Effects of Dimethylsulfoxide (DMSO) and B995 on Growth and Metabolic Products of *Datura innoxia*

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The aerial parts of *Datura innoxia* were sprayed with 2 per cent solutions of DMSO and B995 or a combination of the two. DMSO-treated plants resembled controls in habit. At the final harvest slight increases were found in the concentration of alkaloids in the plant organs but the total alkaloid content per plant was similar to the controls. The total chlorophyll content of the leaf-tops was reduced, while a 23 per cent increase was found in the water-soluble extractive. Significant reductions in height, growth and plant maturity, total alkaloid content, and chlorophyll content were found in the B995-treated plants. The concentration of stem alkaloids was increased 56 per cent at the second week and 90 per cent at the fourth week by the B995. A 14 per cent decrease was found in the alcohol-soluble extractive. When DMSO was combined with B995, the effect appeared to be additive.

In a previous investigation (1) *Datura tatula* was treated with 2% aqueous sprays of dimethylsulfoxide (DMSO) and *N*-dimethylamino succinamic acid (B995) and the following pertinent results were reported: slight reductions in plant weight and total alkaloid content per plant by the DMSO; significant decreases in plant height, total plant weight, and the concentration of root alkaloids by the B995; increased concentration of stem alkaloids by the B995; and potentiation of the inhibitory effects on height, growth, and alkaloid content by the DMSO when it was combined with B995. James and Sciuchetti (2) employed aqueous sprays of 100 to 1000 p.p.m. of B995 on *D. innoxia* (2), and Bennett and Sciuchetti (3) administered aqueous sprays of 1000 p.p.m. of B995 to *D. meteloides*. Significant retardation of stem elongation was not induced at the concentrations of inhibitor employed, although other noteworthy results were found. A characteristic response of sensitive plants to B995 treatment is the retardation of stem elongation (4, 5).

The objectives of this research were (*a*) to study the effects of DMSO and B995 on the growth and alkaloid biogenesis of *D. innoxia* Miller during a 4-week period, (*b*) to ascertain whether DMSO would enhance the growth-retardant effects of B995, (*c*) to determine whether results similar to that previously reported for *D. tatula* or *D. innoxia* would occur, and (*d*) to investigate the effects of the chemicals on the chlorophyll content and the various types of metabolic products produced by the plant as indicated by selective solvent extractions.

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EXPERIMENTAL

Procedure.—*D. innoxia* plants employed in this study were germinated from seeds obtained from plants grown in the Oregon State University drug garden in 1963. The germination of the seeds, transplantation of the seedlings into individual 1-gal. metal containers, and soil composition are described in a previous publication (6). The growth studies were performed under greenhouse conditions at a temperature varying from 70–90° F. throughout the experiment.

On June 30, 1964 (zero time), 72 uniform plants, which were 3 weeks old, were selected and divided into four groups of six plants each, according to the following treatment plan: controls (untreated plants), DMSO-treated plants, B995-treated plants, and plants receiving a combined DMSO–B995 treatment. At this time the plants were labeled according to treatment and randomized on a greenhouse bench.

The treatment solutions were prepared by dissolving 2% (v/v) of DMSO[1] in distilled water, 2% (w/v) B995[2] in distilled water, or by dissolving 2% (w/v) of B995 in a 2% (v/v) DMSO solution. At zero time, the aerial portions of the plants were sprayed with an atomizer to run-off with the previously designated solutions to assure an approximate equal dosage per plant based on a volume to unit area relationship. Specially prepared paper shields 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 plants were harvested at 0, 2, and 4 weeks. At harvest time, each plant was washed immediately, cleaned, and any excess moisture was removed with towels. Then the plant was divided into leaf-tops, stem, and root portions. The organs were cut into small segments, and the fresh weight of each portion was taken immediately. Dry weight determinations, pulverization, and storage of the powdered material were conducted in a manner

[1] The DMSO was supplied through the courtesy of Robert J. Herschler, Crown Zellerbach Corp., Camas, Wash.

[2] B995 was furnished by Mr. James A. Wilkerson, Naugatuck Chemical, Portland, Oreg.

previously described (6). To indicate the degree of maturity of the plants, the capsules were removed from the leaf-tops portions of each plant at the final harvest. After fresh and dry weight determinations were made of the capsules, they again were combined with the leaf-tops of their respective plants before pooling and pulverization according to harvest and group. Also the size of the capsules was evaluated by a point system whereby numerical values were given to the capsules according to size. The total points for each group were then compared with the total points for the control group. Prior to the final harvest, the average stem circumference in centimeters of each plant was determined by measuring the circumference of the main stem at the soil line, at the top below the first main *Y* branch, and midway between these two points. The average of these three readings was taken as the stem circumference.

Analysis for Alkaloids.—Pooled 25-mg. samples of the dried plant parts were analyzed for the concentration of alkaloids by the Brummett-Sciuchetti method (7). Two extractions were made per group, and each of the extractions was analyzed in duplicate. When duplicate determinations did not agree, two further extractions were made for each group.

Total Plant Alkaloids.—The total alkaloids per plant and per plant organ were obtained by multiplying the dry weight of the plant part by the per cent of alkaloids from the alkaloid analysis and expressing the results in milligrams.

Chlorophyll Content.—The chlorophyll analysis was conducted on 0.5-Gm. samples of the dry powdered leaf-tops of each group and expressed as mg./Gm. by a method previously described (6).

Selective Solvent Extraction.—To determine the effects of the treatment on the formation of other types of metabolic products, duplicate 2-Gm. samples of dried leaf-tops obtained at the terminal harvest from each group were selectively extracted in a Soxhlet apparatus in sequence with the following solvents: petroleum ether U.S.P., anhydrous ether C.P., ethyl alcohol U.S.P., and distilled water. Details of the method employed are described in a previous publication (2).

RESULTS

Growth Effects.—The habit of the plants treated with 2% DMSO resembled the controls. The maturity of the plants as indicated by the evaluation for capsule formation, as previously described, was significantly delayed in the B995 and B995–DMSO groups. The numerical values for the groups were: control, 21; DMSO, 20; B995, 2; B995–DMSO, 4. The per cent of control dry weight of capsules for each group were: DMSO, 70%; B995, 6%; and B995–DMSO, 11%. Capsule formation did not occur in any of the plants until after the second week. An increase was noted in the number of lateral shoots in the B995 and B995–DMSO groups.

The height of B995-treated plants was significantly decreased (Fig. 1). The reductions for this group were 58% at the second week and 65% at the fourth week. The figures for the B995–DMSO group were similar. Retardation of stem elongation by B995 was apparent within a week of the first treatment. The average stem circumference was reduced 6% in the DMSO group, 20% in the B995 group, and 19% in the B995–DMSO group.

Plant growth, indicated by fresh and dry weights, was reduced significantly by the B995 treatment (Table I). The decreases in dry weights for the B995 group at the final harvest were 34% in total weight, 23% in the leaf-tops, 69% in the stems, and 9% in the roots. Corresponding figures for the B995–DMSO group were 39, 30, 70, and 11%. The greater reductions in the latter group were due to an additive effect since the dry weights of the DMSO groups were slightly less than the controls.

Effect on Alkaloid Patterns.—Treatment with DMSO induced a higher concentration of alkaloids in the plant organs (Table II). The stems of the B995-treated group indicated a 56% increase in the concentration of alkaloids at the second week and a 90% increase at the fourth week. The concentration of root alkaloids of this group was increased also. Except for the leaf-tops, a similar trend was noted in the plant parts of the B995–DMSO group (Table II).

The total alkaloid content at the terminal harvest of the DMSO group was similar to the controls (Table II). The total alkaloid content per plant at the fourth week was reduced 21% in the B995 group and 31% in the B995–DMSO group. The decreases of 42% in the leaf-tops of the B995–DMSO group, of 57% in the stems of the same group, and of 42% in the stems of the B995 group were considered significant. The reductions were caused by the inhibition of growth by the growth retardant.

Chlorophyll Content.—A significant reduction in the concentration of chlorophyll a and b (mg./Gm.) was noted in the B995 group at the second week. Appreciable decreases in the concentration of chlorophyll b were found in all the treated groups at the fourth week. The total chlorophyll a and b content per leaf-tops at the fourth week was significantly reduced in the B995 and B995–DMSO groups. A similar trend was found at the terminal harvest of the DMSO group.

Selective Solvent Extraction.—No appreciable changes were found in the petroleum ether or ether extracts of the treated groups. A considerable reduction was found in the alcohol extract of the

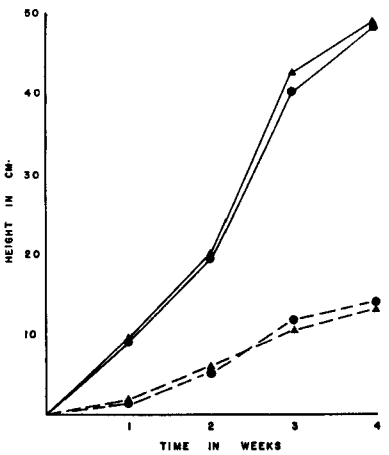

Fig. 1.—Height increase from zero time of *D. innoxia*. Key: ●—●, control; ▲—▲, DMSO; ●- -●, B995; ▲- -▲, B995–DMSO.

TABLE I.—WEIGHT OF *Datura* 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]	2.47	0.21	...	1.65	0.15	...	0.43	0.03	...	0.39	0.03	...
Control, 2	85.2	6.50	...	51.6	4.44	...	21.2	1.13	...	12.4	0.93	...
DMSO, 2	83.3	6.30	97	52.8	4.28	96	17.7	1.09	96	12.8	0.93	100
B995, 2	53.0	4.17	64	39.3	3.20	72	5.6	0.42	37	8.1	0.55	59
B995–DMSO, 2	62.5	5.01	77	45.5	3.84	86	7.1	0.51	45	9.9	0.66	71
Control, 4	209.5	31.6	...	109.3	17.9	...	58.2	9.32	...	42.0	4.41	...
DMSO, 4	187.8	28.9	91	95.4	15.8	89	52.5	8.65	93	39.8	4.47	101
B995, 4	145.3	20.7	66	89.8	13.8	77	17.7	2.86	31	37.8	4.00	91
B995-DMSO, 4	138.9	19.3	61	83.1	12.5	70	16.8	2.83	30	39.0	3.94	89

[a] Mean weights per group of six plants. [b] Mean weights of all four groups which were harvested but not treated at zero time.

TABLE II.—ANALYSIS OF ALKALOIDS IN *D. innoxia*

Treatment and Harvest Time, Wk.	Total Alkaloids,[a] mg./Gm. Leaf-Tops	Total Alkaloids,[a] mg./Gm. Stems	Total Alkaloids,[a] mg./Gm. Roots	Total Alkaloid Content,[b] mg. Per Plant	Total Alkaloid Content,[b] mg. Leaf-Tops	Total Alkaloid Content,[b] mg. Stems	Total Alkaloid Content,[b] mg. Roots
Control, 0	1.03	6.04	2.18	0.37	0.16	0.15	0.06
Control, 2	2.56	3.25	3.92	18.7	11.40	3.67	3.65
DMSO, 2	2.17	3.12	3.60	16.0	9.29	3.40	3.35
B995, 2	2.48	5.08	4.25	12.4	7.94	2.13	2.34
B995–DMSO, 2	2.65	4.07	4.82	15.4	10.20	2.08	3.18
Control, 4	2.40	2.62	4.28	90.6	42.9	24.4	23.3
DMSO, 4	2.58	2.66	5.82	89.8	40.8	23.0	26.0
B995, 4	2.53	4.98	5.55	71.4	35.0	14.2	22.2
B995–DMSO, 4	1.97	3.75	6.85	62.3	24.7	10.6	27.0

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

B995 group. All of the treated groups contained larger amounts of water-soluble extracts than controls. The per cent of increases over controls were 23% for the DMSO group, 33% for the B995 group, and 42% for the B995–DMSO group. These increases were considered significant.

DISCUSSION AND CONCLUSIONS

Treatment of *D. innoxia* with DMSO did not affect the habit of the plant. The height and the rate of maturity of the plant as indicated by the methods for evaluating capsule formation were similar to controls. There was only a slight reduction in the total dry weight of the plant. These trends were similar to that noted in *D. tatula* treated 3 months earlier with a 2% spray of DMSO (1). The concentration of alkaloids in the plant parts was slightly higher than controls. This factor combined with a slight decrease in plant growth resulted in total alkaloid production being similar to the controls. An appreciable decrease in the chlorophyll a content and a significant reduction in the chlorophyll b content were found at the terminal harvest of the DMSO group. The only significant trend demonstrated by the selective solvent extractions was a 23% increase in the water-soluble extractive of the leaf-tops. This would indicate that synthesis of carbohydrate-type compounds was stimulated by DMSO.

The characteristic response of sensitive plants to B995, retardation of stem elongation, was induced by the B995. This was noted within a week of the first treatment and resulted in decreases in height of 58 and 65% at the second and fourth weeks, respectively. Significantly delayed flowering and capsule formation, significant reductions of plant growth indicated by dry weight data, and smaller stems were caused by treatment with B995. Of the plant organs, the stem growth was inhibited the most. The stem dry weight of the B995 group at the final harvest was 31% of controls. It was observed that the number of lateral shoots in the B995 group was considerably greater in the B995 group *versus* controls. Increases in the concentration of alkaloids in the organs of the B995 groups was the general trend. The greatest increases were found in the stems, *viz.*, 56% at the second week and 90% at the fourth week. These increases can be attributed to the delayed maturity of the plants with the retardant. Previous work by us on *Datura* spp. (2, 6–9) has shown that the concentration of stem alkaloids decreases with age. Total alkaloid production was reduced about 21% per plant in the B995 group. This was due mainly to retardation of plant growth. The concentration of chlorophyll a and b as well as the total chlorophyll content of the B995 group were appreciably lower than the control group. No significant changes were found in the petroleum ether or ether extracts of the leaf-tops. A decrease of 14% was noted in the alcohol extract, while a 33% increase was found in the water extract of the B995 group. This suggests that carbohydrate synthesis and/or content was increased by the B995 treatment.

In general, when DMSO was combined with B995, an additive effect rather than potentiation resulted. This is not in agreement with our previous work with *D. tatula* (1). However, the following factors differed between the two investigations. A higher

temperature was employed in this experiment; the season of the year was different, summer *versus* spring; there was more intense illumination fortified by fluorescent lighting for 16 hr. daily in the previous experiment; younger plants (7-week *versus* 9-week at the final harvest) were used in this experiment; the two species were different. Any or all of these variables could account for the fact that an enhancement of the effects of the growth retardant was not noted when DMSO was combined with B995. Cathey (4) and other workers (10–12) have reported differences in the response of plants to growth retardants due to variations in the season of the year, temperature, and light intensity. The effect of the combined B995–DMSO treatment on plant habit and growth, chlorophyll formation, and fractions extracted by various selective solvents paralleled that noted in plants treated with B995 alone. In most cases similar trends were noted in the two groups (increases or decreases) in alkaloid concentration of the plant parts or total alkaloid content. The combination of DMSO with B995 appeared generally to have an additive effect.

The results of this experiment differed markedly from that of James and Sciuchetti (2), who employed concentrations of B995 one-twentieth or less to *D. innoxia* during a 4-week period. The pertinent differences between this experiment and the previous one based on data for the final harvest were: height reduction of 65% *versus* a 10% decrease, significantly delayed flowering and capsule formation *versus* no apparent effect, a 10% reduction in total weight and a 69% decrease in stem weight *versus* no loss in total weight and a 11% decrease in stem weight, a 90% increase in the concentration of stem alkaloids *versus* a 5% increase, a 21% decrease in the total alkaloid content per plant *versus* a 7% increase, a 14% decrease in the alcohol extract and a 33% increase in the water extract *versus* a 26% decrease in the petroleum ether extract, a similar decrease in the alcohol extract, and a 11% reduction in the water extract. These differences indicate that the strength of growth retardant employed can induce an entirely different response in sensitive plants and confirms our previous observation (2) that growth retardants in dilute concentrations can stimulate growth, whereas at high concentrations growth is inhibited. Recent work in our laboratory employing 2% aqueous sprays of B995 on *D. innoxia* and other species of *Datura* and 25 mg. of gibberellic acid either 1 week prior to or following treatment with retardant indicates that B995 has an anti-gibberellin effect.

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Use of 2-Thiobarbituric Acid–Malonaldehyde Reaction as a Measure of Antioxidant Effectiveness in Pharmaceutical Oils

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A method for estimation of antioxidant efficiency in pharmaceutical oils is described. The procedure depends upon the measurement of malonaldehyde which is produced during the oxidative degradation of the oils. The malonaldehyde is condensed with 2-thiobarbituric acid (TBA) to obtain a pink chromogen which is measured spectrophotometrically at 530 mμ. The influence of 0.02 per cent butylated hydroxyanisole, butylated hydroxytoluene, nordihydroguaiaretic acid, propyl gallate, lauryl gallate, gentisic acid, ascorbyl palmitate, and acetone sodium bisulfite upon the formation of malonaldehyde in peanut, sesame, corn, cottonseed, and soya oils is reported.

OXIDATION IS ONE of the more prominent pathways by which pharmaceutical preparations undergo degradation. Fats and oils which can readily undergo oxidative deterioration are used commonly in ointments, creams, suppositories, and injectable solutions. Oxidative decomposition of pharmaceutical formulations containing these materials may occur during or after manufacture, manifesting itself by physical changes in the dosage form, such as texture, color, odor, flavor, or by change in potency of the active ingredients they contain.

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