tallize but was readily converted to a crystalline hydrochloride, which had unusual melting properties: it sintered at 135–136° and remained opaque until it formed a clear melt at 210–215°. The melting properties and elemental analysis suggested identity as **54**·HCl (lit.[26] mp 211° with sintering at 135°), which was supported by ^{1}H NMR analysis. Anal. ($C_8H_{11}NO$·HCl) C, H, N.

Acknowledgment. This investigation was supported by U.S. Army Medical Research and Development Command through Contract No. DA-49-193-MD-2028 and DADA17-69-C-9033. The authors are indebted to Mrs. Martha Thorpe for interpretation of ^{1}H NMR spectral data.

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Antitumor Agents. 16.[1] Steroidal α-Methylene-γ-lactones

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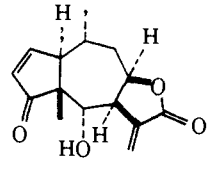
Several novel steroidal α-methylene-γ-lactones and related derivatives have been synthesized as potential steroid alkylating antitumor agents. The synthesis of these compounds involved the convenient Reformatsky-type reaction between ethyl α-(bromomethyl)acrylate and the proper steroidal ketones. In vitro assay for the cytotoxicity of these compounds against the growth of tissue culture cells originating from human epidermoid carcinoma of the larynx (H.Ep.-2) has shown significant activity. Cytotoxicity was improved at least sixfold with the introduction of lipophilic steroidal character. Preliminary in vivo tumor assay also indicated that these compounds were active against Walker 256 carcinosarcoma in rats and were inactive against both L1210 lymphoid leukemia and Ehrlich ascites carcinoma in mice. However, the simple α-methylene-β,β-dicarbethoxy-γ-butyrolactone significantly inhibited Ehrlich ascites tumor growth.

Steroidal alkylating agents, such as phenesterin,[2,3] estradiol mustard,[4–6] and some homoaza steroid mustards,[7] are known for their significant inhibitory activity on growth of various experimental tumors[8] and are currently undergoing clinical trials. During the course of an investigation of the relationship between the sesquiterpene lactone structure and the cytotoxic and antitumor activities, it was found that one of the structural requirements for significant cytotoxicity was an O=C—C=CH_2 system as part of an ester as well as a ketone or lactone.[9–12] This type of system has been observed, for example, in the cytotoxic antitumor agent helenalin (**1**). The ability of the latter system, i.e., an α-methylene-γ-lactone, to act as an alkylating center for the cytotoxic and antitumor sesquiterpene lactones, such as elephantopin, euparotin acetate, and vernolepin, has been demonstrated and ascribed to a rapid Michael-type addition reaction of the biological nucleophiles, such as L-cysteine[13] or sulfhydryl-bearing enzymes, e.g., phosphofructokinase[14] and glycogen synthase.[15] Although syntheses of certain simple α-methylene-γ-lactones and related derivatives as potential antitumor agents have recently been reported,[11,16,17] a survey of the literature[18] revealed no record of any investigation on the synthetic α-methylene-γ-lactone bearing steroids or hormones as alkylating anticancer agents.



1

We decided to combine chemically this α-methylene-γ-

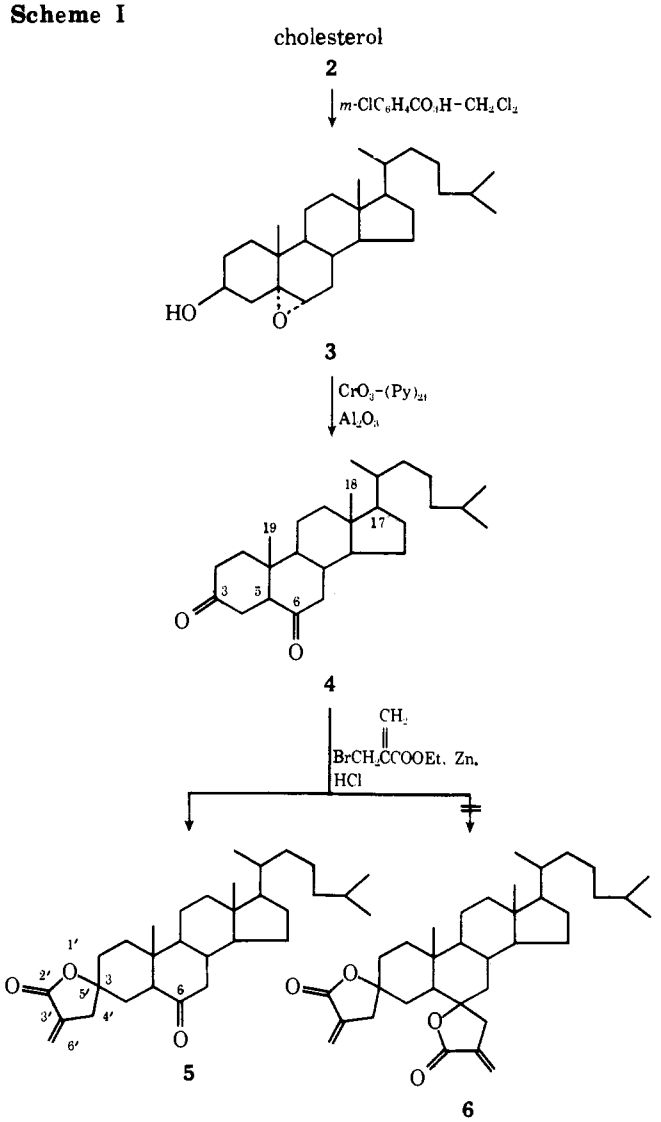
lactone alkylating center with a steroidal or hormonal carrier moiety as a means of obtaining chemotherapeutic agents which might be tumor site specific (e.g., for breast or prostatic cancer). Moreover, since steroidal molecules are known to be lipophilic, it would also be of interest to investigate the effect of the introduction of the lipophilic steroidal moiety upon the cytotoxicity of α-methylene-γ-lactone. The importance of lipophilicity for enhanced cytotoxicity among the naturally occurring sesquiterpene lactones and related derivatives has been demonstrated.[19,20]

Chemistry. The synthesis of α-methylene-γ-lactone bearing bifunctional alkylating steroids or hormones was an initial objective of our efforts since additional alkylating functionalities appear to enhance cytotoxic and antitumor activity significantly.[10,11,19,20] The general approach to the preparation of these steroidal α-methylene-γ-lactones involved a convenient Reformatsky-type reaction between ethyl α-(bromomethyl)acrylate[21] and a cyclohexanone ring of steroids, as reported by Öhler and coworkers[22] as well as Rosowsky and coworkers.[16] Cholestane 5,6α-epoxide (**3**) was prepared from cholesterol (**2**) with *m*-chloroperbenzoic acid according to the literature.[23] Oxidation of **3** with the chromium trioxide–pyridine complex[24] gave, after neutral alumina chromatography, cholestane-3,6-dione (**4**) in 69% yield. Attempted preparation of **4** by an alternative literature method[25] involving the direct oxidation of **2** with sodium dichromate in acetic acid followed by acid isomerization of the resulting intermediate, Δ^4-cholesten-6β-ol-3-one, was unsuccessful. Treatment of **4** with ethyl α-(bromomethyl)acrylate in the presence of activated zinc yielded, after acidic treatment, instead of the bifunctional α-methylene-γ-lactone **6**, the monofunctional lactone **5**. The formation of the α-methylene-γ-lactone ring is assumed to occur at the 3 position of **5** since it is well known that the 3 position is less sterically hindered than the 6 position (Scheme I).

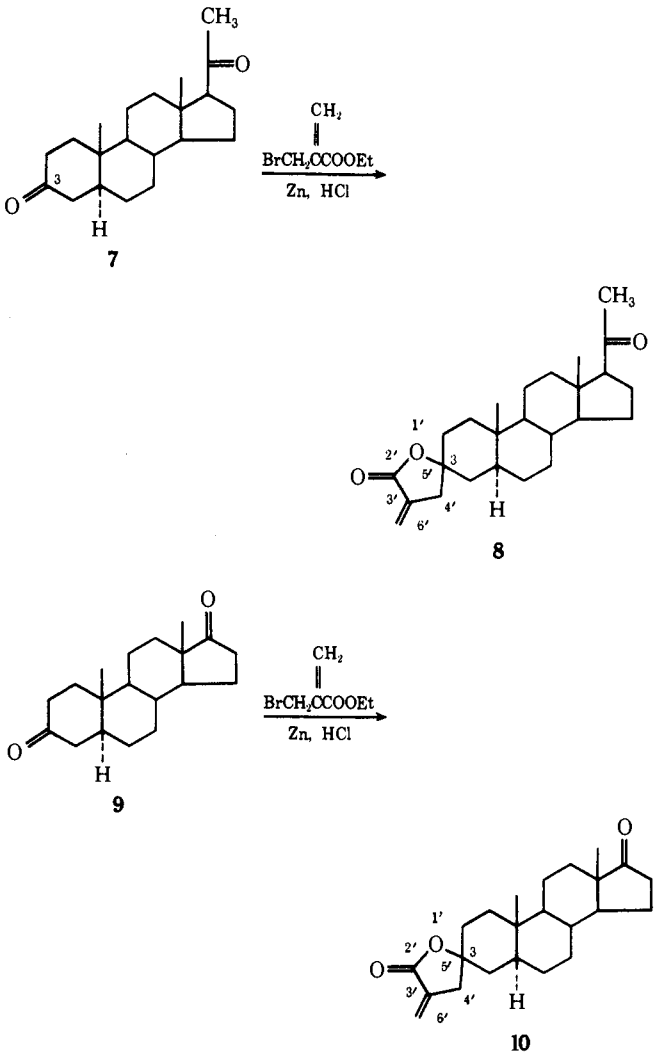
Similar treatment of 5α-pregnane-3,20-dione (**7**) and 5α-androstane-3,17-dione (**9**) with ethyl α-(bromomethyl)acrylate led to the monolactones **8** and **10**,[26,27] respectively (Scheme II). Since no bifunctional α-methylene-γ-lactones could be made at either the 3 and 20, or 3 and 17, positions under these reaction conditions, it was decided to prepare compounds which possess in addition to the essential alkylating center, such as an α-methylene-γ-lactone, an additional conjugated ester side chain, such as a cinnamoyl moiety. This system had been reported previously to enhance the cytotoxicity of the sesquiterpene lactones.[20] 5α-Androstan-17β-ol-3-one (**11**) was esterified with cinnamoyl chloride to yield the corresponding cinnamate (**12**). Compound **12** was then subjected to the foregoing Reformatsky-type reaction to afford the lactone cinnamate **13** (Scheme III).

Other bifunctional analogs related to helenalin (**1**), such as compound **18** which contains in addition to the α-methylene-γ-lactone a β-unsubstituted cyclopentenone ring system, were synthesized according to Scheme IV. 17-Ethy-

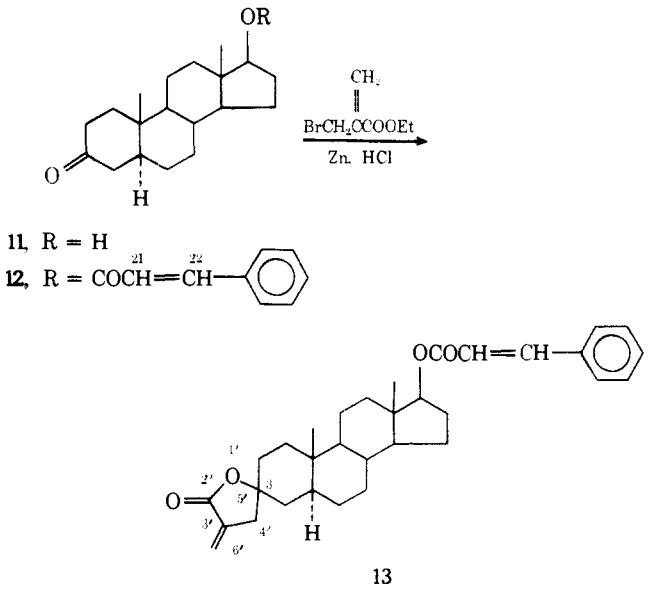
Scheme I



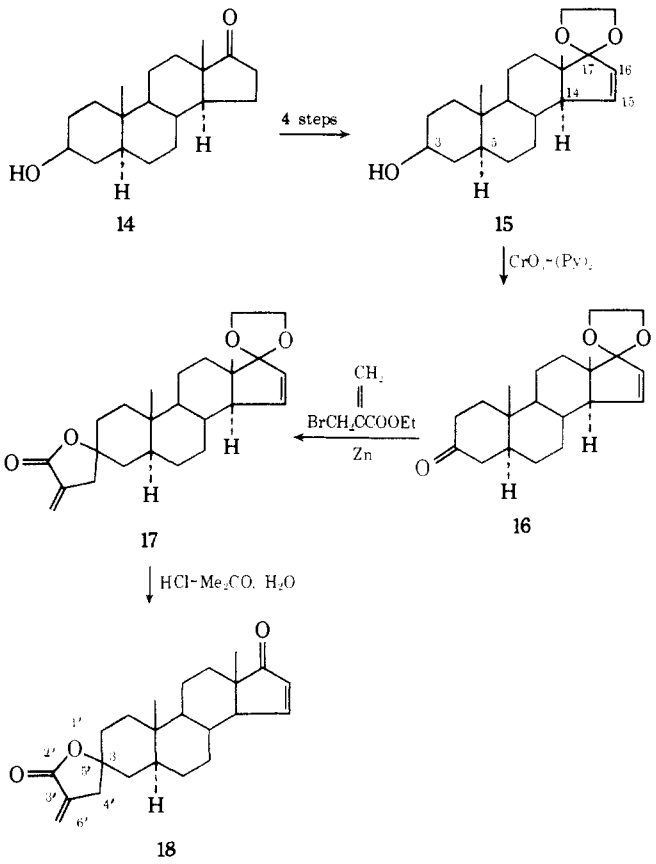
Scheme II



Scheme III



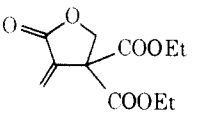
Scheme IV



lenedioxy-5α-androst-15-en-3β-ol (**15**) was synthesized from 5α-androstan-3β-ol-17-one (**14**) by literature methods.[28,29] Compound **15** was oxidized by means of the chromium trioxide–pyridine complex in dichloromethane. Subsequent Reformatsky-type reaction of the resulting ketone **16** with ethyl α-(bromomethyl)acrylate gave the expected ketal lactone **17**. The latter, upon treatment with concentrated hydrochloric acid in acetone, provided the desired bifunctional lactonic cyclopentenone **18** whose spectral data were in accordance with the assigned structure **18**.

Further studies on the structure and stereochemistry of compounds **5**, **8**, **10**, **13**, and **18**, especially the configuration of the spiro linkage of these compounds, are in progress.

The simple α-methylene-β,β-dicarbethoxy-γ-butyrolactone (**19**) was also synthesized according to the method of Piskov[30] for the purpose of structure–activity relationships studies.



19

Biological Results. Compounds prepared in this study were first assayed for their cytotoxicity against the growth of tissue culture cells originating from human epidermoid carcinoma of the larynx (H.Ep.-2) according to a rapid microtiter method previously described.[31] A comparison of the ED_{50} values for the cytotoxicity of the compounds listed in Table I disclosed that all of the steroidal α-methylene-γ-lactones, such as compounds **5**, **8**, **10**, **13**, **17**, and **18**, exhibited approximately equally significant cytotoxicity. Steroids which are lacking the α-methylene-γ-lactone ring (e.g., compounds **3**, **12**, and **14**) were essentially inactive except for compound **7**. An enhancement of the cytotoxicity was not observed in the bifunctional derivative, such as **18**, as well as in the monofunctional derivative with a conjugated ester moiety, such as **13**. However, cytotoxicity was improved at least sixfold with the introduction of the lipophilic steroidal character (e.g., compound **18**) since a simple α-methylene-γ-lactone (e.g., compound **19**) itself was inactive.

As shown in Table II, compounds **1**, **5**, **7**, **8**, **10**, **13**, **17**, **18**, and **19** were also evaluated for experimental antitumor activity against Walker 256 ascites carcinosarcoma in Sprague–Dawley male rats and were found to be all active. Compounds **1**, **5**, **8**, **10**, **13**, **17**, and **18** in the L1210 screen were inactive as antitumor agents. Compounds **1**, **5**, **8**, **10**, and **19** were also screened in Ehrlich ascites carcinoma; however, only **1** and **19** were significantly active (Table III). Further studies on the analogs of **19** are in progress.

Experimental Section

Chemistry. Unless otherwise specified, melting points were determined on a Thomas-Hoover melting point apparatus and were corrected. Ir spectra were determined in chloroform with a Perkin-Elmer 257 grating ir spectrophotometer. NMR spectra were measured in $CDCl_3$ with a Jeolco C-60 HL spectrometer (TMS) and chemical shifts reported in δ (ppm) units: s, singlet; d, doublet; t, triplet; m, multiplet; and the *J* values in hertz. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Silica gel for column chromatography refers to Mallinckrodt SilicAR cc-7 (200–325 mesh); silica gel for preparative TLC refers to Merck silica gel GF-254; and silica gel for the TLC refers to Merck silica gel G developed with chloroform–acetone (3:1) and visualized by spraying with 40% aqueous sulfuric acid and heating. Deactivated neutral alumina refers to neutral alumina AG-7 (100–200 mesh), Bio-Rad, activity grade III. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga.

Cholestane-3,6-dione (4). To a slurry of CrO_3–$(Py)_2$ (9.0 g, 25 mmol) in CH_2Cl_2 (150 ml), under N_2, was added 1.0 g (2.5 mmol) of cholestane 5,6α-epoxide (**3**). The mixture immediately turned brown and precipitated as a tar. TLC indicated complete reaction after 45 min. The mixture was extracted with CH_2Cl_2 and the CH_2Cl_2 extract was washed with saturated $NaHCO_3$, 5% HCl, and H_2O, dried (anhydrous Na_2SO_4), and evaporated under vacuum to yield a yellowish oil. This oil was chromatographed on neutral alumina (3 × 40 cm, grade I) and the column was eluted with benzene to give a colorless crystalline residue (700 mg, 69%) which was recrystallized from EtOH to afford **4**: mp 169–170° (lit.[25] mp 172°); ir (KBr) 1715 cm^{-1} (double strength C=O).

Spiro[(cholestan-6-one)-3,5'-(2'-oxo-3'-methylenetetrahydrofuran)] (5). Cholestane-3,6-dione (**4**, 1.0 g, 2.4 mmol) was dis-

Table I. Cytotoxicity and Physical Constants of Steroidal α-Methylene-γ-lactones and Related Compounds

No.	Formula	Analyses[a]	Mp, °C	Recrystn solvent	ED_{50},[b] μg/ml (H. Ep. - 2)
1	$C_{15}H_{18}O_4$		170–172[c]	Benzene	0.10
3	$C_{27}H_{46}O_2$		144–145[d]	H_2O–Me_2CO	11.00
5	$C_{31}H_{48}O_3$	C, H	184–186	Et_2O	1.35
7	$C_{21}H_{32}O_2$	*e*			0.84
8	$C_{25}H_{37}O_3$	C, H	153–155	CH_2Cl_2–Et_2O	0.89
10	$C_{23}H_{32}O_3$	C, H	183–185	CH_2Cl_2–Et_2O	0.58
12	$C_{28}H_{36}O_3$	C, H	198–200	CH_2Cl_2–Et_2O	8.62
13	$C_{32}H_{40}O_4$	C, H	250–252	CH_2Cl_2–Et_2O	1.55
14	$C_{19}H_{30}O_2$	*e*			>10.00
16	$C_{21}H_{30}O_3$	C, H	201	MeOH	
17	$C_{25}H_{34}O_4$	C, H	200–202	Et_2O–MeOH	1.65
18	$C_{23}H_{30}O_3$	*f*	*g*		1.74
19	$C_{11}H_{14}O_6$		*g*		>10.00

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [b] The values of ED_{50} are used for expressing the potency of cytotoxicity which is the calculated effective dose that inhibits the net cell growth to 50% of control growth. [c] See ref 32. [d] Lit.[23] reported mp 141–143°. [e] Sigma Chemical Co., St. Louis, Mo. [f] *m/e* 354.3192 ($C_{23}H_{30}O_3$). [g] Oil.

Table II. Effects of Steroidal α-Methylene-γ-lactones and Related Compounds on Inhibition of Walker 256 Ascites Tumor Growth

Compound	N[c]	Days survived	T/C[a]
0.05% Tween 80	6	7.8 ± 0.5	
1	6	26.3	316
5	6	11.6	139
7	5	12.4	159
8	5	13.0	166
10	6	12.8	154
13	5	11.2	144
17	5	11.0	141
18	5	12.4	159
19	6	11.7	140
Melphalan[b]	5	23.0	338

[a] A compound is active if it exhibits a T/C of ≥125%.[35] [b] Wellcome Research Laboratories, Research Triangle Park, N.C. [c] *N* is the number of animals per group.

Table III. Effects of Steroidal α-Methylene-γ-lactones and Related Compounds on Inhibition of Ehrlich Ascites Tumor Growth

Compound	N[c]	Survival at 7th day	Ascitocrit	Vol, ml	% inhibn
0.05% Tween 80	6	5/6	30[a]	4.1[a]	
1	6	4/6	0	0.1	99
5	6	3/6	28	5.0	
8	6	6/6	22	4.6	15
10	6	6/6	26	4.4	18
19	6	6/6	2	0.8	98
6-Mercaptopurine[b]	6	6/6	2	0.4	99

[a] Standard deviation on the control ascitocrit was 11.5 and on the volume was 1.2. [b] Sigma Chemical Co. [c] *N* is the number of animals per group.

solved in dry THF (30 ml, freshly distilled from $LiAlH_4$). To this was added activated zinc powder (200 mesh, 211 mg, 3.2 mmol) and ethyl α-(bromomethyl)acrylate (625 mg, 3.2 mmol).[33] The mixture was heated at reflux for 10 hr,[34] cooled, and poured into ice-cold HCl (150 ml) and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (anhydrous Na_2SO_4), and evaporated in vacuo to yield a yellow crystalline residue. Recrystallization from Et_2O furnished 200 mg (17%) of pure **5**: mp 184–186°; ir (KBr) 1753 (γ-lactone) and 1710 cm^{-1} (C=O); NMR 6.24 (1 H, m, H-6′), 5.62 (1 H, m, H-6′), and 2.72 (2 H, m, H-4′).

Spiro[(5α-pregnane)-3,5′-(2′-oxo-3′-methylenetetrahydrofuran)] (8). A mixture of 5α-pregnane-3,20-dione (**7**, 316 mg, 1 mmol), ethyl α-(bromomethyl)acrylate (193 mg, 1 mmol),[33] activated zinc powder (130 mg, 2 mmol), and a trace of *p*-hydroquinone (2 mg) in dry THF (5 ml) was refluxed overnight (16 hr) and cooled. The reaction mixture was poured into 20 ml of ice-cold dilute HCl and extracted with $CHCl_3$ (30 ml). The $CHCl_3$ extract was washed with H_2O, dried, and evaporated in vacuo to give a viscous residue which was purified by preparative TLC [silica gel, benzene–EtOAc (4:1)] to furnish 110 mg (28%) of **8**: mp 153–154°; ir (Nujol) 1753 (γ-lactone), 1713 (C=O), 1668 cm^{-1} (C=C); NMR 6.22 (1 H, t, J = 2.5 Hz, H-6′), 5.60 (1 H, t, J = 2.5 Hz, H-6′), 2.70 (2 H, t, J = 2.5 Hz, H-4′), 2.10 (3 H, s, $COCH_3$), 0.80 (3 H, s, H-19), and 0.60 (3 H, s, H-18).

Spiro[(17-oxo-5α-androstane)-3,5′-(2′-oxo-3′-methylenetetrahydrofuran)] (10). A mixture of 5α-androstane-3,17-dione (**9**, 576 mg, 2 mmol), ethyl α-(bromomethyl)acrylate (390 mg, 2 mmol), activated zinc powder (130 mg, 2 mmol), and *p*-hydroquinone (50 mg) in dry THF (10 ml) was refluxed overnight (16 hr) and cooled. The reaction mixture was worked up in an analogous manner as described for the conversion of **7** to **8** to yield, after recrystallization from CH_2Cl_2–Et_2O, 325 mg (45%) of **10** as colorless needles: mp 183–185°; ir 1758 (γ-lactone), 1740 (C=O), 1670, 1625, and 1610 cm^{-1} (C=C); NMR 6.28 (1 H, t, J = 2.5 Hz, H-6′), 5.65 (1 H, t, J = 2.5 Hz, H-6′), 2.70 (2 H, t, J = 2.5 Hz, H-4′), 0.88 (3 H, s, H-19), and 0.85 (3 H, s, H-18).

17β-Cinnamoyl-5α-androstan-3-one (12). A mixture of 5α-androstan-17β-ol-3-one (**11**, 115 mg, 0.39 mmol) and cinnamoyl chloride (100 mg, 0.6 mmol) in 10 ml of dry benzene was refluxed for 16 hr. After cooling, the reaction mixture was diluted with Et_2O (40 ml), washed with 5% $NaHCO_3$ and H_2O, dried, and evaporated to give an oily residue. Chromatography of this residue on deactivated neutral alumina by use of benzene as an eluent gave **12** as colorless crystals in quantitative yield after one recrystallization from CH_2Cl_2–Et_2O: mp 198–200°; ir 1712 (C=O), 1648 (C=C), 1610, 1588, 1507 cm^{-1} (aromatic C=C); NMR 7.30–7.70 (5 H, m, aromatic protons), 7.75 (1 H, d, $J_{21,22}$ = 16.5 Hz, H-22), 6.45 (1 H, d, $J_{21,22}$ = 16.5 Hz, H-21), 1.02 (3 H, s, H-19), and 0.90 (3 H, s, H-18).

Spiro[(17β-cinnamoyl-5α-androstane)-3,5′-(2′-oxo-3′-methylenetetrahydrofuran)] (13). A mixture of compound **12** (100 mg, 0.24 mmol), ethyl α-(bromomethyl)acrylate (48 mg, 0.24 mmol), activated zinc powder (17 mg, 0.24 mmol), *p*-hydroquinone (2 mg), and dry THF (10 ml) was refluxed overnight (16 hr) and cooled. The reaction mixture was worked up in the exact manner described for compound **8**. The product formed colorless needles (**13**, 70 mg, 60%) after one recrystallization from CH_2Cl_2–Et_2O: mp

250–252°; ir 1758 (γ-lactone), 1713 (C=O), 1672, 1648 (C=C), 1588, 1518 and 1508 cm^{-1} (aromatic); NMR 7.30–7.60 (5 H, m, aromatic), 7.71 (1 H, d, $J_{21,22}$ = 16.5 Hz, H-22), 6.45 (1 H, d, $J_{21,22}$ = 16.5 Hz, H-21), 6.21 (1 H, t, J = 2.5 Hz, H-6'), 5.60 (1 H, t, J = 2.5 Hz, H-6'), 2.65 (2 H, t, J = 2.5 Hz, H-4'), 0.87 (3 H, s, H-19), and 0.82 (3 H, s, H-18).

17-Ethylenedioxy-5α-androst-15-en-3-one (16). A mixture of 17-ethylenedioxy-5α-androst-15-en-3β-ol (**15**, 1.3 g, 4.5 mmol), CrO_3-$(Py)_2$ (13 g, 50.4 mmol), and CH_2Cl_2 (100 ml) was stirred at room temperature. After 6 min, the mixture was filtered and the filtrate was washed with H_2O, dried, and evaporated to yield a residue. This was chromatographed in CH_2Cl_2 on alumina (Brockmann, grade 7) and elution with the same solvent gave **16** as colorless needles (1.0 g, 77%) after one recrystallization from MeOH: mp 201°; ir 1710 (C=O) and 1630 cm^{-1} (C=C); NMR 6.22 (1 H, dd, $J_{15,16}$ = 6.0 Hz, $J_{14,16}$ = 1.5 Hz, H-16), 5.77 (1 H, q, $J_{15,16}$ = 6.0 Hz, $J_{14,15}$ = 3.0 Hz, H-15), 3.95 (4 H, m, $-OCH_2CH_2O-$), 1.05 (3 H, s, H-19), and 0.95 (3 H, s, H-18).

Spiro[(17-ethylenedioxy-5α-androst-15-ene)-3,5'-(2'-oxo-3'-methylenetetrahydrofuran)] (17). To a mixture of compound **16** (940 mg, 2.9 mmol) and activated zinc powder (247 mg, 3.8 mmol) in dry THF (20 ml) was added dropwise a solution of ethyl α-(bromomethyl)acrylate (728 mg, 3.8 mmol) in THF (15 ml). The reaction mixture was heated under reflux for 2 hr. After cooling, it was filtered and the filtrate was evaporated under reduced pressure. The residue was extracted with Et_2O. The Et_2O extract was washed with H_2O, dried, and evaporated to afford a colorless oil (850 mg). Chromatography of this oil over silica gel (30 g) using $CHCl_3$ as eluent followed by one recrystallization from Et_2O–MeOH (1:3) afforded pure **17** as colorless needles (600 mg, 53%): mp 200–202°; ir 1750 (γ-lactone), 1670, 1630 and 1595 cm^{-1} (C=C); NMR 6.25 (1 H, t, J = 2.5 Hz, H-6'), 6.15 (1 H, overlapped dd, $J_{15,16}$ = 6.0 Hz, $J_{14,16}$ = 1.5 Hz, H-16), 5.68 (1 H, overlapped q, $J_{14,15}$ = 3.0 Hz, $J_{15,16}$ = 6.0 Hz, H-15), 5.61 (1 H, t, J = 2.5 Hz, H-6'), 3.93 (4 H, m, $-OCH_2CH_2O-$), 2.68 (2 H, t, J = 2.5 Hz, H-4'), 0.92 (3 H, s, H-19), and 0.85 (3 H, s, H-18).

Spiro[(17-oxo-5α-androst-15-ene)-3,5'-(2'-oxo-3'-methylenetetrahydrofuran)] (18). A solution of compound **17** (220 mg, 0.58 mmol), Me_2CO (10 ml), H_2O (2 ml), and concentrated HCl (1 ml) was allowed to stand at room temperature. After 10 hr, the solution was further refluxed for 10 hr and then evaporated in vacuo. The product was extracted with Et_2O and the Et_2O extract was washed with H_2O, dried (anhydrous Na_2SO_4), and evaporated. The residual oil was purified by preparative TLC (silica gel, developed with $CHCl_3$) to yield the pure **18** as a colorless oil (40 mg, 20%): ir 1755 (γ-lactone), 1705 (cyclopentenone C=O), 1670, 1630, and 1595 cm^{-1} (C=C); NMR 7.78 (1 H, dd, $J_{15,16}$ = 6.0 Hz, $J_{14,15}$ = 2.5 Hz, H-15), ~6.29 (1 H, overlapped m, H-16), 6.23 (1 H, overlapped t, J = 2.5 Hz, H-6'), 5.62 (1 H, t, J = 2.5 Hz, H-6'), 2.69 (2 H, t, J = 2.5 Hz, H-4'), 1.10 (3 H, s, H-19), and 0.79 (3 H, s, H-18). Found for M^+, 354.2192; $C_{23}H_{30}O_3$ requires 354.2195.

In Vivo Tumor Screens. In the Walker 256 ascites carcinosarcoma screen, 10^6 tumor cells were implanted in 80 ± 10 g Sprague–Dawley male rats. Test compounds dissolved in 0.05% Tween–H_2O were injected ip at 2.5 mg/kg/day and the day of death was recorded. Treated/control (T/C) values were calculated according to NIH protocol 1.500.[35]

In the Ehrlich ascites screen, 10^6 cells were implanted on day 0. Test compounds were suspended in 0.05% Tween 80–water and homogenized to obtain a fine suspension. Each compound was injected ip, twice a day (0.5 mg/dose), 33.3 mg/kg/day, to CF_1 male mice (~30 g). On the seventh day the mice were sacrificed and the total volume of the ascites tumor and packed cell volume (ascrit) was determined in order to calculate the percent inhibition.[36]

The L1210 lymphoid leukemia screen (see protocol 1:100 of ref 35) was carried out in DBA/2 male mice (~20 g) who were maintained on 0.5 g/16 oz of cosa terramycin (Pfizer) in the drinking water to deter infection. In this screen 10^5 cells were implanted on day 0. Drugs were administered ip at 15 mg/kg/day. T/C values were calculated from the survival times.[35] 6-Mercaptopurine was used as the internal standard in the Ehrlich and L1210 screens and melphalan in the Walker 256 screen.

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7α-Carboalkoxy Steroidal Spirolactones as Aldosterone Antagonists

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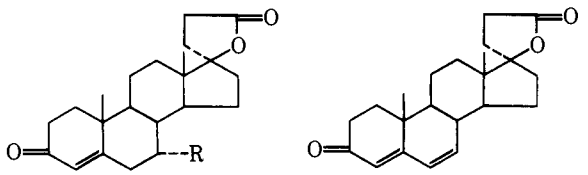
A variety of esters of 17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic acid γ-lactone (**7a**) was synthesized in a sequence using the corresponding 3-oxo-4,6-diene (**2**) as starting material. The methyl (**5**), ethyl (**7c**), and isopropyl (**7e**) esters as well as the C-1 unsaturated methyl ester (**8a**) showed good oral and subcutaneous activity (MED ≤ 0.71 mg) as DCA antagonists in the adrenalectomized rat. The corresponding potassium salts of the opened lactone **10a,b,d** and **11a** appeared to have slightly increased oral potency under these test conditions (MED ≤ 0.41 mg). Some general observations on structure–activity relationships are made.

Aldosterone, synthesized in the adrenal cortex, is a potent mineralocorticoid which plays an important role in regulating the electrolyte composition of the body fluid by promoting the excretion of potassium and retention of sodium ions. An excess of this hormone is observed in such edematous states as congestive heart failure, nephrosis, and cirrhosis of the liver, as well as in primary aldosteronism.[1]

It is well known that many steroids which possess a spirolactone side chain at C-17 are aldosterone antagonists.[2,3] Principal examples of this class of steroid are spironolactone (**1a**, Aldactone) and canrenone (**2**). It is in the treatment of the above-mentioned disorders that spirolactones have their therapeutic value. In addition, spironolactone (**1a**) is receiving increased attention as a hypotensive drug in the treatment of essential hypertension[4] even though excess aldosterone is not directly observed.

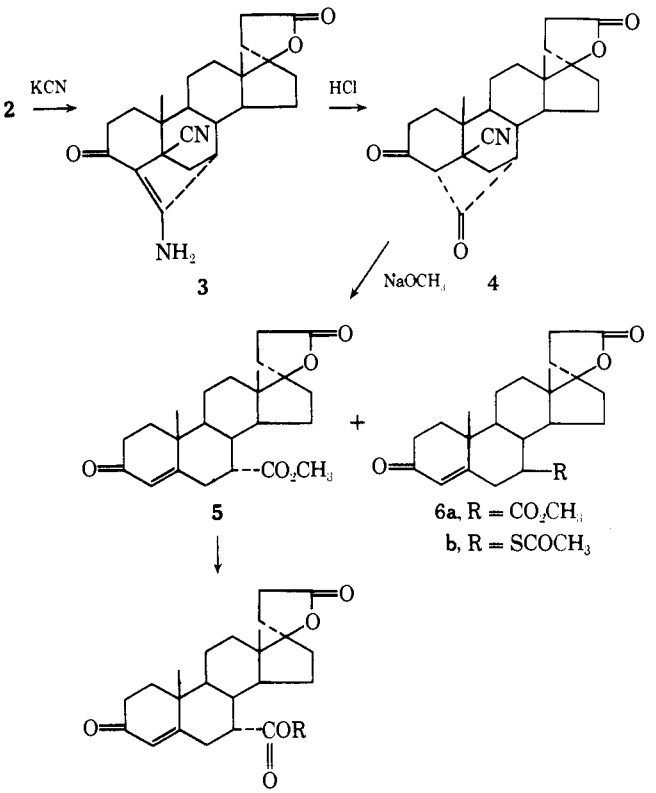


1a, R = SAc
b, R = H

2

In the search for more potent analogs of spironolactone (**1a**), it has been found that introduction of a carboalkoxy function in the 7α position imparts to the steroidal spirolactone good antimineralocorticoid activity and provides a new series of antagonists. This paper describes the synthesis and diuretic activities of these compounds.

Chemistry. The synthetic sequence utilized canrenone (**2**)[3a] as the starting material. It was converted to the methyl ester **5** according to the sequence used earlier by Christiansen and Johnson[5] on Δ^6-testosterone. Treatment of **2** with excess KCN in refluxing aqueous methanol buffered with ethyl acetate gave the steroidal aminomethylidyne compound **3** in yields up to 41%. Although the yield of **3** is modest, and despite the fact that there are several other products in the reaction mixture, the basic nature of **3** allows it to be easily isolated by extraction of the crude reaction product with dilute HCl. Treatment of **3** with dilute HCl on the steam bath under heterogeneous reaction conditions gave the diketone **4**, generally in yields greater than 90%. The diketone **4** was cleaved with sodium methoxide in refluxing methanol to give two compounds. The first and major compound was isolated by direct crystallization and was assigned the structure of the 7α isomer **5**. The second product was isolated in only small yield after chromatogra-



7a, R = H
b, R = CO_2-*i*-C_4H_9
c, R = C_2H_5
d, R = *n*-C_3H_7
e, R = *i*-C_3H_7
f, R = *n*-C_4H_9
g, R = *i*-C_4H_9
h, R = c-C_5H_9
i, R = $CH_2CH{=}CH_2$
j, R = *i*-C_6H_{13}
k, OR = SC_2H_5