An Infrared Absorption Study of Autoxidized Methyl Linoleate[1,2]

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INFRARED absorption studies have been found useful in many investigations, but very little has been reported in the literature concerning the use of infrared spectroscopy in a study of the autoxidation of fats and fatty acids. Gamble & Barnett (1) have examined the infrared absorption of methyl oleate and methyl elaeostearate before and after exposure to ultraviolet light and found strong absorption at 3.4 μ (2900 cm^{-1}), which shifted toward higher frequencies after irradiation with ultraviolet. This they attributed to the introduction of hydroxyl groups. Honn, Bezman, and Daubert (13) have studied the infrared absorption of hydroxy compounds formed in autoxidizing linseed oil. They found a maximum absorption at 2.9 μ (3420 cm^{-1}) which they attributed to hydroxyl, carboxyl, and hydroperoxyl groups.

Experimental

A series of samples of autoxidized methyl linoleate were prepared in the range from peroxide value (PV) 1 to PV 940 m.e./kg. by bubbling air through methyl linoleate at 98°C. The samples were sealed in evacuated ampules and stored in a refrigerator until the time of use, which was a number of months after preparation. Conceivably, further changes could occur in the stored samples, but it is thought the interpretation would remain grossly the same since the absorption curves corresponded well with those obtained in preliminary investigations. For comparative purposes spectra were determined on oxidized methyl linoleate and on a portion of the same sample reduced to a PV of 0 by treatment with KI reagent. The reduced samples were washed with water to remove the acetic acid, dried with sodium sulphate, filtered, and the chloroform removed under reduced pressure.

Spectra were also determined on fractions from an adsorption column on which the oxidized esters were concentrated as reported by Dugan, Beadle, and Henick (2).

The absorption curves were obtained on a Perkin Elmer Recording Spectrometer, using a lithium fluoride prism to give higher resolution than was obtained in preliminary studies with rock salt prisms.

The samples were studied as liquid films in 0.1 mm. and 0.025 mm. thicknesses. Earlier investigations of autoxidized fatty acid esters, including methyl linoleate, showed that principal changes occurred in two regions: at 3400-3550 cm^{-1} where —OH groups absorb and at 1650-1775 cm^{-1} where C = 0 groups absorb. Accordingly, measurements for this study were made in only these two regions. The measurements in the 3400 cm^{-1} region were made on the 0.1 mm. samples. It was necessary to reduce the sample thickness to 0.025 mm. to study the absorption in the 1700 cm^{-1} region. No studies were made of the effect of the autoxidative reaction on the double bonds since the lithium fluoride prism is not effective below 1650 cm^{-1}.

Results and Discussion

The effect of oxidation on the absorption by —OH groups is shown in Fig. 1. A common absorption band exists for all samples observed at 3467-70 cm^{-1} with the lower frequency predominating in the samples most highly oxidized. This band is believed to be due to hydroxyl groups in the molecules. That hydroxyl groups can be formed by an intermolecular reaction between a hydroperoxide group and a double bond in another molecule has been discussed by Farmer (3). Swift and Dollear (4) found that dihydroxy compounds resulted from an interaction of methyl hydroperoxido oleate with oleic acid.

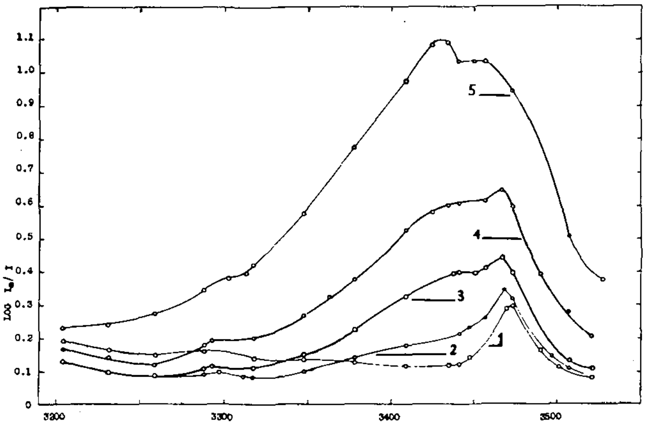
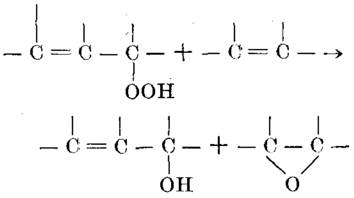


FIG. 1. Infrared absorption curves showing the effect of increasing oxidation in the —OH absorption region.
1. PV 1. 2. PV 240. 3. PV 410. 4. PV 587. 5. PV 940.



Other samples have been observed in which the band was missing or much less intense than in the curve shown for methyl linoleate with a PV of only 1. The intensity of the band in this sample is believed to be due to one of two factors: partial oxidation and breakdown of the hydroperoxide formed during processing of the ester or to interaction during the storage period of the sample. It would appear then that infrared absorption in this region should serve as a criterion of purity of a fat with respect to oxidation effects. A truly fresh fat with no oxidation should exhibit no absorption in this frequency range.

In addition to the band which is common to all samples, there is a broad band which becomes more intense with increasing PV of the samples until it

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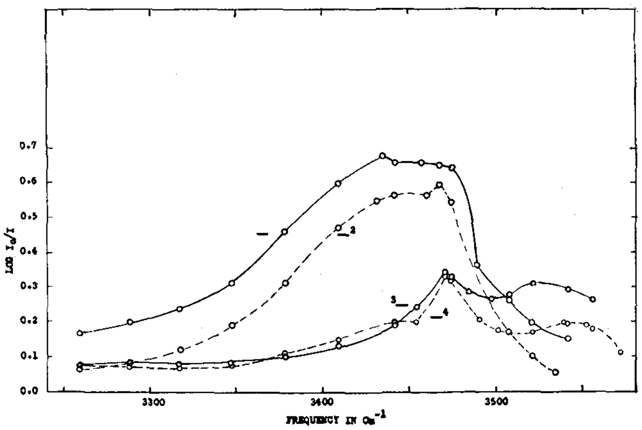
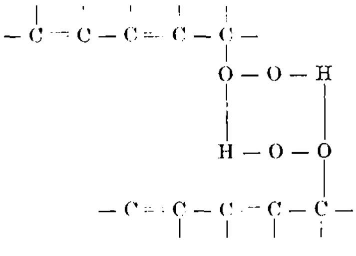


FIG. 2. Effect of HI reduction of autoxidized methyl linoleate in the —OH absorption region.
1. PV 560. 2. PV 205. 3. PV 560 reduced to 0. 4. PV 205 reduced to 0.

is resolved into a true maximum at 3430 cm^{-1} as illustrated in the curve for the ester having a PV of 940 m.e./kg. This maximum shifts from a frequency of about 3445 to 3430 cm^{-1} with increasing peroxide value of the oxidized esters. As will be shown later, this band is believed to be due to the hydroperoxide groups in the molecule and the frequency shift to lower frequencies due to an "associated" state which is enhanced by the increase of both the —OH and —OOH groups in the molecules. This was pointed out by Barnes, Liddel, and Williams (5) who stated, " 'free' hydroxyl vibrations, where the hydrogen is not affected by any atom except the oxygen to which it is bound, have a characteristic absorption between 3700 and 3500 cm^{-1}. If the material is concentrated so that 'hydrogen bonding' can occur, this absorption becomes broader and stronger and moves to lower frequency."

It is believed the association referred to above could occur in a number of ways in molecules having —OOH groups, but since the hydroperoxides are fairly stable substances, they may stabilize by associating in the following manner:



Of course, there may be many other structures and molecules that can contribute to association since the products of autoxidation of methyl linoleate are complex and not all are the simple monohydroperoxides pictured above.

The evidence on which is based the assignment of the band at 3470 cm^{-1} to —OH and at 3430-45 cm^{-1} to —OOH is illustrated in Fig. 2. Two samples of autoxidized methyl linoleate were divided, and a portion of each was reduced with potassium iodide in chloroform-acetic acid as described above. Swift, Dollear, and O'Connor (6) demonstrated that hydroperoxides react with this reagent to form hydroxy-compounds. The oxidized portions, with PV 205 and 560, respectively, gave the typical absorption spectra observed before. The reduced samples however had entirely different structures. The band at 3470 cm^{-1} remained, but the broad intense band at 3430-45 cm^{-1} was completely removed and in each case a new band developed at a higher frequency. The new band from the sample with an original PV of 560 was shifted 10 wave numbers toward lower frequency from that for the sample with an original PV of 205 and it also absorbed more intensely.

The reason for the equal intensity in the reduced samples of the bands at 3470 cm^{-1} is not clear. Other cases have been observed in which the intensity is greater in the reduced sample, which had the greater intensity prior to reduction. It is believed that the absorption at 3470 cm^{-1} is enhanced by the great absorption at the lesser frequency. The intensity of the reduced sample should more nearly indicate the true intensity of the substances absorbing at 3470 cm^{-1} than the absorption which includes the contribution of those substances absorbing at a lesser frequency.

Since the broad intense band at the lower frequency was removed, it apparently was due to the presence of "associated" hydroperoxide groups, but since the band at 3470 cm^{-1} was not removed, it was apparently due to hydroxyl groups in the molecule. The new band formed at the higher frequencies by reduction of the hydroperoxides would then be an —OH band and would be expected to absorb more intensely and at a lesser frequency in the reduced PV 560 sample than in the reduced PV 205 sample. If the assignment of the band at 3470 cm^{-1} to —OH is correct, this pair of curves for the reduced samples would then indicate that resolution had been made between the absorption bands for two similar compounds containing —OH groups on different carbon atoms. These two compounds can be accounted

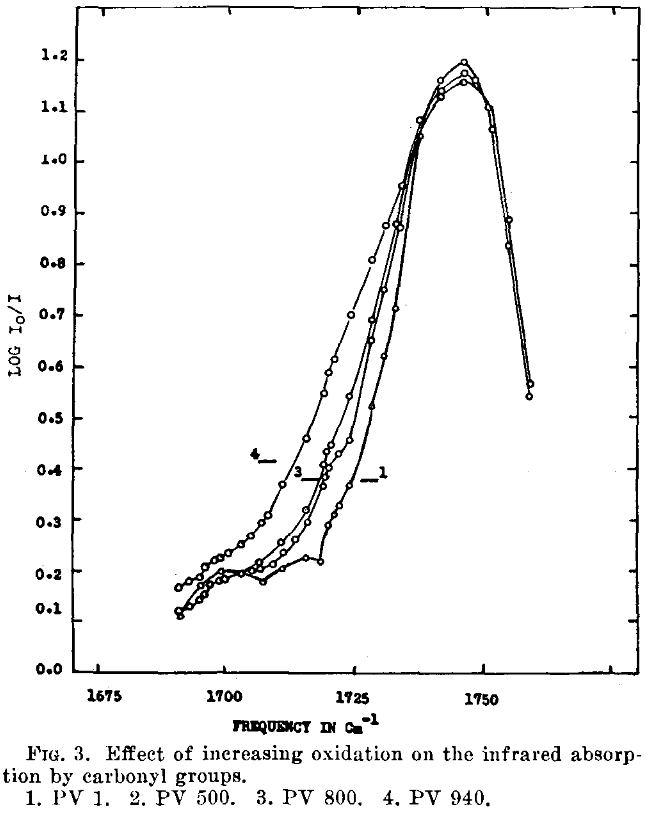


FIG. 3. Effect of increasing oxidation on the infrared absorption by carbonyl groups.
1. PV 1. 2. PV 500. 3. PV 800. 4. PV 940.

for by an acceptance of the concepts of Bolland and Koch (7), who postulated oxidative attack at the α-methylene C atom with resulting formation of three resonance hybrid structures, two of which are conjugated. The monohydroperoxides formed from methyl linoleate would then be at least two-thirds conjugated, and the spectroscopic evidence obtained by them supported this. Bergstrom (8) identified the 9- and the 13-hydroxystearic acids from hydrogenation of autoxidized linoleic acid. Holman (9) has suggested that the conjugated forms can be expected as sole primary oxidation products from autoxidation of methyl linoleate, which thus allows two isomeric monohydroxy conjugated-diene methyl linoleates as expected products from HI reduction of the autoxidized methyl linoleate. It is believed that these two isomeric conjugated diene methyl hydroxylinoleates may well be the compounds which contribute the observed bands.

Substances absorbing at 2760 Å in the ultraviolet have been observed in autoxidized methyl linoleate. They were postulated by Bergstrom (10) to be doubly unsaturated ketones resulting from decomposition of the conjugated-diene hydroperoxides. In later studies Lundberg and Chipault (11) suggested that these substances result from a common precursor of them and the hydroperoxides. These substances should absorb in the infrared in the region where C = O groups absorb.

In an examination of the carbonyl absorption region, absorption curves such as those shown in Fig. 3 were obtained. It is known that the carbonyl group in an ester absorbs generally from 1725-1750 cm^{-1} and aldehyde and ketone carbonyls absorb generally from 1675-1730 cm^{-1}. The esters absorb with such intensity that the absorption due to carbonyl from ketones or aldehydes was detectable only as a shoulder on the ester absorption band. In order to obtain an idea of what the absorption bands due to other carbonyl groupings was like, a plot was made using the intensity measured for the least oxidized sample in the lot (PV = 1) as I_0 and that for each oxidized sample as I. The plot of Log I_0/I vs. frequency in cm^{-1} would then show only those changes due to oxidation of the samples. Such a plot is illustrated in Fig. 4. Note that a minimum now replaces the maximum due to ester carbonyl shown in Fig. 3. A double maximum exists for all samples at 1724 cm^{-1} and at 1733 cm^{-1} with an additional small maximum in some samples at 1755 cm^{-1}. Both of the major maxima increase with increasing oxidation of the samples. It must be noted however that the maximum at 1733 cm^{-1} is initially the higher but increases less rapidly than the one at 1724 cm^{-1} and is finally superseded by the maximum at 1724 cm^{-1} in the sample with a PV of 940. This double maximum indicates the presence in the autoxidized samples of at least two separate carbonyl containing compounds, which are formed in different quantities depending on the state of oxidation of the sample. These two compounds absorbing in the carbonyl region could be the substances which have been found to absorb at 2760 Å part of which enolize with KOH and part of which do not enolize. From a consideration of the characteristics for (C = O) frequencies as outlined by Barnes, Gore, Stafford, and Williams (12), the band at the lower frequency would be the band due to

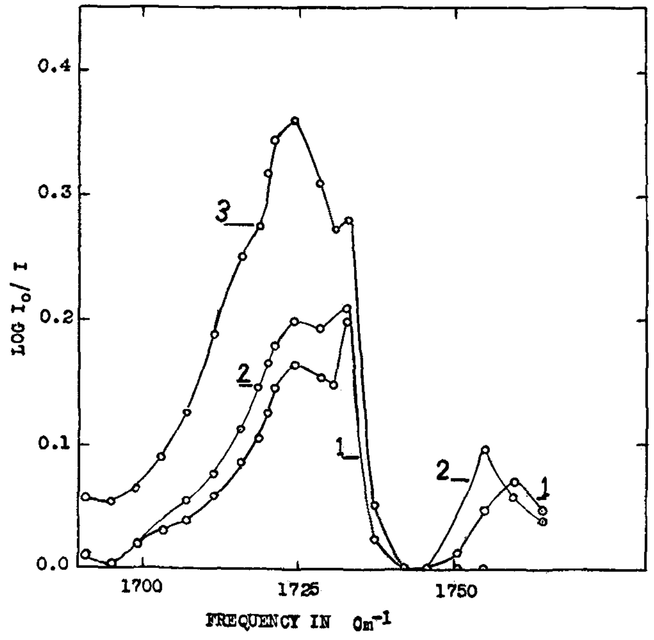


FIG. 4. Absorption by carbonyl groups other than ester carbonyls. *Effect of increasing oxidation.*
1. PV 500. 2. PV 800. 3. PV 940.

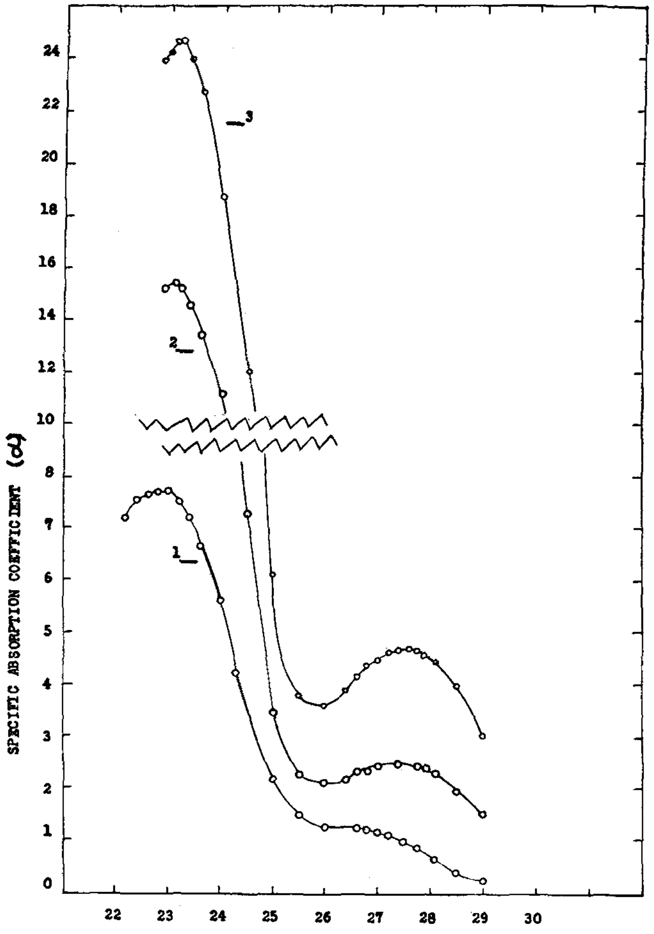


FIG. 5. Ultraviolet absorption spectra of substances eluted from sections of an adsorption column.
1. Top section, PV 1100. 2. Middle section, PV 2900. 3. Bottom section, PV 1300.

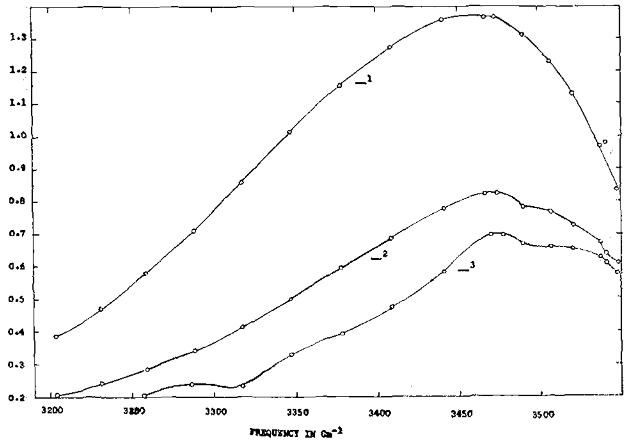


FIG. 6. Absorption by —OH and —OOH of substances eluted from an adsorption column.

1. Top section, PV 1100. 2. Middle section, PV 2900. 3. Bottom section, PV 1300.

ketones in conjugation with a double bond while those at the higher frequency would be non-conjugated. At this time no interpretation other than possible aldehydes from scission products can be offered for the weaker band at 1755 cm^{-1} which seems to be more variable than the others. Reduction with KI reagent produced no consistent changes in the absorption in this region, thus indicating that carbonyl absorption is due to side reactions or further decomposition of the hydroperoxides formed.

An additional study was made in which autoxidized methyl linoleate was chromatographed on a sodium aluminum silicate adsorbent for comparison with observations made previously (2). The column was divided into three fractions, after development of the autoxidized esters on the column with petroleum ether as follows: 1st fraction 0-3 inches, 2nd fraction 3-9 inches, and the 3rd fraction 9-17 inches, the sections beginning at the top. The fractions were eluted with ethanol and ultraviolet absorption spectra determined as shown in Fig. 5. As pointed out in a previous paper (2), the substances adsorbed at the top of the column have the most highly adsorbable substances, that is, those having the most polar groups and the more complex molecules. Those on the middle section have fewer polyoxygenated compounds while those on the lower section have predominantly the fewest polar groups and should be composed of the monohydroperoxides, which would be in molecules with conjugated dienes, and the doubly unsaturated ketones and hydroxy-compounds resulting from intermolecular reaction of the hydroperoxides with other olefinic groups. These postulated locations on the column for the groups mentioned are substantiated by the low broad band at 2300 Å (Fig. 5) for the fraction from the top of the column indicating less conjugated-diene absorption than is shown for the fractions from the middle and bottom sections of the column. Further evidence is given by the intense absorption by the fraction from the bottom section with a sharp maximum at 2315 Å and also by its greater absorption at 2760Å, where doubly unsaturated ketones absorb.

The broad intense absorption band in the 3400-3500 cm^{-1} frequency range (Fig. 6) indicates many hydroxyl and/or hydroperoxyl groups with strong "association" in the fraction from the top section of the adsorption column. This follows from the concept that the most highly oxygenated, polymerized, and complex products would be adsorbed at the top of the column. The fraction from the middle section has the highest PV and has a broader, more intense band than that from the lowest section of the column. Since the fraction from the bottom section was shown to have a high concentration of conjugated dienes by its intense absorption band at 2315 Å, it follows that it should have a high concentration of monohydroperoxides and monohydroxy compounds. This is brought out by the fact that the 3470 cm^{-1} band is present but is still a broad band, and by the inflection which is almost a maximum at 3520 cm^{-1}, characteristic of the —OH band which was shown to exist when —OOH groups were reduced to —OH groups with HI.

An examination of the 1700-1750 cm^{-1} region shows intense carbonyl absorption other than that for the ester carbonyl (Fig. 7). All fractions exhibit maxima at 1724 cm^{-1}. That from the bottom section of the column absorbs most intensely in comparison with maximum absorption (by this fraction) at 2760 Å. The infrared absorption spectra of these fractions thus correlate with the interpretations made from the ultraviolet absorption spectra (2).

Summary

A study has been made of the infrared absorption spectra of autoxidized methyl linoleate in samples ranging from PV 1 to PV 940 m.e./kg. Principal changes occur in the frequency range 3400-3550 cm^{-1} where bonded —OH groups absorb and at 1650-1775 cm^{-1} where $>c = o$ groups absorb.

Two maxima were observed in oxidized samples in the —OH absorption range: one sharp and distinct at 3467-70 cm^{-1} which increased in intensity with in-

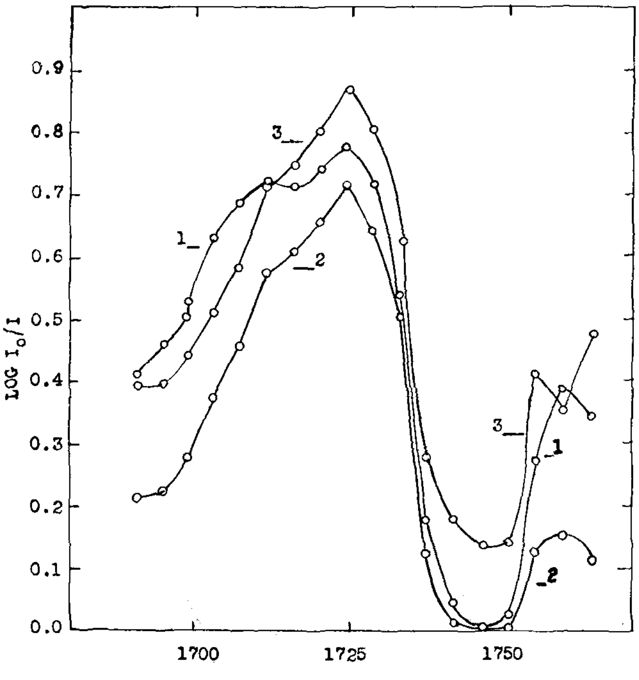


FIG. 7. Absorption by carbonyl groups other than ester carbonyl in substances eluted from adsorption column.

1. Top section, PV 1100. 2. Middle section, PV 2900. 3. Bottom section, PV 1300.

crease in PV and a broad band which increased with increasing PV until it resolved into a true maximum at 3430 cm^{-1}. Reduction of typical oxidized samples with KI reagent resulted in disappearance of the 3430 cm^{-1} band and appearance of a new band above 3500 cm^{-1}. The band at 3430 cm^{-1} was attributed to —OOH groups associated by hydrogen bridging. The band at 3467 cm^{-1} and the band appearing above 3500 cm^{-1} were attributed to —OH groups, the band at the higher frequency resulting directly from reduction of a hydroperoxide.

Absorption due to ketone and aldehyde carbonyl groups appeared only as an indefinite shoulder on the band due to the ester carbonyl. These were resolved by using the intensity of the sample with PV 1 as I_0 and that for the oxidized samples as I. A plot of Log I_0/I then revealed three maxima. These indicate the presence of two and possibly three carbonyl containing substances other than the ester carbonyl in autoxidized methyl linoleate.

Absorption in the two critical frequency ranges of fractions of autoxidized methyl linoleate eluted from an adsorption column correlate with interpretations made from ultraviolet absorption studies of the same substances.

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Determination of Bicarbonate in Soap Products

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CONTINUED need for the accurate determination of bicarbonate in the presence of carbonate and alkali salts such as phosphates and silicates has led to the development of an improved procedure yielding quicker and more accurate results than can be obtained by the procedure originally described by Blank and Boggie (1). The latter procedure, as emphasized in the original paper, is subject to errors due to the solubility of bicarbonate in alcohol and to the decomposition of bicarbonate during the process of isolating the alcohol insoluble material required for the determination.

The method described herewith permits the determination of bicarbonate and carbonate directly in the presence of soap, thus avoiding any loss of bicarbonate due to solubility in alcohol or in virtue of its inherent instability.

Principle of Method

Essentially the method consists in heating the sample containing bicarbonate and carbonate to 200°C. for one-half hour in a dry flask connected to the usual carbon dioxide train. After obtaining the increase in weight of the carbon dioxide absorption tube due to the CO_2 liberated by the decomposition of the bicarbonate, water is added to the contents of the flask to dissolve the sample, the residual carbonate is decomposed by the addition of 20 ml. 1:1 HCl and the CO_2 is evolved by heating as described in the official method of the A.O.C.S. (2).

If bicarbonate is found to be present in a sample, any moisture value obtained by distillation must be corrected by subtracting the amount of water liberated by decomposition of the salt. Each gram of $NaHCO_3$ present yields 0.107 grams of H_2O.

The temperature at which $NaHCO_3$ is quantitatively decomposed has been variously reported (3, 4). More recently Waldbauer, McCann, and Tuleen (5) conclude that any sample of $NaHCO_3$ heated above 150°C. is completely converted into the carbonate. This temperature is considerably less than that formerly regarded as necessary for the quantitative conversion. It has been found in this laboratory that a temperature of 200°C. for a period of one-half hour is sufficient to accomplish the conversion to the extent of about 98%. Figure 1 presents a plot of per cent loss of CO_2 from $NaHCO_3$ against time in minutes at the two temperatures of 150°C. and 200°C.

Apparatus

The apparatus employed in this determination may be any one of the usual CO_2 trains (1, 2, 6) with but one difference. In this procedure the CO_2 evolution flask is heated by means of an electric heating mantle of the usual laboratory type, connected to a pyrometer so that the temperature can be controlled to 200 ± 10°C.

Procedure

Weigh 2.5 to 3.0 grams of the sample into a 300-ml. CO_2 evolution flask. Attach the flask to a carbon dioxide train and bring the absorption tube to constant weight by the slow passage of air through the train for 10 to 15 minutes. When the absorption tube has attained constant weight, attach it to the train and maintain the evolution flask at a constant temperature of 200° ± 10°C. for one-half hour. Turn off the current and continue to draw CO_2 free air through the apparatus for an additional 20 minutes, at the end of which time detach the absorption tube and weigh. The percentage of $NaHCO_3$ present in the original sample may be calculated as follows:

$$\frac{\text{Weight of } CO_2 \times 3.8184 \times 100}{\text{Sample Weight}} = \%\ NaHCO_3$$

Replace the absorption tube in the train. Add distilled water through the entrance funnel attached to the evolution flask until the level is slightly above the lower end of the entry tube. It may be necessary to apply slight suction through the train to accomplish this operation.

Add 20 ml. of 1:1 HCl containing a few drops of methyl orange indicator to the sample in the flask. If the solution in the flask is not distinctly acid, add sufficient additional 1:1 HCl to bring the solution to distinct acidity. Increase the electric current until smooth boiling is effected. After boiling is initiated, the power may be decreased to the point at which it is just sufficient to maintain steady boiling. Boil the solution for 10 to 15 minutes. Turn off the current and continue to draw CO_2 free air through the apparatus for an additional 30