Components of the Hardening Flavor Present in Hardened Linseed Oil and Soybean Oil

J. G. KEPPLER, J. A. SCHOLS,[1] W. H. FEENSTRA and P. W. MEIJBOOM, Unilever Research Laboratory, Vlaardingen, The Netherlands

Abstract

The characteristic hardening flavor which develops in hardened linseed and soybean oils during storage has been concd from hardened linseed oil by stripping with nitrogen. After separating the volatile substances by adsorption chromatography on silica, the fraction containing the hardening flavor has been converted into 2,4-dinitrophenylhydrazones (DNPHs) and separated by means of partition chromatography. On regeneration of the fractions of DNPSs obtained, the characteristic hardening flavor was observed in one specific band. Both by hydrogenation and by oxidation of the free carbonyl the carrier of the flavor was found to be an unsaturated aldehyde; however, not of the α-β unsaturated type. Further separation of the regenerated carbonyls by means of gas-liquid chromatography (GLC) points to a C_9-aldehyde. After synthesis of the 4-,5- and 6-*cis*- and *trans*-nonenals, comparison made it probable that the carrier of the hardening flavor is a mixture of 6-*cis* and 6-*trans*-nonenal, the latter of which has the greatest share in the hardening flavor.

In order to confirm the location of the double bond in the carrier of the hardening flavor a recent isolation technique was applied. The volatile substances from hardened linseed oil were first separated via GLC. After conversion of the carbonyls in question into their DNPHs, the latter have been separated by means of thin-layer chromatography (TLC). By means of IR-analysis and oxidation with osmium tetroxide of the pure derivative, the principal carrier of the hardening flavor has been identified as 6-*trans*-nonenal.

Introduction

For many years investigations have been carried out to identify the hardening flavor which is observed in hardened linseed oil and soybean oil (1–3). Lemon (4–6) has established that on hardening oils containing linolenic acid, "*iso*-linoleic acid" is formed from which the off-flavor(s) develops. In particular 9,15-octadecadienoic acid is considered to be the precursor. In later experiments, Lemon could no longer demonstrate a marked correlation between the amt of *iso*-linoleic acid formed and the intensity of the hardening flavor. Therefore he put forward the hypothesis that in addition an interaction must take place in the hardened oil between the *iso*-linoleic acid and another component (7).

Other research workers, e.g. Daubert et al. (8,9) and Mattill (1) have found that *iso*-linoleic acid is also formed during the hardening of soybean oil. In their opinion the conclusion must be drawn that a group of *iso*-linoleic acids is responsible for the occurrence of the off-flavors in hardened soybean oil and linseed oil.

For some time, the difference in off-flavor between hardened and unhardened soybean oil and linseed oil was not sufficiently distinguished.

Chang (10), however, has made a clear distinction between the two flavors and carried out investigations into the hardening flavor of hardened soybean oil. By means of a degassing technique Chang (11), obtained a flavor conc from hardened soybean oil which conc then was separated into fractions. He found that the characteristic hardening flavor is present in the fractions of the saturated aldehydes and ketones (12).

For a number of years the problem of the hardening flavor has been investigated in our laboratory and it has been established that the hardening flavor is present in the mixture of aldehydes which can be concd from hardened soybean oil and linseed oil.

Procedure and Discussion

Since both hardened soybean and linseed oils develop hardening flavors on storage, it was first investigated what unsaturated fatty acid is responsible for this flavor. Elias (13) has found that on hardening ethyllinolenate using a nickel catalyst, a strong hardening flavor developed in the hardened product. However, this flavor was not observed in hardened ethyllinoleate or ethyloleate. Since both soybean and linseed oil contain linolenic acid and both oils display the hardening flavor, it can be assumed—in accordance with the findings of other research workers (4,8)—that linolenic acid during hardening may be converted into another unsaturated acid from which the hardening flavor in question will be formed by oxidation. As it it known that hardened linseed oil very often displays a more intensive hardening flavor than hardened soybean oil, it was decided to choose hardened linseed oil as starting material. Linteris (14) pointed out that in hardened soybean oil the hardening flavor—in addition to many other flavors—is present in a stronger degree when air is sucked through at 100–130C.

Concentration of the Volatile Material from Hardened Linseed Oil. The above method was applied to our starting material. Air was sucked through portions of 5–10 kg hardened linseed oil (I.V. 110) for 2–3 hr at 130C. The strongly smelling oil was degassed at 200C by passing it slowly through a current of nitrogen at a vacuum of ca. 0.1 mm Hg. Via a spray trap, the entrained substances were passed through four cold traps. The first trap was kept at −10C in order to collect any fatty material carried along, in the second and third (at −80C) the flavor conc was collected, whereas the fourth trap (at −80C) served as a security and did not contain any condensate.

Preliminary Separation of the Flavor Concentrate. Since in the accelerated reversion procedure a great number of other volatile substances are produced, a preliminary separation of the flavor conc was carried

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[1] Present address: R & D Laboratory, DOW Chemical of Canada Ltd., Sarnia, Ontario, Can.

out on an adsorption column of silicagel prepared according to Gordon et al. (15). In order to prevent the flavor components from being destroyed at the surface, the silicagel was moistened with 1–3% H_2O. As eluent light petroleum (bp 40–60C) with gradually increasing percentages of diethyl ether (0,0.5,1,2,5%) was used. In the fractions with 0.5–1% diethyl ether, the characteristic hardening flavor was perceived.

Investigation into the Carbonyl Function. The fractions displaying the hardening flavor were collected and their volume reduced to 10–20 ml by means of careful evaporation of the solvent at low temp. After diluting the solution with diethyl ether, the carbonyls were converted into their DNPHs by refluxing with 2,4-dinitrophenylhydrazine, using acetic acid as a catalyst. After termination of the reaction, the hardening flavor had disappeared. The fact that after careful regeneration of the carbonyls from part of the hydrazones this flavor could be perceived again, confirmed the hypothesis that the carrier of the hardening flavor has a carbonyl function. For this regeneration the following method was used: 1–5 mg DNPHs was dissolved in 1–2 ml 50% acetic acid and then ca. 0.5 ml 40% formaldehyde solution was added. The mixture was refluxed for 5 min, cooled, diluted with 10 ml water and extracted with 1–2 ml light petroleum. The formaldehyde-DNPH formed is practically insoluble in light petroleum. The light petroleum contains the desired carbonyl compounds(s), but also some of the excess formaldehyde and acetic acid. Therefore, the light petroleum solution was washed once or twice with 15 ml water. The carbonyl compound can now be recognized in the light petroleum solution by its typical odor.

Partition Chromatography of the DNPHs. The chromatographic separation according to Kramer and Van Duin (16) by which eight colored zones were obtained showed that the first separation over the silica-column had been incomplete. Portions of each zone were regenerated. On the basis of the smell of the liberated carbonyls, it could be established that the carrier of the hardening flavor was present in the hydrazone band which had a retention volume (R_v) of 112 ml.

In experiments with model substances, it appeared that the hydrazones of *n*-heptanal and 2-octenal had the same R_v values. However, the flavors of these aldehydes are entirely different from those of the hardening flavor in question.

Investigation into the Nature of the Flavor Carrier. The following experiments were carried out with the regenerated carbonyls from part of the DNPHs having a R_v value of 112 ml:

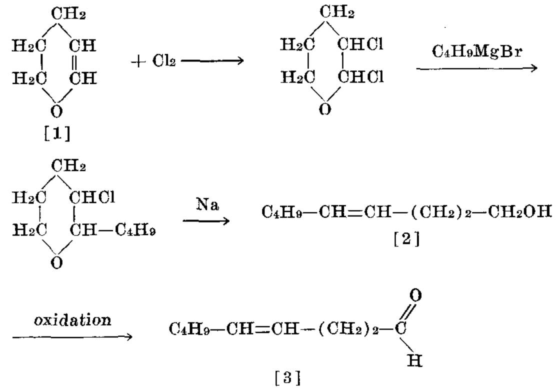
a) A small portion was oxidized with moist silver oxide for 1 hr at 60C (17,18) after which the hardening flavor disappeared completely. From this finding the conclusion was drawn that an aldehyde was present which is even very likely to have the following group $R-CH_2-CH_2-CH_2-\overset{O}{\overset{/\!/}{C}}-H$, since by this method 2-enals and 2,4-dienals cannot so easily be converted into acids, and ketones such as pentyl-vinylketone and pentyl-ethylketone are not sensitive to this type of oxidation.

b) Another portion of the above carbonyls was hydrogenated for 1–2 min at 25C in a solution of light petroleum (bp 40–60C) using palladium as a catalyst. It may be mentioned that under these mild conditions only carbon-carbon double bonds will be saturated and aldehyde groups will remain unaffected (19). The fact that the hardening flavor disappeared indicates that the aldehyde must have an unsaturated character.

c) A larger amt of the fraction of DNPHs with R_v = 112 ml (56 mg) were regenerated by means of the method mentioned before and concd to a small volume (0.5 ml) by freeze drying. This conc was then analyzed by means of GLC, and ca. 90% heptanal and 9% 2-octenal were found. Approx at the place of nonanal, a very small peak was collected which displayed a strong hardening odor. However, the odor of both nonanal and 2-nonenal are quite different from the hardening flavor in question so that these compounds can be ruled out.

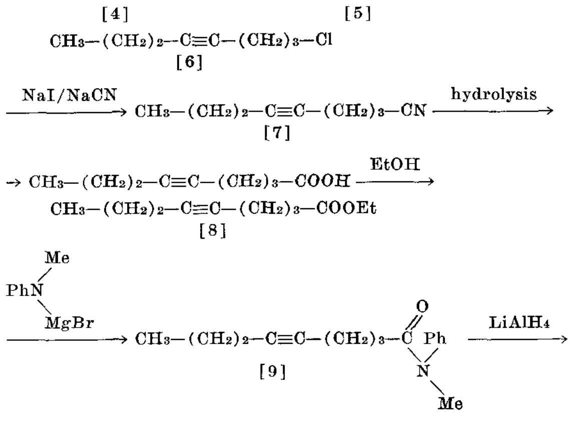
Therefore, it was assumed that an unsaturated C_9-aldehyde was present, having a double bond which is further removed from the carbonyl group than in the case of 2-nonenal. By synthesis of 4-,5- and 6-nonenals and comparing their flavor, flavor potency and R_v of their DNPHs, it was found that 6-*cis* and 6-*trans*-*nonenal are most likely to be the carriers of the typical hardening flavor.*

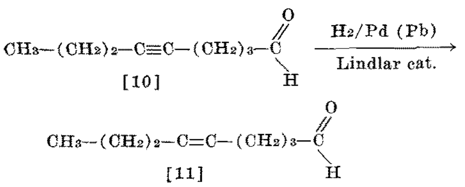
Synthesis of 4-,5- and 6-Nonenals. 4-Nonenal was synthesized starting from dihydropyran (I) (20) according to the following reaction scheme:



A mixture of *cis*- and *trans*-4-nonenols [2] is formed. In our case we could not confirm the findings of Crombie and Harper (21) concerning the exclusive formation of the *trans* compound. The oxidation of the unsaturated alcohol [2] to the unsaturated aldehyde [3] was carried out (i) by slow oxidation with a mixture of bichromate and acetic acid (22), (ii) by rapid oxidation with bichromate-H_2SO_4 (23,24), (iii) by oxidation with tertiary butylchromate (25–27). Although none of these methods was satisfactory, the method mentioned under (i) gave the highest yield (ca. 20%). R_v 4-nonenal-DNPH = 112 ml.

5-cis-Nonenal was prepared from sodium pentyne (4) following the reaction scheme:



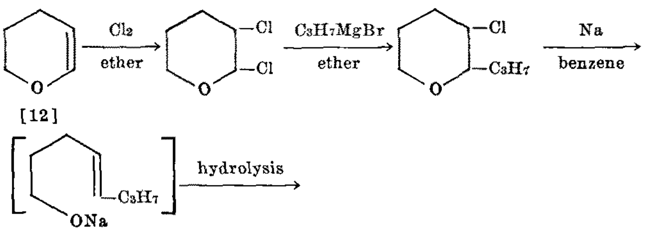


$$\underset{[10]}{\mathrm{CH_3{-}(CH_2)_2{-}C{\equiv}C{-}(CH_2)_3{-}CHO}} \xrightarrow[\text{Lindlar cat.}]{\mathrm{H_2/Pd\ (Pb)}} \underset{[11]}{\mathrm{CH_3{-}(CH_2)_2{-}C{=}C{-}(CH_2)_3{-}CHO}}$$

1-Chloro-3-bromopropane [5] was prepared from allyl chloride with HBr under the influence of UV radiation (28). 1-Chloro-4-octyne [6] was converted into the cyanide [7] via the iodide, and the cyanide [7] hydrolyzed and esterified with ethanol. To prevent formation of a *trans* double bond in the final product [11], the ester [8] was first converted into the corresponding anilide [9] with the Grignard compound of mono-methyl anilide (29). The anilide [9] was reduced with lithium tetrahydrido aluminate (23,24) to 5-nonynal [10], which after purification via the DNPH was hydrogenated with a Lindlar catalyst. In this way exclusively 5-*cis*-nonenal [11] with a yield of 50.7% was formed. R_v 5-nonynal-DNPH = 250 ml, R_v 5-nonenal-DNPH = 112 ml.

In an attempt to hydrogenate [7] directly to [10] by means of lithium tetrahydrido aluminate, no results were obtained.

5-trans-Nonenal was prepared in accordance with the following reaction scheme:



[12]

$$\underset{[13]}{\mathrm{C_3H_7{-}CH\overset{tr}{=}CH{-}(CH_2)_2{-}CH_2OH}} \xrightarrow{\mathrm{PBr_3}}$$

$$\underset{[14]}{\mathrm{C_3H_7{-}CH\overset{tr}{=}CH{-}(CH_2)_2{-}CH_2Br}} \xrightarrow[\mathrm{CH(OC_2H_5)_3}]{\mathrm{Mg}}$$

$$\underset{[15]}{\mathrm{C_3H_7{-}CH\overset{tr}{=}CH{-}(CH_2)_3{-}CH(OC_2H_5)_2}} \xrightarrow{\text{hydrolysis}}$$

$$\underset{[16]}{\mathrm{C_3H_7{-}CH\overset{tr}{=}CH{-}(CH_2)_3{-}CHO}}$$

First 4-*trans*-octen-1-ol [13] was prepared from 2,3-dihydropyran [12] using the method of Crombie and Harper (30). The corresponding bromide [14] was synthesized according to another procedure also described by Crombie (31). In accordance with the procedure of Smith and Nichols (32), the 1-bromo-4-*trans*-octene [14] formed was coupled via the corresponding Grignard compound to ethyl orthoformate. The 5-*trans*-nonenal diethyl acetal [15] obtained was hydrolyzed by the method of Winter (33) into 5-*trans*-nonenal in 74% yield.

6-cis-Nonenal was prepared according to the following scheme (34–36). The last stage in the synthesis was carried out by the method of Franzen and Otto (37).

$$\mathrm{HC{\equiv}CNa + C_2H_5Br} \xrightarrow[\text{dimethyl formamide}]{\text{xylene}} \mathrm{CH_3{-}CH_2{-}C{\equiv}CH + NaBr}$$

$$\mathrm{CH_3{-}CH_2{-}C{\equiv}CH + ICH_2{-}(CH_2)_3{-}CH_2Cl} \xrightarrow[\text{liquid } \mathrm{NH_3}]{\mathrm{NaNH_2}\text{ in}}$$

$$\mathrm{CH_3{-}CH_2{-}C{\equiv}C{-}(CH_2)_4{-}CH_2Cl} \xrightarrow[\text{acetone}]{\text{NaI in}}$$

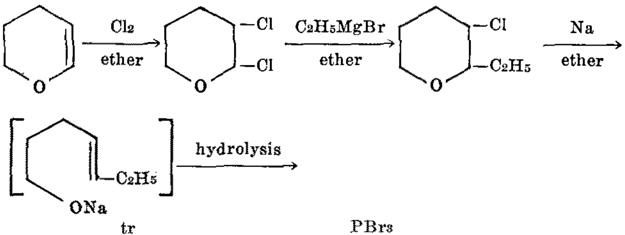
$$\mathrm{CH_3{-}CH_2{-}C{\equiv}C{-}(CH_2)_4{-}CH_2I} \xrightarrow[\text{Lindlar cat.}]{\mathrm{H_2/Pd\ (Pb)}}$$

$$\mathrm{CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}(CH_2)_4{-}CH_2I} \xrightarrow[\mathrm{CHCl_3}]{\mathrm{(CH_3)_3NO}}$$

$$\mathrm{CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}(CH_2)_4{-}CHO}$$

The yield was 35%: ca. 90% of the *cis*-form and ca. 10% of the *trans*-form.

6-trans-Nonenal was synthesized as follows:



$$\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_2{-}CH_2OH} \xrightarrow{\mathrm{PBr_3}}$$

$$\underset{[17]}{\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_2{-}CH_2Br}} \xrightarrow[\mathrm{HCHO}]{\mathrm{Mg}}$$

$$\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_3{-}CH_2OH} \xrightarrow{\mathrm{PBr_3}}$$

$$\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_3{-}CH_2Br} \xrightarrow[\mathrm{CH(OC_2H_5)_3}]{\mathrm{Mg}}$$

$$\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_4{-}CH(OC_2H_5)_2} \xrightarrow{\text{hydrolysis}}$$

$$\mathrm{C_2H_5{-}CH\overset{tr}{=}CH{-}(CH_2)_4{-}CHO}$$

The synthesis was carried out in a manner analogous to that for 5-*trans*-compound with the exception of the intermediate stage to extend the 1-bromo-4-*trans*-heptene [17] by one carbon atom (38) to 5-*trans*-octenol, which now proceeded in the normal way. The yield of 6-*trans*-nonenal was 70%, purity being 99%.

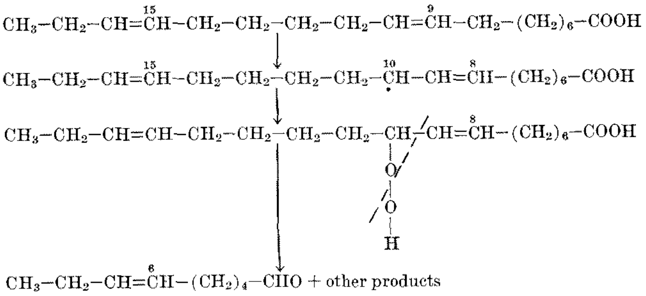
Both 6-*cis*- and 6-*trans*-nonenal have a typical flavor reminiscent of green melon. The flavor potency of the *trans*-form dissolved in paraffin oil is ca. 10 times stronger than that of the synthesized *cis*-form (to be published). Although the type of flavor of 5-*trans*-nonenal is identical with that of 6-nonenal, its flavor potency in paraffin oil is lower than that of 6-*trans*-nonenal by a factor of 100–200 (to be published).

Location of the Double Bond. In order to verify the results obtained so far and to obtain additional proof of the location of the double bond, we applied a modification of the technique of De Jong et al. (40) using TLC with silica impregnated with silver nitrate (41) for the separation of unsaturated DNPHs into various classes. A conc of the hardening flavor was obtained by degassing 50 kg of hardened linseed oil (I.V. 87.6) and by preparative GLC (apparatus: F and M 770, temp 170C, press. 20 lbs/sq in, column length 4.80 m) using polyethylene glycol adipate (20%) as immobile phase. The fraction with the typical hardening flavor and with the same retention time as synthetic 6-*trans*-nonenal (carbon number 9.6) (42) was trapped in a condenser which was cooled with liquid nitrogen. The volatile material was taken up in carbonyl-free light petroleum (bp 40–60C) and the carbonyls converted into their DNPHs (43). The DNPHs were purified by passing over a neutral alumina column using a mixture of light petroleum and an increasing amt of diethyl ether as eluent and then separated on chromatoplates using silica impregnated with silver nitrate as adsorbent (to be published). From these plates two fractions were recovered with the same elution rates as those of 6-*trans*- and 6-*cis*-nonenal. Samples of both fractions displayed the typical hardening flavor after regeneration, whereas in the other fractions this flavor was not present.

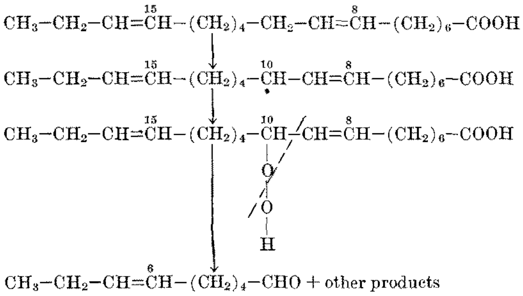
Subsequently, the two DNPH fractions were separated by TLC using silicagel G (ex Merck) (44), and the spots having the same elution rates as the model substances were recovered. After regeneration of the carbonyls from part of the DNPHs, the hardening flavor in question could be perceived again. Finally, both fractions were further purified by passing over an alumina column as described before. For both cases the UV-spectrum showed an absorption maximum at 358 mμ measured in chloroform solution. The absorption at this wave-length is typical of DNPHs of aldehydes with a CH_2-group at the α-position. The amt of material of the fraction showing a chromatographic behaviour similar to that of 6-*cis*-nonenal was too small for further investigation. The IR-spectrum of the *trans* fraction showed an absorption maximum at 970 cm^{-1} which indicates the presence of an isolated *trans* double bond in the molecule. The double bond could be located by means of an oxidation technique using osmium tetroxide. Analysis of the degradation products showed the presence of propanal thus proving the double bond to be situated between carbon atoms 6 and 7 in the nonenal molecule.

Precursors of the Hardening Flavor Components. Assuming that the oxidation mechanism according to Farmer (39) is correct, the precursors of 6-*trans*- and 6-*cis*-nonenal must be 9,15- and/or 8,15-octadecadienoic acids, which may decompose in accordance with the following schemes:

With Allylic Rearrangement

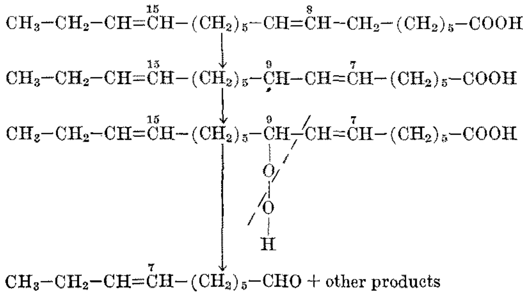


Without Allylic Rearrangement

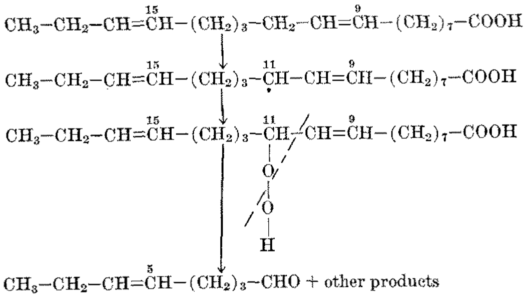


Besides the formation of both 6-*cis*- and 6-*trans*-nonenal from 9,15- and 8,15-octadecadienoic acids other monoenal degradation products may also be formed from the peroxides of these acids:

With Allylic Rearrangement



Without Allylic Rearrangement



By synthesizing 7,15-, 8,15- and 9,15-octadecadienoic acids we could indeed confirm that the 6-nonenals are produced in the very early stages of oxidation. This subject will be dealt with in a future publication.

ACKNOWLEDGMENT

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