Isolation of Two Isomeric 2, 6-Nonadienals and Two Isomeric 4-Heptenals from Beef and Mutton Tallow

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Abstract

By the use of mainly chromatographic and UV spectrophotometric techniques, 4*cis*- and 4*trans*-heptenal (with a "green" odor, characterized in high dilution as tallowy, creamy, butterscotch-like) and 2*trans*,6*cis*- and 2*trans*,6*trans*-nonadienal (with a "green"-cucumber-like and a tallowy odor) were isolated from reverted beef and mutton tallow.

Introduction

TALLOW IS THE HARD FAT of ruminants, and the most important is that of cows and sheep. It has a typical "tallowy" odor, which can be defined as a heavy, sweetish, soapy odor with a slight "greenish" note. It may sometimes have a nauseating effect. Fully refined tallow redevelops this off-flavor in about two weeks. This phenomenon is called flavor reversion, which takes place in the early stages of the fatty acid autoxidation. We studied this flavor reversion process in connection with the quality of products which contained fairly large amounts of this important raw material.

Reversion and Isolation

Off-flavors develop in vegetable oils, marine oils, and hardened fats, the triglycerides of which contain fatty acids that have more than two double bonds or a particular double-bond distribution (1). To ascertain whether tallow flavor reversion originates from some tallow fatty acids or from the unsaponifiable part, tallow fatty acids freed of unsaponifiables were stored in contact with air at room temperature. After one week the fatty acids had the typical tallowy off-flavor whereas the unsaponifiable had not developed any such flavor after storage for a longer period. It was therefore concluded that some of the fatty acids might be responsible for the development of this off-flavor.

Stripping of Tallows. To obtain an off-flavor concentrate, strongly smelling beef or mutton tallow of Australian origin was stripped with a nitrogen stream in a 10-mm Hg vacuum at 160C for several hours. The volatiles were collected in two cold traps; the first was cooled with ethanol/dry ice (−80C), and the second with liquid nitrogen (−196C). The volatiles were dissolved in light petroleum and freed of fatty acids by washing with a 10% solution of sodium hydrogen carbonate. The neutral portion was washed alkali-free with water and dried over sodium sulphate. The light petroleum was then evaporated in vacuum in the cold.

Gas-Liquid Chromatography of Volatiles. About 0.5 µl of beef tallow volatiles were examined at 104C by GLC on a Carlo Erba P-AID/2 f-type chromatograph with double flame ionization detector on a 5-m long, 2-mm diameter column. The pressure of the nitrogen carrier gas was 0.5 atm gauge. Polyethylene glycol adipate (5%) on Celite was used as the immobile phase, and the chart speed was 12 cm/hour. The retention times (Rt) of saturated straight-chain aliphatic aldehydes are given as reference on a separate abscissa (Fig. 1).

Peaks 1, 2, and 3 indicate typical odors, which can be characterized as "green," tallowy, and cucumber-like respectively. The Rt of the first peak is between the Rt's of saturated n-aldehydes with 7–8 carbon atoms. The Rt's of the second and third peak are between the Rt's, corresponding to saturated n-aldehydes with carbon numbers between 10.5 and 11.5.

The substances giving these peaks were separated at 115C into fractions (finally collected in U-shaped capillaries cooled with liquid nitrogen) using semi-preparative gas-liquid chromatography (column length 130 cm, diameter 4 mm, gas pressures 0.8 atm gauge). Nitrogen was used as the mobile phase, and polyethylene glycol adipate (30%) on Celite (120–140) as the immobile phase. The detector was a gas density balance. The first peak was collected in Fraction I, and the other two peaks combined in Fraction II because, under the semipreparative chromatographic conditions applied, there was no distinct difference between the Rt-values of the latter. The contents of the two capillaries were washed with carbonyl-free light petroleum (40–60C) on a Celite/dinitrophenylhydrazine hydrochloride reaction column (2). DNPH formation was established in both fractions.

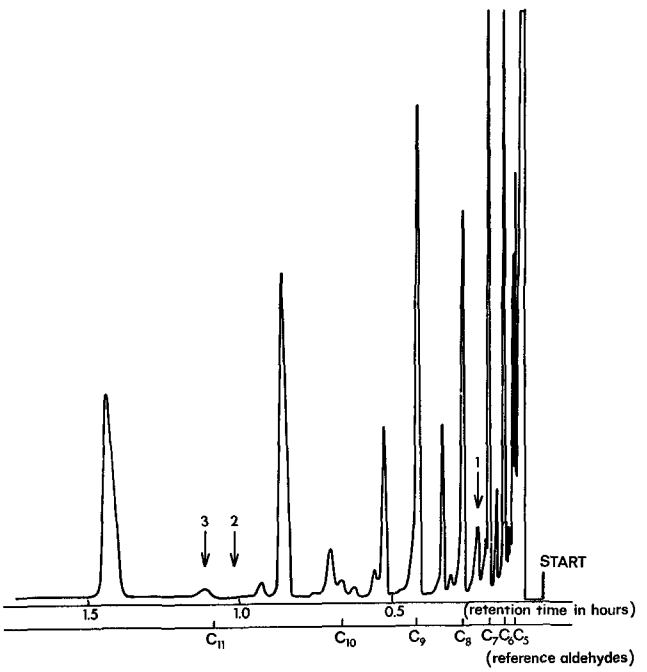

FIG. 1. Gas-liquid chromatogram of beef tallow volatiles.

[1] Presented in part by G. Hoffmann at the 7th ISF-Congress, Hamburg, October 1964.

Separation of Tallow DNPHs

To facilitate analytical determination, carbonyls isolated from autoxidized or reverted fats are frequently transformed into their colored, nonvolatile DNPH derivatives. DNPH-mixtures are separated by various chromatographic techniques, after which the separate DNPHs are classified by UV spectrophotometry. Three classes of aliphatic aldehyde DNPHs are generally distinguished: saturated, 2-unsaturated, and 2,4-unsaturated with a maximum absorption at 356–358, 372–375, and 388–390 nm respectively, measured in chloroform solution.

This classification is far from complete. Any aldehyde DNPH, with one or more isolated double bond(s), will absorb light at 356–358 nm and be classified as saturated. Any dienoic aldehyde, in which the first double bond is conjugated with the imine bond but the second bond is not conjugated with this grouping, will absorb light at 372–374 nm and be classified as 2-unsaturated. A trienoic aldehyde with a 2,4-conjugated double bond next to the imine bond and a third isolated double bond will be classified as 2,4-unsaturated.

Readily detectable aldehydes are formed by the autoxidation of the predominant fatty acids of vegetable and animal oils and fats. Since these aldehydes do not produce tallowy flavor when isolated from the fats, it is quite unlikely that, when present in tallow, they alone would be responsible for the tallowy flavor.

However, on the basis of experience with the carrier flavor of reverted hardened (1) and liquid vegetable oils (3) and on consideration of the successful isolation of the carrier of the tallowy, creamy, butterscotch-like flavor from butterfat (4), it may be postulated that aldehydes quite different from those commonly isolated are responsible for certain specific flavors or off-flavors. It has also been established that some of these specific types of aldehydes have a low flavor threshold (5) and that their concentration is generally low. If the carriers of the tallowy flavor should indeed belong to this class of special aldehydes, more sophisticated analytical techniques will be needed for their isolation.

Therefore the DNPHs obtained were further investigated by the thin-layer chromatography technique developed by Meijboom and Jurriens (6). Silica Gel G, impregnated with 30% silver nitrate, was used as adsorbent and 100% benzene as eluent. By this technique, unsaturated aliphatic aldehyde-DNPHs containing isolated *trans* or *cis*-double bonds form stronger π-complexes with the Ag-atoms than the conjugated double bonds present in 2- and 2,4-unsaturated aldehydes. Saturated aldehyde-DNPHs form very weak π-complexes. Fraction I contained two DNPHs which were strongly complexed with the Ag-atoms besides some weakly complexed DNPHs. Two strongly complexed DNPH spots were detected in Fraction II. These strongly and very strongly complexed DNPHs could not be classified as 2-alkenals or 2,4-alkadienal DNPHs. Therefore they were assumed to correspond with unsaturated aldehydes with isolated *cis*- and trans-double bonds.

After acid hydrolysis of the DNPHs with a 2 N H_2SO_4 solution, followed by steam distillation, the originally assessed odors of the fractions were perceived again, namely "green," tallowy, and cucumber-like.

The four isolated carbonyls were further purified on a Silica Gel G thin-layer plate to remove unknown short-chain contaminations. Hexane/diethyl ether/ethanol (88/10/2) was used as eluent (7). The longest chain compounds (spots with the highest R_f-values in this elution system) were respotted on a silver nitrate-impregnated Silica Gel G plate, on which they retained their original R_f-values.

The maximum absorption of the four DNPHs was measured in a chloroform solution, with which they had been extracted from the Silica Gel G layer. Both spots from Fraction I gave a maximum absorption at 356–358 nm, corresponding to an aldehyde DNPH with a (saturated) methylene group next to the imine bond. The two spots from Fraction II showed a maximum absorption at 373–374 nm corresponding to a 2-unsaturated aldehyde DNPH.

Interpretation of Results

The carbonyls isolated from Fraction I are aldehydes saturated at the 2-position with an isolated double bond in either a *cis*- or a *trans*-configuration. Their carbon number is most probably 7 or 8. The only ordinary unsaturated aldehyde eluting in this GLC interval, 2-hexenal, would not be strongly complexed by the silver nitrate plate if present in this fraction.

The carbonyls isolated from Fraction II are aldehydes unsaturated at the 2-position with the second isolated double bond in the *cis*- or trans-configuration. As their Rt-value on GLC was approximately that corresponding to a saturated n-aldehyde with 11 C-atoms, they most probably have a chain length of 9 C-atoms. Undecanal and 2,4-octadienal, if present, would not be strongly complexed by the silver nitrated plate.

Butterfat definitely contains 4*cis*-heptenal which has a creamy, tallowy, butterscotch-like, "greenish," flavor (4). The similarity of flavor descriptions makes it likely that there may also be 4*cis* and 4*trans*-heptenal although presence of the other three heptenal isomers, 3-, 5-, and 6-heptenals, cannot be excluded.

The 2*trans*,6*cis*-nonadienal (violet leaf aldehyde) has an odor resembling that of cucumber (8,9), but synthetic 2*trans*,6*trans*-nonadienal has a flavor which can be assessed as tallowy (10). All these aldehydes have been synthesized in this laboratory and com-

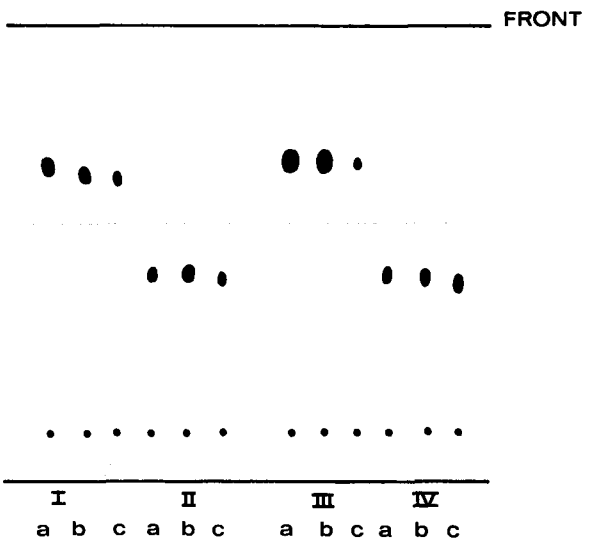

FIG. 2. Comparison of isolated DNPHs from beef tallow with synthesized DNPHs on a (30%) silver nitrate Silica Gel G plate. Eluent: benzene. I. 2*trans*,6*trans*-nonadienal; II. 2-*trans*,6*cis*-nonadienal; III. 4*trans*-heptenal; and IV. 4*cis*-heptenal. a) Synthetic DNPH, b) mixture of synthetic and isolated DNPHs, and c) isolated DNPH.

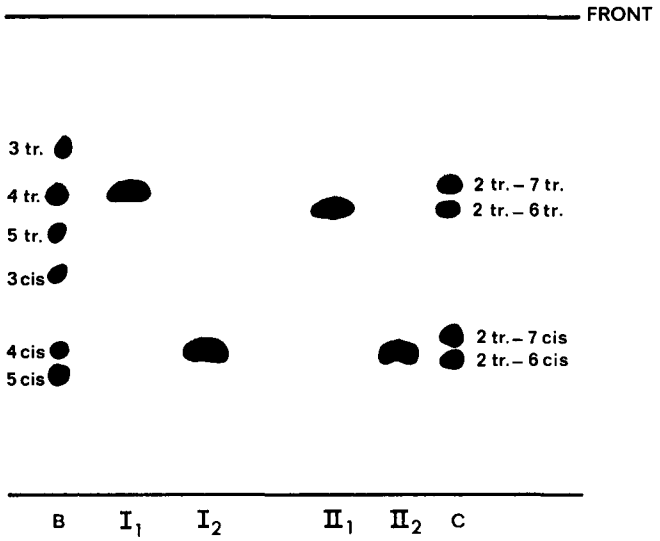

FIG. 3. Comparison of the four isolated aldehyde DNPHs and the different mixtures of the possible isomeric DNPHs of the same chain-length on a silver nitrate Silica Gel G plate. *b*) six isomeric n-heptenals, *c*) four isomeric n-nonadienals. I_1 isolated 4*trans*-heptenal; I_2 isolated 4*cis*-heptenal; II_1 isolated 2*trans*,6*trans*-nonadienal; and II_2 isolated 2*trans*,6*cis*-nonadienal. Layer thickness: 0.25 mm SiO_2 (30% $AgNO_3$). Developing liquid: benzene.

pared in their free and DNPH form with the "unknown" aldehydes. The 2*trans*,6*cis*- and 2*trans*,6*trans*-nonadienals were synthesized according to the method of Jutz (11). The 4*cis*- and 4*trans*-heptenal syntheses were accomplished as described in Reference 12.

Confirmation of Proposed Structures

Four mixtures of DNPHs were made, each consisting of a synthetic DNPH and the corresponding isolated DNPH (4*cis*- and 4*trans*-heptenal, 2*trans*,6*cis*- and 2*trans*,6*trans*-nonadienal). The four individual isolated DNPHs, the four individual synthetic DNPHs, and the four mixtures mentioned above were each spotted onto a thin-layer plate (12 spots on a 30% silver nitrate-impregnated Silica Gel G plate; eluent 100% benzene). In all spottings the three corresponding spots moved on the plate with the same R_f-value (Fig. 2). No separation of the mixtures of the model substance and isolated DNPHs was observed which was regarded as extra proof of the proposed identity.

Moreover the four isolated aldehyde DNPHs and the following model (synthetic) aldehyde DNPH mixtures were brought onto a Kieselguhr G plate, impregnated with Carbowax (13).

The mixtures of model aldehyde DNPHs were: a) a mixture of C 1 to C 12 saturated n-aliphatic aldehyde DNPHs (12 DNPHs); b) a mixture of 3,4 and 5*cis*- and *trans*-heptenal DNPHs (6 DNPHs); and

TABLE I

Relative Retention Times of Aldehydes Investigated

Substance	Rt[a]	Temperature (C)
4*trans*-heptenal	7.42	80
4*cis*-heptenal	7.54	80
2*trans*-nonenal	10.52	104
2*trans*,6*trans*-nonadienal	10.84	104
2*trans*,6*cis*-nonadienal	11.04	104

[a] The relative retention times of the compounds are based on those of the series of saturated, n-aliphatic aldehydes (expressed in carbon number).

TABLE II

Flavor Threshold of Aldehydes in Paraffin Oil

Substance	Odor (mg/kg)	Taste (mg/kg)
4*cis*-heptenal	0.01	0.0016
4*trans*-heptenal	2.3	0.32
2*trans*,6*cis*-nonadienal	0.01	0.002
2*trans*,6*trans*-nonadienal	0.21	0.018

c) a mixture of *trans*,*trans*- and *trans*,*cis*-isomers of 2,6 and 2,7-nonadienal DNPHs (4 DNPHs). The plates were eluted with a petroleum fraction (bp 100–200C).

The four isolated DNPHs and the model aldehyde DNPHs all eluted between the R_f-value of the saturated C 5 and C 6 aldehyde DNPHs (14). The isolated DNPHs and the mixtures b) and c) were re-examined on the silver nitrate Silica Gel G plate used before; eluent 100% benzene. A complete separation of all model heptenals on this plate proved that the two isolated heptenals were really 4*cis*- and 4*trans*-heptenals. The separation of the four model nonadienal DNPHs was also complete although the positionally isomeric 2*trans*,6*cis*- and 2*trans*,7*cis*-nonadienal DNPHs were generally not so sharply separated as the isomeric heptenal DNPHs. The degree of separation depends on the activity of the plate. In this way it could be established that the isomeric nonadienals are of the 2,6-type (Fig. 3).

The identity of the volatiles isolated was proved by comparing their retention times (cf. Table I). These results were obtained on a Carlo Erba type P-AID, 2 column f-type chromatograph with double flame ionization detector with the use of a polar immobile phase under identical conditions.

The flavor threshold (5) of the four aldehydes was determined in paraffin oil. The synthesized aldehydes, purified by gas-liquid chromatography, were used for this purpose. The results are given in Table II.

With the exception of 4 trans-heptenal, the three other aldehydes have a sufficiently low flavor threshold (0.2–0.01 ppm for odor and 0.02–0.002 ppm for taste) to be identified as "greenish," tallowy, and creamy, and distinguished from the other off-flavor volatiles present. It is not yet known whether other volatiles (e.g., 2-nonenal, present in considerable amounts) also contribute to the tallowy odor.

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P. D. Harkes and W. J. Begemann synthesized some of the model aldehydes, and A. C. J. Kroesen recorded the gas chromatograms.

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