The Relationship between the Constitution and the Effect of Chemical Compounds on Plant Growth

2. QUATERNARY AMMONIUM SALTS

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It has been recorded by Jones, Metcalfe & Sexton (1949) that certain quaternary ammonium salts containing the substituted phenoxyethyl radical exert a differential effect upon the germination of oat and rape seeds and it was suspected that the activity of some of them might be dependent upon their quaternary salt function rather than the presence of substituted phenoxyethyl radicals. Some quaternary ammonium salts are known to react readily with compounds containing thiol groups, and in this respect they resemble $\alpha\beta$ -unsaturated ketones and lactones. The bactericidal activity of these ketones and lactones has been attributed to their ability to react with thiols (Geiger & Conn, 1945; Cavallito & Haskell, 1945). Since $\alpha\beta$ -unsaturated lactones are also active in suppressing the germination of seeds (see, for example, Veldstra & Havinga, 1943, 1945) it was considered possible that quaternary ammonium salts which react with thiols, for example, those containing benzyl radicals, might show herbicidal activity. Support for this hypothetical basis for biological activity in quaternary salts containing benzyl radicals was obtained in preliminary experiments which showed that the thiol-dependent enzymes, urease and glyoxalase, were inhibited by several of them. Evidence for the importance of thiol enzymes in plant growth has recently been provided by Thimann & Bonner (1949).

METHODS

Preparation of compounds

The compounds were obtained by the conventional method, by reaction of alkyl or benzyl halides or dimethyl sulphate with a tertiary base (with or without a solvent). Methosulphates were converted to iodides by the addition of NaI or KI to their aqueous solutions. Yields were generally good. The compounds which are new are listed in Table 1, together with their physical characteristics and analytical data. A typical preparation, that of methyldiethylbenzylammonium iodide, is described below.

Methyldiethylbenzylammonium iodide. (i) *Direct method.* Methyl iodide (2 ml., 3.5 mol.) was added to a solution of diethylbenzylamine (1.46 g., 1 mol.) in methanol (10 ml.).

After warming for 15 min. the cooled reaction mixture was treated with ether (35 ml.) and the crystalline precipitate separated, washed with ether and dried. Yield 1.5 g. (55%), m.p. 147–149°. Recrystallization from ethanol or water afforded material, m.p. 149–151°. Alternatively, the base was warmed with methyl iodide without a solvent and the resulting solid purified by recrystallization. (ii) *Through the methosulphate.* Diethylbenzylamine (1.08 g., 1 mol.) and methyl sulphate (2 ml., 3.2 mol.) were dissolved in methanol (10 ml.) and warmed for 0.5 hr. After cooling and addition of ether (40 ml.) the lower oily layer of quaternary salt was washed with ether, dissolved in water (10 ml.) and, after clarification with charcoal, treated with excess of KI. The precipitated quaternary iodide (1.3 g., 64%) was separated and purified, m.p. 149–151°, by recrystallization from water.

Biological methods

The biological test methods, using germinating oat and rape seeds on agar in test tubes, are described by Jones *et al.* (1949). In the tables which follow, two plus signs indicate the highest measure of activity (at least 50% inhibition of root length in more than half the seeds). Control seeds were grown on agar.

RESULTS

The results of routine tests are recorded for comparative purposes in Tables 2–4. Table 2 indicates the effect of varying molecular weight upon the activities of quaternary ammonium salts, using variation of alkyl chain length (the alkyl groups being of the normal series except where otherwise indicated) and using aromatic groups in certain instances.

Attention was next directed (Table 3) towards benzyl compounds, as it was desirable to know whether the benzene ring substituents which are known to increase activity in the phenoxyacetic acid series had similar effects in the quaternary benzyl ammonium salts. We studied the effect of introduction of chlorine or nitro in the aromatic ring and the effect of the fusion of a second ring (giving 1-naphthyl) on the biological activity of quaternary derivatives of trimethylammonium, methyldiethylammonium, morpholinium and piperidinium ions. In each group of compounds the anion was identical.

Table 1. *New compounds prepared*

Compound		Melting point	Analysis	
Cation	Anion		Found	Required
$\text{C}_6\text{H}_5\cdot\text{CH}_2\text{N}^+\text{MeEt}_2$	I^-	149–151°	C, 46.9 H, 6.9	47.2 6.6
$\text{C}_6\text{H}_5\cdot\text{CH}_2\text{N}^+\text{Me}$	I^-	126°	I, 38.9	40.0
$\text{C}_6\text{H}_5\cdot\text{CH}_2\text{N}^+\text{Me}$	I^-	152–156°	I, 40.1	39.9
$p\text{-Cl}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\text{N}^+\text{Me}$	I^-	171°	N, 4.3	4.0
$2:4\text{-Cl}_2\cdot\text{C}_6\text{H}_3\cdot\text{CH}_2\text{N}^+\text{Me}_3$	I^-	208° (sinters 192°)	C, 34.3 H, 4.3	34.7 4.1
$2:4\text{-Cl}_2\cdot\text{C}_6\text{H}_3\cdot\text{CH}_2\text{N}^+\text{MeEt}_2$	I^-	165° (sinters 148°)	C, 38.7 H, 4.9	38.6 4.5
$2:4\text{-Cl}_2\cdot\text{C}_6\text{H}_3\cdot\text{CH}_2\text{N}^+\text{Me}$	I^-	88–90° (with 1 mol. H_2O)	C, 33.5 H, 7.9	33.5 8.1
$1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{Et}_2\text{H}$	Cl^-	214°	C, 71.3 H, 7.9	75.8 8.1
$1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{H}$	Cl^-	233°	C, 68.2 H, 6.7	68.5 6.8
$1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{H}$	Cl^-	229°	C, 72.7 H, 7.5	73.2 7.7

The following new compounds were also made, but were not analysed:

$[p\text{-Cl}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\text{N}^+\text{Me}_3]\text{Cl}^-$, hygroscopic solid; $[2:4\text{-Cl}_2\cdot\text{C}_6\text{H}_3\cdot\text{CH}_2\text{N}^+\text{H}]\text{Cl}^-$, m.p. 102–103°;

$[1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{MeEt}_2]\text{I}^-$, could not be crystallized;

$[1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{Me}]\text{I}^-$, m.p. 200°; $[1\text{-C}_{10}\text{H}_7\cdot\text{CH}_2\text{N}^+\text{Me}]\text{I}^-$, m.p. 206°.

In addition to the above compounds, the following sulphonic acid derivatives were found to be completely inactive at 100 p.p.m.

Attention was finally directed to the effect of variation of substituents on the nitrogen atom of quaternary benzylammonium or substituted benzylammonium ions (Table 4). There was some anion variation dictated by the immediate availability of compounds for test, but sufficient valid comparisons were available to permit an assessment of the effect of structural changes in the cation.

DISCUSSION

Marked herbicidal activity in quaternary salts containing *n*-alkyl chains does not appear until the decyl compound. Higher in the series the activity falls off, at least against rape (Table 2). This is a typical homologous series effect in which the variation in biological activity is largely determined by the physico-chemical properties of the molecule. The highest herbicidal activity occurs when the compounds have marked surface activity as judged by the foaming of aqueous solutions, and in this the bactericidal and fungicidal properties of the same compounds are recalled. There is clearly a maximum activity against rape between C_{10} and

C₁₄ though its position has not been determined with precision. The position of the maximum against oats is not revealed by the results, but it would appear that it is probably at a somewhat greater chain length than in the case of rape. This difference results in the appearance of selective action in the case of the octadecyl compound, which has passed the peak with rape, but is still very active against oats. It is to be noted that molecular weight, as such, is not a factor determining activity. The last three compounds in Table 2, containing naphthyl radicals, are of low activity compared with *n*-decyl- and *n*-dodecyl-trimethylammonium halides, which are of comparable molecular weight.

In Table 3, it is shown that the introduction of chlorine into the benzene ring or substitution of phenyl by α -naphthyl does not cause the enhancement of activity characteristic of the substituted phenoxyacetic acids. Nor does the *p*-nitro group have a marked effect. A further difference from the phenoxyacetic acid series is seen in the sensitivity of oats to the benzyl substituted quaternary salts and the insensitivity of rape. It is interesting to note that, in the groups of compounds recorded in Table 3, activity is but slightly influenced, if at all, by substituents in the benzyl radical, whereas it is very considerably influenced by the other substituents on the nitrogen atom. Thus very little activity is shown by quaternary salts derived from *N*-methylmorpholine or ethyldimethylamine, but considerable activity in those derived from tri-

Table 2. *Activities of homologous quaternary ammonium salts on the germination of rape and oats*

(Activities in this and subsequent tables shown by + and - signs as stated in text (p. 110).)

Compound [Me ₃ N+R]X ⁻		Concentration (p.p.m.)	Activity	
R	X		Rape	Oats
CH ₃	I	100	-	+
C ₂ H ₅	I	50	-	+
C ₃ H ₇	Br	100	-	-
C ₄ H ₉	Br	50	-	-
C ₆ H ₁₃	Br	50	-	-
C ₇ H ₁₅	Br	50	-	-
C ₈ H ₁₇	Br	50	-	+
C ₁₀ H ₂₁	Br	50	++	++
		10	++	+
		1	-	.
C ₁₂ H ₂₅	Br	50	++	++
		10	++	++
C ₁₄ H ₂₉	Br	50	++	++
		10	++	++
C ₁₆ H ₃₃	Br	50	+	++
		10	.	++
C ₁₈ H ₃₇	Cl	50	+	++
C ₃ H ₅ (allyl)	I	50	-	+
C ₆ H ₅	I	50	-	++
		10	.	+
C ₆ H ₅ CH ₂	Cl	50	-	+
compound				
[1-C ₁₀ H ₇ CH ₂ N+Me ₂ R]Cl ⁻	R			
CH ₃		100	-	++
		50	.	+
C ₂ H ₅		100	-	+
C ₃ H ₇		100	+	+

Table 3. *Effect of variation of substituents in the benzyl radical of quaternary ammonium salts on the activities against germinating rape and oats*

Compound		Concentration (p.p.m.)	Activity	
Type	Radical R		Rape	Oats
[Me ₃ N+CH ₂ R]Cl ⁻	C ₆ H ₅	50	-	+
	<i>o</i> -ClC ₆ H ₄	50	-	+
	2:4-Cl ₂ C ₆ H ₃	100	+	++
		50	.	++
		10	.	+
	<i>p</i> -NO ₂ C ₆ H ₄	50	-	+
	1-C ₁₀ H ₇	100	-	++
		50	.	+
[EtMe ₂ N+CH ₂ R]I ⁻	C ₆ H ₅	50	-	+
	2:4-Cl ₂ C ₆ H ₃	100	-	+
	1-C ₁₀ H ₇	100	-	+
N+MeCH ₂ R]I ⁻	C ₆ H ₅	100	-	+
	<i>o</i> -ClC ₆ H ₄	100	-	+
	2:4-Cl ₂ C ₆ H ₃	100	-	+
	<i>p</i> -NO ₂ C ₆ H ₄	100	-	+
	1-C ₁₀ H ₇	100	-	+
N+MeCH ₂ R]I ⁻	C ₆ H ₅	100	-	++
		50	.	++
	<i>o</i> -ClC ₆ H ₄	50	-	++
	1-C ₁₀ H ₇	50	-	++
		10	.	+

Table 4. *Effect of variation of the N-substituents on the activities of quaternary ammonium salts containing benzyl and substituted benzyl radicals against germinating rape and oats*

Type	Compounds		Concentration (p.p.m.)	Rape	Oats
	Radicals R	Anion X			
[C ₆ H ₅ CH ₂ N ⁺ R ₃]X ⁻	Me ₃	Cl ⁻	50	-	+
	Et ₃	I ⁻	50	-	++
			10	.	-
	Me.Et ₂	I ⁻	50	-	+
	Me ₂ .Ph	I ⁻ , Cl ⁻ , Br ⁻ and SO ₄ Me ⁻	50	-	++
			10	-	+
	Me.Et.Ph	I ⁻	100	-	++
			50	.	-
	Me.C ₅ H ₁₀ (piperidine)	I ⁻	50	-	+
	Me.C ₄ H ₈ O (morpholine)	I ⁻	100	-	+
	C ₅ H ₅ (pyridine)	Cl ⁻	100	-	+
[2:4-Cl ₂ C ₆ H ₃ CH ₂ N ⁺ R ₃]I ⁻	Me ₃		100	+	++
			50	.	++
			10	.	+
	Me.Et ₂		100	-	+
	Me.C ₄ H ₈ O (morpholine)		100	-	+
	C ₅ H ₅ (pyridine)		50	-	++
			10	.	+
[1-C ₁₀ H ₇ CH ₂ N ⁺ R ₃]I ⁻	Me ₃		100	-	++
			50	.	+
	Me.Et ₂		100	-	+
	Me.C ₄ H ₈ O (morpholine)		100	-	+
	Me.C ₅ H ₁₀ (piperidine)		100	+	++
			50	.	++
	C ₅ H ₅ (pyridine)		50	-	++
			10	.	+

methylamine and *N*-methylpiperidine. From the selective action and from the absence of surface-active properties in this group of compounds, it is to be concluded that their mode of action is different from that of the normal alkyl compounds recorded in Table 2. In spite of this, the physical properties of the quaternary benzyl compounds are not without significance, as is shown by the complete loss of activity on introduction into the molecule of a sulphonic acid group.

The realization of the decisive part played by the other substituents on the nitrogen atom of quaternary benzylammonium salts prompted further study. The results given in Table 4 provide additional information on the effect of substituents and include also a comparison of the effects of different anions with the same cation. At the concentrations examined these showed no differences. It will be seen that from the point of view of achieving the highest degree of biological activity, pyridine was a much better base than *N*-methylmorpholine or methyldiethylamine in the 2:4-dichlorobenzyl series, but this was not so in the unsubstituted benzyl series. Again, trimethylamine is a favourable base

in the 2:4-dichloro series, but not in the 1-naphthyl series.

Table 5. *Comparison of herbicidal activities with the alkylating activities of quaternary benzyl cations*

(Herbicidal activities measured against oats at 50 p.p.m.)

Cation	Herbicidal activity	Alkylating activity
PhCH ₂ N ⁺ Et ₃	++	Slightly reactive
PhCH ₂ N ⁺ Me ₃	+	Slightly reactive
Ph.CH ₂ N ⁺	+	Slightly reactive
PhCH ₂ N ⁺ Me ₂ Ph	++	Reactive
Ph.CH ₂ N ⁺	+	Reactive
Ph.CH ₂ N ⁺ Me ₃	++	Not reactive

It is of interest to compare the herbicidal activities of the benzyl quaternary salts with their alkylating (benzylating) ability. Although accurate quantitative data on the chemical side are lacking,

the work of Snyder & Speck (1939) and Snyder, Smith & Stewart (1944) on the benzylation of thiol compounds, acetoacetate and malonate provides useful information. From this and the data in Tables 1 and 4, the comparisons given in Table 5 have been made. Thus it can be seen that chemical affinity for a reactive hydrogen does not appear to run parallel with biological activity.

As has been suggested in the case of contact insecticides (Davies & Sexton, 1948), it is probable that biological activity depends upon having the correct degree of chemical reactivity in the group concerned, balanced with physical properties which ensure maximum ease of penetration to the site of action.

After our work had been completed, a paper by Mitchell, Wirwille & Weil (1949) appeared, recording plant-growth regulatory activity in certain quaternary salts containing benzyl and substituted benzyl radicals. Prill, Barton & Solt (1949) have also observed a reduction of the growth of wheat roots by various surface-active compounds of which the most active were two quaternary ammonium salt derivatives, both of which reduced root growth by 50 % at concentrations of 5 p.p.m.

SUMMARY

1. The herbicidal activity of quaternary ammonium salts has been examined by a laboratory seed-germination technique, using rape and oats.

2. In the homologous series of *n*-alkyltrimethyl ammonium salts activity against both species rose as the series was ascended. In the case of rape, activity diminished after C₁₄. In the case of oats the position of the maximum was not determined, but it appeared to be higher.

3. In a series of quaternary salts containing benzyl or substituted benzyl radicals, oat seeds were more susceptible than rape.

4. In contrast to the results in the phenoxyacetic acid series, the herbicidal activity was but little affected by the introduction of substituent groups (e.g. chlorine) into the benzene ring. A sulphonic acid group brought about complete inactivation.

5. The activity was greatly influenced by the nature of the other substituent groups on the nitrogen atom.

6. The results are discussed in relation to the known ability of certain quaternary salts to alkylate thiol groups.

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Fluorescence of Riboflavin and Flavin-adenine Dinucleotide

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The fluorescence of riboflavin has been extensively studied with a view to the determination of this substance. Relatively little work has been done on the variations of this property in the compounds of riboflavin of biological importance such as flavin-adeninedinucleotide (FAD) and the flavoproteins.

The variations in fluorescence under different conditions, and notably after addition of quenchers, can give some indication of the configuration of a

molecule. The following is a study of the fluorescence of riboflavin and FAD from this point of view.

EXPERIMENTAL

The intensity and the degree of polarization of the fluorescent light and the absorption spectrum were determined with solutions of riboflavin and FAD under various conditions.