ice water and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a column of silica gel. The fractions eluted with 50% AcOEt/benzene and 2% MeOH/CH_2Cl_2 were collected to obtain **61** (700 mg, mp 76–77 °C, from AcOEt/*i*-Pr_2O, overall yield 9%): IR (Nujol) 1690, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (3 H, d, J = 7.5 Hz, CH_3), 6.10–8.00 (9 H, m, =CHC, aromatics). Anal. ($C_{14}H_{12}ClNO_2$) C, H, Cl, N.

Acknowledgment. We thank K. Kanazawa and K. Ito for their excellent technical assistances.

Registry No. 1, 104940-90-1; **2**, 104940-89-8; **3**, 104940-93-4; **4**, 104940-91-2; **5**, 104940-92-3; **6**, 104940-86-5; **7**, 104940-94-5; **8**, 104940-95-6; **9**, 104940-87-6; **10**, 104940-88-7; **11**, 104941-07-3; **12**, 108664-23-9; **13**, 104940-96-7; **14**, 108664-24-0; **15**, 108664-25-1; **16**, 108664-26-2; **17**, 104940-99-0; **18**, 104941-01-7; **19**, 108664-27-3; **20**, 104940-98-9; **21**, 104940-97-8; **22**, 108664-28-4; **23**, 108664-29-5; **24**, 108664-30-8; **25**, 104941-04-0; **26**, 108664-31-9; **27**, 108664-32-0; **28**, 108664-33-1; **29**, 108664-34-2; **30**, 108664-35-3; **31**, 108664-36-4; **32**, 98617-94-8; **33**, 104941-05-1; **34**, 104941-08-4; **35**, 13191-28-1; **36**, 108664-37-5; **37**, 24229-73-0; **38**, 104941-10-8; **39**·$C_2H_2O_4$, 108664-38-6; **40**, 108664-39-7; **41**·$C_2H_2O_4$, 108664-41-1; **42**, 108664-42-2; **43**, 104941-12-0; **44**, 104941-13-1; **45**, 108664-43-3; **46**, 108664-44-4; **47**·$C_2H_2O_4$, 108664-46-6; **48**·$C_2H_2O_4$, 108664-47-7; **49**, 108664-48-8; **50**, 108664-49-9; **51**, 108664-50-2; **52**, 108664-51-3; **53**, 108664-52-4; **54**, 108664-53-5; **55**, 90059-70-4; **56**, 108664-54-6; **57**, 108664-55-7; **58**, 104941-03-9; **59**, 104941-02-8; **60**, 6285-05-8; **61**, 108664-56-8; III (R_1 = 4-$MeOC_6H_4$), 100-06-1; III (R_1 = 4-MeC_6H_4), 92-91-1; IV (R_1 = 4-$MeOC_6H_4$), 2632-13-5; IV (R_1 = 4-ClC_6H_4), 536-38-9; V (R_1 = 4-ClC_6H_4, R_2 = T), 58905-19-4; V (R_1 = 4-FC_6H_4, R_2 = T), 58905-21-8; V (R_1 = 4-CNC_6H_4, R_2 = T), 103962-24-9; V (R_1 = 4-MeC_6H_4, R_2 = T), 58905-20-7; V (R_1 = 4-$MeOC_6H_4$, R_2 = T), 89082-07-5; V (R_1 = 4-PhC_6H_4, R_2 = T), 89082-08-6; V (R_1 = 2-furyl, R_2 = T), 108674-95-9; V (R_1 = 2-thienyl, R_2 = T), 108664-57-9; V (R_1 = Me, R_2 = T), 64882-52-6; V (R_1 = *t*-Bu, R_2 = T), 58905-32-1; V (R_1 = 2,4-$Cl_2C_6H_3$, R_2 = PA), 108664-58-0; V (R_1 = Ph, R_2 = P), 952-75-0; V (R_1 = 4-PhC_6H_4, R_2 = P), 13576-81-3; V (R_1 = 2-$MeOC_6H_4$, R_2 = P), 108664-59-1; V (R_1 = 4-$COOMeC_6H_4$, R_2 = P), 108664-60-4; V (R_1 = 2,4-$Cl_2C_6H_3$, R_2 = P), 108664-61-5; V (R_1 = 4-ClC_6H_4, R_2 = PC), 108664-62-6; V (R_1 = 4-ClC_6H_4, R_2 = PM), 108664-63-7; V (R_1 = 4-ClC_6H_4, R_2 = PL), 108664-64-8; V (R_1 = *t*-Bu, R_2 = P), 108664-65-9; V (R_1 = 4-ClC_6H_4, R_2 = PD), 108664-66-0; V (R_1 = 4-ClC_6H_4, R_2 = PY), 108664-67-1; V (R_1 = 4-ClC_6H_4, R_2 = PE), 108664-68-2; V (R_1 = 4-ClC_6H_4, R_2 = Q), 108664-69-3; V (R_1 = 4-PhC_6H_4, R_2 = QA), 108664-70-6; V (R_1 = 4-ClC_6H_4, R_2 = BZ), 108664-71-7; V (R_1 = 4-ClC_6H_4, R_2 = BH), 108664-72-8; V (R_1 = 4-ClC_6H_4, R_2 = QA), 90059-70-4; V (R_1 = 4-MeC_6H_4, R_2 = QA), 90059-68-0; V (R_1 = 4-ClC_6H_4, R_2 = PH), 108664-73-9; V (R_1 = 4-FC_6H_4, R_2 = 2,4-$Cl_2C_6H_3$), 98617-95-9; V (R_1 = 2,4-$Cl_2C_6H_3$, R_2 = 2,4-$Cl_2C_6H_3$), 107680-34-2; V (R_1 = 2-thienyl, R_2 = 4-ClC_6H_4), 67947-51-7; V (R_1 = 2-thienyl, R_2 = Ph), 13196-28-6; V (R_1 = Ph, R_2 = PL), 108674-96-0; V (R_1 = 2-furyl, R_2 = Me), 3194-15-8; VII (R_1 = 4-MeC_6H_4), 5409-63-2; VIII (R_1 = 4-ClC_6H_4, R_2 = T), 81234-31-3; VIII (R_1 = 4-$MeOC_6H_4$, R_2 = T), 108664-74-0; VIII (R_1 = 4-PhC_6H_4, R_2 = T), 81234-79-9; VIII (R_1 = 3-thienyl, R_2 = T), 108664-75-1; VIII (R_1 = 4-PhC_6H_4, R_2 = PA), 108664-76-2; VIII (R_1 = 4-ClC_6H_4, R_2 = P), 108664-77-3; VIII (R_1 = 4-$MeOC_6H_4$, R_2 = P), 108664-78-4; VIII (R_1 = 4-PhC_6H_4, R_2 = P), 108664-79-5; VIII (R_1 = 3-thienyl, R_2 = P), 108664-80-8; VIII (R_1 = 4-ClC_6H_4, R_2 = QA), 108664-81-9; VIII (R_1 = 4-ClC_6H_4, R_2 = NMe_2), 2138-38-7; VIII (R_1 = 4-ClC_6H_4, R_2 = Q), 108664-82-0; VIII (R_1 = 3-thienyl, R_2 = Ph), 108664-83-1; IX (R_1 = 4-ClC_6H_4), 33994-12-6; XI (R_2 = $PhCH_2$), 645-59-0; XI (R_2 = 2,4-$Cl_2C_6H_3$), 6306-60-1; XII (R_2 = 2,4-$Cl_2C_6H_3$), 26923-38-6; XIII (R_2 = 2,4-$Cl_2C_6H_3$), 108664-84-2; TMDAM, 51-80-9; $PhCH_2COCl$, 103-80-0; 2,4-$Cl_2C_6H_3C(=CH_2)COCl$, 108664-85-3; 4-$ClC_6H_4NH_2$, 106-47-8; CH_3CH_2CHO, 123-38-6; 4-FC_6H_4CHO, 459-57-4; 4-$ClC_6H_4COCHBrCH_3$, 877-37-2; morpholine, 110-91-8; 1*H*-1,2,4-triazole, 288-88-0; thiophene, 110-02-1; 1*H*-pyrazole, 288-13-1; 2(1*H*)-quinolinone, 59-31-4; 2-thienylmagnesium bromide, 5713-61-1; 2(1*H*)-pyridinone, 142-08-5.

Progesterone Derivatives That Bind to the Digitalis Receptor: Synthesis of 14β-Hydroxyprogesterone. A Novel Steroid with Positive Inotropic Activity

J. F. Templeton,*† V. P. Sashi Kumar,† D. Cote,⊥ D. Bose,‡,§ D. Elliott,‡ R. S. Kim,‡ and F. S. LaBella*‡

Faculty of Pharmacy, Department of Pharmacology and Therapeutics and Department of Internal Medicine, Faculty of Medicine, University of Manitoba, and St. Boniface Hospital, Winnipeg, Manitoba, Canada. *Received December 22, 1986*

The synthesis of 14-hydroxy-14β-pregn-4-ene-3,20-dione (14β-hydroxyprogesterone) is described. This novel steroid is about 10 times more potent than progesterone and one-tenth as potent as ouabagenin in an [^{3}H]ouabain radioligand binding assay and is the first in a series of progesterone congeners that interact at the cardiac glycoside receptor both to possess the C/D cis ring junction and to enhance contractility of isolated cardiac tissue.

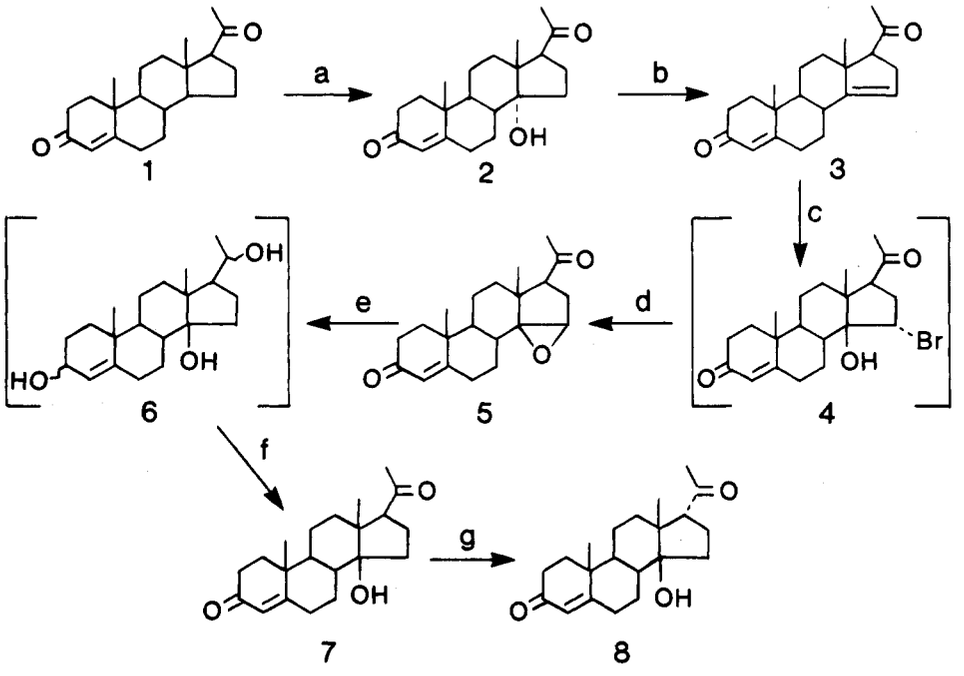
The high-affinity binding of the cardiac glycosides to their biological receptor, i.e., Na^+,K^+-ATPase, is noted, also, for its high degree of structural specificity.[1] In previous studies from our laboratory,[2–6] it was demonstrated that certain derivatives of progesterone are inhibitors of [^{3}H]ouabain binding to cell membrane preparations. The most active congener identified thus far is chlormadinone acetate (17α-acetoxy-6-chloropregna-4,6-diene-3,20-dione), having 3–4 times the potency of ouabagenin. These mammalian steroid derivatives interact at the cardiac glycoside binding site and inhibit Na^+,K^+-ATPase and the sodium pump, and crystallographic studies show important spatial relationships between the C-20 ketone of the progesterone derivatives and the C-23 carboxyl oxygen of the lactone moiety in the cardiac glycoside.[5] However, progesterone and the receptor-active semisynthetic derivatives elicit cardiode-

† Faculty of Pharmacy.
⊥ St. Boniface Hospital.
‡ Department of Pharmacology and Therapeutics.
§ Department of Internal Medicine.

(1) Guntert, T. W.; Linde, H. H. A. in *Cardiac Glycosides*; Greef, K., Ed.; Springer-Verlag: Berlin, 1981; Part I, Chapter 2, pp 13–24.
(2) Chow, E.; Kim, R. S.; LaBella, F. S.; Queen, G. *Br. J. Pharmacol.* **1979**, *67*, 345.
(3) Kim, R. S.; LaBella, F. S.; Zunza, H.; Zunza, F.; Templeton, J. F. *Mol. Pharmacol.* **1980**, *18*, 402.
(4) LaBella, F. S.; Bihler, I.; Kim, R. S. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1057.
(5) LaBella, F. S.; Bihler, I.; Templeton, J.; Kim, R. S.; Hnatowich, M.; Rohrer, D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 2806.
(6) LaBella, F. S.; Bihler, I.; Kim, R. S.; *Nature (London)* **1979**, *28*, 571.

0022-2623/87/1830-1502$01.50/0

Scheme I[a]



[a] (a) *Mucor griseocyanus* (+) 1207a; (b) *p*-TSA/benzene; (c) *N*-bromoacetamide/acetone/H_2O/$HClO_4$; (d) KOH; (e) lithium triethylborohydride/THF; (f) pyridinium dichromate/DMF; (g) 0.5 M methanolic KOH.

pression rather than the positive inotropy expected of cardiac glycoside-like compounds.[4-6] Because a 14β-OH substituent is a common feature of the cardiac glycosides,[1] we now have incorporated this moiety into the progesterone molecule. This substitution increases the potency of progesterone in the cardiac glycoside radioligand binding assay and, more significantly, yields a compound that promotes positive inotropy on isolated cardiac muscle. Thus, 14β-hydroxyprogesterone is remarkably similar to the cardiac glycosides with respect to molecular and cellular sites of action and, specifically, to the enhancement of cardiac contractility, which represents an important basis for the clinical use of the cardiac glycosides.

Results and Discussion

Progesterone (1) (see Scheme I) on incubation with *Mucor griseocyanus* (+) 1207a gave 14α-hydroxyprogesterone (2) as reported.[7,8] 14α-Hydroxyprogesterone was readily dehydrated with *p*-toluenesulfonic acid in benzene to the C-14-unsaturated derivative 3. This olefin was treated with *N*-bromoacetamide and water to give the bromohydrin 4, which was not isolated but further treated with potassium hydroxide to give the epoxide 5. Attempts to convert the bromohydrin 4 to 14β-hydroxyprogesterone (9) with Raney Ni under the conditions described by Wiesner et al.[9] also yielded the epoxide. Treatment of the epoxide with excess lithium triethylborohydride rapidly reduced, as evidenced by TLC, the C-3 and C-20 ketones and more slowly gave reductive opening of the epoxide 5. Neutral oxidation with pyridinium dichromate of the epimeric diols 6 gave 14β-hydroxyprogesterone (7). Reduction of the C-20 ketone removes the possibility of epimerization at C-17, which takes place under basic conditions (8).[10] This conversion from 14α- to 14β-hydroxyproge-

Table I. ^{13}C NMR Spectral Data of Progesterone Derivatives[a]

C	3	5	7	8
1	35.6	35.7	35.9	35.7
2	33.9	33.9	33.9	33.9
3	199.3	199.1p	199.4	199.2
4	24.1	124.3	123.8	124.1
5	170.4	169.5	171.0	170.0
6	32.6	32.2	33.1	32.9
7	(31.4)	27.9	27.9	27.3
8	34.9	33.6	40.0	41.3
9	53.4	52.7	42.9	49.5
10	38.6	38.7	(38.7)	38.6
11	21.9	20.7	20.7	(21.5)
12	41.7	38.9	(38.7)	[30.6]
13	48.1	45.4	49.0	48.0
14	150.1	73.3	84.5	86.3
15	118.1	(60.1)	34.0	[31.2]
16	29.9	27.1	24.9	(20.1)
17	65.1	(60.3)	62.1	61.0
18	18.5	15.7	15.2	18.8
19	17.5	17.7	17.6	17.5
20	209.1	211.5	217.8	210.2
21	(31.4)	29.3	33.4	31.9

[a] Spectra are in $CDCl_3$; chemical shifts are in ppm (Me_4Si internal standard); enclosed numbers in a column are interchangeable or overlapping.

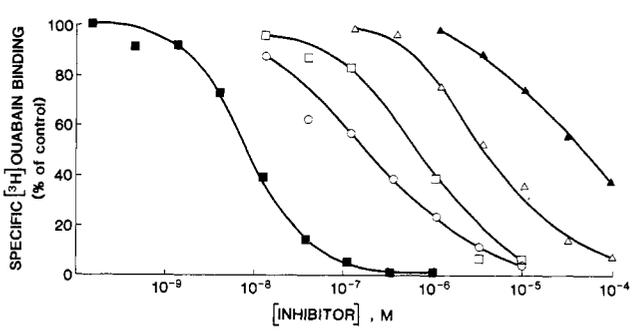


Figure 1. Inhibition of specific binding of [^{3}H]ouabain to cardiac trabecular tissue by various steroids: (■) ouabain, (○) chlormadinone acetate, (□) ouabagenin, (△) 14β-hydroxyprogesterone, (▲) progesterone.

(7) Singh, K.; Sehgal, S. N.; Vezina, C. *Can. J. Microbiol.* **1967**, *13*, 1271.
(8) Templeton, J. F.; Kumar, V. P. S.; LaBella, F. S.; Cote, D. *J. Nat. Prod.*, in press.
(9) Wiesner, K.; Tsai, T. Y. R.; Jaggi, F. J.; Tsai, C. S. J.; Gray, G. D. *Helv. Chim. Acta* **1982**, *65*, 2049.

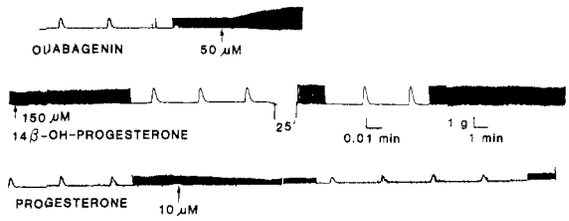


Figure 2. Change in active tension of electrically driven right ventricular trabecula by steroids dissolved in 20 μL of dimethyl sulfoxide and added to the bath at the arrow (↑). Final bath concentrations are indicated.

sterone can be carried out without isolation of intermediates in 32% overall yield. The structures of all derivatives are consistent with the ^{1}H NMR spectra and assignments of the ^{13}C NMR (Table I) have been made with the DEFT sequence[11] and internal consistency with related spectra.[9,12]

In the [^{3}H]ouabain radioligand binding assay, 14β-hydroxyprogesterone is about 10 times more potent than progesterone, $^1/_{10}$ as potent as ouabagenin, and $^1/_{40}$ as potent as chlormadinone acetate, the most potent congener identified thus far (Figure 1). 14α-Hydroxyprogesterone and the 17α-epimer of 14β-hydroxyprogesterone were inactive.

The most biologically significant characteristic of the novel 14β-hydroxyprogesterone is the consistent positive inotropy elicited in isolated cardiac tissue in the two species examined. A representative experiment is shown in Figure 2. In four experiments on isolated dog trabecula 14β-hydroxyprogesterone (150 μM) caused a 29.75 ± 3.17% (mean ± SEM) increase in contractile force. In comparison, progesterone (20 μM) caused 20.23 ± 2.3% depression of contractile force. In the guinea pig heart perfused by the Langendorff method, 14β-hydroxyprogesterone (100 μM) caused a 30% increase in left ventricular developed pressure and at 150 μM a 33% increase in contractile force of isolated guinea pig trabecular muscle. The response to the steroid is slow to develop, in contrast to the prompt stimulatory effect of ouabagenin. Heretofore, the receptor-active congeners, including progesterone and chlormadinone acetate, have usually exerted cardiodepression but, with chlormadinone acetate, occasional transient positive inotropy.[4,5] Additional data on the cardiac glycoside-like actions of 14β-hydroxyprogesterone have been presented in preliminary form[13] and will be presented in detail elsewhere. From Figure 2 it can be seen that, on the isolated ventricular trabecula, progesterone is relatively potent in promptly causing cardiodepression, in comparison to effective concentrations of the cardiostimulant 14β-hydroxyprogesterone.

14β-Hydroxyprogesterone is only about $^1/_{10}$ to $^1/_{15}$ as potent as ouabagenin and only $^1/_{1000}$ to $^1/_{5000}$ as potent as ouabain in the radioligand binding assay. Because of the insolubility of progesterone and its congeners, the true effective concentrations and relative potencies are probably considerably less than those calculated. Perhaps, a glycoside of the steroid would be significantly more potent than the aglycon. The 3-hemisuccinate (unpublished data) or 3-rhamnoside[14] of chlormadinone acetate have both been found to be considerably less potent in the radioligand binding assay; in this instance, the lower potency of the glycoside may reflect the noncardiac glycoside-like configuration of the steroid moiety in chlormadinone acetate.

The cardiac glycoside-like actions of 14β-hydroxyprogesterone support our proposal that naturally occurring compounds, structurally related to progesterone, are likely candidates for putative endogenous digitalis-like hormones.

Experimental Section

Steroid Synthesis. ^{1}H NMR spectra were recorded in deuteriochloroform on a Bruker AM 300 instrument. HPLC was carried out in 1% ethanol/dichloromethane on a Waters μ-Porasil (10 μm) column using a Waters M45 instrument. Preparations were monitored by TLC on silica gel (Merck type 60 H) plates in 25–75% ethyl acetate/hexane followed by spraying with 4% concentrated sulfuric acid and heating 5–10 min at 110 °C to produce a characteristic color. Column chromatography was carried out on silica gel (Merck type 60 H for TLC). Melting points are uncorrected. Chemical microanalyses for carbon and hydrogen are within ±0.3% of the theoretical values.

14α-Hydroxyprogesterone (2). 14α-Hydroxyprogesterone was prepared by incubation of progesterone (1) with *Mucor griseocyanus* (+) 1207a as previously described[7,8] (lit.[7] mp 198–200 °C): mp 204–206 °C from dichloromethane/ethyl acetate; ^{1}H NMR δ 0.79 (3 H, s, C-13 CH_3), 1.20 (3 H, s, C-10 CH_3), 2.12 (3 H, s, C-20 CH_3), 3.22 (1 H, t, J = 8.4 Hz, 17α-H), 5.74 (1 H, d, J = 1.5 Hz, C-4 H).

Pregna-4,14-diene-3,20-dione (3). To a stirred solution of 14α-hydroxyprogesterone (1.0 g, 3.03 mmol) in benzene (150 mL) was added *p*-toluenesulfonic acid monohydrate (0.3 g) and the mixture was maintained at 50–60 °C for 20 min. The mixture was washed with aqueous $NaHCO_3$ to give, on recrystallization from dichloromethane/acetone, pregna-4,14-diene-3,20-dione (0.6 g, 63%): mp 144–146 °C; ^{1}H NMR δ 0.91 (3 H, s, C-13 CH_3), 1.21 (3 H, s, C-10 CH_3), 2.16 (3 H, s, C-20 CH_3), 5.21 (1 H, dd, J = 4 and 8 Hz, C-15 H), 5.76 (1 H, d, J = 2 Hz, C-4 H). Anal. ($C_{21}H_{28}O_2$) C, H.

14,15β-Epoxy-14β-pregn-4-ene-3,20-dione (5). To a solution of pregna-4,14-diene-3,20-dione (259 mg, 0.83 mmol) in 50% acetone–water (13 mL) under argon was added 5% aqueous $HClO_4$ (0.25 mL) followed by *N*-bromoacetamide (134 mg, 0.97 mmol). After 15 min the reaction mixture was made alkaline by dropwise addition of 5% aqueous KOH. Dilution with water and ether extraction gave the 14,15-epoxide (121 mg, 45%): mp 187–188 °C from dichloromethane/methanol; second crop 100 mg (30%), mp 181–185 °C; ^{1}H NMR δ 1.08 (3 H, s, C-13 CH_3), 1.22 (3 H, s, C-10 CH_3), 2.19 (3 H, s, C-20 CH_3), 3.51 (1 H, s, 15α-H), 5.74 (1 H, s, C-4 H). Anal. ($C_{21}H_{28}O_3$) C, H.

14-Hydroxy-14β-pregn-4-ene-3,20-dione (7). The 14β,15β-epoxide **5** (121 mg, 0.37 mmol) in dry tetrahydrofuran (15 mL) under argon was treated with lithium triethylborohydride (1 mL) (Super-Hydride, 1.0 M solution in tetrahydrofuran, Aldrich Chem. Co., Milwaukee, WI). After 2 h of reflux, the reaction mixture was cooled in an ice bath and stirred with 5% NaOH (1 mL) and 30% H_2O_2 (1 mL) for 1 h. The mixture was diluted with water and extracted with dichloromethane to give a product, which was dissolved in dry dimethylformamide (4 mL) and pyridinium dichromate (1.0 g, 2.66 mmol) added. After the mixture was stirred for 10 h, water was added and the mixture extracted with ether, which was washed with dilute HCl and aqueous $NaHCO_3$ to give the 14β-hydroxy compound (83 mg, 73%): mp 180–181 °C from dichloromethane/acetone; ^{1}H NMR δ 1.02 (3 H, s, C-13 CH_3), 1.18 (3 H, s, C-10 CH_3), 2.25 (3 H, s, C-20 CH_3), 2.94 (1 H, dd, J = 4.1, 8.2 Hz, 17α-H), 4.51 (1 H, s, 14β-OH), 5.74 (1 H, d, J = 1 Hz, C-4 H). Anal. ($C_{21}H_{30}O_3$) C, H.

14-Hydroxy-14β,17α-pregn-4-ene-3,20-dione (8). 14β-Hydroxyprogesterone (25 mg) was dissolved in 0.5 M methanolic KOH (5 mL) under argon. After 1 h at 20 °C no change in TLC of the reaction product from the starting material was observed whereas 1 h reflux showed the formation of a second component, which, after dilution with water followed by ether extraction and HPLC separation, gave the C-17 epimer 8 (14 mg, 56%): mp

(10) Danieli, N.; Mazur, Y.; Sondheimer, F. *Tetrahedron* **1966**, *22*, 3189.
(11) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(12) Habermehl, G. C.; Hammann, P. E.; Wray, V. *Magn. Reson. Chem.* **1985**, *23*, 959.
(13) Kobayashi, T.; Bose, D.; Elliott, D.; Templeton, J. F.; Kumar, V. P. S.; Kim, R. S.; LaBella, F. S. *Biophys. J.*, in press.
(14) Repke, K. *Trends Pharmacol. Sci.* **1985**, *6*, 275.

139–141 °C from dichloromethane/acetone; ^{1}H NMR δ 1.18 (3 H, s, C-13 CH_3), 1.26 (3 H, s, C-13 CH_3), 2.16 (3 H, s, C-20 CH_3), 3.25 (1 H, t, J = 9.3 Hz, 17β-H), 5.30 (1 H, d, J = 0.7 Hz, C-4 H). Anal. ($C_{21}H_{30}O_3$) C, H.

[^{3}H]Ouabain Radioligand Binding Assay. Hearts from pentobarbital-anesthetized mongrel dogs were removed and immediately immersed in Krebs–Henseleit buffer (mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.4, $NaHCO_3$ 26, glucose 11) equilibrated with 95% O_2–5% CO_2 at room temperature. Within 60 min the ventricles were chopped with scissors and disintegrated by Polytron treatment (Brinkman Instruments Inc.) in ice-cold 50 mM Tris buffer, pH 7.4. The suspension was passed through a coarse screen to remove the bulk of connective tissue and the filtrate was centrifuged at 34000g for 45 min at 4 °C. The supernatant was discarded and the pellet resuspended in the buffer for radioligand binding assay (see below) and stored at −20 °C. Once thawed, the tissue suspension was used immediately or discarded. The buffer was 45 mM Tris, 5 mM Tris phosphate, 3 mM $MgCl_2$, pH 7.4. Duplicate assay tubes contained 2.0 nM [^{3}H]ouabain in 0.1 mL of buffer, approximately 10 mg of tissue (wet weight) in 0.1 mL of buffer, and steroids (see below). Nonspecific binding (about 10% of total binding) was determined in the presence of 0.1 mM ouabain. Following incubation at 0 °C for 90 min, assay tubes were centrifuged at 2700g for 25 min at 4 °C and the supernatants aspirated off. Pellets were dissolved in 0.3 mL of 2 N KOH and 0.2-mL aliquots dispensed into vials for liquid scintillation counting. Data were expressed as percent of specifically bound [^{3}H]ouabain (i.e., difference between total binding and nonspecific binding). The steroids dissolved in a few microliters of 95% ethanol were added to reaction tubes; this volume of ethanol alone had no effect on specific binding of [^{3}H]ouabain. Similar potencies were obtained for the steroids in the radioligand binding assay when the ethanolic solutions were evaporated to dryness in the reaction tube, tissue suspension was added, and the tube was vortexed for 1 min.

Measurement of Contractility of Isolated Cardiac Tissue. Dogs of either sex, weighing between 5 and 12 kg were anesthetized with sodium pentobarbital (35 mg/kg ic), and the heart was removed. Thin (<1-mm diameter), free-running trabecula were tied to the base of a perspex holder which had embedded electrodes for punctate stimulation. The opposite end of the tissue was connected to a Grass FT-03C isometric force transducer and a Grass Polygraph. A Pulsar 6i stimulator (F Haer Co.) connected to a custom-built computer-controlled programmable pulse generator[15] provided square wave stimuli of 3-ms duration to the trabecula. Stimulus amplitude was adjusted to about 10–20% above threshold. The resulting tension was increased until the maximum active tension in response to electrical stimulation was evoked. The basic stimulus interval was 2000 ms. Tissues were placed in a 10-mL vertical tissue bath containing Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2, and the bath temperature was maintained at 37.0 ± 0.2 °C. The muscle was equilibrated for 1 h prior to the experiment.

Acknowledgment. We are grateful to the Manitoba Heart Foundation for grants to D.B., F.S.L., and J.F.T. F.S.L. is a Career Investigator of the Medical Research Council of Canada. ^{1}H NMR spectra were recorded by Kirk Marat, Department of Chemistry, and HPLC by Diane Smith, Faculty of Pharmacy, University of Manitoba.

(15) Boyechko, G.; Bose, D. *J. Pharmacol. Methods* **1984**, *12*, 45.

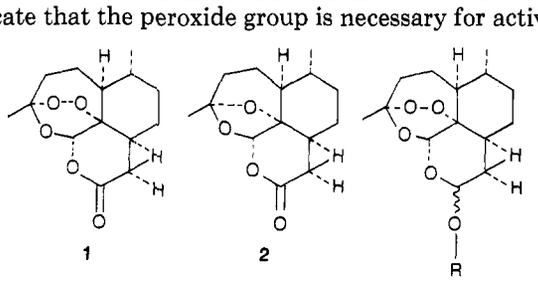
Endoperoxides as Potential Antimalarial Agents

John A. Kepler,*† Abraham Philip,† Y. W. Lee,† H. A. Musallam,‡ and F. Ivy Carroll*†

Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709, and Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307. *Received November 3, 1986*

A number of mono- and bicyclic endoperoxides were prepared and tested for antimalarial activity in search of a simplified analogue of the 5-oxygen-substituted 1,2,4-trioxane ring structure of the naturally occurring antimalarial qinghaosu. The compounds were assayed in an in vitro system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. The most active compound in this assay was 2-[((butyloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (**17a**), which showed an IC_{50} of 100 and 57 ng/mL, respectively. For comparison, qinghaosu exhibits a mean IC_{50} < 3.4 ng/mL.

Artemisinin (qinghaosu, **1**), a novel sesquiterpene lactone antimalarial drug, shows antimalarial activity in both animals and humans against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*.[1-3] Structure–activity relationship studies on **1** show that desoxyqinghaosu (**2**) is inactive whereas dihydroqinghaosu (**3**) and its ether and ester derivatives are active.[1,3] These results indicate that the peroxide group is necessary for activity.



1 **2** **3**

3a, R = H

Since no information about the necessity of the remainder of the qinghaosu molecule is available, we have undertaken an investigation to prepare three types of model compounds for antimalarial evaluation: (1) cyclic peroxides, (2) 1,2,4-trioxanes, and (3) 1,2,4-trioxanes containing a 5-oxygen substituent. These three types of compounds separate the 5-oxa-1,2,4-trioxane ring of **1** into its component parts while maintaining the peroxide function. In this paper we present the syntheses and antimalarial evaluation of members of the first set of compounds, cyclic peroxides.

Chemistry

1,5,5-Trimethyl-6-(3-acetoxybutyl)-3,6-epidioxycyclohexene (**7**) was prepared in order to explore the efficacy of a peroxide group in a simple monocyclic structure for

† Research Triangle Institute.
‡ Walter Reed Army Institute of Research.

(1) Klayman, D. L. *Science (Washington, D.C.)* **1985**, *228*, 1049.
(2) (a) Liu, J.-M.; Ni, M.-Y.; Fan, J.-F.; Tu, Y.-Y.; Wu, Z.-H.; Wu, Y.-L.; Chu, W.-S. *Huaxue Xuebao* **1979**, *37*, 129. (b) Qinghaosu Research Group *Scientia Sinica* **1980**, *23*, 380.
(3) Qinghaosu Antimalaria Coordinating Research Group *Chin. Med. J. (Beijing, Engl. Ed.)* **1979**, *92*, 811.

0022-2623/87/1830-1505$01.50/0