*Phytochemistry*, Vol. 22, No. 2, pp. 589–590, 1983.
Printed in Great Britain.

0031–9422/83/020589–02$03.00/0
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# SHORT REPORTS

# INDOLE-3-ACETIC ACID IN DOUGLAS FIR SEEDLINGS: A REAPPRAISAL

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(*Received* 16 *August* 1982)

**Key Word Index**—*Pseudostuga menziesii*; Douglas fir; Pinaceae; indole-3-acetic acid.

**Abstract**—The use of ring-labelled, pentadeutero IAA as an internal standard in selected ion monitoring analysis of Douglas fir seedlings revealed an estimate of IAA which was nearly an order of magnitude smaller than that reported earlier.

## INTRODUCTION

In an earlier study of the presence of indole-3-acetic acid (IAA) in Douglas fir [*Pseudostuga menziesii* (Mirb) Franco] seedlings, a deuterated analog was used as an internal standard in selected ion monitoring, as provided by combined GC/MS [1]. The deuterium atoms were located on the methylene carbon of the side chain. Magnus *et al.* [2] have more recently demonstrated the labile nature of deuterium at this position. A tetradeuterated analog (4,5,6,7-$d_4$-IAA), however, was shown to be very stable, even under conditions of alkaline hydrolysis of IAA conjugates. They also demonstrated that the pentadeuterated analog (2,4,5,6,7-$d_5$-IAA) lost only a small amount of deuterium from the number two position of the ring during normal work-up. Thus, this deuterated IAA can also be used with confidence under certain conditions of extraction and purification of plant samples. This report describes the use of the pentadeutero IAA in correcting the previously published estimate [1] of the compound in shoot tips of Douglas fir seedlings.

## RESULTS AND DISCUSSION

Representative mass fragmentograms of derivitized IAA isolated from seedling extracts are seen in Fig. 1. In order to get this kind of response it was usually necessary to inject 25–50 ng IAA or $d_5$-IAA into the GC/MS. Recent recoveries have been consistently over 50% and thus sensitivity is no problem. Analyses of eight different populations of Douglas fir seedlings with epicotyls up to 2 cm in length revealed 0.378 ± 0.053 (s.e.) μg free IAA/g fr. wt. This estimate is nearly an order of magnitude smaller than that reported in our earlier study, where $d_2$-IAA was used as an internal standard. Since no exchange between deuterium and hydrogen was seen during injections into the GC/MS used in the earlier study, it is concluded that the higher estimates arose from the loss of deuterium from the analog during the extraction and purification processes. A stronger alkaline solution was also used in partitioning, which most certainly enhanced the exchange. No measurable exchange, however, was observed in the present study when $d_5$-IAA was analysed by mass spectrometry, following all of the steps of the extraction and purification procedures, in the absence of plant extract. The conclusions reached regarding the labile nature of deuterium on the methylene carbon are in accord with those of Magnus *et al.* [2] who demonstrated a significant loss of deuterium from the side chain.

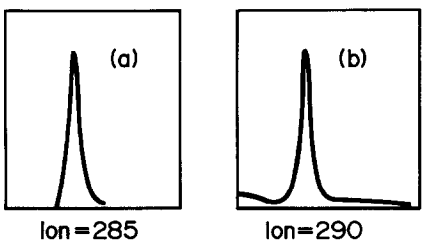


Fig. 1. Fragmentogram obtained from a selected ion-monitoring trace of the trifluoracetylated (a) endogenous IAA ($m/z$ 285) and (b) added $d_5$-IAA ($m/z$ 290).

## EXPERIMENTAL

*Plant materials.* Seeds of Douglas fir were sown in vermiculite which was moistened with a macronutrient soln [3] containing 1 ml/l. micronutrients [4] and 1 ml/l. ferric citrate (1%). Seedlings were grown at ambient room temp. for 3–4 weeks under 1100 lx in a 16 hr photoperiod provided by Gro-Lux bulbs. Shoot tips consisting of cotyledons and epicotyls which ranged in size from a few mm to 2 cm in length were used in the assay.

*Extraction.* Specimens were ground in cold 80% $Me_2CO$ in a chilled mortar. 2,4,5,6,7-$d_5$-IAA (Merck, St. Louis), with 87.5% deuterium on the five carbons, was added as an internal standard usually at 0.88 μg/g fr. wt early in the extraction procedure. The homogenate was sonicated for 2 min, followed by centrifugation at 5000 g for 20 min. The pellet was re-extracted and $Me_2CO$ removed from the combined supernatants *in vacuo* at 35°. The aq. residue was added to 0.2 M Pi buffer, pH 7.1, and partitioned against $Et_2O$ (× 3), the $Et_2O$ phase being discarded. The aq. phase was adjusted to pH 3.0 using 0.1 N HCl, and extracted × 3 with $CH_2Cl_2$. The vol. was reduced and small amounts were transferred to a vial which was dried under a stream of $N_2$. Dried samples were stored at −30°.

*First TLC.* The crude extract was dissolved in $Me_2CO$ and loaded on 10 × 20 cm Bakerflex Polyamide-6 F Gel plates (J. T. Baker) which were developed in MeOH–EtOAc (20:80). Authentic IAA ($R_f$ 0.52) was revealed by the quenching of fluorescence under short-wave UV. In order to protect the

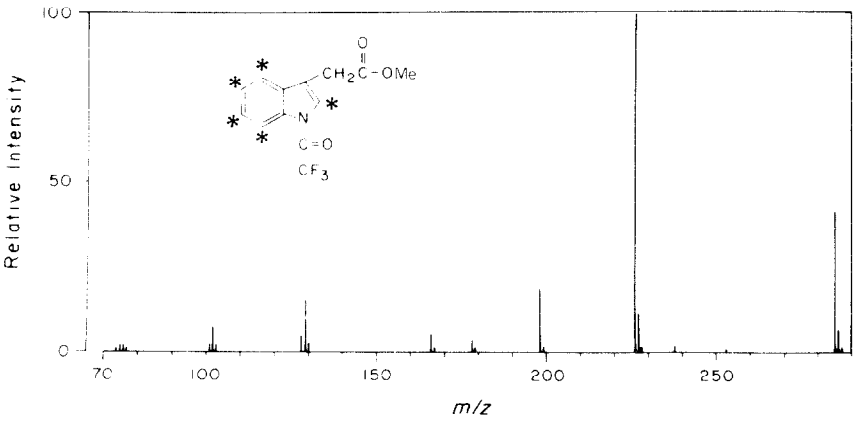


Fig. 2. Mass spectrum of methylated, trifluoroacetylated $d_5$-IAA. Asterisks denote location of deuterium.

internal standard and endogenous IAA, a test strip was removed and examined separately. Strips were sonicated in MeOH, centrifuged and the supernatant passed through a fiber-glass filter and the milky pellet was again extracted. Combined supernatants were reduced in vol. and dried under $N_2$.

*Second TLC.* Dried extracts were methylated with ethereal $CH_2N_2$. The methylated sample was dissolved in $Me_2CO$, separated on TLC; Si gel F-254 (EM Reagents) developed in $CHCl_3$–EtOAc (2:1). Gel from $R_f$ 0.55 ± 0.02 was sonicated in $Me_2CO$ and treated as above.

*GC/MS.* The indolic nitrogen was treated with trifluoroacetic anhydride (TFAA) at room temp. for 1 hr. It is assumed that the TFA generated is removed when $N_2$ is introduced into the vial. MS analyses were performed with a Hewlitt–Packard 5992A. The spectrometer was interfaced with a gas chromatograph by a glass jet separator. Satisfactory results were obtained with a glass column (2 m × 2 mm) packed with 1.5% SP 2250 + 1.95% SP 2401 on Supelcoport 100/120; injection temp. 236°; column temp. 220°; He flow rate of 20 ml/min. The mass spectrometer was operated at 70 eV.

Fully derivatized IAA produced a molecular ion of *m/z* 285 and base peak of *m/z* 226 (Fig. 2). The deuterated analog is five units greater, i.e. 290 and 231, respectively. The benefits of using analogs with this clear MW separation have been described [5, 6]. The molecular ions were used in calculating ratios of $d_5$-IAA IAA. A standard curve of varying ratios was a straight line $y = 0.8926x - 0.6249$. The corrected ratio was used to estimate endogenous IAA, assuming proportionate losses in analog and endogenous IAA throughout the extraction and purification procedures.

*Acknowledgements*—This study was supported by NSF grant PCM 80-2347 and the Dieckmann Chair of Forestry, University of Cincinnati.

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