I. A STUDY OF THE COLOR FORMING REACTION IN ACETYLSALICYLIC ACID-CODEINE TABLETS

II. A SPECTROPHOTOMETRIC METHOD OF ASSAY FOR SPIRITS OF CAMPHOR

by

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PART I

A STUDY OF THE COLOR FORMING REACTION IN ACETYLSALICYLIC ACID-CODEINE TABLETS

INTRODUCTION

Codeine, an important alkaloid, occurs in opium to the extent of 0.1 to 3 per cent. It resembles morphine in its general physiological action although much weaker. The alkylation of the phenolic hydroxyl group of morphine decreases the analgesic, depressant, intestinal, spasmodic, and respiratory activity. Thus codeine requires larger doses to produce the same effects as morphine, but it causes less mental depression and is much less likely to lead to drug addiction. The most extensive use of codeine is for the relief of dry, irritating cough. Codeine phosphate or sulfate may be administered by injection in solution or orally as tablets (1).

For efficient analgesic action, relaxation and sedation, codeine in the form of phosphate or sulfate has often been prescribed with aspirin, caffeine, phenacetin, etc. The preparation of compressed tablets, containing the above ingredients, however, has been rather a problem to many of the manufacturers, as the prepared tablets on aging become spotted and colored. This problem aroused our interest, calling for further investigations on the chemical aspect of the tablets. This paper deals with a study of the effect of pressure, if any, used in compressing the tablets, on the color forming reaction of the tablets, the possible cause of reaction, and an attempt at preparing a stable compressed tablet.

PAST WORK

Various workers have studied the reaction in the aspirin-codeine tablets, and attempts were made to prevent the color formed therein. According to the Swiss Pharmacopeia, these tablets were prepared from 0.5 gram acetylsalicylic acid, 0.02 gram codeine phosphate, 0.5 gram phenacetin, and 0.08 gram starch. However, they turned yellow after a while. Hence Guidini and Sargenti (2) prepared tablets containing 0.5 gram acetylsalicylic acid, 0.016 gram codeine, 0.5 gram phenacetin, 0.2 gram $Al(OH)_3$, 0.05 gram stearic acid, 0.08 gram starch, and 0.03 gram purified talc. These tablets were said to have stayed white for months and to disintegrate in 30-60 seconds in water at 15°.

Jordan and Margaret (3) as early as 1929 concluded from the experiments on preparation of capsules from aspirin, phenacetin and morphine hydrochloride that a chemical combination probably took place between aspirin, phenacetin, and morphine hydrochloride, causing the coloration.

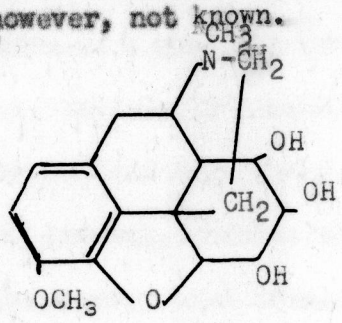
Busse and Patel (4) attempted to prevent the reaction of aspirin-codeine tablets by using ascorbic acid, and it was found that the acid could not prevent the reaction. They were, however, able to prolong the stability of tablets by the addition of powdered sugar or the mixture of powdered sugar and heavy magnesium oxide in these tablets.

Various workers have studied the nature of degradation of codeine and its salts. Pedinelli (5) studied the effects of various substances on codeine, base as well as salts, at 100°, and found that prolonged heating up to 100° or heating at the melting point for a few minutes alone or in the presence of sucrose or formaldehyde did

not have any effect. However, heating above 60° with lactose, glucose, or gum tragacanth destroyed the codeine partially, whereas almost complete destruction occurred on heating with sodium sulfite or hydrogen peroxide.

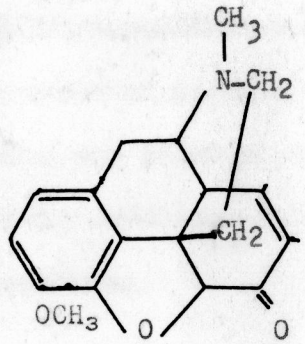
Cahn and Robinson (6) oxidized an ice-cooled 0.5% solution of codeine with one per cent aqueous permanganate leading to the addition of two hydroxyl groups to the ethylenic linking of the molecule at C_7 and C_8, and the product is dihydroxydihydrocodeine (a) which was separated from a dark brown reaction product. The nature of this dark brown residue was, however, not known.

a.



Holmes and Lee (7) oxidized codeine with a solution of chromic anhydride in dilute sulfuric acid and obtained hydroxycodeine which were "rosets of yellow leaflets" with a yield of 15%. The structure of hydroxycodeine was, however, not determined. Moreover, they oxidized acetylcodeine under the same conditions and obtained hydroxycodeine, together with codeinone (b).

b.



David (8) studied some 13 alkaloids with respect to the color changes produced by the action of a mixture of "Magnol" powder (basic magnesium hypochlorite) and acetic acid in contact with concentrated sulfuric acid. Five milligrams of the alkaloid and 3 cc of the reagent (0.2% "Magnol" powder in acetic acid) were rubbed together in a small porcelain mortar with a glass rod to complete solution, then carefully "layered" on 3 cc of concentrated sulfuric acid in a cylinder of Jene glass. The color changes were thereupon observed over a period of 22-23 hours and tabulated. The final reaction for codeine was the formation of a greenish brown ring.

Dietzel and Sollner (9) found that oxygen acts as a weakly oxidizing agent on codeine only after protracted treatment; on heating under pressure, however, codeine suffered important structural changes, forming among other things a hitherto unidentified substance which strongly absorbed ultraviolet rays. They stated that the formation of a pseudocodeine (analogous to pseudomorphine) could not be detected, a fact theoretically to be expected.

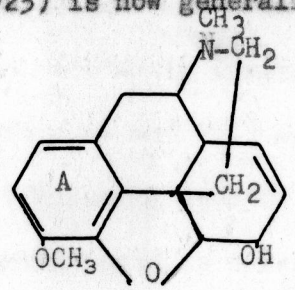
The discoloration of various salts of codeine, codeine in different gases, codeine solutions of varying pH values, solutions at varying temperatures and codeine ester (acetylcodeine) were studied by Dietzel and Stadelmann (10) by exposure to ultraviolet light. Among other findings, it appeared that the discoloration of codeine in ultraviolet light was independent of the solvent or the pH of the solution. The salts of codeine showed the same behavior as the base. The discoloration was based on oxidative action, the rapidity of which was markedly accelerated by short wave ultraviolet light and elevated temperatures.

DISCUSSION

As there had been controversy as to the cause of the color forming reaction in aspirin-codeine tablets, tablets containing different combinations of the ingredients were made and the results were observed. As it was considered that the amount of force used in compressing the tablets might have some effect on the formation or rate of formation of color, the tablets were compressed with different forces.

As it was observed that all tablets containing codeine phosphate were colored after some time, and all others not containing codeine remained white, it was assumed that the change taking place in the tablets was due to the degradation of the codeine molecule. Attention may therefore be given to the chemistry of codeine.

The following structure for codeine, a methyl ether of morphine, due to R. Robinson (1925) is now generally accepted.



From the above formula, it is evident that codeine possesses two functional groups, namely a tertiary amino and an alcoholic hydroxyl, and an ethylenic bond at C_7 to C_8, which are susceptible to oxidation. As a result, the deterioration of aspirin-codeine tablets is probably due to the autoxidation of codeine. Some of the oxidation products were already mentioned in the introduction. Furthermore, ring A, which is a derivative of catechol, might be

oxidized to form a colored degradation product. Deterioration even in the absence of oxygen might also take place by means of peroxidation or polymerization through the oxygen of the oxide grouping which could be reactivated by light and metal ions. This is probably the case when codeine tablets are colored after being compressed with such antioxidants as ascorbic acid.

All autoxidations are caused by free radicals (i. e. molecules or atoms with unpaired electrons) which are capable of starting a chain mechanism. Robertson and Waters (11) in their study of the autoxidation of tetralin divide the autoxidation catalysts into two groups: (i) "initial catalysts" which attack the hydrocarbon, abstracting hydrogen and producing an active radical and (ii) "secondary catalysts," such as salts of copper, cobalt, etc., which promote the decomposition of the hydroperoxide and thereby increase the steady concentration of active radicals. Hence antioxidants may be divided into two types: (i) chain terminators such as ascorbic acid, sodium sulfite, hydroquinone, and (ii) anti-catalysts such as sequestrene and other metal complexing agents.

The fact that ascorbic acid is essentially a chain terminator would probably account for its failure to prevent the deterioration of aspirin-codeine tablets. Kellie and Silva (12) reported that copper and iron in very slight traces catalyzed the irreversible oxidation of ascorbic acid dissolved in ordinary distilled water. Barron et al. (13) showed that the catalytic action of copper on the oxidation of ascorbic acid to dehydroascorbic acid was noticed in concentrations as small as 4.6 micrograms of copper per liter.

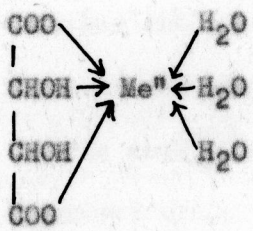
In view of the fact that both ascorbic acid and sodium sulfite

failed to prevent the formation of color in aspirin-codeine tablets, it was assumed that the tablets contain one or more metallic impurities in free, oxide or salt form that might have come from metallic containers. Hence the use of such anti-catalysts as would be able to tie up the metals was considered.

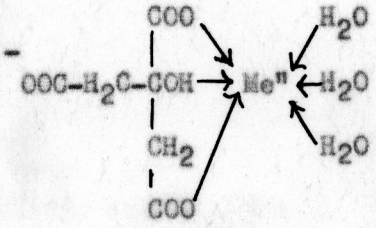
It may also be mentioned here that the protective action of sugar on the oxidation of aspirin-codeine tablets (5) and codeine and its salts (4) is probably due to the anticatalytic action of the carbohydrate. It was reported by Shamrai (14) that the stabilization action of cane sugar on ascorbic acid was due to the exclusion of the catalyzing action of copper traces, by forming copper saccharates. Lieser and Ebert (15) gave the formula for copper complexes with glucose, galactose and fructose as $C_6H_{12}O_8Cu_2$.

Various workers have studied the complexing action of certain organic compounds on metals. Huclin and Stephens (16) reported that in phthalate and phosphate buffers the rate of oxidation of ascorbic acid in solution increased with copper concentration, and that oxalic, malic, citric, and tannic acids inhibited copper catalysis. Martell and Bersworth (17) showed that heavy metal and alkaline earth metal ions reacted with the alkali metal salts of amino acids, such as ethylenediaminetetraacetic acid to form complex ions or chelates. From measurement of the absorption spectra for cupric ion complexes with various substituted carboxylate ions, Klotz et al. (18) observed a marked increase in the formation of complexes of cupric ion with citrate ion. He remarked that the affinity of the organic ion for the metal ion comes not only from electrostatic attraction of the negatively charged substituents but also from the

statistical effect of having three carboxylate groups available to the cation. Sarma (19) also found, from conductometric measurements, that in dilute solutions a complex of copper and tartrate ion in the ratio of 1:1 was formed. It was reported by Bobtelsky and Jordan (20) that salts of hydroxy carboxylic acids like citric and tartaric acids can complex with copper and cobalt salts in aqueous solutions. The structure for such a complex was formulated as follows:



Tartrate



Citrate

where Me" stands for a divalent metal having a coordination number of six.

It was therefore hoped that a codeine salt of such acids would be able to complex with metallic impurities, such as copper or iron, in a similar fashion, and thus prevent the color-forming reaction when it is compressed into tablet form.

EXPERIMENTAL PROCEDURE

Apparatus and Chemicals.

All tablets were compressed with the Stokes model A-3 single punch tablet machine, instrumented for measuring compressional force, and fitted with a 3/8 inch flat-headed punch and die set.

Aspirin, free flow crystals (Mallinckrodt)

Phenacetin, USP (American Pharmaceutical)

Codeine (Mallinckrodt)

Codeine phosphate (Merck)

Citric acid, USP (Merck)

Tartaric acid, USP (Chas. Pfizer and Co., Inc.)

d,l-Malic acid (Eastman Kodak Co.)

Preparation of Codeine Citrate, Tartrate and Malate.

Approximately equimolecular amounts of the base and the acid were dissolved separately in acetone and the two solutions mixed together. The precipitated salt was filtered off. The filtered precipitate was recrystallised from dilute alcohol. The codeine citrate was a white powder (m.p., 174-6°). In the case of the tartrate and malate, both salts were hygroscopic; hence they were discarded.

Titration of Codeine Citrate.

Codeine citrate was titrated with perchloric acid in glacial acetic acid in order to determine the ratio of codeine and citric acid in the salt. Methyl violet (0.2 gram in 100 ml chlorobenzene) was used as an indicator. An approximately decinormal solution of perchloric acid was used and was standardized against potassium acid

phthalate. The analysis of the codeine citrate shows that it is formed from codeine and citric acid in the ratio of 1:1.

Compression of Tablets.

The following formulae were used in compressing the tablets. The formulae were laid out in the same proportion as in commercial tablets; i. e., aspirin, 3½ gr; phenacetin, 2½ gr; and codeine salt, ¼ gr. The weights were increased proportionately so as to fill the 3/8 inch punch used in compressing the tablets.

A.	Aspirin (F. F.)	0.370 gm.	
	Codeine phosphate	0.027 gm.	
B.	Aspirin (Dow)	0.370 gm.	
	Codeine phosphate	0.027 gm.	
C.	Phenacetin	0.270 gm.	weight of each tablet = 0.320 gm.
	Codeine phosphate	0.027 gm.	
	Starch paste 10% q.s.		
D.	Aspirin (F. F.)	0.23 gm.	
	Phenacetin	0.16 gm.	
E.	Aspirin (Dow)	0.23 gm.	
	Phenacetin	0.16 gm.	
F.	Aspirin (F. F.)	0.370 gm.	
	Codeine citrate	0.027 gm.	
G.	Phenacetin	0.270 gm.	weight of each tablet = 0.320 gm.
	Codeine citrate	0.027 gm.	
	Starch paste 10% q.s.		

In mixing the ingredients, the required amounts of each particular formula to make 15 tablets at each compressional level were weighed, and a small amount of each was introduced in a wide-mouthed bottle and shaken. Uniform admixture was thus assured. In formulae A, B, D, E, and F, no starch paste was used, as aspirin, either in crystalline or granulated form, was present in the mixtures, thus enabling them to be easily compressed.

In formulae C and G, 10% starch paste was used, as all the ingredients were in the amorphous state. After the ingredients were thoroughly mixed in the above manner, the homogeneous mixture was moistened with the 10% starch paste until the moist mass could be squeezed into a ball which would not crumble when broken. This mass was then forced through a No. 12 sieve. The granulation was then placed in an oven and allowed to dry. When the granulation was thoroughly dry, it was ground gently in a mortar to obtain finer and more uniform granulation for compression. No disintegrating agent or lubricant was used. Ten tablets of each were compressed, using a 3/8 inch punch with the Stokes tablet machine. The compressional forces used varied from 500 to 3000 pounds per tablet. Formulae A to E were compressed at compressional force levels of 500, 1000, 1500, 2000, 2500, and 3000 pounds per tablet; formulae F and G only at 3000 pounds per tablet.

The compressed tablets were put in separate open vials which were then kept in an oven at $40 \pm 1^{\circ}$. A beaker filled with water was placed in the oven so as to saturate the oven with moisture.

RESULTS AND CONCLUSION

The tablets were observed for any change in color over a period of two months. The results of this observation are shown in Table 1.

From Table 1, it is observed that tablets containing codeine phosphate were all colored. The color was spotted brown. Those prepared with codeine citrate were more stable. The tablets which were compressed at different forces and which were colored showed no difference as regards the color change, except that at the start of the color reaction, the intensity of color developed in tablets compressed at lower forces was slightly less than that developed in tablets compressed at higher forces.

It is also observed that tablets prepared by formula C developed less color than those prepared by formulae A and B. Similarly, tablets prepared by formula G developed less color than those prepared by formula F. This is probably due to the use of starch paste in granulating tablets of formulae C and G, the starch particles forming a protective coating around the codeine salt in the tablets.

It is therefore concluded that the color-forming reaction in aspirin-codeine tablets does not appreciably depend upon the amount of force used in compressing the tablets; it is probably due to the autoxidation of the codeine molecule and can be prevented to a considerable extent by using codeine citrate instead of codeine phosphate.

Table 1. Color Formation in 3/8 Inch Tablets Compressed with Different Forces

Formula	Compressional Force (lbs. per tablet)	Number of weeks the tablets were kept in the oven							
		1	2	3	4	5	6	7	8
A	500	-	+	++	+++	+++	+++	+++	+++
	1000	-	+	++	+++	+++	+++	+++	+++
	1500	-	+	++	+++	+++	+++	+++	+++
	2000	-	++	+++	+++	+++	+++	+++	+++
	2500	-	++	+++	+++	+++	+++	+++	+++
	3000	-	++	+++	+++	+++	+++	+++	+++
B	500	-	+	++	+++	+++	+++	+++	+++
	1000	-	+	++	+++	+++	+++	+++	+++
	1500	-	+	++	+++	+++	+++	+++	+++
	2000	-	++	+++	+++	+++	+++	+++	+++
	2500	-	++	+++	+++	+++	+++	+++	+++
	3000	-	++	+++	+++	+++	+++	+++	+++
C	500	-	-	+	++	++	++	++	++
	1000	-	-	+	++	++	++	++	++
	1500	-	-	+	++	++	++	++	++
	2000	-	+	++	++	++	++	++	++
	2500	-	+	++	++	++	++	++	++
	3000	-	+	++	++	++	++	++	++
D	500	-	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-	-
	1500	-	-	-	-	-	-	-	-
	2000	-	-	-	-	-	-	-	-
	2500	-	-	-	-	-	-	-	-
	3000	-	-	-	-	-	-	-	-
E	500	-	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-	-
	1500	-	-	-	-	-	-	-	-
	2000	-	-	-	-	-	-	-	-
	2500	-	-	-	-	-	-	-	-
	3000	-	-	-	-	-	-	-	-
F	3000	-	-	-	-	-	-	-	++
G	3000	-	-	-	-	-	-	-	+

+ indicates browning
- indicates no change

REFERENCES

(1) Jenkins, G. L. and Hartung, W. H., The Chemistry of Organic Medicinal Products. John Wiley and Sons, New York, 1949.

(2) Guidini, C. and Sargenti, L., Pharm. Acta Helv., 22, 612-13 (1947)

(3) Jordan, C. B. and Margaret, S. K., J. Am. Pharm. Assoc., 18, 810 (1929)

(4) Busse, L. W. and Patel, I. N., M. S. Thesis, University of Wisconsin, 1951.

(5) Pedinelli, M., Ann. Chim. Applicata, 39, 512-22 (1949)

(6) Cahn, R. S. and Robinson, R., J. Chem. Soc., 908-912 (1926)

(7) Holmes, H. L. and Lee, C. C., J. Am. Chem. Soc., 69, 1996-7 (1947)

(8) David, L., Pharm. Ztg., 70, 969-72 (1925)

(9) Dietzel, R. and Sollner, K., Apoth. Ztg., 45, 1030-2 (1930)

(10) Dietzel, R. and Stadelmann, L., Arch. Pharm., 276, 621-33 (1938)

(11) Robertson, A. and Waters, W. A., Trans. Faraday Soc., 42, 208-9 (1946)

(12) Kellie, A. E. and Zilva, S. S., Biochem. J. (London), 29, 1028-35 (1935)

(13) Barron, E. S. G., De Meio, R. H., and Klemperer, F.,J. Biol. Chem., 112, 625-40 (1936)

(14) Shamrai, E. F., Voprosy Pitaniya, 10 (3-4), 42-8 (1941)

(15) Lieser, T. and Ebert, R., Ann. der Chemie, Justus Liebigs, 532, 89-94 (1937)

(16) Huelin, F. E. and Stephens, I. M., Australian J. Sci. Research, 50-7 (1948)

(17) Martell, A. E. and Bersworth, F. C., Proc. Sci. Sect., Toilet Goods Assoc., 10, 26-30 (1948)

REFERENCES

(Continued)

(18) Klotz, I. M., Faller, I. L., and Urquhart, J. M., J. Phys. Coll. Chem., 54, 18-32 (1950)

(19) Sarma, R. N. S., J. Indian Chem. Soc., 27, 683-90 (1950)

(20) Bobtelsky, M. and Jordan, J., J. Am. Chem. Soc., 67, 1824 (1945)

PART II

A SPECTROPHOTOMETRIC METHOD OF ASSAY FOR SPIRITS OF CAMPHOR

INTRODUCTION

Although spirits of camphor have been used for many years, no rapid and accurate method for their assay has been advanced. In this paper, a rapid quantitative spectrophotometric method of analysis is reported.

PAST WORK

All of the assays reported by previous workers have been mostly extraction, gravimetric, volumetric, and a few instrumental types. Some of the typical assay procedures are described below.

Among extraction procedures, one reported by Jumeau (1) involves the salting out of the camphor from hydro-alcoholic solution with saturated lead acetate solution, redissolving the precipitate in ether, and evaporating the ethereal solution. The residue is then weighed. In a similar method reported by Vieth and Bilhuber (2), it is stated that the recovery of camphor varied from 97 to 99 per cent of the theoretical amount. Gori (3) used carbon tetrachloride instead of ether as the extracting liquid. The principal disadvantage in these methods is that camphor, being a substance which volatilizes at all temperatures, may be lost during evaporation of the solvent.

In another similar procedure, presented by Kollo (4), camphor was extracted in a known volume of ether. A 10 ml portion of the ethereal solution was weighed. A blank was run by using 70% alcohol instead of the sample. The concentration of camphor in the sample was determined from the difference between the weights of the ethereal extract of the spirits and of the blank. In this procedure, there is the possibility of extracting varying amounts of alcohol in the ether layer, due to

differences in the alcohol concentration between the samples and the blank, and a change in volume of ether layer, due to the presence of camphor in the sample.

Various workers have reported a gravimetric method of analysis of spirits of camphor by precipitating it with 2,4-dinitrophenylhydrazine and weighing the hydrazone. The latest procedure modified by Conroy (5) and adopted by the N. F. IX, claimed to give a recovery of 99.41 to 99.57 as studied by Corbin and Green (6). However, according to Mitchell (7), the results obtained by various workers show an accuracy for this procedure of no better than 98.5 ± 1.5 per cent. The low results may be due to the steric hindrance around the CO grouping of the camphor molecule and the resultant incomplete reaction, or to decomposition of the hydrazone. The main disadvantage of this method, other than incomplete recovery of the camphor, is that it is time-consuming.

Bryant and Smith (8) reported an acidimetric titration method for the determination of a number of carbonyl compounds, including camphor, by using hydroxylamine hydrochloride and pyridine as reagents. The oximation takes place according to the equation:

$$RCOR' + H_2NOH \cdot HCl \longrightarrow RR'C{=}NOH + HCl + H_2O.$$

The liberated acid was then titrated with a standard alkali. The main drawback to this method is that while simple or active carbonyl compounds react completely at room temperature within a short time, the more stable and sterically hindered carbonyl compounds require several hours at high temperatures. The analytical data reveal that a 0.5 gm sample of dl camphor requires 3½ days in the cold with a recovery of 93.5 per cent and 5 hours at 98-100° with a recovery of 98.8 ± 0.2

per cent of the theoretical amount. Furthermore, in the visual titration of the released acid, the precision and accuracy depend to a large extent on the ability of the analyst to match the end point with that of the blank. Under favorable conditions, the relative error is at least ± 1 per cent.

In a quantitative assay method of spirits of camphor reported by H. Bataille (9), water was added to 10 cc of the sample until a permanent precipitate formed. From the amount of water added, which was called "index of precipitation," the concentration of camphor in the sample was calculated.

An instrumental method, official in the U.S.P. IX and X, was reported by Collins (10). This method was based upon the degree of rotation of a camphor solution as determined by means of a polariscope. The disadvantages of this method are that the presence of optically active impurities would interfere with the determination, that the extent of rotation depends on the composition of the solvent, and that this method cannot be applied to the assay of spirits containing synthetic camphor, which is optically inactive.

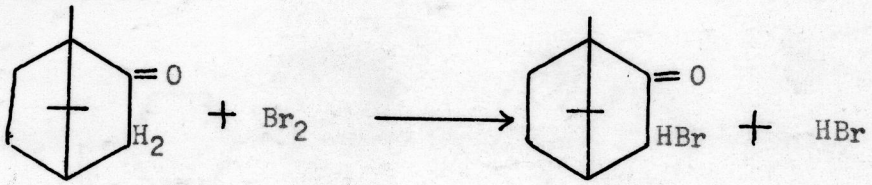
Another instrumental method, involving the use of two physical constants, namely the refractive index and specific gravity, was presented by Flein and Poe (11). This method is relatively simple and rapid, as the concentration can readily be read from a chart constructed from determinations of known samples. However, the authors claim an accuracy for this procedure of about ± 1.8 per cent of the theoretical amount. Difficulties in using this method arise from the fact that refractive index and specific gravity change slowly with changes in camphor concentration. For example, at any constant specific gravity,

the refractive index changes only about 0.0010 units when the relative camphor concentration is varied by 10 per cent.

DISCUSSION

In view of the inconvenience and inaccuracy of the methods hitherto proposed or adopted, a more rapid and accurate method was attempted.

As it has been known that an active methylene group can react with bromine, a bromination procedure for the assay of camphor, which is a cyclic ketone, was contemplated. It has also been known that monobrominated camphor was prepared by the bromination of camphor in methyl alcohol on a water bath for 3 hours with a yield of about 65% after recrystallization (12). Camphor was brominated according to the following reaction:

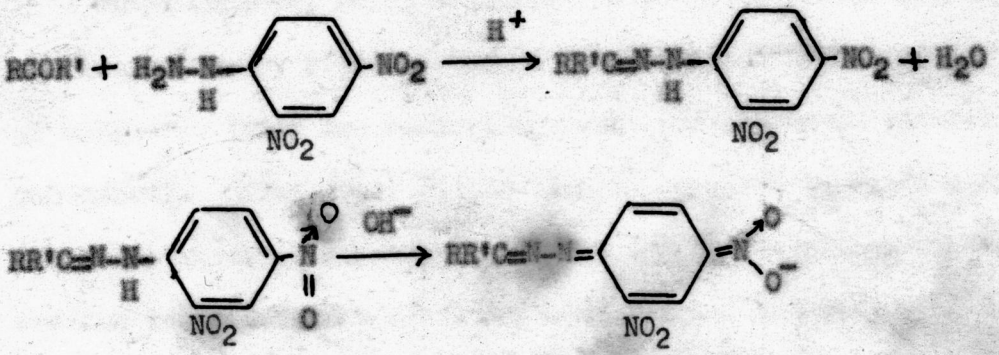


It was hoped that, if camphor could be quantitatively brominated with an excess of bromine solution at room temperature, the assay could be effected by just determining the excess bromine left after the reaction. Hence the following experiment was carried out.

A solution of less than 0.5 gm of camphor was mixed with excess of bromine solution in a 250 ml Erlenmeyer flask; it was protected from light by being wrapped in aluminum foil. Acetic acid and CCl_4 were used as solvents. The mixture was stirred for more than 5 hours or

left overnight. The experiments were performed at room temperature, as both camphor and bromine could be lost at high temperatures. The mixtures were then treated with an excess KI solution, and the liberated iodine titrated with a standard sodium thiosulfate solution. No bromination was found to have taken place. The failure of this experiment was probably due to the fact that camphor very weakly enolized in solution, at least under the circumstances of the experiment. Replacement of hydrogen in the α position to the -CO- might readily take place at an elevated temperature or by using a suitable catalyst.

The next method attempted was the colorimetric 2,4-dinitrophenylhydrazine method. Lappin and Clark (13) reported a general method for the determination of traces of carbonyl compounds. This method is based on the color formed when a 2,4-dinitrophenylhydrazone of a carbonyl compound is treated with a base, according to the following reaction:



The absorbancy of the colored solution is measured at the maximum wavelength. A blank determination is made simultaneously using carbonyl-free methanol in place of the sample.

The method was repeated by using acetone as a sample carbonyl. Two peaks were observed, one at 430 and the other at 530 mu instead of one peak at 476, as presented by Lappin and Clark. In this connection, it

may be mentioned here that Pool et al. (14) reported a similar quantitative procedure by separating the hydrazone with a chromatograph column. From the spectral absorption curves determined over the range 400 to 500 mu for the alkaline solution of the dinitrophenylhydrazones of carbonyl compounds, it was shown that the absorption maxima lies around 430 mu, which is also contrary to the results obtained by Lappin and Clark. However, in this case the blank determination is made by using benzene, which is the eluant. Furthermore, a decrease of 6 per cent in absorbance was observed after the reaction mixture was left standing for one hour. When the experiment was repeated with camphor, the absorption maxima were observed at 410 and 530 mu, and the similar fading in color occurred. For these reasons, the method was discarded. The visible spectra for both acetone and camphor 2,4-dinitrophenylhydrazones are shown in figures 1 and 2.

Since camphor, being a ketone, is known to absorb ultraviolet light, spectrophotometry offers a chance for the quantitative analysis of spirits of camphor. There are several apparent advantages in the use of spectrophotometry. First, very little time is required to assay a sample, since the assay merely consists of diluting the sample with a suitable solvent, reading the absorbance of the diluted sample, and comparing with the absorbance of a solution of known concentration. The ultraviolet absorption spectra of camphor in carbon tetrachloride and absolute alcohol are given in figures 3 and 4.

Spirits of camphor contain about 5 per cent water besides alcohol as solvent. When the absorption of solutions of camphor in different alcohol concentrations was studied, it was observed that both wavelength maxima and extinction coefficients changed with changing solvent

concentrations. As a pure solvent was therefore considered desirable for spectrophotometric determinations, a procedure for extraction of camphor in a non-polar solvent was attempted. One ml of spirits of camphor was mixed with 25 ml carbon tetrachloride and from 25 to 70 ml of water in a separatory funnel. The lower CCl_4 layer was separated and dried over a drying agent, and the concentration of camphor in carbon tetrachloride was determined by measurement of its optical density. This method, though simple, proved to be a failure, as the results of several experiments show an error ranging from + 1.8 to + 3.0 per cent of the theoretical amount. The error was probably due to the loss of either the solvent during the process of shaking the mixture in the separatory funnel, or the influence of a slight amount of alcohol, which might have been extracted in the non-polar solvent.

The results of this experiment are shown in Table 1.

Table 1. Results of Assay of Spirits of Camphor by Extraction and Spectrophotometric Procedure

Water Used, ml.	Drying Agent Used	Error, %
25	anhydrous $MgSO_4$	3.0
50	anhydrous $MgSO_4$	1.8
50	anhydrous K_2CO_3	2.8
60	anhydrous $MgSO_4$	1.83
70	anhydrous $MgSO_4$	2.6

As a result of an observation of changes in the ultraviolet spectrum with difference in the composition of solvent, a study was

made as to the extent of these changes and the possibility of finding a simple method of spectrophotometric analysis. Different alcohol concentrations were prepared, and the absorption maxima and absorbances were studied for solutions of both natural and synthetic camphor. It was observed from such a study that the wavelength maxima shifted towards the shorter wavelength region, and the absorbances increased with increasing concentration of water or decreasing concentration of alcohol in the systems. (See Table 3 and Figures 5 and 6). It was also observed that the change in $E^{1\%}_{1\ cm}$ was fairly rapid, of the order of 0.017 per 1 per cent decrease in alcohol concentration or 1 per cent increase in water concentration in the solvent, from 100% to 90% alcohol (v/v), whereas a similar change below 90% alcohol was about 0.006, one-third as much change as in the former range. Taking into account these facts, it would seem desirable to choose a solvent for diluting the spirits for spectrophotometric measurement in which the alcohol concentration is less than 90%. Since the alcohol concentration of official spirits of camphor is about 85% (official limits range from 80 to 87%), it was considered that no appreciable change in alcohol concentration would take place if the spirits were diluted with 85% alcohol. Furthermore, as the concentration of camphor in official spirits of camphor is 10 per cent (official limits range from 9 to 11 per cent), the spirits must be diluted 50 times so that the absorbances will fall within a range from 0.3 and 0.9, which is roughly the preferred abosrbance working range for best quantitative results with the Beckman spectrophotometer.

EXPERIMENTAL PROCEDURE

Polarimeter from Franz Schmidt and Haensch, Berlin, fitted with a sodium light source and 1 decimeter cell, was used to differentiate between synthetic and natural camphor.

Wavelength maxima were determined with a Cary Recording Spectrophotometer equipped with ultraviolet accessories, using 1 cm silica cells.

Absorbances were measured with a Beckman D. U. Spectrophotometer equipped with ultraviolet accessories, using 1 cm silica cells.

Water content of absolute alcohol was determined by Karl Fischer Titrimeter.

Reagents.

Alcohol, absolute.

2,4-Dinitrophenylhydrazine, the Matheson Co., Inc., East Rutherford, N. J.

The kinds and sources of camphor used were as follows:

(1) Camphor, synthetic, refined, U.S.P., E. I. Du Pont de Nemours and Co., Inc., Wilmington, Delaware.

(2) Camphor, synthetic, U.S.P., W. M. Messer Corp., N. Y.

(3) d-Camphor, Eastman Kodak Co., Rochester, N. Y.

(4) d-Camphor, U.S.P., Magnus, Mabee and Reynard, Inc.

Differentiation of Natural and Synthetic Camphor.

Natural and synthetic camphor were differentiated by melting point determinations, as well as by the optical rotation. Five grams of camphor was weighed in a 50 ml volumetric flask and diluted to volume with U.S.P. alcohol. The optical rotation of the solution was

determined in 1 decimeter cell. The specific rotation of natural camphor was calculated from the following formula:

$$[\alpha]_D^t = \frac{100a}{lc}$$

where

a = the observed rotation in degrees of the liquid at a temperature t, using a sodium light,

l = the length of the tube in decimeters,

c = the concentration of the solution expressed as the number of grams of active substance in 100 ml of solution.

The melting points of synthetic and natural camphor are about 175° and 180° C respectively. The specific rotation of natural camphor used in this assay is 41° at 25°.

Preparation of Various Alcohol Concentrations.

Absolute alcohol (which contained 0.01% water) was used for this purpose. To make x% alcohol concentration, x ml of absolute alcohol was measured from a 100 ml burette into a 100 ml volumetric flask, and distilled water was added to the mark. When alcohol and water are mixed together, a rise in temperature and a contraction in volume take place. The solution was therefore allowed to stand until full contraction had taken place (at room temperature). Then the solution was made to the mark with water.

Purification of Camphor (15).

About 50 grams of camphor was dissolved in about 50 ml concentrated sulfuric acid. The mixture slowly turned yellow and then brown. It was cooled in ice and about 5 drops of concentrated nitric acid were introduced with caution. It was allowed to stand overnight at room

temperature. The mixture was slowly poured into water. The precipitated solid was filtered off, washed with water until it was free from acid, dried in vacuum desiccator, and either crystallized from Skelly C or sublimed as follows:

About 10 grams of camphor was spread out on a 5 inch watch glass, over which a 3 3/4 inch funnel was inverted. The watch glass was heated at about 40°. The first and last grams of the substance were discarded.

Sulfuric Acid Test.

About 0.5 gram of camphor was taken in a test tube and about 1 ml of concentrated sulfuric acid was added. Commercial camphor, both synthetic and natural, gave an immediate discoloration, first turning yellow and then deep brown. Both types of camphor, after purification, gave a colorless solution with sulfuric acid.

Vanillin-Hydrochloric Acid Test (16).

The reagent was prepared by dissolving 1 part of vanillin in 100 parts of 25% hydrochloric acid. About 0.25 gram of camphor was treated with about 1 ml of the reagent and the mixture heated on a steam bath. Synthetic camphor, before or after purification, gave no color reaction, while natural camphor, before purification, gave an intense blue or green coloration within 5 to 10 minutes. Natural camphor, after purification, gave no coloration.

Determination of Wave Length Maxima of Camphor Solutions.

Amounts of camphor varying from about 0.1 to 0.5 gm were weighed into 50 ml volumetric flasks and diluted with the required alcohol solutions to volume. The solutions were then filled into 1 cm silica cells and wavelength maxima determined with the Cary Spectrophotometer

at the neighborhood of the maxima and at the rate of 1 mu per division; the slit width was about 1 mm. The respective alcohol solutions which were used as solvents were used as references.

Determination of Absorbances of Different Solutions of Camphor.

After the wavelength maxima were known by the above procedure, the absorbances of the solutions of camphor were determined with the Beckman Spectrophotometer using 1 cm silica cells and a slit width of 1 mm at the respective maxima of absorption. The results of wavelength maxima and absorbances of solutions of camphor are shown in Table 3 and Figures 5 and 6.

Testing Conformity to Beer's Law.

As it was intended to use 85% alcohol as diluent for the assay of spirits, solutions of camphor with concentrations ranging from about 0.1 to 0.4 per cent in 85% alcohol were prepared and their absorbances determined. Both natural and synthetic camphor, U.S.P., were used for this purpose. The results of this experiment are shown in Tables 4 and 5 and Figure 7.

Preparation of Spirits of Camphor.

Both natural and synthetic camphor were used in this procedure. The amounts of camphor ranging from 4 to 6 grams were weighed into 50 ml volumetric flasks. To the flasks were added 37.5, 40, 42.5, and 45 mls of absolute alcohol. The solutions were then made to volume with water, and mixed.

Assay of Samples.

One ml each of the prepared spirits was measured into a 50 ml

volumetric flask and diluted to the mark with 85% alcohol. The absorbance of the resulting solution was then determined at 288.5 mu, using 85% alcohol as reference. The results of assay on the prepared spirits are shown in Tables 6 and 7.

The samples were also determined by the method given in N. F. IX (17), for the sake of comparison.

RESULTS AND CONCLUSION

The results of the experiments mentioned above are shown in the following tables and graphs.

The visible absorption curves for acetone and camphor as determined by the colorimetric 2,4-dinitrophenylhydrazine method are given in Figures 1 and 2. The absorption curve for acetone was similar to that of camphor, except that the peaks of absorption in the shorter wavelength region were at 430 and 410 mu respectively.

Commercial samples of natural and synthetic camphor give different values of $E^{1\%}_{1\ cm}$, although after purification they give almost the same value. (See Table 2). It follows that measuring the absorbance of camphor from any source is a useful criterion of its purity. Melting points of both types of camphor increase after purification, slightly in the case of natural and significantly in the case of synthetic camphor. The discrepancy may be due to the fact that the commercial samples of both natural and synthetic camphor contain impurities. This is supported by the fact that both types of camphor give positive test with sulfuric acid (15) and that natural camphor gives a positive test with vanillin-hydrochloric acid reagent (16). The discoloration of commercial samples of camphor when tested with concentrated sulfuric acid is probably due to the presence of certain organic impurities which are dehydrated and/or polymerized as sulfuric acid is a strong dehydrating and polymerizing agent. The positive test with vanillin-hydrochloric acid reagent indicates the presence of traces of phenols and/or reactive carbonyl compounds. The possible impurities in natural camphor are terpineol, phellandrene, dipentene, cadinene,

eugenol, cineol, d-pinene, safrol and acetaldehyde, which are the constituents of the oil of camphor (18); in synthetic camphor they are pinene, bornyl chloride, camphene, isobornyl acetate and isoborneol, which are the intermediate products during the technical production of camphor (19). However, these impurities cause no abnormality in the shape of the ultraviolet absorption spectra for both natural and synthetic camphor obtained from commercial sources.

From Table 3 and Figure 5, it is observed that the absorption peak shifts towards the shorter wavelength region as the alcohol concentration decreases. Furthermore, from the same table and Figure 6, it is seen that $E^{1\%}_{1\ cm}$ increases as the alcohol concentration decreases. It is probably due to the formation of a hydrate of camphor in hydroalcoholic solution, which absorbs more strongly than camphor in alcohol. Carbonyl compounds are known to form hydrates and hemiacetals. It has been reported (20) that abnormalities in the ultraviolet absorption spectra of alcohol and aqueous solutions of carbonyl compounds are due to hemiacetal and hydrate formation respectively, the extent of which can be estimated from absorption measurements.

It may also be mentioned here that the absorption spectra of camphor in carbon tetrachloride has its peak at 293 mu, whereas in absolute alcohol it is 290. (See Figures 3 and 4). The difference here may probably be ascribed to the formation of alcoholate of camphor in absolute alcohol.

The change in the rate of increase of $E^{1\%}_{1\ cm}$ as the alcohol concentration varies from 100% to 50% (v/v) may be explained as follows: In 100% alcohol, all camphor is in the form of free carbonyl and an

alcoholate. When a small amount of water is added, a large portion of camphor is converted into a hydrate. This phenomenon is seen up to 90% alcohol concentration. After this, apparently most of the camphor in solution is already present as a hydrate and hence the presence of more water does not cause the hydrate formation as much as in the former case.

From Tables 4 and 5, it can be observed that Beer's law is obeyed roughly within ± 0.7% of the average $E^{1\%}_{1\ cm}$ for concentrations of both natural and synthetic camphor ranging from about 0.1 to 0.4 per cent in 85% alcohol.

It can be seen from the results of assay in Tables 6 and 7 that the difference in alcohol concentration in the spirits of camphor does not affect the assay. The reason for this is due to the dilution of the sample fifty times, so that the final concentration of water or alcohol in the diluted solution remains almost the same as the diluting solvent.

No difficulties were encountered in the assay of camphor in spirits of camphor by the above method except that optical densities of solutions of commercial camphor varied as indicated in Table 2. This method of assay gives results which are within ± 1% of the theoretical amount; the N. F. method gives results which are consistently about 3% too low. It was found that an assay could be completed in about thirty minutes, amounting to a considerable saving in time over the gravimetric procedure.

It may be mentioned here that with all its advantages over other procedures, this method has its disadvantages. The extinction of camphor in the ultraviolet region is extremely low, of the order of 2.2

for one per cent solution, so that a slight amount of impurities in the camphor or in the solvent which absorbs strongly in the same region as camphor would seriously interfere in the results of the experiment. An example of such a preparation is one made with ethyl alcohol which has been denatured with ketones and other compounds which absorb ultraviolet light.

Another disadvantage of the present method is that the result depends upon the kind of solvent. It is clear from Table 3 that poor results may be obtained if close attention is not paid to the solvent conditions used in actual analysis. For instance, the alcohol concentration of the solvent used in this analysis should not lie beyond $85 \pm 1\%$.

Table 2. $E^{1\%}_{1\ cm}$ and Melting Points of Synthetic and Natural Camphor before and after Purification

	SYNTHETIC				NATURAL			
	USP, Du Pont		USP, Messer		Eastman		USP, M. M. R.	
	$E^{1\%}_{1\ cm}$	M. P.	$E^{1\%}_{1\ cm}$	M. P.	$E^{1\%}_{1\ cm}$	M. P.	$E^{1\%}_{1\ cm}$	M. P.
Before purification	2.158	172-6	2.158	172-6	2.224	177.5-179	2.230	178-179
After purification	2.265	178-9	2.264	178-9	2.268	178.5-179.5	2.262	178.5-179.5

Table 3. Wavelength Maxima and $E^{1\%}_{1\ cm}$ of Solutions of Natural Camphor (USP) in Alcohol of Different Concentrations

Alcohol Concentration % v/v	λ_{max} mu	$E^{1\%}_{1\ cm}$
100	290.00	2.075
95	289.50	2.163
90	288.50	2.249
85	288.50	2.262
80	288.25	2.301
75	288.00	2.332
70	287.75	2.360
65	287.50	2.402
60	287.25	2.441
55	287.00	2.460
50	286.50	2.505

Table 4. Relationship between Concentration and Absorbance at 288.5 mu for Solutions of Natural Camphor, USP (M. M. R.) in 85% Alcohol

Concentration of Camphor % w/v	Absorbance in 1 cm cell	$E^{1\%}_{1\ cm}$
0.4294	0.967	2.252
0.3194	0.717	2.245
0.2572	0.585	2.274
0.2147	0.487	2.268
0.1597	0.361	2.260
0.1286	0.292	2.271
		Av. 2.262

Table 5. Relationship between Concentration and Absorbance at 288.5 mu for Solutions of Synthetic Camphor, USP (Messer) in 85% Alcohol

Concentration of Camphor % w/v	Absorbance in 1 cm cell	$E^{1\%}_{1\ cm}$
0.4124	0.937	2.272
0.3026	0.688	2.274
0.2374	0.535	2.254
0.2062	0.468	2.270
0.1513	0.343	2.267
0.1187	0.267	2.249
		Av. 2.264

Table 6. Results of Assay for Spirits of Camphor, Prepared from Synthetic Camphor, USP

Per Cent Camphor in Spirits w/v	Per Cent Alcohol v/v	Camphor Recovered by Spectrophotometric Assay*	Per Cent Error	Camphor Recovered by N. F. Assay	Per Cent Error
7.9221	75	7.9775	0.70	7.7082	-2.4
9.5356	85	9.4603	-0.79	9.2877	-2.6
12.0012	80	12.0912	0.75	11.5812	-3.5

Table 7. Results of Assay for Spirits of Camphor, Prepared from Natural Camphor, USP

Per Cent Camphor in Spirits w/v	Per Cent Alcohol v/v	Camphor Recovered by Spectrophotometric Assay*	Per Cent Error	Camphor Recovered by N. F. Assay	Per Cent Error
8.1001	90	8.1633	0.78	7.8571	-3.0
10.7021	85	10.6486	-0.50	10.4024	-2.8
12.0110	70	12.0386	0.23	11.6266	-3.2

*$E^{1\%}_{1\ cm}$ used in these assays is 2.265, which is the average of the values given in Table 2.

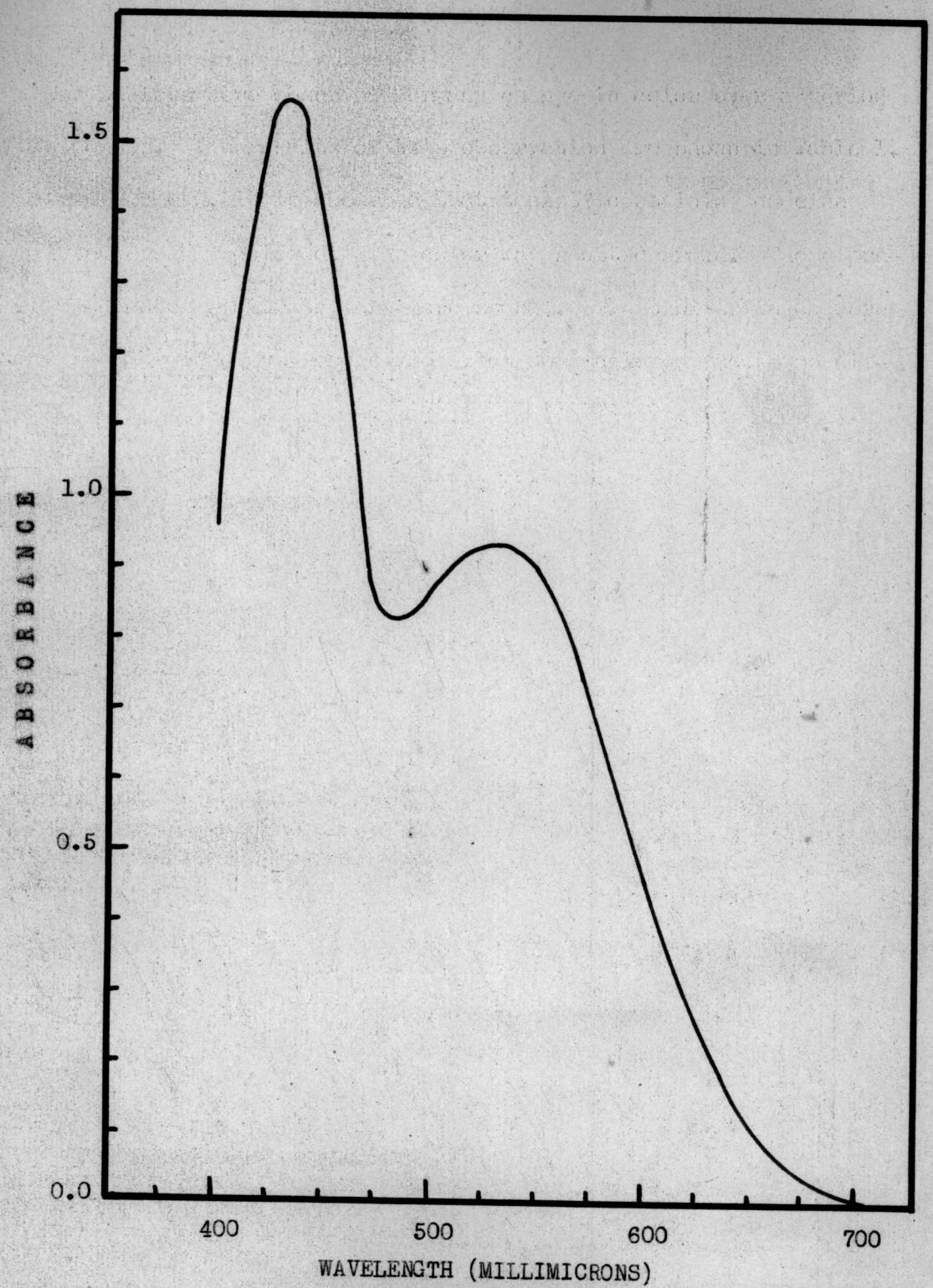


Figure 1. Visible Absorption Spectrum of Acetone 2,4-Dinitrophenylhydrazone (Approximately 1.2 x 10^{-4} Molar)

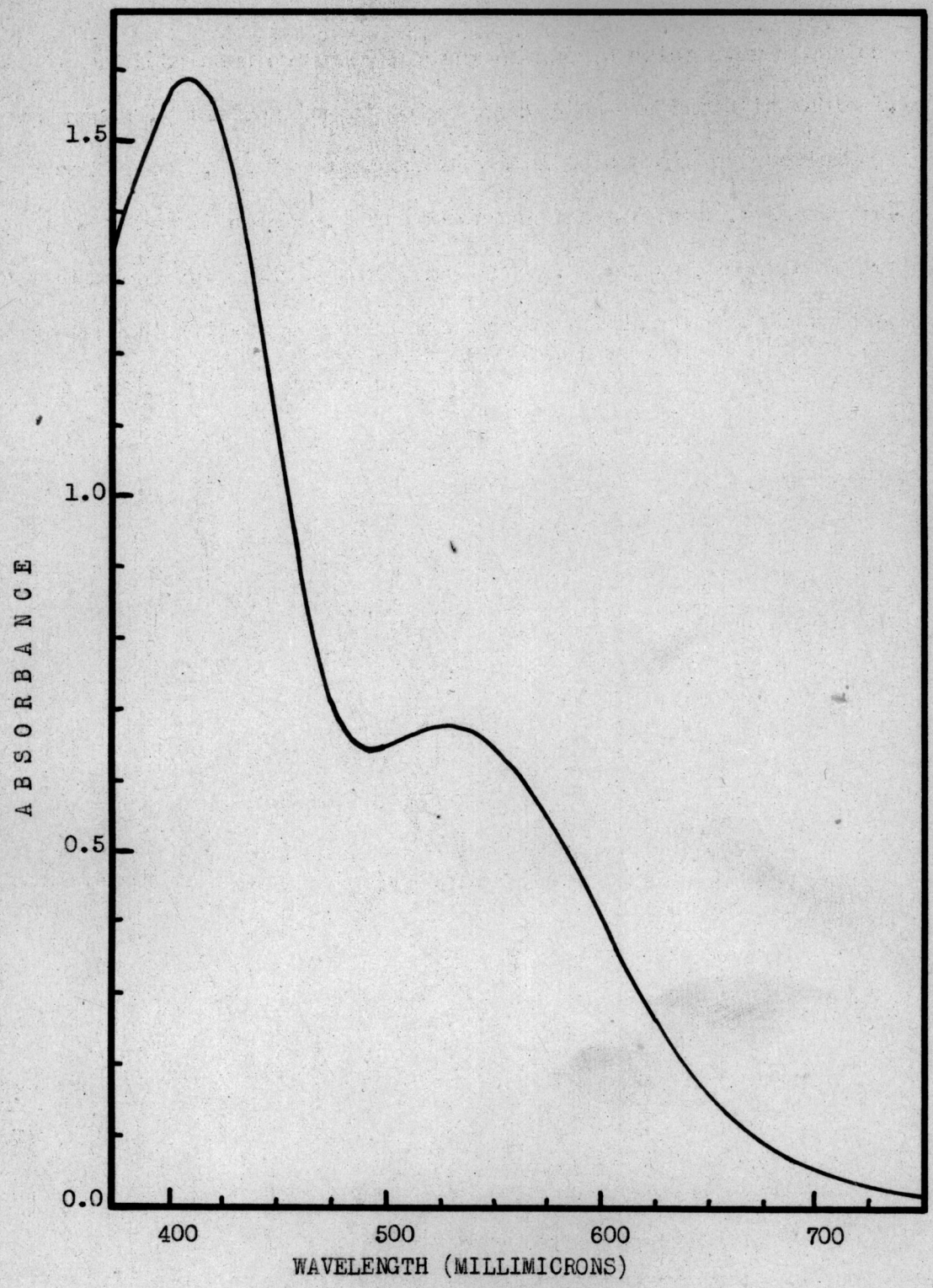


Figure 2. Visible Absorption Spectrum of Camphor 2,4-Dinitrophenylhydrazone (Approximately 1.0 x 10^{-4} Molar)

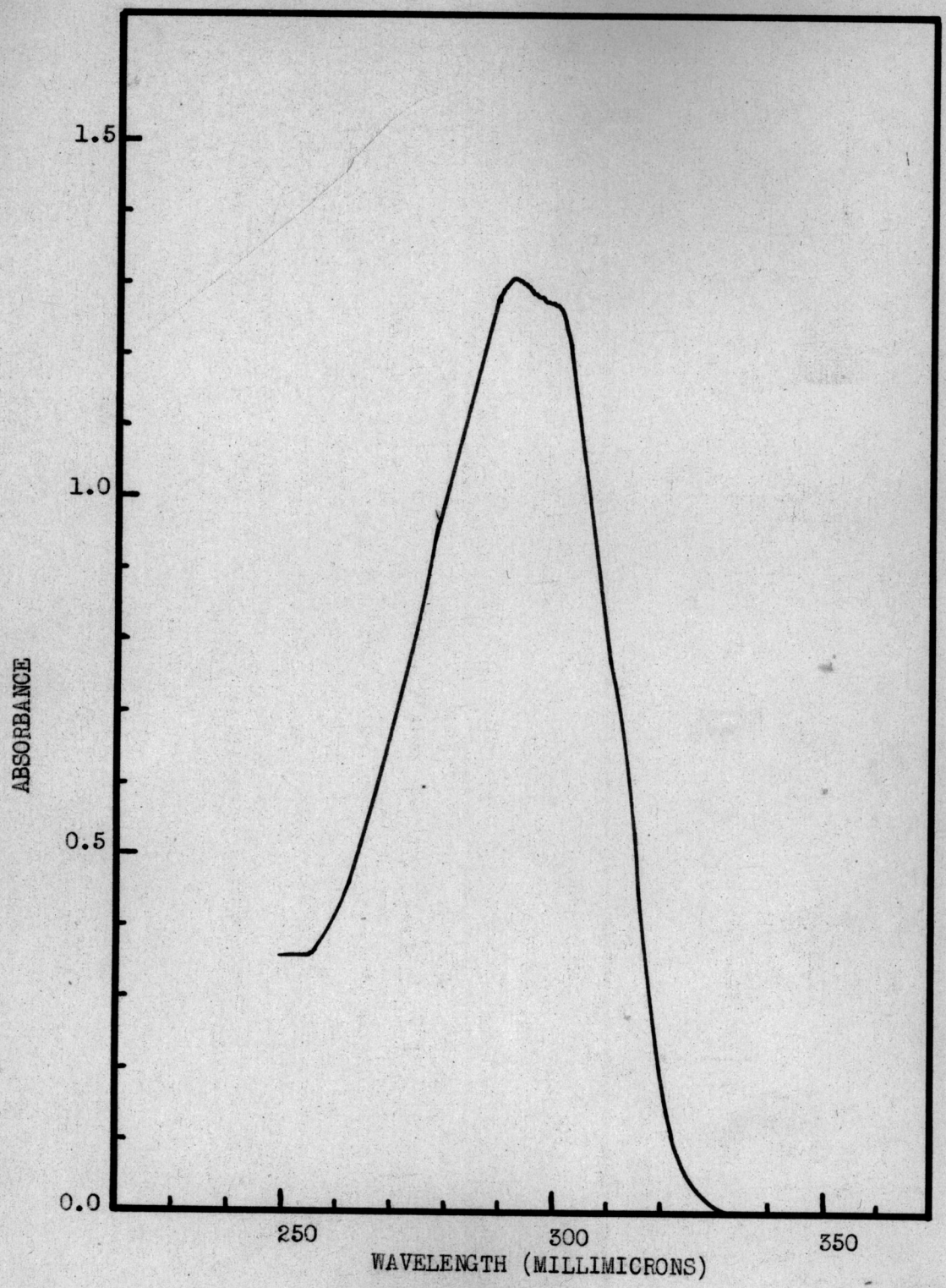


Figure 3. Ultraviolet Absorption Spectrum of Camphor (0.605 per cent) in Carbon Tetrachloride

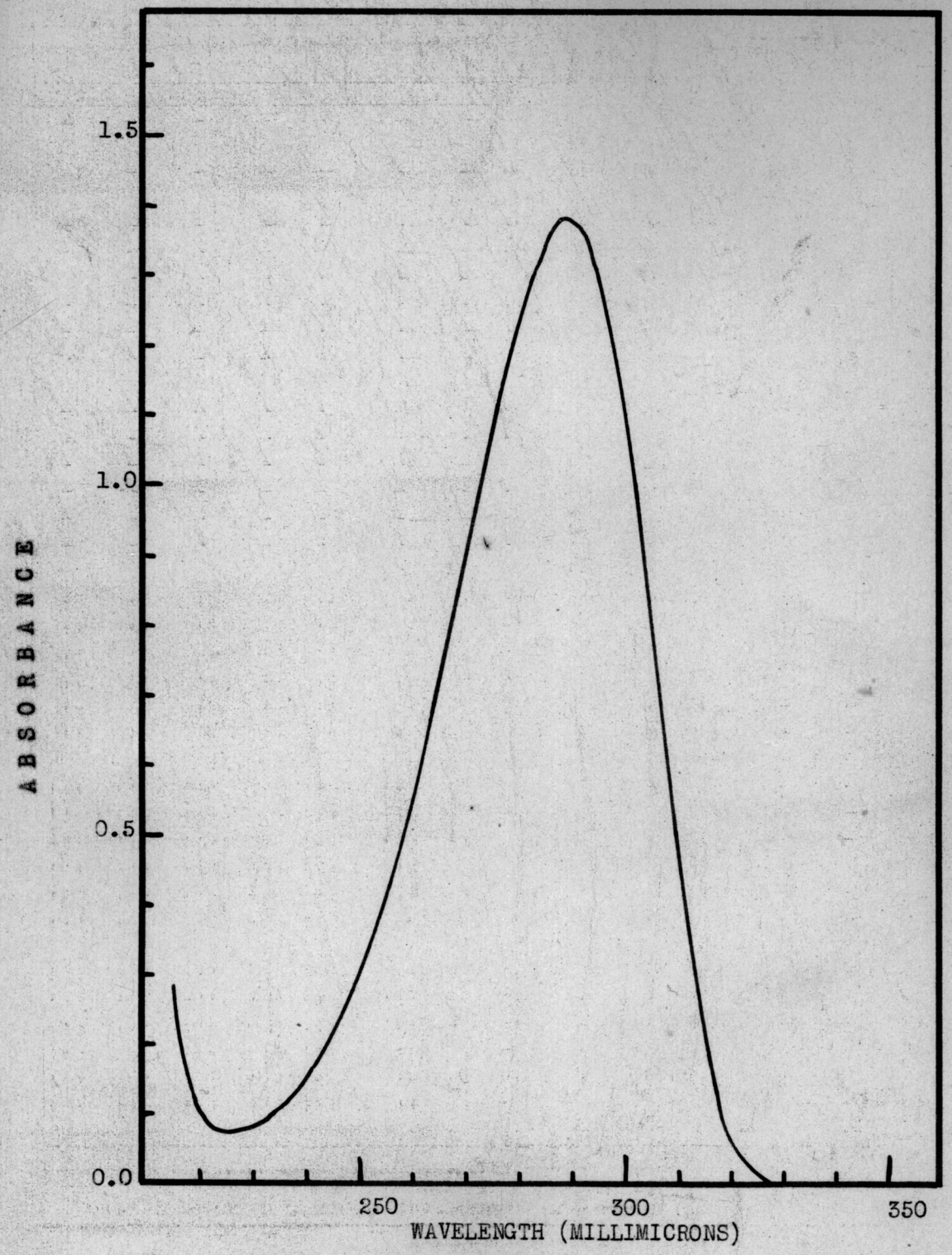


Figure 4. Ultraviolet Absorption Spectrum of Camphor (0.674 per cent) in Absolute Alcohol

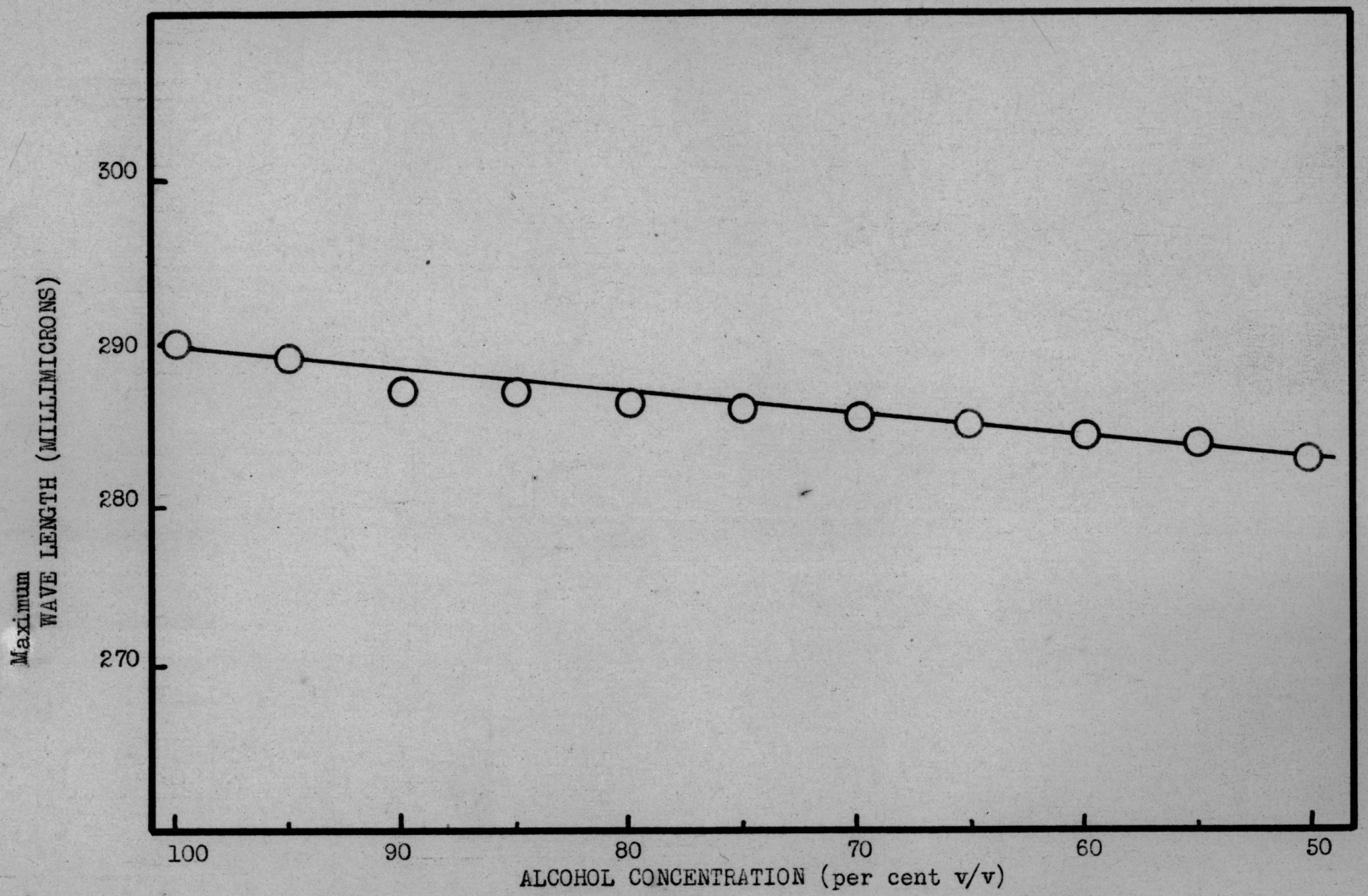


Figure 5. Change in the Absorption Peak of Camphor with Change in Solvent (Alcohol) Concentration

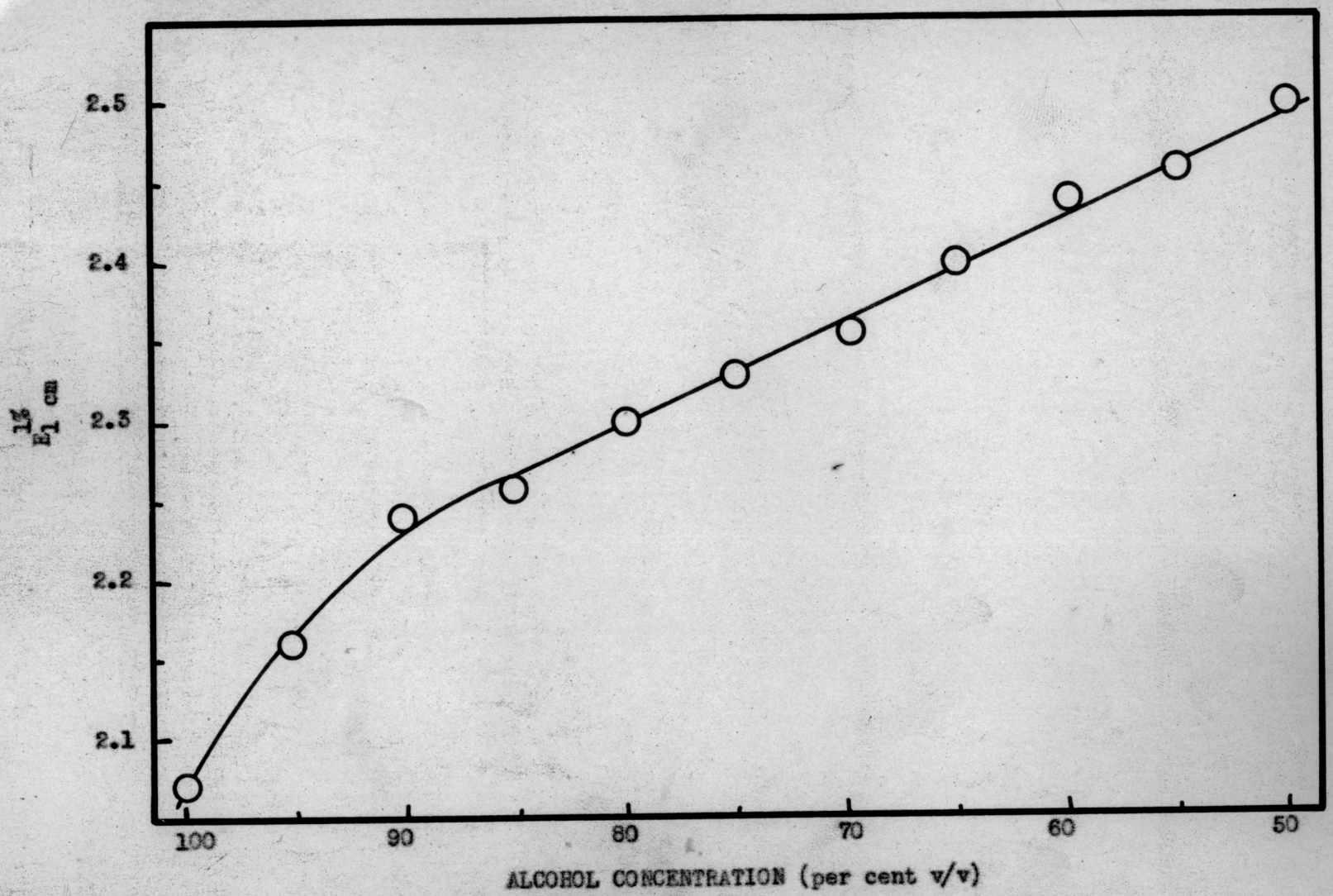


Figure 6. Change in $E_{1\ cm}^{1\%}$ of Solutions of Natural Camphor, USP, with Change in Solvent (Alcohol) Concentration

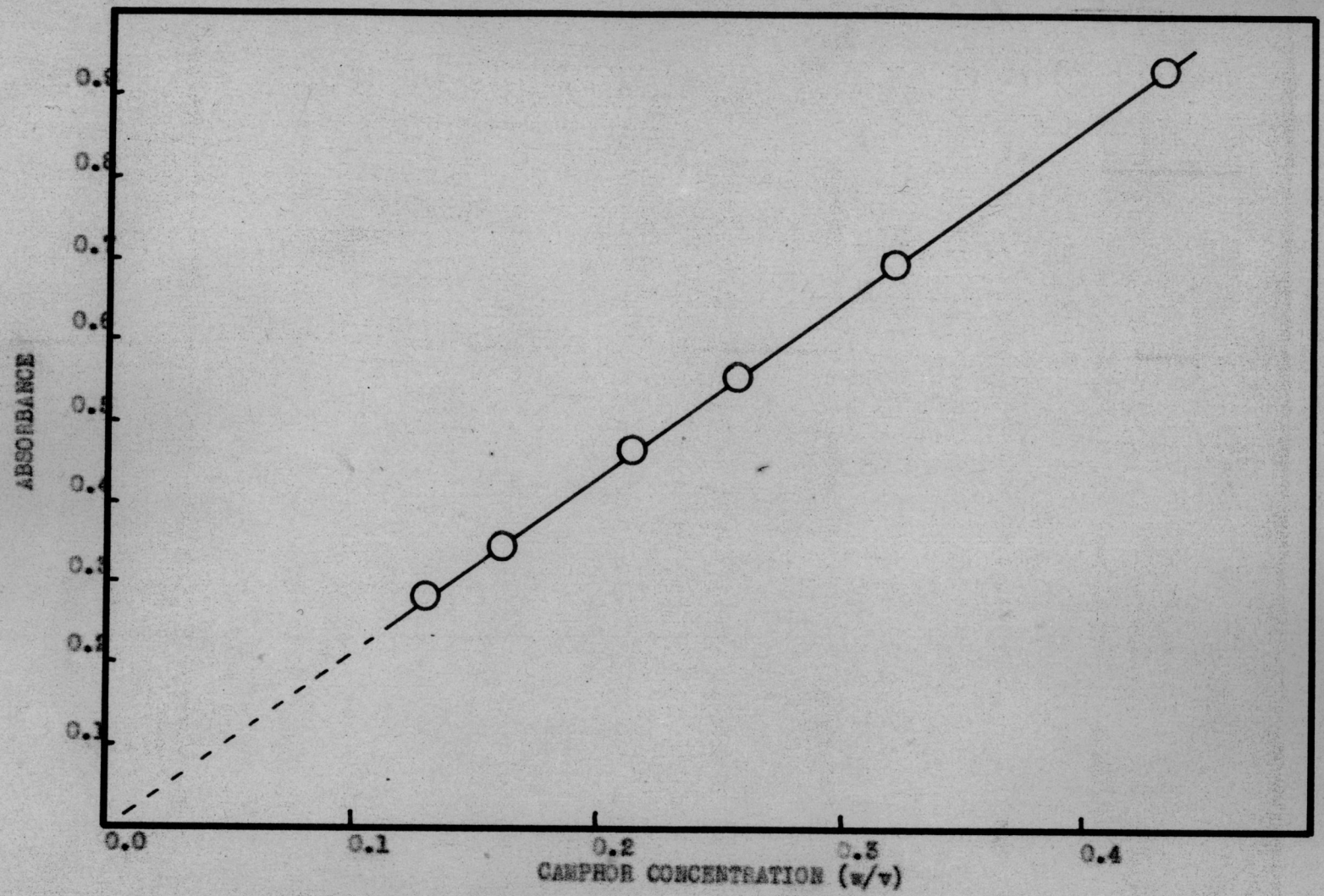


Figure 7. Relationship between Concentration and Absorbance at 288.5 mu for Solutions of Natural Camphor, USP, in 85% Alcohol

REFERENCES

(1) Jumeau, J., Bull. Sci. Pharmacology, 20, 607-8. (1913)

(2) Vieth, F. and Bilhuber, E. A., J. Am. Pharm. Assoc., 13, 636 (1924).

(3) Gori, G., Ann. Chim. Appl., 15, 283-300 (1925).

(4) Kollo, C., Bull. de Chim. Bukarest, 17, 44-48 (1916).

(5) Conroy, H. W., J. Assoc. Offic. Agr. Chemists, 31, 115, 535 (1935).

(6) Corbin, E. N. and Green, M. W., Bull. Nat. Formulary Comm., 16, 151 (1948).

(7) Mitchell, J., Organic Analysis. Vol. 1. Interscience Publishers. New York, 1953.

(8) Bryant, W. M. D. and Smith, M. D., J. Am. Chem. Soc., 57, 57 (1935).

(9) Bataille, H., Bull. Sci. Pharmacology, 19, 407, 548 (1912)

(10) Collins, A. T., J. Ind. Eng. Chem., 4, 514 (1912).

(11) Plein, E. M. and Poe, C. F., Ind. Eng. Chem., Anal. Ed., 16, 168 (1944).

(12) Rojahn, C. A., Deut. Apoth. Ztg., 50, 1527 (1935).

(13) Lappin, G. R. and Clark, L. C., Anal. Chem., 25, 541-2 (1951).

(14) Pool, M. F. and Klose, A. A., J. Am. Oil. Chem. Soc., 28, 215-7 (1951).

(15) Lucas, M. R., Ann. de Physique, 9, 446 (1928).

(16) Bohrisch, C., Pharm. Zentralhalle, 48, 527 (1907).

(17) The National Formulary. 9th ed. (1950). p.116.

REFERENCES

(Continued)

(18) Osol, A. and Farrar, G. E., The Dispensatory of the United States of America, 1950.

(19) Fieser, L. F. and Fieser, M., Organic Chemistry. D. C. Heath and Company. Boston, 1950.

(20) Herold, W., Z. Elektrochem., 39, 566-71 (1933).