Acknowledgment

The authors wish to express their appreciation to C. L. Hoffpauir and V. O. Cirino for many of the analytical determinations reported herein, and to E. A. Gastrock and H. L. E. Vix for the planning and evaluation of this work.

REFERENCES

1. Daugherty, P. M., Sineath, H. H., and Wastler, T. A., Georgia Eng. Expt. Sta. Bull., *17*, 36 pp. (1953).
2. Eaves, P. H., Molaison, L. J., Black, C. L., Crovetto, A. J., and D'Aquin, E. L., J. Am. Oil Chemists' Soc., *29*, 88–94 (1952).
3. Feuge, R. O., Pepper, M. B. Jr., O'Connor, R. T., and Field, Elsie T., J. Am. Oil Chemists' Soc., *28*, 420–426 (1951).
4. Greene, R. A., and Roster, E. O., Botan. Gaz., *94*, 826–828 (1933).
5. Greene, T. G., Hilditch, T. P., and Stainsby, W. J., J. Chem. Soc., *1936*, 1750–1755 (1936).
6. Knoepfler, N. B., and Vix, H. L. E., J. Agr. Food Chem., *6*, 118–121 (1958).
7. MacGee, A. E., Oil Mill Gaz., *52* (2), 17–21, 35–43 (1947).
8. McKinney, R. S., and Jamieson, G. S., Oil & Soap, *13*, 289–292 (1936).
9. Mirov, N. T., Econ. Botany, *6*, 41–47 (1952).
10. Reuther, C. G. Jr., LeBlanc, M. F. H. Jr., Batson, D. M., and Knoepfler, N. B., J. Am. Oil Chemists' Soc., *30*, 28–32 (1953).
11. Warth, A. H., "The Chemistry and Technology of Waxes," 2nd ed., Reinhold Publishing Corporation, New York, N. Y., 1956, pp. 302–307.
12. Wells, F. B., Cereal Chem., *32*, 157–158 (1955).

[Received October 9, 1958]

Isolation and Identification of Carbonyl Compounds Formed by Autoxidation of Ammonium Linoleate

H. T. BADINGS,[1] **Laboratory of Technical Biology, Technical University, Delft, The Netherlands**

VARIOUS STUDIES, involving the volatile decomposition products formed by autoxidation of edible fats and oils, have been carried out during the past few years. Among these products many carbonyl compounds have been identified (4, 7, 8, 9, 12, 14, 15, 16, 19, 20, 21, 22).

It is commonly accepted that the autoxidation of nonconjugated unsaturated fatty acids starts with the formation of α-methylene radicals and that the hydroperoxides produced in the so-called propagation stage decompose to form carbonyl compounds, keto acids, etc. As will be discussed later, these theories imply that specific carbonyl compounds are to be expected as the (primary) products of the decomposition of the hydroperoxides derived from a specific unsaturated fatty acid.

However too complicated a mixture of unsaturated fatty acids has usually been studied. A mixture involves the production of a whole series of carbonyl compounds, each of which may have been formed in several ways. Swift, O'Connor, Brown, and Dollear (22) and Lea (17) have shown that specific carbonyl compounds are to be expected and have, in fact, been found in autoxidizing systems containing linoleate.

Using a method published by van Duin (11), we have studied the main carbonyl compounds produced after a limited autoxidation of a system containing linoleic acid (in the form of ammonium linoleate) as the only fatty acid, with the object of discovering whether these carbonyl compounds in fact were those to be expected on theoretical grounds.

Methods

Autoxidation of Ammonium Linoleate. Linoleic acid of a high degree of purity (prepared from the urea addition compound, giving 99.3% purity) has been used in the work described. A limited autoxidation was carried out according to a method described by Tappel (23). A solution of 0.02 M. ammonium linoleate in 0.1 M. of phosphate buffer of pH 6.9, containing 10^{-4} M. $CuSO_4$ as a catalyst, was subjected to autoxidation in air at 37.2° C., using a Warburg apparatus. After an uptake of ½ mol. O_2 per mol. of linoleate, the reaction was stopped.

Concentration of Carbonyl Compounds. The carbonyl compounds were collected by extracting the reaction mixture three times with 25 ml. of carbonyl-free benzene. This extract was chromatographed, using a column of aluminum oxide containing 5% water; the oxidation products, such as acids and peroxides, were retained. In this way a benzene solution containing the carbonyl compounds free from acids, peroxides, etc., was obtained.

Conversion into D.N.P.-Ones. The conversion of the carbonyl compounds into their D.N.P.-ones (abbr. for 2,4-dinitrophenyl hydrazones) was carried out by adding 0.5 g. of 2,4-dinitrophenyl hydrazine and 2.5 g. of trichloroacetic acid to the benzene solution and leaving the mixture in the dark at 20°C. for 12 hours. The reaction mixture was shaken twice with 5% sodium bicarbonate solution, then three times with water, and dried over anhydrous magnesium sulphate. The benzene was removed by distillation under reduced pressure and a temperature not to exceed 30°C. Finally the D.N.P.-ones were extracted from the residue with four 10-ml portions of carbonyl-free petroleum ether (b.p. 60–80°C.).

Separation of the D.N.P.-Ones. Separation and identification were carried out, using the method of van Duin (10, 11).[2] By partition chromatography on a column with nitromethane on silica gel as the immobile phase and petroleum ether (b.p. 60–80°C.) as the mobile phase, a separation based on the chain length of the carbonyl compounds was carried out. The existence of a rectilinear relation between the logarithms of the retention volmes of the D.N.P.-ones of the single members of various homologous series of carbonyl compounds and the sequence of these members in these series was used for the determination of their chain length.

A further separation of each fraction from the partition column was necessary because D.N.P.-ones of different types of carbonyl compounds may possess the same retention volume in spite of a different chain-length. This was effected by van Duin *(loc. cit.)* by

---

[1] Present address: Netherlands Institute for Dairy Research, N.I.Z.O., Ede, The Netherlands.

[2] This method will be described in more detail in a thesis which will be published in the near future.

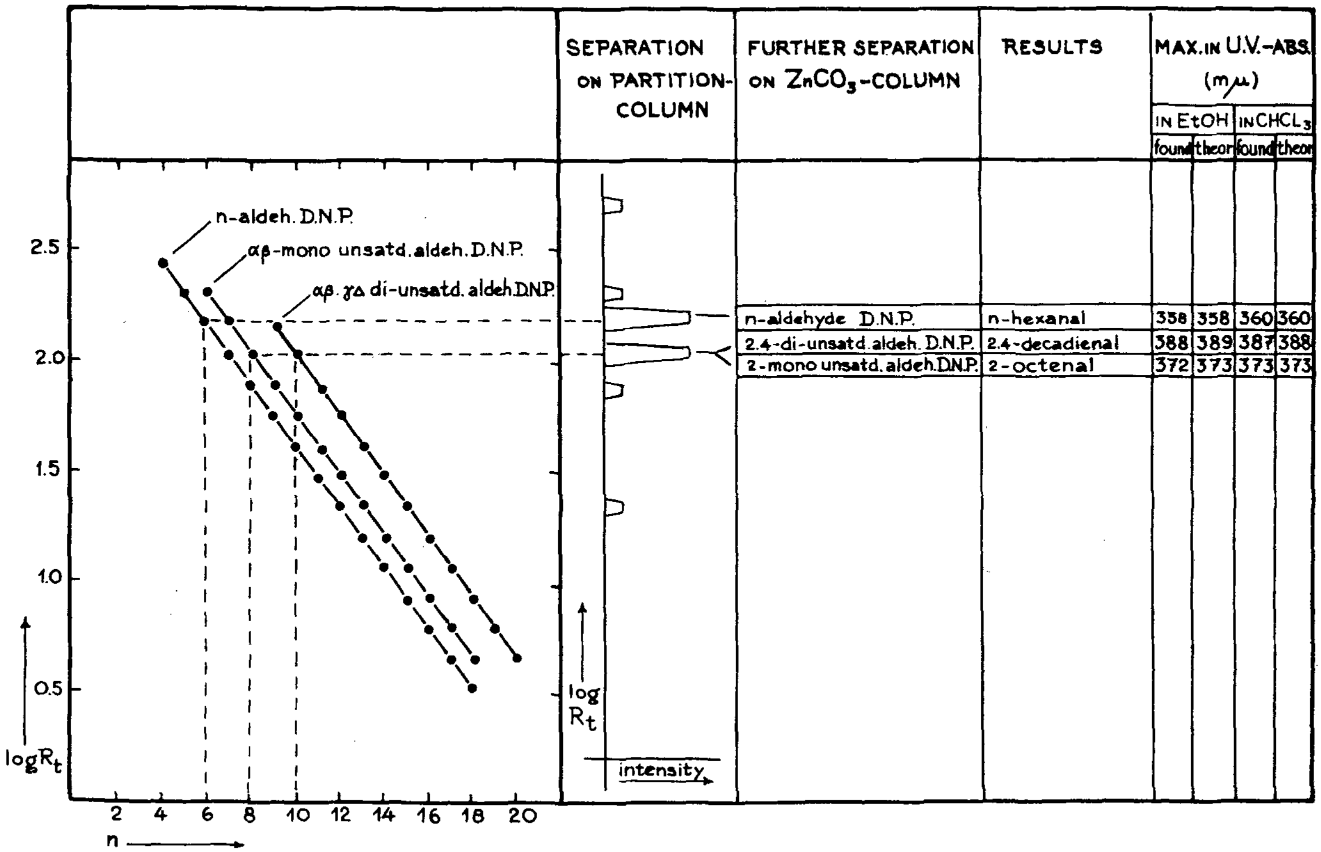

FIG. 1. Separation and identification of D.N.P.-ones.

applying adsorption chromatography on zinc carbonate columns. This method was used at first, but later on the use of chromatoplates, made by applying a slurry of zinc carbonate with 5% starch on a glass plate, was preferred. Addition of pyridine to the petroleum ether-benzene eluent gave an identification chromatogram because the eluted bands assumed characteristic colors.

The data of partition and adsorption separation combined with the determination of U.V.-absorption spectra (5) enabled conclusive identifications to be made.

Results

About 1 g. of linoleic acid was oxidized in the form of ammonium linoleate and treated as described in the preceding paragraph to yield the D.N.P.-ones which were subsequently separated and identified by using the methods mentioned.

The results are summarized in Figure 1. It can be seen that the D.N.P.-ones are mainly concentrated into two bands on the partition column. When eluted on a $ZnCO_3$-adsorption column, Band A appears to consist of a saturated n-aldehyde. The determination of the U.V.-absorption spectrum in both ethanol and chloroform confirms this conclusion. The number of carbon atoms in this saturated n-aldehyde is found by making use of the rectilinear relation between the logarithms of the R_t-values of the D.N.P.-ones of the single members of the saturated n-aldehydes and the sequence of these members in this series. From this we may conclude that Band A consists of the D.N.P.-one of n-hexanal.

In the same way Band B of the partition column has been studied. Further separation on a $ZnCO_3$-adsorption column shows that this band consists of two D.N.P.-ones, namely, of an $\alpha\beta$-unsaturated aldehyde and of an $\alpha\beta, \gamma\delta$-di-unsaturated aldehyde. Determination of the U.V.-absorption spectra in both ethanol and chloroform confirms this observation. By following the same procedure as described for Band A, it is now possible to determine the number of carbon atoms in these carbonyl compounds, using the relation of log R_t ($Vs = 10$) to n pertaining to mono- and di-unsaturated aldehydes, respectively. From this we can conclude that Band B consists of the D.N.P.-ones of 2-octenal and 2, 4-decadienal.

Minor quantities of other D.N.P.-ones were present, but these have not been studied.

Discussion

The question arises whether the carbonyl compounds found are those which, according to existing theories, are to be expected as major oxidation products of linoleic acid.

The mechanism which leads to the formation of hydroperoxides as well as the dismutation of these products to carbonyl compounds is now well understood (1, 3, 13). The hydroperoxides are formed in a chain reaction of radicals, in which the methylene group adjacent to the double bond is involved. In the autoxidation of linoleate the C_{11}-methylene group is preferentially involved in the chain reaction. The formation of three isomeric hydroperoxides is possible (Figure 2). However investigations by Cannon, Zilch, Burket, and Dutton (6) and Privett, Lundberg, Khan, Tolberg, and Wheeler (18) have shown that by far the greater part of the hydroperoxides formed (probably even 100%) consists of the hydro-

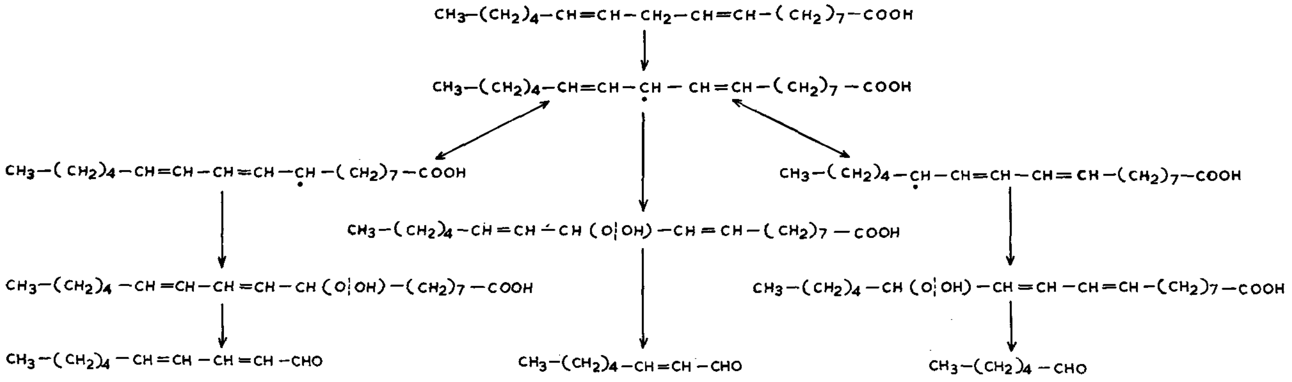

FIG. 2. Formation of hydroperoxides and dismutation to carbonyl compounds.

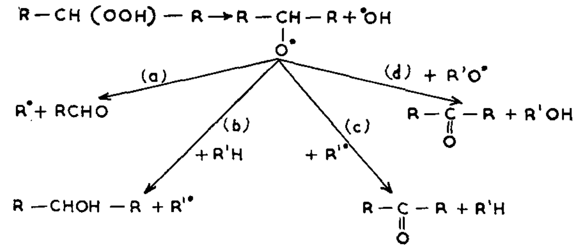

FIG. 3. Dismutation of hydroperoxides.

peroxides with conjugated double bonds. Various explanations are possible for this feature (1, 13).

The dismutation of the hydroperoxides may occur as shown in Figure 3 by Bell (2). According to reaction (a) of this scheme, we expect the formation of 2,4-decadienal and hexanal as dismutation products of the conjugated hydroperoxides. Dismutation of the nonconjugated hydroperoxide would lead to the formation of 2-octenal. However in the investigations mentioned above the presence of this hydroperoxide could not be demonstrated.

It is possible that the nonconjugated ROO•-radical would be formed but because of its less stable configuration would either dismutate, leading to the formaion of 2-octenal or change rapidly into its conjugated isomers. It is also possible that 2-octenal is formed as a result of the decomposition of 2,4-decadienal. Hence it is not clear how 2-octenal is actually formed.

As has been stated in the introduction, Swift, O'Connor, Brown, and Dollear (22) and Lea (17) have shown that specific carbonyl compounds are to be expected and have, in fact, been found in autoxidized systems containing linoleate. Swift, O'Connor, Brown, and Dollear (22) collected the steam-volatile products from autoxidized cottonseed oil. From this they isolated and identified n-hexanal, 2-octenal, and 2,4-decadienal. As linoleic acid is a major constituent of cottonseed oil, our experiments in a system with linoleic acid (in the form of ammonium linoleate) as the only fatty acid are a confirmation that indeed the identified carbonyl compounds have been formed by autoxidation of linoleate.

As far as we know, there is no reason to suppose the existence of a different mechanism of autoxidation for the ester and for the free acid although the rates of autoxidation will differ.

Summary

1. The isolation and identification of carbonyl compounds formed by autoxidation of pure ammonium linoleate (0.02 M. in water) has been carried out.

2. The autoxidation has been performed according to a method described by Tappel (23). A solution of 0.02 M. ammonium linoleate in 0.1 M. phosphate buffer of pH 6.9, containing 10^{-4} M. $CuSO_4$, was subjected to autoxidation at 37.2°C.

3. The isolation and identification of the D.N.P.-ones of the carbonyl compounds have been carried out, using a method of partition and adsorption chromatography published by van Duin (10, 11).

4. It was demonstrated that the principal compounds formed were n-hexanal, 2-octenal, and 2,4-decadienal.

5. The formation of these aldehydes is fully in line with the theories of autoxidation of unsaturated fatty acids (1, 3, 13) and of the dismutation of the hydroperoxides formed (2).

Acknowledgment

The author wishes to express his thanks to H. van Duin, Netherlands Institute for Dairy Research, Ede, The Netherlands, for his valuable suggestions concerning the chromatographic techniques used in this work, and to the Unilever Research Laboratory, Vlaardingen, which kindly supplied the pure methyl linoleate.

REFERENCES

1. Bateman, L., Quart. Revs. (London), *8*, 147 (1954).
2. Bell, E. R., Raley, J. H., Rust, F. F., Seubold, F. H., Vaughan, W. E., Disc. Faraday Soc., *10*, 242 (1951).
3. Bolland, L. J., Quart. Revs. (London), *3*, 1 (1949).
4. Brekke, J., Mackinney, G., J. Am. Oil Chemists' Soc., *27*, 238 (1950).
5. Braude, F. A., Johnes, E. R. H., J. Chem. Soc. (London), 498 (1945).
6. Cannon, J. A., Zilch, K. T., Burket, S. C., Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 447 (1952).
7. Chang, S. S., Kummerow, F. A., J. Am. Oil Chemists' Soc., *30*, 251 (1953).
8. Chang, S. S., Kummerow, F. A., J. Am. Oil Chemists' Soc., *31*, 324 (1954).
9. Coleman, J. E., Knight, H. B., Swern, Daniel, J. Am. Oil Chemists' Soc., *32*, 135 (1955).
10. Duin, H. van, Nature, *180*, 1473 (1957).
11. Duin, H. van, Neth. Milk and Dairy J., *12*, 74, 81 (1958).
12. Fritsch, C. W., Deatherage, F. E., J. Am. Oil Chemists' Soc., *33*, 109 (1956).
13. Holman, R. T., "Progress in Chem. Fats Lipids," vol. II, 51 (1954).
14. Johnson, O. C., Chang, S. S., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *30*, 317 (1953).
15. Kawahara, F. K., Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 372 (1952).
16. Kawahara, F. K., Dutton, H. J., Cowan, J. C., J. Am. Oil Chemists' Soc., *29*, 633 (1952).
17. Lea, C. H., Chem. and Ind. (London), 1303 (1953).
18. Privett, O. S., Lundberg, W. O., Khan, N. A., Tolberg, W. E., Wheeler, D. H., J. Am. Oil Chemists' Soc., *30*, 61 (1953).
19. Schepartz, A. I., Daubert, B. F., J. Am. Oil Chemists' Soc., *27*, 367 (1950).
20. Stapf, R. J., Daubert, B. F., J. Am. Oil Chemists' Soc., *27*, 374 (1950).
21. Swift, C. E., Dollear, F. G., Brown, L. E., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *25*, 39 (1948).
22. Swift, C. E., O'Connor, R. T., Brown, L. E., and Dollear, F. G., J. Am. Oil Chemists' Soc., *26*, 297 (1949).
23. Tappel, A. L., J. Am. Oil Chemists' Soc., *32*, 252 (1955).

[Received November 19, 1958]