[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, MAYO FOUNDATION]

PREPARATION AND ABSORPTION SPECTRA OF STEROIDS WITH 2,4-DINITROPHENYLHYDRAZONO GROUPS AT C-20 AND C-21

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In a study of the bromination of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I) (Fig. 1)[1] it was found that the halogen entered exclusively in position 21, with the formation of two diastereoisomers (1). Recent observations (2, 3) which have shown that the presence of the 2,4-dinitrophenylhydrazono group increases the reactivity of an adjacent atom of halogen prompted us to extend our investigations to the bromination of the 20-(2,4-dinitrophenylhydrazone) of I. If bromine entered the molecule at C-17 the product might be useful in the partial synthesis of 17-hydroxy-11-dehydrocorticosterone. On the other hand, if bromination followed the same pattern as was found previously for the C-20 ketone, the 21-bromo-21-acetoxyl derivatives which would be formed would allow the preparation of steroids with glyoxal and α-keto acid side chains which have not been obtained before.

This group of compounds was also of interest in connection with the correlation between the chemical structure and the absorption spectra of some steroidal 2,4-dinitrophenylhydrazones (4, 5, 6).

It was found that the usual methods could not be applied for the preparation of the 2,4-dinitrophenylhydrazone of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (V) because of the low reactivity of the keto group at C-20. In cold acetic acid with mineral acid present some hydrazone was formed, but under these conditions 2,4-dinitrophenylhydrazine was acetylated rapidly. In methanolic solution in the presence of mineral acid hydrolysis of the 21-acetyl group occurred, and the dinitrophenylhydrazone of the 3-monoacetate (IV) separated in crystalline form. The mother liquor contained the hydrazone of the 3,21-dihydroxy compound (III). Both of these products formed the 3,21-diacetate (V) with acetic anhydride.

The dihydroxyhydrazone (III) was difficultly soluble in most common solvents and tended to crystallize with retention of solvent. One such complex was formed when the hydrazone was crystallized from pyridine. The crystals tenaciously retained 1 molecule of pyridine when dried at 100°. When this compound was dissolved in benzene and the pyridine was removed with acid, titration indicated a 1:1 relation between the hydrazone and pyridine. As with pyridine, the compound also formed a complex with acetic acid. Partial acetylation of III yielded the 21-monoacetate (VI) which was oxidized with chromic acid to give the 3-keto compound (IX). The 20-dinitrophenylhydrazone of the bromine-free steroid 3α,21-dihydroxy-11,20-diketopregnane (VII) and its diacetate (VIII) were also prepared.

The main maxima of the absorption bands of all hydrazones discussed so far—

[1] Roman numerals refer to the structural formulas in Figures 1 and 4.

both in chloroform and in acetone—are listed in Table I. Also included are two compounds with geminal functions at C-21 whose preparation will be outlined. The similarity of the absorption bands of these two compounds with those of monosubstituted hydrazones is apparent from Figure 2. The data in Table I are suggestive of some generalizations: (a) The spectra of the four compounds with an acetoxyl group in position 21 are almost identical. (b) A hydroxyl group

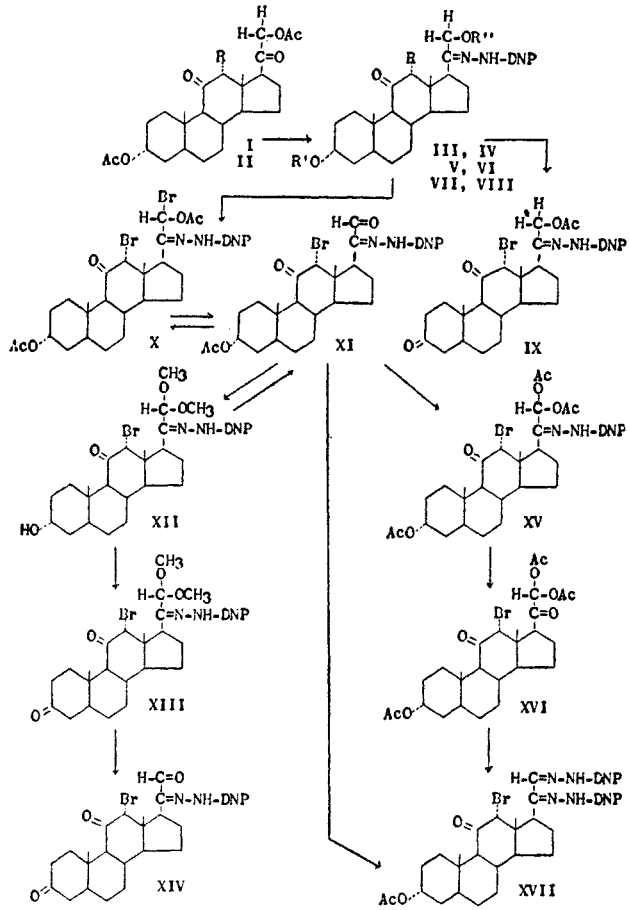

FIG. 1. STRUCTURAL FORMULAS OF COMPOUNDS I THROUGH XVII

I. R = Br; II. R = H; III. R = Br, R′ = R″ = H; IV. R = Br, R′ = Ac, R″ = H; V. R = Br, R′ = R″ = Ac; VI. R = Br, R′ = H, R″ = Ac; VII. R = H, R′ = R″ = H; VIII. R = H, R′ = R″ = Ac.

in position 21 causes a bathochromic shift of 3 to 9 mμ compared with the acetoxyl group. (c) In both types of compounds acetone—compared with chloroform—causes a bathochromic shift of 3 to 4 mμ. (d) The 3,21,21-triacetate (XV) and the 21,21-dimethoxy compound (XII) are very similar to the 21-monosubstituted hydrazones. However, in both compounds acetone does not appear to have a bathochromic effect when compared with chloroform.

The bromination of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (V) was carried out at 0° in dry chloroform with gaseous hydrogen bromide as catalyst, in analogy to the reaction of the ketone I. The product obtained by bromination in chloroform showed an absorption band with maximum at 362.5 mμ, ε = 23,700 in carbon tetrachloride, which was

TABLE I

ABSORPTION SPECTRA OF C-20 HYDRAZONES WITH ONE AND TWO GROUPS AT C-21

NO.	COMPOUND	$\lambda_{max.}$, mμ (chloroform)	ε (chloroform)	$\lambda_{max.}$, mμ (acetone)	ε (acetone)
	A. 21-ACETOXY COMPOUNDS				
V	3α,21-Diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	359	24,600	362	24,000
VI	3α-Hydroxy-21-acetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	359	24,200	362.5	24,600
IX	21-Acetoxy-3,11,20-triketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	358.5	24,100	362	24,700
VIII	3α,21-Diacetoxy-11,20-diketopregnane 20-(2,4-dinitrophenylhydrazone)	359.5	24,000	363.5	24,400
	B. 21-HYDROXY COMPOUNDS				
IV	3α-Acetoxy-21-hydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	362.5	23,100	366	24,500
III	3α,21-Dihydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	366	23,100	369	24,000
VII	3α,21-Dihydroxy-11,20-diketopregnane 20-(2,4-dinitrophenylhydrazone)	367	22,700	371	24,400
	C. GEMINAL COMPOUNDS				
XV	3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	357.5	24,500	358.5	24,400
XII	3α-Hydroxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone)	366	25,300	365	25,600

similar to that of the starting material. Its optical rotation in the same solvent was $[M]_D$ 9,150. When the solution in carbon tetrachloride was boiled under reflux for two hours, hydrogen bromide was not liberated, and the absorption of light and optical rotation remained unchanged. Replacement of the carbon tetrachloride with acetic acid, followed by short heating on the steam-bath, eliminated hydrogen bromide and a yellow 2,4-dinitrophenylhydrazone crystallized

from solution. This compound (XI) showed a characteristic absorption spectrum in chloroform with two maxima, at 370 mμ and 400 mμ (Fig. 3), which at first suggested a mixture of a saturated and an unsaturated hydrazone. Yet the compound was obviously pure and the amount of hydrogen bromide liberated was determined to be about 1 equivalent. Also, when the absorption of light was measured in methanol the two-peak spectrum was replaced with a single band with a maximum at 365 mμ, ϵ = 24,700, characteristic of a saturated hydrazone. The 2,4-dinitrophenylhydrazone of 3β-acetoxy-20-keto-$\Delta^{5,6;16,17}$-pregnadiene[2] was prepared and found to have an absorption band typical for the hydrazone of an α,β-unsaturated ketone ($\lambda_{max.}$ 384 mμ, ϵ = 26,800 in chloroform). It was

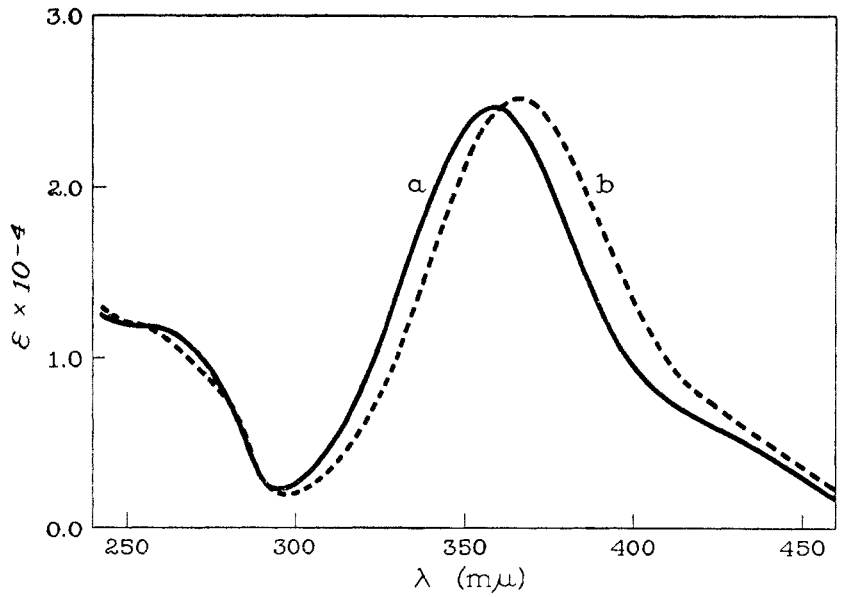

FIG. 2. ULTRAVIOLET ABSORPTION SPECTRA IN CHLOROFORM SOLUTION OF (a): 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone,) (V); (b): 3α-hydroxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XII).

then concluded that bromination had taken place at C-21, that the crystals were the 20-mono-(2,4-dinitrophenylhydrazone) of the glyoxal (XI), and that in methanol a hemiacetal had been formed. This conclusion has found support through formation of several derivatives of the new hydrazone which are described in this paper.

The hydrazone (XI) was also obtained when the 3α-acetoxy-21-hydroxy compound (IV) was brominated. It was likewise formed when IV was treated with selenium dioxide in glacial acetic acid, though the reaction was not as smooth as with bromine.

[2] We are grateful to Dr. Oliver Kamm of Parke, Davis & Company, Detroit, Michigan, for this compound.

The hydrazone XI gave a bisulfite addition compound. With one molar equivalent of 2,4-dinitrophenylhydrazine it formed a bis-dinitrophenylhydrazone with a characteristic three-band absorption spectrum (Fig. 3). The same compound was obtained when 3α, 21,21-triacetoxy-11,20-diketo-12α-bromopregnane (XVI) (1) was allowed to react with one equivalent of 2,4-dinitrophenylhydrazine [there was no indication for the formation of any 20-mono-(2,4-dinitrophenylhydrazone) (XV)].

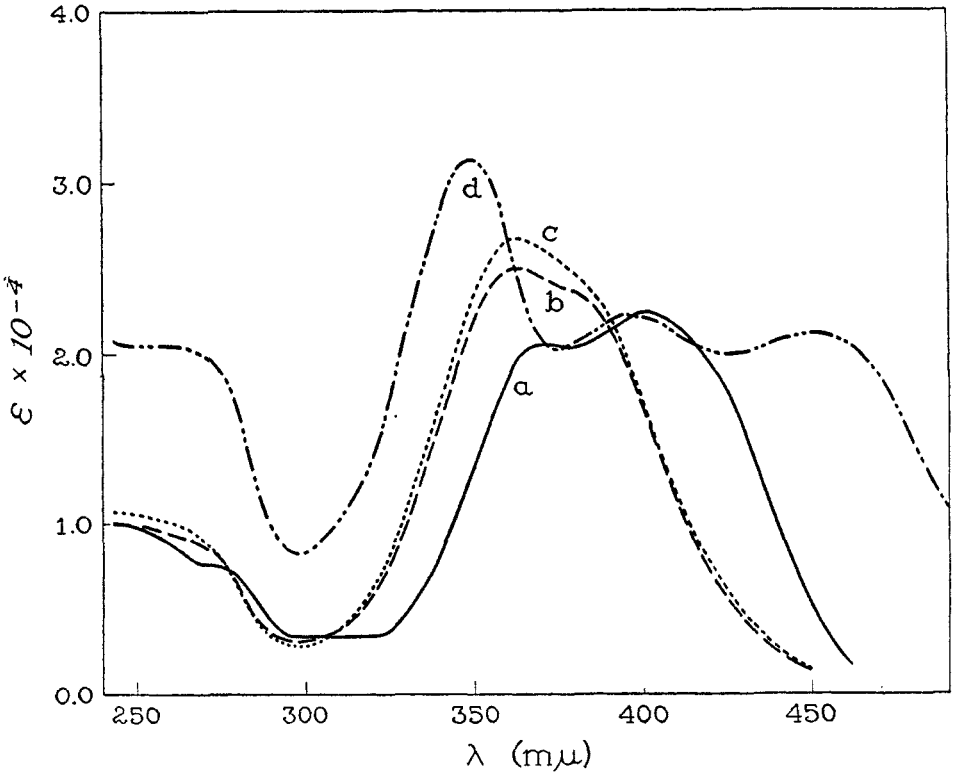

FIG. 3. ULTRAVIOLET ABSORPTION SPECTRA IN CHLOROFORM SOLUTION OF (a): 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI); (b): 3α-acetoxy-11,20-diketo-12α-bromo-21-pregnanoic acid 20-(2,4-dinitrophenylhydrazone) (XVIII); (c): methyl 3α-acetoxy-11,20-diketo-12α-bromo-21-pregnanoate 20-(2,4-dinitrophenylhydrazone) (XIX) and (d): 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20, 21-bis-(2,4-dinitrophenylhydrazone) (XVII).

With acetyl bromide and a little sulfuric acid the hydrazone XI appeared to form a mixture of the 21-diastereoisomeric bromoacetates (X) with a spectrum the same as that of the bromination product of V. Attempts to separate the two C-21 diastereoisomers were not encouraging, apparently owing to the poor crystallizability of the compounds.[3] With acetic anhydride and sulfuric acid a triacetate compound was obtained in amorphous, but apparently pure form.

[3] All 21-disubstituted hydrazones seem to share this property of poor crystallizability.

This was formulated as the 3,21,21-triacetate derivative (XV), since treatment with ozone afforded 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (XVI). With methanol-sulfuric acid XI gave crystals which appeared to be the dimethyl acetal XII in which the acetoxy group at C-3 had been removed. This compound when treated in acetic acid with a little sulfuric acid was converted back to the original hydrazone XI.

The dimethyl acetal XII was oxidized at C-3 to the 21,21-dimethyl acetal 3-ketone (XIII) which crystallized with difficulty. The 21,21-methoxy groups were hydrolyzed at room temperature in acetic acid with a small amount of sulfuric acid to give the 3-keto-21-aldehyde (XIV). The absorption of the latter was identical with that of compound XI.

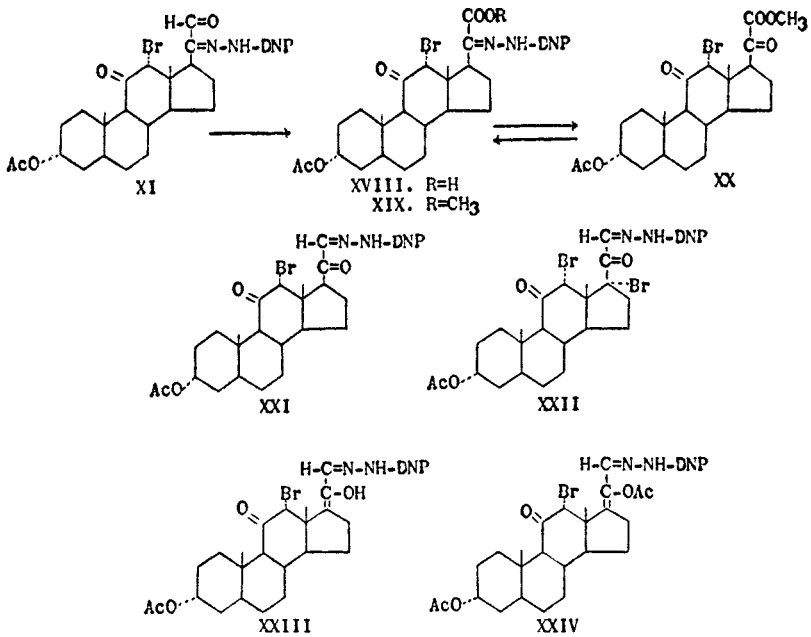

FIG. 4. STRUCTURAL FORMULAS OF COMPOUNDS XVIII THROUGH XXIV

Oxidation of compound XI with hydrogen peroxide in pyridine gave an acid in 70% yield to which the structure of a 21-carboxylic acid (XVIII) (Fig. 4) was assigned. With diazomethane the acid was converted to the methyl ester XIX. Both compounds showed characteristic broad absorption bands (Fig. 3). The acid was found to crystallize from acetone with 1 molecule of the solvent which dissociated only at about 185°, or on recrystallization from chloroform.

To obtain the 20-keto ester, the hydrazone XIX was cleaved with ozone. To our knowledge ozone has not been applied before to split the carbon-nitrogen double bond of hydrazones. While the cleavage of the double bond took place readily even at the temperature of Dry Ice, the separation of the ketone from the colored aromatic fragments presented some difficulty and this is probably the

reason why the yield of the pure keto ester XX was not higher than 50%. (In the reversal of V to I a 70% yield was obtained.) The 20-keto ester XX with 2,4-dinitrophenylhydrazine regenerated the hydrazone XIX.

Since the 2,4-dinitrophenylhydrazones of compounds with a ketone group at C-20 and with a carbonyl function in α-position at C-21, compounds XI, XIV, XVIII, and XIX, all showed characteristic broad bands, it was of interest to prepare the 21-(2,4-dinitrophenylhydrazones) of the glyoxals (XXI, XXII)

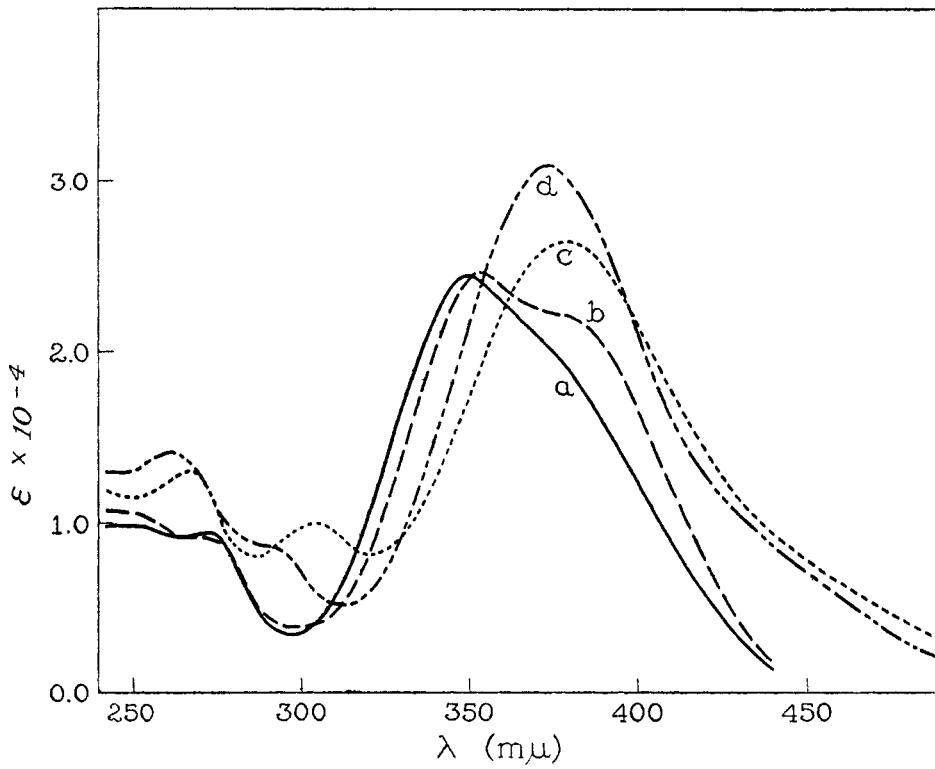

FIG. 5. ULTRAVIOLET ABSORPTION SPECTRA IN CHLOROFORM SOLUTION OF (a): 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XXI); (b): 3α-acetoxy-11, 20-diketo-12α, 17α-dibromopregnan-21-al 21-(2, 4-dinitrophenylhydrazone) (XXII); (c): 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al 21-(2,4-dinitrophenylhydrazone) (XXIII); (d): 3α,20-diacetoxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al 21-(2,4-dinitrophenylhydrazone) (XXIV).

and of the enol derivatives (XXIII, XXIV) (Fig. 5), and investigate their light absorption. The parent compounds have been described recently (1). The preparation of these hydrazones presented no difficulty and will be described in the experimental part. Their spectra are reproduced in Figure 5. It is again apparent that the broad bands of compounds XXI and XXII are due to the carbonyl group in α-position.[4] The enol derivatives XXIII and XXIV, on the other hand,

[4] In methanol the spectra are similar. There is, therefore, no indication for the formation of hemiacetals.

exhibit absorption spectra which are typical for α,β-unsaturated 2,4-dinitrophenylhydrazones. Compound XXIV has a maximum at 373 mμ, ϵ = 30,900 in chloroform, while crotonaldehyde 2,4-dinitrophenylhydrazone has its maximum at 374 mμ in the same solvent (6). A comparison of the hydrazone of the enol (XXIII) and the enol acetate (XXIV) shows that the hydroxyl group at C-20 —as compared with the acetoxy group—has only a small bathochromic effect of 5.5 mμ similar to that which was noted previously in saturated 21-hydroxy-20-hydrazones (Table I), while in the parent aldehydes the difference in the position of the maxima is 36 mμ [282 mμ and 246 mμ (1)]. This is in keeping with the observation that the bands of the 2,4-dinitrophenylhydrazones of conju-

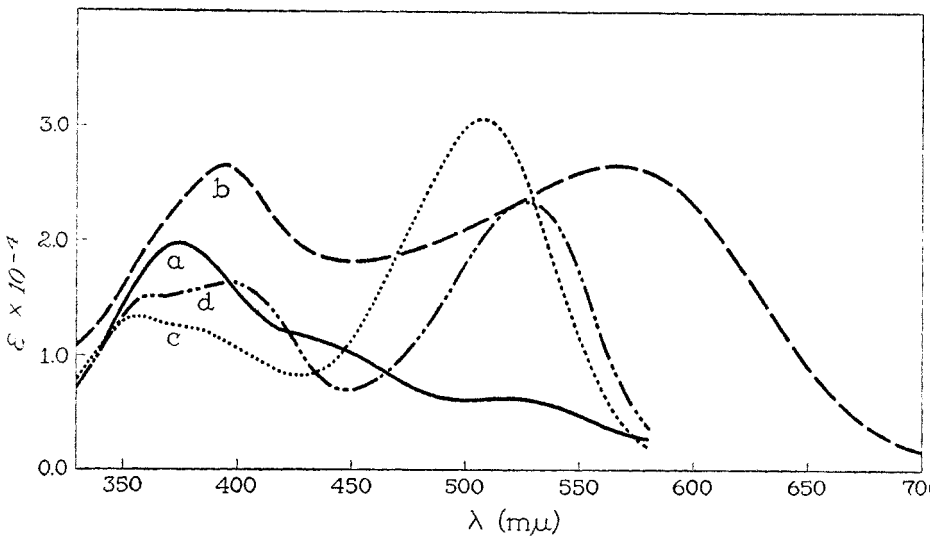

FIG. 6. ULTRAVIOLET ABSORPTION SPECTRA IN 80% AQUEOUS ACETONE WITH 0.002 *M* sodium bicarbonate of (a): 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-dinitrophenylhydrazone (XI); (b): 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20,21-bis-(2,4-dinitrophenylhydrazone) (XVII); (c): 3α-acetoxy-11-20-diketo-12α-bromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XXI); (d): 3α-acetoxy-11,20-diketo-12α,17α-dibromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XXII).

gated dienones and of conjugated enones differ by only 12 to 14 mμ, while those of the parent ketones are 40 mμ apart (6) [see also (4) and (5)].

The mono- and bis-2,4-dinitrophenylhydrazones of the glyoxals XXI, XXII, XVII, and to a lesser degree, XI show one feature which distinguishes them from all other hydrazones studied. Vivid colors (rose with XXI and XXII, purple with XVII, and peach with XI) are produced when a little aqueous sodium bicarbonate or sodium acetate is added to a very dilute acetone solution (1 to 2 mg. %) of these compounds. The sensitivity of compounds XXI and XXII to hydroxyl ions is so remarkable that water alone produces a rose color. The absorption curves of the four compounds in 80% aqueous acetone with 0.002 *M* sodium bicarbonate are given in Figure 6. Probably these spectra are due only in part to the colored forms, for in some curves the original bands can still be

recognized. An increase in the concentration of hydroxyl ions usually increased the intensity of the color (but also made it more fleeting).

The hydrazones of the enol derivatives (XXIII and XXIV) did not show any new bands in the presence of sodium bicarbonate. Furthermore, on acidification the original spectra were restored. (All 2,4-dinitrophenylhydrazones show a small and nonspecific general absorption in the visible spectrum when a little alkali is added, which appears to the eye as a brownish tint; acid reverses this effect.) This indicates that no ketonization took place under the influence of bicarbonate ions.

EXPERIMENTAL

All melting points were taken on the Fisher-Johns apparatus. The absorption spectra were determined in a Beckman quartz spectrophotometer. For optical rotations a steroid concentration of approximately 1% in chloroform or acetone was chosen unless stated otherwise. Some of the compounds described in this paper were analyzed in the laboratory of Merck & Co., Inc., Rahway, New Jersey, and the remainder were analyzed by Mr. J. F. Alicino, Metuchen, New Jersey.

3α,21-Dihydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (III) from 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I). To 25.57 g. of I dissolved in 100 ml. of chloroform, 1 liter of methanol and 17 ml. of concentrated hydrochloric acid were added, and the mixture was allowed to stand at room temperature overnight. The solution was concentrated under reduced pressure to about 800 ml., 9.9 g. of 2,4-dinitrophenylhydrazine was added with stirring, and the yellow mass of crystals which separated (26.1 g., m.p. 250–253°) was washed with methanol.

After concentration of the filtrate a second crop of 2.52 g., m.p. 250–253°, was obtained; total yield, 95%.

After three crystallizations from pyridine-ether the melting point was 253–254°. From the elementary analysis it appeared that the material contained 1 molecule of pyridine.

Anal. Calc'd for $C_{27}H_{35}BrN_4O_7$: C, 53.38; H, 5.81; N, 9.22.
Calc'd for $C_{27}H_{35}BrN_4O_7 \cdot C_5H_5N$: C, 55.97; H, 5.87; N, 10.20.
Found: C, 55.95; H, 5.89; N, 9.80.

To determine the pyridine directly 686.6 mg. of the compound was dissolved in 100 ml. of acetone, and 600 ml. of benzene was added. The mixture was washed with 250 ml. of 0.02 *N* sulfuric acid and with water. The combined aqueous layers were concentrated to about a third, and an aliquot was titrated with 0.10 *N* sodium hydroxide, using Congo Red. The presence of 1 equivalent of pyridine was indicated. The benzene layer after concentration yielded 606 mg. of the 2,4-dinitrophenylhydrazone. For analysis this material was crystallized from chloroform and ligroin; m.p. 256–257° with violent decomposition, $[\alpha]_D$ +30.5° ± 2° (acetone); $\lambda_{max.}$ 366 mμ, ϵ = 23,100 (chloroform); $\lambda_{max.}$ 369 mμ, ϵ = 24,000 (acetone).

Anal. Calc'd for $C_{27}H_{35}BrN_4O_7$: C, 53.38; H, 5.81; N, 9.22.
Found: C, 53.19; H, 5.93; N, 9.28.

3α-Acetoxy-21-hydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (IV) and 3α,21-dihydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (III) from 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I). To 20.46 g. of I dissolved in 1.6 liters of methanol, a solution of 9.60 g. of 2,4-dinitrophenylhydrazine in 1.6 liters of 2 *N* hydrochloric acid in 60% aqueous methanol was added. After three hours at room temperature the mixture was cooled in an ice-bath, and 10.6 g. of the crude 3-monoacetate, m.p. 200–204°, was obtained. After six recrystallizations from chloroform-methanol the compound melted at 221–223° with decomposition; $[\alpha]_D$ +26° ± 2° (chloroform), $[\alpha]_D$ +67° ± 2° (acetone); $\lambda_{max.}$ 362.5 mμ, ϵ = 23,100 (chloroform); $\lambda_{max.}$ 366 mμ, ϵ = 24,500 (acetone).

Anal. Calc'd for $C_{29}H_{37}BrN_4O_8$: C, 53.62; H, 5.74; N, 8.63.
Found: C, 53.30; H, 5.97; N, 8.43.

The chloroform extract of the filtrate from the 3-monoacetate was washed with water, dried, and concentrated. A product, 5.50 g., m.p. 246–247°, was obtained which after crystallization from ethyl acetate melted at 257° with decomposition and was found to be identical with the 3,21-dihydroxy derivative (III).

3α,21-Diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (V) from 3α,21-dihydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (III). After 25.9 g. of III was suspended in 100 ml. of dry pyridine, acetylation was carried out with 50 ml. of acetic anhydride at room temperature. After 18 hours the mixture was poured on ice, and the hydrazone was filtered, washed with water, and dried. One crystallization from hot chloroform-methanol gave orange-colored square platelets; yield, 90%, m.p. 194–195°, $[\alpha]_D$ +50° ± 2° (chloroform); $[\alpha]_D$ +90° ± 2° (acetone); $\lambda_{max.}$ 359 mμ, ϵ = 24,600 (chloroform); $\lambda_{max.}$ 362 mμ, ϵ = 24,000 (acetone).

Anal. Calc'd for $C_{31}H_{39}BrN_4O_9$: C, 53.84; H, 5.68; N, 8.10.
Found: C, 53.94; H, 6.07; N, 8.01.

3α,21-Diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (V) from 3α-acetoxy-21-hydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (IV). A solution of 5.11 g. of IV in 25 ml. of dry pyridine and 10 ml. of acetic anhydride was kept 18 hours at room temperature; then ice was added. The insoluble material was filtered, washed with water, dried, and crystallized from chloroform-methanol. The orange-colored square platelets (3.5 g.) melted at 188–191°, and after one recrystallization melted at 193–194°. When mixed with an authentic sample of V the melting point was not depressed.

3α-Hydroxy-21-acetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (VI) from 3α,21-dihydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (III). A solution of 9.4 g. of III in 70 ml. of pyridine and 1.55 ml. of acetic anhydride was kept at room temperature for three hours. Then chloroform and water were added, and the chloroform solution was washed with dilute hydrochloric acid, with water, and dried. The solvent was removed under reduced pressure, and the residue was crystallized from acetic acid; yield, 7.52 g.; m.p. 125–127°. One recrystallization from methanol gave m.p. 136–138°, $[\alpha]_D$ +32° ± 2° (chloroform); $[\alpha]_D$ +76° ± 2° (acetone); $\lambda_{max.}$ 359 mμ, ϵ = 24,200 (chloroform); $\lambda_{max.}$ 362.5 mμ, ϵ = 24,600 (acetone).

Anal. Calc'd for $C_{29}H_{37}BrN_4O_8$: C, 53.62; H, 5.74; N, 8.63.
Found: C, 53.71; H, 5.97; N, 8.80.

3α,21-Dihydroxy-11,20-diketopregnane 20-(2,4-dinitrophenylhydrazone) (VII) from 3α,21-diacetoxy-11,20-diketopregnane (II). A solution of 4.325 g. of II in 20 ml. of chloroform to which 200 ml. of methanol and 3.4 ml. of concentrated hydrochloric acid were added was kept 18 hours at room temperature. The solution was concentrated under reduced pressure to 100 ml. and addition of 1.98 g. of 2,4-dinitrophenylhydrazine yielded 4.735 g. of a hydrazone, m.p. 250–253°. Two crystallizations from ethyl acetate-petroleum ether gave m.p. 257–258°. The compound was less soluble in acetone and more soluble in ethyl acetate than the corresponding 12α-bromo derivative; $[\alpha]_D$ +10.5° ± 3° ($c \sim 0.33$, chloroform); $[\alpha]_D$ +38° ± 4° ($c \sim 0.25$, acetone); $\lambda_{max.}$ 367 mμ, ϵ = 22,700 (chloroform); $\lambda_{max.}$ 371 mμ, ϵ = 24,400 (acetone).

Anal. Calc'd for $C_{27}H_{36}N_4O_7$: C, 61.35; H, 6.87; N, 10.60.
Found: C, 61.13; H, 7.06; N, 10.62.

3α,21-Diacetoxy-11,20-diketopregnane 20-(2,4-dinitrophenylhydrazone) (VIII) from 3α,21-dihydroxy-11,20-diketopregnane 20-(2,4-dinitrophenylhydrazone) (VII). Acetic anhydride (5 ml.) containing 1 g. of VII was boiled under a reflux for 1.5 hours. The solvent was removed under reduced pressure, and the residue was crystallized from acetone-methanol; yield, 810 mg., m.p. 181–183°; $[\alpha]_D$ 102.5° ± 2° (chloroform); $[\alpha]_D$ 109.5° ± 2° (acetone); $\lambda_{max.}$ 359.5 mμ, ϵ = 24,000 (chloroform); $\lambda_{max.}$ 363.5 mμ, ϵ = 24,400 (acetone).

Anal. Calc'd for $C_{31}H_{40}N_4O_9$: C, 60.77; H, 6.58; N, 9.15.
Found: C, 60.90; H, 6.69; N, 9.05.

21-Acetoxy-3,11,20-triketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (IX) from 3α-hydroxy-21-acetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (VI) Compound VI (650 mg.) was dissolved in 4 ml. of alcohol-free chloroform and 15 ml. of acetic acid. The solution was cooled in an ice-bath, and 1.25 ml. of 2.4 *N* chromic acid in 95% acetic acid was added. After five hours water was added, and the hydrazone was extracted with chloroform. The chloroform solution was washed with water, sodium bicarbonate solution, and with water. The solvent was removed under reduced pressure, and the residue was crystallized (424 mg.) from a small volume of acetic acid. Three crystallizations from dilute acetone then gave material of m.p. 196–197°, $[\alpha]_D$ +31° ± 2° (chloroform); $[\alpha]_D$ +65° ± 2° (acetone), $\lambda_{max.}$ 358.5 mμ, ϵ = 24,100 (chloroform); $\lambda_{max.}$ 362 mμ, ϵ = 24,700 (acetone).

Anal. Calc'd for $C_{29}H_{35}BrN_4O_8$: C, 53.79; H, 5.45; N, 8.65.

Found: C, 54.06; H, 5.57; N, 8.88.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI) from 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (V). After 27.66 g. of V was dissolved in 400 ml. of dry chloroform, bromination was carried out at 0° for 21 hours with a solution of 80 ml. of 1 *N* bromine in chloroform and gaseous hydrogen bromide which was passed through the flask for 15 minutes. The chloroform was concentrated under reduced pressure to a small volume, and 120 ml. of acetic acid was added and concentrated to remove the last traces of chloroform. When the reaction product was placed on the steam-bath a copious mass of yellow crystals separated. The flask was cooled, 360 ml. of acetone was added, and the material which separated was washed with acetone. The first crop weighed 12.224 g., m.p. 267–269°. A second crop (2.435 g.) of equally pure compound was obtained, and when the filtrate was re-treated on the steam-bath for a half hour a third crop of crystals (2.01 g.) separated which was slightly less pure. The total yield was 65 %. The remainder appeared to contain the 20,21-bis-(2,4-dinitrophenylhydrazone). Compound XI was found to be only slightly soluble in acetic acid and acetone, but fairly soluble in chloroform. The analytic sample was recrystallized several times from acetone, but the melting point and absorption spectrum did not change; $[\alpha]_D$ +99° ± 2° (chloroform); $\lambda_{max.1}$ 370 mμ, ϵ = 20,500, $\lambda_{max.2}$ 400.5 mμ, ϵ = 22,200 (chloroform). $\lambda_{max.1}$ 373 mμ, ϵ = 22,200, $\lambda_{max.2}$ 380–392 mμ, ϵ = 22,000 (acetone). $\lambda_{max.}$ 365 mμ, ϵ = 24,700 (methanol).

Anal. Calc'd for $C_{29}H_{35}BrN_4O_8$: C, 53.79; H, 5.45; N, 8.65; Br, 12.34.

Found: C, 53.98; H, 5.67; N, 9.09; Br, 12.81.

The primary product of bromination was also converted into compound XI in 80% aqueous pyridine. After 15 minutes at room temperature the pyridine solution was poured into an excess of mineral acid and ice, and the solid material was filtered and washed with acetone. The yield was similar to that of the first method.

By a third procedure the primary product of bromination, after removal of the chloroform, was dissolved in acetone, some water was added, and the mixture was warmed for a short time on the steam-bath until compound XI began to crystallize.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI) from 3α-acetoxy-21-hydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (IV). Compound IV (650 mg.) was brominated for two hours at 0° in 10 ml. of chloroform, saturated with hydrogen bromide gas, with 2 ml. of 1 *N* bromine in chloroform. The residue, after removal of the chloroform under reduced pressure, was dissolved in acetone, and, with warming, water was added until crystals appeared. Yield, 243 mg.; after three recrystallizations from dilute acetone, m.p. 267–269°. When mixed with compound XI prepared from the 3,21-diacetate there was no depression of the melting point; $[\alpha]_D$ +99° ± 2° (chloroform). The absorption spectra of the two compounds were identical.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI) from 3α-acetoxy-21-hydroxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (IV) with selenium dioxide. A mixture of 325 mg. of IV and 56 mg. of selenium dioxide in 6 ml. of acetic acid was refluxed for one hour. The hydrazone was extracted with chloroform which was washed with water, sodium bicarbonate solution, and water. From the

extinction coefficients of the chloroform solution at 365 mμ and 400 mμ it was calculated that approximately 44% of compound XI had been formed and that 39% of starting material was still present. The remaining 17% was unaccounted for. In a second experiment 325 mg. of IV in 5 ml. of glacial acetic acid was heated with 111 mg. of selenium dioxide at 100° for 15 hours. The spectrum indicated that 62% of compound XI had been formed. Of the starting material, 15% remained unchanged and 23% had been destroyed.

In a third experiment the time of heating was extended to 45 hours, and none of the starting material remained unchanged. The spectrum was essentially that of compound XI with some additional absorption in the shorter wavelength region (below 350 mμ). Calculation indicated the presence of about 60% of compound XI. The solution was brought to dryness under reduced pressure, the residue was dissolved in acetone, treated with activated carbon, and concentrated until crystals separated. After one further recrystallization from acetone, the yield was 50 mg., m.p. 248–256° with decomposition; $\lambda_{max.1}$ 370 mμ, ϵ = 19,500; $\lambda_{max.2}$ 400 mμ, ϵ = 21,500, indicating 96% of compound XI. When 324 mg. of compound XI was refluxed in a mixture of 5 ml. of acetic acid and 5 ml. of chlorobenzene with 111 mg. of selenium dioxide for 72 hours, the absorption curve in chloroform indicated 20% of the original compound XI still present, but no acidic material had been formed.

3α,21-Diacetoxy-11,20-diketo-12α,21-dibromopregnane 20-(2,4-dinitrophenylhydrazone) (X) from 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI). To 100 mg. of XI dissolved in 1 ml. of acetyl bromide was added 1 drop of concentrated sulfuric acid. After 15 minutes at room temperature chloroform and ice were added. The chloroform solution was washed with sodium bicarbonate solution and with water, dried over sodium sulfate, and the absorption spectrum was determined. The single band showed a maximum at 362 mμ, ϵ = 22,400 (chloroform), and indicated that the original aldehyde group had been substituted. The separation of the two diastereoisomers did not appear to be a simple matter, and was not further investigated.

3α-Hydroxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XII) from 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI). To 648 mg. of XI dissolved in 10 ml. of chloroform at room temperature was added 40 ml. of methanol and 1 ml. of concentrated sulfuric acid. After 18 hours fine needle-shaped crystals separated which were washed with water; yield, 475 mg.; m.p. 216–239°, $[\alpha]_D$ +125° ± 2° (chloroform); $[\alpha]_D$ +135° ± 2° (acetone); $\lambda_{max.}$ 366 mμ, ϵ = 25,300 (chloroform); $\lambda_{max.}$ 365 mμ, ϵ = 25,600 (acetone).

Anal. Calc'd for $C_{29}H_{39}BrN_4O_8$: C, 53.46; H, 6.03; CH_3O, 9.53.

Found: C, 53.42; H, 6.07; CH_3O, 8.94.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI) from 3α-hydroxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XII). To 65 mg. of XII dissolved in 2 ml. of chloroform was added a mixture of 3 ml. of acetic acid and 0.15 ml. of concentrated sulfuric acid. After 2.5 hours at room temperature the mixture was washed with water, sodium bicarbonate solution, and with water. The chloroform was removed under reduced pressure, and the residue was cyrstallized from dilute acetone. After two further recrystallizations the material melted at 267–269°, gave the typical absorption of compound XI, and when mixed with a sample of this compound there was no depression of the melting point.

21,21-Dimethoxy-3,11,20-triketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XIII) from 3α-hydroxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XII). To a solution of 326 mg. of XII dissolved in 2 ml. of chloroform and held at 0° in an ice-bath, 7.5 ml. of acetic acid and 0.63 ml. of 2.4 *N* chromic acid in 95% acetic acid were added. After five hours at 0° the solution was diluted with water and extracted with chloroform. The chloroform solution was washed with water, sodium bicarbonate solution, and with water. Removal of chloroform under reduced pressure and addition of methanol afforded a gelatinous product, m.p. 146–155°. This material showed $\lambda_{max.}$ 365 mμ, ϵ = 22,500 (chloroform). Without further purification it was converted to the 21-aldehyde.

3,11,20-Triketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XIV) from 21,21-dimethoxy-3,11,20-triketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XIII). Compound XIII (313 mg.) was dissolved in 18 ml. of acetic acid to which 0.11 ml. of concentrated sulfuric acid was added. After four hours at room temperature water and chloroform were added, the chloroform extract was washed with water, sodium bicarbonate solution, and with water, and was evaporated to dryness under reduced pressure. Addition of acetone to the residue yielded 200 mg. of crystals which after two recrystallizations from dilute acetone melted at 232–234°, $[\alpha]_D$ +76° ± 2° (chloroform); $[\alpha]_D$ +111° ± 2° (acetone); $\lambda_{max.1}$ 370.5 mμ, ϵ = 20,400; $\lambda_{max.2}$ 399.5 mμ, ϵ = 22,200 (chloroform); $\lambda_{max.1}$ 373.5 mμ, ϵ = 22,600; $\lambda_{max.2}$ 380–392 mμ (plateau), ϵ = 22,400 (acetone).

Anal. Calc'd for $C_{27}H_{31}BrN_4O_7$: C, 53.73; H, 5.18; N, 9.28.

Found: C, 53.91; H, 5.43; N, 9.07.

3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane 20-(2,4-dinitrophenylhydrazone) (XV) from 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI). To a suspension of 648 mg. of XI in 10 ml. of acetic anhydride cooled in an ice-bath, 0.2 ml. of concentrated sulfuric acid was added. The hydrazone rapidly dissolved, and after 15 minutes the solution was poured into ice-water. The acetic anhydride was decomposed, and the solid material was washed with water. No satisfactory way of crystallizing the air-dried compound (764 mg.) was found. Repeated partial precipitation from dilute acetic acid yielded fractions which spectrophotometrically appeared to be uniform; m.p. about 130°. Near 180° the physical properties changed, the material resolidified and melted at 259–265° (with strong decomposition). This behavior suggested that the 21-aldehyde was re-formed at about 180°. As a check the resolidified material was dissolved in chloroform, and the quotient of the extinction coefficient at 400 mμ to that at 360 mμ was determined. It was found to be 0.87; the ratio for the triacetate was 0.39 and for the 21-aldehyde was 1.10. The triacetate showed the following rotations: $[\alpha]_D$ +101° ± 2° (chloroform); $[\alpha]_D$ +110° ± 2° (acetone); $\lambda_{max.}$ 357.5 mμ, ϵ = 24,500 (chloroform); $\lambda_{max.}$ 358.5 mμ, ϵ = 24,400 (acetone).

Anal. Calc'd for $C_{33}H_{41}BrN_4O_{11}$: C, 52.87; H, 5.51; N, 7.47.

Found: C, 52.87; H, 5.54; N, 7.46.

3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane (XVI) from its 20-(2,4-dinitrophenylhydrazone) (XV). Ozone (3 molar-equivalents) was passed through a solution of 184 mg. of XV dissolved in 5 ml. of ethyl acetate maintained at a low temperature in an acetone-Dry Ice bath. About 10 ml. of methanol and 2 ml. of a saturated solution of sodium bisulfite were added, and the mixture was refluxed for ten minutes. Water was added, and the mixture was extracted with chloroform; the organic phase was washed with water and evaporated to dryness under reduced pressure. A little methanol added to the residue yielded material which after two recrystallizations and a treatment with activated carbon weighed 28 mg., m.p. 168–169°. The melting point was not depressed when the compound was mixed with an authentic sample of 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20,21-bis-(2,4-dinitrophenylhydrazone) (XVII) from 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI). To a solution of 324 mg. of XI in 50 ml. of chloroform, 99 mg. of dinitrophenylhydrazine and 50 ml. of methanol which contained 0.3 ml. of concentrated sulfuric acid were added. After 15 hours 300 mg. of material had separated. The product was only slightly soluble in all common solvents: it began to char but did not melt at 300°. The compound was dissolved in hot chloroform, and hot ligroin was added until the mixture became slightly turbid. After three such crystallizations the following three bands were observed: $\lambda_{max.1}$ 349 mμ, ϵ = 31,200; $\lambda_{max.2}$ 395 mμ, ϵ = 22,000; $\lambda_{max.3}$ 450 mμ, ϵ = 21,000 (chloroform). $\lambda_{max.1}$ 354 mμ, ϵ = 31,900; $\lambda_{max.2}$ 398 mμ, ϵ = 24,200; $\lambda_{max.3}$ 440 mμ, ϵ = 21,200 (acetone).

Anal. Calc'd for $C_{35}H_{39}BrN_8O_{11}$: C, 50.79; H, 4.75; N, 13.54.

Found: C, 50.66; H, 4.90; N, 13.43.

3α-Acetoxy-11,20-diketo-12α-bromopregnan-21-al 20,21-bis-(2,4-dinitrophenylhydrazone)

(XVII) from 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (XVI). To a solution of 140 mg. of XVI (0.246 millimole) dissolved in 2 ml. of chloroform, 12.5 ml. of a hot solution of 49.5 mg. of 2,4-dinitrophenylhydrazine (0.25 millimole) in methanol and 3 drops of concentrated sulfuric acid were added. After 18 hours orange-colored crystals (71 mg.) separated and were washed with methanol. Concentration of the filtrate under reduced pressure and the addition of a little water gave an additional crop of 25 mg. Both fractions showed the typical three-band spectrum of the bis-2,4-dinitrophenylhydrazone. The yield was 46.5% of the steroid and 93% of the dinitrophenylhydrazine. There was thus no indication for the formation of a mono-2,4-dinitrophenylhydrazone at C-20.

3α-Acetoxy-11,20-diketo-12α-bromo-21-pregnanoic acid 20-(2,4-dinitrophenylhydrazone) (XVIII) from 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 20-(2,4-dinitrophenylhydrazone) (XI). A solution of 16.67 g. of XI dissolved in 200 ml. of pyridine was cooled in an ice-bath. Then 50 ml. of 30% hydrogen peroxide was added, and the temperature was maintained at about 5° for 14 hours. The mixture, which contained a mass of crystals, was poured into an excess of sulfuric acid and ice, and the solid material was washed with water, dried, and extracted with 2.5 liters of acetone. A residue (1.45 g.) remained and was identified as starting material. To the acetone solution, 250 g. of alumina (Harshaw, untreated) was added with stirring. The alumina was filtered off and washed with acetone. Concentration of the acetone yielded 2.78 g. of starting material, a total of 25%. The alumina was eluted with a mixture of 9 parts of acetone and 1 part of acetic acid, and finally with acetic acid. The combined eluates were concentrated under reduced pressure to a small volume, to which chloroform and water were added. The chloroform solution was well washed with water, concentrated to about 100 ml., and diluted with about the same volume of ligroin. After 18 hours at 5°, 7.22 g. of material separated; m.p. 241° (decomposition). Concentration of the mother liquor gave a second crop (1.677 g.), m.p. 224–230° (dec.), which on washing with chloroform yielded 840 mg. of material, m.p. 241° (dec.). Considering the weight of the starting material which was recovered, the yield of acid was 70%.

The acid was recrystallized several times from chloroform-ligroin without change of the melting point; $[\alpha]_D$ +37° ± 2° (chloroform); $[\alpha]_D$ +104° ± 2° (acetone). $\lambda_{max.}$ 364 mμ, ϵ = 24,900 (chloroform). $\lambda_{max.}$ 375.5 mμ, ϵ = 25,500 (acetone).

Anal. Calc'd for $C_{29}H_{35}BrN_4O_9$: C, 52.49; H, 5.32; N, 8.44.
Found: C, 52.60; H, 5.23; N, 8.53.

When crystallized from acetone the compound separated with 1 mole of acetone of crystallization. Such material melted at 185°, resolidified, and melted again at 230–234° with decomposition.

Methyl 3α-acetoxy-11,20)-diketo-12α-bromo-21-pregnanoate 20)-(2,4-dinitrophenylhydrazone) (XIX) from 3α-acetoxy-11,20-diketo-12α-bromo-21-pregananoic acid 20-(2,4-dinitrophenylhydrazone) (XVIII). A suspension of 6.635 g. of XVIII in 100 ml. of chloroform was cooled in an ice bath and esterified with an ether solution of diazomethane. The residue after removal of the solvents was crystallized from chloroform-methanol. The ester (6.1 g.) separated in yellow, feathery crystals, m.p. 147–150°. An additional 617 mg., m.p. 138–145°, was obtained from the mother liquor. For analysis, material from the first crop was recrystallized, m.p. 149–151°; $[\alpha]_D$ +78° ± 2° (chloroform), $[\alpha]_D$ +106° ± 2° (acetone). $\lambda_{max.}$ 363.5 mμ, ϵ = 26,600 (chloroform); $\lambda_{max.}$ 366.5 mμ, ϵ = 27,000 (acetone).

Anal. Calc'd for $C_{30}H_{37}BrN_4O_9$: C, 53.18; H, 5.51; N, 8.27; CH_3O, 4.58.
Found: C, 53.47; H, 5.78; N, 8.49; CH_3O, 4.76.

Methyl 3α-acetoxy-11,20-diketo-12α-bromo-21-pregnanoate (XX) from its 20-(2,4-dinitrophenylhydrazone) (XIX). Compound XIX (3.388 g.) in 50 ml. of ethyl acetate was ozonized for 33 minutes (200% excess O_3) at the temperature of a Dry Ice-acetone bath. The typical absorption spectrum of the starting material had disappeared. The excess ozone was removed with a stream of oxygen, 250 ml. of methanol and 50 ml. of saturated sodium bisulfite solution were added, and the mixture was refluxed for one hour. After the addition of water the mixture was extracted with chloroform, and the organic phase was washed with

water and evaporated to dryness under reduced pressure. Addition of a small volume of methanol gave a product (1.267 g.), m.p. 135–140°. The filtrate, diluted with acetone, treated with activated carbon, and concentrated, gave an additional 449 mg. of keto ester, m.p. 117–132°; total yield of crude compound, 69%. After four recrystallizations from dilute acetone and treatment with activated carbon 1.238 g. of white crystals, m.p. 149–151°, was obtained; $[\alpha]_D$ +30° ± 2° (chloroform); $[\alpha]_D$ +37° ± 2° (acetone).

Anal. Calc'd for $C_{24}H_{33}BrO_6$: C, 57.95; H, 6.69; Br, 16.06; CH_3O, 6.24.

Found: C, 58.05; H, 6.70; Br, 16.23; CH_3O, 6.84.

Note: When the 20-(2,4-dinitrophenylhydrazone) of 3,21-diacetoxy-11,20-diketo-12α-bromopregnane (V) was treated in the same manner with ozone a 70% yield of the 20-ketone, compound I, was obtained.

Conversion of methyl 3α-acetoxy-11,20-diketo-12α-bromo-21-pregnanoate (XX) to its 20-(2,4-dinitrophenylhydrazone) (XIX). Compound XX (100 mg.) was dissolved in 100 ml. of methanol, and 10 ml. of a saturated solution of 2,4.dinitrophenylhydrazine in aqueous-2 *N* hydrochloric acid was added. After 18 hours at room temperature the solution was concentrated and the crystals which separated (125 mg.) were washed with water. One recrystallization from methanol gave a product, m.p. 147–150° which did not depress the melting point of an authentic sample of XIX. The absorption spectra of the two compounds were identical.

Preparation of 3α-acetoxy-11,20-diketo-12α-bromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XXI). To a solution of 485 mg. of 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (1) in 5 ml. of chloroform, 297 mg. of 2,4-dinitrophenylhydrazine and 10 ml. of 80% acetic acid were added and the mixture was shaken for 15 minutes. Excess 2,4-dinitrophenylhydrazine was removed, and the filtrate was diluted with water and extracted with chloroform. The organic phase was washed with water, sodium bicarbonate solution, and water, dried over sodium sulfate, and concentrated to a small volume. Addition of methanol yielded material which was filtered off and washed with methanol. Several crystallizations from chloroform-methanol and from dilute acetone gave feather-like crystals (416 mg.), m.p. 179–180°, which was not changed by further crystallizations; $[\alpha]_D$ +39° ± 2° (chloroform), $[\alpha]_D$ + 37° ± 2° (acetone); $\lambda_{max.}$ 350.5 mμ, ϵ = 24,400 (chloroform); $\lambda_{max.}$ 360 mμ, ϵ = 24,800 (acetone); $\lambda_{max.}$ 358 mμ, ϵ = 24,300 (methanol).

Anal. Calc'd for $C_{29}H_{35}BrN_4O_8$: C, 53.79; H, 5.45; N, 8.65.

Found: C, 53.73; H, 5.64; N, 8.66.

Preparation of 3α-acetoxy-11,20-diketo-12α,17α-dibromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XXII). To a solution of 282 mg. of 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α,17α-dibromopregnane (1) in 2.5 ml. of chloroform, 3 ml. of acetic acid, 0.75 ml. of water and 150 mg. of 2,4-dinitrophenylhydrazine were added and the mixture was shaken. After one half-hour yellow, needle-shaped crystals separated. Once recrystallized from dilute acetone the product melted at 204–205° depending somewhat on the rate of heating; $[\alpha]_D$ +19° ± 2° (chloroform); $[\alpha]_D$ −7° ± 2° (acetone); $\lambda_{max.}$ 353.5 mμ, ϵ = 24,600 (chloroform); $\lambda_{max.}$ 378 mμ, ϵ = 26,700 (acetone); $\lambda_{max.}$ = 380 mμ, ϵ = 25,700 (methanol).

Anal. Calc'd for $C_{29}H_{34}Br_2N_4O_8$: C, 47.94; H, 4.72; N, 7.71; Br, 22.00.

Found: C, 48.04; H, 4.92; N, 7.91; Br, 22.37.

Preparation of 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al 21-(2,4-dinitrophenylhydrazone) (XXIII). First, 200 mg. of 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al (1) was dissolved in 5 ml. of benzene and 5 ml. of acetic acid. Then, 85 mg. of 2,4-dinitrophenylhydrazine was added, and the mixture was shaken for 45 minutes. The solvents were removed under reduced pressure, and the hydrazone which was obtained as crystals from chloroform-ligroin weighed 149 mg.; after two further recrystallizations, m.p. 165–168°. The compound crystallized in red globules which when dry turned to dark orange; $[\alpha]_D$ +68° ± 2° (chloroform); $\lambda_{max.1}$ 267.5 mμ, ϵ = 13,000; $\lambda_{max.2}$ 305 mμ, ϵ = 9,960; $\lambda_{max.3}$ 378.5 mμ, ϵ = 26,400 (chloroform); $\lambda_{max.}$ 387.5 mμ, ϵ = 27,900 (acetone).

Anal. Calc'd for $C_{29}H_{35}BrN_4O_8$: C, 53.79; H, 5.45; N, 8.65.

Found: C, 53.76; H, 5.67; N, 8.79.

At the melting point the color of the crystals was red, but soon turned to yellow. The spectrum of this yellow material showed a maximum at 352 mμ, ε = 21,400, which indicated that the compound had undergone ketonization.

Preparation of 3α,20-diacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al 21-(2,4-dinitrophenylhydrazone) (XXIV). To a solution of 140 mg. of 3α,20-diacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (1) in 2 ml. of benzene, 55 mg. of 2,4-dinitrophenylhydrazine and 1 ml. of acetic acid were added. The mixture was shaken for 45 minutes and the solvents were removed under reduced pressure. The residue was crystallized from a small volume of chloroform and ligroin. The hydrazone (188 mg.) crystallized in short yellow needles, m.p. 260–264°. After one recrystallization in the same manner, the melting point rose to 265–266° and did not change on further treatment; $[\alpha]_D$ +74.5° ± 2° (chloroform); $\lambda_{max.1}$ 261.5 mμ, ε = 14,100; $\lambda_{max.2}$ 289 mμ, ε = 8,600; $\lambda_{max.3}$ 373 mμ, ε = 30,900 (chloroform); $\lambda_{max.}$ 380.5 mμ, ε = 30,700 (acetone).

Anal. Calc'd for $C_{31}H_{37}BrN_4O_9$: C, 53.99; H, 5.41; N, 8.13.
Found: C, 54.32; H, 5.83; N, 7.88.

SUMMARY

Treatment of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane with 2,4-dinitrophenylhydrazine yields the 20-dinitrophenylhydrazone (V). In methanolic hydrogen chloride the 21-acetate may be hydrolyzed and prolonged treatment also removes the 3α-acetyl group. Acetylation of the 3α,21-dihydroxy steroid affords the 3α-hydroxy-21-acetoxy-20-(2,4-dinitrophenylhydrazone). Bromination of V gives the 21-bromo-21-acetate derivative which is converted into the 21-aldehyde-20-(2,4-dinitrophenylhydrazone) (XI) in aqueous acetic acid or aqueous acetone. Acetyl bromide converts XI to the 21-bromo-21-acetate-20-(2,4-dinitrophenylhydrazone).

With methanol and sulfuric acid XI forms the 3α-hydroxy-21,21-dimethoxy acetal 20-(2,4-dinitrophenylhydrazone). The 3α-hydroxyl group may be oxidized to a ketone and the 21-dimethyl acetal hydrolyzed to give the 3-keto derivative of XI. Acetic anhydride and sulfuric acid with XI yield the 21,21-diacetate 20-(2,4-dinitrophenylhydrazone) (XV). Ozone cleaves the hydrazone of XV and affords 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (XVI). With 2,4-dinitrophenylhydrazine XI and XVI form the 20,21-bis-2,4-dinitrophenylhydrazone derivative. Oxidation of XI with hydrogen peroxide and pyridine yields the 21-carboxylic derivative of the 20-(2,4-dinitrophenylhydrazone) which is esterified with diazomethane. This 21-methyl ester 20-(2,4-dinitrophenylhydrazone) (XIX) is cleaved with ozone to give methyl 3α-acetoxy-11,20-diketo-12α-bromopregnanoate (XX). With 2,4-dinitrophenylhydrazine XX is converted to XIX.

A series of 21-(2,4-dinitrophenylhydrazones) of 3α-acetoxy-11,20-diketo-12α-bromo-21-al pregnane have been prepared with the following modifications: 17α-bromo, 20-enol-Δ^{17,20}, 20-enol acetate-Δ^{17,20}. The absorption spectra of the hydrazones at C-20 and C-21 have been determined and certain relationships have been noted.

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Recent advances in this laboratory indicate the possibility that the atom of bromine designated as 17α may be at position 16. This problem is under investigation.