SECTIONS

BOARD OF REVIEW OF PAPERS (SCIENTIFIC SECTION).—*Chairman*, F. E. Bibbins; H. M. Burlage, W. G. Crockett, E. V. Lynn, C. O. Lee, L. W. Rising, L. W. Rowe, Heber W. Youngken, Ralph E. Terry, Carl J. Klemme.

MICROSCOPY OF WAXES AND WAX CONSTITUENTS.*

BY HARRY TAUB AND SAMUEL ZWEIG.[1]

The purpose of this investigation is to develop a rapid method for identification as well as determination of adulterants in the commonly used waxes. Due to the complexity as well as variation in composition, the determination of adulterants in waxes by ordinary chemical or physical means is no easy task. It is, therefore, hoped that a suitable microchemical method may be evolved which will prove useful for this purpose.

Watson (1) was able to detect as little as 0.5% of carnauba wax in beeswax by the change of the crystalline appearance when crystallized from *n*-butyl alcohol. Microchemical work on oils and fats has been done by Rosenthaler (2,3), Greene (4,5), Butcher (6) and Mehlenbacher (7). The procedure for oils is to mix a drop of oil with a drop of reagent on a slide, cover with a cover slip and allow to stand until crystals appear. Due to the difference in consistencies this procedure cannot be applied to waxes. The latter, however, can be dissolved in a suitable solvent at the solution temperature of the particular wax and allowed to cool slowly and spontaneously to room temperature. The crystals are slowly formed and allowed to grow while the solution is cooled. If too rapid cooling or chilling takes place, the crystals formed are small, deformed and not characteristic. Care, therefore, should be taken to allow slow and uniform cooling of all test-tubes in order to obtain duplicable results.

Since most waxes are mixtures of various substances, one would expect to find different types of crystal formations as each constituent comes out of solution. This, however, is not the case, and in many solvents only one type of crystal formation results, *i. e.*, the entire mixture crystallizes in one form depending upon the type of constituents present, the concentration and the reagent used. When crystallized from *n*-butyl alcohol, the waxes act in this manner. On the other hand, when monoamylamine[2] is used as the solvent, well-formed crystals, often of more than one type, are produced. Monoamylamine also has the advantage of being able to react chemically with the free fatty acids present in waxes resulting in formation of monoamylamine esters. The esters of the lower fatty acids such as palmitic, stearic, etc., are extremely soluble and remain in solution. The esters of the higher fatty acids, such as cerotic, melissic, etc., are insoluble, and may crystallize out in their own characteristic crystal forms. It is therefore apparent that monoamylamine, due to its dual nature of solvent and chemical reagent, should be a suitable substance for wax crystallography.

---

* Presented before the Scientific Section, A. PH. A., Minneapolis meeting, 1938.

[1] Columbia University, College of Pharmacy, Dept. of Materia Medica.

[2] Manufactured by Sharples Solvents Corp., Philadelphia, Pa.

PROCEDURE.

The quantity of each wax used varies with the solubility of the individual waxes. It was found that 0.1 Gm. of all waxes except carnauba (0.05 Gm.), and spermaceti (0.5 Gm.) was a satisfactory amount. The waxes are placed in a test-tube of uniform bore, about $^5/_8$" in diameter and 6" long. 5 cc. of monoamylamine or 10 cc. of *n*-butyl alcohol are added. The test-tubes are then placed in a bath which has previously been heated to a temperature sufficient to dissolve the most insoluble wax. This temperature was found to be 65° C. for monoamylamine and 85° C. for *n*-butyl alcohol, at which points the most insoluble carnauba and ozokerite remained in solution. One of the requirements of the experiment is that the temperature of the solutions shall now be allowed to drop to room temperature in the course of about four to five hours. For this a suitable bath is required. Watson (1) describes an apparatus consisting of two beakers and a perforated metal disk, which is probably the most suitable for this purpose. However, the ordinary water-bath of 1500 cc. capacity, filled with 1000 cc. of water and fitted with a perforated metal disk

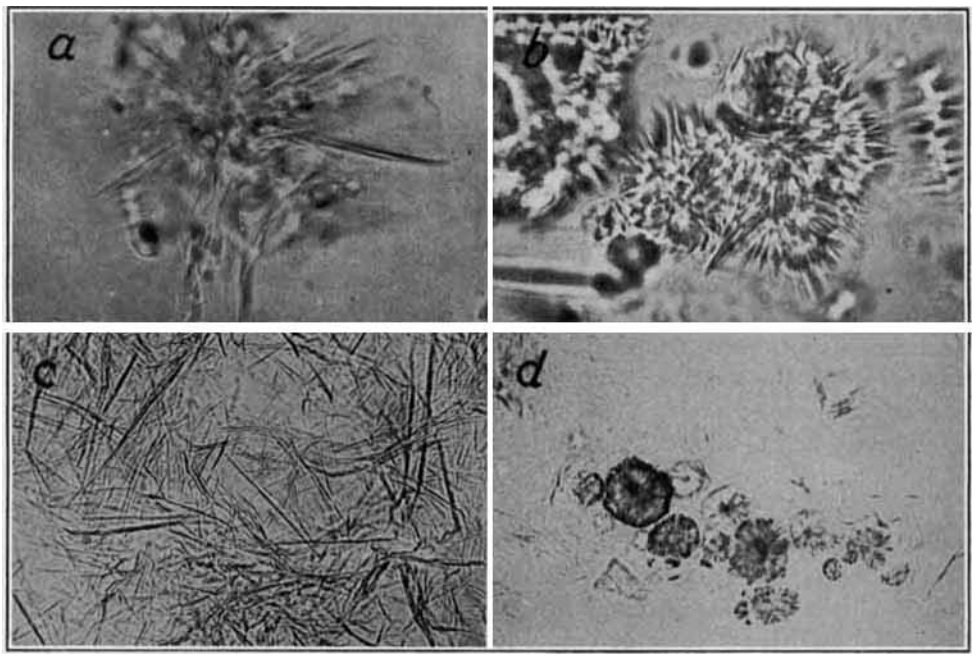

Fig. I.—(a) and (b) Crystals of beeswax from monoamylamine (600 X); (c) Beeswax from *n*-butyl alcohol (600 X); (d) Rosettes of Ozokerite crystallized from monoamylamine (300 X).

capable of supporting 12 test-tubes, was used in the authors' laboratory, and was found very satisfactory. After the solutions come to room temperature they should be allowed to stand for 24 hours before being examined. In all cases, with the exception of candelilla wax, the crystals settle to the bottom of the test-tubes. Then, without disturbing or stirring the sediment too much, a small amount of the solid is removed by means of a long pipette, placed on a slide and examined with the microscope. It was found that violent stirring or pressing down with the cover slip sometimes disintegrated the larger crystal aggregates and therefore this should be avoided as much as possible.

RESULTS.

The photomicrographs accompanying this report do not in all cases represent the entire crystal picture of the wax in question. In some instances, notably candelilla wax, only the characteristic segmented rosettes were photographed and the more abundant needle crystals are not very apparent in the picture. One should, therefore, not rely completely on the photomicrographs but reference should be made to the descriptions that follow.

Ozokerites (Yellow and White).—Crystallize from monoamylamine in the form of spherical compact rosettes consisting of small, thick needles; also individual needles with tendency to aggregate and form a stellate arrangement (Fig. Id).

Beeswax, White and Yellow.—Crystals are of two types when monoamylamine is used: (*a*) Individual, long, slender needles sometimes radiating from a common center (as shown in Fig. Ia). (*b*) Very small spindle-shaped needles aggregated to form either a spherical or wavy arrangement, the latter resembling hairy caterpillars (Fig. Ib).

From butyl alcohol, beeswax crystallizes in the form of long slender acicular crystals haphazardly arranged (Fig. Ic). Some circular amorphous-like bodies are also present.

Candelilla Wax.—In monoamylamine two types of crystals are formed: (*a*) Slender, curved needles, singly or in stellate arrangement, similar to those obtained from butyl alcohol (Fig. IIb). (*b*) Spherical bodies with markings similar to those found in carnauba wax as shown in Fig. IIa.

From butyl alcohol slender curved needles, as described above, are obtained (Fig. IIb).

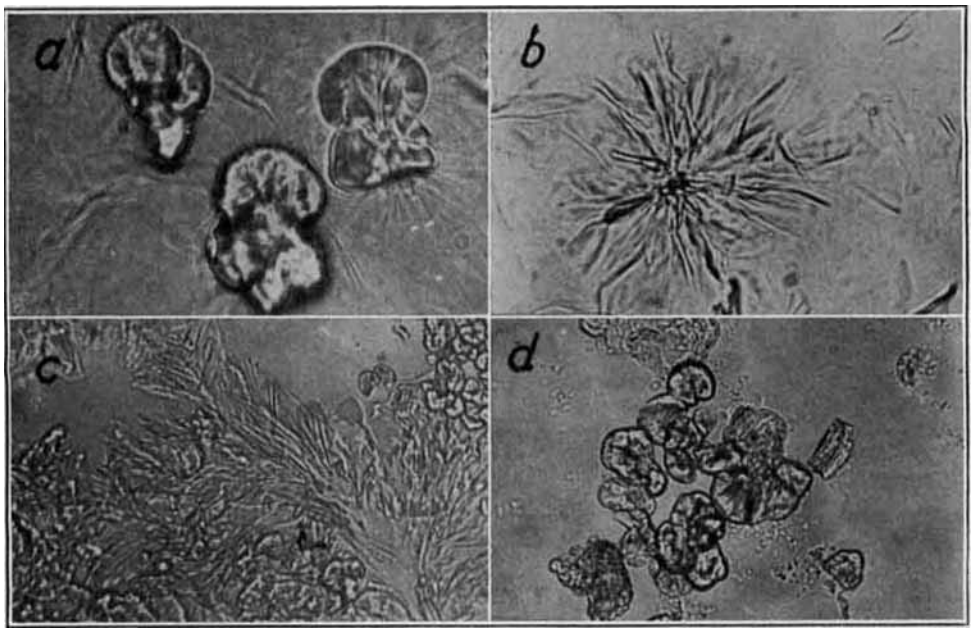

Fig. II.—(a) Candelilla wax from monoamylamine (600 X); (b) Candelilla wax from *n*-butyl alcohol (600 X); (c) Carnauba wax from monoamylamine (300 X); (d) Carnauba wax from *n*-butyl alcohol (300 X).

Carnauba Wax.—Crystallizes from monoamylamine in the form of: (*a*) Long slender needles in the form of sheaves (Fig. IIc). (*b*) Small segmented spherical rosettes with a white wax-like appearance, as in Fig. IId.

From butyl alcohol the crystals are similar to the rosettes obtained from monoamylamine (Fig. IId).

Soap Crystals.—When a solution of KOH in *n*-butyl alcohol is added in small amount to a dilute solution of stearic acid[1] in *n*-butyl alcohol, potassium stearate precipitates out in the form of slender needles haphazardly arranged. However, if the reagent is added in excess to a more concentrated solution of stearic acid in butyl alcohol, a translucent gel is formed, which, when allowed to stand over night, is converted into a crystalline precipitate. These crystal aggregates (Fig. IIIa and b) show a characteristic weave-like formation, with the individual needle crystals crossing each other at an oblique angle and forming an interwoven meshwork.

Palmitic acid and cerotic acid do not produce this type of crystal formation (Fig. IIIc).

---

[1] Commercial triple pressed stearic acid.

Cerotic Acid in Monoamylamine (the compound monoamylamine cerotate is probably formed) shows crystal formations which appear like sections of a square prism, the crystals belonging to the tetragonal system (Fig. IIId). These tend to aggregate and form sheaf-like groups of superimposed crystals.

Wax Mixtures.—As already stated above, waxes, although consisting of mixtures of various substances, usually show but one or at most two types of crystal formations. This phenomenon is also true of mixtures of two or more waxes. It was found that the presence of one wax in another influenced a change in crystal forms; this change consisted of a readily apparent modification of the original crystals. Therefore, although one could not pick out microscopically the characteristics of each individual wax in the mixture, yet, from the change in appearance of the

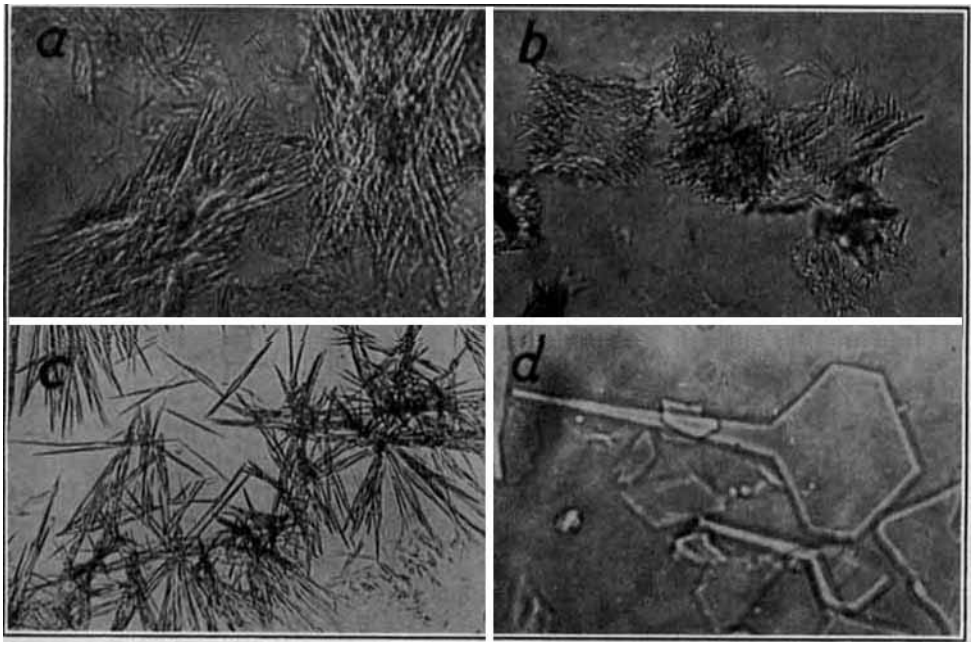

Fig. III.—(a) and (b) Potassium Stearate crystallized from *n*-butyl alcohol after being treated as described (400 X); (c) Potassium Palmitate from *n*-butyl alcohol (200 X); (d) Cerotic acid from monoamylamine (600 X).

crystal structures, the added wax may be inferred. The above facts have been demonstrated for mixtures of two waxes only; further work is being done on more complex mixtures.

The following compositions were prepared and examined:

1. 75% Beeswax+25% Carnauba Wax
2. 25% Beeswax+75% Carnauba Wax
3. 75% Beeswax+25% Candelilla Wax
4. 25% Beeswax+75% Candelilla Wax
5. 75% Beeswax+25% Bleached Montan
6. 25% Beeswax+75% Bleached Montan
7. 75% Beeswax+25% Paraffin
8. 25% Beeswax+75% Paraffin
9. 75% Beeswax+25% Ozokerite
10. 25% Beeswax+75% Ozokerite

RESULTS.

1. 75% Beeswax+25% Carnauba Wax.—Acicular crystals, aggregating around a central axis rather than from a point, giving rise to the caterpillar-like formations described under beeswax (Fig. IIb), but having a rounded outline with almost no projecting needles. The small spherical rosettes are denser, with the outline less serrate, than those of pure beeswax. The carnauba addition is further manifested by the crystals in sheaf segments as shown in Fig. IIc. In general, as will be seen later, the addition of a harder and less soluble wax to beeswax causes a suppression of the fine hairy needles projecting from the rosettes and other aggregate forms of beeswax.

2. 25% Beeswax+75% Carnauba Wax.—The crystal appearance is almost similar to pure carnauba and the presence of beeswax is not very apparent. It therefore follows that although it would be difficult to detect beeswax in carnauba by this method, it should, however, be possible to detect carnauba in beeswax.

3. 75% Beeswax+25% Candelilla Wax.—The typical beeswax crystal aggregate (Fig. Ib) has completely lost its fuzziness. It is now very compact and smooth in outline. Candelilla is apparent from the presence of wax-like spherical or bean-shaped bodies (Fig. IIa). The absence of the sheaf-like structures described in mixture No. 1, differentiates candelilla from carnauba.

4. 75% Candelilla+25% Beeswax.—Nothing very characteristic is apparent. Here again the presence of candelilla in beeswax is detectable but the reverse is not.

5, 6, 7 and 8.—The addition of Bleached Montan or Paraffin to Beeswax results in formation of large rosettes made of short, coarse and distinct acicular crystals. The aggregates similar to beeswax (Fig. Ib) are more pilose in appearance. The crystals in general are much larger than in pure beeswax. The large acicular crystals, which were numerous in beeswax both individually and in rosettes (Fig. Ia), are entirely absent. The effect of adding montan or paraffin to beeswax is opposite to the effect produced by candelilla or carnauba additions.

9. 75% Beeswax+25% Ozokerite.—The 25% ozokerite has an effect on beeswax crystal structures similar to candelilla and carnauba. It can, however, be differentiated from the two by the absence of the characteristics of carnauba and candelilla as described above (mixtures No. 1 and No. 3).

10. 25%Beeswax+75% Ozokerite.—Compact rosette forms typical of ozokerite (Fig. Id) consisting of short needles; also large rosettes consisting of distinct, slender acicular crystals, similar to those found in beeswax (Fig. Ia).

CONCLUSION.

Although at the present stage of the investigation it may be difficult to determine qualitatively all waxes present in an unknown mixture, it should however be possible to identify one or two waxes in such mixture by following the procedure outlined. Other physical or chemical methods such as solubility, melting point, saponification value, acid value, etc., may then be resorted to for positive identification of all ingredients.

SUMMARY.

Wax crystals are obtained by dissolving the wax in a suitable solvent at the solution temperature of the wax in question and allowing to cool spontaneously to room temperature. Although the waxes represent mixtures of several constituents, yet, each wax will tend to produce just one or, at most, two types of crystal formations. Monoamylamine, due to its dual nature of solvent and chemical reagent, was found very suitable for wax crystallography. *n*-Butyl alcohol was also used as the solvent. Some typical crystal formations were obtained with beeswax, carnauba wax, candelilla wax and ozokerite, when monoamylamine was used as the reagent. Odd type of crystal formations were obtained from potassium stearate in *n*-butyl alcohol and from cerotic acid in monoamylamine. In mixtures of waxes some idea of the main constituents present may be obtained from the appearance of the crystal formations.

REFERENCES.

(1) Watson, L. R., *Chemist-Analyst*, 20, 4 (1931).
(2) Rosenthaler, L., *Schweiz. Apoth. Ztg.*, 58, 545–549, 562–567, 578–583 (1920).
(3) Rosenthaler, L., *Microchemie*, 8, 72 (1930).
(4) Greene, L. W., *Am. J. Pharm.*, 109, 67–71 (1937).
(5) Greene, L. W., *Chemist-Analyst*, 25, 79 (1936).
(6) Butcher, C. H., *Ind. Chemist*, 10, 495–497 (1934).
(7) Mehlenbacher, V. C., *Oil and Soap*, 13, 277–282 (1936).