[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, MAYO FOUNDATION]

STEROIDS WITH A GLYOXAL SIDE CHAIN AT C-17 AND RELATED COMPOUNDS

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The bromination of pregnane derivatives with a ketone group at C-20 but without a substituent at C-21 was studied by Marker and associates (1). These authors reported that the first atom of bromine entered position 17, a finding which was confirmed more recently by Plattner and associates (2).

We have studied the bromination of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I)[1] (Fig. 1), an intermediate in the partial synthesis of adrenal hormones (4). It was expected that bromine would enter one of the positions *alpha* to the 20-keto group but whether this would be at C-17 or C-21 could not be anticipated.

When I was treated with 1 mole of bromine in dry chloroform at 0° with gaseous hydrogen bromide as the catalyst, utilization of bromine was complete in four hours and practically all of the material could be separated in crystalline form. Recrystallization from chloroform-ligroin revealed the presence of two compounds which were shown to be isomeric by analysis. The less soluble one, bromide "a," was present in somewhat larger amounts than the more soluble compound. It crystallized in flat plates and showed an optical rotation in chloroform $[\alpha]_D$ +139°. The more soluble compound, bromide "b," crystallized in prismatic needles. Its optical rotation in chloroform was $[\alpha]_D$ −94°.

After separation of bromide "a" from a mixture of the two bromides it was often noted that the rotation of the mother liquors changed in a positive direction and that more bromide "a" could then be separated. Also, if impure bromide "a" was kept in solution its rotation decreased. Hydrogen bromide increased the rate of the mutarotation of both bromide compounds. Figure 2 shows the change in specific rotation of both compounds with 0.10 *N* hydrogen bromide in acetic acid at 33°. Both curves approached a common value which was assumed to be that of an equilibrium mixture of the two bromides or a $\underset{k_b}{\overset{k_a}{\rightleftharpoons}}$ b. With bromide "b" as the starting material, the rate of change in rotation α may be expressed by $\frac{d\alpha}{dt} = ([\alpha]_a - [\alpha]_b)(k_b \cdot b - k_a \cdot a) = k_b' \cdot b - k_a' \cdot a$ where a and b are the amounts of the two bromides—as fractions of 1—present at the time t, k_a and k_b are the velocity constants, and $[\alpha]_a$ and $[\alpha]_b$ are the specific optical rotations of the pure isomers. Integration and substitution of the initial specific rotations of the two compounds gave the equation

$$t = \frac{473.8}{k_a' + k_b'} \log \frac{k_b'}{0.646\, k_b' - 0.354\, k_a' - \frac{k_a' + k_b'}{206} \cdot [\alpha]_D}$$

where $[\alpha]_D$ is the rotation at the time t.

[1] Roman numerals refer to the structural formulas in Figure 1.

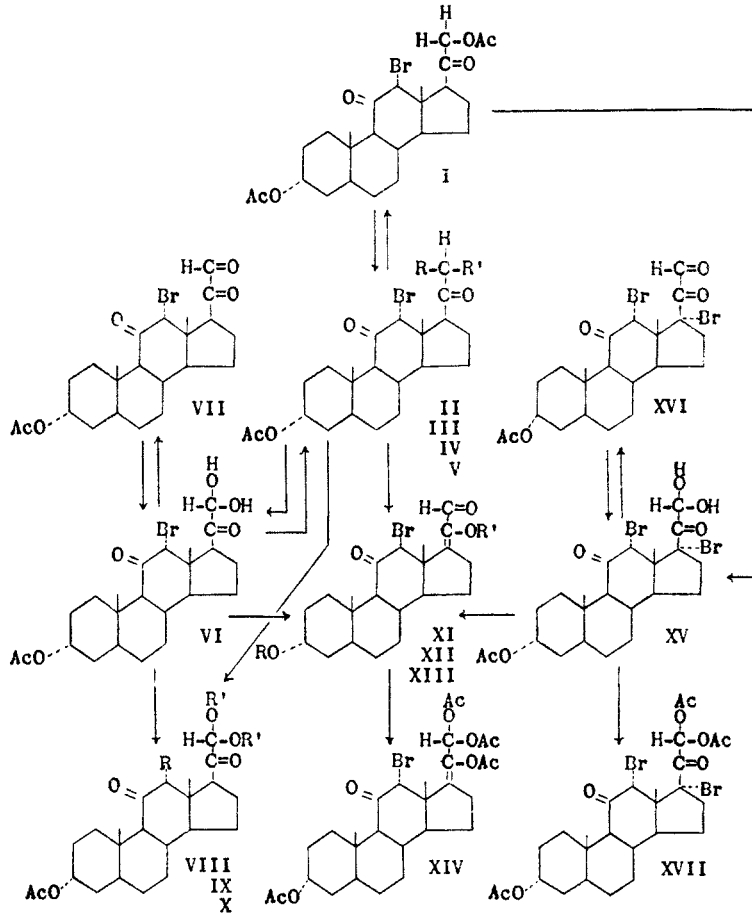


FIG. 1. STRUCTURAL FORMULAS OF COMPOUNDS I THROUGH XVII

II. R = Br; R′ = OAc. III. R = OAc; R′ = Br. IV. R = Cl; R′ = OAc. V. R = OAc; R′ = Cl.

VIII. R = Br; R′ = Ac. IX. R = H; R′ = Ac. X. R = Br; R′ = CH_3.

XI. R = Ac; R′ = H. XII. R = R′ = Ac. XIII. R = R′ = H.

XV, XVI, XVII. The configuration of the bromine at carbon 17 is assigned *alpha* in analogy with similar reactions (3).

With two experimental values, for which t = ∞ and $[\alpha]_D = 0$, the velocity constants were calculated:

$$k_a' = 5.46 \text{ degrees per minute}; \quad k_a = 0.0265 \text{ min.}^{-1}$$
$$k_b' = 9.29 \text{ degrees per minute}; \quad k_b = 0.0451 \text{ min.}^{-1}$$

The equations for the two reactions were,

$$\text{for bromide "a," } t = 32.2 \times \log \frac{5.46}{0.0716[\alpha]_D - 4.07}$$

$$\text{for bromide "b," } t = 32.2 \times \log \frac{9.29}{4.07 - 0.0716[\alpha]_D}$$

It can be seen from Figure 2 that the observed rotations fit the calculated curves, an indication that there is an equilibrium between the two compounds which consists of about 63% bromide "a" and 37% bromide "b" under the experimental conditions used.

It was observed that on longer standing in acetic acid solution with 0.10 *N* hydrogen bromide the optical rotation of both bromides slowly decreased until after 48 hours the value $[\alpha]_D$ +8° was reached. It was found that decrease in rotation was associated with a loss of bromine from the steroid, but the change in chemical structure is not known.

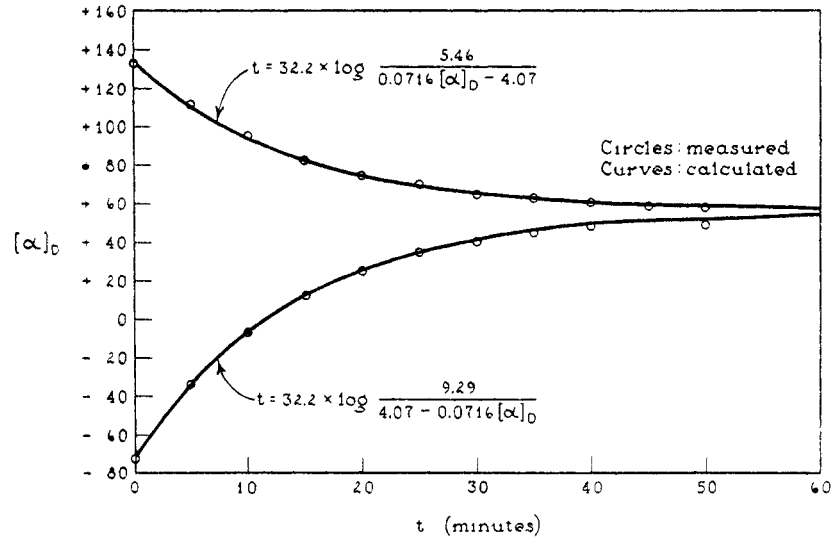


FIG. 2. MUTAROTATION OF BROMIDE "a" AND BROMIDE "b" IN 0.1 *N* HBr-HOAc

The formation of two bromides "a" and "b" and their interconversion through the influence of hydrogen bromide are observations which do not establish the position of the newly entered atom of bromine, although they do suggest that the two compounds may be the C-21 diastereoisomers (II and III). This formulation is supported by the substitution reactions which will be described.

REDUCTION WITH SODIUM IODIDE AND ACETIC ACID

The newly entered atom of bromine in both compounds was rapidly reduced with sodium iodide in glacial acetic acid, and I was recovered in practically quantitative yield.

REPLACEMENT WITH THE ACETOXYL GROUP

When bromide "a" or bromide "b" was dissolved in a mixture of benzene and glacial acetic acid and shaken with silver acetate for two days, a triacetoxy compound was obtained in high yield which appeared to be 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (VIII). With sodium acetate in acetic acid the

same compound was formed but in smaller yields. The triacetoxy steroid reduced both ammoniacal silver nitrate and phosphomolybdic acid (5), but with less intensity than did the ketol acetate (I). Zinc dust and acetic acid converted the triacetoxy compound to the bromine-free derivative (IX), and boiling with methanol in the presence of hydrogen chloride followed by reacetylation gave 3α-acetoxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane (X) in good yield.

REACTION WITH AQUEOUS PYRIDINE

Both bromo compounds "a" and "b" rapidly liberated one equivalent of bromide ion with aqueous pyridine at room temperature. The reaction product could be crystallized almost quantitatively from aqueous acetone or aqueous acetic acid. It had the properties of a hydrated glyoxal and formed yellow solutions in anhydrous solvents such as acetic acid, benzene, and chloroform, but colorless solutions in alcohols or aqueous solvents. The yellow color of the free glyoxal exhibited a weak absorption band at 440 mμ, ϵ = 20. Methyl glyoxal also absorbs light at this wavelength. The steroid glyoxal reduced ammoniacal silver nitrate and liberated iodine from acetic acid-sodium iodide, although at a rate slower than that of bromo compounds "a" and "b." It is, therefore, formulated as 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). The free glyoxal VII has not been obtained in crystalline form. Reich and Reichstein (6) who prepared derivatives of 3α-acetoxy-20-keto-$\Delta^{5,6}$-pregnen-21-al and 3,20-diketo-$\Delta^{4,5}$-pregnen-21-al by the method of Kröhnke and Börner, were also unable to crystallize the free glyoxals.

When the glyoxal hydrate (VI) was treated with methanolic hydrogen chloride, and the reaction product was reacetylated, a dimethyl acetal was obtained which was identical with the compound (X) prepared from the triacetate. With acetic anhydride and a drop of concentrated sulfuric acid VI gave the triacetate (VIII). With acetyl bromide and a trace of sulfuric acid VI yielded a mixture of bromoacetates from which bromide "a" (II) was isolated. The rotation of the reaction product suggested the presence of more than 40% of bromide "b," but when isolation was attempted partial isomerization to bromide "a" occurred. That acetyl bromide had converted the glyoxal hydrate (VI) largely into bromides "a" and "b" was indicated by reduction of the whole product with sodium iodide in acetic acid. This operation gave an excellent yield of I.

When VI was treated with acetyl chloride and all other conditions were the same as with acetyl bromide two isomeric chlorides could be isolated. The specific rotation of chloride "a" (IV) in chloroform was $[\alpha]_D$ +88°. The specific rotation of the more soluble chloride "b" (V) was $[\alpha]_D$ −35°. As in the case of the two bromides (II and III) the formation of chloride "a" is a little more favored than that of chloride "b" (about 54:46).

When the glyoxal hydrate (VI) was dissolved in a mixture of 1 part of pyridine and 4 parts of glacial acetic acid and heated at 60° for 16 hours the reaction product showed an absorption band in ultraviolet light with a maximum at 284 mμ, ϵ = 6,200 (chloroform). Longer heating caused a gradual loss of absorption. The same product was formed at room temperature, but required several days.

Chromatographic separation and recrystallization yielded a compound with an extinction coefficient at 284 mμ, ε = 13,700 (chloroform) (Fig. 3). Its analysis showed it to be isomeric with glyoxal VII.

This product reduced ammoniacal silver solution and showed in alcohol a greenish brown color when a drop of alcoholic ferric chloride solution was added. With tetranitromethane it gave a weakly positive reaction. In chloroform solution 1 mole of bromine was absorbed within 15 minutes. It was concluded that this compound was the enol derivative of the glyoxal: 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al (XI).

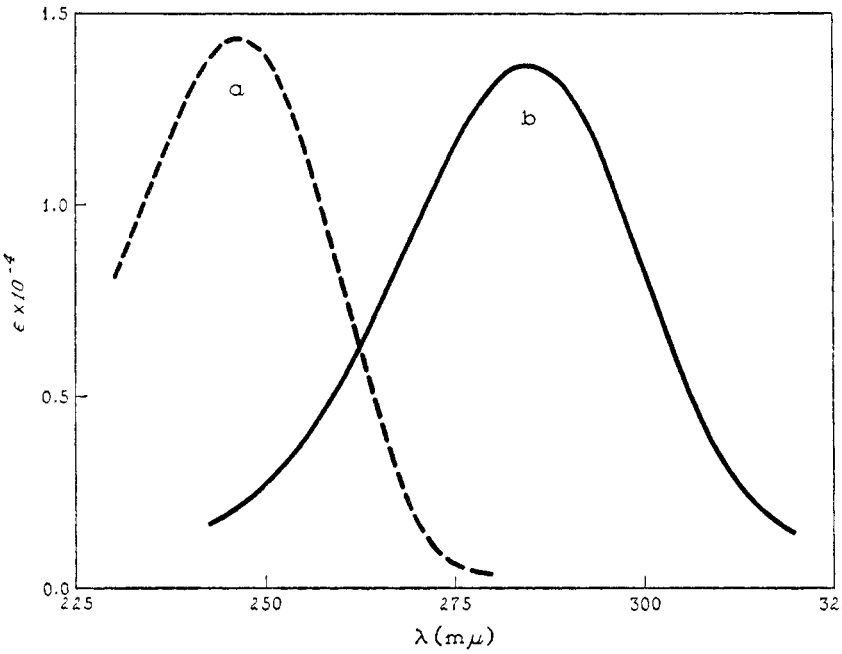


FIG. 3. ULTRAVIOLET ABSORPTION SPECTRA OF (a) 3α,20-diacetoxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al (XII) in ether; (b) 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al (XI) in chloroform.

The absorption data are in agreement with those of the enol forms of α-diketones (7–10). Such enol derivatives may be obtained with alcoholic potassium hydroxide or sodium hydroxide, but this procedure is not applicable to a glyoxal, since it causes immediate rearrangement to the corresponding α-hydroxy acid.

On acetylation with pyridine-acetic anhydride the enol was converted to its acetyl derivative (XII) with characteristic absorption $\lambda_{max.}$ 246 mμ, ε = 14,300 (ether) (Fig. 3). The acetyl derivative (XII) did not give the ferric chloride reaction; it reduced ammoniacal silver nitrate and absorbed bromine, although at a rate much slower than did the free enol.

The shift of the maximal absorption on acetylation from 284 mμ to 246 mμ is in keeping with previous observations on the enol derivatives of α-diketones.

In each case the enol acetate showed the absorption characteristic of an α-substituted α,β-unsaturated ketone (7–10).

It was further observed that the enol acetate (XII) was formed directly from either bromide "a" or bromide "b" with a mixture of pyridine and acetic acid. Spectrophotometric analysis revealed that mixtures of enol and enol acetate were formed, depending on the proportion of acetic acid and pyridine used (Table I).

Solution of the enol acetate in acetic anhydride and addition of a little concentrated sulfuric acid afforded a good yield of a crystalline product which did not give the enol reaction with ferric chloride but absorbed 1 mole of bromine within three hours. The structure of this compound appears to be 3α,20,21,21-tetraacetoxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnene (XIV).

TABLE I

FORMATION OF ENOL AND ENOL ACETATE FROM BROMIDE "a"

SOLVENTS		REACTION PRODUCTS FORMED[d]	
Acetic acid, %	Pyridine, %	Enol, %	Enol acetate, %
0	100	15	0
50	50	36[a]	17[a]
75	25	13[b]	43[b]
90	10	5[c]	35[c]
100	0	0	0

[a] For bromide "b" 33% and 23% respectively were found. [b] After 112 hours the amounts formed were 28% and 30% respectively. [c] After 112 hours the amounts formed were 11% and 31% respectively. [d] After 45 hours at 25°.

Treatment of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I) in acetic acid with a large excess of bromine and a small amount of hydrogen bromide yielded a crystalline dibromo compound which had the characteristics of a hydrated glyoxal. It formed yellow solutions in anhydrous solvents, but colorless solutions in methanol or aqueous solvents. There was an absorption maximum at 423 mμ, ϵ = 40. Analysis and the physical and chemical properties indicated that the structure of the compound was 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α,17α-dibromopregnane (XV).

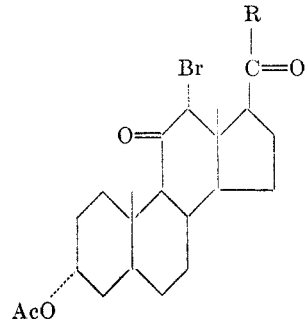
The free glyoxal (XVI) has not been obtained in crystalline form. Acetic anhydride and a little sulfuric acid converted XV into a crystalline derivative to which the structure 3α,21,21-triacetoxy-11,20-diketo-12α,17α-dibromopregnane (XVII) was assigned.

An unexpected rearrangement occurred when XV was heated in aqueous methanol in the presence of a slight molar excess of sodium bisulfite. The product exhibited an absorption maximum at 284 mμ, ϵ = 10,900 (methanol), and closely resembled the "enol" obtained from the glyoxal (VI). However, it did not contain an acetyl group and was apparently formed by reductive removal of the atom of bromine at C-17 with enolization and hydrolysis of the acetyl group at C-3.

A series of 21-substituted 3α-acetoxy-11,20-diketo-12α-bromopregnanes are arranged in Table II in the order of increasing molecular weight. It is apparent that the molecular weights and the molecular rotations[2] increase in the same order and the data suggest a linear relationship. Since all of the compounds are identical except for the substituents at C-21, the assumption is made that the vicinal influence of the side chain at C-17 is negligible and that, therefore, its contribution to the molecular rotation is confined to its own partial rotation.

TABLE II

OBSERVED AND CALCULATED OPTICAL ROTATIONS OF COMPOUNDS WITH DIFFERENT SUBSTITUENTS AT C-21



R	$M^a_{-CO\cdot R}$	$[\alpha]^c_D$ (Found)	$[M]^a_D$ (Found)	REF.	$[M]^a_D$ (Calculated)	$[\alpha]_D$ (Calculated)
CH_3	43.0	25±2°	1,130°	4	1,105°	24.4°
CHO	57.0	26±2°	1,220°	[b]	1,219°	26.0°
CH_2OCH_3	73.1	26±2°	1,260°	4	1,350°	28.0°
$CH(OH)_2$	75.0	28±2°	1,360°	[b]	1,367°	28.1°
$COOCH_3$	87.1	30±2°	1,490°	11	1,465°	29.4°
CH_2OAc	101.1	33±2°	1,690°	4	1,578°	30.8°
$CH(OCH_3)_2$	103.1	30±2°	1,540°	[b]	1,594°	31.0°
CH_2Br	122.0	34±2°	1,810°	4	1,749°	32.8°
$CH(OAc)_2$	159.1	35±2°	1,990°	[b]	2,054°	36.1°

[a] See text for explanation. [b] This paper.

It is further assumed that this contribution is proportional to the weight of the side chain and the total rotation therefore can be separated into two parts:

$$[M]_D = a + bM_{-CO\cdot R}$$

where b is the contribution of the side chain of the molecular weight $M_{-CO\cdot R}$, and a that of the remainder of the molecule. By the method of least squares the constants of this equation were calculated and the equation was obtained:

$$[M]_D = 753 + 8.19\ M_{-CO\cdot R}$$

[2] $[M]_D = \dfrac{[\alpha]_D \times \text{M.W.}}{10}$

In the last column of Table II are given the calculated values for the specific rotations.

EXPERIMENTAL[3]

All rotations were taken in chloroform ($c \sim 1$) unless stated otherwise. All melting points were taken on the Fisher-Johns apparatus. The light absorption curves were determined with a Beckman quartz spectrophotometer.

Bromide "a" (II) and bromide "b" (III) from 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I). Compound I (20.46 g.) was dissolved in 400 ml. of chloroform. At a temperature of 0°, 80 ml. of 1 *N* bromine in chloroform and gaseous hydrogen bromide (for ten minutes) were added. After three hours at 0° the solution was concentrated under reduced pressure to a small volume, chloroform was added and again removed under reduced pressure, and the residue was crystallized in about 95% yield from a large volume of ligroin; $[\alpha]_D$ +34° ± 2°. Crystallization from 40 ml. of chloroform and 200 ml. of ligroin afforded about a half of the material with $[\alpha]_D$ +106°. After five recrystallizations in the same manner the rotation remained constant at $[\alpha]_D$ +139° ± 2°; $[\alpha]_D$ +133° ± 2° ($c \sim 1$, acetic acid). This compound, bromide "a," crystallized in flat plates and melted at 180–182° with strong decomposition.

Anal. Calc'd for $C_{25}H_{34}Br_2O_6$: C, 50.86; H, 5.80; Br, 27.08.

Found: C, 51.03; H, 5.99; Br, 26.41.

From the mother liquor of bromide "a", bromide "b" was obtained by removal of most of the chloroform under reduced pressure and addition of a large volume of ligroin. The specific rotation was $[\alpha]_D$ −38° ± 2°. After ten crystallizations from a small volume of chloroform and much ligroin the rotation was constant at $[\alpha_D$ −94° ± 2°; $[\alpha]_D$ −73° ± 2° ($c \sim 1$, acetic acid). Bromide "b" crystallized in prismatic needles, melted at 147–148° with resolidification and then melted at 167–173° with strong decomposition.

Anal. Calc'd for $C_{25}H_{34}Br_2O_6$: C, 50.86; H, 5.80; Br, 27.08.

Found: C, 50.74; H, 5.92; Br, 27.68.

3α,21-Diacetoxy-11,20-diketo-12α-bromopregnane (I) from bromide "a" (II) and bromide "b" (III). Bromide "a" (100 mg.) was dissolved in 2 ml. of benzene and 5 ml. of acetic acid; 200 mg. of sodium iodide was added with shaking, and after 15 minutes at room temperature the addition of water permitted titration of the liberated iodine. The theoretical amount of 0.10 *N* thiosulfate (3.4 ml.) was required. The steroid was extracted with benzene, the benzene extract was washed with water, sodium bicarbonate solution, again with water, and evaporated to dryness. The residue, crystallized from dilute acetone, weighed 83 mg. (96%) and melted at 162–163°. The melting point was not depressed when the crystals were mixed with an authentic sample of compound I.

When a 50-mg. sample of bromide "b" was treated with acetic acid and sodium iodide, compound I was obtained in a yield of 95%.

3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane (VIII) from bromide "a" (II) and bromide "b" (III) with silver acetate. For this, 590 mg. of either bromide "a" or bromide "b" was dissolved in 5 ml. of benzene and 15 ml. of acetic acid. Silver acetate (334 mg.) was added, and the mixture was agitated at room temperature for 45 hours. The insoluble material was filtered off, chloroform was added, and the organic phase was washed with water, sodium bicarbonate solution, again with water, and dried. The material which remained after removal of the solvents under reduced pressure was crystallized from methanol. Compound VIII was obtained from both bromide "a" and bromide "b" in a yield of almost 90%. The product melted at 169.5–170.5°; $[\alpha]_D$ +35° ± 2°. This derivative

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[3] The compounds described in this paper were analyzed in the laboratories of Mr. J. F. Alicino, Metuchen, New Jersey; Mr. William Saschek, Chicago, Illinois; and Merck & Co., Inc., Rahway, New Jersey.

of glyoxal reduced both ammoniacal silver nitrate and phosphomolybdic acid, but with less intensity than did the ketol acetate (I).

Anal. Calc'd for $C_{27}H_{37}BrO_8$: C, 56.94; H, 6.55; Br, 14.03.

Found: C, 56.80; H, 6.30; Br, 13.75.

3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane (VIII) from bromide "a" (II) and bromide "b" (III) with sodium acetate. As above, 590 mg. of either bromide "a" or bromide "b" was added to 5 ml. of a 0.33 *M* solution of sodium acetate in acetic acid which was heated on the steam-bath for two hours. Water was then added, and the precipitate was dissolved in chloroform. The latter was washed with water, sodium bicarbonate solution, water, and dried. After removal of the chloroform under reduced pressure the residue was recrystallized several times from methanol. The product melted at 169.5–170.5° and did not depress the melting point of VIII when mixed with a sample of this compound obtained by the silver acetate method. With sodium acetate and acetic acid the yield of material was only about 60%. The removal of the bromine was quantitative.

3α,21,21-Triacetoxy-11,20-diketopregnane (IX) from 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (VIII). To 3.0 g. of VIII dissolved in 60 ml. of acetic acid at temperature 15°, 3.0 gm. of zinc dust was added, and after one hour with frequent shaking at room temperature the solution was filtered. The filtrate was diluted with water, extracted with chloroform, and the organic phase, after washing with water, sodium bicarbonate solution, and water, was dried with sodium sulfate and evaporated. The residue crystallized from methanol (short rods), weighed 2.445 g., and melted at 115-116°; $[\alpha]_D$ +117° ± 2°.

Anal. Calc'd for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81.

Found: C, 65.96; H, 7.87.

3α-Acetoxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane (X) from 3α,21,21-triacetoxy-11,20-diketo-12α-bromopregnane (VIII). In 160 ml. of methanol 0.25 *N* with respect to hydrogen chloride, 5.695 g. of VIII was dissolved, and the solution was boiled under reflux for two hours. The solution was cooled, 20 ml. of water containing 10 g. of potassium carbonate was added, and the solvents were concentrated to a small volume and extracted with chloroform. The chloroform solution was washed with water, dried, and evaporated to dryness under reduced pressure. Acetylation of the 3α-hydroxyl group was carried out in the usual way with 20 ml. each of acetic anhydride and pyridine. The material crystallized from dilute methanol in long, fine needles, weighed 4.115 g., and after two recrystallizations from dilute methanol melted at 157–159°; $[\alpha]_D$ +30° ± 2°.

Anal. Calc'd for $C_{25}H_{37}BrO_6$: C, 58.47; H, 7.26; CH_3O, 12.09.

Found: C, 58.56; H, 6.96; CH_3O, 12.00.

3α-Acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI) from bromide "a" (II) or bromide "b" (III). A 236 mg. sample of either bromide "a" or bromide "b" was dissolved in 2 ml. of 80% aqueous pyridine at room temperature. After 15 minutes the solution was poured into 2 ml. of 10 *N* sulfuric acid and ice. The precipitate was filtered off, washed with water, and air-dried. The filtrate contained the theoretical amount of bromide ion. The careful addition of water to a solution of the dry reaction product in acetic acid afforded 142 mg. of small needles and 31 mg. in a second crop; yield, 89%. For analysis the first crop was recrystallized from dilute acetic acid; m.p. 120–140° with decomposition. When placed on the stage at 147° the product melted at 149–151° with decomposition; $[\alpha]_D$ +28° ± 2°.

A freshly prepared solution of VI in chloroform is colorless, but on standing the solution becomes pale yellow and there is a decrease in the specific rotation to +26° ± 2° (calculated as glyoxal). These changes are explained through loss of water from C-21 and formation of the free glyoxal.

Anal. Calc'd for $C_{23}H_{33}BrO_6$: C, 56.91; H, 6.85; CH_3CO, 8.86.

Found: C, 56.80; H, 7.11; CH_3CO, 8.92.

3α-Acetoxy-21,21-dimethoxy-11,20-diketo-12α-bromopregnane (X) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). First, 100 mg. of VI was dissolved in warm benzene, and the solvent was removed under reduced pressure. The residue was treated

in the same manner twice, then dissolved in 4 ml. of 0.25 *N* methanolic hydrogen chloride and boiled under reflux for two hours. The solution was cooled, and an excess of sodium carbonate solution and ice was added. The steroid was extracted with chloroform which was washed, dried over sodium sulfate, and evaporated under reduced pressure. The residue was acetylated in the usual manner with 1 ml. each of acetic anhydride and pyridine. The acetylated product was dissolved in methanol, and water was added until the solution became turbid. After filtration through infusorial earth and addition of more water the acetal (41 mg.) crystallized. One recrystallization from dilute acetone afforded material in the form of hairlike needles which melted at 157–159° and showed no depression of the melting point when mixed with the compound prepared from the triacetate.

3α,21,21-Triacetoxy-11,20-diketo-12α-bromopregnane (VIII) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). When 100 mg. of VI was dissolved in 2 ml. of warm acetic anhydride the solution was yellow due to the formation of the free glyoxal VII. It was cooled in an ice-bath, and 1 drop of concentrated sulfuric acid was added. After a half minute ice was added, the acetic anhydride was decomposed, and the insoluble material was filtered, washed with water, and air-dried. The material was recrystallized twice from a small volume of methanol, m.p. 167–168°; it did not depress the melting point when mixed with the triacetate obtained from the 21-bromo compounds.

Bromide "a" (II) and bromide "b" (III) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). To 1.0 g. of VI dissolved in 10 ml. of acetyl bromide was added 0.2 ml. of concentrated sulfuric acid. After 15 minutes at room temperature the mixture was diluted with 100 ml. of ice-cold chloroform and poured into a separatory-funnel with ice. The mixture was shaken vigorously. The chloroform extract was washed with cold sodium bicarbonate solution and with water, and dried over sodium sulfate. The optical rotation was $[\alpha]_D$ +36° ± 2°, which is the rotation of a mixture of 56% bromide "a" and 44% bromide "b." The chloroform was removed under reduced pressure, and the residue was crystallized from 2 ml. of chloroform and 10 ml. of ligroin. After five recrystallizations from chloroform-ligroin the rotation of the product was $[\alpha]_D$ +137° ± 2°, the melting point was 180–182°, and there was no depression when the crystals were mixed with a sample of bromide "a."

The first mother liquor had the rotation $[\alpha]_D$ −32° ± 2°. This value indicated about 74% of bromide "b." The products of this reaction were quite similar to the mixtures obtained after bromination of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I).

Chloride "a" (IV) and chloride "b" (V) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). After 337 mg. of VI was dissolved in 3 ml. of acetyl chloride, 3 drops of concentrated sulfuric acid were added. After one hour at room temperature the acetyl chloride was decomposed with ice, and the organic material was extracted with chloroform. The chloroform solution was washed with water, sodium bicarbonate solution, again with water, and dried over sodium sulfate. Optical rotation was $[\alpha]_D$ +31° ± 2°. Concentration of the solvent and addition of ligroin afforded 173 mg. of crystals which melted at 160–178°, $[\alpha]_D$ +63°. Several recrystallizations from dilute acetone raised the melting point of chloride "a" to 189–191° and the rotation to $[\alpha]_D$ + 88° ± 2°.

Anal. Calc'd for $C_{25}H_{34}ClBrO_6$: C, 55.00; H, 6.28; Cl, 6.49; Br, 14.64.
Found: C, 55.23; H, 6.10; Cl, 6.35; Br, 14.35.

The mother liquor of the first crystallization was brought to dryness, and the residue was dissolved in chloroform; $[\alpha]_D$ +11° ± 2°. After removal of the solvent, and six crystallizations from dilute acetone, the product melted at 153–154°; $[\alpha]_D$ −35° ± 4° (12.2 mg. in 2.5 ml. of chloroform).

Anal. Calc'd for $C_{25}H_{34}ClBrO_6$: C, 55.00; H, 6.28; Halogen, 21.13 (Cl, 6.49; Br, 14.64).
Found: C, 55.16; H, 6.43; Halogen, 20.55.

3α-Acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI) from chloride "a" (IV). Chloride "a" (29 mg.) was dissolved in 0.5 ml. of 80% aqueous pyridine at room temperature. After 20 minutes benzene was added, and the mixture was washed with sulfuric acid. The acid washings were titrated and found to contain 0.050 milliequivalent of chloride ion (94%).

The benzene extract was washed with sodium bicarbonate solution and water and taken to dryness. The residue crystallized from dilute acetic acid; 23 mg., m.p. 148–149°, $[\alpha]_D$ +28° ± 3°.

3α-Acetoxy-20-hydroxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XI) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α-bromopregnane (VI). A solution of 1.30 g. of VI in 20 ml. of a mixture of 4 parts of acetic acid and 1 part of pyridine was kept 16 hours at 60°; the solution then was poured into an excess of mineral acid and ice. The precipitate was filtered, washed with water, and air-dried. The product, which had a maximal absorption at 284 mμ, ϵ = 6,200 (chloroform), was dissolved in 140 ml. of benzene and passed through a column composed of 44 g. of a 1:1 mixture of magnesium silicate and infusorial earth. Benzene eluted 581 mg. of material, and this fraction after removal of the solvent under reduced pressure was crystallized from methanol. After several recrystallizations from dilute acetone the crystals melted at 190–191°; $[\alpha]_D$ +96° ± 2°, $\lambda_{max.}$ 284 mμ, ϵ = 13,700 (chloroform); $\lambda_{max.}$ 282 mμ, ϵ = 12,500 (methanol).

Anal. Calc'd for $C_{23}H_{31}BrO_5$: C, 59.09; H, 6.69; CH_3CO, 9.21.

Found: C, 58.76; H, 6.94; CH_3CO, 9.79.

When the time of the reaction was varied, the yields, based on the absorption of light at 284 mμ, were 34, 40, 45, and 39% respectively, after 4, 7, 16, and 32 hours.

An increase in the rate of formation of XI was noted when the amount of acetic acid was reduced; with a mixture of equal parts of acetic acid and pyridine at 60° the values were 40 and 45%, respectively, after 4 and 7 hours.

3α,20-Diacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XII) from 3α-acetoxy-20-hydroxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XI). A solution of 234 mg. of XI in 5 ml. of pyridine and 5 ml. of acetic anhydride was kept one hour at room temperature. Then ice and water were added, the acetic anhydride was decomposed, and the insoluble material was extracted with chloroform. The chloroform solution was washed with dilute sulfuric acid, sodium bicarbonate solution, water, and was dried with sodium sulfate. The solvent was removed under reduced pressure, and the residue was crystallized from chloroform-ligroin as leaflets (229 mg.). After two recrystallizations from dilute acetone the melting point was 162–164°, $[\alpha]_D$ +86° ± 2°. The compound in ether showed an absorption band with the maximum at 246 mμ, ϵ = 14,300.

Anal. Calc'd for $C_{25}H_{33}BrO_6$: C, 58.94; H, 6.53; CH_3CO, 16.90.

Found: C, 58.92; H, 6.48; CH_3CO, 16.97.

3α-Acetoxy-20-hydroxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XI) and 3α,20-diacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XII) from bromide "a" (II). Bromide "a" (59.0 mg.) was dissolved in 1.0 ml. of a mixture of 3 parts of acetic acid and 1 part of pyridine. After 45 hours at room temperature benzene was added and the benzene solution was washed with water, dilute hydrochloric acid, water, sodium bicarbonate solution, again with water, and dried over sodium sulfate. The benzene was removed under reduced pressure, and the residue was dissolved in ether. The absorption spectrum showed maxima at 246 mμ and 284 mμ, and indicated that 43% of the bromide "a" (II) had been converted into the enol acetate (XII) and 13% was present as the free enol (XI). When the time of the reaction was extended to five days, the amount of enol acetate generally decreased, that of the enol increased.

3α,20,21,21-Tetraacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnene (XIV) from 3α,20-diacetoxy-11-keto-12α-bromo-Δ^{17,20}-pregnen-21-al (XII). To a solution of 206 mg. of XII in 4 ml. of acetic anhydride, cooled in an ice-bath, 2 drops of concentrated sulfuric acid were added. After a half minute ice was added, the acetic anhydride was decomposed, and the material was filtered, washed with water, and air-dried. The crude product was crystallized from acetone and petroleum ether; after three recrystallizations from dilute acetone, m.p. 154–155° (long needles), $[\alpha]_D$ +60° ± 2°.

Anal. Calc'd for $C_{29}H_{39}BrO_9$: C, 56.96; H, 6.43.

Found: C, 57.11; H, 6.23.

3α-Acetoxy-21,21-dihydroxy-11,20-diketo-12α,17α-dibromopregnane (XV) from 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I). A solution of 10.22 g. of I in 500 ml. of acetic

acid which contained 4.0 g. of bromine and 8.1 g. of hydrogen bromide was kept four days at room temperature. After concentration under reduced pressure to about 75 ml., 50 ml. of 80% acetic acid was added, and the solution was again concentrated. The addition of benzene and water induced the separation of crystals at the interphase. The material (3.4 g.) was purified by recrystallization from dilute acetic acid until the melting point was 206–208° with decomposition when placed on the stage at 203°. $[\alpha]_D$ −34.5° ± 2°. The solution soon turned yellow, owing to formation of the free glyoxal, and the rotation changed to $[\alpha]_D$ −37° ± 2° (calculated as glyoxal).

Anal. Calc'd for $C_{23}H_{32}Br_2O_6$: C, 48.95; H, 5.72; Br, 28.32; CH_3CO, 7.63.

Found: C, 48.84; H, 5.74; Br, 28.20; CH_3CO, 8.27.

3α,21,21-Triacetoxy-11,20-diketo-12α,17α-dibromopregnane (XVII) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α,17α-dibromopregnane (XV). To 50 mg. of XV dissolved in 2 ml. of acetic anhydride, 1 drop of concentrated sulfuric acid was added. After a half minute the mixture was poured on ice, the acetic anhydride was decomposed, the insoluble material was filtered, washed with water, and air-dried. Crystallization first from methanol, then from dilute acetone afforded a product, m.p. 167–169°, $[\alpha]_D$ −24° ± 2°.

3α,20-Dihydroxy-11-keto-12α-bromo-$\Delta^{17,20}$-pregnen-21-al (XIII) from 3α-acetoxy-21,21-dihydroxy-11,20-diketo-12α,17α-dibromopregnane (XV). To 1.128 g. of XV dissolved in 20 ml. of warm methanol, was added 1.04 g. of sodium bisulfite, and the solution was warmed and diluted with 40 ml. of water. The initial turbidity disappeared as the solution became hot. After three to four minutes at the boiling temperature crystals separated which weighed 770 mg.; m.p. 179–180°, $[\alpha]_D$ +90° ± 2°. After several recrystallizations from dilute acetone the melting point was 189.5–191°, $[\alpha]_D$ +101° ± 2°; $\lambda_{max.}$ 284 mμ; ϵ = 10,900 (methanol).

Anal. Calc'd for $C_{21}H_{29}BrO_4$: C, 59.29; H, 6.87.

Found: C, 59.16; H, 6.89; no acetyl present.

SUMMARY

Bromination of 3α,21-diacetoxy-11,20-diketo-12α-bromopregnane (I) in chloroform catalyzed with hydrogen bromide affords the two epimeric forms of the 21-bromo-21-acetate derivative of I.

Both epimers are converted into the 21-diacetate steroid with silver acetate and into the 20,21-glyoxal with aqueous pyridine. Both epimeric forms are reduced to I with sodium iodide in acetic acid.

The glyoxal separates in hydrated form as the 21-dihydroxy derivative. With acetyl bromide and sulfuric acid the glyoxal is converted to the two epimeric 21-bromo-21-acetate compounds derived by bromination of I, and with acetyl chloride the glyoxal forms the corresponding 21-chloro-21-acetate steroids. Methanolic hydrogen chloride converts the glyoxal to the 21-dimethyl acetal and with acetic anhydride and sulfuric acid the 21-diacetate is formed. In a mixture of acetic acid and pyridine the glyoxal forms the 20-enol-$\Delta^{17,20}$ isomer (XI). Acetic anhydride with XI yields the 3α,20-diacetate-$\Delta^{17,20}$ derivative in the presence of pyridine and the 3α,20,21,21-tetraacetate-$\Delta^{17,20}$ compound in the presence of sulfuric acid.

Bromination of the starting material in acetic acid with bromine and hydrogen bromide affords the 17α-bromo derivative of the 20,21-glyoxal hydrate (XVI) which forms the 21,21-diacetate with acetic anhydride and sulfuric acid. The 17-bromohydrated glyoxal (XVI) with sodium bisulfite in aqueous methanol is converted with loss of bromine into the enolic form (XIII).

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Recent advances in this laboratory indicate the possibility that the atom of bromine designated as 17α may be at position 26. This problem is under investigation.