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ERYTHRO-γ-HYDROXYHOMO-L-ARGININE: AN AMINO ACID FROM SEED OF *LONCHOCARPUS COSTARICENSIS*, AND ITS PREFERENTIAL INTERACTION WITH BORATE

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Key Word Index—*Lonchocarpus costaricensis*; *Lathyrus tingitanus*; Leguminosae; *threo*-γ-hydroxyhomo-L-arginine; *erythro*-γ-hydroxyhomo-L-arginine; *erythro*-γ-hydroxy-L-arginine; non-protein amino acid; borate complex.

Abstract—A new non-protein amino acid, *erythro*-γ-hydroxyhomo-L-arginine has been isolated from seed of *Lonchocarpus costaricensis* by exploiting its property of interacting with borate ions. For structural comparisons, *threo*-γ-hydroxyhomo-L-arginine was isolated from seed of *Lathyrus tingitanus* and *erythro*-γ-hydroxyarginine from *Vicia unijuga* by novel procedures. The reasons for the interaction of borate with the *erythro*- but not the *threo*-forms of these amino acids are discussed.

INTRODUCTION

threo-γ-Hydroxyhomoarginine [1] and *erythro*-γ-hydroxyarginine [2] have been known for some years to occur in the legume tribe Vicieae [3–7]. In a recent chemotaxonomic survey of the tribe Tephrosieae, which is characterized by a number of novel guanidino compounds [8], seed of many species of *Lonchocarpus*, *Tephrosia*, *Millettia* and *Derris* were found to accumulate an unknown guanidino amino acid, designated LGX. It closely resembled *threo*-γ-hydroxyhomoarginine (LTX) in its chromatographic and electrophoretic properties, but reacted somewhat differently with Sakaguchi reagent for guanidines [9] and, in particular, had a greatly reduced mobility on electrophoresis in pH 9.2 borate buffer (Table 1).

This paper describes the isolation of LGX from seed of a *Lonchocarpus* species, its characterization as *erythro*-γ-hydroxyhomoarginine and examines the reasons for its ability to complex with borate.

RESULTS AND DISCUSSION

LGX and LTX both crystallized as the free base and gave a purple colour with ninhydrin. Their paper chromatographic and electrophoretic behaviour was very similar but with the notable exception that, on electrophoresis at pH 9.2 in borate buffer, the mobility of LGX relative to that of LTX was approximately halved. LGX also gave a distinctive colour with Sakaguchi reagent (Table 1). Elemental analysis suggested that LGX, like LTX, was a monohydroxy-substituted homoarginine: 2 LGX·H_2O requires C, 39.4; H, 7.8; N, 27.5%; found C, 39.7; H, 7.7; N, 27.6%; 2 LTX·$3H_2O$ requires C, 36.4; H, 8.2; N, 24.2%; found C, 36.7; H, 7.6; N, 24.4%. The structure of LTX has been established previously as *threo*-γ-hydroxyhomoarginine [1].

In order to investigate the substitution pattern of LGX, the ^1H NMR spectra of both compounds were compared. These were very similar both in deuterated water alone and after addition of deuterohydrochloric acid (Table 2).

Table 1. Colour reactions and rates of migration relative to arginine (HVE and PC)

Amino acid	Colour reaction with Sakaguchi reagent	PC	HVE		
		BAW	pH 1.9	pH 3.6	pH 9.2 (borate)
Arginine	Orange-red	100	100	100	100
LGX	Orange-yellow	95	97	102	46
LTX	Orange	94	98	100	105
VUX	Pink	65	97	99	27
Homoarginine	Orange	123	106	96	119

For abbreviations and other details see Experimental.

Table 2. ^1H NMR spectra of LGX and LTX (60 MHz Varian EM 360 spectrometer with sodium 3-trimethylsilyl-1-propane sulphonate as internal standard)

Solvent	Chemical shift (δ) LGX	LTX	Description		Assignment
D_2O	1.50–2.00	1.50–2.00	*m*, 4H		2 × C–CH_2–C (β, δ)
	3.30	3.30	*t* (*J* = 6.8 Hz),	4H	N–CH_2–C (ε)
	3.10–4.08	3.07–4.08	*m*,		N–CH–CO_2 (α)
					CH–O (γ)
D_2O + DCl	1.57–2.27	1.53–2.27	*m*, 4H		2 × C–CH_2–C (β, δ)
	3.30	3.30	*t*, 2H		N–CH_2–C (ε)
	4.20	4.27	*t* (*J* = 6.1 Hz),	2H	N–CH–CO_2 (α)
	3.73–4.40	3.62–4.13	*m*,		CH–O (γ)

The signals at higher field (less than δ 3) were equal in area to those at lower field, compatible with LGX and LTX being either β-, γ- or δ-hydroxyhomoarginine. In both cases it was possible to exclude δ-hydroxyhomoarginine because the ε-CH_2 group appeared as a triplet not a doublet and β-hydroxyhomoarginine because on irradiation of the higher field signals, the α-CH group appeared as a singlet not a doublet. Thus, both LGX and LTX appeared to be γ-hydroxyhomoarginine. However, there are differences between the two spectra, for example in the contour of the higher field multiplet and, thus, the most probable interpretation is that they are stereochemical isomers of γ-hydroxyhomoarginine. Since the structure of LTX is known, LGX was tentatively identified as *erythro*-γ-hydroxyhomoarginine.

The reduced mobility of LGX in borate suggests that it forms a complex with borate anions which decreases its net charge. This would also account for it eluting ahead of arginine from ion-exchange resin in the presence of borate (see isolation procedure). Complex formation was confirmed by CD studies which showed that the ellipticity, associated with LGX but not LTX, in the 190–240 nm region was decreased in the presence of borate (Fig. 1).

γ-Hydroxy acids, hydroxy amines and α-amino acids are all capable of forming complexes with borate [10]. It seems probable, therefore, that given the correct stereochemistry γ-hydroxy-α-amino acids could act as tridentate ligands with a resulting higher affinity than bidentate ligands. Both *threo*- and *erythro*-L-hydroxy-α-amino acids could theoretically exist in two staggered conformations capable of tridentate co-ordination (Fig. 2). However, the *threo*-isomer in conformation II experiences appreciable steric interaction between the δ-CH_2 and the α-amino function and, in conformation I, hydrogen bonding is so highly favoured due to the favourable stereochemistry that the amino function is unlikely to co-ordinate preferentially to boron. The *erythro*-isomer in conformation IV experiences steric constraint between the δ-CH_2 and the CO_2^- and even if this structure were to exist the intramolecular hydrogen bond would render the amino function less likely to act as a ligand for boron. In contrast to I, II and IV, conformation III experiences no serious steric constraints and no intramolecular hydrogen bonds involving the amino function. The stereochemical arrangement of the hydroxyl, carboxylate and amino functions in this conformation is similar to that of the cyclitols [11] and, thus, is likely to form a stable complex with borate. These observations support the assignment of *erythro*-γ-hydroxyhomoarginine to LGX and *threo*-γ-hydroxyhomoarginine to LTX.

In order to confirm this preferred stereochemical interaction, *erythro*-γ-hydroxyarginine isolated from *Vicia unijuga* [6] was subjected to electrophoresis and CD analysis in the presence and absence of borate anions at

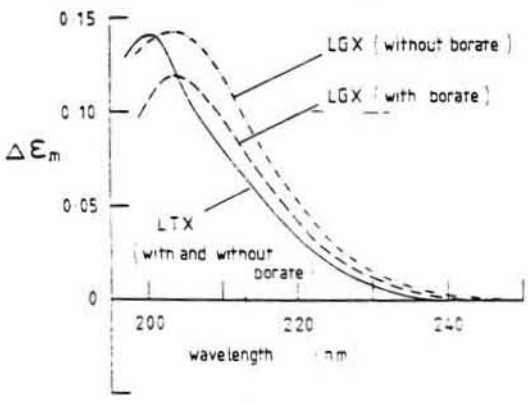

Fig. 1. CD spectrum observed for LTX and LGX with and without the presence of borate.

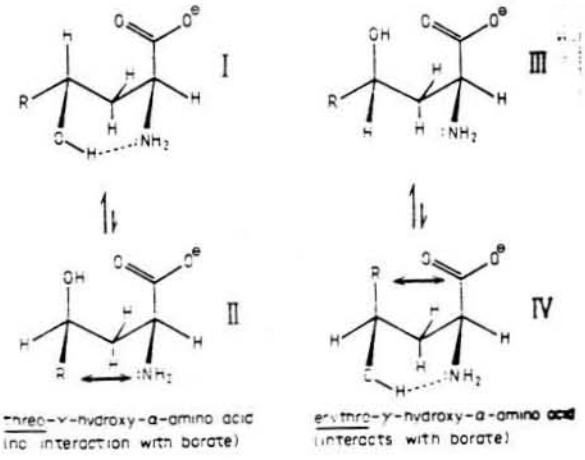

Fig. 2. Theoretical staggered conformations capable of tridentate co-ordination.

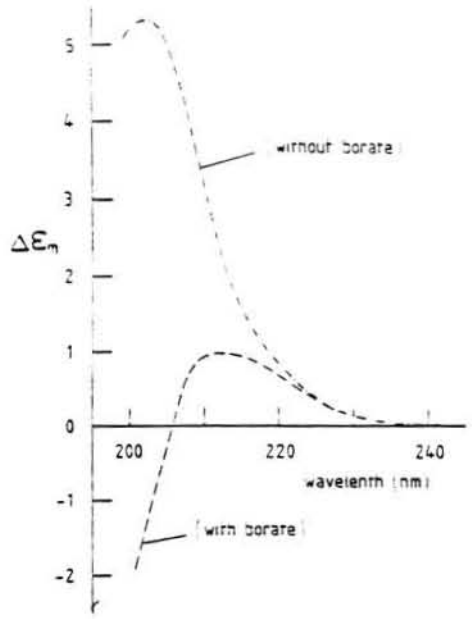

Fig. 3. CD spectrum observed for *erythro*-γ-hydroxyarginine with and without the presence of borate.

pH 9.2. As was observed with LGX, *erythro*-γ-hydroxyarginine possesses a markedly reduced electrophoretic mobility in the presence of borate (Table 1). Borate also induces a large change in the CD spectrum of this diastereoisomer (Fig. 3), thus adding further weight to the stereochemical assignments proposed for the two isomeric γ-hydroxyhomoarginines. The reason for the much larger borate-induced difference in the CD of *erythro*-γ-hydroxyarginine, while similar relative reductions in ionophoretic mobilities are obtained for both *erythro*-γ-hydroxyarginine and LGX, is not immediately obvious. Clearly, a borate complex is obtained in both cases in complete contrast to the situation with LTX. The most probable explanation is associated with the difference in the borate–guanidine intramolecular distances of the two complexes. Their close proximity in γ-hydroxyarginine will permit the formation of an intramolecular salt link, thus largely restricting the motion of the guanidine function. Such restrictions generate large shifts in CD spectra. An analogous salt link, should it form in LGX, might be expected to be weaker and, therefore, less likely to restrict the mobility of the guanidine function.

To date there are no reports of LTX occurring outside the tribe Vicieae and none of LGX outside the Tephrosieae [8]. This mutually exclusive distribution may have some bearing on the biosynthesis of the pyrimidinyl amino acids lathyrine (exclusive to the Vicieae [12]) and tetrahydrolathyrine (exclusive to the Tephrosieae [8]). No studies of the biosynthesis of tetrahydrolathyrine have yet been attempted but there are some conflicting reports as to whether *threo*-γ-hydroxyhomoarginine or orotate is the precursor of lathyrine [4, 13, 14]. In future, investigations of the metabolism of *erythro*-γ-hydroxyhomoarginine should add to our understanding of the biosynthesis of both pyrimidinyl compounds.

EXPERIMENTAL

Isolation of erythro-*γ-hydroxyhomoarginine (LGX).* Mature seed of *Lonchocarpus costaricensis* (Donn. Smith) Pittier was collected in Santa Rosa National Park, Costa Rica, in March 1983.

Finely ground seed (150 g) was defatted with Me_2CO and extracted with 4 × 500 ml 70% aq. MeOH. Pooled filtered MeOH extracts were applied to a column of Amberlite CG-50 (20 × 4.5 cm, NH_4^+ form) equilibrated in 50% aq. MeOH. After washing with 50% aq. MeOH (500 ml) and H_2O (500 ml), elution with 0.25 M NH_4OH (400 ml) gave the basic amino acids. LGX co-eluted with Arg but was thereby separated from tetrahydrolathyrine [15]. The fractions containing LGX and Arg were pooled, evaporated to dryness and the residue dissolved in H_2O (15 ml). This soln was applied to Amberlite CG-50 (30 × 1.5 cm, NH_4^+ form) pre-equilibrated in borate buffer (0.05 M H_3BO_3 adjusted to pH 9.0 with NH_4OH). Passage of the same buffer (200 ml) caused LGX to elute ahead of and quite separately from Arg. Fractions containing LGX were pooled and applied to Amberlite CG-50 (10 × 1 cm, H^+ form). After washing with H_2O (100 ml) to remove borate, LGX was displaced with 0.5 M NH_4OH (50 ml) and evaporated to dryness. The residue was dissolved in 10 ml H_2O and applied to Amberlite CG-400 (15 × 1 cm, OH^- form). LGX was removed with H_2O, lyophilized and recrystallized from H_2O–EtOH. Yield 985 mg.

Isolation of threo-*γ-hydroxyhomoarginine (LTX).* Seed of *Lathyrus tingitanus* L. was obtained from the Royal Botanic Gardens, Kew (Accession No. 060-78-000574 originally collected in southern Spain).

Finely ground seed (120 g) was defatted with Me_2CO and extracted with 4 × 500 ml 70% aq. MeOH. Pooled filtered extracts were applied to Amberlite CG-120 (35 × 2 cm 100–200 mesh, NH_4^+ form) equilibrated in 50% aq. MeOH. After washing with 50% aq. MeOH (500 ml) and H_2O (500 ml), LTX was displaced with 0.5 M NH_4OH (125 ml) ahead of and separate from contaminating homoarginine. Fractions containing LTX were pooled, reduced in vol. to 15 ml under red. pres. and applied to Amberlite CG-50 (10 × 1 cm, NH_4^+ form). LTX was displaced with 0.25 M NH_4OH (40 ml), evaporated to dryness, dissolved in 10 ml H_2O and applied to Amberlite CG-400 (5 × 1 cm, OH^- form). Washing with H_2O removed LTX which was lyophilized and recrystallized from H_2O–MeOH–EtOH. Yield 590 mg.

Isolation of erythro-*γ-hydroxyarginine (VUX).* Seed of *Vigna unijuga* A. Braun was obtained from the Royal Botanic Gardens, Kew (Accession No. 549-82-06275 originally collected in South Korea).

Finely ground seed (100 g) was defatted with Me_2CO and extracted with 3 × 500 ml 70% aq. MeOH. Pooled filtered extracts were applied to Amberlite CG-400 (30 × 1.5 cm, 100–200 mesh, OH^- form) equilibrated in 50% aq. MeOH. Washing with 50% aq. MeOH (200 ml) removed VUX ahead of and separate from contaminating Arg and homoarginine. Fractions containing VUX were pooled, evaporated to dryness, dissolved in 10 ml H_2O and applied to Amberlite CG-50 (10 × 1 cm, NH_4^+ form). After washing with H_2O (100 ml) VUX was displaced with 0.2 M NH_4OH (100 ml). NH_3 was removed under red. pres. and VUX lyophilized and recrystallized from H_2O–MeOH–EtOH. Yield 465 mg.

Chromatography and electrophoresis. Descending PC on Whatman No. 1 paper with *n*-BuOH–HOAc–H_2O (12:3:5) (BAW).

High voltage paper electrophoresis (HVE), Whatman No. 1 paper at 70 V/cm for 30 min. Buffers used routinely were pH 1.9 and 3.6 [16] and pH 9.2 borate (19 g $Na_2B_4O_7 \cdot 10H_2O$ per l.).

Ninhydrin and Sakaguchi location reagents were used as in ref. [9].

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REFERENCES

1. Fujita, Y., Koilonitsen, J. and Witkop, B. (1965) *J. Am. Chem. Soc.* **87**, 2030.
2. Fujita, Y. (1960) *Bull. Chem. Soc. Jpn.* **33**, 1379.
3. Bell, E. A. (1963) *Nature (London)* **199**, 70.
4. Bell, E. A. (1964) *Biochem. J.* **91**, 358.
5. Smith, T. A. and Best, G. R. (1976) *Phytochemistry* **15**, 1565.
6. Bell, E. A. and Tirimanna, A. S. L. (1964) *Biochem. J.* **91**, 356.
7. Suiser, H. and Stute, R. (1974) *Lebensmitt. Wiss. Technol.* **7**, 322.
8. Evans, S. V., Fellows, L. E. and Bell, E. A. (1985) *Biochem. Syst. Ecol.* (in press).
9. Smith, I. (1969) in *Chromatographic and Electrophoretic Techniques*, Vol. I, p. 121. Heinemann-Interscience, London.
10. Steinberg, H. (1964) in *Organoboron Chemistry*, John Wiley, New York.
11. Angyril, J. J. and McHugh, D. J. (1957) *J. Chem. Soc.* **1423**.
12. Bell, E. A. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. and Turner, B. L., eds.) p. 179. Academic Press, London.
13. Hider, R. C. and Jonn, D. I. (1973) *Phytochemistry* **12**, 119.
14. Brown, E. G. and Al-Baidawi, N. F. (1977) *Biochem. J.* **164**, 589.
15. Fellows, L. E., Bell, E. A., Lee, T. S. and Janzen, D. H. (1979) *Phytochemistry*, **18**, 1333.
16. Bell, E. A. and Tirimanna, A. S. L. (1965) *Biochem. J.* **97**, 104.