

## Maple Sirup. XVI. Isolation and Identification of Compounds Contributing to the Flavor of Maple Sirup<sup>a</sup>

### SUMMARY

**A major portion of the flavor was isolated from maple sirup by extraction with chloroform. From this was separated several pure crystalline compounds and several impure materials. Vanillin and syringaldehyde were definitely identified. These are compounds that could come from precursors in the decomposition of lignin, and this possible source of maple flavor is being investigated.**

The distinctive flavor of maple sirup has kept this product selling at premium prices for many years. Many attempts have been made to imitate it with mixtures of vanillin, coumarin, and lovage, and extracts of coffee grounds, fenugreek seed, corn cobs, and hickory bark. Also, the reaction products of some reducing sugars and amino compounds have a maple-like flavor (Nelson, 1928). All, however, fall far short of true maple flavor, which has never been completely characterized. In 1925 Robinson separated a maple flavor fraction from maple sirup with chloroform, ether, or benzene. Skazin (1930) and Nelson (1928) separated vanillin-like products from ether and chloroform extracts. Risi and Labrie (1935) and Findlay and Snell (1935) suggested a relation of maple flavoring compounds to lignin. The most extensive attempt to identify maple flavor was made by Sair and Snell, in 1939. They separated a chloroform extract of a large amount of sirup into several gross fractions by the use of other organic solvents. These isolates, on further fractionation, yielded a number of compounds and mixtures of compounds. None, however, was identified definitely.

The main objective of research on maple products at this laboratory has been the development of modern processing methods

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for making high-quality maple sirup through improved control of the factors influencing flavor development. The earlier program included a study to determine the general chemical composition of maple sap and sirup. The current program contains a more intensive study of the chemical identity and origin of the flavor in maple sirup. This paper reports isolation of a flavor fraction from a good-quality maple sirup, and characterization of some of the compounds contributing to the maple flavor.

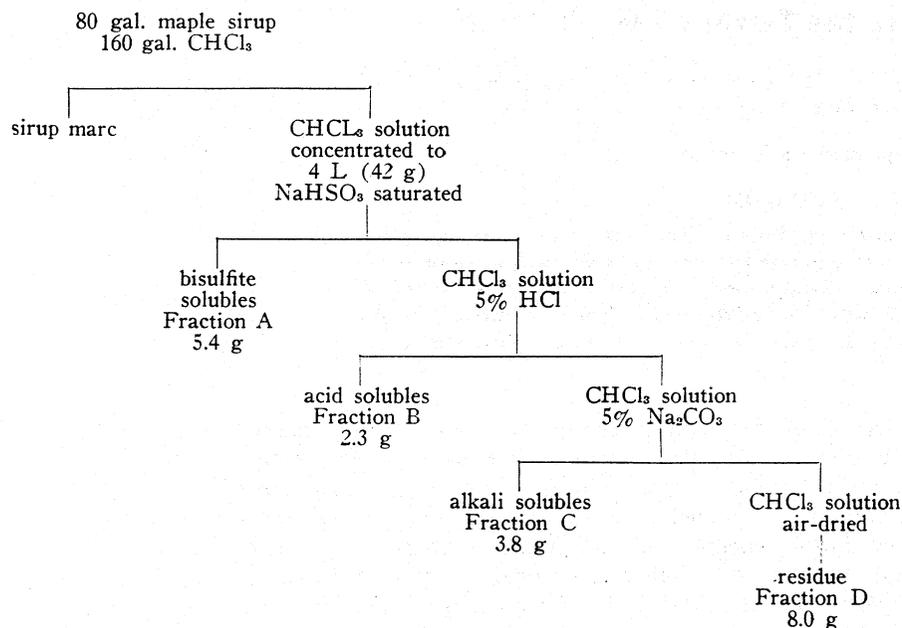
### EXPERIMENTAL

Using information of Sair and Snell (1939) and Nelson (1928) on extraction of maple flavor, chloroform was used to obtain from maple sirup an extract, free of sugar and color, that contained the maple flavor.

**Procedure.** Eighty gallons of maple sirup with distinctive maple flavor was divided into four 20-gal. portions. Each portion was extracted with two successive 20-gal. portions of chloroform. Extraction was accomplished by placing the sirup and chloroform in a 55-gal. drum and stirring for one hour. The stirred mixture was then let stand for several hours or overnight for the sirup and chloroform to separate into two layers, and then re-extracted with the second portion of chloroform. All of the chloroform portions were combined and concentrated at atmospheric pressure to a volume of 4 liters. Determination of the solids in an aliquot of the concentrated chloroform extract showed that 41.6 g of air-dried material had been obtained from the 80 gal. of sirup. The extracted sirup marc was stored for later work on color.

**Fractionation of the Chloroform Extract.** For a preliminary survey of the composition of the

Table 1. Flow sheet, chloroform extraction of maple sirup



chloroform extract, a small aliquot was mixed with silicic acid, dried, and added to the top of a silicic acid column prepared from a slurry of Skellysolve B (mention of trade names does not imply endorsement by the Department over others not named). The column was eluted with a series of solvents giving increasing polarity from Skellysolve B, to chloroform, to ethyl alcohol. A large number of fractions were obtained. This procedure proved impractical because of the time required to obtain adequate amounts of separated materials. Therefore the following procedure was used to separate the original chloroform extract into four gross fractions for further purification in quantities large enough for identification tests.

Three 1000-ml portions of the concentrated chloroform extract were each concentrated on a steam bath to 500 ml. These were extracted by shaking in a separatory funnel with three 200-ml portions of ice-cold saturated sodium bisulfite solution. The extracted solution was held for later treatment. The combined bisulfite extracts were treated with solid sodium bicarbonate until frothing stopped and pH became 7. This decomposed the sulfite addition compounds removed from the original chloroform extract. The resulting solution was extracted with three 200-ml portions of chloroform, and these chloroform extracts were combined and evaporated to 250 ml. This chloroform solution of the sulfite addition compounds was then extracted with three 150-ml portions of ammonium hydroxide (1-10). The ammonium hy-

droxide extract was acidified with hydrochloric acid and re-extracted with chloroform to give a final purified solution of the compounds isolated through sulfite addition (Fraction A) containing 5.4 g of dry matter.

The sulfite-extracted chloroform extract was divided into three 500-ml portions. Each was extracted by shaking with three 250-ml portions of 5% hydrochloric acid. Again the extracted solution was held for further treatment. The combined hydrochloric acid extract was neutralized with sodium carbonate and extracted with chloroform. On air drying, this chloroform extract of the acid-soluble material yielded 2.32 g of a brown sirup (Fraction B).

The acid-extracted original chloroform extract was divided into 500-ml portions and each shaken with three 100-ml portions of 5% sodium carbonate and the water layers combined. This was neutralized with hydrochloric acid and re-extracted with chloroform. The resulting chloroform solution of the alkali-soluble compounds of the original chloroform extract was evaporated to 25 ml on a steam bath and allowed to go to dryness at room temperature, yielding a brown sirup (3.84 g) with a strong maple flavor. This was taken up in chloroform for storage (Fraction C).

The exhaustively extracted original chloroform extract was evaporated to dryness at room temperature and yielded a residue of 8 g (Fraction D). Substantial losses of material occurred dur-

ing fractionation, especially in the bisulfite treatment.

**Compounds Isolated.** a. The bisulfite addition compounds isolated in Fraction A were chromatographed on a column of silicic acid with a solvent of 70% Skellysolve B and 30% chloroform. Two fractions were obtained, both sweet in odor but definitely still impure. The first fraction was rechromatographed on silicic acid with a solvent of 95% Skellysolve B and 5% chloroform. The eluate from this column contained a compound that, when crystallized from chloroform-Skellysolve B, melted sharply at 80°C. The infrared curve for this compound was identical with that of synthetic vanillin. Its 2,4-dinitrophenylhydrazine derivative (DNP) melted at 271–272°C (recorded for the vanillin 2,4-DNP 270°C (Kremers, 1948)). The second fraction contained small faintly yellow crystals that, when purified by crystallization from Skellysolve B-chloroform, melted at 111°C (cor.). The 2,4-DNP derivative had the same melting point, 232°C, as that found for a known syringaldehyde 2,4-DNP. The infrared absorption curve obtained for the crystals was almost identical with that of syringaldehyde (Fig. 1).

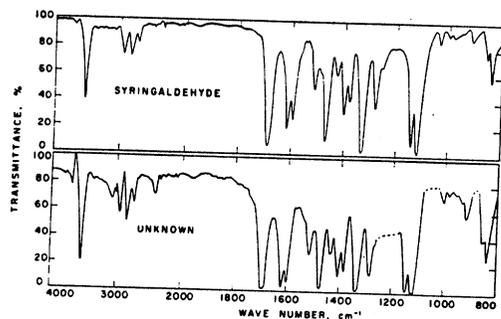


Fig. 1. Infrared absorption curves of syringaldehyde and flavor component.

b. The acid-soluble (Fraction B) part of the flavor isolate had a nut-like aroma. The material was chromatographed on a silicic acid column with a solvent of 37% chloroform and 67% Skellysolve B. The first, faintly yellow, band that was eluted had the intense nutty aroma of this fraction. The material was amorphous and will require further purification for identification. A second band eluted with the same solvent was highly fluorescent. It, too, lacked crystalline structure, and was devoid of aroma. A paper chromatogram showed that

it was not closely related to the sugars. Its infrared absorption curve had many of the bands characteristic of vanillin and syringaldehyde found in the bisulfite fraction (Fraction A). Finally, a residual brown material was stripped from the column with ethanol.

c. The alkali-soluble fraction was the largest of the gross separations except for the residue left in the original chloroform extract after the treatment with bisulfite, acid, and alkali. Of all the fractions of the flavor isolate, only this portion had an intense aroma that closely resembled true maple. It could not be adequately separated into its pure components with the silicic acid chromatographic column used with Fractions A and B. Consequently, further study will be necessary for identification of its components.

d. Fraction D. The residue remaining in the original chloroform isolate after extraction with bisulfite, acid, and alkali was a gummy brown mass with a sweet taste. This will be analyzed further and reported in a subsequent paper.

#### Acknowledgment

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