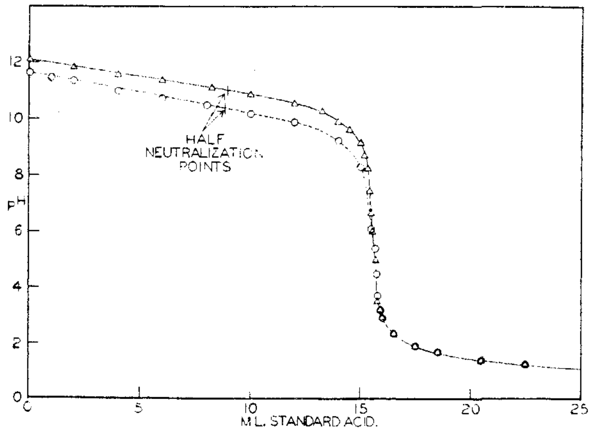
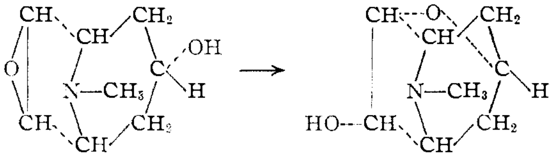


Fig. 1.—Titration curves of tropanol (Δ and solid line) and pseudotropanol (O and dashed line).

It was hoped that the rule of v. Auwers[7] might be applied, although tridimensional models of the molecules reveal that the reference point for *cis–trans* definition is not clear. The densities obtained for liquid tropine and ψ-tropine are $d^{116}{}_{20}$ 1.001 and $d^{116}{}_{20}$ 0.998.

In this connection it may be pointed out that in scopine, I, the basic moiety of hyoscine (scopolamine), the -OH group at position 3 is most probably *trans* to the $\rangle NCH_3$ bridge, since that configuration best explains the easy rearrangement to scopoline, II. Hyoscine frequently occurs together with atropine in various *Atropa* and *Datura* species,[8] suggesting similar stereo-biochemical genesis for the two alkaloids.



I Scopine

II Scopoline

[7] K. v. Auwers, *Ann.*, **410**, 287 (1915); **420**, 84 (1920).

[8] T. A. Henry, "The Plant Alkaloids," The Blakiston Co., Philadelphia, Pa., 1949, p. 65.

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Simultaneous Dissociation of Two Protons. The Acid–Base Equilibria of Porphyrins[1]

BY ROBERT IRVING WALTER[2]

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The four nitrogen atoms of the porphyrin ring system can participate in a series of acid–base equilibria which can be written (PH_2 is the neutral porphyrin molecule)

$$PH_4^{++} \rightleftharpoons PH_3^+ + H^+ \qquad (1)$$

$$PH_3^+ \rightleftharpoons PH_2 + H^+ \qquad (2)$$

$$PH_2 \rightleftharpoons PH^- + H^+ \qquad (3)$$

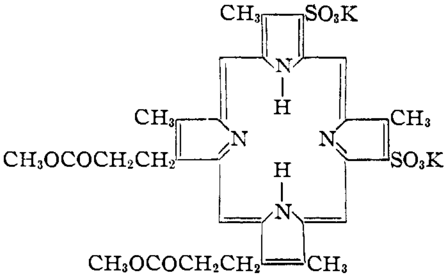
$$PH^- \rightleftharpoons P^= + H^+ \qquad (4)$$

A potentiometric study of equilibria (1) and (2) was reported by Conant, *et al.*,[3] who observed only a single inflection in the titration curve for the addition of two equivalents of acid to PH_2. They assumed $pK_1 = pK_2$, and reported values averaging 2.5 for five porphyrins in glacial acetic acid. A colorimetric study of equilibria (1) and (2) was reported by Aronoff and co-workers,[4] who found only one absorption curve for each of three porphyrins in various sulfuric acid–pyridine mixtures. From the absence of a spectrum intermediate between those of PH_4^{++} and PH_2, they concluded that PH_3^+ could exist over only a limited *p*H range, and assumed that K_1 and K_2 were very nearly equal.

Equilibria (3) and (4) have been studied colorimetrically by McEwen,[5] who found that no difference in acid strength could be detected for the first and second ionization steps of PH_2. He assigned $pK_3 = pK_4 = 16$ for both steps of the ionization of etioporphyrin in methanol.

The authors quoted[3–5] apparently intended to express the conclusions that reactions (1) and (2) occur with equal ease and, similarly, that reactions (3) and (4) occur with equal ease. If this is the case, it is *not* true that $K_1 = K_2$ and $K_3 = K_4$. Instead, due to statistical factors arising in the conventional definition of the dissociation constants, $K_1 = 4K_2$ and $K_3 = 4K_4$[6] (if PH_4^{++} and PH_2 be assumed to dissociate as independent dibasic acids). In such a reaction, the intermediate (PH_3^+ or PH^-) would attain a maximum concentration, when the reaction was half completed, equal to half that of the total porphyrin present.

These results on a number of different porphyrins in a variety of solvents are not strictly comparable, but they suggest an unusual situation in which a tetrabasic acid loses two protons more or less simultaneously in each of two widely separated stages on the *pK* scale. Accordingly, equilibria (1) and (2) have been investigated in an effort to detect the presence of PH_3^+. The water soluble porphyrin chosen for study was the dipotassium salt of 1,3,5,8-tetramethylporphyrin-6,7-dipropionic acid methyl ester-2,4-disulfonic acid



The formula given is thus the species PH_2.

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[1] This material was presented before the Organic Section at the 119th meeting of the American Chemical Society, Cleveland, April, 1951.

[2] School of Chemistry, Rutgers University, New Brunswick, New Jersey.

[3] J. B. Conant, B. F. Chow and E. M. Dietz, THIS JOURNAL, **56**, 2185 (1934).

[4] (a) S. Aronoff and C. A. Weast, *J. Org. Chem.*, **6**, 550 (1941); (b) S. Aronoff and M. Calvin, *ibid.*, **8**, 205 (1943).

[5] W. K. McEwen, THIS JOURNAL, **58**, 1124 (1936).

[6] J. Greenspan, *Chem. Revs.*, **12**, 339 (1933).

Experimental

The porphyrin was prepared from ferriprotoporphyrin chloride and purified chromatographically by a method described elsewhere.[7] The substance actually recovered from the column was the dipotassium salt of the two strong sulfonic acid groups. Aliquots of a standard solution in methanol were air dried and made up in distilled water or aqueous buffers for study. The concentrations of all solutions thus were self-consistent, but involved a constant error estimated to be ±10% because the porphyrin was hygroscopic and only a small sample was weighed.

The buffer used was acetic acid–potassium acetate, with *p*H 4.75 and ionic strength 0.20. Acid solutions were prepared by diluting hydrochloric acid of known concentration.

Absorption of the various solutions was read in the visible region on a Beckman model DU spectrophotometer using 1-cm. corex cells.

Results

Figure 1 shows the absorption spectra of the porphyrin in neutral and acid aqueous solution. These curves differ sufficiently to permit application of the method of continuous variations of Vosburgh and Cooper[8] to the study of the equilibrium

$$PH_2 + nH^+ \rightleftharpoons PH_{2+n}^{n+} \qquad (5)$$

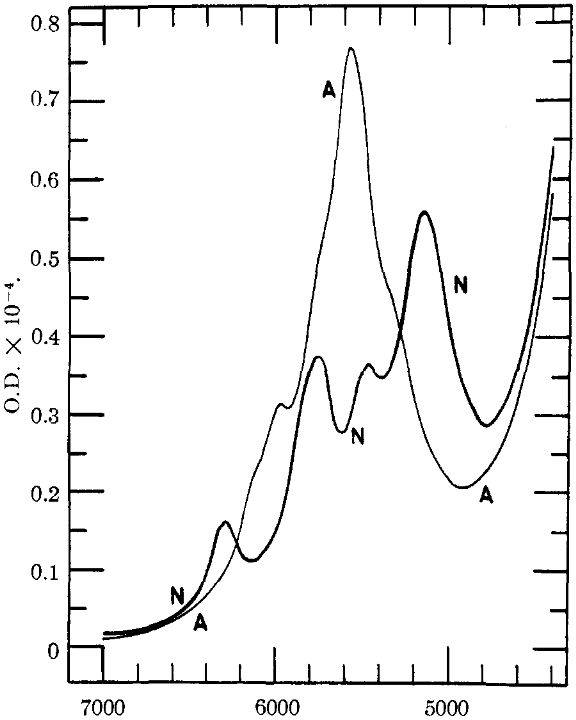

Fig. 1.—Optical densities *vs.* wave lengths in ångströms. The ordinates are calculated for 1 *M* concentration, and thus equal molar extinction coefficients within the estimated concentration error of ±10%: Curve N, absorption of neutral porphyrin, PH_2, in aqueous HOAc–NaOAc buffer, *p*H 4.75, ionic strength = 0.20; curve A, absorption of acid porphyrin, PH_4^{++}, in aqueous HCl, *p*H 0.70, ionic strength = 0.20.

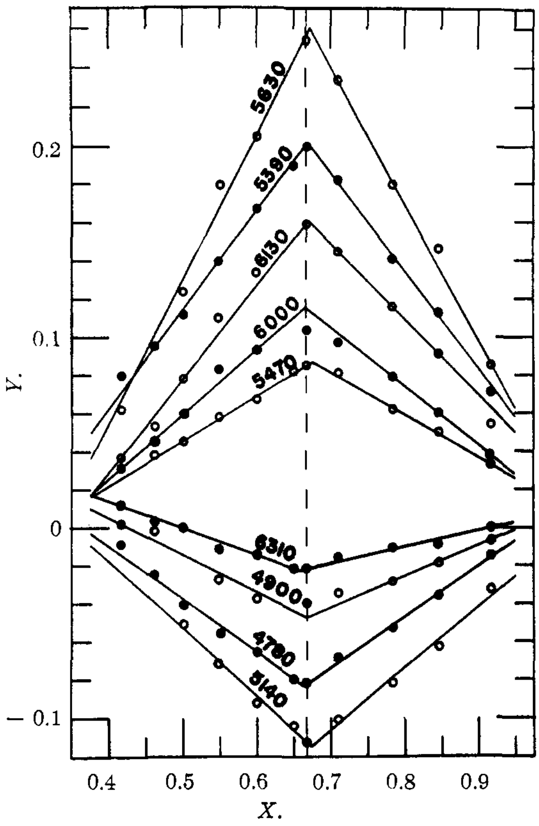

Fig. 2.—Application of the method of continuous variations: Y = observed optical density less that calculated for the porphyrin solution assuming no reaction with acid; X = volume fraction of acid in the mixture of porphyrin and acid solutions.

Figure 2 gives the results, in a plot of the difference function Y against the volume fraction X, of acid solution in a mixture of 0.001 *M* aqueous solutions of porphyrin and hydrochloric acid. Y is defined as the optical density observed less the optical density calculated for the mixed solutions assuming no reaction between the solutes. For the nine wave lengths at which it was determined, the extreme value of Y occurs at $X_{\max} = 0.667 \pm 0.004$, and the value of n in equation (5) above, found from Vosburgh and Cooper's equation (5), is two. (Formation of the intermediate PH_3^+ would result in $X_{\max} = 0.50$.) The transformation observed thus involves equilibria (1) and (2) simultaneously. Since the points in Fig. 2 were obtained both by dilution of porphyrin with acid solution, and by dilution of acid with porphyrin solution, the system is reversible. Furthermore, the sharpness of the maxima and the invariance of $X_{\max}$ for measurements at different wave lengths mean that no intermediate substance PH_3^+ is formed. Stated another way, the two protons add simultaneously to PH_2, within the limits of error of the method.

The Y values in Fig. 2 represent a small difference between two relatively large values of the optical density, so the scatter of the points, including those at the maxima, is within the experimental error of the measurements. Calculation of the minimum concentration at which PH_3^+ could be detected would require assignment of arbitrary values for the extinction coefficient of this substance in aqueous solution, and has not been attempted.

It would be desirable to confirm the result by analysis of the shape of the acid–base titration

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[7] R. I. Walter, *J. Biol. Chem.*, **196**, 151 (1952).

[8] W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 437 (1941).

curve for this substance, so attempts were made to determine the curve potentiometrically. However, the species PH_4^{++} is a sufficiently strong acid that the complete titration curve could not be observed in water, aqueous methanol, or aqueous dioxane. The anhydrous solvents could not be used because the LiCl required to maintain a constant high ionic strength for the accurate determination of curve shape was not sufficiently soluble in them.

Discussion

Although acid-base and oxidation–reduction systems have traditionally been considered from different viewpoints, the exact analogy in principle between the two classes has been discussed by various authors.[9,10] For both systems, the titration curves (potential or *p*H plotted against per cent. reaction) form a family in which the individual members can be identified by their midpoint slopes. Michaelis demonstrated the existence of this family of curves both experimentally and theoretically in his studies of the semiquinones.[11] He showed that the titration curves of lowest midpoint slope, for reactions in which two electrons are transferred simultaneously, are replaced by curves whose midpoint slope increases as conditions are chosen which increase the stability of the semiquinone intermediate in the reaction. The extreme case is the two-step titration curve (with very steep midpoint slope) observed for very stable semiquinone systems.

In the case of the dibasic acids, successive stages of proton transfer almost always occur with increasing difficulty because of the accompanying ionic charge accumulation. As a result, the intermediate stage of ionization is stable and reaches a maximum concentration exceeding 50% that of the total acid–base system at the titration midpoint, and $K_1 >> K_2$. Only in the limiting case of dibasic acids in which the functional groups are separated by long carbon chains do the protons dissociate independently. The intermediate then has the same stability as the completely ionized or completely un-ionized forms, and reaches a maximum concentration of 50% that of the total acid–base system at the midpoint of the titration.[6,10] The acid–base reactions given by equations (1) and (2) *cannot* be of this type, with $K_1 = 4K_2$, since the concentration of intermediate has been shown to be too small to detect, and certainly less than the 50% maximum expected in such a reaction. (If $K_1 = K_2$, as has been assumed previously for the porphyrins, the concentration of PH_3^+ would still reach a maximum equal to 33% that of the total acid–base system.) Thus the intermediate PH_3^+ is less stable than PH_4^{++} or PH_2, and in consequence the second proton is transferred more readily than the first. (The transfer is simultaneous if the concentration of PH_3^+ is zero.) In this case, $K_1 << K_2$, and the individual constants have little meaning. The significant constant is that for the over-all reaction, equation (5), given by $K = K_1K_2$.

One previous example has been reported of proton transfer leading to an intermediate of lower stability, so that the second proton is transferred more readily than the first. Schwarzenbach[10] described a dibasic acid in which the maximum concentration of intermediate was only 22% at the titration midpoint, but his system was too unstable for complete reversibility.

Consideration of the oxidation–reduction analogy has led to the expectation that the intermediate PH_3^+ will be less stable than either PH_4^{++} or PH_2. This is confirmed by consideration of the structures which might reasonably contribute to resonance in the porphyrin system. Assuming fixed N–H bonds, two uncharged and four separated charge structures have been proposed for PH_2, and twelve with no charge separation for PH_4^{++}.[12] The number of such structures depends upon the twofold axis of symmetry in PH_2, and the fourfold axis in PH_4^{++}. Loss of these symmetry elements in PH_3^+ reduces the number of structures contributing to its resonance, and results in its relatively lower stability.

The argument can be repeated for the ionization of PH_2 to give $P^=$, and one would expect in this case also a smaller stability for PH^- than for the other species. (The very close similarity of the absorption spectra of PH_4^{++} and $P^=$ in the case of etioporphyrin[12] indicates that they must be similar electronically.) The ionization of PH_2 could not, however, be studied conveniently by the method of continuous variations, because the *pK* value reported by McEwen[5] would require that the solutions of both porphyrin and hydrogen ion be of the order of 10^{-16} *M* concentration, too low for spectrophotometric work.

Although variation of substituents on the porphyrin ring will alter the magnitude of the acid dissociation constants, their distribution would not be greatly changed. It thus seems probable that the simultaneous dissociation reported here is general for the porphyrin ring system (but not necessarily for reduced ring systems such as the chlorins).

[12] J. G. Erdman and A. H. Corwin, This Journal, **68**, 1885 (1946).

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Studies on Coördination Compounds. VIII. Some Factors Concerning the Effect of the Terminal Groups on the Chelating Abilities of β-Diketones

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RECEIVED FEBRUARY 13, 1953

The general relationships between the stability constants of the β-diketones and their terminal groups have been discussed in a previous paper.[2] Additional constants are given herein which enable a further clarification of the relationships involved to be made.

Experimental

The β-diketones, other than those mentioned previously,

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[9] M. Schubert, *Ann. N. Y. Acad. Sci.*, **40**, 111 (1940).

[10] G. Schwarzenbach and R. Sulzberger, *Helv. Chim. Acta*, **26**, 453 (1943).

[11] L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 437 (1938).

[1] Union Carbide and Carbon Corporation Fellow, 1951–1952; Bell Telephone Laboratories, Murray Hill, New Jersey.

[2] L. G. Van Uitert, W. C. Fernelius and B. E. Douglas, This Journal, **75**, 457 (1953).