reach significant levels in treated cotton plants and in plants (i.e., native grass) grown immediately after post-harvest cultivation in treated area. If such residues are subsequently judged to be potentially harmful, then some limitation on posttreatment use of treated fields may be advisable.

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Capillary Gas Chromatograms of Leaf Volatiles. A Possible Aid to Breeders for Pest and Disease Resistance

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The chemical pest and disease defenses in plants must have been selected among spontaneously emitted substances and substances released or produced after the plant is wounded. Leaf volatiles are the first defense wall which meets an attacker of higher plants. Wound-emitted leaf volatiles from seven tomato cultivars and two wild tomato species were isolated and concentrated by adsorption on Tenax GC. A capillary gas chromatograph, adapted to give reproducible retention time values, has been used to separate the emitted volatiles. Two different data programs were used to graphically present a comparison of the chromatograms. As expected from the breeding history of the tomato, the recorded chemical characters were found to be very homogenous in varieties of *Lycopersicon esculentum*, but *Lycopersicon peruvianum* and *Lycopersicon hirsutum* showed very different component patterns. Resistance breeders work blindly without knowing the biochemical basis of resistance. Methods are needed to screen the chemical diversity in plants as guidance for the broadening of the genetic base in new crop varieties by the incorporation of pest resistance factors. Such chemical emission patterns could be used in breeding programs without identification of the different components.

It is safe to predict that resistant varieties will play an increasingly important part in the control of crop pests and diseases. The gene pool contains sources of resistance to all major groups of plant attackers, even birds and parasitic weeds (Russel, 1978). Most resistant varieties are produced with empirical methods without knowledge of the nature of the resistance or the genetic background. It seems clear that the causes of resistance are dominated by biochemical characters. Without understanding of the basis of resistance, the breeders work blindly. This is a serious problem from several points of view. They can only indirectly select for chemical characters of resistance; i.e., they have to wait for the results of spontaneous and induced attacks. It is especially difficult to breed for a quantitative polygenetic resistance without knowing the causes of the resistance. For the same reason, a valuable and durable horizontal resistance can be masked and lost during the selection for a monogenetically inherited total resistance. Demands have been raised that plant breeders should specify the chemical changes in new varieties to avoid negative health consequences for man and animals from toxic or antinutritional resistance chemicals. Extreme suggestions to stop resistance breeding do not solve this problem as all breeding probably involves chemical changes in the plants. The conclusion is instead that plant breeders need methods to follow the chemical changes which are produced. Such methods can in the first step be designed as pattern comparisons without knowing the chemical identity of the various components. The goal is to screen the patterns of spontaneously emitted substances and substances released or produced after the plant is wounded. The chemical parasite defenses in the plants must have been selected among these substances. There is a need for short words for such recordings. In analogy with terms such as "antennogram" and "encephalogram", we have suggested the somewhat popular words "leakogram" and "woundogram" (Andersson et al., 1979). No technique is available to cover the whole chemical emission pattern of a plant. As a beginning, we have focused our interest on leaf volatiles which are the first defense wall which meets an attacker of higher plants. Capillary gas chromatography is a natural choice of tool to screen plant volatiles.

The pretreatment of the plant samples is very important, in order not to exclude interesting chemicals or not to introduce artefacts. A practical procedure has been designed to isolate and concentrate plant volatiles by adsorption (Andersson et al., 1979; Andersson et al., in press). This paper reports the results of an investigation of wound-emitted leaf volatiles from seven tomato cultivars

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Table I. Investigated Cultivars of Tomato

no.	cultivar	resistance
1	*L. peruvianum*	tobacco mosaic virus (TMV)
2	*L. hirsutum*	TMV
3	*L. peruvianum* × *L. esculentum*	TMV
4	*L. esculentum*, cv. Pendulina	
5	*L. esculentum*, cv. Ida, F_1 hybrid	TMV *Fusarium oxysporum* *Cladosporium fulvum* *Verticillium alboatrum*
6	*L. esculentum*, mother line to no. 5	TMV
7	*L. esculentum*, father line to no. 5	TMV *F. oxysporum* *C. fulvum* *V. alboatrum*
8	*L. esculentum*, cv. College Red (Australia)	*F. oxysporum*
9	*L. esculentum*, line WW 020	*Meloidogyne* sp. TMV *C. fulvum*
10	*L. esculentum*, line WW 012	*F. oxysporum* *C. fulvum* TMV

and two wild tomato species. We have chosen *Lycopersicon esculentum* as a pilot species as the tomato has an interesting cultivation history (Rick, 1978) and has also been a favorite subject for genetic studies resulting in unusually detailed chromosome maps. Wild *Lycopersicon* species all have the same chromosome number and can be hybridized with *L. esculentum*.

EXPERIMENTAL SECTION

Plant Material. Table I lists the tomato cultivars which were selected from the available plant material. Fresh shoots were taken from tomato plants in a green house. Five to ten grams of leaves was cut in ~1-cm broad stripes and immediately put in the adsorption apparatus. Spontaneously emitted volatiles were not recorded because of difficulties in handling the leaves without damaging the glandular hairs.

The Adsorption Apparatus. The adsorption technique and the adsorption apparatus have previously been described (Andersson et al., 1979). For the tomato investigation, 0.3 g of Tenax GC (35–60 mesh) was used as the adsorbent in each sample tube. One end of the glass sample tube (Figure 1) is drawn out to a capillary injection needle, and the opposite end is equipped with a glass cone (Quickfit 7/6) which enables a quick and clean connection to the carrier gas of the gas chromatograph. The adsorption time was 30 min. The sample tubes were cooled and transported to our laboratory where they were kept in a freezer until the analysis.

The Capillary Gas Chromatograph. A Carlo Erba 2900 capillary gas chromatograph (Italy) has been modified to give reproducible retention time values (Andersson et al., 1979). The injector is combined with a desorption oven and a U-shaped cold trap cooled with liquid nitrogen. The heating of the oven and the cold trap is controlled by individual temperature regulators. The chromatograph is equipped with a flame ionization detector. A SCOT column, i.d. 0.5 mm × 55 m, of OV 17 was used and was linearly temperature programmed from 40 to 230 °C at 4 °C/min.

The Integrator. Integration and calculation of the peak area for the different fractions in percent of total peak area are done with an integrator (Hewlett Packard 3385 A). For the purpose of comparison of leaf emission patterns, the integrator should have the capacity to give peak areas and relative retention times for at least 200 fractions.

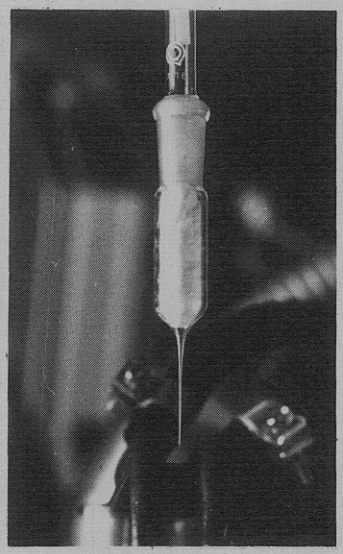

Figure 1. A glass sampling tube.

RESULTS AND DISCUSSION

The analyzed samples are all taken at the same occasion during a period of 120 min (total adsorption time). In order to be able to specify the leaf volatile characteristics of different varieties and species, it is of course also necessary to study the intraindividual and the intragroup variation. For this purpose, a more detailed screening of wound-emitted leaf volatiles from wild tomato species has already been initiated. With these reservations, we believe that the results of this introductory study are worth being published separately. Very little is known about the quantitative contents of allelochemics in different parts of a plant and their variation as a function of plant age and habitat (Feeny, 1976).

Figure 2 gives an example of a chromatogram (cultivar (no. 7) printed by the integrator. The results show that the capillary gas chromatograph must have a large dynamic range. The adsorbent must also have a large capacity to avoid saturation. The results are lists in Table II. The retention time values (t_R) are average values for the same fraction in all the 10 woundograms. By the described instrumentation, the variation in t_R values for a certain fraction can be held within 0.02 min (under 0.1%). It is difficult to use such tables of values for comparisons. In Figure 3 the results are plotted in a cluster diagram where replicate fractions from the 10 tomato cultivars have been grouped together.

Different ways to sum up the chromatographic pattern characteristics in a single comparison number for chemotaxonomic purposes have already been discussed (Andersson et al., 1979). In Figure 4 the tomato chromatograms are compared by using a similarity index developed by one of us (Holman, 1978). In this pseudo-three-dimensional plot it is visualized that the relationships of the wild tomato species (1 and 2) to the other cultivars are weak.

A number of the individual components in Table II have been identified by combined capillary gas chromatography/mass spectrometry. Some of these compounds have previously been identified in other tomato cultivars (Lundgren et al., 1978).

The following characteristics of the chromatographic patterns (Table II and Figure 3) can be noticed. β-Caryophyllene, t_{R_m} = 36.87 min, is the dominating component (51.64%) among the volatile effluents from *Lycopersicon*

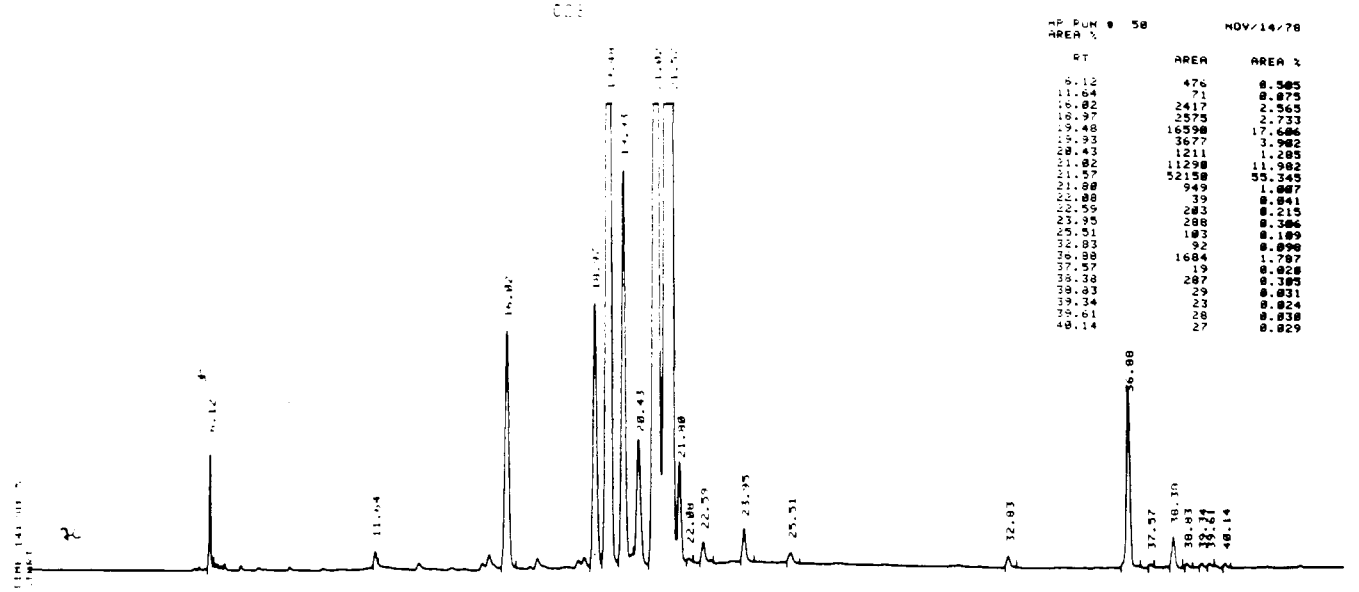

Figure 2. Example of a chromatogram. Cultivar no. 7.

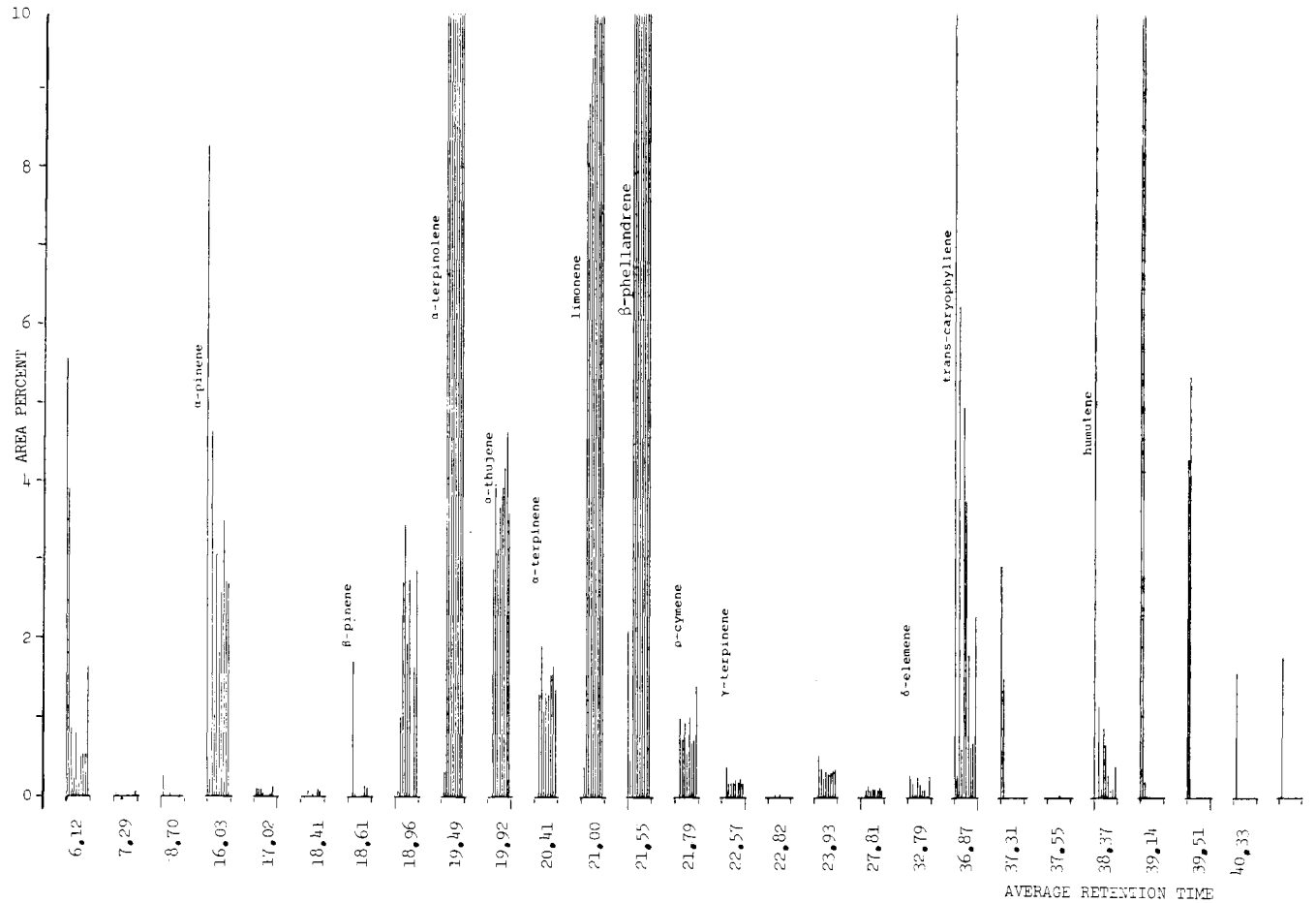

Figure 3. Relative amount of each main fraction in the capillary gas chromatograms plotted with a computer program originally designed for mass spectra.

peruvianum (no. 1). Other main fractions are humulene, t_{R_m} = 38.37 min (13.30%), α-pinene, t_{R_m} = 16.03 min (8.25%), and two unidentified sesquiterpenes, t_{R_m} = 39.14 min (11.80%) and t_{R_m} = 39.51 min (4.25%). *Lycopersicon hirsutum* produces a broader spectrum of volatiles in which β-caryophyllene is almost lacking but an unidentified sesquiterpene, t_{R_m} = 39.14 min (mass spectrum in Figure 5) is quantitatively dominating (82.95%), probably contributing to the strong peculiar odor of this species. β-Phellandrene is the dominating fraction, t_{R_m} = 21.55 min (~50%), in all cultivars of *L. esculentum* (no. 4–10) and also in the hybrid *L. peruvianum* × *L. esculentum* (no. 3) (46.24%).

The peculiar production of allelochemic volatiles in *L. hirsutum* is especially interesting because this species is resistant to more insect species than any other tested tomato species. Removal of the glandular exudate from *L. hirsutum* renders it more susceptible to attack by flea

Table II. Chromatogram Data

av retention time (t_{R_m}), min	area % of tomato cultivar no. 1	2	3	4	5	6	7	8	9	10	identified compd	M_r
6.12	5.56	3.90	0.86	0.28	0.80	0.20	0.51	0.54	0.54	1.63		44
7.29		0.05	0.01						0.01	0.07		
8.70		0.27			0.04				0.01			
16.03	8.25	0.22	4.62	1.34	3.06	1.82	2.57	3.48	2.71	2.68	α-pinene	136
17.02		0.14	0.10	0.10	0.05		0.01	0.03	0.12			
18.41				0.08		0.03		0.10	0.07			
18.61			1.70	0.07				0.14	0.12		β-pinene	136
18.96		0.08	1.03	2.78	3.43	1.93	2.73	0.05	1.63	2.84		134
19.49		0.34	22.81	21.61	19.42	18.68	17.61	22.12	19.19	18.53	α-terpinolene	136
19.92			2.86	3.95	3.12	3.67	3.90	4.14	4.61	3.57	α-thujene	136
20.41			1.30	1.90	1.09	1.31	1.29	1.53	1.64	1.35	α-terpinene	136
21.00		0.39	8.57	8.77	9.35	16.70	11.98	11.94	11.91	10.97	limonene	136
21.55	2.08	0.47	46.24	56.65	51.52	49.41	55.35	53.63	54.16	52.94	β-phellandrene	136
21.79			1.03	0.73	0.93	0.56	1.01	0.68	0.73	1.40	p-cymene	134
22.57			0.40	0.18	0.19	0.19	0.22	0.20	0.24	0.19	γ-terpinene	136
22.82				0.04		0.05						
23.93			0.53	0.37	0.24	0.34	0.31	0.32	0.34	0.36		
27.81			0.10	0.15	0.09	0.12	0.11	0.10	0.13	0.10		
32.79		0.28	0.19	0.04	0.26	0.16	0.10	0.10	0.03	0.27	Δ-elemene	204
36.87	51.64	0.31	6.18	0.64	4.91	3.73	1.79	0.65	0.69	2.26	β-caryophyllene	204
37.31	2.90	1.51										
37.55						0.04	0.02					
38.37	13.30		1.16	0.13	0.89	0.67	0.31	0.11	0.12	0.40	humulene	204
39.14	11.80	82.95	0.23	0.03	0.02							204
39.51	4.25	5.29										
40.33		1.56										

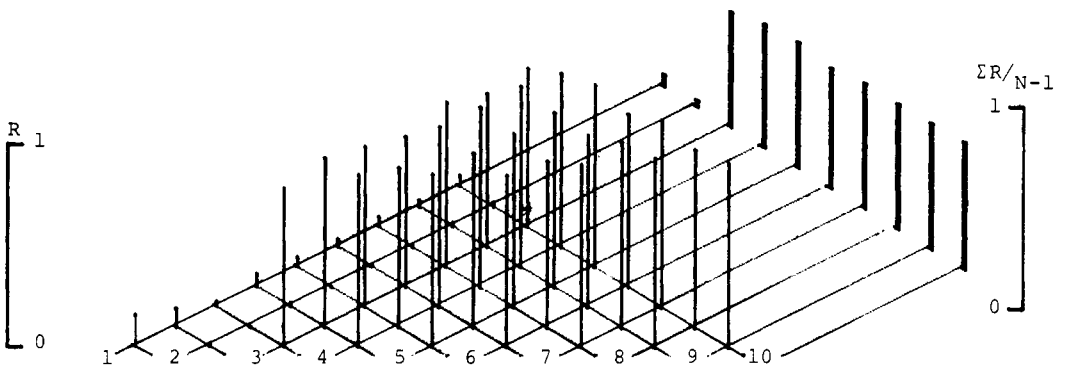

Figure 4. Relationship indices based on comparison of corresponding peaks are calculated for the tomato chromatograms (1–10) according to the formula developed by Holman (1978). R varies from 0 (no similarity) to 1 (total identity). Heavy bars to the right are average relationships to the group as a whole, $\Sigma R/n$.

beetles and by whiteflies (Rick, 1973). Rick (1978) states that the cultivated and wild forms of *L. esculentum* are remarkably homogenous outside of their native region in western South America as a result of genetic drift and natural selection for a limited number of successful genotypes. This homogeneity is confirmed by the recorded chemical characters in our woundograms of *L. esculentum* cultivars no. 3–10, shown in Figures 3 and 4. The results agree well with the prediction, for most of the tomato breeding has been done on morphological characters, visible physiological characters, and certain chemical components concerning fruit quality (Rick, 1975). The importance of the development of methods to recognize chemical characters can be exemplified by the detection of some enzymes in tomato by starch–gel electrophoresis which was followed by a rapid progress in finding the genes coding for the synthesis of these enzymes.

The genetic base of modern crop plants is rather narrow and is becoming even narrower in spite of warnings for increased vulnerability to pest and disease attacks. Chemical diversity is an important defense strategy in plants (Feeny, 1976). We need methods to screen the chemical diversity in order to be able to efficiently broaden

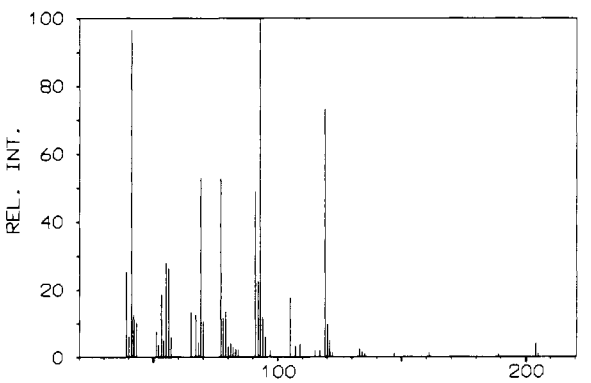

Figure 5. Mass spectrum of an unidentified sesquiterpene (t_{R_m} = 36.14 min) from *L. hirsutum*. Electron impact (EI), 70 eV ionizing energy, and 200 °C ion source temperature.

the genetic base by incorporating pest resistance characteristics into new crop varieties. This investigation is a methodical step on this way. Pattern recognition methods must be developed to correlate chemical characteristics with an observed resistance. Capillary gas chromatography and other separation methods can without laborious and

expensive identifications be used to screen resistance factors in native regions and gene banks. Associational resistance, i.e., a benefit from the chemical defense in other plants, is another part of the natural chemical defense of plants (Atsatt and O'Dowd, 1976). These methods could also be used to mix crops in the most favorable defense combinations.

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Pergillin: A Nontoxic Fungal Metabolite with Moderate Plant Growth Inhibiting Properties from *Aspergillus ustus*

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A new metabolite, $C_{15}H_{16}O_4$, was isolated from cultures of *Aspergillus ustus* found growing on seeds of *Pisum sativum* var. macrocarpon. It was nontoxic to day-old chicks but significantly inhibited wheat coleoptile growth at 10^{-3} and 10^{-4} M. The trivial name pergillin was given to the metabolite.

A further survey of fungi for the production of plant growth inhibiting substances revealed that cultures of *Aspergillus ustus*, found growing on the testa of pea seeds, *Pisum sativum* var. macrocarpon (Oregon Sugarpod), produced a metabolite that significantly inhibited the growth of wheat coleoptiles. The metabolite was nontoxic to day-old chicks.

Certain strains of *A. ustus* have yielded several metabolites toxic to vertebrates. Among those reported are austamide (Steyn, 1971), austidiol (Vleggaar et al., 1974), and the highly complex polyisoprenoid austin (Chexal et al., 1976).

We now report the isolation of a new metabolite, to which we have assigned the trivial name pergillin (I) (Figure 1). The effects induced by pergillin in plants are also described.

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MATERIALS AND METHODS

Production, Isolation, and Purification of Pergillin. *A. ustus* (Bainier) Thom & Church (ATCC accession no. 38849) was isolated from greenhouse-produced pea seeds *P. sativum* var. macrocarpon (cv. Oregon Sugarpod) grown in Georgia. The fungus was cultured on potato-dextrose-agar slants at 26 °C for 14 days and then maintained at 5 °C. Cultures were then transferred to Fernbach flasks (2.8 L), each containing 100 g of shredded wheat, 200 mL of Difco mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose (Kirksey and Cole, 1974) for production of the metabolite. Inoculated flasks were incubated in the laboratory for 12 days at about 26 °C. Then 300 mL of acetone was added to each flask. The substrate and mycelia were macerated with a Super Dispax homogenizer, and the resulting suspension was strained through cheesecloth to remove the pulp. The filtrate was filtered through Whatman No. 1 filter paper on a Buchner funnel, and the clarified filtrate was reduced under vacuum at 50 °C to an aqueous phase. The aqueous phase was extracted twice with 2 volumes of ethyl acetate, and each volume of solvent used was equal to the volume of the aqueous phase. Ethyl acetate extracts were combined, dried over anhydrous sodium sulfate, and reduced in volume under vacuum at 50 °C. This crude extract was placed on a silica gel (70-230 mesh) chromatography column (9.0 × 10 cm) that had been slurry packed in benzene. The extract was eluted stepwise by 1.0 L each of benzene, ethyl ether, ethyl acetate, acetone, and methanol. Each solvent drained to the top of the silica gel before addition of the next sequential