Undoubtedly, a particular steric configuration is required for an inhibitor to approach the reactive site successfully. The configuration assumed by a molecule is the result of the various actions exerted by its different substituents. The substitution of a bulky chlorine atom at the ortho-position of the benzene ring would prevent the ring from assuming certain spatial relationships relative to the linear portion of the molecule. Consequently, the molecule may not have a steric configuration that would enable it to approach the reactive site. This may also explain the decreased inhibitory activity shown by the ortho-chlorinated derivatives.

Until more information becomes available relative to the mechanisms involved in the Hill reaction, it will not be possible to determine where in the reaction, or how, the alkyl N-phenylcarbamates interfere.

Whether interference with photosynthesis is one of the primary ways through which the alkyl N-phenylcarbamates exercise their control over plant growth remains to be ascertained. While interference with this system may not be the main way through which control over growth is exercised, it might be a contributory one. If the carbamates do reach the chloroplasts within the plant, interference with photosynthesis should result. The finding by other workers concerning inhibition of photosynthesis by EC and EPC in microorganisms suggests that similar responses could be expected in higher plants. Some of the carbamates used as herbicides should be even more potent inhibitors of this reaction in microorganisms than the ones available to the earlier investigators.

Correlations such as the ones attempted in this paper between laboratory and greenhouse studies should help elucidate the contribution made by the various parts of a molecule in terms of the over-all effect. Progress in the comprehension of the mechanism of action of herbicidal materials should be accelerated as information of this type becomes available to the investigator.

Acknowledgment

The authors acknowledge the assistance extended by J. L. Hilton, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md., during the preliminary phases of this investigation and the cooperation of J. A. Garman, Fairfield Chemical Division, Food Machinery and Chemical Corp., Baltimore, Md., in supplying most of the carbamates.

Literature Cited

(1) Arnon, D. I., Plant Physiol. 24, 1-15 (1949).
(2) Arnon, D. I., Science 122, 9-16 (1955)
(3) Arnon, D. I., Whatley, F. W., Arch. Biochem. 23, 141-56 (1949).
(4) Aronoff, S., Plant Physiol. 21, 393-409 (1946).
(5) Barker, M., Hunter, L., Reynolds, N. G., J. Chem. Soc. 1948, 874-81.
(6) Blackman, G. E., Parke, M. H., Garton, G., Arch. Biochem. Biophys. 54, 45-54 (1955).
(7) Ibid., pp. 55-71.
(8) Davenport, H. E., Hill, R., Whatley, F. R., Proc. Roy. Soc. (London) 139B, 346-58 (1952).
(9) Freed, V. H., Science 111, 285-6 (1950).
(10) George, D. K., Brian, W. P., Moore, D. H., Garman, J. A., J. Agr. Food Chem. 2, 990-5 (1954).
(11) George, D. K., Moore, D. H., Brian, W. P., Garman, J. A., Ibid., 2, 356-63 (1954).
(12) Hill, R., Nature 139, 881-2 (1937).
(13) Hill, R., Scarisbrick, R., Proc. Roy. Soc. (London) 129B, 238-55 (1940).
(14) Horsfall, J. G., "Principles of Fungicidal Action," Chronica Botanica Co., Waltham, Mass., 1956.
(15) Huggins, M. L., J. Org. Chem. 1, 407-56 (1936).
(16) Macdowall, F. D. H., Plant Physiol. 24, 462-80 (1949).
(17) Norman, A. G., Weintraub, R. L., Natl. Research Council, Natl. Acad. Sci. (U. S.), Chem. Biol. Coördination Center Publ., First Symposium, pp. 46-72 (1951).
(18) Rich, S., Horsfall, J. G., Phytopathology 42, 457-60 (1952).
(19) Rich, S., Horsfall, J. G., Science 120, 122-3 (1954).
(20) Shaw, W. C., Swanson, C. R., Weeds 2, 43-65 (1953).
(21) Spikes, J. D., Lumry, R., Eyring, H., Wayrynen, R. E., Arch. Biochem. Biophys. 28, 48-67 (1950).
(22) Templeman, W. G., Sexton, W. A., Nature 156, 630 (1945).
(23) Templeman, W. G., Sexton, W. A., Proc. Roy. Soc. (London) 133B, 480-5 (1946).
(24) Veldstra, H., Booij, H. L., Biochim. et Biophys. Acta 3, 278-312 (1949).
(25) Warburg, O., Biochem. Z. 100, 230-70 (1919).
(26) Ibid., 103, 188-217 (1920).
(27) Wassink, E. C., Katz, E., Dorrestein, R., Enzymologia 10, 285-354 (1942).
(28) Wassink, E. C., Kersten, J. A. H., Ibid., 11, 282-312 (1945).
(29) Wessels, J. S. C., van der Veen, R., Biochim. et Biophys. Acta 19, 548-9 (1956).

PLANT GROWTH REGULATORS

Preparation and Plant Growth-Regulating Activity of Crude Protein Hydrolyzate Derivatives of DL-2-(2,4-Dichlorophenoxy)-propionic Acid

CHARLES F. KREWSON, JOAN F. CARMICHAEL, and PAUL S. SCHAFFER[1]
Eastern Utilization Research and Development Division, U. S. Department of Agriculture, Philadelphia 18, Pa.
B. C. SMALE and JOHN W. MITCHELL
Crops Research Division, U. S. Department of Agriculture, Beltsville, Md.

THIS STUDY is a continuation of research on amino acid amides of plant grwoth-regulating acids which has been concerned with basic studies on specific puirfied amino acid derivatives (3-6, 8). The objective of the present work

[1] Present address, Central Project Office, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

was to modify advantageously the growth-regulating properties of conventional, regulating compounds utilizing readily available, inexpensive mixtures of amino acids and peptides. These mixtures come from the simple acid, alkaline, or enzymatic hydrolysis of abundant proteins.

Previous preliminary findings with amino acid amides have shown that amino acid coupling with phenoxy-type regulators produces from little or no effect to marked effect upon various growth-regulating properties of this type of compound. On selected test plants this effect depends upon the kind and optical configuration of the amino acid and the kind of phenoxy acid coupled. It also depends upon the specific plant used.

These investigations were undertaken for the purpose of utilizing low-cost protein hydrolyzates made from by-products or readily obtainable protein source materials. DL-2-(2,4-dichlorophenoxy)propionic acid [2-(2,4-DP)], a very active plant growth regulator, was chemically combined with a variety of these materials to determine whether the new products possessed growth-regulating properties that differed qualitatively or quantitatively from the parent acid. In general, coupling of protein hydrolyzates with 2-(2,4-DP) produced derivatives which induced growth responses suggesting the possible commercial use of the former as agents for the preparation of low-volatile amide herbicides. Although the general responses of test plants were somewhat less, corn and cucumber responses were greater, than to the parent acid. Reduction of undesirable formative effects characteristic of phenoxy parent acids may be of interest for other purposes such as induction of fruit development.

Test plants treated with amino acid derivatives of DL-2-(2,4-dichlorophenoxy)propionic acid [2-(2,4-DP)], show wider variability in response than do phenoxy derivatives of other series evaluated. This fact was responsible for the selection of 2-(2,4-DP) for coupling with crude protein hydrolyzate mixtures.

Gentner and Shaw (2) have reported favorably on the selective herbicidal properties of two protein hydrolyzates (A. E. Staley Manufacturing Co., Decatur, Ill.) derivatives of 2-(2,4-DP) prepared by one of the authors. In field tests on 26 crop plants and seven weed species, one of these hydrolyzate derivative products, applied as pre-emergence treatment, was active on small seeded legumes. Both protein hydrolyzate products applied as postemergence sprays showed excellent selective killing activity upon pigweed, mustard, lambsquarters, and broadleaf weeds, with little or no effect on desirable plants such as corn, oats, peanuts, gladiolus, and Sansevieria. These tests, together with those reported here, indicate the practical value of protein hydrolyzate derivative products made from the phenoxy, and possibly other types of plant growth regulators.

Some commercially available protein hydrolyzate mixtures (Hercules Co., Huron Milling Division, Harbor Beach, Mich.) having a known amino acid content were used. In general, these hydrolyzates had been prepared by acid hydrolysis of wheat protein followed by various refinements to yield products with varying amino acid compositions and special flavor characteristics for edible purposes. Composition data on these materials are presented in Table I.

Another group of hydrolyzates (Chemical Concentrates, Division of Baker Industries, Inc., Fort Washington, Pa.) from six protein source materials was used, but the approximate amino acid composition of each of the samples was not determined. Indication of the amino acid content of each protein hydrolyzed is available in the literature (1, 7). The preparation of these hydrolyzates involved an alkaline (lime) treatment carried out under pressure for various time intervals. Hydrolyzate

samples of α-protein from soybean, animal blood, casein, chicken feathers, animal glue, and meat and bone meal were supplied. The purpose in varying the hydrolysis time was to find out if an effect of partial hydrolysis of the protein might be detected in the biological response of test plants. Data on these hydrolyzates are presented in Table II.

For the preparation of the crude protein hydrolyzate derivatives of 2-(2,4-DP) procedures similar to those previously described were employed (3–5). The preparation of the intermediate, DL-2-(2,4-dichlorophenoxy)propionyl chloride, has been described (3). The following description using the protein hydrolyzate HMD-106-B-79 made from wheat is illustrative of the general procedure employed in the preparation of all the protein hydrolyzate 2-(2,4-DP) derivatives.

Preparation of Crude Protein Hydrolyzate Derivatives of 2-(2,4-DP)

To 4.88 grams (0.04M, calculated on a combining weight of 122 from the amino acid composition) of protein hydrolyzate dissolved in 120 ml. of 1N sodium hydroxide (0.12M) and chilled to 5° C. were added dropwise with stirring 30 ml. of a benzene solution containing 10.0 grams (0.04M) of DL-2-(2,4-dichlorophenoxy)propionyl chloride. After 1 hour, the cold mixture was allowed to warm up to room temperature and then extracted three times with diethyl ether (120-, 40-, and 40-ml. portions). The ether extracts were combined and washed once with water, and the water washing was added to the extracted alkaline aqueous solution. To the latter, during mechanical stirring, sufficient 1N hydrochloric acid was added to adjust the alkaline solution to a pH of 2. A brown oily product separated, which became somewhat crystalline after overnight storage in the refrigerator. The supernatant liquid was discarded and the product washed thoroughly with several portions of water. After being dried to constant weight in a vacuum desiccator, the product was thoroughly washed with portions of hot petroleum ether (63° to

70° C.) to remove traces of parent acid. The weight of the vacuum-dried product was 10.88 grams, 78.4% yield. Analytical data on this and other final products prepared from the various wheat gluten hydrolyzates are presented in Table I.

In the preparation of 2-(2,4-DP) derivatives of the hydrolyzates from the crude proteins listed in Table II, techniques similar to those described above were used. To estimate the quantity of 2-(2,4-dichlorophenoxy)propionyl chloride required for a prescribed amount of hydrolyzate solution, it was necessary to assume an average combining weight for the amino acids and peptides present in these hydrolyzates; the value assigned was 120. An average of 33.3% solids [solids less calcium chloride (see Table II)] and an excess of 80% were used to calculate the quantity of hydrolyzate solution to be used for each reaction. The 80% excess was an attempt to adjust for the impurities present in the crude proteins hydrolyzed. In retrospect, this figure appears to have been slightly low in several cases, as indicated by a nitrogen value of 8.44% for the meat and bone meal obtained at a later date.

Discussion of the Chemistry

If the plant growth regulator portion of each product is derived from 2-(2,4-DP) and the latter combines only with amino acids containing one nitrogen atom, then the ratio of chlorine to nitrogen atoms should have an expected value of 2. A small allowance for deviation above this ratio could be made for the possibility of acetylation of the hydroxyl groups of the hydroxy amino acids present, such as hydroxyproline, serine, threonine, and tyrosine. However, the presence of partially hydrolyzed protein in the form of peptide units, multiple nitrogen amino acids, such as arginine, cystine, histidine, and lysine, and the presence of tertiary cyclic nitrogen compounds such as histidine are factors tending to lower the chlorine-nitrogen ratio, apparently restricting the acceptability limit to a maximum of 2. If values higher than 2 for the ratio of chlorine to

Table I. Percentage Composition of Acid-Hydrolyzed Vegetable Proteins[a] and Analytical Data on Their DL-2-(2,4-Dichlorophenoxy)propionic Acid Derivatives

Description	5-SD Tan powder	HVP-A Tan powder	Luxor Light colored paste	Dee-S Brown paste	HMD-106-B-79 Tan powder	E-610 Brown liquid	L-625 Amber liquid	700 Heavy brown liquid	HMD-105-B-78 Brown paste
					Protein Hydrolyzates				
Alanine	3.70	3.70	3.45	3.77	2.50	0.67	1.68	2.79	6.40
Arginine	2.78	2.78	2.60	2.84	...	0.81	1.27	2.10	...
Aspartic acid	1.10	1.10	1.03	1.13	...	1.24	0.50	0.83	...
Cystine	...	...	...	...	...	0.30	Trace	...	...
Glutamic acid[b]	3.14	3.14	2.94	3.21	0.20	1.43	1.43	2.37	0.60
Glycine	6.66	6.66	6.62	6.81	3.00	0.97	3.04	5.03	7.60
Histidine	1.54	1.54	1.44	1.57	...	0.40	0.70	...	...
Leucine and isoleucine	...	...	...	...	21.00	0.80	Trace	...	...
Lysine	0.96	0.96	0.90	0.98	...	0.44	0.44	0.73	...
Methionine	...	...	...	...	3.60	0.27	Trace	...	...
Phenylalanine	3.46	3.46	3.24	3.54	21.00	1.58	1.57	2.61	...
Proline	7.41	7.41	6.92	7.56	22.00	3.93	3.36	5.58	55.80
Serine	...	...	...	...	7.50	1.51	...	...	18.60
Threonine	2.01	2.01	1.89	2.06	4.40	0.81	0.92	1.52	11.00
Tyrosine	...	...	...	...	...	0.38	Trace	...	...
Valine	2.50	2.50	2.34	2.56	10.00	0.84	1.14	1.89	...
Solids	...	...	88.00	88.00	...	38.00	38.00	52.00	...
Chlorides	52.1	53.8	45.0	41.0	...	17.5	17.5	17.0	...
Combining weight of amino acid	113.8	113.8	114.2	113.8	122.0	122.2	114.6	112.3	109.1
					Derivatives				
Yield, %	73.1	15.3	40.8	5.4	78.4	21.0	23.0	38.7	33.6
Chlorine[c] Calcd.	21.43	21.43	21.40	21.43	20.91	21.00	21.39	21.53	21.74
Found	22.91	21.71	19.93	22.40	20.55	21.03	21.49	19.88	21.18
Nitrogen[c] Calcd.	4.23	4.23	4.22	4.23	4.12	4.13	4.22	4.25	4.28
Found	3.56	4.46	3.88	4.25	3.49	4.96	4.40	3.74	3.69
Molecular weight Calcd. (average)	331.9	331.9	332.3	331.9	340.1	340.3	332.7	330.5	327.2
Found	310.0	327.0	355.8	316.6	345.1	337.2	330.0	356.7	335.0
$[\alpha]_D^{25}$ (pyridine)[c]	Levo 10	...	Levo 13.6 ± 0.5	...	Levo 14.7 ± 0.5	...	Levo 18.2 ± 0.7	...	Levo 21.5 ± 1.5

[a] Data supplied by Hercules' Huron Milling Division. [b] As monosodium glutamate. [c] Analyses by J. S. Ard.

Table II. Analytical Data on Alkali-Hydrolyzed Proteins and Their DL-2-(2,4-Dichlorophenoxy)propionic Acid Derivatives

Protein Source	Hydrolysis Time, Hours	Sp. Gr.	pH	Solids, %	Ash, %	Calcium Chloride %	Amount Used,[b] Grams	Product Weight, Grams	Cl,[c] %	N,[c] %	Average molecular weight	Cl/N Ratio	Weight, grams	N,[c] %
			Hydrolyzate Solutions[a]						*Derivative-Mixtures, DM*				*Residues from Petroleum Ether Extraction of DM*	
α-Protein, soy	2	1.205	7.10	43.5	9.0	10	50	4.44	14.36	8.10	494	0.70	0.94	0.68
	4	1.210	7.00	43.5	9.0	9	50	10.87[d]	17.58	6.26	403	1.11	7.24	0.30
	8	1.210	7.10	43.5	9.0	8	50	13.24	17.00	6.45	417	1.04	7.49	0.53
Animal blood	2	1.210	7.15	42.0	12.4	10	25	4.47	15.03	8.26	472	0.75		
	4	1.210	6.90	43.0	12.4	10	25	5.49	17.86	6.63	397	0.72		
	8	1.210	7.00	42.0	13.0	10	25	1.91	18.42	6.39	385	1.06		
Casein	2	1.210	7.20	44.0	11.0	10	50	11.67	16.09	6.93	441	0.92	4.81	0.12
	4	1.210	7.30	44.0	11.0	10	50	14.65	17.55	6.01	404	1.15	3.14	0.20
	8	1.210	7.25	44.0	11.0	9	50	12.48	16.50	6.50	430	1.00	6.31	0.32
Chicken feathers	2	1.210	7.15	46.0	10.0	10	25	2.47	13.68	8.77	518	0.62		
	4	1.210	7.15	45.0	10.5	10	25	2.90	14.15	8.08	501	0.69		
	8	1.210	7.10	46.0	10.5	9	25	3.77	14.69	7.72	483	0.75		
Animal glue	2	1.210	7.00	41.0	12.0	9	75	18.69[e]	20.93	5.00	339	1.65	7.76	0.42
	4	1.210	7.10	41.0	12.0	10	75	6.12[f]	20.31	5.64	349	1.42	3.26	0.96
Meat and bone meal	2	1.210	7.10	40.0	10.4	10	75	15.42	17.78	6.93	399	1.01	7.08	0.40
	4	1.210	7.10	41.0	10.5	10	75	11.11	19.54	5.44	363	1.42	7.11	0.39
	8	1.210	7.10	42.0	10.5	10	75	4.01	19.94	4.83	356	1.63	4.14	0.84

[a] Data supplied by Baker Industries' Chemical Concentrates, Inc.
[b] Represents an 80% excess. Ten grams of 2-(2,4-dichlorophenoxy)propionyl chloride used for every 25 grams of hydrolyzate solution.
[c] Analyses by J. S. Ard.
Optical rotation [d] Levo 6.0. [e] Levo 3.0. [f] Levo 3.3.

nitrogen were obtained, it was assumed that the product was contaminated with parent acid, 2-(2,4-DP). Further extraction with petroleum ether was indicated for such products and this was repeated until the ratio was satisfactory or remained unaltered by such treatment.

In a few cases the petroleum ether extracted material was spot-checked by nitrogen analysis to determine if this solvent was extracting the product excessively. Low nitrogen values (Table II) indicate that this was not the case.

From the chlorine-nitrogen ratios and the average molecular weights of the products prepared from proteins hydrolyzed by alkali it is apparent that complete hydrolysis did not occur. It was approached only in the case of animal glue and the meat and bone meal proteins (Table II). Apparently the resulting mixtures of derivatives may have contained, besides monoamides of the amino acids, some diamide (or polyamide), and ester derivatives, also possibly some purinelike units. In contrast to hydrolysis by acids and enzymes

Table III. Plant Growth-Regulating Activity of Some DL-2(2,4-Dichlorophenoxy)propionic Acid Derivatives of Various Crude Protein Hydrolyzates Tested at 1 or 0.1% Concentrations[a]

Products Tested, Listed by Protein Source of 2-(2,4-DP) Derivatives	Protein Hydrolyzed, Hours	Stem Curvature Be	S	Cu	Growth Inhibition Be	S	Cu	Epinasty Be	S	Cu	Formative Effects Be	S	Cu	1st Internode Be	S	Cu	Hypocotyl Be	S	Cu	Treated Area Be	S	Cu	Coated Sand Stem Curvature B	Cn	Coated Sand Growth Inhibition B	Cn
Parent acid, 2-(2,4-DP)		2[b]	1[b]	0[b]	2[b]	2[b]	3[b]	0[b]	0[b]	0[b]	0[b]	1[b]	2[b]	0[b]	0[b]	0[b]	0[b]	1[b]	0[b]	3[b]	1[b]	0[b]	2	1	3	1
α-Protein, soy	2	3	2	2[b]	3	3	2[b]	0	0	0[b]	0	1	0[b]	1	1	0[b]	2	0	1[b]	2	2	1[b]	0	1	0	2
	4	3	3	3[b]	3	3	3[b]	0	2	1[b]	0[b]	1	1[b]	3	2	0[b]	3	2	0[b]	3	2	0[b]	0	1	0	2
	8	3	2	1[b]	3	3	1[b]	0	1	0[b]	0	1	0[b]	2	2	0[b]	3	0	1[b]	3	2	1[b]	0	0	0	2
Animal blood	2	1	2	3[b]	2	3	3[b]	0	0	0[b]	0	3	1[b]	0	0[b]	0[b]	1	3	0[b]	3	3	1[b]	0	0	0	2
	4	2	2	1	2	3	3	0	0	3	0	3	0	1	0	0	2	3	0	3	3	3	0	0	0	2
	8	3	1	2[b]	3	3	2[b]	0	0	0[b]	0	2	0[b]	2	3	0	2	1	0[b]	3	3	0[b]	0	1	0	2
Casein	2	3	2	1	3	3	1[b]	0	1	0	0[b]	1	0[b]	3	3	0[b]	1	3	0[b]	2	3	0[b]	0	1	0	2
	4	3	1	2[b]	3	3	2[b]	0	0	0[b]	0	2	0[b]	3	0	0[b]	2	1	0[b]	3	1	1[b]	0	1	0	2
	8	3	1	1[b]	3	3	1[b]	0	3	0[b]	0	1	1[b]	3	2	0[b]	1	2	1[b]	3	2	2[b]	0	1	0	2
Chicken feathers	2	1	2	2	2	3	2	0	0	3	0	3	0	0	0	0	1	3	1	3	3	1	0	1	0	2
	4	1	2	3	2	3	3	0	0	3	0	3	0	0	0	0	1	3	0	3	3	2	0	1	0	2
	8	1	2	3	2	3	3	0	0	3	0	3	0	3	0	0	3	3	0	3	3	2	2	0	0	2
Animal glue	2	3	2	2	3	3	2[b]	0	1	0[b]	0	1	0[b]	3	2	0[b]	3	1	1[b]	3	2	1[b]	0	1	2	3
	4	3	2	3	3	2	3[b]	0	0	0[b]	0	1	0[b]	2	2	0[b]	3	2	1[b]	3	1	1[b]	0	1	0	2
Meat and bone meal	2	3	2	2[b]	3	3	2[b]	0	2	0[b]	0	1	0[b]	3	2	0[b]	2	1	0[b]	3	1	0[b]	0	1	1	2
	4	2	1	2[b]	3	3	2[b]	0	0	3[b]	0	2	0[b]	2	0	0[b]	1	1	2[b]	2	1	2[b]	0	2	0	2
	8	3	2	1[b]	3	3	1[b]	0	0	0[b]	0	1	0[b]	3	0	0[b]	1	1	1[b]	2	1	1[b]	0	1	2	2
Vegetable 5-SD		3	1[b]	0[b]	3	3	2[b]	0	0[b]	0[b]	0	1[b]	0[b]	2	1[b]	0[b]	1	0[b]	2[b]	2	0[b]	2[b]	0	0	1	2
HVP-A		2	0	0[b]	2	3	3	0	0	0[b]	0	1	0[b]	0	0	1[b]	0	0	0[b]	2	0	0[b]	0	0	0	3
Luxor		2	3	2	3	2	3	0	0	3	0[b]	0	0[b]	3	1	0	2	1	0	3	1	0	0	2	1	3
Dee-S		2	1	0	3	2	3	0	0	0	0[b]	1	0[b]	3	1	0	3	1	0	3	1	0	0	1	0	2
HMD-106-B-79		2	1	1	3	3	3	0	1	1	0[b]	1	0[b]	3	2	0	3	1	0	3	2	0	0	1	1	2
E-610		2	2	1	3	2	3	0	0	3	0[b]	3	0[b]	3	3	0	2	1	1	3	2	1	0	0	2	1
L-625		3	2	0	3	3	3	0	0	1	0[b]	3	0[b]	3	3	0	3	1	0	3	2	0	0	0	1	2
700		2	3	1	3	3	3	0	1	1	0[b]	3	0[b]	3	1	0	3	1	0	3	1	0	0	0	0	2
HMD-105-B-78		2	3	3[b]	3	3	3[b]	0	0	1[b]	0[b]	2	0[b]	3	3	1[b]	2	3	1[b]	3	3	0[b]	0	1	1	2

[a] Test plants designated as follows: Be, bean; S, sunflower; Cu, cucumber; B, barley; and Cn, corn. Activity designated as 0, no effect; 1, slight effect; 2, moderate effect; and 3, marked effect. Observations made 14 days following application of a mixture of one part Tween 20 and four parts lanolin, this mixture contains 1 or 0.1% of the compound.

[b] Designates that the responses obtained with a mixture containing 1% of the compound was so marked that evaluation of responses listed could not be made and it was necessary to retest the compound at 0.1% concentration.

(enzymically hydrolyzed protein derivatives have been prepared and are being evaluated), alkaline hydrolysis may have racemized many of the L-type assymetries to give proportions of D-isomers ·approaching the 1 to 1 DL- limit. Judging by the low order of activity usually found for the isolated D-isomers (3–5), the expected effect of racemization would be to reduce activity.

Some of the hydrolyzates were unique in composition with especially high proportions of certain amino acids, as, for example, HMD-105-B-78 (Table I) which contained 56% proline and HMD-106-B-79 which was distinctively high in proline, phenylalanine, leucine, and isoleucine. These factors would also furnish a basis for differing biological effects.

Methods Used in Plant Tests

The parent acid, 2-(2,4-DP), and derivatized protein hydrolyzates were evaluated for plant growth–regulating activity using the dicotyledonous plants, Pinto bean, large seeded sunflower, Arlington White Spine cucumber, and the monocotyledonous plants Wong barley and U. S.-13 hybrid corn. The lanolin-assay method was used on the dicots and the coated sand-assay method was used on the monoçots (5).

The degree of growth modification induced by each derivative was estimated and scored according to intensity at intervals of 2, 4, 7, and 14 days following treatment. The responses observed 14 days after treatment are shown in Table III. Scores for the other intervals have been omitted to save space. Responses studied were stem curvature, growth inhibition, epinasty, formative effects, and induced cell proliferation (gall formation). The data are representative, although they do not show the rate of response or relative progressive effectiveness of the compounds tested.

Results of Plant Screening

Coupling of protein hydrolyzates with 2-(2,4-DP) gave products which, in general, induced plant-growth responses. With but few exceptions the magnitude of response of bean, sunflower, and barley to all derivatives was less than it was to the parent acid. Cucumber and corn showed a higher response to the derivatives. In about one third of the test plants observed the magnitude of response was greater than to 2-(2,4-DP) (Table III).

There was no definite correlation between plant responses and the time of hydrolysis for proteins hydrolyzed by alkali.

A summary of each growth-regulating activity with respect to magnitude of response follows, to point out individual product and plant differences.

Stem Curvature. Cucumber response to all derivatives of α-protein, animal blood 2-hour and 8-hour, casein 4-hour and 8-hour, all meat and bone meal, and wheat gluten HMD-105-B-78 was greater than to the parent acid. Bean and sunflower responses were less to all hydrolyzate derivatives than to the parent acid. Barley response was also less with one exception. With the chicken feather 8-hour hydrolyzate product the response was equal to that produced by 2-(2,4-DP). The response of corn varied with the derivatives used. In nine cases it was less, in 15 cases equal to, and in the cases of meat and bone meal and in the vegetable protein (Dee-S) hydrolyzates it was greater than that of the parent acid.

Growth Inhibition. In general, all derivatives were more effective in inhibiting the growth of corn than the parent regulating compound; hence there was a marked increase in activity. This is a unique characteristic in striking contrast to the behavior of some individual D-, DL-, and L-amino acid derivatives of 2-(2,4-DP) previously evaluated (3). Only in the case of cucumber and the use of α-protein 4-hour, animal blood 2-hour, animal glue 4-hour, and wheat

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.

Acknowledgment

The authors acknowledge the advice and counsel of J. S. Ard of the Eastern Regional Research Laboratory in the preparation of this manuscript.

Literature Cited

(1) Block, R. J., Bolling, D., "The Amino Acid Composition of Proteins and Foods," 2nd ed., C C Thomas, Springfield, Ill., 1951.
(2) Gentner, W. A., Shaw, W. C., 1958 Field Results, Processed Rept. CR-6-59, Crops Research Division, Agricultural Research Service, U. S. Dept. of Agriculture, Beltsville, Md., 1959.
(3) Krewson, C. F., Drake, T. F., Mitchell, J. W., Preston, W. H., Jr., J. Agr. Food Chem. 4, 690–3 (1956).
(4) Krewson, C. F., Drake, T. F., Neufeld, C. H. H., Fontaine, T. D., Mitchell, J. W., Preston, W. H., Jr., Ibid., 4, 140–3 (1956).
(5) Krewson, C. F., Neufeld, C. H. H., Drake, T. F., Fontaine, T. D., Mitchell, J. W., Preston, W. H., Jr., Weeds 3, 28–37 (1954).
(6) Krewson, C. F., Saggese, E. J., Carmichael, J. F., Ard, J. S., Drake, T. F., Mitchell, J. W., Smale, B. C., J. Agr. Food Chem. 7, 118–22 (1959).
(7) Lyman, C. M., Kuiken, K. A., Hale, F., Ibid., 4, 1008–13 (1956).
(8) Wood, J. W., Fontaine, T. D., J. Org. Chem. 17, 891–6 (1952).

PLANT REGULATOR TRANSLOCATION

New Plant Regulators That Exude from Roots

JOHN W. MITCHELL, BERNARD C. SMALE, and WILLIAM H. PRESTON, Jr.

Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

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-methoxy-

phenylacetic 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