Inhibition of Nomilin Accumulation in *Citrus limon* Fruit by Naphthaleneacetic Acid

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Radioactive tracer work demonstrated that 1-naphthaleneacetic acid (NAA) inhibits the accumulation of limonoids in detached stem and fruit tissues of mature lemon trees. When 3-cm-long stems with small lemon fruits attached were fed [^{14}C]acetate after having already been treated with 20 ppm NAA for 3 days, there was a 50–100% inhibition of nomilin accumulation in the steam and an 80–100% inhibition in the fruit when compared to the controls. These findings suggest that NAA may have potential as a bioregulator for reduction of bitter limonoids in citrus fruits.

Bitterness due to limonoids in a variety of citrus juices is a major problem of the worldwide citrus industry and has significant negative economic impact. Limonin is the major cause of limonoid bitterness and is widely distributed. Recently, nomilin was also shown to contribute to limonoid bitterness in grapefruit juices (Rouseff, 1982). The chemical structures of limonin and nomilin are given in Figure 1. One of our approaches to this bitterness problems is to develop a preharvest treatment method to reduce bitter limonoids in citrus fruits. Advances in the field of citrus limonoid biochemistry in the last 3 years (Hasegawa et al., 1984a, 1986a; Hasegawa and Herman, 1985, 1986; Herman and Hasegawa, 1985) have shown biosynthetic pathways of the major limonoids and have pointed to the importance of nomilin as being an early precursor of the pathway and the major limonoid accumulated in young citrus seedlings. Furthermore, recent findings in our laboratory have shown that nomilin is biosynthesized in the stem and, from there, translocated to other tissues, including fruit tissues, where further biosynthesis occurs (Hasegawa et al., 1986b). A significant step toward the goal of a preharvest treatment method was our finding that auxins are potent inhibitors of nomilin biosynthesis in young lemon seedlings (Hasegawa et al., 1986c).

In this study we report that 1-naphthaleneacetic acid (NAA), a synthetic auxin, is also a potent inhibitor of nomilin biosynthesis in the stems of mature lemon trees and that the NAA treatment reduces the accumulation of nomilin in the fruit.

MATERIALS AND METHODS

Materials. Fruit-bearing *Citrus limon* trees used in this study were grown at the Pasadena laboratory. Sodium [1-^{14}C]acetate (54 mCi/mmol) was purchased from Dupont New Products, Billerica, MA. Silica gel HLF plates (250-μm thickness) were purchased from Analtech, Newark, DE.

Feeding of Chemicals. A 20 ppm aqueous solution of NAA (pH 6) was fed to a stem on a mature tree about 2 cm away from the base of a small terminal fruit through a set string by a procedure described previously (Hasegawa et al., 1984a). Approximately 2.0 mL of the feeding solution were taken up during 3 days. After 3 days of NAA feeding, small fruits attached to a piece of stem were detached from the tree. Fruits ranged in size from 5 to 9 mm in diameter and 10–17 mm along the longitudinal axis. For each experiment, fruits for the control and treated were carefully taken from the same branch. Detached stems were cut diagonally at 3 cm below the base of the fruit, fed labeled acetate (10–30 μCi) through the cut area, and incubated for 3 days at 22 °C under dark or light (330 footcandles).

Extraction and Analysis. Tissues fed with radioactive material were extracted by procedures designed to extract mainly lactones (Hasegawa et al., 1984a). The extracts were spotted on silica gel plates that were developed routinely with the solvent system EtOAc–cyclohexane (3:2). TLC radiochromatograms were scanned with a Berthold automatic TLC linear analyzer, LB 2832. Total radioactivites of extracts were counted with a Beckman liquid scintillation system, LS-3133P.

Identification of Labeled Metabolites. Nomilin was identified by procedures described previously (Hasegawa et al., 1984a), and other limonoids such as limonin and obacunone were also identified by procedures described previously (Hasegawa and Herman, 1985; Herman and Hasegawa, 1985).

RESULTS AND DISCUSSION

1-Naphthaleneacetic acid (NAA) was used in this study because we had previously shown that this synthetic hormone is a potent inhibitor of limonoid biosynthesis in citrus seedlings (Hasegawa et al., 1986c). Moreover, NAA has been widely used commerically for a number of purposes for treatment of fruit trees such as apple, citrus, pear, and grape (Jolliffe and Coggins, 1970; Looney, 1983). The concentration of NAA, 20 ppm, was chosen on the basis of our previous study (Hasegawa et al., 1986c). We have shown that detached stems are capable of biosynthesizing limonoids from acetate and that limonoids are translocated from stems to the fruit tissues (Hasegawa et al., 1986b). Therefore, small fruits attached to 3-cm stems were detached and used with [^{14}C]acetate in this study.

Figure 2 shows curves of TLC radiochromatograms of the extracts obtained from the fruits treated with or without 20 ppm of NAA. Stem extracts showed similar results on TLC radiochromatograms. The major peak shown in the control was identified as nomilin by the procedures described previously (Hasegawa et al., 1984a). In every case, nomilin was the largest peak in the control under the conditions used. NAA reduced the size of the nomilin peak significantly in both the stem and fruit, showing that NAA inhibits biosynthesis of nomilin in the steam and thereby indirectly reduces the accumulation of nomilin in the fruit. Also, as shown previously of auxins on lemon seedlings (Hasegawa et al., 1986c), NAA appeared to have little effect on nonlimonoid radioactive peaks, suggesting that its inhibitory effect is specific for

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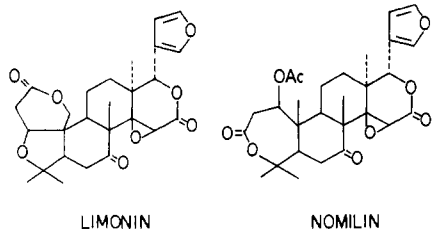

Figure 1. Chemical structures of limonin and nomilin.

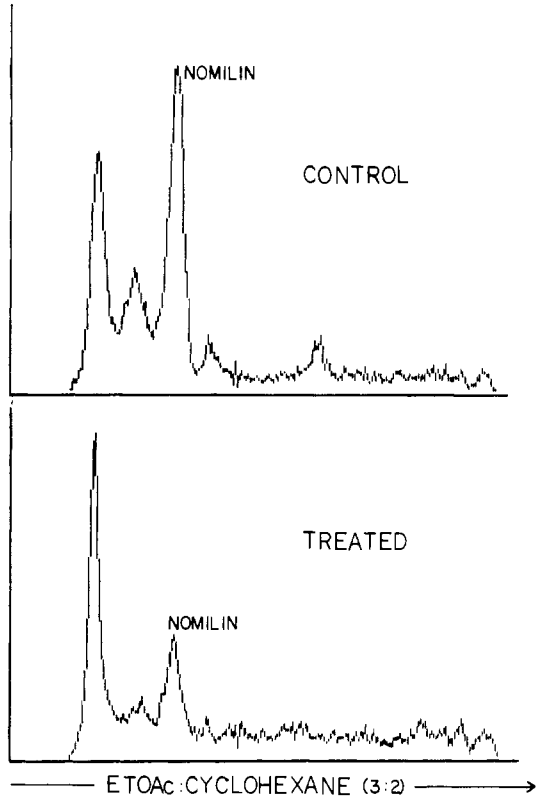

Figure 2. Radiochromatograms of the fruit extracts obtained from detached stem–fruit systems treated with or without 20 ppm NAA (experiment 4).

nomilin among those compounds extracted.

Table I shows the inhibitory effect of 20 ppm NAA on the total amounts of labeled limonoids produced by the stem–fruit system. These results clearly show that NAA inhibits the accumulation of nomilin in the fruit tissues. As shown previously (Hasegawa et al, 1986b,c), this hormone inhibited the biosynthesis of nomilin in the stems and subsequently less nomilin migrated to the fruit tissues.

The amount of incorporation of labeled acetate into nomilin in mature stems was lower than that for young seedlings. The amount of incorporation of the label into nomilin from [^{14}C]acetate in young seedlings was about 3–5% (Hasegawa et al., 1984b). In contrast, the incorporation of ^{14}C label into nomilin using stems and fruits of mature trees amounted to only about 0.06–0.88% in those experiments reported (Table I). A few experiments, where the controls showed less than 0.05% incorporation of ^{14}C label into nomilin, were not used because nonlimonoid radioactive peaks on the radiochromatograms accounted for nearly all of the label, thus making the limonoid peaks very difficult to resolve for routine analysis.

Unlike the seedling experiments (Hasegawa et al., 1984a), there was considerable variation in the extent of incorporation of acetate into nomilin by detached mature stems. However, the clear trend is that NAA inhibits the biosynthesis of nomilin in stems and inhibits the accumulation of nomilin in the fruit tissues. Since nomilin is

Table I. NAA Inhibition of Nomilin Accumulation in Detached Stems and Fruits of *C. limon*

		stem		fruit	
expt	treatment	incorp, cpm	inhibn, %	incorp, cpm	inhibn, %
1	control	61 000		3160	
	treated	0	100	0	100
2	control	76 700		787	
	treated	0	100	0	100
3	control	48 210		710	
	treated	20 277	58	260	64
4	control	121 760		8370	
	treated	57 060	53	1260	85
5	control	30 590		3120	
	treated	0	100	0	100
6	control	62 500		1870	
	treated	0	100	0	100
7	control	25 070		9780	
	treated	0	100	136	99
8	control	54 790		260	
	treated	0	100	0	100
9	control	48 280		3410	
	treated	0	100	0	100
10	control	450 640		81200	
	treated	13 280	97	140	100
11	control	70 250		25 780	
	treated	12 720	82	5560	78
12	control	86 900		16500	
	treated	10 200	88	3300	80

an early precursor in limonoid biosynthesis (Hasegawa and Herman, 1986), the inhibition of nomilin accumulation most likely inhibits the biosynthesis of other limonoids, particularly limonin, the major cause of citrus juice limonoid bitterness. In fact, the conversion of nomilin to other limonoids such as limonin and obacunone in the fruit tissues has been demonstrated (Herman and Hasegawa, 1985).

The results obtained in this and previous studies (Hasegawa et al., 1986c) suggest that NAA may ultimately have use as a bioregulator for reduction of bitter limonoids in citrus fruits. Further studies toward that end are planned.

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Registry No. NAA, 86-87-3; nomilin, 1063-77-0.

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