Determination of Carbonyl Compounds by 2-Diphenylacetyl-1,3-Indandione-1-Hydrazone

Donald J. Pietrzyk and Elsa P. Chan

University of Iowa, Department of Chemistry, Iowa City, Iowa 52240

The use of 2-diphenylacetyl-1,3-indandione-1-hydrazone as an analytical reagent for carbonyl compounds is described. The variables studied in developing the quantitative procedure are reaction times, type of catalysts, heat, solvent, and conditions for separation on thin layers. Carbonyl derivatives are highly colored and fluorescent and can be analyzed by spectrophotometry or fluorescence with the latter being more sensitive. Carbonyl compounds studied include a variety of aldehydes and ketones, several of which are steroids and biologically important compounds. Separation and analysis of mixtures of carbonyl compounds are also possible.

A WIDE VARIETY of hydrazine derivatives have been used for the identification and determination of carbonyl compounds with the most popular reagent being 2,4-dinitrophenylhydrazine. Gravimetric, spectrophotometric, amperometric, polarographic, and other analytical techniques as well as an assortment of chromatographic separation schemes have been used. Often, the most important limitations of these reagents are lack of reagent stability, long reaction times, and poor stoichiometry which is often due to steric hindrance in the carbonyl compound (*1*, *2*).

In 1958 Braun and Mosher (3) introduced 2-diphenylacetyl-1,3-indandione-1-hydrazone, **I**, as a useful reagent for characterizing carbonyl compounds. They observed that the reagent and the carbonyl derivatives were highly colored and fluorescent, reactions were rapid, and quantitative isolated yields were often obtained. These as well as some other observations suggest that **I** would be a useful analytical reagent for carbonyl compounds.

Brandt and Cheronis (*4*) used the fluorescent property in the analysis of acetone. Since the reaction is run on a micro scale, they were also concerned with the reaction rates at this concentration level (*5*). Paper chromatography was used in separation studies of several aldehyde derivatives of the reagent (*6*). More recently Mosher *et al.* (*7*) have reported reaction conditions for the detection of several other functional groups with **I**.

This paper reports our studies on the chemistry and analytical usefulness of 2-diphenylacetyl-1,3-indandione-1-hydrazone. Analytical procedures involving separations, fluorescence, and spectrophotometric measurements are described. Data for typical carbonyl compounds such as conjugated and unconjugated ketones and aldehydes and steroids are reported. The reagent appears to be especially useful, because of its application on the micro level, for the analysis and identification of carbonyl compounds in smog, polluted air, biochemical, and pharmaceutical mixtures.

EXPERIMENTAL

Reagents-Solvents. The carbonyl compounds were obtained from readily available commercial sources. Distillation or recrystallization techniques were used for further purification until boiling or melting points were found to be within the acceptable range.

All the solvents used were of ACS Reagent Grade or Reagent Grade. Chloroform and the solvents used for the synthesis of the hydrazone reagent were specially treated. The solvents were dried by treatment with Linde molecular sieves, size 4A except chloroform which was passed through a column impregnated with 2,4-dinitrophenylhydrazine–H_3PO_4–H_2O (made by grinding 0.5 g of the 2,4-DNP, 6 ml of 85% H_3PO_4, 4 ml of H_2O, and 10 g of celite), treated with $CaCl_2$ distilled, and stored in a dark bottle.

2-Diphenylacetyl-1,3-Indandione-1-Hydrazone. Sodium metal (0.24 mole) is dissolved in 100 ml of dry methyl alcohol in a 1-liter flask. When the reaction is complete, 300 ml of dry benzene are added and the mixture is concentrated to 100 ml by distillation. *o*-Dimethylphthalate (0.2 mole) is added. The temperature is raised to the boiling point at which time 1,1-diphenylacetone (0.20 mole) dissolved in 200 ml of benzene is added dropwise. At the same time the solution is concentrated by distillation to 100 ml. The yellow-brown solution is refluxed for 6 hours. The mixture is cooled, transferred to a beaker, 200 ml of a 10% NaOH solution are added, and stirred overnight. A yellow precipitate which forms is filtered, water washed, and then suspended in water. The suspension is then acidified with HCl and the product 2-diphenylacetyl-1,3-indandione is filtered, dried, and recrystallized from 95% ethanol, mp = 143–144 °C.

Conversion to the hydrazone is performed by the procedure of Braun and Mosher (*3*). Other indandiones are prepared by the same procedure using the appropriate methyl ketone.

Reagent **I** was also obtained from Aldrich Chemical Company.

Thin Layers. The thin layers were of two sources. In one case silica gel layers from Eastman, Chromatogram Sheet No. 6061, was used. In the other, silica gel layers were made in the following manner. SiliCAR TLC-7GF, a silica gel-binder mixture from Mallinckrodt, was mixed with water and the slurry smeared on a 10 × 20 or 20 × 20 cm glass plate. The thickness of the layer is controlled by tape (Mallinckrodt) applied at the edge of the glass. In both cases layers were activated in an oven for about 1 hour prior to use. Alumina layers No. 6063 used were also from Eastman.

Azine-Carbonyl Derivatives. The procedure for reacting a carbonyl compound with the hydrazone reagent is similar to that of Braun and Mosher (*3*). Modifications were necessary according to the structure of the carbonyl compound. For example, reflux times up to a few hours were sometimes required. For those carbonyl compounds which were insoluble in $CHCl_3$ a few drops of methanol were added. If the carbonyl compound has a carboxyl group, ethanol could be used as the solvent and the HCl catalyst left out of the reaction mixture.

(1) S. Siggia, "Quantitative Organic Analysis via Functional Groups," J. Wiley and Sons, Inc., New York, N. Y., Third Ed., 1963.
(2) See review issues—ANAL. CHEM., **40**, (5), (1968) and **41**, (5), (1969).
(3) R. A. Braun and W. A. Mosher, *J. Am. Chem. Soc.*, **80**, 2749, 3048 (1958).
(4) R. Brandt and N. D. Cheronis, *Microchem. J.*, **5**, 110 (1961).
(5) R. Brandt and N. D. Cheronis, *Microchem. Acta*, **3**, 467 (1963).
(6) R. Brandt, J. C. Kouines, and N. D. Cheronis, *Microchem. J.*, **6**, 519 (1962).
(7) W. A. Mosher, I. S. Bechara, and E. J. Pozomek, *Talanta*, **15**, 482 (1968).

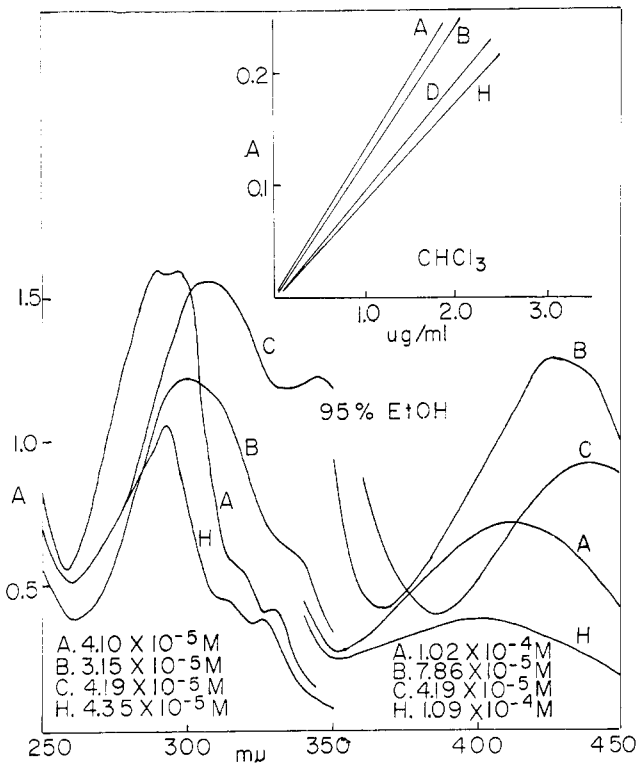


Figure 1. Absorption spectra and calibration curves (UV wavelength) for several azine-derivatives

		λ_{max}	ϵ_{max}
A.	Azine–acetone	297; 412mμ	38,800; 7,060
B.	Azine–benzaldehyde	300; 427mμ	39,000; 15,000
C.	Azine–cinnamaldehyde	308; 435mμ	37,700; 20,950
D.	Azine–acetaldehyde		
H.	Hydrazone reagent	293; 400mμ	24,200; 3,490

A general procedure for derivative preparation is to mix 0.005 mole of the carbonyl compound, 0.0045 mole of hydrazone, 20 to 30 ml of $CHCl_3$, and 2 drops of concentrated HCl. This is then heated at reflux until a deeply colored clear solution is obtained. The hot solution is filtered, cooled, and the product is precipitated by the addition of 20 ml of methyl alcohol. It is then filtered and recrystallized from MeOH–$CHCl_3$ using as little $CHCl_3$ as possible.

Since the hydrazone reagent is also useful for characterizing carbonyl compounds, melting points are included (See Table III).

For quantitative measurements, it is necessary in the above procedure to carefully weigh out the carbonyl sample and to be sure that the hydrazone is used in excess, about 10–15%. A second important modification is to add several molecular sieve particles, Linde size 4A. After the reaction is complete (filtering is not needed), the entire mixture is carefully transferred to a 50-ml volumetric flask and diluted to volume with $CHCl_3$. Aliquots of the solution are then taken either by micropipet or microsyringe.

Separation Procedure. Spots containing about 1 μg of carbonyl derivative in $CHCl_3$ are applied to the thin layer by micropipet or microsyringe (in the order of 5 or 10 μl). Care should be exercised to keep the total area of the spot small and thus portions of the sample are often applied, air dried, and then the remainder is applied. Generally, the spotting was done at 1 to 1 1/2 cm from the bottom of the layer. After allowing the sample solvent to evaporate either of two different developing chambers was used. One type is a glass developing jar with lid (Brinkmann Instruments, Inc., 25-10-20). The container was lined with filter paper with its one edge dipping in the eluting solvent at the bottom of the jar. When the filter paper is fully saturated with eluting solvent, which allows the entire jar to be more efficiently saturated, the thin layer is placed in the jar at an angle, covered, and the chromatographic process is allowed to begin. The second type of developing chamber used was the sandwich type (Brinkmann Model 04-10-17). In both, when the eluting solvent front reaches the designated end mark (at least 3/4 of the layer is used), the chromatographic process was stopped and the layers were air dried. In some cases the developing procedure was repeated several times. Since the compounds are highly colored and fluorescent, their location is determined easily either visually or by a hand UV-lamp. R_f values were computed in the usual way.

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

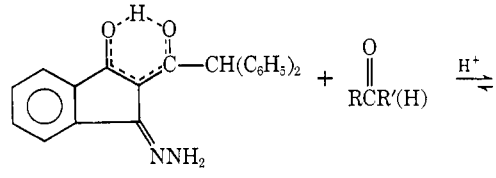
Analytical Procedures. Calibration curves of *A vs.* concentration or *F vs.* concentration were made in the usual way using the prepared carbonyl–azine derivatives as standards. For example, for a benzaldehyde calibration curve a pure sample of benzaldehyde is reacted with the hydrazone and this product after purification etc. is used as the standard for the calibration curve.

After effecting the separation as described, which includes the usual precautions to ensure quantitative results, the layer is dried and the spots are located. Each spot is then carefully scraped off with a spatula and transferred to a 25-ml volumetric flask and diluted to volume with $CHCl_3$. The solid particles of adsorbent are allowed to settle, the absorption or fluorescence is measured, and the carbonyl content is determined from the calibration curve.

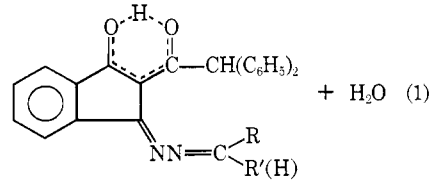
Instruments. Absorption and fluorescent spectra were obtained on the Cary 11 Spectrophotometer and Aminco-Bowman Fluorometer, respectively. For routine measurements, a Beckman Model DU Spectrophotometer and Turner Model 111 Fluorometer with filters No. 110-812 (405 mμ) and No. 110-822 (color specification 58) were used. Melting points were determined for all synthesized compounds and derivatives with a Thomas Hoover Capillary melting point apparatus. Elemental analysis was provided by the University of Iowa Chemistry Department.

RESULTS AND DISCUSSION

The reaction of a carbonyl compound with 2-diphenylacetyl-1,3-indandione-1-hydrazone, **I**, is illustrated in reaction 1. As general nomenclature compound **I** is referred to as the



I



II

hydrazone reagent and **II** as the azine-carbonyl derivative (for acetone it would be called azine-acetone). The keto–enol equilibrium in the structures in reaction 1 is readily established by UV and NMR measurements.

Structure of Reagent. If the —$CH(C_6H_5)_2$ group is replaced by —CH_2CH_3 or —$CH_2(C_6H_5)$ (this is done by using the appropriate methyl ketone in the synthesis of the reagent), the introduction of the hydrazone group is on the side chain

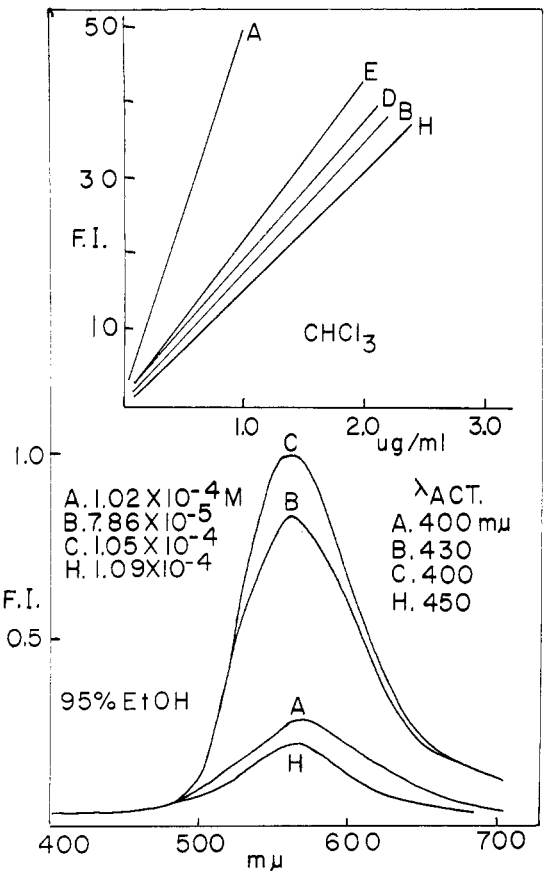


Figure 2. Fluorescent spectra and calibration curves (Turner fluorometer) for several azine derivatives

A. **Azine–acetone**
B. **Azine–benzaldehyde**
C. **Azine–cinnamaldehyde**
D. **Azine–acetaldehyde**
E. **Azine–*p*-chloroacetophenone**
H. **Hydrazone reagent**

rather than on the ring system and the fluorescence is greatly reduced (*3*). Other 2-acyl-1,3-indandiones synthesized and converted to the monohydrazones were the 2-acetyl, 2-propionyl, 2-isobutyryl, and 2-benzoyl derivatives. Fluorescence was found only for the 2-benzoyl derivative, but it was less than for **I**. Azine derivatives of the benzoyl or **I** reagent were easily formed and highly fluorescent with the increase in fluorescence being the largest for the carbonyl derivatives of **I**. Since reagent **I** was more easily prepared, commercially available, and offered more sensitivity, it was selected for further development. Preliminary experiments had shown that **I** was more readily adaptable to quantitative measurements. Other methods of introducing the hydrazone group were also studied. These experiments along with studies of the enol–keto equilibrium will be reported later.

In order to use reagent **I** two important questions had to be answered. First, what are the best experimental conditions for quantitative measurements? Second, how can the fluorescence of the excess reagent be eliminated?

Spectral Properties. The azine derivatives are highly colored and fluorescent. These properties would, therefore, be useful for the final analysis. To establish the absorption and fluorescence characteristics, three azine derivatives (acetone, benzaldehyde, and cinnamaldehyde) were studied. Absorption curves for these compounds and Beer's law plots for these and several others are shown in Figure 1. Figure 2 contains the fluorescent spectra for the three azine derivatives and calibration curves for several other derivatives.

Several conclusions can be made from these data. In general, fluorescence is more sensitive than absorption and it is possible to analyze the carbonyls at a lower concentration level (about a factor of 10). Although absorption can be used, all analyses reported were done by fluorescence because of the increased sensitivity. For the best accuracy calibration curves must be made for each azine-derivative. If the derivatives are very similar, for example azine–hexaldehyde and –heptaldehyde, the calibration curves can be used interchangeably. Third, a large excess of the hydrazone reagent will interfere in the analysis since it absorbs and fluoresces at similar wavelengths as the derivatives.

Reaction Variables. According to reaction 3, several reaction conditions can be varied. These include catalyst, heat, and reaction time. Potentially, solvent is also a variable. However, the solubility of **I** and the azine derivative is the deciding factor. Chloroform is the best solvent for both the reactants and products. In some cases small amounts of methyl alcohol are used to dissolve the carbonyl sample if it is not soluble in $CHCl_3$.

The reaction between benzaldehyde and **I** was studied using HCl, H_2SO_4, $HC_2H_3O_2$, $HClO_4$, and *p*-toluenesulfonic acid as catalysts. A 30-second heating time was used (no heating for $HClO_4$) and the fluorescence of the different solutions compared. Conversion of the derivative was favored by the mineral acids. The most convenient acid to use was HCl. Excessive amounts of HCl interfere only in that water is introduced into the mixture. For example, azine acetophenone was refluxed for 3 hours in $CHCl_3$ in the presence of HCl without change in fluorescence.

Reaction time varied and appears to depend on the structure of the carbonyl sample. Fortunately, there are two properties which can be used to indicate the progress of the reaction. First, the hydrazone, some of which exists as the hydrochloride salt is not completely soluble in $CHCl_3$. As the reaction is carried out, the precipitate dissolves. Also, and more useful, the color of the reaction mixture undergoes an easily seen color change from an initial light yellow to an orange to red-orange.

Deeper colors are observed for the carbonyl samples that have conjugation in their structure. For simple-unhindered aldehydes and ketones 10 to 20 minute heating is all that is required, while for the more conjugated systems 1 to 3 hours were used. Excessive heating in moderation does not appear to affect the stoichiometry through undesirable side-reactions.

In the early stages of the development of the quantitative procedure, conversion of a carbonyl compound to the azine varied in the range 75–90%. The formation of the azine, reaction 1, is an equilibrium reaction and under the original experimental conditions the Keq, although large, apparently is not sufficiently large enough to ensure quantitative conversion. Addition of acid catalyst will only affect the rate while change of solvent is limited by the solubility properties. The problem was attacked by considering ways of removing water from the reaction; it was anticipated that this would be a sufficient driving force toward quantitative conversion.

Several drying agents were examined by putting these in the reaction mixture. Quantitative conversion was obtained. Of all tested, molecular sieves (4A) offered the most advantages; primarily they were the easiest to use. To ensure that the sieve was not trapping or absorbing the azine derivative, azine heptaldehyde (1 μg/ml) was dissolved in 25 ml of $CHCl_3$ and the sieve added. The fluorescence of the solution remained constant up to the maximum time measured,

Table I. Thin-Layer Data for Azine-Derivatives and Hydrazone Reagent in Pure Solvents

	Azine–acetone		Hydrazone reagent	
	Comment[a]	Spot travel, cm[b]	Comment[a]	Spot travel, cm[b]
Methyl alcohol	Tailing	6.9; 7.8	Symmetrical	6.5; 7.1
Ethyl alcohol	Tailing	5.7; 7.0	Symmetrical	6.5; 7.6
Isopropyl alcohol	Tailing	5.2; 6.6	Symmetrical	5.4; 6.9
Benzene	Elongated tailing	5.5; 7.1	No movement	
Chloroform	Symmetrical	6.5; 6.8	Symmetrical	3.7; 7.1
Water	No movement		No movement	
Hexane	No movement		No movement	

[a] Shape of zone after chromatographic process.
[b] First number refers to distance from origin to the end of the tail while second number refers to distance solvent front has traveled.

Table II. R_f Values for Azine-Derivatives in Mixed Solvents

	Eluent mixture			
Derivative	$EtOA_c$:C_6H_{14} 1:3	$CHCl_3$:C_6H_{14} 1:1	Et_2O:C_6H_{14} 1:1	C_6H_6:$CHCl_3$ 1:1
Azine–acetone	0.58	0.23	0.58	0.50
Azine–acetaldehyde	0.57	0.30	0.58	0.50
Azine–benzaldehyde	0.73	0.36	0.71	0.62
Azine–acetophenone	0.71	0.33	0.71	0.57
Azine–*p*-chloroacetophenone	0.76	0.33	0.71	0.62

15 hr. In addition the same reading was obtained whether no, or anywhere from 1 to 14 4A-sieve pellets were in the flask. Similar results were obtained with $CaCl_2$. Since the sieves easily settle in the reaction mixture in contrast to $CaCl_2$, the sieves were used in the general quantitative procedure.

Separation. The second question that needed to be answered was how to remove the interference of the hydrazone since it fluoresces and absorbs in the same regions as the azine derivatives (see Figures 1 and 2). It is possible to isolate the azine derivative through precipitation (*3*). However, this procedure does not appear attractive because of the difficulty in handling such small quantities. A more desirable technique is a chromatographic separation method. The reasons for this are twofold. First, the azine derivative is separated from the excess hydrazone. Second and most important is that it might be possible to separate also the azine derivatives in those cases where carbonyl mixtures are being analyzed. This is desirable since the different azine derivatives fluoresce and absorb in a similar wavelength region. Without a separation it is possible only to determine total carbonyl content.

A rapid and attractive method of separation is thin-layer chromatography, TLC. Preliminary experiments were with silica gel and alumina thin-layers and with a wide variety of solvents and solvent mixtures. The properties sought were fixation of the hydrazone at the origin and movement of the azine derivatives in sharply defined spots. Azine–acetone, –benzaldehyde, and –heptaldehyde were the model compounds and the solvents studied were methyl–, ethyl–, and isopropyl–alcohol, benzene, chloroform, hexane, diethyl ether, ethyl acetate, and water. The preliminary experiments in light of the desired properties illustrated two important points. First, separations were better using silica gel and, second, the more useful solvents were the nonpolar solvents or mixtures of polar and nonpolar solvents.

The solvent mixture that appears to be the most useful is $CHCl_3$–hexane. However, other solvent mixtures can be used and the final decision rests on the structure of the azine derivative. Table I summarizes the observations for pure solvent while Table II lists some data for mixed solvents.

Although data are shown for only one solvent ratio, several were actually studied and from these data several conclusions can be made. For the $CHCl_3$–hexane mixtures higher $CHCl_3$ content causes the hydrazone to migrate slowly and the azine derivative to follow the solvent front. On the other hand, higher hexane ratios reduce the azines R_f values while still retaining the hydrazone at the origin.

Increasing the concentration of ethyl alcohol, benzene, or ethyl acetate when mixed with hexane has the same effect as increasing the concentration of $CHCl_3$. If methyl alcohol: H_2O (8:1), which was used as eluting agent in qualitative studies on paper (*6*), is used, tailing for the azine derivative is observed. As the water content is increased, movement of both the azine derivative and hydrazone reagent is reduced. For H_2O at 50% or higher, no movement of either is observed.

The quantitative nature of the entire procedure was verified in the following way. The preliminary chromatographic experiments had already illustrated that separations are possible. To answer the question of whether sample addition and spot removal was quantitative, the following experiments were performed. First, a standard solution of an azine derivative in $CHCl_3$ and an appropriate fluorescence calibration curve were prepared. Different aliquots were spotted on a thin layer by microsyringe and allowed to dry. The spots were carefully removed by a scrapping technique and the solid material was transferred to a volumetric flask, diluted to volume, and the fluorescence read. Quantitative spot removal was found for all cases. The only precaution is that the undissolved thin-layer material be allowed to settle before attempting any measurements. Also, since the derivative is fluorescent, the spot zone is more easily defined under UV light and thus it is easier to scrape off only the necessary portion.

The next step was to test the chromatographic procedure. In this case an azine derivative was spotted at the starting point and the chromatographic process started. When the $CHCl_3$–hexane eluting agent reached the termination point the chromatographic process was stopped and the TL was air-dried. The spot was scraped off and the analysis completed. Quantitative results for different sample sizes were found.

The final test was to start with known weights of carbonyl compounds, prepare the azine derivative, carry out the TL separation, and test for quantitative recovery based on carbonyl compound taken. If the optimum conditions of catalyst, heat, reaction time, and eluting mixture as previously established are used, quantitative results are obtained.

R_f Values. A wide variety of organic molecules of biochemical, pharmaceutical, medicinal, and synthetic use contain the carbonyl functional group. Often these are in mixtures. Any successful analytical procedure will rest on whether the chosen analytical reaction is selective for one of the carbonyl compounds in the mixture or whether a separation scheme is employed prior to analysis. If the latter is the case, the separation in part is determined by the rest of the structure of the molecule.

By examining R_f values for pure azine derivatives, the eluting conditions for separation of carbonyl mixtures can be established. Table III lists these data.

An extension of the data is possible. The magnitude of the R_f values is affected by the composition of the eluting agent and thus the values can be altered by changes in the composition of the eluting agent. Tables I and II provide information which allows prediction of the direction of R_f change upon using different eluting solvents. Second, it is an easy and common chromatographic technique to carry out development once, allow the plate to dry, and repeat the eluting procedure. This process can be repeated many times. Finally, since TLC is relatively simple and fast, some preliminary experiments based on the R_f data presented here are easily done. Therefore, although R_f data were collected for other eluting conditions, they will not be reported here.

The collecting of the R_f data revealed several important points. For those carbonyl compounds with conjugation or steric problems, reaction times were generally longer. For example, quantitative conversion was not found for benzophenone even after heating in excess of 24 hours (about 35% conversion). While for compounds containing 2 or more carbonyl groups, it was questionable in some cases as to whether all carbonyl groups were converted to the azine or not. When acetyl acetone was used, color changes in the reaction mixture occurred rapidly and from elemental analysis it was obvious that both carbonyl groups were converted to azines. On the other hand cortisone acetate and hydrocortisone acetate yielded azine derivatives in which the 3 carbonyl groups were not completely converted to azines. (R_f values of about 0 for $CHCl_3$–hexane were found for the isolated material, respectively.) For the azine carbonyl derivatives with —OH or —COOH groups, small R_f values (often at or very near the origin) were found for $CHCl_3$–hexane mixtures. Improvement in separations, increase in R_f, can be obtained by including some polar solvent in the mixture. Most successful are benzene–ethanol or $CHCl_3$–hexane–ethanol mixtures. It should be remembered, however, that incorporation of the polar solvent in the mixture also causes slight movement of the unreacted hydrazone reagent. Experiments also demonstrated that R_f values were independent of concentration at least for a modest concentration range.

Quantitative Separations. In establishing the optimum conditions, quantitative results were obtained for several individual carbonyl compounds such as benzaldehyde, heptaldehyde, chloral hydrate, and testosterone. Since the different carbonyl azine derivatives vary in their R_f values, it remained to check on whether quantitative separations of carbonyl mixtures was also possible. Several synthetic mixtures were made by mixing accurately weighed carbonyl compounds.

Table III. R_f Values for Azine–Carbonyl Compounds

Compound	Mp, °C	R_f[b]
Aliphatic aldehydes		
Formaldehyde	169–170[a]	0.50
Acetaldehyde	181–182[a]	0.45
Propionaldehyde	137–138	0.50
Butyraldehyde	164.5–165.5[a]	0.55
Valeraldehyde	154.5–156	0.57
Hexaldehyde	137–138	0.59
Heptaldehyde	132–133	0.63
2-Ethyl hexaldehyde	131–132	0.70
Substituted benzaldehydes		
Benzaldehyde	239–240[a]	0.30
p-Hydroxy-	333–335	0
p-Nitro-	297–298[a]	0.07
p-Dimethylamino-	281–282.5[a]	0.12
p-Methoxy-	227–228	0.14
p-Chloro-	256–257	0.29
2,4-Dichloro-	248–249	0.41
Substituted acetophenones		
Acetophenone	243–245[a]	0.20
p-Hydroxy-	329–330	0
p-Amino-	269–270	0
p-Methoxy-	247–248	0.10
p-Nitro-	298–299[a]	0.05
p-Chloro-	275–276	0.22
p-Bromo-	273–274	0.21
Steroids		
Testosterone	189–190	0.03
Androstan-17-one	158–160	0.48
Cholestan-3-one	218–219	0.53
Androstan-3,17-dione	231–234 dec	0.06
Estrone	193–194.5	0.06
Dehydroisoandrosterone	185–186	0.03
Androstanolone	266–267	
Biological ketones and aldehydes		
Oxalacetic Acid	248–250	0
3-Oxoglutaric Acid	220–221	0.13
2-Ketoglutaric Acid	166–166.5	0.09
Pyruvic Acid	251–252	0
Phenylpyruvic Acid	173–175	0.24
p-Hydroxyphenylpyruvic acid	200.5–202.5	0.03
Pyridoxal	255–258 dec	0.033
Miscellaneous carbonyl compounds		
Acetone	226–227[a]	0.23
9-Acridone	250–251	0.01
d-Carvone	222–224	0.71, 0.64[c]
l-Carvone	223.5–224.5	0.71, 0.64[c]
Chalcone	182–184	0.36
d-Camphor	261–262	0.03
Benzophenone	219–220	0.35
Dibenzylketone	146–147	0.39
Acetyl acetone	266–267	0.04
Ethyl acetoacetate	165–166	0.07
Chloral hydrate	206–207	0.70
Cinnamaldehyde	225–226[a]	0.45
Paraldehyde	181–182[d]	

[a] Derivatives reported before (*3*).
[b] A mixture of 1:1 $CHCl_3$:hexane was used for aliphatic aldehydes, substituted benzaldehydes and acetophenones, 9-acridone, and acetone. All the rest are for 2:1 $CHCl_3$:hexane mixtures.
[c] Derivative separates into 2 distinct spots.
[d] Produces azine–acetaldehyde derivative.

Table IV. Data for Analysis of Carbonyl Compounds

Sample	Compound	Eluting conditions	Concentration, μg Taken	Found
1	Benzaldehyde	1:1 $CHCl_3$:hexane	0.452	0.445
			0.723	0.732
			0.903	0.900
2	Heptaldehyde	1:1 $CHCl_3$:hexane	0.725	0.670
			0.966	0.960
			1.69	1.600
3[a]	Chloral hydrate	2:1 $CHCl_3$:hexane	11.39	11.12
4	Acetaldehyde	1:1 $CHCl_3$:hexane	10.80	10.86
	Benzaldehyde		7.04	7.05
5	Acetaldehyde	1:1 $CHCl_3$:hexane	0.535	0.515
	Propionaldehyde	or 2:1 $CHCl_3$:hexane	0.417	0.410
	Butyraldehyde		0.489	0.450
	Valeraldehyde		0.442	0.445
	Hexaldehyde		0.466	0.830
	Heptaldehyde		0.415	
	2-Ethyl hexaldehyde		0.372	0.360
6[b]	Acetaldehyde (a)	1:1 $CHCl_3$:hexane	1.20	1.18
	Propionaldehyde (b)	or 2:1 $CHCl_3$:hexane	1.32	1.31
	Butyraldehyde (c)		1.08	1.04
	Valeraldehyde (d)		1.08	1.15
	Hexaldehyde (e)		1.02	1.72
	Heptaldehyde (f)		0.798	
	2-Ethyl hexaldehyde (g)		0.684	0.640
7[a]	Benzaldehyde	2:1 $CHCl_3$:hexane (0.26)[c]	4.56	4.75
	p-Anisaldehyde	(0.38)	9.14	9.08
	p-Nitrobenzaldehyde	(0.62)	9.76	9.90
8[a]	*p*-Aminoacetophenone	2:1 $CHCl_3$:hexane (0.04)[c]	3.95	3.63
	p-Bromoacetophenone	(0.41)	3.98	4.03
	p-Methoxyacetophenone	(0.72)	3.84	3.88
9[d]	Testosterone	2:1 Benzene:ethanol	6.48	6.45
			12.96	12.63
10[d]	Testosterone	5:1 Benzene:ethanol	5.39	5.23
	Cholestan-3-one	2:1 $CHCl_3$:hexane	13.54	13.07
	Androstan-17-one	2:1 $CHCl_3$:hexane	8.68	7.75

[a] Average of 4 measurements.
[b] Mixture run at three other concentrations; in the range 0.8, 1.1, and 2.0 μg.
[c] R_f values for 2:1 $CHCl_3$:hexane.
[d] Average of 3 measurements.

The data in Table IV involve conversion of the carbonyl compounds to azine derivatives, their separation, removal from the TL, and final measurement by fluorescence.

The concentrations listed in Table IV are weights of azine-derivatives, calculated on the basis of the weight of carbonyl taken except for samples 4 and 5, that were applied to the TL. Actual carbonyl weights used in the reaction with the hydrazone reagent were in the 20–50 mg range. This reaction mixture was diluted to a known volume and then sampled.

For samples 4 and 5, the mixture was made from azine–aldehyde derivatives. This illustrates that quantitative results are possible for the aldehydes on the basis of separation and spot removal. Spots are easily outlined under UV light and only for hexaldehyde and heptaldehyde is it difficult to differentiate the spots. Thus, these two were analyzed as a sum. This is possible because the calibration curves for the two are identical. A multiple pass (3 or 4 times) and 2-dimensional chromatographic techniques were employed for samples 5 and 6. Observing the spots under a UV lamp is a convenient guide as to the number of passes that is needed. Quantitative separation for the aliphatic aldehydes is faster for the 2:1 CCl_3H:hexane mixture. Figure 3 illustrates a typical TL separation.

For testosterone special eluting conditions had to be used. Since its R_f value is very small, the testosterone azine derivative would be contaminated by unreacted reagent. If excessive amounts of reagent are not used, the fluorescence due to the unreacted reagent is small enough and quantitative results can be obtained. However, if the benzene–ethanol eluting agent is used, the testosterone derivative will migrate easier while the unreacted reagent stays at the origin. For separation of the 3 steroids, sample 10, elution was first done with $CHCl_3$–hexane. Azine–cholestan-3-one and azine–androstan-17-one migrated down the layer and the spots after

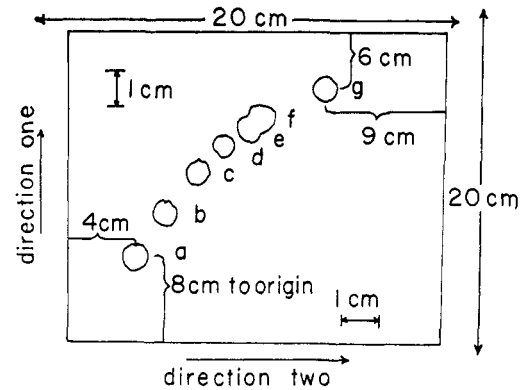


Figure 3. Thin-layer separation of an aldehyde mixture by 2-dimensional chromatography; see Sample 6 in Table IV for details

For 1:1 $CHCl_3$:hexane direction 1 was repeated 4 times while direction 2 was repeated 3 times. For 2:1 $CHCl_3$:hexane direction 1 was repeated 3 times while direction 2 was repeated 2 times.

drying were carefully scrapped off. Elution was repeated except that EtOH–benzene was used long enough to move the testosterone–azine derivative from the origin.

A series of 4 samples containing benzoic acid, phenol, aniline, and benzamide, respectively, and *p*-nitrobenzaldehyde was prepared. Quantitative conversion of the aldehyde to the azine was found and none of the compounds interfered in the analysis.

Products from the reaction of glucose and xylose with the hydrazone reagent were isolated. However, they were not identified and at this point it does not appear that the reagent is useful for the analysis of sugars.

Because of the fluorescent nature of the azine product, the reaction is also a very useful qualitative reaction. Derivatives for 60 carbonyl compounds have already been reported (*3*) while this paper reports data for an additional 40 compounds. Furthermore, the hydrazone reagent is not limited to the common organic aldehydes and ketones. In addition to the various types of carbonyl compounds studied in this report, naturally occurring keto carotenoids and carotenals have been isolated as azine-derivatives (*8*).

RECEIVED for review August 1, 1969. Accepted October 13, 1969. Presented before the 17th Detroit Anachem Conference, Detroit, Michigan, September 1969. Financial Assistance from the National Science Foundation (GP 3957) is gratefully acknowledged.

(8) H. Thommen, *Int. Z. Vitaminforsch.*, **37**, 175 (1967); *C.A.*, **67**, 105323.

Iron(III)–(II) Couple in Acetonitrile

Oxidation of Thiocyanate by Iron(III)

Byron Kratochvil and Robert Long

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

The formal reduction potential of the iron(III)–(II) couple in acetonitrile is estimated to be 1.57 ± 0.05 volts *vs.* a silver–0.01*M* silver nitrate in acetonitrile reference. Titrations of thiocyanate with iron(III) gave inflections at iron to thiocyanate ratios of 0.19 and 1.00, corresponding to formation of an iron(III)–thiocyanate complex followed by oxidation of thiocyanate to thiocyanogen. The scope of titrations with iron(III) in acetonitrile is discussed.

IN A NONAQUEOUS SOLVENT the effects of acid concentration, ionic strength, and complexation on a half-reaction may be much greater than in water, especially if ion association is promoted by a solvent of low dielectric constant. In addition, specific solvation of ions is likely, with the result that the potential difference between any two half-reactions generally varies appreciably upon going from one solvent to another. This difference in relative reduction potentials can be analytically useful, as has been shown for the copper(II)–(I) couple in acetonitrile. In this case the stabilization of copper(I) relative to copper(II) allows use of copper(II) as an analytical oxidant and as a reagent for investigating the oxidation of organic compounds (*1–5*).

Polarographic data on reactions of metal ions in acetonitrile indicate that iron(III) should also be an effective oxidant (*6*). We report here a study of the iron(III)–(II) couple in acetonitrile, along with a potentiometric and spectrophotometric investigation of the reactions of iron(III) and iron(II) with thiocyanate. A brief survey of the analytical scope of titrations with iron(III) in acetonitrile is included.

(1) B. Kratochvil, D. A. Zatko, and R. Markuszewski, ANAL. CHEM., **38**, 770 (1966).
(2) B. Kratochvil and D. A. Zatko, *ibid.*, **40**, 442 (1968).
(3) D. A. Zatko and B. Kratochvil, *ibid.*, p 2120.
(4) P. Quirk and B. Kratochvil, 157th National Meeting, American Chemical Society, Minneapolis, Minn., April 1969.
(5) H. C. Mruthyunjaya and A. R. Vasudeva Murthy, ANAL. CHEM., **41**, 186 (1969).
(6) I. M. Kolthoff and J. F. Coetzee, *J. Amer. Chem. Soc.*, **79**, 1852 (1957).

EXPERIMENTAL

Reagents and Apparatus. Acetonitrile was purified by an adaptation of the method of O'Donnell, Ayres, and Mann (*7*). About 2500-ml batches of technical-grade acetonitrile (Matheson, Coleman and Bell, Norwood, Ohio) were distilled rapidly from 30 g of potassium permanganate and 30 g of sodium carbonate. The distillate was acidified with several drops of concentrated sulfuric acid to remove ammonia formed in the previous step, vacuum distilled rapidly, and then distilled again at high reflux in a 30-plate column at 10 ml per hr. The first liter and the last 200 ml were discarded. Solvent purified in this way showed no absorption in the ultraviolet down to 200 mμ and no oxidizable impurities could be detected polarographically at 2.0 V *vs.* a silver–0.1*M* silver nitrate in acetonitrile reference. Karl Fischer titrations showed the water content to be in the range of 1 to 2 × 10^{-4}*M*. Where this amount interfered, acetonitrile prepared as outlined above was stirred with calcium hydride and filtered in a drybox immediately before use; in this way a water concentration less than 5 × 10^{-5}*M* was achieved. This level corresponds to one drop of Karl Fischer reagent per 50-ml sample. The end point was followed electrometrically with a Metrohm Model 436E automatic titrator. Dry acetonitrile avidly scavenges water from its surroundings; accordingly the water content began to increase immediately on removal of the calcium hydride, even in a closed container in a drybox. Therefore the water content of solutions prepared from very dry solvent was always considerably higher than the minimum level.

Hydrated iron(II) perchlorate was prepared from perchloric acid and electrolytic iron. Hydrated iron(III) perchlorate (nonyellow) was used as received (G. F. Smith Chemical Co., Columbus, Ohio, and Alfa Inorganics, Beverly, Mass.). Anhydrous silver perchlorate was prepared by reacting silver carbonate with a slight excess of perchloric acid, evaporating, filtering, and drying under vacuum at about 60 °C.

Anhydrous iron(II) perchlorate, $Fe(ClO_4)_2 \cdot 6CH_3CN$, was prepared by azeotropic distillation of acetonitrile solutions

(7) J. F. O'Donnell, J. T. Ayres, and C. K. Mann, ANAL. CHEM., **37**, 1161 (1965).