ASSAY OF THYMOL AND CARVACROL

BY

HERMAN FREDERICK SCHWARZ

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The first work on the action of Iodine on phenols in alkaline solution was accomplished by Messinger and Vortman at Aachen in 1889.(1)

They noticed that on the addition of alkalies and iodine to solutions of phenols, colored flocculent precipitates were thrown down. They afterwards proved that this chemical action took place quantitatively and would be very useful in the titative estimation of phenols.

Among other phenols they studied the action of iodine on thymol in alkaline solution. When iodine in potassium iodide solution is added, a dark red flocculent precipitate is thrown down, the thymol and iodine combining to form a diiodide of dithymol. F~~rom~~our molecules of alkali must be present to every molecule of thymol. This red thymol iodide was found to give off a portion of its iodine on warming or upon standing in the dry condition, and passing from the dark red to the yellow variety, which is weaker in iodine.

On account of the variable amount of iodine present in the compounds above named, analysis of the thymol iodide which they made gave a percentage of iodine much too low.

The amorphous red thymol iodide, they found melted at 110 C., giving off iodine at the same time. It is insoluble in water and sparingly soluble in alcohol, while chloroform and ether dissolve it readily.

Upon evaporation of the ethereal solution, the red thymol iodide remains behind as an amorphous mass. Upon boiling with water, it loses iodine and passes over into a light yellow amorphous iodine compound. A similar change takes place on boiling with aqueous alkaline solution, or digestion with aqueous solutions of sulphur dioxide, sodium sulphite, or sodium thiosulphate.

The yellow compound acts in a similar manner towards solvents. It is odorless and melts at 165 C. It dissolves to a slight extent on boiling with potassium hydroxide solution. On treating the cold and filtered solution with iodine , red thymol iodide again separates.

According to Carius method they found the yellow thymol iodide to contain 45.5% of iodine.

For the preparation of the red thymol iodide at least 2 molecules of potassium hydroxide must be present. If there is less thymol precipitates with the red thymol iodide. Such products form soft masses, having lower melting points than thymol iodide. If the soft mass is distilled with water vapor, thymol passes over and the remaining resinous portion, upon boiling with potassium hydroxide, liberates still more thymol. If the remainder is dissolved in ether and the solution treated with alcohol, a light yellow, nearly white, flocculent thymol iodide separates out. If the red thymol iodide is prepared with a sufficient excess of alkali, and kept in closed receptacles, it will keep its color for many months, but if kept in a closed damp vessel it will turn yellow in a very short time (several hours). If protected from light, even exposed to air, it keeps its color several weeks.

These two thymol iodides stand in very close relation to each other. Upon reduction of the yellow iodide in alkaline solution by means of sodium amalgam or zinc dust, no thymol is regenerated. They think it is a diodide of a dithymol, or a very similar compound. After reduction the alkaline solutions were acidulated when a white flocculent precipitate was thrown down, which was purified by repeated precipitation out of its alcoholic solution by means of water.

These white sealy crystals were found to be insoluble in water very easily soluble in alkalies and in alcohol to form yellow colored solutions.

As Messinger and Vortman suspected this compound to be a dithymol, they made a dithymol according to Dianin's process (2) by means of ferric alum solution on thymol. Both compounds had the same properties and the same melting points. Dianin's preparation, he claimed, had the melting point of 165.5 C., while their preparation and the one prepared according to Dianin melted at 135 C. The chemical analysis gave no results which agreed with dithymol. The average of the constituents found were 75.6 carbon and 8.6% hydrogen. As dithymol should have 87.26% carbon and 8.72% hydrogen, the above percentages would correspond better with an oxydithymol which contains 76.41% carbon and 8.29% hydrogen. The analytical results of the red dithymol dioidide would also agree better with the preparation of this latter reduction product although the synthetical examination gave that one molecule of thymol used four atoms of iodine, that therefor probably only 2 atoms were taken up. The chemical analysis of the freshly prepared red thymol diiodide showed it to contain 48 to 52 per cent of iodine. This amount would give three atoms of iodine to every molecule of thymol. Both compounds (red and yellow) can be considered derivati~~ons~~ves of dithymol. The yellow variety on account of its insolubility in alkalies, according to Zincke, may be a diketodiiodide, while the red may be a partly keto compound and partly iodooxyl derivative.

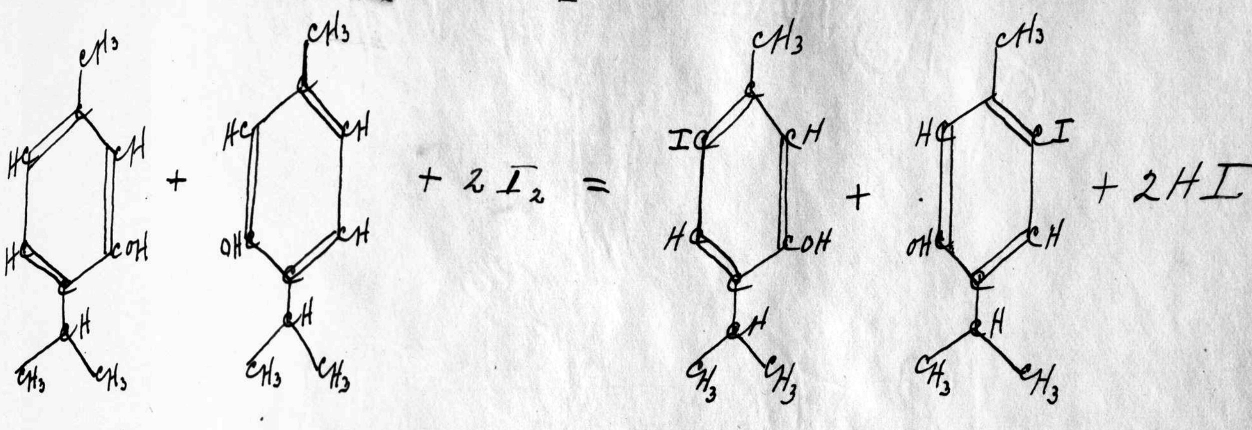
As to the position of the two iodine atoms in the yellow variety, they assume that the two atoms are in the same position as the iodine in the mono thymol iodide prepared by Willgerodt,(3) and when this compound was treated with alkali and iodine, it furnished their red thymol diodide, one molecule of the above monothymol iodide used up two atoms of iodine.

That this compound is identical with their red thymol diiodide, proves that one atom of iodine in the thymol molecule in the red as well as in the yellow thymol diiodide has the same position as in the monothymol iodide prepared by Willgerodt.

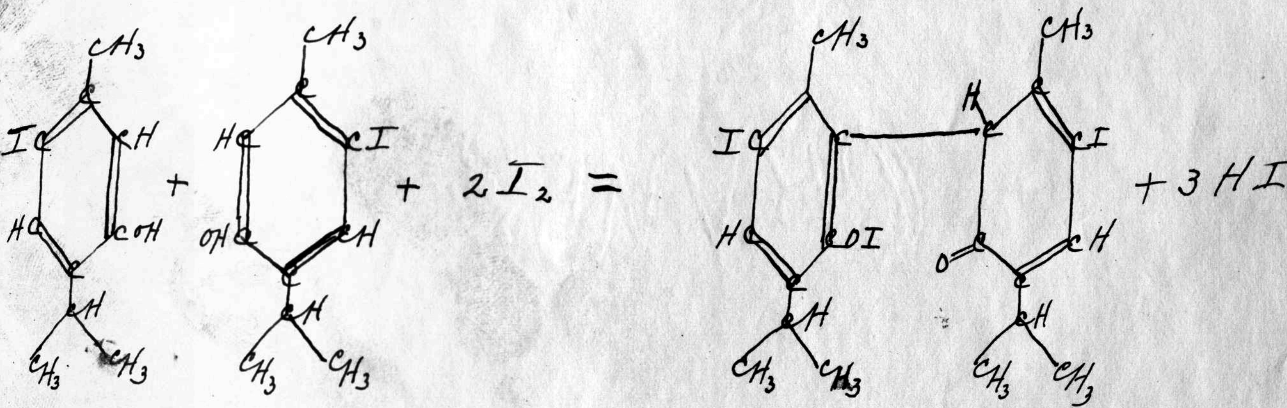
Upon analysis the above yellow thymol diiodide was found to contain 47.24% of iodine.

The preparation of the red thymol diiodide according to the preceeding is explained in the following formula:

I

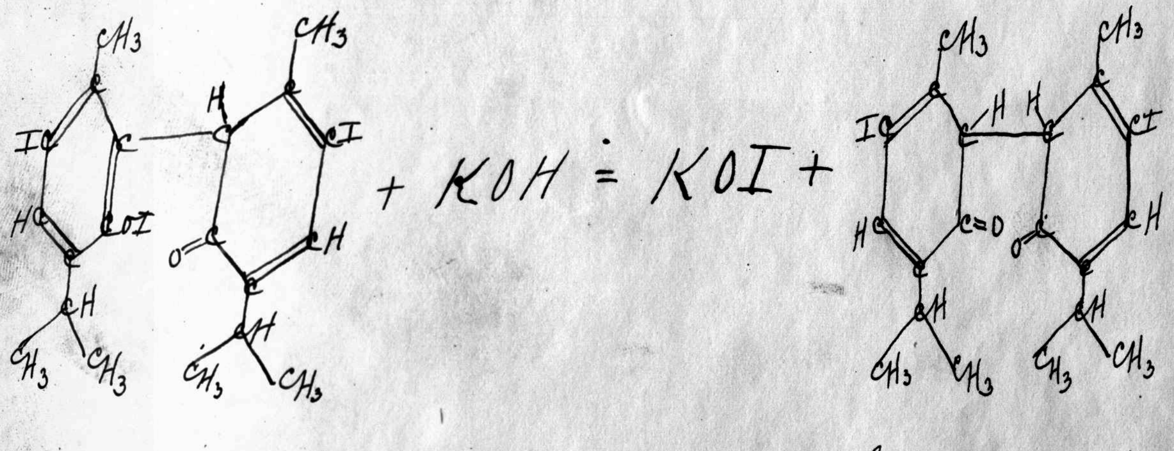


ii



red thymol iodide

change of red to yellow iodide.



yellow thymol iodide.

Quantitative experiments showed that by the changing of the red to the yellow thymol diiodide about one atom of iodine to one molecule of the compound stepped out. A portion of the red preparation, treated with hypoiodide of potassium and dilute sulphuric acid, was distilled with water vapor. Upon analysis 12.36% of iodine were found.

A portion of the red preparation was boiled with a standard solution of potassium hydroxide, the solution was then filtered and the excess of potassium hydroxide titrated with standard sulphuric acid. According to this estimation 12.33% of iodine had stepped out. According to the above comparison 18% of iodine should have stepped out, but knowing that the red preparation could not be weighed before it decomposed some, the above result may still be considered sufficient proof for the corrections of the equation given above. With their method they could not obtain the monothymol iodide described by Willgerodt; upon the addition of iodine to an alkaline thymol solution. Even when the thymol is in great excess, the first drop of iodine solution will form a red precipitate.

They also found that the isomer of thymol, namely carvacrol, gave a yellow precipitate with iodine and alkali, which however they did not examine.

A year after the above work was accomplished, Messinger and Vortman made the following thymol estimations in known solutions.(4)

The factor by which the amount of iodine consumed must be multiplied to calculate the amount of thymol present is the following:

$$\frac{\text{1 molecule thymol}}{\text{4 atoms of Iodine}} - \frac{149.66}{506.16} - 0.2956772$$

The thymol (about .1 to .3 gr) was dissolved in sodium ~~by~~hydroxide solution (four molecules of the sodium hydroxide to one molecule of thymol). To the alkaline solution decinormal iodine was added in excess. It was then acidulated and the excess of iodine was titrated by means of the decinormal sodium thiosulphate solution. Their results agreed very closely with the amount of thymol dissolved in the sodium hydroxide solution.

Amount dissolved	Amount found
0.2874 gm	0.2902 gm
0.1964 "	0.1947 "
0.3878 "	0.3863 "
0.3832 "	0.3798 "
0.1293 "	0.1288 "

Among the phenols that occur in volatile oils, thymol and carvacrol are not the least important. The only way according to which the quantity of a phenol in an oil was estimated, until recently, was either by fractional distillation or by shaking out the phenol with aqueous alkali. The latter method for example has been employed in assaying oils of monarda distilled from plants collected at different seasons of the year, also in controlling the commercial value of such oils, as oil of thyme. There can be but little doubt that the second method is preferable to the first not only because it will admit of using smaller quantities for assay, but also because on the whole it may beconsidered more accurate. Nevertheless it has shortcomings which at times may render it useless for absolute determination. Thus for example, Kremers and Schreiner, call attention to the fact that even five per cent solution of caustic alkali will dissolve other constituents of the oil than phenol. Especially is this true of old oils containing a large percentage of resinous substance, thus rendering the method at times worse than worthless for the phenol assay of volatile oils. For these reasons the work of Messinger and Vortman was applied to the assay of thymol and carvacrol in volatile oils. The experience so far gained as to its applicability has been entirely in connection with the oils of *Monarda punctata* and *Monarda fistulosa*.

E. Kremers and Oswald Schreiner devised the following method for the estimation of these phenols in volatile oils.(6) A rather unsatisfactory method had been used before, which gave only a comparative percentage of phenol between the different oils. The trouble was, that the five or ten per cent alkali with which the oil was shaken, dissolved other constituents besides the phenol, thus giving a result which was too high. In this method the difference by volume of the oil taken and that remaining after shaking with the alkali was calculated as phenol. It was soon noticed that the results varied with the strength of the solution, as well as with the quantity of the solution with which the oil was shaken. Furthermore when such an alkaline solution was diluted with water a lower percentage of phenol was found.

These facts clearly showed that the alkaline solution also removed some other constituents from the oil. As the iodine compounds are most stable and most easily made, they of course would be looked to, to furnish a method for the quantitative estimation of the phenols.

As for every molecule of thymol present, there should be at least four molecules of alkali, this ratio cannot be obtained in volatile oils which contain a variable amount of phenol.

Experiments were then made using different percentage strength solutions of alkali in which known weights of thymol had been dissolved. It was found that five per cent alkali gave the best results, and also that a moderate excess of the alkali could be used.

Slightly different results were obtained when a more dilute solution of alkali was used, the results coming out somewhat higher. The method of titration was similar to

Messinger and Vortman's except that an excess of alkali was always employed.

To estimate the thymol in volatile oils a known quantity of the oil was repeatedly shaken with a five per cent potassium hydroxide solution until all the thymol had been removed. The amount of thymol in the alkaline solution was then determined by titration with $\frac{N}{10}$ iodine solution. In order to compare this method with the solution method before described the volume of oil that remained after shaking out the phenol was always noticed. For this purpose a Mohr burette with glass stopcock, graduated to 1/10 C.C. was fitted with a glass stopper at the top, so that the oil could be shaken with the alkaline solution. The oil was diluted with an equal volume of a low boiling fraction of petroleum ether. The advantage is that the alkaline solution is not as likely to dissolve the other constituents. The separation of oil and solution is also much more rapid and complete. After separation had taken place, the remaining volume of oil could be read off accurately. The alkaline solution was then allowed to run off into a graduated flask and the remaining oil treated with fresh solution. This operation was repeated till all the thymol had been removed. By proceeding in this way both methods could be applied to the same quantity of oil at the same time. The method in detail is as follows:

From a weighed flask of oil pour out about five cubic centimeters into a glass stoppered burette graduated to 1/10 cubic centimeters. Weigh the flask again, the difference gives the weight of oil used for the experiment. Add about an equal volume of petroleum ether and then note the volume of the mixture carefully. A little water can be added so as to bring the oil up to the scale of graduations on the burette Now add the five per cent potassium hydroxide solution and shake vigorously for a short time, then allow to stand until separation is complete. Note the decrease in the volume of the oil and allow the alkaline solution to run into a 100 c.c. graduated flask. This operation is repeated several times, until no further decrease in the volume of the oil takes place, or better still, until the oil no longer shows Flückiger's test for thymol. To make this test put a few drops of the oil into a test tube, add about twenty drops of chloroform and a small quantity of solid sodium hydroxide and apply heat. If thymol is still present a red color will appear. When all the thymol has been removed notice the volume of the remaining oil carefully. The difference between the volumes of oil before and after shaking with the alkali gives the volume of thymol dissolved. From this the percentage by volume is readily calculated. The alkaline solution of thymol is made up to 100 c.c. or 200 c.c. as the case may require using the five per cent alkali as diluent. To ten cubic centimeters of this solution in a graduated 500 c.c. flask decinormal iodine solution in slight excess is added. The thymol is precipitated as a dark reddish-brown iodine compound. In order to know when a sufficient quantity of iodine has been added, remove a few drops from the flask to a test-tube containing some dilute hydrochloric acid. When sufficient iodine is present the color of the solution shows the presence of iodine, otherwise the solution is milky due to the separation of thymol. The solution is now made slightly acid with dilute hydrochloric acid and diluted too 500 c.c. From this 100 c.c. are filtered off and the excess of iodine determined by titration with decinormal sodium thiosulphate.

The number of cubic centimeters required, multiplied by five, and deducted from the number of cubic centimeters decinormal iodine added gives the number of cubic centimeters of iodine required by the thymol.

$$C_{10}H_{14}O + 4I + 2NaOH = C_{10}H_{12}I_2O + 2NaI + 2H_2O.$$

Every cubic centimeter of decinormal iodine solution equals 0.0037415 grams of thymol. Knowing the quantity of thymol in the alkaline solution, the percentage in the original oil is readily found. Twenty-five per cent solutions of pure thymol in pure limonene and pure cymene were made and subjected to the above method of estimation, 28% and 30% respectively were obtained by the solution method and 25.1% and 25.4% respectively were obtained by the iodine method. The estimation of carvacrol was carried out in a similar manner, the only difference being that the acidulation of the alkaline solution was made later than in thymol estimation. After an excess of iodine was run in, the solution was made up to 500 c.c., 100 c.c. were then filtered off and acidulated. The excess of iodine was estimated by means of decinormal sodium thiosulphate solution. The rest of the process and calculation was carried out exactly like that in the estimation of thymol.

The method was tried with the following result, at the same time a comparison being made with the old method of shaking out the phenol:

		Iodine method.		Solution method.	
Oil from	Sp Gr.	Thymol	Carvacrol	Thymol	Carvacrol
Thyme		56%		65%	
Monarda fistulosa	0.939		58.26%		70%
Monarda fistulosa	0.927		53.90%		65%

Kremers and Melzner(7) made some estimations of carvacrol in several samples of oil of monarda fistulosa. The averages are the following:

Samples of Oil.	Carvacrol found by iodine method.	Carvacrol found by solution method
1.	52.15%	64 %
2.	54.35%	66 %
3.	58.24%	70 %
4.	58.22%	71 %
5.	53.35%	64.5%
6.	54.36%	65.5%

EXPERIMENTAL PART.

On attempting the assay of thymol it was noticed that there is always a portion of free iodine present, which does not combine with the thymol, and is very misleading in that, if tested for free iodine by dropping a few drops of the solution into dilute hydrochloric acid, it will give a yellow coloration. In place of the latter test carbon bisulphide was also employed. It was noticed that when no free iodine was present, the carbon bisulphide was not colored pink but became cloudy in appearance. If the least amount of iodine was present which had not combined though thymol was in excess, the carbon bisulphide was colored pink.

For closer examination of this action when different quantities of iodine were employed, the following table will be of some value. Five grams of thymol and eight grams of potassium hydroxide were dissolved in sufficient water to make 500 c.c. of solution. This solution contained one molecule of thymol to every four molecules of potassium hydroxide. 10 c.c. of this solution were used for every titration with decinormal iodine solution. Thus 10 c.c. contained 0.100 grams of thymol.

	ess of found the whole sol.	$\frac{N}{10}$ I used up by thymol	Thymol found	% of thymol	Remarks
52.79	23.80	28.99	0.10846	108.46	
48.08	19.35	28.73	0.10749	107.49	
45.3	16.65	28.65	0.10719	107.19	
45.05	16.55	28.50	0.10663	106.63	Yellow color dropped HCl dil.
45.03	16.60	28.43	0.10637	106.37	
45.02	16.65	28.37	0.10615	106.15	
44.93	16.45	28.48	.10656	106.56	
44.91	16.70	28.21	.10555	105.55	
43.52	15.10	28.42	.10633	106.33	
42.18	13.95	28.23	.10562	105.62	
40.50	12.80	27.70	.10364	103.64	Very slight yellow no turbidity.
40.16	12.30	27.86	0.10424	104.24	Very slight yellow faint with CS_2
40.10	11.90	28.20	0.10551	105.51	
40.17	11.65	28.52	0.10670	106.70	
40.11	11.65	28.46	0.10648	106.48	
39.02	10.60	28.42	0.10633	106.33	
35.13	7.15	27.98	0.10468	104.68	Gave blue color with starch before acidulation.

35.07	7.30	27.77	0.10390	103.90	
35.06	7.35	27.71	.10367	103.67	
35.06	7.60	27.46	0.10274	102.74	
34.95	7.20	27.75	0.10382	103.82	
36.10	8.25	27.85	0.1042	104.20	
32.37	5.25	27.12	0.10147	101.47	
31.36	4.35	27.01	0.10106	101.06	Pink color with CS_2
30.08	3.10	26.98	0.10094	100.94	do
29.38	3.00	26.38	0.09870	98.70	slight pink with
28.48	1.90	26.58	0.09945	99.45	
27.30	1.03	26.27	0.09829	98.29	
27.07	0.50	26.57	0.09941	99.41	
26.96	1.34	25.62	0.09586	95.86	
26.84	0.88	25.96	0.09712	97.12	
25.01	0.70	24.31	0.09096	90.96	

From the above table we see that no matter how much iodine has been added, a test for the same will give an affirmative result. Two tests for free iodine were applied. The one was to add a drop or two of the solution to dilute hydrochloric acid. A yellow coloration without turbidity signified free iodine. The other test was to add a drop or two of the solution to some dilute hydrochloric acid and then shake this with a little carbon bisulphide. A pink coloration of the carbon bisulphide took place if free iodine was present. This latter test was also misleading as stated before. When large excesses of iodine were used, the results were proportionately much higher. This it is supposed was due to secondary reactions taking place. With decrease of iodine used the percentage also decreased. Within the limit of two to five cubic centimeters in excess the best results were obtained, but an excess must be present or the results will be too low. Excess of acid does not change the result any.

In making the estimation of thymol and carvacrol in volatile oils containing variable amounts of these phenols it is advisable to add a sufficient large excess of iodine to show a very strong coloration of iodine and no turpidity when a drop or two are added to dilute hydrochloric acid. If this is done it will be found that an excess of at least 10 c.c. has been added. The operation is then repeated with a new quantity using a smaller excess of iodine each time until there is an excess of about 3 cc. of iodine present in the whole quantity.

Calculating from this the amount of phenol present, will give a result within one per cent of the true amount.

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Prof. of Pharm. Chemistry.