

## Studies on *Mucuna* Species of Sri Lanka

### I. The L-DOPA Content of Seeds

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**Abstract :** Several local selections of *Mucuna* were investigated for L-DOPA content. The most promising type was a selection of *Mucuna atterrima* which contained about 4.5% L-DOPA in its seed. The L-DOPA could be recovered at 80% efficiency provided the seeds are fresh and used immediately after powdering. Climatic factors appear to have little or no effect on L-DOPA content and succeeding generations appear not to vary significantly from the parent plant with respect to this parameter.

#### 1. Introduction

3-(3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) is a drug, used for some years in the treatment of Parkinson's disease.<sup>3</sup> More recently this compound has found application in the treatment of mental disorders.<sup>8</sup> As a result of its importance as a drug considerable attention has been paid to its occurrence in plant material.

L-DOPA was first isolated from *Vicia faba* as early as 1913.<sup>5</sup> In 1971, Daxenbichler *et al*,<sup>3</sup> screened 724 species (from 447 genera and 135 families) and found L-DOPA only in 4 legumes species (*Mucuna*, *Dolichos*, *Baptisia* and *Vicia*). Previously L-DOPA had been reported in *Lupinus*,<sup>3</sup> *Euphorbia*,<sup>7</sup> *Robinia*,<sup>6</sup> and *Sarothamnus*<sup>6</sup> species. However, all studies including that of Bell and Janzen<sup>2</sup>, showed that only *Mucuna* had sufficient levels for commercial exploitation. The studies of Bell and Janzen<sup>2</sup> showed that 6 species of *Mucuna* had L-DOPA contents of more than 5%. Further, Daxenbichler<sup>3</sup> screening plants from 5 different countries (3 continents) reported that *M. atterrima* selections had L-DOPA levels of 4% to 5%.

In this study, local *Mucuna* species have been investigated for the first time for L-DOPA content. The primary aim being to collect information on the factors affecting L-DOPA content of *Mucuna* seeds in order to study the feasibility of setting up an industry in Sri Lanka, for the commercial extraction of L-DOPA.

#### 2. Experimental

##### 2.1. Plant Material

The original seed material was collected from different parts of the country either during field surveys or through intermediate sources. All types containing viable seeds were grown in experimental plots located at the CISIR. The plants were grown under controlled conditions using supports for the climber to ascend.

## 2.2. Identification of Plant Material.

A major problem of this study was the lack of authoritative identification of the plant material. Very little documentation on the taxonomy of *Mucuna* is available, both in Sri Lanka and abroad. However with the limited quantity of available data, 4 of the collected varieties were identified. They were (1) CISIR code No. A (see results)—a *M. atterrima* selection (2) CISIR code No. L—a *M. utilis* selection, (3) CISIR code No. R—a *M. deringian* selection. (4) CISIR code No. B. *M. nivia*.

For this reason, all varieties are described here with code numbers. A detailed description of the plants have been communicated elsewhere.<sup>1</sup> All plant specimens have been sent to Kew, Royal Botanic Gardens, who due to controversy in classification have advised the authors to retain CISIR classification number and herbarium specimens. Experimental plants are also being maintained at the CISIR.

## 2.3. Extraction of L-DOPA.

L-DOPA was extracted using 0.25% acetic acid as described previously.<sup>9</sup>

## 2.4. Assay of L-DOPA.

L-DOPA was assayed by the method of Daxenbichler<sup>4</sup> as modified by Pieris.

## 2.5. Recovery of L-DOPA.

L-DOPA was recovered after deproteinising, concentrating under vacuo, decolourising and further concentration. This concentrate yields crude L-DOPA on cooling. The purity of the recovered L-DOPA was of the order of 90% (determined by UV absorption<sup>4</sup>).

## 2.6. Purification of L-DOPA.

L-DOPA was purified by recrystallisation from a water-ethanol mixture. The purity of the product was at least 98%. The UV and IR absorption spectra were identical with that of authentic L-DOPA (obtained by courtesy of Dr. N. R. Farnsworth, Academy of Sciences, USA). Thin layer chromatography (cellulose) with ethyl acetate: acetic acid: water (10:3:6) as solvent gave a purple spot with ninhydrin reagent with a R<sub>f</sub> value identical to authentic L-DOPA. The melting point of the L-DOPA (isolated) was 282°C (lit. 283°C to 286°C).

## 3. Results

### 3.1. Selection

Seeds of 19 different *Mucuna* plants from varying parts of the country were collected and analysed for L-DOPA content, by the UV Absorption method.<sup>3</sup> These selections fell into at least seven different morphological types. Results, shown in Table 1, clearly illustrate the wide variation in L-DOPA content of the selections.

TABLE 1. L-DOPA Content of Different Selections of Seeds.

Mucuna CISIR Code No.	Colour	Average seed Weight (g)	Percentage L-DOPA (on dry weight)
A ( <i>M. atterrima</i> )	Black	1.20	4.2
B ( <i>M. nivea</i> )	Grey	1.00	2.5
B.1	Greyish brown	1.10	1.6
B.2	Black	0.90	2.3
C	Black	0.85	2.8
D	Mottled brown on grey	0.75	2.2
E	Mottled brown on grey	0.70	3.5
F	Black	0.80	2.2
G	Black	N.D.	4.4
H	Black	N.D.	3.7
I	Black	0.85	3.8
J	Black	0.65	3.4
L ( <i>M. utilis</i> )	Black	1.00	4.1
N	Mottled brown on grey	1.15	4.2
O	off-white	0.90	2.8
P	Black	0.95	4.5
Q	Brown-black	0.70	2.3
R	Mottled black	0.80	2.7
( <i>M. deringiana</i> )	on grey		
T	Black	0.80	4.1

N.D. -- Not determined.

### 3.2. Recovery of L-DOPA.

Efficiency of recovery of L-DOPA varied to a great extent and appeared to depend on a number of factors mainly,

- (a) age of seeds
- (b) L-DOPA content of the seeds.

When the seeds were old, low recoveries were obtained and in all these cases the cotyledons were discoloured and extracts therefore highly coloured. Both factors are illustrated by results shown in Table 2.

TABLE 2. Factors affecting L-DOPA Recovery

Batch Code	L-DOPA content (%)	Recovery (%)
Fresh seeds		
A	4.2	81
J	3.4	76
Q	4.3	80
B.1	1.6	26
B	2.5	17
C	2.8	25
*Old Seeds		
G	4.4	No Recovery
H	3.7	17
N	4.2	29

\*Old seeds refer to the seeds that are discoloured and at least six months old. The fresh seed was obtained directly from the tree.

Another factor that affects both L-DOPA content and recovery is the time that elapses between powdering of seeds, and their extraction with acetic acid. Losses of the order of 15% (one week) and 30% (3 to 4 weeks) have been observed.

The maturity of the seeds used and their mode of drying (sun drying or oven drying) had no effect on both L-DOPA content and recovery.

In an industrial process, materials of construction are of considerable importance. Metals were found to have a profound adverse effect on L-DOPA recovery; use of Cu, Zn, Fe, Ni, Sn in fine powdered form all caused oxidation of L-DOPA and therefore low recoveries. The only metal used which did not effect recovery adversely was Al.

### 3.3. Effect of Propagating Seeds of known- L-DOPA content under different Conditions.

The variation in climatic conditions appeared to have little effect on the average L-DOPA content. Plants of the new generation planted at CISIR had approximately the same L-DOPA content as the respective parent plant. Studies showed that L-DOPA content does not vary much in the first two generations. When subjected to detailed study *M. atterrima* showed the following. In the first generation when the individual plants were grown under different conditions which resulted in different habits and L-DOPA content ranged from 3.0% to 4.8%. It is interesting to note that the plants grown on supports gave values of 4.5, 4.2 and 4.8 while the plant allowed

to trail along the ground (without a support) gave an L-DOPA content of only 3.4%. In the next generation 21 plants were studied and only 3 of them showed abnormal L-DOPA contents. Two were low (3.4% and 3.6%) while one plant gave seeds with a high L-DOPA content of 6.1%. Mean L-DOPA content of the seeds of separate plants was  $4.4 \pm 0.5$ .

#### 4. Discussion

Although the presence of L-DOPA in *Mucuna* has evoked some interest due to its possible role as a chemical barrier to insect attack<sup>10</sup> the main investigations on this subject has been nearly confined to the extraction of this compound for use as a drug.

While considerable data on the L-DOPA levels of several tropical varieties of the species is available, the local *Mucuna* varieties have not been investigated previously. This is indeed surprising as the seeds of many local varieties (notably varieties similar to B and R) have been used for some time in Ayurvedic medicine in Sri Lanka.

The primary aim of this study was to determine the L-DOPA content of local varieties. Here, L-DOPA content of the seeds of the varieties collected (excluding seed coat) was determined by the UV absorption method. It should be noted that the L-DOPA value given includes the tetra-hydro isoquinolines reported by Daxenbichler<sup>4</sup> (which are generally present at levels of less than 0.3%).

Only 6 selections contained more than 4% L-DOPA. Of these, the high seed yielding type A was selected for further study. This plant was found to produce 0.5 kg to 1 kg seed per plant when planted 2 meters apart on supports. It had been identified as a variety of *M. atterrima*.<sup>1</sup>

Studies showed that L-DOPA content did not vary significantly with both (i) the location of cultivation and (ii) from generation to generation. The other factor that appeared to affect L-DOPA levels was the habit of the plant where it was found that supports are necessary for both high yields of seed and high L-DOPA content. The only other feature worthy of note was that one plant produced seeds with a rather high L-DOPA content (6%). Maturity of the seed (unlike previously reported) had no effect on L-DOPA levels.

The optimum conditions of extraction (including acetic acid strength and volume and time of extraction) have been reported previously.<sup>9</sup> It is now reported that L-DOPA can be extracted at 80% efficiency using this method, provided the seeds are fresh and are used soon after powdering. It is very likely that L-DOPA is lost through oxidation on keeping.

Conclusions reached from this study include the point that the local requirement of L-DOPA (approx. 100 kg) can be produced by the cultivation of a mere 10 acres of this plant, the only major problem encountered so far being the susceptibility of most *Mucuna* varieties to a mosaic virus.

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