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3-[2-AMINO-2-IMIDAZOLIN-4(5)-YL]ALANINE (ENDURACIDIDINE) AND 2-[2-AMINO-2-IMIDAZOLIN-4(5)-YL] ACETIC ACID IN SEEDS OF *LONCHOCARPUS SERICEUS*

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Key Word Index—*Lonchocarpus sericeus*; Leguminosae; 2-(2-amino-2-imidazolin-4(5)-yl)acetic acid; 3-(2-amino 2-imidazolin-4(5)-yl) alanine; enduracididine; isolation; MS; PMR; ^{13}C-NMR.

Abstract—3-[2-Amino-2-imidazolin-4(5)-yl]alanine (enduracididine) and 2-[2-amino-2-imidazolin-4(5)-yl] acetic acid have been isolated from seeds of *Lonchocarpus sericeus*. The concentration of each compound was *ca* 0.5% of the fresh seed weight.

INTRODUCTION

The legume *Lonchocarpus sericeus* (Poir), H.B. and K., is a native of the West Indies and tropical America, but is also found in tropical West Africa extending from Senegal to Angola. Various parts of the plant are reported to have insecticidal or piscicidal properties, and bark extracts are used to treat parasitic skin infections. The fruit is considered to be poisonous [1]. The seeds were examined in our laboratory as part of a programme concerned with the distribution of potentially physiologically active compounds in the Leguminosae. Preliminary screening by PC revealed the presence of unidentified compounds designated B (basic) and N (neutral), each giving a characteristic deep blue–purple colour with pentacyanoaquoferriate (PCF), a reagent known to give colours with some aromatic amines [2] and guanidine derivatives [3]. This paper describes the isolation and purification of both B and N, and their characterization respectively as 3-[2-amino-2-imidazolin-4(5)-yl]alanine, a non-protein amino acid previously reported as a component of the peptide antibiotics enduracidin [4] and minosaminomycin [5] and given the trivial name enduracididine, and 2-[2-amino-2-imidazolin-4(5)-yl]acetic acid.

RESULTS AND DISCUSSION

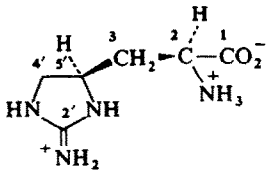
On the basis of elementary analysis, NMR, IR, MS, and chemical reactions, the structures of B and N were deduced to be as shown in Scheme 1. Both N and B (as the monohydrochloride) crystallized as colourless needles. B migrated with arginine on paper ionophoresis at pH 1.9 and 3.6. N moved slower than B at pH 1.9 and was uncharged at 3.6. B reacted as an α-amino acid [6] and both could be readily detected (limit of sensitivity *ca* 1 μg) on paper with the PCF reagent. The possibility that both were derivatives of 2-amino-2-imidazoline was suggested by our observation that if synthetic 2-aminoimidazole [7] is reduced with H_2 and Pt black to the 4,5-dihydro-derivative (2-amino-2-imidazoline), the latter gives a deep blue–purple colour with PCF reagent almost identical to that given by B and N. The ^{13}C and ^{1}H NMR spectra of both compounds (see Experimental) showed the presence of a common cyclic structure. Less easily explained were the dimers seen in the field desorption MS of both B and N which do not appear to be the result of covalent bonding, since the molecules would be unlikely to break in a symmetrical manner. Moreover, any covalent dimer involving the carboxylate function would destroy the free rotation of the side chain about carbon 5′ of the imidazoline ring, a property implicit in the NMR spectra of both compounds. A more likely explanation is that both B and N form a dimer ion under the conditions of field desorption.

The stereochemistry of B is identical to that of enduracididine [4] as shown by PMR and optical rotation. The absolute configuration of enduracididine has recently been shown to be 2*S*, 5′*R* (Scheme 1) [8]. The stereochemistry of the C5′ carbon in N is assumed to be identical to that in B.

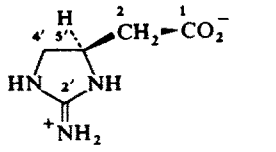
To our knowledge this is the first report of the occurrence of 2-[2-amino-2-imidazolin-4(5)-yl]acetic acid in nature, and the first report of enduracididine as a free amino acid. It is noteworthy that the 2-amino-2-imidazoline nucleus is found linked to a monoterpene in the alkaloid chaksine, isolated from *Cassia absus* [9], and that 2-aminoimidazole has recently been isolated from the seeds of *Mundulea sericea* [10]. That γ-hydroxyarginine occurs with B and N in the seeds of *L. sericeus* suggests that B is formed from γ-hydroxyarginine by internal cyclization and elimination of a molecule of water, in a manner analagous to the postulated internal cyclization of γ-hydroxyhomoarginine prior to the formation of lathyrine in *Lathyrus tingitanus* [11]. N could then be formed from B by deamination and decarboxylation.

The discovery of two 2-amino-2-imidazoline derivatives adds to the growing list of 'unusual' nitrogen-rich low MW compounds in seeds which may act both as nitrogen stores and as antimetabolites in potential predators [12]. Since both B and N are possible analogues of several intermediates of primary biochemical pathways, they are of potential pharmacological interest. There is

currently much interest in the pharmacology of imidazoline derivatives; e.g. derivatives of 2-imidazoline are known for their antihypertensive [13] and antidiuretic effects [14].



(B)



(N)

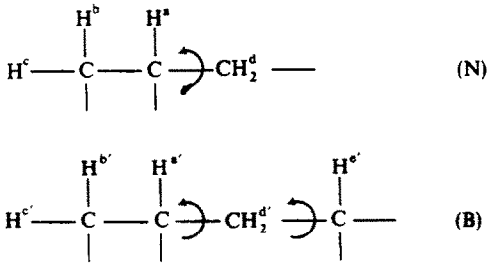
Scheme 1.

EXPERIMENTAL

Isolation of N and B. (Column effluents were monitored throughout for N and B using PCF reagent). (i) Ground seed (375 g) was extracted for 17 hr in a Soxhlet apparatus with CH_2Cl_2 (1 l.), and the extract discarded. The seed powder was shaken for 48 hr with 70% EtOH, (5 l.). After filtering, the extract was applied directly to a column (20 × 45 cm) of anion exchange resin (Dowex 2 × 8) in the OH^- form at 5 ml/min. Both N and B passed through with the effluent which was fed directly onto a column (20 × 4.5 cm) of cation-exchange resin (Amberlite CG-50) in the H^+ form at 5 ml/min. B was retained on the column and N passed through with the effluent, from which it was subsequently isolated, (see iii). (ii) The Amberlite CG-50 column was washed with 70% EtOH, (0.5 l.) and H_2O, (2 l.), and B was eluted with 1 M HOAc (1.2 l.). This eluate was concd to 100 ml in a rotary evaporator. The concentrate (pH 5) was applied to a column (15 × 4.5 cm) of cation-exchange resin (Dowex 50 × 8) in the H^+ form. The column was washed with H_2O, (50 ml), 70% EtOH, (0.5 l.), and finally H_2O (0.5 l.) before B was eluted with 2 M NH_4OH, (0.5 l.). The eluate was concd *in vacuo* at 50° leaving a yellow oil. This oil contained B together with traces of arginine and γ-hydroxyarginine which were identified by their R_f values and reactions with Sakaguchi's reagent on paper [15]. The oil was dissolved in H_2O, (10 ml), and applied to a column (25 × 1 cm) of Amberlite CG-50, in the NH_4^+ form. The column was washed with H_2O, (20 ml). On passing 0.2 M NH_4OH (1 ml/min) through the washed column, B was eluted free of Arg and γ-OH Arg in the first 20–25 ml. After removal of NH_3 under red. pres. B was dissolved in H_2O and the pH adjusted to 3 with HCl. This soln was taken to dryness, the residue suspended in a minimum of warm MeOH, filtered, and crystallization was achieved by adding EtOH and cooling at 4°. The salt was recrystallized twice. Yield. 0.6 g. (iii) The effluent containing N (from step (i)) was fed directly onto a column (20 × 4.5 cm) of Dowex 50W-x8, 20–50 mesh, in the H^- form at 5 ml/min. After washing with 70% EtOH, (0.5 l.), and H_2O, (1 l.), N was eluted with 2 M NH_4OH. After removal of NH_3 under red. pres., a yellow oil remained which was dissolved in a minimum vol. of MeOH and filtered. EtOH–Et_2O, (1:1) was added until the soln became cloudy and crystals separated on standing at 4° which were recrystallized in the same way. Yield: 0.7 g.

PC and high voltage ionophoresis. Descending PC using solvents *n*-BuOH–HOAc–H_2O (12:3:5), (BuAc), and 80% PhOH–EtOH–H_2O–aq.NH_3 (18 M) (120:40:10:1), (Phe Eth Am) HVE using buffers of pH 1.9 and 3.6 as described in [15]. Ninhydrin, PCF and Sakaguchi location reagents were used as in [3].

Analysis of N (see Fig. 1). Found: C, 41.55; H, 6.44; N, 28.92; Calc. for $C_5H_9O_2N_3$: C, 41.95; H, 6.29; N, 29.37%. Field desorption MS (70°) showed two major ions at *m/e* 287 (2M + 1), and *m/e* 144, (M + 1). The percentage of dimer in the spectrum (see Discussion) decreased from almost 100% to zero as the source temp. was raised. Both the 2 M + 1 ion and the M + 1 ion were accompanied by traces of ions at *m/e* 243 and *m/e* 100 respectively, indicating loss of CO_2 and hence the presence of a carboxyl group. IR (solid, nujol mull) showed sharp $\rangle C{=}O$ vibrations at 1698 cm^{-1} (saturated aliphatic carboxylic acid) and broad NH^+ vibration at 3000 cm^{-1}. ^{13}C NMR, (D_2O soln, TMS int. stand.) revealed 5 carbon atoms; 3 aliphatic at 43.7, 48.7 and 53.7 ppm, assigned respectively to C2, C4′ and C5′, one adjacent to heteroatoms at 147.5 ppm (C2′) and one at 180 ppm (carbonyl, C1), Application of off resonance technique confirmed that C2 and C4′ are methylene, C5′ is methine, and C1 and C2′ are not attached to protons. PMR (100 mHz, D_2O soln, TMS int. stand.) revealed proton (a), octuplet centred at 4.3 ppm; proton (b), *t* centred at 3.75 ppm; proton (c) *q* centred at 3.3 ppm; protons (d), 2(2 protons), *d* centred at 2.5 ppm (Scheme 2). Calculated $J_{ad} = J_{ac} = 7$Hz; $J_{bc} = J_{ab} = 10$ Hz. Since $J_{ab} = J_{bc}$, proton (b) observed as triplet. Application of spin decoupling confirmed the structure given.



(N)

(B)

Scheme 2.

Analysis of B (see Scheme 1). (i) Found: C, 34.42; H, 6.56; N, 26.85; Cl, 16.54. Calc. for $C_6H_{12}O_2N_4 \cdot HCl$: C, 34.53; H, 6.24; N, 26.86; Cl, 17.03%. (ii) field desorption MS (70°) showed 2 major ions at *m/e* 345, (2 M + 1), and *m/e* 173, (M + 1), indicating dimer formation as for N (above). (iii) IR analysis (solid, nujol mull) showed sharp $\rangle C{=}O$ vibration at 1680 cm^{-1} (similar to that of arginine) and broad NH^+ vibration centred on 3100 cm^{-1}. (iv) ^{13}C NMR (D_2O soln, TMS int. stand.) revealed 5 carbon atoms; 4 aliphatic at 38, 48.7, 53.7 and 54 ppm, assigned respectively to C3, C4′, C5′ and C2, and one adjacent to heteroatoms at 147.5 ppm, (C2′). The carboxylate carbon was not observed. (v) PMR, (100 mHz, D_2O soln, TMS int. stand.) revealed protons (a′) and (e′), (2 protons), complex splitting pattern centred at 4.25 ppm; proton (b′), *q* centred at 3.75 ppm; proton (c′), *q* centred at 3.35 ppm; protons (d′), (2 protons), *t* centred at 2.1 ppm; protons (a′) and (e′) could not be resolved. Calculated $J_{a'b'} \neq J_{b'c'}$, ($J_{a'b'} = 9$ Hz, $J_{b'c'} = 10$ Hz), (indicating a slight difference in the planarity of the rings of B and N), hence both proton (b′), (coupled to (a′) and (c′)) and proton (c′) (coupled to (a′) and (b′)) are observed as quartets (Scheme 2). This spectrum is almost identical to that reported for enduracididine monoxalate [4]; a shift of the proton (e′) may arise from the different pH values used. However, since the coupling constants and the relative positions of protons (a′), (b′), (c′) and (d′) are identical,

Table 1. R_f values and colour reactions of B, N and arginine

	R_f BuAc	R_f Phe Eth Am	Ninhydrin	PCF	Sakaguchi
B	13	64	+	+	–
N	40	75	–	+	–
Arg	13	58	+	–	+

it is certain that B is the same stereoisomer (2*S*, 5′*R*) as enduracidine. This was confirmed by the specific rotation of B + 62° in 0.1 M HCl, that of enduracididine being reported as + 63.3° [4].

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REFERENCES

1. Irvine, F. R. (1961) *Woody Plants of Ghana*. Cambridge University Press, London.
2. Feigl, F. (1958) *Spot Tests in Organic Analysis*. Elsevier, London.
3. Smith, I. (1960) *Chromatographic and Electrophoretic Techniques*. Heinemann-Interscience, London.
4. Horii, S. and Kameda, Y. (1968) *J. Antibiotics* **21**, 665.
5. Iinuma, K., Kondo, S., Maeda, K. and Umezawa, H. (1975) *J. Antibiotics* **28**, 613.
6. Larsen, P. O. and Kjaer, A. (1960) *Biochim. Biophys. Acta* **38**, 148.
7. Storey, B. T., Sullivan, W. W. and Moyer, C. L. (1964) *J. Org. Chem.* **29**, 3118.
8. Tsuji, S., Kusomoto, S. and Shiba, T. (1975) *Chem. Letters* 1281.
9. Weisner, K., Valenta, Z., Hurlbert, B. S., Bickelhaupt, F. and Fowler, L. R. (1958) *J. Am. Chem. Soc.* **80**, 1521.
10. Fellows, L. E., Bell, E. A. and King, G. S. (1977) *Phytochemistry* **16**, 1399.
11. Bell, E. A. and Przybylska, J. (1965) *Biochem. J.* **94**, 35p.
12. Bell, E. A. (1976) *FEBS Letters* **64**, 29.
13. Claxton, I., Palfreymann, M. G., Poyser, R. H. and Whiting, L. J. (1976) *European J. Pharmacol.* **37**, 179.
14. Deitchmann, D. and Gomoll, A. W. (1973) *Proc. Soc. Exp. Biol. Med.* **144**, 203.
15. Bell, E. A. and Tirimanna, A. S. L. (1964) *Biochem. J.* **91**, 356.