

## The Identification of Methylcyclopentenolone and Other Compounds in Maple Sirup Flavor Extract

### SUMMARY

A number of compounds in the flavor-containing chloroform extract of maple sirup have been isolated and identified. A major constituent not previously isolated by gas chromatography was acetol. Other constituents present in lesser concentrations which have not previously been reported were acetoin, ethyl vanillate, syringoyl methyl ketone, and methylcyclopentenolone.

In a previous study utilizing gas chromatography (Underwood and Filipic, 1963) the authors reported the presence of vanillin, syringaldehyde, and dihydroconiferyl alcohol in a chloroform flavor extract of maple sirup. Since these compounds were the major fractions separated by the gas chromatographic techniques used, sufficient materials for infrared identification were easily obtained. By using a much larger amount of the chloroform extract for gas chromatography, quantities of several other compounds sufficient for identification were obtained. One of these was identified as methylcyclopentenolone, which is reported to impart a maple flavor to simple sirup (Dow Chemical Company, 1956). This paper is a report on the components identified in the chloroform extract and of the methods by which they were isolated and characterized.

### EXPERIMENTAL PROCEDURE AND RESULTS

Twenty gallons of commercial maple sirup, U. S. Grade A, were extracted twice with 20-gallon volumes of chloroform according to the procedure described by Underwood *et al.* (1961). The combined extracts were concentrated to 200 ml by evaporation at atmospheric pressure. This concentrate was then shaken with 800 ml of ether to precipitate the ligneous material. The supernatant liquid was concentrated to 40 ml, thus resulting in a 2,000:1 concentration of the chloroform-ether-soluble components in the maple sirup.

A 500- $\mu$ l portion of the final concentrate was injected into an F & M chromatograph, model 720, using the instrumental conditions shown in Table 1. The chromatogram obtained is shown in Fig. 1. Portions of this chromatogram are shown in greater detail in Figs. 2 and 3. Except for the chloroform solvent, the entire eluate from 70 such injections was collected in 24 traps inserted in the exit port at the retention times indicated by the dashed lines in Figs. 2 and 3. The traps were 4-mm OD glass U tubes immersed in a dry ice-acetone bath.

The decision as to the retention time at which the collection of a new fraction should begin was based on the consideration that all fractions would be rechromatographed. Therefore, eluates showing incompletely resolved peaks were collected in one trap for greater efficiency. For the same reason, eluates represented by a succession of peaks of low

Table 1. Gas chromatographic operating conditions.

Columns	Dual, stainless steel, 1/4-inch OD, 4 ft long
Packing	20% Carbowax 20M on 60-80-mesh acid-washed Chromosorb W
Detector	Thermal conductivity
Temperatures	Injector port, 145°C. Column Oven, 4 min at 50°C. 56 min from 50°C to 240°C (approx. 3.5°/min) 40 min at 250°C
	Detector 250°C
Flow rate	50 ml He/min
Attenuation	4X

intensity were also collected in another single trap. Since the individual components of the higher-boiling fractions had been efficiently condensed in the past, they were collected in individual traps, no matter how small the peaks representing them. Since the highest-boiling fractions had been identified previously, all materials eluting at temperatures above those for vanillin were collected in one trap.

The eluate collected in each trap was removed by washing with ether and chloroform. The first seven of the resulting 24 solutions were then rechromatographed on the same Carbowax column to retain the better resolution afforded by a polar substrate for the oxygenated materials present in maple sirup extract. The remaining 17 fractions were rechromatographed on an SE-30 column to reduce the contamination of these higher-boiling eluates with column bleed. The various components of all fractions were collected in  $2 \times 100$ -mm capillary tubes cooled with powdered dry ice. As soon as each eluate was obtained, the tubes were sealed with a micro torch.

**Identifications.** Each identification of the component collected was based on comparison of the infrared spectra and of retention times on both 20M and SE-30 columns with standards. The IR spectra of the most volatile compounds were obtained in sealed micro cells, those of intermediate volatility in micro mull plates, and the remainder in micro KBr discs. The discs were prepared by rinsing the contents of the capillary collection tube onto the surface of a micro agate mortar. After evaporation of the chloroform solvent, the thin film of solute was taken up in 15 mg of powdered KBr by grinding for about 2 min. Pellets of 3 mm diameter were made with a Beckman No. 16585 die. These pellets were placed in a Perkin-Elmer  $4\times$  beam condenser mounted in a Beckman IR-8 spectrophotometer. To obtain ultraviolet spectra, the discs were simply dissolved in sufficient water.

Since the first 11 fractions were liquid, sealed cells were used to obtain the spectra of the isolated components. The very large peak of fraction 1 was due to the ether added to the chloroform concentrate. Fraction 2 was identified as ethyl acetate, and fraction 3 as ethyl alcohol. The eluate represented by the large peak between fractions 3 and 4 was due to the chloroform solvent and was not collected. Fraction 4 was water. None of the components in fraction 5 were identified.

There was a shoulder on the ascending slope of the major peak in fraction 6 that was more clearly evident on rechromatographing, as shown between the dashed lines in Fig. 4. This shoulder, fraction 6B, was shown to result from the presence of acetoin. The main constituent, fraction 6C, was shown to be acetol. The infrared spectrum of the

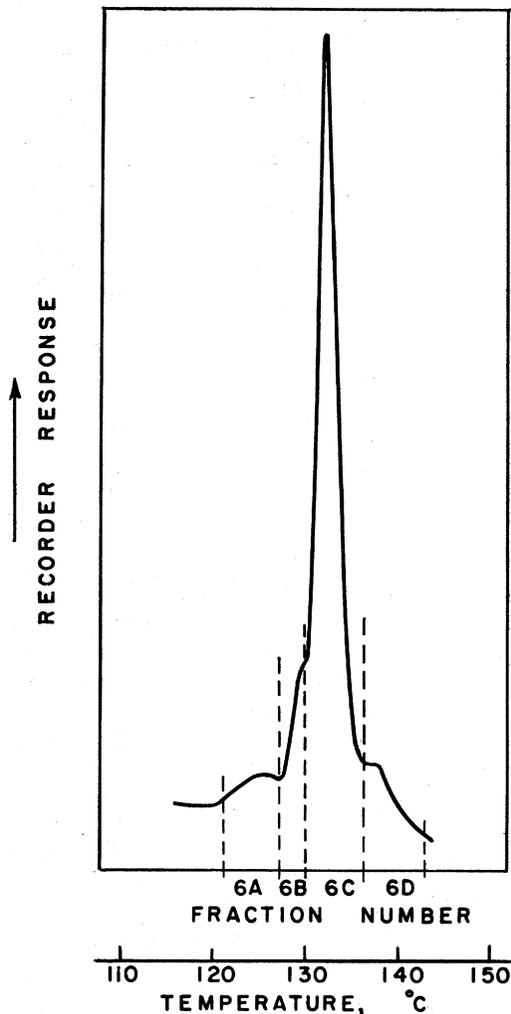


Fig. 4. Rechromatography of fraction 6 on Carbowax 20M.

sample (fraction 6C) is compared with that of acetol in Fig. 6. In this case sufficient material was available to obtain a nuclear magnetic resonance spectrum using a micro cell. This spectrum (Fig. 7) is consistent with the structure of acetol. It has peaks resulting from methyl, hydroxyl, and methylene protons in the peak area ratio of 3:1:2. The lack of coupling indicates that protons are not present on adjacent carbon atoms.

The material recovered in fractions 7 through 11 was insufficient to permit identification. The remaining fractions (12-24) consisted of materials so low in volatility that KBr discs were used to obtain their infrared spectra.

Fraction 12 yielded a crystalline material as the major component on rechromatography. In the process of making a micro KBr disc from it a dis-

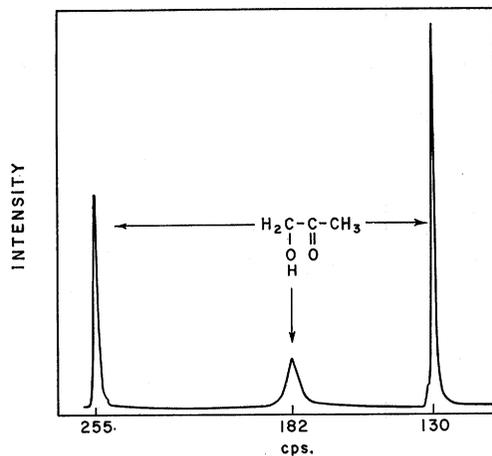


Fig. 7. The nuclear magnetic resonance spectrum of fraction 6C.

final peak, which eluted above 200°C, is due to dihydroconiferyl alcohol. The presence of high-boiling constituents in this fraction probably resulted from a cold spot in the discharge end of the detector, causing condensation of eluates in the exit port. Then, as the temperature of the carrier gas increased during programming, these components would volatilize, causing all subsequent fractions to contain materials of a wide boiling range. They were, however, richer in those components which were eluting from the column at that time.

Fraction 15 was indicated by its infrared spectrum to be a long-chain fatty acid (about C<sub>8</sub>), and the infrared spectrum of 16 closely resembled that of the glyceride, diacetin. In fractions 17 through 22 the ratio of component to contaminants even after being rechromatographed was so low that identification by infrared was not possible. This contamination was probably a combination of column bleed and other decomposition products.

Fraction 23 contained vanillin, and the last two peaks in fraction 24 represent syringaldehyde and dihydroconiferyl alcohol, all compounds previously identified.

Fraction 24 was rechromatographed at a much higher injection temperature (280°C) to completely volatilize the higher-boiling components. Based on infrared spectra, guaiacol, 2,6-dimethoxy phenol, ethyl vanillate, and syringoyl methyl ketone were identified. Identification of the syringoyl methyl ketone, a dicarbonyl of yellow color, was based on a comparison with the spectrum given by Pearl (1959). The maximum column temperature was not maintained long enough to elute this diketone in a single chromatogram. Its presence was detected only because of the long series of sequential runs.

Identifications of the compounds in the different eluted fractions are summarized in Table 2.

## DISCUSSION

Three of the major constituents of the concentrated chloroform extract of maple sirup (vanillin, syringaldehyde, and dihydroconiferyl alcohol) have been identified previously. Underwood *et al.* (1961) found vanillin and syringaldehyde in column chromatography. In 1963, using gas chromatography, they found dihydroconiferyl alcohol. Acetol, which was tentatively identified by Underwood *et al.* (1956) in the form of a dinitrophenylhydrazine derivative, was not observed in the gas chromatographic procedure used in 1961, no doubt because it decomposes at 146°C and was destroyed by the high injection temperatures then used. This current study, using lower injection temperature, resulted in identification of

Table 2. Compounds identified in GLC studies.

Fraction No. <sup>a</sup>	Compounds <sup>b</sup>
1	Ether
2	Ethyl acetate
3	Ethyl alcohol
4	Water
6B	Acetoin
6C	Acetol
12	Methylcyclopentenolone
13	Butylated hydroxytoluene (BHT)
14	Phenol
15	A long-chain fatty acid
16	Diacetin
23	Vanillin
24	Ethyl vanillate
	Syringaldehyde
	Dihydroconiferyl alcohol
	Syringoyl methyl ketone
	Guaiacol <sup>c</sup>
	2,6-dimethoxyphenol <sup>c</sup>

<sup>a</sup> From original chromatogram on 20M column.

<sup>b</sup> Compounds were identified after rechromatography of each fraction by infrared spectrophotometry and comparison of retention times on Carbowax 20M and SE-30. Other means of identification were ultraviolet spectrophotometry for methylcyclopentenolone and phenol, mass spectrometry for butylated hydroxytoluene, and nuclear magnetic resonance spectrometry for acetol.

<sup>c</sup> Probable degradation products resulting from use of a higher injection temperature. Retention times indicate that these compounds should have been isolated in fractions 13 to 16 if they were initially present in the extract.

characteristic flavor of maple sirup, is 3-methylcyclopent-2-en-2-ol-1-one. Fig. 10 shows the three possible tautomeric forms of this compound. Bredenberg (1959) has shown that this compound occurs as structure 1, both in the solid state and in solution. Methylcyclopentenolone is sold commercially under the trademark "Cyclotene." It is used in compounding flavors and is said to improve the taste of maple, walnut, or pecan mixtures.

Methylcyclopentenolone can be formed by boiling wood at 100°C in caustic solution (Enkvist, 1954; Linberg and Enkvist, 1953) and also by refluxing glucose and acetone in an aqueous alkaline medium (Fray, 1961). Maple sap is a dilute sugar solution containing among its organic constituents small amounts of soluble lignins. As the sap is converted into sirup by boiling, the solution passes through an alkaline phase (Hayward and Pederson, 1946; Willits *et al.*, 1952), being at a maximum pH of about 9 for some time. Thus, conditions exist under which this cyclic ketol can be formed.

The presence of acetoin and ethyl alcohol also raised the possibility that a fermentation may have occurred in the sap from which this sirup was made. Syringoyl methyl ketone can also arise from the ethanolysis or fermentation of lignin (Brauns and Brauns, 1960).

The ethyl alcohol, in turn, could account for the presence of the esters ethyl acetate and ethyl vanillate, since maple sirup is known to contain acetic acid (Nelson, 1928) and vanillic acid (Risi and Labrie, 1935). On the other hand, hydrolysis of such esters could account for the presence of ethyl alcohol and the acids. Some of the compounds isolated—ethyl acetate, ethyl alcohol, and phenol—are ubiquitous materials. These three components could be contaminants or

#### METHYLCYCLOPENTENOLONE

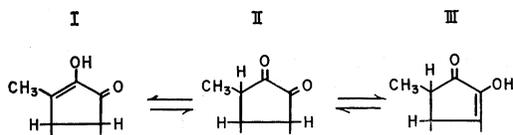


Fig. 10. Possible tautomeric forms of methylcyclopentenolone.

artifacts introduced during the processing of the maple sirup or in the analytical procedures.

The compounds guaiacol, 2,6-dimethoxyphenol, and syringoyl methyl ketone were isolated from a single analysis made on the SE-30 column in which the injection temperature was raised to 280°C (compared to 145°C, used previously). It is quite possible that they are the products of thermal degradation of higher-molecular-weight materials. Guaiacol, however, has been previously reported as a constituent of maple sirup (Risi and Labrie, 1935). It, as well as vanillin and syringaldehyde, could result from the alkaline hydrolysis of lignin (Brauns and Brauns, 1960).

Commercial fats and oils are frequently used by maple sirup producers as antifoaming agents. Isolation of the fatty acid, glyceride, and antioxidant (BHT) is clear evidence that such an agent was present in the batch of maple sirup analyzed in this study. The ability to isolate and identify a trace constituent, the antioxidant, added in trace amounts to the sirup as an antifoaming agent, illustrates the sensitivity that can be obtained in combining gas chromatography with infrared spectrophotometry.

#### REFERENCES

- Brauns, F. E., and D. A. Brauns. 1960. The chemistry of lignin. Supplement Volume. Academic Press, New York and London, pp. 460, 719 and 392.
- Bredenberg, J. B. 1959. The enol structure of 3-methylcyclopentane-1,2-dione. *Acta Chem. Scand.* **13**, 1733.
- Dow Chemical Company. 1956. Information sheet on Cyclotene.
- Enkvist, T. 1954. Formation of methylcyclopentenolone by digestion of spruce wood or galactose with sodium hydroxide solutions at 100°C. *Acta Chem. Scand.* **8**, 51.
- Fray, G. I. 1961. The formation of 3 (or 5)-methylcyclopent-2-en-2-ol-1-one from acetone. *Tetrahedron* **14**, 161.
- Gianturco, M. A., A. S. Giammarino, and R. G. Pitcher. 1963. The structure of five cyclic diketones isolated from coffee. *Tetrahedron* **19**, 2051.
- Hayward, F. W., and C. S. Pederson. 1946. Some factors causing dark-colored maple sirup. *N. Y. State Agr. Expt. Sta., Geneva, Bull.* **718**.