loss of information and greatly simplified the subsequent calculations.

Each compound was screened initially to determine the dose range in which it was effective. Four linearly spaced doses including zero were then chosen, and estimates were made of the median effective doses of oxotremorine when administered intravenously 15 min after intraperitoneal injection of the test compound. The "up-and-down" method for small samples described by Dixon[11] was employed to estimate the median effective doses, using a logarithmic series of doses of oxotremorine with a spacing of 0.1 in the $\log_{10}$ dose scale and a nominal sample size of 5. When the median effective dose of oxotremorine was plotted against the dose of the test compound used for premedication, most compounds gave results characteristic of competitive antagonism (Figure 2). The intercept on the abscissa provides an estimate of the dose of antagonist which doubles the median effective dose of oxotremorine, and was determined by a weighted regression analysis to allow for the fact that the standard error of the estimate is constant on the log-dose scale.[11] Some compounds showed no significant linear regression, and are recorded as inactive at the doses tested.

Mydriatic activity was estimated by measuring the pupillary diameter of mice in groups of 5, both before and 15–20 min after the i.p. injection of the test compound. The measurements were made under standard lighting conditions with a binocular microscope fitted with calibrated eyepiece. The mydriatic dose was estimated graphically as that required to double the pupil size relative to the control.

Acetylcholine antagonism was measured in isolated guinea pig ileal strips suspended in oxygenated Krebs solution at 38°. Contractions were recorded isotonically at 1-g tension, using a Collins displacement transducer and potentiometric recorder. A series of cumulative dose–response curves was obtained using acetylcholine only; these were then repeated in the presence of a test compound at concentrations increasing in the ratio 1:3:10:30.... The preparation was allowed to equilibrate with each new concentration for 30 min before the ACh dose–response curve was obtained.

(11) W. J. Dixon, *J. Amer. Statist. Ass.*, **60**, 967 (1965).

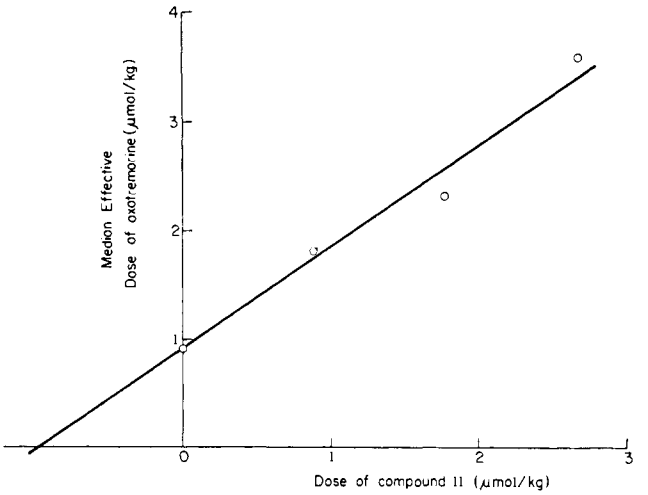

Figure 2.—Median effective dose of oxotremorine, estimated by the "up and down" method for small samples, plotted against dose of 11 used for premedication, showing typical competitive antagonism isobole.

In the case of competitive antagonists, the Ach concentration giving a 50% response was estimated by interpolation at each concentration of antagonist, and the antagonist concentration producing a twofold block of acetylcholine was estimated graphically. For noncompetitive antagonism, the effective concentration is recorded as that which reduces the maximum response to ACh by 50%.

Acknowledgment.—Part of this work has been supported by the Swedish Medical Research Council (Project B69-13X-533-05B) which is gratefully acknowledged.

Modified Cardenolides. V.[1a] Replacement of the C-17 Lactone Substituent by Alkylating Groups[1b]

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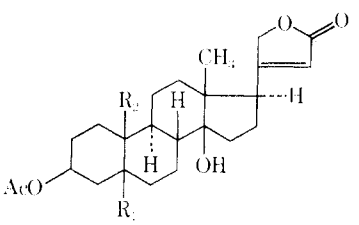
The synthesis of a variety of potential alkylating substances derived from cardiac aglycones is described. For example, ozonolysis of digitoxigenin acetate gives 3β,14,21-trihydroxy-5β,14β-pregnan-20-one 3-acetate which is converted into the 21-chloroacetate and 21-iodoacetate. Biological evaluation in the usual cat toxicity test shows that some of these compounds have appreciable cardiotoxicity, but tests for cardiotonic activity are negative.

The discovery of the cardioactivity of 3β,5β,14β,19,-21-pentahydroxypregnan-20-one 3,19-diacetate 21-iodoacetate (**13**) in cats[1a] prompted us to prepare a number of related structures to investigate this structural lead systematically. The basis for the design of these compounds was the hypothesis that alkylation of an essential nucleophilic group on the receptor is required for drug action, and that the α,β-unsaturated lactone group in cardenolides, and the iodoacetate function in **13**, respectively, perform this function.

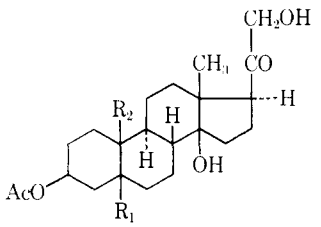
(1) (a) For a preliminary communication containing a small part of this work, see paper IV in this series: M. E. Wolff, W. Ho, and H. H. Chang, *J. Pharm. Sci.*, **57**, 1450 (1968); (b) This research was supported in part by Public Health Service Grant (HE-09578) from the National Heart Institute, U. S. Public Health Service.

It is known that 17α-lactones derived from cardenolides are inactive in the usual tests for cardioactivity, and it was desired to determine whether this loss of activity would also be seen in the cardiotoxic C-21 substituted alkylating agents.

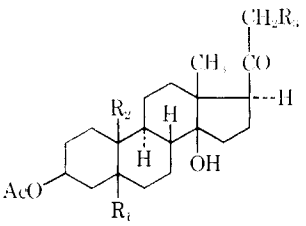
The characteristic C/D *cis* fusion of cardiac aglycones is considered to be important for biological activity. To obviate the necessity for the synthesis of C/D *cis* steroids from ordinary C/D *trans* steroids, the naturally occurring cardiac glycosides strophanthin and digitoxin were used as starting materials in planning the syntheses. Without disturbing the steroid nucleus, the butenolide ring was replaced with functional groups which were capable of reacting with SH groups.



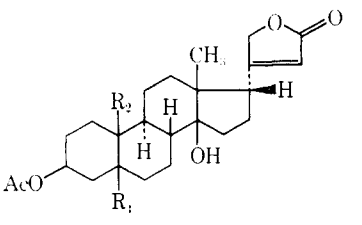
1. $R_1 = H$; $R_2 = CH_3$
2. $R_1 = OH$; $R_2 = CH_2OAc$



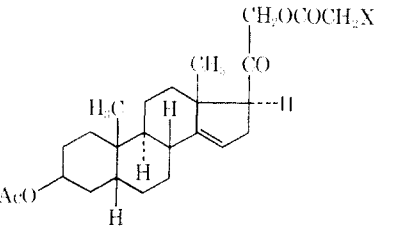
3. $R_1 = H$; $R_2 = CH_3$
4. $R_1 = OH$; $R_2 = CH_2OAc$



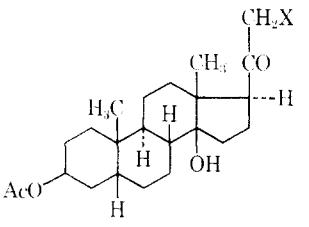
5. $R_1 = H$; $R_2 = CH_3$; $R_3 = AcO$
6. $R_1 = H$; $R_2 = CH_3$; $R_3 = ClCH_2CO_2$
7. $R_1 = H$; $R_2 = CH_3$; $R_3 = ICH_2CO_2$
8. $R_1 = H$; $R_2 = CH_3$; $R_3 = CH_2{=}CHCO_2$
9. $R_1 = H$; $R_2 = CH_3$; $R_3 = C_2H_5CO_2$
10. $R_1 = H$; $R_2 = CH_3$; $R_3 = HOOC(CH_2)CO_2$
11. $R_1 = OH$; $R_2 = CH_2OAc$; $R_3 = AcO$
12. $R_1 = OH$; $R_2 = CH_2OAc$; $R_3 = ClCH_2CO_2$
13. $R_1 = OH$; $R_2 = CH_2OAc$; $R_3 = ICH_2CO_2$
14. $R_1 = OH$; $R_2 = CH_2OAc$; $R_3 = CH_2{=}CHCO_2$



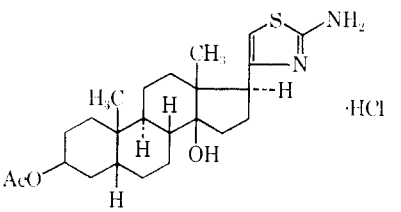
21. $R_1 = H$; $R_2 = CH_3$
22. $R_1 = OH$; $R_2 = CH_2OAc$



17. X = Cl
18. X = I



15. X = Cl
16. X = I



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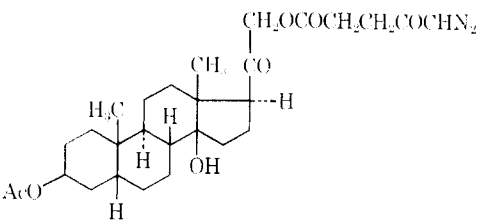

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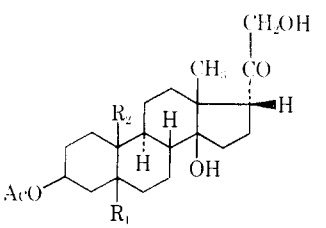
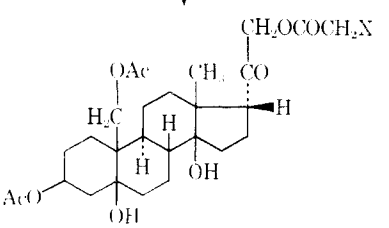
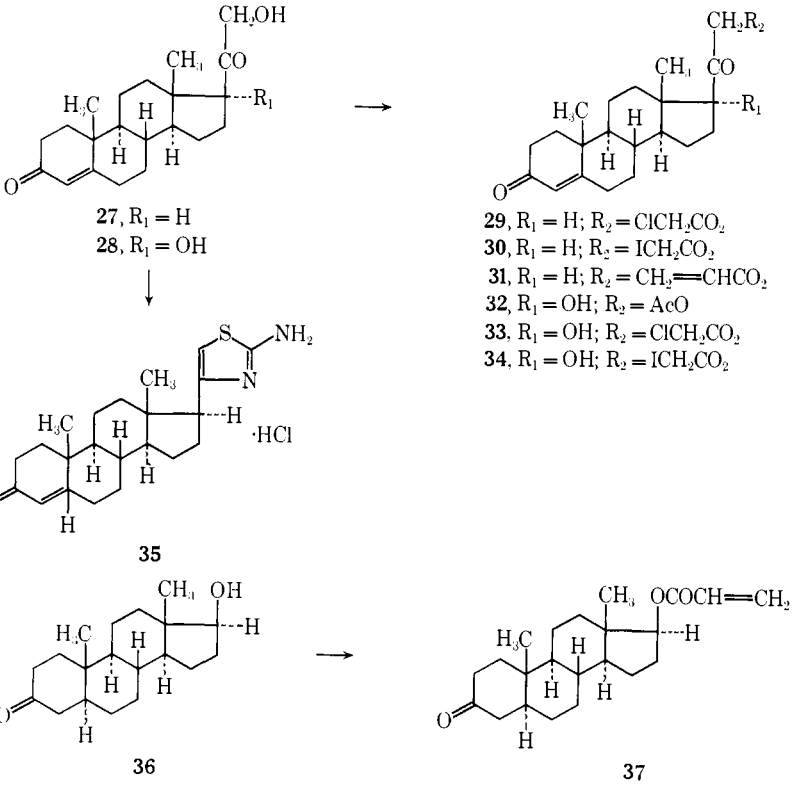


23. $R_1 = H$; $R_2 = CH_3$
24. $R_1 = OH$; $R_2 = CH_2OAc$



25. X = Cl
26. X = I



Strophanthidol 3,19-diacetate (**2**), obtained from reduction of strophanthidin with $NaBH_4$ followed by acetylation, was allowed to react with O_3 at $-70°$ in EtOAc solution and the resulting ozonide was decomposed with Zn dust in glacial HOAc. The crude 21-glyoxalate of **4** was hydrolyzed selectively with $KHCO_3$ in aqueous MeOH solution to afford **4**. The acetoxy groups at C-3 and C-19 were not hydrolyzed under these conditions. Acetylation of **4** gave **11** in which the carbonyl moiety of the 21-acetoxy group spatially resembles the carbonyl of the butenolide ring in cardiac aglycones.

For C/D *cis* steroids, the 17β-H compounds are known to be more stable than their 17α-H isomers. Digitoxigenin was readily converted into its more stable 17β-H isomer, allodigitoxigenin, by refluxing in DMF solution with sodium tosylate and NaOAc.[2] Application of the ozonolysis degradation scheme in analogous fashion gave the corresponding 17α-pregnane derivatives. Similarly, in the strophanthidol series, the corresponding 17α-substituted compounds were obtained.

Treatment of **4** with chloroacetic anhydride produced the corresponding 21-chloroacetate ester **12**. Reaction temperature and times were critical. Initially, what appeared to be dehydrated compounds were produced but with control of temperature and time, the reaction was made rather clean by monitoring with tlc. Treatment of **12** with NaI in Me_2CO afforded **13**. The iodoacetoxy group, a well-known SH reagent, was thus introduced in a proper spatial relationship to the steroid nucleus. Similarly, by starting with digitoxigenin, the corresponding 21-acetate **5**, chloroacetate **6**, and iodoacetate **7** were obtained. By checking with tlc, treatment of **3** with chloroacetic anhydride produced **6** first, and under higher reaction temperature and longer time, **17** was obtained. The chemical shifts of the angular Me protons, 19-H_3 (0.99 ppm), 18-H_3 (0.91 ppm) of **17** roughly agreed with the calculated values according to the "additivity" rule.[3] Moreover, a broad peak corresponding to the 15-H at 5.15 ppm eliminated the possibility of a $\Delta^{8(14)}$ compound.

A diazo ketone derivative **20**, also a potential alkylating drug, was prepared from **3** by treatment with succinic anhydride to give **10** followed by conversion into the diazo ketone.

A number of other alkylating functions were also introduced into **3** and related compounds. Thus, treatment of **3** with acrylic anhydride in pyridine afforded the 21-acrylate, in which the unsaturated acrylate could combine with a nucleophilic group. Again, α-halo ketones in which the halide could alkylate the receptor, were obtained by tosylation of **3** and conversion of the crude product into chloro ketone **15** by refluxing with LiCl in Me_2CO. Treatment of **15** with NaI readily gave iodo ketone **16**. As the 2-aminothiazole derivative **35** has been reported to possess digitalis-like cardiac activity,[4] the related compound **19** was prepared by refluxing **15** with thiourea in Me_2CO.

To obtain related compounds *lacking* the stereochemistry of the cardiac aglycones but having a similar basic structure deoxycorticosterone and 11-desoxy-17-hydroxycorticosterone were converted into haloacetates, acrylates, and a 2-aminothiazole derivative.

Biological Testing.[5]—The following test methods (see Experimental Section) were used: (a) toxicity in cats

(2) J. H. Russel, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **43**, 167 (1960).

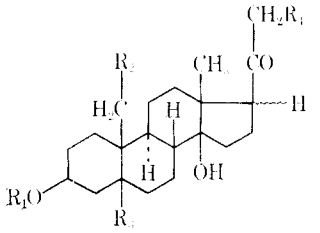
(3) R. F. Zürcher, *ibid.*, **44**, 1380 (1961); **46**, 2054 (1963).

(4) J. W. Ralls, M. Grove, and C. G. Bergstrom, U. S. Patent 2,793,207; *Chem. Abstr.*, **52**, 464 (1958).

(5) We are indebted to Professor Bert Katzung and Mr. Charles Lee for many discussions regarding the biological testing.

Table I

Chemical and Biological Properties of Substituted 14β-Hydroxypregnan-20-ones



									Digitoxigenin 3-acetate		
									Chemical data		
No.	Starting material	Method	R_1	R_2	R_3	R_4	17-H	Mp (°C)	Recrystn solvn[a]	$[\alpha]_{589}^{20}$ (°C (c, % solvn)[b]
24	22	A	Ac	OAc	OH	OH	β	109–112	EA	+3 (1, $CHCl_3$)B
4	2	A	Ac	OAc	OH	OH	α	120–122	E	+71 (1, $CHCl_3$)B
25	24	B	Ac	OAc	OH	$ClCH_2CO_2$	β	104–108	A–PE	−12 (1, $CHCl_3$)B
12	4	B	Ac	OAc	OH	$ClCH_2CO_2$	α	109–111	E	+51 (0.5, $CHCl_3$)B
26	25	C	Ac	OAc	OH	ICH_2CO_2	β	82–85	A–PE	+3 (0.5, $CHCl_3$)B
10	3	D	Ac	H	H	$HO_2C(CH_2)_2CO_2$	α	118–120	EA	+32 (1, $CHCl_3$)B
14	3	E	Ac	OAc	OH	$CH_2{=}CHCO_2$	α	162–164	A	+47 (1, $CHCl_3$)B
20	10	F	Ac	H	H	$N_2CHCO(CH_2)_2CO_2$	α	90–95	A	
13	12	C	Ac	OAc	OH	ICH_2CO_2	α	150–155	E	+49 (1, $CHCl_3$)B
6	3	B	Ac	H	H	$ClCH_2CO_2$	α	145–146	E	+18 (EtOH)B
7	6	C	Ac	H	H	ICH_2CO_2	α	116–117	E	+26 (EtOH)J
15	3	G	Ac	H	H	Cl	α	175–176	A–E	
16	15	C	Ac	H	H	I	α	208–212	A–E	
8	3	E	Ac	H	H	$CH_2{=}CHCO_2$	α	152–154	A–E	+59 (EtOH)B
23	21	A	Ac	H	H	OH	β	156	E	−28 (EtOH)J
9	3	H	Ac	H	H	$CH_3CH_2CO_2$	α	105–107	A–H	
11	4	c	Ac	OAc	OH	OAc	α	198–202	A	+67 (1, $CHCl_3$)B
5	3	c	Ac	H	H	OAc	α	184–185	A–E	

[a] EA, EtOAc; E, Et_2O; A, Me_2CO; PE, petroleum ether, bp 30–60°; H, hexane. [b] See ref 7b. [c] Prepared by acetylation with

was measured by the method of Chen;[1a,6] (b) isolated rabbit atria; (c) inhibition of ouabain-sensitive Na^+ efflux from human red cells. These experiments were kindly performed by Dr. Ian Glynn, Physiological Laboratory, University of Cambridge. (d) The effect on transport ATPase was observed by Dr. H. J. Portius and Professor K. R. H. Repke, Institute of Biochemistry, German Academy of Sciences, Berlin-Buch/GDR; their results will be published elsewhere in detail.

Results

The data from the cat toxicity test indicate that the iodoacetate derived from strophanthidol (**13**) has much greater cardioactivity than the simple acetate **11** (Table I). The steroid nucleus contributes to this activity in a specific way as shown by the smaller activity (Table III) of iodoacetate **34** and the small ratio between the activities of acetate **32** and iodoacetate **34**. Other compounds were tested in only single cats and therefore no structure–function comparisons can be made. However, when tested in the isolated rabbit atrium, which measures cardiotonic activity directly, no evidence of cardiotonic activity in any of the compounds was seen. In addition there was a lack of activity of these compounds in the ion movement test or the ATPase test.

Further investigation of this lead was discontinued when it was realized that the observed cardioactivity was a cardiotoxic, rather than a cardiotonic effect. One useful conclusion that can be drawn from this experience is that the time-honored cat test is useless as a screen for cardiotonic substances, except when dealing with cardenolides and bufadienolides as such.

Experimental Section[7]

Method A (Ozonolysis). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate (3).—A solution of 2.0 g of digitoxigenin 3-acetate (1) in 150 ml of EtOAc was cooled to −70°, and O_3 was passed in for 12 min. The colorless solution turned deep blue. O_2 was passed for 30 min to expel excess O_3 in the solution whereupon the blue color disappeared. EtOAc was removed under vacuum and the residue was dissolved in 50 ml of HOAc. To the HOAc solution, 2.0 g of Zn powder was added and the suspension was stirred at 27° for 2 hr. It was filtered and the filtrate was evapd under vacuum. A small amount of ice–water was added to the residue and the mixture was made slightly alk with K_2CO_3.

(6) P. G. Henderson and K. K. Chen, *J. Med. Chem.*, **8**, 577 (1965) and ref cited therein.

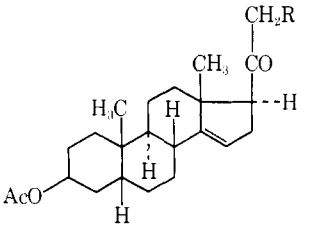
(7) (a) Melting points were determined with a Thomas–Hoover apparatus equipped with a corrected thermometer. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Nmr spectra were obtained at a field strength of 60 MHz on samples in $CDCl_3$ solution on a Varian A-60A instrument using TMS as internal standard. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values; (b) Optical rotations were obtained in a 0.5-cm tube with a Rudolph Photoelectric polarimeter (R), in a 0.05-cm cell with a Bendix automatic polarimeter (B), or in a 0.1-dm cell with a Jasco ORD/UV-5 instrument (J); (c) It is a pleasure to acknowledge the help of Dr. Sharad Gupte in the synthesis of large samples of material for biological testing and in the synthesis of compound **9**.

		Biological data						
		Cat LD	ATPase inhibition		Inhibition of ouabain-sensitive sodium efflux		Rabbit atria test	
			Concn		Concn		Concn	Inotropic
		(mg/kg)	(μM)	%	(g/ml)	%	(mg/ml)	activity
		0.5	1.2	−50	10^{-6}	100	1×10^{-2}	+
					10^{-7}	83		
Formula	Analyses				10^{-8}	31		
$C_{24}H_{38}O_8$	C, H		100	±0				
$C_{25}H_{38}O_8 \cdot H_2O$	C, H						$1.0–1.8 \times 10^{-2}$	0
$C_{27}H_{39}ClO_9$	C, H		100	±0				
$C_{27}H_{39}ClO_9$	C, H, Cl	2.15						
$C_{27}H_{39}IO_9$	C, H		100	+5				
$C_{27}H_{40}O_8$	C, H						$0.4–1.8 \times 10^{-2}$	0
$C_{28}H_{38}O_9$	C, H	3.83						
$C_{28}H_{40}O_7N_2$	C, H, N		50	±0			$0.4–1 \times 10^{-2}$	0
$C_{27}H_{39}IO_9$	C, H, I	1.37 ± 0.26						
$C_{25}H_{37}ClO_6$	C, H, Cl	3.8	10	−10				
$C_{25}H_{37}IO_6$	C, H, I	1.71	10	−20	10^{-1}	18	$4–10 \times 10^{-2}$	0
$C_{23}H_{29}ClO_4$	C, H, Cl		100	−25				
$C_{23}H_{33}IO_4$	C, H, I	5.4	50	±0				
$C_{26}H_{38}O_6$	C, H	6.3	10	−10	10^{-1}	22	$4–10^{-2}$	0
$C_{23}H_{36}O_5$	C, H							
$C_{26}H_{40}O_6$	C, H							
$C_{27}H_{40}O_9$	C, H	15.59 ± 2.28	100	+7				
$C_{25}H_{38}O_6$	C, H	10.0	10	−15	10^{-1}	33	0.4×10^{-2}	0

Ac_2O–Py.

Table II

Chemical and Biological Properties of Substituted Pregn-14-enes



No.	Starting material	Method	R	Mp (°C)	Recrystn[a] solvn	Formula	Analyses	Cat LD (mg/kg)
17	3	I	$ClCH_2CO_2$	132–134	E	$C_{23}H_{35}ClO_5$	H, Cl[b]	
18	17	C	ICH_2CO_2	136–137	E	$C_{25}H_{35}IO_5$	C, H, I	3.08

[a] E, Et_2O. [b] C: Calcd 66.58; found 66.08.

The solution was then made slightly acidic (pH 6–7) with dil HCl. The solid ppt was collected to afford 1.9 g of white powder. The white powder was dissolved in 100 ml of MeOH and 2 g of $KHCO_3$ in 50 ml of H_2O was added. The mixture was stirred at 27° for 16 hr, neutralized with a small amount of dil HCl, and the MeOH was removed under reduced pressure. The ppt was collected and recrystd.

Method B (Chloroacetylation). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-Chloroacetate (6).—A mixture of 0.5 g of **3** and 2.5 g of $(ClCH_2CO)_2$ was melted and allowed to react for 10 min in a H_2O bath. After cooling, a small amount of ice–water was added and the mixture was neutralized slowly with $KHCO_3$. Et_2O was added to dissolve the gum, and the Et_2O layer was washed with H_2O, dried (Na_2SO_4), and evapd. The residue was recrystd.

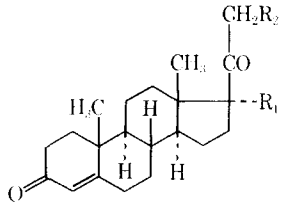
Method C (Iodide Displacement). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-Iodoacetate (7).—A solution of 0.2 g of **6** and 0.1 g of NaI in 20 ml of Me_2CO was refluxed for 30 min. After cooling the Me_2CO was removed under reduced pressure. Ice–water was added to the residue and the product was extracted with Et_2O. The Et_2O layer was washed with dil $Na_2S_2O_3$ solution and H_2O, dried (Na_2SO_4), and evapd. The residue was recrystd.

Method D (Hemisuccinate Formation). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-Hemisuccinate (10).—A solution of 0.3 g of **3** and 0.3 g of succinic anhydride in 10 ml of C_5H_5N was kept at 23° for 18 hr. It was diluted with H_2O and the ppt was collected, washed with H_2O, dried (Na_2SO_4), and evapd. The residue was recrystd.

Method E (Acrylate Formation). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-Acrylate (8).—To a solution of 0.89 g of **3** in 10 ml of C_5H_5N was added 1 ml of acrylic anhydride and the mixture was allowed to react at 27° for 4 hr. The solvent was removed under vacuum. The residue was dissolved in Me_2-

TABLE III

CHEMICAL AND BIOLOGICAL PROPERTIES OF SUBSTITUTED 3,20-DIOXO-4-PREGNENES

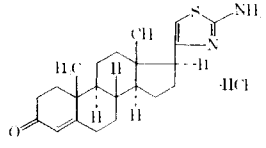
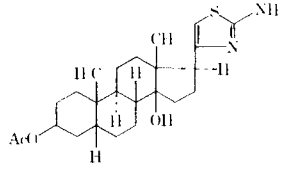
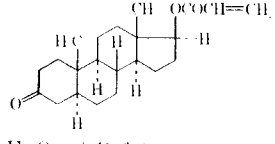


No.	Starting material	Method	R_6	R_1	Mp (°C)	Recrystn solvn[a]	$[\alpha]_{589}^{22}$ (°) (c, % solvn)[b]	Formula	Analyses	Cat LD (mg/kg)	ATPase inhibitions Concn (μM)	%	Inhibition of ouabain-sensitive sodium efflux— Concn (g/ml)	%
29	27	B	H	$ClCH_2CO_2$	221–223	A–E	+203 (EtOH)B	$C_{23}H_{31}ClO_4$	C, H, Cl					
30	29	C	H	ICH_2CO_2	130–131 dec	A	+121 (EtOH)B	$C_{23}H_{31}IO_4$	C, H, I					
33	28	B	OH	$ClCH_2CO_2$	219–220	A	+110 (1, $CHCl_3$)R	$C_{23}H_{31}ClO_5$	C, H, Cl		50	−20		
34	33	C	OH	ICH_2CO_2	196–197	A	+102 (1, $CHCl_3$)R	$C_{23}H_{31}IO_5$	C, H, I	5.08 ± 1.75	50	±0	10^{-6}	12
31	27	E	H	$CH_2{=}CH{-}CO_2$	151	A–E	+222 (EtOH)B	$C_{24}H_{32}O_4$	C, H	10.9				
32	*c*	*d*	OH	OAc	232–236	A		$C_{23}H_{32}O_5$	C, H	11.8 ± 1.2	50	+0		

[a] A, Me_2CO; E, Et_2O. [b] See ref 7b. [c] 11-Deoxy-17-hydroxycorticosterone. [d] Prepared by acetylation with Ac_2O Py.

TABLE IV

CHEMICAL AND BIOLOGICAL PROPERTIES OF 17β-SUBSTITUTED STEROIDS

No.	Starting material	Method	Structure	Mp (°C)	Recrystn solvn[a]	Formula	Analyses	Cat LD mg/kg	Rabbit aorta test Concn (mg)	Rabbit aorta test Inotropic activity
35[b]		J		290	M	$C_{22}H_{30}N_2O_3S \cdot HCl$		>20.5		
19	15	J		241–242	M	+24 (EtOH)J	$C_{24}H_{36}N_2O_3S \cdot HCl$	C, H, N, S, Cl	5.5	2×10^{-2}	0
37	36	E		147–148	A–E	+58 (EtOH)B	$C_{22}H_{32}O_3$	C, H	10.3		

[a] M, MeOH; A, Me_2CO; E, Et_2O. [b] Ref 4.

CO and passed through a column of 30 g of Florisil using Me_2CO as eluent to remove polymeric impurities. Me_2CO was removed under pressure and the residue was recrystd.

Method F (Diazo Ketone Formation). 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-(5-Diazo-4-oxo)pentanoate (6).—To a suspension of 0.2 g of **10** in 15 ml of dry C_6H_6 was added 15 mg of NaOH. After the mixture had been stirred for 0.5 hr at 23°, 3 drops of C_5H_5N was added. The mixture was chilled in an ice bath and 1.6 ml of $(COCl)_2$ was added. The resulting mixture was stirred for 30 min and then concd under reduced pressure. The residue was mixed three times with 10-ml portions of dry C_6H_6 and then evapd to dryness. After the residue was further dried under high vacuum it was dissolved in 10 ml of C_6H_6. The solution was filtered and the filtrate added to an Et_2O solution of CH_2N_2 cooled in an ice bath. The reaction mixture was allowed to warm to 23°. The solvent was allowed to evaporate in the hood overnight. The residue was purified by preparative tlc and recrystd.

Method G. 21-Chloro-3β,14-dihydroxy-5β,14β-pregnan-20-one 3-Acetate (15).—To a solution of 0.4 g of **3** in a mixture of 2.7 ml of EtOH-free $CHCl_3$ and 0.3 ml of C_5H_5N was added 0.38 g of *p*-TsCl. After 20 hr at 27°, 300 ml of $CHCl_3$ was added, and the solution was washed with dilute HCl, 5% $NaHCO_3$ solution, and H_2O, then dried (Na_2SO_4), and evapd under reduced pressure. The residue was a mixture of 21-tosylate and 21-chloro compounds. It was dissolved in 30 ml of Me_2CO, 0.2 g of LiCl was added, and the mixture was refluxed for 30 min and cooled. The Me_2CO was evapd under reduced pressure and the residue was dissolved in 300 ml of $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried (Na_2SO_4), and evapd under reduced pressure. The residue was recrystd.

Method H. 3β,14,21-Trihydroxy-5β,14β-pregnan-20-one 3-Acetate 21-Propionate (9).—A solution of 0.2 g of **3** and 0.8 ml of $(EtCO)_2O$ in 5 ml of C_5H_5N was allowed to react at 25° for 24 hr. It was then poured into H_2O, and the product was collected, washed with H_2O, dried, and recrystd.

Method I. 3β,21-Dihydroxy-5β-pregn-14-en-20-one 3-Acetate 21-Chloroacetate (17).—A melt of 0.5 g of **3** and 2.5 g of $(ClCH_2CO)_2O$ was allowed to react at 80° in an oil bath for 4 hr. After cooling, a small amount of ice–water was added and the mixture was neutralized slowly with $KHCO_3$. The product was extracted with Et_2O, and the Et_2O layer was washed with H_2O, dried (Na_2SO_4), and evapd. The residue was recrystd.

Method J. 3β,14-Dihydroxy-17β-(2′-amino-4′-thiazolyl)-5β,-14β-androstane 3-Acetate Hydrochloride (20).—A solution of 0.11 g of **15** and 0.33 g of thiourea in 10 ml of Me_2CO was refluxed for 3 hr. After cooling, the ppt was collected and recrystd.

Biological Methods

Determination of Cardiotonic Activity in Isolated Rabbit Atria.—Stunned rabbits were exsanguinated by carotid section and the left atrium was obtained and separated from fat and septal tissue. The base of the atrium was impaled on a hooked stimulating electrode. The preparation was maintained at 35.5° in a 50-ml bath containing low Ca^{2+} Feigen's solution: NaCl, 72 g; KCl, 3.36 g; $NaHCO_3$, 4.80 g; glucose, 16 g; $CaCl_2 \cdot 2H_2O$, 2.35 g; H_2O q.s. 8.1. The opposite border of the atrium was hooked to a thread attached to an FT-03C Grass force displacement transducer. Sufficient tension was placed on the muscle to obtain maximal responses. A Grass square wave stimulator Model S-6 was set to deliver repetitive rectangular pulses (5-msec duration) at a frequency of 1/sec. The voltage was set at 20% above threshold (*ca.* 1 V). The response was recorded on a Grass polygraph. The tissue was allowed to stabilize for 30 min and the test compounds were administered dissolved in a vehicle of DMSO (0.1–0.5 ml in the bath).

Inhibition of Ouabain-Sensitive Sodium Efflux from Human Red Cells.—Human red cells loaded with ^{24}Na were suspended in physiological medium at 37°. The hematocrit was about 5%. The total and ouabain-sensitive efflux of Na^+ was measured during 40 min in the presence of various test substances.