

Amine Derivatives for Pesticide Residue Analysis

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Amines such as those obtained by the hydrolysis of many common pesticides generally react rapidly and quantitatively with either 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene or $\alpha,\alpha,\alpha,4$ -tetrafluoro-3-nitrotoluene in alkaline solution to produce substituted anilines. These derivatives readily undergo gas chromatography and may be detected quantitatively

in amounts down to 50 picograms by the electron-capture detector. Specific examples of application to pesticide residue analysis are molinate in water, EPTC in sugar beets, and aniline derived from pyrazon or IPC. Alcohols present in samples and solvents interfere and must be removed prior to derivatization.

A rather large proportion of the pesticides now in common use contain the amine function or may be readily converted to amines. This characteristic is true whether the chemicals in question are employed as insecticides, herbicides, fungicides, or in other ways (Table I). Hydrolysis, under either acidic or basic conditions, is the most common method for conversion.

Hydrolysis followed by quantitative determination of the generated amine has been widely employed in both the formulation analysis and residue analysis of pesticides. Secondary aliphatic amines generally are determined colorimetrically by the copper-dithiocarbamate method; analysis of Bidrin residues through dimethylamine formation is an example (Murphy *et al.*, 1965). Aromatic amines may be determined by diazotization and coupling (Montgomery and Freed, 1959), by reaction with the phenol-hypochlorite reagent (Gard and Rudd, 1953), or by gas chromatography (Henkel, 1965).

The gas chromatographic behavior of simple aliphatic amines has been examined (Burchfield and Storrs, 1962; Szymanski, 1964), but neither sensitivity nor resolution generally has been adequate for residue analysis. However, several types of amine derivatives have been employed successfully. McCurdy and Reiser (1966) described the trifluoroacetyl derivatives of higher aliphatic amines; Tilden *et al.* (1968) examined 4-bromobenzoyl derivatives; and 2,4-dinitrophenyl derivatives have been reported to undergo gas chromatography (Day *et al.*, 1966).

The related dialkylaniline herbicides trifluralin and benefin also have been quantitatively determined by gas chromatography (Kerr *et al.*, 1964). The present paper describes and compares the formation and utility of nitroanilines of this type for the quantitative determination of amines as applicable to pesticide residue analysis.

EXPERIMENTAL

Chemicals and Apparatus. Solvents were reagent grade, redistilled shortly before use; other common chemicals also were reagent grade. Solvents such as acetone must be free of alcohols and deionized water must not contain dis-

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Table I. Amines from Pesticides

Class	Example	Amine
N-Methylcarbamate	Carbaryl	CH_3NH_2
Dithiocarbamate salt	SMDC	CH_3NH_2
Phosphoramidate	DMPA	$(\text{CH}_3)_2\text{CHNH}_2$
N-Phenylcarbamate	IPC	$\text{C}_6\text{H}_5\text{NH}_2$
N,N'-Dimethylcarbamate	Dimetilan	$(\text{CH}_3)_2\text{NH}$
Urea	Monuron	$(\text{CH}_3)_2\text{NH}$
Amide	Bidrin	$(\text{CH}_3)_2\text{NH}$
Dithiocarbamate ester	CDEC	$(\text{C}_2\text{H}_5)_2\text{NH}$
Thiocarbamate	EPTC	$(\text{C}_3\text{H}_7)_2\text{NH}$
		NH
Guanidine	Dodine	$\text{C}_{12}\text{H}_{25}\text{NH}-\text{C}-\text{NH}_2$
Imidazoline	Glyodin	$\text{C}_{20}\text{H}_{40}\text{N}_2$

solved amines; distillation generally provided satisfactory solvents.

Pesticide samples were analytical standards donated by the respective manufacturers. Organic reagents were commercial products of the highest available purity. 2,4-Dinitrofluorobenzene and 4-chloro-3-nitrobenzotrifluoride (Aldrich Chemical Co.); 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene and $\alpha,\alpha,\alpha,4$ -tetrafluoro-3-nitrotoluene (J. T. Baker Chemical Co.); and 1-chloro-2,4-dinitrobenzene (Eastman Organic Chemicals) were used as received. These names were designated by the individual suppliers; the accepted nomenclature is 1-fluoro-2,4-dinitrobenzene, 4-chloro- α,α,α -trifluoro-3-nitrotoluene, 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene, $\alpha,\alpha,\alpha,4$ -tetrafluoro-3-nitrotoluene, and 1-chloro-2,4-dinitrobenzene, respectively.

Gas chromatographic analysis was conducted with an Aerograph Model 600B gas chromatograph equipped with either a tritium electron-capture detector or hydrogen flame ionization detector. In most instances, a 10-foot $\times$ 1/8-inch o.d. stainless steel column was employed, packed with either 3% SE-30 silicone gum or 3% FFAP (free fatty acid phase) on HMDS-treated 60- to 80-mesh Chromosorb G. Temperatures ranged from 150° to 205° C. at gas flow rates of 30 ml. per minute.

Preparation of Standard Derivatives. Into a 100-ml. round-bottomed flask fitted with a reflux condenser were introduced about 0.2 gram of either 4-chloro-3-nitrobenzo-

trifluoride (1 millimole) or 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene (0.7 millimole) and 20 ml. of redistilled acetone. An excess of the appropriate amine (about 0.5 gram) was added followed by 20 ml. of 0.1M sodium tetraborate solution, and the mixture was boiled under reflux for one hour. About 150 ml. of water was added, the mixture was extracted with several 15-ml. portions of methylene chloride or chloroform, the organic layers were combined and dried over anhydrous sodium sulfate, and solvent was removed by evaporation. Solid derivatives were purified by recrystallization from ethanol, while liquid derivatives were purified by preparative gas chromatography. C, H, and N content agreed closely with the calculated values.

Procedure A: Molinate in Water. One liter of water was acidified with a few drops of concentrated hydrochloric acid and extracted with four 50-ml. portions of hexane, and the hexane extracts were combined.

An aliquot (20 ml.) of the hexane extract was transferred to a 40-ml. glass-stoppered centrifuge tube, 0.5 ml. of concentrated sulfuric acid was added, and the mixture was shaken. Most of the upper organic phase was removed with a pipet and discarded; the lower acid layer was heated in a hot-water bath at 85° C. for 20 minutes, cooled in ice, diluted to 5-ml. volume, and neutralized with 30% aqueous potassium hydroxide solution (phenolphthalein end-point). After addition of 2 drops more of the base, the solution was shaken with 5 ml. of benzene, and the lower aqueous layer was drawn off and discarded. The benzene extract was shaken with 1 ml. of 0.1M aqueous hydrochloric acid, the benzene layer was removed and discarded, and the acidic solution of amine hydrochloride was mixed with 2 drops (about 0.15 ml.) of a 15 mg. per ml. solution of $\alpha,\alpha,\alpha,4$ -tetrafluoro-3-nitrotoluene or 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene in acetone, 2 ml. of 0.1M sodium tetraborate solution, 0.2 ml. of 0.1M potassium hydroxide solution, and 5 ml. of acetone.

The mixture was heated in a hot-water bath at about 55° C. for one to two hours, the contents of the tube were washed into a 100-ml. volumetric flask with distilled water, and 1.0 ml. of hexane was introduced. The flask was shaken, the phases allowed to separate, and the flask was filled to volume with water. An aliquot of the upper hexane layer was withdrawn with a microsyringe for injection into the gas chromatograph. One microliter of this hexane solution was equivalent to 100 μ l. of water and contained 1.54 nanograms of mononitro derivative or 1.75 nanograms of dinitro derivative when the original water was fortified with molinate at the 0.01 p.p.m. level.

Procedure B: EPTC in Sugar Beets. A 100-gram sample of sugar beet root was cut into small pieces and homogenized in a Waring Blendor with several hundred milliliters of distilled water. More water was added to make a total volume of approximately 1 liter, and the mixture was transferred to a 4-liter flask, acidified with 25 ml. of glacial acetic acid, and distilled. Distillation was terminated after about 400 ml. of distillate had been collected; concentrated hydrochloric acid (5 drops) was added to the distillate to neutralize any free amines present, and after extraction twice with 25-ml. portions of hexane, the extracts were combined and held for analysis.

A 10-ml. aliquot of the hexane solution was treated

exactly as described in the second and third paragraphs of the procedure for molinate. One microliter of the final hexane extract represented 20 mg. of sugar beet and contained 315 picograms of mononitro derivative when the crop sample was fortified with EPTC at the 0.01 p.p.m. level.

Procedure C: Improved Procedure for Aniline Analysis. An aniline sample, such as that obtained by hydrolysis of pyrazon [5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone] or IPC (isopropyl *N*-phenylcarbamate), was concentrated in the form of its salt by acidification with concentrated hydrochloric acid, extraction of neutral and acidic impurities with ether, and evaporation of the aqueous layer under reduced pressure. The residue was taken up in about 1 ml. of water, transferred to a 100-ml. volumetric flask, and sufficient 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene (15 mg. per ml. in acetone) was added to provide at least a 10-fold molar excess over the amount required by stoichiometry. Ten milliliters of 0.1M sodium bicarbonate solution and 1 ml. of hexane were added, the mixture was shaken mechanically at room temperature for one hour, water was added to volume, and an aliquot of the upper hexane layer was withdrawn in a microsyringe for injection into the gas chromatograph. One microliter of this solution contained 3.51 nanograms of dinitro derivative per microgram of total aniline originally present.

RESULTS AND DISCUSSION

The reaction of 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) with amino acids has become well established in protein chemistry. It proceeds rapidly in the presence of an acid-receptor to provide high yields of the corresponding dinitrophenylamino acids. The reagent also reacts with the amines resulting from the hydrolysis of pesticides; these dinitrophenyl derivatives are stable, intensely colored crystalline solids exhibiting rather high melting points (Table II).

A variety of other reactive aromatic halides combine similarly with primary and secondary amines. For example, the 2,6-dinitro-4-trifluoromethyl (DNT) anilines formed by reaction of 4-chloro- α,α,α -trifluoro-3,5-dinitrotoluene (I) with amines proved to be similar in appearance and melting point to the corresponding 2,4-dinitrophenyl (DNP) compounds, while the less polar 2-nitro-4-trifluoromethyl (MNT) analogs formed from $\alpha,\alpha,\alpha,4$ -tetrafluoro-3-nitrotoluene (II) and the appropriate amines were obtained as low-melting solids or liquids (Table II). These derivatization reactions are generalized in Figure 1, although the amines could represent any of those exemplified in Table I.

Gas chromatography of these trifluoromethyl compounds provided much greater detector response, better resolution, and shorter retention times than the series of DNP homologs (Figure 2). In most instances, MNT derivatives were detected with somewhat greater sensitivity than the corresponding DNT derivatives. As in the case of the DNP derivatives, DNT and MNT derivatives of *N,N*-dialkylamines exhibited the shortest retention times. Comparison of retention times relative to the *N,N*-diethyl compounds (Table III) indicated that the higher-melting DNT derivatives generally eluted much more rapidly and in a tighter group than did their MNT analogs. In both

Table II. Nitrophenyl Derivatives: Melting Points

R—			
	M.P., °C	M.P., °C	M.P., °C
CH ₃ NH—	176	86	78.5
(CH ₃) ₂ N—	87	126	Liquid
C ₂ H ₅ NH—	113	72	59.5
(C ₂ H ₅) ₂ N—	69 (80)	95	Liquid
C ₃ H ₇ NH—	95	124	52
(C ₃ H ₇) ₂ N—	41	49	Liquid
C ₂ H ₅ N— 	82	87	Liquid
 —	103	95	Liquid
 —NH—	156	119.5	84

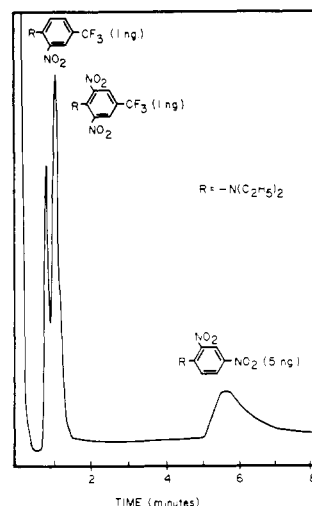


Figure 2. Gas chromatogram of nitrophenyl derivatives

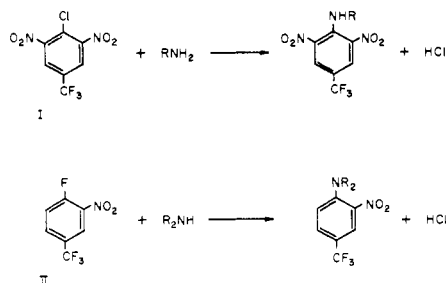


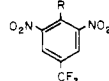
Figure 1. Reaction of DNT and MNT reagents with amines

series, a distinct separation appeared between the derivatives of amines bearing six aliphatic carbons or less and those with aromatic rings or larger numbers of aliphatic carbons (Figure 3).

Primary and secondary amines with up to six carbons provided derivatives which chromatographed well at 170° to 180° C. on a variety of columns. The preferred column packings were either 3% SE-30 or 3% FFAP on silanized Chromosorb G, but other silicone columns also provided good separation and resolution. At these temperatures, the electron-capture detector permitted measurement of about 50 picograms of the more volatile derivatives; the less volatile group required a temperature of about 200° C. for sharp resolution, and quantitative measurements could not be assured with samples of less than about 200 picograms of derivatives.

Application of DNT and MNT derivatives to herbicide residue analysis may be exemplified by the determination of molinate (Ordram, *S*-ethyl hexahydro-1*H*-azepine-1-carbothioate) (III) in water (Figure 4). One-liter samples were acidified to fix any amines already present, extracted with hexane, and an aliquot of the organic layer was re-extracted into concentrated sulfuric acid. The acid solution was heated for 20 minutes at 85° C. to effect hydrolysis, neutralized, and the amine (IV) was extracted into benzene and subsequently isolated by re-extraction with dilute hydrochloric acid. This acid solution was treated with an

Table III. Nitrophenyl Derivatives: Relative Retention Times^a

R—		
(C ₂ H ₅) ₂ N—	1.00	1.00
(CH ₃) ₂ N—	0.95	1.20
(C ₃ H ₇) ₂ N—	1.46	1.46
C ₂ H ₅ NH—	1.82	1.54
CH ₃ NH—	2.57	1.80
C ₃ H ₇ NH—	1.75	2.04
C ₂ H ₅ N— 	4.08	3.52
 —	3.58	5.17
 —NH—	4.70	7.42

^a Ten-foot column, 4% SE-30 on 60- to 80-mesh Chromosorb G, nitrogen flow 30 ml. per minute, 200° C.

acetone solution of the appropriate derivatizing agent, borate buffer, and acetone; the reaction was permitted to proceed at 50° to 55° C. for one hour, the derivative was extracted into hexane, and aliquots of the organic layer were analyzed by gas chromatography. In a typical determination, 1 μl. of the hexane solution represented 100 mg. of the water sample and contained 1.54 nanograms of 1-[2'-nitro-4'-(α,α,α-trifluoromethyl)phenyl]hexamethylenimine (the MNT derivative from 1.0 nanogram of molinate) or 1.75 nanograms of the DNT derivative (V), 1-[2',6'-dinitro-4'-(α,α,α-trifluoromethyl)phenyl]hexamethylenimine, when fortified at the 0.01 p.p.m. level; recoveries ranged from 95 to 105%.

Figure 5 represents a typical gas chromatogram of the MNT derivative from 0.44 nanogram of molinate. Although several minor peaks were observed, they did not interfere with the analysis. However, when the DNT derivative was employed (Figure 6), many extraneous peaks appeared. Although analysis still was possible because

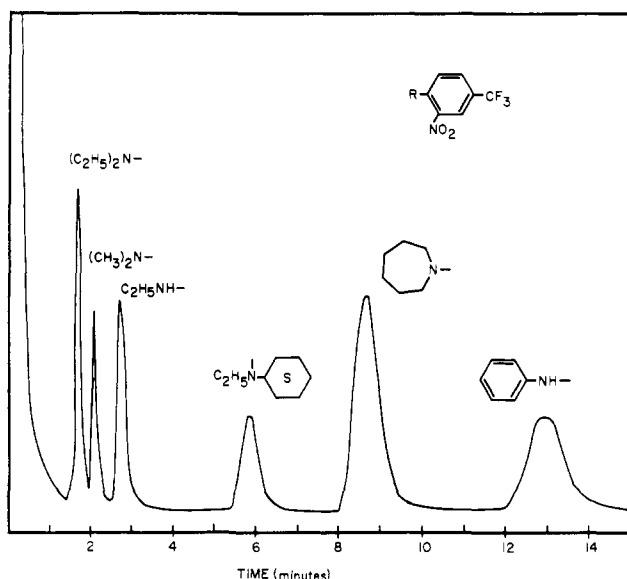


Figure 3. Gas chromatogram of a mixture of MNT derivatives

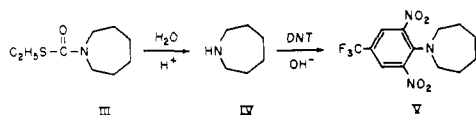


Figure 4. Formation of a DNT derivative from molinate

of the relatively long retention time of this hexamethylenimine derivative, the determination of other amines might be seriously affected.

Extraneous peaks could be ascribed to unreacted reagent and its phenolic hydrolysis product, but these were determined to fall so close to the solvent peak as to cause no interference. Eventually it was found that, like dinitrofluorobenzene (Day *et al.*, 1966; Whalley, 1950), the reagents react rapidly with alcohols, the amine under analysis serving to catalyze the reaction. Careful redistillation of the reagent grade acetone employed in the procedure almost completely removed the undesired peaks (Figure 7). An analytical determination of EPTC (Eptam, *S*-ethyl *N,N*-dipropylthiocarbamate) (VI) in sugar beet roots was conducted by the same general procedure employed for molinate in water with very satisfactory results (Figures 7 and 8); in a typical measurement, 1 μ l. of the final hexane solution represented 20 milligrams of sugar beet and provided 315 picograms of α,α,α -trifluoro-2-nitro-*N,N*-dipropyl-*p*-toluidine (VII) when fortified at the 0.01 p.p.m. level with EPTC. Recoveries ranged from 70 to 95% depending on the period allowed for reaction with the reagent. Any satisfactory existing method for the generation or isolation of amines could be employed; for instance, the volatile amine often could be generated by alkaline hydrolysis and distilled or swept with nitrogen into a trap containing dilute acid, as in the case of aniline formed by hydrolysis of IPC (VIII) (Figure 9).

The various amines reacted with the reagents at different rates (Table IV), although essentially identical results were obtained in conversions to either MNT or DNT deriva-

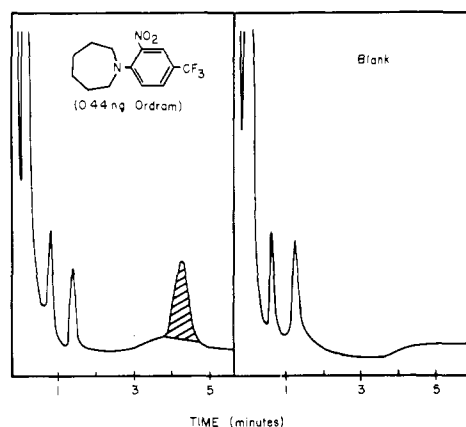


Figure 5. Gas chromatogram of the MNT derivative from molinate (Ordram) in water

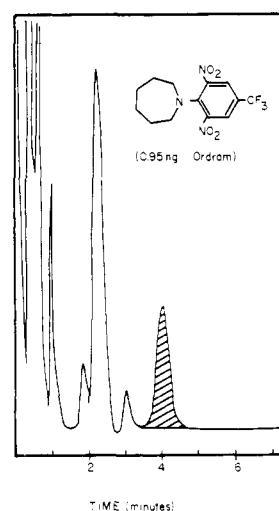


Figure 6. Gas chromatogram of the DNT derivative from molinate (Ordram) in water

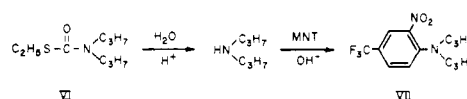


Figure 7. Formation of an MNT derivative from EPTC

tives. The presence of catalytic amounts of a strong organic base (triethylamine) generally did not increase the rate appreciably. The conversion of aniline to 2,6-dinitro-4-(α,α,α -trifluoromethyl)diphenylamine became quantitative when derivatization by the DNT reagent in dilute bicarbonate or borate solutions was conducted at room temperature rather than at 55°, but the MNT reagent produced less than 5% conversion at the lower temperature. Ethyl cyclohexylamine did not produce satisfactory results under any of a wide variety of conditions.

The DNT and MNT derivatives offer many advantages over existing methods for the quantitative determination of amines. The high degree of sensitivity of the electron-capture detector to these compounds permits analysis of

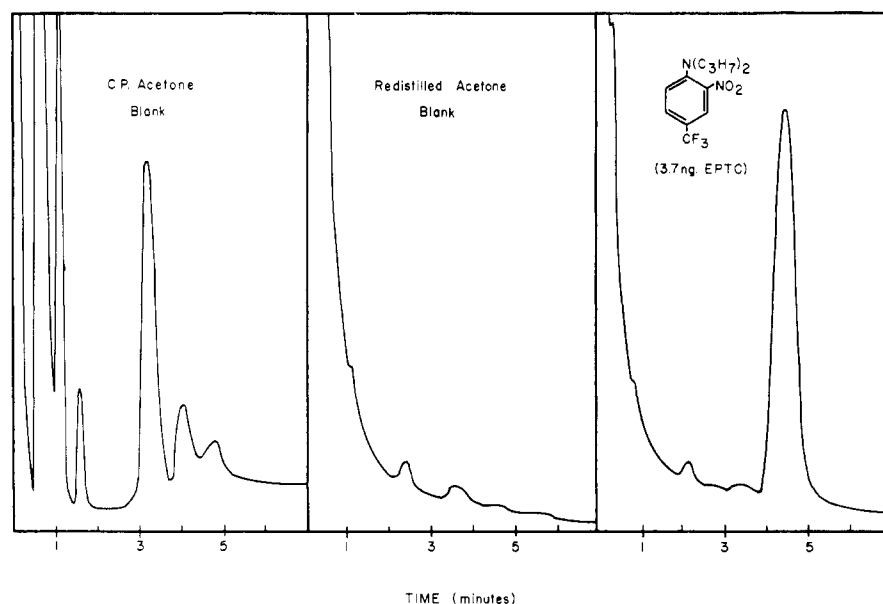


Figure 8. Gas chromatogram of the MNT derivative from EPTC (Eptam) in sugar beets

very small quantities of amines or, in residue analysis, a more convenient sample size. These derivatives also provide an increased amount of compound for detection; 31 nanograms of methylamine is transformed into 265 nanograms of DNT derivative. Almost all present analytical methods for aliphatic amines are nonspecific; the use of derivatives permits sufficient specificity for the analysis of mixtures and also provides a certain degree of identification of the constituents. Volatile aliphatic amines are fixed in a low volatility, highly stable form. The derivatives are insoluble in water but soluble in all types of organic solvents. Behavior on gas chromatog-

raphy is greatly improved over that of the parent compounds themselves and many other derivatives.

The highly colored DNT and MNT derivatives are readily adaptable to paper and thin-layer chromatography—as little as 100 nanograms may be detected visually on a TLC plate. Their high extinction coefficients permit adaptation to colorimetric analysis, while the ease of gas chromatography and large increase in nitrogen content provide for sensitive analysis with element-specific detectors.

In addition to their facile reaction with primary and secondary amines, under appropriate conditions DNT and MNT derivatives may be obtained from alcohols, thiols, phenols, dithiocarbamic acids and their salts, and certain inorganic ions such as cyanide. Further application of these reactions to organic microanalysis will be reported at a later date.

Table IV. Amine Conversion to MNT Derivatives

Amine	Per Cent Conversion ^a			
	0.1M bicarbonate	0.1M borate buffer	Bicarbonate/triethylamine ^b	Borate/triethylamine ^b
Methyl	78	71	71	83
Dimethyl	100	100	100	100
Ethyl	89	100	77	100
Diethyl	100	100	100	100
<i>n</i> -Propyl	100	100	100	100
Di- <i>n</i> -propyl	100	100	100	100
Phenyl	12	25	18	16
Cyclohexyl	92	79	84	112
<i>N</i> -Ethyl cyclohexyl	36	50	25	32
Hexamethylenimine	100	100	100	100

^a One hour, 55° C., 10 μ moles of amine salt, 100 μ moles of reagent, 3 ml. of buffer, 5 ml. of acetone.

^b Triethylamine, 5 mg. in 50 μ l. of acetone.

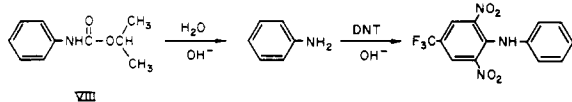


Figure 9. Formation of a DNT derivative from IPC

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