Stereochemical Aspects and Metabolite Formation in the *in Vivo* Metabolism of the Psychotomimetic Amine, 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane

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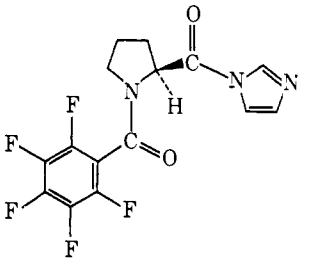
Our interest in structure–metabolism relationships of pharmacologically active 1-phenyl-2-aminopropanes has led to *in vivo* investigations of the fate of the psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (1). Compound 1 was resolved *via* its *o*-nitrotartranilate salts and the absolute configurations were found to be (*S*)-(+) and (*R*)-(−). Determination of the enantiomeric composition of unmetabolized amine excreted in the urine of rabbits treated with racemic 1 established the *R*/*S* ratio to be equal to or greater than 1. With the aid of 1-^{14}C (labeled at the benzylic position) it has been established that 1 was extensively metabolized by the rabbit and that of several suspected metabolites only 1-(2,5-dimethoxy-4-carboxyphenyl)-2-aminopropane was excreted to any great extent.

Since an appreciation of the biochemical events associated with psychotomimesis may further our understanding of altered CNS function and mental disease,[1] a number of studies designed to elucidate the mechanisms of action of psychotomimetic agents have been reported.[2] In an effort to explore metabolic parameters which may contribute to the dramatic differences in pharmacological activity observed for structurally similar 1-phenyl-2-aminopropane (amphetamine) derivatives,[3-5] we have initiated a program to compare the metabolic fate of selected members of this series. In this paper we report our results on the *in vivo* metabolism of the potent psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (1)[6,7] in rabbits.

As with several 1-phenyl-2-aminopropane derived drugs,[8] the pharmacological activities of the (+) and (−) forms of 1 differ, the psychotomimetic activity apparently residing in the (−) isomer.[9] Stereoselective metabolic processes are also well documented[10] and in the case of amphetamine have been implicated in the greater CNS stimulant activity of the *S* enantiomer.[11,12] As part of the present study we have examined the stereochemical control factors operating in the *in vivo* metabolism of 1 by determining the concentrations of **1a** and **1b**, the *R* and *S* enantiomers of 1, respectively, in the urine of animals treated intraperitoneally with racemic 1.

Methods. The enantiomeric composition of urinary 1 was calculated from a knowledge of the ratio of **1a** to **1b** and the total amount of **1a** and **1b** present in a particular sample. The ratio of enantiomers was determined by a glpc analysis of the diastereomeric amides **3a** and **3b** formed by the nonstereoselective condensation of the mixture of **1a** and **1b** with the chiral acid (*S*)-(−)-*N*-pentafluorobenzoylprolyl-1-imidazolide (**2**).[13] The total amount of **1a** and **1b** excreted was estimated by an isotope dilution analysis based on a knowledge of the amount and specific activity of the ^{14}C-labeled 1 administered to the animal and the specific activity of recovered 1 after dilution with a known amount of carrier 1 and purification *via* the *N*-benzoyl derivative **10**.

The assignment of the absolute configuration of 1 was achieved by first resolving the amine *via* the salts obtained with (−)- and (+)-*o*-nitrotartranilic acids[14] (**4a** and **4b**, respectively). The physicochemical and optical properties of the resolved amine hydrochlorides agree well with those recently published by Nichols, *et al.*, for the hydrochloride salts of (−)- and (+)-1 obtained by an asymmetric synthesis.[15] A comparison of the CD curves of the *N*-salicylidine derivatives **5a** and **5b** with the corresponding curves of the *N*-salicylidene derivatives of (*R*)-(−)- and (*S*)-(+)-amphetamine[16] established the absolute configuration of 1 to be (*R*)-(−)- and (*S*)-(+)-1a and -1b, respectively. The diastereomeric amide **3a** derived from 1a had the shorter retention time on SE-30 and OV-17 glpc columns.



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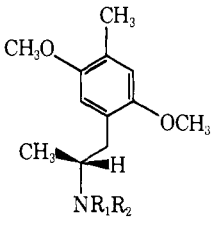
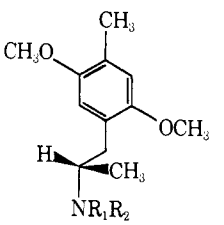
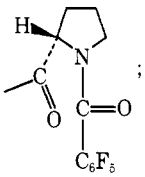


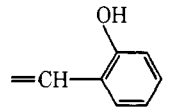
a



b

1, $R_1 = R_2 = H$

3, R_1 =  ; $R_2 = H$

5, R_1, R_2 = 

10, $R_1 = -CC_6H_5$; $R_2 = H$

The ^{14}C label introduced into amine 1 was located at the benzylic side-chain carbon in order to avoid loss of the label resulting from side-chain metabolic cleavage. *N*-Methylformanilide-*carbonyl*-^{14}C (**6**), prepared from *N*-methylaniline and $H^{14}COOH$, was employed in the conversion of 2,5-dimethoxytoluene (**7**) to 2,5-dimethoxy-4-methylbenzaldehyde-*carbonyl*-^{14}C (**8**). The absence of coupling of the two aromatic proton signals in the nmr spectrum of 8 established the para orientation of the newly introduced formyl group. The conversion of 8 to ^{14}C-labeled 1 followed the well-established phenylnitropropene route[17] except that AlH_3[18] instead of $LiAlH_4$ was

Table I. Enantiomeric Composition of Urinary Amine 1[a]

Dose, mg/kg	Urinary collection, hr	% dose excreted	% unchanged present	Enantiomeric composition of recovered amine (R/S)
5	0–24	75	2.0	1.0
	24–28	6	<0.5	
12.5	0–24	66	0.9	1.0
	24–28	13	<0.5	
21	0–24	54	0.6	1.7
	24–28	17		
22	0–20	*b*	*b*	1.4
	20–48	6	<0.5	1.4
29	0–48	74	1.0	1.1

[a]Male Dutch rabbits (1.5–2.0 kg) were injected ip with racemic 1-^{14}C and the urine was collected. Per cent of dose excreted is in terms of total radioactivity administered. Isotope dilution analyses provided the per cent of unchanged 1 expressed in terms of excreted ^{14}C. Enantiomeric composition was determined by derivatization with the prolyl reagent 2 and glpc analysis. [b]Leakage in metabolic cage prevented quantitative estimation.

employed to reduce 1-(2,5-dimethoxy-4-methylphenyl)-2-nitropropene-*1*-^{14}C (9) since inconsistent yields were obtained with $LiAlH_4$. The final product, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane-*1*-^{14}C hydrochloride (1-*1*-^{14}C·HCl), was obtained in 15% overall yield and had a specific activity of 96.4 μCi/mmol.

Results

Stereochemical Composition of Urinary 1. Dutch male rabbits were administered 1-^{14}C intraperitoneally and the urine was collected and analyzed in 0–24- and 24–48-hr fractions when possible. The salient data are summarized in Table I. At the various doses studied, an average of about 75% of the administered radioactivity was excreted after 48 hr. However, 2% or less of the excreted activity could be accounted for as unchanged drug. Differences in enantiomeric composition (R/S excreted > 1) were observed when relatively large amounts of racemic 1 were administered. To determine if metabolic or chemical racemization of 1a and/or 1b was occurring, the pure enantiomers were studied. However, glpc analysis with the prolyl reagent of the urinary amine excreted in each case established that no racemization had occurred. Thus, these data clearly establish that stereoselective processes are involved in the *in vivo* metabolism of 1 although, because of the small percentage of unmetabolized drug excreted, it is difficult to assess their quantitative significance. We do know that with rabbit liver homogenates the R/S ratios of unmetabolized amine 1 range from 1.5 (30% metabolized) to 4.0 (63% metabolized),† suggesting that stereochemically sensitive events may be of quantitative significance in the *in vivo* metabolism of 1.

Metabolite Identification. We have prepared several suspected metabolites of 1 and, by isotope dilution analyses, have determined their concentrations in the urine obtained from rabbits receiving 1-^{14}C. One would expect *a priori* that side-chain alterations would be most sensitive to the configuration about the chiral center. Therefore, the oxidative metabolism of 1 to the ketoxime 11 and the ketone 12 was considered to be a potentially important pathway. It has been reported that treatment of phenyl-2-propanone ketoxime with acid results in its rapid conversion to the corresponding ketone.[20] Consequently, acid treatment of the urine aliquot and isotope dilution analysis should provide an estimation of the combined amounts of ketone 12 and ketoxime 11. Synthetic ketone 12 was prepared by reduction of the nitrostyrene 9 with iron filings and acetic acid.[21] Less than 10% of the radioactivity administered could be accounted for in the neutral fraction isolated from the 24-hr urine and the recrystallized ketone was not radioactive.

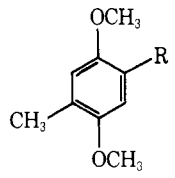
It has been suggested[10] that phenyl-2-propanone, a major metabolite of amphetamine in the rabbit, may be further metabolized to benzoic acid. We prepared the corresponding compound 2,5-dimethoxy-4-methylbenzoic acid (13) by oxidation of aldehyde 8 with potassium permanganate. The glycyl derivative 14 was also synthesized and the urinary concentrations of both compounds were estimated by isotope dilution analysis. In both instances essentially no radioactivity was observed in the reisolated and recrystallized substances. Thus, we were unable to establish side chain metabolism as a major pathway.

In an attempt to determine the extent of oxidative metabolism of the C-4 methyl substituent of compound 1 in the rabbit, a pathway already established for this amine in the rat[7b] and also described for other compounds containing arylmethyl groups,[22,23] we undertook the synthesis of the C-4 hydroxymethyl 15 and C-4 carboxy 16 derivatives of compound 1. The preparation of the 1-(2,3-dimethoxy-4-carboxyphenyl)-2-aminopropane (16) has been reported by Ho and coworkers.[7a] However, these workers did not characterize the amino acid itself. Since our isotope dilution analysis required the pure amino acid, a modified procedure for the preparation of this compound was followed. The known phthalimidoaldehyde 17 was oxidized under basic conditions with silver oxide to the corresponding carboxyl compound. The reaction conditions also led to the partial hydrolysis of the imide moiety to yield the *N*-phthaloyl acid 18. Subsequent hydrolysis with HCl yielded after crystallization the pure amino acid 16. Isotope dilution analysis through the benzamide derivative 19 of compound 16 established that this material represented 38% of the total dose or 51% of the total 24-hr urinary radioactivity.

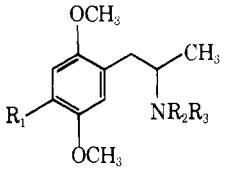
The possibility that the hydroxymethyl derivative 15 is a major urinary metabolite of amine 1 was also considered. The synthesis of the hydroxymethyl compound was attempted by sodium borohydride reduction of the phthalimidoaldehyde 17 in aqueous base. This reaction yielded the *N*-phthaloylhydroxymethyl intermediate 20 as expected. Attempted acid cleavage of the phthaloyl group gave a product with an nmr spectrum lacking the signal for the benzyl alcohol methylene protons and exhibiting an unexpected singlet at δ 3.90 ppm, suggestive of a diphenylmethane moiety. Attempted crystallization of the crude reaction product was unsuccessful. Treatment of the isolate with benzoyl chloride, however, gave a solid which by chemical ionization mass spectral analysis showed a parent ion (MH^+) at 611, consistent with a structure composed of two molecules of the desired alcohol 15 minus the elements of formaldehyde and water. The nmr, mass spectral, and microanalytic data are consistent with the bis-benzoylated self-condensation product 23. The original adduct 22 presumably arises from an acid-catalyzed condensation reaction to form intermediate 21 which then loses formaldehyde.[24] Prolonged treatment of the intermediate *N*-phthaloyl alcohol 20 with base did provide the desired alcohol. Isotope dilution analysis was accomplished *via* the bis-benzoyl derivative 24. Less than 0.4% of the administered radioactivity could be assigned to this compound.

Based on the results of these studies, it is clear that as

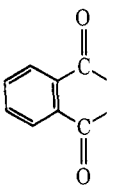
†P. S. Callery and N. Castognoli, Jr., unpublished results.



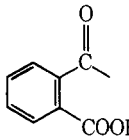
11, R = $CH_2C(=NOH)CH_3$
12, R = $CH_2C(=O)CH_3$
13, R = COOH
14, R = $C(=O)NHCH_2COOH$



15, $R_1 = CH_2OH$; $R_2 = R_3 = H$
16, R_1 = COOH; $R_2 = R_3 = H$

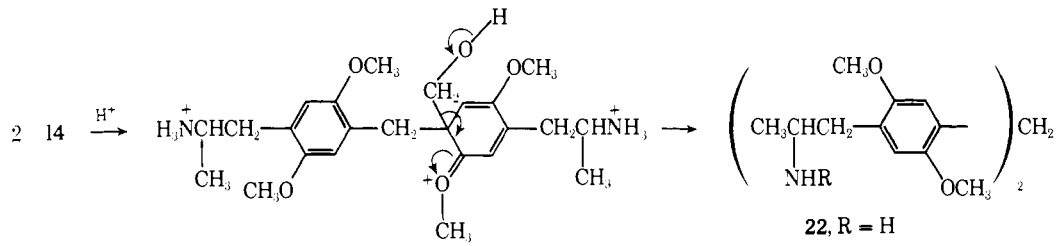


17, R_1 = CHO; R_2, R_3 =



18, R_1 = COOH; R_2 = (A); $R_3 = H$

19, R_1 = COOH; $R_2 = C_6H_5CO$; $R_3 = H$
20, $R_1 = CH_2OH$; R_2 = A; $R_3 = H$
21, $R_1 = C_6H_5C(=O)OCH_2$; $R_2 = C_6H_5C(=O)$; $R_3 = H$



21

22, R = H
23, R = $C(=O)C_6H_5$

with amphetamine the rabbit metabolizes amine 1 extensively. Unlike amphetamine, however, the side-chain oxidative pathway, leading to the formation of the benzyl methyl ketone 12 and benzoic acid derivative 13, is a minor process. Instead, oxidation of the toluene methyl group represents a principal metabolic route. Additional details are required to account for the remaining 60% of the administered radioactivity and current efforts are being directed to this question. Furthermore, the potential qualitative and quantitative significance of stereosensitive processes in the metabolism of 1 suggested by the greater amount of **1b** *vs.* 1a excreted by the rabbit is under study.

Experimental Section

Unless otherwise stated, reactions were run under a N_2 atmosphere and solvents were removed under vacuum on a rotary evaporator. Melting points were determined with a Thomas-Hoover Uni-Melt stirring oil capillary tube melting point apparatus and are uncorrected. Gas–liquid partition chromatography was performed on a Varian Aerograph Model 2100-00 Life Sciences gas chromatograph. U-Shaped 2 m × 2 mm i.d. Pyrex columns packed with 3% SE-30 or 3% OV-17 on acid washed, DMCS-treated Chromosorb W were used at a column temperature of 185° for SE-30 and 245° for OV-17. Purity of radioactive compounds was verified by tlc on alumina (Eastman Chromatogram 6063) with benzene or benzene-acetic acid (5:1) and by scanning the chromatogram with a Varian-Berthold Model LB2722-10 radio scanner. Liquid scintillation counting of samples in 10 ml of Aquasol (New England Nuclear) was performed on a Packard Tricarb Model 3375. All values were corrected for efficiency by toluene-^{14}C internal standard (New England Nuclear) or by automatic external standard. Glpc peak intensities were measured on a Du Pont Curve Resolver Model 310 or by cutting and weighing. The infrared spectra were measured with a Perkin-Elmer Model 337 infrared spectrophotometer. Proton magnetic resonance spectra were determined at 60 MHz with a Varian Model A-60A pmr spectrometer. The chemical shifts are expressed in δ values (ppm) relative to either TMS or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. In the presentation of the pmr spectra, the following notations are used: s = singlet, d = doublet, and comp m = complex multiplet.

The chemical ionization mass spectra[25] were obtained with a modified AEI Model MS902 double focus mass spectrometer equipped with a direct inlet system. Isobutane was used as the reactant gas at 0.7 Torr with a source temperature of 210°. Electron-impact mass spectra were obtained by direct insertion on an AEI MS-12 operated at a resolving power of 1000, accelerating potential 8 kV, ionizing potential 50 eV, and source temperature 200°.

***N*-Methylformanilide-*carbonyl*-^{14}C (6).** A mixture of formic acid-^{14}C (0.92 g, 19.9 mmol, 2 mc) and *N*-methylaniline (4.3 g, 40.2 mmol) was allowed to react at room temperature in the dark for 84 hr. The reaction mixture was transferred to a short-path still and water and unreacted formic acid were removed at 100–110° (760 mm). The product distilled at 110–120° (5 mm). Further purification was achieved by partitioning the distillate between Et_2O (50 ml) and 10% HCl (10 ml). After extracting the aqueous layer a second time with Et_2O, the combined extracts were dried (Na_2SO_4) and removed under vacuum. Short-path distillation provided pure 6 (1.39 g, 11.5 mmol, 52%): bp 110–120° (5 mm).

2,5-Dimethoxy-4-methylbenzaldehyde-7-^{14}C (8). To a stirred solution of 6 (1.39 g, 11.5 mmol) was added dropwise freshly distilled $POCl_3$ (1.8 g, 11.8 mmol) followed 1 hr later by 2,5-dimethoxytoluene (7, 3.0 g, 19.7 mmol). The stirred reaction mixture was maintained at 70° for 4 hr. After cooling, 10% NaOAc (15 ml) was added and the mixture was stirred at room temperature overnight. The reddish brown precipitate which separated was extracted into Et_2O (2 × 50 ml). The combined Et_2O layers were evaporated, and to the residue was added $NaHSO_3$ reagent (32% $NaHSO_3$ in 20% aqueous EtOH). After 5 min of vigorous shaking, 250 ml of H_2O was added and the resulting solution was washed with Et_2O (200 ml). The pH of the aqueous layer was adjusted to 12 with 10% NaOH and the solution was extracted with Et_2O (2 × 125 ml). The combined Et_2O layers were dried (Na_2SO_4) and evaporated to yield 1.17 g of pure aldehyde 8 (6.5 mmol, 56.5%): mp 82–83° (lit.[26] mp 84–85°); pmr ($CDCl_3$) δ 2.26 (s, $ArCH_3$), 3.80 and 3.87 (s, 6, OCH_3), 6.80 and 7.23 (s, 2, Ar H), 10.73 ppm (s, CHO).

1-(2,5-Dimethoxy-4-methylphenyl)-2-nitropropene-*1*-^{14}C (9). A mixture of 8 (1.17 g, 6.5 mmol), NH_4OAc (0.23 g, 3.0 mmol), and nitroethane (3 ml, 40 mmol) was held at reflux for 2 hr. After cooling the mixture was concentrated to dryness and the residue was recrystallized from 2-propanol–water to give pure 9 (1.15 g, 4.9 mmol, 75%): mp 85–87°; pmr ($CDCl_3$) δ 2.29 (s, $ArCH_3$), 2.43 (s, $NCCH_3$), 3.86 (s, 6, OCH_3), 6.82 (s, 2, Ar H), 8.30 ppm (s, olefinic H). *Anal.* ($C_{12}H_{15}NO_4$) C, H, N.

(*R,S*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane-*1*-^{14}C Hydrochloride (1·HCl). Into a 250-ml three-neck flask equipped with a magnetic stirrer, pressure equalizing dropping funnel, and dry N_2 system were placed $LiAlH_4$ (0.68 g, 18 mmol) and freshly distilled anhydrous THF (50 ml). The system was purged with dry N_2 and cooled to 3°. To this cooled, stirred sus-

pension was slowly added 100% H_2SO_4 (0.93 g, 9 mmol). The resulting mixture was stirred for 30 min at 3° to complete reagent formation following which a 100-ml dry THF solution of compound **9** (0.82 g, 4.5 mmol) was added over a 30-min period. After an additional 2 hr at 3° followed by 4 hr at room temperature, 1.6 ml of distilled water was added carefully to destroy the excess reagent, followed by 1.6 ml of 15% NaOH and 4.8 ml of water. To complete salt formation, 9.0 ml of 15% NaOH was added and the mixture was stirred for 15 min and then filtered. The filter cake was digested 30 min with Et_2O and the organic solvents were combined and dried over K_2CO_3 and concentrated to give the free amine. The crude product in 100 ml of anhydrous Et_2O was saturated with dry HCl and after cooling, the Et_2O was decanted and the solid hydrochloride was crystallized from 2-propanol to yield pure amine hydrochloride (0.55 g, 2.25 mmol, 64.3%): mp 185–188° (lit.[26] mp 184–185°); 96.4 μCi/mmol (8.8×10^5 dpm/mg). The benzamide **10** used in the isotope dilution analyses was obtained by stirring a mixture of unlabeled **1** (0.5 g, 2.04 mmol), benzoyl chloride (0.5 ml, 4.0 mmol) in $CHCl_3$ (5 ml), and 5% NaOH (20 ml) for 12 hr. The $CHCl_3$ was separated and the aqueous layer was extracted with 10 ml of $CHCl_3$. The combined, dried ($MgSO_4$) organic solvents were removed to give crude **10** (0.60 g, 1.9 mmol, 94%, mp 152–160°). Pure (*R,S*)-1-(2,5-dimethoxy-4-methylphenyl)-2-benzamidopropane (**10**, 0.38 g, 1.2 mmol, 61%) was obtained by crystallization from 2-propanol: mp 169–170°; pmr ($CDCl_3$) δ 1.30 (d, J = 6.5 Hz, CCH_3), 2.20 (s, $ArCH_3$), 2.77–3.05 (comp m, CH_2), 3.77 (s, OCH_3), 3.82 (s, OCH_3), 3.97–4.65 (comp m, NCH), and 6.50–7.90 ppm (comp m, 7, Ar H); ir (KBr) ν_{max} 1645 cm^{-1} (C=O). *Anal.* ($C_{19}H_{23}NO_3$) C, H, N.

Resolution of (*R,S*)-1. The free base **1** (4.1 g, 20.0 mmol) [obtained by extracting (3×50 ml of Et_2O) a solution of **1**·HCl (5.0 g, 20.4 mmol) in aqueous 10% NaOH (25 ml)] in absolute EtOH (50 ml) was treated with a solution of (+)-*o*-nitrotartranilic acid[14] (2.70 g, 10.0 mmol) in 95% EtOH (75 ml) and the resulting mixture was allowed to stand overnight at room temperature. The salt which formed (4.7 g) was shown to be 70% *S* (see under *In Vivo* Studies for procedure used to determine enantiomeric composition). After four recrystallizations from 95% EtOH the enantiomerically pure salt was obtained (1.4 g, 3.10 mmol, 31%). The free base was liberated by extracting a pH 10 solution of the tartranilate salt with Et_2O (3×50 ml). After drying over K_2CO_3, HCl gas was passed through the Et_2O to precipitate the HCl salt of **1b** (0.56 g, 2.3 mmol, 11.5%): mp 198–200° (lit.[15] mp 204–205°); $[\alpha]^{25}D$ +17.2° (*c* 2.5, H_2O) (lit.[15] +17.2°). *Anal.* ($C_{12}H_{19}NO_2 \cdot HCl$) C, H, N. The combined mother liquors containing amine **1** enriched with **1a** were worked up to yield the pure base which was treated with (−)-*o*-nitrotartranilic acid and the resulting salt was recrystallized four times to yield the enantiomerically pure product in 30% yield. The hydrochloride salt of **1a** (obtained in 14% yield) had mp 200–202° (lit.[15] mp 204–205°); $[\alpha]^{25}D$ −17.6° (*c* 2.5, H_2O) (lit.[15] −17.2°). *Anal.* ($C_{12}H_{19}NO_2 \cdot HCl$) C, H, N.

Preparation of Salicylidene Derivatives. The following general procedure was used. A solution of the resolved amine (10 mmol) and salicylaldehyde (10 mmol) in dry C_6H_6 was heated under reflux for 45 min with continuous removal of H_2O (Dean-Stark trap). The yellow oil obtained after removal of the solvent was crystallized from absolute EtOH to give the pure products in 20–25% yield. The melting points of salicylidines obtained from (*R*)- and (*S*)-1-phenyl-2-aminopropane (amphetamine) were 57.5–58.5° (lit.[16] mp 58–60°). (*R*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane-*N*-salicylidene (**5a**) had mp 58–59.5°. *Anal.* ($C_{19}H_{23}NO_3$) C, H, N. (*S*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane-*N*-salicylidene (**5b**) had mp 58.5–61°. *Anal.* ($C_{19}H_{23}NO_3$) C, H, N.

Circular dichroism spectra were determined in 95% EtOH at 25° at 1 mg/100 ml using a Jouan dichrograph instrument. Molecular elipticities [θ] are reported for the highest and lowest wavelengths measured as well as for peaks and troughs.

(*S*)-1-Phenyl-2-aminopropane-*N*-salicylidene: $[\theta]_{470}$ 0, $[\theta]_{400}$ +2840, $[\theta]_{359}$ +1580, $[\theta]_{314}$ +14,400, $[\theta]_{276}$ +3470, $[\theta]_{252}$ +28,400, $[\theta]_{235}$ 0.

(*R*)-1-Phenyl-2-aminopropane-*N*-salicylidene: $[\theta]_{470}$ 0, $[\theta]_{400}$ −2050, $[\theta]_{358}$ −473, $[\theta]_{313}$ −12,900, $[\theta]_{276}$ −2370, $[\theta]_{251}$ −27,000, $[\theta]_{235}$ 0.

(*S*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane-*N*-salicylidene (**5b**): $[\theta]_{470}$ 0, $[\theta]_{402}$ +7250, $[\theta]_{353}$ +3520, $[\theta]_{311}$ +27,700, $[\theta]_{283}$ +3520, $[\theta]_{251}$ +17,000, $[\theta]_{283}$ +3520, $[\theta]_{251}$ +17,000, $[\theta]_{235}$ +11,600.

(*R*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane-*N*-salicylidene (**5a**): $[\theta]_{470}$ 0, $[\theta]_{402}$ −5800, $[\theta]_{353}$ −1450, $[\theta]_{311}$ −26,300, $[\theta]_{283}$ −1450, $[\theta]_{251}$ −17,000, $[\theta]_{235}$ −7245.

1-(2,5-Dimethoxy-4-methylphenyl)-2-propanone (12). Into a 500-ml, three-neck, round-bottom flask equipped with a heating mantle, mechanical stirrer, and condenser were placed powdered iron (10.4 g, 186 mmol) and glacial HOAc (20 ml). The mixture was heated to reflux and 1-(2,5-dimethoxy-4-methylphenyl)-2-nitropropene (4.9 g 20.7 mmol) was added as the solid. The mixture was held at reflux with vigorous stirring for an additional 2 hr and then was filtered by suction through a bed of wet Celite. The solids were washed with 300 ml of H_2O and 300 ml of Et_2O. The aqueous phase was twice extracted with 100-ml portions of Et_2O and the combined organic extracts were washed with 2×100 ml of saturated K_2CO_3 solution, dried over $MgSO_4$, and concentrated to give 3.3 g of red oil which was distilled under vacuum [111–115° (0.5 mm)] to give a pale green solid. Crystallization from C_6H_6 gave 2.8 g (13.5 mmol, 65%) of colorless crystals: mp 57–59° (lit.[7b] mp 49–51°, C_6H_{14}); pmr ($CDCl_3$) δ 2.12 (s, CH_3), 2.23 (s, $ArCH_3$), 3.63 (s, CH_2), 3.74 (s, 6, OCH_3), 6.65 (s, Ar H), and 6.72 ppm (s, Ar H); ir (KBr) ν_{max} 1730 cm^{-1} (C=O); electron-impact mass spectrum, m/e 208 M^+ (51), 165 (100), 135 (53), 28 (47).

2,5-Dimethoxy-4-methylbenzoic Acid (13). To a stirred suspension of 2,5-dimethoxy-4-methylbenzaldehyde (**8**, 7.2 g, 40 mmol) in 150 ml of H_2O maintained at 70–80° was added a solution of $KMnO_4$ (9.0 g, 56 mmol) in 180 ml of H_2O over a 45-min period. After an additional hour, the pH was adjusted to 10 with aqueous 10% NaOH and the resulting solution filtered hot. The MnO_2 precipitate was washed with 3×20 ml of hot H_2O. Upon cooling of the combined filtrates, unreacted aldehyde separated and was collected. Acidification of this filtrate with 10% HCl gave the acid **13** (4.0 g, 21 mmol, 51% based on starting aldehyde). Crystallization from water provided the analytical sample: mp 122–124°; pmr ($CDCl_3$) δ 2.28 (s, $ArCH_3$), 3.84 (s, OCH_3), 4.04 (s, OCH_3), 6.92 (s, Ar H), 7.55 (s, Ar H), 9.67 ppm (s, COOH); ir ($CHCl_3$) ν_{max} 1740 cm^{-1} (C=O). *Anal.* ($C_{10}H_{12}O_4$) C, H, N.

***N*-2,5-Dimethoxybenzoylglycine (14).**‡ To a cooled solution of the above benzoic acid **13** (1.0 g, 5.1 mmol) in anhydrous THF (15 ml) was added dropwise under N_2 with stirring over a 1-hr period a solution of carbonyldiimidazole (0.83 g, 5.1 mmol) in anhydrous THF (20 ml). After an additional 1.5-hr period of stirring in the cold, glycine (0.57 g, 7.7 mmol) was added and the resulting mixture was stirred for 6 days at room temperature. Removal of the THF under vacuum left an oily residue which in 20 ml of hot EtOH left solid, unreacted glycine. After filtration and removal of solvent, the residue in 20 ml of CH_2Cl_2 was extracted with 3 *M* K_2CO_3 (3×10 ml). The extract was washed once with CH_2Cl_2 (35 ml) and then the pH was adjusted to 2 with 10% HCl. Standing overnight at room temperature gave a white solid which was collected and combined with a second crop obtained after cooling the filtrate. Recrystallization from C_6H_6 provided 0.69 g (2.7 mmol, 53%) of pure **14**: mp 184–185°; pmr ($CDCl_3$) δ 2.28 (s, $ArCH_3$), 3.86 (s, OCH_3), 3.97 (s, OCH_3), 4.33 (d, J = 6 Hz, CH_2; after exchange with D_2O d collapses to s), 6.80 (s, Ar H), 8.71 (t, NH; exchange with D_2O), 10.3 ppm (s, COOH; exchanges with D_2O); ir (KBr) ν_{max} 3350 (NH), 1740 (carboxyl C=O), 1650 cm^{-1} (amide carbonyl); uv (H_2O) λ_{max} 305 nm (ε 4800), 241 (9800). *Anal.* ($C_{12}H_{15}NO_5$) C, H, N.

(*R,S*)-1-(2,5-Dimethoxy-4-carboxy)-2-aminopropane Hydrochloride (16·HCl). A mixture of the phthalimidoaldehyde **17** (5.0 g, 14.2 mmol), prepared by the method of Ho, *et al.*,[7a] Ag_2O (6.38 g, 27.5 mmol), and NaOH (5.07 g, 127 mmol) in 85 ml of H_2O was stirred overnight at room temperature. After heating for 1.5 hr on a steam cone, the reaction mixture was allowed to cool and was then filtered. At 0°, the filtrate was acidified with 10% HCl to pH 1 and the resulting white precipitate (4.3 g, 11.1 mmol, 78%, mp 178–181°) was crystallized from EtOH to yield pure (*R,S*)-*N*-(2-carboxybenzoyl)-2,5-dimethoxy-4-(2-aminopropyl)benzoic acid (**18**, 2.9 g, 7.5 mmol, 53.5%): mp 186–188°; pmr (DMSO-d_6) δ 1.13 (d, J = 6.5 Hz, CCH_3), 2.65–3.05 (comp m, CH_2), 3.77 (s, 6, OCH_3), 3.95–4.65 (comp m, NCH), 7.0–7.96 ppm

‡Attempted condensation of the acid chloride of **13** with glycine gave instead of the desired amide **14** a yellow solid, mp 147–149° (EtOH), which proved to be 2,5-dimethoxy-3-carboxy-6-methylphenyl 2,5-dimethoxy-4-methylphenyl ketone (**1**): nmr ($CDCl_3$) δ 2.3 (s, 6, $ArCH_3$), 3.80 (s, 6, OCH_3), 3.83 (s, 6, OCH_3), 6.82 (s, 2 Ar H), 7.50 ppm (s, 2 Ar H); ir (KBr) λ_{max} 1780 (ketone C=O), 1720 cm^{-1} (carboxyl C=O); uv (EtOH) λ_{max} 332 nm (ε 9650), 251 (15,000); uv (EtOH–NaOH) λ_{max} 302 nm (ε 7000), 280 sh (14,200); electron-impact mass spectrum (rel intensity) m/e 374 (MH^+, 100), 330 ($M - CO_2$, 20). *Anal.* ($C_{20}H_{22}O_7$) C, H, N.

(comp m, 6, Ar H); ir (KBr) ν_{max} 1720 (carboxyl C=O), 1650 cm^{-1} (amide C=O). *Anal.* ($C_{20}H_{21}NO_7$) C, H, N. A suspension of the above amido acid (1.2 g, 3.1 mmol) in 25 ml of 10% HCl was heated at reflux for 3 hr and after cooling the mixture was washed with 3 × 40 ml of EtOAc. Removal of the H_2O *in vacuo* yielded a brown oil which upon trituration with Et_2O gave crude solid amino acid HCl salt (0.83 g, 3.0 mmol, 98%, mp 80–135°). Crystallization from CH_3CN provided pure **16** as its HCl salt (0.2 g, 0.73 mmol, 23%): mp 196–198°; pmr (D_2O) δ 1.47 (d, J = 6.5 Hz, CCH_3), 2.85–3.30 (comp m, CH_2), 3.65–4.1 (comp m, NCH), 4.03 (s, 6, OCH_3), 7.10 (s, Ar H), and 7.28 ppm (s, Ar H); ir (KBr) ν_{max} 1730 cm^{-1} (C=O). *Anal.* ($C_{12}H_{18}NO_4Cl$) C, H, N, Cl. The benzamide **19** of the amino acid **16** (0.2 g, 0.7 mmol) was prepared with benzoyl chloride (0.2 g, 1.7 mmol) according to Ho, *et al.*[7a] The pure benzamide (0.16 g, 64%) had mp 183–184° (lit.[7a] 185–186°); pmr (DMSO-d_6) δ 1.30 (d, J = 6.5 Hz, CCH_3), 2.85–3.10 (comp m, CH_2), 3.83 (s, 6, OCH_3), 4.10–4.65 (comp m, NCH), 6.98–8.00 ppm (comp m, 7, Ar H).

(*R,S*)-*N*-(**2-Carboxybenzoyl**)-1-(**2,5-dimethoxy-4-hydroxymethylphenyl**)-**2-aminopropane** (**20**). A solution of $NaBH_4$ (0.36 g, 9.5 mmol) in 1 *N* NaOH (45 ml) was stirred with the *N*-phthalimidoaldehyde 17[7b] (3.0 g, 8.4 mmol) at room temperature for 3 hr, followed by heating on a steam cone for 2 hr. After cooling, the suspension was filtered and the filtrate was acidified to pH 1 with 10% HCl. The white precipitate which formed was collected (2.6 g, 85%, mp 156–159°). Recrystallization from absolute EtOH–Et_2O gave 1.4 g (3.8 mmol, 46%) of pure **20**: mp 171–172°; pmr (DMSO-d_6) δ 1.14 (d, J = 6.5 Hz, CH_3), 2.62–2.98 (comp m, $ArCH_2C$), 3.73 and 3.78 (s, 6, OCH_3), 4.52 (s, $ArCH_2O$), 6.80–7.95 ppm (comp m, 6, Ar H). *Anal.* Calcd for $C_{20}H_{23}NO_6$: C, 64.33; H, 6.21; N, 3.75. Found: C, 63.86; H, 6.30; N, 3.82.

Bis[2,5-dimethoxy-4-(2-aminopropyl)phenyl]methane (**22**). A suspension of the phthaloyl alcohol **20** (0.86 g, 2.2 mmol) in 10% HCl (25 ml) was held at reflux for 2 hr. After cooling, the mixture was extracted with EtOAc (2 × 50 ml). The combined EtOAc layers were dried (Na_2SO_4) and evaporated to give an oil (0.5 g, 1.3 mmol, 57%), the nmr of which showed the presence of mainly compound **22**: pmr ($CDCl_3$) δ 1.10 (d, J = 6.0 Hz, 6, CH_3), 1.72 (s, 4, NH), 2.35–2.82 (comp m, 4, CH_2), 2.89–3.38 (comp m, 2, CHN), 3.68 (s, 6, OCH_3), 3.79 (s, 6, OCH_3), 3.91 (s, $ArCH_2Ar$), 6.69 ppm (s, 4, Ar H). Compound **22** was further characterized as its bis-benzoyl derivative **23** prepared as described above for amine 1 and crystallized from EtOH: mp 196–198°; pmr ($CDCl_3$) δ 1.30 (d, J = 6.5 Hz, 6, CH_3), 2.65–3.03 (comp m, 4, CH_2), 3.65 and 3.74 (s, 6, OCH_3), 3.90 (s, $ArCH_2Ar$), 4.01–4.66 (comp m, 2, CHN), 6.51–7.83 ppm (comp m, 14, Ar H). *Anal.* ($C_{37}H_{42}N_2O_6$) C, H, N.

(*R,S*)-1-(**2,5-Dimethoxy-4-hydroxymethylphenyl**)-**2-aminopropane** (**18**). The hydroxymethyl derivative **20** (177 mg, 0.47 mmol) in 20% NaOH (35 ml) was heated under reflux for 18 hr (N_2 atmosphere). After cooling, the reaction mixture was extracted with $CHCl_3$ (3 × 20 ml) and the extract was washed once with H_2O and then dried (K_2CO_3). Removal of the solvent gave an oil (35 mg, 0.16 mmol, 38%) which crystallized on standing: mp 90–92° (lit.[7a] mp 92–95°). The bis-benzoyl derivative, compound **24**, used in the isotope dilution analysis, was prepared in 82% yield in pyridine with benzoyl chloride and crystallized from EtOH: mp 162–163°. *Anal.* ($C_{26}H_{27}NO_5$) C, H, N.

In Vivo **Studies.** Male Dutch rabbits (6 months, 1.5–2.0 kg) housed in a metabolic cage were given ip racemic 1-^{14}C in normal saline in doses ranging from 5 to 29 mg/kg (4.4×10^6–25.6×10^6 dpm/kg). For stereochemical determinations, the 0–24- and 24–48-hr urine collections were divided into two parts, a 10% aliquot for isotope dilution analysis and the remainder for determining the enantiomeric composition of unchanged amine. In the study used to estimate metabolite formation, 1-^{14}C (8.36 mg, 7.36×10^6 dpm) was administered to a 1.5-kg rabbit. The 0–24-hr urine collection (41 ml, 1.01×10^6 dpm or 74.9% of the administered dose) was diluted to 50 ml with H_2O and then frozen in 5-ml aliquots which were subsequently used for the various isotope dilution analyses.

Enantiomeric Composition Determinations. The urine sample to be analyzed (pH adjusted to 12 with 10% NaOH) was extracted with hexane (3 × 40 ml) and the combined extracts were back extracted with 10% HCl (2 × 10 ml). The combined HCl extracts were treated with 10% NaOH (to pH 12) and were extracted with hexane (3 × 40 ml). After removal of the solvent and azeotropic drying of the residue with dry C_6H_6 (10 ml), the residue was treated with the prolyl reagent **2** (0.5–1 mg) in dry benzene (0.1 ml). After gentle warming, the mixture was analyzed by glpc to determine the ratio of **3b** to **3a** from which the enantiomeric composition was calculated. When urine obtained from a rabbit not receiving amine 1 was analyzed in this way, no peaks were observed where **3a** and **3b** elute from the column.

Radiochemical Isotope Dilution Analyses for Urinary Metabolites and Amine 1. (a) Amine 1. To a solution of carrier 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (1, 15–25 mg weighed accurately) in 10% (5 ml) of the urine sample was added 5% NaOH (5 ml) and the resulting mixture was extracted with 3 × 15 ml of Et_2O. The combined Et_2O layers were evaporated and the residue was treated with $CHCl_3$ (5 ml), 5% NaOH (5 ml), and benzoyl chloride (0.1 ml). The mixture was stirred at room temperature overnight and then the $CHCl_3$ layer was separated and the aqueous layer extracted with $CHCl_3$ (10 ml). The combined $CHCl_3$ layers were dried ($MgSO_4$) and evaporated to leave the benzamide **10** which after two recrystallizations from 2-propanol (mp 169–170°) reached a constant specific activity which was used to calculate the per cent of recovered amine (Table I).

(b) Hydroxymethyl Metabolite 18. To a 10-ml (20%) aliquot of the urine was added carrier **18** (32.9 mg) following which the pH was adjusted to 13 with 10% NaOH and the mixture extracted with Et_2O (5 × 25 ml). The combined, dried (K_2CO_3) organic extracts were evaporated to dryness *in vacuo*. The residue in dry pyridine (1 ml) was treated with benzoyl chloride (0.1 ml) and the mixture was warmed on a steam cone for 30 min. The $CHCl_3$ was washed with 5% Na_2CO_3 (2 × 5 ml), dried ($MgSO_4$), and removed under vacuum to leave a yellow oily residue which crystallized from EtOH. After three recrystallizations (mp 161–162°) the net dpm/mg remained constant and represented 0.4% conversion to compound **18**.

(c) 1-(2,5-Dimethoxy-4-carboxyphenyl)-2-aminopropane (**16**). To a solution of carrier **16** (200.0 mg) in 10% of the urine sample was added 10% NaOH (5 ml) and benzoyl chloride (0.5 ml) and the resulting mixture was then stirred at room temperature overnight. The mixture was filtered and the filtrate made to pH 1 with 10% HCl. The precipitate which formed was collected and washed with petroleum ether (bp 30–60°, 100 ml). The remaining near white solid was then recrystallized three times from EtOH (mp 183–184°) to constant specific activity. The metabolite represented 38% of the administered dose.

(d) 2,5-Dimethoxy-4-methylbenzoic Acid (**13**). To a mixture of carrier **13** (200.0 mg) and 10% (5 ml) of the urine sample was added an equal volume of concentrated HCl and the resulting mixture was then held at reflux for 1 hr. Upon cooling a precipitate developed which after two recrystallizations from H_2O (mp 122–124°) contained no radioactivity.

(e) *N*-2,5-Dimethoxybenzoylglycine (**14**). To a 10-ml aliquot of urine was added carrier **14** (103.1 mg) plus 10% NaOH (0.5 ml) to aid dissolution. The pH was then adjusted to 1 with 10% HCl and the mixture extracted with EtOAc (3 × 10 ml). Removal of the dried ($MgSO_4$) solvent gave a solid residue which after four recrystallizations from C_6H_6 (mp 184–185°) showed a constant specific activity from which it was calculated that compound **14** accounted for no more than 1% of the administered radioactivity.

(f) 1-(2,5-Dimethoxy-4-methylphenyl)-2-propanone (**12**). A mixture of carrier **12** (150.0 mg) in 5 ml of the urine sample was treated with an equal volume of concentrated HCl and the resulting solution was stirred at room temperature overnight. The mixture was then extracted with 3 × 20 ml portions of Et_2O. The combined Et_2O layers were dried ($MgSO_4$) and evaporated to leave an oil which after two recrystallizations from hexane (mp 57–59°) was found to contain no radioactivity.

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Bis-Basic-Substituted Polycyclic Aromatic Compounds. A New Class of Antiviral Agents.[1,2] 2. Tilorone and Related Bis-Basic Ethers of Fluorenone, Fluorenol, and Fluorene

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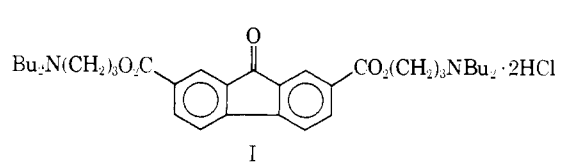
Tilorone hydrochloride, 2,7-bis[2-(diethylamino)ethoxy]-9*H*-fluoren-9-one (11), was found to prolong survival of mice infected with lethal challenges of encephalomyocarditis (EMC) virus. It was effective by oral as well as subcutaneous administration. It showed broad-spectrum antiviral activity and was later found to induce interferon in mice. Tilorone was selected from a series of congeners that was synthesized to determine structure–activity correlations. These indicated that fluorenol and fluorene analogs were much less effective than fluorenones, that thioethers showed less activity than corresponding ethers, that the 2,6- and 2,5-substituted isomers of tilorone were also active, and that elongation of the side chains and increase of molecular weight of the dialkylamine substituent led to decreased oral activity. Monoalkamine ethers showed very little or no activity.

In the first paper of this series,[2] discovery of the antiviral activity of bis(3-dibutylaminopropyl) 9-oxo-9*H*-fluorene-2,7-dicarboxylate dihydrochloride (I) and related bisalkamine esters of fluorenone-, fluorenol-, and fluorenedicarboxylic acids was reported. It also contains an introduction to this series of papers with a general discussion of our extensive synthetic work on bis-basic-substituted polycyclic aromatic compounds prepared to determine those structural features that give optimum biological properties to members of this new class of antiviral agents. Several presentations on this subject have been given.[1,3,4]



I

In this paper, we wish to report the synthesis and evaluation of the antiviral activity of a series of bis-basic ethers of fluorenone, fluorenol, and fluorene. This series includes tilorone hydrochloride (11), the first member of this class of antiviral agents to be reported.[5,6]

Chemistry. Tilorone and related bisalkamine ethers (Table I) were prepared from 2,7-dihydroxy-9*H*-fluoren-9-one (VI) as shown in Scheme I. By the method of Cour-