Factors Affecting Analytical Values of Beeswax and Detection of Adulteration[1]

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ABSTRACT

Various factors that could affect analytical values for beeswax, and so also detection of adulteration, have been investigated. Ester value determination was checked using synthetic monoesters. Gas liquid chromatographic analysis of overheated wax confirmed that free acids decreased on heating and also showed loss of unsaturated hydrocarbons and of monoesters. The saponification cloud point detected as little as 1% of a paraffin mp 83 C (chain length C_{20}-C_{60}) but only 6% or more of a paraffin mp 53 C (chain length C_{20}-C_{35}). Gas liquid chromatographic analysis of the hydrocarbon fraction of waxes containing these paraffins detected 1% of either paraffin, but only the low melting paraffin was estimated accurately. The presence of 2.5% of carnauba wax in beeswax was detected and estimated by gas liquid chromatography.

INTRODUCTION

The range of analytical values that characterize pure beeswax is now generally accepted (1), but various factors that might affect these values have not been studied extensively. Analytical values usually considered important for beeswax are acid value, ester value, ratio number and saponification cloud point. Determination of acid value has not been difficult, but conditions for ester value determination, particularly solvent and reaction time, have been investigated many times (1). Most investigators tried to find conditions that gave reproducible maximum values for a particular beeswax sample. No pure compounds with known ester values were used.

Prolonged heating of beeswax at 150 C or higher causes a decrease in acid value and an increase in ester value (2,3). Free acids presumably react with hydroxy mono- and polyesters, which form ca. 12% of the wax (4). The effect of relatively mild overheating, such as might occur in practice, was not studied. The saponification cloud point (5) is a reliable method of detecting paraffin adulteration in many cases (1,6), but the relationship between cloud point and paraffin composition was not examined.

This paper reports investigations of ester value determination using synthetic long chain esters, and the effect of mild heating on acid and ester value and also on the gas liquid chromatographic (GLC) composition. The detection of low and high melting paraffin in beeswax by saponification cloud point and by GLC is also described.

EXPERIMENTAL PROCEDURES

Acid and ester values were determined by the U.S.P. method for carnauba wax (7).

Synthesis of Long Chain Esters

Octadecyl octadecanoate, triacontyl hexadecanoate and triacontyl triacontanoate were prepared by esterification of the appropriate alcohol with the appropriate acid chloride (4). The C_{30} acid was synthesized as described by Hünig and Buysch (8); C_{30} alcohol was prepared by reduction of the ethyl ester of C_{30} acid with $LiAlH_4$ in tetrahydrofuran. The C_{46} ester, after crystallization from chloroform, had mp 73-74 C. $C_{46}H_{92}O_2$ calculated: C, 81.58; H, 13.69. Found: C, 81.61; H, 13.71. The C_{60} ester, after crystallization from butyl acetate, had mp 91-92 C. $C_{60}H_{120}O_2$ calculated: C, 82.49; H, 13.85. Found: C, 82.64; H, 14.20.

Heating of Beeswax

Beeswax (25 g) was heated in an open beaker (150 ml) in an electric oven. Saponification cloud point was determined by the U.S.P. method (9).

Paraffin mp 53 C was from a candle intended for a coffee pot heater. Paraffin mp 83 C was from a sheet of wax, pressed out like foundation comb, intended for home candle making. Melting points were determined by the open capillary method (10).

Hydrogenation of Beeswax

Beeswax (15 g) was hydrogenated over 5% palladium on 2 g charcoal in 150 ml ethyl acetate at 75 C and 2000 psi for 6 hr. The solution of product was filtered through a Seitz filter to remove traces of catalyst which might affect the cloud point. The cloud point was 63.0 C.

GLC analysis, using Dexsil 300 as liquid phase, was carried out as previously described (11).

RESULTS AND DISCUSSION

Determination of Ester Value

Many investigations of ester value were concerned with finding the most suitable solvent. Ethanol was the usual solvent and is still specified in many pharmacopeias (9).

TABLE I

Effect of Heat on Analytical Values and Composition of Beeswax

Time, hr	Temperature, C	Wt loss, %	Acid value	Ester value	Ratio no.	Hydrocarbons, %	Free acids, %	Long chain esters, %
0	0	0	19.1	73.5	3.84	14.6	14.7	36.3
24	100	0.09	17.7	75.1	4.24	14.7	14.1	37.3
90	100	0.16	16.3	76.0	4.66	15.6	13.7	38.1
170	100	0.28	16.2	77.6	4.79	15.1	11.3	37.4
3	150	0.16	17.4	74.5	4.29	13.4	14.0	36.8
24	150	3.70	11.2	87.9	7.88	8.5	6.0	17.3
1	200	1.51	16.1	76.7	4.76	11.4	12.8	37.7
3	200	4.92	12.4	83.0	6.70	9.7	9.0	32.0

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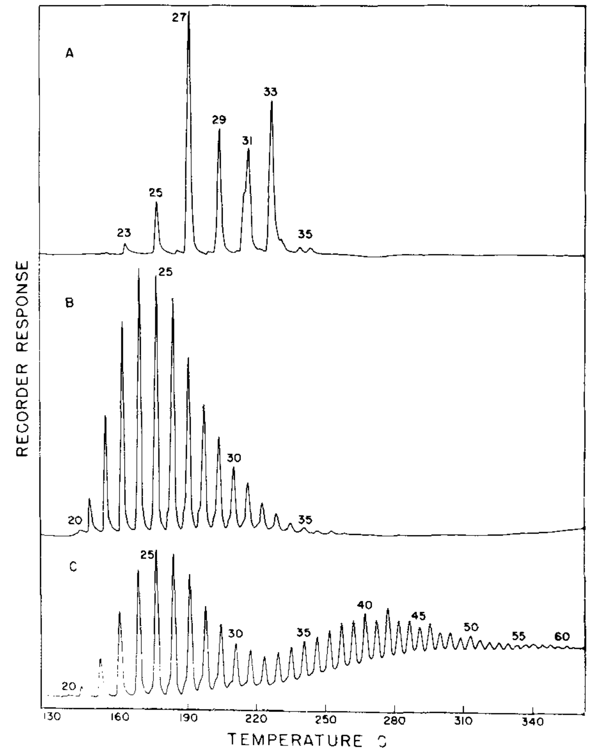

FIG. 1. Gas liquid chromatographic separation of: (A) pure beeswax hydrocarbons; (B) paraffin mp 53 C; and (C) paraffin mp 83 C.

Though saponification is probably complete, ethanol has the disadvantage that wax components precipitate during back titration of excess alkali, making end point determination difficult. Addition of xylene, first recommended to increase the rate of saponification of carnauba wax (12), gave a clear solution at the end point. A xylene-ethanol mixture was specified for ester value determination of beeswax in the German Pharmacopeia in 1926 (13). A toluene-isopropanol mixture is now specified for ester value of carnauba wax in U.S.P. XVIII (7) and for ester value of beeswax by American Wax Importers and Refiner's Association (14) and was the method used in this investigation.

Determination of ester values of pure synthetic C_{36}, C_{46} and C_{60} monoesters gave the following results, with theoretical values in parentheses: C_{36}, 103.4 (104.50); C_{46}, 81.9 (82.86); C_{60}, 65.0 (64.23). The agreement between found and theoretical values showed that the method was satisfactory. The C_{46} ester, triacontyl hexadecanoate, is a natural component of beeswax, forming ca. 27% of the monoesters or nearly 10% of the whole wax (4). As a further test of the method, two samples previously analyzed by White (1) using ethanol as solvent were satisfactorily reanalyzed: no. 1, 74.7 ([1] gives 75.2); and no. 59, 76.7 ([1] gives 75.3).

Effect of Heat on Analytical Values and Composition

TABLE II

Effect of Paraffin on Saponification Cloud Point of Beeswax

Wax, %		Saponification cloud point, C
Pure beeswax		62.6
+ Paraffin mp 53 C	2.5	62.6
	5.0	64.0
	6.0	66.2
	7.5	70.2
+ Paraffin mp 83 C	0.5	62.6
	1.0	71.2
	2.5	77.0

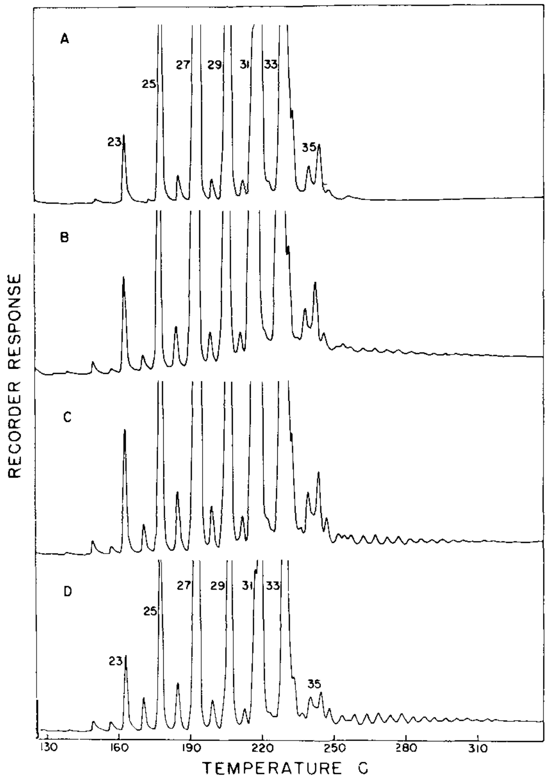

FIG. 2. Gas liquid chromatographic separation of: (A) beeswax hydrocarbons; (B) beeswax hydrocarbons containing 6% of paraffin mp 83 C; (C) beeswax hydrocarbons containing 14.8% of paraffin mp 83 C; and (D) beeswax hydrocarbons containing 28.5% of paraffin mp 83 C.

Results of heating beeswax at various temperatures and times are shown in Table I. Heating at 100 C for 24 hr causes measurable changes, the small increase in ester value and small decreases in acid value produce an amplified increase in ratio number. Further heating could produce wax with still acceptable acid and ester values but with ratio number above the specified value of 4.30 (14). Continued heating at 100 C caused further changes in the same direction, and GLC showed that after 168 hr the free acid percentage had fallen to 11.3, from 14.7. Heating at higher temperatures for short periods, 150 C for 3 hr and 200 C for 1 hr, caused relatively small changes in acid and ester values, which was confirmed by the GLC results. GLC also showed some decrease in hydrocarbon content due to breakdown of unsaturated hydrocarbons.

Heating at 200 C for 3 hr or 150 C for 24 hr caused a large decrease in hydrocarbon content (ca. 65% of unsaturated hydrocarbons had disappeared). These two sets of conditions had similar results showing that at the higher temperatures a much shorter heating time produced the same effect. In addition, after 24 hr at 150 C monoester content had fallen to half its original value. Part of the decrease in monoesters is probably due to polymerization of unsaturated esters (oleic acid formed 11% of acids of monoesters [4]). Interesterification with hydroxypolyesters might also have occurred, but if so an increase in free alcohols would be expected. However reanalysis after acetylation showed a free alcohol content of only 1%. Increase in ester value on heating is attributed to incorporation of free acids into di- and polyesters and not to formation of monoesters. Clearly beeswax underwent extensive chemical changes as a result of this treatment; physically the color changed from pale yellow to reddish brown and the wax became much more brittle. The results of heating at 150 C for 24 hr are similar to those obtained by Sibelien (2) by heating at 150 C for 48 hours; however the ester values did not show the large and unexpected changes reported by Buchner (3).

TABLE III

Gas Liquid Chromatographic Composition of Hydrocarbons in Whole Beeswax-Paraffin (mp 53 C) Mixtures and in Beeswax Hydrocarbon-Paraffin (mp 53 C) Mixtures

Chain length	Pure beeswax hydrocarbons	Whole wax +1% paraffin	Hydrocarbons +6.3% paraffin	Whole wax +3% paraffin	Hydrocarbons +18.1% paraffin	Whole wax +6% paraffin	Hydrocarbons +40% paraffin
21	0.1	0.4	0.3	0.5	0.5	0.8	0.9
22	---	0.3	0.3	0.6	1.0	1.5	2.4
23	1.8	2.5	2.3	3.1	3.4	4.4	5.8
24	---	1.2	0.9	2.1	2.6	4.0	5.9
25	6.5	7.2	7.1	7.6	8.1	8.9	10.1
26	0.6	0.9	1.4	1.7	2.8	2.1	5.7
27	31.4	27.5	29.6	26.3	27.9	24.1	22.7
28	0.4	0.7	0.7	1.3	1.4	2.4	3.0
29	18.4	16.2	17.2	15.2	16.2	14.0	13.1
30	0.3	0.3	0.5	0.8	0.8	1.5	1.5
31	20.5	19.8	19.5	18.9	17.7	17.0	13.5
32	---	---	---	---	---	---	0.9
33	18.4	22.0	18.7	21.0	16.4	18.6	13.6
35	1.6	1.0	1.5	0.9	1.2	0.7	0.9
Added paraffin[a]							
Calculated from gas liquid chromatographic results	---	6.9	7.4	12.6	16.1	22.4	38.3
Per cent paraffin in hydrocarbons of whole wax samples[b]	---	6.3	---	17.1	---	29.9	---

[a] Expressed as a percentage of hydrocarbons.

[b] Calculated by assuming pure beeswax contained 15% hydrocarbons.

Saponification Cloud Point Determination

This test, which specifies that the solution obtained by saponifying a 3 g sample of beeswax in 30 ml 0.5N ethanolic potassium hydroxide should not be cloudy above 65 C (9,14), depends basically on the poor solubility of long chain ($>C_{20}$) hydrocarbons in hot ethanol. Solubility decreases with increasing chain length so that sensitivity of the test will depend on composition of the paraffin adulterant. Two samples of paraffin in commercial use, mp 53 and 83 C, were selected to investigate this effect. GLC analysis of these in comparison with beeswax hydrocarbons is shown in Figure 1. The low melting paraffin contained only C_{20}-C_{35} hydrocarbons. These were also present in the high melting paraffin but in addition there were C_{36}-C_{60} components; the reasons for the baseline rise in the C_{35}-C_{45} region which was always observed with this paraffin are not clear. Hydrocarbon fractions from 10 adulterated beeswax samples, obtained from beeswax processors, all contained paraffin similar to the high melting type.

The saponification cloud points of mixtures of beeswax with small amounts of the two paraffins were determined. The results in Table II show that 6% of low melting paraffin was detectable, but 5% only raised the cloud point to 64 C and 2.5% had no effect. High melting paraffin, however, was much more easily detected; 1% raised the cloud point to 71.2 C and only 0.5% was not detected. Therefore the cloud point test will detect high melting paraffin satisfactorily but will not detect 5% or less of low melting paraffin. An ester value below 70 could indicate the presence of 5% of paraffin but only if the original ester value was not above 73.5. Pure beeswax can have values in the range of 70-72 (15), so that ester values in this range do not necessarily indicate that paraffin is present.

Since beeswax hydrocarbons contain very little even-numbered hydrocarbons (Fig. 1) (15-17), it should be possible to detect and estimate paraffin, especially low melting paraffin, in beeswax by GLC either of whole wax or of the isolated hydrocarbon fraction. GLC analysis of mixtures of beeswax hydrocarbons (obtained by column chromatography [4] with high melting paraffin) is shown in Figure 2. The mixtures contained 6, 14.8 and 28.5% paraffin and are what would be obtained by column chromatography of beeswaxes containing ca. 1, 2.5 and 5.6% paraffin (assuming beeswaxes to have an average natural hydrocarbon content of 15% [1,15]). Clearly 6% of this paraffin in beeswax hydrocarbons can be detected by the series of small peaks beyond C_{35}. Analysis of mixtures of high melting paraffin with whole beeswax did not give satisfactory results because much of the GLC response of the paraffin consisted of small peaks in the C_{35}-C_{60} region which were obscured by large beeswax ester peaks.

Figure 1 shows that, in the C_{20}-C_{35} region, composition of petroleum paraffin is symmetrical about the largest peak, whereas that of beeswax hydrocarbons is not. The principal even-numbered hydrocarbons of beeswax are C_{26} and C_{28}, adjacent to the major C_{27} peak (15,16). An approximate estimate of the percentage of paraffin in beeswax hydrocarbons can be made by making two assumptions: (a) Even-numbered hydrocarbons are all due to paraffin; and (b) the percentage of odd-numbered hydrocarbons due to paraffin can be calculated by interpolating figures between those of the two neighboring even-numbered hydrocarbons. When this method was applied to the above mixtures containing 14.8 and 28.5% paraffin, the apparent paraffin contents were 6.5 and 11.5%, respectively. The low figures are presumably due to underestimation of C_{35}-C_{60} components and show that, while high melting paraffin can be detected by GLC, it cannot be estimated accurately.

More quantitative results were obtained with mixtures containing low melting paraffin. Hydrocarbon composition and percentage of paraffin was estimated for mixtures with whole wax and with hydrocarbons only and gave the results in Table III. With hydrocarbon mixtures percentage of added paraffin agreed well with theoretical values but whole wax mixtures gave low results probably because C_{30}-C_{35} hydrocarbons were underestimated. Thus percentages of low melting paraffin which are not detected by the saponification cloud point can be detected and estimated by GLC analysis of the hydrocarbon fraction.

Other factors considered that might affect the cloud point were percentage of unsaturated hydrocarbons in beeswax hydrocarbons and the effect of prolonged overheating. The hydrocarbons of a Czechoslovakian beeswax

(16) and of a Canadian beeswax (11) both contained ca. 30% of unsaturated components, and another 20 Canadian beeswaxes examined by the same method contained similar amounts. It is possible that some beeswaxes contain only saturated hydrocarbons, as was reported for an Australian beeswax (17). A greater proportion of saturated hydrocarbons might be expected to raise the cloud point, but a completely hydrogenated beeswax had the same cloud point (63.0 C) as before hydrogenation. The cloud point of beeswax that had been heated at 150 C for 24 hr, and thus had undergone considerable chemical change (see above), was also unaffected.

Effects of Other Waxes

The effect of wax of other races of bees was also considered. Wax of African bee, *Apis mellifera adansonii*, has analytical values similar to those of wax of the usual bee in Europe and North America *(A. Mellifera)* (18). GLC analysis of a sample of wax of *A. mellifera adansonii* (from Brazil) showed it to be very similar to Canadian beeswax, the only noticeable difference being a smaller proportion of unsaturated C_{31} hydrocarbon.

Bees from China and the Indian subcontinent, *A. indica, A. florea* and *A. dorsata,* produce wax with lower acid values (5-10) and higher ester values (80-100) (18). GLC analysis of components of a Chinese beeswax (19) and of whole waxes from *A. indica* and *A. florea* (A.P. Tulloch, unpublished work) showed mainly quantitative differences from ordinary beeswax. The principal hydrocarbon was C_{27} and the principal monoester C_{46}; both components were present in greater proportions than in wax of *A. mellifera.* Mixtures of these waxes with ordinary beeswax would not be easily detected by GLC.

Adulteration of beeswax by carnauba wax has occurred in the past and a test based on the appearance of wax crystals obtained from butanol solution can detect, but presumably not estimate, 0.5% of carnauba wax (20) (carnauba wax cannot be detected by the cloud point test [6]). Carnauba wax contains ca. 10% of free alcohols (21) and GLC analysis of whole wax, treated with diazomethane and acetylated, showed that C_{32} was the major alcohol. GLC analysis of mixtures of beeswax and carnauba wax, treated as above, showed that 2.5% carnauba wax can be detected and estimated. The acetylated C_{32} alcohol gives a peak between C_{32} and C_{34} methyl esters similar in size to the C_{35} hydrocarbon peak or ca. 0.25% of the wax. The C_{50}-C_{60} monoesters of carnauba wax are partly obscured by beeswax diester peaks and cannot be used to estimate the amount of carnauba wax present.

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