Analysis of Whole Beeswax by Gas Liquid Chromatography[1]

ABSTRACT

Gas liquid chromatographic analysis of unfractionated beeswax (treated with diazomethane) using Dexsil 300 as liquid phase gives a good separation of hydrocarbons, free fatty acids as methyl esters, and long chain monoesters. These components can be estimated using internal standards and together account for ca. 65% of the wax. Petroleum hydrocarbon or fatty acid adulterants can be detected. Unsaturated hydrocarbons are partially resolved from the corresponding saturated compounds.

Beeswax, which consists mainly of mono-, di-, poly- and hydroxy esters, free acids and hydrocarbons, has been fractionated by column chromatography and the individual fractions analyzed by gas liquid chromatography (GLC) (1-4); but a more rapid method was needed to compare large numbers of samples. Detection and estimation of adulterants such as petroleum hydrocarbons and stearic acid would also be useful.

Previously (A.P. Tulloch, unpublished work) beeswax was treated with diazomethane to convert free fatty acids to methyl esters and analyzed by GLC using a silicone SE-30 column, but the methyl esters were not separated from hydrocarbons with three more carbon atoms. It has now been found that GLC analysis using the carboranesiloxane Dexsil 300 (Analabs Inc., North Haven, Conn.) as liquid phase gives a good separation of hydrocarbons, methyl esters and long chain monoesters. A typical separation is illustrated in Figure 1, the beeswax used was part of the same sample previously analyzed by column fractionation (2). The gas chromatograph was a Hewlett-Packard model 402 with flame ionization detectors; columns were 3 ft x 1/8 in. stainless steel packed with 60-80 mesh, acid-washed and silanized, Chromosorb W coated with 1.5% Dexsil 300. Temperature was programed from 125 to 375 C at 3 C/min, and the flow rate was 60 ml helium per minute.

In Figure 1 the very small peak between C_{22} methyl ester and C_{27} hydrocarbon is presumably due to C_{26} hydrocarbon. The separation is such that any unusual increase in the amount of even numbered hydrocarbons, indicating the presence of petroleum adulterants, would be readily observed. It is also of interest that saturated and unsaturated hydrocarbons are partly separated. Comparison of Figure 1 with GLC analysis of the same wax after hydrogenation (in ethyl acetate over 5% Pd on charcoal) showed that the earlier eluted shoulder of the C_{31} hydrocarbon peak is the unsaturated component, the small, later eluted shoulder of the C_{33} peak is the saturated component, and the small C_{35} peak, between C_{30} and C_{32} methyl ester peaks, is due to unsaturated hydrocarbon. The shorter retention times, usual for unsaturated components on nonpolar columns, agree with these designations. Calcu-

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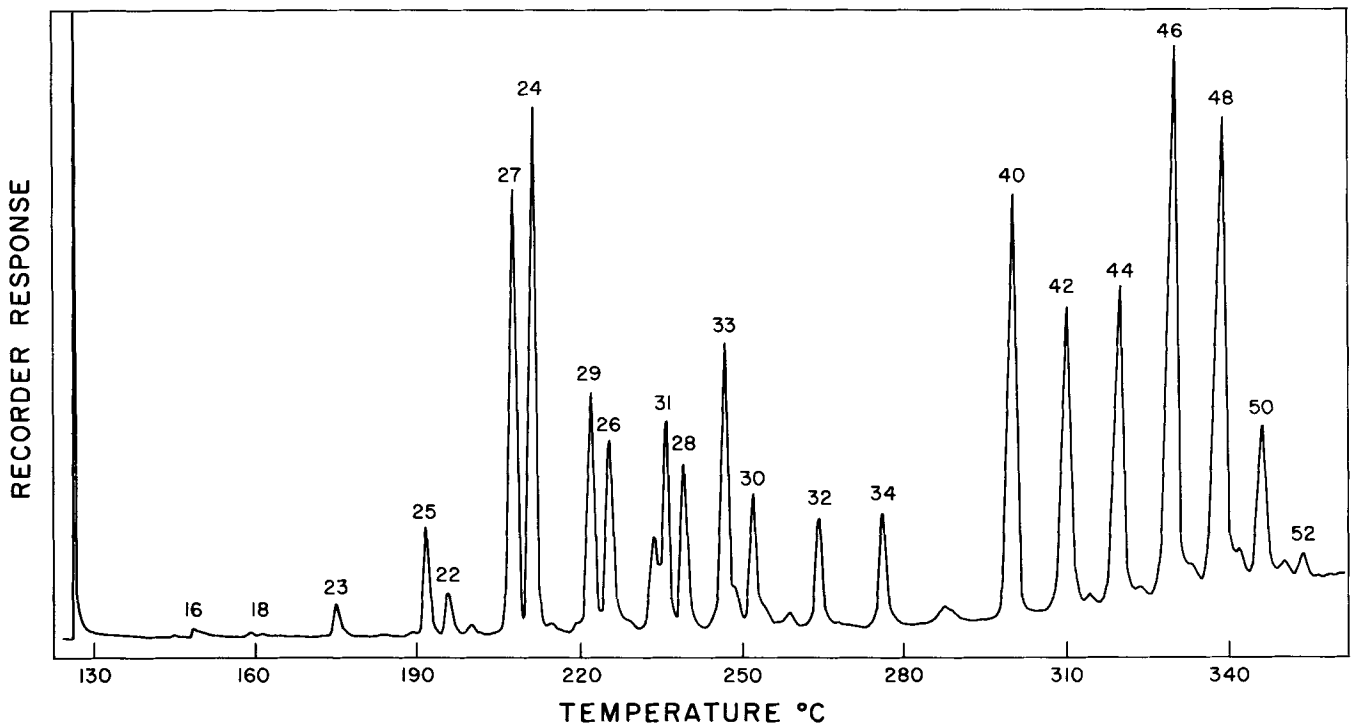

FIG. 1. Gas liquid chromatographic separation of a typical sample of Canadian beeswax after treatment with diazomethane. Peaks with even carbon numbers from 16-34 are fatty acid methyl esters, peaks with odd numbers from 23-33 are hydrocarbons and peaks with even numbers from 40-52 are long chain monoesters.

lations from Figure 1 show that 30% of the hydrocarbons are unsaturated, and the composition of the unsaturated portion is 24% C_{31}, 73% C_{33} and 3% C_{35}. Streibel et al. (1) have reported 32% unsaturated hydrocarbons in Czechoslovakian beeswax with major components 2% C_{29}, 26% C_{31}, 60% C_{33} and 2% C_{35}.

The composition of free acid methyl esters, calculated from Figure 1, is similar to that found earlier (2). In particular, as can be seen easily, the amounts of C_{16} and C_{18} acids are very small. Analysis using a large sample showed that the small peak between C_{34} methyl ester and C_{40} monoester has two partly resolved components, C_{36} methyl ester and C_{38} monoester in the approximate ratio 1:2. During column separation (2), the free acids are spread over a number of fractions so that some could be lost or the shortest chain acids could be retained by the column; the present method avoids these difficulties. Addition of stearic acid to beeswax would be very easily detected and estimated, since extremely little is present naturally.

The composition of the monoesters agreed very closely with that found before (2). Hydroxy esters, diesters and acid monoesters (as methyl esters) did not seem to interfere but may cause the rise in baseline. These esters of secondary alcohols give a much poorer response than esters of primary alcohols (2).

By adding a mixture of three internal standards, eicosane, methyl eicosanoate and octadecyl eicosanoate and comparing their areas with the total areas of hydrocarbons, methyl esters and monoesters, respectively, the percentages of these three groups of components in beeswax were calculated. The results agreed well with those found earlier (2) (in parentheses): hydrocarbons, 13.2% (14.0%); free acids, 13.2% (11.9%); and monoesters, 37.5% (34.7%). It should be noted that, though an acid value can be calculated from the free acid composition, it is lower than that found (16.9 compared to 19.0) because of the presence of acid mono- and polyesters (2).

Thus the amounts in the wax and the composition of the three major groups of components, ca. 65% of whole beeswax, can be obtained from one GLC analysis. The method should be suitable for comparing the compositions of a large number of waxes of different origin and for detecting and estimating adulterants.

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Laundry Soil-Suspending Activity of Certain Microbial Polysaccharides[1]

ABSTRACT

Certain extracellular microbial anionic heteropolysaccharides and phosphorylated mannans suspend laundry soil comparably with carboxymethyl cellulose. These biodegradable biopolymers have other properties compatible with components commonly used in detergent formulations.

Efficient laundering of cotton fabrics by use of synthetic detergents requires additives that hold the soil in suspension or prevent its redeposition, or both (1). Sodium carboxymethyl cellulose (CMC) acts in both ways: it is the standard for comparison of soil-suspending activity and is the most widely used antiredeposition agent (1). Other hydrophilic colloids that serve one of these functions, but usually not both, have been cellulosic derivatives or synthetic polymers (1). These, like CMC, are nonbiodegradable.

We have found that certain microbial anionic heteropolysaccharides and phosphorylated mannans compare favorably with CMC in tests for soil-suspending activity both in the absence and the presence of an anionic surfactant, using a water-based substrate, Aquablak B. These biopolymers, the extracellular occurrence of which permits production on an industrial scale (2), are biodegradable and compatible with most commonly used components of detergent formulations.

The microbial polysaccharides tested are identified in Table I. They are products of pilot plant-scale preparations or are derived from these by further treatment in the laboratory and have a 98% or higher purity. Samples tested are from different preparations of the respective strains. Three samples of commercial CMC served as controls; they were designed for soap and detergent formulations and were used without correction for possible inert material. Each value shown is the average of six determinations.

Tests for soil-suspending power were made by a modified procedure based on the methods of Bayley and Weatherburn (6) and of Weatherburn et al. (7). The soil-suspending agent, 2.5 g, was dissolved in 1 liter of water of 300 ppm hardness using a high-sheer Brookfield counter rotating stirrer. Aquablak B, 0.15 g, weighed on a 2 in. x 2 in. sheet of Saran Wrap, was added to the contents of the 1 liter stainless steel beaker in a Terg-O-Tometer and stirred in the Terg-O-Tometer until the carbon was removed. Four unsoiled cotton swatches 2.5 in. x 3.25 in. (Test Fabrics, Inc.) were added, and the suspension was stirred (110 cpm) for 20 min at 60 C. The swatches were withdrawn, rinsed in tap water and ironed dry. Reflectance was measured with a Photovolt meter relative to MgO as 100, and the per cent soil-suspending power was calculated from the average reflectance of the swatches before and after testing, as follows:

$$\% SP = \frac{R_{after} - R_{blank}}{R_{unsoiled} - R_{blank}} \times 100$$

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