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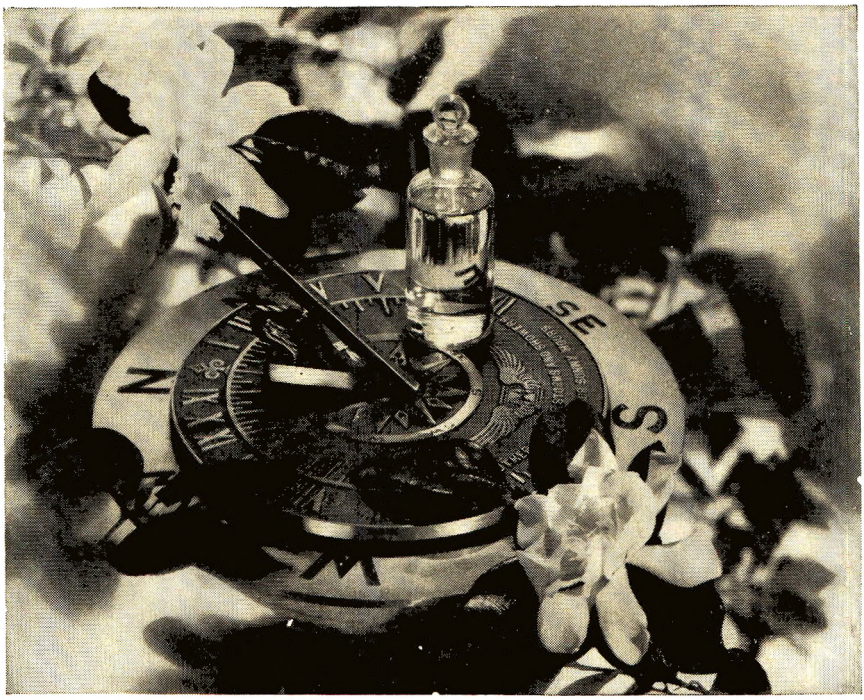

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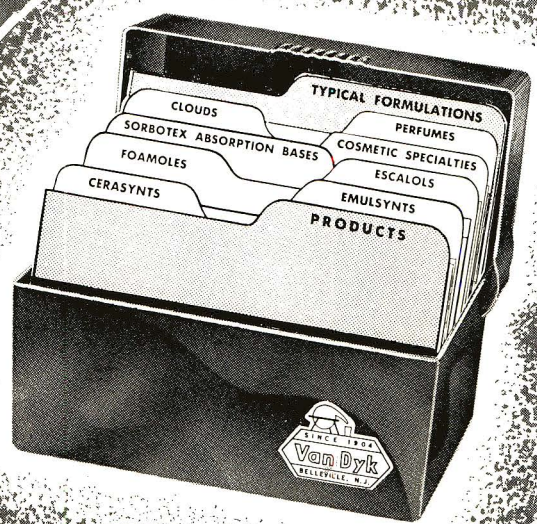

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THE POTENTIAL OF ENZYMES FOR TOPICAL APPLICATION

By T. Cayle, Ph.D.*

Presented January 9, 1963, New York City

Abstract

Examination of the skin and cutaneous disorders from the standpoint of potential substrates for a variety of enzymes leads to the conclusion that these highly specific, mildly acting reagents will probably find a place amongst the more important active ingredients available to the cosmetic chemist.

In addition to a recognition of the enzyme-substrate relationship that is so important to the successful use of enzymes, the cosmetic chemist must have an understanding of the possible interplay between an enzyme and the vehicle in which it is formulated, and *vice versa*. Although enzymes can be extremely useful, attention is drawn to the fact that they should not be considered a panacea. A number of areas which appear to offer some exciting new approaches to the cosmetic industry are discussed.

I. Introduction

No matter what his specialization, at one time or another during the career of every chemist, he is exposed to some of the unique properties of enzymes. He is made aware of enzyme specificity, rapidity of action, mild operating conditions, etc., and it is easy to understand why the formulating cosmetic chemist should turn to these reagents as potentially important members of his armamentarium. The object of the following discussion is to bring into perspective some of the current thinking concerning the potential of enzymes for topical application. The cosmetic chemist views the skin and its appendages as a part of the body to be maintained and to be beautified. The enzyme chemist, on the other hand, views the integument as a continuous source of varied substrates, many of which are exceedingly refractory to enzyme attack. The successful application of his product, the enzyme, is based on the objective scrutiny of those who expect it to perform a particular task, and this it can only do if the skin lends itself to attack. Therefore, the enzyme chemist is more interested in the chemical changes than in the morphological changes that occur during keratinization of the epidermis: the conversion of a "normal"

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cellular protein to a form that is completely insoluble and extremely refractory to enzymatic degradation.

Synthesis of this "new" protein form takes place in the active basal layer, with a period of "curing" required as the cells migrate toward the periphery. The keratin precursors are stabilized and made three dimensionally rigid by virtue of the inter- and intra-molecular disulfide linkages so abundant in the keratin product. The degree of rigidity and, therefore, the ability of the molecule to withstand enzymatic hydrolysis, is directly correlated with the number of disulfide linkages present, as well as with the extent of hydrogen bond formation between protein chains.

Table I shows the half-cystine content of several keratins and that found in several "typical" soluble proteins. Hair and nails are examples of so-called "hard" keratin and represent the type of material most refractory

TABLE I.—COMPARISON OF HALF-CYSTINE CONTENT OF SEVERAL KERATINS WITH THAT FOUND IN SEVERAL SOLUBLE PROTEINS

	Half-Cystine Content, Moles per 10^5 g. Protein
Keratin	
Wool*	92–114
Human Hair*	138–150
Epidermis*	19–32
Collagen†	0
Casein†	3
Ovalbumin†	13

* (1, p. 32).
† (2, p. 278).

to hydrolysis and having the highest content of stabilizing disulfide groups. "Soft" keratin is represented by epidermis and is said to contain about 20% of a water-soluble protein which is electrophoretically identical to keratin.

The higher half-cystine content of hard keratin is indicated by the sulfur content of approximately 5%, whereas it is only approximately 1% in the case of soft keratin (2).

The epidermis is the origin of accessory structures known as skin appendages, i.e., hair, sebaceous glands, sweat glands (apocrine and eccrine) and nails.

In 1961, 40% of the dollar volume (3) spent on toilet goods was devoted to products designed to maintain, retain, diminish or glorify these accessory appendages. In addition, almost one fifth of all diseases of the skin seen by dermatologists were diagnosed as acne (4), a syndrome associated primarily with hair follicles and sebaceous glands.

The approximate composition of sebum, the secretion of the sebaceous gland, is given in Table II. The predominantly lipid nature of this

TABLE II.—THE APPROXIMATE COMPOSITION OF SEBUM*

Free, unsaturated fatty acids	20%
Triglycerides	25%
Fatty acid esters	25%
Cholesterol (free & combined)	5%
Hydrocarbons, including squalene	15%

* (5, p. 362).

material suggests the enzyme that might be useful in treating conditions associated with seborrhea.

Other skin appendages will not be discussed here, except to mention that the nail is composed of hard keratin and the associated cuticle of soft keratin.

II. AREAS AMENABLE TO ENZYME TREATMENT

There are many "conditions" that fall within the realm of the cosmetic chemist that appear to be amenable to treatment with enzymes. The following list is not meant to be inclusive but represents some of the more obvious areas of high interest.

A. Acne
B. Hair removal and conditioning
C. Skin softener
D. Dandruff
E. Oral hygiene

Each of these will be examined from the standpoint of availability as a substrate for known enzyme systems.

A. Acne

Based on a recent survey of the ten most common skin diseases (4), acne represents almost 20% of all diagnoses by dermatologists, and may be observed in up to 80% of adolescents (6, p. 273).

According to Rothman (7), "Natural juvenile acne has two main pathogenetic factors. One is sebaceous-gland hyperplasia, and the other is excessive follicular keratinization at the orifice, which occludes the pore and hinders the expulsion of sebum."

In addition to the varying quantities of sebum present in the comedones, one usually finds keratinized cellular debris. Usually the clinical picture is complicated by secondary bacterial infections brought about by squeezing, scratching, etc. Frequently sub-surface papules associated with increased inflammatory exudate are present (8).

If one were to examine this clinical picture from the standpoint of potential enzyme substrates, it would become apparent that there are at least four major focal points of attack.

1. Sebum. A lipolytic complex should be quite useful in softening and reducing the sebaceous component blocking the follicular pore.

2. Keratin. The presence of a keratinaceous plug as one of the main causes of mechanical obstruction makes this component a prime target. Enzymes that will aid in the penetration and removal of this material can probably be found among the wide variety of proteolytic systems available. The keratin present in a comedone is of the soft variety and may thus be more amenable to attack than the type found in hair. The question of "keratinase" activity will be discussed below.

3. Bacterial and Cellular Metabolic Waste Products. A secondary bacterial infection results in a pustule which contains bacterial and cellular metabolic waste products in addition to sebum and keratin. Probably the most important of these, for this discussion, is the nucleic acid which contributes toward the viscosity of the follicular plug. Deoxyribonucleases should be useful in this area.

4. Inflammation. The recent success of many enzymatic anti-inflammatory preparations may provide the impetus for dermatologists to try similar therapy in those acne conditions which warrant such treatment. While this application would be systemic and, therefore, outside the area of immediate interest to the cosmetic chemist, it is included here because of its obvious relationship to the topical treatment of this syndrome.

The use of enzymes to hydrolyze the specific substrates making up the primary acne lesions offers an additional important benefit. Destruction of the comedone plug in which microörganisms may be imbedded should provide for enhanced activity of topically applied antibiotics by making the microbial invaders more available to these agents.

In extremely severe cases of acne where lesions have a necrotic overlay, a mild debridement type of activity may be indicated. In such cases, particular proteolytic enzymes could be topically applied which would serve to remove the necrotic tissue, thereby allowing the healthy granulation underneath to function unimpeded. This also would serve to bring other active medicinal agents into intimate contact with the affected area.

B. Hair Removal and Conditioning

The use of enzymes as a depilatory or as an aid in the waving or straightening of hair has long been of extreme interest to the cosmetic chemist. Judgment as to the potential of enzymes for such use must be based on a knowledge of the chemistry of hair.

As indicated above, hair is composed of hard keratin, and, as such, is completely insoluble in water and refractory to enzyme attack.

According to Harry (5, p. 571), the "ideal" depilatory is one which would have no odor, would remove hair in about one minute, could be used regularly without causing irritation, and could replace the daily task of shaving. There are no preparations currently marketed that fulfill all of these requirements. It has been the belief of many that a true keratinase

will be the ideal depilatory. In the opinion of the author this is a possibility but it is not a probability.

An enzymatic depilatory could act in one of two ways; the hair could be hydrolyzed to the point where it becomes solubilized, as with thioglycolate and sodium sulfide, or the follicle could be attacked, loosening the hair, as with sodium sulfite. With either method, the insolubility of the native keratin (assuming the keratinaceous lining of the follicle is degraded in the second case) would prevent an enzyme from attacking it with the dispatch required of a useful cosmetic preparation. In addition, if an enzyme was active enough to destroy the stable structure of hair keratin rapidly, it would more than likely attack the skin.

Goddard and Michaelis (9, 10) were able to show that the most stable of keratins becomes susceptible to proteolysis subsequent to reduction by a number of reducing compounds such as those indicated in the following reactions:

$$R{-}S{-}S{-}R + 2HS{-}CH_2COOH \rightarrow 2R{-}SH + (S{-}CH_2COOH)_2$$

$$R{-}S{-}S{-}R + HCN \rightarrow R{-}SH + R{-}S{-}CN$$

$$R{-}S{-}S{-}R + 2Na_2S \rightarrow 2R{-}SNa + Na_2S_2$$

In these illustrations thioglycolic acid, hydrogen cyanide and sodium sulfide all provide for the reduction of the indicated disulfide.

It should also be noted that forms of keratin normally most stable can be converted to a susceptible substrate for a number of proteolytic enzymes by oxidation (9) and mechanical disruption (2, p. 176). In other words, there are a variety of treatments to which keratin can be subjected which result in transforming this stable protein into substrate for ordinary proteases. This type of information, coupled with the fact that reports in which "true" keratinase activity is described are subject to alternative interpretations for the observations that are recorded, has convinced many enzyme chemists that the activity attributed to "true" keratinases may, in reality, be ascribable to ordinary proteolytic enzymes acting on a modified keratin substrate.

It may thus be possible to modify hair characteristics by the judicious combination of chemical treatment and proteolysis. Whether this could result in rapid enough depilatory activity is difficult to predict. However, the effects of such treatment on hair conditioning (waving, straightening, dying, etc.) might well be worth investigation by the cosmetic chemist.

C. Skin Softener

Reference here is made to those conditions which result from a build-up of keratin at the skin surface, whether by an increase in the rate of keratinization without concomitant sloughing off of the horny layer at a comparable

rate or by retention of the stratum corneum, yielding a layer of keratinaceous material greater than normal.

Calluses and corns would also fall into the general category of hyperkeratosis, caused by the stimulation of the epidermis by intermittent pressure.

The soft nature of this epidermal keratin could enable it to serve as a substrate. While the appropriate enzyme may not currently be available, product research and development should ultimately provide a suitable system.

D. Dandruff

Dandruff is defined as the scales that flake off from the outer horny layer of the skin of the scalp. They are keratinaceous and many times impregnated with an oily film resulting from the seborrhea which so often accompanies the dermatitis.

Here is another area where the potential for enzyme treatment is high, and the general approach discussed for skin softening should be applicable for dandruff. Because an enzyme in a cream could be applied to the scalp and allowed to remain in contact with the substrate for a relatively long period of time, this area of interest offers a high probability of success.

E. Oral Hygiene

It is generally accepted that in order to keep caries and halitosis at a minimum, food particles must not be allowed to remain trapped between the teeth for an extended period of time.

Salivary amylase undoubtedly serves to reduce the carbohydrate content in the mouth by converting insoluble forms of the foodstuff to soluble, ingestible forms which are readily swallowed.

The use of the proper proteolytic enzyme in a toothpaste, or preferably in a troche, could serve several functions. Proteinaceous material trapped

TABLE III.—COMPOSITION OF DENTAL PLAQUE*

	g./100 g. of Dry Plaque
Inorganic	
Ca	2.7
Mg	0.6
PO_4	3.8
Total ash	10.6
Nitrogen (micro-Kjeldahl)	
Total	12.6
Protein nitrogen	11.1 (× 6.25 = 69.4)
Free amino acids	11.6
Lipid	1.9
Carbohydrate	
Glucose	3.5–5
Pentose	ca. 1.0
Hexosamines	<0.1

* (11)

between the teeth would fall ready prey to such activity and would be solubilized and removed from the site where fermentation and putrefaction could take place with the resultant acid and odor production.

Another important application of enzymes in the area of oral hygiene is concerned with plaque formation. Dental plaque is composed primarily of protein, as shown in Table III. The low carbohydrate content of plaque is not surprising, since, as mentioned above, one would expect the salivary amylase to prevent the accumulation of such insoluble carbohydrates as starch.

One day after deposition on the teeth, plaque becomes quite difficult to remove by toothbrush alone. It is likely that a troche containing the proper proteolytic enzyme can be used to diminish the rate of plaque formation. Prevention of the initial deposition of protein by enzymatically degrading this foodstuff should inhibit plaque formation.

III. Formulations

There are few special requirements that have to be met when choosing an enzyme for a formulation and the wide assortment of enzymes currently available (or available in the future) serves to ease the burden on the formulator.

An enzyme should be considered as merely another active ingredient that is present in a product. However, often the choice of enzyme dictates the composition of the topical preparation.

A. Enzyme Selection

Some of the factors important in enzyme selection are the following:

1. Nature of Substrate. That the nature of the substrate imposes restrictions on the formulator is obvious, but this is slightly complicated by the fact that there are many enzymes from which one could choose to act on a particular class of substrate. For example, the term "proteolytic" covers a multitude of enzymes, and it is important to know other factors possibly influencing enzyme activity that are associated with the substrate in question.

2. pH of the Environment. For most topical applications the pH range of the skin is used as the basic frame of reference. While it is known that the pH of the skin varies throughout the body, it is generally agreed that the range is pH 4.2–5.6 (5, p. 16). Enzymes should be chosen that are active at these pHs; otherwise the formulation must be buffered to the pH at which activity will be exhibited. Irritation of the skin may, of course, result if the formulation is buffered at a poorly tolerated pH.

It is also important to consider the pH requirements of *each* enzyme if a mixture is employed. There is no point in attempting to combine enzymes with different requirements in the same formulation. Fortunately, the

wide variety of these catalysts available from microbial sources allows the formulator a large degree of freedom in this area.

3. Stability Characteristics of the Enzyme. In addition to the pH-*activity* characteristics of an enzyme, the formulator must be cognizant of the pH-*stability* qualities as well. Selection must be based on a knowledge that the enzyme would be stable in a formulation in the pH range 4.2–5.6. Other factors will be discussed below which contribute to the longevity of an enzyme in a formulation.

B. Dosage Form

Enzymes can be incorporated in all of the typical dosage forms used by the cosmetic chemist, e.g., ointments, lotions, etc. However, this does not mean that *all* enzymes can be incorporated into *all* existing ointments and lotions. The following are some of the areas of special concern:

1. Enzyme Compatibility. Formulators will immediately recognize the importance of avoiding basic incompatibilities between the enzymes and other components in a formulation. It should be noted, though, that the form an ointment, lotion or emulsion might take could be responsible for the stability characteristics of an enzyme. Many enzymes are susceptible to oxidation, resulting in loss of activity, and most are relatively unstable in solution in an aqueous medium in the absence of stabilizers.

It has been our experience that incorporation, as a suspension, in a non-aqueous vehicle provides for excellent product shelf life. The main difficulty with this approach is associated with the need for the suspended enzyme to leach out, as a solubilized agent, following application to the skin. If this does not occur, the enzyme will remain suspended, and its activity will not be available. On the other hand, if it leaches out of the oil phase too readily, then the shelf life of the product could suffer.

Since the distribution of an ingredient is inherently more uniform when it is in solution rather than suspension, it may be advisable to seek ways to include the enzymes as stable components of the aqueous phase of an emulsion. It is sometimes possible to prepare stable preparations without resorting to the suspension technique by protecting the enzyme in the aqueous phase by such polyhydric compounds as sorbitol or glycerol, and this, in turn, is made the internal phase of a water-in-oil emulsion. In this way the oil also acts as a protective coating around the enzyme-containing aqueous phase.

Of course, it is also possible to incorporate enzymes into a completely nonaqueous ointment. In addition to the drawback that greasy, inelegant cosmetic preparations have, the lack of any moisture in the formulation could hinder or prevent the proper functioning of the enzyme. Unless an extraneous source of moisture is applied, the enzyme must depend on body exudates to supply the necessary water for enzyme solubilization and for

the hydrolytic reactions to take place. This is more likely to occur when the enzyme is applied to a wound which is actively discharging serum, etc. However, for most cosmetic applications, free tissue water will be absent and must therefore be supplied as part of the formulation.

2. Base Compatibility. This refers to the other side of the coin; the stability of the emulsion in the presence of the enzymes. One cannot expect to obtain a stable emulsion with a lipid base in the presence of a lipolytic enzyme, nor can one expect a surfactant that is an ester to withstand the hydrolytic activity of the esterases. So many commercial enzyme products contain esterase activity present, either as nonspecific contamination or as part and parcel of proteolytic activity, that the selection of the proper emulsifying agent may become a major problem to the formulator. He must not only be certain that the surfactant is compatible with the enzyme but must be concerned with the reverse condition, as well. In some cases switching from an ester to an ether is all that may be necessary, whereas in others the search for the appropriate emulsifier could become a major research project.

3. In Vivo Activity. An ingredient in an ointment can react only at the interface between the skin and the base. One might then expect *in vivo* enzyme activity to be a function of enzyme concentration up to the point where the interface becomes saturated. In some instances this could be modified by the proper selection of a hydrophilic or lipophilic base.

A hydrophilic ointment should serve to attract moisture into a nonaqueous vehicle so that all of the enzyme at the interface would be reactive at any one time. A lipophilic base might be expected to keep at least part of the enzyme inactive throughout a period of contact by preventing the uptake of water.

Both types of bases can serve useful purposes, if we assume that a given enzyme would be equally stable in both. If we wanted most rapid action it would appear that the hydrophilic ointment would be more suitable. On the other hand, the lipophilic base would offer a degree of "sustained release" by virtue of the enzyme's inability to hydrate all at once.

4. In Vitro Activity. Usually the activity of a reagent *in vitro* is not considered if it can be shown to function properly *in vivo.* In the case of enzymes, however, a label claim can be made for a product only if the active ingredient can be assayed.

It has been our experience that the dosage form can be a critical factor in determining whether an enzyme can be quantitatively recovered from a product. More often than not, if the enzyme cannot be recovered under the optimum conditions in our laboratory, it will not be available as an active agent *in vivo.*

As might be expected, vehicles with high Hydrophilic-Lipophilic Balance (HLB) pose the least problem. On the other hand, mixtures that have to

be extracted with a fat solvent in order to free the enzyme create the greatest concern. One example of such a problem is the inability to distinguish between poor recovery due to poor stability, or due to the technique used to separate the water soluble protein from the lipophilic base. The proper selection of the type of ointment, as well as the components with which it is made, can spell the difference between being able to demonstrate both *in vitro* and *in vivo* activity and having a preparation with the enzyme so bound as to make it virtually useless.

And finally, it should also be recognized that an enzyme can be chemically, as well as physically, complexed to some components of cosmetic preparations, due to the large number of reactive groups present on all proteins. An example of this particular type of problem is the complexing of certain types of enzymes with carboxymethylcellulose, which appears to occur *via* an ion exchange-type of mechanism. Sometimes reactions of this nature can be minimized by adjusting pH or preferentially complexing the proteins with reagents that will not interfere with the activity, or the physical stability, of a cosmetic preparation.

C. *Areas of Questionable Potential*

The cosmetic chemist most certainly will become better acquainted with the desirable features of enzymes that make them so suitable for cosmetic purposes. This does not mean that enzymes will become the panacea; there are many areas in which nonenzymatic methods are, and will remain, superior.

For example, it would take a most remarkable enzyme to function as a cuticle remover as effectively as do the inorganic alkalies currently in use. Also, as indicated above, an enzyme with the characteristics of the "ideal" depilatory more than likely does not exist.

The areas of most questionable potential are those in which the demand is for an agent to react instantaneously on an insoluble substrate. The critical parameter as far as the enzymes are concerned is the insoluble substrate with which the cosmetic chemist is so often concerned. Under the proper conditions enzymes can perform in the desirable fashion, if the substrate is readily available. Unfortunately, many of the conditions with which the cosmetic chemist must deal are those that are least compatible with the requirements of the enzymes. However, by adjusting some of these, such as the extension of contact time, choice of suitable dosage form, etc., some of the drawbacks can be overcome.

In those areas where nonenzymatic reagents may be more economical and function as well as or better than their enzymatic counterparts, it would be pointless to consider an enzyme as the active ingredient. However, there are areas in which enzyme specificity would offer such advantages (e.g., in cases of "oily skin" it may be possible to remove specific

portions of sebum which would otherwise be completely removed with fat solvents) that the development of such products would be well worth the added effort.

IV. Summary

Whether or not an enzyme can be used for a given topical application depends on the nature of the cutaneous disorder as well as the limitations associated with the enzyme activity. To a large extent, the physiology and anatomy of the skin dictate the degree of success an enzyme preparation might enjoy as a cosmetic or therapeutic agent. (The distinction between "cosmetic" and "therapeutic" has become extremely difficult to define and is in all probability more a question of semantics than of practical importance.)

The enzyme group that has proved to be quite useful is the proteases, some of which have more suitable specificities than others. In addition, the search by suppliers for new enzymes active on the more refractory substrates continues.

Recent studies of enzyme formulations suitable for topical use have shown that such dosage forms are relatively easy to handle. However, the choice of base, surface active agent, etc., is important to provide for a stable formulation. The choice of base is also important in determining the ease with which the enzyme present can be assayed. Since enzymes can act only in an aqueous environment, proper vehicle selection is also critical for the proper activity *in situ*.

The mild conditions associated with the enzymatic process, dovetailed with the characteristics of healthy and pathological skin, provide for optimism as far as the potential of enzymes for topical application is concerned. While all of the requirements of the cosmetic chemist cannot be met with currently available products, enzyme research and development is being conducted with these in mind.

(Received February 8, 1963)

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ODOR AND ISOMERISM IN OLFACTIVE CHEMICALS

By E. H. Eschinasi, Ph.D.*

Presented February 28, 1963, Meeting, New England Chapter

Abstract

This paper describes some typical cases of odor variations as functions of isomeric changes in certain known species of olfactive chemicals. Selected examples are used to illustrate the theoretical and practical importance of the isolation and stereospecific synthesis of pure odorant compounds.

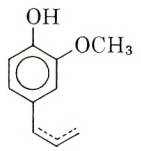
The olfactive evaluation of essential oils, isolates and aromatic chemicals leads quite often to divergent and sometimes conflicting results for the organic chemist working in this field of challenging problems. Indeed, few are the chances of encountering the same absolute odor likeness in different samples of the same essential oil or isolate. Variation due to geographic origin, seasonal changes and different means of extraction, handling and preparation of the essential oils and their related products are chief contributors to odor variance.

Major differences in odor found in similar isolates of varying origin have been traced to the presence of different minor constituents originally present in their respective parent oils. The complete removal of these so-called impurities from the isolates is often difficult and seldom even desirable. In other instances, however, odor variations could be related to fluctuations of some specific stereoisomeric olfactive species.

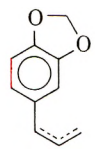
With the remarkable progress made in analytical and preparative techniques in chemistry, especially in the field of gas-liquid chromatography, it became possible, in the last decade, to prepare isolates and synthetic compounds of unusual chemical purity. The use of preparative chromatographic columns, equivalent to fractionating columns having an efficiency of more than thousand plates, afforded for the first time a practical and rapid separation of pure stereoisomers. The availability of chemicals of reliable structures contributed to a greater measure of objectivity and reliability in their olfactive evaluation. As a result, the study of the relationship between odor and chemical structure advanced a step further and helped, in some instances, to dramatize the effect of isomerism on odor variations.

* The Givaudan Corp., Delawanna, N. J.

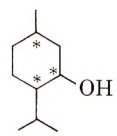
The existence of isomers in aromatic chemicals and their contribution to the olfactive distinctiveness has long been known. The eugenols, safroles, menthols, ionones and many others have been known for quite some time, and their use in the aromatic industry is quite well established.



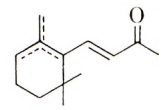
Eugenols



Safroles



Menthols



Ionones

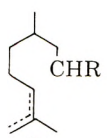
Continuing advances in the gas-liquid chromatographic techniques have made possible the resolution and separation of many pure isomeric species and simultaneously promoted the search for new stereospecific methods of synthesis.

Although the nature of the relation between odor perception and chemical structure remains as complex as ever (1), some measure of comfort is taken from the knowledge that real progress can be achieved in this field with research now carried out with absolutely pure olfactive chemicals of known conformation and structure.

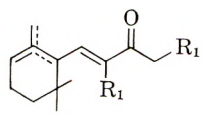
Some of the odor variations and their relation to isomerism will be reviewed and the synthesis of a number of pure olfactive isomeric species will be described.

A few typical examples have been chosen to illustrate the odor differences between isomers and to emphasize their economic importance. The examples include the citronellyl and rhodinyl series (I), the methylionones (II), the irones (III), the Nerones®* (2) (IV), the rosenoxides (V) and the hexenols (VI and VII).

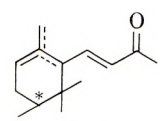
R = H_2,HOH, O, HO Acyl. R_1 = H, CH_3



(I)



(II)



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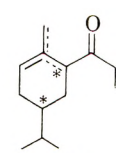
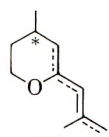
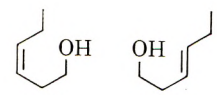


(IV)



(V)



(VI) (VII)

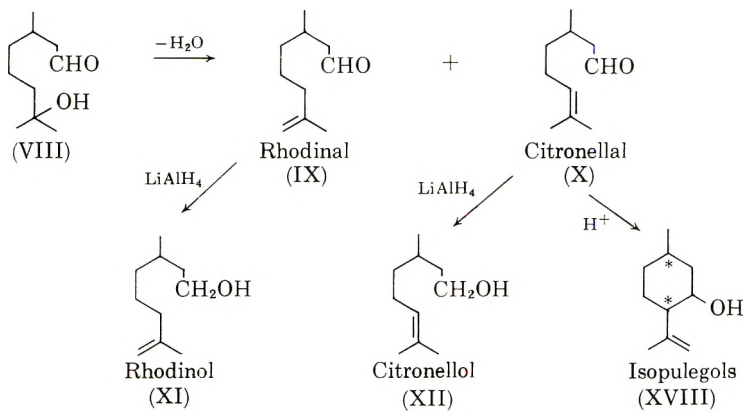
* Nerone is a registered trademark of The Givaudan Corp.

These examples demonstrate the general types of isomerism encountered in this field, namely:

1. C—C double bond isomerism as in I, II, III and IV with alkene bonds in different position along the molecule
2. Skeletal isomerism as in II and III with the substituents in different positions along the molecule
3. Cis-trans isomerism as in III, IV and V with different spatial arrangement of substituents around a fixed or cyclic structure
4. Cis-trans isomerism like VI and VII with different geometrical arrangement of the alkene bond substituents

An important contribution to the solution of the many stereochemical problems has been made through the study of the conformational analysis of the cyclohexane ring and its derivatives. The menthols, Nerones, irones and rosenoxides provide an excellent illustration of the theoretical and practical benefits derived from such studies. The synthesis of (neo) α-irone and cis-α-nerone to be described provide solid proof of the practical benefit derived from the knowledge of conformational analysis.

This discussion can be conveniently started with one of the long disputed cases of isomerism which involved the rhodinol-citronellol structures.



The early work of Barbier and Loquin (3) reporting the formation of rhodinol (XI), an isomer of citronellol (XII) with a marked and finer rose odor, started the old and well publicized controversy on the citronellol-rhodinol structures (4, 5). Subsequently, Verley (4) and other workers (6) proposed a structure for rhodinal (IX), an aldehyde obtained from the dehydration of hydroxycitronellal (VIII), different from that of citronellal (X). Substantial work based on ozonolysis and spectroscopic data (7) was since undertaken to decide on the correct structures. In this case the picture was unfortunately clouded by the easy conversion of citronellal (X) into isopulegols (XIII) which possess the same methylenic group as rhodinal (IX) and, therefore, yielded similar degradation products on

ozonolysis. The issue remained undecided for a number of years, leading finally to the belief that rhodinal and citronellal as well as their corresponding alcohols and derivatives consisted of inseparable mixtures of the two structural forms (8).

Recently (9) the citronellol-rhodinol controversy was definitely settled when it was demonstrated that both citronellol and rhodinol were capable of individual and separate existence. The syntheses of pure rhodinal and its derivatives were achieved, and no spontaneous equilibration between the rhodinyl and the citronellyl forms was ever noticed.

Both rhodinal and citronellal were reduced by the Wolff-Kishner method to 2,6-dimethyl-1-octene and 2,6-dimethyl-2-octene, respectively. The ozonolysis of the olefin from rhodinal afforded 6-methyl-2-octanone and that from citronellal, 4-methyl-hexanol. The ozonolysis of rhodinol yielded 6-methyl-8-hydroxy-2-octanone whereas ozonolysis of citronellol resulted in a complex mixture of cyclic derivatives of 4-methyl-6-hydroxy-hexanal (9). Olfactive tests confirmed the more fragrant and subtle rosy character of the rhodinyl in comparison with the citronellyl series. Rhodinol and its esters as well as rhodinal in particular blend exceedingly well with rose and muguet compositions.

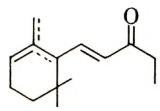
It is worthwhile mentioning in this connection that the first gas-liquid chromatographic studies with the rhodinyl and citronellyl series met with one of the few instances of failures. Indeed, the two isomeric species always appeared in the chromatograms as single unresolved peaks. Only the discovery of the unusual stability of rhodinal toward mild acids (9), as compared to the easy cyclization of citronellal into isopulegols under the same conditions, made it possible to devise an adequate chromatographic analysis of a mixture of both aldehydes.

Mixtures of citronellal, rhodinal and isopulegols were analyzed by gas-liquid chromatography using alternatively a neutral and an acidic column. Rhodinal was resolved as a distinct peak over an acidic Celatom FM73 column (9), from the common peaks of isopulegols which included the citronellal. In a neutral Chromosorb W column, on the other hand, both rhodinal and citronellal showed up as a single unresolved peak distinct from those of the isopulegols. The amount of citronellal was easily deduced by difference. Today the use of capillary chromatographic columns, coupled with the recently available ion detectors, has increased the sensitivity of the chromatographs resulting in a better separation of the citronellyl and rhodinyl species.

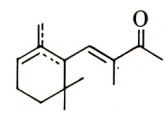
The commercial availability of a pure grade of (citronellal-free) rhodinal is based on its resistance to acid cyclization and its separation from isopulegols through boration. The chemical solution of the citronellol-rhodinol problem has not as yet affected the custom, prevalent in the trade, of using the term, "rhodinol," to describe a grade of *levo*-citronellol ob-

tained from geranium oil as distinct from a grade of citronellol isolated from citronella oil. It is quite possible, in view of the recent work on the composition of geranium oil, by Naves and his co-workers (10), that some minor constituents of the geranium oil including rosenoxide present in rhodinol of the trade contribute markedly to its superior fragrance and greater olfactive value when compared with the common grade of citronellol from citronella oil.

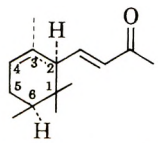
In the field of the methylionones and irones an increasing demand for the supply of pure isomers has been evident for some time; also certain preferred mixtures of isomers of the methylionones are highly prized by perfumers for their fine odors. The right combination of isomers is apparently at the base of the secret of their commercial success. Here,



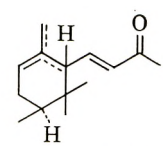
Methyl-Ionones (XV)



Isomethyl-Ionones (XIV)



α-Irone (cis) (XVI)



Iso-α-Irone (trans) (XVII)

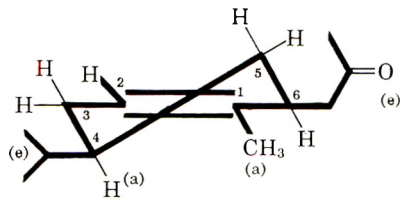
isomerism includes double bond isomerism (group 1), skeletal isomerism (group 2) and spatial cis-trans conformations (group 3).

The practical value of isomethylionones has been emphasized in a recent patent by Beets and V. Essen (11) on the ways of preparation of the more fragrant α-isomethylionone (XIV), which possesses a finer violet note than methylionones (XV). Specific conditions for the reaction of citral with 2-butanone are reported which are said to favor the aldol condensation with the methylene group of the 2-butanone.

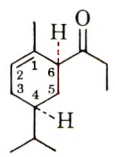
In addition to the orignal work of Haarman and Reimer (12) on the preparation of the methylionones, extensive studies have been carried out by Naves, Kitchens (13) and others regarding the effect of various cyclizing agents on ionones and their derivatives. Of particular interest is the desirable effect of boron trifluoride on the cyclization of trans-pseudoirones reported by Naves (14) to yield mainly α-irone (XVI). Under the same conditions, both 85% phosphoric acid and 62% sulfuric acid yield iso-α-irone (XVII) as the major constituent. Besides their skeleto-isomeric differences with the methylionones, steric differences exist between the α- and iso-α-irones in the cis-trans spatial arrangements of the ring substituents in the 2 and 6 positions (15). As reported by Naves (16), the olfactive value of the α-irone, which recalls iris odor, supersedes by far that of

the iso-α-irone, which recalls the more common methylionones. The cis- and trans-(neo)-arrangements of the butenone side chain do not seem to affect appreciably the odor of the α- and iso-α-irones, which are both assumed to acquire the most stable trans- or neo-configuration.

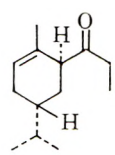
An interesting case involving a spatial cis-trans isomerism of the substituents (group 3) is well illustrated by the case of Nerone® (XVII), 1-(p-menthen-6-yl)-1-propanone (2).



Nerone (XVIII)



cis
$\alpha_D = -133°$
$n_D = 1.4711$
$d_4 = 0.9087$



trans
$\alpha_D = +44°$
$n_D = 1.4736$
$d_4 = 0.9110$

When originally synthesized the ratio of cis- and trans-Nerone at equilibrium was approximately 2:1. Upon separation, it was found that the olfactive properties of the cis-isomer were far superior to those of the trans-isomer. Indeed, the cis-configuration imparted the product with a very pleasant and fragrant odor recalling bergamott and citrus leaves, while the trans-Nerone was bland and almost odorless.

The conformational analysis applied to the cyclohexane ring of Nerone requires the two large substituents (isopropyl and propanone groups) of the cyclohexene ring to lie in the equatorial position (e) for the more stable cis-configuration. The conformation of the trans-isomer, on the other hand, assigns to one of its two substituents an axial (a) and less stable spatial configuration.

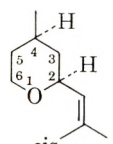
This was indeed the case and was confirmed by the lower constants (density, refractive index and lower boiling point) of the more expanded and stable cis-configuration as compared to the higher constants of the puckered and less stable trans-structure.

From past knowledge of conformational analysis a practical and successful conversion of the trans-isomer into the more desirable and stable cis-isomer could be expected by disturbing the thermodynamic equilibrium of

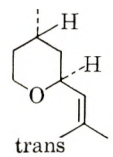
the isomeric species. This was indeed achieved by taking advantage of the greater thermal stability of the cis-isomer. This conversion was easily followed by observing the reversal of the optical rotation of the *dextro*-trans isomer ($\alpha_D = +44°$) into the *levo*-cis-isomer ($\alpha_D = -133°$). Some irreversible formation of the β-isomer as a by-product of the trans-cis-arrangement was accompanied by loss of optical activity through racemization resulting from the migration of the alkene bond. Here again, as in the ionone series, the β-Nerone isomer did not possess an odor of particular interest.

A similar type of spatial cis-trans isomerism occurs in rosenoxide present in rose and geranium oils (17). As in the case of Nerone this isomerism is the result of a cis-trans spatial configuration of the 2,4 substituents of the tetrahydropyrane ring.

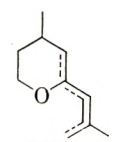
LEAF ALCOHOL



(XIX)



(XX)



(XXI)

The cis-isomer (XIX) which is the major component (80–85%) of the natural rosenoxide happens to possess the finest odor characteristics. By analogy with parent cyclohexane derivatives the conformational analysis of this meta substituted tetrahydro pyrane assigns its substituents (methyl and methylpropenyl groups) to the equatorial position for a most stable cis-configuration. Other double-bond isomers of rosenoxide (XXI), and in particular the vinyl ether isomers, show little olfactive value unless readily converted to cis-rosenoxide.

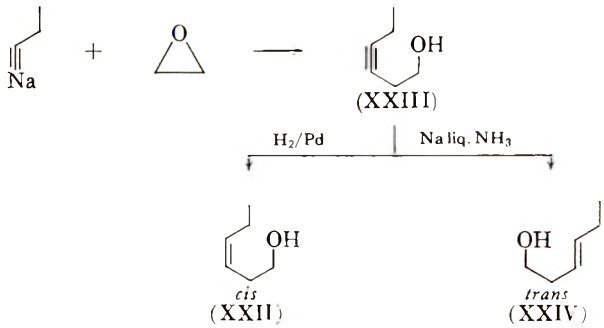
Finally, leaf alcohol, a valuable component in violet compositions, illustrates the last case of a typical alkene (cis-trans) isomerism (group 4). Ever since the olfactive importance of leaf alcohol, cis-hex-3-en-1-ol (XXII), was recognized its structure was the subject of intensive research (18) which culminated in its preparation.

Its synthesis was achieved by Stohl, Sondheimer and other workers (19) through the selective palladium hydrogenation of the corresponding hex-3-yn-1-ol (XXIII). The latter was obtained through the condensation of 1-butyne sodium with ethylene oxide. The trans-hex-3-en-1-ol (XXIV) obtained by the sodium/liquid ammonia reduction of the corresponding hexynol has no great olfactive value and does not possess the green leafy fragrance almost indispensable in violet compositions.

The theoretical and practical benefits derived from the knowledge gained through the stereochemical studies of olfactive substances is obvious. The

ROSENOXIDES

Hex-3-en-1-ol



proper use of sophisticated instrumentation in the field of gas-liquid chromatography, nuclear magnetic resonance, infrared and ultraviolet spectroscopy, coupled with the necessity of devising ingeneous stereospecific syntheses, constitute a constant challenge exciting to meet for those active in the field of research in olfactive chemicals.

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CHEMICAL STRUCTURE AND ANTIMICROBIAL ACTIVITY OF BIS-PHENOLS. III. BROAD SPECTRUM EVALUATION OF HEXACHLOROPHENE AND ITS ISOMERS

By William S. Gump, Ph.D., and George R. Walter, Ph.D.*

Abstract

Symmetrical methylenebis-(trichlorophenol) isomers being either 2,2′-, 3,3′- or 4,4′- with regard to the methylene bridge were investigated for their antimicrobial activity against a broad spectrum of bacteria, molds and yeasts. There are 10 possible isomers, including hexachlorophene; two of them, 2,2′-methylenebis(3,5,6-trichlorophenol) and 4,4′-methylenebis(3,5,6-trichlorophenol), whose preparation would have been very difficult, had been omitted from the study. Maximum activity against bacteria was shown by the 2,2′-methylenebis-phenols; 2,2′-methylenebis(3,4,5-trichlorophenol) was found to be generally more potent than hexachlorophene and the other 2,2′-isomer. Great variations between the 3,3′-isomers were noted; the 4,4′-isomer was definitely the least active of the compounds tested. Against the dermatophytes, the activity of the isomers was found to be of the same magnitude. None of the bis-phenols was active against *C. albicans* or *P. ovale* at a concentration of 100 μg./ml.

A previous investigation pertained to the bacteriostatic and fungistatic properties of a number of bis-phenols and a discussion of the relationship between chemical structure and antimicrobial activity (1). This work was then extended to an evaluation of the bactericidal properties of a selected group of the same bis-phenols in the presence of a synthetic anionic surfactant (2).

Initially, 65 compounds were investigated for activity against three microörganisms. It would seem to be of interest, from the viewpoint of structural specificity and biological activity, to evaluate the closely related methylenebis (trichlorophenol) isomers against a broader spectrum.

There are ten possible symmetrical configurations of the methylenebis-(trichlorophenols) being either 2,2′-, 3,3′- or 4,4′- with regard to the methylene linkage. Two isomers, 2,2′-methylenebis(3,5,6-trichlorophenol) and 4,4′-methylenebis(3,5,6-trichlorophenol), have been omitted because of the complicated preparation of the starting material, 2,3,5-trichlorophenol (3, 5). Furthermore, condensation of 2,3,5-trichlorophenol with

* Sindar Corporation, Delawanna, N. J.

TABLE I—METHYLENEBIS(TRICHLOROPHENOL) ISOMERS

No.	Isomer	M.p. °C.	Analyses, % Chlorine† found
1	2,2′-methylenebis(3,4,6-trichlorophenol)*	161–163	....
2	2,2′-methylenebis(4,5,6-trichlorophenol)	220–222	51.9
3	2,2′-methylenebis(3,4,5-trichlorophenol)	193.5–195	52.1
40	3,3′-methylenebis(2,4,6-trichlorophenol)	208–211	51.8
65	3,3′-methylenebis(2,4,5-trichlorophenol)	239–241	52.2
66	3,3′-methylenebis(2,5,6-trichlorophenol)	184–186	52.2
67	3,3′-methylenebis(4,5,6-trichlorophenol)	240–243	51.7
42	4,4′-methylenebis(2,3,6-trichlorophenol)	166–167.5	52.3

* Sample of commercial hexachlorophene, U.S.P. (Sindar), manufactured according to U. S. Patent 2,812,365 (1957).

† Calculated for $C_{13}H_4O_2Cl_6$: Cl, 52.3%.

formaldehyde would lead to a mixture of the 2,2′-, 4,4′- and 2,4′-methylenebis compounds whose separation would be extremely difficult.

The methylenebis(trichlorophenols) are listed in Table I with their melting points and chlorine contents. The organisms employed for microbiological evaluation are given in Table II.

EXPERIMENTAL

I. Chemical

Pure 2,4,5-trichlorophenol, 2,3,4-trichlorophenol and 2,3,6-trichlorophenol were kindly provided by Hooker Chemical Corporation, Niagara Falls, N. Y. 3,4,5-Trichlorophenol was prepared from pract.2,6-dichloro-4-nitroaniline (Matheson Co., East Rutherford, N. J.) as described

TABLE II—ORGANISMS EMPLOYED FOR THE EVALUATION OF METHYLENEBIS(TRICHLOROPHENOL) ISOMERS

Name	American Type Culture Collection No.
Staphylococcus aureus	6538
Staphylococcus epidermidis	155
Bacillus subtilis	9372
Bacterium ammoniagenes	6871
Proteus vulgaris	9920
Escherichia coli	11229
Salmonella typhosa	6539
Pseudomonas aeruginosa	....
Pseudomonas fluorescens	11251
Shigella sonnei	....
Klebsiella pneumoniae	10031
Trichophyton mentagrophytes	9129
Trichophyton rubrum	10218
Microsporum audouini	11347
Candida albicans	10231
Pityrosporum ovale	....
Clostridium tetani	8033
Clostridium perfringens	8009
Clostridium sporogenes	11437
Chlorella vulgaris	9765

by Johary, *et al.* (4) and Tiessens (5). 6-Bromo-2,3,4-trichlorophenol (6) was obtained by bromination of 2,3,4-trichlorophenol in acetic acid solution; similar procedures yielded 6-bromo-2,4,5-trichlorophenol (6) from 2,4,5-trichlorophenol and 4-bromo-2,3,6-trichlorophenol (7) from 2,3,6-trichlorophenol.

Bis-phenols were obtained by condensation of the monophenols with formaldehyde in the presence of ethylene dichloride and 20% oleum or 96% sulfuric acid. Reaction ortho or para to the hydroxyl group of the phenols proceeds rapidly whereas linkage in the meta position requires considerably more time. The 3,3′-methylenebis compounds with an unsubstituted ortho or para position (Nos. 65, 66, 67 in Table I) were synthesized by blocking these positions in the trichlorophenols with bromine, condensing the bromotrichlorophenols with formaldehyde and removing the bromine from the 3,3′-methylenebis(bromotrichlorophenols) with zinc dust in potassium hydroxide solution (8). The crude bis-phenols were purified by repeated crystallization from various solvents. No efforts were made to obtain maximum yields. Specific details for the preparation of each of the isomers are as follows:

2,2′-Methylenebis(4,5,6-trichlorophenol), No. 2

A mixture of 50 g. of 2,3,4-trichlorophenol, 125 ml. of ethylene dichloride and 20 g. of tech. oleum (20–23% SO_3) was stirred and heated at 55–60°. Paraformaldehyde (4.1 g.) was added over a period of one hour, and the resultant mixture was refluxed for two hours. After cooling to room temperature, crushed ice and water were carefully added, and the solvent and unreacted trichlorophenol were removed by steam distillation. The crude product was filtered, washed with water and dried (42 g.). The substance was recrystallized from 900 ml. of ethylene dichloride using Filtrol® (Filtrol Corp., Los Angeles, Calif.) as a decolorizing agent; white needles (31 g.) were obtained.

2,2′-Methylenebis(3,4,5-trichlorophenol), No. 3

A solution of 4 g. of 37% aqueous formaldehyde in 6 ml. of methanol was added dropwise to a stirred mixture of 20 g. of 3,4,5-trichlorophenol, 70 ml. of ethylene dichloride and 20 ml. of 96% sulfuric acid over a period of two hours. The batch was then heated to 60° for two hours. After dilution with water, the solvent and the unchanged trichlorophenol were removed by steam distillation. The remaining solid was filtered, washed with water and dried (17 g.). A slurry was made with 100 ml. of acetone to separate the desired substance from an acetone-insoluble by-product of higher molecular weight. The acetone slurry was filtered and the bis-phenol precipitated by dilution with water. The pure substance (7 g.) was obtained by recrystallization of the dried precipitate from a small amount of toluene with the aid of Filtrol.

4,4'-Methylenebis(2,3,6-trichlorophenol), No. 42

Condensation of 2,3,6-trichlorophenol (50 g.) with paraformaldehyde was performed in the same manner as described for compound No. 2, except that the mixture was refluxed for 4 hours. Crystallization of the crude material (46 g.) from 150 ml. of ethylene dichloride with addition of Filtrol yielded 34 g. of white, fine needles.

3,3'-Methylenebis(2,4,6-trichlorophenol), No. 40

The procedure was similar to that used for the preparation of compound No. 2. Paraformaldehyde (5.6 g.) was added to a mixture of 70 g. of 2,4,6-trichlorophenol, 150 ml. of ethylene dichloride and 35 g. of oleum. The mixture was refluxed for twenty hours and processed by steam distillation as described above. The crude bis-phenol (64 g.) was crystallized from 250 ml. of toluene with addition of Filtrol. A low purity product which softened from 190° and melted at 198–200° was obtained (37.6 g.); it was therefore recrystallized from 145 ml. of acetic acid and then from 120 ml. of ethylene dichloride which yielded 9.1 g. of white, crystalline powder.

3,3'-Methylenebis(2,4,5-trichlorophenol), No. 65

1A. 3,3'-Methylenebis(6-bromo-2,4,5-trichlorophenol). A mixture of 105 g. of 6-bromo-2,4,5-trichlorophenol, 200 ml. of ethylene dichloride, and 105 g. of oleum was stirred and heated to 75°. A slurry of 5.5 g. of paraformaldehyde in 50 ml. of ethylene dichloride was added over a period of one hour. Stirring and refluxing were continued for twenty-four hours. The batch was processed as before; recovery of unchanged 6-bromo-2,4,5-trichlorophenol amounted to 33 g. The crude material was crystallized from 730 ml. of methanol plus 175 ml. of dioxane with the addition of Nuchar® (West Virgina Pulp and Paper Co., New York, N. Y.) The resulting 61 g. were recrystallized from 960 ml. of toluene plus 100 ml. of dioxane, Filtrol being used for decolorization. A light, tan-colored product (48 g.) was obtained with a m.p. of 275–278°.

1B. 3,3'-Methylenebis(2,4,5-trichlorophenol). A solution of 48 g. of 1A in 400 g. of potassium hydroxide and 2000 ml. of water was heated to 85° and 105 g. of zinc dust added with vigorous stirring during a period of two hours. Agitation and heating at 85–90° were continued for five hours. The mixture was filtered, and ice was added to the filtrate, which was made acid to Congo Red paper with hydrochloric acid. The precipitate was filtered, washed with water and crystallized from 600 ml. of methanol and 150 ml. of water. The solution was held at −10° overnight, after which the resulting product (16.1 g.; m.p. 235–238°) was recrystallized from 600 ml. of petroleum naphtha (b.p. 120–135°) and 80 ml. of dioxane. Yield: 13 g. of white, crystalline powder.

3,3'-Methylenebis(2,5,6-trichlorophenol), No. 66

IIA. 3,3'-Methylenebis(4-bromo-2,5,6-trichlorophenol). Condensation of 4-bromo-2,3,6-trichlorophenol (105 g.) with paraformaldehyde was conducted as described for IA. Crystallization of the crude bis-phenol from 420 ml. of methanol and 190 ml. of water with addition of Nuchar yielded 76 g. of dark material, m.p. 204–212°. Recrystallization from 300 ml. of petroleum naphtha plus 300 ml. of toluene, with Filtrol as decolorizing agent, and then from a mixture of 100 ml. of carbon tetrachloride and 250 ml. of ethylene dichloride (plus Filtrol) yielded 30 g. of a tan-colored product, m.p. 222–225°.

IIB. 3,3'-Methylenebis(2,5,6-trichlorophenol). Debromination of 30 g. of IIA was performed in the same manner as that of IA. The crude substance (19 g.; m.p. 138–148°) was crystallized from 100 ml. of methanol and 50 ml. of water (plus Nuchar). The first fraction (5.8 g.; m.p. 169–175°) was recrystallized from 40 ml. of toluene and then from 30 ml. of methanol. White, small crystals (2.7 g.) were obtained.

3,3'-Methylenebis(4,5,6-trichlorophenol), No. 67

IIIA. 3,3'-Methylenebis(2-bromo-4,5,6-trichlorophenol). 2-Bromo-4,5,6-trichlorophenol (60 g.) was converted into the bis-phenol as described for IA. The crude product (39 g.) was twice crystallized from methanol plus dioxane; 27 g. of satisfactory material, melting at 294–295°, was obtained.

IIIB. 3,3'-Methylenebis(4,5,6-trichlorophenol). Treatment of the alkaline solution of IIIA with zinc yielded 16.6 g. of crude material which was first dissolved in boiling 80% v./v. aqueous methanol; 1.5 g. remained insoluble. On cooling the filtrate, 9.1 g. (m.p. 211–214°) was obtained. Subsequent crystallization from 300 ml. of petroleum naphtha, from 160 ml. of toluene (plus Filtrol), and finally from 20 ml. of methanol yielded 2.3 g. of the pure substance.

II. Microbiological

Each of the compounds was dissolved in alcohol (specially denatured No. 30) to make a 1% stock solution. Twofold serial dilutions of the stock solution were made in alcohol, and 0.2 ml. of each dilution level was added to a tube containing 20 ml. of 50° molten agar. The tube was swirled thoroughly to distribute the test compound and the contents were poured into a sterile plate. Hardened plates were surface-inoculated with an Accu-Drop dispenser (Scientific Products, Flushing, L. I., N. Y.). Agar employed for bacteria was Dextrose Tryptone Extract Agar (Difco Laboratories, Inc., Detroit, Mich.); Sabouraud's Dextrose Agar (Difco) was used for yeasts and molds. The American Type Culture Collection liquid

medium and Fluid Thioglycollate (Difco) broth for the Chlorella and anaerobic bacteria, respectively, were also employed.

Inocula for the aerobic bacteria consisted of one drop (0.007 ml.) of a 1–100 distilled water dilution of a 24-hour, 35°, Tryptic Soy Broth (Difco) culture onto the surface of the agar plates. Inoculum for *Chlorella vulgaris* consisted of one drop of a 1–100 dilution of a 5-day, 25° culture to 10 ml. of broth containing the test compound. Anaerobic bacterial inocula consisted of one drop of a 1–100 dilution of a 72-hour, 35° broth culture to 15 ml. of broth containing the test compound. Yeast inoculum was one drop per plate of a 1–100 water dilution of a heavy cell suspension prepared by the addition of 10 ml. of distilled water to a 72-hour agar slant. Mold inocula were prepared by suspending the conidia from the surface growth of a 7-day slant in 20 ml. of distilled water with no further dilution.

Incubation for aerobic bacterial plates was 35° for forty-eight hours, at the end of which the level completely inhibiting growth was recorded. Anaerobic tubes were incubated for fourteen days at 35°, after which bacteriostatic levels were recorded. Yeast results were noted after four days incubation at 30° and mold results after fourteen days at 30°. Inhibition of Chlorella was recorded at the end of thirty days incubation at 25° over two 30-inch fluorescent tubes at a distance of 20 inches.

Because of inherent error in the procedure, tests were performed in triplicate on different days for aerobic bacteria. Tests with all other organisms (cf. Table II) were performed in duplicate on different days. The average inhibitory levels, calculated as the geometric mean of the values obtained, are reported in Table III.

TABLE III—ANTIMICROBIAL SPECTRA OF METHYLENEBIS(TRICHLOROPHENOL) ISOMERS. (MINIMAL INHIBITORY LEVEL, μG./ML.)

Microorganism	Compound (by Number)							
	1	2	3	40	65	66	67	42
S. aureus	0.93	0.61	0.39	10	2.5	20	1.56	25
S. epidermidis	0.93	0.78	0.23	12.5	2.4	20	1.56	25
B. subtilis	0.19	0.39	0.19	3.9	1.9	9.9	1.56	15.6
B. ammoniagenes	0.39	0.48	0.19	15.6	2.4	40	1.56	31.5
P. vulgaris	3.9	1.9	3.9	25	*	63	10	63
E. coli	25	*	12.5	*	*	*	*	*
S. typhosa	40	*	10	*	*	*	*	*
Ps. aeruginosa	25	*	50	*	*	*	*	*
Ps. fluorescens	0.23	0.61	0.19	12.5	2.4	31.5	1.56	25
Sh. sonnei	40	*	9.9	*	*	*	*	*
K. pneumoniae	50	*	15.6	*	*	*	*	*
T. mentagrophytes	1.74	3.12	3.12	8.9	8.9	6.25	4.4	6.25
T. rubrum	4.4	3.12	8.9	8.9	3.1	1.56	1.74	9.9
M. audouini	3.12	1.74	3.12	3.12	4.4	3.12	8.9	4.4
C. albicans and P. ovale	*	*	*	*	*	*	*	*
Cl. tetani	0.19	0.39	0.09	1.56	0.27	1.74	0.19	4.4
Cl. perfringens	0.78	0.39	0.55	1.74	0.55	6.25	0.39	6.25
Cl. sporogenes	0.78	0.55	0.55	1.56	0.55	6.25	0.55	6.25
C. vulgaris	3.12	4.4	8.9	*	*	17.8	4.4	17.8

* Denotes growth at 100 μg./ml.

Discussion

It may be noted from Table III that each of the isomers tested was found to be biologically active and that the activity varied over a wide range in certain instances. Compound No. 42, which has the methylene bridge in the 4,4′-positions, was found to be the least active, which is in agreement with previous findings (1, 9). Of the 3,3′-methylenebis compounds, the weakest was No. 66, followed by No. 40. Why these compounds were decidedly inferior to Nos. 65 and 67 cannot be explained at this time. The most active 3,3′-isomer was No. 67 in which the three chlorine atoms are adjacent to each other. It was shown previously (1, 9) that maximum antibacterial activity of halogenated bis-phenols was observed with compounds containing the 2,2′-methylene bridge; the isomers of hexachlorophene are no exception to this rule. However, this is apparently true only for bacteria; against the dermatophytes the activity of the isomers was found to be of the same magnitude. Hexachlorophene and four of its isomers were very active against the Clostridia tested; less active were Nos. 42 and 66, which were also the poorest against the aerobic bacteria. A similar pattern was observed with *Chlorella vulgaris*. Hexachlorophene and all isomers consistently lacked activity against the two yeasts, *Candida albicans* and *Pityrosporum ovale*.

Major differences in bacteriostasis may be noted with the Staphylococci and other Gram-positive organisms, compound No. 3 being of outstanding activity. It might be of interest to note that compound No. 3 is the only isomer which has no chlorine substituents adjacent to the hydroxyl groups. Only hexachlorophene and one of the 2,2′-isomers (compound No. 3) demonstrated activity against the more resistant Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhosa*, *Pseudomonas aeruginosa*, *Shigella sonnei* and *Klebsiella pneumoniae*. *Proteus vulgaris* and *Pseudomonas fluorescens* were generally found to be extremely susceptible to all three 2,2′-isomers.

Summary

The broad spectrum antimicrobial activity of hexachlorophene and of seven of its nine isomers was investigated. Maximum activity against bacteria was shown by the 2,2′-methylenebis-phenols; 2,2′-methylenebis-(3,4,5-trichlorophenol) was found to be generally more potent than hexachlorophene and the other 2,2′-isomer. Great variations between the 3,3′-isomers were noted; the 4,4′-isomer was definitely the least active of the compounds tested. Against the dermatophytes the activity of the isomers was found to be of the same magnitude. None of the bis-phenols was active against *C. albicans* or *P. ovale* at a concentration of 100 μg./ml.

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TRANSPARENT EMULSIONS

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Abstract

The formulation of clear cosmetic "lotions" and "gels" is discussed in terms of Schulman's theory of microemulsions. Conditions required for the preparation of these microemulsions include a metastable negative interfacial tension and a liquid condensed interphase. It is shown that conventional emulsifying agents of both nonionic and ionic types can be used to prepare these systems. Agents are selected to condense and to expand the interfacial film. When these materials are present in the proper proportions, as determined by a "titration" procedure, a transparent emulsion results.

I. Introduction

There appears to be a growing interest on the part of cosmetic chemists in transparent fluid and gel cosmetic products containing appreciable amounts of both oil and water. There are several reasons for this interest. *First*, the industry is constantly searching for new products that are both useful and have cosmetic appeal. Certainly, a crystal-clear liquid or gel is elegant and has a different appearance from an opaque product. *Second*, the transparent products may have increased utility, because of the likelihood that absorption into the skin will be promoted by the extremely small size of the dispersed droplets. *Third*, for the formulator, there is the advantage that these systems are easily prepared, and they are thermodynamically stable. Further, they can be opacified, so that they look like conventional creams and lotions.

Lanolin derivatives are commonly used to prepare transparent cosmetic products, with excellent results. The intent of this paper is to show that other emulsifying agents may also be used and to present a theoretical basis for the formulation of these products.

Microemulsions

The transparent fluid and visco-elastic systems under discussion are treated here as microemulsions. The nature of microemulsions and the conditions under which they will form have been discussed by Schulman and co-workers (1–3). However, it should not be assumed that this is

* Foster D. Snell, Inc., New York 11, N. Y.

the only valid description of these transparent compositions. Solubilization in concentrated surfactant solutions as described by Lawrence (4) and by Winsor (5–9) may be equally applicable because no valid method is known for distinguishing between microemulsions and solubilized systems. These different approaches have been reviewed recently (10).

The microemulsions of Schulman are said to have a droplet diameter in the range of 100 to 600 Å. In comparison, spherical anionic micelles in aqueous solution have a diameter of about 50 Å., while nonionic micelles are double this size. Solubilization swells the micelles and their diameter can easily fall in the range of microemulsion droplets. Both solubilization and microemulsion formation occur spontaneously, without the necessity for homogenizing or colloid milling. The resulting systems are thermodynamically stable. Since the diameter of the droplets is less than $^1/_4$ the wavelength of light, the systems are transparent.

The advantage in using microemulsion theory to describe these systems is that the theory has been developed to the point of providing a satisfactory basis for the formulation of transparent cosmetic products containing high proportions of both oil and water.

The essential aspects of microemulsion theory are as follows. (1) The combination of emulsifying agents and their concentration must be such as to produce a metastable negative interfacial tension. (2) The emulsifier interphase must not be too highly condensed. (3) Molecules of the oil phase must be able to interpenetrate or associate with the mixed interfacial film constituting the interphase. These three features are discussed in more detail below.

Negative Interfacial Tension: In order to form microemulsions, the concentration of emulsifiers must be greater than that required to reduce the oil-water interfacial tension to zero. Under these conditions the interfacial tension must have a metastable negative value. This would cause droplets to break up spontaneously and would also stabilize the dispersed phase and prevent phase separation. As the emulsified droplets become smaller, the interfacial area increases and the emulsifier would become depleted by adsorption until the interfacial tension increases to zero, constituting the equilibrium condition.

An appropriate combination of emulsifying agents will give a lower interfacial tension than either component used alone. For example, a suitable combination consists of an anionic surfactant with a long-chain fatty acid or fatty alcohol. A similar effect is obtained with a combination of water-soluble and water-insoluble nonionic emulsifying agents.

The metastable negative interfacial tension cannot be measured directly, since the interface emulsifies spontaneously. However, it can be calculated directly if a counter tension is placed on the interfacial measuring device to prevent surface breakup.

If a long-chain fatty acid or fatty alcohol is spread on water, the surface tension γ_f can be defined as

$$\gamma_f = \gamma_{wa} - \pi$$

where γ_{wa} is the surface tension of pure water and π is the surface pressure of the long-chain polar compound. If the surface area is maintained constant, and an anionic surfactant is injected into the aqueous phase, the surface pressure will be found to increase. If a high ratio of nonpolar oil molecules to emulsifier molecules is now added to the system, it will form a mixed film. Upon compression of the mixed film on a Langmuir trough, some of the oil molecules will be squeezed out and will spread on the mixed monolayer in the form of a thin oil film, about 150 to 500 Å. thick. The tension at the newly formed oil-air interface holds the interface together and permits measurement of the surface pressure, from which the interfacial tension can be calculated.

Liquid Interphase Required: In addition to a negative interfacial tension, the mixed interfacial film must not be too highly condensed, or a microemulsion will not form. Aqueous solutions used in the preparation of microemulsions generally contain from 10 to 40% of emulsifying agents. The micelles present in these concentrated aqueous solutions have a lamellar structure, and this structure can be considered as the interphase between oil and water. In the case of emulsions of the type stabilized by cholesterol and a straight-chain alkyl sulfate, the interphase is strongly condensed. It cannot assume a large enough curvature to form droplets that are smaller than about one micron in diameter.

One method of converting a highly condensed interphase into a liquid interphase is to add an alcohol of medium chain length to the system. The addition of an alcohol containing five to eight carbon atoms to an aqueous solution containing lamellar ionic micelles will cause the micelles to swell almost without limit in both water and oil. Interpenetration of the micelles by the medium chain-length alcohol results in a liquid interphase. If the chain length of the alcohol is greater than ten carbon atoms, interpenetration of the micelles by the alcohol also occurs. However, the interphase is strongly condensed, and the micelles do not swell. The interphase can also be made less strongly condensed by raising the temperature or by using an ionic surfactant with a large counter ion, as in the case of an alkanolamine soap.

Interpenetration of Nonpolar Oil: The third and final requirement, according to Schulman, is that the nonpolar oil must interpenetrate and associate with the interfacial film. The oils that can be used to form microemulsions with a given emulsifier combination must be structurally similar to the emulsifiers and of equal or smaller hydrocarbon chain length. For example, if the emulsifier consists of the combination of alkanolamine oleate and oleyl alcohol, it will not form a microemulsion with benzene.

A transparent emulsion will form if the oleyl alcohol is replaced by p-methylcyclohexanol, which is structurally similar to benzene. The alkanolamine oleate-oleyl alcohol combination will form microemulsions with nonpolar oils having a hydrocarbon chain length of 18 carbon atoms or less. If the hydrocarbon chain length of the oil is greater than 18 carbon atoms, a microemulsion will not form. If the alcohol chain length is now increased so that it is greater than that of the hydrocarbon, a microemulsion will again form.

II. Experimental and Results

In Table I are shown the compositions of three O/W emulsions prepared by heating while stirring with a spatula until uniform, followed by cooling. Emulsion 1A consists of isopropyl palmitate emulsified in a 45% aqueous solution of Tween 60.®* At room temperature 1A is a white,

Table I.—O/W Emulsions

	Parts By Weight		
	1A	*1B*	*1C*
Tween 60	20	20	20
Isopropyl palmitate	20	20	20
Water	25	25	25
Span 80		6.5	6.5
2-Ethylhexanediol-1,3			6.5
Appearance	opaque gel	translucent gel	transparent fluid

semi-translucent gel. A thin film shows blue and orange Tyndall colors. The appearance of the gel suggests that droplets of about 1 or 2 μ diameter, typical of macroemulsions, as well as much smaller droplets are present.

In 1B, Span 80®* has been added to the system. The emulsion is a translucent gel showing blue and orange Tyndall colors. The larger size droplets characteristic of macro emulsions are essentially missing. The Tyndall colors suggest that the particles are of the order of $^1/_4$ the wavelength of light.

A medium chain-length alcohol, 2-ethylhexanediol-1,3 (6.12®)†, was added to 1B to give 1C. This addition resulted in a crystal-clear viscous fluid. The absence of Tyndall colors shows that the droplets are now smaller than $^1/_4$ the wavelength of light.

The interpretation of these results in terms of microemulsion theory is as follows. Tween 60 alone gives an expanded, rather than a condensed, surface film. It cannot produce a negative interfacial tension, and therefore will not form a microemulsion. The addition of Span 80 results in a condensed surface film and negative interfacial tension. However,

* Registered trade names of Atlas Chemical Industries, Wilmington, Del.
† Registered trade name of Union Carbide Corp., New York, N. Y.

the surface film is too highly condensed to produce the high curvature necessary for the formation of completely transparent systems. The added 6.12 penetrates the interfacial film and increases its fluidity, permitting a higher curvature and the formation of smaller droplets. It may be noted that the emulsion breaks if too much 6.12 is added.

Table II shows how the appearance of these emulsions changes with temperature based on the first appearance or disappearance of haze. Results appear to be reproducible to within 1°C. Emulsion 2A became crystal clear at 80°C and remained clear to 90°C, the highest temperature of the experiment. Clarification of this emulsion on heating was to be expected. Tween 60 dehydrates and becomes less water soluble with an increase in temperature. The reduced solubility tends to the formation of a condensed film. At the same time, the elevated temperature reduces the effect of van der Waals interaction between the hydrocarbon chains of the Tween 60 molecules. The result is a liquid condensed film and microemulsion formation.

TABLE II.—EFFECT OF FILM MODIFIERS ON TRANSPARENCY RANGE

	Parts By Weight				
	2A	*2B*	*2C*	*2D*	*2E*
Tween 60	20	20	20	20	20
Isopropyl palmitate	20	20	20	20	20
Water	25	25	25	25	25
Span 80		6.5	13	13	13
2-Ethylhexanediol-1,3				2	4
Transparent Range, °C	80–>90	74–>90	62–>90	33–>90	<20–77

The addition of Span 80 to emulsion 2A reduces the temperature required for the clarification of the emulsion. Thus, with 10% of Span 80 added to 2A the emulsion became transparent at 74°C. With 20% of Span 80 added, clarification occurred at 62°C. Since the presence of Span 80 results in a condensed interface, it is only necessary to heat the emulsion sufficiently to reduce molecular interaction to give a liquid condensed interphase. Since it is not known which Tween-Span ratio gives the more condensed film, the ratio that will produce clearing at the lower temperature cannot be predicted.

Small additions of 6.12 to emulsion 2C, containing both Tween and Span, result in transparency at decreasing temperatures, until transparency occurs at room temperature and below. However, these clear emulsions cloud when heated. The 6.12 expands the condensed film to give conditions suitable for the formation of a microemulsion. Upon heating, the interfacial film expands to the point where it is no longer condensed, and the emulsion becomes unstable. The addition of too much 6.12 has exactly the same effect.

TABLE III.—EFFECT OF FILM MODIFIERS ON TRANSPARENCY RANGE

	Parts By Weight		
	3A	*3B*	*3C*
Tween 60	20	20	20
Mineral oil	20	20	20
Water	25	25	25
Span 80		6.5	6.5
2-Ethylhexanediol-1,3			4
Appearance	opaque gel	opaque gel	transparent fluid
Transparency Range, °C	none	74–>90	<20–>90

One requirement specified by Schulman for the formation of a microemulsion is that the chain length of the nonpolar oil should not be greater than the hydrocarbon chain length of the emulsifiers. Isopropyl palmitate presumably meets this requirement since the fatty acid group has only 16 carbon atoms; Carnation®* mineral oil probably does not.

Table III is similar to Table II, with Carnation mineral oil substituted for isopropyl palmitate. Emulsion 3A, without Span 80 or 6.12, does not clarify even when heated to 90°C, suggesting that it is more difficult to form a microemulsion with the longer chain oil. However, the effects of addition of Span 80 and 6.12 are quite similar to those obtained with isopropyl palmitate. It should also be noted that the addition of 6.12 causes a substantial reduction in the viscosity of the emulsion. This is consistent with general observations to the effect that a highly condensed interphase gives a stiff emulsion.

A number of experiments were conducted by first forming a transparent emulsion, adding water or mineral oil until the emulsion remained turbid upon heating and cooling, and then adding increments of emulsifier. After each addition of emulsifier, the emulsion was heated, to effect more rapid emulsification, and then cooled. If the emulsion did not remain transparent, additional emulsifier was added. The results of these experiments are summarized in Table IV. The ingredients in each clear composition are expressed as a ratio to the amount of Tween 60 present. Referring to the O/W emulsions at the top of the table, it can be seen that the ratio of Span 80 to Tween 60 varies from 0.32 to 0.88. The only significant correlation between this ratio and that of the other components present is the 6.12 to Tween 60 ratio: The larger the ratio of 6.12 to Tween 60, the higher the ratio of Span 80 required to effect clarification. This finding is consistent, since the Span 80 addition condenses the interphase, and the 6.12 addition expands it.

In the case of W/O emulsions, the Span 80 is the predominant emulsifier at the interface, and the Tween 60 is the condensing agent. Increasing the ratio of Tween 60 to Span 80 requires a corresponding increase in 6.12 content.

* Registered trade name of Sonneborn Chemical and Refining Co., Div. of Witco Chemical Co., New York, N. Y.

TABLE IV.—RATIO OF COMPONENTS IN TRANSPARENT EMULSIONS

	Tween 60	Span 80	6.12	Water	Oil
		Transparent O/W Emulsions			
A	1	0.32	0.21	1.2	1.0
B	1	0.44	0.16	1.7	2.0
C	1	0.51	0.21	1.2	2.5
D	1	0.53	0.24	1.3	2.9
E	1	0.53	0.24	1.8	2.9
F	1	0.65	0.32	1.2	1.0
G	1	0.65	0.32	1.9	1.0
H	1	0.65	0.32	1.2	2.6
I	1	0.88	0.32	3.2	1.0
		Transparent W/O Emulsions			
J	1	1.5	0.16	1.7	4.7
K	1	0.90	0.21	1.2	3.5
L	1	0.90	0.21	1.2	5.3

It is of interest to compare HLB values for microemulsion and macroemulsion formation. The range of Span-Tween ratios in Table IV for O/W microemulsions correspond to an HLB range of 10.0 to 12.3. A recent Atlas bulletin (11) lists a required HLB for mineral oil O/W emulsions as 11 ± 1, and the two values are consequently in good agreement. In the case of the W/O microemulsions of Table IV calculated HLB values are in the range of 8.5 to 9.9. In contrast, the same bulletin gives a required HLB of 3 to 8 for W/O emulsions.

Formation of microemulsions with anionic emulsifiers is analogous to that with nonionic emulsifiers. Consequently, only one example is shown in Table V. A 32% aqueous triethanolamine stearate solution was prepared. Two parts of this soap solution was heated with one part of isopropyl palmitate. At the pH of this soap solution (pH 8.1), the micelles are highly condensed and very little of the isopropyl palmitate is taken into the micelles. Even with heating and vigorous stirring, most of the oil remains as a separate phase. Upon the addition of about 3 to 6% of 6.12, the soap micelles swell to take up all of the isopropyl palmitate and form an opaque O/W emulsion. The addition of a small amount of oleyl alcohol then condenses the interphase sufficiently to form a transparent emulsion.

TABLE V.—O/W EMULSIONS CONTAINING SOAP

	Parts By Weight		
	5A	*5B*	*5C*
Triethanolamine stearate	9.5	9.5	9.5
Water	20.5	20.5	20.5
Isopropyl palmitate	15.0	15.0	15.0
2-Ethylhexanediol-1,3		3.0	6.0
Oleyl alcohol			0.5
Appearance	2 separate phases	Soap solution swells to take up oil, opaque	Transparent above 25°C

III. Discussion

The theory of microemulsion formation can be useful in many ways for the preparation of cosmetic creams and lotions.

1. The theory provides a rationale for the preparation of transparent lotions and gel.

2. Since these transparent products are thermodynamically stable, they have a decided advantage over conventional emulsions. The addition of an opacifying agent to a transparent emulsion will give the appearance of a conventional lotion or cream.

3. Macro emulsions can be readily prepared without the use of homogenizers and other high shear equipment by first dispersing the internal phase as a microemulsion. Upon subsequent dilution with the solute of the continuous phase, and cooling, the size of the dispersed droplets increases to a value consistent with the final concentration of emulsifying agents.

4. Because of the small size of the dispersed droplets in a transparent emulsion, biologically active ingredients (in the dispersed phase) may be absorbed more readily. Thus, these systems may be the preferred vehicles for topical application. However, a word of caution is necessary because conventional cosmetic ingredients may prove to be not quite so innocuous when applied in this manner.

IV. Summary

The experimental study has shown that the conditions required for the preparation of transparent emulsions can be explained on the basis of the physical state of the interphase. If the interphase is expanded or too highly condensed, a transparent emulsion will not form. These results are consistent with Schulman's microemulsion theory. Only one deviation from the theory was observed in that transparent emulsions can be formed even though the hydrocarbon chain length of the nonpolar oil is greater than that of the emulsifying agents. This deviation does not detract from the theory but is simply an extension of experimental findings that broadens the applications of the theory.

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BOOK REVIEWS

FUNDAMENTALS OF KERATINIZATION, edited by E. O. Butcher and R. F. Sognnaes. Publication No. 70, American Association for the Advancement of Science, Washington, D. C. 1962. 189 pages, illustrated and indexed. Price $6.50 ($5.75 to AAAS members).

This small volume comprises papers presented at a symposium held by the American Association for the Advancement of Science in December, 1960. Most of these papers are original, and this volume is, therefore, of major interest to the workers in the field of keratin.

The first paper in this book, by Matoltsy, is concerned with the role of keratohyalin in keratinization of mammalian epidermis. On the basis of histological studies, the author concludes that epidermal cells keratinize independently of one another and that tonofilaments and keratohyalin granules are differentiation products formed for incorporation into the elaborated keratin.

In another interesting chapter, Szabó concludes that there is a difference between the embryonic and adult basal cells of the epidermis: In the embryonic stage, these cells are self-differentiating, whereas basal cells in the adult epidermis are not self-differentiating.

Rhodin and Reith, in their papers on the ultra structure of keratin again, return to the role of keratohyalin in keratinization. The purpose of keratohyalin has been in dispute ever since it was first discovered, and various workers have interpreted their observations differently. Mercer and his group have always maintained that keratohyalin is transformed into fibrils. These authors assign to keratohyalin a somewhat different role and indicate that keratohyalin aggregates with tonofilaments only during the formation of soft keratin. In contrast, the formation of hard keratin and of amorphous keratin do not seem to require keratohyalin.

Cosmetic chemists will be interested in the papers from a number of groups concerned with the influence of Vitamin A on keratinization. Bern and Lawrence conclude that the effect of Vitamin A on keratinizing epithelium varies as a result of dosage. On the other hand, Parnell and Sherman conclude that Vitamin A can be utilized directly by excised tissue and stimulates tissue mitotic activity at moderate dosage levels.

One of the most interesting observations in this volume concerns the one by Watson on the extracellular position of dental enamel. Watson concludes on the basis of some beautiful electron photomicrographs that there is a cell membrane between the ameloblast and the enamel, indicating that enamel is formed extracellularly. Similarly, the last chapter of this book by Piez demonstrates that the protein matrix of enamel from human teeth, at least on the basis of amino acid composition, is not a true keratin and instead resembles collagen. It is equally interesting that this protein normally cannot be isolated unless the teeth have been treated with formaldehyde.

This volume, though relatively small, contains a wealth of interesting information and should be read and consulted by all interested in skin, hair, or teeth.—Martin M. Rieger, Warner-Lambert Research Institute.

COLLAGEN, edited by N. Ramanathan. Interscience Publishers, New York, N. Y. 1962. 579 pages, illustrated. Price $20.

Collagen is a compilation of papers presented in person or by correspondence at a Symposium in Madras, India, in November of 1960. Sponsoring agency was the Central Leather Research Institute, Council of Scientific and Industrial Research.

The symposium is divided into three main divisions: Structural Studies; Medical and Biochemical Studies; and Physical-Chemical and Technological Studies. Virtually every aspect of the theory and evidence concerning structure, physical chemistry and biological properties of collagen is discussed. Clinical and pathological conditions of collagen are not stressed.

Many aspects of this volume are outstanding and impressive. For one thing, the list of major contributors numbers 58, and they are drawn from all over the world and both sides of the Iron Curtain, including the United States, Russia, South Africa and India. The caliber of these investigations is very high and generally oriented toward the physical-chemical and molecular level. This reviewer, having served on the S. C. C. Special Award Committee for four years, was struck by the fact that not one of these numerous original investigators was ever nominated for the special award. It can be concluded that either interest in collagen or the amount of basic scientific reading by Society members is deplorably low. It is gratifying that this year's Award winner, Dr. Jerome Gross, is extensively quoted by many of the authors in *Collagen*.

The highlights of the book are an excellent review of protein structure by G. N. Ramachandran, a thorough evaluation of the correlation of structure theory with X-ray diffraction and other evidence, O. Kratky's work correlating X-ray diffraction patterns with age of human tendon collagen, E. Kuhnke's presentation of collagen as a basis of tendon tissue function, and, especially for cosmetic chemists, K. T. Joseph's study on the influence of biological aging on the stability of skin collagen in albino rats. Each chapter has an extensive bibliography and an often penetrating discussion section. English is used throughout, although many of the original manuscripts had to be translated from other languages. Despite this, not more than a half-dozen errors in text were found by this reviewer. Beautifully clear pictorial representation of collagen structure, electron micrographs and other tables abound.

On the negative side, the lack of chapter sequential numbering and a subject index should be mentioned. A definite lack of continuity results from inclusion of a few papers on narrowly limited aspects of collagen. Occasionally, lack of sufficient introduction to a subject detracts from reader understanding.

Over-all, this is a valuable summation of current status of collagen research for the basic researcher in this field, especially the physical biochemist. For the cosmetic development chemist, this should be an interesting contribution to his understanding of skin, its structure and properties.—H. E. Jass, Revlon, Inc.

SURFACE ACTIVITY AND DETERGENCY by K. Durham. Macmillan & Co. Ltd., London. 1961. 250 pages, illustrated and indexed. Price $8.00.

This book presents a brief account of the basic physical chemistry of detergent systems and examines detergent action from a fundamental standpoint. It is an excellent summation of the state of our knowledge and is primarily written for people working on and familiar with theories of detergency. The book's major weakness is that it was written by five contributors, making for discontinuity of presentation and style and leading to needless repetition.

Five of the nine chapters of this volume are contributed by Durham. His opening chapter, *Properties of Detergent Solutions*, is poorly organized. The author covers micelle formation very briefly, discusses adsorption phenomena in more detail, and then quite abruptly describes in detail the properties of nonionic detergents. No such detailed treatment is given to the equally important anionic detergents. The author discusses the use of Gibbs' adsorption equations but admits it is much less difficult to use Gibbs' formulae than to understand them.

Chapter 2, *Aggregation in Detergent Solutions*, by Garrett is an excellent review of the theory of micelle formation. The chapter is very well written and well documented, with references to the basic work in this field. Particularly interesting is the discussion of the controversy between the "spherical" *versus* the "lamellar" micelle schools of thought.

Another chapter by Durham, *Wetting*, is a thorough job and, in contrast to Chapter 1, is very well organized. The different types of wetting are discussed, as are the equations associated with the wetting of smooth and rough porous and nonporous surfaces. One of the very interesting topics covered is the influence of fabric structure on its surface wettability. The duck's feather is a classic natural example of a water repellent structure, and apparently, its water repellency is due to the spatial arrangement of its fibers rather than any waterproofing agent (preen gland oil) on the feather.

In Chapter 4, *Dirt Removal*, by Jones, a good review of the theory of oily and particulate dirt is given. The author presents data which clearly show how the composition and particle size of the soil affect the efficacy of detergents. The effect of builders on dirt removal is well presented, and it is interesting to learn that only soils contaminated with metal-ions respond to the action of builders. A number of inconsistencies have crept into the text: Data presented in Table 4 on page 89 indicate that carbon soil is easier to remove than vacuum cleaner dust, yet the data shown in Figs. 4.7 and 4.8 are completely reversed. Moreover, the values for the per cent detergency of given detergents vary by more than 50%, depending on whether one refers to the tables or the graphs.

The effect of temperature on detergency is treated briefly, and very little information is given relating the efficacy of detergents to temperature, chemical structure or molecular weight.

In Chapter 5, *Effects of Detergents on Redeposition*, Durham covers the mechanism of detergency. The discussion of the effect of the ionic double layer as a barrier to coalescence and the theories by which oily and particulate soils are removed is in large measure a repeti-

tion of what has appeared in Chapter 4.

The chapter contributed by Stevenson, *The Ancillary Effects in Detergent Action*, contains many beautiful plates showing soil removal from fibers. Aside from these, this chapter is primarily a repetition of earlier chapters. The role of foam in detergency is treated very briefly. The author states that low foaming nonionic detergents appear to be as efficient as their foaming counterparts but gives no substantiating data. Surprisingly, the subject of alcohols and of long chain alkanolamides, which are widely used as detergency improvers, receives almost no mention.

Lawrence prefaces his contribution, *Polar Interaction in Detergency*, with the following statement: "When I was asked to give a lecture on this series, I said that I knew nothing about the subject of the title nor, so far as I was aware, did anyone else." This reviewer is inclined to agree with Dr. Lawrence, and for all practical purposes this chapter could have been omitted from the text. The author talks (literally—in the first person) about the solubility of organic substances in aqueous soap solutions in a disjointed and rambling fashion. Many graphs on binary and ternary systems are presented which are probably very clear to experts in this field, but very little effort is made to make them understandable to those who would like to become experts. The reader is certainly entitled to a more lucid explanation than statements such as: "As usual in the smectic mesophase, the texture is not one of simple layers, but that of focal conics of Dupin's cyclides," appearing on page 169. The fact that detergents remove polar soil by penetration is covered in the last four pages of a 34-page chapter.

The last two chapters, *Kinetics of Adsorption and Its Relation to Detergency* and *Evaluation of Detergent Efficiency*, both by Durham, are brief but adequate. The latter rounds out the picture by discussing conventional and radioactive methods of evaluating dishwashing, metal cleaning and fabric detergency.

This reviewer believes that people working with surface active materials in the fields of detergency and more specifically laundry and dishwashing detergents will find this book a good review and summation of the pertinent knowledge in the field today. However, this book is probably of very limited value to the cosmetic chemist whose major interests lie in the use of surfactants as emulsifiers, solubilizers and as detergents for skin and hair.—Charles Fox, Warner-Lambert Pharm. Co.

BY-LAWS OF SOCIETY OF COSMETIC CHEMISTS

(Incorporated under the laws of Delaware)

As Amended May 20, 1962.

ARTICLE I

NAME, OFFICES, OBJECT AND CORPORATE SEAL

SECTION 1. *Name.* The name of the corporation is Society of Cosmetic Chemists, hereinafter called the SOCIETY.

SECTION 2. *Offices.* The principal office of the SOCIETY unless otherwise ordered by the Board of Directors shall be at 317–325 South State Street, Dover, Kent County, Delaware, and the name of the resident agent in charge thereof shall be The Prentice-Hall Corporation Systems, Inc., whose address is 317–325 South State Street, Dover, Kent County, Delaware. The SOCIETY may also have offices at such other places as the Board of Directors may from time to time designate.

SECTION 3. *Objects.* Its objects are fully set forth in its Certificate of Incorporation, which, briefly defined, are to establish a medium for the dissemination of scientific knowledge of the Toilet Goods Industry and to improve the professional standing of scientists in the fields of cosmetics and perfumery. No profit or private benefit shall inure to any persons from the income or property of the SOCIETY.

Its purposes shall be limited to scientific, professional, educational, social, or charitable activities.

SECTION 4. *Corporate Seal.* The SOCIETY shall have a corporate seal which shall consist of two concentric circles, between which shall be the name of the SOCIETY, and in the center shall be inscribed the year of its incorporation and the words "Corporate Seal, Delaware."

ARTICLE II

MEMBERSHIP

SECTION 1. *Membership.* The SOCIETY shall consist of three classes of Members, namely: *Active* Members, *Honorary* Members, and *Emeritus* Members. Persons interested in the objects of the SOCIETY shall be eligible for Membership as defined in ARTICLE II, SECTIONS 2, 3, and 4, of these By-laws.

SECTION 2. *Active Membership.* The Board of Directors may elect to Active Membership persons who have (1) majored in the fields of Chemistry, Pharmacy, Chemical Engineering, Medicine, Physics, or other related sciences, and are recipients of degrees from accredited colleges or universities; or, (2) matriculated for not less than two years in an accredited college or university with recognized credit in the above stated fields of Science and who, thereafter, have been engaged in a technical capacity in the Toilet Goods Industry for not less than five years; or, (3) been deemed eligible upon examination of their qualifications by the Board of Directors. However, no more than two applicants shall be accepted in any one year under clause (3).

To be eligible for Active Membership, applicants shall qualify in accordance with one of the three stated specifications; shall file with the Secretary of the SOCIETY an application endorsed by three Members of the SOCIETY who are qualified to do so by right of full Membership privileges; and, shall pay the initial stated annual dues. If approved by the Board of Directors, they shall be elected to Active Membership by the majority vote of the Directors present at the

meeting at which their names are presented.

SECTION 3. *Honorary Membership.* The SOCIETY shall have the power to confer Honorary Membership upon such persons as may be deemed worthy, who shall be recommended by and receive the majority vote of the Board of Directors. An Honorary Member shall be entitled to all the privileges of an Active Member for life, with exemption from payment of dues, but shall not be entitled to the privilege of vote or of holding office. However, conferral of Honorary Membership on an Active Member shall not deprive him of his right to vote and hold office.

SECTION 4. *Emeritus Membership.* Any member who has reached the age of sixty years, has retired from active, remunerative work and who has been a Member for ten years, may through request or by nomination in his behalf, transfer to Emeritus Membership by application to the Secretary of the SOCIETY.

The Secretary shall then refer such request or nomination to a Committee composed of the Secretary, Treasurer, and the Chairman of the Membership Committee, and upon its favorable recommendation and approval by the Board of Directors such Member shall be entitled to the designation Emeritus Member and to all the privileges of an Active Member for life, with exemption from payment of dues, but an Emeritus Member shall not be eligible for election as an Officer or Director.

SECTION 5. *Termination of Membership.* The voluntary resignation of any Active Member shall become effective immediately upon receipt by the Secretary of such request in writing from the Member.

SECTION 6. *Termination of Privileges.* All rights, powers, privileges, obligations or duties of a Member, Director or Officer shall cease upon the death, resignation, or other termination of such Member, Director or Officer from the rolls of the SOCIETY.

SECTION 7. *Renewal of Membership.* Any active Member who shall resign while in good standing may be restored by request in writing to the Secretary of the SOCIETY and by payment of the stated annual dues for that year in which he requests reinstatement.

ARTICLE III

MEETINGS

SECTION 1. *Annual Meeting.* Each year, the Board of Directors, by a majority vote, shall set the Annual Meeting on a date in December to be held at the principal office of the SOCIETY or at such other time and place as the Board of Directors shall designate. Notice of not less than two weeks before the date of such meeting shall be mailed by the Secretary to each Member at his recorded address stating the object of such meeting.

SECTION 2. *Special Meetings.* A special meeting of the SOCIETY may be held at any time or place upon the call of the President or Secretary, provided not less than two weeks notice is sent by the Secretary to each Member at his recorded address stating the object of such meeting.

SECTION 3. *Quorum.* Not less than fifty Members of the SOCIETY shall form a quorum at any Annual or Special Meeting of the SOCIETY at which business is transacted.

SECTION 4. *Voting Privilege at Meetings.* At all meetings of the SOCIETY each Member in good standing shall be eligible to cast one vote in person. Any Member in arrears for dues shall not be eligible to vote.

All questions, presented for action, except those for which decision is regulated by statue, shall be determined by a majority vote of the eligible Members present.

ARTICLE IV

GOVERNING BODY

SECTION 1. *Board of Directors.* The governing body of the SOCIETY shall be known as the Board of Directors which shall consist of the Officers, namely: President, President-elect, Secretary, Treasurer, four Elected Directors and, to represent the established Chapters, the Chairman of each established Chapter or in his absence his designate delegate who is a Member of the respective Chapter.

All Chairmen of Chapters, or in their absence their designate delegates, shall enjoy the temporary status of Directors of the SOCIETY only while attending a meeting of the Board of Directors, but the quorum of the Board of Directors shall not be affected in any manner by the absence of any or all representation from the established Chapters.

SECTION 2. *Authority.* The Board of Directors shall have control over the affairs of the SOCIETY, including the direction and management of its activities and the control and disposal of its property and funds. It shall have the powers and authority especially conferred upon it by the Certificate of Incorporation and by the By-laws including such right, power and authority as may be exercised by the SOCIETY in its privileges as a nonprofit corporation organized under and subject to the laws of the State of Delaware, in the provisions of the Certificate of Incorporation, and in the By-laws of the SOCIETY.

SECTION 3. *Election.* The Officers and those Elected Directors elected each year, in accordance with ARTICLE VI, SECTION 3, of these By-laws, shall take office at the close of the Annual Meeting each year; and, except for the Elected Directors or when appointed to fill an unexpired term, shall serve for one year or until successors are duly elected and take office.

Elected Directors shall serve for two years or until successors are duly elected and take office. The Directors shall be so grouped that two shall be elected and two retired each year, and such retired Elected Directors shall be eligible for re-election to such office for not more than one additional term.

No member may serve as President-elect for more than three terms of office.

The Secretary and Treasurer shall be eligible to re-election to such office for not more than four consecutive terms, following which there shall be a lapse of at least one year before they may again become eligible for election to such office.

SECTION 4. *Filling Vacancies.* Whenever for any reason a vacancy shall occur on the Board of Directors, the remaining Members of the Board of Directors shall have the power to elect a Member of the SOCIETY to fill such vacancy until the next annual election.

SECTION 5. *Limitation of Privileges.* All Officers and Members of the Board of Directors shall be Members of the SOCIETY.

No Member of the Board of Directors shall receive any remuneration for service performed for the SOCIETY but upon prior authorization by the Executive Committee may be allowed reimbursement for expenses incurred for attendance at meetings or when performing duties as a Member of the Board of Directors.

ARTICLE V

BOARD OF DIRECTORS AND DEFINED COMMITTEES

SECTION 1. *Regular Stated Meeting.* The Board of Directors shall hold at least two regular meetings in each calendar year. Notice of such stated meeting shall not be necessary if such meeting is convened immediately following the Annual Meeting. Five elected Members of the Board of Directors shall constitute a quorum.

SECTION 2. *Special Meetings.* A special meeting of the Board of Directors may be called by the President at any time. A special meeting of the Board shall be called by the President or the Secretary at any time upon the request of two of its elected Members.

Notice of all special meetings to be held by the Board shall be sent to each Member of the Board of Directors, including the Chairman of established Chapters, not less than one week prior to the stated meeting.

SECTION 3. *Procedure.* The Board of Directors shall hold its regular or special meetings at the stated principal office of the SOCIETY or at such other place as it may designate. The Board of Directors may transact any business pertaining to the SOCIETY at any of its meetings.

Except wherein these By-laws require an otherwise vote by the Board of Directors, any action taken by a majority vote of the Members of the Board of Directors present at any meeting duly called and convened shall have full force and effect.

SECTION 4. *Advisory Committee.* This Committee shall consist of the President, the President-elect, and the five most recent active Past-Presidents who are Members of the SOCIETY. The most recent active Past-President shall serve as Chairman.

The Advisory Committee shall have the privilege to initiate matters pertinent to the welfare of the SOCIETY and shall consider such matters referred to it by the Board of Directors for study. It shall make appropriate recommendation to the Board of Directors.

SECTION 5. *Executive Committee.* In the Interim period of the meetings of the Board of Directors, this Committee, consisting of a majority of the Board of Directors, may meet at the call of the President. The President shall serve as Chairman. This Com-

mittee shall have all the powers of and act in lieu of the Board of Directors, provided such action is taken by the unanimous vote of those present and that a report of its actions is submitted at the next meeting of the Board of Directors.

SECTION 6. *Finance Committee.* This Committee shall consist of the President-elect, who shall serve as Chairman; the Chairman of the Advisory Committee; and two Elected Directors appointed by the President. Three members shall constitute a quorum.

The Finance Committee shall study the Annual Budget of the SOCIETY and of each Chapter, as prepared and submitted by the respective Treasurers not later than November 1st of each year, and shall make appropriate recommendation to the Board of Directors for its considered action at its next regular meeting.

The Finance Committee shall consider and recommend appropriate action for any financial matters of the SOCIETY and of the Chapters referred to it by the Board of Directors for study.

The Treasurers of the SOCIETY and of each Chapter shall have the privilege of attendance at such meetings of the Finance Committee when the budget estimates are being considered, but they shall not have the power of vote.

ARTICLE VI

PROCEDURE FOR NOMINATIONS AND ELECTIONS

The President-elect, Secretary, Treasurer and the four Elected Directors of the SOCIETY shall be chosen and elected in accordance with the provisions of this ARTICLE of these By-laws.

SECTION 1. *Nominating Committee.* The President shall appoint three Members to serve as a Nominating Committee, two of whom shall be from the Membership at large, and such appointment shall be made not later than June 1st of each year.

SECTION 2. *Nominations.* Prior to September 15th, the Secretary shall send to each member of the Society a nomination ballot on which the Member may write in for each office the name of one Society Member and for Elected Directors the names of not more than two Society Members.

The Member shall seal his ballot in a plain envelope marked "Ballot" and shall enclose this envelope in a sealed envelope bearing his handwritten signature; and, to be valid, it must be returned to and received by the Secretary of the SOCIETY not later than October 5th of each year.

The Secretary shall then meet with the Nominating Committee to open these envelopes and count the total returns for each Member proposed and prepare an Election Ballot as directed in SECTION 3 of this ARTICLE of these By-Laws.

Any interested Member of the SOCIETY may be an observer to these proceedings, provided that such Member makes no attempt to influence the Nominating Committee or to interfere with its stated functions.

SECTION 3. *Preparation of the Election Ballot and Method of Balloting.* For each Office there may be not more than two candidates, and for Elected Directors not more than four candidates.

The list of candidates for each office shall include the name of the consenting member who has received the largest number of votes for that office on the nomination ballot, provided that the candidate so selected received the highest number of votes above five per cent of the total membership of the SOCIETY.

The Nominating Committee may place in nomination at least one candidate for each office in addition to those determined by the nomination ballots; and it shall be the duty of the Nominating Committee to name one candidate for each office when none is determined by the nomination ballots, as herein provided.

The list of candidates for Elected Directors shall include the names of the two consenting members who shall have received the largest number of votes for Elected Directors on the nomination ballots, provided that these two candidates have received the highest number of votes above three per cent of the total membership of the SOCIETY. It shall be the duty of the Nominating Committee to make certain that there are at least two candidates on the election ballot.

If any nominee shall receive nominating votes sufficient to entitle him to be a candidate for more than one elective position, the Nominating Committee shall notify the nominee, and the Committee in consultation with the nominee shall decide that office for which he shall be a candidate.

Under the title of each position on the election ballot the Nominating Committee shall list alphabetically the names of the candidates therefor, without any other designation. The Nominating Committee shall certify to the Secretary that each person whose name appears on the election ballot has consented to hold office if elected.

The Secretary shall arrange for the printing of the election ballot as received from the Nominating Committee and shall send a copy to each member of the SOCIETY prior to October 25th. To be valid such ballot must be returned and received by the Secretary not later than November fifteenth. Election ballots so received shall be the only ballots which shall be counted.

Three tellers, none of whom is a candidate for office, shall be appointed by the President. The tellers shall receive the election ballots from the Secretary; if deemed necessary they shall verify the signatures against the master list of Members. The tellers shall count the votes and deliver to the Secretary all election ballots in a sealed package, together with a signed report certifying the number of votes for each name on the Election Ballot.

These proceedings may be observed by any interested Member of the SOCIETY, provided the Member does not interfere with the business of the tellers.

The candidates receiving the highest vote for each Office and the two candidates receiving the highest number of votes for Directors shall be declared elected. In the case of a tie vote, the Advisory Committee shall elect from the tied candidates. The results of the election shall be announced by the President at the Annual Meeting.

The Election Ballots, packaged and sealed shall remain in the custody of the Secretary until the next election unless surrendered to the tellers, by order of the Board of Directors, for the purpose of verifying the votes for the election of any officers. Any candidate shall have the right to demand a recount within fourteen days after announcement of the results has been made at the Annual Meeting.

Article VII

Officers

Section 1. The Officers of the SOCIETY shall be a President, President-elect, Secretary and Treasurer, all of whom shall be Members of the Board of Directors.

Section 2. The Board of Directors may appoint other Officers and Agents who may reside and/or act anywhere in the world. Appointed Officers need not be members of the Board of Directors. Appointed agents need not be Active or Honorary Members of the Society or of the Board of Directors. Such appointed Agents shall hold their offices for such term or terms and shall exercise such powers and perform such duties and receive such compensation for their services as shall be determined by the Board of Directors. The appointment by the Board of any person to be an Agent of the SOCIETY shall not necessarily confer upon such appointee Active or Honorary Membership in the SOCIETY.

Section 3. Except for the office of President, vacancy in any office by reason of death, resignation, removal, disqualification, or otherwise may be filled by the Board of Directors for the unexpired portion of the term, in accordance with Article IV, Section 4, of these By-laws.

Article VIII

Powers and Duties of Officers

Section 1. *President.* The President shall be the chief executive Officer of the SOCIETY. He shall preside at all meetings of the Board of Directors and at all meetings of the Members of the SOCIETY. He shall have general supervision, direction, and active management of the business and affairs of the SOCIETY. He shall direct the performance of all orders and resolutions as issued and adopted by the Board of Directors. He shall execute all contracts, deeds, bonds, and other instruments in writing as authorized by the Board of Directors in the name of the SOCIETY. He shall have the general powers of supervision and management usually vested in the Office of President of a nonprofit corporation under the laws of Delaware.

Section 2. *President-elect.* In the absence of the President, the President-elect shall exercise all the functions of the President. He shall serve as Chairman of the Finance Committee. He shall keep the Policy Manual up to date and shall distribute a copy to each Committee Chairman in January of each year.

Section 3. *Secretary.* The Secretary shall keep the permanent records and minutes of the meetings of the SOCIETY and of the Board of Directors, which minutes shall

be signed by him. He shall keep the Membership roll of the Active Members, a separate Membership roll of the Honorary Members and shall properly record all newly-elected Members. He shall be responsible for and have access to all records of the SOCIETY, and of its Chapters upon demand by the Board of Directors, and to its Corporate Seal, which he shall affix and attest to as directed by the Board of Directors. He shall perform all such duties as are associated with the Office of a secretary of a nonprofit corporation under the laws of Delaware.

SECTION 4. *Treasurer.* The Treasurer shall have the custody of all of the funds and property of the SOCIETY. He shall take such steps as may be necessary to collect moneys due and payable to the SOCIETY. When necessary and proper he shall endorse on behalf of the SOCIETY all checks, notes, or other obligations and evidences of money payable and received by the SOCIETY or coming into his possession, and shall deposit the funds arising therefrom together with all other funds of the SOCIETY coming into his possession, in such banks as may be selected as the depositories of the SOCIETY, or properly care for them in such manner as the Board of Directors may direct. He shall have access to the financial records of the Chapters. He shall prepare the annual budget estimate of the SOCIETY and shall submit it to the Finance Committee not later than November 1st of each year.

Whenever required by the Board of Directors or by the President, he shall exhibit a complete and true statement of his cash account, of the securities, and other property in his possession, custody, and control. He shall enter regularly in the books-of-accounting belonging to the SOCIETY, to be kept by him for such purpose, an accurate account of all money received and paid by him on account of the SOCIETY together with all other business transactions. He shall perform all duties which are associated with the Office of Treasurer of a nonprofit corporation under the laws of Delaware. The Treasurer shall be bonded. Each year he shall cause to have published the audited Annual Report of the financial status of the SOCIETY.

SECTION 5. *Assistant Secretary* and *Assistant Treasurer.* The Secretary may appoint one or more Assistant Secretaries and the Treasurer may appoint one or more Assistant Treasurers who may, but need not, be Members of the SOCIETY and shall not on account of their appointment to such positions be constituted Members of the SOCIETY. Upon order by the Secretary any Assistant Secretary may sign any document requiring the signature of the Secretary of the SOCIETY and may affix the corporate seal thereto. The Assistant Secretaries, Assistant Treasurers, and other Agents of the SOCIETY shall be under the direct supervision of the person to whom they are appointed Assistant or Agent thereof, unless otherwise provided by the Board of Directors.

ARTICLE IX

FISCAL YEAR

SECTION 1. The Fiscal year of the SOCIETY shall commence on the first day of January in each year and shall terminate on the thirty-first day of December.

ARTICLE X

DUES

SECTION 1. The annual dues of Active Members shall be of such amount as the Board of Directors shall determine and shall be due and payable on or before January 1st of each year.

Beginning October 1st of each year the annual dues of Active Members elected during the last three months of each calendar year shall be accepted as payable for the year beginning on January 1st of the next year. Any Member who fails to pay his dues for one year shall be dropped upon three months notice by the Treasurer but may, on payment of his indebtedness and with the approval of the Board of Directors, be restored to full Membership.

SECTION 2. Honorary Members and Emeritus Members shall be exempt from payment of dues.

ARTICLE XI

DUTIES AND CONDUCT

SECTION 1. Contravention of the By-laws and the rules of this SOCIETY, or unprofessional or unethical conduct as described in the code of ethics, shall subject the offender to censure, suspension, or expulsion, as determined by the vote of the Executive Committee, provided the accused shall have had at least two weeks' notification in writing from the Secretary of the

SOCIETY stating the charges preferred.

SECTION 2. Charges against a Member shall not be presented to the SOCIETY but shall be submitted in writing to the Secretary, to be submitted to the Executive Committee within one week, who shall then, within one month thereafter, satisfy itself of the validity of such charges. If this Committee deems the accusation warrants consideration, the Secretary shall transmit to the accused a copy of the charges and cite him to appear before such Committee on a specific date to make answer in his own behalf. Should he fail to appear in person or to be represented by attorney after a second notice (sent 30 days later), the Committee shall proceed with the trial, and its action shall be final in all cases. The decision and recommendations of this Committee, together with any or all evidence upon which its conclusions have been based, shall be sealed and kept on file by the Secretary. The Executive Committee shall report (1) that the charges are not sustained; or (2) that the charges are sustained in whole or in part and that the accused be (a) censured; (b) suspended for a definite time; or (c) expelled. Censure, suspension or expulsion from the SOCIETY shall require the unanimous vote of the Executive Committee.

SECTION 3. The trial shall be conducted in private executive session of the Executive Committee.

SECTION 4. A Member suspended for a stated period of time shall automatically be reinstated at the expiration of that time.

SECTION 5. Unless authorized by the Board of Directors, Members of the SOCIETY shall not knowingly or willfully allow the name or seal of the SOCIETY or its assets to be used by any person who is not a Member of the SOCIETY. The name of the SOCIETY shall not be used in any way by any Member to further or foster the advertising of a Member or a nonmember.

SECTION 6. No debts shall be incurred on behalf of the SOCIETY by any Officer of the SOCIETY, or of a Chapter, nor by any Member unless authorized by the Board of Directors or by such authority as is delegated by the Treasurer.

ARTICLE XII

STANDING COMMITTEES

SECTION 1. At each Annual Meeting or as soon thereafter as may be convenient, the President shall appoint the Chairman of each of the following standing committees. The Chairman shall then, with the approval of the President, appoint the personnel of his stated committee.

Arrangements	Placement
International Affairs	Publications
Laboratory Methods	Public Relations
Library	Scientific Program
Medal Award	Seminar
Membership	Special Award

The President and the Secretary shall have the privilege of attending all meetings of the Committees.

Each Committee shall use the Policy Manual for guidance of its duties.

ARTICLE XIII

INTERNAL ORGANIZATION

SECTION 1. The SOCIETY shall be further governed by such standing rules and regulations as shall be recommended by the Board of Directors and voted upon by a majority of the Members of the SOCIETY present and voting at the next regular meeting of the Members of the SOCIETY. These rules and regulations shall be binding on all Members.

ARTICLE XIV

BY-LAWS AND AMENDMENTS

SECTION 1. An official copy of these By-laws shall be kept in the custody of the Secretary who shall make the proper alterations in this copy whenever these By-laws are amended.

Suggestions for amendments to these By-laws may originate in (1) The Advisory Committee, (2) the Board of Directors, or (3) a petition presented to the Secretary and signed by not less than twenty-five Members of the SOCIETY in good standing. The Advisory Committee shall formulate all such suggested amendments and submit them to the Board of Directors, together with a statement of approval or disapproval. If the Board of Directors by a majority vote approves the proposed amendment, the Secretary shall mail a copy of such proposed amendment together with an explanation and a dated ballot to each Member of the SOCIETY entitled to vote. To be valid such ballot shall be returned to the Secretary of the SOCIETY not later than thirty days from the date stated on the ballot. The proposed amendment shall be adopted if

approved by a majority vote of the ballots returned.

Any proposed amendment not approved by the Board of Directors within ninety days from the time it is submitted to the Secretary or the Advisory Committee may be brought to vote of the Membership in the aforementioned manner by a petition signed by not less than seventy-five members in good standing.

Article XV

Parliamentary Authority

The rules contained in Robert's "Rules of Order," current revised edition, shall govern the actions of the Society of Cosmetic Chemists in all cases to which they are applicable and in which they are not inconsistent with the Certificate of Incorporation and the By-laws of the SOCIETY.

Article XVI

Chapters

Section 1. The SOCIETY shall have the right to establish local Chapters in the United States, its territories and possessions, as well as in foreign countries.

Section 2. Each Chapter shall have the following officers: Chairman, Chairman-elect, Secretary, and Treasurer. The Offices of Secretary and Treasurer, or Chairman-elect and Treasurer may be held by one person, but the Offices of Chairman-elect, Secretary and Treasurer may not be held by one person.

Section 3. All Chairmen of Chapters shall enjoy the status of Directors of the SOCIETY only while attending a meeting of the Board of Directors, but the quorum of the Board of Directors shall not be affected in any manner by the absence of any or all Chapter Chairmen.

The Chairman of a Chapter may delegate his powers described in this Section to his designate delegate, who is a Member of the respective Chapter, to attend the meeting of the Board of Directors.

Section 4. In the absence of the Chairman, the Chairman-elect shall exercise all functions of the Chairman.

Section 5. Each Chapter shall draw up a set of By-laws under which, following approval by the Board of Directors of the SOCIETY, it shall be governed. These By-laws shall be derived from, and be adapted to, the requirements of the individual Chapters except that no provision of such By-laws shall be in contravention to any provision of the By-laws of the SOCIETY, either in fact or in spirit. Proposed amendments to, the By-laws of a Chapter shall be submitted to the Board of Directors of the SOCIETY for approval before adoption by a Chapter.

Section 6. The Board of Directors shall have the right to amend the By-laws of any Chapter if it deems such action necessary for the protection of the SOCIETY. A unanimous vote of the elected Board of Directors is required in support of such amendment.

Section 7. Each Chapter shall file a set of its By-laws with the Secretary of the SOCIETY.

Section 8. When election of Chapter Officers occurs the Chapter Secretary shall notify the Secretary of the SOCIETY of results of such election giving the names, residence and business addresses of the elected Officers.

Section 9. The Treasurer of each Chapter shall prepare and submit an Annual Budget estimate to the Finance Committee of the SOCIETY not later than November first of each year.

Upon approval by the Board of Directors of the SOCIETY of the annual budget of each Chapter, a check shall be transmitted by the Treasurer of the SOCIETY to the Treasurer of the Chapter on February first of each year for $5.00 of the annual dues paid into the SOCIETY by each Member affiliated with that Chapter. This provision shall not be retroactive in any respect. This arrangement shall apply only to the Chapters in the United States, its territories and possessions, but not to Chapters in foreign countries, with whom special arrangements shall be made individually.

Prior to February first of each year, the Treasurer of each Chapter shall notify the Finance Committee of any unspent moneys remaining in the accounts of the Chapter. The Finance Committee of the SOCIETY shall have the privilege of recommending to the Board of Directors that such sums over and above $500.00 be applied to the succeeding annual budget of the Chapter or be returned to the treasury of the SOCIETY.

Section 10. Chapter status may be granted to not less than twelve Members who shall apply to the SOCIETY.

Section 11. Each member of the Society

may designate his wish to become a member of any one Chapter. Such written declaration, forwarded to the Secretary of the Society, shall entitle that Chapter to receive $5.00 of each annual membership fee thereafter paid into the Society Treasury by that member while he is a member of that Chapter. A member may change his affiliation to another Chapter by notifying the Secretary of the Society of his wish to do so. At least once every three years the Secretary of the Society shall check each member's preference for Chapter membership by asking him to indicate on a suitable questionnaire either the Chapter of his choice or his desire not to be a member of any Chapter. The Secretary of the Society shall notify the Society Treasurer of any transfers and shall notify the Chapter Secretaries of any transfers of members affecting their Chapters. A member may maintain membership in Chapters in addition to the one he has designated as his primary choice by paying directly to the Treasurer of each of the other Chapters of his choice the $5.00 annual fee for Chapter membership.

SECTION 12. The Board of Directors of the Society shall have the right to revoke the charter of any Chapter which is inactive for a period of one year or is deemed to be operating to the detriment of the Society. Within two months after receipt by the Secretary of the Society of a letter signed by three Society members, stating that any one Chapter has been inactive for one year or is operating to the detriment of the Society, and setting forth supporting details, a meeting shall be held by the Advisory Committee which the officers of the Chapter concerned shall be invited to attend in order to discuss the charges. The Advisory Committee shall report to the Board of Directors at the next Board meeting, recommending the action to be taken. The Board may then revoke the charter of the Chapter by a unanimous vote of all Board members present, excepting any who are officers of the Chapter concerned.

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3. Electron Microscopy

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ACUTE EYE APPLICATION—ALBINO RABBITS

Each Chemoderm was tested in the rabbit eyes according to Draize, J.H.(5). Results: No eye responses of any consequence were produced. (11)

SKIN SENSITIZATION STUDIES—ALBINO GUINEA PIGS

A modified technique was used, Landsteiner, Jacobs (6), Draize, Woodward, and Calvery (7). Results: Unmistakable sensitization was not produced by any material tested. Possible sensitization was noted in three of six positive control animals, using known skin sensitizer. (11)

TISSUE CULTURE STUDIES, IN VITRO

Using human conjunctival cells (Chang) and primary explants of chick heart fibroblasts, cytotoxic studies were conducted. Results: The Chemoderms studied were tolerated at considerably higher concentrations than mercuric chloride and on a comparative tolerance level to that of oil of peppermint. (11) (12)

HUMAN SKIN PHOTOSENSITIZATION

Photodynamic Chemoderm studies were conducted using natural sunlight intensity 514.5 gram calories per square c.m. Challenge tests were conducted on the same skin site 3 weeks after initial exposure. Results: Chemoderms did not produce evidence of inflammation and/or pigmentation on either initial or challenge application. The control material produced inflammation and pigmentation. (11) (13)

REPEATED INSULT PATCH TESTING—HUMANS

Using 4 times the normal perfume level in cosmetics; procedures (Draize-Shelanski) Lehman, A. J., (8) and with patch modification (3). Results: No Chemoderm produced primary irritation, skin fatigue, or skin sensitization. (11)

USE PATCH TEST—HUMANS

Chemoderms were put in 6 nationally known and widely used cosmetics; cold cream, dry skin cream, hand lotion, four shades of lipstick, powder and make-up. Several hundred women under medical and dermatological supervision used the six cosmetics for three weeks. Fifteen days later they were challenge patch tested. Procedures: (11) (Traub-Tusing-Spoor) (9) Schwartz and Peck (10). Results: Initial and challenge patches revealed no evidence of primary irritation or skin sensitization. It is concluded that these substances can be considered safe for general use in consumer products. (11)

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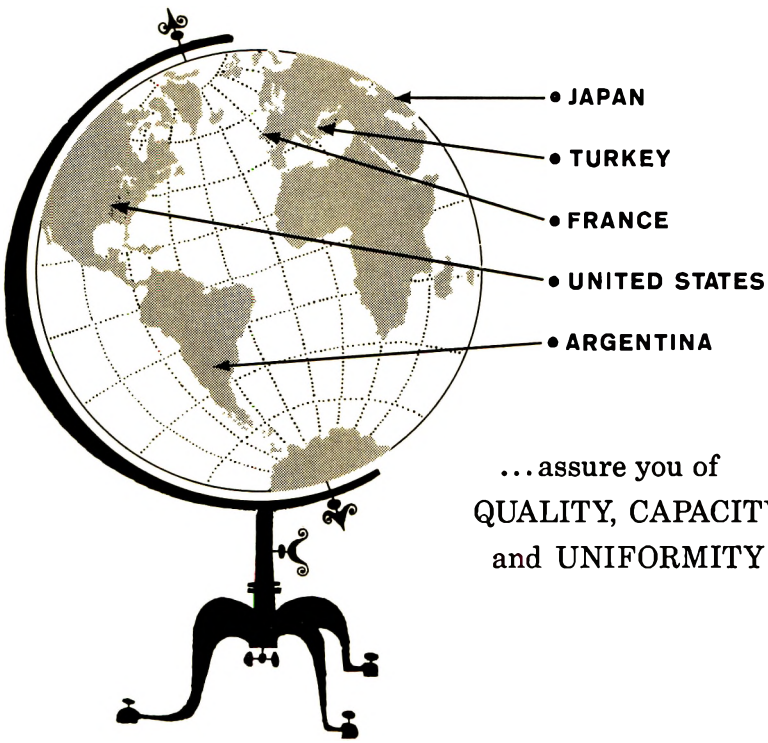

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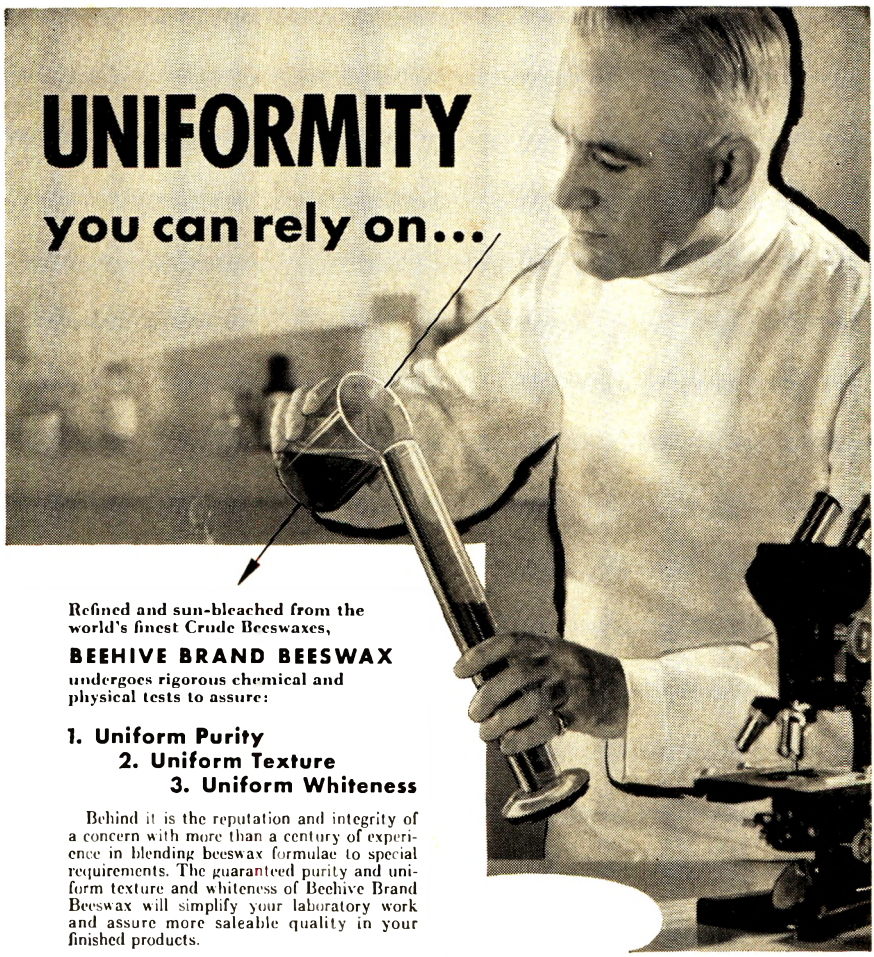

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