Some Carbonyl Flavor Compounds of Oxidized Soybean and Linseed Oils[1]

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Abstract

Evidence is presented that 2,3-pentanedione, as well as diacetyl, contributes to the buttery flavor found in the early stages of oxidation of soybean oil. Components with a fishy and potatoey flavor were found in distillates from soybean oil that had a fishy or paint-like flavor. Linseed oil proved a reliable source of these flavor compounds. The fishy compound was identified as *cis*-4-heptenal and the potatoey compound is probably 2,4-pentadienal. Mechanisms for the production of these compounds are suggested.

Introduction

Previous investigations on oxidized soybean oil have suggested several compounds (1–4) that may make important contributions to the flavor. This paper presents evidence that 2,3-pentanedione, *cis*-4-heptenal and 2,4-pentadienal also contribute to the flavor of oxidized soybean oil.

Methods

Soybean and linseed oil were obtained from commercial sources. Although some batches of soybean oil stored at room temperature quickly produce the buttery, fishy and paint-like flavors studied in these experiments, other batches require longer storage and more drastic conditions to yield these flavors. Buttery flavor was usually produced in soybean oil by storage in diffuse light for 10–20 days at room temperature. The addition of a few parts per million of cupric palmitate seemed to hasten the development of this flavor. Fishy and paint-like flavors were produced in resistant batches of oil by storage for three to six months in the dark at room temperature or by incubation at 37 to 50 C in partly sealed containers for two to three weeks. Linseed oil was used as received.

Volatile materials were distilled from the soybean oil by high-vacuum distillation in a Rota-film still at room temperature (2,5). Linseed oil was deodorized in an all glass apparatus similar to that of Schwab and Dutton (6) for 0.5 hr at 180–230 C at 50 μ pressure. The gas chromatography and flavor evaluation were carried out as described previously (3) except that mineral oil was substituted for corn oil as a tasting medium. Petroleum ether (bp 60 C) was purified by the method of Schwartz and Parks (7). Other solvents were reagent grade.

2,4-Dinitrophenylhydrazones (2,4-DNPH) were prepared by bubbling the fractionated vapor of the flavor compound from the gas chromatograph into a solution of 2,4-dinitrophenylhydrazine in tetrahydrofuran-acetic acid (99:1) or in 2 N hydrochloric acid. Or, the reagent in 2 N hydrochloric acid was added directly to the distillation cold trap. When tetrahydrofuran was used, it was alway freshly distilled over sodium metal. After reaction, the tetrahydrofuran was evaporated under reduced pressure, and petroleum ether was used to extract the desired monocarbonyl-2,4-DNPH and leave the unreacted hydrazine. When aqueous reagent was used, the precipitated 2,4-DNPHs were filtered off, washed and extracted with petroleum ether, chloroform or ethyl acetate, or both, to recover the desired 2,4-DNPH.

Appropriate preparative thin layer chromatography (TLC) was carried out on the extracts and followed by careful comparative TLC for identification. Preparative TLC of the monocarbonyls was generally carried out on Gelman TLC media of silica gel or alumina, or both (Gelman Instrument Co., Ann Arbor, Mich.). For identification purposes, poured plates of Silica Gel HR (Brinkman Instruments, Inc., Great Neck, N.Y.) were used. For separation according to class (aldehyde, ketone, vinyl ketone), alumina media developed in 4% diethyl ether in petroleum ether was used. For separation according to degree of unsaturation, alumina or silica-gel media impregnated with a 25% silver nitrate solution and dried at room temperature were developed in 30% (alumina) or 16% (silica gel) diethyl ether in petroleum ether. The use of silver nitrate TLC plates facilitated the removal of unsaturated monocarbonyls from dicarbonyls. For separation according to chain length, alumina or silica gel media impregnated with 10% 2-phenoxyethanol in acetone and dried were developed in petroleum ether. Dicarbonyl-2,4-DNPHs were separated according to Cobb (8).

Cis-4-heptenal was prepared by the method of Havercamp Begemann and Koster (9), and 2,4-pentadienal was prepared according to Pippen and Nonaka (10). 2,3-Pentanedione and diacetyl were obtained commercially.

Results and Discussion

Buttery Flavor

Buttery flavors are usually encountered in the early stages of the oxidation of soybean oil at room temperature. Although diacetyl is present in soybean oil and certainly reproduces the buttery flavor sensation, more than one buttery component seems present in some soybean oils. When gas chromatograms on butanediol succinate of concentrates rich in buttery flavor were evaluated organoleptically, they gave the flavor sequence: buttery, vinyl ethyl ketone, buttery, and finally green. The retention time of the first buttery component agreed with that of diacetyl. It was possible that the second buttery fraction was simply a tailing of the diacetyl on the column. One can often distinguish the aroma of a strong flavor compound well before and after the emergence of the main peak. Injection of pure diacetyl under the same conditions, however, revealed that its aroma was never detectable at the retention time of the second buttery component found in the distillate. The second buttery component had a retention time close to that of pentanal, while diacetyl had a retention time close to that of butanal on butanediol succinate. This suggested that the second buttery compound might be

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[1] Journal Paper No. J-6183 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1517.

2,3-pentanedione. This compound has flavor quite similar to diacetyl and a retention time that matched that of the second buttery fraction. TLC of the DNPHs prepared via the aqueous reagent revealed materials that traveled with the bis-2,4-DNPHs of 2,3-pentanedione as well as diacetyl. The ultraviolet spectrum of the derivatives recovered from thin layer plates resembled that of dicarbonyls, but that corresponding to the 2,3-pentanedione was not pure enough to distinguish the number of alkyl groups attached to the α-diketo chromophore (Hammond and Bird, unpublished results).

Fishy Flavor

Soybean oil sometimes develops a flavor resembling that of fish oils or drying oils, such as linseed oil. Although this sometimes occurs at room temperature, these flavors are more characteristic of oils oxidized at 37–50 C or higher, and are typical problems in soybean oil used in frying operations. Flavor distillates obtained from such oils have not reproduced the flavors of the original oils satisfactorily when the distillates were mixed with the appropriate amount of mineral oil. Gas chromatography of such flavor distillates, however, has revealed two compounds not found in soybean oil in less advanced stages of flavor deterioration. These flavors are potent enough to be obvious when the distillate is diluted out to its concentration in the original soybean oil, so they must make a contribution to the fishy and paint-like flavors of the oil. The two components were given the designations potatoey and fishy from their resemblance to the aroma of pan-fried potatoes and fish oils.

We have had considerable difficulty in getting soybean oil to produce this flavor stage consistently, although soybean oil oxidized at 37–50 C always contains some of these two flavor components. The resemblance of the flavor to drying oils suggested linseed oil as a possible source, and this has proved a much more abundant and consistent source of the fishy and potatoey flavor. Deodorizer distillates from raw linseed oil were always rich in the flavor. The assumption that the compounds found in linseed oil are the same as those found in soybean oil is based on (a) the identical retention times of the flavor compounds by gas chromatography on both polar and nonpolar columns, (b) the unanimous agreement by the flavor panel that they produced the same sensations, and (c) similar migrations during preliminary purification of derivatives by TLC.

The fishy-flavored compound has a retention time on butanediol succinate between that of *n*-heptanal and *n*-octanal. On Apiezon L, it moved slightly slower than *n*-heptanal. TLC of the 2,4-DNPH (prepared via tetrahydrofuran reagent) was simplified because it spontaneously regenerated the fishy flavor. The fraction responsible for the flavor moved markedly slower than heptanal- or octanal-2,4-DNPH on silver ion media, but on silica gel media, it traveled with these saturated aldehydes. These properties suggested that the compound was a seven carbon aldehyde with a double bond not conjugated with the carbonyl group. The occurrence of the fishy-flavored compound in linseed and soybean oil suggested that linolenic acid was the precursor and that it should have a *cis* double bond 3 carbons from the methyl end of the chain. Synthetic *cis*-4-heptenal had the same retention time and flavor as the unknown. The 2,4-DNPH of the unknown traveled with that of *cis*-4-heptenal in all the thin layer systems. The 2,4-DNPHs of 2-heptenal, 3-*cis*-heptenal, 3-*trans*-heptenal, 4-*cis*-heptenal and 4-*trans*-heptenal were all separated by TLC on silver ion impregnated silica gel under our conditions. In view of the chromatographic separations obtained by Meijboom and Jurriens (11), *cis*-4-heptenal seems the only possibility. The *cis*-4-heptenal-2,4-DNPH also had IR and UV spectra identical to that of the unknown.

Cis-4-heptenal-2,4-DNPH forms two isomers that separate on silver ion, silica gel media. These are presumed to be the syn and anti isomers. If one band is isolated from a thin layer plate and rechromatographed, the two bands are again obtained. Both the known and unknown showed this phenomenon. Although syn and anti isomers have been reported before (12), we have previously encountered only one of these forms in our laboratory. Evidently, *cis*-4-heptenal-2,4-DNPH is more prone to form these isomers than are other aldehydes. Storage in liquid nitrogen at all times except during actual manipulation significantly retarded development of this isomerism. Synthetic *cis*-4-heptenal-2,4-DNPH crystals are stable; after the compound has been subjected to TLC, however, it will, like the isolated material, spontaneously regenerate the flavor.

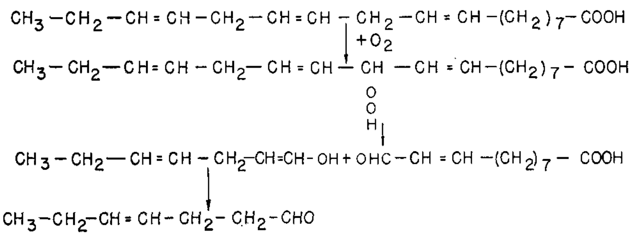
Potatoey Flavor

This flavor compound also traveled between *n*-heptanal and *n*-octanal by gas chromatography on butanediol-succinate columns. It came out just before the fishy-flavored compound. On Apiezon L, it traveled close to *n*-hexanal. The aroma was destroyed by 2,4-dinitrophenylhydrazine reagent, but the flavor could not be regenerated from the hydrazones (13–15). The large shift in retention time relative to saturated aldehydes, when polar and nonpolar gas chromatography columns were used, suggests 2,4-dienals. The shift is larger than that occurring with 2-enals. The longer chain dienals have an oily aroma, but 2,4-pentadienal has a distinctly different aroma, which our panel identified as the same as that of the potatoey compound. The retention time of the 2,4-pentadienal on both butanediol succinate and Apiezon L was similar to that of the unknown. 2,4-Pentadienal forms a 2,4-DNPH that will not regenerate the aroma of the free aldehyde by the usual treatments. Thus, pentadienal has the properties of the potatoey compound found in soybean and linseed oils, but we have not been able to identify a 2,4-DNPH of this compound from soybean or linseed oils. This is attributed to the small amounts of this compound present in the oil.

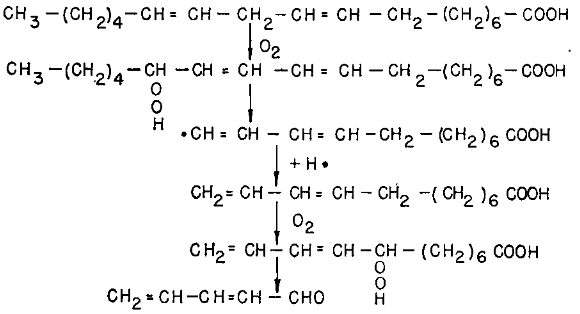
Havercamp Begemann (private communication) has suggested that 2,3-pentadione might arise from the breakdown of one of the cyclic diperoxides produced in the oxidation of linolenic acid (16) but no satisfactory mechanism for the production of diacetyl in oxidized oils has been suggested.

Workers at Unilever have found *cis*-4-heptenal in oxidized butter and tallow (9,17). They have proposed that this compound must arise from a classical peroxide decomposition and have found evidence in milk fat for several fatty acids of unusual structure that might be precursors of this compound (18). The occurrence of this compound in linseed and soybean oils suggests that there is probably a route to this compound from ordinary linolenic acid. Scheme 1 shows a possible mechanism. Although the occurrence of the nonconjugated peroxide postulated here has been questioned (19), this kind of structure has been evoked to explain the production of 2-octenal

SCHEME 1



SCHEME 2



from linoleate (1). The Unilever workers have described the flavor of *cis*-4-heptenal as buttery (9).

2,4-Pentadienal arises from linoleic acid according to previous work from our laboratory (20). One supposes that this would require the scission of the fatty acid chain at two points. A possible mechanism for this is shown in Scheme 2. The order of attack by oxygen and peroxide breakdown could vary. Other fatty acids capable of giving conjugated hydroperoxides might also be precursors for 2,4-pentadienal.

ACKNOWLEDGMENT

2,4-Dinitrophenylhydrazones of various 7 carbon aldehydes by J. G. Keppler. Supported in part by a PHS Research Grant UI-00090 from the National Center for Urban and Industrial Health, Public Health Service.

REFERENCES

1. Hoffmann, G., "Lipids and Their Oxidation," Edited by H. W. Schultz, E. A. Day and R. O. Sinnhuber, Avi Publishing Co., Inc., Westport, Conn., 1962, p. 215–229.
2. Hill, F. D., and E. G. Hammond. JAOCS *42*, 1148–1150 (1965).
3. Seals, R. G., and E. G. Hammond, Ibid. *43*, 401–403 (1966).
4. Krishnamurthy, R. G., T. H. Smouse, B. D. Mookherjee, B. R. Reddy and S. S. Chang, J. Food Sci. *32*, 372–374 (1967).
5. El-Negoumy, A. M., D. M. Miles and E. G. Hammond, J. Dairy Sci. *44*, 1047–1056 (1961).
6. Schwab, A. W., and H. J. Dutton, JAOCS *25*, 57–59 (1964).
7. Schwartz, D. P., and O. W. Parks, Anal. Chem. *33*, 1396 (1961).
8. Cobb, W. Y., J. Chromatog. *14*, 512–513 (1964).
9. Havercamp Begemann, P., and J. C. Koster, Nature *202*, 552 (1964).
10. Pippen, E. L., and M. Nonaka, J. Org. Chem. *23*, 1580–1582 (1958).
11. Meijboom, P. W., and G. Jurriens, J. Chromatog. *18*, 424–426 (1965).
12. Edwards, A. N., Jr., Ibid. *22*, 29–35 (1966).
13. Keeney, M., Anal. Chem. *29*, 1489–1491, (1957).
14. Ralls, J. W., Ibid. *32*, 332–336 (1960).
15. Bassette, R., and E. A. Day, JAOCS *37*, 482–483 (1960).
16. Havercamp Begemann, P., W. J. Woestenburg and S. Leer, J. Agric. Food Chem. *16*, 679–684 (1968).
17. Hoffmann, G., and P. M. Meijboom, JAOCS *45*, 468–470 (1968).
18. de Jong, K., Fette Seifen Anstrich. *69*, 277-279 (1967).
19. Frankel, E. N., "Lipids and Their Oxidation," Edited by H. W. Schultz, E. A. Day and R. O. Sinnhuber, Avi Publishing Co., Inc., Westport, Conn., 1962, p. 64–73.
20. El-Negoumy, A. M., M. S. de Puchal and E. G. Hammond, J. Dairy Sci. *45*, 311–316 (1962).

[Received March 18, 1969]