• *Technical*

3-*cis*-Hexenal, the "Green" Reversion Flavor of Soybean Oil

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By means of gas-liquid-chromatography the neutral volatile products obtained by stripping oxidized soybean oil were fractionated.

The fraction with ''green bean'' odor (a well-known off-flavor of soybean oil) was investigated. By the application of various methods it was possible to isolate the flavor carrier proper and, in spite of its small amount, to complete the analysis to a point at which the structure 3-*cis*-hexenal seemed highly probable. Synthesis and subsequent comparison with the natural product confirmed the correctness of the assumption.

n-Hexanal and 3-*trans*-hexenal, also found in this fraction, play no significant role in the reversion flavor.

IN THE LAST FEW YEARS the reversion of soybean oil has been the subject of many investigations, which were induced by the economic importance of this oil. Fundamental work carried out in the United States has proved that linolenic acid is the precursor of the reversion flavors (1). The chemical structure of these off-flavors has also been investigated, and it is generally accepted that most of them have a carbonyl function.

In a recent investigation by von Pezold (2) the volatile, light-petroleum-soluble products isolated from reverted or rancid soybean oil were separated into fractions by means of partition chromatography and countercurrent distribution. The flavors of these fractions were partly recognized as the characteristic soybean oil off-flavors, well known to the members of a trained panel. In these fractions many aldehydes, belonging to the homologous series of saturated, $\alpha\beta$-, and $\alpha\beta$, $\gamma\delta$-unsaturated aliphatic aldehydes, were identified by means of their dinitrophenylhydrazones (DNPHs). From model experiments with esters of linoleic and linolenic acid it was concluded that 2,4-heptadienal, and/or 2,4-octadienal, both originating from the autoxidation of linolenic acid, play a decisive role in causing the reversion flavor of soybean oil.

The present investigation deals with the isolation and identification of a special off-flavor (odor), not identified earlier, to which the descriptive name ''green beans'' is given.

Isolation and Identification of the ''Green Bean'' Flavor

It may be assumed that the reversion of oils containing linolenic acid is caused by oxidation. On storage of such oils, reversion off-flavors will soon develop, which are organoleptically perceptible at such low concentrations that they cannot be detected with current analytical methods. In order to collect the volatile substances in analytically detectable amounts, soybean oil was subjected to oxidation by air at high temperature. In this way a fairly large amount of volatile decomposition products as well as of the compounds responsible for the reversion off-flavors was formed. These products were liberated from water and fatty acids and were separated on a gas-liquid-chromatography (GLC) column into fractions.

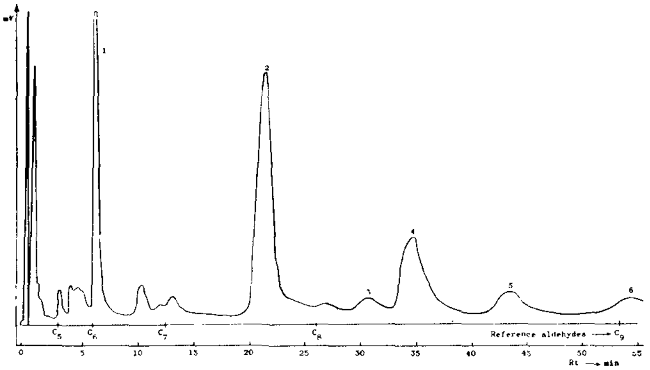


FIG. 1. GLC of soybean oil stripping products. Column—length 900 mm.; diameter 4 mm.; carrier, Celite 545; immobile phase, silicone oil (30%, Dow-Corning); mobile phase, nitrogen. Temperature 93°C.; gas-pressure 24.5 cm. Hg; gas-rate 29 ml./min. For reference the retention times (Rt-values) of a homologous series of saturated aliphatic aldehydes are given on a second abscissa.

The chromatogram is shown in Figure 1. The Rt-values and corresponding characteristic odors belonging to the major peaks are recorded in Table I. The first fraction was more intensely studied. The Rt-value is equal to that of normal hexanal, but the latter compound has no ''green bean'' flavor. Infrared (IR) analysis indicated the presence of mainly short-chain aldehydes with the characteristic frequencies of 1733 and 2730 cm.$^{-1}$ for the aldehyde group and of 725 cm.$^{-1}$ for the $-(CH_2)_4$-group.

TABLE I

Rt-Values and Odors of the GLC Fractions of the Neutral Volatile Matter from Oxidized Soybean Oil

Fraction number	Rt at 93°C. (min.)	Odor description
1	6[a]	Green beans
2	20	Brown beans
3	29	Rotten apples
4	33	Rancid hazelnut
5	42	Brown beans
6	53	Citrus

[a] Rt equal to that of n-hexanal.

To confirm the presence of carbonyl compounds, the 2,4-dinitrophenylhydrazones (DNPHs) of Fraction 1 (dissolved in light petroleum) were prepared (3). It was found that the light petroleum solution of the DNPHs was odorless after the forming of the DNPHs.

Upon hydrolysis of a small portion of the DNPH-derivatives and steam distillation of the liberated carbonyl compounds the typical ''green bean'' odor could be detected again. This was taken as a proof of the carbonyl function in the relevant compound.

By paper chromatography (4) of the DNPHs four spots were obtained: two heavy spots having the same Rf-values as n-hexanal and n-pentanal and two weak spots corresponding to n-butanal and propanal, respectively (Table III, Column 2). After treatment with alcoholic KOH the spots turned brown, the typical color for saturated aldehydes, or more correctly, for aldehydes not having a 2,3-double bond. Partition chromatography of the same DNPHs on the nitromethane/silicagel column (5) gave four distinct bands. The retention-volumes (Rv) and the ultraviolet (UV) absorption maxima are given in Table II.

TABLE II

Partition Chromatography of the DNPHs of the GLC Fraction Having "Green Bean" Odor

Band	Rv (ml.)	$\lambda_{max.}$ (mμ, $CHCl_3$)	Paper chromatogram corresponding with that of DNPH of
1	190	359	n-hexanal
2	320	360	n-pentanal, 3-*trans*-hexenal
3	540	Not measured Very weak	....
4	600	Not measured Very weak	....

TABLE III

DNPH-Spots of the Original Fraction and Ozonolyzed "Green Bean" Band 1b

Reference DNPH of	DNPHs in original fraction	DNPHs in ozonolyzed Band 1b
ethanal	....	w
propanal	w	s
n-butanal	w	....
n-pentanal	s	....
n-hexanal	s	....
n-heptanal	....	....

w = weak; s = strong.

After hydrolysis and distillation of Band 1 the "green bean" odor was again noticeable, proving that, besides hexanal, the unknown substance was also present in this band.

To get further separation the DNPHs of Band 1 were chromatographed over alumina. Two bands, 1a and 1b could be obtained. On being subjected separately to the nitromethane/silicagel column (5), the Rv and $\lambda_{max.}$ values for each band were the same as and were equal to those of hexanal DNPH (Table II, Band 1).

Paper chromatography (4) revealed that both bands also had the same Rf-value as hexanal DNPH. After hydrolysis and distillation Band 1a gave the typical hexanal odor, Band 1b the relevant "green bean" odor; the latter seems to be much stronger than the odor of n-hexanal. The isolation of the odor carrier had thus been achieved.

On the basis of these facts it may be concluded that the "green bean" odor carrier is an aldehyde which has possibly not more than six carbon atoms and is saturated at the α-β position. If unsaturated, the double bond must be situated between the third and fourth or between the fourth and fifth carbon atoms.

(In the IR spectra there was no indication for the presence of a vinyl group.)

A portion of Band 1b was directly (consequently in the form of DNPH) ozonolyzed (6). The ozonides were decomposed, and the volatile decomposition products were distilled in dinitrophenylhydrazine-reagent. The DNPHs formed were chromatographed on paper (7). The main component gave a spot at the propanal-position. There was an impurity at the ethanal position but no spot at a higher aldehyde position. Treatment with alcoholic KOH produced again the brown color typical for saturated aldehydes (Table III, Column 3).

From the presence of the strong propanal DNPH-spot as chief ozonolysis decomposition product it can be deduced that the isolated unknown flavor carrier is presumably 3-hexenal. This aldehyde can be derived from oxidized linolenic acid, according to the theories of Farmer (8) and has actually been predicted as a possible decomposition product of linolenic acid by Broderick (9). On theoretical considerations (see Discussion) it was concluded that this 3-hexenal must possess the *cis* stereoconfiguration. After synthesis of 3-*cis*-hexenal the properties were indeed found to correspond completely to the isolated "green bean" compound from oxidized soybean oil.

Experimental

Oxidation and Analysis. An amount of refined and deodorized soybean oil (4 kg.) was oxidized for 4 hrs. at 120°C. in a strong current of air. The volatile products were condensed in a spiral cooled in dry ice/alcohol. The oil was subsequently stripped at 170°C. and 10 mm. Hg by passing through nitrogen gas for 6 hrs.

The total condensate, consisting of water, a small amount of lower fatty acids, and neutral substances, was extracted with light petroleum. The extract was neutralized with 10% aqueous sodium hydrogen carbonate, washed neutral with water, and dried over anhydrous sodium sulphate. Subsequently the solvent was evaporated *in vacuo,* using a freeze-drying apparatus. The residue was fractionated on a GLC-column (Figure 1 and Table I); the fractions were separately collected in a trapping system after the detector (gas-density balance).

The liberation of the DNPHs was carried out by boiling a few mg. of them with 2–3 ml. of 2 N sulphuric acid in a small all-glass distillation apparatus. The condenser is provided with an adapter and submerges beneath the surface of 2 ml. of light petroleum in a test-tube. After hydrolysis the volatile carbonyls are distilled with the water vapor and collected in the light petroleum. DNPHs with melting points far above 100°C. are liberated by the method of Keeney (10).

The micro-ozonolysis of DNPHs (6) was carried out as follows. 0.02–2 mg. of DNPH-compound is dissolved in 1–3 ml. of carbon tetrachloride. A mixture of O_3/O_2 is blown through the solution at 0°C. until it becomes colorless. The carbon tetrachloride is evaporated *in vacuo,* and the residue is dissolved in water. This solution, to which some crystals of ferrous sulphate are added to decompose the ozonides, is boiled, and the liberated volatile carbonyls are stripped by a stream of nitrogen and collected in two washing-bottles filled with dinitrophenylhydrazine reagent. The DNPHs formed are chromatographed (7).

For IR analyses a Unicam S.P. 130 double-beam grating spectrophotometer was employed and for UV analyses a Unicam 500 S.P. UV spectrophotometer.

Synthesis of the DNPH of 3-cis-Hexenal. For the synthesis of the DNPH of 3-*cis*-hexenal, at first, 3-hexynal was prepared according to the method of Herbertz (11). 1-Methoxy-1-buten-3-yne (b.p. 62°C. at 80 mm.; $n^{20/D}$ 1.4778) was converted into the 4-sodium compound with sodium amide. By treatment of the product formed with diethyl sulphate, 1-methoxy-1-hexen-3-yne was obtained (b.p. 98–100°C. at 75 mm.; $n^{25/D}$ 1.4868; yield 46%).

Upon partial hydrogenation with a palladium catalyst, according to Lindlar (12), 1-methoxy-hexa-1,3-

diene was formed (b.p. 75–78°C. at 75 mm.; $n^{25}/_D$ 1.4659; yield 70%). From GLC analysis it appeared that this substance contained 80% of the product. IR analysis (Figure 2) proved the presence of the *cis-trans* conjugated double bonds (945 and 980 cm.$^{-1}$). Acid hydrolysis of 1-methoxy-hexa-1,3-diene and immediate DNPH-formation of the aldehyde formed was achieved by using the dinitrophenylhydrazine-celite column (3).

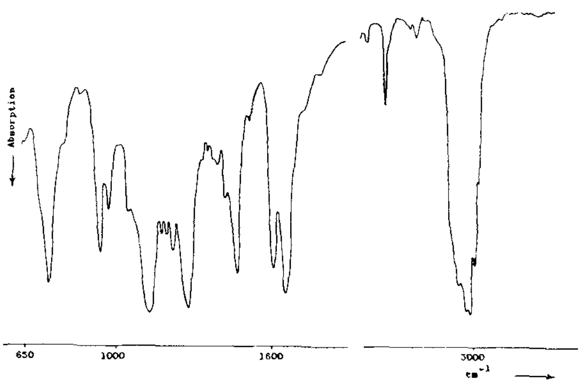


FIG. 2. Infrared spectrum of 1-methoxy-hexa-1,3-diene *(trans, cis).*

Partition chromatography of the synthetic 3-*cis*-hexenal-DNPH gave three bands on the nitromethane/silicagel column (5). Paper chromatography (4) of Band 1 (the major component) gave a spot corresponding with n-hexanal-DNPH and with the DNPH of the isolated natural product (cf. Table II). IR analysis of this band indicated the presence of a monounsaturated aldehyde (Figure 3a) and showed no *trans* absorption at 970 cm.$^{-1}$ The IR spectrum of authentic 3-*trans*-hexenal-DNPH (synthesized also in our laboratory) is very similar (Figure 3b) but has a very strong *trans* absorption at 970 cm.$^{-1}$ Band 1 was rechromatographed on alumina and eluted with light petroleum/ethyl ether (49:1).

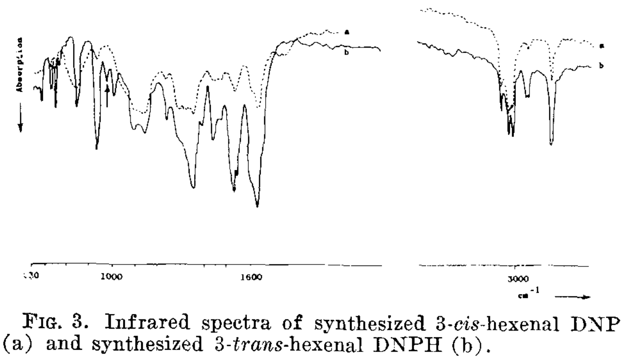
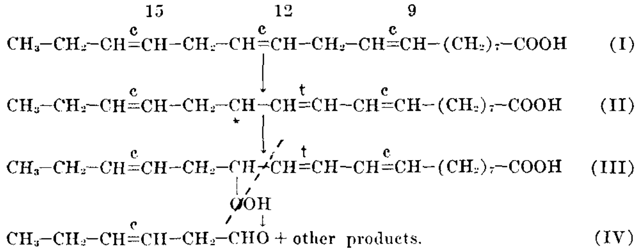


FIG. 3. Infrared spectra of synthesized 3-*cis*-hexenal DNPH (a) and synthesized 3-*trans*-hexenal DNPH (b).

No impurities were found, and it was concluded that during the hydrogenation step of the synthesis no n-hexanal had been formed as a by-product. Ozonolysis (6) of this DNPH and paper chromatography (7) of the DNPH of the decomposition product obtained by ozonolysis again gave propanal. This confirmed the expected position of the double bond. After hydrolysis of Band 1 and distillation the "green bean" odor was perceptible. The odor could not be distinguished from that of the natural product isolated from reverted soybean oil.

Discussion

As has been already mentioned, the formation of 3-hexenal from oxidized linolenic acid is readily explained by the theoretical considerations of Farmer (8). The *"cis"* configuration can be predicted by applying the rules of Nichols *et al.* (13) to linolenic acid. The reaction scheme may be illustrated as follows:



$$\overset{15}{}\quad \overset{12}{}\quad \overset{9}{}$$

$$CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}CH_2{-}CH\overset{c}{=}CH{-}CH_2{-}CH\overset{c}{=}CH{-}(CH_2)_7{-}COOH \qquad (I)$$

$$CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}CH_2{-}\underset{*}{C}H{-}CH\overset{t}{=}CH{-}CH\overset{c}{=}CH{-}(CH_2)_7{-}COOH \qquad (II)$$

$$CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}CH_2{-}\underset{OOH}{\underset{|}{C}H}{-}CH\overset{t}{=}CH{-}CH\overset{c}{=}CH{-}(CH_2)_7{-}COOH \qquad (III)$$

$$CH_3{-}CH_2{-}CH\overset{c}{=}CH{-}CH_2{-}CHO + \text{other products.} \qquad (IV)$$

In the initial stage of the oxidation an hydrogen atom can be abstracted from the linolenic acid molecule (I) at the C11-atom leading *via* an allylic rearrangement to give radicals such as (II). This gives the hydroperoxide (III) as a primary oxidation product. The hydroperoxide can decompose in a number of ways, one of these leading to 3-*cis*-hexenal (IV).

Apart from 3-*cis*-hexenal, Fraction 1 (Table I) contains n-hexanal (in Band 1 of Table II) and by the same sequence of analysis 3-*trans*-hexenal (in Band 2 of Table II). The formation of n-hexanal from oxidized linoleic acid is obvious; an attempt to explain the presence of 3-*trans*-hexenal would seem presumptuous at this stage of the investigation. At any rate the odor of Fraction 1 is dominated by 3-*cis*-hexenal. It is however clear that 3-*cis*-hexenal is not the sole reversion flavor of soybean oil. Table I records other characteristic odors, which are at present under investigation.

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