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SHORT REPORTS

α-(HYDROXYMETHYL)SERINE FROM *VICIA PSEUDO-OROBUS*

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Key Word Index—*Vicia pseudo-orobus*; Leguminosae; seeds; α-(hydroxymethyl)serine; α-alkyl amino acid.

Abstract—α-(Hydroxymethyl)serine was isolated and characterized from the seeds of *Vicia pseudo-orobus* (Leguminosae).

INTRODUCTION

During the isolation of N^δ-benzoyl-L-ornithine, N^δ-benzoyl-*threo*-γ-L-hydroxyornithine [1, 2] and L-*threo*-γ-hydroxycitrulline [3] from the seeds of *Vicia pseudo-orobus* Fish. et Mey., additional unusual ninhydrin-positive substance was detected. We isolated it and identified it with α-(hydroxymethyl)serine, which has not been reported before as a natural product.

RESULTS AND DISCUSSION

Isolation was carried out by a standard column chromatography with cellulose powder and two different solvent systems. Elementary analysis agreed with the formula $C_4H_9NO_4$. Its optical inactivity and resistance to L-amino acid oxidase suggested the structure, α-(hydroxymethyl)serine, immediately. ^{1}H NMR and ^{13}C NMR spectra supported the structure. α-(Hydroxymethyl)serine was synthesized according to Otani and Winitz [4] and the comparison of its IR and NMR spectra, as well as the behaviour on TLC, with those of the natural amino acid, was satisfactory.

Except for a few cyclic amino acids, 1-aminocyclopropane-1-carboxylic acid in pears and apples [5], 3-amino-3-carboxypyrrolidine in Cucurbitaceae [6], and 1-amino-D-proline, as a component of linatine, a vitamin B_6 antagonist from flaxseed [7], alkyl amino acids are encountered very rarely in nature. Thus, α-aminoisobutyric acid in an antibiotic of *Paecilomyces* [8], and in horse muscle [9], and α-methylserine in amicetin [10], have been reported all in bound forms. In the studies on the metabolism of α-methylserine occurring in the antibiotic amicetin, Wilson and Snell [11] isolated a bacterium from soil, that grew with α-methyl-DL-serine or α-(hydroxymethyl)serine as sole source of carbon and nitrogen. Subsequently they prepared from it a stereospecific α-methylserine hydroxymethyltransferase which catalyses the cleavage of (+)-α-methylserine to D-alanine or α-(hydroxymethyl)serine to D-serine, respectively. Its properties suggest a possible mechanism of biosynthesis of (+)-α-methylserine in amicetin. Natural α-(hydroxymethyl)serine might be formed in a similar way.

EXPERIMENTAL

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

Spectroscopy. ^{1}H NMR and ^{13}C NMR were taken by JEOL-GX-400 FT-NMR spectometer with DSS (0.00 ppm) and dioxane (67.40 ppm) as internal standards, respectively.

Isolation. Fraction of the acidic and neutral amino acids prepared from 416 g seeds [1] was fractionated on a cellulose column (117 × 4.8 cm) using *n*-BuOH–HOAc–H_2O (63:10:27). The concomitant asparagine was removed by the second column (145 × 2.0 cm) and *i*-PrOH–HOAc–H_2O (80:4:20). Yield of crude crystals: 460 mg. Recrystallized from EtOH–H_2O, mp 244° (decomp.). Optically inactive. Found: C, 35.12; H, 6.88; N, 10.19. Calc. for $C_4H_9NO_4$: C, 35.55; H, 6.71; N, 10.37%. ^{1}H NMR (400 MHz, D_2O): δ3.77 (2H, *d*, $J = 12.2$ Hz, H-3 and H-4), 3.90 (2H, *d*, $J = 12.2$ Hz, H-3 and H-4). ^{13}C NMR (100 MHz, D_2O): δ173.06 (C-1), 68.10 (C-2), 62.35 (C-3 and C-4).

Synthesis. α-(Hydroxymethyl)serine was synthesized from cupper glycine and formaldehyde according to Otani and Winitz [4]. Yield 38%. Recrystallized from EtOH–H_2O, mp 245° (decomp.). Found: C, 35.23; H, 7.12; N, 10.25; Calc. for $C_4H_9NO_4$: C, 35.55; H, 6.71; N, 10.37%.

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IDENTIFICATION OF THE TOXIC PRINCIPLE OF *CNESTIS GLABRA* AS METHIONINE SULPHOXIMINE

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Key Word Index—*Cnestis glabra*; Connaraceae; neurotoxin; glabrin; structural determination; toxic amino acid; methionine derivative; methionine sulphoximine.

Abstract—Glabrin, the toxic principle of *Cnestis glabra* isolated from root bark, was identified as *S*-(3-amino-3-carboxypropyl)-*S*-methyl sulphoximine (methionine sulphoximine) by spectroscopic and chemical means. The natural occurrence of this toxic methionine derivative is reported for the first time.

INTRODUCTION

The toxic principle of *Cnestis glabra*, a neurotoxic compound occurring in all parts of the plant and temporarily named glabrin, was isolated from root barks in 0.4 % yield [1] and some of its physicochemical and biological properties were studied [1, 2]. We found that glabrin, appearing as a white, solid compound, has a M_r less than 500. It is heat-stable, soluble in water, but not in usual organic solvents. The compound has no aromatic group in its structure (no retention on activated charcoal and no absorption in UV). It reacts positively with ninhydrin and behaves as methionine on an amino acid analyser. These physicochemical properties preclude glabrin from belonging to some classes of well-known toxic natural compounds of low M_r occurring in the plant kingdom, such as alkaloids. Glabrin could be a non-aromatic amino acid, possibly a methionine derivative. This hypothesis was partially supported by the fact that a number of unusual toxic amino acids are well known in higher plants [3, 4]. In the present paper we report the spectral and chemical data which led us to the identification of glabrin as methionine sulphoximine.

RESULTS AND DISCUSSION

Mass spectrometry showed glabrin to have $[M+1]^+$ at m/z 181, in agreement with the formula $C_5H_{12}N_2O_3S$ obtained from high-resolution mass spectrometry. The carbon atoms gave rise to five signals in the ^{13}C NMR spectrum: δ27.18 (t, $J = 130$ Hz), 44.08 (q, $J = 140$ Hz), 54.98 (t, $J = 145$ Hz), 56.00 (d, $J = 145$ Hz), 175.96 (s). The last signal is indicative of a C=O function, probably that of a carboxyl group, as was suggested by the broad absorption between 2250 and 3500 cm^{-1} in the IR spectrum.

The ^{1}H NMR spectrum of glabrin exhibited a one-proton triplet at δ3.89 ($J = 7.5$ Hz), a two-proton multiplet centred at 2.37, a two-proton multiplet centred at 3.43, and finally a methyl singlet at 3.17. This pattern is similar to that of methionine, the conspicuous difference being the chemical shifts of the signals and especially that of the methyl group, which suggested a new environment for the sulphur atom in the natural compound.

No change was observed on an amino acid analyser when performic acid treatment was attempted to oxidize the sulphur atom, thus suggesting a tetracoordinate