Preliminary investigations indicated that the crystal system of **5a** was orthorhombic. Lattice parameter and intensity data were collected on an Enraf-Nonius CAD-4 automatic X-ray diffractometer fitted with a liquid N_2 low-temperature device. The receiving aperture, which had a variable width of $(4.0 + 0.86\theta)$ mm and a constant height of 5 mm, was located 173 mm from the crystal. The intensities of three reflections, remeasured after every 200 reflections, showed no significant variation during the time of data collection.

Positions of the non-hydrogen atoms were refined by a full-matrix least-squares routine using anisotropic thermal parameters for the non-hydrogen atoms.[22] Scattering factors were obtained from *International Tables for X-ray Crystallography*.[23] An analysis of the variance, after refinement of the data, revealed no systematic variance of $\sum\omega||F_o| - |F_c||^2$ with either sin θ or F.[24]

(22) Sheldrick, G. M. *Shelx-76. Program for Crystal Structure Determination*; University Chemical Laboratory: Cambridge, England, 1976.
(23) Ibers, J. A.; Hamilton, W. C. *International Tables for X-Ray Crystallography*; Kynoch: Birmingham, 1974; Vol. 4.

Acknowledgment. We gratefully acknowledge partial support of this work by the DHHS, National Institute of Heart, Lung, and Blood, Grant HL-32191. Grateful acknowledgment is also due to the American Heart Association, Oklahoma Affiliate, Tulsa Section, for partial early support of the work via a grant-in-aid (OK-84-G-3). B.J.S. is grateful for partial support via a grant from the Veterans Administration. We express our gratitude to Joy Merritt, Senior Editor, Nomenclature Section of Chemical Abstracts, for aid in naming these compounds.

Registry No. **1a**, 106947-09-5; **1b**, 106947-17-5; **2**, 106947-10-8; **3**, 93126-32-0; **4a**, 106947-11-9; **4b**, 106947-18-6; **5a**, 106947-12-0; **5b**, 106947-19-7; **6**, 106947-14-2; **7**, 106947-15-3; **8**, 106947-16-4; benzylamine, 100-46-9; formaldehyde, 50-00-0; benzaldehyde, 100-52-7; 2-phenethylamine, 64-04-0.

Supplementary Material Available: Atomic coordinates and anisotropic factors for **1a** and **5a** (6 pages). Ordering information is given on any current masthead page.

(24) Reference 23, p 71.

Flavones. 1. Synthesis and Antihypertensive Activity of (3-Phenylflavonoxy)propanolamines without β-Adrenoceptor Antagonism[1]

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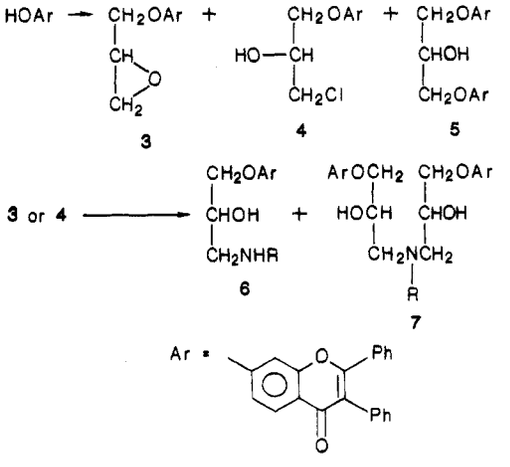
The synthesis of a series of (3-phenylflavonoxy)propanolamines is described. These compounds were evaluated for potential antihypertensive activity in spontaneously hypertensive rats, as well as for in vivo and in vitro evidence of β-adrenoceptor antagonism. Some of the compounds of this series exhibited effective antihypertensive properties but did not antagonize β-adrenergic receptors. These active compounds represent a unique series of effective antihypertensive agents that, despite possessing structural characteristics typical of β-blockers, does not have β-adrenergic receptor blocking activity.

Ahlquist's concept of classifying adrenergic receptors into two distinct groups, α and β,[2] provided the basis for the emergence of a new class of drugs, β-adrenergic blocking agents, which have made a substantial impact on the understanding and the treatment of a variety of disease states. Propranolol, a well-known β-adrenergic blocking drug, was first introduced for the treatment of angina and later was observed to show significant antihypertensive activity in patients.[3,4] The discovery of this antihypertensive activity has led to the use of β-blockers as primary or secondary drugs in the treatment of hypertension.[5-8]

Despite the considerable attractiveness of β-adrenoceptor antagonists for treatment of hypertension, there is good rationale to attempt to extend the utility of β-adrenergic antagonists by incorporating desirable ancillary antihypertensive properties. For instance, prizidilol has been developed as a β-adrenoceptor antagonist having additional vasodilatory properties.[9] Also noteworthy has been the realization of labetalol, a new class of antihypertensive agent having both α- and β-adrenoceptor blocking properties,[10,11] whose antihypertensive efficacy is greater than that of simple β-adrenergic antagonists.[12] Based on these encouraging findings, our research efforts focused on developing a class of agents having both β-adrenoceptor antagonist and vasodilatory properties.

Scheme I



Our rationale for the design of compound **1** and its analogues was based on recordil (**2**), a chromone derivative,

(1) Wu, E. S. C.; Davidson, T. A.; Borrelli, A. R. *Abstracts of Papers*, 182nd National Meeting of the American Chemical Society, New York, NY; American Chemical Society: Washington, DC, 1981; MEDI 9.
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(4) Prichard, B. N. C.; Gillam, P. M. S. *Am. J. Cardiol.* **1966**, *18*, 387.

† Department of Organic Chemistry.
‡ Department of Pharmacology.

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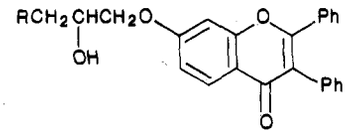
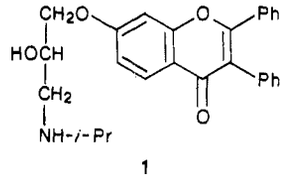
Table I. (3-Phenylflavonoxy)propanolamines



no.	R	formula[a]	recrystn solvent	mp, °C[b]/yield[c]	dose, mg/kg, po	pretreatment SBP ± SE (mmHg)	% fall in SBP[d] 2 h	5 h	7.5 h	24 h	β-receptor affinity: K_i,[e] M	% inhibn depressor response[f]
1	NH-*i*-C_3H_7	$C_{27}H_{27}NO_4$·HCl·H_2O[h]	MeOH	160–161/80	8	198 ± 5.8	*	10	8	*	$>5 \times 10^{-5}$	0[g]
					17	195 ± 4.9	*	14	20	11		
8	NH-*n*-C_3H_7	$C_{27}H_{27}NO_4$	*i*-PrOH	140–142/62	8	191 ± 2.8	*	12	16	10	$>5 \times 10^{-5}$	0[k]
					17	198 ± 3.4	*	11	20	14		
9	NH-c-C_3H_5	$C_{27}H_{25}NO_4$	*i*-PrOH	138–139/44	8	188 ± 3.1	7	*	10	*	$>5 \times 10^{-5}$	0
					17	193 ± 3.0	10	23	18	*		
10	NH-c-C_5H_9	$C_{29}H_{29}NO_4$·HCl·$1.5H_2O$[h,i]	*i*-PrOH	162–163/87	75	200 ± 2.6	10	23	23	18	NA	0
11	$NHCH_2$-$CH{=}CH_2$	$C_{27}H_{25}NO_4$	*i*-PrOH	119/82	75	197 ± 1.5	*	10	11	9	NA	0
12	NH-*sec*-C_4H_9	$C_{28}H_{29}NO_4$	*i*-PrOH	139–139.5/98	75	210 ± 7.2	12	18	19	19	NA	0
13	N(CH_3)-*n*-C_3H_7	$C_{28}H_{29}NO_4$·maleate	*i*-PrOH	135–137/90	75	199 ± 4.6	7	9	10	18	NA	0
14	$NHC(CH_3)_3$	$C_{28}H_{29}NO_4$·HCl	*i*-PrOH	157–158/87	75	196 ± 4.0	*	*	*	*	$>5 \times 10^{-5}$	NA
15	NH-*n*-C_4H_9	$C_{28}H_{29}NO_4$	MeOH–$CHCl_3$	125–26/43	75	199 ± 2.5	*	*	11	*	$>5 \times 10^{-5}$	NA
16	NH-*n*-C_5H_{11}	$C_{29}H_{31}NO_4$	*i*-PrOH	119–121/81	75	191 ± 2.3	*	*	*	*	NA	NA
17	NH-*n*-C_6H_{13}	$C_{30}H_{33}NO_4$·HCl·H_2O[h,j]	*i*-PrOH–EtAc	120–121/60	75	200 ± 2.2	*	*	*	*	NA	NA
18	NH-c-C_6H_{11}	$C_{30}H_{31}NO_4$	*i*-PrOH	153–154/91	75	216 ± 8.0	*	10	13	16	NA	NA
19	(propranolol)				75	212 ± 3.0	*	*	*	*	6.5×10^{-9}	100[l]

[a] Elemental analyses were within 0.4% of theory unless otherwise noted. [b] Melting points are not corrected. [c] Isolated yield from epoxide **3**. [d] Five male rats per dosage group. Percentage falls in systolic blood pressure were recorded at the indicated hour after dosing. Values in the table are statistically significant ($p \geq 0.05$) relative to control values; asterisks indicate percentage falls in systolic blood pressure were not statistically significant. [e] β-Receptor binding was determined in rat brain membrane fraction with [^{3}H]dihydroalprenolol. [f] Determined in SHR by inhibition of the depressor response of isoproterenol (0.25 mg/kg, iv) per group. Four male SHR were used. Dose administered (with base factor corrections) was 150 mg/kg, po. NA indicates that compounds were not tested. [g] No inhibition at 10 mg/kg, iv. [h] The water content was determined by Karl Fischer titration. [i] H: calcd, 6.41; found, 5.90. [j] Cl: calcd, 6.73; found, 7.38. [k] **8** did not inhibit isoproterenol-induced tachycardia in normotensive rats at 150 mg/kg, which was about a 10 times higher dose than needed to lower blood pressure in SHR. Four rats were used. [l] The inhibition of isoproterenol depressor response by propranolol hydrochloride at the dose of 10 mg/kg was 100% (SBP and diastolic blood pressure) at 5 h and 50% (SBP) or 72% (DBP) at 24 h.

which exhibits coronary vasodilating effects.[13] Stereochemically and electronically, the chromone ring system resembles the naphthalene nucleus in propranolol. By



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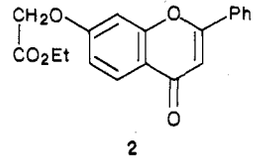

2

attaching a β-blocking side chain to the 7-position of the chromone nucleus, it was hoped that the resulting compound **1** and its analogues would produce desirable antihypertensive effects. Interestingly, the resulting compounds exhibited marked antihypertensive activity but did not possess significant β-adrenoceptor antagonism.

Chemistry

The general synthesis of compound **1** and its analogues is shown in Scheme I. 7-Hydroxy-2,3-diphenylchromone[14] was synthesized by heating a mixture of sodium benzoate, benzoic anhydride, and ω-phenylresacetophenone, which was, in turn, prepared from resorcinol and benzyl cyanide by a modified literature method.[15] The hydroxychromone was then allowed to react with epichlorohydrin in the presence of alcoholic sodium hydroxide. The product isolated contained a mixture of the epoxide **3**, the chlorohydrin **4**, and the dialkylated alcohol **5**. The ratio of **3** and **4** varied depending upon the base and solvent used. For example, the reaction gave the epoxide **3** as the major product in the presence of NaOH in aqueous ethanol, while the chlorohydrin **4** was isolated as the major product in aqueous sodium hydroxide.[16] In this process, the dialkylated product **5** was a major side product. Even when a 20-fold excess of epichlorohydrin was used, 10–20% of **5** was still formed. Addition of the sodium salt of 7-hydroxy-3-phenylflavone to a heated alcoholic solution containing epichlorohydrin did not minimize the yield of **5**. As **5** was quite insoluble, most of it could be removed either by trituration with methylene chloride or an alcohol. The purified epoxide **3** or the total reaction product from epoxidation was heated under reflux with amines to afford

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(11) Louis, W. J.; McNeil, J. J.; Drummer, O. H. *Drugs* **1984**, *28* (Suppl 2), 16.
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(13) Riseman, J. E. F.; Koretsky, S.; Altman, G. E. *Am. J. Cardiol.* **1965**, *15*, 220.
(14) Baker, W.; Robinson, R. *J. Chem. Soc.* **1925**, *127*, 1981.
(15) Chapman, E.; Sephen, H. *J. Chem. Soc.* **1923**, *123*, 404. Ethyl acetate was used as a solvent instead of anhydrous ether.
(16) The detailed studies will be published elsewhere.

6. The reaction of the chlorohydrin 4 with amines was quite sluggish under refluxing alcoholic conditions; however, it reacted readily in Me_2SO. The major side product of the ring-opening reaction was the tertiary amine 7,[17] a dialkylated product.

Biological Results and Discussion

The compounds in Table I were tested for antihypertensive activity and β-adrenergic antagonism in male spontaneously hypertensive rats (SHR). The data in Table I represent the percentage decrease in systolic blood pressure for the drug-treated group relative to the value for the untreated control. The percentage inhibition of the depressor response of isoproterenol for the drug-treated group compared to vehicle-treated rats gauged the extent of in vivo β-adrenoceptor antagonism. In vitro assessments were also made of the affinity of test compounds for β-adrenergic receptors.

In this series, the most active compounds in lowering systolic blood pressure of SHR were the *n*-propyl-substituted compound 8 and the cyclopropyl-substituted compound 9. Both compounds were active in lowering blood pressure at 8 mg/kg, po; however, 8 exhibited a much longer duration of activity than did 9. The isopropyl analogue 1 was very potent, and it had a long duration when tested orally at 17 mg/kg. A decrease in antihypertensive activity was noticed when the *n*-propyl substituent was replaced with the allyl group (11, about $^1/_{10}$ as active as 8), or when the size of the N-cyclic substituent was increased (e.g., 9, 10, and 18). Further branching of *N*-isopropyl (1) to *N*-*tert*-butyl (14) led to a decrease of the activity. While the *n*-propyl analogue 8 was the most active in this series and the *sec*-butyl analogue 12 showed a weak antihypertensive activity in SHR, *n*-butyl, *n*-pentyl, and *n*-hexyl analogues, 15, 16, and 17, respectively, did not exhibit any activity at 75 mg/kg, the highest dose tested. These results suggest that the optimal size for *N*-alkyl substituents is three carbons. The tertiary amine 13 exhibited a marginal activity.

All of these compounds are structurally similar to the classical β-blockers, i.e., they possess an aromatic ring substituted by a 3-(alkylamino)-2-hydroxypropoxy side chain. In our laboratory the classical β-blockers do not lower blood pressure in the SHR model, although it has been occasionally reported that such agents may be marginally effective in this model.[18] However, compounds 1, 8–13, 15, and 18 at ≤75 mg/kg, po, were effective in this model, and furthermore, 1 and 8–13 did not inhibit the depressor response of isoproterenol even at 150 mg/kg, po. In addition isoproterenol-induced tachycardia, when measured, was not altered. For example, compound 8 did not inhibit tachycardia at all even at 150 mg/kg, po, this dose being about 10 times higher than the dose needed to lower blood pressure. To further characterize the lack of β-adrenoceptor antagonism, the inhibition constants of 1, 8, 9, 14, and 15 were measured in a β-receptor radioligand binding assay. Inhibition constants were all 4 orders of magnitude greater than that of propranolol, suggesting that these compounds have very weak β-antagonist properties.

Significant differences in the structure–activity relationships also exist between these compounds and other series of β-blockers. Usually the most effective β-blockers contain the branched alkyl N-substituents, such as isopropyl and *tert*-butyl, and a marked reduction in effect is seen with straight-chain substituents. In our series, however, the antihypertensive effect was most pronounced for the *n*-propyl compound 8, the isopropyl compound 1[19] was less effective, and the *tert*-butyl compound 14 did not show the activity at 75 mg/kg. Thus, despite containing structural characteristics of β-adrenergic antagonists, these compounds do not exhibit β-antagonist properties, but still are effective antihypertensive agents.

The compounds of this series usually possessed antihypertensive effects of long duration when tested at doses higher than the minimally active ones. No indication of cumulative effects was observed after SHR were dosed with compound 8 orally for 18 consecutive days. Pharmacodynamic evaluation of 8 in rats failed to show any α-adrenergic blocking activity, as indicated by unaltered pressor responses to intravenous challenges of epinephrine (2 μg/kg), administered at 4 h after oral dosing with the test compound.

Although the mechanism by which these compounds decrease blood pressure remains to be determined, the results clearly indicate that it is not due to β-adrenergic antagonism. Preliminary studies with selected members of this series have found that antihypertensive effects may be associated with depletion of catecholamines in cardiovascular tissue. For instance, compound 8 (35 mg/kg, po) reduced arterial pressure and also depleted myocardial norepinephrine (NE) to below detectable levels, as measured by high-pressure liquid chromatography with electrochemical detection;[20,21] the control NE value was 0.32 ± 0.03 μg/g, compared to the treatment value of less than 0.07 μg/g.[22] In contrast, compound 16 (75 mg/kg, po) did not lower arterial pressure or appreciably deplete cardiac catecholamines. Although these cursory findings are far from conclusive, it is known that the antihypertensive effect of flavodilol [7-[2-hydroxy-3-(*n*-propylamino)propoxy]flavone], a closely related flavone analogue, is associated with depletion of peripheral catecholamines.[23,24] Thus, the possibility exists that the antihypertensive activity of these (diphenylchromonoxy)propanolamines is related to modulation of sympathetic function by reducing noradrenergic neurotransmitter stores available for release during neuronal depolarization.

Experimental Section

The melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were determined in the indicated solvent on a Varian EM360A NMR spectrometer at the ambient operating temperature with tetramethylsilane as internal standard for proton spectra unless

(17) A tertiary amine 7 (R = n-C_3H_7) was fully identified. This compound was isolated as white prisms (about 1–5% yield): mp 137–139 °C (i-PrOH–PhCH$_3$); IR 3420, 1625 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 0.50–2.10 (m, 9 H, CH_2CH_3; the higher integration may be due to the presence of toluene and Me_2SO-d_6), 2.10–2.80 (m, 11 H, $(CH_2)_2NCH_2$; integration is off due to overlapping with Me_2SO-d_6), 4.05 (br s, 6 H, OCH_2CHO), 5.00 (br s, 1 H, OH), 6.70–8.10 (m, 26 H, aromatic H. Anal. Calcd for $C_{51}H_{45}NO_8$ (containing 0.8% of toluene and 1.37% of water): C, 75.64; H, 5.77; N, 1.71. Found: C, 75.92; H, 5.80; N, 1.65.

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(19) Compound 1 was also shown to be active at 17, 35, and 75 mg/kg, po in the DOCA hypertensive rat model.

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(22) The detailed methodology will be published soon. The methodology was presented in ref 23 and 24.

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(24) Blosser, J. C.; Kaiser, F. C.; Kinsolving, C. R.; Parker, R. B.; McCreedy, S. A.; Milgate, T. E.; Watkins, B. E. *Pharmacologist* **1985**, *27*(3), 261.

otherwise stated. Chemical shifts are given in ppm units, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded on a Nicolet MX-1 Fourier transform infrared spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tn, and were within ±0.4% of the theoretical value when indicated by symbols of the element unless otherwise noted.

ω-Phenylresacetophenone. A solution of resorcinol (51.6 g, 0.469 mol) and benzyl cyanide (46.9 g, 0.400 mol) in ethyl acetate (190 mL) was placed in a three-necked, round-bottomed flask equipped with a gas dispersion tube, a mechanical stirrer, and an air condenser topped with a drying tube. Anhydrous zinc chloride (37.5 g, 0.275 mol) was added to the solution. A stream of dry hydrogen chloride gas was passed into the stirred suspension over a period of 5.5 h, while the temperature was maintained below 20 °C. HCl gas was introduced rapidly at first to saturate the solvent and then in a slow gentle stream. In 1.5 h, a very thick suspension was formed. Stirring was continued for 1 more hour at the end of HCl introduction. The imine hydrochloride was filtered and washed with ethyl acetate until the filtrate was no longer bright red (about 200 mL of ethyl acetate was used); 82.5 g (78% yield), mp 210–215 °C.

The imine hydrochloride (82.5 g) was suspended in 250 mL of water and heated at 95–100 °C with stirring for 1 h. A pale brown oil separated out and solidified rapidly upon seeding. The solid was collected and washed with water to give 68 g (75%) of white crystals, mp 113–117 °C (lit.[15] mp 115 °C).

7-(2,3-Epoxypropoxy)-3-phenylflavone. Epichlorohydrin (120 mL, 1.53 mol) was added to a solution of 7-hydroxy-3-phenylflavone (47.1 g, 0.15 mol), which was prepared from ω-phenylresacetophenone according to the Baker and Robinson procedure,[14] in an aqueous ethanol solution (300 mL of 95% alcohol and 225 mL of water) containing 6.0 g of sodium hydroxide. The resulting solution was heated at 75 °C for 1.5 h and cooled to 45 °C. At this point, some crystals were added, and oiling occurred. The oil was stirred with 45 mL of 95% ethanol for 3 h at room temperature. The white solid that formed was filtered and washed with 150 mL of 50% aqueous ethanol; 41.6 g (75% yield). The product was purified by silica gel column chromatography, eluting with 10% ether/CH_2Cl_2. Three products were isolated: the epoxide **3** (56% yield), the chlorohydrin **4** (2%), and the dimer **5** (9%). Epoxide **3**: mp 146.5–147.5 °C (MeOH); IR (KBr) 1630, 1600, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60–3.00 (m, 2 H, CH_2 of the epoxide), 3.10–3.50 (br s, 1 H, CHO), 3.80–4.50 (m, 2 H, CH_2OAr), 6.7–7.7 (m, 12 H, aromatic H), 8.12 (dd, J = 9, 2 Hz, 1 H, C_5H). Anal. ($C_{24}H_{18}O_4$) C, H, O. Chlorohydrin **4**: mp 160–160.5 °C (EtOH); IR (KBr) 3400, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.05 (m, 1 H, exchangeable with D_2O, OH), 3.70 (br d, J = 4 Hz, 2 H, CH_2Cl), 4.18 (br s, 3 H, OCH_2CHO), 6.7–7.7 (m, 12 H, aromatic H), 8.12 (dd, J = 9, 2 Hz, 1 H, C_5 H). Anal. ($C_{24}H_{19}ClO_4$) C, H, Cl, O. Dimer **5**: mp 265–268 °C (DMF); IR (KBr) 3400, 1622 cm^{-1}; ^1H NMR ($CDCl_3$ + trifluoroacetic acid) 4.30–5.00 (m, 5 H, OCH_2CHCH_2O), 6.20–8.60 (m, 26 H, aromatic H). Anal. Calcd for $C_{45}H_{32}O_7$ (containing 0.52% of DMF and 0.30% of water): C, 78.48; H, 4.75; N, 0.1; O, 16.59. Found: C, 77.94; H, 4.94; O, 17.12.

Synthesis of 6. The preparation of **1** is prresented as an example of the general synthesis of **6** as shown in Scheme I.

7-[2-Hydroxy-3-(isopropylamino)propoxy]-3-phenylflavone Hydrochloride (1). A white suspension of relatively pure 7-(2,3-epoxypropoxy)3-phenylflavone (27.78 g, 75 mmol), isopropylamine (18.8 mL), and methanol (150 mL) was heated under nitrogen at 55 °C until disappearence of the starting epoxide was complete (about 3 h). The white precipitate (1.09 g) in the cooled reaction mixture was filtered off, and the filtrate was evaporated to a golden syrup. Anhydrous ether was added, and white crystals (28.07 g) were obtained. Purification by chromatography over silica gel eluting with 2% MeOH–CH_2Cl_2 yielded 24.05 g of white crystals, mp 152–154 °C (75%).

The amine thus obtained was dissolved in 100 mL of MeOH and treated with a saturated solution of hydrogen chloride in ethanol until the pH of the solution was 1. The hydrochloride salt was precipitated by addition of anhydrous ether. A white solid was collected and was recrystallized from MeOH–ether (also decolorized with a small amount of activated charcoal) to produce 21.6 g (83%) of pure white crystals: mp 164–166 °C; IR (KBr) 3400, 2800–2300, 1630 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.32 (d, J = 6 Hz, 6 H $CHMe_2$), 2.8–3.9 (br s, 3 H, CH_2NHCH), 4.30 (br s, 3 H, OCH_2CHO), 6.05 (br d, J = 4 Hz, 1 H, exchangeable, OH), 6.70–8.20 (m, 13 H, aromatic H), 9.00 (br s, 2 H, exchangeable, NH and HCl). Anal. ($C_{27}H_{27}NO_4 \cdot HCl \cdot H_2O$) C, H, N, O; Cl: calcd, 7.33; found, 7.95.

7-[2-Hydroxy-3-(*n*-propylamino)propoxy]-3-phenylflavone (8). (a) A pale yellow solution of the chlorohydrin **4** (7.32 g, 18 mmol) and *n*-propylamine (8.63 g, 12 mL, 146 mmol) in Me_2SO (9 mL) was heated at 80 °C for 14 h and cooled. Water (150 mL) was added to the reaction mixture. The yellow precipitate was collected and washed with water. The crude product (7.39 g) was recrystallized from aqueous methanol to give 5.00 g of golden prisms (65% yield).

(b) A similar reaction was performed except Me_2SO was replaced with 95% ethanol. The reaction was monitored by TLC (10% MeOH–CH_2Cl_2) and it was found that no reaction occurred after refluxing for 6 h.

Indirect Blood Pressure Measurement in Unanesthetized Rats. Male spontaneously hypertensive rats of the Wistar-Kyoto strain were used. Arterial systolic blood pressure was measured with an indirect tail cuff method. Prior to each set of measurements, the rats, five per treatment group, were heated in an environmental chamber at 35–37 °C for 15–20 min to raise body temperature slightly to allow the pulse in the dilated caudal artery to be easily detected. In all cases, at least three consecutive measurements were obtained, and the average was reported as the systolic blood pressure for a particular rat at a specific time. Pretreatment measurements were made in the afternoon before an experiment that began on the following morning. Only rats with a systolic pressure of 180 mmHg or higher were used. All doses of test compounds that were administered were corrected to deliver 100% of the free base. All test compounds were suspended in a 4% aqueous Clearjel solution, adjusted to a volume of 1% of body weight. Statistical analysis of the results was performed by using analysis of variance.

Isoproterenol-Response Inhibition. SHR, three animals in each treatment group, were dosed orally with 150 mg/kg of the various test compounds, as a suspension in 4% Clearjel. Four hours after dosing, generally the time of near-optimal antihypertensive response of active compounds, the rats were anesthetized with sodium pentobarbital (45 mg/kg, ip), and the left femoral artery and vein were cannulated. Arterial pressure was then continually monitored from the arterial cannula. The acute vasodepressor response, and occasionally tachycardiac response, was assessed following intravenous administration of 0.25 μg/kg of isoproterenol in 0.1 mL of saline. Potential ability of a test compound to inhibit isoproterenol responses was evaluated by comparison to responses of rats that had been treated orally with the Clearjel vehicle.

In vivo assessment of possible antihypertensive mechanisms of active compounds must be made at the time of appreciable blood pressure reduction, which in this instance was 4–5 h after oral dosing. This consideration is essential to allow sufficient time for adequate oral absorption of compounds and consequent onset of pharmacological effects.

β-Adrenoceptor-Affinity Studies. Affinity to β-adrenergic receptors was measured as described by Bylund and Snyder.[25] After removal of the cerebellum, rat brains were homogenized with a Brinkman Polytron in ice-cold 50 mM Tris·HCl, pH 8.0, in a volume equal to 20 times the brain weight, and centrifuged at 50000*g* for 15 min. The pellet was resuspended and recentrifuged as above and the final pellet was resuspended in 50 volumes of the Tris buffer. The receptor binding assay consisted of 0.5 mL of homogenate (0.4–0.6 mg of protein), 1 nM of [^{3}H]dihydroalprenolol ([^{3}H]DHA, 50 Ci/mmol, New England Nuclear), compound to be tested, and 50 mM Tris buffer (pH 8.0) in a final volume of 1 mL. To measure nonspecific binding, 10^{-4} M *l*-alprenolol tartrate was substituted for the test compound. After a 20-min incubation, reaction was terminated by pouring onto a Whatman GF/B filter under vacuum. The filters were washed with 5 mL of ice-cold Tris buffer and counted by liquid

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scintillation. Specific binding of [^{3}H]DHA (binding in the absence of any added compounds minus that in the presence of 20 μM *l*-alprenolol) was approximately 65 fmol/mg protein. In a typical experiment the total specific binding was 7–8% of the free [^{3}H]DHA and the nonspecific binding was 50% of the total specific binding. The affinity of a compound was estimated by competition binding with [^{3}H]DHA with 5–10 different concentrations of each compound and the IC_{50} (concentration at which specific [^{3}H]DHA binding is inhibited by 50%) was determined visually from a semilog plot. The apparent K_i was calculated according to the formulation of Cheng and Prusoff:[26] $K_i = IC_{50}/(1 + L/KD)$, where K_i is the inhibition constant of the test compound, L is the concentration of [^{3}H]DHA, and the KD is the dissociation constant for [^{3}H]DHA (approximately 1 nM).

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Registry No. 1, 84858-29-7; 1·HCl, 84858-18-4; **3**, 84858-17-3; **4**, 106820-96-6; **5**, 106820-97-7; **7** (R = Pr), 87272-33-1; **8**, 87272-33-1; **9**, 84858-38-8; **10**, 84858-53-7; **10**·HCl, 84858-54-8; **11**, 84858-51-5; **12**, 84858-55-9; **13**, 84858-56-0; **13**·maleate, 84858-57-1; **14**, 106820-99-9; **14**·HCl, 106821-05-0; **15**, 106821-00-5; **16**, 106821-01-6; **17**, 106821-02-7; **17**·HCl, 106821-06-1; **18**, 106821-03-8; $PhCH_2CN$, 140-29-4; $PhCO_2COPh$, 93-97-0; *i*-$PrNH_2$, 75-31-0; $PrAlH_2$, 107-10-8; $CH_2{=}CHCH_2NH_2$, 107-11-9; *s*-$BuNH_2$, 13952-84-6; MeNHPr, 627-35-0; *t*-$BuNH_2$, 75-64-9; $BuNH_2$, 109-73-9; $CH_3(CH_2)_4NH_2$, 110-58-7; $CH_3(CH_2)_5NH_2$, 111-26-2; 7-hydroxy-3-phenylflavone, 18651-11-1; resorcinol, 108-46-3; ω-phenylresacetophenone imine hydrochloride, 106821-04-9; ω-phenylresacetophenone, 3669-41-8; epichlorohydrin, 106-89-8; cyclopropanamine, 765-30-0; cyclopentanamine, 1003-03-8; cyclohexanamine, 108-91-8.

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Structure–Activity Relationship in PAF-acether. 3.[1] Hydrophobic Contribution to Agonistic Activity

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The synthesis of some selected PAF-acether homologues with an alkoxy-chain length from C_1 to C_{20} in position 1 is described. All agonist activities are closely correlated among themselves and with the calculated fatty-chain hydrophobicity. After a discussion on recent published results and comparison with our data, we conclude that the ether oxide function is absolutely essential at the glycerol 1-position for potent agonist activity and that potency correlates well with hydrophobicity parameters. We indicate the importance of steric and configurational constraints.

Since its structure was elucidated in 1979 via hemisynthesis from plasmalogens,[2,3] platelet activating factor (PAF-acether) Ib + Ic (natural configuration *R*) (Figure 1) has been increasingly studied for its remarkable biological activities, mainly platelet aggregation,[4] bronchoconstriction,[5] and hypotension.[6] Its biosynthesis was found to occur in a variety of inflammatory cells:[7] basophils,[8] macrophages,[9] neutrophils,[10,11] and platelets themselves.[4] Research interest is now focused on the study of the relationship between specific chemical moieties of this molecule and its biological activities.[12-16]

Here, we report the synthesis of some selected linear-ether-chain homologues of PAF-acether, from C_1 to C_{20}, by a minor modification of the methods we have already described.[17-19] We also studied correlations between lipophilicity of the ether chain and agonistic activities such as platelet aggregation (on washed platelets (WP) and platelet-rich plasma (PRP)), hypotension, bronchoconstriction, and thrombocytepenia.

Chemistry

The PAF-acether homologues described in this paper are listed in Figure 1. Alkylacetylglycerophosphocholines I were prepared by using modified versions of our already published procedures[17-19](Scheme I).

Glyceryl ethers 1 were obtained from the potassium salt of 1,2-*O*-isopropylideneglycerol and the corresponding alkyl methanesulfonate (R = C_6–C_{20})[20] or by condensing 1,2-*O*-isopropylideneglycerol with alkyl iodides (R = C_4H_9 and

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