

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%.

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Formation of Meisenheimer Complexes in Dinitroaniline Plant Growth Regulators

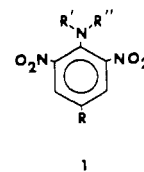
Randall C. Hall¹ and Ching S. Giam*

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 sub-

stituent. 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).



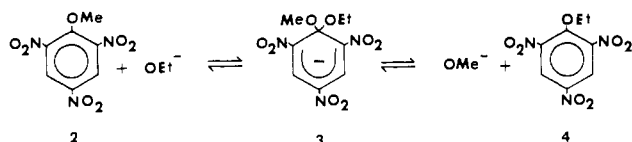
Since there does not appear to be a common functional group which is responsible for the biological activity of the dinitroanilines, it is possible that activity is derived from the electron deficient nature of the aromatic nucleus. The dinitroanilines fall into a class of compounds known as Meisenheimer 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-

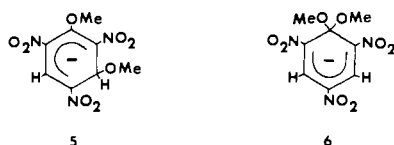
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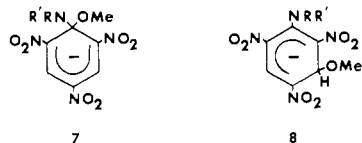
ety of other Meisenheimer intermediates have now been confirmed by nmr studies (Foster and Fyfe, 1965; Gold and Rochester, 1964; Servis, 1967; Caveng *et al.*, 1967).



There is considerable evidence that C-1 intermediates such as 3 are not formed directly, but are formed by the rearrangement of an initial kinetically favored C-3 intermediate (Crampton, 1969; Foster and Fyfe, 1966). Thus, when sodium methoxide is added to 2,4,6-trinitroanisole and the nmr spectrum taken immediately after mixing, two doublets which can be assigned to the ring protons of 5 are observed. These doublets soon disappear, however, and a singlet corresponding to the ring protons of 6 is seen (Crampton and Gold, 1965; Servis, 1965). Servis (1967) suggested that initial attack of the methoxide ion occurs at the 3 position to form 5 which then rearranges to 6.



Similar C-1 and C-3 intermediates (7 and 8, respectively) have also been observed in the reaction of picramides with sodium methoxide (Crampton and Gold, 1964, 1965; Farmer, 1959; Gold and Rochester, 1964; Mackie, 1962). Servis (1965) has reported the nmr spectrum for 7, R = R' = Et, and Crampton and Gold (1967) have reported a number of "stable" 8.



The reactions of 4-substituted-2,6-dinitro-*N,N*-di-*n*-propylanilines with sodium methoxide were investigated to determine the generality of Meisenheimer intermediate formation in the dinitroaniline plant growth regulators, and thus determine the feasibility of a nucleophilic interaction scheme with the plant system.

EXPERIMENTAL SECTION

The dinitroanilines used in this study were synthesized as previously reported (Hall and Giam, 1972). Meisenheimer complexes were formed by adding 0.9 equiv (unless otherwise specified) of methanol-free sodium methoxide in dimethyl sulfoxide (Me₂SO) to approximately 60 mg of a dinitroaniline dissolved in 0.5–0.7 ml of Me₂SO and contained in an nmr tube. Nmr spectra were recorded on a Varian HA 100-MHz instrument equipped with a Hewlett-Packard Model 200 ABR auxiliary audiooscillator. Chemical shifts were measured relative to external tetramethylsilane and corrected to internal tetramethylsilane.

RESULTS AND DISCUSSION

Reactions are observed between sodium methoxide and 4-substituted-2,6-dinitro-*N,N*-di-*n*-propylanilines (9) which contain substituents that vary from the strongly electron withdrawing nitro group to the electron donating amino group. Characteristic nmr spectra consistent with structure 10 were observed for all compounds studied except the amino and carboxyl substituted compounds (9, R = NH₂ and R = COOH) and are reported in Table I. There is a very good linear correlation between the shifts of the ring protons of the parent (9, H_a) and the proton at the unsaturated site of the Meisenheimer complex (10,

Table I. Nmr Chemical Shift Data for Meisenheimer Complexes of 4-Substituted-2,6-dinitro-*N,N*-di-*n*-propylanilines^a

| R | Shift (δ) | | |
|---------------------------------|----------------|----------------|----------------|
| | H _a | H _b | H _c |
| OCH ₃ | 7.73 | 6.06 | 5.42 |
| CH ₃ | 7.95 | 6.54 | 5.36 |
| <i>i</i> -Pr | 7.97 | 6.68 | 5.49 |
| <i>t</i> -Bu | 8.06 | 6.72 | 5.64 |
| H | 8.11 | 7.06 | 5.64 |
| Cl | 8.32 | 7.15 | 5.60 |
| <i>p</i> -NO ₂ Ph | 8.48 | 7.75 | 6.03 |
| CON(Me) ₂ | 8.21 | 7.26 | 5.81 |
| CF ₃ | 8.47 | 7.59 | 5.75 |
| CN | 8.64 | 7.84 | 5.64 |
| SO ₂ CH ₃ | 8.55 | 7.93 | 6.05 |
| NO ₂ | 8.88 | 8.52 | 6.28 |

^a Solvent was dimethyl sulfoxide; see structures 9 and 10 for H_a, H_b, and H_c positions.

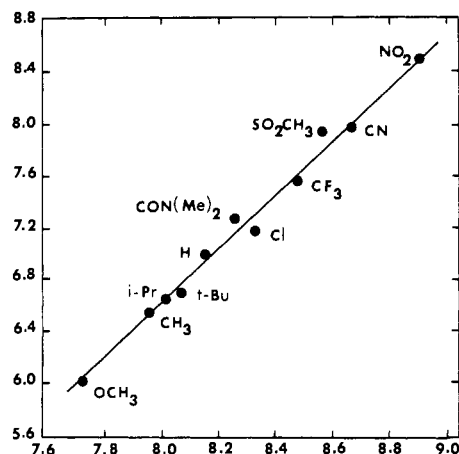
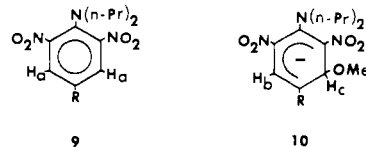


Figure 1. Plot of the ring proton shift (b) at the unsaturated site of the C-3 Meisenheimer complexes of 4-substituted-2,6-dinitro-*N,N*-di-*n*-propylanilines vs. the ring proton shifts (a) of the parent.

H_b), which is shown in Figure 1. This linear relationship, which can be used to predict the chemical shifts of the protons of other intermediates, indicates that the factors affecting the shifts of the ring protons of the parent and the complex are similar. The slope of the line in this correlation is 2.00, which indicates that the ring proton at the unsaturated site in the complex is twice as sensitive to the influence of substituents as the aromatic protons in the parent. The shifts of the ring protons at the saturated site (10, H_c) in the Meisenheimer complexes are only slightly affected by the electronic nature of the substituent.



Addition of sodium methoxide (methanol free) to a Me₂SO solution of a dinitroaniline (1) containing a substituent with a Hammett's σ_p value of 0.23 (value for chloro) or greater causes a decrease in the intensity of the resonance of the ring protons of the parent and the formation of two new upfield resonances corresponding to the ring protons of 10. At this time the color of the solution changes from a light orange to a dark red. The addition of sodium methoxide to a dinitroaniline containing a substituent with a σ_p value less than 0.23, however, produces a somewhat different reaction. Addition of small quantities

of sodium methoxide (ca. 0.1 equiv) causes a decrease in height and broadening of the original ring proton resonances. This resonance usually totally disappears within 5–10 min without the formation of any new resonances. The solution immediately turns from a light red to a dark green (looks almost black) as soon as the sodium methoxide is added. Additional sodium methoxide (0.3–1.0 equiv) causes the appearance of two new upfield resonances which are characteristic of the ring protons of **10**. When these new resonances appear, the color of the solution changes to a dark red. The amino compound (**9**, R = NH₂) turns a dark green with the addition of sodium methoxide and the original ring proton resonance disappears. However, no new resonances are observed in an excess of sodium methoxide (ca. 3.0 equiv) nor is there a further change in the color of the solution. The reaction of this compound is probably complicated by abstraction of an amino hydrogen (Crampton, 1969), which would deactivate the compound toward nucleophilic attack. Furthermore, if an intermediate corresponding to **10** were formed there is a possibility that it might have some radical character, and, hence, not be easily seen by "normal" nmr techniques. This is observed for the corresponding carboxy compound (X, R = COOH). The addition of either water or sodium bicarbonate to a methanolic solution of this compound causes a broadening and eventual disappearance of the proton resonances. There is also a corresponding change in color from light orange to dark red; however, no new resonances appear.

On the basis of observed nmr spectra and the known chemistry of similar compounds it can be concluded that the reaction of dinitroaniline plant growth regulators with a nucleophile to produce intermediates represented by **10** is a general reaction of 4-substituted-2,6-dinitro-*N,N*-dipropylanilines which do not have ionizable substituents (e.g., COOH, NH₂). The reaction pathway leading to **10**, as observed by nmr, can vary depending upon the elec-

tronic nature of the substituent. It is interesting to note that activity is very low (Hall, 1971) for compounds such as **9**, R = COOH or NH₂, which do not readily form Meisenheimer intermediates. On the basis of the generality of Meisenheimer intermediate formation, it can be conceived that activity of the dinitroanilines is derived from their nucleophilic reactivity. The possible role of these intermediates will be presented when detailed structure-activity relationships have been concluded.

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Synthesis of L-N-[(1-Naphthyl)acetyl]glutamic Acid

Theodore R. C. Tonkinson* and Mark L. Brenner

L-N-[(1-Naphthyl)acetyl]glutamic acid was synthesized. The synthesis involved coupling the dibenzyl ester of L-glutamic acid with 1-naphthaleneacetic acid in the presence of Woodward's reagent (*N*-ethyl-5-phenylisoxazolium-3'-sulfonate). The final product was obtained when toluene was

removed from the glutamic acid moiety by hydrogenolysis. Assay by gas-liquid chromatography-mass spectrometry, infrared and ultraviolet spectroscopy, and thin-layer chromatography confirmed the purity and structure of the final product.

While investigating conversion rates by leaf tissue of 1-naphthaleneacetic acid (NAA) to *N*-[(1-naphthyl)acetyl]aspartic acid a compound was discovered which was thought to be *N*-[(1-naphthyl)acetyl]glutamic acid (NAGlu) (Brenner and Tonkinson, 1974). NAGlu was synthesized in order to confirm the identity of the natural product and characterize some of its properties. Coupling of the NAA and the glutamic acid was accomplished using a carboxyl activating reagent described by Woodward *et al.* (1961). The two-step sequence involved in the coupling and isolation of the end product by partitioning makes this procedure quite simple and direct.

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PROTECTION OF CARBOXYL GROUPS OF GLUTAMIC ACID

The dibenzyl ester of the L-glutamic acid was prepared according to Good's (1956) modification of a method developed by Ciperia and Nicholls (1955). The resulting benzenesulfonate salt of the ester was recrystallized from a 1:4 methanol-ether solution before conversion to the HCl salt. The yield of the HCl salt was 27%. The low yield was possibly due to formation of γ -lactam under the refluxing conditions and to dimer formation in carbon tetrachloride.

Dibenzyl esterification was confirmed by an infrared spectrum of the L-dibenzyl glutamate-HCl. Absorption at 690 and 725 cm⁻¹ indicated the presence of benzene rings and that at 1200 cm⁻¹ indicated an ester linkage.