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2-AMINO-4-CARBOXYPYRIMIDINE IN SEEDS OF *LATHYRUS TINGITANUS*

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Key Word Index—*Lathyrus tingitanus*; Leguminosae; 2-amino-4-carboxypyrimidine; lathyrine; non-protein amino acids; pyrimidine metabolism.

Abstract—2-Amino-4-carboxypyrimidine, earlier identified as a substrate of the enzyme lathyrine synthase, and precursor of the pyrimidine moiety of the non-protein amino acid lathyrine, has been shown to be present in seeds of *Lathyrus tingitanus*. This pyrimidine, the natural occurrence of which has not previously been demonstrated, was obtained by sequential chromatography and electrophoresis, and identified by co-chromatography and co-electrophoresis with an authentic sample. Identification was confirmed by UV-absorption spectrophotometry and FAB-mass spectrometry. Demonstration of the presence of 2-amino-4-carboxypyrimidine in seeds of *L. tingitanus* consolidates previous studies elucidating the pathway of lathyrine biosynthesis.

INTRODUCTION

The non-protein amino acid lathyrine, which has the structure β-(2-aminopyrimidin-4-yl)alanine [1], is produced by various *Lathyrus* species [2–4]. Biosynthesis of this pyrimidinyl amino acid was shown to involve a preformed pyrimidine nucleus originating from the orotate pathway *via* uracil [5]. Subsequent studies with enzymic extracts, obtained from seedlings of *Lathyrus tingitanus*, identified the immediate precursor as 2-amino-4-carboxypyrimidine [6]. The enzyme catalysing its conversion to lathyrine, which involves replacing the 4-carboxyl group with an alanine residue [6], has been partially purified and its properties described [7]. The present report concerns an investigation into, and confirmation of, the natural occurrence of 2-amino-4-carboxypyrimidine in seeds of *L. tingitanus*.

RESULTS AND DISCUSSION

Dry seeds of *L. tingitanus* (250 g) were milled to a fine powder and then homogenized for 3 min in 0.3 M perchloric acid at 0–4° using an electric blender. The homogenate was centrifuged at 12 000 *g* for 20 min at 4° and the supernatant retained. After re-extracting the debris a further three times in the same way, all the supernatants were combined. The pH was adjusted to pH 7.2 with KOH and after standing overnight at 4°, again centrifuged to remove precipitated $KClO_4$. The supernatant was treated with prepared Norit PN.5 charcoal (5 g) and the suspension stirred continuously for 15 hr at 4°. After centrifuging, the charcoal was suspension was centrifuged and the eluate retained. Elution was repeated three times and the combined eluates filtered through a pre-washed Millipore Durapore membrane (por. 5 μM) to remove traces of charcoal. The extract was evaporated to dryness *in vacuo* at 30° and the residue redissolved in 5 ml of aq. ethanol (25% v/v) for chromatography.

Together with reference samples of 2-amino-4-carboxypyrimidine, the extract was chromatographed on paper (Whatman 3MM) as a band in butan-1-ol–HOAc–H_2O (12:3:5). The UV-light absorbing band of R_f 0.38, which corresponded with the reference samples was eluted from 6 replicate sheets and rechromatographed in EtOH–NH_3–H_2O (8:1:1). The band of R_f 0.40, which again corresponded with the reference samples, was eluted. Following concentration *in vacuo* of the eluate from three replicate chromatograms, it was further fractionated by HV electrophoresis on Whatman 3MM paper at pH 2.0 (HCO_2H–HOAc buffer) in a gradient of 60 V cm^{-1}. Reference samples of 2-amino-4-carboxypyrimidine were run alongside. The UV-absorbing band behaving similarly to the reference sample, and which migrated towards the cathode at 2.0 cm hr^{-1}, was eluted for further examination. During subsequent co-chromatography and co-electrophoresis with authentic samples in the two solvent systems and the electrophoretic systems described above, the product behaved identically.

UV-absorption spectrophotometry in aqueous solution showed the extracted compound to exhibit the same spectral characteristics as those of the reference sample (λ 320 nm, λ 261 nm at pH 1 and λ

evaporation *in vacuo* for mass spectrometry. FAB-mass spectrometry of the sample in a 10% glycerol matrix gave prominent peaks at *m/z* (rel. int.) 115.2 (20) $[GroNa]^+$, 140.2 (55) $[MH]^+$, 185.2 (100) $[Gro_2H]^+$, 207.1 (6) $[Gro_2Na]^+$, 232.1 (16) $[MGroH]^+$, 277.1 (14) $[Gro_3H]^+$.

Demonstration of the natural occurrence of 2-amino-4-carboxypyrimidine in seeds of *L. tingitanus* consolidates previous studies in this laboratory [5–7] showing that the compound is converted enzymically into lathyrine and that this is the main biosynthetic route by which lathyrine arises in plants.

EXPERIMENTAL

Materials. Seeds of *Lathyrus tingitanus* were provided by Mr R. Isherwood and Mr M. Roberts of the University of Wales Swansea, Botanic Garden. Before extraction, seeds were finely ground in a microhammer mill (Glen Creston, Stanmore). Norit PN.5 charcoal was prepared for use by boiling with 6 M HCl until the filtrate was colourless, washing with H_2O until free from Cl^- ($AgNO_3$) and air-drying at 140° for 15 hr.

Preparation of 2-amino-4-carboxypyrimidine. The compound was prepared by alkaline $KMnO_4$ oxidation of 2-amino-4-methylpyrimidine as described in ref. [8] and recrystallized from aq. EtOH (needles; mp 267°, decomp.).

Mass spectrometry. Positive ion FABMS spectra were obtained with a VG ZAB-2F mass spectrometer using Xe (8 keV). Samples (3 μl) were in 10% glycerol-H_2O and the accelerating potential was 8 kV.

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