

## Studies on *Mucuna* Species of Sri Lanka

### II. Determination of the Tetrahydroisoquinoline Content of Seeds

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**Abstract :** A method of quantitative analysis of the major tetrahydroisoquinolines (THIQ) of *Mucuna* species using tlc and the imine reaction with ninhydrin is reported. Using the test the methyl and non-methyl THIQ content of 6 selections of *Mucuna* have been determined separately. Results show that 4 selections had approximately 1% H-THIQ (non-methyl) while the levels in the other 2 selections were relatively low. Only two selections contained a significant level of Me-THIQ. It appears significant that those varieties with high THIQ content are preferred for use in Ayurveda in Sri Lanka for lowering blood pressure and that these compounds are structurally similar to tetrahydroisoquinolines which have already been reported to have such effects on blood pressure.

#### 1. Introduction

Although the component of major interest in *Mucuna* species is L-DOPA 3- (3, 4, dihydroxyphenyl - L- alanine), recently several investigators have shown interest in the tetrahydroisoquinoline derivatives (THIQ) present in this genus. Daxenbichler *et al*, in 1972<sup>5</sup> reported the presence of 3, 6, 7-dihydroxy 1-methyl- 1, 2, 3, 4 tetrahydroisoquinoline - 3 - carboxylic acid (I, Me-THIQ) in *Mucuna deringiana* while Bell *et al*<sup>3</sup> reported the presence of the non-methyl derivative i.e. 6, 7 dihydroxy- 1, 2, 3, 4 tetrahydroisoquinoline - 3 - carboxylic acid (II, H- THIQ) in the seeds of *Mucuna mutisiana*. Daxenbichler and others in 1972<sup>6</sup> also reported the presence of both tetrahydroisoquinolines (THIQ) in 10 selections of *Mucuna* species, *M. atterrima*, *M. deringiana*, *M. gigantea*, *M. holtonii*, *M. mutisiana*, *M. prurita*, *M. sloanii* and *M. urens*.

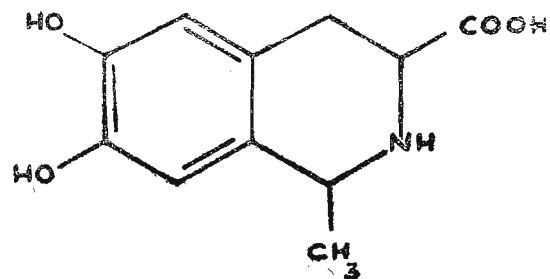
In this study we report: (1) the presence of these THIQ in 6 local selections of *Mucuna*, (2) the quantitative analysis of H-THIQ and Me-THIQ using thin layer chromatography and the ninhydrin reaction with imines, (3) the use of this procedure to estimate the THIQ content of these 6 selections.

#### 2. Experimental and Results

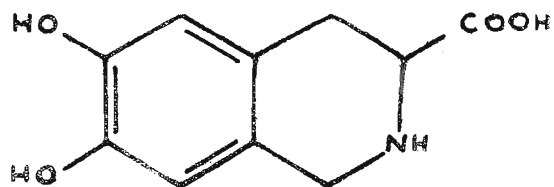
##### 2.1. Plant Material

Plant material was obtained from the CISIR experimental plot. Data on code numbers<sup>10</sup> and identification<sup>2</sup> have been previously reported.

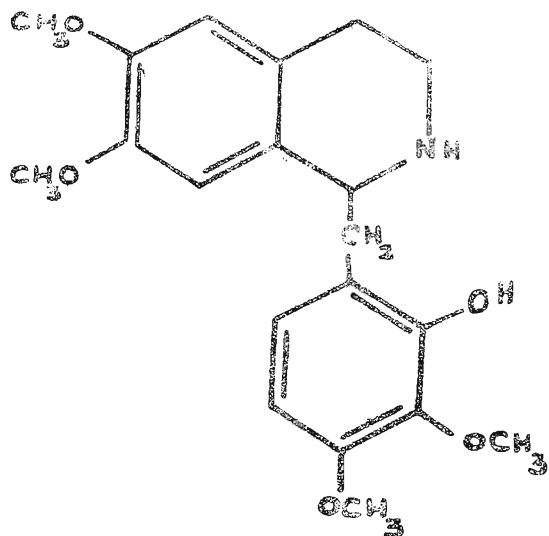
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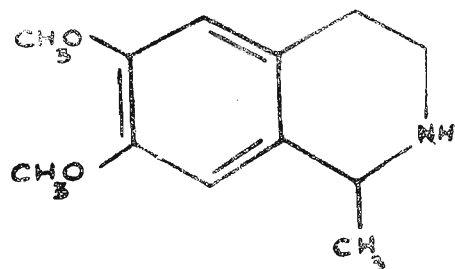
I



II



III



IV

Figure 1. Some naturally occurring tetrahydroisoquinolines

- I. 1-methyl, 3-carboxy, 6,7-dihydroxy, tetrahydroisoquinoline (Me-THIQ)
- II. 3-carboxy, 6,7-dihydroxy, tetrahydroisoquinoline (H-THIQ)
- III. Papavarine
- IV. Salisolidine.

## 2.2. Preparation of Standards

3-6, 7-dihydroxy, 1, 2, 3, 4, tetrahydroisoquinoline -3-carboxylic acid (H-THIQ) and its 1-methyl derivative were synthesized from L-DOPA by the method described by Bell *et al.*<sup>3</sup>

## 2.3. Extraction of Seed.

Fresh (whole) seeds of 6 *Mucuna* species were dried to about 10% moisture and finely ground. The flour was extracted with dilute acetic acid as follows. A known weight of seed (10g to 50g) was allowed to stand at room temperature for about 18h with 9 times its weight of 0.25% acetic acid with periodic mixing.<sup>10</sup> The supernatant was separated by filtration and the residue washed with acetic acid. The residue on extraction with 50% ethanol containing, 0.5% ascorbic acid did not show the presence of THIQ. The filtrate was deproteinised by boiling and filtration.

## 2.4. Thin layer Chromatography

Thin layer chromatography was performed on the above extract using cellulose (MN 600 cellulose) plates (500  $\mu$  thickness) with n butanol : acetic acid : water (4:1:3) as solvent. The plates were sprayed with 1% ninhydrin and heated in an oven for exactly 20 mins at 100°C. The Rf values of the methyl and nor methyl tetrahydroisoquinolines were 0.57 and 0.49 respectively.

## 2.5. Quantitative Estimation of the THIQ

The THIQ standards (15 to 75  $\mu$ g) were chromatographed on the same plate as the extract (10  $\mu$ l). After spraying with ninhydrin and developing the spots the areas of the (yellow) spots were carefully calculated and a standard curve of concentration vs square root spot area plotted. While plots of conc. vs spot area and conc. vs log spot area did not give straight lines, the above mentioned plot resulted in a straight line from which the concentration of the unknown was determined. In all determinations, the standards were run on the same plate as the unknown and separate calibration curves plotted. The standard deviation of the experimental determination was of the order of 10%. It was observed that H-THIQ gave a much deeper colour with ninhydrin than an equimolar amount of Me-THIQ. However, spot areas did not have the same degree of variance.

## 2.6 Adaptation of test for screening

It was observed that on covering the sprayed and heated chromatography plate with glass plates, all the purple - violet spots of the amino acids disappeared and the yellow THIQ spots increased in intensity. By this method small quantities of these compounds may be detected. It was also found that a third yellow spot with ninhydrin was present at a Rf value of 0.25 while L-DOPA gave a Rf value of 0.30.

### 2.7. THIQ content of some selections of *Mucuna*.

Using the method of estimation, 6 selections of *Mucuna* species were subjected to THIQ assay. Results (Table 1) showed that all varieties contained these compounds but to varying extents. The *M. atterrima* selection planned for use for the extraction of L-DOPA contained very small quantities. While selections B, P, Q and R contained high levels of H-THIQ, the selection Q also contained Me-THIQ in larger quantities than the other selections which had only traces of this compound.

TABLE 1. THIQ content of Selections of *Mucuna* Species

CISIR Code No.	Identity	Me-THIQ	H-THIQ	Total 283 nm* absorbing material (as % L-DOPA)
A	<i>M. atterrima</i>	trace	trace	4.4
B	<i>M. nivea</i>	Not detected	0.87(1.04)	2.5
P	<i>M. species</i> (unidentified)	0.33	0.60 (0.96)	4.5
Q	<i>M. species</i> (unidentified)	1.09	0.92 (1.03)	2.3
L	<i>M. utilis</i>	Not detected	0.40 (0.21)	3.8
R	<i>M. deringiana</i>	trace	1.00(0.97)	2.7

Some *Mucuna* species have yet to be identified.

\*from data from paper of Pieris *et al.*<sup>10</sup>

Data in parenthesis are the values obtained from a second batch of seeds.

### 2.8. Isolation of THIQ

Attempts to isolate the THIQ by sephadex G-10 gel chromatography by the method described by Daxenbichler<sup>6</sup> and by dry cellulose chromatography were not successful.

### 3. Discussion

The seeds of *Mucuna* species have from ancient times been used for medicinal purposes in the indigenous medical systems of many cultures. In India, *M. puriens* played a prominent role in medical folk lore.<sup>8</sup> In ancient Brazil, *M. bracheata* was extensively used for numerous conditions ranging from crocodile and snakebite, sores and syphilis to cancer.<sup>7</sup> While in Hawaii, the seed of *M. gigantea* has been used as a laxative.<sup>9</sup> The *Mucuna* species have also played a major role as a source of L-DOPA which is used in western medicine as a drug for the treatment of Parkinson's disease and mental disorders. Therefore, it is not at all surprising that the seeds of this plant are also used in the Ayurvedic system of medicine in Sri Lanka. However, its use in this country has mainly been in the medicinal preparations used for the treatment of nervous disorders and high blood pressure. The Sri Lankan example being the only instance that the seed is reported to be used for hypertension. What is still more significant is that the variety preferred in Ayurveda contains low L-DOPA content but high tetrahydroisoquinoline content.

Another point of possible significance is that 5,6-dihydroxy tetrahydroisoquinolines are reported to have similar physiological activity. For example, papavarine (III) has been reported to have a negative effect on arterial blood pressure<sup>1</sup> and salsolidine (IV) has been used for the same purpose.<sup>4</sup> These compounds are derivatives of 6, 7 dihydroxy - 1, 2, 3, 4, tetrahydroisoquinoline.

The significance of this study is manifold:

- (1) A local *Mucuna* species has been studied for the presence of THIQ for the first time.
- (2) A new method of quantitative estimation has been reported. The only other method of estimation reported previously for this naturally occurring THIQ, being the weighing of the isolated compounds.
- (3) For the first time the THIQ (methyl and nor methyl) have been estimated separately. Of the six selections the methyl form was present in more than trace quantities in only two varieties.
- (4) Results have shown that varieties used in Ayurveda have high levels of THIQ (nearly 1.0%) but low L-DOPA content.
- (5) TLC studies have shown the presence in one species of another imino acid which is likely to be related to the natural THIQ. The point of relatedness being deduced from the fact that the same spot was present in the impure synthesized standard.

#### Acknowledgements

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