Plant Growth Regulating Activity of Substituted Phthalate Esters

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Nine esters of phthalic and related acids inhibited growth and induced chlorosis in meristematic tissues of several plant species. Among the 29 compounds tested, the propyl esters of the following acids are listed in order of decreasing activity: 3-methylphthalic; 3-nitrophthalic; 3-bromophthalic; 1,2,3-benzenetricarboxylic; 2-bromoisophthalic; phthalic; and 2,3,4-pyridinetricarboxylic. The butyl and allyl esters of 1,2,3-benzenetricarboxylic acid are less active than the corresponding tripropyl ester. The 1,2- and 1,2,3-substitution patterns were necessary for this particular combination of growth regulating activities. The active phthalates effectively inhibited the growth of tobacco, beans, corn, and *Coleus* when applied as emulsions. The growth inhibition was accompanied by an absence of chlorophyll in the developing parts of the treated plants. The effects were localized, with no evidence of translocation.

Buta and Steffens (1970) studied the effectiveness of alkyl esters of halogenated benzoic acids as contact plant growth inhibitors. But they questioned the activity of the corresponding nonhalogenated benzoates. Several preliminary experiments indicated slight activity of butyl benzoate as a contact type of regulator. Dibutyl phthalate had been reported to stimulate slightly the growth of rice plants, but had no activity in other plant bioassay systems (Isogai and Komoda, 1972). Studies on a series of benzene carboxylic acids, such as the various phthalic acids, and their esters have not been published. Therefore, this study was undertaken to determine whether these compounds have any significant plant growth regulating activity and to relate structure to activity by use of several intact plant systems.

EXPERIMENTAL SECTION

Organic Compounds. The carboxylic acids (Table I) were obtained commercially or prepared by permanganate oxidation of suitable precursors. The esters were prepared by the usual methods and purities checked with ir and GLC.

Biological Assay Procedures. *Nicotiana tabacum* cv. Xanthi-nc plants (6 weeks old) were used for the most extensive tests (Marth and Mitchell, 1964). The upper portions of the intact plants were sprayed to runoff with emulsions of test compounds containing 1% Tween-20 and 2.5% methyl isobutyl ketone. Four plants were used per treatment and each treatment was repeated a minimum of three times. The concentrations of emulsified compounds tested were 1×10^{-4}, 1×10^{-3}, 5×10^{-3}, 1×10^{-2}, 2×10^{-2}, and 3×10^{-2} *M*. The apical portions of the treated plants were harvested after 14 days and the weight of new growth compared to that of the control plants.

Phaseolus vulgaris L. cv. Greenpod 407 plants (7 days old) were treated by spraying to runoff with emulsions of test compounds at concentrations of 1×10^{-4} to 1.5×10^{-2} *M*. Other experiments involved dropping measured quantities of emulsified compounds on the apex of the test plant. *Coleus* sp. (30 days old) were also sprayed with emulsions of the test compounds at a concentration of 1×10^{-2} *M*. *Zea mays* L. cv. Gold Cup (14 days old) plants were treated by spraying with emulsified compounds or by dropping emulsions at 1×10^{-2} *M* into the central leaf whorl of the plants.

The possible translocation of the compounds found to be active was studied by applying 0.01 ml of emulsified tripropyl 1,2,3-benzenetricarboxylate at 3×10^{-2} *M* within a 1-cm diameter lanolin ring constructed on the upper surface of small expanding leaves of *Nicotiana* cv. Xanthi plants. Another experiment involved the injection of the same ester emulsion at 1×10^{-2} *M* into the stems of Xanthi plants just below the apex by use of a hypodermic syringe. Ten to twenty microliters of emulsion could be introduced in this manner in 3 days by intermittent injections.

RESULTS AND DISCUSSION

An unexpected response to the application of the tripropyl ester of 1,2,3-benzenetricarboxylic acid at 1×10^{-2} *M* was the development of extensive chlorosis with concomitant growth inhibition on the test *Nicotiana* plants 3 days after treatment. The chlorosis appeared to be confined to parts of the plants that were meristematic when treated. At this concentration, 1×10^{-2} *M*, the plant apex remained chlorotic (albinistic) and much inhibited in growth for at least 14 days (Figure 1). Eventually growth resumed; the newly developed leaves were normal in size and apparent chlorophyll content. The lower leaves that were present and had responded to the tricarboxylate ester treatment expanded somewhat but remained chlorotic. If lower concentrations of the ester, 1×10^{-3} or 5×10^{-3} *M*, were sprayed on the test plants, growth inhibition was not significant and only a variegated pattern of chlorotic tissue was observed on the expanding leaves. Ester concentrations less than 1×10^{-3} *M* caused no visible response to treatment. Concentrations such as 2×10^{-2} or 3×10^{-2} *M* resulted in the same apical chlorosis and growth inhibition as seen with 1×10^{-2} *M* treatments.

To examine the structural requirements of related compounds that might cause this type of plant growth regulating activity, a number of structurally similar compounds were synthesized and applied to *Nicotiana* plants at 1×10^{-2} *M* (Table I). The compounds considered to be active caused an initial apical chlorosis and the degree of activity of the various compounds could be related to the amount of growth inhibition found after 14 days. The apices of plants that had been treated with propyl 3-methylphthalate or corresponding nitro or bromo esters were essentially chlorotic and very little growth occurred within the 14-day test period. Variegated apices resulted from treatments with compounds that caused 60% or less growth inhibition.

Tripropyl 1,2,3-benzenetricarboxylate or dipropyl 3-methylphthalate were applied also to beans, corn, and *Coleus* at the 1×10^{-2} *M* concentration used with tobacco. The same pattern of chlorosis and growth inhibition of the meristematic tissues of these plant species was observed. A dropwise application of 20 μg of tripropyl 1,2,3-benzenetricarboxylate applied as a 1×10^{-2} *M* emulsion to the apex of a bean plant before the second internode had elongated caused the new growth to be chlorotic. A concentration of

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Table I. Inhibition of Growth of *Nicotiana* by Chlorosis-Inducing Phthalates[a]

Compound	% inhibition[b]
Dipropyl 3-methylphthalate	100
Dipropyl 3-nitrophthalate	90
Dipropyl 3-bromophthalate	87
Tripropyl 1,2,3-benzenetricarboxylate	74
Dipropyl 2-bromoisophthalate	60
Dipropyl phthalate	48
Tributyl 1,2,3-benzenetricarboxylate	40
Triallyl 1,2,3-benzenetricarboxylate	32
Tripropyl 2,3,4-pyridinetricarboxylate	30

[a] Structurally related compounds that did not induce chlorosis: dipropyl 3-aminophthalate; dipropyl 3-hydroxyphthalate; dipropyl isophthalate; dipropyl 2-nitroisophthalate; dipropyl terephthalate; hexapropyl benzenehexacarboxylate; pentapropyl benzenepentacarboxylate; propyl 2-nitro-3-methylbenzoate; pyrogallol tripropionate; tetrapropyl 1,2,3,4-benzenetetracarboxylate; tetrapropyl 1,2,3,5-benzenetetracarboxylate; triethyl 1,2,3-benzenetricarboxylate; trimethyl 1,2,3-benzenetricarboxylate; tripentyl 1,2,3-benzenetricarboxylate; 1,2,3-tripropoxybenzene; tripropyl 1,2,4-benzenetricarboxylate; tripropyl 1,3,5-benzenetricarboxylate; tripropyl 1,2,3-naphthalenetricarboxylate. [b] Plants were sprayed with emulsions of the test compounds at 10^{-2} *M*. The inhibited (chlorotic) apical portions of the plants were harvested and weighed 14 days after treatment. Percentage of inhibition expressed is (control minus test/control) × 100.

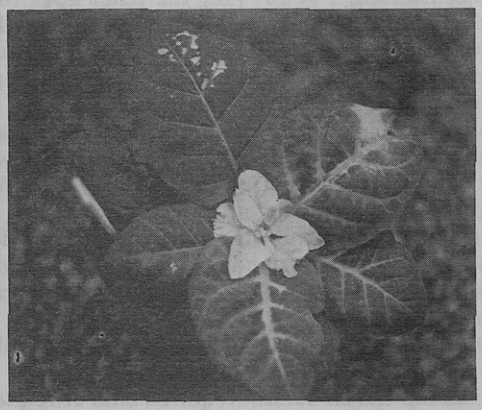

Figure 1. *Nicotiana* Xanthi treated with tripropyl 1,2,3-benzenetricarboxylate at 1×10^{-2} *M* (spray).

30 μg of the ester applied to the apex was sufficiently toxic to completely inhibit further growth and caused the death of the plant.

No translocation of the tripropyl 1,2,3-benzenetricarboxylate was observed using the application of active compound within the lanolin ring constructed on expanding tobacco leaves. The tissue contacted by the chemical within the ring became chlorotic while the surrounding tissue developed apparently normally. Lack of translocation is also suggested by the lack of response of tissues developed after application as seen in the spray treatments.

Applied in high concentrations, lipids such as the benzoate esters usually cause some phytotoxic effects such as bud destruction or contact damage to leaves (Ashton and Crafts, 1973). Applications of the specific active compounds listed in Table I inhibit growth and cause chlorosis at concentrations so low that nonspecific structural damage to plant tissues is not obvious.

Plant growth regulation is related to certain structural characteristics of the benzoates. Esterification of the benzoic acids enhances penetration through the plant cuticle. Unesterified acids cause no growth regulation. Also important is the length of the alkyl chain of the esters, since penetration is maximized with the 3- and 4-carbon chain lengths of a homologous C_1–C_{10} alkyl series (Buta and Steffens, 1971). If the chain length of the alkyl moiety of the ester is increased, as for example, tridecyl 1,2,3-benzenetricarboxylate, the activity is lost. Benzoic acid derivatives cause various types of plant growth regulating activity (Ashton and Crafts, 1973; Hanson, 1973). Nevertheless, the particular combined effects observed with the substituted phthalates presented in Table I were not published previously.

The chlorosis and growth inhibition of meristematic tissues appear when the benzene ring has relatively inert substituents (–CH_3, –Br, –NO_2, or –CO_2R) at the 1,2,3 positions on the ring. Activity was not detected if biologically labile groups such as OH or NH_2 were substituents on phthalic acids. The arrangement of the substituents within the 1,2,3 grouping is important, as can be seen by more activity with Br substitution in dipropyl 3-bromophthalate than in dipropyl 2-bromoisophthalate. Activity is lost by substitution at more than the 1,2,3 locations on the benzene ring. This loss, plus the substituent effects discussed above, suggests that structural requirements for activity are rather specific. Sites of activity responsive to these esters appear to be located in the meristematic and young differentiating tissues of plants. However, the mode of action of these compounds is not known and the relationship between the growth inhibition and inhibition of chlorophyll development has not been studied extensively.

Biological activity of phthalate esters has been studied extensively in animals (Jaeger and Rubin, 1970). Phthalate esters at low concentrations may act as teratogens or mutagens in some animals (Autian, 1973). The presence of these esters in biological samples appears to be due to contamination, since little evidence is available to support the identification of phthalates as natural products, especially in plants (Mathur, 1974). Certain phthalates were previously reported to be slightly active as plant growth regulators in bioassays. However, the results reported here indicate that the nine esters related to the phthalates (Table I) significantly inhibited growth and reduced chlorophyll levels in intact plants. Modification of the phthalate structure to the monosubstituted compounds with the 1,2,3-substitution pattern resulted in greatly enhanced activity causing growth inhibition and chlorosis.

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