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Influence of Enol Ether Amino Acids, Inhibitors of Ethylene Biosynthesis, on Aminoacyl Transfer RNA Synthetases and Protein Synthesis

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ABSTRACT

The analogs of rhizobitoxine, aminoethoxyvinylglycine (AVG) (L-2-amino-4-2'-aminoethoxy-*trans*-3 butenoic acid) and methoxyvinylglycine (MVG) (L-2-amino-4-methoxy-trans-3-butenoic acid), that are potent inhibitors of ethylene biosynthesis at 0.1 millimolar also inhibited protein synthesis and charging of tRNA especially at 1 millimolar and higher concentrations. The saturated analog of MVG inhibited ethylene synthesis while the saturated analog of AVG did not. Both saturated AVG and MVG inhibit methionyl- and leucyl-amino acyl-tRNA synthetase. Because of the inhibition of amino acid metabolism in plant tissues by these rhizobitoxine analogs caution is advised in interpreting the results obtained with concentrations of compounds above 0.1 millimolar.

Recently rhizobitoxine[3] and related enol ether amino acids have been shown to inhibit ethylene production from methionine in higher plants (6, 9). Rhizobitoxine was first isolated from root nodules produced by *Rhizobium japonicum* in soybean, *Glycine max* (L.) Merr., wherein it causes symptoms of rhizobial-induced chlorosis (8). The aminoethoxy analog (AVG) of rhizobitoxine was isolated from a *Streptomyces* strain and inhibited the growth of three *Bacilli* species and of *S. cellulosae* (10). The methoxy analog (MVG) or rhizobitoxine was discovered in a fermentation broth of *Pseudomonas aeruginosa* and inhibited the growth of *Bacillus sp.* (13).

All three unsaturated enol ether amino acids inhibit the biosynthesis of ethylene in apple fruit and other plant tissues (6) with a few exceptions (2). Rhizobitoxine was also shown to inhibit β-cystathionase (4, 11), while the methoxy analog, MVG, inhibited cytoplasmic pig heart aspartate aminotransferase (12). The inhibition of ethylene biosynthesis by these amino acids has been assumed to occur by irreversible binding with the ethylene biosynthesis enzyme system. For example, the inhibition of bud growth by AVG, after release from dormancy, was attributed to the inhibition of ethylene production during bud growth (14). Inasmuch as these enol ether amino acids are analogs of amino acids it was of interest to determine whether they also inhibit protein synthesis and other aspects of amino acid metabolism. These possible side effects would be important when interpreting the results of ethylene biosynthesis inhibitor studies. Here we present evidence that these analogs also inhibit protein synthesis to various extents in *Penicillium digitatum*, apple tissue slices, and soybean axes. In addition these compounds interfere with aminoacylation of transfer ribonucleic acids (tRNAs).

MATERIALS AND METHODS

Chemicals. Aminoethoxy (AVG) and methoxy (MVG) analogs of rhizobitoxine and their saturated counterparts (AAEB and AMB, respectively) were gifts from Dr. A. Stempel, Hoffmann LaRoche, Nutley, N.J. The structures of the four enol ether amino acid analogs tested for their effects on protein synthesis and aminoacylation of tRNAs are given (Fig. 1). Labeled amino acids were obtained from New England Nuclear Corp.[4]

tRNA (18.4 A_{260} units/mg) from *Escherichia coli* was obtained from Sigma Chemical Company; glutamic acid-specific tRNA from *E. coli* MRE 600 was obtained from Boehringer Mannheim, Germany; *E. coli* aminoacyl-tRNA synthetase (specific activity 55 units/mg protein) was obtained from Miles Laboratories, Elkhart, Ind., and trasylol from Mobay Chemical Co., New York.

Test Organisms and Tissues. *P. digitatum* Sacc. ATC 10077 was grown in a modified Pratt medium at 25 C for 4 days as described previously (3). Apple slices from postclimacteric Golden Delicious fruit, prepared as described previously (7), were incubated in 3 ml of 0.6 M sorbitol-10 mM Tris-HCl (pH 7.5). Soybean (*G. max* L. cv. Kent) seed axes were used after imbibition with water containing 10 μg/ml chloramphenicol for 3 h at 25 C (1).

Ethylene Measurement. Apple slices (0.5 g) were incubated in 25-ml Erlenmeyer flasks containing 3 ml 0.8 M sorbitol, 10 mM Mes (pH 6.0), 30 μg chloramphenicol, 15 μg fungizone and inhibitors. Gas samples for ethylene determination were measured at intervals and flasks were flushed with air and resealed. Ethylene was measured by gas chromatography (7).

***In Vivo* Incorporation of Amino Acids into Trichloroacetic Acid-insoluble Materials.** *P. digitatum* cultures (1-2 g fresh weight), apple slices (1 g fresh weight), and soybean axes (10, equivalent to about 100 mg) were incubated with or without the enol ether amino acids in buffer or water solutions containing 0.5 μCi/ml of one of the following labeled substrates: L-[U-^{14}C]leucine (320

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[3] Abbreviations: rhizobitoxine: 2-amino, 3-hydroxypropoxyvinylglycine (2-amino-4-(2'-amino-3-hydroxypropoxy) *trans* 3-butenoic acid); AVG: aminoethoxyvinylglycine (L-2-amino-4-(2'-aminoethoxy)-*trans*-3-butenoic acid); MVG: methoxyvinylglycine (L-2-amino-4-methoxy-*trans*-3-butenoic acid); AAEB: L-2-amino-4 (2'-aminoethoxy)-butanoic acid; AMB: L-2-amino-4-methoxy-butanoic acid; CHI: cycloheximide.

[4] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available.

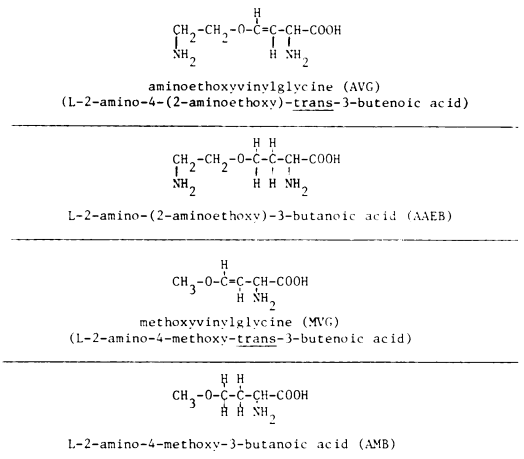

FIG. 1. Structure of enol-ether amino acid analogs.

mCi/mmol), L-[methyl-^{14}C]methionine (15.2 mCi/mmol); L-[U-^{14}C]glutamate (235 mCi/mmol), L-[^{35}S]methionine (1,150 Ci/mmol), and L-[U-^{14}C]glycine (102 mCi/mmol). Each treatment had three replicates. The incubation tubes or flasks were sealed with rubber stoppers to which filter paper wicks (Whatman No. 3), wetted with 10 N NaOH to absorb CO_2, were fixed with stainless steel pins. After an incubation period of 0.75 or 1.5 h at either 30 C (for apple and soybean) or 20 C (for *P. digitatum*) the filter paper wicks were transferred to scintillation vials containing 1 ml hydroxide of hyamine and allowed to mix for 30 min. Then 5 ml of Liquifluor (New England Nuclear) was added and radioactivity determined in a Packard Tri-Carb spectrometer. The tissue samples were removed from the incubation flasks, washed with tap water, extracted with 10% (w/v) ice-cold trichloroacetic acid, and filtered under vacuum on Whatman GF/C filters. The residue on the filters was washed several times with several portions of cold 5% trichloroacetic acid, ethanol, and ether. The dried Liquifluor was added, and radioactivity determined.

Preparation of Crude tRNA Synthetase. All operations were carried out at 2 C. Dry soybean axes (150 mg), apple slices, or *P. digitatum* mycelial pellets were frozen in liquid N_2 and ground by a mortar and pestle to a powder. The 5 ml of the extraction medium containing 5% glycerol, 1 mM EDTA, 150 mM NaCl, 10 mM $MgSO_4$, 5 mM β-mercaptoethanol, 200 Kalleikrein inactivator units of trasylol, 20 mM Tris-Mes (pH 8.0), and 5 mg of washed bentonite, were added to the powder, and the frozen material thawed. The thawed mixture was homogenized, and the homogenate centrifuged at 30,000*g* for 20 min. The supernatant was recentrifuged at 100,000*g* for 90 min. The top two-thirds of the supernatant was removed and stored at −80 C until used. From this material endogenous substrates were removed either by dialysis for 4 h against 20 volumes of the extraction buffer minus bentonite, or by filtration through a Sephadex G-25 column equilibrated with the extraction buffer minus bentonite.

Protein was determined in the 10% trichloroacetic acid precipitates of the samples by fluorometry with fluorescamine (2).

Assay for tRNA Synthetases in 100,000*g* Supernatants from Soybean, Apple Slices, and *P. digitatum*. The assay was based on the amino acyl-tRNA synthetase-mediated conversion of radioactive acid-soluble amino acids into trichloroacetic acid-insoluble product. The same amino acids used in the *in vivo* experiments were used except that L-[methyl-^{3}H]methionine (200 mCi/mmol) was used in place of [^{14}C]methionine. Saturating and noninhibitory ATP concentrations and time periods of incubation for obtaining linear rates of reaction were determined in preliminary experiments. The standard assay mixture contained: 67 mM Tris-HCl (pH 7.4), 6.7 mM $MgCl_2$, 2 mM ATP, *E. coli* tRNA (1 *A* unit), 0.3 or 1.5 μM tRNA synthetase, plus or minus inhibitor, and water to 0.3 ml. Synthetase activity toward each amino acid was determined separately. The control reaction mixtures were run in the absence of ATP and/or tRNA. The reaction was initiated by addition of the enzyme and terminated after incubation for varying times at 30 C, by addition of 100 μl of ice-cold 100% trichloroacetic acid. After 30 min in ice, the precipitate was collected on Whatman GF/C filters, washed three times with 10 ml trichloroacetic acid, twice with 10 ml ethanol-petroleum ether (1:1), and once with 10 ml petroleum ether. Alternatively, aliquots were removed at various time intervals for spotting on Whatman 3MM discs, and after 15 s transferred to a magnetically stirred ice-cold solution of 10% trichloroacetic acid. The filter paper discs were carried through a twice repeated washing procedure each lasting 15 to 20 min. The solvent sequence for the wash was: 10% trichloroacetic acid, 5% trichloroacetic acid, ethanol, ethanol-petroleum ether (1:1), and petroleum ether. The dried filters were transferred to scintillation vials to which 5 ml of Liquifluor was added and radioactivity determined. The activity of *E. coli* aminoacyl-tRNA synthetase was similarly determined, according to Kelmers *et al.* (6).

RESULTS

Effects of AAEB and AMB on Ethylene Production by Apple Slices. Ethylene production by apple slices was not greatly affected by AAEB at any of the concentrations tested (Table I). However, AMB did inhibit ethylene production by 25 to 29% at 0.1 mM and more at 1 mM.

Effects on *in Vivo* Incorporation of Amino Acids into Trichloroacetic Acid-insoluble Materials and CO_2. The effects of MVG and AVG (Fig. 1) on the incorporation of labeled methionine, glutamate, leucine, and glycine into labeled CO_2 and labeled trichloroacetic acid-precipitable materials (protein) in *P. digitatum*, apple, and soybean axes in 0.75 h are shown in Table II. In *P. digitatum* the incorporation of L-methionine, L-leucine, and L-glycine into trichloroacetic acid-precipitable fractions was inhibited 27, 24, and 5%, respectively, by 1 mM MVG, and 21, 49, and 44%, respectively, by 1 mM AVG (Table IIA). The conversion of the labeled substrates into labeled CO_2 increased from about 7 to 38% with both inhibitors.

In apple slices, the incorporation of L-methionine, L-glutamate, and L-leucine into acid precipitates was inhibited 28, 72, and 36%, respectively, by 1 mM MVG, and 38, 62, and 22%, respectively, by 1 mM AVG (Table IIB). However, while MVG inhibited the incorporation of L-glycine by 30%, AVG increased its incorporation by about 17% (Table IIB). The only significant effect on the conversion of the amino acids into CO_2 by the two inhibitors was an inhibition of CO_2 evolution from [^{14}C]glutamate (about 30%) and a slight stimulation from [^{14}C]leucine in the presence of AVG.

In soybean axes, the inhibitors had little effect on the incorporation of labeled methionine into acid-precipitable fractions or CO_2 but the incorporation of glutamate, leucine, and glycine was inhibited 56, 38, and 32%, respectively, by 1 mM MVG (Table

Table I. *Effect of Saturated Enol Ether Amino Acids on Ethylene Production by Apple Slices*

Treatment	AAEB (mM)		
	0.01	0.1	1.0
h		*% of control*[1]	
0–2	101	102	99
2–4	102	102	95
4–6	96	101	92
	AMB		
0–2	100	92	67
2–4	96	75	46
4–6	95	71	53

[1] Controls ranged from 26 to 94 nl/g·h except for one experiment when Granny Smith apples were used. These controls ranged from 6.6 to 12.7 nl/g·h.

Table II. *Incorporation of Amino Acids into CO_2 and Trichloroacetic Acid-Precipitable Fractions in Presence and Absence of Enol Ether Amino Acid Inhibitors*

Amino Acid	Inhibitor (1 mM)	% of Initial Radioactivity Taken Up from Incubation Mixture	Labeled CO_2	Incorporation into Trichloroacetic Acid-Precipitable Fraction	
			cpm/h	*cpm/h × 10^{-3}*	*% Inhibition*
A. *P. digitatum*[1]					
[^{14}C]Methionine	None	19	2018	884.37	0
	MVG	16	2162	641.96	27
	AVG	24	2678	700.51	21
[^{14}C]Leucine	None	10	1494	380.95	0
	MVG	19	1645	287.82	24
	AVG	15	1629	195.78	49
[^{14}C]Glycine	None	16	37	96.51	0
	MVG	17	51	91.03	5
	AVG	15	51	54.06	44
B. Apple[2]					
[^{14}C]Methionine	None	12	1067	16.67	0
	MVG	7	1073	11.95	28
	AG	6	1109	10.34	38
[^{14}C]Glutamate	None	4	784	1.41	0
	MVG	11	542	0.39	72
	AVG	8	553	0.54	62
[^{14}C]Leucine	None	5	1756	36.01	0
	MVG	11	1792	23.09	36
	AVG	13	1936	27.97	22
[^{14}C]Glycine	None	12	63	9.79	0
	MVG	11	59	6.81	30
	AVG	5	60	11.46	−17
C. Soybean Axes[3]					
[^{14}C]Methionine	None	25	344	3.12	0
	MVG	21	340	2.85	9
	AVG	29	341	3.25	−4
[^{14}C]Glutamate	None	5	318	0.16	0
	MVG	2	274	0.07	56
	AVG	2	279	0.07	56
[^{14}C]Leucine	None	30	153	8.75	0
	MVG	22	134	5.46	38
	AVG	33	153	9.19	−5
[^{14}C]Glycine	None	28	46	7.01	0
	MVG	18	43	4.80	32
	AVG	20	44	5.65	19

[1] Each labeled amino acid was taken up in water and 5 ml of the labeled amino acid (2.5 μCi) solution was added to 4-day-old mycelia grown by shake cultures. Data of incorporation into trichloroacetic acid-precipitable material are given per g dry weight of the fungus.

[2] Each labeled amino acid was taken up in 0.6 M sorbitol and 10 mM Tris-HCl (pH 7.5), and 3 ml of this solution (1.5 μCi) added to 1 g of apple slices (postclimacteric fruit). Data on the incorporation into trichloroacetic acid-precipitable material are given per g fresh weight.

[3] Each labeled amino acid solution was added to a final volume of 1.5 ml (1.5 μCi). Data on incorporation into trichloroacetic acid-precipitable material are given per 10 imbibed soybean axes (100 mg).

IIC). In contrast, 1 mM AVG had little influence on the incorporation of leucine, but inhibited that of glutamate and glycine by 56 and 19%, respectively. Whereas the degrees of inhibition by 1 mM AVG and 1 mM MVG on the inhibition of CO_2 from glutamate and leucine varied between 12 and 14% there was no effect on the conversions of methionine or glycine to CO_2 (Table IIC).

The uptake of the label from the different amino acids in the incubation media was affected differently by the inhibitors (Table II). These differences in uptake did not correlate with the effects on incorporation of the amino acids into proteins or into labeled CO_2. Results of a study comparing the effects of 0.01 and 0.1 mM AVG and 0.01 mM CHI on ethylene and protein synthesis are shown in Table III. AVG at 0.1 mM and cycloheximide (0.01 mM) inhibited incorporation of [^{35}S]methionine into acid-insoluble material while 0.01 mM AVG had little effect. In the time period studied only AVG inhibited ethylene synthesis. In addition to inhibiting incorporation of label, AVG at 0.1 mM also reduced the amount of radioactivity accumulating in the trichloroacetic acid-insoluble fraction, whereas 0.01 mM AVG had little or no effect. After correcting for uptake difference, 0.1 mM AVG still inhibited incorporation of radioactivity 15%, after 2-h incubation. CHI at 0.01 mM had virtually no influence on ethylene production during the 2 h, but inhibited incorporation of label into the trichloroacetic acid-precipitable fraction about 90%, even after only 30 min of incubation.

Effects of Amino Acid Analogs on Aminoacyl-tRNA Synthetase Activity. We attempted to study whether or not the enol ether amino acid analogs inhibit protein synthesis by competing with the endogenous amino acids for tRNAs during charging. Preliminary experiments showed that storage at −80 C, of the 100,000 supernatant fractions of apple, *P. digitatum*, and soybean axes considerably increased their activity in aminoacylation of tRNA. The time of storage for obtaining optimal activation varied with the amino acid used as the substrate; we seemed to be dealing with different enzymes specific for different amino acids. Such differences in activities were further observed when BSA was used in the assay media; its presence either stimulated or inhibited the charging process. Results for both apple and *P. digitatum* supernatant fraction varied greatly. We therefore carried out detailed experiments with soybean enzyme extracts which were much less variable, and also with a commercial preparation of amino acyl-tRNA synthetase from *E. coli.*

The effects of AAEB, AMB, AVG, and MVG on the activities of methionyl- and leucyl-tRNA synthetases in the 100,000*g* supernatant of soybean axes homogenates are shown in Table IV. All four analogs were inhibitory at 1 mM but were generally less inhibitory and in one case slightly stimulatory at concentrations of 0.5 mM and less. AMB was the most effective inhibitor. The inhibitory effects of the analogs were reversed in all cases when the amino acid concentrations in the reaction mixtures were raised by five times (data not shown).

Table III. *Effect of CHI and AVG on Ethylene and Protein Synthesis of Apple Fruit Tissue*

Treatment[1]	Incubation	Ethylene	Incorporation		
			Insol.	Total uptake	Insol. corrected for equal uptake
	h	*nl/g·h*	*cpm × 10^{-3}/g*		
Control	0.5	80.3	24.8	228.5	24.8
0.1 mM AVG		58.4	13.7	138.2	22.3
0.01 mM AVG		68.6	26.9	206.3	29.3
0.01 mM CHI		85.4	2.5	226.4	2.5
Control	1.0	73.7	47.3	331.0	47.3
0.1 mM AVG		42.7	30.4	222.3	45.3
0.01 mM AVG		40.1	51.7	316.7	54.0
0.01 mM CHI		64.9	4.2	323.5	4.5
Control	2.0	65.3	110.3	498.1	110.3
0.1 mM AVG		24.4	63.3	336.5	94.1
0.01 mM AVG		25.9	103.3	457.7	120.1
0.01 mM CHI		61.3	7.7	474.8	8.1

[1] One g of apple discs was incubated in 3 ml of 0.8 M sorbitol, 10 mM Mes (pH 6.0), 100 mM $CaCl_2$, 10 μg/ml chloroamphenicol, and 0.5 μCi/ml L-[^{35}S]methionine (1, 150 Ci/mmol).

Table IV. *Effects of Enol Ether Amino Acids on Activity of Aminoacyl-tRNA Synthetase of Soybean Axes*

The enzyme source was the 100,000*g* supernatant fraction of soybean axes. Assay mixture (0.3 ml) is as described under "Materials and Methods." The concentration of each amino acid used was 0.3 μM per reaction mixture. Specific activities are given under "Materials and Methods."

Substrate	Inhibitor	Aminoacylation of tRNA							
		Specific Activity				Relative Activity			
		Inhibitor Conc. (mM)							
		0	0.1	0.5	1.0	0	0.1	0.5	1.0
		pmol/mg protein				*% of control*			
L-[^{3}H]Methionine	AVG	1.2		1.3	0.7	100		108	58
	AAEB	1.2		1.1	0.8	100		92	67
	MVG	1.2	1.1	0.6	0.7	100	92	50	58
	AMB	1.2	0.9	0.5	0.1	100	75	42	8
L-[^{14}C]Leucine	AVG	12.8		9.3	7.6	100		72	60
	AAEB	12.8		9.4	9.9	100		73	77
	MVG	12.8	7.7	6.7	4.7	100	60	52	36
	AMB	12.8	6.9	3.9	3.4	100	54	30	26

Table V. *Effects of Enol Ether Amino Acids on Activity of E. coli Aminoacyl-tRNA Synthetases*

Substrate	Inhibitor	Aminoacylation of tRNA							
		Specific activity[1]				Relative activity			
		Inhibitor Conc. (mM)							
		0	0.6	3.0	6.0	0	0.6	3.0	6.0
		pmol/enzyme unit·5 min				*% of control*			
L-[^{3}H]Methionine	AVG	2.06	2.06	2.09	2.04	100	100	100	99
	AAEB	2.06	2.21	1.97	1.93	100	107	96	94
	MVG	2.06	2.05	2.14	1.94	100	99	104	94
	AMB	2.06	1.90	1.44	0.98	100	92	70	48
L-[^{14}C]Leucine	AVG	1.20	1.17	0.88	0.82	100	97	73	68
	AAEB	1.20	0.72	0.79	0.80	100	60	66	67
	MVG	1.20	0.74	0.71	0.59	100	62	59	49
	AMB	1.20	0.95	0.53	0.45	100	79	44	37

[1] pmol of amino acid incorporated into trichloroacetic acid-insoluble material per unit of enzyme in 5 min.

In contrast to soybean axes, aminoacyl-tRNA synthetase, the methionyl-accepting activity of *E. coli* enzyme was markedly inhibited only by AMB and the concentration of the inhibitor required for a significant inhibition was several times greater than that effective for soybean. None of the other analogs were significantly inhibitory up to a tested concentration of 6 mM (Table V). The leucyl-accepting activity of the *E. coli* enzyme was by comparison much more inhibited by all four analogs. The glutamyl-accepting activity of the soybean and the bacterial enzyme was low. The bacterial system increased appreciably when glutamic acid tRNA was included in the assay mixture, but the activity was unaffected by any of the four analogs tested (data not shown). Like the synthetase from soybean, that from *E. coli* was not inhibited by the analogs when the amino acid concentration was raised five times to 1.5 μM.

DISCUSSION

The study demonstrates that analogs of rhizobitoxine inhibit protein synthesis (trichloroacetic acid-precipitable fractions) by inhibiting to various degrees the incorporation of some specific amino acids into protein in *P. digitatum*, apple fruit slices, and soybean axes. One of the steps at which these inhibitors act may be the aminoacylation of tRNAs. Since the inhibitory effect on the aminoacylation reaction was reversed by higher substrate concentrations the inhibitors appear to inhibit reversibly by acting as competitive inhibitors for aminoacyl-tRNA synthetase. Our findings suggest that whether or not protein synthesis is inhibited by the analogs depends on the size and composition of the endogenous amino acid pool. A possible factor contributing to the apparent inhibition of protein synthesis would be a change in uptake of some amino acids in the presence of the amino acid analogs (*e. g.* glutamate). In tests with the various tissues there appeared to be no consistent correlation between changes in the uptake of labeled amino acids and changes in protein synthesis (trichloroacetic acid-precipitable fraction).

MVG, AMB, and AAEB generally were more potent than AVG in inhibiting the aminoacyl-tRNA synthetase. The inhibition by these compounds is probably due to their structural resemblance to methionine and leucine. One cause of rhizobial-induced chlorosis (8) in soybean by rhizobitoxine and the inhibition of growth of *Bacillus sp.* (13) by MVG, may, therefore be that it inhibits protein synthesis and charging of tRNAs in the host cells.

Comparison of the differences between protein synthesis and ethylene biosynthesis (6) with respect to inhibition by the enol ether amino acid analogs suggests that a double bond (Table I) between carbons 3 and 4 and the type of moiety on the enol O_2 on carbon 4 are important only in the inhibition of ethylene biosynthesis. Another significant difference between the inhibition of protein synthesis and ethylene biosynthesis relates to concentration of the inhibitors. Concentrations (0.1–0.01 mM) which were or would appear to be virtually ineffective in inhibiting aminoacyl-tRNA synthetase for methionine charging, potently inhibit ethylene biosynthesis (6). On the other hand CHI which strongly inhibited protein synthesis within 30 min had no effect on ethylene production by apple tissue in a 2-h incubation period. Consequently, inhibition of ethylene biosynthesis by AAEB at concentrations of 0.1 to 0.01 mM is not or is only marginally associated with inhibition of protein synthesis. Because of other possible adverse effects, concentrations of enol ether amino acid analogs above 0.1 mM should not be used to inhibit ethylene biosynthesis in higher plants.

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LITERATURE CITED

1. ANDERSON JD 1977 Adenylate metabolism of embryonic axes from deteriorated seeds. Plant Physiol 59: 610–614
2. BAKER JE, M LIEBERMAN, JD ANDERSON 1978 Inhibition of ethylene production in fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol 61: 610–614
3. BOHLEN P, S STEIN, W DAIRMAN, S UDENFRIEND 1973 Fluorometric assay of protein in the nanogram range. Arch Biochem Biophys 155: 213–220
4. CHALUTZ E, M LIEBERMAN 1977 Methionine-induced ethylene production by *Pencillium digitatum*. Plant Physiol 60: 402–406
5. GIOVANELLI J, LD OWENS, SH MUDD 1971 Mechanism of inhibition of spinach β-cystathionase by rhizobitoxine. Biochim Biophys Acta 227: 671–684
6. KELMERS AD, GD NOVELLI, MP STULBERG 1965 Separation of tRNA by reverse phase chromatography. J Biol Chem 240: 3979–3987
7. LIEBERMAN M, AT KUNISHI, LD OWENS 1974 Specific inhibitors of ethylene production as retardants of the ripening process in fruits. Colloq Inter CNRS No. 238. Facteurs et Regulation de la Maturation des Fruits, Paris, pp 161–170
8. MATTOO AK, M LIEBERMAN 1977 Localization of the ethylene-synthesizing system in apple tissue. Plant Physiol 60: 794–799
9. OWENS LD, S GUGGENHEIM, J HILTON 1968 Rhizobium-synthesized phytotoxin: an inhibitor of β-cystathionase in *Salmonella typhimurium*. Biochim Biophys Acta 158: 219–225
10. OWENS LD, M LIEBERMAN, AT KUNISHI 1971 Inhibition of ethylene production by rhizobitoxine. Plant Physiol 48: 1–4
11. OWENS LD, DA WRIGHT 1965 Rhizobial-induced chlorosis in soybeans: isolation and production in nodules and varietal specificity of the toxin. Plant Physiol. 40: 927–930
12. PRUESS DL, JP SCANNELL, M KELLETT, HA AX, J JANECEK, TH WILLIAMS, A STEMPEL, J BERGER 1974 Antimetabolites produced by microorganisms. X. L-2-Amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid. J Antibiotics 27: 229–233
13. RANDO RR 1974 B Unsaturated amino acids as reversible inhibitors. Nature 250: 586–587
14. SCANNELL JP, DL PRUESS, TC DENNY, LH SELLO, TH WILLIAMS, A STEMPEL 1972 Antimetabolites produced by microorganisms. V. L-2-Amino-4-methoxy-*trans*-3-butenoic acid. J Antibiotics 24: 122–127
15. ZIMMERMAN RH, M LIEBERMAN, OC BROOME 1977 Inhibitory effect of rhizobitoxine analog on bud growth after release from dormancy. Plant Physiol 59: 158–160