result in the formation of a new wave, but it also shifted the half-wave potentials. The first reduction wave of *p*-nitroacetanilide at 1.53 mg. per gram of NaI occurred at −0.114 volt and upon the addition of gelatin, the half-wave potential shifted to −0.124 volt. Similar results were observed for *m*- and *p*-nitroaniline. The second reduction wave showed less dependence on the effect of gelatin.

A discussion of the possible effect of gelatin on the reduction of nitro compounds in this system will be published at a later date.

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Spectrophotometric Determination of Formaldehyde and Formaldehyde-Releasing Compounds with Chromotropic Acid, 6-Amino-1-naphthol-3-sulfonic Acid (J Acid), and 6-Anilino-1-naphthol-3-sulfonic Acid (Phenyl J Acid)

EUGENE SAWICKI, THOMAS R. HAUSER, and SYLVESTER McPHERSON

Robert A. Taft Sanitary Engineering Center, U. S. Department of Health, Education, and Welfare, Cincinnati 26, Ohio

▶ Three highly selective procedures for the determination of formaldehyde and formaldehyde - releasing compounds are introduced. In all cases xanthylium cationic or dicationic dyes are formed. The procedures have sensitivities approximately two and one-half times that of the chromotropic acid method. A sensitive thermochromic blue spot test for formaldehyde with 6 - amino - 1 - naphthol - 3 - sulfonic acid is described, and nine different methods for the determination of formaldehyde are compared. The interference of formaldehyde-releasing compounds is discussed.

The spectrophotometric determination of formaldehyde with chromotropic acid has been described in many papers since the original observations of Eegriwe (*5*). By the same procedure or through the use of higher temperatures or longer heating times, many formaldehyde-releasing compounds give a positive purple color and thus can be determined (*1*, *4*). A formaldehyde-releasing compound is defined as any organic compound which is hydrolyzed or oxidized in warm sulfuric acid under test conditions to give formaldehyde as one of the products. Other organic compounds, such as formic acid, can be reduced to formaldehyde (*6*) or, like methanol, can be oxidized to formaldehyde (*2*) and then determined by the chromotropic acid procedure.

Recently the 2-hydrazinobenzothiazole (*9*), the 2-hydrazinobenzothiazole - *p* - nitrobenzenediazonium tetrafluoroborate (*11*), and the 3 - methyl - 2 - benzothiazolone hydrazone (*10*) procedures for aldehydes were studied. All of these methods can be used to determine formaldehyde. The advantages and disadvantages of these various methods in the determination of formaldehyde are discussed and are compared with new methods.

EXPERIMENTAL

Reagents and Apparatus. Chromotropic acid was purified by recrystallization from water, as some commercial samples contained only 50% of the acid. The reagent solution used was 1.0% chromotropic acid in concentrated sulfuric acid. The solution was stable for at least 1 day but darkened slightly on further standing.

6-Amino-1-naphthol-3-sulfonic acid (J acid; K & K Laboratories, Inc., Jamaica, N. Y.) was purified by washing with boiling dimethylformamide, filtering, washing the cake with acetone, dissolving the cake in boiling aqueous potassium hydroxide, treating with charcoal, filtering hot, reprecipitating with hydrochloric acid, filtering, and again washing the cake with acetone. The reagent solution used was 0.2% in concentrated sulfuric acid and was stable for at least 2 days.

6-Anilino-1-naphthol-3-sulfonic acid (Phenyl J acid; K & K Laboratories, Inc., Jamaica, N. Y.) was purified in the same manner as the 6-amino derivative. The reagent solution used was 0.1% in concentrated sulfuric acid and was stable for at least 2 days.

Sodium metabisulfite was a 20% aqueous solution containing 1 ml. of concentrated sulfuric acid per 100 ml. of solution.

Formaldehyde was a 39.7% ACS reagent solution from Matheson, Coleman, and Bell. The solution was analyzed by the gravimetric method of Yoe and Reid (*14*).

In the preliminary spectrophotometric experimentation, a Beckman Model B spectrophotometer was used, and in all quantitative analyses, a Cary Model 11 recording spectrophotometer with 1-cm. cells was used.

Chromotropic Acid Procedures. The spectrophotometric procedures of West and Sen (*13*) (Procedure A) and Bricker and Johnson (*3*) (Procedure B) were applied to compounds containing combined formaldehyde. The spectral data obtained from these procedures are recorded in Table I.

J Acid Procedures. PROCEDURE A. Two milliliters of aqueous test solution and 5 ml. of 0.2% reagent solution are mixed in a 10-ml. volumetric flask without control of the heat of mixing. After the mixture has cooled to room temperature, it is diluted to 10 ml. with concentrated sulfuric acid. A positive test gives a yellow color, whereas a blank produces no color. The absorbance is de-

Table I. Determination of Formaldehyde and Formaldehyde-Releasing Compounds with Chromotropic, Phenyl J Acid, and J Acid

Compound	Chromotropic Acid, Procedure A λ max, mμ	Chromotropic Acid, Procedure A ε × 10⁻³	Chromotropic Acid, Procedure B λ max, mμ	Chromotropic Acid, Procedure B ε × 10⁻³	Phenyl J Acid λ max, mμ	Phenyl J Acid ε × 10⁻³	J Acid, Procedure A λ max, mμ	J Acid, Procedure A ε × 10⁻³	J Acid, Procedure B λ max, mμ	J Acid, Procedure B ε × 10⁻³
Formaldehyde	578	15.7	578	15.7			468	21.0	468	21.0
	480	8.3	480	8.3	660	51.4	376	13.0	376	13.0
Formaldehyde hydrazone	...	...	578	14.7			467	24.0	467	25.5
	...	...	480	8.4	655	34.5	450s[b]	22.5	450s	24.5
Formaldehyde 2,4-dinitrophenylhydrazone	578	10.9	578	19.1			375	15.0	375	17.0
	480	6.9	480	11.3	655	19.5	467	21.5	467	24.0
							450s	20.5	450s	22.5
							375	18.0	375	19.0
Piperonal	578	5.0	578	12.9			467	19.0	467	20.6
	480	2.8	480	7.4	660	43.9	450s	17.9	450s	19.5
							375	21.0	374	22.4
Piperonylic acid	578	15.3	578	16.8			465	21.5	467	21.5
	480	8.0	480	8.9	660	52.5	450s	20.0	450s	20.0
							375	15.0	375	15.0
β-Piperonylacrylic acid	578	12.1	578	17.3			467	18.0	467	19.4
	480	7.2	480	10.5	660	22.8	450s	17.0	450s	18.5
							375	17.5	375	16.0
sym-Trioxane	578	44.0	...	...			467	60.2	467	60.6
	480	25.7	...	...	660	141.9	450s	56.4	450s	57.0
							376	39.2	376	39.4
Anisyl alcohol	578	3.3	578	8.8			467	1.0	467	2.2
	480	1.7	480	4.9	Neg.		450s	1.0	450s	2.2
							376	0.8	376	1.8
Cinnamyl alcohol	...	...	578	2.3						
	...	...	480	2.2	Neg.					
Dextrose	578	0.43	578	2.3			463	3.5	468	4.8
	480	0.33	480	2.3	660	6.6	450	3.5	450	4.9
							375	3.1	375	4.3
Pyruvaldehyde	...	...	578	3.6			467	0.2	467	0.3
	...	...	480	5.2	Neg.		450s	0.2	450s	0.3
							375	0.3	375	0.3
Glyoxal	578	1.1	578	5.9			468	2.6	467	3.0
	480	0.8	480	4.2	660	5.0	450s	2.5	450s	2.9
							376	2.0	376	2.3
sym-Trithiane	578	1.4	578	51.6			468	2.0	468	22.0
	480	0.8	480	26.8	655	5.0	450s	1.9	450s	20.5
							375	1.3	376	16.0
Biacetyl	578	1.5	578	3.4			468	1.4	468	2.3
	480	0.8	480	2.0	666	0.9	450s	1.3	450s	2.1
							376	1.1	376	1.7
Glyoxylic acid hydrate	578	0.6	578	1.3						
	480	0.6	480	1.3	Neg.					
Hexamethylenetetramine	578	28.8	578	28.6			468	51.0	468	54.0
	480	15.0	480	15.6	660	122.5	450s	49.0	450s	51.5
							376	33.5	376	38.5
N,N'-Methylene-bis-acrylamide	578	9.9	578	16.9			467	19.0	467	19.8
	480	5.4	480	8.4	660	39.3	450s	18.2	450s	19.0
							376	12.7	376	13.2
Isosafrole	...	...	578	7.7						
	...	...	480	6.9	645	5.0				
Glycolic acid	578	~0.4	578	13.4			467	0.2	467	0.6
	480	~0.3	480	7.4	Neg.		447s	0.2	447s	0.6
							374	0.2	374	0.4
Acetaldehyde	Neg.		Neg.		Neg.		468[a]	0.05	468[a]	~0.05
Propionaldehyde	Neg.		Neg.		Neg.		468[a]	0.4	468[a]	~0.4
Acrolein	Neg.		Neg.		Neg.		468[a]	0.5	468[a]	~0.5

[a] Not a wavelength maximum. No bands above 400 mμ. [b] s = shoulder.

termined at 468 mμ. The color is stable for at least 1 day.

PROCEDURE B. This procedure is similar to Procedure A. After the test and reagent solutions are mixed, however, the mixture is heated for 30 minutes on a boiling water bath and then is cooled to room temperature before being diluted with sulfuric acid. Again, a positive test is indicated by a stable yellow color, while the blank produces no color.

PROCEDURE C. Two milliliters of aqueous test solution and 5 ml. of 0.2% reagent solution are mixed in a 25-ml. volumetric flask. The mixture is allowed to cool to room temperature, and then is diluted to 25 ml. with 50% aqueous ammonium acetate with additional cooling. The absorbance at 612 mμ is determined within 10 minutes. A blue color denotes a positive test. The blank is colorless.

PROCEDURE D. This method is similar to Procedure C. After the test and reagent solutions are mixed, however, the mixture is heated on a boiling water bath for 30 minutes and then is cooled to room temperature before it is diluted to 25 ml. with a 50% aqueous ammonium acetate solution. The absorbance is determined at 612 mμ within 10 minutes.

SPOT TEST PROCEDURE. In a small test tube (1 × 7.5 cm.) is placed 1 drop (0.03 ml.) of the test solution followed by 5 drops of reagent solution. The mixture is heated for 5 minutes on a boiling water bath, cooled, and then diluted with 10 drops of ammonium acetate solution. A positive test is indicated by a blue color, which is discharged after the addition of one drop of sodium metabisulfite solution. Heating this colorless mixture in a microflame regenerates the blue color which again disappears on cooling. This thermochromic phenomenon can be repeated. The blank is colorless. The identification limit for formaldehyde with the blue color test is 0.03 μg.

Phenyl J Acid Procedure. Two milliliters of aqueous test solution is placed in a 25-ml. volumetric flask,

Table II. Detection and Determination of Formaldehyde and Formaldehyde-Releasing Compounds with J Acid in Aqueous Sulfuric Acid

Compound	Procedure C λ max, mμ	Procedure C ε × 10⁻³	Procedure D λ max, mμ	Procedure D ε × 10⁻³	Spot test I.L., μg.
Formaldehyde	612	34.0	612	34.0	0.03
Glycolic acid	612	0.2	612	7.5	11
sym-Trioxane	612	98.0	612	96.0	0.01
β-Piperonylacrylic acid	612	4.4	612	14.1	0.2
Piperonal	612	17.0	612	34.0	0.06
Formaldehyde-2,4-dinitrophenylhydrazone	580	12.6	580	11.6	0.1
Formaldehyde hydrazone	590	20.5	590	23.9	0.03
Piperonylic acid	612	33.8	612	34.8	0.3
N,N-Methylene-bis-acrylamide	585	8.3	585	9.9	0.08
Glyoxal	600	3.0	600	3.2	0.2
Anisyl alcohol	Neg.	Neg.	600	2.6	0.4
Dextrose	612	2.6	612	3.4	0.5
Hexamethylenetetramine	612	71.3	612	66.3	0.01
Biacetyl	612	0.2	612	0.6	0.7
Isosafrole	Neg.	Neg.	610	3.0	Neg.
sym-Trithiane	Neg.	Neg.	605	23.8	0.2
Pyruvaldehyde	612	0.3	612	0.3	0.5

and 5 ml. of the reagent solution is added while the mixture is cooled at ice water temperatures. After the heat of mixing has subsided, the mixture is heated on a boiling water bath for 30 minutes, cooled, and then diluted to 25 ml. with methyl Cellosolve (2-methoxyethanol). A blank prepared in this manner is colorless. The absorbance is read at 660 mμ. The color intensity is stable for 24 hours.

In the chromotropic acid procedure, a purple monocationic dibenzoxanthylium dye is formed in sulfuric acid, as the aromatic hydroxyl group is less basic than the aromatic amino group in J acid and thus does not add a proton in sulfuric acid to form a dication analogous to the dicationic dibenzoxanthylium dye obtained from J acid. In the phenyl J acid procedure a mixture of dicationic and monocationic salts is formed, as the anilino group is intermediate in basicity between the hydroxyl and amino groups. Consequently less water is necessary to form the blue monocation.

The structures of the colored chromogens in the reaction between formaldehyde and either chromotropic acid, J acid, or phenyl J acid have been proved by Kamel and Wizinger (*7*). These workers state that the specificity of the reaction of formaldehyde with these reagents is a result of the steric effect of the two sulfonic acid groups.

RESULTS AND DISCUSSION

J Acid Procedures. The spectral data (wavelength maxima and molar absorptivities) and spot test identification limits for all compounds are listed in Tables I and II. Whenever any compound tested in any of the procedures was water insoluble, it was dissolved in 95% ethyl alcohol.

Variables in the spectrophotometric procedures were investigated. In all the procedures developed, formaldehyde was used as the test substance, and measurements of the other compounds tested were determined by these procedures. In Procedures A and C, the heat of mixing was sufficient to give complete reaction for formaldehyde. Procedure B was devised for the compounds that yield formaldehyde on heating. An example is piperonal, which gave a molar absorptivity of 5000 in Procedure A and 12,900 in Procedure B. The concentration of the reagent used in Procedures A and B was set at 0.2%. Halving or quadrupling the reagent concentration had little effect on the absorbance; when the reagent concentration was increased, the blank became more yellow. The stability of the final yellow solution was satisfactory for quantitation in Procedures A and B, as no increase or decrease in absorbance was observed after the solution was allowed to stand for 19 hours. The reproducibility of Procedures A and B was shown by the Beer's law study, which showed a linear relationship from 1.5 to 32 μg. of formaldehyde per 10 ml. of final solution over an absorbance range of 0.1 to 2.2.

Acetaldehyde, propionaldehyde, and acrolein reacted in Procedures A and B, had no distinctive absorption spectral bands above 400 mμ, and had molar absorptivities at a wavelength of 468 mμ of 50, 400, and 500, respectively. Although Procedures A and B are highly selective for formaldehyde and for compounds giving formaldehyde, and give essentially negative results with other aliphatic aldehydes, fairly large amounts of the aliphatic aldehydes, obviously would interfere. In the other procedures, these aldehydes do not interfere.

A blue color appeared when the yellow solution from Procedure A or B was diluted with water. If water alone was used as the diluting solvent, a rapidly fading, slightly turbid solution resulted. If methanol or ethanol was used as the diluting solvent, an unstable color resulted. When 25% aqueous ammonium acetate was used, a solution was produced with a molar absorptivity of 21,000, which from an initial absorbance of 1.60 decreased only 0.01 absorbance unit in 10 minutes. If the concentration of the ammonium acetate was increased to 50%, a molar absorptivity of 31,000 was obtained. This solution was stable for 10 minutes but faded 0.1 absorbance unit in 2½ hours from an initial absorbance of 2.36. Other aqueous solutions tried as diluting solvents were trisodium phosphate, potassium acetate, ammonium sulfate, and ammonium oxalate. None of these gave satisfactory results. The reagent concentration and heating time used in Procedures A and B gave maximum results in Procedures C and D. In Procedures C and D, Beer's law was obeyed from 2.2 to 48 μg. of formaldehyde per 25 ml. of final solution. A final volume of 25 ml. was selected because the blue color was formed in the test after the addition of approximately 11 ml. of the ammonium acetate solution. Thus, 25 ml. was the next largest convenient volume with which to work.

Spot Test Procedure. Added heat was needed for color development in this procedure, since the heat of mixing of such small volumes was not sufficient to cause reaction. The thermochromic property of this test is very interesting and can be repeated until all the sodium metabisulfite is discharged, at which time the test solution will remain blue. The concentration limit obtained in the detection of formaldehyde was 1 to 1,000,000.

Phenyl J Acid Procedure. The spectral data obtained for formaldehyde-releasing compounds are recorded in Table I. In the development of this procedure formaldehyde was used as a standard and then was applied to the other compounds containing combined formaldehyde. The variables in the spectrophotometric procedure were investigated. When the aqueous test solution was mixed with the reagent solution, the heat of mixing affected the final molar absorptivity of the blue color. If the heat of mixing was not controlled, in most (not all) cases a green solution of varying intensity was obtained. When ice water cooling was used during mixing of the test solution and reagent, a yellow solution resulted. If the green color was allowed to form on mixing, the final intensities were erratic. Results were reproducible, however,

when the test solution and reagent were mixed at ice water temperatures.

Heating the reaction mixture longer than 30 minutes decreased the absorbance very slightly whereas heating less than 20 minutes appreciably decreased the absorbance. Heating at 123° to 125° C. for 10 minutes gave approximately the same results as heating on a water bath for 30 minutes. Longer periods of heating at 123° to 125° C. produced a pink color in the blank. When the volume of the reagent was doubled while the concentration of the reagent was halved, no appreciable change in absorbance was noted. Methyl Cellosolve was used as the diluting solvent because it gave a colorless blank. The use of water as a diluent resulted in precipitation; the use of 50% ammonium acetate did not augment the final results. Methanol was not used because of the difficulty in mixing. The blue color formed in the standard procedure was stable for 24 hours; in this time a decrease of only 0.02 absorbance unit from an initial absorbance of 1.60 was observed. Beer's law was obeyed in the phenyl J acid procedure from 1.4 to 31 μg. per 25 ml. of final solution.

The reagent concentration used in the test was not the concentration that gave the highest intensity of color. A reagent concentration of 0.05% gave approximately an 8 to 10% increase in molar absorptivity, but Beer's law did not hold for formaldehyde concentrations greater than 12 μg. per 25 ml. of final solution. Consequently, the concentration of the reagent had to be increased.

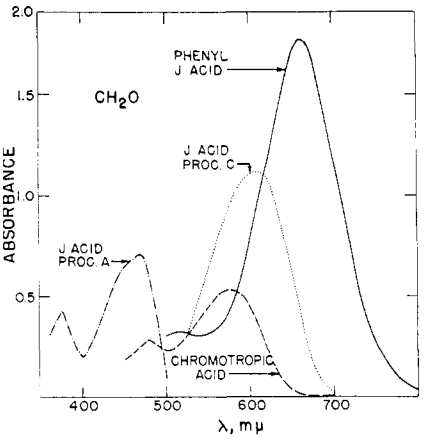

Figure 1. Visible absorption spectra of chromogens obtained in the determination of formaldehyde (final concentration 3.38 × 10^{-5} M) with chromotropic (– – –), J acid Procedure A (–·–·–), J acid Procedure C (·····), and phenyl J acid (——)

Figure 1 shows a comparison of the strikingly different absorption spectra obtained in the chromotropic acid, the J acid, and the phenyl J acid methods for the spectrophotometric determination of formaldehyde.

COMPARISON OF VARIOUS PROCEDURES

The results from some of the better known procedures for the determination of formaldehyde are compared in Table III. The sensitivity of a spectrophotometric method is affected by two factors—the dilution factor and the molar absorptivity (based on the compound analyzed) of the final chromogen. The closer the dilution factor is to one and the higher the molar absorptivity the greater the sensitivity of a procedure. The procedures are compared briefly in the following paragraphs.

Schiff Test. This test has one of the best dilution factors but also has the lowest absorptivity and the longest reaction time, about 2 hours, of any of the procedures. The lower the concentration limit and the dilution factor, the more sensitive is a procedure. The Schiff test also does not satisfactorily follow Beer's law, whereas the other procedures do.

Phenylhydrazine Procedure. This procedure has a fairly high absorptivity. The blank is colorless. It has the poorest dilution factor of any of the procedures tested however, and other aliphatic aldehydes react in the procedure. The method could be improved further.

2-Hydrazinobenzothiazole Method. This is the most versatile of the procedures considered. Spot paper, spot plate, silica gel, and spectrophotometric tests for formaldehyde in solution or as a gas are available. In the spot plate method, 0.01 μg. of formaldehyde can be detected at a concentration as low as 1 to 3,000,000. The blank has a light-green color. Other aliphatic aldehydes react with the reagent but at only about 4% the intensity of formaldehyde.

2-Hydrazinobenzothiazole–*p*-Nitrobenzenediazonium Fluoborate Procedure. This procedure has not been thoroughly investigated. Aliphatic, aromatic, and heterocyclic aldehydes readily react in the method.

3-Methyl-2-benzothiazolone Hydrazone Procedure. Quantitatively,

Table III. Spectrophotometric Determination of Formaldehyde

Procedure	Ref.	λ max, mμ	ε × 10^{-3}	Absorptivity, μg.$^{-1}$ ml. cm.$^{-1}$	Concn. limit,[a] p.p.m.	Dilution[b] factor	Color stability, min.	Beer's law followed	Sensitivity[c]	Reproducibility[d]	Speed, min.[e]
Schiff	(*8*)	550	3.5	0.12	0.83	5	~30	No	0.7	Very good	122
Phenylhydrazine	(*12*)	520	34.2	1.14	0.09	42	15	Yes	0.8	...	32
HBT[f]	(*9*)	582	48.0	1.60	0.06	10 or 20	20	Yes	4.8	Very good	20
HBT + NBD[g]	(*10*)	610	24.0	0.80	0.13	10	...	...	2.4	Poor	30
MBTH[h]	(*11*)	670	65.0	2.17	0.05	10 or 20	>40	Yes	6.5	Very good	10
Chromotropic acid, A or B	...	578	15.7	0.52	0.19	10	>24 hrs.	Yes	1.6	Very good	5
J acid, A or B	...	468	21.0	0.70	0.14	5	>24 hrs.	Yes	4.2	Very good	5 (Proc. A)
J acid, C or D	...	612	34.0	1.13	0.09	12.5	10	Yes	2.7	Very good	7
Phenyl J acid	...	660	51.4	1.71	0.06	12.5	>24 hrs.	Yes	4.1	Very good	33 (Proc. C)

[a] For absorbance = 0.10 in a 3-ml. cell with a light path length of 1 cm.
[b] Essentially final volume/test solution volume.
[c] Sens. = $\frac{\epsilon \times 10^{-3}}{\text{dilution factor}}$. Higher numerical values denote greater sensitivity.
[d] Per cent deviation < 2 = very good; 2 to 5 = good, 5 to 10 = fair; > 10 = poor.
[e] Speed = total time for an analysis.
[f] HBT = 2-hydrazinobenzothiazole.
[g] NBD = 4-nitrobenzenediazonium fluoborate.
[h] MBTH = 3-methyl-2-benzothiazolonehydrazone.

this is the most sensitive procedure for the determination of formaldehyde. It has a wide versatility in qualitative and quantitative methods. Other aliphatic aldehydes react just as readily as formaldehyde with the reagent. The reagent in the procedure has to be pure. Otherwise, the presence of impurities such as 3-methyl-2-benzothiazolone azine in the reagent can result in a final turbid solution. However, purification is readily accomplished (*10*).

Chromotropic Acid Procedure. This procedure is highly selective for formaldehyde; however, formaldehyde-releasing compounds, such as piperonylic acid, anisyl alcohol, and dextrose, also react.

J Acid Procedures A and B. These procedures appear to be highly selective for formaldehyde. Formaldehyde-releasing compounds also react. Any compound that gives a color in sulfuric acid would interfere in the procedure. Very large amounts of aliphatic aldehydes interfere. Although these procedures are not so highly selective as the C and D procedures, they are somewhat more sensitive because of their low dilution factor.

J Acid Procedures C and D. These procedures are highly selective for formaldehyde. Compounds containing combined formaldehyde will also react. These procedures are about twice as sensitive as the chromotropic acid method for equivalent dilution factors.

Phenyl J Acid Procedure. This procedure is also highly selective for formaldehyde. Compounds containing combined formaldehyde can react. The sensitivity of this procedure is approximately two and one-half times that of the chromotropic acid procedure.

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Simultaneous Determination of Morphine, Codeine, and Porphyroxine in Opium by Infrared and Visible Spectrometry[1]

KLAUS GENEST and CHARLES G. FARMILO

Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ont., Canada

▶ **Morphine and codeine in opium can be rapidly and accurately determined simultaneously by infrared spectrometry in carbon tetrachloride after quantitative instantaneous acetylation. The minor alkaloid porphyroxine can be estimated in the presence of other opium alkaloids by a specific color reaction. Results for morphine and codeine compare favorably with those obtained by a liquid-liquid extraction method. Absolute values for porphyroxine in opium are given for the first time. The method is recommended for use in determination of the origin of opium for the UN opium research program and has 95% confidence limits for morphine, codeine, and porphyroxine of ±0.26, ±0.28, and ±0.0083, respectively.**

ALL KNOWN METHODS for the determination of morphine and codeine in opium require separation of the two alkaloids. Many methods are based on the classical liquid-liquid extraction or include chromatographic techniques (*14, 17, 19, 22, 23, 25*). The final determination is made by gravimetric, titrimetric, or spectrophotometric methods. Quantitative paper chromatography by strip densitometry (*16, 21*) and after elution (*24*) was used. A gas chromatographic procedure, which improves the separation step considerably is not yet quantitative (*6, 7, 20*). Another method based on the observation that morphine and codeine show different fluorescence spectra after treatment with sulfuric acid was not applied to opium (*4*). Porphyroxine was analyzed separately in samples of opium by another method (*8, 11*). Earlier, from our laboratory, a method for the simultaneous infrared analysis of narcotine, thebaine, and papaverine in opium was published (*2*).

Morphine cannot be analyzed directly by infrared spectrometry because of its poor solubility in suitable solvents; diacetylmorphine, however, is soluble in carbon tetrachloride. The catalyzed quantitative transformation of morphine and codeine by acetic anhydride rapidly yields diacetylmorphine and acetylcodeine at room temperature which can be assayed simultaneously by infrared spectrometry. The object of this paper is to describe an infrared method for the simultaneous determination of morphine and codeine as acetyl derivatives and a spectrophotometric method for porphyroxine in the presence of other opium alkaloids in opium.

EXPERIMENTAL

Apparatus. A Perkin-Elmer Model 221 infrared spectrophotometer and a Beckman DK 2 recording spectrophotometer were used.

Reference Standards and Reagents. Diacetylmorphine (121.8 to 243.8 mg.) and acetylcodeine (11.4 to 57.0 mg.) were dissolved in anhydrous carbon tetrachloride and made up to 25 ml. in volumetric flasks which were protected from light. Porphyroxine (0.26 mg.) was dissolved in methanol and, after treatment with 2*N* hydrochloric acid (for details of procedure see Porphyroxine), diluted to 10 ml. with methanol.

MECKE'S REAGENT. Selenious acid (0.5 gram) was dissolved in concen-

[1] Third part of the series "The Determination of the Origin of Opium" (*2, 3*).