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TETRAHYDROLATHYRINE: A NEW AMINO ACID FROM SEEDS OF *LONCHOCARPUS COSTARICENSIS*

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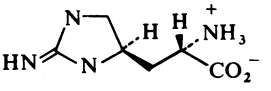
Key Word Index—*Lonchocarpus costaricensis*; Leguminosae; tetrahydrolathyrine; 2(*S*)-3(2-amino-1,4,5,6-tetrahydropyrimidin-4-yl)alanine; non-protein amino acid.

Abstract—A new non-protein amino acid, tetrahydrolathyrine (2(*S*)-3(2-amino-1,4,5,6-tetrahydropyrimidin-4-yl)alanine), has been isolated from seeds of *Lonchocarpus costaricensis*.

INTRODUCTION

Seeds of *Lonchocarpus costaricensis* (Donn. Smith), Pittier were obtained from the mature newly dehisced fruits of healthy adult trees growing in the deciduous forest in Santa Rosa National Park, Guanacaste Province, Costa Rica. They were mature, dormant and viable and were examined in our laboratories as part of a programme concerned with the distribution of potentially physiologically active compounds in the Leguminosae.

It has been reported [1] that of 28 New World species of *Lonchocarpus* examined, the seeds of 20 accumulate the non-protein amino acid 3(2-amino-2-imidazolin-4-yl)alanine (enduracididine) (**1**). *L. costaricensis was*



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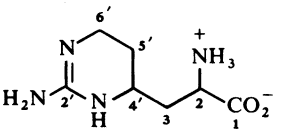
found to accumulate no enduracididine, but did contain *ca* 1% of an unknown basic amino acid designated LCX which resembled enduracididine in its behaviour when subjected to paper ionophoresis and ion exchange chromatography. This compound failed, however, to give the blue-purple colour with pentacyanoaquoferriate reagent (PCF) [2] which is characteristic of enduracididine. This paper describes the isolation and characterization of LCX as tetrahydrolathyrine.

RESULTS AND DISCUSSION

LCX crystallized in colourless needles as the monohydrochloride. It gave a purple colour on paper with ninhydrin but failed to react with the Sakaguchi, PCF, diacetyl and Jaffe reagents. Elementary microanalysis, EI-MS($P^+ = M^+ - H_2O$) and FD-MS($P^+ = M^+ + 1$) showed that LCX hydrochloride had the formula $C_7H_{14}N_4O_2.HCl$. Its EI-MS was identical to that of the mixed diastereomers of tetrahydrolathyrine (**2**)



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produced by catalytic hydrogenation of racemic lathyrine [3]. It was not possible to separate LCX from the mixed diastereomers of tetrahydrolathyrine by TLC in 3 different solvent systems. The 360 MHz ^1H NMR spectrum of LCX was interpreted in terms of the tetrahydrolathyrine structure with the help of double irradiation experiments, the results of which are presented in Table 1. Further ^1H NMR evidence for the identity of LCX with one diastereomer of the synthetic tetrahydrolathyrine is shown in Fig. 1. In Fig. 1 the 360 MHz spectra of LCX and of the reduced product of racemic lathyrine are compared. LCX can be seen to be a component of the spectrum of the mixture of diastereomers derived from racemic lathyrine. ^{13}C NMR spectra of LCX and the mixed diastereomers of tetrahydrolathyrine in unbuffered D_2O were very similar, and an interpretation of the spectrum given by LCX is presented in Table 2.

LCX is optically active having $[\theta]_{210}^{22°}$ + 6300 (0.1 N HCl *c* 0.00687) and this large positive CD indicates [4] that LCX is a *S*-α-amino acid (**3**) like lathyrine itself (**5**). The stereochemistry of LCX at C-4′ is unknown.

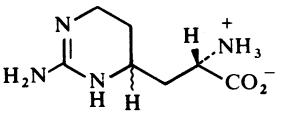

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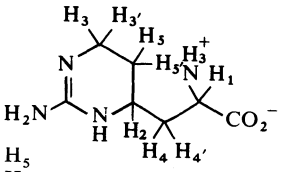
Table 1. 360 MHz spectrum of LCX and assignments

Assignments	δ	J(Hz)* (calculated [10])	Double resonance experiments: Point of irradiation → Point of effects
			
H_5	1.85	$J_{5,5'} = -14.16$	
$H_{5'}$	2.20		
H_4	2.10	$J_{4,4'} = -14.41$	
$H_{4'}$	2.45		
$H_3, H_{3'}$	3.42, 3.35	$J_{3,3'} = -14.28; J_{3,5} = 8.20$ $J_{3,5'} = -0.60; J_{3',5} = -0.79$ $J_{3',5'} = 6.21$	
H_2	3.85	$J_{2,4'} = 6.72; J_{2,4} = 6.46$ $J_{2,5'} = 5.25; J_{2,5} = 6.47$	
H_1	4.23	$J_{1,4} = 6.90; J_{1,4'} = 7.16$	

* The standard deviation for the calculated J is 0.08 Hz.

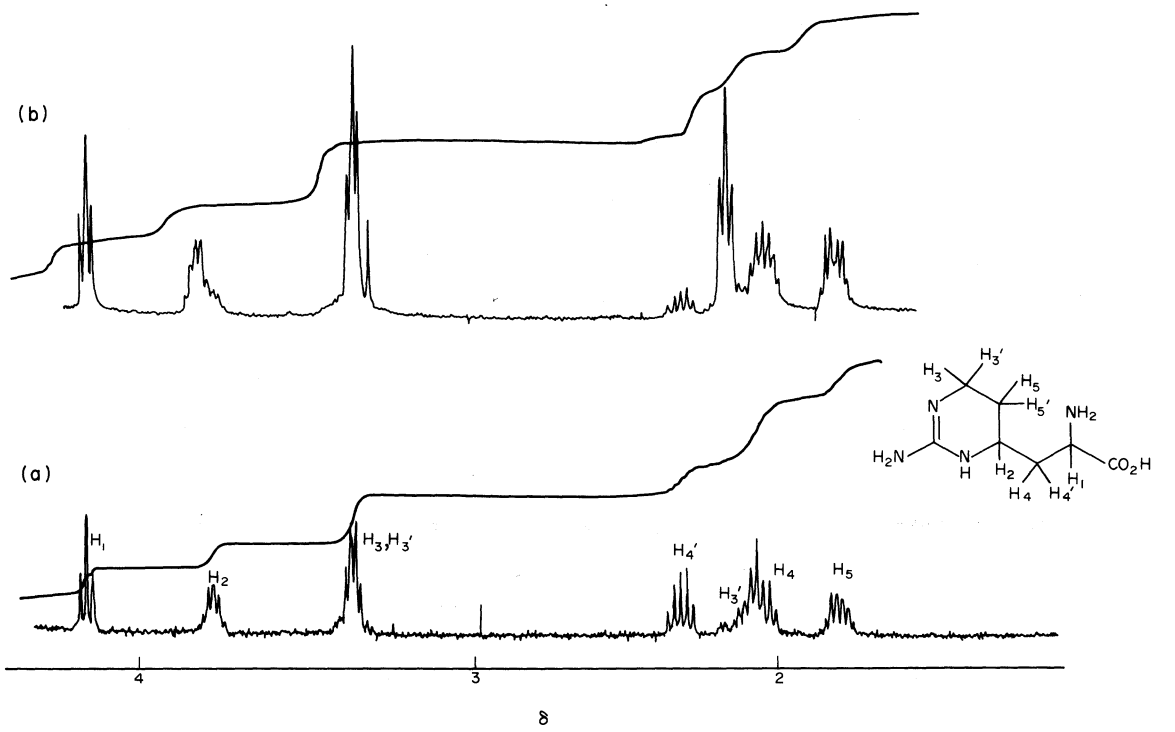
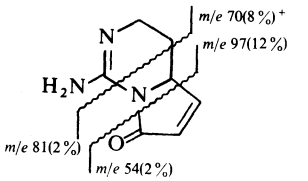


Fig. 1. ^{1}H NMR spectrum of LCX (a) and the mixed diastereomers of tetrahydrolathyrine (b) at 360 MHz.

The EI mass spectrum of LCX showed no $M^+ - CO_2$, CO_2H or HCO_2H and no M^+, instead, the parent ion at *m/e* 168 corresponded to $M^+ - H_2O$. This is not the case with lathyrine which, as expected, shows a prominent $M^+ - CO_2H$ fragment ion. The EI-MS of LCX can be explained in part by the loss of both H_2O and NH_3 to give the ion (**4**) at *m/e* 151, which further fragments as indicated.

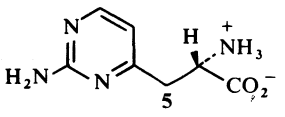
To our knowledge, this is the first report of tetrahydrolathyrine as a naturally occurring amino acid, although lathyrine itself has been found in a number of *Lathyrus* species [5].



4 *m/e* 151(23%)

Table 2. 25 MHz ^{13}C NMR spectrum and assignments of LCX for a D_2O solution

Assignment	δ_c, Multiplicity in proton coupled spectrum; J_{CH} (Hz)
5′	26.5, *t*; 131
3, 6′	38.1, 37.9, *ts*; 125 and 148
4′	47.9, *d*; 147
2	53.8, *d*; 143
2′	155.3, *s*
1	175.8, *s*



The biosynthesis of lathyrine has aroused interest since radioactive tracer studies have shown that it can be formed both by the internal cyclization of 4-hydroxyhomoarginine and from pyrimidine precursors [6, 7]. It has been postulated that synthesis from 4-hydroxyhomoarginine in *Lathyrus* species proceeds via 4-oxohomoarginine and 5,6-dihydrolathyrine which may then be oxidized to lathyrine [8]. If this hypothesis is correct then a modified pathway in *L. costaricensis* involving the reduction rather than the oxidation of 5,6-dihydrolathyrine may lead to the synthesis of tetrahydrolathyrine. Although the discovery of tetrahydrolathyrine in a genus taxonomically somewhat remote from *Lathyrus* was unexpected, such are the compound's properties that it may have escaped detection in other genera.

The seeds of *L. costaricensis* are preyed on by the larvae of two species of bruchid beetle, *Ctenocolum tuberculatum* (Motsch.) and *Ctenocolum crotonae* (Fähr.). As many non-protein amino acids are toxic to insects [9], it is possible that these two species are capable of circumventing the potentially toxic effects of tetrahydrolathyrine in their diet. This possibility is being investigated.

EXPERIMENTAL

Isolation of LCX. Finely ground seed (170 g) was shaken for 48 hr at room temp. with 0.1 N HCl in MeOH (1 l.). After filtration the extract was concd under red. pres. to 0.5 l. and H_2O (0.5 l.) added. The ppt. formed was removed by centrifugation and the supernatant applied to a column (15 × 5 cm) of strongly acidic ion-exchange resin (Amberlite IR-120, × 8) in the H^+ form prepared in 50% MeOH. LCX was retained on the resin, which was washed with 50% MeOH (0.5 l.), 0.1 N HCl in MeOH (0.5 l.) and H_2O (1 l.). After removing weakly basic material with 0.6 N HCl (1 l.) and washing with H_2O, LCX was displaced from the column with 1 M NH_4OH (400 ml). The ammoniacal soln was taken to dryness on a rotary evaporator at 40° and the residue dissolved in H_2O (5 ml). This soln was applied to a column (20 × 2 cm) of Amberlite CG-120, 100–200 mesh, in the NH_4^+ form. The column was washed with H_2O (0.2 l.) and LCX eluted with 0.2 N NH_4OH. The first 120 ml which contained the LCX were evapd to dryness under red. pres. The residue was dissolved in a minimum of warm MeOH and crystallization was achieved by the addition of EtOH and cooling. Yield: 70 mg monohydrochloride. This was re-crystallized twice from aq. Me_2CO, mp 258–259°. (Found: C, 37.6; H, 6.9; N, 25.1; Cl, 16.0. $C_7H_{15}N_4O_2Cl$ requires: C, 37.8; H, 6.9; N, 25.2; Cl, 16.0%). $[\alpha]_D^{25}$ −18.9° (H_2O *c* 0.175), CD in text. IR γ_{max}^{nujol} cm^{-1}: 3300–3100 (br), 1690, 1675, 1630, 1610, 1550, 1500. ^1H and ^{13}C NMR spectra were determined for D_2O plus DCl solns with Na-2,2,2,2-tetradeutero-3-(trimethylsilyl) propionate (TSP) as external standard. See Fig. 1a and Tables 1 and 2. EI-MS. LCX and hydrogenated lathyrine (see below) were very similar and showed the following features: *m/e* 168 (7%), 151 (23), 98 (5), 97 (12), 96 (13), 70 (8), 69 (6), 56 (10), 55 (11), 44 (46), 43 (33), 42 (8), 41 (6), 40 (10), 38 (34), 37 (4), 36 (100), 35 (12). FD-MS. 187 (100%), 169 (17), 142 (16), 107 (22), 58 (23), 53 (10).

Hydrogenation of lathyrine. Racemic, synthetic lathyrine (0.30 g, 1.6 mmol) was dissolved in 50% aq. MeOH (30 ml). To the soln was added conc HCl (1.5 ml) and 5% Pd/C (0.3 g). The mixture was hydrogenated at room temp. and pres. until H_2 uptake ceased (1.75 hr). The reaction mixture was filtered and evapd *in vacuo* and the residue recrystallized from H_2O–Me_2CO to yield the mixed diastereomers of tetrahydrolathyrine hydrochloride (0.25 g, 70%) mp 218–250° decomp. (lit. [3] 252–254°). (Found: C, 37.8; H, 6.8; N, 25.2; Cl, 15.9. $C_7H_{15}N_4O_2Cl$ requires: C, 37.8; H, 6.7; N, 25.2; Cl, 16.0%). ν_{max}^{nujol} cm^{-1}: 3400–3100 (br), 1670, 1630, 1550, 1500. The ^1H NMR spectrum is shown in Fig. 1b and its ^{13}C NMR spectrum (D_2O) was very similar to that of LCX (Table 1).

TLC was carried out on Si gel F_{254} plates. LCX and synthetic tetrahydrolathyrine gave single spots (visualized with ninhydrin) with identical R_f values: 0.06 (BuOH–H_2O–HOAc, 4:1:1), 0.46 (PhOH–NH_4OH (0.88), 3:1), 0.1 (*n*-PrOH–NH_4OH (0.88), 7:3).

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