acetyl-α-chymotrypsin fall on the line established by the measurements for diisopropylphosphoryl-α-chymotrypsin. When monoacetyl-α-chymotrypsin is deacylated at pH 6.0, the resulting molecule has the same optical rotatory dispersion parameters as α-chymotrypsin. This indicates that the changes in the optical rotatory dispersion parameters are reversible and intimately related to the formation and breakdown of the enzyme-substrate intermediate, monoacetyl-α-chymotrypsin.

The effect of temperature on the specific rotation of α-chymotrypsin, diisopropylphosphoryl-α-chymotrypsin, and monoacetyl-α-chymotrypsin is demonstrated in Fig. 2. In the absence of other data $(\alpha)_{340}$ can only be considered as one measure of the thermal stability of the enzymes. For each experiment the same protein solution was used throughout the temperature range. Measurements were made until the solutions became turbid, which occurred at a different temperature in the case of each protein. It can be seen that the specific rotation of monoacetyl-α-chymotrypsin and diisopropylphosphoryl-α-chymotrypsin is considerably less temperature dependent than the specific rotation of α-chymotrypsin.

These experiments demonstrate that the formation of diisopropylphosphoryl-α-chymotrypsin or monoacetyl-α-chymotrypsin is accompanied by significant changes both in the optical rotatory dispersion parameter K', and in the temperature stability of the molecules. Previous experiments[3-7] have demonstrated that the formation of monoacetyl-α-chymotrypsin or diisopropylphosphoryl-α-chymotrypsin is accompanied by spectral changes and in the case of diisopropylphosphoryl-α-chymotrypsin, by increased stability toward unfolding in 8 M urea at pH 7.0. These data are consistent with the occurrence of structural changes in the formation of monoacetyl-α-chymotrypsin or diisopropylphosphoryl-α-chymotrypsin. Significant changes in the optical rotatory dispersion parameter K', with only minor changes in λ_c, suggest changes in side group interactions rather than in helical content. While a full interpretation of these data must await a more complete understanding of both the theory of optical rotation and the structure of the enzyme, this conclusion is in agreement with the data presented previously[7] on the characterization of the spectral changes which accompany the formation of diisopropylphosphoryl-α-chymotrypsin or monoacetyl-α-chymotrypsin.

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THE H_- SCALE AND THE ACIDITY OF AROMATIC AMINES

Sir:

We wish to report the establishment of a self-consistent H_- scale for four systems: water, pyridine–water, sulfolane (tetramethylene sulfone)–water and dimethyl sulfoxide–water, each containing tetraalkylammonium hydroxide. H_- de-

scribes the ability of a solvent to remove a proton from a neutral acid and is defined as[1]

$$H_- = -\log \frac{a_{H^+} f_{A^-}}{f_{HA}} = pK_a + \log \frac{[A^-]}{[HA]}$$

H_-, in dilute aqueous solutions, thus becomes identical with pH. The scale was established using the Hammett stepwise technique[1b] with the concomitant determination of the acidities of twenty-three substituted anilines and diphenylamines. Previous work on strongly basic solutions has been reported by Schwarzenbach and Sulzberger[2] (aqueous alkali), Deno[3] (hydrazine–water), Schaal[4] (hydrazine–water, ethylenediamine–water and other systems), and Langford and Burwell[5] (sulfolane–water plus base). However, several discrepancies in both pK_a and H_- exist among their published works. Our results for sulfolane–water containing tetramethylammonium hydroxide are in general agreement with those of Langford and Burwell[5] for the somewhat different system, sulfolane–water containing phenyltrimethylammonium hydroxide.

The acid ionizations were measured spectrophotometrically at 25° and all the indicators listed below showed an instantaneous, reversible, spectral change on addition of base. Those compounds which appeared to have an anomalous ionization behavior were rejected in establishing the scale. In most cases the neutral molecule had little or no absorption at the λ_{max} of the ion. For a given solvent pair variations in the solvent composition had only a small effect on the spectra of the ions.

The compound which bridges the region from dilute aqueous solution to the mixed solvents is 2,4,6-trinitroaniline whose pK_a is 12.20 in aqueous buffers, in good agreement with the value of Schaal.[4b] The pK_a values listed in Table I are averaged values from several solvents (e.g., 4,4-dinitrodiphenylamine; pK_a = 14.09 in pyridine–water–tetramethylammonium hydroxide, 14.15 in aqueous benzyltrimethylammonium hydroxide, 14.00 in sulfolane–water–tetramethylammonium hydroxide). Those compounds which ionize completely in dilute aqueous solution (pK_a values from 2 to 10) have been included because the good correlation existing between acid strength and structure over a range of some 16 logarithmic units helps anchor the scale in the dilute aqueous region. These correlations together with a study of the effects of varying the concentration of hydroxide ion and the identity of the cation will be described in subsequent publications.

Figure 1 shows the variation of H_- with solvent composition[6] for the systems pyridine–water,

(1) (a) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, Inc., New York, N. Y., 1940, p. 269; (b) ibid., p. 264; (c) M. A. Paul and F. A. Long, Chem. Revs., 57, 1 (1957).

(2) G. Schwarzenbach and R. Sulzberger, Helv. Chim. Acta, 27, 348 (1944).

(3) N. C. Deno, J. Am. Chem. Soc., 74, 2039 (1952).

(4) (a) R. Schaal and G. Gadet, Compt. rend., 251, 2176 (1960); (b) R. Schaal, J. Chim. Phys., 52, 784 (1955); (c) R. Schaal and P. Favier, Bull. soc. chim., 2011 (1959).

(5) C. H. Langford and R. L. Burwell, J. Am. Chem. Soc., 82, 1503 (1960).

(6) The plots of log [A⁻]/[HA] for different indicators against solvent composition were found to be parallel, at a given composition in all cases reported here. Above 60 mole per cent. pyridine, in pyri-
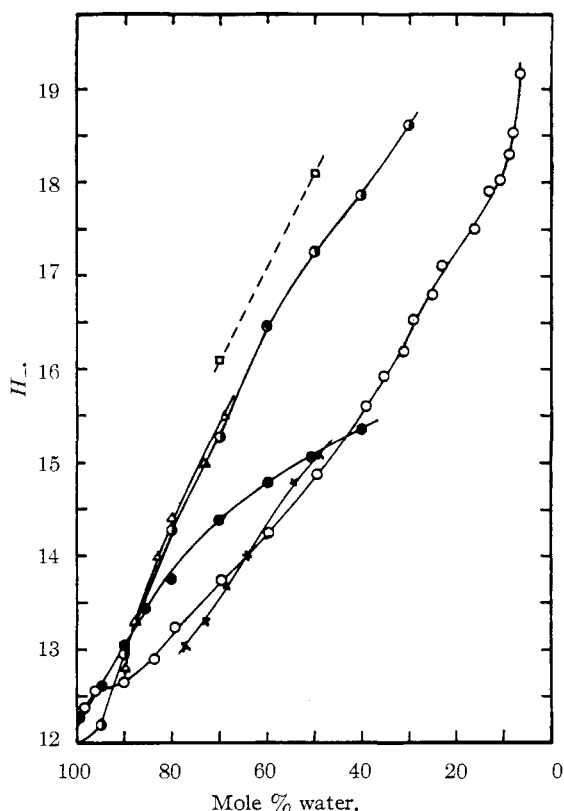
Fig. 1.—Various of basicity with mole per cent. of water in the following mixed solvents: ●, pyridine–water–tetramethylammonium hydroxide (0.011 M); ◐, dimethylsulfoxide–water–tetramethylammonium hydroxide (0.011 M); ○, sulfolane–water–tetramethylammonium hydroxide (0.011 M); ✕, hydrazine–water; △, ethylenediamine–water; □, pyridine–water–benzyltrimethylammonium hydroxide (0.96 M)

sulfolane–water, dimethyl sulfoxide–water[7] (each containing added base), hydrazine–water, and ethylenediamine–water. The H_- values for the latter two systems, which do not contain added hydroxide, were calculated with the optical absorption data of Schaal[4] but using the pK_a values reported herein. It is interesting to note that the effect of reducing the water concentration in the relatively inert solvents (pyridine, dimethyl sulfoxide, sulfolane), each containing added strong base, is not greatly different from that for the systems containing only a weak base (hydrazine, ethylenediamine) as one of the solvent pairs. Strongly basic solutions also can be obtained using water alone as the solvent. Thus, 2.4 molar aqueous benzyltrimethylammonium hydroxide has an H_- value of 16.2.

We believe the foundations of the present H_- scale are firm for three reasons. First, a great many indicators have been used in establishing the scale. Second, unlike previous investigators we have not based our scale on the acidity of p-

dine–water mixtures, however, the slopes deviated greatly owing, presumably, to the decreased dielectric constant of the media.

(7) The very high basicity of dimethyl sulfoxide–water–base solutions is in accord with the kinetic work of Cram, et al. (D. J. Cram, B. Rickborn, C. A. Kingsbury and P. Haberfield, J. Am. Chem. Soc., **83**, 3678 (1961)).

TABLE I
INDICATOR ACIDITIES

Substituted diphenylamines[a]	pK_a	Substituted anilines	pK_a
2,4,6,2',4',6'-Hexanitro	2.63	2,4,6-Trinitro	12.20
2,4,6,2',4'-Pentanitro	6.72	6-Bromo-2,4-dinitro[b]	13.63
2,4,6,4'-Tetranitro	8.88	2,4-Dinitro[b]	15.00
2,4,6,3'-Tetranitro	9.15	2,6-Dichloro-4-nitro	15.55
2,4,6-Trinitro	10.38	2,5-Dichloro-4-nitro	16.05
2,4,2',4'-Tetranitro	10.82	4-Chloro-2-nitro[b]	17.22
2,4,6-Trinitro-4'-amino	10.82	2-Nitro	17.88
2,4,4'-Trinitro	12.35	4-Nitro[b]	18.37
2,4,3'-Trinitro	12.65		
2,4-Dinitro	13.84		
4,4'-Dinitro	14.08		
2,4-Dinitro-4'-amino	14.64	3-Nitrocarbazole	14.10
4-Nitro[b]	15.90		
2-Nitro	17.57		

[a] Diphenylamine, itself, is less than half ionized in 95% dimethyl sulfoxide–5 mole per cent. water containing 0.011 molar tetramethylammonium hydroxide, based on the optical density obtained in anhydrous ethylenediamine containing benzyltrimethylammonium hydroxide. [b] The pK_a values of these five compounds have been determined previously by Langford and Burwell (ref. 5). Except for 2,4-dinitroaniline the agreement is very good (the deviations range from 0.01 to 0.14 pK unit).

nitrobenzyl cyanide which, as will be shown in a subsequent publication, is a most unsatisfactory indicator because of its anomalous ionization equilibria. Third, several solvent systems all yield the same pK_a for a given indicator. This strongly supports the validity of the H_- scale as a measure of solvent basicity.[8]

(8) All the indicators used in this work were structurally similar and it remains to be seen whether or not the difficulties which appear to occur with the H_0 scale when structurally dissimilar indicators are used also occur with H_-. Cf. Ref. 1c and R. W. Taft, Jr., J. Am. Chem. Soc., **82**, 2965 (1960).

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THE CONSTITUTION OF HELMINTHOSPORAL

Sir:

The isolation and characterization of helminthosporal, a toxin produced by the fungus *Helminthosporium sativum*, has been reported recently.[1] This toxin is responsible for a widespread seedling blight, foot and root rot and leaf spot of cereals in North America, which during the last 25 years has resulted, in Western Canada, in an estimated loss of the order of $50,000,000.[2] We now wish to report evidence which reveals it to be a modified sesquiterpenoid and leads to the expression (1a) for this substance.

Helminthosporal, $C_{15}H_{22}O_2$, contains two aldehyde functions, one of which is α,β-unsaturated.[1] Since there are no other points of unsaturation in the molecule the substance, is, therefore, bicyclic. The derived acid (1b) obtained by silver oxide

(1) P. de Mayo, E. Y. Spencer and R. W. White, Can. J. Chem., **39**, 1608 (1961); cf. R. A. Ludwig, Can. J. Botany, **35**, 291 (1957).

(2) B. J. Sallans, Can. Plant Disease Survey, **38**, 11 (1958).