

Spectrometric Measurements.—Ultraviolet and visible spectra were measured on a Cary Model 11 recording spectrophotometer using matched silica cells of path length 1.00 ± 0.01 cm. Pure solvent was used in the reference cell. Solutions of the perchlorate salts were prepared in anhydrous acetonitrile (refluxed and distilled from phosphorus pentoxide). Solutions of 7-dimethyl-

aminoflavylum chloride were prepared using a solvent mixture of methanol-water (50:50). Beckman and Coleman buffer packages were employed to produce buffered solutions having the approximate pH values shown. The solutions were permitted to stand at room temperature for 24 hr. before their spectra were taken so that equilibrium might be established.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGY AND JOHN HARRISON LABORATORY OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA, AND CHEMISTRY DEPARTMENT, DREXEL INSTITUTE OF TECHNOLOGY, PHILADELPHIA, PENNA.]

Mechanistic Investigations of Porphyrin Syntheses. I. Preliminary Studies on *ms*-Tetraphenylporphin¹

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Investigations of the effect of various solvents, metallic cations, anions, temperature, time, and other conditions of reaction upon the homogeneous phase condensation of pyrrole and benzaldehyde to *ms*-tetraphenylporphin have offered considerable insight into the mechanism of this reaction. They also provide a basis for an extended thermodynamic and kinetic study, and also for a relatively rapid, readily purified, large yield reaction for the preparation of this material.

The biological importance of porphyrin-like materials such as hemin, chlorophyll, and vitamin B₁₂, coupled with their unusual and striking physical and chemical properties, make them both interesting and important objects of research. Many biochemical investigations have been devoted to studying the enzyme-catalyzed mechanism of biosynthesis of porphyrins.² An understanding of these biosynthetic reactions would be abetted by a further knowledge of the mechanism of some similar, simpler organosynthetic reactions. Such studies would be of interest in themselves and could also provide the improved routes of synthesis necessary to extended physical chemical and solid state investigations of these materials. With such objects in mind we have carried out preliminary researches on three parent compounds: porphin, tetraazaporphin, and *ms*-tetraphenylporphin (hereafter abbreviated as TPP). The preliminary TPP results are reported here.

TPP has previously been synthesized by the direct condensation of pyrrole and benzaldehyde in sealed tubes, the reaction taking place at 140 to 220° over 24 to 48 hr. and yielding 4 to 5% of TPP with variable amounts of tetraphenylchlorin (TPC) dependent upon temperature, concentration of reactants, etc.³⁻⁶ Addition of zinc acetate to these sealed tube reaction mixtures increases the yield to 10 to 11% (based on pyrrole).⁶ An increase of porphyrin yield in the presence of metallic cations has been noted in a variety of other porphyrin condensation reactions.⁷⁻¹² However, sealed tube TPP condensations are inconvenient for thermo-

dynamic and kinetic investigations for a variety of obvious reasons. Therefore a preliminary study of possible simpler condensation conditions was undertaken.

Results

Rothemund and Menotti found that TPP was slowly formed by refluxing pyrrole, benzaldehyde, and pyridine in methanol for several days at atmospheric pressure.⁴ They also obtained what is now recognized as TPC in admixture with the TPP from this reaction. Therefore, to initiate this present investigation, a number of solvents both with and without various metallic salts were refluxed with pyrrole and benzaldehyde and the time courses of the reactions followed spectrophotometrically using the extinction data of Dorrough, *et al.*^{13,14}

The usual procedure was to add 0.005 mole of redistilled pyrrole (approximately 0.35 ml.) and 0.005 mole of redistilled benzaldehyde (approximately 0.50 ml.) to 250 ml. of either the refluxing solvent or refluxing solvent containing 0.00125 mole of the metallic salt (reagent grade employed without further purification). Aliquots were then drawn at timed intervals and the spectra from 350 to 650 m μ were recorded, after appropriate dilutions, on either a Beckman DK-1 or a Bausch and Lomb Spectronic 505. Spectra in the Soret region were often rerun using a quartz spacer to diminish the usual 1.00-cm. path length to 0.100 cm. Although the yields (based on pyrrole) *vs.* time were routinely calculated from this spectrophotometric data, a few reactions were checked by chromatographic separation and weighing of the dried product. Such controls are necessitated by the frequent presence in the reaction mixtures of both highly scattering materials and by-products spectrally similar to the desired products. The yields obtained by the two methods always agreed to within the expected experimental errors (spectrophotometric yields were usually about 2-3% higher).

Porphyrins, metalloporphyrins, or mixtures of metallo derivatives and free bases or acid salts of porphyrins and chlorins were obtained with the following salt-

(1) This research has been supported by U. S. Army Signal Corps Research Grant DA-SIG-36-039-61-G9 and U. S. Army Research Office (Durham) Grant DA-31-124-ARO(D)-101.

(2) See part II, "Conference on Hemoglobin," Publication 557, National Academy of Sciences-National Research Council, Washington, D. C., 1958, pp. 66-140, for review and further sources. Also see "Porphyrin Biosynthesis and Metabolism," Ciba Foundation Symposium, Little, Brown, and Co., Boston, Mass., 1955.

(3) P. Rothemund, *J. Am. Chem. Soc.*, **61**, 2912 (1939).

(4) P. Rothemund and A. R. Menotti, *ibid.*, **63**, 267 (1941).

(5) S. Aronoff and M. Calvin, *J. Org. Chem.*, **8**, 205 (1943).

(6) R. N. Ball, G. D. Dorrough, and M. Calvin, *J. Am. Chem. Soc.*, **68**, 2278 (1946).

(7) D. W. Thomas and A. E. Martell, *ibid.*, **78**, 1335 (1956).

(8) H. Corwin and V. L. Sydow, *ibid.*, **75**, 4484 (1953).

(9) A. W. Johnson and I. T. Kay, *J. Chem. Soc.*, 2418 (1961).

(10) R. P. Linstead and M. Whalley, *ibid.*, 4839 (1952).

(11) N. Fukada, *Bull. Chem. Soc. Japan*, **34**, 884 (1961).

(12) S. Krol, *J. Org. Chem.*, **24**, 2065 (1959).

(13) G. D. Dorrough, J. R. Miller, and F. M. Huennekens, *J. Am. Chem. Soc.*, **73**, 4315 (1951).

(14) G. D. Dorrough and F. M. Huennekens, *ibid.*, **74**, 3974 (1952).

solvent combinations: $\text{Cu}(\text{CH}_3\text{COO})_2$ in acetic acid, in methanol, in ethanol, in dioxane, in dimethylformamide; CuCl_2 in acetic acid, in benzene; CuSO_4 in acetic acid; $\text{Co}(\text{CH}_3\text{COO})_2$ in acetic acid, in benzene; $\text{Ni}(\text{CH}_3\text{COO})_2$ in acetic acid, in benzene; $\text{Mn}(\text{CH}_3\text{COO})_2$ in acetic acid, in benzene; FeCl_2 in acetic acid; CrCl_2 in acetic acid; $\text{Zn}(\text{CH}_3\text{COO})_2$ in methanol, in acetic acid; $\text{Ca}(\text{CH}_3\text{COO})_2$ in acetic acid; $\text{Ba}(\text{CH}_3\text{COO})_2$ in acetic acid, in benzene; $\text{Pb}(\text{CH}_3\text{COO})_2$ in acetic acid, in methanol; $\text{Mg}(\text{CH}_3\text{COO})_2$ in acetic acid; $\text{Hg}(\text{CH}_3\text{COO})_2$ in acetic acid, in benzene, in methanol; $\text{Ag}(\text{CH}_3\text{COO})$ in acetic acid, in benzene, in dimethylformamide. Products were also detected with the following solvents in the absence of any metallic salts: acetic acid; benzene acidified with monochloroacetic acid, trifluoroacetic acid, sulfuric acid, or phosphoric acid; methanol acidified with acetic acid; acetone acidified with monochloroacetic acid.

For the metallic salt-solvent reactions, yields were generally less than 1%, except for the reactions in acetic acid. Very small yields could frequently be detected by the characteristic Soret excited fluorescence of porphyrins. In the salt-acetic acid reactions, the yields ranged from 1% (Ag) to 35% (Cu). Usually, the alkaline earth metals gave yields less than 10% and the transition metals gave yields greater than 10%. The low yield reactions were frequently accompanied by the heavy deposition of a copious precipitate on the walls of the reaction vessel. The "no salt" reactions, in general, gave yields in excess of 20% to as high as 40%. The reaction is clearly favored by acidic media both in the presence and absence of metallic salts.

All the salt-solvent reactions had the following general characteristics: (1) the yield passed through a maximum, *i.e.*, the concentration of porphyrin increased to a maximum and then began to decrease (this was shown to be an actual loss of product and not just precipitation, etc.); (2) the maximum yield was generally attained in 8-10 hr.; (3) the yield increased linearly with time up to the maximum (from about 2 to 75% of the reaction to maximum yield followed a linear time course, giving zero-order kinetics for the formation of porphyrin in this phase of the reaction); (4) the relatively slow appearance of Soret absorption was always preceded by the rapid appearance (often within a few minutes in acid media) of absorption bands in the region of 475-500 $\text{m}\mu$ and the intensity of this "intermediate" band always decreased proportionately with time as the Soret band increased in intensity; (5) chlorin was generally observed either during the course of reaction or as a product admixed with porphyrin; (6) the loss of porphyrin after maximum yield did not follow any simple order behavior; (7) in several experiments, particularly those with the transition metal salts, reproducibility of a given maximum yield was frequently quite erratic; *e.g.*, cupric acetate-acetic acid yields varied from 18 to 35% over five experiments. Despite these occasional erratic results in the reproducibility of the maximum yield for the salt-solvent reactions, however, the zero-order rates of formation of product were quite reproducible. The "no salt" reactions gave the same characteristics as the salt-solvent reactions, except that the yield did not decrease after reaching its maximum value and was

reproducible, in contrast to the frequently erratic "maximum" yields for the salt-solvent reactions. The "no salt" reactions are therefore generally preferable for preparative work.

Following the lines indicated by these preliminary experiments, several further studies were made. First, further quantitative checks were made upon the spectrophotometric yield. Using the reaction conditions and concentrations described above, the cupric acetate-acetic acid mixture was allowed to react for 6 hr., giving an observed spectrophotometric yield of 30% at the time the reaction was stopped. The solvent was removed by vacuum distillation and the solid residue was Soxhlet extracted with water for 12 hr. to remove water-soluble copper compounds (eluate gave no visible reaction with ammonia after 12 hr.). The remaining solid material was then dried and a copper analysis performed on this crude material, giving a 28% yield based on the percentage of copper in the total weight of the residue.¹⁵ The remaining residue was then chromatographed on Florex with chloroform (Florex is an extruded fuller's earth manufactured by the Floridan Co., Inc., Warren, Pa.). The yield calculated from the weight of the dried Cu-TPP thus obtained was 25%. A yield of Cu-TPC amounting to another 4% based upon pyrrole was also obtained. The chromatographed Cu-TPP gave 99.4% of its expected percentage of Cu (% Cu: expected, 9.40%; observed, 9.35% (average of 3 samples)).

Similarly, a "no salt"-acetic acid condensation was checked. Refluxing was continued for 10 hr. at which point a spectrophotometric yield of 41% was obtained. Solvent was removed under vacuum and the solid residue dissolved in chloroform and then chromatographed with chloroform on Florex. The porphyrin came through the column first, followed by a small band of chlorin, leaving a black residue on the top of the column. The porphyrin fraction was collected, solvent removed, dried under vacuum, and the resulting material weighed; yield 301 mg. of TPP or 38% based upon pyrrole. The yield of the chromatographically separated TPC was spectrophotometrically obtained as 1.7% based on pyrrole. The spectrum of the chromatographed TPP in pyridine was identical in extinction and peak position with that given in the literature for the free base.¹³ Thus, to within the experimental errors, it can be seen that the spectrophotometric yield is a reasonable quantitative measure of the total porphyrin material produced.

A series of kinetic studies was then undertaken, using the previous reaction conditions and concentrations, and the spectrophotometric yield *vs.* time to calculate k_0' , the observed zero-order rate of formation of porphyrin. The temperature was uniformly maintained to within 1° by using oil baths, instead of the previously employed heating mantles, and also by the use of magnetic stirring both in the bath and the reaction vessel. The results are shown in Table I and the data for the cupric acetate-acetic acid reaction at different temperatures are also shown graphically in Fig. 1.

The acidified benzene and acetone reactions in Table I contained 10 g. of acid/250 ml. of solution. Pyrrole

(15) This analysis was performed by Mr. J. L. Harris using a modified EDTA titration method developed in this laboratory for metalloporphyrin analyses. The material was first decomposed with hot nitric acid and two independent samples were taken. The reproducibility was $\pm 1/4\%$.

TABLE I
OBSERVED ZERO-ORDER RATE CONSTANTS OF FORMATION

Solvent	Salt	T, °C.	k_0' , moles l. ⁻¹ sec. ⁻¹
Acetic acid	None	22	1.3×10^{-9}
Acetic acid	Cu(CH ₃ COO) ₂	22	1.3×10^{-9}
Acetic acid	Cu(CH ₃ COO) ₂	60	1.3×10^{-8}
Acetic acid	Cu(CH ₃ COO) ₂	93	6.0×10^{-8}
Acetic acid	Cu(CH ₃ COO) ₂	118	1.3×10^{-7}
Acetic acid	None	118	1.1×10^{-7}
Acetic acid	CuSO ₄	118	1.0×10^{-7}
Acetic acid	CuSeO ₄	118	1.0×10^{-7}
Acetic acid	CuBr ₂	118	0.6×10^{-7}
Acetic acid	CuCl ₂	118	1.2×10^{-7}
Acetic acid	Pb(CH ₃ COO) ₂	118	0.3×10^{-7}
Acetic acid	Mg(CH ₃ COO) ₂	118	0.3×10^{-7}
Acetic acid	Ba(CH ₃ COO) ₂	118	0.5×10^{-7}
Acetic acid	Co(CH ₃ COO) ₂	118	1.0×10^{-7}
Methanol	Zn(CH ₃ COO) ₂	64	1.0×10^{-9}
Benzene-ClCH ₂ COOH	None	80	5.0×10^{-8}
Benzene-CF ₃ COOH	None	80	1.0×10^{-9}
Acetone-ClCH ₂ COOH	None	57	5.0×10^{-11}

and benzaldehyde refluxed in unacidified benzene for 48 hr. gives no porphyrin products, but the yields in acidified benzene were about 35-40%.

Inspection of the results in Table I shows that all the acetic acid results at 118° are almost identical, k_0' only spanning a fourfold range. Therefore, although the nature of the solvent itself and the "acidity" of the solvent strongly affect the reaction, variation of anions and cations for the metallic salt-solvent reactions seem only to affect k_0' to a secondary extent in a given solvent. In fact, to within the experimental errors the cupric acetate-acetic acid and "no salt"-acetic acid reactions give identical formation kinetics.

Therefore observed activation parameters may be calculated for these two reactions from the temperature data given. The enthalpy of activation calculated from these results is $\Delta H^* = 15 \pm 1$ kcal./mole and the observed entropy of activation is $\Delta S^* = -60 \pm 5$ e.u. In order to check that the zero-order rate might reflect surface catalysis due to the glass, the room temperature cupric acetate-acetic acid reaction was rerun in a polyethylene vessel; the zero-order rate observed was 1.0×10^{-9} mole liter⁻¹ sec.⁻¹ at 22°. To within our expected experimental errors this rate is identical with the previous data and therefore surface catalysis does not seem to be of primary importance in the formation reaction.

A "no salt" reaction was next studied at reflux in formic acid, both a strong acid and a strong reductant. Shortly after the initiation of the reaction, an "intermediate" spectrum with a large peak at 488 m μ and shoulders at 460 and 550 m μ was obtained. As the reaction continued this material disappeared, without any evidence for the formation of either chlorin or porphyrin material accompanying its decomposition. Eventually the solution became colorless.

A cupric acetate-acetic acid reaction was then run at reflux while compressed air was slowly bubbled through the reaction mixture. This was done in order to ascertain the effect of an enhancement of the oxidizing atmosphere on the course of the reaction. The maximum yield reached was only 0.02% and after 24 hr. of reaction the Cu-TPP formed had practically all but disappeared.

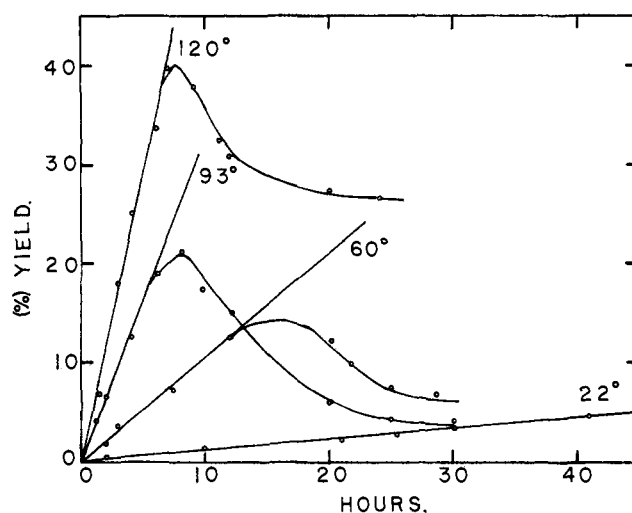


Fig. 1.—Time course of cupric acetate-acetic acid TPP condensation at 22, 60, 93, and 120°. To preserve clarity of presentation, all data taken within the first hour at each temperature have been excluded and also the data beyond 45 hr. for the 22° reaction. The 22° reaction reaches a maximum of 10% at 90 hr. and then begins to decrease very slowly in yield with time. The yield given is the spectrophotometric yield.

The acidified benzene reactions afforded an opportunity to establish that water was one of the products in the over-all thermodynamic reaction. The solvent was thoroughly dried over activated alumina for 1 week and then the monochloroacetic acid-benzene reaction was rerun at reflux with freshly distilled reagents and with a capillary Dean-Stark trap attached to the condenser. A yield of 50% (the highest ever obtained for a TPP condensation) was obtained with 36 hr. of reaction. Water amounting to 108% of that expected for this 50% yield based on 7 H₂O/TPP (see below) was collected and identified in the trap. The calculation allows for the solubility of water in benzene.¹⁶ Approximately 80% of this collected water was trapped within the first 20 min. of reaction, thus coinciding with the formation of the 480 m μ "intermediate." Therefore the water in excess of the theoretically expected amount could be accounted for by the fraction of "intermediate" that forms products other than porphyrin. This water also amounts to 95% of that expected for this 50% yield based on 7 benzaldehyde/TPP and therefore the identity of the oxidant in the reaction is equivocal.

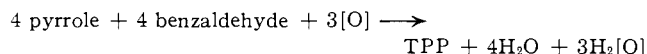
Another experiment bearing on the chemistry of the reaction was carried out to ensure that the 480 m μ "intermediate" was not the "polypyrrole" usually obtained when pyrrole stands in acidified solution¹⁷; 0.005 mole of freshly distilled pyrrole was refluxed for 10 hr. in 250 ml. of acetic acid and a spectrum taken of the resulting material. It gave absorption peaks at 480 and also at 435 and 410 m μ . However, the extinction under comparable dilutions was $1/100$ to $1/500$ of that of any of the reaction mixtures. As the composition and structure of this material have never been established, a molar extinction coefficient cannot be calculated. It should be noted that addition of cupric acetate to this material did not appreciably alter its spectrum. However, the results obtained strongly

(16) A. S. Seidel, "Solubilities," 2nd Ed., D. Van Nostrand Co., Inc., New York, N. Y., 1919, p. 745.

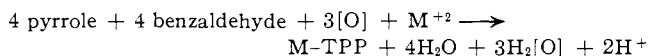
(17) A. A. Morton, "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1961, p. 66.

indicate that the reaction "intermediate" is not the usually acid-generated "polypyrrole."

Discussion.—The over-all thermodynamic reaction with the simplest stoichiometry that can be written in the absence of metallic salts is



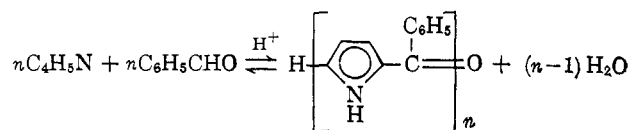
while the simplest over-all formation reaction in the presence of metallic salts is



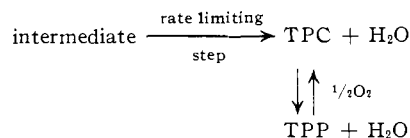
where [O] represents 2 oxidizing equivalents of any suitable oxidant and the reactions are considered as essentially irreversible. While [O] might be identified with either benzaldehyde or $1/2\text{O}_2$ on the basis of the experiment with the Dean-Stark trap, we consider $1/2\text{O}_2$ the more likely reactant species. Since the supply of O_2 would be diffusion limited to different extents in different experiments (dependent upon dimensions of the apparatus used, etc.), such an identification would explain the variable ratios of chlorin to porphyrin frequently observed in several otherwise identical or similarly performed syntheses.

As the 480 $m\mu$ absorbing species decreases in concentration proportionately as the porphyrin concentration increases, it has been assumed that it represents a reaction intermediate. On the basis of its spectral characteristics we have tentatively identified it as a mixture of polypyrrolylphenylmethanes of varying chain lengths and degrees of oxidation.^{18,19} An acid-catalyzed reaction of pyrrole and benzaldehyde to form such products and water seems very reasonable. Further speculations on such reactions are best left until further experimental observations are available.

The following reaction scheme is therefore postulated for the formation reaction in the absence of metallic salts



and for the case $n = 4$ in the presence of oxygen



This proposed mechanism would explain both the kinetic and chemical observations, including the identical kinetic behavior in the presence and absence of metal salts. Closure of the straight chain into the macrocyclic ring of the product with elimination of water would be expected to be rate limiting and therefore could yield a pseudo-zero-order reaction of the characteristics obtained. Since this closure represents a relative increase in order of this system, it is in keeping with the observed negative change in the entropy of activation (*i.e.*, $\Delta S^* = -60$ e.u.) for rate of formation of porphyrin. Chain tetrapyrrole cyclizations to

porphyrins have been previously described.^{9,20} The conversion of Cu-TPC to Cu-TPP with atmospheric oxygen, in particular, has also been reported.^{21,22} It is of interest to note in this connection that purification of our products by prolonged Soxhlet extractions, rather than chromatography, has frequently led to a conversion of the chlorin present to porphyrin. Of further interest is the observation that this oxidation is accelerated in sunlight and therefore shows the expected photosensitivity.²²

The mechanism proposed for the formation of porphyrin products in the presence of metallic cations is essentially that above, except that the metallo-TPC and metallo-TPP are among the observable species present. One might anticipate that metallic cations could promote the rate-limiting ring closure reaction by chelation of the intermediate species into a favorable steric configuration. This does indeed seem to be the case, as indicated by the fact that the zero-order constant is dependent to some extent upon the particular cations present and, in fact, is fastest for the cupric ion, a species known to form square-planar complexes reasonably readily. Similar conclusions were drawn in a previous study.⁹

However, it should be noted that the formation reaction is equally fast in the absence of any metallic cations and the yields are both reproducible and higher. Therefore, interestingly enough, the over-all effect of metallic cations seems to be to decrease the yield of the over-all reaction. Such a decrease in yield could arise from several sources. For example, metallic cations could decrease the yield by precipitating intermediates from the homogeneous phase where the reaction seems to occur. There is some evidence for such precipitation reactions.

In view of the fact that metalloporphyrins are known to serve as oxidation-reduction catalysts in biological systems, a more likely explanation, particularly for the transition metals, of the decrease in yield with metallic cations might be the autocatalytic oxidative destruction of metalloporphyrin. The presence of such a "destruction" reaction would explain several observed features of the metallic cation experiments, such as the lack of simple order behavior beyond the maximum yield and, also, the variability of the maximum yield. The maximum yield would occur when the rate of formation of porphyrin equalled its rate of autocatalytic oxidative destruction. This latter, on the other hand, would depend upon the oxygen supply, which being diffusion limited would vary from experiment to experiment dependent upon rate of reflux, dimensions of the apparatus, etc. Such circumstances would give the observed reproducible rate of formation with a variable maximum yield and a complex rate of loss of product after maximum. Two further observations support this suggestion. Firstly, the higher the maximum yield, generally, the higher the ratio of chlorin to porphyrin in the cupric acetate-acetic acid experiments run at reflux, indicating a decreased oxygen content during a reaction resulting in a relatively higher yield. Secondly, the higher the maximum yield, the faster the rate of initial rate of decrease of porphyrin after maximum as would be expected in a case of auto-

(18) C. H. Gray, "The Bile Pigments," Methuen, London, 1953, p. 26 ff.

(19) R. Lemberg and J. W. Legge, "Hematin Compounds and Bile Pigments," Interscience Publishers, Inc., New York, N. Y., 1949, p. 95 ff.

(20) A. H. Corwin and E. C. Coolidge, *J. Am. Chem. Soc.*, **74**, 5196 (1952).

(21) M. Calvin, R. H. Ball, and S. Aronoff, *ibid.*, **65**, 2259 (1943).

(22) F. M. Huennekens and M. Calvin, *ibid.*, **71**, 4031 (1949).

catalysis. This suggestion is also further supported by the "bubbled air" cupric acetate-acetic acid experiment. Clearly, however, the interpretation of a given reaction, particularly the temperature dependence, in such circumstances as postulated would be a very complicated affair, as oxidation reactions occur at several steps in the proposed mechanism. Therefore one can only conclude that the above is a reasonable explanation of the observations for the case of the reactions in the presence of metallic salts.

The decreased yield of porphyrin in the presence of metallic salts of these acidic condensations carried out

in a relatively oxidizing atmosphere indicates that there are essential differences in the mechanism of these reactions and those previously observed to show an increase of yield in the presence of metallic cations under other conditions. For example, such cation-increased yields were observed either in relatively high temperature melts,^{8,11} basic media,^{8,10} relatively reducing atmospheres under pyrolytic conditions,^{6,7} or in the presence of free-radical generators.¹² The continued and extended physical chemical and kinetic investigations of these reactions will prove both interesting and informative.

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Mass Spectrometry in Carbohydrate Chemistry. Diethyl Dithioacetal and Dithioacetal Peracetates

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The mass spectra of various monosaccharide diethyl dithioacetal peracetates and of D-fructose diethyl dithioacetal pentaacetate are discussed. A detailed interpretation of the fragmentation processes which these molecules undergo is presented; it is supported by the mass spectra of analogous peracetates prepared with acetic anhydride-*d*₆. The molecular weight can readily be obtained from the molecular ion peak. It is possible to relate the characteristics of the mass spectra to the position of deoxy groups in these molecules and to distinguish between aldoses and ketoses.

Introduction

The potential importance of mass spectrometry as a technique for structure determination in the carbohydrate field has recently been demonstrated. The mass spectra of the peracetates of hexoses and pentoses¹ and their partially O-methylated derivatives,² of the O-isopropylidene derivatives of hexoses and pentoses,³ and of various carbohydrate methyl ethers^{4,5} have been interpreted in terms of molecular weight, ring size, variation in the type and degree of substitution, ketose or aldose structure, and stereochemistry. The structure of a newly isolated di-O-isopropylidene derivative of D-galactose was determined by applying this technique.³

Dithioacetal derivatives are very useful for the characterization of monosaccharides because of their stability and ease of preparation. They also are useful intermediates for the preparation of acyclic derivatives of sugars. The peracetates of these compounds, which usually are crystalline, are sufficiently volatile and thermally stable to be introduced directly into a conventional inlet system of the mass spectrometer.

Since the diethyl dithioacetal peracetates are monosaccharide derivatives in the acyclic structure, their mass spectra need not be interpreted in terms of ring size and exhibit only minor intensity differences with a change in stereochemistry. Structural differences which express themselves in the size of substituents and of the entire molecule and in the position of the carbonyl group lead to significant variation in their mass

spectra; this is borne out in the following discussion by a comparison of the mass spectra of the corresponding diethyl dithioacetal peracetate derivatives of hexoses, pentoses, deoxyhexoses, and of a diethyl dithioacetal hexose pentaacetate.

The mass spectra of typical representatives of these derivatives are presented and interpreted. The fragmentation schemes proposed have been substantiated by the mass spectra of deuterated analogs, obtained by acetylation with anhydride-*d*₆. Perhaps the most valuable aspect of these mass spectra is the presence of a sizable molecular ion peak which allows direct determination of the molecular weight. In all the mass spectra of carbohydrate derivatives published previously, the molecular weight must be determined from a fragment peak resulting from the loss of a substituent on a carbon atom of the pyranose or furanose ring^{1,2,4,5} or from the loss of a methyl group from an O-isopropylidene ring.³

The mass spectra of D-arabinose diethyl dithioacetal tetraacetate (I, Fig. 1) and its *d*₁₂-analog (II, Fig. 2) will be discussed in detail to elucidate the fragmentation processes characteristic of this class of compound. This shall be followed by a discussion of the influence of adding a -CH₂OCOCH₃ group to form a ketohexose or an aldohexose and of adding a CH₂- group to form a 2- and a 6-deoxyaldohexose.

D-Arabinose Diethyl Dithioacetal Tetraacetate (I, Fig. 1).—The peak at *m/e* 424(436)⁶ corresponds to the molecular weight of this compound. Elimination of a molecule of acetic acid¹ from the molecular ion forms fragment 364(373). The peak at *m/e* 363(375) arises from the fission of a carbon-sulfur bond in the molecu-

(1) K. Biemann, D. C. DeJongh, and H. K. Schnoes, *J. Am. Chem. Soc.*, **85**, 1763 (1963).

(2) D. C. DeJongh and K. Biemann, *ibid.*, **85**, 2289 (1963).

(3) D. C. DeJongh and K. Biemann, *ibid.*, **86**, 67 (1964).

(4) N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Zolotarev, *Tetrahedron*, **19**, 2209 (1963).

(5) K. Heyns and H. Scharmann, *Ann.*, **667**, 183 (1963).

(6) Throughout this article, the *m/e* assignments are followed by parentheses containing the location of the peak in the mass spectrum of the deuterated analog, when known.