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SHORT REPORTS

ABSOLUTE CONFIGURATION OF N^{δ}-BENZOYL-γ-HYDROXYORNITHINE FROM *VICIA PSEUDO-OROBUS*

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Key Word Index—*Vicia pseudo-orobus*; Leguminosae; seeds; *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine; configuration; synthesis.

Abstract—A pair of diastereoisomers of N^{δ}-benzoyl-γ-hydroxy-L-ornithine was synthesized. By comparison with the two synthetic compounds, the natural amino acid isolated from *Vicia pseudo-orobus* seeds was found to be identical with *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine.

INTRODUCTION

A new acyl amino acid, N^{δ}-benzoyl-γ-hydroxyornithine, was isolated from seeds of *Vicia pseudo-orobus* [1]. It was suggested that the configuration of the α-carbon of this amino acid is L$_S$ in view of its optical property, whereas that of the γ-carbon was undecided.

In the present study, the L$_S$-configuration for the natural product was ascertained by the use of L-amino acid oxidase. A pair of diastereoisomers with the L$_S$-configuration on the α-carbon was synthesized to establish that on the γ-carbon of the natural amino acid. On the basis of the data presented here, we assign the absolute configuration of the natural product as *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine.

RESULTS AND DISCUSSION

Erythro- and *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithines were synthesized by benzoylation of the corresponding γ-hydroxy-L-ornithines [2] according to the usual procedure [3]. Both natural and synthetic compounds were subjected to oxidative deamination by L-amino acid oxidase from Habu snake venom. TLC analysis showed that all three isomers were degraded completely to the corresponding oxidation products.

By comparison with the two synthetic L-isomers, the natural amino acid was found to be identical with *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine with respect to mp, $[\alpha]_D$, amino acid analysis and ^{1}H NMR. Analogously to N^{δ}-acetyl-γ-hydroxyornithines [4], the diastereoisomers of N^{δ}-benzoyl compound were clearly separated from each other using an automatic amino acid analyser with the *threo* isomer eluting faster than the *erythro* isomer. Further evidence was obtained by ^{1}H NMR for the identity of the natural compound as the *threo*-L$_S$-isomer. The spectrum obtained for the *threo* isomer was identical with that previously described for the natural compound and clearly different from that of the *erythro* isomer. The above data indicated that the natural amino acid is *threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine.

It is noteworthy that three kinds (amidino, carbamoyl and benzoyl) of derivatives of γ-hydroxyornithine have been isolated as natural products. γ-Hydroxyarginine isolated from sea cucumber has the *erythro*-L$_S$-configuration [5], whereas N^{δ}-benzoyl-γ-hydroxy-ornithine in this study is the *threo*-L$_S$-isomer. However, the complete configuration of γ-hydroxyarginine [6, 7] and γ-hydroxycitrulline [8] from seeds of other Leguminosae has not been determined so far.

EXPERIMENTAL

General. Mps are uncorr. The elution times of the amino acids on an automatic amino acid analyser are given as R_t. TLC was carried out on Si gel PF_{254} with *n*-BuOH–HOAc–H_2O (4:1:1). Spots were detected under UV.

Natural N^{δ}-benzoyl-γ-hydroxyornithine. This amino acid was isolated from seeds of *V. pseudo-orobus* [1]. R_t: 134 min. Other properties were as reported in ref. [1].

Synthesis of threo- *and* erythro-*N^{δ}-benzoyl-γ-hydroxy-*L$_S$-*ornithines.* Each isomer of γ-hydroxy-L-ornithine · HCl (92 mg) was converted into the corresponding N^{δ}-benzoyl derivative by the method of ref. [3], provided that Na_2CO_3 was used. The products were recrystallized from H_2O–EtOH. *Threo*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine, yield 68 mg (54%), mp 206° (decomp.), $[\alpha]_D^{20}$ +6.0° (0.1 M NaOH; *c* 2). (Found: C, 57.07; H, 6.43; N, 10.98. Calc. for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.11%.) R_t: 134 min. ^{1}H NMR (100 MHz, D_2O): 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*, $-C^{\beta}H_2-$); identical to the natural product. *Erythro*-N^{δ}-benzoyl-γ-hydroxy-L$_S$-ornithine, yield 84 mg (67%), mp 215° (decomp.), $[\alpha]_D^{20}$ −3.0° (0.1 M NaOH; *c* 2). (Found: C, 57.03; H, 6.46; N, 10.98%.) R_t: 141 min. ^{1}H NMR (100 MHz, D_2O): δ 7.7 (5 H, *m*, aromatic), 4.2 (1 H, *m*, $-C^{\gamma}H-$ or $-C^{\alpha}H-$), 3.8 (1 H, *m*, $-C^{\alpha}H-$ or $-C^{\gamma}H-$), 3.5 (2 H, *d*, $-C^{\delta}H_2-$), 1.7–2.3 (2 H, *br m*, $-C^{\beta}H_2-$).

Oxidation with L-amino acid oxidase. To an aq. soln of N^{δ}-benzoyl-γ-hydroxyornithine (2.5 mg/0.5 ml), L-amino acid oxidase (0.26 unit/0.5 ml) in 0.02 M NH_4Ac buffer, pH 7.2, was added and the mixture incubated at 37° for 20 hr with vigorous stirring. The reaction was terminated by acidification (HOAc) and heating. After centrifugation, an aliquot of the supernatant was analysed by TLC. R_f values were 0.52 for benzoyl amino acids and 0.79 for oxidation products. All N^{δ}-benzoyl derivatives (natural and synthetic) were degraded completely. L-Amino acid

oxidase (0.2 unit/mg protein) used was a partially purified prepn from Habu snake (*Trimeresurus flavoviridis*) venom [9] and its stereospecificity was confirmed as being L-directed from the fact that a prepn of N^{δ}-benzoyl-γ-hydroxy-D-ornithine was not oxidized by the enzyme under the conditions described above.

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L-*THREO*-γ-HYDROXYCITRULLINE FROM *VICIA PSEUDO-OROBUS*

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Key Word Index *Vicia pseudo-orobus*; Leguminosae; L-*threo*-γ-hydroxycitrulline.

Abstract—L-*Threo*-γ-hydroxycitrulline was isolated and identified from the seeds of *Vicia pseudo-orobus*. The structure was clarified from the results of elementary analysis, ^{1}H NMR spectrum, enzymic deamination and comparison of the hydrolysis product with the authentic *threo*- and *erythro*-γ-hydroxyornithine.

INTRODUCTION

Previously we reported the isolation and characterization of N^{δ}-benzoyl-L-ornithine and N^{α}-benzoyl-L-γ-hydroxyornithine from the seeds of *Vicia pseudo-orobus* [1]. Subsequently the configuration of the two asymmetric carbon atoms of the latter amino acid was unequivocally determined as L-*threo*-form by comparison with the synthetic samples [2, 3]. The seeds of *V. pseudo-orobus* contain still other ninhydrin-positive substances yielding on hydrolysis γ-hydroxyornithine. One of these proved now to be L-*threo*-γ-hydroxycitrulline.

RESULTS AND DISCUSSION

By the use of cellulose CC we obtained γ-hydroxycitrulline from the neutral and acidic amino acid fraction. The result of elementary analysis was in good agreement with the formula $C_6H_{13}N_3O_4$. It gave a brownish violet coloration with ninhydrin and the colour turned to normal violet with time. Ehrlich reagent yielded a yellow colour just as in the case of citrulline. Though on strong alkaline hydrolysis it gave a mixture of *threo*- and *erythro*-γ-hydroxyornithine, only the former was detected in the mild alkaline hydrolysis products. Also, strong acid yielded only *threo*-γ-hydroxyornithine. Epimerization of γ-hydroxyornithine is known to occur more rapidly in alkaline than in acidic solution [2]. Further, ^{1}H NMR spectrum of the natural γ-hydroxycitrulline was very similar to that of N^{α}-benzoyl-L-*threo*-γ-hydroxy ornithine and different from that of the *erythro*-form [1, 3]. An experiment with L-amino acid oxidase ascertained that our isolate belongs to the series of L-amino acids.

γ-Hydroxycitrulline was first identified as a natural product by Bell and Tirimanna from *Vicia fulgens* and *V. unijuga* but not isolated [4]. The first isolation was carried out by Inatomi *et al.* from young seeds of *Vicia faba* [5]. The stereochemical nature, however, was not studied.

EXPERIMENTAL

Plant. Seeds of *Vicia pseudo-orobus* Fisch. et Mey. were the same as previously reported [1].

Ehrlich reagent. To the solution of 1 g *p*-dimethylaminobenzaldehyde in 30 ml EtOH, 30 ml conc HCl and 180 ml *n*-BuOH were added.

Isolation. Syrup of the neutral and acidic amino acid fraction from the seeds (416 g) [1] was fractionated on a cellulose column (117 × 4.8 cm) using *n*-BuOH–HOAc–H_2O (63:10:27). γ-Hydroxycitrulline and asparagine were displaced together from the column. Asparagine crystallized first on addition with EtOH from the concentrated fractions and was removed. Me_2CO was then added dropwise to the mother liquor and γ-hydroxycitrulline was obtained (530 mg). It was purified by recrystallization × 4 from EtOH–H_2O, mp 186–189° (decomp.) [cf. lit. [5] 185–187° (decomp.)]. $[\alpha]_D^{25}$ +8.0 (2 N HCl; *c* 1) [cf. lit. [5] $[\alpha]_D^{20}$ +4.5° (2 N HCl; *c* 2)]. Found: C, 37.54; H, 7.10; N, 21.83. Calc. for $C_6H_{13}N_3O_4$: C, 37.69; H, 6.85; N, 21.98%. ^{1}H NMR (100 MHz,

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