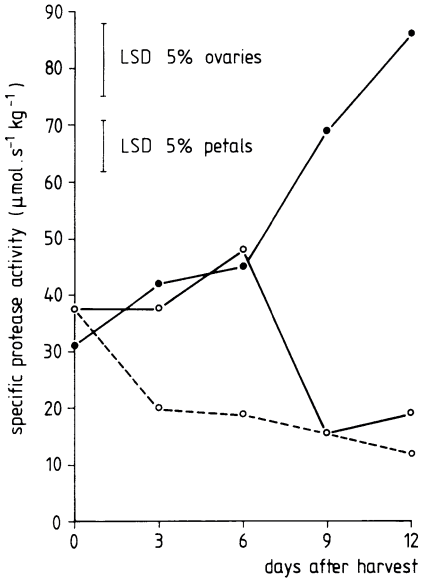

Fig. 1. Specific catalytic activity of protease in ovaries (○) from cut 'Sonia' roses treated with ethylene at a concentration of 12 ppm (---), and from untreated controls (—) for a 12-day period after harvest. Protease activity in petals (●) from treated and untreated flowers (which did not differ). Means were calculated from triplicate samples.

lowed by a least significant difference (LSD) test at the 5% level of probability. ANOVA showed a significant effect of ethylene treatment and of days after harvest, and an interaction of these two factors on protease activity in ovaries. LSD for ovaries was computed on the basis of the interaction. Thus, the LSD test was used for separation of means from both classes of ovaries pertaining to a given day after harvest and for separation of means within the same class of ovaries on different days after harvest. An F test did not indicate any significant effect of ethylene treatment of cut roses on protease activity in petals, but it indicated a significant effect of days after harvest. Thus, the LSD test for petals was computed for separation of means pertaining to different days after harvest.

Ethylene treatment of cut roses affected protease activity in ovaries but not in petals. Protease activity in ovaries from treated flowers decreased from cutting until the 3rd day, and then remained at the same level until the 12th day. The activity from the controls was constant during the first 6 days, decreased until day 9, and remained at that level until day 12.

Protease activity in petals from both treatments was constant during the first 6 days and increased steadily until the 12th day. We cannot explain why the ethylene treatment affected protease activity in ovaries but not in petals.

In the control flowers, an increase in protease activity in petals coincided with a decrease in activity in ovaries. Such simultaneous changes in activity were not detected in ethylene-treated flowers nor in either treated or control cut roses from plants grown hydroponically (3). Thus, it appears that there is no causal relationship between activities in the two parts of the flower.

In ethylene-treated flowers, the sepals were reflexed immediately after treatment. On day 6, the control flowers also had reflexed sepals. On day 9, the outer petals from ethylene-treated flowers were bluish. Otherwise, no differences were observed between treated and control flowers. Petal abscission occurred after 12 days.

Literature Cited

1. Gorin, N., B. van Dongen, and G. Vos. 1986. Protease activity in extracts of petals from cut roses cv. Sonia during vase life. Acta Hort. 181:81–85.
2. Lukaszewska, A. 1986. The effect of benzyladenine and ethephon on soluble protein content and invertase activity in wilting cut roses cv. Carina. Acta Hort. 181:87–92.
3. Lukaszewska, A., F.J. Pérez-Zúñiga, and N. Gorin. 1985. Effect of ethylene on protease activity in extracts of petals and ovaries from intact or cut roses cv. Sonia grown hydroponically. A preliminary note. Sprenger Institute, Wageningen, Netherlands. Rpt. 2305.
4. Moss, D.W. 1983. Nomenclature and units in enzymology, p. 8–14. In: H.U. Bergmeyer, J. Bergmeyer, and M. Grabl (eds.). Methods of enzymatic analysis, 3rd edition, Vol. I. Verlag Chemie Weinheim, Basel, Switzerland.
5. Woltering, E.J. and E.P. Sterling. 1986. Design for studies on ethylene production of ornamental products. Acta Hort. 181:483–488.

HortScience 22(1):97–99. 1987.

Synthesis and Biological Activity of [^{14}C]IAA Ethyl Ester

Rebecca L. Darnell, L. Carl Greve, and George C. Martin
Pomology Department, University of California, Davis, CA 95616

Additional index words. *Fragaria ×ananassa*, strawberry, fruit growth

Abstract. **A simple procedure for synthesizing and purifying the [^{14}C]ethyl ester of IAA (Et-IAA) is described. This auxin has been found to stimulate parthenocarpic fruit set in day-neutral strawberries (*Fragaria ×ananassa* Duch. 'Fern'), which are nonresponsive to various other auxins. Et-IAA may prove useful in eliciting physiological responses in systems shown previously to be auxin-nonresponsive. Chemical name used: 1*H*-indole-3-acetic acid (IAA).**

Application of auxins to receptacles of June-bearing and ever-bearing strawberry cultivars induce parthenocarpic fruit set. The range of auxin compounds capable of stimulating parthenocarpy in these cultivars include IAA (7), 1*H*-indole-3-butanoic acid (IBA) (7, 11), 1-naphthaleneacetic acid (NAA) (1, 2, 7), and 2-naphthoxyacetic acid (2-NOA) (7, 10, 11). Experiments performed (unpublished results) indicate that day-neutral strawberry cultivars ('Hecker', 'Brighton', 'Fern', and 'Selva') do not set fruit in response to the previously mentioned auxins. The only auxin found to date that induces parthenocarpy in these day-neutral strawberries is Et-IAA.

Early work on identification of auxins in plant tissues indicated that Et-IAA was a natural constituent of both corn and apple endosperm, which may be the factor responsible for fruit set (8, 9). Later, it was reported that the presence of Et-IAA in the plant extracts was an artifact of the extraction procedure, and there was no endogenous Et-IAA present (3). There have been no further reports, to our knowledge, of the presence of this auxin in plant tissues.

In this study, a simple method for synthesizing [^{14}C]Et-IAA is described. The demonstration of an auxin response with Et-IAA in day-neutral strawberries, combined with the availability of the radiolabeled compound, may stimulate new investigations of auxin-mediated physiological responses in previously auxin-nonresponsive systems.

Synthesis of [^{14}C]IAA ethyl ester. The procedure followed was a modification of Jackson's tryptophol synthesis method (6). A total of 250 μCi (1 Ci = 37 GBq) of 2-[^{14}C]IAA (Amersham, specific activity 2.04 GBq/mmol) in 5.0 ml toluene was dried under a stream of N_2 prior to the addition of 3.0 ml of 75% ethanol. The resulting solution was brought to 1.0 N with HCl and stirred in the dark for 24 hr. The reaction was stopped by the addition of 0.35 ml of 10.0 N NaOH (bringing the pH to 6.0) and the solution was dried under vacuum. Distilled water was added to the reaction vial, and the products were partitioned three times against H_2O-saturated ethyl acetate. The aqueous phase was discarded, and the ethyl acetate phases containing [^{14}C]Et-IAA were combined and dried under vacuum.

Purification, identification, and quantification. The products were resuspended in 100% methanol, sonicated, and brought to 20% methanol with the addition of distilled H_2O. The solution was centrifuged in an Eppendorf centrifuge for 10 min at 15,600× *g*, and the supernatant was decanted and subjected to fractionation on a 10 × 150 mm

Received for publication 7 Apr. 1986. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

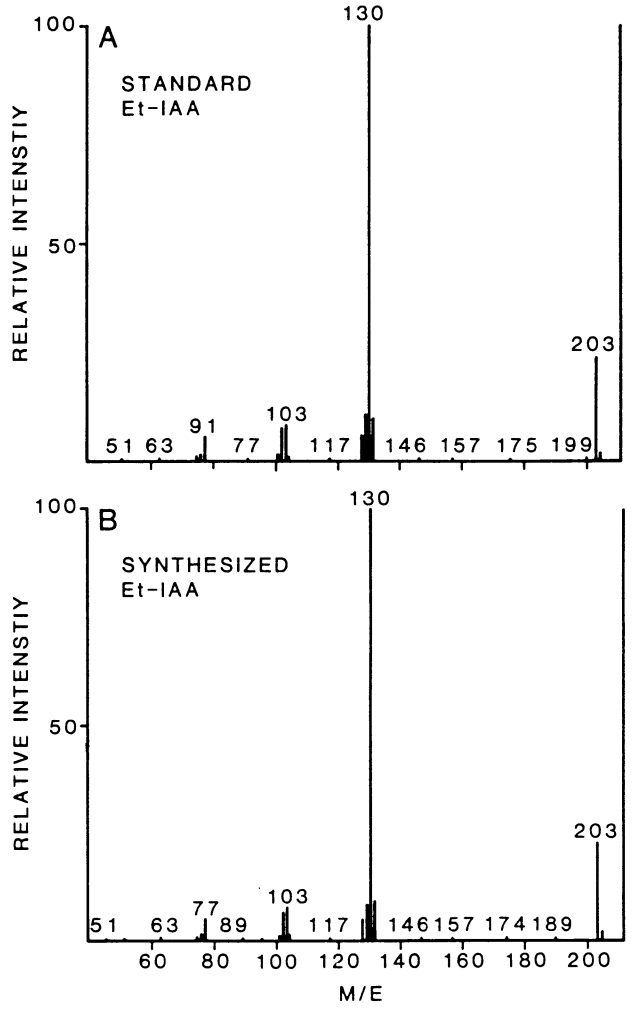

Fig. 1. Electron ionization mass spectra of standard (**A**) and synthesized (**B**) Et-IAA. Major ions shown are *m/e* 203 (molecular ion of Et-IAA), *m/e* 130 (base peak, representing loss of the $-COOCH_3$), *m/e* 103 (representing fragmentation of the indole ring), and *m/e* 77 (benzene). Note that product identification was confirmed using unlabeled IAA in the identical synthesis procedure. Spectra were generated in a Finnigan 3200 electron ionization GC–MS using a 30-m DB-1 column and a temperature program of 100° to 250°C, 10°/min; injector temperature of 250°; and He linear velocity of 35 $cm \cdot s^{-1}$. The mass spectrometer was set at 70 eV, 20 μA, with scan speed of 1.2 sec/decade and source temperature of 80°. M/E = *m/e*.

stainless steel ODS-18 semi-preparative column by HPLC (LDC/Milton Roy). Separation of the products was performed using a 30-min linear gradient of 20–100% methanol in distilled H_2O at a flow rate of 3 $ml \cdot min^{-1}$. The [^{14}C]Et-IAA fraction (as determined by comparison of retention times with a cold Et-IAA standard supplied by Sigma) was collected and dried under vacuum. The residue was resuspended in 100% methanol and aliquots removed for identification and quantification. Product identification was confirmed by comparison with the Et-IAA standard on a Finnigan 3200 gas chromatography–mass spectrometer (GC–MS) using electron ionization. The product was quantified by comparison with an Et-IAA standard curve generated with a Beckman quartz spectrophotometer with wavelength set at 280 nm.

Physiological activity. One-year-old day-neutral strawberry plants were planted in 15-cm pots in the greenhouse in 1 soil : 1 peat : 1 sand (by volume), and fertilized with 1 g of Osmocote (14N–6P–12K) slow-release fertilizer. After inflorescence development the plants were moved to a growth chamber equipped with mercury halide and fluorescent lamps maintained at a light intensity of 440 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The temperature in the chamber was held at 21.6° ± 0.3°C during the 13-hr day cycle and at 17.7° ± 0.2° during the night cycle. Flowers were emasculated 1 day prior to opening and the following treatments applied: 1) hand-pollinated, with pollen of the same cultivar; 2) nonpollinated control; or 3) Et-IAA. Et-IAA (2.5×10^{-3} M) in aqueous solution containing 2% dimethyl sulfoxide (DMSO) and buffered at pH 6.8 was applied to the receptacles by dipping for 10 sec. The nonpollinated control treatment consisted of dipping receptacles in a 2% DMSO aqueous solution buffered at pH 6.8. Fruit set and growth in the three treatments were determined by measuring receptacle length.

The synthesis of [^{14}C]IAA ethyl ester is simple, fast, and requires only standard laboratory glassware and reagents. HPLC analysis revealed four UV absorbing peaks, of which the presumptive Et-IAA peak constituted the majority of the area. GC–MS spectra of the standard Et-IAA and the synthesized Et-IAA were identical (Fig. 1 A and B). Quantification of the product using UV absorption indicated a reaction yield of 43%. This yield was confirmed by liquid scintillation spectrophotometry.

Preliminary time course studies indicated that the reaction can continue in the dark at room temperature for up to 48 hr without decreasing yield. After that time, however, yield of Et-IAA begins to decrease. After 72 hr in the dark, yield of Et-IAA is ≈24%.

Various other synthesis methods were tried, but discarded on the basis of lower yield. Among these was the use of thionyl chloride ($SOCl_2$) to convert IAA into the acid chloride, followed by reaction of the acid chloride with ethanol to form the ethyl ester. Theoretically, these are essentially irreversible reactions that go to completion and the use of $SOCl_2$ is convenient for the formation of the acid chloride, since other products formed are gases and therefore easily separated from the acid chloride (5). However, HPLC analysis of the IAA–Cl derivative revealed the presence of more than 40 peaks in the reaction mixture. Further reaction of the acid chloride with ethanol to form the ester also resulted in numerous peaks upon HPLC analysis, rendering preliminary identification of the desired product extremely difficult. This synthesis method was then discarded in favor of direct esterification of the acid, which has the advantage of being a single-step synthesis.

Various manipulations of the direct esterification method were performed. Initially, the reactants (IAA and ethanol, with HCl as an acid catalyst) were refluxed at 90°C, and aliquots taken hourly to determine yield. Refluxing for 6 hr gave the maximum yield of Et-IAA (22%). Refluxing in the dark had no effect on yield. Since heating is known to enhance the rate of degradation of acidic IAA solutions (4, 12), direct esterification of IAA at room temperature was tried. This method resulted in the highest yield (43%, as reported earlier) and was therefore the method of choice.

Et-IAA induces fruit set and growth in day-neutral strawberries (Fig. 2). Fruit set of the Et-IAA-treated receptacles ranged from 75% to 100%, while set in the pollinated treatment ranged from 95% to 100%. Growth of the auxin-treated fruit keeps pace with growth of the pollinated fruit during the early part of the developmental cycle. However, growth of the auxin-treated fruit begins to slow between 6 and 9 days after treatment, although these fruit mature and ripen simultaneously with pollinated fruit. This decrease in the growth rate of the Et-IAA-treated fruit is in agreement with work done on auxin-induced set and growth of June-bearing and everbearing strawberry cultivars (1, 10), where auxin-induced fruit growth ceased 10–12 days after the initial auxin application. Growth could be restimulated by additional applications of auxin to the receptacle, suggesting that a continuous supply of auxin is needed for continued growth.

The present study demonstrates an easy method for synthesizing and purifying

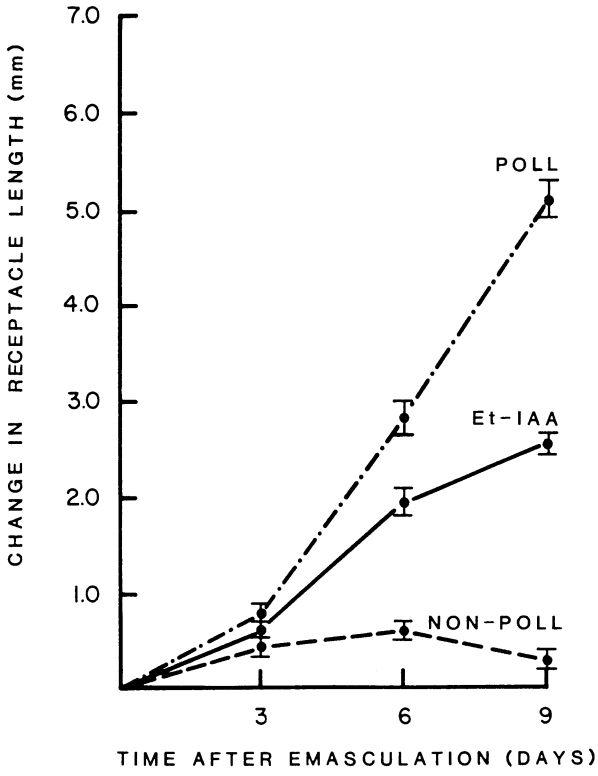

Fig. 2. Effects of pollination, nonpollination, and Et-IAA on fruit growth of 'Fern' strawberry. (SE is indicated, n = 10)

[^{14}C]Et-IAA. It also demonstrates that Et-IAA is a biologically active auxin that is capable of inducing parthenocarpic fruit set in strawberry cultivars that were thought to be auxin nonresponsive with respect to set. Whether the ester itself is biologically active or the ester conjugation prevents the degradation of IAA, thereby allowing the free acid to reach the sight of action, is unknown.

Literature Cited

1. Archbold, D.D. and F.G. Dennis, Jr. 1985. Strawberry receptacle growth and endogenous IAA content as affected by growth regulator application and achene removal. J. Amer. Soc. Hort. Sci. 110:816–820.
2. Beech, M.G. 1983. The induction of parthenocarpy in the strawberry cultivar 'Redgauntlet' by growth regulators. J. Hort. Sci. 58:541–545.
3. Fukui, H.N., J.E. DeVries, S.H. Wittwer, and H.M. Sell. 1957. Ethyl-3-indole acetate: an artefact in extracts of immature corn kernels. Nature (London) 180:1205.
4. Galston, A.W. and W.S. Hillman. 1961. The degradation of auxin. Encyl. of Plant Physiol. 14:647–670.
5. Gee, M. 1965. Methyl esterification of nonvolatile plant acids for gas chromatographic analysis. Anal. Chem. 37:926–928.
6. Jackson, R.W. 1930. A synthesis of tryptophol. J. Biol. Chem. 88:659–662.
7. Mudge, K.W., K.R. Narayanin, and B.N. Poovaiah. 1981. Control of strawberry fruit set and development with auxins. J. Amer. Soc. Hort. Sci. 106:80–84.
8. Redemann, C.T., S.H. Wittwer, and H.M. Sell. 1951. The fruit-setting factor from the ethanol extracts of immature corn kernels. Arch. Biochem. Biophys. 32:80–84.
9. Teubner, F.G. 1953. Identification of the auxin present in apple endosperm. Science 118:418.
10. Thompson, P.A. 1969. The effect of applied growth substances in development of the strawberry fruit: II. Interactions of auxins and gibberellins. J. Expt. Bot. 20:629–647.
11. Thompson, P.A. 1967. Promotion of strawberry fruit development by treatment with growth regulating substances. Hort. Res. 7:13–23.
12. Yamakawa, T., O. Kurashashi, K. Ishida, S. Kato, T. Kodama, and Y. Minoda. 1979. Stability of indole-3-acetic acid to autoclaving, aeration and light illumination. Agr. Biol. Chem. 43:879–880.