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.

Acknowledgment. We thank Dr. M. L. Maddox, J. Nelson, and L. Kurz of Syntex Analytical Research for obtaining and interpreting spectroscopic data.

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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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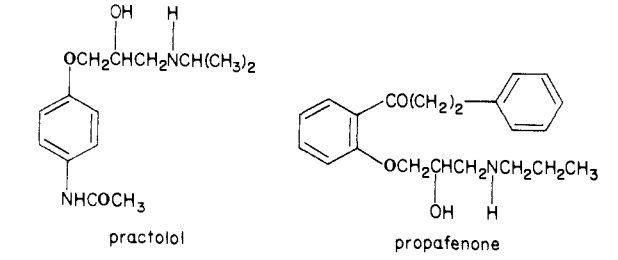
The antiarrhythmic efficacy of 17β-amino- and 17β-amino-16α-hydroxyestratrien-3-ols and 3-acetates (group 1) was compared with the efficacy of corresponding 3-[2-hydroxy-3-(isopropylamino)propyl] and 3-[2-hydroxy-3-(*tert*-butylamino)propyl] ethers (group II), substituents which are usually associated 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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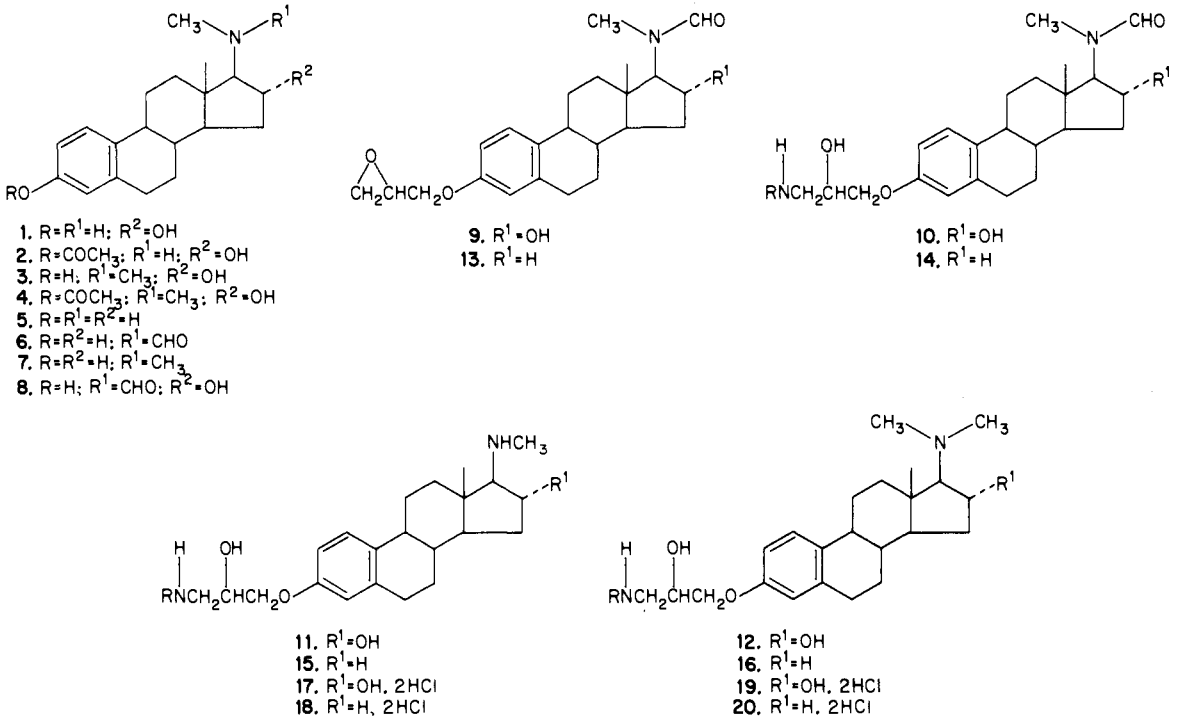
Chart II[a]



[a] For compounds 10–12 and 14–20: **a**, R = $CH(CH_3)_2$; **b**, R = $C(CH_3)_3$.

experimental models. Again, these compounds can be classified as class I agents. Since drugs that block β-adrenoceptors also exert marked antiarrhythmic actions both in animals and man (class II action),[7] it was felt worthwhile to investigate whether incorporation of a chemical moiety (2-hydroxy-3-(isopropylamino)propoxy), which in conjunction with an aromatic ring is normally associated with β-adrenoceptor blockade, would further enhance the antiarrhythmic activity of this series. The known β-adrenoceptor blocking agent practolol and also propafenone, which combines both class I and II activity,[8] were also investigated for comparative purposes. The structures of these two compounds are shown in Chart I.

Chemistry. The syntheses of 17β-(methylamino)- and 17β-(dimethylamino)estratriene-3,16-diols (**1** and **3**) and their 3-acetates (**2** and **4**) (Chart II) have been described by us recently,[9] while the 17β-methylamino compound **5** was described some time ago by Japanese workers,[10] who claimed antibiotic activity. The dimethylamino compound **7** was readily obtained from the methylamine **5** by lithium aluminum hydride reduction of the intermediate *N*-formyl derivative **6**.

Table I. Activity of Test Compounds against Aconitine-Induced Arrhythmias in Mice and Concentrations Required To Reduce MFF of Isolated Guinea Pig Atria by 25%[a]

compd	salt	mouse aconitine ED_{50}, mg/kg	guinea pig atria EC_{25}, μg/mL
3-ols and acetates			
1	maleate	5.9 (5.7–6.2)	1.67 (1.18–2.15)
2	maleate	9.0 (6.5–12.7)	5.06 (2.90–6.91)
3	maleate	11.5 (5.3–20.1)	1.86 (1.30–2.42)
4	maleate	6.5 (3.0–12.8)	0.99 (0.71–1.28)
5	maleate	8.2 (3.3–13.1)	2.09 (1.80–2.41)
7	HCl	4.4 (2.3–8.9)	1.65 (1.85–2.89)
propafenone		24.3 (13.1–30.3)	1.85 (1.46–2.27)
3-ethers			
17a	2HCl	inactive	21.0 (16.9–25.4)
19a	2HCl	inactive	13.7 (10.3–16.4)
17b	2HCl	inactive	38.6 (34.1–43.0)
19b	2HCl	inactive	17.1 (15.3–18.9)
18a	2HCl	inactive	11.9 (10.2–13.7)
20a	2HCl	inactive	16.1 (14.2–18.5)
18b	2HCl	inactive	25.8 (19.6–31.6)
20b	2HCl	inactive	24.1 (20.9–27.3)

[a] 95% confidence limits given in parentheses.

The method of building up the 3-amino-2-hydroxypropyl ether group on a phenol has been well established.[11] 17β-(Methylamino)estratriene-3,16α-diol **1** was considered to be a convenient starting material and was converted to the epoxypropyl ether **9**, after protection of the amino function as the *N*-formyl derivative **8**. The epoxide was then opened with isopropylamine and *tert*-butylamine respectively to give the 3-(3-amino-2-hydroxypropyl) ethers **10a** and **10b** as a mixture of diastereoisomers, which were hydrolyzed to the 17-(methylamino)-16-hydroxy compounds **11a** and **11b** or reduced with lithium aluminum hydride to the 17-(dimethylamino)-16-hydroxy compounds **12a** and **12b**. The corresponding 16-deshydroxy-17-amino compounds **15a**, **15b**, **16a**, and **16b** were similarly prepared from amine **5** via intermediates **13** and **14**. All amino

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Table II. Effects of the 3-ol Estratrienes 1 and 3 on Transmembrane Action Potentials of Guinea Pig Atria[a]

compd	concn, μg/mL	n	RMP, mV	AP, mV	APD_{50} ms	APD_{90}, ms	MRD, V/s
controls		40	71.3 ± 0.8	86.7 ± 1.1	51.5 ± 0.5	83.5 ± 1.3	125.1 ± 5.2
1	1.0	40	72.8 ± 0.8	85.8 ± 1.3	49.2 ± 1.1	83.6 ± 1.6	98.4 ± 4.3**
	2.0	40	75.8 ± 0.8**	86.3 ± 1.1	45.5 ± 0.9**	80.2 ± 1.8	86.5 ± 5.4**
	4.0	40	72.1 ± 0.8	81.5 ± 1.0*	44.5 ± 1.0**	80.6 ± 1.9	62.4 ± 2.5**
controls		40	69.4 ± 0.9	86.6 ± 0.9	40.3 ± 2.0	81.4 ± 2.6	146.4 ± 6.2
3	2.0	30	70.1 ± 1.1	78.9 ± 1.7**	29.6 ± 1.3**	70.9 ± 1.5*	86.0 ± 4.8**
	4.0	40	71.8 ± 0.9	73.5 ± 1.6**	29.0 ± 0.8**	74.2 ± 1.0	60.1 ± 3.8**
	8.0	39	69.3 ± 0.7	69.9 ± 1.1**	43.5 ± 1.0	98.9 ± 2.9**	30.5 ± 1.7**
controls		49	69.3 ± 0.8	84.7 ± 1.4	36.9 ± 1.1	79.8 ± 1.4	145.3 ± 6.9
19a	2	50	71.0 ± 0.8	83.8 ± 2.0	26.0 ± 1.1**	69.8 ± 1.3**	139.5 ± 5.9
	4	50	70.4 ± 0.8	81.6 ± 1.0	20.7 ± 0.9**	64.8 ± 1.8**	124.0 ± 5.7
	8	45	69.1 ± 0.5	79.5 ± 1.1*	19.5 ± 1.1**	64.9 ± 2.6**	139.3 ± 7.6

[a] (*) $p < 0.01$ and (**) $p < 0.001$ denote significant differences from the control values (according to Student's t test). Each result is the mean ± SEM of results obtained from n cells using five preparations.

compounds were converted to a water-soluble salt, viz. hydrochloride (or dihydrochloride) or maleate, for pharmacological testing.

It is possible that partial separation of diastereoisomers may have occurred during isolation and/or crystallization of the free bases or their salts. However, examination of the NMR spectra of these compounds was not helpful in resolving this problem, since the peak due to the proton attached to the new chiral center was very broad and was not always separated from other peaks in the spectrum.

Biological Results and Discussion

Activity of 17β-Amino- and 17β-Amino-16α-hydroxyestratrien-3-ols or 3-Acetates. All the compounds tested were highly active against aconitine-induced arrhythmias in mice and were more active than propafenone (Table I). In addition, all the compounds showed comparable activity to that of propafenone in reducing the maximum following frequency (MFF) of isolated guinea pig atrium (Table I). There were no marked differences in potency between any of the steroids in either of these tests.

The mouse aconitine test is relatively specific for class I antiarrhythmic activity.[12] Compounds 1 and 3 were therefore chosen for electrophysiological study and the results (Table II) of these tests revealed that both compounds induce a dose-dependent reduction in the maximum rate of phase 0 depolarization (MRD) of the cardiac cellular transmembrane action potential. This change is accompanied by a decrease in action potential height (AP) in the absence of a reduction in resting membrane potential (RMP) and indicates a decrease in the intensity of the fast inward sodium current. Thus, these compounds appear to behave like classical class I antiarrhythmic agents.[13] Effects on the action potential duration (APD) were variable; 1 produced a dose-dependent reduction in the time taken to reach 50% repolarization (APD_{50}) but did not significantly change the time taken to reach 90% repolarisation (APD_{90}). The lower concentrations of 3 (2 and 4 μg/mL) also shortened APD_{50} whereas the higher concentration was without effect. APD_{90} was shortened by the lowest concentration of 3 used but lengthened by the high concentration. However, these effects on APD are unlikely to contribute to antiarrhythmic efficacy.

We found no evidence of β-adrenoceptor blockade by the 3-ols or 3-acetates. This is supported by the observation that neither 2 nor 3, in doses that antagonized arrhythmias evoked by coronary artery ligation in the rat (Table III), reduced isoprenaline-induced tachycardia in these animals (Table IV). In addition, whereas the β-adrenoceptor blocking agents practolol and propafenone (which also has class I activity) caused parallel rightward

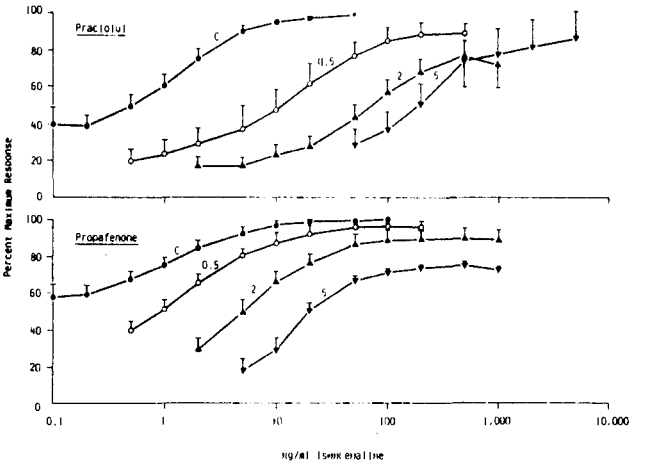

Figure 1. Log dose/response lines to isoprenaline, for positive inotropism in guinea pig atria, in the absence and in the presence of the β-adrenoceptor blocking agents practolol and propafenone. The concentrations (micrograms/milliliter) of antagonists used are shown above the curves. Each point is the mean ± SEM obtained from six preparations. C denotes control.

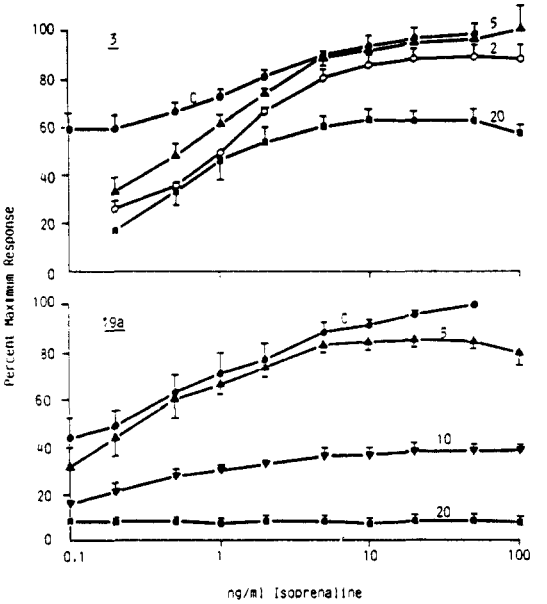

Figure 2. Log dose/response lines to isoprenaline, for positive inotropism in guinea pig atria, in the absence and in the presence of **19a** and **3**. The concentrations (micrograms/milliliter) of test drugs used are shown above the curves. Each point is the mean ± SEM of results obtained from six preparations.

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Table III. Antagonism of the Development of Ventricular Fibrilloflutter (VF) Evoked by Coronary Artery Ligation[a]

	3-ols or 3-acetates					3-substituted ethers			
treatment	mg/kg	*n*	% VF	approx. ED_{50}, mg/kg	treatment	mg/kg	*n*	% VF	approx. ED_{50}, mg/kg
controls		32	81		controls		33	82	
1	0.02	6	83		**19a**	1.0	8	13***	<1.0
	0.05	6	33*	0.04	**17b**	0.05	6	67	
	0.1	6	17**			1.0	8	25**	0.7
2	0.2	7	29*	<0.2		2.0	6	0***	
3	0.2	9	33*		**19b**	0.5	9	44	
	0.5	8	13***	0.15		1.0	8	38*	0.5
	2.0	3	0*			2.0	7	0***	
propafenone	0.2	10	50		**18a**	2.0	7	0***	<2.0
	0.5	8	50	0.7	**20a**	1.0	7	29*	
	1.0	8	25**			2.0	7	14**	<1.0
					18b	2.0	7	0***	<2.0
					20b	0.05	8	38*	
						0.1	8	25**	0.05
						1.0	8	0***	
					practolol	2	5	80	
						5	7	43	5.6
						10	7	29*	

[a] (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ using the χ^2 test denote significant differences from the appropriate control group. *n* is the number of animals used in each group.

Table IV. Effects of Test Compounds on Heart Rate and on Isoprenaline-Induced Tachycardia in the Anesthetised Rat[a,d]

	heart rate (beats/min)				
treatment (dose, mg/kg)	pretreatment	posttreatment (i.e. preisoprenaline)	% change[b]	heart rate (beats/min), 1 min post-isoprenaline	max % increase[c]
controls	439 ± 3	413 ± 18	−5.9	460 ± 14	11.4*
2 (0.2)	403 ± 12	359 ± 14	−10.9*	392 ± 9	9.2*
3 (0.5)	442 ± 18	434 ± 14	−1.8	490 ± 8	12.9**
propafenone (1.0)	443 ± 16	391 ± 16	−11.7*	445 ± 14	13.8*
19a (2.0)	394 ± 8	366 ± 15	−7.1	366 ± 17	0
17b (2.0)	415 ± 20	294 ± 27	−29.2**	302 ± 24	3.0
19b (2.0)	393 ± 26	325 ± 16	−17.3*	340 ± 14	4.6
18a (2.0)	407 ± 24	315 ± 19	−22.6*	318 ± 16	1.0
20a (1.0)	405 ± 27	387 ± 20	−4.4	393 ± 14	1.6
18b (2.0)	387 ± 21	335 ± 17	−13.4*	335 ± 17	0
20b (1.0)	390 ± 13	363 ± 18	−6.9	376 ± 13	3.4
practolol (5.0)	459 ± 31	351 ± 32	−23.5*	377 ± 33	7.4

[a] The results are the mean ± SEM of 7–12 observations. [b] Percent change reflects the mean change in heart rate recorded 14 min after administration of test drugs. [c] Percent increase is the mean increase in heart rate evoked by isoprenaline. [d] (*) $p < 0.05$ and (**) $p < 0.01$ denote significant differences between pretreatment and posttreatment values and between pre-isoprenaline and post-isoprenaline values.

shifts of log dose/response curves to isoprenaline for positive inotropism in isolated atria (Figure 1), **3** (2–5 μg/mL) was without effect (Figure 2). These concentrations of **3** reduced MFF and induced marked class I electrophysiological actions in atrial tissue. Only a relatively high concentration of **3** (20 μg/mL) caused a moderate depression of the maximum inotropic response to isoprenaline, an action shared by propafenone, and probably attributable to membrane stabilization. Thus, unlike practolol or propafenone, the estratrien-3-ols and acetates are unlikely to exert β-adrenoceptor blocking actions.

Of interest was the observation that, in antiarrhythmic doses in the rat, propafenone also failed to modify isoprenaline-induced tachycardia, suggesting that its main action in this model does not involve β-receptors.

Effects of 17β-Amino- and 17β-Amino-16α-hydroxy-3-[2-hydroxy-3-(isopropylamino)propoxy]-estratrienes or the Corresponding 3-*tert*-Butylamino Compounds. Like practolol, none of these compounds was active in the mouse aconitine test at doses of up to 50 mg/kg (Table I). Practolol also failed to reduce MFF of isolated guinea pig atria at concentrations of up to 300 μg/mL whereas all the steroidal compounds showed some activity in this test (Table I). However, potency, compared to the 3-ols, was reduced by a factor of between 5 and 10. This is clearly seen when the 3-ols **1**, **3**, **5**, and **7** are compared with their 3-[2-hydroxy-3-(isopropylamino)propoxy] analogues **17a**, **19a**, **18a**, and **20a**. Replacement of the 3-(isopropylamino)propyl group with a 3-(*tert*-butylamino)propyl group, to give **17b**, **19b**, **18b**, and **20b**, if anything, further reduced ability to modify MFF.

The isopropylamino compound **19a** was selected for electrophysiological study (Table II). Unlike its 3-hydroxy analogue **3**, **19a**, in similar concentrations, failed to reduce MRD, indicating a lack of class I activity. Thus, addition of the 3-amino-substituted ether would appear to markedly reduce class I activity. Since practolol is not only inactive in the aconitine test but is also inactive in reducing MFF, the observed weak activity of these compounds in the latter test may reflect some residual membrane stabilization at high concentrations. It is also worthy of note however that some β-adrenoceptor blocking agents, e.g. propranolol but not practolol, show local anesthetic properties at high concentrations.

As in the case of **1** at all concentrations and **3** at the lower concentrations, **19a** shortened both APD_{50} and APD_{90}. In this context it is of interest that 17β-estradiol also shortens APD of atrial tissue.[14] Despite the lack of activity of the 3-ether compounds in the above tests, all of these compounds were active in reducing the severity of ligation-induced arrhythmias in the rat (Table III). The

(14) De Beer, E. L.; Keizer, H. A. *Steroids* **1982**, *40*, 223.

results obtained are insufficient to draw any conclusions regarding structure/activity relationships. However, comparison of the approximate ED_{50} values of the 17-dimethylamino compounds **3** (the 3,16-diol), **19a** (the 16-hydroxy-3-[2-hydroxy-3-(isopropylamino)propyl] ether), and **19b** (the corresponding 3-(*tert*-butylamino)propyl ether) does not reveal any marked potency differences. Interestingly, **20b** (the 16-desoxy-3-[2-hydroxy-3-(*tert*-butylamino)propyl] ether) was approximately 10 times more potent than the corresponding 3-(isopropylamino)-propyl ether **20a** and displayed a similar degree of potency to the 3,16-diol 1. All the compounds tested were at least as potent as propafenone and more potent than practolol. Since a number of other β-adrenoceptor blocking agents are known to be active in this test,[15] it is tempting to suggest β-adrenoceptor blockade as the mechanism of action of the substituted ethers. Support for this comes from the observations that all these compounds, unlike the 3-ols or 3-acetates, reduced or abolished (by 60–100%) isoprenaline-induced tachycardia in the rat (Table IV), while the β-adrenoceptor blocking agent practolol reduced the response by 35%. Both practolol and propafenone induced a moderate but sustained bradycardia. However, it is unlikely that a similar action of the compounds under study contributed to inhibition of isoprenaline-induced tachycardia. Reduced heart rate was most pronounced in response to **17b** and **18a** while the 3-ol **3** and the 3-substituted ethers **19a**, **20a**, and **20b** were without marked or sustained negative chronotropic actions. Thus, bradycardia does not appear to depend on the nature of the 3-substituent. In view of these results, the 3-[2-hydroxy-3-(isopropylamino)propyl] ether **19a** was selected for comparison with practolol and propafenone for study of potential β-adrenoceptor blocking activity in isolated driven guinea pig atria. While both practolol and propafenone induced a parallel rightward shift of the log dose/response lines to isoprenaline (Figure 1), indicating competitive antagonism, **19a** failed to produce a parallel shift and markedly depressed the maximum response to isoprenaline (Figure 2). The 3-[2-hydroxy-3-(*tert*-butylamino)propyl] ether **17b** induced similar effects (not shown). Such marked depression is unlikely to be due to membrane stabilization since it was seen in response to a relatively low concentration of **19a** (10 μg/mL). A concentration of 8 μg/mL of this agent failed to exert any class I activity and 14 μg/mL was required to reduce MFF by 25%. The most likely explanation of these results is that **19a** noncompetitively inhibits isoprenaline. The effect of **19a** on the shape of the dose/response curves could be explained by one of several mechanisms. Compounds **19a** and **19b** may be irreversibly bound to β-receptors. In the absence of spare receptors, irreversible binding would lead to depression of the maximum response and depression of the slope of the log dose/response line. However, the absence of spare receptors in cardiac tissue seems unlikely. Noncompetitive inhibition of isoprenaline may also result from binding of the antagonist at a point on the receptor but remote from the active site (allosteric inhibition) or may be due to inhibition of one of the intermediate steps in the chain of events leading from β-adrenoceptor activation to cardiac contraction. Our results cannot distinguish between these possibilities but suggest a functional or noncompetitive β-adrenoceptor blocking action.

Conclusions

17β-Amino- or 17β-amino-16α-hydroxyestratrienes, bearing a 3-ol or a 3-acetate, exert pronounced antiarrhythmic activity, probably mediated by inhibition of the fast inward sodium current in cardiac cells. Substitution at the 3-position with 2-hydroxy-3-(isopropylamino)propyl or 2-hydroxy-3-(*tert*-butylamino)propyl groups markedly reduces this class I activity while conferring noncompetitive β-adrenoceptor blocking activity. However, such substitution does not offer any obvious advantage over the parent compounds.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus. IR spectra were recorded with a Perkin-Elmer 457 spectrometer. UV spectra were recorded for solutions in EtOH with a Perkin-Elmer 402 spectrometer. Optical rotations were measured for 1% solutions in $CHCl_3$ unless otherwise stated. ^1H NMR spectra were recorded at 60 MHz with a Perkin-Elmer R12B spectrometer or at 100 MHz with a Varian Associates XL-100A-12FT spectrometer. Solutions of products were dried over anhydrous Na_2SO_4.

General Method for Conversion of Free Base to Dihydrochloride Salt. A solution of the free base in MeOH (15–20 vol) was treated with a solution of HCl gas (10%) in MeOH. After 5 min the solution was evaporated to dryness and the resulting salt was recrystallized from suitable solvents (vide infra).

17β-(*N*-Formyl-*N*-methylamino)estra-1,3,5(10)-triene-3,16α-diol (8). To a stirred suspension of 17β-(methylamino)-estra-1,3,5(10)-triene-3,16α-diol (1; 13.5 g, 0.045 mol) in EtOH (67.5 mL) and ethyl formate (135 mL) was added sodium metal (2.25 g, 0.097 mol) in small pieces. The reaction mixture became warm and then cooled slowly to room temperature. After 1 h, further sodium metal (2.25 g) was added. Two hours later, MeOH (100 mL) was added to dissolve most of the solid material, and the mixture was brought to pH 6 by the addition of 5 M HCl and then poured into stirred water (3 L). The product was isolated by filtration and recrystallized from CH_2Cl_2/MeOH to give the formamide 8 (12.1 g, 82%): mp 272–276 °C; $[\alpha]_D$ ±0° (pyridine); IR (KCl) ν 3270, 3200 (OH), 1650 cm^{-1} (NCHO); NMR (C_5D_5N) δ 0.71 (3 H, s, 13-Me), 3.06 (3 H, s, NMe), 4.75–5.2 (1 H, m, 16β-H), 5.5–6.6 (1 H, br m, OH), 7.0, 7.12, 7.15, 7.22, 7.38 (3 H, aromatic protons), 8.62 (1 H, s, CHO), 10.5–11.4 (1 H, br m, OH). Anal. ($C_{20}H_{27}NO_3$) C, H, N.

17β-(*N*-Formyl-*N*-methylamino)estra-1,3,5(10)-trien-3-ol (6). To a stirred suspension of the 17β-methylamino compound **5** (25 g, 0.088 mol) in formamide (250 mL) was added formic acid (125 mL). The mixture was heated to boiling, by which time the steroid had dissolved. After 1 h, the mixture, which contained precipitated product, was cooled to room temperature and poured into stirred water (1875 mL). The gelatinous solid was filtered off, washed with water, and dried under vacuum to give the formamide **6** (25.3 g), which was used without further purification: IR (KCl) ν 3150 (OH), 1642 cm^{-1} (NCHO); NMR (C_5D_5N) δ 0.59 (3 H, s, 13-Me), 2.90 (3 H, s, NMe), 6.98, 7.10, 7.16, 7.21 (3 H, aromatic protons), 8.44 (1 H, s, CHO).

3-[(2,3-Epoxypropyl)oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-trien-16α-ol (9). To a stirred solution of the formamide 8 (11.5 g, 0.035 mol) in dimethylformamide (287.5 mL) was added epichlorohydrin (11.5 mL, 0.124 mol), followed by sodium methoxide (2.07 g, 0.038 mol). Further portions of sodium methoxide (2.07, 1.04, and 2.07 g) were added after 1, 2, and 24 h, respectively. After 26 h further epichlorohydrin (5.75 mL) was added, and after 30 h, the mixture was poured into stirred water (1500 mL).

The precipitated solid was filtered to give the crude product (11.9 g), which was crystallized from CH_2Cl_2/acetone to give epoxypropyl derivative **9** (10.9 g, 81%): mp 185–198 °C; $[\alpha]_D$ +2.7° (pyridine); IR (KCl) ν 3360 (OH), 1655 cm^{-1} (NCHO); NMR (C_5D_5N) δ 0.69 and 0.73 (3 H, 2 s, 13-Me), 2.60–2.90 (2 H, m, sharp, epoxide CH_2), 2.94 and 3.03 (3 H, 2 s, NMe), 3.20–3.65 (1 H, m, epoxide CH), 3.65–4.55 (2 H, m, $-CH_2O-$), 4.70 (1 H, exchangeable, OH), 4.90 (1 H, br m, 16β-H), 6.55–7.40 (3 H, m, 4-H, 2-H, and 1-H), 8.52 and 8.57 (1 H, 2 s, NCHO). Anal. ($C_{23}H_{31}NO_4$) C, H, N.

3-[(2,3-Epoxypropyl)oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-triene (13). By a similar procedure to

(15) Parratt, J. R.; Campbell, C.; Fagbemi, O. In "Catecholamines and the Heart"; Delius, W., Ed.: Springer: Berlin, 1981; p 269.

that described for **9**, formamide **6** (10 g, 0.031 mol) was converted to the epoxypropyl derivative **13** (11.5 g, 97%): mp 113–117 °C; $[\alpha]_D$ +19.3°; IR (CH_2Cl_2) ν 1660 cm^{-1} (NCHO); NMR ($CDCl_3$) δ 0.77 (3 H, s, 13-Me), 2.93 (3 H, s, NMe), 2.6–2.9 (m), 3.2–3.6 (m), 3.7–4.4 (m) (5 H, 3-propyloxy protons), 6.68, 6.80, 6.85, 7.15, 7.30 (3 H, aromatic protons), 8.26 (1 H, s, CHO). Anal. ($C_{23}H_{31}NO_3$) C, H, N.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-trien-16α-ol (10a). A solution of epoxypropyl derivative **9** (12 g, 0.031 mol) in isopropylamine (120 mL) was heated to reflux for 30 h. The solution was evaporated under reduced pressure to give the crude isopropylamino derivative **10a** as a gum (14.2 g), which was carried to the next stage without purification.

A sample that crystallized from MeOH/ether had the following: mp 91–95 °C; $[\alpha]_D$ +3.8°; IR (CH_2Cl_2) ν 3600 and 3380 (OH, NH), 1662 cm^{-1} (NCHO); NMR ($CDCl_3$) δ 0.76 (3 H, s, 13-Me), 1.07 (6 H, d, J = 6 Hz, Me_2CH), 2.97 and 3.04 (3 H, 2 s, NMe), 3.95 (2 H, s, $-CH_2O-$), 4.02 (1 H, br m, $-CH_2CH(OH)CH_2O-$), 4.70 (1 H, m, br 16β-H), 6.55–7.40 (3 H, m, 4-H, 2-H, and 1-H), 8.18 and 8.25 (1 H, 2 s, NCHO). Anal. ($C_{26}H_{40}N_2O_4$) C, H, N.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-triene (14a). By a similar procedure to that described for **10a**, the epoxypropyl derivative **13** (11.4 g, 0.035 mol) was converted to the isopropylamino compound **14a** (6 g, 48%): mp 123–130 °C; $[\alpha]_D$ +15.9°; IR (CH_2Cl_2) ν 3580, 3300 (OH, NH), 1660 cm^{-1} (NCHO); NMR ($CDCl_3$) δ 0.74 (3 H, s, 13-Me), 1.07 (6 H, d, J = 6 Hz, Me_2CH), 2.80 (2 H, m, CH_2N), 2.91 (3 H, s, NMe), 3.1–3.7 (1 H, m, $-CH_2CH(OH)CH_2O-$), 3.94 (2 H, br s, $-CH_2O-$), 6.57–7.37 (3 H, m, 4-H, 2-H, and 1-H), 8.23 (1 H, s, CHO). Anal. ($C_{26}H_{40}N_2O_3$) C, H, N.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(methylamino)estra-1,3,5(10)-trien-16α-ol (11a). To a stirred solution of the crude isopropylamino derivative **10a** (6.0 g, 0.014 mol) in MeOH (60 mL) under nitrogen was added 10 M KOH solution (30 mL), and the mixture was heated to reflux for 2 h, then cooled, and poured into stirred water (300 mL).

The precipitated solid was filtered, washed with water, and dried, to give crude 17β-methylamino compound **11a** (4.9 g), which was carried to the next stage without purification.

The crude product which could not be crystallized had the following: mp 72–79 °C; IR (KCl) ν 3295 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.77 (3 H, s, 13-Me), 1.10 (6 H, d, J = 6 Hz, Me_2CH), 2.58 (3 H, s, NMe), 3.98 (2 H, s, $-CH_2O-$), 4.00 (2 H, br m, 16β-H and $-CH_2CH(OH)CH_2O-$), 6.69 (1 H, s, 4-H), 6.76 and 7.24 (2 H, AB q, J = 9 Hz, 2-H and 1-H).

The dihydrochloride **17a**, crystallized from MeOH/2-propanol, had the following: mp 265–277 °C dec; $[\alpha]_D$ +38.0° (MeOH); IR (KCl) ν 3340 (OH), 2760 cm^{-1} (NH_2^+); NMR (Me_2SO-d_6) δ 0.85 (3 H, s, 13-Me), 1.28 and 1.30 (6 H, 2 d, J = 6 Hz, Me_2CH), 2.68 (3 H, s, NMe), 3.95 (2 H, d, J = 5 Hz, $-CH_2O-$), 4.21 and 4.37 (2 H, 2 br m, 16β-H and $-CH_2CH(OH)CH_2O-$), 5.40 (1 H, d, J = 5 Hz, exchangeable, OH), 5.85 (1 H, br m, exchangeable, OH), 6.68 (1 H, s, 4-H), 6.74 and 7.21 (2 H, AB q, J = 9 Hz, 2-H and 1-H), 9.02 (4 H, br m, 2-NH_2^+). Anal. ($C_{25}H_{42}N_2O_3Cl_2 \cdot H_2O$) C, H, N, Cl.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(methylamino)estra-1,3,5(10)-triene (15a). By the method described for 11a, the *N*-formyl-*N*-methylamino derivative **14a** was converted into the *N*-methylamino derivative **15a**: mp 80–96 °C; $[\alpha]_D$ +64.3°; IR (CH_2Cl_2) ν 3570, 3320 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.72 (3 H, s, 13-Me), 1.06 (6 H, d, J = 6 Hz, Me_2CH), 2.45 (3 H, s, NMe), 2.80 (3 H, m, $CH(OH)CH_2N$), 3.94 (2 H, br s, $-CH_2O-$), 6.64 (1 H, s, 4-H), 6.73 and 7.20 (2 H, AB q, J = 8 Hz, 2-H and 1-H). Anal. ($C_{25}H_{40}N_2O_2$) C, H, N.

The dihydrochloride **18a**, crystallized from MeOH/ether, had the following: mp 272–288 °C dec; $[\alpha]_D$ +63.1° (Me_2SO); IR (KCl) ν 3320 (NH_2^+), 3040–2380 cm^{-1} (series of peaks - HCl salt); NMR (Me_2SO-d_6) δ 0.85 (3 H, s, 13-Me), 1.27 and 1.29 (6 H, 2 d, J = 6 Hz, Me_2CH), 3.35 (3 H, s, NMe), 3.94 (2 H, d, J = 6 Hz, $-CH_2O-$), 4.20 (1 H, br m, $CH_2CH(OH)CH_2$), 5.87 (1 H, m, exchangeable, OH), 6.68 (1 H, s, 4-H), 6.72 and 7.21 (2 H, AB q, J = 6 Hz, 2-H and 1-H), 8.90 (4 H, br m, 2-NH_2^+). Anal. ($C_{25}H_{42}N_2O_2Cl_2$) C, H, N, Cl.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(dimethylamino)estra-1,3,5(10)-trien-16α-ol (12a). To stirred THF (76 mL) under nitrogen and cooled in an ice bath was added lithium aluminum hydride (2.85 g, 0.075 mol) in portions. A solution of crude *N*-formyl-*N*-methylamino compound **10a** (5.7 g, 0.013 mol) in THF (30 mL) was added slowly and washed in with THF (8 mL). The mixture was heated to reflux for 1 h, then cooled in an ice bath, and treated carefully with a mixture of CH_2Cl_2 (57 mL) and EtOAc (28.5 mL), followed by water (5.7 mL).

Cooling was stopped, and the mixture was stirred for a short time and then filtered. The precipitate was washed with boiling 1:1 CH_2Cl_2/THF and then the filtrate was evaporated to a gum (5.0 g). The product was crystallized from CH_2Cl_2/ether (twice) and then CH_2Cl_2/hexane (twice) to give the 17β-dimethylamino compound **12a** (1.9 g), which was still impure but was carried to the next stage.

This product had the following: mp 150–156 °C; IR (KCl) ν 3340 and 3130 cm^{-1} (OH, NH); NMR (C_5D_5N) δ 0.89 (3 H, s, 13-Me), 1.04 (6 H, d, J = 6 Hz, Me_2CH), 2.44 (6 H, s, NMe_2), 4.27 (2 H, s, $-CH_2O-$), 4.35 [2 H, br m, 16β-H and $-CH_2CH(OH)CH_2-$], 6.72–7.38 (3 H, m, 4-H, 2-H, and 1-H).

The dihydrochloride **19a**, purified by crystallization from EtOH/EtOAc, had the following: mp 220–229 °C; $[\alpha]_D$ +27.3° (MeOH); IR (KCl) ν 3330 (OH), 2695 cm^{-1} (NH_2^+ and NH^+); NMR (Me_2SO-d_6) δ 0.97 (3 H, s, 13-Me), 1.28 and 1.29 (6 H, 2 d, J = 6 Hz, Me_2CH), 2.88 (6 H, s, NMe_2), 3.95 (2 H, d, J = 5 Hz, $-CH_2O-$), 4.22 and 4.41 [2 H, 2 br m, 16β-H and $-CH_2CH(OH)CH_2-$], 5.51 (1 H, d, J = 5 Hz, exchangeable, OH), 5.87 (1 H, d, J = 5 Hz, exchangeable, OH), 6.69 (1 H, s, 4-H), 6.74 and 7.20 (2 H, AB q, J = 9 Hz, 2-H and 1-H), 8.72, 9.09, and 10.59 (3 H, 3 br m, NH_2^+ and NH^+). Anal. ($C_{26}H_{44}N_2O_3Cl_2 \cdot 1.5H_2O$) C, H, N, Cl.

3-[[2-Hydroxy-3-(isopropylamino)propyl]oxy]-17β-(dimethylamino)estra-1,3,5(10)-triene (16a). By the method described for **12a** the *N*-formyl-*N*-methylamino derivative **14a** was converted to the 17-dimethylamino compound **16a**, which was noncrystalline: IR (CH_2Cl_2) ν 3600 cm^{-1} (OH); NMR ($CDCl_3$) δ 0.83 (3 H, s, 13-Me), 1.02 and 1.12 (6 H, 2 s, Me_2CH), 2.24 (6 H, s, NMe_2), 3.96 (2 H, br s, $-CH_2O-$), 6.67–7.31 (3 H, m, 4-H, 2-H and 1-H).

The dihydrochloride **20a**, recrystallized from EtOH/ether, had the following: mp 215–227 °C dec; $[\alpha]_D$ +44.9° (Me_2SO); IR (KCl) ν 3300 (OH), 3040–2420 cm^{-1} (series of peaks - HCl salt); NMR (Me_2SO-d_6) δ 0.96 (3 H, s, 13-Me), 1.27 and 1.28 (6 H, 2 d, J = 8 Hz, Me_2CH), 3.35 (6 H, s, NMe_2), 3.94 (2 H, d, J = 6 Hz, $-CH_2O-$), 4.20 [1 H, br m, $CH_2CH(OH)CH_2O$], 5.86 (1 H, d, J = 5 Hz, exchangeable, OH), 6.68–7.24 (3 H, m, 4-H, 2-H, and 1-H), 8.50–9.30 and 10.20 (3 H, br m, NH_2^+ and NH^+). Anal. ($C_{26}H_{44}N_2O_2Cl_2 \cdot 0.75H_2O$) C, H, N, Cl.

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-trien-16α-ol (10b). A suspension of epoxypropyl derivative **9** (11.2 g, 0.029 mol) in *tert*-butylamine (112 mL) was heated to reflux with stirring. The steroid dissolved completely only after the addition of further *tert*-butylamine (56 mL after 1 day and another 56 mL after 2 days). After 6 days, the solution was evaporated under reduced pressure to leave crude *tert*-butylamino compound **10b** as a solid (14.0 g). This product could not be crystallized and therefore was carried to the next stage without purification.

The product had the following: mp 81–109 °C; IR (CH_2Cl_2) ν 3665, 3600, 3490 (OH, NH), 1660 cm^{-1} (NCHO); NMR ($CDCl_3$) δ 0.73 (3 H, s, 13-Me), 1.08 (9 H, s, Me_3C), 2.93 and 3.02 (3 H, 2 s, NMe), 3.88 (2 H, s, $-CH_2O-$), 3.93 [1 H, br m, $-CH_2CH(OH)CH_2O-$], 4.63 (1 H, br m, 16β-H), 6.53–7.37 (3 H, br m, 4-H, 2-H, and 1-H), 8.13 and 8.18 (1 H, 2 s, NCHO).

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(*N*-formyl-*N*-methylamino)estra-1,3,5(10)-triene (14b). In a similar manner to that described for **10b**, the epoxypropyl derivative **13** was converted to the *tert*-butylamino compound **14b**: mp 142–149 °C; $[\alpha]_D$ +22°; IR (CH_2Cl_2) ν 3580, 3420, 3320 (OH, NH), 1660 cm^{-1} (NCHO); NMR ($CDCl_3$) δ 0.75 (3 H, s, 13-Me), 1.09 (9 H, s, Me_3C), 2.24 (1 H, exchangeable), 2.91 (3 H, s, NMe), 3.93 (2 H, s, $-CH_2O-$), 6.66–7.29 (3 H, m, 4-H, 2-H, and 1-H), 8.24 (1 H, s, NCHO). Anal. ($C_{27}H_{42}N_2O_3$) C, H, N.

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(methylamino)estra-1,3,5(10)-trien-16α-ol (11b). By the method

described for **11a**, the *N*-formyl-*N*-methylamino derivative **10b** was converted to the methylamino compound **11b**, which could not be purified by crystallization.

The product had the following: mp 108–125 °C; IR (KBr) ν 3295 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.73 (3 H, s, 13-Me), 1.10 (9 H, s, Me_3C), 2.01 (1 H, exchangeable), 2.45 (3 H, s, NMe), 3.93 (2 H, s, $-CH_2O-$), 3.95 [2 H, br m, 16β-H and $-CH_2CH(OH)CH_2O-$], 6.56–7.32 (3 H, br m, 4-H, 2-H, and 1-H).

The dihydrochloride **17b**, crystallized from EtOH, had the following: mp 272–300 °C dec; $[\alpha]_D$ +42.0° (MeOH); IR (KCl) ν 3350 and 3185 (OH), 2750 cm^{-1} (NH_2^+); NMR (Me_2SO-d_6) δ 0.83 (3 H, s, 13-Me), 1.34 (9 H, s, Me_3C), 2.69 (3 H, s, NMe), 3.97 (2 H, d, J = 5 Hz, $-CH_2O-$), 4.20 and 4.36 [2 H, 2 br m, 16β-H and $-CH_2CH(OH)CH_2O-$], 5.38 (1 H, d, J = 5 Hz, exchangeable, OH), 5.86 (1 H, br m, exchangeable, OH), 6.68 (1 H, s, 4-H), 6.74 and 7.20 (2 H, AB q, J = 8 Hz, 2-H and 1-H), 9.00 (4 H, br m, 2-NH_2^+). Anal. ($C_{26}H_{44}N_2O_3Cl_2 \cdot 0.25H_2O$) C, H, N, Cl.

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(methylamino)estra-1,3,5(10)-triene (15b). By the method described for **11a**, the *N*-formyl-*N*-methylamino derivative **14b** was converted to the 17β-methylamino compound **15b**, which was noncrystalline: IR (CH_2Cl_2) ν 3320, 3150 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.73 (3 H, s, 13-Me), 1.11 (9 H, s, Me_3C), 2.45 (1 H, exchangeable), 2.56 (3 H, s, NMe), 3.94 [4 H, br s, $-CH_2O-$; 16β-H; $-CH_2CH(OH)CH_2O-$], 6.66–7.28 (3 H, m, 4-H, 2-H, and 1-H).

The dihydrochloride **18b**, crystallized from MeOH/2-propanol, had the following: mp 298–305 °C dec; $[\alpha]_D$ +57.6° (MeOH); IR (KCl) ν 3330 (OH, NH), 2970–2400 cm^{-1} (series of peaks - HCl salt); NMR ($CDCl_3$) δ 1.02 (3 H, s, 13-Me), 1.52 (9 H, s, Me_3C), 2.78 (3 H, s, NMe), 3.99 (2 H, m, $-CH_2O-$), 4.60 [1 H, m, $-CH_2CH(OH)CH_2O-$], 6.59–7.10 (3 H, m, 4-H, 2-H, and 1-H). Anal. ($C_{26}H_{44}N_2O_2Cl_2$) C, H, N, Cl.

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(dimethylamino)estra-1,3,5(10)-trien-16α-ol (12b). By the method described for **12a**, the *N*-formyl-*N*-methylamino derivative **10b** was converted to the crude 17β-dimethylamino compound **12b**: mp 120–150 °C; IR (CH_2Cl_2) ν 3670, 3600, 3360 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.83 (3 H, s, 13-Me), 1.08 (9 H, s, Me_3C), 2.34 (6 H, s, NMe_2), 3.73–4.33 [2 H, br m, 16β-H and $-CH_2CH(OH)CH_2O-$], 3.92 (2 H, s, $-CH_2O-$), 6.62 (1 H, s, 4-H), 6.71 and 7.16 (2 H, AB q, J = 8 Hz, 2-H and 1-H).

The dihydrochloride **19b**, crystallized from EtOH, had the following: mp 242–258 °C dec; $[\alpha]_D$ +37.4° (MeOH); IR (KCl) ν 3300 (OH), 2710 cm^{-1} (NH_2^+ and NH^+); NMR (Me_2SO-d_6) δ 0.96 (3 H, s, 13-Me), 1.34 (9 H, s, Me_3C), 2.88 (6 H, s, NMe_2), 3.97 (2 H, d, J = 5 Hz, $-CH_2O-$), 4.20 and 4.39 [2 H, 2 m, 16β-H and $-CH_2CH(OH)CH_2O-$], 5.48 (1 H, d, J = 5 Hz, exchangeable, OH), 5.86 (1 H, d, J = 5 Hz, exchangeable, OH), 6.70 (1 H, s, 4-H), 6.75 and 7.20 (2 H, AB q, J = 8 Hz, 2-H and 1-H), 8.58, 9.06, and 10.45 (3 H, 3 br m, exchangeable, NH_2^+ and NH^+). Anal. ($C_{27}H_{46}N_2O_3Cl_2 \cdot 0.5H_2O$) C, H, N, Cl.

3-[[2-Hydroxy-3-(*tert*-butylamino)propyl]oxy]-17β-(dimethylamino)estra-1,3,5(10)-triene (16b). By the method described for **12a**, the *N*-formyl-*N*-methylamino derivative **14b** was converted to the noncrystalline 17β-dimethylamino compound **16b**: IR (CH_2Cl_2) ν 3590, 3350 cm^{-1} (OH, NH); NMR ($CDCl_3$) δ 0.83 (3 H, s, 13-Me), 1.10 (9 H, s, Me_3C), 2.24 (6 H, s, NMe_2), 3.93 (2 H, s, $-CH_2O-$), 6.65–7.28 (3 H, m, 4-H, 2-H, and 1-H).

The dihydrochloride **20b**, crystallized from MeOH/2-propanol, had the following: mp 270–280 °C dec; $[\alpha]_D$ +39.2° (MeOH); IR (KCl) ν 3350 (OH), 2970–2400 cm^{-1} (series of peaks - HCl salt); NMR (Me_2SO-d_6) δ 0.97 (3 H, s, 13-Me), 1.36 (9 H, s, Me_3C), 2.79 (6 H, s, NMe_2), 4.00 (2 H, m, $-CH_2O-$), 4.22 [1 H, m, $-CH_2CH(OH)CH_2O-$], 5.89 (1 H, d, J = 5 Hz, exchangeable, OH), 6.66 (1 H, s, 4-H), 6.71 and 7.16 (2 H, AB q, J = 8 Hz, 2-H and 1-H), 8.80, 9.40, and 10.54 (3 H, m, exchangeable, NH_2^+ and NH^+). Anal. ($C_{27}H_{46}N_2O_2Cl_2 \cdot 1.5H_2O$) C, H, N, Cl.

Biological Procedures. Aconitine-Induced Arrhythmias in Mice. Compounds (given intraperitoneally) were assessed for ability to delay the development of arrhythmias induced by intravenous administration of aconitine to anesthetised mice.[12] At least three different doses of test compounds were administered to separate groups of animals (n = 6–10) and ED_{50} values, defined as the dose of test compound required to raise the arrhythmogenic dose of aconitine by 50% above that required in controls, were calculated.

Isolated Guinea Pig Left Atria. Compounds were tested for ability to reduce the maximum frequency at which isolated guinea pig left atrial preparations would follow a driving stimulus (MFF). Left atria were set up in an organ bath containing Krebs solution, at 35 °C, gassed with carbogen. Isometric contractions elicited by electrical stimulation were recorded and test drugs were added cumulatively to the bath. MFF determinations were made 6 min after addition of each test concentration. An EC_{25} value for each compound was calculated from results obtained from six preparations.

In a separate series of experiments, cumulative log dose/response lines to isoprenaline for its positive inotropic effect in preparations driven at 2 Hz were obtained before and 30 min after the addition of selected test compounds. Each preparation received only one test drug concentration and each concentration was tested on six preparations. Control data were pooled.

Acute Coronary Artery Ligation in Rats. The effects of test compounds on arrhythmias evoked by acute coronary artery ligation in rats were determined.[16] Carotid arterial pressure and a standard lead II ECG were recorded continuously. A left thoracotomy was performed in anesthetised, artificially ventilated male Wistar rats and a fine silk suture was placed under the main left coronary artery. The animal was allowed to stabilize. Test drugs or vehicle were given intravenously and after 14 min isoprenaline (10 μg/kg) was administered. One minute later the ligature was tightened and the ensuing incidence of VF was noted. Surviving animals were terminated after 30 min. In these animals heart rate (obtained from the ECG) was determined immediately prior to drug administration and 14 min after administration (i.e. immediately before isoprenaline administration) to assess any direct effects of the drug on cardiac rate. Heart rates before and after treatment were compared by using the Student's t test. One minute after isoprenaline administration, the rate was again determined to assess any effects of drug treatment on the expected positive chronotropic response to isoprenaline. Responses to isoprenaline in drug-treated animals were compared with the response in control animals by using the Student's t test. Each group contained 7–12 animals.

Electrophysiological Studies. Electrophysiological studies were performed on guinea pig left atria pinned to the base of a recording chamber, superfused with Krebs solution, gassed with carbogen, and maintained at a temperature of 35 ± 0.5 °C. The tissue was electrically stimulated, and transmembrane action potentials were recorded by using conventional microelectrode techniques. Selected drugs were added to the superfusate and action potentials were recorded before and 30–40 min after commencement of drug perfusion.

Registry No. 1, 80158-12-9; **2**, 80177-82-8; **3**, 80177-55-5; **4**, 98902-96-6; **5**, 99031-77-3; **6**, 98902-97-7; **7**, 98902-98-8; **8**, 80177-54-4; (3*R*)-**9**, 98902-99-9; (3*S*)-**9**, 98903-21-0; (3*R*)-**10a**, 98903-00-5; (3*S*)-**10a**, 98903-22-1; (3*R*)-**10b**, 98903-01-6; (3*S*)-**10b**, 98903-23-2; (3*R*)-**11a**, 98903-02-7; (3*S*)-**11a**, 98903-24-3; (3*R*)-**11b**, 98903-25-4; (3*S*)-**11b**, 98903-03-8; (3*R*)-**12a**, 98903-04-9; (3*S*)-**12a**, 98903-26-5; (3*R*)-**12b**, 98903-27-6; (3*S*)-**12b**, 98903-05-0; (3*R*)-**13**, 98903-06-1; (3*S*)-**13**, 98903-28-7; (3*R*)-**14a**, 98903-29-8; (3*S*)-**14a**, 98903-07-2; (3*R*)-**14b**, 98903-30-1; (3*S*)-**14b**, 98903-08-3; (3*R*)-**15a**, 98903-09-4; (3*S*)-**15a**, 98903-31-2; (3*R*)-**15b**, 98903-32-3; (3*S*)-**15b**, 98903-10-7; (3*R*)-**16a**, 98903-33-4; (3*S*)-**16a**, 98903-11-8; (3*R*)-**16b**, 98903-34-5; (3*S*)-**16b**, 98903-12-9; (3*R*)-**17a**, 98903-13-0; (3*S*)-**17a**, 98903-35-6; (3*R*)-**17b**, 98903-14-1; (3*S*)-**17b**, 98903-36-7; (3*R*)-**18a**, 98903-37-8; (3*S*)-**18a**, 98903-15-2; (3*R*)-**18b**, 98903-38-9; (3*S*)-**18b**, 98903-16-3; (3*R*)-**19a**, 98903-39-0; (3*S*)-**19a**, 98903-17-4; (3*R*)-**19b**, 98903-40-3; (3*S*)-**19b**, 98903-18-5; (3*R*)-**20a**, 98903-41-4; (3*S*)-**20a**, 98903-19-6; (3*R*)-**20b**, 98903-42-5; (3*S*)-**20b**, 98903-20-9; $(CH_3)_2CHNH_2$, 75-64-9; $(CH_3)_3CNH_2$, 75-31-0; (±)-epichlorohydrin, 13403-37-7.

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