the waste material from the processing of upholstering fibers, it may prove profitable for some industrial organization to investigate this plant as a possible source for a hard natural wax.

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Manuscript received October 28, 1952.

Effects on Plant Growth of Some Compounds with Structural Similarities to Maleic Hydrazide[1]

Victor A. Greulach

Department of Botany,
University of North Carolina, Chapel Hill

Investigation of the effects on plant growth of compounds structurally related to maleic hydrazide should help clarify the mechanism of maleic hydrazide inhibition of plant growth. Maleic acid, maleimide, and the cyclic hydrazides and imides of related dicarboxylic acids should be particularly useful, although other hydrazides, hydrazines, and hydrazine itself might yield information of value. The difficulty of obtaining many of these compounds has been at least partially responsible for lack of reports on their effects on plant growth. This paper deals with the first of a series of experiments on the effects of such compounds on plant growth. Additional compounds in the groups mentioned are being investigated as they become available, not only for comparison with maleic hydrazide, but also for possible interesting effects which they may have on plant growth.

The effects of hydrazine derivatives, other than maleic hydrazide, on plant growth have been investigated little if at all, but there have been a number of studies on the effects of maleic acid and maleates on both plants and animals. Most investigators seem to agree that maleic acid is a respiratory inhibitor in animals and microorganisms, though some have reported it to be utilized as a respiratory substrate (*1, 2*). Annau (*3*) claims that maleic acid competes with fumaric and succinic acids for enzyme surfaces without fulfilling the functions of the latter acids and Weil (*4*) reported that maleic acid inhibits the Krebs cycle but not glycolysis. Copisarow (*5, 6*) found that maleic acid dissolved in etherial oils (but not in water) inhibited the sprouting of potatoes, the ripening of apples, and the growth of fungi on both. Isaacs (*7*) confirmed its effect on fruit ripening, but reported that skin injuries by the acid made the fruits more susceptible to fungi. English *et al.* (*8*) stated that maleic acid is slightly effective as a traumatin. Bonner and Galston (*9*) found that maleic acid had a slight inhibiting effect on plant growth, while Lundegårdh (*10*) reported that maleic as well as fumaric acid was utilized by wheat roots and accelerated their respiration. Thimann and Bonner (*11*) found that maleic as well as other organic acids counteracted iodoacetate growth inhibition. Krishnamurti and Subrahmanyan (*12*) found that maleic acid and a variety of other compounds inactivated the milk-clotting and protease enzymes of fig latex by affecting active —SH groups in the enzymes. Since Greulach and Atchison (*13*) and a number of subsequent investigators have found maleic hydrazide to be an antimitotic in plants, it is particularly interesting that Friedman *et al.* (*14, 15*) found maleic acid to be an antimitotic in chick fibroblasts and later (*16*) reported maleimide and citraconimide to be even more effective antimitotics, whereas succinimide was inactive. They suggest that antimitotic effectiveness was directly proportional to the rate of —SH uptake by these compounds.

Although hydrazides other than maleic have not been used on higher plants, Grunberg and Schnitzer (*17*) and others have found isonicotinic hydrazide and its derivatives to inhibit growth of the tuberculosis bacillus and claim it to be effective in the treatment of tuberculosis.

In the experiments reported on here bean and sunflower plants two weeks old and tomato plants three weeks old were dipped in solutions of the following compounds: maleic hydrazide, maleic acid, diformyl hydrazine, phenylhydrazine hydrochloride, succinic hydrazide, succinimide, isonicotinic hydrazide (rimifon), and 1-isonicotinyl 2-isopropyl hydrazide (marsilid).[2] Succinic and fumaric acids were also used for comparison with possible maleic acid effects, although all three acids were actually applied as their sodium salts to avoid pH effects. With the following exceptions, all solutions were 0.015 *M*: (1) in one experiment with beans 0.03 *M* diformyl hydrazine was used, (2) the quite insoluble succinic hydrazide was applied as a saturated solution of around 3×10^{-4} *M*, (3) succinic hydrazide was also applied to one series of plants at 4000 ppm in lanolin applied to the under surface of one leaf of each plant, with plain lanolin being used on the controls. Dreft was added to each solution at a concentration of 0.025% as a wetting agent. A minimum of six plants was used per treatment, the plants being maintained in a greenhouse in porous clay pots. Weekly observations and measurements of height were made, and "t" values were calculated to determine the significance of the differences of the means.

The effects of the various compounds on the growth

[1] This report is one of a series from a research program aided in part by a grant from the Carnegie Foundation for the Advancement of Teaching.

[2] The maleic hydrazide (as the diethanolamine salt) was supplied through the courtesy of the Naugatuck Chemical Company, the rimifon and marsilid by the Hoffmann-LaRoche Corporation, and the succinic hydrazide, succinimide, and diformyl hydrazine were synthesized by the Chemistry Department of the University of North Carolina under the direction of Arthur Roe.

TABLE 1

MEAN GROWTH OF PLANTS FOR 28 DAYS FOLLOWING TREATMENT WITH COMPOUNDS HAVING AFFINITIES WITH MALEIC HYDRAZIDE

(All solutions were 0.015 *M* except as noted)

Compound	Growth as percentage of growth of controls				
	Beans, 1	Beans, 2	Beans, 3	Tomatoes	Sunflowers
Maleic hydrazide	14.0*	15.2*	15.7*	10.7*	11.7*
Succinic hydrazide solution†	—	53.1*	74.5*	125.2	—
Succinic hydrazide in lanolin‡	—	58.6*	80.0*	139.8‖	—
Diformyl hydrazine§	137.0‖	99.3	74.6*	136.9‖	—
Phenylhydrazine hydrochloride	—	—	3.4*	15.7*	—
Isonicotinic hydrazide (rimifon)	—	—	76.5*	61.0*	93.3
1-Isonicotinyl 2-isopropyl hydrazide (marsilid)	—	—	73.4*	72.9*	60.9*
Succinimide	164.0*	120.7	—	129.1	—
Sodium maleate	185.0*	128.9	—	122.3	—
Sodium succinate	105.0	114.5	—	114.6	—
Sodium fumarate	107.0	115.9	—	125.2	—

* Significant at the 1% level or less.
† About 3×10^{-4} *M*.
‡ 4000 ppm.
§ 0.03 *M* in bean-3 experiment.
‖ Significant at the 5% level.

in height of the plants is summarized in Table 1. Except for phenylhydrazine hydrochloride, none of the compounds caused growth inhibition comparable with that brought about by maleic hydrazide. The phenylhydrazine was apparently quite toxic, causing death of the younger parts of all stems, but subsequent to the time the data in the table were taken, all these plants resumed growth from lateral buds. The new growth was normal except for reduced leaf size. Significant growth inhibition was also caused by succinic hydrazide (in beans but not tomatoes), 0.03 *M* (but not 0.015 *M*) diformyl hydrazine, rimifon, and marsilid. Although inhibition of sunflowers by rimifon 28 days after treatment was not significant, there was a highly significant growth inhibition 14 days after treatment followed by rapid recovery.

Only rimifon and marsilid produced formative effects at all similar to those caused by maleic hydrizide, the marsilid effects being more pronounced in both beans and sunflowers than the rimifon, while neither brought about formative effects in the tomatoes. The principal effects in beans were emarginate leaflet apexes, extra leaflets, and downward curled margins on some leaflets. The sunflower leaf margins were much more extensively curled, many leaves were emarginate, and marsilid reduced leaf size to about half that of comparable control leaves. The leaves which developed subsequent to treatment with marsilid were very narrow, accuminate, wrinkled, and distorted. On many of the older leaves of plants treated with marsilid a roughly oval chlorotic spot developed almost across the base of the blade, and the tissue just above this was unusually dark green. About half of the sunflower plants treated with marsilid lost apical dominance, followed by branching. Marsilid also produced deformities of the flower heads similar to those following maleic hydrazide treatment. Both marsilid and rimifon reduced the number of flowers and fruits of the beans plants significantly, but neither blocked reproductive development as does maleic hydrazide. Succinic hydrazide and phenylhydrazine hydrochloride also reduced but did not block reproductive development of the bean plants.

The leaves of all bean, but not tomato, plants dipped in diformyl hydrazine turned white on the upper surface within a week following treatment, followed by necrotic spots and eventual death of the leaves. Leaves developing subsequent to treatment were not affected. The white surface proved to be due to the loosening of the epidermis from the mesophyll. The bean plants treated with succinic hydrazide looked quite different from those treated with maleic hydrazide, being spindly, generally chlorotic, and somewhat wilted. This indicates that it did not act in a manner comparable with maleic hydrazide, and in view of the low solubility of succinic hydrazide and its failure to affect the tomato plants adversely, it is possible that the effects on beans were due to some other factor, associated with the succinic hydrazide treatments. Further study of this compound is needed.

All plants treated with succinimide, 0.015 *M* diformyl hydrazine, and maleate, as well as with succinate and fumarate, and the tomato plants treated with succinic hydrazide had a greater mean height than the controls, and in the first experiment with beans the differences for maleate and succinimide were highly significant. The plants treated with the compounds mentioned also appeared to be sturdier and darker green than the controls even in experiments in which the differences in height were not significant, indicating that these compounds were being utilized by the plants.

Although the present preliminary data do not warrant any extensive generalizations, they do indicate that further investigation of various hydrazine derivatives as plant growth inhibitors should prove to

be fruitful. On the basis of the results presented here and some of those presented elsewhere, especially by Lundegårdh (*10*), malate (or maleic acid) does not appear to be a plant growth inhibitor, but may actually be utilized by at least some plants. However, in view of the inhibitory effect of maleic acid on animal respiration and mitosis, the possibility that under certain conditions it may act in a similar capacity in plants cannot be discarded. The rather conflicting results with maleic acid could possibly be due to its enzymatic conversion into related naturally occurring metabolic acids in at least some plants under certain conditions.

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Manuscript received October 29, 1952.

The Reduction of Vitamin B_{12}

Richard N. Boos, Jean E. Carr, and John B. Conn

Research Laboratories, Merck & Co., Inc., Rahway, New Jersey

We have carried out a titrimetric reduction of cyanocobalamin with chromium (II) ethylenediamine tetraacetate complex in a specific application of a new general analytical technique to be described in detail elsewhere. Here we shall list some observations concerning the nature of reduced vitamin B_{12} which are at variance with those reported by Diehl *et al.* (*1, 2*).

In 0.1 *M* sodium enta, pH 9.5, cyanocobalamin gives a polarogram comprising a single reductive wave, $E_{1/2} = -1.021$ v vs saturated calomel electrode (25°). When this solution is titrated amperometrically with standard 0.1 *N* chromium (II) chloride under rigorous exclusion of oxygen, the diffusion current corresponding to the above wave diminishes linearly with volume of titrant; at the same time an *anodic* wave, $E_{1/2} = -0.311$ v vs saturated calomel electrode appears whose diffusion current reaches maximum development

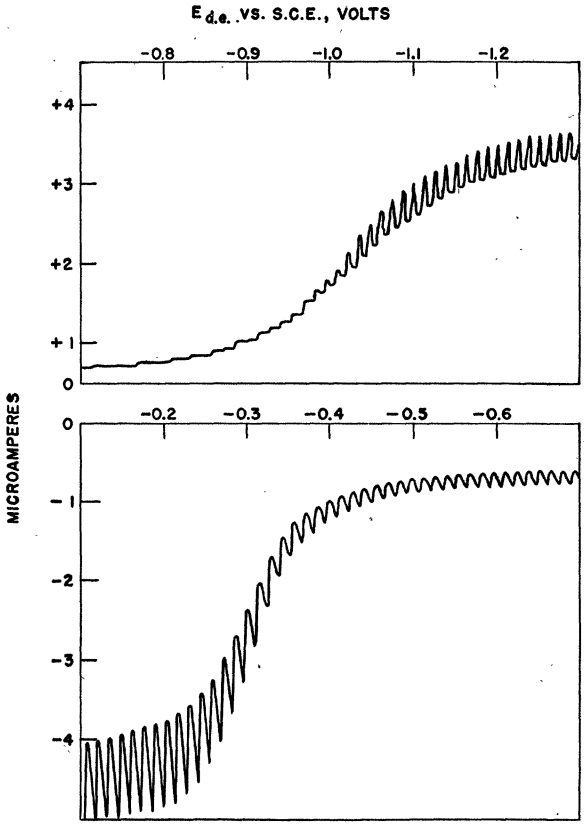

FIG. 1. Top, cathodic wave of vitamin B_{12}; bottom, anodic wave of reduced vitamin B_{12}.

coincident with the vanishment of the cyanocobalamin wave. During this operation the color of the solution changes from red to brown. Polarograms of cyanocobalamin and its reduction product are shown in Fig. 1; titrimetric data on replicate determinations are given in Table 1, and polarographic data in Table 2.

It is evident that cyanocobalamin and its reduction

TABLE 1*

Cyanocobalamin (mg)	Milliequivalents of Cr^{++}	Equivalent weight of cyanocobalamin
13.62	0.01017	1341
2.224	0.001679	1342

* The sample of cyanocobalamin used in this investigation was 99.8% pure by solubility analysis on dry basis; drying loss was 22.8%. All data were obtained on hydrous crystals and corrected accordingly.

TABLE 2

		Cyanocobalamin	Reduction product
$E_{1/2}$ v vs S.C.E.		−1.021 (1.362 mg/cc)	−0.311 (0.3094 mg/cc)
$I_d/Cm^{2/3}t^{1/6}$	(C in mg/cc)	0.219	0.318