Cell growth at each dose level was expressed as a percentage of growth (protein) in control wells and the dose resulting in 50% inhibition of growth was determined.

Antileishmanial Evaluation. Human macrophage cultures were derived from the monocytes of the peripheral blood of normal human volunteers by methods previously described.[31] After being infected with amastigotes of *Leishmania tropica* WR 401 (NIH 173), infected macrophage cultures in 0.1 mL of culture medium were exposed to a constant dose of pyrazolo[3,4-*d*]pyrimidine nucleoside for 6 days. The culture medium used was RPMI-1640 (GIBCO Laboratories, Grand Island, NY) containing 10% heat-activated fetal calf serum (GIBCO Laboratories), penicillin (50 U/mL), and streptomycin (50 μg/mL). After 6 days the number of amastigotes per 100 macrophages in control (non-drug-treated) cultures and experimental cultures was determined by counting 100–200 Giemsa-stained macrophages in each culture. The number of macrophages per culture was estimated by counting 20 representative fields for each culture. In initial experiments, drug doses of 0.01–1.0 μM were employed. Generally, the drug dosage was increased in subsequent experiments until macrophage toxicity (see below) or a dose of at least 70 μM was achieved.

Enumeration of Data. The number of *Leishmania* amastigotes per 100 macrophages surviving in drug-treated cultures was expressed as a percentage of the number in simultaneously cultivated controls. The concentration of drug calculated to eliminate 50% of amastigotes compared to controls (the 50% effective dose [ED_{50}]) was determined by nonlinear regression analysis[32] of the results of each experiment. For drugs for which the dose–response curve was so flat that statistical analysis could not be performed, the ED_{50} was estimated by inspection of the data.

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Registry No. 1, 16220-07-8; **3a**, 54738-73-7; **3b**, 5387-84-8; **4**, 14215-97-5; **5a**, 90914-30-0; **5b**, 90914-33-3; **5c**, 90914-37-7; **6a**, 90914-31-1; **6b**, 90914-34-4; **6c**, 90914-32-2; **7**, 72760-85-1; **8**, 90914-35-5; **9**, 90914-36-6; **10a**, 90914-38-8; **10b**, 90914-39-9; **10c**, 90914-40-2; 11, 90914-41-3; **12**, 90414-39-4; **13a**, 90914-42-4; **13b**, 90914-44-6; **14**, 83255-86-1; **15**, 90914-43-5; **16**, 90914-45-7; **17**, 90914-52-6; **18a**, 90914-46-8; **18b**, 90914-47-9; **18c**, 90914-48-0; **18d**, 90914-49-1; **19**, 90914-51-5; **20**, 90914-50-4; formamide, 75-12-7; methylamine, 74-89-5; diethylamine, 124-40-3.

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Synthesis and Antihypertensive Activity of 6,7-Disubstituted *trans*-4-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols

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A series of novel 6,7-disubstituted *trans*-3,4-dihydro-2,2-dimethyl-4-pyrrolidino-(or piperidino)-2*H*-1-benzopyran-3-ols was prepared and tested for antihypertensive activity in the conscious spontaneously hypertensive rat (SHR) and compared with certain of their monosubstituted analogues. The potent blood pressure lowering activity of the 6-monosubstituted compounds was enhanced by incorporation of an acetylamino or amino group at C(7) and that of the 7-nitro-substituted compound by incorporation of an amino (but not an acetylamino group) at C(6). The combination of 6-nitro or 6-cyano with 7-(acetylamino) or 7-amino groups and 6-amino with 7-nitro groups in *trans*-4-pyrrolidino- or -4-piperidino-2,2-dimethyl-2*H*-1-benzopyranols conferred superior antihypertensive activity to hydralazine and to the calcium antagonist, nifedipine, in SHR. The synthetic route to these compounds involves the conversion of 2*H*-1-benzopyrans to bromohydrins that were treated with pyrrolidine or piperidine. Preparation of the 6-cyano-7-amino analogue was accomplished when 6-cyano-7-[(trifluoroacetyl)amino]-2,2-dimethylbenzopyran was used as starting material.

In a series of monosubstituted *trans*-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols,[1] two structural features were found to maximize antihypertensive activity. The first of these was a strong electron-withdrawing aromatic substituent, such as nitro or cyano located at either C(6) or C(7), and the second that the 4-amino group was incorporated in a pyrrolidine or piperidine ring. An extension to that work was the synthesis of *trans*-3,4-dihydro-2,2-dimethyl-4-(1-pyrrolidinyl)-2*H*-1-benzopyran-3-ols and *trans*-3,4-dihydro-2,2-dimethyl-4-(1-piperidinyl)-2*H*-1-benzopyran-3-ols containing substituents located at both C(6) and C(7). Several disubstituted compounds were thus prepared and evaluated in the spontaneously hypertensive rat (SHR) as this model was subsequently found to be more sensitive to the monosubstituted aminobenzopyranols (**1–6**, see Table I) than the deoxycorticosterone acetate (DOCA)/saline treated hypertensive rat used in the initial study.[1] Hydralazine and nifedipine were included as standard antihypertensive agents since in the previous study[1] the monosubstituted aminobenzopyranols were shown to have a vasodilator action.

Chemistry. Convenient starting materials for the synthesis of the 6,7-disubstituted compounds described in Table II are the 2,2-dimethyl-2*H*-1-benzopyrans that stem

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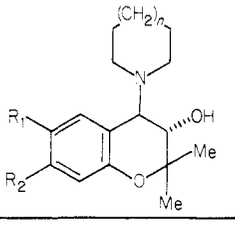
0022-2623/84/1827-1127$01.50/0

Table I. 6- or 7-Monosubstituted-3,4-dihydro-2,2-dimethyl-*trans*-4-(1-piperidinyl)- or -4-(1-pyrrolidinyl)-2*H*-benzopyran-3-ol Salts



no.	R_1	R_2	n	dose,[a] mg/kg	max fall in BP, mmHg[b] (mean ± SEM) DOCA[c]	SHR	no. of rats (SH)
1[d]	NO_2	H	0	1.0	52 ± 7	52 ± 8	6
				0.3		32 ± 4	6
2[e]	NC	H	0	1.0	43 ± 7	61 ± 5	5
				0.3	15 ± 4	41 ± 4	6
3[d]	H_2NCO	H	1	10.0	9 ± 4	109 ± 8	6
				3.0		76 ± 4	6
				1.0		24 ± 3	6
4[e]	H_2N	H	0	100.0	6 ± 8		
				10.0		10 ± 5[f]	6
5[d]	MeCONH	H	1	100.0	4 ± 6	42 ± 7	6
				10.0	6 ± 8	19 ± 4	6
6[d]	H	NO_2	0	3.0	90 ± 11		
				1.0	44 ± 9	83 ± 12	6
				0.3		17 ± 4	6
		hydralazine[d]		10.0	113 ± 10	89 ± 6	12
				3.0	65 ± 12	51 ± 3	14
				1.0	32 ± 8	42 ± 3	12
		nifedipine		3.0	51 ± 2	62 ± 2	6
				1.0	33 ± 2	30 ± 8	6
				0.3	20 ± 6	13 ± 6	5

[a] Compounds were given orally to rats, and doses are expressed as base. [b] Systolic blood pressure was measured indirectly at intervals from 1 to 6 h. [c] Results from ref 1. [d] HCl salt. [e] $MeSO_3H$ salt. [f] Rise in blood pressure recorded for compound 4 in SHR.

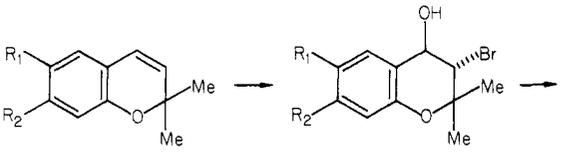
from the 6-acetylamino-7-nitrobenzopyran reported previously.[1] The appropriate benzopyrans were subjected to the synthetic route shown in Scheme I, using previously described conditions, although the epoxides were not usually isolated, and the final products (Table II) were generally purified as hydrochloride or methanesulfonate salts.

The route is exemplified by the synthesis of the 6-(acetylamino)-7-nitro-3,4-epoxy compound **27** from the 6-(acetylamino)-7-nitrobenzopyran via the bromohydrin **23** and its subsequent reaction with pyrrolidine and piperidine to furnish compounds **7** and **8**, respectively, which gave the 6-amino-7-nitro compounds **9** and **10**, respectively, on hydrolysis.

4-Aminobenzopyran-3-ols **11–13** were prepared from 2,2-dimethyl-7-nitrobenzopyran[1] via reduction and acetylation to the 7-(acetylamino)benzopyran **17** followed by nitration to give the 7-(acetylamino)-6-nitrobenzopyran **18**. This was subjected to the sequence depicted in Scheme I via bromohydrin **26** to give compound **11** and was followed by acid hydrolysis to give compound **13**. Compound **12** was prepared in a similar fashion.

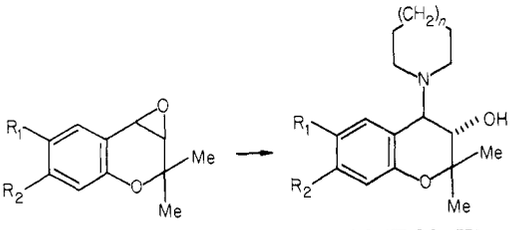
The preparation of the 4-pyrrolidinylbenzopyran-3-ol **16** follows the sequence of compounds **19** → **20** → **22** → **25** → **16**. The formation of the 6-cyano-7-nitrobenzopyran **19** could not be accomplished directly by cyanide treatment of the diazonium salt of 6-amino-2,2-dimethyl-7-nitro-2*H*-1-benzopyran.[1] However this diazonium salt could be converted to the 6-iodo-7-nitrobenzopyran and treated immediately with cuprous cyanide to provide the required compound **19**. Use of the 7-(acetylamino)-6-cyanobenzopyran **21** in the route shown in Scheme I resulted in the 7-(acetylamino)-6-cyano product **14**. This was purified by column chromatography as the free base and pharmacologically evaluated as such, since on treatment with 5 N HCl followed by basification, compound **14** was converted to the tricyclic quinazolinone **28** (Scheme I; an example of a facile route to quinazolinones[2] discovered in

Scheme I



	R_1	R_2
17	H	MeCONH
18	NO_2	MeCONH
19	NC	NO_2
20	NC	NH_2
21	NC	MeCONH
22	NC	CF_3CONH

	R_1	R_2
23	MeCONH	NO_2
24	NC	MeCONH
25	NC	CF_3CONH
26	NO_2	MeCONH



	R_1	R_2
27	MeCONH	NO_2

7-16 (Table II)

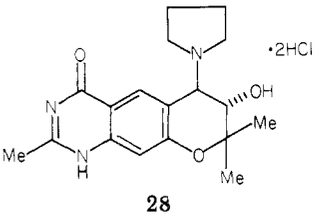


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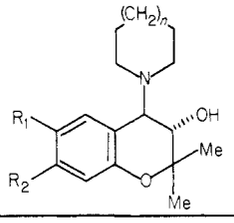
Table II. 6,7-Disubstituted-3,4-dihydro-2,2-dimethyl-*trans*-4-(1-piperidinyl)-2*H*-1-benzopyran-3-ol and 6,7-Disubstituted-3,4-dihydro-2,2-dimethyl-*trans*-4-(1-pyrrolidinyl)-2*H*-1-benzopyran-3-ol Salts



no.	R_1	R_2	n	yield, %	mp, °C	recryst solvent[a]	formula	anal.[b]	dose, mg/kg po	max fall in BP in mmHg,[c] (mean ± SEM)
7	MeCONH	NO_2	0	25	138–143	E_1	$C_{17}H_{23}N_3O_5 \cdot HCl$	C, H, Cl; N[d]	10.0	99 ± 3
									1.0	25 ± 4
8	MeCONH	NO_2	1	24	200–204	E_2	$C_{18}H_{25}N_3O_5 \cdot MeSO_3H$	C, H, N, S	10.0	89 ± 13
									1.0	44 ± 6
9	NH_2	NO_2	0	27	241–243	E_2	$C_{15}H_{21}N_3O_4 \cdot HCl$	C, H, N, Cl	1.0	71 ± 3
									0.3	83 ± 5
									0.1	22 ± 8
10	NH_2	NO_2	1	73	243–244	E_1–E_2	$C_{16}H_{23}N_3O_4 \cdot MeSO_3H$	C, H, N, S	1.0	67 ± 2
									0.3	42 ± 10
11	NO_2	MeCONH	1	5	177–181	E_1–E_2	$C_{18}H_{25}N_3O_5 \cdot MeSO_3H$	C, H, N	0.3	108 ± 6
									0.1	78 ± 6
12	NO_2	NH_2	0	13	193–195	E_1–E_2	$C_{15}H_{21}N_3O_4 \cdot MeSO_3H$	C, H; N[e]	0.1	122 ± 7
									0.03	47 ± 11
13	NO_2	NH_2	1	60	179–182	E_1–E_2	$C_{16}H_{23}N_3O_4 \cdot MeSO_3H$	C, H, N	0.3	111 ± 4
									0.1	110 ± 4
									0.03	71 ± 5
									0.01	14 ± 6
14	NC	MeCONH	0	49	175–176	E_3–P	$C_{18}H_{23}N_3O_3 \cdot 0.3H_2O$	C, H, N	0.3	149 ± 5
									0.1	36 ± 8
15	H_2NCO	MeCONH	1	12	210–213	E_1–E_2	$C_{19}H_{27}N_3O_4 \cdot HCl \cdot H_2O$	C, H, N	1.0	50 ± 23
									0.3	34 ± 6
16	NC	NH_2	0	48	173–174	E_1–E_2	$C_{16}H_{21}N_3O_2 \cdot HCl \cdot H_2O$	H, N, Cl; C[f]	0.3	115 ± 20
									0.1	54 ± 12
									0.03	40 ± 20[g]

[a] E_1 = Et_2O, E_2, = EtOH, E_3 = EtOAc, P = 60–80 °C petroleum ether. [b] Analyses for the elements indicated were within ±0.4% of the theoretical values. [c] Systolic blood pressure was measured indirectly at intervals from 1 to 6 h in groups of six SH rats per compound. [d] N: calcd, 10.89; found, 10.03. [e] N: calcd, 10.42; found, 9.46. [f] C: calcd, 56.27; found, 56.94. [g] Three rats used.

our laboratories). When the 7-(acetylamino)-6-cyano-4-(1-piperidinyl)benzopyranol, obtained from the bromohydrin **24** by the action of piperidine, was treated with dilute acid, hydrolysis of the cyano group occurred, leading to the 6-(aminocarbonyl)-7-(acetylamino) compound **15**. Both quinazolinone formation and cyano group hydrolysis were obviated by the use of a 7-[(trifluoroacetyl)amino] group as in compounds **22** and **25** and thence directly to the 6-cyano-7-amino-4-(1-pyrrolidinyl)benzopyranol **16** by the action of pyrrolidine.

Results and Discussion

Compounds were evaluated for oral antihypertensive activity in the SHR. Systolic blood pressure, recorded indirectly from the tail, was determined before dosing and at various time intervals during the ensuing 6 h. Maximum falls in blood pressure obtained for all compounds (Tables I and II) occurred at 1 or 2 h postdose with some recovery to the predose level of blood pressure being evident at 6 h.

For comparative purposes, the 6- and 7-monosubstituted compounds **1–6** were evaluated in both SHR and the DOCA rat. The compounds possessed antihypertensive potency (see Table I), which was generally greater in the SHR than that observed in the DOCA rat.[1] Also included in Table I are the responses to the two standard reference antihypertensive drugs hydralazine and nifedipine, which had similar antihypertensive potency in both SHR and DOCA rat.

The initial 6-(acetylamino)-7-nitro compounds **7** and **8** (see Table II) were less active than the 7-nitro compound **6**, but hydrolysis of the acetylamino moiety as in compounds **9** and **10** resulted in increased potency both with respect to compounds **7** and **8** and compound **6**. Similarly, introduction of a 7-amino substituent into the 6-nitro compound **1**, as in compounds **12** and **13** resulted in increased potency, and these compounds were more active than the corresponding 6-amino-7-nitro compounds **9** and **10**. The progress of monosubstituted compound **2** in the clinic prompted the synthesis of the 7-amino analogue **16**, which was more potent than compound **2**.

Although the addition of a 6-(acetylamino) substituent diminished the activity of the 7-nitro compound **6**, insertion of such a group at C(7) adjacent to a 6-nitro, -cyano, or -carboxamido group as in compounds **11**, **14**, and **15**, respectively, augmented the antihypertensive activity of the respective monosubstituted compounds **1**, **2**, and **3**.

The contribution of the acetylamino and amino substituents to the activity of the title compounds possessing an electron-withdrawing group at C(6), and in the case of the amino group, at C(7), is not clear, as the two groups do not appear to be particularly active when used indi-

(2) Showell, G. A. *Synth. Commun.* **1980**, *10*, 241.
(3) Protons at C(5) and C(8) were assigned by the small couplings between protons at C(8) and C(4), seen as broadening of the signals and first reported by Arnone et al.: Arnone, A.; Cardillo, G.; Merlini, L.; Mondelli, R. *Tetrahedron Lett.* **1967**, 4201.

vidually in the monosubstituted reference compounds **4** and **5**. Intramolecular hydrogen bonding, present in acetylamino nitro and amino nitro compounds is not likely to be important since the cyano–amino and cyano–acetylamino combinations that cannot undergo hydrogen bonding are equally effective. It is more likely that the electronic effects of the electron-withdrawing groups, particularly nitro and cyano located at C(6), are modified by the inclusion of an adjacent amino group in such a way as to increase the antihypertensive potency.

This work demonstrates that in SHR, the antihypertensive activity of *trans*-4-aminobenzopyran-3-ols, which is associated with the presence of a strong electron-withdrawing group at C(6), can be enhanced by combination of such a substituent with a 7-amino group as in compounds **12**, **13**, and **16**. Compound **16** is 10 times more potent than compound **2** (currently in clinical study) and 30 times more potent than the standard antihypertensives, hydralazine and nifedipine, in the SHR model.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus. Both melting points and boiling points are uncorrected. IR, NMR, and mass spectra, which were in agreement with the structures cited, were recorded on a Perkin-Elmer 197, a Varian EM 360A at 60 MHz, a Varian CFT-20 at 80 MHz, or a JEOL GX 270, and an AEI MS9 instrument at 70 eV, respectively. The petroleum ether used was that having a boiling point of 60–80 °C.

7-(Acetylamino)-2,2-dimethyl-2*H*-1-benzopyran (17). 2,2-Dimethyl-7-nitro-2*H*-1-benzopyran[1] (40.0 g, 0.195 mol) and electrolytic Fe (88 g, 1.58 mol) in glacial HOAc (200 mL) and Ac_2O (120 mL) were stirred and heated at 120 °C for 16 h. The reaction mixture was cooled, diluted with H_2O, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Filtration and evaporation gave a crude gum, which was chromatographed on silica gel, by using EtOAc–petroleum ether mixtures in a gradient elution. Chromatographically homogeneous fractions were combined and recrystallized from petroleum ether to give **17** (5.27 g, 12.5%) as needles: mp 80–81 °C. Anal. ($C_{13}H_{15}NO_2$) C, H, N.

7-(Acetylamino)-2,2-dimethyl-6-nitro-2*H*-1-benzopyran (18). Fuming HNO_3 (1.70 mL, 18 mmol) was added dropwise at 0 °C to compound **17** (5.17 g, 23.8 mmol) dissolved in stirred glacial HOAc (29 mL). The reaction mixture was diluted with H_2O and extracted with EtOAc. The crude material (6.63 g) so obtained was chromatographed on silica gel by using EtOAc–petroleum ether mixtures. The least polar component, compound **18**, was recrystallized from EtOH as yellow needles (1.80 g, 35%): mp 148–150 °C; NMR ($CDCl_3$) δ 1.47 [s, 6 H, $C(Me)_2$], 2.28 (s, 3 H, COMe), 5.72 (d, 10, H-3), 6.33 (d, 10, H-4), 7.93 (s, H-5), 8.25 (s, H-8)[3]. Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

6-Cyano-2,2-dimethyl-7-nitro-2*H*-1-benzopyran (19). Concentrated H_2SO_4 (400 mL) was added to a cooled solution of 6-amino-2,2-dimethyl-7-nitro-2*H*-1-benzopyran[1] (20.0 g, 0.091 mol) in glacial HOAc (800 mL) and the solution was stirred at 8 °C. Nitrosylsulfuric acid [prepared by dissolving $NaNO_2$ (6.26 g, 0.091 mol) in cold concentrated H_2SO_4 (200 mL), the mixture being warmed to dissolve the solid then recooled to about 4 °C] was added to the solution while the temperature was maintained at below 12 °C. The dark viscous reaction mixture was stirred with cooling for another 1 h and was then poured into an ice-cooled solution of KI (15.27 g, 0.092 mol) in H_2O (200 mL). Toluene (800 mL) was added to dissolve the resulting precipitate and the mixture stirred for 20 min in an ice bath and then for 18 h at room temperature. The reaction mixture was diluted with H_2O (500 mL), and the organic phase was separated, washed with H_2O, dried, and evaporated to give 6-iodo-2,2-dimethyl-7-nitro-2*H*-1-benzopyran (11.84 g, 39%) as a red gum: NMR ($CDCl_3$) δ 1.43 [s, 6 H, $C(Me)_2$], 5.79 (d, 10, H-3), 6.25 (d, 10, H-4), 7.28 (s, H-8), 7.51 (s, H-5).[3]

The crude iodo compound (11.74 g, 35 mmol), used directly, was heated under reflux in dry pyridine (450 mL) containing CuCN (3.20 g, 36 mmol) for 8 h. The mixture was evaporated to half volume and added to H_2O (1 L). Extraction via EtOAc followed by chromatography on silica gel with EtOAc–petroleum ether mixtures gave compound **19** (4.00 g, 49%). Recrystallization of a small sample from EtOAc–60–80 °C petroleum ether gave **19** as orange crystals: mp 154–155 °C; IR (Nujol) 2220, 1530, 1330 cm^{-1}; NMR ($CDCl_3$) δ 1.51 [s, 6 H, $C(Me_2)$], 5.89 (d, 10, H-3), 6.34 (d, 10, H-4), 7.40 (s, H-5), 7.62 (s, H-8).[3] Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

7-Amino-6-cyano-2,2-dimethyl-2*H*-1-benzopyran (20). Compound **19** (2.29 g, 9.9 mmol) and electrolytic Fe (1.94 g, 35 mmol) in glacial HOAc (100 mL) were stirred at 100 °C for 1 h. Dilution with H_2O and extraction via EtOAc gave compound **20** (1.98 g, 99%). The analytical sample of mp 137–138 °C was obtained as yellow crystals after two recrystallizations from EtOAc–petroleum ether: NMR ($CDCl_3$) δ 1.39 [s, 6 H, $C(Me)_2$], 4.20–4.52 (br m, 2 H exchangeable, NH_2), 5.48 (d, 10, H-3), 6.10 (s, H-8) overlapping 6.16 (d, 10, H-4), 6.97 (s, H-5),[3] IR (Nujol) 3340, 3240, 2220 cm^{-1}. Anal. ($C_{12}H_{12}N_2O$) H, N; C: calcd, 71.98; found, 71.31.

7-(Acetylamino)-6-cyano-2,2-dimethyl-2*H*-1-benzopyran (21). Compound **20** (1.88 g, 9.4 mmol) was stirred vigorously in Ac_2O (30 mL) and EtOH (80 mL) at room temperature for 8 h. Evaporation and recrystallization from EtOAc–petroleum ether gave compound **21** (2.20 g, 97%) as yellow crystals: mp 136–137 °C; IR ($CHCl_3$) 3410, 2200, 1700 cm^{-1}. Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

6-Cyano-2,2-dimethyl-7-[(trifluoroacetyl)amino]-2*H*-1-benzopyran (22). Compound **20** (3.18 g, 15.9 mmol) in $CHCl_3$ (50 mL) was added to a stirred solution of 4-(dimethylamino)-pyridine (3.91 g, 0.04 mol) and $(CF_3CO)_2O$ (4.51 mL, 31.9 mmol) in $CHCl_3$ (50 mL) at room temperature during 10 min. The red solution was stirred with heating under reflux for a further 2 h, cooled, washed with water, dried, and evaporated to give compound **22** (4.43 g, 94%) as yellow crystals from EtOAc–petroleum ether: mp 87–88 °C; IR (Nujol) 3280, 2210, 1730 cm^{-1}. Anal. ($C_{14}H_{11}N_2O_2F_3$) C, H, N.

***trans*-3-Bromo-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-4-ols (23–26).** Freshly recrystallized NBS (0.205 mol) was added in one portion to a vigorously stirred solution of the benzopyrans (0.1 mol) in Me_2SO (40 mL) and H_2O (0.2 mol). After the exothermic reaction, stirring was continued for an additional 0.5 h, followed by pouring into H_2O and extraction via EtOAc. The EtOAc was washed with H_2O and brine, dried, and evaporated, leaving the crude bromohydrins. Recrystallization gave the following. Compound **23** (95%): mp 198–200 °C (EtOH). Anal. ($C_{13}H_{15}N_2O_5Br$) C, H, N. Compound **24** (56%): mp 191–192 °C (EtOAc–petroleum ether). Anal. ($C_{14}H_{15}N_2O_3Br$) C, H, N. Compound **25** (88%): mp 183–184 °C (EtOAc–petroleum ether). Anal. ($C_{14}H_{12}N_2O_3BrF_3$) C, H. N. Compound **26** (95%): mp 205–206 °C (EtOH). Anal. ($C_{13}H_{15}N_2O_5Br$) H, Br; C, N: calcd C, 43.47; N, 7.80; found C, 43.99; N, 8.35.

6-(Acetylamino)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-7-nitro-2*H*-1-benzopyran (27). Bromohydrin **23** (14.02 g, 0.039 mol) and NaOH pellets (14 g, 0.35 mol) were stirred in dioxane (750 mL) and H_2O (140 mL) at room temperature for 3 h. The solution was evaporated to half volume and diluted with H_2O (1 L). Extraction via EtOAc and recrystallization from EtOH gave **27** as a yellow solid (5.92 g, 55%): mp 156–158 °C; NMR ($CDCl_3$) δ 1.27 [s, 3 H, $C(Me_2)$], 1.60 [s, 3 H, $C(Me_2)$], 2.25 (s, 3 H, COMe), 3.53 (d, 4, H-3), 3.98 (d, 4, H-4), 7.62 (s, 1 aromatic proton), 8.77 (s, 1 aromatic proton). Anal. ($C_{13}H_{14}N_2O_5$) C, H, N.

***trans*-4-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol Salts.** The epoxides (0.10 mol) or the bromohydrins (0.05 mol) and pyrrolidine or piperidine (0.11 mol) were heated under reflux in EtOH (250 mL) for 18–48 h. Cooling and evaporation of solvent gave the crude amino alcohols, which with the exception of compound **14**, were purified by acid–base treatment. The salts were prepared by dissolving the bases in EtOH and dry Et_2O followed by addition of 1 equiv of the acid. The yields and properties of the amino alcohol salts are presented in Table II.

Compound **14** (see Table II) was purified by chromatography on silica gel with EtOAc–petroleum ether mixtures by using a gradient elution technique: NMR ($CDCl_3$) δ 1.22 [s, 3 H, $C(Me_2)$], 1.50 [s, 3 H, $C(Me)_2$], 1.72–2.04 (m, 4 H, NCH_2CH_2), 2.22 (s, 3 H, COMe), 2.57–3.14 (m, 5 H, NCH_2 and includes 1 exchangeable H), 3.58 (d, 10, H-3), 3.95 (d, 10, H-4), 7.43 (br s, 2 H, NH and

1 aromatic proton), 7.79 (s, 1 aromatic proton); mass spectrum, m/z 329.18 ($M^+ - 0.3H_2O$, 0.8), 257 (retro-Diels–Alder ion, 100); exact mass calcd for $C_{18}H_{23}N_3O_3$, 329.17.

The crude amino alcohol obtained from bromohydrin **24** by the action of piperidine as above was taken up in an EtOAc–H_2O–HCl mixture. Separation of the layers and basification of the aqueous layer with Na_2CO_3 solution followed by extraction with EtOAc and preparation of the hydrochloride salt as above furnished compound **15** (see Table II).

Compound **16** (see Table II) was obtained directly from the bromohydrin **25** (1.55 g, 3.9 mmol) by the action of refluxing pyrrolidine (15 mL, 0.18 mol) during 30 min. Purification was achieved by acid–base extraction and salt formation as above: NMR (Me_2SO-d_6) δ 1.05 [s, 3 H, $C(Me)_2$], 1.42 [s, 3 H, $C(Me)_2$], 1.83–1.96 (m, 4 H, NCH_2CH_2), 2.93–2.99 (m, 2 H, NCH_2), 3.36–3.49 (m, 2 H, NCH_2) 3.97 (d, 10, H-3), 4.50 (d, 10, H-4), 6.21 (s, H-8), 8.26 (s, H-5).

Hydrolysis of 6-(Acetylamino) or 7-(Acetylamino) Amino Alcohols. The appropriate amino alcohol as the free base (1.2 mmol), 5 N HCl (4 mL), and EtOH (10 mL) were heated under reflux on a water bath for 3 h. Dilution with H_2O (200 mL) and basification with 10% aqueous NaOH and extraction via EtOAc furnished crude 6-amino or 7-amino compounds, which were purified as described in the previous experiment as their salts (**9**, **10**, **12**, **13**; see Table II).

***trans*-1,6,7,8-Tetrahydro-7-hydroxy-2,8,8-trimethyl-6-(1-pyrrolidinyl)-4*H*-pyrano[3,2-*g*]quinazolin-4-one Dihydrochloride (28).** The 7-(acetylamino)-6-cyano compound **14** (0.44 g, 1.3 mmol) was stirred vigorously with 5 N HCl (40 mL) in EtOAc (40 mL) for 10 min at room temperature. The mixture was basified with 10% aqueous NaOH, and the layers were separated. The EtOAc was washed with H_2O and brine and dried over anhydrous $MgSO_4$. Filtration and evaporation gave a light brown foam (0.43 g), which was dissolved in EtOH and treated with Et_2O–HCl. The resulting precipitate was collected and recrystallized from aqueous EtOH to give the quinazolinone **28** (0.25 g, 47%): mp 258–260 °C; IR (KBr) 1715, 1660 cm^{-1}. Anal. ($C_{18}H_{23}N_3O_3 \cdot 2HCl$) C, H, N.

Pharmacological Testing. Hypertensive Rats. The results (see Table I) obtained in DOCA/saline hypertensive rats are quoted from ref 1, and the method for inducing this type of experimental hypertension is described therein.

In the present study (see Tables I and II) all of the test compounds and the standard drugs were evaluated for antihypertensive activity in conscious spontaneously hypertensive rats (14–24 weeks old), derived from the Japanese (Okamoto) strain. Animals with systolic blood pressure ≥ 180 mmHg (1 mmHg ≈ 133 Pa) were considered to be hypertensive.

Systolic blood pressure was recorded by the tail-cuff method using a W + W B.P. recorder, Model No. 8002. For all measurements of blood pressure, the rats were held in restraining cages in a heated environment (33.5 ± 0.5 °C), and each determination was the mean of at least six recordings. Blood pressure measurements were made prior to the oral administration of test compound and at intervals for up to 6 h postdose.

All compounds were administered (via on oral dosing needle placed in the esophagus) as a solution or suspension in 1% w/v methylcellulose solution. Doses are expressed as free base.

With use of the above procedure, vehicle alone typically has little or no effect on blood pressure apart from a slight reduction (by 5–10%) at 6 h postdose.

Registry No. 1, 86824-10-4; **2**, 86824-32-0; **3**, 65018-84-0; **4**, 86824-61-5; **5**, 86824-54-6; **6**, 86824-27-3; **7**, 78939-11-4; **7** (free base), 90867-39-3; **8**, 78939-08-9; **8** (free base), 78939-07-8; **9**, 78939-16-9; **9** (free base), 90867-40-6; **10**, 78939-13-6; **10** (free base), 78939-12-5; **11**, 79014-15-6; **11** (free base), 79014-14-5; **12**, 79014-20-3; **12** (free base), 79014-19-0; **13**, 79014-17-8; **13** (free base), 79014-16-7; **14**, 79014-28-1; **15**, 79014-30-5; **15** (free base), 79014-29-2; **16**, 79014-34-9; **16** (free base), 79014-33-8; **17**, 79014-11-2; **18**, 79014-12-3; **19**, 79014-24-7; **20**, 79014-25-8; **21**, 79014-26-9; **22**, 79014-31-6; **23**, 78939-05-6; **24**, 79014-27-0; **25**, 79014-32-7; **26**, 79014-13-4; **27**, 78939-06-7; **28**, 79714-18-4; 2,2-dimethyl-7-nitro-2*H*-1-benzopyran, 64169-76-2; 6-amino-2,2-dimethyl-7-nitro-2*H*-1-benzopyran, 64169-75-1; 6-iodo-2,2-dimethyl-7-nitro-2*H*-1-benzopyran, 79014-23-6.

Steroids. 2. Synthesis of C-18 Functionalized Steroids via the Smith–Hughes Route

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The total synthesis of a series of racemic C-18 functionalized steroids was carried out in a search for novel estrogen- and/or progestin-receptor agonists or antagonists. The target compound 3,18-dihydroxyestra-1,3,5(10)-triene (**2**), 13-(2-oxopropyl)gona-4-en-3-one (**3**), 13-(1-hydroxy-1-prop-2-ynyl)gona-4-en-3-one (**4a** and **4b**) and 13-(1-acetoxy-2-oxo-1-propyl)gona-4-en-3-one (**5**) are position isomers of the highly biologically active estradiol, progesterone, norethindrone, and 17-acetoxyprogesterone, respectively. Nevertheless the synthetic C-18 functionalized steroids **3**–**5** showed little activity in the Clauberg and anti-Clauberg assays. Compound **2** showed no antagonism in the postcoital assay despite the fact that it exhibited weak but measurable in vitro receptor-binding activity.

Although both naturally occurring and synthetic steroids functionalized at C-18 are known,[1] their number is rather small in comparison to those in other steroid classes. One of the main reasons for this is the somewhat greater synthetic difficulty of introducing substituents at this position as opposed to other locations in the molecule. As a consequence, the effect that changes in substitution at C-18 have on biological activity has not received extensive study.

We became interested in this particular class because of our search for steroid agonists and/or antagonists of both estradiol and progesterone. In particular the development of antagonists in the latter class would represent a new and perhaps more suitable means of regulating mammalian fertility at the level of estrus control.

The basic concept that led to the steroid syntheses described in this paper was the idea that if the C-17 functionality of a steroid were moved to C-18, then there was

(1) Besides the Apocynaceae alkaloids and aldosterone, examples of naturally occurring C-18 functionalized steroids are the holothurian sapogenins (Singh, H.; Pereira, V., Jr.; Parashar, V. V. *Indian J. Pharmacol.* **1965**, *27*, 150. Heftmann, E. *Lloydia* **1967**, *30*, 209), batrachotoxinin A, (Tokuyama, T.; Daly, J.; Witkop, B.) and 18-hydroxyesterone (Loke, K. H.; Marrian, G. F.; Johnson, W. S.; Meyer, W. L.; Camerru, D. D. *Biochim. Biophys. Acta* **1958**, *28*, 214. Karle, J. L.; Karle, J. *J. Am. Chem. Soc.* **1968**, *90*, 1917). For a series of synthetic 18-functionalized progesterone derivatives, see: Auel, R. A. M.; Freerksen, R. W.; Walt, D. S. *Steroids* **1978**, *31*, 2232.

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