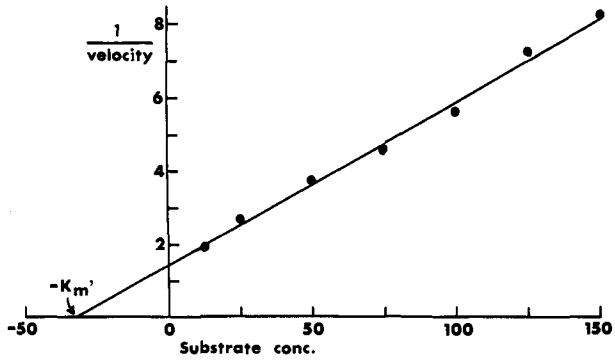


Figure 1. Substrate inhibition of PNMT by 3,4-dichlorophenylethanolamine. The reciprocal of velocity in pmoles/min is plotted *vs.* micromolar concentration of 3,4-dichlorophenylethanolamine. All concentrations were chosen to be in the range of inhibition by excess substrate.

Table II. Self-inhibition by PNMT Substrates at High Concentrations

Substrate	π^b	Concentration range studied, m*M*	$-\text{Log } K_m'$ value[a]
2-Chlorophenylethanolamine	0.59	0.5–8	1.72
2-Fluorophenylethanolamine	0.04	0.5–8	1.81
Phenylethanolamine		4–20	2.26
4-Fluorophenylethanolamine	0.14	0.2–3.2	2.38
3,4-Dihydroxyphenylethanolamine	−1.13	0.2–1.6	2.57
4-Hydroxyphenylethanolamine	−0.61	0.2–3.2	2.74
3-Bromophenylethanolamine	0.91	0.025–0.15	4.12
3,4-Dichlorophenylethanolamine	1.38	0.01–0.15	4.49
4-Bromophenylethanolamine	0.90	0.01–0.15	4.62

[a] K_m' value was determined graphically as shown in Figure 1. Units of K_m' values were in molar substrate concentration. [b] *Cf.* Table I.

consistent in their behavior. Whereas 3-methoxy-4-hydroxyphenylethanolamine (normetanephrine) had an observed $-\log K_m$ value (3.50) in good agreement with the calculated value (3.63), both 4-hydroxyphenylethanolamine (octopamine) and 3,4-dihydroxyphenylethanolamine (norepinephrine) were better substrates than the equation predicted. The $-\log K_m$ values observed for octopamine and norepinephrine were 5.00 and 4.90, respectively, whereas the calculated values were only 3.58 and 2.95, respectively. These compounds resembled the amphetamines[6] in that the 4-hydroxyl contributed to binding with PNMT in a manner over and above its effect on lipophilicity.

The negative contribution calculated by Fujita and Ban for the 4-hydroxyl group arose largely because norepinephrine had low activity according to the data they used, *e.g.*, much lower than phenylethanolamine itself. Paradoxically, the reason for the low relative activity of norepinephrine in those conditions is because its affinity for the enzyme is so great; the high substrate concentration was further above the optimum concentration and hence inhibition by excess substrate was greater with norepinephrine than with any of the other substrates in their list.

Figure 1 illustrates the inhibition that occurs at excess substrate levels in the case of 3,4-dichlorophenylethanolamine. The plot of the velocity reciprocal *vs.* substrate concentration permits calculation of a K_m' value.[9] The K_m' is a measure of the inhibition by excess substrate just as K_m is a measure of the substrate activity. Other phenylethanolamines were studied, and the data were plotted as in Figure 1. The values were fitted to a straight line by linear regression analysis, and the K_m' value was determined as the x intercept. Table II lists the calculated $-\log K_m'$ values for nine PNMT substrates. The $-\log K_m'$ values roughly paralleled the K_m values. Excluding the compounds in Table II with ortho or with hydroxyl substituents, we correlated the $-\log K_m'$ values for the remaining five compounds according to eq 2. The square of the correlation coefficient was

$$-\log K_m' = -1.484\pi^2 + 3.813\pi + 2.094 \quad (2)$$
$$(\pm 0.323) \quad (\pm 0.765) \quad (\pm 1.031) \quad (\pm 0.262)$$

0.96. Note that in this case the fit was improved by adding the π^2 term. On the basis of these limited data, it appears that the self-inhibition requirements are about the same as the substrate requirements. An optimum π value for self-inhibition, based on the derivatives of eq 2, is about 0.84. 4-Hydroxyl compounds again have higher $-\log K_m'$ values than would be predicted. That observation is consistent with our finding with amphetamines as inhibitors.[6]

References

(1) T. Fujita and T. Ban, *J. Med. Chem.*, 14, 148 (1971).
(2) J. Axelrod, *J. Biol. Chem.*, 237, 1657 (1962).
(3) J. Axelrod, *Pharmacol. Rev.*, 18, 95 (1966).
(4) R. W. Fuller and J. M. Hunt, *Biochem. Pharmacol.*, **14**, 1896 (1965).
(5) R. W. Fuller, B. J. Warren, and B. B. Molloy, *Biochim. Biophys. Acta*, 222, 210 (1970).
(6) R. W. Fuller, J. Mills, and M. M. Marsh, *J. Med. Chem.*, **14**, 322 (1971).
(7) G. N. Wilkinson, *Biochem. J.*, **80**, 324 (1961).
(8) R. W. Fuller and J. M. Hunt, *Anal. Biochem.*, **16**, 349 (1966).
(9) M. Dixon and E. C. Webb, "Enzymes," Academic Press, New York, N. Y., 1958.
(10) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

Are Calculated Electron Populations Suitable Parameters for Multiple Regression Analyses of Biological Activity?

P. R. Andrews

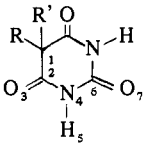
Department of Physical Biochemistry, John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory 2601, Australia.

Received March 27, 1972

A major objective of medicinal chemistry is identification of the salient structural properties of biologically active molecules. Hansch in particular[1] has sought quantitative correlations between activity and molecular properties by applying linear multiple regression analysis to combinations of substituent constants in free energy relationships similar to the Hammett equation. He has commonly employed hydrophobic, steric, and electronic constants, but has suggested[2] replacing the latter with the electron populations on atoms chosen during regression analysis of data from molecular orbital calculations.

The calculation of atomic electron populations by suitably partitioning molecular wave functions is familiar for π-electron systems, and many other structures have become accessible to quantum chemistry through the introduction of semiempirical all-valence-electron techniques such as extended Hückel theory (EHT)[3] and the complete neglect of

Table I. Substituent Constants and Net Atomic Charges (EHT) of Barbiturates



		Substituent constants			Atomic charges						
R	R'	Hansch[a] π constant	Taft[b] polar constant, σ^*	Taft[b] steric parameter, E_s	1	2	3	4	5	6	7
H	H	0.00	0.98	2.48	−0.1852	1.2985	−1.3310	−0.4304	0.3323	1.4582	−1.3427
C_2H_5	C_2H_5	2.00	−0.20	−0.14	0.0476	1.2785	−1.3286	−0.4334	0.3322	1.4588	−1.3422
C_2H_5	C_6H_5	2.77	0.50	*c*	0.0495	1.2745	−1.3107	−0.4314	0.3323	1.4563	−1.3426

[a]See ref 12. [b]See ref 13. [c]Varies with orientation (Kutler and Hansch[14]).

Table II. Regression of EHT Atomic Charges (y) on CNDO/2 Atomic Charges (x)

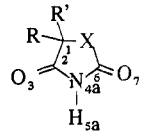
		Regression coefficients		Standard deviation				
Atom[a]	Order[b]	Slope	Intercept	Slope	Intercept	n	r	P
All		3.42	0.00	0.06	0.02	133	0.982	$\ll\ll$0.001
1	1	3.44	0.06	0.35	0.03	19	0.922	$\ll$0.001
6	1	2.59	0.34	0.40	0.17	19	0.844	$\ll$0.001
2	2	1.67	0.67	0.55	0.19	19	0.593	<0.01
4a	2	2.26	0.10	0.45	0.10	14	0.823	<0.001
4b	2	2.53	0.18	0.71	0.13	5	0.899	<0.05
7	2	0.18	−1.27	0.16	0.06	19	0.263	>0.05
3	3	0.31	−1.23	0.11	0.04	19	0.564	~0.01
5a	3	−0.44	0.39	0.17	0.03	14	0.599	<0.05
5b	3	1.68	−0.23	0.48	0.05	5	0.896	<0.05
8	4	−0.09	0.11	0.05	0.00	15	−0.410	>0.05

[a]Atom numbers refer to diagrams I and II in the text. [b]Number of bonds between atom and nearest variable substituent.

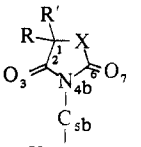
differential overlap approximation (CNDO/2),[4] as well as the increased scope of *ab initio* calculations. However, although substituent constants usually span a reasonable numerical range, variations in the net charge† calculated for a specified atom in a series of related molecules could be so small that realistic error margins would prevent a significant estimate of biological relevance. For example, consider the substituent constants and net atomic charges (EHT) given for three barbiturates in Table I. Each of the substituent constants covers a substantial range, as does the net charge for atom 1, but the variations in charge on atoms 2-7 are comparatively small. Nevertheless, such variations, regardless of their size, represent potential sources of correlation in multiple regression analyses, especially in view of the large number of alternative parameters involved. It is therefore important to determine whether or not the calculated variations have any physical significance.

Computed charge distributions may be tested by comparison with experimental dipole moments, and it has been found that reasonable quantitative values are predicted by *ab initio*[5] and CNDO/2[6,7] calculations, and by EHT with the use of a scaling factor.[8] Chemical shifts (^{13}C) have also been correlated with atomic charges from both EHT[9] and CNDO/2[10] calculations, and atomic charges for nitrogen (CNDO method) follow the same trends as inner electron binding energies.[11] However, these experimental techniques are not sufficiently precise to reflect such small variations in atomic charge as those which EHT attributes to the substituent changes given in Table I. Consequently, the validity of these variations may only be tested by comparing the predictions of various molecular orbital methods. This procedure is not entirely satisfactory, and substantial qualitative agreement is clearly no more than a minimal requirement.

The noniterative EHT method increases bond polarity and net charge approximately threefold, but excellent overall correlation has been observed with CNDO/2 results for a multiatomic sample,[8] and an equally good correlation can be demonstrated between the CNDO/2[6] and *ab initio*[5] results of Pople, *et al.*, over a series of molecules. However, the deviations from collinearity will naturally be magnified if atomic species are considered individually, and could overwhelm real charge variations due to remote structural changes. The correlations between CNDO/2 and EHT or *ab initio* charges have therefore been examined for different atomic species at varying distances from substituent changes in groups of related molecules.



I



II

The results of least-squares analysis of the net atomic charges calculated by EHT and CNDO/2 for the numbered atoms in the molecular series I and II‡ are presented in Table II, where the overall correlation obtained previously[8]

†Net charge is the atomic electron population minus the core charge on the nucleus. In the EHT and CNDO/2 calculations the 1s electrons are included in the core.

‡The molecules in series I were barbituric acid, 5,5-diethylbarbituric acid, glutarimide, β-methyl-β-ethylglutarimide, hydantoin, 1-methylhydantoin, 5-methylhydantoin, 5-phenylhydantoin, 5-ethyl-5-phenylhydantoin, 5,5-dimethyloxazolidine-2,4-dione, oxazolidine-2,4-dione, succinimide, 3-phenylsuccinimide, and 3-methyl-3-phenylsuccinimide. Series II was comprised of *N*-methylglutarimide, 3-methylhydantoin, 3,5,5-trimethyloxazolidine-2,4-dione, 3,5-dimethyl-5-ethyloxazolidine-2,4-dione, and 1-methylsuccinimide. Some of the calculated atomic charges have been published;[7] others are available from the author.

Table III. Regression of *ab Initio* Atomic Charges (y) on CNDO/2 Atomic Charges (x)

Atom[a]	Order[b]	Regression coefficients		Standard deviation		n	r	P
		Slope	Intercept	Slope	Intercept			
All		1.11	0.01	0.05	0.01	80	0.923	$\ll\ll$0.001
1 $H_3C-\mathit{C}-X$	1	1.02	−0.05	0.06	0.02	7	0.991	<0.001
2 H_3C-C-X	2	1.45	0.05	0.44	0.02	7	0.827	<0.05
3 H_3C-C-X	3	0.61	0.00	0.19	0.01	9	0.772	<0.05
4 $H_2C=\mathit{C}-X$	1	1.50	−0.11	0.03	0.01	4	0.9996	<0.001
5 $H_2C=C-X$	2	1.47	−0.11	0.13	0.02	4	0.992	<0.01
6 $\mathit{H}_2C=C-X$	3	0.98	0.06	0.13	0.00	6	0.967	<0.01
7 $O=\mathit{C}-X$	1	1.69	−0.19	0.21	0.07	5	0.978	<0.01
8 $\mathit{O}=C-X$	2	1.53	0.11	0.27	0.07	5	0.956	~0.01

[a]Relevant atom given in italics; X represents variable substituent groups. [b]See Table II.

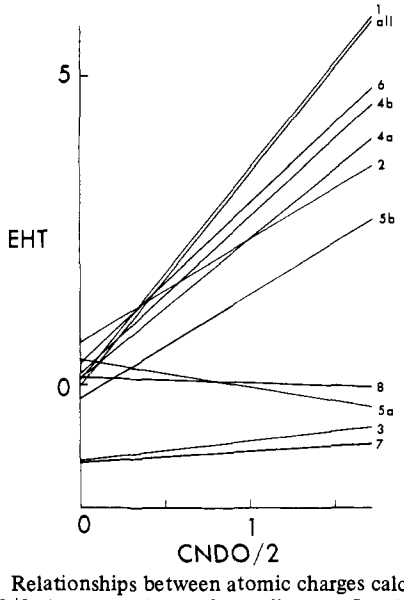


Figure 1. Relationships between atomic charges calculated by EHT and CNDO/2. Atom numbers refer to diagrams I and II in the text.

is included for comparison. It is clear from the probabilities given for the correlation coefficient, r, that the correlation is best for atoms 1 and 6, which are bonded directly to variable substituents. However, the influence of separation from these groups is not otherwise apparent, since the correlation is significant ($P < 0.05$) for all atoms except numbers 7 and 8, which are separated by two and four bonds, respectively, from the nearest variable substituent. Further information is provided by graphical comparison of the regression lines, which fall into two distinct groups (Figure 1). The larger group is bunched around the overall regression and includes atoms which are from one to three bonds distant from the variable substituents. The other group comprises four lines with slopes close to zero, reflecting comparatively small variations in the calculated EHT charges. These four atoms are between two and four bonds distant from the variable substituents, but differ from the first group in that they all terminate chains, being bonded to only one other atom.

The CNDO/2[6] and *ab initio*[5] studies of Pople, *et al.*, do not include as many closely related molecules, but provide sufficient data§ for the analysis given in Table III. The correlations are significant ($P < 0.05$) for all the atoms, which are from one to three bonds distant from the nearest molecular modification. The regression lines, *including* those of the terminal hydrogens and carbonyl oxygen, all lie in the vicinity of the general *ab initio*-CNDO/2 regression.

Discussion

For the multiatomic sample of *ab initio* and CNDO/2 data the highly significant correlation ($P \ll\ll 0.001$) and the unit positive slope (1.11) of the regression line demonstrate that the two methods predict similar distributions of charge between different atomic species, and the calculated dipole moments[5] provide reasonable evidence for the quantitative accuracy of these estimates. For individual atoms the significant and moderately self-consistent correlations between the CNDO/2 and *ab initio* techniques provide circumstantial evidence# for the view that either method may be employed to qualitatively predict charge variations in atoms separated by up to three bonds from a molecular modification.

With the proviso that the EHT charges are exaggerated approximately threefold, the same conclusions arise from the EHT-CNDO/2 comparison for all but the terminal hydrogen and oxygen atoms. In these instances EHT predicts essentially random variations in charge with respect to those calculated by CNDO/2, although the latter agree substantially with *ab initio* results in similar circumstances. Thus, although the data considered are by no means comprehensive, it appears that EHT should not normally be employed to compute charge variations due to substituent alterations.

It is concluded that both the CNDO/2 and *ab initio* methods of Pople, *et al.*, but not EHT, are probably adequate for predicting qualitative charge variations in atoms separated by as many as three bonds from a molecular modification. These calculated charges could reasonably be included in multiple regression analyses designed to elucidate biological activity.

References

(1) M. S. Tute, *Advan. Drug Res.*, **6**, 1 (1971).
(2) C. Hansch, *Annu. Rep. Med. Chem.*, **1966**, 347 (1967).
(3) R. Hoffman, *J. Chem. Phys.*, **39**, 1397 (1963).
(4) J. A. Pople and G. A. Segal, *ibid.*, **44**, 3289 (1966).
(5) W. J. Hehre and J. A. Pople, *J. Amer. Chem. Soc.*, **92**, 2191 (1970).
(6) J. A. Pople and M. S. Gordon, *ibid.*, **89**, 4253 (1967).
(7) P. R. Andrews, *J. Med. Chem.*, 12, 761 (1969).
(8) P. R. Andrews, *Theor. Chim. Acta*, 14, 261 (1969).
(9) J. E. Bloor and D. L. Breen, *J. Phys. Chem.*, 72, 716 (1968).

§The atomic charges used here are those reported[5,6] for methane, ethane, ethylene, acetylene, propene, propyne, methyl fluoride, fluoroform, fluoroethane, 1,1,1-trifluoroethane, fluoroethylene, fluoroacetylene, methanol, formaldehyde, acetaldehyde, ketene, formic acid, ammonia, hydrogen cyanide, methyl cyanide, formamide, and nitromethane.

#For the multiatomic sample the variations in atomic charge result largely from differences in atomic properties (*e.g.*, ionization potential) between neighboring atoms, but for a particular atom in a series of related molecules the variations reflect broader environmental effects, so that other factors may assume greater importance. The agreement between the correlations for the single and multiatomic samples is therefore not definitive.

(10) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **90**, 3543 (1968).
(11) J. M. Hollander, D. N. Hendrickson, and W. L. Jolly, *J. Chem. Phys.*, **49**, 3315 (1968).
(12) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1968).
(13) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 556.
(14) E. Kutler and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).

A Novel Synthesis of L-(−)-α-Methyl-3,4-dimethoxyphenylalanine

Kurt Freter,* Manfred Götz, and Karl Grozinger

Pharma-Research Canada Ltd., Pointe Claire 730, Quebec, Canada. *Received March 24, 1972*

Numerous synthetic approaches to L-(−)-α-methyldopa manifest the importance of this antihypertensive drug. The original processes utilize variations of the classical amino acid syntheses, starting from 3,4-dimethoxyphenylacetone.[1-3] More recent and also more elaborate syntheses introduce the amino group in the penultimate step by either displacing halogen with ammonia,[4] by Curtius rearrangement[5] or by Schmidt reaction.[6] In all these cases the racemic α-methyldopa has to be resolved with optically active acids or bases or by selective crystallization.[7] Weinges, *et al.*,[8] were able to obtain, however, in an elegant asymmetric Strecker synthesis, α-methyldopa in optically pure form.

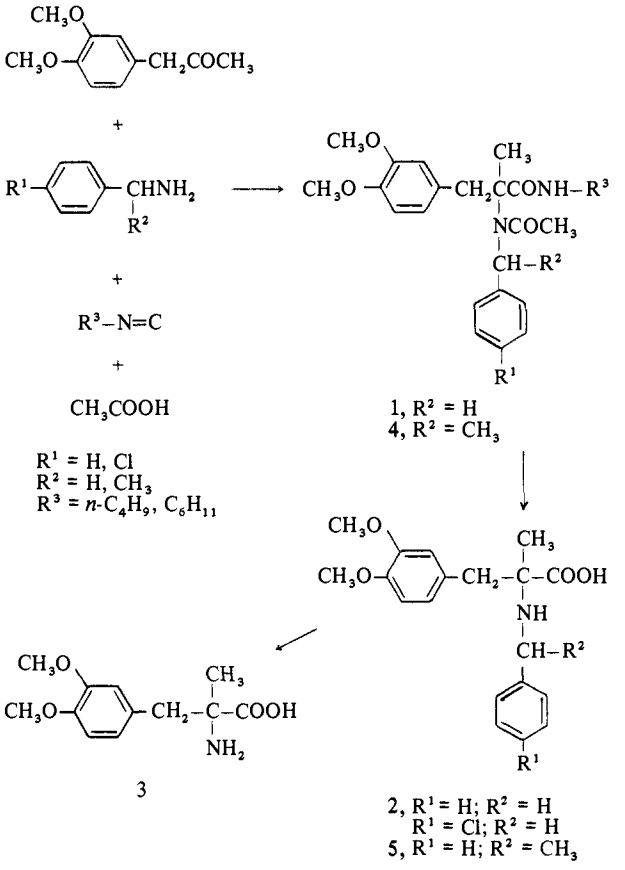
We wish to report a novel synthesis of 3,4-dimethoxyphenylalanine, the precursor of α-methyldopa, unrelated to those reported previously, which combines the simplicity of the Ugi reaction[9] with a facile separation of the resulting diastereoisomeric L-(or D)-α-methylbenzyl-DL-amino acids.

Model studies with optically inactive materials showed that the four component reaction (Scheme I) easily yielded the benzylamino acids (**2**) after saponification of the uncharacterized α-acetylamino acid amides (**1**). The DL-α-methyl-3,4-dimethoxyphenylalanine (**3**) was obtained by hydrogenolytic cleavage.

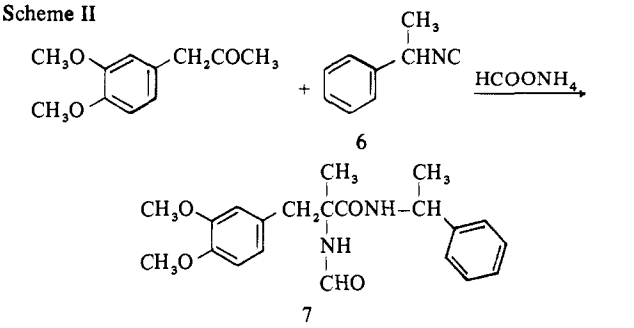
This reaction sequence was repeated with L-(−)-α-methylbenzylamine, and the resulting diastereoisomers (**4**) were—again without isolation—saponified to the amino acid (**5**). From the mixture of diastereoisomeric hydrochlorides of **5**, one isomer crystallized in optically pure form as judged by the specific rotation, which remained unchanged on recrystallization. On hydrogenolysis of the methylbenzyl moiety the desired L-(−)-α-methyl-3,4-dimethoxyphenylalanine (**3**) was obtained, which demonstrates that the crystalline diastereoisomer **5** had the LL configuration.

The loss of the optically active benzylamine on hydrogenolysis is clearly a disadvantage of this method. It therefore appeared attractive to introduce the second asymmetric center with an optically active isonitrile (**6**). On hydrolysis of the Ugi product (**7**), optically active amine could be recovered, which then could be reconverted into the isonitrile (Scheme II). This approach proved to be unrewarding, however, in view of persistent low yields in all reaction steps and was therefore abandoned.

Scheme I



Scheme II



Experimental Section

DL-*N*-**Benzyl-α-methyl-3,4-dimethoxyphenylalanine** (**2**, R^1 = H). A mixt of 3,4-dimethoxyphenylacetone (38.8 g, 0.2 mole), benzylamine (21.4 g, 0.2 mole), and C_6H_6 (200 ml) was refluxed under stirring for 2 hr; at that time H_2O sepn in a Dean-Stark tube had stopped. The solvent was evapd *in vacuo*, and the residue stirred with butylisonitrile (16.6 g, 0.2 mole) and AcOH (12 g, 0.2 mole) in MeOH (150 ml) at room temp for 1 week. The mixt was evapd to dryness, and the residue heated with H_2SO_4 (30%) (100 ml) to reflux for 24 hr. H_2O (200 ml) was added, and the soln washed with $CHCl_3$. On neutralization, the amino acid pptd. It was recrystd from water: yield 20 g (30.5%); mp 233–235°. *Anal.* ($C_{19}H_{23}NO_4$) C, H, N.

DL-*N*-**(4-Chlorobenzyl)-α-methyl-3,4-dimethoxyphenylalanine** (**2**, R^1 = Cl). This compd was obtained exactly as **2**, R^1 = H, using 4-chlorobenzylamine and cyclohexylisonitrile: yield 27.6%; mp 234–236°. *Anal.* ($C_{19}H_{22}ClNO_4$) C, H, Cl, N.

DL-α-**Methyl-3,4-dimethoxyphenylalanine** (**3**-DL). Both compds (**2**) were hydrogenated in EtOH with Pd/C (5%) at room temp and 25 psi in the usual way. The amino acid crystd as the hydrochloride monohydrate: yield 75.0%; mp 223–225°.

DL-α-**Methyl**-*N*-L-(−)-α-**methylbenzyl-3,4-dimethoxyphenylalanine** (**5**-DL-L). This compd was prepd like **2**, using 3,4-dimethoxyphenylacetone (38.8 g, 0.2 mole), L-(−)-α-methylbenzylamine (24.2 g, 0.2 mole), and *n*-butylisonitrile (16.6 g, 0.2 mole): yield 30.5 g (44.5%); mp 212–214°; $[\alpha]^{25}D$ −44° (*c* 0.5, 0.5 *N* NaOH). *Anal.* ($C_{20}H_{25}NO_4$) C, H, N.

L-α-**Methyl**-*N*-L-(−)-α-**methylbenzyl-3,4-dimethoxyphenylalanine Hydrochloride** (**5**-L-L·HCl). The above mixt of diastereoisomers (13.1 g) was converted to the hydrochlorides in EtOH (150 ml) with dry HCl. On concn and trituration with Et_2O the pure