centrated hydrochloric acid. The ether and aqueous phases were separated, the aqueous phase extracted several times with ether and the combined ether extracts dried over Drierite. After the solvent and the low boilers were removed at atmospheric pressure, the residue was distilled in vacuum to give *sym*-diphenyldifluoroethylene (24.2 g., 37%, b.p. 120–123° at 2 mm., m.p. 73.8–74.2° from 60–70° petroleum ether; *Anal.*[6] Calcd. for $C_{14}H_{10}F_2$: C, 77.78; H, 4.63; F, 17.59; mol. wt., 216. Found: C, 77.96; H, 4.57; F, 17.11; mol. wt., 222, 225 (using a McCoy apparatus and benzene as the solvent)); benzophenone (28.7 g., 52%, b.p. 125–127° at 2 mm., m.p. 48.2–48.8°, 2,4-dinitrophenylhydrazone, m.p. 237–239°)[7] and triphenylfluoroethylene (2.3 g., 6%, b.p. 150–155° at 2 mm., m.p. 103.6–104.2° from 60–70° petroleum ether; *Anal.*[6] Calcd. for $C_{20}H_{15}F$: C, 87.59; H, 5.47; F, 6.93; mol. wt., 274. Found: C, 87.23; H, 5.83; F, 7.02; mol. wt., 286, 287).

Reaction of Phenyllithium with Tetrafluoroethylene.—The apparatus used in this reaction was the same as that in the previous experiment except that the water-cooled condenser was replaced by a Dry Ice condenser charged with a slurry of Dry Ice and ether as the coolant. Dry powdered sodium perfluoropropionate (0.3 mole, 55.8 g.) was pyrolyzed by heating the flask containing it to 250–290° in a metal-bath. The tetrafluoroethylene[4] thus formed was passed in succession through a safety trap, two traps containing 30% aqueous potassium hydroxide (to trap the carbon dioxide formed during the pyrolysis), a trap containing concentrated sulfuric acid (to remove any moisture), a second safety trap and then into the reactor containing 0.9 mole of phenyllithium. After the tetrafluoroethylene was added, the Dry Ice condenser was replaced by a water-cooled condenser and the mixture then was refluxed for 30 minutes. The reaction was then processed as described above to give 35.2 g. (55%) of *sym*-diphenyldifluoroethylene, m.p. 73.7–74.2°, and 10.2 g. (13%) of triphenylfluoroethylene, m.p. 103–104.2°. Mixed melting points between each of these compounds and the corresponding compounds prepared in the previous experiment showed no depression.

Oxidation of *sym*-Diphenyldifluoroethylene.—To 0.027 mole (4.3 g.) of potassium permanganate, dissolved in 200 ml. of water, was added 0.02 mole (4.3 g.) of *sym*-diphenyldifluoroethylene. The reaction mixture was stirred and heated at 100° for six hours, stirred for 20 hours at room temperature and then made acid with concentrated hydrochloric acid. The mixture was extracted with several portions of ether, the combined ether extracts dried over Drierite and the solvent distilled. The crystalline residue was extracted with boiling water to give an aqueous solution and a residual oil. On cooling the aqueous solution there was obtained 1.8 g. (37%) of benzoic acid, m.p. 120.6–121.2° alone and when mixed with an authentic sample. The oil crystallized on standing to give 1.2 g. (28%) of recovered *sym*-diphenyldifluoroethylene, m.p. 73.6–74.2°.

(6) Analysis by Clark Analytical Laboratory, Urbana, Ill.

(7) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., Third Edition (1948).

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If an Enzyme–Substrate Modifier System Exhibits Non-competitive Interaction, then, in General, its Michaelis Constant is an Equilibrium Constant

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Recently, Botts and the author[1] derived the expression for the steady-state reaction velocity, v = d(products)/dt, in the system shown below. Here E stands for enzyme ($[E_0]$ for total enzyme concentration), S for substrate, and Y for any rate-modifying substance ("modifier") *e.g.*, H^+, an inhibitor, an activator, etc. The expression is

$$\frac{v([S],[Y])}{[E_0]} = \frac{k_2\bar{K}_1[S] + k_2'K_0'\bar{K}_1[Y][S] + k_2'(\Delta_{ES}/\Delta) + k_2'(\Delta_{SEY}/\Delta)}{1 + \bar{K}_1[S] + K_0[Y] + K_0'\bar{K}_1[Y][S] + (\Delta_{EY} + \Delta_{ES} + \Delta_{SEY})/\Delta} \quad (1)$$

where

$$r = k_2/k_{-1};\ r' = k_2'/k'_{-1} \quad (2)$$

$$\bar{K}_1 = k_1/(k_{-1} + k_2) \quad (3)$$

$$\mathfrak{D} = (r - r')k'_{-1}K_0'\bar{K}_1[Y][S] \quad (4)$$

$$\Delta = k_{-0}k'_{-1}(1 + r')\{k_0'[Y] + k_{-1}(1 + r)\} + k'_{-0}k_{-1}(1 + r)\{k_1'[S] + k_{-0}\} \quad (5)$$

$$\Delta_{EY} = -k'_{-0}k_{-1}(1 + r)\mathfrak{D} \quad (6)$$

$$\Delta_{ES} = k_{-0}k'_{-0}\mathfrak{D} \quad (7)$$

$$\Delta_{SEY} = k_{-0}\{k_0'[Y] + k_{-1}(1 + r)\}\mathfrak{D} \quad (8)$$

Since all the perturbation terms in equation 1 contain $\mathfrak{D}$ as a factor, $v/[E_0]$ assumes simple forms whenever $\mathfrak{D} = 0$. We have remarked[1] that $\mathfrak{D}$ will vanish in, (a) the absence of modifier, *i.e.*, [Y] = 0, whence equation (1) reduces to the familiar Michaelis–Menten equation, (b) the case of "competitive" interaction, *i.e.*, $K'_0 = K'_1 = 0$, and (c) the coincidence that $r = r'$. We have also reported that equation 1 will reduce to that of "non-competitive" interaction if both $K_0 = K'_0$ (or, because of free energy balance, $K_1 = K_1'$), and the enzyme–substrate reactions are at quasi-equilibrium, *i.e.*, $r \to 0$, and $r' \to 0$. It is the purpose of this note to examine the inversion of these latter conditions.

A plot of $1/v$ against $1/[S]$ will be straight throughout the range of [S] if and only if the perturbation terms of equation 1 vanish. Simple algebraic arguments show that, in turn, this will be so if and only if $\mathfrak{D}$ vanishes. Therefore, the straightness of the plot may be taken to mean that $\mathfrak{D}$ is, for some reason, equal to zero. As is well-known, the intercept of the plot on the $(1/v)$-axis will be independent of [Y] in "competitive" interaction. If the system does not meet this test, then we can presume that $\mathfrak{D}$ vanishes for reasons other than (a) or (b) above. We may then ask if varying [Y] varies the intercept and the slope of the plot by the same factor. If it does not, then it is likely that the system is characterized by the coincidence (c). If varying [Y] does affect intercept and slope by the same factor, then we may presume that $K_0 = K'_0$ (and $K_1 = K'_1$), and that the system is governed by the equation

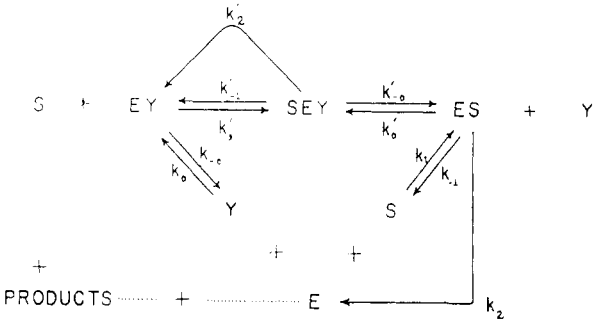
$$\frac{1}{v([S],[Y])} = \left\{\frac{1 + K_0[Y]}{v(\infty, 0) + K_0[Y]v(\infty, \infty)}\right\}\left\{1 + \frac{1}{\bar{K}_1[S]}\right\} = \left\{\frac{(1 + K_0[Y])v(\infty, 0)}{v(\infty, 0) + K_0[Y]v(\infty, \infty)}\right\}\frac{1}{v([S], 0)} \quad (9)$$

We suggest that compliance with equation 9 be taken as the operational definition of "non-competitive" interaction. Clearly, "non-competitive inhibition" is a special case wherein $v(\infty, \infty) = 0$.

The foregoing remarks show that for a system exhibiting non-competitive interaction, $\mathfrak{D} = 0$, but in general $K'_0\bar{K}_1[Y][S] \neq 0$; in other words, the quantity, $k'_{-1}(r - r') = 0$. This quantity vanishes in just three cases: I, the coincidence (c),

(1) D. J. Botts and M. F. Morales, *Trans. Faraday Soc.*, **49**, 696 (1953).

above; II, the coincidence of three conditions[2]: total inhibition ($k'_2 = 0$); $k'_1 \rightarrow 0$, and $k'_{-1} \rightarrow 0$; K'_1, finite and different from zero. III, *The enzyme–substrate reactions at quasi-equilibrium, i.e.,* $r \rightarrow 0$ and $r' \rightarrow 0$, or $\bar{K}_1 \rightarrow K_1$. Compliance with equation 9 is experimentally ascertainable; suppose a system complies, what can be inferred about its state? Under which of these three cases does it fall? Although Case I is a mathematical possibility, it is, on chemical grounds, a freak, for it requires that the presence of the modifier affect two quite different processes (a desorption involving only secondary forces, and a reaction involving chemical bonds) by exactly the same factor. The improbability of Case I would be exaggerated by the demonstration that the system was non-competitive with respect to more than one modifier. Case II would also be freakish, particularly if (as is frequently the case) the modifier is H^+ or some other small ion; moreover, Case II has some experimentally identifiable characteristics. Particularly, Case II *cannot* hold if the rate-modification is an *activation* ($k'_2 \neq 0$). Even if it is a total inhibition the postulated low values of k'_1 and k'_{-1} should become evident in order-of-addition experiments, *i.e.*, in principle, the initial rate of the system (E + S) + Y should perceptibly decelerate to that of the system (E + Y) + S. It is for these reasons that we believe Case III to be the common explanation of non-competitive interaction, and that we are led to the proposition entitling this note. Because the interpretation of the "Michaelis Constant" ($1/\bar{K}_1$) is a recurring problem in quantitative enzymology we feel that the present conclusions may be of some practical use.



Considerations similar to the foregoing have been developed in the past. Some years ago, Hearon[3] pointed out that the practice[4] of treating reversible thermal deactivation of luciferase by replacing, in the velocity expression, $[E_0]$ with $[E_0]/(1 + K_D)$, where K_D was the equilibrium constant of deactivation, was tantamount to assuming that the enzyme-substrate reaction was at quasi-equilibrium. Since the reaction system for deactivation is homomorphic to the present one, and since *multiplication* of the S-function by $1/(1 + K_D)$ is the mathematical indication that S-binding and deactivation are (somewhat unrealistically[5]) assumed to be independent[1] or "non-competitive," Hearon's conclusions are entirely consistent with the considerations of the present note. Our own suggestions[5] that (in 0.6 *M* KCl, *p*H 7.0) the myosin–ATP combination was probably at quasi-equilibrium because 10^{-3} *M* Ca^{++} and 10^{-3} *M* Mg^{++} alter k_2 without changing the apparent $\bar{K}_1$ (reciprocal slope of the $(1/v) - (1/[S])$ plot multiplied by $1/v_{max}$ is also supported by the present considerations, especially because Ca^{++} is a non-competitive *activator* of the system.[6] Finally we take the opportunity to acknowledge the interesting paper of Segal, *et al.*,[7] which, although not specifically concerned with the present problem, does treat the general problem of modifier kinetics in terms of a reaction scheme very similar to that employed by Botts and the author.[1]

I am very indebted to Drs. Sidney Bernhard and Keith Laidler for valuable discussions of this problem.

(2) Case II is sometimes fortuitously invoked by *omitting* the reaction, $EY + S \underset{k'_1}{\overset{k'_{-1}}{\rightleftarrows}} SEY$, and "solving" the resulting reaction system forthwith. The conditions set forth here—and first pointed out to the author by Professor Keith Laidler—are the rigorous equivalent of this omission, but they are more enlightening, as we shall see presently.

(3) Personal communication.

(4) H. Eyring and J. L. Magee, *J. Cell. Comp. Physiol.*, **20**, 169 (1942).

(5) L. Ouellet, K. J. Laidler and M. F. Morales, *Arch. Biochem. Biophys.*, **39**, 37 (1952).

(6) We know from personal communication that this is the conclusion reached independently by Dr. S. Watanabe.

(7) H. L. Segal, J. F. Kachmar and P. D. Boyer, *Enzymologia*, **15**, 187 (1952).

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Methylphosphonic Diamide[1]

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No diamides of aliphatic phosphonic acids containing unsubstituted amino groups are recorded in the literature. v. Hofmann[2] attempted to prepare methylphosphonic diamide by the reaction of methylphosphonic dichloride with ammonia, but the resulting mixture of inorganic salts and the desired diamide was not separated readily.

It was found that methylphosphonic diamide can be prepared easily by the reaction of methylphosphonic dichloride and ammonia in chloroform according to the equation

$$CH_3P(O)Cl_2 + 4NH_3 \longrightarrow CH_3P(O)(NH_2)_2 + 2NH_4Cl$$

This mixture cannot be separated by extraction with hot chloroform, although the diamide is fairly soluble in this solvent. The ammonium chloride was converted to diethylamine hydrochloride which is very soluble in chloroform, and the methylphosphonic diamide crystallized from the chloroform solution in an almost pure state.[3]

In aqueous solution the diamide forms characteristic precipitates with silver, mercury, copper and lead ions. In an excess of Cu^{++} or Pb^{++} ions the

(1) This article is based on work performed under Project 116-B of The Ohio State University Research Foundation, sponsored by the Olin Mathieson Chemical Corp., Baltimore, Md.

(2) A. W. v. Hofmann, *Ber.*, **6**, 307 (1873).

(3) R. Klement and O. Koch, *Chem. Ber.*, **87**, 338 (1954), recommend this procedure for the isolation of phosphonic triamide.