was maintained at an internal temperature close to 170° under nitrogen for six hours in a flask equipped with a reflux condenser. The excess diethylaminoacetonitrile was then removed at water pump pressure and the residue was submitted to distillation at the oil pump. After a small forerun of indole, indoleacetonitrile was collected at 144–152° (0.03 mm.) or 160–165° (0.2 mm.) (lit.[7] 157° (0.2 mm.)) as a viscous, slightly cloudy liquid weighing 5.2–6.9 g. (33–44%). The picrate[7] melted at 128–129° without recrystallization (lit.[7] 127–128°) and hydrolysis of the nitrile with 20% aqueous potassium hydroxide[7] yielded the acid, m.p. 162–164° (dec.) in 85% yield (lit.[7] m.p. 164–165° (dec.), yield 86%). We were unable to find a solvent for the reaction, aromatic hydrocarbons or aliphatic alcohols having been proved unsuitable. Attempts to scale up the preparation led to a drop in yield.

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The Estimation of the Relative Activities of a Series of Specific Substrates[1]

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It is generally recognized that when the rate of disappearance of the specific substrate S_t in the system

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \ldots \quad (1)$$

is given by equation (2), where $K_S = (k_2 + k_3)/k_1$, a plot of $\ln [S]_0/[S]_t$ *versus* t will at particularly

$$-d[S]/dt = k_3[E][S]/(K_S + [S]) \quad (2)$$

low concentrations of $[S]_0$ when K_S is large relative to $[S]_0$ approximate that of a first-rrder reaction, that a plot of $([S]_0 - [S]_t)$ *versus* t will at particularly high concentrations of $[S]_0$ when K_S is small relative to $[S]_0$ approximate that of a zero-order reaction.

Despite the apparent success in relating the activities of several series of specific substrates on the basis of their respective approximate first-order constants determined at a single and arbitrary initial specific substrate concentration, *i.e.*, on the basis of so-called first-order proteolytic coefficients,[3–6] it is clear from the investigations of Neurath and his co-workers[7–9] that this practice is basically unsound and should be abandoned.

In an attempt to devise a more rational procedure for the comparison of the activities of a series of specific substrates Neurath, *et al.*,[7–9] suggested that the approximate first-order constants be extrapolated to zero initial specific substrate concentrations, *i.e.*, where the so-called maximum first-order proteolytic coefficient C^1_{max} is defined by equation (3).

$$2.3\, C^1_{max} \doteq k_3/K_S \quad (3)$$

There are two limiting cases for equation (3): I, where $k_3 \gg k_2$, $K_S \doteq k_3/k_1$ and $C^1_{max} \doteq k_1$; and II, where $k_3 \ll k_2$, $K_S \doteq k_2/k_1$ and $C^1_{max} = k_1k_3/k_2$. It is obvious that in case I C^1_{max} is in no way related to the susceptibility of ES to subsequent reaction being clearly the constant for the reaction depicted in equation (4). Thus, if

$$E_f + S_f \xrightarrow{k_1} ES \quad (4)$$

it can be shown that for all specific substrates being compared $k_3 \gg k_2$ then values of C^1_{max} can be used to compare the rates with which these specific substrates will combine with a given enzyme present in a particular reaction system recognizing of course that independent evidence must be provided to show that all of the specific substrates are reacting with the same catalytically active site if the results are to be interpreted in this manner.

For case II C^1_{max} is directly proportional to k_3, which in many instances, but not in all, can be taken as an index of the suceptibility of ES to subsequent reaction, and inversely proportional to the dissociation constant k_2/k_1 of ES. Thus, in this case C^1_{max} can only lead to a somewhat ambiguous estimate of the relative activity of a series of specific substrates and cannot be used to estimate on one hand the affinity of the enzyme for a particular specific substrate, or set of specific substrates, and on the other the susceptibility to subsequent reaction of the corresponding enzyme–substrate complexes. An example of the confusion that can arise through the use of C^1_{max} values to estimate the relative activity of a series of specific substrates where $k_3 \ll k_2$ is given immediately below.

If it is assumed with some justification[10,11] that the molecular weight of α-chymotrypsin is *ca.* 22,000 and its nitrogen content is *ca.* 16%, then for acetyl-L-tryptophanamide[12] $k_3 = 0.029$ sec.$^{-1}$, $K_S = 0.0053$ M and $C^1_{max} = 5.5$ M^{-1} sec.$^{-1}$, and for acetyl-L-phenylalaninamide[13] $k_3 = 0.047$ sec.$^{-1}$, $K_S = 0.034$ M and $C^1_{max} = 1.4$ M^{-1} sec.$^{-1}$ For these two specific substrates it is probable that in both instances $K_S \doteq k_2/k_1$[13,14] and consequently C^1_{max} is to be interpreted in terms of case II above. The fact that C^1_{max} for acetyl-L-phenylalaninamide is but *ca.* 1/4 of that for acetyl-L-tryptophanamide is not due to a greater susceptibility to hydrolysis of the enzyme–substrate complex arising from acetyl-L-tryptophanamide than that arising from acetyl-L-phenylalaninamide since the respective k_3 values, *i.e.*, 0.029 and 0.047 sec.$^{-1}$ actually predict the reverse situation, but rather to the far greater affinity of the enzyme for acetyl-L-tryptophanamide than for acetyl-L-phenylalaninamide as indicated by the K_S values of 0.0053 and 0.034 M, respectively. Thus in the absence of any knowl-

(1). Supported in part by a grant from Eli Lilly and Company.

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(6) E. L. Smith in J. B. Sumner and K. Myrbäck, "The Enzymes," Academic Press, Inc., New York, N. Y., 1951, p. 793 *et seq.*

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edge of the pertinent K_S and k_3 values it is clear that values of C^1_{max} for case II provide no more than an ambiguous estimate of relative activity in which the contributions due to K_S and those due to k_3 can not be separately evaluated. It should be noted that past experience has shown that there is no basis for the assumption that in an extended series K_S is approximately constant and k_3 is the important variable or *vice versa*.[13]

While it is true that in the system depicted in equation (1) there is no ambiguity arising in the interpretation of C°, *i.e.*, the true zero-order coefficient, since it is directly proportional to k_3, it is obvious that valid comparisons cannot be made between C° values on one hand and C^1_{max} values on the other.

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Bromomagnesium Salts of Dialkyl Phosphites as Intermediates in the Synthesis of Substituted Hydroxymethyl Phosphonic Acid Esters

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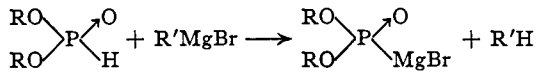
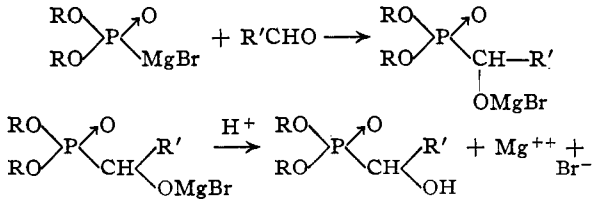
Recent reports[1-4] on the base-catalyzed condensation of dialkyl phosphites with carbonyl compounds to yield α-hydroxy phosphonates prompt us to report preliminary work on their synthesis by the use of bromomagnesium derivatives of dialkyl phosphites prepared *in situ*. These derivatives may be prepared by the addition of an ether solution of a Grignard reagent to an ether solution of a dialkyl phosphite according to the equation



Refluxing the bromomagnesium derivative with an aldehyde or ketone and decomposing the reaction mixture with saturated ammonium chloride solution or dilute acid yields after drying and vacuum distillation the α-hydroxyphosphonate.

The reactions leading to the formation of the α-hydroxyphosphonates may be written in a manner analogous to the reaction of Grignard reagents with carbonyl compounds.



The acidity of dialkyl phosphites is well known,[5] magnesium salts,[6] in addition to others, having been prepared and while cognizance of this is taken in the replacement of alkoxy groups of dialkyl phosphites by alkyl groups from Grignard reagents,[7] apparently no previous attempt has been made to prepare and utilize halomagnesium derivatives for synthetic purposes.[8]

The *in situ* prepared bromomagnesium salts are stable on refluxing in diethyl ether, do not react with alkyl halides (*n*-butyl bromide and methyl iodide) on refluxing in diethyl ether and react with oxides and acyl halides in addition to ketones and aldehydes. These and other reactions are under investigation.

Experimental

Ethane Evolution from Ethylmagnesium Bromide and Diethyl Phosphite.—A solution of 1.38 g. (0.010 mole) of diethyl phosphite in 6 ml. of anhydrous ether was placed in a 3-necked flask of 20-ml. capacity. The flask was connected *via* a small cold-water condenser to two gas burets arranged in series. After displacement of air by ether vapors, 6.65 ml. (0.010 mole) of a 1.50 *M* solution of ethylmagnesium bromide in anhydrous ether was added dropwise with shaking over a period of 20 minutes at room temperature. The evolved gas was collected in the first buret and then transferred to the second buret where its volume was measured at room temperature and atmospheric pressure. The confining liquid in both burets was 85% phosphoric acid. Blank determinations indicated essentially complete absorption of ether vapors. The volume of gas collected, reduced to standard conditions, was 218 ml. (97.4% of theory).[9]

***In situ* Preparation of the Bromomagnesium Salt of Dibutyl Phosphite.**—Into a 3-necked flask, equipped with sealed stirrer, dropping funnel, reflux condenser and calcium chloride drying tubes, a solution of 38.8 g. (0.2 mole) of dibutyl phosphite in 50 ml. of anhydrous ether was placed. A solution of 0.2 mole of ethylmagnesium bromide in 100 ml. of anhydrous ether was then added dropwise with stirring and cooling in an ice-salt mixture. Toward the end of the addition, the reaction mixture separated into two layers, the lower a thick oil. In the case of diethyl phosphite a pasty mass separated which on continued stirring dispersed and two layers formed. The intact reaction mixture or that prepared by reverse addition under the same conditions was used as a source of the bromomagnesium salt in subsequent experiments.

Attempts to isolate the bromomagnesium derivative of diethyl phosphite by separation of the lower layer, washing with ether and drying in high vacuum yielded a white, hygroscopic powder whose analysis consistently failed to agree with the calculated values and from which no empirical formula could be calculated.

Anal. Calcd. for $C_4H_{10}O_3BrMgP$: C, 19.82; H, 4.11; Br, 33.12; Mg, 9.95; P, 12.83. Found: C, 13.65; H, 5.61; Br, 26.56; Mg, 18.90; P, 11.50.

Stability of the Bromomagnesium Salt of Dibutyl Phosphite to Reflux in Ether.—After preparation of the bromomagnesium salt, using the quantities and conditions indicated above, the cooling bath was removed and the reaction mixture was refluxed for three hours. After standing overnight at room temperature decomposition of the salt was effected by shaking the reaction mixture with 100 ml. of cold

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(7) G. M. Kosolapoff and R. M. Watson, THIS JOURNAL, **73**, 4101 (1951), use a Grignard to dialkyl phosphite ratio of 3.3:1 for the preparation of phosphinous acids intermediate in a synthesis of phosphinic acids.

(8) During the preparation of this manuscript, A. N. Pudovik, *Zhur. Obschchei Khim.*, **22**, 109 (1952); *C. A.*, **46**, 11099 (1952), prepared, *in situ*, the bromomagnesium salts of diethyl and dibutyl phosphite and treated these with 1-alkoxy-5-chloro-3-pentenes and 1-alkoxy-3-chloro-4-pentenes to give the corresponding 1-alkoxy-5-(dialkylphosphono)-3-pentenes.

(9) Pudovik, footnote 8, using almost identical quantities as described above, on addition of diethyl phosphite to ethylmagnesium bromide found the evolution of ethane to be 32.4%.