TIME VARIANTS IN THE ASSAY OF OIL OF PEPPERMINT.*

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INTRODUCTION.

Several times during the commercial analysis of samples of peppermint oil, the acetylation mixture was permitted accidentally to reflux for more than one hour, the time specified by the United States Pharmacopœia. The possible effect of the prolonged heating on the menthol content of the oil prompted the investigation of the effects of longer or shorter reflux periods in the acetylation of the oil with acetic anhydride and anhydrous sodium acetate. Obviously, the effect of longer and shorter periods of heating in the saponification or alkaline hydrolysis of the acetylated oil with alcoholic potassium hydroxide solution could be studied at the same time.

In a previous paper (1) the effect of varying the amount of alcoholic potassium hydroxide solution and the degree of hydrolysis at various temperatures has been studied. The velocity constants for the saponification of menthyl acetate and acetylated peppermint oil at different temperatures have been reported (1), (2).

EXPERIMENTAL PROCEDURE.

A bulked sample of raw peppermint oil collected during the 1937 season was used in this work. Adequate portions of the oil (about 75 cc.) were refluxed with acetic anhydride and anhydrous sodium acetate over a sand-bath for periods varying from fifteen minutes to three hours. The time was noted from the beginning of boiling until the flask was removed from the sand-bath.

TABLE I.—TOTAL PER CENT MENTHOL IN ACETYLATED OIL.

Sample No.	Saponification Time (Minutes).	Time of Acetylation. 15 Min.	30 Min.	45 Min.	60 Min.	90 Min.	120 Min.	180 Min.
		Per Cent Menthol.						
1	15	47.01	46.73	46.41	46.85	47.56	46.16	46.72
2	15	47.86	47.08	45.74	46.73	47.23	46.21	48.96
3	30	47.91	47.56	46.67	47.46	47.81	46.63	48.05
4	30	47.03	47.32	47.14	47.37	48.20	46.95	47.76
5	45	48.71	47.57	48.00	48.13	48.57	48.78	50.05
6	45	48.87	47.76	47.71	48.12	48.52	49.00	50.04
7	60	49.03	48.45	48.58	48.51	48.77	49.48	49.14
8	60	50.03	48.40	48.62	48.54	48.49	49.27	49.69
9	90	49.64	48.97	49.90	48.82	49.49	50.28	49.34
10	90	49.53	47.69	49.61	49.99	49.63	49.88	49.56
11	120	49.65	49.07	49.27	49.91	49.80	49.81	50.00
12	120	50.53	48.14	49.37	50.33	50.09	49.94	50.12
Average		48.82	47.89	48.08	48.40	48.68	48.53	49.12
Deviation from arithmetic mean of 84 samples, 48.50%		+0.32	−0.62	−0.42	−0.10	+0.18	+0.03	+0.62
Standard Deviation for 7 averages from arithmetic mean 48.50%		0.39%						

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After washing with water and diluted sodium carbonate T.S., and drying over calcium chloride, duplicate 5-cc. portions, accurately weighed, of the acetylated oil, were treated with 50-cc. portions of half-normal alcoholic potassium hydroxide and refluxed for varying lengths of time, from fifteen minutes to two hours, the period of refluxing being noted in the same manner as in the acetylation process. After cooling, the samples were titrated with half-normal sulfuric acid, using phenolphthalein as indicator. From this data and the per cent of ester, previously determined, the per cent of menthol in each sample was calculated. From these figures the arithmetic mean and the mean deviation for the entire lot of samples as well as for each group of samples were calculated. See Table I. Table II shows the deviation from the arithmetic mean for each sample, the number

TABLE II.—DEVIATION OF PERCENTAGES FROM THE AVERAGE MENTHOL CONTENT, 48.50%.

Sample No.	Saponification Time (Minutes).	Time of Acetylation. 15 Min.	30 Min.	45 Min.	60 Min.	90 Min.	120 Min.	180 Min.	Mean Deviation Based upon Saponification Time.
1	15	−1.49	−1.77	−2.09	−1.65	−0.94	−2.34	−1.78	1.72
2	15	−0.64	−1.42	−2.76	−1.77	−1.27	−2.29	+0.46	1.51
3	30	−0.58	−0.94	−1.83	−1.04	−0.69	−1.87	−0.45	1.06
4	30	−1.47	−1.18	−1.36	−1.13	−0.30	−1.55	−0.74	1.10
5	45	+0.21	−0.93	−0.50	−0.37	+0.07	+0.28	+1.55	0.56
6	45	+0.37	−0.74	−0.79	−0.38	+0.02	+0.50	+1.54	0.62
7	60	+0.53	−0.05	+0.08	+0.01	+0.27	+0.98	+0.64	0.36
8	60	+1.53	−0.10	+0.12	+0.04	−0.01	+0.77	+1.19	0.54
9	90	+1.14	+0.47	+1.40	+0.32	+0.99	+1.78	+0.84	0.99
10	90	+1.03	−0.81	+1.11	+1.49	+1.13	+1.38	+1.06	1.14
11	120	+1.15	+0.57	+0.77	+1.41	+1.30	+1.31	+1.50	1.14
12	120	+2.03	−0.36	+0.87	+1.83	+1.59	+1.44	+1.62	1.39
Mean Deviation		1.01	0.78	1.14	0.95	0.72	1.37	1.11	..
Number of samples with Deviation less than 1%		5	9	6	5	8	4	5	..
Mean Deviation for 84 samples		1.01							

of samples with deviations less than 1%, the mean deviation for each group of samples, based both upon saponification time and acetylation time, and the mean deviation for the 84 samples. The arithmetic mean for the 84 samples is 48.50%.

DISCUSSION.

In the determination of menthol in commercial samples of mint oil it is sometimes difficult to get concordant results from two or more laboratories. A communication from an official of one of the large essential oil companies mentions briefly one of the reasons for these differences. "We carry the back titration to the absence of red color. At times two different operators may think this takes place at slightly different points which would cause some difference in their results. We have found it hard to obtain consistent checks when the same samples are tested in different laboratories and for that reason we usually allow up to 1% for experimental error on menthol determinations."

This would help to explain the deviations from the average per cent of menthol obtained in this work. Inasmuch as all of the titrations in this investigation were performed by one dividiaul who, within the limits of visual error, titrated all

samples to the same end-point, other factors must help to explain the deviations in menthol percentages.

From the data listed in Table II, it would seem that the time of acetylation may vary within broad limits, while the time of saponification had best be varied between 45 minutes and 60 minutes to give results which do not deviate abnormally from the mean. It has been observed in earlier work (1) that at 50° C., acetylated peppermint oil is 80% hydrolyzed in 50 minutes and 84% hydrolyzed in 60 minutes. At steam-bath temperatures it is reasonable to assume that the reaction would go almost to completion in the same length of time. It will be observed that in those cases where the oil was saponified for periods shorter than 45 minutes the deviation was usually negative, indicating incomplete saponification, and that in the case of those samples heated for more than 60 minutes the deviation was positive, indicating, it is believed, that side reactions involving potassium hydroxide take place, thereby showing in the final calculation an erroneous per cent of menthol. While experimental evidence is not yet available to prove the contention, it is believed that resinification or polymerization of certain constituents, evidenced by a darkening of the reaction mixture, is induced by prolonged heating with potassium hydroxide, and that in the reaction some base is used up, thereby leading to erroneous results.

REFERENCES.

(1) Baldinger, L. H., JOUR. A. PH. A., 27, 581 (1938).
(2) Baldinger, L. H., *Ibid.*, 26, 208 (1937).

A COMPARATIVE STUDY OF THE ANTISEPTIC PROPERTIES OF OFFICIAL PREPARATIONS. A MODIFICATION OF THE REDDISH CUP METHOD FOR VOLATILE SUBSTANCES.*

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The purpose of this investigation is to study the inhibitory properties of several official preparations using Reddish's original method (1).

EXPERIMENTAL.

Staphylococcus aureus was grown at 37° C. in broth containing 1% Armour's Peptone, 0.5% Leibig's Beef Extract and 0.5% sodium chloride, the p_H being adjusted to 6.8. 0.1 ml. of a 24 hour old culture was thoroughly mixed with 15 to 20 ml. of nutrient agar (composed of 1% Armour's Peptone, 0.5% Leibig's Beef Extract, 0.5% sodium chloride and 1.5% bacto-agar, the p_H adjusted to 7.2–7.4) at 45° C., the mixture poured into a sterile petri dish, and allowed to harden. A disk was cut out in the agar by means of a sterile cork borer, 1.5 cm. in diameter. The disk was removed with sterile forceps and any cracks or crevices sealed with one or two drops of melted agar. After the agar cup was prepared six drops of the liquid antiseptic to be tested were placed in the cup and the plate incubated under an unglazed porcelain top for 24 to 48 hours at 37° C. Ointments, pastes and salves were melted. Enough of the melt was used to obtain a complete peripheral contact.

If the preparation is antiseptic or inhibitory a circular zone of clear agar surrounds the cup; the unaffected organisms continue to grow outside this zone. The distance from the edge of the cup to the edge of the zone is read in mm. This distance is a measure of the inhibitory (or penetra-

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