Phytochemistry, Vol. 26, No. 2, pp. 327–328, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

DETECTION OF ABSCISIC ACID, INDOLE-3-ACETIC ACID AND INDOLE-3-ETHANOL IN SEEDS OF *DALBERGIA DOLICHOPETALA*

ANA MARIA MONTEIRO*, GÖRAN SANDBERG† and ALAN CROZIER‡

Department of Botany, The University, Glasgow G12 8QQ, U.K.; †Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden

(*Received* 30 *May* 1986)

Key Word Index—*Dalbergia dolichopetala*; Leguminosae; seeds; abscisic acid; indole-3-acetic acid; indole-3-ethanol; HPLC; GC-MS.

Abstract—Abscisic acid (ABA) and indole-3-acetic acid (IAA) have been identified by GC-MS in a purified acidic EtOAc fraction from seeds of *Dalbergia dolichopetala* while GC-SIM was used to detect indole-3-ethanol (IEt) in a neutral EtOAc extract. GC-MS and GC-SIM analyses of neutral and acidic EtOAc fractions obtained after base hydrolysis indicated that the seeds also contained ester conjugates of ABA and IAA. Oxindole-3-acetic acid (OxIAA), indole-3-carboxylic acid (ICA) and indole-3-methanol (IM) were not detected in any of the extracts that were investigated.

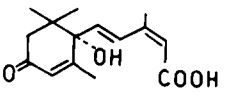
INTRODUCTION

The 'cerrado' is a region of arid, savanna-like vegetation occupying 25% of the total area of Brazil. Following germination, the development of many 'cerrado' plants, such as *Kielmeyera corimbosa* (sp) Mart., *Magonia pubescens* St. Hil. and *Dalbergia dolichopetala*, is characterized by rapid root elongation accompanied by a relatively slow rate of shoot growth (see [1]). It has been suggested that this growth pattern, which is thought to be an adaptation to help germinating seedlings reach the deep water table, may be controlled by auxins [2]. This paper reports on an analysis of endogenous indoles and ABA in mature seeds of the woody legume *Dalbergia dolichopetala*.

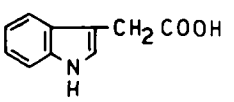
RESULTS AND DISCUSSION

A methanolic extract from 700 g of mature, dry *Dalbergia* seeds was reduced to dryness, dissolved in 100 ml 0.1 M, pH 8 Pi buffer and purified by polyvinylpyrrolidone (PVP) column chromatography before neutral and acidic EtOAc fractions were obtained. The residual aqueous phase was adjusted to pH 11 with 2 M NaOH and incubated at 90° for 1 hr. The sample was then cooled and partitioned against EtOAc at pH 8 and 2.7. The alkali treatment results in the hydrolysis of both IAA ester conjugates [3], and ABA glucosyl ester [4] and the cleaved products are recovered in the EtOAc extracts.

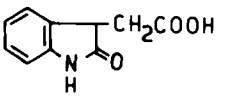
The neutral and acidic EtOAc extracts obtained before and after base hydrolysis were purified on silica gel Sep-Pak before reversed and normal phase HPLC where fractions corresponding to the retention volumes of ABA (1), IAA (2), OxIAA (3), ICA (4), IEt (5) and IM (6) were collected. Each fraction was then methylated and/or silylated to form trimethylsilyl (TMS) derivatives before being analysed by GC-MS in the scanning and selected ion monitoring (SIM) modes.



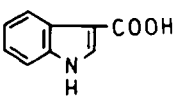
ABSCISIC ACID (1)



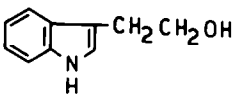
INDOLE-3-ACETIC ACID (2)



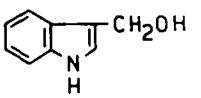
OXINDOLE-3-ACETIC ACID (3)



INDOLE-3-CARBOXYLIC ACID (4)



INDOLE-3-ETHANOL (5)



INDOLE-3-METHANOL (6)

The GC-MS data obtained are summarized in Table 1. Full scan spectra established the presence of both ABA and IAA in the acidic EtOAc fraction while GC-SIM revealed trace amounts of IEt in the neutral EtOAc extract. Neither procedure was able to detect OxIAA, ICA or IM. GC-MS and GC-SIM analyses of the EtOAc fractions obtained after base hydrolysis indicated the release from conjugated forms of a significant amount of ABA and a relatively small quantity of IAA. *Dalbergia dolichopetala* thus differs from other legumes such as *Glycine max* and *Phaseolus vulgaris* as the mature seed of

*Permanent address: Departmento de Fisiologia Vegetal, Universidade de Campinas, 13100 Campinas, São Paulo, Brazil.

‡To whom correspondence should be addressed.

Table 1. Summary of GC-MS and GC-SIM analyses of EtOAc fractions obtained (A) before base hydrolysis and (B) after base hydrolysis of an extract from seeds of *Dalbergia dolichopetala*

Compound	Characteristic ions*	A		B	
		Neutral	Acidic	Neutral	Acidic
ABA-Me	278 (2), 190 (100), 162 (59)	—	FS, SIM	—	FS, SIM
bis-TMS-IAA	319 (15), 304 (4), 202 (100)	—	FS, SIM	—	FS, SIM
tris-TMS-OxIAA	407 (12), 290 (100), 202 (20)	—	n.d.	—	n.d.
bis-TMS-ICA	305 (100), 290 (60), 256 (80)	—	n.d.	—	n.d.
bis-TMS-IEt	305 (25), 216 (7), 202 (100)	SIM	—	n.d.	—
bis-TMS-IM	291 (100), 276 (8), 202 (80)	n.d.	—	n.d.	—

FS—full scan mass spectrum, SIM—selected ion monitoring (3 ions), n.d.—not detected.
*m/z values with relative intensities in brackets.

these species contain relatively large pools of IAA ester conjugates [3]. In the current investigation there was no evidence of the hydrolysis of conjugates of OxIAA, ICA, IEt and IM.

EXPERIMENTAL

Plant material. Mature, dry seeds of *Dalbergia dolichopetala* were collected in the 'cerrado' near Brasilia, Brazil, in July 1985.

Extraction and partitioning. D. dolichopetala seeds (700 g dry wt) were homogenized in 2 l. of cold MeOH containing 0.02 M Na diethyldithiocarbamate as an antioxidant. After extraction for 2 hr at 4°, the extract was filtered and reduced to dryness. The sample was dissolved in 200 ml 0.1 M, pH 8 Pi buffer, centrifuged in order to remove debris and applied to a 300 × 20 mm i.d. PVP column which was eluted with 100 ml of Pi buffer. The 300 ml eluent was collected and saturated with $(NH_4)_2SO_4$. After cooling to 4°, precipitated proteins were removed by centrifugation at 12000 *g* for 15 min and the supernatant partitioned × 5 against 1/3 vols EtOAc. The aq. phase was then adjusted to pH 2.7 and again partitioned against EtOAc. The neutral and acidic EtOAc extracts were dried with Na_2SO_4 and reduced to dryness *in vacuo*. After partitioning at pH 2.7, the aq. phase was adjusted to pH 11 with 2 M NaOH and incubated at 90° for 1 hr before being cooled to *ca* 25° and partitioned against EtOAc at pH 7.0 and 2.7 as described above.

Silica gel Sep-Pak. The four EtOAc extracts were purified individually using silica gel Sep-Paks (Waters Associates). The Sep-Paks were prewashed with 10 ml 0.5 M NOAc and 5 ml hexane and equilibrated with EtOAc–hexane (1:4). Samples were applied to the Sep-Pak cartridge in 5 ml EtOAc–hexane (1:4) and eluted with 5 ml EtOAc–hexane (7:3) which was collected and reduced to dryness under a stream of N_2.

HPLC. Semi-purified extracts were introduced off-column, via a Valco injector valve with a 250 μl loop, at a flow rate of 0.8 ml/min, using a liquid chromatograph consisting of two Gilson 302 pumps and an Apple II gradient controller. Column eluent was monitored with either a Spectra Physics SF 970 fluorimetric detector (excitation 285 ± 5 nm, emission 360 ± 10 nm) or an LDC absorbance monitor (254 nm). Reversed phase HPLC utilized a 125 × 4 mm i.d. 5 μm Lichrosorb C18 column. Acidic extracts were eluted isocratically with 45% MeOH in 1% HOAc while a 25 min gradient of 20–70% MeOH in 0.01 M, pH 6.5 Pi buffer was used for neutral samples. Fractions corresponding to the R_fs of IAA, OxIAA, IEt, IM, ICA and ABA were collected, reduced to the aq. phase and partitioned × 5 against Et_2O. The Et_2O fractions were combined, reduced to dryness, dissolved in 100 μl 20% hexane in EtOAc and subjected to normal phase HPLC on a 250 × 4 mm i.d. 5 μm Nucleosil CN column, eluted with a 25 min gradient of 20–80% EtOAc in hexane. Glacial HOAc (1%) was added to the mobile phase when acidic extracts were chromatographed. Fractions corresponding to the R_fs of the compounds of interest were collected and reduced to dryness under a stream of N_2. To avoid column memory effects from standards a blank injection was carried out immediately prior to the analysis of all extracts.

GS-MS. Following reversed and normal phase HPLC, the purified samples were either methylated in ethereal diazomethane or silylated in 100 μl CH_3CN–bis(trimethylsilyl)trifluoroacetamide (BSTFA) (1:1, v/v), at 70° for 15 min. The derivatized extracts were reduced to dryness and dissolved in 30 μl *n*-hexane before being analysed by GC-MS on an HP 5890 gas chromatograph linked via a direct capillary inlet to an HP 5970B mass selective detector equipped with an HP 9000 computer system. Samples were injected in the splitless mode (2 min splitless time) at 225° onto a 25 m × 0.31 mm i.d. cross-linked methyl silicone capillary column with a 0.52 μm film thickness. The column temperature was initially held at 60° for 3 min, then programmed at 30°/min to 130° followed by a 7°/min gradient to 235°. The interface temperature was maintained at 250°. The He carrier gas flow rate was 1 ml/min. The retention time for air in the system was 63 sec. In the scanning mode positive ion EIMS were recorded at 70 eV. When SIM was utilized three characteristic ions in the mass spectrum of the compound of interest were monitored (see Table 1). In all instances blank injections were carried out prior to the analysis of extracts.

Acknowledgements—A.M.M. was supported by a postdoctoral fellowship from CNPq (Brazil). G.S. acknowledges financial assistance from the Swedish Council for Forestry and Agricultural Research. A.C. and G.S. would also like to thank the British Council for their support.

REFERENCES

1. Rizzini, C. T. (1985) *Ann. Mo. Bot. Gdn* **52**, 410.
2. Poggiani, F. (1971) in *III Simpósio sobre o Cerrado*, p. 201. E. Blücher, USP, São Paulo.
3. Bandurski, R. S. and Schulze, A. (1977) *Plant Physiol.* **60**, 211.
4. Neill, S. J., Horgan, R. and Heald, J. K. (1983) *Planta* **157**, 371.