was necessary to carry out the process long after the extract was colored, since the removal of pungency is slow and does not parallel that of color. From 4 Kg. of waste was obtained about 800 cc. of oleoresin. This was mixed with twice its volume of heavy liquid petrolatum and extracted three times, using 1500 cc. of 57 per cent alcohol each time. The alcohol was removed by distillation, the aqueous residue was completely extracted by ether, and the solvent was removed again by distillation. The residual crude substance was mixed with 25 Gm. of purified lithium hydroxide and 1500 cc. of water, and the whole was boiled for about 10 minutes with vigorous stirring. After this had stood for 24 hours and had been filtered, carbon dioxide was run in to saturation, giving a fine, crystalline precipitate. This was dried in a desiccator and crystallized repeatedly from hot petroleum benzin, in which it is only slightly soluble. The total yield of highly purified crystals was 8.81 Gm. or 0.22 per cent. It is estimated that the total amount present in the tabasco waste is about 0.4 per cent.

The crystalline product was completely identified as capsaicine. It gave the color reaction with ammonium vanadate or vanadium oxychloride in acetone. The corrected melting point was found to be 64.8°. When it was intimately mixed with an equal amount of the purified substance from capsicum which melted at the same temperature, this mixture was found to have the identical melting point.

Tests with smaller amounts of material indicated that the whole tabasco pepper contains about 0.4 per cent of capsaicine. We conclude that the latter is distributed uniformly throughout the fruit and that the waste is just as pungent as the whole peppers. This was confirmed by organoleptic tests, which also indicated that either contains about four times as much pungency as Capsicum U. S. P. In the literature the latter is said to yield 0.007 to 1.0 per cent, but actually no one has apparently made an accurate determination; the most reliable statements place the content at about 0.1 per cent. In any event, tabasco peppers or any portion of them contains at least as much and probably several times this.

CONCLUSION

The recommendation is accordingly made herewith that the domestic tabasco pepper be considered for inclusion in the Pharmacopœia in place of capsicum, or at least that the monograph be revised to include tabasco pepper.

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The Assay for Menthol*

By Charles O. Wilson†

It has been the experience of the author that the United States Pharmacopœia XI assay for menthol in oil of peppermint gave very faulty results and according to the literature, other workers have also found this to be true. Since the method now used was first introduced by Power and Kleber (1) there has been a continued effort to remedy some of its more obvious sources of error. This subject has been under investigation by the author for some time and a report was practically completed when Brignall (2) published his work on oil of peppermint, covering the same method of approach. The evidence here offered, therefore, serves to confirm Brignall's findings.

Elliott (3) has pointed out the difficulty of completely removing the excess acetic anhydride from the acetylized oil. He suggested that one add 10 cc. of water to the acetylized oil and boil the mixture for ten or fifteen minutes to break up the acetic anhydride, then wash several times with dilute sodium carbonate solution to remove the acetic acid. Nelson (4) states that the reagents used should be of the best quality and that the sodium acetate must be perfectly dry for best results. In 1939 Baldinger (5) carried out experiments on oil of peppermint, varying the time of both acetylation and saponification. The saponification time for most consistent results was found to be forty-five to sixty minutes. He also found that the acetylation time may vary greatly, that in some cases fifteen minutes was sufficient. The author has

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also obtained satisfactory results when acetylation time has been only fifteen or twenty minutes. A method suggested by Regilyant (6), using one per cent phosphoric acid as a catalyst in acetic anhydride was tried and gave good results when acetylation time was only ten minutes.

Considerable trouble has been encountered in the estimation of the end-point after saponification, both for ester content and total menthol. The practice now in use, using phenolphthalein T.S., U. S. P. XI as indicator, requires the titration to be carried out until no color remains in the mixture. As stated by Baldinger (5), checks of one per cent are considered satisfactory by some volatile oil companies for the menthol assay. To overcome this difficulty, Nelson (4) suggested adding thirty to forty drops of phenolphthalein as indicator. Both he and Baumgarten (7) recommended diluting with water prior to titration and indicated very satisfactory results. Baumgarten (7) tried methylene blue as an indicator but states that dilution with water was better. The following procedure for the estimation of ester content in oil of peppermint involves dilution with water.

EXPERIMENTAL

Samples of oil were assayed for ester content using the U. S. P. XI method and the modified method suggested here.

The oil was weighed and saponified in the usual manner, but after refluxing and partially cooling, the mixture was diluted with 50 cc. of distilled water, allowed to stand for about five minutes and then titrated with 0.5*N* sulfuric acid, using two or three drops of phenolphthalein T.S. as indicator. Table I shows the comparison of the U. S. P. XI and the modified method. If allowed to stand a few minutes after the addition of water, the mixture tends to clear up and the red coloration is less noticeable.

Table I

Sample	U. S. P. XI, Per Cent Esters	Modified U. S. P. XI, Per Cent Esters
1*a*	7.0	7.1
1*b*	6.9	7.1
1*c*	7.0	7.1
2*a*	10.0	10.3
2*b*	10.1	10.2
2*c*	10.0	10.2

The end-point, using the U. S. P. XI method, was very difficult to distinguish and in some cases it was practically impossible to notice a definite change of color. Results expressed in Table I show that the addition of water (4), (7) does affect the results, as an average of 0.16 per cent more esters are found by its use. It is thought that these results are very satisfactory, as only one-third of the indicator is required and the end-point is very sharp and is easily observed.

In past years, Verley and Bolsing (8), Freed and Wynne (9), Delaby, *et al.* (10), and Rabaté (11) have used acetylizing mixtures of acetic anhydride and pyridine to determine primary and secondary alcohols. The following method is based on their findings.

In a tared 125-cc. Erlenmeyer flask weigh accurately about 2 Gm. of oil of peppermint. Add 5 cc., accurately measured, of acetylizing agent (one part acetic anhydride and four parts of anhydrous pyridine) and reflux in boiling water for thirty minutes, using a water-cooled condenser. The pyridine is made sufficiently anhydrous by distilling and collecting that portion of the distillate coming over between 114° and 116° C. Through the condenser add about 30 cc. of warmed distilled water and continue heating for fifteen minutes. This causes the complete transformation of the excess acetic anhydride to acetic acid. Allow the system to cool and before disconnecting the Erlenmeyer flask rinse the condenser once with 15 cc. of distilled water. Care should be exercised to keep the flask stoppered to prevent loss of any volatile acetic acid. The contents of the flask are now titrated with 0.5*N* alcoholic potassium hydroxide, using two drops of phenolphthalein T.S., U. S. P. XI as an indicator. The end-point is very sharp.

A blank determination must also be carried out and the number of cubic centimeters of 0.5*N* alcoholic potassium hydroxide required to neutralize the acetic acid produced by 5 cc. of the acetylizing agent noted.

The number of cubic centimeters of alcoholic potassium hydroxide required for the blank, minus the number of cubic centimeters required for the sample, multiplied by 0.0781,[1] equals the number of grams of free menthol present. The percentage of total menthol may be obtained by multiplying the percentage of esters by 0.7880 and adding to the percentage of free menthol.

The acetylizing agent was found to be stable for about one week, but after that time low results were obtained by its use. After two weeks, only results of 93 per cent or less were obtained. Light causes a darkening of the reagent, so it must be stored in a dark place. Pyridine, after being

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[1] One gram of menthol requires 0.6535 Gm. of acetic anhydride in reacting to form one molecule of menthyl acetate and one molecule of acetic acid. The acetic acid formed is 0.3844 Gm. and is equal to 12.8 cc. of 0.5*N* alcoholic potassium hydroxide. Therefore, 1 Gm. of menthol, divided by 12.8, equals 0.0781 Gm. of menthol per cc. of 0.5*N* alcoholic potassium hydroxide.

rendered anhydrous, is suitable for use after several months, if kept protected from light and tightly stoppered.

Samples of oil of peppermint were assayed for menthol by the U. S. P. XI and the suggested method. Results are in Table II.

Table II

Sample	Per Cent Esters, Modified Method	U. S. P. XI, Total Menthol, Per Cent	U. S. P. XI, Free Menthol, Per Cent	Suggested Method, Per Cent Free Menthol
1	7.1	52.1	46.5	43.6
2	10.2	52.0	44.0	41.0
3	5.2	50.2	46.1	43.3
4	5.6	51.6	47.2	44.7
5	7.3	52.3	46.6	43.8
6	6.1	50.5	45.7	42.5

The data show that the suggested method gave an average free menthol content of 2.8 per cent lower than that of the U. S. P. XI method. A cause for this difference is partially explained in Tables III, IV and V.

The minimum requirement of the U. S. P. XI for oil of peppermint is 50 per cent total menthol. Brignall (2) found 43.0 per cent free menthol by his method equivalent to the U. S. P. XI requirement, while in the author's work 43.3 per cent of free menthol was found.

Solutions of purified menthol in rectified oil of turpentine U. S. P. XI were used to test the accuracy of this method as compared with that of the one now in the U. S. P. XI. Blank determinations by the U. S. P. XI method on the rectified oil of turpentine gave average findings of 0.3 per cent esters, calculated as menthyl acetate, 1.2 per cent total alcohols calculated as menthol, and 1.0 per cent free menthol. No free alcohols were found in rectified oil of turpentine by the suggested method. In Table III are recorded the findings of method comparison, taking into account the alcohols found in the controls.

Table III

Sample	Calculated Per Cent Menthol	U. S. P. XI Method, Per Cent Total Menthol Minus 1.2%	Per Cent Free Menthol Minus 1.0%	Suggested Method, Per Cent of Menthol
1	8.1	9.1	8.9	8.0
2	17.7	18.8	18.6	18.1
3	34.2	35.8	35.6	34.4
4	69.3	70.5	70.3	69.1
5	85.1	86.3	86.1	85.3

The data in Table III indicate that the suggested method gives better determinations than the U. S. P. XI procedure. The percentages of menthol found by the two methods do not differ to the same extent as they did in Table II. It appears then that some potassium hydroxide is consumed in other than ester saponification (5) and/or other constituents of the oil are acetylized by the official procedure (Table IV) and/or small errors are present, causing high results, due to the manipulations in the U. S. P. XI procedure and, as noted by Table V, the suggested method is more exact than that of the U. S. P. XI.

The fact that by the U. S. P. XI method 1.2 per cent total alcohols are found in rectified oil of turpentine and none are accounted for by the suggested method indicates that some non-alcoholic constituents are acetylized by the U. S. P. XI procedure. Experiments were carried out to compare the two methods of acetylization, that of the U. S. P. XI, using acetic anhydride and anhydrous sodium acetate and that of the suggested method, using acetic anhydride and pyridine. Oil of peppermint was assayed for total menthol, following the U. S. P. XI procedure but differing only in the method of acetylizing the oil. Table IV expresses the results found.

Table IV

Sample	Per Cent of Esters	Per Cent Total Menthol, U. S. P. XI	Acetic Anhydride and Pyridine, Per Cent Total Menthol, U. S. P. XI
1	7.1	52.1	50.0
2	10.2	52.0	49.5
3	5.2	50.2	47.8
4	5.6	51.6	48.5
5	7.3	52.3	50.1

It does appear, then, by the lower percentage of menthol recorded when the acetylant is acetic anhydride and pyridine, that other constituents are acetylized, and ultimately expressed as menthol, by the U. S. P. XI method. An average of 2.4 per cent less total menthol was found when the acetylant was acetic anhydride and pyridine. If in Table II and Table IV the average differences in procedures are compared (Table II, 2.8 per cent; Table IV, 2.4 per cent) it could be concluded that the greatest source of error in the U. S. P. XI procedure is the acetylization of some constituent or constituents other than menthol.

Assay Applied to Pure Menthol.—The present method as given by the Association of Official Agricultural Chemists (12) gives high results. Nelson (4) points out that a probable reason is the difficulty of washing the acetylized menthol free of acetic anhydride and this was also observed by the author. Table V shows the results of the determination carried out on purified menthol.

Table V

Sample	Calculated Per Cent Menthol	A. O. A. C. Method, Per Cent Menthol	Suggested Method, Per Cent Menthol
1	100.0	104.6	100.3
2	100.0	101.7	99.9
3	100.0	101.4	100.1
4	100.0	102.2	100.2
5	100.0	102.3	99.8

The suggested method appears to give more true results than that of the A. O. A. C. determination and requires much less time. Only one gram samples were used, whereas five gram samples are required by the A. O. A. C.

SUMMARY

A method is suggested which is suited for the determination of free menthol in oil of peppermint and in menthol. The time required by the suggested method is about one-half of that for the U. S. P. XI method. The saponification step of the U. S. P. XI method, known to be a source of error, is eliminated, as is the washing of the acetylized oil with dilute sodium carbonate solution. An acetylant mixture of acetic anhydride and pyridine, itself quite stable, is used, esterifying the menthol in thirty minutes. Some evidence is presented which indicates that the U. S. P. XI method of acetylization affects other constituents of oil of peppermint besides menthol.

In the determination of esters in oil of peppermint, the addition of water after saponification was found to produce more accurate results, as only one-third of the amount of indicator is required and the end-point is very sharp and easily observed.

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Some of the Constituents of the Tuber of Coqui (*Cyperus rotundus* L.). III.—The Sugars

*By Conrado F. Asenjo**

The purpose of the present communication is to report on the sugars present in the alcoholic extractive obtained from the tubers of *Cyperus rotundus* L.

EXPERIMENTAL

Extraction and Clarification of the Molasses.—After having subjected the tubers to the process of extraction by continuous percolation with petroleum ether and ether, the marc left was subjected to extraction with 95 per cent alcohol. The alcoholic extractive, a dark brown syrup, amounted to 17.7 per cent of the air-dried tubers. Only part of this syrup was soluble in water; the rest, a resinous mass, remained insoluble. The water-soluble portion reduced Fehling's reagent very readily. After slowly evaporating the water at a temperature of not over 50° C., a dark brown molasses remained which amounted to 50 per cent of the original alcoholic extractive. The molasses thus obtained was clarified by treating with basic lead acetate, neutral lead acetate and, finally, with vegetable charcoal. The excess lead was always precipitated with H_2S. The resulting molasses had a light caramel color.

Proximate Composition of the Molasses.—Reducing sugars were determined by the Drefren-O'Sullivan method (1), and amounted to 51 per cent of the molasses. Reducing sugars, after acid hydrolysis, 55 per cent. Non-reducing sugars by difference, 4 per cent. Aldoses determined by the Willstäter-Schudel titration (2), 41.7 per cent. As the molasses gave a positive Seliwanoff's test, the difference of 9.3 per cent between total reducing sugars and aldoses is probably made up of ketoses. The per cent of water in the molasses was 20 per cent. By difference, then, there was 25 per cent of substances which were neither sugars nor water in the clarified molasses.

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