

# Source of Aromatic Compounds in Maple Sirup Flavor

J. C. UNDERWOOD AND V. J. FILIPIC

Eastern Regional Research Laboratory, Eastern Utilization Research and Development Division  
Agricultural Research Service, U. S. Department of Agriculture  
Philadelphia, Penna. 19118

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## SUMMARY

A sugar-free maple flavor concentrate made by extracting maple sirup with chloroform has been found to contain vanillin, syringaldehyde, and dihydroconiferyl alcohol, aromatic compounds related to lignin. When a chloroform extract of the sap of the maple tree is subjected to alkaline hydrolysis and nitrobenzene oxidation, the amounts of vanillin and syringaldehyde increase. The amount of dihydroconiferyl alcohol in this sap extract increases upon alkaline hydrolysis and then decreases upon subsequent nitrobenzene oxidation. Nitrobenzene oxidation of lignin-like material in the sap yields vanillin as the chief reaction product.

## INTRODUCTION

Earlier work by the authors has revealed a number of aromatic compounds in the chloroform extracts of both maple sap and sirup (Underwood *et al.*, 1961; Underwood and Filipic, 1964; Filipic and Underwood, 1964). Compounds such as vanillin and syringaldehyde were found in both sap and sirup, while coniferyl aldehyde, coumarin, and 2,6-dimethoxybenzoquinone were identified only in sap extracts. Dihydroconiferyl alcohol was present only in sirup. A material with many properties of lignin was also found in both sap and sirup. Findlay and Snell (1935) reported a lignin in sap. This paper reports the results of studies on the relationship of these aromatic chloroform-soluble compounds in maple sap to those of the same character found in maple sirup.

## EXPERIMENTAL

**Preparation of maple flavor extracts.** *Maple sirup.* A chloroform extract was prepared from a good-quality, flavorful maple sirup according to the procedure described by Underwood *et al.* (1961). Briefly, it consisted of extracting by stirring for 4 hr one volume of sirup with two successive and equal volumes of chloroform. The solvent layers were separated from the sirup, combined, and evaporated at room temperature in an open vessel across the top of which was blown a draft of air. The evaporation was stopped when precipitation began to occur in the concentrate.

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*Maple sap.* Again, a chloroform extract of sap was obtained free of sugars and color. Because of the minute amounts of compounds to be isolated and the need to minimize microorganism contamination, the special apparatus and procedure described by Filipic and Underwood (1964) were used. Maple sap, immediately after exuding from the trees, was collected in plastic tubing and transported to special extractors installed in the sugar grove. The sap was extracted by bubbling it up through a layer of chloroform in the extractor. The chloroform extract was concentrated as described for the sirup extract.

**Alkaline hydrolysis of the sap extract.** To 5 ml of the concentrated chloroform extract in a 125-ml Erlenmeyer flask, 5 ml of 1N NaOH was added and the mixture heated on a steam bath to remove the  $\text{CHCl}_3$ . Then the remaining aqueous solution was heated under reflux on a steam bath for 3 hr. At the end of the heating period, the solution was acidified with 1N HCl. This solution was extracted with two 10-ml portions of chloroform by shaking in a separatory funnel. The  $\text{CHCl}_3$  extracts were combined and evaporated at room temperature to 5 ml. The gas chromatograms of the original and of the hydrolyzed  $\text{CHCl}_3$  extracts are shown in Fig. 1. Chromatograms in Figs. 1 and 2 in this paper were obtained with an Aerograph A-350 (no endorsement implied) with a 4-ft- $\frac{3}{4}$ -in. column packed with 20% Carbowax 20M on 60-80-mesh acid-washed Chromosorb W and with a dual thermal-conductivity detector. Injection temperature was 270°C, detector temperature was 280°C, and the temperatures of the columns were programmed from 90 to 240°C and then held at 240°C until the last peak was obtained (about 65 min). Flow rates were 100 ml helium per min. Chromatograms of Figs. 3 and 4 were obtained with an F and M Model 720. Con-

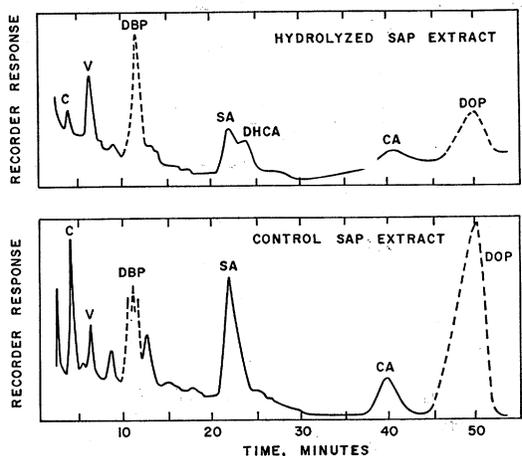


Fig. 1. Gas chromatograms showing the effect of alkaline hydrolysis of chloroform extract of maple sap.

C—coumarin  
 V—vanillin  
 DBP—dibutyl phthalate  
 DHCA—dihydroconiferyl alcohol  
 CA—coniferylaldehyde  
 DOP—dioctyl phthalate  
 SA—syringaldehyde

ditions were the same as for the Aerograph A-350 except that injection temperature was 150°C, flow rate was 50 ml per min, and column length was 6 ft.

**Oxidation of the sap extract.** The identification of ligneous material in maple sap suggested at once that it could be the precursor of aromatic compounds in maple sirup. Since the alkaline nitrobenzene oxidation reaction has been used by

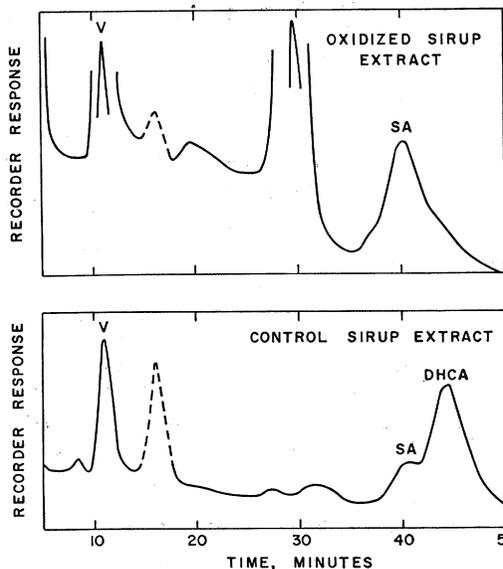
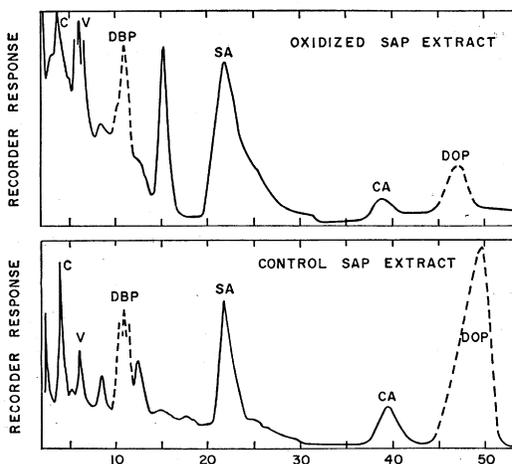
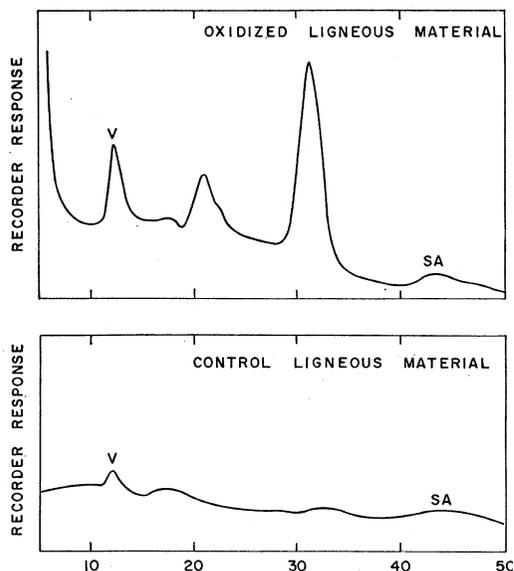


Fig. 3. Gas chromatograms to show the effect of nitrobenzene oxidation of a chloroform extract of maple sirup. Peak designations same as Fig. 1.

many people to evaluate wood lignins according to the breakdown products obtained, this treatment was applied to the sap extract. The micro procedure of Stone and Blundell (1951) was used. To 5 ml of the  $\text{CHCl}_3$  extract of sap in the nickel chamber of a Parr bomb was added 2 ml of 2N



NaOH and the mixture heated on a steam bath with stirring to remove the chloroform. Then 0.1 ml of nitrobenzene was added, the bomb was closed, and the mixture was heated 3 hr in an oven at 160°C. After the bomb was allowed to cool, the contents were transferred to a beaker using 5 ml of 2*N* HCl and enough additional acid to make the mixture acidic. The mixture was then extracted with two 10-ml portions of CHCl<sub>3</sub> and the combined CHCl<sub>3</sub> extracts evaporated at room temperature to 1 ml. Fig. 2 shows the gas chromatograms of the original CHCl<sub>3</sub> sap extract and the nitrobenzene-oxidized extract.

**Oxidation of the chloroform extract of sirup.** When maple sirup made by the usual commercial process is further heat-treated (Porter *et al.*, 1952) the flavor is increased. This would indicate that not all of the precursors of maple flavor are converted during the normal sap evaporating process. To determine whether any of the unreacted flavor precursors in sirup are extracted by chloroform, an aliquot of the sirup chloroform extract was oxidized by the previously described nitrobenzene oxidation procedure. Gas chromatograms of the oxidized and untreated sirup extracts are shown in Fig. 3.

**Oxidation of the ligneous material in sap.** Ten ml of the CHCl<sub>3</sub> extract of sap were transferred to a centrifuge tube and 40 ml of diethyl ether added. The precipitate formed was separated by centrifugation and washed twice with diethyl ether. The precipitate (ligneous material) was dissolved in 15 ml of chloroform, and this was evaporated to 10 ml to adjust the concentration of the lignin to that in the original CHCl<sub>3</sub> extract. Five ml of the lignin solution was subjected to nitrobenzene oxidation. Fig. 4 compares gas chromatograms of the oxidized and unoxidized lignin.

## RESULTS AND DISCUSSION

When maple sirup is extracted with chloroform the resulting marc (extracted sirup) is devoid of maple flavor. However, heat treatment of the marc according to the high-flavoring process of Willits and Porter (1950) or by autoclaving at 15 lb pressure will produce more flavor. This indicates that CHCl<sub>3</sub> does not remove all of the precursors of maple flavor in maple sap and sirup. This should be kept in mind as the results of these studies are discussed.

No single chemical compound has yet been identified which will give maple flavor, as has been done with benzaldehyde of almond flavor or the anthranilates of grape flavor.

Instead, current research tends to indicate that maple flavor is a composite of at least several flavorful compounds. In previous papers the authors have shown that the chloroform extract of maple sirup (flavor fraction) contains a number of such compounds.

The higher-boiling compounds in gas chromatographic fractionation of the flavor extract include vanillin, syringaldehyde, and dihydroconiferyl alcohol. In preliminary studies on the precursors of these and other possible aromatic compounds as yet unidentified, a number of compounds have been separated from sap and identified as coumarin, vanillin, coniferylaldehyde, syringaldehyde, and lignin (Filipic and Underwood, 1964). These could be the precursors of the aromatics and/or other flavor compounds of maple sirup. Even though vanillin and syringaldehyde were found in trace amounts in sap, the 30-fold concentration resulting from evaporation of the sap to sirup could account only in part for the appreciable amounts found in sirup. It is also possible that the traces of these compounds found in sap result from interactions which occur when the chloroform extract is passed through the gas chromatographic column. This source will be checked in future work. Therefore, the large amounts of vanillin and syringaldehyde in sirup can best be accounted for by other mechanisms, as described in the following paragraphs.

**Alkaline hydrolysis of the CHCl<sub>3</sub> extract of sap.** The effect of alkaline hydrolysis of the chloroform extract of sap is shown in Fig. 1. Only the portions of the chromatograms showing the peaks representing the higher-boiling compounds are shown. The more volatile, lower-boiling components are not yet identified and not considered in this paper. Also, the chromatograms show a few peaks that have not been identified. The peaks for dibutyl phthalate and dioctyl phthalate, identified by dotted lines, are artifacts since they seem to occur in the chloroform extract as impurities picked up from the plastic equipment used in extraction and concentration. Hydrolysis of the extract caused some decrease in the amounts of coumarin and coniferylaldehyde, with a

marked decrease in syringaldehyde. The vanillin content was unchanged, while the dihydroconiferyl alcohol shows a pronounced increase. These hydrolytic-induced changes could and likely do occur in maple sap as it is being concentrated by boiling at atmospheric pressure to sirup. The sap becomes quite alkaline, rising to pH 8-9 during the early stages of the boiling period and then dropping slowly to about neutrality (pH 6.8-7.2) as the boiling process continues.

**Nitrobenzene oxidation of the sap extract.** The nitrobenzene oxidation is carried out in alkaline solution producing double action (hydrolysis and oxidation) on the extract. The chromatograms in Fig. 2 again show the hydrolytic action of the alkali by causing a decrease in the ester and coumarin content of the extract. The other action, the oxidative effect of the nitrobenzene, caused a significant increase in the amount of syringaldehyde and a *very pronounced* increase in vanillin. The compound represented by the large peak between the dibutyl phthalate and syringaldehyde is formed by the oxidation treatment. It may be an artifact, but this will be established by further work.

**Nitrobenzene oxidation of the ligneous material in maple sap.** Finding a lignin-like material in maple sap introduced the possibility that this might be the source of the precursor of some of the aromatic compounds in maple sirup. Therefore, this material was subjected to nitrobenzene oxidation. The chromatograms in Fig. 4 show that a significant amount of vanillin is formed by oxidation of the lignin in maple sap, but that only a trace of syringaldehyde is so produced. Also, the chromatograms of the oxidized sap lignin show the formation of several compounds yet to be identified.

**Nitrobenzene oxidation of a chloroform extract of maple sirup.** Fig. 3 shows the products obtained from nitrobenzene oxidation of the chloroform extract of maple sirup. Here, as with the sap extract, the nitrobenzene oxidation caused a pronounced increase in vanillin, with a lesser, though significant, increase in syringaldehyde and a decrease in dihydroconiferyl alcohol. This formation

of vanillin explains, in part at least, the development of additional maple flavor by controlled heating of maple sirup.

From these studies the vanillin found in the sirup can come from several constituents of the sap, first by the concentration of the trace amount of vanillin in the sap, and secondly, by oxidation of the coniferyl aldehyde, the ether-insoluble ligneous material, and/or the dihydroxyconiferyl alcohol formed during the early boiling of the maple sap when it is definitely alkaline.

The syringaldehyde in the sirup appears to be formed by an oxidative reaction from the ether-soluble fraction of the chloroform extract of maple sap. Fig. 1 shows that the peak representing syringaldehyde decreased when the  $\text{CHCl}_3$  extract was refluxed with 1N NaOH, and Fig. 2 shows that syringaldehyde increased when the extract was oxidized with nitrobenzene.

The dihydroconiferyl alcohol was produced by a hydrolytic reaction and, once formed, was susceptible to oxidation, as illustrated in Figs. 1 and 3.

These data indicate that the major aromatic compounds identified in chloroform extracts of maple sirup can be formed from compounds in maple sap by hydrolytic and oxidative reaction mechanisms. However, these aromatic compounds constitute only a part of the total maple flavor; no one of these has all of the characteristics of the flavor known as maple. Such a compound may be discovered among the lower-boiling components of the chloroform extract, or this specific maple flavor compound might be altered by chloroform extraction and have to be isolated by some other technique.

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