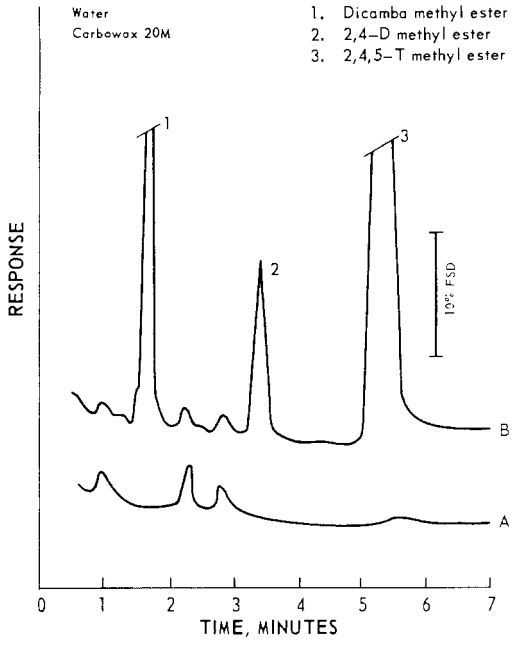


Figure 7. Gas chromatograms of acid–ether extracts of water and water fortified with dicamba, 2,4-D, and 2,4,5-T, after methylation without cleanup, as separated on Carbowax 20M at 231°: (A) blank water; (B) fortified water representing a recovery of 56% of dicamba at 0.05 ppm, 94% of 2,4-D at 0.05 ppm, and 103% of 2,4,5-T at 0.05 ppm.

Water Samples. The analysis of herbicides as methyl esters in tap water was performed on acid–ether extracts without cleanup. Water fortified at 0.05 ppm with the herbicides showed a recovery of 56% for dicamba, 94% for 2,4-D, and 103% for 2,4,5-T with a Carbowax 20M column at 231° (Figure 7B). No interferences were observed with the control water sample (Figure 7A). Similar results were obtained with an OV-17/QF-1 column. The results on the recovery of these herbicides as obtained with two columns (OV-17/QF-1 and Carbowax 20M) are summarized in Table V, from which it could be surmised that the water samples could be analyzed satisfactorily with the described method. No interferences were experienced with the Carbowax 20M column for all three herbicides. However, on the OV-17/QF-1 column, 2,4-D showed a maximum interference of 20 ppb while 2,4,5-T and dicamba were free from interference.

Table V. Per Cent Recovery of Herbicides from Fortified Tap Water

	Dicamba		2,4-D		2,4,5-T	
Gc column	0.05 ppm	0.20 ppm	0.05 ppm	0.20 ppm	0.05 ppm	0.20 ppm
OV-17/QF-1[a]	66	69	48	75	76	75
Carbowax 20M[b]	56	78	94	82	103	102

[a] 216°. [b] 231°.

In conclusion, the acid–ether method involving diethyl ether extraction at low pH followed by partition, *n*-butylation or methylation, Florisil cleanup, and ^{63}Ni gc with an OV-17/QF-1 or Carbowax 20M column has been found to give a sensitivity of 0.05 ppm or better for the simultaneous analysis of 2,4-D, 2,4,5-T, and dicamba residues in soil. The *n*-butylation provided better recovery of dicamba than that obtained by methylation. The procedure developed is also applicable without cleanup to water samples containing similar levels of these herbicides.

ACKNOWLEDGMENT

The author thanks W. R. McDowell for technical assistance.

LITERATURE CITED

McKone, C. E., Hance, R. J., *J. Chromatogr.* **69,** 204 (1972).
Woodham, D. W., Mitchell, W. G., Loftis, C. D., Collier, C. W., *J. Agr. Food Chem.* **19,** 186 (1971).
Woolson, E. A., Harris, C. I., *Weeds* **15,** 168 (1967).
Woolson, E. A., Reichel, W. L., Young, A. L., Ensor, P. D. J., presented at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, Division of Pesticide Chemistry, Paper No. 91.

Received for review February 28, 1973. Accepted February 20, 1974. Presented at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 10–14, 1972. Contribution No. 748 from the Chemistry and Biology Research Institute, Canada Department of Agriculture.

Chromatographic Parameters of the Bisquaternary Herbicides, Paraquat and Diquat

Charles W. Sharp* and Emile M. Lores, Jr.

Analysis of bisquaternary amines by thin layer chromatography represents a special problem. The diacetate, dichloride, and (mixed) acetate-chloride salts are resolved following development with a solvent composed of *n*-butyl alcohol, acetic acid, and water. Formation of the acetate salts could be suppressed by the addition of chloride ions to the system. However, from among several systems examined, solvents containing benzene–*n*-pentyl alcohol–methanol–1 *N* HCl, either 1:1:2:1 or 1.3:4:8:8, in combination with 300 MN cellulose thin layers are particularly suitable for the chromatography of paraquat, diquat, and perhaps other bisquaternary amines. This permitted the resolution of paraquat from diquat, as well as from impurities present in a technical grade of paraquat.

Deaths resulting from accidental ingestion of paraquat (1,1′-dimethyl-4,4′-bipyridinium) and the prevalent use of these bisquaternary amine herbicides, including diquat (1,1′-ethylene-2,2′-dipyridinium) and morfamquat [1,1′-bis(3,5-dimethylmorpholinocarbonylmethyl)-4,4′-bipyridinium], have stimulated an interest as to their possible environmental impact (Calderbank, 1968; Sharp *et al.*, 1972; "Touching up the Paraquat Picture," 1972; Kimbrough, 1973). Efforts to evaluate the effects of residues and any potential metabolite of these compounds require,

Pathologic Physiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.

in part, a suitable separation and detection system. Chromatographic systems such as those used for choline and other quaternary amines were inadequate (Whittaker, 1963; Bayzer, 1964; Taylor, 1964). Modifications of these systems resulted in the formation of multiple spots not unlike that previously observed in the chromatography of monoquaternary compounds in the presence of two different anions (Crocker, 1959; Way *et al.*, 1966; Mazzei and Lederer, 1968).

This report indicates several conditions to be fulfilled in designing a satisfactory system for the chromatographic analysis of paraquat, diquat, or other bisquaternary amines.

EXPERIMENTAL SECTION

Materials. Paraquat dichloride (>99%), a gray, granular form of paraquat dichloride (96%) and designated grade b, diquat dichloride, and morfamquat dichloride were from Imperial Chemical Industries (ICI). A commercial preparation of paraquat dichloride was obtained from Chevron Chemical Co. and [^{14}C]paraquat dichloride came from Amersham-Searle. 4,4′-Dipyridyl dichloride came from Aldrich Chemical Co.

Paraquat diacetate was prepared for use as a chromatographic standard by passing paraquat dichloride (>99%) through the acetate form of Dowex AG-1-X8 resin, 100–200 mesh.

Chromatography. Tlc plates of silica gel G and 300 MN cellulose of 250-μ thickness on glass were obtained from Analtech, Inc. In some cases, cellulose plates were loaded with NaCl prior to use. The plates were developed with the NaCl solution indicated, immersed in the same solution, and then dried at 110° for 1 hr. Prior to use, both the silica and cellulose plates were heated at 110° for 20 min. Whatman grade 20 paper was also used. To load the paper with NaCl, 20 × 20 sheets were dipped in 1.5 *M* NaCl and dried at room temperature.

Solvents were prepared within 1 hr of use. Ascending chromatography of 15 cm required 1 hr for the cellulose or silica plates and 5 hr for paper. Spots were visualized by uv, by exposure to iodine vapor, or by spraying with Dragendorff's reagent (Stahl, 1969).

Radioactive Analysis. Radioactivity on tlc plates was assayed following the scraping of successive 6-mm zones into vials with a device obtained from Analabs Co. (Snyder and Kimble, 1965). Three- or six-millimeter sections of paper chromatograms were cut and placed in the counting vials. Then 20 ml of the scintillation mixture (4 g of 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene, 80 g of naphthalene, 600 ml of toluene, and 400 ml of ethylene glycol monomethyl ether) was added to each vial and radioactivity was counted in a Beckman LS 250 scintillation spectrometer.

Spectral Analyses. Thin films of the amine salts in methanol were coated on NaCl plates, and the infrared spectra were obtained with the Perkin-Elmer Model 621 spectrometer. Ultraviolet and visible spectra of the bisquaternary compounds in 0.05 *M* potassium phosphate (pH 9) were obtained following dithionite reduction (Sharp *et al.*, 1972). The formation of a complex, measured at 570 mμ, between a fresh 1:50 dilution of Dragendorff's reagent and the amine salts was used to quantitate the components isolated by chromatography.

RESULTS AND DISCUSSION

Chromatography of Bisquaternary Compounds in the Presence of Acetic Acid. Several spots, a–d, appear on chromatograms of paraquat dichloride developed with *n*-butyl alcohol, acetic acid, and water (Figure 1, lines 1 and 2). Spot d accounted for <0.5% of the Dragendorff-positive material and was not observed on chromatograms of grade b or ^{14}C-labeled paraquat. Similar to the results of Funderburk *et al.* (1966) and Slade and Smith (1967) we did not observe spots a, b, or c (R_f ≤0.2) utilizing a solvent containing <15% acetic acid. However, increasing the concentration of acetic acid increased the R_f value of spot c to 0.8, while the R_f of spot a did not exceed 0.25. The solvent composition utilized in Figure 1 permitted optimal resolution of these components.

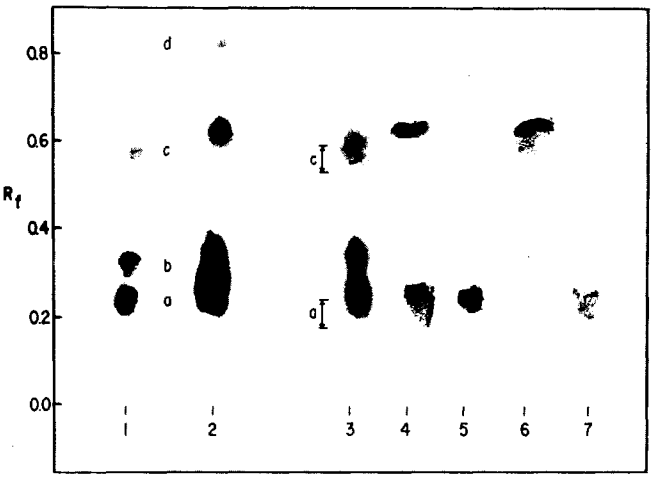


Figure 1. Chromatographic behavior of paraquat on untreated paper, developed with *n*-butyl alcohol–acetic acid–water (12:8:5, v/v/v). Chromatograms 1 and 2 were obtained from 10 and 50 μg of paraquat dichloride, respectively. Areas a and c were cut from unsprayed replicates of chromatogram 3, following application of 20 μg of paraquat dichloride. These were extracted either with 0.1 *M* acetic acid or 0.05 *M* HCl, centrifuged, the supernatant transferred, and the solvents removed by lyophilization. These residues were redissolved in 40 μl of methanol for rechromatography. Chromatograms 4 and 5 are, respectively, the acetic acid and HCl extracts of area a; chromatograms 6 and 7 are, respectively, the acetic acid and HCl extracts of area c. The spots were visualized with Dragendorff's reagent.

The identity of these spots is revealed following rechromatography of extracted spots a and c (Figure 1, lines 3–7). These results suggest that spot a represents paraquat dichloride while spot c represents paraquat diacetate. The similarity of the R_f values and the infrared spectra of spot c to those of the diacetate salt (see Experimental Section) confirm this conclusion. Spot b is presumed to represent the mixed acetate–chloride salt of paraquat.

The amount of salt in spot c varied inversely with the amount of the bisquaternary salt applied (Figure 2A). The amount chromatographed ranged in amounts from 150 to 1 μg, and the quantity of ^{14}C in spot c ranged from as little as 1% to as much as 30% with the remainder in spots a + b. The quantity of ^{14}C in each spot following extraction was quantitatively confirmed as paraquat by dithionite reduction (see Experimental Section).

Chromatography of bisquaternary halides in the presence of acetic (or formic) acid can be improved by the addition of chloride ion to the developing system. In two examples (Figure 2B), spot a contained 96% of the [^{14}C]paraquat. Also, chromatography may be carried out on cellulose thin layers impregnated with 0.5 *M* NaCl and developed with *n*-butyl alcohol–acetic acid–water (3:3:1, v/v/v). Here, a single spot at R_f 0.4 contained 98% of the radioactivity of 1 μg of [^{14}C]paraquat. As on paper, several spots appeared when NaCl was not added, and also excessive streaking occurred.

In all these systems, diquat dichloride behaved identically with paraquat, except that no spot d was observed.

Other Chromatographic Systems. The most satisfactory chromatographic conditions for the bisquaternary compounds were obtained with some of the A systems (Table I) on paper or cellulose thin layers. On these chromatograms, a single Dragendorff sensitive spot was ob-

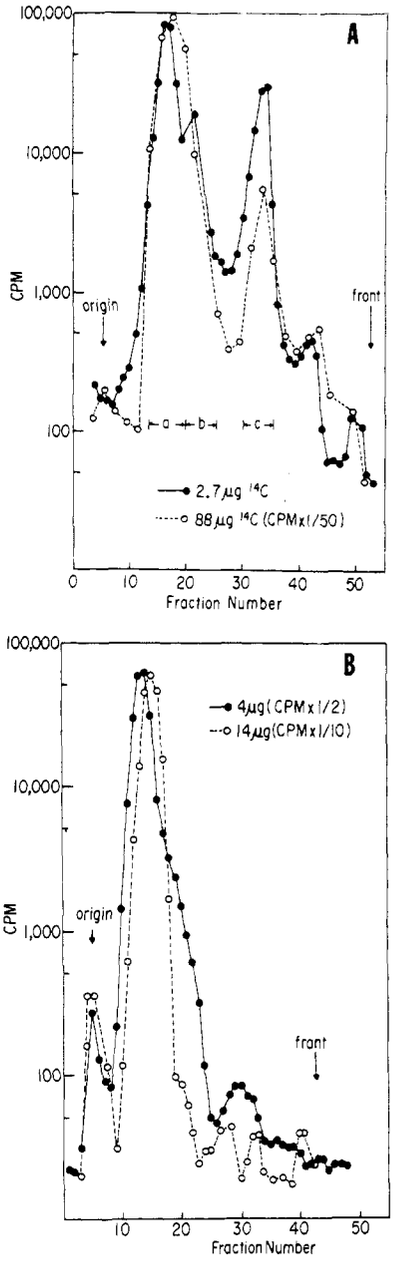


Figure 2. Distribution of radioactivity in chromatograms of [^{14}C]paraquat dichloride: (A) on untreated paper following development with *n*-butyl alcohol–acetic acid–water (12:8:5, v/v/v); (B) (---○) on paper impregnated with 1.5 *M* NaCl and the same solvent as in A; (—●) on untreated paper developed with *n*-butyl alcohol–acetic acid–0.03 *M* HCl (12:8:5, v/v/v).

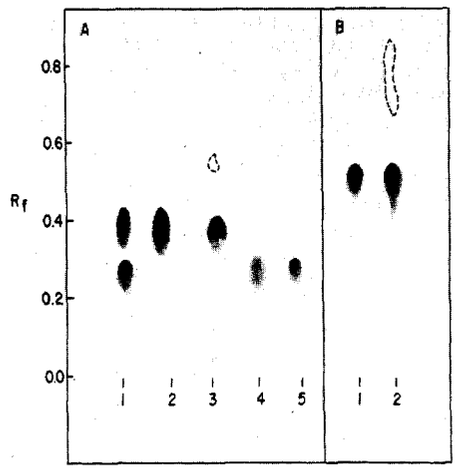


Figure 3. Chromatography of paraquat, diquat, and 4,4′-dipyridyl on cellulose thin layers. (A) Solvent was benzene–*n*-pentyl alcohol–methanol–1 *M* HCl (1:1:2:1, v/v/v/v): (1) 50 μg of paraquat dichloride plus 35 μg of diquat dichloride; (2) 50 μg of paraquat dichloride; (3) 35 μg of grade b paraquat dichloride; (4) 35 μg of 4,4′-dipyridyl dichloride; and (5) 35 μg of diquat dichloride. (B) Solvent was *n*-butyl alcohol–acetic acid–water (3:3:1, v/v/v) and the thin layers were impregnated with 0.15 *M* NaCl: (1) 10 μg of paraquat dichloride; (2) 10 μg of grade b paraquat dichloride. Shaded areas are compounds that reacted with Dragendorff's reagent and areas encircled by dashed lines are compounds that fluoresced under uv light.

tained which contained >96% of the radioactivity of 1 μg of [^{14}C]paraquat dichloride. The R_f of this spot varied from 0.2 to 0.7 depending on the ratio of the solvent mixture (group A, Table I). Although resolution on paper is comparable to that on cellulose thin layers, the latter is superior because (1) the development time is shorter and (2) paraquat is resolved from diquat (Figure 3A). Chromatographic systems, Table I, group A, no. 2, 3, and 4, also resolve a bulky bisquaternary compound morfamquat, with an $R_f \geq 0.8$, from either paraquat or diquat.

Paraquat or diquat was eluted as uniform spots on paper with at least three other chromatographic systems (systems B, C, and D, Table I). A satisfactory R_f value of 0.5 was obtained with system B. However, a second front midway in the chromatogram, containing material sensitive to Dragendorff's reagent, and the presence of a second anion diminish the applicability of system B.

When silica gel thin layers were used, either (1) low R_f values were obtained when many of the above solvent systems were used or (2) streaking occurred. The latter was obtained in systems with a high concentration of methanol (*e.g.*, system E, Table I) or with another system previously reported by Slade (1966) (system F in Table I).

Solvents containing a high concentration of a strong

Table I. R_f Values of Paraquat, Diquat, and 4,4′-Dipyridyl in Several Different Chromatographic Systems

Group	Solvent system (vol ratios)	Thin layer (tlc) or paper chromatography	R_f of compound		
			Paraquat	Diquat	4,4′-Dipyridyl
A-1	Benzene–*n*-pentyl alcohol–methanol–1 *N* HCl (14:7:14:5)	Cellulose (tlc)	0.19	0.11	0.11
A-2	Benzene–*n*-pentyl alcohol–methanol–1 *N* HCl (1:1:2:1)	Cellulose (tlc)	0.37	0.27	0.27
A-3	Benzene–*n*-pentyl alcohol–methanol–1 *N* HCl (1.3:4:8:8)	Cellulose (tlc)	0.67	0.57	0.56
A-4	Benzene–*n*-pentyl alcohol–methanol–1 *N* HCl (1.3:4:8:8)	Paper	0.55	0.49	0.45
A-5	Benzene–*n*-pentyl alcohol–methanol–1 *N* HCl (14:7:14:5)	Paper	0.16	0.11	0.10
B	*n*-Butyl alcohol–ethanol–5 *M* ammonium acetate (4:3:3)	Paper	0.5	0.49	1.0
C	2-Propanol–ethanol–1 *N* HCl (3:3:2)[a]	Paper	0.19	0.14	
D	*n*-Butyl alcohol–ethanol–56% phenol (in water) (2:1:2)	Paper	0.2	0.13	
E	*n*-Butyl alcohol–methanol–6 *N* HCl (3:3:2)[b]	Silica gel (tlc)	0.20	0.20	
F	5 *M* NH_4Cl[b]	Silica gel (tlc)	0.15	0.23	0.09
G	*n*-Butyl alcohol–5 *N* HCl (2:23)	Silica gel (tlc)	0.50	0.57	
H	*n*-Pentyl alcohol–2 *N* HCl (3:97)	Silica gel (tlc)	0.45	0.50	

[a] Used to test radiochemical purity of paraquat by Amersham-Searle. [b] Pronounced streaking occurred with these systems.

acid are more suitable for silica gel chromatography of bisquaternary compounds. For example, these compounds remain at the origin following development with a solvent of 5 *N* HCl–*n*-butyl alcohol (1:8). However, as previously observed by Pate and Funderburk (1965), a suitable R_f was obtained with a solvent composed of the acid saturated with an alcohol (system G or H, Table I). Using these latter systems, only one spot is observed with either grade b or 99% paraquat. The apparent limitation of silica gel thin layers for the chromatography of bisquaternary salts is probably due to a strong interaction of these compounds with anionic sites on the supporting media.

To detect any impurities present with paraquat, grade b paraquat and 4,4′-dipyridyl were used. At least two components in grade b paraquat were resolved from paraquat (Figure 3). One spot at the origin absorbed uv light and was Dragendorff positive; another, with an R_f greater than paraquat diacetate, fluoresced under uv light and was Dragendorff negative. In addition, two minor Dragendorff positive components with low R_f values are present in the material obtained from Chevron (not shown) and used agriculturally. These impurities are detectable in all chromatographic systems of group A. However, none of these impurities were detected with system G or H of Table I.

4,4′-Dipyridyl, a precursor in the synthesis of paraquat (but not diquat), was resolved from paraquat using several chromatographic systems (*e.g.*, group A, Table I). The amount of this precursor present in paraquat (99%) was determined to be <0.5%.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the suggestions and discussion contributed by Drs. Phillip Albro, Herbert S. Posner, and William E. Wilson, and the gifts of reagent grades of diquat, morfamquat, and paraquat from Imperial Chemical Industries.

LITERATURE CITED

Bayzer, H., *Experientia* **20,** 233 (1964).
Calderbank, A., *Advan. Pest Contr. Res.* **8,** 127 (1968).
Crocker, C., *J. Chromatogr.* **2,** 115 (1959).
Funderburk, H. H., Jr., Negi, N. S., Lawrence, J. M., *Weeds* **14,** 240 (1966).
Kimbrough, R. D., *J. Amer. Med. Ass.* **223,** 692 (1973).
Mazzei, M., Lederer, M., *J. Chromatogr.* **37,** 292 (1968).
Pate, D. A., Funderburk, H. H., Jr., *Proc. S. Weed Conf.* **18,** 612 (1965).
Sharp, C. W., Ottolenghi, A., Posner, H. S., *Toxicol. Appl. Pharmacol.* **22,** 241 (1972).
Slade, P., *Weed Res.* **6,** 158 (1966).
Slade, P., Smith, A. E., *Nature (London)* **213,** 919 (1967).
Snyder, F., Kimble, H., *Anal. Biochem.* **11,** 510 (1965).
Stahl, E., "Thin Layer Chromatography," 2nd ed, Springer-Verlag New York, New York, N. Y., 1969.
Taylor, E. H., *Lloydia* **27,** 96 (1964).
"Touching up the Paraquat Picture," *Food Cosmet. Toxicol.* **10,** 700 (1972).
Way, J. L., Brady, W. T., Tong, H. S., *J. Chromatogr.* **21,** 280 (1966).
Whittaker, V. P., *Handb. Exp. Pharmakol.* **15,** 1 (1963).

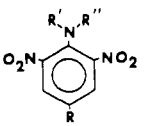
Received for review October 25, 1973. Accepted December 19, 1973.

Formation of Meisenheimer Complexes in Dinitroaniline Plant Growth Regulators

Randall C. Hall[1] and Ching S. Giam*

The reactions of 4-substituted-2,6-dinitro-*N,N*-di-*n*-propylanilines with sodium methoxide are characterized by the formation of "stable" Meisenheimer intermediates. The reaction path leading to these intermediates was found to depend upon the electronic properties of the 4 substituent. Compounds with substituents more electron withdrawing than hydrogen appeared to follow one reaction path, while compounds with substituents more electron donating than hydrogen proceeded by a different reaction path.

In the majority of plant growth regulators the functional group responsible for biological activity is readily recognized. Thus, removal of the carboxymethyleneoxy, carboxy, carbamyl, chloroacetyl, and urea groups in the phenoxyacetic acid, benzoic acid, phenyl carbamate, chloroacetamide, and urea plant growth regulators, respectively, results in a loss of activity. The functional moiety of the dinitroanilines (1) is not readily apparent, however. A number of substituents (R) in the 4 position, encompassing a wide range of chemical properties, yield active compounds (Soper, 1966). Activity is still retained when the nitro group in the ortho position is replaced by the ring nitrogen of the corresponding pyridine compounds (Soper, 1964) and even by the electron donating amino group (Soper, 1969). Significant variation of the amino group is also possible (Soper, 1963, 1966; Gentner, 1966, 1970; Malichenko *et al.*, 1968), and in some cases the amino group can be replaced by the hydrazino group (Soper, 1964).



1

Since there does not appear to be a common functional group which is responsible for the biological activity of the dinitroanilines, it is possible that activity is derived from the electron deficient nature of the aromatic nucleus. The dinitroanilines fall into a class of compounds known as Meisenheimer compounds, which are characterized by their reaction with nucleophiles to form "stable" σ complexes.

Meisenheimer (1902) first observed σ complexes; he obtained the same red salt by treating either 2,4,6-trinitroanisole (2) with potassium ethoxide or 2,4,6-trinitrophenetole (4) with potassium methoxide. Meisenheimer assigned 3 for its structure. The structures of 3 and a vari-

Department of Chemistry, Texas A&M University, College Station, Texas 77843.
[1] Present address: Department of Entomology, Purdue University, Lafayette, Ind. 47907.