

SUMMARY

The Gross and Smith colorimetric method for the determination of rotenone and deguelin has been improved. Sulfuric acid has been substituted for nitric acid, the concentration of the alcoholic potassium hydroxide has been reduced by diluting with water, and the nitrite necessary to produce the color is added as sodium nitrite in the alcoholic potassium hydroxide. The stability, hue, and depth of color produced by the rotenone as well as that produced by the reagents themselves can be easily controlled. This combination of reagents practically eliminates fading and increases the sensitivity of the test 20 times.

DETERMINATION OF THUJONE IN ABSINTH-TYPE LIQUEURS

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No reagent has been found that will yield a compound sufficiently insoluble in alcohol or with sufficient stability to withstand the treatment necessary to separate thujone from the large proportions of alcohol contained in absinth-type liqueurs.

Unfortunately, ketones do not react in the uniform manner expected of aldehydes, so that any predictions as to their behavior often go awry. It is also difficult to obtain pure substances, and therefore supposedly standard solutions are often not really standard.

Semicarbazide is the reagent used most frequently for the purpose of holding back thujone. However, the experiments of the writer indicate that thujone semicarbazone is not sufficiently stable for the purposes of a quantitative method.

The qualitative method in use today, that of Enz,¹ involves a modification of the Legal test.² The details follow:

To 500 cc. of absinth add 1 cc. of freshly distilled aniline and 1 cc. of sirupy phosphoric acid, and reflux 30 minutes on the steam bath. Distil off two 100 cc. portions, reject the first, and test the second for thujone as follows:

Add 0.5 gram of semicarbazide hydrochloride and 0.6 gram of anhydrous sodium acetate (or 1.0 gram of the crystallized salt) and allow the mixture to stand overnight. Distil off the alcohol at as low a pressure as possible. Steam distil to remove essential oils and other volatile material, collect, and reject about 15 cc. of distillate. Wash down the condenser with a little alcohol and with water. Cool the sample, add 1 cc. of $H_2SO_4(1+1)$, and again steam distil, this time collecting 20 cc. of distillate in a cylinder. Pour the distillate into a small separatory funnel, and add 20 cc. of ether, using the receiver as the measure. Shake and separate the ether solution. Add 10 cc. of 65 per cent alcohol and allow the ether to evaporate spontaneously. When all the ether has evaporated, note the odor of the residue. The odor of thujone will be apparent if 2 mg. or more is present in the solution, provided it is

¹ *Schweiz. Wochschr.*, 49, 337, 507 (1911).

² *Rocques, Ann. chim. anal.*, 13, 227 (1908).

not masked by the presence of other odoriferous substances. Make the modified Legal test as follows:

To the solution obtained as directed above, add 1 cc. of 10 per cent zinc sulfate solution and 0.25 cc. of freshly prepared sodium nitroprusside solution (0.1 gram per cc. of H₂O). Slowly, with constant stirring, add 2 cc. of 5 per cent sodium hydroxide solution. Allow to stand one or two minutes. Add 1.5 cc. of glacial acetic acid and mix. A precipitate of raspberry red color (resembling the alcohol precipitate of a red fruit juice) shows the presence of thujone. A negative test is shown by a similar precipitate having an appearance similar to that of the alcohol precipitate from apple jelly or other light colored fruit.

The above procedure was studied critically. First the modified Legal test was applied to solutions of known quantities of thujone in 10 cc. of 65 per cent alcohol. The results are given in Table 1.

TABLE 1.—*Results, modified Legal test*

THUJONE PRESENT	TEST	THUJONE ODOR
<i>mg.</i>		
1	Doubtful	None
1	Positive	None
2	Positive	Slight
2	Doubtful	Slight
5	Strongly positive	Strong
5	Strongly positive	Strong
10	Strongly positive	Strong
10	Strongly positive	Strong

Known quantities of thujone were then placed in the steam distillation apparatus, and 25 cc. of water was added. The solution was steam distilled, and 20 cc. of distillate was collected. The distillate was extracted with ether, and the test was made on the ether solution. The results are given in Table 2.

TABLE 2.—*Recovery of thujone by steam distillation*

THUJONE PRESENT	THUJONE ODOR	TEST
<i>mg.</i>		
1	None	Doubtful
1	None	Doubtful
2	Slight	Positive
2	Slight	Positive
5	Strong	Strongly positive
5	Strong	Strongly positive

These experiments show that positive results may be expected from the modified Legal test if 2 mg. or more of thujone is present in the solution when steam distilled, and also that the odor of thujone can be detected when 2 mg. or more is present in the 10 cc. of solution to be tested.

For convenience the semicarbazide reagent was made up by dissolving 10 grams of semicarbazide hydrochloride and 12 grams of anhydrous sodium acetate in water and diluting to 100 cc. Known quantities of thujone were added to 100 cc. portions of 80 per cent alcohol, 5 cc. of semicarbazide reagent was added to each, and the mixtures were allowed to stand overnight. The next day the alcohol was distilled off by means of a Widmer distilling apparatus at a pressure of about 60 mm., the distilling flask being heated with a water bath. The temperature of the vapors was about 35° C. When only about 15 cc. of liquid remained, the flask was disconnected from the Widmer column,¹ about 10 cc. of water was added, and the flask was connected to a steam distillation apparatus and steam distilled. About 15 cc. of distillate was collected in a 25 cc. graduated cylinder. The receiver was then removed, and while the distilling flask was allowed to cool, the condenser was washed down with two small portions (about 10 cc. each) of alcohol and two portions of water. The washings were rejected. About 1 cc. of H₂SO₄ (1+1) was then added to the distilling flask, and the contents were mixed. The liquid was again steam distilled, and 20 cc. of distillate was collected in a 25 cc. graduated cylinder. The distillates were extracted with 20 cc. of ether, 10 cc. of 65 per cent alcohol was added to the ether extract, and the mixture was allowed to evaporate spontaneously until the odor of ether could no longer be detected. The modified Legal test was then applied to the residue after its odor had been noted. The results are given in Table 3.

TABLE 3.—*Thujone held back as semicarbazone during removal of alcohol*

THUJONE PRESENT	1ST DISTILLATION BEFORE ACIDIFYING		2ND DISTILLATION AFTER ACIDIFYING	
	ODOR	TEST	ODOR	TEST
<i>mg.</i>				
1	None	Negative	None	Doubtful
2	None	Negative	Doubtful	Positive
3	None	Negative	Thujone	Positive
5	None	Negative	Thujone	Positive
10	Thujone	Slightly positive	Strong thujone	Positive
20	Doubtful	Negative	Strong thujone	Positive
50	Thujone	Doubtful	Strong thujone	Positive

The data in Table 3 show that thujone semicarbazone has sufficient stability to withstand the removal of alcohol and essential oils and to give a positive test for thujone in the proper fraction when 2 mg. is present, although 3 mg. or more must be present before the odor of thujone is apparent in the fraction. The data also show that when 10 mg. or more

¹ *Helv. Chim. Acta*, 7, 59 (1924).

of thujone is present, a sufficient quantity may be lost in the steam distillation before the thujone semicarbazone is decomposed with acid, so that thujone may be detected both by odor and by the modified Legal test in that distillate. This behavior precludes the possibility of using semicarbazide as a means of holding back thujone quantitatively.

Next, known quantities of thujone were added to 500 cc. portions of 50 per cent alcohol and distilled slowly. Two 100 cc. fractions were collected, each of which was treated in the same manner as were the solutions in the previous experiment. The results are given in Table 4.

TABLE 4.—*Detection of thujone in solutions by the modified Enz method*

THUJONE PRESENT	FRACTION	1ST DISTILLATION BEFORE ACIDIFYING		2ND DISTILLATION AFTER ACIDIFYING	
		THUJONE ODOR	TEST	THUJONE ODOR	TEST
<i>mg.</i>					
1	a	None	Negative	None	Negative
	b	None	Negative	None	Negative
2	a	None	Negative	None	Negative
	b	None	Negative	Doubtful	Negative
3	a	None	Negative	Slight	Doubtful
	b	None	Doubtful	None	Positive
5	a	None	Negative	Slight	Negative
	b	None	Positive	Slight	Positive
10	a	Doubtful	Negative	Slight	Positive
	b	Slight	Positive	Slight	Positive
20	a	None	Negative	Slight	Negative
	b	None	Negative	Distinct	Sl. pos.
50	a	None	Negative	Distinct	Positive
	b	Doubtful	Positive	Strong	Positive
100	a	Doubtful	Positive	Strong	Positive
	b	Distinct	Positive	Strong	Positive

The data in Table 4 show that when the modified Enz method is used a positive reaction for thujone may be expected in the proper fraction when 3 mg. or more is present in the sample. In most cases the odor of the fraction appears to be as good an index of the presence of thujone as is the test, but the test and odor may serve as confirmatory evidence of the presence of thujone, especially when less than 10 mg. is present.

The experience of the writer indicates that less than 3 mg. of thujone gives a positive test at times but not always. Before the Widmer column was obtained a variety of kinds of apparatus was used for removing alcohol, but the results were irregular. In some cases a positive test would be obtained when 2 mg. was present, and sometimes negative tests were obtained when 5 mg. or more thujone was present.

In the testing of commercial samples of absinth-type liqueurs, the color test is sometimes more or less obscured by an olive green color of unknown origin.

The minimum and maximum quantities of thujone to be expected in absinth were calculated from the literature. A study of books on the manufacture of cordials shows that when they are made from essences from 0.6 to 1.0 gram of wormwood oil is added for each 10 liters of absinth. As wormwood oil is reported to contain from 3 to 45 per cent of thujone, the thujone in absinth may vary from 1.8 mg. to 45 mg. per liter.

The available recipes indicate that when absinth is made from the plant, from 25 to 32 grams of the herb *artemisia absinthium* are extracted for each liter of the liqueur. Since about 0.3 per cent of essential oil containing from 3 to 45 per cent of thujone is obtained from the herb, it may be expected that absinth made from the herb will contain from 2 to 34 mg. of thujone in one liter. The study of the modified Enz method recorded here would indicate that while positive tests may be expected on absinth-type liqueurs containing the larger quantities of oil or extracted herb, many of these products made with smaller quantities of oil or herb extract would escape detection.

SUMMARY

While the present available procedures for the detection of thujone in absinth-type liqueurs can be depended upon to give positive tests when 5 mg. or more of the ketone is present, the fact that the test is negative does not show conclusively that the liqueur does not contain thujone in quantities which may be regarded as significant from a pharmacological point of view.

ADDITIONAL REFERENCES

- (1) Anonymous, Determinations Required under the Swiss Absinth Law. *Veröffentl. kais. Gesundheits.*, 34, 1205 (1910).
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- (3) Cuniasse, Characterization of the Essence of Absinth. *Bull. soc. chim.* (4) 1, 279 (1907).
- (4) Delage, The Masking of the Ketone Reaction for Essential Oils in Absinth and Similar Liqueurs. *Ann. fals.*, 16, 412 (1923).
- (5) Duparc and Monnier, Identification of Thujone in Liqueurs. *Ann. chim. anal.*, 13, 378 (1908).
- (6) Phillipe and von Fallenburg, The Work of H. Enz on the Testing of Thujone in Absinth. *Schweiz. Wochschr.*, 49, 418 (1911).
- (7) Ronnet, Analysis of Commercial Absinth. *Ann. fals.*, 3, 477 (1910).
- (8) Schaffer and Phillipe, Investigation of and Standards for Absinth and its Substitutes. *Mitt. Lebensm. Hyg.*, 1, 1 (1910).