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A Chemical and Pharmacological Comparison of the Menthols

By A. Richard Bliss, Jr.,* and H. Bryson Glass†

Many users of menthol are unaware of the fact that there are several menthols. The literature on menthol most commonly available is limited chiefly to "Menthol, U. S. P.," which is defined as "An alcohol obtained from oil of peppermint or other mint oils or prepared synthetically" (1). In manufacturing quarters the common impression is that there are two menthols, *viz.*, (a) "natural menthol" and (b) "synthetic menthol," and little attention has been paid to the fact that there are eight possible menthols, and that six of them have been isolated and characterized.

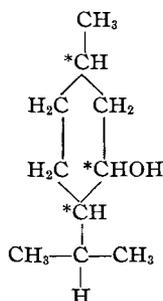
A major objective of the studies herewith reported was the determination of any measurable differences in the pharmacodynamic activities and in the toxicities of these menthols which might affect their employment as ingredients of creams, ointments, lotions, douches, nasal sprays, mouth washes, dusting powders, tooth pastes, shaving creams, cough drops, etc. Through the courtesy of a manufacturer,¹ generous supplies of the menthols used in these studies were obtained.

Until recently the world supply of menthol was obtained from Japanese peppermint oil produced from *Meniha arvensis*. According to Trease (2), Japanese peppermint oil is derived from *M. canadensis*, *var. piperascens*. *Meniha canadensis*, having a chromosome number of 27, is derived from *M. arvensis* (36 chromosomes) and *M. aquatica* (18 chromosomes). According to this writer, before the foregoing facts were elucidated, the Japanese plant was brought to England by Christy, and its affinity to

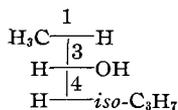
M. arvensis being recognized by Holmes, he named it *M. arvensis*, *var. piperascens* (3), (4). Numerous attempts have been made to produce menthol from mint oil grown in other parts of the world, but the menthol content of these oils is too low to permit commercial production. A stereoisomeric menthol, *l*-menthol is the well-known U. S. P. material (1). As a general rule, organic compounds containing an asymmetric carbon atom which occur in plants are found in the optically active state. Usually, *only one* stereoisomer of a given compound is found.

Several synthetic menthols have been on the market for a number of years, but they have not been identical with the natural product, being optically inactive and of lower melting point than the natural product. These synthetic menthols also differ greatly from the natural in odor, taste and "cooling effect."

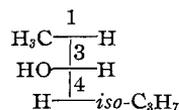
A very brief and uninvolved consideration of a portion of the chemistry of menthol will serve to explain these differences. From the constitution of menthol as developed by Beckmann, Semmler, Widman and others (5), it is evident that menthol contains three asymmetric carbon atoms (designated by *) as shown in the following structural formula:



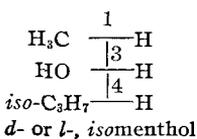
and is capable, therefore, of existence in *eight optically active forms, viz.:*



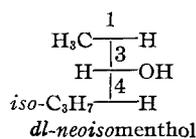
d-, or *l*-, menthol



d-, or *l*-, neomenthol



d- or *l*-, isomenthol



dl-neoisomenthol

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These forms having the same chemical constitution, but differing in the spatial arrangement of groups around the asymmetric carbon atoms, are known as stereoisomers, or enantiomorphs. Representation of three-dimensional figures on paper is difficult, but using the conventional diagrams for stereoisomers it is obvious from the above that there are eight possible menthols.

Stereoisomers containing one, and only one, asymmetric carbon atom show identical physical properties in the *d*- and *l*-forms, also in the *dl*- (racemic) form, except for their effect on plane polarized light. Likewise their chemical reactions are identical. Stereoisomers containing two, or more, asymmetric carbon atoms may differ from each other in their physical properties, such as melting point, solubility, etc., and their rates of reaction are different, although their chemical reactions are the same. Vapor pressures, however, appear to be independent of configuration in such closely related molecules, and therefore their boiling points are very close together.

Of the eight possible menthols, six have been isolated and characterized, the remaining two have not been prepared.

Stereoisomer ^a	Melting Point ° C.	Specific Rotation [α] _D
<i>d</i> -Menthol	43.0	+50.10
<i>l</i> -Menthol	43.0	-49.44
<i>dl</i> -Menthol	34.0	0.0
<i>d</i> -Neomenthol	-17.0	+19.69
<i>l</i> -Neomenthol	..	-19.72
<i>dl</i> -Neomenthol	51.0	0.0
<i>d</i> -Isomenthol	81.5	+27.00
<i>l</i> -Isomenthol	80.5	-24.10
<i>dl</i> -Isomenthol	53.5	0.0

^a See (5).

Ordinarily when an asymmetric carbon atom is formed, outside of living matter, the chance of the two possible configurations is equal, and the resulting compound is a racemic mixture of the *d*- and *l*-forms. Because of this fact synthesis of menthol from derivatives of benzene (thymol, cresol, etc.) produce a mixture of stereoisomers from which it is impractical to isolate any single isomer. At least one such mixture of menthols has been on the market for a number of years and is optically inactive.

The use of a *d*- or *l*-form of an optically active raw material in organic syntheses usually results in a so-called "asymmetric synthesis" in which the stereoisomers of the resulting compound (containing a *new* asymmetric carbon atom) are formed in *unequal* amounts. Thus, by using *d*-citronellal as the raw material for menthol a mixture of three menthols, instead of eight, is obtained. It is a fortunate fact that *l*-menthol is one of the three produced, and even more fortunate that the amount of *l*-menthol is greater than the amount of the other two isomers combined. It is because of this fact of fortune that the syntheses of *l*-menthol is possible.

Synthetic Menthol Process.—By the distillation of citronella oil, *d*-citronellal (3,7-dimethyloctene-7-al) is obtained and serves as the raw material for the synthesis of U. S. P. or *l*-menthol. This aldehyde is then made to undergo an intramolecular aldol condensation, involving a hydrogen atom alpha to an ethylenic linkage (6), (7), (8), (9), (10).

The *iso*-pulegols so obtained are converted into a mixture of menthols by hydrogenation over nickel. From this mixture of menthols, predominantly *l*-menthol, has been isolated *d*-neomenthol and *d*-isomenthol. The crude material has an optical rotation of from -17° to -19° . By suitable esterification of this material the *l*-menthol is removed as a solid from the liquid menthyl esters. This separation is never complete due to the solubility of the *l*-menthyl ester in the liquid esters, even at temperatures well below 0° .

The *l*-menthyl ester is recrystallized from alcohol to remove traces of other menthyl esters adhering to the crystal surfaces. The *l*-menthol is then regenerated by hydrolyzing with caustic soda, washed with hot water, and, after drying, distilled. In this way *l*-menthol having a melting point of 42.5° and $[\alpha]_D -48^{\circ}$ to -49° is obtained. Since this material solidifies to a mass of hard, very fine crystals, it is necessary to put it into the customary form of large needle-like crystals by recrystallizing it from a hydrocarbon solvent (the use of an alcohol as the solvent produces very fine, long needles).

The liquid menthyl esters, as an unseparated mixture, are then hydrolyzed with caustic soda to give a mixture of *l*-menthol, *d*-isomenthol and *d*-neomenthol. These menthols have been isolated from the mixture and obtained in a pure state. None of the other five isomers has been detected. This is not surprising inasmuch as the original asymmetric carbon atom $R-\overset{*}{C}H-(CH_3)-CH_2-CHO$ of the raw material has not been involved in any structural change during the synthesis. Since optical rotation measurements are additive it is reasonable to assume, though not certain, that this mixture of menthols is made up of equal amounts of the three stereoisomers which have been isolated from the mixture originally obtained. A mixture of equal parts of the three pure stereoisomers should give an optical rotation of $[\alpha]_D -2.75^\circ$, whereas the actual mixture obtained in the production of *l*-menthol has a rotation of $[\alpha]_D -2^\circ$ to -4° .

Comparison of the Three Stereoisomers.—The differences in physical properties may be seen from the table given. It is interesting to observe the difference in odors of the three stereoisomers; *d*-isomenthol has a slightly camphor-like odor, almost odorless; *d*-neomenthol has a musty, choking odor; *l*-menthol has a clean, "lifting" odor very much like that of growing mint plants, quite different from that of the natural menthol from Japan. The Japanese menthol is crystallized from the natural mint oil,² and very appreciable quantities adhere to, and are occluded by, the menthol crystals. The odor of the Japanese menthol can be reproduced very closely by spraying *l*-menthol with approximately 0.03% of its weight of mint oil.

The esterification rate of *d*-neomenthol is much slower than that of *l*-menthol. Under identical conditions of temperature and concentration, *l*-menthol shows 43.4% esterification while *d*-neomenthol shows 41.2%. Upon continuing the reaction for several hours *l*-menthol gives 66.5% esterification, whereas *d*-neomenthol gives only 53.4%.

² The following compounds have been identified from mint oil: pinene, limonene, cadinene, phellandrene, menthone, menthyl acetate, *isovaleric* aldehyde and acid, cineol, etc.

Complete esterification can be obtained by using high concentrations of the reacting acid and protracted times of reaction.

EXPERIMENTAL

The Menthols Studied.—

Menthol A: U. S. P. Menthol obtained from oil of peppermint.

Menthol B: Synthetic U. S. P. Menthol or *l*-menthol.

Menthol C: Liquid menthol consisting of approximately 66% of *l*-menthol, 16% of *d*-neomenthol and 16% of *d*-isomenthol.

Menthol D: Liquid menthol "Extra" consisting of approximately equal quantities of *l*-menthol, *d*-neomenthol and *d*-isomenthol.

Menthol E: Menthol 30° obtained from liquid menthol, "Extra" by distillation, and consisting principally of *l*-menthol and *d*-isomenthol contaminated with a small amount of *d*-neomenthol. Observed rotation, -11° .

Menthol F: Liquid menthol consisting of a small amount of *d*-neomenthol mixed with *l*-menthol and *d*-isomenthol. Observed rotation, -5.5° .

Menthol G: *d*-Neomenthol.

Menthol H: *d*-Isomenthol.

Effects on Human Skin.—Forty-three subjects of both sexes, ranging in age from 17 years to 45 years, and representing the various types of complexion and skin textures, were employed. Each menthol, in the form of a 20% solution in olive oil, was applied by brisk inunction for 15 minutes to the forearms of the subjects. Carefully measured quantities of the oily solutions were applied so that the quantitative factors were exactly the same. The subjects were unaware of the identity of the menthol each individual employed in each experiment.

The intensity, rapidity of development and duration of (a) hyperemia, (b) a burning or smarting sensation, (c) a sensation of coolness and (d) partial anesthesia or numbness were employed as the bases of comparison because of the fact that these are the effects upon which the use of menthol in the types of preparations referred to above depend.

Menthols *A*, *B* and *C* produced in the vast majority of subjects a more rapidly appearing, intense and lasting sensation of coolness, followed by a somewhat greater degree of numbness than with the other menthols; and the smarting sensation and hyperemia produced were slightly less intense and lasting, and developed somewhat more slowly than the other samples. Between menthol *A* and menthol *B*, on the one hand, and menthol *C*, on the other, the oily solutions of this menthol concentration demonstrated practically no estimable differences.

In the cases of menthols *D*, *E*, *F*, *G* and *H*, the results obtained presented the reverse in intensity, rapidity of development and duration of action when compared with menthols *A*, *B* and *C*, in that

a smarting sensation accompanied by hyperemia appeared more rapidly, and were more intense and of longer duration; whereas the cooling sensation and subsequent numbness appeared more latent and of lesser intensity and duration.

In no case did the local irritation pass beyond the rubefacient stage. It was noted that the youngest subjects, the females, those with soft finer skin textures, especially the blonde subjects, were more susceptible, and that with these types the hyperemia and smarting sensation were somewhat more pronounced, but consistent among the members of the same types and ages; this applied also to the duration of these actions. Urinalyses were made in the cases of a portion of the subjects in order to determine whether any of the menthols had been absorbed from the oily solutions applied to the skin; the results were all negative.

Effects on Human Nasal and Oral Mucous Membranes.—Sixteen subjects of both sexes were employed. The several samples of menthol were applied separately in the form of (a) a 0.5% solution in light liquid petrolatum, (b) a 0.2% solution in the same solvent and (c) a 0.1% solution in physiological salt solution, by means of a dropper or atomizer to the nasal passages, and the solutions permitted to run down into the throat. Applications were made three times a day, and two-day periods were permitted to elapse between the application periods in order to insure complete recovery from any effects produced by previous applications. The same quantities of each of the solutions were used by all subjects.

The 0.5% solutions of all the menthols in light liquid petrolatum were all irritating to the nasal and mucous membranes of all subjects, although menthols *A*, *B* and *C* appeared to be a trifle less irritating than the others to several of the subjects. In the cases of the 0.2% solutions of the menthols in light liquid petrolatum, menthols *A*, *B* and *C* were non-irritating or almost non-irritating with most of the subjects; the remainder, *D*, *E*, *F*, *G* and *H*, seemed to be slightly irritating to most of the subjects. The 0.1% aqueous solutions were non-irritating to most of the subjects in the cases of menthols *A*, *B*, *C* and *F*, but somewhat slightly irritating in the cases of the other menthols.

The Toxicities on Albino Rats.—Pure bred, healthy albino rats, which were kept on the standard diet throughout the experimentation, were used. Equal numbers of the sexes were employed, and at least six adult rats of approximately the same age were employed in each individual experiment. Accurately prepared solutions of the menthols in U. S. P. olive oil were injected, and the quantities in mg. per Gm. of rat required to produce marked symptoms of poisoning, as well as the quantities required to insure death, were determined and compared. All of the menthols studied are capable of producing the characteristic symptoms of poisoning when administered in adequate dosage, and will produce death when fatal quantities are injected.

All samples produced the same types of symptoms. In the event the toxic dose was not too large (4 mg. per Gm. of rat body weight), the animals recovered. The differences in rapidity of action and in toxic doses were rather slight. Toxic symptoms appeared somewhat more slowly with menthols *A*, *B* and *C*. From a quantitative standpoint, menthols *A*, *B*, *C*, *D*, *E*, *F* and *G* seemed to be very slightly less toxic than menthol *H*.

The Toxicities on Rabbits.—Healthy adult rabbits of approximately the same age and weight were employed. Equal numbers of both sexes were used, and all animals were kept on the standard diet. The intravenous injection of the several menthols was followed by practically the same effects in each case, there being very little measurable qualitative or quantitative differences among the menthols studied.

From the results observed in the cases of human subjects and the experimental animals employed, it appears that from a practical standpoint there is very little measurable difference in the toxicity of the several menthols investigated; they appear to be identical qualitatively, and even in the case of menthol *H*, as compared with the others, the quantitative difference in toxicity is very slight. In less than lethal doses there is very little difference. Practically no significance can be attached to the differences observed in the cases of both types of experimental animals.

From the observed effects on the human skin it appears that these menthols are qualitatively equal; but that in the event the "cooling effect" is of major importance in a preparation, menthols *A*, *B* and *C* possess a slight advantage over the others; and in the event the local irritant properties are of major importance, menthols *D*, *E*, *F*, *G* and *H* possess at least a slight theoretical advantage.

In view of the fact that there is an interesting difference in the costs of the several menthols studied, it is obvious that the manufacturer who is interested in this item will investigate the practical adaptability of these menthols to his individual needs.

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The Isolation and Identification of Alpha- and Beta-Amyrin from the Bark of *Viburnum Opulus**

By J. L. Powers† and W. E. Powers‡

In the course of a chemical examination of the bark of *Viburnum opulus*,¹ a considerable quantity of a wax-like material soluble in petroleum ether (b. p. 60–75°) was obtained. This, upon saponification and subsequent removal of the soap, yielded a yellowish colored unsaponifiable portion amounting to nearly 50% of the weight of the wax. The unsaponifiable portion, after several recrystallizations from alcohol, with the aid of charcoal, became nearly colorless, but did not possess a reasonably sharp melting point. This material produced the characteristic color reactions of sterols (1), (2), (3), (4). However, the quantity obtained seemed to preclude the possibility of its consisting entirely of a mixture of sterols.

Attempts to make a separation of the material through fractional crystallization of the acetates or benzoates were unsuccessful. Finally digitonin was used to precipitate all sterols precipitable by that reagent. The fraction which was not precipitated by the digitonin, as was expected, constituted the major portion of the mixture. It now melted at 165–172°, even after many recrystallizations from various solvents. After several attempts to make a further separation, it was found that after benzoylating the mixture a separation could be made by means of low-boiling petroleum ether (b. p. 30–40°). Two benzoates, one melting at

194–195° and constituting the major portion, and the other melting at 230–232° and constituting the minor portion, were obtained. The first benzoate upon hydrolysis yielded an alcohol melting at 182–184°; the second, an alcohol melting at 191–193°. Carbon and hydrogen determinations upon the alcohols and upon the benzoates indicated that probably both alcohols possessed the formula $C_{30}H_{50}O$. It seemed reasonable to expect that these might be known compounds, and it was found that the physical and chemical properties of the first isomer were in fairly close agreement with those given in the literature for α -amyrin, while the properties of the second were in close agreement with those given for β -amyrin. Moreover, it was found that apparently the best method of separation of the mixed amyryns was that described by Horrmann and Firzlafl (5), through the benzoates by means of pentane.

In order to test the correctness of the assumption that both α - and β -amyrin were present in *Viburnum opulus* bark, these compounds were isolated from Manila Elemi Gum,² converted into the corresponding acetates, benzoates and nitrobenzoates, and compared with corresponding derivatives of the alcohols obtained from the bark of *Viburnum opulus*. The alcohols and derivatives of them from both sources were quite similar in physical and chemical properties, and it is believed that *Viburnum opulus* bark undoubtedly contains both amyryns.

The amyryns were first obtained from Elemi Gum by Rose (6), and were first separated into two modifications by Vesterberg (7) through fractional crystallization of the acetates from ligroin. More recent work, relating to the structure of the amyryns, has been done by Ruzika (8), (9), Brunner (10), Dieterle and Salomon (11), Rollett (12) and others (5), (13), (14), (15). Despite the extensive literature on the amyryns, there is little to indicate that they are widely distributed in plants. They have been reported as constituents of *Asclepias syriaca* (16), of the latex from the milk tree, *Bros-*

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² Purchased from the S. B. Penick Company.