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Periodate-Permanganate Oxidations. IV. Determination of the Position of Double Bonds in Unsaturated Fatty Acids and Esters[1, 2]

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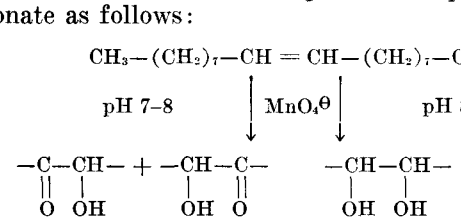
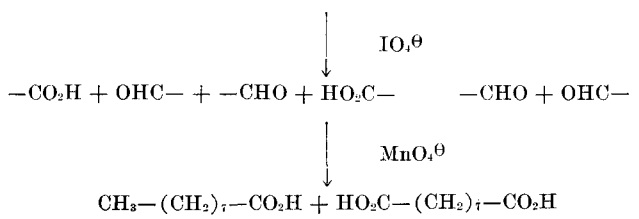
THE POSITION of double bonds in unsaturated fatty acids, and alkenes in general, has been determined mainly by permanganate oxidation or by ozonolysis. In 1950 Haverkamp and co-workers (2) demonstrated that these oxidation reactions do not adhere strictly to the following expected reaction scheme:

$$R{-}CH{=}CH{-}(CH_2)_n{-}CO_2H + 4\,O \longrightarrow R{-}CO_2H + HO_2C{-}(CH_2)_n{-}CO_2H \qquad (I)$$

By means of an elegant chromatographic method for determining homologous dicarboxylic acids in the C_7 to C_{13} range, these authors found that lower homologues are produced in addition to the expected dicarboxylic acid. The much more tedious method of hydroxylating the unsaturated compound with peracid, followed by periodate or lead tetraacetate cleavage and subsequent oxidation of the aldehydes, also did not lead to quantitative results. Best results were obtained with potassium permanganate in glacial acetic acid (according to Armstrong and Hilditch [1]) or with ozonolysis, but 5 to 8% of lower degradation products were still obtained.

The presence of 5% or more of lower homologous dicarboxylic acids can be misleading in the qualitative and quantitative interpretation of such oxidative results, and a method which adheres strictly to reaction I is highly desirable. The results obtained in this work show that such a quantitative oxidation is possible with the periodate-permanganate reagent (3, 4). This reagent, which consists of a dilute solution of sodium *meta*-periodate containing catalytic amounts of potassium permanganate, was shown to react with oleic acid in the presence of potassium carbonate as follows:





Although two different reaction routes occurred, the end-products were the same. The very mild conditions at pH 7–8 appear to be conducive to the quantitative oxidation of the intermediate aldehydes to carboxylic acids since practically theoretical yields of azelaic and pelargonic acids were obtained after complete oxidation (3). The over-all reaction may be written as

$$R{-}CH{=}CH{-}(CH_2)_n{-}CO_2H + 4IO_4\theta \xrightarrow{MnO_4\theta} R{-}CO_2H + HO_2C{-}(CH_2)_n{-}CO_2H + 4\,IO_3\theta$$

which is equivalent to reaction I. The crucial feature of the reaction is that, in the pH range, from about 5 to 10 periodate is capable of regenerating the permanganate. The limiting reaction conditions under which this reaction scheme was strictly adhered to were: temperatures below 40°C., a pH range of about 6 to 9, reaction times of less than 1 week's duration, and concentrations not higher than twice those described in the experimental part.

Since Haverkamp *et al.* (2) have shown by means of partition chromatography that lower homologues were formed in permanganate oxidations, the analysis of the oxidation products obtained from the periodate-permanganate oxidation of oleic acid was repeated by this method. The method was extended to cover the analysis of monocarboxylic acids as well. Where tailing occurred (which could, of course, be due to the presence of small amounts of the next lower homologue), the respective fractions were rechromatographed. These experiments showed conclusively that, within the error of the method (± 2%), the expected acidic end-products were formed quantitatively and that no lower degradation products were formed.

In order to show that the method is a more general one, the reaction with several other unsaturated fatty

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[2] Issued as Paper No. 214 on the "Uses of Plant Products" and as N.R.C. No. 3882.

acids was examined. With compounds which dissolved in the dilute, weakly alkaline reaction mixture, quantitative results were obtained readily. The higher unsaturated fatty acids or methyl esters did not give satisfactory results because of their insolubility in the reagent. This problem was overcome with some compounds by use of a modified reagent which contains pyridine or dioxane (4) and hence increases the solubility of the lipid. However highly insoluble or less reactive esters, such as methyl oleate or triolein, did not give quantitative results under these improved conditions.

Experimental

Materials. The acids used for the standardization of the chromatographic columns were purified by established distillation and crystallization techniques to give chromatographically pure compounds. Undecanedioic and brassylic acids were prepared in small amounts by periodate-permanganate oxidation of eicosenoic and erucic acid, respectively.

All solvents used in the chromatographic experiments were distilled over solid potassium hydroxide. Pyridine was purified by permanganate oxidation and distillation over solid potassium hydroxide.

The chromatographic absorbent was Mallinckrodt's 100-mesh, analytical grade silicic acid, which was washed twice with distilled water (the finer particles remaining in suspension after 3 hrs. of settling time were discarded), dried overnight at 110–120°C. and finally in a vacuum oven at 100–110°C. for 3 hrs. The finely powdered product was stored *in vacuo*.

Chromatographic Columns (Type A.) This column is suitable for the analysis of dicarboxylic acids in the C_5 to C_{13} range and is a modification of the one described by Haverkamp and co-workers (2).

A 5-g. aliquot of silicic acid was mixed thoroughly in a mortar with 3.5 ml. of the aqueous phase, obtained by equilibrating in a separatory funnel 10 vol. of benzene with 7 of methanol and 3 of water. Enough of the benzene phase was added immediately to give a thin suspension. A small cotton plug was placed at the constriction of a 25-ml. or 50-ml. burette and a few ml. of benzene phase were added. With the stopcock open, the silicic acid slurry was poured into the burette and allowed to settle. The slurry was stirred with a wire for removal of air bubbles and more even settling. The column was then packed under a pressure of 10 to 15 cm. Hg, and the excess benzene phase was allowed to flow through the column until the meniscus just disappeared at the top of the column. A 50- to 100-mgm. sample of the acids to be analyzed was dissolved in a minimum of benzene phase (0.5 to 2.0 ml.; if the solution was incomplete, a few drops of methanol were added) and carefully applied to the column with a pipette. Pressure was applied to allow this aliquot to flow into the column. The flask in which the acids were dissolved was washed twice with 0.5-ml. benzene phase; each aliquot was applied separately to the column. The column was then carefully filled with benzene phase to the 0-ml. mark, and sufficient pressure (5 to 25 cm. Hg) was applied to give a flow of 0.3 to 0.5 ml. per minute. Fractions of 0.5 or 1.0 ml. were collected and titrated under nitrogen to the phenolphthalein end point. After 50 or 60 ml. had been titrated, the fractions were increased to 2.0 ml. This column gave a quantitative separation (99% ± 2% recovery) of sebacic, azelaic, suberic, and pimelic acids. For higher acids (C_{13} to C_9) the column was doubled in weight whereas for lower acids (C_9 to C_5) it was halved. All monocarboxylic acids in the C_6 to C_{18} range emerged as a narrow band after 4 to 8 1-ml. fractions had been collected. Lower monocarboxylic acids overlapped with some of the dicarboxylic acid bands, but evaporation to dryness of the sample before application to the column removed these more volatile acids quantitatively.

(Type B.) This column is suitable for the analysis of monocarboxylic acids in the C_6 to C_{18} range and is a modification of the one described by Zbinovsky (5).

The procedure was the same as described above except that the rate of flow was already satisfactory with no or very little pressure. The solvent mixture was 100 parts Skellysolve B (petroleum ether b.p. 66–67°C.), 9 parts of methyl-cellosolve (b.p. 121–122°C.), and 1 part water. Six grams of silicic acid were treated with 5.0-ml. polar phase to give a column which was satisfactory for 10–50 mgm. of a mixture of monocarboxylic acids in the C_6 to C_{10} range. The increasing or decreasing of the column length gave satisfactory results for the higher or lower acids.

The positions of the various acids on these columns were in the same order as described in the literature and often coincided closely with the fraction numbers reported (2, 5).

Oxidation of Unsaturated Fatty Acids. The procedure was similar to the one described previously (3). In a typical experiment 0.1412 g. (0.5 mM.) oleic acid were dissolved in 100 ml. of water containing 0.207 g. of (1.5 mM.) potassium carbonate. This solution was added at room temperature to 100 ml. of a solution consisting of 0.834 g. (3.9 mM.) of sodium *meta*-periodate and 1.0 ml. of 0.1 M potassium permanganate solution. The reaction was stopped after 20–24 hrs. by adding with cooling 10 ml. of 10% sulfuric acid and enough sodium bisulfite to reduce all periodate, iodate, and iodine to iodide (as can readily be seen by the disappearance of free iodine). This reaction mixture was then extracted continuously with ether for 16 to 20 hrs. The ether extract was divided into two equal fractions, one of which was evaporated to dryness. The residue was taken up in a minimum of benzene phase and applied to type A column for chromatographic analysis. The other fraction was titrated with 0.05 *N* methanolic sodium hydroxide to the phenolphthalein end-point and then evaporated to dryness. The residual salts were dissolved in a minimum of aqueous ethanol and transferred quantitatively to a small test tube. After evaporating all solvents, the salts were acidified by adding 2 to 3 drops of 50% *ortho*-phosphoric acid and a few drops of Skellysolve B. The test tube was slowly rotated until the fatty acids were completely regenerated. To this suspension 0.5 to 1.0 ml. of Skellysolve B and 0.5 to 1 g. of anhydrous sodium sulfate were added. After standing for at least 5 hrs., the Skellysolve B solution was withdrawn and applied to type B column. The recovery of monocarboxylic acids in the C_6 to C_{10} range after washing the test tube twice with 0.5 ml. aliquots of Skellysolve B was 99%, but most of the dicarboxylic acids remained in the test tube.

When the unsaturated fatty acid to be analyzed did not dissolve in the aqueous solution of potassium carbonate, the concentrations were halved (*e.g.*, with

elaidic acid) or, if this did not give satisfactory results, the acid was dissolved in 10 to 20 ml. of pyridine and then added to the carbonate solution (*e.g.*, with erucic acid). The latter procedure was also used for methyl esters; the amount of potassium carbonate was reduced to 1 mM. The analytical procedure was the same, except that considerably more sulfuric acid was added at the end of the reaction to bind the pyridine and thus ensure the complete reduction to iodide.

Analysis and Identification of Oxidation Products. When the results from the titration of individual fractions from the chromatographic analysis showed a single peak, *i.e.*, a single acid, these fractions were combined, evaporated to dryness, and the acid regenerated. Dicarboxylic acids were identified by melting point and mixed melting point and monocarboxylic acids as their hydrazides. These results confirmed the identity of an acid, as was already deduced from its position on the chromatographic column.

Results and Discussion

Oleic, elaidic, eicosenoic, and 10-undecenoic acids gave quantitative (98% of theory, or better) results in the aqueous reaction mixture. 10-Undecenoic acid yields formaldehyde and carbon dioxide besides sebacic acid (3). In this work only the amount and purity of sebacic acid was determined. When oleic acid was oxidized for 3 or 4 days, the results were essentially the same as for 1-day reaction time.

Linoleic acid reacted too fast at the normal concentrations and reduced the permanganate at a spread greater than its rate of regeneration by the periodate. Greater dilution, or a slow addition of the postassium salt to the oxidant, gave a satisfactory rate of reaction, and from the reaction mixture azelaic and caproic acids were isolated in practically quantitative amounts. No malonic acid was isolated, indicating that the central portion of the linoleic acid molecule was extensively degraded, possibly because the intermediate malon aldehydes were degraded faster than they were oxidized to malonic acid. Also malonic acid itself was slowly oxidized by the reagent. All conclusions must therefore be drawn from the yield of azelaic and caproic acids.

Erucic acid was too insoluble to give satisfactory results in the aqueous reaction mixture, even at higher dilution. The addition of 5 to 10% of pyridine increased the solubility sufficiently to allow the reaction to proceed normally, and from the oxidized sample brassylic and pelargonic acids were isolated in near quantitative yields. Similar results were obtained with oleic acid in this medium when the reaction time was two days or less. Although pyridine itself is slowly oxidized by the reagent, no interference with the analytical procedure was experienced. Methyl linoleate reacted to completion in media containing only 5 to 10% of pyridine, giving the expected degradation products in a 95–96% yield. Apparently the rapid formation of the intermediate diols and ketols increased the solubility sufficiently to allow the reaction to proceed normally even though it was initially heterogeneous.

Concentrations of 20% or more pyridine were necessary to give a homogeneous reaction mixture with methyl oleate and triolein, and it was of interest to determine whether these and other water-insoluble esters could also be analyzed satisfactorily in such media. Although the reaction appeared to proceed normally, in that the permanganate was not decolorized, quantitative results no longer were obtained even with oleic acid. A more detailed study of the periodate-permanganate reagent in media containing 10% and more pyridine, and possibly other solvents, is necessary to decide whether or not a quantitative result is possible with methyl esters and triglycerides. This will be the subject of another publication.

Summary

The reaction of the periodate-permanganate reagent with unsaturated fatty acids, including oleic, elaidic, eicosenoic, 10-undecenoic, and linoleic acids proceeded smoothly to give the expected end-products in quantitative yields. The latter were conveniently isolated and determined by partition chromatography on silicic acid columns in 10 to 100 mgm. quantities.

Some compounds, which were unreactive due to insolubility in the reagent, gave satisfactory results when the media contained 5 to 10% of pyridine. This was true also for methyl linoleate even though solution was incomplete. However methyl oleate and triolein did not give quantitative results even in media containing 20% or more of pyridine in which the esters were completely dissolved.

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