To a stirred solution of 8.0 g of X in 80 ml of dry tetrahydrofuran was added, at room temperature and over a 30-min period, 100 ml of the above disiamylborane stock solution and the resulting mixture was allowed to stir at room temperature for 2 hr. The reaction mixture was then treated with 10 ml of saturated sodium sulfate solution and the precipitated inorganic salts were removed by filtration. The filtrate was evaporated to near dryness and the residue was diluted with 300 ml of ether–methylene chloride (3:1) The organic layer was washed with saturated sodium chloride solution and then with water. The organic solution was dried (Na_2SO_4) and concentrated to dryness to afford a colorless oil which was dissolved in benzene and chromatographed on a column (400 g) of silicic acid (400-ml fractions). The fractions eluted with 30% ethyl acetate–benzene and then pure ethyl acetate were concentrated and dissolved in benzene and chromatographed again using a 250-g column of silicic acid (250-ml fractions). The fractions eluted with 40% ethyl acetate–benzene, followed by pure ethyl acetate, were combined and concentrated. The residue was crystallized once from ether–hexane and then from methylene chloride–ether to give 1.94 g of XIII, mp 166.5–168° (vacuum). The mother liquors were combined and chromatographed as described above. Two crystallizations gave an additional 0.44 g, mp 167.5–169° (vacuum, total yield 30%). Crystallization from ether–hexane gave the analytical sample: mp 167–169° (vacuum), $[\alpha]^{25}D$ −42.2° (*c* 1.0, $CHCl_3$), λ_{max}^{KBr} 2.97 μ (no carbonyl absorption), $\lambda_{max}^{CHCl_3}$ 2.77 and trace (about 2%) carbonyl absorption at 5.83 μ, nmr ($CDCl_3$) τ 5.03 (C_{17} proton) and 6.03 (ethylene ketal protons).

Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.60; H, 9.92.

17ξ-Hydroxy-16-oxa-5α-androstan-3-one (XIV).—A solution of 2.25 g of XIII in 225 ml of tetrahydrofuran and 50 ml of 3 *N* hydrochloric acid was allowed to stand at room temperature for 3 days. Most of the solvent was then removed under vacuum and the residue was diluted with 250 ml of water. The resulting mixture was extracted with ether–methylene chloride (3:1) and the organic layers were washed with water, dried (N_2SO_4), and evaporated. The residue was dissolved in benzene and filtered through a short column (20 g) of silicic acid. The fractions eluted with 20% ethyl acetate–benzene and pure ethyl acetate were combined and evaporated. The residue was crystallized twice from methylene chloride–ether–hexane to give 1.04 g of crude XVI, mp 155.5–159° dec (vacuum). An additional 0.221 g, mp 158–160.5° dec (vacuum), was obtained from the mother liquors. Both samples were combined and crystallized once from acetonitrile to give 0.763 g (39%) of XIV: mp 161.5–163° dec (vacuum), $[\alpha]^{25}D$ −28.3° (*c* 1.1, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.77 and 5.85 μ, nmr ($CDCl_3$) τ 5.03 (doublet, J = 3.5 cps; this doublet collapses to a singlet upon addition of D_2O).

Anal. Calcd for $C_{18}H_{28}O_3$: C, 73.93; H, 9.65. Found: C, 73.97; H, 9.91.

Registry No.—IIa, 7785-89-9; IIb, 10022-25-0; III, 7785-90-2; IV, 7785-91-3; V, 7785-92-4; VI, 7785-93-5; VII, 7785-94-6; VIII, 10028-43-0; IX, 7785-95-7; X, 7785-96-8; XI, 7785-97-9; XII, 7785-98-0; XIII, 7785-99-1; XIV, 7786-00-7.

Acknowledgment.—We are indebted to Dr. Al Steyermark and his staff for the microanalyses, and to Dr. V. Toome, Mr. S. Traiman, and Dr. Floie M. Vane for the ultraviolet, infrared, and nmr spectra, respectively. We are also indebted to Mr. R. Vass for technical assistance.

The Absolute Configuration at C-20 in 11-Oxo-3,20,21-trihydroxy Steroids[1]

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The C-11 and C-21 oxygen atoms of 3α,20,21-trihydroxy-5β-pregnan-11-one, mp 233–234.5°, were removed by reactions which did not alter the configuration at C-20. The product was identical with 5β-pregnane-3α,20β-diol, a substance of known absolute configuration at C-20. It follows that the absolute configuration at C-20 in the original compound is β.

In a previous paper,[2] 3α,20-diacetoxy-11-oxo-5β-pregnan-21-oic acid (mp 199–200°) was shown to have the same C-20 configuration as a compound designated 3α,20β,21-trihydroxy-5β-pregnan-11-one[3] (IV). The initial assignment[4] of configuration at C-20 in the latter compound was based in part on application of the rule which states that the acetylation increments in optical rotatory values for 20β-hydroxy steroids are strongly positive and that those for 20α-hydroxy steroids are either negative or weakly positive.[5] Evidence subsequently obtained from several other lines of investigation[5] has been in agreement with the configuration which was assigned originally. By this method of correlation, the C-20 configuration in compound IV appeared to be the same as that in 5β-pregnane-3α,20β-diol.[4]

However, the use of optical rotatory values is not valid for assignment of configuration at the C-20 position in some circumstances.[6]

Consequently, we wished to correlate the C-20 configuration of 3α,20β,21-trihydroxy-5β-pregnan-11-one (IV) (and thus that of 3α,20-diacetoxy-11-oxo-5β-pregnan-21-oic acid,[2,6] mp 199–200°) with that of 5β-pregnane-3α,20β-diol by a direct procedure, independent of correlations derived from measurement of optical rotatory values. The objective was to remove the C-11 and C-21 oxygen functions from compound IV by use of reactions which would not disturb the configuration at C-20 and to determine whether the product was identical with 5β-pregnane-3α,20β-diol. That inversion of configuration at C-20 has not occurred during the process of removal of the oxygen functions at C-11 and C-21 can be assumed if (1) one uses well-known reactions which ordinarily do not cause inversion of configuration at the asymmetric center of interest, (2) one removes each function by two or more different pathways, and (3) the different pathways lead to the same product. This conclusion should then be on as firm a basis as is the absolute configuration which has been assigned to 5β-pregnane-3α,20β-diol[7] through

(1) This investigation was supported in part by Research Grant AM-5452 from the National Institutes of Health, U. S. Public Health Service.

(2) V. R. Mattox and Wiley Vrieze, *J. Org. Chem.*, **29**, 3158 (1964).

(3) L. H. Sarett, *J. Am. Chem. Soc.*, **71**, 1165 (1949).

(4) L. H. Sarett, *ibid.*, **71**, 1175 (1949).

(5) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp 612–618.

(6) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1779 (1963).

(7) Reference 5, p 338.

correlation *via* numerous intermediates to D-tartaric acid.

The "arbitrary" configuration at C-20 in 5β-pregnane-3α,20β-diol had been assigned by Marker and associates[8] on the basis that 5β-pregnane-3α,20α-diol was the natural compound and that 5β-pregnane-3α,-20β-diol was the epimer which was formed in greater yield by catalytic reduction of the corresponding C-20 ketone to the alcohol. More recently the "absolute" configuration at C-20 of 5β-pregnane-3α,20β-diol (mp 237°) has been determined.[7] Fortunately, the arbitrary configuration which was assigned originally corresponds to the Fischer convention for designating the actual arrangement in space of the groups at C-20. According to the Fischer convention,[7,9] this substance is called 5β-pregnane-3α,20β-diol.

Removal of the C-11 Oxygen Function.—For removal of the C-11 oxygen function by two different processes, it was expedient to synthesize compound III and to convert it into compound V by pathway III to IV to V and by pathway III to VI to VIII to V.

Reduction of 12α-bromo-3α,21-diacetoxy-5β-pregnane-11,20-dione (II) with sodium borohydride followed by removal of the acetyl groups by acid hydrolysis gave a mixture which was difficult to purify by crystallization. After column chromatography in formamide–chloroform in the presence of borax,[10] the pure 3α,-20β,21-triol (III) was obtained in 79% yield. Removal of the 12α-bromine atom from III by zinc in acetic acid gave the known 3α,20β,21-trihydroxy-5β-pregnan-11-one[3] (IV) and thereby established identical C-20 configurations in III and IV.

Removal of the 11-oxo substituent from chromatographically pure triolone (IV) was accomplished by a modified Wolff–Kishner procedure. By using diethylene glycol as the solvent and rigorously excluding moisture,[12] V was obtained from IV in 70% yield. During isolation of V by column chromatography, no 20α epimer (5β-pregnane-3α,20α,21-triol) could be detected in the reaction mixture.

The other pathway for converting the 11-oxo-12α-bromotriol (III) into the 11-deoxytriol (V) is *via* the bromohydrin (VI) and the Δ^{11}-triol (VIII). Treatment of either the 11-keto-12α-bromotriol (III) or the 11-keto-12α-bromoketol (I) with lithium borohydride[13] gave the bromohydrin (VI) as the principal crystalline product. The reaction product (possibly complexed with borate) was always obtained as a gum which was difficult to crystallize. However, crystals could be obtained by stirring it mechanically in a mixture of chloroform and 1 *N* hydrobromic acid. Under these conditions the bromohydrin (VI) was obtained from I in about 47% yield. After crystalline material had been obtained it could be recrystallized readily from any of several solvents.[14]

The bromohydrin (VI) could be prepared also from the Δ^{11}-triol (VIII). Acetylation of the Δ^{11}-triol gave a product which could not be induced to crystallize but which was chromatographically homogeneous. Addition of HOBr to this Δ^{11}-triol triacetate (IX), under conditions described by Sarett,[15] gave the amorphous bromohydrin (VII) which, upon acid hydrolysis, yielded the same bromohydrin (VI) as was obtained by reduction of I and of III.

That the C-11 hydroxyl group in the bromohydrin (VI) was in the β orientation was shown by catalytic debromination to the previously prepared tetrol[2] (X) which gave a triacetate on acetylation under mild conditions. The triacetate (XI) was oxidizable to the known 3α,20β,21-triacetoxy-5β-pregnan-11-one[2] with chromic acid in acetic acid at 15°.

The elimination of HOBr from the bromohydrin (VI) to give the Δ^{11}-triol (VIII) proved to be difficult. A small amount of Δ^{11}-triol (VIII) was formed by treatment of the bromohydrin with zinc in glacial acetic acid or zinc in aqueous acetic acid at room temperature, but most of the bromohydrin was recovered unchanged. When the zinc–acetic acid–steroid mixture was heated, the steroid was partially acetylated without removal of most of the HOBr. Powdered zinc in boiling methanol removed HOBr slowly and so did alcoholic chromous chloride. The best method found employed zinc in a mixture of *n*-butyl alcohol and 10 *N* sulfuric acid at the reflux temperature. After chromatography of the complex reaction mixture the Δ^{11}-triol (VIII) was obtained from VI in 22% yield.

The Δ^{11}-triol (VIII) was reducible with platinum oxide in acetic acid to the corresponding saturated compound (V) which has been obtained from IV by Wolff–Kishner reduction. This 20β-triol (V) has been prepared and characterized by Lewbart[16] and Schneider.

Efforts to convert the tetrol (X) into the triol (V) *via* the $\Delta^{9(11)}$-triol (XII) were unsuccessful. 5β-Pregnane-3α,11β,20β,21-tetrol (X) was acetylated to give the triacetate (XI) which could not be induced to crystallize. It was dehydrated in acetic acid–boron trifluoride to give the 9(11)-unsaturated derivative (XIII) which, upon hydrolysis, yielded the crystalline, unsaturated triol (XII). Attempts to hydrogenate the 9(11) double bond of XII and of its triacetate (XIII) using Adams platinum oxide in acetic acid at 1 atm and also using platinum supported on charcoal failed. The 9(11) double bond of XII was not reduced in

(8) R. E. Marker, O. Kamm, E. L. Wittle, T. S. Oakwood, E. J. Lawson, and J. F. Laucius, *J. Am. Chem. Soc.*, **59**, 2291 (1937).

(9) L. F. Fieser and M. Fieser, *Tetrahedron*, **8**, 360 (1960).

(10) Schneider and Lewbart[11] reported that a borate buffer is useful for improving the chromatographic resolution of mixtures of steroids which have glycol functions associated with the side chain and are epimeric at C-20. In this laboratory, chromatography systems which contained borax were particularly useful for checking the homogeneity of several steroidal glycols which have been prepared. The use of a 3% solution of borax in formamide as the stationary phase and chloroform as the mobile phase made possible the chromatographic separation, on a preparative scale, of III from a slower moving contaminant and also of V from a less mobile component (presumably its 20α epimer). Compound V had been prepared by borohydride reduction of the corresponding 20-ketone and both C-20 isomers should have been formed. The chromatographic system of borax–formamide–chloroform is not stable; it should be used soon after it is prepared if the beneficial effect of borax is to be obtained.

(11) J. J. Schneider and M. L. Lewbart, *Federation Proc.*, **22**, 468 (1963); *Tetrahedron*, **20**, 943 (1964).

(12) D. H. R. Barton, D. A. J. Ives, and B. R. Thomas, *J. Chem. Soc.*, 2056 (1955).

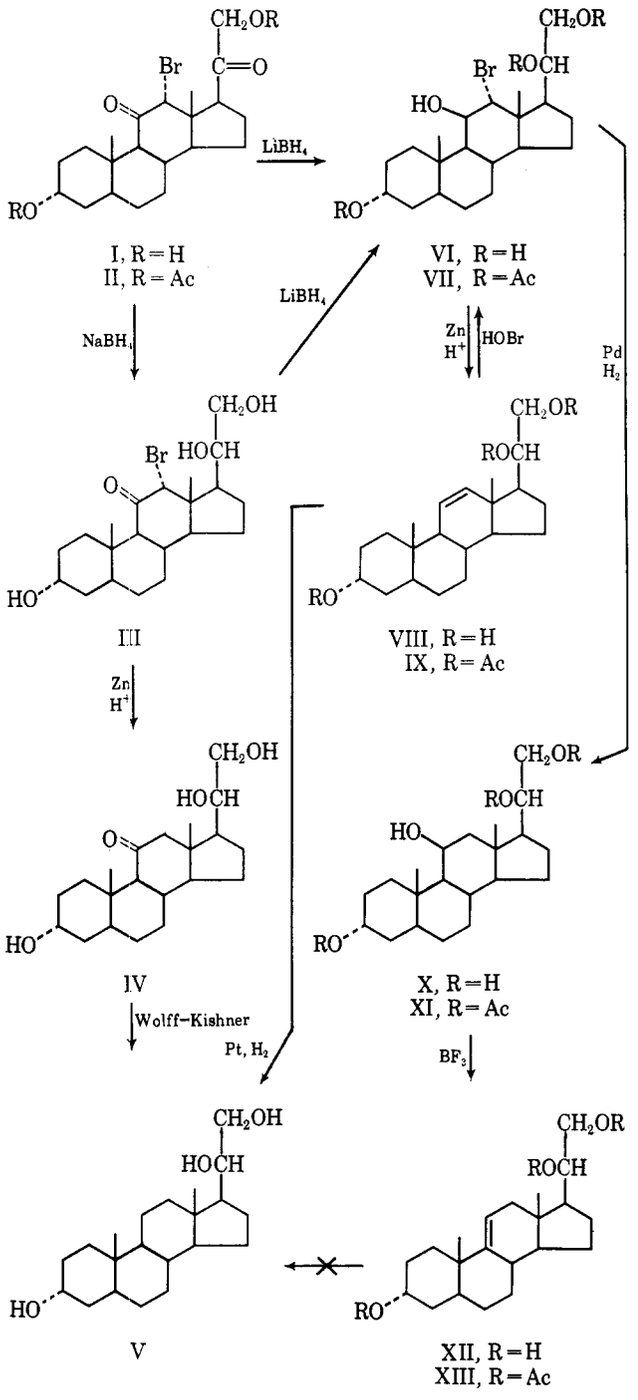
(13) D. Taub, R. D. Hoffsommer, and N. L. Wendler, *J. Am. Chem. Soc.*, **79**, 452 (1957).

(14) Whereas compound VI was homogeneous, as judged by chromatography in systems A, B, D, E, F, and G, it gave two spots (R_f 0.10 and 0.21) when chromatographed in system F with borax. By chromatographing compound VI in system F with various molar ratios of borax to steroid (the range extended from one boron atom per four steroid molecules up through 200 boron atoms per one steroid molecule), it was possible to show that formation of two spots was dependent on a particular borax–steroid ratio rather than on the presence of an impurity in compound VI. These results will be published elsewhere.

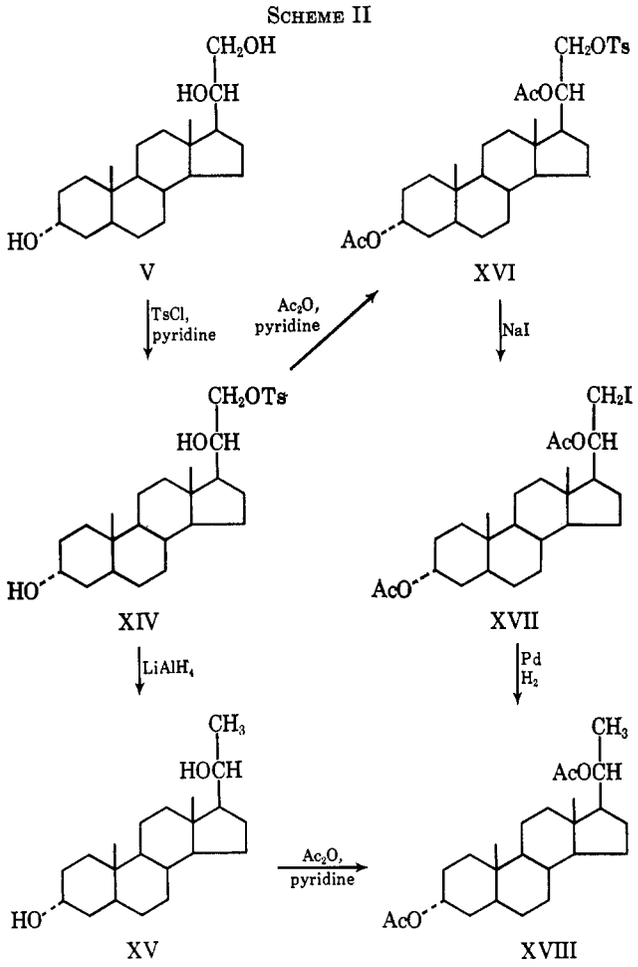
(15) L. H. Sarett, *J. Biol. Chem.*, **162**, 613 (1946).

(16) We are indebted to Dr. M. L. Lewbart for a sample of 5β-pregnane-3α,20β,21-triol and of its 20α epimer.

SCHEME I



SCHEME II



alcohol with Adams catalyst and hydrogen at 150° and 17 atm.[17] (See Scheme I.)

Removal of the C-21 Hydroxyl Group.—The 3α,20β,-21-triol (V), upon treatment with tosyl chloride and pyridine, gave a mixture of tosylates from which pure 5β-pregnane-3α,20β,21-triol 21-tosylate (XIV, Scheme II) was obtained by chromatography. Treatment of the 21-tosylate with lithium aluminum hydride in boiling ether gave predominantly 5β-pregnane-3α,20β-diol (XV); a trace of 5β-pregnane-3α,20β,21-triol (V) was formed. 5β-Pregnane-3α,20α-diol was not formed in detectable amount. The 5β-pregnane-3α,20β-diol was acetylated to yield a diacetate with properties identical with those of 5β-pregnane-3α,20β-diol diacetate. Since the configuration of the hydroxyl group at C-20 is not disturbed in the transformation of the triol (V) to the tosylate (XIV) or in the conversion of the tosylate to pregnanediol (XV) these reactions establish that the 5β-pregnane-3α,20,21-triol of mp 212–213° contains a 20β-hydroxyl group.

It was possible to remove the oxygen function from C-21 of the tosylate (XIV) in another manner. Acetylation of the tosylate gave the 3,20-diacetyl derivative (XVI). The C-21 tosyloxy group was replaced with iodine by treatment with sodium iodide in acetic anhydride.[18] Subsequently, the iodine atom at C-21 was removed by hydrogenolysis in the presence of palladium and ethyl morpholine to yield 5β-pregnane-3α,20β-diol diacetate (XVIII).

The correlation of the C-20 configuration of 3α,20,21-trihydroxy-5β-pregnan-11-one (IV), mp 233–234.5°, with that of 5β-pregnane-3α,20β-diol by the series of reactions which has been described shows conclusively that the absolute configuration of the C-20 hydroxyl group in the former compound is β. From previous work[2] it follows that the configuration of the oxygen function at C-20 in 3α,20-diacetoxy-11-oxo-5β-pregnan-21-oic acid, mp 199–200°, is also β.

The assumption that the 11-oxo group, which is not in the immediate vicinity of C-20, would not abolish the dextrorotatory effect which was produced by acetylation of the 20β-hydroxy group[4] in the 11-deoxy-5β-pregnane-20β-ols is fully verified. However, marked alterations in structure of that part of the molecule

(17) We are indebted to Dr. R. M. Dodson for use of the high-pressure hydrogenation apparatus.

(18) W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 137 (1942).

adjacent to the 20β-hydroxyl group—a change in orientation of the pregnane side chain[19] from 17β to 17α or the conversion of C-21 to a carboxyl group[6]—make the rule inapplicable.

Experimental Section

Paper chromatography was performed at room temperature by the descending method. The following solvent systems were used: A, toluene–ethyl acetate–methanol–water (18:2:10:10); B, isooctane–toluene–methanol–water (225:275:400:100); C, isooctane–toluene–methanol–water (350:150:400:100); D, benzene–*t*-butyl alcohol–methanol–water (10:1:4:6); E, isooctane–*t*-butyl alcohol–water (50:25:45); F, chloroform–formamide; G, butyl acetate–formamide–water; H, kerosene with 2:1 ethanol–water. In systems F and G the paper was impregnated with 40% formamide in acetone; in system H, 40% kerosene in acetone was used. For system G the mobile phase was *n*-butyl acetate saturated with formamide–water (1:1). When borax was used with systems A to E the paper was dipped in a solution of 2% borax in water and dried for 30 min in air before the steroid was applied.[11,20] When borax was used in systems F and G the paper was impregnated by drawing it through a freshly prepared solution which was made by dissolving 3.0 g of finely powdered borax in 100 ml of formamide at room temperature and adding 150 ml of methanol. Steroids were detected by dipping the papers in 4% phosphomolybdic acid in absolute alcohol and heating at 80–90° for 5–10 min. When borax was present the papers had to be treated two to four times to obtain satisfactory color development.

Analyses were by Mr. Joseph F. Alicino, Metuchen, N. J. Rotations were taken in methanol. Melting points were taken on a Fisher-Johns apparatus and are corrected.

12α-Bromo-3α,20β,21-trihydroxy-5β-pregnan-11-one (III) from II.—12α-Bromo-3α,21-diacetoxy-5β-pregnane-11,20-dione[21] (5.11 g, 10.0 mmoles) was dissolved in 200 ml of warm 95% ethanol and the solution was cooled to 25°. Some of the steroid crystallized. A solution of 1.5 g of sodium borohydride in 100 ml of 95% ethanol was added to the vigorously stirred suspension of steroid during 3 min. The temperature did not rise above 25°; all of the steroid dissolved within 3 min. Ten minutes later the solution was placed in an ice bath and 4.0 ml of glacial acetic acid was added at such a rate that the temperature remained below 25°. The solution was concentrated to a syrup and 100 ml of chloroform and 15 ml of water were added. The aqueous phase was separated and washed with two 50-ml portions of chloroform. The aqueous phase contained 0.97% of the theoretical amount of bromide ion. The combined chloroform extracts were washed with water, filtered through anhydrous sodium sulfate, and evaporated to an oil *in vacuo*.

A. Hydrolysis.—The residue was dissolved in 27 ml of chloroform, and 93 ml of methanol, 7 ml of water, and 11 ml of concentrated hydrochloric acid were added. After 24 hr, 20 g of sodium acetate in 25 ml of water was added and the solution was concentrated *in vacuo* to about 10 ml and diluted with 40 ml of water. The solution was extracted with three 300-ml portions of chloroform. The organic phase was washed with dilute sodium hydroxide and water and taken to dryness *in vacuo*. The crude product contained a small amount of material (presumably the 20α isomer) which was not readily removed by crystallization. The relative chromatographic mobilities of the by-product and chief product were as follows: system B (1:1.36), system F (1:1.17), system F with borax (1:2.7), system G with borax (1:2.0).

B. Chromatography.—Finely ground borax ($Na_2B_4O_7 \cdot 10H_2O$) was dissolved in formamide to give a 3% solution. The freshly prepared formamide–borax solution was used to equilibrate chloroform (about 1.6% by volume of this solution was required to give two phases) for the mobile phase for the chromatogram. Hyflo Super Cel (700 g) was suspended in 5300 ml of mobile phase and while the mixture was being stirred vigorously with a mechanical stirrer 280 ml of 3% borax in formamide was added. The mixture was packed in a 9.5-cm diameter glass cylinder to give a column 26 cm high.

The steroid was dissolved in a mixture of 7.5 ml of 3% borax in formamide and 15 ml of chloroform. It was mixed thoroughly with 18 g of Hyflo Super Cel and the mixture was packed evenly on top of the column. The column was eluted with mobile phase and 21-ml fractions were collected. Aliquots of selected fractions were analyzed by paper chromatography. Fractions 85–259 were combined, washed with dilute sodium hydroxide and water, and concentrated *in vacuo*. A total of 3.39 g (79%) of chromatographically pure 12α-bromo-3α,20β,21-trihydroxy-5β-pregnan-11-one was obtained. It crystallized from ethyl acetate with solvent of crystallization. Loss of weight at 100° (1 mm) was 9.1%; calcd for 0.5 mole of ethyl acetate, 9.34%, mp (on apparatus at 160°) 177–178°, mp (on apparatus at 181°) 183–184°, $[\alpha]D$ −20 ± 2°.

Anal. Calcd for $C_{21}H_{33}BrO_4$: C, 58.74; H, 7.75; Br, 18.61. Found: C, 58.74; H, 7.84; Br, 18.24.

3α,20β,21-Trihydroxy-5β-pregnan-11-one (IV) from III.—Zinc dust (200 mg) was added in four portions at 15-min intervals to a solution of 115 mg of 12α-bromo-3α,20β,21-trihydroxy-5β-pregnan-11-one in 2.0 ml of glacial acetic acid while the mixture was being shaken mechanically. After 30 min the solution was filtered and concentrated to dryness. The residue was distributed between ethyl acetate and water. The organic phase was taken to dryness and the residue gave 84 mg (89%) of crystals (mp 233–234.5°) from aqueous methanol. This product did not depress the melting point of the triolone prepared by alkaline hydrolysis of 3α,20β-diacetoxy-21-hydroxy-5β-pregnan-11-one.[2]

5β-Pregnane-3α,20β,21-triol (V) from IV.—One gram of sodium was added to 50 ml of dry, redistilled diethylene glycol and the solution was warmed until the sodium had dissolved. Throughout the entire procedure, rigorous precautions were taken to exclude moisture from the reaction mixture. Ten milliliters of the solution was added to 309 mg of chromatographically pure (system F with borax) 3α,20β,21-trihydroxy-5β-pregnan-11-one, the solution was heated to 150°, and dry hydrazine (10 ml of 95% hydrazine plus 10 g of sodium hydroxide refluxed 3 hr)[12] was distilled into the mixture until the solution refluxed freely at 150°. The solution was refluxed at this temperature overnight, the hydrazine was then distilled off until the temperature in the flask increased to 210°, and the solution was refluxed for 24 hr and cooled. Water (30 ml) was added and the semicrystalline product was collected, washed with water, dissolved in methanol, and filtered. The residue from the filtrate (263 mg) contained no material with the chromatographic mobility of starting material (IV) or of 5β-pregnane-3α,20α,21-triol[16] in systems A, F, and G with borax; the principal product migrated at the same rate as 5β-pregnane-3α,20β,21-triol. During chromatography on paper in system F, 5β-pregnane-3α,-20β,21-triol[16] migrated 1.07 as fast as its 20α epimer;[16] in system F with borax the 20β compound migrated 2.35 as fast as the 20α substance.

The residue was chromatographed on Celite (200 g plus 80 ml of formamide which contained 2.4 g of borax) with chloroform saturated with formamide as the mobile phase. Crystallization of the appropriate fraction from ethyl acetate gave 206 mg (70% yield) of product which melted at 212.5–213°, did not depress the melting point of 5β-pregnane-3α,20β,21-triol, and had an infrared spectrum identical with that of this substance.[16]

5β-Pregnane-3α,20β,21-triol (V) from VIII.—A solution of 62 mg of 5β-pregn-11-ene-3α,20β,21-triol in 10 ml of glacial acetic acid was shaken with 25 mg of Adams platinum oxide until the catalyst coagulated and hydrogen uptake ceased (6 min). An additional 25 mg of catalyst was added and shaking was continued until uptake of hydrogen ceased. The mixture was then treated with a third 25-mg portion of catalyst in the presence of hydrogen. The spent catalyst was removed and the product was crystallized from methyl ethyl ketone. It contained a small amount of impurity which migrated chromatographically (system B, 15 hr) at the same rate as starting material (and 0.81 the rate of the main product) and which was not readily removed by crystallization. By chromatography on a 1.8 × 34-cm column containing 50 g of Celite and 25 ml of the heavier phase of system B, 35 mg of pure triol V was obtained. The triol, crystallized from ethyl acetate, melted at 212.5–213.5° and did not depress the melting point of a sample of 5β-pregnane-3α,20β,21-triol[16] which had been prepared by sodium borohydride reduction of 3α,21-dihydroxy-5β-pregnan-20-one. The infrared spectra of the two samples of triol V were identical.

12α-Bromo-5β-pregnane-3α,11β,20β,21-tetrol (VI) from I.—A solution of 12α-bromo-3α,21-dihydroxy-5β-pregnane-11,20-dione[21]

(19) D. M. Glick and H. Hirschmann, *J. Org. Chem.*, **27**, 3212 (1962).

(20) We are indebted to Dr. John J. Schneider for directions for use of borax with Bush-type chromatography systems.

(21) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 2001 (1963).

(4.85 g, 10 mmoles) in 80 ml of benzene was concentrated *in vacuo* to dryness to remove acetone of crystallization from the steroid. The residue was dissolved in 475 ml of dry tetrahydrofuran in an atmosphere of hydrogen and cooled to −11° in a flask fitted with a thermometer, magnetic bar, and separatory funnel. A solution of 1.76 g of lithium borohydride in 125 ml of tetrahydrofuran was added during 25 min; the temperature was kept below 0°. The mixture was stirred at about 0° for 4 hr and then cooled to −10°. A mixture of 15 ml of acetic acid plus 117 ml of water was added slowly with stirring and the solution was concentrated *in vacuo* to 80 ml; 100 ml of water was added and the solution was again concentrated to 80 ml to remove all tetrahydrofuran. Water (100 ml) was added and the solvent was decanted from the gum. The decantate contained 3.8 mmoles (38% of theory) of bromide ion. To the gum, 235 ml of chloroform and 25 ml of water were added and the solution was stirred mechanically. When no crystals had formed after 30 min, 25 ml of 1.0 *N* aqueous HBr was added and stirring was continued. Crystals started to form immediately. After 7 hr the crystals were collected and washed with chloroform and with water, yield 2.03 g (47%). A sample for analysis was prepared by recrystallization from methanol: mp 199–200°, $[\alpha]_D$ +60° ± 2°.

Anal. Calcd for $C_{21}H_{35}BrO_4$: C, 58.46; H, 8.17. Found: C, 58.22; H, 8.45.

12α-Bromo-5β-pregnane-3α,11β,20β,21-tetrol (VI) from III.—Chromatographically pure (system F with borax) 12α-bromo-3α,20β,21-trihydroxy-5β-pregnan-11-one (2.14 g) was treated with lithium borohydride as described in the previous paragraph except that the hydrogen bromide was omitted when the gummy product was stirred with chloroform. The product, dried at 100°, weighed 481 mg (22%), mp 187–189°. After crystallization from methanol the melting point was 197–197.5°. Its infrared spectrum was identical with that of VI described in the previous paragraph.

12α-Bromo-5β-pregnane-3α,11β,20β,21-tetrol (VI) from VIII.—A sample of 5β-pregn-11-ene-3α,20β,21-triol (56 mg) was acetylated in 1.0 ml each of acetic anhydride and pyridine during 16 hr at room temperature. It was not possible to crystallize the acetate (IX). The product was homogeneous by thin layer chromatography on silica gel G (methylene chloride with 3% *t*-butyl alcohol, R_f 0.49, detection with iodine vapor) and by paper chromatography in system H (16 hr, detection with phosphomolybdic acid).

A. Addition of HOBr.—A solution of 60 mg of amorphous 5β-pregn-11-ene-3α,20β,21-triol triacetate in a mixture of 1.4 ml of *t*-butyl alchol and 0.35 ml of water was cooled to 15°. Forty milligrams of N-bromoacetamide and 0.35 ml of 0.8 *N* sulfuric acid were added.[15] After 8 min an excess of aqueous sodium bisulfite was added and the solution was washed with 10 ml of water and extracted with chloroform. The chloroform phase was washed with water and taken to dryness *in vacuo*.

B. Hydrolysis.—The residue (VII) was dissolved in a mixture of 2.6 ml of chloroform, 9.0 ml of methanol, 0.7 ml of water, and 1.1 ml of concentrated hydrochloric acid. After 36 hr water was added, the solution was extracted with chloroform, and the extract was washed and concentrated to dryness.

C. Chromatography.—The residue was chromatographed on 36 g of Celite (which was impregnated with 10.8 ml of formamide) and the product was eluted with chloroform saturated with formamide. The bromohydrin weighed 24 mg. It migrated chromatographically[14] on paper in systems A, B, F, and G (and in systems F and G in the presence of borax) at the same rate as VI which had been prepared from I and from III. The product, crystallized from methanol, melted at 195–196.5°. The infrared spectrum of the substance in Nujol was identical with that of VI which was prepared from the bromo ketone (III).

5β-Pregn-11-ene-3α,20β,21-triol (VIII) from VI.—To a solution of 390 mg of 12α-bromo-5β-pregnane-3α,11β,20β,21-tetrol in 18 ml of butyl alcohol–10 *N* sulfuric acid (4:1) was added 775 mg of powdered zinc. While being stirred vigorously the solution was heated under reflux for 15 min and then cooled. The zinc was filtered off and the filtrate was diluted with 10 ml of water and extracted with chloroform. The aqueous phase contained 85% of the theoretical amount of Br. The organic phase was washed with an excess of sodium bicarbonate and with water and was taken to dryness. Paper chromatography of an aliquot of the product (system B, developed for 15 hr) showed the presence of five substances. The residue was chromatographed on a 1.8 × 34 cm column of 50 g of Celite containing 25 ml of the heavier phase of the system, isooctane–benzene–methanol–water (225:275:400:100). The triolene (VIII) began to appear after 720 ml of effluent had been collected, 67 mg, yield, 22%. The sample for analysis was crystallized from methyl ethyl ketone: mp 190.5–191°, $[\alpha]_D$ +36 ± 2°. It was chromatographically homogeneous in system F with borax (R_f 0.59) and in system G with borax (R_f 0.43).

Anal. Calcd for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.45; H, 10.11.

5β,Pregnane-3α,11β,20β,21-tetrol (X) from VI.—A solution of 100 mg of 12α-bromo-5β-pregnane-3α,11β,20β,21-tetrol (0.233 mmole) in 20 ml of 95% ethanol plus 0.20 ml of ethyl morpholine was shaken with 200 mg of $Pd(CaCO_3)_x$[22] in an atmosphere of hydrogen until the catalyst separated from the support and coagulated and the uptake of hydrogen ceased. The solution was shaken under hydrogen with five successive 400-mg portions of catalyst in an attempt to remove all of the halogen. Then it was filtered and taken almost to dryness. The residue was mixed with chloroform and the solution was washed with water and taken to dryness. Paper chromatography of an aliquot in system D indicated starting material (R_f 0.56) and 5β-pregnane-3α,11β,-20β,21-tetrol (R_f 0.33) in a ratio of about 1:10. Crystallization from methyl ethyl ketone gave 48 mg (59%) of material which melted at 203.5–204.5° (homogeneous on chromatography in system G with added borax, R_f 0.18) and did not depress the melting point of 5β-pregnane-3α,11β,20β,21-tetrol[2] which had been prepared by reduction of 3α,21-dihydroxy-5β-pregnane-11,20-dione. The infrared spectra of the two samples of tetrol were identical.

5β,Pregn-9(11)-ene-3α,20β,21-triol (XII) from X.—A solution of 353 mg of 5β-pregnane-3α,11β,20β,21-tetrol[2] in 3.0 ml each of acetic anhydride and pyridine stood at room temperature for 18 hr. Ice was added, the product was extracted with chloroform, and the solution was washed with dilute hydrochloric acid, sodium bicarbonate solution, and water, and taken to dryness. Persistent attempts to crystallize the product (XI) ended in failure.

A. Dehydration.—To the residue in 25 ml of glacial acetic acid was added 1.0 ml of boron trifluoride etherate at room temperature. After 18 hr the mixture was worked up in the manner described in the previous paragraph. The product (XIII) could not be crystallized.

B. Hydrolysis.—A solution of the residue in 5.7 ml of methanol and 1.3 ml of 4.0 *N* methanolic potassium hydroxide was refluxed for 10 min, diluted with 3.7 ml of water, and refluxed for an additional 5 min. Water was added to the point of turbidity and crystals formed (309 mg, 92%, mp 213–215°) as the solution cooled. After recrystallization from methyl ethyl ketone the product (XII) melted at 216–217°, $[\alpha]_D$ +33 ± 2°. The product was chromatographically homogeneous in solvent systems B, F with borax (R_f 0.33), and G with borax (R_f 0.47).

Anal. Calcd for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.78; H, 10.23.

5β-Pregnane-3α,20β,21-triol 21-(*p*-Toluenesulfonate) (XIV) from V.—A precooled solution of 209 mg (1.10 mmoles) of *p*-toluenesulfonyl chloride in 2.0 ml of dry pyridine was added to 337 mg (1.00 mmole) of 5β-pregnane-3α,20β,21-triol and the resulting solution was maintained at 0° for 4.5 hr. Water was added and after 20 min the mixture was extracted with chloroform. The extract was washed with dilute hydrochloric acid, sodium hydroxide solution, and water and then taken to dryness *in vacuo*. Crystals (114 mg, mp 180–181°) were obtained from ethyl acetate. The residue from the filtrate was chromatographed on a 1.8-cm diameter column of Hyflo Super Cel (50 g) which contained 25 ml of stationary phase from system C; 6.8-ml fractions were collected. Two relatively mobile substances (combined weight 156 mg) were eluted in fractions 9-19 and 21–31 but were not characterized. Fractions 42–96 yielded 45 mg of crystals (mp 177–180°) and brought the total yield of 21-tosylate to 44%. Finally, toluene was used as the mobile phase and 58 mg (17%) of 5β-pregnane-3α,20β,21-triol was recovered. A sample of 21-tosylate was purified from ethyl acetate: mp 181–182°, $[\alpha]_D$ +16 ± 2°.

Anal. Calcd for $C_{28}H_{42}O_5S$: C, 68.54; H, 8.63; S, 6.53. Found, C, 69.15; H, 8.57; S, 6.52.

5β-Pregnane-3α,20β-diol (XV) from XIV.—A solution of 400 mg of lithium aluminum hydride in 25 ml of absolute ether was added slowly to a vigorously stirred solution of 100 mg of 5β-

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pregnane-3α,20β,21-triol 21-(*p*-toluenesulfonate) and the mixture was refluxed for 4 hr. An excess of ethyl acetate was added slowly and then 30 ml of 2 *N* hydrochloric acid was added. The ether phase was washed with water until neutral, filtered, and concentrated to dryness. Paper chromatography of an aliquot (system B, 2.5 hr) showed a principal product with the mobility of 5β-pregnane-3α,20β-diol (R_f 0.73) and a trace of material with the mobility of 5β-pregnane-3α,20β,21-triol (R_f 0.21). Crystallization from ethanol gave 39 mg (60%) of product, mp 234–235°, which did not depress the melting point of 5β-pregnane-3α,20β-diol; the infrared spectra of the two samples were identical. A second crop of crystals (7 mg, 11%), mp 230–233°, brought the yield to 71%. Aliquots of the crystals and mother liquor were chromatographed for 44 hr on paper impregnated with 30% propylene glycol in acetone and developed with cyclohexane. No 5β-pregnane-3α,20α-diol was detected in the crystals or mother liquor. Standard 5β-pregnane-3α,20β-diol moved 19.2 cm; 5β-pregnane-3α,20α-diol moved 11.6 cm.

5β-Pregnene-3α,20β-diol Diacetate (XVIII) from XIV. A. Acetylation.—A solution of 491 mg (1.00 mmole) of 5β-pregnane-3α,20β,21-triol 21-(*p*-toluenesulfonate) in 2.0 ml each of acetic anhydride and pyridine was heated on a steam bath for 6 min, cooled, and diluted with water. The mixture was extracted with chloroform and the chloroform solution was washed with dilute hydrochloric acid, dilute sodium hydroxide, and water and then taken to dryness *in vacuo*. It was not possible to obtain crysals from the product (XVI).

B. Treatment with Sodium Iodide.—A solution of the material in chloroform was evaporated to dryness *in vacuo;* 25 ml of acetic anhydride and 600 mg of sodium iodide were added. Ten milliliters of acetic anhydride was evaporated and then the solution was heated under reflux for 1 hr and concentrated almost to dryness. The residue was dissolved in chloroform and the solution was washed with water and concentrated to dryness *in vacuo*. Efforts to obtain crystals were not successful.

The residue was chromatographed in a 3.6-cm diameter column which contained 360 g of silica gel and 180 ml of *t*-butyl alcohol.[23] The product was eluted with 1% *t*-butyl alcohol in methylene chloride (11-ml fractions). Fractions 37–54 gave 408 mg of residue (XVII) which could not be induced to crystallize but which appeared to be homogeneous by chromatography on paper in system H for 24 hr.

C. Catalytic Deiodination.—Half of product XVII (204 mg) was dissolved in 20.0 ml of methanol and the solution was shaken in an atmosphere of hydrogen in the presence of 2.0 ml of dry ethyl morpholine and 400 mg of $Pd(CaCO_3)_x$[22] until uptake of hydrogen ceased (45 min). Then it was retreated with hydrogen and an additional 400 mg of catalyst. The solution was filtered and concentrated; the residue was taken into benzene and the solution was washed with water. The aqueous phase contained 0.315 mmole (82% of theory) of iodide ion. The residue from the organic phase was chromatographed on 90 g of silica gel plus 45 ml of *t*-butyl alcohol[23] in a 1.8-cm diameter column and eluted with 1% *t*-butyl alcohol in methylene chloride. Fractions (4.8 ml each) 24–42 gave 124 mg of residue which yielded crystals (114 mg, mp 112.5–113°) from aqueous alcohol. The product did not depress the melting point of authentic 5β-pregnane-3α,20β-diol diacetate (mp 112.5–113°); the infrared spectra of the two samples were identical.

5β-Pregnane-3α,20β-diol Diacetate (XVIII) from XV.—Acetylation of 5β-pregnane-3α,20β-diol, mp 234–235° (prepared from XIV by treatment with $LiAlH_4$), in acetic anhydride–pyridine yielded a product which melted at 111.5–112.5° (lit.[3] mp 112–113°) and which did not depress the melting point of 5β-pregnane-3α,20β-diol diacetate. Its infrared spectrum was identical with that of authentic 5β-pregnane-3α,20β-diol diacetate.

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Synthesis of C-3 Ureido Steroids[1]

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3α-Ureido-5α-androstan-17-one, 3α-ureido-5β-androstan-17-one, and their 3β epimers have been synthesized. The stereospecific synthesis of the 3-aminoandrostan-17β-ols was first carried out. Carbamylation of the amino group was achieved with nitrourea or trichloroacetoisocyanate and oxidation of the 17β-hydroxy group afforded the 3-ureido-17-keto steroids. 3α-Ureido-5α-androstan-17-one was also prepared by an alternate route from the ethylene ketal of 3α-azido-5α-androstan-17-one.

In recent years there has been great interest in the biological activity of nitrogenous steroids from natural sources and from partial synthesis.[3] In general the nitrogen atom in the naturally occurring steroidal alkaloids is present as an alkamine which may vary from simple primary amine to complex tertiary amine. It was therefore interesting that an ureido steroid was isolated from human urine.[4] The compound was obtained after administration of 11β-hydroxy-Δ⁴-androsten-17-one and was characterized as 3α-ureido-11β-hydroxy-Δ⁴-androsten-17-one. It was subsequently demonstrated that the ureido steroid was formed at pH 5 from the allylic metabolite, 3α,11β-dihydroxy-Δ⁴-androsten-17-one, and urea present in the urine.[5] In order to study the biological properties as well as chemical and physical properties of this novel type of steroids, the synthesis of the epimeric saturated 3-ureido-11-deoxy-17-keto steroids has been investigated.

The stereospecific synthesis of the isomeric C-3 amino 5α- and 5β-steroids was examined as a first stage in the synthesis of ureido derivatives. It is well known that cyclic oximes upon reduction with sodium and alcohol afford equatorial amines whereas axial amines are obtained by catalytic hydrogenation. The synthesis of 3β-amino-5α-androstan-17β-ol (Ia) and its 3α-amino epimer IIa from 3-oximino-5α-

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