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Identification of Traumatin, a Wound Hormone, as 12-Oxo-*trans*-10-dodecenoic Acid[1]

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ABSTRACT

12-Oxo-*trans*-10-dodecenoic acid (*trans*-10-ODA) is an oxidation product of polyunsaturated fatty acids in plant tissues. The structural similarity of *trans*-10-ODA and traumatic acid, a compound considered to be a wound hormone, suggested that *trans*-10-ODA might be a precursor of traumatic acid. Both *trans*-10-ODA and traumatic acid were active in the Wehnelt bean assay. The results were more consistent with *trans*-10-ODA than with traumatic acid. Cucumber (*Cucumis sativus* L. var. National Pickling) hypocotyls also showed a growth increase following treatment with *trans*-10-ODA, which suggested that *trans*-10-ODA has a more general influence on plant development than previously ascribed to traumatic acid.

Runner beans (*Phaseolus vulgaris* L. var. Kentucky Wonder) were analyzed for the presence of endogenous *trans*-10-ODA and traumatic acid. These are the beans from which traumatic acid was originally isolated in 1939. They contained *trans*-10-ODA but no traumatic acid. Young beans were a better source of *trans*-10-ODA than older beans and an increase in the esterified form of *trans*-10-ODA with age may have been due to a conversion of the free acid to the esterified form. The amount of endogenous *trans*-10-ODA increased when bean pod tissue was sliced and wounded. Rapid stirring and the presence of oxygen increased autooxidation of *trans*-10-ODA to traumatic acid in runner beans, which indicated that the compound identified as traumatic acid is formed by autooxidation of *trans*-10-ODA and that *trans*-10-ODA is a natural compound with growth-regulating properties.

Enzyme extracts of runner beans synthesized *trans*-10-ODA from linoleic acid. No enzymic synthesis of traumatic acid was observed even when cofactors were added to the reaction mixture. This confirmed the conclusion that traumatic acid is formed by autooxidation of *trans*-10-ODA.

The concept of plants producing a chemical substance in response to wounding was suggested in the 19th century by Wiesner, but it was not until 1921 that Haberlandt (8) demonstrated the presence of a wound hormone in potato tubers. Wehnelt (25) developed a quantitative assay for wound hormone activity using the parenchymous lining of the seed chambers of immature bean pods. A drop of test solution resulted in the formation of an intumescence of dividing and enlarging cells on the surface of the seed chamber. Bonner and English (2, 3) used the height of the intumescence produced in the Wehnelt bean test as a measure of activity for the assay of various fractions of bean extract during the isolation and purification of wound hormone. The hormone was named traumatin (1). English *et al.* (4, 5) crystallized traumatin from runner bean extracts, and identified it as *trans*-2-dodecenedioic acid and named it traumatic acid.

Traumatic acid has been shown to induce tumors in green tomatoes (22) and to accelerate abscission of cotton petioles (20). The internodes of *Ricinus communis* demonstrated a wound response to traumatic acid (18). However, many other tissues have failed to respond to traumatic acid, and Keller (11) and Haagen-Smit and Viglierchio (7) reported that crude extracts of beans and citrus fruit were much more active in the Wehnelt assay for wound hormones than was traumatic acid.

Vick and Zimmerman (24) recently reported the presence of a hydroperoxide lyase enzyme in watermelon seedlings that converted 13-hydroperoxy-linoleic acid to *trans*-10-ODA[2] and hexanal. Tressl and Drawert (23) proposed that *trans*-10-ODA was formed from linoleic acid and 13-hydroperoxy-linoleic acid in homogenates of climacteric and postclimacteric bananas. Because of the similarity of the structures, *trans*-10-ODA is a possible precursor of traumatic acid via a dehydrogenase. We investigated this possibility by repeating the work of English *et al.* (4, 5) concerning the isolation and physiological activity of traumatic acid.

MATERIALS AND METHODS

General Conditions. All organic solvents and buffers for enzyme extraction were deoxygenated by bubbling N_2 through them for 20 min. Samples were extracted and stored under N_2. Enzyme incubations and assays were performed at 22 C. Organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure in a 30 C water bath.

Growth Conditions. Runner beans (*Phaseolus vulgaris* L. var. Kentucky Wonder, brown seed) were planted in pots containing Vermiculite and watered with one-third strength Hoagland nutrient solution. Plants were grown in a greenhouse at 21 to 24 C with a 16-h photoperiod.

Wehnelt Bean Assay for Traumatin Activity. Immature runner beans (9–12 cm) were used in the assay described by Bonner and English (2). For use in test solutions, traumatic acid (Aldrich Chemical Co.)[3] or *trans*-10-ODA purified from incubations of 5-day-old watermelon seedlings with linoleic acid (24) was dissolved in 0.1 to 0.15 ml acetone and the solution injected into 0.05 M K-phosphate (pH 7.4) to provide final concentrations of 10^{-6} M and 10^{-4} M. The acetone was then evaporated under N_2 and the solution applied in 5-μl aliquots to bean seed chambers. The *trans*-10-ODA solutions were prepared daily. The control solution was 0.05 M K-phosphate (pH 7.4). For preparation of the bean test solution, immature beans were crushed in a mortar with a pestle,

[1] This work was conducted in cooperation with the North Dakota Agricultural Experiment Station, Paper No. 892.

[2] Abbreviations: *trans*-10-ODA: 12-oxo-*trans*-10-dodecenoic acid; MOX: methoxylamine.

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filtered through four layers of cheesecloth and diluted with two parts water.

Cucumber Hypocotyl Assay. The assay described by Moore (16) was also used to test the growth regulating activity of *trans*-10-ODA. A test solution of 0.707 mM *trans*-10-ODA in 0.05% Tween 20 (polyoxyethylene [20] sorbitan monolaurate) solution was applied in 1- or 10-μl aliquots to the shoot tip of cucumber (*Cucumis sativus* L. var. National Pickling) seedlings. A standard solution of 10^{-6} M IAA in 0.05% Tween 20 solution and a control solution of 0.05% Tween 20 solution were applied in 10-μl aliquots.

Endogenous Lipids of Runner Beans. Immature runner beans (6–10 cm) were crushed in a French pressure cell under 10,000 p.s.i. The liquified tissue was diluted with deoxygenated water (1:1, v/v), acidified to pH 2.5, and extracted overnight with an equal volume of petroleum ether. Pentadecanoic acid (100 μg in 1 ml methanol) was added as an internal standard. The organic layer was decanted, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The extracts were esterified with diazomethane and then divided into two parts, one to be analyzed for traumatic acid and the other for *trans*-10-ODA. The latter was derivatized with MOX reagent (Pierce Chemical Co.) to stabilize the α,β-unsaturated carbonyl. A flow chart for the analysis of endogenous bean lipids is shown in Figure 1.

Preparation of Bean Enzyme Extracts. Beans of 6 to 10 cm were chopped in small pieces, placed in a mortar, frozen with liquid N_2, and ground to a powder. The powder was gently stirred into 0.05 M K-phosphate (pH 6.0) containing Polyclar AT. The mixture was filtered through cheesecloth and centrifuged at 12,000*g* for 20 min. The supernatant was used for enzyme assays and incubations with linoleic acid.

Enzyme Assays. Lipoxygenase was measured by the absorption at 234 nm of the conjugated diene of the hydroperoxide product. The reaction mixture contained 2.9 ml 0.05 M K-phosphate (pH 6.0), 0.02 ml linoleic acid (Nu Chek Prep, Inc.) substrate solution (21), and 0.1 ml bean extract. The reaction was initiated by the addition of the substrate.

Hydroperoxide-metabolizing enzymes were measured by the decrease in *A* at 234 nm of the hydroperoxide. The hydroperoxide substrate solution was prepared by incubation of 1.2 mg soybean lipoxygenase (Sigma Chemical Co.) with 0.6 ml linoleic acid substrate solution in 30 ml deionized H_2O for 1 h. The reaction mixture contained 2.4 ml 0.5 M K-phosphate (pH 6.0), 0.5 ml hydroperoxide substrate solution, and 0.1 ml bean extract. The reaction was initiated by the addition of the extract.

Aldehyde dehydrogenase (12) was assayed by measurement of the change in *A* of nicotinamide adenine dinucleotides at 340 nm. The reaction mixture contained 2.5 ml of 0.05 M K-phosphate (pH 6.0), 0.02 ml linoleic acid substrate solution, 0.3 ml bean extract, and 0.1 ml NAD^+ or $NADP^+$ (1 mg/ml in buffer).

Incubations of Bean Extract with Linoleic Acid. Bean extract (48 ml) was incubated with 20 ml linoleic acid substrate solution in 300 ml 0.05 M K-phosphate (pH 6.0). Incubations used to investigate the oxidation of *trans*-10-ODA to traumatic acid contained 100 ml buffer, 5 ml linoleic acid substrate solution, and 25 ml enzyme extract with or without 10 mg NAD^+ or $NADP^+$. The heat-denatured reaction contained bean extract that had been placed in a boiling water bath for 10 min.

After incubation of the reactions for 2 h at 22 C, the mixtures were acidified to pH 2.5, saturated ammonium sulfate solution (1:2, v/v) was added, and the products were extracted twice with petroleum ether for 18 to 24 h. The organic extracts were concentrated under reduced pressure and esterified with diazomethane.

Product Identification. Endogenous lipids and products of incubations were analyzed with a Varian Aerograph Series 1700 gas chromatograph equipped with a glass column (3 m × 2 mm i.d.) packed with 3% OV-25 on 100 to 120 mesh Gas-chrom Q (Applied Science Laboratories, Inc.). The column was temperature-programed from 190 to 230 C at 1 degree/min with a carrier flow rate of 20 ml/min. The gas chromatograph was attached to a Hewlett-Packard 3380A recording integrator. The products of incubations were applied to thin layer plates spread with Silica Gel H (Applied Science Laboratories, Inc.) at a thickness of 250 μm, and developed three times in a solvent system composed of petroleum ether, diethyl ether, and glacial acetic acid (85:15:1, v/v). The UV absorbing band with an R_F of *trans*-10-ODA was scraped and analyzed by combined GC/MS. A Varian Aerograph chromatograph connected to a Varian/MAT CH5-DF mass spectrometer with an attached data system was used.

RESULTS

Wehnelt Bean Assay for Traumatin Activity. The assay described by Bonner and English (2) was used to test the physiological properties of traumatic acid and *trans*-10-ODA. Both compounds induced formation of an intumescence on the seed chamber by both cell division and elongation (25). A high contrast photograph of a control section and a section treated with *trans*-10-ODA is shown in Figure 2. The treated seed chamber shows a triangular section of darker cells below the intumescence. This area is edged with a zone of light colored cells that separate the cells which were in contact with the *trans*-10-ODA from those not

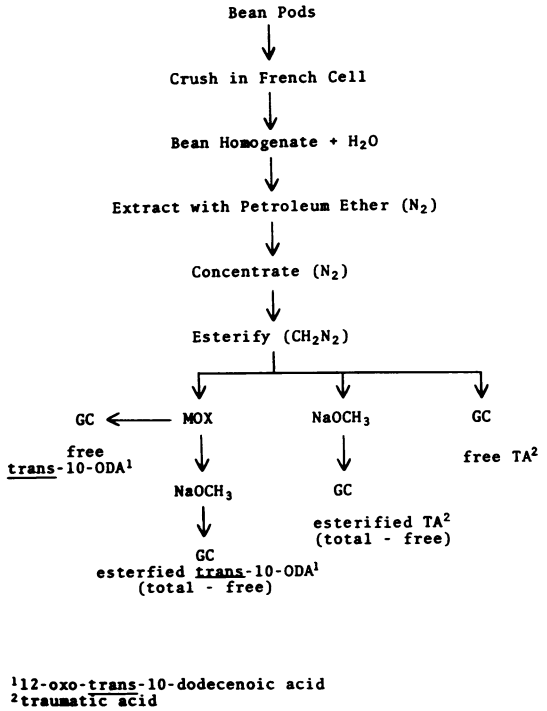

FIG. 1. Flow diagram for analysis of lipids in runner bean pods.

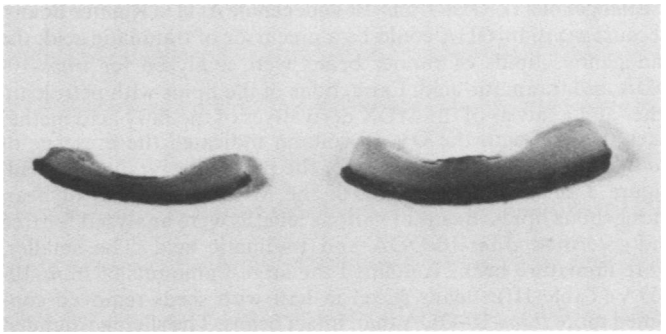

FIG. 2. Bean pod sections treated with buffer (left) and 10^{-6} M *trans*-10-ODA (right) and incubated at 25 C for 64 h.

Table I. Bean intumescence assay for traumatin activity.

Bean pod length, cm	Height of intumescence, mm				
	Buffer control	trans-10-ODA[1]		TA[2]	
		10^{-6} M	10^{-4} M	10^{-6} M	10^{-4} M
9-10[3]	0.19 ± 0.06	0.28 ± 0.05**	0.37 ± 0.06**	0.27 ± 0.08*	0.25 ± 0.06*
11-12[4]	0.23 ± 0.07	0.30 ± 0.08*	0.35 ± 0.05**	0.30 ± 0.05*	0.26 ± 0.07
combine 9-10 + 11-12	0.21 ± 0.06	0.29 ± 0.06**	0.36 ± 0.05**	0.28 ± 0.06**	0.25 ± 0.06*

[1] 12-oxo-trans-10-dodecenoic acid
[2] traumatic acid
[3] mean of 10 observations ± std dev, 64 hour incubation
[4] mean of 8 observations ± std dev, 64 hour incubation
** significantly different from control at 1% level
* significantly different from control at 5% level

in contact with the compound. The light zone around the intumescence was observed in all bean seed chambers treated with *trans*-10-ODA and with traumatic acid.

The seed chambers of 9- to 10-cm bean pods showed the greatest response to test compounds and the seed chambers from 11- to 12-cm bean pods were also quite responsive (Table I). Beans of less than 8 cm or more than 12 cm showed little response in the bean assay. Applications of 10^{-6} M and 10^{-4} M *trans*-10-ODA induced responses that were statistically different from control responses at the 1% significance level. Responses of applications of 10^{-6} M traumatic acid were also significantly different at the 1% level, while responses with 10^{-4} M traumatic acid were significantly different at the 5% level. Traumatic acid treatments yielded less consistent results than *trans*-10-ODA treatments.

Cucumber Hypocotyl Assay. Several other assays for growth hormone activity were tested in an attempt to verify the growth-promoting effect of *trans*-10-ODA. Intact cucumber seedlings showed a significant increase in the length of hypocotyls when treated with *trans*-10-ODA (Table II), but the effect was not as great as that of IAA, which is known to influence growth of the hypocotyls.

The growth-inducing properties of *trans*-10-ODA were not confirmed in other assays. The *Avena* coleoptile growth assay (15) yielded no significant result nor did a pinto bean second internode assay or a pea epicotyl assay. Assays that measured bean epicotyl elongation and sunflower hypocotyl elongation yielded results that approached statistical significance at the 10% level. All of these assays showed extensive variability between replicates. In addition, these assays required that some part of the plant be excised, which may have resulted in formation of endogenous *trans*-10-ODA. Light quality, age of tissue, and other test conditions were not optimized for these assays. Thus, the results were inconclusive.

Endogenous 12-Oxo-*trans*-10-dodecenoic Acid in Runner Beans. Because *trans*-10-ODA could be a precursor of traumatic acid, the endogenous lipids of runner beans were analyzed for *trans*-10-ODA and traumatic acid. Extractions of the lipids with petroleum ether and analysis of the MOX derivatives of the fatty acid methyl esters by GC with the OV-25 column indicated the presence of *trans*-10-ODA but no evidence for the presence of traumatic acid. Figure 3 shows a GC tracing of the MOX derivatives of bean endogenous lipids. Beans of various lengths were analyzed for free and esterified *trans*-10-ODA and traumatic acid. The smaller, more immature beans contained the largest amounts of *trans*-10-ODA (Table III). Beans sliced in half with seeds removed contained more *trans*-10-ODA than intact beans. The slicing wounded the tissues and the results showed that *trans*-10-ODA increased when the tissue was wounded. No traumatic acid was detected in any of these sliced or intact tissues.

Table II. Growth enhancement by indole acetic acid and 12-oxo-trans-10-dodecenoic acid in cucumber hypocotyls.

Treatment	Increase in length, mm[1]
Control	1.00 ± 0.73
IAA[2] (0.0175 µg)	2.45 ± 0.83**
trans-10-ODA[3] (0.15 µg)	1.70 ± 0.86*
trans-10-ODA[3] (1.5 µg)	1.60 ± 0.82*

[1] mean of 20 observations ± std dev
[2] indole acetic acid
[3] 12-oxo-trans-10-dodecenoic acid
** significantly different from control at 1% level
* significantly different from control at 5% level

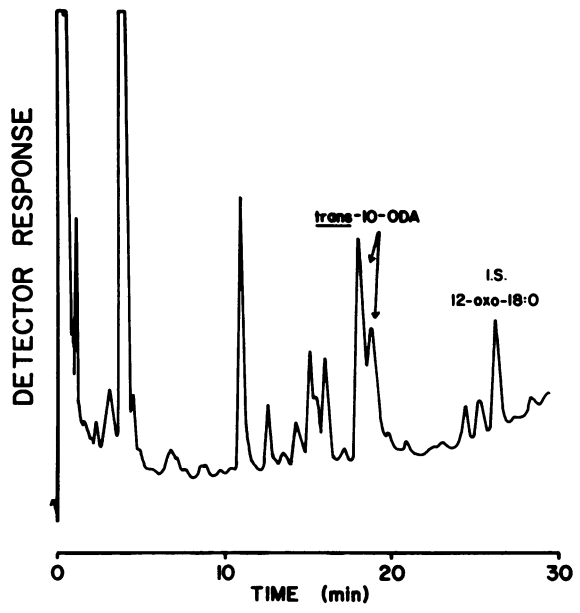

FIG. 3. Gas chromatographic tracing of methyl ester and methoxime derivatives of lipids from runner bean pods extracted with petroleum ether. Internal standard was pentadecanoic acid; components were separated on an OV-25 column.

The older beans contained more esterified *trans*-10-ODA than younger beans treated in a similar manner (Table III). This indicated that *trans*-10-ODA may be converted from the free form to the esterified form as the tissue ages. The nature of the ester moiety was not determined.

Table III. Endogenous 12-oxo-trans-10-dodecenoic acid and traumatic acid in runner beans.

Bean pod condition	Bean pod length, cm	trans-10-ODA[1] µg/g fresh wt		TA[2] µg/g fresh wt	
		Free[3]	Ester[3]	Free[3]	Ester[3]
Intact	6-7	15.7 ± 5.1		0	0
"	7-10	1.6 ± 0.2	3.8 ± 0.1	0	0
Sliced with beans removed	8	22.0 ± 2.5	2.1 ± 2.4	0	0
"	10	8.2 ± 1.1	5.9 ± 1.8	0	0

[1] 12-oxo-trans-10-dodecenoic acid
[2] traumatic acid
[3] mean of 3 observations ± std dev

Traumatic acid was found during this study of lipids from runner beans when special precautions were not taken to prevent oxidation. Four extractions of bean tissue were prepared under different conditions to demonstrate this autooxidation. The first sample was sealed under a N_2 atmosphere and extracted with slow stirring; the second sample was also sealed under N_2 but was stirred rapidly; the third sample was left open to the room atmosphere and stirred rapidly; the fourth sample was sealed under an O_2 atmosphere and stirred rapidly. The decrease in the ratio of *trans*-10-ODA to traumatic acid (Table IV) indicated that stirring and the presence of O_2 increased oxidation of *trans*-10-ODA to traumatic acid.

The structures of *trans*-10-ODA and traumatic acid from bean extracts exposed to O_2 were confirmed by GC/MS analysis of the methyl ester derivatives. The mass spectrum of the MOX derivative of *trans*-10-ODA was identical to that of the compound produced by watermelon (Fig. 4). The mass spectrum showed peaks at m/e 255 (M), and m/e 224 (M-31). The mass spectrum of traumatic acid was identical to that of commercial traumatic acid (Fig. 5). The spectrum showed peaks at m/e 225 (M-31), m/e 196 (M-CH_3OOCH), m/e (M-2 CH_3OH), m/e 164 (M-CH_3OOCH-CH_3OH).

Enzymic Synthesis of 12-Oxo-*trans*-10-dodecenoic Acid from Linoleic Acid by Bean Extracts. Bean enzyme extracts were prepared and assayed for lipoxygenase and hydroperoxide-metabolizing enzymes. Both were present and demonstrated good activity. The extracts were then incubated with linoleic acid substrate solution. The products were esterified and analyzed by GC, TLC, and GC/MS. A GC tracing of the products is shown in Figure 6. No traumatic acid was produced by the crude enzyme extract, but *trans*-10-ODA was one of the products formed. A compound with the retention time of a ketol of linoleic acid was the major product of the reaction. The esterified products were spotted on thin layer plates, developed four times, and the UV absorbing band with an R_F value of *trans*-10-ODA was scraped from the plates and eluted. The components of the band were analyzed by GC/MS and the presence of *trans*-10-ODA was confirmed. Figure 7 shows the mass spectrum which contained peaks at m/e 194 (M-CH_3OH) and m/e 166 (M-CH_3OOCH). This same spectrum was obtained with *trans*-10-ODA which was purified from a watermelon preparation. This mass spectrum differs slightly from that which we published earlier for *trans*-10-ODA (24). The earlier spectrum was obtained with a mass spectrometer utilizing a heated, solid probe for sample introduction. The heating of the probe was undoubtedly responsible for the rearrangements and losses obtained in the previous spectrum. We have repeatedly and reproducibly seen the mass spectrum shown in Figure 7 for *trans*-10-ODA produced from a number of different plant tissue sources and on two different GC/MS instruments.

The enzymic conversion of *trans*-10-ODA to traumatic acid via an aldehyde dehydrogenase was investigated by spectrophotometric assays and by analysis of reaction products from incubations of bean enzyme extracts with linoleic acid in the presence of cofactors. The spectrophotometric assays showed no change in *A* at 340 nm when either NAD^+ or $NADP^+$ was added. Gas chromatographic analysis of the products of the incubations failed to detect any traumatic acid formed as a product of the enzymic reactions.

Table IV. Effect of oxygen and stirring on autoxidation of 12-oxo-trans-10-dodecenoic acid to traumatic acid.

Extraction conditions	trans-10-ODA[1]/TA[2]
Stir slowly[3], N_2 atmosphere	77.43
Stir fast, N_2 atmosphere	70.89
Stir fast, air atmosphere	64.32
Stir fast, O_2 atmosphere	60.56

[1] 12-oxo-trans-10-dodecenoic acid
[2] traumatic acid
[3] 16 h

DISCUSSION

trans-10-ODA is an oxidation product of polyunsaturated fatty acids in plants. The structural similarity of *trans*-10-ODA and traumatic acid suggested that *trans*-10-ODA might be a precursor of traumatic acid. Experiments presented here did not support this hypothesis. Both *trans*-10-ODA and traumatic acid were active in the Wehnelt bean assay for traumatin activity. The observed activity of *trans*-10-ODA and traumatic acid was probably due to their structural similarities. Haagen-Smit and Viglierchio (7) reported that lauric acid, a 12-carbon saturated monocarboxylic acid, also exhibited activity in the bean assay for traumatin activity, which indicated that a 12-carbon chain with a carboxyl group could be required for recognition of a compound as a wound hormone. Other fatty acids have also demonstrated plant hormone activity. Fatty acid esters and fatty acids have promoted growth of pea epicotyl sections and increased the activity of GA_3 and IAA (19). An unusual polyhydroxy polyketo 12-carbon fatty acid, phaseolic acid, demonstrated growth-regulating properties in several bioassays (17).

As Bonner and English stated (2), a large and constant supply of beans for the bean intumescence assay was essential. It was difficult to obtain significant numbers of uniformly firm, immature, dark green bean pods from plants grown in a greenhouse. The beans that gave the best responses in the assay were picked when the plants first began to bear fruit. Beans produced by older plants did not respond as well. The activity of the beans was also affected by the amount of sunlight the plants received, even

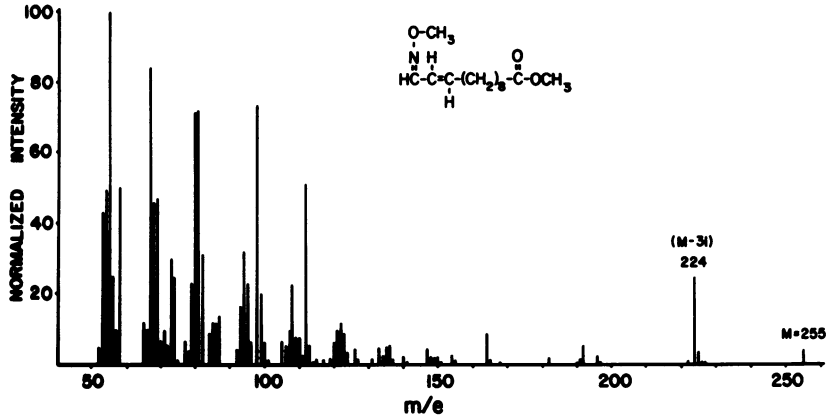

FIG. 4. Mass spectrum of methyl ester and methoxime derivative of *trans*-10-ODA isolated from runner bean pods.

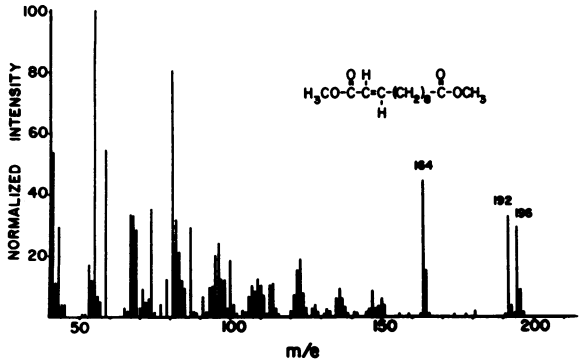

FIG. 5. Mass spectrum of dimethyl ester of traumatic acid formed by autooxidation of *trans*-10-ODA in runner bean pod extracts.

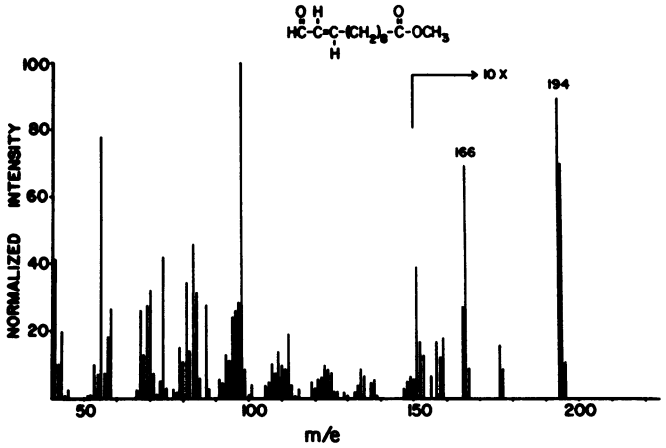

FIG. 7. Mass spectrum of methyl ester of *trans*-10-ODA formed from linoleic acid by extracts of runner bean pods.

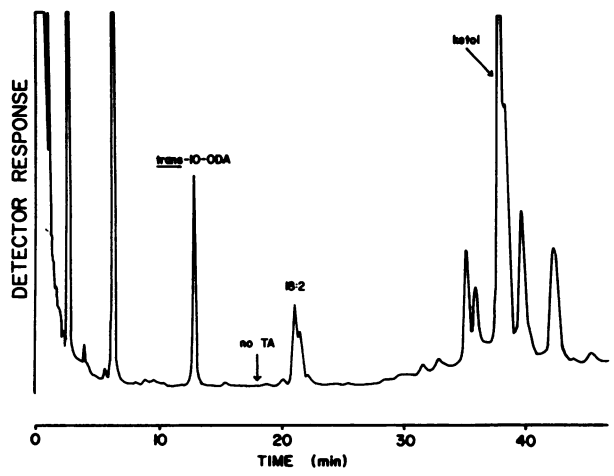

FIG. 6. Gas chromatographic tracing of methyl ester products formed from linoleic acid by extracts of runner bean pods separated on an OV-25 column.

though plants were supplemented with artificial light. Beans produced in July responded better than beans produced in November. Another disadvantage of the bean assay was that 3 months were required for plants to produce fruit under our growth conditions.

The source of seed was also a major factor influencing the activity of the beans in the traumatin assay. Kentucky Wonder brown seeds obtained from Gurney Seed Company, Yankton, S.D. did not respond to test compounds or to the liquid from crushed bean pods. Kentucky Wonder brown seeds obtained from Northrup King and Company, Minneapolis, Minn. did respond to either test solutions or the liquid from crushed bean pods, although not to the extent described by Bonner and English (2). They reported obtaining intumescences of 3 mm when diluted liquid from crushed bean pods was applied, while a maximum of only 0.8 mm was obtained in the present study. The most probable explanation for these results is that a change has occurred in the genetic composition of commercially available Kentucky Wonder beans that has decreased the response to exogenous traumatin.

The positive results of the cucumber hypocotyl assay suggested that *trans*-10-ODA has a more general influence on development than previously ascribed to traumatin, and that the influence is not limited to bean pod tissue. This would be expected if wound metabolism is not qualitatively different from normal metabolism and a change in hormone ratio is responsible for the accelerated rate of cell division and differentiation (13).

In the future, growth-inducing activity of *trans*-10-ODA should be assayed with intact tissues such as those of the cucumber hypocotyl. A method for synthesis of large amounts of *trans*-10-ODA and a better method for application to the tissue to prevent loss by decomposition or nonspecific interactions are necessary for continued study of the physiological role of *trans*-10-ODA in plants.

If traumatic acid is a wound hormone, one would expect it to be present in tissues after wounding even if it is not present in healthy tissues. The absence of traumatic acid in any of the test situations reported here suggested that the compound isolated by English *et al.* (5) may have been formed from the oxidation of *trans*-10-ODA during their isolation procedure because no precautions were taken to keep the extract in an inert atmosphere during the isolation of traumatin. At one point in the procedure the extract was dissolved in base, which could have resulted in the

loss of any free *trans*-10-ODA that had not been oxidized to traumatic acid. English and Bonner (3) stated that the substance they isolated originally appeared to be monobasic, but the final product was dibasic. They also suggested that loss of hormone activity during the purification may have been due to alteration of the hormone to a closely related inactive substance. For these reasons, *trans*-10-ODA appears to be a compound with wound hormone properties and traumatic acid is produced by autooxidation of *trans*-10-ODA. Incubations with cofactors showed that *trans*-10-ODA was not enzymically converted to traumatic acid and supported the conclusion that traumatic acid arises solely from autooxidation of *trans*-10-ODA.

In addition to *trans*-10-ODA, hydroperoxide lyase produces hexanal from linoleic acid and hexenal from linolenic acid (24). Numerous studies have cited the production of these 6-carbon aldehydes from various fruit, leaf, and seed tissues (6, 10, 14). These volatile aldehydes and their corresponding alcohols are responsible for much of the odor and flavor properties associated with various food products. Although some of these aldehydes can arise by autooxidative processes, it seems probable that a majority of them are produced via endogenous hydroperoxide lyase activity which increases following wounding (cutting, slicing, macerating, or grinding) of the tissue. The enzyme appears to be essential in the production of flavor in tea leaves (9). The broad distribution of hexanal and hexenal in plant tissues suggests that hydroperoxide lyase and *trans*-10-ODA are also present in numerous plant tissues. The occurrence and formation of *trans*-10-ODA in other plant tissues along with the development of simplified methodology for the quantitation of oxo-acids are the subject of future publications.

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