be concluded that the Diels-Alder adduct of divinyl sulfone and eleostearic acid exists as a transannular sulfone (III).

Summary

The reaction of divinyl sulfone with *alpha*-eleostearic acid has been studied. The reaction product was found to be monomeric, consisting of only one divinyl sulfone and one eleostearic moiety. Contrary to expectations, the adduct contained only two ethylenic linkages rather than three and the addition product is considered to be a transannular sulfone.

Acknowledgment

The authors are indebted to R. T. O'Connor for interpretation of the infrared curves and to L. E. Brown for the elemental analyses.

REFERENCES

1. Hoffmann, J. S., O'Connor, R. T., Magne, F. C., and Bickford, W. G., J. Am. Oil Chemists' Soc. *32*, 533–538 (1955).
2. Hoffmann, J. S., O'Connor, R. T., Magne, F. C., and Bickford, W. G., J. Am. Oil Chemists' Soc., *33*, 410–414 (1956).
3. Bickford, W. G., Hoffmann, J. S., Heinzelman, Dorothy C., and Fore, S. P., J. Org. Chem., *22*, 1080–1083 (1957).
4. Placek, L. L., and Bickford, W. G., J. Am. Oil Chemists' Soc., *36*, 463–466 (1959).
5. Alder, K., Rickert, H. F., and Windemuth, E., Ber., *71*, 2451–2461 (1938).
6. Teeter, H. M., O'Donnell, J. L., Schneider, W. J., Gast, L. E., and Danzig, M. J., J. Org. Chem., *22*, 512–514 (1957).
7. Hoffmann, J. S., O'Connor, R. T., Heinzelman, Dorothy C., and Bickford, W. G., J. Am. Oil Chemists' Soc., *34*, 338–342 (1957).
8. Ramsey, L. L., and Patterson, W. I., J. Assoc. Offic. Agr. Chemists, *31*, 139–150 (1948).
9. Pack, F. C., Planck, R. W., and Dollear, F. G., J. Am. Oil Chemists' Soc., *29*, 227–228 (1952).
10. Kaufmann, H. P., "Studien auf dem Fettgebiet," p. 23, Verlag Chemie, Berlin, 1935.
11. Findlay, A., "Practical Physical Chemistry," 7th ed., p. 125, Longmans, Green and Company, New York, 1941.
12. Placek, L. L., Magne, F. C., and Bickford, W. G., J. Am. Oil Chemists' Soc., *36*, 651–652 (1959).
13. Kohler, E. P., and Potter, H., J. Am. Chem. Soc., *57*, 1316–1321 (1935).
14 Alexander, J. R., and McCombie, H., J. Chem. Soc., *1931*, 1913–1918.
15. Freiman, A., and Sugden, S., J. Chem. Soc., *1928*, 263–269.
16. Sutton, L. E., New, R. G. A., and Bentley, J. B., J. Chem. Soc., *1933*, 652–658.
17. Weitz, E., and Scheffer, A., Ber., *54*, 2327–2344 (1921).

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Morro Seed Oil[1]

MARIO LEWY VAN SÉVEREN, Servicio Cooperativo Agrícola Salvadoreño-Americano, Centro Nacional de Agronomía, Santa Tecla, El Salvador

THE MORRO TREE, *Crescentia alata* HBK, which belongs to the family *Bignoniaceae*, grows to a height of 4 meters and produces globose fruits that are borne directly on trunk and branches. These fruits, 15 cm. in diameter, are brownish yellow when ripe, contain a pulp with a characteristic agreeable odor, and have a sweetish taste that is very palatable to livestock. The seeds, which are contained in the pulp, are dark brown and flat, have a heart shape, and are 8 mm. long, 6 mm. thick; the hull is thin and the kernel yellowish white.

The average weight per indivudual seed is 40 mg., 73% of which represents the kernel and 27% the hull. With an average of 8% moisture the seeds contain 31% oil. They are utilized in El Salvador in the preparation of refreshments (called "horchata"), to which they impart their particular agreeable aroma. The seeds are sold in the markets for these purposes.

The Morro tree grows wild and is usually found growing in heavy clay soils (grumosols usually 1 or 2 meters deep over hardpan), which in spite of their level topography are not adapted to most other agricultural uses. These soils, which constitute about 3% of the agricultural land of El Salvador and which occupy large areas in other Central American countries, are generally used only for unimproved pastures consisting of native grasses. During the rainy season such soils become water-logged and, during the dry season, so dry that large cracks appear. Morro trees seem to be the climax vegetation on such soils.

The northernmost point of distribution of the Morro is Mexico while the southernmost is Colombia and Venezuela (4,5,6). The only known uses so far are that the pulp is eaten by livestock, the seeds are used for refreshments, and to a limited extent the hard epicarp is used as containers or cups by rural people.

As the fruits are attached to the trunk and branches, harvesting is done by hand. Some of the ripe fruits fall, and even though cattle can break the hard shell, the fruits are usually cut in halves and the pulp is left for the cattle to feed on.

The analysis of the whole pulp is shown in Table I.

TABLE I

Analysis of the Whole Pulp, Including Seeds, of Ripe Morro Fruits [a]

	%
Moisture	73.43
Proteins [b]	14.60
Fat [b]	13.00
Crude fiber [b]	6.75
Carbohydrates (by difference) [b]	56.35

[a] Determined by Official Methods of the A.O.A.C. (2).
[b] On dry-matter basis.

Laboratory Procedure. Hulls of the Morro seed can be separated from the kernel relatively easily by aspiration because they do not adhere to the kernel when dry.

Laboratory samples of Morro seed oil were prepared by pressing the ground kernels in a Carver hydraulic press at a temperature of 100°C. and at a pressure of 5,000 p.s.i. The moisture content of the seed when pressed was approximately 8%. Expression of the oil was easier when the moisture was increased to 10%.

Oil extracted with petroleum ether had about the same characteristics including color and flavor as the pressed oil.

[1] A contribution from Servicio Cooperativo Agrícola Salvadoreño-Americano, a technical agricultural service organization for El Salvador, operated jointly by the Government of El Salvador and the International Cooperation Administration.

No refining test was made as the organoleptic properties permit the use of the unrefined oil after filtering and, where necessary, neutralization. According to Squibb *et al.* (7), the oil has a coefficient of digestibility of 96.4 as determined on rats.

Characteristics of the Oil. The color of the oil, whether extracted by pressure or solvent, is light yellow in color, is a liquid at room temperature, and has the strong odor and flavor of olive oil.

TABLE II

Characteristics of Morro Seed Oil [a]

Characteristics	Value
Specific gravity 25/25°C.	0.913
Refractive index $N_D^{40°}$	1.4616
Free fatty acids as % oleic	1.1
Iodine value	90.2
Hehner value	4.–
Saponification value	190.–
Unsaponifiable matter %	1.21

[a] All characteristics were determined by the Official and Tentative Methods of the American Oil Chemists' Society (1).

Table II shows the characteristics of the oil, and Table III gives the fatty acids composition.

Morro seed oil resembles both olive oil and cotton seed oil, in composition. It differs specially in the amount of linolenic acid present. The acid is found in lesser quantities than is found in soybean oil. Calabash seed, *Crescentia cujete* (a closely related species), produces an oil the characteristics of which are similar to those of Morro seed oil (3).

TABLE III

Composition of Morro Seed Oil [a] (expressed as percentage of fatty acids)

Constituent	%
Oleic	61.8
Linoleic	15.0
Linolenic	2.3
Saturated	16.6
Conjugated diene	0.16

[a] Determined by A.O.C.S. Method Cd7-48 (1).

Discussion

The storage of Morro seed presents no special problems. One must take care against insect infestation as the seed may be attacked by the almond moth, *Ephestia cautella* (Wlkr).

The residual cake is rich in proteins and can be used both for human and animal consumption. Table IV gives the composition of the cake.

TABLE IV

Composition of the Residual Cake of Morro Seed [a]

Constituent	%
Moisture	10.25
Protein	40.60
Fat	8.16
Ash	6.69
Crude fiber	20.72
Nitrogen-free extract	13.58

[a] Determined as prescribed in Official Methods of Analysis, A.O.A.C. (2).

As the cake has the same flavor as the seed, it could be used for the preparation of beverages in the same manner as the seed.

Summary

The seeds of the *Crescentia alata*, known as Morro in El Salvador and other countries of Central America, contains 31% oil. This oil is light yellow with a strong odor and flavor of olive oil and does not need refining.

A fatty acid analysis of Morrow seed oil showed 61.8% oleic, 15.0% linoleic, 2.3% linolenic, and 16.6% saturated acids.

REFERENCES

1. American Oil Chemists' Society, Official and Tentative Methods, 2nd ed. revised, Chicago, 1957.
2. Association of Official Agricultural Chemists, Official Methods of Analysis, 8th ed., Washington, 1955.
3. Smith, B. A., and Dollear, F. G., J. Am. Oil Chemists' Soc., *24*, 52–54 (1947).
4. Standley, P. C., "Flora of Costa Rica," Field Museum of Natural History (Chicago), Pub. 392 Bot. Ser., Vol. 18 (1937).
5. Standley, P. C., "Trees and Shrubs of Mexico," Contrib. U. S. Natl. Herb., Vol. 23, Smithsonian Institution, Washington, D. C. (1920–26).
6. Standley, P. C., y Calderón, S., "Lista Preliminar de Plantas de El Salvador," 2nd ed., Universidad de El Salvador (1941).
7. Squibb, Robert L., Love, H. T., and Wyld, M. K., J. Nutrition, *44* (4), 547–553 (1951).

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Malonaldehyde Production During the Controlled Oxidation of Pure, Unsaturated Fatty Acids[1]

BASIL G. TARLADGIS[2] and BETTY M. WATTS,[3] Department of Food and Nutrition, Florida State University, Tallahassee, Florida

THE MODIFICATION of the 2-thiobarbituric acid (TBA) method to allow the quantitative determination of malonaldedyde in rancid foods (6) enables independent workers to compare quantitatively TBA numbers obtained from different foods and to correlate them with taste-panel results. These comparisons would have more significance however if quantitative data existed also on the TBA values of pure unsaturated fatty acids, which are believed to be responsible for the production of malonaldehyde in rancid foods containing them.

Wilbur *et al.* (8) followed the effects of ultraviolet radiation and different catalysts on the TBA-chromogen production from unsaturated fatty acids and their esters. No independent measurement of fat oxidation was used. TBA values of the catalyzed

[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 2014 in the series of papers approved for publication.
[2] Present address: Central Food Research Laboratories, Libby McNeill and Libby Company, Blue Island, Ill.
[3] Some of this work was conducted at the American Meat Institute Foundation during the period of the Frank C. Vibrans Fellowship held by this author during the summer of 1958.

oxidation of the fatty acids showed that linolenic acid reached the highest value, followed by arachidonic and linoleic. TBA values of oleic acid were negligible.

Kenaston, Wilbur, *et al.* (2) in a more recent paper have compared the extent of oxidation of unsaturated fatty acids as measured by peroxides, aldehydes, diene conjugation, and the TBA method. Although their TBA method was not quantitative, they were able to show that this method is more sensitive and precise than peroxide for linolenic and linoleic acids, at least for the early stages of oxidation that they have followed.

Studies in our laboratories on TBA values of cooked meats and fishery products over the past several years have frequently shown a fall of TBA from earlier higher values (7, 9). Such experimental evidence suggests that TBA values reach a peak during storage, which may be correlated with oxygen uptake. If this is true, then the malonaldehyde precursor does not accumulate as a stable end-product.

Similar studies have also shown that fish products in sealed cans reached TBA numbers in the range 6–9 and maintained these values over long storage periods (7). After the cans were opened, the TBA numbers increased to peaks of 30–40. It is possible therefore that oxygen is necessary not only for the production of the malonaldehyde precursor but also for its decomposition.

The TBA reaction mixture from several food products gives another peak at 450 mμ. This peak is not observed with malonaldehyde-TBA chromogen, but it was noted with unsaturated fatty acids (7). Landucci *et al.* (4, 5) have postulated that the TBA-reductone chromogen undergoes rearrangement in several steps upon standing, giving rise not only to a 450 mμ peak but to other peaks also. If this is true, then absorption at 538 mμ (malonaldehyde-TBA complex) should decrease upon standing and the 450 mμ peak should increase or other peaks should appear.

The purpose of this study is to measure quantitatively the production of malonaldehyde during the controlled oxidation of pure, unsaturated fatty acids and correlate it with their off-odor. Absorption at 450 mμ, obtained when the oxidation products of the fatty acids are reacted with TBA, will be explored also.

Materials and Methods. Pure oleic, linoleic, linolenic, and arachidonic acids were obtained from the Hormel Institute. The fatty acids were emulsified in 0.1 M phosphate buffer pH 6.0, using 0.2 g. of Tween 20 (Atlas Powder Company) in 30 ml. of buffer. The concentration of fatty acids was 0.1 mmol per ml.

In the case of oleic acid, 1% linoleic acid was added to the oleic, before the preparation of the emulsion, and 1 p.p.m. of copper in the form of copper sulfate was added to the emulsion in order to speed up the oxidation. The concentration of oleic acid was also 0.1 mmol per ml.

The oxidation was carried out in the Warburg apparatus at 45°C. Three ml. of the emulsions were used in each Warburg vessel. The contents of individual vessels (0.3 mmol of fatty acid) were removed at various stages during the oxidation, made up to 100 ml. with distilled water and sufficient 2:1 HCl to bring the pH to 1.5 and distilled as described by Tarladgis *et al.* (6). The TBA reaction was performed on the distillates, and the TBA numbers were expressed as mg. of malonaldehyde in 1,000 g. of fatty acid. Absorption of the TBA reaction mixture was

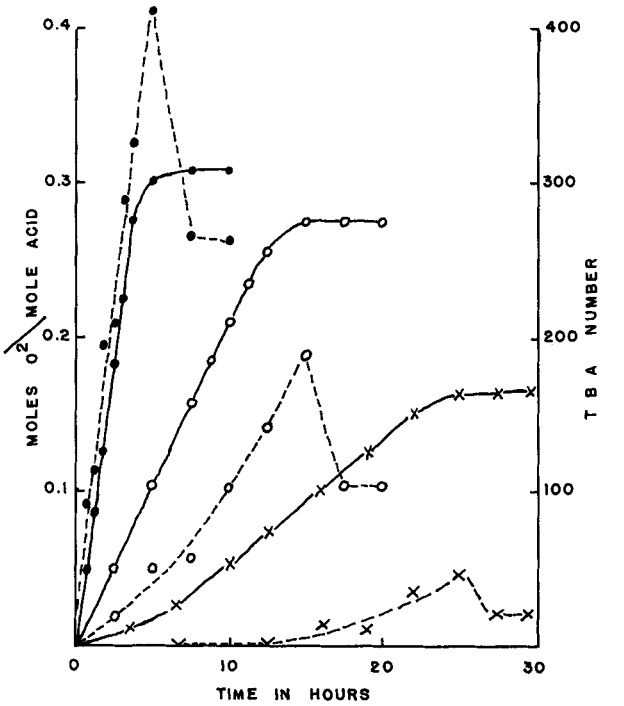

FIG. 1. Oxygen uptake of unsaturated fatty acids and production of malonaldehyde.

Oxygen uptake (———) TBA number (- - -)

● — Linolenic acid
○ — Linoleic acid
X — Oleic acid (catalyzed)

read not only at the malonaldehyde peak at 538 mμ but at 450 mμ also. The readings at these wavelengths were repeated at intervals up to 10 days.

Odors of the distillates obtained for the TBA test at various oxidation levels were compared with each other, with distillates from meat having high tissue rancidity, and with distillates from 1,1,3,3-tetraethoxypropane (yielding malonaldehyde upon acid hydrolysis).

Results. Figure 1 shows the oxygen uptake and malonaldehyde produced by oleic, linoleic, and linolenic acids oxidized under normal atmospheric conditions. The TBA numbers of all acids reached a peak at about the time that the rate of oxidation began to decline. There was very little decline in the TBA numbers following the peak.

It is believed that the oxidation of linoleic and linolenic acids in these experiments was inhibited in the latter stages by depletion of oxygen within the Warburg vessels. The same reason may account for the leveling off of the TBA numbers after the peak is reached. The experiments were repeated therefore, exactly as before, but under an atmosphere of oxygen, *i.e.*, pure oxygen was flushed through the vessels each time the manometers were reset. Instead of oleic acid, in the oxidation of which oxygen was not believed to be a limiting factor, arachidonic acid was included in this series of experiments. The results are shown in Figure 2.

The shapes of these curves are similar to those obtained by Holman and Elmer (1) and Kern *et al.* (2), but the oxidation of the fatty acids was followed to completion. Peak TBA numbers of linolenic are double those of linoleic and approximately 24 times higher than the values obtained in a catalyzed oxidation of oleic acid. TBA numbers of arachidonic lay between

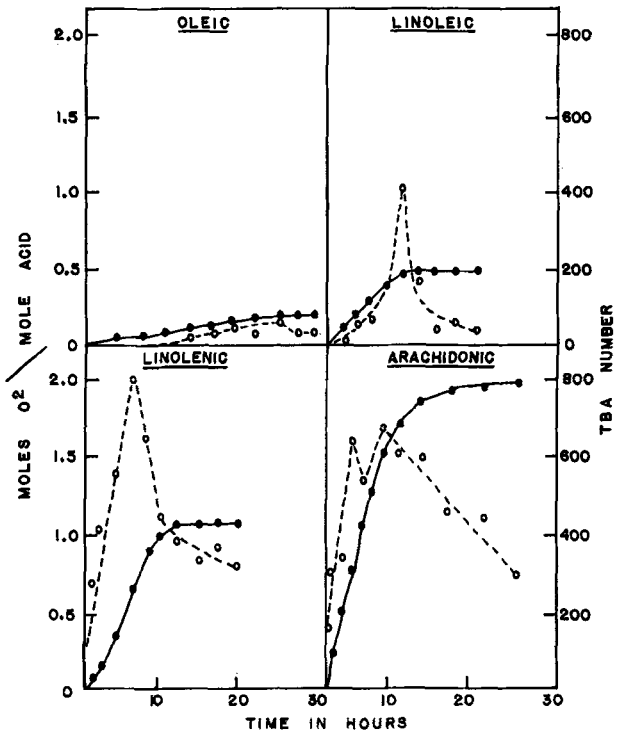

FIG. 2. Oxidation of pure, unsaturated fatty acids in excess of oxygen at 45°C.
TBA number - - - - Oxygen uptake ———

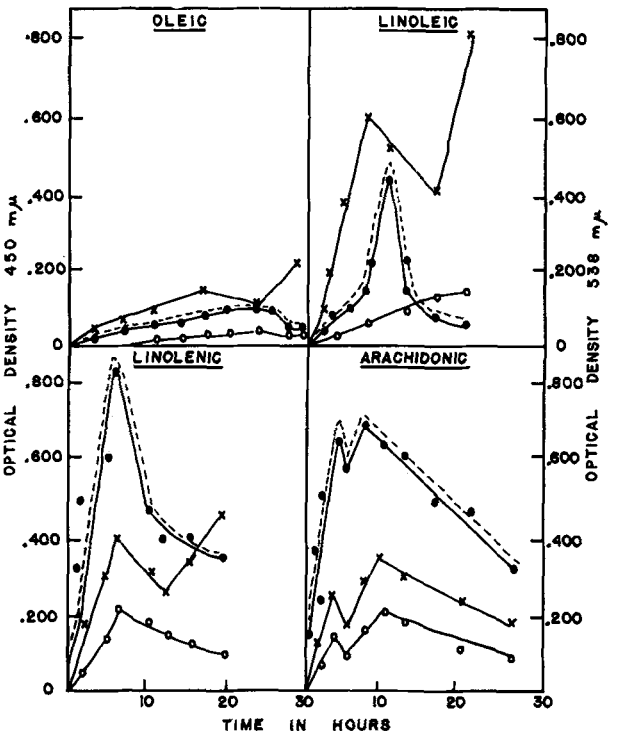

FIG. 3. Comparison of absorption at 450 mμ and 538 mμ of TBA-fatty acid reaction mixture. The abscissa represents progressive oxidation in the Warburg (for oxygen uptakes refer to Figure 2).
Initial optical density at 538 ●-●-●
Initial optical density at 450 ○-○-○
Optical density at 450 after TBA reaction mixture had stood 120 hrs. X-X-X
Optical density at 538 after the TBA reaction mixture had stood 120 hrs. ----------

those of linolenic and linoleic, respectively. This is in agreement with the findings of Wilbur *et al.* (8) for the ultraviolet radiation-catalyzed oxidation of the same fatty acids. In the early stages of oxidation, of particular concern in many food products, the malonaldehyde produced by linolenic and arachidonic acids is many times that produced by linoleic acid. This is in agreement with the findings of Kenaston *et al.* (2).

Comparison of Figures 1 and 2 shows the limiting effect of oxygen depletion on oxygen uptake and TBA numbers of linoleic and linolenic acids. The increase of the peak TBA numbers of linolenic acid is not proportional to the increase of the oxygen uptake. This, as well as the lower TBA numbers of arachidonic acid, is believed to be caused by polymerization of these two highly unsaturated fatty acids. The formation of a yellow insoluble polymer was observed in the Warburg vessels at the late stages of oxidation. This polymer was removed by the strong acid used in the distillation-TBA method and included in the quantity to be distilled.

Odor of Distillates. With distillates from linoleic, linolenic, and arachidonic acids the odor was at a maximum when the TBA numbers were at their peaks and declined in intensity beyond the peak. Distillates from oleic acid did not have much of an odor difference, and the odor intensity was much lower.

Qualitatively the odor of the distillates from arachidonic and linoleic acid resembled those from the rancid meat. The odor of the linoleic acid distillate was still detectable after diluting 1:10,000. The odors of the distillates from linolenic and oleic acids closely resembled the odor of malonaldehye; the former was much stronger than the latter.

Absorption at 450 mμ. Figure 3 compares the absorption at 450 mμ and 538 mμ of the TBA-fatty acids reaction products. Progressively increasing absorption at 450 mμ is observed with all acids when the TBA chromogen is allowed to stand at room temperature. After several days the solution appears yellow rather than pink in color. The total absorption at 450 mμ becomes much higher than at 538 mμ in the cases of oleic and linoleic acids.

During this period of standing, optical density at 538 mμ increased only about 10%, and this during the first two days of standing. No peaks were observed at any other wavelength.

Discussion

The above experiments show clearly that malonaldehyde production during the oxidation of pure unsaturated fatty acids under controlled conditions follows very closely their oxygen uptake, reaching a peak at the same time the oxygen uptake starts declining. It becomes evident therefore that the malonaldehyde precursor does not accumulate as a stable end-product but that, after reaching the peak, more precursor is destroyed than produced. Oxygen seems to be a limiting factor not only for the oxidation of the fatty acids but for the destruction of this precursor also.

The distillates from different foods and from pure unsaturated fatty acids contain a compound which reacts with TBA to give a peak at 450 mμ. This peak is not observed with pure malonaldehyde solutions, and the compound responsible for it has not been identified. It has been shown that, upon standing of the TBA-fatty acid reaction mixtures, this peak progres-

sively increases while the peak at 538 mμ (malonaldehyde) increases very slightly. This is thought to be due to the slow liberation of a compound, which reacts with the excess TBA present in the solution to give rise to the complex absorbing at 450 mμ. It is not due to the rearrangement of the malonaldehyde-TBA Complex, as postulated by Landucci (4), since there is no decrease in absorption at 538 mμ.

Summary

The quantitative production of malonaldehyde during the oxidation of pure, unsaturated fatty acids under controlled conditions, has been measured and correlated with their off-odor. It was found that malonaldehyde does not accumulate as a stable end-product of fat oxidation but reaches a peak at the same time that oxygen uptake begins declining. Oxidation products of all of the fatty acids investigated reacted slowly with TBA to give a compound absorbing at 450 mμ in addition to the malonaldehyde-TBA complex absorbing at 538 mμ.

REFERENCES

1. Holman, R. T., and Elmer, O. C., J. Am. Oil Chemists' Soc., *24*, 127 (1947).
2. Kenaston, C. B., Wilbur, K. M., Ottolenghi, A., and Bernheim, F., J. Am. Oil Chemists' Soc., *32*, 33 (1955).
3. Kern, U. W., und Willersin, H., Makrom. Chem., *15*, 1 (1955).
4. Landucci, J. M., Bull. Soc. Chi. France, *22*, 857 (1955).
5. Landucci, J. M., Pouradier, J., and Durante, M. A., Compt. Rend., *149*, 919 (1955).
6. Tarladgis, B. G., Watts, Betty M., Younathan, M. T., and Dugan, L. R. Jr., J. Am. Oil Chemists' Soc., *37*, 44 (1960).
7. Unpublished data from this laboratory.
8. Wilbur, K. M., Bernheim, F., and Shapiro, O. W., Arch. Biochem., *24*, 305 (1949).
9. Younathan, M. T., and Watts, Betty M., Food Res., *25*, 4 (1960).

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• *Letter to the Editor*

Preparation of 8t,10t-Octadecadienoic Acid

IN THE JANUARY ISSUE of this Journal Gupta and Kummerow (1) report the preparation of 8t,10t-octadecadienoic acid by the bromination of oleic acid with N-bromosuccinimide and free bromine, followed by debromination with zinc in ethanol. Their method results in a 15–20% yield and therefore represents a considerable improvement over previous procedures, making this acid as easily accessible as the corresponding isomers of 9,11- and 10,12-octadecadienoic acids.

Their review of previous literature however indicates that they were not aware of the fact that this acid, which Smit (2) originally thought to be an isomer of 9,11-octadecadienoic acid and which was later identified as the 10,12-compound by von Mikusch (3), has also been prepared by us from methyl oleate and methyl elaidinate *via* allylic bromination, followed by dehydrobromination with collidin (4). They furthermore refer to the work of Schmidt and Lehmann (5) and, like these authors, have obtained a lower-melting product thought to be a 9,11-octadecadienoic acid. This work however has been rechecked by von Mikusch (3), with the result that no evidence was obtained for the formation of the supposed 9,11-octadecadienoic acid melting at 32–33° and that this product evidently was an eutectic mixture of higher melting *trans,trans*-acids. It is therefore unlikely that the small amount of conjugated acid, m.p. 33–34°, obtained by Gupta and Kummerow is 9,11-octadecadienoic acid.

In this connection it seems appropriate to supplement further the list of known conjugated octadecadienoic acids given by the recent authors by referring to the two *cis,trans*-isomers, which have also been obtained pure and identified, *i.e., trans,cis*-10,12-octadecadienoic acid, m.p. 23°, and *cis,trans*-9,11-octadecadienoic acid, m.p. 20°.

The former was first described by Nichols *et al.* (6) and independently by von Mikusch, who thought this compound had a *cis,cis*-structure (7) but later accepted the view of Nichols *et al.* (8). Nichols *et al.* also obtained a fraction, m.p. −6 to 3°, which was thought to be the impure *cis,trans*-9,11-isomer. By a similar procedure, *i.e.,* extensive crystallization of alkali-isomerized linoleic acid, von Mikusch obtained the pure *cis-trans*-9,11-octadecadienoic acid, m.p. 20°, $n^{25/D} = 1.4810$. The structure of both of these compounds was characterized by diene and pandiene values and by the maleic anhydride adducts, which may well serve for identification purposes. Adducts for the three positional isomers have the following melting points:

M.A.-adduct of 8,10-octadecadienoic acid m.p. 110°C.

M.A.-adduct of 9,11-octadecadienoic acid m.p. 94.5°C.

M.A.-adduct of 10,12-octadecadienoic acid m.p. 102°C.

J. D. VON MIKUSCH, Unilever Research Laboratory (p.A. F. Thörl's V. H. Ölfabriken), Hamburg-Harburg, Germany

REFERENCES

1. Gupta, S. C., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *37*, 32–34 (1960).
2. Smit, W. C., Rec. trav. chim., *49*, 539–551 (1930).
3. von Mikusch, J. D., J. Am. Oil Chemists' Soc., *29*, 114–115 (1952).
4. von Mikusch, J. D., Fette und Seifen, *54*, 751–754 (1952).
5. Schmidt, H., and Lehmann, A., Helv. Chim. Acta, *33*, 1494–1502 (1950).
6. Nichols, P. L., Herb, S. F., and Riemenschneider, R. W., J. Am. Chem. Soc., *73*, 247–252 (1951).
7. von Mikusch, J. D., Lack- und Farbenchemie, *6*, 15–22 (1) (1952).
8. von Mikusch, J. D., Off. Dig. Pt. Varn. Prod. Clubs, *1956*, 44–70 (1).

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