorded so far for this type of reaction (*cf.* 14). The present syntheses of the two ketones IXa and IXb are new, and while both substances have been prepared by a variety of methods (10, 11), only the partial Clemmensen reduction (10a) of allopregnane-3,20-dione to allopregnan-20-one appears to be superior with respect to yield and availability of starting materials.

In view of its comparative simplicity, the hydrogenolysis of Δ^4-3,6-diacetoxy steroids to saturated, desoxyallo steroids may at times prove to be the method of



choice. Various, obvious reaction paths may be formulated to account for the production of VIII and IX from VII and the intermediate formation of an allylic rearrangement product such as XII must be considered as one of them.

EXPERIMENTAL[5]

Δ^{16}-Allopregnene-3β,6α-diol-20-one (III). 22-Isoallospirostane-3β,6α-diol 3,6-diacetate (chlorogenin diacetate) (Ib) (12), m.p. 155–157°, was converted in 76–80% yield into Δ^{20}-allofurostene-3β,6α,26-triol (IIa) by heating with three to four times its weight of acetic anhydride in a sealed tube at 200° for ten hours, followed by methanolic alkali saponification; m.p. 263–266°, $[\alpha]_D^{20}$ +30.7° (pyridine). Reported (2): 56% yield, m.p. 268–270°. The furostene derivative (10 g.) was acetylated by refluxing for one-half hour with 50 cc.

[5] All melting points are uncorrected. Unless noted otherwise, rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are indebted to the Srtas. Paquita Revaque and Maria Eugenia Frontana for these measurements and to Srta. Amparo Barba of our Microanalytical Department for the analyses.

of acetic anhydride and distilling to dryness *in vacuo*. The residual triacetate IIb in 350 cc. of acetic acid was oxidized at room temperature with a solution of 10 g. of chromium trioxide in 100 cc. of 80% acetic acid. After the usual work-up, including saponification with either boiling methanolic potassium hydroxide or dilute, methanolic carbonate solution[6] the residue was chromatographed on ethyl-acetate washed alumina and all fractions exhibiting an ultraviolet absorption maximum at 238–240 mμ were combined. One recrystallization from hexane acetone afforded 4.26 g. (70%) of crystals with m.p. 135–140°, ultraviolet absorption maximum at 238 mμ (log ε 3.82). A Girard separation removed a small amount of non-ketonic material and several recrystallizations from hexane-acetone raised the m.p. to 143–145°; on cooling the melt and remelting, the m.p. was 202–205°, $[\alpha]_D^{20}$ +68.8, u.v. maximum at 240 mμ (log ε 4.09).

Anal. Calc'd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70.

Found: C, 75.63; H, 9.70.

Allopregnane-3β,6α-diol-20-one (IV). A solution of 2.0 g. of the unsaturated ketone III in 120 cc. of ethyl acetate was shaken with 0.4 g. of 5% palladium-on-barium sulfate catalyst in an atmosphere of hydrogen until the gas uptake stopped. Filtration, evaporation of the solvent and recrystallization from acetone gave 1.7 g. of allopregnane-3β,6α-diol-20-one (IVa); m.p. 202–203°, $[\alpha]_D^{20}$ + 94.9° [lit. (3), m.p. 208–210°]. Acetylation with acetic anhydride-pyridine, followed by crystallization from pentane gave a nearly quantitative yield of *allopregnane-3β,6α-diol-20-one 3,6-diacetate* (IVb), m.p. 96–99°. The analytical sample was obtained from hexane, m.p. 101–102°, $[\alpha]_D^{20}$ +88.4°.

Anal. Calc'd for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15.

Found: C, 71.55; H, 9.35.

Pregnane-3β,5α,6β-triol-20-one 3,6-diacetate (VIa). The present procedure was adapted from that used recently (6) for the conversion of chloesterol to cholestane-3β,5α,6β-triol.

Twenty grams of Δ⁵-pregnen-3β-ol-20-one (Va) was heated on the steam-bath with 120 cc. of 90% formic acid for five minutes, cooled to room temperature, and then treated with 20 cc. of 30% hydrogen peroxide in small portions care being taken that the temperature remained below 35°. A gummy precipitate, which formed initially, dissolved within 20 minutes and crystals started to appear soon thereafter. After six hours at room temperature, water was added, the product collected, and then refluxed for 15 minutes with 200 cc. of methanol, 20 cc. of water, and 6 g. of potassium hydroxide. Evaporation of the methanol under reduced pressure, dilution with water, and filtration afforded 16.4 g. (78%) of pregnane-3β,5α,6β-triol-20-one, m.p. 237–245°, which was satisfactory for the next step. Recrystallization from methanol-chloroform raised the m.p. to 252–255°, $[\alpha]_D^{20}$ +58.8° [reported (5, 13) m.p. 256–258°].

Acetylation of the unrecrystallized triol (13.8 g.) with acetic anhydride and pyridine afforded after one recrystallization 14.4 g. (83%) of the *3,6-diacetate* VIa with m.p. 212–215°, $[\alpha]_D^{20}$ −7.2° [reported (5) m.p. 217–219°].

Δ⁴-Pregnene-3β,6β-diol-20-one 3,6-diacetate (VIIa). The dehydration was accomplished by adding 1.65 cc. of thionyl chloride to an ice-cold solution of 5.5 g. of the 5-hydroxy-3,6-diacetate VIa in 25 cc. of pyridine. After five minutes at 0° the product was precipitated with water (5.18 g., 98% yield, m.p. 145–150°) and once recrystallized from acetone-hexane; yield, 4.05 g. (76%), m.p. 155–157°. Further recrystallization yielded the analytical sample, m.p. 156–158°, $[\alpha]_D^{20}$ +44.4°.

Anal. Calc'd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71.

Found: C, 72.07; H, 8.65.

Pregnane-3β,5α,6β,21-tetrol-20-one 3,6,21-triacetate (VIb). The performic acid hydroxylation was carried out exactly as above using 12 g. of Δ⁵-pregene-3β,21-diol-20-one 21-acetate (Vb), 70 cc. of 90% formic acid, and 12 cc. of 30% hydrogen peroxide. The crude and still

[6] No difference in yield was observed in this instance. The chromatographic separation and spectrophotometric analysis excluded the presence of any appreciable amount of 16-methoxy derivative [*cf.* Fukushima and Gallagher, *J. Am. Chem. Soc.*, **72**, 2306 (1950)].

moist product after dilution with water was boiled for one-half hour in a current of nitrogen with 8 g. of potassium carbonate, 150 cc. of methanol, and 30 cc. of water. After addition of saturated salt solution, the precipitate was filtered, dried, and acetylated by heating with acetic anhydride—pyridine. Crystallization from hexane-acetone gave 10.66 g. (68%), m.p. 164–170°, which was raised on further recrystallization to 173–175°, $[\alpha]_D^{20}$ +5° (acetone) [reported (4) m.p. 175.5–176°, $[\alpha]_D$ +3.5° (acetone)].

Δ^4-Pregnene-3β,6β,21-triol-20-one 3,6,21-triacetate (VIIb). (*a*) *By dehydration of pregnane-3β,5α,6β,21-tetrol-20-one 3,6,21-triacetate* (VIb). Application of the above described dehydration method to 2.4 g. of VIb led to 2.12 g. (92%) of slightly yellowish product, m.p. 138–141°. Recrystallization from hexane—acetone, using Norit, gave the analytical sample of VIIb as rosettes of colorless needles, m.p. 143–144°, $[\alpha]_D^{20}$ +49°. The substance gave no color with tetranitromethane.

Anal. Calc'd for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07.

Found: C, 68.52; H, 8.05.

(*b*) *By lead tetraacetate oxidation of Δ^4-pregnene-3β,6β-diol-20-one 3,6-diacetate* (VIIa). A solution of 4.0 g. of the 3,6-diacetate VIIa in 100 cc. of c.p. glacial acetic acid and 1.5 cc. of acetic anhydride was treated dropwise over a period of three hours at 70° with a solution of 7.6 g. of lead tetraacetate in 75 cc. of acetic acid. After an additional three hours at 70° and 12 hours at room temperature, the mixture was evaporated to near dryness *in vacuo*, diluted with water, and extracted with ether. Two recrystallizations of the ether residue from hexane—acetone yielded 1.76 g. (39%) of the triacetate VIIb, m.p. 136–140°, $[\alpha]_D^{20}$ +45.2°. Further recrystallization gave m.p. 142–143° and no depression was observed on admixture with a specimen prepared according to (a). Chromatography of the mother liquors on 70 g. of ethyl acetate—washed alumina and elution with hexane-benzene (7:3) afforded 0.95 g. (24%) of recovered starting material, m.p. 151–153°.

Δ^{16}-Allopregnen-20-one (X). A solution of 10 g. of 22-isoallospirostan (desoxytigogenin) (XI) (8, 9) was heated with 35 cc. of acetic anhydride for ten hours in a sealed tube at 200°. A small sample after alkaline saponification and crystallization from ether-hexane gave crystals of Δ^{20}-allofurosten-26-ol, m.p. 128–130°, $[\alpha]_D^{20}$ +30°. The remaining part of the solution was oxidized as described above for the 3,6-diacetoxy derivative IIb and the crude oxidation product was saponified with 4.6 g. of potassium bicarbonate, 40 cc. of methanol, and 12 cc. of water. Recrystallization from methanol gave 1.92 g. (26%) of colorless crystals of the unsaturated ketone X, m.p. 155–157°, $[\alpha]_D^{20}$ +43°, ultraviolet maximum at 240 mμ, (log ε 4.10).

Anal. Calc'd for $C_{21}H_{32}O$: C, 83.94; H, 10.74.

Found: C, 83.97; H, 10.76.

Allopregnan-20-one (IXa). The unsaturated ketone X (800 mg) in 30 cc. of ethyl acetate and 100 mg. of 5% palladium-on-charcoal catalyst took up one mole of hydrogen in 40 minutes at room temperature and atmospheric pressure (570 mm.). Filtration of the catalyst, evaporation to dryness, and recrystallization from methanol gave 0.72 g. (90%) of allopregnan-20-one (IXa) as colorless plates, m.p. 133–135°, $[\alpha]_D^{20}$ +99° [reported (10) m.p. 128–130° to 136–139°, $[\alpha_D]$ +102°].

Anal. Calc'd for $C_{21}H_{34}O$: C, 83.38; H, 11.33.

C, 83.73; H, 11.53.

Allopregnan-21-ol-20-one 21-acetate (IXb). A mixture of 0.58 g. of allopregnan-20-one (IXa), 15 cc. of acetic acid, 0.25 cc. of acetic anhydride, and 0.81 g. of lead tetraacetate was heated for eight hours at 70° and then allowed to stand at room temperature for 12 hours. Dilution with water, extraction with ether, and recrystallization of the ether residue from methanol-chloroform gave 0.45 g. (65%) of the ketol acetate IXb, m.p. 192–195°, $[\alpha]_D^{20}$ +100°. The analytical sample was twice recrystallized and then sublimed in a high vacuum, m.p. 201–202°, $[\alpha]_D^{20}$ +98° [reported (11) m.p. 200°, $[\alpha]_D^{20}$ +101.8°].

Anal. Calc'd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07.

Found: C, 76.83; H, 9.89.

Hydrogenation of Δ^4-pregnene-3β,6β-diol-20-one 3,6-diacetate (VIIa). A solution of 2 g. of

Δ^4-3,6- diacetate VIIa in 50 cc. of ethyl acetate was shaken in an atmosphere of hydrogen with 150 mg. of 5% palladium-on-charcoal for six hours at which time the gas uptake had ceased and corresponded to 2.6 moles of hydrogen. Filtration, evaporation to dryness, and several recrystallizations from hexane led to 0.13 g. of allopregnan-3β-ol-20-one 3-acetate, m.p. 141–143°, $[\alpha]_D^{20}$ +94.9°, identical with an authentic sample. Evaporation of the hexane filtrates to dryness and recrystallization from methanol gave 0.5 g. of allopregnan-20-one (IXa), m.p. 133–135°, $[\alpha]_D^{20}$ +98°, which proved to be identical with the specimen prepared above by hydrogenation of Δ^{16}-allopregnen-20-one (X). The mother liquors were saponified by boiling for 30 minutes with 1 g. of potassium hydroxide and 25 cc. of methanol and on dilution with a small amount of water gave 0.78 g. of crystals, m.p. 178–185°, identified as allopregnan-3β-ol-20-one (VIIIa) m.p. 192–195°, $[\alpha]_D^{20}$ +93° (0.44 g.) after recrystallization from hexane-acetone, by comparison with an authentic specimen (m.p. 194–196°, $[\alpha]_D^{20}$ +98°). Chromatography of the mother liquors gave an additional 0.39 g. of allopregnan-20-one, m.p. 127–131°. This hydrogenation, therefore, yielded[7] 61% of allopregnan-20-one (IXa) and 36% of allopregnan-3β-ol-20-one (VIIIa).

When carried out as above but using acetic acid instead of ethyl acetate, there was isolated 46% of allopregnan-20-one and only 10% of allopregnan-3β-ol-20-one. With acetic acid and platinum oxide catalyst, four moles of hydrogen were absorbed in 1½ hours. After oxidation with chromium trioxide, there was obtained 57% of allopregnan-20-one.

Hydrogenation of Δ^4-pregnen-3β,6β,21-triol-20-one 3,6,21-triacetate (VIIb) A solution of 1 g. of triacetate VIIb and 100 mg. of palladium-on-charcoal catalyst in 30 cc. of acetic acid absorbed 2.8 moles of hydrogen in four hours. The residue after filtration of the catalyst and evaporation to dryness amounted to 0.78 g., m.p. 145–170°. Chromatography on 25 g. of ethyl acetate—washed alumina yielded two distinct fractions. The hexane eluates after recrystallization from chloroform—methanol gave 0.28 g. (37%) of allopregnan-21-ol-20-one 21-acetate (IXb), m.p. 198–200°, $[\alpha]_D^{20}$ +94°, identical with the specimen prepared above by lead tetraacetate oxidation of IXa. The benzene-hexane and benzene eluates were combined and recrystallized repeatedly from hexane-acetone, whereupon 0.22 g. (25%) of allopregnan-3β,21-diol-20-one (VIIIb) 3,21-diacetate was obtained, m.p. 150–151°, undepressed upon admixture with an authentic specimen, $[\alpha]_D^{20}$ +82° [reported (14) m.p. 152–153.5°.

SUMMARY

Marker's degradation (3) of 22-isoallospirostane-3β,6α-diol (chlorogenin, Ia) has been repeated and the intermediates characterized.

Δ^5-Pregnen-3β-ol-20-one has been converted by performic acid hydroxylation to pregnane-3β,5α,6β-triol-20-one 3,6-diacetate (VIa) which was readily dehydrated to Δ^4-pregnene-3β,6β-diol-20-one 3,6-diacetate (VIIa). Hydrogenation of this substance afforded allopregnan-3β-ol-20-one (VIIIa) and allopregnan-20-one (IXa). The latter was synthesized independently by degradation of 22-isoallospirostan (XI) (desoxytigogenin).

Application of the same reaction sequence to Δ^5-pregnen-3β,21-diol-20-one 21-acetate (Vb) afforded Δ^4-pregnen-3β,6β,21-triol-20-one 3,6,21-triacetate (VIIb), which could also be obtained from the corresponding 20-ketone VIIa by lead tetraacetate oxidation. The behavior of the unsaturated triacetate VIIb on

[7] In one experiment, in addition to VIIIa and IXa, about 10% of Δ^5-pregnen-3β-ol-20-one (Va) was isolated. This substance, which might be one of the precursors in the formation of VIIIa, in turn might arise by hydrogenolysis of the 6-acetoxy group of VIIa, followed by a shift of the double bond, or by hydrogenolysis of a rearrangement product such as XII.

hydrogenation duplicated that of the diacetate VIIa. One of the hydrogenolysis products, allopregnan-21-ol-20-one 21-acetate (IXb) was also synthesized by lead tetraacetate oxidation of allopregnan-20-one (IXa).

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