• *Short Communication*

Factors Governing the Extent of Overoxidation in Permanganate Oxidation of Unsaturated Fatty Acids

Abstract

The extent of overoxidation of half-esters of dicarboxylic acids is governed mainly by the nature and proportions of the oxidizing agent employed and not by reaction temperatures. In procedures which produce overoxidation, this occurs mainly at the time of the scission of the double bonds and no method is known to prevent it. The acetone permanganate procedure overoxidizes monocarboxylic acids (MCA), dicarboxylic acids (DCA) and half esters of DCA. The acetic acid-acetone permanganate procedure overoxidizes DCA but not MCA half-esters of DCA or azelaoglycerides. The DCA corresponding to the first double bond in unsaturated fatty acids (UFA) can be isolated quantitatively if the esters or triglycerides are oxidized by the latter procedure.

The double bond nearest the carboxyl in the unsaturated fatty acid molecule may be designated the first double bond (FDB). It is of fundamental interest to ascertain whether all the individual unsaturated fatty acids present in natural fats from particular biological species contain the FDB in the same site or position in relation to the carboxyl, and if not, to determine the proportions of acids having the FDB in particular sites. This is the object of first double bond site (FDBS) distribution analysis.

To obtain a fully reliable picture of FDBS distribution the UFA has first to be broken down by techniques which can effect quantitative scission of all double bonds present. Ozonolysis procedures cannot produce this quantitative scission as components present in lesser proportions may be selectively or even predominantly used in the formation of the large proportions of resinification products usually observed in this procedure (1).

There is no resinification in permanganate oxidation procedures and quantitative scission of double bonds can be obtained under proper conditions, e.g., in acetone permanganate (2) and acetic acid acetone permanganate (3) procedures. Quantitative scission is perhaps not attainable for UFA in the periodate permanganate technique (4). Permanganate oxidation procedures, however, sometimes produce chain degradation (overoxidation) of the MCA and DCA generated by chain scission, this problem has been comprehensively reviewed a number of times with an exhaustive list of references (5). The factors governing this overoxidation are not fully understood as yet, but among them the factors governing the overoxidation of DCA are of vital importance in FDBS distribution analysis.

It is generally believed that, for the same technique, overoxidation is less extensive or even absent at lower reaction temperatures. This was tested as follows. Mixed fatty acid esters of *Sarcostigma kleinii* seed fat were oxidized by the acetone-permanganate procedure in 0.5% acetone solution, in the presence of 0.1% K_2CO_3 (to maintain the pH alkaline from the beginning), at refluxing temperature (56 C) and at room temperature (22 C), using 10 g permanganate per gram of ester. At 56 C the oxidation was complete in 5 hr while at 22 C it required four days shaking for complete absorption of permanganate. The products were processed as usual with sodium bisulphite and dilute sulphuric acid, extracted with diethyl ether and lower dibasic acids removed by several washings with water. The residue was hydrolyzed with alcoholic KOH and the resulting MCA + DCA mixture separated into MCA and DCA by partitioning between hexane and water (4); the MCA were further separated into higher saturated acids (HSA) and lower saturated acids (LSA) by Bertram separation (3). The same yield of HSA (43–44%) of the iodine value below 1 indicated completeness of the oxidation at both temperatures. Reactions at both temperatures gave the same yield of DCA (19%) with the same eq. wt. of 90 as against 26% yield of DCA of eq. wt. 95–96 obtained by acetic acid-acetone-permanganate oxidation of the same esters with the same proportions of permanganate. Therefore reaction temperatures are not very important in determining the extent of overoxidation; the latter is determined mainly by the relative proportions of the permanganate used. When oxidation is conducted at 22 C with smaller quantities of permanganate, the proportions and iodine values of HSA increase showing the incompleteness of oxidation; with 5 g permanganate/g ester at 22 C the HSA showed iodine value of 16–18 against 72 for the original esters. No appreciable inhibition of overoxidation can therefore be effected by lowering reaction temperatures.

The above esters were subjected to acetone-permanganate oxidation as already described for 5, 10 and 50 hr and the DCA and HSA fractions isolated and examined as before. The HSA amounted to 43–44% and had eq. wt. 284–85 in all cases, therefore the HSA esters do not undergo any overoxidation with this technique. The DCA amounted to 19%, 17% and 13% respectively but showed eq. wt. 90 throughout, compared to 26% DCA of eq. wt. 95–96 obtained by 5 hr oxidation by acetic acid-acetone-permanganate technique. Changes in composition of DCA produced by overoxidation were therefore limited to the first few hours of the oxidation. After the attainment of this constant composition DCA mixture, further changes are limited to a decrease in the amount of DCA present. This leads to the probability that, in cases where the oxidation procedures used were not fully standardized, it is only this constant composition DCA mixture which has been isolated and analyzed and not the true DCA corresponding to FDBS. The weights of DCA fractions thus form the most reliable criterion by which the extent of overoxidation can be measured.

These results also bring out an entirely unexpected and surprising feature of overoxidation. In the first 5 hr of oxidation, the amount of DCA lost by overoxidation amounts to 7% on ester basis, while it is only about 2% in the next 5 hr and continues at the rate of about 1% every 10 hr for the next 40 hr. This indicates that, for reasons which are unknown at present, the overoxidation is extremely rapid at the time of scission of the double bonds and falls off to a comparatively very slow rate once the scission is over. The initial rates can naturally differ for different UFA. No method is known at present for inhibiting or slowing down this very rapid overoxidation of DCA (and probably also of MCA) at the time of scission of the double bonds. It is therefore obvious that if the DCA corresponding to FDBS is to be isolated without any overoxidation, a technique has to be selected which will not produce any detectable overoxidation. This must be established by gravimetric methods involving isolation of DCA under proper conditions, i.e., after differing periods of oxidation.

The mixed acid esters of *S. kleinii* seed fat were oxidized by the acetic acid-acetone-permanganate technique for 5, 10 and 50 hr and DCA and HSA fractions isolated as before. The DCA fraction remained constant at 26% and its eq. wt. remained unchanged at 95–96; the HSA amounted to 43–44% (eq. wt. 284–85) in all cases. Therefore this technique does not produce overoxidation of half-esters of dicarboxylic acids since oxidation of UFA esters will give rise directly to half-esters of dicarboxylic acids.

Possible overoxidation of the dicarboxylic acids in the azelaoglycerides is of importance in connection with the azelaoglyceride analysis technique. The original *S. kleinii* fat was therefore oxidized by the above technique for 5, 15, 25 and 50 hr; 10, 15, 21 and 36 parts of permanganate were used per gram of fat. The products were processed as reported elsewhere (6). The insoluble azelaoglycerides amounted to 70.3–70.9%, irrespective of length of oxidation, and contained 21–22% DCA of eq. wt. 95–96 in all cases. Therefore no overoxidation of DCA in azelaoglycerides takes place when fats are oxidized by the acetic acid-acetone-permanganate procedure.

Proof that free HSA, either alone or in the presence of large proportions of UFA, do not undergo any overoxidation by acetic acid-acetone-permanganate procedure was recorded earlier (7). This is quite similar to the resistance shown by half-esters of dicarboxylic acids now recorded. This would normally apply to LSA as well, and this observation made possible the direct determination of HSA in mixed fatty acids by oxidation methods (7). Direct oxidation of mixed fatty acids for FDBS distribution analysis can be performed if the DCA fraction of oxidation products is also stable towards overoxidation. The free carboxylic group however, acts as a reactive center of degradation, as shown by the example that saturated hydrocarbons are fully resistant towards hot aqueous alkaline permanganate, whereas the HSA are extensively degraded by the same reagent (7). Similarly, proof that an increase in the number of free carboxylic groups in the molecule can increase the overoxidizability of the molecule is provided by the fact that oxalic and malonic acids are readily oxidized by aqueous acidic permanganate, although the corresponding monocarboxylic acids, acetic and propionic, are very resistant to the same reagent. Dicarboxylic acids will thus be more susceptible to overoxidation than the corresponding monocarboxylic acids, but whether this increased susceptibility will make them liable to overoxidation by acetic acid-acetone-permanganate cannot be predicted. Therefore the overoxidizability of DCA fractions of eq. wt. 95–96 from *S. kleinii* fat was tested by means of this procedure. In 5 hr oxidation, using 5 g permanganate per gram of acid there is only 80% recovery of acids with eq. wt. 90 as against 98–100% recovery of acids with unchanged eq. wt. in blank runs. The technique cannot therefore be used for direct oxidation of free UFA in determining FDBS distribution.

The increased overoxidizability of DCA as compared to MCA can be readily demonstrated by any technique producing overoxidation, e.g., the acetone-permanganate technique. When HSA (eq. wt. 280) is oxidized by this technique with 5 parts permanganate, oxidation products recoverable by ether extraction amounted to 88%, but when DCA (eq. wt. 95–96) is similarly oxidized with the same proportions of permanganate, products recoverable with ether extraction are decreased to about 80%. In view of this highly enhanced overoxidizability of DCA, the suitability of particular oxidation techniques for FDBS distribution analysis can be conveniently determined by a single reaction with pure palmitic or stearic acid at any convenient temperature below 96 C. According to this test, significant overoxidation of stearic acid is produced by aqueous alkaline-permanganate (7), acetone-permanganate (2) and periodate-permanganate (8) techniques. The acetic acid-acetone-permanganate technique (3) alone passes this test. However the susceptibility of free DCA to overoxidation by this technique has to be overcome by oxidizing the acids in ester or triglyceride form.

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