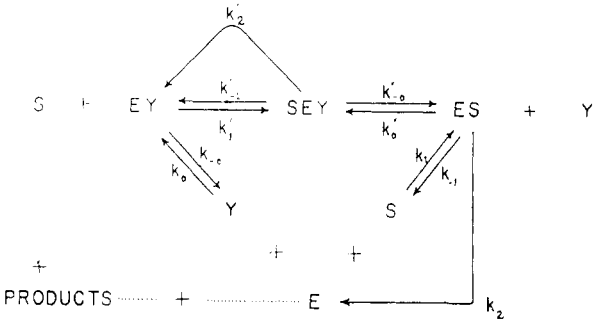
above; II, the coincidence of three conditions[2]: total inhibition ($k'_2 = 0$); $k'_1 \rightarrow 0$, and $k'_1 \rightarrow 0$; K'_1, finite and different from zero. III, *The enzyme–substrate reactions at quasi-equilibrium, i.e.,* $r \rightarrow 0$ and $r' \rightarrow 0$, or $\bar{K}_1 \rightarrow K_1$. 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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RECEIVED FEBRUARY 3, 1955

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.

precipitates dissolve, but after some minutes reprecipitation occurs. The composition of the silver salt corresponds to $(CH_3P(O)NH_2OAg)_2 \cdot AgNO_3$. The diamide hydrolyzes readily on exposure to the atmosphere; the crystals liquefy forming diammonium methyl phosphonate, but after some time the liquid resolidifies as ammonium hydrogen methyl phosphonate. Aromatic phosphonic acid diamides, such as $C_6H_5P(O)(NH_2)_2$ and tetra-alkyl-substituted aliphatic phosphonic acid diamides, such as $CH_3P(O)[N(C_2H_5)_2]_2$, are reported to be much more stable to hydrolysis.[4]

Experimental

To absolute chloroform (800 ml.), which had been saturated at −10° with dry ammonia gas, was added 38 g. of freshly distilled methylphosphonic dichloride (b.p. 63°, 14 mm.) dropwise over a period of two hours with stirring at −10 to 0°; the passage of ammonia was continued during the addition. The precipitated mixture of methylphosphonic diamide and ammonium chloride (48 g.) was collected rapidly on a Büchner funnel with a sintered-glass plate and immediately stored in a vacuum desiccator over phosphorus pentoxide. The finely powdered material, suspended in a mixture of 200 g. of anhydrous chloroform and 50 g. of anhydrous diethylamine, was refluxed gently until solution was effected and the hot solution was filtered to remove a small amount of impurities. When the filtrate was cooled to −15°, methylphosphonic diamide crystallized as large plates, which were collected on a sintered-glass plate (yield 21 g., 73.5%) and recrystallized from methanol containing 3% ether; m.p. 128–129° (sealed capillary, dec.).

Anal. Calcd. for CH_7N_2OP: C, 12.78; H, 7.45; N, 29.80; P, 32.98. Found: C, 12.86; H, 7.57; N, 29.49; P, 32.76.

Methylphosphonic diamide (0.7 g.) was dissolved in a small amount of methanol. Upon the addition of a solution of silver nitrate in 80% aqueous methanol, a white amorphous precipitate was formed. After decanting and thorough washing with methanol, the compound was dried *in vacuo* over phosphorus pentoxide prior to analysis.

Anal. Calcd. for $Ag_3C_2H_{10}N_3O_7P_2$: Ag, 56.14; N, 7.33; P, 10.80. Found: Ag, 55.35; N, 7.25; P, 10.94.

Methylphosphonic diamide (1.3563 g.), exposed to air, increased in weight as follows:

Hours	48	60	72	96	108	120
Increase, %	36.7	38.0	35.0	24.2	20.5	20.4

Calcd. increase for $CH_3P(O)(ONH_4)_2$ 38.4%, for $CH_3P(O)(OH)ONH_4$ 20.2%.

The end product ammonium hydrogen methylphosphonate was recrystallized from 80% ethanol as rhombic pyramids.

Anal. Calcd. for CH_8NO_3P: C, 10.81; H, 7.21; N, 12.61; P, 27.90. Found: C, 10.41; H, 7.19; N, 12.69; P, 27.43.

An aqueous solution of ammonium hydrogen methylphosphonate was neutralized with dilute ammonium hydroxide and silver nitrate added. The white precipitate, disilver methylphosphonate, which was dried over P_4O_{10} *in vacuo* gave almost the same silver value as the product prepared by Michaelis and Kraehne[5] from methylphosphonic acid and silver nitrate. The analytical data of both silver salts agree better with the calculated values for disilver methylphosphonate monohydrate than with those for the anhydrous salt.

Anal. Calcd. for $CH_3PO_3Ag_2 \cdot H_2O$: Ag, 67.89; P, 9.75. Found: Ag, 68.19; P, 9.75. Found by Michaelis and Kraehne: Ag, 68.91.

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(4) A. Michaelis, *Ann.*, **293**, 215 (1896); **326**, 164 (1903).

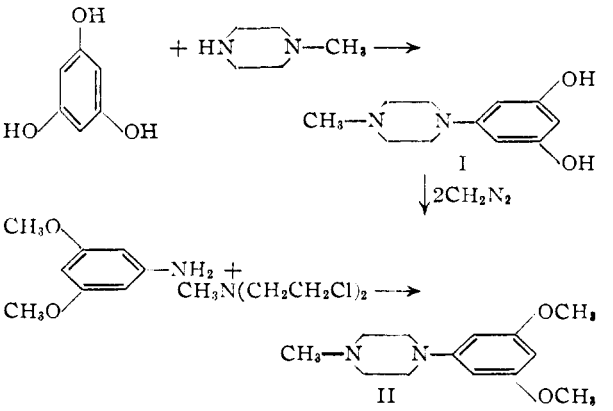
(5) A. Michaelis and B. Kraehne, *Ber.*, **31**, 1054 (1898), give the calculated silver value for $CH_3PO_3Ag_2$ as 69.72%; it should be 71.99%, and the phosphorus value 10.33%.

The Reaction of Phloroglucinol with 1-Methylpiperazine

BY ARMIGER H. SOMMERS AND JAMES D. BARNES

RECEIVED MARCH 21, 1955

Phloroglucinol has been reported to react with such cyclic amines as piperidine and piperazine to form either tertiary amines with loss of water[1] or molecular compounds corresponding to a 1:1 ratio of the reagents.[2] We have found that the reaction of phloroglucinol with 1-methylpiperazine results in the formation of 1-methyl-4-(3′,5′-dihydroxyphenyl)-piperazine (I). This material was converted by diazomethane to the corresponding dimethyl ether, 1-methyl-4-(3′,5′-dimethoxyphenyl)-piperazine (II), which was also prepared by the reaction of 3,5-dimethoxyaniline with N,N-bis-(β-chloroethyl)-methylamine. The structural identity



of the products was established by their conversion to the same maleate and methiodide salts. Attempts to demethylate II using hydrobromic acid yielded only tars from which no phenolic amine could be isolated.

Acknowledgment.—We thank Mr. E. F. Shelberg and members of the Microchemical Laboratory for the analyses reported here.

Experimental

1-Methyl-4-(3′,5′-dihydroxyphenyl)-piperazine (I).—A mixture of 16.2 g. (0.1 mole) of phloroglucinol dihydrate, 10 g. (0.1 mole) of 1-methylpiperazine and 50 ml. of anhydrous toluene was boiled under a reflux condenser and water trap for 17 hours, when 4 cc. of water had been collected. The solid which was collected and dried weighed 18 g. and melted at 228–235°. It was stirred in dilute hydrochloric acid and the mixture was filtered. The filtrate was made just basic with 5% sodium hydroxide solution, and the resulting solid was recrystallized from methanol. This gave 13.5 g. of powdery solid, m.p. 236–241°. A sample of this material which was sublimed twice melted at 248–249° after preliminary darkening.

Anal. Calcd. for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.75; N, 13.45. Found: C, 63.67; H, 7.58; N, 13.26.

The **dihydrochloride** salt obtained by evaporation of a solution of the base in dilute hydrochloric acid was recrystallized from methanol to give fine needles, m.p. 257–258°.

Anal. Calcd. for $C_{11}H_{18}Cl_2N_2O_2$: C, 46.98; H, 6.45; N, 9.96. Found: C, 46.66; H, 6.25; N, 10.03.

When this salt was sublimed at 0.2 mm., a crystalline

(1) M. P. Schmidt and O. Süs, German Patent 639,125, *C. A.*, **31**, 1632 (1937).

(2) G. Sanna and A. Sorarù, *Rend. seminar facoltà sci. univ. Cagliari*, **12**, 34 (1942); *C. A.*, **38**, 5501 (1944).