Anal. Calcd. for $C_{11}H_8O_4NI_3$: C, 22.04; H, 1.35. Found: C, 21.66, 21.74; H, 1.61, 1.42.

Anhydro 2-Acetylamino-3,5-diiodobenzoic Acid (Procedure No. 5).—2-Amino-3,5-diiodobenzoic acid (57.8 g.) was refluxed 30 minutes with 260 ml. of acetic anhydride containing 3 drops of sulfuric acid. Upon cooling, the anhydro form of 2-acetylamino-3,5-diiodobenzoic acid separated in large crystals; weight 58.5 g. (95% yield), m.p. 196–197.5°. Recrystallized from acetic acid, the product melted over a range from 197–214° but after crystallizing again from acetic anhydride it melted at 199–201° (dec.). It is probable that crystallization from acetic acid partially opens the ring of the inner anhydride. The material from acetic anhydride was analyzed.

Anal. Calcd. for $C_9H_5O_2NI_2$: C, 26.2; H, 1.20. Found: C, 26.35, 26.06; H, 1.50, 1.53.

2-(*n*-Caproylamino)-3,5-diiodobenzoic Acid (Procedure No. 6).—2-Amino-3,5-diiodobenzoic acid (9.7 g.) was mixed with 50 ml. of dioxane and 8 g. (8.4 ml.) of *n*-caproyl chloride and refluxed two hours. Hydrogen chloride ceased to be liberated after 1.5 hours. The mixture was then diluted with 50 ml. of water, warmed to 70–80° and sodium hydroxide solution was added, maintaining the mixture alkaline to phenolphthalein until all of the solid had dissolved (12 min.). The solution was diluted with 150 ml. of water, treated with decolorizing carbon, filtered again and precipitated hot (65–70°) from a volume of 6 l. by adding dilute hydrochloric acid to *p*H 4. The solid was filtered from the hot solution, washed and dried at 70°; weight 11.1 g. (90.5% of theory) melting at 205.5–206.5° (dec.).

Anal. Calcd. for $C_{13}H_{15}O_3NI_2$: C, 32.00; H, 3.08; neut. equiv., 487. Found: C, 32.30, 32.59; H, 3.19, 3.51; neut. equiv., 482.

2-Acetylamino-3,5-diiodobenzoic Acid (Procedure No. 7).—Ten grams of anhydro 2-acetylamino-3,5-diiodobenzoic acid was stirred with 50 ml. of dioxane and 50 ml. of water at room temperature and sodium hydroxide solution (35° Bé) was added drop by drop to maintain the mixture alkaline to phenolphthalein. The mixture consumed 3 ml. of the sodium hydroxide solution in 30 minutes. It was stirred an additional 10 minutes without *p*H change. The mixture was diluted with 100 ml. of water and made acid to litmus with acetic acid. No precipitate was formed, indicating the absence of unacetylated acid. The product was precipitated by hydrochloric acid, filtered, washed, and dried at 105°. It weighed 9.7 g. (93.4% yield) and melted at 225–227°. Recrystallized from acetic acid it melted at 228.5–230°.

Anal. Calcd. for $C_9H_7O_3NI_2$: C, 25.1; H, 1.62; neut. equiv., 430.8. Found: C, 25.14, 24.73; H, 1.81, 1.79; neut. equiv., 430.

3-Formylamino-2,4,6-triiodobenzoic Acid (Procedure No. 8).—3-Amino-2,4,6-triiodobenzoic acid (257.5 g., 0.5 mole) was suspended in 87% formic acid (1750 ml.) in a 5-l. flask fitted with stirrer, dropping funnel and thermometer. During 1.5 hours 1500 ml. of acetic anhydride was added, the mixture being well-stirred and maintained between 60 and 70°. A new and bulkier precipitate formed during the reaction. One thousand ml. of water was added slowly to decompose the excess acetic anhydride. The crude product, obtained by filtration, weighed 234 g. (86%). The product was purified by recrystallization from ethanol; over-all yield 71%. It melted at 252° (dec.). Analysis showed 69.0% iodine compared to the theoretical 70.5%. Repeated recrystallization failed to effect further purification.

3-Caproylamino-2,4,6-triiodobenzoic Acid (Procedure No. 9).—3-Amino-2,4,6-triiodobenzoic acid (103.0 g., 0.2 mole) was suspended in toluene (1100 ml.) in a 2-liter flask equipped with stirrer and condenser. Heat was applied and 250 ml. of toluene was distilled from the reaction mixture to remove any water. The condenser was set for reflux and 40 ml. (0.3 mole) of caproyl chloride was added. The reaction mixture was heated under reflux for one hour, cooled and the product separated by filtration, washed with ether and recrystallized from alcohol-water; over-all yield 65%. Calcd. neut. equiv., 613; found, 610.

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[CONTRIBUTION FROM THE DEPARTMENTS OF RADIATION BIOLOGY AND BIOCHEMISTRY, THE UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE & DENTISTRY]

The Ozonolysis of Substituted Maleic Acid Imides and its Application to the Degradation of Porphyrins[1]

BY JONAS E. RICHMOND AND KURT I. ALTMAN

RECEIVED FEBRUARY 28, 1952

This paper describes a method for the decomposition of the oxidation products of protoporphyrin IX, by means of ozonolysis. The substituted maleic acid imides studied here form stable, crystalline ozonides which may be reductively decomposed, leading to identifiable α-keto acids.

In the course of investigations concerned with various phases of porphyrin metabolism using C^{14}-labeled precursors,[2] the need has arisen for a method for the degradation of the oxidative break-down products of such tetrapyrroles as protoporphyrin and chlorophyll. The oxidative degradation of porphyrins and related substances with chromic acid results in the formation of substituted maleic acid imides[3] whose further degradation is often desirable in order to determine the isotope concentration of individual carbon atoms in the porphyrin ring system. Although other approaches to this problem have been found feasible,[4] the method to be reported here[5] is accompanied by higher over-all yields.

The degradation of substituted maleic acid imides has been accomplished by formation of the corresponding stable ozonides with subsequent reductive decomposition of these ozonides according to the method described by Fischer and co-workers.[6] This approach is facilitated by the fact that the ozonides in question are crystalline solids which can be prepared in good yield. Owing to the relatively greater stability of the ozonides of substituted maleic acid imides, milder methods of decomposition were unsuccessful and the aforementioned

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[1] This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

[2] K. I. Altman, G. W. Casarett, R. E. Masters, T. R. Noonan and K. Salomon, *J. Biol. Chem.*, **176**, 319 (1948); K. I. Altman, L. L. Miller and J. E. Richmond, *Arch. Biochem.*, **29**, 447 (1950); **36**, 399 (1952).

[3] H. Fischer and H. Orth, "Die Chemie des Pyrrols," Bd. II, 1. Hälfte, Akademische Verlagsgesellschaft m.b.H., Leipzig, 1937, p. 366.

[4] J. Wittenberg and D. Shemin, *J. Biol. Chem.*, **185**, 103 (1950).

[5] Preliminary Report: K. I. Altman and J. E. Richmond, *Abstr. XIIth Internat. Congr. Pure and Applied Chem.*, p. 70 (1951).

[6] F. G. Fischer, H. Düll and L. Ertel, *Ber.*, **65**, 1467 (1932).

reductive decomposition had to be resorted to. This method led to the isolation of the expected keto acids (Va, b). The course of the degradation procedure is shown in Fig. 1.

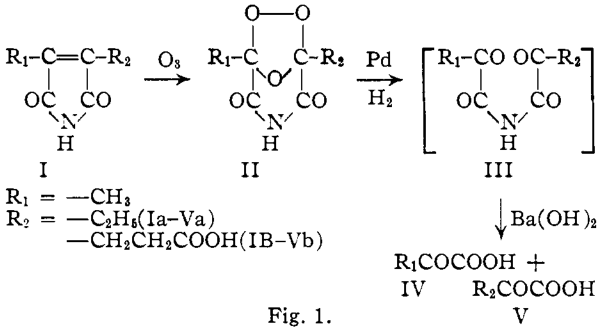

Fig. 1.

Although one of the ozonides (Ia) in question has been reported,[7] no information concerning ozonide Ib and the decomposition of these ozonides appears to be available in the literature. The oily intermediate III (Fig. 1) was not isolated in pure form prior to alkaline hydrolysis since satisfactory yields could be obtained without this additional step of purification.

Experimental

Preparation of Methylethylmaleic Acid Imide and Hematinic Acid Imide (Ia,b).—These compounds were prepared by chromic acid oxidation of protoporphyrin IX as described by Muir and Neuberger.[8]

Preparation of the Ozonides (II).—Ozonization was carried out by passing ozone produced in a high-voltage ozone generator[9] through a chloroform solution of Ia or Ib (100 mg. in 100 ml. of chloroform) at a rate of 1.8 meq. per minute for 30 minutes. Both ozonides could be crystallized after concentrating the chloroform solution to a small volume in a vacuum desiccator. These products were recrystallized several times from chloroform–petroleum ether. The crystalline material separated in colorless plates with m.p. 83.5–84.5° (ozonide of methylethylmaleic acid imide) and 124–125.5° (ozonide of hematinic acid imide). All melting points were determined with the melting point block. In the case of both substituted maleic acid imides studied, the ozonides were obtained in 95% yield.

The ozonide of hematinic acid imide was further characterized by determination of the neutral equivalent: calcd. 231, found 229.

The structural arrangement of the oxygen atoms in the ozonide grouping is, of course, not proven by the degradation procedure employed, and the Staudinger formula has been adopted merely as a convenient representation. Criegee[10] has pointed out that crystalline, stable ozonides are very rare and that most presumed stable ozonides are actually dimers involving a ketoperoxide-like structure. However, it has been postulated recently[11] that five-membered ring systems, such as the substituted maleic acid imides, form stable, crystalline ozonides. The degradation of the ozonides leads to α-keto acids whose formation could not be accounted for by parent compounds other than those shown in Fig. 1. This is considered to constitute evidence for the proposed structure of the two ozonides, shown in Fig. 1.

Reductive Decomposition of the Ozonides.—Attempts to decompose ozonides of type II by addition of water, various concentrations of sulfuric acid, zinc and hydrochloric acid, or by the addition of hydroquinone in trace amounts to any of these reaction mixtures, proved unsuccessful. However, the procedure described below led to satisfactory results.

Twenty milligrams of the ozonide was taken up in 50 ml. of dry ethyl acetate to which was added 10 mg. of Pd-$CaCO_3$ catalyst. Hydrogen gas was bubbled through the reaction mixture at atmospheric pressure and at 25° for one hour. The reaction mixture was then centrifuged, the supernatant liquid removed, and the residue extracted several times with ethyl acetate. Upon evaporation of the ethyl acetate *in vacuo*, an oily residue was obtained which presumably is identical with intermediate III.

Hydrolysis of N-Acylpyruvamide (III).—The oily residue III was transferred to a tube containing 50 ml. of saturated aqueous barium hydroxide. This tube was sealed and shaken continuously at room temperature for 24 hours after which the solution was acidified and extracted with ethyl ether several times. The solvent was evaporated *in vacuo* and the α-keto acids converted to their corresponding 2,4-dinitrophenylhydrazone derivatives which were obtained in 80% yield with reference to the parent compound. The resulting mixture of 2,4-dinitrophenylhydrazones was separated by means of column chromatography on Super-cel, essentially as described by Le Page.[12] It was found advantageous to introduce slight modifications in the solvent systems used for the development of the chromatogram in order to obtain adequate separation of the 2,4-dinitrophenylhydrazone of pyruvic acid from that of α-ketobutyric acid. After chromatography each α-keto acid was obtained in the pure state in 70–75% yield.

The derivatives of the α-keto acids (0.1–0.5 mg.) were dissolved in 3–10 ml. of ethyl ether saturated with water and put on a Super-cel column (50–100 cm. × 2.4 cm.). Le Page Solution I (10 ml.) was now added, followed by 10 ml. of a solution identical with Solution II of Le Page, except that the concentration of hydrochloric acid was increased to 2.2 *N*. It was found that better resolution of the chromatogram was obtained if the composition of Le Page Solution III was modified as follows: 2.5 ml. of ethanol, 3.0 ml. of 2.2 *N* HCl, 0.1 ml. of chloroform and 100 ml. of ethyl ether saturated with water. This solution was used to develop the chromatogram. The yellowish zones containing the compounds in question were separated by cutting them out of the column and the separated bands were then eluted with ethyl acetate. Each fraction was rechromatographed on Super-cel using the modified procedure of Le Page described above.

The purity of the phenylhydrazones of the α-keto acids isolated by the technique described was established by determining the melting points and the mixed melting points. The latter procedure was carried out with authentic samples which had been purified by the same chromatographic procedure employed for the ozonolysis products. The following melting point data were obtained:

2,4-Dinitrophenylhydrazone derivative	M.p.[a] of ozonolysis product, °C.	M.p.[a] of authentic sample, °C.
Pyruvic acid	217–218	217–218
α-Ketobutyric acid	199–200	199–200
α-Ketoglutaric acid	222–223	222–223

[a] All melting points were determined with the melting point block. The mixed melting points were identical with those of the ozonolysis product and the authentic sample.

The small quantities of α-keto acids separated in these experiments demonstrate the applicability of this method at a level which is often encountered in biochemical work. However, quantities of α-keto acid derivatives 20–100 times greater than those reported in this paper have been separated by this method. There appears to be no reason why even larger quantities could not be separated by the procedure outlined provided that a sufficiently large column is used.

Infrared Absorption Spectra.[13]—The infrared absorption spectra of the ozonides prepared are compared with their respective parent compounds (Ia,b) and are shown in Figs. 2 and 3. These spectra were obtained from solid films of the compounds deposited on silver chloride plates. The absorption peaks at 3.0 μ are probably referable to the $-NH$ group as well as to a lesser extent to the $-CH_3$ and $-C_2H_5$ group, whereas the vibrational frequency of the

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[7] H. Fischer and M. Deželić, *Z. physiol. Chem.*, **222**, 270 (1933).

[8] H. M. Muir and A. Neuberger, *Biochem. J.*, **45**, 163 (1949).

[9] The authors are indebted to Dr. Carl J. Claus of the Department of Chemistry of this University for making available to them an ozone generator.

[10] R. Criegee, *Fortschr. chem. Forschg.*, **1**, 508 (1950).

[11] R. Criegee, personal communication.

[12] C. A. Le Page, *Cancer Research*, **10**, 393 (1950).

[13] The authors are greatly indebted to Dr. L. T. Steadman and Mr. A. Levy for carrying out the determinations of the infrared spectra.

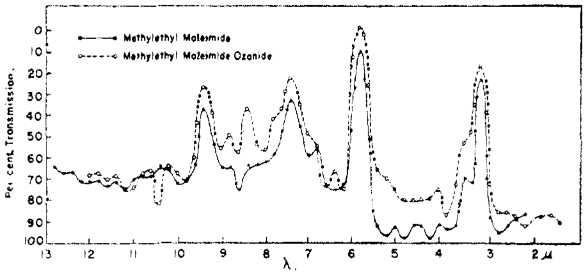

Fig. 2.—Infrared absorption spectra of methylethylmaleimide and methylethylmaleimide ozonide.

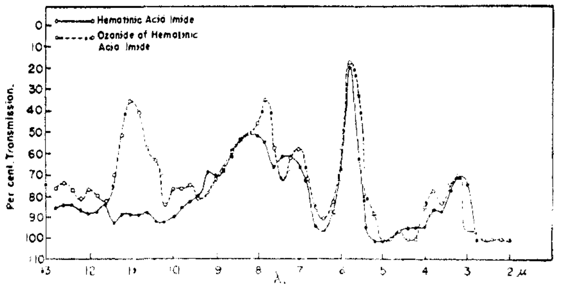

Fig. 3.—Infrared absorption spectra of hematinic acid imide and the ozonide of hematinic acid imide.

imide –CO and of the –CO of the carboxyl group of hematinic acid imide may be identified with the absorption band at 5.8 μ. It is also likely that the –C–O– groups of the ozonide make a contribution to the spectral band at this frequency.[14] The absorption band exhibited by methylethylmaleic acid imide and its ozonide at 7.3–7.5 μ is probably due to deformation vibrations of the $-CH_3$ group.[15] Hematinic acid imide has a comparable absorption band between 7.2–7.4 μ whereas in the case of the corresponding ozonide there is a shift of this band to the region of 6.8–7.2 μ. Both ozonides possess an absorption band in the region between 7.7–8.5 μ. It appears likely that the -C–O- grouping of the ozonide contributed to this vibrational frequency as has been observed for ozonides in solution.[14] At 9.3–9.6 μ an intense absorption band is observed with methylethylmaleic acid imide and its ozonide although hematinic acid imide and its ozonide fail to show such a band. It seems probable, particularly in view of the absence of this band in hematinic acid imide and its ozonide, that the $-CH_2CH_3$ grouping contributes to this absorption.[16] It should, furthermore, be noted that a striking difference exists between hematinic acid imide ozonide and its parent compound, inasmuch as the former compound exhibits a strong absorption band between 10.7–11.2 μ. Although the nature of the structural configuration responsible for this band has not been established, it is possible that the association of H with the –O–O–grouping might lead to a structure closely related to the O–O–H grouping to which Shreve, *et al.*,[17] have assigned the absorption band at approximately 12 μ. Hydrogen bonding involving the H atom of the carboxyl group and the ozonide configuration could only occur in the case of hematinic acid imide ozonide, thus explaining the absence of a band in the 12 μ region for methylethylmaleic acid imide ozonide.

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[14] E. Briner, *et al.*, *Helv. Chim. Acta*, **35**, 340, 345, 353 (1952).

[15] H. W. Thompson, *J. Chem. Soc.*, 238 (1948).

[16] K. F. Luft, *Angew. Chem.*, **19B**, 2 (1947).

[17] O. D. Shreve, M. R. Heether, H. B. Knight and D. Swern, *Anal. Chem.*, **23**, 277 (1951).

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[Contribution from Havemeyer and Chandler Laboratories, Columbia University]

The Oxidative Bisdecarboxylation of α,β-Dicarboxylic Acids[1]

By W. von E. Doering,[2] Milton Farber and Adnan Sayigh

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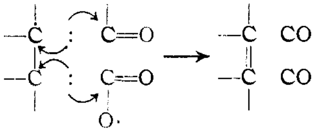
Lead dioxide has been discovered to oxidize α,β-dicarboxylic acids and their anhydrides to carbon dioxide and the corresponding α,β-olefin. Although the yields are only about 25% of theoretical, the new reaction offers degradative and synthetic possibilities.

The search for a reagent which would remove two adjacent carboxyl groups in a single operation was initiated when many of our schemes projected for the synthesis of 1-substituted bicyclic compounds seemed unfeasible for lack of such a reaction. That a diamagnetic or paramagnetic diradical of the structure I should decompose to two molecules of carbon dioxide and the olefin was the basic working hypothesis. In the electrolysis of succinic acid and fumaric acid to carbon dioxide and ethylene and acetylene, respectively,[3,4] and in the decomposition of succinyl and fumaryl peroxides to the same respective olefins[5] one has examples of the desired reaction and support for the hypothesis. When applied to substituted succinic acids the electrolysis has so far been quite unsatisfactory. Extension of the peroxide decomposition to hexahydrophthaloyl peroxide was successful in that cyclohexene in variable yield was obtained but the peroxide, which was prepared according to Vanino and Thiele,[6] was extremely unstable and could not be purified.



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Investigation of tetravalent lead compounds as reagents to effect the desired reaction was prompted by their use in the cleavages of glycols and α-hydroxy acids, two reactions which resemble the projected reaction rather closely. The lead tetraacetate cleavage of glycols is not only structurally similar but has been shown to proceed through a

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[1] Taken in part from a dissertation submitted by Milton Farber in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

[2] Department of Chemistry, Yale University, New Haven, Connecticut.

[3] A. Kekule, *Ann.*, **131**, 79 (1864).

[4] See also J. Peterson, *Z. physik. Chem.*, **33**, 698 (1900); F. Fichter and A. Petrovitch, *Helv. Chim. Acta*, **24**, 549 (1941).

[5] F. Fichter and A. Fritsch, *ibid.*, **6**, 329 (1923).

[6] L. Vanino and E. Thiele, *Ber.*, **29**, 1724 (1896).