stirred at 5 °C for 2 h; then the CH_2Cl_2 and two additional 175-ml charges of CH_2Cl_2 were distilled from the reaction. $AlCl_3$ (80 g, 0.60 mol) and CH_2Cl_2 (200 ml) were added to the reaction which was warmed slowly to reflux, refluxed for 2.5 h, then quenched in ice (600 g) and concentrated HCl (75 ml), extracted with Et_2O (800 ml), washed with H_2O, back extracted into 5% NaOH (3 × 100 ml), and acidified with HCl. The product was filtered, rinsed with water, dried, and used in step B without further purification.

Step B. (2,3-Dichloro-4-acetylphenoxy)acetic Acid. In an N_2 atmosphere Na (3.79 g, 0.165 g-atom) was dissolved in EtOH (450 ml). 2,3-Dichloro-4-acetylphenol (30.75 g, 0.15 mol) and ethyl bromoacetate (30.06 g, 0.18 mol) were added; the reaction was refluxed for 2 h, heated with 5% KOH (350 ml), and refluxed an additional hour, and the EtOH was distilled. The aqueous solution was acidified with HCl, extracted into Et_2O (4 × 300 ml), and dried (Na_2SO_4), and the Et_2O was evaporated to dryness. Recrystallization from xylene (500 ml) gave 32.2 g (85%) of the product: mp 154–156 °C. Anal. ($C_{10}H_8Cl_2O_4$) C, H, Cl.

Step C. [2,3-Dichloro-4-(1-hydroxyethyl)phenoxy]acetic Acid. To a stirred, ice-cooled suspension of (2,3-dichloro-4-acetylphenoxy)acetic acid (10.5 g, 0.04 mol) in H_2O (350 ml) was added a solution of KBH_4 (4.0 g, 0.074 mol) in H_2O (200 ml) over a period of 1 h. The reaction mixture was acidified with HCl and the product was collected and recrystallized: mp 145–147° (from H_2O). Anal. ($C_{10}H_{10}Cl_2O_4$) C, H, Cl.

Step D. Compound 4. Absolute EtOH (4.6 g) in a round-bottomed flask was cooled in a dry ice bath and then treated with P_2O_5 (5.7 g). The flask was warmed slightly and [2,3-dichloro-4-(1-hydroxyethyl)phenoxy]acetic acid (5.3 g) was added. The reaction mixture was heated 3 h on a steam bath and treated with ice water, the product was extracted into Et_2O and dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by recrystallization. Anal. ($C_{10}H_8Cl_2O_3$) C, H, Cl.

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Synthesis and Antimineralocorticoid Activities of Some 7α-Cyano and 7α-Alkoxycarbonylamino Steroidal Spirolactones

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The synthesis and antimineralocorticoid potencies of several steroidal spirolactones bearing novel nitrogenous substituents in the 7α position are reported. These substituents include the cyano, the isocyanato, and the alkoxycarbonylamino groupings. The nitrile **1b** and the *N*-carbomethoxy compound **1h** showed good antimineralocorticoid potency (MED ≤ 0.79 mg) on subcutaneous administration to adrenalectomized rats.

We have recently reported[1] that substitution of a carboalkoxy function in the 7α position of the steroidal spirolactone (e.g., **1a**) yielded a series of compounds possessing strong antimineralocorticoid potency on both subcutaneous and oral administration to adrenalectomized rats. This finding prompted us to survey the effects on potency brought about by other types of functionality in this position. This communication describes the synthesis and antimineralocorticoid potencies of those structures bearing the cyano, the isocyanato, and the alkoxycarbonylamino groups in this 7α position. Although our earlier publication reported only esters at this position, the substituents reported herein are all nitrogen bearing. In addition, the isocyanato and the cyano groups have a linear geometry and, thus, depart quite drastically from the branched-type substituents heretofore described.

Synthesis. Dienones **2a** and **2b**[2] served as convenient starting materials for the synthesis of the 7α-cyano compounds in the normal and 19-nor series, respectively. Michael addition to this system was effected by treating these steroids with 1 equiv of KCN in the presence of 1 equiv of HOAc in aqueous 98% Me_2SO on the steam bath. This reaction was run in a pressure bottle to prevent the escape of any HCN generated during the course of the reaction. Although this procedure gave only modest to poor yields of **1b** (38%) and **1c** (17%), the use of only 1 equiv of KCN effectively suppressed the formation of any bis adduct previously noted in this type of system.[1,3] A minimum amount of H_2O and only 1 equiv of HOAc were employed to minimize the possibility of any hydrolysis of the nitrile group during the reaction.[4]

The stereochemistry of the cyano group in **1b** was determined by its NMR and CD spectra according to the methods described earlier.[1] The NMR spectrum shows the equatorial proton on C-7 as a complex multiplet centered at about 3.03 ppm. This represents a shift of approximately 0.2 ppm downfield from the chemical shift of the C-7 proton in the 7α-carboalkoxy series but such a shift is not unreasonable because of the greater electronegativity of –CN relative to $-CO_2R$.[5] Were this proton in an axial position as in the epimeric 7β-cyano compound, the C-7 proton would be expected to appear at higher field.[6]

The CD spectrum of **1b** showed it to have a molecular ellipticity (θ) of –2389°. Like **1a**, this value is considerably more positive than θ for **1d** (–4110°)[1] and, therefore, according to the octant rule, the stereochemistry of this substituent is clearly α.[7]

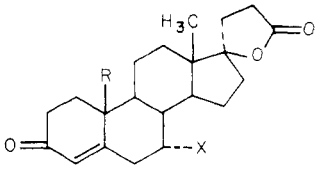
Two derivatives of **1b** were also synthesized. The C-1 unsaturated nitrile **4** was prepared by treatment of **1b** with dichlorodicyanobenzoquinone in refluxing benzene. For purposes of oral administration, the water-soluble potassium salt of the γ-hydroxy acid corresponding to **1b** was also prepared (**3**).

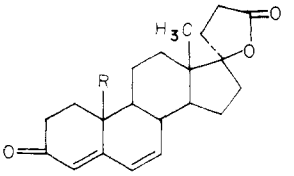
The isocyanate **1g** was synthesized from the previously reported mixed anhydride **1e**[1] by treatment of this

Table I. DCA Blocking Potencies

Compd	MED[a] sc[b]	ig[c]
1a[e]	0.33	0.71
1j[f]	0.33	0.48
1b	0.39	>2.4
1c	1.3	>2.4
1g	>2.4	>2.4
1h	0.79	2.0
1i	>2.4	>2.4
3[d]		2.3
4	2.3	>2.4

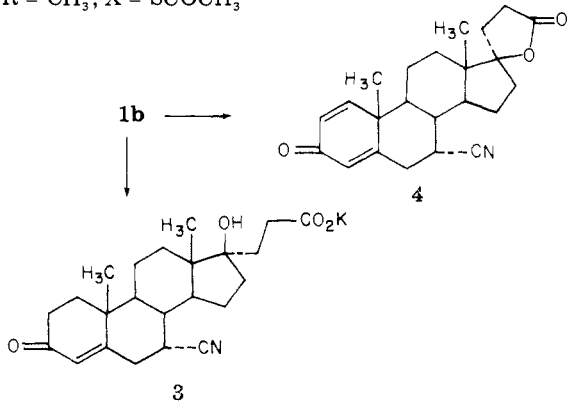
[a] Median effective dose (mg/rat) necessary for 50% inhibition of urinary electrolyte effects induced by administration of DCA. Rats weighed 150–200 g. See text. [b] Subcutaneous administration. [c] Intragastric administration. [d] Administered in saline solution. All other compounds were administered in corn oil. [e] See ref 1. [f] See ref 6.





1a, R = CH_3; X = CO_2CH_3
b, R = CH_3; X = CN
c, R = H; X = CN
d, R = CH_3; X = H
e, R = CH_3; X = CO_2CO_2-*i*-Bu
f, R = CH_3; X = CON_3
g, R = CH_3; X = NCO
h, R = CH_3; X = $NHCO_2CH_3$
i, R = CH_3; X = $NHCO_2C_2H_5$
j, R = CH_3; X = $SCOCH_3$

2a, R = CH_3
b, R = H



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compound with NaN_3 in aqueous acetone at 0°. The resulting azide **1f** was unstable and samples of it were always contaminated with isocyanate **1g**. Consequently, this material was converted completely to **1g** by pyrolysis in boiling benzene. Isocyanate **1g** was in turn converted to the methyl (**1h**) and ethyl (**1i**) carbamates by treatment with MeOH and EtOH, respectively.

Biological Data. The compounds were assayed in a 4-h test in groups of four adrenalectomized rats, each animal being treated subcutaneously with 12 μg of deoxycorticosterone acetate (DCA) and 2.5 ml of isotonic saline solution prior to administration of the test compound.[8] The median effective dose (MED) for anti-DCA activity was established by determining the dosage (mg/rat) necessary for 50% inhibition of urinary electrolyte effects (i.e., increase in Na–K ratio) of administered DCA. Test results for both the spirolactones and the potassium salt are shown in Table I.

Only compounds **1b** and **1h** exhibited reasonable potency on subcutaneous (sc) administration under these test conditions. At least in the case of **1b**, this potency level approached that observed for both the 7α-carbomethoxy compound **1a**[1] and spironolactone **1j**.[9] However, on intragastric (ig) administration at the standard level of 2.4 mg/rat, no activity was observed with **1b** and only weak activity was seen with **1h**. Compound **1b** was also converted to the potassium salt of the corresponding hydroxy acid **3** and to its C-1 unsaturated analogue **4**, but neither modification resulted in any significant improvement in oral potency. Indeed, in the case of **4**, the potency on sc administration was diminished as well. The 19-nor analogue of **1b**, namely compound **1c**, was less potent than **1b** on sc administration.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Unless otherwise noted, NMR spectra were taken in $CDCl_3$ on a Varian A-60A or a Varian T-60 spectrometer using tetramethylsilane as an internal standard. Ultraviolet spectra were obtained in MeOH on a Beckman DK-2A. Infrared spectra were obtained in $CHCl_3$ on a Beckman IR-12. Optical rotations are specific rotations taken in $CHCl_3$ on a Perkin-Elmer Model 141 polarimeter. ORD–CD curves were obtained in dioxane on a Durrum-Jasco J-20 ORD/CD spectropolarimeter. Potassium analyses were obtained by atomic absorption spectrophotometry on a Perkin-Elmer instrument, Model 403. Other elemental analyses were also obtained on a Perkin-Elmer instrument, Model 240.

7α-Cyano-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic Acid γ-Lactone (1b). A mixture of dienone **2a**[2] (19.99 g, 58.7 mmol) and KCN (4.28 g, 65.7 mmol) in aqueous 98% Me_2SO (200 ml) was treated with 2.5 ml of glacial HOAc (2.63 g, 43.8 mmol) and allowed to stand at room temperature overnight in a pressure bottle. The dark-colored mixture was then heated on the steam bath for 2 h and after standing another 24 h at room temperature was poured onto ice water to give a brown solid. This precipitate was filtered, washed with H_2O by decantation, and air-dried on the filter to give a tan solid. After washing again with a minimum amount of cold MeOH to remove some of the brown color, it was recrystallized from MeOH to give 9.43 g of crude 1b. A second recrystallization gave 8.13 g (37.7%), mp 228–230°. An analytical sample was prepared by further recrystallization of a sample of this material from MeOH: mp 228–230°; λ_{max} 235 nm (ε 15350); $[\alpha]_D$ +71° (*c* 1.008); $[\alpha]_{365}$ +21° (*c* 1.008); ν 1680, 1768, 2240 cm^{-1}; NMR 5.88 (C-4), 3.03 (C-7), 1.22 (C-19), and 1.00 ppm (C-18). Anal. ($C_{23}H_{29}NO_3$) C, H, N.

7α-Cyano-17-hydroxy-3-oxo-19-nor-17α-pregn-4-ene-21-carboxylic Acid γ-Lactone (1c). A mixture of dienone **2b** (3.91 g, 12 mmol) and KCN (840 mg, 12.9 mmol) in aqueous 98% Me_2SO (30 ml) was treated with 0.75 ml of glacial HOAc (790 mg, 13.2 mmol) in a pressure bottle. After standing at room temperature for 18 h, the reaction was heated on the steam bath for 7 h.

The dark brown solution was poured onto ice to give a tan precipitate which in turn was washed with water four times by decantation and dried on the steam bath to give 3.38 g of a crude brown solid. TLC (50% EtOAc–C_6H_6) showed this material to consist of starting material (minor) and a new more polar product (major). This material was treated with decolorizing carbon in boiling MeOH and on concentration 1.07 g of a gray precipitate was obtained. Recrystallization of this material from MeOH gave 0.71 g (16.8%) of analytically pure 1c: mp 239–244°; $[\alpha]_D$ +20° (*c* 1.000); $[\alpha]_{365}$ −272° (*c* 1.000); λ_{max} 233 nm (ε 17000); ν 1765, 1670, 2240, and 1622 cm^{-1}; NMR 5.98 (C-4), 3.07 (C-7), and 1.02 ppm (C-18). Anal. ($C_{22}H_{27}NO_3$) C, H, N.

Potassium 7α-Cyano-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylate (3). A slurry of analytically pure 1b (0.73 g, 1.99 mmol) in MeOH (40 ml) was treated with 2.7 ml of 0.73 N KOH solution. The resultant mixture was warmed gently on the steam bath to effect dissolution and then let stand at room temperature overnight under nitrogen.

The yellow reaction solution was heated at 40–50° for 40 min. Removal of the solvent in vacuo yielded a yellow gum that solidified on scratching under ethyl ether. This solid was filtered and air-dried to give 0.81 g (96%) of **3** as a yellow powder: λ_{max}

241 nm (ε 14800); ν (KBr) 2240, 1680, and 1585 cm^{-1}; NMR (D_2O) 5.93 (C-4), 3.28 (C-7), 1.23 (C-19), 0.90 ppm (C-18). Anal. ($C_{23}H_{30}NO_4K$) K.

7α-Isocyanato-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic Acid γ-Lactone (1g). To a stirred, cold (0°) solution of anhydride **1e** (5.30 g, 10.9 mmol) in anhydrous acetone (100 ml) was added a solution of NaN_3 (2.3 g, 35.4 mmol) in H_2O (15 ml). A white precipitate formed rapidly and the mixture was stirred at 0° for 30 min. The reaction was concentrated in vacuo and filtered to give 2.7 g of a white solid whose infrared spectrum showed it to consist of a mixture of the acid azide and the corresponding isocyanate. This solid was dissolved in benzene (150 ml) and the solution refluxed with stirring under nitrogen for 1 h. Concentration of the reaction solution in vacuo gave a crystalline solid which was recrystallized twice from ethyl ether to give 1.2 g (29%) of **1g**, mp 158–160°. An analytical sample was obtained by one further recrystallization from ethyl ether: mp 161–162°; ν 2280, 1779, 1680, and 1628 cm^{-1}; $[\alpha]_D$ +38° (*c* 1.000); $[\alpha]_{365}$ −115° (*c* 1.000); NMR 5.95 (C-4), 3.81–4.03 (C-7), 1.22 (C-19), 1.00 ppm (C-18). Anal. ($C_{23}H_{29}NO_4$) C, H, N.

7α-Cyano-17-hydroxy-3-oxo-17α-pregna-1,4-diene-21-carboxylic Acid γ-Lactone (4). A solution of 1b (3.68 g, 10 mmol) and dichlorodicyanobenzoquinone (2.72 g, 12 mmol) in benzene (130 ml) was refluxed with stirring for 26 h. Solvent was removed in vacuo and the red residue was dissolved in CH_2Cl_2 (400 ml). The organic layer was extracted six times with 2% aqueous Na_2SO_3 solution and twice with saturated NaCl solution and dried (Na_2SO_4, $MgSO_4$). The solvent was removed in vacuo to give 2.3 g of a light yellow foam which was recrystallized from MeOH to give 1.37 g (37%) of analytically pure **4**: mp 258–263°; λ_{max} 241 nm (ε 17700); $[\alpha]_D$ +45° (1.000); $[\alpha]_{365}$ +129° (1.000); ν 1779, 1678, 1636, and 2245 cm^{-1}; NMR 7.09 (d, J = 11 Hz, C-1), 6.29 (d, J = 11 Hz, C-2), 6.20 (broad singlet, C-4), 3.14 (broad, C-7), 1.29 (C-19), 1.03 ppm (C-18). Anal. ($C_{23}H_{27}NO_3$) C, H, N.

17-Hydroxy-7α-(methoxycarbonyl)amino-3-oxo-17α-pregn-4-ene-21-carboxylic Acid γ-Lactone (1h). Crude azide **1f** (1.86 g, 4.52 mmol), prepared according to the procedure described above from anhydride **1e** (4.0 g, 8.2 mmol) and NaN_3 (1.17 g, 18 mmol), was dissolved in benzene (150 ml) and the solution refluxed for 3 h. The solvent was removed in vacuo and the residue dissolved in MeOH and let stand at room temperature for 3 days. The solution was concentrated in vacuo and the residue treated with ethyl ether to give a white crystalline solid. This material was recrystallized from ethyl acetate–Skellysolve B and dried at 110° (0.2 mmHg) to give 1.67 g (88.9%) of analytically pure **1h**: mp 229–231°; $[\alpha]_D$ +1.0° (*c* 0.995); ν 1772, 1730, 1675, 1622, and 3440 cm^{-1}; λ_{max} 241 nm (ε 14000); NMR 5.79 (C-4), 4.89 (C-7), 3.69 (–OCH_3), 1.25 (C-19), and 1.00 ppm (C-18). Anal. ($C_{24}H_{33}NO_5$) C, H, N.

7α-(Ethoxycarbonyl)amino-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic Acid γ-Lactone (1i). Crude azide (0.51 g, 1.24 mmol), prepared in the usual manner, was dissolved in benzene (50 ml) and the solution refluxed with stirring under nitrogen for 3.5 h. The solvent was removed in vacuo, the residue dissolved in absolute EtOH, and this solution refluxed for 8 h. After standing overnight at room temperature, the solvent was removed in vacuo and the resulting white foam recrystallized from ethyl ether to give 272 mg (51.1%) of analytically pure **1i**: mp 122–125°; $[\alpha]_D$ +0.9° (*c* 1.128); λ_{max} 241 nm (ε 13300); ν 1775, 1728, 1680, and 1625 cm^{-1}. Anal. ($C_{25}H_{35}NO_5$) C, H, N.

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On the Use of Fibonacci Searches in Structure–Activity Studies

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The Fibonacci search is applicable only to response surfaces that increase smoothly toward the optimum from both sides; the search will fail on response surfaces on which there is superimposed noise.

Two recent papers[1,2] have advocated the use of a Fibonacci search[3,4] to locate the most biologically active compound in a series of analogues between set limits in a predetermined number of steps. It should be pointed out that the Fibonacci search is applicable only to response surfaces that increase smoothly toward the optimum from both sides; the search will fail on response surfaces on which there is superimposed noise (arising either from experimental error—e.g., in the determination of biological activity—or from anomalous behavior—e.g., of drugs). The high efficiency of the Fibonacci search is obtained by excluding certain regions of the factor domain (e.g., log P values) from further search; it is this same feature of the search that causes it to fail in the presence of noise.

To illustrate, consider the graphical presentation (Figure 1) of the 22 data points (numbered 0–21) in Table IV of Santora and Auyang.[2] Point numbers 8 and 13 are circled and represent the initial two experiments of the Fibonacci search. On the basis of these two results, the search proceeds to evaluate point 16 and in so doing excludes from future search points 0–7 (oversize dots). The consequences, in this case, are not serious.

Consider, however, a different data set in which the first two points of Figure 1 are not included but two additional