Digitoxigenin 3-O-β-D-Furanosides†

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The syntheses of the title compounds were accomplished by Koenig–Knorr condensation of acylated furanoses with digitoxigenin followed by basic hydrolysis of protecting groups. In this manner the riboside, 5-amino-5-deoxyriboside, 3,6-anhydroglucoside, and 3,6-dideoxy-3,6-iminoglucoside of digitoxigenin were prepared. These compounds as well as several of the synthetic intermediates showed weak to moderate cardiotonic activity.

Variation of the sugar moiety of cardiac glycosides affects inotropic potency, toxicity, and pharmacokinetic properties. It is interesting to note, however, that, among the vast number of reported natural and synthetic glycosides,[1-3] there is but one example of a cardenolide furanoside. This is the α-L-arabinofuranoside of digitoxigenin reported by Schwabe and Tschiersch.[4] In order to ascertain what effect a five-membered sugar ring may have on inotropy, we have synthesized some digitoxigenin ribofuranosides, 3,6-anhydroglucofuranosides, and 3,6-iminoglucofuranosides.

Synthesis

The Koenigs–Knorr condensation[5] of tri-O-acetyl-D-ribosyl bromide and digitoxigenin (1) in the presence of mercuric cyanide in acetonitrile produced the tri-O-acetyl-β-D-ribofuranoside (3) in 81% yield. During condensation, participation of the 2-O-acetyl function should lead to a product having the β configuration.[1a] This was confirmed by ^{1}H NMR spectroscopy wherein $J_{1',2'}$ for 3 was <1 Hz. Subsequent deacetylation of 3 using methanolic ammonia gave the free ribofuranoside (4) in 66% yield (Scheme I)

Only a few cardiac glycosides incorporating an amino sugar are known.[6-9] However, it has been suggested that an amino sugar can increase the potency and improve the therapeutic index over other glycosides.[10] In order to investigate the effect an amine-substituted furanose moiety might have on cardiac activity, we undertook the synthesis of 3-O-(5-amino-5-deoxy-β-D-ribofuranosyl)digitoxigenin (10). 1,2,3-Tri-O-acetyl-5-O-tosyl-β-D-ribofuranose (5)[11] was brominated with use of hydrogen bromide and then condensed with digitoxigenin in a manner analogous to that used for the riboside. Unfortunately, the presence of the tosyl function had a deleterious effect on the reaction, with the glycoside 6 being isolated in only a 20% yield from a complex mélange of products. The ^{1}H NMR of this glycoside displayed a $J_{1',2'}$ coupling of <1 Hz, confirming the β configuration.

To avoid the condensation reaction of a bromo sugar tosylate, the riboside 4 was treated with p-toluenesulfonyl chloride in pyridine. The slow addition of 2 equiv of reagent optimized the selective sulfonylation of the primary alcohol. Subsequent displacement of the tosylate with azide ion occurred readily on heating 7 with lithium azide in dimethylformamide, affording a 93% yield of the 5'-azido derivative 8. For an additional confirmation of structure, compounds 6 and 8 were each converted to a common intermediate. The tosylate of 6 was displaced with azide ion to furnish 3-O-(2,3-di-O-acetyl-5-azido-5-deoxy-β-D-ribofuranosyl)digitoxigenin (9), which was also made by acetylation of the azido diol 8.

The lipophilicity of cardiac glycosides plays an important role in determining their toxicity and pharmacokinetics.[12-14] An interesting contrast in biological activity may be displayed by an amino glycosyl cardenolide and its fully aceylated derivative. Therefore, the 5'-aminoribofuranoside 10 was first obtained by hydrogenation of the azide 8 in the presence of Raney nickel and then acetylated with use of acetic anhydride in pyridine. Although the free amino glycoside 10 was crystalline, its acetylated derivative 11 remained amorphous and resisted all attempts to free it from last traces of water.

As a further modification of the furanose moiety, 3-O-(3,6-anhydro-β-D-glucofuranosyl)digitoxigenin (18) was synthesized (Scheme II). Such a rigid carbohydrate structure may be more specific for a cardiac glycoside receptor and, furthermore, has some resemblance to the 6'-deoxy-3'-O-methylpyranoses present in such naturally occurring glycosides as nerifolin, odoroside, and honghelin.

The requisite 1,2,5-tri-O-acetyl-3,6-anhydro-D-glucofuranose (14) was synthesized by treatment of 1,2-O-isopropylidene-3-O-(p-tolylsulfonyl)-α-D-allofuranose[15] (12) with sodium methoxide in methanol to form the anhydro "bridge". Following hydrolysis of the acetonide using Dowex 50 (H^+) ion-exchange resin and acetylation with acetic anhydride in pyridine, 14 was isolated as a viscous syrup in 84% overall yield. We were concerned that during the removal of the acetonide of 13, isomerization to the pyranose might have occurred, even though this is contrary to the expected behavior.[16] To allay these fears, 14 was

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(1) For reviews see: (a) Zorbach, W. W.; Bhat, K. V. *Adv. Carbohydr. Chem.* **1966**, *21*, 273. (b) Meyer, K. *Planta Med.* **1971**, *4* (suppl), 2. (c) Thomas, R.; Boutagy, J.; Gelbart, A. *J. Pharm. Sci.* **1974**, *63*, 1649.
(2) Chen, K. K. *J. Org. Chem.* **1970**, *13*, 1029.
(3) Chen, K. K. *J. Org. Chem.* **1970**, *13*, 1035.
(4) Schwabe, K.; Tschiersch, B. *Pharmazie* **1982**, *37*, 827.
(5) Zorbach, W. W.; Valiaveedan, G. D.; Kashelikar, D. V. *J. Org. Chem.* **1962**, *27*, 1766.
(6) Stevens, C. L.; Ransford, G. H.; Gutowski, G. E. *Abstr. Pap. —Am. Chem. Soc.* **1968**, 156th, MED 109.
(7) Meyer zu Reckendorf, W.; Wassiliadou-Micheli, N.; Machleidt, H. *Arch. Pharm. (Weinheim, Ger.)* **1970**, *303*, 17.
(8) Meyer zu Reckendorf, W.; Wassiliadou-Micheli, N.; Bischof, E. *Chem. Ber.* **1971**, *104*, 1.
(9) Peterson, R. U.S. Pat. 4088757, 1978.
(10) Cook, L. S.; Caldwell, R. W.; Nash, C. B. *J. Cardiovasc. Pharmacol.* **1979**, *1*, 551 and references cited therein.
(11) Kanazawa, T.; Satô, T. *Nippon Kagaku Zasshi* **1959**, *80*, 200; *Chem. Abst.* **1961**, *55*, 6385h.
(12) Takiura, K.; Yuki, H.; Okamoto, Y.; Takai, H.; Honda, S. *Chem. Pharm. Bull.* **1974**, *22*, 2263.
(13) Runge, T. M. *Am. Heart J.* **1977**, *93*, 248.
(14) Smith, T. W. *N. Engl. J. Med.* **1973**, *288*, 719.
(15) Verheyden, J. P. H.; Richardson, A. C.; Bhatt, R. S.; Grant, B. D.; Fitch, W. L.; Moffatt, J. G. *Pure Appl. Chem.* **1978**, *50*, 1363.
(16) Haworth, W. N.; Owen, L. N.; Smith, F. *J. Chem. Soc.* **1941**, 88.

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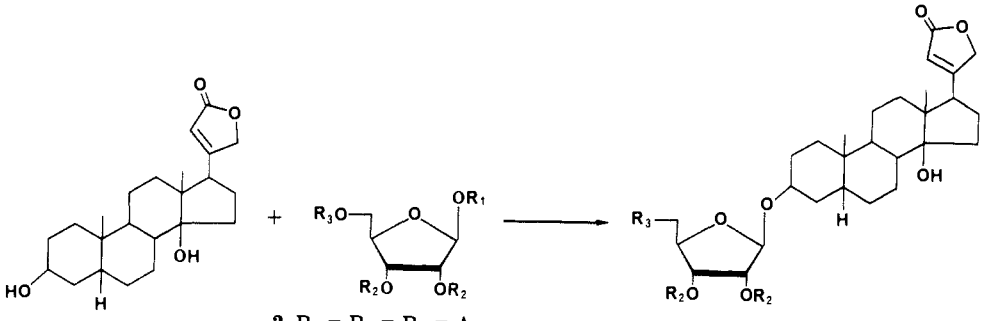
Scheme I

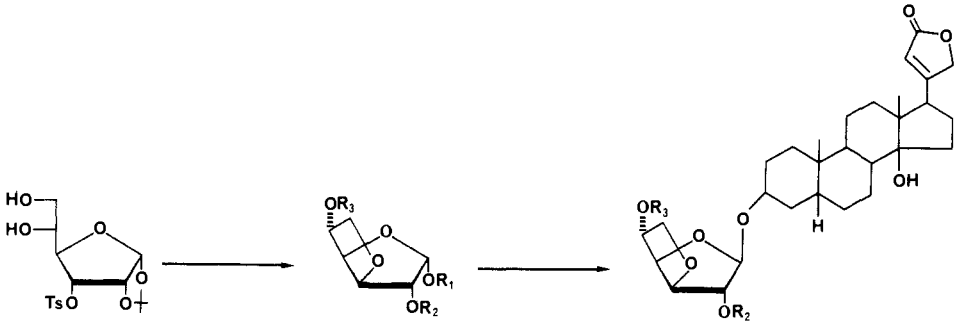


1

2, $R_1 = R_2 = R_3 = Ac$
5, $R_1 = R_2 = Ac$; $R_3 = Ts$

3, $R_2 = Ac$; $R_3 = OAc$
4, $R_2 = H$; $R_3 = OH$
6, $R_2 = Ac$; $R_3 = OTs$
7, $R_2 = H$; $R_3 = OTs$
8, $R_2 = H$; $R_3 = N_3$
9, $R_2 = Ac$; $R_3 = N_3$
10, $R_2 = H$; $R_3 = NH_2$
11, $R_2 = Ac$; $R_3 = NHAc$

Scheme II



12

13, $R_1, R_2 = >C(CH_3)_2$; $R = H$
14, $R_1 = R_2 = Ac$
15, $R_1, R_2 = >C(CH_3)_2$; $R_3 = Ac$
16, $R_1 = R_2 = H$; $R_3 = Ac$

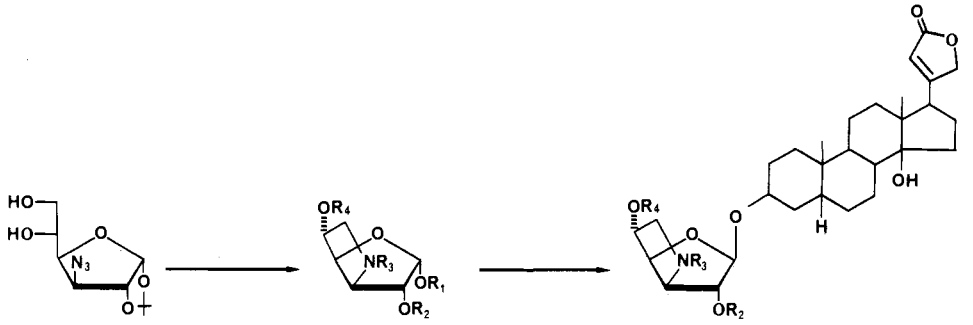
17, $R_2 = R_3 = Ac$
18, $R_2 = R_3 = H$

made unambiguously by first protecting the 5-hydroxyl of **13** as the acetate, then removing the acetonide with Dowex 50 (H^+) resin, and acetylating the product with acetic anhydride in pyridine. This furnished a sample of 1,2,5-tri-*O*-acetyl-3,6-anhydro-D-glucofuranose (**14**), which was identical with that made by the first route. The peracetyl sugar **14** was brominated with use of hydrogen bromide in dichloromethane. When the bromo sugar was heated with digitoxigenin in acetonitrile in the presence of mercuric cyanide, a 66% yield of 3-*O*-(2,5-di-*O*-acetyl-3,6-anhydro-β-D-glucofuranosyl)digitoxigenin (**17**) was isolated. This product was then deprotected with methanolic ammonia to give the glycoside **18** in 72% yield. The β configuration at the anomeric position was confirmed by the absence of H-1′ coupling in the ^1H NMR spectrum.

In order to further explore the biological consequences of nitrogen/oxygen substitutions, the analogue having a 3,6-imino bridge was constructed. The carbohydrate portion, suitably derivatized for condensation with the steroid, was obtained by classical procedures from the readily accessible 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**19**).[17-19] Thus, **19** was treated successively, and without isolation of intermediates, with *p*-toluenesulfonyl chloride in pyridine, Raney nickel in a hydrogen atmosphere, sodium acetate in refluxing ethanol, and benzyl chloroformate. With the exception of the final carbamoylation, this procedure closely parallels that of Meyer zu Reckendorf.[19] The resulting 3,6-[(benzyloxycarbonyl)imino]-3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**20**) was obtained in overall yields ranging from 35% to 44%. An efficient alternative synthesis of this compound has been reported by Yamada et al.[20] Hydrolysis of the acetonide **20** using Dowex 50 (H^+) ion-exchange resin followed by acetylation with acetic anhydride in pyridine gave 1,2,5-tri-*O*-acetyl-3,6-[(benzyloxycarbonyl)imino]-3,6-dideoxy-D-glucofuranose (**21**) in quantitative yield. Although it may be possible to couple this derivative to the steroid, later removal of the carbobenzoxy protecting group could be problematic. The catalytic hydrogenation necessary to effect this cleavage would likely also reduce the butenolide ring. Therefore, the carbobenzoxy group was removed by hydrogenation over palladium on charcoal and replaced with a trifluoroacetyl group by reaction with trifluoroacetic anhydride in ether. The product, 1,2,5-tri-*O*-acetyl-3,6-dideoxy-3,6-[(trifluoroacetyl)imino]-D-glucofuranose (**22**), was isolated as a colorless syrup in a 95% yield over the two steps (Scheme III). The fully acetylated sugar **22** could now be brominated with hydrogen bromide in dichloromethane and condensed with digitoxigenin in the presence of mercuric cyanide to give 3-*O*-[2,5-di-*O*-acetyl-3,6-di-

(17) Verheyden, J. P. H.; Repke, D. B.; Tompkins, T. C.; Moffatt, J. G. *ACS Symp. Ser.* **1980**, *No. 125*, 209.
(18) Brimacombe, J. S.; Bryan, J. G. H.; Husain, A.; Stacey, M.; Tolley, M. S. *Carbohydr. Res.* **1967**, *3*, 318.
(19) Meyer zu Reckendorf, W. *Chem. Ber.* **1968**, *101*, 3802.
(20) Yamada, M.; Tachibana, K.; Kuroda, T.; Sakakibara, T. *Carbohydr. Res.* **1983**, *112*, 189.

Scheme III



19

20, R_1, R_2 = >C(CH$_3$)$_2$; R_3 = Cbz; R_4 = H
21, R_1 = R_2 = R_4 = Ac; R_3 = Cbz
22, R_1 = R_2 = R_4 = Ac; R_3 = TFA

23, R_2 = R_4 = OAc; R_3 = TFA
24, R_2 = R_3 = R_4 = H

Table I. Inotropic Effects on Guinea Pig Atria[a]

compd	n[b]	dose (μM) producing % increase in developed tension of 50	75	dose (μM) producing negative inotropy (NI) or arrhythmia (A)
digitoxigenin	5	0.38	0.64	2.5 (NI)
digoxin	2	0.42	0.82	2.5 (NI)
3	2	1.9	3.0	*c*
4	2	0.36	0.64	2.5 (NI)
8	1	0.25	0.43	1.0 (NI) (A)
9	2	0.30	0.44	2.5 (NI)
10	2	0.26	0.35	5.0 (NI) (A)
17	8	0.39	0.53	1.0 (NI) (A)
18	2	10	*c*	*c*
23	1	0.54	0.65	1.0 (NI), 0.75 (A)
24	2	0.50	0.86	2.0 (NI), 1.5 (A)

[a] See Experimental Section for details. [b] Number of trials. [c] Minimal activity.

deoxy-3,6-[(trifluoroacetyl)imino]-β-D-glucofuranosyl]digitoxigenin (**23**) in 48% yield. Deprotection of the glycoside **23** with methanolic ammonia resulted in a 45% yield of the desired 3-*O*-(3,6-dideoxy-3,6-imino-β-D-glucofuranosyl)digitoxigenin (**24**). Again the absence of coupling of the 1′-H signal in the ^{1}H NMR of compound **24** confirmed the β configuration of the glycoside.

Biological Results and Discussion

Structure–activity relationship (SAR) studies on the glycosidic moiety of cardiac glycosides have compared the effect of various functional groups, length of a polysaccharide chain, and configuration at the anomeric center. However, except for the single report of digitoxigenin 3-*O*-α-L-arabinofuranoside,[4] these comparisons have been limited to pyranosides.

Since the hydroxyl functions of the sugar are important contributors to cardiac glycoside receptor binding, the presence of a five-membered sugar ring could have a significant effect on inotropic activity. The spatial relationships among the hydroxyls are quite different from those of a six-membered ring and the ridgidity of the system is enhanced. Furthermore, recent articles[22-25] have emphasized the importance of the 4′-OH of the glycoside for receptor binding and the enhancement of activity when the hydroxyl is in the equitorial position. If Drieding models of a β-pyranoside and a β-D-furanoside are compared, the glycosidic oxygen, the carbons in positions 4 and 5, and the ring oxygen of the pyranose are nearly superimposable with the glycosidic oxygen, carbons in positions 5 and 4, and the ring oxygen of the furanose, respectively. Rotation around the 4–5 carbon bond of the furanoside would allow a 5-substituent to closely approximate the position of either an axial or equitorial substituent at position 4 of the pyranoside. This suggests that the 5′-position of a digitoxigenin β-D-furanoside may be as influential in determining activity as the 4′-position of digitoxigenin β-D-pyranosides.

Examination of the inotropic activities of the ribosides (see Table I) suggests that they are at least as active as digoxin or digitoxigenin. The 5′-azido-5′-deoxyfuranoside **8** and the 5′-amino-5′-deoxyfuranoside **10** are the most potent compounds with measured activities approximately twice that of digoxin. Two other ribosides, **4** and **9**, are equipotent to digoxin, while the fully acetylated riboside **3** is nearly 10 times less active. This loss of activity with acetylation is consistent with the results of Brown and Thomas[22] who have reported decreasing activity with an increasing degree of acetylation for a number of cardiac glycosides.

The formation of a 3′–6′ "bridge" over the furanose ring as in compounds **17**, **18**, **23**, and **24** results in a decrease in activity. The 3,6-anhydroglucofuranoside **18** was the

(21) Stevens, J. D.; Ness, R. K.; Fletcher, H. G. *J. Org. Chem.* **1968**, *33*, 1806.
(22) Brown, L.; Thomas, R. *Arzneim.-Forsch.* **1983**, *33*, 814.
(23) Fullerton, D. S.; Kihara, M.; Deffo, T.; Kitatsuji, E.; Ahmed, K.; Simat. B.; From, A. H. L.; Rohrer, D. C. *J. Med. Chem.* **1984**, *27*, 256.
(24) Rohrer, D. C.; Kihara, M.; Deffo, T.; Rathore, H.; Ahmed, K.; From, A. H. L.; Fullerton, D. S. *J. Am. Chem. Soc.* **1984**, *106*, 8269.
(25) Chiu, F. C. K.; Watson, T. R. *J. Med. Chem.* **1985**, *28*, 509.

least active of all the compounds tested (0.04 the activity of digoxin). Oddly, the acetylated derivative of 18 (compound 17) remained moderately active. Probably, by forming the 3′,6′-anhydro bridge, the 5′-OH is ridgedly held in an orientation less favorable to receptor binding than that which it can achieve when it is free to rotate about the 4′–5′ carbon bond. In fact, on reexamination of the Drieding models, the 5′-OH of the 3′,6′-anhydrofuranoside is now directed away from the 4′-equitorial position of a superimposed pyranoside. Although this is a tempting hypotheses, other reasons for the decrease in activity could be the additional steric hindrance across the β face of the sugar, the decrease in acidity of the 5′-OH as it becomes a secondary OH, the absence of a 3′-OH, or the increase in ridgidity of the entire system.

The substitution of an amine function for a hydroxyl group has been reported to sometimes potentiate cardenolide activity.[10] In our series of compounds, the presence of an amine caused no or only slight enhancement of activity. It is interesting to note that when the 5′-OH of the riboside 4 is substituted with an azido group (compound 8) a slight increase in activity is obtained.

Estimates of toxicity were made by observing the onset of arrhythmias or negative intropy. The toxicity paralleled inotropy for all compounds except the 5′-amino-5′-deoxy riboside 10, which was one of the least toxic analogues yet had substantial inotropic activity. The corresponding 5′-hydroxy riboside 4 is twice as toxic and slightly less active. This seems to support the premise that an amino sugar can improve the therapeutic index of cardiac glycosides.

Although it appears that the substitution of a furanose for the pyranose moiety of a cardiac glycoside does not, in itself, lead to an increase in potency or a decrease in toxicity, digitoxigenin furanosides offer new opportunities for delineating the role that the sugar plays in determining biological activity.

Experimental Section

General Methods. Nuclear magnetic resonance spectra were recorded on Varian HA-100 (^{1}H NMR, 100 MHz) and Bruker WM-300 (^{1}H NMR, 300) spectrometers and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Mass spectra (MS) were recorded on a Finnigan MAT CH7 spectrometer operating in the direct inlet mode. Some elemental analyses were obtained from Dr. A. Bernhardt, Elbach über Engelskirchen, while most instrumental analyses were performed by the staff of the Syntex Analytical Research Division. All chromatographic purifications were carried out on silica gel. Melting points were determined on a hot-stage microscope and are corrected.

3-*O*-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)digitoxigenin (3). A solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide [from 2.5 g, 8.0 mmol of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (2)[21]], digitoxigenin (1) (1.5 g, 4.0 mmol), and mercuric cyanide (4.0 g, 16 mmol) in acetonitrile (200 mL) was stirred at 60 °C for 2 h and then evaporated to dryness. A filtered solution of the residue in $CHCl_3$ was washed with saturated aqueous sodium bicarbonate, 30% aqueous potassium iodide, and water. The dried organic phase was evaporated and the residue was chromatographed on a column of silica gel (400 g) with chloroform–acetone (12:1), giving 2.1 g (83%) of 3 as a homogeneous white foam: ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.82 (br s, 1 H, H-22), 5.31 (t, J = 5 Hz, 1 H, H-3′), 5.17 (d, J = 5 Hz, 1 H, H-2′), 5.04 (s, 1 H, H-1′), 4.73, 4.98 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 4.03–4.30 (m, 3 H, H-4′, H-5′), 2.03–2.09 (m, 9 H, OAc), 0.92 (s, 3 H, H-19), 0.86 (s, 3 H, H-18). Anal. ($C_{34}H_{48}O_{11}$) C, H.

3-*O*-β-D-Ribofuranosyldigitoxigenin (4). A solution of 3 (1.95 g, 3.08 mmol) in saturated methanolic ammonia (20 mL) was stored at room temperature for 18 h and then evaporated to dryness. The residue was purified on a column of silica gel (200 g) with chloroform–methanol (19:1) and then crystallized from ethanol/water, giving 0.95 g (61%) of 4: 223–224 °C; ^{1}H NMR (100 MHz, Me_2SO-d_6) δ 5.85 (br s, 1 H, H-22), 4.89 (m, 2 H, H-21), 4.78 (br s, 1 H, H-1′), 0.85 (s, 3 H, H-19), 0.76 (s, 3 H, H-18); MS, 506 (M^+), 357, 203 (base). Anal. ($C_{28}H_{42}O_8$) C, H.

3-*O*-[5-*O*-(*p*-Tolylsulfonyl)-β-D-ribofuranosyl]digitoxigenin (7). Additions of a 1.0 M solution of *p*-toluenesulfonyl chloride in pyridine (0.80 mL) to a stirred solution of 4 (810 mg, 1.6 mmol) in pyridine (12 mL) were made at times 0, 1 h, 2 h, and 4 h. After a total of 5.75 h the reaction was quenched with methanol and evaporated. The residue was chromatographed on a column of silica gel (120 g) with chloroform–methanol (30:1), giving 646 mg (61%) of 7 as a homogeneous white foam: ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.72 (d, J = 8 Hz, 2 H, Ar H), 7.27 (d, J = 8 Hz, 2 H, Ar H), 5.81 (br s, 1 H, H-22), 4.94 (s, 1 H, 1′-H), 4.72, 4.97 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 2.40 (s, 3 H, Ar CH_3), 0.87 (s, 3 H, H-19), 0.84 (s, 3 H, H-18). Anal. ($C_{35}H_{48}O_{10}S$) C, H.

3-*O*-(5-Azido-5-deoxy-β-D-ribofuranosyl)digitoxigenin (8). A solution of 7 (528 mg, 0.80 mmol) and lithium azide (120 mg, 2.4 mmol) in dimethylformamide (7.0 mL) was heated for 6 h at 80 °C and then evaporated to a viscous syrup. The syrup was dissolved in ethanol, treated with charcoal, filtered, reevaporated, and crystallized from ethanol/water to give 346 mg of 8. The mother liquors were purified by preparative TLC with chloroform–methanol (9:1) to furnish an additional 47 mg after crystallization and bringing the total yield of 8 to 393 mg (93%): mp 139–142 °C; IR (KBr) 2120 (N_3) cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.84 (br s, 1 H, H-22), 5.02 (s, 1 H, H-1′), 4.74, 5.00 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 3.90–4.25 (m, 4 H, H-3, H-2′, H-3′, H-4′), 0.90 (s, 3 H, H-19), 0.85 (s, 3 H, H-18); MS, 531 (M^+), 375 (base), 357. Anal. ($C_{28}H_{41}N_3O_7$) C, H, N.

3-*O*-(5-Amino-5-deoxy-β-D-ribofuranosyl)digitoxigenin (10). A mixture of 8 (212 mg, 0.4 mmol) and Raney nickel (1.4 g as an ethanol wet paste) in methanol (20 mL) was stirred for 1.5 h under 1 atm of hydrogen and then filtered and the filtrate evaporated to a syrup. After triturating the syrup with ethyl acetate, the residue was crystallized from water to give 117 mg of 10. The mother liquors of crystallization were combined with the ethyl acetate washings and purified by preparative TLC using two elutions with chloroform–methanol (4:1). Isolation of the major band followed by crystallization from H_2O gave 22 mg more of 10, for a total of 139 mg (69%): mp 169–173 °C dec; ^{1}H NMR (100 MHz, Me_2SO-d_6) δ 5.84 (br s, 1 H, H-22), 4.88 (br s, 2 H, H-21), 4.78 (s, 1 H, H-1′), 0.85 (s, 3 H, H-19), 0.76 (s, 3 H, H-18); MS, (NH_3–CI), 506 (MH^+), 375, 357, 203 (base). Anal. ($C_{28}H_{43}NO_7 \cdot H_2O$) C, H, N.

3-*O*-(5-Acetamido-2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)digitoxigenin (11). A solution of 10 (60 mg, 0.12 mmol) and acetic anhydride (1.0 mL, 10.6 mmol) in pyridine (2.0 mL) was stirred at 21 °C for 3.5 h before being evaporated to a viscous syrup. Purification of the residue by preparative TLC using chloroform–methanol (19:1) gave 52 mg (70%) of 11 as a homogeneous foam: ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.97 (br s, 1 H, NH), 5.83 (br s, 1 H, H-22), 5.10–5.24 (m, 2 H, H-2′, H-3′), 5.02 (s, 1 H, H-1′), 4.74, 5.00 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 2.07 and 2.02 (s's, 6 H, OAc's), 1.94 (s, 3 H, NAc), 0.92 (s, 3 H, H-19), 0.85 (s, 3 H, H-18). Anal. ($C_{34}H_{49}NO_{10} \cdot {}^1/_4H_2O$) C, H, N.

3-*O*-[2,3-Di-*O*-acetyl-5-*O*-(*p*-tolylsulfonyl)-β-D-ribofuranosyl]digitoxigenin (6). Dry hydrogen bromide was bubbled through a 0 °C solution of 1,2,3-tri-*O*-acetyl-5-*O*-(*p*-tolylsulfonyl)-D-ribofuranose (5)[11] (150 mg, 0.333 mmol) in dichloromethane (3.0 mL) for 15 min. The solution was left at 0 °C for 1 h and then was brought to room temperature and evaporated to a syrup. The syrup was coevaporated twice with toluene (2.0 mL) and the residue dissolved in acetonitrile (2.0 mL). One half of the bromo sugar solution was added to a 60 °C stirred solution of digitoxigenin (1) (60 mg, 0.16 mmol) and mercuric cyanide (170 mg, 0.70 mmol) in acetonitrile (10 mL). After 20 min the rest of the bromo sugar was added and heating continued for an additional 3 h. After evaporation of the solvent, a filtered solution of the residue in chloroform was washed with aqueous sodium bicarbonate, 30% aqueous potassium iodide, and water. The dried organic phase was evaporated and the residual syrup purified by preparative TLC using three elutions with ethyl acetate–benzene (3:7) to give 24 mg (20%) of 6 as a homogeneous, colorless glass. Also, recovered was 23 mg (38%) of unreacted

digitoxigenin. Compound **6**: ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.74 (d, J = 8, 2 H, Ar H), 7.29 (d, J = 8, 2 H, Ar H), 5.81 (br s, 1 H, H-22), 5.05–5.25 (m, 2 H, H-2′, H-3′), 4.99 (s, 1 H, H-1′), 4.71, 4.97 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 2.40 (s, 3 H, Ar CH_3), 2.04 and 1.97 (s's, 6 H, OAc's), 0.88 (s, 3 H, H-19), 0.85 (s, 3 H, H-18). Anal. ($C_{39}H_{52}O_{12}S$) C, H.

3-O-(2,3-Di-O-acetyl-5-azido-5-deoxy-β-D-ribofuranosyl)digitoxigenin (9). (a) A solution of **6** (38 mg, 0.05 mmol) and lithium azide (3 mg, 0.06 mmol) in dimethylformamide (0.3 mL) was stirred at 80 °C for 16 h. More lithium azide (3 mg, 0.06 mmol) was added and heating continued for another 2 h before evaporation of solvent. Purification of the residue by preparative TLC using two elutions with ethyl acetate–benzene (7:3) gave 16 mg (52%) of **9** as a homogeneous foam: IR (neat) 2100 (N_3) cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.83 (br s, 1 H, H-22), 5.15–5.32 (m, 2 H, H-2′, H-3′), 5.06 (s, 1 H, H-1′), 4.73, 4.99 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 2.08 and 2.02 (s's, 6 H, OAc's), 0.92 (s, 3 H, H-19), 0.85 (s, 3 H, H-18). Anal. ($C_{32}H_{45}N_3O_9$) C, H, N.

(b) A solution of **8** (53 mg, 0.1 mmol) and acetic anhydride (0.1 mL, 1.0 mmol) in pyridine (0.5 mL) was kept at 21 °C for 16 h before evaporating to a syrup. The residue was purified by preparative TLC using two elutions with chloroform–acetone (9:1) which gave 50 mg (77%) of **9** as a homogeneous foam identical with the product described above.

1,2,5-Tri-O-acetyl-3,6-anhydro-D-glucofuranose (14). (a) A suspension of 1,2-*O*-isopropylidene-3-*O*-(*p*-tolylsulfonyl)allofuranose[15] (**12**) (1.12 g, 3.0 mmol) in methanolic sodium methoxide (0.125 N, 29 mL) was heated at reflux for 5 h and then evaporated to give **13** as a syrupy solid. Water (20 mL) and Dowex AG-50 X8 (H^+) cation-exchange resin (8 mL wet) were added, and the mixture was stirred at 60 °C for 3 h. The filtered reaction mixture was evaporated to a syrup which was dried by coevaporation first with toluene (3 × 25 mL) and then with pyridine (25 mL). A solution of the residue and acetic anhydride (4.0 mL, 42 mmol) in pyridine (20 mL) was kept at 21 °C for 3 h before evaporation in vacuo. The resulting syrup was dissolved in chloroform (25 mL) and the solution extracted with water (2 × 20 mL). The dried ($MgSO_4$) organic phase was concentrated to a syrup which was chromatographed on a column of silica gel (100 g) with benzene–ethyl acetate (85:15) as eluent. The 1:1 anomeric mixture of pure **14** was isolated as 720 mg (84%) of a colorless, viscous syrup: ^{1}H NMR (100 MHz, $CDCl_3$) δ 6.47 (d, J = 4 Hz, 0.5 H, H-1α), 6.13 (s, 0.5 H, H-1β), 4.50–5.15 (m, 4 H, H-2α and -β, H-3α and -β, H-4α and -β, H-5α and -β), 3.68–4.14 (m, 2 H, H-6α and -β), 2.06 (m, 9 H, OAc's). Anal. ($C_{12}H_{16}O_8$) C, H.

(b) A solution of 3,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose (**13**) (200 mg, 1 mmol, obtained as above), acetic anhydride (0.5 mL, 5.3 mmol), and dry pyridine (2.0 mL) was kept at 21 °C for 16 h before evaporating to a syrup. The residue was purified by preparative TLC using chloroform–acetone (19:1) as eluent to give 190 mg (79%) of **15** as a colorless syrup: ^{1}H NMR (100 MHz) δ 5.93 (d, J = 3.5 Hz, 1 H, H-1), 5.17–4.87 (m, 2 H, H4, H-5), 4.57 (d, J = 3.5 Hz, 1 H, H-2), 4.48 (d, J = 3.5 Hz, 1 H, H-3), 3.84 (m, 2 H, H-6), 1.45 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3). A solution of **15** (100 mg, 0.41 mmol) in dioxane (0.5 mL) was added to a suspension of Dowex AG-50 X8 (H^+) cation-exchange resin (0.5 mL) in water (1.0 mL) and stirred at 60 °C for 2 h. After filtration, the solution was evaporated to dryness. The residue was purified by preparative TLC ($CHCl_3/CH_3OH$, 9:1) and 43 mg (51%) of **16** was isolated as a colorless, viscous syrup: ^{1}H NMR (100 MHz) δ 2.07 (s, 3 H, OAc). Compound **16** (40 mg, 0.2 mM) was dissolved in dry pyridine (0.5 mL) and allowed to react at 21 °C for 16 h with acetic anhydride (0.2 mL, 2.1 mmol) before concentrating by evaporation. The residue was purified by preparative TLC (C_6H_6/EtOAc, 7:3) to give 54 mg (95%) of **14** as a colorless syrup which was identical in all respects with that prepared by Method (a).

3-O-(2,5-Di-O-acetyl-3,6-anhydro-β-D-glucofuranosyl)digitoxigenin (17). Dry hydrogen bromide was bubbled through a 0 °C solution of **14** (419 mg, 1.45 mmol) in dichloromethane (5 mL) for 20 min. The mixture was kept at 0 °C for 1 h and then evaporated in vacuo. After coevaporation with toluene (4 × 5 mL) the residue was dissolved in acetonitrile (2 mL). Three equal portions of this solution were added at 0.5-h intervals to a 60 °C, stirred solution of digitoxigenin (**1**) (280 mg, 0.75 mmol) and mercuric cyanide (750 mg, 3.0 mmol) in acetonitrile (30 mL). After a total of 4 h the reaction mixture was evaporated to dryness. A filtered solution of the residue in chloroform was washed with saturated aqueous sodium bicarbonate, 30% aqueous potassium iodide, and water. The dried ($MgSO_4$) organic phase was evaporated and the residue was purified by preparative TLC using chloroform–methanol (97:3) to give 280 mg (62%) of **17** as a homogeneous white foam. Also isolated was 40 mg (14%) of unreacted digitoxigenin. Compound **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88 (br s, 1 H, H-22), 5.12 (s, 1 H, H-2′), 5.03 (s, 1 H, H-1′), 4.78–5.03 (m, 4 H, H-21, H-4′, H-5′), 4.56 (d, J = 5 Hz, 1 H, H-3′), 2.10 (s, 3 H, OAc), 2.09 (s, 3 H, OAc), 0.94 (s, 3 H, H-19), 0.87 (s, 3 H, H-18). Anal. ($C_{33}H_{46}O_{10}$) C, H.

3-O-(3,6-Anhydro-β-D-glucofuranosyl)digitoxigenin (18). A solution of **17** (250 mg, 0.415 mmol) in methanolic ammonia (5.0 mL) was kept at 21 °C for 8.5 h and then evaporated to dryness. The residue was crystallized from ethanol to give 85 mg of **18** in one crop. Purification of the mother liquors by preparative TLC using chloroform–acetone (7:3) gave another 69 mg of **18** after crystallization from ethanol and increased the total yield to 72%: mp 241–243 °C; ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 5.90 (br s, 1 H, H-22), 4.99 (s, 1 H, H-1′), 4.88, 4.99 (ABX, J = 18 and 1.5, 2 H, H-21), 4.52 (t, J = 4.5, 1 H, H-4′), 4.22 (d, J = 4.5 Hz, 1 H, H-3′), 3.86 (d, J = 4 Hz, 1 H, H-2′), 0.87 (s, 3 H, H-19), 0.78 (s, 3 H, H-18); MS, 518 (M^+), 357, 69 (base). Anal. ($C_{29}H_{42}O_8$) C, H.

3,6-[(Benzyloxycarbonyl)imino]-3,6-dideoxy-1,2-O-isopropylidene-α-D-glucofuranose (20). To an ice-cold solution of 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**19**)[17] (490 mg, 2.0 mmol) in pyridine (2.0 mL) was added a solution of *p*-toluenesulfonyl chloride (490 mg, 2.6 mmol) in chloroform (1.0 mL). The mixture was kept at 20 °C for 16 h before being poured into water (20 mL) and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated to 0.90 g of viscous syrup. A solution of the syrup in ethanol (25 mL) was stirred for 1 h in the presence of Raney nickel (4.0 g of ethanol wet paste) under 1 atm of hydrogen. The filtered solution was evaporated to 0.64 g of a white solid, which was dissolved in ethanol (35 mL) containing anhydrous sodium acetate (0.5 g). After refluxing for 2 h, the solution was evaporated to a semisolid. A mixture of the residue, benzyl chloroformate (0.7 mL, 5 mmol), sodium carbonate (265 mg, 2.5 mmol), water (30 mL), and acetone (10 mL) was stirred at 20 °C for 45 min. The acetone was evaporated off and the remaining aqueous solution diluted with water (10 mL) and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated and the residual syrup was chromatographed on a column of silica gel (100 g), eluting with 200 mL of chloroform, 400 mL of chloroform/acetone (19:1), and then 400 mL of chloroform/acetone (9:1), giving 290 mg (44%) of **20** as a homogeneous, colorless, viscous syrup: ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.32 (s, 5 H, phenyl), 5.32 (d, J = 3.5 Hz, 1 H, H-1), 4.60–4.83 (m, 2 H, H-2, H-4), 4.28 (d, J = 3.5 Hz, 1 H, H-3), 4.04–4.17 (m, 1 H, H-5), 1.47 (s, 3 H, CH_3), 1.28 (s, 3 H, CH_3). Anal. ($C_{17}H_{21}NO_6$) C, H, N.

1,2,5-Tri-O-acetyl-3,6-[(benzyloxycarbonyl)imino]-3,6-dideoxy-D-glucofuranose (21). A mixture of **20** (1.18 g, 3.5 mmol) and Dowex AG-50 X8 (H^+) cation-exchange resin (5.0 mL) in water (10 mL) and dioxane (2.5 mL) was stirred at 60 °C for 2.5 h. After filtration, the solution was evaporated and the residue dried by coevaporation with first toluene and then pyridine. To a solution of the resulting oil in pyridine (25 mL) was added acetic anhydride (2.5 mL, 26 mmol). After stirring for 3 h at 20 °C, the mixture was evaporated and chromatographed on a column of silica gel (100 g), using chloroform–acetone (19:1), giving 1.38 g (93%) of syrupy **21** as a 1:1 anomeric mixture: ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.32 (s, 5 H, phenyl), 6.45 (d, J = 5 Hz, 0.5 H, H-1α), 6.11 (d, J = 3.5 Hz, 0.5 H, H-1β), 2.05 (m, 9 H, OAc's). Anal. ($C_{20}H_{23}NO_9$) C, H, N.

1,2,5-Tri-O-acetyl-3,6-dideoxy-3,6-[(trifluoroacetyl)imino]-D-glucofuranose (22). A solution of **21** (1.32 g, 3.14 mmol) in ethanol (75 mL) was stirred for 1 h under 1 atm of hydrogen in the presence of 10% palladium on carbon (200 mg). The catalyst was removed by filtration and the filtrate evaporated to a syrup, which was dried by coevaporation with toluene (3 × 15 mL). To the residue were added diethyl ether (75 mL) and trifluoroacetic anhydride (2.0 mL, 14.2 mmol). After remaining

at 21 °C for 2.5 h, the solution was washed with saturated, aqueous sodium bicarbonate (25 mL) and water (25 mL). The dried ($MgSO_4$) ether phase was evaporated to 1.12 g (93%) of viscous, syrupy **22**, which was nearly pure and was suitable for direct use. An analytical sample was prepared by preparative TLC using chloroform–acetone (19:1): ^1H NMR (100 MHz, $CDCl_3$) δ 6.49 (d, J = 4.5 Hz, 0.5 H, H-1α), 6.18 (br s, 0.5 H, H-1β), 2.02–2.10 (m, 9 H, OAc's). Anal. ($C_{14}H_{16}NO_8F_3$) C, H, N.

3-*O*-[2,5-Di-*O*-acetyl-3,6-dideoxy-3,6-[(trifluoroacetyl)imino]-β-D-glucofuranosyl]digitoxigenin (23). Dry hydrogen bromide was bubbled for 20 min through a 0 °C solution of **22** (986 mg, 2.58 mmol) in dichloromethane (20 mL). After remaining at 0 °C for 1 h, the mixture was evaporated and then coevaporated with toluene (3 × 10 mL). A solution of the resulting syrup, digitoxigenin (**1**) (375 mg, 1 mmol), and mercuric cyanide (1.3 g, 5.15 mmol) in acetonitrile (50 mL) was heated at 60 °C for 24 h. Following evaporation, a filtered solution of the residue in $CHCl_3$ was washed with saturated aqueous sodium bicarbonate, 30% aqueous potassium iodide, and water. The dried ($MgSO_4$) organic phase was evaporated and the residue chromatographed on a column of silica gel (200 g), using first 4% acetone in chloroform (2 L) and then 5% acetone in chloroform (1 L) to give 338 mg (48%) of **23** as a homogeneous, white foam: ^1H NMR (300 MHz, $CDCl_3$) δ 5.87 (br s, 1 H, H-22), 4.81, 5.00 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 5.17 (s, 1 H, H-2′), 5.14 (s, 1 H, H-1′), 5.00–5.10 (m, 2 H, H-4′, H-5′), 4.54 (d, J = 5 Hz, 1 H, H-3′), 2.14 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 0.91 (s, 3 H, H-19), 0.87 (s, 3 H, H-18). Anal. ($C_{35}H_{46}NO_{10}F_3$) C, H, N.

3-*O*-(3,6-Dideoxy-3,6-imino-β-D-glucofuranosyl)digitoxigenin (24). A solution of **23** (100 mg, 0.144 mmol) in methanolic ammonia (2 mL) was left at 20 °C for 16 h before concentrating and purifying by preparative TLC using chloroform–methanol (4:1). The resulting solid was crystallized from methanol/water to give 35 mg (45%) of **24** as a hydrate: mp 199–200 °C dec; ^1H NMR (300 MHz, Me_2SO-d_6 δ 5.90 (br s, 1 H, H-22), 4.87, 4.97 (ABX, J = 18 and 1.5 Hz, 2 H, H-21), 4.91 (s, 1 H, H-1′), 4.40 (t, J = 4.5, 1 H, H-4′), 3.75 (s, 1 H, H-2′), 3.43 (d, J = 4.5 Hz, 1 H, H-3′), 0.87 (s, 3 H, H-19), 0.77 (s, 3 H, H-18); MS, 517 (M^+), 357, 203 (base). Anal. ($C_{29}H_{43}NO_7.H_2O$) C, H, N.

Biological Assay.[26] Left atria isolated from white, male guinea pigs (250–450 g) were suspended in a tissue chamber and bathed with a Krebs–Henselit solution consisting of the following (in mM concentrations): NaCl, 118.2; KCl, 4.6; CaCl, 2.0; $NaHCO_3$, 24.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0. The bathing medium was continuously aerated with a 95%/5% O_2/CO_2 gas mixture and maintained at a temperature of 29–30 °C. The atria were stimulated with rectangular pulses at a frequency of 1 Hz, duration of 5 ms, and voltage 1.5 times that of threshold. A resting tension was applied to the atria that was 75% of the resting tension that yielded a maximal developed tension. Compounds were exposed to the tissue in a cumulative concentration fashion allowing 30 min of contact time at each level.

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(26) Thomas, R.; Boutagy, J.; Gelbart, A. *J. Pharmacol. Exp. Ther.* **1974**, *191*, 219.

Antiarrhythmic Activity of 17β-Aminoestratrienes. Comparison of 3-ols and 3-Acetates with the Corresponding 3-(3-Amino-2-hydroxypropyl) Ethers

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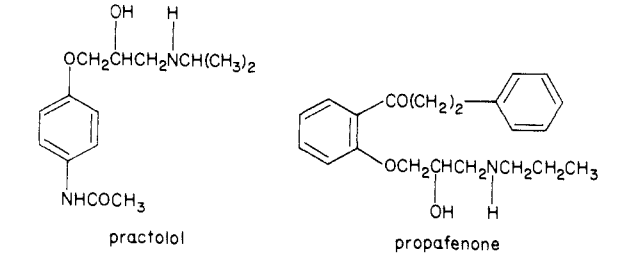
The antiarrhythmic efficacy of 17β-amino- and 17β-amino-16α-hydroxyestratrien-3-ols and 3-acetates (group 1) was compared with the efficacy of corresponding 3-[2-hydroxy-3-(isopropylamino)propyl] and 3-[2-hydroxy-3-(*tert*-butylamino)propyl] ethers (group II), substituents which are usually associated with β-adrenoceptor blocking activity. Group I compounds exerted potent antiarrhythmic activity against both aconitine-induced arrhythmias in mice and ischemia-induced arrhythmias in rats and reduced the maximum following frequency of isolated guinea pig atria. Electrophysiological studies indicated that their mechanism of action is due to an ability to reduce the fast inward sodium current in cardiac cells (class I antiarrhythmic action). Group II compounds were inactive in the aconitine and atrial tests and electrophysiological studies confirmed that they were devoid of class I activity. However, these compounds, like both class I antiarrhythmic and β-adrenoceptor blocking drugs, were active against ischemia-induced arrhythmias. Group II compounds, unlike group I compounds, exerted nonspecific β-adrenoceptor blocking actions, which may account for their activity in the rat test. It was concluded that introduction of the 3-substituted ether group did not confer any advantage over the parent 3-ol or 3-acetate compounds.

A number of amino steroids have emerged that possess interesting antiarrhythmic activity. These compounds include 3α-amino-2β-hydroxy-5α-androstan-17-one hydrochloride (Org 6001),[1] several 3-amino-2-hydroxy and 2-amino-3-hydroxy isomers of Org 6001,[2] methyl 2β-ethoxy-3α-hydroxy-11α-[(3-methylbutyl)amino]-5α-androstane-17β-carboxylate hydrochloride,[3] and both 3-methoxy-16α-(methylamino)estra-1,3,5(10)-trien-17β-ol hydrochloride and its enantiomer.[4] None of these compounds has important hormonal effects and their antiarrhythmic actions reside in an ability to inhibit the fast inward sodium current in cardiac cells (class I action).[2,4-6] The present study concerns a series of 17β-aminoestratrienes, which we have synthesized and have found to possess marked antiarrhythmic activity in a number of

Chart I



† Department of Pharmacology.
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(1) (a) Hewett, C. L.; Savage, D. S. *J. Chem. Soc. C* **1968**, 1134. (b) Marshall, R. J.; Winslow, E. *Gen. Pharmacol.* **1981**, *12*, 315.

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