Phytochemistry, Vol. 21, No. 3, pp. 525–530, 1982.
Printed in Great Britain.

0031-9422/82/030525-06$03.00/0

QUANTIFICATION OF INDOL-3-YL ACETIC ACID IN PEA AND MAIZE SEEDLINGS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

JAMES R. F. ALLEN, LAURENT RIVIER and PAUL-EMILE PILET

Institute of Plant Biology and Physiology of the University, 6 Place de la Riponne, 1005 Lausanne, Switzerland

(*Received* 12 *May* 1981)

Key Word Index—*Pisum sativum*; *Zea mays*; Leguminosae; Gramineae; hormone; indol-3-yl acetic acid; GC/MS; deuterium label.

Abstract—A procedure is described for the identification and quantification of IAA in plant tissues by GC/MS analysis of the *N*-heptafluorobutyryl ethyl ester of IAA using [$^2H_5$]IAA as an internal standard. The detection limit is *ca* 3 pmol IAA/tissue sample. By using this method, IAA levels of 30–90 pmol/g fr. wt were obtained for dark-grown *Pisum sativum* epicotyls and 71–199 pmol/g fr. wt for dark-grown *Zea mays* seedlings. When either methanol or ethanol was used as extraction solvent, some esterification of IAA during sample preparation was observed. No evidence for the natural occurrence of methyl or ethyl esters of IAA in *Pisum sativum* seedlings was found.

INTRODUCTION

The importance of hormones in the growth and differentiation of plants has long been recognized [1]. If the role of hormones is to evaluated comprehensively, e.g. in gravireaction [2], then accurate and precise measurements of the endogenous hormones within plant tissues are essential [3]. The hormone, indol-3-yl acetic acid (IAA), occurs in pmol/g fr. wt levels in plant tissues and the low amounts in tissue extracts relative to impurities make it difficult to quantify accurately. A wide variety of techniques have been used for the quantification of IAA, including bioassay, radioimmunoassay and assorted physico-chemical detectors (see ref. [4]), the most frequently used of which is spectrophotofluorimetry [5].

One of the most sensitive and specific techniques for plant hormone analysis currently available is that of GC/MS using the mass spectrometer focused to monitor selected ions (SIM) characteristic of the compound under investigation. The error involved in quantification due to losses occurring during extraction and purification of the tissue extracts can be minimized by the use of an appropriate internal standard [6]. The specificity of detection of SIM permits discriminate measurement of both IAA and the internal standard at the same time. Analysis by SIM of IAA in plant tissues has previously been made using a structural analogue as an internal standard [7]. More recently, stable isotope-labelled compounds have come to be recognized as the best choice of internal standard since these are most closely related to the compound of interest [8]. Within this laboratory, a GC/MS–SIM method for analysis of the plant hormone, abscisic acid, using a ^2H-labelled internal standard has been developed [9]. [^2H]IAA, substituted in the methylene group of the C-3 side chain has also been used for the analysis of IAA by SIM (see ref. [10]). Potentially, compounds containing three or more ^2H atoms possess the advantage of providing no interference between the internal standard and the compound of interest. Recently, procedures for the synthesis and use as internal standards of [$^2H_4$]- and [$^2H_5$]IAA, labelled in the indole ring have been described [11].

The development and evaluation of a GC/MS procedure using [$^2H_5$]IAA as an internal standard and 5-methyl-indol-3-yl acetic acid (5-MeIAA) as a carrier, for the routine quantification of IAA in pmol amounts is described and the results obtained from the analysis of etiolated pea and maize seedlings using this method are presented.

RESULTS AND DISCUSSION

One of the principal difficulties in the precise determination of the trace quantities of IAA in plant tissues has been the high losses of IAA incurred during extraction and purification of extracts for analysis [13, 14]. These losses are usually attributed to degradation of the compound, especially during sample concentration and drying down. Iino *et al.* [15] reported that the use of high purity solvents and an anti-oxidant greatly improves the recovery during purification. In the present study, final recoveries of endogenous IAA and the internal standard were routinely 30–40% after extraction and purification, with losses occurring at all stages of the procedure. The losses during sample purification can be reduced by the inclusion of 5-MeIAA as a carrier. In addition, the presence of [$^2H_5$]IAA as internal standard systema-

tically corrects for variation in recovery between samples since the ^{2}H-labelled analogue possesses similar chemical characteristics to those of the endogenous compound.

In preliminary investigations it was found that a single-stage derivatization procedure using pentafluoropropionic anhydride and pentafluoropropanol (PFPA–PFPOH, 4:1), two reagents previously used to provide a simultaneous esterification and *N*-actylation of IAA [16], caused a rapid deuterium exchange within [$^2H_5$]IAA, manifested as a marked increase in the $^2H_4$:$^2H_5$ ratio. This was most probably due to the highly acidic conditions in the derivatization medium causing exchange at the C-2 position on the indole ring. In view of this, extremes of pH and, in particular, acidic conditions, should be avoided when using [$^2H_5$]IAA. No detectable exchange of ^{2}H located in the methyl group of the side chain of [$^2H_2$]IAA was observed when using this derivatization procedure [16].

A variety of derivatives of IAA have been used for GC analyses, the choice of which is determined principally by the detector (see ref. [17]). A further consideration in the choice of derivatives are their stability, and alkyl esters of IAA are stable enough to permit prep. TLC to be carried out with relatively little loss. Esterification of IAA by diazoalkanes is a rapid and mild procedure with a high yield and can be carried out on impure extracts. Although esters of IAA can be analysed by GC directly [11, 18], a subsequent *N*-acylation was carried out in the present study. In general the specificity of SIM increases at higher *m/z* values [8] and thus perfluorinated *N*-acyl derivatives provide a further advantage to that of increased volatility. The use of heptafluorobutyrylimidazole (HFBI) for *N*-acylation of IAA-ester has been reported to be slow and incomplete [17]. In the present study, a 30–40% reaction yield was obtained when pmol quantities of IAA-Et were derivatized for 2–3 hr at 85° with HFBI alone. However, determination of the yield by GC/MS and confirmation by monitoring the recovery of radioactivity from [^{3}H]IAA following partitioning after the reaction showed that in the presence of 20–50% (v/v) dry pyridine, *N*-acylation was essentially complete after incubation with HFBI at 85° for 2–3 hr. No ^{2}H exchange in [$^2H_5$]IAA was detected when using this relatively mild derivatization procedure. The electron impact (EI) mass spectra of IAA–HFB–Et and [$^2H_5$]IAA–HFB–Et showed the typical fragmentation pattern of 3-substituted indole carboxylic acids [7, 19] with a prominent M^+ and major fragment ions arising from the β-cleavage fragment, the quinolinium ion and fluorinated acyl fragments (Fig. 1). The presence of an additional methyl group in the carrier, 5-MeIAA, resulted in its derivative making only a minor contribution to the channels monitored [7] and caused it to elute from the GC column *ca* 50 sec after the IAA–HFB–Et peaks, thus providing no interference in the analyses.

By using the procedure described, a compound was isolated from extracts of both pea and maize seedlings which co-chromatographed on TLC with IAA-Et and, following acylation, eluted as a sharp peak by GC/MS at the same retention time as IAA–HFB–Et, producing ions at *m/z* 399 and 326, corresponding to

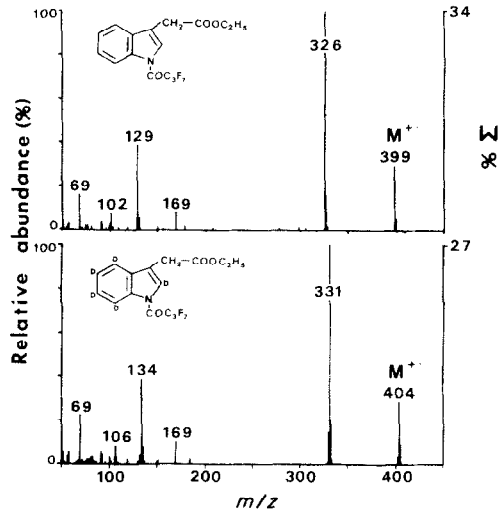

Fig. 1. EI/MS of the HFB–Et derivative of IAA and [2, 4, 5, 6, 7-$^2H_5$]IAA at an ionization potential of 70 eV.

the M^+ and the β-cleavage fragment, with a relative intensity within ± 1% that of authentic IAA–HFB–Et (Fig. 2). Due to the small quantities of endogenous IAA present in the extracts, identification by GC/MS was only possible by SIM which is 2–3 orders of magnitude more sensitive than full spectral scanning.

The accuracy and precision of plant hormone analyses has been appraised by Reeve and Crozier [4] who have attempted to assess their reliability objectively in terms of information theory by allocating *bits* of information to the data obtained from chromatographic and physico-chemical detection. The information provided is thus determined by the efficiency of chromatographic separation and the specificity of the method of detection, and can be compared to a theoretical 140 *bit* minimum value required to attain confidence in identification [4]. Thus, using SIM (4 *bits*/channel), the information content can be determined as: $4\phi n + \phi$ *bits*, where ϕ represents the chromatographic 'peak capacity' and n the number of channels monitored. With the limited number of prominent ions in the EI spectrum, the value obtained is largely determined by ϕ and this varies with the GC conditions and the duration of

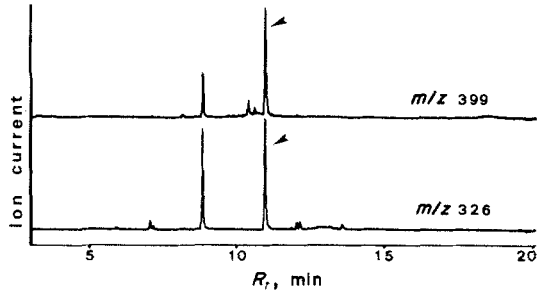

Fig. 2. Selected ion chromatogram of extract from *Pisum* epicotyls using a GC oven program to cause IAA–HFB–Et to elute at 11 min (peaks arrowed). Each channel is normalized independently to the most intense peak.

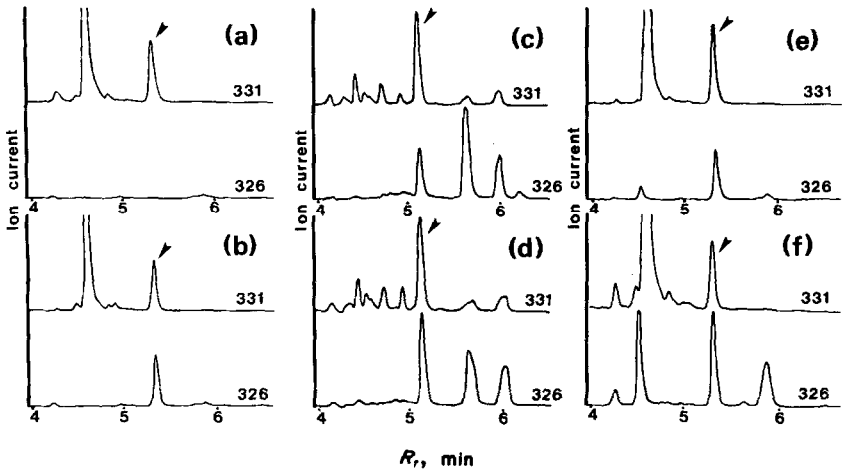

Fig. 3. Selected ion chromatograms from extracts of: (a) Standard of 250 pmol [$^2H_5$]IAA only; (b) standard of 250 pmol IAA and 250 pmol [$^2H_5$]IAA; (c) *Zea* coleoptile; (d) *Zea* mesocotyl; (e) dark-grown *Pisum* epicotyl; (f) light-grown *Pisum* epicotyl. Arrows indicate the peaks from [$^2H_5$]IAA-HFB-Et.

monitoring. On the capillary columns used in the present study, values for ϕ of 30–100 were obtained with up to 10 contaminant peaks due to impurities being observed (Figs. 2 and 3). This provides 110–460 *bits* of information per channel and thus meets the criteria proposed for reliable identification of endogenous IAA in maize and pea seedlings. In practice, the difficulties involved in determining the information yielded by physico-chemical purification and analysis of plant extracts render appraisal by information theory only approximate.

Quantification of the endogenous IAA in the tissue extracts was made by reference to a calibration plot derived from standards subjected to the same purification procedure as extracts and prepared afresh for each series of samples (Fig. 4). The plot of $^2H_0/^2H_5$ was linear over the concentrations determined, passing through the point of origin due to the absence of interference and producing a slope close to that expected from the known isotopic purity of the internal standard (theoretical value: 1.25). The slope derived from the β-cleavage fragment ions was consistently lower than that from the M^+s, an effect apparently due to the presence of ^2H on the relative ion intensity. An impurity was found to be present in the carrier solution of 5-MeIAA which chromatographed closely with IAA in TLC and GLC and which caused some interference in the calibration plot. This limited the amount of carrier which could be added without causing interference, to a 5 nmol/sample. The addition of the carrier appeared to augment recovery, though it was not clear at which stage its presence was most effective. Calibration plots and the amounts of internal standard used were adjusted according to the quantity of endogenous compound expected from the tissues. The lower limit of quantification of endogenous IAA in the tissues, determined from the confidence interval derived from the calibration plots was *ca* 3 pmol/extract. The coefficient of variation in replicate analyses of standards was found to be 2–4%, whilst in replicate samples of eight whole pea seedlings each it was 11.5% (Table 1). This shows that the

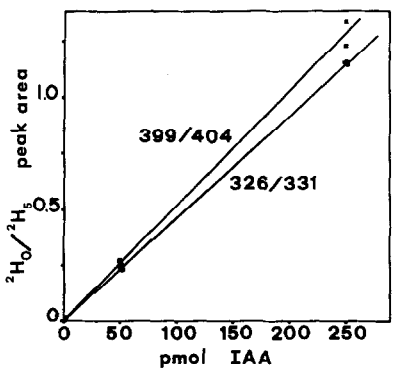

Fig. 4. Typical calibration plot of IAA against the peak area ratios derived from the M^+ and β-cleavage fragment ions, using 250 pmol [$^2H_5$]IAA as an int. standard.

principal source of error in the determinations derives from endogenous variation in IAA content in the plant material.

Additional sources of error in determinations of IAA can derive from the formation as artefacts of methyl or ethyl esters of IAA during preparation of samples for analysis and from their natural occurrence in plant material. In the present study, it was found that when 250 pmol IAA and 250 pmol [$^2H_5$]IAA in 8 ml 80% methanol or ethanol were reduced to the aqueous phase either by vortex evaporation at 50° or by evaporation under a stream of N_2 at 40°, a low, but significant, amount of esterification took place. Using methanol as a solvent, up to 10% of the IAA was converted to the methyl ester, whilst using ethanol, up to 2% of ethyl ester was formed. A similar degree of esterification was observed to take place in alcoholic plant extracts. From the $^2H_0/^2H_5$ ratio of the esters it was estimated that formation was due predominantly to esterification of the free acid and not to transesterification of IAA conjugates present in the tissue extracts (cf. ref. [20]). In practice, the possibility of errors arising from alcoholysis can be circumvented

Table 1. Precision of IAA quantification in standards and extracts of *Pisum* epicotyls by monitoring the β-cleavage fragment ion ratios

	n	Mean ± s.e.	Coefficient of variation (%)
IAA standards			
500 pmol	4	505 ± 9.8*	3.9
250 pmol	6	250 ± 2.0*	2.0
Tissue extract	6	82 ± 3.8†	11.5

*pmol IAA/sample.
†pmol IAA/g fr. wt in extracts of eight entire epicotyls each.

by making a judicious choice of extraction solvent and can be minimized by the inclusion of an internal standard. In the present study, although methanol was used as an extraction solvent, it was assumed that any losses due to methanolysis were corrected for by the presence of [$^2H_5$]IAA as internal standard. As an additional precaution, the ethyl ester of IAA was prepared for analysis by derivatization with diazoethane.

The ethyl and methyl esters of IAA were found to partition readily from aqueous solution into organic solvents at both acid and basic pH and thus the solvent partitioning procedure employed, involving a back partition into alkaline phosphate buffer, effectively excluded esters present in the initial extracts. The possibility that these esters of IAA exist naturally in the plant tissues was checked in pea seedlings. Thus, using 250 pmol [$^2H_5$]IAA-Et, synthesized by esterification of [$^2H_5$]IAA with diazoethane as an internal standard, 1–2 g fr. wt dark grown pea epicotyl tissue was homogenized in either methanol or ethanol and subjected to a single partitioning against ether and the ether phase purified by TLC. No evidence of the presence of either the methyl or ethyl ester was found in the extracts (limit *ca* 1 pmol/g fr. wt) when using ethanol or methanol respectively as extraction solvents. The ease with which the corresponding esters are formed during sample purification as artefacts suggests that caution should be employed in attributing their presence in extracts to their natural occurrence *in vivo* [21–23].

Additional sources of error in the determination of IAA in plant tissues lie in the possibility of IAA formation as an artefact, during extraction and purification, either by decarboxylation of endogenous indol-3-yl pyruvic acid [15] or by the hydrolysis of IAA conjugates present within the tissues [27]. Although the extent of the contribution from these sources in the present study was not determined, the possibility of artefactual formation was minimized by the use of small quantities of plant material, by working through the purification procedure rapidly and avoiding extremes of temperature and pH.

The levels of endogenous IAA previously determined in etiolated seedling tissues by spectrophotofluorimetry and UV spectroscopy (58–205 pmol/g fr. wt in maize [15, 24]; 41–200 pmol/g fr. wt in pea [25–27]) are within the range determined here by GC/MS (Tables 2 and 3). The levels of IAA in pea seedling internodes decline with age down the epicotyl (Table 3) resulting in a gradient, thus confirming previous results obtained by bioassay [28]. In maize seedlings the apical part of both the coleoptile and mesocotyl contain higher levels of IAA than

Table 2. Levels of IAA in dark-grown *Zea* seedlings. Values are derived from extracts of 15 seedlings each

	Fr. wt (mg/segment)	IAA level (pmol/segment)	IAA level (pmol/g fr. wt)
Coleoptile*			
Apex (0–1.2 cm)	20.4	4.07	199
	24.5	4.07	166
Base (1.2–2.4 cm)	27.5	3.47	126
	32.2	3.47	108
Mesocotyl			
Apex (0–2.5 cm)	81.5	10.13	124
	83.1	10.47	126
Base (2.5–5.0 cm)	84.1	6.33	75
	84.8	6.00	71

*Primary leaf removed.

Table 3. Levels of IAA in dark-grown *Pisum* epicotyls. Values are derived from extracts of 15 seedlings each

Internode	Fr. wt (mg/segment)	IAA level (pmol/segment)	(pmol/g fr. wt)
Apical	67	5.71	85.1
	67	6.06	90.4
Median	161	8.64	53.7
	173	9.64	55.8
Basal	165	6.07	36.7
	171	6.58	38.5

the respective basal parts (Table 3). A gradient of IAA has previously been found by bioassay to exist in cereal coleoptiles [1], though the levels in etiolated mesocotyls has not been determined previously. At the time of harvest, growth was nearing completion in the coleoptile, though still vigorous in the subtending mesocotyl. The levels of IAA therefore, show only an approximate correlation with the distribution of growth and this suggests that other factors are controlling the growth of these tissues at this developmental stage.

EXPERIMENTAL

Plant material. Seeds of *Zea mays* var. LG-11 (Assoc. des Sélectionneurs, Lausanne, Switzerland) and *Pisum sativum* var. Alaska (Sharpes, Sleaford, U.K.) were soaked in running H_2O for 8 hr, planted in moist Vermiculite and grown in complete darkness or under white light at $25 \pm 1°$. Maize seedlings were harvested after 5 days and pea seedlings after 8 days.

Extraction and purification procedure. Plant material (0.5–2.5 g fr. wt) was harvested in dim green light. Excised tissues were diced into 4 mm sections and transferred directly to tubes containing the homogenization medium in an ice–H_2O bath. Tissues were immediately homogenized for 30 sec. with a Polytron® homogenizer (Kinematica, Lucerne, Switzerland) in a medium which comprised 6.5 ml MeOH, containing 500 or 250 pmol [$^2H_5$]IAA and 5 nmol 5-MeIAA. After steeping at 4° for 90 min the homogenates were centrifuged at 1800 *g* for 5 min and the supernatants transferred to 10 ml conical glass tubes and reduced to the aq. phase by evaporation at 50 mm Hg at 50° in a vortex evaporator (Buchler, Fort Lee, U.S.A.). The remaining aq. phases were transferred to 10 ml round-bottomed tubes, to which 3 ml Et_2O and 0.15 ml 2M HCl were added, the tubes capped with PTFE-lined screw tops and tumbled for 10 min by rotation at 8 rpm. After low speed centifugation, the aq. phases were removed by pasteur pipette and the Et_2O phases were partitioned (twice) against 2 ml 200 mM K_2HPO_4 buffer (pH > 8). The combined buffer phases were acidified by the addition of 0.45 ml 2M HCl and partitioned against 5 ml Et_2O. The final Et_2O phases were transferred by pasteur pipette to conical glass tubes and reduced to *ca* 0.5 ml under a stream of N_2 at 40°. The residues were alkylated by incubation in an ice–H_2O bath with 0.15 ml EtOH and 1.5 ml ethereal diazoethane for 15 min. The residues were then blown to dryness under a stream of N_2 and taken up in 100 μl EtOH for TLC or dried more thoroughly for subsequent derivatization by HFBI by azeotroping in 500 μl EtOH–toluene (9 : 1) followed by evaporation to dryness under a stream of N_2.

TLC. The EtOH solns of the alkylated phases were loaded as 15 mm streaks onto 250 μm Si gel F-254/366 plates (Woelm, Eschwege, West Germany) using 40 nmol 5-MeIAA–Et spots at the sides of the plate as markers, and developed in either hexane–EtOAc (1 : 1) or $CHCl_3$–MeOH (93 : 7). The markers, running at similar R_fs to IAA–Et (R_f 0.6 ± 0.1) were visualized under UV_{254} and the corresponding regions on the plate scraped off and eluted with 1.5 ml MeOH, in pasteur pipettes containing cotton wool support plugs. The eluates were blown to dryness under a stream of N_2 at 40°.

HFBI derivatization. To the dried residues in glass-stoppered tubes 50 μl dry pyridine + 50 μl HFBI were added. After incubation for 3 hr at 85° the tubes were chilled, 400 μl hexane and 1.0 ml 0.5 N H_2SO_4 were added and vortexed for 20 sec. Following low speed centrifugation, the aq. phases were removed by pasteur pipette and discarded. The hexane phases were repartitioned against 1 ml H_2O and transferred to conical glass tubes. Prior to analysis the contents were reduced to *ca* 40 μl under a stream of N_2.

GC/MS. Analysis was carried out on a low resolution quadrupole instrument under computer control (model 5985 A; Hewlett-Packard, Palo Alto, U.S.A.). Splitless injections of 2 μl were made onto a 10 or 25 m × 0.3 mm OV 101 WCOT glass or fused silica capillary column connected either directly or by an open split interface to the ion source. Typical GC operating conditions were: injector 250°, GC oven 110° programmed at 30°/min to 220°, He *ca* 2 ml/min. The MS was tuned by reference to perfluorotributylamine using the Autotune program with typical operating conditions being: ionization potential 70 eV, emission current 300 μA, ion source 200°. For quantitative analyses, the instrument was operated in the SIM program, monitoring *m/z* 326.1, 331.1, 399.0 and 404.0 simultaneously with a dwell time of 50 msec on each ion. Prior to analyses the retention time and fine focus were checked by injection of *ca* 300 pg of [$^2H_5$]IAA–HFB–Et. Though frequently checked, no evidence of 'memory effect' from previous injections was observed. Under these conditions, IAA–HFB–Et had a retention time of 5–7 min.

Standards and calibration procedure. Quantification of the endogenous IAA in the extracts was made by reference to calibration plots derived from a series of standards routinely subjected to the same purification procedure. Standards containing 5 nmol 5-MeIAA and either 500 or 250 pmol [$^2H_5$]IAA together with known amounts of 0–500 pmol IAA exhibited a linear relationship between the amount of IAA present and the $^2H_0/^2H_5$ ratio derived from either the M^+ or β-cleavage fragment ion peak areas (Fig. 4). Recovery of endogenous IAA and the int. standard were routinely estimated by homogenizing replicate samples of plant material,

in the homogenization medium containing, in addition, 5.0×10^5 dpm [^{3}H]IAA (sp. act. 29 Ci/mmol; CEA, Gif-sur-Yvette, France). Typical values for the recovery in the final hexane phase, determined by liquid scintillation counting, were 30–40% when the TLC stage was omitted. Similar values were obtained when recovery was calculated separately by reference to the peak areas derived from standards containing known amounts of added IAA–Et and analyses by GC/MS.

Chemicals. All solvents were of analytical grade and were re-distilled prior to use. Diazoethane in Et_2O was prepared by synthesis from *N*-ethyl-*N*-nitrosourea [12] and was used at a concn of 30–80 mM in Et_2O, calculated by titration, and stored at −20° prior to use. [2, 4, 5, 6, 7-$^2H_5$]IAA synthesized by Merck, Sharp & Dohme (Canada) was obtained from Professor R. P. Pharis of the University of Calgary, Canada. Analysis of the isotopic purity of the HFB–Et derivative by GC/MS showed the following isotope distribution: $^2H_0 < 0.1\%$; $^2H_1 < 0.1\%$; $^2H_2 < 1\%$; $^2H_3$ 1.3%; $^2H_4$ 11.2%; $^2H_5$ 81.7%; $^2H_6$ 5.5%. ^2H was located principally in the indole ring with a small amount of incorporation in the methylene group of the side chain. Solutions of IAA and [$^2H_5$]IAA were made up in MeOH (5×10^{-6} M) and stored at −20°. GC/MS analysis by SIM using freshly-prepared solutions as reference standards showed that no detectable breakdown or esterification of IAA or ^2H exchange occurred during 6 months storage in MeOH.

Acknowledgement—James Allen wishes to thank the Royal Society for support from a European Fellowship during the period of this study.

REFERENCES

1. Went, F. W. and Thimann, K. V. (1937) *Phytohormones.* Macmillan, New York.
2. Pilet, P.-E. (1980) in *Plant Growth Substances*, 1979, (Skoog, F., ed.) p. 450. Springer, Berlin.
3. Reinhold, L. (1978) in *Phytohormones and Related Compounds—A Comprehensive Treatise* (Letham, D. S., Goodwin, P. B. and Higgins, T. J. V., eds.) Vol. 2, p. 251. Elsevier, Amsterdam.
4. Reeve, D. R. and Crozier, A. (1980) in *Hormonal Regulation of Development I, Encyclopaedia of Plant Physiology* (MacMillan, J. ed.) Vol. 9, p. 203. Springer, Berlin.
5. Pilet, P.-E., Elliott, M. C. and Moloney, M. M. (1979) *Planta* **146**, 405.
6. Millard, B. J. (1978) in *Quantitative Mass Spectrometry in Life Sciences II* (de Leenheer, A. P., Roncucci, R. R. and van Peteghem, C., eds.) p. 83. Elsevier, Amsterdam.
7. Rivier, L. and Pilet, P.-E. (1974) *Planta* **120**, 107.
8. de Leenheer, A. P. and Cruyl, A. A. (1980) in *Biochemical Applications of Mass Spectrometry* (Waller, G. R. and Dermer, O. C., eds.) Suppl. Vol. 1, p. 1169. Wiley, New York.
9. Rivier, L. and Pilet, P.-E. (1981) *Phytochemistry* **20**, 17.
10. Allen, J. R. F., Rebeaud, J. E., Rivier, L. and Pilet, P.-E. in *Proceedings of 4th International Conference on the Use of Stable Isotopes* (Schmidt, H. L., Heinzinger, K. and Förstel, H., eds.) Elsevier, Amsterdam (in press).
11. Magnus, V., Bandurski, R. S., Schulze, A. (1980) *Plant Physiol.* **66**, 775.
12. Darbre, A. (1978) in *Handbook of Derivatives for Chromatography* (Blau, K. and King, G., eds.) p. 39. Heyden, London.
13. Mann, J. D. and Jaworski, E. G. (1970) *Planta* **92**, 285.
14. Little, C. H. A., Heald, J. K. and Browning, G. (1978) *Planta* **139**, 133.
15. Iino, M., Yu, R. S.-T. and Carr, D. J. (1980) *Plant Physiol.* **66**, 1099.
16. Allen, J. R. F. and Baker D. A. (1980) *Planta* **148**, 69.
17. McDougall, J. and Hillman, J. R. (1978) *J. Exp. Botany* **26**, 741.
18. Bayer, M. H. (1964) *Plant Physiol.* **44**, 267.
19. Jamieson, W. D. and Hutzinger, O. (1970) *Phytochemistry* **9**, 2029.
20. Milborrow, B. V. and Mallaby, R. (1975) *J. Exp. Botany* **26**, 741.
21. Stowe, B. B. (1959) *Prog. Chem. Org. Nat. Comp.* **17**, 248.
22. Pilet, P.-E. (1961) *Les Phytohormones de Croissance*, p. 183. Masson, Paris.
23. Bearder, J. R. (1980) in *Hormonal Regulation of Development I, Encyclopedia on Plant Physiology* (Macmillan, J., ed.) Vol. 9, p. 9. Springer, Berlin.
24. Epstein, E., Cohen, J. D. and Bandurski, R. S. (1980) *Plant Physiol.* **65**, 415.
25. Mousedale, D. M. A., Butcher, D. N. and Powell, R. G. (1978) in *Isolation of Plant Growth Substances* (Hillman, J. R., ed.) p. 27. Cambridge University Press, Cambridge.
26. Liebermann, M. and Knegt, E. (1977) *Plant Physiol.* **60**, 475.
27. Bandurski, R. S. and Schulze, A. (1977) *Plant Physiol.* **60**, 211.
28. Scott, T. K. and Briggs, W. R. (1963) *Am. J. Botany* **50**, 652.