Isolation and Identification of 2-Pentenylfurans in the Reversion Flavor of Soybean Oil[1]

STEPHEN S. CHANG, GUO-HUI SHEN,[2] JIAN TANG,[2] QI ZHANG JIN,[3] HUANG SHI,[4] JAMES T. CARLIN[5] and CHI-TANG HO, Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

ABSTRACT

The volatile flavor compounds in a reverted soybean oil with a peroxide number of 6.0 meq/kg were isolated by semicontinuous countercurrent vacuum steam distillation. Based upon the gas chromatographic retention times and mass spectra of the four synthesized 2-pentenylfurans, it was found that *cis*- and *trans*-2-(1-pentenyl)furans and a mixture of *cis*- and *trans*-2-(2-pentenyl)-furans are present in reverted soybean oil. At least the greater portion of 2-(2-pentenyl)furans identified was *cis*-isomer. These compounds may contribute to the reversion flavor of soybean oil.

INTRODUCTION

The search for the mechanism of the formation of the reversion flavor in soybean oil has been conducted since 1936 (1-3). Smouse and Chang (4) systematically identified 71 compounds in the volatiles of a typical reverted-but-not-rancid soybean oil. They found that 2-pentylfuran is primarily responsible for the beany and grassy flavor of soybean oil. Chang et al. (5) postulated that 2-pentylfuran can be formed from linoleate through autoxidation. The initial step of the proposed mechanism involved the formation of a 10-hydroperoxide of linoleate (5). Recently, it was reported that the reaction of linoleate with singlet oxygen resulted in the formation of 10-hydroperoxide in a relative amount of 17% (6). Furthermore, there are increasing evidence that singlet oxygen may be important in initiating normal free radical autoxidation of unsaturated fats (7,8). All of these tend to support the proposed mechanism of Chang et al.

In 1978 (9), it was proposed that by using the mechanism of Chang et al. for the formation of 2-pentylfuran, linolenate would produce four unsaturated pentenylfurans. They are *cis* and *trans* forms of 2-(1-pentenyl)furans and 2-(2-pentenyl)furans. These four compounds were then synthesized and organoleptic evaluations suggested that they could contribute to the reversion flavor of soybean oil (9,10). Most recently, Lomanno and Nawar (11) identified 2-pentenylfuran in the volatiles of thermally oxidized linolenate.

The present paper is an attempt to ascertain whether or not these 2-pentenylfurans are present in a typical reverted soybean oil and therefore could contribute to its reversion flavor.

EXPERIMENTAL PROCEDURES

Preparation of Reverted Soybean Oil

The method used was that described by Chang et al. (12). This method involves placing commercially refined and deodorized soybean oil into one-gallon-size clear glass jars, filled to the neck and tightly capped. They were stored for 35 days under diffused daylight at room temperature. Twenty-one gallons of soybean oil treated in this manner developed a reverted-but-not-rancid flavor. The reverted soybean oil had a peroxide number of 6.0 meq/kg, and an organoleptic flavor score of 5.3.

Organoleptic Evaluation of the Oils

Organoleptic evalutions of the reverted soybean oils before and after the flavor isolation were conducted by a panel of eight trained, experienced members.

A scale of 1 to 10 was used for scoring of the samples. A score of 10 was for a completely bland sample, and a score of 1 was for a sample with a strong, repulsive flavor.

The samples (5 mL) were served at 60 C in glass creamers. To maintain the temperature during the evaluation period, the creamers were set in holes drilled into heavy aluminum blocks preheated to 60 C.

Synthesis of the Four 2-Pentenylfurans

A mixture of *cis*- and *trans*-2-(1-pentenyl)furans were synthesized according to the method described previously (9). They were purified and separated into pure compounds by gas chromatography (GC) (9). *cis*-2-(2-Pentenyl)-furan and *trans*-2-(2-pentenyl)furan were synthesized separately as pure compounds, as described previously (10). In the present study, a modified method as described below was used for the preparation of *cis*-2-(2-pentenyl)furan.

Preparation of cis-*2-penten-1-ol.* 8.4 g (0.1 mol) of 2-pentyn-1-ol in 10 mL of hexane was hydrogenated by using 0.3 g of 10% Pd-$BaSO_4$ as the catalyst under 1 atm at room temperature (5 drops of quinoline were added). A total of 2450 mL of hydrogen was absorbed in 5 hr. Filtration and solvent removal gave *cis*-2-penten-1-ol.

Preparation of cis-*1-bromo-2-pentene.* To 9 g of phosphorus tribromide (0.1 mol) in 12 mL of hexane and 2 drops of dry pyridine, 8.2 g of *cis*-2-penten-1-ol (0.1 mol) in 10 mL of hexane and 1.5 mL of dry pyridine was added dropwise at ca. 0 C. After the addition, the mixture was stirred for 1 hr at room temperature. The mixture was then neutralized with 10% of sodium carbonate solution, and extracted with diethyl ether. The ether extract was dried over anhydrous sodium sulfate and the solvent

---

[1] Presented at the 73rd AOCS annual meeting, Toronto, 1982.

[2] Present address: Department of Chemical Engineering, Wuxi Institute of Light Industry, Wuxi, People's Republic of China.

[3] Present address: Scientific Research Institute of Fragrance and Flavor Industry, Ministry of Light Industry, Shanghai, People's Republic of China.

[4] Present address: Scientific Research Institute of Fermentation Industry, Ministry of Light Industry, Beijing, People's Republic of China.

[5] Present address: Pepsico, Inc., Valhalla, New York, NY 10595.

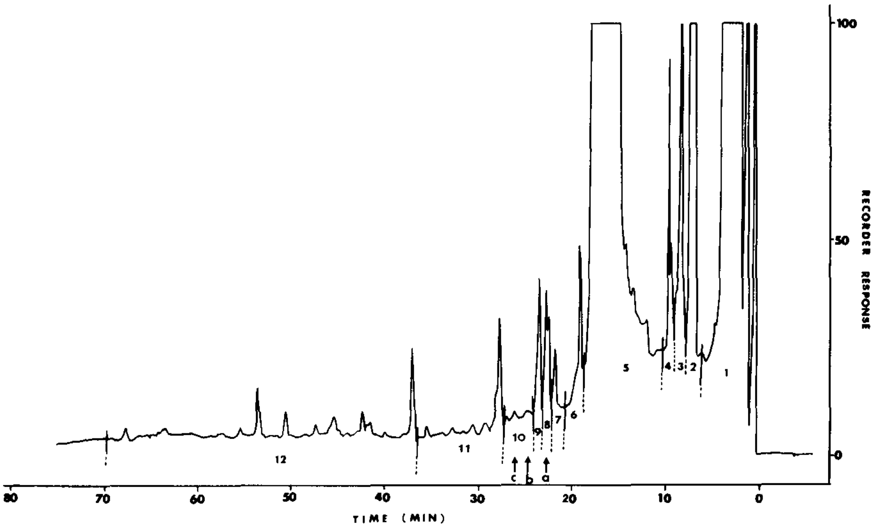

FIG. 1. Initial preparative gas chromatogram of the reverted soybean oil volatiles by 10% OV-17 column. (a) Retention time of *cis*- and *trans*-2-(2-pentenyl)furan. (b) Retention time of *cis*-2-(1-pentenyl)furan. (c) Retention time of *trans*-2-(1-pentenyl)furan.

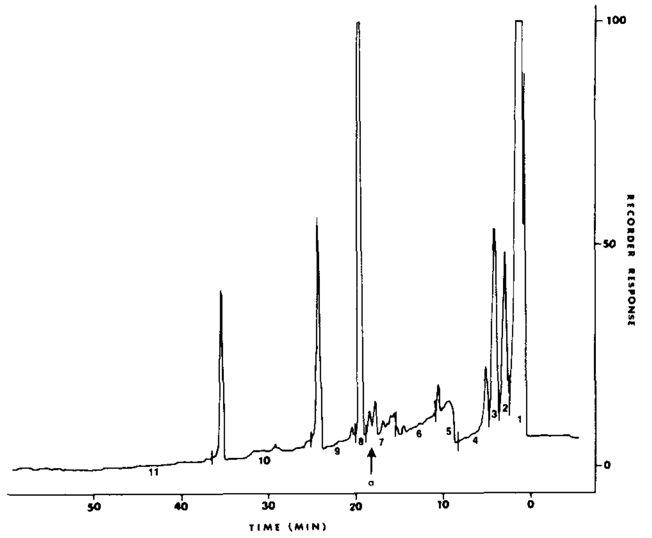

FIG. 2. Second gas chromatogram of fraction 8 by 10% OV-351 column. (a) Retention time of *cis*- and *trans*-2-(2-pentenyl)furan.

removed with the use of a rotary evaporator. The yield was 6 g.

Preparation of cis-*2-(2-pentenyl)furan.* To 26 mL of 1.7 M solution of *n*-butyl lithium in hexane at −25 C under nitrogen, 3.5 g (0.05 mol) of redistilled furan was added dropwise. After addition, the mixture was stirred for 4 hr at −15 C. To the mixture, 6 g of *cis*-1-bromo-2-pentene (0.04 mol) was added. The solution was stirred for 1 hr after addition, then at room temperature for 3.5 hr. The mixture was poured over crushed ice, the layers separated, and the aqueous layer extracted with diethyl ether. The combined organic layers were dried with anhydrous sodium sulfate. After the removal of the ether, distillation gave the desired product, bp 33–36 C/0.7 mm Hg.

Isolation of Volatile Flavor Compounds from Reverted Soybean Oil

The volatile flavor compounds were isolated from the reverted soybean oil with the use of the semicontinuous countercurrent vacuum steam distillation process as described by Chang (13). The oil was heated to 95 C continuously in the heat exchanger and was metered into the Oldershaw column at a rate of 0.3 L/hr under a vacuum of 0.02 mm Hg.

Volatiles from 21 gal of the reverted soybean oil were collected in a series of cold traps cooled with dry ice. The condensate collected in the traps was washed out and extracted with analytical grade ethyl ether. The ethyl ether extract (2500 mL) of the condensate was dried with anhydrous sodium sulfate and then concentrated to a volume of 50 mL with the use of a 30-plate Oldershaw column. It was finally concentrated to a volume of 4 mL with a spinning band still.

Fractionation of the Flavor Isolate

The initial preparative gas chromatography of the isolated volatiles from reverted soybean oil was performed on a Beckman GC-5 gas chromatograph equipped with a thermal conductivity detector, fitted with a 1/8 in od × 12 ft stainless steel column, packed with 10% OV-17 on 80–100 mesh Chromosorb WHP. The flow rate was 30 mL/min. The column temperature was held at 50 C for 6 min, then increased to 220 C in 64 min. The chromatogram was divided into 12 broad fractions (Fig. 1). Each broad fraction was successively collected according to the method of Thompson et al. (14).

Fractions 8 and 10, which possibly contain *cis*- and *trans*-2-(2-pentenyl)furans and *cis*- and *trans*-2-(1-pentenyl)-furans, respectively, were subjected to a second fractionation. A 1/8 in od × 12 ft stainless steel column packed with 10% OV-351 liquid stationary phase on 60–80 mesh Chromosorb WHP was used for the second fractionation. Helium flow rate was 30 mL/min. The column temperature was held at 50 C for 6 min and then programmed to a final temperature of 220 C in 64 min. The chromatogram of fraction 8 was divided into 11 subfractions (Fig. 2). The chromatogram of fraction 10 was divided into 13 subfractions (Fig. 3). Each subfraction was again successively collected in a hairpin trap.

Mass Spectrometry

By using the four authentic 2-pentenylfurans, it was determined that *cis-* and *trans*-2-(2-pentenyl)furans would be in fractions 8-7 and *cis-* and *trans*-2-(1-pentenyl)furans would be in fraction 10-9. So, only these two fractions were subjected to mass spectrometric analysis.

Mass spectrometry was performed on a Du Pont 21-490 mass spectrometer which was interfaced by a jet separator to a Varian Moduline 2700 gas chromatograph equipped with an FID detector. A 10 ft X 1/8 in od stainless steel column, packed with 10% OV-351 on 60-80 mesh Chromosorb W AW DMCS was used for the analysis. The flow rate was 30 mL/min. The column temperature was held at 50 C for 6 min, then increased by 4 C/min to a holding temperature of 220 C. Ionization voltage was 70 eV.

RESULTS AND DISCUSSION

Isolation of Volatile Flavor Compounds from Reverted Soybean Oil

The volatile flavor compounds isolated from the reverted-but-not-rancid soybean oil with a peroxide number of 6.0 meq/kg and an organoleptic flavor score of 5.3 had a strong, true reversion odor and flavor. After isolation the organoleptic flavor score of the oil was evaluated to be 7.1. Since the peroxide number of the oil remained as 6.0 meq/kg before and after the steam stripping, it is reasonable to conclude that no further decomposition with the formation of volatile decomposition products as artifacts, took place during the isolation process.

Preparative Gas Chromatrography of the Flavor Isolate

The initial preparative gas chromatograms of the reverted soybean oil isolated on an OV-17 column is shown in Figure 1. The retention times of the four authentic 2-pentenylfurans, as analyzed by gas liquid chromatography (GLC) under the same conditions as the flavor isolate, were: *cis-* and *trans*-2-(2-pentenyl)furans, 22.0 min; *cis*-2-(1-pentenyl)furan, 23.5 minutes and *trans*-2-(1-pentenyl)furan, 24.5 min. The reversion flavor isolate was then spiked with each of the four authentic 2-pentenylfurans separately and the spiked samples were analyzed by GLC. The gas chromatograms indicated that *cis-* and *trans*-2-(2-pentenyl)furans would be found in fraction 8 while *cis-* and *trans*-2-(1-pentenyl)furans would be found in fraction 10 if they were present in the flavor isolate. Thus, fractions 8 and 10 were repeatedly collected. They were gas chromatographed for the second time on an OV-351 column.

Figures 2 and 3 show the second gas chromatograms of fractions 8 and 10, respectively. Fraction 8 was spiked with *cis-* and *trans*-2-(2-pentenyl)furans and analyzed by GLC. The gas chromatogram indicated that they should be in the subfraction, 8-7, if they were present in the reverted soybean oil. Similarly, *cis-* and *trans*-2-(1-pentenyl)furans would be in subfraction, 10-9, if they were present in the reverted soybean oil. Subfractions 8-7 and 10-9 were then repeatedly collected and analyzed by GC-mass spectrometry.

Identification of 2-Pentenylfurans in Reverted Soybean Oil

The retention times of four authentic 2-pentenylfurans were determined under the same GC-mass analytical conditions as for the subfractions, 8-7 and 10-9. *cis*-2-(2-pentenyl)furan and *trans*-2-(2-pentenyl)furan had the same retention times of 19.5 minutes. *cis*-2-(1-pentenyl)furan had a retention time of 22.5 min and *trans*-2-(1-pentenyl)furan had a retention time of 24.0 min. During the analysis of samples 8-7 and 10-9, the highest sensitivity was used to obtain the mass spectra when the retention times reached the retention times of 2-pentenylfurans.

The gas chromatogram of subfraction 8-7 is shown in Figure 4. There was a large peak in the area between 15.7

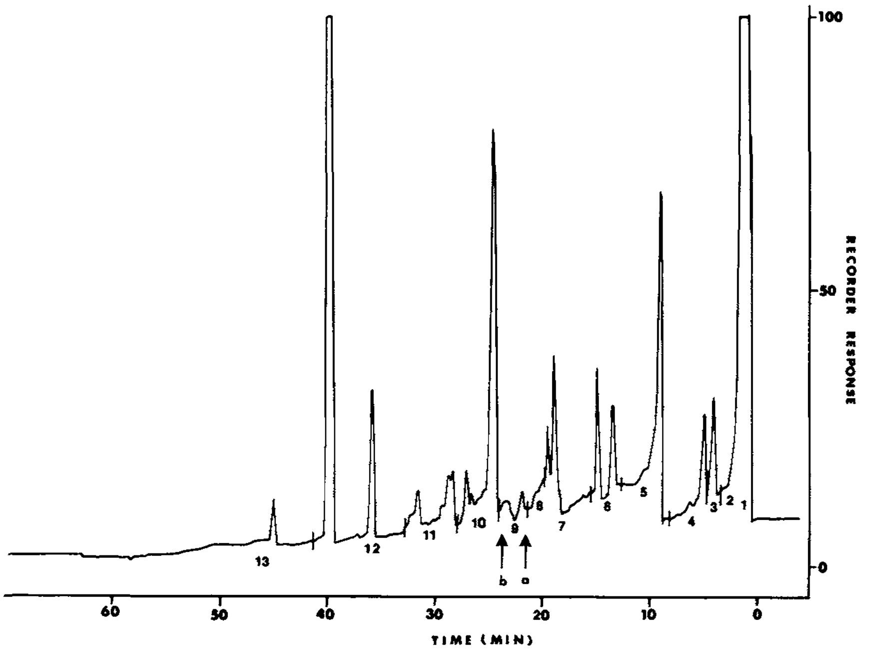

FIG. 3. Second gas chromatogram of fraction 10 by 10% OV-351 column. (a) Retention time of *cis*-2-(1-pentenyl)furan. (b) Retention time of *trans*-2-(1-pentenyl)furan.

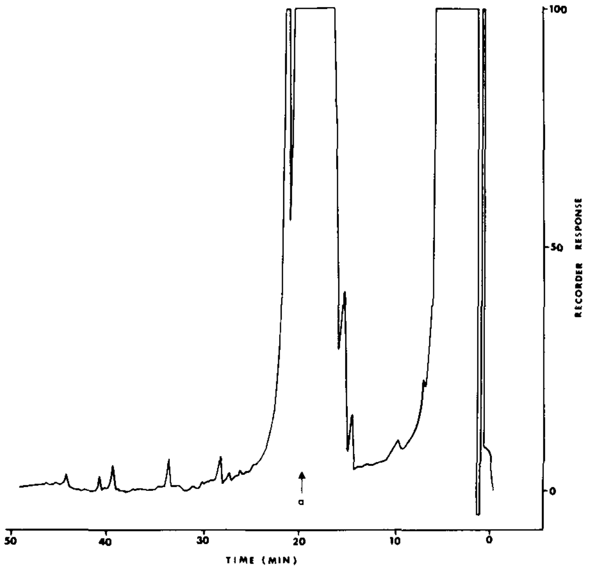

FIG. 4. Gas chromatogram of subfraction 8-7 on GC-mass analysis. (a) Retention time of *cis*- and *trans*-2-(1-pentenyl)furan.

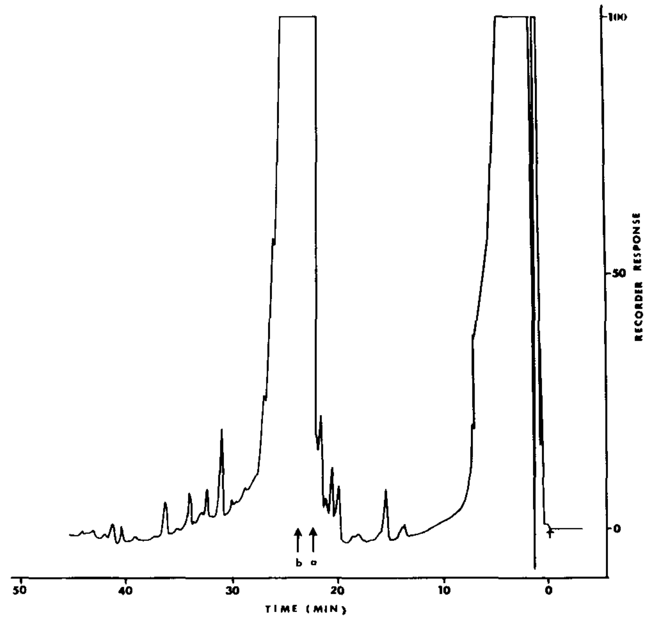

FIG. 5. Gas chromatogram of subfraction 10-9 on GC-mass analysis. (a) Retention time of *cis*-2-(1-pentenyl)furan. (b) Retention time of *trans*-2-(1-pentenyl)furan.

minutes and 20.8 minutes which was identified as 2-octanone by mass spectrometry. The mass spectra obtained at the retention time of 19.5 min showed all the peaks of a mixture of *cis*- and *trans*-2-(2-pentenyl)furans plus the peaks of 2-octanone. Therefore, the presence of the 2-(2-pentenyl)furans would be covered by the dominant 2-octanone if the retention times of the authentic samples were not previously determined. Table I shows the comparison of the mass spectra of 2-(2-pentenyl)furan and of the synthesized *cis*-2-(2-pentenyl)furan and *trans*-2-(2-pentenyl)-furan. It is obvious from this table that the major portion of the 2-(2-pentenyl)furans identified in subfraction 8-7 is *cis*-isomer.

The gas chromatogram of subfraction 10-9 is shown in

TABLE I

Mass Spectra of Suspected 2-(2-Pentenyl)Furans and of Synthesized *cis*-2-(2-Pentenyl)Furans and *trans*-2-(2-Pentenyl)Furan

Compound	Retention time	Mass spectrum
A mixture of *cis*- and *trans*-2-(2-pentenyl)-furans identified in reverted soybean oil	19.5 min	68(100%), 107(32%), 136(28%), 94(26%)
Synthesized *cis*-2-(2-pentenyl)furan		68(100%), 107(75%), 136(57%), 94(51%)
Synthesized *trans*-2-(2-pentenyl)furan		107(100%), 136(91%), 94(69%), 79(63%)

TABLE II

Mass Spectra of Suspected and of Synthesized *cis*-2-(1-Pentenyl)Furan and *trans*-2-(1-Pentenyl)Furan

Compound	Retention time	Mass spectrum
cis-2-(1-pentenyl)furan identified in reverted soybean oil	22.5 min	107(100%), 79(67%), 94(55%), 136(48%)
Synthesized *cis*-2-(1-pentenyl)furan		107(100%), 79(36%), 94(27%), 136(19%)
trans-2-(1-pentenyl)furan identified in reverted soybean oil	24.0 min	107(100%), 79(84%), 136(71%), 94(39%)
Synthesized *trans*-2-(1-pentenyl)furan		107(100%), 79(30%), 94(22%), 136(17%)

Figure 5. Again, there was a large peak in the area between 22.0 and 28.0 min. This large peak was identified as 1-dodecene by its mass spectrum. The mass spectra (Table II) obtained at the retention time of *cis*-2-(1-pentenyl)furan (22.5 min) and *trans*-2-(1-pentenyl)furans (24.0 min) showed all the peaks of *cis*- and *trans*-2-(1-pentenyl)furans, respectively, plus the peaks of 1-dodecene. Therefore, the presence of *cis*- and *trans*-2-(1-pentenyl)furan would be covered by the dominant 1-dodecene if the retention times of the authentic samples were not previously determined.

We have positively proven that *cis*- and *trans*-2-(1-pentenyl)furans and a mixture of *cis*- and *trans*-2-(2-pentenyl)-furans are present in a reverted soybean oil. Therefore, linolenate could oxidize to form 2-pentenylfurans which could then contribute to the characteristic beany and grassy flavor of soybean oil.

ACKNOWLEDGMENTS

New Jersey Agricultural Experiment Station Publication No. D-10503-1-82, supported by State Funds. Preliminary work has been performed by M.S. Smagula. We wish to thank J.B. Shumsky for secretarial aid.

REFERENCES

1. Durkee, M.M., Ind. Eng. Chem. 28:898 (1936).
2. Chang, S.S., and F.A. Kummerow, JAOCS 30:251 (1953).
3. Chang, S.S., and F.A. Kummerow, JAOCS 31:324 (1954).
4. Smouse, T.H., and S.S. Chang, JAOCS 44:509 (1967).
5. Chang, S.S., T.H. Smouse, R.G. Krishnamurthy, B.D. Mookherjee and B.R. Reddy, Chem. Ind. 1926 (1966).
6. Frankel, E.N., W.E. Neff and T.R. Bessler, Lipids 14:961 (1979).
7. Rawl, M.R., and P.J. van Santen, JAOCS 47:135 (1970).
8. Terao, J., and S. Matsushita, JAOCS 54:234 (1977).
9. Ho, C.-T., M.S. Smagula and S.S. Chang, JAOCS 55:233 (1978).
10. Smagula, M.S., C.-T. Ho and S.S. Chang, JAOCS 56:516 (1979).
11. Lomanno, S.S., and W.W. Nawar, J. Food Sci., 47:744 (1982).
12. Chang, S.S., K.M. Brobst, H. Tai and C.E. Ireland, JAOCS 38:671 (1961).
13. Chang, S.S., JAOCS 38:669 (1961).
14. Thompson, J.S., W.A. May, M.M. Paulose, R.J. Peterson and S.S. Chang, JAOCS 55:897 (1978).

[Received July 1, 1982]