H. Cordes and R. F. Hirschmann for their encouragement during the course of this study, to Dr. T. Verhoeven for the preparation of nitrile 12, to Dr. W. C. Randall and J. P. Moreau for analytical support, to Dr. D. W. Cochran and J. S. Murphy for the ^{1}H NMR spectra, and to M. Z. Banker for manuscript preparation.

Supplementary Material Available: ^{1}H NMR data for biphenylcarboxaldehydes 3 and 3-biphenylylpropenals 4 plus atomic parameters for 100(+) (8 pages). Ordering information is given on any current masthead page.

Synthesis and Investigation of the β-Adrenoceptor Agonist and Platelet Antiaggregatory Properties of 1,7,8-Trisubstituted 2,3,4,5-Tetrahydro-1*H*-2-benzazepine Analogues of Trimetoquinol

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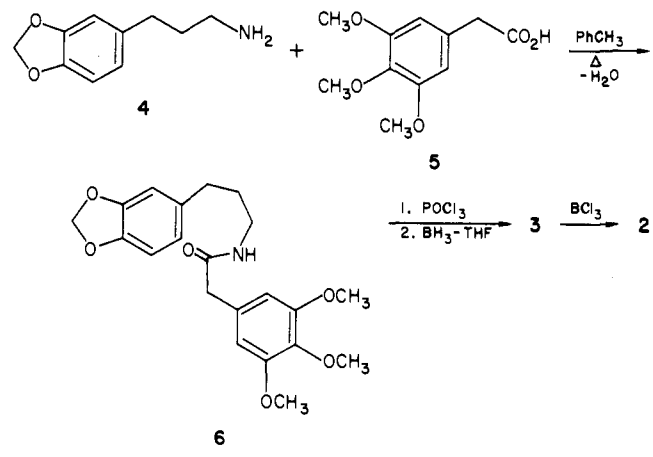
The synthesis and biological evaluation of 7,8-dihydroxy (2) and 7,8-methylenedioxy (3) analogues of 1-[(3,4,5-trimethoxyphenyl)methyl]-2,3,4,5-tetrahydro-1*H*-2-benzazepine on β-adrenoceptor systems and human platelets were undertaken and compared with trimetoquinol (TMQ, 1). Whereas 1 is a potent β-adrenoceptor agonist in guinea pig atria and trachea (pD_2 = 8.2), analogue 2 was marginally effective at relaxing guinea pig tracheal smooth muscle (pD_2 = 4.4) and inactive as an agonist on guinea pig atria. Analogues 2 and 3 were inhibitors of phospholipase C (PLC; from *Clostridium perfringens*) induced and secondary wave of ADP-induced aggregation responses and inactive against low-dose thrombin-induced or stable endoperoxide (U46619) induced human platelet aggregation. Against ADP-induced serotonin secretion, 3 was 9-fold more active than analogue 2. Further, the rank order of TMQ isomers and 3 as inhibitors of PLC-induced platelet aggregation, serotonin secretion, and phosphatidylinositol degradation was identical (3 > (*S*)-(−)-1 > (*R*)-(+)-1). The results suggest that these compounds are blocking the action of PLC by interfering with phosphatidylinositol turnover in platelet membranes. The inhibition of ADP-induced responses in human platelets by analogues 2 and 3 also suggests a site of inhibition at a level of arachidonic acid release. Thus, ring expansion of 1 as in the benzazepine analogues 2 and 3 has allowed us to develop selective inhibitors of platelet function that lack significant β-adrenoceptor activity.

Trimetoquinol (1) is a potent β-adrenoceptor agonist[1,2] and platelet antiaggregatory drug.[3,4] Our laboratory has investigated the effect of structural modification of 1 with the goal of obtaining highly selective β_2-adrenergic agonists and antiplatelet agents.[5-10] To our knowledge, no investigation, other than our own, has been carried our to establish the effect of enlargement of the tetrahydroisoquinoline ring to a tetrahydro-1*H*-2-benzazepine on β-adrenergic activity. Recently, we have reported[11] that 2 and 3 were found to be more potent than 1 as inhibitors of bacterial phospholipase C (PLC) induced human platelet aggregation. In the following discussion, the synthesis and more extensive biological evaluation of 2 and 3 in human platelets and of 2 in β_1- and β_2-adrenergic systems is reported.

Scheme I



Chemistry. The synthesis of compound 2 involved the preparation of *N*-[[3,4-(methylenedioxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (6) from 3-[3,4-(methylenedioxy)phenyl]-1-aminopropane (4) and 3,4,5-trimethoxyphenylacetic acid (5). Compound 6 was then cyclized via a Bischler–Napieralski ring closure and converted to 2 with BCl_3 as illustrated in Scheme I.

Of importance in the synthetic strategy was the preparation of compound 4. The original preparation of 4 involved reduction, via hydrogenation or sodium amalgam, of 3,4-(methylenedioxy)cinnamic acid to 3-[3,4-(methylenedioxy)phenyl]-1-propionic acid.[12] The propionic acid

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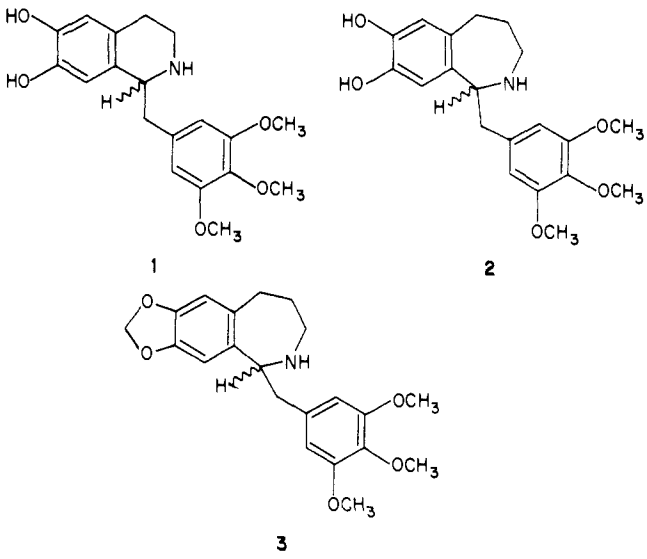


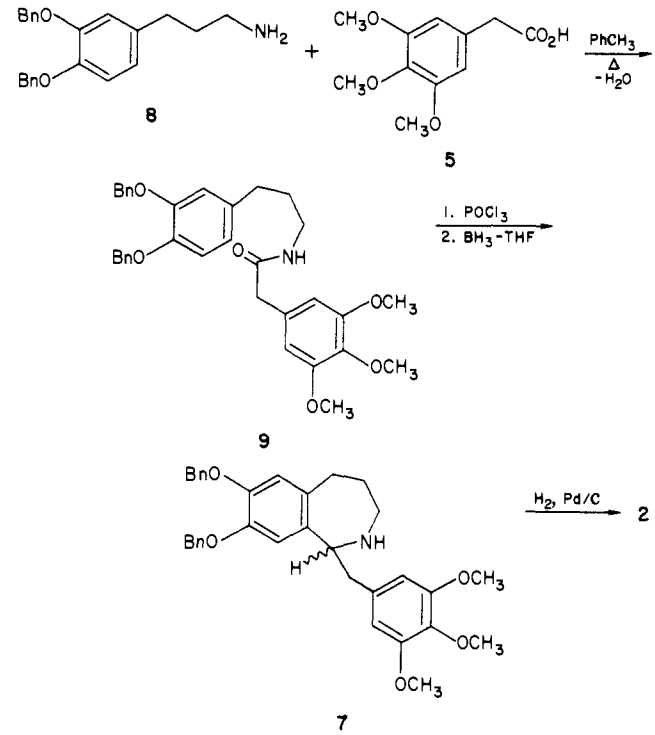
was converted into 3-[3,4-(methylenedioxy)phenyl]-1-propanamide[12] by ethyl chloroformate and ammonia.[13] This amide was reduced with diborane to amine **4**.[14] As an alternative preparation of **4**, the method of Kabalka et al.[15] was incorporated into the synthetic scheme. The method, accomplished in one pot, involved treating 3-[3,4-(methylenedioxy)phenyl]-1-propene (safrole) with diborane in dry THF for 1 h, and then aqueous ammonium hydroxide and commercial grade bleach were added. Amine **4** was then isolated from the reaction mixture. The amine **4** was heated at reflux with **5** in toluene, with removal of water, for 3 days to give amide **6** in 75% yield. When amide **6** was heated with $POCl_3$ in CH_3CN for 5 h, reduced with diborane in dry THF for 9 h, and chromatographed (silica gel, 5% MeOH/EtOAc, 1% NH_4OH), **3** was obtained in very poor yield. The yield of **3** could not be increased by other reagents, for example with P_2O_5[16] or $AlCl_3$.[17] Treatment of **3** with BCl_3 provided **2** in a 43% yield.

An alternative pathway in the preparation of **2** is illustrated in Scheme II. Initially, the preparation of tetrahydro-1*H*-2-benzazepine (**7**) required the synthesis of amine **8**.[18] The amine **8** was prepared by the treatment of 3-[3,4-bis(benzyloxy)phenyl]propionic acid with ethyl chloroformate and ammonia[13] followed by diborane reduction. The amine **8** was condensed with **5** to give N-[3-[3,4-bis(benzyloxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (**9**). Bischer–Napieralski ring closure of **9** and reduction with diborane gave **7** in 58% yield. Catalytic reduction of the bis(benzyloxy) groups gave catechol **2** in a 75% yield.

Biological Results and Discussion

Previous work from our laboratory[2,4-10] and others[1,3] has shown that **1** is both a potent β-adrenoceptor stimulant and inhibitor of human platelet function. In our studies, compound **2** was inactive as an agonist in guinea pig atria at concentrations from 1×10^{-9} to 3×10^{-5} M and caused relaxation of guinea pig tracheal strips at $>3 \times 10^{-6}$ M with

Scheme II



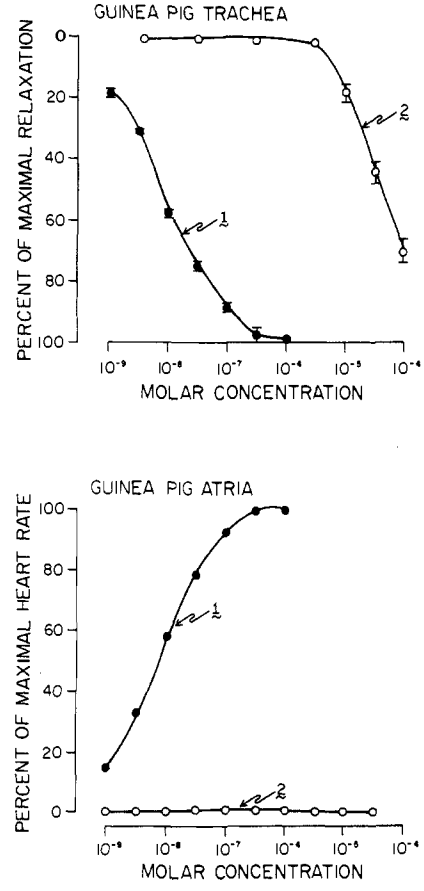


Figure 1. Comparative concentration-dependent actions of **1** and **2** on guinea pig trachea (upper panel) and atria (lower panel). Data represent the mean ± SEM of $N = 4$–8. Key: **1** (●) and **2** (○).

a pD_2 of 4.4 (Figure 1). In guinea pig atria, addition of **2** at 10^{-5} M did not block the chronotropic responses to isoproterenol. By contrast, **1** is a potent agonist on guinea pig atria and trachea having pD_2 values of 8.15 and 8.18, respectively. Thus, ring expansion of **1** as in analogue **2**

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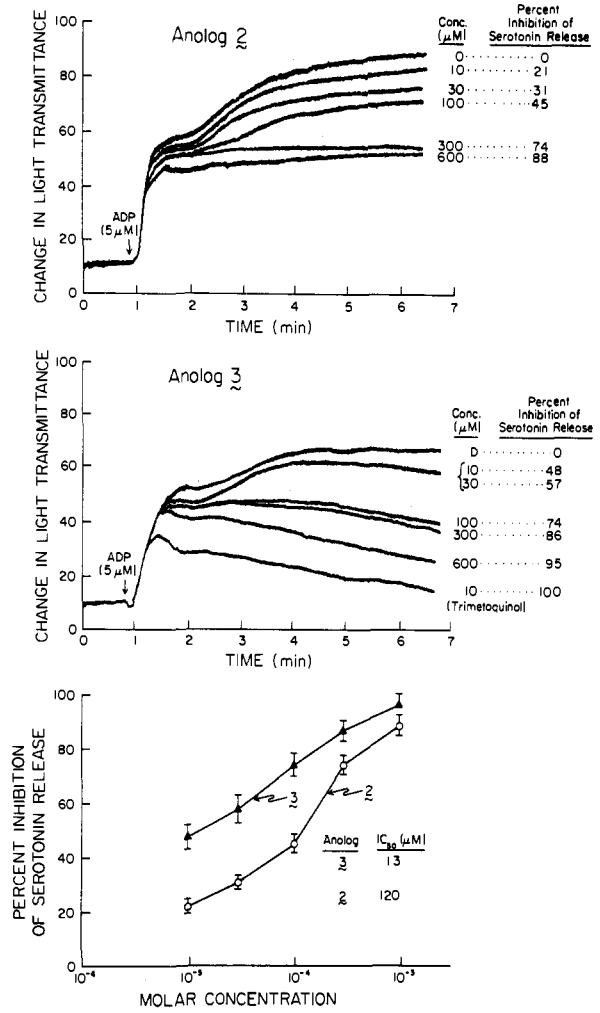

Figure 2. Concentration-dependent inhibition of ADP (5 μM) induced human platelet aggregation and serotonin secretion by benzazepine analogues **2** (upper panel) and **3** (middle panel) and comparative inhibition of serotonin release induced by ADP (lower panel). Trimetoquinol (10 μM) is included as a comparison standard inhibitor (middle panel). Results are representative of five to six donors.

has produced a reduction or abolition of β-adrenoceptor agonist activity.

We previously showed that **1** is a potent inhibitor of the prostaglandin-dependent pathway of platelet activation,[4] and both **2** and **3** blocked the secondary wave of ADP-induced platelet aggregation and the secretion of serotonin (Figure 2). Analogue **3** was 2.2- and 9-fold more potent than **2** as an inhibitor of ADP-induced aggregation and serotonin secretion, respectively.

In an earlier report,[11] we observed that analogue **3** was a more potent inhibitor of bacterial phospholipase C (PLC) induced human platelet aggregation than **1**. This PLC-mediated pathway is independent of released ADP or prostaglandin biosynthesis.[19] Our studies show that both analogues **2** and **3** are more effective than **1** as inhibitors of platelet aggregation induced by PLC (Table I and Figure 3) whereas neither analogue was found to block low-dose thrombin (0.03 U/mL) or stable prostaglandin H_2 endoperoxide (U46619) stimulated platelet aggregation (Table I). An identical rank order of inhibition of **3** > (*S*)-(−)-**1** > (*R*)-(+)-**1** was seen for the blockade of PLC-induced aggregation and serotonin secretion. IC_{50} values of 3, 50, and 100 μM were observed for **3**, (*S*)-(−)-**1**, and (*R*)-(+)-**1**, respectively, against PLC-induced serotonin release (data not presented).

Table I. Inhibitory Activity of Trimetoquinol Isomers and Benzazepine Analogues against Aggregation Induced by Various Agonists in Human Platelets

	IC_{50} (μM) ± SEM[a]			
inducer (concn)	(*S*)-(−)-TMQ	(*R*)-(+)-TMQ	**3**	**2**
thrombin (0.03 U/mL)	50 ± 2	150 ± 10	[b]	[b]
PLC (0.05 U/mL)	50 ± 7	175 ± 5	3 ± 2	30 ± 6
U46619 (2 μM)	11 ± 4	0.14 ± 0.1	[b]	[b]

[a] Inhibitory concentration 50 = IC_{50}. Values are the mean ± SEM (N = four to six donors). [b] No inhibitory activity at concentrations up to 400 μM.

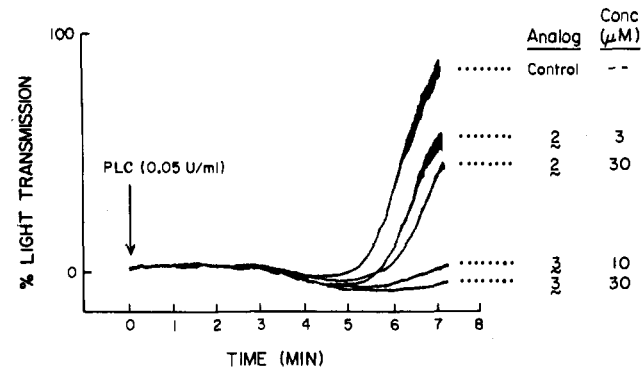

Figure 3. Concentration-dependent inhibition of phospholipase C (0.05 U/mL) induced human platelet aggregation by analogues **2** and **3**. Data are representative of five donors.

Many stimuli of human platelet activation increase the breakdown of polyphosphatides in membranes, leading to the formation of diacylglycerol, phosphatidic acid, and inositol phosphates.[20-23] The time course of phosphatidylinositol (PI) breakdown by bacterial PLC and inhibition of PI degradation by **3** and isomers of **1** are given in Figure 4. The rank order of inhibitory potency (IC_{50} values) against PLC-induced PI breakdown was **3** (10 μM) > (*S*)-(−)-**1** (100 μM) > (*R*)-(+)-**1** (>100 μM). This is the same order as against PLC-induced aggregation and [^{14}C]serotonin secretion, strongly suggesting a role for PI turnover by PLC as an arachidonic acid independent mechanism of platelet activation.

On the basis of this data, we have shown that the profile of antiaggregatory actions of **2** and **3** differs from the isomers of **1**. We showed that the isomers of **1** blocked PLC- and thrombin-induced aggregation with the same stereoselectivity[24] whereas analogues **2** and **3** were only inhibitors of PLC-induced aggregation (Figure 3 and Table I). Similarly, only the isomers of **1** blocked the aggregatory responses to U46619 in human platelets. It should be noted that **2** and **3** also blocked ADP-induced responses in human platelets with the same degree and rank order of potency as against PLC-induced platelet activation. Since **3** does not block arachidonic acid induced platelet aggregation[19] and interferes selectively with the secondary wave of ADP-induced aggregation and serotonin release (Figure 2), we propose that this compound inhibits this

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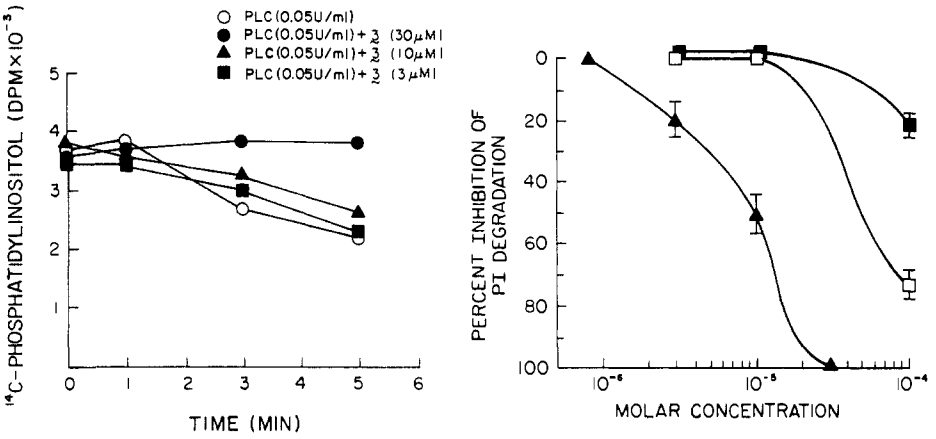

Figure 4. Time course (left panel) and concentration-dependent inhibitory (right panel) effect of **3** and isomers of **1** on phospholipase C (0.05 U/mL) induced phosphatidylinositol (PI) degradation in washed human platelets. Platelets prelabeled with [^{14}C]arachidonic acid were preincubated with varying concentrations of (*R*)-(+)-TMQ (■), (*S*)-(−)-TMQ (□), or **3** (▲) for 3 min prior to the addition of PLC (0.05 U/mL). [^{14}C]PI was measured at varying times (left panel) or at 5 min (right panel) after PLC addition as described in Biological Methods. Each value is the mean ± SEM of three experiments.

prostaglandin dependent pathway at the level of phospholipase A_2 activation and/or arachidonic acid release. This evidence suggests that **2** and **3** act as selective inhibitors of this PLC-dependent platelet activation (a prostaglandin independent pathway) and at an early stage of arachidonic acid release of the prostaglandin dependent pathway of platelet activation. Further studies are needed to establish whether the antagonism of ADP- or PLC-induced platelet activation by **2** and **3** are mediated by a common or differing mechanisms of action. Taken collectively, however, these data clearly indicate that the inhibitory action of **2** and **3** against these inducers differs from that of the isomers of **1**.

The mechanism by which bacterial PLC causes platelet activation is not known. However, it has been shown that PLC acts on the platelet surface by hydrolyzing a very small fraction of the plasma membrane phospholipid[25] and subsequently generates a phosphorylated protein (40 000 daltons) which may be linked to platelet secretory and aggregatory responses.[26] Furthermore, in the concentrations used in these studies, PLC does not require ADP or thromboxane A_2 to produce aggregation or cause platelet lysis.[11,19] It is proposed that PLC may perturb the platelet surface in some way that triggers PI turnover, aggregation, and secretion. The mechanism by which the isomers of **1** and analog **3** inhibit PLC-induced events is also not known. However, our results indicate that this inhibitory effect is related to the blockade of PI degradation (Figure 4) and is independent of thromboxane A_2 and phosphatidic acid production.[19] In addition, by the use of a synthetic substrate for PLC, (*p*-nitrophenyl)phosphorylcholine,[27] we have found that neither isomer of **1** nor analogue **3** was a direct inhibitor of bacterial PLC activity (data not presented). Thus, it is likely that the isomers of **1** modify the hydrolysis of PI by an action at the level of the PI-specific PLC in platelets.

In summary, these agents blocked PLC-induced PI degradation with the same stereoselectivity and order of potency **3** > (*S*)-(−)-**1** > (*R*)-(+)-**1** as they blocked platelet aggregation and secretion. These results suggest that the turnover of PI may play an important role in the mechanism of activation of the PLC pathway of platelet activation. Therefore, these compounds represent a chemically novel class of antiplatelet agents capable of interfering with turnover of membrane phospholipids. Moreover, enlargement of the ring system of **1** as in **2** and **3** virtually abolished β-adrenoceptor activity, suggesting that a high degree of pharmacological selectivity (antiaggregatory vs. β-adrenoceptor activity) with these drugs is achievable with use of the tetrahydro-1H-2-benzazepine ring system.

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Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared data were collected on a Beckman 4230 spectrophotometer. The NMR spectra were recorded on a Bruker HX-90E or a IBM 270 spectrometer with tetramethylsilane as the internal standard. The mass spectra were determined on a DuPont Model 491 mass spectrometer. Chemical analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN. TLC was performed on silica gel 60 F_{254} precoated aluminum-backed plates from EM Reagents. Column chromatography was performed on silica gel 60, 70–230 mesh, from EM Reagents. All organic solvents were appropriately dried prior to use.

***N*-[[3,4-(Methylenedioxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (6).** 3-[3,4-(Methylenedioxy)phenyl]-1-aminopropane hydrochloride[14] (10 g, 47 mmol) was dissolved in water (100 mL). Potassium carbonate (6.9 g, 47 mmol) was added and the mixture was extracted with $CHCl_3$ (2 × 100 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to an oil. The oil was dissolved in toluene (200 mL) and 3,4,5-trimethoxyphenylacetic acid (10.67 g, 47 mmol) was added. The mixture was heated at reflux for 3 days with removal of H_2O by a Dean–Stark trap. After the mixture was cooled, it was concentrated under reduced pressure and the residue was dissolved in $CHCl_3$. The solution was washed with 10% HCl (2 × 200 mL), 10% Na_2CO_3 (2 × 200 mL), brine (2 × 200 mL), and H_2O (2 × 200 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to a solid. The solid was recrystallized from EtOAc to give 13.6 g of **6** (75%): mp 103–104 °C; IR (KBr) 3290 (NH), 1635 (C=O); ^{1}H NMR ($CDCl_3$) δ 1.73 (m, 2 H, Ar $CH_2CH_2CH_2$), 2.5 (m, 2 H, Ar$CH_2CH_2CH_2$), 3.23 (m, 2 H, CH_2NH), 3.48 (s, 2 H, Ar CH_2CO), 3.53 (s, 9 H, 3 × Ar OCH_3), 5.47 (br, 1 H, NH), 5.90 (s, 2 H, OCH_2O), 6.48 (s, 2 H, 2 × Ar H), 6.54–6.70 (m, 3 H, 3 × Ar H). Anal. ($C_{12}H_{25}NO_6$) C, H, N.

1-[(3,4,5-Trimethoxyphenyl)methyl]-7,8-(methylenedioxy)-2,3,4,5-tetrahydro-1*H*-2-benzazepine (3). *N*-[3-[3,4-(Methylenedioxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (1.5 g, 3.88 mmol) and $POCl_3$ (1.08 mL, 11.6 mmol) in CH_3CN (80 mL) were heated at reflux for 5 h. The mixture was cooled and concentrated to a oily residue. The residue was dissolved in THF (40 mL) and cooled to 0 °C and 1 M diborane in THF (80 mL) was added over a period of 0.5 h. The mixture was heated at reflux for 9 h and cooled to 0 °C and MeOH (15 mL) was added slowly. The mixture was concentrated to an oil

and the oil was redissolved in MeOH (repeated three times). The oily residue which formed was purified by column chromatography on silica gel (5% MeOH/EtOAc, 1% aqueous NH_4OH) to give an oil which solidified on standing and was recrystallized from MeOH to yield 0.2 g of **3** (15%): mp 128–128.5 °C; IR (neat) 3400 (NH), 1590 (aromatic); ^1H NMR (free base, $CDCl_3$) δ 1.50–1.73 (m, 3 H, Ar $CH_2CH_2CH_2$ and NH), 2.65–3.40 (m, 6 H, Ar CH_2-CH_2CH_2 and Ar CH_2CHN), 3.85 (s, 9 H, 3 × Ar OCH_3), 4.05 (m, 1 H, Ar CH_2CHN), 5.90 (s, 2 H, OCH_2O), 6.47 (s, 2 H, 2 × Ar H), 6.66 (s, 1 H, 1 × Ar H), 6.74 (s, 1 H, 1 × Ar H). Anal. ($C_{21}H_{25}NO_5$) C, H, N.

1-[(3,4,5-Trimethoxyphenyl)methyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2-benzazepine (2). Method A. To 1-[(3,4,5-trimethoxyphenyl)methyl]-7,8-(methylenedioxy)-2,3,4,5-tetrahydro-1*H*-2-benzazepine (37.1 mg, 0.1 mmol) in a CH_2Cl_2 solution was added a 1 M solution of BCl_3 in CH_2Cl_2 (0.2 mL, 0.2 mmol) under argon atmosphere. After the mixture stirred for 5 h at room temperature, it was quenched with MeOH (0.2 mL) and stirred an additional 1 h at room temperature. The mixture was evaporated in vacuo and the residue was crystallized from EtOH–H_2O to give 17 mg of **2** (43%).

Method B. 1-[(3,4,5-Trimethoxyphenyl)methyl]-7,8-bis(benzyloxy)-2,3,4,5-tetrahydro-1*H*-2-benzazepine (300 mg, 0.5 mmol) was added to 10% Pd/C (300 mg) in EtOH (50 mL). The mixture was hydrogenated at 40 psi and room temperature overnight. The catalyst was removed by filtration through Celite and the filtrate was concentrated under reduced pressure. The residue was crystallized from EtOH–Et_2O and recrystallized from EtOH to give 154 mg of **2** (75%): mp 195–196 °C; IR (KBr, HCl salt) 3200 (NH), 1595 (aromatic); ^1H NMR (CD_3OD, HCl salt) 1.90 (m, 2 H, Ar $CH_2CH_2CH_2NH$), 2.90–3.60 (m, 6 H, Ar $CH_2CH_2CH_2N$ and Ar CH_2CHN), 3.72 (s, 3 H, 1 × Ar OCH_3), 3.76 (s, 6 H, 2 × Ar OCH_3), 4.60 (m, 1 H, Ar CHN), 6.44 (s, 2 H, 2 × Ar H), 6.53 (s, 1 H, 1 × Ar H), 6.70 (s, 1 h, 1 × Ar H). Anal. ($C_{20}H_{25}NO_5$·HCl) C, H, N.

***N*-[3-[3,4-Bis(benzyloxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (9).** 3-[3,4-Bis(benzyloxy)phenyl]-1-aminopropane[18] (2.7 g, 7.8 mmol) and 3,4,5-trimethoxyphenylacetic acid (2.3 g, 7.8 mmol) in toluene (80 mL) were heated at reflux for 3 days with removal of H_2O by a Dean–Stark trap. The mixture was cooled and concentrated under reduced pressure and the residue was dissolved in $CHCl_3$ (100 mL). The $CHCl_3$ solution was washed with 10% HCl (2 × 100 mL), H_2O (100 mL), 10% Na_2CO_3 (2 × 100 mL), water (2 × 100 mL), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to give a solid which was crystallized from EtOAc to give 2.7 g of **9** (61%): mp 100–102 °C; IR (KBr) 3310 (NH), 1640 (C=O); ^1H NMR ($CDCl_3$) δ 1.74 (m, 2 H, Ar $CH_2CH_2CH_2NH$), 2.48 (t, 2 H, Ar $CH_2CH_2CH_2N$), 3.18 (m, 2 H, CH_2NH), 3.46 (s, 2 H, Ar CH_2CO), 3.83 (s, 9 H, 3 × Ar OCH_3), 5.12 (s, 4 H, 2 × Ar CH_2O), 5.45 (br, 1 H, NH), 6.44 (s, 2 H, 2 × Ar H), 6.51–6.92 (m, 3 H, 3 × Ar H), 7.30–7.50 (m, 10 H, 10 × Ar H). Anal. ($C_{34}H_{37}NO_6$) C, H, N.

1-[(3,4,5-Trimethoxyphenyl)methyl]-7,8-bis(benzyloxy)-2,3,4,5-tetrahydro-1*H*-2-benzazepine (7). Phosphorous oxychloride (1.76 g, 11.5 mmol) was added to *N*-[3-[3,4-bis(benzyloxy)phenyl]propyl]-3,4,5-trimethoxyphenylacetamide (2.12 g, 3.8 mmol) in CH_3CN (40 mL). The mixture was heated at reflux for 5 h, cooled, and concentrated to an oily residue. The residue was dissolved in THF (20 mL) and cooled to 0 °C. A 1 M solution of diborane in THF (60 mL) was added slowly over a period of 0.5 h. The mixture was heated at reflux overnight and cooled to 0 °C and MeOH (5 mL) was added slowly. The solution was concentrated under reduced pressure to give an oil which again was dissolved in MeOH and concentrated under reduced pressure to give an oil which solidified on standing. The solid was crystallized from absolute EtOH to give 1.2 g of **7** (58%): mp 91.5–93 °C (free base); IR (free base, neat) 3320 (NH), 1595 (aromatic); ^1H NMR (free base, $CDCl_3$) δ 1.70 (m, 2 H, Ar $CH_2CH_2CH_2NH$), 2.20 (br, 1 H, NH), 2.40–3.35 (m, 6 H, Ar$CH_2CH_2CH_2N$ and Ar CH_2CHN), 3.82 (s, 6 H, 2 × Ar OCH_3), 3.84 (s, 3 H, 1 × Ar OCH_3), 4.04 (dd, 1 H, Ar CHN, J = 9.3, 5.3 Hz), 5.06 (s, 2 H, 1 × Ar CH_2O), 5.12 (s, 2 H, 1 × Ar CH_2O), 6.42 (s, 2 H, 2 × Ar H), 6.78 (s, 1 H, 1 × Ar H), 6.80 (s, 1 H, 1 × Ar H), 7.23–7.55 (m, 10 H, 10 × Ar H). Anal. ($C_{34}H_{37}NO_5$) C, H, N.

Biological Methods. Materials. Phospholipase C from *Clostridium perfringens* and ADP were obtained from Sigma Chemical Co. (St. Louis, MO). [^{14}C]Serotonin (57 mCi/mmol) was supplied by Amersham (Arlington Heights, IL). [^{14}C]Arachidonic acid was supplied by New England Nuclear (Boston, MA). Chromatography paper (Whatman No. 1) and TLC plates (Linear K, LK6 250) were obtained from Whatman Chemical Separations Inc. (Clifton, NJ). U46619 was a gift from Dr. John Pike (Upjohn Laboratories, Kalamazoo, MI). The stereoisomers of trimetoquinol (TMQ), 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) were a gift from Dr. Yoshio Iwasawa, Tanabe Seiyaku Co. Ltd. (Tokyo, Japan).

β-Adrenergic Activities. Guinea pigs of each sex (weighing 300–500 g) were employed in all experiments. The isolation and procedures for testing of each compound in isolated guinea pig atria and trachea were identical with those described previously.[8,9] Responses were recorded on a Grass (Model 7) polygraph via FT-03 force displacement transducers. Cumulative dose–response curves were obtained according to the method of van Rossum.[28] Each drug concentration was added only after the effects of the previous concentration reached a maximum and remained constant. The final maximum concentration was considered to be that response at which further addition of a higher concentration of the testing compound did not increase the effect.

Platelet Antiaggregatory Activities. Blood was collected from normal human volunteers who reported to be free of medication for at least 10 days prior to blood collection. Washed platelets were prepared and suspended in a modified Tyrode's solution, pH 7.4, as previously described.[11] The platelet count was adjusted to 3×10^8/mL for aggregation, secretion, and biochemical studies. Platelet aggregation studies were performed according to the turbidometric method of Born[29] as modified by Mustard et al.[30] in a Payton Model 600 dual-channel aggregometer.

Secretion of the contents of platelet dense granules was measured by monitoring the release of radioactivity from platelets prelabeled with [^{14}C]serotonin as previously described.[11]

For studies of phospholipid metabolism, platelets were labeled with [^{14}C]arachidonic acid (0.5 μCi/mL). Platelet-rich plasma was incubated with the labeled arachidonic acid for 2 h at 37 °C. The platelet suspension was then washed as above.

In metabolism and aggregation experiments, platelets were incubated at 37 °C for 3 min prior to the addition of aggregating agents. This time served as the preincubation period for all compounds added to modify aggregation or phospholipid metabolism. Metabolism experiments were performed at 37 °C with each incubation containing 3×10^8 platelets in 0.5 mL. Incubations were stopped by addition of 1.8 mL of $CHCl_3$/MeOH (1:2, v/v) and extracted according to the method of Bligh and Dyer.[31] The phases were split by adding 0.6 mL of chloroform and 0.6 mL of 2 M KCl solution containing 5 mM EDTA. The lower $CHCl_3$ phase was removed and evaporated under nitrogen. Dried lipids were redissolved in $CHCl_3$ and spotted on formaldehyde-impregnated paper for separation of PI from other phospholipids. PI was separated on formaldehyde-treated paper by descending development using the upper phase of a mixture of 1-BuOH/CH_3CO_2H/H_2O/Et_2O (80:20:100:10 by volume).[32]

Lipids were visualized by iodine vapor and spots were scraped from the silica plates or cut from the formaldehyde-treated paper, placed in scintillation vials with an emulsion-type scintillation solution (Thrift-solve, Kew Scientific, Columbus, OH), and counted by liquid scintillation spectrometry.

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