[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. XVI. ACETYLATION STUDIES ON 3β,5-DIHYDROXY-14-ISO-17-ISO-21-NORPREGNANE-19,20-DIOIC ACID AND ITS ETHYL ESTERS[1]

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The conversion of strophanthidin into 19-norprogesterone and 19-nor-11-desoxycorticosterone was reported from this laboratory in 1944 (1).[2] Both products were amorphous. It was assumed that they were mixtures of stereoisomers, the isomerism involving in particular carbon atom 10. In analogy to conclusions drawn by Swiss investigators on structurally related compounds, carbon atoms 14 and 17 were tentatively assigned the iso-configurations (3). The 19-norprogesterone was found to possess high progestational activity (4). In the meanwhile other workers (5) have prepared by a different route a crystalline, stereochemically pure 19-norprogesterone with normal configurations at carbon atoms 14 and 17, which is likewise a very potent gestagen.

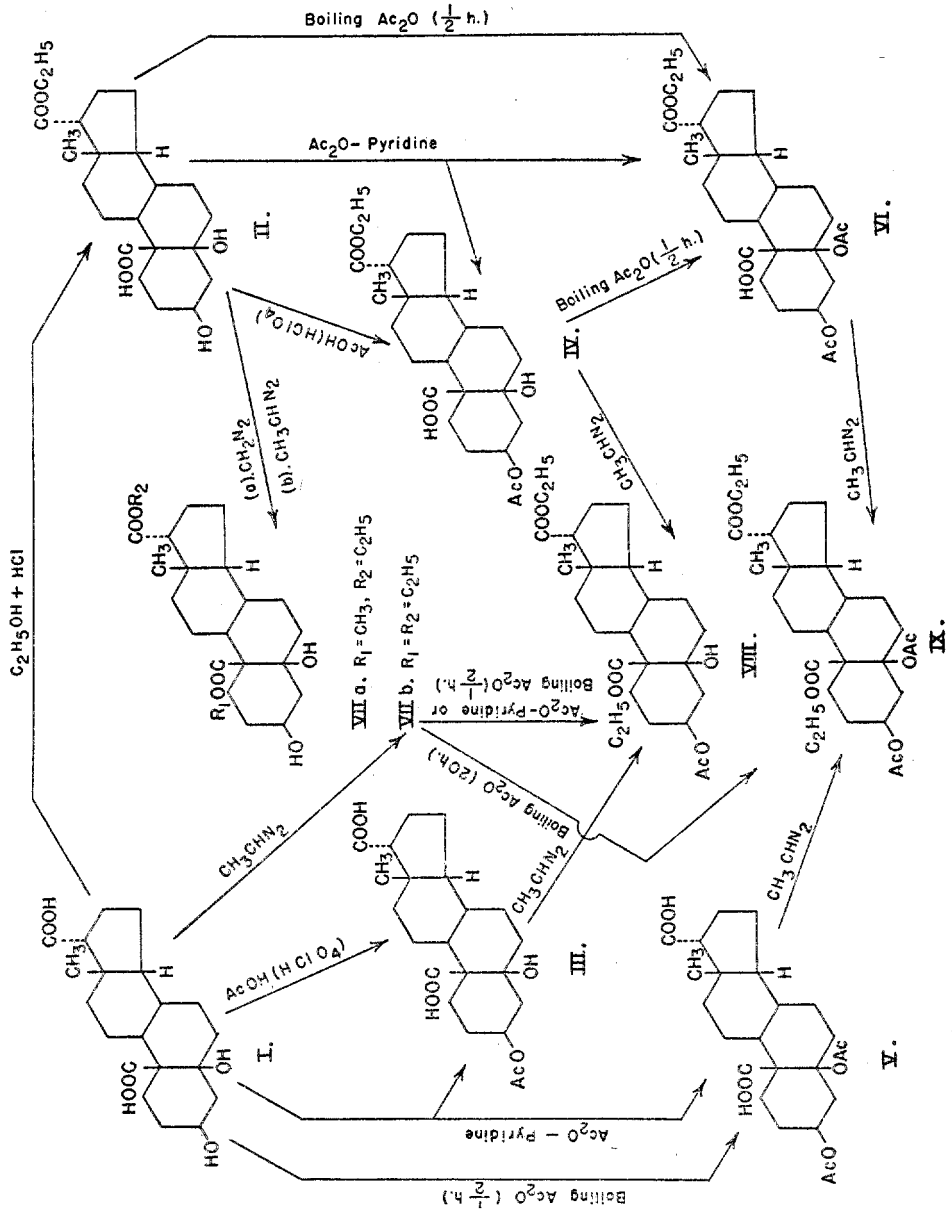
A reinvestigation of the previous route of preparation has been under way in this laboratory with the aim of preparing a stereochemically uniform 19-norprogesterone and related compounds of the 14-iso-17-iso series. A key intermediate in the sequence of transformations was 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (I) which had been characterized by the dimethyl ester (6) and the 20-ethyl ester (II) (2). From the latter (II), the 19-methyl-20-ethyl ester (VIIa) and the diethyl ester (VIIb) have now been prepared with diazomethane and diazoethane respectively. VIIb has also been obtained by the action of diazoethane on I.

In the earlier work (1), the dicarboxylic acid I had been acetylated by two different methods. Reaction of I with pyridine and acetic anhydride at room temperature had yielded a crystalline product melting at 219–222°, whereas treatment of I with boiling acetic anhydride had furnished an amorphous product. On the basis of the analytical results, the interpretation was advanced with reservations that these products represented the 3,5-diacetate (V) and the 3-monoacetate (III) respectively, a conclusion now shown to be erroneous. It is now known that both III and V are hygroscopic when dry and require special precautions on analysis (9).

Inasmuch as the amorphous product from the reaction of I with boiling acetic anhydride served as an intermediate in the preparation of 19-norprogesterone and 19-nor-11-desoxycorticosterone, and especially because all subsequent intermediates in these preparations were also amorphous, a reinvestigation of the acetylation of I has been made. In order to obtain conclusive evidence, it became

[1] This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service.

[2] The original designations were 10-norprogesterone and 10-nor-11-desoxycorticosterone. The present terminology is in better agreement with the established nomenclature (*cf.* 2).


Boiling Ac_2O ($\frac{1}{2}$ h.)
Ac_2O– Pyridine
C_2H_5OH + HCl
CH_3CHN_2
AcOH (HClO$_4$)
Ac_2O – Pyridine
Boiling Ac_2O ($\frac{1}{2}$ h.)
Boiling Ac_2O (20 h.)
Ac_2O-Pyridine or Boiling Ac_2O ($\frac{1}{2}$ h.)
VII a. $R_1 = CH_3$, $R_2 = C_2H_5$
VII b. $R_1 = R_2 = C_2H_5$
I.
II.
III.
IV.
V.
VI.
VIII.
IX.

necessary to study also the acetylation of the 20-ethyl ester (II) and of the diethyl ester (VIIb).

Because of the results obtained on treating I and II with acetic anhydride and pyridine (*vide infra*), a milder acetylating agent was sought. Treatment at room temperature with glacial acetic acid in the presence of a catalytic amount of perchloric acid was found to be sufficiently gentle to acetylate only the 3-hydroxyl group of I and II. When applied to the dicarboxylic acid I, this method gave 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (III), melting at 255–256°. The product crystallized readily and its identity was confirmed by titration. It was reconverted into I by saponification under mild conditions.

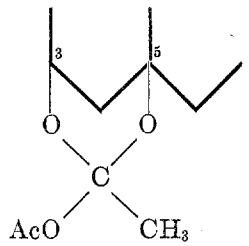
The product obtained from I with boiling acetic anhydride, previously described as amorphous, has now been secured in crystalline form. This substance, melting at 195°, crystallized very slowly and with difficulty even after purification. It was recognized as 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 3,5-diacetate (V) from which I could be regenerated by gentle saponification.

Reaction of I with acetic anhydride and pyridine at room temperature yielded a crystalline mixture of III and V. In a number of experiments, repeated recrystallization yielded material with a constant melting point at 207°, which may be a molecular compound. On chromatography of this product, approximately equal amounts of the pure 3-monoacetate (III) and 3,5-diacetate (V) were obtained. Crystallization of a mixture of equal parts of III and V yielded material with a constant melting point at 208°.

Acetylation of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (II) gave results analogous to those obtained with I. Reaction of II with glacial acetic acid in the presence of perchloric acid at room temperature yielded 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IV), which was reconvertible into II by mild saponification. Reaction of II with boiling acetic anhydride produced a good yield of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester 3,5-diacetate (VI). The identical diacetate (VI) was also obtained by refluxing IV with acetic anhydride. Mild saponification of VI regenerated II, but an attempt to effect a partial deacetylation of VI failed. Treatment of II with pyridine and acetic anhydride at room temperature gave an amorphous reaction product which was subjected to chromatographic separation. Two pure crystalline products were isolated and identified as IV and VI.

There seems to be no instance in the literature of the acetylation of a 5β-hydroxyl group. It has been stated (8) that "the 5β-hydroxyl group present in many cardiac aglycones is not acylable". In contrast to this view, both the formation and saponification of the 3,5-diacetates V and VI proceeded under surprisingly mild conditions. The structures V, VI, and IX have been assigned although it is not certain that these compounds are normal 3,5-diacetates rather than 3,5-orthodiacetates (*cf.* 7). The nomenclature adopted for the diacetates is purposely noncommittal.

It is clear, however, that the acetylation of the 5β-hydroxyl group in I and II is greatly facilitated by the presence of the free carboxyl group at position 10, for the 5-hydroxyl group of the diethyl ester (VIIb) proved to be considerably



3,5-Orthodiacetate.

more resistant to acetylation. Only the 3-monoacetate (VIII) was obtained when VIIb was treated with acetic anhydride and pyridine at room temperature or with acetyl chloride and dimethylaniline in refluxing chloroform. By the latter method, the diacetate of 3β,5-dihydroxycholestane had been prepared (12). Refluxing VIIb with acetic anhydride for one-half hour gave only the 3-monoacetate (VIII), whereas refluxing for three hours gave a mixture containing mainly VIII and a trace of the 3,5-diacetate (IX). Twenty hours' refluxing with acetic anhydride gave a mixture of products consisting principally of the 3,5-diacetate (IX).

The 3-monoacetate VIII was also obtained on treatment of either III or IV with diazoethane.[3] Similarly, reaction with diazoethane of both V and VI yielded the diester-diacetate IX. In fact, IX was most readily prepared in this way from VI. By means of these conversions, the identities of the acetylation products of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (I) are now firmly established.

EXPERIMENTAL

The melting points were determined with the Fisher-Johns melting-point apparatus. The readings are sufficiently near the true melting points so that no corrections have been made. Unless stated otherwise, the microanalyses were carried out by Dr. E. W. D. Huffman, Denver 2, Colorado, on samples which were dried *in vacuo* over phosphorus pentoxide at 80–90°.

3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 19-methyl-20-ethyl ester (VIIa). The 20-ethyl ester (II) (50.0 mg.) upon treatment with diazomethane yielded, after recrystallization of 57.9 mg. of crude product from acetone-petroleum ether and methanol-water, 42.2 mg. of diester, m.p. 170–171°. $[\alpha]_D^{20}$ +37° (20.37 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.76° ±0.02°).

Anal. Calc'd for $C_{23}H_{36}O_6$ (408.52): C, 67.62; H, 8.88.
Found: C, 67.58; H, 8.65.

3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid diethyl ester (VIIb). (*a*) *From the diacid* (I). The 19,20 diacid (I) (20.0 mg.) upon treatment with diazoethane[4]

[3] Similarly, treatment of IV with diazomethane yielded a substance which did not crystallize. Mild hydrolysis of this product with 0.1 *N* ethanolic potassium hydroxide at room temperature yielded VIIa.

[4] Prepared from ethylnitrosourea according to the Organic Syntheses directions for diazomethane (11).

yielded, after recrystallization of 23.7 mg. of crystals from ether-petroleum ether, 16.8 mg. of colorless prisms, m.p. 120°. These were identical with material obtained under (*b*).

(*b*) *From the mono-ethyl ester* (II). The 20-ethyl ester (II) (50.0 mg.) upon treatment with diazoethane[4] yielded, after recrystallization from methanol-water (shining plates) and ether-petroleum ether, 42.4 mg. of large, rectangular prisms, m.p. 121°. $[\alpha]_D^{27}$ +28° (11.98 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.34° ±0.02°).

Anal. Calc'd for $C_{24}H_{38}O_6$ (422.54): C, 68.22; H, 9.06.

Found:[5] C, 68.24; H, 8.94.

Acetylation of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (I) *A. By treatment with glacial acetic acid in the presence of a catalytic amount of perchloric acid: 3β-Acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid* (III). To 50.0 mg. of I (m.p. 262°) in 1 cc. of glacial acetic acid was added approx. 0.01 cc. of 60% perchloric acid and the solution was then kept at room temperature for two days. The mixture was evaporated *in vacuo* (below 0°) to about one-half of its initial volume After diluting with 10 cc of water, the resulting precipitate was extracted with ethyl acetate. This solution was washed with water and was then thoroughly extracted with cold *N* sodium bicarbonate and water. The bicarbonate and subsequent water extracts were combined and acidified to Congo Red with 6 *N* hydrochloric acid. After extracting with ethyl acetate, drying over sodium sulfate, and evaporating, 58.4 mg. of white brittle foam resulted. Recrystallization from acetone-petroleum ether and from acetone-ether gave 37.5 mg. of clusters of short needles with the constant m.p. 255–256°. Additional, less pure material resulted from the mother liquors. $[\alpha]_D^{20}$ +73° (10.99 mg. in 2.0 cc. of chloroform, containing 3 drops of ethanol; *l*, 2 dm., α +0.80° ±0.03°; not corrected for crystal solvent).

Anal. Calc'd for $C_{22}H_{32}O_7$ (408.48): C, 64.68; H, 7.90.

Found:[6] C, 64.61; H, 7.93.

Titration and saponification: A solution of 9.66 mg. of the above III in 1 cc. of neutral ethanol was titrated to a phenolphthalein end point with 0.00825 *N* sodium hydroxide. Consumed: 5.76 cc. Calc'd as 2 equiv. for $C_{22}H_{32}O_7$: 5.73 cc. A total of 10.00 cc. of 0.00825 *N* sodium hydroxide was then added and the solution was kept at room temperature for one week. Back titration indicated a total consumption of 8.69 cc. of 0.00825 *N* sodium hydroxide. Calc'd as 3 equiv. for $C_{22}H_{32}O_7$: 8.60 cc. The titration mixture was finally acidified to Congo Red. Clusters of small needles deposited over a period of several days; 4.2 mg.; m.p. 250–251°; no depression of m.p. with authentic specimen of I.

A second sample of III (10.99 mg.) was titrated with 0.00825 *N* sodium hydroxide (6.38 cc. required; calc'd 6.52 cc.) and the solution was immediately acidified to Congo Red whereupon a crystalline precipitate slowly formed (10.8 mg.). Recrystallization from acetone-petroleum ether gave 8.7 mg. of rosettes of needles; m.p. 257–258°; identified by mixture m.p. as unchanged III.

B. By refluxing with acetic anhydride: 3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 3,5-diacetate (V). A total of 50.0 mg. of I was suspended in 0.5 cc. of redistilled acetic anhydride and the mixture was refluxed (bath at 140–150°) for ½ hour. After the addition of 0.5 cc. of glacial acetic acid and 0.3 cc. of water the heating was continued on a water-bath for one hour.[7] The mixture was evaporated to dryness *in vacuo* (below 0°) yielding a white brittle foam. This was taken up in ether and extracted with *N* sodium bicarbonate. The bicarbonate extract was made acid to Congo Red with 6 *N* hydrochloric acid. After extracting with ether, drying over sodium sulfate, and evaporating, 58.2 mg. of white brittle foam resulted. This was chromatographed over 40 g. of silica

[5] As a precautionary measure special drying (9) was performed though it proved unnecessary.

[6] Special drying (9) was essential.

[7] When this step was omitted neutral material, probably consisting of mixed anhydrides, was obtained, from which V could be prepared by heating with acetic acid and water.

gel[8] (washed with water and dried at 180° for 24 hours); diam. of column 16 mm.; elution time of each 40-cc. fraction, 10–20 minutes. Fractions 1–6, chloroform;[9] fractions 7–12, chloroform-ether (39:1); fractions 13–18, chloroform-ether (19:1); fractions 19–29, chloroform-ether (7:1); fractions 30–37, chloroform-ether (3:1); fractions 38–40, chloroform-ether (1:1); fractions 41–42, ether; fraction 43, methanol. Recovery: All fractions were colorless resins or foams: fractions 1–20, 2.8 mg.; fractions 21–26, 4.2 mg.; fractions 27–29, 4.2 mg.; fractions 30–32, 26.9 mg.; fractions 33–41, 18.4 mg.; fractions 42–43, 0.0 mg. total recovery, 56.4 mg.

The residues from fractions 30 through 32 were recrystallized from methanol-water, yielding clusters of short needles; 19.6 mg.; m.p. 194–195°. The mother liquors were combined with the residues from fractions 27 through 29 and 33 through 41. Recrystallization from methanol-water gave 19.3 mg. of additional crystalline material; m.p. 193–194°. $[\alpha]_D^{21}$ +63° (7.00 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.44° ±0.03°; not corrected for crystal solvent).

Anal. Calc'd for $C_{24}H_{34}O_8$ (450.51): C, 63.98; H, 7.61.

Found:[6] C, 63.81; H, 7.55.

Titration: A solution of 11.19 mg. of V (m.p. 192–193°) in 1 cc. of neutral ethanol was titrated to a phenolphthalein end point with 0.01087 *N* sodium hydroxide. Consumed: 4.41 cc. Calc'd as 2 equiv. for $C_{24}H_{34}O_8$: 4.57 cc. On acidifying to Congo Red, short needles formed slowly (7.6 mg.; m.p. 195°) consisting of unchanged V.

Saponification: A solution of 10.0 mg. of V in 1.0 cc. of 0.0954 *N* ethanolic potassium hydroxide (4.3 equiv. for $C_{24}H_{34}O_8$) was kept at room temperature for 17 hours. After evaporating the mixture to dryness below room temperature, dissolving the residue in 5 cc. of water, and acidifying to Congo Red with 6 *N* hydrochloric acid, the reaction product was extracted with ether. The solvent was dried over sodium sulfate and evaporated, leaving 9.2 mg. of amorphous material which gave from acetone-petroleum ether 3.4 mg. (first crop) of clusters of small, shining needles; m.p. 261–262°; identified as I by mixture m.p.

C. With pyridine-acetic anhydride: 3β-Acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (III) *and 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid, 3,5-diacetate* (V). To 50.0 mg. of I in 0.5 cc. of pyridine was added 0.25 cc. of acetic anhydride. After keeping the mixture at room temperature overnight, 0.25 cc. of acetic acid and 0.1 cc. of water were added.[7] This mixture was heated on the steam-bath for one hour and was then evaporated *in vacuo* below room temperature, yielding a white brittle foam. The foam was dissolved in ether, washed free of pyridine with *N* hydrochloric acid and water, and then extracted with *N* sodium bicarbonate and water. After acidifying the combined bicarbonate extracts to Congo Red, extracting with ether, drying (sodium sulfate), and evaporating, 60.7 mg. of white brittle foam was obtained. Crystallization from ether-petroleum ether gave 52.2 mg. of granular crystals; m.p. 205–207°. Repeated recrystallization did not raise the m.p. above 207°.

A 23.4-mg. sample of this material was chromatographed over 20 g. of silica gel[8] (washed with 1% ammonia and water, then dried at 180°); diam. of column 16 mm.; elution time per 40-cc. fraction, 15–20 minutes. Fractions 1–3, chloroform;[9] fractions 4–6, chloroform-ether (39:1); fractions 7–14, chloroform-ether (14:1); fractions 15–24, chloroform-ether (37:3); fractions 25–27, chloroform-ether (7:1); fraction 28, chloroform-ether (3:1); fraction 29, chloroform-ether (1:1); fraction 30, ether; fraction 31, methanol. Recovery: Fractions 1–13, 0.6 mg. of resin; fractions 14–18, 9.5 mg. of resin; fractions 19–24, 8.9 mg. of crystals; fractions 25–30, 3.7 mg. of crystals; total recovery, 22.1 mg.

The residues from fractions 14 through 18 were combined and crystallized from methanol-

[8] Davison Silica Gel, lot 23-08-X.926, The Davison Chemical Corporation, Baltimore 3, Md.

[9] Dried over calcium chloride and distilled.

water, yielding 5.9 mg. of clusters of short needles; m.p. 196° with effervescence (when placed on the block at 190°; when heating is begun at lower temperatures, the m.p. is 1 to 4 degrees lower). This product was identified as 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 3,5-diacetate (V).

The residues from fractions 19 through 24 were combined and recrystallized from acetone-petroleum ether, yielding 5.6 mg. of long, silky needles; m.p. 256–257°; identified by mixture m.p. as 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (III). The mother liquor from this material was combined with the residues from fractions 25 through 30 and the total was recrystallized from acetone-petroleum ether, giving 5.5 mg. additional III; m.p. 252–254°.

Acetylation of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (II). *A. By treatment with glacial acetic acid in the presence of a catalytic amount of perchloric acid: 3β-Acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester* (IV). To 50.0 mg. of II (m.p. 201°) in 1 cc. of glacial acetic acid was added a droplet of 60% perchloric acid and the mixture was kept at room temperature for 46 hours. After concentrating *in vacuo* to about one-half of the original volume (below 0°), 10 cc. of ice-water was added, causing a curdy, white precipitate which was extracted with ether. The ether solution was washed with water and was subsequently extracted with two 5-cc. portions of cold *N* sodium bicarbonate[10] and two 5-cc. portions of water. After drying over sodium sulfate and evaporation of the ether, 46.1 mg. of white brittle foam (a) resulted. The bicarbonate extracts were cooled in ice and made acid to Congo Red with 6 *N* hydrochloric acid. After extracting with ether, drying (sodium sulfate), and evaporating to dryness, 10.9 mg. of brittle foam (b) was obtained. Crystallization of (a) from ether-petroleum ether and from methanol-water gave 30.5 mg. of short, colorless needles with the constant m.p. 191–192°. $[\alpha]_D^{26}$ +61° (9.37 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.57° ±0.06°).

On chromatographing the product over aluminum oxide, it behaved like a uniform substance. It was eluted by two consecutive combinations of chloroform and methanol (19:1, 3:1). By this procedure the m.p. was raised to 194°.

Anal. Calc'd for $C_{24}H_{36}O_7$ (436.53): C, 66.03; H, 8.31.

Found:[5] C, 65.90; H, 8.32.

Titration: A solution of 10.70 mg. of IV (m.p. 190–191.5°) in 1.0 cc. of neutral ethanol was titrated to a phenolphthalein end point with *0.01087 N sodium hydroxide. Consumed:* 2.22 cc. Calc'd as 1 equiv. for $C_{24}H_{36}O_7$: 2.25 cc. On acidifying to Congo Red, a mass of fine needles (10.2 mg.) formed, consisting of IV. Potentiometric titration of IV in 50% ethanol at 26° indicated that the acid dissociation constant is approximately 2.3×10^{-7}.

Saponification: A solution of 9.18 mg. of IV (m.p. 190°) in 1.00 cc. of 0.0807 *N* ethanolic potassium hydroxide (3.8 equiv.) was kept at room temperature overnight. After acidifying to Congo Red and working up in the usual fashion, 8.0 mg. of brittle foam was obtained. Crystallization from ether-petroleum ether gave 6.2 mg. of clusters of needles; m.p. 200–201°; identified as II by mixture m.p.

B. By refluxing with acetic anhydride: 3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester 3,5-diacetate (VI). A solution of 50.0 mg. of II (m.p. 201°) in 0.5 cc. of redistilled acetic anhydride was refluxed (bath at 145°) for 30 minutes. After adding 0.5 cc. of glacial acetic acid and 0.5 cc. of water, the mixture was heated on a steam-bath for one hour and then evaporated to dryness *in vacuo* (below 0°). After dissolving the residue (white brittle foam) in 20 cc. of ether, the solution was extracted with *N* sodium carbonate and then with water. The cooled carbonate extract was made acid to Congo Red with 6 *N* hydrochloric acid and was then extracted with ether. The ether extract was dried over sodium sulfate and evaporated, leaving 49.8 mg. of white brittle foam. Crystallization from methanol-water gave a mass of long, thin needles. Repeated recrystallization gave 38.6 mg.

[10] The reaction product (IV) is a very weak acid. Hence only a part of it was extracted by the sodium bicarbonate.

with the sharp m.p. 189°. $[\alpha]_D^{26.5}$ +37° (20.12 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.74° ± 0.03°).

Anal. Calc'd for $C_{26}H_{38}O_8$ (478.56): C, 65.25; H, 8.00.

Found:[5] C, 65.18; H, 7.91.

The identical compound (VI) was also obtained from 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IV) as follows: A solution of 20.0 mg. of IV in 0.5 cc. of redist. acetic anhydride was refluxed (bath at 140–145°) for ½ hour and then, after the addition of 0.5 cc. of glacial acetic acid and 5 drops of water, was heated on a steam-bath for an additional period of ½ hour. The reaction mixture was worked up as in the preceding experiment. From the carbonate extract there was isolated 20.2 mg. of foamy acidic material. Crystallization from methanol-water gave 18.2 mg. of short needles; m.p. 183–184°; no depression of m.p. when mixed with an authentic specimen of VI.

Saponification: A solution of 20.1 mg. of VI in 2.0 cc. of 0.0954 *N* ethanolic potassium hydroxide was kept at room temperature overnight. After removal of most of the ethanol *in vacuo* the residue was diluted with water. The aqueous solution was made acid to Congo Red and extracted with ether and the ether phase was in turn extracted with *N* sodium carbonate. From the latter, 18.5 mg. of foamy, acidic material was obtained. Crystallization from ether-petroleum ether and methanol-water yielded 5.5 mg. of crystalline II; m.p. 199–200°; no depression of m.p. on admixture with authentic II. From the mother liquors 4.2 mg. of VI was isolated; m.p. 183–185°; identified by mixture m.p.

Attempted partial saponification: A solution of 11.2 mg. of VI in 0.45 cc. of 0.0988 *N* ethanolic potassium hydroxide (95% of 2 equiv. for $C_{26}H_{38}O_8$) was kept at room temperature overnight, was subsequently heated on a steam-bath for ½ hour, and was worked up in the usual fashion. By recrystallizing the acid fraction from methanol-water, 7.3 mg. of fine needles was obtained; m.p. 184–185°; identified as starting material (VI) by mixture m.p.

C. With pyridine-acetic anhydride: 3β-Acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IV) *and 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester 3,5-diacetate* (VI). To 100.0 mg. of II in 0.5 cc. of pyridine was added 0.5 cc. of acetic anhydride and the mixture was kept at room temperature for 20 hours. After the addition of about 10 g. of ice and acidification to Congo Red with 6 *N* hydrochloric acid, the gummy precipitate was extracted with ether. The ether solution was washed twice with 3 cc. of cold *N* hydrochloric acid, once with 5 cc. of water, and finally with two 5-cc. portions of cold *N* sodium bicarbonate,[11] followed by two 5-cc. portions of water. After drying over sodium sulfate and evaporating, the ethereal phase yielded 115.3 mg. of white brittle foam. A sample (53.3 mg.) of this product was chromatographed over 3 g. of aluminum oxide [activity III–IV (10); diam. of column, 6 mm.; time of elution of each 30-cc. fraction, 15–30 minutes]. The first 22 fractions (consisting of petroleum ether-benzene, benzene, benzene-ether, and ether-chloroform (5:1, 2:1, 1:1) combinations) yielded no appreciable residues (total 2.7 mg.).

Fractions 23 through 26 (ether-chloroform 1:2 and pure chloroform) yielded 13.9 mg. of colorless resin. This material was dissolved in 0.5 cc. of methanol to which water was added until the solution became turbid. After seeding with VI, clusters of fine needles separated: 11.5 mg.; m.p. 185–186°. There was no depression of the melting point when mixed with an authentic specimen of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester 3,5-diacetate (VI).

Fractions 27 through 30 [chloroform and chloroform-methanol (300:1 and 60:1)] yielded 11.6 mg. of resinous residues which were not investigated. Fractions 31 through 34 [chloroform-methanol (60:1, 29:1, 14:1, 5:1)] eluted 22.4 mg. of colorless resin. Crystallization from ether-petroleum ether gave 8.2 mg. of shining needles; m.p. 190–192°; no depression of m.p. when mixed with an authentic specimen of IV.

[11] The reaction products (IV and VI) are very weak acids. Hence only a small part (8.7 mg.) of the acid material was extracted by the sodium bicarbonate.

3β-Acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid diethyl ester (VIII) *A. From 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid diethyl ester* (VIIb). (*a*) *by treatment with pyridine-acetic anhydride.* To 42.4 mg. of VIIb in 1 cc. of pyridine was added 0.5 cc. of acetic anhydride. The mixture was kept at room temperature overnight and was then evaporated to dryness *in vacuo* (room t.). The resulting sirupy residue was taken up in ether, washed with cold *N* hydrochloric acid and water, dried (sodium sulfate), and evaporated to give 46.2 mg. of brittle foam. It was chromatographed over 5.0 g. of aluminum oxide [activity III (10); diam. of column, 6 mm.; time of elution of each 50-cc. fraction, 30 minutes]. The first 8 fractions (petroleum ether and petroleum ether-benzene combinations) yielded only traces of material (total 0.9 mg.). Fractions 9 through 14 [five benzene fractions and one benzene-ether (4:1) fraction] gave a total of 38.8 mg. of resinous residues which, by repeated recrystallization from methanol-water, gave 34.4 mg. of fine needles with constant m.p. 130°. $[\alpha]_D^{27}$ +54° (11.27 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.61° ±0.04°).

Anal. Calc'd for $C_{26}H_{40}O_7$ (464.58): C, 67.21; H, 8.68.

Found:[5] C, 67.09; H, 8.41.

The remaining fractions (15–18) (total wt., 5.2 mg.) yielded from ether-petroleum ether 2.8 mg. of crystalline starting material; m.p. 119°.

(*b*) *By refluxing with acetic anhydride.* (*A*) *refluxing for ½ hour.* A solution of 14.7 mg. of VIIb in 0.5 cc. of redist. acetic anhydride was refluxed (bath at 140–150°) for ½ hour and, after the addition of 5 drops of water and 0.5 cc. of glacial acetic acid, was heated on a steam-bath for one hour. Evaporation of the reaction mixture *in vacuo* (below 0°) yielded 16.1 mg. of white brittle foam which was recrystallized several times from petroleum ether and from methanol-water, yielding 9.8 mg. of long, slender needles, m.p. 126–127°. There was no depression of the m.p. on admixture with an authentic specimen of VIII.

(*B*) *Refluxing for 3 hours.* A solution of 10.0 mg. of VIIb in 0.5 cc. of redist. acetic anhydride was refluxed for 3 hours and was then evaporated to dryness *in vacuo*. Crystallization of the residue from methanol-water gave a mass of long, slender needles; 9.6 mg.; m.p. 64–65°. The crystals and the residue from the mother liquor were combined and the total was dissolved in petroleum ether and cromatographed over 4 g. of aluminum oxide [activity III (10)]. Nothing was eluted by petroleum ether or petroleum ether-benzene combinations, but 240 cc. of pure benzene eluted a total of 8.5 mg. as a single band. Crystallization from methanol-water gave 6.3 mg. of very small needles melting at 65–67° (X).

Anal. Calc'd for $C_{26}H_{40}O_7$ (464.58): C, 67.21; H, 8.68.

Calc'd for $C_{28}H_{42}O_8$ (506.62): C, 66.38; H, 8.36.

Found:[5] C, 66.70; H, 8.62.

This product (X) was apparently a mixture of the 3-monoacetate (VIII) and the 3,5-diacetate (IX). The analytical figures are in agreement with this conclusion and the determination of the infrared absorption spectra[12] of pure VIII, pure IX, and X indicated that X consisted essentially of the 3-monoacetate (VIII), contaminated by a small amount of the 3,5-diacetate (IX). Crystallization of a mixture of 5.0 mg. of VIII and 5.0 mg. of IX from methanol-water yielded 9.0 mg. of small, apparently uniform needles which melted at 69–71°.

(*c*) *By treatment with acetyl chloride and dimethylaniline.* To a solution of 10.0 mg. of VIIb in 2 cc. of chloroform were added 0.1 cc. of dimethylaniline and 0.1 cc. of acetyl chloride. The mixture was refluxed for four hours and was then evaporated *in vacuo*. The viscous residue was taken up in ether and the solution was washed with *N* hydrochloric acid, water, and *N* sodium carbonate. After drying over sodium sulfate and evaporating the ether, the resinous residue (11.4 mg.) was dissolved in petroleum ether and chromatographed over 4 g. of aluminum oxide [activity III (10); diam of column 6 mm.]. Nothing was eluted by petroleum ether-benzene combinations, but the first three benzene fractions (each 40 cc.) yielded 7.8 mg. of resinous residues. Crystallization from methanol-water gave 7.4 mg. as a mass of needles; m.p. 122–126°; identified as VIII by mixture m.p.

B. From 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (III) *with*

[12] Carried out in the laboratories of Dr. K. Dobriner, The Sloan-Kettering Institute for Cancer Research, New York City.

diazoethane. When 5.0 mg. of III (m.p. 255–256°) was treated with diazoethane[4] and the product was recrystallized from methanol-water it gave needles; wt. 3.8 mg.; m.p. 125–127°; no depression of the m.p. when mixed with an authentic specimen of VIII.

C. From 3β-acetoxy-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IV) *with diazoethane.* When 5.0 mg. of IV (m.p. 190–191.5°) was treated with diazoethane,[4] and the product was recrystallized from petroleum ether and then from dilute methanol, it gave a mass of colorless needles; wt. 4.9 mg.; m.p. 127–128°; no depression of the m.p. when mixed with an authentic specimen of VIII.

3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid diethyl ester 3,5-diacetate (IX). *A. From 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid diethyl ester* (VIIb) *by refluxing with acetic anhydride (20 hours).* The monoethyl ester (II) (50.0 mg.) was treated with diazoethane and converted to a crystalline residue of VIIb which was dissolved in 2 cc. of redist. acetic anhydride. This solution was refluxed for 20 hours (bath at 140–150°) and was then evaporated to dryness *in vacuo,* leaving a resinous, yellow residue. By dissolution in ether and extraction with *N* sodium carbonate and water the color was removed, and after drying over sodium sulfate and evaporating the ether 51.1 mg. of white brittle foam was obtained. This material was dissolved in petroleum ether-benzene (3:2) and chromatographed over 5.0 g. of aluminum oxide [activity III (10); diam. of column 6 mm.]. Petroleum ether-benzene combinations and pure benzene eluted a total of 24.9 mg. Crystallization of this material from methanol-water yielded a total of 15.3 mg. of thin plates melting in the range 121–125°. There was no depression of the melting point on admixture with authentic 3,5 diacetate (IX).

B. From 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 3,5-diacetate (V) *with diazoethane.* Compound V (7.0 mg., m.p. 195°) was treated with diazoethane[4] and the resinous residue was crystallized several times from methanol-water; very small needles; wt. 3.8 mg. m.p. 117–118°; no depression of the m.p. when mixed with authentic IX.

C. From 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester 3,5-diacetate (VI) *with diazoethane.* Compound VI (5.0 mg., m.p. 189°) was treated with diazoethane[4] and the residue of brittle foam was crystallized from methanol-water, yielding 4.1 mg. of colorless platelets; m.p. 123.5°; no depression of the m.p. on admixture with authentic IX.

IX was also prepared from II by acetylation (refluxing with acetic anhydride; *vide supra*) and subsequent treatment of the crude VI with diazoethane. Thus, 50.0 mg. of II (m.p. 200–201°) gave a first crop of 48.9 mg. of crystalline IX. Repeated crystallization from methanol-water and from petroleum ether yielded 39.0 mg. of thin plates; m.p. 126°. $[\alpha]_D^{26.5}$ +31° (21.23 mg. in 2.0 cc. of chloroform; *l*, 2 dm., α +0.66° ±0.03°).

Anal. Calc'd for $C_{28}H_{42}O_8$ (506.62): C, 66.38; H, 8.36.

Found:[5] C, 66.16; H, 8.17.

Saponification: A solution of 21.2 mg. of IX (m.p. 126°) in 2.0 cc. of 0.0954 *N* ethanolic potassium hydroxide (4.3 equiv.) was kept at room temperature for 20 hours. The mixture was concentrated to a small volume *in vacuo* and, after the addition of 1 cc. of water, was made acid to Congo Red with 6 *N* hydrochloric acid and extracted with ether. The ether was washed with *N* sodium carbonate, dried over sodium sulfate, and evaporated, yielding 17.2 mg. of crystalline residue. Recrystallization from methanol-water gave 15.4 mg. as a mass of needles; m.p. 119–120°; no depression of the m.p. when mixed with an authentic specimen of VIIb.

Attempts to effect a partial deacetylation of the diacetate IX were unsuccessful. Treatment of IX with one equivalent of 0.1 *N* alcoholic potassium hydroxide or with 0.1 *N* alcoholic hydrogen chloride yielded only unchanged IX.

SUMMARY

1. The acetylation reactions under various conditions of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (I) and its 20-ethyl ester (II) and diethyl ester (VIIb) have been studied.

2. On treatment at room temperature with glacial acetic acid containing a trace of perchloric acid, I and II yielded exclusively the corresponding 3-monoacetates (III and IV respectively).

3. Reaction of I and II with boiling acetic anhydride ($\frac{1}{2}$ hour) yielded exclusively the corresponding 3,5-diacetates (V and VI respectively).

4. When treated with acetic anhydride and pyridine at room temperature, I yielded a mixture of III and V, and II gave a mixture of IV and VI.

5. Treatment of the diethyl ester VIIb with acetic anhydride and pyridine, with acetyl chloride and dimethylaniline in refluxing chloroform, or with boiling acetic anhydride ($\frac{1}{2}$ hour), yielded in each case only the diethyl ester 3-monoacetate (VIII). Longer refluxing of VIIb with acetic anhydride gave mixtures of VIII with the diethyl ester 3,5-diacetate (IX).

6. By reaction with diazoethane, the diethylester 3-monoacetate (VIII) has been prepared from III and from IV, and the diethyl ester 3,5-diacetate (IX) has been prepared from V and from VI, thus interrelating the acetylation products of I, II, and VIIb.

7. The presence of a free carboxyl group at position 10 in I and II greatly facilitates acetylation of the 5β-hydroxyl group.

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