Synthesis of 20α- and 20β-Acetamido, Amino, Nitro and Hydroxy Derivatives of 14-Hydroxy-5β,14β-pregnane 3β-Glycosides: Pregnanes that Bind to the Digitalis Receptor

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Synthesis of 20α- and 20β-acetamido-, amino-, nitro- and hydroxy-3β-glycoside (α-L-rhamnopyranoside and tris-β-D-digitoxoside) and genin derivatives of 14-hydroxy-5β,14β-pregnane together with the C-20 oxime, hydrazone and amidinohydrazone is described from digitoxin. Ortho esters were also isolated. Structures were established by NMR measurements. These compounds have been shown to bind to the digitalis receptor of heart muscle. The 20β derivatives were consistently more potent than are the corresponding 20α compounds. The 20β-nitro α-L-rhamnoside derivative proved to be the most potent. Receptor binding data are given and structure–activity relationships are presented.

Certain pregnanes and related steroids bind to the cardiac glycoside recognition site on Na^+, K^+-ATPase and inhibit the enzyme (the sodium pump) in membranes, cells and tissues.[1] Previously we have shown that the 20β-hydroxy substituent can replace the α,β-unsaturated γ-lactone of the cardiac glycosides by effectively binding to the ouabain binding site of heart muscle and the C-20 alcohol can still retain positive inotropic activity. As part of our structure–activity investigation of these pregnanes we compared 20α- and 20β-amino and -nitro, 20α-hydroxy and related derivatives with the corresponding 20β-hydroxy derivative for binding potency in a [^{3}H]ouabain radioligand binding assay.[2] We report here on the synthesis of the C-20 oxime, 20α- and 20β-acetamido-, 20α- and 20β-amino-, 20α- and 20β-nitro- and 20α- and 20β-hydroxy-5β,14β-pregnan-3β-yl α-L-rhamnopyranosides and the C-20 oxime, hydrazone, amidinohydrazone, and the 20ξ-amino- and 20ξ-nitro-5β,14β-pregnane 3β-tris-β-D-digitoxosides (Schemes 1–6). Receptor-binding data are compared and the products' structure–activity relationships discussed. Structures were established by NMR methods.

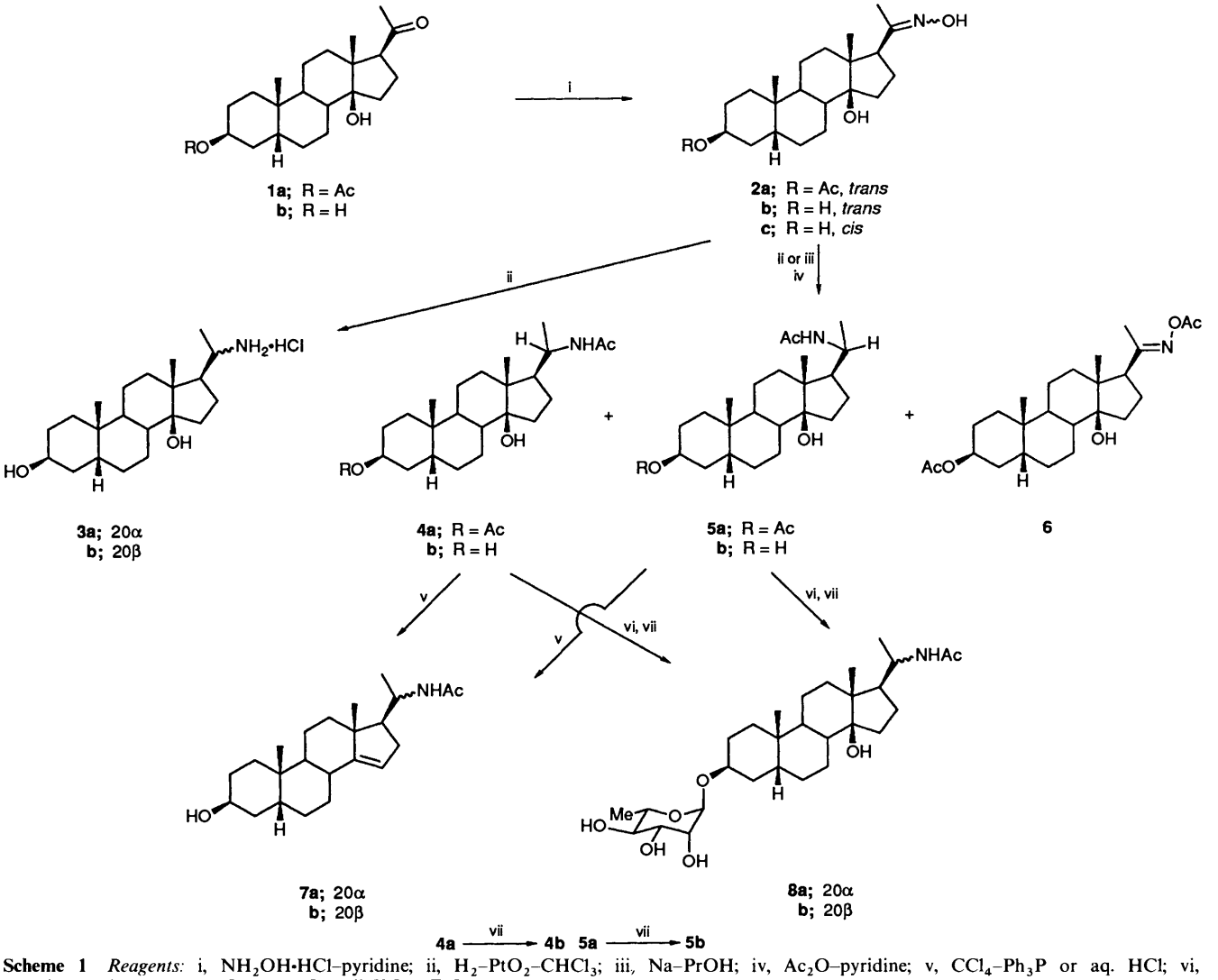
Results and Discussion

3β-Acetoxy- **1a** and 3β-hydroxy- **1b** 14-hydroxy-5β,14β-pregnan-20-one were prepared as previously reported.[3] Treatment of ketone **1b** with hydroxylamine gave the *trans* (*anti*)-oxime **2b** together with a minor product assigned the *cis* (*syn*) structure **2c** (Scheme 1). The *trans* stereochemistry was assigned to the major product by analogy with the C-20 oxime in the 14α-series.[4] The *trans*-oxime **2a** was isolated from similar treatment of compound **1a**. Hydrogenation of the *trans*-oxime **2b** with PtO_2 in ethanol containing a trace of chloroform[5] gave a mixture of the 20α- **3a** and 20β- **3b** amine hydrochlorides (1:6), which were separated by crystallization and flash chromatography. Acetylation of the amine hydrochlorides **3a** and **3b** and separation by flash chromatography gave the 20α- and 20β-acetamide **4a** and **5a**. A small amount of the *trans* oxime diacetate **6** was also obtained from acetylation of the unreduced starting oxime **2a**. Selective hydrolysis of the acetamides **4a** and **5a** also gave the 3β-alcohols **4b** and **5b**. Reduction of the *trans*-oxime **2a** with sodium in propan-1-ol[6] gave, after acetylation and selective hydrolysis without purification of intermediates, the 3β-alcohols **4b** and **5b** in approximately equal amounts.

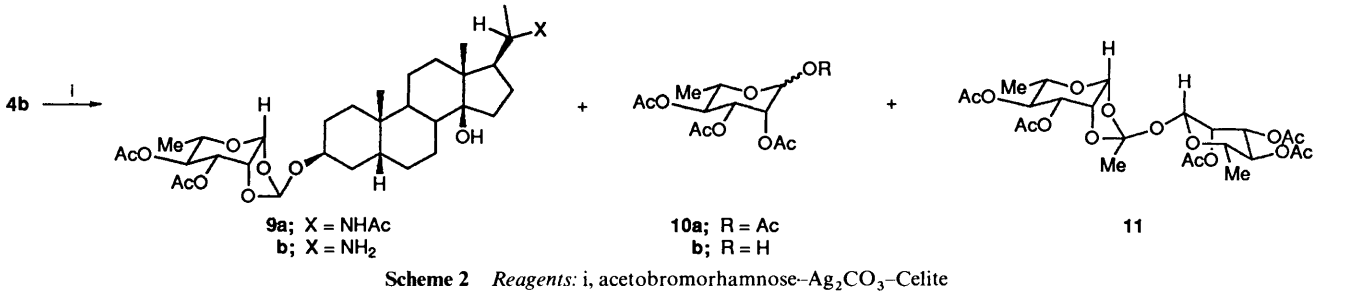
Initial attempts to prepare the α-L-rhamnopyranoside of the alcohol **4b** by using 2,3,4-tri-*O*-acetylrhamnopyranosyl bromide (hereafter referred to as acetobromorhamnose) and Fetizon's reagent[7] gave instead the steroid ortho ester **9a** together with the 20α-amine ortho ester **9b** (Scheme 2). Several reagent by-products were also isolated as epimeric mixtures based on their NMR spectra, namely tetra-*O*-acetyl-L-rhamnopyranose **10a**, 2,3,4-tri-*O*-acetyl-L-rhamnopyranose **10b** and the rhamnose ortho ester **11**. The *exo* isomer formed results typically from attack of the ortho ester alkoxy group on the least hindered side of the intermediate dioxolenium ion.[8] Dependence of ortho ester formation on reaction conditions has been previously observed.[9] The structures of the products **9a**, **9b** and **11** were established from NMR studies (see below). However, when the 20α-acetamide alcohol **4b** was treated with acetobromorhamnose and mercury(II) cyanide[10] the acetamido α-L-rhamnoside **8a** was obtained; similar treatment of the 20β-acetamide alcohol **5b** gave the corresponding acetamide α-L-rhamnoside **8b**. Attempts to hydrolyse the 20α- and 20β-acetamide **4b** and **5b** were unsuccessful. Dehydration of the acetamide alcohols (**4b** on treatment with triphenylphosphine–CCl_4[11] and **5b** on HCl work-up) gave the C-14 unsaturated derivatives **7a** and **7b**.

Attempts to protect the amine while allowing preparation of the C-3 glycoside proved unsuccessful. For example, when a mixture of the 20α- and 20β-amine epimers **3a** and **3b** was treated with trifluoroacetic anhydride (TFAA) in pyridine the C-14 unsaturated derivatives **12a** and **12b** were obtained (Scheme 3). Oxidation of the mixture of alcohols **3a** and **3b** with dimethyldioxirane,[12] followed by acetylation, yielded the 20α-nitro **14a** and 20β-nitro **14b** derivatives, in low yield, together with the 20β-nitro-3-ketone **13**. Treatment of the 20β-nitro derivative with iron filings in acetic acid[13] gave the 20β-amine **15**.

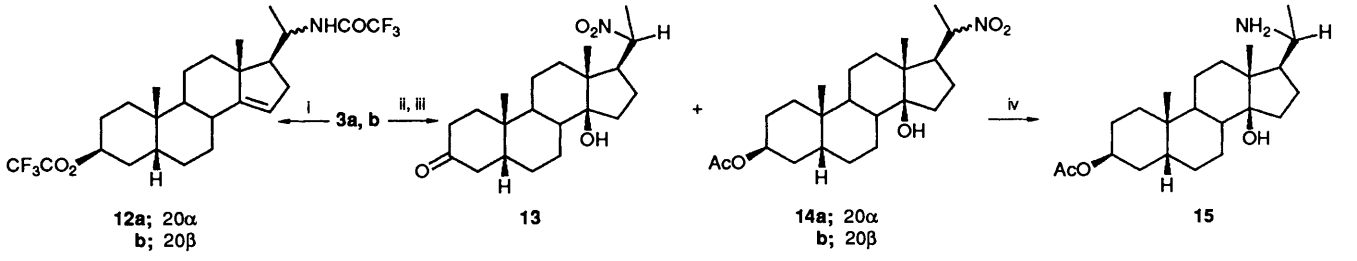
Digitoxigenin α-L-rhamnoside (evomonoside) tribenzoate **16** was treated with ozone and zinc–acetic acid as described previously[3] to give the ketone **17** together with the 20β-alcohol **18** (Scheme 4). Reduction of a keto group to an alcohol group under these Clemmensen-type conditions is unusual as the alcohol is not considered to be an intermediate in the Clemmensen reduction.[14] The C-3 ketone was reduced to the hydrocarbon under these conditions.[3] Hydrolysis of the tribenzoate **18** gave the rhamnoside **19**, while pyridinium dichromate (PDC) oxidation yielded the 20-ketone **17**.[15] This route to diol **19** (and ketone **17**) is different from that previously reported.[15]



Scheme 1 *Reagents:* i, NH_2OH·HCl–pyridine; ii, H_2–PtO_2–$CHCl_3$; iii, Na–PrOH; iv, Ac_2O–pyridine; v, CCl_4–Ph_3P or aq. HCl; vi, acetobromorhamnose–$Hg(CN)_2$–MeCN, vii, KOH–EtOH



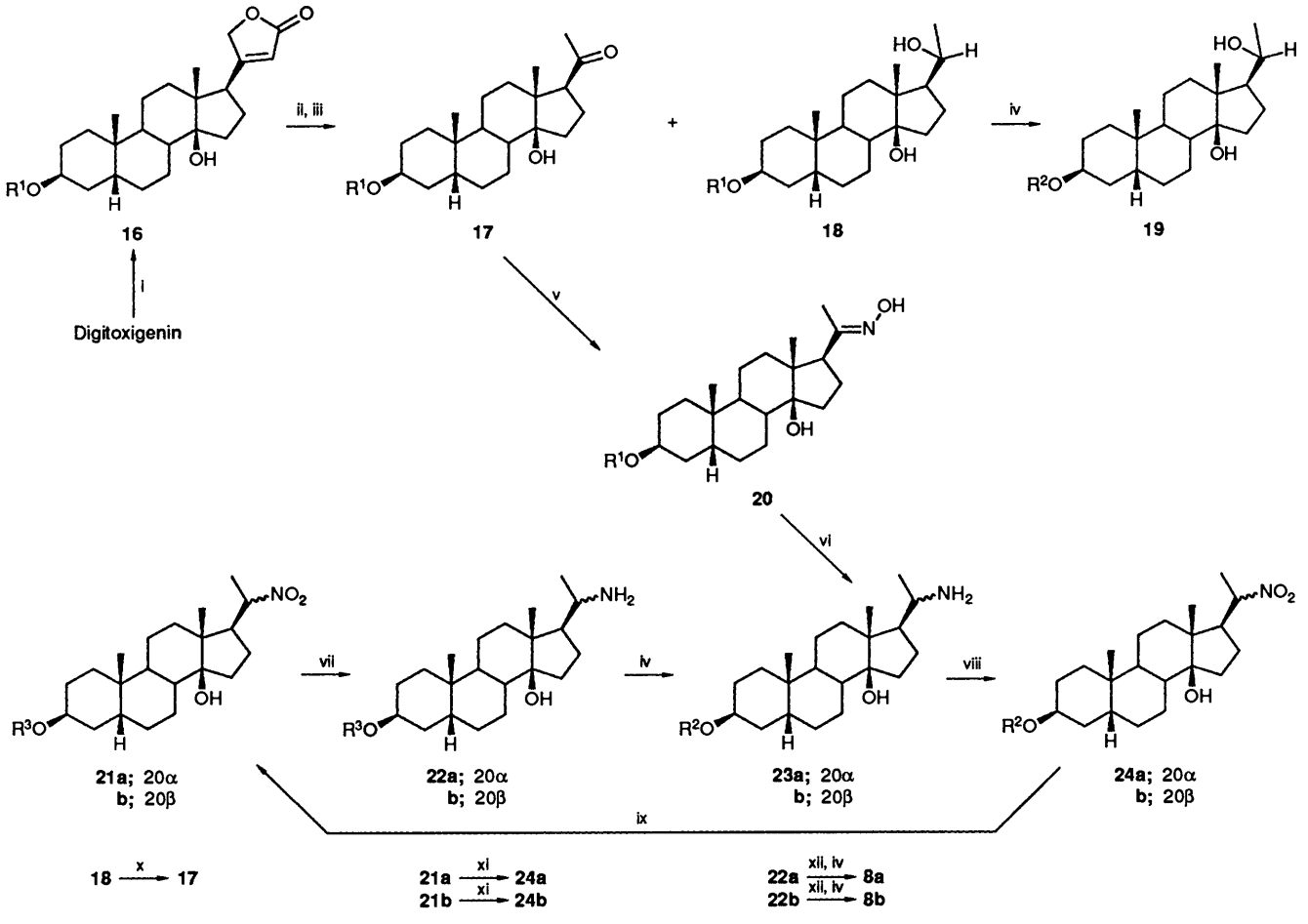
Scheme 2 *Reagents:* i, acetobromorhamnose–Ag_2CO_3–Celite



Scheme 3 *Reagents:* i, TFAA–pyridine; ii, dimethyldioxirane–Me_2CO; iii, Ac_2O–pyridine; iv, Fe–HOAc

Formation of the *trans*-oxime **20** from ketone **17**, followed by reduction with sodium in propan-1-ol, gave an epimeric mixture of the 20ξ-amines **23a/b**, which on oxidation with dimethyldioxirane gave the 20ξ-nitro epimers **24a/b**. Neither the amine nor the nitro epimers could be efficiently separated by flash chromatography.

Acetylation of the 20ξ-nitro mixture **24a** and **24b**, followed by flash chromatography, gave the pure 20α-nitro and 20β-nitro tri-



Scheme 4 *Reagents:* i, tri-*o*-benzoylrhamnopyranosyl bromide–$ClCH_2CH_2Cl$–$Hg(CN)_2$; ii, O_3–CH_2Cl_2; iii, Zn–HOAc; iv, NH_3–MeOH; v, NH_2OH·HCl–pyridine; vi, Na–PrOH; vii, Fe–HOAc; viii, dimethyldioxirane–Me_2CO; ix, Ac_2O–pyridine; x, PDC; xi, Et_3N–aq. MeOH; xii, Ac_2O–DMAP–Et_2O.

O-acetyl-α-L-rhamnosides **21a** and **21b**. Mild hydrolysis of either compound **21a** or **21b** with triethylamine in aq. methanol gave the 20β-nitro rhamnoside **24**, demonstrating the easy epimerization of the 20α-nitro epimer.[16] It was therefore necessary to use a different route to the 20α-nitro α-L-rhamnoside **24a** from the triacetate **21a**. Treatment of the 20α- and 20β-nitro derivatives **21a** and **21b** with iron in acetic acid gave the corresponding 20α- and 20β-amines **22a** and **22b**, which on hydrolysis yielded the 20α- and 20β-amino α-L-rhamnosides **23a** and **23b**. Subsequent oxidation of each amine with dimethyldioxirane yielded the 20α- and 20β-nitro α-L-rhamnosides **24a** and **24b**. This route was also required to obtain the 20α-amino α-L-rhamnoside **23a**. Acetylation of the 20α-amine **22a** and 20β-amine **22b** followed by alkaline hydrolysis gave the 20α- and 20β-acetamido α-L-rhamnosides **8a** and **8b**, respectively, which showed identical properties with those obtained earlier. Correlation of the C-20 stereochemistry of these rhamnosides with that determined for the genins **4a** and **5a** established the C-20 configuration for all the derivatives (see below).

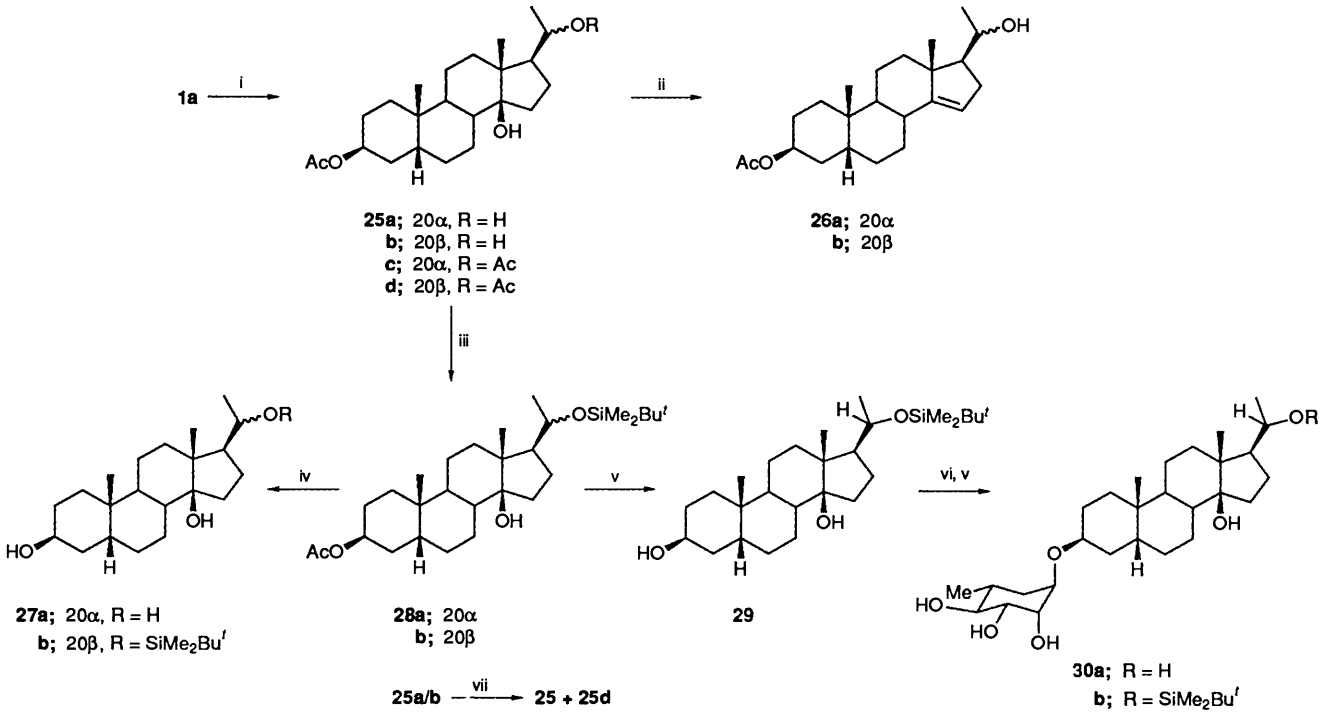
The 20-ketone **1a** was reduced with sodium borohydride in ethanol to give the C-20 alcohols **25a** and **25b** in the ratio 1:1.8 (20α:20β) based on their C-20 proton signals at δ 4.0 (Scheme 5). Lindig has reported a ratio of 1:4 (20α:20β) in a similar reduction.[17] Flash chromatographic separation gave pure diols **25a** and **25b** in 30 and 40% yield, respectively. Acetylation of the C-20 alcohols gave the 3β,20α-diacetate **25c** and the 3β,20β-diacetate **25d**. Dehydration with triphenylphosphine–carbon tetrachloride[11] gave the C-14 unsaturated 20α- **26a** and 20β-**26b** alcohols, respectively.

The 20ξ-alcohols **25a/b** were protected as the 20ξ-silyl ether **28a/b**, which after separation gave separate epimers **28a** and **28b**. When a mixture of the silyl ethers **28a/b** was treated with lithium aluminium hydride (LAH) in diethyl ether unexpected desilylation of the 20α-silyl ether **28a** but not the 20β-silyl ether **28b** occurred as well as deacetylation to give the 3β,14β,20α-triol **27a** and the 20β-silyl ether **27b**. Treatment of 20α-silyl ether **28a** with LAH also gave triol **27a**. However, alkaline hydrolysis of the 20α-silyl ether **28a** gave the 3β-alcohol **29**. This alcohol on reaction with acetobromorhamnose and Fetizon's reagent, followed by basic hydrolysis, gave the 20α-hydroxy rhamnoside **30a** and the 20α-silyl ether rhamnoside **30b**, some loss of the silyl group having occurred during the glycosylation reaction.

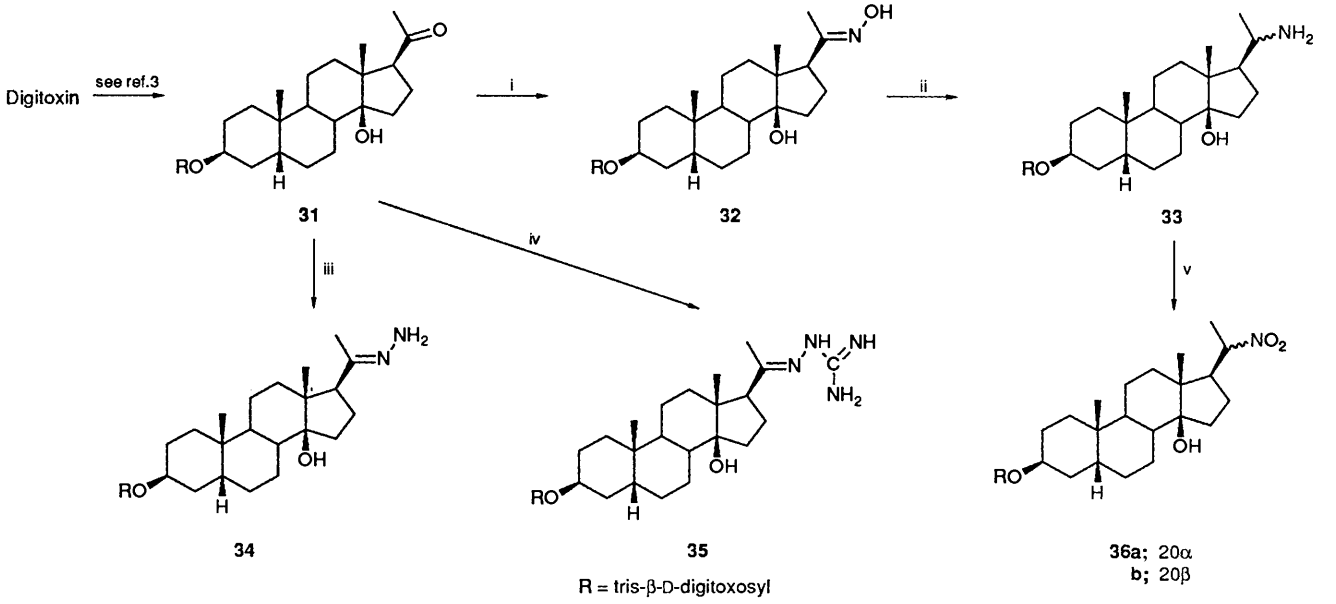
The trisdigitoxoside 20-ketone[3] **31** was converted into the *trans*-oxime **32**, which was reduced with sodium in propan-1-ol to a mixture (1:1) of the 20ξ-amines **33** (Scheme 6). Oxidation of the amine with dimethyldioxirane yielded the 20ξ-nitro epimers **36**. The ketone **31** was converted into the 20-hydrazone **34** and the amidinohydrazone **35**, which were assigned the *trans* structure by analogy with the oxime **32**.

Nuclear Magnetic Resonance Analyses.—Steroid structures, except for the C-20 configuration, were established by ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectral analysis. ^{13}C NMR assignments are based on published data,[18] polarization transfer[19] and internal consistency. COSY,[20] CH correlation,[21] and nuclear Overhauser effect (NOE) measurements[22] were performed as indicated in the Tables.

The configuration of the ortho esters **9a** and **11** was



Scheme 5 *Reagents:* i, $NaBH_4$–EtOH; ii, Ph_3P–CCl_4; iii, Bu^tMe_2SiCl–imidazole–DMF; iv, LAH–Et_2O; v, aq. KOH–EtOH; vi, aceto-bromorhamnose–Ag_2CO_3–Celite; vii, Ac_2O–pyridine



Scheme 6 *Reagents:* i, NH_2OH·HCl–pyridine–NaOAc; ii, Na–PrOH; iii, NH_2NH_2–Et_3N–EtOH; iv, aminoguanidine hydrogen carbonate–NaOH–EtOH; v, dimethyldioxirane–Me_2CO

established as follows (see Tables 1 and 3). The axial orientation of 1′-H in **9a** and **11** was confirmed by the NOE observed at 3′-H and 5′-H when 1′-H was irradiated; a typical 1–3 diaxial interaction. 2′-H has an axial–equatorial coupling to 1′-H. An NOE between the 4′-H and the ortho ester methyl determines the methyl configuration. Both 3′-H and 5′-H show diaxial couplings to 4′-H. An NOE was observed between the ortho ester methyl and the 4′-H in ortho esters **9a** and **11**, confirming the configuration of the quaternary ortho ester carbon. Other NOE measurements shown in Table 3 are consistent with the assigned structures. A similar NOE would be highly unlikely for the alternative configuration. The structure of the amino compound **9b** was inferred from correspondence of its ^1H NMR with that of the acetamido compound **9a**.

The C-20 stereochemistry for the 20α- and 20β-alcohol **25a** and **25b** and hence the glycoside **19** was consistent with earlier assignments.[18] Further NMR analysis was required which would employ coupling constants and NOE measurements to determine both the configurational and conformational structures necessary to establish firmly the configuration of the remaining 20α- and 20β-epimer.[23] Owing to the rotational freedom about the C-17/C-20 bond, it is not possible to solve the conformational and configurational problems independently. The three-bond coupling between 17-H and 20-H coupled with NOE measurements between 20-H and C-13 methyl can be used to locate the position of 20-H uniquely. Care was taken to ensure that the observed couplings were not subject to the effects of virtual coupling.[24] The size of the NOE observed between the C-13 and C-20 methyls was then used to determine the spatial orientation of the C-20 methyl. Other NOE

Table 1 Chemical shifts (*J* in Hz)[a]

Compd.	10-Me	13-Me	3-H[b]	20-H[c]	20-NHCO*Me*	20-Me	5′-Me	Others
2a	0.95	0.96	5.07			1.91		2.04 (s, 3-OAc), 2.35 (m, 17-H), 6.39 (s, 14-OH), 10.24 (s, =NOH)
2b[d]	0.94	0.97	4.08			1.93		2.46 (dd, *J* 5.0, 9.2, 17-H)
3a[e]	0.97	0.97	4.04	3.46 (ddd, *J* 1.5, 6.7, 13.5)		1.25, (d, *J* 6.7)		
3b[e]	1.02	1.09	4.08	3.55 (ddd, *J* 1.5, 6.7, 13.5)		1.41 (d, *J* 6.7)		
4a	0.94	0.95	5.06	3.71	1.88	1.16 (d, *J* 6.4)		2.03 (s, 3-OAc), 7.64 (d, *J* 3.2, 20-N*H*Ac)
4b	0.93	0.94	4.12	3.72	1.90	1.16 (d, *J* 6.4)		7.68 (br s, 20-N*H*Ac)
5a	0.95	1.07	5.05	3.81	1.91	1.21 (d, *J* 6.8)		2.03 (s, 3-OAc), 7.00 (d, *J* 6.1, 20-N*H*Ac)
5b	0.96	1.07	4.09	3.75	1.92	1.21 (d, *J* 6.7)		
6	0.96	0.97	5.08					2.05 (s, =NOAc), 2.06 (s, 3-OAc), 2.60 (m, 17-H), 3.40 (s, 14-OH)
7a[d]	0.97	0.98	4.05	4.09	1.93	1.15 (d, *J* 6.5)		5.13 (br s, 15-H)
7b	0.94	0.90	4.08	4.13	1.94	1.10 (d, *J* 6.3)		5.12 (br s, 15-H), 5.25 (d, *J* 8.8, 20-N*H*Ac)
8a[d]	0.95	0.98	3.96	3.67	1.93	1.27 (d, *J* 6.0)	1.17 (d, *J* 6.4)	3.39 (t, *J* 9.4, 4′-H), 3.67 (m, 5′-H), 3.72 (dd, *J* 3.4, 9.4, 3′-H), 3.81 (dd, *J* 1.5, 3.1, 2′-H)
8b[d]	0.96	1.04	3.95	3.85	1.93	1.27 (d, *J* 6.3)	1.15 (d, *J* 6.0)	3.39 (t, *J* 9.4, 4′-H), 3.67 (m, 5′-H), 3.73 (dd, *J* 3.3, 9.5, 3′-H), 3.85 (m, 2′-H), 4.80 (s, 1′-H)
9a[e]	0.96	0.98	4.11	3.67	1.92	1.20 (d, *J* 6.4)	1.18 (d, *J* 6.7)	1.71 (s, CMe), 2.08 (s, 2 × OAc), 3.67 (m, 5′-H), 4.61 (dd, *J* 2.3, 4.2, 2′-H), 4.97 (t, *J* 9.7, 4′-H), 5.19 (dd, *J* 4.2, 9.9, 3′-H), 5.48 (d, *J* 2.2, 1′-H)
9b[e]	0.99	1.02	4.10	3.51		1.29 (d, *J* 6.6)	1.19 (d, *J* 6.0)	1.70 (s, CMe), 2.08 (s, 2 × OAc), 3.65 (m, 5′-H), 4.60 (dd, *J* 2.3, 4.3, 2′-H), 4.97 (t, *J* 9.7, 4′-H), 5.18 (dd, *J* 4.3, 9.8, 3′-H), 5.47 (d, *J* 2.2, 1′-H)
12a	1.00	0.97	5.29	4.23		1.26 (d, *J* 6.5)		5.15 (br s, 15-H), 6.05 (d, *J* 8.2, 20-N*H*Acyl)
12b	1.00	0.90	5.29	4.18		1.21 (d, *J* 6.4)		5.18 (br s, 15-H), 6.01 (d, *J* 8.8, 20-N*H*Acyl)
13	0.97	0.87		4.71		1.46 (d, *J* 6.7)		2.28 (dd, *J* 5.3, 14.5, 4β-H), 2.41 (m, 17-H), 2.59 (d, *J* 14.2, 4α-H)
14a	0.96	1.03	5.05	4.91		1.57 (d, *J* 6.4)		2.04 (s, 3-OAc)
14b	0.94	0.88	5.06	4.73		1.49 (d, *J* 6.7)		2.04 (s, 3-OAc), 2.42 (m, 16β-H)
15	0.96	1.15	5.15	3.37		1.45 (d, *J* 6.6)		2.05 (s, 3-OAc)
16	0.89	1.03	4.08				1.35 (d, *J* 6.0)	2.80 (m, 17-H), 4.23 (m, 5′-H), 4.81 and 5.10 (each d, J_{AB} 18.1, 21-H_2), 5.10 (d, *J* 1.3, 1′-H), 5.62 (dd, *J* 1.8, 3.2, 2′-H), 5.67 (t, *J* 9.9, 4′-H), 5.87 (dd, *J* 3.4, 10.2, 3′-H), 5.88 (s, 22-H), 7.23–8.12 (Ph)
17	0.99	1.03	4.07			2.24 (s)	1.34 (d, *J* 6.3)	2.91 (dd, *J* 9.1, 4.2, 16β-H), 4.23 (m, 5′-H), 4.32 (s, 14-OH), 5.10 (d, *J* 1.3, 1′-H), 5.62 (dd, *J* 1.7, 3.8, 2′-H), 5.66 (t, *J* 9.9, 4′-H), 5.87 (dd, *J* 3.4, 10.1, 3′-H), 7.23–8.12 (m, Ph)
18	1.04	1.21	4.07	3.86		1.27 (d, *J* 6.4)	1.34 (d, *J* 6.2)	4.23 (m, 5′-H), 5.10 (br s, 1′-H), 5.63 (m, 2′-H), 5.66 (t, *J* 9.9, 4′-H), 5.86 (dd, *J* 3.3, 10.1, 3′-H), 7.23–8.12 (m, Ph)
20	0.98	1.04	4.07			1.95 (s)	1.35 (d, *J* 6.3)	2.40 (m, 17-H), 4.24 (m, 5′-H), 5.10 (d, *J* 1.3, 1′-H), 5.63 (dd, *J* 1.5, 3.3, 2′-H), 5.66 (t, *J* 9.9, 4′-H), 5.87 (dd, *J* 3.4, 10.1, 3′-H), 9.04 (br s, =NOH), 7.23–8.12 (Ph)
21a	0.96	1.03	3.94	4.91		1.57 (d, *J* 6.4)	1.19 (d, *J* 6.3)	1.99, 2.05, 2.15 (3 s, 3 × OAc), 3.91 (m, 5′-H), 4.80 (d, *J* 1.5, 1′-H), 5.05 (t, *J* 9.9, 4′-H), 5.19 (dd, *J* 1.7, 3.4, 2′-H), 5.32 (dd, *J* 3.4, 10.0, 3′-H)
21b	0.94	0.88	3.94	4.73		1.49 (d, *J* 6.7)	1.19 (d, *J* 6.3)	1.98, 2.05, 2.14 (3 s, 3 × OAc), 2.42 (m, 17-H), 3.92 (m, 5′-H), 4.80 (d, *J* 1.5, 1′-H), 5.05 (t, *J* 9.9, 4′-H), 5.18 (dd, *J* 1.7, 3.4, 2′-H), 5.32 (dd, *J* 3.4, 10.0, 3′-H)
22a	0.97	0.94	3.93	3.32		1.22 (d, *J* 6.3)	1.17 (d, *J* 6.3)	1.97, 2.03, 2.13 (3 s, 3 × OAc), 3.88 (m, 5′-H), 4.79 (br s, 1′-H), 5.03 (t, *J* 9.9, 4′-H), 5.19 (dd, *J* 1.5, 3.1, 2′-H), 5.32 (dd, *J* 3.4, 10.1, 3′-H)
22b	0.97	1.14	3.95	3.28		1.42 (d, *J* 7.1)	1.19 (d, *J* 6.3)	1.98, 2.05, 2.13 (3 s, 3 × OAc), 3.90 (m, 5′-H), 4.81 (d, *J* 1.2, 1′-H), 5.05 (t, *J* 9.9, 4′-H), 5.21 (dd, *J* 1.6, 3.3, 2′-H), 5.33 (dd, *J* 3.5, 10.0, 3′-H)
23a[e]	0.97	0.98	3.94	3.47		1.23 (d, *J* 6.1)	1.22 (d, *J* 6.1)	3.36 (t, *J* 9.4, 4′-H), 3.64 (m, 5′-H), 3.77 (d, 2′-H), 3.68 (dd, *J* 3.2, 9.4, 3′-H), 4.78 (br s, 1′-H)
23b[e]	0.95	1.05	3.93	3.43		1.34 (d, *J* 6.6)	1.22 (d, *J* 6.3)	3.36 (t, *J* 9.5, 4′-H), 3.63 (m, 5′-H), 3.68 (d, *J* 3.4, 9.4, 3′-H), 3.76 (dd, *J* 1.7, 3.4, 2′-H), 4.75 (d, *J* 1.7, 1′-H)

Table 1 (*continued*)

Compd.	10-Me	13-Me	3-H[b]	20-H[c]	20-NHCO*Me*	20-Me	5′-Me	Others
24a[f]	0.86	0.91	3.79	4.85		1.49 (d, *J* 6.3)	1.09 (d, *J* 6.2)	3.16 (t, *J* 9.3, 4′-H), 3.54 (m, 3′-H), 4.60 (s, 1′-H)
24b[f]	0.84	0.73	3.80	4.43		1.41 (d, *J* 6.6)	1.09 (d, *J* 6.2)	2.03 (m, 17-H), 3.08 (t, *J* 9.3, 4′-H), 3.32 (m, 5′-H), 3.39 (dd, *J* 3.3, 9.2, 3′-H), 3.50 (dd, *J* 1.8, 3.2, 2′-H), 4.47 (s, 1′-H)
25a	0.96	1.02	5.06	4.02		1.10 (d, *J* 6.3)		2.03 (s, 3-OAc)
25b	0.97	1.19	5.07	3.85		1.26 (d, *J* 6.6)		2.04 (s, 3-OAc)
25c	0.97	0.89	5.08	4.99		1.23 (d, *J* 6.3)		2.05 (s, 3-OAc), 2.08 (s, 20-OAc)
25d	0.95	0.95	5.07	4.93		1.18 (d, *J* 6.1)		2.01 (s, 20-OAc), 2.04 (s, 3-OAc)
26a	0.91	0.98	5.05	3.88		1.24 (d, *J* 6.0)		2.04 (s, 3-OAc), 5.18 (s, 15-H)
26b	0.98	1.02	5.05	3.92		1.18 (d, *J* 6.3)		2.04 (s, 3-OAc), 5.31 (s, 15-H)
27a	0.96	1.03	4.11	4.02		1.10 (d, *J* 6.3)		
28a	0.98	0.96	5.06	4.13		1.15 (d, *J* 6.3)		0.14 (s, $SiMe_2$), 0.91 (s, CMe_3), 2.04 (s, 3-OAc)
29	0.97	0.94	4.11	4.08		1.12 (d, *J* 6.3)		0.12 (s, $SiMe_2$), 0.89 (s, CMe_3)
30a[d]	0.93	1.01	3.93	3.98		1.08 (d, *J* 6.3)	1.26 (d, *J* 6.0)	3.34 (t, *J* 9.4, 4′-H), 3.66 (m, 5′-H), 3.74 (dd, *J* 3.8, 9.5, 3′-H), 3.83 (q, *J* 1.6, 3.3, 2′-H), 4.80 (d, *J* 1.3, 1′-H)
30b[d]	0.98	1.01	3.95	4.17		1.18 (d, *J* 6.6)	1.27 (d, *J* 6.3)	0.16, 0.17 (2 s, $SiMe_2$), 0.93 (s, CMe_3), 3.38 (t, *J* 10.9, 4′-H), 3.67 (m, 5′-H), 3.74 (dd, *J* 3.3, 9.4, 3′-H), 3.81 (dd, *J* 1.7, 3.2 2′-H), 4.80 (m, 1′-H)
32[d,g]	0.94	0.93	4.04			1.93		2.45 (dd, *J* 5.0, 9.0, 17-H)
33[d,g,h]	0.95	0.95	4.04	~3.45[i]		~1.25 (m)[j]		
34[d,g]	0.94	0.89	4.04			1.86		2.47 (dd, *J* 5.0, 9.0, 17-H)
35[d,g]	0.95	0.94	4.04			1.99		2.52 (dd, *J* 4.5, 9.0, 17-H)
36a[d,g]	0.95	1.03	~4.04[i]	4.85[i]		1.58 (d, *J* 6.4)		
36b[d,g]	0.93	0.87	~4.04[i]	~4.45[i]		1.49 (d, *J* 6.7)		2.39 (dd, *J* 9.0, 18.0, 16β-H)

[a] For solutions in $CDCl_3$ ($SiMe_4$ internal standard) unless otherwise indicated on a Bruker AM 300 instrument. [b] Broad singlet. [c] Multiplet. [d] In $CDCl_3$–CD_3OD (1:1). [e] In CD_3OD. [f] In $(CD_3)_2SO$. [g] Trisdigitoxoside spectra **32**–**36a**, **36b** are in agreement with data reported in ref. 3. [h] Major isomer. [i] Obscured by trisdigitoxoside, see ref. 3. [j] Obscured by 6′,6″,6‴-H_3 signals.

measurements, such as those from the C-20 methyl to 16β-H or from 20-H to 17-H, were occasionally observed and were consistent with the proposed stereochemistries and conformations. When necessary, identification of the protons involved was accomplished with standard two-dimensional NMR techniques (COSY)[20] and inverse C–H correlation.[25] For some compounds the NOEs were measured in a reciprocal fashion by irradiating, for example, both C-20 and C-13 methyls in turn and observing the enhancement of the other methyl. In other cases measurement from both methyls was not possible owing to overlap of one of the methyl groups with other resonances. In all cases interpretation of the NOE results was done by comparing data from both epimers. Complete coupling constant and NOE data, together with a detailed description of the techniques involved, will be published separately.[23]

Receptor Binding.—14β,20β-Dihydroxy-5β,14β-pregnan-3β-yl α-L-rhamnopyranoside **19** binds strongly to the cardiac glycoside recognition site of heart muscle[15] (see Table 4) whereas the 20α-epimer **30a** binds less well. Comparison of the 20α- and 20β-acetamido **8a** and **8b**, the 20α- and 20β-amino **23a** and **23b** and the 20α- and 20β-nitro **24a** and **24b** pairs shows a similar relationship. Restricted rotation around the C-17–C-20 bond limits the space in which the polar group is projected from the steroid skeleton. The stereochemistry appears to be more favourable in the 20β than in the 20α derivatives. The 20α- and 20β-amino compounds can interact through donor and acceptor hydrogen bonding to the active site in an analogous manner to the alcohols. Unlike the alcohols and amines the 20α- and 20β-nitro derivatives cannot form donor hydrogen bonds, indicating that an acceptor bond is formed with a hydrogen atom of the enzyme. Owing to their different spacial requirements potent binding of these groups most likely occurs with receptor groups different from those interacting with the unsaturated lactone in the cardiac glycosides. Clearly a planar conjugated π-bonded structure is not required for highly potent binding to occur.

While the genin derivatives are very much less potent than the glycosides the relationship of greater potency for the 20β over 20α derivatives remains the same, *e.g.* the 20β-acetamido- **5a** over the 20α-acetamido- **4a**, the 20β-nitro- **14b** over the 20α-nitro **14a**, and the 20β-alcohol **25b** over the 20α-alcohol **25a** derivatives. Little difference is observed between the 20α- and 20β-acetamido 3β-acetates **4a** and **5a** and the corresponding 3β-alcohols **4b** and **5b**. The C-3 ketone **13** binds only weakly.

We have shown that the trisdigitoxoside is less potent than the rhamnoside for the 20β-alcohol **19**[15] and this relationship apparently holds for the amino and nitro derivatives **33** and **36** also. Pregnane derivatives of this type, unlike the corresponding cardenolides, show K^+-sparing diuresis, a desirable property for cardiotonic substances as it increases their margin of safety.[26] Other C-20 groups tested in the RBA which showed moderately strong receptor-binding potency were the oxime **32**, the hydrazone **34** and the amidinohydrazone **35**. C-20 Amidinohydrazone genin derivatives have been shown to possess cardiotonic properties.[27]

Experimental

^1H and ^{13}C NMR spectra, except for compounds **10a**, **10b** and **11**, are reported in Tables 1 and 2. *J*-Values are given in Hz.

Table 2 ^{13}C chemical shifts[a]

Carbon	Compounds										
	2a	**2b**	**3a**[b]	**3b**[b]	**4a**	**4b**	**5a**	**5b**	**6**	**7a**[c]	**7b**
1	30.50[m]	29.77	30.87	30.80	30.41	29.63	30.44	29.66	30.50	30.11	29.69
2	25.07	27.92	28.58[m]	28.48[m]	25.01	27.85	25.01	27.93	25.07	28.01	27.93
3	70.59	67.05	67.67	67.59	70.36	66.86	70.39	66.85	70.51	67.00	66.95
4	30.56[m]	33.44	33.83[n]	34.14	30.41	33.24	30.44	33.34	30.50	33.50	33.30
5	36.97	36.19	37.47	37.34	36.78	35.96	36.80	36.02	36.92	36.84	36.33
6	26.47	26.61	27.89[m]	27.64[m]	26.35	26.50	26.41	26.57	26.42[m]	26.78	26.20
7	21.07[n]	21.11[m]	22.12[o]	21.81[n]	20.95[m]	20.95[m]	20.70[n]	20.74[m]	21.30[n]	24.48	23.69
8	40.01	40.10	41.75	41.55	41.20	41.26	41.24	41.33	40.12	40.04	39.41
9	35.25	35.10	36.43	36.45	35.71	35.58	35.70	35.60	35.42	35.64	34.97
10	35.13	35.41	36.48	36.67	35.14	35.39	35.14	35.42	35.17	35.49	35.26
11	21.24[n]	21.37[m]	22.49[o]	22.24[n]	21.31[m]	21.43[m]	21.24[m]	21.38[m]	21.49[n]	22.22	21.93
12	39.53	39.67	41.04	39.75	41.20	41.26	42.29	42.41	40.12	42.60	42.73
13	48.96	49.00	~50[p]	~50[p]	46.82	46.83	47.35	47.38	49.33	46.96	47.10
14	85.90	85.88	85.26	87.03	86.01	86.26	86.04	86.24	85.29	155.70	155.45
15	33.79	33.79	34.26[n]	32.80	31.85	31.84	32.09	32.16	33.62	117.04	116.34
16	27.68	27.56	19.77	20.61	21.41[m]	21.43[m]	25.86	25.88	26.21[m]	34.96	34.60
17	56.79	56.85	57.49	60.75	54.27	54.24	55.05	55.13	56.86	58.96	58.69
18	15.45	15.49	15.78	15.69[o]	13.94	13.95	16.39	16.43	15.56	17.03	13.95
19	23.84	23.90	23.61	24.27	23.58[n]	23.70[n]	23.56[n]	23.61[n]	23.80	23.57	23.47[m]
20	162.43	162.79	46.06	54.34	46.33	46.41	49.36	49.10	168.02	47.14	46.49
21	17.51	17.44	24.41	15.36[o]	19.35	19.30	20.23	20.27	17.63	21.40	19.30
1′											
2′											
3′											
4′											
5′											
6′											
OCO*Me*	21.50				21.46		21.46		21.49		
OCO*Me*											
OCO*Me*	170.66										
OCOMe					170.60		170.60		170.63		
OCOMe											
OCOMe											
NHCO*Me*					23.66[n]	23.47[n]	23.69[n]	23.76[n]	19.55	22.55	23.70[m]
NHCOMe					170.25	170.70	169.60	169.66	171.41	170.99	168.59

Table 2 (*continued*)

Carbon	Compounds													
	8a[c]	**8b**[c]	**9a**[b,d,e]	**12a**[f]	**12b**[f]	**13**	**14a**[e]	**14b**[e]	**15**	**16**[g,h]	**17**[g]	**18**[c,g]	**20**[g]	**21a**[i]
1	30.00[m]	30.03[m]	32.24[m]	30.02[m]	30.02[m]	37.13	30.51	30.49	30.48[m]	30.42	30.55	30.59	30.57	30.31
2	27.04	27.13	27.72	24.78	24.77	36.71	25.08	25.10	25.05	26.44	26.51[m]	26.33[m]	26.51[m]	26.51
3	72.55	72.55	69.80	76.23	76.24	213.00	70.42	70.43	74.47	73.20	73.36	73.43	73.45	72.88
4	30.76[m]	30.77[m]	31.42[m]	30.08[m]	30.08[m]	42.13	30.51	30.49	30.53[m]	29.60	29.67	29.71	29.67	29.48
5	37.17	37.16	36.90	36.91	36.91	43.71	36.90	36.88	36.96	36.37	36.58	36.60	36.59	36.34
6	26.76	26.83	27.94	25.81	25.82	26.60	26.42	26.46	26.45	26.51	26.60[m]	26.56[m]	26.67[m]	26.37
7	21.35[n]	21.19[n]	22.40	23.73	23.74	20.79[m]	20.62	20.72	20.45[n]	21.20[m]	21.59[n]	21.52[n]	21.18[n]	20.59
8	41.13	41.39	42.04	39.55	39.63	41.26	41.19	41.52	40.39	41.79	40.02	40.82	40.13	41.14
9	36.06	36.12	38.02	34.96	34.85	36.64	35.53	35.73	35.51	35.74	35.33	35.78	35.41	35.54
10	35.54	35.57	36.21	34.45	34.98	35.24	35.22	35.22	35.18	35.26	35.28	33.38	35.28	35.21
11	21.71[n]	21.69[n]	22.51	21.94	21.69	20.98[m]	21.14	21.28	21.33[n]	21.37[m]	20.94[n]	20.68[n]	21.39[n]	21.17
12	41.50	42.38	42.30	42.27	41.85	40.02	40.61	40.21	40.78	40.03	39.40	41.72	39.72	40.62
13	47.50	47.46	49.85	47.07	46.78	47.23	47.26	47.21	48.22	49.59	49.36	47.72	49.01	47.18
14	85.67	86.07	86.52	154.82	154.83	85.86	86.09	86.15	85.03	85.49	84.96	85.64	85.88	86.11
15	31.50	31.57	33.31	116.60	116.56	31.42	32.16	31.43	31.94	33.14	33.98	32.19	33.82	32.06
16	21.79	26.19	22.65	34.91	34.10	24.56	24.79	24.59	25.74	26.86	24.94	26.94[m]	27.56	24.85
17	54.66	55.02	55.62	58.20	58.19	51.98	54.38	52.06	54.39	50.93	62.42	56.77	56.87	54.30
18	13.98	15.99	14.68	17.35	16.92	13.70	16.02	13.69	16.31	15.76	15.40	16.39	15.52	16.00
19	23.70	23.83	23.21	23.38	23.38	22.57	23.76	23.74	23.74	23.75	23.88	23.92	23.94	23.70
20	47.01	49.46	47.90	27.61	47.90	88.76	87.71	86.80	51.10	174.52	217.68	71.94	162.75	87.75
21	18.73	19.90	20.69	20.74	21.12	20.34	21.26	20.34	21.50	73.42	33.36	23.37	17.70	21.20
1′	98.79	98.72	98.49							95.78	95.77	95.84	95.81	95.60
2′	71.69	71.73	77.88							70.14	71.57	71.63	71.58	70.54
3′	71.95	71.91	72.16							71.53	70.17	70.25	70.21	69.29
4′	72.23	73.26	71.98							71.95	72.04	72.09	72.06	71.29
5′	69.02	69.96	70.13							66.91	66.88	66.94	66.87	66.50
6′	17.29	17.39	17.78							17.67	17.70	17.75	14.47	17.40
OCO*Me*							21.53	21.53						20.73
OCO*Me*														20.82
OCO*Me*														20.98
OCO*Me*														
OCOMe							170.65	170.67						169.61
OCOMe														170.01
OCOMe														170.24
NHCO*Me*	22.67	22.50	24.29											
*NHC*OMe	172.9	171.17	173.15											

Table 2 (*continued*)

Carbon	Compounds														
	21[b,i]	**22a**[i]	**22b**[i]	**23a**[b,e]	**23b**[b,e]	**24a**[j]	**24b**[j]	**25a**	**25b**	**25c**	**25d**	**26a**	**26b**	**27a**	**28a**
1	30.30[m]	30.40[m]	30.41[m]	31.61	31.83	30.80[m]	30.66[m]	30.52[m]	30.52	30.55	30.49	30.51[m]	30.54[m]	29.43	30.63[m]
2	26.55[n]	26.37[n]	36.39[n]	27.47	27.52	26.47[n]	26.48[n]	25.03	25.05	25.10	25.08	25.07	25.12	27.31	25.17
3	73.07	73.10	73.06	73.59	73.60	72.05	72.04	70.52	70.53	70.54	70.48	70.65	70.68	66.35	70.75
4	29.57[m]	29.52[m]	29.53[m]	30.93	30.86	30.30[m]	30.32[m]	30.46[m]	30.52	30.55	30.49	30.43[m]	30.44[m]	32.86	30.71[m]
5	36.33	36.48	36.48	38.05	38.14	36.40	36.42	36.91	36.94	36.97	36.90	37.20	37.23	35.84	37.10
6	26.37[n]	26.54[n]	26.62[n]	27.71	27.90	26.05[n]	26.06[n]	26.36	26.25	26.43	26.48	26.14	26.15	26.34	26.52
7	20.69[o]	19.12	20.47	21.95	22.07	20.23	20.38[o]	21.25[n]	20.54[m]	21.51[m]	21.30[m]	23.92	23.95	20.65	21.22
8	41.49	40.43	40.32	41.83	42.29	~40[p]	~40[p]	40.58	40.67	39.81	41.52	39.68	39.78	40.07	39.81
9	35.75	35.42	35.43	36.88	36.88	34.67	34.74	35.46	35.55	35.23	35.69	34.77	34.97	35.07	35.24
10	35.20	35.18	35.24	36.33	36.38	34.85	34.85	35.13	35.18	35.23	35.19	35.06	35.09	35.07	35.28
11	21.33[o]	21.32	21.17	22.27	22.53	20.89	20.89[o]	21.47[n]	21.32[m]	21.07[m]	20.80[m]	21.61	21.82	20.98	21.48
12	40.23	39.80	40.98	40.42	42.54	39.50	39.51	39.98	41.55	41.19	41.67	41.69	42.80	39.63	41.07
13	47.16	47.97	48.24	~50[p]	~50[p]	46.79	46.95	47.58	47.64	47.27	46.67	46.41	47.29	47.41	47.29
14	86.11	84.19	84.89	86.35	86.23	84.34	84.15	84.78	85.42	84.42	85.73	154.81	155.51	84.47	83.48
15	31.39	32.47	32.00	32.95	32.49	29.42	29.43	32.52	32.13	32.12	31.72	116.79	116.29	31.95	32.54
16	24.52	26.54[n]	25.96	20.02	23.59	25.00	23.80	18.09	26.47	20.26	25.08	33.56	33.72	17.80	18.78
17	52.03	55.22	54.84	55.63	54.95	53.70	51.90	56.20	56.61	54.14	54.31	60.38	58.69	55.84	57.32
18	13.64	15.22	16.42	15.40	15.94	16.06	14.01	14.88	16.31	14.56	15.15	17.33	17.55	14.44	15.02
19	23.70	23.78	23.77	24.31	24.37	23.77	23.80	23.75	23.76	23.79	23.75	23.63[n]	23.77[n]	23.43	23.91[n]
20	86.72	45.85	51.04	47.74	51.24	88.17	86.72	65.59	71.89	71.58	74.28	69.18	69.49	65.05	68.66
21	20.24	22.02	20.63	19.85	17.32	20.89	19.93	22.04	23.32	18.92	19.29	23.55[n]	23.58[n]	21.32	23.33[n]
1′	95.70	95.63	95.64	99.84	99.85	98.24	98.27								
2′	70.56	70.53	70.56	72.91	72.93	70.79	70.78								
3′	69.29	69.30	69.34	72.63	72.54	71.09	71.11								
4′	71.28	71.34	71.35	74.13	74.08	71.15	71.17								
5′	66.51	66.41	66.46	69.97	70.01	68.60	68.63								
6′	17.40	17.37	17.41	17.92	17.92	17.86	17.89								
OCO*Me*	20.75							21.47	21.49	21.51	21.50	21.50	21.52		21.56
OCO*Me*	20.82									21.44	21.62				
OCO*Me*	20.98														
O*C*OMe	170.01							170.64	170.66	170.66	170.63	170.65	170.65		170.70
O*C*OMe	170.25									169.56	170.38				
O*C*OMe	170.25														
NHCO*Me*															
NH*C*OMe															

Table 2 (*continued*)

	Compounds								
Carbon	**29**	**30a**	**30b**	**32**[c,k]	**33**[c,k]	**34**[c,k]	**35**[c,k,l]	**36a**[c,k]	**36b**[c,k]
1	29.84	30.05[m]	30.10[m]	30.41[m]	30.19[m]	30.27[m]	30.34[m]	30.54	30.15
2	27.79	27.07	27.09	27.10[n]	27.14[n]	29.96	27.01[n]	26.82[m]	26.82[m]
3	66.96	72.50	72.58	73.73	73.15	73.22	73.29	73.15	73.15
4	33.38	30.85[m]	30.88[m]	30.82[m]	30.62[m]	30.70[m]	30.76[m]	30.54	30.15
5	36.24	37.11	37.17	37.13	36.95	37.02	37.08	36.90	36.90
6	26.63	26.92	26.91	27.24[n]	26.88[n]	27.14	27.17[n]	27.04[m]	27.04[m]
7	21.13	21.34	21.73	21.67[o]	21.80[o]	21.52[n]	21.54[o]	20.58[n]	21.07[n]
8	39.64	40.71	40.00	41.19	40.10	40.83	40.54	41.25	41.26
9	35.44	35.92	35.62	36.10	35.75	35.88	35.87	36.07	35.95
10	35.08	35.59	35.72	35.76	35.50	35.61	35.68	35.54	35.54
11	21.52	21.68	21.89	22.00[o]	22.37[o]	21.88[n]	21.92[o]	20.95[n]	21.68[n]
12	41.03	40.28	41.19	40.53	42.51	40.22	40.19	41.24	40.74
13	47.23	48.08	47.59	~50[p]	50.14	~50[p]	~50[p]	47.77	47.67
14	83.81	85.09	84.79	86.30	85.57	86.20	86.70	86.28	86.35
15	32.40	32.60	32.67	34.08	32.18	34.09	34.37	31.45	31.21
16	18.74	18.46	19.03	27.24	29.98	27.36	27.85	25.45	24.75
17	57.19	56.50	57.45	57.39	56.73	59.56	59.32	54.62	52.55
18	14.92	15.06	15.05	15.85	16.61	15.68	15.86	16.30	14.03
19	23.88	24.08	24.02	24.13	23.89	24.00	24.02	23.78	23.78
20	68.68	65.70	68.86	163.87	49.65	159.94	164.84	88.56	87.35
21	23.22	21.88	23.28	16.46	10.67	17.22	20.77	21.28	20.34
1′		98.51	98.61						
2′		71.85	71.82						
3′		71.88	71.88						
4′		73.37	73.33						
5′		68.79	69.25						
6′		17.63	17.53						

[a] For solutions in $CDCl_3$ ($SiMe_4$ internal standard) unless indicated otherwise on a Bruker AM300 instrument. [b] In CD_3OD. [c] In $CDCl_3$–CD_3OD (1:1). [d] 20.61, 20.69 (2 × *Me*CO), 171.67, 171.70 (2 × Me*C*O), 24.29 (*Me*CO_3), 125.53 (Me*C*O_3). [e] Assignments based on 2D analysis. [f] 114.64 (q, *J* 285.7), 115.85 (q, *J* 288.0) (2 × *C*F_3CO); 155.94 (q, *J* 37.7), 156.98 (q, *J* 29.6) (2 × CF_3*C*O). [g] The tribenzoate is in agreement with digitoxigenin tri-*O*-benzoylrhamnoside **16**: 165.55, 165.68, 165.77 (3 × C=O), 129.18, 129.28, 129.42 (C-1), 129.59, 129.67, 129.83 (6 × C-2), 128.21, 128.36, 128.52 (6 × C-3), 133.00, 133.22, 133.36 (3 × C-4). [h] 117.61 (C-22), 174.47 (C-23). [i] The triacetate is in agreement with digitoxigenin tri-*O*-acetylrhamnoside: 20.75, 20.82, 20.98 (3 × *Me*CO), 170.01, 170.25, 170.25 (3 × Me*C*O). [j] $(CD_3)_2SO$. [k] The trisdigitoxoside is in agreement with digitoxin: 95.91 (1′) 99.39, 99.56 (1″, 1‴), 37.40, 37.76, 38.47 (2′, 2″, 2‴), 67.07, 61.21, 68.15 (3′, 3″, 3‴), 82.73, 83.02 (4′, 4″), 74.45 (4‴), 68.60, 68.73 (5′, 5″), 70.20 (5‴), 18.11, 18.18, 18.23 (6′, 6″, 6‴). [l] 159.03 [=N–NH*C*(=NH)NH_2]. [m–o] Chemical shifts are interchangeable within a column. [p] Obscured by solvent signals.

Table 3 NOE measurements as percentage enhancements[a]

Proton irradiated in **9a**	Observed NOE						
	1′	2′	3′	4′	5′	6′	O_3CMe
1′		6	1.4	0	4	0	0
2′	9		6	0	0	0	0
5′	5	0	5	4		6	0
O_3CMe	0.3	0.1	0	1.2	0		
in **11**							
1′		8	0.5	0	6	0	0
2′	11		13	0	0	0	0
O_3CMe	0.6	0.3	0	2.6	0		

[a] For solutions in $CDCl_3$ on a Bruker AM500 instrument.

Table 4 Potency of 14-hydroxy-5β,14β-pregnane derivatives in a [^{3}H]ouabain radioligand binding assay (RBA)[a]

Compd.	Substituent 3β	20α	20β	Inhibitory conc (IC_{50}/nmol dm^{-3})
4a	OAc	NHAc	H	14 000
5a	OAc	H	NHAc	1100
4b	OH	NHAc	H	12 600
5b	OH	H	NHAc	1500
14a	OAc	NO_2	H	424 000
14b	OAc	H	NO_2	10 200
25a	OAc	OH	H	41 000
25b	OAc	H	OH	8000
13	carbonyl	H	NO_2	10 000
8a	α-L-rhamnoside	NHAc	H	1800
8b	α-L-rhamnoside	H	NHAc	450
30a	α-L-rhamnoside	OH	H	1600
19	α-L-rhamnoside	H	OH	75
23a	α-L-rhamnoside	NH_2	H	115
23b	α-L-rhamnoside	H	NH_2	72
24a	α-L-rhamnoside	NO_2	H	940
24b	α-L-rhamnoside	H	NO_2	45
32	tris-β-D-digitoxoside	*trans*=N–OH		1300
33[b]	tris-β-D-digitoxoside	20ξ-amino		300
36[b]	tris-β-D-digitoxoside	20ξ-nitro		450
34	tris-β-D-digitoxoside	$=NNH_2$		5200
35	tris-β-D-digitoxoside	$=NNH(=NH)NH_2$		200

[a] IC_{50} represents the concentration that inhibits binding of [^{3}H]ouabain by 50% and is obtained from a complete concentration/inhibition curve. Digitoxigenin and digitoxin give values of 20 and 8 nmol dm^{-3}, respectively. [b] Approximately 1:1 (20α:20β).

TLC was carried out in the following solvent systems on silica gel (Merck type 60H): acetone–diethyl ether, ethyl acetate–light petroleum (35–60 °C), or methanol–methylene dichloride mixtures (genins); methanol–methylene dichloride (glycosides); and chloroform–methanol–diethylamine (100:10:0.75) (C-20 amines), and compounds were visualized by dipping of the plates in 5% sulfuric acid–ethanol followed by heating at 120 °C. Flash chromatography was carried out on silica gel (Merck type 60 for column chromatography) unless otherwise stated. M.p.s were measured on a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were performed by Mr. W. Baldeo, School of Pharmacy, University of London, England.

3β-*Acetoxy*-14-*hydroxy*-5β,14β-*pregnan*-20-*one* trans-*Oxime* **2a**.—A solution of the 20-ketone **1a** (210 mg), prepared as reported in ref. 3, in 95% ethanol (9 cm^3)–pyridine (1.5 cm^3) was refluxed with hydroxylamine hydrochloride (210 mg) for 2 h and diluted with ice–water (100 cm^3). The precipitate was filtered off to give the trans-*oxime* **2a** (170 mg), m.p. 233–236 °C (from aq. MeOH) (Found: C, 70.6; H, 9.6; N, 3.35. $C_{23}H_{37}NO_4$ requires C, 70.55; H, 9.5; N, 3.6%).

3β,14-*Dihydroxy*-5β,14β-*pregnan*-20-*one* trans **2b** *and* cis-*Oxime* **2c**.—Following the procedure described above the 20-ketone **1b** (550 mg) gave the 20-oximes, which were separated by flash chromatography. Elution (70% ethyl acetate–light petroleum) gave the trans-20-*oxime* **2b** (528 mg), m.p. 124–126 °C (from methylene dichloride–light petroleum) (Found: C, 72.1; H, 10.0; N, 4.0. $C_{21}H_{34}NO_3$ requires C, 72.4; H, 9.8; N, 4.0%). Further elution gave the cis-20-*oxime* **2c** (31 mg), m.p. 170–172 °C (from acetone–light petroleum) (Found: C, 70.8; H, 10.0; N, 3.4. $C_{21}H_{34}O_3N{\cdot}0.5H_2O$ requires C, 70.55; H, 9.9; N, 3.9%).

20α-*Amino*- **3a** *and* 20β-*Amino*-5β,14β-*pregnane*-3β,14-*diol Hydrochloride* **3b**.—A solution of the 20-oxime **2b** (300 mg) in abs. ethanol (25 cm^3) containing chloroform (0.5 cm^3) was hydrogenated over PtO_2 (125 mg) at 3 atm for 2 days until no starting material remained (TLC). The PtO_2 was filtered off and the filtrate was evaporated to give the 20α-amine·HCl **3a** (18 mg), m.p. 258–260 °C (decomp.) (from MeOH). The residue from the mother liquor was separated by flash chromatography by elution [$CHCl_3$–MeOH–NH_4OH (132:12:0.9)] to give the 20β-*amine·HCl* **3b** (130 mg), m.p. 272–274 °C (from methylene dichloride–light petroleum) (Found: C, 67.75; H, 10.3; N, 3.8; Cl, 9.6. $C_{21}H_{37}NO_2{\cdot}HCl$ requires C, 68.0; H, 10.05; N, 3.8; Cl, 9.5%).

20α-*Acetamido*- **4a** *and* 20β-*Acetamido*-14-*hydroxy*-5β,14β-*pregnan*-3β-*yl Acetate* **5a** *and* 20-trans-*Acetoxyimino*-14-*hydroxy*-5β,14β-*pregnan*-3β-*yl Acetate* **6**.—The 20-oxime **2b** (400 mg) was hydrogenated as described above and the 20ξ-amine products **3a**/**3b** were treated with pyridine (5 cm^3) and acetic anhydride (5 cm^3) for 18 h at room temperature. Work-up and flash chromatography gave, on elution [acetone–ethyl acetate–light petroleum (40:18:42)], the 20-oxime acetate **6** (24 mg), m.p. 161–163 °C (from acetone–light petroleum) (Found: C, 69.1; H, 9.2; N, 3.1. $C_{25}H_{39}NO_5$ requires C, 69.25; H, 9.05; N, 3.2%). Further elution gave the 20β-*acetamide* **5a** (54 mg), m.p. 210–211.5 °C (from acetone–light petroleum) (Found: C, 71.3; H, 10.1; N, 3.0. $C_{25}H_{41}NO_4$ requires C, 71.6; H, 9.85; N, 3.3%) and the 20α-*acetamide* **4a** (290 mg), m.p. 244–245.5 °C (from acetone–light petroleum) (Found: C, 71.3; H, 9.9; N, 3.25%).

20α-*Acetamido*- **4b** *and* 20β-*Acetamido*-5β,14β-*pregnane*-3β,14-*diol* **5b**.—The 20α-acetamide **4a** (80 mg) was dissolved in ethanol (6 cm^3), 0.5 mol dm^{-3} aq. potassium hydroxide (6 cm^3) was added, and the mixture was stirred overnight at room temperature. The mixture was acidified to pH 5 with glacial acetic acid, extracted with methylene dichloride and the combined extracts were washed successively with water and saturated aq. sodium hydrogen carbonate to give the 20α-*acetamide diol* **4b** (34 mg), m.p. 235–236 °C (from acetone–light petroleum) (Found: C, 73.0; H, 10.2; N, 3.9. $C_{23}H_{39}NO_3$ requires C, 73.2; H, 10.4; N, 3.7%).

The 20β-acetamide **5a** (564 mg) was hydrolysed as described above for compound **4a**, to give the *diol* **5b** (354 mg), m.p. 239–242 °C (from methylene dichloride–light petroleum) (Found: C, 73.1; H, 10.8; N, 3.4%).

The preparation of epimers **4b** and **5b** was also carried out without separation of intermediates as follows: the 20-ketone **1a** (1.6 g) was treated with hydroxylamine hydrochloride (1.6 g) as described above to give the crude oxime (m.p. 227–232 °C), which was refluxed in propan-1-ol (100 cm^3), and sodium (4 g) was added in portions during 2 h. The mixture was concentrated, diluted with water, and extracted with methylene dichloride to give the 20ξ-amine base of compounds **3a**/**b**. The 20-amines were treated with pyridine (20 cm^3) and acetic anhydride (20 cm^3) for 18 h to give the 20-acetamides **4a**/**5a** (3.2 g), which were treated with 0.5 mol dm^{-3} aq. potassium hydroxide–abs. ethanol for 18 h and worked up as described

above to give a residue, which was separated by flash chromatography. Elution (50% acetone–light petroleum) gave the 20α-acetamide **4b** (553 mg), m.p. 230–234 °C, and the 20β-acetamide **5b** (601 mg), m.p. 235–240 °C.

20α-Acetamido- **7a** *and* 20β-*Acetamido*-5β-*pregn*-14-*en*-3β-*ol* **7b**.—The 20α-acetamide **4b** (120 mg) and triphenylphosphine (208 mg) was dissolved in acetonitrile (4 cm^3), a few drops of carbon tetrachloride were added, and the mixture was left for 4 days at room temperature. TLC showed that some starting material still remained. The reaction mixture was evaporated and diluted with water, and the water phase was extracted with methylene dichloride to give a residue, which was separated by flash chromatography. Elution (5% methanol–methylene dichloride) gave (i) triphenylphosphine oxide (79 mg), m.p. 155–158 °C (from acetone–light petroleum) (lit.,[28] 156 °C); (ii) 14-*ene*-20α-*acetamide* **7a** (49 mg), m.p. 201–203 °C (from methanol–acetone–light petroleum) (Found: C, 76.5; H, 10.4; N, 4.15. $C_{23}H_{37}NO_2$ requires C, 76.8; H, 10.4; N, 3.9%). Starting material **4a** (16 mg), m.p. 195–200 °C, was recovered.

When the 20β-acetamide **5a** (50 mg) was treated with 0.5 mol dm^{-3} aq. potassium hydroxide–ethanol as described above for compound **5b** and 5% hydrochloric acid was used instead of acetic acid for neutralization, the dehydration product, 20β-*acetamide*-14-*ene* **7b** (21 mg), m.p. 245–247 °C (from acetone–light petroleum) was obtained (Found: C, 76.6; H, 10.6; N, 3.8%).

20α-Acetamido- **8a** *and* 20β-*Acetamido*-3β-(α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-14-*ol* **8b**.—(a) *From compounds* **4b** *and* **5b**. The 20β-amide **5b** (184 mg) was refluxed in acetonitrile (30 cm^3) until it was dissolved and the solution was then cooled to room temperature. Acetobromorhamnose,[29,30] m.p. 65–66 °C (352 mg), and mercury(II) cyanide[10] (252 mg) were added and the solution was stirred at room temperature for 1 h, when saturated aq. sodium hydrogen carbonate (15 cm^3) was added and the mixture was stirred for a further 50 min and then the aqueous phase was extracted with toluene. The combined toluene layers were washed with water and the residue obtained on evaporation was dissolved in 0.5 mol dm^{-3} aq. KOH–ethanol and left overnight. Brine was added and the mixture was extracted with tetrahydrofuran (THF) and the residue obtained on evaporation was subjected to flash chromatography to give on elution (35% acetone–light petroleum) starting material **5b** (76 mg), m.p. 235–240 °C (from methylene dichloride–light petroleum) and 20β-*acetamide rhamnoside* **8b** (20 mg), m.p. 267–268 °C (from methanol–acetone–light petroleum) (Found: C, 65.0; H, 9.5; N, 2.7. $C_{29}H_{49}NO_7 \cdot 0.5H_2O$ requires C, 65.4; H, 9.5; N, 2.6%).

Following the same procedure the 20α-amide **4b** (218 mg) gave starting material **4b** (60 mg) and the 20α-*acetamide rhamnoside* **8a** (25 mg), m.p. 294–296 °C (from methanol–acetone–light petroleum) (Found: C, 65.4; H, 9.4; N, 2.8%).

(b) *From compounds* **22a** *and* **22b** (*preparation given below*). To a suspension of 20α-amino triacetate **22a** (45 mg) in anhydrous diethyl ether (5 cm^3) were added 4-(dimethylamino)-pyridine (DMAP) (5 mg) and acetic anhydride (0.25 cm^3) and the mixture was stirred for 2 h until reaction was complete by TLC. Diethyl ether (40 cm^3) was added and the solution was washed with brine, evaporated, and the residue was dissolved in methanol (10 cm^3), 10% ammonia gas–methanol (3 cm^3) was added, and the mixture was stirred overnight. After evaporation the residue was recrystallized to give the 20α-acetamide rhamnoside **8a** (8 mg), m.p. 292–295 °C (from methanol–acetone–hexane) (mixed m.p. 293–296 °C), which was identical (^1H and ^{13}C NMR) with that obtained from compound **4b**.

Similar treatment of 20β-amine triacetate **22b** (45 mg) gave, from flash chromatography and elution [chloroform–methanol–diethylamine (100:10:0.75)], compound **8b** (9 mg), m.p. 263–267 °C (from acetone–hexane), identical (^1H and ^{13}C NMR) with that obtained from compound **5b**.

20α-Acetamido- and 20α-*Amino-14-hydroxy*-5β,14β-*pregnan*-3β-*yl* 3,4-*Di*-O-*acetyl*-α-L-*rhamnopyranose*-1-C,2-C-*diyl ortho-acetates* **9a** *and* **9b**, *Tetra*-O-*acetyl*-α-L-*rhamnopyranose* **10a**, 2,3,4-*Tri*-O-*acetyl*-ξ-L-*rhamnopyranose* **10b**, *and* 3,4-*Diacetyl*-α-L-*rhamnopyranose*-1-C,2-C-*diyl* 2,3,4-*Tri*-O-*acetyl*-β-D-*rhamnopyranosyl Orthoacetate* **11**.—To a vigorously stirred solution of the 20α-amide **4b** (100 mg) and Fetizon's reagent (1.2 g) in methylene dichloride (15 cm^3) was added, in one portion, a solution of acetobromorhamnose[29,30] (0.8 g) in methylene dichloride (10 cm^3). After the mixture had been stirred for 0.5 h, the solid was removed by filtration through a Celite pad. The filtrate was washed with saturated aq. sodium hydrogen carbonate and flash chromatographed to give, on elution (50% diethyl ether–light petroleum), epimeric mixtures of tetra-*O*-acetylrhamnose **10a** (54 mg); $\delta_H(CDCl_3)$ (α-isomer) 1.22 (d, *J* 6.2, 6-H_3), 1.99, 2.05, 2.15 and 2.16 (4 s, 4 × OAc), 3.92 (m, 5-H), 5.11 (t, *J* 9.9, 4-H), 5.24 (dd, *J* 1.9 and 3.5, 2-H), 5.30 (dd, *J* 3.5 and 10.0, 3-H) and 6.00 (d, *J* 1.7, 1-H); (β-isomer) 1.29 (d, *J* 6.2, 6-H_3), 3.67 (m, 5-H), 5.83 (d, *J* 1.1, 1-H); and 2,3,4-tri-*O*-acetylrhamnopyranose **10b** (238 mg),[30] $\delta_H(CDCl_3)$ (α-isomer) 1.16 (d, *J* 6.3, 6-H_3), 1.95, 2.03 and 2.11 (3 s, 3 × Ac), 4.11 (m, 5-H), 4.96 (m, 4-H), 5.02 (d, *J* 1.8, 1-H), 5.19 (dd, *J* 1.9 and 3.4, 2-H) and 5.31 (dd, *J* 3.4 and 10.1, 3-H); (β-isomer) 4.99 (s, 1 H).

Further elution (80% diethyl ether–light petroleum) gave the *ortho ester* **11** (102 mg), m.p. 161–164 °C (from diethyl ether–methylene dichloride–light petroleum), $\delta_H(CDCl_3)$ 1.22 (d, *J* 5.7) and 1.24 (d, *J* 5.4) (2 × 6-H_3), 1.74 (s, O_3CMe), 1.97, 2.04, 2.05, 2.11 and 2.16 (5 × OAc), 3.51 (m, 2 × 5-H), 4.51 (dd, *J* 2.8 and 3.6, 2-H), 4.93–5.14 (m, 1-H, 2 × 3-H and 2 × 4-H) and 5.33 (d, *J* 2.4, 2-H) and 5.41 (d, *J* 2.4) (1-H); δ_C 17.41 and 17.69 (2 × C-6), 20.62, 3 × 20.78 and 20.92 (5 × OC*Me*), 24.79 (O_3C*Me*), 69.51, 69.59, 69.93, 70.13, 70.24, 70.86 and 70.98 (C-2 and 2 × C-3, -4 and -5), 75.67 (C-2 ortho ester), 91.71 and 97.43 (2 × C-1), 134.74 (O_3*C*Me) and 3 × 169.75, 170.05, 170.31 and 170.45 (6 × *C*OMe) (Found: C, 51.6; H, 6.3. $C_{24}H_{34}O_{15}$ requires C, 51.3; H, 6.1%).

Elution (2% methanol–methylene dichloride) gave the 20α-*acetamido orthoacetate* **9a** (59 mg), m.p. 210–212 °C (from methanol–diethyl ether) (Found: C, 64.5; H, 8.6; N, 2.1. $C_{35}H_{55}NO_{10}$ requires C, 64.7; H, 8.5; N, 2.2%). The 20α-amino orthoacetate **9b** (20 mg) was obtained as a non-crystalline gum.

20α- **12a** *and* 20β-*Trifluoroacetmido*-5β-*pregnan*-14-*en*-3β-*yl Trifluoroacetate* **12b**. To pyridine (2 cm^3) stirred under argon at 0 °C was added dropwise TFAA (0.8 cm^3); the mixture showed pH 7–8 (wet pH paper). A solution of the 20ξ-amines **3a**/**b** (84 mg), prepared as described above, in pyridine (1 cm^3) was added dropwise and the mixture was stirred for 1 h until reaction was complete by TLC. Diethyl ether (50 cm^3) was added and the ether layer was washed successively with cold, saturated aq. sodium hydrogen carbonate and brine. The residue was separated by flash chromatography. Elution (10% diethyl ether–light petroleum) gave *compound* **12a** (10 mg), m.p. 208–210 °C (from diethyl ether–hexane) (Found: C, 58.8; H, 6.6; N, 2.6. $C_{25}H_{33}F_6NO_3$ requires C, 58.9; H, 6.5; N, 2.8%) and its *isomer* **12b** (13 mg), m.p. 203–205 °C (from diethyl ether–hexane) (Found: C, 58.9; H, 6.7; N, 2.7%).

14-*Hydroxy*-20α- **14a** *and* 20β-*nitro*-5β,14β-*pregnan*-3β-*yl Acetate* **14b** *and* 14-*Hydroxy*-20β-*nitro*-5β,14β-*pregnan*-3-*one* **13**.—0.1 Mol dm^{-3} dimethyldioxirane–acetone solution was prepared as described by Adams *et al.*[31] To stirred dimethyl-

dioxirane–acetone solution (20 cm^3) was added a solution of the 20ξ-amine **3a/b** (300 mg) in methanol (2 cm^3) at room temperature and the mixture was left overnight. After evaporation the residue was treated with a mixture of pyridine (1 cm^3) and acetic anhydride (1 cm^3) overnight. After the usual work-up, the residue was separated by flash chromatography. Elution (40% diethyl ether–light petroleum) gave 20α-*nitro compound* **14a** (28 mg), m.p. 172–175 °C (from acetone–diethyl ether–hexane) (Found: C, 67.8; H, 9.1; N, 3.6. $C_{23}H_{37}NO_5$ requires C, 67.8; H, 9.15; N, 3.4%).

Further elution gave *isomer* **14b** (59 mg), m.p. 203–204 °C (from acetone–hexane) (Found: C, 67.9; H, 9.2; N, 3.6%) and the *ketone* **13** (11 mg), m.p. 167–169 °C (from aq. MeOH) (Found: C, 69.3; H, 9.2; N, 4.0. $C_{21}H_{33}NO_4$ requires C, 69.4; H, 9.15; N, 3.85%).

20β-*Amino*-14-*hydroxy*-5β,14β-*pregnan*-3β-*yl Acetate* **15**.—Iron filings (250 mg) were washed with 4% HCl, the acid was decanted, and the powder was rinsed twice with water, twice with acetic acid, and acetic acid (3 cm^3) was added. To the stirred suspension was added a solution of 20β-nitro compound **14b** (60 mg) in acetic acid (1 cm^3) and the mixture was brought to reflux under argon for 1.5 h until reduction was complete by TLC. The mixture was filtered, adjusted to pH 9–10 (pH paper) with 10% aq. NaOH, and extracted with ethyl acetate, and the extract was evaporated to give, after flash chromatography and elution [chloroform–methanol–diethylamine (100:10:0.75)], the title compound **15** (24 mg) as a non-crystalline gum.

14-*Hydroxy*-3β-(*tri*-O-*benzoyl*-α-L-*rhamnopyranosyloxy*)-5β,14β-*card*-20(22)-*enolide* (*Evomonoside* α-L-*Rhamnopyranoside Tribenzoate*) **16**.—To a stirred solution of digitoxigenin[3] (374 mg) and tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide[32] (1.08 g) in ethylene dichloride (25 cm^3) was added finely powdered mercury(II) cyanide[10] (506 mg). After 3 h, during which time dry argon was bubbled through the mixture to remove hydrogen cyanide, the mixture was filtered through Celite and the filter was washed with methylene dichloride (100 cm^3). The combined filtrate was washed successively with 20% aq. KI, saturated aq. $NaHCO_3$, and water, and evaporated to give a residue, which was subjected to flash chromatography. Elution (35% ethyl acetate–light petroleum) gave the *rhamnoside tribenzoate* **16** (772 mg), m.p. 225–226 °C (from acetone–light petroleum) (Found: C, 72.4; H, 6.9. $C_{50}H_{56}O_{11}$ requires C, 72.1; H, 6.8%).

A similar reaction carried out on digitoxigenin (374 mg) as above with Fetizon's reagent gave compound **16** (204 mg) and digitoxigenin (256 mg recovery).

3β-(*Tri*-O-*benzyl*-α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnane*-14,20β-*diol* **18** *and* 14-*Hydroxy*-3β-(*tri*-O-*benzoyl*-α-L-*rhamnopyraosyloxy*)-5β,14β-*pregnan*-20-*one* **17**.—A solution of evomonoside tribenzoate **16** (350 mg) in methylene dichloride (50 cm^3) was cooled to −60 °C in a solid CO_2–acetone-bath. A stream of ozone was passed into the solution until reaction was complete by TLC (*ca.* 1 h) and excess of ozone was removed by a stream of nitrogen. Zinc (2.5 g) and acetic acid (10 cm^3) were added and the mixture was brought to room temperature. The solvent was removed under reduced pressure at 40 °C and the residue was dissolved in acetic acid (20 cm^3); zinc powder (3.5 g) was added and the mixture was shaken overnight, filtered, and washed with methylene dichloride. The filtrate was washed successively with water and saturated aq. sodium hydrogen carbonate to give, after flash chromatography and elution (40% acetone–hexane), the 20-*ketone* **17** (228 mg), m.p. 198–202 °C (from aq. MeOH) (Found: C, 72.7; H, 7.2. $C_{48}H_{56}O_{11}$ requires C, 72.7; H, 7.1).

Further elution gave the 20β-alcohol **18** (61 mg) as a non-crystalline gum.

3β-(α-L-*Rhamnopyranosyloxy*)-5β,14β-*pregnane*-14,20β-*diol* **19**.—The 20β-hydroxy tribenzoate **18** (200 mg) obtained from compound **16** was stirred with 5% ammonia gas–methanol (17 cm^3) overnight, and after concentration and extraction the residue was purified by flash chromatography. Elution (10% methanol–methylene dichloride) gave compound **19** (136 mg), m.p. 205–207 °C (decomp.) (from aq. MeOH) (lit.,[15] 243–246 °C). The ^1H and ^{13}C NMR data were consistent, as was the RBA, with those reported earlier.[15]

14-*Hydroxy*-3β-(*tri*-O-*benzoyl*-α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-20-*one* **17** *from Compound* **18**.—A solution of the 20β-hydroxy tribenzoate **18** (535 mg) and PDC (537 mg) in methylene dichloride (25 cm^3) was stirred at room temperature until oxidation was complete by TLC (14 h). Diethyl ether (100 cm^3) was added and the mixture was filtered through a Celite pad. The ether filtrate was washed with water and evaporated to give the 20-ketone **17** (445 mg), m.p. 195–200 °C (from aq. MeOH).

14-*Hydroxy*-3β-(*tri*-O-*benzoyl*-α-L-*rhamnopyranosyloxy*-5β,14β-*pregnan*-20-*one* trans-*Oxime* **20**.—The 20-ketone **17** (100 mg) was treated with hydroxylamine hydrochloride (100 mg) as described for compound **2a**, to give the trans-*oxime* **20** (41 mg), m.p. 206–208 °C (from aq. EtOH) (Found: C, 71.2; H, 7.1; N, 2.0. $C_{48}H_{57}NO_{10}$ requires C, 71.35; H, 7.1; N, 1.7%).

20α-**21a** *and* 20β-*Nitro*-3β-(*tri*-O-*acetyl*-α-L-*rhamnopyranosyl*-*oxy*)-5β,14β-*pregnan*-14-*ol* **21b**.—To a refluxing solution of the *trans*-oxime **20** (600 mg) in propan-1-ol (30 cm^3) was added sodium (1.2 g) in small portions during 2 h. The solution was adjusted to pH 9–10 (pH paper) with 4% HCl, water (50 cm^3) was added, and the mixture was extracted with ethyl acetate to give the 20ξ-amine isomers (165 mg). The 20ξ-amine (140 mg) was oxidized with dimethyldioxirane–acetone solution (5 cm^3) as described for compounds **14a** and **14b** to give the 20ξ-nitro isomers **24a/b** (101 mg). The 20ξ-nitro compound **24/b** (60 mg) was treated with pyridine (0.5 cm^3) and acetic anhydride (0.5 cm^3) as described above for compounds **4a** and **5a**. Flash chromatography (60% diethyl ether–hexane) gave the 20α-*nitro triacetate* **21a** (18 mg), m.p. 186–188 °C (from diethyl ether–hexane) (Found: C, 62.3; H, 8.0; N, 2.0. $C_{33}H_{51}NO_{11}$ requires C, 62.15; H, 8.1; N, 2.2%).

Further elution gave the 20β-*nitro triacetate* **21b** (19 mg), m.p. 175–177 °C (from diethyl ether–hexane) (Found: C, 62.1; H, 7.9; N, 2.45%).

20α-*Amino*- **22a** *and* 20β-*Amino*-3β-(*tri*-O-*acetyl*-α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-14-*ol* **22b**.—Following the procedure described above for compound **15**, the 20α-nitro triacetate **21a** (50 mg) gave the 20α-amino triacetate **22a** (45 mg). The 20β-nitro triacetate **21b** (100 mg) similarly gave the 20β-amino triacetate **22b** (55 mg). Both were obtained as non-crystalline gums from purification by flash chromatography on silica by elution with chloroform–methanol–diethylamine (100:10:0.75).

20α-*Amino*- **23a** *and* 20β-*Amino*-3β-(α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-14*ol* **23b**.—The 20β-amine **22a** (34 mg) was dissolved in methanol (5 cm^3), 10% ammonia gas–methanol (1.5 cm^3) was added, and the mixture was stirred under argon for 18 h. After evaporation the residue was recrystallized to give *title compound* **23a** (17 mg), m.p. 243–245 °C (decomp.) (from methanol–diethyl ether) (Found: C,

60.6; H, 9.7; N, 2.3. $C_{27}H_{47}NO_6 \cdot 3H_2O$ requires C, 60.5; H, 10.0; N, 2.6%).

Following the same procedure compound **22b** (55 mg) gave the *title compound* **23b** (16 mg), m.p. 251–252 °C (decomp.) (from methanol–diethyl ether) (Found: C, 60.4; H, 9.8; N, 2.4%).

20α-*Nitro*- **24a** *and* 20β-*Nitro*-3β-(α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-14-*ol* **24b**.—(a) *From compounds* **23a** *and* **23b**. The 20α-amino rhamnoside **23a** (31 mg) was oxidized with dimethyldioxirane–acetone solution (10 cm^3) as described above, to give the 20α-*nitro rhamnoside* **24a** (7 mg), m.p. 274–276 °C (from methanol–acetone–hexane) (Found: C, 59.6; H, 8.9; N, 2.6. $C_{27}H_{45}O_8N \cdot 2H_2O$ requires C, 59.2; H, 9.0; N, 2.6%).

Following the same procedure, the 20β-amino rhamnoside **23b** (40 mg) gave the 20β-*nitro rhamnoside* **24b** (15 mg), m.p. 258–262 °C, having identical ^1H and ^{13}C NMR with the sample obtained from hydrolysis of compounds **21a** and **21b** (Found: C, 59.0; H, 8.9; N, 2.6%).

(b) *From compounds* **21a** *and* **21b**. The 20β-nitro triacetate **21b** (80 mg) was dissolved in a solution of methanol (7 cm^3), triethylamine (3.5 cm^3, freshly distilled), and water (0.25 cm^3), and the mixture was stirred under argon for 3 days until hydrolysis was complete by TLC. The excess of solvent was evaporated under reduced pressure at room temperature, and flash chromatography and elution (7.5% methanol–methylene dichloride) gave compound **24b** (58 mg), m.p. 260–263 °C (from acetone–diethyl ether) (Found: C, 59.1; H, 8.9; N, 2.7%).

Following the same procedure, the 20α-nitro triacetate **21a** (80 mg) was hydrolysed to give compound **24b** (20 mg), m.p. 257–261 °C (from acetone–diethyl ether), mixed m.p. 258–262 °C with the sample obtained from compound **23b** (Found: C, 59.3; H, 8.9; N, 2.5%).

14,20α- **25a** *and* 14β,20β-*Dihydroxy*-5β,14β-*pregnan*-3β-*yl Acetate* **25b**.—To a stirred solution of the 20-ketone **1a**[3] (526 mg) in 20% aq. ethanol (34 cm^3) was added sodium borohydride (70 mg). After 30 min at room temperature no starting material remained (TLC). Following acidification with 12% acetic acid the mixture was extracted with methylene dichloride to give, from flash chromatography and elution (35% ethyl acetate–light petroleum), **25a** (155 mg), m.p. 210–213 °C (from acetone–light petroleum) (lit.,[17] 212–214 °C) and **25b** (209 mg), m.p. 167–169 °C (from acetone–light petroleum) (lit.,[17] 168–168.5 °C).

14-*Hydroxy*-5β,14β-*pregnane*-3β,20α-*diyl* **25c** *and* -3β,20β-*diyl Diacetate* **25d**.—The mixture of compounds **25a**/**b** (50 mg) obtained as described above from compound **1a** was treated with acetic anhydride (0.5 cm^3) and pyridine (0.5 cm^3) for 18 h to give, after flash chromatographic separation (25% ethyl acetate–light petroleum), the non-crystalline 3β,20α-diacetate **25c** (9 mg) and the 3β,20β-*diacetate* **25d** (26 mg), m.p. 144–146 °C (from aq. MeOH) (Found: C, 71.4; H, 9.4. $C_{25}H_{40}O_5$ requires C, 71.4; H, 9.6%).

20α- **26a** *and* 20β-*Hydroxy*-5β-*pregn*-14-*en*-3β-*yl Acetate* **26b**.—Following the procedure described from compound **7a** the 20α-alcohol **25a** (58 mg) was treated with triphenylphosphine in carbon tetrachloride and acetonitrile to give *compound* **26a** (27 mg), m.p. 143–145 °C (from acetone–light petroleum) (Found: C, 76.4; H, 10.1. $C_{23}H_{36}O_3$ requires C, 76.6; H, 10.1%).

Similarly, substrate **25b** (40 mg) gave the 20β-*isomer* **26b** (15 mg), m.p. 160–161 °C (from acetone–light petroleum) (Found: C, 76.4; H, 10.1%).

5β,14β-*Pregnane*-3β,14,20α-*triol* **27a** *and* 20β-(tert-*Butyldimethylsiloxy*)-5β,14β-*pregnane*-3β,14-*diol* **27b**.—The silyl ethers **28a**/**b** (see below) (105 mg) were dissolved in diethyl ether (15 cm^3) and LAH (40 mg) was added. After 0.5 h acetone and water were added and the mixture was adjusted to pH 8 (pH paper) with dil. HCl. Extraction with diethyl ether gave, after flash chromatography (35% ethyl acetate–light petroleum), the 20β-silyl ether **27b** (40 mg), m.p. 170–172 °C (from acetone–light petroleum) (lit.,[1] 171–173 °C), and the triol **27a** (17 mg), m.p. 210–212 °C (lit.,[17] 208–213 °C).

The 20α-silyl ether **28a** (50 mg) in anhydrous diethyl ether was refluxed with LAH (20 mg) for 4 h. Excess of acetone was added and the pH was carefully adjusted to pH 8 with 0.1 mol dm^{-3} HCl and extracted with diethyl ether to give triol **27a** (12 mg), m.p. 213–215 °C (from ethanol–methylene dichloride–light petroleum).

20α- **28a** *and* 20β-(tert-*Butyldimethylsiloxy*)-14β-*hydroxy*-5β,14β-*pregnan*-3β-*yl Acetate* **28b**.—The 20ξ-alcohols **25a**/**b** (1 g) obtained from reduction with $NaBH_4$ as described above were treated with Bu^tMe_3SiCl (1 g) and imidazole (1 g) in dry dimethylformamide (DMF) (30 cm^3) and the solution was stirred for 16 h at room temperature. Work-up as before gave, after flash chromatography and elution (10% ethyl acetate–light petroleum), *compound* **28a** (342 mg), m.p. 185–187 °C (from diethyl ether–acetone) (Found: C, 70.6; H, 10.4. $C_{29}H_{52}O_4Si$ requires C, 70.7; H, 10.6%) and compound **28b**[1] (370 mg), m.p. 175–178 °C (from diethyl ether–light petroleum) (Found: C, 70.4; H, 10.6%).

20α-(tert-*Butyldimethylsiloxy*)-5β,14β-*pregnane*-3β,14-*diol* **29**.—The 20α-silyl ether **28a** (310 mg) was treated with 0.5 mol dm^{-3} KOH–abs. ethanol (20 cm^3) at room temperature for 4 h (TLC). The mixture was diluted with water and extracted with methylene dichloride to give the *silyl ether* **29** (82 mg), m.p. 135–137 °C (from diethyl ether–light petroleum) (Found: C, 70.85; H, 11.2. $C_{27}H_{50}O_3Si \cdot 0.5H_2O$ requires C, 70.6; H, 11.2%).

3β-(α-L-*Rhamnopyranosyloxy*)-5β,14β-*pregnane*-14,20α-*diol* **30a** *and* 20α-(tert-*Butyldimethylsiloxy*)-3β-(α-L-*rhamnopyranosyloxy*)-5β,14β-*pregnan*-14*ol* **30b**.—The 20α-silyl ether **29** (130 mg) was treated with acetobromorhamnose (1.0 g) in methylene dichloride and Fetizon's reagent (1.6 g) as described for compounds **9a** and **9b**. After work-up the residue was treated with 0.5 mol dm^{-3} aq. KOH–abs. ethanol (10 cm^3) for 14 h, when brine (40 cm^3) was added and the mixture was extracted with methylene dichloride. The residue was separated by flash chromatography, which on elution (5% methanol–methylene dichloride) gave the *silyl ether* **30b** (15 mg), m.p. 228–230 °C (from aq. MeOH) (Found: C, 65.2; H, 10.5. $C_{33}H_{61}O_7Si \cdot 5H_2O$ requires C, 65.4; H, 10.2%) and *compound* **30a** (22 mg), m.p. 273–275 °C (from aq. MeOH) (Found: C, 64.3; H, 9.8. $C_{27}H_{46}O_7 \cdot H_2O$ requires C, 64.8; H, 9.7%).

14-*Hydroxy*-3β-(*tris*-β-D-*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* trans-*Oxime* **32**.—To a stirred solution of the methyl ketone[3] **31** (200 mg) (prepared as described in ref. 3) in a mixture of 95% ethanol (20 cm^3) and pyridine (5 cm^3) was added a mixture of hydroxylamine hydrochloride (400 mg) and sodium acetate (286 mg) in water (5 cm^3). After 2 h under reflux the mixture was cooled, and diluted with methylene dichloride. The organic layer was washed with dil. hydrochloric acid to give the trans 20-*oxime* **32** (142 mg), m.p. 252–255 °C (from chloroform–acetone) (Found: C, 63.5; H, 8.9; N, 2.0. $C_{39}H_{65}NO_{12}$ requires C, 63.3; H, 8.85; N, 1.9%).

20ξ-*Amino*-3β-(*tris*-β-D-*digitoxosyloxy*)-5β,14β-*pregnan*-14-*ol* **33**.—A solution of the oxime **32** (275 mg) in propan-1-ol (20 cm^3) was brought to reflux under argon and sodium (1.02 g) was added in small pieces over a period of 135 min. The solution was then cooled, and diluted with methylene dichloride, and the

organic layer was washed thoroughly with water to give the 20ξ-*aminopregnane trisdigitoxoside* **33** (115 mg), m.p. 205–209 °C (from diethyl ether) (Found: C, 64.4; H, 9.4; N, 1.8. $C_{39}H_{67}NO_{11}$ requires C, 64.5; H, 9.3; N, 1.9%).

14-*Hydroxy*-3β-(*tris*-β-D-*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* 20-*Hydrazone* **34**.—The methyl ketone [3] **31** (250 mg) was refluxed with 85% hydrazone (1 cm^3) and triethylamine (freshly distilled, 6.6 cm^3) in 95% ethanol (20 cm^3). After 2 h the solvents were evaporated off and several crystallizations gave the *hydrazone* **34** (120 mg), m.p. 220–242 °C (decomp.) (from diethyl ether) (Found: C, 64.2; H, 9.5; N, 3.9. $C_{39}H_{70}N_2O_{10}$ requires C, 64.4; H, 9.7; N, 3.85%).

14-*Hydroxy*-3β-(*tris*-β-D-*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* 20-*Amidinohydrazone* **35**.—The methyl ketone [3] **31** (100 mg) and aminoguanidine hydrogen carbonate (100 mg) in 95% ethanol (10 cm^3) were heated to reflux with sodium hydroxide (30 mg) under argon for 6 h, when TLC (10% methanol–methylene dichloride) indicated no starting material remained. The mixture was extracted with methylene dichloride and the organic layer was washed with water to give, after two crystallizations, the 20-*amidinohydrazone* **35** (38 mg), m.p. 248.5–252 °C (from diethyl ether–methanol) (Found; C, 60.0; H, 8.7; N, 6.9. $C_{40}H_{68}N_4O_{11}\cdot H_2O$ requires C, 60.2; H, 8.6; N, 7.0%).

20ξ-*Nitro*-3β-(*tris*-β-D-*digitoxosyloxy*)-5β,14β-*pregnan*-14-*ol* **36**.—A 0.1 mol dm^{-3} dimethyldioxirane–acetone solution [31] (26 cm^3) was added dropwise to a stirred solution of the 20ξ-aminopregnane **33** (350 mg) in methylene dichloride (25 cm^3) at room temperature. After 15 min, TLC (10% methanol–methylene dichloride) showed no starting material remained. The solution was evaporated at ~40 °C on a rotary evaporator to give a residue, which was purified by flash chromatography. Elution (3% methanol–methylene dichloride) gave fractions of the 20ξ-*nitropregnane* **36** (157 mg), m.p. 215–223 °C (from diethyl ether) (Found: C, 62.0; H, 8.7; N, 1.8. $C_{39}H_{65}NO_{13}$ requires C, 62.0; H, 8.7; N, 1.85%).

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References

1 J. F. Templeton, V. P. S. Kumar, D. Bose and F. S. LaBella, *J. Med. Chem.*, 1989, **32**, 1977.
2 E. Chow, R. S. Kim, F. S. LaBella and G. Queen, *J. Pharmacol.*, 1979, **67**, 345.
3 J. F. Templeton, Y. Ling, J. Jin, M. A. Boehmer, T. H. Zeglam and F. S. LaBella, *J. Chem. Soc., Perkin Trans. 1*, 1991, 823.
4 E. P. Oliveto in *Organic Reactions in Steroid Chemistry*, ed. J. Fried and J. A. Edwards, Van Nostrand Reinhold, New York, 1972, vol. 2, p. 142.
5 J. A. Secrist and M. W. Logue, *J. Org. Chem.*, 1972, **37**, 335.
6 H. Spreitzer, G. Buchbauer and C. Puringer, *Tetrahedron*, 1989, **45**, 6999.
7 L. Brown, J. Boutagy and R. E. Thomas, *Arzneim-Forsch.*, 1981, **31**(II), 1059.
8 R. H. DeWolfe, *Carboxylic Ortho Acid Derivatives*, Academic Press, New York, 1970, ch. 5.
9 K. Igarashi, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 272.
10 F. X. Jarreau and J. J. Koenig, *U.S. Pat.* 4 885 280, 1989 (*Chem. Abstr.*, 1990, **112**, 179606h).
11 F. Theil, C. Lindig and K. Repke, *Z. Chem.*, 1980, **20**, 372.
12 R. W. Murray, S. N. Rajadhyaksha and L. Mohan, *J. Org. Chem.*, 1989, **54**, 5783.
13 T. Neilsen, *J. Org. Chem.*, 1962, **27**, 1998.
14 E. Vedejs, *Org. React.*, 1975, **22**, 401.
15 J. F. Templeton, P. Setiloane, V. P. S. Kumar, Y. Tan, T. H. Zeglam and F. S. LaBella, *J. Med. Chem.*, 1991, **34**, 2778.
16 E. H. Massey, H. E. Smith and A. W. Gordon, *J. Org. Chem.*, 1966, **31**, 684.
17 C. Lindig, *J. Prakt. Chem.*, 1983, **325**, 587.
18 G. C. Habermehl, P. E. Hamman and V. Wray, *Magn. Reson. Chem.*, 1985, **23**, 959.
19 D. M. Dodderell, D. P. Pegg and M. T. Bendall, *J. Magn. Reson.*, 1982, **48**, 323.
20 W. P. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 2229.
21 A. Bax and G. Morris, *J. Magn. Reson.*, 1981, **42**, 501.
22 J. K. M. Sanders and J. D. Mersh, in *Progress in Nuclear Magnetic Resonance Spectroscopy*, eds. J. W. Emsley, J. Feeney and L. H. Sutcliffe, Pergamon Press, Oxford, 1982, vol. 15, p. 161.
23 K. Marat, J. F. Templeton and Yangzhi Ling, submigged to *Magn. Reson. Chem.*
24 K. Marat, J. F. Templeton and V. P. S. Kumar, *Magn. Reson. Chem.*, 1987, **26**, 25.
25 D. Neuhaus, J. Keeler and R. Freeman, *J. Magn. Reson.*, 1985, **61**, 553.
26 J. F. Templeton, S. V. P. Kumar, D. Bose, D. D. Smyth, R. S. Kim and F. S. LaBella, *Can. J. Physiol. Pharmacol.*, 1988, **66**, 1420.
27 A. Gelbart and R. Thomas, *J. Med. Chem.*, 1978, **21**, 284 and references therein.
28 *Dictionary of Organic Compounds*, 5th cumulative supplement, Eyre and Spottiswoode, London, 4th edn., 1969, p. 954.
29 G. M. Bebault, G. G. S. Dutton and C. K. Warfield, *Carbohydr. Res.*, 1974, **34**, 174.
30 E. Fischer, M. Bergmann and A. Rabe, *Ber. Dtsch. Chem. Ges., Teil B*, 1920, **53**, 2362.
31 W. Adams, Y. Y. Chan, D. Cremer, J. Gauss, D. Schentzow and M. Schindler, *J. Org. Chem.*, 1987, **52**, 2800.
32 R. K. Ness, H. G. Fletcher and C. S. Hudson, *J. Am. Chem. Soc.*, 1951, **73**, 296.

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