2-*trans*,4-*cis*,7-*cis*-Decatrienal, the Fishy Off-Flavor Occurring in Strongly Autoxidized Oils Containing Linolenic Acid or ω3,6,9, etc., Fatty Acids[1]

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ABSTRACT

A possible decomposition product of oils containing unsaturated fatty acids with the double bond pattern ω3,6,9 or ω3,6,9, etc., is 2-*trans*,4-*cis*, 7-*cis*-decatrienal. It has therefore been investigated whether this trienal is present in strongly autoxidized linolenic acid, soybean, linseed and fish oils. Via a degassing technique the carbonyls from the samples of oils heated in a closed system under nitrogen were collected and separated as their 2,4-dinitrophenylhydrazones (DNPHs) by thin layer chromatography (TLC). The presence of 2-*trans*,4-*cis*,7-*cis*-decatrienal in the samples was positively established by TLC, UV absorption and mass spectroscopy of its DNPH. All analytical data established the complete identity of the isolated DNPH with a synthesized 2,4,7-decatrienal-DNPH. The free aldehyde obtained on hydrolysis of the alkatrienal-DNPH in question had the same flavor as authentic 2-*trans*,4-*cis*,7-*cis*-decatrienal, having a fish or rather a whale oil flavor.

INTRODUCTION

In the period 1919-36, many investigators (1-4) studied the occurrence of a fishy off-flavor in autoxidized butter and were of the opinion that trimethylamine or its oxide was responsible for this off-flavor in stored butter. In the period 1944-47, however, Van der Waarden (5,6) established that this opinion was false; he made a suggestion, based partly on experimental evidence, that possibly unsaturated aldehydes with the double bond not in conjugation with the carbonyl group might be responsible for the off-flavor. Later Pont et al. (7), Forss et al. (8), Stark and Forss (9) and El-Negoumy et al (10) carried out investigations in the same direction. Badings (11) stated that 4-*cis*-heptenal, previously isolated by Haverkamp Begemann and Koster (12) from cold-storage butter, can be characterized as having a fish or whale oil flavor, if present in a concentration of 15 μg/kg or higher. Haverkamp Begemann and Koster, however, found that the flavor of 4-*cis*-heptenal in high dilution (1.5 μg/kg) is cream-like or butterscotch-like.

A whale oil flavor can also be observed in strongly autoxidized soybean (13) and linseed oils (14), and, more easily, in autoxidized fish oils. From the work of Frankel et al. (15) we know that mainly four monohydroperoxides of linolenic acid with the peroxide group at the 9th, 12th, 13th and 16th positions in the chain will be formed by autoxidation. Decomposition of the isomeric hydroperoxides would result in the formation of 2,4,7-decatrienal, 2,4-heptadienal, 3-hexenal and propanal, respectively. Evidence for all four aldehydes is given in the literature (16-18,38). We have concentrated our work on the isolation and identification of 2-*trans*,4-*cis*,7-*cis*-decatrienal in autoxidized oils containing linolenic acid or other higher unsaturated fatty acids with the double bond pattern ω3,6,9, etc.

EXPERIMENTAL PROCEDURES

Preparation of Carbonyl-Free Hexane

Hexane was saturated with ozone at -70 C and then shaken several times with concentrated sulfuric acid, each

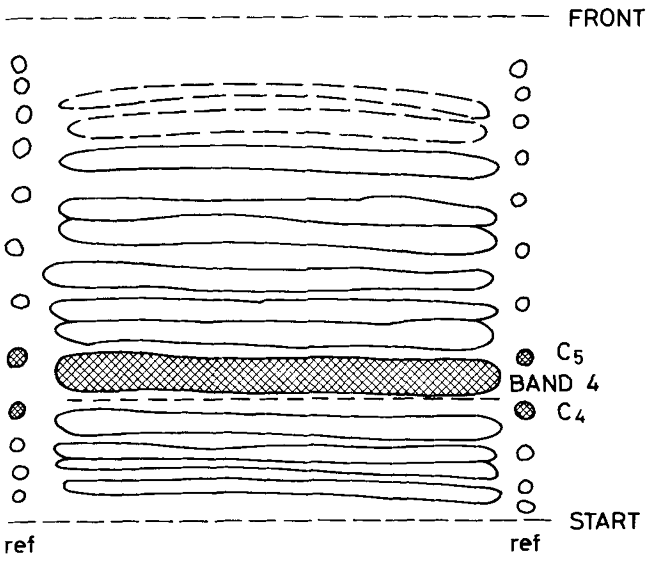

FIG. 1. Separation of 2,4-dinitrophenylhydrazones of carbonyls from strongly autoxidized soybean oil by partition thin layer chromatography; adsorbent: 33.3% Carbowax 400 on Kieselguhr G. Mobile phase: light petroleum (bp 100-120 C). Ref.: DNPHs of C_1-C_{12} alkanals.

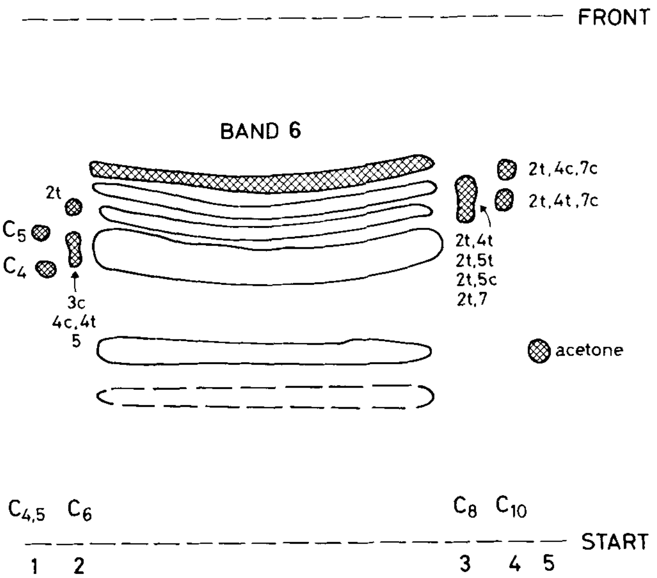

FIG. 2. Separation of band 4 of Figure 1; adsorbent: Silica Gel G, mobile phase: CCl_4/hexane/ethyl acetate 10:2:1 v/v/v. Refs: DNPHs of (1) butanal and pentanal; (2) hexenals; (3) octadienals; (4) decatrienals; (5) acetone.

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[1] Presented by J.B.A. Stroink at the ISF Congress, Göteborg, Sweden, June 1972.

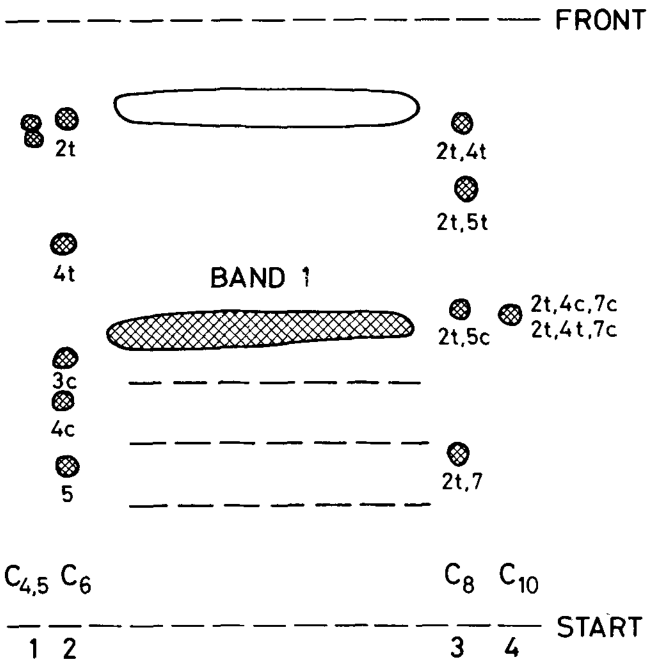

FIG. 3. Separation of band 6 of Figure 2; adsorbent: Silica Gel G impregnated with 30% $AgNO_3$, mobile phase: benzene. Refs.: see (1-4) in Figure 2.

time using an acid-hexane ratio of 1:3 in a separatory funnel until the acid layer remained colorless, washed with distilled water, dried over anhydrous magnesium sulfate and finally distilled. Subsequently 30 g Al_2O_3 ("active neutral" from Merck) in purified hexane was introduced as a slurry into a chromatographic column with an internal diameter of 2 cm, forming a 10 cm layer of Al_2O_3. Above this layer, celite impregnated with 2,4-dinitrophenylhydrazine dissolved in 2 N HCl (19) was introduced as a hexane slurry. The amount of hexane needed for the experiments was percolated three times over this column as an additional measure to make it carbonyl-free.

Conversion of Carbonylic Volatiles into Their 2,4-Dinitrophenylhydrazones (DNPHs)

About 100-500 g soybean oil with a peroxide value of 30 meq/kg was first heated in a stoppered flask in a nitrogen atmosphere at 100 C for 1 hr to decompose the peroxides and was subsequently degassed (20) at 40 C for 5 hr at a pressure $<10^{-5}$ mm Hg. The volatile decomposition products were trapped in a U-tube which was cooled at -180 C with liquid nitrogen and was subsequently dissolved in ca. 200 ml carbonyl-free hexane. This solution was brought onto a 2,4-dinitrophenylhydrazine reaction column prepared according to Schwartz and Parks (21). During slow elution with a sufficient amount of carbonyl-free hexane, the volatile carbonyls were converted into their DNPHs. The hexane DNPH solution was evaporated in vacuo, and the residue was taken up in a small volume of freshly distilled diethyl ether.

Thin Layer Chromatography (TLC) of the DNPHs

For the separation and identification of the DNPHs from different autoxidized oils containing linolenic acid or polyunsaturated fatty acids with the double bond pattern ω3,6,9, etc., our previously described sequence of TLC analyses was used (22).

a) *Partition TLC on Carbowax 400-Kieselguhr G (Fig. 1):* The DNPH mixture from soybean oil, dissolved in freshly distilled diethyl ether, was spotted as a small band onto a Kieselguhr G chromatoplate impregnated with 33.3% Carbowax 400 (23). On both sides of this band, a mixture of alkanal-DNPHs (C_1-C_{12}) was applied as reference material.

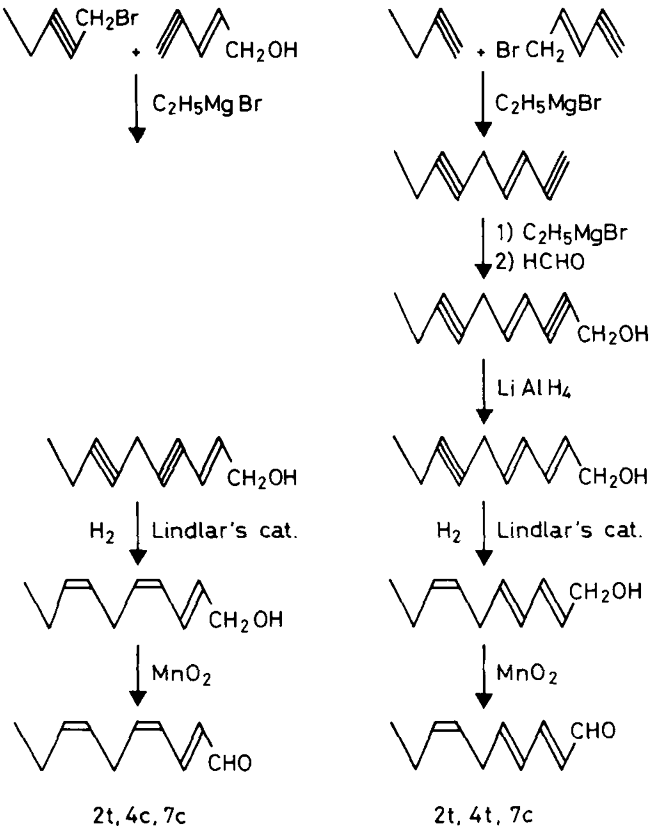

FIG. 4. Syntheses of 2-*trans*,4,7-*cis*-decatrienals.

After development with light petroleum (bp 100-120 C) as eluant, many yellow-to-orange bands were obtained. After spraying the chromatogram with dilute alcoholic alkali, the DNPHs underwent their characteristic color change, as described by Lappin and Clark (24). The purple band 4, which originally had an orange color, probably contained the DNPH of 2,4,7-decatrienal, and was scraped off the plate and extracted with freshly distilled diethyl ether. This procedure was repeated several times to obtain enough material for further analyses. To dispose of the dissolved Carbowax present in the pooled ether extract, this ether solution was washed three times with distilled water and finally dried with anhydrous $Na_2SO_4/MgSO_4$. To ascertain whether the dried ether DNPH solution contained the 2-*trans*,4-*cis*,7-*cis*-decatrienal, the TLC separations described under *b*), *c*) and *d*) were applied to band 4.

b) *Adsorption TLC on Silica Gel G (Fig. 2):* The DNPHs from *a*), in a small amount of diethyl ether, were applied as a narrow band onto an ordinary silica chromatoplate between the reference materials. These were acetone-DNPH and DNPH mixtures of butanal and pentanal, of isomeric straight chain hexenals and of octadienals and decatrienals. All these DNPHs have nearly the same Rf value on the Carbowax plate (23) used in *a*). After ascending chromatography with carbon tetrachloride-hexane-ethyl acetate 10:2:1 v/v/v as mobile phase (25), six distinct bands were obtained. The upper band 6 (with the same Rf value as authentic 2-*trans*,4-*cis*,7-*cis*-decatrienal-DNPH) was scraped off and extracted with freshly distilled diethyl ether.

c) *Argentation TLC on Silica Gel G-AgNO₃ (Fig. 3):* Band 6 from *b*) was brought onto a silica plate impregnated with 30% $AgNO_3$, and the same reference substances as under *b*) were applied to both sides. By ascending chromatography, with benzene as eluant (26), several bands were obtained. The distinct band 1 had, also in this TLC system, the same Rf value as the model substance 2-*trans*,4-*cis*,7-*cis*-decatrienal-DNPH. After extraction of this band with distilled diethyl ether, measurement of the UV absorption spectrum in chloroform showed three λ_{max} at 266, 303

TABLE I

Analytical and UV Spectral Data

Compound	Yield, %	Boiling point, C (mm Hg)	Melting point, C	n_D^t	Gas liquid chromatography purity, %	UV spectrum λmax, nm	UV spectrum (E)	UV spectrum Measured in
2*t*-Decen-4,7-diynol	55.0	103-105 (2)	—	1.5230^{25}	99.5		—	
2*t*,4*c*,7*c*-Decatrienol	—	91-93 (0.45)	—	1.5015^{25}	96	234	21,700	EtOH
2*t*,4*c*,7*c*-Decatrienal as such	80.0	65-67 (0.4)	—	1.5402^{25}	96.5	278	21,600	EtOH
2*t*,4*c*,7*c*-Decatrienal DNPH	—	—	51-52	—	—	268	17,500	$CHCl_3$
						307	11,200	
						390	36,600	
3*t*-Nonen-1,6-diyne	47.5	35-38 (0.8)	—	1.4932^{20}	97.5		—	
4*t*-Decen-2,7-diynol	28.7	105-107 (0.5)	—	1.5198^{20}	99.5		—	
2*t*,4*t*-Decadien-7-ynol	92.8	—	—	1.5123^{20}	90		—	
2*t*,4*t*,7*c*-Decatrienol	92.4	88-91 (0.9)	—	1.5060^{20}	84		—	
2*t*,4*t*,7*c*-Decatrienal as such	75.0	—	—	1.5345^{20}	85	273	21,600	EtOH
2*t*,4*t*,7*c*-Decatrienal DNPH	—	—	111.5-113	—	—	268	21,500	$CHCl_3$
						306	17,700	
						397	37,000	

and 390 nm which are characteristic for DNPHs of 2,4,-alkadienals.

d) *Rechromatography on Carbowax 400-Kieselguhr G:* Band 1 from c) was reapplied to a 33.3% Carbowax 400-Kieselguhr G plate, which proved (23) that the isolated alkatrienal-DNPH in question emerged as only one band between the DNPHs of butanal and pentanal.

Synthesis of 2-*trans*,4-*cis*,7-*cis*- and 2-*trans*,4-*trans*,7-*cis*-decatrienals (Fig. 4)

(a) Following the procedure of Osbond et al. (27) the di-Grignard derivative of 2-*trans*-penten-4-ynol (28) was coupled with 1-bromo-2-pentyne (29) giving 2-*trans*-decen-4,7-diynol. Partial hydrogenation of the latter compound in the presence of Lindlar's catalyst (30) resulted in the formation of 2-*trans*,4-*cis*,7-*cis*-decatrienol. Since the semi-hydrogenated product contained some under- and over-hydrogenated compounds, it was subjected to counter-current distribution. The purified decatrienol was oxidized with "active" manganese dioxide according to the method of Attenburrow et al. (31), giving 2-*trans*,4-*cis*,7-*cis*-decatrienal. The red-brown DNPH derivative was prepared according to a modification of the method of Winter and Gautschi (32), in which ultimately, instead of water, water-ethanol 2:1 v/v was added; it was recrystallized from 90% aqueous ethanol. For analytical data see Table I (upper half) and II (left-hand side).

(b) 1-Bromo-2-*trans*-penten-4-yne, prepared from 2-*trans*-penten-4-ynol according to Henbest et al. (33), was coupled with the Grignard derivative of 1-butyne (34), using the method of Eǧe et al. (35) to give 3-*trans*-nonen-1,6-diyne. A Grignard reaction between this compound and depolymerized paraformaldehyde, according to the method of Taylor and Strong (36) gave 4-*trans*-decen-2,7-diynol. Treatment with lithium aluminium hydride in ether (37) resulted in 2-*trans*,4-*trans*-decadien-7-ynol. Subsequent partial hydrogenation (30) and oxidation (31), as described under (*a*), afforded 2-*trans*,4-*trans*,7-*cis*-decatrienol and the corresponding aldehyde, respectively. Its DNPH was brick-red. For analytical data see Table I (lower half) and II (right-hand side).

RESULTS AND DISCUSSION

Isolation of carbonyls and separation of their DNPHs, as described under Experimental Procedures, were carried out separately for strongly autoxidized linolenic acid, linseed

TABLE II

IR and Mass Spectral Data and NMR Parameters[a]

Specification	2-*trans*,4,7-*cis*-Decatrienal 4-*cis*	2-*trans*,4,7-*cis*-Decatrienal 4-*trans*
IR (bands in cm^{-1})		
2-*trans*,4-Dienal	1682,1635,1595,1145 1120,1007,987,962,855	2745,1692,1645,1605 1165,1125,1017,993
Isolated *cis*	3018,720	3020,730
$-C_2H_5$	2970,795	2980,798
NMR (δ-value, 220 MHz, CCl_4)		
$-CH_3$	0.99*t*/7.4 (3)	0.98*t*/7.5 (3)
$-CH_2-$	2.09*q*/7.4 (2)	2.03*q*/7.5 (2)
$\overset{c}{=}\overset{\vert}{C}-CH_2-\overset{\vert}{C}=$	3.05*t*/7.4 (2)	2.91*t*/6.3 (2)
$-CH\overset{c}{=}CH-$	5.26*dt*/10.8,7.4 (1) 5.44*dt*/10.8,7.4 (1)	5.27*dt*/10.5,6.3 (1) 5.45*dt*/10.5,7.5 (1)
$-CH=\overset{\vert}{C}-\overset{\vert}{C}\overset{t}{=}CH-$	5.85*dt*/10.8,7.4 (1) 6.06*dd*/15.3,7.8 (1)	5.96*dd*/15.5,7.5 (1) 6.11*dt*/15,6.3 (1)
$=CH-CH\overset{t}{=}$	6.21*dd*/11.5,10.8 (1) 7.39*dd*/15.3,11.5 (1)	6.26*dd*/15,10 (1) 6.93*dd*/15.5,10 (1)
-CHO	9.55*d* 7.8 (1)	9.52*d*/7.5 (1)
Mass (m/e)		
Parent peak found (calculated)	150 (150)	150 (150)

[a] δ-Values followed by J or J_1 and J_2 values. Abbreviations used as follows: *d* = doublet, *t* = triplet, *q* = quintet, *dd* = doublet of doublets, *dt* = doublet of triplets.

and fish oils by which the occurrence of 2-*trans*,4-*cis*,7-*cis*-decatrienal in these products could be established. Because of a sufficient amount obtained via TLC, the molecular weight of the alkatrienal-DNPH could be determined in duplicate by means of mass spectroscopy, as an additional proof of its identity. Besides, the position of the isolated double bond of the alkatrienal-DNPH in question could be located by means of an oxidation technique using osmium tetroxide. Analysis of the degradation products showed the presence of propanal, proving the isolated double bond to be situated between carbon atoms 7 and 8 in the alkatrienal molecule.

The flavor of the isolated compound as free aldehyde has been compared with that of synthesized 2-*trans*,4-*cis*,7-*cis*-decatrienal in a dilution of 10 mg/kg paraffin oil (the alkatrienal-DNPH was hydrolyzed via a micro steam distillation technique in the presence of 4 N H_2SO_4 to give the free aldhyde in aqueous solution). We assessed both samples as having the same fishy or cod liver-like flavor. Contrary to our findings, Badings (38) characterized the flavor of both 2-*trans*,4-*cis*,7-*cis*- and 2-*trans*,4-*trans*,7-*cis*-decatrienal, isolated from autoxidized methyl linolenate, as being sliced beans. For the synthesized *trans* isomer we only assessed—besides a less pronounced fish or whale oil flavor—a sweet, greeny, cucumber- and melon-like flavor.

It can be concluded that the presence of 2-*trans*,4-*cis*,7-*cis*-decatrienal among the decomposition products in strongly autoxidized linolenic acid, and further in soybean, linseed and fish oils can be proved via combined TLC techniques, location of the double bond in isolated position, UV and mass spectroscopy. The aldehyde should thus be considered as an important carrier of fish or whale oil flavor, present in oils containing linolenic acid or unsaturated fatty acids having an $\omega3,6,9$, etc., double bond pattern.

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