This simple study leads to manifold consequences which may be advantageously utilized by the formulator and researcher. Control of solubility as a function of dielectric constant variation might have many applications such as increasing the solubility of drugs by using additives such as sucrose or other sugars. On the other hand, decreased solubility may also be useful in a kinetic sense. Since a drug, such as aspirin suspension, will only degrade depending upon the amount of drug in solution, decreased solubility could lead to increased stability to some degree. This approach could be quite useful for aspirin, sulfonamides, antibiotics, and other common pharmaceutical suspensions.

Preliminary work indicates that methocel and other viscosity-inducing agents in solution have dielectric constants higher than that of pure water. These vehicles also possess the proper consistency for physical suspension, stability, and palatability. Increased chemical stability *via* decreased solubility or increased polarity (dielectric constant) is a definite possibility. Studies along these lines are being conducted in these laboratories and results will be reported in future communications.

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Effects of Various Growth Regulators on *Datura meteloides*

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Datura meteloides D.C. was treated during a 4-week period with Amo-1618, CCC, AMAB, Phosphon, B995, and CO-11. Growth was not significantly affected by the treatments. The concentration of alkaloids was increased 26 per cent in the roots of the Amo-1618 group and 17 per cent in the roots of the Phosphon group. The total alkaloid content was increased 25 per cent in the leaf-tops of the CO-11 group, 32 per cent in the roots of the Amo-1618 group, and 19 per cent in the roots of the B995 group. Increases of about 18 per cent in chlorophyll concentration were found in the Phosphon, B995, and CO-11 groups. The results of a selective solvent extraction of the leaf-tops are reported.

NUMEROUS CHEMICAL COMPOUNDS have been recently reported to cause a marked decrease in stem and petiole elongation (1–9). These compounds can be conveniently classified into three chemical groups, *viz.*, the quaternary ammonium compounds, the quaternary phosphonium compounds, and a few selected organic acids. Members of the first group include 4-hydroxy-5-isopropyl-2-methylphenyl trimethylammonium chloride, 1-piperidine carboxylate (Amo-1618), (2-chloroethyl) trimethylammonium chloride (CCC), and allyltrimethylammonium bromide (AMAB). Reduction of internode length resulting in shorter plants has been reported in various plants by treatment with Amo-1618 (1–6), CCC (4, 6, 7), and AMAB (6, 7). CCC and AMAB have been shown to increase the alkaloid content of *Nicotiana tabacum* L. and decrease that of *N. rustica* L. (6).

A phosphonium compound which has been reported to inhibit plant growth is 2,4-dichlorobenzyltributylphosphonium chloride (Phosphon) (2, 3, 6, 8, 9). This compound and the three aforementioned ones are considered to be antigibberellins (1, 3, 6, 9–11). Lockhart (12) concludes that Phosphon and CCC retard stem elongation by partially blocking the system which provides gibberellin to the growth mechanism. Among the organic acids reported to retard plant growth are *N*-dimethylaminomaleamic acid (CO-11) and *N*-dimethylaminosuccinamic acid (B995) (13). These two compounds are closely related to maleic hydrazide (MH), which is considered by some to be an inhibitor of auxin (14, 15).

Since these compounds with reported growth-retarding activity had not been tested on *Datura* spp., it was decided to determine their effects on the growth and synthesis of certain chemical constituents of *Datura meteloides*. In addition, since some of these compounds appear to be antigibberellins and since gibberellin treatment generally reduces the concentration of alkaloids in many medicinal plants (16, 17), it was of interest to note whether a "growth retardant" would cause an increase in the concentration of alkaloids of the plants.

EXPERIMENTAL

Procedure.—Seventy uniform plants were grown under greenhouse conditions in a soil mixture composed of 1 part sand, 2 parts sandy loam, and 10 Gm. of organic fertilizer[1] per gallon can. On July 12, 1962, *Daturas* which were about 34 days old were labeled according to the following plan for treatment: Amo-1618-treated plants, CCC-treated plants, AMAB-treated plants, CO-11-treated plants, B995-treated plants, Phosphon-treated plants, and control (untreated) plants. Each of the above groups consisted of 10 plants. On that date the plants were randomly arranged on a greenhouse bench, and treatment was instituted. Treatments

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[1] Organic Morcrop, Chas. Lilly Co., Seattle, Wash. *Anal.* —5% total nitrogen, 3% available phosphate, 2% available potash.

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

Treatment	Leaf-Tops mg./Gm.	Leaf-Tops Control, %	Stems mg./Gm.	Stems Control, %	Roots mg./Gm.	Roots Control, %
Control	4.18	...	3.33	...	5.95	...
Amo-1618	3.78	90.3	3.43	103.0	7.48	125.6
AMAB	4.00	95.7	3.20	96.2	6.42	107.9
Phosphon	4.55	108.9	3.80	114.3	6.98	117.2
CCC	4.55	108.9	3.32	99.9	6.13	102.9
B-995	3.73	89.1	3.48	104.5	6.58	110.5
CO-11	4.60	110.1	3.32	99.9	6.45	108.4

[a] Alkaloids were calculated as scopolamine.

TABLE II.—TOTAL ALKALOID CONTENT[a] OF *Datura*

Treatment	Leaf-Top mg.	Leaf-Top Control, %	Stems mg.	Stems Control, %	Roots mg.	Roots Control, %	Per Plant mg.	Per Plant Control, %
Control	18.68	...	13.39	...	15.65	...	47.72	...
Amo-1618	17.50	93.7	14.89	111.1	20.64	131.9	53.03	111.1
AMAB	19.08	102.1	13.86	103.5	17.78	113.6	50.72	106.3
Phosphon	21.43	114.2	14.78	110.4	16.12	103.0	52.33	109.7
CCC	21.79	116.6	14.11	105.5	16.31	104.2	52.21	109.4
B-995	17.68	94.6	13.57	101.3	18.56	118.6	49.81	104.4
CO-11	23.32	124.82	13.78	102.9	17.22	110.0	54.32	113.8

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

TABLE III.—CHLOROPHYLL CONTENT[a] IN LEAF-TOPS OF *Datura* Plants

Treatment	Chlorophyll *a* mg./Gm.	Chlorophyll *a* Control, %	Chlorophyll *b* mg./Gm.	Chlorophyll *b* Control, %
Control	2.54	...	2.27	...
Amo-1618	2.17	81.9	2.07	91.2
AMAB	2.26	89.0	2.09	92.1
Phosphon	3.00	118.1	2.74	120.8
CCC	2.64	103.9	2.28	100.4
B995	2.92	115.0	2.52	111.0
CO-11	2.73	107.4	2.23	98.2

[a] Based upon the analysis of 1-Gm. samples from a pool of 10 plants per group.

with Amo-1618, CCC, AMAB, CO-11, and B995 consisted of spraying the plants to run-off with aqueous solutions containing 1000 p.p.m. of the chemicals.[2] Paper shields were employed to prevent the spray from contacting the soil. Due to its phytotoxicity when applied to the aerial parts of plants (3), the Phosphon[3] was applied in the form of a soil drench. Each plant of this group received 100 mg. of the chemical. Subsequent applications of the chemicals were made at weekly intervals for a period of 4 weeks. The habit of the plants was observed daily during this time. Height measurements were taken at 2-day intervals. On the day prior to harvesting (August 11) the plants were examined for the number of flowers, the number and size of capsules, the number of internodes, and the stem diameter. The division of the plants into their morphological parts at harvest time, fresh and dry weight determinations, pulverization, and storage of the powdered material were conducted in a manner previously described (18).

Growth Effects.—Statistical analysis of height and dry weight data indicated no significant changes from the treatments. However, the B995 and CO-11 groups were about 90 and 94%, respectively, as tall as controls. This would indicate slight retardation of stem elongation. No significant changes were noted at harvest time in the number of flowers and capsules or in the stem diameters of the variously treated groups.

Analysis for Alkaloids.—The alkaloid analyses, performed on pooled samples by the Brummett-Sciuchetti method (17), indicated variable trends (Table I). Two extractions were made per group, and each group was analyzed in duplicate. When duplicate determinations did not agree, two further extractions were performed on each group. The concentration of alkaloids in the roots of the Amo-1618 group increased about 26%. Increased concentrations, varying from 9 to 17%, were found in the organs of the Phosphon group (Table I). Likewise, increases of approximately 10% were noted in the roots of the B995 group and in the leaf-tops of the CO-11 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 obtained from the alkaloid analyses and expressing the results in milligrams (Table II). The following increases were noted in total alkaloid content: 32% in the roots of the Amo-1618 group, 25% in the leaf-tops of the CO-11 group,

[2] The authors are indebted to Dr. Paul C. Marth, Crops Protection Research Branch, U.S.D.A., Beltsville, Md., for the supply of Amo-1618, CCC, AMAB, and CO-11 employed in this study and to Dr. J. A. Riddell, Naugatuck Chemical Co., Betheny, Conn., for the supply of B995.
[3] The technical grade of Phosphon was furnished through the courtesy of Dr. Charles R. Downing, Agr. Chem. Group, Virginia-Carolina Chemical Corp., Richmond, Va.

19% in the roots of the B995 group, and 17% in the leaf-tops of the CCC group. It is of interest to note that all organs of the AMAB, Phosphon, CCC, and CO-11 groups indicated higher total alkaloid content than the controls.

Chlorophyll Content.—The analysis for chlorophyll content was conducted by a modification (17) of the method described by Gjerstad (19). A minimum of two duplicate determinations was carried out on each sample. Although no apparent macroscopic differences were observed in the green color of the leaves of the treated groups, an effect was found when the leaf-tops were analyzed for the concentrations of chlorophyll *a* and *b* (Table III). Plants treated with Amo-1618 exhibited about a 19% reduction in chlorophyll *a* and a 9% decrease in chlorophyll *b*. Decreases of a smaller magnitude were also found in the AMAB group. Significant increases of about 20% were noted for both pigments in the Phosphon group. The B995 group demonstrated a similar trend.

Selective Solvent Extraction.—To determine the effects of the growth regulators on various other plant constituents, an abbreviated selective solvent extraction was performed by following the general sequence of solvents proposed by Dragendorff (20). The method employed 2-Gm. samples of dried leaf-tops material. This material was completely extracted in a Soxhlet continuous extraction apparatus with the following sequence of solvents: petroleum ether U.S.P., anhydrous ether C.P. grade, alcohol U.S.P. (92.3% w/w), and distilled water. The weights of the dry extracts were obtained by evaporation of the solvent on a water bath followed by oven drying at 48.5° for 24 hours. No significant differences were found in the petroleum ether or ether extractives of the treated groups. However, the alcohol-soluble extractives of the Phosphon and CO-11 groups were increased over controls 37 and 25%, respectively. The general trend was a higher content of alcohol-soluble extractive in all treated groups. Increases of 18, 25, and 44% were noted in the water-soluble extractives of the CCC, Amo-1618, and CO-11 groups, respectively. A 17% decrease was noted in the Phosphon group.

DISCUSSION AND CONCLUSIONS

The principal response of sensitive plants to "growth retardants" is a reduction in internode length, resulting in shorter plants. Plants from all treated groups were shorter than controls. There appeared to be an apparent retardation of growth, indicated by the reduced height of the treated groups. However, when height and dry weight data were subjected to statistical analysis, the differences were insignificant.

An interesting aspect of the experiment was an increased concentration of alkaloids in many of the organs of the treated groups. The 26% increase in the roots of the Amo-1618 group was considered significant. All treated groups synthesized more total alkaloids per plant. The total alkaloids in the leaf-tops were increased about 15, 17, and 25% in the Phosphon, CCC, and CO-11 groups, respectively. The total alkaloids of the roots were increased by all treatments. The increases of 32% in the Amo-1618 group and 19% in the B995 group were considered significant. Previous investigations demonstrated that gibberellin treatment usually induces a reduction in the concentrations of alkaloids in the aerial parts of *Daturas* (16–18). Several workers (1, 3, 6, 9–11) consider Amo-1618, CCC, AMAB, and Phosphon to be antigibberellins. Thus, it appears that there is a relationship between gibberellin action, growth, and alkaloid synthesis. In this experiment some of the growth retardants increased alkaloid synthesis. This suggests that the effects of some of the chemicals employed were antigibberellin in nature regarding alkaloid synthesis. This tends to confirm the reports of other investigators that some of these compounds are antigibberellin in nature (1, 3, 6, 9–11).

Of the chemicals employed, Amo-1618 and Phosphon induced the greatest changes in plant composition. The former caused a 26% increase in root alkaloid concentration, a 32% increase in total root alkaloid content, a 25% increase in the water-soluble extractive, and a 19% decrease in the concentration of chlorophyll *a*. Phosphon induced increases in the concentration of alkaloids in all the plant organs and caused an increased total alkaloid content in the morphological parts of the plant. Furthermore, about a 20% increase was found in the chlorophyll *a* of this group and a 37% increase in the alcohol-soluble extractive. Appreciable differences from controls were also noted in the CO-11, CCC, and B995 groups. The following increases were found in the CO-11 group: 25% in the total leaf alkaloids, 25% in the alcohol-soluble extractive, and 44% in the water-soluble extractive. All of the organs of the CCC group indicated increased total alkaloid content. Increases of 17% in total leaf alkaloid content and 18% in the water-soluble extractive were noted. A higher concentration of chlorophyll *a* and a 19% gain in total root alkaloids were found in the B995 group.

It is the authors' opinion that more pronounced effects, especially on internode elongation, might have been induced if a five to tenfold increase in the concentration of the chemicals had been employed or if the plants had been treated and harvested at an earlier stage of plant development.

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