C\(\text{CH}_3\), 2.5 (10 H, m, NCH\(_2\)), 3.0 (1 H, NTCH\(_2\)), 3.56 (2 H, t, CH\(_2\)O); IR (CHCl\(_3\)), 3460, 3440, 1697 cm\(^{-1}\).

Pharmacology Methods. Animals. Experiments were performed on male Spraque-Dawley rats housed in air-conditioned quarters. The room was lighted between 0700 and 1900 h daily and was maintained at a temperature of 24 ± 2 °C.

Materials. In addition to the test compounds, the following drugs were used: 6-hydroxydopamine hydrobromide (Aldrich Chemical Co., Inc.) and bromocriptine methanesulfonate (generous gift of Sandoz Pharmaceuticals). The doses used were calculated as the free base. The compounds were dissolved in distilled water or suspended in distilled water with a few drops of Tween 50 (2-3 drops/10 mL). Fresh solutions were prepared on the day of the experiment.

6-OHDA-Induced Hypokinesia in Rats. Details of the lesioning procedure and behavioral testing were recently described.\(^{38}\) Briefly, the rats (approximately 280 g) were anesthetized with sodium pentobarbital and placed in a Stoelting stereotaxic instrument. 6-OHDA (25 µg/4 µL) was injected bilaterally into the anterolateral hypothalamus\(^{14}\) by using the DeGroot\(^{18}\) brain atlas. Four days postoperatively, the rats were placed into an open field, the floor of which was divided into 36 squares (11.5 x 11.5 cm). The rats were observed for a 2-min period, and only rats with almost total akinesia were used. Drug effect was evaluated in the course of six 2-min test periods, 15, 30, 45, 60, 90, and 120 min after the sc administration of the tropanylpiperazines and 2, 3, 4, 5, 6, and 7 h after the sc administration of bromocriptine. The placement of all four limbs in one square was taken as one ambulation score. The results are expressed as cumulative ambulation scores, which are the sums of the scores obtained during the 2-min observation periods.

Rotational Behavior in Unilaterally 6-OHDA-Lesioned Rats. The body weights of the rats were approximately 250 g at the time of the stereotaxic operation. During the course of the subsequent rotational experiments, the rats were housed individually and received about 20 g of food per day, which maintained their body weight between 350 and 400 g.

The lesioning procedure was based upon the method of Unge stedt\(^{19}\) utilizing the modifications of Pycock and Marsden.\(^{20}\) The rats were anesthetized with sodium pentobarbital, 40 mg/kg ip, and immobilized in a Stoelting stereotaxic instrument. Unilateral injections of 6-OHDA (8 µg in 3 µL delivered at a rate of 1 µL/min) were made into the left ascending median forebrain bundle (MFB) in the lateral hypothalamus using the stereotaxic coordinates of the De Groot\(^{18}\) brain atlas (A, +4.6; L, 1.9; V, -2.7). 6-OHDA was made up in distilled water containing 0.2 µg/µL of ascorbic acid and kept in ice throughout the injection procedure.

Three to four weeks after lesioning, the rats were tested for rotational behavior in response to apomorphine, 0.25 mg/kg sc. Rats that turned 8-10 times per minute during peak activity were selected for further drug trials.

Rotational behavior was determined in automatically recording rotometers, details of which were recently described.\(^{39}\) Groups of four to eight rats were injected sc with the test compounds and then placed immediately into the rotometer. Rotational behavior was continuously recorded until its cessation. The results are expressed as total number of turns.

Acknowledgment. The authors express their appreciation to Dr. G. Shilling and his associates for microanalytical and spectral data. The skillful assistance of M. Asselin and J. Csakvary in the chemical synthesis and N. Lapeyre and Ida Vozella in the pharmacological studies is gratefully acknowledged.

Registery No. 4, 80100-65-1; 5, 80100-67-9; 6, 89746-93-3; 7, 80100-70-5, 8; 80100-66-7; 8 maleate, 89748-94-1; 9, 80100-78-1; 10, 80100-77-2; 11, 89746-92-5; 11-HCl, 80100-82-9; 12, 80100-75-0; 13, 80100-61-4; 13 maleate, 80100-62-5; 14, 89746-95-3; 14 maleate, 89746-97-4; 18, 80100-92-9; 19, 80100-83-0; 19 (amine derivative), 80100-88-5; 20, 80100-84-1; 21, 80100-89-6; 22-HOAc, 89746-99-9; 23, 80100-95-4; 24, 89745-00-2; 25, 89747-01-3; 25 maleate, 89747-02-4; 26, 89747-03-5; 26 maleate, 89747-04-8; 27, 89747-06-7; 28, 89746-43-6; 29, 89746-43-5; 29-HI, 89746-45-6; 29, 89747-08-8; 30, 89747-07-9; 35, 80101-15-1; 36, 80101-56-0; 37, 80101-24-2; 37 maleate, 80101-28-6; 38, 80101-41-3; 39, 80101-16-2; 40, 80101-17-3; 40 maleate, 80101-18-4; 41, 80101-24-2; 42, 80101-03-1; 42 maleate, 80101-02-8; 43, 80101-00-4; 44, 89746-98-7; 44 maleate, 89746-99-8; 45, 80101-14-0; 46, 80101-96-5; 47, 80101-22-0; 48, 89747-08-0; 48-HBr; 80101-23-1; 2-methoxyphtalimide, 2101-42-0; 2-chlorophtalimide, 2959-48-3; pipperazine, 110-83-0; 1-(2-aminoethyl)pipperazine, 140-31-8; 1-ptyl-butylpiperazine, 38216-72-7; N-(2-bromoethyl)-phthalimide, 574-98-1; quinuclidine hydrochloride, 38986-05-6; 1-(2-oxo-3,7-cycloheptatrien-1-yl)quinuclidinium chloride, 89747-09-1; 4-chloro-1-methylpipperidine, 5570-77-4.

Sulfur Analogues of Psychotomimetic Agents. 3. Ethyl Homologues of Mescaline and Their Monothio Analogue\(^{1}\)

Peyton Jacob III\(^{1}\) and Alexander T. Shulgin\(^{4}\)

3787 Highland Road and 1483 Shulgin Road, Lafayette, California 94549. Received October 17, 1983

All possible monothio analogues of the mono-, di-, and triethoxy homologues of mescaline have been synthesized and pharmacologically evaluated in man. Modifications at the ring position para to the ethylamine chain, either with a sulfur atom, a longer alkyl chain, or both, lead to compounds of high central nervous system activity. The 4-n-propoxy and 4-n-butoxy homologues and their corresponding 4-thio analogues were also synthesized and pharmacologically evaluated in man. Modifications at the ring position para to the ethylamine chain, either with a sulfur atom, a longer alkyl chain, or both, lead to compounds of high central nervous system activity. The 4-n-propoxy and 4-n-butoxy homologues and their corresponding 4-thio analogues were also synthesized and pharmacologically evaluated. The propyl homologues retain high potency, but a butyl group (either with or without a sulfur atom) leads to a decrease in activity. The m-ethyl or m-thio analogues retain some central action but the diethoxy and especially the triethoxy homologues are relatively inactive as psychotomimetic drugs.

Although, mescaline (1a) has rather low psychotomimetic potency, its simple structure, together with its complex and well-characterized psychological intoxication profile, has made it a desirable paradigm for structure-activity relationship inquiries. Human clinical studies of homologues of 1a (Chart I) have centered on three structural positions: (a) alkylation of the primary amine function, (b) alkylation of the position α to this amine group, or (c) homologation of the p-methoxy group. N,N-Dimethylmescaline (Trichocerine) is reported to be of reduced potency, showing little, if any central activity even at twice the effective dosage of 1a.\(^{1}\)

\(^{1}\) For paper 2 in this series, see ref 24.

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The α-methyl homologue (3,4,5-trimethoxyamphetamine, TMA) has about twice the potency of 1a but further elongation of the α-substituent results in the loss of activity.\(^3\) Homologation at the 4-position [to give 1c (escaline) and 1j (proscaline)] increases the potency by almost an order of magnitude.\(^4\) This fact, together with the reported increases in potency realized by the replacement of the oxygen atoms of 1a with sulfur\(^6\) to give 3-thio-4-thiomescaline (proscaline)\(^{6}\) increases the potency by almost 12 times 1a. The target compounds were prepared through the intermediacy of either an appropriately substituted phenylacetonitrile or benzaldehyde.

Those phenethylamines with a sulfur atom in the 4-position were prepared from an appropriate \(m\)-diaxoxybenzene (Scheme I). Lithiation with butyllithium-\(m\)-tetramethylethylenediamine, followed by reaction with a dialkyl disulfide,\(^7\) provided the corresponding thioethers. Bromination of 4 led to 5, which were converted to the acetonitriles 6 via a benzyne reaction with the lithium salt of acetonitrile.\(^8\) Reduction of 6 with \(\text{AlH}_3\) yielded the corresponding amines 3.

The synthesis of 3,5-dihydroxy-4-methoxyphenethylamine (15f) was accomplished by Mannich condensation of 2,6-dimethoxynaphthalene with dimethyloxalyl and formaldehyde to give the \(N,N\)-dimethylenzylamine 7a, which was quaternized with methyl iodide and reacted with sodium cyanide in DMF to give the nitrile 7c.\(^8\) Methylation and

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**Table I. Relative Psychotomimetic Potencies of 1-3a-k Arranged by Structural Differences**

<table>
<thead>
<tr>
<th>Heteroatom</th>
<th>No Sulfur</th>
<th>Meta</th>
<th>Para</th>
</tr>
</thead>
<tbody>
<tr>
<td>no ethyls</td>
<td>1a</td>
<td>1°</td>
<td>12</td>
</tr>
<tr>
<td>1 ethyl</td>
<td>1b (N = 11, (T = 28)) 6</td>
<td>2a(^6)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>meta</td>
<td>2b (N = 8, (T = 16)) 6</td>
<td>3a(^6)</td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>2c (N = 8, (T = 18)) 6</td>
<td>3b (N = 2, (T = 8)) 4</td>
</tr>
<tr>
<td></td>
<td>assim</td>
<td>2d (N = 2, (T = 8)) 6</td>
<td>3c (N = 6, (T = 19)) 6</td>
</tr>
<tr>
<td>2 ethyls</td>
<td>sym</td>
<td>2e (N = 2, (T = 5)) 2</td>
<td>3e (N = 2, (T = 9)) 4</td>
</tr>
<tr>
<td></td>
<td>3 ethyls</td>
<td>2f (N = 2, (T = 7)) 6</td>
<td>3f (N = 2, (T = 7)) &lt;1</td>
</tr>
<tr>
<td>1 propyl</td>
<td>meta</td>
<td>2g (N = 2, (T = 5)) 2</td>
<td>3h (N = 2, (T = 8)) &lt;1</td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>2h (N = 2, (T = 5)) 2</td>
<td>3i (N = 2, (T = 5)) 6</td>
</tr>
<tr>
<td>1 butyl</td>
<td>3j (N = 2, (T = 5)) 6</td>
<td>3k (N = 2, (T = 11)) 3</td>
<td>3</td>
</tr>
</tbody>
</table>

---

**Chart I**

![Chart I](image)

**Scheme I**

![Scheme I](image)

**Scheme II**

![Scheme II](image)
Sulfur Analogues of Psychotomimetic Agents

Scheme III

Scheme IV

reduction with AlH₃ yielded the desired amine 1f (Scheme II). Gallic acid was the starting material for the triethoxy homologe (1h) of mescaline. Ethylation,⁸ followed by reduction, provided the benzyl alcohol 9a, which was converted to the chloride 9b with concentrated HCl. Displacement of the chloride with cyanide, followed by reduction with AlH₃, provided the amine 1b.

All of the remaining phenethylamines were prepared through the corresponding benzaldehydes (Scheme III). The bromination of either vanillin or bourbinal ortho to the phenolic hydroxyl group,¹⁰-¹² followed by O-alkylation, led to the aldehydes 10, which were converted to the Schiff's bases 11 with cyclohexylamine. Metal-halogen exchange¹¹,¹² with butyllithium in ether gave the corresponding lithio derivatives, which were reacted either with a dialkyl disulfide to yield 12 or with butyl borate, followed by hydrogen peroxide oxidation, to give 13a,b.¹³ These phenolic aldehydes were alkylated with the appropriate alkyl iodide to give 13c-e.

Evidence for the structural assignments given to aldehydes 12 and 13 was obtained from the conversions

Scheme V

outlined in Scheme IV. Bromination of vanillin has been reported by several authors to occur in the 5-position. By analogy, one would expect bromination of bourbinal to occur in the 5-position as well. That this was indeed the case was shown by the conversion of both vanillin and bourbinal to 3-ethoxy-4,5-dimethoxybenzaldehyde¹⁴ as shown in Scheme IV. Had bromination occurred at any other position, two distinct products would have been obtained.

In one case, replacement of bromine with an alkylthio group was shown to take place without rearrangement. Lithiation of 11a, followed by reaction with dimethyl disulfide, provided 3,4-dimethoxy-5-(methylthio)benzaldehyde, which was identical (mp and mmp) with that prepared from the cyclic thiacarbamate⁶ (see Scheme IV). It is reasonable to assume that the analogous conversions of 11a-d to 12a-g also occurred without rearrangement.

Two routes were used to convert aldehydes 12 and 13c-e to the desired phenethylamines (Scheme V). Condensation with nitromethane yielded nitrostyrenes 14, which were reduced to amines 1 and 2 with AlH₃. In some cases, difficulties were encountered in the synthesis of 14, and, consequently, an alternate approach was utilized. Wittig condensation with methylenetriphenylphosphorane provided the corresponding styrenes, which were hydroborated with disiamylborane and then iodinated to give the phenethyl iodides.¹⁵ Reaction with potassium phthalimide in DMF gave the N-phthalimido derivatives 15, which were cleaved with hydrazine in butanol to provide amines 1 and 2. Although several steps were employed, it was possible to convert aldehydes 12 or 13 to the crystalline phthalimide derivatives 15 without purification of any intermediates.

Pharmacology and Discussion

Some earlier reports have described both animal and human pharmacological properties of several of the sulfur-free compounds. In vitro pharmacological studies have been made on the 4-substituted homologues 1e,j,k as serotonin agonists¹⁴ and on 1c,e¹⁵ and 1h¹⁶ as enzyme inhibitors. Animal studies have been reported on the frog¹⁷ and cat¹⁸,¹⁹ for 1e and 1. In human studies, it has been reported that

(10) Brink, M. Acta Univ. Lund., Sect. 2 1965, no. 6, 1.
homologation of the 4-methoxy group of mescaline leads to compounds of increased potency. Leminger\textsuperscript{4} reported that both the 4-ethoxy and the 4-allyloxy analogues are psychotomimetic in man. Braun et al. confirmed the activity of the 4-ethoxy analogue and reported the central nervous system (CNS) activity of the 4-propoxy homologue 1j.\textsuperscript{5}

This report describes the preliminary psychopharmacology of all the possible ethoxy homologues of mescaline, as well as all monothio analogues thereof, in normal human subjects. In addition, the propyl and butyl homologues of the more potent compounds were prepared and evaluated.

From the quantitative point of view, an increase in the length of the 4-alkyl group led first to an increase in potency, followed by an abrupt drop (Table II). In parallel with previous studies involving homologation of an alkyl group attached directly to the aromatic system,\textsuperscript{19} the 2- and 3-carbon chains were more potent than the 1-carbon counterpart. With the sulfur heteroatom, the maximum potency was at the two-carbon length, with 4-(ethylthio)-3,5-dimethoxyphenethylamine (3e) being the most potent compound in this study, at 20 times the potency of mescaline. With both oxygen and sulfur in the 4-position, the activity dropped abruptly with a four-carbon chain. Homologation or sulfur replacement at the meta position led to less dramatic changes. Replacement of a m-oxygen with sulfur generally produced a modest increase in potency, although in one case (2d) CNS activity was decreased. Incorporation of ethyl groups into the 3- and 4-positions, with or without sulfur, resulted in somewhat higher potencies relative to mescaline. Those homologues with ethyl groups in the 3- and 5-positions or with three ethyl groups were all less potent than mescaline or were without any observed psychotomimetic activity altogether.

These relationships are shown pictorially in Figure 1. Where there is a redundancy due to symmetry (i.e., 3-thio = 5-thio), the data are duplicated; where the observed value is <1, the column has no height; and where the compound was not evaluated, only a dot appears. The terms "mono-, di-, and trialkyl" refer to substitution patterns in excess of the methoxy group; i.e., monoalkyl designates a monoethyl-, monopropyl-, or monobutyl- dimethyl homologation of the oxygen or sulfur atoms.

Qualitatively, a distinctly different picture emerges, with no immediate relationship obvious between the absolute potency and the descriptions of psychological effect. The oxygenated analogues 1e and 1j produce, during the period of maximum intoxication, less sensory distortion (visual synthesis and color intensification) than is characteristic of mescaline, but a larger degree of neurological hyperre- flexia (exaggerated reflex and mild tremor). In contrast, metacecaline (1b) was remarkably similar to mescaline in both sensory and interpretive content, as well as in its low potency. With a sulfur atom located in the 4-position, the nature of action was relatively independent of chain length. Thioescaline (3c), the most potent of the materials described in this report, is one of the longest lasting, with CNS effects still apparent some 15 h following ingestion. It is especially rich in fantasy potential and, despite some early signs of parasympathomimetic stimulation (facial flushing and nausea), produced a state of perceptual distortion, tactile responsiveness, and mood enhancement (euphoria and easy humor) that closely resembled that of mescaline. The homologue 3j, as well as compounds 2e, 2g, and 3e, produced somatic changes, including dizziness and hyperreflexia, that overshadowed the psychological syndrome. All compounds with three ethyl groups, with or without sulfur, appeared to be without central or peripheral activity. Of those compounds that were assayed in the more extended studies (N\textsuperscript{2}6), there was a consistent chronology of a plateau of CNS intoxication extending from the 2nd h to 4–6th h following ingestion, followed by a gradual recovery of a preexperimental baseline between the 9th and 12th h. The longer-lived exception, 3c, was mentioned above. All compounds consistently produced mydriasis, but no noteworthy cardiovascular changes (pulse and blood pressure).

**Experimental Section**

Melting points were determined on a Mel-temp apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Aculab-2 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN, and were within 0.4% of the theoretical values, except where otherwise specified. All distillations (unless otherwise specified) were conducted bulb-to-bulb in a Kugelrohr apparatus (Aldrich Chemical Co.) in vacuo.

2,6-Diethoxythioanisole (4f). To a solution of 16.6 g of m-diethoxybenzene (100 mmol) in 200 mL of petroleum ether (30–60 °C) there was added 12.1 g (105 mmol) of tetramethylthiuram disulfide (TMEDA). The reaction mixture was cooled to 0 °C with external ice-water and then there was added 60 mL of 1.6 m BuLi in hexane. The granular precipitate was warmed to room temperature, stirred for 0.5 h, cooled again to 0 °C, and then treated with 9.45 mL of dimethyl disulfide (5% excess). The granular solids became creamy with the evolution of heat. After stirring for 1/2 h at ambient temperature, the reaction mixture was poured into 600 mL of dilute H2SO4. The aqueous phase was extracted with 2 250 mL portions of ether. The organic extracts, combined with the filtered solids, were evaporated to dryness, yielding 16.9 g (80% of theory) of white solids, mp 68–71 °C. Recrystallization from methylecyclopentane yielded glistening needles, mp 71.5–72 °C. Anal. (C10H18O2S) C, H.

In the same manner, the corresponding thioureas 4a–e, g were prepared from the appropriate meta-diether and the appropriate dialkyl disulfide:

<table>
<thead>
<tr>
<th>Compound</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>45–46</td>
<td>96</td>
</tr>
<tr>
<td>4b</td>
<td>110–115</td>
<td>95</td>
</tr>
<tr>
<td>4c</td>
<td>130–140</td>
<td>86</td>
</tr>
<tr>
<td>4d</td>
<td>95–105</td>
<td>95</td>
</tr>
<tr>
<td>4e</td>
<td>26–27</td>
<td>82</td>
</tr>
</tbody>
</table>

and the THF solution was basic to damp pH paper. The solids was added 0.78 mL of 100% H2SO4 cooling, the excess hydride was cautiously destroyed with 2-bromobenzylamine. The mixture was added to 200 mL of water containing 1 g of sodium bicarbonate, and the colorless resulting oil was taken up in 1 L of dilute sulfuric acid. The slightly turbid suspension was extracted with 2 × 75 mL of CH2Cl2, which were discarded. The aqueous phase was made basic with 5% NaOH, and again extracted with 2 × 75 mL of CH2Cl2. These latter were pooled, and the solvent was removed in vacuo to provide a residual yellow oil. Bulb-to-bulb distillation at 0.4 mmHg yielded a yellow oil fraction with bp 135–160 °C, which was dissolved in 20 mL of 2-propanol and neutralized with concentrated HCl to external, damp, universal pH paper. The formed solids were dissolved by bringing the 2-propanol to a boil on the steam bath and, following the addition of 80 mL of boiling ether, the product was obtained as white crystals. This was filtered washed with a 2-propanolether mixture, followed by ether, and then air-dried to constant weight. The product weighed 1.5 g; yield 43%. Anal. (C6H5ClNO2S) C, H.

In the same manner, the corresponding phenethylamines 3a,b,c,d,e,f,g,h,i,j,k,l,m were prepared from the appropriate 4. [Note: the lettering of compounds 4–6 (Scheme I) correspond to substitution patterns that are different for 3 (Table I).]

Compound 3a: bp 110–120 °C (0.3 mmHg); yield 91%. Anal. (C6H5ClNO2S) C, H.

Compound 3b: bp 113–121 °C (0.3 mmHg) (HCl salt); white crystals; mp 165–165 °C. Anal. (C6H5ClNO2S) C, H.

Compound 3c: (from 6a): bp 112–135 °C (0.2 mmHg) (HCl salt); white crystals obtained as a hydrate; mp 101–106. The anhydrous form, white solids, had, after drying at 100 °C for 24 h, in vacuo, mp 167–168 °C. Anal. (C6H5ClNO2S) C, H.

Compound 3d: (from 6b): bp 135–150 °C (0.3 mmHg); yield 94%. Anal. (C6H5ClNO2S) C, H.

Compound 3e: (from 6c): bp 130–140 °C (0.2 mmHg); providing white crystals; mp 177–178 °C. Anal. (C6H5ClNO2S) C, H.

Compound 3f: (from 6d): bp 137–137 °C (0.3 mmHg); yield 164–165 °C. Anal. (C6H5ClNO2S) C, H.

Compound 3g: (from 6e): bp 140–155 °C (0.4 mmHg). Anal. (C6H5ClNO2S) C, H.

Compound 3h: white crystals; mp 154–155 °C. Anal. (C6H5ClNO2S) C, H.

Compound 3i: (from 6f): bp 140–155 °C (0.4 mmHg). Anal. (C6H5ClNO2S) C, H.

Compound 3j (from 6g): bp 150–160 °C (0.5 mmHg). Anal. (C6H5ClNO2S) C, H.

Compound 3k (from 6h): bp 130–150 °C (0.5 mmHg). Anal. (C6H5ClNO2S) C, H.

Grinding under cold methylcyclopentane provided a white solid: mp 35.5–35.7 °C. Anal. (C6H7NO) C, H. C: calculated, 82.12; found, 82.56.

Compound 6a: bp 140–160 °C (0.25 mmHg); yield 50% of a product perhaps 80% pure by TLC.

Compound 6b: bp 130–150 °C (0.2 mmHg); providing white solids from cold methanol; mp 65–66 °C; yield 50%. Anal. (C6H7NO) C, H.

Compound 6c: bp 140–150 °C (0.3 mmHg); yield 85%. Anal. (C6H7NO) C, H.

Compound 6d: bp 130–150 °C (0.2 mmHg); providing white solids from cold methanol; mp 65–66 °C; yield 50%. Anal. (C6H7NO) C, H.

Compound 6e: bp 140–150 °C (0.3 mmHg); yield 25% of an 80% pure product by TLC.

3,5-Diethoxy-4-(methylthio)phenethylamine (3f). To a 1 M solution of LiAlH4 in the THF (30 mL, 30 mmol) stirred magnetically and cooled with an external ice-water bath there was added 0.78 mL of 100% H2SO4 (15.0 mmol) as a concentrated H2SO4 and fuming H2SO4. After 15 min of stirring in the ice bath, a solution of 3.0 g of 6f (12 mmol) in 15 mL of anhydrous THF was added over 5 min. The reaction mixture was then filtered, the filtrate was washed with water, and the colorless resulting oil was taken up in 1 L of dilute sulfuric acid. The slightly turbid suspension was extracted with 2 × 75 mL of CH2Cl2, which were discarded. The aqueous phase was made basic with 5% NaOH, and again extracted with 2 × 75 mL of CH2Cl2. These latter were pooled, and the solvent was removed in vacuo to provide a residual yellow oil. Bulb-to-bulb distillation at 0.4 mmHg yielded a yellow oil fraction with bp 135–160 °C, which was dissolved in 20 mL of 2-propanol and neutralized with concentrated HCl to external, damp, universal pH paper. The formed solids were dissolved by bringing the 2-propanol to a boil on the steam bath and, following the addition of 80 mL of boiling ether, the product was obtained as white crystals. This was filtered washed with a 2-propanolether mixture, followed by ether, and then air-dried to constant weight. The product weighed 1.5 g; yield 43%. Anal. (C6H5ClNO2S) C, H.
3,5-Diethoxy-4-methoxybenzenethiolamine (1f). In a manner analogous to the described synthesis of 3f above, 8 was redissolved to 1f, which distilled at 120–140 °C (0.5 mmHg) and gave an overall yield of 66%. mp 186–187 °C. Anal. (C12H19N03) C, H.

3,4,5-Triethoxybenzyl Chloride (9b). The ethyl ester of 3,4,5-triethoxybenzoic acid (9; 18.9 g, 80 mmol) in 25 mL of THF was added, with good stirring, to a suspension of 8.0 g of LAH in 150 mL of THF. The mixture was kept at a reflux for 24 h and cooled, and the excess hydride was destroyed with 2-propanol. There was then added sufficient 25% NaOH until the solids were substantially white and easily filterable. After filtration, the filter cake was washed with 2-propanol, the washes were combined with the mother liquor, and the solvents were removed in vacuo, yielding 12.2 g of an oil, which distilled [120–140 °C (0.4 mmHg)] to yield 8.6 g of a colorless product that spontaneously crystallized. This had a mp of 29–30 °C and (by infrared) was free of the ester carbonyl at 1709 cm⁻¹, but it showed a broad hydroxyl stretch centered at 3280 cm⁻¹. [23] Compound 11b: bp 150–157 °C (0.2 mmHg); mp 60–61 °C from methanol; yield 90%. Anal. (C12H18BrNO3) C, H.

In the same manner, the corresponding Schiff’s bases 11a–c were prepared from the corresponding aldehydes. Compound 11a: bp 146–160 °C (0.2 mmHg); yield 87%. Anal. (C12H18BrNO3) C, H.

3,4,5-Triethoxybenzyl Cyanide (9c). To a solution of 4.5 g of 9b (17.4 mmol) in 10 mL of DMF there was added 5 g of NaN₃. After 1 h of heating on the steam bath, the reaction mixture was melting point showed an initial sintering at 165 °C, followed by gradual heating to reflux, which effected redissolution of the DMF there was added 31 g of ethyl iodide (200 mmol) and 32 g of a purple color and a heavy precipitate. The mixture was reduced to lh, which distilled at 115–135 °C (0.4 mmHg) and gave an overall yield of 66%. mp 186–187 °C. Anal. (C12H19N03) C, H.

In a manner analogous to the described synthesis of 3f above, 9c (3.6 g, 14.5 mmol) was suspended in 30 mL of concentrated HCl, and the mixture was quenched in 700 mL of water, made strongly basic, and extracted with 2× 50 mL of DMF, and the extracts were pooled, and the solvent was removed in vacuo. The residue (8.8 g of a viscous yellow oil) was distilled [128–140 °C (0.3 mmHg)] to yield a colorless oil that did not crystallize. Anal. (C12H18BrNO3) C, H.

3-Bromo-4,5-diethoxybenzaldehyde (10d). A solution of 4.7 g of 10d (32 mmol) in 150 mL of anhydrous ether was cooled to –90 °C, resulting in a slight cloudiness. There was then added 25 mL of 1.6 N BuLi (40 mmol) in hexane, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S (48 mmol) over 20 min, leading to an increased cloudiness and viscous suspension. The temperature was slowly raised to room temperature over an additional hour. The reaction mixture was then quenched with 400 mL of dilute HCl. The organic phase was separated, the solvent was removed, and the aqueous phase was added to the residues, and the mixture was heated on the steam bath for 2 h. The cooled aqueous mixture was extracted with CH₂Cl₂ (× 100 mL), the extracts were pooled, and the solvent was removed in vacuo. The residue (11.0 g of an amber oil) was distilled [130–150 °C (0.2 mmHg)] to give a colorless oil that crystallized: mp 72–73 °C; yield 68%. Recrystallization from methanol gave white crystals, mp 55–57 °C. Anal. (C12H16O₃S) C, H.

In the same manner, the corresponding benzaldehydes 12a–f were prepared with either ethyl disulfide or diethyl disulfide from the Schiffs’s bases 11a–d. Compound 12a (from 11a and Me₂S): bp 135–145 °C (0.2 mmHg); yield 55%. Anal. (C12H18O₃S) C, H.

In the same manner, the corresponding benzaldehydes 12a–f were prepared with either ethyl disulfide or diethyl disulfide from the Schiffs’s bases 11a–d. Compound 12a (from 11a and Me₂S): bp 135–145 °C (0.2 mmHg); yield 55%. Anal. (C12H18O₃S) C, H.

3,4-Diethoxy-5-ethylthio)benzaldehyde (12g). A well-stirred solution of 11.5 g of 11d (52 mmol) in 150 mL of anhydrous ether was cooled to –90 °C, resulting in a slight cloudiness. There was then added 25 mL of 1.6 N BuLi (40 mmol) in hexane, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S (48 mmol) over 20 min, leading to an increased cloudiness and viscous suspension. The temperature was slowly raised to room temperature over an additional hour. The reaction mixture was then quenched with 400 mL of dilute HCl. The organic phase was separated, the solvent was removed, and the aqueous phase was added to the residues, and the mixture was heated on the steam bath for 2 h. The cooled aqueous mixture was extracted with CH₂Cl₂ (× 100 mL), the extracts were pooled, and the solvent was removed in vacuo. The residue (11.0 g of an amber oil) was distilled [130–150 °C (0.2 mmHg)] to give a colorless oil that crystallized: mp 72–73 °C; yield 68%. Recrystallization from methanol gave white crystals, mp 55–57 °C. Anal. (C12H16O₃S) C, H.

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In the same manner, the corresponding benzaldehydes 12a–f were prepared with either ethyl disulfide or diethyl disulfide from the Schiffs’s bases 11a–d. Compound 12a (from 11a and Me₂S): bp 135–145 °C (0.2 mmHg); yield 55%. Anal. (C12H18O₃S) C, H.

3-Bromo-4,5-diethoxybenzaldehyde (10d). A solution of 4.7 g of 5-hydroxyveratraldehyde (15a; 26 mmol; see ref 11) and 21 g of propyl bromide in 75 mL of dry acetone was treated with 6.0 g of powdered KI and 7.0 g of powdered anhydrous K₂CO₃. After stirring at reflux for 15 h, the reaction mixture was quenched in 750 mL of water, made strongly basic, and extracted with 3× 250 mL of petroleum ether. The extracts were pooled and washed with 5% base, which removed most of the color. After the solvent was removed, the residue (8.8 g of a viscous yellow oil) was distilled [133–145 °C (0.15 mmHg)] to yield 4.5 g (78% of theory) of a pale yellow oil that did not crystallize. It was employed directly for the synthesis of the nitrostyrene (q.v.), which was characterized and analyzed.

Compounds 11a–f were isolated in a similar manner, the use of ethyl iodide gave rise to 13b: mp 110–118 °C (0.25 mmHg); mp 45–49.5 °C from cyclohexane; yield 58% of theory. Anal. (C13H22ClN03) C, H.

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Sulfur Analogues of Psychotomimetic Agents

**3,4-Diethoxy-5-methoxy-@-nitrostyrene (14c).** A solution of 6.4 g of 13c (28.5 mmol) in 40 mL of CH3NO2 was treated with 0.5 g of ammonium acetate and brought to reflux on the steam bath. In this preparation, as in all the following examples, it is mandatory to evaluate the reaction’s progress frequently by TLC and to stop when the aldehyde has been consumed. On silica gel, with CH2Cl2 as a moving phase and employing UV for detection, the product nitrostyrene has the highest Rf (usually with a deeply colored fluorescence), the starting aldehyde is slightly lower (always with a white fluorescence), and the unwanted byproducts are yet lower toward the origin. At 1 h the solvent nitromethane was removed vacuo, and the residual oil spontaneously recrystallized. Recrystallization from 40 mL of boiling methanol yielded 3.0 g of yellow plates: yield 39%; mp 89–90 °C. Anal. (C13H17NO3) C, H.

In the same manner, the corresponding nitrostyrenes 14a–b, d–h were prepared from the appropriate aldehyde 12 or 13.

**Compounds 14a (from 13c):** fine yellow needles from methanol; mp 89.5–90 °C; yield 38%. Anal. (C13H17NO3) C, H.

**Compounds 14b (from 13d):** yellow crystals from methanol or cyclohexane; mp 73–81 °C with a trace of unmelting residue; yield 53%. Anal. (C13H17NO3) C, H.

**Compounds 14d (from 12a):** yellow crystals obtainable as polomorphs; mp 80–82 °C from methanol, 109–110 °C from cyclohexane; yield 43%. Anal. (C13H17NO3) C, H.

**Compounds 14e (from 12b):** bright yellow crystals from methanol; mp 92–93 °C; yield 46%. Anal. (C13H17NO3) C, H.

**Compounds 14f (from 12c):** yellow crystals from methanol; mp 99–99 °C; yield 33%. Anal. (C13H17NO3) C, H.

**Compounds 14g (from 12d):** yellow crystals from methanol; mp 102.5–104 °C; yield 3%. Anal. (C13H17NO3) C, H.

**Compounds 14h (from 12e):** yellow crystals from methanol; mp 78.5–79 °C; yield 17%. Anal. (C13H17NO3) C, H.

**3,4-Diethoxy-5-methoxyphenethylamine (1e).** A well-stirred solution of 3.0 g of LiAlH4 in 100 mL of THF, under He and at 0 °C, was treated with 2.1 mL of 100% H2SO4 (prepared from concentrated H2SO4 and fuming H2SO4) dropwise over 10 min. The AlH3 solution was allowed to come to room temperature, and a solution of 0.5 g of 1e (13.1 mmol) in 30 mL of THF was added with external cooling as needed to control the exothermic reaction. Following the addition, the reaction mixture was held at reflux for 1 h and cooled, and the excess hydride was destroyed with 2-propanol. There was then added 10% NaOH until the solids were white and granular. The reaction mixture was filtered, and the solids were washed with 2-propanol. The mother liquor and the washings were combined, and the solvents were removed to yield a yellow oil, which was treated with 100 mL of dilute H2SO4. The cloudy suspension was extracted with 2 × 75 mL of CH2Cl2, and the aqueous phase was made basic with NaOH, and reextracted with 2 × 75 mL of CH2Cl2. These extracts were combined, the solvent was removed, and the residue was distilled [110–120 °C (0.4 mmHg)] to give 2.0 g of a colorless liquid. This was dissolved in 7 mL of 2-propanol, neutralized with concentrated HCl to external dampened pH paper (32 drops required), and then treated with 50 mL of anhydrous ethyl alcohol. After several minutes, crystallization started. Another 50 mL of ether was added, and the mixture was standing for several additional minutes the product was removed by filtration, washed with ether, and air-dried to constant weight. The product weighed 1.25 g: yield 35%; mp 142–143 °C. Anal. (C13H17NO3) C, H.

In the same manner, the corresponding phenethylamines with structure 1 or 2 were prepared from the nitrostyrenes 14a, b, d–f.
Covalent poly(L-lysine) conjugates of methotrexate (MTX) have been studied in several laboratories\textsuperscript{1-10} as a means of achieving drug uptake by pinocytosis as opposed to the usual mechanism of carrier-mediated MTX active transport, and they have given promising therapeutic results against human solid-tumor xenografts in nude mice.\textsuperscript{3} From the available evidence in neoplastic and non-neoplastic cell lines in culture, it appears that the conjugates are internalized in micropinocytic vesicles that coalesce into larger vacuoles and ultimately fuse to protease-rich secondary lysosomes. The conjugates themselves are ineffective as dihydrofolate reductase inhibitors,\textsuperscript{2} but degradation of the poly(L-lysine) backbone by the lysosomal proteases yields small fragments that exert typical antifolate effects\textsuperscript{68} upon being expelled into the cytoplasm.

Cellular uptake of the conjugates is much more rapid than the uptake of MTX, especially when the cells are MTX resistant by virtue of a transport defect.\textsuperscript{1,6,8} Moreover, the uptake of MTX-poly(L-lysine), unlike that of MTX, is

**Psychopharmacological Assays.** The screening and human potency determinations, the experimental protocols, and the basis of determining effective dosages were essentially those described in detail in earlier studies.\textsuperscript{24} Briefly, trials were initiated in normal adult subjects at levels assumed to be inactive (generally 0.5 mg, orally), and the assay levels were increased, at appropriate intervals, in increments of about 1:6:1. With the confirmed establishment of threshold levels (levels at which the chronology of action was certain but the qualitative nature not clearly defined), assays were expanded to a larger group of volunteers, all experienced with a broad spectrum of psychotropic drugs. All potency values were determined in at least two subjects, but five products (1b, 2b, 2e, 3e, and 1) were sufficiently interesting to warrant broader evaluation. The number of subjects (N) and number of trials (T) are summarized in Table I. The qualitative aspects of these studies are summarized under Results and Discussion.


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**Methotrexate Analogues. 23. Synthesis, Dihydrofolate Reductase Affinity, Cytotoxicity, and in Vivo Antitumor Activity of Some Putative Degradation Products of Methotrexate–Poly(L-lysine) Conjugates**

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Derivatives of methotrexate (MTX) in which the γ-carboxyl group is joined to the ε-amino group of L-lysine, L-lysyl-L-lysine, or L-lysyl-L-lysyl-L-lysine, respectively, were prepared for evaluation of their dihydrofolate reductase (DHFR) affinity, their ability to retard cell growth in culture, and their antitumor activity in vivo. These small lysine derivatives of MTX are of interest as putative breakdown products of MTX–poly(L-lysine). Inhibition of DHFR in a cell-free assay was decreased only 3-fold relative to MTX, indicating that γ-substitution by up to three lysines is well tolerated for binding. On the other hand, toxicity toward L1210 murine leukemia cells in culture decreased up to 120-fold relative to MTX as the lysines increased in number from one to three, suggesting that uptake across the cell membrane becomes difficult when positively charged lysines are at the γ-position. Growth inhibition of H35 rat hepatoma cells was decreased 40- to 60-fold relative to MTX, but in H35R\textsuperscript{K4} cells, which have normal DHFR content but are 180-fold MTX resistant by virtue of a transport defect, the lysine derivatives were only 3- to 7-fold less toxic than MTX. When the adducts were given to L1210 leukemic mice by twice-daily injection for 10 days, an increase in life span (ILS) of 80-100% was observed at 40 mg/kg (equivalent to 20-30 mg/kg of MTX). MTX itself, on the same schedule, gave a 100% ILS at 0.5 mg/kg. The low in vivo activity of the mono-, di-, and trilysine adducts suggests minimal systemic hydrolysis to free MTX.

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