Anal. Calcd for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07. Found: C, 69.48; H, 9.72.

2-Methyl-3-oxocyclohexylacetic Acid (IXi′) (Ravid and Ikan, 1974a) (bp 175° (0.2 mm); yield, 89%): ir (liquid) 3050–3100, 1710, 1410, 1310, 1220, 1170 cm^{-1}; NMR (CCl_4) δ 1.04 (3 H, d), 1.33–2.75 (1.0 H, m), 9.0 (1 H, s). Anal. Calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.27; H, 7.96.

Methyl (2-Methyl-3-oxocyclohexyl)acetate (IXj′) (Ravid and Ikan, 1974a) (bp 145–147° (0.2 mm)): ir (liquid) 1735, 1710, 1440, 1345, 1320, 1260, 1225, 1165 cm^{-1}; NMR (CCl_4) δ 1.03 (3 H, d), 1.3–2.6 (10 H, m), 3.56 (3 H, s); semicarbazone, mp 175–177°. Anal. Calcd for $C_{11}H_{19}N_3O_3$: C, 54.76; H, 7.94; N, 17.41. Found: C, 54.63; H, 8.08; N, 17.09.

2-Methyl-3-pentylcyclohexan-1-one (IXk′) (Ravid and Ikan, 1974a) (bp 85° (0.2 mm); yield, 63%): ir (liquid) 1710, 1460, 1379, 1145, 955 cm^{-1}; NMR (CCl_4) δ 0.92 (6 H, m), 1.08–1.72 (10 H, m), 1.92–2.26 (6 H, m); semicarbazone, mp 159–160°. Anal. Calcd for $C_{13}H_{25}N_3O$: C, 65.23; H, 10.53; N, 17.55. Found: C, 65.20; H, 10.34; N, 17.87.

2-Methyl-3-dimethylmalonylcyclohexan-1-one (IXl′) (Ravid and Ikan, 1974a) (bp 112–114° (0.3 mm); yield, 78%): ir (liquid) 1735, 1712, 1440, 1230, 1160, 1010 cm^{-1}; NMR (CCl_4) δ 1.05 (3 H, d), 1.50–2.59 (8 H, m), 3.55 (1 H, d), 3.65 (6 H, s). Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.49; H, 7.49. Found: C, 59.70; H, 7.00.

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Mode of Action of 2-Hydroxycyclohexyl Quaternary Ammonium Plant Regulators

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A direct correlation between enzyme inhibition and plant growth retardation has been demonstrated for 10 *n*-alkyl derivatives of 2-hydroxycyclohexyldimethylammonium bromide. Enzyme inhibition data were obtained using both butyrylcholinesterase from human blood serum and acetylcholinesterase isolated from mung bean (*Phaseolus aureus*). Growth retardant activity was determined for alfalfa (*Medicago sativa* L., Hairy Peruvian), cucumber seed radicles (*Cucumis sativus* L., Marketer), and grapefruit seed radicles (*Citrus paradisi* Macf., Duncan). Maximum serum butyrylcholinesterase inhibition occurred when the alkyl chain attached to nitrogen had 12 carbons while the maximum for bean acetylcholinesterase inhibition occurred at a chain length of 14 carbons. The three growth tests showed maximum activities for those compounds in which the *n*-alkyl group had 11 to 13 carbons. The inhibition of bean root cholinesterase was competitive and reversible. These correlations between growth regulation and enzyme inhibition suggest that these quaternary ammonium derivatives may act on an enzyme-mediated step essential to plant growth.

There is evidence that certain quaternary ammonium growth regulators retard plant growth by blocking GA biosynthesis (Lang, 1970). Fall and West (1971) purified Kaurene synthetase from cell-free extracts of the fungus *Fusarium moniliforme* L. and showed inhibition of the enzyme by Amo-1618, Phosfon D, Phosfon S, and several quaternary ammonium derivatives of (+)-limonene. Riov and Jaffe (1973a) recently reported the isolation of a cholinesterase (ChE) from mung bean (*Phaseolus aureus*) roots and its irreversible, noncompetitive inhibition by Amo-1618 (Riov and Jaffe, 1973b). They also have shown an excellent correlation between bean ChE inhibition and retardation of the growth of secondary bean roots for a number of plant growth regulators (Riov and Jaffe, 1973c). A correlation has been reported between growth retardant activity and serum ChE inhibition for a series of quaternary ammonium derivatives of (+)-limonene (Newhall, 1969, 1971).

We have studied the correlation between both human blood serum ChE and bean root ChE inhibition and growth retardation for a new series of ten plant growth regulators derived from 2-dimethylamino-1-cyclohexanol (Newhall, 1974).

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MATERIALS AND METHODS

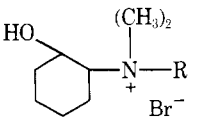
Compound Preparation. The synthesis and characterization of the ten quaternary ammonium derivatives used in this study have been reported (Newhall, 1974).

Sources of Enzymes. Human blood serum diluted 1 to 10 was used as a source of serum ChE. Mung bean ChE was isolated from roots of 12-day-old light-grown seedlings as previously described (Riov and Jaffe, 1973a).

Enzyme Assays. Serum ChE inhibition was determined using standard Warburg manometric techniques (Newhall, 1969). Bean root ChE inhibition was measured photometrically using a modification of the method of Ellman et al. (1961) (Riov and Jaffe, 1973a).

Growth Tests. Three bioassays were used to determine the regulant activities of the ten test compounds. The most sensitive test was based on the radicle growth of cucumber Marketer seed at concentrations of the test compound between 0.5 m*M* and 20 μM. This bioassay procedure, as well as a second test based on the growth response of Hairy Peruvian alfalfa seedlings, has been described previously (Newhall, 1969, 1971). In the third growth test, grapefruit seeds, after removal of both seed coats, were placed in Petri dishes on Whatman No. 1 paper wetted with various con-

Table I. Enzyme and Plant Growth Inhibition of Quaternary Ammonium Derivatives of 2-Dimethylamino-1-cyclohexanol



No. of carbons in R	Serum ChE inhibition, I_{50}, mM	Bean ChE inhibition, I_{50}, mM	Cucumber growth inhibition, I_{50}, mM	Alfalfa growth inhibition, I_{50}, mM	Grapefruit growth inhibition, I_{50}, mM
7	0.37	5.2	0.32	40	14.0
8	0.107	2.6	0.22	14	7.9
9	0.064	1.8	0.035	12	4.8
10	0.023	0.95	0.055	9.5	3.3
11	0.009	0.82	0.016	6.6	2.2
12	0.007	0.36	0.048	6.2	1.4
13	0.019	0.27	0.033	14	1.0
14	0.052	0.11	0.12	11	1.4
15	0.050	0.75	0.044	17	5.6
16	0.065	0.27	0.19	12	3.8

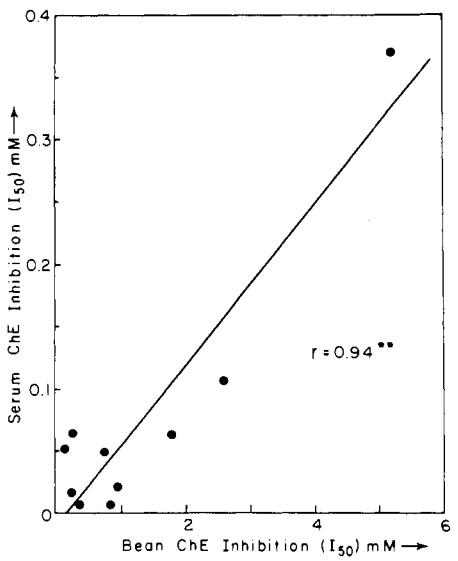


Figure 1. Correlation between bean ChE and serum ChE inhibition for ten *n*-alkyl derivatives of 2-hydroxycyclohexyldimethylammonium bromide.

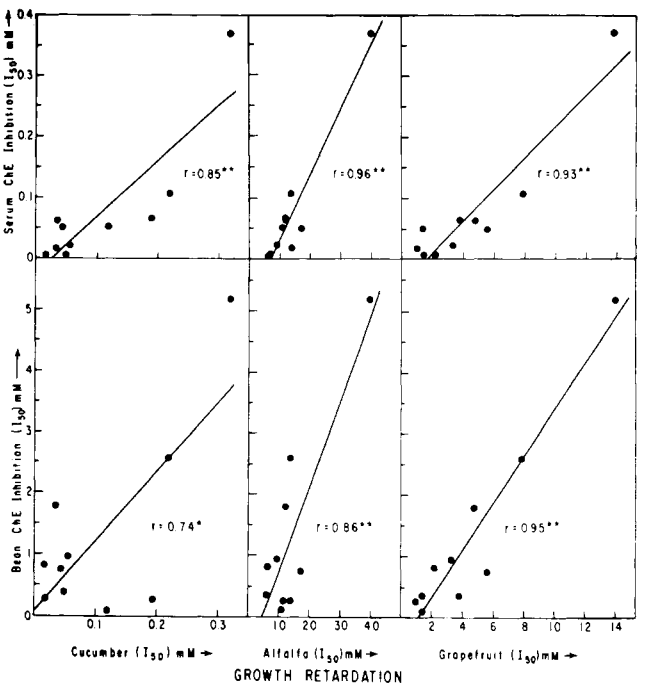


Figure 2. Correlations between bean ChE, serum ChE, and growth retardation of cucumber, alfalfa, and grapefruit.

centrations of test compound. Radicle lengths were measured after 14 days incubation in the dark at 25°.

RESULTS

The general formula of the ten test compounds is shown in Table I. R represents a saturated, straight chain of from 7 to 16 carbons. Concentrations of test compounds giving 50% enzyme inhibition or 50% growth retardation are recorded as I_{50}'s in millimoles per liter (mM). These I_{50} values were determined from semilog plots of concentration against enzyme inhibition or growth retardation as previously described (Newhall, 1971).

Serum ChE inhibition is plotted in Figure 1 against bean ChE inhibition for the ten quaternary ammonium derivatives (Table I). The line of best fit and correlation coefficient (r = 0.94**) were calculated by linear regression analysis. Double asterisks indicate statistical significance at the 99% level of confidence.

The upper three graphs in Figure 2 are plots of serum ChE inhibition against (from left to right) cucumber, alfalfa, and grapefruit growth retardation for the ten test compounds. The lower three graphs are similar plots for bean ChE inhibition. Lines of best fit and the correlation coefficients obtained by linear regression analysis are indicated in each graph. The single asterisk for bean ChE inhibition vs. cucumber growth retardation (r = 0.74*) indicates statistical significance at the 95% level of confidence.

A double reciprocal Lineweaver–Burk plot of the heptyl derivative (Table I) at two concentrations in the presence of bean ChE is shown in Figure 3. Each concentration was assayed for enzyme inhibition in the presence of four concentrations of the substrate, acetylthiocholine (ATCh): 4.6 $\times 10^{-4}$ M, 2.3 $\times 10^{-4}$ M, 1.15 $\times 10^{-4}$ M, and 5.57 $\times 10^{-5}$ M. Catalytic velocity was calculated as percent total activity at the highest substrate concentration. The line equations and therefore the intercepts and the slopes were obtained by linear regression analysis. Since this plot of l/velocity vs. l/substrate concentration shows an intercept for the uninhibited enzyme and the two concentrations of the heptyl

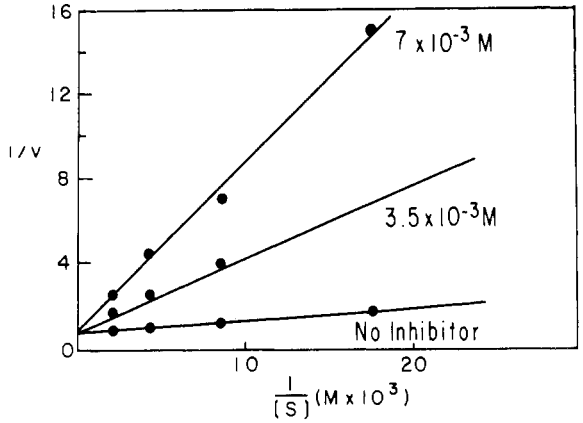


Figure 3. Lineweaver–Burk plot of the *n*-heptyl derivative (Table I) (V = % × 10^{-2} activity at highest substrate concentration).

derivative on the ordinate (Figure 3) inhibition is competitive.

To test for reversibility of inhibition, the dodecyl compound (Table I) at a concentration of 8.2×10^{-4} M and bean root ChE were dialyzed overnight against 4 l. of 0.01 M potassium phosphates (pH 7.0). Activity was compared to another aliquot of enzyme, similarly diluted and dialyzed overnight. The activity of the ChE plus the dodecyl compound after dialysis and the activity of the enzyme dialyzed alone were identical. Therefore, the inhibition is reversible.

DISCUSSION

In view of the striking correlation between inhibition of serum ChE and growth retardation previously reported (Newhall, 1969) for a number of *n*-alkyl and benzyl quaternary ammonium derivatives of (+)-limonene, it is not surprising that a similar correlation can be demonstrated for the structurally related quaternary ammonium derivatives of 2-dimethylaminocyclohexanol. The equally good correlation shown between inhibition of bean ChE and growth retardation of three test plants indicates that the two enzymes, although from very different sources, may have similar active sites. It has been shown that, unlike Amo-1618, the action of these new growth regulators on bean ChE is competitive and reversible.

The specific site(s) of action of these and other growth regulators is not known, although we can indicate several alternative explanations for their action. Since the growth retardant activity of these new plant regulators is effectively counteracted by added GA (unpublished data), they may interfere with GA biosynthesis. This would be a logical assumption since they are structurally related to the quaternary ammonium derivatives of (+)-limonene which have been shown to inhibit two enzyme-mediated reactions essential to GA biosynthesis (Fall and West, 1971). Alternatively, these growth regulators may exert their effect by interacting with a hypothetical receptor molecule or an allosteric binding site of some protein. Other enzymes which have been suggested as possible target sites for growth regulators are choline kinase (Tanaka and Tolbert, 1966), bean ChE (Riov and Jaffe, 1973a–c), and the transport system for choline (Infante and Kinsella, 1973; Tanaka and Tolbert, 1966).

The direct correlations demonstrated (Figures 1 and 2) between inhibition of two hydrolase enzymes and growth retardation of cucumber, alfalfa, and grapefruit provide strong presumptive evidence for an enzymatic mode of action by these ten quaternary ammonium compounds in plants.

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