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Structural and Conformational Analogues of L-Methionine as Inhibitors of the Enzymatic Synthesis of S-Adenosyl-L-Methionine

II. Aromatic Amino Acids

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SUMMARY

COULTER, A. W., LOMBARDINI, J. B., AND TALALAY, PAUL: Structural and conformational analogues of L-methionine as inhibitors of the enzymatic synthesis of *S*-adenosyl-L-methionine. II. Aromatic amino acids. *Mol. Pharmacol.* **10**, 305–314 (1974).

A series of aromatic amino acids structurally related to L-methionine were synthesized as potential inhibitors of the enzymatic formation of *S*-adenosyl-L-methionine by partially purified preparations of ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) of bakers' yeast, *Escherichia coli*, and rat liver. 2-Amino-5-phenylpentanoic acid was a considerably better inhibitor of the liver enzyme than 2-aminopentanoic acid (norvaline) but produced no inhibition of the yeast and *E. coli* enzymes. (*E*)-2-Amino-5-phenyl-4-pentenoic and 2-amino-5-phenyl-4-pentynoic acids were superior in their inhibitory activity to the corresponding chain-saturated analogues. The phenyl group thus imparts a favorable influence on the inhibitory power. *S*-Phenyl-DL-homocysteine is an even more potent inhibitor of the liver enzyme, but is also devoid of activity in the microbial enzyme systems. *O*-Phenyl-DL-homoserine displays similar properties, but was not quite as effective as the sulfur analogue. In order to evaluate the effect of electron-donating and electron-withdrawing substituents on the phenyl ring, the following compounds were synthesized: *O*-(*p*-fluorophenyl)-DL-homoserine, *O*-(*p*-chlorophenyl)-DL-homoserine, *O*-(*p*-bromophenyl)-DL-homoserine, *O*-(*p*-nitrophenyl)-DL-homoserine, *O*-(*p*-methoxyphenyl)-DL-homoserine, *O*-(*p*-methylphenyl)-DL-homoserine, *O*-(*m*-chlorophenyl)-DL-homoserine, *O*-(*m*-bromophenyl)-DL-homoserine, *O*-(*m*-nitrophenyl)-DL-homoserine, and *O*-(*m*-methoxyphenyl)-DL-homoserine. When the inhibitory potencies of these compounds (expressed as pI_{50} values) were analyzed in terms of the Hammett σ values of the substituents, the inhibitory potency was correlated with the positive magnitudes of the σ values. If the anomalous nitro compounds are excluded, the Hammett *sigma-rho* equation $pI_{50} = 1.45\sigma + 1.82$ is obtained by least-squares linear regression analysis. This finding suggests that the electron-withdrawing substituents are most favorable for inhibition, and that the creation in the analogue of a partial positive charge at the heteroatom bearing the phenyl group may resemble most closely the transition state of

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the enzymatic reaction, in which the thioether group of L-methionine is converted to the positively charged sulfonium group of *S*-adenosyl-L-methionine. *O*-Methyl-DL-homoserine is a strong inhibitor of the adenosyltransferases of yeast, *E. coli*, and rat liver, and especially powerful for the *E. coli* enzyme. Since it has been shown that this compound inhibits the growth of certain microorganisms and viruses, as well as displaying certain specific toxicities in rodents, the possibility should be considered that these effects are due to inhibition of the adenosyltransferase reaction.

INTRODUCTION

This paper describes the synthesis and evaluation of a series of aromatic amino acids designed as inhibitors of the enzymatic synthesis of *S*-adenosyl-L-methionine by partially purified preparations of ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) of bakers' yeast, *Escherichia coli*, and rat liver. These studies are part of a systematic effort to obtain inhibitors of this reaction with a high degree of potency and tissue selectivity. In accompanying papers, we report on related series of aliphatic (1) and cyclic (2) analogues of L-methionine that possess similar inhibitory properties. The rationale for the design of such amino acids, and their effects on tissue levels of L-methionine and *S*-adenosyl-L-methionine, have been set out in detail elsewhere (3–5).

The aromatic compounds examined include terminal phenyl derivatives of the saturated and unsaturated aliphatic amino acids described in a companion paper (1). In addition, we have synthesized a series of *O*-phenylhomoserine and *S*-phenylhomocysteine derivatives in which the aromatic rings have been substituted with electron-withdrawing or electron-donating groups. The influences of these groups on the inhibitory potency of the analogues have been interpreted in terms of the Hammett *sigma-rho* relationship (6).

EXPERIMENTAL PROCEDURE

The materials and methods used in these experiments have been described (1). The inhibitory potencies of all analogues were examined with partially purified preparations of adenosyltransferases[2] of bakers' yeast, *E. coli*, and rat liver (1, 5). The concentrations of inhibitors required to produce 50% inhibition of enzyme activity (I_{50} values) were determined at L-methionine concentrations of 37.5 μM by the graphical method of Dixon (7).

Amino Acid Analogues

The structures and inhibitory potencies of all amino acid analogues are shown in Tables 1–3, in which each compound tested for inhibitory activity is assigned a Roman numeral. Table 4 lists all compounds and intermediates synthesized, together with their physical constants.

Commercial. L-Phenylalanine (I), L-tyrosine (II), and DL-homoserine (XI) were supplied by Schwarz/Mann. DL-Homocysteine (VII) and *O*-ethyl-DL-homoserine (XIII) were supplied by Pfaltz and Bauer.

Gifts. *S-n*-Propyl-DL-homocysteine (VIII) and *S-n*-butyl-DL-homocysteine (IX) were synthesized according to published procedures (8), and kindly supplied by Dr. H. M. Kolenbrander, Central College, Pella, Iowa.

Syntheses

General. Melting points were determined on a Kofler hot-stage melting point apparatus and are uncorrected. All new compounds were analyzed for C, H, N or C, H, halogen and were within ±0.4% of the theoretical values. Infrared, nuclear magnetic resonance, and mass spectra of all compounds were consistent with the assigned structures.

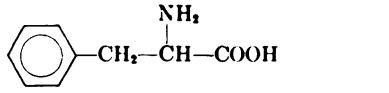
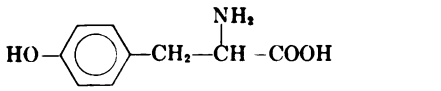
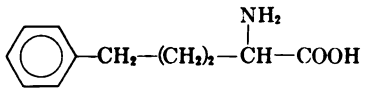
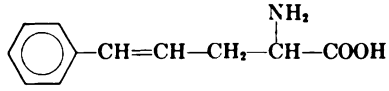
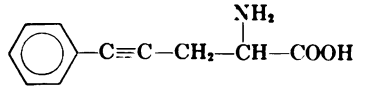
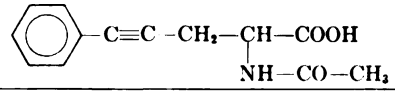
Preparation of L-amino acids. Acylase I hydrolysis of the DL-2-acetamido amino acid according to the general procedure of Green-

[2] The abbreviations used are: adenosyltransferase, ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6); I_{50}, concentration of inhibitor required to achieve 50% reduction in adenosyltransferase activity under specified conditions; pI_{50}, $\log_{10}(1/I_{50})$, where the I_{50} value is in molar units.

TABLE 1

Inhibitory potencies of ω-phenyl-substituted aliphatic amino acid analogues on ATP:L-methionine S-adenosyltransferases of yeast, E. coli, and rat liver

The adenosyltransferase activities were measured at 37.5 μM L-methionine as described (1).

Compound No.	Structure	Configuration at C-2	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	mM	mM	mM
I		L	35	I[a]	I	I
II		L	4.4	I	I	44[b]
III		L	10.0	I	I	17[b]
IV		L	3.9	I	I	12[b]
V		DL	2.5	I	I	13[b]
VI		DL		14.4[b]	45[b]	4.6

[a] Compounds were designated as I (inactive) if less than 10% inhibition was observed at the maximum concentration that could be tested because of limitations of solubility.

[b] These values were obtained by graphical extrapolation, and are not bracketed by experimental observations.

stein and Winitz (9) afforded the L-amino acid (Table 4).

Previously described amino acids. Compounds III, IV, and V were prepared according to the procedure of Schlögl (10). Compound X was prepared by the method of Armstrong and Lewis (11). DL-Methoxinine (XII) (12, 13) was prepared by Dr. H. Doshan of this laboratory, by condensation of methanol with 2-propenaldehyde, followed by Strecker synthesis.

General method for preparation of diethyl acetamidomalonate derivatives. The appropriate alkyl or aryl bromide (14–16) (10% excess) in absolute ethanol was added to a stirred solution of diethyl acetamidomalonate in absolute ethanol containing an equivalent amount of sodium ethoxide. The mixture was refluxed for 16–24 hr. The ethanol was removed under reduced pressure, and the resulting oil was extracted with ether. The combined ether extracts were washed successively with water and saturated sodium chloride solution, and the ether was dried over sodium sulfate. Upon evaporation of the ether, the resulting oil usually crystallized. If crystallization did not occur, the oil was chromatographed on a silica gel column and the isolated product was crystallized from ethyl acetate–petroleum ether; yields were 30–70% (Table 4).

General method for preparation of N-acyl-amino acids. The diethyl acetamidomalonate derivative was refluxed for 4 hr in a 5-fold excess of 2.5 N sodium hydroxide, cooled, and acidified to pH 2 with 6 N hydrochloric

TABLE 2

Inhibitory potencies of S-substituted homocysteine derivatives on ATP:L-methionine S-adenosyltransferases of yeast, E. coli, and rat liver

The adenosyltransferase activities were measured at 37.5 μM L-methionine as described (1).

Compound No.	Structure $R—S—CH_2—CH_2—\underset{\displaystyle NH_2}{\underset{\vert}{CH}}—COOH$ R =	Configuration at C-2	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	*mM*	*mM*	*mM*
VII	H—	DL	20	44[a]	34[a]	46[a]
VIII	$CH_3—CH_2—CH_2—$	DL	8.3	I[b]	I	I
IX	$CH_3—CH_2—CH_2—CH_2—$	DL	4.2	I	I	I
Xa		DL	4.0	I	I	11.5[a]
Xb		L	4.0	I	I	13.5[a]
Xc		D	4.0	I	I	12[a]

[a] These values were obtained by graphical extrapolation, and are not bracketed by experimental observations.

[b] Compounds were designated as I (inactive) if less than 10% inhibition was observed at the maximum concentration that could be tested because of limitations of solubility.

acid. The dicarboxylic acid which precipitated was collected and decarboxylated by refluxing for 4 hr in water. The solution was adjusted to pH 2 with 6 N hydrochloric acid and cooled to 5°, and the *N*-acylamino acid was collected and crystallized from ethanol–water; yields, 50–75% (Table 4).

General method for acid hydrolysis. The diethyl acetamidomalonate derivative (1 mmole) was refluxed for 4–5 hr in 5 ml of 6 N hydrochloric acid. The solution was adjusted to pH 5 with 10 N sodium hydroxide, and the precipitated amino acid was collected and crystallized from hot water; yields, 45–95%.

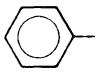
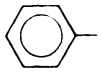
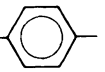
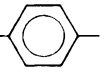
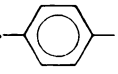
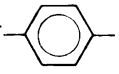
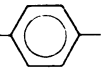
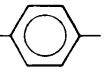
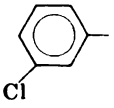
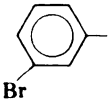
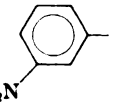
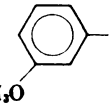
RESULTS AND DISCUSSION

ω-Phenyl-substituted aliphatic amino acids. Although L-norvaline is a rather poor inhibitor of the adenosyltransferases [I_{50} = 144, 45, and 124 mM for the yeast, *E. coli*, and rat liver systems, respectively (1)], the introduction of a terminal phenyl group, as in L-2-amino-5-phenylpentanoic acid (III, Table 1), led to considerable enhancement of the inhibitory activity for the liver enzyme (I_{50} = 17 mM). At the same time, this structural modification totally abolished the inhibitory properties for the two microbial enzyme systems. Since the introduction of terminal olefins and acetylenes considerably enhanced the inhibitory properties of L-norvaline (1), these structural modifications were combined with a ω-phenyl group, and (*E*)-2-amino-5-phenyl-4-pentenoic (IV) and 2-amino-5-phenylpentynoic (V) acids were prepared. The unsaturated phenyl derivatives were both considerably better inhibitors of the liver enzyme (Table 1, I_{50} = 12 mM for L-IV and 13 mM for DL-V) than their parent compounds lacking the phenyl group [I_{50} = 65 mM for DL-2-amino-4-pentenoic acid and I_{50} = 20.5 mM for DL-2-amino-4-pentynoic acid (1)]. Moreover, just as in the case of the saturated analogues, the 5-phenyl olefin and acetylene were with-

TABLE 3

Inhibitory potencies of O-substituted homoserine derivatives on ATP:L-methionine S-adenosyltransferases of yeast, E. coli, and rat liver

The adenosyltransferase activities were measured at 37.5 μM L-methionine as described (1).

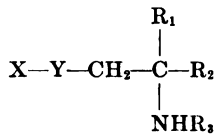
Compound No.	Structure R—O—CH_2—CH_2—CH(—NH_2)—COOH R =	Configuration at C-2	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	mM	mM	mM
XI	H—	DL		64	52	60
XII	CH_3—	DL		13	2	7
XIII	CH_3—CH_2—	DL	35	I[a]	I	I
XIVa		DL	7.5	I	I	23[b]
XIVb		L	3.2	I	I	26[b]
XV	F— 	DL	13.5	I	I	22[b]
XVI	Cl— 	DL	4.3	I	I	6.1[b]
XVII	Br— 	DL	4.0	25[b]	I	4.4[b]
XVIII	O_2N— 	DL	4.8	I	I	5.1[b]
XIX	CH_3O— 	DL	6.6	I	I	36[b]
XX	CH_3— 	DL	4.0	I	I	15[b]
XXI	 Cl	DL	8.8	28[b]	I	5.2
XXII	 Br	DL	4.4	16[b]	I	3.2
XXIII	 O_2N	DL	7.6	I	I	8.7
XXIV	 CH_3O	DL	28.9	93[b]	I	14.6

[a] Compounds were designated as I (inactive) if less than 10% inhibition was observed at the maximum concentration that could be tested because of limitations of solubility.

[b] These values were obtained by graphical extrapolation, and are not bracketed by experimental observations.

TABLE 4

Structures and physical constants of aromatic amino acids and intermediates in their synthesis



Compound No.	Steric configuration	X	Y	R_1	R_2	R_3	Melting point
							°C
		C_6H_5—	—CH_2CH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	85–86
	DL	C_6H_5—	—CH_2CH_2—	—H	—CO_2H	—$COCH_3$	143–145
	DL	C_6H_5—	—CH_2CH_2—	—H	—CO_2H	—H	216–224 (d)[a]
III	L	C_6H_5—	—CH_2CH_2—	—H	—CO_2H	—H	209–211[b]
	(*E*)	C_6H_5—	—CH=CH—	—CO_2Et	—CO_2Et	—$COCH_3$	84–85
	(*E*)-DL	C_6H_5—	—CH=CH—	—H	—CO_2H	—$COCH_3$	146–147
	(*E*)-DL	C_6H_5—	—CH=CH—	—H	—CO_2H	—H	211 (d)[c]
IV	(*E*)-L	C_6H_5—	—CH=CH—	—H	—CO_2H	—H	197–200[d]
		C_6H_5—	—C≡C—	—CO_2Et	—CO_2Et	—CHO	54[e]
V	DL	C_6H_5—	—C≡C—	—H	—CO_2H	—H	215–220 (d)[f]
VI	DL	C_6H_5—	—C≡C—	—H	—CO_2H	—$COCH_3$	126
		C_6H_5—	—SCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	67–68
Xa	DL	C_6H_5—	—SCH_2—	—H	—CO_2H	—H	210–212 (d)[g]
	DL	C_6H_5—	—SCH_2—	—H	—CO_2H	—$COCH_3$	160–162[h]
Xb	L	C_6H_5—	—SCH_2—	—H	—CO_2H	—H	215–217 (d)[i]
Xc	D	C_6H_5—	—SCH_2—	—H	—CO_2H	—H	211–214 (d)[i]
XII	DL	CH_3—	—OCH_2—	—H	—CO_2H	—H	220–225 (d)[k]
		C_6H_5—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	61–63
XIVa	DL	C_6H_5—	—OCH_2—	—H	—CO_2H	—H	210–217 (d)
	DL	C_6H_5—	—OCH_2—	—H	—CO_2H	—$COCH_3$	125–126
XIVb	L	C_6H_5—	—OCH_2—	—H	—CO_2H	—H	215–230 (d)[l]
		p-FC_6H_4—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	68–69
XV	DL	*p*-FC_6H_4—	—OCH_2—	—H	—CO_2H	—H	190–200 (d)
		p-ClC_6H_4—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	83–84
XVI	DL	*p*-ClC_6H_4—	—OCH_2—	—H	—CO_2H	—H	205–212 (d)
		p-BrC_6H_4—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	80
XVII	DL	*p*-BrC_6H_4—	—OCH_2—	—H	—CO_2H	—H	271–274 (d)
		p-$NO_2C_6H_4$—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	100–101
XVIII	DL	*p*-$NO_2C_6H_4$—	—OCH_2—	—H	—CO_2H	—H	197–199 (d)
		p-$CH_3OC_6H_4$—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	55–56
XIX	DL	*p*-$CH_3OC_6H_4$—	—OCH_2—	—H	—CO_2H	—H	221–224 (d)
		p-$CH_3C_6H_4$—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	56–57
XX	DL	*p*-$CH_3C_6H_4$—	—OCH_2—	—H	—CO_2H	—H	230–232 (d)
		m-ClC_6H_4—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	57–59
XXI	DL	*m*-ClC_6H_4—	—OCH_2—	—H	—CO_2H	—H	197–205 (d)
		m-BrC_6H_4—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	62–63
XXII	DL	*m*-BrC_6H_4—	—OCH_2—	—H	—CO_2H	—H	203–206 (d)
		m-$NO_2C_6H_4$—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	115–118
XXIII	DL	*m*-$NO_2C_6H_4$—	—OCH_2—	—H	—CO_2H	—H	197–199 (d)
		m-$CH_3OC_6H_4$—	—OCH_2—	—CO_2Et	—CO_2Et	—$COCH_3$	50–53
XXIV	DL	*m*-$CH_3OC_6H_4$—	—OCH_2—	—H	—CO_2H	—H	197–204 (d)
	DL	*p*-BrC_6H_4—	—SCH_2—	—H	—CO_2H	—H	223–233 (d)

[a] Ref. 10, mp 206–209°.

out inhibitory effects on the yeast and *E. coli* enzymes. Although strict comparisons of inhibitory activities could not be made, since all the optical isomers were not available, it may be inferred from these findings that the previously established relationship that progressive unsaturation at C-4 improves the inhibitory power of aliphatic C_5 amino acids (1, 3, 4) also applies to their 5-phenyl derivatives. It should be noted that although the introduction of the phenyl group enhances the inhibitory potency and restricts it to the liver enzyme, the unsubstituted aromatic ring is not as inhibitory as a methyl group [e.g., for DL-2-amino-5-phenyl-4-pentynoic acid (V) I_{50} = 13 mM, whereas that for DL-2-amino-4-hexynoic acid is 3.7 mM for the liver enzyme (1)]. In this connection it would be of interest to examine the optimum location of the phenyl group by preparing 4-phenyl and 6-phenyl derivatives of the shorter and longer homologous aliphatic amino acids. We know that the phenyl group may not be placed 2 carbon atoms closer to the amino and carboxyl termini, since phenylalanine (I) is quite inactive. However, in this context, it may be recalled that elongation or contraction of the chain between the α-carbon and the sulfur atom of L-methionine destroys substrate properties without leading to significant inhibitory activities (3, 4).

The terminal phenyl group augments the inhibitory activity for the liver enzyme while completely abolishing any effect on the yeast and *E. coli* enzymes. Since the ω-phenyl compounds are not strict structural or electronic analogues of methionine, we are led to believe that their enhanced inhibitory properties may be ascribed to a hydrophobic interaction with a complementary region on the liver enzyme, which is not present or available for binding in the isofunctional enzymes of microbial origin. This finding provides clues for the achievement of selective toxicity, and the tissue specificity of these compounds among animal tissues deserves examination.

S-Substituted homocysteine derivatives. DL-Homocysteine (VII, Table 2) is only a mediocre inhibitor of the adenosyltransferases. Methylation and ethylation of the thiol group of homocysteine provide the natural substrate (L-methionine) and ethionine, respectively. Ethionine is converted to *S*-adenosylethionine by the yeast and liver adenosyltransferases, but it is a very poor substrate or inhibitor for the *E. coli* enzyme (3). Further enlargement of the *S*-alkyl group to *S*-*n*-propyl- (VIII) or *S*-*n*-butyl- (IX) DL-homocysteine results in compounds that are neither substrates nor inhibitors of the enzymes. Thus, moderately sized alkyl groups alone are insufficient to produce significant hydrophobic binding to the enzyme. D- and L-*S*-Phenylhomocysteines (X) proved to be moderately good inhibitors of the liver adenosyltransferase (I_{50} = 11.5–13.5 mM). Like the ω-phenyl aliphatic amino acids, the *S*-phenylhomocysteines displayed strict selectivity for the liver enzyme and were not inhibitory for the microbial enzymes examined. With a view to exploiting the desirable, electron-withdrawing properties of substituents (see below), *S*-(*p*-bromophenyl)-DL-homocysteine was synthesized, but unfortunately this compound proved to be extremely water-insoluble and thus could not be evaluated in the enzyme systems.

O-Substituted homoserine derivatives. DL-

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[b] $[\alpha]_D^{25}$ +22.2° (*c*, 1, in 1 N HCl).
[c] Ref. 10, mp 213–215°.
[d] $[\alpha]_D^{25}$ −18.7° (*c*, 1, in 1 N HCl).
[e] Ref. 10, mp 60–62°.
[f] Ref. 10, mp 215–218°.
[g] Ref. 11, mp 229–232°.
[h] Ref. 11, mp 164–165°.
[i] $[\alpha]_D^{25}$ +29.2° (*c*, 1, in 1 N HCl); ref. 11, mp 234–236° (d), $[\alpha]_D^{20}$ +30.3° (*c*, 1, in 1 N HCl).
[j] $[\alpha]_D^{25}$ −29.0° (*c*, 1, in 1 N HCl); ref. 11, mp 233–236° (d), $[\alpha]_D^{24}$ −30.2° (*c*, 1, in 1 N HCl).
[k] Refs. 12 and 13, mp 220–250 (d).
[l] $[\alpha]_D^{25}$ −15.2° (*c*, 0.5, in H_2O).

Homoserine (XI, Table 3) is a poor enzyme inhibitor and is somewhat inferior to DL-homocysteine (VII, Table 2), as might be expected, since the sulfur derivative is clearly a closer structural and electronic analogue of L-methionine. *O*-Methyl-DL-homoserine (DL-methoxinine, XII) is a powerful inhibitor of all the three adenosyltransferases, but is especially effective in the *E. coli* system. DL-Methoxinine was first synthesized by Roblin *et al.* (12), who reported its inhibition of the growth of certain microorganisms. This effect was antagonized by L- but not D-methionine (12, 13). In high concentration, methoxinine also inhibited the growth of vaccinia virus in tissue culture (17). In rats, the feeding of DL-methoxinine resulted in weight loss, renal toxicity, and lowering of the lipid content of animals maintained on a high-fat, low-protein diet (18). The possibility should be considered that the antimicrobial effects and some of the toxic effects of methoxinine may be due to the inhibition of the adenosyltransferases. Even a modest increase in size of the alkyl group, e.g., *O*-ethyl-DL-homoserine (ethoxinine, XIII), destroys inhibitory activity, although the low solubility of XIII in aqueous systems might have obscured a low level of inhibitory potency.

O-Phenylhomoserine (XIV, Table 3) is a moderately good inhibitor, which, as in the case of the already mentioned phenyl aliphatic and *S*-phenylhomoserine derivatives, shows a high degree of selectivity for the liver enzyme. A series of *meta*- and *para*-substituted halo, nitro, methyl, and methoxyl derivatives of *O*-phenyl-DL-homoserine were synthesized with a view to evaluating the influences of the electron-donating or electron-withdrawing properties of substituents on the inhibitory potency, in accordance with the well-known principles established by Hammett (6). These compounds (XV–XXIV, Table 3) differed considerably in their inhibitory strengths. All of them resembled the parent compound, in that they were either very weakly inhibitory or inactive with the *E. coli* and yeast enzymes in comparison to their more powerful inhibitory effects on the liver enzyme. Substitution in the *para* position on the phenyl moiety of *O*-phenyl-DL-homoserine by electron-withdrawing groups enhanced the inhibitory potency. Thus, in the halogen-substituted series, *O*-*p*-bromophenyl-DL-homoserine (XVII, $\sigma = +0.232$) is a better inhibitor than the *O*-*p*-chloro (XVI, $\sigma = +0.227$) compound, and the latter compound in turn is much better than *O*-*p*-fluorophenyl-DL-homoserine (XV, $\sigma = +0.062$). Nitration of the phenyl moiety in the *para* position also favors electron withdrawal and results in a potent inhibitor (XVIII). In contrast, the electron-donating *p*-methoxyl group (XIX, $\sigma = -0.268$) detracts from the inhibitory potency of *O*-phenyl-DL-homoserine. The electron-donating *p*-methyl group (XX, $\sigma = -0.170$) enhances the inhibitory power of *O*-phenyl-DL-homoserine.

A series of *meta*-substituted *O*-phenyl derivatives of homoserine were also synthesized and showed behavior similar to the *para*-substituted analogues. The most electron-withdrawing groups on the phenyl ring in general produced the best inhibitors. The inhibitory potency of these substituted *O*-phenyl-DL-homoserines decreased in the following order: *m*-bromo, *m*-chloro, *m*-nitro, and *m*-methoxyl.

These findings were analyzed according to the well-known *sigma-rho* equation of Hammett (6) by plotting the pI_{50} value [i.e., $\log_{10}(1/I_{50})$] against the σ values assigned to each substituent according to its tendency to act as an electron-donating or electron-withdrawing group (Fig. 1). A reasonable correlation appears to exist between the pI_{50} values and the positive magnitude of the σ values for all the substituents, with the exception of the *m*- and *p*-nitrophenyl derivatives, which are clearly anomalous. This deviant behavior of nitrophenyl groups has been observed in several other Hammett analyses of enzyme inhibitors (19–21). If the nitrophenyl derivatives are excluded, a least-squares linear regression analysis of the nine remaining compounds leads to the following Hammett equation: $pI_{50} = 1.45\sigma + 1.82$ (with a correlation coefficient $r = 0.883$). In an addendum to this paper, Hulbert (22) has obtained an almost perfect correlation between pI_{50} values and a combination of Hammett σ and Hansch π values.

The positive correlation between the pI_{50}

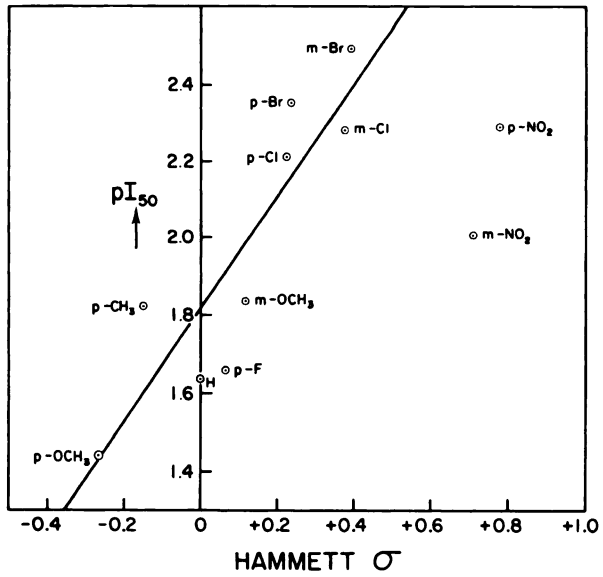

FIG. 1. *Analysis according to Hammett sigma-rho relationship of inhibitory potencies of a series of substituted O-phenyl-DL-homoserine derivatives for ATP:L-methionine adenosyltransferase of rat liver*

The pI_{50} values [$\log_{10}(1/I_{50})$] are plotted as a function of the Hammett σ values for each substituent. The I_{50} values were obtained as described under EXPERIMENTAL PROCEDURE and are expressed in units of *molarity*. The σ values were assigned according to Hammett (6). The line was drawn on the basis of a least-squares regression analysis of all I_{50} and σ values except those for the nitro derivatives. The derived equation is $pI_{50} = 1.45\sigma + 1.82$. The correlation coefficient, r, is 0.883 ($r^2 = 0.780$).

values and the electron-withdrawing tendency of the aromatic substituents in this group of compounds suggests that the creation of a partially positive charge at the heteroatom bearing the aromatic ring favors binding of the inhibitor to the enzyme. This finding might be related to the fact that in the enzymatic reaction the thioether sulfur atom of L-methionine bearing two pairs of free electrons is converted to a sulfonium group (*S*-adenosyl-L-methionine) carrying a net positive charge. Thus the transition state of the enzymatic reaction might involve the development of a partially positive charge in the topographical neighborhood of the heteroatom. A formal positive charge on the sulfur atom does not appear to favor binding to the enzyme, since the sulfonium derivative, *S*-methyl-L-methionine, is a quite poor inhibitor (I_{50} = 55 mM, inactive, and 90 mM for the yeast, *E. coli*, and liver enzymes). The last-mentioned compound is, of course, closely related structurally to the reaction product. Thus these inhibitors appear to act as transition-state rather than product-analogue inhibitors. It is to be noted that even the introduction of the most powerful electron-withdrawing groups did not lead to inhibitors superior to those encountered among the aliphatic (1) and the carbocyclic or heterocyclic analogues (2).

Enantiomeric specificity. In the accompanying papers (1, 2) it has been noted that among all reasonably powerful inhibitors that occur in enantiomeric pairs, the inhibitory potency appears to reside in one member of each such pair. Among the *O*-phenylhomoserines (XIV) and *S*-phenylhomocysteines (X), no enantiomeric specificity could be detected. It might be assumed that the hydrophobic interaction of the phenyl ring outweighs the importance of the correct steric fit in the region of the α-carbon of the amino acid, or that in fact the electrostatic interactions of the COO^- and NH_3^+ groups of the amino acids with the complementary groups on the enzyme are very weak, or do not occur. Because of this lack of steric preference, the substituted

O-phenyl-DL-homoserines (Table 3) were not subjected to optical resolution.

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