Synthesis and Structure−Activity Relationships of 17β-Substituted 14β-Hydroxysteroid 3-(α-L-Rhamnopyranoside)s: Steroids That Bind to the Digitalis Receptor

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Received December 30, 1996[⊗]

The preparation of 17β-substituted 14β-hydroxysteroid C-3 α-L-rhamnopyranosides is described. These derivatives have a 14β,20-ether, 14β,20-lactone, or 17β-CH_2CH_2OH, $-CH_2CH_2NH_2$, $-CH{=}CHNO_2$(*E*), $-CH{=}CHCOOH$(*E*), $-CH(OH)CH_2NO_2$(*R*), $-CH(OMe)CH_2NO_2$(*R*), $-CH_2CH_2COOH$, or $-CH(OH)CH_2NH_2$(*R*) group. Derivatives were assayed in a radioligand binding assay for [^{3}H]ouabain in membranes from canine heart muscle. The digitalis "receptor" comprises isoenzymes of the ion-pumping enzyme, Na^+,K^+-ATPase. The 17β-$CH{=}CHNO_2$(*E*), 17β-$CH{=}CHCOOH$(*E*), and 17β-$CH(OMe)CH_2NO_2$(*R*) derivatives were the most potent and equivalent to ouabain with low-nanomolar IC_{50} values. The very potent binding affinity of the disubstituted compound 17β-$CH(OMe)CH_2NO_2$(*R*) further demonstrates that 17β-unsaturated substitution is not required for potent binding affinity. This observation may be of value in the separation of cardiotonic and cardiotoxic effects. Tosylation of the 17β-CH_2OH, prepared from the 17β-CHO by lithium aluminum hydride reduction, yielded the 14β,17β-ether. Synthesis of the 17β-CH_2COOH gave the epimeric 14α,17α- and 14β,17β-lactones. Structures have been established by NMR analysis.

Introduction

During our investigation into the structure−activity relationships of 14β-hydroxy-21-norpregnanes and 14β-hydroxypregnanes possessing digitalis-like activity the 20-amino- and 20-nitro-14β-hydroxy-3β-(α-L-rhamnopyranosyloxy)-21-nor-5β-pregnane and 21-nitro-14β-hydroxy-3β-(α-L-rhamnopyranosyloxy)-5β-pregnane were shown to bind strongly to the digitalis receptor of heart muscle in a radioligand binding assay.[1,2] Isosteric and isoelectronic analogues of these amino and nitro compounds, together with alcohol and carboxylic acid derivatives, have been synthesized. The 20,21-disubstituted and 20-unsaturated 14β-hydroxypregnane α-L-rhamnopyranosides have been prepared, and their digitalis receptor binding affinity has been determined. The nature of the 17β-pregnane side chain has been shown to have a major influence on binding affinity.[1−3] We report here on the chemical synthesis and receptor binding affinity of these derivatives as measured in a radioligand binding assay.[4]

Results and Discussion

Chemistry. The 17β-aldehyde **1**, obtained from degradation of the 17β-unsaturated lactone present in the natural cardiac glycosides, is a key synthetic intermediate in the synthesis of C-17 steroid derivatives.[2] Modification of our earlier procedure increased the overall yield of the 17β-aldehyde **1** from digitoxigenin from 27% to 86%.[2]

Attempts to convert the 20-alcohol[2] **2**, obtained by lithium tri-*tert*-butoxyaluminum hydride (LTBAH) reduction of the aldehyde **1**, into the 20-tosylate **3,** a suitable leaving group for substitution and chain extension, led to rapid intramolecular cyclization to the 14β,-20-ether **4a** (Scheme 1). Hydrolysis of the benzoate esters in the oxide **4a** with NH_3/MeOH gave the rhamnopyranoside **4b**.

A number of methods are available for the extension of an aldehyde by one carbon unit.[5,6] The synthetic route to the alcohol **8** shown in Scheme 1 was chosen because of its simplicity and the stability of other functional groups to the reagents employed.[5−10] 2-Chloro-1,3-dithiane was prepared as described by Arai and Okai[7] and converted into 1,3-dithiane 2-triphenylphosphonium chloride as described by Kruse *et al*.[8] Condensation of the aldehyde **1** with the ylid generated from the phosphonium salt followed by hydrolysis with Hg^{II}-Cl_2[9] gave two isomeric products (**6** and **7a**) which were separated by chromatography. The major compound was identified as the 17β-isomer of the lactone **7a** and the minor product the 17α-isomer **6**. Hydrolysis of the 17β-isomer with NH_3/MeOH gave the rhamnopyranoside **7b**. The 17β-lactone **7a** was reduced with lithium aluminium hydride (LAH), to give the 14β,21-diol **8**. The 14β-hydroxyl group is ideally located for cyclization to a 5- or 6-membered ring. The small amount of the 17α-isomer **6** obtained may result from epimerization of the aldehyde **1** by the sodium ethoxide used in the reaction to produce the intermediate ketene dithioacetal. Formation of the 14α,17α-lactone **6** may result, after initial epimerization at C-17, from carbonium ion formation at C-14 induced by the acidic $HgCl_2$/MeCN/H_2O conditions. C-17 epimerization decreases steric crowding of the 14-OH and, perhaps also through anchimeric assistance by the carboxyl group, favors carbonium ion formation. The carboxylic acid would be favorably located for intramolecular cyclization to the lactone. While generally not reactive the sterically hindered

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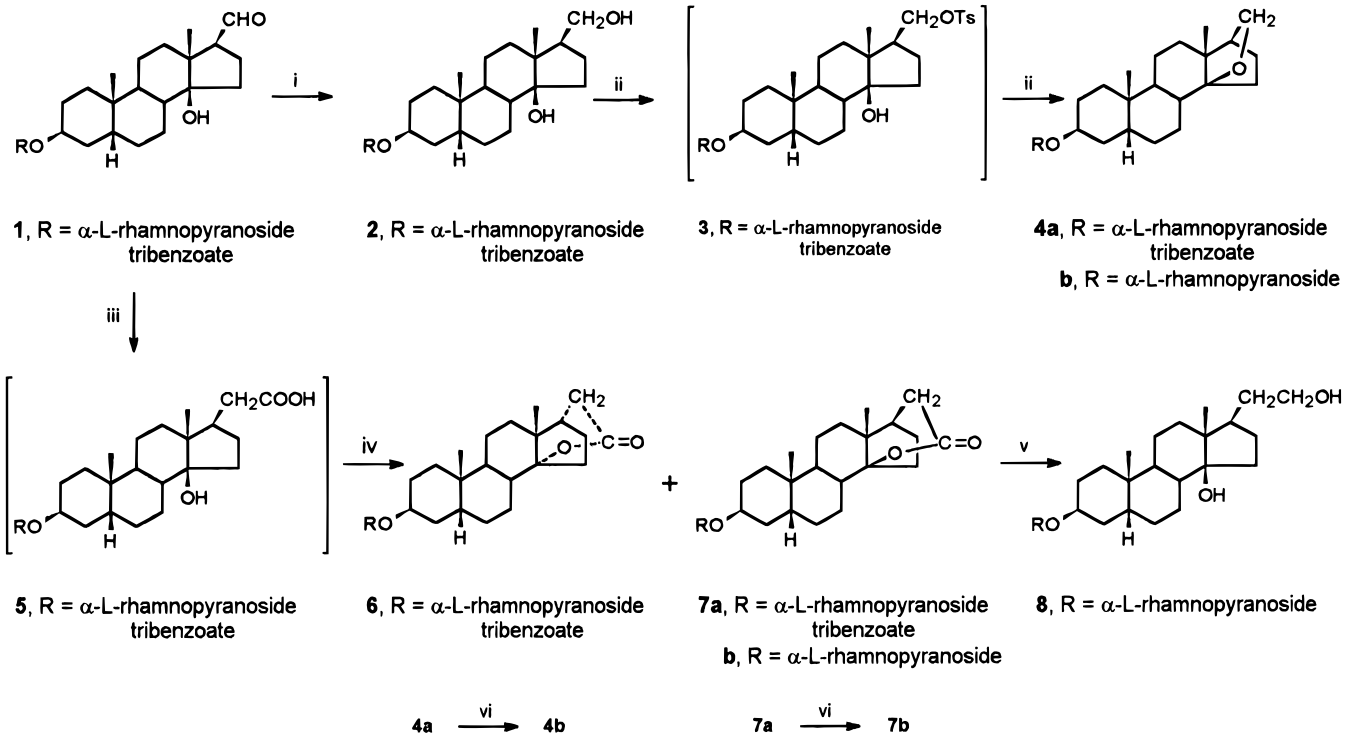
⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1997.

S0022-2623(96)00880-1 CCC: $14.00

Scheme 1[a]



[a] (i) LTBAH/Et_2O; (ii) TsCl/pyridine; (iii) NaOMe/EtOH/PhP_3 + 1,3-dithiane Cl^-; (iv) $HgCl_2/CH_3CN/H_2O$; (v) LAH/Et_2O; (vi) NH_3/MeOH.

tertiary 14-alcohol readily undergoes these intramolecular cyclizations.

Condensation of the aldehyde **1** with nitromethane in the presence of KF gave only one isomer, the 20(*R*)-nitrol **9a** (Scheme 2).[10] This stereoselectivity may result from steric hindrance by the 13-Me group and hydrogen bonding between the 14β-OH and the 17β-CHO, which orients the aldehyde so that approach of the nitromethane carbanion occurs to the *re* face of the carbonyl bond.

Attempts to hydrolyze the benzoate groups in **9a** with NH_3/MeOH yielded a mixture of products containing aldehyde signals in the ^1H NMR spectrum. The 2-nitrol group can undergo a retro-aldol reaction in base.[11] To avoid this reaction the 20-hydroxyl group in **9a** was protected as the silyl ether **10**. Employing the conventional method (*t*-BuMe_2SiCl/imidazole/DMF) preparation of the silyl ether failed. However, when the nitrol **9a** was treated with *tert*-butyldimethylsilyl triflate and triethylamine, the noncrystalline silyl ether **10** was obtained.

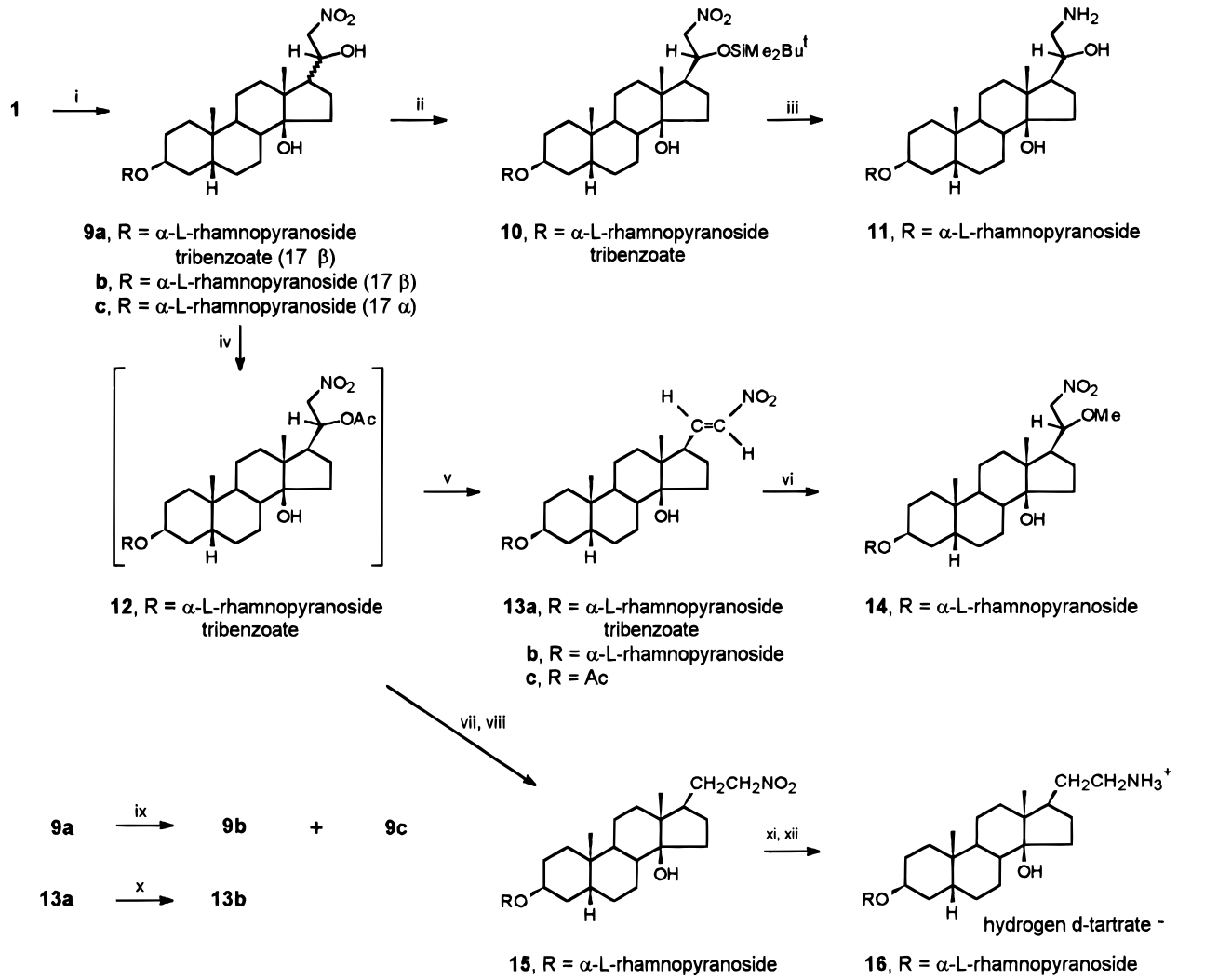
In 1974, Eberlein *et al.*[12] reported that when the 17β-aldehyde trisdigitoxose tetraacetate obtained from digitoxin was condensed with nitroethane in the presence of NaOMe/MeOH for 3 h, the 17β-(2-nitro-1-hydroxypropyl) glycoside was obtained as two pairs of diastereoisomers.[12] When similar conditions were applied to the condensation of the 17β-aldehyde tribenzoate **1** with nitromethane, the major product obtained was the nitrol **9b** together with the 17α-epimer **9c** as a minor product. When the condensation reaction was carried out for a longer time (16 h) the proportion of 17α-epimer **9c** increased. This result is indicative of reversible aldehyde condensation with nitromethane and epimerization of the 17β-aldehyde followed by nitromethane condensation (Scheme 3).

Dornow *et al.*[13] reported an improved yield of aminol on LAH reduction of a nitrol when a solution of LAH was added to the nitrol rather than the reverse. The nitrol **9a** was therefore reduced in this way to give the aminol **11**. The silyl-protected nitrol **10** was also reduced with LAH in refluxing Et_2O to give the aminol **11** because it has been reported that the silyl derivative can protect the nitrol group from bond cleavage.[14] However, in both cases the yield of the aminol **11** was low.

The 17β-CH=C(CH_3)NO_2(*E*) trisdigitoxoside, prepared by Eberlein *et al.*,[12] showed weak digitalis-like activity on heart muscle. However, because of the high reactivity of the nitroethylene group, it proved necessary to have a methyl group at C-22 to increase the stability of nitroethylene in order to prepare the glycoside.[12] Nevertheless, despite the instability of the unsubstituted olefin, we have been able to synthesize the unsubstituted derivative **13b** by the following procedure.

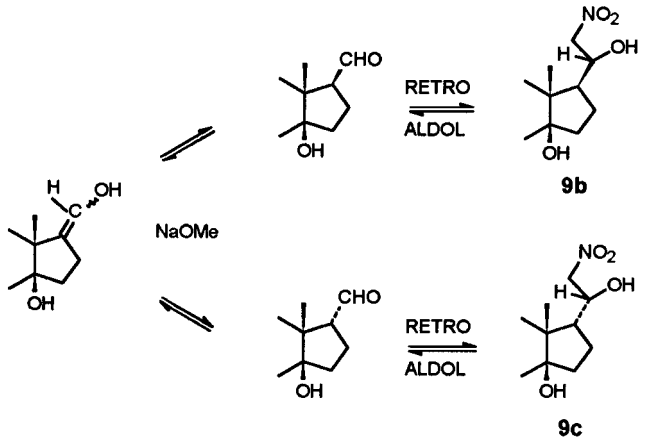
The nitrol **9a** was acetylated to give the intermediate nitrol acetate **12**, a compound prone to elimination, which formed the nitroethylene **13a** during chromatographic purification on SiO_2. Attempts to hydrolyze the benzoate groups in **13a** with NH_3/MeOH caused a series of changes probably due to the reactivity of the nitroethylene group.[12] We therefore studied the hydrolysis by NaOMe in more detail and found that although hydrolysis of the benzoates took place very rapidly so did Michael addition of MeO^- at C-20. When the nitroethylene **13a** was treated with 0.25 M NaOMe/MeOH in an ice−water bath, the 20(*R*)-methoxy derivative **14** was separated as the main product. Steric requirements favor selective addition to the double bond from the *re* face. When **13a** was treated with 0.25 M *t*-BuOK/*t*-BuOH for 10 min, the bulky *t*-BuO^- group

Scheme 2[a]



[a] (i) $MeNO_2$/KF; (ii) *t*-$BuMe_2SiSO_3CF_3$/Et_3N; (iii) LAH/Et_2O; (iv) Ac_2O/DMAP; (v) SiO_2; (vi) NaOMe/MeOH; (vii) $NaBH_4$/EtOH; (viii) NH_3/MeOH; (ix) $MeNO_2$/NaOMe; (x) *t*-KOBu/*t*-BuOH; (xi) H_2/PtO_2/HOAc; (xii) *d*-tartaric acid.

Scheme 3. Enolization/Aldol/Retro-Aldol Equilibria



retarded Michael addition as expected and the rhamnopyranoside **13b** was obtained in 32% yield.

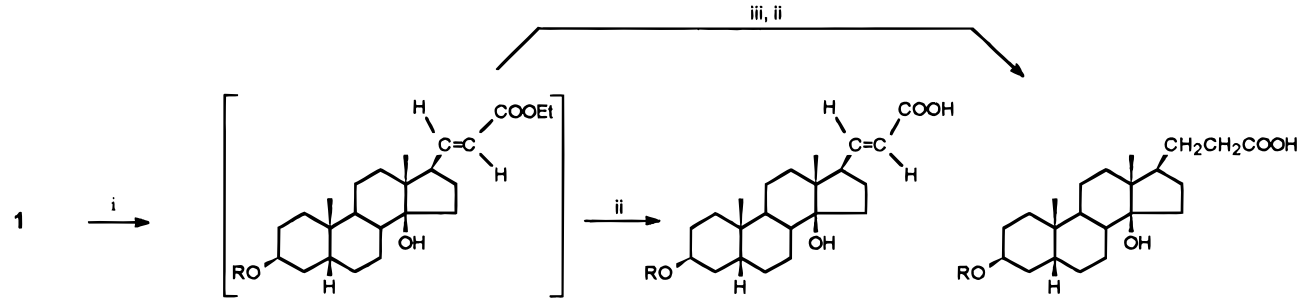
The 21-nitro compound **15** was prepared from the nitrol **9a** via the intermediate **12** as described in ref 2. Compound **15** was hydrogenated in glacial HOAc at 5 atm with PtO_2 as catalyst to give the 21-amine which was isolated as the hydrogen tartrate salt **16**.

The aldehyde **1** reacted readily with the anion of [(ethoxycarbonyl)methyl]diethylphosphonate to give the α,β-unsaturated ester **17** (Scheme 4).[15] Hydrolysis of the esters in compound **17** gave the acid **18**. The ester **17** was hydrogenated at 5 atm with PtO_2 as catalyst to give the saturated ester, which was hydrolyzed to give the saturated acid **19**. The proposed mechanism for the phosphonate modification of the Wittig reaction suggests that only the *E* isomer is produced under these conditions.[16]

Nuclear Magnetic Resonance Structural Assignments. Structures of all synthetic compounds were established from the ^1H and ^{13}C NMR and are in agreement with published data.[1,2] Complete 2D assignments (HSQC and COSY) were performed on compounds **6**, **7a**, **9a**−**c, 13a, 14** and **18**.

The structure of **4a** was established by comparison of the ^1H and ^{13}C NMR with compound **2** which showed the expected changes to the C-20 methylene protons. Compound **6** showed a relatively small NOE (*ca.* 2%) from the C-13 methyl to the C-20 protons, in contrast to **7a** (4%). However, a substantial NOE was observed from the C-13 methyl group to H-17 (6.0%), with a corresponding NOE observed from the C-13 methyl (3.5%). These data clearly establish that the C-17 side chain in **6** has α stereochemistry. The C-14 signal in the 14α,17α-isomer **6** at 85.76 ppm, unlike the 14β,17β-isomer **7a** at 87.09 ppm, clearly distinguishes between

Scheme 4[a]



17, R = α-L-rhamnopyranoside tribenzoate **18**, R = α-L-rhamnopyranoside **19**, R = α-L-rhamnopyranoside

[a] (i) $(EtO)_2POCH_2COOEt$/NaH; (ii) NaOMe/MeOH; (iii) H_2/PtO_2/EtOH.

the isomers. The β-face stereochemistry of the lactone **7a** was established by the observation of NOEs from the C-13 methyl to the low-field C-20 proton (4.1%) and from the low-field C-20 proton to the C-13 methyl group (4.5%). Only a relatively small NOE (<2%) was observed from the C-13 methyl group to H-17 which gave further confirmation of the stereochemistry. The structures of both lactone isomers **6** and **7a** were determined unambiguously using 2D-NMR techniques.

The 21-alcohol **8** showed comparable spectra to the 21-nitro derivative **15** which is in agreement with the structure proposed. In nitrol **9c** *J* (17,20) is very small (*ca.* 1 Hz) indicating a gauche arrangement of H-17 and H-20. Irradiation of the C-13 methyl showed NOEs to H-20 (8.8%), H-8 (3.4%), and H-12β (1.9%) and indicates a β stereochemistry for the C-17 side chain.

The silyl derivative **10** shows a value of 1.5 Hz for *J* (17,20) indicating a gauche arrangement of H-20 and H-17. Irradiation of H-20 resulted in a 1.6% NOE of the C-13 methyl group, while irradiation of the C-13 methyl group resulted in a 5.5% NOE of H-20, clearly indicating that H-20 is anti to C-16 as opposed to anti to C-13. Irradiation of the C-13 methyl also resulted in a 2.4% NOE of the low-field C-20 Me_3Si. Irradiation of the low-field Me_3Si resulted in a 0.9% NOE of the C-13 methyl. The NOEs observed between the C-13 methyl and the C-20 Me_3Si indicate that the C-20 *t*-$BuMe_2Si$ group must be anti to H-17. With the location of H-20 already established, this is sufficient to determine the C-20 stereochemistry as *R*.[17] NOE for this compound was determined in benzene-d_6 to separate the side chain proton shifts. The 20(*R*) configuration was therefore established for **9a** (and **9b**) based on the silyl ether **10**.

The *J* (20,21) coupling of 13.3 Hz in the nitroethylene **13a** indicates a trans rather than a cis arrangement of H-20 and H-21. This assignment was confirmed by the absence of a detectable NOE between the two protons. Irradiation of H-21 resulted in a 1.7% NOE to H-17, while irradiation of the C-13 methyl group resulted in a 4.2% NOE to H-20. These data, along with a *J* (17,-20) of 11.5 Hz suggest that the C-17 side chain adopts a conformation in which H-17 is anti to H-20. This analysis establishes the *E* stereochemistry for **13a**.

A value of 1.5 Hz was observed for *J* (17,20) in the methoxy derivative **14**. Irradiation of the C-13 methyl resulted in NOEs to H-20 (13.5%) and C-20 (3.65%). Irradiation of the C-20 methoxy resulted in NOEs to H-20 (6.1%) and the C-13 methyl (1.5%). These data establish that H-20 is anti to C-16 and that the C-20 methoxyl is anti to H-17. The C-20 stereochemistry is thus *R*. NOE measurements involving the C-21 protons were not possible because of overlap with the solvent peak.

Table 1. [^{3}H]Ouabain Radioligand Assay Potency of 17β-Substituted 14β-Hydroxypregnane 3β-(α-L-Rhamnopyranoside)s[a,b]

no.	17β	IC_{50}, nM, ± SE
4b	14β,20-ether	2200 ± 320
7b	14β,20-lactone	NA[c]
8a	$-CH_2CH_2OH$	660 ± 70
9c	$-CH(OH)CH_2NO_2(R)$	4730 ± 420
11	$-CH(OH)CH_2NH_2(R)$	3220 ± 250
13b	$-CH{=}CHNO_2(E)$	8.3 ± 2.2
14	$-CH(OMe)CH_2NO_2(R)$	30 ± 7.5
16[d]	$-CH_2CH_2NH_2$	1890 ± 220
18	$-CH{=}CHCOOH(E)$	13 ± 2.2
19	$-CH_2CH_2COOH$	230 ± 30
20[e]	$-CH_2CH_2NO_2$	45.5 ± 8.2

[a] IC_{50} represents the concentration that inhibits binding of [^{3}H]ouabain by 50%. [b] Digitoxin IC_{50} = 8 nM. [c] Not active, >100 μM. [d] Hydrogen tartrate salt. [e] See ref 2.

The *E* geometry of the 20,21-double bond in **17** and **18** was evident from the coupling constant of the doublet (J = 15.5 Hz) for H-21. In the unsaturated acid **18** the 15.5 Hz value for *J* (20,21) and the lack of any observable NOE between these protons clearly indicate a trans arrangement of H-20 and H-21.[15] Irradiation of H-21 resulted in a 3% NOE to H-17, while irradiation of H-17 resulted in a 7.4% NOE to H-21. Irradiation of the C-13 methyl group resulted in a 7.5% NOE to H-20. *J* (17,-20) is 10.7 Hz. This coupling and the NOE data suggest that the side chain adopts a conformation in which H-17 is anti to H-20 as seen in **13a**.

Radioligand Receptor Binding and Structure−Activity Relationships. The relatively weak binding affinity obtained for the cyclic ether **4b** (see Table 1) nevertheless shows that, as has been observed previously, neither the unsaturated lactone[1,2] nor the 14β-hydroxyl group of the natural cardiac glycosides are essential for strong receptor binding.[3] Similarly the binding affinity values of **14**, **19**, and **20** show that an unsaturated system at C-17 is not required for strong receptor binding although considered necessary for strong positive inotropy[3,18] (see below). Examination of 3β-rhamnopyranosyl-14β-amino-5β-pregnan-20(*R*)-ol (LND 623) indicates that the saturated pregnane side chain is adequate for strong inotropic activity.[19] The lack of significant affinity observed for the 6-membered 14β,17β-lactone ring **7b** shows that this substituent

interferes with receptor binding, more so than the smaller, and less polar, 5-membered ether ring in **4b**.

The derivatives which are isosteric with the naturally occurring unsaturated lactone ring, **13b** and **18**, show binding affinity approaching that of the natural cardiac glycosides (*ca.* 8 nM). Thomas *et al.*[20] demonstrated that bioisosteres of the unsaturated lactone ring such as the esters −CH=CHCOOMe(*E*) showed positive inotropy while the acid −CH=CH-COOH(*E*) was inactive. The nitroethylene **13b** and the unsaturated carboxylic acid **18** show very strong binding affinity. In both compounds **13b** and **18**, the NMR results clearly demonstrate that the C-17 side chain and its polar substituent are orientated so that C-21 eclipses H-17.[21] This places the polar C-21 substituent in a similar location to that of the C-23 carbonyl in the cardiac glycosides and supports the proposal that optimum receptor binding requires a polar group in this location and a positive center at C-20.[3] Thomas *et al.*[3] correlated positive inotropy with the size of the positive charge induced at C-20 based on its chemical shift which places **13b** (C-20 138.92 ppm) among the potentially least potent inotropic compounds. However, while consistently the 20-methyl glycoside analogue of **13b**, synthesized by Eberlein *et al.*,[12] showed only weak cardiotonic activity, this may be due to steric factors which do not permit the 17-substituent to take up the most favorable conformation. The saturated analogues, **19** and **20**, of **18** and **13b**, respectively, while binding strongly to the receptor, bind somewhat more weakly than **13b** and **18**. Thomas *et al.*[3,18] have shown that inotropic activity was almost completely abolished upon saturation of the C-20 double bond in the 17β-acrylate, whereas these results indicate that receptor binding affinity is much less affected.

The saturated 21-nitro-20(*R*)-methoxy-substituted derivative **14**, unlike the corresponding 21-nitro-20(*R*)-alcohol **9c**, binds strongly, comparable to the unsubstituted 21-nitro derivative **20**. The 21-amino-20(*R*)-alcohol **11**, like the 21-nitro-20(*R*)-alcohol **9c**, shows weak binding affinity. The 21-amino derivative **16** shows weak binding compared with the much stronger binding affinity of the 21-nitro derivative **20**. The 21-alcohol **8a** binds more strongly than the 21-amino derivative **16** but less strongly than the 21-nitro derivative **20**. Differences in receptor binding may result from competition between the hydrogen-bonding donor molecules **8a** and **16** and the acceptor molecule **20**. These results are in agreement with the demonstration by Repke and co-workers[22] that the 5β,14β-androstane-3β,14-diol is the basic steroid structure of the cardiac glycosides possessing cardiotonic activity.

Conclusions

Pregnane 3β-α-L-rhamnopyranoside derivatives have been synthesized from digitoxigenin. Some derivatives show receptor binding affinity comparable to the naturally occurring cardiac glycosides as measured in a radioligand binding assay. Unsaturation at C-20 is not required for strong receptor binding affinity. Some synthetic pregnane glycosides have been shown to possess an improved margin of safety compared with the naturally occurring cardiac glycosides.[23−25] Pharmacological studies of compounds **13b**, **14**, **18**, and **20** may provide structure−activity relationship evidence to permit differentiation between cardiotonic and cardiotoxic effects.[3]

Experimental Section

General. All ^{1}H and ^{13}C NMR survey spectra were recorded on a Bruker AM300 spectrometer, while homonuclear correlation (COSY), heteronuclear correlation (HSQC), and nuclear Overhauser effect (NOE) difference spectra were recorded on a Bruker AMX500 instrument as described previously.[26] All *J* values are reported in hertz (Hz). Samples were *ca.* 50 mM solutions in 5 mm tubes in the solvents reported. For samples in $CDCl_3$ the residual $CHCl_3$ peak in the solvent (δ_H 7.26 ppm, δ_C 77.0 ppm) was used as the internal reference; for other solvents Me_4Si was used as an internal reference. All spectra were run at 300 K. Carbon spectra were classified as to multiplicity with the DEPT technique.[27] Complete ^{1}H and ^{13}C NMR assignments are reported for the tribenzoate **13a** and α-L-rhamnopyranoside **14**; for the remaining compounds only data relevant to the C-17 side chain are given. Reactions were monitored by TLC on silica gel plates (Merck type 60H) and visualized by dipping in 4% H_2SO_4 in EtOH followed by heating at 120−150 °C. Flash column chromatography (FCC) was carried out on silica gel (Merck type 60 for column chromatography) in the solvent systems indicated. LP refers to petroleum fractions with bp 35−60 °C. Solutions were dried using anhydrous Na_2SO_4. Melting points were measured on a Kofler type hot-stage apparatus and are uncorrected.

3-[(Tri-*O*-benzoyl-α-L-rhamnopyranosyl)oxy]-17β-(hydroxymethyl)-5β-androstane-3β,14β-diol (2). To a stirred solution of **1**[28] (200 mg, 0.25 mmol) in dry Et_2O (20 mL) was added LTBAH (254 mg, 1.3 mmol) for 2 h when TLC (25% acetone/LP) showed no starting material. CH_2Cl_2 was added and the mixture washed with 1 M HCl. After evaporation FCC of the residue on elution with 25% acetone/LP gave the noncrystalline **2** (145 mg, 57%) which was sufficiently pure by TLC and ^{1}H NMR for use in the following reaction: ^{1}H NMR ($CDCl_3$) δ 1.00 and 1.02 (2s, 6H, 10-, 13-Me), 3.43 (dd, J = 2.5, 10.3, 1H, 20-H), 3.72 (d, J = 10.3, 1H, 20-H); ^{13}C NMR ($CDCl_3$) δ 21.31 or 21.71 (16), 51.28 (17), 14.75 (18), 62.31 (20).

3β-[(Tri-*O*-benzoyl-α-L-rhamnopyranosyl)oxy]-17β-methylene-5β-androstane 14β,20-Epoxide (4a). 2 (130 mg, 0.17 mmol) was treated with *p*-toluene sulfonyl chloride (100 mg, 0.53 mmol) in pyridine (2 mL) and allowed to stand at 20 °C for 16 h; CH_2Cl_2 (50 mL) was added and the mixture washed with 1 M HCl, saturated $NaHCO_3$, and water. After evaporation FCC (elution with 10% acetone/LP) gave **4a** (70 mg, 54%): mp 207−210 °C (Et_2O/LP); ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H, 13-Me), 1.09 (s, 3H, 10-Me), 3.47 (d, J = 7.5, 1H, 20-H), 3.97 (ddd, J = 3.2, 3.2, 7.3, 1H, 20-H); ^{13}C NMR ($CDCl_3$) δ 25.65 (16), 46.16 (17), 15.32 (18), 73.37 (20). Anal. ($C_{47}H_{54}O_9$) C, H.

3β-(α-L-Rhamnopyranosyloxy)-17β-methylene-5β-androstane 14β,20-Epoxide (4b). 4a (90 mg, 0.12 mmol) in MeOH (5 mL) was treated with 10% NH_3/MeOH (5 mL) at 0 °C for 14 h. After evaporation, the residue on FCC (elution with 7.5% MeOH/CH_2Cl_2) gave **4b** (45 mg, 85%): mp 249−251.5 °C (MeOH/acetone/LP); ^{1}H NMR (CD_3OD) δ 0.97 and 1.00 (2s, 6H, 10-, 13-Me), 3.48 (d, J = 7.3, 1H, 20-H), 3.95 (m, 2H, 20-H); ^{13}C NMR (CD_3OD) δ 26.47 (16), 47.41 (17), 15.65 (18), 74.17 (20). Anal. ($C_{26}H_{42}O_6$) C, H.

14α-Hydroxy-3β-[(tri-*O*-benzyoyl-α-L-rhamnopyranosyl)-oxy]-5β,17α-pregnane-21-carboxylic Acid 14,21-Lactone (6) and 14β-Hydroxy-3β-[(tri-*O*-benzoyl-α-L-rhamnopyranosyl)oxy]-5β-pregnane-21-carboxylic Acid 14,21-Lactone (7a). To the 1,3-dithaine-2-triphenylphosphonium chloride[7,8] (180 mg, 0.36 mmol) in absolute ethanol (2.5 mL) under Ar was added with vigorous stirring 0.25 M ethanolic NaOEt (1.8 mL, 0.45 mmol). The reaction mixture immediately became bright yellow, and a precipitate formed. After 5 min a solution of **1** (240 mg, 0.31 mmol) suspended in absolute ethanol (l mL) was added in one portion. The solid dissolved slowly on stirring which was continued for 2 h when TLC (20% acetone/LP) showed that reaction was complete. Water (100 mL) was added, the mixture extracted with CH_2Cl_2, and the organic layer washed with water, dried, and evaporated. The

residue, without further purification, was taken up in CH_3CN/water (5:l) (12 mL), $Hg^{II}Cl_2$ (400 mg, 1.50 mmol) added, and the mixture stirred for 18 h when TLC indicated that hydrolysis was complete.[9] The reaction mixture was filtered through a Celite pad which was washed with acetonitrile and on evaporation the residue was taken up in CH_2Cl_2 and dried. After evaporation the residue on FCC (elution with 25% EtOAc/LP) gave fractions (23 mg) which on recrystallization yielded **6** (12 mg, 5%): mp 205−207 °C (Et_2O/LP); ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 3H, 10-Me), 1.38 (s, 3H, 13-Me), 2.31 (d, J = 18.2, 1H, 20-H), 2.48 (t, J = 9.5, 1H), 2.90 (dd, J = 9.3, 18.1, 1H, 20-H); ^{13}C NMR ($CDCl_3$) δ 33.49 or 35.62 or 37.36 (16, 20), 43.28 (17), 18.77 (18), 177.14 (21). Anal. ($C_{48}H_{54}O_{10}$) C, H.

Further elution yielded fractions (143 mg) which gave **7a** (92 mg, 37%): mp 214−216 °C (Et_2O/LP); ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 6H, 10-, 13-Me), 2.41 (d, J = 19.7, 1H, 20-H), 2.87 (dq, J = 2, 4, 19, 1H, 20-H); ^{13}C NMR ($CDCl_3$) δ 27.48 (16), 42.14 (17), 14.63 (18), 31.07 or 32.29 (20). Anal. ($C_{48}H_{54}O_{10}$) C, H.

14β-Hydroxy-3β-(α-L-rhamnopyranosyloxy)-5β-pregnane-21-carboxylic Acid 14,21-Lactone (7b). 7a (55 mg, 0.069 mmol) was stirred in MeOH (7 mL) and l0% NH_3/MeOH (5 mL) for 16 h under Ar when TLC (12% MeOH/CH_2Cl_2) indicated that hydrolysis was complete. On evaporation the residue was immediately recrystallized to give **7b** (22 mg, 66%): mp 271−274 °C (acetone/LP); ^{1}H NMR (DMSO-d_6) δ 0.94 and 0.95 (2s, 6H, 10-, 13-Me), 2.35 (d, J = 19.2, 1H, 20-H), 2.77 (dq, J = 2.4, 4.8, 18.9, 1H, 20-H), 4.46 (d, J = 4.5, ROH), 4.64 (dd, J = 5.3, ROH), 4.69 (dd, J = 5.3, ROH); ^{13}C NMR (DMSO-d_6) δ 26.78 (16), 41.43 (17), 14.18 (18), 38.24 (20), 170.73 (21). Anal. ($C_{27}H_{42}O_7$) C, H.

3β-(α-L-Rhamnopyranosyloxy)-5β-pregnane-14β,21-diol (8). To a solution of **7a** (170 mg, 0.22 mmol) in dry Et_2O (15 mL) was slowly added a solution of LAH (180 mg, 4.7 mmol) in dry Et_2O (30 mL). After refluxing under Ar for 1 h the stirred mixture was cooled in an ice−water bath, and water (0.2 mL), 15% NaOH (0.18 mL), and again water (0.5 mL) were added to give a granular precipitate.[29] After evaporation the residue was extracted by reflux with MeOH (3 × 45 mL) for 30 min. The mixture was filtered through Celite and evaporated. The residue on FCC (elution with 12% MeOH/CH_2Cl_2) gave **8** (31 mg, 30%): mp 227−230 °C (MeOH/acetone); ^{1}H NMR (CD_3OD) δ 0.94 and 0.95 (2s, 6H, 10-, 13-Me), 3.43 (m, 1H, 21-H), 3.58 (m, 1H, 21-H); ^{13}C NMR (CD_3OD) δ 28.44 (16), 47.26 (17), 15.92 (18), 38.68 (20), 62.45 (21). Anal. ($C_{27}H_{46}O_7$) C, H.

3β-[(Tri-*O*′-Benzoyl-α-L-rhamnopyranosyl)oxy]-21-nitro-5β-pregnane-14β,20(*R*)-diol (9a). 1 (1.19 g, 1.3 mmol), freshly distilled nitromethane (400 mg, 6.4 mmol), and KF (94 mg, 1.0 mmol) were stirred in dry 2-PrOH (20 mL) for 16 h.[10] The mixture was diluted with CH_2Cl_2 (100 mL) and washed with water. The residue from evaporation on FCC (elution with 20% acetone/LP) gave fractions of the noncrystalline **9a**[2] (460 mg, 38%) which was sufficiently pure by TLC (25% acetone/LP) and ^{1}H and ^{13}C NMR for use in the following reactions: ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 3H, 10-Me), 1.11 (s, 3H, 13-Me), 4.23 (m, H, 21-H), 4.42 (dd, J = 8.6, 11.4, 1H, 21-H), 4.52 (dd, J = 4.2, 8.7, 1H, 20-H); ^{13}C NMR ($CDCl_3$) δ 27.48 (16), 42.14 (17), 14.63 (18), 31.29 or 32.07 (20).

21-Nitro-3β-(α-L-rhamnopyranosyloxy)-5β-pregnane-14β,20(*R*)-diol (9b) and 21-Nitro-3β-(α-L-rhamnopyranosyloxy)-5β,17α-pregnane-14β,20(*R*)-diol (9c). To a solution of **1** (470 mg, 0.60 mmol) and freshly distilled nitromethane (400 mg, 6.6 mmol) in MeOH (10 mL) was added slowly 4 M NaOMe/MeOH (3.0 mL, 12 mmol). After stirring for 3.5 h at 20 °C, the mixture was carefully adjusted to pH 8 (indicator paper) with 1 M HCl in MeOH while cooled in an ice−water bath. EtOAc (150 mL) was added and the mixture washed with aqueous $NaHCO_3$ and water. After evaporation, the residue was crystallized to give **9b** (61 mg, 19%): mp 195−196 °C (acetone/LP); ^{1}H NMR (CD_3OD) δ 0.96 and 1.06 (2s, 6H, 10-, 13-Me), 4.32−4.49 (3H, 20-H, 21-H_2); ^{13}C NMR (CD_3OD) δ 19.64 (16), 53.36 (17), 15.21 (18), 68.84 (20), 81.67 (21). Anal. ($C_{27}H_{45}O_9N\cdot H_2O$) C, H, N.

The mother liquor on FCC (elution with 7.5% MeOH/CH_2Cl_2) gave **9b** (140 mg, 44%): mp 194−196 °C (acetone/LP). Further elution gave **9c** (25 mg, 8%): mp 196−197 °C (acetone/LP); ^{1}H NMR (CD_3OD) δ 0.95 (s, 10-Me), 1.12 (s, 13-Me), 4.23 (ddd, J = 1.9, 9.9, 9.9, 20-H), 4.32 (d, J = 9.9, 21-H_B), 4.55 (dd, J = 2.0, 9.9, 21-H_A); ^{13}C NMR (CD_3OD) δ 24.43 (16), 51.97 (17), 20.47 (18), 72.21 (20), 82.85 (21). Anal. ($C_{27}H_{45}0_9N\cdot H_2O$) C, H, N.

20(*R*)-(*tert*-Butyldimethylsiloxy)-21-nitro-3β-[(tri-*O*-benzoyl-α-L-rhamnopyranosyl)oxy]-5β-pregnan-14β-ol (10). To a solution of **9a** (200 mg, 0.24 mmol) in CH_2Cl_2 (10 mL) containing Et_3N (261 mg, 2.6 mmol), cooled in an ice−water bath under an Ar atmosphere, was added slowly *tert*-butyldimethylsilyl triflate (283 mg, 1.1 mmol), and stirring was continued for 1.5 h when TLC (20% acetone/LP) indicated reaction was complete. CH_2Cl_2 (150 mL) was added, and the organic layer was washed with water to give a residue which on FCC (elution with 12% acetone/LP) gave the noncrystalline **10** (116 mg, 51%) which was sufficiently pure by TLC and ^{1}H and ^{13}C NMR for use in the following reaction: ^{1}H NMR ($CDCl_3$) δ 0.93 (s, 3H, 13-Me), 1.03 (s, 3H, 10-Me), 4.48 (m, 3H, 20-H, 21-H_2); ^{13}C NMR ($CDCl_3$) δ 19.41 (16), 52.68 (17), 14.90 (18), 39.90 (20), 79.12 (21).

21-Amino-3β-(α-L-rhamnopyranosyloxy)-5β-pregnane-14β,20(*R*)-diol (11). From 9a: To a stirred solution, under Ar, of **9a** (500 mg, 0.60 mmol) in dry Et_2O (50 mL) under reflux was added LAH (600 mg, 16 mmol) in dry Et_2O (100 mL) over 15 min, and reflux was continued for a further 75 min when TLC [$CHCl_3$:MeOH:Et_3N (100:40:3)] indicated that reaction was complete. To the stirred, cooled (ice bath) mixture were added slowly water (0.4 mL), 15% NaOH (0.4 mL), and again water (1.2 mL), and was stirring continued for a further 20 min to form a granular precipitate.[29] The residue from evaporation of the Et_2O was extracted by 40 min reflux with MeOH (3 × 50 mL) and filtered through silica gel, and the filtrate was evaporated and subjected to FCC. Elution with $CHCl_3$:MeOH:Et_3N (100:40:3) gave **11** (50 mg, 17%): mp 225−229 °C (MeOH/Et_2O); ^{1}H NMR (CD_3OD) δ 0.96 (s, 3H, 10-Me), 1.04 (s, 3H, 13-Me), 2.80 (m, 2H, 21-H_2), 3.93 (m, 1H, 20-H); ^{13}C NMR (CD_3OD) δ 19.44 (16), 53.81 (17), 15.38 (18), 67.60 (20), 45.71 (21). Anal. ($C_{27}H_{47}O_7N\cdot 2.5H_2O$) C, H, N.

From 10: 10 (190 mg, 0.18 mmol) was added to a solution of LAH (400 mg, 10.5 mmol) in Et_2O (45 mL) and the mixture refluxed for 1.5 h. Workup as above gave **11** (27 mg, 30%): mp 224−228 °C (MeOH/Et_2O).

14β-Hydroxy-21-nitro-3β-[(tri-*O*-benzoyl-α-L-rhamnopyranosyl)oxy]-5β-pregna-20,21-diene (13a). 9a (250 mg, 0.30 mmol), Ac_2O (1 mL), and 4-(*N*,*N*-dimethylamino)pyridine (10 mg) in dry Et_2O (5 mL) were stirred for 14 h to give **12**. Et_2O (50 mL) was added and the mixture washed with excess aqueous $NaHCO_3$ and water. The residue on FCC (elution with 20% acetone/LP) gave **13a** (113 mg, 46%): mp 242−244 °C (acetone/Et_2O); ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, 10-Me), 1.04 (s, 3H, 13-Me), 4.08 (br s, 1H, 3-H), 5.10 (d, J = 1.2, 1H, 1′-H), 5.63 (dd, J = 1.8, 3.4, 1H, 2′-H), 5.87 (dd, J = 3.4, 10.1, 1H, 3′-H), 5.67 (t, J = 9.9, 1H, 4′-H), 4.23 (m, 1H, 5′-H), 1.35 (d, J = 6.3, 1H, 6′-H), 6.85 (d, J = 13.3, 1H, 21-H), 7.25−8.12 (m, 16H, 20-H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 30.47 (1), 26.47 (2), 73.22 (3), 29.64 (4), 35.29 (5) (interchangeable numbers), 26.52 (6), 20.78 (7), 41.86 (8), 35.80 (9) (interchangeable numbers), 36.45 (10), 21.24 (11), 38.26 (12), 50.04 (13), 85.92 (14), 32.85 (15), 26.83 (16), 49.72 (17), 15.82 (18), 23.80 (19), 137.73 (20), 148.35 (21), 95.80 (1′), 70.17 (2′), 71.56 (3′), 71.99 (4′), 66.93 (5′), 17.70 (6′). Anal. ($C_{48}H_{55}O_{11}N$) C, H, N.

14β-Hydroxy-21-nitro-3β-(α-L-rhamnopyranosyloxy)-5β-pregn-20-ene (13b). 13a (272 mg, 0.33 mmol) was added in one portion to a vigorously stirring solution of 0.256 M *t*-BuOK/*t*-BuOH at 20 °C, and stirring was continued for 10 min. The reaction mixture was then adjusted to pH 7−8 (indicator paper) with 1 M HCl in MeOH and evaporated to give a residue which on FCC (elution with 7.5% MeOH/CH_2Cl_2) gave **13b** (54 mg, 32%): mp 215−216 °C (MeOH/H_2O); ^{1}H NMR (CD_3OD) δ 0.95 (s, 3H, 10-Me), 0.88 (s, 3H, 13-Me), 6.99 (d, J = 13.3, 1H, 21-H), 7.53 (dd, J = 11.4, 13.4, 1H, 20-H); ^{13}C NMR (CD_3OD) δ 27.86 (16), 51.06 (17), 15.40 (18), 138.92 (20), 149.80 (21). Anal. ($C_{27}H_{43}O_8N$) C, H, N.

14β-Hydroxy-21-nitro-3β-(α-L-rhamnopyranosyloxy)-5β-pregn-20-ene (13b). 13a (272 mg, 0.33 mmol) was added

in one portion to a vigorously stirred solution of 0.256 M *t*-BuOK/*t*-BuOH (27 mL) at 20 °C and stirring continued for 10 min. The reaction mixture was then adjusted to pH 7−8 (indicator paper) with 1 M HCl in MeOH and evaporated to give a residue which on FCC (elution with 7.5% MeOH/CH_2Cl_2) gave **13b** (54 mg, 54%): mp 215−216 °C (MeOH/H_2O).

20(*R*)-Methoxy-21-nitro-3*β*-(α-L-rhamnopyranosyloxy)-5*β*-pregnan-14*β*-ol (14). **13a** (140 mg, 0.17 mmol) was stirred in 0.25 M NaOMe/MeOH (5 mL) in an ice−water bath for 3.45 h when TLC (7.5% MeOH/CH_2Cl_2) showed formation of one major component. The reaction mixture was neutralized to pH 7−8 (indicator paper) with 1 M HCl and evaporated to give a residue which on FCC (elution with 7.5% MeOH/CH_2Cl_2) yielded **14** (40 mg, 28%): mp 212−213 °C (MeOH/Et_2O); ^{1}H NMR (CD_3OD) δ 0.97 and 1.00 (2s, 6H, 10-, 13-Me), 3.94 (br s, 1H, 3-H), 4.76 (d, J = 1.2, 1H, 1′-H), 3.76 (dd, J = 1.5, 3.1, 1H, 2′-H), 3.68 (m, 1H, 3′-H) (overlapping signals), 3.37 (t, J = 9.4, 1H, 4′-H), 3.68 (m, 1H, 5′-H) (overlapping signals), 1.23 (d, J = 6.2, 1H, 6′-H), 4.10 (m, 1H, 20-H), 4.56 (m, 1H, 21-H), 4.73 (m, 1H, 21-H); ^{13}C NMR (CD_3OD) δ 31.66 (1), 27.52 (2), 73.62 (3), 30.89 (4), 38.17 (5), 27.87 (6), 22.14 (7), 41.16 (8), 36.42 (9), 36.36 (10), 22.63 (11), 41.13 (12), ∼50 (13), 85.59 (14), 33.39 (15), 20.26 (16), 53.58 (17), 15.25 (18), 24.39 (19), 79.70 (20), 78.03 (21), 99.84 (1′), 72.93 (2′), 72.53 (3′), 74.09 (4′), 70.00 (5′), 17.98 (6′). Anal. ($C_{28}H_{47}O_9N$) C, H, N.

21-Amino-3*β*-(α-L-rhamnopyranosyloxy)-5*β*-pregnan-14*β*-ol *d*-Hydrogen Tartrate (16). **15** (300 mg), prepared from **9a** as described in ref 2, in glacial HOAc (30 mL) containing PtO_2 (150 mg) was shaken with H_2 at 5 atm for 16 h when TLC [$CHCl_3$:MeOH:Et_3N (100:30:1)] indicated reaction was complete. The mixture was filtered through a Celite pad and evaporated. The residue was triturated with Et_2O, to give the 21-amine (270 mg, 95%) as a pale yellow noncrystalline powder, showing one component on TLC and no extraneous signals in the ^{1}H NMR. The 21-amine (100 mg, 0.20 mmol) and *d*-tartaric acid (30 mg, 0.20 mmol) were dissolved in MeOH (2 mL), and Et_2O was added slowly until the solution became turbid, to give **16** (83 mg, 53%): mp 203−206 °C; ^{1}H NMR (5% D_2O/CD_3OD) δ 0.93 (2s, 6H, 10-, 13-Me), 2.81 (m, 1H, 21-H), 2.96 (m, 1H, 21-H), 4.42 (s, 2H, tartrate); ^{13}C NMR (5% D_2O/CD_3OD) δ *ca*. 27.5 (16), 47.91 (17), 15.59 (18), 32.32 (20), 40.57 (21), 74.00 and 176.97 (tartrate). Anal. ($C_{31}H_{53}O_{12}N\cdot 4H_2O$) C, H, N.

14*β*-Hydroxy-3*β*-[(tri-*O*-benzoyl-α-L-rhamopyranosyl)-oxy]-5*β*-androstane-17*β*-acrylic Acid Ethyl Ester (17). [(Ethoxycarbonyl)methyl]diethylphosphonate (500 mg, 2.23 mmol) in diglyme (5 mL) was added over 5 min to NaH (120 mg, 50% oil suspension, 2.5 mmol) suspended in diglyme (3 mL) and the mixture stirred at 20 °C for 20 min until H_2 evolution had ceased.[16] A solution of **1** (500 mg, 0.655 mmol) in diglyme (5 mL) was slowly added and the mixtured stirred for a further 20 min. TLC (25% acetone/LP, developed twice) showed that no starting material remained. EtOAc (100 mL) was added and the mixture washed with water to give a residue which on FCC (elution with 15% acetone/LP) gave the noncrystalline **17** (450 mg, 80%) which was sufficiently pure by TLC and ^{1}H and ^{13}C NMR for use in the following reactions.

14*β*-Hydroxy-3*β*-(α-L-rhamnopyranosyloxy)-5*β*-androstane-17*β*-acrylic Acid (18). **17** (357 mg, 0.44 mmol) was taken up in 0.25 M NaOMe/MeOH (27.5 mL) and cooled in an ice−water bath for 45 min when TLC (10% MeOH/CH_2Cl_2) indicated that reaction was complete. The mixture was adjusted to pH 7−8 (indicator paper) with 1.4 M HCl. After evaporation, the residue was purified by FCC (on elution with 10% MeOH/CH_2Cl_2) which gave **18** (67 mg, 28%): mp 121−124 °C (118 °C shrinking) (acetone/LP); ^{1}H NMR (CD_3OD) δ 0.95 (s, 3H, 10-Me), 0.85 (s, 3H, 13-Me), 2.32 (m, 1H, 17α-H), 7.19 (dd, J = 10.6, 15.5, 1H, 20-H); ^{13}C NMR (CD_3OD) δ 28.05 (16), 55.52 (17), 14.60 (18), 157.22 (20), 120.11 (21). Anal. ($C_{28}H_{44}O_8\cdot 2H_2O$) C, H.

14*β*-Hydroxy-3*β*-(α-L-rhamnopyranosyloxy)-5*β*-androstane-17*β*-propionic Acid (19). **17** (480 mg, 0.56 mmol) in absolute EtOH (20 mL) containing 10% Pd/C as catalyst was shaken with H_2 at 5 atm for 14 h. The mixture was filtered through Celite and the residue from evaporation dissolved in 0.25 M NaOMe/MeOH (16 mL) and cooled in an ice−water bath for 45 min. Workup and FCC (elution with 10% MeOH/CH_2Cl_2) gave **19** (54 mg, 18%): mp 102−105 °C (acetone/LP); ^{1}H NMR (CD_3OD) δ 0.94 (s, 3H, 13-Me), 0.95 (s, 3H, 10-Me), 2.13 (m, 1H, 21-H), 2.33 (m, 1H, 21-H); ^{13}C NMR (CD_3OD) δ 29.19 (16), 50.84 (17), 15.70 (18), 31.18 or 34.28 (20), 31.18 or 34.28 (21). Anal. ($C_{28}H_{46}O_8$) C, H.

[^{3}H]Ouabain Radioligand Binding Assay. [^{3}H]Ouabain binding to dog heart microsomes was determined in the presence of steroids added in 5 μL of ethanol as previously described.[4]

Acknowledgment. We thank the Medical Research Council of Canada and the Heart and Stroke Foundation of Manitoba for financial support. The Bruker AM300 and AMX500 instruments were funded by the Natural Sciences and Engineering Council of Canada with additional support from the Manitoba Research Council (AM300), The University of Manitoba Research Board (AM300), The University of Manitoba (AMX500), The University of Winnipeg (AMX500), and Lakehead University (AMX500). We are grateful to Willson Caetano for assistance with the experimental work. F. S. LaBella is a Career Investigator of the Medical Research Council of Canada.

Supporting Information Available: Tables containing ^{1}H and ^{13}C NMR spectral data (5 pages). Ordering information is given on any current masthead page.

References

(1) Templeton, J. F.; Ling, Y.; Zeglam, T. L.; Marat, K.; LaBella, F. S. Synthesis of 20α and 20*β*-Acetamido-, Amino-, Nitro-, and Hydroxy-Derivatives of 14-Hydroxy-5*β*,14*β*-pregnane 3*β*-Glycosides: Pregnanes that Bind to the Digitalis Receptor. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2503−2517 and references therein.

(2) Templeton, J. F.; Ling, Y.; Zeglam, T. H.; Marat, K.; LaBella, F. S. Synthesis of 20-Hydroxy-, 20-Amino-, 20-Nitro-, 14-Hydroxy-21-nor-5*β*,14*β*-pregnane C-3 Glycosides: Structure-Activity Relationships of Pregnanes that Bind to the Digitalis Receptor. *J. Med. Chem.* **1993**, *36*, 42−45.

(3) Thomas, R.; Gray, P.; Andrews, J. Digitalis: Its Mode of Action, Receptor, and Structure-Activity Relationships. *Adv. Drug Res.* **1990**, *19*, 311−562.

(4) Hnatowich, M.; LaBella, F. S. Endogenous Digitalis-like Factors: Comparison of in vitro Biological and Immunological Activities of Peptide and Steroid Candidates. *Eur. J. Pharmacol.* **1984**, *30*, 385−394.

(5) Martin, S. F. Synthesis of Aldehydes, Ketones, and Carboxylic Acids from Lower Carbonyl Compounds by C-C Coupling Reactions. *Synthesis* **1979**, 633−663.

(6) Grobel, B. T.; Seebach, D. Umpolung of the Reactivity of Carbonyl Compounds through Sulfur-Containing Reagents. *Synthesis* **1977**, 357−402.

(7) Arai, K.; Oki, M. Evidence for Ionic Dissociation of 2-Chloro-1,3-dithiane in Various Solvents. *Tetrahedron Lett.* **1975**, 2183−2186.

(8) Kruse, C. G.; Broekhof, N. L. J. M.; Wijsman, A.; van der Gen, A. Synthetic Applications of 2-Chloro-1,3-dithiane, Preparation of Ketene Dithioacetals. *Tetrahedron Lett.* **1977**, 885−888.

(9) Corey, E. J.; Shulman, J. I. The Application of Lithium Reagents fron (1-Methyl)alkylphosphonate Esters to the Synthesis of Ketones. *J. Org. Chem.* **1979**, *35*, 777−780.

(10) Wollenberg, R. H.; Miller, S. J. Nitroalkane Synthesis. A Convenient Method for Aldehyde Reductive Nitromethylation. *Tetrahedron Lett.* **1978**, 3219−3222.

(11) March, J. *Advanced Organic Chemistry*, 4th ed.; John Wiley and Sons: New York, 1992; p 939.

(12) Eberlein, W.; Heider, J.; Machleidt, H. Steroidal Cardenolides: II. Replacement of the Butenolide Ring of Cardiac Glycosides by Unbranched Open Chain π-Eectron Systems. *Chem. Ber.* **1974**, *107*, 1275−1284.

(13) Dornow, A.; Gellrich, M. Aliphatic Nitro Compounds. X. Reduction of Aliphatic Nitrocompounds with Lithium Aluminium Hydride. *Ann.* **1955**, *594*, 177−184.

(14) Colvin, E. W.; Seebach, D. Silyl Nitronates: Improved Nitroaldol Reactions and Reductive Routes to 2-Aminoalcohols. *J. Chem. Soc. Chem. Commun.* **1978**, 689−690.

(15) Boutagy, J. S.; Thomas, R. Cardenolide Analogues. 2. Synthesis of C17*β*-unsaturated Esters and Derivatives. *Aust. J. Pharm. Sci.* **1972**, *NS1*, 67−75.

(16) Wadsworth, D. H.; Schupp, O. E.; Seus, E. J.; Ford, J. A. The Stereochemistry of the Phosphonate Modification of the Wittig Reaction. *J. Org. Chem.* **1965**, *30*, 680−685.
(17) Marat, K.; Templeton, J. F.; Ling, Y. NMR Study of Conformation and Configuration in C-20-Substituted 5β,14β-Pregnanes, 5β-Pregn-14-enes and 21-Nor-5β,14β-Pregnanes. *Magn. Reson. Chem.* **1993**, *31*, 17−22.
(18) Thomas, R.; Boutagy, J.; Gelbart, A. Cardenolide Analogs. V. Cardiotonic Activity of Semisynthetic Analogs of Digitoxigenin. *J. Pharmacol. Exp. Ther.* **1974**, *191*, 219−231.
(19) Maxient, J. M.; Berrebi-Bertrand, I.; Lelievre, L. G. Inhibition of Cardiac (Na^+,K^+)-ATPase isozymes by LND 623. *Biochem. Pharmacol.* **1991**, *42*, S223−S224.
(20) Smith, P.; Brown, L.; Boutagy, J.; Thomas, R. Cardenolide Analogues. 14. Synthesis and Biological Activity of Glucosides of C17β-Modified Derivatives of Digitoxigenin. *J. Med. Chem.* **1982**, *25*, 1222−1226.
(21) Hintsche, R.; Megges, R.; Pfeiffer, D.; Portius, H. J.; Schonfeld, W.; Repke, K. R. H. Biological Potency and Solution Conformation of Cardenolides Determined by a New ^{1}H NMR Method. *Eur. J. Med. Chem.* **1985**, *20*, 9−15.
(22) Schonfeld, W.; Weiland, J.; Lindig, C.; Masnyk, M.; Kabat, M. M.; Kurek, A.; Wicha, J.; Repke, K. H. R. The Lead Structure in Cardiac Glycosides is 5β,14β-androstane-3β,14-diol. *Arch. Pharmacol.* **1985**, *329*, 414−426.
(23) Smyth, D. D.; Templeton, J. F.; Kumar, V. P. S.; Yan, Y.; Widajewicz, W.; LaBella, F. S. Digitaloid Pregnanes Promote Potassium-sparing Diuresis in the Guinea Pig. *Can. J. Pharmacol. Physiol.* **1992**, *70*, 723−727.
(24) Maxient, J. M.; Bertrand, I. B.; Lelievre, L. G.; Fenard, S. Efficacy and Safety of the Novel Na^+,K^+-ATPase Inhibitor 20R 14β-Amino 3β-rhamnosyl 5β-pregnan 20β-ol in a Dog Model of Heart Failure. *Arzneim.-Forsch.* **1992**, 1301−1305.
(25) Weiland, J.; Schwabe, K.; Hubler, D.; Schonfeld, W.; Repke, K. R. H. Glycosidation of Chlormadinol Acetate Alters its Actions on Na^+,K^+-transporting ATPase and Cardiac Contractility: A Contribution to the Endogenous Digitalis Problem. *J. Enzyme Inhib.* **1987**, *2*, 31−36.
(26) Templeton, J. F.; Ling, Y.; Lin, W,; Pitura, R. J.; Marat, K.; Bridson, J. Novel Insertion, Rearrangement and Addition Products from Dihalocarbene Reaction with 5(10)-Unsaturated Steroids. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1149−1158.
(27) Doddrell, D. M.; Pegg, D. P.; Bendall, M. T. Distortionless Enhancement of NMR Signals by Polarization Transfer. *J. Magn. Reson.* **1982**, *48*, 323−327.
(28) The aldehyde **1** was prepared as described in ref 2 except for the following modifications which increased the overall yield from 27% to 86%. Evomonoside tribenzoate was prepared as in ref 2 and used without FCC purification. Workup of the LTBAH product was carried out by washing with 1 M HCl instead of $NaHCO_3$ to avoid formation of an emulsion. Reaction time for the $NaIO_4$ cleavage was shortened to 30 min; longer reaction time markedly decreased the yield.
(29) Micovic, V. M.; Mihailovic, M. L. J. The Reduction of Acid Amides with Lithium Aluminum Hydride. *J. Org. Chem.* **1953**, *18*, 1190−1200.

JM960880L