gluten HMD-105-B-78 hydrolyzate derivatives were responses equal to that produced by the parent acid. All other products had less effect on the growth inhibition of bean, sunflower, cucumber, and barley than did 2-(2,4-DP).

Epinasty. Only on cucumber did hydrolyzate products induce a greater effect than did 2-(2,4-DP). Products affecting this response were those prepared from α-protein 4-hour, meat and bone meal 4-hour, and wheat gluten HMD-105-B-78 hydrolyzates. A few products did induce an equal response in some plants, but the majority induced less response.

Formative Effects. No hydrolyzate products induced formative effects exceeding those of the parent acid. The hydrolyzates produced less formative effects than did the parent acid in the case of cucumber.

Cell Proliferation at the First Internode. Three derivatives induced a slightly greater effect than 2-(2,4-DP). These were wheat gluten 5-SD acting on sunflower, and wheat gluten Luxor and HMD-105-B-78 acting on cucumber. On the cucumber hypocotyl, nine products induced cell proliferation from slight to moderate as compared with the parent acid which induced none. These derivatives were the α-protein 2-hour and 8-hour, animal glue 2-hour and 4-hour, meat and bone meal 4-hour and 8-hour and the wheat gluten 5-SD and HMD-105-B-78 hydrolyzates. On the treated area only a slight to moderately increased cell proliferation was induced on cucumber by α-protein 2-hour and 4-hour hydrolyzates and by all the meat and bone meal hydrolyzates. There was less response to the majority of other derivatives than to the parent acid.

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PLANT REGULATOR TRANSLOCATION

New Plant Regulators That Exude from Roots

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The plant growth regulator α-methoxyphenylacetic acid is absorbed, translocated throughout the plant, and exuded from the roots. The regulating efficacy of this compound is associated with the L (+) stereoisomer. Three new halogen-substituted forms of the acid, *m*-chloro-, *m*-fluoro-, and *p*-fluoro-, proved to be very effective plant regulators. These were exuded from roots of treated plants in readily detectable amounts. Four other ring-substituted forms of the acid also exhibited marked growth-regulating properties, but these compounds were not exuded from roots of treated plants in detectable amounts. Root exudation was governed by the number and position of substitutions in both the aromatic and the aliphatic portions of the reference compound, α-methoxyphenylacetic acid.

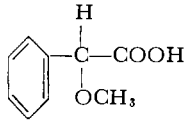
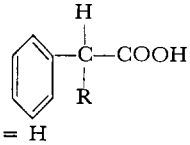
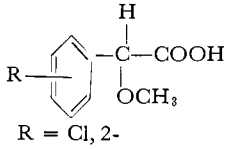
The plant regulator α-methoxyphenylacetic acid (*5*) has been reported to be readily absorbed and translocated by many kinds of plants (*3*). This methoxy compound when applied to leaves or stems of some herbaceous plants is translocated readily downward to the roots and exuded. Root exudation of the methoxy regulator is detectable, because the exuded compound may be absorbed by roots of nearby untreated plants in amounts sufficient to induce foliar growth modification (*4*). Similar root exudation of certain chlorinated benzoic acids has also been reported (*1*). Linder *et al.* (*2*) recently described the quantitative aspects of the absorption, translocation, and exudation by roots of carbon-14–labeled α-methoxyphenylacetic acid.

Because the translocation of organic compounds down stems into roots, and exudation of these compounds in easily detectable amounts are of interest in connection with pest control, the absorption and translocation, plant-regulating properties, and the exudation from roots of the stereoisomers of α-methoxyphenylacetic acid and a wide variety of related compounds were studied. This paper summarizes the results obtained.

Methods

A paste containing 1% of each compound was prepared by dissolving 12.5 mg. of the substance in 0.25 gram of Tween 20 (sorbitol derivative, Atlas Powder Co., Wilmington, Del.). One gram of melted lanolin was then added and thoroughly mixed. Ten Pinto bean plants—two plants per 4-inch pot—grown in composted soil were selected

Table I. Plant-Regulating Properties and Translocatability of Stereoisomers of α-Methoxyphenylacetic Acid and Structurally Related Compounds Applied to Pinto Bean Plants

Compound Designation	Name	Structural Formula	Transient Foliar Effects[a]	Movement out of Roots	Cell Proliferation[b] Local	Cell Proliferation[b] Systemic	Formative Effects[c]	Growth Inhibition[d]
		Stereoisomers						
A	DL-α-Methoxyphenylacetic acid (6)[e]		+++[f]	+++	+	+++	+++	+++
B	D-α-Methoxyphenylacetic acid		0	0	0	0	0	0
C	L-α-Methoxyphenylacetic acid		+	+++	++	+++	+++	+++
		α-Carbon Substitutions						
								
D	Phenylacetic acid	R = H	+	0	+	0	0	0
E	Hydratropic acid	R = CH_3	0	0	0	0	0	0
F	Mandelic acid	R = OH	0	0	0	0	0	0
G	α-Ethoxyphenylacetic acid (6)	R = OC_2H_5	+++	0	+	++	+++	+
		Substitutions on Phenyl Ring						
								
H	o-Chloro-α-methoxyphenylacetic acid (ammonium salt)	R = Cl, 2-	0	0	+++	0	+++	+
I	m-Chloro-α-methoxyphenylacetic acid (ammonium salt)	R = Cl, 3-	++	++	+++	+++	+++	++++
J	2,4-Dichloro-α-methoxyphenylacetic acid (6)	R = Cl, 2,4-	++	0	+++	0	+++	+++
K	3,4-Dichloro-α-methoxyphenylacetic acid (6)	R = Cl, 3,4	++	0	+++	+++	++	++++
L	p-Bromo-α-methoxyphenylacetic acid	R = Br, 4-	+++	0	++	++	+++	+++
M	m-Fluoro-α-methoxyphenylacetic acid (ammonium salt)	R = F, 3-	+	++	++	+++	+++	+++
N	p-Fluoro-α-methoxphenylacetic acid	R = F, 4-	0	+++	+++	+++	+++	+++
O	m-Nitro-α-methoxyphenylacetic acid	R = NO_2, 3-	+++	0	+++	+++	+++	+++
P	α-o-Dimethoxyphenylacetic acid	R = OCH_3, 2-	+	0	0	0	+++	++
Q	α-2,3-Trimethoxyphenylacetic acid (ammonium salt)	R = OCH_3, 2,3-	0	0	+++	+++	+++	+++
R	α-3,4-Trimethoxyphenylacetic acid	R = OCH_3, 3,4-	0	0	0	0	0	0

[a] Temporary upward or downward curling of primary leaves.
[b] Cell proliferation involving the treated region of stem (local) or extending above and below this region (systemic).
[c] Modification of trifoliate leaves.
[d] Inhibition of stem and leaf growth following application of the compound.
[e] Racemic mixtures were used except where otherwise designated.
[f] Slight, moderate, marked, and complete designated by +, ++, +++, and ++++, respectively.

for uniformity and used in a greenhouse to test each preparation. Primary leaves of selected plants were 3 to 4 cm. wide and their trifoliate leaves were still folded in the terminal buds. A small portion of the paste containing approximately 125 γ of a compound was applied as a band 3 to 5 mm. wide and about 1 mm. thick around the stem of one plant of each pair. The band was placed about midway between the first and the second node. Comparable pairs of plants to which no chemicals were applied were used as controls. The plants were then grown for 7 to 10 days, and growth responses of plant pairs were periodically evaluated by comparing them with untreated plant pairs.

Results and Discussion

The D (−) and L (+) isomers of α-methoxyphenylacetic acid were markedly different in their plant-regulating properties; the L (+) form was very active, while the D (−) form had no apparent effect on the test plants (B and C, Table I.) The racemic mixture, A, resulted in responses similar to those induced by the L (+) form. Responses due to treatment with the methyl ester of α-methoxyphenylacetic acid were identical with those induced by the racemic mixture of the parent acid.

Complete loss of the slight regulating activity of phenylacetic acid, D, resulted from substitution of a methyl or a hydroxyl group for one of the hydrogen atoms associated with the α-carbon E, F. Replacement of one of the hydrogens associated with the α-carbon by an ethoxy group, G, however, increased the regulating activity substantially over that of phenylacetic acid, but did not impart the root-exudation characteristic associated with the methoxy group substitution, A.

m-Chloro-, I, *m*-fluoro-, M, and *p*-fluoro-, N, substituents in the phenyl ring of α-methoxyphenylacetic acid resulted in marked plant regulation and, in addition, they were exuded by roots of the treated plants in readily detectable amounts. *o*-Chloro-, H, *p*-bromo-, L, 2,4-dichloro-, J, and 3,4-dichloro-, K,

phenyl ring substitutions in the reference compound, α-methoxyphenylacetic acid, on the other hand, exhibited moderate or marked plant-regulating activity as did *m*-nitro-, O, substitution. These compounds were not exuded by the roots of treated plants in biologically detectable amounts. Ring positions 3 and 4, therefore, show limited flexibility in the type of substituent that can be used to replace the hydrogen without interfering with the ability of the roots to exude the compound. α-Aminophenylacetic acid, the ethyl ester of α-cyanophenylacetic acid, α-methoxydiphenylacetic acid, α-phenylbutyric acid, *p*-isopropyl-α-methoxyphenylacetic acid (ammonium salt), and 4-methyl-α-methoxyphenylacetic acid (ammonium salt) were only slightly active or were inactive and these compounds could not, therefore, be critically evaluated for root exudation.

α - 2,3 - Trimethoxyphenylacetic acid (ammonium salt), Q, possessed regulating activity equal to or greater than that of α-methoxyphenylacetic acid, but lacked the root-exudation characteristic. In contrast, α-3,4-trimethoxyphenylacetic acid, R, possessed no detectable regulating activity and the α-*o*-dimethoxy-substituted compound, P, tested possessed only slight activity.

With respect to α-methoxyphenylacetic acid, loss of activity also resulted when a methanethiol group or a chlorine atom was substituted for the methoxy group in the side chain. Reduced activity likewise occurred when a methyl group was substituted in the *o*-, *m*-, and the 2,3,6-positions on the ring. Alkoxy groups, except 2,3-dimethoxy-, reduced the regulating activity when used as the following ring substituents: *o*-ethoxy, *o*-methoxy, 3,4-diethoxy, and 3,4-dimethoxy. The 3,4-methylenedioxy substitution reduced the activity slightly. Complete loss of or a decrease in regulating activity resulted when the phenyl ring was replaced with the following structures: a methyl group, the 2-furyl group, 2-thienyl group, or the α-naphthyl group.

The data presented in this paper show that of those compounds tested, relatively few changes in either the aromatic or the aliphatic portion of α-methoxyphenylacetic acid resulted in compounds that retained both the marked plant-regulation and root-exudation characteristics of the acid.

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SULFUR DETERMINATION

Nitric-Perchloric Acid Oxidation for Sulfur in Plant and Animal Tissues

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The available methods for the determination of total sulfur in plant and animal tissue were found to be either cumbersome or of low degree of precision. A simple procedure utilizing nitric and perchloric acid oxidation was developed, which gives precise and accurate results for sulfur comparable with those obtained by the AOAC magnesium nitrate method on a great variety of plant and animal products, and the amino acids, cystine, cysteine, and methionine. Sulfur recoveries in standard materials were within 2% of theory. The same sample preparation can serve for the determination of calcium, magnesium, potassium, sodium, and phosphorus, as well as sulfur. The sulfate was determined gravimetrically as barium sulfate, but the prepared solution can be adapted for the indirect flame spectrophotometric or any other desired method of sulfur determination.

A SURVEY OF RECENT LITERATURE in the agronomic and botanical fields showed that for total sulfur in plants, the AOAC magnesium nitrate method (*1*) was in common use. This presents a rather anomalous situation in view of the fact that the wet-ashing of plant materials by nitric-perchloric acids has found wide acceptance in the mineral analysis of plants, which in some instances has included determinations of sulfur. The long-drawn-out digestion technique frequently used in studies on sulfur undoubtedly has been the greatest deterrent to the use of the nitric-perchloric acid procedure for sulfur. On the other hand, some workers reported satisfactory sulfur results on plant materials using short-time digestion periods.

The objective of the present study was to re-examine critically the factors of time and temperature in the nitric-perchloric acid oxidation procedure with a view to redefining the conditions of this procedure, so that total sulfur could be determined in the same sample preparation that would be used for the mineral analysis of organic materials. Any special sulfur procedures, and particularly those that introduce high salt concentrations or analytical reagents that would interfere with the determinations of the mineral elements in the sample, would be outside the present objectives.

Experimental

The experimental work was designed primarily to define adequately the time and temperature factors in the final stage of perchloric acid digestion for routine oxidation of plant and biological materials in 1-gram quantities. Flexibility in operating conditions, such as size of sample, reaction container, and heating devices, greatly influences the accuracy as well as the acceptability of a