A FURTHER STUDY ON THE COMPOSITION OF AMERICAN TUNG OIL WITH SPECIAL REFERENCE TO THE LINOLEIC ACID CONTENT

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Abstract

The complete separation of elaeostearic acid from linoleic acid by the irradiation and crystallization of tung oil fatty acids was found difficult if not impossible. The application of several cold alkaline permanganate oxidation procedures to samples of tung oil fatty acids indicated the presence of less than one per cent of linoleic acid in tung oil. A study of the reaction of maleic anhydride with alpha elaeostearic, beta elaeostearic acid, and with the alpha elaeostearic acid glyceride present in tung oil showed that this reagent does not react with them quantitatively but only to 86.6 per cent of the theoretical amount. It was found that the elaeostearic acid content of a tung oil can be calculated by dividing its diene value by 78.4, the determined diene value of pure alpha elaeostearic acid. In this way it was calculated that two samples of American tung oil contained 85.5 and 89.4 per cent of elaeostearic acid. It was found that alpha elaeostearic acid and the mixed fatty acids from tung oil when exposed to the air quickly underwent a change to form an extremely sticky material whose use as an adhesive for insecticides will probably soon be determined.

ABOUT two years ago, the results of a prevouis investigation (*ibid., 12,* 92 (1935)) of expressed American tung oil were reported by the authors. It will be observed that in this paper the percentages of fatty acids present in the tung oil were derived by using the thiocyanogen value, the calculated iodine number, and the quantity of oleic acid as indicated from the dihydroxy stearic acid isolated from experiments made according to the well known Lapworth and Mottran oxidation procedure. During that investigation no evidence was obtained of the presence of any linoleic acid, although attention was given at that time to this possibility.

Recently, H. P. Kaufmann and J. Baltes (Berichte, *69, 2676* (1936), who investigated samples of tung oil from several different sources, reported the presence of over 9 per cent of linoleic acid. In order to remove the elaeostearic acid, the mixed fatty acids of tung oil in a petroleum ether solution were exposed to ultra-violet rays three different times for 2½ hours. After each treatment the solution was cooled intensely and the beta acid removed by filtration. The residual acids in the final filtrate were brominated in the usual manner and a tetrabromide was finally obtained which melted at 113.5-114° and there was no depression of the melting point when mixed with pure linoleic tetrabromide.

As it appeared doubtful to us whether elaeostearic acid could be completely removed under these conditions, it was believed preferable to apply the modified Hazura alkaline permanganate method described by T. G. Greene and T. P. Hilditch (Biochem. J. *29,* 1555 (1935)) to the residual acids remaining after the removal of as much as possible of the elaeostearic acid in the beta form. These authors (loc. cit.) have shown that this oxidation method is capable of yielding about 38.5 per cent of the total linoleic acid present as recoverable tetrahydroxy stearic acids.

An experiment was made using 37.2 grams of fatty acids, separated from tung oil, which were dissolved in about 75 cc. of petroleum ether. After the addition of several milligrams of iodine as a catalyst, the mixture was irradiated for two hours and a half. Sufficient petroleum ether was added so that when the solution was heated, all of the beta acid was dissolved, leaving a very small quantity of oxidation products which were removed by filtration. The filtrate was placed in the refrigerator overnight. After filtering off the beta acid, the solution was concentrated to a small volume and cooled as before, which resulted in a further recovery of this acid. This treatment was repeated three times. The residual acids (4.2 g.) remaining after the removal of the solvent from the final filtrate gave a refractive index at 40° of 1.4809, whereas that for linoleic acid alone at this temperature is but 1.4614. Also, it should be kept in mind that the residual acids contain oleic and saturated acids which give refractive indices well below that of linoleic acid. Therefore it is evident that the mixture still contains a notable quantity of elaeostearic acid.

The residual fatty acids were then dissolved in an aqueous solution of potassium hydroxide and treated with a solution of potassium permanganate in accordance with the modified Hazura procedure. The solution was decolorized with sulphur dioxide and acidified with concentrated hydrochloric acid. The solid fatty acids were collected on a filter and treated with petroleum ether to remove the saturated acids, then with a small quantity of ethyl acetate to dissolve the dihydroxy acids. The undissolved precipitate was fractionally crystallized from ethyl alcohol. The first crop of crystals which weighed 0.1174 grams melted at 173.5°. A combustion analysis gave C 62.08 per cent, H 10.44 per cent, calculated for $C_{18}H_{36}O_6$——C 62.02 per cent and H 10.41 per cent. A second crop was obtained which weighed .0572 grams and melted at 159°; by analysis it gave C 61.90 per cent and H 10.64 per cent. The total quantity of tetrahydroxy acids separated was equivalent to 0.95 per cent of linoleic acid in the original oil on the assumption, as previously mentioned, that the yield of hydroxy acids is equivalent to 38.5 per cent of the total linoleic acid present, when using the modified Hazura method. The acid filtrate from the Hazura oxidation was neutralized and evaporated to dryness. This residue was extracted several times with ethyl alcohol. The alcoholic extracts were combined and evaporated to dryness. The residue was acidulated with 1:4 hydrochloric acid and the fatty acids extracted with ethyl ether. The dried ethereal solution was evaporated, yielding 0.2805 grams of residual acids which had a strong odor of valeric acid. This residue was treated with petroleum ether and the insoluble acids collected on a filter. Attempts to crystallize this material from various solvents failed. It gave a neutralization equivalent of 167.2 and the following results by combustion: C 62.20%, H 9.24% (calculated for $C_9H_{16}O_3$, mol. wt. 172.1, C 62.8% and H 9.30%). As this acid failed to react with acetyl chloride, obviously the third oxygen is not in the hydroxyl form.

The alcohol insoluble residue was acidified with dilute hydrochloric acid, then extracted with ethyl ether. The ethereal solution was washed free of mineral acid, dried with anhydrous sodium sulfate and evaporated to dryness, yielding 0.4083 grams of residue. This material was insoluble in water and petrolic ether, but very soluble in chloroform. Attempts to crystallize this substance were not successful, nor could the acetyl derivative be formed. It gave a neutralization equivalent of 128 by titration and analysis of C 60.20, H 8.79, calculated for $C_{13}H_{22}O_5$, mol. wt. 258.2, C 60.50, H 8.53.

In our recent paper on Japanese tung oil (Oil and Soap, *14,* 2 [1937]), it was shown that the proportions of elaeostearic and oleic acids in oils of the genus Aleurites could be calculated from the Rosenmund-Kuhnhenn iodine value and the thiocyanogen value, provided that there was no linoleic acid present. Even in the presence of linoleic acid these calculations hold good for the saturated and oleic acids, but the value X in the equations will yield the content of elaeostearic plus linoleic acid. American tung oil was found to have an iodine value of 162.0 (R. and K.) and a thiocyanogen value of 82.75. Inserting these values in the two equations give the following results:

(1) $182.4X + 89.9Y = 16200$
(2) $91.2X + 89.9Y = 8275$

$X = 86.8\%$ of elaeostearic and linoleic acids
$Y = 3.89\%$ oleic acid
Saturated acids $= 95.6 - (86.8 + 3.89 + 0.47) = 4.44\%$
Found 4.40% (Bertram)

A volumetric modification of the Lapworth and Mottram oxidation procedure which was recently devised in this laboratory was applied to known quantities of oleic, linoleic, linoleic acids, and to the mixed fatty acids from tung oil. The method is based upon the titration of the potassium salts of the fatty acid in a very dilute cold alkaline solution with an approximately 1 per cent aqueous solution of potassium permanganate. It was calculated that one gram of oleic acid would require 37.35 cc. of 1 per cent solution of potassium premanganate.

In the first experiment, 0.331 grams of Kahlbaum's oleic acid was dissolved in 100 cc. of 0.5 per cent potassium hydroxide solution, 800 cc. of water were added and the mixture transferred to an evaporating dish, 10 inches in diameter, and cooled to 10° C. The standard permanganate solution was added slowly, during which the solution was vigorously stirred. The solution throughout the titration was maintained at 10°. As the end point was approached, from time to time, a small portion (about 1 cc.) of the solution was pipetted and placed in a test tube. The test portion was acidified with acetic acid and filtered to remove the precipitated manganese. The end point of this titration was reached when the acidified filtrate of a test portion showed a faint pink color. The titration required 13.0 cc. of permanganate solution. It was calculated that this weight of oleic acid would consume 12.6 cc. of the permangante solution if the reaction stopped with the formation of dihydroxy stearic acid. Applying this method to the mixed fatty acids of Pataua palm oil (OIL & SOAP, *11,* 208 [1934]), which contains 80.7 per cent of oleic acid and 3.7 per cent linoleic acid, it was found that 0.3250 grams of mixed acids required 11.0 cc. of the permanganate solution. On the basis of the composition of the fatty acids, it was calculated that the portion taken would consume 11.1 cc. of permanganate solution if the respective reactions were completed at the formation of the di and tetrahydroxy stearic acids. A third experiment was made with 0.3245 grams of the unsaturated fraction of fatty acids separated from a sample of apricot kernel oil (OIL & SOAP, *10,* 147 [1933]), consisting of 66.9 per cent of oleic acid and 33.1 per cent of linoleic acid, required 17.0 cc. of permanganate solution. It was calculated that this quantity of fatty acids would require 16.3 cc. of permanganate solution.

Applying this method to 0.1990 grams of pure beta-elaeostearic acid (M. P. 71.5°), 43 cc. of the permanganate solution was used, which is 5.67 times the quantity required by an equal weight of oleic acid. Another experiment in which 0.1032 grams of alpha-elaeostearic acid (M.P. 47°) was taken required 19.5 cc. of permanganate solution, which is about 5.0 times that used by the same weight of oleic acid.

The fatty acids of tung oil, weighing 0.1090 grams, were found to require 19.0 cc. of the permanganate solution. These mixed fatty acids contained 0.47 per cent of unsaponifiable matter, 4.40 per cent of saturated acids, 4.15 per cent of oleic acid, leaving 90.98 per cent of other unsaturated acids. Two equations can now be formulated with which the approximate percentage of linoleic and elaeostearic acids can be calculated as follows:

(3) $37.35 \times$ weight of sample $\times (x + 2y + 5z) =$ cc. of 1% $KMnO_4$ Sol.
(4) $x + y + z =$ proportions of unsaturated acids in samples.
$x =$ proportion of oleic acid in sample of mixed fatty acids which was calculated by the use of the thiocyanogen and R and K iodine number equations previously given.
$y =$ proportion of linoleic acid.
$z =$ proportion of elaeostearic acid.

The solution of these equations (3 and 4) indicates that the mixed fatty acids of this tung oil contain 90.44 per cent of elaeostearic acid and 0.58 per cent of linoleic acid. On the basis of the original oil, the results are as follows: saturated acids 4.4 per cent, oleic acid 3.9 per cent, linoleic acid 0.6 per cent and elaeostearic acid 86.3 per cent.

The reaction of potassium permanganate with linolenic acid was also examined. For this purpose the unsaturated acids from lumbang oil were used. These consisted of 30.3 per cent of oleic, 45.8 per cent of linoleic and 24.1 per cent of linolenic acid. It was found that 0.2185 grams of the sample consumed 18.0 cc. of the standard permangante solution. As it has previously been shown that oleic and linoleic acids react with theoretical amount of oxygen required to form the di and tetrahydroxy stearic acids, it was possible to calculate the quantity of oxygen used by the linolenic acid. It was found that it consumed four times as much oxygen as that by an equivalent weight of oleic acid.

The consumption of oxygen by linolenic, alpha-elaeostearic and beta-elaeostearic acids in the alkaline oxidation procedure was found to be 4.0, 5.0 and 5.7 times as much as that consumed by oleic acid. This would indicate that, in addition to the formation of the hexahydroxy compound, there is a decomposition of these acids at the double bonds with the formation of smaller molecular weight acids. In the modified Hazura procedure, which is somewhat similar to this procedure, we were able to obtain evidence of the formation of two oxygenated acids, a monocarboxylic nine carbon acid and a dicarboxylic thirteen carbon acid, as well as traces of valeric acid.

In connection with the separation of alpha-elaeostearic acid, we were particularly impressed with the great rapidity with which this acid underwent a change, forming an extremely sticky compound. This change was found to be accelerated by increased temperature and by exposure to sunlight, but took place more slowly even at icebox temperature in the dark. This sticky material, which exhibited certain water-resistant properties, appeared promising for use as an adhesive. It is understood that there is a need for such a compound to be used in the application of insecticides and fungicides so that they will stick to the vegetation.

In several recent investigations on tung oil (Kaufmann and Baltes, loc. cit.; B. A. Ellis and R. A. Jones, Analyst *61,* 812 [1936]), the elaeostearic acid was estimated by the use of the diene value. The percentage of elaeostearic acid was much lower than that obtained in this study or that given in our previous article dealing with the composition of tung oil (loc. cit.). Their calculations depend upon the assumption that the reaction of alpha-elaeostearic acid glycerides in tung oil with maleic anhydride reagent is quantitative. Our inability to find such large quantities of oleic and linoleic acids in tung oil, as already shown by us, raises the question as to the quantitative character of the diene method when applied to the examination of tung oil.

In order to investigate this matter, an experiment was made in which 8.85 grams of tung oil fatty acids were heated for several hours with 3.0 grams of maleic anhydride in a sealed tube, placed in a steam bath. After cooling, the tube was opened, the contents were transferred to a flask containing distilled water and allowed to stand over night. Then the insoluble residue was filtered and washed free from maleic acid. The residue was repeatedly digested with petroleum ether so as to remove all of the fatty acids which had not reacted with the maleic anhydride. After the removal of the solvent, the recovered fatty acids weighed 1.79 grams, which is equivalent to 19.5 per cent of the fatty acids originally taken for this experiment. The refractive index at 20° C. of these acids was 1.4800, which indicated that the mixture contained a considerable quantity of elaeostearic acid. They gave a diene value of only 5.3, which indicated that the larger part of the elaeostearic acid present was in a form not capable of combining with maleic anhydride.

In view of the fact that it had been previously shown, as already mentioned, that alpha and beta elaeostearic acids do not react in the same manner with a dilute alkaline permanganate solution, it appeared desirable to compare the reactions of these two acids with maleic anhydride. For this purpose, the pure alpha acid which melted at 48.6° was prepared from Florida tung oil according to the method suggested by B. H. Nicolet, J. Amer. Chem. Soc., *43, p.* 938 (1921). The thiocyanogen value of the acid was 91.4 (calculated 90.5) and the iodine number with the Rosenmund and Kuhnhenn reagent reacting for 30 minutes was 181.3 (calculated 181.0). By following the method of Kaufmann and Baltes, a diene value of 78.4 was obtained, whereas the calculated value is 90.5. It was found that the pure beta acid melting at 70.4° gave a thiocyanogen value of 91.2, an iodine number (R. and K.) of 182.4, and a diene value of 78.4.

The use of the diene value of tung oils for the estimation of their elaeostearic acid content obviously depends upon the quantitative reaction of the alpha (acid) glycerides with maleic anhydride. It has already been shown that the beta (acid) glyceride does react in a quantitative manner with the reagent. This was confirmed by the present investigators who found that the pure beta (acid) glyceride melting at 61.80° gave a diene value of 87.0; the calculated value being 87.2.

On account of the difficulty of isolating the unstable alpha acid glycerides in a pure condition, it was considered preferable to determine its reaction with maleic anhydride by indirect means. First, the diene value was determined upon a sample of the tung oil and found to be 67.0. Then the mixed fatty acids were separated from this oil, taking every precaution to protect them from change. The diene value of these acids amounted to 69.3.

If the alpha (acid) glycerides in the oil reacted to the same extent with the reagent as did the alpha acid present in the mixed acids, then the latter should give a decidedly higher diene value than that of the original oil. A comparison of the diene values of the mixed fatty acids and the oil show conclusively that the reaction of the reagent is closely similar in both cases. Therefore, the elaeostearic acid content of a tung oil can be ascertained by dividing the diene value of the oil by 78.4 (the determined diene value of pure alpha elaeostearic acid). On this assumption, it was calculated that this oil from the 1934 tung fruit crop contains 85.5 per cent of elaeostearic acid. Similarly, it was estimated that a sample of tung oil from the 1936 crop produced in Pearl River County, Mississippi which gave a diene value of 70.1, contained 89.4 per cent of elaeostearic acid.

SUMMARY

The complete removal of elaeostearic acid from tung oil fatty acids by irradiation and crystallization was found difficult if not impossible. The permanganate oxidation of the residual fatty acids from such a treatment by the modified Hazura procedure yielded small amounts of tetrahydroxy stearic acids, indicating the present of 0.95 per cent of linoleic acid in this tung oil.

A volumetric modification of the Lapworth and Mottram alkaline permanganate oxidation procedure has been worked out and applied to known quantities of oleic, linoleic, linolenic acids, and to the mixed fatty acids from tung oil. Equations were formulated for this reaction from which the approximate percentage of linoleic and elaeostearic acids in tung oil can be calculated. Application of this formula indicated the presence of 0.6 per cent linoleic acid and 86.3 per cent of elaeostearic acid in the tung oil studied.

A study of the reaction of maleic anhydride with alpha elaeostearic acid, beta elaeostearic acid, and with alpha elaeostearic acid glycerides present in tung oil showed that this reagent does not react with them quantitatively but only to 86.6 per cent of the theoretical amount. The elaeostearic acid content of a tung oil can be calculated by dividing its diene value by 78.4, the determined diene value of alpha elaeostearic acid. In this way it was calculated from their diene values that two recent samples of tung oil from Florida and Mississippi contained 85.5 and 89.4 per cent of elaeostearic acid.

It was found that alpha elaeostearic acid and the mixed fatty acids from tung oil when exposed to the air quickly underwent a change to form an extremely sticky material which may prove useful as an adhesive and its use for insecticides and fungicides will probably soon be determined.