Cardenolide Analogues. 7. Synthesis and Biological Activity of Some New Steroidal Guanylhydrazones

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The synthesis, proof of structure, and biological activity of some new steroidal 17β-formyl guanylhydrazones are described. The guanylhydrazones of nondigitalis-like steroids inhibited myocardial Na^+,K^+-ATPase but had only a depressant effect on myocardial contractility. By comparison, the corresponding guanylhydrazone of a digitalis-like steroid gave a positive inotropic effect in concentrations that also inhibited Na^+,K^+-ATPase. The nondigitalis-like guanylhydrazones also inhibited membrane Mg^{2+}-ATPase and this may infer that the compounds act nonspecifically by membrane stabilization rather than by interaction with stereoselective receptors. Biological activity was determined in the guinea pig.

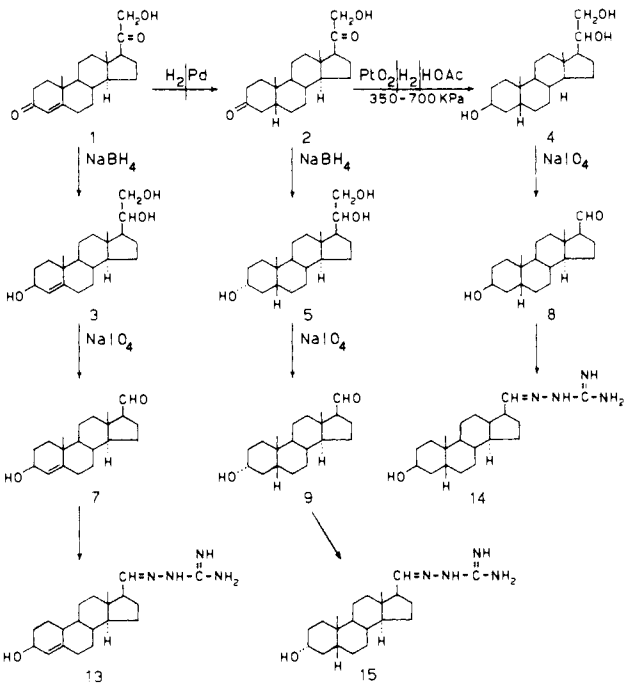
In 1964 Kronenberg and co-workers showed that the 3,20-bis(guanylhydrazones) of prednisolone and related compounds had potent digitalis-like activity.[1-3] Many such compounds were subsequently synthesized, and their structure–activity relationships (SAR) have been reviewed.[4,5] Although evidence for an improved therapeutic ratio was obtained for some of these compounds,[3] their digitalis-like activity was too brief for useful therapeutic activity.[3] The compounds remain of interest because of their relevance to SAR analyses and to speculation about the nature of the digitalis receptor.[5] Unlike the cardiac glycosides, the stereochemistry of the steroidal bis(guanylhydrazones) does not appear to be a critical factor for biological activity.[4] This could mean that the steroidal bis(guanylhydrazones) are bound to the receptor mainly through the two cationic guanylhydrazone groups with little or no contribution from the steroid ring system.

We have shown[5] that it is possible to replace the lactone of digitoxigenin with a 17β-formyl guanylhydrazone moiety to give a hybrid compound (**18**) which has digitalis-like activity. This raised the question of whether a 17β-formyl guanylhydrazone group was sufficient to confer digitalis-like activity regardless of the stereochemistry of the steroid ring system. We therefore synthesized four steroidal guanylhydrazones (**13–16**) which differed from **18** principally by the fact that they lacked the 14β-OH and the C/D cis ring junction. These structural features are known to be essential for significant biological activity among the cardiac glycosides, although there are other groups of compounds which lack these features and yet show digitalis-like activity.[5] This report describes the synthesis and proof of structure of four steroidal 17β-formyl guanylhydrazones and includes some preliminary data on their biological activity.

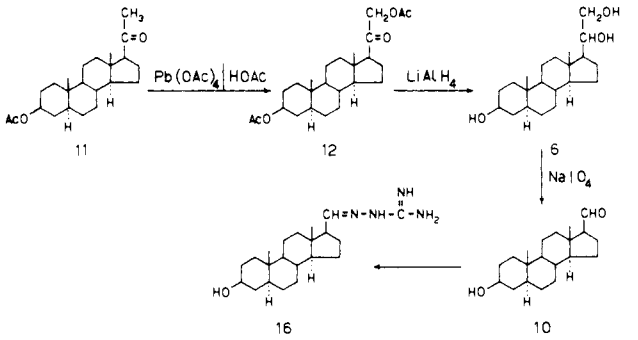
Chemistry. The guanylhydrazones were made by reacting aminoguanidine with appropriate 17β-formyl steroids as shown in Schemes I and II. The formyl groups were generated by periodate fission of the corresponding 20,21-diols.

3β,20,21-Trihydroxypregn-4-ene (**3**) was prepared by $NaBH_4$ reduction of deoxycorticosterone (**1**). Although it was expected that $NaBH_4$ reduction of the 20-keto group would result in a mixture of 20α- and 20β-alcohols, no attempt was made at separation. Catalytic hydrogenation of **1** over Pd/C at atmospheric pressure gave predominantly the A/B *cis*-21-hydroxy-3,20-dioxo-5β-pregnane (**2**). $NaBH_4$ reduction of **2** gave mainly the equatorial alcohol, 3α,20,21-trihydroxy-5β-pregnane (**5**). Catalytic hydrogenation of **2** at increased pressure over PtO_2 gave predominantly the axial alcohol 3β,20,21-trihydroxy-5β-pregnane (**4**). The 5α-steroid triol **6** was obtained by acetoxylation of 3β-acetoxy-20-oxo-5α-pregnane (**11**), followed by treatment of the resulting 3β,21-diacetoxy-20-oxo-5α-pregnane (**12**) with $LiAlH_4$ to give 3β,20,21-trihydroxy-5α-pregnane (**6**).

Scheme I



Scheme II



The 17β-formyl group was generated by periodate fission of the 20,21-diols **3–6**. The aldehyde function gave rise to a strong absorption at 1725 cm^{-1} in the IR spectra and, in the NMR spectra, gave a doublet at δ 9.81–9.87 ($J_{17,20}$ = 3 Hz due to the aldehyde proton coupled to the C-17 proton).

Free base guanylhydrazones were obtained by reacting the 17β-formyl steroids with aminoguanidine bicarbonate in the presence of NaOH. The IR spectra of compounds **13–16** displayed –OH and –NH absorptions in the 3400–

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Table I. Biological Activity of Steroidal Guanylhydrazones Compared with Digitoxigenin (17) on Guinea Pig Myocardium[a]

Compd	Concn, M	% inhibn of membrane ATPase		Effects on atrial contractility	
		Na^+,K^+-ATPase	Mg^{2+}-ATPase	% change in isometric force of contraction	Positive inotropic act.
13	5×10^{-5}	54	30[b]	−18	Absent
14	5×10^{-5}	55	55	−45	Absent
15	5×10^{-5}	37	45	−10	Absent
16	5×10^{-5}	40	43[b]	−50	Absent
17	2×10^{-6}	60	0	+130	Present
18	1×10^{-5}	20	0	+120	Present
	5×10^{-5}	53	0	Toxic	

[a] All biological data shown were taken from log dose-response curves. [b] Inhibition of Mg^{2+}-ATPase by 13 and 16 reached a maximum value at about the levels shown and was then not further affected by increasing concentrations of drug.

3200-cm^{-1} region and broad bands at 1620 and 1650 cm^{-1} attributable to -C=N and -NH groupings. The NMR spectra were determined in pyridine-d_5 or in a mixture of pyridine-d_5–Me_2SO-d_6 because of the limited solubility of the guanylhydrazones in nonpolar solvents. A broad multiplet representing four D_2O exchangeable protons at δ 4.51–6.00 ($W_{h/2}$ = 11–35 Hz) was assigned to the $-NHC(NH)NH_2$ protons. The proton at C-20 appeared further downfield as a doublet centered at δ 7.82–8.17 ($J_{17,20}$ = 6 Hz).

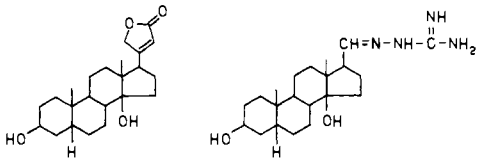
The chemistry employed in the synthesis of the aldehydes 7–10 and in the subsequent condensation reactions is unlikely to cause epimerization at C-17. The assignment of β-orientation at C-17 is substantiated by the position of the 19-CH_3 and the 18-CH_3 signals in the NMR spectra. For 17α epimers, these signals would be expected to be almost coincidental, thereby giving rise to a single diffuse band, or for the 18-CH_3 signal to appear downfield from the 19-CH_3 signal. The reactions used to reduce the 4,5 double bond and the 3-keto group are considered to be stereoselective and give predominantly one stereoisomer.[8]

The stereochemistry of the A/B ring junction was confirmed by the relative chemical shifts of the C-19 methyl protons. In the Δ^4 series, the C-19 methyl group lies in a deshielding cone arising from the diamagnetic effect of the 4,5 double bond, whereas the C-19 methyl groups of the saturated series are shielded by the diamagnetic effects of the ring bonds which are 2,3 related to the substituent. In the 5α series, the C-19 methyl group will be further shielded due to the fact that it bears an axial relationship to both rings A and B, whereas in the 5β series the C-19 methyl group is axial with respect to ring B but equatorial with respect to ring A. These considerations lead to the expectation that chemical shifts for the C-19 methyl group will increase in the order: 5α series < 5β series < Δ^4 series. This order has been confirmed in the androstane series[6] and was found for the assigned structures in the present study.

The stereochemistry of the hydroxyl group at C-3 was confirmed by the chemical shift and width of the NMR signal of the C-3 proton. It would be expected that the quasiequatorial protons of the Δ^4 series and the equatorial protons of the remaining series would be deshielded and show larger chemical shifts than the axial C-3 protons of the saturated A-ring steroids.

The size of the dihedral angle is considered to be the major factor determining the magnitude of vicinal coupling constants in rigid ring systems.[7] Large vicinal coupling constants are expected between protons that are approximately diaxial whereas much smaller splittings are expected between protons that are diequatorial or axial-equatorial.[7] On this basis, large coupling constants ($W_{h/2}$) were expected and found for the axial protons of the 3α-OH-5β series and 3β-OH-5α series and for the quasiequatorial proton of the 3β-OH-Δ^4 series. The value obtained for all these protons was about 20 Hz except for compounds 5 and 6 where the signal was superimposed on the narrower signals from the C-20 and C-21 protons.

Biological Activity. The biological activity of compounds 13–18 is summarized in Table I. It will be noted that all compounds, except for 17, have a guanylhydrazone group attached to the 17β position of the steroid.



17 18

Compound 17 is digitoxigenin and has an α,β-unsaturated lactone at C-17. It should be further noted that the guanylhydrazone 18 has the same steroid system as digitoxigenin.

All the guanylhydrazones, regardless of their steroid system, inhibited Na^+,K^+-ATPase to about the same extent. There were thus no major SAR features with respect to inhibition of Na^+,K^+-ATPase except that activity was greatest in those guanylhydrazones which possessed both 3β-OH and 5β-H functions. However, those guanylhydrazones with steroid systems different from that of digitoxigenin showed reduced specificity since they also inhibited ouabain-insensitive Mg^{2+}-ATPase. Furthermore, 13 and 16 differed from 14 and 15 in that the former produced only partial inhibition of Mg^{2+}-ATPase; with these compounds, inhibition reached a maximum of 30–45% and then became independent of drug concentration. This effect was not due to insolubility at higher concentrations since such concentrations continued to give a dose-related inhibition of Na^+,K^+-ATPase. Compounds 14 and 15 have a cis A/B ring junction, whereas those compounds which gave only partial inhibition of Mg^{2+}-ATPase lack this feature; 13 has a double bond between C-4 and C-5 and thus has a quasiplanar junction between rings A and B, and 16 has a trans A/B ring junction. These results suggest the existence of at least two forms or states of ouabain-insensitive membrane bound Mg^{2+}-ATPase, and the compounds may prove useful as tools for studying this enzyme system.

Although all the guanylhydrazones inhibited Na^+,K^+-ATPase, only 18 produced a positive inotropic effect on the isolated guinea pig atrium. All the guanylhydrazones which possessed a nondigitalis type steroid (namely, 13–16) gave only a negative inotropic effect. In high enough concentrations (10^{-4} M) these compounds completely

abolished atrial contractility. This effect was quite different from the negative inotropic effect associated with toxic doses of digitalis-like compounds. On atrial preparations, digitalis-like compounds produce a bell-shaped dose–response curve. The ascending portion of the curve represents the positive inotropic effect. This curve inflects at the onset of toxicity and the tissue arrests at or shortly after this point. If, however, the stimulating voltage is increased, the tissue will continue to contract but with a dose-related decrease in force of contraction. In the case of the guanylhydrazones **13–16** there was only a negative inotropic effect over the concentration range 10^{-8}–10^{-4} M, and this effect was sustained without the need to increase the stimulating voltage.

These results appear to be at variance with the generally accepted theory that the cardiotonic effects of digitalis occur as a result of the interaction which leads to inhibition of Na^+,K^+-ATPase. All of the guanylhydrazones inhibited the enzyme; yet, only **18**, which has the same steroid system as digitoxigenin, gave rise to a positive inotropic effect. However, the fact that the guanylhydrazones **13–16** also inhibited ouabain-insensitive Mg^{2+}-ATPase as well as certain other membrane-associated enzymes (unpublished results) may infer that the compounds are acting by nonspecific membrane stabilization rather than by interaction with the stereoselective receptor that mediates the activity of digitalis.

Experimental Section

Chemistry. General Methods. Melting points were determined on a Kofler hot block and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 21 double beam spectrophotometer or on a Unicam SP200G spectrophotometer. NMR spectra were recorded by a Varian A-60 instrument using Me_4Si (δ 0.00) as internal standard. The values of δ (ppm) are quoted for the 60-MHz instrument. Mass spectra were obtained on an AEI MS 9 instrument operating at 12 or 70 eV ion current with the source several degrees below the melting point of the compounds. Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Analytical samples were dried at 40–50 °C (0.5 mmHg) over P_2O_5 and paraffin wax for 24 h. Where water of crystallization was indicated, the samples were further dried at elevated temperatures for 24 h. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values.

Synthesis of 21-Hydroxy-3,20-dioxo-5β-pregnane (2). 21-Hydroxy-3,20-dioxopregn-4-ene (**1**) (0.2 g, 0.6 mmol) dissolved in CH_2Cl_2 (6.5 mL) was added to a prehydrogenated suspension of Pd (10%) on charcoal (0.06 g) in MeOH (6.5 mL) and H_2O (0.63 mL). The mixture was shaken under H_2 at atmospheric pressure for 20 min at room temperature. The catalyst was removed by filtration. H_2O (5 mL) was added and most of the solvent was removed under reduced pressure at 25 °C. The solid that then separated was collected, washed with H_2O, and recrystallized from MeOH–H_2O to yield **2** (0.16 g, 0.48 mmol, 80%), mp 152 °C. The identity of **2** was established by comparison of physical constants (mixture melting point, IR, and NMR) with those of an authentic sample.

Synthesis of 3β,20,21-Trihydroxypregn-4-ene (3). A solution of **1** (5.0 g, 15 mmol) in THF (60 mL) and H_2O (20 mL) was treated for 30 min at room temperature with excess $NaBH_4$ (2.5 g, 60 mmol). Excess $NaBH_4$ was decomposed by careful addition of HOAc. Most of the THF was removed under reduced pressure at 25 °C and the solid which then separated was collected, washed with H_2O, and recrystallized from EtOH–Me_2CO to yield **3** (3.9 g, 11.5 mmol, 77%): mp 159–164 °C; mass spectrum m/e 334 (M^+); ν max (Nujol) 3320 (br, –OH), 1660 (C=C), 1040, 960, 860 cm^{-1}; NMR (pyridine-d_5) δ 0.96 (s, 18-CH_3), 1.05 (s, 19-CH_3, 3.91 (m, $W_{h/2}$ = 7.5 Hz, 20-CH_2), 4.5 (m, $W_{h/2}$ = 19 Hz, 3-CH), 5.5 (m, $W_{h/2}$ = 33 Hz, underwent exchange with D_2O, 3,20,21-OH), 5.75 (m, $W_{h/2}$ = 3.5 Hz, 4-CH). Anal. ($C_{21}H_{34}O_3$) C, H.

Synthesis of 3β,20,21-Trihydroxy-5β-pregnane (4). To a solution of **2** (1.4 g, 4.2 mmol) in glacial HOAc (35 mL) was added 0.33 g of PtO_2 as catalyst. The mixture was hydrogenated for 3 h at room temperature at a pressure of 350–700 kPa. The catalyst was then removed by filtration and most of the glacial HOAc was removed under reduced pressure at 50 °C. The solid that separated was collected, dried, and recrystallized twice from EtOH–Me_2CO to give **4** (0.7 g, 2.1 mmol, 50%). The analytical sample was recrystallized twice from EtOH: mp 212 °C (lit.[9] mp 212 °C); mass spectrum m/e 336 (M^+); ν max (Nujol) 3360–3240 (–OH), 1100, 1070, 1065, 1030, 1000, 950, 880 cm^{-1}; NMR (pyridine-d_5) δ 0.90 (s, 18-CH_3), 1.00 (s, 19-CH_3), 4.00 (m, $W_{h/2}$ = 6 Hz, 20-CH, 21-CH_2), 4.48 (m, $W_{h/2}$ = 6 Hz, 3-CH), 5.2 (m, $W_{h/2}$ = 40 Hz, underwent exchange with D_2O, 3,20,21-OH). Anal. ($C_{21}H_{36}O_3$) C, H.

Synthesis of 3α,20,21-Trihydroxy-5β-pregnane (5). A solution of **2** (2.0 g, 6.0 mmol) in THF (24 mL) and H_2O (8 mL) was treated with excess $NaBH_4$ (1.0 g, 24 mmol) for 1 h at room temperature. Excess $NaBH_4$ was decomposed by careful addition of HOAc. Most of the THF was removed under reduced pressure at 25 °C and the separated solid was collected, washed with H_2O, and recrystallized from EtOH–Me_2CO and EtOH–Me_2CO–H_2O to give **5** (1.2 g, 3.6 mmol, 60%): mp 209.5–210.5 °C (lit.[9] mp 210–211 °C); mass spectrum m/e 336 (M^+); ν max (Nujol) 3500–3200 (–OH), 1260, 1095, 1055, 970, 880 cm^{-1}; NMR (pyridine-d_5) δ 0.91 (s, 18-CH_3), 0.96 (s, 19-CH_3), 3.92 (m, $W_{h/2}$ = 13 Hz, 3-CH, 20-CH, 21-CH_2), 5.22 (m, $W_{h/2}$ = 6 Hz), and 5.85 (m, $W_{h/2}$ = 12 Hz, underwent exchange with D_2O, 3,20,21-OH). Anal. ($C_{21}H_{36}O_3$) C, H.

Synthesis of 3β,21-Diacetoxy-20-oxo-5α-pregnane (12). To a solution of 3β-acetoxy-20-oxo-5α-pregnane (**11**) (5.0 g, 13.9 mmol) in glacial acetic acid (200 mL) was added $Pb(OAc)_4$ (11.0 g, 41.5 mmol) and Ac_2O (2.5 mL), and the mixture was heated at 68–70 °C for 48 h. Excess $Pb(OAc)_4$ was decomposed by addition of ethylene glycol (30 mL) and allowed to stand at 68–70 °C for 2 h. Most of the glacial HOAc was removed under reduced pressure at 68–70 °C and the solid that separated was collected, washed with H_2O, dried, and recrystallized from C_6H_6–*n*-pentane to yield **12** (2.5 g, 6.0 mmol, 43%): mp 150–152 °C (lit.[10] mp 152–153 °C); mass spectrum m/e 418 (M^+); ν max (Nujol) 1755, 1740 (–C=O), 1250 (C–OAc), 1090, 1070, 1050, 1000, 970, 860 cm^{-1}; NMR ($CDCl_3$) δ 0.67 (s, 18-CH_3), 0.85 (s, 19-CH_3), 2.05 (s, 3-$OCOCH_3$), 2.16 (s, 21-$OCOCH_3$), 4.51, 4.83 (pair of doublets, $J_{20,21}$ = 16.7 Hz, 21-CH_2). The gem protons at C-21 are not equivalent because of restricted rotation about the C-20–C-21 bond.

Synthesis of 3β,20,21-Trihydroxy-5α-pregnane (6). $LiAlH_4$ (1.0 g, 24 mmol) suspended in dry THF (30 mL) was added slowly to **12** (2.4 g, 5.8 mmol) dissolved in dry THF (100 mL). The mixture was stirred at room temperature for 1 h. Excess $LiAlH_4$ was decomposed by careful addition of EtOAc. The bulk of the solvent was removed under reduced pressure at 25 °C. H_2O (150 mL) was added and the mixture was extracted with Et_2O (4 × 250 mL). The Et_2O extract was washed with H_2O, dried with anhydrous Na_2SO_4, and then evaporated to yield crystalline **6** (1.6 g, 4.8 mmol, 83%): mp 208–210 °C (lit.[9] mp 212 °C); mass spectrum m/e 336 (M^+); ν max (Nujol) 3380 (br, –OH), 1095, 1050, 970, 900, 800 cm^{-1}; NMR (DMF-d_6) δ 0.77 (s, 18-CH_3), 0.81 (s, 19-CH_3), 3.47 (m, $W_{h/2}$ = 17.5 Hz, 3-CH, 20-CH, 21-CH_2), 4.36 (m, $W_{h/2}$ = 18.5 Hz, underwent exchange with D_2O, 3,20,21-OH). Anal. ($C_{21}H_{36}O_3$) C, H.

Synthesis of 17β-Formyl Steroids (7–10). The appropriate triol (**3**, **4**, **5**, or **6**) (1.5–3.9 g, 5–13 mmol) was dissolved in MeOH (100–200 mL) and treated at room temperature for 90 min with an equal weight of $NaIO_4$ dissolved in H_2O (20–50 mL). The $NaIO_3$ formed during the reaction was removed by filtration, and most of the MeOH was removed under reduced pressure at room temperature. The solid that separated was collected, washed with H_2O, and reprecipitated from the solvent shown in Table II. Nomenclature of aldehydes synthesized by the above method: 3β-hydroxy-17β-formylandrost-4-ene (**7**), 3β-hydroxy-17β-formyl-5β-androstane (**8**), 3α-hydroxy-17β-formyl-5β-androstane (**9**), 3β-hydroxy-17β-formyl-5α-androstane (**10**). Physical data for compounds **7–10** are shown in Table II.

Synthesis of 17β-Guanylhydrazones (13–16). The appropriate 17β-formyl steroid (**7**, **8**, **9**, or **10**) (0.11–0.4 g, 0.4–1.3 mmol) was dissolved in EtOH (22–80 mL) and treated with an excess of aminoguanidine bicarbonate (0.135–0.48 g, 1.15–4.0 mmol) and NaOH (0.045–0.16 g, 1.15–4.0 mmol) dissolved in H_2O (14–60

Table II. Analytical and Spectral Data

Compd	Mp, °C (solvent)	Yield, %	Formula	Analyses	Spectral data
7	55–57[b] (MeOH-H_2O)	92	$C_{20}H_{30}O_2$		IR 3390 (OH), 2710 (17-CH), 1725 (CO), 1670 cm^{-1} (C=C); NMR ($CDCl_3$) 0.78 (s, 18-CH_3), 1.06 (s, 19-CH_3), 4.15 (m, $W_{h/2}$ = 20 Hz, 3-CH), 4.3 (m, $W_{h/2}$ = 3.3 Hz, 4-CH), 9.81 (d, $J_{17,20}$ = 3 Hz, HCO); mass spectrum *m/e* 302 (M^+)
8	146–148 (Me_2CO)	96	$C_{20}H_{32}O_2$		IR 3380 (OH), 2720 (17-CH), 1725 cm^{-1} (CO); NMR ($CDCl_3$) 0.77 (s, 18-CH_3), 0.99 (s, 19-CH_3), 4.14 (m, $W_{h/2}$ = 6.7 Hz, 3-CH), 9.87 (d, $J_{17,20}$ = 3 Hz, HCO); mass spectrum *m/e* 304 (M^+)
9	57–59[b] ($MeCO_2Et$)	90	$C_{20}H_{32}O_2$		IR 3400 (OH), 2730 (17-CH), 1725 cm^{-1} (CO); NMR ($CDCl_3$) 0.75 (s, 18-CH_3), 0.93 (s, 19-CH_3), 3.63 (m, $W_{h/2}$ = 20 Hz, 3-CH), 9.82 (d, $J_{17,20}$ = 3 Hz, HCO); mass spectrum *m/e* 304 (M^+)
10	90[b] ($MeCO_2Et$)	88	$C_{20}H_{32}O_2$		IR 3450 (OH), 2730 (17-CH), 1725 cm^{-1} (CO); NMR ($CDCl_3$) 0.76 (s, 18-CH_3), 0.83 (s, 19-CH_3), 3.56 (m, $W_{h/2}$ = 20 Hz, 3-CH), 9.87 (d, $J_{17,20}$ = 3 Hz, HCO); mass spectrum *m/e* 304 (M^+)
13	204–205 (MeOH)	76	$C_{21}H_{34}N_4O\cdot H_2O$	C, H, N	IR 3480, 3360, 3240 (OH, NH), 1600–1650, 1560 cm^{-1} (NH, C=N); NMR (pyridine-d_5) 0.72 (s, 18-CH_3), 1.00 (s, 19-CH_3), 4.45 (m, $W_{h/2}$ = 20 Hz, 3-CH), 5.65 (m, $W_{h/2}$ = 6.0 Hz, 4-CH), 5.07 [m,[a] $W_{h/2}$ = 27 Hz, –NHC(NH)NH_2], 7.92 (d, $J_{17,20}$ = 6.0 Hz, 20-CH); mass spectrum *m/e* 358 (M^+)
14	195–198 (MeOH)	65	$C_{21}H_{36}N_4O$	C, H, N	IR 3600, 3460 (NH, OH), 1640, 1600, 1570 cm^{-1} (NH, C=N); NMR (pyridine-d_5) 0.70 (s, 18-CH_3), 0.89 (s, 19-CH_3), 4.35 (m, $W_{h/2}$ = 5.3 Hz, 3-CH), 6.0 [m,[a] $W_{h/2}$ = 10.7 Hz, –NHC(NH)NH_2], 8.18 (d, $J_{17,20}$ = 6.0 Hz, 20-CH); mass spectrum *m/e* 360 (M^+)
15	145–146 (MeOH-H_2O)	65	$C_{21}H_{36}N_4O\cdot 0.5H_2O$	C, H, N	IR 3500, 3350 (NH, OH), 1600, 1540 cm^{-1} (NH, C=N); NMR (pyridine-d_5) 0.74 (s, 18-CH_3), 0.99 (s, 19-CH_3), 3.83 (m, $W_{h/2}$ = 20 Hz, 3-CH), 5.49 [m,[a] $W_{h/2}$ = 35 Hz, –NHC(NH)NH_2], 7.89 (d, $J_{17,20}$ = 6.0 Hz, 20-CH); mass spectrum *m/e* 360 (M^+)
16	238–240 (MeOH)	77	$C_{21}H_{36}N_4O$	C, H, N	IR 3600, 3500 (NH, OH), 1650–1600, 1570 cm^{-1} (NH, C=N); NMR (pyridine-d_5–Me_2SO-d_6) 0.70 (s, 18-CH_3), 0.80 (s, 19-CH_3), 3.71 (m, $W_{h/2}$ = 20 Hz, 3-CH), 4.51 [m,[a] $W_{h/2}$ = 10.7 Hz, –NHC(NH)NH_2], 7.82 (d, $J_{17,20}$ = 6.0 Hz, 20-CH); mass spectrum *m/e* 360 (M^+)

[a] Exchangeable with D_2O. [b] Material probably amorphous.

mL). The mixture was refluxed for 15 min, allowed to cool, and filtered. During the removal of EtOH at reduced pressure, crystalline solid separated. This solid was collected, washed with H_2O, and recrystallized from the solvent shown in Table II. Nomenclature of compounds synthesized by the above method: 3β-hydroxy-17β-formylandrost-4-ene guanylhydrazone (**13**), 3β-hydroxy-17β-formyl-5β-androstane guanylhydrazone (**14**), 3α-hydroxy-17β-formyl-5β-androstane guanylhydrazone (**15**), 3β-hydroxy-17β-formyl-5α-androstane guanylhydrazone (**16**). Physical data for compounds **13**–**16** are shown in Table II.

Biological Methods. Inhibition of Phosphohydrolase Activity. Na^+,K^+-ATPase and Mg^{2+}-ATPase were prepared from guinea pig myocardium as previously described.[11] The crude membrane preparations contained 5–10 units of each enzyme activity. Units of activity were calculated as micromoles of inorganic phosphate liberated per milligram of protein per hour. Incubations were carried out at 37 °C for 30 min as previously described.[11] Mg^{2+}-ATPase activity (with and without drug) was determined in the absence of Na^+ and K^+ and was ouabain-insensitive. Na^+,K^+-ATPase activity was determined by subtracting Mg^{2+}-ATPase activity from total phosphohydrolase and was completely inhibited by 10^{-4} M ouabain. Na^+,K^+-ATPase activity in the presence of drug was obtained by subtraction of Mg^{2+}-ATPase activity in the presence of drug from total phosphohydrolase activity in the presence of the same concentration of drug. All measurements were corrected for nonenzymatic hydrolysis of ATP. The rate of release of inorganic phosphate in the absence of drug was pseudo-first-order when followed for 60 min. The total amount of substrate consumed in 30 min in the absence of drug was less than 15% of the total available. Time dependence effects on rate in the presence of drug were not examined.

Dose–response curves for the test compounds were determined over a drug concentration range of 10^{-8}–10^{-4} M. All determinations were carried out in duplicate. The data shown in Table II were taken from the dose–response curves.

Determination of Inotropic Activity. Inotropic activity was determined using the left auricle of the guinea pig as previously described.[12] The auricle was suspended in Krebs–Henseleit solution at 32 °C and was gassed with O_2 containing 5% CO_2. The resting tension was about 1 g. The auricle was stimulated by a rectangular pulse (10-ms duration) at a frequency of 100/min. The voltage was set at 20% above threshold. Changes in isometric contraction were calculated with reference to changes in control atria using the protocol described previously.[12] Cumulative dose–response curves for the test compounds were determined over a drug concentration range of 10^{-8}–10^{-4} M. Each dose–response curve determination was repeated four to six times. The data shown in Table I were taken from plots of pooled dose–response curve determinations.

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Metabolism of *cis*-Δ^4-15(*S*)-15-Methylprostaglandin $F_{1\alpha}$ Methyl Ester in the Rat

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The metabolic transformation and excretion of tritium-labeled *cis*-Δ^4-15(*S*)-15-methylprostaglandin $F_{1\alpha}$ methyl ester (**1**) have been investigated in rats after chronic and single dose oral administration. Three metabolites have been identified from a partially purified urinary extract. They were the *cis*-Δ^4-15-methylprostaglandin $F_{1\alpha}$ (**2**), 15-methyl-2,3-dinorprostaglandin $F_{1\alpha}$ (**3**), and 15-methyl-2,3,4,5-tetranorprostaglandin $F_{1\alpha}$ (**4**). The excretion of the drug-related species was significantly slower than that of the natural prostaglandin $F_{2\alpha}$. The slow excretion rate and the presence of unchanged *cis*-Δ^4-15-methylprostaglandin $F_{1\alpha}$ in urine suggested that the metabolically protected prostaglandin could persist in the body and, therefore, exhibit longer duration of action.

Although natural prostaglandins possess many potent and diverse biological activities, they usually have a short duration of action due to their rapid metabolism and excretion. PGE_2 and $PGF_{2\alpha}$ administered intravenously in humans disappear from the peripheral circulation in a few seconds.[1] The bulk of the administered drug is excreted usually within a few hours.[2] The administered prostaglandins are completely degraded into various metabolites before excretion.

Studies of prostaglandin metabolism in this laboratory[3,4] and others[5-7] have defined the major routes of metabolism involved: (a) oxidation of the C-15 hydroxyl group, (b) reduction of the C-13 double bond, (c) β-oxidation of the carboxyl acid side chain, (d) ω-hydroxylation and oxidation, and (e) reduction of the C-15 keto back to hydroxyl groups. Urinary metabolites isolated from natural prostaglandin treated animals or human subjects usually reflect the combined results of several degradative reactions. The urinary metabolite patterns vary depending on animal species used and physiological state, as well as the dosage or route of administration.[8]

The major catabolic pathways that cause the rapid disappearance of active prostaglandins are the prostaglandin 15-hydroxyl dehydrogenase and the β-oxidation system. Both enzyme systems are widely distributed in various tissues and organs. The lung is especially enriched in prostaglandin 15-hydroxyl dehydrogenase and is believed to be the primary site for the removal of circulating PG.

Many chemical modifications of PG have been designed to circumvent these metabolic pathways and prolong the duration of their pharmacological actions. The 15-methyl and 16,16-dimethyl analogues of PGE_2 and $PGF_{2\alpha}$[9,10] which are not substrates of prostaglandin 15-hydroxyl dehydrogenase are more potent and longer in duration than natural PG. Green et al.[11] reported that shifting the Δ^5 double bond of $PGF_{2\alpha}$ to the Δ^4 position considerably retards the degradative action of the β-oxidation system. The Δ^4-$PGF_{2\alpha}$ is still a substrate of prostaglandin dehydrogenase and is degraded to the 15-keto derivatives in the same way as the naturally occurring Δ^5 compound.

Recently, Johnson and Nidy[12] reported the *cis*-Δ^4-15-methyl analogue of $PGF_{2\alpha}$. This compound is slightly less active than $PGF_{2\alpha}$ as a pressor agent in the rat in stimulating gerbil colon smooth muscle but four times more active than $PGF_{2\alpha}$ as an abortifacient in hamsters. The compound is not a substrate for prostaglandin 15-hydroxyl dehydrogenase and should partially resist β-oxidation. In this communication we will describe the excretion and metabolism of this compound in the rat.

Experimental Section

Materials. [11-^{3}H]-*cis*-Δ^4-15(*S*)-15-Methylprostaglandin $F_{1\alpha}$ methyl ester was kindly synthesized by Dr. E. W. Yankee. The specific activity of the final product was 154 μCi/mg and the radioactive purity was greater than 93%. The reference compounds, *cis*-Δ^4-15(*S*)-15-methylprostaglandin $F_{1\alpha}$ methyl ester, *cis*-Δ^4-15(*S*)-15-methylprostaglandin $F_{1\alpha}$, 15(*S*)-15-methyl-2,3-dinorprostaglandin $F_{1\alpha}$, and 15(*S*)-15-methyl-2,3,4,5-tetranorprostaglandin $F_{1\alpha}$ lactone, were made available by members of the Experimental Chemistry Unit of The Upjohn Company.

Silicic acid (SilicAR CC-4) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Sephadex LH-20 was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, N.Y.). The prepacked preparative silica gel 60 column was purchased from EM Laboratories, Inc. (Elmsford, N.Y.). Amberlite XAD-2 resin was purchased from Rohm and Hass (Philadelphia, Pa.). Bis(trimethylsilyltrifluoroacetamide) with 1% trimethylsilane and Trisil Z were obtained from Pierce Chemical Co. (Rockford, Ill.). Glass redistilled solvents were purchased from Burdick and Jackson Laboratories, Inc. (Muskegon, Mich.).

Methods. Drug Administration and Sample Collection. A. Single Dose Oral Administration. Four female Sprague–Dawley rats (Upjohn strain) weighing approximately 200 g were fasted overnight. Each rat was given a single 0.28-mg dose of [^{3}H]-*cis*-Δ^4-15(*S*)-15-methylprostaglandin $F_{1\alpha}$ methyl ester (**1**) (1.24×10^7 dpm) in distilled water via a gastric tube. The rats were housed in stainless steel metabolism cages with free access to food and water. Urine and feces samples were collected at appropriate intervals for a total of 54 h.

B. Accumulation of Metabolites. Fifteen rats were housed in metabolism cages for 17 days. Each morning the rats were administered a 0.25-mg oral dose of **1** containing 1×10^7 dpm of radioactivity. Urine samples were collected daily, pooled, and stored at −70 °C.

Extraction and Chromatographic Methods. The unchanged drug and metabolites were isolated from urine using the Amberlite XAD-2 column procedure as described previously.[4] The recovery of radioactivity was greater than 95%. The crude extract was partially purified by silicic acid column chromatography (200 g, SilicAR CC-4 column) with ethyl acetate–heptane (8:2) as solvent. The bulk of the radioactivity was readily eluted with this solvent. A small amount of polar components was eluted with ethyl acetate–methanol (9:1). The overall radioactivity recovery was approximately 95%.

The separation of radioactivity components in the partially purified urine extract was carried out by reversed phase partition column chromatography. Sephadex LH-20 was used as the solid support, and the solvent mixture C-38 consisting of isooctyl alcohol–chloroform–methanol–water (15:15:114:86) was the partition system. Sephadex LH-20 (60 g) was shaken with 60 mL

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