l-Dopa Recovery from *Mucuna* Seed

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A simple hot water extraction and ion exchange procedure gave excellent recoveries of *l*-dopa from seed of nine species of *Mucuna*. Use of a modified dextran column as an additional step permitted recovery of nearly all the *l*-dopa present in the seed. Yields from eight species examined ranged from 3.1 to 6.1% of the mature seed.

In a screening survey for 3-(3,4-dihydroxyphenyl)-L-alanine (*l*-dopa) in seed from more than 1000 species in 135 plant families by Daxenbichler *et al.* (1971), the only seed found to have enough *l*-dopa to suggest possible use as a commercial source came from the genus *Mucuna* (Leguminosae). Assays for *l*-dopa in defatted, air-dried seed meal from 11 species of *Mucuna* ranged from 3.1 to 6.7%. In several species, Bell and Janzen (1971) found 5.9–9.0% *l*-dopa in the 60–85% of the seed that remained after removal of the seed coat. Bell *et al.* (1971) isolated *l*-dopa in 3.9% yield from ground seed of *M. mutisiana*. A patent has been issued (Wysong, 1966) for recovery of *l*-dopa from velvet beans, members of the genus *Mucuna* formerly sometimes classified in the genus *Stizolobium*. The patent reported the yield of *l*-dopa as 1.9% of the seed. Our alternate method of recovery produces greater yields of *l*-dopa from several species of *Mucuna*.

MATERIALS AND METHODS

The whole seed was ground in a Wiley mill equipped with a 2-mm screen. Samples of 100 g of ground seed were extracted with hot distilled water three times. The extraction mixture was heated by a steam bath and stirred manually at 10-min intervals. The first extraction was for 60 min with 1500–2000 ml of water. The extraction mixture was cooled to near room temperature by placing the container in cold water and stirring its contents. The meal solids were then separated by centrifuging and the supernatant liquid was decanted and saved. The meal residue was suspended in 1500 ml of hot water for 40 min. After again cooling and centrifuging, the meal residue was reextracted with 750 ml of hot water for 20 min. The measured volume of the combined extracts was usually about 4 l. An aliquot (0.001 of the total extract) was diluted to 100 ml with ethanol, and *l*-dopa was measured by the ultraviolet (uv) absorption of a filtered portion. A sample of the wet meal residue, equivalent to about 0.5 g of dry meal, was assayed for unextracted *l*-dopa by addition of 3 ml of 0.1 *N* HCl and subsequent extraction and uv measurement as described by Daxenbichler *et al.* (1971).

Between 400 and 450 ml of Bio-Rad AG1X2 (50–100 mesh) resin was placed in a 4-cm column, converted to the OH^- form with 1 *N* NaOH, and washed with water until the effluent was neutral. The entire extract from the meal was then passed through this ion exchange column, followed by 1–2 l. of wash water. Because extracts from *M. holtonii*, *M. mutisiana*, *M. sloanei*, and *M. urens* were viscous, their passage through the column was facilitated by applying a slight amount of air pressure. The materials retained by the column were eluted with 10% acetic acid solution, and the eluate was collected in 200-ml volumetric flasks. Aliquots from the flasks were diluted with ethanol, and the *l*-dopa content was measured by uv absorption.

Usually only three flasks contained the *l*-dopa, and these were combined and concentrated to about 100 ml. After the concentrate remained overnight in a refrigerator, the first crop of crystals (Table I) was collected by filtration. The mother liquor and washings from the first crystallization were concentrated to a volume of about 40 ml and again left overnight in the refrigerator for additional crystallization. The second crop was filtered off, and the mother liquor and washings were applied to a 5 × 100 cm Sephadex G-10 column. The components were eluted from the column with water at a flow rate of 70 ml/hr and collected in three tubes per hour. The column eluate was passed through a recording refractometer and measured against a water reference. Samples after the void volume (615 ml) were examined by thin-layer chromatography (tlc), as described by Daxenbichler *et al.* (1971), with ninhydrin as the detection reagent. Contents of appropriate tubes were combined into fractions on the basis of tlc and peaks on the refractometer chart.

The uv absorption (230–310 nm) of the various fractions was measured, and these measurements provided from one fraction an estimate of the final amount of *l*-dopa and from another fraction an estimate of the total of the two tetrahydroisoquinoline compounds reported by Bell *et al.* (1971) and Daxenbichler *et al.* (1972). All preparations of *l*-dopa and of the tetrahydroisoquinoline compounds were also examined by infrared (ir) spectroscopy and by paper chromatography or tlc. The paper chromatography was performed on Whatman No. 1 paper with the upper layer of 1-butanol–acetic acid–water (4:1:3 v/v) as solvent.

Ir spectra were taken in KBr wafers with a Perkin-Elmer Model 337 spectrophotometer. Uv measurements were made with a Beckman Model DK-2a recording spectrophotometer. The recording refractometer was from Waters Associates. A Cary Model 60 recording spectropolarimeter was used for the optical rotation measurements.

RESULTS AND DISCUSSION

Most of the *l*-dopa obtained came from the first crystallization after concentrating the eluate from the anion exchange column (Table I). Although the preparations were off-white to tan in color, their ir spectra were identical to that of authentic *l*-dopa. No impurities were detected by tlc or paper chromatography. Some impurities noted in the ir spectra of some second-crop preparations were not detected with ninhydrin on either tlc or paper chromatography. Conditions for the first and second crop preparations were selected simply to give the largest amount of *l*-dopa easily after the anion exchange separation step and to give about 40 ml of final

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Table I. *Mucuna* Seed Composition and *l*-Dopa Isolation Data, Percent of Air-Dried Seed

Species	Moisture	Oil	Nitrogen	THIQ[a]	*l*-Dopa				
					Assay[b]	Crystallization: First crop	Crystallization: Second crop	Fraction from Sephadex column	Total recovered
M. aterrima	7.5	3.3	4.4	0.32	4.4	1.76	0.61	0.94	3.31
M. deeringiana (Early Jumbo)	11.0	4.6	4.1	0.17	3.6	2.28	0.02	0.76	3.06
M. deeringiana (90-Day Speckle)	8.2	4.4	4.1	0.46	4.2	1.58	0.66	0.89	3.13
M. gigantea	10.4	6.9	2.7	0.09	3.7	2.04	1.21	0.53	3.78
M. holtonii	10.7	4.2	2.6	0.16	6.1	4.84	0.70	0.59	6.13
M. holtonii[c]	10.3	2.0	3.9	0.55	8.9	4.92	1.15	0.78	6.85
M. mutisiana	10.1	3.6	2.8	0.41	4.7	3.57	0.21	0.80	4.58
M. prurita	11.1	4.7	4.5	0.17	4.8	2.99	0.57	0.38	3.94
M. sloanei	6.9	4.9	2.6	0.23	5.3	2.30	2.05	0.48	4.83
M. urens	10.8	5.0	2.2	0.36	4.9	3.84	0.51	0.57	4.92

[a] Weight of tetrahydroisoquinoline fraction (see text) from Sephadex G-10 column. [b] Calculated from determination on oil-free meal. [c] Immature seed.

mother liquor for application to the Sephadex column. As shown in Table I, significant additional amounts of *l*-dopa (0.4–0.9% of the seed) were recovered by use of the Sephadex G-10 column.

All meal residues were tested for unextracted *l*-dopa, but the dilute HCl extract showed little uv absorption. Since its maximum was at 280 instead of at 283 nm as found for *l*-dopa, we believe it contained no significant amount of *l*-dopa. If attributed to *l*-dopa, the amount present was never equivalent to more than 0.1% of the seed.

Small-scale preparations of *l*-dopa were made from seed of *M. aterrima*, *M. deeringiana*, *M. gigantea*, and *M. holtonii*, using only anion exchange column and crystallization steps. Rotation measurements for these materials, $[\alpha]_D$ − 11.9° to $[\alpha]_D$ − 12.7°, agreed well with literature reports for *l*-dopa; for example, $[\alpha]_D$ − 12.0° reported by Sealock (1949).

Uv assays indicated 6.1–6.7% *l*-dopa in mature seed of *M. holtonii* and as high as 13.8% in immature seed. In seed taken from a single plant in El Salvador 4 months before mature seed was harvested from the same plant, the assay showed 8.9% *l*-dopa present; 6.9% was recovered by isolation. The mature seed, however, proved to be about seven times as large as this selection of immature seed (7500 g/1000 seed and 1130 g/1000, respectively) and about 12 times as large as the most immature sample from the same vine (630 g/1000, 13.8% *l*-dopa by uv, insufficient sample for recovery study). Although the highest recovery (Table I) was from immature seed of *M. holtonii*, this apparent advantage is more than offset by the greater amount of *l*-dopa per seed at maturity.

Moisture, oil, and nitrogen were determined on the ground seed (Table I). A high nitrogen value does not necessarily indicate a high *l*-dopa content; those species of the *Stizolobium* type appear to be richer in nitrogen than other species but not in *l*-dopa.

Pilot plant studies and detailed cost analysis would be required before the commercial feasibility of the procedure described here can be determined. However, extraction is quick and complete with only hot water, and both the anion exchange resin and Sephadex G-10 may be reused many times in the recovery operation without obvious decrease in effectiveness. The anion exchange column must be regenerated to the OH^- form and washed to neutrality before reuse, but no such regeneration is necessary with the Sephadex column.

Many other components were detected in the fractions that were eluted from the Sephadex column before *l*-dopa; no attempt was made to identify all of them. All fractions were screened for uv absorption, however, to detect possible sources of interferences with determination of *l*-dopa in the initial assays. Only minor amounts of uv absorption were encountered in fractions preceding *l*-dopa except for one peak that often appeared just before it on the refractometer recordings. This peak was observed with preparations from *M. holtonii*, *M. mutisiana*, *M. sloanei*, *M. urens*, and *M. gigantea*. The material responsible for this peak was not detected by ninhydrin on tlc but did show uv absorption, λ_{max} 260 nm (H_2O) from *M. gigantea* and λ_{max} 266 nm (H_2O) from the others. A crude preparation of the fraction from *M. holtonii* contained nearly 17% nitrogen, but the material was not further investigated.

Following the elution of *l*-dopa, all preparations gave one additional peak on the refractometer chart. This fraction gave a yellow-brown spot with ninhydrin on tlc and migrated at a rate very similar to that of *l*-dopa. The amount of the compound when calculated as *l*-dopa from the uv absorption agreed closely with the weight that is listed for the tetrahydroisoquinoline fraction in Table I. This fraction from all species examined was found by paper chromatography to contain both the L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline isolated by Bell *et al.* (1971) from *M. mutisiana* and the methylated derivative isolated from *M. deeringiana* by Daxenbichler *et al.* (1972). Since the uv absorption spectra of these compounds show a maximum at 282 nm in acid solution, they are presumed to be included in initial *l*-dopa estimates.

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Nutritional Characteristics of Marine Food Fish Carcass Waste and Machine-Separated Flesh

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The yield and nutritional characteristics of whole carcass waste, machine-separated flesh, and bone–skin fractions of six marine food fish were determined. Yields of flesh from whole carcass waste ranged from 48.9 to 60.2% and resulted in a 43.7–55.3% recovery of the total protein, 42.7–66.0% of the fat, and 13.3–21.6% of the ash. Levels of phosphorus, calcium, strontium, manganese, boron, and chromium largely associated with bone and skin were greatly reduced in the flesh fractions over levels found in the whole carcass wastes. Concentrations of potassium, sodium, and iron were higher in the flesh fractions. Calcium/phosphorus and potassium/sodium ratios were generally lower in separated-flesh fractions. Machine separation of bone and skin markedly improved the quality of protein in carcass waste. Protein efficiency ratio values for separated flesh fractions were significantly higher than values for whole carcass waste.

Demersal species of marine food fish represent a sizeable food resource. Oregon landings alone in 1969 amounted to over 21 million pounds of round weight (Fish Commission of Oregon, 1971). Using as a basis an average fillet yield of 30%, the remaining 70% waste would represent 14.7 million pounds of material. At a mean protein content of 14%, the waste from landings in Oregon alone would represent well over 2 million pounds of protein.

Presently, this waste material is being utilized for mink and pet food, for the preparation of low-grade fish meal, and for crab bait. The lower protein and high mineral content of this waste tends to limit its scope of utilization and value.

During the last 20 years machines have been developed in Japan (Tanikawa, 1963) and in the Scandinavian countries which remove edible flesh from bone and skin in a coarsely minced form. More recently, equipment which accomplishes the same objective has been developed in the United States. Machine separators have been recently evaluated as a means of recovering flesh from dressed fish (Miyauchi and Steinberg, 1970) and from dressed and filleted fish carcasses (King and Carver, 1970).

The purpose of this investigation was to determine the yield of flesh which could be obtained from carcass waste by machine separation of bone and skin and to evaluate the effect of machine separation on the composition and nutritional characteristics of the separated fractions. Emphasis was placed on the nutritional characteristics of the protein and mineral fractions.

EXPERIMENTAL

Carcass waste was obtained by random selection from two commercial filleting plants. Approximately 200 lb of carcasses and associated waste was collected for each of six different species of fish that varied greatly in their anatomical features.

A portion (45 lb) was ground through a 0.75-in. plate using a dual-cut meat chopper and then passed through a high-speed mill equipped with a head possessing 0.012-in. openings. The remaining fraction (155 lb) was passed through a Yanagiya Fish Separator ("miny" model) equipped with a perforated rotating drum with 4-mm openings. The head and collar portions of species (rockfish and ling cod) that could not be accommodated by the laboratory scale separator were passed once through a 0.75-in. plate prior to processing. Separated flesh and bone–skin fractions were milled. The three milled samples for each species (whole carcass waste, separated flesh, and bone–skin fraction) were each thoroughly mixed for 20 min with a mechanical mixer. Milled whole carcass waste and the separated flesh fraction were dried using a laboratory atmospheric double-drum drier. Both dry and wet samples were vacuum sealed in moisture vapor-proof packaging material and held at −35°C prior to analysis.

Proximate analyses were carried out on wet, milled samples. Samples for mineral analyses were dried in vacuum at 65°C and passed through a 1-mm sieve of a high speed mill with Dry Ice. Drum-dried samples used to determine protein efficiency ratio (PER) values were mixed with an equal weight of cornstarch and passed through a 0.02-in. sieve of a high speed hammer mill. The use of Dry Ice and the mixing of drum-dried samples with cornstarch was necessary to assure thorough milling of samples high in fat.

Moisture, ash, protein (total N × 6.25), fat, and PER values were determined according to A.O.A.C. procedures (1965a, b, c, d, and e, respectively). Mineral elements were determined by WARF Institute, Inc., Madison, Wis., using direct reading emission spectroscopy methods described by Christensen *et al.* (1968). Data were analyzed by analysis of variance and the differences in PER value means tested by the least significant difference (LSD) method.

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