stirred and refluxed (3 hr), then filtered, and the filtrate was evaporated to obtain a crystalline residue. The bulk of the product was obtained by treating the cake of K_2CO_3 with hot H_2O, and filtering off the insoluble fraction. This was combined with the residue from Me_2CO evaporation, and recrystallized from MeOH giving 95 g (50%) of colorless crystals, mp 158–160° dec, λ_{max}^{MeOH} 269 mμ (ϵ_{max} 26,300). *Anal.* ($C_{18}H_{19}ClNO$) C, H, N.

1-(4-Chlorophenoxymethyl)isoquinoline (8).—1-(4-Chlorophenoxymethyl)-3,4-dihydroisoquinoline (10 g) in $CHCl_3$ (200 ml) was treated with *m*-chloroperbenzoic acid (13 g, 2.5 equiv).[28] The mixture was then heated to reflux (4 hr), cooled, washed (saturated $NaHCO_3$, H_2O), dried, and evaporated to a brown oil. The oil was taken up in a little C_6H_6 and chromatographed on neutral alumina (300 g, 2.5 × 40 cm column) eluting with 1:1 Et_2O–C_6H_5. Evaporation of the first 2000 ml of eluate gave a waxy substance (200 mg) which was discarded. The next 3000 ml yielded 2.2 g (22%) of the desired free base, mp 91–92°, converted to the hydrochloride by adding HCl in Et_2O to a solution in Et_2O. Recrystallization (EtOH–Et_2O) gave colorless crystals: mp 183°; $\lambda_{max}^{MeOH-1\% HCl}$ 230, 278, 335 mμ (ϵ_{max} 47,500, 4490, 5570). *Anal.* ($C_{16}H_{22}ClNO \cdot HCl$) C, H.

N-(1,1-Dimethyl-2-phenethyl)-4-chlorophenoxyacetamide.—4-Chlorophenoxyacetonitrile (33.5 g, 0.2 mole) was dissolved in AcOH with stirring. A mixture of concentrated H_2SO_4 (50 ml) and AcOH (25 ml) was added at room temperature, followed by 1,1-dimethyl-2-phenylethanol (34.0 g, 0.2 mole). The mixture was stirred and heated to 70° for 0.5 hr and then stoppered and left at room temperature for 2 hr. H_2O (250 ml) was added and the solution was neutralized with Na_2CO_3 and extracted with Et_2O, and the extract was dried and evaporated to yield an oil which solidified. Two recrystallizations from petroleum ether (bp 40–60°) gave 18.4 g (30%) of colorless crystals, mp 66–67°. *Anal.* ($C_{18}H_{20}ClNO$) C, H, N.

3-(4-Chlorophenyl)propiothiomorpholide.—4-Chloropropiophenone (20.0 g), sulfur (4.8 g), and morpholine (15.5 g) was stirred and refluxed for 17 hr. After cooling, the mixture was poured into warm EtOH (75 ml), whereupon brown crystals separated. Recrystallization from EtOH (charcoal) and then from EtOH–H_2O gave 12.0 g (37%) of yellow crystals, mp 98–100°. *Anal.* ($C_{13}H_{16}ClNOS$) C, H, N.

3-(4-Chlorophenyl)propionic Acid.—The thiomorpholide (9.0 g) was hydrolyzed by refluxing in a mixture of AcOH (20 ml), H_2O (45 ml), and concentrated H_2SO_4 (3 ml) for 5 hr. The crude product was precipitated by pouring into H_2O (180 ml), and the precipitate was digested with hot 5% NaOH (250 ml) for 4 hr. This gave a yellow solution and dark red oil. The yellow solution was decanted from the oil and acidified (HCl) to give a light yellow solid. Two recrystallizations from C_6H_6 gave 3.0 g (49%) of colorless plates, mp 115–116°. *Anal.* ($C_9H_9ClO_2$) C, H.

N-Phenethyl-3-(4-chlorophenyl)propionamide.—3-(4-Chlorophenyl)propionic acid (35.0 g) and phenylethylamine (23.0 g, 1 equiv) were refluxed in dry toluene (1500 ml) for 48 hr, with azeotropic removal of H_2O. Evaporation of the toluene left a brown residue, recrystallized (charcoal) from C_6H_6–petroleum ether (bp 100–120°) and then twice from petroleum ether (bp 100–120°) to give 30.0 g (55%) of colorless needles, mp 119–121°. *Anal.* ($C_{17}H_{18}ClNO$) C, H, N.

(28) The use of 1 equiv of *m*-chloroperbenzoic acid gave a yield of only 3.5%. Other components of the mixture have not yet been investigated.

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The Inhibition of Viral Neuraminidase by 1-Phenoxymethyl-3,4-dihydroisoquinolines. II. A Hansch Analysis

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A multiple regression analysis on a series of 1-(*para*- and *meta*-substituted phenoxymethyl)-3,4-dihydroisoquinolines, using the methods described by Hansch, relates various substituent constants to inhibition of the viral enzyme neuraminidase. A highly significant relationship has been shown to exist between enzyme inhibition and the hydrophobicity constant, π, and also between enzyme inhibition and group dipole moment, μ. The significance of terms in μ and μ^2 is discussed; these terms are believed to represent dipole–charge (μ) and dipole–induced dipole (μ^2) interactions between drug and receptor. The Hansch analysis has thus been used as a diagnostic tool rather than in any predictive sense and affords evidence as to the nature of the receptor site on the enzyme, or enzyme–substrate complex.

In continuing our work on structure–activity relationships in the 1-phenoxymethyl-3,4-dihydroisoquinoline series,[1] we realized that here was the ideal situation for study of drug–receptor interaction using the methods described by Hansch and his coworkers,[2] based on a linear free-energy relationship between relative biological response and various substituent constants, these being used as parameters in a multiple regression analysis. The choice of parameters is arbitrary and the statistical method allows the testing of each of the corresponding coefficients by the application of a *t* test. A semiempirical approach has been tried here, using first the constants found by Hansch to be generally useful, namely the hydrophobic bonding constant π and the Hammett constant σ.[2b] This report describes how we have applied these constants, and then tried others, namely group dipole moment μ and polarizability α, which we had reason to believe could be especially significant to the analysis of a simple interaction between drug and receptor *in vitro*.

Method.—The standard equation to be solved in multiple regression analysis is of the form of eq 1

$$y = k_1x_1 + k_2x_2 + k_3x_3 + \cdots + k \qquad (1)$$

where y represents relative biological response and where x_1, x_2, x_3, ... are substituent constants. A computer program was written for the solution of this equation by the method of least squares, to provide the regression coefficients k_1, k_2, k_3, ... the constant k, the multiple correlation coefficient, and the standard

(1) Part I: M. S. Tute, K. W. Brammer, B. Kaye, and R. W. Broadbent, *J. Med. Chem.*, **13**, 44 (1970).

(2) (a) C. Hansch, *Ann. Rept. Med. Chem.*, 348 (1967); (b) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964); (c) T. Fujita, J. Iwasa, and C. Hansch, *ibid.*, **86**, 5175 (1964).

TABLE I
SUBSTITUENT CONSTANTS AND OBSERVED AND CALCULATED NEURAMINIDASE INHIBITION OF 1-PHENOXYMETHYL-3,4-DIHYDROISOQUINOLINES[a]

No.	Function	σ[b]	π[c]	μ[d]	μ_V	μ_H	α[e]	—Log (1/C)— Obsd	Calcd[f]	Δ log (1/C)[f]	Calcd Log (1/C)[g]	Δ log (1/C)[g]
1	4-NO_2	0.78	0.50	−4.01	−4.01	0.00	6.68	2.9031	2.9155	0.0124	2.9207	0.0176
2	4-Br	0.27	1.13	−1.57	−1.57	0.00	7.68	2.7670	2.8337	0.0667	2.8430	0.0760
3	4-CN	0.66	0.14	−4.05	−4.05	0.00	4.86	2.8386	2.8251	−0.0135	2.8197	−0.0189
4	4-Cl	0.23	0.93	−1.60	−1.60	0.00	4.84	2.8069	2.7806	−0.0263	2.7832	−0.0237
5	4-F	0.06	0.31	−1.48	−1.48	0.00	0.03	2.6345	2.6092	−0.0253	2.5930	−0.0415
6	H	0.00	0.00	0.00	0.00	0.00	0.00	2.5768	2.5515	−0.0253	2.5521	−0.0247
7	4-Me	−0.17	0.48	0.35	0.35	0.00	4.63	2.6819	2.7066	0.0247	2.7325	0.0506
8	4-OMe	−0.27	−0.12	1.28	0.31	1.24	6.14	2.6198	2.5409	−0.0789	2.5465	−0.0733
9	4-OH	−0.37	−0.87	−1.60	0.00	1.60	1.70	2.2441	2.3160	0.0719	2.2888	0.0447
10	4-OEt	−0.24	0.38	1.28	0.31	1.24	11.03	2.6498	2.6763	0.0265	2.6978	0.0480
11	4-OPr	−0.25	0.88	1.28	0.31	1.24	15.66	2.7905	2.8117	0.0212	2.8491	0.0586
12	4-OBu	−0.32	1.38	1.28	0.31	1.24	20.29	2.7852	2.9470	0.1618		
13	4-CMe_3	−0.20	1.68[h]	0.35	0.35	0.00	18.52	3.1487	3.0315	−0.1172	3.0956	−0.0531
14	3-Me	−0.07	0.56	0.35	0.18	−0.30	4.63	2.7825	2.7152	−0.0673	2.7385	−0.0440
15	3-F	0.34	0.47	−1.48	−0.74	1.28	0.03	2.6655	2.6494	−0.0161	2.6484	−0.0171
16	3-Cl	0.37	1.04	−1.60	−0.80	1.39	4.84	2.8182	2.8028	−0.0154	2.8189	0.0007

[a] The compounds were prepared and characterized by the methods described in part 1.[1] Yields and physical constants are omitted to conserve space. [b] σ values are taken from the table for substituted phenols by G. B. Barlin and D. D. Perrin, *Quart. Rev.* (London), **290,** 75 (1966). [c] π values are from the phenol system.[2c] [d] Values for group dipole moment were taken from the table by L. E. Sutton in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, N. Y., 1955, p 395. [e] Values of α were calculated from tables of electronic polarizability given by Y. K. Syrkin and M. E. Dyatkina, ref 9, p 201. [f] Calculated using eq 10. [g] Calculated using eq 12. [h] Taken from the table for substituted anilines by T. Fujita and C. Hansch, *J. Med. Chem.*, **10,** 991 (1967).

deviation about the regression line as well as providing tests of significance for the coefficients.

For the expression of relative biological activity, y was written

$$y = \log (1/C)$$

where C is the molar concentration of compound required to elicit a standard biological response.

A Lineweaver–Burk plot on representative compounds had shown[1] that inhibition was of the noncompetitive type, and it was therefore considered to be the result of binding to an allosteric site. Dose–response curves for all compounds were of a typical sigmoid shape, being linear from 20 to 60% inhibition. In order that dose should be linearly related to response for all compounds at the concentration C, we chose 40% (rather than the more usual 50%) inhibition as the standard biological response in this instance, this being in the middle of the linear range.

Table I contains data on 16 molecules for which substituent constants are available. Most molecules are substituted in the phenyl ring *para* to the ether function, but **14**, **15**, and **16** have *meta* substituents. From previous work[1] it seemed that these could be included in the same analysis without applying any correction for steric influences, and it was later found that the results obtained supported this assumption.

Results and Discussion

Using π, π^2, and σ eq 2 was derived from a least-squares fit of the data in Table I. Here n represents

$$\log (1/C) = 0.244\pi + 0.003\pi^2 + 0.156\sigma + 2.587$$
$$(n = 16, r = 0.883, s = 0.099) \quad (2)$$

the number of data points used in the regression, r is the multiple correlation coefficient, and s the standard deviation from regression. A π^2 term is believed to characterize the importance of an optimum partition coefficient for passage of a drug through cellular membranes,[2b] and as in this simple *in vitro* system no membranes or transport phenomena are involved, it was not surprising to find by a t test that the coefficient in π^2 was insignificant.

It was also found that the coefficient in σ did not reach the $p = 0.05$ confidence level, but the coefficient in π was highly significant ($p < 0.01$) indicating the importance of some hydrophobic interaction.[3]

Equation 3 shows the result of omitting the insignificant π^2 and σ terms and including only a term in π.

$$\log (1/C) = 0.253\pi + 2.592$$
$$(n = 16, r = 0.834, s = 0.108) \quad (3)$$

The coefficient in π is still highly significant and the over-all correlation is good, but only 70% of the variance in the data is accounted for by this term alone.

The term in σ having failed to account for any of the variation in activity, other parameters were considered, which could possibly be used to describe electronic effects influencing the binding between drug and receptor. Gill,[4] in reviewing the various binding forces, observed that the energy of interaction between a single fixed charge and a permanent dipole can contribute significantly to intermolecular interactions. The magnitude of such interaction is governed by the relationship of eq 4 where e is the charge on one molecule and

$$E = (Ne\mu \cos \theta)/D(b^2 - d^2) \quad (4)$$

μ the dipole moment of the interacting molecule, N is the Avogadro constant, and D the dielectric constant. The distance b is that between charge and center of dipole, and the distance d is that between the two centers of charge in the dipole. The angle θ is that between the

(3) G. Némenthy, *Angew. Chem. Intern. Ed. Engl.*, **6,** 195 (1967).
(4) E. W. Gill, *Progr. Med. Chem.*, **4,** 39 (1965).

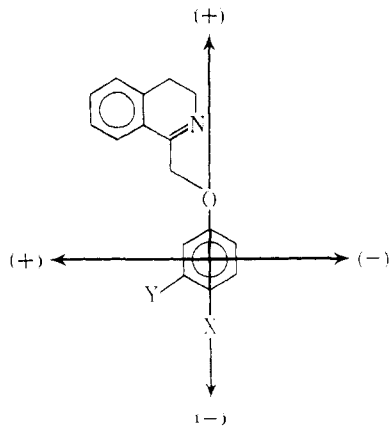

Figure 1.—Resolution of group dipole moment.

line joining the two centers of charge in the dipole and the line joining the center of the dipole to the charge.

Now if e is a charge on the receptor and μ a dipole in the drug molecule, then once hydrophobic binding forces have brought drug and receptor into close proximity, b and d will be of comparable magnitude and a significant electrical interaction will occur. In a series of drug molecules interacting with the same receptor, the values of b, d, and θ can be assumed to remain constant provided there are no gross steric differences between members of the series. Any change of dipole moment in passing from one member of the series to another is then linearly related to a change of charge–dipole interaction energy. Linear energy changes can be treated by eq 1, and so it was decided to include group dipole moment as one parameter.

Since dipole moment is a vector quantity, it was necessary to resolve each group contribution into two components, and consider them as separate parameters. The values of μ in Table I have therefore been resolved into the components μ_V (along the vertical axis OX, through the oxygen atom O and the *para*-substituent X) and μ_H (the horizontal component, at 90° to the OX axis). The μ_V component was treated as of negative sign in the direction O to X, and the μ_H component of negative sign in the direction from left to right. The *meta* substituents were assumed to occupy the position Y (Figure 1).[5] The vector angles given by Lumbroso and Marschalk[6] were used in order to resolve the group dipoles for hydroxy and alkoxy substituents.

Other drug–receptor interactions discussed by Gill include dipole–dipole and dipole–induced dipole interactions, and it seemed that a term in μ^2 might account for the latter; since the magnitude of an *induced* dipole is dependent upon the magnitude of the *inducing* dipole, the energy of interactions could well depend upon μ^2 (eq 5–7).

$$\mu(induced) = k\mu(inducing) \quad (5)$$

$$E = k'\mu(induced)\mu(inducing) \quad (6)$$

$$= k''\mu^2(inducing) \quad (7)$$

A much more elegant treatment of the forces of interaction between two dipolar molecules is to be found in the work of McFarland,[7] who has used an equation relating energy of interaction of two dipolar molecules to their dipole moments and polarizability values. If one again considers a series of molecules interacting with the same receptor, then eq 8 is applicable, where

$$E = k\mu^2 + k'\alpha \quad (8)$$

μ and α represent group dipole moment and group polarizability. From the derivation of this equation,[7] it is apparent that the constant k' depends on the magnitude of the dipole moment of the receptor, so that if this is zero then a term in μ^2 would not be expected to have a large regression coefficient and may in fact be insignificant. These considerations led to the inclusion of the parameters μ_V, μ_H, and α in the data of Table I.

Using π together with all the dipole moment parameters, eq 9 was derived. This is a highly significant relationship in π ($p < 0.001$) and also significant in μ_V^2 ($p < 0.02$). The μ_V term does not quite reach significance at the $p = 0.05$ level, but the μ_H^2 and μ_H terms have *no* significance at all. This was confirmed by omitting terms in μ_H^2 and μ_H and deriving eq 10, which is not significantly different from eq 9 in correlating the data. In eq 11 a term in α was included, but although the multiple correlation coefficient was slightly improved, this was undoubtedly due to the fact of inclusion of an extra variable rather than to any significance that variable may have had, for the coefficient in α was negative in sign (which is incompatible with the derivation of eq 8) and did not reach significance at the $p = 0.05$ level. Equation 10 was therefore the most significant overall relationship accounting for 88% of the variance in the data.

$$\log(1/C) = 0.258\pi + 0.069\mu_V - 0.030\mu_H + 0.029\mu_V^2 - 0.011\mu_H^2 + 2.591$$
$$(n = 16,\ r = 0.949,\ s = 0.073) \quad (9)$$

$$\log(1/C) = 0.271\pi + 0.062\mu_V + 0.030\mu_V^2 + 2.552$$
$$(n = 16,\ r = 0.937,\ s = 0.074) \quad (10)$$

$$\log(1/C) = 0.354\pi + 0.140\mu_V + 0.049\mu_V^2 - 0.011\alpha + 2.593$$
$$(n = 16,\ r = 0.954,\ s = 0.066) \quad (11)$$

$$\log(1/C) = 0.303\pi + 0.088\mu_V + 0.036\mu_V^2 + 2.552$$
$$(n = 15,\ r = 0.971,\ s = 0.052) \quad (12)$$

In examining the differences between log (1/C) (observed) and log (1/C) as calculated using eq 10 (Table I), it was seen that the differences were generally small, but that there was an abnormally large difference for the observation on compound **12**. This compound has the longest alkyl chain in the series, the substituent being a butoxy group. Compound **11**, with a propoxy substituent, fits the data very well as do the ethoxy- and methoxy-substituted compounds (**10**, **8**). It is likely therefore that the terminal CH_3 of the butoxy substituent in **12** normally lies outside the lipophilic

(5) See the argument in part I,[1] from which it was concluded that a *meta* substituent prefers position Y rather than the alternative obtained by rotation of the phenyl ring through 180° about the OX axis.

(6) H. Lumbroso and C. Marschalk, *J. Chim. Phys.*, **49**, 385 (1952).

(7) J. W. McFarland, *et al.*, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968; also several private communications.

(8) Y. K. Syrkin and M. E. Dyatkina, "Structure of Molecules and the Chemical Bond," Butterworth and Co. Ltd., London, 1950, p 478.

area of the receptor, and hence plays no part in hydrophobic binding. When this compound was omitted from the analysis, leaving 15 data points, eq **12** was derived in which all points now fitted very closely.

Equation 12 represents the relative neuraminidase inhibition for the series, accounted for in terms of hydrophobic binding (π term significant at the $p < 0.001$ level), and in terms of electronic interaction represented by a μ_v term ($p < 0.01$) and a μ_v^2 term ($p < 0.001$). These terms together account for 94% of the variance in the data.

It now remains to discuss the meaning of this result in terms of the drug–receptor interaction. The Hammett σ constant denotes the electron availability from a substituent, and hence its influence on acid or base strength at any part of the molecule. As a term in σ is not significant, it may be concluded that variation in the basicity of the N atom of the 3,4-dihydroisoquinoline ring does not significantly alter binding to the enzyme. This result agrees with previous experimental data[1] showing that inhibition of the enzyme is constant over the range of pH 5.5–7.0, *i.e.*, is independent of the degree of ionization of the base.

It has been supposed that a term in μ would indicate dipole–charge interaction between drug and receptor. This must remain speculative in the absence of further evidence, but if it is as supposed, the finding that only the vertical component μ_v is significant places the charge on the receptor at a small angle (θ in eq 4) to the vertical, OX axis. The sign of the coefficient in μ_v is positive (eq 12), and so is consistent with an *anion* situated along the OX axis beyond X (Figure 1).[9] The anion in question could be on a peptide residue (aspartic or glutamic acid) in the enzyme, or it could be the ionized CO_2H of N-acetylneuraminic acid in the substrate, the rate of breakdown of the complex between enzyme and substrate to give products being under allosteric control by the inhibitor molecule. This is in accord with the observed kinetics of inhibition[1] and also allows a ready explanation of the finding that with a different substrate (sialolactose instead of collocalia mucoid) the 1-phenoxymethyl-3,4-dihydroisoquinolines will stimulate rather than inhibit enzyme activity.[10]

Interaction of a dipole on the drug with a charge (ionized CO_2H) on the receptor is an explanation of the dependence on a μ_v term which is still consistent with there being no change in inhibition over the range of pH 5.5–7.0; for the degree of ionization of CO_2H would not change significantly over this range of pH, the pK_a of the CO_2H being more than one pH unit below pH 5.5. The aspartic acid β-carboxyl group has a pK_a of 3.86,[11] the glutamic acid γ-carboxyl group has a pK_a of 4.25,[11] and the N-acetylneuraminic acid carboxyl group has a pK_a of 2.6.[12]

The possibility was considered that the μ_v and μ_v^2 terms should be treated together, as describing some complex dependence of binding on the total dipole moment of the molecule, or on the dipole component of the substituted phenoxy group. If this were the case, one might suppose that the minimum value of μ_v (−1.22 D), obtained by partial differentiation of eq 12, would bear some simple relationship to the dipole moment component along the OX axis of the parent molecule (**6**, X = H). Measurement of the dipole moment[13] of **6** did not reveal any such relationship, so the μ_v and μ_v^2 terms probably represent quite independent binding forces.

The dependence of enzyme inhibition on a μ_v^2 term but not on a term in α presumably means that the molecule of inhibitor is inducing a dipole in the receptor, but the receptor is such that no large dipole is correspondingly induced in the molecule. This follows from the derivation of eq 8. The fact that a μ_H^2 term is not significant is consistent with the idea that the receptor becomes polarized (supports an induced dipole) preferentially in one direction, which would be expected.

A part of the receptor has thus been characterized as an *anion*, another part or parts as being (i) hydrophobic (π term highly significant), (ii) polarizable (μ_v^2 term highly significant), and (iii) nondipolar (α term not significant).

Of the amino acid residues present in proteins, several (tryptophan, phenylalanine, tyrosine, methionine, alanine) could participate in hydrophobic interaction, but of these only phenylalanine is also both polarizable and nondipolar. It is possible that the aromatic ring of phenylalanine may be involved in hydrophobic binding to the phenyl ring of the inhibitor.

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(9) The data are also consistent with a *cation* along OX beyond O, but this was dimissed as being highly unlikely due to steric crowding in the vicinity of the N atom. The protonated N itself cannot be responsible (*i.e.*, an intramolecular interaction) since pH variation has no effect on enzyme inhibition.

(10) Professor G. Belyavin, private communication.

(11) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966.

(12) L. Svennerholm, *Acta Chem. Scand.*, **10**, 694 (1956).

(13) Dipole moment measurements were kindly undertaken by Professor A. R. Katritzky. The conformation indicated in Figure 1 was confirmed. the unsubstituted compound (X, Y = H) having a net dipole (benzene, 25°) of 2.65 D at an angle of 87 ± 2° to the OX axis. The component along the OX axis would be −0.13 D (conformational study to be published).