Synthesis and Microbial Toxicity of Dinitrobutadienes and Related Compounds

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Various nitro- and dinitrobutadienes have been synthesized and their fungicidal and bactericidal properties determined. Of the compounds screened, 1,4-dibromo- and 1,4-dichloro-1,4-dinitro-1,3-butadiene and 2,5-dinitro-1,3-hexadiene were the most active.

Many examples of the fungicidal and bactericidal properties of nitroölefins containing aliphatic, aromatic, or heterocyclic residues have been reported (Bluestone, 1959; Eckstein, 1958; Iwai, 1963; McGowan *et al.*, 1948; Nagawa, 1963; Pianka, 1963; Pyne, 1963; Robertson, 1958; Schales and Graefe, 1952; Stevenson *et al.*, 1963; Vecchi and Melone, 1963; Yamanaka *et al.*, 1962; Zsolnai, 1961). None has achieved widespread use. The fungicidal properties of 1,4-dinitro-1,3-butadiene, **1**, and related substances have not been reported previously. Certain of these materials have been found to be highly active against various fungi of economic importance in agriculture. Details of their synthesis and biological activity are described.

CHEMISTRY

The materials studied in this work are summarized, together with their biological properties, in Table I. Compound **1**, 1,4-dinitro-1,3-butadiene, was prepared by a modification of a method reported previously (Carroll, 1966; Carroll *et al.*, 1966; Novikova *et al.*, 1960). Compound **1** has also been prepared by the dehydrochlorination of 1,4-dinitro-2,3-dichlorobutane (Perekalin and Lerner, 1959) and by the simultaneous bromination-dehydrobromination of the disodium salt of 1,4-dinitrobutene-2 (Lipina *et al.*, 1963). The synthesis of **1** developed in this work (Equation 1) is convenient for a large scale preparation. It is not necessary to separate the diastereoisomeric diols **2** prior to acetylation since both lead to the same dinitrobutadiene (Carroll, 1966). The acetylation of **2** with isopropenyl acetate (Equation 1) is somewhat slower than is the reaction involving acetyl chloride but has the advantage of a simplified work-up. Water and acetic acid must be removed from the diacetate. This problem is minimized by the use of isopropenyl acetate. The base-catalyzed conversion of the diacetate to **1** usually proceeds in a straightforward manner once initiated, but the induction period is somewhat erratic, apparently depending on several factors, *inter alia*, the absence of moisture or acetic and efficient stirring.

Mechanistic considerations lead to the prediction that **1** is the *trans*, *trans* isomer. The validity of this prediction is supported by the following data. The NMR spectrum in *d*-acetone consists of a single peak while in *d*-benzene the spectrum becomes a symmetrical grouping of peaks characteristic of an A_2B_2 system (Carrol *et al.*, 1966; Jackman, 1959). The symmetry of this spectrum is indicative of the symmetry of the molecule, either *cis*, *cis*, or *trans*, *trans* but not *cis*, *trans* (Bothner-by and Harris, 1965; Braye, 1963). Infrared spectra of **1** are relatively simple showing, *inter alia*, intense bands assignable to a *trans*-CH=CH— [Fegley *et al.*, 1957; Viehe and Franchimont, 1964; Sadtler Commercial Spectra: β-nitrostyrene (No. 3301), *p*,β-dinitrostyrene (No. 3322), and *o*,β-dinitrostyrene (No. 3320)]. The ultraviolet spectrum of **1** (Carroll *et al*, 1966) is consistent with a conjugated system (Braye, 1963; Gillam and Stern, 1957). On the grounds of the sterochemistry of the synthetic reaction and these spectral data, the *trans*, *trans* structure is assigned to **1**.

The report (Vasileva *et al.*, 1961) that **1** possesses a dipole

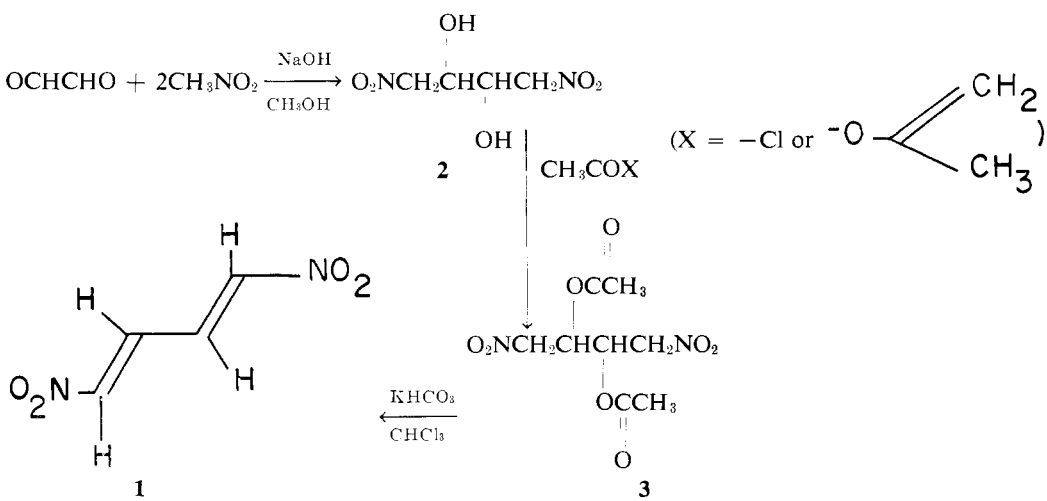

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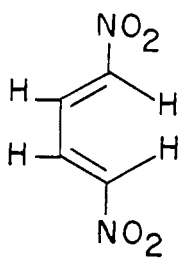
Table I. Microbiocidal Effectiveness of Various Dinitrobutadienes and Related Compounds in Preliminary Tests[a]

Compound No.	Structure	Agar Incorporation Tests[b]			Soil Surface Mycelial Growth Tests[b]				Foliage Disease Tests[b]			
		Sa	Ea	An	Pd	Rs	Fo	Sr	As	Cl	Ep	Up
1	$(O_2NCH{=}CH{-})_2$	5	...	5	...	4	1	...	1	1	1	5
2	$(O_2NCH_2CH(OH){-})_2$	1	...	1	...	1	1	...	1	1	1	1
3	$(O_2NCH_2CHC(OCOCH_3){-})_2$	3	...	5	...	3	5	...	4	1	1	5
4	$(O_2NC(Br){=}CH{-})_2$	5	5	5	5	5	4	...	4	5	1	1
5	$(O_2NC(Cl){=}CH{-})_2$	...	5	...	1	1	4	...	5	5	1	1
6	$O_2N(CHBr)_4NO_2$	1	5	1	2	1	3	...	1	1	1	1
8	$(O_2NC(CH_3){=}CH{-})_2$	1	5	1	5	4	5	5	4	4	1	1
9	$(O_2NC(C_2H_5){=}CH{-})_2$	1	1	1	5	4	5	5	1	4	1	1
10	$(O_2NCH(CH_3)CH(OCOCH_3){-})_2$	1	1	1	1	1	3	...	4	5	1	1
11	$C_6H_5CH{=}CH{-}CH{=}CHNO_2$	1	5	3	4	3	4	4	4	1	1	1
14	$C_6H_5CH{=}CH{-}CH{=}CBrNO_2$	1	5	5	3	3	4	...	4	1	1	1
15	$cis\text{-}O_2NC(CH_3){=}C(CH_3)NO_2$	1	1	1	3	2	5	...	1	1	1	4
16	$(CH_3)_3CCH{=}CBrNO_2$	5	5	5	5	4	4	5	1	1	1	1
17	$Cl_3CCH{=}CBrNO_2$	5	5	5	2	4	4	...	1	1	1	1
Standards												
	$C_6H_5CH{=}CHNO_2$	5	...	5	5	5	5	5	1	...	1	1
	$C_6H_5CH{=}CBrNO_2$	5	...	5	4	5	5	5	1	...	1	1
	$C_6H_5CH{=}CClNO_2$	5	...	5	4	4	5	5	1	...	1	1

[a] Effectiveness ratings are complete control (5) to no control (1). [b] Identification of test organisms:
Bacteria
Sa = *Staphylococcus aureus*
Ea = *Erwinia amylovora*
Fungi
An = *Aspergillus niger*
Pd = *Pythium debaryanum*
Rs = *Rhizoctonia solani*
Fo = *Fusarium oxysporum f. lycopersici*
Sr = *Sclerotium rolfsii*
As = *Alternaria solani*
Cl = *Colletotrichum lagenarium*
Ep = *Erysiphe polygoni*
Up = *Uromyces phaseoli*

moment of 0.71 D. is not consistent with structure **1** but is explicable by an equilibrium between 1 and **c**. Such an equilibrium, about 4% in the "*S-cis*" form (Smith and Massingell, 1961) has been observed with 1,3-butadiene (Hine, 1956), but the dipole moment of 1,3-butadiene is indistinguishable from zero (Hannay and Smyth, 1943; Wienges *et al.*, 1966). In the case of **c** the dipole moment



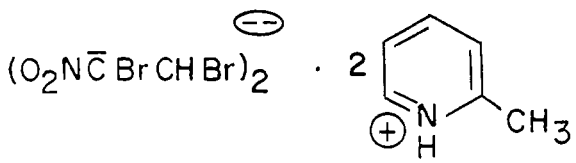
c

would be significantly greater than in the "*S-cis*" form of butadiene.

The dinitrodiene **1** was used as the starting material in the preparation of 1,4-dibromo- and 1,4-dichloro-1,4-dinitro-1,3-butadiene, **4** and **5**, respectively. Compound **4** was prepared by a modification of a published method (Carroll *et al.*, 1966). The crude residue from the addition of bromine to **1** was used directly in the dehydrobromination reaction. On one occasion, a solid isomer of the tetrabromo compound, **6**, was isolated and its general structure was verified. It is apparently identical with a material obtained from the bromination of **1** in acetic acid (Lipina and Perekalin, 1963).

For the quantities of **4** required, an agent other than Florisil (Carroll *et al.*, 1966) was desired for the dehalogenation step. After the evaluation of several candidates, 2-picoline was selected.

When 2-picoline was added to a solution of **6** in benzene, a yellow solid separated. On the basis of elemental analyses, spectral properties, and the fact that it recrystallized as a discrete compound, the yellow solid is assigned structure **7**. Upon heating in water, this material gave rise to **4**. The elimination step in this reaction apparently requires a polar medium. In



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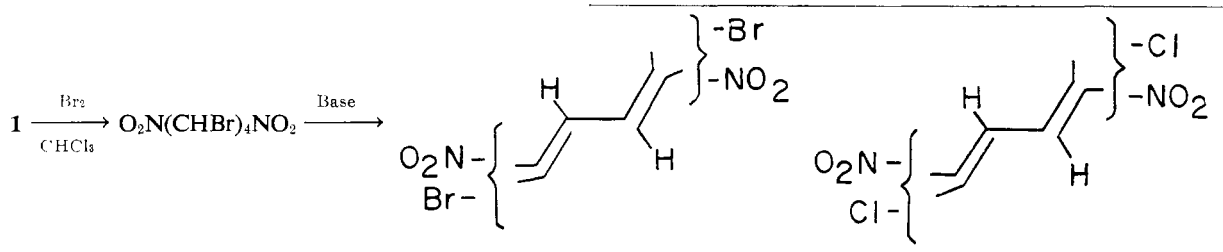
the dehydrobromination reaction involving potassium acetate in benzene, **4** is isolated directly. Recrystallization of **6** from methanol has also been reported to give **4** (Lipina *et al.*,

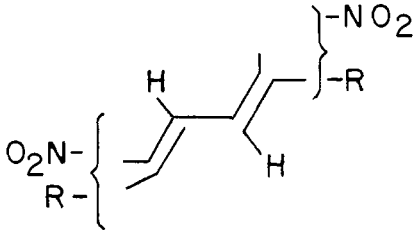


6 **4** **5**

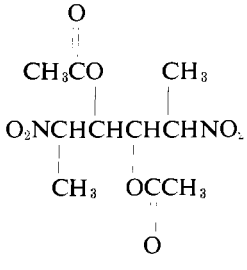
1963). The spectral properties of **4** are in excellent agreement with those reported (Carroll *et al.*, 1966).

Attempts to prepare compound **5** by a method similar to that employed for **4** were unsuccessful; **5** was prepared by the reported method (Carroll *et al.*, 1966).

The geometry of **4** and **5** is uncertain but their simple spectra and sharp melting point indicate that they may be monoisomeric. The alkyl analogs, **8** and **9**, of **4** and **5**, were prepared according to Equation 1, substituting nitroethane or nitropropane for nitromethane. As in the case of **4** and **5**, the geometric relationships in 2,5-dinitro-2,4-hexadiene, **8**, and 3,6-dinitro-3,5-octadiene, **9**, are not known although the NMR spectrum of **8** indicates a symmetrical structure. The intermediate diacetate, **10**, was also of interest in the biological studies.



8 (R = CH_3)
9 (R = C_2H_5)



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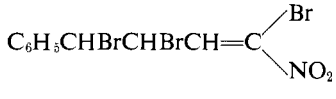
The reaction of cinnamaldehyde with nitromethane gave **11** (Kochetkov and Dudzkina, 1958), which upon treatment with bromine gave the tetrabromo derivative **12** which could be dehydrobrominated to the tribromo compound **13** as the main product.

$$C_6H_5CH{=}CHCH{=}CHNO_2 \xrightarrow{2Br_2} C_6H_5(CHBr)_4NO_2 \longrightarrow$$

11 **12**



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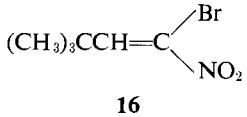
The structural assignments are based on infrared and NMR spectral studies and elemental analyses.

A second product frequently encountered when crude **12** was dehydrobrominated was 1-bromo-1-nitro-4-phenyl-1,3-butadiene, **14**, which undoubtedly arose from 1,2-dibromo-1-nitro-4-phenylbutene-3, a likely coproduct with **12** from the addition of bromine to **11**.

The nitroölefin **16** was prepared from pivaldehyde with nitromethane after Equation 1 with a subsequent bromination–dehydrobromination as in the preparation of **4**. Compound **17** was prepared in an analogous manner from chloral and nitromethane.



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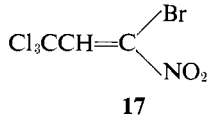

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The dinitrodienes decompose explosively upon heating and have shown a high degree of shock sensitivity. Their decomposition is characterized by high energy release.

BIOLOGICAL RESULTS

The compounds prepared in this work were evaluated in a series of standard primary screening tests designed to detect bactericidal and fungicidal activity. Toxicity to bacteria was detected by an agar incorporation test and fungitoxicity by agar incorporation, a soil mycelial growth test, and a foliage disease control evaluation. In these tests, certain of the butadiene derivatives were toxic to a gram-negative and gram-positive species of bacteria and several fungi (Table I). Included in Table I for comparison are ω-nitrostyrene and its ω-bromo and ω-chloro derivatives, a proprietary bactericide, and several fungicides.

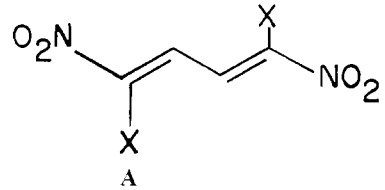
Compounds **4**, **16**, and **17** showed excellent to moderate activity as bactericides. Although several substances inhibited the mycelial growth of test fungi in soil, in more practical experiments none of these compounds were effective in protecting germinating seedlings from attack by plant pathogenic soil fungi. The reason for this reduction in apparent activity is not known. Such a reduction in activity is not uncommon in going from the relatively sensitive soil mycelial growth tests to the more stringent germination tests which require control of the pathogen in the presence of a host.

In the foliar tests, the dinitrodihalobutadienes **4** and **5**, the hexadiene **8**, and the diacetoxyhexane **10** were quite effective when compared to the other compounds in Table I. When this activity was quantitated (Table II), compounds **4**, **5**, and **8** were relatively effective against *A. solani* (tomato early blight), *C. laganerium* (cucumber anthracnose), and *V. inaequalis* (apple scab) in laboratory tests. The diester **10** was not evaluated against apple scab but compared favorably with **4**, **5**, and **8**, in control of *A. solani* and *C. langanerium*. In field tests where higher rates were required for control of apple scab infections, **4** and **5** elicited phytotoxicity on the apple trees. Compound **8** was a more effective and less phytotoxic fungicide than either **4** or **5** in field tests.

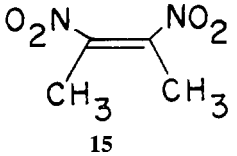
The biological activity of **10**, the diester precursor of **8**, is of interest since it closely parallels the activity of **8** and suggests, therefore, an *in vivo* conversion of **10** to **8**.

The mechanism by which the dinitrobutadienes exert their fungicidal activity is not known. In this regard it is interesting to note that the mechanism of activity of the β-nitrostyrenes has been related to the presence of the olefinic linkage activated by a nitro group (Clark *et al.*, 1963; Zsolnai, 1961). The antifungal activity of these nitroölefins seems to parallel their ability to participate in Michael-type addition reaction with various nucleophiles (Clark *et al.*, 1963).

Considering the basic structure A with the results in Tables I and II, maximum activity against *C. laganerium* and *A. solani* is observed when X is Cl, Br, or CH_3. When X is H or C_2H_5, activity is negligible.



A



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cis-2,3-Dinitrobutene-2, **15**, (Plaut, 1952) a compound with similarities to **8**, demonstrates no activity relative to the foliar fungi but shows good activity against Fusarium, a soil organism. Compound **11**, the vinylog of β-nitrostyrene, is not considerably different from that material in fungicidal activity although it is not as effective a bactericide. The tribromo derivative of **11**, **13**, shows essentially the same activity as **11**, as does the 1-bromo analog, **14**. The reasons for the rather specific requirements for X relative to activity are not obvious. Substitution of a Cl or a Br in β-nitrostyrene (Table I) does not enhance the activity against the foliage fungi under consideration. The lack of activity of **16** and **17**

Table II. Quantitation of Microbiocidal Properties (ED_3 Values) of Active Compounds in Table I[a]

Compound No.	Agar Incorporation Tests[b]			Soil Tests[c]				Foliage Disease Tests[d]			
	Sa	Ea	An	Pd	Rs	Fo	Sr	As	Cl	Up	Vi
1			<6		>38					1000	
3			~6			19		>100		100	
4	<6	~60	12	<19	>38	38	27	32	30		<60
5						75		44	20		60
8				>50	>50	38	>50	100	32		<60
9				>50	>50	20	50		30		
10								44	30		
11	>250		>250	>50		>50	>50	100			
14			60			>50		100			
15						22				1000	
16	120	120	60	>50	>50	25	18				
17	120	120	120		>50	31					
$C_6H_5CH{=}CHNO_2$	>6		>6	<19	<19	7	>38				
$C_6H_5CH{=}CBrNO_2$	6		>6	<19	<19	7	>38				
$C_6H_5CH{=}CClNO_2$	<6		>6	<19	<19	19	>38				
Hexachlorophene	6	6									
Copper 8-quinolinolate			<6								
Zineb								4	4		
Cyprex								12			20
PCNB					7						
Mylone				19			25				
Captan						19		14	40	40	

[a] Vi = *Venturia inaequalis*, other test organisms are identified in Table I.
[b] ED_3 values are parts of chemical per million parts of agar media required to give a control rating of 3 (see footnote *a*, Table I).
[c] ED_3 values are pounds of chemical per 6 inch acre of soil required to give a control rating of 3 (see footnote *a*, Table I).
[d] ED_3 values are parts of chemical per million parts of water required to give a control rating of 3 (see footnote *a*, Table I).

shows that bulk or electronegativity in the group attached to the 1-nitro-bromo ethylene group does not alone determine activity.

EXPERIMENTAL SECTION

WARNING: THE DINITROBUTADIENES, **1** AND **8**, DECOMPOSE EXPLOSIVELY UPON HEATING AND SHOW A HIGH DEGREE OF SHOCK SENSITIVITY. LARGE-SCALE PREPARATIONS SHOULD BE AVOIDED.

Chemistry. The melting points are uncorrected. The infrared spectra were obtained using a Baird Atomic AB-2 recording infrared spectrophotometer. The NMR spectra were obtained on a Varian A-60 instrument using tetramethylsilane as the internal standard. Elemental analyses were performed by Union Carbide European Research Associates, Brussels Belgium.

1,4-DINITRO-2,3-BUTANEDIOL, **2**. The general procedures of Novikova *et al.* (1960) and Carroll (1966) were followed with certain modifications. A solution of 1 mole of sodium hydroxide in sufficient water to make 72 ml. and 72 ml. (0.5 mole) of glyoxal were added dropwise at the same rate to a well-stirred solution of 350 ml. each of methanol and nitromethane at 5° to 10° C. When addition was complete, the heavy suspension which had formed was stirred at 0° for 40 minutes and then neutralized to pH 5 with sulfur dioxide *without* prior dilution with water while the temperature rose to about 34° C. The insoluble materials which separated during acidification were collected, washed with cold nitromethane, and the combined filtrates were chilled to 0° and then filtered through Filter-cel. A series of *in vacuo* concentrations and filtrations gave relatively pure isomeric fractions: 30 grams, m.p. 126–28° [Carroll (1966), 135–135.5°; Novikova *et al.* (1960) reports for *meso* 134°] and 23.5 grams, m.p. 82–83° [Carroll (1966), 101–102°; Novikova *et al.* (1960) reports for *racemic* 89.5°] for a total yield of 61%. The infrared spectra of these fractions agreed with the proposed structures.

1,4-DINITRO-2,3-DIACETOXYBUTANES, **3**. The Acetyl Chloride/Acetic Acid Method. Using the general method of Carroll (1966) 1 mole of predominantly *meso* **2** in 1 liter of acetic acid was reacted with 5.3 moles of acetyl chloride. When the initial reaction was complete, the mixture was heated slowly to 85° C. and then allowed to cool to room temperature after which it was poured into 1 liter of ice water and the resulting solid collected and washed thoroughly with cold water. The damp product was dissolved in methylene chloride, the aqueous layer separated, and the organic solution was dried over sodium sulfate and finally evaporated *in vacuo* to give 230 grams (87%) of white solid, m.p. 88–90° [Carroll (1966), *meso*, 91.5–92°].

The Isopropenyl Acetate Method. A solution of 180 grams (1 mole) of predominantly *meso* diol in 300 ml. of isopropenyl acetate and 1 ml. of concentrated HCl was gently refluxed overnight. After cooling at room temperature, the dark solution was seeded and allowed to crystallize. The mixture was then diluted with an equal volume of hexane with stirring and, after about 2 hours, was filtered to give 210 grams of product, m.p. 88–89.5°. Addition of 200 ml. of hexane to the filtrate followed by chilling gave 20 grams more of product, m.p. 87.5–89.5°; total yield 87%.

In each case, the infrared spectrum was in agreement with a reference scan.

trans,trans-1,4-DINITRO-1,3-BUTADIENE, **1**. Using the procedure of Novikova *et al.* (1960), to a solution of 41 grams (0.155 mole) of **3** in 1200 ml. of chloroform (AR) was added 0.1 gram of potassium bicarbonate and the mixture was heated at reflux with *vigorous* stirring. The progress of the reaction could be followed quite conveniently by infrared spectral changes. The starting diester (in chloroform) showed two sharp bands, one at 5.65 μ (C=O, ester) and the other at 6.4 μ (—NO_2). As the reaction proceeded the 5.65-μ band diminished and was gradually replaced by a band at ~5.8 μ (C=O, acetic acid) while the band at 6.4 μ diminished in intensity and a second band appeared at 6.55 μ (NO_2,

conjugated). When spectral changes were no longer apparent, the reaction was discontinued. During the course of the reaction the solution darkened. When the reaction was complete, the mixture was cooled at room temperature and then chilled to give 15 grams (89%) of product, m.p. 146–48° [Carroll *et al.* (1966), m.p. 146–47°].

The product obtained from this reaction appeared to be isomerically homogeneous by TLC and possessed the following spectral properties: ultraviolet $\lambda_{max}^{CH_3OH}$ 281 mμ ($\epsilon = 2.1 \times 10^4$), Carroll *et al.* (1966), report 281 mμ ($\epsilon = 1.85 \times 10^4$); NMR (*d*-acetone) shows a singlet at δ 7.90, while in *d*-benzene the spectrum consists oı a symmetrical complex multiplet of 12 peaks centered at δ 6.2. The reported NMR spectrum (Carroll *et al.*, 1966) consists of two singlets at δ 7.52 and δ 7.55, respectively, solvent unspecified; $\lambda_{max}^{1\%\ KBr}$ (μ) 6.12 (C=C stretching), 6.6 and 7.4 (conjugated NO_2), 10.1 or 10.61 (—CH=CH—, trans,) and two unassigned strong bands at 11.83, and 13.35, and a medium intensity band at 14.95; $\lambda_{max}^{6\%CH_3CN}$ (μ) 6.2 (C=C stretching), 6.55 and 7.42 (conjugated NO_2), 10.15 or 15.55 (CH=CH, trans) with unassigned bands at 11.88 and 15.02.

1,4-Dibromo-1,4-dinitro-1,3-butadiene, **4.** Bromination of **1.** 1,2,3,4-Tetrabromo-1,4-dinitrobutane, **6.** To a suspension of 7.2 grams (0.05 mole) of **1** in 200 ml. of chloroform was added, dropwise with stirring over a period of 30 minutes, 16 grams (0.1 mole) of bromine. After 3 hours, the reaction mixture was homogeneous and, after 18 hours, was concentrated *in vacuo* at 25°; the residual oil crystallized upon scratching. Two recrystallizations from heptane gave 9 grams (39%) of white solid, m.p. 79–80°. This material did not absorb between 200 and 400 mμ. The NMR spectrum (*d*-chloroform) consisted of two sets of doublets at δ 6.12 (—$CHBrNO_2$) and δ 5.08 (—CHBr—). $\lambda_{max}^{10\%KBr}$ (μ) 3.3 and 3.35 (CH), 6.38 and 7.48 (NO_2) and broad absorption at ~17 (C—Br).

Anal. Calcd for $C_4H_4Br_4N_2O_4$: C, 10.35; H, 0.87; N, 6.04. Found: C, 10.57; H, 1.38; N, 5.54.

The Dehydrobromination of Tetrabromodinitrobutane. A solution of 0.1 mole of tetrabromodinitrobutane in sufficient benzene to give 100 ml. was prepared by adding 14.6 grams (0.1 mole) of **1** portionwise to 32 grams (0.2 mole) of bromine in 80 ml. of benzene. When the reaction was complete, the resulting solution was filtered to remove a small amount of flocculent material and the volume of the filtrate was adjusted to 100 ml.

To 10 ml. of the above solution was added 1.96 grams (0.02 mole) of potassium acetate and the mixture was stirred for 4 hours at room temperature when TLC, benzene liquid phase, indicated a complete conversion to **1.** The inorganic material was collected and the filtrate was evaporated by an air-stream. The resulting residue was recrystallized from heptane to give 2.0 g. (67%) of product, m.p. 125–26.5°.

Under the same conditions for 18 hours, 10-ml. aliquots treated with equivalent quantities of calcium carbonate, potassium carbonate, or magnesium oxide gave no reaction.

Both 2,6-lutidine and 2-picoline were effective dehydrobrominating agents and their use is illustrated in the following reaction involving 2-picoline.

The Large-Scale Preparation of 1,4-Dibromo-1,4-dinitro-1,3-butadiene, **4.** To a suspension ot 68 grams (0.48 mole) of **1** in 1 liter of chloroform was added 152 grams (0.95 mole) of bromine over a period of 2 hours during which time the reaction temperature reached 47°. After about 5 hours, the temperature began to decrease and the reaction mixture was a light amber solution which, upon concentration *in vacuo* at 25°, gave a dark oil assumed to be a quantitative yield of tetrabromo compound.

This crude product was dissolved in 1 liter of benzene, cooled to 10°, and treated dropwise with stirring with 88.6 grams (0.95 mole) of 2-picoline in 100 ml. of benzene (maximum temperature 17°). The discrete yellow solid which formed during the addition was collected after 1 hour at 10° and dried as much as possible on the filter. This yellow solid was then heated and stirred at 80° in 2 liters of water for 20 minutes after which time the insoluble material was collected and dried. Recrystallization from heptane (30 ml./gram) gave 114 grams (85%) of **4,** m.p. 126.5–27.5° [Carroll *et al.* (1966) report m.p. 123.5–25.5°]. The following spectral data were obtained: $\lambda_{max}^{1\%\ KBr}$ (μ) 3.25 (=CH), 6.2(C=C), 6.52 and 7.61 (conjugated NO_2), 11.18 (R_2C=CHR), 16.8 (C—Br); ultraviolet λ_{max}^{MeOH} = 339 mμ ($\epsilon = 1.18 \times 10^{-4}$); NMR (*d*-chloroform) showed a singlet at δ 8.22.

The bis-(2-Picoline) Salt of 1,2,3,4-Tetrabromo-1,4-dinitrobutane, **7.** A sample of the yellow solid initially formed during the preparation of **4** was isolated and air dried, m.p. 116–17° (dec.). Recrystallization from methylene chloride by slow addition of hexane gave yellow needles, m.p. 113° (dec.), $\lambda_{max}^{1\%\ KBr}$ (μ) 3.81 (NH); 6.18, 6.22, and 6.35 (arom. C=C?); 6.49 and 7.57 (NO_2).

Anal. Calcd for $C_{16}H_{18}Br_4N_4O_4$; C, 29.56; H, 2.79; N, 8.62; O, 9.85. Found: C, 29.57; H, 2.76; N, 8.75; O, 9.98.

2,5-Dinitro-2,4-hexadiene, **8,** and 3,4-Diacetoxy-2,5-dinitrohexane, **10.** Using the procedure for compound **2,** 38.5 grams (0.5 mole) of nitroethane and 36.5 grams (0.25 mole) of 40% glyoxal were reacted to give a residual oil which was treated with excess acetyl chloride in acetic acid to give a 58% yield of the isomeric diacetates **10,** in two portions, which possessed melting point ranges of 130–37° and 85–98° (toluene), $\lambda_{max}^{1\%\ KBr}$ (μ) 5.71 (C=O), 6.43 and 7.35 (NO_2), 8.35 (C—O), 9.63 or 9.67 (acetate) and were generally characteristic of different mixtures of isomeric materials.

The diene **8** (apparently the same isomer in each case) was prepared from either isomeric mixture of **10** in yields of 30 to 60% using the method previously described for **1.** Recrystallization from chloroform gave **8,** m.p. 165–66°, $\lambda_{max}^{1\%\ KBr}$ (μ) 3.25 (=CH—), 3.35 and 7.2 (CH_3), 6.15 (conjugated C=C), 6.6 and 7.75 (conjugated NO_2); λ_{max}^{MeOH} 310 mμ ($\epsilon = 1.5 \times 10^{-4}$), NMR (*d*-chloroform) δ 7.68 2H singlet (=CH—) and δ 2.45 6 H singlet ($—CH_3$).

Anal. Calcd for $C_6H_8N_2O_4$: C, 41.86; H, 4.68; O, 37.18. Found: C, 42.01; H, 5.17; O, 36.85.

3,6-Dinitro-3,5-octadiene, **9.** Using the methods for **8** but substituting nitropropane for nitroethane, there was obtained 2 grams (20%) of product, m.p. 96–98°. The infrared spectrum agreed with the structure.

Anal. Calcd for $C_8H_{12}N_2O_4$: C, 47.99; H, 6.04; O, 31.97. Found: C, 48.06; H, 6.08; O, 32.12.

1,2,3,4-Tetrabromo-1-nitro-4-phenylbutane, **12.** To a solution of 17.5 grams (0.1 mole) of 1-nitro-4-phenyl-1,3-butadiene **11** (Kochetkov and Dudzkina, 1958) in 200 ml. of chloroform was added dropwise, with stirring, 33.7 grams (0.21 mole) of bromine. When addition was complete, stirring was continued overnight and the reaction mixture was then concentrated *in vacuo* to a solid residue which was extracted with 3 × 50 ml. of boiling toluene. After a gravity filtration, this extract was chilled to give 20 g. (41% yield) of white solid, m.p. 163.5–165°. The NMR spectrum ($CDCl_3$) was highly complex and was generally in agreement with the proposed structure; aromatic H : aliphatic H = 5/4. The

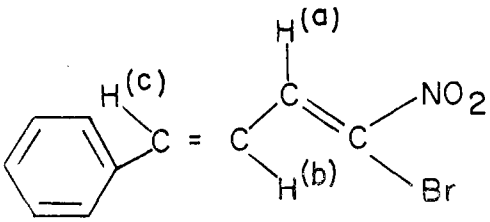
infrared spectrum was in agreement with the proposed structure showing, *inter alia*, a non-conjugated nitro group.

Anal. Calcd for $C_{10}H_9Br_4NO_2$: C, 24.27; H, 1.83; N, 2.83. Found: C, 24.58; H, 1.96; N, 2.92.

1,3,4-TRIBROMO-4-PHENYL-1-NITROBUTENE-3, **13**. To a suspension of 2.5 grams (0.005 mole) of **12** in 200 ml. of acetic acid was added portionwise with stirring 0.82 gram (0.1 mole) of sodium acetate. During the addition a 5° temperature increase was noted. The mixture was then heated at 50° for 19 hours with stirring. After filtration the filtrate was poured with stirring into 75 ml. of ice water. The yellow solid which separated was collected on the filter and, after drying, was recrystallized from 75 ml. of hexane to give finally 1.22 grams (60%) of product, m.p. 137.5–39°, $\lambda_{max}^{1\%\ KBr}$ (μ) 3.25 (=CH—); 3.47 (CHBr); 6.12 (C=C); 6.49, 7.6 (NO_2); 10.6 (C=C). The NMR spectrum (*d*-acetone) showed a 5H complex multiplet between δ 7.3 and δ 7.8. (arom. H); 6 lines (3 doublets?) between δ 5.3 and δ 5.85. (—CHBr—, 2H); a 1H doublet at δ 8.12 (—CH=CBrNO_2).

Anal. Calcd for $C_{10}H_5Br_3NO_2$: C, 29.02; H, 1.95; N, 3.38. Found: C, 29.01; H, 1.90; N, 3.32.

1-BROMO-1-NITRO-4-PHENYL-1,3-BUTADIENE, **14**. To a solution of 49.5 grams (0.1 mole) of crude **12** in 300 ml. of benzene was added 18.6 grams (0.2 mole) of 2-picoline dropwise with stirring. A slight temperature increase was noted. When this subsided, the mixture was heated at 60 to 65° for 3 hours and then cooled and 150 ml. of water was added. After stirring for 30 minutes, the organic layer was separated from the aqueous layer (and some black tar) and was dried over sodium sulfate. The benzene solution was concentrated *in vacuo* at room temperature and the residual oil solidified at room temperature. This solid was thoroughly washed with 100 ml. of boiling hexane, and this solution filtered through charcoal. The charcoal cake was washed with 3 × 20 ml. of boiling hexane and the combined hexane solutions were allowed to crystallize at room temperature to give a yellow solid. Concentration of the filtrate to 80 ml. with subsequent cooling gave a second portion which, combined with the first portion gave 4 grams of crude **14,** m.p. 86–89.5°. Recrystallization from hexane gave 1 38 g. of **14,** m.p. 85.5–91°, $\lambda_{max}^{1\%\ KBr}$ (μ) 3.30 (=CH—); 6.22, 6.32 (C=C<); 6.6, 7.6, 7.7, 7.75 (conjugated NO_2); 10.3 (*trans* C=C); 15.0, 17.5 (C—Br?). The NMR spectrum ($CDCl_3$) shows the following peaks:



δ 7.15–7.5, complex multiplet (5 arom. H); δ 8.14, a doublet with fine splitting (H (*a*)); H (*b*) and H (*c*) occur as an AB pair further split into doublets centered at δ 6.9 and δ 7.2, respectively.

Anal. Calcd for $C_{10}H_8BrNO_2$: C, 47.27; H, 3.17; N, 5.51. Found: C, 47.26; H, 3.02; N, 5.38.

1-BROMO-1-NITRO-3,3,3-TRICHLOROPROPENE, **17**. Using the general procedure for **4,** a solution of 19.1 grams (0.1 mole) of 1-nitro-3,3,3-trichloropropene (Brown and Burkett, 1953) in 500 ml. of chloroform was reacted with 16 grams (0.1 mole) of bromine in 25 ml. of chloroform. As in the case of **4,** the chloroform was replaced with benzene and this solution was treated with 9.4 grams (0.1 mole) of 2-picoline over a period of 40 minutes. A light yellow gum separated. Treatment of the benzene solution with 50 ml. of water with subsequent stirring and finally separating, drying, and distilling the benzene layer gave finally 23 grams of pale yellow liquid, b.p. 91–93°/4 mm. Redistillation through a 6-inch column packed with 1/4-inch triple turn glass helices gave 17 grams (63%) of product, b.p. 86–87°/4 mm. The NMR spectrum (*d*-chloroform) showed a singlet at δ 8.38, λ_{max}^{Capil} (μ) 3.23 (=CH—); 6.15 (C=C); 6.4, 7.59 (NO_2); 12.2 (R_2C=CHR); 16 and/or 16.9 (C—Br).

Anal. Calcd for $C_3HBrCl_3NO_2$: C, 13.39; H, 0.37; O, 11.88. Found: C, 13.93; H, 0.50; O, 11.58.

1-BROMO-1-NITRO-3,3-DIMETHYLBUTENE-1, **16**. To a solution of 17.2 grams (0.2 mole) of pivaldehyde and 12.2 grams (0.2 mole) nitromethane in 100 ml. of absolute ethanol was added dropwise with stirring and cooling (2 to 7°) over a period of 40 minutes a solution of 4.6 grams (0.2 atom) of sodium in 75 ml. of absolute ethanol. The resulting slurry was stirred for 30 minutes after addition was complete and then 12 grams (0.2 mole) of acetic acid in 50 ml. of ethanol was added and the reaction mixture was stirred for 1 hour, filtered, and the filtrate was concentrated *in vacuo* to give 17 grams of light oil. This was dissolved in 50 ml. of glacial acetic acid and treated with 50 grams (excess) acetyl chloride. A vigorous evolution of hydrogen chloride was noted and when this had subsided, the mixture was heated at reflux for 2 hours and then cooled to room temperature and poured into 200 ml. of ice water. The oil which separated was extracted out with 100 ml. of ether and the ether solution was dried over potassium carbonate. This was then filtered and the filtrate concentrated *in vacuo* to give 19 grams of yellow oil. The infrared spectrum was in agreement with the nitroester structure.

The nitroester was converted to the olefin using the method for **3** to **1** involving 100 ml. of chloroform and 0.1 gram of potassium bicarbonate. Distillation of the reaction mixture after filtration gave 9 grams of nitroölefin, b.p. 81–83°/13 mm.

This olefin in 100 ml. of chloroform was reacted with 11.2 grams (0.07 mole) of bromine, after the method of **1** to **6,** except that the reaction flask was wrapped with aluminum foil to exclude light and 0.5 ml. of concentrated HCl was used as the catalyst. Concentration gave a residue which was converted (using the method of **17**) to 8.7 grams (21%) of **16,** b.p. 87–88°/5 mm. The NMR spectrum (*d*-chloroform) shows a 9H singlet at δ 1 31 and a 1H singlet at δ 7.80, λ_{max}^{Capil} (μ) 3.32 (=CH); 3.37, 3.43, (CH_3); 6.5 (C=C), 7.64 (NO_2); 12.6 (R_2C=CHR); 15.5 (C—Br).

Anal. Calcd for $C_6H_{10}BrNO_2$: C, 34.63; H, 4.85; N, 6.74. Found: C, 35.17; H, 4.64; N, 6.58.

Biological Methods and Materials. The microbiocidal efficacy of these compounds was measured by using agar incorporation, soil mycelial growth, and foliage disease control tests. The compounds were formulated at 5000 p.p.m. by dissolving 620 mg. in 25 ml. of acetone containing 0.1% Triton X-155 (a nonionic alkyl aryl polyether alcohol surfactant, Rohm & Haas Co.) and 100 ml. of water. Serial dilutions of this formulation were made with water to obtain the concentrations used in the following tests:

Microbial Agar Incorporation Test. Bacteria were cultured on Difco nutrient agar and *A. niger* was cultured on Difco potato dextrose agar. Two milliliters of chemical formulated at 1000 p.p.m. were added to 18 ml. of sterile, melted medium at 50 to 60° and thoroughly mixed and then poured into sterile Petri plates. When the agar had solidified, these plates were streaked with bacteria (or fungi) from one-week-old cultures using a transfer loop and streaking from

the center of the dish outward in a spoke-like fashion. The final concentration of chemical was 100 p.p.m. The inoculated dishes rated as follows: 5 = no growth, 3 = moderate growth, 1 = heavy growth equal to untreated controls. Compounds which received a rating of 5 in the preliminary tests were retested at lower dosages to quantitate the observed activity. The control ratings thus obtained for each dosage were plotted on semi-logarithmic paper, a straight line was fitted by eye to the plots and the index of effectiveness was taken as the concentration at which the straight line crossed the middle grade of 3 (ED_3 values).

Soil Surface Mycelial Growth Test. Norfolk sandy loam soil was mixed with fungal inoculum of the desired species grown on corn meal–sand medium and then placed in 4-ounce paper cups. A 20-ml. volume of a 250-p.p.m. dispersion of the test compound was poured on the surface of each of two cups containing the inoculated soil. The final concentration of chemical in the soil was equivalent to 50 pounds per acre. The treated cups were incubated for two days at 21° C. and 95% relative humidity and then the surface growth was visually rated as follows: 5 = no growth, 4 = one or two colonies, 3 = surface one-half covered with colonies, 2 = surface three-fourth covered with colonies, 1 = growth equal to untreated controls. Compounds which received a rating of 4 or 5 in the preliminary mycelial growth tests for control of *Fusarium* or *Sclerotium* were retested at lower dosage and ED_3 values were derived from the control ratings which were obtained.

Soil Disease Tests. Compounds which received a rating of 4 or 5 on *Pythium* or *Rhizoctonia* were evaluated for control of damping-off diseases of peas and cotton, respectively. For these determinations, soil which had been inoculated with the pathogen was transferred to 4-inch clay pots and treated with 75 ml. of the standardly prepared aqueous dispersion of the chemical. Three test dosages and two replicates per dosage for each compound were tested. Following an incubation period of 2 days at 21° C. and 95% relative humidity the pots were seeded with peas (for the Pythium test) and cotton (for the Rhizoctonia test) and returned to the incubator until the seeds germinated. Approximately 7 days after planting, a count was made of the number of disease-free seedlings per pot. The average per cent germination was converted to numerical ratings as follows: 5 = 90 to 100% healthy seedlings, 4 = 70 to 89, 3 = 50 to 69, 2 = 25 to 49, 1 = 0 to 24.

ED_3 values for each compound were derived from these ratings.

Foliage Disease Control Tests. Protectant disease control evaluations were conducted with tomato early blight (*Alternaria solani*) and cucumber anthracnose (*Colletotrichum lagenarium*). The plants were first sprayed to run-off with formulated chemical on a modified Campbell turntable (Sousa and Spurr, 1967; 1968). After the plants dried, they were inoculated with the appropriate fungal spore suspension. Eradicant disease control evaluations were made with bean powdery mildew (*Erysiphe polygoni*), bean rust (*Uromyces phaseoli*), and apple scab (*Venturia inaequalis*) by inoculating the plants 24 hours prior to applying the chemicals. For tomato early blight, cucumber anthracnose, and bean powdery mildew, a 100 p.p.m. of chemical dispersion in water was applied and for bean rust similar applications containing 1000 p.p.m. of chemical were made. Following an appropriate incubation period, the disease control effectiveness of the treatments was rated as follows: 5 = 100% control (no disease), 4 = infection, 3 = moderate infection, 1 = heavy infection equal to untreated controls. Compounds which received a rating of 4 to 5 in preliminary tests were retested at lower dosages in order to quantitate the observed activity. ED_3 values were derived from these ratings.

Several of the compounds which showed good control of early blight of tomato or of cucumber anthracnose were evaluated as apple scab eradicants employing methods similar to those described for the other foliar pathogens.

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