Sesquiterpene Lactones in Virus-Resistant Lettuce

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Lactuca saligna and *Lactuca virosa* are two wild lettuce species that show multiple virus resistance, and both have been successfully used in breeding programs for the introduction of virus resistance into commercial lettuce, *Lactuca sativa*. These two wild *Lactuca* species contained three major sesquiterpene lactones, i.e. lactucin, 8-deoxylactucin, and lactucopicrin, as measured by gradient RP-HPLC. *L. saligna* contained 103, 372, and 79 μg/g, respectively, and *L. virosa* contained 257, 173, and 1733 μg/g, respectively, of these three lactones that are present in both free and glycoside bound forms. The commercial lettuce varieties Montello (*L. sativa*) and Saladcrisp, derived from *L. sativa* × *L. saligna*, had very low levels of lactucin and lactucopicrin and were devoid of 8-deoxylactucin. Five breeding lines of lettuce, bred from the same interspecies cross as var. Saladcrisp, were also devoid of 8-deoxylactucin and contained between 0 and 60% as much lactucin or lactucopicrin as their parent strains. One lettuce breeding line, 93-426, contained only 18 μg/g of lactucin and was devoid of both lactucopicrin and 8-deoxylactucin, but this line is resistant to three viruses. This suggests that the level and types of these sesquiterpenene lactones present in lettuce have little effect on virus resistance. Nevertheless, lettuce breeders using wild *Lactuca* as resistant parents should be cognizant of their high levels of sesquiterpene lactones.

Keywords: *Lettuce; Lactuca sativa; Lactuca saligna; sesquiterpene lactones; virus resistance; lactucin; lactocpicrin; 8-deoxylactucin*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) (Compositae) is a vegetable that has an increasing yearly demand probably due to its use as a healthy, low caloric, salad component part of meals. Commercial production in the United States is in excess of 205 000 tons annually, but there is also a great deal of lettuce produced in home gardens. Lettuce originated in the Mediterranean region and has been cultivated since at least 4500 B.C. (Lindqvist, 1960). It was introduced to the New World by Columbus in 1494 but as a nonheading type that quickly formed a seedstalk and apparently did not succeed in establishing lettuce as a permanent food crop. Head lettuce was first reported in 1543 (Helm, 1954). Crisphead (iceberg) varieties predominate in U.S. markets, especially for long distance shipping, but romaine (cos), butterhead, and leaf types are also produced, and various types of these varieties, e.g. redleaf, are becoming quite popular.

The objective of numerous lettuce breeding programs worldwide is multiple virus resistance. Pesticides are ineffective against viruses. There are at least 11 separate viral diseases of lettuce for which genetic sources of resistance or tolerance have been found (Robinson and Provvidenti, 1993). Wild *Lactuca* species are being used in lettuce breeding programs as sources of resistance to viruses and other diseases.

Sesquiterpene lactones are C_{15} terpenoids, known as "bitter principles", with over 500 types present in Compositae plants. These terpenoids have shown antibiotic, cytotoxic, and allergenic properties (Burnett et al., 1978). The sesquiterpenoid lactone lactucin is the main bitter principle of the latex of the wild lettuce *Lactuca virosa* (Barton and Narayanan, 1958). The objective of this study was to analyze two viral-resistant, wild *Lactuca* species (*L. saligna* and *L. virosa*) and some commercial and new lettuce varieties and breeding lines, bred from these wild species (Robinson and Provvidenti, 1993), for their major sesquiterpene lactones.

MATERIALS AND METHODS

Lettuce. *L. saligna* PI 261653 from Portugal is a wild species that is resistant to at least three viruses of lettuce, as well as to a nematode, several fungi, a bacterium, and the cabbage looper insect. The crisphead variety Saladcrisp, the first lettuce variety from a cross with this species (*L. sativa* × *L. saligna*), is resistant to cucumber mosaic virus (CMV) (Provvidenti, et al., 1980). Another wild lettuce species, *Lactuca virosa,* is also resistant to at least two viruses (Bos and Huijberts, 1989; Provvidenti et al, 1984), *Sclerotinia* fungal disease (Abawi et al., 1980), and the leaf aphid (Eenink et al., 1982). Var. Vanguard was bred from a commercial letttuce, *L. sativa*, crossed with *L. virosa* (Thompson and Ryder, 1961). Resistance to lettuce mosaic virus (LMV) was obtained by numerous backcrosses to develop var. Vanguard 75. Montello has no wild *Lactuca* species in its pedigree and is tolerant to most viruses but shows resistance to the bacterial disease corky root rot (CRR). The lettuce breeding lines tested were 93-295, the F4 generation of var. Saladcrisp × Montello, and resistant to CMV and CRR; 93-400, the F7 generation of var. Montello × (Vanguard 75 × *L. saligna* PI 261653), resistant to CMV; 93-413, the F6 generation of var. Vanguard 75 × Saladcrisp, and resistant to CMV and LMV; 93-426 and 93-436, are lettuce lines from the same original cross with var. Vanguard 75 and *L. saligna* PI 261653 but are derived from different F3 plants. Line 426 is resistant to CMV, LMV, and broad bean wilt virus (BBWV), while line 436 is resistant only to CRR.

Materials and Standards. Pure standards of lactucin and lactucopicrin were kindly provided by Professor Dr. K. H.

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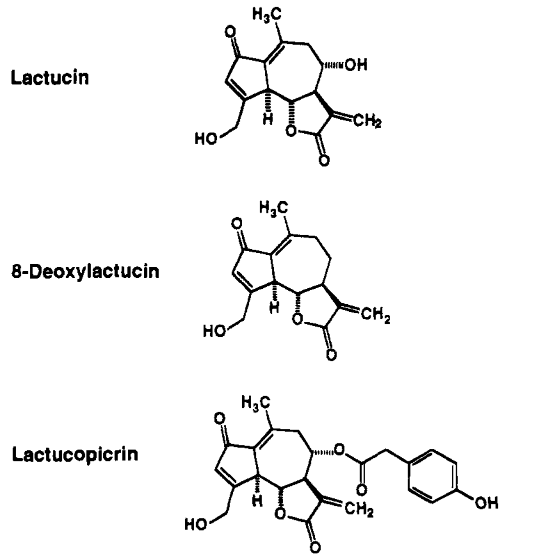

Figure 1. Structures of the sesquiterpene lactones in letttuce.

Gensch, Freie Universität Berlin, Germany. The sample of 8-deoxylactucin was a gift from Professor Dr. T. Miyase, University of Shizuoka, Japan. The enzyme cellulase (EC 3.2.1.4) and the internal standard, santonin, were purchased from Sigma (St. Louis, MO). Solid phase extraction cartridges were C_{18} Sep-Pak (Millipore Corp., Milford, MA). The TLC plates, PE SIL G/UV, 200 μm layer, UV fluorescence at 254 nm were purchased from Whatman Ltd. (Maidstone, Kent, England). All other chemical reagents and solvents were of analytical grade.

Sample Preparation. Fresh lettuce was frozen (−40 °C), lypholized, powdered, and stored at −20 °C subsequent to chemical analysis. A 500 mg dried, powdered lettuce sample was used in preparation for determining the sesquiterpene lactones according to the schematic diagram of Figure 2. All fractions (Fr-1, Fr-2, Fr-3, Fr-4, and Fr-5) were qualitatively observed by TLC using 100% ethyl acetate under UV light. The sesquiterpene lactones were present in Fr-3 and Fr-4. These fractions, dissolved in 1 mL of either 10% or 80% methanol (Figure 2), were each divided into two portions. One portion of each fraction was evaporated to dryness and redissolved in 1 mL of the cellulase enzyme solution (10 mg of cellulase/mL of water) that will hydrolyze glycoside-bound sesquiterpene lactones. This portion, containing both the free and previously glycoside-bound sesquiterpene lactones in the solution, was then incubated at 37 °C for 2 h with stirring. The solvent was evaporated and then made up to 500 μL. This was the hydrolyzed or total lactone portion, while the unhydrolyzed portions contained both the free and glycoside-bound sesquiterpene lactones. Both of these portions from Fr-3 and Fr-4 were used in quantification of the sesquiterpene lactones.

High-Pressure Liquid Chromatography (HPLC) Analysis. Sesquiterpene lactones were analyzed by gradient RP-HPLC. The mobile phase was water/acetonitrile (90:10), changing linearly over 20 min at a flow rate of 1.5 mL/min to 0:100. The peaks were identified by comparison of relative retention times (t_R lactones/t_R santonin, internal standard) of pure compounds. The pure compounds had been previously diluted in methanol with increasing concentrations. All three sesquiterpene lactones showed a correlation coefficient of higher than 0.99 of concentration versus peak areas.

The systems hardware consisted of a Ranin Rabbit HP pump, a 20 μL sample loop, and a 5 μL (22 cm × 4.6 mm i.d.) column (Brownlee Labs) at room temperature with a Gilson Holochrome UV−Vis variable-wavelength detector. The detection of the lactones was monitored at 262 nm. Data analysis was done by the Ranin Dynamax HPLC Method Manager which controlled both the operation of the HPLC system and the acquisition, analysis, and editing of the generated data.

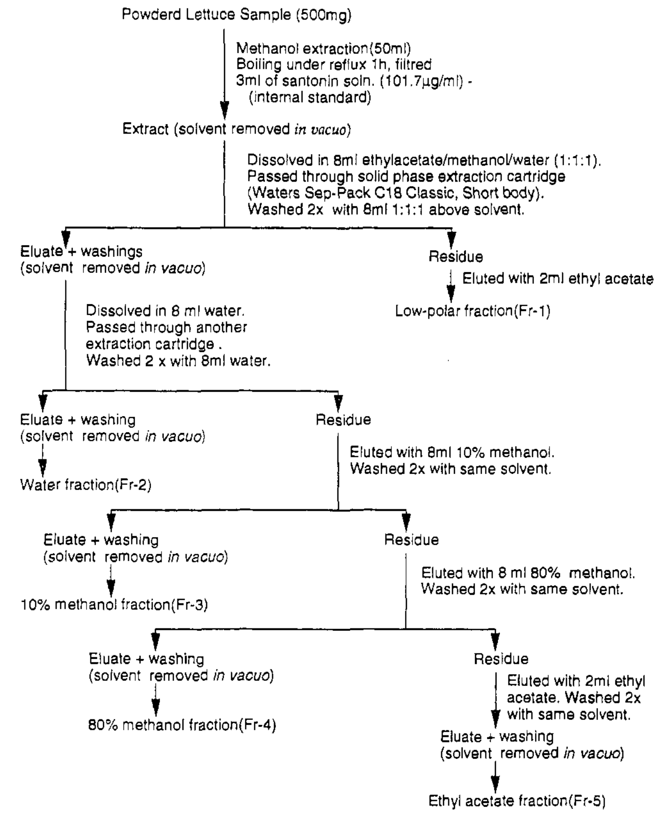

Figure 2. Diagram of lettuce sample preparations. Fr, fractions used for checking sesquiterpene lactones. These compounds were found in fractions 3 and 4 (Fr-3, Fr-4).

RESULTS AND DISCUSSION

The multiple disease and insect resistance of the two wild *Lactuca* species is of great importance for lettuce breeding, but could be cause for concern if any of the resistance factors are toxic or physiologically bioactive to the consumer. Lactucin was first discovered in the latex of *L. virosa*. The dried latex, known as "lactucarium" or lettuce opium, had been used throughout Europe as a hypnotic for centuries (Crosby, 1963). After more than a century of repeated investigation, the structure of lactucin was reported by Barton and Narayanan (1958) as shown, with the two other major sesquiterpene lactones in lettuce, in Figure 1. Figure 3 shows the HPLC chromatograms of the sesquiterpene lactone standards. The three standards were separated on the reversed-phase column in the order of lactucin, 8-deoxylactucin, lactucopicrin, and the internal standard, santonin.

All *Lactuca* taxa had sesquiterpene lactones present in Fr-4 (Figure 2). There was some bound 8-deoxylactucin only in Fr-3 of *L. saligna*. Indeed, as seen in Table 1, this species contained the highest level of 8-deoxylactucin with over 40% glycoside-bound. The other wild lettuce species, *L. virosa*, contained 8-deoxylactucin but only at about 47% of the level seen in *L. saligna*. Lactucin and lactucopicrin were over 2- and 20-fold higher, respectively, in *L. virosa* than in *L. saligna*. The commercial lettuce varieties Montello and Saladcrisp and all of the numbered lettuce breeding lines were very much lower in lactucin and latucopicrin and devoid of 8-deoxylactucin. In this study only the total lactucin level is reported (Table 1); problems with extraction and drying (Schenck, 1966) perhaps resulted in difficulty with separation of the free and glycoside-bound portions. Price et al. (1990) observed that most of the sesquiterpenes in lettuce were in the glycoside-bound form and

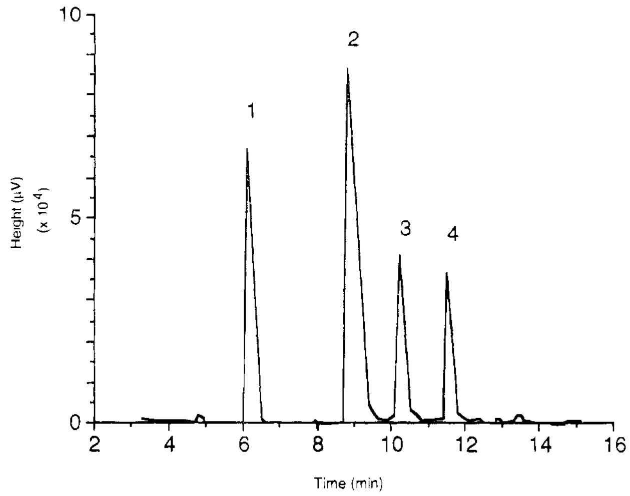

Figure 3. Chromatograms of the sesquiterpene lactone standards: 1, lactucin; 2, 8-deoxylactucin; 3, lactucopicrin; 4, internal standard, santonin.

Table 1. Mean Levels (±SEM) of Sesquiterpene Lactones[a] in Various Lettuce Cultivars and Species

species/ cultivar	lactucin	8-deoxylactucin	lactucopicrin
L. saligna	103.1 ± 5.4	372.1 ± 1.1 (43.8)[b]	79.0 ± 2.1 (27.1)
L. virosa	256.9 ± 8.1	173.5 ± 1.2 (33.5)	1732.7 ± 20.9 (37.7)
Montello	4.9 ± 0.1	0	10.1 ± 0.6 (14.2)
Saladcrisp	5.9 ± 0.4	0	6.6 ± 0.2 (52.4)
93-436	7.3 ± 0.3	0	4.5 ± 0.2 (23.3)
93-426	17.7 ± 1.4	0	0
93-413	11.2 ± 1.5	0	21.3 ± 0.8 (26.3)
93-400	0	0	7.7 ± 1.0 (55.8)
93-295	30.4 ± 2.3	0	47.0 ± 2.8 (19.5)

[a] µg/g dry wt. [b] (% of bound lactone).

that bitterness was related to lactucin glycoside in both lettuce and chicory (*Cichorium intybus*). However, in their studies the lettuce or chicory extracts were partitioned between water and chloroform with free sesquiterpene lactones assumed to be present only in the chloroform layer. Free sesquiterpenes also may have been present in the water layer.

Both wild species of *Lactuca* exhibit disease and insect resistance. The relatively high levels of sesquiterpene lactones were thought to account for these multiple disease and insect resistance traits. Other kinds of sesquiterpenes are known to be involved with insect resistance (Burnett et al., 1978). Specifically for viruses, *L. saligna* is resistant to CMV, prevalent in New York (Provvidenti et al., 1980), and *L. virosa* is resistant to BBWV, which also occurs in New York lettuce fields (Provvidenti et al., 1984). Var. Saladcrisp is resistant to CMV pathotype L-1 but tolerant to strain L-2. The accession of *L. saligna* tested is resistant to pathotype L-1, but some accessions are also tolerant to L-2 (Robinson and Provvidenti, 1993). It is of interest to note that the lettuce breeding line 93-426 containing about 18 µg/g of lactucin, but devoid of both 8-deoxylactucin and lactucopicrin (Table 1), is resistant to three viruses, CMV, LMV, and BBWV. Line 93-295, as compared to the other lines, contained the largest levels of both lactucin and lactucopicrin and is resistant to both CMV and the bacterial disease CRR. Thus, these data suggests that CMV resistance, and possibly resistance to other viruses, is not dependent on the relatively high levels of sesquiterpene lactones.

In conclusion, this study shows quite high levels of three major sesquiterpene lactones in two multiple virus resistant wild species of *Lactuca* that have been used in lettuce breeding programs. Lettuce lines that have *L. saligna* in their background contained much smaller amounts of lactucin and lactucopicrin than their parent stock and are devoid of 8-deoxylactucin. The high sesquiterpene lactone content of *L. saligna* and *L. virosa*, whether or not associated with viral resistance, should still be of concern to lettuce breeders and consumers.

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LITERATURE CITED

Abawi, G. S.; Robinson, R. W.; Cobb, A. C.; Shail, J. W. Reaction of lettuce germplasm to artificial inoculation with *Sclerotina minor* under greenhouse conditions. *Plant Dis.* **1980**, *64*, 668–671.

Barton, D. H. R.; Narayanan, C. R. Sesquiterpenoids. Part X. The constituents of lactucin. *J. Chem. Soc.* **1958**, 963–971.

Bos, L.; Huijberts, N. Lettuce big vein in the Netherlands: screening for resistance. In *Recent Advances Vegetable Virus Research*, Abstract, International Society Horticultural Science, 6th Conference, **1989**; p 36.

Burnett, W. C.; Jones, S. B.; Mabry, T. J. The role of sesquiterpene lactones in plant-animal coevolution. In *Biochemical Aspects of Plant & Animal Coevolution*; Harborne, J. B., Ed.; Academic Press: London, 1978; pp 233–257.

Crosby, D. G. The organic constituents of food. I. Lettuce. *J. Food Sci.* **1963**, *28*, 347–355.

Eenink, A. H.; Groenwold, R; Dieleman, F. L. Resistance of lettuce (*Lactuca*) to the leaf aphid *Nasonovia ribis nigri*. *Euphytica* **1982**, *31*, 569–570.

Helm, J. *Lactuca sativa* in morphologisch-systematischer Sicht. *Kulturpflanze* **1954**, **2**, 72–129.

Lindqvist, K. On the origin of cultivated lettuce. *Hereditas* **1960**, *46*, 319–350.

Price, K. R.; Dupont, M. S.; Shepherd, R.; Chan, H. W-S.; Fenwick, G. R. Relationship between the chemical and sensory properties of exotic salad crops—coloured lettuce (Lactuca sativa) and Chicory (Cichorium intybus). *J. Sci. Food Agric.* **1990**, *53*, 185–192.

Provvidenti, R; Robinson, R. W.; Shail, J. W. A source of resistance to a strain of cucumber mosiac virus in Lactuca saligna L. *HortScience* **1980**, 15, 528–529.

Provvidenti, R.; Robinson, R. W.; Shail, J .W. Incidence of broad bean wilt virus in lettuce in New York State and sources of resistance. *HortScience* **1984**, *19*, 569–570.

Robinson, R. W.; Provvidenti, R. Breeding lettuce for viral resistance. In *Resistance to Viral Disease of Vegetables: Genetics & Breeding*; Kyle, M.,Wallace, D., Eds.; Timber Press: Portland, OR, 1993; pp 61–79.

Schenck, G. Pharmacology of nitrogen-free bitter principles of Lactuca virosa: destruction of these substances during extraction and drying. *CNS Drugs Symposium* **1966**, 112–117.

Thompson, R. C.; Ryder, E. C. Description and pedigrees of nine varieties of lettuce. *USDA Tech. Bull.* **1961**, *No. 1244* 19 pp.

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