TABLE II. Steric Strain Energies, $\Delta\Delta E_R$, (kcal/mole) of Amine-BMe_3 Addition Compounds

Amine	$\Delta\Delta E_R$ kcal/mole
n-$C_3H_7NH_2$	0.0
n-$C_4H_9NH_2$	0.0
iso-$C_3H_7NH_2$	1.4
s-$C_4H_9NH_2$	1.6
t-$C_4H_9NH_2$	6.6
Et_2NH	6.1
Et_3N	17

that the S value is an exponential function of $\Delta\Delta E_R$, and log S is linearly related to $\Delta\Delta E_R$, as shown in Fig. 2. These relations can be expressed by the following empirical equations:

$$S_\alpha = 28.9\, e^{-0.21\Delta\Delta E_R} \qquad S_\beta = 11.6\, e^{-0.16\Delta\Delta E_R}$$

where S_α and S_β= Shift parameters of α- and β-H, respectively, induced by Eu(DPM)$_3$
$\Delta\Delta E_R$= Steric strain energy (kcal/mole) of the amine-BMe_3 addition reaction

Thus there seems to be an analogy between the coordination scheme of the shift reagent and BMe_3 on the lone pair of electrons on the nitrogen, and the contribution of the steric strain is probably a major factor in determining the magnitude of the S value. Details of this works will be published elsewhere.

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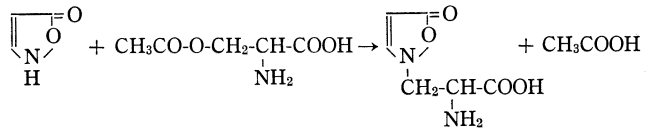
Enzymic Synthesis of 2-Alanyl-3-isoxazolin-5-one from O-Acetylserine and Isoxazolin-5-one by Pisum Seedling Extracts

In recent years two UV-sensitive heterocyclic amino acids, that may be regarded as β-substituted alanines, have been isolated by Lambein, *et al.* from pea (*Pisum sativum*) seedlings.[1,2] These amino acids are not present in the dry seeds, but they can be detected after germination for two or three days. The structures assigned on the basis of chemical and spectroscopic methods were 2-alanyl-3-isoxazolin-5-one (I) and 2-(β-D-glucopyranosyl)-4-alanyl-3-isoxazolin-5-one.

1) F. Lambein, N. Schamp, L. Vandendriessche, and R. Van Parijs, *Biochem. Biophys. Res. Comm.*, **37**, 375 (1969).
2) F. Lambein and R. Van Parijs, *Biochem. Biophys. Res. Comm.*, **40**, 557 (1970).

Recent reports by Murakoshi, *et al.*[3,4] showed that synthesis of β-substituted alanines such as mimosine, β-pyrazolylalanine and S-alkyl-cysteines was catalyzed by enzyme preparations from *Leucaena* and *Mimosa*, *Citrullus*, and legume and *Allium* seedlings, respectively; each synthesis involved the condensation of the appropriate heterocyclic compound or mercaptan with an alanyl moiety arising from O-acetylserine. We have now extended these observations and shown that 2-alanyl-3-isoxazolin-5-one (I) may be synthesized from O-acetylserine (III) and isoxazolin-5-one (II)[5] by an analogous reaction catalyzed by exrtacts of pea seedlings (*Pisum sativum* and *P. arvense*); neither serine nor O-phosphoserine could serve as a donor of the alanyl fragment.



II III I

Fig. 1

Crude enzyme preparations were obtained from seedlings of *P. sativum* or *P. arvense*, grown in the dark for 4 days at 30°; extraction procedures were carried out at about 4°. After removing the testas, seedlings were homogenized in 0.1 M potassium phosphate buffer, pH 7.5, containing 0.5% 2-mercaptoethanol (0.5 ml/g seedlings). The extract was pressed through fine nylon and centrifuged at 25000 ***g*** for 30 min. The clear supernatant was applied to a column of Sephadex G-25 (fine), equilibrated with 0.1 M potassium phosphate buffer, pH 7.5, to give a protein-containing solution free from low mol. wt. substances, which was used directly as the enzyme preparation.

Reaction mixtures used to demonstrate 2-alanyl-3-isoxazolin-5-one formation contained O-acetylserine or O-acetylserine-3-^{14}C (5 μmoles, 0.5 μCi), isoxazolin-5-one (25 μmoles) and 0.2 ml enzyme preparation (equivalent to 0.4 g seedlings fresh weight) in a final volume of 0.4 ml (normally maintained at pH 7.3 by 0.1 M potassium phosphate buffer). Mixtures were incubated at 30° for appropriate periods (100 min) when the reaction was terminated by the addition of 3 vol. ethanol. Precipitated protein was removed by centrifuging, and aliquots of the residual supernatant were examined chromatographically for the presence of 2-alanyl-3-isoxazolin-5-one.

Control mixtures lacking either II or III were examined simultaneously: I was not detected after incubation of such mixtures, nor when an enzyme preparation (boiled for 15 min) was employed.

The product obtained in these enzymic experiments was characterized as 2-alanyl-3-isoxazolin-5-one[1] by paper chromatographic comparison with authentic material using the following solvent systems: 1, pyridine–butan-1-ol–water–acetic acid (40: 20: 20: 5, v/v); 2, propan-2-ol–formic acid–water (80: 4: 20, v/v). The *Rf* values for 2-alanyl-3-isoxazolin-5-one obtained in these solvents were 0.35 and 0.25, respectively, whilst O-acetylserine exhibited the following *Rf* date; 0.46 and 0.47, respectively. Under the same conditions, serine moved at *Rf*' s of 0.28 and 0.33, respectively. Therefore, 2-alanyl-3-isoxazolin-5-one was well separated from O-acetylserine and serine when chromatograms were developed in solvent

3) I. Murakoshi, H. Kuramoto, J. Haginiwa, and L. Fowden, *Phytochem.*, **11**, 177 (1972).
4) It is tentatively found in our laboratory that the enzymes which utilize O-acetylserine and serine O-sulphate as a substrate for S-alkyl-cysteine formation in a number of higher plants are different (I. Murakoshi, A. Yamazaki, and J. Haginiwa, presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 7, 1972).
5) Isoxazolin-5-one was synthesized by the method of F. De Sarlo, C. Dini, and P. Lacrimini (*J. Chem. Soc.*, **1971**, 86).

1 or 2. This method indicated clearly the presence of a product, reacting positively with ninhydrin (a pale orange-brown which faded gradually), that was inseparable from added authentic 2-alanyl-3-isoxazolin-5-one.

Product formation was also determined by ^{14}C-incorporation from O-acetylserine-3-^{14}C into I. Radioactivity associated with each ninhydrin-positive substance present after reaction was determined by scanning chromatograms using a gas-flow 4 π radiochromatogram scanner (Aloka PCS-2B, Tokyo).

Further proof that the reaction product was 2-alanyl-3-isoxazolin-5-one was obtained using alkaline degradation followed by acid hydrolysis, when α,β-diaminopropionic acid was present among the degradation products. This behaviour is in agreement with that observed by Lambein, *et al.*[1] for the authentic compound, and for other N-substituted 3-isoxazolin-5-ones by De Sarlo, *et al.*[6,7]

The addition of pyridoxal phosphate to reaction mixtures caused neither stimulation nor inhibition of I formation.

The enzyme had a pH optimum of 7.3 by using 0.1 M potassium phosphate buffer. The optimum II/III ratio for I synthesis was about 6: 1.

The amount of I formed from II and III was proportional to time over a period of at least 100 min and reached a maximum value (about 10% yield) at about 120 min.

A more detailed investigation of the synthetase from *Pisum* responsible for production of 2-alanyl-3-isoxazolin-5-one is in progress in our laboratory.

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6) F. De Sarlo, L. Fabbrini, and G. Renzi, *Tetrahedron*, **22**, 2989 (1966).
7) F. De Sarlo and G. Renzi, *Tetrahedron*, **22**, 2995 (1966).