tank may cause these slight variations. If possible, the number of broken seals and opened containers should be kept to a minimum.

In conclusion, one may say that although general hints such as have been given above can be of service in the buying of essential oils, there is a lot to be said for the good will and the good name of the seller, and the old adage that the high standing of the firm and high reputation on the label is as good a guarantee of quality as one can obtain from chemical tests.

THE EFFECT UPON EMULSIONS OF THE HYDROXY COMPOUNDS IN BEESWAX

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Work is described in which the role of the hydroxy compounds in beeswax is demonstrated. It is found that these play an important part in the emulsification behaviour of the wax and attention is drawn to the resultant value of the acetyl value as a beeswax constant.

A STUDY of the literature does not produce a very lucrative yield of information on the composition of beeswax. The following information has been gleaned from various sources.

1. "Chemically, beeswax is composed of myricyl palmitate, cerotic and homologous acids with small amounts of hydrocarbons, cholesterol esters and ceryl alcohols, pollen and resins. The presence of resins in undue amounts renders bleaching difficult. The free fatty acid content is an important factor in emulsifiability.

Properties :

Melting point	145–158° F.
Specific gravity	0·952–0·975
Acid number	16·6–20·7
Saponification number	90–96
Unsaponifiable matter	52–56%
Iodine number	4–12
Acetyl number	15·1
Ester number	72–78
Ester-acid ratio	3·6–4·2
Colour	white, yellow, brown
Refractive index (75° C.)	1·4398–1·4451
Odour	honey
Dielectric constant	3·1–3·3
Effective A.C. conductivity	70–86
Volume resistivity	0·9–1·5

*Polak and Schwarz (England) Ltd., Enfield, Middlesex.

When beeswax is heated to 150–250° C., the acid number decreases while the ester number and saponification number increase. Continued heating causes re-esterification or estolide formation with decrease of ester and saponification numbers."

2.

"Esters of long chain acids	72%
Cholesteryl esters	0·8%
Lactones	0·6%
Free acids	13%
Hydrocarbons	12%
Water	2%

The esters have a saponification equivalent of about 700 with a melting point of 63° C. and contain :

33% myricyl palmitate
12% myricyl palmitoleate
12% myricyl cerotate
9% lacceryl palmitate
6% myricyl hydroxypalmitate
(myricyl alcohol $C_{30}H_{61}OH$)

Free acids equivalent 377. Melting point 78° C.
Neocerotic $C_{25}H_{50}O_2$
Cerotic $C_{27}H_{54}O_2$
Montanic $C_{29}H_{58}O_2$
Melissic $C_{31}H_{62}O_2$

Hydrocarbons mainly hentriacontane $C_{31}H_{44}$*
Melting point 68·7° C."

* Probably an error, Karrer gives $C_{31}H_{64}$.

Most specifications for beeswax quote acid and ester values with emphasis on the ratio number (ester value divided by acid value), but there is a notable absence of acetyl values.

This work was prompted by the thought that certain hydroxy compounds (alcohols and polyglycols) play an important part in emulsification technique. For example, cetyl alcohol, woolwax alcohols and glyceryl monostearate, when used with soaps or alcohol sulphates, act as efficient oil-in-water emulsifiers. Now beeswax has quite amazing properties when employed in emulsions. For instance, in the presence of a base, whether caustic alkali, triethanolamine, borax or lime water, emulsions of unusual properties are obtained. Its properties in this direction are very well known, even if its exact function is not.

It seems unlikely that when a base—say, triethanolamine or caustic soda—is reacted with beeswax to produce an emulsion the total emulsifying effect depends only on the soap formed. Borax, when used as the reacting

base, undoubtedly introduces added complications, but these are outside the consideration of this paper. The point in question is, have we an association between soap formed from the beeswax acids and hydroxy compounds present in the wax ?

It will be seen that the presence in beeswax of alcohols or compounds which contain hydroxy radicals has been suggested in the literature. The amount of such substances is represented by an acetyl value of 15·1 and the presence of ceryl alcohol $C_{27}H_{55}OH$ has been suggested. (Paul Karrer gives $C_{26}H_{53}OH$ for this alcohol.) It has also been suggested that hydroxy acids and alcohols are present.

An obvious step in the investigation of the influence of alcohols present in beeswax on the emulsifying properties of the wax would be to try the effect of blocking the free hydroxy groups by, for example, acetylation. There are certain pitfalls in this procedure due to the fact that heat tends to reduce the acid value and increase the ester value of the wax, presumably by a process of esterification or lactonisation. It would be possible to attempt complete acetylation in the presence of a solvent to reduce heat effects, but this would introduce the complication of removing the water-immiscible solvent from the wax. In any case, the boiling point of acetic anhydride is only 138° C. at normal pressure.

The following procedure of acetylation was adopted : 300 gm. of beeswax were gently heated until molten and acetic anhydride added slowly with stirring until 200 ml. had been added The mixture was heated under reflux for one hour. During this time the temperature rose from 119° C. to a maximum of 139° C. The acetylated product was allowed to cool and 300 ml. of boiling water added and the whole stirred for 10 minutes This was allowed to separate and the water-layer removed. The mass was washed six times with hot water and after the last washing, at which point the wash-water was neutral to phenolphthalein, the water was allowed to thoroughly separate. The last traces of water were removed under reduced pressure. Below are shown certain analytical figures for the original and the acetylated wax.

	Original Wax	*Acetylated Wax*
Melting point	63·4° C.	63·0° C.
Acid value	17·9	16·6
Ester value	75·2	90·6
Ratio Number $\frac{(E.V}{A.V)}$	4·20	5·46

CALCULATION OF ACETYLISABLE FREE-ALCOHOL IN BEESWAX

$$\text{Free alcohol} = \frac{(\text{acetylated ester value} - \text{ester value}) \times \text{molecular weight of the alcohol.}}{0{\cdot}42 \times (1336 - \text{acetylated ester value})}$$

$$= \frac{15{\cdot}4 \times 214{\cdot}4}{0{\cdot}42 \times 1245{\cdot}4}$$

= 6·3% calculated as myristyl alcohol or
7·1% calculated as cetyl alcohol.

During acetylation, besides the reaction represented by :

$$ROH + (CH_3CO)_2O \rightarrow ROOCCH_3 + CH_3COOH$$

we might anticipate reactions of the acid-exchange type.

$$ROOCC_{15}H_{31} + CH_3COOH + [(CH_3CO)_2O] \rightarrow ROOCCH_3 + C_{15}H_{31}COOH + [(CH_3CO)_2O]$$

regarding the acetic anhydride $[(CH_3CO)_2O]$ as a catalyst.

Such a reaction would increase the ester value without increasing the molecular proportion of esters present

$$\left(\frac{\text{Number of molecules of esters}}{\text{Total number of molecules}}\right)$$

However, reactions of this type would also increase the acid value by the introduction of further fatty acid (e.g., palmitic acid), but both these effects would be reduced by the increase in bulk due to introduction of acetate radicals. If no significant increase in acid value occurs as a result of acetylation, then it appears safe to assume that this type of acid-exchange occurs only on a negligible scale and hence the calculation of the acetylisable free alcohols is valid.

From the above discussion it would appear that we have prepared a sample of beeswax in which the free hydroxy radicals have been converted to the corresponding acetate.

Comparison of Beeswax and its Acetylation Product

If we have achieved our object when acetylating the beeswax, we can now undertake experiments designed to determine the effect of removing the free hydroxyl radicals. Emulsions of a typical cold cream type were prepared as shown in Table I.

TABLE I

Constituents parts by weight	CREAM							
	A	B	C	D	E	F	G	H
Beeswax	14·0	—	14·00	—	—	—	—	—
Acetylated Beeswax	—	14·0	—	14·00	14·0	14·00	14·0	14·00
Mineral Oil	50·0	50·0	50·00	50·00	49·0	49·00	49·0	49·00
Water, distilled	35·0	35·0	35·00	35·00	35·0	35·00	35·0	35·00
Borax	1·0	1·0	0·93	0·93	1·0	0·93	1·0	0·93
Cetyl alcohol	—	—	—	—	1·0	1·00	—	—
*Ethylene oxide-cetyl alcohol condensate	—	—	—	—	—	—	1·0	1·00

* Stated by the manufacturer to contain twelve molecules of ethylene oxide, but this figure is probably on the low side.

The creams were made by heating the beeswax or acetylated beeswax in the mineral oil to 65° C. The borax and water were also heated to 65° C. and added to the oil phase at a controlled speed. The rate and time of stirring was constant for each cream, which was poured into jars at a temperature of 45° C. When cetyl alcohol or ethylene oxide condensate was included this was incorporated in the oil phase. Creams A and B may be regarded as "standard" types, the borax in creams C and D was reduced to 0·93 parts to allow for the drop in acid value in the acetylated wax (17·9 to 16·6). Cetyl alcohol and ethylene oxide/cetyl alcohol condensate were added to investigate the effect of re-introducing hydroxyl-containing compounds to the acetylated wax. One part of cetyl alcohol to 14 parts of beeswax corresponds roughly to the figure calculated from the acetyl value of the wax. All creams were made in triplicate.

*p*H readings on creams A, B, E and G were taken, resulting as shown in Table II.

TABLE II

pH Readings of Creams A, B, E and G

Cream	*p*H
A	8·7 to 8·8
B	8·7 to 8·8
E	8·5
G	8·8 to 8·9

These tests were made mainly to ensure absence of free acetic acid in the acetylated creams. The low figure for cream E (containing cetyl alcohol) is of interest, if difficult to explain. The cream containing ethylene oxide condensate (cream G) has a slightly higher *p*H than cream A and cream B. It might be worth pointing out that cetyl alcohol is not soluble in water, while the condensate is.

Tests for phase type (w/o or o/w) were undertaken by three methods, i.e conductance-bridge, colouring with both oil- and water-soluble dyes, and by the water and oil dilution method. The results were not conclusive and would be misleading to record.

Assessment of the Creams

1. *By visual examination.*

In no case was the effect of reducing the borax from 1·0 to 0·93 significant, so the main examination was confined to creams A, B, E and G.

(a) *observations during manufacture.*

The ease of emulsification was greatest with cream E, closely followed by cream A, with creams B and G about equal. Nevertheless, all creams made easily enough.

(*b*) *The viscosity or "body" of the cream.*

When poured at 45° C. cream G was very thin, cream E was perhaps

less viscous than creams A and B at this stage. After a few weeks standing in the capped jars there was, perhaps, little difference in the firmness of creams A, B and E, but cream G was very soft.

(*c*) *Surface effect.*

Examined when cold, creams A and G were the most shiny, followed by B and then E. There was, however, not a great deal of difference between the extremes.

(*d*) *Texture.*

When the surface of the cream was disturbed, cream G had the closest grain, immediately followed by cream A. Cream B came next, with cream E rather more granular.

(*e*) *Examined after* 3 *months standing in screw-capped jars.*

Cream A still had a shiny surface and did not move round in the jar when stirred (slipping in the container indicates lubrication of the vessel-sides due to free liquid). Cream B had a dull surface and appeared to have lost water and in addition readily slipped round the jar. Cream E had lost water from the surface but did not rotate when stirred. Cream G remained a good, shiny cream but was very soft.

(*f*) *Examined after* 6 *months.*

Cream A did not appear to have lost water from the surface and had not shrunk in the jar. Cream B had a dull surface, almost transparent, with considerable shrinkage from the sides of the jar. Cream E had suffered considerable water loss and was almost transparent but had not shrunk from the sides. Cream G remained a good emulsion but very soft.

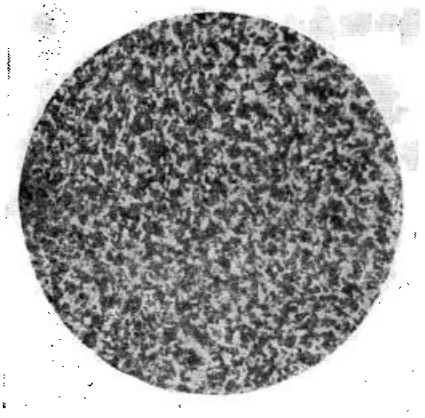
(*g*) *Effect of introducing an oil-soluble dye to creams A and B.*

During the microscopical examination of the creams the effect of adding dyes to both water and oil phase had been studied. Although no useful results were obtained one observation is worth recording. Creams A and B had been made using a soluble red dye in the oil phase. Cream A (coloured) remained stable but cream B (coloured) broke very rapidly.

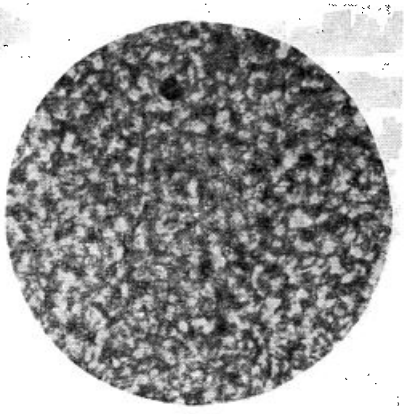
(*h*) *Microscopical examination.*

The specimens were prepared by spreading the creams as uniformly as possible on the slide and lowering the cover-slip with gentle pressure and without sliding. The magnification in each case was 720 diameters. Three slides of each cream were prepared and each was photographed. The creams examined were A, B, E and G, these being photographed on the same day of making and after three and six months standing in screw-top jars. Photographs I, II, III and IV were taken on the day the creams were made, photographs V, VI, VII and VIII after the creams had stood for three months, and photographs IX, X, XI and XII after six months.

Photograph I (cream A) shows a fine state of division, while photograph II (cream B) has a much larger particle size. Photograph III (cream E), which demonstrates the effect of added cetyl alcohol, shows a close packing



I.



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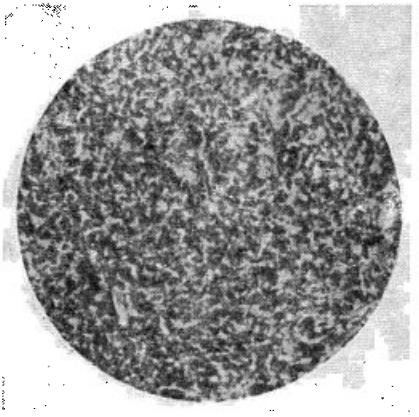
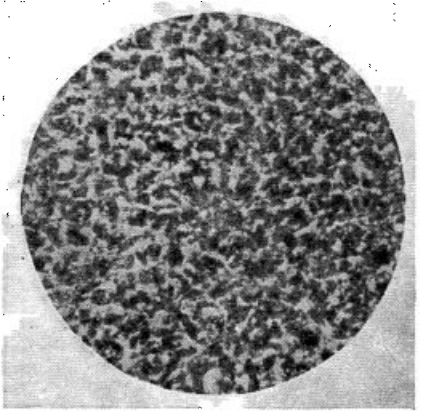
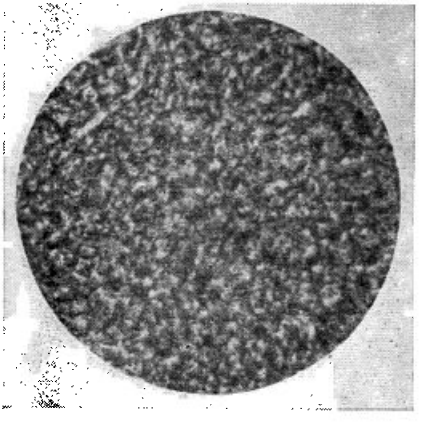


III.



IV.



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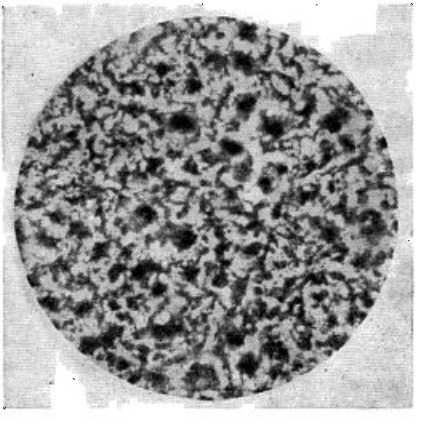

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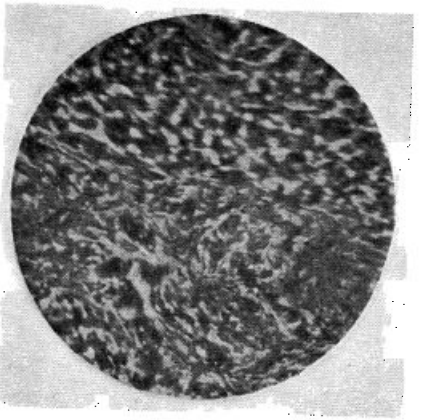
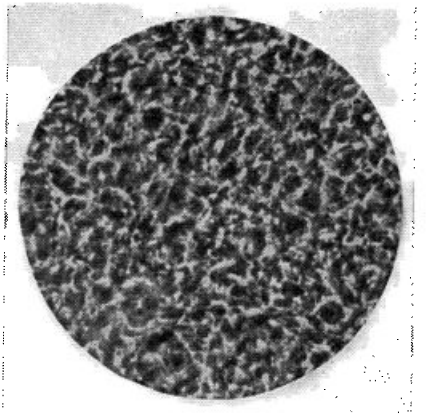
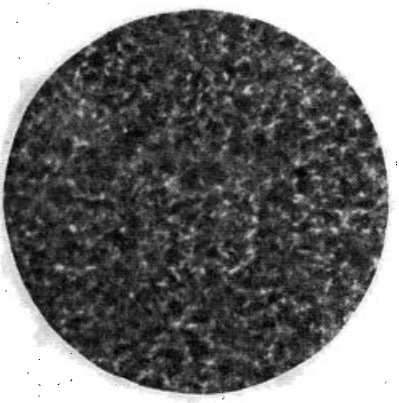


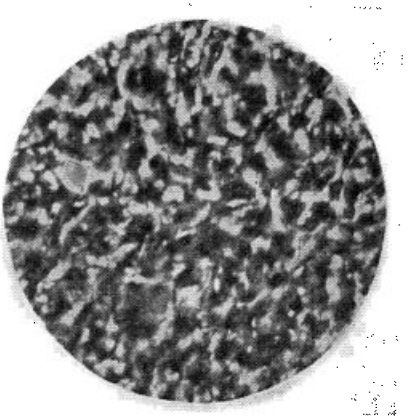
VII.



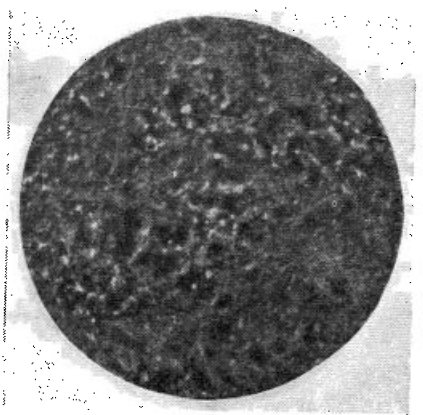
VIII.



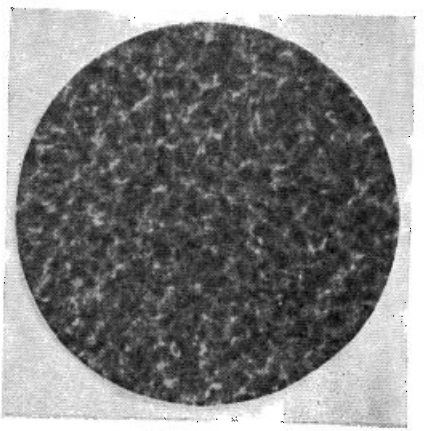
IX.



X.



XI.



XII.

of the particles, but the particles are less uniform in shape than those in creams A or B. Photograph IV (cream G) shows a more uniform particle shape and size than cream E but the emulsion is coarser than cream A (photograph I).

On examining the photographs taken when the creams were three months old, photograph V (cream A) shows little change from photograph I and indicates that cream A has retained its fine emulsion characteristics after three months. Photograph VI indicates a larger particle size when compared with photograph II and indicates a deterioration of the emulsion with time.

Photograph VII was difficult to obtain as a representative picture due to continuous movement of the particles under the microscope. It will be seen that the picture shows in the upper half a coarse emulsion and in the lower half a somewhat blurred effect.

Photograph VIII appears very similar to photograph IV, indicating little change in the emulsion on standing. After six months standing and allowing for the difference in intensities of the prints, cream A has changed but little, cream B has suffered further breakdown, cream E is of large particle size and cream G has not greatly changed.

From the microscopical examination it is evident that creams made from acetylated beeswax are coarser and less stable than those made from untreated beeswax. The effect of adding cetyl alcohol to the acetylated beeswax cream is, initially, to produce an improved emulsion as judged by particle size. On standing, however, this particular cream changed in consistency and was no longer uniform. The effect of adding a cetyl alcohol/ethylene oxide condensate to the acetylated wax was to reduce particle size in the emulsion, and this cream remained uniform after six months standing. The particle size of this emulsion was not as small as that in cream A made from untreated beeswax.

Beeswax/Lime-water Emulsion

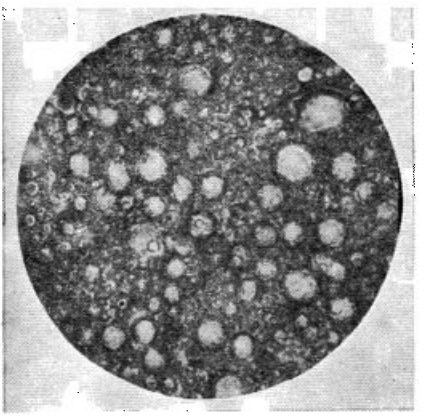
To carry the investigation a stage farther, two creams of the w/o type were made to the following formulæ :

	Cream J	*Cream K*
Beeswax	4	—
Acetylated beeswax	—	4
Mineral oil	46	46
Lime water B.P.	25	25
Tap water	25	25

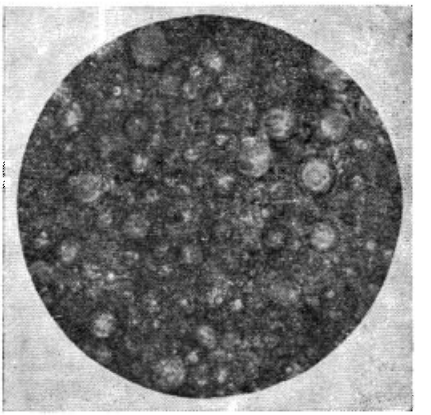
These were made by heating the beeswax or acetylated wax in the mineral oil to 40° C. The aqueous phase also at 40° C. was added to the oil phase with controlled stirring. Each emulsion was divided into two portions and one portion was placed three times through a laboratory homogeniser.

During manufacture the emulsions differed only slightly and there was a suggestion that the emulsion based on acetylated wax was rather "stiffer" and less white. Emulsion stability as measured by centrifuging did not appear to differ in either cream, homogenised or unhomogenised.

Photograph XIII shows the homogenised emulsion made from formula J and photograph XIV the homogenised emulsion made according to formula K.



XIII.



XIV.

It will be seen that there is little difference in the structure of the two emulsions.

Effect Upon Viscosity

At an earlier stage of this work attempts had been made to study the effect of acetylation upon the viscosity of beeswax solutions. Although the results were of no real significance, one set may be worth recording. Unfortunately, beeswax does not produce a clear solution in mineral oil and rapidly settles on standing. Ordinary beeswax settles much more rapidly than the acetylated product. Four "solutions" were prepared as below :

	1	2	3	4
Mineral oil	95·0	95·0	95·0	95·0
Beeswax	5·0	—	—	—
Acetylated beeswax	—	5·0	5·0	5·0
Cetyl alcohol	—	—	0·35	—
Cetyl alcohol/ethylene oxide condensate	—	—	—	0·35

Viscosities were taken by measuring the rate of flow through a standard capillary at 25° C., mineral oil giving a time of 89 seconds under these conditions.

	Flow time in seconds.	
"Solution"	*Immediately*	*After 24 hours (re-stirred)*
1	95	105
2	120	135
3	116	122
4	109	119

As might be expected, the presence of hydroxyl radicles tends to reduce solubility in mineral oil and to reduce the viscosity of the resulting "solution." The addition of hydroxyl-containing products to the acetylated wax tends to reduce the viscosity, but not to the figure given by the untreated wax "solution."

Absorption Spectra Examination

The U.V. spectra of beeswax before and after acetylation was measured in cyclohexane. As might have been anticipated, acetylation hardly changed the spectrum, which was completely uncharacteristic and from which no information could be obtained. Unfortunately, no opportunity was afforded to study the I.R. absorption spectra, which might well provide detailed information on the nature and distribution of the functional groups in beeswax.

Discussion of Results

When the free hydroxyl radicles present in beeswax constituents are blocked by acetylation the properties of the wax are modified in certain directions. Acetylation naturally brings about a fall in acid value and an increase in ester value and, consequently, a considerable increase in the ratio number (E.V./A.V.). The melting point of the acetylated wax is very slightly higher than the untreated wax.

Typical cold creams based on beeswax and borax show changed properties when the beeswax is replaced by acetylated wax. The *p*H of creams A and B are the same. During the manufacture of creams A and B little or no differences were noted but, on cooling, the cream containing untreated wax was rather more shiny and the grain more fine. After standing in screw-top jars cream A (untreated wax) remained shiny, did not appear to have lost water from the surface, had not shrunk and, in fact, appeared to have retained its original fine emulsion characteristics. Cream B, on the other hand, had deteriorated considerably, having become dull and translucent on the surface, shrunk from the sides of the jar and lost water. As evidenced

by the effect of adding an oil soluble dye to the creams, the cream made from acetylated beeswax is susceptible to certain added ingredients, this effect not being apparent in the cream made from the untreated beeswax.

Emulsions of the w/o type based on beeswax and lime water are not materially effected when the beeswax is replaced by acetylated wax.

The limited solubility of beeswax in mineral oil is somewhat increased by acetylation and the viscosity of the dispersion is enhanced. In attempting to assess the effect of adding compounds which contain hydroxyl groupings to the acetylated beeswax there are obvious difficulties to be considered. In the first place, the exact nature of the beeswax "alcohols" are not known or at any rate not available in the literature. They may be hydroxy acids, hydroxy alcohols, any one of a variety of alcohols or, indeed, a combination of these types. The selection of cetyl alcohol was an obvious step, as this approximates to the straight chain fatty alcohols which are mentioned in the literature. The ethylene oxide condensate was selected as a contrast to the oil-soluble cetyl alcohol.

The Effect of Reintroducing Hydroxy Compounds to Acetylated Beeswax

(*A*) *Cetyl alcohol*

When used in conjunction with acetylated beeswax as in cream E, the *p*H of this cream is 8·5 compared with 8·7 to 8·8 for creams A and B. If anything, the presence of cetyl alcohol enhances the ease of manufacture of the cold cream, but tends slightly to reduce the shiny surface and to cause a more granular structure.

On standing in jars the presence of cetyl alcohol appeared to have pre-prevented shrinkage but did not inhibit water loss from the cream. In the early stages cetyl alcohol, judged from the microscopical examination, seems to have improved the emulsion based on acetylated wax, but on standing the cream is far from uniform.

(*b*) *Cetyl alcohol/ethylene oxide condensate.*

The above product appears slightly to increase the *p*H of the cream based on acetylated beeswax. Its most significant effect is the reduction in viscosity of the cream. The improvement in the acetylated beeswax cream due to the presence of the ethylene oxide condensate is most marked so far as particle size and stability are concerned.

Summary and Conclusions

It is well known that additions of certain hydroxyl-containing compounds to surface-active agents often result in improved emulsifying performance. The acids present in beeswax form soaps with bases and it is possible that the alcohols present in the wax form complexes with the soap to produce

emulsifiers which are responsible for the peculiar properties of beeswax. Regrettably, little published information on the nature of "alcohols" in beeswax is to be found.

In order to determine the effect of the beeswax alcohols on emulsions made from this wax the free hydroxyl groups were blocked by acetylation. Although we are now dealing with a changed ratio value it has been shown that acetylated beeswax lacks many of the admirable emulsifying properties of the untreated wax. A cold cream made from the acetylated wax possesses larger particle size and is relatively unstable when compared to the normal cold cream.

To some extent the performance of this cream can be improved by re-introducing compounds which contain hydroxyl radicles. It would appear that cetyl alcohol is too oil-soluble and the ethylene oxide condensate too water soluble adequately to replace the alcohols removed by acetylation.

Further work (unpublished) has shown that a careful selection of other hydroxyl compounds used in their correct proportions in conjunction with acetylated beeswax will lead to a cream quite as good as the cream made from the untreated beeswax. In fact, it is fair to say that the additions have, in some respects, resulted in creams with improved properties. Cream A, which may be regarded as a "normal" cold cream, can be greatly improved in many ways, e.g., smaller particle size, attractive appearance, inhibition of water-loss, stability of the emulsion, by the addition of small amounts of hydroxyl-containing materials. The fact that acetylation does not greatly affect the properties of beeswax as a w/o emulsifier is not surprising. The formation of calcium soaps with the beeswax acids results in a reasonably efficient w/o emulsifier and its properties would not be greatly influenced by small additions of alcohols.

The above work indicates that the rather unusual emulsifying properties of beeswax depend to some extent upon the presence of alcohols or, at any rate, compounds which contain hydroxyl radicals. This effect does not appear to be generally recognised and certainly little attention is given to it in the literature. It is therefore suggested that more attention could be given to the acetyl value of beeswax during analysis and general estimation of its properties.

I acknowledge with thanks the laboratory experiments undertaken by Mrs. P. Mitchell and Miss E. Parmenter, and the general help and advice given by Mr. Geoffrey Pickthall, especially as far as the photomicrography is concerned.