(2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE AND RELATED COMPOUNDS AS PLANT GROWTH SUBSTANCES
II. EFFECT ON GROWTH OF WHEAT[1,2]

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In this series of publications, the action of (2-chloroethyl)trimethylammonium chloride and certain other compounds structurally related to the quaternary ammonium cation, $(CH_3)_3N^+CH_2\text{-}CH_2X$, are described as a class of plant growth substances. In the first paper (1), compounds were examined for biological activity by an assay with wheat seedlings which involved the measurement of the distance between the bases of the first two leaf blades. Three very active and closely related compounds were discovered in which X was a chloro, bromo or $=CH_2$ group, namely, (2-chloroethyl)trimethylammonium chloride, (2-bromoethyl)trimethylammonium bromide and (2,3-*n*-propylene)trimethylammonium bromide.[3]

The growth of wheat plants after various treatments with the three most active derivatives is described in this paper. The most characteristic growth change was a reduction in height of plants accompanied by an increase in stem diameter. In most respects the growth alterations after treatment with derivatives related to (2-chloroethyl)trimethylammonium chloride were the opposite from that obtained with gibberellin, and in addition, these effects were reversed by gibberellin. In fact, the actions of gibberellin and (2-chloroethyl)trimethylammonium chloride on the growth of plants have been found to be mutually antagonistic.

MATERIALS AND METHODS

The three quaternary ammonium salts used in the present investigation were synthesized by the reaction of trimethylamine with the corresponding dihalide, i.e., 1,2-dibromoethane for preparing (2-bromoethyl)-trimethylammonium bromide (1).

For routine experiments 15 to 18 wheat seedlings (*Triticum vulgare* of variety Thatcher unless otherwise specified) were grown in 8 in. pots with a sand and loam soil mixture. Greenhouse temperatures were 56° F ± 3 at night and 60° to 70° F during the day except as indicated otherwise. All data were obtained from experiments which were conducted during the winter and spring when light intensity in the greenhouse was low. Supplementary light from 500 watt incandescent bulbs extended the day length to 16 hours. Since the chemicals were neutral salts, aqueous solutions were used in the tests. Seedling plants were treated once at 11 days after planting the seed, at which time the second leaf was emerging. For soil treatments, 500 ml of a designated molarity of chemical was poured onto the soil. For spray treatments, solutions containing about 0.01 % aerosol OT as wetting agent were sprayed onto the leaves of the seedlings until the leaves were wet. Seed treatment consisted of placing seeds overnight on filter paper moistened with a solution of the chemical and then planting them immediately in untreated soil. Gibberellin was applied only by spray treatment to the leaves of seedling plants.

Two weeks after the soil or spray treatment, the distance from the base of the first leaf blade to the base of the second leaf blade for each plant was measured in millimeters. The data are average values from measurements on at least 15 plants. The distance between the second and third leaf blades at the time of the first measurement was rapidly increasing, and thus, this length was not consistent even in the controls. Therefore, four weeks after treatment, the total stem height of the plants from the soil to the base of the uppermost leaf blade was also recorded in some experiments.

Plants from a few treatments were kept to maturity to observe later growth patterns and yield of grain. In these cases, 12 in pots were used and Hoagland nutrient solution was applied about every ten days.

RESULTS AND DISCUSSION

TREATMENTS AND GENERAL EFFECT ON GROWTH: When wheat plants were treated once with either (2-chloroethyl)trimethylammonium chloride, (2-bromoethyl)trimethylammonium bromide or (2,3-*n*-propylene)trimethylammonium chloride or bromide, the major growth difference was the development of

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[3] In the literature these compounds have also been referred to as derivatives of choline. Thus, (2-bromoethyl)-trimethylammonium bromide has been called bromocholine bromide and (2-chloroethyl)trimethylammonium chloride would be called chlorocholine chloride.

TABLE I

LENGTH OF THATCHER WHEAT PLANTS AFTER TREATMENT WITH (2-CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE OR (2-BROMOETHYL) TRIMETHYLAMMONIUM BROMIDE*

TREATMENT	2 WEEKS MM	4 WEEKS MM
None	34	260
Soil application of (2-chloroethyl) trimethylammonium chloride		
10^{-2} M	−3	78
10^{-3} M	1	91
10^{-4} M	7	139
10^{-5} M	15	153
10^{-6} M	23	200
Soil application of (2-bromoethyl) trimethylammonium bromide		
10^{-3} M	5	75
10^{-4} M	9	131
10^{-5} M	13	171
10^{-6} M	18	188
Leaf spray of (2-bromoethyl) trimethylammonium bromide		
10^{-2} M	6	...
10^{-3} M	9	...
5×10^{-4} M	14	...

* The measurements at 2 weeks after treatment were the distance between the bases of the first and second leaf blades. The values at 4 weeks after treatment represented the total stem length to the base of the uppermost leaf. Values are the average from 15 to 18 plants.

plants with shorter and thicker stems than in untreated plants (all figures and tables) and the leaves were of darker green color. Growth effects from the three compounds were the same and they were active over the same range of concentrations. Most of the experiments were repeated with all three of the compounds, but only one chemical name, (2-chloroethyl)-trimethylammonium chloride, is used in this manuscript to represent this group of three compounds.

Soil treatment of seedlings was effective after one application of 10^{-2} to 10^{-6} M solutions (table I). For (2-chloroethyl)trimethylammonium chloride, a 10^{-6} M solution is equivalent to 0.13 ppm. From one application of a 10^{-4} to 10^{-6} M solution to the soil 11 days after planting the seed, the distances between the bases of all leaf blades of Thatcher wheat plants were substantially reduced in length through their early vegetative growth cycle, but not at the time of heading. Corresponding to the decrease in stem length, the width of stems from treated plants was thicker than stems on control plants. The shorter and thicker stems resulted in wheat plants which grew very erect with no tendencies toward lodging.

Earlier treatments than used in the spray and soil experiments reported here caused the stems to be shorter than recorded in the tables. Solutions of 10^{-3} M or stronger reduced the first interleaf distance essentially to zero. Applications of 10^{-2} M solutions so reduced the elongated growth of the plant that the base of the second leaf remained below the base of the first leaf as indicated by a negative value in the table. As a result, the base of the second leaf grew out through the sheath of the older first leaf. At 10^{-2} M concentration total growth of the plants as measured by weight was at first reduced but there were no other toxicity symptoms.

The compounds could also be applied as a spray to the leaves (table I), in which case 10^{-2} M solutions were not toxic and the lowest concentration for effectiveness was in the range of 10^{-5} M. One soil application at the same molarity was more effective than one spray treatment, probably because large volumes of dilute solutions could be applied to the soil as a more continuous source.

After any of the chemical treatments, including 10^{-2} M solutions, and during the final growth of plants at the time of heading, the upper portion of the stems elongated to produce a plant of height nearer the untreated controls. Nevertheless, mature plants which had been treated once as seedlings with greater than 10^{-5} M concentrations of the compounds remained shorter than control plants.

Treated plants were by visual inspection darker green in color, and (2,3-*n*-propylene)trimethylammonium bromide was very effective in producing this response. Detailed results concerning the increase in chlorophyll content of leaves from a variety of treated plants will be presented in another paper. With wheat plants the stockier and compact appearance of the plants also gave the impression of greener plants.

The leaves of treated plants were also shorter in length and broader in width (fig 1). In a series of measurements 3½ weeks after one treatment of plants with 10^{-3} M (2-bromoethyl)trimethylammonium bromide, the last fully developed leaf of treated plants averaged more than 9 mm in width, while the same leaf on the control plants was 7 mm wide. This growth pattern in the leaves was consistent with the development of shorter and thicker stems. This measurement of leaves was not used for routine assay because of wide biological variation among plants and the fact that the numerical values were not as greatly different as those values on stem length.

SEED TREATMENT: Seeds were placed overnight on filter paper moistened with an aqueous solution of (2-chloroethyl)trimethylammonium chloride and then planted the next morning in untreated soil. Plants were grown at day temperatures of 75° to 85° F and in high light intensities obtained in May. The appearance of these plants was similar to that obtained from treatment of seedlings. The height and wet weight of the tops after 26 days are given in table II. The results indicate that seed treatment is an effective method for applying the chemical.

TILLERING: There was a pronounced and earlier tillering of the young wheat plants (table III) within a few days to two weeks after treatment with the chemical compounds. In greenhouse experiments in the winter, the control plants developed tillers near the time of heading, and as a result, the tillers had an

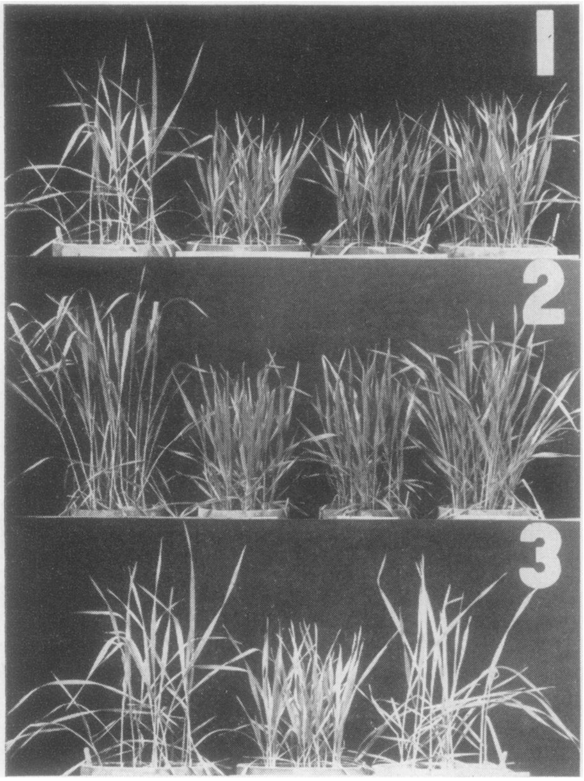

Fig. 1. Thatcher wheat seedlings 2 weeks after one soil treatment with (2-chloroethyl)trimethylammonium chloride as described in the text: from left to right, no treatment, 10^{-3}, 10^{-4}, and 10^{-5} M.

Fig. 2. Thatcher wheat 4 weeks after the same treatment as in figure 1.

Fig. 3. Combined effect of (2-bromoethyl)trimethylammonium bromide and gibberellin on Thatcher wheat. 10^{-3} M (2-Bromoethyl)trimethylammonium bromide was applied once to the soil of the two pots on the right 11 days after planting. Plants in the pot on the far right were sprayed with 100 ppm gibberellin 8 days later, and this photograph was taken after another 6 days or 14 days after the first treatment.

Table II

Height and Weight of Wheat after Seed Treatment with (2-Chloroethyl)trimethylammonium Chloride*

Treatment	1st to 2nd leaf MM	Total height** MM	Wet wt/ plant GM
0	19	72	0.7
10^{-1} M	7	42	0.8
10^{-2} M	8	48	0.8
10^{-3} M	11	52	0.7

* The method of treatment is described in the text. Measurements were made 26 days after planting the seed.
** From the soil to the base of the uppermost leaf.

appearance of a resurgent growth. In the treated plants almost all the tillers appeared shortly after treatment and grew almost as rapidly as the main culm, while there was correspondingly little or no tillering later during heading. This early tillering by treated plants was part of a growth pattern contributing to the appearance of bushier plants than the controls. Thus, the appearance of there being more plants in the treated pots (figure 1) was caused by the early tillering. The number of plants in each pot was the same, but the taller control plants without tillers appeared more thinly planted.

Uniformity of Growth: The number of leaves was not altered by the chemical treatments and the time of appearance of the early leaves was not appreciably delayed. Later leaves generally appeared 1 to 3 days after the corresponding leaf on the control plants. The treated plants were much more uniform in height than control plants (fig 1) and in time of heading of the grain. This uniformity in height of treated plants was accentuated by the fact that the tillers in the treated plants developed at nearly the same rate as the first stalk rather than developing later.

Rapidity and Persistence of Effect: The visual changes in the growth of wheat after one treatment with these compounds developed slowly over a period of two weeks. A difference in stem length between controls and treated plants could first be detected after three to five days. The persistence of the effects from one application of the compounds for weeks afterwards suggested that these substances were not rapidly destroyed in the plant and were translocated into the new leaves.

Wet Weight, Maturation, and Crop Yield: When low concentrations of (2-bromoethyl)trimethylammonium bromide were applied to the soil, there were no differences in wet or dry weight of the tops of Thatcher or Russell wheat plants 40 days after planting as compared with the controls, even though the treated plants were shorter in height. The wet weight of young seedlings also was not altered during growth after prior seed treatment, even though the growth pattern was strikingly different (table II).

Experiments on maturation of treated wheat plants were conducted in the greenhouse, but the results were only suggestive because of restrictions on growth induced by the pot and by insufficient light. There was no decrease in yield from treatments sufficient to reduce height of the plant, and in fact, some of the greenhouse experiments indicated an increase in yield by plants treated with 10^{-5} to 10^{-6} M solutions. This increase was accounted for by a higher weight per kernel of grain, since the total number of kernels per head and the total number of heads of grain per plant did not vary. All these experiments on growth suggested that the action of the chemical was to alter the developmental pattern rather than rate of growth, and thus the compounds have been called growth substances.

Table III

Effect on Tillering*

Treatment	No. of tillers/No. of plants**
0	3/16, 7/15, 3/16
10^{-3} M (2-chloroethyl)trimethylammonium chloride	20/16
10^{-4} M (2-chloroethyl)trimethylammonium chloride	20/18
10^{-3} M (2,3-*n*-propylene)trimethylammonium bromide	23/16
10^{-4} M (2,3-*n*-propylene)trimethylammonium bromide	19/16
10^{-3} M (2-bromoethyl)trimethylammonium bromide	21/18, 14/15
10^{-3} M (2-bromoethyl)trimethylammonium bromide + 100 ppm gibberellin***	1/17, 1/16
10^{-3} M (2-bromoethyl)trimethylammonium bromide + 100 ppm gibberellin†	11/16

* Tillers were counted 3 weeks after treatment with chemicals which were applied as described in the text.
** Different results for the same treatment were from different pots of plants.
*** 100 ppm gibberellin was sprayed on the plants either at the time of application of (2-bromoethyl)trimethylammonium bromide or up to eight days later with the same results.
† These plants were sprayed with gibberellin 14 days after the treatment with (2-bromoethyl)trimethylammonium bromide.

Treatments with these compounds also caused a delay in the time of final ripening of wheat plants in the greenhouse for at least two reasons. The control plants headed two to four days before the treated plants. In addition, during a two-week period after heading, when the wheat kernels were maturing, the treated plants remained greener than the control plants which slowly turned yellow. Thus, there was in greenhouse experiments an extended period during which the treated plant appeared more capable of furnishing the ripening seed extra carbohydrates.

Temperatures: In one series of experiments wheat plants were grown under three different conditions of temperatures: A. 65° to 70° F during the day and 55° to 58° F at night; B. 70° to 75° F during the day and 65° to 68° at night; C. about 85° F and above during the day and about 80° F at night. For good growth of wheat it is usually considered that night temperatures of 55° F or lower are necessary. When applied by soil treatment to plants at all three temperatures, (2-bromoethyl)trimethylammonium bromide had the same effect of decreasing the distance between the leaf bases, though the growth of the plants at the highest temperature was poor. The results suggest the possibility that the compounds may be beneficial to some plants by preventing severe elongation from the effect of high night temperatures. Although the compounds were most effective near the optimum growth temperature, they may be useful when applied at high temperatures where satisfactory growth of wheat cannot normally be obtained.

Light Intensity: The growth pattern obtained after treatment with (2-chloroethyl)trimethylammonium chloride is, in general, characteristic of growth in high intensity light. In this respect, these chemical compounds reversed the elongated growth pattern obtained in low light intensity. In total darkness or light intensities of about 10 ft-c, however, the compounds were without effect.

All the experiments reported in this paper were performed from November through February, during which months light intensity and total light obtained in Michigan were low. The compounds were retested during April when the light intensity in the greenhouse was much higher, and the conclusions were the same. At the higher light intensity obtained in April, however, the magnitude of the effect with the same concentration of the chemicals was somewhat less, since the distance between the bases of the first leaf blades of the control plants was less in high light intensity.

Other Grains: Since the chemicals were only tested extensively with Thatcher wheat, a spring wheat, a few other grains were treated. (2-Bromoethyl)trimethylammonium bromide was about equally effective on another spring wheat, Russell and the chemical was active also on Genesee, a winter wheat. (2-Bromoethyl)trimethylammonium bromide caused Montcalm barley and Gary oats to grow with shorter internodes (table IV), but the effects were less than on spring wheats. The treated barley plants in the greenhouse were sturdier and greener than the untreated controls.

Combined Treatments of (2-Chloroethyl)trimethylammonium Chloride and Gibberellin:

Table IV

Distance Between Bases of 1st and 2nd Leaf Blades of Barley and Oats after Treatment with (2-Bromoethyl)trimethylammonium Bromide*

Treatment	Montcalm barley mm	Gary oats mm
0	50	42
10^{-2} M	23	21
10^{-3} M	29	32

* Measurements were made 2 weeks after treatment of 11 day old seedlings.

The plant growth promoting action of the (2-chloroethyl)trimethylammonium chloride type of compound seemed to be by inspection opposite to the action of gibberellin. Gibberellin promoted stem elongation and the development of spindly plants, while (2-chloroethyl)trimethylammonium chloride produced shorter stems and stockier plants. Both classes of compounds by themselves were effective at low concentrations, though (2-chloroethyl)trimethylammonium chloride was active at as low as 0.1 ppm on the wheat, whereas 10 ppm of gibberellin was necessary to cause an appreciable effect by itself. After gibberellin treatment, leaves were lighter green than controls; after (2-chloroethyl)trimethylammonium chloride, the leaves were darker green. Certain combinations of the two chemical treatments produced plant growth of nearly normal appearance since the effects from one chemical were nullified by the other.

When excess gibberellin and (2-chloroethyl)trimethylammonium chloride were applied simultaneously to young Thatcher wheat seedlings, the action of gibberellin completely dominated so that there was no decrease in the height of the plants which had been treated with both compounds compared to plants treated with gibberellin only (table V, experiment A). Therefore, lower concentrations of gibberellin with excess (2-chloroethyl)trimethylammonium chloride were tried. At 10 ppm of gibberellin, 10^{-2} M (2-chloroethyl)trimethylammonium chloride prevented most of the elongation obtained from the gibberellin treatment. Similar results were obtained with 10 ppm gibberellin and 10^{-4} M solutions of the compounds. The action of gibberellin was particularly effective for increasing the stem length between the bases of the first two leaf blades, and (2-chloroethyl)-trimethylammonium chloride was only partially effective in preventing this elongation. At 10 ppm or less of gibberellin the action of (2-chloroethyl)trimethylammonium chloride dominated after about one to two weeks, so that the distance between the bases of the second and third leaf blades was very short. These results indicate that (2-chloroethyl)trimethylammonium chloride prevented or inhibited the growth expression from 10 ppm gibberellin. The results also illustrate loss of the gibberellin effect a few days after treatment and the persistence of one treatment of (2-chloroethyl)trimethylammonium chloride. One ppm of gibberellin by itself was without visible effect on the wheat, but 1 ppm of gibberellin was still able to reverse a substantial portion of the effect from excess (2-chloroethyl)trimethylammonium chloride.

Gibberellin reversed the effects of a (2-chloroethyl)trimethylammonium chloride treatment many days after the treatment with the 2-chloroethyl derivative (table V, experiment B and fig 3). Eight days after treatment with (2-chloroethyl)trimethylammonium chloride alone, short plants had developed as illustrated by the middle pot in figure 3. These plants

TABLE V

COMBINED APPLICATIONS OF (2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE AND GIBBERELLIN TO THATCHER WHEAT SEEDLINGS

TREATMENT	DISTANCE BETWEEN LEAF BASES AFTER 2 WKS		TOTAL STEM HEIGHT AFTER 4 WKS
	1ST-2ND	2ND-3RD	
	MM	MM	MM
Exp. A (simultaneous treatments)			
None	37	43	
100 ppm gibberellin	58	49	
100 ppm gibberellin + 10^{-2} M (2-chloroethyl)trimethylammonium chloride	60	44	
10 ppm gibberellin	48	47	
10 ppm gibberellin + 10^{-2} M (2-chloroethyl)trimethylammonium chloride	37	4	
1 ppm gibberellin	38	43	
1 ppm gibberellin + 10^{-2} M (2-chloroethyl)trimethylammonium chloride	18	7	
10^{-2} M (2-chloroethyl)trimethylammonium chloride	3	0*	
Exp. B			
None	34	32	260
10^{-3} M (2-bromoethyl)trimethylammonium bromide	5	0*	112
10^{-3} M (2-bromoethyl)trimethylammonium bromide + 100 ppm gibberellin simultaneously	55	24	258
10^{-3} M (2-bromoethyl)trimethylammonium bromide + 100 ppm gibberellin 3 days later	18	36	244
10^{-3} M (2-bromoethyl)trimethylammonium bromide + 100 ppm gibberellin 14 days later	5	0*	269

* The third leaf was present but the leaf base had not emerged from the sheath of the second leaf.

quickly and completely reverted to a normal or elongated plant after one application of gibberellin. When gibberellin was applied, elongation occurred most between the bases of leaf blades which were then forming, but also renewed growth took place all along the wheat stem if heading had not occurred.

At the time of heading of wheat which had been treated only with (2-chloroethyl)trimethylammonium chloride, elongation of the plants occurred so that the final height of the heads of grain was near that of the controls. It is possible that the production of natural gibberellin-like material during heading reversed the effect of the (2-chloroethyl)trimethylammonium chloride.

Experiments on the mutual antagonistic effect of gibberellin and (2-chloroethyl)trimethylammonium chloride on the growth of plants have not been explored in crop production. In greenhouse experiments, wheat, which had been treated with both gibberellin and (2-cholorethyl)trimethylammonium chloride so that growth in height was about normal, appeared greener and stouter than the untreated plants. Another possibility would be to initiate earlier lateral development by treatment with (2-chloroethyl)trimethylammonium chloride, and then with gibberellin stimulate normal or tall growth of both the main plant and the lateral developments. Early tillering produced by (2-chloroethyl)trimethylammonium chloride was prevented by simultaneous application of gibberellin (table III), but it would be impossible to reverse this effect if the gibberellin were applied after the tillers had become well established.

Summary

Compounds of the structure $(CH_3)_3N^+CH_2\text{-}CH_2X$ were active as plant growth substances when X was a Cl, Br or $=CH_2$ group. Solutions of (2-chloroethyl)trimethylammonium chloride, (2-bromoethyl)-trimethylammonium bromide and (2,3-*n*-propylene)-trimethylammonium bromide or chloride were effective from 10^{-2} to 10^{-6} M when poured onto the soil or used in nutrient culture. Though the chemicals were most effective when applied to the soil, they could also be sprayed on the leaves, or added by soaking of seeds before planting. One treatment was effective on the total growth of the plant. The most characteristic growth alterations were shorter and thicker stems, broader and greener leaves, earlier and stronger tillering, and more uniform growth. These alterations in growth occurred without a change in wet or dry weight. The growth changes were similar to those produced by high light intensity and the opposite from those caused by gibberellin. Gibberellin reversed the action of these compounds on growth and they reversed the effect of gibberellin.

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