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Synthesis of 15-Oxo- and Δ^{16}-14β-Isobufadienolides[1]

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In order to clarify the structure–activity relationship, synthesis of the titled compounds has been undertaken. The acid–cleavage of 14α,15α-epoxyisobufadienolide (I) followed by oxidation with chromium trioxide gave the 14β-hydroxy-15-ketone (III). The ring opening of the β-epoxide (IV) and subsequent epimerization at C-14 resulted in formation of 15-oxo-14β-isobufadienolide (VI). Upon treatment with N-bromosuccinimide and then with lithium chloride or sodium iodide 14β,17α- and 14α,17β-isobufadienolides (IX and XIII) were led to Δ^{16},$\Delta^{14,16}$ and Δ^{16}-14β,15β-epoxy derivatives (X, XV and XVI), respectively.

The structure–activity relationship of the cardiotonic steroids has recently been investigated and in consequence the structural factors essential for physiological activity has been elucidated.[3] However, significance of the linked position of the unsaturated lactone ring to the steroid nucleus has not yet fully been clarified, although we reported the preparation of 14β,15β-epoxyisobufadienolide in the preceding paper.[4] In addition, with regard to the configurational requirement at C-14 and C-17 the potency of Δ^{16}-14β-isobufadienolides appeared to be of particular interest. The present paper describes the synthesis of the titled compounds to obtain the more precise knowledge on the relationship between structure and physiological potency.

An initial attempt was made on the synthesis of 15-oxo-14β,17β-isobufadienolides employing two epimeric 14,15-epoxides[4] as the starting compounds. The opening of the α-oxido ring with mineral acid was first undertaken. On brief exposure to perchloric acid under mild conditions 14α,15α-epoxy-17β-isobufadienolide (I) underwent hydrolytic cleavage to give the 14,15-*trans*-glycol (II) in satisfactory yield. The configuration of the hydroxyl groups was tentatively assignable, since the mode of ring opening was sufficiently substantiated with the analogous compounds.[5] Indeed, oxidation with chromium trioxide–pyridine complex[5e] gave the 14β-hydroxy-15-ketone (III), whose structure was reasonably assigned by the nuclear magnetic resonance spectral evidence. The 18-methyl proton resonated at 1.03 ppm, which was in good accord with the expected chemical shift (1.01 ppm) derivable from the values of 15-oxo-14β,17β-isobufadienolide (VI) and 14β-hydroxyl group by the additivity rule.[6] The 3-hydroxylic compound should be obtained by acidic hydrolysis[7] of III.

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[1] This paper constitutes Part VII of the series "Studies on Cardiotonic Steroid Analogs"; Part VI: T. Nambara, K. Shimada, T. Nemoto, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **18**, 1658 (1970).

[2] Location: *Aobayama, Sendai.*

[3] *a*) Ch. Tamm, "Proceeding of the 1st International Pharmacological Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p. 11; *b*) T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1967); *c*) S. Imai, H. Murase, M. Katori, M. Okada, and T. Shigei, *Jap. J. Pharmacol.*, **15**, 62 (1965); *d*) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).

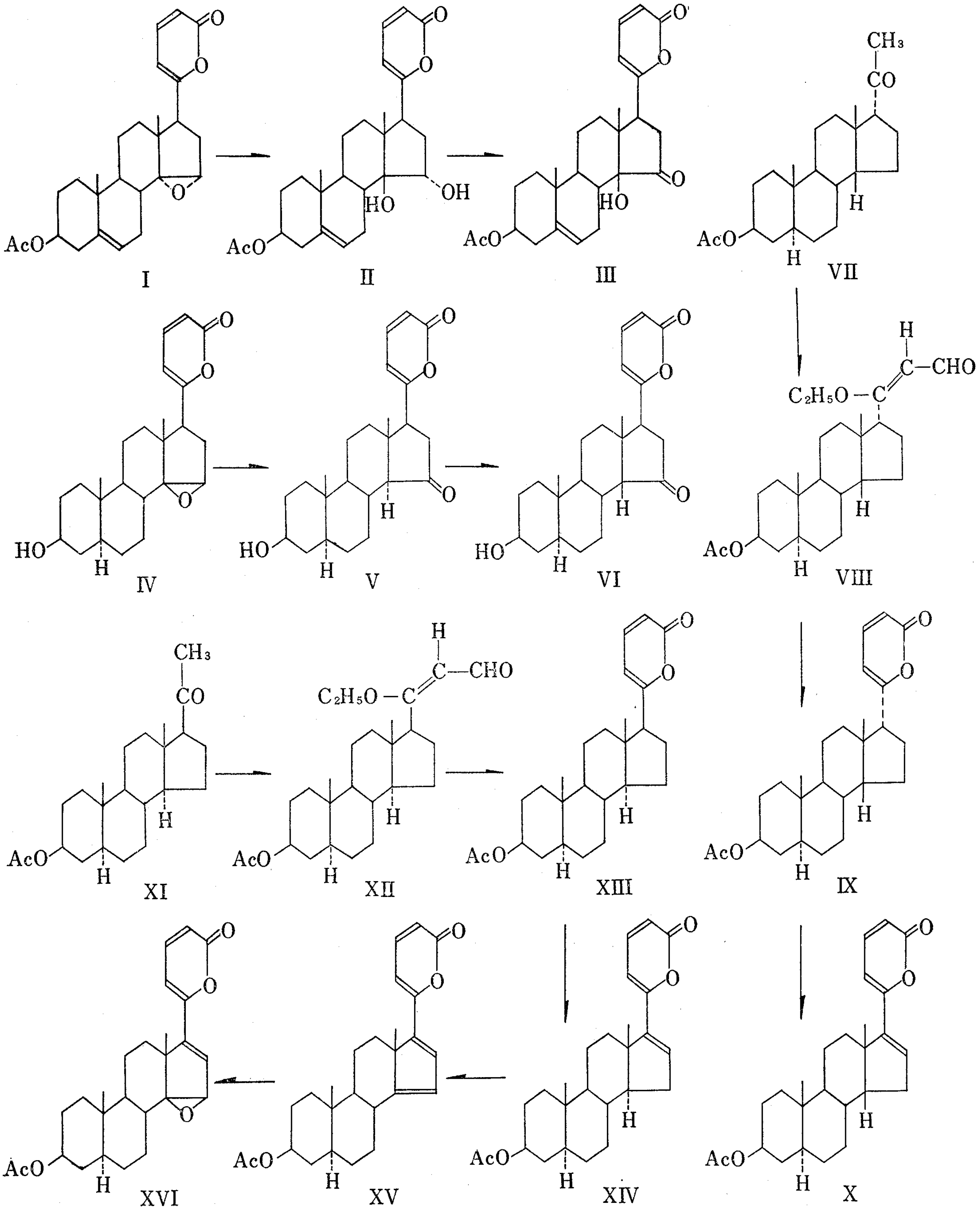
[4] T. Nambara, K. Shimada, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **18**, 453 (1970).

[5] *a*) H. Hasegawa, Y. Sato, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **9**, 409 (1961); *b*) H. Ishii, *ibid.*, **9**, 411 (1961); *c*) H. Ishii, T. Tozyo, and D. Satoh, *ibid.*, **10**, 645 (1962); *d*) *Idem, ibid.*, **11**, 576 (1963); *e*) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).

[6] N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964, p. 14.

[7] N. Danieli, Y. Mazur, and F. Sondheimer, *J. Am. Chem. Soc.*, **84**, 875 (1962); *idem*, *Tetrahedron*, **22**, 3189 (1966).

The acid cleavage of the oxido ring was then performed with the epimeric β-epoxide (IV). Upon treatment with perchloric acid in acetone IV was readily transformed into 15-oxo-14α-isobufadienolide (V). Being refluxed in methanolic hydrogen chloride for the prolonged period, V was epimerized with ease to yield an equilibrium mixture, from which the thermodynamically more stable 15-oxo-14β-isobufadienolide (VI) could be isolated by preparative thin-layer chromatography.



$Ac = CH_3CO-$

Chart 1

The next project was directed to the preparation of Δ^{16}-14β-isobufadienolide. For this purpose 3β-acetoxy-5α,14β,17α-pregnan-20-one (VII) was taken as the starting material. Condensation with orthoformate in the manner as previously reported[4,8] gave the unsaturated aldehyde (VIII), which on treatment with malonic acid was led to the α-pyrone derivative (IX). Allylic bromination with N-bromosuccinimide in carbon tetrachloride and subsequent dehydrobromination with lithium chloride in dimethylformamide[9] afforded the desired Δ^{16}-14β-isobufadienolide (X).

In a similar fashion 3β-acetoxy-5α-pregnan-20-one (XI) was converted into 14α,17β-isobufadienolide (XIII) by way of the unsaturated aldehyde (XII). Upon repeated dehydrogenation by treatment with N-bromosuccinimide and then with lithium chloride or sodium iodide XIII was led to the $\Delta^{14,16}$-diene derivative (XV) through the Δ^{16}-monounsaturated compound (XIV). When XV was exposed to a limited amount of per-acid, the reagent attacked the Δ^{14}-double bond selectively to furnish the desired 14β,15β-epoxy- Δ^{16}-isobufadienolide (XVI), whose characteristic ultraviolet absorption (λ_{max} 243, 325 mμ) justified putting the oxido ring at C-14 and C-15. The configuration was assignable on the basis of several findings regarding the epoxidation of the $\Delta^{14,16}$-diene system.[10]

It has already been reported that 15-oxocardenolide exhibited somewhat less potency than digitoxigenin, but was definitely active.[3d] It is hoped that the biological assay of the above-mentioned compounds together with 14,15-epoxyisobufadienolides may clarify the significance of the attached position of α-pyrone ring to the steroid nucleus.

Experimental[11]

6-(3β-Acetoxy-14β,15α-dihydroxyandrost-5-en-17β-yl)-2-pyrone (II)——To a solution of 6-(3β-acetoxy-14α,15α-epoxyandrost-5-en-17β-yl)-2-pyrone[4] (I) (24 mg) in acetone (8 ml) was added 2% $HClO_4$ (2 ml) and the resulting solution was allowed to stand at room temperature for 2 days. The solution was diluted with ether, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.25) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave II (10 mg) as colorless needles. mp 226—228°. $[\alpha]_D^{22}$ 0° (c=0.12). *Anal.* Calcd. for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.53; H, 7.92. NMR (4% solution in $CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 2.02 (3H, s, 3β-$OCOCH_3$), 4.35 (1H, d, J=6 cps, 15β-H), 4.50 (1H, m, 3α-H), 5.45 (1H, m, 6-H), 6.10 (1H, d, J=6 cps, 23-H), 6.15 (1H, d, J=4 cps, 21-H), 7.25 (1H, q, J=6,4 cps, 22-H).

6-(3β-Acetoxy-14β-hydroxy-15-oxoandrost-5-en-17β-yl)-2-pyrone (III)——To a solution of II (30 mg) in pyridine (0.3 ml) was added a solution of CrO_3 (35 mg) in pyridine (0.3 ml) under ice-cooling and the resulting solution was allowed to stand at room temperature for 12 hr. The solution was diluted with ether, washed with H_2O, 5% HCl and H_2O successively and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (1:1) as developing solvent. The adsorbent corresponding to thespot *Rf* (0.50) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave III (13 mg) as colorless leaflets. mp 237—240°. $[\alpha]_D^{13}$ −60.2° (c=0.08). *Anal.* Calcd. for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32. Found: C, 70.71; H, 7.32. NMR (4% solution in $CDCl_3$) δ: 0.99 (3H, s, 19-CH_3), 1.03 (3H, s, 18-CH_3), 2.03 (3H, s, 3β-$OCOCH_3$), 4.65 (1H, m, 3α-H), 5.45 (1H, m, 6-H), 6.15 (1H, d, J=9 cps, 23-H), 6.27 (1H, d, J=6 cps, 21-H), 7.25 (1H, q, J=9,6 cps, 22-H).

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[8] T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968); T. Nambara, K. Shimada, S. Goya and N. Sakamoto, *ibid.*, **18**, 617 (1970).

[9] G. Bach, J. Capitaine, and Ch. R. Engel, *Can. J. Chem.*, **46**, 733 (1968).

[10] *a*) M. Okada and Y. Saito, *Steroids*, **6**, 645 (1965); *b*) Pl. A. Plattner, L. Ruzicka, H. Heusser, and E. Angliker, *Helv. Chim. Acta*, **30**, 385 (1947); *c*) T. Nambara, S. Goya, J. Goto, and K. Shimada, *Chem. Pharm. Bull.* (Tokyo), **16**, 2228 (1968).

[11] All melting points were taken on a micro hot-stage apparatus and are uncorrected. The optical rotations were measured in $CHCl_3$ unless otherwise specified. The ultraviolet and infrared spectral measurements were run on Hitachi Model EPS-2U and JASCO Model IR-S spectrophotometers, respectively. The nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer at 60 Mc using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, q=quartet, and m=multiplet.

6-(3β-Hydroxy-15-oxo-5α-androstan-17β-yl)-2-pyrone (V)——To a solution of 6-(3β-hydroxy-14β,15β-epoxy-5α-androstan-17β-yl)-2-pyrone⁴⁾ (IV) (28 mg) in acetone (3 ml) was added $HClO_4$ solution (0.1 ml) (0.1 ml of 70% $HClO_4$ dissolved in 2 ml of acetone) and the resulting solution was refluxed for 7 min. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.20) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave V (15 mg) as colorless prisms. mp 250—253°. $[\alpha]_D^{14}$ 0° (c=0.06, MeOH). *Anal.* Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 75.10; H, 8.43. NMR (4% solution in $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 3.55 (1H, m, 3α-H), 5.98 (1H, d, J=6 cps, 23-H), 6.18 (1H, d, J=9 cps, 21-H), 7.25 (1H, q, J=6,9 cps, 22-H). IR ν_{max}^{KBr} cm^{-1}: 1708 (δ-lactone ring C=O), 1732 (5-membered ring C=O), 3585 (OH).

6-(3β-Hydroxy-15-oxo-5α,14β-androstan-17β-yl)-2-pyrone (VI)——A solution of V (15 mg) in MeOH (3 ml) containing 1 drop of conc. HCl was refluxed for 1.5 hr. The reaction mixture was diluted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.18) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave VI (10 mg) as colorless prisms. mp 245—247°. $[\alpha]_D^{20}$ +18.7° (c=0.11). *Anal.* Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 74.91; H, 8.33. NMR (4% solution in $CDCl_3$) δ: 0.77 (3H, s, 19-CH_3), 1.03 (3H, s, 18-CH_3), 3.55 (1H, m, 3α-H), 5.98 (1H, d, J=6 cps, 23-H), 6.18 (1H, d, J=9 cps, 21-H), 7.25 (1H, q, J=6,9 cps, 22-H).

3β-Acetoxy-20-ethoxy-21-formyl-5α,14β,17α-pregn-20-ene (VIII)——To a solution of 3β-acetoxy-5α,14β,17α-pregnan-20-one¹⁰ᵇ⁾ (VII) (250 mg) in ethyl orthoformate (5 ml) was added a few drops of $HClO_4$ dropwise under ice-cooling over a period of 20 min. After addition of several drops of pyridine to decompose the perchlorate, the resulting solution was extracted with ether. The organic layer was washed with cold 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up a crystalline product was obtained. Recrystallization from MeOH gave VIII (200 mg) as colorless leaflets. mp 151—154°. $[\alpha]_D^{20}$ +135.3° (c=0.10, MeOH). *Anal.* Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 75.09; H, 9.79.

6-(3β-Acetoxy-5α,14β-androstan-17α-yl)-2-pyrone (IX)——To a solution of VIII (250 mg) in pyridine (5 ml) were added a trace of morpholine and malonic acid (125 mg) and the resulting solution was refluxed for 4 hr. The solution was extracted with ether, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (9:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.59) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave IX (60 mg) as colorless leaflets. mp 161—162°. $[\alpha]_D^{20}$ +55.5° (c=0.15). *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.60; H, 8.80. NMR (4% solution in CCl_4) δ: 0.82 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 1.95 (3H, s, 3β-$OCOCH_3$), 4.65 (1H, m, 3α-H), 5.85 (1H, d, J=3 cps, 23-H), 6.05 (1H, d, J=6 cps, 21-H), 7.25 (1H, q, J=6,3 cps, 22-H).

6-(3β-Acetoxy-5α,14β-androst-16-en-17-yl)-2-pyrone (X)——To a solution of IX (50 mg) in CCl_4 (5 ml) was added N-bromosuccinimide (25 mg) and the resulting solution was irradiated with two tungsten lamps (500 W) in N_2 atmosphere for 15 min. The cooled solution was filtered and the precipitate was rinsed with CCl_4. Evaporation of the combined filtrates *in vacuo* gave a yellow syrup (50 mg). To a solution of this crude product in DMF (5 ml) was added LiCl (25 mg) and refluxed under a current of N_2 for 1 hr. The resulting solution was diluted with ether and washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (9:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.50) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave X (10 mg) as colorless needles. mp 190—191°. $[\alpha]_D^{20}$ +93.1° (c=0.12). *Anal.* Calcd. for $C_{26}H_{34}O_4$: C, 76.06; H, 8.34. Found: C, 75.73; H, 8.40. UV λ_{max}^{EtOH} mμ (ε): 242 (7900), 338 (10200). NMR (4% solution in $CDCl_3$) δ: 0.83 (3H, s, 19-CH_3), 1.29 (3H, s, 18-CH_3), 1.95 (3H, s, 3β-$OCOCH_3$), 4.65 (1H, m, 3α-H), 6.05 (1H, d, J=6 cps, 23-H), 6.15 (1H, d, J=3 cps, 21-H), 6.45 (1H, m, 16-H), 7.25 (1H, q, J=6,3 cps, 22-H).

6-(3β-Acetoxy-5α-androstan-17β-yl)-2-pyrone (XIII)——In the same manner as described in IX 3β-acetoxy-20-ethoxy-21-formyl-5α-pregn-20-ene (XII)¹²⁾ (300 mg) was treated with malonic acid (150 mg), pyridine (5 ml) and a trace of morpholine. The crude product obtained was submitted to the preparative TLC using benzene-AcOEt (10:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.42) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave XIII (70 mg) as colorless prisms. mp 238—240°. $[\alpha]_D^{26}$ +14.2° (c=0.11). *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.50; H, 8.96.

6-(3β-Acetoxy-5α-androst-16-en-17-yl)-2-pyrone (XIV)——In the same manner as described in X a solution of XIII (136 mg) in CCl_4 (10 ml) was treated with N-bromosuccinimide (70 mg). The crude product obtained was further treated with LiCl (60 mg) in DMF (5 ml). On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (10:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.42) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave

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12) J. P. Dusza, J.P Toseph, and S. Bernstein, U. S. Patent 3201425 (1965) [*C.A.*, **63**, 18231 e (1965)].

XIV (50 mg) as colorless needles. mp 178—180°. $[\alpha]_D^{27}$ $+41.7°$ ($c=0.16$). *Anal.* Calcd. for $C_{26}H_{34}O_4$: C, 76.06; H, 8.34. Found: C, 76.12; H, 8.56. UV λ_{max}^{EtOH} mμ (ε): 239 (9800), 339 (12000). NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 19-CH_3), 0.99 (3H, s, 18-CH_3), 2.00 (3H, s, 3β-$OCOCH_3$), 4.65 (1H, m, 3α-H), 6.10 (1H, d, $J=6$ cps, 23-H), 6.25 (1H, m, 21-H), 6.57 (1H, m, 16-H), 7.25 (1H, m, 22-H).

6-(3β-Acetoxy-5α-androsta-14,16-dien-17-yl)-2-pyrone (XV)——To a solution of XIV (90 mg) in CCl_4 (10 ml) was added N-bromosuccinimide (45 mg) and the resulting solution was irradiated with two tungsten lamps (500 W) under a current of N_2 for 1 hr. The cooled solution was filtered and the precipitate was rinsed with CCl_4. Evaporation of the combined filtrates *in vacuo* gave a yellow syrup. To a solution of this crude product (90 mg) in acetone (5 ml) was added NaI (80 mg) and refluxed for 4 hr in N_2 atmosphere. The reaction mixture was diluted with ether and washed with 5% $Na_2S_2O_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work–up the residue obtained was submitted to the preparative TLC using benzene–AcOEt (9:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.30) was eluted with AcOEt–CH_2Cl_2 (1:1). Recrystallization of the eluate from MeOH gave XV (30 mg) as colorless needles. mp 177—178°. $[\alpha]_D^{22}$ $+388.7°$ ($c=0.08$). *Anal.* Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90. Found: C, 76.39; H, 7.91. UV λ_{max}^{EtOH} mμ (ε): 278 (4990), 381 (25900). NMR (4% solution in CCl_4) δ: 0.95 (3H, s, 19-CH_3), 1.19 (3H, s, 18-CH_3), 1.93 (3H, s, 3β-$OCOCH_3$), 4.55 (1H, m, 3α-H), 5.80—6.10 (3H, m, 15-H, 21-H, 23-H), 6.95—7.30 (2H, m, 16-H, 22-H).

6-(3β-Acetoxy-14β,15β-epoxy-5α-androst-16-en-17-yl)-2-pyrone (XVI)——To a solution of XV (18 mg) in $CHCl_3$ (3 ml) was added a solution of perbenzoic acid (14 mg) in $CHCl_3$ and the resulting solution was allowed to stand at 4° for 24 hr. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with 5% $Na_2S_2O_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work–up the residue obtained was submitted to the preparative TLC using benzene–AcOEt (9:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.25) was eluted with AcOEt. Recrystallization of the eluate from AcOEt gave XVI (10 mg) as pale yellow prisms. mp 240—243°. $[\alpha]_D^{23}$ $+186.7°$ ($c=0.11$). *Anal.* Calcd. for $C_{26}H_{32}O_5$: C, 73.56; H, 7.60. Found: C, 73.09; H, 7.75. UV λ_{max}^{EtOH} mμ (ε): 243 (4500), 325 (6300). NMR (4% solution in $CDCl_3$) δ: 0.87 (3H, s, 19-CH_3), 1.35 (3H, s, 18-CH_3), 2.03, (3H, s, 3β-$OCOCH_3$), 3.80 (1H, s, 15α-H), 4.65 (1H, m, 3α-H), 6.15 (1H, d, $J=4$ cps, 23-H), 6.18 (1H, m, 21-H), 6.75 (1H, s, 16-H), 7.25 (1H, m, 22-H).

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