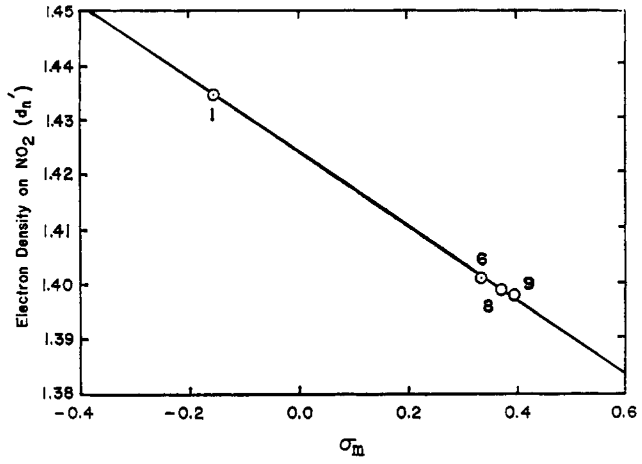

Figure 18.—The relationship between d_n' and σ_m for 4-substituted 2-nitroanilines. Numbers refer to compounds listed in Table V.

ionized water. The spectra of these five solutions were measured against reference solutions of aqueous ethanol of the same concentration.

The pK_A values were calculated from the equation[7b] where A

$$pK_A = H_0 - \log \frac{A - A_1}{A_2 - A}$$

is the absorbance of the solution containing varying concentrations of aqueous $HClO_4$, A_1 is the absorbance of the solution in 70% $HClO_4$, A_2 is the absorbance of the base solution, and H_0[31] is the acidity function.

Polarographic Reductions of Some 4-Substituted 2-Nitroanilines.—A 0.005 M solution (10 ml.) of IV in 75% aqueous ethanol plus 10 ml. of 1 M KNO_3 solution were diluted to 100 ml. with deionized water. A portion of this solution was transferred to the cathode compartment of a three-compartment polarography cell, the center compartment containing 1 M KNO_3 solution, and the anode compartment containing a saturated calomel electrode. The compartments were separated by plugs of agar. The solution of IV was purged with nitrogen and the polarogram was obtained with a Sargent Model XXI polarograph. The reduction half-wave potentials shown in Table II were obtained by the point method.[32]

Infrared Spectral Studies and Measurement of Intensities of 4-Substituted 2-Nitroanilines.—The N-H stretching bands of IV were measured with a Beckman IR-9 infrared spectrometer. A variable path length cell was initially used to balance out solvent absorption. Matched absorption cells of the sandwich type containing sodium chloride prisms were used.

A 0.05 M solution of IV was prepared in spectroquality chloroform. The absorption spectra were measured over a wavenumber range of 50 cm.$^{-1}$ on either side of the band center. The N-H stretching band was expanded five times.

The absorption intensity, I, was obtained from the relation[33]

$$I = \frac{K}{cl}\left[\log\left(\frac{T_0}{T}\right)_{\nu_{\max}}\right]\Delta\nu_{1/2}$$

The area under the curve was obtained by the triangular method, *i.e.*, the peak height times the band width at one-half the peak height. The results are shown in Tables III and IV.

Acknowledgment.—This study was supported in part by a research grant from the Robert A. Welch Foundation. Helpful discussions of the results with Dr. Edward A. Meyers are gratefully acknowledged. The statistical calculations were performed by the Data Processing Center, Texas Engineering Experiment Station, College Station, Texas, on an IBM-709 computer.

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[31] M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).

[32] H. H. Willard, L. L. Merritt, Jr., and J. A. Dean, "Instrumental Methods of Analysis," D. Van Nostrand Co., Inc., Princeton, N. J., 1958, p. 544.

[33] D. A. Ramsay, *J. Am. Chem. Soc.*, **74**, 72 (1952). From Table III, the value of K equals 1.57.

Mass Spectra of Porphyrins and Chlorins

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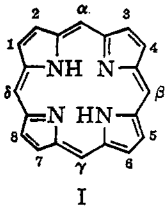
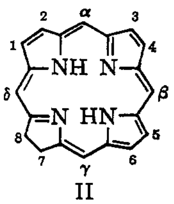
Received May 16, 1965

The mass spectra of several porphyrins and chlorins of synthetic and biological interest have been obtained and interpreted. These compounds fragment only peripherally as one would expect of a large aromatic system. Substituents in the 7- and 8-positions in chlorins are more labile than pyrrole ring substituents. γ-Substituents are even more labile and are lost with the transfer of one and two hydrogens to the tetrapyrrole system, if possible. A steric effect is observed in the loss of γ-substituents. The spectra of compounds containing additional five- and six-membered carbocyclic rings, including methyl phaeophorbide, were obtained and interpreted. The directions of ring closure predicted for two isomeric chlorins in Woodward's chlorophyll synthesis were confirmed. The mass spectra of several compounds containing six-membered lactone rings are discussed. Mechanisms are proposed for several of the types of fragmentations observed.

Porphyrins (I) and chlorins (II) are very difficult compounds to handle in the laboratory. Many of them are very complex and quite reactive. The verification of their structures has always been a major problem, since many classical methods cannot always give unambiguous results. Infrared and ultraviolet absorption spectra provided some information, but it was not always definitive. Recently n.m.r. spectroscopy has been shown to be a useful tool for investigating the structures of these compounds,[1] but many interesting tetrapyrrole compounds are not soluble enough to give good spectra. Additional methods are very much needed in this field because of the immense biological importance of these compounds in both plants and animals. The chlorins are in many ways quite similar to the porphyrins, but in others they are quite different.[2] See Table I for compounds studied.



I



II

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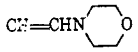
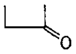
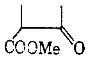
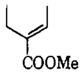
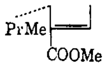
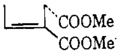
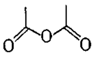
[1] R. B. Woodward and V. Skaric, *J. Am. Chem. Soc.*, **83**, 4676 (1961).

[2] R. B. Woodward, *Ind. chim. belge*, **27**, 1293 (1962).

TABLE I

COMPOUNDS STUDIED

Compd.	Substituent at position[a]				
	2	6	7	8	γ
Porphyrins					
III	Et	Et	Me	Et	...
IV	Et	H	Pr	Me	...
V	Et	PrMe	PrMe	Me	...
VI	Et	H	PrMe	Me	Me
VII	Et	COOEt	Et	Me	Et
VIII	PrEt	COOEt	PrEt	Me	Me
IX	PrEt	COOEt	PrEt	Me	Et
X	PrEt	COOEt	H	Me	PrEt
XI	PrEt	COOEt	PrEt	Me	PrEt
XII	PrA	COOMe	PrMe	Me	CH=CHCOOMe
XIII	PrA	COOMe	PrMe	Me	
XIV	PrA	COOMe	PrMe	Me	C≡CCOOMe
XV	PrA	COOMe	PrMe	Me	CHO
Chlorins[b]					
XVI	V	COOMe	PrMe	Me	H
XVII	V	COOMe	PrMe	Me	COOMe
XVIII	V	COOMe	PrMe	Me	CH_2COOMe
XIX	V	COOMe	PrMe	Me	CHO
XX	V	COOMe	PrMe	Me	COCOOMe
XXI	PrA	COOMe	PrMe and COCOOMe	Me	CHO
XXII	V		PrMe	Me	See 6
XXIII	V		PrMe	Me	See 6
XXIV	V	COOMe		Me	See 7
XXV	PrA	COOMe		Me	See 7
XXVI	PrA		PrMe	Me	See 6
XXVII	V		PrMe	Me	See 6

[a] Me is CH_3, Et is C_2H_5, Pr is CH_2CH_2COOH, PrA is $CH_2CH_2NHCOCH_3$, and V is $CH=CH_2$. All compounds have 1,3,5-methyl groups and a 4-ethyl group. [b] The 7- and 8-substituents are *trans* in all but XXI, XXIV, and XXV.

A mass spectrum requires only 10–50 μg. of material if the compound is reasonably volatile. One would expect that porphyrins would not decompose spontaneously under high vacuum at the temperatures necessary for volatilization because of the great stability of the 18 π-electron system. Only a very small vapor pressure, 10^{-6} torr, is needed to obtain a spectrum if the compound is introduced directly into the source. The temperatures required for volatilization were about 360–480°. In general the spectrum obtained consisted of a strong peak for the molecular ion followed by a series of fragment peaks all containing the intact tetrapyrrole nucleus. A relatively strong doubly charged spectrum was observed in most cases, and in a few cases some triply charged peaks were also observed.

The mass spectra of simple porphyrins containing only pyrrole ring substituents are consistent with Hood's[3] work on nickel etioporphyrin I (III). Only peripheral substituents were lost, and the fragmentation occurred primarily one carbon atom away from the ring. Almost every peak in each of the spectra was easily assignable to the loss of some combination of substituents. The spectrum of mesoporphyrin dimethyl ester (V), Figure 1, is representative of these compounds. The free acids decomposed thermally before vaporizing sufficiently. In chlorins the major losses, as one would expect, are the 7- and 8-substituents cleaving adjacent to the ring.

Substituents in the γ-position behave quite differently from ring substituents under electron impact as well as chemically.[1] The loss of γ-substituents has been observed in some chemical reactions, indicating that they are quite labile. Unless the γ-substituent contains a carbon–carbon double bond in conjugation with the tetrapyrrole system, the largest peak(s) is for its loss. The γ-substituents are lost adjacent to the nucleus not allylically, and, if they are available, one or two hydrogens are transferred to the π system. In a γ-ethyl porphyrin of mass 694, IX, the largest fragment peak is for the loss of 27 from the molecular ion. An accurate mass measurement showed this to be 667.348 corresponding to $C_{39}H_{47}N_4O_6$ (the loss of

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(3) A. Hood, E. G. Carlson, and M. J. O'Neal in "Encyclopedia of Spectroscopy," G. L. Clark, Ed., Reinhold Publishing Corp., New York, N. Y., 1960, p. 616.

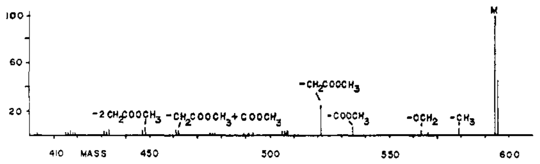


Figure 1.—Mass spectrum of mesoporphyrin dimethyl ester (V).

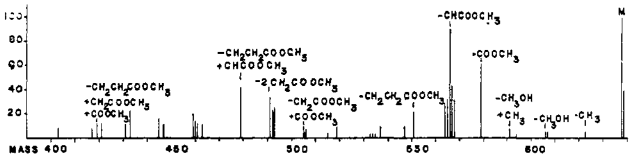


Figure 2.—Mass spectrum of trimethyl chlorin e_6 (XVII).

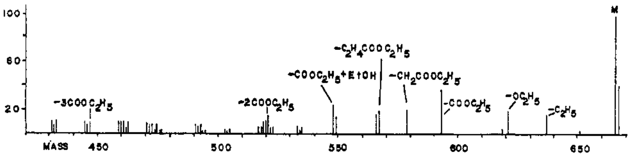


Figure 3.—Mass spectrum of compound X.

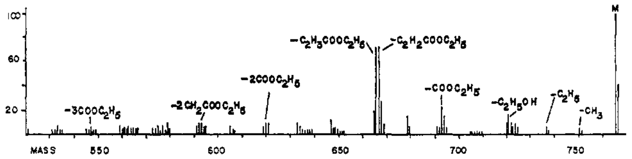


Figure 4.—Mass spectrum of compound XI.

C_2H_3) which requires 667.349. Metastables were observed for the losses of 27, 28, and 29 from the molecular ion. In chlorins also the major loss is the γ-substituent; for an example, see the spectrum of trimethyl chlorin e_6 (XVIII), Figure 2.

γ-Substituents with α-carbonyl groups are lost in three ways. In a γ-$COCOOCH_3$-chlorin, XX, peaks were observed at 564.272 (relative intensity 70%) for the loss of $HCOCOOCH_3$ which requires 564.274, 565.279 (98%) for the loss of $COCOOCH_3$ which requires 565.281, and 566.288 (100%) for the loss of $COCOOCH_2$ which requires 566.289; the molecular ion peak had a relative intensity of only 7% in this compound. If the γ-substituent contains a carbon–carbon double or triple bond, the loss of the γ-substituent is not very facile. In the compound with a γ-acrylic ester, XII, a weak peak (less than 10%) was observed for the loss of $C_2HCOOCH_3$ at 623.309; $C_{36}H_{41}N_5O_5$ requires 623.310. In an analogous compound with a morpholine group replacing the ester, XIII, a much stronger peak (66%) was observed at 623.311. Weak metastables were visible for these preceding transitions. This difference in intensity was interpreted as a steric effect. Two other pairs of compounds were available to test this hypothesis. In the γ-methyl compounds, VI and VIII, substitution of COOEt for H on the 6-position causes the intensity of the M − 15 peak relative to the molecular ion to increase by a factor of 2.5. In the γ-propionic acid ethyl ester compounds, X and XI, the change from a 7-H to a 7-propionic acid ethyl ester causes an increase in the intensity of the peaks for the loss of the γ-substituent by a factor of about 4 (Figures 3 and 4).

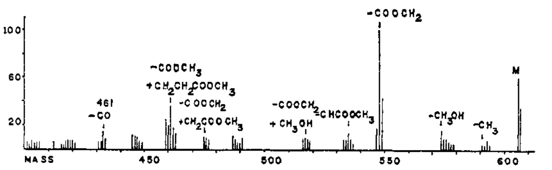


Figure 5.—Mass spectrum of methyl phaeophorbide *a* (XXIII).

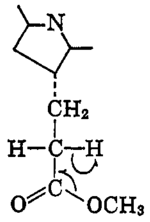


Figure 6.—Loss of CH_3OH.

Examination of molecular models of these compounds demonstrates the magnitude of this crowding at the bottom of the planar or almost planar porphyrin system quite effectively.

Some Biologically and Synthetically Interesting Compounds.—Some of the most important of these compounds are the phaeophorbides *a* and *b* obtained by the removal of magnesium and the hydrolysis of the phytyl ester group from chlorophylls *a* and *b*. One of the simplest phaeophorbides is methyl pyrophaeophorbide *a* (XXII), obtained by heating methyl phaeophorbide *a* (XXIII). The mass spectrum of this material is quite simple; there is a peak for the loss of the propionic acid methyl ester group from the 7-position, a peak for the loss of methyl, and a relatively large peak for the loss of $CH_2CH_2COOCH_3$ plus 28, the CO from the ketone group. This loss of CO occurs only after another substituent has been lost. A similar situation is observed in the mass spectrum of methyl phaeophorbide *a* (XXIII), Figure 5. The peak at *m/e* 558 arises from the loss of $COOCH_2$ from the β-keto ester. The molecular ion peak is about 60% of the intensity of the 558 peak. This reaction might be primarily thermal, but some of it is probably due to electron impact since other losses are observed from the molecular ion at *m/e* 606, and since most of the major peaks are due to the loss of another substituent in addition to $COOCH_2$. A peak is present for the loss of $COOCH_2$ plus $CH_2CH_2COOCH_3$ plus CO.

An additional six-membered ring is found in neopurpurin 4 (XXIV). The mass spectrum of this compound is quite interesting in that no very large peaks arise from the loss of just one substituent, although there are no very labile substituents. The largest fragment peaks are at *m/e* 469, 470, 471, and 457, 458, 459. The second set probably arise from the loss of $HCOOCH_3$ plus $COOCH_3$, two $COOCH_3$, and $COOCH_3$ plus $COOCH_2$. The other group is the loss of $C_4H_{10}O_3 \pm H$; the 470 peak could be the loss of $COOCH_3$ plus CH_3OH plus CH_3 or some other similar combination. This complex fragmentation might be caused by the tendency of both the cyclohexadiene ring and the chlorin system to aromatize fully to $4n + 2\pi$ electron systems.

Chlorins Containing Six-Membered Lactone Rings.—Chlorins with a lactone formed between the 6- and γ-substituents are useful synthetic intermediates.

The basic compound in this series, XXVII, is derived from chlorin p_6. In the mass spectrum of this compound there is a huge peak for the loss of the 7-substituent, $CH_2CH_2COOCH_3$, at *m/e* 491 which is over twice the intensity of the molecular ion peak. The next largest fragment peak is at *m/e* 447 for the loss of CO_2 from the 491 fragment. Metastables are observed for both of these transitions. The pattern of the spectrum changes radically if one of the carbonyl oxygens is replaced by another functional group. In the cyano lactone the peak for the loss of the 7-substituent is only one-third of the molecular ion peak, and, in the compound with a hydrogen in place of the nitrile, only one quarter. When instead this group is methoxyl, the spectrum is again quite different. In this case two isomeric compounds were obtained. In the spectrum of each the largest peaks were for the loss of the methoxyl group: in one as OCH_3 and in the other as CH_3OH, OCH_3, and OCH_2. Both spectra show peaks for the loss of $CH_2CH_2COOCH_3$ in addition to the loss of the methoxyl group. The only hydrogen available for a transfer through either a four- or six-membered transition state is on the 7-position of the chlorin. This suggests that the compound that loses CH_3OH has the methoxyl on the inside. This also explains the loss of OCH_2, since the methoxyl group is in a position similar to a γ-substituent. The compound which loses OCH_3 must then have the methoxyl on the outside of the lactone ring. The synthesis of these compounds makes it improbable that these compounds are a *cis–trans* pair, since one would expect that the *cis* and *trans* would both be formed and that they would be difficult to separate. The loss of CO_2 is not clearly observable in these compounds as it is in the dicarbonyl compound, but there is a peak at *m/e* 533.255 corresponding to the loss of $C_2H_5O_2$, CH_3OH plus CHO.

Determination of the Direction of Ring Closure and Chlorin Formation.—In his magnificent total synthesis of chlorophyll[4,5] Woodward performed a cyclization reaction on a porphyrin and isolated two isomeric purpurins, one in very good yield, which he showed to be the desired product, and the other in a very small amount. He assigned[5] the major component the structure with the ring closed at the 7-position, XXV, and he considered the minor component to be cyclized at the 6-position, XXVI, on the basis of its chemical and physical properties. The mass spectra of these two compounds clearly confirm his assignment. The cyclization reaction produces a quaternary carbon atom and, as is well known,[6] this makes the loss of aliphatic substituents and/or rearrangements very facile. In the compound cyclized at C-7 there is a propionic acid methyl ester group in this position, while in the one cyclized at C-6 there is a carbomethoxy group. In the mass spectrum of each of these compounds the largest fragment peak is due to the loss of this quaternary group. The remainder of each of the spectra shows the expected fragmentations, which are in each case totally consistent with the assigned structure.

Three products were isolated from another chlorin-forming reaction. The n.m.r. spectrum clearly showed that a five-membered ring had closed. The mass spectra showed that two of them were closed at C-7 and one at C-6. The spectrum of one of the C-7 isomers had a peak for the loss of CH_3CH_2COOEt from the molecular ion. This could easily occur if the propionic acid ester and the 8-H are *trans*, and can eliminate to form a porphyrin system. This reaction is not observed in the *cis* chlorins related to chlorin e_6 by synthesis.

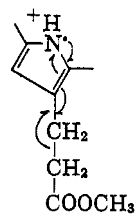


Figure 7.—Allylic cleavage.

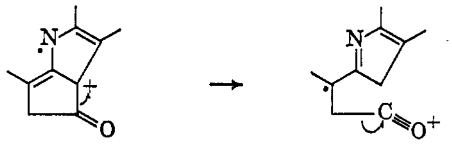
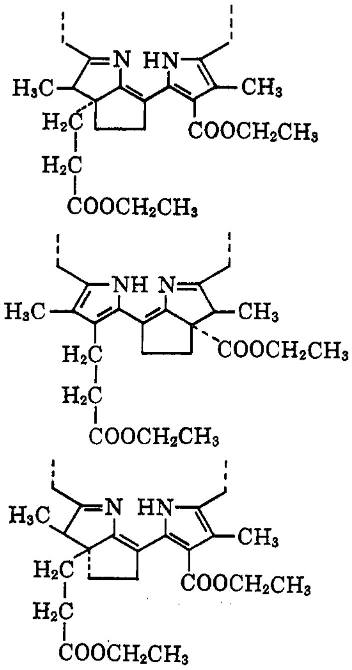


Figure 8.—Loss of CO from phaeophorbides.



Mechanisms of Fragmentations in Porphyrins and Chlorins.—Many of the fragmentations observed in porphyrins and chlorins correspond to commonly known reactions.[6] Most functional groups fragment in their characteristic manners. For instance, the loss of methanol from propionic and acetic acid methyl esters is an excited state rearrangement with a four-membered transition state (Figure 6). The loss of CH_2COOR or $CH_2NHCOCH_3$ from a pyrrole ring position is just an allylic cleavage (Figure 7). Fragmentation at a tertiary carbon combined with allylic cleavage leads to the loss of the 7- and 8-substituents in chlorins. In these systems the positive charge probably tends to localize on the four nitrogens of the pyrrole rings. CO and CO_2 are lost from compounds with additional rings by the breaking of two bonds. In the phorbide

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(4) R. B. Woodward, *et al.*, *J. Am. Chem. Soc.*, **82**, 3800 (1960).

(5) R. B. Woodward, *Pure Appl. Chem.*, **2**, 383 (1961).

(6) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

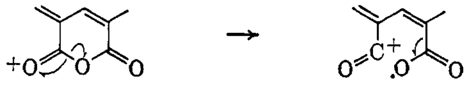

Figure 9.—Loss of CO_2 from lactone.

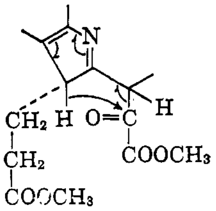

Figure 10.—Loss of $HCOCOOCH_3$.

systems CO is lost only after the loss of another substituent. A possible mechanism for this loss is given in Figure 8. The loss of CO_2 is a somewhat similar reaction (Figure 9), but requires the presence of a group adjacent to the oxygen which can stabilize a positive charge.

Cleavage α to the ring system is observed in the loss of γ-substituents. This is expected in compounds in which the γ-substituent is a simple functional group like CHO or COOR,[7] but it also occurs in these compounds when the γ-substituent is an acetic or propionic acid ester group. In a few unusual cases this type of fragmentation has been shown to occur.[8] However, the loss of a γ-substituent with hydrogen on a carbon near the ring exhibits a novel feature; one or two hydrogens are transferred from the substituent to the tetrapyrrole system. This double hydrogen transfer is even observed when the γ-substituent is an ethyl group. This is obviously a fairly complex reaction and it is not understood at the present time. In the compound XX the loss of $HCOCOOCH_3$ can be explained by the transfer of a hydrogen through a six-membered transition state (Figure 10), producing a porphyrin ion from the chlorin ion, which explains the relatively large peak (35%) for the loss of $HCOCOOCH_3$ plus CH_2COOCH_3. The chlorin ion would be expected to lose $CH_2CH_2COOCH_3$ much more easily than CH_2COOCH_3.

Experimental Section

The compounds used in this study were all either commercial materials or were synthesized by Professor R. B. Woodward and his co-workers.[2,4,5] Many of them are described by Woodward[4,5] and Fischer.[9]

The mass spectra were obtained on an AEI MS9 double-focussing mass spectrometer. The samples were inserted directly into the source by the use of a vacuum-lock probe.[10] Temperatures of 360–480° were needed to evaporate these compounds. The results of accurate mass measurements are reported in the text; heptacosafluorotributylamine[11] was used as a mass standard for the high-resolution work. The ionizing energy was maintained at 70 e.v.

Acknowledgment.—The author would like to thank Professor R. B. Woodward for generously donating the compounds used in this study and for his encouragement and advice, and would also like to thank Dr. M. Barber of AEI Ltd., Manchester, for his many helpful discussions and suggestions. This work was supported by the National Institutes of Health.

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[7] H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, pp. 3, 11.

[8] See ref. 7, p. 259.

(9) H. Fischer and H. Orth, "Die Chemie des Pyrrols," II Band, Hälfte 1 and 2, Akademische Verlagsgesellschaft, Leipzig, 1937 and 1939.

(10) M. Barber, R. M. Elliott, and T. O. Merren, International Symposium on Mass Spectrometry, Paris, 1964.

(11) Obtained from Peninsular ChemResearch Inc., Gainesville, Fla.

Solvent Effects. II. The Influence of Aqueous Dimethyl Sulfoxide on Ester Saponification Reactions

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Received April 30, 1965

The rates of alkaline hydrolysis of seven ethyl esters have been determined in aqueous dimethyl sulfoxide (DMSO) solvent mixtures of 0.20 to 0.65 mole fraction of DMSO. The rate constant increase for the branched esters is proportional to the DMSO content over the entire solvent composition range and is attributed to electrostatic effects. The rate constant increase for the straight-chain esters is also sensitive to the DMSO content of the solvent, but exhibits enhanced sensitivity in the solvent composition region of a molar excess of DMSO. The influence of solvent upon the nonbranched ester reaction is attributed to electrostatic effects in the solvent mixtures with a molar excess of water and to a combination of electrostatic and specific solvation effects in solvent mixtures with a molar excess of DMSO.

The dependence of a quantitative structure–reactivity correlation of esters upon solvent was reported in the previous paper of this series.[1] The observed effect on the reaction constants was interpreted in terms of increased importance of steric interference to solvation in aqueous dimethyl sulfoxide relative to aqueous ethanol. The investigation of the alkaline hydrolysis of ethyl acetate[1] and ethyl benzoate[2] in aqueous dimethyl sulfoxide mixtures also revealed a significant solvent effect which was in qualitative agreement with the Hughes–Ingold theory.[3] In addition, the appearance of an activation energy minimum at approximately 0.45 mole fraction of dimethyl sulfoxide was observed with both esters.

In order to gain further insight concerning the observed solvation effects, the importance of various other solvent interaction mechanisms need to be established, particularly anion desolvation[4] and electro-

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(1) D. D. Roberts, *J. Org. Chem.*, **29**, 2714 (1964).

(2) D. D. Roberts, *ibid.*, **29**, 2039 (1964).

(3) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 345–350.

(4) A. J. Parker, *Quart. Rev.* (London), **16**, 163 (1962).