calculated in microamperes (columns 4 and 7). These figures are compared with actual weights of the gases determined by Method A (columns 2 and 5) and the corresponding total thermal conductivity readings observed.

DISCUSSION

Collectively these results show substantially correct recoveries of the two gases by Method A or B, either individually or in admixture without mutual interference. The additive relationship of thermal conductivity values, at least for low concentrations of the mixed gases, is demonstrated.

For the most accurate work, especially at low concentrations of SO_2F_2 (below 20 mg. per liter), Method B is to be preferred, since the calculation is made independently from that for CO_2.

In Method A the calculation for either gas depends on the precise conversion of carbonate to bicarbonate without loss of CO_2 from solution. Some small variation in successive estimations arising from this may be caused by difference in rate of titration or by a local concentration of excess acid during titration. End points with the methyl red–bromocresol green indicator used in Method B are sharper when titrating with 0.02*N* $Ba(OH)_2$ than when using 0.05*N* reagent in Method A.

The curves in Figure 3 show that the rate of reaction of SO_2F_2 depends on the strength of the absorbent reagent. Rate of reaction would probably increase with temperature, but this also increases the possibility of initiating the partial further reaction of Equation 1, invalidating the quantitative determination. For calibration purposes, a reaction time of 24 hours at room temperature is considered satisfactory, with an adequate excess of reagent present.

In large scale practical fumigation, where results must be obtained quickly in order to make adjustments during the fumigation period, a reasonable estimate can be made by titration of samples after 2 hours. For example, with 0.5*N* NaOH as absorbent, about 93% recovery of SO_2F_2 is obtained (Figure 3). However, the principal value of the methods described lies in the calibration and support of field instruments of the thermal conductivity type, and in the measurement of concentrations of SO_2F_2 (by Method B) from 0.5 to 10 mg. per liter, used in toxicity studies. At the lower end of this concentration range, the correction required for CO_2 at normal atmospheric level can amount to 50% of a thermal conductivity meter reading. In fumigating insect infested stored products, enhanced CO_2 concentrations up to 3% in air, or 100 times normal, may be encountered in the interstitial space, so results obtained using a thermal conductivity meter without a CO_2 trap must be treated with circumspection.

ACKNOWLEDGMENT

The author is indebted to E. E. Kenaga and the Dow Chemical Co., Midland, Mich., for providing a supply of sulfuryl fluoride. Much of the analytical work was carried out by Catherine A. O'Gorman.

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Five New Methods for the Spectrophotometric Determination of Alkylating Agents Including Some Extremely Sensitive Autocatalytic Methods

Application to Air Pollution

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▶ The presence of alkylating agents in the air and in various air pollution sources has been demonstrated with the new reagents. Because of the wide range of toxic effects caused by alkylating agents, a more thorough investigation of the composition of the air in terms of alkylating agents is necessary. Four new sensitive reagents for the determination of alkylating agents are introduced. A much more sensitive 4-(4-nitrobenzyl)-pyridine procedure is described for the determination of the relative reactivity of over 90 alkylating agents. A new type of spectrophotometric method for the analysis of organic compounds involves an autocatalytic reaction for determining alkyl iodides in trace quantities. For example, in the 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone procedure for the determination of 1-iodobutane a molar absorptivity of 1,500,000 can be obtained. The determination limit is about 50 nanograms of 1-iodobutane. These reagents can be used to determine very low concentrations of iodine and iodine precursors through an autocatalytic reaction. For example, in the determination of iodine with 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone a molar absorptivity of 6,000,000 can be obtained. The determination limit for iodine with this reagent is 15 nanograms. Several new methods use the new reagents for the detection of acylating agents. The relative activity of many functional groups is discussed.

MANY alkylating agents have been shown to be toxic to the animal body, as lachrymators, sternutators, or vesicants (*13*), mutagens (*10*), nerve gases (*9*), antimitotic agents (*11*), cocarcinogens (*12*), or carcinogens (*5*). Many alkylating agents also are considered to be potential war gases (*9*, *13*). A very large number of references are available on the toxic, mutagenic, and carcinogenic effect on man and animal of aliphatic halides, organic phosphates, and other alkylating agents, many of which are used as insecticides (*1*).

The presence of any of these compounds in the atmosphere could be considered a potential human hazard. An alkylating agent of the carcinogenic type is probably present in the air of some communities, as indicated by the

carcinogenicity of some of the aliphatic fractions of airborne particulates (*6*). Ozonized gasoline has been shown to be carcinogenic to animals (*8*).

The most versatile reagent for the determination of alkylating agents has been 4-(4-nitrobenzyl)pyridine (*3*). With this reagent a procedure has been described for the determination of ethyleneimines, α-halogenated esters, diethyl sulfate, alkyl iodides, bromides, and chlorides; of various phosphorus, silicon, and nitrogen chlorides; and of nitrogen and sulfur mustard gases (*3*). Higher-molecular-weight nitrogen mustard compounds have also been determined by this procedure (*4, 7*).

4-(4-Nitrobenzyl)pyridine has been used for the determination of benzoyl chloride (*3*) and phosgene (*2*).

A modification of the literature procedure to give much greater sensitivity is presented in this paper. In addition, four new easily prepared reagents are introduced for the determination of alkylating agents.

EXPERIMENTAL

Analyses are by the Galbraith Laboratories, Inc., P. O. Box 4187, Knoxville 21, Tenn.

Synthesis of 4-Pyridinecarboxaldehyde 4 - Nitrophenylhydrazone. A minimum volume of a hot methanolic solution of *p*-nitrophenylhydrazine, (m.p. 161–2° C., 9.05 grams, 0.059 mole), was added to 25 ml. of a methanolic solution of 4-pyridine carboxaldehyde (6.32 grams, 0.059 mole). Two to three drops of concentrated hydrochloric acid was added as a catalyst. The reaction was allowed to proceed for 1 hour. The precipitate was crystallized from *o*-dichlorobenzene and then from 2-methoxyethanol. The yield of yellow cottony needles (m.p. 259–61° C. *cor.*) ranged from 22 to 29%. Calculated for $C_{12}H_{10}N_4O_2$: C, 59.5; H, 4.16; N, 23.1. Found: C, 59.2; H, 4.13; N, 22.9.

Synthesis of 4-Pyridinecarboxaldehyde 2-Benzothiazolylhydrazone. A hot solution of 2-hydrazinobenzothiazole (1.93 grams, 0.117 mole) in a minimal volume of 2-methoxyethanol and methanol (1:1) was added to 25 ml. of a methanolic solution of 4-pyridinecarboxaldehyde (15 grams, 0.14 mole). Two to three drops of concentrated hydrochloric acid was added. After 1 hour of standing, the precipitate was recrystallized from aqueous 2-methoxyethanol. The yield of yellow crystalline hydrazone (m.p. 236–7° C. cor.) was 19.5 grams or 66%. Calculated for $C_{18}H_{10}N_4S$: C, 61.4; H, 3.96; S, 12.6. Found: C, 61.2; H, 4.15; S, 12.7.

Synthesis of 4-Acetylpyridine 4-Nitrophenylhydrazone. A hot solution of 4-nitrophenylhydrazine (7.7 grams, 0.05 mole) in a minimal volume of methanol was added to a solution of 4-acetylpyridine (6.1 grams, 0.05 mole) in a minimal volume of methanol. Two drops of concentrated hydrochloric acid was added. The mixture was allowed to stand overnight. The precipitate was filtered, washed with ether, and then crystallized from aqueous 2-methoxyethanol. The yield of fluffy yellow needles (m.p. 257–8.5° C. cor.) was 9.1 grams or 71%. For analysis a small portion was crystallized from aqueous dimethylformamide; the product melted at 258.5–9° C. cor. Calculated for $C_{13}H_{12}N_4O_2$: C, 60.9; H, 4.72; N, 21.9. Found: C, 60.7; H, 4.53; N, 21.7.

Synthesis of 4-Acetylpyridine 2-Benzothiazolylhydrazone. A hot solution of 2-hydrazinobenzothiazole (8.3 grams, 0.05 mole) in a minimal volume of 2-methoxyethanol–methanol (1:1) was added to a methanolic solution of 4-acetylpyridine (6.1 grams, 0.05 mole). Two drops of concentrated hydrochloric acid was added. The mixture was allowed to stand overnight. Crystallization from aqueous 2-methoxyethanol gave 10.8 grams (81% yield) of pale brownish crystals (m.p. 174–5° C.). Calculated for $C_{14}H_{12}N_4S$: C, 62.7; H, 4.51; N, 20.9. Found: C, 62.4; H, 4.45; N, 20.6.

Synthesis of *N*-Ethyl-4-(4-nitrobenzyl)pyridinium *p* - Toluenesulfonate. A mixture of 4-(4-nitrobenzyl)pyridine (2.14 grams, 0.01 mole) and ethyl *p*-toluenesulfonate (2.00 grams, 0.01 mole) was gradually heated on an open flame for a few minutes until the homogeneous blue liquid suddenly turned brown. The tarry mixture was dissolved in a minimum amount of hot acetone and then enough hot carbon tetrachloride was added so that the compound would crystallize once the solution cooled. The yield of light yellow needles (m.p. 137–8.5° C. cor.) was 1.98 grams or 48%. Calculated for $C_{21}H_{22}N_2O_5S$: C, 60.9; H, 5.30; N, 6.70. Found: C, 60.6; H, 5.46; N, 6.56.

Miscellaneous Reagents. 4-(4-Nitrobenzyl)pyridine was obtained from the Aldrich Chemical Co. The alkylating agents were obtained in the purest form possible from the Aldrich Chemical Co., Inc., Milwaukee 10, Wis., Distillation Products Industries, Rochester 3, N. Y., K and K Laboratories, Inc., Jamaica 33, N. Y., and Laboratory Services, Inc., Cincinnati 9, Ohio. The purest grades of acetophenone and dimethylformamide were obtained from Laboratory Services and distilled before use.

Cary recording spectrophotometers, Models 11 and 14, were used for quantitative analysis.

Reagent Solutions. 4-(4-Nitrobenzyl)pyridine, 50% in acetophenone. All the pyridine reagents can crystallize out on standing. In that case it is necessary to warm the solution to dissolve the crystals and then to cool to room temperature before proceeding with the analysis. The solution is stable for several months.

4 - Pyridinecarboxaldehyde 4 - nitrophenylhydrazone, 1.5% in acetophenone. The solution is stable for several months.

4-Pyridinecarboxaldehyde 2-benzothiazolylhydrazone, 3% in acetophenone - dimethylformamide (v.:v., 7:3). Concentrated hydrochloric acid (0.01 ml.) is added to 100 ml. of this solution. The solution is stable for at least a month.

4-Acetylpyridine 4-nitrophenylhydrazone, 1.5% in acetophenone–dimethylformamide (v.:v., 17:3). The solution is stable for several months.

4-Acetylpyridine 2-benzothiazolylhydrazone, 3% in acetophenone. The solution is stable for several months.

Triethylamine, 2% in acetophenone. The solution is prepared fresh each day.

Cyclohexylamine, 0.5, 1.0, and 2.0% in acetophenone. These solutions turn light yellow after 4 to 5 hours of standing; they are prepared fresh each day.

Spectrophotometric Procedures. 4 - (4 - NITROBENZYL)PYRIDINE PROCEDURE. To 1 ml. of acetophenone test solution was added 1 ml. of the 50% reagent solution. The mixture was heated for 3½ minutes at 180° C. in an *o*-dichlorobenzene bath, quickly cooled under the tap, and then diluted to 4 ml. with 0.5% cyclohexylamine in acetophenone. The absorbance was read immediately at the wavelength maximum.

The somewhat analogous procedures with other reagents are described in Table I.

The data obtained with various alkylating agents are compared in Tables II, III, and IV.

All analyses were done in 12-ml. centrifuge tubes, each fitted with a

Table I. Procedures for Determination of Alkylating Agents

Reagent	Reagent, %	Time at 180° C., min.	Base,[a] %
4-(4-Nitrobenzyl)pyridine[b]	50	3.5	0.5
4-Pyridinecarboxaldehyde 4-nitrophenylhydrazone	1.5	30	1
4-Pyridinecarboxaldehyde 2-benzothiazolylhydrazone[c]	3	15	2
4-Acetylpyridine 4-nitrophenylhydrazone	1.5	30	2
4-Acetylpyridine 2-benzothiazolylhydrazone	3	15	2

1 ml. test solution, 1 ml. reagent.

[a] In all cases dilute to 4 ml. with basic solution. Cyclohexylamine was used as base in all procedures except 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone, where triethylamine was used.

[b] More detailed procedure given in experimental section. Except for differences cited in the table, procedures are similar.

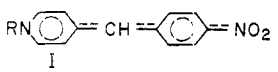
[c] Two-ml. reagent with this compound.

cork stopper large enough to fit snugly into the neck of a 3-necked 1-liter flask that contained refluxing *o*-dichlorobenzene. A reflux condenser was attached to the middle neck of the flask. With this setup a sample and a blank were heated with boiling *o*-dichlorobenzene at the same time.

Detection of Acylating Agents. One drop of acetophenone test solution is placed on filter paper (impregnated with an acetone solution of 0.05% 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone) and dried. Positive results are shown by a purple color which fades within several minutes. If a negative result is obtained, the paper is waved over triethylamine fumes. Some acylating agents need this last step to give positive results.

REACTION MECHANISMS

The structures of the chromogens obtained with the five reagents are:



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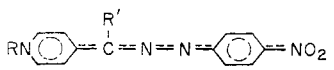
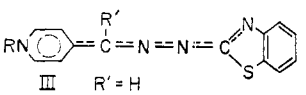


II R′ = H
R′ = CH_3



III R′ = H
R′ = CH_3

The mechanism in the formation of the chromogen from the reaction of an alkylating agent with 4-(4-nitrobenzyl)-pyridine has been described (*4*). In the procedures described in this paper for the determination of alkyl iodides the mechanism is much more complicated, for the reaction is autocatalytic. For example, the pure ethyl-*p*-toluenesulfonate salt obtained from 4-(4-nitrobenzyl)pyridine has a molar absorptivity of about 33,000 in alkaline solution, while in the analytical procedure molar absorptivities of around 120,000 are obtained with alkyl iodides. The mechanism, especially in relation to the limiting reaction, has not been completely elucidated, but enough information has been assembled to resolve the main reactions.

In this respect the following facts are of value. Iodine, hydrogen iodide, and soluble tetraalkylammonium iodides will react just like the alkyl iodides. The chromogen salt, once formed, tends to form an equilibrium in a hot solution with the dye and acid, as shown by the color of the solution. Cooling shifts the equilibrium toward salt formation, as shown by the change to a lighter color. The autocatalytic reaction cannot be carried on for an extended period at 180° C., for eventually the chromogen

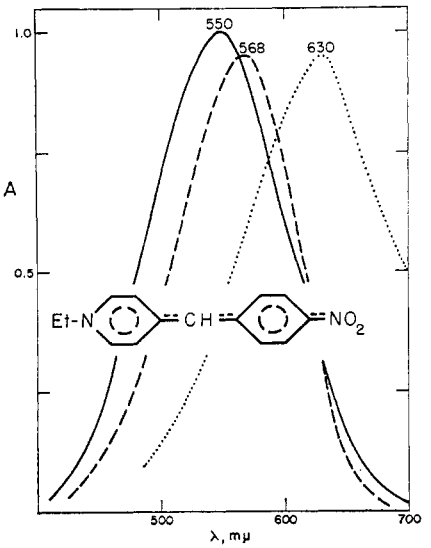
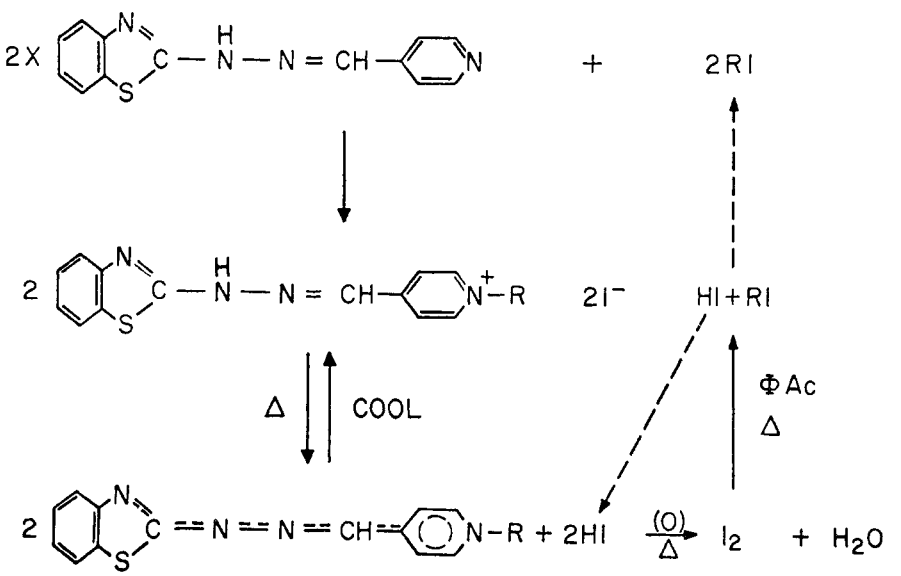


Figure 1. Visible absorption spectra of *N*-ethyl-4-(4-nitrobenzyl)pyridinium *p*-toluenesulfonate (3×10^{-5}M)

—— In acetone containing 7.5% triethylamine
– – – In acetophenone containing 0.25% cyclohexylamine
... In water-methanol (3:1) containing 7.5% tetraethylammonium hydroxide

begins to decompose faster than it is formed and the solvent acetophenone, or a by-product, starts to react with the reagent to form a colored product.

Since 2-iodoacetophenone is believed to be the key intermediate from which the high molar absorptivity is obtained, the presentation is simplified by letting 2-iodoacetophenone equal RI. Essentially the same mechanism would apply to the determination of any alkyl iodide with any of the reagents. Cooling followed by the addition of alkali stops the reaction at the III stage. On the basis of all this information the following mechanism is postulated:



III

The chromogen obtained from the addition product of ethyl-*p*-toluenesulfonate with 4-(4-nitrobenzyl)pyridine shows a fairly drastic change in absorption spectrum with change in solvent (Figure 1). This type of solvent effect could be expected with the analogous addition products from the other alkylating agents. This phenomenon could prove to be of value in future analytical work with the reagents described in this paper.

DISCUSSION

Variables were investigated for the determination of 1-iodobutane in each of the recommended procedures. In the analysis of any of the other alkylating agents the best procedure for optimum results could be determined readily by investigating a few variables. Acetophenone was chosen as the analytical solvent because the higher temperature of reaction possible with this solvent makes the autocatalytic effect of overwhelming importance. The molar absorptivity values were in some cases approximately 30 times higher than the theoretical.

4 - (4 - Nitrobenzyl)pyridine Procedure. Increasing the concentration of reagent increased the absorbance (Figure 2). Optimum intensities were obtained by heating at 180° C. for $3\frac{1}{2}$ to 15 minutes. Heating longer than 5 minutes gave a darker blank. Heating at lower temperatures gave lower absorbances (Figure 3). Although the recommended procedure was used with all halides, optimum results with some were obtained at lower temperatures (Figure 3). Cyclohexylamine was a more satisfactory base than benzylamine, triethylamine, piperazine, or decahydroquinoline. Optimum intensities were obtained with 0.2

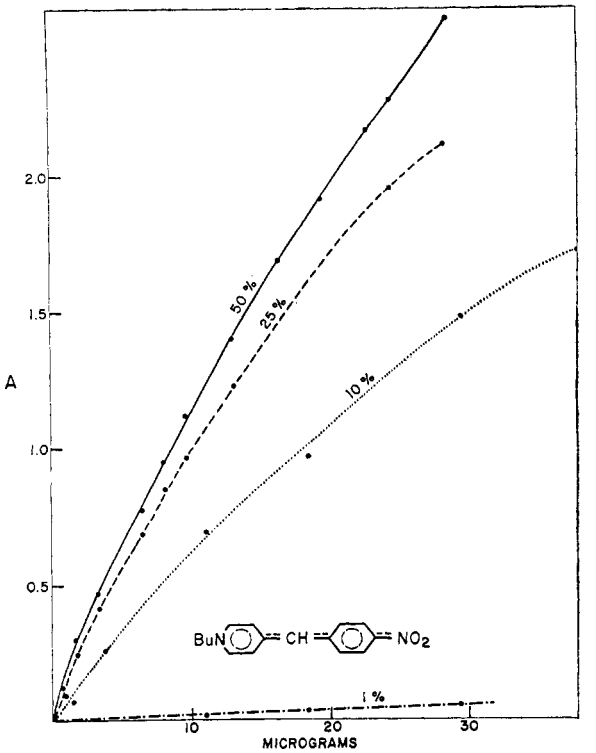


Figure 2. Absorbance at wavelength maximum *vs.* concentration in micrograms of 1-iodobutane

Reagent 4-(4-nitrobenzyl)pyridine
—— 50% reagent
– – – 25% reagent
... 10% reagent
—·—· 1% reagent

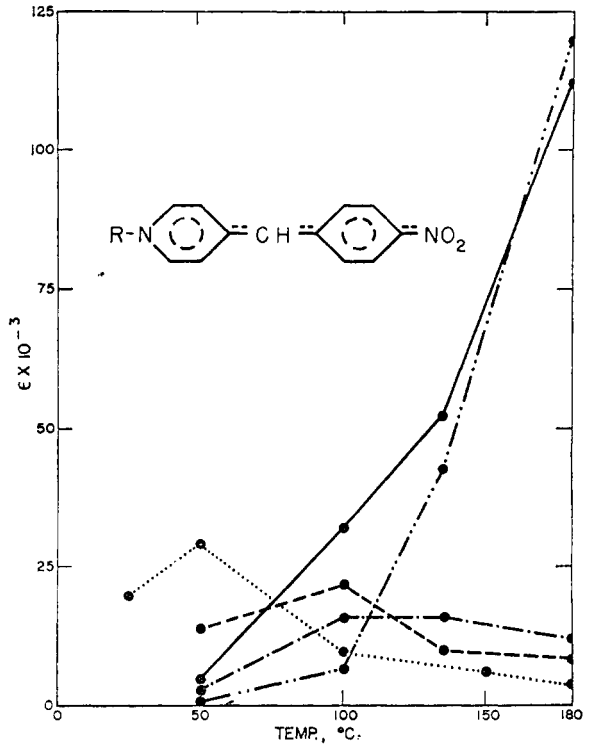


Figure 3. Effect of temperature on absorbance obtained at wavelength maximum in 4-(4-nitrobenzyl)-pyridine procedure

—— Iodoacetic acid, 5 × 10^{-6}M
—·— 1-Iodobutane, 10^{-5}M
... 2-Bromoacetophenone, 5 × 10^{-5}M
– – – 4-Nitrobenzyl bromide, 5 × 10^{-5}M
—·—· Bromoacetic acid, 3 × 10^{-5}M

to 1.0% cyclohexylamine. The color intensity gradually decreased with time, as shown for 1-iodobutane and 1-bromobutane (Figure 4).

The change in absorbance with concentration is shown in Figures 2 and 5. Greater relative intensity per unit weight of compound was obtained at the lower concentrations. The same type of absorbance-concentration curve is given by each of the other reagents also. As expected, because of the major role of iodine in the autocatalytic mechanism, iodine reacted much more intensely than iodobutane (Figure 5).

The relative intensity obtained with iodides, bromides, and chlorides is approximately 84:12:1 (Table II). Primary halides gave a more intense color than secondary halides. The substitution of a second bromine or chlorine atom on the same carbon atom decreased the intensity. The intensity of the color obtained with iodoform is approximately 450 times that obtained with bromoform; chloroform gave an essentially negative reaction. Cyclopentyl iodide or bromide gave a more intense color than the analogous cyclohexyl derivatives. Alkyl sulfates, methanesulfonates, *p*-toluenesulfonates, and trifluoroacetates gave positive results with the reagent.

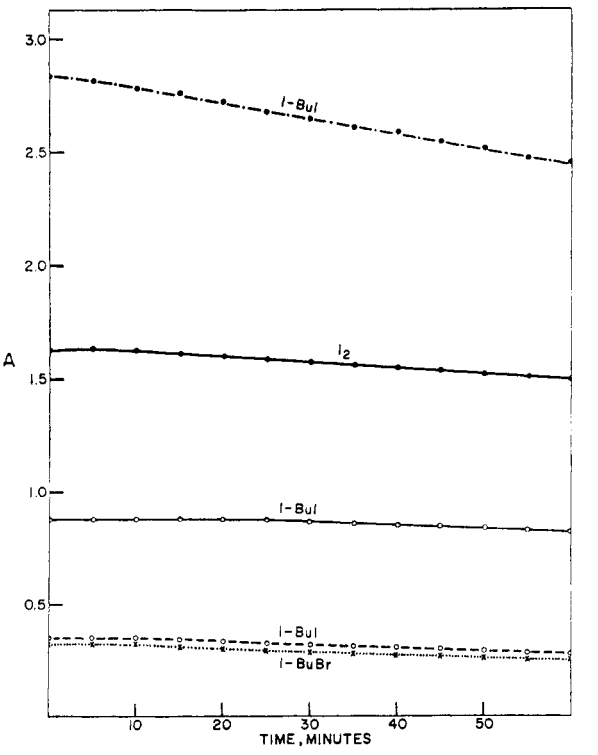


Figure 4. Absorbance at wavelength maximum *vs.* time

4-(4-nitrobenzyl)pyridine procedure
—·—· 1-Iodobutane, 5 × 10^{-5}M
····· 1-Iodobutane, 5 × 10^{-6}M
... 1-Bromobutane, 2.1 × 10^{-5}M
4-Pyridinecarboxaldehyde 2-benzothiazolylhydrazone procedure
○— 1-Iodobutane, 10^{-6}M
●— Iodine, 4.5 × 10^{-7}M

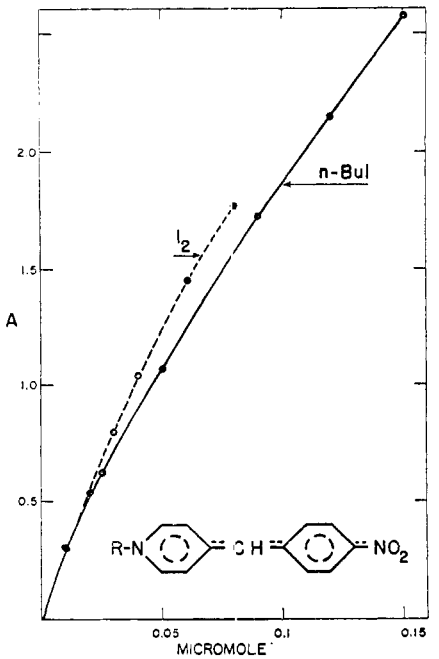


Figure 5. Absorbance at wavelength maximum vs. concentration in 4-(4-nitrobenzyl)pyridine procedure

—— 1-Iodobutane
– – – Iodine

Negative results were obtained with hexachloroethane, *sym*-tetra-chloroethane, bromobenzene, iodobenzene, nitrosodimethylamine, acrolein, nitroethane, *n*-butyl nitrate, paraformaldehyde, dihydroxyacetone, benzyl alcohol, 1-octadecanol, propylene glycol, octadecane, 1-octadecene, cyclohexene oxide, 2-chloropyridine, *o*-dinitrobenzene, *p*-dinitrobenzene, methyl oleate, 2-methylmercaptobenzothiazole, benzoic acid, nonanoic acid, and benzo[*a*]pyrene. The fluoro-, chloro-, and bromo-1,1,1-trihaloethanols gave very weak to negative results.

4-Pyridinecarboxaldehyde 4-Nitrophenylhydrazone Procedure. The absorbance increased as the reagent concentration was increased. The chosen 1.5% concentration was near the saturation point. Optimum intensities were obtained with 30 minutes of heating. The intensities were approximately 5% lower with 20 or 35 minutes of heating. Higher intensities were obtained with cyclohexylamine than with triethylamine. Optimum results were obtained with 1 to 4% of the base. The blanks were lighter with the lower concentrations of base. The absorbance had to be read within 10 minutes; it faded from an initial value of 1.14 to 1.02 in 55 minutes. The change in absorbance with concentration is shown in Figure 6.

The spectrophotometric data on the determination of 1-iodobutane with this reagent are given in Table III. A representative group of other types of alkylating agents that react with this reagent is shown in Table IV.

Table II. Determination of Alkylating Agents with 4-(4-Nitrobenzyl)pyridine

Compound	Concentration molarity × 10^4	$\lambda_{max.}$	$\epsilon \times 10^{-3}$
Iodomethane	0.55	558	69
Iodoform	0.12	558	91
Iodoethane	0.68	558	64
	6.8	558	48
1-Iodopropane	0.08	558	115
2-Iodopropane	0.1	558	103
1-Iodobutane	0.01	558	120
	0.15	558	81
2-Iodobutane	0.125	558	48
2-Iodo-2-methylpropane	0.25	558	79
1-Iodo-2,2-dimethylpropane	0.0625	556	118
1-Iodohexane	0.0625	556	125
1-Iodoheptane	0.0625	556	122
1-Iodo-octane	0.0625	556	125
1-Iododecane	0.025	556	120
1-Iodododecane	0.0625	556	123
1-Iodohexadecane	0.0625	556	123
1-Iodo-octadecane	0.0625	556	123
1,1-Dibromopropane	0.19	560	2.4
1-Bromobutane	1.7	572	17
2-Bromobutane	6.8	572	5.2
1-Bromopentane	1.25	572	18
2-Bromopentane	4.0	572	7.6
1,5-Dibromopentane	0.19	566	31
3-Bromohexane	1.6	572	4.8
1-Bromoheptane	1.18	572	20
2-Bromoheptane	1.5	572	6.8
1-Bromo-octane	1.25	572	17
2-Bromo-octane	1.6	572	8.8
1-Bromononane	0.4	572	28
1-Bromodecane	1.1	572	18
1-Bromododecane	0.2	572	20
1-Bromotetradecane	0.2	572	20
1-Bromohexadecane	1.0	572	18
1-Bromo-octadecane	0.2	572	18
1-Chloro-2,2-dimethylpropane	2.1	572	0.67
1-Chloro-octane	1.6	572	1.4
2-Chloro-octane	29.3	572	0.2
3-Iodopropene	0.134	556	113
3-Bromopropene	0.41	560	9.0
3-Chloropropene	0.93	560	6.1
3-Bromopropyne	2.4	560	13
2-Bromo-1-butene	13	560	2.8
2-Bromo-2-butene	140	550	0.2
β-Bromostyrene	2.5	575	0.08
Iodocyclopentane	0.095	560	81
Iodocyclohexane	0.0625	560	21
Bromocyclopentane	0.35	572	8.4
Bromocyclohexane	21	575	0.5
Bromocycloheptane	3.1	575	3.5
Bromocyclo-octane	0.21	567	2.1
2-Bromomethyloxirane	1.3	569	21
2-Chloromethyloxirane	6.8	610	2.6
1-Phenyloxirane	0.5	572	4.0
1-Phenoxymethyloxirane	0.25	572	4.4
1,2-Bis-chloromethyloxirane	3.1	560	4.3
2-Bromoacetophenone	0.5	558	3.6
4′-Phenyl-2-bromoacetophenone	0.25	561	6.0
9-Bromofluorene	0.98	560	4.6
2-Nitro-9-bromofluorene	2.5	558	2.1
4-Nitrobenzyl bromide	0.5	554	8.6
4-Nitrobenzal bromide	2.5	568	1.4
Benzyl chloride	0.22	561	7.9
2,4-Dinitrochlorobenzene	0.25	547[a]	12
2,4-Dinitrofluorobenzene	0.25	547[a]	12
Picryl chloride	2.5	550[a]	1.3
2-Nitrophenylsulfenyl chloride	2.5	559	1.0
2,5-Dichloro-*p*-benzoquinone	2.3	560	2.4
Chloranil	0.6	560	7.0
2-Chloromethyl-5-hydroxy-γ-pyrone	0.25	542	12.8
Cyanuric chloride	0.35	490	40
Iodoacetic acid	0.05	558	112
3-Iodopropionic acid	0.25	560	58
Bromoacetic acid	0.30	570	12
2-Bromopropionic acid	0.19	570	18
3-Bromopropionic acid	1.03	572	7.6
2-Bromobutyric acid	0.28	570	18
3-Bromobutyric acid	2.0	570	3.4
2-Bromopentanoic acid	1.4	572	21
Diethylbromomalonate	1.28	560	11
Chloroacetic acid	0.30	575	10
Difluoroacetic acid	1.3	569	0.4

(*Continued on page 1484*)

Table II. (*Continued*)

Compound	Concentration molarity × 10^4	$\lambda_{max.}$	$\epsilon \times 10^{-3}$
Dibromoacetic acid	0.6	562	8
Dimethyl sulfate	0.27	568	7.8
Diethyl sulfate	0.95	570	11
Methyl *p*-toluenesulfonate	0.25	570	15
Ethyl *p*-toluenesulfonate	0.25	570	12
Ethylmethanesulfonate	0.55	570	22
Butyl trifluoroacetate	0.575	570	2.4
Butyl heptafluorobutyrate	2.5	570	2.8
Benzyl thiocyanate	0.205	560	13
Triphenyl methyl perchlorate	5.3	566	2.7
3-Methyl-2-methylthiobenzothiazolium-*p*-toluenesulfonate	2.5	562	8.4
O,O-Dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate	0.25	572	8.0
Diazinon	0.70	571	12
Malathion	0.72	565	4
Iodine	0.01	556	135

[a] Broad band.

4-Pyridinecarboxaldehyde 2-Benzothiazolehydrazone Procedure. Optimum intensities were obtained with 4% reagent. The presence of some dimethylformamide was necessary to obtain this high a concentration. A 3% concentration of reagent was chosen because the blank was lighter and the color intensity obtained with iodobutane was only slightly lower. Maximum intensities were reached at 10 to 20 minutes of heating. Slightly higher intensities were obtained with 2% base. It was necessary to add a slight amount of hydrochloric acid to the reagent; otherwise the slight basicity of the dimethylformamide hindered the reaction.

The change of the absorbance with time is shown for the determination of iodobutane and iodine in Figure 4. The change of absorbance with the concentration of iodobutane and iodine is shown in Figure 7. This figure clearly shows the autocatalytic effect. With ethyl methanesulfonate and ethyl *p*-toluenesulfonate Beer's law is obeyed (Figure 8); with 1-bromo-octadecane there is a slight deviation from Beer's law.

4-Acetylpyridine 4-Nitrophenylhydrazone. Optimum results were obtained with 1.5% reagent in acetophenone-dimethylformamide (v.:v. = 17:3). In acetophenone alone the concentration of reagent and the resultant absorbance obtained with iodobutane were low. With an increased concentration of dimethylformamide in the reagent the absorbance increased but the blank became much darker. Optimum intensities were obtained after 20 to 35 minutes of heating at 180°, with

Table III. Comparison of Various Reagents for Determination of 1-Iodobutane

Reagent	Concn. analyzed, μg.	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$	Sensitivity[a]	Detn. limit, μg.[b]	Rel. std. dev.	Color stability, min.	Proc. time, min.
4-(4-Nitrobenzyl)pyridine (*3*)	12.9	565	7.3	2.6	43		~2	50
4-(4-Nitrobenzyl)pyridine	0.74	558	120	30	0.6	4.3	1	5
4-Pyridinecarboxaldehyde 4-nitrophenylhydrazone	14.8	625	62	15.5	1.3	4.8	10	33
4-Pyridinecarboxaldehyde 2-benzothiazolylhydrazone	0.30	559	1100	275	0.05	7.5	~15	18
4-Acetylpyridine 4-nitrophenylhydrazone	1.48	642	133	33.3	0.88	8.3	50	33
4-Acetylpyridine 2-benzothiazolylhydrazone	1.48	567	160	40	0.52	15	10	18

[a] Sensitivity = $\frac{\epsilon \times 10^{-3}}{\text{dilution factor}}$.

[b] Total micrograms of 1-iodobutane in test solution giving absorbance of 0.1 in 1-cm. cell.

Table IV. Determination of Some Alkylating Agents with Reagents

Compound	4-(4-Nitrobenzyl)pyridine μg.	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$	4-Pyridinecarboxaldehyde 4-nitrophenylhydrazone μg.	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$	4-Pyridinecarboxaldehyde 2-benzothiazolylhydrazone μg.	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$
Iodoform	19	558	91	80	625	37	0.8	560	696
Iodoacetic acid	3.7	558	112	22	625	64	0.22	560	1130
1-Iodo-octadecane	9.5	556	123	39	625	62	0.39	560	1480
1-Bromo-octadecane	17.3	570	18	173	622	13	17.3	555	27
1-Chloro-octadecane	116	575	1.5	1390	625	0.17	1390	556	1.4
Ethyl methanesulfonate	27.4	570	22	20	625	27	20	555	15
Ethyl *p*-toluenesulfonate	20	573	14	4	625	33	40	556	15

Compound	4-Acetylpyridine-4-nitrophenylhydrazone			4-Acetylpyridine 2-benzothiazolylhydrazone		
Iodoform	8	640	112	0.8	565	160
Iodoacetic acid	0.22	642	137	2.2	567	146
1-Iodo-octadecane	0.39	642	190	3.9	567	161
1-Bromo-octadecane	17.3	640	33	17.3	563	21
1-Chloro-octadecane	1390	642	0.7	1390	566	0.78
Ethyl methanesulfonate	20	640	35	20	567	30
Ethyl *p*-toluenesulfonate	40	642	38	40	567	37

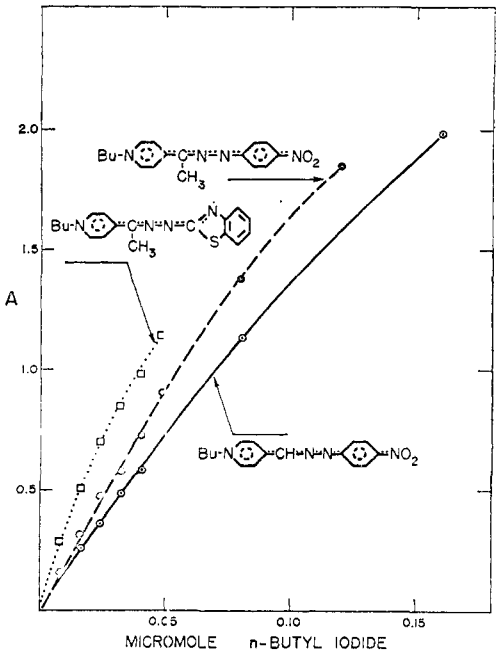


Figure 6. Absorbance at wavelength maximum vs. concentration of 1-iodobutane

—— 4-Pyridinecarboxyaldehyde 4-nitrophenylhydrazone procedure
– – – 4-Acetylpyridine 4-nitrophenylhydrazone procedure
... 4-Acetylpyridine 2-benzothiazolylhydrazone procedure

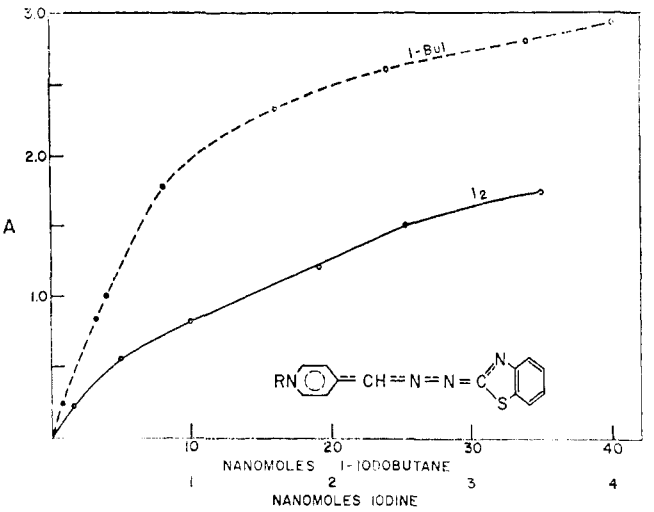


Figure 7. Absorbance at wavelength maximum vs. concentration in 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone procedure

– – – 1-Iodobutane
—— Iodine

2% cyclohexylamine. Lower concentrations gave lower intensities, and higher concentrations gave darker blanks. The color intensity at the wavelength maximum is stable for at least 50 minutes. The relation between absorbance and concentration of iodobutane is shown in Figure 6. As with the other reagents, the stronger autocatalytic effect is found at lower concentrations of iodobutane.

4-Acetylpyridine 2-Benzothiazolylhydrazone. With 10% reagent higher absorbances were obtained. A much lighter blank (and a slightly lower absorbance) was obtained with 3% reagent. Optimum intensities were obtained with 10 to 20 minutes of heating at 180°. Maximum intensities were obtained with 2% triethylamine or cyclohexylamine. The color intensity at the wavelength maximum faded from an initial value of 0.39 to 0.35 in 45 minutes. Stable readings were obtained from 5 to 15 minutes after the last addition. The change in absorbance with the concentration of 1-iodobutane is shown in Figure 6.

Detection of Acylating Agents. Filter paper impregnated with 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone has a shelf life greater than 20 days. 4-Acetylpyridine 2-benzothiazolylhydrazone could be used also as the impregnated reagent. This reagent gave identification limits fairly similar to that obtained in Table V. However, the colors were bluer. 4 - Pyridinecarboxaldehyde 4 - nitrophenylhydrazone, 4-acetylpyridine, 4-nitrophenylhydrazone, and 4-(4-nitrobenzyl)pyridine could be used as reagents also. The first two compounds gave unstable violet to purple colors; the latter reagent gave a stable yellow color with benzoyl chloride as previously reported (3). Acetone, methyl ethyl ketone, chloroform, carbon tetrachloride, tetrahydrofuran, or ether can be used instead of acetophenone as the test solvent.

The following compounds gave yellow colors in the spot test: trichloroacetyl chloride, 3,5-dinitrobenzoyl chloride, thionyl chloride, trifluoroacetic anhydride, phthalic anhydride, maleic anhydride, and succinic anhydride. 1-Iodobutane, benzoic acid, and nonanoic acid gave negative results.

Table V. Detection of Acylating Agents with 4-Pyridine Carboxaldehyde 2-Benzothiazolyl Hydrazone

Compound	Ident. limit, μg.
Acetyl chloride	0.15
Chloroacetyl chloride	0.1
Dichloroacetyl chloride	0.5
Succinyl chloride	0.2
Phenylacetyl chloride	0.1
Nonanoyl chloride	0.06
α-Furoyl chloride	0.3
Benzoyl chloride	0.25
4-Nitrobenzoyl chloride	0.5
2-Ethoxybenzoyl chloride	0.1
Anisoyl chloride[a]	0.2
Cinnamoyl chloride	0.2
1-Naphthoyl chloride	0.1
4-Phenylazobenzoyl chloride	0.2
Acetic anhydride[a]	50
Methanesulfonic anhydride[b]	1
p-Toluenesulfonyl chloride[b]	7
p-Nitrobenzenesulfonyl chloride[b]	20
Methanesulfonyl chloride[a,b]	7
Trichloromethanesulfonyl chloride[b]	15
2-Bromoacetophenone	6
Cyanogen bromide [a]	0.4
Cyanuric chloride[c]	0.05
p-Nitrobenzyl bromide[d]	2
2-Chloroacetophenone[d]	30
Diphenylphosphoryl chloride[a]	30
1-Methyl-2-iodoquinolinium methosulfate[a,e]	2

[a] Contact with triethylamine fumes gives best results for these compounds.
[b] Took 5 to 15 minutes for color to form.
[c] Blue-green color.
[d] Drop test solution onto impregnated paper, heat 5 minutes at 100° C., add drop of triethylamine, and read color.
[e] Test solvent is acetone containing 0.025% water.

COMPARISON OF METHODS

The new 4-(4-nitrobenzyl)pyridine procedure reported is based on a highly useful report describing the discovery and use of this chemical for the determination of alkylating agents (3). It was believed that the sensitivities could be improved considerably. This was confirmed by use of the modified procedure, with which the molar absorbances are approximately 10 to 150 times as great as those obtained by the literature method. This improvement in sensitivity was found to hold even

for the relatively insensitive chloroalkanes. For example, 1-chlorobutane or 1-chloropentane gave a molar absorptivity of approximately 10 by the literature procedure (*3*), while 1-chlorooctane gave 1400 by the modified procedure.

Hydrogen iodide, iodide salts soluble in acetophenone, and halogenating agents such as iodine and, to a lesser extent, bromine give false positives in all the methods. For example, iodine reacts in the 4-(4-nitrobenzyl)pyridine procedure to give a band at 556 mμ with a molar absorptivity of 135,000 (Table II).

The sensitivity of the new reagents for the determination of 1-iodobutane is reported in Table III. These reagents give molar absorptivities which are 8 to 150 times greater than those obtained by the 4-(4-nitrobenzyl)pyridine literature procedure.

The procedures for the determination of iodo alkylating agents reported in this paper are the first autocatalytic methods developed in our laboratory. These autocatalytic methods apparently constitute a new type of organic spectrophotometric procedure hitherto unreported in the literature. Their potentiality for extremely high sensitivities should prove to be most useful in trace analysis.

The most sensitive autocatalytic reagent for iodides is 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone. A molar absorptivity of 1,500,000 can be obtained at lower concentrations of iodide with this reagent.

In the 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone procedure a molar absorptivity of 6,000,000 can be obtained at lower concentrations of iodine (Figure 7). In the 4-(4-nitrobenzyl)pyridine procedure for the determination of iodine, molar absorptivities ranging from 90,000 to 135,000 can be obtained, the latter at lower concentrations of iodine. These results show clearly that iodine and iodine precursors can be determined by any of the reagents described in this paper.

Further work is to be done in the development of these and other reagents for the determination of alkylating agents. It is believed that the method for chlorides could be made 100 times more sensitive by converting the chloride to iodide. It is believed that the reagents can be applied in modified procedures to the determination of ketones, acids, alcohols, and olefins. More selective reagents are to be developed.

New methods for the detection of extremely active acylating agents have been developed which also might be eventually useful in the identification of microgram amounts of carboxylic acids separated by chromatography of the acid fraction of airborne particulates. Further work is needed in stabilizing the colors and modifying the procedures for acid analysis. Although 4-(4-nitrobenzyl)pyridine gives a stable yellow color with benzoyl chloride, this reagent would be difficult to use with the bright yellow acid fraction of airborne particulates.

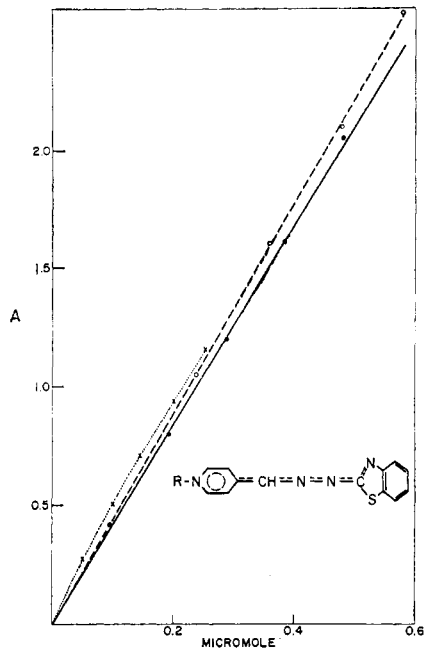


Figure 8. Absorbance at wavelength maximum vs. concentration in 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone procedure

—— Ethylmethane sulfonate
– – – Ethyl-*p*-toluenesulfonate
... 1-Bromo-octadecane

Based on color intensities obtained at 180° C. the approximate relative activity of functional groups with the reagent 4-(4-nitrobenzyl)pyridine can be summarized as follows: $I_2 > RCH_2I > R_2CHI >$ ethyl methane-sulfonate $\geq RCH_2Br \geq$ methyl *p*-toluenesulfonate $\geq$ methyl sulfate $> R_2CHBr >$ butyl trifluoroacetate $> RCHBr_2 > RCH_2Cl > R_2CHCl = CHBr_3 > CHCl_3$. Based on the rate of reaction found at room temperature with the reagent 4-pyridinecarboxaldehyde 2-benzothiazolyl hydrazone, the approximate relative activity of the more reactive functional groups can be summarized as follows: $ArCOCl = RCOCl > RSO_2Cl > ArSO_2Cl >$ 2-bromoacetophenone > 2-chloroacetophenone > benzyl chloride $> RCH_2I$.

APPLICATION

Because so many alkylating agents are toxic, it was of interest to determine whether alkylating agents could be present in various types of atmospheric particulate fractions. Tests were performed on 4 to 40 mg. of sample. With the 4-(4-nitrobenzyl)pyridine procedure, aliphatic fractions of particulate samples obtained from submarine air, auto exhaust fumes, and urban atmospheres gave a positive test for alkylating agents. The last sample was a composite from communities all over the United States.

Aromatic, acidic, and neutral oxygenated fractions obtained from urban airborne particulates gave positive results. A neutral fraction from cigarette smoke tar gave a positive test in the 4-acetylpyridine 4-nitrophenylhydrazone procedure. This procedure was used with the more highly colored samples.

A composite benzene-soluble fraction of the airborne particulates from about 200 widely spaced American communities was analyzed and found to have an average molecular formula of $C_{32.4}H_{48}O_{3.3}N_{0.16}S_{0.085}X_{0.065}(OR)_{0.12}$, where X represents chloride, bromide, and iodide reported in terms of chloride, while OR represents alkoxy groups reported in terms of the methoxy group. The average molecular weight of this material is 460. A test for alkylating agents with the 4-acetylpyridine 4-nitrophenylhydrazone procedure gave positive results.

From the wavelength maxima obtained in these various tests on atmospheric fractions, it was obvious that iodides were at the most a minor constituent.

In conjunction with gas chromatography—e.g., electron capture detector methods—and other types of separation methods, more selective spectral methods need to be developed for the determination of alkylating agents. A more thorough investigation of the composition of the air in terms of alkylating agents is necessary, since so many of these chemicals are toxic to human beings and other forms of life.

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