Characterization of the Carbonyl Compounds in Reverted Soybean Oil[1, 2, 3]

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Abstract

Twenty-one mono-carbonyl compounds were identified in a reverted but not rancid soybean oil with a peroxide number of 2.7 meq/kg and a flavor score of 6.0. Six of the carbonyl compounds were saturated methyl ketones, ten were saturated aldehydes and five were 2-enals. The amount of 2,4-dienals in the reverted soybean oil was too small to be fractionated and identified. One fraction of the 2,4-dinitrophenylhydrazones of mono-carbonyl compounds obtained was different in chemical properties from those of the four known classes of compounds; viz. saturated aldehydes, saturated ketones, 2-enals, and 2,4-dienals. The chemical identity of this fraction has not been completely established.

The technique used for the identification of the mono-carbonyl compounds consisted of essentially five steps: (a) passing a hexane solution of the soybean oil through a celite column, which was homogeneously impregnated with 2,4-dinitrophenylhydrazine and phosphoric acid, in order to convert quantitatively all the carbonyl compounds in the oil into their 2,4-dinitrophenylhydrazones; (b) removing the soybean oil from the solution with a Seasorb-Celite Column; (c) removing decomposition products and 2,4-dinitrophenylhydrazones of ketoglycerides with an Alumina Column; (d) separating, by adsorption chromatography, the 2,4-dinitrophenylhydrazones of mono-carbonyl compounds into four classes; viz. saturated ketones, saturated aldehydes, 2-enals, and 2,4-dienals; and (e) identifying the individual components in each class of compounds by the Rf values of liquid-liquid partition chromatography.

Introduction

THE RELATIONSHIP between the carbonyl compounds produced by oxidation and the reversion flavor of soybean oil has been studied extensively (1,2,3,4). The procedure generally used for the characterization of carbonyl compounds, consists of fractionation of the mixed 2,4-dinitrophenylhydrazones by adsorption chromatography and subsequent identification of the pure fractions by their melting points, and percentage compositions of carbon, hydrogen and nitrogen. Since it requires approximately 3 mg of one fraction for a complete identification, previous workers usually autoxidized the soybean oil to a stage far beyond reversion in order to produce a large quantity of volatile decomposition products.

By this technique, acetaldehyde, propanal, hexanal, crotonaldehyde, 2-pentenal, 2-heptenal, and 2,4-decadienal were identified as the major components in the volatile decomposition products obtained from highly autoxidized soybean oil. Whether these carbonyl compounds are responsible for reversion or rancidity is, however, difficult to ascertain. Furthermore, it has been reported recently that a compound present in a concentration below its threshold of organoleptic detection may contribute a flavor when other flavor compounds are also present (5). Therefore, an analysis of all the carbonyl compounds present in a slightly autoxidized soybean oil is necessary in order to establish the relationship between carbonyl compounds and reversion flavor.

Recently, Schwartz developed an ingenious procedure for the quantitative conversion of the carbonyl compounds in an oil into their 2,4-dinitrophenylhydrazones without the necessity of separating the carbonyl compounds from the oil (6,7). The 2,4-dinitrophenylhydrazones were then purified and fractionated by adsorption chromatography and identified by Rf values from liquid-liquid partition chromatography. A carbonyl compound present in an oil in a concentration as low as 0.1 ppm can be determined by this method. The present paper reports the use of this technique for the characterization of the mono-carbonyl compounds in a reverted soybean oil.

Experimental

Materials Used. Soybean oil used in this experiment was alkaline refined, water washed, bleached, and deodorized at 180C under a reduced pressure of 0.05 mm Hg with 5% by weight of water. The deodorized oil had a peroxide number of 0.2 meq/kg and an organoleptic flavor score of 8.5. It was immediately aged in a tightly closed Pyrex glass bottle filled to the neck for three weeks at room temperature under diffused daylight. The aged oil had a peroxide number of 2.7 meq/kg, an organoleptic flavor score of 6.0, and was reverted but not rancid.

Solvents used in this experiment were purified according to the procedure of Schwartz and Parks (8). Celite, Seasorb, and Alumina were used without further purification.

Conversion of Carbonyl Compounds into Their 2,4-Dinitrophenylhydrazones. Sufficient hexane was added to 20 g of soybean oil to give a volume of 100 ml. This solution was passed through a column (2.5 × 25 cm) which was packed with 10 g of Celite (analytical grade). The Celite was homogeneously impregnated with 0.5 g of 2,4-dinitrophenylhydrazine, 6 ml of 85% phosphoric acid and 4 ml of water. The column was then washed with hexane until the effluent showed no absorption at 335, 340, 355, and 370 mμ. Ten columns were run simultaneously in this experiment. All the effluents and washings were combined to form Fraction 1. This fraction contains 2,4-dinitrophenylhydrazones of all carbonyl compounds and ketoglycerides, some decomposition products, and the soybean oil.

Purification of 2,4-Dinitrophenylhydrazones. Fraction 1 was passed through a column (4 × 40 cm) which was packed with a mixture of 30 g each of Seasorb and Celite 545. The column was then washed with 100 ml of hexane. All the components in Frac-

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tion 1, except the soybean oil, were adsorbed on this column as a dark blue band. The soybean oil passed through the column with hexane as effluent. The Seasorb-Celite column was then eluted with 100–200 ml of 25% nitromethane in chloroform. The effluent, Fraction 2, contained all the 2,4-dinitrophenylhydrazones of mono-carbonyl compounds. It was still contaminated with some decomposition products and a trace of soybean oil. The 2,4-dinitrophenylhydrazones of dicarbonyl compounds and the major portion of the decomposition products remained at the top of the Seasorb-Celite column as a dark blue band.

Fraction 2 was freed from the solvent by being heated on a water bath under a current of nitrogen. The residue was dissolved in as small an amount as possible of hexane, and the solution was passed through a column (2.5 × 40 cm) which was packed with 60 g of alumina hydrated with 6% of water. Hexane was then used to wash the column in order to remove the last trace of soybean oil as effluent. The 2,4-dinitrophenylhydrazones of mono-carbonyl compounds were then eluted from the column with a mixture of equal volumes of benzene and hexane.

The 2,4-dinitrophenylhydrazones of known mono-carbonyl compounds yielded a single band on the Alumina column. However, when reverted soybean oil was used, two bands, A and B, were formed and eluted (Fig. 1). All decomposition products and 2,4-dinitrophenylhydrazones of ketoglycerides remained at the top of the Alumina column as an orange band.

Class Separation. The effluents containing bands A and B, respectively, were freed from solvents by being heated on a water bath under a current of nitrogen. The residue containing band A was dissolved in chloroform. This solution was passed through a column (3 × 30 cm) packed with a mixture of 15 g each of Seasorb and Celite 545. After the chloroform solution was completely adsorbed on the column, 15 ml of chloroform were used for washing the container of the solution and the column. The dark grey band formed at the top of the column was developed with 2% methanol in chloroform. When 2,4-dinitrophenylhydrazones of known carbonyl compounds were used, four distinct bands corresponding to four different classes of carbonyl compounds, viz., saturated methyl ketones, saturated aldehydes, 2 enals, and 2,4-dienals were obtained. These four bands could be successively eluted from the class separation column by increasing the concentration of methanol in chloroform (Fig. 2). Each band was identified by its characteristic absorption maxima with chloroform and hexane as solvents (Table I).

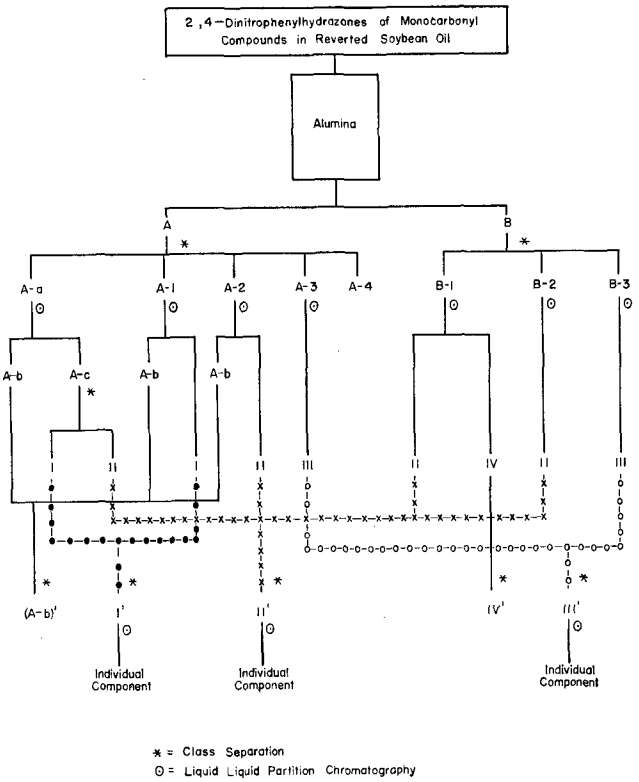

FIG. 1. Fractionation of mono-carbonyl compounds in reverted soybean oil by chromatography of their 2,4-dinitrophenylhydrazones.

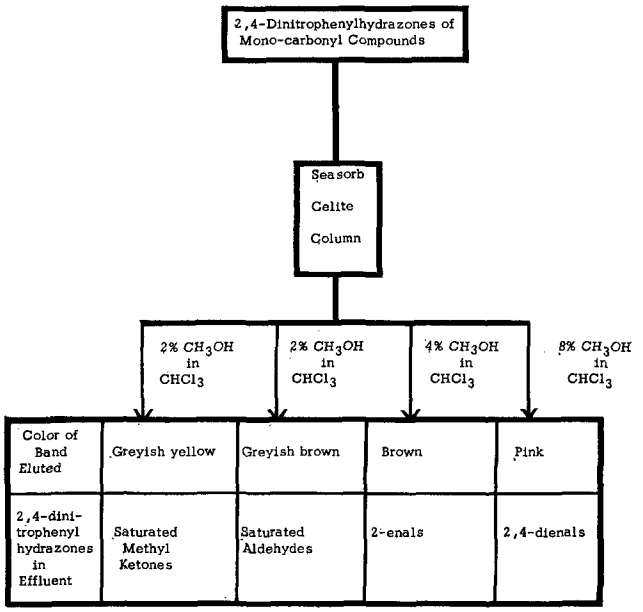

FIG. 2. Class separation of the 2,4-dinitrophenylhydrazones of mono-carbonyl compounds.

When band A was subjected to the elution procedure of class separation, an additional dark grey band A-a was obtained in addition to the four bands A 1, A 2, A 3, and A 4 corresponding respectively to the 2,4-dinitrophenylhydrazones of saturated methyl ketones, saturated aldehydes, 2-enals, and 2,4- dienals. The band A 4 was not sufficient in amount for further characterization. The dark grey band, A-a, showed absorption maxima of 370 mμ in hexane and 380 mμ in chloroform. There was so much of band A-a that it contaminated bands A 1, A 2, A 3, and A 4. Therefore, each of these fractions was further purified by the method of liquid-liquid partition chromatography.

Band B was then fractionated by the same class separation procedure. It yielded only three bands, B 1, B 2, and B 3, corresponding to saturated methyl ketones, saturated aldehydes and 2-enals respectively. The band B 1 was of such a relatively large amount that it contaminated bands B 2 and B 3. Therefore, each of these fractions was also further purified by

TABLE I

Absorption Maxima of 2,4-Dinitrophenylhydrazones of Mono-Carbonyl Compounds

2,4-dinitrophenylhydrazone \ Solvent	Hexane (mμ)	Chloroform (mμ)
Saturated methyl ketones	340	360
Saturated aldehydes	335	355
2-enals	355	370
2,4 dienals	370	390
IV'	340	360
(A-b)'	370	380

TABLE II
Carbonyl Compounds Identified in Reverted-but not Rancid Soybean Oil

Carbon number	Satd. methyl ketones μ moles/ 100 g oil	Satd. aldehydes μ moles/ 100 g oil	2-enals μ moles/ 100 g oil
C_{13}	.....	0.106	0.018
C_{12}	.....	.....	0.040
C_{11}	.....	.....	0.018
C_{10}	.....	0.052	.....
C_9	.....	0.035	0.030
C_8	0.037	0.262	.....
C_7	0.054	0.252	0.040
C_6	0.160	0.105	.....
C_5	0.143	0.104	.....
C_4	0.052	0.100	.....
C_3	.....	0.096	.....
C_2	.....	0.061	.....
Total amounts	0.446	1.173	0.146

the method of liquid-liquid partition chromatography.

Liquid-Liquid Partition Chromatography. When the 2,4-dinitrophenylhydrazones of a single class of mono-carbonyl compounds were chromatographed on a column (2.5 × 40 cm) packed with 25 g of celite using 33 ml of acetonitrile and 0.5 ml of water as the stationary phase and hexane as the mobile phase, each compound could be separated, identified by its Rf value, and its quantity determined by its absorbance at the wave length of its absorption maximum (9). In order to further purify the four bands obtained from fraction A by class separation, viz. A-a, A 1, A 2, and A 3, each was subjected to the liquid-liquid partition chromatography into 200 tubes of 5 ml of effluent each (Fig. 1). The absorption maxima of each tube were determined with a Beckman DU Spectrophotometer. Band A-a contained a red crystalline substance, A-b, besides some 2,4-dinitrophenylhydrazones of saturated aldehydes and ketones collected as Fraction A-c. This Fraction, A-c, was rechromatographed by the procedure of class separation. The band corresponding to 2,4-dinitrophenylhydrazones of saturated ketones was collected as Fraction I, and that of saturated aldehydes as Fraction II. The red crystalline substance was eluted in the first five to ten tubes, and had an absorption maximum in chloroform different from those of the 2,4-dinitrophenylhydrazones of all the four known classes of mono-carbonyl compounds (Table I). The first five to ten tubes from the liquid-liquid partition chromatography of bands A 1 and A 2 also contained this red crystalline substance. These tubes were combined with Fraction A-b. The 2,4-dinitrophenylhydrazones of saturated ketones obtained from band A 1 were combined with Fraction I and those of saturated aldehydes obtained from band A 2 were combined with Fraction II. Band A 3 contained only 2-4-dinitrophenylhydrazones of 2-enals and was collected as Fraction III.

The three bands obtained from Fraction B by class separation, viz., B 1, B 2, and B 3 were purified in exactly the same manner by liquid-liquid partition chromatography. Band B 1 contained a relatively large amount of 2,4-dinitrophenylhydrazones of ketones in tubes 160–180, which were combined as Fraction IV, and 2,4-dinitrophenylhydrazones of saturated aldehydes, which were combined with Fraction II. Band B 2 contained only 2,4-dinitrophenylhydrazones of saturated aldehydes and was combined with Fraction II. Band B 3 contained only 2,4-dinitrophenylhydrazones of 2-enals and was combined with Fraction III.

Each of these five fractions obtained, viz. I, II, III, IV, and A-b was then rechromatographed by the procedure of class separation. A sharp clear band was then obtained from each fraction; I′ as 2,4-dinitrophenylhydrazones of saturated ketones from the combined I, II′ as saturated aldehydes, III′ as 2-enals, IV′ as a ketone, and (A-b)′ as the red crystalline unknown substance.

Each of the fractions I′, II′ and III′ was then analyzed qualitatively and quanitatively for its individual components by the procedure of liquid-liquid partition chromatography. Fractions (A-b)′ and IV′ were subjected to infrared spectral analyses for chemical characterization.

Results and Discussion

A total of twenty carbonyl compounds was identified in a reverted but not rancid soybean oil with a peroxide number of 2.7 meq/kg and an organoleptic flavor score of 6.0. The quantitative relationships among these carbonyl compounds were shown in Table II. Besides these, Fraction IV′ was identified by its infrared absorption spectrum, melting point, and mixed melting point as the 2,4-dinitrophenylhydrazone of acetone. The amount of this fraction was significantly higher than that of the other carbonyl compounds.

Fraction (A-b)′ has not been completely identified. Its absorption maxima in hexane and chloroform (Table I) as well as its Rf values during class separation and liquid-liquid partition chromatography (Fig. 2) were all different from those of the 2,4-dinitrophenylhydrazones of the four classes of known carbonyl compounds. Its infrared absorption spectrum (Fig. 3)

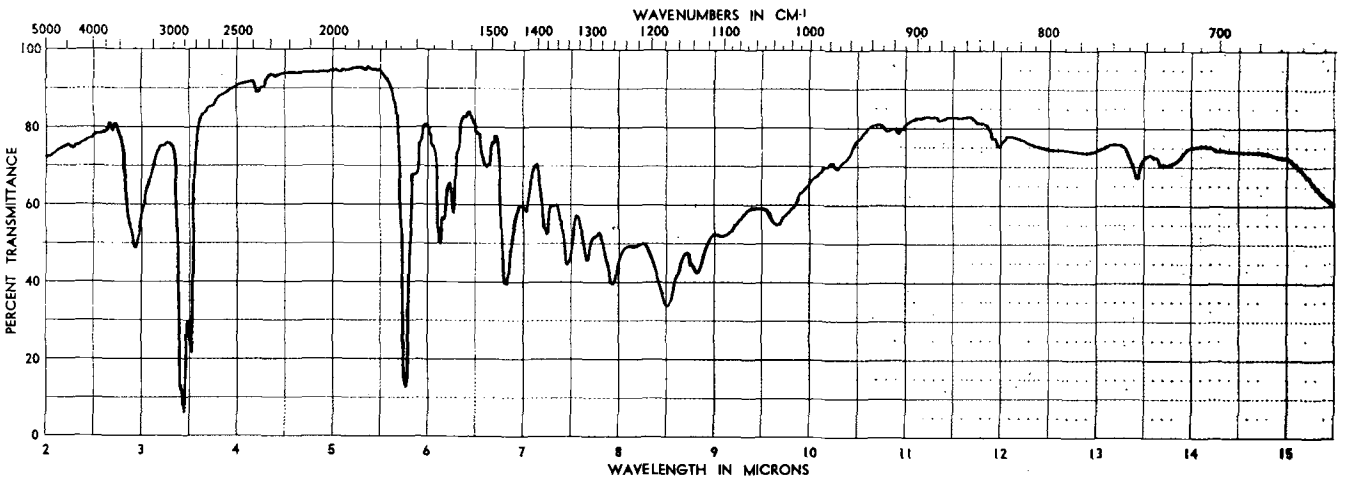

FIG. 3. Infrared absorption spectrum of fraction (A-b)′

suggested that this fraction might be the 2,4-dinitrophenylhydrazone of a compound with a keto group near the end of a long chain ester. The spectrum showed rather weak hydrazone bands. Its strong carbonyl band at 5.8 μ and the band at 8.5 μ indicated the presence of an ester group. The strength of the C-H stretching band at 3.4 μ suggested considerable aliphatic hydrogen which indicated a long chain attached to the hydrazone. The peaks between 7 and 8 μ showed more splitting than was the case with the 2,4-dinitrophenylhydrazone of a long chain aldehyde, thus leading to the possibility of at least one short chain attached to the hydrazone group.

The mechanisms for the formation of saturated aldehydes and 2-enals by the autoxidation of unsaturated fatty esters have been postulated by various investigators (10,11) based upon the hydroperoxide theory of Farmer et al. (12). The mechanism for the formation of ketones was proposed by Bell et al. (13).

$$R-CH(OOH)-R \longrightarrow R-\underset{\underset{O.}{|}}{CH}-R + .OH$$

$$R-\underset{\underset{O.}{|}}{CH}-R + R'H \longrightarrow R-CHOH-R + R'.$$

$$R-\underset{\underset{O.}{|}}{CH}-R + R'. \longrightarrow R-\underset{\underset{O}{||}}{C}-R + R'H$$

or

$$R-\underset{\underset{O.}{|}}{CH}-R + R'O. \longrightarrow R-\underset{\underset{O}{||}}{C}-R + R'OH$$

It should be noted that a carbonyl compound may contribute to the flavor of an oil at an extremely low concentration, often less than 1 ppm. Modern techniques of flavor chemistry have enabled us to isolate and identify carbonyl compounds which are present in fats and oils at such low concentrations. On the other hand, our knowledge of the numbers and locations of double bonds in unsaturated fatty acids in natural fats and oils is far below this accuracy. Therefore, in order to postulate adequately the mechanism for the formation of all carbonyl compounds, our knowledge of fatty acid composition and location of double bonds must first be significantly enriched.

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REFERENCES

1. Chang, S. S., and F. A. Kummerow, JAOCS *31*, 324–7 (1954).
2. Stapf, R. J., and B. F. Daubert, *Ibid. 27*, 374–7 (1950).
3. Kawahara, F. K., and H. J. Dutton, *Ibid. 29*, 372–7 (1952).
4. Hoffmann, G., *Ibid. 38*, 1–3 (1961).
5. Nawar, W. W., and I. S. Fagerson, Food Tech. *11*, 107–9 (1962).
6. Schwartz, D. P., O. W. Park, and Mark Keeney, Anal. Chem. *34*, 669–71 (1962).
7. Schwartz, D. P., H. S. Haller, and Mark Keeney. Presented at the Am. Chem. Soc. meeting in Atlantic City, N. J., 1959.
8. Schwartz, D. P., and O. W. Parks, Anal. Chem. *33*, 1396–8 (1961).
9. Corbin, E. A., D. P. Schwartz, and Mark Kenney, J. Chromatography, *3*, 322–9 (1960).
10. Evans, C. D., Proceedings Flavor Chemistry Symposium, Camden, N. J., 123–46 (1961).
11. Mark Keeney, Symposium on Foods: Lipids and Their Oxidation, AVI Publ. Co., Westport, Conn., 1962, pp. 77–81.
12. Farmer, E. H., G. F. Bloomfield,, A. Sundarlingam, and D. A. Sutton, Trans, Faraday Soc. *38*, 348–56 (1942).
13. Bell, E. R., J. H. Raley, F. F. Rust, F. H. Seubold, and W. E. Vaughan, Discussions Faraday Soc. *10*, 242–9 (1951).

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Thin-Layer Chromatography of Brain Phospholipids[1,2]

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Abstract

Thin-layer chromatography systems are described for separation of most tissue phospholipids and sphingolipids. Acidic lipids such as phosphatidyl serine and cerebroside sulfate are resolved without streaking on several types of modified silica gel.

Introduction

THIN-LAYER chromatography is especially valuable for the separation of lipids (1,2). However, some phospholipids do not chromatograph well (3) on systems reported to date (4–12). The greatest difficulty is encountered with acidic lipids which show marked tailing on silica gel. Mangold (1,13) has overcome this by adding ammonium sulfate to the stationary phase. Aldehydogenic lipids are split on this stationary phase. In the present investigation, a number of modifications of stationary and mobile phases are described which are designed to minimize streaking of acidic lipids. In addition to previously described chloroform-methanol-ammonia solvents, the effects of impregnation of silica gel with acidic and basic substances are reported. The basic plates give well-defined spots for acidic compounds. An ammonia-containing solvent system was also reported recently by Skidmore and Entenman (14).

Materials and Methods

Thin-layer plates were prepared as described by Stahl (15), using 30.0 g Silica Gel G[3] and 60 ml water. Modified plates were prepared with 27.0 g Silica Gel G and 3.0 g of ammonium sulfate, sodium acetate, oxalic acid, or potassium hydroxide dissolved in 60 ml water, or 20 ml of a saturated solution of sodium borate in 60 ml water.

Lipid samples were obtained by fractionation of rat, monkey, and human brain lipids on combinations of diethylaminoethyl cellulose (16,17), silicic acid-silicate (16–18), and Unisil[4] silicic acid columns. Monkey liver mitochondria lipids were fractionated on a Unisil[4] silicic acid column. Samples were applied to thin-layer plates in a stream of nitrogen using a Hamilton syringe. Plates were developed in unlined, equilibrated tanks until the solvent front was 10 cm above the starting points.

Two indicators have been found to be most useful for phospholipids. Plates are first sprayed with a ninhydrin reagent and heated for 15 min at 110C, then stained with iodine. The plates are exposed to

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[3] C. A. Brinkman and Co., Inc., 115 Cutter Mill Road, Great Neck, Long Island, N.Y. (manufactured by E. Merck, A. G., Darmstadt, Germany).
[4] Clarkson Chemical Co., Inc., Williamsport, Pa.