Mucuna News

Developing Multiple Uses for a Proven Green Manure/Cover Crop

Update on the Progress of the Project
"Increasing Mucuna's Potential as a Food and Feed Crop"

CENTER FOR COVER CROPS INFORMATION AND SEED EXCHANGE IN AFRICA (CIEPCA)

Third issue, July 2001

Progress of the Project

By R.J. Carsky and M. Eilittä

These past two months have seen the initiation of the project activities and researchers trying to solve problems as they arise, often in communication with each other. Project participants are now working on their activities and for the next newsletter we hope to have progress reports from several of them.

We are particularly grateful to R. Myhrman and N. Szabo for their continued advisory role in L-dopa analyses. While L-dopa analysis has proved to be a challenge in many countries, we believe that these limitations will be overcome. In those countries where equipment exists, local analysis will be possible through continued technical backstopping by R. Myhrman and N. Szabo. In countries where equipment is limiting, we will try to arrange for samples to be sent to laboratories with equipment for and experience in L-dopa analysis. In addition to their correspondence with investigators who have been having troubles with L-dopa analyses, R. Myhrman and N. Szabo are collaborating on a multi-part series on the analytical methods in the *Mucuna News*. This issue presents the second part by R. Myhrman which reviews several extraction and quantitation methods, giving special emphasis on methods successfully utilized in Judson College over the years.

We also have two new activities in West Africa that we are highlighting in this issue. In Northern Benin, Groupe d'Actions de Recherches et d'Echanges pour le Développement Durable (GARED) will conduct an adoption study of the utilization of *Mucuna* as animal feed; this issue gives some background on the extension work conducted. In Nigeria, researchers affiliated with the University of Ibadan and IITA will conduct a nutritional characterization of the 12 *Mucuna* accessions available at IITA.

This issue of the bulletin also describes Ph.D. project of E.N. Nyambati in Kenya on *Mucuna* hay as a feed for dairy cows and research and dissemination efforts by Natural Resources Institute (NRI) in Ghana on various *Mucuna* types. We are grateful for the articles supplied by R. Ahlonsou and B. Loko, by R. Myhrman, by E.N. Nyambati, and for the materials supplied by L. Kiff and H. Loos. As always, our thanks to the MOIST-CIIFAD of Cornell University for posting our newsletter to the Internet.

Please note that during the summer, M. Eilittä will be relocating to Abidjan, Ivory Coast and will only have erratic email access July 1-August 10. B. Carsky will be on home leave from 19 July to 26 August. The next *Mucuna News* will be out in October.

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This bulletin is available through the CIEPCA website hosted by MOIST-CIIFAD (http://ppathw3.cals.cornell.edu/mba_project/CIEPCA/home.html).

If you are interested in posting news or inquiries in this bulletin, please contact Marjatta Eilittä.

Project Update

G by E Trial Underway

Seed for the 2001-2002 Genotype by Environment Trial has been sent to the collaborators and the trial has been planted in most locations already. Our Mexican collaborators, J. Castillo in Yucatan and R. Quiroga in Chiapas, were extremely persistent in their efforts to obtain the seeds through the Mexican customs, and the seeds were finally released to them. S. Temple at U. C. Davis and the CIEPCA team at Cotonou, Benin have observed very low germination of the 'Deeringiana' accession.

L. Capo-chichi, the coordinator of the trial, has led the development of the protocols for the trial management and sampling; both of those have been sent to the collaborators. The Rockefeller Foundation has already released additional funds for the trial to IITA. For further information regarding the trial, please contact L. Capo-chichi (email: cludovic@acesag.auburn.edu).

Adoption of Mucuna as Animal Feed in Northern Benin

By R. Ahlonsou and B. Loko, GARED

As a part of the *Mucuna* project, Groupe d'Actions de Recherches et d'Echanges pour le Développement Durable (GARED) is undertaking a study to determine adoption rates and factors impacting adoption of *Mucuna* in Eastern Borgou, Republic of Benin.

The Livestock Development Project in Eastern Borgou (PDEBE), Republic of Benin, was created to promote animal husbandry and settle cattle growers who move about with their cattle. The Eastern Borgou region is characterised by a unimodal rainfall pattern (1,000-1,200 mm per annum) with very little rain from October to April. The natural vegetation offers little fodder resources, especially during the dry season. To remedy this situation the PDEBE has undertaken extension activities with fodder crops and particularly with *Mucuna.* In the following, such activities are briefly presented.

The work initiated in 1994-95 with pre-extension campaigns in which slide shows were utilized to present legumes such as *Mucuna* as high potential fodder crops and seeds were distributed to farmers who wished to grow *Mucuna*, provided that they would reimburse half the quantity of seeds received. The farmers were encouraged to plant *Mucuna* either in June (planting at the bottom of trees in yam fields for seed production) or in late July-early August (typically intercropped with maize or sorghum for fodder production; at times sole-cropped). *Mucuna* plants grown around trees were highly productive and some produced up to one hundred pods each. The *Mucuna* crop planted in July-August was harvested before flowering, then dried and stored for use as hay. Some pastoralists and agro-pastoralists preferred to grow *Mucuna* as a sole crop instead of intercropping it with cereals. Farmer acceptance of *Mucuna* was high.

Problems encountered have included damage to *Mucuna* from a fungus that attacks the stems. To control this fungus the *Mucuna* seeds were treated with the fungicide Macozeb. Another limitation raised by the farmers was the frequently poor germination of the seeds. Following evaluation sessions organised by participating pastoralists and agro-pastoralists, recommendations were made for better management to solve the problems encountered and to stimulate adoption.

Various research activities have accompanied the extension exercise. One experiment involved *Mucuna* variety, planting date, and planting density. The results obtained indicate that appropriate dates for the planting of *Mucuna* are between July 22nd and August 5th; *Mucuna* var. *cochinchinensis* should be recommended for late planting; mixing seeds of the two varieties (*utilis* and *cochinchinensis*) can help solve problems relating to interruptions in rainfall, and intercropping *Mucuna* with maize reduces the yield of maize.

In 2000, in spite of the difficult financial situation confronting the PDEBE, some extension activities were carried out to respond to numerous requests from target groups excited about the successful experience with *Mucuna*. One hundred pastoralists and agro-pastoralists received a total of 470 kg of seeds of *Mucuna* var. *rajada* for free. Photo on page 9 shows *Mucuna* seeds being used in southern Benin for the popular *"Adji"* game.

For further information, please contact the authors through A. Eteka of CIEPCA (c.eteka@cgiar.org).

Nutritional Evaluation of Mucuna Types Available at IITA

Another new activity of the *Mucuna* project is a nutritional evaluation of the *Mucuna* varieties available at IITA. The study will evaluate both physical characteristics and chemical composition of the twelve varieties available at IITA. The study will be conducted by I. E. Ezeagu, Nutritional Biochemist, affiliated with Department of Animal Science, University of Ibadan, G. Tarawali, Consultant Scientist, IITA,

and B. Maziya-Dixon, Food Technologist, IITA. Both physical characteristics and chemical composition will be evaluated. Physical characteristics to be evaluated include:

- Grain hardiness
- Kernel dimensions
- Hull content
- Bulk and true density
- Swelling index

Chemical evaluation will include:

- Proximate Composition: moisture, crude protein, fat, ash, CF, starch and total sugars
- Major and minor minerals
- Anti-nutrients: trypsin inhibitory activity, lectins, tannins, phytic acid, total and soluble oxalates, hydrocyanic acid.
- Energy

For further information, contact I. E. Ezeagu and G. Tarawali (g.tarawali@cgiar.org).

Other News

Mucuna Work in Ghana

In Ghana, there have been a number of interesting activities on *Mucuna*'s food and feed uses. In the country there is traditional utilization of various *Mucuna* species as a food, and the *Mucuna* project hopes to learn more about it in a planned food survey. Several projects have also worked with *Mucuna* for soil improvement, and some of them have incorporated aspects of food utilization in their work.

Natural Resources Institute, a UK-based organization, has been active since 1994 in the Brong Ahafo region of Ghana. One component of their work was research and extension of several green manure species (including *Mucuna*) and animal manures as soil ameliorants on dry season vegetable farms. That project has now come to an end, but currently, GTZ Sedentary Farming Project and The University of Science and Technology at Kumasi are continuing cover crop/ green manure research.

The group worked with four *Mucuna* types, differentiated by seed color (creamy white, light mottled, dark mottled, and shiny black), whose life cycles range from 90 to 300 days to harvest. In the Brong Ahafo region there is also a wild *Mucuna* type with abundant, long stinging hairs on the pod and white seed, which is used for thickening soups. Human contact results in an intensely itchy dermatitis. The group sent the seed of all five varieties to R. Myhrman at Judson College for L-dopa analysis; results are provided in Table 1. The local stinging variety seemingly has a markedly lower L-dopa content, something that can perhaps be used in future efforts to utilize *Mucuna* as a food and feed.

Table 1. L-dopa content of various accessions of *Mucuna* in Ghana.

Accession	L-Dopa (%w/w)	95% confidence level
Local variety (*Adua apia*)	2.17a	0.03
White seed	3.12b	0.05
Light mottled seed	4.34c	0.04
Dark mottled seed	4.50d	0.02
Black seed	5.64e	0.05

For the past activities of the Natural Resources Institute, contact Lizz Kiff (E.K.Kiff@greenwich.ac.uk. For current activities of the GTZ project, contact Heinz Loos (gtzsun@ncs.com.gh).

Supplementing Dairy Cows with Mucuna

By E.N. Nyambati, KARI/Univ. of Florida

Inadequate and low quality feed resources and declining soil fertility limit smallholder dairy production and crop yields in the tropics. In the high and medium rainfall regions such as the highlands of northwestern Kenya, mixed farming based on high yielding fodder grasses, for example napiergrass (*Pennisetum purpureum* var. Bana), has high potential for improving both the quantity and quality of feed available throughout the year. However, as napiergrass matures, its crude protein concentration decreases. Under these systems, the main constraint to higher productivity is the inadequacy of feed, especially during the dry season when the quality of available feed is low. Feed resources for mixed crop-livestock smallholder production systems may be improved by integrating legumes for improving the nutritive value of feeds and soil fertility.

At the Kenya Agricultural Research Institute in Kitale, northwestern Kenya, we conducted a study in 1999-2000 to determine the effect of supplementing dairy cows with *Mucuna* and lablab [*Lablab purpureus* (Sweet) cv Rongai] hay to a basal diet of napiergrass. This study forms a part of the Ph.D. thesis of the author, which will be completed in 2002.

In the study, we measured various characteristics of lactating dairy cows, such as dry matter intake, apparent dry matter digestibility, milk yield and milk composition, quality and quantity of manure, body condition score, and weight gain. Eight multiparous Friesian cows were used in two 4 x 4 Latin squares with four dietary treatments. Napiergrass was the basal diet and supplement treatments included two legume hays and a

commercial dairy meal that were fed at iso-nitrogenous levels to meet the CP requirement of a 350 kg lactating cow producing 10 kg milk; the control was a basal diet of napiergrass. The chemical composition of the *Mucuna* and lablab hays in comparison to the commercial feed is shown in Table 1.

Preliminary results indicate that while *Mucuna* has potential as a protein supplement, its beneficial impacts are poorer than those of commercial dairy supplements. Both *Mucuna* and lablab hay as well as the commercial protein supplement significantly ($P<0.01$) increased total dry matter intake (130, 136 and 142 g $kg^{-0.75}$, respectively) compared to the basal diet (108 g $kg^{-0.75}$). Supplementation also increased apparent dry matter digestibility, daily milk production, and mean body condition score. Although supplementation had no effect ($P>0.05$) on manure quality, it significantly ($P<0.01$) increased the quantity of nutrients excreted. There was no indication that supplementation improved body weight gain. In most cases, commercial dairy supplement improved dairy cow performance more than did the two legume hays. Such commercial supplements are not, however, easily available to the smallholder dairy farmers in Kenya and elsewhere in the tropical countries mainly due to their high cost. Low-cost alternatives such as legume hay therefore hold promise for improved dairy production in these systems.

For further information, please contact E. Nyambati (emn@gnv.ifas.ufl.edu).

Table 1. Constituents of *Mucuna* and lablab hay.

Constitutuents	*Mucuna* hay	Lablab hay	Dairy meal
DM (%)	88.3	88.4	91.4
N (% of DM)	2.71^{b}	2.60^{bc}	2.86^{c}
CP (% of DM)	16.95^{bc}	16.25^{b}	17.85^{c}
Lignin (% of DM)	11.36	8.58	3.83
Total polyphenol (% of DM)	2.37	1.88	1.32
P (% of DM)	0.24^{b}	0.28^{c}	0.75^{d}
Ca (% of DM)	1.47^{b}	1.47^{b}	1.05^{c}
DE (Kcal kg^{-1} DM)	2.73	2.89	2.94

Analyzing for L-dopa. Part II. Alternate Methods of Extraction and Quantitation by HPLC Using Absorbance Detectors

By R. Myhrman, Judson College, Illinois, USA

Background

In the first part of this series (*Mucuna News,* Second issue, April 2001*)*, N. Szabo outlined a general approach to the analysis of L-dopa in plant material, and presented a quantitation method developed in her laboratory at the University of Florida that utilizes a high performance liquid chromatograph (HPLC) and a mass spectrometer (MS). This combination is here denoted as LC-MS. She also presented a modified version of the acidic extraction method described by Brain (1976).

As noted by Dr. Szabo, LC-MS represents the theoretical ideal for identifying and quantifying L-dopa in samples of either plant or animal origin, and represents a standard against which results from other methods can be verified by comparison. However, the required instrumentation is very expensive to purchase and maintain. (Complete systems with MS detectors are typically $150,000-200,000).

In this article, we will first describe alternate methods of extracting L-dopa from plant samples. We will remind the reader of the modification of Brain's acidic extraction method that was presented by N. Szabo, and then describe a method developed by us at Judson College (water extraction), and methods utilized by L. St-Laurent et al. (ultrasonication in water) and by P. Siddhuraju and K. Becker (ultrasonication in HCl). All four methods have been used successfully in L-dopa extraction, and the choice depends on the equipment and chemicals available to the investigator and on his/her preference.

We will then present an overview of methods for the quantitation of L-dopa in material derived from plants, and present in detail the method that we have used at Judson College over the years, namely an HPLC method utilizing an absorbance detector. The required instrumentation, as detailed below, is much less expensive to purchase and maintain than the instrumentation for LC-MS, and yet is well suited for the routine analysis of a wide variety of light-absorbing substances such as L-dopa.

In subsequent issues of *Mucuna News*, we will review methods by which the L-dopa content of plant samples can be estimated by using a spectrophotometer in place of an HPLC system. This method is relatively cheap and utilizes equipment that is more widely available, but

requires care in sample preparation and data interpretation if it is to give more than a rough estimation of the L-dopa content of the plant material. Nevertheless, it has been successfully employed in screening studies. In subsequent issues, we will also discuss the application of HPLC systems with various detectors to the determination of L-dopa in samples of animal origin, given the importance of evaluating the amount of L-dopa in milk, meat, or eggs obtained from animals consuming *Mucuna*-based feed, and in the breast milk of humans consuming *Mucuna*-based foods.

Methods for extracting L-dopa from plant material

A. Boiling in HCl (N. Szabo, *Mucuna News,* Second issue, April 2001)
As previously indicated by Dr. Szabo, this method is considered to be direct, rugged, and exhaustive. It should be noted that in the method described by Brain (1976), the samples are extracted twice, once with boiling HCl and ethanol, the second time with ethanol alone. In the modified procedure described by Dr. Szabo, the sample is also extracted twice, but with HCl and ethanol both times.

B. Boiling in water (R. Myhrman, Forthcoming.)
This method, which we use routinely in our laboratory at Judson College, utilizes four extractions with boiling water rather than one or two extractions with HCl and ethanol. To verify the completeness of extraction using water, we have periodically subjected samples of treated material to further extraction with 0.1 M HCl, and have observed no additional release of L-dopa. The water method avoids the hazards associated with boiling acid solutions, including the release of acidic vapors into the air of the laboratory, and seems to remove less extraneous material from the sample.

Procedure (typically done in triplicate for each sample):
1) Weigh out 0.1875 grams of powdered seed into a 16mm(O.D.) x 100 mm glass culture tube (screw cap style).
2) Add 5 mL of distilled water, cap the tube, and vortex in several bursts (or agitate vigorously by hand) until the flour is suspended.
3) Remove the cap, and insert the tube in the boiling water bath for 6 minutes.
4) Replace the cap, and centrifuge for 2 minutes in a tabletop centrifuge. Transfer the supernatant to a 100 mL volumetric flask
5) Repeat steps 2 through 4 three additional times (for a total of four extractions), and combine the extracts in the volumetric flask. (During the final extraction, boil for 10 minutes instead of 6.)
6) Dilute the combined extracts to 100 mL, and mix gently (since L-dopa is oxygen-sensitive) but thoroughly.
7) Filter 3-4 mL of the solution through a 0.45 µm nylon filter (25 mm diameter).

Notes:
1. The extraction procedure is best done in subdued light due to the photosensitivity of L-dopa.
2. Samples from other plant parts (e.g. leaves) may require more than 5 mL of water.

C. Ultrasonication in water (St. Laurent et. al., Forthcoming)
This method was devised several years ago by Francois Lorenzetti, then at the University of Ottawa, Canada, as a rapid alternative to methods (such as A and B above) that require boiling and centrifugation.

Procedure:
1) Combine 0.1 g of powdered seed and 15 mL of distilled water in a 25 mL Corning® vial with stirring.
2) Immerse the vials in a sonication bath for 5 minutes. (No additional extraction was observed using 10 minutes of sonication.)
3) Filter the extracts through 0.45 µm nylon filters to prepare them for chromatography.

D. Ultrasonication in HCl (Siddhuraju and Becker, 2001)
This method includes features of Methods A and C.

Procedure:
1) To 100 mg of finely ground and defatted seed, add 5 mL of 0.1 N HCl, and stir for 10 minutes at room temperature.
2) Subject the mixture to sonication (Ultraturrax T25 at 20,500 min^{-1} for 30 seconds in an ice bath), then stir for 1 hour at room temperature.
3) Collect the supernatant by centrifugation (13,000 rpm, 15 minutes).
4) Perform two additional extractions, and combine the three supernatants.
5) Filter the solution through a 0.2 µm glass filter.

Methods for quantifying L-dopa in plant material following extraction

A. LC-MS (Separation of the sample into its individual components by high performance liquid chromatography, followed by identification and quantitation of each component by mass spectrometry). (N. Szabo, *Mucuna News,* Second issue, April 2001)
Although the cost is high (complete systems are typically $150,000-200,000), a mass spectrometer gives more information than any of the other detectors that are commonly incorporated into liquid chromatography systems. In addition to measuring the amount of each

substance present, the MS can provide positive identification of a substance without requiring that the analyst have an authentic sample available for comparison. In addition, because the mass spectrum of each substance is unique, the instrument can measure the individual components of challenging samples, such as plasma, even if a complete separation of all substances is not possible.

B. HPLC with absorbance detectors (e.g. R. Myhrman, Forthcoming)

Since L-dopa readily absorbs light of about 280 nm wavelength, absorbance detectors represent an effective and relatively economical means for quantifying the substance as it elutes from a chromatography column. Either fixed- or variable-wavelength detectors can be used for routine analyses in cases where the chromatographic column is able to produce a clean separation of the L-dopa from other substances that absorb near 280 nm. However, the more expensive variable-wavelength or "tunable" detectors are more sensitive as well as more versatile, and are therefore better suited for measuring the very low concentrations of L-dopa which are the goal in preparation of food and feed.

The ideal absorbance detector is the photodiode array ("PDA" or "DAD"). These collect an entire absorbance spectrum at selected intervals (typically one per second) as the separated components of a sample emerge from the chromatograph. This makes possible the simultaneous determination of several substances of interest with a single chromatographic run, regardless of the wavelengths at which they absorb. A PDA detector also allows the analyst to recognize the presence of other absorbing substances that may have emerged at the same time as the substance of interest. A correction can then be applied to avoid positive errors in the reported concentration.

In general, absorbance detectors are relatively inexpensive, easy to use and maintain, and suitable for the analysis of a wide variety of samples.

In the U.S., an HPLC system with comparable features to an LC-MS system, but with a variable-wavelength ("tunable") absorbance detector and no mass spectrometer, costs about $30,000 – 35,000. Systems in this price range can run batches of samples unattended, and are capable of changing the composition of the solution entering the chromatography column during the separation stage of the analysis. The latter feature, called "gradient elution," makes possible the analysis of more complicated mixtures, and also facilitates cleaning and maintenance of the chromatography column. Systems with PDA detectors are priced in the $40,000 – 50,000 range. Adding a tunable detector to an existing system costs between $5,000 – 10,000, while a PDA with the required software is about $20,000.

In situations in which budget limitations are severe, entry-level HPLC systems with absorbance detectors can be assembled for as little as $10,000 –15,000. Those at the upper end of this range are more sensitive and offer better opportunity for subsequent upgrading.

The method that we have most often utilized at Judson College for the analysis of L-dopa in plant material by HPLC is as follows:

Instrumentation: Two Rainin HX pumps with pressure monitor controlled by Dynamax software (Macintosh). Waters 717+ autosampler set at minimum syringe draw rate; sample compartment temperature 15 °C. Waters 991M photodiode array detector with Waters Millennium software, or PerkinElmer 200 tunable absorbance detector with Varian Star software. The column is thermostatted at 30 °C, and a 0.5 µm inline filter is fitted between the autosampler and the guard.

Column: Zorbax StableBond SB-C18, 4.6 x 150 mm, 3.5 µm particles, Part # 863953-902, fitted with corresponding guard, Part # 820950-920. (Source: MAC-MOD Analytical, 127 Commons Court, Chadds Ford, PA 19317, 1-800-441-7508)

Mobile phase: Component A: 0.1 M phosphoric acid, 1 mM 1-octanesulfonic acid (Sigma O-8380), 2 mM disodium EDTA, adjusted to pH 3.0 with NaOH prior to final dilution with water of 18 megohm resistivity. (The octanesulfonic acid is an “ion-pairing reagent” which increases the affinity of the column for L-dopa, thereby improving the separation.) Component B: HPLC grade methanol

Elution: 90% A, 10% B at 1.0 mL/min for 15-20 minutes. Injection volume: 40 microliters. Standard: L-dopa (Sigma D-9628), 1.00 mM in water. Detection wavelength: 279 nm.

Note:
After every twenty or thirty runs, water is substituted for the "A" component of the mobile phase, and the column is flushed with a gradient to 100% methanol. The column must then be flushed in mobile phase for at least one hour at 1 mL/min to allow the column to re-equilibrate with the ion-pairing reagent.

C. HPLC with other detectors

While absorbance detectors have been used with success by many investigators for the analysis of L-dopa in material derived from plants, other options are available, including fluorescence and electrochemical detectors. These other options must be considered when designing an analysis for L-dopa in samples of animal origin, and will be discussed in a subsequent issue of *Mucuna News*.

D. Spectrophotometry

Before HPLC systems came into common use, the determination of L-dopa in plant material was typically performed by measuring the absorbance of the treated sample in a spectrophotometer. This method formed the basis for the extensive surveys conducted by Daxenbichler *et al.* in the early 1970's. Those workers were able to demonstrate an excellent correlation with results obtained using an amino acid analyzer, which was in fact an early example of a specialized HPLC system. While the required equipment is more readily available and much less expensive than that for HPLC, considerable care must be exercised in the preparation of samples and in the interpretation of data.

We will discuss methods based on spectrophotometry in a subsequent issue of *Mucuna News*.

Discussion

Extraction methods

The methods detailed above and in Part I of this series (*Mucuna News,* Second issue, April 2001) all rely on release of L-dopa from plant material into water or an ethanol-water mixture by the use of heat or ultrasonic agitation, with or without the presence of 0.1M HCl and a prior defatting step.

Comparisons of boiling and sonication in water (Methods B and C) in our laboratory have produced results agreeing within 3% of each other (e.g. L-dopa percentages of 1.00 and 1.03), with relative standard deviations (the standard deviation divided by the mean) for 11 or 12 replications of less than 1% for boiling, and about 2% for sonication. We have also observed very good agreement for parallel samples that were treated by boiling in our lab, but by sonication in the lab of J. T. Arnason at the University of Ottawa in Canada.

We also find good agreement in our lab between results from samples treated with or without HCl by either boiling or sonication, especially in cases where the amount of L-dopa in the sample is very low.

In summary, any of the above extraction methods can produce accurate results, so the choice is largely a matter of personal preference and available equipment. However, one cannot help but be impressed by the simplicity and rapidity of the Lorenzetti method (C), and we have used it in screening situations where fine distinctions between samples are not required, and where the larger standard deviation of the sonication method is therefore acceptable.

Quantitation Methods

Columns and conditions:

The literature reveals that the typical column for analysis of L-dopa by HPLC is an “octadecyl” or “C18,” and that columns from several different manufacturers have been successfully utilized. Simple acetonitrile/water mobile phases (the solution that carries the sample through the column), as well as more complicated phosphate-based mixtures, have been employed with either isocratic (constant composition) or gradient elution. For LC-MS systems, simple mobile phases result in more efficient operation of the mass spectrometer.

On some brands of columns, the elution time of L-dopa is markedly increased, and the separation thereby improved, by adding an ion-pairing reagent such as octanesulfonic acid to the mobile phase, as illustrated in the method detailed above which is employed at Judson College.

At Judson, the aqueous component of our mobile phase also includes a phosphate buffer for pH control, and a substance called EDTA that minimizes effects from metal ions that might be present in the sample. While this solution takes time to prepare, it results in a well-controlled environment inside the chromatography column where the mixture is being separated into its individual components. We have found that we can run large batches of samples without the need to make individual pH adjustments, and do so with a high degree of reproducibility.

Detectors:

Chromatographic detectors are described as specific or non-specific. Specific detectors respond to only certain compounds, and can therefore measure particular components in complicated mixtures even if the components of the mixture are not completely separated by the chromatography column. On the other hand, they cannot even detect the presence of many substances.

Non-specific detectors respond to all, or nearly all, compounds in a mixture, but are unable to determine the amount of the component of interest if the separation is not complete, as is often the case.

The ideal detector would have both characteristics; it would miss nothing, but be able to specifically measure anything. A mass spectrometer comes as close as possible to this ideal, but at a high price.

Absorbance detectors represent an attractive alternative. Since many compounds absorb light, and do so at different wavelengths, a tunable absorbance detector can be adjusted so be specific for different compounds. A PDA detector, on the other hand, is both non-specific

and specific. It simultaneously detects all compounds that absorb light anywhere within its range, but can then present data at any wavelength that is equivalent to that of a tunable detector set to that wavelength.

While absorbance detectors have worked well for the determination of L-dopa in samples from plants, detectors of greater specificity are generally required for the analysis of samples of animal origin. MS is one possibility; less expensive alternatives will be discussed in subsequent issues.

For further information, please contact R. Myhrman (rmyhrman@judson-il.edu)

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Selected Bibliography of Mucuna

Introduction

In this issue, we will present articles that specifically focus on L-dopa and alkaloids in *Mucuna*. Please note that many articles presented in the previous issues (which focused on nutritional characterization of *Mucuna* seeds) also include information on *Mucuna*'s anti-nutritional compounds. As in Part I, we will list the most recent articles first.

As always, if you are unable to locate any of these materials please contact M. Eilittä (meilitta@hotmail.com). Additionally, please inform her of any significant materials not listed here.

Part II. L-dopa and Alkaloids in Mucuna

Balaban, M.O. and A.A. Teixeira. Potential home and industrial process treatments to reduce L-Dopa in *Mucuna* bean. Forthcoming. Food and Feed From *Mucuna*: Current Uses and the Way Forward. Proceedings of a workshop held in Tegucigalpa, Honduras, April 26-29, 2000. CIDICCO, Honduras. (Please watch future issues of this bulletin for further information.)

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Photo 1. *Mucuna* seeds being used for the popular "*Adji*" game in southern Benin. Photo *by* R. Carsky.