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A REAPPRAISAL OF THE FREE AMINO ACIDS IN SEEDS OF *CROTALARIA JUNCEA* (LEGUMINOSAE)

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Key Word Index—*Crotalaria juncea*; Leguminosae; non-protein amino acids; δ-hydroxynorleucine.

Abstract—The seeds of *Crotalaria juncea* have been reported at different times to contain β-hydroxy-*N*-methyl-DL-norvaline and δ-hydroxynorleucine, which are isomers. We detected only one non-protein amino acid in seeds obtained from various sources; it has been isolated and its identity as δ-hydroxynorleucine confirmed.

INTRODUCTION

Crotalaria juncea (Leguminosae) is cultivated in the Indian subcontinent as a source of fibre [1]. The seeds are used medicinally [2] and the green parts are toxic to horses and sheep [3, 4]. Seeds of *C. juncea* have been reported to contain β-hydroxy-*N*-methyl-DL-norvaline [5] and δ-hydroxynorleucine (5-hydroxy-2-amino-hexanoic acid) [6], the last-named also occurring in *C. tetragona* Roxb. [7].

Chromatographic studies in this laboratory revealed the presence of only one major non-protein amino acid in extracts of *C. juncea* seeds obtained from four different sources. This amino acid designated NJ has been isolated and its identity as δ-hydroxynorleucine confirmed.

RESULTS AND DISCUSSION

When seeds of *C. juncea* were extracted with either 70% aqueous ethanol or with methanol (the solvent reported to extract β-hydroxy-*N*-methyl-DL-norvaline [5]) the extracts were found to contain major concentrations of only one non-protein amino acid. This amino acid was isolated by ion-exchange chromatography. The compound formed a chelate with cupric ions showing it to be an α-amino-α-carboxylic acid [8], and it failed to react as an *N*-methyl compound with *p*-nitrobenzoyl chloride [9].

The MS spectrum showed a parent peak at *m/e* 147 (Found: 147.0900; calc. for $C_6H_{13}NO_3$: 147.0895). The base peak at *m/e* 84 indicated the loss of COOH + H_2O from the parent molecule (Found: 84.0811; calc. for $C_5H_{10}N$: 84.0813). A peak at *m/e* 74 (intensity = 63.5%) corresponded to a $CH(NH_2)COOH$ fragment (Found: 74.039; calc. for $C_2H_4NO_2$: 74.0242). There was no peak at *m/e* 89, which would be expected if a $CH(NH—CH_3)COOH$ fragment had been produced.

The PMR spectrum in D_2O showed a doublet δ 1.18 (3H, *d*, *J* = 6 Hz, C-6), attributable to a CH_3 group split by a lone proton, multiplets δ 1.52 (2H, *m*, C-3) and 1.84 (2H, *m*, C-4)) attributable to two CH_2 groups, each split by a CH_2 group and a lone proton (one occurred further down field than the other attributable to its proximity to an OH group), and a multiplet δ 3.81 (2H, *m*, C-2 and C-5) attributable to a CH group split by a CH_3 and a CH_2 group plus a CH group split by a COOH and a CH_2 group. These peaks have the relative intensities 3:2:2:2. Irradiation of the low field multiplet reduced the high field doublet to a singlet, a further indication that the CH_3 signal is split by a lone proton.

The IR spectrum of the amino acid lactone (in EtOH) showed a major peak at 1749 cm^{-1}, which indicated a δ-lactone [10] confirming that the OH and COOH groups of the molecule are separated by a 4-carbon chain. The amino acid is optically inactive and sublimes with decomposition at 228–232° (open bench).

EXPERIMENTAL

Paper chromatography and high voltage paper ionophoresis. Finely ground seed was shaken with 70% EtOH (100 mg/ml) for 65 hr. 120 µl supernatant was subjected to 2D PC on Whatman No. 1 paper by ascent. Solvents used were *n*-BuOH–HOAc–H_2O (12:3:5) followed by PhOH–H_2O (4:1, w/v) in the presence of NH_3 [9]. Samples were also chromatographed on Whatman No. 1 paper by descent in PhOH–EtOH–H_2O (3:1:1, w/v/v) + 1 vol. 0.88 NH_3 added before use and MeOH–H_2O–Py (20:5:1, v/v/v) [9]. High voltage paper ionophoresis was carried out on 30 µl supernatants at pH 1.9 and 3.6 as previously described [11].

Isolation of NJ. C. juncea seeds (6395 g) were ground in a blade-action mill, and extracted with 14 l. 70% EtOH. 1 l. Amberlite IR-120 (H^+) resin sealed in Visking Dialysis Tubing (36/32 inch dia) was suspended in the supernatant. The resin was replaced every 48 hr for 2 months, and the seed material was stirred daily.

The 'loaded' resin was washed with 2 × 1 l. portions of 2 M NH_3, and the washings were pooled and evapd to dryness *in vacuo* at 25°. The solid (212.6 g) was dissolved in 200 ml H_2O and placed on an Amberlite IR-120 (H^+) column (4.5 × 114 cm, bed vol. = 1.82 l.). Neutral and acidic amino acids were displaced with 8.5 l. 2 M Py. The NJ fractions were evapd to dryness *in vacuo* at 25°, and NJ was crystallized from hot EtOH–water. The amino acid was recrystallized until pure (yield = 9.39 g). Seed residue (200 g) was dried and further extracted with 100 ml 95% MeOH for 48 hr. This extract was subjected to 2D PC and HVE at pH 1.9 and 3.6.

Synthesis of lactone. NJ (60 mg) was heated at 70° in 20 ml 2 M HCl for 90 min. The lactone was dehydrated over solid NaOH.

Quantitative determination of δ-hydroxynorleucine. Samples were subjected to HVE on paper at pH 1.9, the papers were developed with cadmium-ninhydrin reagent for 21 hr [12], and the colour intensities were determined at 505 nm after elution of the coloured pigment with MeOH. Values of 1–2% of dry seed wt were obtained.

Seed identification. All *C. juncea* samples used were compared by PC and high voltage paper ionophoresis with 2 accessions for which vouchers are held by the Royal Botanic Gardens, Kew.

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