Sulfur Analogues of Psychotomimetic Agents. 3. Ethyl Homologues of Mescaline and Their Monothio Analogues

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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. 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 diethyl and especially the triethyl 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.

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. Homologation at the 4-position (to give 1c (escaline) and 1j (proscaline)) increases the potency by almost an order of magnitude. This fact, together with the reported increases in potency realized by the replacement of the oxygen atoms of 1a with sulfur to give 3-thio-mescaline (3a), suggested the synthesis of the sulfur analogues of 1c and homologues thereof.

There are five possible ethoxy homologues of 1a (i.e., 1b, c, e, f, h), and these allow a total of 12 possible monothio analogues to exist (2b–h and 3b, c, e, f, h). These compounds were all synthesized and pharmacologically evaluated in man. The 3- and 4-propyl and the 4-buty1 homologues of 1a, along with the 4-thio analogues of the latter two, were also synthesized and pharmacologically evaluated.

**Chemistry.** The target compounds were prepared through the intermediary of either an appropriately substituted phenylethylamine or benzaldehyde.

Those phenethylamines with a sulfur atom in the 4-position were prepared from an appropriate m-dialkoxyphenylacetonitrile or benzaldehyde.

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

<table>
<thead>
<tr>
<th>no sulfur</th>
<th>meta</th>
<th>one sulfur</th>
<th>para</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ethyl</td>
<td>1a</td>
<td>2a&lt;sup&gt;6&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>2 ethyls</td>
<td>1b&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2b (N = 8, T = 16)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2c (N = 2, T = 8)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3 ethyls</td>
<td>1c&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2c (N = 8, T = 18)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2d (N = 2, T = 8)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1 propyl</td>
<td>1h (N = 2, T = 6)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2e (N = 2, T = 5)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1 butyl</td>
<td>1j (N = 9, T = 19)</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2f (N = 2, T = 7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2h (N = 2, T = 5)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3g (N = 2, T = 9)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3h (N = 2, T = 8)</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Human potency of mescaline = 1 MU, which represents the potency of a compound relative to that of mescaline. It is derived by dividing the effective dose of mescaline by the effective dose of the compound in question, both determined in man. Values from ref 6. <sup>b</sup>Total number of subjects. <sup>c</sup>Total number of trials. <sup>d</sup>Value from ref 4. <sup>e</sup>Reference 5 gives MU = 6. This revision reflects a broader clinical evaluation.

**Chart I**

![Chart I](image)

**Scheme I**

![Scheme I](image)

**Scheme II**

![Scheme II](image)


dehydes

phenolic aldehydes were alkylated with the appropriate sulfur analogues

a dialkyl disulfide to yield by hydrogen peroxide oxidation, to give alkyl iodide to give through the corresponding benzaldehydes (Scheme 111). Schiffs' bases exchanging with butyllithium in ether gave the corresponding lithio derivatives, which were reacted either with Schiff's bases or with cyclohexylamine. Metal-halogen exchange followed by 0-alkylation, led to the aldehydes which were converted to the chlorides, which were reduced to amines with concentrated HCl. Displacement of the chloride with cyanide, followed by reduction with AlH₃ yielded the desired amine 11f (Scheme II). Gallic acid was the starting material for the triethoxy analogue, 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-dimethoxybenzaldehyde 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 thiocarbonate (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 methyleneetriphenylphosphorane provided the corresponding styrenes, which were hydroborated with disiamylborane and then iodinated to give the phenethyl iodides. 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 studies of several of the sulfur-free compounds. In vitro pharmacological studies have been made on the 4-substituted homologues 1e, j, k as serotonin agonists and on 1e, f, j as enzyme inhibitors. Animal studies have been reported on the frog and cat for 1e and 1. In human studies, it has been reported that

(10) Brink, M. Acta Univ. Lund. Sect. 2 1963, no. 6, 1.
homologation of the 4-methoxy group of mescaline leads to compounds of increased potency. Leminger 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 11.5

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-allyl group led first to an increase in potency, followed by an abrupt drop (Table II). In parallel with previous studies involving homologation of an allyl group attached directly to the aromatic system, 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-5,5-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., monoaalkyl designates a monoethyl- or monopropyl-, or monobutyl-dimethyI 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 synethsis and color intensification) than is characteristic of mescaline, but a larger degree of neurological hyperrhesis (exaggerated reflex and mild tremor). In contrast, metaxascine (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 (3e), 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 ≥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 Acculab-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.

2,4-Diethoxythionianiso (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 tetramethylthiurea (TMEDA). The reaction mixture was cooled to 0 °C with external ice-water and then there was added 60 mL of 1.5 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 solids were filtered, and the aqueous phase was extracted with 2 volumes of ether. The organic extracts, combined with the filtered solids, were evaporated to dryness, yielding 16.9 g (80% of theory) of white m-diethoxy-3-(methylthio)-1-bromobenzene (5f). Recrystallization from methylcyclopentane yielded glistening needles, mp 71.5–72 °C. Anal. (C10H12O2S) C, H.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
<th>Anal. C, H</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>45–46</td>
<td>96</td>
<td>(C10H12O2S) C, H</td>
</tr>
<tr>
<td>4b</td>
<td>110–115</td>
<td>95</td>
<td>(C10H12O2S) C, H</td>
</tr>
<tr>
<td>4c</td>
<td>100–105</td>
<td>95</td>
<td>(C10H12O2S) C, H</td>
</tr>
<tr>
<td>4d</td>
<td>95–105</td>
<td>95</td>
<td>(C10H12O2S) C, H</td>
</tr>
<tr>
<td>4e</td>
<td>86</td>
<td>86</td>
<td>(C10H12O2S) C, H</td>
</tr>
</tbody>
</table>

2,4-Diethoxy-3-(methylthio)-1-bromobenzene (5f). A solution of 21.2 g (100 mmol) of 4f in 200 mL of methylene chloride was treated with 16.5 g (3% excess) of bromine in 100 mL of
and the THF solution was basic to damp pH paper. The solids aqueous NaOH until the formed solids were granular and white, requiring about 2 mL. There was then added 15% propanol, to the 30 mL of LiAlH4 in the THF (30 mmol) stirred magnetically and cooled with an external ice-water bath there was the immediate formation of a deep red color. Stirring followed, over the course of about 1 min, by 11.6 g of reduced directly to the amine. Samples obtained as solids were perhaps 80% pure by TLC. Grinding under cold methylcyclopentane provided a white solid: Compound 5a: bp 105-115 °C (0.15 mm Hg); yield 87%. Anal. (C13H24Br2O4S) C, H. Compound 5b: bp 112-120 °C (0.3 mm Hg); yield 94%. Anal. (C13H24Br2O4S) C, H. Compound 5c: bp 125-140 °C (0.4 mm Hg); yield 83%. Anal. (C13H24Br2O4S) C, H. Presumably, 5d is a mixture of two positional isomers.

In the same manner, the corresponding bromobenzenes 5a-e, g were prepared from the appropriate 4 and elemental bromine: Compound 5a: bp 105-115 °C (0.15 mm Hg); yield 87%. Anal. (C13H24Br2O4S) C, H. Compound 5b: bp 112-120 °C (0.3 mm Hg); yield 94%. Anal. (C13H24Br2O4S) C, H. Compound 5c: bp 125-140 °C (0.4 mm Hg); yield 83%. Anal. (C13H24Br2O4S) C, H. Presumably, 5d is a mixture of two positional isomers.

[3,5-Diethoxy-4-(methylthio)phenyl]acetonitrile (6f). To a solution of dichloromethane (160 mmol) in the hexane there was added 100 mL of a hexane solution of BuLi (1.6 M, 160 mmol). After 15 min of stirring, the viscous suspension was diluted with 200 mL of rigidly dry THF, and the lower suspension that remained was cooled externally to 0 °C. There was then added 4.0 mL of CH3CN (80 mmol) in one portion, followed, over the course of about 1 min, by 11.8 g of HSO3 (40 mmol). There was the immediate formation of a deep red color. Stirring was continued for 0.5 h, and then the reaction contents were poured into dilute sulfuric acid, the phases were separated, and the aqueous fraction was extracted with 2 × 75 mL of CH3Cl. The combined organic fractions were dried over K2CO3, the solvent was removed in vacuo, and the residue was distilled in vacuo. The first fraction [125-145 °C (0.25 mm Hg)] contained (by TLC) starting materials and nonpolar byproducts. A second fraction [bp 100-110 °C (0.2 mm Hg)] provided 27.3 g of a pale yellow oil. Distillation [120-125 °C (0.25 mmHg)] yielded 36.2 g of a pale yellow oil. Distillation [120-125 °C (0.25 mmHg)] was (by TLC) about 90% pure nitrile 

In the same manner, the corresponding phenylacetonitriles 6a-g were prepared from the appropriate 5.

Compound 5f: bp 112-122 °C (0.3 mm Hg); yield 75%. Anal. (C13H24Br2O4S) C, H. Presumably, 5g is a mixture of two positional isomers.

Compound 6a: bp 150-170 °C (0.35 mm Hg); yield 28%. Anal. (C13H24Br2O4S) C, H. Compound 6b: bp 150-170 °C (0.3 mm Hg); yield 40%. Grindings under cold methycyclopentane provided a white solid: mp 35.5-37.5 °C. Anal. (C13H24Br2O4S) C, H. Compound 6c: bp 150-170 °C (0.3 mm Hg); yield 36%.

In the same manner, the corresponding phenethylamines 6a-j were prepared from the appropriate 6. (Note: the lettering of compounds 4-6 (Scheme I correspond to substitution patterns that are different for 3 (Table I).)

Compound 3b: bp 132-140 °C (0.4 mm Hg) (HCl salt); white crystals; mp 164-165 °C. Anal. (C13H26ClNO3S) C, H, N. Compound 3c: bp 112-135 °C (0.2 mm Hg) (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. (C13H26ClNO3S) C, H, N. Compound 3d (from 6b): bp 125-130 °C (0.3 mm Hg); yield 35%.

In the same manner, the corresponding pyrazolines 7a-d were prepared from the appropriate 5.

Compound 7a: bp 137-157 °C (0.3 mm Hg). 3j.HCl: white crystals; mp 182-183 °C. Anal. (C13H26ClNO3S) C, H, N. Compound 7b: bp 112-125 °C (0.3 mm Hg); yield 43%. Anal. (C13H26ClNO3S) C, H, N. Compound 7c (from 6b): bp 135-150 °C (0.3 mm Hg); yield 35%.

Compound 3e (from 6b): bp 124-140 °C (0.3 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3f (from 6b): bp 100-110 °C (0.35 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3g (from 6b): bp 112-135 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3h: bp 112-135 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N.

In the same manner, the corresponding phenethylamines 6a-j were prepared from the appropriate 6. (Note: the lettering of compounds 4-6 (Scheme I correspond to substitution patterns that are different for 3 (Table I).)

Compound 3b (from 6d): bp 132-140 °C (0.4 mm Hg) (HCl salt); white crystals; mp 164-165 °C. Anal. (C13H26ClNO3S) C, H, N. Compound 3c (from 6d): bp 112-135 °C (0.2 mm Hg) (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. (C13H26ClNO3S) C, H, N. Compound 3d (from 6d): bp 125-130 °C (0.3 mm Hg); yield 35%.

Compound 3f (from 6d): bp 124-140 °C (0.3 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3h: bp 112-125 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3i: bp 112-135 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 3j: bp 112-135 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N.

In the same manner, the corresponding phenethylamines 6a-g were prepared from the appropriate 6.

Compound 6a: bp 150-170 °C (0.35 mm Hg); yield 28%. Anal. (C13H26ClNO3S) C, H. Compound 6b: bp 150-170 °C (0.3 mm Hg); yield 40%. Grindings under cold methylcyclopentane provided a white solid: mp 35.5-37.5 °C. Anal. (C13H26ClNO3S) C, H. Compound 6c: bp 150-170 °C (0.3 mm Hg); yield 36%.

In the same manner, the corresponding pyrazolines 7a-d were prepared from the appropriate 5.

Compound 7a: bp 137-157 °C (0.3 mm Hg). 3j.HCl: white crystals; mp 182-183 °C. Anal. (C13H26ClNO3S) C, H, N. Compound 7b: bp 112-135 °C (0.2 mm Hg). Anal. (C13H26ClNO3S) C, H, N. Compound 7c (from 6b): bp 135-150 °C (0.3 mm Hg); yield 35%.

In the same manner, the corresponding pyrazolines 7a-d were prepared from the appropriate 5.
After 1 h of heating on the steam bath, the reaction mixture was not improved by recrystallization from hexane: IR 2249 cm⁻¹.

3,4,5-Triethoxybenzyl Chloride (9b). The ethyl ester of 3,4,5-triethoxybenzoic acid (9; 16.9 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 [110-140 °C (0.4 mmHg)] to yield 8.6 g of a colorless product that spontaneously crystallized. This had a m.p. of 29-30 °C and (by infrared) was free of the ester carboxyl at 1709 cm⁻¹, but it showed a broad hydroxyl stretch centered at 3280 cm⁻¹. The compound was obtained by distillation of a small portion [bp 105-115 °C (0.2 mmHg)] to give a colorless oil that crystallized: yield 7.2 g.

Without further purification, this benzyl alcohol (9a) was suspended in 30 mL of concentrated HCl, and the mixture was heated briefly on the steam bath, cooled to room temperature, and treated with 75 mL of CH₂Cl₂ and 75 mL of water.

The phases were separated, the aqueous phase was removed, the solvent was added in vacuo, yielding 10.2 g of an off-white oil, which distilled at 112-125 °C (0.4 mmHg) to yield 7.5 g of a colorless product that spontaneously crystallized: mp 34-37 °C; yield (from the starting ester) 48%. An analytical sample from hexane had a m.p. of 37.5-38.5 °C, which when mixed with 9a (mp 29-30 °C) produced an oil.

3,4,5-Triethoxybenzyl Cyanide (9e). To a solution of 4.5 g of 9b (17.4 mmol) in 10 mL of DMSO there was added 5 g of NaCN. After 1 h of heating on the steam bath, the reaction mixture was added to 100 mL of stirred water, yielding an oil that set up as a slight darkening.

There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening. There was then added 5.8 g of Et₂S₂ (48 mmol) over 20 min, leading to an increase in the temperature, and the mixture was stirred for 15 min. There was a slight darkening.
pound essentially as described in ref 11 for the methoxy counter-
part: bp 115–116 °C (0.4 mmHg); mp 75–175 °C from cy-
clohexane. Anal. (C12H15NO) C, H. A stirred solution of this
product in 75 mL of CH2Cl2 was treated with 5 mL of methyl
iodide, followed by 3.0 g of KOH. The reaction mixture was
refluxed on the steam bath for 1 h. The product was distilled
from 95 to 110 °C (10 mmHg), yielding 9.2 g (93% yield) of
an almost colorless oil: mp ~ 20 °C. Anal. (C12H17NO) C, H.
3,4-Diethoxy-5-(methylthio)phenyl-2-phthalimido-
ethane (15a). To 200 mL of dry THF, under He and well stirred,
there was added 16.2 g of methyltriphosphophenyl bromide
(45 mmol), and the temperature was brought down to 0 °C
with external ice-water. There was then added 30 mL of 1.6 N BuLi
in hexane (48 mmol), leading to a clearly visible solution, and the
reaction mixture was stirred at room temperature. Then there
was added dropwise a solution of 7.0 g of 12e (29.2 mmol) in 50 mL
of THF. After the addition was complete, the reaction mixture was
refluxed on the steam bath for 1 h. The reaction mixture was
quenched in 800 mL of water, the hexane layer was separated,
and the aqueous layer was extracted with 2 × 75 mL of petroleum
ether. Removal of the solvent yielded 12.0 g of a pale amber oil
(crude styrene), which was used directly in the next step.
A solution of 6.0 mL of BBr3·SiCl3 in 45 mL of THF was
cooled to 0 °C and treated with 12.6 mL of 2-methyl-2-butene.
This was allowed to stand and come to room temperature over
the course of 1 h. To this mixture was added the above crude
styrene, and the mixture was stirred for an additional hour. The
excess hydride was destroyed with methanol (about 2 mL needed;
air was rigidly excluded); then there was added 11.4 g of I2 (45
mmol), followed by a solution of 2.4 g of NaOH in 30 mL of boiling
methanol, over the course of 10 min. This was followed by
sufficient 25% NaOH (about 4 mL) to discharge the color of the
excess iodine. The reaction mixture was quenched in 500 mL of
water, and the residual color was removed with Na2S2O3 (about
4 g). After extraction with 3 × 100 mL of petroleum ether, the
organoiodides were combined, and the solvent was removed in
vacuo to yield the alkyl iodide as a crude pale yellow oil.
To this crude product there was added 90 mL of DMP and 12 g
potassium phthalimide, and the mixture was held at reflux for
1/2 h. The mixture was then added to 500 mL of 5% NaOH,
the phases were separated, and the aqueous phase was extracted
with 2 × 75 mL of ether. The organic extracts were combined,
separated organic phase, the solvents were removed in vacuo,
and then all volatiles were removed by heating to 170 °C at 0.2 mmHg.
The pot residues were ground under an equal volume of methanol
to yield tan solids, which upon recrystallization from methanol
(1 mL/g) yielded white crystals: mp 83–84 °C; yield 3.4 g, 30%
overall from the starting aldehyde. Anal. (C12H19NO3S) C, H.
In the same manner, the corresponding phthalimides 15b–d
were prepared from the appropriate aldehyde 12.

Compound 15b (from 12e): white, wet needles from ethanol; mp
81–82 °C; yield 23%. Anal. (C12H19NO3S) C, H.

Compound 15c (from 12f: fine, white crystals from methanol; mp
85.5–86.5 °C; yield 30%. Anal. (C12H19NO3S) C, H.

3,4-Diethoxy-5-(methylthio)phenyl-2-phthalimido-
ethane (2a). A solution of 3.2 g of 15a in 150 mL of 1-butanol was
added to 20 mL of 66% hydrazine and heated on the steam bath for 2 h.
The reaction mixture was then quenched in 600 mL of dilute
H2SO4, and the two layers were separated. The butanolic layer
was extracted with 2 × 100 mL of dilute H2SO4, and these
extracts were combined with the aqueous layer, which was then extracted
with 2 × 75 mL of CH2Cl2. The aqueous fraction was then made
basic with 25% NaOH and extracted with 3 × 75 mL of CH2Cl2,
the extracts were pooled, and the solvent was removed in vacuo.
The resulting crude product was distilled [140–145 °C (0.3
jugates are internalized in micropinocytotic vesicles that means of achieving drug uptake by pinocytosis as opposed transport, and they have given promising therapeutic re-

Products of Methotrexate-Poly(L-lysine) Conjugates


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Derivatives of methotrexate (MTX) in which the β-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(ε-lysine). Inhibition of DHFR in a cell-free assay was decreased only 3-fold relative to MTX, indicating that ε-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 β-position. Growth inhibition of H35 rat hepatoma cells was decreased 40- to 60-fold relative to MTX, but in H35Ro.3 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 to 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.

Covalent poly(L-lysine) conjugates of methotrexate (MTX) have been studied in several laboratories1−10 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.6 From the available evidence in neoplastic6,8,10 and non-neoplastic5,15 cell lines in culture, it appears that the conjugates are internalized in micropinocytotic vesicles that coalesce into larger vacuoles and ultimately fuse to protease-rich secondary lysosomes. The conjugates themselves are ineffective as dihydrofolate reductase inhibitors,2 but degradation of the poly(L-lysine) backbone by the lysosomal proteases yields small fragments that exert typical antifolate effects8 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.1,8,9 Moreover, the uptake of MTX–poly(ε-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.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 es-