The Metabolism of Hormones during Seed Germination and Dormancy

II. THE METABOLISM OF 8-14C-ZEATIN IN BEAN AXES[1,2]

Ernest Sondheimer and Dong-Sun Tzou
Department of Chemistry, Plant Physiology Biochemistry Group, SUNY College of Forestry at Syracuse University, Syracuse, New York 13210

ABSTRACT

8-14C-Zeatin is taken up rapidly and is extensively metabolized by excised bean axes during a 12-hour incubation at 26 C. Most of the radioactivity is found in the 80% ethanol soluble fraction and consists of zeatin, zeatin riboside, zeatin-5'-ribotide, as well as corresponding dihydrozeatin derivatives. The characterization of 14C-dihydrozeatin included crystallization to constant specific radioactivity. No cleavage of the zeatin side chain to adenine, hypoxanthine, their ribosides, or glycylpurine was detected. Dihydrozeatin has been previously isolated from yellow lupin seeds, and our experiments indicate that it can be derived through reduction of the side chain from preexisting cytokinin. While the total amount of zeatin metabolized is not affected by growth-inhibiting concentrations of abscisic acid or cycloheximide, the conversion to dihydrozeatin derivatives is curtailed. Although somewhat less effective than zeatin and zeatin riboside, dihydrozeatin and dihydrozeatin riboside also counteract the abscisic acid-induced growth inhibition.

Evidence is available which supports the supposition that the regulation of germination and dormancy in embryonic plants is brought about through opposing hormonal actions. Cases involving ABA, gibberellins, and cytokinins are well documented and have been summarized by Amen (2), Wareing (19), and Addicott (1). While very little is known about the actual mechanisms of plant hormonal interactions, at least three different explanations can be invoked. For example, one hormone may influence the rate of synthesis or degradation of another growth regulator. Or, one hormone may affect the availability of another through an influence on transport rates or membrane characteristics. If it is assumed that plant hormones must bind to macromolecules before exerting a physiological action, then, through allosteric effects, a third possibility for interaction is provided. These considerations prompted us to study the metabolism of 14C-labeled hormones during dormancy and germination and to determine the effects of other plant growth regulators on the metabolism of the labeled hormone. In this paper we report on the metabolism of 8-14C-zeatin in axes excised from beans, a tissue where germination does not involve the breaking of dormancy.

Excised axes from seeds of *Phaseolus vulgaris* imbibe water rapidly, go through a lag phase, and then initiate fresh weight increase at a linear rate (16). At 26 C the growth phase starts 4 to 5 hr after imbibition and continues for at least 10 hr. This growth is inhibited by ABA and while exogenously added zeatin does not affect the normal growth rate, it does lead to a partial reversal of the ABA-induced inhibition (18). Because of these relations, we studied the metabolism of 8-14C-zeatin alone and in the presence of growth-inhibiting concentrations of ABA in order to determine whether ABA affects zeatin metabolism. The effect of cycloheximide, a growth inhibitor in bean axes (17) and a general inhibitor of protein synthesis, on 8-14C-zeatin metabolism was also determined.

MATERIALS AND METHODS

8-14C-Zeatin. This compound was synthesized by the procedure of Shaw et al. (15). 6-Chloropurine-8-14C, 4.3 mg (Calbiochem, nominal specific radioactivity 3.6 mc/mmole) and 7.3 mg of *trans*-4-amino-2-methyl-2-buten-1-ol sulfate were dissolved in 0.27 ml of 1-butanol and 0.03 ml of triethylamine. This mixture was heated in a sealed tube at 130 C for 90 min, cooled, and concentrated. The radioactive product was isolated and purified by paper chromatography with solvent systems a, b, and c, Table I. The isolated material was chromatographically homogeneous by radiographic and ultraviolet criteria and has the same R_F values and absorption spectrum as unlabeled zeatin. The yield was 70%, and the specific radioactivity was 2.95 mc/mmole based on a molar extinction coefficient, $\epsilon_{270\ nm} = 16,500$.

Zeatin-9,β-riboside was synthesized by the procedure of Shaw et al. (15) and (RS)-dihydrozeatin-9,β-riboside was prepared from the above compound by catalytic hydrogenation (9). That the dihydrozeatin-9,β-riboside was free of zeatin-9,β-riboside could be established by mass spectrometry. 2iP[3] and 2iPA were synthesized by condensation of 3-methyl-2-butenylamine with 6-chloropurine and its riboside respectively (8). 6-Glycylpurine was prepared from 6-chloropurine and glycine (3). R_F values for the above and other purines used in this study are shown in Table I.

Tissue Incubation. Information on the growth characteris-

[3] Abbreviations: MAK: methylated albumin kieselguhr; 2iP: 6-(3-methyl-2-butenylamino)purine; 2iPA: 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine.

tics of excised bean axes, *Phaseolus vulgaris* L. var. White Marrow fat, has been published (16). The incubation medium consisted of 0.05 M HEPES buffer, pH 7.0, containing 50 μg/ml of chloramphenicol plus the test compounds. 8-¹⁴C-Zeatin was administered by adding 97 μg, 2.9 × 10⁶ dpm, in 3 ml of buffer to 1.0 g of dry axes, and incubating in a small tube for 1.5 hr at 26 C. The mixture was transferred to a 1-liter flask, 17 ml of buffer plus appropriate test compound was added, and incubation was continued with shaking at 26 C for a total of 12 hr. This procedure assures high uptake of the 8-¹⁴C-zeatin since the axes imbibe almost three times their weight of water during the initial 1.5 hr. Even in the presence of ABA or cycloheximide, 70 to 80% of the total recovered radioactivity was found in the axes at the end of the 12-hr incubation.

The axes were filtered on cheesecloth, washed with Hepes buffer, surface dried, and weighed. The washings and incubation medium were combined, counted, and in several cases, chromatographed. The axes were ground in a Sorvall omnimixer at 0 C with 20 ml 80% (v/v) ethanol, centrifuged at 15,000 g for 5 min, and the supernatant decanted. The residue was re-extracted three more times. The combined extracts were concentrated below 30 C in a rotary evaporator, and the residue was taken up in 3 ml of water. The solution was extracted five times with 1-ml portions of 1-butanol. The butanol extracts, which contained the radioactive N-bases and most of the ¹⁴C-containing ribosides, were combined and concentrated for counting and chromatography with solvents a and b, Table I. The aqueous phase, which contained all of the radioactive low molecular weight phosphates and a portion of the ribosides, was also concentrated and used for chromatography with solvent system a, Table I.

Counting Procedures. The radioactive compounds were located on the paper chromatograms by autoradiography on Kodak no-screen x-ray film. The chromatograms were cut into at least 25 pieces, each piece immersed in 10 ml of scintillation fluid, 4 g of Omnifluor per liter toluene, in standard counting vials and counted in a Nuclear Chicago Model 6850 liquid scintillation spectrometer. For the ¹⁴C-ribosides, the counts from the chromatograms of the aqueous and butanol extracts were combined. For the counting of the aqueous solutions, adequate amounts of Beckman Bio-solv solubilizer in 10 ml of scintillation fluid was added. Samples were counted in a Nuclear Chicago Model 6850 or Packard Tri-carb Model 3375 liquid scintillation spectrometer and cpm were converted to dpm with quenching corrections.

Characterization of Radioactive Compounds. In addition to comparisons of R_F values of the radioactive compounds to those of standards, several chemical and enzymatic procedures were used. The radioactive compounds in a particular area were eluted from the paper chromatograms with water or 80% (v/v) ethanol, concentrated, and treated as described below.

Periodate oxidation under the conditions developed by Weith and Gilman (20) results in high conversion of ribosides to the free N-bases. The material to be oxidized is treated with 0.05 ml of water, 0.05 ml of 1 M cyclohexylamine in 2.5 M sodium malonate, pH 8.0, and 0.05 ml of 0.05 M sodium periodate. The solution was held at 45 C for 90 min, cooled, and spotted directly on paper for chromatography. The procedure was also used with the radioactive ribotides.

Permanganate oxidation was used in the detection of radioactive compounds with saturated side chains. To the test compound, 0.01% of aqueous potassium permanganate was added dropwise until a pink color persisted for at least 30 sec. The solution was spotted and chromatographed with solvent system a, Table I. While the R_F of the dihydrozeatin, its riboside, and ribotide are unaffected by this treatment, zeatin and its

Table I. *R_F Values of Zeatin, Zeatin Riboside, Zeatin-5'-Ribotide, and Related Compounds on Whatman No. 3 MM Paper*

Compound	Solvent Systems[1]				
	a	b	c	d	e
Zeatin and D,L-dihydrozeatin	0.79	0.71	0.67	0.55	0.62
2iP	0.89	0.86	0.92	...	...
Adenine	0.55	0.45	0.32	0.46	0.23
Guanine	0.30	0.03	...	...	...
Hypoxanthine	0.45	0.25	...	...	...
6-Glycylpurine	0.55	0.04	0.00	0.50	0.02
Zeatin-9-β-D-furanosylriboside and D,L-dihydrozeatin-9-β-D-furanosylriboside	0.72	0.37	0.54	0.48	0.55
2iPA	0.86	0.22	0.86	...	0.84
Adenosine	0.49	0.11	0.21	0.27	0.18
Guanosine	0.30	0.02	...	...	...
Inosine	0.22	0.04	...	...	...
Zeatin-5'-ribosylphosphate and D,L-dihydrozeatin-5'-ribosylphosphate	0.31	0.00	0.00	...	0.00
5'-AMP	0.15	0.00	0.00	...	...
5'-IMP	0.15	0.00	0.00	...	...

[1] a: 1-Butanol, acetic acid, water (12:3:5); b: water saturated 1-butanol on 0.03 M pH 8.4 borate impregnated paper; c: ethyl acetate, 1-propanol, water (4:1:2, upper phase); d: 1-butanol, formic acid, water (10:4:5, upper phase), e: 1-butanol, ammonia, water (86:5 as 28% ammonium hydroxide:14).

derivatives yield smaller R_F values. The percentage of dihydro compounds was obtained by dividing the count in the area with unchanged R_F value by the total count from the permanganate-treated material.

Enzymatic hydrolysis was used to establish the presence of phosphate esters. To the test compound dissolved in 0.1 ml of 0.1 M tris-HCl buffer, pH 8.6, was added 0.02 ml of 0.02 M magnesium chloride and 2.8 units *E. coli* alkaline phosphatase (Worthington Biochemical Corp.). After 4 hr at 37 C, the hydrolysate was spotted on paper and chromatographed with solvent system a or b, Table I. To distinguish between 3' and 5' phosphates, the test compound was dissolved in a total volume of 0.2 ml consisting of 0.5 M glycine-NaOH buffer, pH 8.5, 0.05 M magnesium chloride, and 0.1 mg of 5'-nucleotidase (Sigma Chemical Co. Grade II, 18 units/mg). After 25 min storage at 37 C, the mixture was chromatographed with solvent system a or b.

Additional proof for the presence of ¹⁴C-dihydrozeatin in the bean axes was obtained by crystallization to constant specific radioactivity with zeatin and dihydrozeatin. Axes that had been incubated with 8-¹⁴C-zeatin were extracted with 80% (v/v) ethanol as above and chromatographed with solvent a; the material with R_F = 0.8 was eluted, rechromatographed on borate impregnated paper with solvent b, and the material with R_F = 0.7 eluted. This fraction was oxidized with permanganate, chromatographed in solvent a, and the substances with R_F = 0.8 eluted, yielding 1.05 × 10⁵ dpm. Half of this material was crystallized three times with 14 mg D,L-dihydrozeatin from aqueous acetonitrile at −20 C, and half with 12.6 mg zeatin at O C using water as the solvent.

Nucleic acids were isolated and fractionated on MAK columns as described by Walton *et al.* (18).

Table II. 8-14C-Zeatin Metabolism in Bean Axes during 12-hr Incubation at 26 C

Addend[1]	Growth Inhibition	Percentages 8-14C-Zeatin and Conversion Products Recovered						Zeatin Converted	Dihydrozeatin and Riboside and Ribotide Formed
		Zeatin	Dihydro-zeatin	Zeatin riboside	Dihydro-zeatin riboside	Zeatin ribotide	Dihydro-zeatin ribotide		
	%							%	%
None	...	6	9	11	14	13	17	92	57
40 µM (RS)-ABA	45	7	8	16	9	22	13	91	40
Cycloheximide 2.5 µg/ml	100	5	5	33	7	21	4	93	21

[1] Incubation conditions were as described in "Materials and Methods." Each set contained the same amount of 8-14C-zeatin.

RESULTS

Characterization of the 8-14C-Zeatin Conversion Products. Uptake of 8-14C-zeatin by bean axes is rapid and after 12-hr incubation at 26 C, extensive conversion to other low molecular weight products has occurred. Fractionation of the axes homogenate with aqueous phenol and chromatography on MAK columns failed to give clear evidence for the presence of 14C-nucleic acids. All further work, therefore, dealt with the alcohol soluble fraction since it contained 85% of the radioactivity taken up. The major radioactive compounds in this extract were shown to be zeatin, dihydrozeatin, their ribosides and ribotides.

The aqueous alcohol soluble 14C-containing substances were separated by 1-butanol extractions and paper chromatography. The radioactive compounds in the 1-butanol layer separated particularly well on borate-impregnated paper with water-saturated 1-butanol. One spot, $R_F = 0.37$ cochromatographs with zeatin riboside in four different solvent systems and the other, $R_F = 0.71$ with zeatin in four systems. Up to now it has not been possible to separate zeatin and dihydrozeatin chromatographically (7). However, they can be distinguished after permanganate oxidation. This procedure, while confirming the presence of 14C-zeatin, also showed the presence of a high proportion of radioactive permanganate-resistant material with $R_F = 0.71$. The permanganate-resistant, radioactive material with $R_F = 0.71$ was isolated and shown to be 14C-dihydrozeatin by dividing it into half and crystallizing with zeatin and dihydrozeatin. After three crystallizations the specific radioactivity of the zeatin-containing sample had decreased from 4100 dpm to 500 dpm. In the sample containing unlabeled dihydrozeatin, the specific radioactivity remained almost constant after three crystallizations, dropping from 3600 dpm to 3200 dpm. Since the radioactive material with $R_F = 0.71$ remaining in the incubation medium was oxidized almost completely by permanganate, it is concluded that 14C-dihydrozeatin is formed enzymatically inside the axes and that very little, if any, leaches out. Permanganate-resistant radioactive compounds which cochromatograph with zeatin riboside and zeatin ribotide before hydrolysis and with dihydrozeatin afterwards were also found, and these have been assumed to be the respective dihydrozeatin derivatives. Verification that the substances with $R_F = 0.37$ are ribosides was obtained by treatment with periodate and cyclohexylamine, a procedure that leads to formation of N-bases from their respective ribosides (20). Chromatography of the periodate-treated material resulted in complete loss of radioactivity at $R_F = 0.37$ and appearance of radioactivity in the area where zeatin and dihydrozeatin are found.

The major radioactive compounds remaining in the aqueous phase after butanol extraction were identified as the ribosyl-5'-phosphates of zeatin and dihydrozeatin. The compounds did not move from the origin with basic chromatographic solvents, but could be separated with acidic developing systems. With solvent a, Table I, a major spot with $R_F = 0.31$ and a minor spot with $R_F = 0.07$ were obtained. Treatment of both materials with phosphatase resulted in conversion to compounds that cochromatograph with zeatin riboside. From a comparison of the R_F values of these compounds with those of AMP and ADP it seems likely that the material with $R_F = 0.31$ consists of monophosphates and that the slower moving substances are di- or triphosphates. Evidence that the compounds with $R_F = 0.31$ are 5'-phosphates comes from two sources. Commercial 5'-nucleotidase does not catalyze the hydrolysis of 3'-ribotides at an appreciable rate, but did bring about rapid conversion of the phosphates with $R_F = 0.31$ to zeatin and dihydrozeatin ribosides. Also, periodate-dicyclohexylamine treatment of the phosphates yields compounds which cochromatograph with zeatin. As model experiments with 5'-AMP and 3'-AMP confirmed, these results are incompatible with a 3'-phosphate structure assignment for the zeatin derivatives.

The extracts from the 12-hr-old bean axes also contained several minor radioactive compounds which have not been identified. A number of possible zeatin conversion products were tentatively eliminated by chromatographic comparisons of these minor radioactive compounds to reference substances. These are: 2iP, 2iPA, 6-glycyladenine, adenine, adenosine, AMP, hypoxanthine, inosine, and inosinic acid.

Effects of ABA and Cycloheximide on 8-14C-Zeatin Metabolism. The percentages of radioactive zeatin, dihydrozeatin, their ribosides and ribotides found after 12-hr incubation are shown in Table II. This table also shows the effects of ABA and cycloheximide on zeatin conversions. In all three cases slightly more than 90% of the radioactivity is found in the zeatin conversion products. The actual amount of zeatin converted to ribosides and ribotides is not strongly influenced by ABA or cycloheximide, even though both compounds were used at growth-inhibiting concentrations. However, these inhibitors led to a marked decrease in the amounts of dihydrozeatin, riboside, and ribotide. This is particularly noticeable in the presence of cycloheximide.

Effects of Zeatin, Dihydrozeatin, and Their Ribosides on the ABA-induced Growth Inhibition. Since extensive conversion of zeatin to dihydrozeatin, zeatin riboside, and dihydrozeatin riboside occurs in the bean axes, the relative ability of these substances to antagonize the ABA-induced growth inhibition was determined.

Table III shows that the presence of a ribosyl moiety is of minor significance and that saturation of the side chain decreases the activity significantly only at the lowest concentration. Dihydrozeatin and its riboside were tested as the racemates but are assumed to occur naturally in an optically pure

form. The zeatin and dihydrozeatin ribotides were not available at sufficiently high concentration for testing.

DISCUSSION

Two types of metabolic modification are observed on incubation of bean axes with 8-^{14}C-zeatin, saturation of the zeatin side chain, and addition of ribosyl or ribosyl phosphate moieties. If cleavage of the purine side chain occurs, it must be of minor significance since neither ^{14}C-adenine, ^{14}C-hypoxanthine, nor their ribosides or ribotides were deleted. This differs from the results obtained with benzyladenine and 2iP where side chain degradation was observed (5, 12).

Although dihydrozeatin has been isolated from yellow lupin seeds (7), neither dihydrozeatin riboside nor its ribotides have been reported as naturally occurring compounds. Our results indicate that the saturated compounds can be derived from zeatin. On the basis of Miura and Miller's report (14) that zeatin is derivable from 2iP, one can suggest the following biogenetic sequence: 2iP → zeatin → dihydrozeatin. The ability to convert the isopentenyl alcohol side chain of zeatin or its ribosyl derivatives to saturated analogues does not appear to be universal. When 8-^{14}C-zeatin was administered to excised embryos from Fraxinus americana seeds for 24 hr, extensive conversion to zeatin riboside and zeatin ribotides took place in the absence of any reduction of the isopentenyl alcohol side chain (Tzou and Sondheimer, unpublished results). The most obvious explanation for the decrease in the proportion of dihydrozeatin compounds in the presence of cycloheximide is based on the known ability of this antibiotic to interfere with de novo synthesis of proteins. However, since cycloheximide may also interfere in energy transfer in some tissues (10) the decreased availability of reducing agents, such as NADH or NADPH, may also contribute to the decreased proportion of compounds with saturated side chains.

Two paths are known for the conversion of purines to ribosides and ribotides. The N-base can react with 1-pyrophosphoryl ribosyl-5-phosphate to yield a 5'-ribotide which can then be hydrolyzed to the riboside or converted to a di- or triphosphate. Or the riboside may form first by a reaction between the N-base and ribose-1-phosphate, followed by ribotide formation. Either or both paths may be operative in the synthesis of zeatin and dihydrozeatin ribosides and ribotides in bean axes. Chen and Hall (4) report that the 2iP moieties of certain t-RNA species are formed by addition of the side chain to polymeric precursor t-RNA. Hydrolysis of the tRNA would then yield the ribosides and ribotides. Since tRNA species which contain cis-zeatin moieties are also known (6), it is conceivable that zeatin riboside and zeatin 5'-ribotide could also be formed through this route, although it would also require a cis-trans rearrangement. Our results, while giving no information on the existence of this route, do suggest that zeatin and dihydrozeatin ribosides and ribotides can be formed by direct addition of ribosyl units to purines that already contain a 5-carbon alcohol side chain.

Since only about 10% of the radioactivity found in the 80% ethanol-soluble fraction is still present as zeatin 12 hr after the start of incubation, a decision on which compounds are hormonally active is not easily reached. Skoog and coworkers (9) found that in the tobacco callus assay, dihydrozeatin has only one-tenth the activity of zeatin and zeatin 9-β-D-riboside is less active than zeatin. In the bean axes the ribosides are at least as effective as the free N-bases and the dihydroderivatives almost as good in their ability to antagonize the ABA effects as the unsaturated compounds. However, nothing is known concerning their rates of interconversion and it there-

Table III. *Reversal of (RS)-ABA-induced Growth Inhibitions by Cytokinins*

Concn. Cytokinin[1]	% Reversal of (RS)-ABA-Inhibition			
	Zeatin	(RS)-Dihydrozeatin	Zeatin riboside	(RS)-Dihydrozeatin riboside
M				
1×10^{-7}	25	10	25	0
1×10^{-6}	45	30	55	40
4×10^{-6}	45	40	50	40

[1] A control containing the appropriate cytokinin gave the base value from which the percentage of reversal was calculated. (RS)-ABA was used at a concentration of 3.9×10^{-5} M and gave 80% growth inhibition during 12 hr at 26 C.

fore is not clear which compounds are active. The major effect of ABA on the zeatin conversions is to lead to a decrease in the proportion of saturated compounds. Since this does not explain the observed growth antagonism, it is suggested that the mode of interaction between these two growth regulators is not related to the types of zeatin conversions discussed here.

While ^{14}C-cytokinin metabolism has been studied previously, this is the first report on ^{14}C-zeatin conversions. Of the earlier studies, the one by Martin (11) is of particular interest. ^{14}C-Phenyl-labeled 6-N-benzyladenine was fed to a cytokinin-requiring strain of tobacco callus tissue culture. After 24 hr, 50% of the labeled cytokinin found in the cells was in the barium-precipitable fraction and the remaining material was about equally divided between the free base and 6-N-benzyladenosine. Within a 12-hr period, 90% of the supplied cytokinin was taken up by the cells. To the extent that comparisons are possible, one may conclude that the metabolism of natural and synthetic cytokinins are similar. This resemblance is observed in tissues that require exogenous cytokinins as well as those that do not.

LITERATURE CITED

1. ADDICOTT, F. T. AND J. L. LYON. 1969. Physiology of abscisic acid and related substances. Annu. Rev. Plant Physiol. 20: 139–164.
2. AMEN, R. D. 1968. A model of seed dormancy. Bot. Rev. 34: 1–32.
3. CARTER, C. E. 1956. Synthesis of 6-succinoaminopurine. J. Biol. Chem. 223: 139–146.
4. CHEN, C. M. AND R. H. HALL. 1969. Biosynthesis of N^6- (Δ^2-isopentenyl) adenosine in the transfer nucleic acid of cultured tobacco pith tissue. Phytochemistry. 8: 1687–1695.
5. CHEN, C. M., D. M. LOGAN, B. McLENNAN, AND R. H. HALL. 1968. Studies on the metabolism of a cytokinin, N^6- (Δ^2-isopentenyl) adenosine. Plant Physiol. 43: 5–18.
6. HALL, R. H., L. CSONKA, H. DAVID, AND B. McLENNAN. 1967. Cytokinins in the soluble RNA of plant tissues. Science. 156: 69–71.
7. KOSHIMIZU, K., T. KUSAKI, T. MITSUI, AND S. MATSUBARA. 1967. Isolation of a cytokinin, (−)-dihydrozeatin, from immature seeds of Lupinus luteas. Tetrahedron Lett. 14: 1317–1320.
8. LEONARD, N. J. AND T. FUJII. 1964. The synthesis of compounds possessing kinetin activity. The use of a blocking group at the 9-position of adenine for the synthesis of 1-substituted adenines. Proc. Natl. Acad. Sci. U.S.A. 51: 73–75.
9. LEONARD, N. J., S. M. HECHT, F. SKOOG, AND R. Y. SCHMITZ. 1969. Cytokinins: Synthesis, mass spectra, and biological activity of compounds related to zeatin. Proc. Natl. Acad. Sci. U.S.A. 63: 175–182.
10. MACDONALD, I. R. AND R. J. ELLIS. 1969. Does cycloheximide inhibit protein synthesis specifically in plant tissues? Nature 222: 791–792.
11. MARTIN, J. H. 1969. A study of cytokinin metabolism. Ph.D. thesis. Diss. Abstr. Int. B: 30 (3) 1000–1001.
12. McCALLA, D. R., D. J. MOORE, AND D. J. OSBORNE. 1962. The metabolism of a kinin, benzyladenine. Biochim. Biophys. Acta 55: 522–528.
13. MILBORROW, B. V. 1967. The identification of (+)-abscisin II, (+) dormin, in plants and measurements of its concentrations. Planta 76: 93–113.
14. MIURA, G. A. AND C. O. MILLER. 1969. 6- (γ,α-Dimethylallylamino) purine as a precursor of zeatin. Plant Physiol. 44: 372–376.

15. SHAW, G., B. M. SMALLWOOD, AND D. V. WILSON. 1966. Purines, pyrimidines and imidazoles. Part XXIV. Syntheses of zeatin, a naturally occurring adenine derivative with plant cell division-promoting activity, and its 9-β-D-ribofuranoside. J. Chem. Soc. (C) : 921-924.

16. WALTON, D. C. 1966. Germination of *Phaseolus vulgaris*. I. Resumption of axes growth. Plant Physiol. 41: 298-302.

17. WALTON, D. C. AND G. S. SOOFI. 1969. Germination of *Phaseolus vulgaris*. III. The role of nucleic acid and protein synthesis in the initiation of axis elongation. Plant Cell Physiol. 10: 307-315.

18. WALTON, D. C., G. S. SOOFI, AND E. SONDHEIMER. 1970. The effects of abscisic acid on growth and nucleic acid synthesis in excised embryonic bean axes. Plant Physiol. 45: 37-40.

19. WAREING, P. F., J. GOOD, AND J. MANUEL. 1968. Some possible physiological roles of abscisic acid. *In:* F. Wightman and G. Satterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa. pp. 1561-1579.

20. WERTH, H. C. AND P. T. GILMAN. 1967. Structural analysis of polynucleotides by sequential base elimination. The sequence of the terminal decanucleotide fragment of the ribonucleic acid from bacteriophage f2. J. Amer. Chem. Soc. 89: 5473-5474.