

by comparing the report of Fritz *et al.* (1970) which says that the iron in enriched breakfast cereal and enriched flour had relative availabilities of 43 to 32%, respectively, with the present findings that the iron in commercially processed milk-based liquid infant formulas has an availability of 138 to 143%. This suggests a substantially lower dietary iron requirement when the dietary iron source is an iron-fortified processed infant formula, compared to when it is other food sources of iron.

With information on the biological availability of a variety of iron salts in processed infant formulas now available, it can be concluded that iron salts other than ferrous sulfate can be used in infant formula products and other milk-based and soy isolate liquid nutrition products with the assurance that they furnish absorbable iron.

#### ACKNOWLEDGMENT

The authors thank Charles Cutteridge for help in preparing the experimental products and Frank Beach for assisting with the care of the animals.

#### LITERATURE CITED

- Amine, E. K., Hegsted, D. M., *J. Nutr.* **101**, 1575 (1971).  
 Council on Foods and Nutrition, *J. Amer. Med. Ass.* **203**, 6 (1968).  
 Fritz, J. C., Pla, G. W., Roberts, T., Boehne, J. W., Hove, E. L., *J. Agr. Food Chem.* **18**, 647 (1970).  
 Gross, S., "Iron Nutrition in Infancy," Report of the Sixty-Second Ross Conference on Pediatric Research, Columbus, Ross Laboratories, 1970, p 89.  
 Hodson, A. Z., *J. Agr. Food Chem.* **18**, 947 (1970).  
 National Academy of Sciences-National Research Council, "Recommended Dietary Allowances," Revised 1968, Publ. No. 1694, Washington, D. C., 1968.  
 Niccum, W. L., Jackson, R. L., Stearns, G., *Amer. J. Dis. Child.* **86**, 553 (1953).  
 Pla, G. W., Fritz, J. C., Boehne, J. W., *Fed. Proc.* **30**, 461 (1971).  
 Steel, R. G. D., Torre, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York, N. Y., 1960.  
 Theuer, R. C., Kemmerer, K. S., Martin, W. H., Zoumas, B. L., Sarett, H. P., *J. Agr. Food Chem.* **19**, 555 (1971).  
 Wasserman, R. H., Lengemann, F. W., *J. Nutr.* **70**, 377 (1960).

Received for review July 27, 1972. Accepted December 27, 1972.

## trans-2-Nonenal: Coffee Compound with Novel Organoleptic Properties

Thomas H. Parliment,\* William Clinton, and Richard Scarpellino

A compound which imparts a fresh-brewed woody character to roasted and ground coffee was isolated by gas chromatography and shown to be trans-2-nonenal. Organoleptic evaluation was utilized extensively during the fractionation to fol-

low the desired component. The organoleptic properties of 2-nonenal in soluble coffee and in water are described and the importance of this compound to the flavor of other natural materials is discussed.

Although the volatile composition of coffee has been studied for many years, great strides have been made in the last few years. The advent of new instrumental methods of analysis and the reduction in required sample size have led to the identification of an impressive list of chemicals (Friedel *et al.*, 1971; Gianturco, 1967; Stoffelsma *et al.*, 1968; Stoll *et al.*, 1967), which extends our knowledge of the chemistry of coffee.

For some time, coffee tasting experts have agreed that there is a note in brewed coffee, absent in soluble coffee, which contributes an effect described as "woody." None of the previously identified coffee aroma chemicals possess this characteristic. This note is found in steam distillates of roasted coffee beans; however, the complexity of coffee aroma concentrates hampered earlier efforts to characterize and isolate the compound responsible. In the course of our efforts to identify this woody flavor in coffee aroma we became aware that a similar flavor note existed in the volatile fraction of bell peppers. Since the volatiles of peppers are a much less complex material than roasted coffee, initial efforts were directed toward isolating this note from a distillate of bell peppers. After the compound responsible for the "woody" effect had been identified in peppers, similar techniques were used in the present study on coffee. This report describes the isolation and characterization of the compound responsible for the "woody" flavor in coffee and gives a description of its organoleptic properties.

#### PROCEDURE

**Preparation of Aroma Concentrate.** Eighteen kilograms of coarsely ground roasted Arabica coffee beans were steam distilled at atmospheric pressure. A total of 13 l. of aqueous distillate was collected and this was extracted with a total of 10 l. of diethyl ether in small portions. The ethereal extract was concentrated by distillation through a 20-cm Vigreux column to a volume of about 5 ml.

**Separation Scheme.** Gas chromatography was used throughout in the purification of the desired component.

A preliminary crude separation was made by preparative scale gas chromatography using an Aerograph Auto-prep Model 713 equipped with a flame ionization detector. Gas chromatographic collections were made on an equal time basis as described previously (Parliment, 1971) using the Aerograph 5-ml collector bottles and collecting at 2-min intervals. Gas chromatographic conditions are described in Table I, Purification Step No. 1. Each of the fractions obtained was evaluated by mixing a small quantity of the trapped material in soluble coffee and the samples were tasted. By this procedure, the desired effect was shown to be associated with the gas chromatographic fraction which eluted at 7 to 9 min. This fraction was still quite complex, as shown by rechromatography on other gas chromatographic columns containing dissimilar liquid phases.

By trial and error it was shown that rechromatography on three successive liquid phases was required to yield the chemically pure component with the desired effect. The procedures used for each of these three further purification steps were similar. In each case the separation was

\*General Foods Technical Center, White Plains, New York 10625.

**Table I. Gas Chromatographic Conditions for Four Steps of Purification**

Purification step no.	Column <sup>a</sup>		Helium flow rate, ml/min	Program rate, °C	Retention time possessing effect, min
	Liquid phase, %	Length X diameter			
1.	SE-52, 20%	6 ft X 1/4 in.	70	180° isothermal	7-9
2.	DEGS, 10%	10 ft X 1/4 in.	70	150° isothermal	6-7.5
3.	Carbowax 20M, 10%	6 ft X 1/8 in.	30	80 to 200° at 5°/min	11-12
4.	OV-225, 15%	6 ft X 1/8 in.	30	100 to 200° at 5°/min	10.8

<sup>a</sup> In all cases, solid support used was Anakrom ABS, 80/90 mesh.

performed in a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector. For the second separation (purification step 2, Table I), a small aliquot of the crude fraction was separated on the DEGS column and the effluent from the exit port was bubbled into a series of 25-ml portions of soluble coffee, changing the trapping medium each time the recorder indicated the elution of a different component. By taste, it was evident that the desired component was associated with the retention time of 6 to 7.5 min. Having located the woody note, the entire sample was further purified by repetitive gas chromatography on the DEGS column, collecting the 6 to 7.5 min region by condensation in a dry ice-cooled melting point capillary.

In similar fashion, two more stages of gas chromatographic purification were required to yield a pure product, as indicated by gas chromatography on other selective phases and by mass and infrared spectroscopy. The actual gas chromatographic operating conditions for the latter two stages of purification are summarized in Table I. Throughout these separations, evaluation of the separated components in soluble coffee was performed in order to follow the location of the desired component.

**Spectrometric Analysis.** An infrared spectrum of the isolated compound was run as a thin film between sodium chloride plates using a 4X refracting beam condenser in a Perkin-Elmer 521 infrared spectrophotometer.

A high-resolution mass spectrum of the isolated component was run on a CEC-21-110 double-focusing mass spectrometer using an ionizing voltage of 70 eV.

**Organoleptic Evaluation.** As indicated above, the location of the desired component during separation on different gas chromatographic liquid phases was done by bubbling the gas chromatographic effluent into soluble coffee and noting the resultant effect.

After the woody component had been identified as 2-nonenal, the organoleptic properties of this chemical were studied. Commercial 2-nonenal (Compagnie-Parento, Croton-on-Hudson, N. Y.) was purified by preparative scale gas chromatography and a standard solution was pre-

pared. This solution was evaluated in soluble coffee as well as in water at various concentrations by an expert profile evaluation panel (Cairncross and Sjoström, 1950).

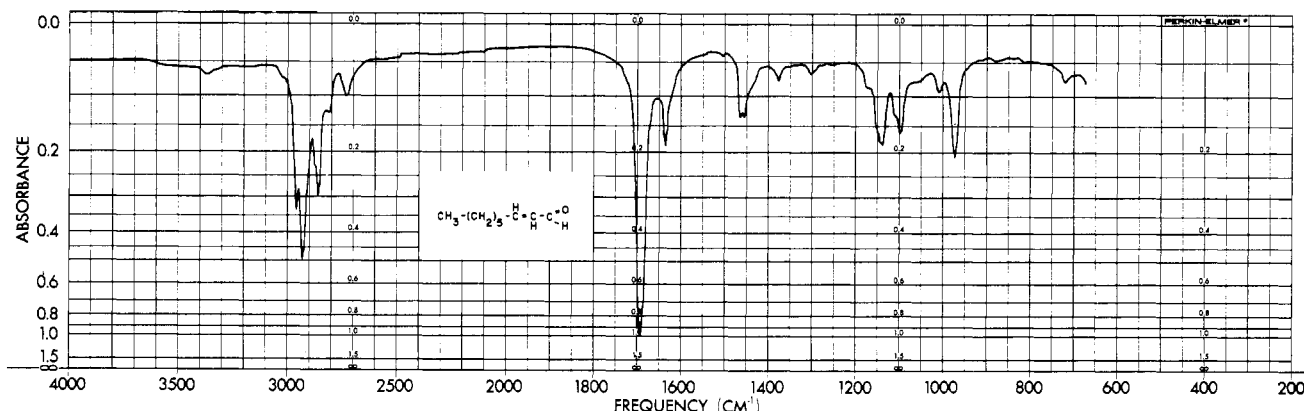
**RESULTS AND DISCUSSION**

The infrared spectrum (Figure 1) indicates that this component is an  $\alpha,\beta$ -unsaturated aldehyde. The abbreviated mass spectrum is given in Table II and is formed by selecting the two largest peaks in each 14 mass unit interval throughout the spectrum, as suggested by Hertz *et al.* (1971). This spectrum is consistent with a nine-carbon unsaturated aldehyde, mol wt = 140. Taken together, this information indicated the isolated component to be 2-nonenal. The configuration about the double bond of both the isolated component and the synthetic nonenal was shown to be trans by the presence of a strong infrared band at 965  $\text{cm}^{-1}$  and the absence of a band near 690  $\text{cm}^{-1}$ . The characterization of the isolated component as 2-nonenal was most surprising since this is a well-known chemical whose organoleptic properties have been described as "orris-like," fatty, slightly green, and waxy (Arcander, 1969). Nevertheless, all spectral and chromatographic properties of *trans*-2-nonenal agreed with those of the component isolated from coffee.

When 2-nonenal was evaluated at 1 ppb in soluble coffee by a profile evaluation panel, the effect was that of adding a desirable woody, fresh brewed flavor to the brew. In addition, this compound reduced the undesirable caramel, acid, and astringent notes of soluble coffee.

The effect of 2-nonenal at various levels in soluble coffee is indicated in Table III. From this, it is evident that the desirable level of 2-nonenal in coffee is extremely low, at levels of from 0.2 to 2 ppb. Also shown in Table III are the responses elicited when nonenal was evaluated in water at various levels. From this it is evident the flavor progresses from woody, to fatty and oily, to cucumber.

As early as 1958 it was shown that 2-nonenal has a flavor threshold in the low ppb range (Lea and Swoboda, 1958). Forss *et al.* (1962) indicated it possesses an oily or tallowy character at low (1 ppb) levels and a cucumber



**Figure 1. Infrared spectrum of *trans*-2-nonenal.**

Table II. Abbreviated Mass Spectrum of *trans*-2-Nonenal

Mass interval	Mass	Intensity
34-47	41	48
	43	38
48-61	55	84
	57	43
62-75	69	76
	70	97
76-89	83	100
	84	66
90-103	96	54
	97	28
104-117	109	8
	111	14
118-131	122	13
	123	6
132-145	138	8
	140	3

character at higher (10 ppb) levels and that it, along with other nonadienals, is responsible for the characteristic flavor of cucumbers. Buttery *et al.* (1968) reported that 2-nonenal has a threshold of about 0.1 ppb and that it makes a substantial contribution to the total odor impact of carrot root oil. Recently, 2-nonenal has been characterized as the compound responsible for the typical "hardening flavor" which occurs in certain linoleic and oleic acid-containing oils such as peanut, cottonseed, and sunflower after hardening and refining (Feenstra and Meijboom, 1971). 2-Nonenal has been reported in diverse food products such as tomato (Buttery *et al.*, 1971), beef (Liebich *et al.*, 1972), cranberry (Anjou and von Sydow, 1967), and muskmelon (Kemp *et al.*, 1972), but its importance to the overall character impact of these products was not discussed. It had not been reported to be a constituent of bell peppers; however all spectrometric and chromatographic properties of the component which we isolated from bell peppers in our preliminary investigation and of 2-nonenal are in agreement. Due to the extremely low threshold (approximately 0.1 ppb) and the ease of formation from unsaturated fatty acids (Feenstra and Meijboom, 1971), it is likely that 2-nonenal contributes its subtle effect to numerous natural products.

The technique of noting aromas of components as they elute from the gas chromatograph is well recognized (Dravnieks and O'Donnell, 1971). The concept of bubbling selected components into a trapping medium and noting the effect by tasting has not received as much attention for defining organoleptically important compounds. Such a trapping procedure has certain advantages over noting aromas "on-the-fly" as they elute from the chromatograph: the samples can be evaluated at the investigator's convenience; various samples can be compared directly against each other; several investigators can evaluate each sample; and unusual effects may only be evident when tasted in solution. Although we used coffee as a trapping medium, other appropriate media, including water, can be used. For example, when 2-nonenal was bubbled into cranberry juice at a level of 1 ppb, there was a dramatic reduction in the normal astringent character of the juice, without the addition of any objectionable notes. This effect could not have been predicted from the aroma of 2-nonenal.

Table III. Organoleptic Evaluation of 2-Nonenal at Various Levels

Concentration, ppb	Evaluation in	
	Soluble coffee <sup>a</sup>	Water <sup>b</sup>
0.2	Detectable subtle flavor	Slightly plastic-like
0.4	Subtle woody	Woody
1.0	Woody, smoother	Woody
2.0	Woody, groundsy	Woody
8.0	High woody impact	Fatty
16.0	Tallowy	Fatty
30.0	Burnt, fatty	Unpleasant oily, fatty
40.0	Burnt, fatty, rancid	Unpleasant fatty
1000		Strong cucumber

<sup>a</sup> Evaluated at 140-160°F. <sup>b</sup> Evaluated at room temperature.

The woody flavor note of *trans*-2-nonenal is an important constituent of regular coffee and particularly of coffee prepared in a household percolator. It helps significantly to balance other flavor notes—specifically the acid or sour, caramel, and winey buttery notes. The discovery of this compound has opened other experimental routes in the flavor compounding field for coffee and many other foodstuffs and beverages.

In summary, several points can be made in regards to the work herein described. 1. The preliminary investigation of a material less complex than coffee in order to isolate the woody flavor note. 2. The importance of utilizing organoleptic evaluation in following the location of the indicated compound during the separation procedure. 3. The significance of a low level of 2-nonenal in coffee in order to achieve the desired flavor.

#### ACKNOWLEDGMENT

The authors thank Michael Kolor for obtaining the mass spectrum.

#### LITERATURE CITED

- Anjou, K., von Sydow, E., *Acta Chem. Scand.* **21**, 945 (1967).  
 Arctander, S., "Perfume and Flavor Chemicals," S. Arctander, Publisher, Montclair, N. J., 1969, Monograph 2356.  
 Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R., Ling, L. C., *J. Agr. Food Chem.* **16**, 1009 (1968).  
 Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C., *J. Agr. Food Chem.* **19**, 524 (1971).  
 Cairncross, S., Sjostrom, L., *Food Technol.* **4**, 308 (1950).  
 Dravnieks, A., O'Donnell, A., *J. Agr. Food Chem.* **19**, 1049 (1971).  
 Feenstra, W. H., Meijboom, P. W., *J. Amer. Oil Chem. Soc.* **48**, 684 (1971).  
 Forss, D. A., Dunstone, E. A., Ramshaw, E. H., Stark, W., *J. Food Sci.* **27**, 90 (1962).  
 Friedel, P., Krampfl, V., Radford, T., Renner, J. A., Shephard, F. W., Gianturco, M. A., *J. Agr. Food Chem.* **19**, 530 (1971).  
 Gianturco, M. A., in "The Chemistry and Physiology of Flavors," Schultz, H. W., Day, E. A., Libbey, L. M., Ed., AVI Publishing Co., Westport, Conn., 1967, pp 431-449.  
 Hertz, H. S., Hites, R., Biemann, K., *Anal. Chem.* **43**, 681 (1971).  
 Kemp, T. R., Stoltz, L. P., Knavel, D. E., *J. Agr. Food Chem.* **20**, 196 (1972).  
 Lea, C. H., Swoboda, P. A., *Chem. Ind.* 1289 (1958).  
 Liebich, H. M., Douglas, D. R., Zlatkis, A., Muggler-Chavan, F., Donzel, A., *J. Agr. Food Chem.* **20**, 96 (1972).  
 Parliment, T. H., *J. Chromatogr.* **58**, 283 (1971).  
 Stoffelsma, J., Sipma, G., Kettenes, D. K., Pypker, J., *J. Agr. Food Chem.* **16**, 1000 (1968).  
 Stoll, M., Winter, M., Gautschi, F., Flament, I., Willhalm, B., *Helv. Chim. Acta* **50**, 628 (1967).

Received for review November 21, 1972. Accepted February 16, 1973.