

Chromatographic Determination of Hydrocarbons in Beeswax

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The hydrocarbon content of beeswax is a potentially useful analytical tool. Values for hydrocarbon appear in the literature from about 1890 (1). Two general methods have been most used, those of A. and P. Buisine (5) and of Leys (4). The former converted alcohols in the unsaponifiable fraction to acids by heating with potash-lime; hydrocarbons were the remaining petroleum ether-soluble material. In the Leys procedure, the hydrocarbons in the unsaponifiable fraction are separated by their insolubility in hot fuming hydrochloric acid-amyl alcohol. Vizern and Guillot (9) have proposed extracting the dry saponified wax with petroleum ether, and then acetylating the extract to remove small amounts of higher alcohols. All of these methods require considerable manipulation.

A chromatographic determination of paraffin-type hydrocarbons in carnauba wax, using alumina, is described by the ASTM (7). Curylo and Zalewski (3) have reported the analyses of 24 samples of beeswax from Poland, using chromatography on silica gel. Rosenberg (8) has described a chromatographic procedure for hydrocarbon content of waxes; he also used silica gel.

Newburger (6) described the use of alumina columns for separating hydrocarbons from cosmetic mixtures of beeswax and spermaceti. All material from a saponified mixture except hydrocarbon was found to be retained on the column. Using this work as a basis, Bruening (2) developed a quantitative procedure for determining hydrocarbon in cosmetic creams. He noted that it was not necessary to saponify beeswax before adsorption.

We have applied this procedure to the analysis of beeswax and mixtures of beeswax and microcrystalline wax. The volumes of solvents had to be modified slightly to obtain consistent hydrocarbon values for the micro-

crystalline waxes and mixtures. The method has been applied to the determination of hydrocarbon content of 60 samples of authentic beeswax and 20 samples of commercial microcrystalline wax. The complete results and analytical constants of the beeswax appear elsewhere (11).

METHOD

Materials

Alumina.—Alorco¹ Activated Alumina, Grade F-20, 80-200 mesh (Aluminum Company of America). Heat in an open pan in an oven at 190-200°C for 15 hours, cool somewhat in air, and store in closed containers.

Petroleum ether.—ACS grade, boiling range 30-65°C. Redistill if any interfering residue is left on evaporation.

Column.—A glass column, 16 mm o.d. × 300 mm with a 200 ml bulb on top and a Teflon stopcock on the lower end.

Pack the column as described by Bruening: place a pledget of cotton in the bottom and fill the tube with petroleum ether. Add the alumina to form a column 175 mm high, after light packing with about 5 pounds air pressure.

Procedure

Place 0.75 g sample (weighed to 0.1 mg) of beeswax (or sufficient sample to provide 100-125 mg hydrocarbon) in a 400 ml beaker, and melt on a steam bath. Add 250 ml petroleum ether, mark solvent level on outside, cover, and boil until wax is completely dissolved. Make volume to the 250 ml line, cool, and transfer the solution to the column. Regulate flow rate (gravity) to the fastest dropwise rate, collecting in a 400 ml beaker. Periodically transfer eluate to a weighed 50 ml beaker containing 6 glass beads and begin evaporating it on the steam bath. Do not allow beaker to cool after evaporation begins. After the sample is adsorbed, wash all residues into the column with 100 ml petroleum ether, then wash the column with 150 ml solvent (total

* Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

¹ Mention of trade names in this paper does not imply endorsement by the U.S. Department of Agriculture over similar products not named.

volume 5(9) ml). Evaporate all eluate to dryness, place it under a heat lamp for 5 minutes, cool for 15 minutes, and weigh. Weight of residue divided by sample weight gives hydrocarbon content.

Freezing Point of Isolated Hydrocarbon.—

This is most conveniently determined in a capillary. Capillary methods described in the literature for melting or freezing points of waxes (10) use open tubes, U-tubes, or closed tubes. Difficulty may be encountered when the tube, attached to a thermometer and immersed in a water bath, is heated. Frequently the sample may be displaced from the closed tube or may move upward in the open tube near the melting point. This point of movement is not reproducible, is not the melting point, and depends on the depth of immersion of the capillary and the depth of wax in the tube. Introduction of wax into the bottom of a closed tube is difficult. To eliminate these objections, let 3–5 mm of wax solidify in an open capillary and fill the remaining volume with water. (Proper positioning of the relative water levels in the tube and bath will allow the sample to remain in the tube after melting.) Fix the capillary to a thermometer graduated in 0.2° C divisions and immerse in a water-filled 25 × 110 mm test tube, then in turn immerse the test tube in a 1 L beaker filled with water. Stir the water by a slow air stream. Judge the freezing point of the hydro-

Table 1. Reproducibility of chromatographic determination of beeswax hydrocarbon^a

Sample	Hydrocarbon	
	Amount in Beeswax, %	Freezing Point ^b , °C
45	13.68, 13.67	55.4, 55.1
47	15.31, 15.31	55.1, 55.1
48	14.32, 14.33	55.2, 54.8
49	13.64, 13.63	56.0, 55.8
50	14.80, 14.74	55.7, 55.4
51	14.30, 14.30	55.3, 55.2
52	14.60, 14.65	55.6, 55.3
53	14.11, 14.09	55.4, 55.3
54	15.48, 15.46	55.7, 54.6
55	13.73, 13.74	55.5, 55.0
57	13.67, 13.63	55.0, 55.0
58	15.02, 15.02	54.7, 54.5
59	14.04, 14.04	55.2, 55.2
61	14.92, 14.92	54.2, 53.9
62	15.46, 15.48	54.4, 54.3
s	0.016	0.17
C _v	0.11%	0.31%

^a Each value is a single determination.

^b These values are those of the individual samples.

Table 2. Determination of beeswax hydrocarbon by two analysts^a

Sample No.	Hydrocarbon, %		Freezing Point, °C	
	Analyst A	Analyst B	Analyst A	Analyst B
3	12.29	12.61	56.0	55.9
4	17.09	17.21	53.8	53.4
6	12.92	12.95	55.9	55.8
22	13.82	14.08	55.6	55.6
s	0.15		0.15	
C _v	1.06%		0.27%	

^a Each value is average of duplicates.

carbon to be the first appearance of cloud in the melted sample.

Results and Discussion

The reproducibility of the hydrocarbon values and of the freezing points of the isolated hydrocarbon may be seen in Table 1, where data from 15 consecutive determinations, in duplicate, are listed. The standard deviation of the hydrocarbon determination is 0.016% ($C_v = 0.11\%$) and for the freezing point determination is 0.17°C ($C_v = 0.31\%$). Table 2 shows the results obtained by two analysts for four samples run about six months apart. Here the standard deviation between analysts is 0.15% in hydrocarbon and 0.15°C in freezing point. Coefficients of variation are 1.06% and 0.27%.

Composition of the Isolated Hydrocarbon.

—An infrared spectrum showed no structure other than that due to long-chain aliphatic hydrocarbon. The iodine numbers of two samples of beeswax hydrocarbon were 17.6 for Sample 41 (old comb wax) and 22.7 for Sample 43 (cappings wax).

Lewkowitsch (4) has reported beeswax hydrocarbons to melt at 49.5–59.5°, with iodine number of 20–22. Vizern and Guillot (9) report values for 12 preparations of 18.7–28.1.

Gas Chromatography.—The hydrocarbon fraction from a sample of old comb wax was subjected to gas-liquid partition chromatography. The column was an 8 ft. length of stainless steel tubing, 0.180 i.d., packed with "Chromosorb" 42–60 mesh coated with diethylene glycol succinate-adipate polyester (20%); column temperature was 225°C, helium carrier gas flow 40 p.s.i.g. Sample size

Table 3. Aliphatic hydrocarbons in beeswax

N-Hydrocarbon	Amount Present, %	
	Hydrocarbon Basis	Whole Wax Basis
Below C ₁₉	trace	—
C ₁₉	0.26	0.035
C ₂₀	0.01	0.001
C ₂₁	0.63	0.086
C ₂₂	0.29	0.040
C ₂₃	3.78	0.516
C ₂₄	0.29	0.040
C ₂₅	11.6	1.58
C ₂₆	1.21	0.165
C ₂₇	40.7	5.56
C ₂₈	1.42	0.194
C ₂₉	25.2	3.44
C ₃₀	trace	—
C ₃₁	14.5	1.98
C ₃₃	trace	—

was approximately 1 microliter. Detector was a 4 filament thermal conductivity cell, current 200 milliamperes.

The results are given in Table 3. These data may be compared with the compilation given by Warth (10, p. 92), where the C₃₁ hydrocarbon is reported in the highest concentration. Gas chromatography was not used to determine the values cited by Warth. It is noteworthy that the even-numbered hydrocarbons are definitely present, though at far lower concentrations than those with an odd number of carbon atoms. The log of the relative retention times of all hydrocarbons (including C₂₀, C₂₆, C₂₈, C₃₂ added for identification purposes) fell on a straight line when plotted against chain length.

Summary

A rapid, reproducible procedure for the determination of hydrocarbon in beeswax and mixtures with other materials is described. Only hydrocarbons remain unadsorbed when a solution of beeswax in light

petroleum ether is passed through a column of activated alumina. The freezing point of the isolated hydrocarbon is a useful constant for beeswax analysis. The beeswax hydrocarbons were analyzed by gas chromatography.

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REFERENCES

- (1) *Allen's Commercial Organic Analysis*, 5th Ed., Vol. II, pp. 337, 339, Blakiston Co., Philadelphia, 1924.
- (2) Bruening, C. F., *This Journal*, **39**, 391 (1956).
- (3) Curylo, J. and Zalewski, W., *Pszczel. Zes. Nauk*, **1**, 105 (1957), reported in *Apicultural Absts.* 37/60 (*Bee World*, **41**, 27 (1960)).
- (4) Lewkowitsch, J., *Chemical Technology and Analysis of Oils, Fats, and Waxes*, 6th Ed., Vol. I, p. 615, 617, Macmillan and Co., London, 1922.
- (5) *Ibid.*, p. 619.
- (6) Newburger, S. H., *J. Soc. Cosmetic Chemists*, **1**, 95 (1948); *This Journal*, **31**, 670 (1948).
- (7) *1958 Book of ASTM Standards*, D-1342-57, 10, 1372, Am. Soc. Testing Materials, Philadelphia, 1958.
- (8) Rosenberg, G. F. von, *Fette, Seifen, Anstrichmittel*, **59**, 28 (1957).
- (9) Vizern and Guillot, *Chimie & Industrie*, Spec. No., 932 (April 1934).
- (10) Warth, A. H., *The Chemistry and Technology of Waxes*, 2nd Ed., p. 602, Reinhold Pub. Corp., New York, 1956.
- (11) White, J. W., Jr., Riethof, M. L., and Kushnir, I., *This Journal*, **43**, 781 (1960).