INHIBITION OF PLANT GROWTH BY PHENETHYLAMINES AND TETRAHYDROISOQUINOLINES

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ABSTRACT.—Growth inhibitory effects in a bean second internode bioassay were shown by a preliminary screening of those phenethylamines and tetrahydroisoquinolines (and their derivatives) that have been found to occur in peyote and other cacti. Some compounds showed phytotoxicity at all concentrations, whereas others induced toxic effects only when their concentrations were above the tolerance limits of plant growth inhibition. Dopamine hydrochloride and the methiodides of candicine and trichocerine were also evaluated in a sorghum bioassay for growth-inhibiting properties. An attempt was made to interpret the results on the basis of structure-activity relationships.

Several alkaloids containing phenethylamine and tetrahydroisoquinoline moieties are constituents of Cactaceae and other plant families (1). They elicit various physiological responses including hallucinogenic effects. For centuries, Indians of north central Mexico and the southwestern United States used the small peyote cactus in medicine, as an amulet and as a hallucinogenic religious sacrament (2). Mescaline, a peyote constituent, appears to have psychological benefits (3, 4). Peyocactin and hordenine account for the reported antimicrobial activity (5).

Alkaloids appear to be important constituents of plants and their occurrence as secondary metabolites is widespread. According to Swain (16), they constitute about one-half of the low-molecular-weight secondary metabolites (10,000) in higher plants and fungi. A few of them are known to possess plant growth-regulating activity. For example, tomatine, an alkaloid from tomato and other *Lycopersicum* species is a potent growth inhibitor (7). Other alkaloids with growth-regulating activity also have been reported (8).

In our search for natural products with plant growth-inhibiting activity, a series of substituted phenethylamines and tetrahydroisoquinolines and their synthetic analogs was examined for biological activity. Several naturally occurring compounds were isolated from peyote and other cacti. Some of the compounds were tested as their hydrochlorides or quaternary ammonium salts. The physiological activity of these compounds was measured by their ability to retard cell elongation and cell division in a standardized plant bioassay system. These types of activity are commonly used to classify substances as plant growth inhibitors (e.g. abscisic acid). Assays to demonstrate the activity of some of the compounds, especially catecholamines, have been carried out in other test systems (9) in which epinephrine, norepinephrine, dopamine and 3,4-dihydroxymandelic acid show a synergistic response when applied with gibberellins. In this paper, we report

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our preliminary evaluation of the effect of the peyote alkaloids and related compounds on plant growth.

MATERIALS AND METHODS[3]

PREPARATION OF TEST COMPOUNDS.—The procedures for isolation, characterization and synthesis of peyote alkaloids and their derivatives have been reviewed (1, 10). The test candidates were obtained following the published procedures.

BIOASSAYS.

1. *Bean Second Internode Bioassay.*—This bioassay (11) was used to evaluate the growth-regulating activity of alkaloids. Plants were grown in growth rooms (temperature, 25–30°; light, 7.5 Klux for 12 hrs); 6-day-old pinto bean seedlings with second internodes 2-mm long were treated with the test compounds (50 μg/plant and 150 μg/plant) in 250 μg lanolin (tables 1 and 2). The control plants were treated with lanolin alone. After 4 days, the treated and control plants were compared and the percent of decrease or increase in internode elongation relative to the controls was used as a measure of the activity of the growth-inhibiting substances.

Unless otherwise stated, the phenethylamines and tetrahydroisoquinolines were evaluated at 50 and 150 μg concentrations. Only those compounds that exhibited growth inhibition are presented in tables 1 and 2.

2. *Sorghum Bioassay.*—"Rio" sorghum seedlings were grown in petri dishes on moist sterilized cotton in a growth room at 25° under continuous cool white fluorescent light (7.5 Klux). Test compounds (40 μl; concn. 1 mg/1 ml) were applied directly to the roots of each 3-day-old seedling per dish. Measurements were taken after 4 days and the growth of test plants was compared with that of controls grown in water. The percent growth inhibition of the whole plant (from the root level to the top of the plant) was measured.

In both assays, necrosis was the criterion of phytotoxicity.

RESULTS

A number of phenethylamines (table 1) and tetrahydroisoquinolines (table 2) were assayed on beans to evaluate their plant growth-inhibiting activity. The phenethylamines include mono-, di- and trioxygenated compounds which appear to originate from phenylalanine or tyrosine (1, 2). They include the derivatives (methyl and/or benzyl ethers) of tyramine (mono-oxygenated), dopamine (di-oxygenated) and substituted 3,4,5-trihydroxyphenethylamines. The 47 compounds were tested as free bases, hydrochlorides and quaternary ammonium salts (e.g. methiodides). The tetrahydroisoquinolines include those compounds that are trioxygenated at C-6, C-7 and/or C-8 and are biogenetically derived from trioxygenated phenethylamine progenitors (12). Among the 50 tetrahydroisoquinolines (free bases and their salts) tested, only 16 compounds showed growth-inhibiting activity in the preliminary screen (table 2).

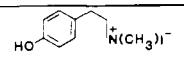
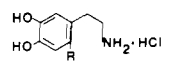
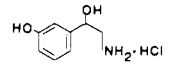
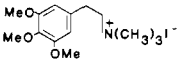
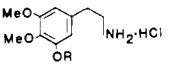
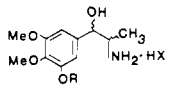
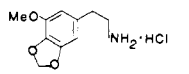
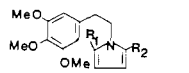
The bean bioassay shows a response to several types of biologically active materials. The treated internode is particularly sensitive to gibberellins and similar compounds, the response being cell elongation. It will also respond to a cell division promoter such as brassinolide at the microgram or submicrogram level (13). In the present tests, however, the response of the bean to essentially all tested compounds was one of inhibition of cell elongation and in many cases inhibition of terminal shoot growth. Abscisic acid (ABA), a natural growth-inhibiting hormone, inhibits elongation of the treated internode in this test system. In a typical experiment, ABA at 10 μg/plant inhibits internode growth by 82% without phytotoxicity (14).

[3]*Abbreviations:* GA_3, Gibberellic acid A_3; IAA, Indole-3-acetic acid; ABA, Abscisic acid; AMO-1618, *N,N,N,2*-Tetramethyl-5(1-methylethyl)-4-[(1-piperidinylcarbonyl)oxy]benzenaminium chloride; chlormequat, 2-(Chloroethyl)trimethylammonium chloride or CCC or chlorocholine chloride or cycocel.

Since results varied and because only a few plants were used in each test in this study, the results are expressed as a range of activity on a 0–100% scale (tables 1 and 2): 0–25% activity designated as +; 26–50% as ++; 51–75% as +++, and 76–100% as ++++. Zero percent activity refers to no growth inhibition and 100% indicates complete inhibition of internode elongation. If the compounds showed very strong inhibition (e.g. +++ and ++++), they were tested at a wide concentration range (0.01–50 μg) to determine the concentration at which growth inhibition would be maximal (fig. 1).

A sorghum bioassay was used to evaluate those compounds that showed

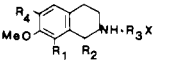
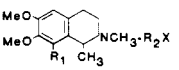
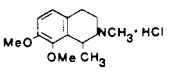
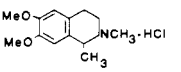
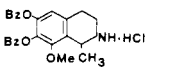
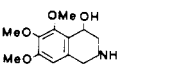
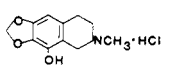
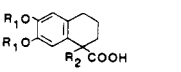
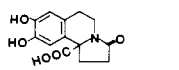
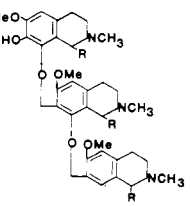
Table 1. Plant Growth-Inhibiting Activity of Phenethylamines and Their Derivatives in Bean Second Internode Bioassay[a]

Compound	Structure	Biological Activity 50μg	Biological Activity 150μg	Comments[b]
1. Candicine Iodide		++++	++++	N at 50 and 150μg
2a. Dopamine-HCl (R=H)		++	+	Tested only at 1 and 10μg
b. 6-Hydroxydopamine-HCl (R=OH)		++	++	--
3. Norphenylephrine-HCl		++	O	--
4. Trichocerine-MeI		++++	++++	N at 50 and 150μg
5a. 3-*Demethylmescaline*-HCl (R=H)		++++	++++	N at 50 and 150μg
b. 3-(Benzyloxy)-4,5-dimethoxy-phenethylamine-HCl (R=Bz)		+++	+	--
6a. β-Hydroxy-α-methylmescaline-HCl (R=CH3, X=Cl)		++	++	N at 150μg
b. β-Hydroxy-α-methylmescaline-oxalate (R=CH3, X=oxalate)		++	+	--
c. β-Hydroxy-α-methylmescaline-D-tartrate (R=CH3, X=tartrate)		++	+	--
d. β-Hydroxy-α-methyl-3-demethylmescaline-oxalate (R=H, X=oxalate)		+	++	N at 150μg
7. 3-Methoxy-4,5-(methylenedioxy)-phenethylamine-HCl		+	++	--
8a. Peyonine (R_1=H, R_2=COOH)		+	–	Tested only at 10μg
b. Peyonine methyl ester (R_1=H, R_2=$COOCH_3$)		+	–	Tested only at 10μg
c. Decarboxypeyonine (R_1=R_2=H)		+++	++	--

a. 6-Day old pinto bean plants were treated with the test compounds at 50μg/plant and 150μg/plant, in lanolin; measurements, after 4 days. Control plants treated with lanolin – 3 replicates for each concentration. Inhibition was measured on 0–100 scale. Range of activity: +, 0–25; ++, 26–50; +++, 51–75; ++++, 76–100%. Biological activity based on 2 repetitive experiments.

b. N-necrosis (indicates phytotoxicity).

Table 2. Plant Growth-Inhibiting Activity of Tetrahydroisoquinolines and Their Derivatives in the Bean Second Internode Bioassay+

Compound	Structure	Biological Activity 50µg	Biological Activity 150µg	Comments *
1a. Salsoline-HCl (R_1=H, R_2=Me, R_3X=HCl, R_4=OH)		++	+++	--
b. Anhalinine-HCl (R_1=R_4=OMe, R_2=H, R_3X=HCl)		+	b	Tested at 10 and 50µg N at 50µg
c. Anhalinine-MeI (R_1=R_4=OMe, R_2=H, R_3X=MeI)		b	b	N at 50 and 150µg
d. (±)-O-Methylanhalonidine-HCl (R_1=R_4=OMe, R_2=Me, R_3X=HCl)		++	+++	--
2a. Pellotine-HCl (R_1=OH, R_2X=HCl)		++	+	--
b. Pellotine-MeI (R_1=OH, R_2X=MeI)		+	+++	Tested at 10 and 50µg N at 50µg
c. O-Methylpellotine-MeI (R_1=OMe, R_2 X=MeI)		++	+++	N at 50µg
3. (±)-Tepenine-HCl		++	b	N at 150µg
4. Carnegine-HCl		++ b	b b	Tested at 10 and 50µg N at 50 and 150µg
5. 1-Methyl-6,7-dibenzyloxy-8-methoxytetrahydro-isoquinoline-HCl		++	++	Tested at 25 and 100µg
6. 4-Hydroxydesmethyl-tehaunine		+	+	N at 150µg
7. 8-Hydroxy-2-methyl-6,7-methylenedioxy-tetrahyroisoquinoline-HCl		+	+	a
8a. 6,7-Dihydroxytetrahydro-isoquinoline-1-carboxylic acid (R_1=R_2=H)		++	++	a
b. 1-Methyl-6,7-dihydroxy-tetrahydroisoquinoline-1-carboxylic acid (R_1=H, R_2=CH_3)		+++	+	a
9. 1,2,3,5,6,10b-Hexahydro-8,9-dihydroxy-3-oxopyrrolo[2,1-a]-isoquinoline-10b-carboxylic acid		++	++	a
10. Pilocerídine		+++	++	Tested at 10 and 50µg

+ 6-Day old pinto bean plants were treated with the test compounds at 50µg/plant and 150µg/plant, in lanolin; measurements, after 4 days. Control plants treated with lanolin - 3 replicates for each concentration. Inhibition was measured on 0-100 scale. Range of activity:+, 0-25; ++, 26-50; +++,51-75;++++, 76-100%. Biological activity based on 2 repetitive experiments.

* N-necrosis (indicates phytotoxicity).
a-concentration at 150µg or above shows slight growth promotion
b-complete necrosis (100% inhibition of internode growth)

growth inhibition of 76–100% (++++). Dopamine-HCl, candicine-iodide and trichocerine-MeI were studied in this system at 1 mg/ml concentration (table 3).

The results shown in table 1 indicate that several phenethylamines induce growth-inhibition in the bean second internode bioassay (inactive compounds are not listed in table 1). A few generalizations based on the structure-activity relationship can be drawn: hydrochlorides and quaternary ammonium salts (methiodides) were generally more active than the free bases; of mono-oxygenated phenethylamines, candicine-iodide was the only active compound; of di- and tri-oxygenated compounds, dopamine-HCl, trichocerine-MeI, 3-demethyl mescaline-HCl and decarboxypeyonine showed the greatest activity while the others showed moderate activity; replacement of the hydroxy group with a methoxy group showed no apparent effect on activity. Compounds containing methylenedioxy groups (e.g. compound 7, table 1) may require a higher concentration to induce maximum activity; several compounds at concentrations above 150 μg showed phytotoxicity. The optimum concentration for effective inhibition is about 10–100 μg.

TABLE 3. Growth-inhibiting activity of dopamine-HCl, candicine iodide and trichocerine-MeI in sorghum bioassay.

Treatments	Plant height[a] (mm)
Controls	45.89±0.70 a
Dopamine-HCl	40.44±0.88 b
Candicine iodide	33.22±0.97 d
Trichocerine-MeI	36.22±1.12 c

[a]The means were tested for difference at the 1% probability level by Duncan's multiple range test. Such differences are indicated by different letters beside the means. Values denote shoot growth from the seed coat to the tip of the second leaf 4 days after treatment.

In the tetrahydoisoquinoline series (table 2), salsoline-HCl (**1a**), (±)-*O*-methylanhalonidine-HCl (**1d**), pellotine-MeI (**2b**) and *O*-methylpellotine-MeI (**2c**) showed strong inhibition at high concentrations (150 μg). Anhalinine as the hydrochloride (**1b**) and methiodide (**1c**) exhibited phytotoxicity (50 and 150 μg), but the hydrochloride showed slight growth inhibition at 10 μg. Among the other compounds including the free bases, dihydroxy acid (**8a**) and piloceridine (**10**) showed activity (50 and 150 μg). However, they were phytotoxic at 150 μg. Other compounds in table 2 showed moderate inhibition (+ and ++). As with phenethylamines (table 1), phytotoxicity was also noted in the tetrahydroisoquinolines when the concentration exceeded 150 μg. We conclude that oxygenation tends to increase the growth-inhibiting activity: tri- di- mono-oxygenation. In general, most of the compounds showed increased activity with increasing concentration in the given concentration range; they then caused phytotoxicity above the concentrations required for inducing growth inhibition (tables 1, 2).

The growth-inhibition results (tables 1, 2) showed that the activity of peyote alkaloids is categorized into two types: 1) Most of the compounds follow a concentration-dependent growth inhibition. 2) A few compounds inhibit strongly

at low concentrations; the activity gradually decreases with increasing concentration and finally diminishes at very high concentrations (some show slight stimulation).

Both types of inhibition patterns were demonstrated clearly in experiments with dopamine-HCl, trichocerine-MeI and candicine iodide (figure 1). Trichocerine-MeI and candicine iodide showed a normal linear inhibition pattern. In contrast, dopamine-HCl at low concentration showed maximum biological activity which gradually diminished with increasing concentrations. Similar observations (not shown) were made with compounds 7, 8a, 8b, and 9 of the tetrahydroisoquinoline series (table 2).

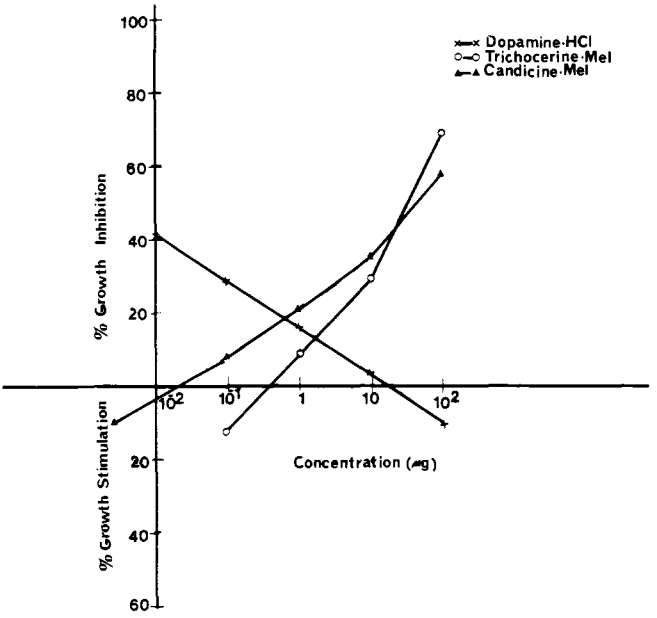


FIG. 1. Percent growth inhibition of dopamine-HCl, trichocerine-MeI and candicine iodide at various concentrations in the bean second internode bioassay.

DISCUSSION

Interpretation of the structure-activity relationship is difficult without knowing the site and mode of action of the materials. It is not clearly understood why the growth inhibition by a few compounds (e.g. dopamine-HCl) at relatively low concentrations is greater than that inhibition found at higher concentrations (above 10 μg). This is contrary to the generally accepted phenomenon that growth inhibition is concentration dependent and should give a linear relationship within a given concentration range. A majority of compounds (e.g. trichocerine-MeI and candicine iodide) exhibit the usual growth inhibition pattern and show phytotoxicity above that concentration range (150 μg).

High concentrations of some of the compounds (tetrahydroisoquinolines in general, and salsoline and (±)-*O*-methylanhalonidine hydrochlorides in particular) were needed to exhibit growth-inhibiting properties. This may be due to slow uptake at the site of action and/or slow metabolism, as was shown in previous

growth inhibition studies (15). This is particularly true in the case of salts (free bases vs. their methiodides) which could serve as alkylating (methylating) agents (16). Further, these salts may be absorbed much faster than the free bases because of their solubility in water.

These changes, particularly with derivatives, may affect permeability and penetrability, not only into cells but also to active sites. Any speculation upon the role of these compounds should also include their effect on cell division in addition to their effect on cell enlargement.

Some of the peyote and related constituents may play a regulatory role comparable to that of abscisic acid. The present study simply suggests the potential for these and similar compounds in the field of plant growth regulation. Furthermore, the activity shown by the test system indicates that these compounds may have a growth-regulatory role in the cactus and other plants in which they occur naturally (1–5). However, there is no evidence available for such an *in vivo* role of these alkaloids.

There is some similarity between these growth effects and those produced by AMO-1618 and chlormequat, and several quaternary ammonium derivatives (+)-limonene (17, 18, 19). For the latter, the onium ion in quaternary ammonium salts is responsible for biological activity (16). Moreover, the onium pole is susceptible to nucleophilic attack which often leads to the production of tertiary amines. This may explain why the salts are more active than the free bases. Correlations between growth regulation and enzyme (acetylcholinesterase) inhibition suggest that the peyote alkaloids and their derivatives may act on an enzyme-mediated step essential to plant growth (20).

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