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N^{δ}-BENZOYL-L-ORNITHINE AND N^{δ}-BENZOYL-L-γ-HYDROXYORNITHINE FROM *VICIA PSEUDO-OROBUS*

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Key Word Index—*Vicia pseudo-orobus*; Leguminosae; non-protein amino acids; N^{δ}-benzoyl-L-ornithine, N^{δ}-benzoyl-L-γ-hydroxyornithine.

Abstract—Two new non-protein amino acids, N^{δ}-benzoyl-L-ornithine and N^{δ}-benzoyl-L-γ-hydroxyornithine have been characterized from the seeds of *Vicia pseudo-orobus*.

INTRODUCTION

Many non-protein amino acids have been identified from seeds of *Vicia* species and their distribution in the genus has been shown to be of taxonomic significance [1]. More recently the possible physiological roles of non-protein amino acids as toxins in the natural environment has been considered [2].

We report now the isolation and characterization of N^{δ}-benzoyl-L-ornithine and N^{δ}-benzoyl-L-γ-hydroxyornithine from the seeds of *Vicia pseudo-orobus* Fisch. et Mey. These amino acids have not been reported previously as natural products.

RESULTS AND DISCUSSION

Fractionation of the amino acid fraction from the seeds of *Vicia pseudo-orobus* revealed the presence of several uncommon amino acids, two of which were isolated as pure crystals by successive use of Dowex-50 and cellulose powder.

N^{δ}-*Benzoyl-L-ornithine* (**1**). The yield of the crude crystals was *ca* 0.03% of the dried seeds. Identification was based on the results of elementary analysis and by comparison of UV and IR spectra, the value of $[\alpha]_D$ and chromatography (TLC) with data of an authentic sample.

N^{δ}-*Benzoyl-L-γ-hydroxyornithine* (**2**). The yield was 0.3%. The results of elementary analysis agreed with the formula $C_{12}H_{16}N_2O_4$. The UV spectrum was the same as **1** and acid hydrolysis gave benzoic acid. Although the isolation of γ-hydroxyornithine from the hydrolysis products was unsuccessful, ^{1}H NMR spectroscopy confirmed the structure of the benzoyl derivative. A positive shift of the $[\alpha]$ value in alkaline solution suggests that **2** also belongs to the L series of amino acids. Study is now in progress to determine the configuration of the other asymmetric carbon atom at C-4.

It is of biochemical and physiological interest that benzoyl derivatives of ornithine and hydroxyornithine are accumulated in considerably high concentrations in this species. Paper chromatography showed that the accumulation of **1** and/or **2** occurs in young seedlings as well as mature seed coats, stems and leaves.

Except for hippuric acid (benzoylglycine) and ornithuric acid (dibenzoylornithine), there is no other evidence for the natural occurrence of benzoyl amino acids. Free γ-hydroxyornithine was identified by Bell and Tirimanna from the seeds of *Vicia onobrychoides* L. and *V. unijuga* Braun by paper chromatography and ionophoresis [3]. Lentil seeds (*Lens culinaris* Med.) also contain this amino acid [4]. On the other hand, N^{δ}-acetylornithine is well known from various plant species [5–7]. We surveyed carefully for the possible occurrence of free ornithine and especially γ-hydroxyornithine in the basic amino acid fraction of this species. The results were, however, negative.

EXPERIMENTAL

Plant material. Seeds of *Vicia pseudo-orobus* Fisch. et Mey. were collected in October 1978, in Oshino Village, Yamanashi Prefecture. The identification was made by Professor H. Ohashi of the Tohoku University. The vouchers have been deposited in the Herbarium of the Department of Botany, Faculty of Science, University of Tokyo.

Isolation. The seeds (416 g) were separated from the seed coats, powdered in a mill, soaked ×2 in 80% EtOH for a few days, and the mixture refluxed ×5 each for 30–60 min. The combined extract (30 l.) was passed through a column of Amberlite IR-120B (H^+) (400 ml) and the amino acids were eluted with 2 N NH_4OH (4 l.). The conc. eluate was applied to a column of Dowex 50 × 8 (H^+, 200–400 mesh, 3 × 94 cm). The neutral and acidic amino acids were eluted with 1 M pyridine and concd to a syrup, which was fractionated on a cellulose column (4.8 × 117 cm) with *n*-BuOH–HOAc–H_2O (63:10:27). Pure samples were obtained by recryst. from hot H_2O.

N^{δ}-*Benzoyl-L-ornithine* (**1**), 115 mg, mp 205–213° (decomp.), $[\alpha]_D^{20}$ +22.1° (*c* 1, 3 N HCl), lit. [8] $[\alpha]_D^{10}$ +19.8° (*c* 2.2, 3 N HCl). UV: $\lambda_{max}^{H_2O}$ 228 nm, ε = 11 500. Found: C, 60.09; H, 6.77; N, 11.62. Calc. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86%. The authentic sample was prepared by the partial hydrolysis of ornithuric acid [9], mp 291° (decomp.), UV: $\lambda_{max}^{H_2O}$ 228 nm, ε = 11 500. Found: C, 60.93; H, 7.00; N, 11.80. N^{α}-Benzoyl-L-ornithine, prepared also from ornithuric acid [9], could be distinguished clearly from N^{δ}-isomer by cellulose-TLC, solvents: *iso*-PrOH–HCOOH–H_2O (20:1:5), EtOH–NH_4OH (7:3) and PhOH–H_2O (25:8).

N^{δ}-*Benzoyl-L-γ-hydroxyornithine* (**2**), 1.41 g, mp 205–206° (decomp.). $[\alpha]_D^{20}$ −3.5° (*c* 1.4, H_2O), +6.0 (*c* 2, 0.1 M NaOH). UV: $\lambda_{max}^{H_2O}$ 228 nm, ε = 11 100. Found: C, 57.49; H, 6.57; N, 11.05. $C_{12}H_{16}N_2O_4$ requires: C, 57.13; H, 6.39; N, 11.10%. ^{1}H NMR: δ 7.7 (5 H, *m*, aromatic), 4 (2 H, *m*, 2–CH–), 3.5 (2 H, *d*, $-C^{\delta}H_2-$), 2.1

(2 H, *m*, $-C^{\beta}H_2-$). Hydrolysis: 20 mg of **2** were dissolved in 1 ml 1.5 N HCl and heated in a sealed ampoule at 100° for 21 hr. On cooling crystals separated, mp 120–123°. No depression of mp was observed in a mixture with benzoic acid and IR and UV spectra also agreed with those for benzoic acid.

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VOLATILE COMPONENTS OF SUGAR BEET LEAVES

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; sugar beet leaves; volatile components.

Abstract—Extracts of both young and old sugar beet plants were obtained using a modified Likens and Nickerson apparatus. Constituents were identified by GC/MS, and using selected ion monitoring it was shown that the previously determined phenylacetonitrile was probably not of glucosinolate origin. Some unsaturated aldehydes, alcohols and derivatives (enzymic lipid degradation products) were formed to greater extents by the younger leaves, but otherwise such quantitative differences were relatively few and generally random. An interesting range of chlorinated compounds was obtained only from the older plants; a pesticide origin is suggested.

INTRODUCTION

During a recent examination of sugar beet leaves for constituents possessing auxin-like activity [1], an interesting array of volatile components was observed. These have now been studied in more detail and results are reported here. The previously determined growth substance, phenylacetonitrile [1], could conceivably originate from a glucosinolate precursor, and this would be noteworthy since such occurrence would be rather unexpected in a plant so distant from the Cruciferae. Therefore, a specific search was undertaken in appropriate extracts of sugar beet leaves for other products of glucosinolate degradation, in particular isothiocyanates. The previous study had also shown the nitrile to be present to a greater extent in extracts of young leaves compared with those of mature leaves [1], and indeed this might be expected with a growth-promoting substance. This finding was further tested in this work, and other volatile components were also assessed for this behaviour.

RESULTS AND DISCUSSION

Table 1 lists the components identified in extracts of both young and old sugar beet plants. The relative percentage abundance of each component in a sample is given, together with an assessment of absolute concentration of a fr. wt basis. Where no quantitative data are given in the table this means that the component in question was not detected in that sample. Most constituents were positively identified from their mass spectra, and their spectra agreed with the literature within experimental error (i.e. instrumental variability). All spectra have been adequately reported before so none is given here. Some constituents were only partially characterized and a few remain unidentified.

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