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# 3-ISOXAZOLIDONE FROM JACK BEAN SEEDLINGS

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**Key Word Index**—*Canavalia ensiformis*; Leguminosae; jack bean; 3-isoxazolidone; L-canavanine; L-canaline; pentacyanoammoniferrate reagent; Jaffe reagent.

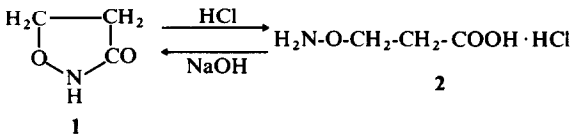
**Abstract**—3-Isoxazolidone has been isolated from jack bean seedlings.

## INTRODUCTION

It is well known that L-canavanine, a principal free amino acid of seeds of jack bean, *Canavalia ensiformis* (L.) DC., forms a magenta colour with pentacyanoammoniferrate reagent (PCAF) [1–3]. In the course of a study of the principal of jack bean seedlings, we have found a new PCAF-positive compound. This compound gave a blue colour with PCAF at neutral pH. The present paper describes the isolation of this compound and its characterization as 3-isoxazolidone. The relationship between 3-isoxazolidone and L-canavanine is also discussed. 3-Isoxazolidione has previously been reported as a parent ring of the antibiotic cycloserine [4–6]. To our knowledge, this is the first report of the occurrence of 3-isoxazolidone in nature.

## RESULTS AND DISCUSSION

Compound **1** (3-isoxazolidone) is an unstable colourless oily substance. On treatment with 1 M HCl, **1** formed colourless needles (**2**) which developed an orange colour with Jaffe reagent, the reagent known to give colour with aminoxy compounds and creatinine [7–9]. From elemental analysis, colour reaction, melting point and chromatographic comparison with an authentic sample, **2** was identified as 3-aminoxypropionic acid hydrochloride. On treatment with 0.5 N NaOH, **2** was cyclized to the original compound **1** (3-isoxazolidone) as shown in Scheme 1.




1 2

Scheme 1. Interconversion of 3-isoxazolidone (**1**) and 3-aminoxypropionic acid hydrochloride (**2**).

In order to elucidate the relationship between 3-isoxazolidone and L-canavanine, changes of these compounds at various stages of growth were determined. The results are shown in Fig. 1. 3-Isoxazolidone is absent

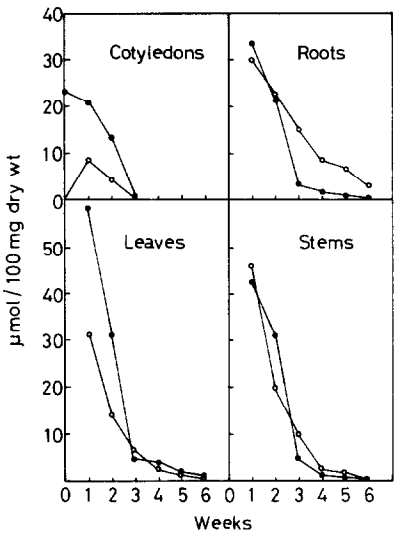
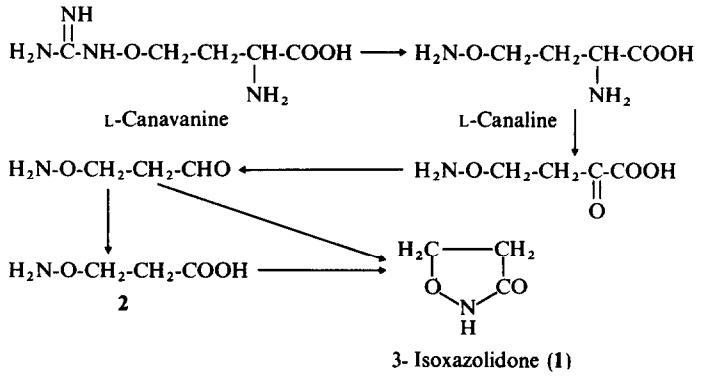



Fig. 1. Changes of 3-isoxazolidone (○) and L-canavanine (●) in jack bean growth. Each point represents the average of duplicate determinations. After 3 weeks' growth the cotyledons were abscised.

in jack bean seeds, but it appeared rapidly during the first week's growth of the seedlings and then decreased. L-Canavanine, which is a predominant amino acid of jack bean seeds, rapidly decreased on germination. In the later stages of growth, both 3-isoxazolidone and L-canavanine became minor components. Rosenthal reported that during the early period of growth, much of the L-canavanine in the cotyledons of jack bean is transported to the growing regions of the plant and converted to L-canaline and urea [10]. 3-Isoxazolidone appears in high concentrations in stems, leaves and roots, but not in cotyledons. From these results, it seems that 3-isoxazolidone must be a metabolite of L-canaline via L-canavanine. On the metabolism of L-canaline, Rosenthal proposed a canaline–urea cycle in which *O*-ureido-L-homoserine is formed by carbamylatin of L-canaline [11, 12]. However, *O*-ureido-L-homoserine was not




Scheme 2. Postulated metabolic pathways of L-canavanine.

detected in jack bean extracts and the metabolism of L-canaline has not been fully resolved at this time. If transamination, decarboxylation and cyclization occurred, L-canaline could be converted into 3-isoxazolidone as shown in Scheme 2. Further, 3-isoxazolidone may be degraded rapidly to 3-oxypropionic acid and ammonia, or propionic acid and hydroxylamine.

Eight weeks after sowing, the first flowers shrivelled and young pods appeared. In young pods (6 cm long) 3-isoxazolidone appeared markedly and gradually decreased with the pods growth as shown in Fig. 2. In young pods, the content of L-canavanine was low, whereas it increased with growth and showed a maximum in 14-week-old plants. Then both 3-isoxazolidone and L-canavanine disappeared in the senescing pods from 24-week-old plants. On the other hand in maturing seeds, L-canavanine accumulated gradually. In maturing seeds, the content of 3-isoxazolidone was low and disappeared in the fully matured seeds. Williams and Hunt suggested that L-canavanine is synthesized in the pod and transported to the maturing seed [13]. Rosenthal showed that the ovary wall is the reservoir for seed L-canavanine [14]. From the above observations, it seems that 3-isoxazolidone could be an intermediate for L-canavanine biosynthesis as well as a metabolite of L-canavanine.

## EXPERIMENTAL

*PC.* Ascending PC was carried out on Toyo No. 50 paper using 2 solvents: solvent I, *n*-BuOH–HOAc–$H_2O$ (4:1:5); solvent II, *n*-PrOH–$H_2O$ (7:3) Pentacyanoammoniferrate reagent: PCAF (0.5%) was prepared by the method of ref. [1]. Jaffe reagent: Sprayed first with 2 N NaOH, followed by 1% picric acid soln.

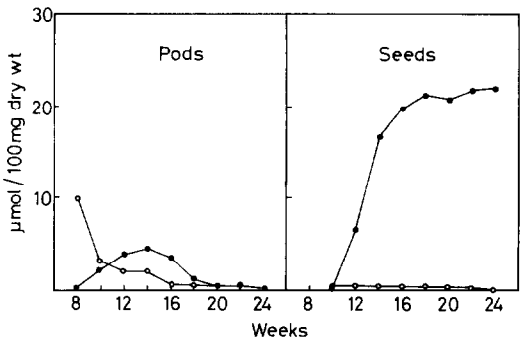



Fig. 2. Changes of 3-isoxazolidone (○) and L-canavanine (●) in pods and seeds of maturing jack bean fruits. Each point represents the average of duplicate determinations.

*Isolation of compound* 1. Fresh seedlings (650 g) grown under light for 1 week at 26–27° in moist sand were homogenized with $H_2O$ (600 ml). To the homogenate, MeOH (1.5 l.) was added and stirred thoroughly. The mixture was allowed to stand for 20 hr at 4° and filtered through cheesecloth. The residue was re-extracted with 60% aq. MeOH (1.5 l.) for 3 hr at 4° and filtered through cheesecloth. The combined filtrate was clarified by centrifugation and the supernatant was evapd *in vacuo* to half vol. The extract prepared as above was passed through a column (3.5 × 60 cm) of Amberlite IR-120 ($H^+$ form). After washing with $H_2O$ (1 l.), the effluent was applied directly onto a column (3.5 × 58 cm) of Amberlite IR-45 ($OH^-$ form). The column was washed with $H_2O$ (1 l.) and the effluent was discarded. Compound **1** was retained on the column which was eluted with 0.5 M HOAc (3 l.). This eluate was concd *in vacuo* to dryness. The residue was dissolved with MeOH (30 ml) and insoluble material was removed by centrifugation. The MeOH soln was concd *in vacuo* to dryness. The residue was dissolved with $H_2O$ (10 ml) and the soln was applied to a column (3.5 × 35 cm) of Dowex 1-X4, 200–400 mesh ($OAc^-$ form). The column was eluted with $H_2O$ at 10 ml/20 min. The eluate was collected in 10 ml fractions which were monitored for **1** using PCAF. Fractions containing **1** (Nos. 24–68) were pooled and evapd *in vacuo* to dryness. Yield: 1.2 g. Hygroscopic, colourless oil. Soluble in $H_2O$ and MeOH, insoluble in $C_6H_6$, $Et_2O$ and EtOAc. In mineral acids this compound was more labile. Found: C, 40.70; H, 5.85; N, 15.11. Calc. for $C_3H_5O_2N \cdot 1/6\ H_2O$: C, 40.00; H, 5.96; N, 15.55%. $^1H$ NMR (100 MHz, $D_2O$, TMS): $\delta$ 4.41 (2H, *t*, $J$ = 9 Hz, $-O-C\underline{H}_2-$), 2.79 (2H, *t*, $J$ = 9 Hz, $-C\underline{H}_2-CO-$). MS *m/e* (rel. int.): 88 (19), 87 [$M^+$] (72), 55 (100), 28 (35), 27 (39). PC: $R_f$ 0.65 (solvent I), 0.77 (solvent II). PCAF (blue); Jaffe (negative); ninhydrin (negative).

*Formation of* **2** *from* **1** *by acid hydrolysis.* Compound **1** (1 g) was dissolved in M HCl (50 ml) and heated at 100° for 1 hr. This soln was evapd *in vacuo* to dryness. The residue was dissolved in $H_2O$ and re-evaporated. This procedure was repeated until all excess HCl had been removed. After standing at 4°, the crystals were dissolved in a min vol. of MeOH and $Et_2O$ was added until the soln became cloudy. Precipitated crystals were further recrystallized in the same way. Yield of **2**: 0.2 g. Colourless needles, mp 150–151°. Found: C, 25.53; H, 5.86; N, 10.00; Cl, 25.15. Calc. for $C_3H_7O_3N \cdot HCl$: C, 25.45; H, 5.69; N, 9.90; Cl, 25.05%. PC: $R_f$ 0.25 (solvent I), 0.59 (solvent II). Jaffe (orange); PCAF (pale blue); ninhydrin (negative). $R_f$, mp and colour reaction of **2** were identical to those of an authentic sample of 3-aminoxypropionic acid HCl which was synthesized by the method of ref. [5].

*Formation of* **1** *from* **2** *with* NaOH. Compound **2** (2–3 mg) was dissolved in 1 drop of $H_2O$ and then 1 drop of 0.5 M NaOH was

added. After standing for 30 min, the reaction mixture was spotted on paper. Both **2** and authentic 3-aminoxypropionic acid HCl, treated as above, showed the same $R_f$ and colour reactions. PC: $R_f$ 0.65 (solvent I), 0.77 (solvent II). PCAF (blue); Jaffe (negative); ninhydrin (negative). These $R_f$ and colour reactions were identical with **1**.

*Determination of 3-isoxazolidone and L-canavanine.* Finely ground lyophilized plant tissue (100 mg) was shaken with 0.1 M HOAc (5 ml) for 5 min and kept for 20 hr at 4°. The mixture was neutralized with 0.1 M $NH_4OH$ (5 ml). After centrifugation, the supernatant was passed through a column (1 × 20 cm) of Amberlite IR-120 ($H^+$ form) and the column washed with $H_2O$ (40 ml). 3-Isoxazolidone was passed through with the effluent and L-canavanine was retained on the column. (i) *3-Isoxazolidone.* The effluent (50 ml) was neutralized at pH 7 with 4% $NH_4OH$ and concd *in vacuo* to dryness. The residue was dissolved in 66 mM KNaPi buffer (pH 7) (5 ml). To the buffer (0.5–3 ml) was added 0.5% PCAF (0.5 ml) and the whole made up to 5 ml with buffer. After 15 min, the blue colour was measured at 670 nm. This colour was stable in light for at least 1 hr. The concn of 3-isoxazolidone was read from a standard curve prepared with a known amount of 3-isoxazolidone. (ii) *L-Canavanine.* L-Canavanine which was retained on the column was eluted with 4% $NH_4OH$. The eluate (50 ml) was concd *in vacuo* at 40° to dryness. The residue was dissolved in the buffer (pH 7) (5 ml). To the buffer (0.5–3 ml) was added 0.5% PCAF (0.5 ml) and the whole was made up to 5 ml with buffer. After 40 min the magenta colour was measured at 522 nm as described in refs. [1–3].

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