Preparation of 7-Hydroxy-2-oxoindolin-3-ylacetic Acid and its [$^{13}C_2$], [5-n†-^{3}H], and [5-n-^{3}H]-7-*O*-Glucosyl Analogues for Use in the Study of Indol-3-ylacetic Acid Catabolism

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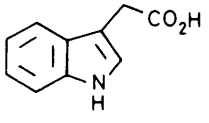
An improved synthesis of 7-hydroxy-2-oxoindolin-3-ylacetic acid (**14**) *via* the base-induced condensation reaction between oxalate esters and 7-benzyloxyindolin-2-one is described. 7-Benzyloxyindolin-2-one was prepared in four steps and 50% overall yield from 3-hydroxy-2-nitrotoluene. The yield of the title compound (**14**) from 7-benzyloxyindolin-2-one was 56%. This route was used to prepare 7-hydroxy-2-oxoindolin-3-yl[$^{13}C_2$]acetic acid (**15**) in 30% yield from [$^{13}C_2$]oxalic acid dihydrate. The method could not be extended to the preparation of the corresponding [$^{14}C_2$]-compound. However, an enzyme preparation from *Zea mays* roots catalysed the conversion of carrier-free [5-n-^{3}H]indol-3-ylacetic acid with a specific activity of 16.7 Ci mmol^{-1} to a mixture of 7-hydroxy-2-oxo[5-n-^{3}H]indolin-3-ylacetic acid (**16**) and its [5-n-^{3}H]-7-*O*-glucoside (**18**) in *ca.* 3 and 40% radiochemical yield respectively. The glucoside (**18**) was converted into the 7-hydroxy compound (**16**) in 80% yield by means of β-glucosidase.

Investigations of the catabolism of the plant growth hormone indol-3-ylacetic acid (IAA) (**1**) in *Zea mays* have, to date, revealed two steps. IAA is first oxidised at C-2 to give 2-oxoindolin-3-ylacetic acid (OxIAA) (**13**).[1,2] OxIAA is then converted into the corresponding 7-hydroxy-OxIAA7-*O*-β-D-glucoside (**17**)[3,4] presumably *via* the non-conjugated compound (7-hydroxy-OxIAA) (**14**).‡ To extend these investigations we required sources of unlabelled, and both stable- and radio-isotope-labelled 7-hydroxy-OxIAA. One synthetic route to the unlabelled compound has been described,[3] but the yield was only 5% based on 3-hydroxy-2-nitrotoluene (3-methyl-2-nitrophenol) (**2**), largely due to the lack of efficient methods for the transformation of indol-3-ylacetic acids into oxoindol-3-ylacetic acids. Further, this method was not readily adaptable to the preparation of isotopically labelled samples. The present paper describes a new synthesis of 7-hydroxy-OxIAA suitable for the preparation of [$^{13}C_2$]-labelled samples. The use of 7-benzyloxyindolin-2-one (**7**) as a synthetic intermediate avoids the low-yield oxidation of a substituted indol-3-ylacetic acid. Attempts to extend this method to a micro-scale preparation of (7-hydroxy-2-oxoindolin-3-yl)-[$^{14}C_2$]acetic acid and a biochemical preparation of (7-hydroxy-2-oxo[5-n-^{3}H]indolin-3-yl)acetic acid are described.

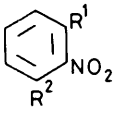


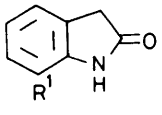
(**1**)

Results and Discussion

7-Benzyloxyindolin-2-one (**7**) was prepared in four steps from commercially available 3-hydroxy-2-nitrotoluene (**2**). Benzylation of compound (**2**) was accomplished in 93% yield with 1.1 mol equiv. each of sodium hydride and benzyl bromide in refluxing tetrahydrofuran (THF). The resultant 3-benzyloxy-2-nitrotoluene (**3**) was converted into the corresponding phenylpyruvic acid (**4**) with 2.2 mol equiv. each of potassium ethoxide and diethyl oxalate, with alkaline work-up, essentially as described by Stoll *et al.* for the isomeric 4-benzyloxy compound.[5] The reaction afforded *ca.* 60% of the desired product plus an almost quantitative recovery of unconsumed starting material (**3**) which could be recycled such that the overall yield of this step after two operations was *ca.* 84%.

Conversion of the pyruvic acid (**4**) into the analogous acetic acid (**5**) by means of aqueous alkaline hydrogen peroxide proceeded in 84% yield as previously reported.[6] The reaction was more conveniently performed on the basic solution resulting from the pyruvate preparation, after removal of unchanged starting material.





(**2**) R^1 = OH, R^2 = Me

(**3**) R^1 = OCH_2Ph, R^2 = Me

(**4**) R^1 = OCH_2Ph, R^2 = CH_2COCO_2H

(**5**) R^1 = OCH_2Ph, R^2 = CH_2CO_2H

(**6**) R^1 = H

(**7**) R^1 = OCH_2Ph

The reductive cyclisation of 3-benzyloxy-2-nitrophenylacetic acid (**5**) to the corresponding 7-benzyloxyindolin-2-one (**7**) was achieved, in 76% yield (after flash chromatography), using *ca.* 40 mol equiv. of zinc metal in refluxing glacial acetic acid. Several other reagents were tried and found to be unsatisfactory. Thus $TiCl_3$, $(NH_4)_2S$, Zn–EtOH, H_2–PtO_2, and $Na_2S_2O_4$ were all poor despite literature precedent for their use in the reduction of aromatic nitro compounds. Ek and Witkop reported [7] the use of $FeSO_4$–NH_3 for this reduction (in 70% yield). In our hands the Zn–AcOH method was cleaner and more reproducible.

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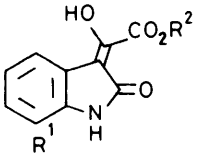
† 'n' Stands for nominal as used by Amersham International M.C., to mean presumed position of radiolabel according to their method of synthesis.

‡ 7-Hydroxy-OxIAA (**14**) has recently been identified as a naturally occurring component of *Zea mays* see: P. Lewer and R.S. Bandurski, *Phytochemistry*, 1987 in press.

The condensation of 7-benzyloxyindolin-2-one (**7**) with dibenzyl oxalate was effectively promoted by several bases. Use of 1.5 mol equiv. of either sodium hydride or sodium 4-methyl-2,6-di-t-butylphenolate[8,9] (NaBHT) gave an 85% yield of the benzyl glyoxylate (**12**) in either case. This, on hydrogenation in glacial acetic acid–conc. sulphuric acid, afforded 7-hydroxy-OxIAA (**14**) in 61% yield after flash chromatography.

In the condensation reaction between indolin-2-one (**6**) and dibenzyl oxalate, use of 1.5 mol equiv. of sodium metal afforded the benzyl glyoxylate (**9**) as previously reported.[10] However, use of 3 mol equiv. of sodium metal resulted in the formation of acid (**8**), which became the sole product if the reaction time was extended from 3-h to 22-h. The 250 MHz ^{1}H n.m.r. spectra of the acid (**8**) and the benzyl ester (**9**) each showed a doublet at δ 7.75 assignable to 4-H, deshielded from its position in the spectra of indolin-2-one (**6**) and of OxIAA (**13**) by the exocyclic enolic glyoxylate system.* Further, the acid (**8**) was converted (EtOH–TsOH; 18 h reflux) into the known ester (**10**),[12,13] which showed a broad singlet at δ 7.74 in its 250 MHz ^{1}H n.m.r.



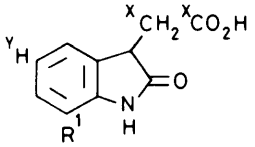
(**8**) $R^1 = R^2 = H$

(**9**) $R^1 = H$, $R^2 = CH_2Ph$

(**10**) $R^1 = H$, $R^2 = Et$

(**11**) $R^1 = OCH_2Ph$, $R^2 = H$

(**12**) $R^1 = OCH_2Ph$, $R^2 = CH_2Ph$



(**13**) X = 12, Y = 1, $R^1 = H$

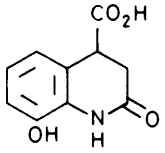
(**14**) X = 12, Y = 1, $R^1 = OH$

(**15**) X = 13, Y = 1, $R^1 = OH$

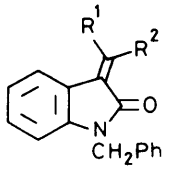
(**16**) X = 12, Y = 3, $R^1 = OH$

(**17**) X = 12, Y = 1, $R^1 = O$-β-D-glucose

(**18**) X = 12, Y = 3, $R^1 = O$-β-D-glucose

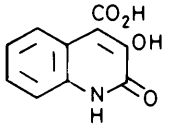


(**19**)



(**20**) $R^1 = H$, $R^2 = CO_2H$

(**21**) $R^1 = CO_2H$, $R^2 = H$



(**22**)

spectrum.† Hydrogenation of the acid (**8**) at 3.5 atm. over 10% Pd–C (in glacial acetic acid–conc. sulphuric acid) gave OxIAA (**13**) in 92% yield.

In view of the improved yield of OxIAA from the glyoxylic acid (**8**) upon hydrogenation, improvements in the synthesis of compounds (**13**) and (**14**) were made. Thus, dibenzyl oxalate was replaced by diethyl oxalate, thereby eliminating the preparation[15] of the former, with increased yields of each of the condensation products. Sodium ethoxide was used as the base and the *in situ* hydrolysis of the ethyl esters gave the glyoxylic acids (**8**) (95%) and (**11**) (80%) in one step from the respective indolinones. Finally, hydrogenation of the 7-benzyloxy acid (**11**) afforded 70% of pure 7-hydroxy-OxIAA after flash chromatography.

The preparation of 7-hydroxy-OxI[$^{13}C_2$]AA {(7-hydroxy-2-oxoindolin-3-yl)[$^{13}C_2$]acetic acid} (**15**) containing 99 atom-% ^{13}C was achieved using [$^{13}C_2$]oxalic acid dihydrate as the labelled starting material. This was converted into its dibenzyl ester in 71% yield (after flash chromatography) with triethylamine–benzyl bromide. The benzyl ester was condensed with 7-benzyloxyindolin-2-one under similar conditions to those used in the synthesis of the unlabelled compound (but see below), and the hydrolysis products were hydrogenated to give 7-hydroxyOxI[$^{13}C_2$]AA (**15**). The product was most effectively purified by DEAE-Sephadex chromatography. The yield for the three steps was 30% based on starting [$^{13}C_2$]oxalic acid dihydrate.

During the preparation of 7-hydroxy-OxI[$^{13}C_2$]AA (**15**) the condensation reaction between dibenzyl [$^{13}C_2$]oxalate and 7-benzyloxyindolin-2-one was found to proceed in good yield only when 2 mol equiv. of base (NaH) and a stoicheiometry of 1.2:1 oxalate:indolinone were used, in contrast to the conditions used during the large-scale preparations. On the scale required to prepare carrier-free 7-hydroxy-OxI[$^{14}C_2$]AA (37 μCi = *ca.* 300 nmol) the initial benzylation of [$^{14}C_2$]oxalic acid dihydrate was accomplished in 65% yield (after purification by t.l.c.) under the same conditions as used for the [$^{13}C_2$] preparation. However, we were unable to achieve reaction of the labelled ester with 7-benzyloxyindolin-2-one despite extensive variations in the base used, the reaction stoicheiometry, or the temperature, and with attention to the exclusion of moisture and oxygen.

While the synthetic work was in progress, high specific activity [5-n-^{3}H]IAA became commercially available. This material was previously prepared by an enzyme-catalysed

* The u.v. spectra of the (2-oxoindolin-3-yl)glyoxylates examined during this work showed these compounds to exist in the *enol* form.[10] Further, in the 250 MHz ^{1}H n.m.r. spectra, the 4-H signal was present as a doublet at δ *ca.* 7.7—7.8 in each of the non-7-benzyloxy glyoxylates, and in the region δ 7.47—7.55 in the 7-benzyloxy analogues. By comparison of the chemical shifts of the 4-H in the spectra of these compounds with those of analogues having defined stereochemistry [*e.g.* (**20**) and (**21**)],[11] (see also P. A. Chopard, R. F. Hudson, and R. J. G. Searle, *Tetrahedron Lett.*, 1965, 2357; A. M. Fahmy, M. Z. A. Badr, Y. S. Mohamed, and F. F. Abdel-Latif, *J. Heterocycl. Chem.*, 1984, **21**, 1233) the *cis* stereochemistry (*i.e.* carbonyl carbon adjacent to lactam carbonyl) was assigned to compounds (**8**)—(**12**) inclusively. In this connection it is noteworthy that the introduction of a 3′-hydroxy group causes no change in the chemical shift of 4-H in OxIAA (unpublished data from this laboratory).

† The preparation of the ethyl ester (**10**) proved to be essential in the identification of acid (**8**) since the m.p. of a plausible alternative compound, the carbostyril (**22**), is reported as 257—258 °C;[12] however, that of its ethyl ester is 227—228 °C.[12] Repeated crystallisation of the sample of acid (**8**) derived from the indolin-2-one–dibenzyl oxalate–3 equiv. Na reaction did not raise the m.p. above 256—257 °C, thus throwing some initial doubt upon its identity in view of the previously reported m.p.s of 265 °C[12,13] and 259—262 °C.[14]

conversion[16] of [5-n-^{3}H]tryptophan. Therefore, the conversion of high specific activity [5-n-^{3}H]IAA (**1**) into the required tritiated compound (**16**) by means of *Zea mays* was investigated.

Incubation of [5-n-^{3}H]IAA with root segments from *Zea mays* was performed with a large surface area exposed to the atmosphere. Previous experiments[17] indicated that this might be important in order to achieve conversion of IAA by root segments. Under these conditions, all substrate was consumed in less than 6 h (*cf.* refs 3, 4, 18), although an optimum yield of the 7-hydroxy-OxIAA 7-*O*-glucoside (**18**) required a 24 h incubation. After incubation, and removal of the aqueous phase, the roots were extracted using methanol. The aqueous phase contained neither substrate (**1**) nor either of the oxidation products (**16**) or (**18**) [by analysis using reverse-phase high performance liquid chromatography (h.p.l.c.)] and was discarded. The methanol extract, when chromatographed on a DEAE-Sephadex column, yielded either two or three radioactive components, depending on the preparation: fractions 1—20 (*ca.* 10% of total recovered), 34—44 (< 5%), and 50—66 (85—90%). Only the latter component contained useful material; the others were discarded.

The major DEAE-Sephadex fraction was then chromatographed by reverse-phase h.p.l.c. using the elution conditions (B) described in the Experimental section, which separate 7-hydroxy-OxIAA 7-*O*-β-D-glucoside, 7-hydroxy-OxIAA, and OxIAA. Three corresponding peaks of radioactivity were generally observed in the proportions *ca.* 11:1:3 respectively.

Preparation of the 7-hydroxy-OxIAA (**16**) was achieved by incubation of the glucoside (**18**) with a commercial β-glucosidase from almonds.[3] After termination of the reaction, the product was purified by h.p.l.c. under the same conditions as used above.

Identification criteria for these two [^{3}H]-compounds were as follows: (*a*) The [^{3}H]-glucoside (**18**) yielded 7-hydroxy[^{3}H]-OxIAA (**16**) upon treatment with β-glucosidase. (*b*) Treatment of both the [^{3}H]-glucoside (**18**) and the 7-hydroxy[^{3}H]OxIAA (**16**) with 2M-hydrochloric acid at 100 °C for 2 h yielded a major [^{3}H]-compound which co-chromatographed on h.p.l.c. with authentic 8-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid, 8-OH-THQCA, (**19**). Each of these compounds were analysed using three different h.p.l.c. systems as described in the Experimental section.

Both the [^{3}H]-glucoside (**18**) and the 7-hydroxy[^{3}H]OxIAA (**16**), when freshly prepared, were of greater than 98% radiochemical purity as determined by h.p.l.c. Storage under a nitrogen atmosphere in nitrogen-purged 50% aq. EtOH prevented otherwise rapid decomposition to unidentified polar products.

Experimental

General Procedures.—M.p.s were determined using a Fisher-Johns apparatus and are uncorrected. ^{1}H N.m.r. spectra were recorded using a Bruker WM-250 spectrometer. Chemical shifts are reported in p.p.m. relative to internal Me_4Si for spectra recorded in $CDCl_3$ or $(CD_3)_2CO$, or external Me_4Si where spectra were recorded in $(CD_3)_2SO$ or C_5D_5N. I.r. and u.v. spectra were recorded using Perkin-Elmer 237B and Cary 15 spectrophotometers. Electron-impact probe mass spectra were obtained at 70 eV on a Finnigan 4000 instrument. Microanalyses were performed by Spang Microanalytical Laboratory, Star Route 1, Box 142, Eagle Harbour, MI 49951. Compounds on analytical t.l.c. plates (Merck silica gel 60 F-254; 0.25 mm) were visualised both under u.v. light and by exposure to iodine vapour. Benzene was distilled from sodium. THF was freshly distilled from the sodium ketyl of benzophenone. Radioactive fractions from chromatography were located by liquid scintillation counting of aliquots on a Beckman LS-7000 instrument. H.p.l.c. was performed using a Varian 5000 instrument. Retention times (at 1.0 ml min^{-1} flow rate) for various compounds are given in the Table.

Table. H.p.l.c. retention times (min) for compounds described in text (nd—not determined).

Compound	System A	System B	System C
7-OH-OxIAA-glu (**17**), (**18**)	7.5	14—14.5	11—12
7-OH-OxIAA (**14**) (**16**)	9.8	20.1	12.4
OxIAA (**13**)	13.5	26.6	nd
IAA (**1**)	20.4	38.4	nd
8-OH-THQCA (**19**)	9.5	20.7	15.2
Decomposition products	4—5	3—4 + 5—6	17—19

Solvent systems

A: Partisil-10 ODS,* 4.6 × 250 mm, 0—5 min: isocratic EtOH–H_2O–AcOH (10:90:0.5); 5—50 min: gradient: from initial conditions to EtOH–H_2O–AcOH (70:30:0.5).

B: Hamilton 5μ PRP-1,* 4.6 × 250 mm, gradient as for A.

C: Partisil-10 SAX,* 4.6 × 250 mm, 0—20 min: gradient of 0—10% AcOH in 50% aq. EtOH.

* In series with 4.6 × 50 mm guard column of CoPell-ODS.

7-Benzyloxyindolin-2-one. (**7**).—3-Benzyloxy-2-nitrophenylacetic acid (**5**) (2.76 g) was dissolved in glacial AcOH (60 ml) and the solution was refluxed. Zinc powder (26.1 g) was added during 25 min and the mixture was refluxed for a further 60 min. After cooling and filtration of the mixture, the residue was washed with several portions of hot AcOH, and the filtrate was concentrated to dryness. The residue was purified by flash chromatography on a 14 × 3.5 cm column of 60—200 mesh silica gel with CH_2Cl_2 as eluant; 20 fractions (25 ml each) were collected, and fractions 6—16 were pooled. Concentration of this material under reduced pressure afforded 7-benzyloxyindolin-2-one (**7**) as an off-white solid (1.75 g), m.p. 151—151.5 °C (needles from aqueous acetone) (lit.,[7] 153—154 °C from benzene) (Found: C, 75.3; H, 5.6; N, 5.8. Calc. for $C_{15}H_{13}NO_2$: C, 75.3; H, 5.5; N, 5.9%); δ_H (250 MHz; $CDCl_3$) 3.47 (2 H, s), 5.08 (2 H, s), 6.81—6.95 (3 H, m), 7.29—7.43 (5 H, m), and 8.71 (1 H, br s); *m/z* 239 (M^+, 10%).

General Method for Preparation of Benzyl Glyoxylates.—The benzyl esters (**9**) and (**12**) were prepared from the appropriate indolinones (**6**) or (**7**), dibenzyl oxalate[15] (1.1 mol equiv.), and a suitable enolate-generator. The latter was either sodium metal (1.3 mol equiv. in benzene) for (**9**) only,[10] NaBHT (in THF), or NaH (in THF). When using either NaH or NaBHT, a solution of the indolinone (**6**) or (**7**) in THF was added to a stirred solution of the base (1.5 mol equiv.) in THF, and the mixture was stirred for a further 1.25 h before addition of the dibenzyl oxalate. In all cases the resultant solution was then stirred for 3 h at 25 °C and poured into water, the organic solvent was removed under reduced pressure at 25 °C, and the remaining aqueous phase was acidified to pH 2 (2M-HCl). The precipitate which formed was isolated by vacuum filtration, then was washed successively with water and hexane, and dried in a vacuum desiccator over Na_2SO_4. This procedure gave products with physical properties as below.

Compound (**9**): m.p. 201—202 °C (from acetone) (lit.,[10] 205—206 °C); δ_H [250 MHz; $(CD_3)_2SO$] 5.31 (2 H, s), 6.95 (1 H, d, *J* 7 Hz), 6.96 (1 H, t, *J* 7 Hz), 7.10 (1 H, t, *J* 7 Hz), 7.36—7.39 (3 H, m), 7.45—7.48 (2 H, m), 7.72 (1 H, d, *J* 7 Hz), and 10.7 (*ca.* 2 H, br s); $\lambda_{max.}$(EtOH) 263 (ε 16 320 dm^3 mol^{-1} cm^{-1}) and 328 nm (8 420); *m/z* 295 (M^+, 3%).

Compound (**12**): m.p. 192—193.5 °C (from acetone) (Found: C, 71.7; H, 4.7; N, 3.4. $C_{24}H_{19}NO_5$ requires C, 71.8; H, 4.8; N, 3.5%); δ_H [250 MHz; $(CD_3)_2SO$] 5.20 (2 H, s), 5.33 (2 H, s), 6.92 (2 H, m), and 7.29—7.54 (11 H, m); *m/z* 401 (M^+, 1.2%).

Use of 3 mol equiv. of sodium in the reaction between indolin-2-one (**6**) and dibenzyl oxalate (1.1 mol equiv.) for 22 h with work-up as above resulted in the formation of the glyoxylic acid (**8**) in 40% isolated yield (based on the starting oxoindoline), m.p. 256—257 °C (from acetone) (lit.,[12] 262—265 °C; lit.,[13] 265 °C; lit.,[14] 259—262 °C); δ_H [250 MHz; $(CD_3)_2SO$] 7.00 (1 H, d, *J* 7.5 Hz), 7.09 (1 H, dt, J_1 7.8, J_2 1 Hz), 7.25 (1 H, dt, J_1 7.8, J_2 1 Hz), 7.85 (1 H, d, *J* 7.5 Hz), and 11.6 (1 H, br s); $\lambda_{max.}$(EtOH) 270 (14 840 dm^3 mol^{-1} cm^{-1}) and 326 nm (10 130); *m/z* 205 (M^+, 48%).

Hydrogenation of (2-Oxoindolin-3-yl)glyoxylates.—Hydrogenation of compounds (**8**), (**9**), (**11**), and (**12**) was performed as described in ref. 10, except that the product was extracted with EtOAc in each case. The extract was washed with an equal volume of water, dried ($MgSO_4$), filtered, and concentrated to dryness. The following reactions were performed in this way: physical parameters refer to the product.

(**9**) ⟶ (**13**) (92% yield), m.p. 140—141 °C (from acetone—benzene) (lit.,[3] 141—143 °C; lit.,[10] 147 °C); δ_H [250 MHz; $(CD_3)_2CO$] 2.75 (1 H, d, *J* 17 Hz), 2.93 (1 H, d, *J* 17 Hz), 3.68 (1 H, m), 6.86 (1 H, d, *J* 7.5 Hz), 6.95 (1 H, t, *J* 7.5 Hz), 7.17 (1 H, d, *J* 7.5 Hz), 7.22 (1 H, t, *J* 7.5 Hz), and 10.4 (*ca.* 1 H, br s); *m/z* 191 (M^+, 22%).

(**12**) ⟶ (**14**). The residue from the general procedure was purified by flash chromatography; compound (**12**) (500 mg) yielded a residue (200 mg) which was eluted through a 12 × 2 cm column of 230—400 mesh silica gel with EtOAc–AcOH (98:2); 25 fractions (3.5 ml each) were collected, and fractions 9—13 were pooled. Concentration of this material afforded compound (**14**) as an off-white solid (160 mg), 173—178 °C (melted with yellowing, and resolidified), m.p. 225 °C (decomp.) (from acetone–benzene) (lit.,[3] 189—191 °C) (Found: C, 58.1; H, 4.4; N, 6.6 Calc. for $C_{10}H_9NO_4$: C, 58.0; H, 4.4; N, 6.8%); δ_H (250 MHz; C_5D_5N)[19,20] 3.16 (1 H, dd, J_1 16.2, J_2 8.5 Hz), 3.53 (1 H, dd, J_1 16.2, J_2 4.2 Hz), 4.26 (1 H, dd, J_1 7.4, J_2 4.2 Hz), 6.95 (1 H, dd, J_1 8.0, J_2 7.3 Hz), 7.08 (1 H, dt, J_1 8.0, J_2 1.1 Hz), and 7.22 (1 H, dt, J_1 8.4, J_2 1.1 Hz); $\nu_{max.}$(Nujol) 3 165br, 3 500—2 400, 3 060, 3 040, 1 714, 1 684, 1 648, 1 595, 1 335, 1 230, 1 193, 922, 773, 751, and 730 cm^{-1}; *m/z* 207 (M^+, 33%), 189 (5), 162 (35), 161 (100), 148 (4), 144 (8), 134 (14), 133 (34), 116 (11), 105 (19), 104 (16), 92 (13), 85 (18), 77 (13), and 65 (10).

(**8**) ⟶ (**13**) (92% yield), physical parameters described above.

(**11**) ⟶ (**14**). Flash chromatography of the residue as described above yielded the product in 70% yield.

Ethyl (2-Oxoindolin-3-yl)glyoxylate (**10**).—The ester was prepared as in ref. 13 (96% yield); by reaction of indolin-2-one with NaH (1.5 and equiv.) followed by diethyl oxalate 1.1 mol equiv. in THF (99% yield); or by quantitative ethylation of the acid (**8**) with EtOH–*p*-TsOH. In each case the product had m.p. 186—187 °C (from acetone) (lit.,[12] 187—188 °C; lit.,[13] 185—187 °C); δ_H [250 MHz; $(CD_3)_2SO$] 1.30 (3 H, t, *J* 7 Hz), 4.31 (2 H, q, *J* 7 Hz), 6.97 (2 H, br q, *J* 7 Hz), 7.14 (1 H, br t, *J* 7 Hz), 7.74 (1 H, br s), and 10.8 (1 H, br s); $\lambda_{max.}$(EtOH) 251sh (11 290 dm^3 mol^{-1} cm^{-1}), 259 (14 470), 270sh (9 600), and 328 nm (6 360); *m/z* 223 (M^+, 23%).

Hydrolysis of (2-Oxoindolin-3-yl)glyoxylate Esters.—The ester (**9**), (**10**), or (**12**) was suspended in 2M-NaOH (*ca.* 100 ml g^{-1} ester) at 25 °C, and the mixture was stirred until t.l.c. indicated complete consumption of starting material. The solution was cooled to 0 °C, and the precipitated product was isolated by vacuum filtration, washed successively with water and hexane, and dried in a vacuum desiccator over Na_2SO_4.

(**9**) ⟶ (**8**) gave a 94% yield of product identical with that from the reaction of indolin-2-one with dibenzyl oxalate promoted by sodium metal (3 mol equiv.).

(**12**) ⟶ (**11**). Owing to poor water-solubility of benzyl (7-benzyloxy-2-oxoindolin-3-yl)glyoxylate (**12**), a small quantity of THF was added to aid solution. Reaction yielded the *acid* (**11**) (85%) with m.p. 207—209 °C (needles from acetone) (Found: C, 65.4; H, 4.3; N, 4.5; $C_{17}H_{13}NO_5$ requires C, 65.6; H, 4.2; N, 4.5%); δ_H [250 MHz; $(CD_3)_2SO$] 5.22 (2 H, s), 7.05 (2 H, m), 7.30—7.42 (3 H, m), and 7.47—7.55 (3 H, m); *m/z* 311 (M^+, 12.2%).

(**10**) ⟶ (**8**) gave a 95% yield of product with physical properties as above.

Preparation of Dibenzyl [$^{13}C_2$]*Oxalate.*—[$^{13}C_2$]Oxalic acid dihydrate (100 mg) was dissolved in CH_3CN–acetone (1:1 v/v; 3.5 ml), and Et_3N (0.24 ml) was added during 5 min to the stirred solution. After the mixture had been stirred for a further 40 min, benzyl bromide (0.375 ml) was added during 5 min and the mixture was refluxed for 3.5 h, then kept at 25 °C overnight. The product was taken to dryness under a stream of nitrogen, and the residue was dissolved in saturated aq. $NaHCO_3$ (50 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The extract was washed with water (50 ml), dried ($MgSO_4$), filtered, and concentrated to give an oily solid (400 mg). This was purified by flash chromatography on a 2 × 13.5 cm column of 230—400 mesh silica gel. Material was eluted from the column with 50 ml aliquots of solvent; fractions from hexane (1 × 50 ml) then EtOAc–hexane mixtures (50 ml) with the following compositions (v/v): 5:95, 10:90, 15:85, 20:80, 30:70, 40:60, and 50:50 were collected. Dibenzyl [$^{13}C_2$]oxalate (151 mg) was recovered from fractions 4 and 5. Physical properties were as for the unlabelled material, δ_H (250 MHz; $CDCl_3$) 5.30 (2 H, dd, J_1 16, J_2 6.4 Hz) and 7.38 (5 H, m).

Preparation of (7-Hydroxy-2-oxoindolin-3-yl)[$^{13}C_2$]*acetic Acid* (**15**).—A mixture of dibenzyl [$^{13}C_2$]oxalate (145 mg) and 7-benzyloxyindolin-2-one (**7**) (106 mg) in THF (total 2 ml) was added quickly to stirred sodium hydride–oil (38 mg of 60% dispersion) under dry nitrogen. The mixture was stirred at room temperature for 7 h, after which time t.l.c. indicated complete consumption of the indolinone. At this time 2M-NaOH (10 ml) was added and the mixture was stirred vigorously for a further 3 h at 25 °C. The mixture was ice-cooled, diluted to 40 ml total volume, acidified to pH 1, and extracted with EtOAc (3 × 40 ml). The organic phase was washed with water (40 ml), filtered, and concentrated to give an orange solid (216 mg).

The crude product from above was taken up in AcOH–H_2SO_4 (99.5:0.5) and hydrogenated at 3.5 atm. for 12 h over 10% Pd–C (200 mg). The product, isolated as described for the unlabelled synthesis, was an off-white solid (59 mg), which was purified on a column of DEAE-Sephadex (acetate form; 12 × 0.5 cm). The column was eluted with 50% EtOH (40 ml; 10 × 4 ml fractions), then with a gradient of 0—5% AcOH in 50% aq. EtOH (200 ml; 1.5 ml fractions). Fractions 43—58 were pooled and concentrated to afford (7-hydroxy-2-oxoindolin-3-yl)[$^{13}C_2$]acetic acid (**15**) (49 mg) as a white solid with the same physical properties as the unlabelled material, δ_H (250 MHz; C_5D_5N) 3.16 (1 H, dddd, J_1 129, J_2 16.2, J_3 8.5, J_4 6.7 Hz), 3.53 (1 H, dddd, J_1 129, J_2 16.2, J_3 6.9, J_4 4.2 Hz), 4.26 (1 H, ddd, J_1 7.4, J_2 5.4, J_3 4.2 Hz), 6.95 (1 H, dd, J_1 8.0, J_2 7.3 Hz), 7.08 (1 H, dt, J_1 8.0, J_2 1.1 Hz), 7.22 (1 H, dt, J_1 8.4, J_2 1.1 Hz), and 12.28 (1 H, br s); *m/z* 209 (M^+, 39%), 208 (1), 164 (11), 163 (25), 162 (100), 135 (12), 134 (27), 117 (9), 106 (13), 105 (15), 92 (11), and 78 (12).

Preparation of Compounds (**18**) *and* (**16**) *using* Zea mays *Root Segments.*—[5-n-^{3}H]Indol-3-ylacetic acid, specific activity 16.7

Ci mmol^{-1}, was obtained from Amersham International p.l.c. as an EtOH solution with an activity 800 μCi ml^{-1}. This was diluted with water to a concentration of *ca.* 30 μCi ml^{-1} and stored in the dark at 5 °C. The solution was stable, as determined by h.p.l.c. analysis, for at least 3 months.

Zea mays seedlings cv. Silver Queen (W. Atlee Burpee Co.) were sterilised and germinated as described in ref. 3. Both 3-and 4-day-old root preparations gave approximately the same metabolism of IAA. Two batches of 75 root segments, each 2 cm in length, were cut from 2—3 mm behind the root tips. Each batch was placed in a 400 ml beaker (i.d. *ca.* 8 cm) containing water (4 ml) and the aqueous substrate solution (2 × 950 μl; a total of 56.4 μCi) was added. Each batch was incubated in darkness at 30 °C for 24 h whilst being gently shaken, the aqueous phase was then removed, and the roots were washed with water (3 × 5 ml; 3 × 1 min). The combined aqueous phase was centrifuged and the supernatant was concentrated. The radioactivity recovered was 3.0 μCi. The roots were extracted with MeOH (20 ml per batch) in darkness at 2 °C for *ca.* 18 h, the extract was removed, and the roots were washed with MeOH (3 × 10 ml). The combined organic phase was centrifuged and the supernatant was concentrated. The radioactivity recovered was 41.0 μCi.

The total MeOH extract was applied to a 10 × 0.8 cm column of DEAE-Sephadex in the acetate form, made up and pre-eluted with 50% aq. EtOH (20 ml). The extract was eluted first with 50% aq. EtOH (30 ml), then with a gradient of 0—5% AcOH in 50% aq. EtOH (200 ml); 1.5 ml fractions were collected. Fractions 1—20 contained a total of 3.0 μCi; fractions 50—66 contained 31.5 μCi.

The major peak of radioactive material from DEAE-Sephadex chromatography was further purified by reverse-phase h.p.l.c. under conditions B (see Table). Appropriate fractions were pooled, and contained radioactivity as follows: *t* 12.0—17.0 min, 21.7 μCi; *t* 19.0—22.0 min, 1.8 μCi; *t* 26.0—29.0 min, 5.7 μCi.

The [^{3}H]-glucoside (**18**) from above was lyophilised twice from water, and a solution of β-glucosidase (Sigma) in 0.05M-KH_2PO_4–citrate buffer (0.5 ml; pH 5.0) [concentration *ca.* 1 mg ml^{-1}] was added. The mixture was incubated at 25 °C for 2 h, then the reaction was terminated by addition of EtOH (2.5 ml) and the precipitated protein was removed by centrifugation. The supernatant was dried under reduced pressure and purified by reverse-phase h.p.l.c. under conditions B (see Table). The yield of 7-hydroxy-[5-n-^{3}H]OxIAA (**16**) was 14.5 μCi [67% based on amount of (**18**) used]. A further 2.79 μCi was recovered as the decomposition products described in the Discussion section. In subsequent experiments, yields of 7-hydroxy[^{3}H]-OxIAA were improved to *ca.* 80% by purging the solution of the product immediately with nitrogen, and storage at −30 °C before and after h.p.l.c. purification. These figures correspond to radiochemical yields of the [^{3}H]-glucoside (**18**) and 7-hydroxy[^{3}H]OxIAA (**16**) of 38.5 and 30.8% respectively, based on [^{3}H]IAA used.

The specific activity of the 7-hydroxy[^{3}H]OxIAA (**16**) [and therefore that of the glucoside (**18**)] was estimated to be 1.0 Ci mmol^{-1}, from the u.v. absorption at 254 nm and liquid scintillation counting of the h.p.l.c. eluant.

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