Table II. Comparison of Chromatographic and Colorimetric Assays for Diosgenin

Sample	Per Cent Monohydroxysapogenin by Chromatography	Per Cent Diosgenin by Colorimetric Assay
1	87	88.5
2	90	90.8
3	87	88.8
4	82	84.0
5	93	92.3

the assay to have a standard deviation of 1.1%. The color produced reaches a maximum intensity after 5 minutes and is stable for an additional 30 minutes. A comparison of the results obtained by the two procedures is given in Table II.

DISCUSSION

Treatment of diosgenin, kryptogenin acetate, and yamogenin with perchloric acid yields a yellow color immediately. These sapogenins all have Δ^5 unsaturation. Chlorogenin which has an H and OH in the 5 and 6 positions, respectively, is presumably dehydrated, giving rise to Δ^5 unsaturation. The dehydrated compound then reacts to give the yellow chromogen observed upon standing. Kammogenin differs from diosgenin in that there are OH groups in both the 2 and 3 positions and a keto group in the 12 position. The visible absorption spectrum of kammogenin, treated with perchloric acid, shows maximum absorption at 450 and 565 mμ with minimum absorption at 505 mμ. A compound such as gentrogenin was not available to resolve the question of the influence of the OH group in the 2 position of a compound such as kammogenin in giving rise to the additional absorption maximum observed with this compound.

The colorimetric procedure described has also been used for the determination of dehydroisoandrosterone. Plots of absorbance *vs.* concentration are linear for concentrations of 25 to 250 μg.

The crude diosgenin samples assayed by reaction with perchloric acid yielded results generally higher than those obtained by the chromatographic procedure. The chromatographic assay has a standard deviation of 2%. The observed deviations between the two assays are reasonable as the colorimetric assay is a measure of all Δ^5 unsaturated compounds while the chromatographic assay is a measure of the monohydroxysapogenin content of the sample. The time of assay for the colorimetric procedure is approximately 30 minutes compared to 8 hours for the chromatographic assay which makes the perchloric acid procedure valuable for routine use.

ACKNOWLEDGMENT

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Microdetermination of Formaldehyde in Air

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▶ A procedure is described for determining the concentration of formaldehyde in air. Formaldehyde is collected by drawing air at a rate of 1 c.f.m. through an impinger containing 0.005*N* hydrochloric acid. The collecting efficiency of this solution is 72% with a standard deviation of 5%. A magenta color is produced by adding Schiff's reagent to the formaldehyde solution; acetone increases the depth and stability of the color. The absorbance is measured in a spectrophotometer at 560 mμ. The method is sensitive to 0.1 μg. of formaldehyde per ml. of collecting solution. With a sampling period of 1 hour, a concentration as low as 5 p.p.b. in air can be determined. Typical results for urban air are presented. Diurnal variations in formaldehyde concentration are found, with peaks occurring during the morning and late afternoon.

ALDEHYDE concentrations ranging up to 1 p.p.m. by volume have been found in the air of a few large cities. In most urban areas which have been surveyed, the average level is less than 0.1 p.p.m. The greater part of the aldehydes present in the atmosphere come from the incomplete combustion of fuels, gasoline, and refuse material. In Los Angeles air, formaldehyde constitutes somewhat less than half of the total aldehydes and ketones present (*11*). No limits for the emission of formaldehyde to the outdoor air have been found in air pollution control legislation, but in a recent Russian paper, a maximum allowable concentration of 0.035 mg. per cubic meter in ambient air has been suggested (*12*).

Several methods for determining formaldehyde in air have been developed. Goldman and Yagoda (*6*) used 1% sodium bisulfite solution as an absorbing agent, and determined the formaldehyde by an iodine titration procedure. Phenylhydrazine hydrochloride solution has been employed as a collecting medium (*10*), but owing to discoloration of this reagent, its use is limited to air samples of not more than 40 liters. Barnes and Speicher (*3*) bubbled air through a 1.25% potassium hydroxide solution, and used Schryver's method (*10*) to determine the absorbed formaldehyde. Jacobs, Eastman, and Shepard (*9*) modified this method and were able to determine a minimum of 10 μg. of formaldehyde in 12 ml. of test solution. West and Sen (*16*) investigated the factors influencing the reaction of chromotropic acid with formaldehyde, and devised a spectrophotometric method for trace amounts.

The present paper describes a method which is sufficiently sensitive to determine low concentrations of formaldehyde in air. The formaldehyde is collected in slightly acidified water and determined by a colorimetric procedure, using acetone (*8*) and Schiff's reagent (*4, 5, 15*). This reagent gives a favorable ratio of volume of sample taken for analysis to volume of reagents added. Water has been suggested (*1*) as an absorbing medium, and was investigated further, as sodium bisulfite and potassium hydroxide interfere with color development. Formaldehyde was found to be more stable in weak hydrochloric acid than in water. The efficiency of the weak acid solution to collect formaldehyde was determined

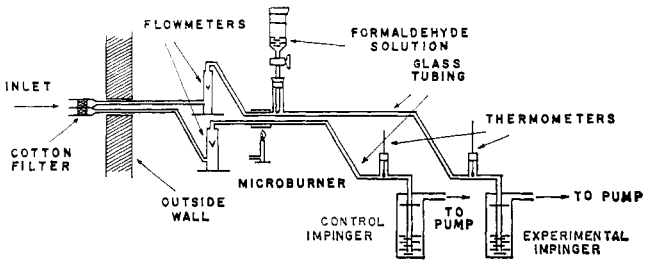

Figure 1. Apparatus for determining impinger efficiency

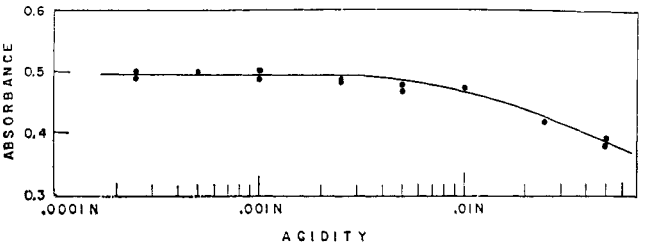

Figure 2. Effect of acidity on color development

to be 72% at an air sampling rate of 1 c.f.m.

EXPERIMENTAL

Reagents. BASIC FUCHSIN. A satisfactory grade of dye was obtained from the British Drug Houses (Canada), Ltd., Toronto, under the name fuchsin, basic, standard stain.

SCHIFF'S REAGENT. Place 0.5 gram of basic fuchsin in a mortar, add a few drops of water, and grind to a fine paste. Wash into a beaker and dilute to 500 ml. with water. Filter the solution into a 500-ml. reagent bottle. Add 4.69 grams of sodium metabisulfite and stir until dissolved. Let stand 15 minutes and then add 17 ml. of 6*N* hydrochloric acid. Allow the solution to stand overnight before use. Keep the bottle well stoppered and away from light. Under these conditions, the reagent is stable for several months.

STANDARD FORMALDEHYDE SOLUTIONS. Using formalin, prepare an aqueous solution containing 1 mg. of formaldehyde per ml. Dilute this solution with 0.005*N* hydrochloric acid to bring the concentration to 5 μg. per ml. Solutions should be made up just prior to use. The strength of the formalin may be determined by the method of Ripper (*13*) or Romijn (*14*).

Procedure. Draw air through an impinger containing 60 to 75 ml. of 0.005*N* hydrochloric acid, at a rate of 1 c.f.m. If the air to be sampled is thought to contain less than about 50 p.p.b. of formaldehyde, a volume of about 60 cubic feet should be taken.

Table I. Results of Efficiency Tests Using Two Impinger Types

Vol. of Absorbing Soln., Ml.	HCHO Evapd., μg.	Sampling Period, Min.	% Recovery
	Impinger I (*7*)		
75	80	60	67 71 76
60	80	60	69 74 74
60	40	30	63 75 78
60	20	15	62 70 75
	Impinger II (*17*)		
60	300	60	64 75 75 79
60	80	60	70 73 75 83
60	20	60	64 66 76 77
60	10	60	66 72 72 74

Mean recovery 72 ± 5%

As rubber absorbs formaldehyde, it is advisable to use a glass air-inlet line, which contains a plug of cotton or glass wool to filter out particulate matter.

After sampling, measure the volume of solution remaining in the impinger and transfer an aliquot, up to 19 ml., to a 25-ml. volumetric flask. Add known amounts of a 5-μg.-per-ml. formaldehyde solution to each of three 25-ml. volumetric flasks. Bring the volumes of these solutions and the aliquot, if less than 19 ml., to 19 ml. with 0.005*N* hydrochloric acid, and add 1 ml. of acetone and 5 ml. of Schiff's reagent; stopper, mix, and let stand. Approximately 70% of the maximum color intensity is developed in the first 3 hours. If the test solution contains less than 10 μg. of formaldehyde, let the color develop for a longer period, preferably overnight. Read the absorbances against a reagent blank in a spectrophotometer at a wave length of 560 mμ. Plot the number of micrograms of formaldehyde in the color standards against the absorbance readings, to obtain a working curve for determining the amount of formaldehyde in the aliquot. Calculate the amount present in the collecting medium from the amount found in the aliquot, and multiply the whole by 1.39, to correct for the 72% collecting efficiency.

Evaluation of Collecting Efficiency. The efficiency of 0.005*N* hydrochloric acid to collect formaldehyde from air was determined, using two types of Greenburg-Smith impingers. One was the Hatch, Warren, and Drinker modification (*7*), which may be operated for sampling periods of varying duration. The other instrument was the Wilson modification (*17*), which operates automatically, allowing hourly samples to be obtained over a 24-hour period. The method for finding the collecting efficiency at an air flow rate of 1 c.f.m. was the same for both instruments.

Two identical assemblies were set up having adjacent inlets, to obtain comparable samples of outdoor air (Figure 1). A plug of cotton wool was placed in each inlet to filter out particulate matter. One assembly served as a test unit in which a known amount of standard formaldehyde solution was evaporated, drop by drop, at the rate of 1 ml. per hour. To ensure continuous evaporation, the incoming air was heated to a temperature of about 32° C. The second assembly, which served as a control, was used to determine the amount of formaldehyde in the incoming air.

At the completion of each test, the volume of solution remaining in each impinger was measured, and it was found that about 25% had evaporated during a 1-hour test. Each solution was analyzed, and the amount of formaldehyde in the control was subtracted from that in the test. The percentage recovery was taken as the collecting efficiency. The results are shown in Table I.

Under the experimental conditions, the efficiency of collection may be taken as 72% with a standard deviation of 5%, in the range of 10 to 300 μg. of formaldehyde, when sampling air at the rate of 1 c.f.m. The efficiency is the same for different sampling periods, and for the two impingers used.

RESULTS AND DISCUSSION

Factors Influencing Color Development. When Schiff's reagent is added to formaldehyde solutions, the amount of color produced depends upon the acidity, temperature, time, and formaldehyde concentration. The effect of each of these factors was determined by adding 1 ml. of acetone and 5 ml. of Schiff's reagent to 19-ml. solutions containing known amounts of formaldehyde and hydrochloric acid. Absorbance readings, using a 1-inch light path, were taken by means of a Bausch & Lomb Spectronic 20 spectrophotometer.

Figure 2 shows the relationship between absorbance and acidity in the range 0.0001 to 0.06*N*, using solutions containing 15 μg. of formaldehyde. The color intensity is the same in acid concentrations up to about 0.01*N*. The color intensity diminishes, and the color of the solutions gradually changes from purplish-red to purple between 0.01 and 0.10*N*.

To determine the effect of temperature, solutions containing 15 μg. of formaldehyde were held for 3 hours, after the addition of Schiff's reagent, at temperatures from 15° to 35° C. The results, plotted in Figure 3, show that absorbance increases with temperature, which confirms the findings of Atkinson (*2*). No advantage was taken of this fact to increase the sensitivity,

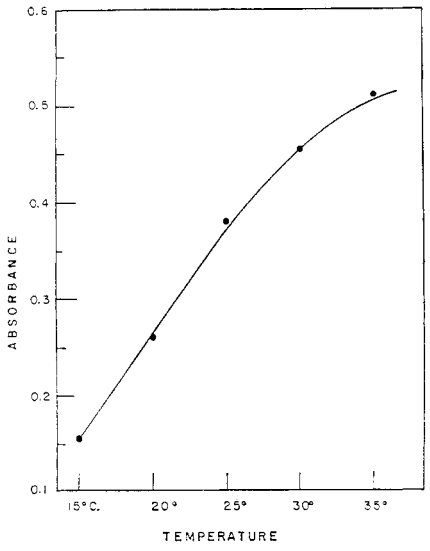

Figure 3. Effect of temperature on color development

as reagent blanks became colored at the elevated temperatures.

Figure 4 illustrates the effect of time on color development. The data were obtained by reading the absorbance of solutions containing up to 25 μg. of formaldehyde, at several intervals after adding the reagents. After an initial time lapse, the rate of color development was greatest during the first 3 hours. The color reached a maximum after about 13 hours, persisted for several hours, and then faded slowly. Figure 4 shows, also, that the absorbance is not directly proportional to the concentration.

As temperature and time affect the rate of color development, and Beer's law is not obeyed, it is necessary to run standards at the same time as the unknowns.

Sensitivity and Precision. Using a 1-inch light path, an absorbance reading of about 0.05 is obtained with 2 μg. of formaldehyde. This is considered to be the limit of sensitivity of the method, and corresponds to a concentration of 0.1 μg. per ml. in the collecting solution, if the maximum aliquot of 19 ml. is taken for analysis. A level of 5 p.p.b. of formaldehyde in air can be determined if 60 cubic feet are sampled.

The precision of the analytical method, using test solutions containing 5 and 10 μg. of formaldehyde, is ±8 and ±4%, respectively.

Stability of Dilute Formaldehyde Solutions. As samples are usually analyzed at various time intervals after collection, the stability of formaldehyde in water and in weak hydrochloric acid was investigated. Solutions containing 5 μg. of formaldehyde per ml. in 0.005*N* hydrochloric acid were stable for 3 days, when kept in the dark. Formaldehyde is more stable in weak acid solution than in water.

Interfering Substances. ACROLEIN. A sample of acrolein, containing hydroquinone as a stabilizer, was distilled, and three drops of the distillate were collected in 10 ml. of water contained in a 25-ml. volumetric flask. The flask was weighed before and after collection, water was added to the mark, and the acrolein concentration calculated. The solution was then diluted with water to bring the concentration to 65 μg. per ml. Up to 2-ml. amounts of this solution were added to 15 ml. of 0.005*N* hydrochloric acid solutions which contained from 0 to 20 μg. of formaldehyde. Acrolein gave a color with Schiff's reagent, and interfered when present in greater amounts than formaldehyde. The stability and shade of the color were comparable to that given by formaldehyde. The results obtained are presented in Table II. Dilute acrolein solutions, containing no preservative, were stable for 24 hours.

OTHER ALDEHYDES. The effect of Schiff's reagent on acetaldehyde, in weak hydrochloric acid, was investigated. A purplish color was produced which reached a maximum intensity almost immediately, and then gradually faded over a period of several hours. With an acetaldehyde-formaldehyde ratio of 10 to 1, a +20% error was found when the absorbance was read after 3 hours, and a +2% error when read the following day. The interference was approximately proportional to the amount of acetaldehyde present. Propionaldehyde and *n*-butyraldehyde, present in tenfold excess, increased the absorbance by about 2%.

SULFUR DIOXIDE. The effect of sulfur dioxide on color development was determined by adding known amounts of sodium metabisulfite to 15 ml. of 0.01*N* hydrochloric acid solutions containing 10 μg. of formaldehyde. Conversion of the sodium metabisulfite to sulfur dioxide and sodium chloride was assumed. The results, presented in Table III, show that sodium metabisulfite in amounts up to 0.5 mg. in the acidic medium, does not interfere. When more is present, the results are low. If it is desired to sample where concentrations of sulfur dioxide in air are high, interference can be eliminated by a preliminary oxidation with iodine. This may be done as follows: Add dropwise to the aliquot taken for analysis, a slight excess of 0.02*N* iodine, and 1 drop to each of the standards. Decolorize the iodine with 1 drop of 0.025*N* sodium thiosulfate and proceed as in the method.

OXIDES OF NITROGEN. The addition of sodium nitrite to 15 ml. of 0.01*N* hydrochloric acid solutions containing 15 μg. of formaldehyde intensified the color developed by Schiff's reagent, and gave high results, as shown in Table IV. Conversion of sodium nitrite to sodium chloride and oxides of nitrogen, in the acidic medium, was assumed.

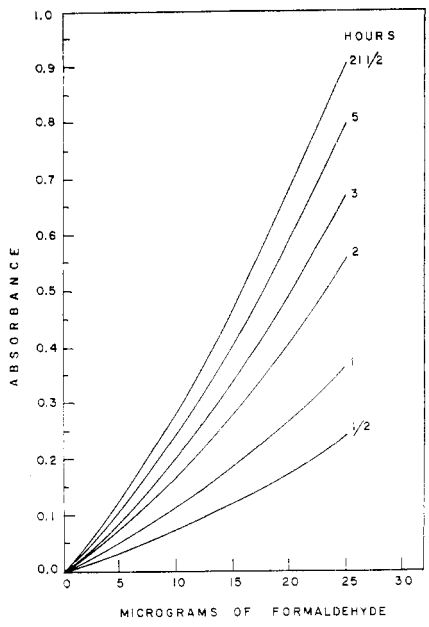

Figure 4. Effect of time on color development

Table II. Effect of Acrolein

Ratio of Acrolein to Formaldehyde	% Error
1:1	+ 3
2:1	+ 7
4:1	+14
8:1	+28
10:1	+34

Table III. Effect of Sulfur Dioxide

Na Metabisulfite Taken, Mg.	HCHO Taken, μg.	HCHO Found, μg.	% Error
0.2	10	10.2	+ 2
0.5	10	9.8	− 2
0.9	10	9.1	− 9
1.4	10	8.4	−16
1.8	10	8.1	−19
2.3	10	6.6	−34

Table IV. Effect of Oxides of Nitrogen

Sodium Nitrite Taken, Mg.	HCHO Taken, μg.	HCHO Found, μg.	% Error
0.5	15	16.0	+ 7
1.0	15	17.0	+13
1.5	15	17.4	+16
2.0	15	18.2	+21

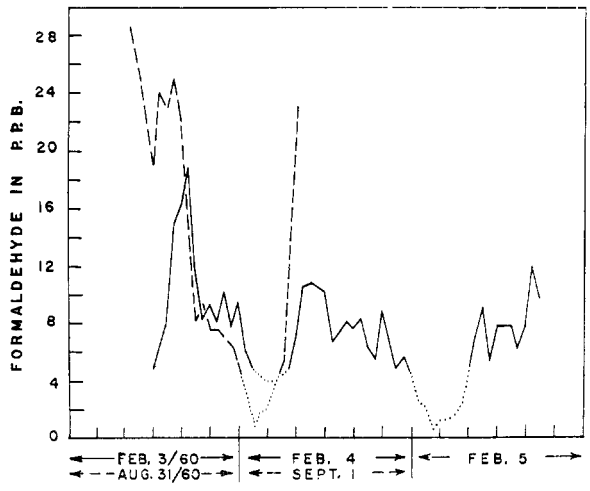

Figure 5. Formaldehyde levels in urban air

OTHER SUBSTANCES. No significant interference occurred with 20-mg. amounts of formic acid (90%), glacial acetic acid, methanol, or ethanol.

Formaldehyde Content of Urban Air. Samples were taken at hourly intervals throughout the day in a commercial area in Toronto, Canada. The sampling site was located at street level, near a busy intersection. Two series of results are presented in Figure 5. Diurnal variations occurred on all days on which the tests were run. The concentrations were greatest during the morning and late afternoon, and least during the early hours of the morning.

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Spot Test Microdetermination of DDT and Its Related Compounds in Biological Materials

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▶ **The nitrated product of DDT displays four distinct colors successively when treated with 20% alcoholic potassium hydroxide, and five distinct colors successively when acetone is added also. This reaction occurs for amounts as small as 1 μg. These tests are applied in the field of forensic toxicology for the identification of DDT and its related compounds, extracted from biological materials of human origin.**

SEVERAL colorimetric methods have been described for the determination of DDT (*3*, *13*) and its nitrated product. Schechter *et al.* (*12*) used methanolic sodium methylate with a benzene solution of the nitrated product for the production of intense colors with a sensitivity of 10 μg. of DDT. Several variations have been suggested for the Schechter method (*4*, *7*, *8*). Alessandrini (*1*) substituted 1*N* alcoholic potassium hydroxide, while Iling and Stephenson (*5*) used alcoholic potassium hydroxide and ammoniacal hydroxylamine for sodium methylate. Amsden and Walbridge (*2*) substituted isopropylamine for sodium methylate.

Luis (*6*) obtained a residue of insecticidal properties from Stas-Otto extracts of the viscera of a poisoned person, which was confirmed as DDT after purification by chromatography on alumina (*1*). The production of color in solution is slow, requires warming, and the method is not sensitive when microquantities are present. Prickett *et al.* (*9*) described a modified Schechter method for the determination of DDT or methoxychlor with a lower limit of 5 μg. of either compound in 5 to 6 grams of fat.

In cases of poisoning by DDT, often only trace amounts are left in biological materials, as the major part is either destroyed or removed from common toxicological specimens by the following means: vomit; degradation in the metabolism to related compounds; total degradation to simpler compounds and excretion through the kidneys and intestines; storage in fatty tissues, etc. Therefore, a definite chemical method was needed by which microquantities of DDT and its decomposition products could be isolated from biological materials and identified.

The purpose of this paper is to present a simple method for the isolation and identification of very small quantities of DDT and its metabolized products present in biological specimens, while examining several DDT poisoning cases of legal significance. No special apparatus or technique is involved. Specimens that are encountered are vomited matter, stomach wash, urine, excreta, stomach with its contents, intestines with their contents, liver, kidney, spleen, and other organs. They are expected to be preserved in rectified spirit for toxicological analysis. However, they are sometimes putrified, because of improper preservation by the personnel who collect the specimens or natural decomposition prior to preservation, and such specimens are analyzed by the modified method with a slight decrease in sensitivity. The principal steps in the method are: extraction of DDT and related com-