Pyrolysis Studies. Controlled Thermal Degradation of Mesoporphyrin[1]

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Levy and co-workers[2] found that pyrolysis of porphyrins at 800–900° caused extensive fragmentation of the porphyrin nucleus, as indicated by the products reported: acetonitrile, ethanol, propionitrile, and an assortment of simple hydrocarbons. We have investigated thermal decomposition of the porphyrin nucleus under less drastic conditions than those previously reported. The major organic products obtained from thermal decomposition of mesoporphyrin at several temperatures over the range 400–780° were the various alkylpyrroles summarized in Table I. In addition to alkylpyrroles, small amounts of acetonitrile and propionitrile were obtained as well as moderate yields of methane, ethylene, and ethane. The yield of hydrocarbons and nitriles increased with temperature. Thermal decomposition products of mesoporphyrin at lower temperatures (400–600°) were the same as those formed in reductive degradation.[3] These characteristic products—pyrroles **6**, **7**, **9**, and **10**, formed by cleavage of the porphyrin at the methene bridge positions only—amounted to 92% of the alkylpyrroles formed at 410°. As the temperature of pyrolysis was elevated, the yield of other less characteristic alkylpyrroles such as the various mono-, di-, tri-, and tetramethylpyrroles and pyrrole itself, increased. Figure 1 shows the effect of temperature on the relative amounts produced of the four characteristic pyrroles. Total yields of the various alkylpyrroles are also plotted as a function of temperature. Relative amounts of volatile products increased rapidly with temperature, while that of alkylpyrroles reached a maximum at about 550°. The best yield of alkylpyrroles obtained was about 1.4 moles of alkylpyrrole/mole of porphyrin pyrolyzed. This amounts to an approximately 50% yield since pyrolysis of 1 mole of mesoporphyrin, carried out in an inert medium, can lead to 2.6–2.9 moles of alkylpyrroles. This range of values is obtained by considering the hydrogen required to go from the porphyrin to the more reduced pyrroles. Nonvolatile residues remaining in the pyrolysis chamber and tubing preceding the column consisted mostly of carbon. Some mesoporphyrin was converted to etioporphyrin by decarboxylation and deposited on cooler portions of the pyrolysis chamber. Spectral examination of the residue (ultraviolet and visible) failed to show any dipyrrylmethanes or rearranged porphyrins that might be suspected as possible intermediates in pyrrole formation.

TABLE I
ALKYLPYRROLE PRODUCTS OF MESOPORPHYRIN PYROLYSIS

		Moles of product/mole of 3-ethyl-4-methylpyrrole					
		Temp., °C.					
No.	Compd.	420	475	510	570	630	770
1	Pyrrole	0.01	0.01	0.02	0.03	0.10	0.13
2	3-Methylpyrrole	0.02	0.02	0.07	0.08	0.15	0.19
3	2-Methylpyrrole			0.08	0.07	0.18	0.26
4	Dimethylpyrroles[a]	0.16	0.24	0.41	0.39	0.53	1.02
5	Trimethylpyrroles[a]	0.08	0.11	0.11	0.12	0.39	0.62
6	3-Ethyl-4-methylpyrrole ("opsopyrrole")	1.00	1.00	1.00	1.00	1.00	1.00
7	2,4-Dimethyl-3-ethylpyrrole pyrrole ("cryptopyrrole")	0.56	0.62	0.69	0.74	0.51	0.46
8	Tetramethylpyrrole	N.d.[b]	N.d.	N.d.	N.d.	0.22	0.69
9	2,3-Dimethyl-4-ethylpyrrole ("hemopyrrole")	0.75	0.88	0.92	0.86	0.76	0.53
10	2,3,5-Trimethyl-4-ethylpyrrole ("phyllopyrrole")	0.70	0.66	0.63	0.56	0.55	0.45

[a] Isomers not separated. [b] Not detected.

Results of mesoporphyrin pyrolysis were reproducible and independent of sample size over the range studied (0.5–20 mg.). Best results were obtained in the temperature range 420–580° where runs could be duplicated with a precision of 5–8%. At higher temperatures results were considerably less reproducible. To investigate the possibility of incomplete degradation, especially at higher temperatures, modified experiments were carried out to check the procedure. A gold baffle was inserted between sample and column to provide a longer path through the pyrolysis hot zone. The baffle caused a less characteristic pyrolysis at temperatures above 550°. At lower temperatures the gold baffle appeared to effect little change in the pattern of thermal decomposition. To check product stabilities, samples of 2,4-dimethyl-3-ethylpyrrole were pyrolyzed over the same temperature range as mesoporphyrin. At temperatures above 560° this compound

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[1] This paper represents results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif., under Contract NAS-7-100, sponsored by the National Aeronautics and Space Administration.

[2] R. L. Levy, H. Gesser, E. A. Halevi, and S. Saidman, *J. Gas Chromatog.*, **2**, 254 (1964).

[3] H. Fischer and H. Orth, "Die Chemie des Pyrrols," Vol. 2, part 1, Akademie-Verlagsgesellschaft, Leipzig, 1937, p. 48; A. S. Holt, D. W. Hughes, H. J. Kende, and J. W. Purdie, *J. Am. Chem. Soc.*, **84**, 2835 (1962).

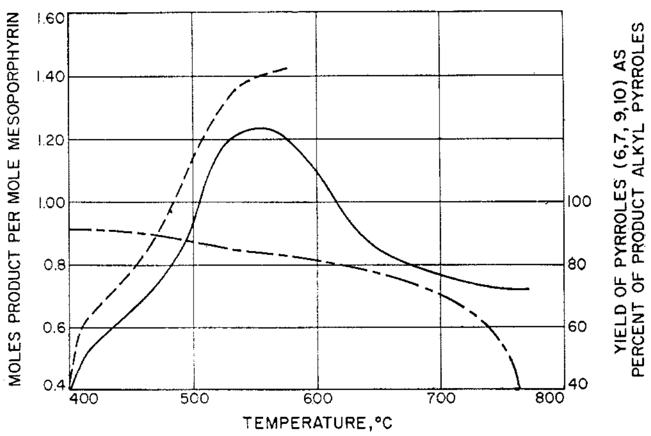

Figure 1.—Pyrolysis of mesoporphyrin as function of temperature: ----, moles of organic products detected (approximate) per mole of mesoporphyrin pyrolyzed; ———, moles of alkylpyrrole (total, compounds **1–10**) formed per mole of mesoporphyrin pyrolyzed; — — —, per cent of pyrroles **6**, **7**, **9**, and **10** (characteristic products) of total alkylpyrroles produced.

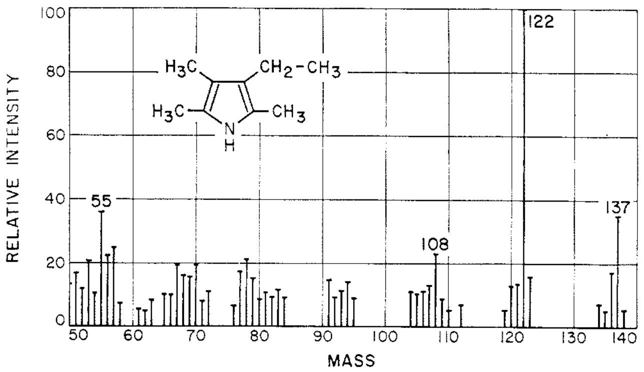

Figure 2.—Mass spectrum of 2,3,5-trimethyl-4-ethylpyrrole.

gave considerable amounts of dimethylpyrrole and monomethylpyrrole.

We also studied pyrolysis of mesoporphyrin in a sealed tube.[4] Samples were sealed in tubes both under vacuum and in hydrogen (450–500 torr at 20°) and heated at 400° for extended periods (*ca.* 1 hr.). The residue remaining in the tube was analyzed by gas chromatography and the gaseous products were analyzed by mass spectrometry. No effects attributable to hydrogen were observed. Products of the sealed tube pyrolysis at 400° were the same as those produced by pyrolysis in dynamic systems at the same temperature.

Mass spectra of several alkylpyrroles have been reported.[5] In the course of this investigation the mass spectra of additional alkylpyrroles were examined. The features mentioned by Budzikiewicz, *et al.*,[5] were also observed with these pyrroles. For C-methylpyrroles such as 2,3,5-trimethylpyrrole and 2,3,4,5-tetramethylpyrrole, the M − 1 peak is the most intense with only a moderate M − 15 peak. For pyrroles having C-ethyl substituents, 3-ethylpyrrole, 2,3-dimethyl-4-ethylpyrrole, and 2,3,5-trimethyl-4-ethylpyrrole (Figure 2), the base peak is at M − 15, presumably from loss of methyl from the ethyl group. From these differences in mass spectra it was easy

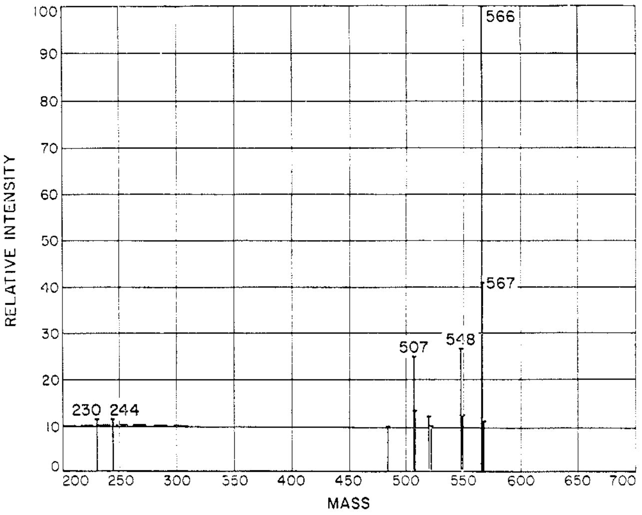

Figure 3.—Mass spectrum of mesoporphyrin IX.

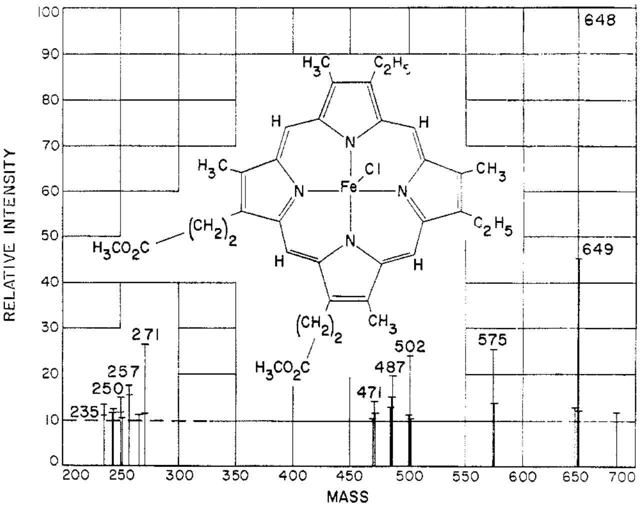

Figure 4.—Mass spectrum of ferric mesoporphyrin IX chloride dimethyl ester.

to distinguish between such isomeric pairs as 2,3,4,5-tetramethylpyrrole and 2,4-dimethyl-3-ethylpyrrole. Mass spectra of isomers having the same types of alkyl substituents are essentially identical and cannot be used to distinguish among the different choices.

Direct solid introduction systems, recently available for mass spectrometers, have provided a means of analysis for porphyrins and other high molecular weight materials. The spectra of mesoporphyrin IX (**11**) (Figure 3) and ferric mesoporphyrin IX chloride dimethyl ester (**12**) (Figure 4) were obtained using a direct introduction system.[6] These spectra are similar to previously reported spectra of nickel and copper etioporphyrins.[7] Relatively high stability of porphyrin positive and double positive ions gives rise to little fragmentation of the porphyrin nucleus. Chloride ion is readily lost from **12** (few fragment peaks contain chloride), but the relative intensity of the peak at 648 (M − Cl) indicates that the iron porphyrin cation may be even more stable than those of metal-free porphyrins. The most prominent fragment peaks in the spectrum of **12** are those formed by cleavage of the

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[4] We wish to thank Dr. E. T. Roux for assistance in this portion of the investigation.

[5] H. Budzikiewicz, C. Djerassi, A. H. Jackson, G. W. Kenner, D. J. Newman, and J. M. Wilson, *J. Chem. Soc.*, 1949 (1964).

[6] We wish to thank Mr. Martin Elliott of Associated Electronics Industries, Ltd., for running these samples.

[7] Hood, Carlson, and O'Neal, "Encyclopedia of Spectroscopy," Vol. I, Clark, Ed., Reinhold Publishing Corp., New York, N. Y., 1960, p. 616.

ester at the β-position with loss of one and two $CH_2CO_2CH_3$ units to give peaks at M − 73 and M − 146, respectively. These peaks amount to about 25% each of the base peak. These data may be compared with spectra obtained for similarly substituted pyrroles[8] (obtained by pyrolysis of mesoporphyrin dimethyl ester) where the M − 73 peak is the base peak and the molecular ion is only 20–30% as intense.

The molecular ion is the base peak in the mass spectrum of mesoporphyrin IX. The most intense fragment peaks are those at M − 18 and M − 59 corresponding to loss of water and $-CH_2COOH$, respectively. Doubly charged ion peaks are also observed, but no prominent peaks attributable to fragmentation of the porphyrin ring into pyrrole subunits have been detected.

The results of this work emphasize the utility of controlled pyrolysis as an adjunct to direct mass spectrometry. The molecular formula for a complex organic compound can often be obtained simply from a careful analysis of its high-resolution mass spectrum. The high-resolution mass spectrum of mesoporphyrin gives molecular weight and molecular formula; the fragmentation pattern indicates the high stability of the compound. Controlled pyrolysis selectively degrades the porphyrin into pyrrole subunits which can be readily identified and used in determining the structure of the parent porphyrin. Whereas oxidative and reductive degradation procedures require 50–250 mg. of material,[9] complete analysis by pyrolysis and mass spectrometry can be accomplished with as little as 1–2 mg. of porphyrin. Techniques developed with mesoporphyrin are being extended to other porphyrins and their metal complexes. Preliminary results indicate that selective cleavage to pyrroles occurs over the same temperature range for a wide variety of metal-free porphyrins and chlorins.

Experimental Section

Materials.—Mesoporphyrin was prepared from commercial ferric protoporphyrin IX chloride by alkaline hydrogenation.[10] Mesoporphyrin dimethyl ester and its ferric complex were prepared by the procedure of Erdman and Corwin.[11] Porphyrin compounds were analyzed by electronic absorption and mass spectrometric methods. The identity and purity of pyrrole standards were checked by n.m.r. and gas chromatographic analysis.

Pyrolysis–Gas Chromatography.—The sample to be pyrolyzed was weighed into a thin-walled, quartz capillary tube (*ca.* 1 in. in length). The sample tube was placed in a stainless steel U tube (1/8 × 6 in., 0.012-in. wall) with the open end of the quartz tube at the bottom of the U tube. The position of the sample tube was fixed by an open quartz tube, placed above it. The U tube containing the sample was connected to a Loenco heated sample valve and pyrolyzer assembly. The sample valve was connected directly to the injection port of a Loenco Model 70 Hi-Flex dual-column gas chromatograph. The heat-sink furnace of the assembly was placed over the U tube for 2 min. and the sample was pyrolyzed in a stream of helium flowing at 50 cc./min. Pyrolysis products were separated satisfactorily on columns of silicone gum XE-60 or Carbowax 20M on Chromosorb G support. Stainless steel and glass columns (6 ft. × 0.25 in.) were used interchangeably. Columns were operated isothermally at temperatures of 50–200°. No additional products were detected by continuing elution for several hours at 200°. A hot-wire thermal-conductivity detector was calibrated with standard pyrroles: 2,3-dimethylpyrrole, 2,4-dimethylpyrrole, and 2,4-dimethyl-3-ethylpyrrole. A plot of detector response *vs.* alkylpyrrole molecular weight was used to obtain quantitative values for the pyrolysis products detected. Separated products were trapped, according to their volatility, in liquid nitrogen cooled traps equipped with high-vacuum stopcocks or, in the case of most alkylpyrroles, in looped capillary tubes immersed in liquid nitrogen or Dry Ice–acetone.

Mass Spectra.—Mass spectra of chromatographic fractions were obtained with a Consolidated Electrodynamics Corp. mass spectrometer no. 21-103C. Fractions of high volatility were analyzed using a metal and glass inlet system. The less volatile alkylpyrroles, usually oils or solids, were introduced through an all-glass inlet system heated to 230°. The ionizing energy was 70 e.v. and the ionizing current 10–50 μa. Porphyrin mass spectra were obtained on an Associated Electrical Industries, Ltd., mass spectrometer, Model No. MS-9.[6]

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[8] D. G. Whitten and K. E. Bentley, unpublished results.

[9] J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., New York, N. Y., 1962, p. 152.

[10] E. W. Baker, M. Ruccia, and A. H. Corwin, *Anal. Biochem.*, **8**, 512 (1964).

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Perchloric Acid Catalyzed Acylations. Enol Lactonization and Enol Acetylation of Steroids[1]

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The use of perchloric acid as catalyst for enol acetylation was first described by Barton, *et al.*,[2] who used carbon tetrachloride as solvent and obtained good yields of 17(20)-enol acetates. Subsequently, other workers[3] employed this system for the enol acetylation of 3-keto steroids and related compounds. Perchloric acid catalyzed acetylation by acetic anhydride in ethyl acetate has been used by Schenk and Fritz[4] in a method for the quantitative determination of alcohols, phenols, thiols, and amines, and Whitman and Schwenk[5] used perchloric acid with acetic anhydride and acetic acid for the acetylation of hydroxy steroids.

The enol lactonization of steroidal 4-nor-3,5-seco-5-oxo-3-oic acids (**1**) is an essential step in the synthesis of 3- or 4-^{14}C-labeled steroids[6] as well as of 4-hetero steroids.[7] The conversion of δ-keto acid **3** to enol lactone **4** is an essential step in a recent total synthesis of steroids.[8] Other workers have employed refluxing acetyl chloride–acetic anhydride or acetic anhydride–sodium acetate for this conversion with varying degrees of success.[9]

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[5] B. Whitman and E. Schwenk, *J. Am. Chem. Soc.*, **68**, 1865 (1946).

[6] A. Murray and D. L. Williams, "Organic Syntheses with Isotopes," part I, Interscience Publishers, Inc., New York, N. Y., 1958, p. 1051.

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[9] (a) R. B. Turner, *J. Am. Chem. Soc.*, **72**, 579 (1950); (b) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, *ibid.*, **74**, 4223 (1952); (c) M. Gut, *Helv. Chim. Acta*, **36**, 906 (1953).