[CONTRIBUTION FROM THE DIVISION OF CHEMICAL RESEARCH, G. D. SEARLE & CO.]

Steroidal Aldosterone Antagonists. IV[1]

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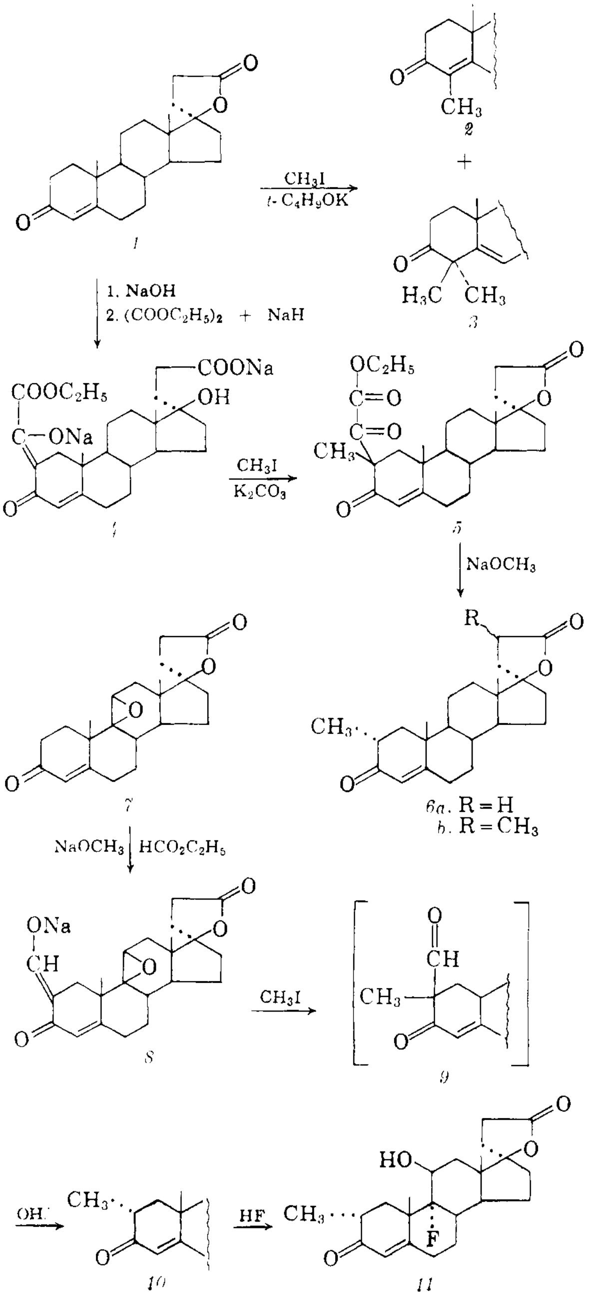
Several nuclear methyl derivatives of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone (1) were prepared and studied as aldosterone antagonists.

Previous studies[1] have shown that certain modifications and substitutions in the basic model structure 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone[1b] (*1*) have produced interesting and important changes in aldosterone blocking properties. In the present paper we have examined the effects of introducing a methyl group into the steroid nucleus of *1* at positions 2, 4, 6, 7, and 16.

Our interest in 2-methyl derivatives stemmed from the observation by Hogg *et al.*[2] that 2α-methylcortisol and its 9α-fluoro derivative showed a striking increase in mineralocorticoid activity over the nonmethylated analogs. Indeed 2α-methyl-9α-fluorocortisol is one of the most potent mineralocortocoids known, considerably exceeding aldosterone as a sodium retaining and potassium excreting agent. According to the classical principles of competitive antagonism,[3] one might expect that the analogous 3-(2α-methyl-3-oxo-9α-fluoro-11β,17β - dihydroxy - 4 - androsten - 17α - yl)propanoic acid lactone (*2*) would likewise possess a high degree of affinity for the target cells, but, because of appropriate alteration of the molecule at C-17, would be a potent aldosterone antagonist.

The improvement in activity resulting from methylation of the anti-inflammatory corticoids at the 6α[4]- and 16α-positions[5] suggested the study of the corresponding modifications in our series of 17-spirolactones. The pronounced enhancement of progestational activity resulting from the introduction of a 6α-methyl group in 17α-acetoxyprogesterone[6] lent further interest to this approach.

For the most part the synthesis of these compounds proceeded in normal ways. Direct treatment of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone (*1*) with methyl iodide gave a 5:1 mixture of mono- and dimethylated products (*2*) and (*3*) which were separated by chromatography. The crude sodium salt resulting from saponification of the lactone (*1*) was condensed with ethyl oxalate to give the enolate (*4*). This, on



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(1) (a) E. A. Brown, R. D. Muir, and J. A. Cella, *J. Org. Chem.*, **25**, 96 (1960); (b) J. A. Cella, E. A. Brown, and R. R. Burtner, *J. Org. Chem.*, **24**, 743 (1959).

(2) J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *J. Am. Chem. Soc.*, **77**, 6401 (1955).

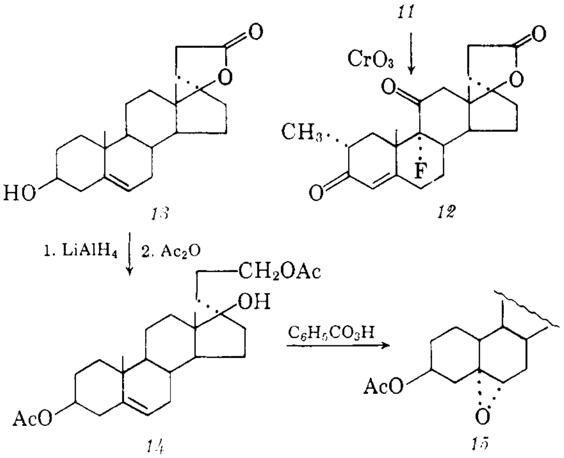
(3) D. W. Wooley, *Physiol. Revs.*, **27**, 308 (1947).

(4) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek, and J. A. Hogg, *J. Am. Chem. Soc.*, **78**, 6213 (1956).

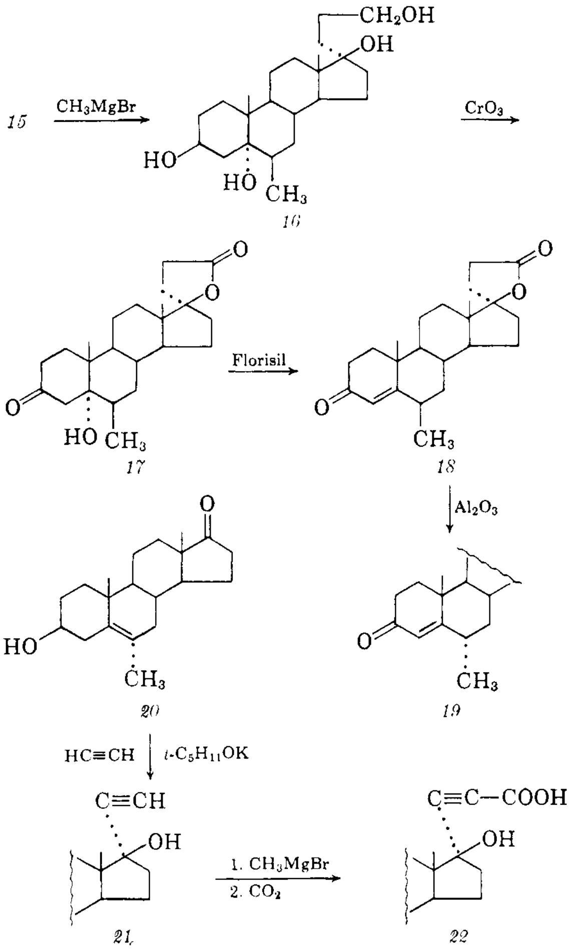
(5) G. E. Arth, D. B. R. Johnston, John Fried, W. W. Spooncer, D. R. Hoff, and L. H. Sarett, *J. Am. Chem. Soc.*, **80**, 3160 (1958).

methylation and subsequent treatment with sodium methoxide, yielded the 2α-methyl derivative (*6a*). The 2α,21-dimethyl compound (*6b*) which was obtained in small amount in this reaction sequence, presumably arose from some unsaponified lactone which condensed with ethyl oxalate at both positions. The NMR spectra of compounds *6a* and *6b* confirmed the number and secondary character of the methyl groups introduced.

The 2α-methyl-9,11-epoxide (*10*) was prepared by methylation of the crude hydroxymethylene derivative (*8*) obtained from the epoxy lactone (*7*). The fluorohydrin was obtained from a mixture which undoubtedly contained the 2α,21-dimethyl derivative as well. The NMR spectrum of compound *11*, however, defined it as a monomethylated compound. The ultraviolet spectrum of the free enol corresponding to the crude salt (*8*) showed that C-2 was the principal site of condensation,[6b] thus assuring that the single methyl group of the fluorohydrin (*11*) was located at this same position.



The 6α-methyl spirolactone (*19*) was synthesized by two different routes. In the first the methyl group was introduced by treatment of the 5α,6α epoxide (*15*) with methylmagnesium bromide. Prior reduction of the lactone ring and acetylation of the diol was necessary to prevent attack by the Grignard reagent and to provide a sufficiently soluble derivative. The Grignard adduct (*16*) was oxidized and dehydrated to give the 6β-methyl compound (*18*) which was epimerized to the α-derivative (*19*) by heating with alumina in benzene.



In the second route 3β-hydroxy-6-methyl-5-androsten-17-one (*20*), prepared from 6-methyl-16-dehydropregnenolone[7] *via* Beckmann rearrangement, was ethynylated,[8] converted to the propiolic acid (*22*) and hydrogenated to give the lactone (*23*). Oppenauer oxidation furnished the 6α-methyl spirolactone (*19*) directly. This oxidation was surprisingly sluggish and required prolonged treatment to obtain 75% conversion (estimated by ultraviolet absorption). A small amount of 3-(3-oxo-6α-methyl-6β,17β-dihydroxy-4-androsten-17α-yl)propanoic acid lactone was formed as a by-product. Dehydrogenation of *19* by selenium dioxide or chloranil produced the 1,4-diene (*24*) and the 4,6-diene (*25*), respectively.

Treatment of the dieneone[9] (*26*) with methylmagnesium bromide in the presence of cuprous chloride yielded the expected 1,6 addition product (*27*) as the sole product. The α-configuration of the

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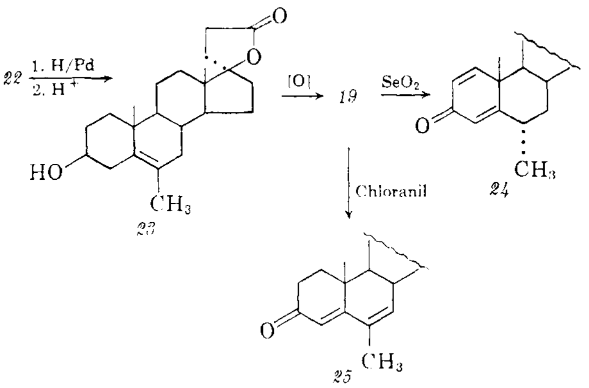
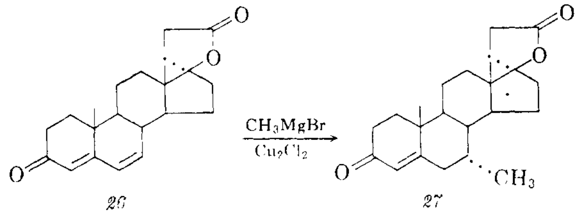
(6) (a) J. C. Babcock, E. S. Gutsell, M. E. Herr, J. A. Hogg, J. C. Stucki, L. E. Barnes, and W. E. Dublin, *J. Am. Chem. Soc.*, **80**, 2904 (1958). (b) The spectrum of this material was nearly identical to those reported for 2-hydroxymethylenetestosterone and 2-hydroxymethylenecholestenone, F. L. Weisenborn, D. C. Remy, and T. L. Jacobs, *J. Am. Chem. Soc.*, **76**, 552 (1954). It differed markedly from the spectrum of 3-(3β,17β-dihydroxy-21-hydroxymethylene-5-androsten-17α-yl)propanoic acid lactone which displayed a single absorption band at 277 mμ in the ultraviolet.

(7) D. Burn, B. Ellis, V. Petrow, I. A. Stuart-Webb, and D. M. Williamson, *J. Chem. Soc.*, 4092 (1957).

(8) The 3β,17β-dihydroxy-6-methyl-17α-ethynylandrost-5-ene was previously prepared by V. Grenville, D. K. Patel, V. Petrow, I. A. Stuart-Webb, and D. M. Williamson, *J. Chem. Soc.*, 4105 (1957), by a different method.

(9) J. A. Cella and R. C. Tweit, *J. Org. Chem.*, **24**, 1109 (1959).

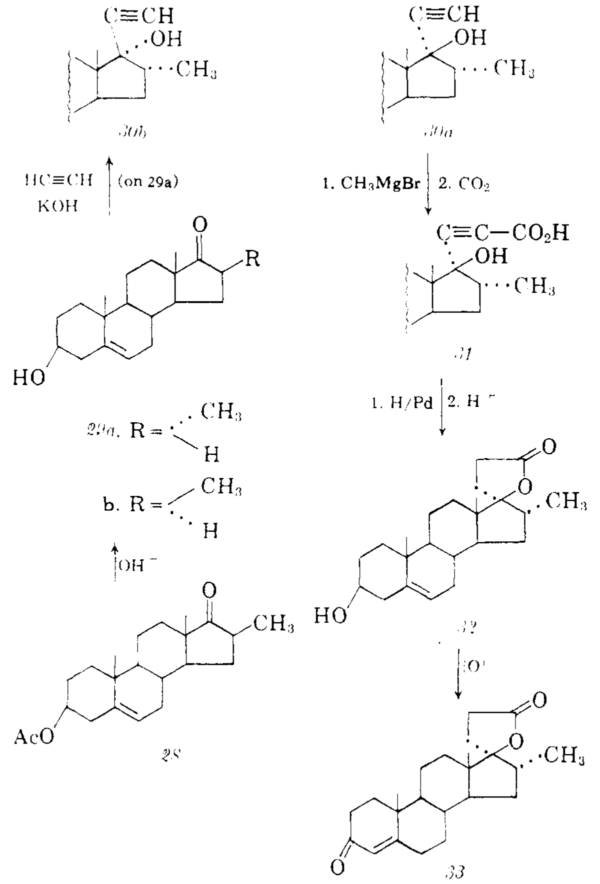




methyl group at C-7 is based upon the close analogy with the work of Campbell and Babcock.[10]

Saponification of 3β-acetoxy-16β-methyl-5-androsten-17-one (*28*)[11] under mild conditions gave two products, the principal one being the 16β-methyl isomer (*29b*) since acetylation with acetic anhydride and pyridine regenerated the parent acetate. The minor isomer must therefore be the 16α-methyl derivative (*29a*) which was further obtained as the sole product of saponification under more vigorous conditions.

Ethynylation of the ketone (*29a*) produced two isomeric ethynyl derivatives, m.p. 208–212° and 170–175°, which required chromatographic separation. This was expected, since examination of a molecular model shows that the directive influence of the 13β-methyl group is nearly offset by that of the 16α-methyl group. The fact that two C-17 isomers were obtained further supports the 16α-configuration of the methyl group in *29a* since a 16β[12]-methyl group would hinder front-side attack. We have tentatively assigned the 17α-configuration to the higher melting ethynyl derivative (*30a*) on the basis of its less negative rotation.[13] The isolation of the 17β-ethynyl compound (*30b*) in pure state was so tedious that its investigation was not further pursued. Compound *30a* was transformed by steps analogous to those previously described to the 16α-methyl spirolactone (*33*).



The biological studies reported in Table I were conducted by Dr. C. M. Kagawa and Mr. Robert Jacobs of these laboratories. It has been demonstrated that a definite and proportional relation-

TABLE I

DESOXYCORTICOSTERONE ACETATE BLOCKING POTENCIES M.E.D.[a]

Compound	Subcutaneous	Oral
1	0.26	19.0
2	>2.4	>2.4
3	>2.4	—
6b	>1.2	>1.2
11	0.26	0.9
12	0.34	>0.5
18	0.28	1.5
19	1.2	0.76
24	0.45	2.6
25	>2.4	—
27	0.72	—
33	>0.4	—

[a] M.E.D. is the minimal effective dose (mg./rat) which, when used with 12 μg. of DOCA in adrenalectomized rats, produces the same urinary sodium-potassium ratio as that which results from the use of 6 μg. of DOCA alone.

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(10) J. A. Campbell and J. C. Babcock, *J. Am. Chem. Soc.*, **81**, 4069 (1959).

(11) (a) P. Julian, E. Meyer, and H. Printy, *J. Am. Chem. Soc.*, **70**, 3872 (1948); (b) M. Romero, J. Romo, and J. Lepe, C. A., **48**, 5202 (1955); (c) The assumption by these investigators that the methyl group at C-16 was in the β-configuration has been strongly reinforced by recent studies on the corresponding 11β-hydroxy series by D. Taub, R. Hoffsommer, H. Slates, C. Kuo, and N. Wendler, *J. Am. Chem. Soc.*, **82**, 4012 (1960).

(12) It should be noted that two attempts to ethynylate 16β-methyldehydroisandrosterone (*29b*) were unsuccessful, starting material being recovered in each case. This effect may be attributed to the steric hindrance of the 17-carbonyl function by the 16β-methyl group which would disfavor an easily reversible reaction such as ethynylation.

(13) T. Reichstein and Ch. Meystre, *Helv. Chim. Acta*, **22**, 728 (1939).

ship exists between the blocking effects of typical steroidal spirolactones when tested on rats treated with aldosterone and with desoxycorticosterone acetate (DOCA).[14] The more available desoxycorticosterone acetate was employed as the sodium retaining agent throughout this work.

It is evident from these data that the introduction of a methyl group at the various positions studied failed to enhance the parenteral activity of the prototype (*1*), although in several instances there was a noticeable increase in oral potency.

EXPERIMENTAL

3-(3-Oxo-4-methyl-17β-hydroxyl-4-androsten-17α-yl)propanoic acid lactone (*2*). A hot solution of the spirolactone (*1*) (3.65 g.) in *t*-butyl alcohol (50 ml.) was added all at once to a boiling solution of potassium *t*-butoxide prepared by dissolving potassium (0.62 g.) in *t*-butyl alcohol (30 ml.). A solution of methyl iodide (1.82 g.) in the same alcohol (100 ml.) was added dropwise over a 2-hr. period to the refluxing mixture and then it was cooled to room temperature, acidified with concd. hydrochloric acid, and diluted with water (200 ml.).[15] The *t*-butyl alcohol was removed by vacuum distillation of the homogeneous solution and the organic material was extracted into benzene. The extracts were washed, dried, and then applied to a chromatography column containing silica gel (400 g., Davidson 923, 80–200 mesh). Solid 4,4-dimethyl compound (*3*), (0.38 g., 10%) was eluted with ethyl acetate–benzene (1:20). Recrystallization from isopropyl ether gave the analytical sample, m.p. 218–220°, $[\alpha]_D$ −42° chloroform; $\lambda_{max}^{CHCl_3}$ 5.76, 5.85, 6.02, 7.23 μ.

Anal. Calcd. for $C_{24}H_{34}O_3$: C, 77.79; H, 9.25. Found: C, 77.92; H, 9.01.

Solid 4-methyl derivative (*2*), (2.10 g.; 55%) was eluted with ethyl acetate-benzene (1:9). The analytical sample was obtained by crystallization from acetone-water, m.p. 83–88°, with resolidification and remelting at 142–144.5°, $[\alpha]_D$ +99°, chloroform, $\lambda_{max}^{CH_3OH}$ 250 mμ, ε 16,000.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.52; H, 9.15.

Starting material (1.9 g., 33%) was recovered by elution of the column with ethyl acetate-benzene (15:85).

3-(2α-Methyl-3-oxo-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone (*6a*) *and 3-(2α,21-dimethyl-3-oxo-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone* (*6b*). A solution of the spirolactone (*1*) (10.4 g.) and sodium hydroxide (1.21 g.) in methanol (400 ml.) was heated at reflux for 1.5 hr. and then distilled to dryness in vacuum. Benzene (200 ml.) was added and the mixture again taken to dryness. The residue slurried in dry benzene (400 ml.) was treated with ethyl oxalate (25 ml.) and a 50% dispersion of sodium hydride in mineral oil (5.0 g.). After stirring at laboratory temperature for 72 hr. methanol (10 ml.) was added and then after an additional hour water (1 l.). The layers were separated and the aqueous phase acidified and extracted with ethyl acetate. These extracts were washed, dried, and evaporated to leave the enol in the form of a yellow resin.

The crude enol was treated with methyl iodide (25 ml.) and anhydrous potassium carbonate (20 g.) in acetone (300 ml.) and the mixture heated at reflux for 16 hr. At the end of this time water (1 l.) was added and the acetone removed by vacuum distillation. The organic material was separated from the aqueous phase by decantation and was then dissolved in methanol (300 ml.) and refluxed 5 hr. with a solution of potassium hydroxide (15 g.) in water (50 ml.). After cooling, the mixture was diluted to faint turbidity with water and acidified to *p*H 2 with concd. hydrochloric acid. After 0.5 hr. the methanol was removed by vacuum distillation and the product extracted with benzene. After drying, this benzene solution was applied to a column containing silica gel (1000 g.).

A mixture (3.0 g.) of compounds *6a* and *6b* was eluted with ethyl acetate-benzene (1:9). The dimethyl compound (*6b*) was obtained from the early fractions by repeated crystallization from acetone and acetone–isopropyl ether, m.p. 241–244°, $[\alpha]_D$ + 69° (chloroform), $\lambda_{max}^{CH_3OH}$ 239 mμ, ε 16,000; NMR 4.300, 8.707, 8.783, 8.717, 8.833, 8.950, 8.967, 9.050 τ,[15b] $\lambda_{max}^{CHCl_3}$ 5.67, 5.99, 6.17 μ.

Anal. Calcd. for $C_{24}H_{34}O_3$: C, 77.79; H, 9.25. Found: C, 77.68; H, 9.02.

The 2α-methyl derivative (*6a*) (0.90 g.) was obtained by crystallization of the later fractions from acetone–isopropyl ether, m.p. 191–192°. The analytical sample melted at 195–197°, $[\alpha]_D$ + 82°, $\lambda_{max}^{CHCl_3}$ 5.64, 5.98, 6.15 μ; $\lambda_{max}^{CH_3OH}$ 239 mμ, ε 16,300; NMR 4.292, 8.795, 8.837, 8.950, 9.020 τ.[15b]

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.63; H, 9.03.

3-(2α-Methyl-3-oxo-9α-fluoro-11β,17β-dihydroxy-4-androsten-17α-yl)propanoic acid lactone (*11*). To a solution of 1.5 g. of 3-(3-oxo-9,11β-oxido-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*7*)[1a] in 25 ml. of dry benzene was added 1.3 g. of sodium methylate and 2.0 ml. of ethyl formate. Dry benzene was then added to a total reaction mixture volume of 60 ml. and the slurry was allowed to stand at laboratory temperature for 72 hr. The reaction mixture was then filtered and the product on the funnel was washed with ether and air-dried to yield 3.3 g. of crude hydroxymethylene derivative (*8*) which was used directly in the next step. Acidification of a sample of this material gave a product showing $\lambda_{max}^{CH_3OH}$ 250 mμ, ε 8300; 310 mμ, ε 4600.

A reaction mixture of 3.3 g. of crude *8*, 2.13 g. of freshly ignited potassium carbonate, 3.2 ml. of methyl iodide and 27 ml. of dry acetone was stirred and refluxed for 23 hr. It was then cooled and extracted with 300 ml. of ether. The ether solution was washed once with water and then with 1*N* sodium hydroxide until the washings were colorless. The solution was finally washed with water, dried over sodium sulfate, and evaporated *in vacuo* to a residue of 1.5 g. of amorphous yellow solid. To this residue was added 10 ml. of methanol and a solution of 1.0 g. of sodium hydroxide in 1.0 ml. of water. After refluxing the resulting solution for 10 min. it was diluted with 50 ml. of water and then carefully acidified to *p*H 6 with dilute acetic acid. The precipitate was collected, washed with water, and dried to yield 800 mg. of crude 2α-methyl derivative (*10*) which could be used directly in the next reaction.

To a cold (−30°) solution of 11.5 g. of hydrogen fluoride in 25 g. of redistilled tetrahydrofuran was added 950 mg. of *10*. The resulting reaction mixture was stirred for several minutes and maintained at −30° for 6 hr. It was then diluted with 100 ml. of water and the precipitate collected, washed with water, dried, and recrystallized from acetone to yield 110 mg. of the desired 9α-fluoro spirolactone (*11*), m.p. 305–307° dec., $\lambda_{max}^{CH_3OH}$ 238 mμ, ε 14,500; NMR 8.392, 8.750, 8.825 τ.[15b]

Anal. Calcd. for $C_{23}H_{31}FO_4$: C, 70.74; H, 8.00. Found: C, 70.60; H, 7.98.

3-(2α-Methyl-3,11-dioxo-9α-fluoro-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*12*). A solution of 160 mg. of *11* in 8 ml. of pyridine was added slowly with mixing to a pyridine–chromic acid complex prepared from 160 mg. of chromium trioxide and 4 ml. of pyridine. During the addition and for 15 min. thereafter, the reaction temperature was

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(14) C. M. Kagawa, J. A. Cella, and C. G. Van Arman, *Science*, **126**, 1015 (1953).

(15) (a) See N. W. Atwater, *J. Am. Chem. Soc.*, **82**, 2847 (1960), for an extension of this method of methylation to other steroids. (b) The NMR data was kindly determined for us by Dr. Neal McNiven of the Worcester Foundation for Experimental Biology. The spectra were obtained with the sample dissolved in deuterated chloroform at 60 mc. using tetramethyl silane as an internal standard and are reported in terms of τ.

maintained at 20°. After standing overnight at laboratory temperature it was transferred slowly into a two-phase solvent mixture of 50 ml. of ethyl acetate and 25 ml. of water. The ethyl acetate layer was separated and combined with one ethyl acetate extract of the aqueous layer. The combined ethyl acetate extracts were washed successively with water, dilute hydrochloric acid, and water. After drying over sodium sulfate, the solvent was evaporated *in vacuo* and the residue was recrystallized from ethyl acetate–hexane to yield 41 mg., m.p. 270–271° dec., $\lambda_{max}^{CHCl_3}$, 5.63, 5.75, 5.95 μ.

Anal. Calcd. for $C_{23}H_{29}FO_4$: C, 71.11; H, 7.53. Found: C, 70.90; H, 7.68.

3-(3β,17β-Dihydroxy-5-androsten-17α-yl)propanol. A solution of 82 g. of 3-(3β,17β-dihydroxy-5-androsten-17α-yl)-propanoic acid lactone (*13*) was reduced in tetrahydrofuran (1.2 l.) with lithium aluminum hydride (30 g.) at reflux temperature for 16 hr. The product was purified by successive trituration with chloroform (1.5 l.), methanol (1.0 l.), and chloroform (0.5 l.). The resulting solid was then dried at 65° for 18 hr. The infrared (5% in chloroform) displayed no band in the region of 5–6 μ. This material was used without further purification.

Diacetate of 3-(3β,17β-dihydroxy-5-androsten-17α-yl)propanol (*14*). The triol (81 g.) was stirred with pyridine (560 ml.) and acetic anhydride (96 ml.) at 85° for 30 min. The resulting mixture was filtered while hot and was then poured into hot water (3 l.). The granular solid which separated was collected and washed with water, 99 g. This diacetate was azeotroped dry with benzene and then used directly in the next step. A sample for analysis was obtained by recrystallization from aqueous methanol. This sample, after being dried at 78° (0.1 mm.) for 24 hr., melted at 103–106°; $\lambda_{max}^{CHCl_3}$, 2.74 and 5.76 μ.

Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.16; H, 9.47.

Diacetate of 3-(3β,17β-dihydroxy-5α,6α-epoxyandrostan-17α-yl)propanol (*15*). A solution of perbenzoic acid in benzene (475 ml., containing 0.0727 g./ml. of perbenzoic acid) was added (5 min.) to a solution of the diacetate (99 g.) in benzene (750 ml.). The mixture was stirred and was then allowed to stand for 3 hr. The reaction mixture was washed with 5% sodium carbonate and then with water. Removal of the solvent followed by seven recrystallizations of the residue from chloroform-hexane gave the α-epoxide as platelets; 17.6 g., m.p. 140–141°, $[\alpha]_D$ − 68° (chloroform).

Anal. Calcd. for $C_{29}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.65; H, 8.68.

A second crop was obtained by crystallization of the material in the mother liquors; 12.1 g., m.p. 138.5–142°.

3-(3β,5α,17β-Trihydroxy-6β-methylandrostan-17α-yl)propanol (*16*). A solution of the α-epoxide (29.7 g.) in tetrahydrofuran (200 ml.) was added slowly with stirring to a solution of methylmagnesium bromide (3*M*) in ethyl ether (800 ml.). This mixture was stirred and distilled until the temperature of the escaping vapors was 59°. The resulting mixture was then stirred at reflux temperature for 18 hr. The reaction mixture was cooled and then poured onto ice (2 kg.) with stirring. The product was extracted into methylene chloride. The methylene chloride solution was washed with dilute hydrochloric acid and then with water. Removal of the solvent gave a crystalline residue which was recrystallized four times from aqueous methanol to give the 5α-hydroxy-6β-methyl compound; 4.4 g., m.p. 218–223°.

Anal. Calcd. for $C_{23}H_{40}O_4$: C, 72.59; H, 10.60. Found: C, 72.62; H, 10.58.

The diacetate was prepared with acetic anhydride in pyridine; m.p. 145–147°, $[\alpha]_D$ − 31.5° (chloroform).

Anal. Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.81; H, 9.32.

3-(3-Oxo-5α,17β-dihydroxy-6β-methylandrostan-17α-yl)-propanoic acid lactone (*17*). The Grignard product (*1b*) (0.96 g.) was oxidized in acetone (50 ml.) with a solution of chromic anhydride in aqueous sulfuric acid (3.5 ml.). Four recrystallizations of the product from methanol gave the ketol (*17*) as platelets; 0.70 g.; m.p. 245–248.5°, $[\alpha]_D$ − 25° (chloroform); $\lambda_{max}^{CHCl_3}$ 5.65, 5.83, and 10.84 μ.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.66; H, 9.02.

3-(3-Oxo-6β-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*18*). A solution of the ketol (*17*) (200 mg.) in benzene (40 ml.) was stirred and refluxed with Florisil (2.00 g.) for 4 hr.[16] The mixture was filtered and the Florisil was washed with ethyl acetate. Removal of the solvents from the combined filtrates gave a crystalline solid. Three recrystallizations from aqueous acetone gave the 6β-methyl compound; 72 mg.; m.p. 189.5–191.5°, $[\alpha]_D$ + 21° (chloroform); $\lambda_{max}^{CH_3OH}$ 241 mμ, (ε 15,600); $\lambda_{max}^{CHCl_3}$ 5.67, 6.02, 6.26, and 11.43 μ.

3-(3-Oxo-6α-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*19*). A solution of the ketol (*18*) (500 mg.) was stirred at reflux temperature in benzene (100 ml.) with Woelm basic aluminum oxide (5.00 g.) for 30 min.[16] The mixture was filtered, the solid washed with benzene, and the solvents were removed from the combined filtrates. Recrystallization of the residue from aqueous methanol and then from ethyl acetate-isopropyl ether (Charcoal) gave the 6α-methyl compound; 120 mg., m.p. 152.5–155.5°. This product was identical (identical infrared spectra; no depression of the melting point on admixture) with the compound prepared from 6-methyldehydroepiandrosterone.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.25, H, 9.07.

3β-Hydroxy-6-methyl-5-androsten-17-one (*20*). (a) *Preparation of the oxime.* A solution of 50 g. of 3β-acetoxy-6-methyl-5,16-pregnadien-20-one, 11.7 g. of hydroxylamine hydrochloride, and 53 ml. of pyridine in 200 ml. of alcohol was refluxed for 0.5 hr. About one half of the solvent was distilled and the residual hot solution was diluted with 1 l. of water. The crude oxime was collected on a filter, rinsed well, and dried at 70° for 24 hr. (53.3 g.). A sample for analysis, after crystallization from ethyl acetate, melted at 199–201°. $\lambda_{max}^{CHCl_3}$ 2.79, 5.80, 7.27, and 7.95 μ.

Anal. Calcd. for $C_{24}H_{37}NO_3$: C, 74.38; H, 9.62. Found: C, 74.61; H, 9.50.

(b) *Beckmann rearrangement.* *p*-Acetylaminobenzenesulfonyl chloride (51.6 g.) in 103 ml. of dry pyridine was added during a 20-min. period at −10° to −5° to a stirred solution of 43 g. of the crude oxime in 116 ml. of dry pyridine. The brown mixture was stirred at 0–5° for 1.0 hr. and then for 3.0 hr. with occasional cooling to keep the temperature below 20°. Following the addition of 200 ml. of water, 300 ml. of mixed solvent was distilled under reduced pressures from the mixture. A solution of 70 ml. of concd. sulfuric acid in 600 ml. of water was added, after which the volume was adjusted to a total of 850 ml. by addition of water. In order to hydrolyze the ketimine, the mixture was heated and stirred vigorously at 80° for 45 min. After cooling to 15°, the granular brown ketone was collected on a filter, rinsed well with water, triturated with 1 l. of 2% sodium bicarbonate, collected again, rinsed, and dried. The crude ketone (37 g.) was crystallized from 220 ml. of methanol to yield 31 g. of small colorless crystals, m.p. 149–150°. Petrow *et al.*[8] report the same melting point.

The acetoxy ketone (30 g.) was saponified by refluxing with 7.5 g. of potassium hydroxide in 150 ml. of 90% methanol under nitrogen for 1.0 hr. The crude carbinol (*20*) (26 g., m.p. *ca.* 140°) was recovered by dilution with water. A sample for analysis melted at 144–146° after crystallization from methanol $\lambda_{max}^{CHCl_3}$ 2.77, 5.77, and 7.27 μ.

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.45; H, 9.75.

3β,17β-Dihydroxy-6-methyl-17α-ethynyl-5-androstene (*21*). Thirty-two grams of potassium was dissolved under a nitrogen atmosphere in 800 ml. of refluxing *t*-amyl alcohol

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(16) R. H. Bible and N. W. Atwater, *J. Org. Chem.*, **26**, 1336 (1961).

(dried over calcium hydride). After the addition of 150 ml. of dry toluene, the cloudy solution was cooled to 0° and saturated with acetylene. Then 31 g. of the crude keto steroid (*20*) was added all at once as a dry powder, after which the rapidly stirred mixture was treated with a moderate stream of acetylene at 0° for 8 hr. After standing overnight in a stoppered flask in the refrigerator, the mixture was diluted with 750 ml. of 10% ammonium chloride solution. The solvent layer was separated, the aqueous phase was extracted with *t*-amyl alcohol, and the combined solutions were washed with saturated salt solution. Most of the solvent was vacuum distilled and the last traces were removed by a brief steam distillation. The crude ethynyl derivative (*21*) was collected on a filter, rinsed and dried (30.5 g., m.p. *ca.* 180–210°). The infrared spectrum indicated the presence of a small amount of unchanged ketone. Consequently, the crude product was treated with 30 g. of Girard's T reagent to remove 2.0 g. of the starting ketone. The crude ethynyl compound thus obtained (28.3 g., m.p. 200–210°) was crystallized from 425 ml. of acetonitrile (Darco) to yield 21 g. of *21*, melting at 211–215°. Petrow *et al.*[8] reported a m.p. of 213–215°. Concentration of the mother liquor gave an additional 5.6 g. of less pure material.

3-(3β,17β-Dihydroxy-6-methyl-5-androsten-17α-yl)propynoic acid (*22*). A solution of 25.2 g. of the acetylene derivative (*21*) in 250 ml. of tetrahydrofuran (distilled over methylmagnesium bromide) was added during a 10-min. period to a stirred solution of 250 ml. of 3*M* methylmagnesium bromide in tetrahydrofuran (700 ml.). The mixture was refluxed with good stirring for 20 hr. (positive RMgX color test), cooled to room temperature, and then treated with carbon dioxide under slight positive pressure with rapid stirring for 24 hr. After hydrolysis with ice cold 1*N* sulfuric acid, the entire mixture was subjected to vacuum distillation to completely remove the organic solvent. The residual crude acid was collected on a funnel, rinsed free of mineral acid, and dried (27 g.). The acid was suspended in 75 ml. of boiling chloroform for 5 min. and the hot suspension was filtered. The insoluble material was washed on the filter with two 25-ml. portions of boiling chloroform. The chloroform-insoluble acid (*22*) thus obtained weighed 21.7 g. and melted at about 225° dec. Crystallization from 100 volumes of butanone afforded tiny white crystals, m.p. unchanged. $\lambda_{\max}^{\mathrm{KBr}}$ 2.87, 2.96, 4.49, 5.10 (Broad), 5.83, 7.28, and 8.1 μ.

Anal. Calcd. for $C_{23}H_{32}O_4 \cdot {}^1/_2CH_3COC_2H_5$: C, 73.44; H, 8.93. Found: C, 73.56; H, 8.48.

3-(3β,17β-Dihydroxy-6-methyl-5-androsten-17α-yl)propanoic acid lactone (*23*). The chloroform-extracted crude acetylenic acid (*22*) (21.5 g.) was dissolved in 320 ml. of anhydrous alcohol containing 1.0 equivalent of triethylamine and then hydrogenated at atmospheric pressure over 2.15 g. of 5% palladium-on-charcoal. Hydrogen uptake stopped abruptly after the theoretical amount had been absorbed (1.0 hr.). The filtered solution was concentrated to about 0.5 volume *in vacuo* and then treated with 15 ml. of 6*M* hydrochloric acid. A precipitate formed which quickly redissolved in the warm solution. Immediate dilution with water precipitated the lactone (*23*) as a somewhat tacky solid which was taken up in chloroform, washed free of mineral acid and recovered by evaporation of the solvent. The residual yellow glass (20 g.) slowly crystallized. A sample for analysis melted at 152–154° after crystallization from ethyl acetate–isopropyl ether. $\lambda_{\max}^{\mathrm{CHCl_3}}$ 2.74, 5.63, and 7.29 μ.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.77; H, 9.80.

3-(3-Oxo-6α-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*19*). A solution of 18 g. of aluminum isopropoxide in 250 ml. of dry toluene was added during 5 min. to a stirred, refluxing solution of 15 g. of the 3-ol-5-ene derivative (*23*) and 80 ml. of cyclohexanone in 600 ml. of dry toluene. Refluxing was continued for 40 min. whereupon the mixture was cooled, treated with 100 ml. of water, and then made strongly acid with 6*N* sulfuric acid. The solvent layer was separated, washed with saturated salt solution, and each was back-extracted with toluene. The combined toluene solutions were steam distilled exhaustively, the residue was cooled and finally extracted with chloroform. Removal of the solvent gave 15 g. of a light brown glass which resisted crystallization (ultraviolet spectrum indicated approximately 90% conjugated ketone). The crude ketone was chromatographed over 1 kg. of silica gel and the 10% ethyl acetate-90% benzene eluates were combined (7.9 g. of solid fractions) and crystallized from ethyl acetate-isopropyl ether to yield 5.3 g. of the pure 6α-methyl ketone (*19*), m.p. 149–150°. This product was identical in all respects (mixed melting point, infrared spectrum, and $[\alpha]_D$) with that obtained above by epimerization of the 6β-methyl compound (*18*).

3-(3-Oxo-6α-methyl-6β,17β-dihydroxy-4-androsten-17α-yl)-propanoic acid lactone. The peak fractions from the 50% ethyl acetate–50% benzene eluate were combined (1.069 g.), triturated with isopropyl ether, and crystallized twice from ethyl acetate to afford 320 mg. of tiny cubes, m.p. 245–248°. $\lambda_{\max}^{\mathrm{KBr}}$ 2.89, 5.63, 5.97, 6.23 μ; $\lambda_{\max}^{\mathrm{CH_3OH}}$ 237 mμ, ϵ 14,150.

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.62; H, 8.56.

3-(3-Oxo-6α-methyl-17β-hydroxy-1,4-androstadien-17α-yl)-propanoic acid lactone (*24*). A stirred mixture of 2.0 g. of *19*, 0.76 g. of selenium dioxide, and 0.5 ml. of acetic acid in 50 ml. of *t*-butyl alcohol was refluxed for 13 hr. The mixture was cooled, filtered through a Celite pad, and reduced to a red-brown oil under vacuum. The oil was dissolved in methylene chloride (50 ml.) and again filtered through a Celite pad. The methylene chloride was removed under nitrogen. The residue was dissolved in benzene and chromatographed over 100 g. of silica. A crystalline solid was eluted with 15% ethyl acetate–85% benzene which still contained traces of selenium. The solid (756 mg.) was chromatographed again using a fraction collector and eluting with 10% ethyl acetate–90% benzene. The crystalline material obtained was recrystallized from acetone and then from ethyl acetate to give 240 mg. of the dienone (*24*), m.p. 223–226°.

Anal. Calcd. for $C_{23}H_{30}O_3$: C, 77.93; H, 8.53. Found: C, 77.56; H, 8.45.

3-(3-Oxo-6-methyl-17β-hydroxy-4,6-androstadien-17α-yl)-propanoic acid lactone (*25*). A solution of 1.00 g. of 3-(3-oxo-6α-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*19*), 1.00 g. of chloranil, and 25 mg. of *p*-toluenesulfonic acid monohydrate in 100 ml. of xylene was refluxed for 3.5 hr. The total reaction mixture was chromatographed on 55 g. of silica gel. The excess chloranil was eluted with 250 ml. of benzene. This was followed by dihydrochloranil, eluted with 500 ml. of 5% ethyl acetate in benzene. The 6-dehydro product was eluted with 1 l. of 15% ethyl acetate in benzene. Crystallization from ethyl acetate–isopropyl ether gave 735 mg. of 3-(3-oxo-6-methyl-17β-hydroxy-4,6-androstadien-17α-yl)propanoic acid lactone (*25*), m.p. 203–205°. The analytical sample, m.p. 204–205°, was obtained by recrystallization from aqueous acetone. $\lambda_{\max}^{\mathrm{KBr}}$, 5.66, 6.03, 6.18, 6.34 μ; $\lambda_{\max}^{\mathrm{CH_3OH}}$ 288 mμ, ϵ 22,600; $[\alpha]_D$ + 29.5° (chloroform).

Anal. Calcd. for $C_{22}H_{30}O_3$: C, 77.93; H, 8.53. Found: C, 78.11; H, 8.49.

3-(3-Oxo-7α-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (*27*). A solution of 10.5 g. of 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propanoic acid lactone (*26*)[9] and 300 mg. of cuprous chloride in 75 ml. of tetrahydrofuran (distilled from methylmagnesium bromide) was cooled under a cover of nitrogen. There was added with stirring over a period of 5 min. a solution of 25 ml. of 3*M* methylmagnesium bromide (Arapahoe), 150 ml. of tetrahydrofuran, and 600 mg. of cuprous chloride. The solution turned from yellow to brown, reverting to yellow at the end of addition. After an additional 5 min. of stirring the solution was poured into a slurry of ice and 30 ml. concd. hydrochloric acid.

The mixture was extracted with a total of 1.8 l. of methylene chloride, washed with water, 10% sodium bicarbonate solution, and water until neutral. After it was dried over anhydrous sodium sulfate, the methylene chloride was removed under vacuum leaving a residue weighing 11.35 g. A sample of this residue when scanned in the ultraviolet region showed absorption peaks at 242 mμ and 286 mμ indicating Δ^4-3-keto conjugation and starting dieneone. A calculation based on these absorption peaks showed that the Δ^4-3-keto material was present to the extent of 62.5% and starting material to the extent of 10.4%.

The residue was dissolved in petroleum ether (b.p. 60–68°) and subjected to column chromatography using 200 g. of silica gel and collecting 400-ml. fractions. Fractions 16–56 of the benzene-ethyl ether (24:1) eluate were pooled and recrystallized from alcohol yielding 2.4 g. of 3-(3-oxo-17β-hydroxy-7α-methyl-4-androsten-17α-yl) propanoic acid lactone, m.p. 169–171°, $[\alpha]_D + 82.0°$ (chloroform) $\lambda_{max}^{CH_3OH}$ 241.5 mμ (ε 16,400).

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.19; H, 9.02.

16α- and 16β-Methyl-3β-hydroxy-5-androsten-17-one (29a and 29b). To a solution of 9.6 g. of 16β-methyldehydroisoandrosterone acetate (*28*)[11] in 700 ml. of methanol was added a solution of 2.5 g. of potassium hydroxide in 22 ml. of water and after a 25-min. reflux period the solvent was evaporated *in vacuo* until an oily separation was noted. Refrigeration produced crystals which were collected on a funnel and washed with water. The crude product (8.3 g.) was recrystallized from 40 ml. of acetonitrile to give 6.15 g. of the 16β-isomer (*29b*), m.p. 160–168°, $[\alpha]_D + 6.8°$ (chloroform), $\lambda_{max}^{CHCl_3}$ 2.76, 5.77 μ.

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.25, H, 9.99.

By concentrating the mother liquor 900 mg. of an isomeric product was obtained, m.p. 133–134°, $[\alpha]_D + 8.9°$ (chloroform); $\lambda_{max}^{CHCl_3}$, 2.75, 5.75 μ. This is the 16α-methyl derivative (*29a*).

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.11; H, 9.98.

Saponification of 13.0 g. of 16β-methyldehydroisoandrosterone acetate in 950 ml. of methanol with more concd. alkali (6.8 g. potassium hydroxide in 30 ml. of water) and a 30-min. reflux period produced 9.0 g. of (*29a*), m.p. 132–135°, upon recrystallization from acetonitrile.

Reacetylation of the higher melting isomer (major product from the milder saponification) in pyridine and acetic anhydride gave on recrystallization from methanol a product melting at 142–144°. The melting point was undepressed when mixed with 16β-methyldehydroisoandrosterone acetate (*29b*).

17α- and 17β-Ethynyl-3-hydroxy-16α-methyl-5-androsten-17-one (30a and 30b). A solution of 130 ml. of diethylene glycol dimethyl ether and 9.4 ml. of diethylene glycol monoethyl ether was stirred vigorously with a metal paddle stirrer and heated to 135° under an atmosphere of nitrogen. Twenty-six grams of 85% potassium hydroxide pellets was added all at once and the source of heat was removed, allowing the reaction mixture to cool to 40°. An ice-salt bath was then applied to the reaction mixture and when the reaction temperature had reached 0°, nitrogen atmosphere was discontinued and acetylene was introduced over the surface of the reaction mixture.

After 30 min. of acetylene passage, 2.65 g. of *29a* dissolved in 20 ml. of diethylene glycol dimethyl ether was added. Acetylene passage was continued for an additional 4 hr. and then the reaction mixture was poured into 1.2 l. of water. The suspension was acidified to *p*H 6 with dilute acetic acid. The solid was collected on a funnel and subjected to chromatographic separation on a column of silica gel. Elution with 10% ethyl acetate plus 90% benzene produced two absorption peaks as successive fractions were collected. Collective recrystallization from acetonitrile of fractions about the first peak gave a product (*30a*) melting at 208–212°, $[\alpha]_D - 88.0°$ (chloroform), $\lambda_{max}^{CHCl_3}$ 2.75, 3.03 μ.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.82. Found: C, 80.36; H, 9.92.

Collective recrystallization from acetonitrile of fractions about the second peak produced an isomer (*30b*) melting at 170–175°, $[\alpha]_D - 108.6°$ (chloroform), $\lambda_{max}^{CHCl_3}$ 2.76, 3.03 μ.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.82. Found: C, 80.16; H, 9.68.

3-(3β,17β-Dihydroxy-16α-methyl-5-androsten-17α-yl)propynoic acid (31). To a stirred solution of 6 ml. (0.018 mole) of ethylmagnesium bromide in ethyl ether and 8 ml. of tetrahydrofuran was slowly added a solution of 1.2 g. of *30a* in 20 ml. of tetrahydrofuran. The boiling point of the reaction mixture was raised to 55–60° by distillation of solvent and then the reaction mixture was allowed to stir and reflux for 18 hr. Following this period an additional 2 ml. (0.006 mole) of the Grignard reagent was added and reflux was maintained for three more hours. The reaction mixture was cooled to room temperature under an atmosphere of nitrogen and 25 ml. of tetrahydrofuran was added. Continuing to stir the reaction mixture, carbon dioxide was passed over its surface for 20 hr. It was then transferred into 150 ml. of water containing 3 g. of concd. sulfuric acid. A viscous oil separated and solidified on standing. It was collected on a funnel and washed free of acid with water. The yield of crude product was 1.2 g., dec. 194–208°. It was further purified by trituration with 15 ml. of boiling carbon tetrachloride to yield 1.1 g. of the pure acid (*31*), dec. 212°, λ_{max}^{KBr} 2.86, 4.50, 5.38, 6.00 μ.

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 73.74; H, 8.46.

3-(3β,17β-Dihydroxy-16α-methyl-5-androsten-17α-yl)propanoic acid lactone (32). One gram of *31* with 0.3 g. of triethylamine in 15 ml. of absolute ethanol was subjected to hydrogenation at laboratory temperature and pressure over 0.1 g. of 5% palladium-on-carbon. Hydrogen uptake ceased in 3 hr. when 93% of the theoretical amount of hydrogen had been absorbed. The solution was filtered and concentrated to a solid residue *in vacuo*. The residue was dissolved in 10 ml. of methanol, and 0.7 ml. of concd. hydrochloric acid and 0.5 ml. of water were added. The solution was allowed to stand at room temperature for 1 hr. and then transferred to 100 ml. of water. The solid which separated was collected on a funnel and washed with water. It was recrystallized from ethyl acetate to yield 450 mg. of the lactone (32), m.p. 247–253°, $[\alpha]_D - 57.7°$ (chloroform), $\lambda_{max}^{CHCl_3}$ 2.77, 5.67 μ.

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.19; H, 9.55.

3-(3-Oxo-16α-methyl-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (33). To a solution of 350 mg. of *32* in 15 ml. of dry toluene was added 1.5 ml. of cyclohexanone. This reaction mixture was heated to distill 1 ml. of solvent and then a solution of 350 mg. of aluminum isopropoxide in 5 ml. of dry toluene was added. The reaction mixture was allowed to stir and reflux for 20 min., and then it was cooled and hydrolyzed by adding 5 ml. of water and then dilute sulfuric acid to acidity. Distillation with steam removed the organic solvents and produced a solid product which was collected, washed with water, and recrystallized from ethyl acetate plus isopropyl ether and then again from ethyl acetate to yield 120 mg. of the lactone (*33*), m.p. 163–170° after drying *in vacuo* at 78°, $[\alpha]_D + 93°$ (chloroform), $\lambda_{max}^{CH_3OH}$ 241 mμ ε 16,300, $\lambda_{max}^{CHCl_3}$ 5.66, 6.00, 6.10 μ.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.61; H, 8.97.

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