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# δ-ACETYLORNITHINE AS A MAJOR NITROGEN STORAGE COMPOUND IN *BISTORTA BISTORTOIDES*

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**Key Word Index**—*Bistorta bistortoides*; Polygonaceae; American bistort; Alpine plants; nitrogen storage; non-protein amino acids; δ-acetylornithine.

**Abstract**—δ-Acetylornithine was found to vary seasonally and in response to nitrogen availability in the rhizomes of the alpine herb, *Bistorta bistortoides* (Pursh). δ-Acetylornithine accounts for 38% of the free amino acid pool and up to 12% of the total nitrogen in rhizomes. It is concluded that this compound plays an important role in nitrogen storage in this species.

## INTRODUCTION

Although δ-acetylornithine has been found in various plants [1–5] and assumed to have a nitrogen storage role [6], there has been no evidence beyond its occasional high concentrations in seeds and taproots that it is actually utilized as such. In the course of studying luxury consumption (uptake of a nutrient beyond what is required for immediate growth) and storage of nitrogen in the alpine herb, *Bistorta bistortoides* (Pursh), large quantities of a non-protein amino acid were found in the rhizome [7]. Herein we identify this compound as δ-acetylornithine (δ-AO), and provide evidence that this compound is synthesized to accommodate luxury consumption of nitrogen and is stored over winter to provide nitrogen for shoot expansion in the growing season.

## RESULTS AND DISCUSSION

HPLC analysis of the free amino acid fraction from the rhizome gave rise to a peak that was not matched by any protein amino acid. The compound was isolated by PC. Acid hydrolysis of the isolated compound produced an amino acid that was identified by HPLC as ornithine. The identity of the original substance was verified as δ-AO by $^1$H NMR. This compound accounted for 38% of the free amino acid pool in both treatments, and varied seasonally from 4 to 8% of the total rhizome nitrogen in non-fertilized plants, and from 6 to 12% in fertilized plants.

Amino acids in the rhizome and total nitrogen in the leaves and flowers were quantified in plants collected from $NH_4NO_3$-fertilized and non-fertilized control plots on Niwot Ridge (elev. 3600 m) in the Colorado Rocky Mountains throughout the growing season (May–Oct). The fertilized plants had consistently higher concentrations of δ-AO than the non-fertilized plants ($P = 0.008$), and the concentrations varied seasonally, being highest at the beginning and end of the season, and lowest at the middle of the season when shoots were fully expanded ($P = 0.0062$) (Table 1). We conclude that δ-AO is mobilized from the rhizome to support shoot growth at the beginning of the season, and once again accumulates in the rhizome at the end of the season as N is retranslocated from senescing leaves. The excess N taken up by the fertilized plants is stored partly in the form of δ-AO. Arginine is present in rhizomes in roughly equimolar quantities to δ-AO, though no ornithine or urea was detected [7]. This suggests the possibility that, in addition to its role in nitrogen storage, δ-AO could serve to stabilize arginine by inhibiting the activity of arginase (the enzyme that catalyses the degradation of arginine to ornithine and urea). Ornithine has been shown to inhibit arginase from rat liver competitively [8], and arginase from *Bacillus brevis* non-competitively [9]. If the acetyl derivative of this amino acid also has this effect, then its increased stability [10] would make it ideal for this purpose. Alternatively, δ-AO additionally appears to function as a compatible solute in some halophilic bacteria [11].

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Table 1. Concentrations (mmol $kg^{-1}$ dry mass) of $\delta$-AO in rhizomes of N-fertilized and non-fertilized plants at the beginning, middle, and end of the growing season

| Treatment | Beginning (June 1) | Middle (July 6) | End (Sept 3) |
|---|---|---|---|
| Non-fertilized | 30.17 ± 9.02 | 8.48 ± 6.12 | 17.90 ± 2.20 |
| Fertilized | 100.26 ± 34.50 | 22.75 ± 7.67 | 54.94 ± 8.18 |

*Values are means ± standard error. Significance was tested with two way ANOVA, with fertilization treatment and date as class variables ($n = 29$). Five replicate 1 $m^2$ plots were fertilized with biweekly additions of 2 g $NH_4NO_3$ dissolved in 5 l of deionized water. The surrounding meadow served as a control.

## EXPERIMENTAL

The compd was extracted from frozen rhizomes ground in liquid $N_2$ in 80% MeOH, isolated using PC with a solvent system of PhOH–$H_2O$ (4:1, w/w). Crystals were rinsed with $Et_2O$. The presence of urea in the original extracts was tested after complexation with diacetyl monoxime [12]. Acid hydrolysis was performed in 6 M HCl, at 100° for 24 hr. HPLC analysis (1 ml $min^{-1}$; gradient: 0.1 M NaOAc (pH 7.5)–MeOH–THF (180:19:1) to MeOH to $H_2O$ at room temp.; column: 25 cm × 4.6 mm, 5 $\mu$m APEX $C_{18}$; Abs 330 nm after O-phthaldialdehyde derivatization). $^1$H NMR (299.94 MHz, $D_2O$; int. st.: TMS); $\delta$1.37 (2H, *m*, H-4), 1.63 (2H, *m*, H-3), 1.74 (3H, *s*, H-7), 2.97 (2H, *t*, H-5), 3.50 (1H, *m*, H-2).

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