

## Gas Chromatographic Identification of Components in Maple Sirup Flavor Extract

By J. C. UNDERWOOD and V. J. FILIPIC (Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia 18, Pa.)

**Three major components in the chloroform extract of the flavor constituents of maple sirup have been separated by gas chromatography and identified as vanillin, syringaldehyde, and dihydroconiferyl alcohol from their infrared spectra.**

Previous publications (1, 2) have described the isolation and identification of some components in chloroform extracts of maple sirup which contain the flavor components. By classic chemical techniques and column chromatography, vanillin and syringaldehyde have been identified in this extract, but other constituents indicated as bands on silicic acid columns remain unknown. Further, the chromatographic techniques used did not give the separation required for the quantitative analytical determination of these compounds.

A method that would identify and quantitatively measure constituents of maple flavor has long been needed. This information would be used to guide research on the improvement of the quality of maple sirup, the development of sirup for specific end uses, and the detection of adulteration.

In 1961 a gas chromatographic procedure (3) was reported that successfully separated for identification a large number of the constituents in vanilla extracts. The similarity of many of the compounds in the vanilla extract to the vanillin and syringaldehyde found in maple flavor extracts led the Associate Referee to initiate work on the application of gas chromatographic techniques to the separation and identification of the components of maple flavor extracts. The results of preliminary studies are reported in this paper.

### Experimental

*Preparation of maple flavor extract.*—Using the information of Sair and Snell (4), Nelson

(5), and Underwood, *et al.* (2) on the extraction of the flavor from maple sirup with chloroform, an extract free of color and sugar but containing the flavor was obtained. The extraction procedure used in the present study was described in the publication by Underwood, *et al.* Briefly, it consisted of stirring a volume of sirup with two successive and equal volumes of chloroform for several hours. After the stirring, the combined chloroform extract was concentrated at room temperature until precipitation began to occur.

To obtain still higher concentrations for gas chromatography the flavor components were further concentrated by treatment of the extract with diethyl ether. One volume of the concentrated chloroform extract was mixed with 3 volumes of diethyl ether and the precipitate was removed by filtration through a fritted glass filter. The filtrate was again concentrated to the point of precipitation, and portions were used for the gas chromatographic separation.

*Gas chromatographic fractionation of the chloroform extract.*—The Aerograph Model A 350,<sup>1</sup> a chromatograph which can be temperature-programmed, was used in these studies. The columns were ¼" o.d. stainless steel coils 5 feet long containing 20 wt % of substrate on 60/80 mesh acid-washed Chromosorb W. The injector port and detector oven were kept above 300°C. With the silicone polymer SE-30 as the substrate and 100 μl of extract, the optimum conditions of helium flow rates and temperature programming were 50 ml/min. and 10°C/min. The chromatogram obtained for the temperature range of 60–300°C is shown in Fig. 1. The two major high-boiling components were collected in medicine droppers inserted in the sample stream outlet. Several runs were required to collect the amounts needed for the infrared spectra.

Columns containing the polar substrate Carbowax 20 M were also used to separate the components in the chloroform extract. The

<sup>1</sup> Mention of a specific company or product does not constitute endorsement by the Department over other companies or products not mentioned.

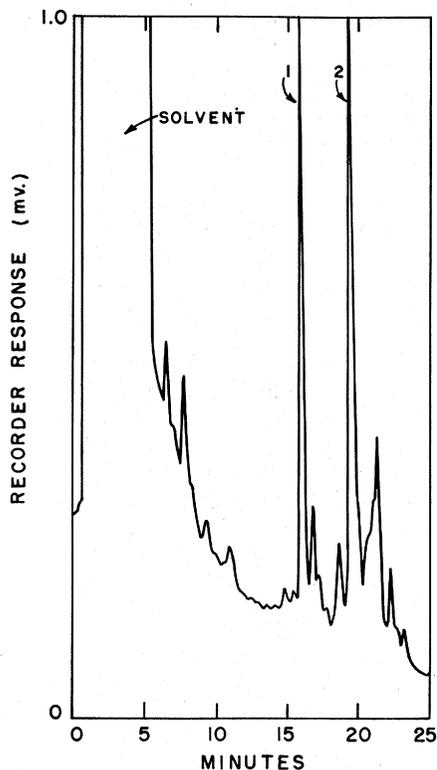


Fig. 1—Chromatogram of maple flavor extract on SE-30 at 50 ml helium per minute and temperature programmed from 60°C at 10°/min.

major components of the chloroform extract were retained more strongly by this substrate. To reduce retention time the column was operated isothermally at 245°C (higher temperatures lead to excessive bleeding of the substrate) and the flow rate was increased to 290 ml/min. Otherwise, the experimental conditions were the same as those used with the SE-30 columns. The chromatogram obtained in 25 minutes is shown in Fig. 2. It can be seen that there are three major peaks; the last two are not completely resolved. The material represented by peak 3 was collected for 6 successive runs and used to obtain an infrared spectrum.

This fraction was collected for a period of approximately 3 minutes during each run, beginning with the emergence of the third major peak; this is indicated by the two dotted lines in Fig. 2.

### Results

The first major component (peak 1, Fig. 1 and Fig. 2) eluted from both the SE-30 and

Carbowax 20 M columns had the characteristic odor of vanillin. Vanillin standards were chromatographed and gave identical retention times. In addition, the infrared curve of this component was identical with that of synthetic vanillin.

Since both vanillin and syringaldehyde had been previously identified in the chloroform extracts of maple sirup, it was suspected that the other major fraction (peak 2, Fig. 1) separated by the SE-30 column was syringaldehyde. A syringaldehyde standard gave the same retention time. However, the infrared curve of this isolate was quite different from that of pure syringaldehyde. This discrepancy suggested that the fraction was either a major component of the chloroform extract not previously identified or that it was a mixture of syringaldehyde and some other material.

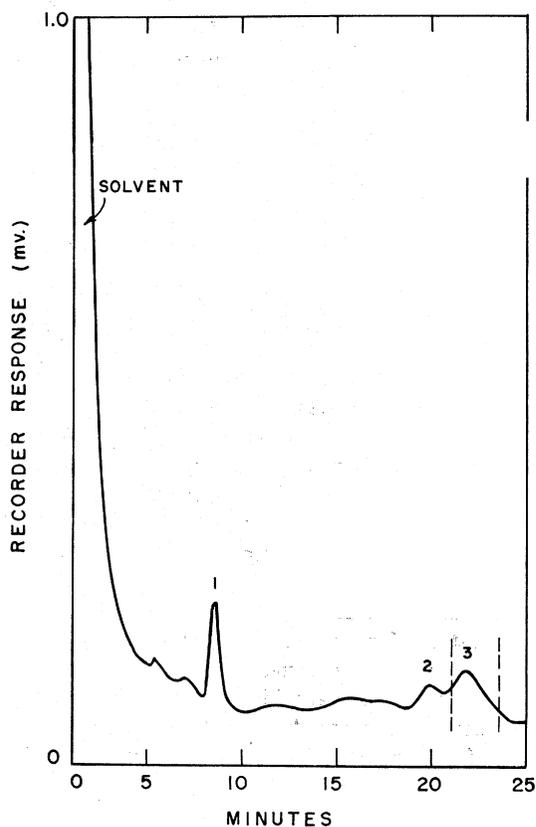


Fig. 2—Chromatogram of maple flavor extract on Carbowax 20 M at 290 ml helium per minute and a column temperature of 245°.

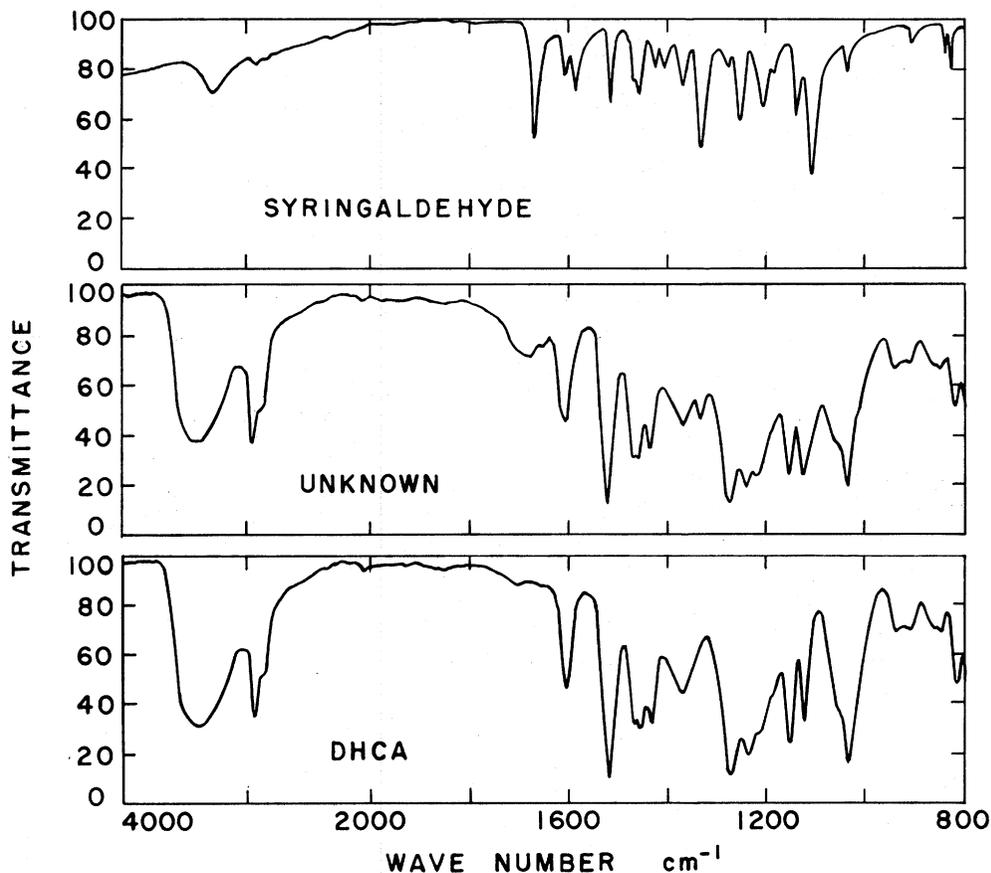


Fig. 3—Infrared spectra of syringaldehyde, fraction 3 (Fig. 2), and dihydroconiferyl alcohol.

When the chromatogram was obtained with Carbowax 20 M, the appearance of three major peaks, rather than two, tended to confirm that peak 2 of Fig. 1 represented a mixture of two compounds. The retention time of syringaldehyde standard was identical with that of peak 2 of Fig. 2. Peak 3 therefore probably contained the unknown component present in the impure fraction obtained from the SE-30 column.

The infrared spectrum of this impure fraction was compared with those reported by Pearl (6) for a group of model lignin compounds. These comparisons showed that dihydroconiferyl alcohol (DHCA) could be the unknown component. A sample of DHCA (kindly supplied by Dr. I. A. Pearl of the Institute of Paper Chemistry) had the same retention time of Carbowax 20 M as the third major peak and increased the height of this peak when added to maple sirup

extract. The infrared spectrum of this third component is compared (Fig. 3) with that obtained for syringaldehyde and DHCA. The spectrum of the unknown (peak 3, Fig. 2) is identical with that of DHCA except for the presence of bands at wave numbers of  $1675\text{ cm}^{-1}$  and  $1335\text{ cm}^{-1}$  and the broadening and intensification of a band at  $1125\text{ cm}^{-1}$ . These are the points at which the three major bands in the spectrum of syringaldehyde occur. The presence of some syringaldehyde is not surprising because of the poor resolution of peaks 2 and 3.

#### Summary and Recommendation

Three major components in the chloroform extract of the flavor compounds of maple sirup have been separated by gas chromatography and identified by their infrared spectra. Quantitative gas chromatographic methods for one or more of these

components are being developed. Should the content of any of these components prove to be reasonably constant, these methods might then be used to detect adulteration. It is also possible that the concentration of these components is related to flavor intensity. Meanwhile, efforts will be continued to isolate and identify lesser components present in solvent extracts (see Fig. 1) that may very well contribute far more to flavor.

It is recommended that the work on the identification of the flavor components of maple sirup be continued.

#### Acknowledgment

We gratefully acknowledge the work of J. S. Ard, who obtained the infrared spectra of the sub-milligram amounts of material isolated in this work.

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This report of the Associate Referee was presented at the Seventy-sixth Annual Meeting of the Association of Official Agricultural Chemists, Oct. 15-17, 1962, at Washington, D.C.

This recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D, and was accepted by the Association. See *This Journal*, **46**, 107, 108 (1963).