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SHORT COMMUNICATION

L-DOPA AND L-3-CARBOXY-6,7-DIHYDROXY-1,2,3,4-TETRAHYDROISOQUINOLINE, A NEW IMINO ACID, FROM SEEDS OF *MUCUNA MUTISIANA**

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Abstract—The isolation of L-DOPA and a new naturally occurring imino acid, L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, from the seeds of *Mucuna mutisiana* is described. The structure of the imino acid has been confirmed by synthesis.

INTRODUCTION

L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) was first isolated from *Vicia faba* in 1913.[1,2] In 1937, Damodaran and Ramaswamy reported that the seeds of another legume, *Mucuna pruriens*, contained much higher concentrations of L-DOPA and from these they obtained the amino acid in quantities equivalent to 1·5% of the seed weight.[3]

Although L-DOPA occurs in species of other legume genera[4,5] and other plant families,[6,7] concentrations exceeding 5% of the dry tissue weight have only been reported in the seed embryos of six species of *Mucuna*.[8] Additional evidence which suggests that the accumulation of high concentrations of L-DOPA in seeds may be a peculiarity of this one genus, has been provided recently by Daxenbichler *et al.*[9] In a survey of 511 species representing 7 families and 246 genera they found major concentrations of L-DOPA in the seeds of only 4 species, all of which belonged to the genus *Mucuna*.

While L-DOPA was found to be the major free amino acid present in all the extracts of *Mucuna* seeds previously analysed, other ninhydrin-reacting compounds (including two 'unknowns') were detected by paper chromatography and high-voltage electrophoresis.[8] The most concentrated of these 'unknowns' gave a bright yellow with ninhydrin but otherwise resembled L-DOPA in giving a green colour with ferric chloride solution and darkening rapidly in alkaline solution.

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The present paper describes the isolation of L-DOPA from seeds of *Mucuna mutisiana* and also of the principal 'unknown' present in the same seeds, its characterization and synthesis.

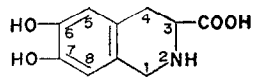
RESULTS AND DISCUSSION

L-DOPA has been isolated from seeds of *M. mutisiana* in 3·9% yield. An 'unknown' compound giving a yellow colour with ninhydrin which was detected by chromatography and high-voltage electrophoresis in seed extracts of *M. andreana*, *M. holtoni*, *M. pruriens*, *M. sloanei*, *M. urens* and *M. deeringiana* (*Stizolobium deeringianum*) has also been isolated from seeds of *M. mutisiana*. The UV spectrum of the compound showed maxima at 282 nm (cf. L-DOPA 282 nm) and 210 nm when determined in acid solution. Like L-DOPA the 'unknown' gave a green colour on paper when sprayed with $FeCl_3$ solution and darkened rapidly when treated with 2 N NaOH. On electrophoresis it moved as a neutral amino acid but failed to react as an α-amino monocarboxylic acid when treated successively on paper with cupric ions and ninhydrin;[10] these findings suggested that the 'unknown' was either an N substituted amino acid or an imino acid. The yellow colour which it gave with ninhydrin, reminiscent of the colour given by proline with ninhydrin, favoured the second possibility. The presence of at least one asymmetric centre was indicated by the strongly laevo-rotatory character of the molecule in acid solution.

Elemental analysis established the empirical formula of the unknown as $C_{10}H_{11}NO_4$. High resolution mass spectrometry showed that this was the molecular formula. Besides an intense M-1 peak, an abundant fragment with the composition $C_9H_{10}NO_2$ expected for the corresponding amine ion[11] of the amino acid was observed. The formula for the unknown corresponds to an amino acid with one more carbon atom than DOPA and also one more ring or double bond in the structure.

The NMR spectrum of the amino acid in acid solution showed a pair of singlets at 6·77 and 6·73 ppm (1 H each) which would be expected for two single protons para to one another in an aromatic ring. This pattern and the absence of any vinyl proton signal suggested that the additional element of unsaturation was accounted for by an additional ring. A multiplet at 3·24 ppm (2H) indicated the presence of a methylene group coupled to a proton at lower field. A complex multiplet at 4·4 ppm (3H) was consistent with an uncoupled methylene group and a single proton coupled to the higher field methylene group. In alkaline solution, all peaks were shifted up field but in such a manner that the overlap of the one and two proton signals was removed, confirming the above assignment. Only the inner four lines of the X part of the ABX system were observed and the AB lines were not well resolved, preventing a complete analysis.

On the basis of this chemical and physical evidence the 'unknown' was tentatively identified as L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (I).



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The synthesis of the racemic form of this compound from DL-DOPA and formaldehyde has previously been described[12] and its m.p. recorded as 277° (10° lower than that of the compound isolated from *M. mutisiana*). The synthesis was repeated using L-DOPA as the starting material and the product gave no depression of m.p. when mixed with the natural compound. The IR, UV, NMR and mass spectra of the natural and synthetic materials were identical and the specific rotations showed good agreement.

This imino acid is presumably formed from L-DOPA in the plant though the origin of the additional carbon atom (I) has not yet been established. The related compound 1-methyl-3-carboxy-6-hydroxy-1,2,3,4-tetrahydroisoquinoline which has been isolated from *Euphorbia myrsinites* L. is thought to be derived from *m*-tyrosine by condensation with acetaldehyde or its equivalent.[13]

EXPERIMENTAL

Isolation of L-*DOPA and* L-3-*Carboxy*-6,7-*dihydroxy*-1,2,3,4-*tetrahydroisoquinoline*

Finely ground seed (200 g) of *M. mutisiana* was shaken with acetone (1 l.) for 24 hr at 20° to remove lipids and pigments. The de-fatted seed was then extracted by shaking with 50% ethanol (1 l.) containing 0·5% (w/v) of ascorbic acid for 24 hr at 20°. The extraction was repeated four times and the combined extracts were concentrated to 200 ml under reduced pressure at less than 35°.

After standing for 24 hr at 4° the solid (crude L-DOPA) which had separated from the concentrated solution, was removed by filtration and recrystallized from hot water (3·6 g) m.p. 282–283 (lit.[3] 281) $[\alpha]_D^{20}$ −11·6 (*ca.* 1·0; 4% HCl lit.[2] −12·0). Elemental composition ($C_9H_{11}NO_4$) and molecular weight (197) were determined by high resolution mass spectrometry. The mass spectrum of isolated material was identical with that of authentic L-DOPA as were the UV, IR and NMR spectra.

The mother liquor which contained further L-DOPA, together with other amino acids, was applied to a column (90 × 3 cm) of weakly basic ion-exchange resin (Amberlite-CG 4B) in the acetate form. The amino acids were eluted with 0·05M HOAc (500 ml) followed by 0·1M HOAc. The first 75 ml of eluate contained no amino acids. The next 650 ml contained L-DOPA and traces of basic amino acids. The next 1000 ml contained the yellow reacting 'unknown' together with traces of L-DOPA. The effluent containing the L-DOPA and traces of basic amino acids was taken to dryness at reduced pressure at less than 35°. The solid residue was recrystallized from hot water to give a further 4·4 g of L-DOPA (total yield 3·9%).

The effluent containing the 'unknown' was evaporated to dryness in the same way and the residue, which proved very sparingly soluble in hot water, was recrystallized from 20% (v/v) HOAc (4·4 g) m.p. 286–288°; $[\alpha]_D^{25}$ −114·9 (*ca.* 1·65; 20% HCl) Fd: C, 57·13; H, 5·48; N, 6·52; Calc. for $C_{10}H_{11}NO_4$: C, 57·43; H, 5·30; N, 6·70%.

Mass Spectrum

The high resolution mass spectrum of the isolated compound was recorded photographically using a Du Pont (CEC) 21–110 high resolution mass spectrometer. The recorded mass spectrum was measured using a Grant plate reader-PDP 8-I system.[14] The determined masses were within 2 milli-amu of the masses calculated for the elemental compositions reported (M^+ determined 209·0680, calculated for $C_{10}H_{11}NO_4$ 209·0688).

The mass spectra of some simple tetrahydroisoquinolines have been reported by Baldwin *et al.*[15] A strong retro-Diels-Alder fragment was observed for those compounds. While such a fragment was observed for the new imino acid (*m/e* 136, $C_8H_8O_2$), it was not a prominent peak in the spectrum.

UV and IR Spectrum

The UV spectrum determined in 20% HCl showed maxima at 282 and 210 nm. The IR spectrum, determined using the KBr disc method showed bands at: 3400 cm^{-1} (2·94 μ) (OH stretching frequency of OH groups); 3330 to 2220 cm^{-1} (3–4·5 μ) (—NH and —CH stretching frequency of NH and CH groups); 1610 cm^{-1} (6·21 μ) (—C=O stretching frequency of COOH group).

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NMR Spectra

NMR spectra were determined with a Varian A-60 spectrometer at ambient temperatures (DSS as internal standard). All signals are reported as ppm in δ values and the coupling constants in Hz. Symbols s and m represent singlet, and multiplet respectively 2 N CF_3COOD in D_2O: 6·77 or 6·73 s, 1H —C(5), 6·77 or 6 73 s, 1H —C(8); 4·4 m, 3H —C(1), C(3); 3·24 m, 2H —C(4). 2 N NaOD in D_2O: 6·40 or 6·27 s, 1H —C(5); 6·40 or 6·27 s, 1H —C(8); 3·76 s, 2H —C(1); 3·35 m, 1H —C(3); 2 69 m, 2H —C(4). The signals for H_3,H_{4a} and H_{4b} are an ABX multiplet. The AB multiplet was not well resolved ($J_{AX} + J_{BX}$ = 15·5 Hz). Sample concentration was 10% (w/v) for each determination

Synthesis of L-3-*Carboxy*-6,7-*dihydroxy*-1,2,3,4-*tetrahydroisoquinoline*

L-DOPA (400 mg) was dissolved in water (20 ml) and to the solution was added 0·35 ml of 40% formaldehyde solution. The mixture was stirred and kept at 30° for 72 hr. The reaction mixture was then taken to dryness and the residue was recrystallized from 20% (v/v) HOAc. Yield 240 mg, m.p. 285–287, $[\alpha]_{25}^{D} = -110{\cdot}5$ (c = 1·67; 20% HCl). No depression of m.p. was found when this compound was mixed with the natural product. The UV, IR, NMR and high resolution mass spectra of the two compounds were identical in all respects.

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