Effects of β-Hydroxyethylhydrazine on Growth and Alkaloid Formation of *Datura stramonium*

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The aerial parts of *Datura stramonium* were sprayed with aqueous solutions of β-hydroxyethylhydrazine (BOH) in strengths of 1, 0.01, and 0.0001 per cent. Treatment with 1 per cent of BOH reduced the height 54 per cent and the total dry weight 67 per cent. Malformations which were induced in the plants are described. The formation of flowers was inhibited. The concentration of leaf-tops alkaloid was decreased 45 per cent, while root alkaloid was increased 61 per cent. The total alkaloidal content per plant was only 26 per cent of controls. The habit of the groups treated with 0.01 and 0.0001 per cent of BOH resembled the controls. The concentration of alkaloids of the 0.01 per cent BOH group was decreased 17 per cent in the leaf-tops, but increased 161 per cent in the roots. The total alkaloidal content of the roots was 230 per cent of controls. In the 0.0001 per cent BOH group increases of 11, 103, and 209 per cent were found in the concentration of alkaloid of the leaf-tops, stems, and roots, respectively. The total alkaloidal content of this group was increased 31 per cent per plant, 99 per cent in the stems, and 219 per cent in the roots.

GOWING AND Leeper (1) in 1955 demonstrated that β-hydroxyethylhydrazine (BOH)[1] induced flowering in pineapples. In subsequent work these authors (2) tested 39 hydrazine derivatives for induction or inhibition of flowering and toxicity to the pineapple plant and found that BOH was one of six hydrazine derivatives which forced the pineapple to flower. The first effect of BOH was retardation of stem elongation which then was followed by floral initiation (3, 4). Reed (5) has indicated that BOH forces flowering in pineapple plants, possibly by reducing auxin concentration. This compound also was found to be a potent inhibitor *in vivo* of animal diamine oxidase (5).

B995, known chemically as *N*-dimethylaminosuccinamic acid, can also be considered to be a hydrazine derivative, *viz.*, the 1,1-dimethylhydrazide of succinic acid. Reed (6) has indicated that inhibition of shoot elongation in dwarf and tall peas by B995 was correlated with the inhibition of the oxidation of tryptamine-2-^{14}C to indoleacetaldehyde-2-^{14}C in homogenates prepared from epicotyls of young plants treated with B995. Previous investigations (7, 8) in this laboratory have shown that B995 caused significant reductions in height, growth, and the total alkaloid content of *Datura tatula* and *D. innoxia*. Plant maturity was notably delayed. It was also found that a 2% aqueous spray of B995 caused significant increases in the concentration of stem alkaloids.

B995 has induced interesting effects on the growth of various *Datura* species, and since the chemical structures of this compound and BOH are closely related, it was decided to determine the effects of various concentrations of BOH on the growth and alkaloid synthesis of *D. stramonium* L. Furthermore, BOH has not been reported to have been tested on medicinal plants.

EXPERIMENTAL

Growth of Plants.—*D. stramonium* plants employed in this investigation were germinated from seeds obtained from plants grown in the Oregon State University drug garden in 1961. The germination of the seeds, transplantation of the seedlings into individual 1-gal. metal containers, and soil composition are described in a previous publication (9). The growth studies were performed under greenhouse conditions at temperatures varying from 85° F. at night to 95° F. during the day. A humid condition was maintained in the greenhouse.

Treatment Plan.—A total of 72 five-week-old plants was selected on July 1, 1965 (zero time), for uniformity as to size and height from a pool of 100 plants grown in individual 1-gal. metal containers. The experimental period which started at this time lasted 4 weeks. Three harvests were made—at 0, 2, and 4 weeks. Each harvest consisted of 6 plants from each of the 4 groups, *viz.*, control plants (untreated plants), plants treated with 1% of BOH, those treated with 0.01% of BOH, and plants receiving treatment with 0.0001% of BOH. The first harvest, at zero time, however, consisted of 24 plants which were considered controls since none had been treated. At zero time, the plants were labeled according to treatment and randomized on a greenhouse bench.

The treatment solutions were prepared on a v/v basis from a stock preparation of β-hydroxyethylhydrazine.[2] At zero time, the aerial portions of the plants were sprayed to run-off with an atomizer with the previously designated solutions to assure an approximate equal dosage on a volume-to-unit area relationship. Specially prepared paper shields

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[1] Marketed as Omaflora by Industrial Chemicals Division of Olin Mathieson Corp., New York, N. Y.
[2] The BOH was generously supplied by Dr. S. I. Cohen, E. R. Squibb and Sons, Division of Olin Mathieson Chemical Corp., New York, N. Y.

prevented the solutions from entering the soil. Treatment according to group was conducted in a separate room to prevent cross-contamination. The plants received four weekly treatments. Height measurements were taken twice weekly, and the plants were observed periodically for any morphological changes.

The dosage regimens employed in this investigation were determined by a preliminary experiment performed 3 months earlier on *D. stramonium* and *D. meteloides*. Groups of 5 plants from each species received biweekly treatments by spraying to run-off for 5 weeks with the following strengths (v/v) of BOH: 1, 0.1, 0.01, 0.001, 0.0001%, and distilled water (controls). The 1% strength of BOH drastically inhibited plant growth and was phytotoxic which was manifested by the appearance of necrotic areas on the leaf blades. In *D. stramonium* the total fresh weight of the plants was only 8.7% of controls, and the average height of the treated plants at the end of the fifth week was only 6.6 cm. *versus* 27.3 cm. for controls. In *D. meteloides* growth was even more drastically inhibited, the total fresh weight being only 1.2% of controls. In this species the average height of the treated group at the end of the fifth week was 5.4 cm. *versus* 29.8 cm. for controls. The plants receiving the 0.1% strength of BOH were shorter than controls, and growth (fresh weight) was decreased 15 and 45% in *D. stramonium* and *D. meteloides*, respectively. The plants treated with 0.01 and 0.001% of BOH closely resembled the controls, whereas a slight increase was noted in height and growth in those receiving the weakest concentration (0.0001%) of BOH. Thus, it was decided to employ weekly treatments instead of biweekly treatments, and to use the previously designated concentrations of the chemical.

Measurements and Harvesting.—Height measurements were taken twice weekly from the soil line to the apex of the highest leaf. At harvest time each plant was immediately washed and cleaned, and the moisture was removed with towels. The plant then was divided into leaf-tops, stem, and root portions. The leaf-tops and stems were cut into small segments, and the fresh weight of each portion was taken immediately. Dry weights were taken after drying 48 hr. in a forced-air drier at 60° C. The plant parts were then ground to a No. 40 powder in a Wiley mill, pooled according to treatment and harvest, and stored in colored glass containers in a desiccator until subsequent analyses were performed.

Alkaloid Analysis.—The dried plant parts, employing pooled samples, were assayed for alkaloid concentration, expressed as scopolamine, according to the Brummett-Sciuchetti method (10). Two extractions were made per group, and each of the extracts was analyzed in duplicate. When duplicate determinations did not agree within a reading of 0.005 absorbance, two further extractions and analyses were made for each group. The total alkaloidal content per plant and per plant organ was obtained by multiplying the dry weight of the plant by the per cent of alkaloids obtained from the alkaloid analyses, and expressing the results in milligrams.

RESULTS

Growth Effects.—Plants treated with 1% of BOH were drastically inhibited in growth. Deformative effects were also noted. The height of this group was only 46% of the controls at the terminal harvest (Fig. 1). The newly formed leaves of this group appeared to be slightly chlorotic, whereas the older leaves were a darker green in color. The young leaves also became curled and wrinkled, and a characteristic yellow splotching was noted among the veins of this group as the leaves matured (Fig. 2). The newly formed leaves were smaller and narrower than similarly formed ones of the controls. Contrasted with this, the older leaves of the group treated with 1% of BOH were thicker than the controls. Numerous necrotic areas varying from 15 to 30 mm. in diameter were noted usually in the upper portion of the blade of these leaves. The width of the blades and petioles of the older leaves

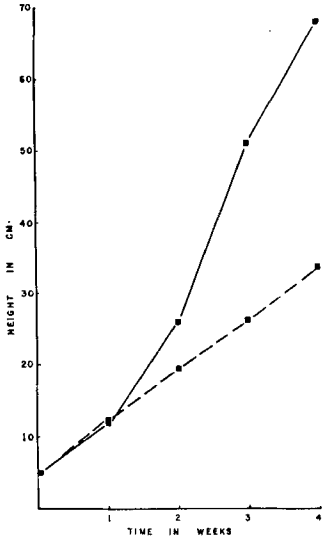

Fig. 1.—Effect of 1% BOH on height of *D. stramonium*. Key: ————, control; — — —, 1% BOH.

Fig. 2.—Habit of plants at final harvest. Key: X, untreated (control); A, 1% BOH.

TABLE I.—WEIGHT OF *D. stramonium* PLANT PARTS[a]

Treatment and Harvest Time, Wk.	Total Wt. Fresh, Gm.	Total Wt. Dry, Gm.	Total Wt. Control Dry Wt., %	Leaf-Tops Fresh, Gm.	Leaf-Tops Dry, Gm.	Leaf-Tops Control Dry Wt., %	Stems Fresh, Gm.	Stems Dry, Gm.	Stems Control Dry Wt., %	Roots Fresh, Gm.	Roots Dry, Gm.	Roots Control Dry Wt., %
Control, 0[b]	0.84	0.07	...	0.46	0.05	...	0.17	0.01	...	0.21	0.01	...
Control, 2	45.7	3.31	...	30.5	2.42	...	8.2	0.47	...	7.0	0.42	...
1.0% BOH,[c] 2	33.0	2.27	69	22.3	1.61	67	5.7	0.36	77	5.0	0.30	71
0.01% BOH, 2	38.0	2.45	74	24.2	1.74	72	7.3	0.37	79	6.5	0.34	81
0.0001% BOH, 2	43.6	2.93	89	28.8	2.09	87	9.8	0.51	109	5.0	0.33	79
Control, 4	171.5	18.81	...	68.0	7.54	...	74.8	8.80	...	28.7	2.47	...
1.0% BOH, 4	84.8	6.19	33	49.5	3.83	51	24.8	1.63	19	10.5	0.73	30
0.01% BOH, 4	155.7	16.91	90	61.3	6.57	87	69.2	8.16	93	25.2	2.18	88
0.0001% BOH, 4	157.1	18.19	97	63.3	7.01	93	67.3	8.62	98	26.5	2.56	104

[a] Mean weight per group of 6 plants. [b] Mean weight of all 24 plants which were harvested but not treated at zero time. [c] BOH is β-hydroxyethylhydrazine.

TABLE II.—CONCENTRATION OF ALKALOIDS OF STRAMONIUM PLANT PARTS[a]

Treatment and Harvest Time, Wk.	Leaf-Tops Alkaloids,[b] mg./Gm.	Leaf-Tops Control, %	Stems Alkaloids,[b] mg./Gm.	Stems Control, %	Roots Alkaloids,[b] mg./Gm.	Roots Control, %
Control, 0[c]	1.78	...	3.04	...	1.72	...
Control, 2	2.56	...	0.89	...	0.99	...
1% BOH, 2	2.38	93	1.11	125	1.80	182
0.01% BOH, 2	2.56	100	0.99	111	0.67	68
0.0001% BOH, 2	2.07	81	1.22	137	0.94	95
Control, 4	3.84	...	1.04	...	0.36	...
1% BOH, 4	2.12	55	1.09	105	0.58	161
0.01% BOH, 4	3.18	83	1.07	103	0.94	261
0.0001% BOH, 4	4.27	111	2.11	203	1.11	309

[a] Based on pooled samples of six plants per group. [b] Total alkaloids calculated as scopolamine. [c] Consisted of all four groups which were harvested but not treated at zero time.

TABLE III.—TOTAL ALKALOIDAL CONTENT[a] OF STRAMONIUM PER PLANT AND ORGAN

Treatment and Harvest Time, Wk.	Per Plant mg.	Per Plant Control, %	Leaf-Tops mg.	Leaf-Tops Control, %	Stems mg.	Stems Control, %	Roots mg.	Roots Control, %
Control, 0	0.14	...	0.08	...	0.04	...	0.02	...
Control, 2	7.04	...	6.20	...	0.42	...	0.42	...
1% BOH, 2	4.77	68	3.83	62	0.40	95	0.54	129
0.01% BOH, 2	5.05	72	4.45	72	0.37	88	0.23	55
0.0001% BOH, 2	5.26	75	4.33	70	0.62	148	0.31	74
Control, 4	38.99	...	28.95	...	9.15	...	0.89	...
1% BOH, 4	10.32	26	8.12	28	1.78	19	0.42	47
0.01% BOH, 4	31.67	81	20.89	72	8.73	95	2.05	230
0.0001% BOH, 4	50.96	131	29.93	103	18.19	199	2.84	319

[a] Calculated from dry weight and alkaloid analyses data; per plant = leaf-tops + stems + roots.

of this group was about 60% of the controls. The leaves of this group were extremely brittle. This brittleness was noted after the second treatment with 1% of BOH and became more pronounced with each subsequent treatment. Great care had to be taken in handling these plants since the leaves would break off from the plant simply by brushing against them. This characteristic was not noted in the other groups. The formation of floral buds and flowers did not occur in the 1% BOH group. On the other hand, the number of floral buds and flowers of the two groups receiving the weaker strengths of BOH was similar to the controls. The habit of the plants receiving treatments with 0.01 and 0.0001% of BOH in general resembled the controls.

Statistical analysis[3] of the height data indicated that the height of the plants treated with 1% of BOH was depressed significantly (at the 1% confidence level) as soon as 2 weeks following application of the chemical. The two weaker concentrations of BOH did not significantly alter the height of the plants.

The growth of the plants as indicated by dry weight data was markedly inhibited by treatment with 1% of BOH (Table I). Statistical analysis of the dry weight data indicated that with the lower concentrations (0.0001 and 0.01%) of BOH there was little difference in growth between treated and

[3] The statistical analyses of height and dry weight data were performed by Dr. R. G. Petersen, Oregon State University, Corvallis.

untreated plants. At a concentration of 1% of BOH, plant growth was depressed. This inhibition of growth became more pronounced with time. In considering the individual plant parts, the effect of 1% of BOH on the depression of growth was least pronounced in the roots and most pronounced on plant height. At the terminal harvest, total dry weight, leaf-top dry weight, and stem dry weight were significantly decreased at the 1% confidence level and root weight at the 5% confidence level.

Effect on Alkaloid Patterns.—At the terminal harvest the concentration of alkaloids of the group treated with 1% of BOH was reduced 45% in the leaf-tops, but increased 61% in the roots (Table II). Similar figures for the 0.01% BOH groups were a decrease of 17% and an increase of 161%. The concentration of stem alkaloids was not affected in either group. Significant increases in the concentration of alkaloids were found in the group treated with 0.0001% of BOH, *viz.*, twofold in the stems and threefold in the roots (Table II).

The total alkaloidal content of the plants receiving treatment with 1% of BOH was significantly decreased (Table III). At the terminal harvest, the total alkaloidal content per plant was only 26% of the controls. All of the plant organs indicated a significant reduction. This was due primarily to the inhibition of growth. Decreases of 19% per plant and 28% in the leaf-tops were noted in the total alkaloidal content of the group treated with 0.01% of BOH, while an increase of 130% was found in the roots. Significant increases of 31% in the leaf-tops and of 219% in the roots of the group receiving the 0.0001% treatment were noted.

DISCUSSION AND CONCLUSIONS

Treatment of *D. stramonium* with 1% of BOH definitely inhibited plant growth. Deformative effects were noted in the leaves. These were manifested by a curling and wrinkling of the newly formed leaves and the appearance of a characteristic yellow splotching among the veins of the leaves of the treated plants. The latter indicates a damaged root system which would limit growth. This suggests that the 1% strength of BOH was a growth inhibitor in *D. stramonium* rather than a growth retardant as has been indicated for B995 (3, 7, 8). Although BOH and B995 are closely related chemically, treatment of *Daturas* with approximately the same concentration of each chemical does not produce the same effect. The effects on the plants treated with 1% of BOH more closely resemble those noted when *Daturas* have been treated in this laboratory with maleic hydrazide or its ester. Other unusual effects noted in this group included the appearance of necrotic areas in the upper portion of the older leaves which had a reduction of about 40% in the width of their blades and petioles compared with leaves from untreated plants, an exceptional brittleness of the leaves, and inhibition of floral bud formation. Flowers did not appear in any of the plants of this group, while the formation of floral buds and flowers in the groups receiving 0.01 and 0.0001% of BOH approximated the controls.

The total alkaloidal content of the 1% BOH group was about one-fourth that of the controls at the final harvest. This was due primarily to the inhibition of growth, although the 45% reduction in the concentration of leaf-tops alkaloids was a contributing factor. The 61% increase in root concentration of alkaloids did not offset the latter since the roots represent slightly more than 10% of the total plant weight compared with 50% for the leaf-tops.

The habit of the plants treated with 0.01 and 0.0001% of BOH generally resembled the controls. Noteworthy was the effect that the treatments with the weaker strengths of BOH had on the concentrations of alkaloids in the plant organs. The concentrations of leaf alkaloids in the group treated with 0.01% of BOH was 83% of controls compared with 111% for the 0.0001% BOH group. This concentration was intermediate between the latter and the group treated with 1% of BOH. The concentration of root alkaloids was increased 161% in the 0.01% BOH group and 209% in the 0.0001% BOH group. This suggests that the synthesis and/or accumulation of alkaloids in this organ was significantly increased by the two weaker strengths of BOH. The only treatment which affected the concentration of stem alkaloids was that with 0.0001% of BOH which resulted in a twofold increase. Due to the phenomenal increase in the concentration of alkaloids in the stems and roots of this group, the total alkaloidal content per plant was increased 31%, that of the stems increased 99%, and that of the roots increased 219%. These increases were considered significant. No precise explanation is presently forthcoming as to why BOH in all three of the strengths employed significantly increased the concentration of root alkaloids.

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