Effect of Dimethylsulfoxide Alone and Combined with *N*-Dimethylamino Succinamic Acid (B995) or (2-Chloroethyl)trimethylammonium Chloride (CCC) on the Growth and Alkaloid Biosynthesis of *Datura tatula*

By LEO A. SCIUCHETTI and ALVIN E. BORN

Dimethylsulfoxide (DMSO), B995, and CCC were administered in the form of a spray to the aerial parts of *Datura tatula*. DMSO did not affect plant height, but caused reductions in plant weight and the total alkaloid content per plant. The concentration of alkaloids in the roots was decreased. The growth retardants, B995 and CCC, induced significant reductions in plant height and total plant weight. Generally, the concentration of alkaloids in the stems was increased, while that in the roots was decreased. The inhibitory effects on height, growth, and alkaloid content were greater in those plants receiving the combined treatment with DMSO and retardant than in those treated with retardant alone. Phytotoxicity was noted in the plants treated with CCC alone or when combined with DMSO.

DIMETHYLSULFOXIDE (DMSO) is currently receiving extensive coverage in the lay press concerning its potential use in medicine, plant pathology, and as a systemic carrier of chemicals. DMSO has been employed as a solvent in biochemical procedures (1) and as an agent for the protection of mammalian cells against freezing damage (2). In plant and animal studies the chemical has enhanced the penetration of substances into tissues (3). It has been reported to be effective in the treatment of musculoskeletal injuries and inflammations (4, 5). A review of the literature indicated that no work has been performed with DMSO on medicinal plants.

A wide variety of plants respond to treatment with the growth retardents, *N*-dimethylamino succinamic acid (B995) and (2-chloroethyl)trimethylammonium chloride (CCC) (6, 7). A characteristic response of sensitive plants to these growth regulators is the retardation of stem elongation (7). Cathey (7) has reviewed adequately the physiology of B995, CCC, and other growth retarding chemicals. Previous work performed employing aqueous sprays of 100 to 1000 p.p.m. of B995 on *Datura innoxia* (8) and 1000 p.p.m. of B995 and CCC on *D. meteloides* (9) did not induce significant retardation of stem elongation, although other noteworthy results were found.

The objectives of this investigation were (*a*) to determine the effects of DMSO on the growth and alkaloid biogenesis of *D. tatula* Linné, (*b*) to ascertain whether higher concentrations of B995 and CCC than previously employed on closely related species would cause significant retardation of stem elongation and/or alter the growth and alkaloid formation of the plant, and (*c*) to determine whether DMSO would potentiate or enhance any effects induced by the retardants when combined with the latter.

Received September 2, 1964, from the School of Pharmacy, Oregon State University, Corvallis.
Accepted for publication October 12, 1964.
Research paper No. 466, Department of Pharmacognosy, School of Pharmacy, Oregon State University, Corvallis.

EXPERIMENTAL

Procedure.—*D. tatula* plants used in this study 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 (10). The growth studies were performed under greenhouse conditions with the temperature controlled at 24° (±3°). A 16-hour day was provided by placing a series of fluorescent lamps 90 cm. above the greenhouse bench tops. The series of lamps were arranged in the ratio of one Sylvania F40cw (cool white) to three Sylvania F40wwx (deluxe warm white). This lighting was supplemented by the daylight normally present in the greenhouse.

On March 24, 1964 (zero time), 36 uniform plants were selected and divided into nine groups of four each, according to the following treatment plan: controls (untreated plants); DMSO, 2%; DMSO, 5%; B995, 0.5%; B995, 2%; B995, 0.5% plus DMSO, 2%; B995, 2% plus DMSO, 2%; CCC, 0.5%; CCC, 0.5% plus DMSO, 2%. The DMSO[1] solutions were 2% or 5% (v/v) solutions of the chemical in distilled water. The B995[2] was dissolved in distilled water to prepare the designated strength of solution. The 0.5% CCC solution was prepared by dilution of Cycocel,[3] which contains 11.8%

[1] The supply of DMSO was furnished through the courtesy of Robert J. Herschler, Crown Zellerbach Corp., Camas, Wash.
[2] B995 was supplied by Dr. J. A. Riddell, Naugatuck Chemical Co., Bethany, Conn.
[3] Cycocel was furnished through the courtesy of Clark R Amen, American Cyanamid Co., Corvallis, Ore.

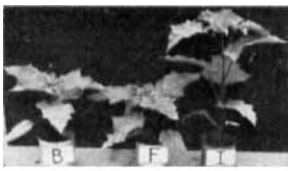

Fig. 1.—Apparent potentiation of growth retardation by combined DMSO-B995 treatment. Key: B, 2% B995; F, 2% B995 + 2% DMSO; I, control.

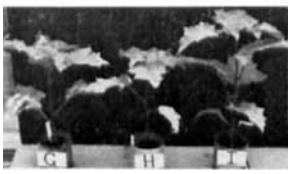

Fig. 2.—Habit of plants treated with CCC and CCC + DMSO. Key: G, 0.5% CCC; H, 0.5% CCC + 2% DMSO; I, control. Note necrotic areas on leaves of treated plants.

of CCC. The solutions consisting of retardant and DMSO were prepared by using a 2% (v/v) solution of DMSO as the solvent. The plants were labeled according to treatment and randomly arranged on the greenhouse bench.

At zero time, the aerial parts of the plants were sprayed with an atomizer to run-off with the previously designated solutions in order to assure an approximate equal dosage per plant based on a volume to unit area relationship. Treatment according to group was performed in a separate room to prevent cross-contamination. Specially prepared paper shields prevented the solutions from entering the soil. The plants received five weekly treatments. Height measurements were taken twice weekly, and the plants were observed periodically for any morphological changes. The concentrations of DMSO were determined by a preliminary experiment on *D. stramonium* when DMSO solutions in the strength of 0.1, 0.5, 1, 5, and 10% were sprayed onto the plants. Severe burning of the leaves, followed by necrosis, was induced by the 10% solution.

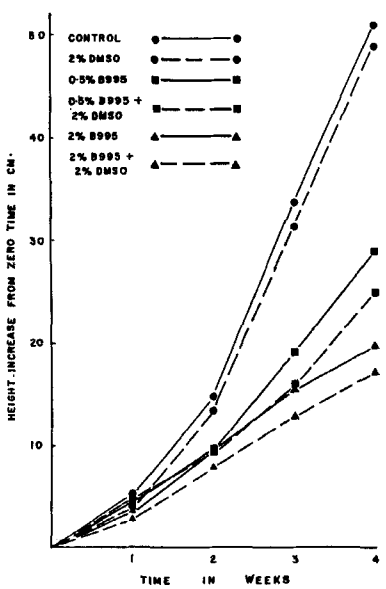

Fig. 3.—Height increase of plants treated with DMSO, B995, and B995 + DMSO.

The maximum strength of DMSO that did not cause phytotoxicity was 5%. Thus, the 2% and 5% concentrations were selected for this study. Since significant growth retardation was not induced by B995 and CCC in the concentrations used in our previous investigations with *Datura* spp. (8, 9), the strengths of the retardants in this experiment were increased five- to twentyfold over that employed previously.

The 64-day-old plants were harvested after a 32-day observation period. The division of the plant into its morphological parts at harvest time, fresh and dry weight determinations, pulverization, and storage of the powdered material were conducted in a manner previously described (10).

Analysis for Alkaloids.—Pooled 25-mg. samples of the dried plant parts were analyzed for the concentration of alkaloids by the Brummett-Sciuchetti method (11).

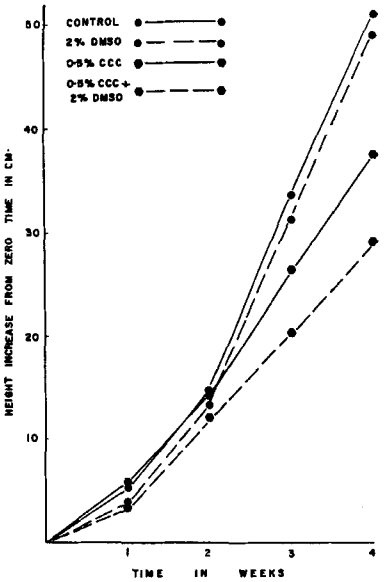

Fig. 4.—Height increase of plants treated with CCC and CCC-DMSO combination.

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 obtained from the alkaloid analyses and expressing the results in milligrams.

RESULTS

Growth Effects.—The habit of the plants treated with 2% DMSO appeared to be similar to the controls. The 5% strength of DMSO caused a slight burning of the leaf margins 4 days after the second treatment. Leaf areas of 2 to 3 cm. in diameter would turn yellow following further treatment and become necrotic within 2 days. A slight degree of phytotoxicity was also noted in the newly formed buds of this group. Some of the small buds would turn yellow and fall from the plant. Light brown streaks parallel to the vascular bundles were found on some of the juvenile buds. Both DMSO groups, however, did produce some flowers and small capsules prior to harvest time.

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

Treatment	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	157.3	25.34	...	68.3	10.23	...	53.9	11.09	...	35.1	4.02	...
DMSO, 2%	140.2	22.11	87.3	57.7	8.56	83.7	47.0	9.88	89.1	35.5	3.67	91.3
DMSO, 5%	136.0	21.19	83.6	47.3	7.19	70.3	53.7	10.47	94.4	35.0	3.53	87.8
B995, 0.5%	153.2	20.72	81.8	66.0	8.40	82.1	34.9	7.10	64.0	52.3	5.21	129.6
B995, 2%	123.1	18.89	74.5	58.7	9.14	89.4	25.9	5.98	53.9	38.5	3.77	93.8
B995, 0.5% + DMSO, 2%	131.8	19.59	77.3	64.4	9.04	88.4	31.3	6.88	62.0	36.1	3.67	91.3
B995, 2% + DMSO, 2%	115.5	17.03	67.2	60.4	8.77	85.7	24.1	5.20	46.9	31.0	3.06	76.1
CCC, 0.5%	127.0	17.35	68.5	58.3	6.99	68.4	42.8	7.75	69.9	25.9	2.61	64.9
CCC, 0.5% + DMSO, 2%	80.3	10.44	41.2	30.9	3.81	37.3	29.1	4.66	42.0	20.3	1.97	49.0

[a] Mean weight per group of four plants.

TABLE II.—CONCENTRATION OF ALKALOIDS[a] IN *Datura* PLANT PARTS

Treatment	Leaf-Tops Alkaloids, mg./Gm.	Leaf-Tops Control, %	Stems Alkaloids, mg./Gm.	Stems Control, %	Roots Alkaloids, mg./Gm.	Roots Control, %
Control	3.50	...	2.00	...	1.48	...
DMSO, 2%	2.60	74	2.30	115	1.25	84
DMSO, 5%	3.78	108	2.00	100	1.10	74
B995, 0.5%	4.23	121	2.30	115	1.18	80
B995, 2%	3.38	97	2.75	138	1.40	90
B995, 0.5% + DMSO, 2%	3.38	97	2.38	119	1.10	74
B995, 2% + DMSO, 2%	3.38	97	2.60	130	1.70	116
CCC, 0.5%	4.23	121	2.30	115	1.48	100
CCC, 0.5% + DMSO, 2%	4.38	125	2.00	100	1.33	90

[a] Calculated as scopolamine.

Internode elongation was retarded to a greater extent in the plants receiving the higher concentration of B995. Except for a squatter-appearing plant, the habit of plants treated with this growth retardant resembled the controls. The plants treated with a combination of DMSO and B995 were inhibited in growth to a greater extent than those treated with the retardant alone (Fig. 1). The DMSO apparently potentiated the growth retardation induced by the B995. Growth retardation was greater in the combined treatment group receiving the higher concentration of B995. Phytotoxicity appeared within 2 days of the first CCC treatment. When DMSO was combined with CCC, the phytoxicity was increased (Fig. 2). Large yellow areas would appear on the leaves of the treated groups within a few hours of treatment (Fig. 2). The entire leaf became necrotic within 2 days and fell off. The newly formed leaves of these plants were smaller and narrower than controls.

Significant reductions of internode elongation were induced by the growth retardants (Figs. 3 and 4). The height of the group treated with 0.5% of B995 was about 57% of controls, and that of the 2% B995 group was about 39% of controls. The plants treated with 5% of DMSO were slightly taller than controls, whereas those treated with 2% of DMSO were slightly shorter. Neither DMSO treatment significantly affected internode elongation. On the other hand, growth retardation was potentiated or enhanced by the DMSO when it was combined with the retardant. Both B995 groups receiving the combined treatment were shorter than the groups treated with B995 alone (Fig. 3). The group treated with 0.5% B995 plus 2% DMSO was 49% as tall as controls; the group receiving 2% each of DMSO and B995 was 34% as tall as controls. A significant reduction in height was also induced by the CCC treatment (Fig. 4). However, growth retardation was not so great in this group as in either of the B995 groups. The group receiving a 0.5% CCC treatment was 74% as tall as controls, while the height of the group treated with 0.5% CCC plus 2% DMSO was 57% of controls. The potentiation by the DMSO was of a greater magnitude with the CCC than with B995.

Plant growth, indicated by fresh and dry weights, was reduced significantly by the growth retardants (Table I). The higher concentration of B995 inhibited growth to a greater extent than the lower concentration. The total dry weights of the groups receiving the combined DMSO and B995 treatments were considerably less than those treated with the retardant alone. Plant growth was also depressed by the DMSO treatments (Table I). The total dry weight of the 2% DMSO group was 87% of controls, and that of the 5% DMSO group was 84% of controls. Plant growth was inhibited to a greater extent by the CCC treatment than by the B995 treatment. The depressant effect on growth was potentiated when DMSO was combined with CCC. The plants of this group weighed the least of all treated groups (about 42% of controls).

Effect on Alkaloid Patterns.—Variable results were found in the concentration of alkaloids in the leaf-tops (the aerial portions of the plant exclusive of the stems) of the treated groups (Table II). Increases of about 21% were found in the 0.5% B995 and 0.5% CCC groups and 25% in the group treated with 0.5% CCC plus 2% DMSO. The concentra-

TABLE III.—TOTAL ALKALOID CONTENT[a] (mg.) OF *D. tatula*

Treatment	Per Plant — Total Alkaloids	Per Plant — Control, %	Leaf-Tops — Total Alkaloids	Leaf-Tops — Control, %	Stems — Total Alkaloids	Stems — Control, %	Roots — Total Alkaloids	Roots — Control, %
Control	64.0	..	35.8	..	22.2	...	6.0	...
DMSO, 2%	49.6	77	22.3	62	22.7	102	4.6	77
DMSO, 5%	51.9	81	27.1	76	20.9	94	3.9	65
B995, 0.5%	57.9	90	35.5	99	16.3	73	6.1	102
B995, 2%	52.6	82	30.9	86	16.4	74	5.3	88
B995, 0.5% + DMSO, 2%	51.0	80	30.6	86	16.4	74	4.0	67
B995, 2% + DMSO, 2%	48.3	75	29.6	83	13.5	61	5.2	87
CCC, 0.5%	51.3	80	29.6	83	17.8	80	3.9	65
CCC, 0.5% + DMSO, 2%	28.6	45	16.7	47	9.3	42	2.6	43

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

TABLE IV.—DRY MATTER CONTENT[a] AND SHOOT–ROOT RATIOS[b] OF *D. tatula*

Treatment	Dry Matter, %	Shoot–Root Ratio
Control	16.1	5.31
DMSO, 2%	15.8	5.03
DMSO, 5%	15.6	5.00
B995, 0.5%	13.6	2.97
B995, 2%	15.3	4.01
B995, 0.5% + DMSO, 2%	14.5	4.34
B995, 2% + DMSO, 2%	14.7	4.57
CCC, 0.5%	13.7	5.65
CCC, 0.5% + DMSO, 2%	13.0	4.30

[a] Dry matter content calculated from mean weight per plant per group in the following manner: (total dry wt. × 100)/total fresh wt. = % dry matter. [b] Calculated from mean weight per plant per group.

tion of alkaloids in the stems of the treated groups was generally increased. The highest increase, 38%, was found in the group treated with 2% of B995. On the other hand, the concentration of alkaloids in the roots of the treated groups was reduced generally (Table II).

The total alkaloid content per plant of the treated groups was markedly reduced (Table III). The greatest reduction (55%) was found in the group receiving the combined CCC and DMSO treatment. The inhibitory effect on total alkaloid production per plant was more pronounced in the groups receiving the combination of DMSO and retardant than when retardant was employed alone (Table III). Significant decreases were found in the total alkaloid content in the leaf-tops and roots of both DMSO groups.

DISCUSSION AND CONCLUSIONS

The DMSO treatments did not affect the height of the plants but did inhibit plant growth, since decreases of about 15% were found in the total dry weights of both DMSO groups. The dry matter content and shoot–root ratios were less in them than in the controls (Table IV). The higher strength of DMSO (5%) induced a slight degree of phytotoxicity. This was manifested in the plants after the second treatment by a burning of the leaf margins, the appearance of small necrotic areas on the leaves, and a brown pigmentation parallel to the vascular bundles in some of the juvenile buds. About a 20% decrease in the total alkaloid content per plant was found in the DMSO groups, due primarily to an inhibitory effect on growth rather than an effect on the concentration of alkaloids in the plant organs. However, appreciable decreases were found in the concentration of root alkaloids of both DMSO groups.

The highest degree of retardation of stem elongation occurred in the B995 groups. These plants were shorter and squatter than controls. Otherwise, their general habit resembled the controls. The effect of B995 on internode elongation is the reverse of that noted in *Datura* spp. treated with gibberellin (10–12). Plant growth, indicated by total dry weight, was inhibited by the growth retardant. Of the plant organs, stem weight was reduced the most. The greatest decrease (46%) was found in the plants with the higher strength of B995. The dry matter content and shoot–root ratios were markedly decreased in the B995 groups (Table IV). The ratios of top–root (shoot–root) of other plants have also been reduced by similar-acting growth retardants (13, 14). Increases in the concentration of alkaloids of the aerial parts was generally found in the B995 groups. This effect of increased concentrations in the aerial parts of *Datura* treated with a growth retardant is the reverse of that usually encountered with gibberellin treatment (8, 10–12). This reversal of the gibberellin effect and that previously described concerning internode elongation suggest that the action of the growth retardant is antigibberellin in nature. The total alkaloid content per plant of the B995 groups was markedly reduced. This was due mainly to the decreased growth of the treated plants.

Phytotoxicity appeared in plants treated with 0.5% of CCC. This was not the case when plants were treated with 0.5% or 2% of B995. A significant reduction of internode elongation was caused by the chemical. However, this reduction was not so great as that noted with B995. Plant growth, indicated by dry weights, was depressed to a greater extent in the CCC group than in the B995 groups. The dry matter content per plant was markedly reduced in the CCC group. A study of leaf-top data showed that this dry matter content of the CCC group was about one-third that of the controls. The concentration of alkaloids in the organs of the CCC group was equal to or higher than that found in the controls. Nevertheless, the total alkaloid content per plant was reduced 20%. This was due to inhibition of plant growth.

A potentiation or enhancement of the effect of the growth retardant was apparent when 2% DMSO was combined with the retardant. This was true whether B995 or CCC was the chemical employed.

Retardation of stem elongation and inhibition of plant growth were greater with the combination of retardant and DMSO than when the retardant was employed alone. The phytotoxicity of the CCC was increased with DMSO. The total alkaloid content per plant of the groups receiving the combined treatment was reduced to a greater extent than when retardant was used alone. The greatest decrease, 55%, was found in the group treated with CCC plus DMSO.

B995 is preferred to CCC when growth retardation by the spray method is desired in *Datura* spp. Phytotoxicity is lacking with B995 solutions up to a 2% strength (20,000 p.p.m.), and the inhibition of plant growth (dry weight) is not so drastic as that noted with CCC. DMSO appears to potentiate the response of plants to the retardant. In all cases, the dry matter content of the treated groups was less than the controls. This indicates that the biosynthesis and/or accumulation of carbohydrates, proteins, or similar components were depressed. The shoot–root ratios of the treated groups were generally less than controls. Some of the effects noted on plants treated with either retardant suggest that the mechanism of action of the growth retardant is antigibberellin in nature.

REFERENCES

(1) Rosenkrantz, H., *et al.*, *Cancer Chemotherapy Rept.*, (No. 31) September 1963.
(2) Lovelock, J. E., and Bishop, M. W. N., *Nature*, **183**, 1394(1959).
(3) Jacob, S. W., Bischel, M., and Herschler, R. J., *Current Therap. Res.*, **6**, 193(1964).
(4) Rosenbaum, E. E., and Jacob, S. W., *Northwest Med.*, **63**, 167(1964).
(5) Rosenbaum, E. E., and Jacob, S. W., *ibid.*, **63**, 227 (1964).
(6) Riddell, J. A., *et al.*, *Science*, **136**, 391(1962).
(7) Cathey, H. M., *Ann. Rev. Plant Physiol.*, **15**, 271(1964).
(8) James, R. P., and Sciuchetti, L. A., THIS JOURNAL, **53**, 1093(1964).
(9) Bennett, J. H., and Sciuchetti, L. A., *ibid.*, **53**, 1254 (1964).
(10) Sciuchetti, L. A., *ibid.*, **53**, 61(1964).
(11) Brummett, R. E., and Sciuchetti, L. A., *ibid.*, **49**, 274(1960).
(12) Sciuchetti, L. A., *ibid.*, **50**, 981(1961).
(13) Wittwer, S. H., and Tolbert, N. E., *Am. J. Botany*, **47**, 560(1960).
(14) Cathey, H. M., and Piringer, A. A., *Proc. Am. Soc. Hort. Sci.*, **77**, 608(1961).

In Vitro Adsorption of Some Anticholinergic Drugs by Various Antacids

By SEYMOUR M. BLAUG and MILTON R. GROSS*

The adsorption of some anticholinergic drugs from aqueous solution by six different antacids was studied. The results obtained were plotted and interpreted according to the Langmuir adsorption isotherm. The adsorptive power of the six antacids varied with the anticholinergic drug being studied. Of the nine anticholinergics investigated, atropine sulfate, methantheline bromide, propantheline bromide, and oxyphenonium bromide were adsorbed to the greatest extent. Magnesium trisilicate showed the highest adsorptive capacity of the antacids studied. The reversibility of the adsorption process was studied using methantheline bromide and propantheline bromide. Magnesium trisilicate retained the largest amount of either of the two drugs. The effect of pH and ionic strength on the adsorption of methantheline bromide and propantheline bromide was investigated.

IN MODERN therapeutics, anticholinergic drugs are often administered in combination with antacids. The adsorption effect of certain antacids on atropine and other anticholinergics has been reported in the literature. Schloss (1) noted the adsorption and destruction of atropine by magnesium oxide. Dey and Haar (2) found that after grinding belladonna extract or powdered belladonna leaves with magnesium oxide, not all the atropine was recovered and that the amounts recoverable decreased with time. Heubner and Haas (3) found that when belladonna extract was combined with bismuth subnitrate or magnesium oxide, the loss in atropine activity was 93–97%. However, even before destruction, atropine was so firmly held that only a slight amount was released by 0.1 *N* hydrochloric acid. Experiments carried out on rats showed that when atropine was administered with adsorbing powders, half of the activity was retained (4) at the most.

The effect of dry aluminum hydroxide gel and dihydroxy aluminum aminoacetate on the acetylcholine action of atropine has been evaluated (5). Both were found to decrease the activity of atropine. Ogakurayama *et al.* (6) found that oral administration of preparations containing adsorbents with atropine must be avoided since the alkaloid was not released in the stomach or intestine and thus was ineffective.

Seifter *et al.* (7) evaluated the effect of aluminum hydroxide gel and hydrated alumina powder on the intensity and duration of action of

Received March 9, 1964, from the College of Pharmacy, State University of Iowa, Iowa City.
Accepted for publication October 6, 1964.
* Fellow of the American Foundation for Pharmaceutical Education. Present address: Johnson and Johnson Research Center, New Brunswick, N. J.