Mississippi-Grown *Cannabis sativa* L. IV: Effects of Gibberellic Acid and Indoleacetic Acid

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Abstract □ Four-week-old *Cannabis sativa* L. plants were treated with 1 ml. of gibberellic acid or indoleacetic acid at concentrations up to 250 p.p.m. The treatment was repeated at weekly intervals for 3 weeks. These plants were harvested 1 week after the last treatment. Gibberellic acid caused a significant increase in height and a decrease in the weight of leaves and Δ^9-tetrahydrocannabinol content. Indoleacetic acid produced no significant changes.

Keyphrases □ *Cannabis sativa* L.—effects of gibberellic and indoleacetic acids on plant growth and Δ^9-tetrahydrocannabinol content □ Δ^9-Tetrahydrocannabinol content in *Cannabis sativa* L.—effects of gibberellic and indoleacetic acids □ Gibberellic acid—effect on plant growth and Δ^9-tetrahydrocannabinol content of *Cannabis sativa* L. □ Indoleacetic acid—effect on plant growth and Δ^9-tetrahydrocannabinol content of *Cannabis sativa* L. □ Plant growth regulators—effects of gibberellic and indoleacetic acids on *Cannabis sativa* L.

Many interesting effects upon plants have been demonstrated in recent years with the gibberellins and other plant hormones. The general effects on growth are increased internodal elongation, resulting in taller and spindlier plants. Apical dominance, a growth feature attributed to auxins, is strongly enhanced by the gibberellins (1–3). Gibberellins are reported to decrease alkaloid concentrations in some species (4–6) and increase the concentration of sugars and glycosides in *Digitalis lanata* (7).

Few studies on the cultivation of *Cannabis sativa* L., especially of the drug type, have been reported. At the time this investigation was initiated, there were no reports of the effects of plant hormones on this species. During this study, a group reported (8) that gibberellic acid increased stem length, the percentage of male plants, and the length of the growing season and that a combination of gibberellic acid and colchicine delayed flowering about 20 days. No data on the effects upon yield or concentration of Δ^9-tetrahydrocannabinol (III) were presented, and data on the quantities of gibberellic acid (I) and colchicine used were not included in the abstract.

The objective of this investigation was to determine the effects of gibberellic acid (I) upon the growth and Δ^9-tetrahydrocannabinol (III) content of *C. sativa* L. Since it has been postulated that gibberellins act by increasing the diffusible auxin in plants (9), indoleacetic acid (II) was included in the study.

EXPERIMENTAL

Procedure—Plants employed in this study were of a Mexican strain[1] which is high in III and hence of the drug type (10). Seeds were cultivated in rows 1 m. in width. When the plants were 4 weeks old, they were thinned, allowing 50 cm. between plants.

The plants were divided into nine groups at random. Each group contained 20 plants. These groups included one control group, four kinds of treatments with I[2], and four kinds of treatments with II[2]. The four treatments with each growth substance were 25 p.p.m., 25 p.p.m. × *F*, 250 p.p.m., and 250 p.p.m. × *F*, in which *F* is a factor correlating the height of the plant at the time of application and its height at the time of the first application. One milliliter of the designated concentration of growth substance was applied by a pipet on the uppermost leaves of the treated plants. In the case of the *F* treatments, *F* equaled the number of milliliters applied. Height measurements were taken prior to the application of growth substances and then at weekly intervals. Plants were harvested 1 week after the third application (fourth measurement) which occurred before flowering.

At harvesttime, each plant was uprooted and the roots were thoroughly washed. Plants were divided into leaf, stem, and root portions. Fresh weights were determined immediately in the field. The respective plant parts were then wrapped separately in cheesecloth, marked, and placed in a warm air (50°) circulating dryer. Dry weights were determined. The dried leaves were pooled according to treatment, manicured through a 1-mm. sieve, and thoroughly mixed to a homogeneous mixture.

Analysis—Samples of the pooled manicured leaf samples were assayed for III. One gram of the leaf material was extracted by shaking overnight with 95% ethanol. The ethanolic extract was evaporated under reduced pressure and redissolved in 1 ml. of 95% ethanol containing 10 mg. of the internal standard, 4-androstene-3,17-dione. GLC analysis was carried out using an instrument[3] equipped with a flame-ionization detector and a stainless steel column 0.31 cm. × 3.1 m. (0.125 in. × 10 ft.) packed with 2% OV-17 on 100–200-mesh Gas Chrom Q. Helium was used as a carrier gas at 40 ml./min. The oven temperature was kept at 210°, while the inlet and detector temperatures were 250 and 280°, respectively. The areas under the peaks were directly correlated with the concentration of the compound using a calculated correction factor (11).

The samples were then spotted on silica gel GF plates, developed in two solvent systems [benzene and benzene–hexane–diethylamine (25:10:1)], and then sprayed with diazotized benzedine spray reagent for qualitative comparison of the cannabinoids present (12).

RESULTS

Remarkable changes in branching habit and height were observed in the plants treated with I (Fig. 1). In general, the branching characteristics of the Mexican strain, which is usually bushy with two or more main branches close to the base, demonstrated apical dominance. The plants acquired one main stem with longer internodes and fewer leaves. At the 25-p.p.m. concentration, the plants were healthy and the stems were strong enough for adequate support. At the higher concentrations of 25*F*, 250, and 250*F*, the plants were tall and spindly and had poor support. Furthermore, necroses occurred in the leaves of many of these latter plants.

Treatment with II did not produce any noticeable changes in growth pattern. *T* tests on uncorrelated data were carried out on the height measurements (Table I) and indicated that there was no difference between the groups before treatment, no difference after

[1] Second generation seed harvested in one of the marijuana gardens of the University of Mississippi were used. Original seed was collected near Acapulco, Mexico, and furnished by Dr. John Scigliano, National Institute of Mental Health.

[2] Gibberellic acid (10% potassium salt) and indoleacetic acid (highest purity) were purchased from LaPine Scientific Co., Chicago, Ill., Irwington, N. Y., and Berkeley, Calif.

[3] Beckman GC-5 or GC-45.

Figure 1—*Effect of indoleacetic acid and gibberellic acid on the height and branching habit of marijuana plants.*

treatment with various concentrations of II, and a significant increase in treatments with I utilizing the 0.05 significance level.

Weight data of all plant parts were analyzed statistically using the same test (Table II). There was no effect due to the treatments with II. Treatments with I showed a significant decrease in the fresh and dry weights of the leaves at the 0.05 significance level.

It was unexpected not to find an increase in the stem weights. This indicated that there was no actual increase in the stem growth, but that rather the habit of growth was changed from bushy to tall nonbranched.

The concentration of 1.04% of dry weight of III in the control was considered as the population mean (Table III). The standard deviation of this population was found to be 0.1% of dry weight, since this represented the limitations of the sensitivity of the procedure and sampling techniques. Utilizing the above parameters and the significance level of 0.05, the concentration of III of the treated plants was tested for significance. There was a significant decrease in the two factorial treatments of I. These data agree also with the dry weight and height data. The *F* treatments caused a more pronounced effect than expected. This could be interpreted as due to the larger volume of applied solution which is more evenly distributed and better absorbed in the plant and, consequently, acts on more sites producing more pronounced effects.

TLC revealed no qualitative changes in the cannabinoids present in the plants.

DISCUSSION AND CONCLUSIONS

A typical gibberellin effect characterized by increased internodal elongation, apical dominance, and taller plants was noted. Compound I also caused a decrease in the fresh and dry weights of the

Table I—Height[a] of Plants

Elapsed Days between Measurements	Control	I 25	I 25*F*	I 250	I 250*F*	II 25	II 25*F*	II 250	II 250*F*
0	45	46	45	39	38	38	38	42	39
7	64	75	77	67	65	56	52	59	58
14	83	116	128	99	106	76	72	77	74
21	125	167	185	156	176	124	116	118	119

[a] Average in centimeters per group of 20 plants.

Table II—Weights[a] of *C. sativa* Plant Parts[b]

Treatment	Leaves		Stems		Roots		Total Weight/Plant	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Control	303.8	89.0	255.5	62.8	71.8	15.4	631.1	165.5
I 25	176.6	55.8	258.0	67.8	58.4	12.4	497.0	135.7
I 25*F*	133.9	44.4	261.4	66.3	53.7	11.2	448.9	119.3
I 250	139.3	47.1	216.8	50.35	52.3	9.5	408.0	106.9
I 250*F*	113.5	41.1	268.0	61.1	50.6	11.0	436.2	111.4
II 25	270.6	82.6	236.9	60.8	73.0	14.1	574.2	157.6
II 25*F*	237.5	74.6	207.3	47.98	55.8	10.92	496.3	134.6
II 250	286.8	87.8	247.7	58.8	66.0	13.7	600.1	160.4
II 250*F*	291.5	87.0	247.2	60.8	63.8	13.7	601.2	161.8

[a] In grams. [b] Average of 20 plants.

Table III—Concentration[a] of Δ^9-Tetrahydrocannabinol

	Gibberellic Acid				Indoleacetic Acid			
Control	25	25F	250	250F	25	25F	250	250F
1.04	0.90	0.60	0.89	0.65	0.78	0.96	0.90	0.91

[a] Average percent dry weight of two samples.

leaves and in the content of III. There was no effect on the stem weight, which suggests that the significant increase in height was due to a change in the growing habit from a branching plant to one main stem.

The concentrations of II used were not high enough to induce any effect on the plants. Similar results were observed on *Hyoscyamus niger* L., where concentrations of 10 and 50 p.p.m. of I induced significant effects on rosette plants while concentrations up to 500 p.p.m. of II applied in the same manner failed to induce any stem elongation.

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Kinetics of Hydrolysis of Hypoglycemic 1-Acyl 3,5-Dimethylpyrazoles

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Abstract □ Other investigators suggested that various 1-acyl 3,5-dimethylpyrazoles might owe their hypoglycemic activity to a nonenzymatic hydrolysis *in vivo* to the potent compound 3,5-dimethylpyrazole. As a test of this hypothesis, relative rates of hydrolysis at pH 2.0 and 6.7 (37.6°) were determined for a representative series of compounds covering a wide range of hypoglycemic potencies. No correlation between hydrolysis rate and activity was observed. 3,5-Dimethylpyrazole-1-carboxamide and 3,5-dimethylpyrazole-1-*N*,*N*-dimethylcarboxamide possess equivalent biological activity; the former was rapidly hydrolyzed (half-life about 1 hr. at pH 2.0 and 6.7), whereas the latter was totally stable. Differences in biological activity reflect intrinsic potencies of the various compounds or differences in their absorption and/or metabolism.

Keyphrases □ 3,5-Dimethylpyrazoles, 1-acyl series—kinetics of hydrolysis, hydrolysis rate–hypoglycemic activity correlation □ Hypoglycemic activity–hydrolysis rate correlation—1-acyl 3,5-dimethylpyrazoles, kinetics of hydrolysis □ Hydrolysis rates—1-acyl 3,5-dimethylpyrazole series, correlated with hypoglycemic activity, kinetics

Wright *et al.* (1) reported the hypoglycemic activities of a large number of pyrazoles in the intact, fasted, glucose-primed rat. The most active compounds were 3,5-dimethylpyrazole (I) (2) and various 1-acyl derivatives. Preliminary experiments showed that 3,5-dimethylpyrazole-1-carboxamide (II) was readily hydrolyzed to I at pH's encountered *in vivo* in the GI tract and the blood. As a consequence, it was suggested that the various 1-acyl derivatives of I might owe their activities either to nonenzymatic hydrolysis *in vivo* or to metabolic transformation to I (1). To test the hypothesis of nonenzymatic conversion, relative rates of