TABLE II —STABILITY OF CHLORPHENIRAMINE N-CYCLOHEXYLSULFAMATE IN AQUEOUS SOLUTION AT 50° FOR 2 MONTHS

pH	Original, mg./ml.	Two Months— mg./ml.	Appearance	% Loss
4	3.24	3.30	Clear	0
5	4.05	3.46	Clear	15
6	3.65	3.20	Clear	13
7	3.85	2.62	Sediment	32

SUMMARY AND CONCLUSIONS

1. The N-cyclohexylsulfamic acid salts of dextromethorphan and chlorpheniramine were prepared and characterized.

2. The salts were found to have greatly improved bitterness thresholds over that of the commonly occurring salts of the compounds.

3. When submitted to accelerated aging conditions, the compounds appeared to have good stability provided they were maintained at optimum pH levels.

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Influence of Kinetin and Gibberellic Acid on the Growth and Alkaloid Patterns in *Datura meteloides*

By DARRYL G. AMBROSE† and LEO A. SCIUCHETTI

Datura meteloides D.C. was administered four weekly-doses of 25 mcg. of kinetin (K) or gibberellic acid (GA). Different growth responses were induced by each treatment. Significant increases in height were noted in plants treated with GA whereas those treated with K were shorter than controls. Considerable increases in dry weights were noted in the GA-treated plants while reductions were observed in K-treated plants. The concentration of alkaloids in the organs of plants treated with GA was markedly reduced but no significant changes occurred with K. The total alkaloid content per plant was less in each of the treated groups but total stem alkaloids were greater in those treated with GA. The per cent of chlorophyll a and b was decreased by GA but increased by K.

PREVIOUS INVESTIGATIONS with kinetin, 6-(furfurylamino)-purine, and gibberellic acid (GA) indicate that these substances induce a profound effect on plant growth. Kinetin (K) was isolated and synthesized in 1955 by Miller, *et al.* (1, 2). This growth regulator has been shown to affect cell division (1, 3), cell enlargement as well as cell division (3, 4), root development (5, 6), shoot formation (7), and seed germination (8–10). Kinetin substituted for the light requirement of *Lemna minor* (11) and caused a modification of sporeling ontogeny in *Marsilea vestita* (12). K-treatment of lupin embryos *in vitro* caused about a twofold increase in alkaloid synthesis (13). Mothes, *et al.*, have shown that K affects nitrogen metabolism (14). Soluble nitrogen compounds in excised tobacco leaves were drawn from other leaf areas or added from the outside by K (14). A review of the literature has indicated that research has not been performed on the effects of K on the growth and alkaloid biogenesis in *Datura meteloides*.

The metabolic effects of GA on many plants (15–17), as well as on some medicinal plants (18), have been well established. The concentration of alkaloids in the plant organs was reduced and stem weight was increased in *Datura stramonium* (18–21), *Atropa belladonna* (19, 20), *Hyoscyamus niger* (22), and tobacco (23, 24). Increased height and variable results on leaftop, and root growth have been reported (18–24).

In view of the fact that K had not been employed on *Datura* species, it was decided to investigate the effect of this substance on the growth pattern, alkaloid formation, and chlorophyll content of *Datura meteloides*. It was further desired to ascertain whether this plant would respond to a GA treatment in a manner similar to that previously reported for *Datura stramonium*, and, also, to observe whether K and GA would induce similar metabolic effects.

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EXPERIMENTAL

Procedure.—*Datura meteloides* plants employed in this study were grown under greenhouse conditions. Seeds were germinated in flats containing a soil mixture of two parts of loam and one part of sand. Approximately 50 Gm. of complete organic fertilizer[1] was incorporated into the soil of each flat. On July 5, 1960, 72 14-day-old seedlings were transplanted into individual 1-gallon metal containers that had been filled with the sandy loam mixture. About 5 Gm. of complete fertilizer had been thoroughly mixed into the soil of each container prior to transplantation. The 72 seedlings were placed on eight different benches in the greenhouse. Nine plants were arranged on each bench by use of a randomization table (25). Nine plants each of *Datura tatula*, *Atropa belladonna*, and *Hyoscyamus niger* were also randomly arranged with *Datura meteloides* on each of the eight benches. The other species were being investigated simultaneously by other workers. Each species was labeled according to the following plan for treatment and harvest: three groups of 24 each were designated as controls, K-treated plants, and GA-treated plants; of the 24 plants within each group, eight each were harvested at 0, 2, and 4 weeks. The division of the plant into its morphological parts during each harvest, fresh and dry weight determinations, pulverization, and storage of the powdered material were conducted in a manner described in a previous publication (19).

On July 14 (zero time), the 16 plants for the K group and the corresponding plants of the GA group designated for the last two harvests were treated with a single 25-mcg. dose of the specific growth regulator. Treatments were continued hereafter at weekly intervals. The dose of the chemical was delivered from a micro pipet onto the surface of the youngest unfolding leaf by applying 0.1-ml. portions of the respective aqueous solutions. The GA[2] solution was prepared freshly from a stock alcoholic solution of GA by dilution with distilled water. The K solution was freshly prepared before each treatment by dissolving 2.5 mg. of kinetin in approximately 3 ml. of a stock potassium hydroxide solution having a pH of 11.2. This solution was back titrated with dilute sulfuric acid ($10^{-3}N$) to a pH of 6.5. Then, enough distilled water was added to make 10 ml. At this time, initial height measurements were made on all plants and the first harvest of eight plants from each group was made. Height measurements were taken weekly thereafter.

Growth Effects.—The K-treated plants and the controls resembled each other in habit and were more bushy than the GA-treated plants (Fig. 1). At the final harvest, some of the plants treated with K appeared darker in color than the controls. Typical gibberellin-effects were noted in the GA-treated group, such as greater internodal elongation, increased height, and a spindlier appearance (Fig. 1). The leaves of the GA-treated plants appeared slightly chlorotic initially, whereas the stems demonstrated an apparent chlorosis at the final harvest.

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

[2] The GA employed in this study was furnished through the courtesy of Dr. Edwin F. Alder, Agricultural Research Center, Eli Lilly and Co., Greenfield, Ind.

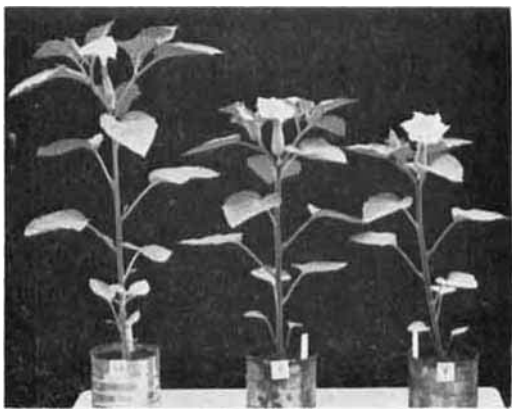

Fig. 1.—Effect of K and GA treatments on the growth of *Datura meteloides*. GA., Gibberellic acid; C, control; K, kinetin. Plants at the final harvest were about 7 weeks old.

Height measurements indicated that plants treated with K were shorter than controls throughout the observation period while the GA-treated plants grew progressively taller (Fig. 2). Treatment with GA induced height increases greater than 40% from the second through fourth weeks.

Fresh and Dry Weights.—The treatment with K caused less growth at the final harvest, as indicated by fresh and dry weight data (Table I). The final dry weights were about 86, 80, and 96% that of controls for leaves-tops, stems, and roots, respectively (Table I). The decreased growth of this group became apparent between the second and fourth weeks. On the other hand, a general favorable response on growth was induced by treatment with GA. The total dry weight per plant of this group attained its maximum gain (about 15%) at the last harvest (Table I). The largest increase in dry weight was noted in the stems. About a 40%

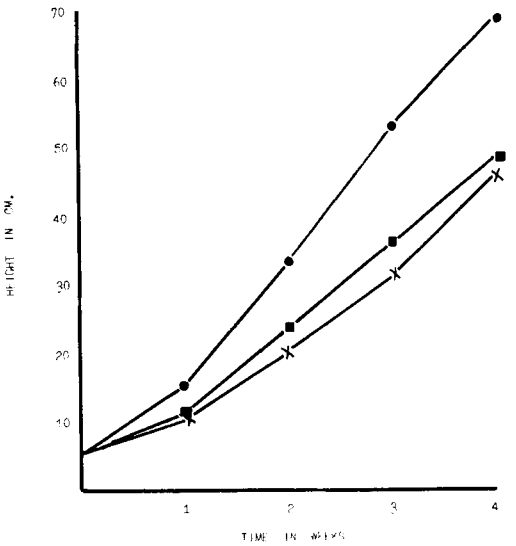

Fig. 2.—Height measurements of *Datura* plants. ■—■, Controls; ×—×, 25 mcg. kinetin; ●—●, 25 mcg. GA.

TABLE I.—WEIGHTS OF *Datura* PLANT PARTS (AV./PLANT/GROUP)

Treatment and Harvest Time, wk.	Total Weight			Leaves-Tops			Stems			Roots		
	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %
Control, 0	1.12	0.16	...	0.83	0.12	...	0.14	0.02	...	0.15	0.02	...
Kinetin, 0[a]	1.19	0.16	103.2[b]	0.86	0.12	102.6[b]	0.16	0.02	110.5[b]	0.17	0.02	100.0
GA, 0[a]	1.21	0.16	98.1[b]	0.87	0.11	97.4[b]	0.15	0.02	100.0[b]	0.19	0.02	100.0
Control, 2	37.23	4.01	...	20.36	2.59	...	7.76	0.71	...	9.11	0.71	...
Kinetin, 2	39.11	4.13	103.0	21.52	2.68	103.7	8.13	0.70	97.8	9.46	0.75	105.8
GA, 2	39.34	4.46	111.4	19.56	2.68	103.7	10.93	0.99	139.3	8.85	0.79	111.3
Control, 4	98.77	14.07	...	43.95	6.83	...	29.19	4.52	...	25.63	2.72	...
Kinetin, 4	87.15	12.08	85.9	38.38	5.87	86.0	24.05	3.60	79.7	24.72	2.61	95.8
GA, 4	108.26	16.15	114.8	47.09	7.25	106.2	37.37	6.35	140.4	23.80	2.56	93.8

[a] These plants had no treatment. [b] Figures obtained by calculations from weights taken in mg., tabulated data are rounded at second decimal place.

TABLE II.—TOTAL ALKALOID CONTENT[a] (MG.) OF *Datura meteloides*

Treatment and Harvest Time, wk.	Per Plant		Leaves-Tops		Stems		Roots	
	Total Alkaloids	% of Controls	Total Alkaloids	% of Controls	Total Alkaloids	% of Controls	Total Alkaloids	% of Controls
Control, 0	0.32	...	0.15	...	0.10	...	0.07	...
Control, 2	8.73	...	4.40	...	2.28	...	2.05	...
Kinetin, 2	8.43	96.6	4.03	91.6	2.23	97.8	2.17	105.9
GA, 2	7.76	88.9	3.49	79.3	2.38	104.4	1.89	92.2
Control, 4	31.30	...	10.92	...	10.85	...	9.53	...
Kinetin, 4	26.85	85.8	8.81	80.7	8.64	79.6	9.40	98.6
GA, 4	28.43	90.8	8.70	79.7	12.06	112.0	7.67	85.5

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

TABLE III.—CHLOROPHYLL A AND B CONTENT OF *Datura* AERIAL PARTS

Treatment	Leaves-Tops				Stems			
	2 Weeks		4 Weeks		2 Weeks		4 Weeks	
	Per cent	Control, %	Per cent	Control, %	Per cent	Control, %	Per cent	Control, %
				Chlorophyll a				
Control	0.538	...	0.223	...	0.037	...	0.013	...
Kinetin	0.545	101.3	0.255	114.3	0.040	108.1	0.016	123.1
GA	0.473	87.9	0.220	98.7	0.034	91.9	0.012	92.3
				Chlorophyll b				
Control	0.420	...	0.163	...	0.035	...	0.014	...
Kinetin	0.410	97.6	0.198	121.5	0.042	120.0	0.015	107.1
GA	0.348	82.9	0.158	96.9	0.035	100.0	0.010	71.4

increase in stem weight was noted at the second and fourth weeks. The leaves-tops gained up to 15% in weight during the same period. Only at the final harvest was root dry weight less than controls.

Analysis for Alkaloids.—The alkaloid concentration, calculated as scopolamine, was determined by the Brummett-Sciuchetti method (20). The concentration of alkaloids (mg./Gm.) in the organs of the K-treated plants approximated that of the controls, while the concentration in the GA-treated plants was considerably lower in all plant parts throughout the stages of growth (Fig. 3.). The reductions of about 25% in the leaves-tops and the stems at the second and fourth weeks were considered significant.

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 total alkaloid content generally was markedly reduced in the leaves-tops and stems by the K treatment. The reductions, compared with controls, amounted to about 20% at the final harvest. This was due mainly to decreased growth of these organs. No significant changes occurred in the roots of K-treated plants. Treatment with GA caused about a 20% reduction in the alkaloid content of the leaves-tops at each harvest and about a 15% decrease in the roots at the final harvest. Due to increased stem growth, the GA-treated plants showed gains in total stem alkaloids at each harvest (Table II).

Chlorophyll Content.—The chlorophyll analysis was conducted by a method described by Gjerstad (26). His method was modified by employing dried material, 1-Gm. samples of leaves-tops and 5 Gm. of stems, and using a Soxhlet extraction apparatus. The chlorophyll was completely extracted from the powdered material with a 20% water in acetone solvent. The results of the chlorophyll analysis are shown in Table III. The per cent of chlorophyll a and b in the leaves-tops and stems of the K-treated plants was markedly increased whereas that of the GA-treated plants was reduced considerably (Table III).

DISCUSSION AND CONCLUSIONS

Specific metabolic effects were noted in the plants treated with kinetin. Although the K-treated plants generally appeared similar to the controls in habit, several differences were noted. The treated plants were shorter than controls throughout the observation period and the leaves appeared darker at the final harvest. Growth of the aerial parts was retarded, but this did not occur until a period of time between the second and fourth weeks. The fresh and dry weights of the aerial parts were reduced up to 20% that of the controls at the final harvest. These observations indicate that there was a delayed response to the kinetin. It is altogether possible that a significant reduction in weight would have occurred had this experiment been allowed to continue for a longer period of time. The concentration of alkaloids was not appreciably altered in the plant parts by the K-treatment. The decreased alkaloid content per plant, per leaves-tops, and per stem at the final harvest was due primarily to the adverse effect of kinetin on growth. A most interesting aspect observed from the K-treatment was an increase in the per cent of chlorophyll in the aerial parts. The greatest increase was at the final harvest. This confirms the observations that the leaves generally appeared darker green in color at the final harvest. The fact that the increased per cent of chlorophyll generally directly paralleled the decrease in plant growth, as shown by the dry weight data, might indicate that there was not an increased capacity for chlorophyll synthesis by the K-treated plants; instead, the higher per cent of chlorophyll was probably due to concentration. Future histological work should clarify this phenomenon.

Datura meteloides responded as do other solanaceous plants when treated with GA (18–22). The following characteristic gibberellin-effects were noted: taller and spindlier plants, greater internodal elongation, slight chlorosis of the leaves and stems, significantly increased stem growth, and decreased concentration of alkaloids. Growth was stimulated by GA since increases in dry weights were generally noted throughout the observation period. In spite of a favorable effect on growth, the decreased concentration of alkaloids in the organs resulted in about a 10% reduction in total alkaloid content per plant at the final harvest. The reduced chlorophyll per cent in the aerial parts of the treated plants confirms our observations which were reported previously that GA-treated plants appeared chlorotic (18–20). The decreased chlorophyll per cent in the GA-treated plants is also in agreement with that reported for spearmint (27), but is not in agreement for that reported for peppermint (26) and foxglove (28). In this experiment, as well as the one reported for spearmint (27), the decrease in chlorophyll per cent was accompanied by appreciable increases in growth. This would suggest that the apparent paleness of leaves from gibberellin treatment is most likely due to dilution rather than a direct effect on chlorophyll synthesis.

Kinetin generally induced a different effect than did GA. The following differences were noted (K *vs.* GA): shorter and bushier plants *vs.* taller and spindly plants; decreased growth *vs.* increased growth; no change in alkaloid concentration in the plant organs compared with controls *vs.* appreciable decreases; a delayed response following treatment *vs.* a rapid response. These differences suggest that K and GA possess different modes of action in various metabolic processes involved in plant growth and biosynthesis.

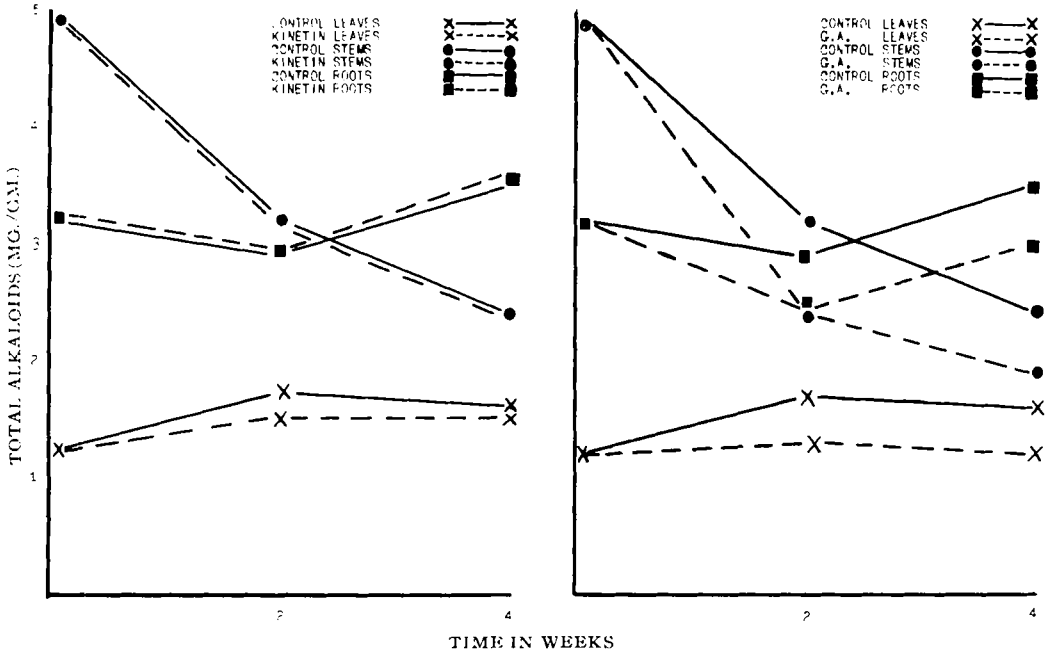

Fig. 3.—Alkaloid pattern of kinetin and gibberellic acid-treated plants. Left, K-treated; right, GA-treated.

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Separation and Identification of Sympathomimetic Amines by Gas-Liquid Chromatography

By E. BROCHMANN-HANSSEN and A. BAERHEIM SVENDSEN

A number of commonly used sympathomimetic amines have been subjected to gas chromatographic analysis on low-loaded columns. With the exception of certain isomers, most compounds can be separated on a column of silicone rubber SE-30. Many of the amines investigated react with ketones and the products produce sharp, symmetrical peaks on the gas chromatogram. Ephedrine and pseudoephedrine can be separated and identified on the basis of the difference in the rate of reaction with acetone. Monohydroxyphenols show strong adsorptive effects as the free bases but can readily be gas chromatographed as the acetone derivatives. The dihydroxyphenols are converted to the triacetyl derivatives which are treated with hexamethyldisilazane prior to the gas chromatographic analysis.

OF THE HUNDREDS of sympathomimetic amines which have been synthesized or isolated from natural sources, about 25 find widespread use in medical practice (1). Many methods for their identification have appeared in the literature (2–9). The official products are usually identified by color reactions, precipitation reactions, or on the basis of the melting ranges of the free amines, their salts, or prepared derivatives. Although these methods often lack specificity, they are usually satisfactory when the problem is to confirm the identity of a single compound which is available in sufficient amount. When mixtures of several amines are involved, as is often the case in pharmaceutical preparations, a separation is usually necessary before the components can be identified. In biological work, the problem is further complicated by the limited amounts available and by the presence of amines which are normal products of the animal body or formed by decomposition of biological material. Another complication stems from the fact that the metabolic products of certain sympathomimetic amines are themselves used as drugs (10, 11).

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Paper chromatography in combination with the use of several different spray reagents has recently been found to be a valuable method for problems in toxicology involving sympathomimetic amines (12).

In a preliminary communication, the authors showed that a number of widely used sympathomimetic amines could be separated by gas-liquid chromatography (13). The details of this study, which has been extended to include a larger number of compounds, are reported in the present paper.

EXPERIMENTAL

A Barber Colman model 15 gas chromatograph equipped with an argon β-ionization detector (radium 226) was used for the experimental work. The colums were glass U-tubes, 6 to 8 feet in length and having an inner diameter of 3 mm. The solid support materials were Gas-Chrom P, 100 to 140 mesh, and Chromosorb W, 60 to 80 mesh, both of which were washed with concentrated hydrochloric acid and methanolic potassium hydroxide and treated with hexamethyldisilazane (14). The stationary phases were applied by means of a solution in toluene or butanone as described by Horning, *et al.*, (15). The amines were introduced with a Hamilton microliter syringe as 1.0 μl. of a 0.5 to 1.0% solution of the free bases in chloroform, acetone, or butanone. A number of the amines were found to