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X-ray single-crystal structure of 2(*S*),4(*R*)-4-hydroxyarginine from *Lens culinaris* seeds

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Abstract

A new method was developed to isolate, as the hydrochloride salt, 4-hydroxyarginine from *Lens culinaris* seeds. The structure and absolute configuration of the compound were determined by single-crystal X-ray crystallography and circular dichroism.

Keywords: *Lens culinaris*; Leguminosae; Seeds; X-ray crystal structure; Circular dichroism; 2(*S*),4(*R*)-4-Hydroxyarginine

1. Introduction

Free 4-hydroxyarginine was first isolated from sea cucumber, *Polycheira ruescens* (Fujita, 1959), and sea anemone, *Anthrophopleura japonica* (Makisumi, 1961). More recently, it was identified as a component of polyphenolic proteins in the adhesive plaques of the marine mussel, *Mytilus edulis* (Papov, Diamond, Biemann, & Waite, 1995). Three years after its discovery in animals, Bell and Tirimanna reported the presence of 4-hydroxyarginine in the seeds of 17 species of *Vicia* and isolated the compound, as the lactone, from *Vicia sativa* (Bell & Tirimanna, 1963, 1964). Sulser and Sager (1976) subsequently isolated 4-hydroxyarginine from seed of *Lens culinaris* which, like *V. sativa*, belongs to the tribe *Viciacae* of the Leguminosae. A survey of non-protein amino acids present in the seeds of 310 species representing 29 genera of a second tribe of the Leguminosae, the *Tephosieae*, showed 4-hydroxyarginine to be widely distributed among species of that tribe also (Evans, Fellows, & Bell, 1985).

The absolute configurations of the compounds obtained from *V. sativa* and *L. culinaris* seed were not established at the time of their isolation. However, Evans, Fellows, Janzen, Chambers, and Hider (1985) reported that the electrophoretic mobility of *erythro*-γ-hydroxyarginine (i.e. 2(*S*),4(*R*)-4-hydroxyarginine) isolated from *V. unijuga* was markedly reduced in the presence of borate ions. While studying the guanidino components of extracts of *L. culinaris seed*, we observed a similar phenomenon with the compound previously identified as 4-hydroxyarginine (Sulser & Sager, 1976), suggesting that this compound too was the *erythro* (i.e. 2*S*,4*R*) isomer. The interest that exists in the pharmacology of guanidino compounds with respect to nitric oxide synthesis prompted us to isolate 4-hydroxyarginine from *L. culinaris* seed and to determine the crystal structure and absolute configuration of the compound.

2. Results and discussion

The ^{1}H NMR spectrum of 2(*S*),4(*R*)-4-hydroxyarginine showed a doublet of doublets for the C(2) proton, due to coupling with two non-equivalent C(3) methylene protons. The C(3) methylene protons give two sets of eight-line multiplets at the highest field. The coupling constants are 2.8 Hz (*cis*), 8.1 Hz (*trans*) and 14.8 Hz (*geminal*) protons. The C(4) proton gave an eight-line multiplet by coupling with non-equivalent C(3) and C(5) methylene protons, with two coupling constants (*cis* = 3.4 Hz and *trans* = 7.0 Hz) The C(5) methylene protons appeared as two sets of four-line multiplets with different patterns, again showing different coupling by *cis* (3.7 Hz) and *trans* (7.5 Hz) protons. ^{13}C NMR resonances

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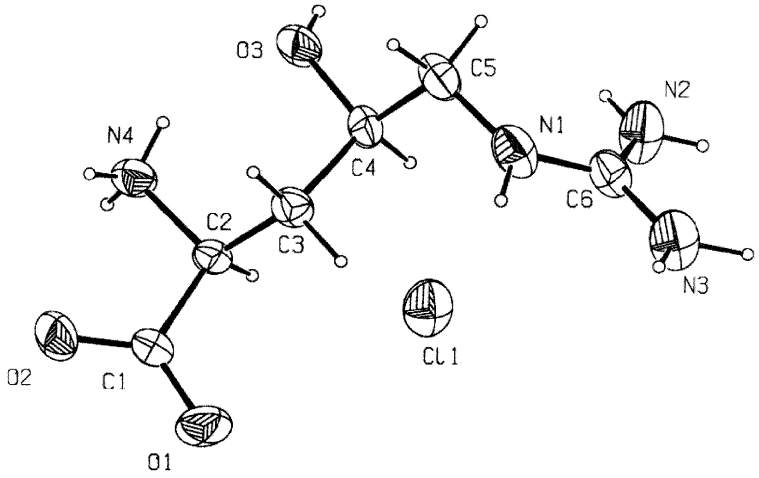

Fig. 1. Structure of 2(*S*),4(*R*)-4-hydroxyarginine·HCl.

appeared as a six-line spectrum corresponding to the six non-equivalent carbon atoms in the molecule. Chemical shift assignments are given in the Section 3.

The X-ray crystal structure was determined using single crystals and was refined by the least-squares method to give a final $R=4.15\%$. The molecular structure is shown in Fig. 1 and the selected bond lengths and angles are given in Table 1.

The bond distance between H_3N^+–C (1.487(5) Å) is close to that reported for *S*-2-amino-3-methylaminopropanoic acid (1.491(4) Å) (Davis, Hursthouse, Motevalli, O'Brien, & Nunn, 1991), *S*-2,4-diaminobutanoic acid (1.494(1) Å) (Hinazumi & Mitsui, 1971), lysine (1.482 Å), ornithine (1.491 Å) (Ueki, Ashida, Sasada, & Kakudo, 1969) and Schiff bases prepared from salicylaldehyde and *S*-2-amino-3-methylaminopropanoic acid (1.476 (3) Å) and *RS*-2,4-diaminobutanoic acid (1.494 (5) Å) (Mahmood, Malik, O'Brien, & Nunn, 1998). The C–C bond distances (1.52–1.53 Å) are almost identical to those in lysine (1.52 Å) and ornithine (1.53 Å), which are usually accepted as standard values. No significant change in C–CO_2 bond distance or angle was noticed. The bond lengths of N(1)–C(6) (1.333(6) Å), N(2)–C(6) (1.314(6) Å) and N(1)–C(6) (1.331(6) Å) are similar to, but slightly shorter than, the usual single bond between nitrogen and carbon atoms (1.45–1.48 Å). The bond distance of C(4)–O(3) (1.421(5) Å) was within the usual range of carbon–oxygen single bonds.

Table 1
Selected bond lengths (Å) and angles (°) for 2(*S*),4(*R*)-4-hydroxyarginine·HCl

O(1)–C(1)	1.244(5)
O(2)–C(1)	1.237(5)
O(3)–C(4)	1.421(5)
N(1)–C(6)	1.333(6)
N(1)–C(5)	1.455(6)
N(2)–C(6)	1.314(6)
N(3)–C(6)	1.331(6)
N(4)–C(2)	1.487(5)
O(3)–C(4)–C(3)	107.7(3)
O(3)–C(4)–C(5)	107.4(3)
C(3)–C(4)–C(5)	111.2(4)
O(3)–C(4)–H(4A)	112.0(2)
C(3)–C(4)–H(4A)	110.0(2)
C(5)–C(4)–H(4A)	109.0(2)
N(2)–C(6)–N(3)	118.9(4)
N(2)–C(6)–N(1)	120.8(4)

The packing diagram (Fig. 2) reveals both intermolecular and intramolecular hydrogen bonds. The most significant intramolecular hydrogen bonding is through C(2)NH_3^+ protons to the C(4)OH oxygen atom and to a deprotonated carboxylate oxygen atom. Intermolecular hydrogen bonding involves the chloride ion of the hydrochloride salt bound to a proton on each terminal nitrogen atom of the guanidino group of one amino acid molecule, the C(5)NH group of a second molecule and the C(2)NH_3^+ of a third. There is also intermolecular hydrogen bonding between carboxylate oxygen atoms and hydrogen atoms on C(4)OH, and terminal guanidino nitrogen atoms.

The specific rotation for *S*-arginine was $[\alpha]_D=+1.23^\circ$ and for 4-hydroxyarginine $[\alpha]_D=+1.37^\circ$. The absolute configuration of 4-hydroxyarginine was confirmed as the *S*-isomer by measurements of circular dichroism.

3. Experimental

3.1. Isolation

Finely ground *L. culinaris* seed (500 g dehusked culinary quality) was defatted by extracting with Me_2CO (0.5 l) and MeOH (0.5 l), each for 48 h in a rotatory shaker

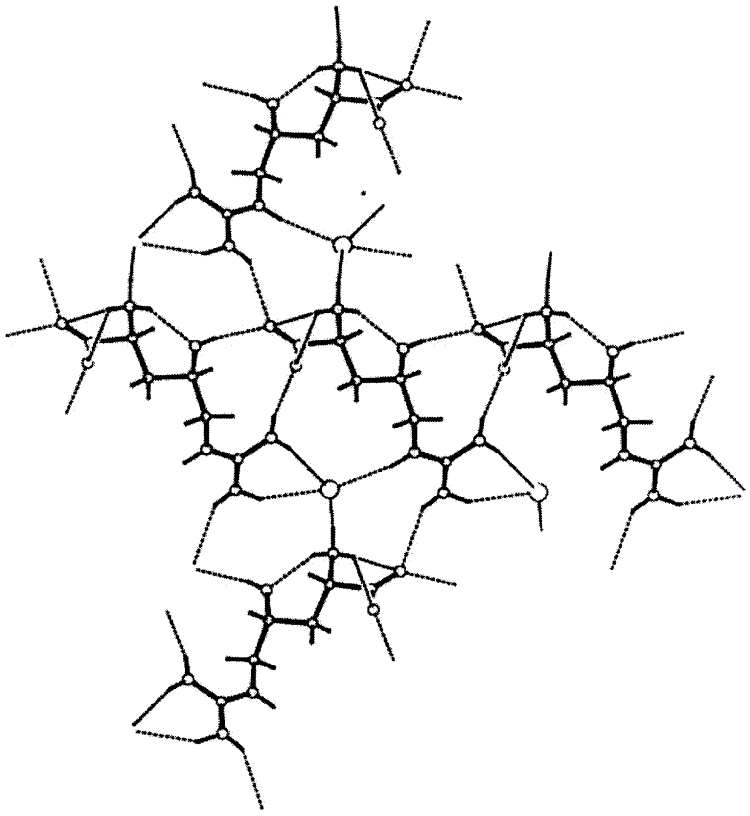

Fig. 2. Packing diagram of 2(*S*),4(*R*)-4-hydroxyarginine·HCl.

at ambient temp. The residue was extracted with 70% (v/v) aq. MeOH (1.5 l) and 50% (v/v) aq. MeOH (1.5 L) over a period of 8 days. The combined MeOH extracts were filtered and concd by rotatory evap. (40°C). The oily residue obtained was dissolved in minimal H_2O and the concentrate passed through a column (5×35 cm) of Dowex-1 (acetate form, 20–50 mesh); the column was washed with H_2O until the effluent was ninhydrin-negative. The effluent and combined washings were passed through a column (7×42 cm) of Amberlite IRC-50 (H^+-form, 100–200 mesh), which was washed with H_2O as before. Basic amino acids were eluted using 2 M NH_4OH soln and Sakaguchi-positive frs were combined and concd by rotatory evap. (40°C) to give an oily residue (2 g). The combined frs were dissolved in minimal H_2O and passed through a column (2×45 cm) of Dowex-50 (NH_4^+-form, 100–200 mesh), which was washed with H_2O (1 l). Amino acids were eluted with 0.1 M NH_4OH soln. Sakaguchi-positive frs were combined and concd by rotatory evap (40°C). The white residue obtained (ca. 0.4 g) was recrystallised as the HCl salt from aq. EtOH. The twice-recrystallised colourless crystals were used for analytical analysis. Microanalysis were carried out by the Imperial College Analytical Service (found for the monohydrochloride salt: C, 32.17; H, 6.51; N, 24.47%; calculated: C, 31.63; H, 7.08; N, 24.71%). Specific rotation and circular dichroism were determined by the EPSRC National Chiroptical Spectroscopy Centre, King's College London.

3.2. X-ray crystallography

The single-crystal method was used. Measurements were made on samples mounted in glass capillaries and intensity data was collected with a CAD4 diffractometer in $\omega/2\theta$ scan mode with graphite-monochromated MoK_α radiation, as described previously (Davis et al., 1991). Absorption corrections were made with the ψ-scan method (North, Phillips, & Mathews, 1968). The unit-cell parameters were determined by a least-squares refinement on diffractometer angles for automatically centered reflections. The structures were solved by the heavy-atom method using the SHELXS program package (Sheldrick, 1986) and refined anisotropically (all non-hydrogen atoms) by a full-matrix least-square on F^2, using the SHELXL-93 program (Sheldrick, 1993). All hydrogen atoms were calculated geometrically (riding model) using the AFIX command of the SHELXL-93 program. The program ORTEP-3 (Farrugia, 1997) was used for drawing molecular structures. Crystal data and details of the intensity measurements and refinements are given in Table 1. Atomic co-ordinates, thermal parameters and bond lengths and angles have been deposited

at the Cambridge Crystallographic Data Center (CCDC).

3.3. NMR

^{1}H NMR were recorded on a Bruker AM 400 pulsed Fourier transform instrument, using D_2O as solvent. ^{13}C NMR (in D_2O) were recorded on the same instrument at 100 MH. ^{1}H chemical shift assignments were δ 1.90 (two sets of d/d/d, 2H, $^{2}J_{H-H}=14.8$ Hz, $^{3}J_{H-H(trans)}=8.1$ Hz, $^{3}J_{H-H(cis)}=2.8$ Hz; C($3H_2$), δ 3.21 (2 sets of d/d, 2H, $^{2}J_{H-H}=14.6$ Hz, $^{3}J_{H-H(trans)}=7.5$ Hz, $^{3}J_{H-H(cis)}=3.7$ Hz); C($5H_2$), 3.75 (d/d, 1H, $^{3}J_{H-H(trans)}=8.0$ Hz, $^{3}J_{H-H}=5.6$ Hz, C(2H), 4.02 (d/d, 1H, $^{3}J_{H-H(trans)}=7.0$ Hz, $^{3}J_{H-H}=3.4$ Hz, C(4H). ^{13}C NMR (D_2O, 100 MHz): δ 177.2 (COO), 60.2 (C4), 71.3 (C6), 56.3 (C2), 49.8 (C5), 37.0 (C3).

3.4. Absolute configuration

Comparative measurements of optical rotation were made of *S*-arginine and the isolated product. The absolute configuration of 4-hydroxyarginine was confirmed by measuring the circular dichroism between 240 and 180 nm. Spectra were broadly similar for both *S*-arginine and 4-hydroxyarginine, with a dominant positive band at 205 nm at pH values >7 for 4-hydroxyarginine (207 nm for *S*-arginine).

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