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Chemotaxonomic significance of hydroxylated pipecolic acids in Central American *Inga* (Fabaceae: Mimosoideae: *Ingeae*)

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Abstract

Hydroxylated pipecolic acid derivatives of 47 species of *Inga* were analyzed primarily from herbarium leaf samples supplemented by field collections. *Inga* species are noted for their morphological variability, which has resulted in considerable taxonomic confusion. Pipecolic acid chemistry appears to be a relatively stable character within species, and the large number of pipecolic acid derivatives translates to greater intraspecific diversity in chemical patterns (18 distinct hydroxypipecolic acid patterns) than previously found in related genera. This chemical diversity makes pipecolic acids particularly useful in solving taxonomic problems in this genus; problems such as identification of distinct species with convergent morphology and identification of mislabeled herbarium specimens. Chemistry provides data independent of morphology with which to assess recent changes in nomenclature (some of which are supported, others not) and may also be useful in the assessment of hybridization and in delimitation of proper species boundaries. At present our chemical knowledge is too fragmentary to allow strong statements regarding relationships above the species level, but there is a trend for taxa traditionally grouped on morphological criteria to share similar chemistries.

Keywords: *Inga* Fabaceae Mimosoideae pipecolic nonprotein amino acid chemotaxonomy

1. Introduction

The genus *Inga* has nearly 400 species, making it one of the larger genera of mimosoid legumes (Elias, 1978). The genus is restricted to the American tropics with

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origins in the low-elevation rainforests of Brazil and extensions north to central Mexico (Leon, 1966). The move into Central America has been accompanied by significant changes in habitat; *Inga* appears to be the only member of its tribe (Ingeae) to venture into high-elevation cloud forests. Species of *Inga* are difficult to delimit due to extraordinary intraspecific variation (Leon, 1966). Sousa's (1993) recent treatment in preparation for the *flora mesoamericana* added 13 new species and reduced 32 others to synonymy; at present there are 81 species of *Inga* (33 endemics) recognized in the Central American flora. There is a great deal of confusion surrounding nomenclature and many species are regarded as only tentative pending further study. With morphological variation making taxonomic treatment difficult, secondary chemistry may provide unique insights into delimitation of species boundaries and relationships among taxa.

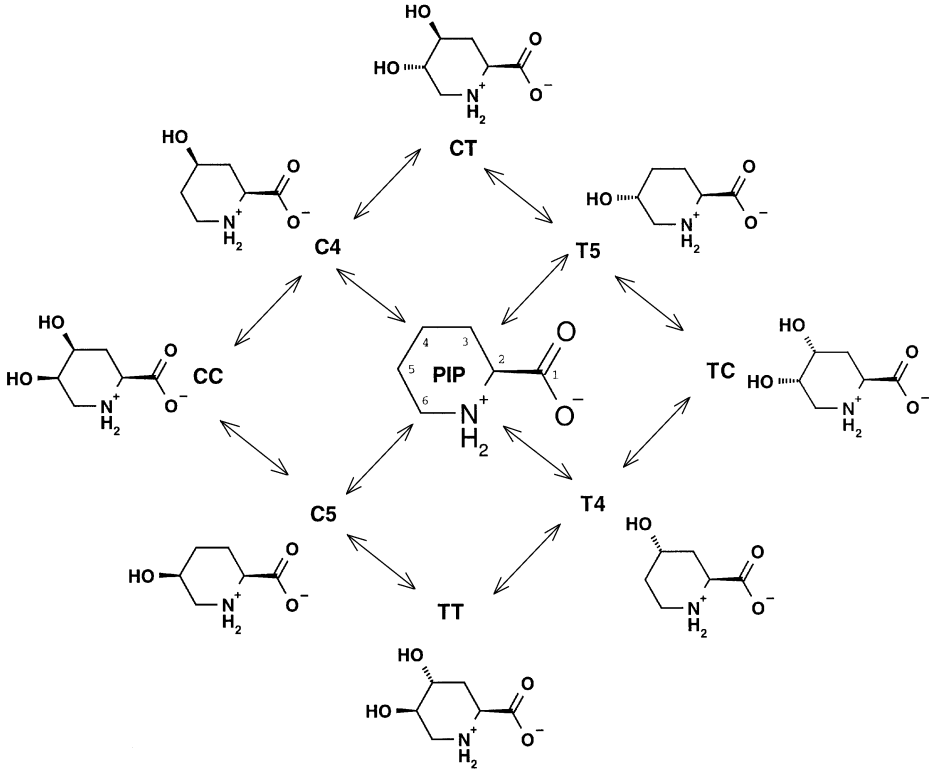

Fig. 1. Biosynthetic pathways to hydroxypipecolic acids. Pipecolic acid is derived from lysine via cyclization. Monohydroxypipecolic acids are synthesized from pipecolic acid; dihydroxypipecolic acids are synthesized from monohydroxy-precursors with the same absolute stereospecificity. A unique enzyme catalyzes each reaction and all reactions are reversible. Only one monohydroxypipecolic acid precursor is required for synthesis of a dihydroxypipecolic acid. Abbreviations: PIP pipecolic acid; C4 *cis*-4-hydroxypipecolic acid; CT 2,4-*cis*-4,5-*trans*-4,5-dihydroxypipecolic acid; C5 *cis*-5-hydroxypipecolic acid; CC 2,4-*cis*-4,5-*cis*-4,5-dihydroxypipecolic acid; T5 *trans*-5-hydroxypipecolic acid; TC 2,4-*trans*-4,5-*cis*-4,5-dihydroxypipecolic acid; T4 *trans*-4-hydroxypipecolic acid; TT 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid.

Pipecolic acid derivatives are promising candidates for chemotaxonomic work (Morton and Romeo, 1990). There are eight possible mono- and dihydroxy stereoisomers of pipecolic acid with hydroxyls at carbons four and/or five (Fig. 1). The biosynthetic reactions involved in the production of 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid (TT) are known from a radiolabel tracer study (Swain and Romeo, 1988); TT can be synthesized in a reversible reaction from either monohydroxy precursor (2,4-*trans*- or 2,5-*cis*- monohydroxypipecolic acid). The remaining pathways are inferred from distribution patterns where dihydroxypipecolic acids are only found in conjunction with the appropriate monohydroxy precursors and they are assumed to be equally stereospecific (Romeo and Morton, 1994). Most hydroxylated pipecolic acid derivatives are found only in the Mimosoideae and are restricted to a few genera (Romeo, 1989). Rare derivatives including *trans*-4-acetylaminopipecolic acid (Marlier et al., 1979), *trans*-4-methoxypipecolic acid (Morton et al., 1991) and other uncharacterized imino acids are known from *Inga* but their biosynthesis has not been examined. Derivatives of pipecolic acid have been used in chemotaxonomic studies of other Mimosoid legumes (Krauss and Reinbothe, 1973; Evans et al., 1977; Bell, 1978; Romeo, 1984, 1986) but no other genus has the diversity of pipecolic acid structures found in *Inga*. Hydroxylated pipecolic acids are known to be stable characters of species in closely related genera (Romeo and Morton, 1994) and preliminary studies in *Inga* suggested that a chemotaxonomic treatment would be feasible (Morton and Romeo, 1990). The objectives of this study were to use pipecolic acid chemistry to evaluate recent changes in nomenclature and assess proposed relationships using independent chemical evidence. The sampling strategy involved relatively intensive sampling of a few species with subsequent samples distributed across many species to survey the extent of interspecific variation.

2. Materials and methods

Plant material: small samples ($\sim$100 mg) were obtained from the New York botanical garden herbarium or other sources where noted (Appendix A). In total, 181 herbarium specimens representing 44 species were examined with one to 23 samples/species. Herbarium specimens were chosen to span the natural geographical and elevational ranges of each species and to span the range of variation in age since collection. An additional 52 unvouchered field samples from five species (three not included in the NYBG) were collected in Costa Rica (Morton and Romeo, 1990).

Extraction: Extracts were prepared using a method modified from Singh et al. (1973). Samples were ground in a mortar and pestle, extracted (x3) 8 h with a 2.0 ml volume of MeOH:$CHCl_3$:H_2O 12:5:1 (total extraction time 24 h, total volume 6.0 ml). The combined extract was separated into organic and aqueous phases by addition of 1.0 ml $CHCl_3$ and 1.5 ml H_2O. The aqueous upper layer was collected and dried under a stream of air at 30°C. Imino acids were redissolved in 0.5 ml 25% EtOH for storage and analysis.

Identification: Extracts were analyzed on Whatman #1 paper using high-voltage electrophoresis (HVE) in one direction (30 min at 4400 V in pH 1.9 buffer [37.5 ml

88% formic acid: 147 ml glacial acetic acid: 1820 ml deionized H_2O]). Most extracts were further analyzed using a two-dimensional system of HVE (45 min, same buffer) followed by butanol : formic acid: H_2O (150 : 34 : 16) for 10–12 h at 90° orientation. The 1-D system provided sufficient resolution for simple patterns, the 2-D system was used for chemically complex samples and was sufficient to resolve all but the comparatively uncommon HVE "Back Band" dihydroxies (2,4-*trans*-4,5-*cis*-dihydroxypipecolic acid = TC and 2,4-*cis*-4,5-*trans*- dihydroxypipecolic acid = CT).

Visualization: Ninhydrin was the primary means of detection (2% acetone dip, 2–3 min. at 100°C). Following ninhydrin reaction, compounds were observed under natural light and long wave (366 nm) UV. Imino acids were identified by their ionic mobilities, R_f values, co-electrophoresis and co-chromatography using authentic markers, and specific color reactions with ninhydrin. Unknowns were tested with Dragendorff's, Ehrlich's and Isatin reagents (Smith, 1969).

3. Results and discussion

Paper chromatography is a traditional method for rapid assessment of imino (heterocyclic nitrogen-containing) acids and is particularly useful in resolving complex stereospecific mixtures of pipecolic acid derivatives (Rosenthal, 1982). Imino acids have several qualities which make them particularly suitable for chemotaxonomic study: they accumulate to high levels so minimal amounts of plant material are needed (the 100 mg needed can often be found in the loose material envelope of a vouchered specimen), they show minimal degradation with age, extraction is quick and inexpensive, and they can be stored for extended periods. Work with herbarium material provides distinct advantages including: identification of plant samples is assured, as subsequent revisions are undertaken the actual samples that have been chemically investigated remain, it is far less expensive than mounting expeditions for plant material, and more taxa can be assayed over wider geographical areas (Phillipson, 1982).

Fig. 1 illustrates the biosynthesis of hydroxylated pipecolic acids; each dihydroxypipecolic acid is synthesized from one or both monohydroxy-precursors. The presence of any pipecolic acid derivative is due to a specific enzyme responsible for its synthesis. All reactions are readily reversible with the exception of lysine to pipecolic acid, which highly favors the formation of pipecolic acid (Swain and Romeo, 1988). The chromatographic system used could not resolve the "back band" dihydroxies (CT and TC) but in the absence of either monohydroxy precursor of CT (C4 or T5) the identity of the backband dihydroxy was assumed to be the more common TC if T4 was present (CT is extremely rare).

3.1. Chemical stability within species

The first step in determining the utility of pipecolic acid derivatives in chemotaxonomy was to sample several *Inga* species intensively. Preliminary work based on field collected samples suggested that chemistry may be useful but was hampered by a lack

of available specimens and difficulty in identifying sterile material in the field (Morton and Romeo, 1990). This study made use of herbarium materials in an attempt to increase sample sizes and avoid problems associated with misidentified specimens. Species chosen for more intensive sampling were *I. cocleensis* (11), I. *densiflora* (19), *I. eriocarpa* (23), *I. hayesii* (8), and *I. sapindoides* (22). Specimens were chosen to represent the greatest range of diversity in terms of range, habitat, morphology, and age since collection in an attempt to detect chemical variation should it exist.

Chemical distribution patterns are presented in traditional tabular format for individuals of three species (Tables 1–3) to illustrate the range of intraspecific variation encountered. Accumulation is scored as major ($>0.5\%$ dry weight) or minor ($\sim 0.1\%$ dry weight) based on the size and intensity of chromatographic spots. *Inga sapindoides* Willd. (Table 1, Fig. 2 pattern #4) is noted even among *Inga* for its extensive morphological variation that has resulted in a large number of binomials (for synonyms see Leon, 1966; Sousa, 1993). Samples were chosen from every Central American country and span the elevational range from 100–1300 m. If chemical variation existed, this species and these samples were intended to identify it. This species is characterized by the presence of PIP, T4, C5, (T5 is variable), TT and BB

Table 1
Inga sapindoides imino acid distribution

Sample	PIP	T4	C4	T5	C5	TT	CC	BB	T4AP	T4MP	Y1	Loc	Year
190	+	+ +		~	+	+ +		+ +			+ +		1967
10030	+	+			+	+ +		+			+	CR	1896
45784	+ +	+		+ +	+	+		+			+	CR	1926
17957	+	+ +		~	+	+ +		+ +			+ +	CR	1913
1104	+ +	+ +		+	+	+ +		+			+	CR	1983
1891	+ +	+ +			+			+ +			+	CR	1939
5816	+	+ +		~	+	+		+			+	CR	1985
3986	+	+ +		~	+	+		+ +			+	CR	1976
6905	+ +	+ +		~	+	+ +		+ +			+ +	ES	1958
23088	+ +	+ +		~	+	+ +		+ +			+ +	ES	1922
171	+ +	+ +		+	+	+		+ +			+ +	ES	1921
489	+ +	+ +			+	+		+ +			+ +	PN	1908
1190	+	+ +		+ +	+	+		+ +			+	PN	1982
8569	+ +	+ +		+	+ +	+		+			+ +	HN	1938
18390	+ +	+ +		~	+	+		+ +			+ +	HN	1966
15235	+ +	+ +		+	+	+		+ +			+ +	GT	1980
10021	+ +	+ +		~	+	+ +		+ +			+ +	GT	1927
8078	+	+ +		~	+	+		+ +			+ +	BZ	1973
3043	+	+ +		~	+	+		+			+	MX	1967
1788	+	+		~	+	+		+			+	CR	1964
5265	+ +	+ +		+	+	+		+			+ +	PN	1968
23641	+ +	+ +		~	+	+		+				CR	1986

Note: Sample numbers are those of the collector (see Appendix A). Column heading abbreviations are the same as Fig. 1 except BB (back band) which could be TC, CT, or both and Y1 which is an uncharacterized imino acid. Loc. is country of origin; CR = Costa Rica, ES = El Salvador, PN = Panama, HN = Honduras, GT = Guatemala, BZ = Belize, MX = Mexico. Year is date collected. + + major accumulator, + minor accumulator, ~ unable to assess due to overlap of large amounts of T4. This pattern of amino acids corresponds to #4 of Fig. 2.

Table 2
Inga eriocarpa imino acid distribution

Sample	PIP	T4	C4	T5	C5	TT	CC	BB	T4AP	T4MP	Y1	year
250	+ +	+ +			+	+	+	+ +				1895
24956	+ +	+ +			+	+	+	+				1953
9248	+	+ +			+	+	+ +	+ +				1930
136	+ +	+ +			+	+	+ +	+				1866
5527	+	+ +			+	+	+	+ +				1934
1842	+ +	+ +			+	+	+	+ +				1927
8977	+	+ +			+	+	+	+				1936
456	+ +	+ +			+	+	+	+				1932
1701	+	+ +			+	+	+	+				1897
22	+	+ +			+	+	+	+				1936
9089	+	+ +			+	+	+ +	+				1936
14181	+	+ +			+	+	+	+				1910
23523	+	+ +			+	+	+	+				1965
4032	+ +	+			+	+	+	+				1971
668	+ +	+ +			+	+	+ +	+				1930
14125	+	+			+	+	+	+	+			1939
9259	+	+ +			+	+	+	+ +	+			1936
2724	+	+ +			+	+	+ +	+	+			1967
3425	+ +	+ +			+ +	+	+	+	+			1981
9997	+ +	+ +			+ +	+	+	+	+			1937
10074	+ +	+ +			+ +	+	+	+ +	+			1937
3981	+	+ +			+ +	+	+	+ +	+			1933
1437	+ +	+ +			+	+	+	+	+			1897

Note: Sample numbers are those of the collector (see Appendix A). Column heading abbreviations are the same as Fig. 1 except BB (back band) which could be TC, CT, or both and Y1which is an uncharacterized imino acid. *Inga eriocarpa* is endemic to Mexico. Year is date collected. + + major accumulator, + minor accumulator. This pattern of amino acids corresponds to #3 of Fig. 2.

(TC and/or CT but most likely TC since T5 is undetectable in several samples leaving no precursor for CT) and an uncharacterized imino acid. Kite (1997) confirms this pattern and notes that the uncharacterized imino acid is a novel dihydroxypipecolic acid. Quantitative variation is evident among *I. sapindoides* samples but the overall qualitative pattern is stable and unique to this species. Lack of the novel imino acid in one sample is most likely an artifact related to the high limits of detection for yellow compounds in paper chromatography. T5 is highly variable, limiting the value of this particular compound as a character in this species. The overall pattern of PIP, T4, C5, (T5), TT, TC, with the dihydroxypipecolic acid distinguishes *I. sapindoides* and may serve as a diagnostic character for the species.

Inga eriocarpa Benth. (Table 2, Fig. 2 #3) is endemic to Mexico and was considered by Leon to be a local subspecies of the more wide-ranging *I. vera* (Leon, 1966). Sousa (1993) differed in placing *I. eriocarpa* as a variant of *I. oerstediana* or possibly as an *oerstediana* × *vera* hybrid (p. 267). The pipecolic acid pattern of *I. eriocarpa* is distinct from *I. spuria* (previously considered a subspecies of *I. vera*, Fig. 2 #1) and it matches *I. oerstediana* except for the tendency toward variation in T4AP in eriocarpa vs. T4MP in oerstediana (Fig. 2 #3). This suggests that *I. eriocarpa* is a valid species independent of *I. oerstediana* and is not consistent with a hybrid origin. These

Table 3
Inga densiflora imino acid distribution

sample	PIP	T4	C4	T5	C5	TT	CC	BB	T4AP	T4MP	Y1	Loc	Year
2418	+ +	+			+ +	+ +						MX	1987
17601	+ +	+ +			+	+ +						CR	1974
25583	+	+ +			+ +	+ +						CR	1984
1316	+ +	+ +			+ +	+ +						CR	1989
46799	+ +	+ +			+ +	+ +						CR	1979
3070	+ +	+ +			+ +	+ +						MX	1987
8415	+	+ +			+	+ +						NC	1978
3030	+ +	+ +			+ +	+ +						NC	1982
1173	+ +	+ +			+ +	+ +						NC	1961
10285	+ +	+ +			+ +	+ +						PN	1987
651	+ +	+ +			+ +	+ +						PN	1928
6232	+ +	+ +			+ +	+ +						PN	1972
9128	+ +	+ +			+ +	+				+		CR	1973
642	+ +	+			+	+				+ +		CR	1984
17186	+				+ +	+ +						CR	1933
5458	+				+ +	+ +						CR	1927
6634	+				+ +	+ +						CR	1929
17049	+				+ +	+ +						CR	1933
5469	+				+ +	+ +						CR	1927

Note: Sample numbers are those of the collector (see Appendix A). Column heading abbreviations are the same as Fig. 1 except BB (back band) which could be TC, CT, or both and Y1which is an uncharacterized imino acid. Loc. is country of origin; MX = Mexico, CR = Costa Rica, NC = Nicaragua, PN = Panama. Year is date collected. + + major accumulator, + minor accumulator. The patterns of amino acids correspond to #1 and #8 of Fig. 2.

samples include one of the oldest *Inga* specimens housed in the NYBG (Bilimek 136 collected in 1866) which had an imino acid profile indistinguishable from recent specimens.

Inga densiflora Benth. (Table 3, Fig 2 #1 and #8) shows two hydroxylation patterns; densiflora-A with PIP, T4, C5, TT in 14 of 19 individuals two of which also add T4MP, and densiflora-B comprised of five samples all collected by Brenes between 1927 and 1933 which lack both T4 and T4MP. *Inga densiflora*, like *I. sapindoides*, is a highly variable species morphologically (Leon 1966; Sousa, 1993). I suspect that the extreme morphological variation in this case is an artifact of an improperly delimited species boundary, but it may also represent human influence as this and many *Inga* species are cultivated for their edible fruits and as shade trees in coffee plantations. The main densiflora samples (densiflora-A) are in agreement with densiflora collections taken by Kite (1997). The potential utility of chemistry is well illustrated as it serves here as a pointer toward interesting or problematic areas in need of further study. In a preliminary investigation, "densiflora" specimens exhibited four distinct patterns of pipecolic acid derivatives (Morton and Romeo, 1990). Specimens collected in the Monte Verde cloud forest showed consistency among themselves (PIP, C5, T5, CC, TT, and TC) yet fail to match the pattern of any known *Inga* species. In Fig. 2 (#6) these are designated *I. montealegrei* following the description of a highland variant of *I. densiflora* mentioned (p. 304) by Leon (1966) though their true identity remains uncertain. Other patterns seen in field collections of

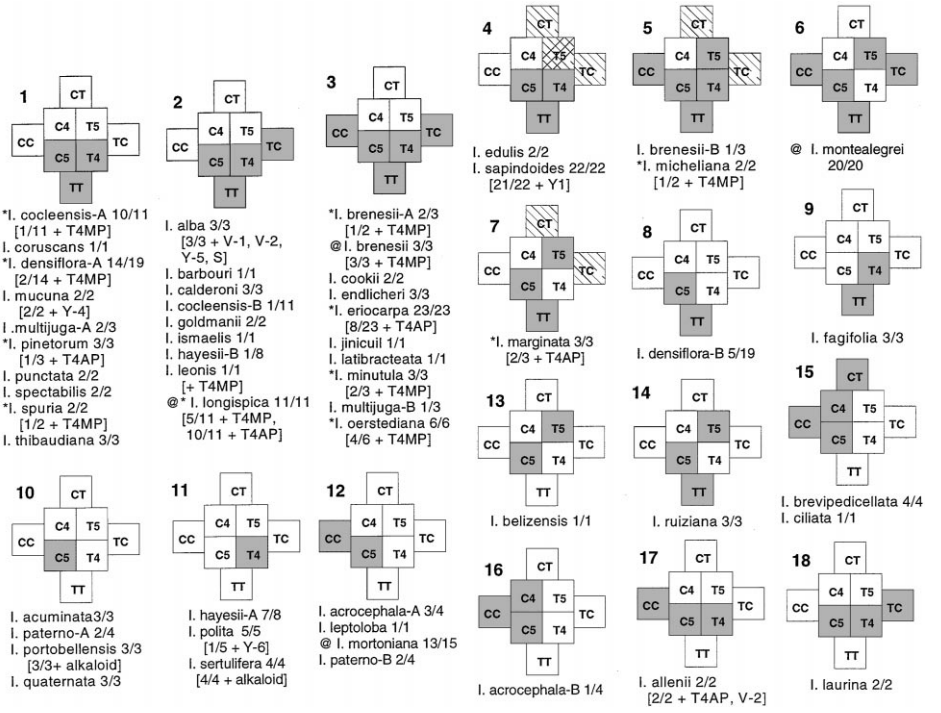

Fig. 2. *Inga* hydroxypipecolic acid patterns. Each 'petal' diagram represents the accumulation of a group of hydroxypipecolic acid derivatives; species with the illustrated pattern are listed beneath the diagram. Shading denotes the presence of a compound, see Fig. 1 for abbreviations. Hatching in CT and TC indicates the presence of one or both compounds; the exact identity was not determined. Hatching in T5 indicates variability. Two numbers follow each species; the numerator represents the number of specimens with the pattern shown above and the denominator represents the total number of specimens examined. An asterisk indicates variability in either T4MP or T4AP and the number of samples containing T4MP or T4AP is shown below in brackets; brackets are also used to note the presence of unidentified compounds (see Appendix B). A letter following the species name (− A or − B) denotes variation in hydroxypipecolic acid pattern. The symbol @ designates unvouchered samples characterized in a preliminary study. Each pattern is numbered to facilitate cross-referencing in the text.

lowland "densiflora" were also unique and also not typical of herbarium *I. densiflora* which is an indication that field determinations (based on sterile material) in that study were incorrect. This suggests that other studies based on field determinations of these "densiflora" are similarly taxonomically suspect (Koptur, 1984a, b, 1985) as the same populations of trees used in Koptur's studies were sampled for my field collection.

The remaining two intensively sampled species both show a tendency toward one defining pattern with limited variation. *Inga cocleensis* Pittier (Fig 2 #1 and #2) is characterized by the pattern PIP, T4, C5, TT in 10 of 11 samples (one also has T4MP) but the last sample adds a second dihydroxy, TC. *Inga hayesii* Benth. has a simple pattern of PIP plus T4 in 7 of 8 (Fig. 2 #11) samples but the Lumer 1358 sample is very different with PIP, T4, C5, TT, TC (Fig. 2 #2). The Lumer 1358 specimen was

collected in Costa Rica while the remaining seven were Panamanian. This distinction between Costa Rican and Panamanian samples is also evident in *I. multijuga* (Fig. 2 #1 and #3). The odd specimens probably indicate misidentifications, not uncommon with sterile material of morphologically variable species.

Does the evidence presented above warrant the conclusion that each *Inga* species can be characterized by a stable chemical pattern? The evidence is suggestive but enough variation was detected to encourage caution. There are several examples where a trait obviously exhibits variable expression. The most notable cases of variation involve T4MP and T4AP; species showing variation in these compounds are preceded by an asterisk in Fig. 2. In the preliminary study, T4MP correlated with high elevation indicating that this character has an ecological component, which would reduce its chemotaxonomic value (Morton and Romeo, 1990). T4AP is similarly most often detected in high-elevation species such as *I. longispica* (Fig. 2 #2, 10 of 11 individuals) and *I. eriocarpa* (Fig. 2 #3, 8 of 23) but is also found in lowland specimens of *I. allenii* (Fig. 2 #17) and *I. marginata* (Fig. 2 #7). Elevation data is missing on many specimens so the relationship with elevation remains speculative. T5 is variable in *I. sapindoides* and *I. edulis* (Fig. 2 #4) but in most cases T5 is consistently expressed at relatively high levels. The implication here is that as with any morphological character, multiple samples should be surveyed and the extent of variation monitored. Each species sampled intensively does appear to have an identifiable pattern of hydroxypipecolic acids. It is highly likely that some herbarium specimens are improperly identified. This must be stated somewhat cautiously as all species sampled more than four times show some degree of variation. The recognition that any kind of character (including chemical data) is potentially suitable to give its own contribution to the species definition (Sbordoni, 1993) may be profitably considered in future studies of *Inga*.

3.2. Trends

Fig. 2 graphically represents observed chemical patterns in the data. Each shaded square denotes presence of a compound, unlike Tables 1–3 this makes no reference to quantity. Each "petal diagram" reflects the hydroxylation pattern observed in the species listed below. All *Inga* species contain pipecolic acid so PIP is not illustrated. The inner four squares represent monohydroxies. On each side of the inner square of four monohydroxies is an outer square overlapping two of them. As illustrated in Fig. 1, each dihydroxy can be synthesized from either of two monohydroxy precursors, or from both. The petal diagram reflects the biosynthetic pathway; each dihydroxy is touching its two potential monohydroxy precursors. Hatching in CT/TC indicates that the exact identity of the "back band" was not determined, crisscross hatching in T5 denotes variable expression. Additional compounds that may be of future interest are noted in brackets beneath the species name, Appendix B lists some properties of the unknowns. Each species is followed by two numbers (X/Y) to denote the number of samples showing that pattern (X)/ the total number of samples (Y). A preceding asterisk denotes variability in either T4MP or T4AP;

a letter (− A or − B) indicates two distinct hydroxypipecolic acid patterns for that species.

Because the biosynthetic pathway for the production of hydroxylated pipecolic acid derivatives is known, it should be possible to make statements regarding relatedness of taxa; more closely related species are more likely to share similar enzymes and pathways. Particularly where morphology is variable, chemicals with known biosynthetic pathways may prove valuable in reconstructing phylogenies and may serve to establish the orientation of character states in cladistic analyses so long as there are sufficient numbers of chemical patterns to allow distinction. The "petal diagram" concept (Fig. 2) for a group of biosynthetically related compounds is capable of delivering more information than standard one-dimensional tabular formats (Tables 1–3) and relationships are more easily discerned. At present, our knowledge of chemistry within the genus is too fragmentary to allow strong assertions to be made regarding relatedness. These Central American species are at the expanding edge of the range of the group and information is needed from ancestral South American representatives to provide greater structure to the overall pattern (see Kite, 1997).

The diversity of non-protein imino acids in *Inga* is extraordinary with 18 distinct patterns for the 47 species and many more patterns are possible (Kite, 1997 adds nine patterns). It appears that the basic or "core" pattern within the tribe Ingeae includes the structures PIP, T4, C5, TT (Fig. 2 #1). In other genera of the Ingeae tribe this core has been minimally altered; *Zapoteca* has only the core pattern for the nine species surveyed and *Calliandra* has nine patterns with over 100 species surveyed (Romeo and Morton, 1994). Preliminary work with the *Pithecellobium* complex indicates that it also shares this same core with considerable variation (Morton and Grimes, unpublished data; Kite, 1997). *Inga* shows loss of imino acids in some taxa but the primary trend is toward gain of additional structures, many of which have not yet been identified. Ten species exhibit the core pattern, another nine add the dihydroxy TC (Fig. 2 #2), and nine more add a third dihydroxy, CC (Fig 2 #3). Species exhibiting these core patterns account for nearly two-thirds of the species so far examined. Species showing loss of pipecolic acid derivatives seem, in several cases, to be replacing them with other chemicals including alkaloids (see Appendix B). Due to the tremendous interspecific variation, pipecolic acid derivatives have great potential to resolve taxonomic problems. This is especially significant given the notable variation in morphology. The presence of such a large number of distinct patterns should make it possible to discern unrelated taxa with convergent morphologies, as I suspect is the case with *I. densiflora.*

Chemistry may also find application in the identification of hybrids; though never attempted with imino acids, it has been attempted with flavonoids (Wyatt and Hunt, 1991) and other compounds (see Alston and Turner, 1963). The descriptions of *Inga* species by various authors often mention hybrid swarms and species groups that resemble syngameons as defined by Grant (1981). Is there any evidence of hybridization in the chemical data? One possible example was discussed above under *I. eriocarpa* and evidence would suggest that it is not a hybrid. Pipecolic acid chemistry does provide evidence for one possible example of hybridization. Sousa

(1993) considers *I. brevipedicellata* Harms to be within *I. acrocephala* Steudel. Chemically, *I. acrocephala*-A (Fig. 2 #12) has a very simple pattern characterized by small amounts of PIP, and large quantities of C5 and CC (quantitative data not shown). *Inga brevipedicellata* (Fig. 2 #15) has similar quantities of PIP, C5, and CC but also adds C4 and a small amount of the extremely rare CT which is otherwise known only from four *Calliandra* species and *Derris eliptica* (Marlier et al., 1972; Romeo and Morton, 1994). Of particular interest is *I. acrocephala*-B (Fig. 2 #16) which has an intermediate chemistry and contains C4. If hybridization were to take place between species with different chemical patterns, some form of complementation might reasonably be expected and the levels of CT (expressed at low levels in *I. brevipedicellata*) may not be present in sufficient quantities to detect. The fact that this is the only identifiable case would argue against the importance of hybridization as a general phenomenon but in cases where hybrids are suspected, chemistry should be able to provide valuable clues.

Another area where chemistry should prove useful is in evaluating the position of taxa whose validity is in doubt. Several species have been extensively modified during revision. One such species is *I. oerstediana* Benth. As more material has become available and extensive intergradation noted, its definition has expanded to include a large number of older binomials, for synonyms see (Leon, 1966; Sousa, 1993). Sousa considered *I. minutula* (Schery) T.S. Elias (Fig. 2 #3) and *I. endlicheri* (Kuntze) J.F. Macbr. (Fig. 2 #3) to be synonyms of *I. oerstediana* so three specimens of each were sampled and both matched the chemical pattern of *I. oerstediana* (Fig. 2 #3) including the split T4MP pattern in *I. minutula*. Chemistry, in this case, may be used to support a more conservative interpretation of *I. oerstediana* that includes both endlicheri and minutula. *Inga densiflora* was discussed previously; the differences in chemistry there suggest that the current interpretation of *I. densiflora* may be too broad and chemistry may be useful in future studies to aid in delimiting proper species boundaries.

In cases of taxonomic confusion, chemistry may help to resolve conflicts. For example, *I. fagifolia* has two authors and two descriptions: *I. fagifolia* (L.) Willd. ex Benth., Trans. Linn. Soc. London 30: 607, 1875- was transferred to *I. laurina*, and *I. fagifolia* Don, Gen. Hist. 2: 391, 1832– to *I. ruiziana* (Sousa 1993) but in sampling labeled specimens of all three species, each was found to be distinctly different (Fig. 2 #9, #14, and #18). This would indicate that the species *I. fagifolia* is a valid entity regardless of problems with nomenclature and chemistry may aid in sorting specimens. There are many examples where a specimen has recently been moved to a different species. Samples previously designated *I. heterophylla* have recently been divided among *I. polita* Killip (Fig. 2 #11) and *I. sertulifera* DC (Fig. 2 #11). This division is supported by chemical evidence, though both species show a pattern based on PIP and T4, *I. sertulifera* contains an unidentified alkaloid that serves to distinguish it. Chemical evidence does not always support recent changes. There is confusion regarding the *I. acrocephala* (Fig. 2 #12 and #16) to *I. allenii* (Fig. 2 #17) boundary and the sample Croat 68964 was transferred from allenii to acrocephala but this specimen has a distinctive allenii pattern. The *I. multijuga*-B specimen (Fig. 2 #3) was previously labeled *I. oerstediana* and chemically it resembles

I. oerstediana (Fig. 2 #3) and it is distinct from the typical multijuga profile (Fig. 2 #1).

Other notes regarding Fig. 2 data and application of chemistry. *Inga leptoloba* (Fig. 2 #12) has been considered a variant of *I. punctata* (Fig. 2 #1) for a number of years (Leon, 1966) but the one *I. leptoloba* specimen assayed was chemically distinct. *Inga recordii* has been grouped into *I. thibaudiana* (Fig. 2 #1), a move supported by chemistry (Leon, 1966). Leon also noted confusion regarding the distinction between *I. thibaudiana* and *I. multijuga* that in my analysis share the same chemical pattern. *Inga allenii* appears to be somewhat confused; specimen Knapp 1153 was recently transferred from allenii (Fig. 2 #17) to *I. barbouri* (Fig. 2 #2) which is supported but Croat 68964 to *I. acrocephala* (Fig. 2 #12) is not supported. Sousa (1993) notes a number of taxa he considers to be close relatives, some of which gain support from similarities in chemistry: *I. latibracteata* and *I. oerstediana* (Fig. 2 #3), *I. leonis* and *I. brenesii* (Fig. 2 #2 and 3), *I. mortoniana* and *I. acrocephala* (Fig. 2 # 12), *I. pinetorum* and *I. punctata* (Fig. 2 #1), *I. longispica* and *I. coruscans* (Fig. 2 #1 and 2), *I. allenii* and *I. mortoniana* (Fig. 2 #17 and 12).

3.3. What is a species?

The question of whether to segregate species pairs such as those differing in the presence/absence of T4MP or T4AP into distinct species is worthy of study. How species are defined in *Inga* and how this holds up when morphology is highly variable is problematic. If chemistry is indeed a conserved character, would chemical differences alone warrant specific designation? At present probably not, but this does point to the need for information on reproductive isolating mechanisms in *Inga*. Simola's finding that non-protein amino acids (e.g. canavanine) can form effective hybridization barriers between various papilionoid taxa and her speculation that the ability to synthesize a novel amino acid may be of evolutionary significance in the isolation of new species may be relevant to mimosoids like *Inga* and should be explored (Simola, 1967).

3.4. Ecological considerations

Members of the Mimosoideae are more common in lowland tropical rainforests, especially near lakes and rivers, and are generally absent at higher elevations (Elias, 1978). *Inga* is a major exception to this rule. The transition into the mountains of Central American was presumably difficult ecologically. The mountains of western Panama and Costa Rica are separated from the Colombian Andes by a gap of 600 km, and another 200 km lowland gap exists in Nicaragua isolating the more northern mountains (Gentry and D'Arcy, 1986). Moving northward along this patchwork of high- and low-elevation habitats surely played a role in shaking up the genome resulting in the locally high levels of endemism and extreme morphological variation in Central American *Inga*. One unusual aspect of *Inga* biology that has remained constant and likely constrains *Inga* habitat is the viviparous embryo (Leon, 1966). It

order to establish, a seedling must land in a suitable wet habitat and in Central America this condition is met in the cloud forest, facilitating the transition into montane habitats.

In addition to the difficulties in adapting to environmental changes, herbivores may have presented a significant challenge to any *Inga* adapting to the montane environment (cloud forests). *Inga* sp. are noted for their complex extra-floral nectaries, an indication of long association with ants (but see Keeler, 1985). Ants defend *Inga* species in the lowlands but in the wetter cloud forests ant defense breaks down (Koptur, 1984a, 1985). Herbivore diversity does not decrease with elevation to the extent that ant diversity is decreased (Janzen, 1973). The result is generally higher herbivore pressure on ant-plants found in wet mountain habitats (Koptur, 1985). One possible solution to the herbivore problem would be the evolution of alternative defenses as documented in other systems such as *Acacia* (Rehr, et al., 1973) and *Bixa* (Bentley, 1977). Proliferation of pipecolic acid derivatives may represent an increased reliance on chemical defense in response to breakdown of the traditional ant-based,

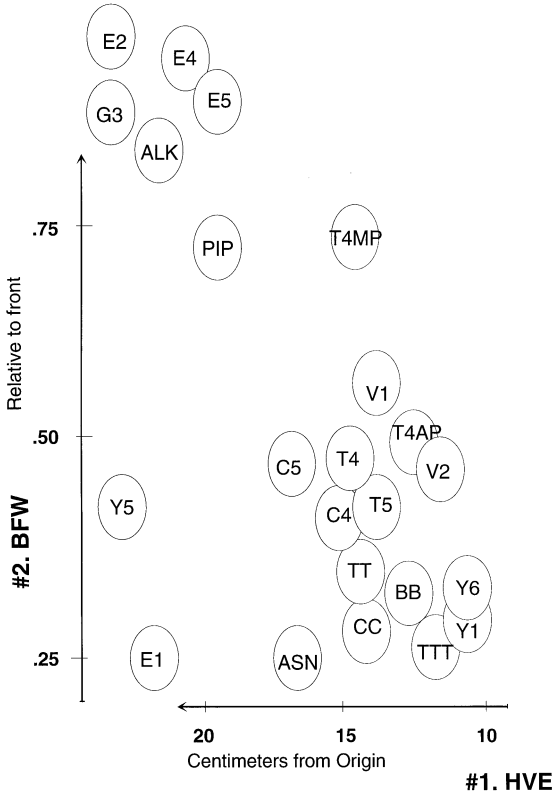

Fig. 3. Relative mobilities of imino acids with paper chromatography.

physical defense. It is of interest to note that *I. oerstediana* (Morton #276) housed in the USF herbarium lacks the extra-floral nectaries that are normally highly developed in this species.

3.5. General comments

This study shows that several species, such as *I. sapindoides*, with remarkable morphological variability do have consistent chemical patterns and appear to be valid species. Other species with considerable morphological variation, such as *I. densiflora*, do not show consistent chemical patterns and this is likely the result of an improperly delimited species boundary. Kite (1997) has extended this work with a larger study that includes many South American specimens using a more sensitive GC-MS identification. Our results are generally in good agreement though he found numerous examples of minor amounts of pipecolic acid derivatives that would be undetectable using paper chromatography. There are a large number of complementary chemicals which are at present unidentified but which may also be incorporated in future chemotaxonomic treatments, Appendix B and Fig. 3 are intended to assist other researchers considering a similar course of study. Kite (1997) has identified numerous N-methylated pipecolic acid derivatives and novel hydroxyprolines that are likely to correspond to many of the unknowns presented here.

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Appendix A. Sample list

The first collector's name and number identify each specimen. Additional information including year collected, country of origin, and elevation are shown if noted by the collector. Letters in column 1 refer to chemical patterns shown in Fig. 2 (listed in column 3). An asterisk in column 1 denotes specimens possessing T4MP or T4AP. In notes, 'ex:' refer to older specific names given to the specimen. [§]Refers to specimens housed in the University of South Florida herbarium, [‡] specimens are in the Herbario Nacional de Costa Rica, San Jose.

(*Table of Appendix starts on opposite page.*)

	Species	Fig. 2	Collector info	Year	Loc	Elev (m)	Notes
a	acrocephala	12	Hammel 14340	1985	cR	500	ex: pezizifera
a	acrocephala	12	burger 8841	1973	cR	200	ex: coruscans
a	acrocephala	12	Grayum 3603	1984	cR	1350	ex: mortoniana
b	acrocephala	16	Kennedy 2009	1972	cR	200	ex: coruscans
	acuminata	10	Hartshorn 1302	1973	cR	200	
	acuminata	10	Foster 2976	1980	PN	50	
	acuminata	10	Opler 1593	1972	cR	45	
	alba	2	Hammel 11662	1982	cR	100	ex: pezizifera
	alba	2	Smith 357	1981	cR	100	ex: pezizifera
	alba	2	Proctor 27032	1966	Nc	65	ex: coruscans
	allenii	17	Gomez 21111	1984	cR	600	
	'acrocephala'	17	croat 68964	1988	PN	80	should be allenii
	barbouri	2	Knapp 1153	1981	PN	20	ex: allenii
	belizensis	13	contreras 6653	1967	GT		
a	brenesii	3	breedlove 48739	1980	MX	1380	
a*	brenesii	3	brenes 4985	1926	cR		
b	brenesii	5	Jimenez 1200	1963	cR	1275	
	brevipedicellata	15	breedlove 58460	1982	MX	1170	
	brevipedicellata	15	Tenorio 14518	1988	GT	450	
	brevipedicellata	15	cedillo 2859	1984	MX		
	brevipedicellata	15	Purpus 8400	1919	MX		
	calderoni	2	calzada 9057	1982	MX	900	
	calderoni	2	Molina 16681	–	GT	600	
	calderoni	2	Matuda 18092	1948	MX	700	
	ciliata	15	Herrera 2317	1988	cR	150	
a	cocleensis	1	Molina 8275	1957	HN	2100	
a	cocleensis	1	Gentle 6724	1949	HN		
a	cocleensis	1	McDowell 718	1982	cR	100	
a	cocleensis	1	Standley 72586	1939	GT	18	
a	cocleensis	1	McPherson 11951	1988	PN	500	
a	cocleensis	1	Moreno 23008	1984	Nc	70	

Continued overleaf

Appendix A. continued

	Species	Fig. 2	Collector info	Year	Loc	Elev (m)	Notes
a	cocleensis	1	Saunders 627	1980	HN		
a	cocleensis	1	Gentle 5214	1945	HN		
a	cocleensis	1	Moreno 24986	1984	Nc	20	
a*	cocleensis	1	correa 556	1968	PN		
b	cocleensis	2	Molina 11799	1963	HN	500	ex: multijuga
	cookii	3	Gentle 6507	1948	HN		
	cookii	3	Molina 25533	1970	HN	1700	
	coruscans	1	Duke 4986	1962	PN		
a	densiflora	1	Ventura 2418	1987	MX	550	
a	densiflora	1	Taylor 17601	1974	CR	1150	
a	densiflora	1	Davidse 25583	1984	CR	1300	
a	densiflora	1	Lumer 1316	1982	CR	1275	
a	densiflora	1	Croat 46799	1979	CR	800	
a	densiflora	1	Ventura 3070	1987	MX	700	
a	densiflora	1	Stevens 8415	1978	NC		
a	densiflora	1	Araquistain 3030	1982	NC	200	
a	densiflora	1	Bunting 1173	1961	NC		
a	densiflora	1	McPherson10285	1987	PN	700	
a	densiflora	1	Proctor 651	1928	PN		
a	densiflora	1	Gentry 6232	1972	PN		
a*	densiflora	1	Burger 9128	1973	CR	800	
a*	densiflora	1	Haber 642	1984	CR	1350	
b	densiflora	8	Brenes 17186	1933	CR		
b	densiflora	8	Brenes 5458	1927	CR		
b	densiflora	8	Brenes 6634	1929	CR		
b	densiflora	8	Brenes 17049	1933	CR		
b	densiflora	8	Brenes 5469	1927	CR		
	edulis	4	Schmalzel 1332	1983	PN	1000	
	edulis	4	McPherson 9618	1986	PN		
	endlicheri	3	Molina 13632	1964	HN	1200	
	endlicheri	3	Bunting 881	1961	NC	50	
	endlicheri	3	Standley 63131	1939	GT	1500	

	eriocarpa	3	Palmer 250	1895	MX	
	eriocarpa	3	Matuda 27956	1953	MX	1800
	eriocarpa	3	Hinton 9248	1930	MX	1150
	eriocarpa	3	Bilimek 136	1866	MX	
	eriocarpa	3	Hinton 5527	1934	MX	
	eriocarpa	3	Mexia 1842	1927	MX	1500
	eriocarpa	3	Hinton 8977	1936	MX	
	eriocarpa	3	Fröderstrom 456	1932	MX	1700
	eriocarpa	3	Rose 1701	1897	MX	
	eriocarpa	3	Matuda 22	1936	MX	
	eriocarpa	3	Hinton 9089	1936	MX	660
	eriocarpa	3	Rose 14181	1910	MX	
	eriocarpa	3	McVaugh 23523	1965	MX	
	eriocarpa	3	Dieterle 4032	1971	MX	
	eriocarpa	3	Lyonnet 668	1930	MX	
*	eriocarpa	3	Hinton 14125	1939	MX	100
*	eriocarpa	3	Hinton 9259	1936	MX	1100
*	eriocarpa	3	Ton 2724	1967	MX	750
*	eriocarpa	3	Fryxell 3425	1981	MX	
*	eriocarpa	3	Hinton 9997	1937	MX	400
*	eriocarpa	3	Hinton 10074	1937	MX	1150
*	eriocarpa	3	Hinton 3981	1933	MX	1340
*	eriocarpa	3	Rose 1437	1897	MX	
	fagifolia	9	Allen 1688	1939	PN	
	fagifolia	9	Matuda 1862	1937	MX	
	fagifolia	9	Croat 6119	1968	PN	
	goldmanii	2	Croat 7211	1969	PN	0
	goldmanii	2	Grijalva 1382	1982	NC	500
a	hayesii	11	Croat 13000	1971	PN	
a	hayesii	11	Dwyer 1743	1961	PN	
a	hayesii	11	Nee 11649	1974	PN	70
a	hayesii	11	Croat 14666	1971	PN	
a	hayesii	11	Miller 732	1983	PN	
a	hayesii	11	Churchill 4264	1984	PN	0
a	hayesii	11	Croat 14912	1971	PN	

Continued overleaf

Appendix A. continued

	Species	Fig. 2	Collector info	Year	Loc	Elev (m)	Notes
b	hayesii	2	Lumer 1358	1982	CR		
	ismaelis	2	Dorantes 2806	1974	MX	130	ex: sapindoides
	jinicuil	3	Breedlove 41 753	1976	MX	2100	ex: paterno
	latibracteata	3	Purpus 10 700	1926	MX		
	laurina	18	Cochrane 11 684	1990	MX	600	
	laurina	18	DeWit 11	1941	Cult.		
	leonis	2	Sytsma 1883	1980	PN	1200	
	leptoloba	12	Breedlove 24 240	1972	MX	300	
	marginata	7	Hammel 14 190	1985	CR	1000	
*	marginata	7	Grayum 4527	1984	CR	20	
*	marginata	7	Haber 1802	1985	CR		
	micheliana	5	Molina 21 730	1968	ES	1800	ex: hintonii
*	micheliana	5	vanderWerff 7167	1985	PN	1300	ex: hintonii
	minutula	3	Croat 38 287	1976	PN	100	
*	minutula	3	Schmalzel 1151	1982	PN		
*	minutula	3	Allen 1972	1939	PN		ex: oerstediana
	mucuna	1	Dwyer 6847	1967	PN		
	mucuna	1	Croat 6858	1968	PN		
a	multijuga	1	Croat 22 464	1973	PN	100	
a	multijuga	1	Liesner 144	1973	PN	20	
b	multijuga	3	Davidse 30 907	1986	CR	5	ex: oerstediana
	oerstediana	3	Hartshorn 1071	1972	CR	100	
	oerstediana	3	Leon 2816‡	1966	CR	720	
*	oerstediana	3	McPherson 7930	1986	PN		
*	oerstediana	3	Molina 26 118	1971	HN	1600	
*	oerstediana	3	Morton 276§	1992	CR	1550	
*	oerstediana	3	11683‡	1898	CR	1800	
a	paterno	10	Marquez 663	1976	MX	430	
a	paterno	10	Standley 21 756	1922	ES		
b	paterno	12	Gomez 22 744	1984	CR		
b	paterno	12	Grimes 2724	1985	MX		ex: jinicuil
	pinetorum	1	Fernandez 1050	1982	MX	20	ex: punctata

	pinetorum	1	431[§]				
*	pinetorum	1	Gentle 4149	1942	HN		
	polita	11	Mori 7722	1975	PN	400	ex: heterophylla
	polita	11	McPherson 6721	1986	PN	500	ex: heterophylla
	polita	11	McPherson 8166	1986	PN	350	ex: heterophylla
	polita	11	McPherson 11 895	1987	PN	200	ex: heterophylla
	polita	11	McPherson 7878	1985	PN	850	(+Y-6)
	portobellensis	10	Gentry 8777	1973	PN	100	
	portobellensis	10	McPherson 8217	1986	PN		
	portobellensis	10	Nee 9572	1974	PN	90	
	punctata	1	Nee 7379	1973	PN	45	
	punctata	1	Grijalva 3619	1984	NC	100	
	quaternata	10	Hartshorn 1326	1973	CR		
	quaternata	10	Gomez 24 115	1984	CR	600	
	quaternata	10	McPherson 10 325	1987	PN	650	
	ruiziana	14	Bunting 888	1961	NC	50	
	ruiziana	14	McPherson 7931	1986	PN		
	ruiziana	14	Schmalzel 1154	1982	PN		
	sapindoides	4	Dwyer 190	1967		400	pavoniana
	sapindoides	4	Tonduz 10 030	1896	CR		pavoniana
	sapindoides	4	Standley 45 784	1926	CR	600	pavoniana
	sapindoides	4	Tonduz 17 957	1913	CR	1170	pavoniana
	sapindoides	4	Chacon 1104	1983	CR	100	pavoniana
	sapindoides	4	Smith 1891	1939	CR	750	pavoniana
	sapindoides	4	Grayum 5816	1985	CR	550	pavoniana
	sapindoides	4	Utley 3986	1976	CR		pavoniana
	sapindoides	4	Allen 6905	1958	ES	700	pavoniana
	sapindoides	4	Standley 23 088	1922	ES	650	pavoniana
	sapindoides	4	171	1921	ES		pavoniana
	sapindoides	4	Williams 489	1908	PN	800	pavoniana
	sapindoides	4	Schmalzel 1190	1982	PN		pavoniana
	sapindoides	4	Yuncker 8569	1938	HN		pavoniana
	sapindoides	4	Molina 18 390	1966	HN	800	pavoniana
	sapindoides	4	Dwyer 15 235	1980	GT	1300	pavoniana
	sapindoides	4	10021	1927	GT		pavoniana

Continued overleaf

Appendix A. continued

	Species	Fig. 2	Collector info	Year	Loc	Elev (m)	Notes
	sapindoides	4	sousa 3043	1967	MX	100	pavoniana
	sapindoides	4	Gentry 8078	1973	BZ	100	pavoniana
	sapindoides	4	Sousa 3043	1967	MX	100	pavoniana
	sapindoides	4	Jimenez 1788	1964	CR	250	pavoniana
	sapindoides	4	Croat 5265	1968	PN		pavoniana
	sapindoides	4	Standley 23641	1922	ES	650	(lacks THP)
	sertulifera	11	Gentry 1897	1971	PN		ex: heterophylla
	sertulifera	11	Kennedy 1811	1972	PN		ex: heterophylla
	sertulifera	11	Croat 13 842	1971	PN		ex: heterophylla
	sertulifera	11	Molina 17 747	1966	CR	550	ex: heterophylla
	spectabilis	1	Croat 15 044	1971	PN		
	spectabilis	1	Croat 14 080	1971	PN		
	spuria	1	LeDoux 2096	1975	GT	25	ex: vera
*	spuria	1	Castro 154	1985	HN	1850	ex: vera
	thibaudiana	1	819[§]				recordii at USF
	thibaudiana	1	Nee 9693	1974	PN	150	
	thibaudiana	1	Gentle 8138	1954	GT		

Appendix 1A

Supplemental samples, not vouchered. #indicates the number of plants sampled. Samples are preceded by an @ in Fig. 2; *Inga punctata* matched the herbarium specimens and were not listed again.

Species	Fig 2.	#	Elevation (m)	Collector
brenesii	3	3	1300–1520	Morton
longispica	2	11	1300–1650	Morton
montealegrei	6	20	1400–1650	Morton
mortoniana	12	13	1400–1650	Morton
punctata	1	5	950–1380	Morton

Appendix B

Color reactions of imino acids and unknowns

Cmpd	Source plant	UV366	Ninhydrin	Nin. + UV	Misc
ASN		–	blue–gray	absorbs	asparagine stnd
PIP		–	violet	pink	
T4		–	yellow	pink	isatin gray
C5		–	violet	brick red	
C4		–	yellow	pink	
T5		–	violet	brick red	
TT, CC, TC, CT		–	yellow fades to blue-gray	brick red	
TTT		–	yellow	yellow	
T4MP		–	yellow fades to gray	pink	isatin gray
T4AP		–	violet	pink	
Y1	sapindoides	–	yellow	wk yellow	novel dihydroxy?
ALK	sertulifera and portobellensis	–	–	–	Dragendorff's + orange
Y4	mucuna	–	yellow	wk yellow	
Y5	alba	–	yellow	str yellow	
Y6	polita	–	yellow	orange	Ehrlich's purple
V1	alba	–	violet	yellow	
V2	alba and allenii	–	violet	pink	same compound?
S	alba	–	purple	–	sulphur aa?
E1	portobellensis	yellow	–	–	Ehrlich's purple
E2	portobellensis	–	–	–	Ehrlich's purple
E4	portobellensis	yellow	–	–	no Ehrlich's
E5	sertulifera and portobellensis	blue	–	–	no Ehrlich's
G3	portobellensis	yellow	yellow fades to gray	light blue	Ehrlich's purple

Appendix B, Fig. 3. Color reactions and relative mobilities of imino acids and unknowns.

This diagram represents the specific color reactions and relative locations of imino acids and unknowns in the 2-D paper chromatographic solvent system used. The first dimension used high-voltage electrophoresis (45 min at 4400 V in pH 1.9 buffer [37.5 ml 88% formic acid: 147 ml glacial acetic acid: 1820 ml deionized H_2O]). For the second dimension, the paper was dried and cut for mounting onto racks for ascending chromatography using butanol : formic acid : H_2O (150 : 34 : 16) for 10–12 h at 90° orientation. Ninhydrin was the primary means of detection, unknowns are further characterized with isatin, Dragendorff's, and Ehrlich's reagents plus observations in natural and UV366 light. Source plants for unknowns are listed, see also Fig. 2. *Inga sertulifera* and *portobellensis* may share one alkaloid in common; *portobellensis* has a second with similar Rf that reacts slowly with Dragendorff's. The 'E' compounds of *portobellensis* have native fluorescence and several react with Ehrlich's reagent. The alkaloid containing *sertulifera* group has several native fluorescent compounds that do not react with Ehrlich's but are otherwise similar to those of the alkaloid containing *portobellensis*. The sulphur compound in *I. alba* had low mobility in the first dimension (< 10 cm) and was not included in dimension $\#2$. The location of asparagine, which has a distinctive (for primary amino acids) blue color, is included for reference.

References

Alston, R.E. Turner, B.L., 1963. Biochemical studies of hybrids. Biochemical Systematics. Prentice-Hall, Englewood Cliffs, N.J., 295–326.

Bell, E.A., 1978. Non-protein amino acids in the Leguminosae. International Legume Conference, Royal Botanic Gardens, Kew, Crown.

Bentley, B.L., 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). J. Ecol. 65' 27–38.

Elias, T.S., 1978. Mimosoideae. International Legume Systematics Conference, Royal Botanic Gardens, Kew, England, Crown.

Evans, C.S., Qureshi, M.Y., Bell, E.A., 1977. Free amino acids in the seeds of *Acacia* species. Phytochemistry 16, 565–570.

Gentry, J.L., D'Arcy, W.G., 1986. In: D'Arcy, W.G., Solanaceae of Mesoamerica. Solanaceae: Biology and Systematics. Columbia University Press, New York, pp. 15–26.

Grant, V., 1981. Plant Speciation. Columbia University Press, New York.

Janzen, D.H., 1973. Sweep samples of tropical foliage insects: effects of seasons, vegetation types, elevation, time of day, and insularity. Ecology 54(3), 687–708.

Keeler, K.H., 1985. Extrafloral nectaries on plants in communities without ants: Hawaii. Oikos 44, 407–414.

Kite, G.C., 1997. Non-protein amino acid chemistry. In: Pennington, T.D. (Ed.), The Genus Inga: Botany. The Royal Botanic Gardens, Kew, 35–70.

Koptur, S., 1984a. Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants. Ecology 65(6), 1787–1793.

Koptur, S., 1984b. Outcrossing and pollinator limitation of fruit set: breeding systems of neotropical *Inga* trees (Fabaceae: Mimosoideae). Evolution 38(5), 1130–1143.

Koptur, S., 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevational gradient. Ecology 66(5), 1639–1650.

Krauss, G.-J., Reinbothe, H., 1973. Die freien aminosauren in samen von Mimosaceae. Phytochemistry 12, 125–142.

Leon, J., 1966. Central American and West Indian species of *Inga* (Leguminosae). Ann. Missouri Bot. Gard. 53(3), 265–359.

Marlier, M., Dardenne, G.A., Casimir, J., 1972. 4,5 dihydroxy-L-pipecolique a partir de *Calliandra haematocephala*. Phytochemistry 11, 2597–2599.

Marlier, M., Darsdenne, G., Casimir, J., 1979. 2S,4R-carboxy-2-acetylamino-4-piperidine dans les fuelles de *Calliandra haematocephala*. Phytochemistry 18, 479–481.

Morton, T.C., Romeo, J.T., 1990. Preliminary chemical investigations of Costa Rican *Inga* species based on nonprotein amino acids. Bulletin IGSM 18: 112–123.

Morton, T.C., Zektzer, A.S., Rife, J.P., Romeo, J.T., 1991. *Trans*-4 methoxypipecolic acid, an amino acid from *Inga paterno*. Phytochemistry 30(7), 2397–2399.

Phillipson, J.D., 1982. Chemical investigations of herbarium material for alkaloids. Phytochemistry 21(10), 2441–2456.

Rehr, S.S., Feeney, P.P, Janzen, D.H., 1973. Chemical defenses of Central American non-ant Acacias. J. Animal. Ecol. 42, 405–416.

Romeo, J.T., 1984. Preliminary chemotaxonomic investigations of Colombian *Calliandra* species based on nonprotein imino acids. *Bull. IGSM* 12, 19–23.

Romeo, J.T., 1986. Distribution of nonprotein imino and sulphur amino acids in *Zapoteca*. Ann. Missouri Bot. Gard. 73, 764–767.

Romeo, J.T., 1989. Heterocyclic amino and imino acids (mimosine, azetidine-2-carboxylic acid, and pipecolic acid). In: Toxicants of Plant Origin. vol. 3, Cheek. P. R., (Ed.), CRC Press, Boca Raton, FL, 3: 125–139.

Romeo, J.T., Morton, T.C., 1994. Nonprotein amino acids of the Ingeae: taxonomic and ecological considerations. In: Sprent, J.I., McKey, D., Advances in Legume Systematics *5:* The Nitrogen Factor, Royal Botanic Gardens, Kew, England, vol. 5, 89–99.

Rosenthal, G.A., 1982. Plant Nonprotein Amino and Imino Acids: Biological, Biochemical, and Toxicological Properties. Academic Press, New York.

Sbordoni, V., 1993. Molecular systematics and the multidimensional concept of species. Biochem. Syst. Ecol. 21(1), 39–42.

Simola, L.K., 1967. The effect of some non-protein amino acids on pollen germination and pollen-tube growth in five species of the vicieae. Planta 77, 287–297.

Singh, T.N., Aspinall, D., Paleg, L.G., Boggess, S.F., 1973. Stress metabolism I. Nitrogen metabolism and growth in the barley plant during water stress. Aust. J. Biol. Sci. 26, 45–56.

Smith, I., 1969. Chromatographic and Electrophoretic Techniques 3rd ed. Interscience Publishers, Bath.

Sousa, M., 1993. El genero *Inga* (Leguminoseae: Mimosoideae) del sur de Mexico y Centroamerica, estudio previo para la Flora Mesoamericana. Ann. Missouri Bot. Gard. 80(1), 223–269.

Swain, L.A., Romeo, J.T., 1988. Metabolism of pipecolic acid derivatives in *Calliandra* and *Zapoteca*. Phytochemistry 27(2) 397–399.

Wyatt, R., Hunt, D.M., 1991. Hybridization in North American *Asclepias*. II. Flavonoid evidence. Syst. Bot. 16(1), 132–142.