Analysis of Indole-3-Acetic Acid Metabolites from *Dalbergia dolichopetala* by High Performance Liquid Chromatography-Mass Spectrometry[1]

Anders Östin, Ana Maria Monteiro, Alan Crozier, Einar Jensen, and Göran Sandberg*

Department of Forest Genetics and Plant Physiology, The Swedish University of Agricultural Sciences, S-901 83
Umeå, Sweden (A.Ö., G.S.); Department of Plant Physiology, Universidade Estadual de Campinas,
13081 Campinas, S.P., Brazil (A.M.M.); Department of Botany, School of Biological Sciences,
The University, Glasgow G12 8QQ, United Kingdom (A.C.); and Department of Plant Physiology,
Institute of Biology and Geology, University of Tromsö, N-9001 Tromsö, Norway (E.J.)

ABSTRACT

A mixture of [2-$^{14}C_1$] and [$^{13}C_6$]indole-3-acetic acid was applied to the cotyledons of 6-day-germinated seeds of "jacarandá do cerrado" (*Dalbergia dolichopetala*) and after 8 hours the seeds were extracted. Analysis of the fractionated extract by reversed-phase high performance liquid chromatography-radiocounting revealed the presence of five radiolabeled metabolite peaks (I–V). After further purification, the individual peaks of radioactivity were analyzed by combined high performance liquid chromatography-steel filter-fast atom bombardment-mass spectrometry. The metabolite fraction V was found to contain [$^{14}C_1$,$^{13}C_6$]indole-3-acetylaspartic acid and unlabeled indole-3-acetylglutamic acid. Analysis of the metabolite fraction II revealed the presence of dioxindole-3-acetylaspartic acid and putative dioxindole-3-acetylglutamic acid as well as putative benzene ring-hydroxylated derivatives of oxindole-3-acetylaspartic acid and oxindole-3-acetylglutamic acid. There was no evidence of significant incorporation of label from [2'-$^{14}C_1$] or [$^{13}C_6$]indole-3-acetic acid into any of these conjugated indoles.

Dalbergia dolichopetala is a woody legume from the Brazilian cerrado. Mature dry seeds contain indole-3-ethanol, IAA (Fig. 1, I)[2], and small quantities of IAA ester conjugates (11). During the initial 24 h of germination, there is an increase in the size of the endogenous IAA pool, which then declines markedly over the next 5 d (12). The 6-d-old germinating seeds convert both [$^2H_5$]L-tryptophan and [$^2H_5$]indole-3-ethanol to IAA. The metabolism of [$^{14}C_1$]IAA leads to the

production of IAAsp[3] (II) and several unidentified, polar, nondecarboxylated products (13).

Application of [$^{14}C_1$]IAAsp to germinating *Dalbergia* seed results in the rapid accumulation of a polar, water-soluble metabolite and only trace amounts of IAA (13). This is in keeping with the situation in *Vicia faba* seedlings, where [$^{14}C,^3H$]IAAsp applied to cotyledons is converted primarily to DiOxIAAsp (III) and 3-Gluc-OxIAAsp (IV), with the formation of IAA being at best a minor event (21). Likewise, [^{14}C]IAAsp applied to cotyledons of *Glycine max* L. is rapidly metabolized to compounds other than IAA (4), and only a small amount of IAA is released following application of IAAsp to stem sections of *Phaseolus vulgaris* (2). Thus, the role of IAAsp in these species is apparently unlike that of arabinoside and galactoside conjugates of IAInos (V) in *Zea mays* (8, 9), because its main function does not appear to be that of a storage product that can be hydrolyzed to provide the plant with a ready supply of IAA (13).

This paper reports on a study of the metabolism of a mixture of [2'-$^{14}C_1$]IAA and [$^{13}C_6$]IAA by germinating seed of *D. dolichopetala* in which purified samples were analyzed by HPLC-MS in an attempt to identify polar, nondecarboxylated metabolites of IAA and IAAsp.

MATERIALS AND METHODS

Plant Material

Seeds of "jacarandá do cerrado" (*Dalbergia dolichopetala*, also known as *D. violacea* and *D. miscolobium*) collected in the cerrado reserve of Itirapina, São Paulo, Brazil in April

[2] Throughout the text, boldface Roman numerals refer to the structures depicted in Figure 1. Such designations should not be confused with the lightface Roman numerals used to identify metabolite peaks in Figure 2.

[3] Abbreviations: IAAsp, indole-3-acetylaspartic acid; DiOxIAA, dioxindole-3-acetic acid; DiOxIAAsp, dioxindole-3-acetylaspartic acid; DiOxIAGlu, dioxindole-3-acetylglutamic acid; FAB, fast atom bombardment; 3-Gluc-OxIAAsp, 3-(O-β-D-glucosyl)-oxindole-3-acetylaspartic acid; 7-Gluc-OxIAA, 7-(O-β-D-glucosyl)-oxindole-3-acetic acid; HPLC-RC, high performance liquid chromatography-radiocounting; IAGlu, indole-3-acetylglutamic acid; IAInos, 2-O-indole-3-acetyl-*myo*-inositol; [MH]⁺, molecular ion; 7-OH-OxIAA, 7-hydroxy-oxindole-3-acetic acid; OxIAA, oxindole-3-acetic acid; OxIAAsp, oxindole-3-acetylaspartic acid; OxIAGlu, oxindole-3-acetylglutamic acid.
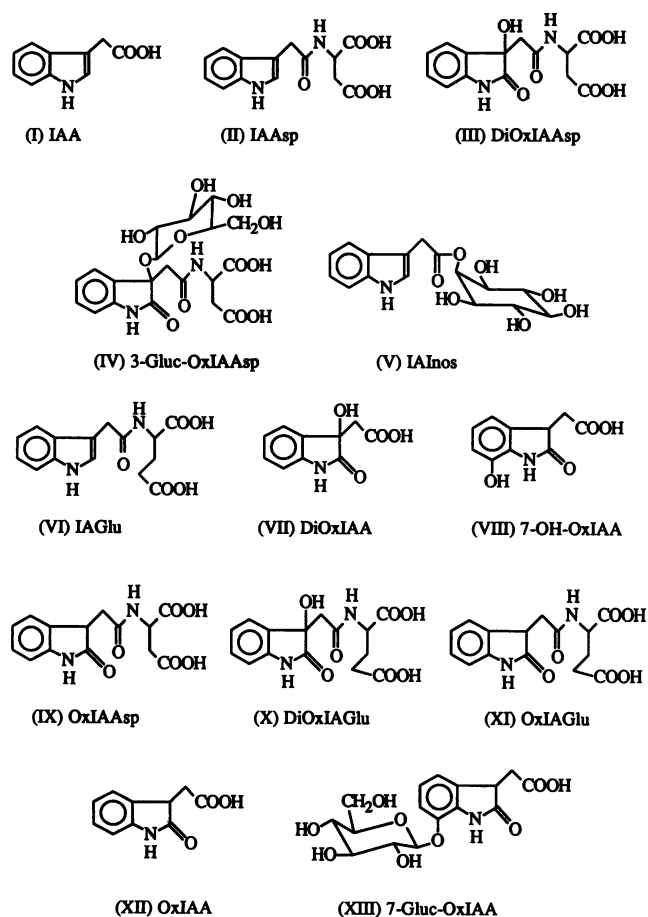
Figure 1. Structures of IAA and related compounds (**I-XIII**). Compounds are referred to by boldface Roman numerals in the text.

and May 1988, were soaked in running tap water for 3 h then placed on damp filter paper in 10-cm glass Petri dishes. They were maintained in a growth room at 25°C with a 24-h photoperiod supplied by Osram "warm white" and daylight fluorescent tubes and incandescent bulbs providing a radiation flux of about 50 $W \cdot m^{-2}$. Isotopically labeled IAA was applied to the germinating seed 6 d after soaking when about 30 cm of root growth was evident and the cotyledons had started to green.

Feeding Conditions

[2'-^{14}C$_1$]IAA (170 × 10^6 dpm, specific activity 54 mCi mmol^{-1}) (Amersham International plc, Amersham, Buckshire, UK) was mixed with [^{13}C$_6$]IAA (270 μg) (Cambridge Isotope Laboratories, Cambridge, UK), dissolved in 850 μL of 50% aqueous ethanol, and 5-μL droplets were applied to the cotyledons of 170 6-d germinated *Dalbergia* seeds incubated in the conditions described above. After an 8-h metabolism period, the seeds were frozen in liquid nitrogen prior to extraction with methanol.

Extraction and Partitioning

The frozen plant material was homogenized and extracted twice, for 30 min, with 500 mL of ice-cold methanol contain-

ing 5 mM sodium diethyldithiocarbamate (6). The methanolic extracts were combined, filtered through cellulose powder, reduced to dryness in vacuo, dissolved in 100 mL of 0.1 M phosphate buffer, pH 8.0, slurried with insoluble PVP (7), filtered, and partitioned against three 100-mL volumes of ethyl acetate. The aqueous phase was then adjusted to pH 2.5 with H$_2$SO$_4$ and partitioned against 50-mL volumes of ethyl acetate. The acidic ethyl acetate extracts were combined, treated with anhydrous sodium sulfate to remove water, and reduced to dryness in vacuo. The neutral ethyl acetate extracts were treated in a similar manner. The residual aqueous phase was frozen and lyophilized. Radioactivity in aliquots of the aqueous phase and the two ethyl acetate extracts was estimated by liquid scintillation counting. Aliquots of all three extracts were also examined by HPLC-RC.

Reversed-Phase Flash Chromatography

Reversed-phase flash chromatography of the aqueous phase and the acidic ethyl acetate extract was carried out on a 20-mm i.d. column, with the slurry packed with 15 g of preparative C$_{18}$ support (50–150 μm) (Waters Associates, Milford, MA) to give a bed height of about 150 mm. The samples, dissolved in 1 mL of methanol to which was added 100 mL of 0.5% aqueous acetic acid, were applied to the column, which was then eluted stepwise, at about 10 mL min^{-1}, with 100-mL volumes of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 50% methanol in 0.5% aqueous acetic acid. Successive 50-mL fractions were collected and aliquots taken for liquid scintillation counting. After examination by HPLC-RC, selected fractions were combined, reduced to dryness in vacuo, and further purified by reversed-phase HPLC.

HPLC

Solvents were delivered at a flow rate of 1 mL min^{-1} by an Altex liquid chromatograph (Altex Scientific Inc., Berkeley, CA). Samples were injected via an Altex 210 sample injection valve with a 1-mL loop. Ion suppression reversed-phase HPLC utilized a 250 × 4.6 mm i.d. 5 μm ODS Hypersil column (Capital HPLC Specialists, Bathgate, Lothian, UK) that was eluted with a 25-min, 10 to 60% gradient of methanol in 0.5% aqueous acetic acid. Samples were also analyzed isocratically using methanol-0.5% aqueous acetic acid mixtures. Column eluate was directed first to an LC 871 absorbance monitor (Pye Unicam, Cambridge, UK) operating at 280 nm, then to a 9701 radioactivity monitor (Reeve Analytical Ltd., Glasgow, UK) with a 200-μL heterogeneous flow cell packed with cerium-activated lithium glass scintillator (19, 20). Signals from both detectors were processed by a 27000 interactive data system with LC-Spring software (Reeve Analytical).

HPLC-MS

Partially purified fractions were analyzed utilizing either a capillary or a microbore reversed-phase HPLC column linked to a double focusing mass spectrometer. In the case of the capillary system, a 590 pump (Waters Associates) was used to deliver a mobile phase of methanol:water:acetic acid

(30:70:1, v/v/v), containing 1% glycerol as a matrix, at a flow rate of 5 μL min^{-1}. The inlet of a 300 × 0.32-mm i.d. capillary column packed with 5 μm C_{18} (LC Packings, Amsterdam, The Netherlands) was inserted directly into a model 7520 injection valve with a 200-nL loop (Rheodyne, Cotati, CA). The outlet of the capillary HPLC column was linked via fused silica tubing (1 m × 50 μm i.d.) to a frit-FAB HPLC-MS interface attached to the ion source of a SX102 double focusing mass spectrometer (JEOL Ltd., Tokyo, Japan). The ion source temperature was 50°C, and ions were generated with a beam of 5 kV xenon atoms at an emission current of 20 mA. The mass spectrometer acceleration voltage was 8 kV and the slits were set to provide a resolution of 1000. The signal from the mass spectrometer was processed by a JEOL JMA-DA6000 data system, and positive ion spectra were recorded at a rate of 3 s per scan with a cyclic time of 3.2 s for a mass range of 50 to 800 amu.

With the microbore HPLC system, the liquid chromatograph consisted of two M510 pumps with micropump heads, controlled by a M680 gradient controller (Waters Associates) and a Rheodyne 752 injection valve with a 1-μL loop. A mobile phase of methanol:water:acetic acid (10:90:1, v/v/v) in a 1% glycerol matrix was delivered at a flow rate of 100 μL min^{-1} to a 100 × 1 mm i.d. column packed with a YMC ODS 5-μm support (Yamura Chemical Laboratory Co. Ltd., Kyoto, Japan). Eluent emerging from the microbore column was passed to a JEOL pneumatic splitter and the flow directed to the frit-FAB LC interface reduced to 4 μL min^{-1}. The mass spectrometer was operated as described above, except that the ion source was at 65°C.

RESULTS

A mixture of [2'-^{14}C$_1$]IAA (170 × 10^6 dpm) and [^{13}C$_6$]IAA was applied in aqueous ethanol to the cotyledons of 170 6-d-germinated *D. dolichopetala* seeds. After an 8-h metabolism period, the seeds were extracted with methanol and 130 × 10^6 dpm of radioactivity recovered. Analysis of an aliquot of the methanolic extract by HPLC-RC revealed the presence of residual IAA along with metabolites I through V (Fig. 2A). Similar metabolic profiles had been obtained in an earlier study in which metabolite V had been identified as IAAsp by combined GC-MS (13).

The methanolic extract was reduced to dryness in vacuo and neutral and acidic ethyl acetate fractions were obtained along with an aqueous fraction. The neutral ethyl acetate extract was discarded because it was very impure and contained only 3 × 10^6 dpm of radiolabeled components that were present in the other two fractions in much larger amounts. The acidic ethyl acetate extract (57 × 10^6 dpm) and the aqueous fraction (27 × 10^6 dpm) were examined by HPLC-RC. The major component in the ethyl acetate fraction was metabolite V, whereas metabolite II was the main radiolabeled component in the aqueous extract. Relatively small amounts of metabolites III and IV were present in both samples (Fig. 1, B and C).

The acidic ethyl acetate and aqueous extracts were fractionated by preparative reversed-phase flash chromatography. After examination of aliquots by HPLC-RC, fractions containing individual metabolite peaks were combined and
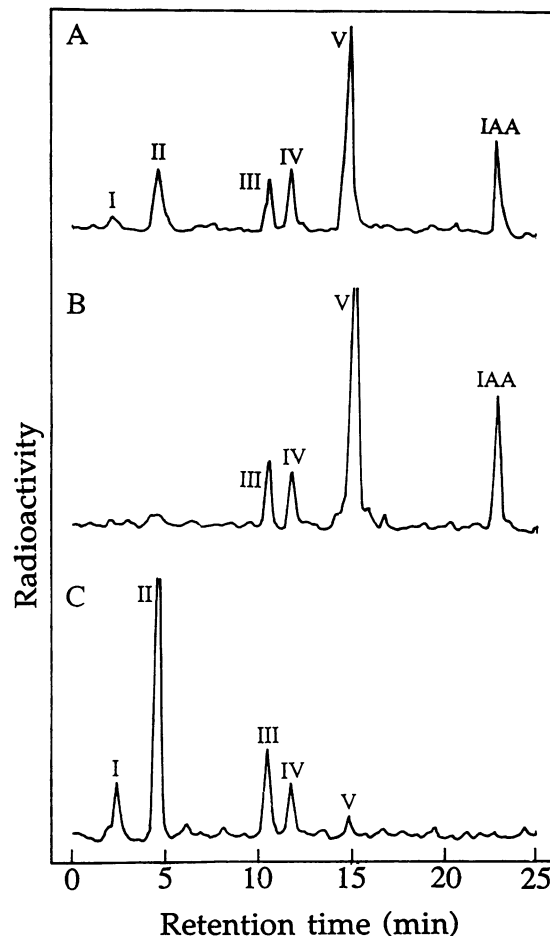

Figure 2. Gradient elution, reversed-phase HPLC-RC of [2'-^{14}C$_1$] IAA and [^{13}C$_6$]IAA metabolites from 6-d-germinated seeds of *D. dolichopetala*. A, Methanolic extract; B, acidic ethyl acetate extract; C, aqueous fraction.

reduced to dryness in vacuo prior to further purification by isocratic reversed-phase HPLC-RC, after which the following quantities of radiolabeled metabolites were recovered: metabolite I, 2.7 × 10^6 dpm; metabolite II, 6.5 × 10^6 dpm; metabolite III, 20.7 × 10^6 dpm; metabolite IV, 0.2 × 10^6 dpm; metabolite V, 6.5 × 10^6 dpm. The semipurified metabolites were next analyzed by HPLC-MS.

HPLC-MS Analysis of Metabolite V Fraction

The extracted ion chromatograms and mass spectra obtained from HPLC-MS analysis of the metabolite V fraction are illustrated in Figure 3, A through C. The mass chromatograms indicate the presence of two major peaks that corresponded to IAAsp (II) and IAGlu (VI) (Fig. 3A). The full-scan FAB mass spectra that were obtained confirmed these identifications (Fig. 3, B and C). The IAAsp spectrum contains a characteristic m/z 291 [MH]$^+$ and a m/z 130 quinolinium ion. Fragments associated with both of these ions, namely m/z 293/297 and m/z 132/136, provide evidence of ^{14}C$_1$ and ^{13}C$_6$ labeling (Fig. 3B). However, the intensities of m/z 293 and 132 relative to m/z 291 and 130, respectively, indicate
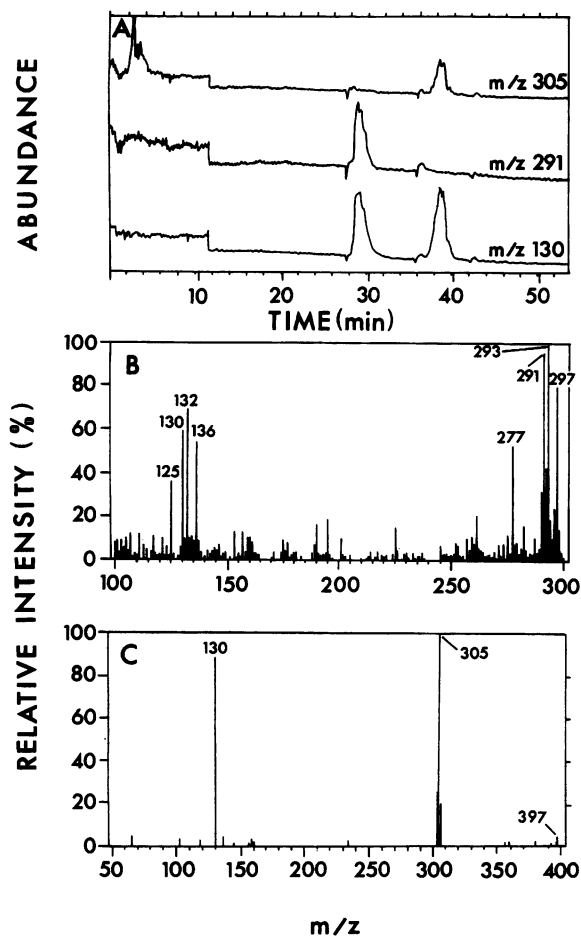
Figure 3. Frit-FAB capillary HPLC-MS analysis of metabolite V fraction. A, Extracted ion chromatograms; B, mass spectrum of [$^{14}C_1$,$^{13}C_6$]IAAsp; C, mass spectrum of IAGlu.

an overabundance of these fragments that is probably due to effects associated with subtraction of the glycerol background.

The dominant fragments in the IAGlu spectrum are, similarly, the [MH]$^+$ and quinolinium ion at m/z 305 and 130 (Fig. 3C). In addition, there is a small but diagnostic [MH]$^+$ glycerol adduct at m/z 397. Fragments indicative of $^{14}C_1$ and $^{13}C_6$ labeling were not present in the IAGlu spectrum.

HPLC-MS Analysis of Metabolite II Fraction

Data obtained from microbore HPLC-MS analysis of the metabolite II fraction are presented in Figures 4 and 5, A through D. The extracted ion chromatograms show four major peaks (A–D) (Fig. 4). The mass spectrum obtained from peak B (Fig. 5B) is that of DiOxIAAsp (III) in which the major fragments are the m/z 323 [MH]$^+$, m/z 306/7, and a m/z 146 2-oxoquinolinium ion. Although the mass spectra from peaks A, C, and D (Fig. 5A, C, and D) do not correspond with those of any of the available indole standards, the positive ion FAB fragmentation patterns do provide evidence of the identity of the compounds involved.

Mass spectra of hydroxylated oxindole derivatives of IAA are diagnostic in that they can be used to distinguish between dioxindoles, in which C-3 of the pyrrole ring is hydroxylated, and oxindoles with a hydroxyl group attached to the benzene ring (17). The dioxindoles, such as DiOxIAA (VII) and Di-OxIAAsp (III), yield spectra containing a dominant m/z 146 2-oxoquinolinium ion (see Fig. 5B), whereas the spectra of benzene ring-hydroxylated oxindoles, such as 7-OH-OxIAA (VIII), are characterized by the presence of a strong m/z 162 hydroxy-2-oxoquinolinium ion, with the m/z 146 fragment being either small or absent. In both instances, an initial loss of a hydroxyl group, as either oxygen or H_2O, results in the appearance of [MH-16]$^+$ and/or [MH-18]$^+$ fragments in FAB spectra (17). On this basis, the mass spectrum in Figure 5A, containing a m/z 323 [MH]$^+$ and a m/z 415 [MH]$^+$ glycerol adduct together with major ions at m/z 305 and 162 and a minor fragment at m/z 146, is likely to be that of a "ring"-hydroxylated derivative of OxIAAsp (IX), the exact locus of the ring hydroxyl group being, as yet, undetermined.

The mass spectra illustrated in Figure 5, C and D are very similar indeed to those of the aspartate derivatives in Figure 5, A and B, except that the analogous [MH]$^+$, [MHG]$^+$, [MH-16]$^+$, and [MH-18]$^+$ fragments are increased by 14 amu. This implies that they are glutamate rather than aspartate indole conjugates. More specifically, the spectra indicate the presence of DiOxIAGlu (X) (Fig. 5D) and a benzene ring-hydroxylated derivative of OxIAGlu (XI) (Fig. 5C). The mass spectra illustrated in Figure 5, A through D demonstrate an absence of significant incorporation of label from [$^{14}C_1$]IAA and [$^{13}C_6$] IAA into DiOxIAAsp and the putative DiOxIAGlu and benzene ring-hydroxylated derivatives of OxIAAsp and OxIAGlu.

HPLC-MS Analyses of Metabolite I, III, and IV Fractions

HPLC-MS analyses of the metabolite I, III, and IV fractions did not yield any mass spectra recognizable as either potential metabolites or derivatives of IAA.
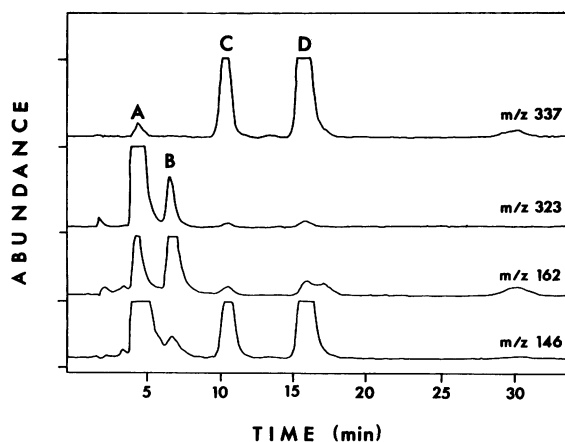

Figure 4. Frit-FAB microbore HPLC-MS analysis of metabolite II fraction. Extracted ion chromatograms.
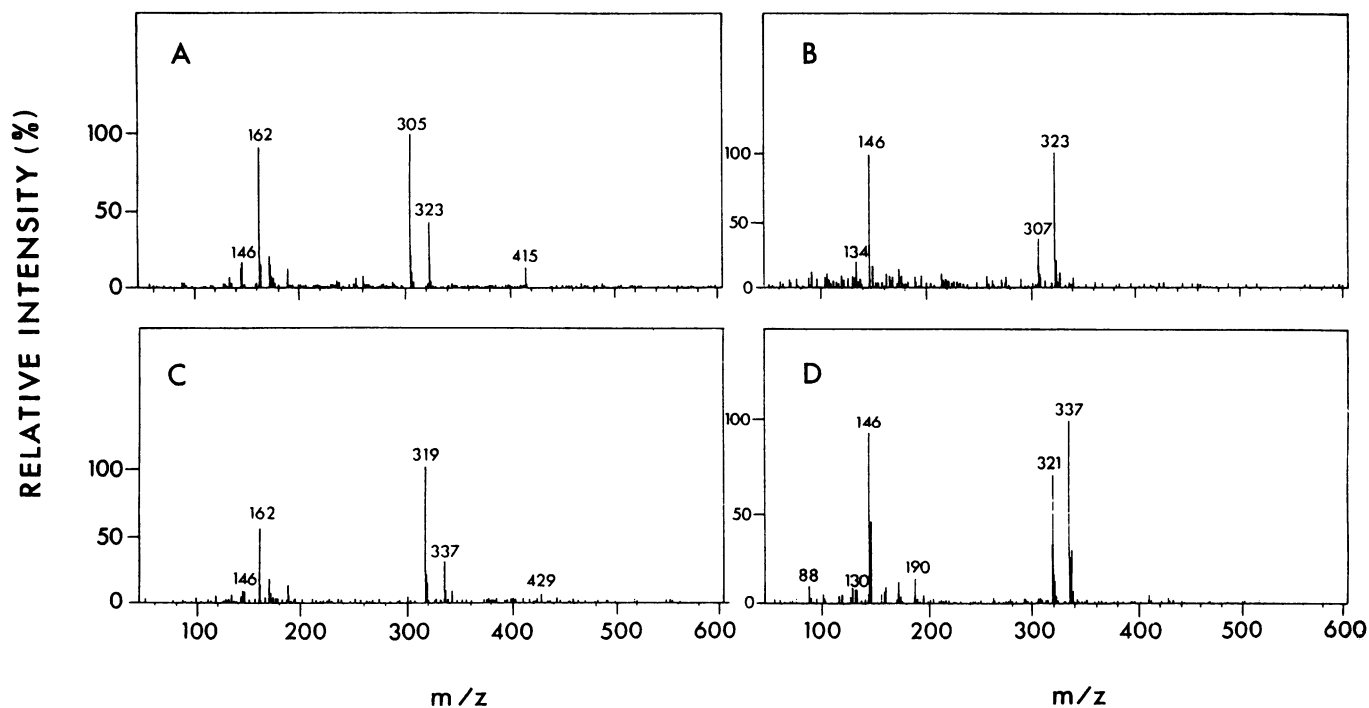
Figure 5. Frit-FAB microbore HPLC-MS analysis of metabolite II fraction. A, Mass spectrum of ion chromatogram peak A (see Fig. 3); B, mass spectrum of ion chromatogram peak B; C, mass spectrum of ion chromatogram peak C; D, mass spectrum of ion chromatogram peak D.

DISCUSSION

Eight hours after the application of $[2'-^{14}C_1]$IAA and $[^{13}C_6]$ IAA to cotyledons of 6-d-germinated *Dalbergia* seed, most of the substrate was converted to five radiolabeled metabolite peaks (I–V) that were resolved by HPLC-RC. The major components were metabolites II and V. Analysis of the metabolite V fraction by HPLC-MS identified $[^{14}C_1,^{13}C_6]$IAAsp and unlabeled IAGlu. The ion chromatograms in Figure 4 suggest that the two amino acid conjugates were present in broadly similar amounts. The lack of detectable isotope labeling in the spectrum of IAGlu therefore implies that the preferred route for the conversion of IAA is via IAAsp rather than IAGlu.

A previous study has shown that IAAsp is converted rapidly to metabolite II (13). In the present investigation, HPLC-MS analysis of the metabolite II fraction demonstrated the presence of DiOxIAAsp and putative DiOxIAGlu and benzene ring-hydroxylated derivatives of OxIAAsp and OxIAGlu. Despite the presence of significant amounts of radioactivity in the metabolite II fraction, there was no evidence of $^{14}C_1,^{13}C_6$ labeling in the spectra of any of these compounds. This may indicate that IAA and IAAsp are converted to other components. Arguably, it is more likely to be a consequence of the oxindole and dioxindole amino acid derivatives having large endogenous pools and a relatively slow rate of turnover.

Although IAAsp is now being detected in plant extracts with increasing frequency (1, 3–5, 16), there are relatively few reports on the metabolism of IAAsp. Data obtained with *Populus tremula* cuttings suggest an IAA → IAAsp → Ox-

IAAsp pathway (18), whereas in *Vicia faba* seedlings, Di-OxIAAsp and 3-Gluc-OxIAAsp are both derived from IAAsp, with OxIAA probably acting as an initial intermediate (21). In the present study with *Dalbergia* seedlings, although the individual steps in the metabolic sequence have yet to be confirmed, the available evidence is suggestive of an IAAsp → OxIAAsp pathway with OxIAAsp acting as a branch point, giving rise to both DiOxIAA and a ring-hydroxylated OxIAAsp derivative. In addition, there would appear to be a parallel route involving glutamate rather than aspartate conjugates.

In summary, the evidence obtained with several dicotyledonous species indicates that the metabolism of IAA initially involved the formation of an amino acid conjugate that is subsequently oxidized to yield oxindole, dioxindole, and, in some instances, glycosyl derivatives. Except for an absence of amino acid conjugation, similar pathways appear to operate in the monocot *Zea mays* as IAA is converted to OxIAA (XII) as the first step in a route leading to 7-Gluc-OxIAA (XIII) (10, 14, 15).

ACKNOWLEDGMENTS

LITERATURE CITED

1. **Andersson B, Sandberg G** (1982) Identification of endogenous N-(3-indole-acetyl) aspartic acid in Scots pine (*Pinus sylvestris* L.) by combined gas chromatography-mass spectrometry using

high performance liquid chromatography for quantification. J Chromatogr **238:** 151–156

2. **Bialek K, Meudt WJ, Cohen JD** (1983) Indole-3-acetic acid (IAA) and IAA conjugates applied to bean stem sections. IAA content and growth response. Plant Physiol **73:** 130–134

3. **Cohen JD** (1982) Identification and quantitative analysis of indole-3-acetyl-L-aspartate in seeds of *Glycine max* L. Plant Physiol **70:** 749–753

4. **Cohen JD, Baldi B** (1983) Studies on endogenous indole-3-acetyl-L-aspartate during germination of soybeans. Proc Plant Growth Regul Soc Am **10:** 117–122

5. **Cohen JD, Ernstsen A** (1991) Indole-3-acetic acid and indole-3-acetylaspartate isolated from seeds of *Heracleum laciniatum* Horn. Plant Growth Regul **10:** 95–101

6. **Ernstsen A, Sandberg G, Crozier A** (1986) Effects of sodium diethyldithiocarbamate, solvent, temperature and plant extracts on the stability of indoles. Physiol Plant **68:** 513–522

7. **Glenn JL, Kuo CC, Durley RC, Pharis RP** (1972) Use of insoluble polyvinylpyrrolidone on the purification of plant extracts and chromatography of plant hormones. Phytochemistry **172:** 47–57

8. **Hall PJ, Bandurski RS** (1987) [^{3}H]Indole-3-*myo*-inositol hydrolysis by extracts of *Zea mays* L. vegetative tissues. Plant Physiol **80:** 374–377

9. **Komoszynski MA, Bandurski RS** (1986) Transport and metabolism of indole-3-acetyl-*myo*-inositol-galactoside in seedlings of *Zea mays*. Plant Physiol **80:** 961–964

10. **Lewer P, Bandurski, RS** (1987) Occurrence and metabolism of 7-hydroxy-2-indolinone-3-acetic acid in *Zea mays*. Phytochemistry **26:** 1247–1250

11. **Monteiro AM, Crozier A, Sandberg G** (1987) Detection of abscisic acid, indole-3-acetic acid and indole-3-ethanol in seeds of *Dalbergia dolichopetala*. Phytochemistry **26:** 327–328

12. **Monteiro AM, Crozier A, Sandberg G** (1988) Endogenous hormones, germination and early seedling growth of *Dalbergia dolichopetala*. J Plant Physiol **132:** 762–765

13. **Monteiro AM, Crozier A, Sandberg G** (1988) The biosynthesis and conjugation of indole-3-acetic acid in germinating seed and seedlings of *Dalbergia dolichopetala*. Planta **174:** 561–568

14. **Nonhebel H, Bandurski RS** (1984) Oxidation of indole-3-acetic acid and oxindole-3-acetic acid to 2,3-dihydro-7-hydroxy-2-oxo-1H indole-3-acetic acid-7'-*O*-β-D-glucopyranoside in *Zea mays* seedlings. Plant Physiol **76:** 979–983

15. **Nonhebel H, Kruse LI, Bandurski RS** (1985) Indole-3-acetic acid catabolism in *Zea mays* seeds. Metabolic conversion of oxindole-3-acetic acid to 7-hydroxy-2-oxindole-3-acetic acid-7'-*O*-β-D-glucopyranoside. J Biol Chem **260:** 12685–12689

16. **Nordström A-C, Eliasson L** (1991) Levels of endogenous indole-3-acetic acid and indole-3-acetylaspartic acid during adventitious root formation in pea cuttings. Physiol Plant **84:** 599–605

17. **Östin A, Moritz T, Sandberg G** (1992) Liquid chromatography-mass spectrometry of indole-3-acetic acid and indole-3-acetic acid derivatives. Biol Mass Spectrom **21:** 292–298

18. **Plüss R, Jenny T, Meier H** (1989) IAA-induced adventitious root formation in greenwood cuttings of *Populus tremula* and formation of 2-indolone-3-acetylaspartic acid, a new metabolite of exogenously applied indole-3-acetic acid. Physiol Plant **75:** 89–96

19. **Reeve DR, Crozier A** (1983) HPLC radioactivity monitors—fact and fiction. Lab Pract **32:** 59–60

20. **Sandberg G, Crozier A, Ernstsen A** (1987) Indole-3-acetic acid and related compounds. *In* L Rivier, A Crozier, eds, The Principles and Practice of Plant Hormone Analysis, Vol 2. Academic Press, London, pp 169–301

21. **Tsurumi S, Wada S** (1986) Dioxindole-3-acetic acid conjugate formation from indole-3-acetylaspartic acid in *Vicia* seedlings. Plant Cell Physiol **27:** 1513–1522