Synthesis, β-Adrenergic Activity, and Platelet Antiaggregatory Activity of a Positional Isomer of Trimetoquinol: 1-(2′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline

Duane D. Miller,* Josef F. Bossart, Joseph R. Mayo, and Dennis R. Feller

Divisions of Medicinal Chemistry and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.
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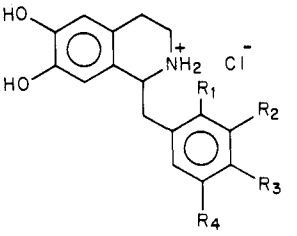
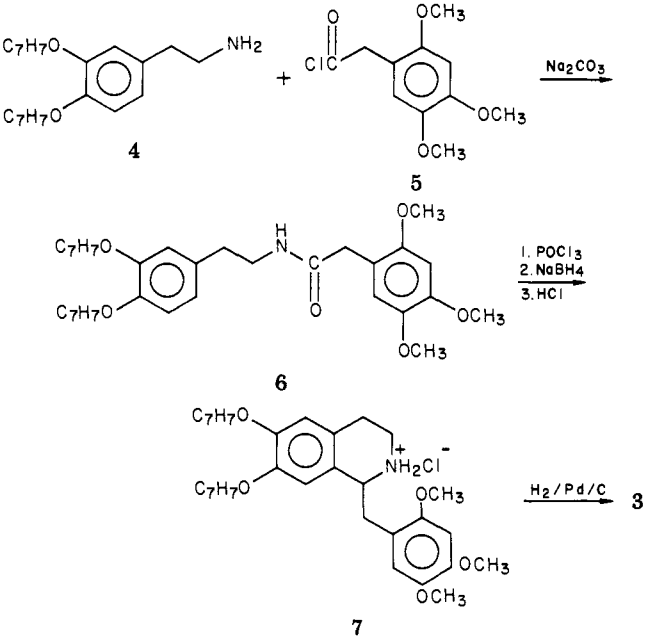
A positional isomer of trimetoquinol (1), 1-(2′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (3), was synthesized and found to possess less β-adrenergic activity than 1 in isolated guinea pig atrial and tracheal preparations. The analogue 3 was an effective antiaggregatory agent in human and rabbit platelet-rich plasma preparations, while 1 was effective only as an inhibitor of arachidonic acid induced aggregation in human platelets. These findings indicate that both qualitative and quantitative differences in biological activity have occurred as a result of changing the position of the methoxy groups on the 1-benzyl substituent of 1.

The potent bronchodilating activity of trimetoquinol (1) is well-known.[1] Considerable differences exist in the biological activity of the optical isomers of trimetoquinol and related tetrahydroisoquinolines.[2-7] It is known that the (*S*)-(−) isomer of trimetoquinol is the most potent β-adrenergic stimulant,[2-5] while the (*R*)-(+) isomer is the most potent platelet antiaggregatory agent.[6,7] In an attempt at determining the structural requirements of tetrahydroisoquinolines for adrenergic activity and as an aid in the development of selective adrenergic drugs, we have been investigating structural modifications of trimetoquinol.[5,8-10] Of the possible positional isomers in which the methoxy groups can be varied on the one benzyl substituent of 1, only 1-(2′,3′,4′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (HCl, 2) has been reported.[11] We now report the synthesis of a second positional isomer of 1, 1-(2′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (HCl, 3), the biological activity of this compound in β-adrenergic systems, and its interesting antiaggregatory activity in comparison to 1.



1, R_1 = H; R_2 = R_3 = R_4 = OCH_3
2, R_1 = R_2 = R_3 = OCH_3; R_4 = H
3, R_1 = R_3 = R_4 = OCH_3; R_2 = H

Scheme I



Chemistry. The synthesis of 1-(2′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was accomplished through a sequence very similar to that we have reported for the synthesis of 1.[5] The *N*-[[3,4-(dibenzyloxy)phenyl]ethyl]-2′,4′,5′-trimethoxyphenylacetamide was prepared by condensing 2,4,5-trimethoxyphenylacetyl chloride (**5**) with 2-[3,4-(dibenzyloxy)phenyl]ethylamine (**4**) according to the procedure of Cava and Buck[12] (see Scheme I). The acid chloride **5** was prepared from 2,4,5-trimethoxyphenylacetic acid[13] by treatment with thionyl chloride in benzene. The amide **6** was allowed to undergo the Bischler–Napieralski reaction using $POCl_3$ in acetonitrile to give the dihydroisoquinoline. This product was reduced with $NaBH_4$ to give the protected tetrahydroisoquinoline, which was isolated as the hydrochloride salt **7**. Hydrogenolysis of **7** using 10% Pd/C was carried out to give the desired trimetoquinol analogue 3.

Biological Results and Discussion. The comparative β-adrenoceptor activities, in vitro, of trimetoquinol (1) and the 2,4,5-trimethoxy analogue 3 were examined in guinea pig trachea and atria (Figure 1). As presented, trimetoquinol (1) possessed potent β-adrenoceptor actions in the tracheal (pD_2 = 7.8) and atrial (pD_2 = 8.8) preparations

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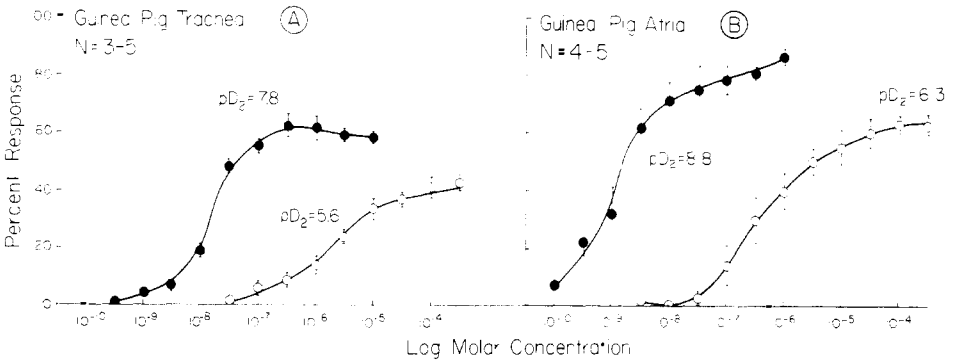

Figure 1. Log dose–response curves for (±)-trimetoquinol (1; ●) and the (±)-2,4,5-trimethoxy analogue (3; ○) on guinea pig tracheal and atrial preparations, in vitro. Key: frame A, guinea pig trachea; frame B, guinea pig atria. Each value represents the mean percent response of N = 3–5 ± SEM as indicated by the vertical bars.

and was 158- and 317-fold more effective as an agonist than analogue 3, respectively. The maximal β-adrenoceptor response produced by trimetoquinol (1) and analogue 3 was less than that found for the standard agonist, (−)-isoproterenol. Therefore, 1 and analogue 3 may be characterized as partial agonists in these preparations.

Yamato et al.[11] reported that 1-(2′,3′,4′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was less active than 1 as a bronchodilating agent. Coupled with the results of our studies with the 1-substituted 2,4,5-trimethoxybenzyl analogue (3) of 1, these findings strongly suggest that optimal β-adrenergic activity for 1-substituted tetrahydroisoquinolines is dependent upon the presence of an intact 3,4,5-trimethoxybenzyl group.

Recent studies have indicated that the blockade of collagen, thrombin, arachidonic acid (AA), or epinephrine mediated aggregation by 1 may be related to an antagonism of prostaglandin action.[14,15] In particular, it has been proposed that 1 may interfere with aggregation induced by cyclic endoperoxides or thromboxane A_2 formed from endogenous or exogenous AA. Using AA as an inducer of platelet aggregation, the comparative inhibitory activities of 1 and analogue 3 were investigated in human and rabbit platelet-rich plasma (PRP) preparations (Figure 2). Analogue 3 exhibited a dose-dependent and near maximal inhibition of AA-induced aggregation in both preparations, whereas trimetoquinol (1) was only an effective inhibitor of aggregation induced by AA in human platelets. Comparing potency, trimetoquinol (1) was 13-fold more potent than analogue 3 as an inhibitor of the aggregation induced by AA in human PRP.

Indomethacin, a known inhibitor of platelet prostaglandin synthesis,[16] was shown to block AA-induced aggregation in both human and rabbit PRP preparations (data not presented). Because of the qualitatively different results obtained with 1 and 3 on AA-induced aggregation, we are continuing to investigate the mode of action of these two compounds in human and rabbit platelet preparations.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. Spectral data were obtained using a Beckman 4230 spectrophotometer. Nuclear magnetic resonance spectra (NMR) were recorded either on a Varian A-60A (60 MHz) or Bruker HX-90E NMR spectrometer (90 MHz) in the pulse mode. Mass spectra were obtained with a Dupont Model 21-491 double-focusing mass spectrometer. Elemental analyses

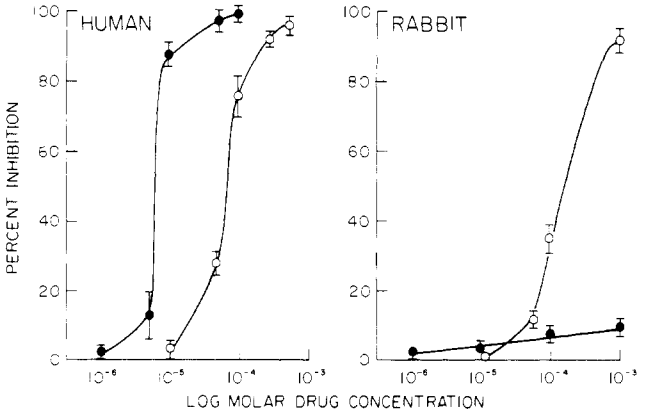

Figure 2. Inhibitory activity of (±)-trimetoquinol (1; ●) and the (±)-2,4,5-trimethoxy analogue (3; ○) against arachidonic acid induced aggregation in human and rabbit platelet-rich plasma preparations. Each value represents the mean percent inhibition of N = 3–5 ± SEM as indicated by the vertical bars. The concentration of arachidonic acid was 0.17 and 1 mM in rabbit and human PRP preparations, respectively.

were performed by Galbraith Laboratories, Inc., Knoxville, TN.

***N*-[[3,4-(Dibenzyloxy)phenyl]ethyl]-2′,4′,5′-trimethoxyphenylacetamide (6).** To a solution of 2 g (0.009 mol) of 2,4,5-trimethoxyphenylacetic acid in 100 mL of benzene was added 2 mL of thionyl chloride. The stirred solution was heated under reflux for 3 h. The mixture was then evaporated in vacuo to give 2.12 g of the acid chloride 5 [IR (neat) 1800 cm^{-1}; COCl]. The acid chloride was then dissolved in 50 mL of $CHCl_3$ and added dropwise, with mechanical stirring, to a mixture of 3.7 g (0.01 mol) of amine 4 in 100 mL of $CHCl_3$ and 2.0 g of $NaHCO_3$ in 100 mL of water. After the mixture was stirred at room temperature for 15 h, the $CHCl_3$ layer was separated and washed successively with 10% hydrochloric acid, saturated aqueous $NaHCO_3$, and water. After drying (Na_2SO_4), the filtrate was evaporated to give a brown oil which crystallized from benzene–ether. Recrystallization of the solid from benzene–ether gave 2.5 g of white solid amide 6. The remaining mother liquor was evaporated in vacuo and placed on a silica gel column using ether–ethyl acetate (7:3) and another 1.5 g of the amide 6 (82% yield) was isolated: mp 124–125 °C. Anal. ($C_{33}H_{35}NO_6$) C, H, N.

1-(2′,4′,5′-Trimethoxybenzyl)-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline (HCl, 7). A mixture of 1 g (1.8 mmol) of the amide 6 in 40 mL of acetonitrile, along with 452 mg of $POCl_3$ (2.9 mmol), was refluxed under nitrogen for 2 h. The mixture was allowed to cool to room temperature, the excess solvent and $POCl_3$ was evaporated in vacuo, and the resulting residue was dissolved in 100 mL of $CHCl_3$ and washed with aqueous 10% NaOH and water. After drying ($MgSO_4$), the filtrate was evaporated in vacuo to give 924 mg of an oil [IR (neat) 1625 cm^{-1}; C=N]. The oil was dissolved in 20 mL of MeOH and to this was added slowly 1 g of $NaBH_4$. The mixture was allowed to stir for 18 h at room temperature. The solvent was removed in vacuo, taken up in 50 mL of $CHCl_3$, washed with aqueous 10% NaOH

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and water, and dried ($MgSO_4$). The $CHCl_3$ layer was evaporated in vacuo to give 920 mg of an oil (97% yield). The oil (750 mg) was placed in a saturated HCl solution of MeOH, the solution was evaporated in vacuo, and the resulting solid was recrystallized from EtOH–EtOAc–Et_2O to give 450 mg of **7** (HCl): mp 184–185 °C. Anal. ($C_{33}H_{36}NO_5Cl$) C, H, N.

1-(2′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (HCl, 3). A solution of 400 mg (0.7 mmol) of **6** and 300 mg of 10% Pd/C in 200 mL of ethanol was hydrogenated on a Parr apparatus at 40 psi for 12 h. The reaction mixture was filtered, the solvent was removed in vacuo to a small volume and ether was added, and the resulting solid, 190 mg (71% yield), was collected. A small portion was recrystallized from ethanol–ether to give crystals, mp 256 °C. Anal. ($C_{19}H_{24}NO_5Cl$) C, H, N.

Biological Testing. Isolated Tracheal Strip Preparation.[5] Guinea pigs of either sex weighing 300–500 g were killed by a sharp blow on the head. The trachea of each animal was isolated and cleaned free of fatty tissue. From each guinea pig two spiral tracheal strips were prepared and mounted in a 12-mL jacketed muscle chamber containing a physiological solution maintained at 37 °C, through which a mixture of 95% O_2–5% CO_2 was bubbled. Drug-induced effects were recorded on a Grass polygraph (Model 7C) via a force-displacement transducer. Strips were allowed to equilibrate for 1–1.5 h before each experiment under a tension of 1 g. Carbachol (3×10^{-7} M) was used to increase the tone of each preparation, and cumulative dose–response curves were obtained for each drug. Individual plots of tracheal relaxation, expressed as a percent of the maximum relaxation obtained with 10^{-5} M (–)-isoproterenol added at the end of each experiment vs. log molar concentration of each drug, were prepared and the ED_{50} values determined individually. In all biological experiments, the ED_{50} values represent the concentration of each agonist required to produce a response equal to one-half of its maximal response in the appropriate system.

Isolated Right Atrial Preparation.[5] The atrium was dissected from extraneous tissue and placed in a 12-mL jacketed muscle bath. The atrium was allowed to equilibrate for a 1-h period in a physiological solution maintained at 37 °C, through which a mixture of 95% O_2–5% CO_2 was bubbled. The increase in atrial rate was recorded on a Grass polygraph (Model 7C) via a force-displacement transducer.

In each experiment, the atrium was exposed to a test dose of a drug and the atrial rate recorded during a 3-min period. Individual recordings were made at 1- and 3-min intervals. Cumulative dose–response curves were obtained for each analogue. The data were plotted on a log scale and the chronotropic responses expressed in terms of the maximum response obtained in the presence of 10^{-5} M (–)-isoproterenol added at the end of each experiment. ED_{50} values were determined from individual plots.

Platelet Aggregation. Human blood was taken by venipuncture from volunteers who reported being free of aspirin-containing medication for at least 14 days. Rabbit blood was collected by arterial puncture from the ear. The whole blood was combined and mixed with 3.8% trisodium citrate (9:1, v/v). Platelet-rich plasma was then prepared by centrifugation at 200*g* for 10 min at room temperature and used within 2 h of isolation. Platelet-poor plasma was obtained by centrifuging platelet-rich plasma for 10 min at 4000*g*. Platelet aggregation was monitored at 37 °C by nephelometry in a Chrono-log aggregometer (Model 330; Haverton, PA) with constant stirring at 1100 rpm. Platelets were incubated for 2 min at 37 °C prior to the initiation of aggregation. This time period also served as the incubation interval for modulators of the system. In all experiments, the minimal concentration of aggregating agent that produced irreversible aggregation was used.

Drugs. Stock solutions of arachidonic acid (NuChek Prep, Elysian, MN) were prepared in absolute ethanol and all other drugs in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.05% metabisulfite.

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Central Nervous System Activity of 7-Substituted 1-Azaphenoxathiin Analogues and Their Oxidation Products

Arthur J. Elliott,*

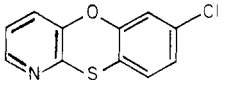
Medicinal Chemistry Department

Norman Eisenstein, and Louis C. Iorio

Pharmacology Department, Schering-Plough Research Division, Bloomfield, New Jersey 07003. *Received September 7, 1979*

A number of 7-substituted 1-azaphenoxathiins and their sulfone oxidation products have been synthesized and screened for central nervous system activity. Some of the compounds have antidepressant activity, with the most active, 7-(trifluoromethyl)-1-azaphenoxathiin 10,10-dioxide (**8**), having similar potency to imipramine.

Recently, Martin et al.[1-6] reported the synthesis and central nervous system (CNS) depressant activity of 7-chloro-1-azaphenoxathiin (**1**) and related compounds. The



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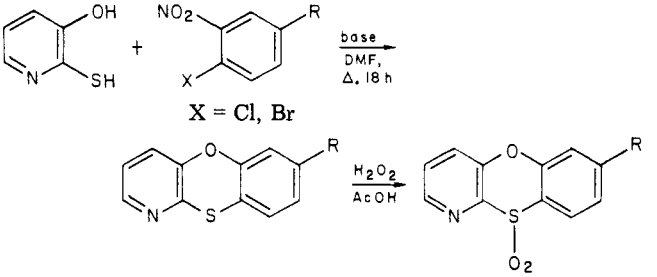
claim of phenothiazine-like antipsychotic activity for **1** was made on the basis of loss of spontaneous motor activity and drug-induced hypothermia in mice.[1,5] We report here our studies on this interesting ring system and the results obtained in five biological screens designed to uncover CNS activity.

Scheme I



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