one, m.p. 112–114°. Finally elution with 2:1 benzene–petroleum ether gave 106 mg. of crystals of XIII which were recrystallized from petroleum ether and melted at 148–150°.

The analytical sample was obtained from the same solvent; m.p. 150–152, $[\alpha]_D$ +110, $\lambda_{max}^{C_2H_5OH}$ 238 mμ (ε 12,000).

Anal. Calcd. for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14; Br, 19.5. Found: C, 61.48; H, 7.24; Br, 19.4.

B. From 15β,17α-Dibromo 16-Ketone VI.—A solution of 50 mg. of VI in 6 cc. collidine and 4 cc. dimethylformamide was refluxed for 15 min. It was then diluted with ether which was then washed with dilute hydrochloric acid and water. The residue remaining after drying and evaporating the solvent was crystallized from petroleum ether to give needles, m.p. 146–150°, identical in all respects with the 3β-acetoxy-17ξ-bromo-5α-androst-14-ene-16-one (XIII).

C. From 15α,17β-Dibromo 16-Ketone XI.—A small amount (15 mg.) of XI was dehydrobrominated as above. The product (6 mg.) was identical by mixed melting point with XIII. The infrared spectrum confirmed this identity, except in that a persistent impurity was also present as shown by a small carbonyl band at 1764 cm.$^{-1}$.

3β-Acetoxy-15,17,-dibromo-5α-androst-14-ene-16-one (XIV).—A 100-mg. sample of the tribromo ketone X in 8 cc. of collidine and 5 cc. of dimethylformamide was refluxed for 15 min. The reaction was worked up in the manner described above to give a reddish oil which was chromatographed on alumina. Elution with 50% benzene in petroleum ether gave 67 mg. of crystals which were recrystallized from methanol to give m.p. 180–183°.

The analytical sample melted at 182–184°, $[\alpha]^{25}_D$ +84, $\lambda_{max}^{C_2H_5OH}$ 262 mμ (ε 10,000).

Anal. Calcd. for $C_{21}H_{28}O_3Br_2$: C, 51.66; H, 5.78. Found: C, 51.52; H, 5.74.

3β-Acetoxy-5α,14β-androstane-16-one (XV).—A 100-mg. sample of the 17ξ-bromo-5α-androst-14-ene-16-one XIII was hydrogenated in ethanol over 5% palladium-on-charcoal. Within 10 min. 12 ml. of hydrogen was taken up. The catalyst was filtered off, the solvent was evaporated, and the residue crystallized from dilute methanol to show a melting point 144–148°. The material was identical in all respects with 3β-acetoxy-5α,14β-androstane-16-one (XV) obtained by a different route.[10]

17ξ-Bromo-5α-androst-14-ene-3β,16ξ-diol (XVI).—A 100-mg. sample of 3β-acetoxy-17ξ-bromo-5α-androst-14-ene-16-one (XIII) was dissolved in ether and treated for 1 hr. with lithium aluminum hydride at 0°. The conventional work-up gave 64 mg. of prisms from petroleum ether–acetone, m.p. 145–148° with decomposition.

Anal. Calcd. for $C_{19}H_{29}O_2Br$: C, 61.79; H, 7.91. Found: C, 61.37; H, 8.17.

14β-Androstane-3β-ol (XVII). A. From 17ξ-Bromo-5α-androst-14-ene-3β,16ξ-diol (XVI).—A 50-mg. sample of VI in ethanol was hydrogenated over 5% palladium-on-charcoal for 5 min. After filtering off the catalyst and evaporating the solvent the residue was crystallized from petroleum ether to give needles, m.p. 148–150°.

The analytical sample of XVII melted 148–150°.

Anal. Calcd. for $C_{19}H_{32}O$: C, 82.54; H, 11.66. Found: C, 82.17; H, 11.34.

B. From 3β-Acetoxy-5α,14β-androstane-17-one.—A 10-mg. sample of 3β-acetoxy-5α,14β-androstane-17-one was reduced by the Huang-Minlon procedure. The product obtained was recrystallized from petroleum ether as needles, m.p. 148–151°, identical in all respects with the 5α,14β-androstane-3β-ol obtained in method A.

Acknowledgment.—The author is grateful to Dr. T. F. Gallagher for his interest and encouragement in this work. He would like to thank Mrs. Beatrice Gallagher and staff for the infrared spectra. The technical assistance of Rosemarie Lehman and Richard Phillion is also gratefully acknowledged.

Bromination of Phenolic Steroids. I. Substitution of Estrone and 17β-Estradiol in Ring A[1]

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Received August 11, 1961

Both the 2- and 4-bromo as well as the 2,4-dibromo derivatives of estrone and 17β-estradiol have been synthesized and characterized. Conditions which produce exclusively one isomer in high yield are described.

Although phenols are brominated smoothly in high yield, this reaction has not been systematically applied to the phenolic steroids. Girard[2] prepared a monobrominated equilenin, but did not determine its structure. Marrian and Haslewood[3] found that the methyl ethers of estrone (I) and estriol consumed one mole of bromine in iodine number titrations (using BrI) forming monobromo compounds of undetermined structure. Woodward[4] synthesized 2,4-dibromoestradiol (IV) in 68% yield by treating estradiol (II) with *N*-bromoacetamide in alcohol. Recently, Tomson and Horwitz[5] synthesized 2- and 4-bromoestrone methyl ethers from the previously described nitro compounds[6] *via* the Sandmeyer reaction. We wish to report experiments which now enable one to brominate I and II in either the 2- or 4-position in high yield and purity. In addition, the 2,4-dibromo compound may be obtained in high yield with little substitution in the nonaromatic rings.

Aromatic dibromination occurs with *N*-bromosuccinimide (NBS) in refluxing chloroform, in

(1) Supported in part by Grant P-235 from the American Cancer Society.

(2) A. Girard, G. Sandulesco, A. Fridenson, and J. J. Rutgers, *Compt. rend.*, **195**, 981 (1932).

(3) G. F. Marrian and G. A. D. Haslewood, *J. Soc. Chem. Ind.*, **51**, 277T (1932).

(4) R. B. Woodward, *J. Am. Chem. Soc.*, **62**, 1625 (1940).

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(6) H. Werbin and C. Holloway, *J. Biol. Chem.*, **223**, 651 (1956).

TABLE I

BROMINATION OF ESTRONE AND 17β-ESTRADIOL

Reactant	Product	% Yield[a]
Estrone (I)	2-Bromoestrone (V)	85[b]
Estrone (I)	4-Bromoestrone (VII)	90,[c] 87[d]
Estrone (I)	2,4-Dibromoestrone (III)	90,[e] 90,[f] 78[g]
Estradiol (II)	2-Bromoestradiol (VI)	75[b]
Estradiol (II)	4-Bromoestradiol (VIII)	85[d]
Estradiol (II)	2,4-Dibromoestradiol (IV)	90,[e] 84,[f] 78[g]

[a] Yield of product after crystallization. [b] Br_2, glacial acetic acid, Fe, 17°. [c] Br_2, 80% aqueous acetic acid, Fe, 20°. [d] NBS, CCl_4, reflux 1 hr. [e] Br_2, glacial acetic acid, 17°. [f] NBS, $CHCl_3$, reflux 1 hr. [g] NBS, C_2H_5OH, 25°, 18 hr.

absolute ethanol at room temperature or with bromine in glacial acetic acid at 17° (Table I). The last method is preferred, since the reaction is so rapid as to be essentially a titration. At 17° further bromination is inappreciable. Small amounts of light brown oil, which increase in amount with increasing reaction temperature, are always obtained. No unchanged starting material was detected at the end of the reaction.

Addition of a small amount of finely divided iron powder to the reaction mixture blocks 4-bromination and the 2-bromo derivative is formed exclusively. The yields are slightly less than those experienced with dibromination. We have no explanation for this action of iron. It is not due to the effect of iron (III), which might be formed *in situ*, for ferric chloride (10^{-3} *M*) completely blocks the reaction and starting material is recovered quantitatively. If the reaction temperature is raised to room temperature, evolution of a gas from the surface of the iron particles becomes visible; the product in this case is an oil which does not crystallize. That iron blocks the entry of bromine rather than selectively removing one bromine from the dibrominated product, as sometimes happens,[7] is supported by the fact that iron has no effect upon the dibrominated product, even in boiling acetic acid.

2-Bromination is critically dependent on the bromine used. The experiments reported here were performed with Mallinckrodt bromine contained in sealed glass ampoules. When we tried to repeat these experiments using either Baker or Mallinckrodt bromine in screw cap bottles, the yield of 2-bromo product was always inferior to that obtained with bromine from glass ampoules. In addition, bromine from screw cap bottles changed rapidly after opening the bottle, and in a few days 2-bromination became impossible although 4-bromination and 2,4-dibromination still occurred readily.

Brominations conducted in aqueous acetic acid follow a different course: I yields only 4-bromoestrone (VII) while II produces 2-bromoestradiol (VI) in 40% yield. 4-Bromoestradiol (VIII) is obtained by reaction with NBS in refluxing carbon tetrachloride. Use of excess NBS does not lead to IV, but a serious reduction in yield of 4-bromo compound does occur. NBS may also be used in the synthesis of VII.

Assignment of positions for the monobromo compounds is based on the synthesis of V from 2-nitroestrone[6] by reduction of the nitro group, diazotization of the amine, and replacement of the diazonium group with bromine by means of the Sandmeyer reaction. In addition, all brominated estrone compounds were reduced to the corresponding 17β-estradiol derivatives by treatment with sodium borohydride.

Additional confirmation of our assignments may be obtained by comparison of the ultraviolet spectra of the nitro-[6] and bromoestrones (Table II). Both 4-nitro- and 4-bromoestrone exhibit approximately the same absorption as the parent compound. On the other hand, both 2-nitro- and 2-bromoestrone show a bathochromic shift with intensification of absorption. As would be expected, the same shift is shown by III. The bromoestradiols are closely comparable to the bromoestrones except in the case of the 2-bromo compounds. No bathochromic shift or intensification of absorption occurs in the case of VI. Unfortunately, Werbin and Holloway did not include 2-nitroestradiol in their series so no comparison can be made.

For analytical purposes the reaction products may be separated by gradient elution chromatography on alumina. Advantage was taken of this fact in order to demonstrate the selectivity of these reactions. When 0.1 μg. of I was brominated with an excess of bromine-82 under conditions selected for 4-bromination, all of the organically bound radioactivity appeared under the peak resulting from gradient elution chromatography of carrier VII. No radioactivity appeared under the peaks given by nonradioactive V and III. Other isotopic experiments showed that 2-bromination is also highly selective.

Larger quantities might be separated by solubility differences since the 2-bromo compounds are more soluble than the 4-bromo derivates in benzene or in ethyl ether. The former, once brought into solution by slight warming, will not crystallize for several minutes, thus allowing the other isomer to be filtered off.

The specificity of the reactions presented above is difficult to explain. Ross[8] investigating solvent effects in the reactions of NBS, has shown that reaction in nonpolar solvents, such as carbon tetrachloride or benzene, produced side-chain bromination whereas in propylene carbonate, a highly polar solvent, nuclear bromination occurred. In our case only the latter was observed, even in the presence of carbon tetrachloride, as evidenced by the failure to form a precipitate

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(8) S. D. Ross, M. Finkelstein, and R. C. Peterson, *J. Am. Chem. Soc.*, **80**, 4327 (1958).

TABLE II

Estrone	M.P.[b]	Empirical Formula	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Ultraviolet λ_{max}^{alc}	Ultraviolet (abs.) ϵ	Opt. Rot. $[\alpha]^{27}D$ $CHCl_3$	Infrared (CS_2) (cm.$^{-1}$) 17-C=O	Infrared (CS_2) (cm.$^{-1}$) Acetate
2-Bromo (V)	193–194	$C_{18}H_{21}O_2Br$	61.90	61.64	6.06	6.08	286	3810	141	1727	
2-Bromo acetate	146–147	$C_{20}H_{23}O_3Br$	61.39	61.19	5.92	5.71	274	1090	112	1727	1773
2-Bromo DNP[a]	270 (dec.)	$C_{24}H_{26}O_5N_4Br$	N = 10.56	N = 10.44							
4-Bromo (VII)	264–265	$C_{18}H_{21}O_2Br$	61.90	61.63	6.06	6.00	281	2170	136	1727	
4-Bromo acetate	204–205	$C_{20}H_{23}O_3Br$	61.39	61.18	5.92	5.70	274	1450	109	1727	1776
4-Bromo DNP	288 (dec.)	$C_{24}H_{26}O_5N_4Br$	N = 10.56	N = 10.51							
2,4-Dibromo (III)	225–226	$C_{18}H_{20}O_2Br_2$	50.49	50.28	4.71	4.45	291 sh285	2800	133	1730	
2,4-Dibromo acetate	184–185	$C_{20}H_{22}O_3Br_2$	51.08	51.00	4.72	4.56	273	1020	107	1730	1783
2,4-Dibromo DNP	280 (dec.)	$C_{24}H_{25}O_5N_4Br_2$	N = 9.20	N = 9.13							
17β-Estradiol										17-Acetate	
2-Bromo (VI)	156–157	$C_{18}H_{23}O_2Br$	61.54	61.23	6.60	6.43	281	2320	132		
2-Bromo 3,17-diacetate	162–163	$C_{22}H_{27}O_4Br$	60.70	61.00	6.25	6.13	269	530	109	1721	1773
4-Bromo (VIII)	207–208	$C_{18}H_{23}O_2Br$	61.54	61.38	6.60	6.38	283	2240	129		
4-Bromo 3,17-diacetate	143–144	$C_{22}H_{27}O_4Br$	60.70	60.50	6.25	6.12	275	1280	103	1721	1776
2,4-Dibromo (IV)	218–219	$C_{18}H_{22}O_2Br_2$	50.25	50.14	5.15	5.20	291 sh286	2850	122		
2,4-Dibromo 3,17-diacetate	167–168	$C_{22}H_{26}O_4Br_2$	51.38	51.38	5.10	5.10	273	750	106	1712	1779

[a] Dinitrophenylhydrazone. [b] Corrected.

upon standing in the presence of alcoholic silver nitrate solution overnight.

Experimental

2-Bromination. To 300 ml. of glacial acetic acid was added 1.0 g. (3.7 mmoles) of I or II. Solution was facilitated by heating on a steam bath. After the reaction mixture had been cooled in an ice bath, 10–15 mg. of powdered iron was added, and stirring with a magnetic stirrer was commenced. At the point of incipient freezing a 5% solution (v./v.) of bromine in glacial acetic acid was added portionwise by means of a dropping funnel over a period of 2–5 min. until the reaction mixture no longer decolorized the added bromine. After stirring for an additional 5 min., the reaction mixture was poured into 1 l. of ice and water. The precipitated 2-bromo compound was filtered, washed with water, and crystallized alternately from 95% ethanol and from chloroform and petroleum ether.

2,4-Dibromination.—The procedure above was followed except that the powdered iron was omitted.

4-Bromoestrone (VII). Method A.—The procedure for 2-bromination is followed except that the acetic acid contains 15–20% water. A mixture of monobromo isomers may be separated by gradient elution chromatography on neutral Woelm alumina containing 11% moisture in a 1 × 7 cm. (7 g.) column. The polar solvent, consisting of 6% (v./v.) absolute ethanol in benzene, was dropped into the mixing chamber, containing initially 300 ml. of petroleum ether–benzene, 3:1, at an influx-efflux ratio of 1:6. The flow rate was 2 ml./min. Under these conditions V and VII are completely separated (elution volumes of 100 and 118 ml., respectively). In one instance bromination was performed with bromine-82.[9] Before chromatography 1 mg. each of V and VII were added to the radioactive preparation. All of the radioactivity was eluted with VII.

Method B.—A mixture of 1.0 g. (3.7 mmoles) of I, 0.69 g. (3.9 mmoles) of NBS, and 100 ml. of carbon tetrachloride was refluxed for 1 hr. Solution was complete in about 15 min. The solvent was removed under reduced pressure, and the brown oil was dissolved in 20 ml. of ether. After the ether solution had been washed with water, the solvent was evaporated, and the residue was crystallized as previously described.

4-Bromoestradiol (VIII).—This can be made in good yield only by method B.

Acetates.—All products were acetylated by dissolving the steroid in the minimum amount of pyridine (about 0.5 ml./50 mg. steroid) and adding 5 volumes of acetic anhydride. After standing overnight at room temperature, the acetates were isolated and crystallized.

2,4-Dinitrophenylhydrazones.—To a solution of 100 mg. of steroid in 75 ml. of 95% ethanol was added 200 mg. of 2,4-dinitrophenylhydrazine and 1 ml. of concd. hydrochloric acid. This was refluxed for 2 hr. Upon removal of one half of the alcohol, the hydrazone precipitated.

Reduction of Bromoestrones.—To 100 mg. of bromoestrone dissolved in 25 ml. of 95% ethanol was added a large excess (*ca.* 50 mg.) of sodium borohydride dissolved in 0.5 ml. of water. After 1.5 hr. 125 ml. of water was added, and the product was extracted and crystallized. Melting points and mixed melting points with the corresponding bromoestradiol compounds produced by direct bromination demonstrated a direct relationship.

2-Bromoestrone (from 2-Nitroestrone).—2-Nitroestrone (2 g., 6.2 mmoles), m.p. 173–174° corr., was reduced and diazotized according to the procedure of Kraychy and Gallagher[10] except that hydrobromic acid was used instead of hydrochloric acid. The diazotized 2-aminoestrone was then added to 100 ml. of boiling hydrobromic acid (47%) containing 1.5 g. of freshly prepared cuprous bromide. After boiling for 10 min., the reaction mixture was cooled, diluted with water, and extracted with chloroform. Crystallization yielded 2-bromoestrone, m.p. 193–194° corr., in 45–50% yield. Mixed melting point with 2-bromoestrone prepared by direct bromination showed no depression.

(9) Details of this procedure, which was presented at the Endocrine Meeting, June 22–24, 1961, will be published elsewhere.

(10) S. Kraychy and T. F. Gallagher, *J. Biol. Chem.*, **229**, 519 (1957).

Infrared and ultraviolet spectra also confirmed the identity of the two compounds.

Physical Measurements.—Rotations were measured on a Rudolph Model 200A optical rotatory dispersion apparatus at the following settings: wave length, 589 mμ; slit width, 0.50 mm.; prism angle, 0.5°; temperature, 27°; source, sodium lamp. All solutions were made in chloroform. Infrared spectra were recorded on a Baird double beam instrument, Model ABB-2, ultraviolet spectra on a Beckman recording spectrophotometer, Model DK-2. Melting points were taken on a Fisher-Johns apparatus and are corrected.

Synthesis of 6α-Fluoromethyl Steroids

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Received November 22, 1961

The syntheses of 6α-fluoromethylprednisolone and the 9α-fluoro analog are reported, based on the application of the "oxo" reaction to 17,20;20 21-bismethylenedioxy-5-pregnene-3β,11β-diol 3-acetate (II), followed by conversion of the 6α-hydroxymethyl group to the fluoromethyl group by the action of potassium fluoride on the 6α-tosyloxymethyl derivative.

The modification of the hydrocortisone molecule, with the object of improving the anti-inflammatory activity, has led to the preparation of methyl and halogen analogs of steroids.[1] Other structural changes have included the introduction of double bonds, hydroxyl groups, and ring enlargement or contraction.[1] Since the substitution at C-6 by both methyl and fluorine to give the 6α-substituted derivatives has led to an enhancement of biological activity, it was decided to introduce other groups at this position. A valuable method for the introduction of substituents at C-6 is the application of the hydroformylation or "oxo" reaction to a steroidal 5,6-double bond, which has been shown to give a 6α-hydroxymethyl-5α-pregnane derivative,[2] *cis* addition occurring from the α-side of the molecule. The present work describes the conversion of the 6α-hydroxymethyl intermediates to the corresponding fluoromethyl derivatives and the elaboration of these latter to derivatives of prednisolone.[3]

The general utility of the bismethylenedioxy (BMD) blocking group for the preparation of cortical analogs has been amply demonstrated in the synthesis of 5,[4] 6,[5] 7,[6] 9[7] and 11[8]-methylated steroids. It seemed desirable, therefore, to prepare a Δ^5-steroid with the cortical side chain protected as its bismethylenedioxy derivative. Accordingly, cortisone was converted to cortisone BMD (I) as described earlier.[9] This latter was treated with isopropenyl acetate under acidic conditions to give the corresponding enol acetate[10] which on prolonged reduction with sodium borohydride, followed by reacetylation at C-3 with acetic anhydride in pyridine gave 17,20;20,21-bismethylenedioxy-5-pregnene-3β,11β-diol 3-acetate (II). Reaction of II with carbon monoxide and hydrogen at 91 kg./cm.^{2} total pressure in the presence of cobalt carbonate at 180° for eighteen hours gave 17,20;20,21-bismethylenedioxy-6α-hydroxymethyl-5α-pregnane-3β,11β-diol 3-acetate (III. R = H) in 54% yield, isolated readily by direct crystallization. The configuration of the "oxo" product is assigned the 5α,6α configuration by analogy with the previous examples. Conversion of this alcohol to the 6α-fluoromethyl derivative followed established methods.[11] Reaction of the corresponding tosylate ester (III. R = $SO_2C_6H_4CH_3$) with anhydrous potassium fluoride in diethylene glycol at 205–215° for one hour effected replacement with fluorine and at the same time partial hydrolysis of the 3-acetate. The ester hydrolysis was completed by a separate treatment with base to give 17,20;20,21-bismethylenedioxy-6α-fluoromethyl-5α-pregnane-3β,11β-diol (IV). An Oppenauer oxidation of the 3β-alcohol IV gave the corresponding 3-ketone (V), which on treatment with selenium dioxide in the presence of acetic acid,[12] introduced two double bonds into ring A to give 6α-fluoromethylprednisolone BMD (VI). Removal of the bismethylenedioxy pro-

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