these ratios across the peak, the values are within the limits of experimental error. These results therefore strongly indicate that the isolated protein is homogeneous, consisting only of native visual pigment.

Autoradiographic analysis revealed a discrete reaction band which extended across the width of each outer segment of the rod near its base (Fig. 2). This indicated that protein formed during the period immediately after injection was now concentrated in a disc-shaped constituent of the outer segments, presumably in membranous discs which had been displaced from the base of the outer segment by newer discs formed in the 7 days after the injection.

Thus we have isolated purified, radioactively labeled visual pigment from retinal rod outer segments after injection of labeled amino acids in adult frogs. Since visual pigment is considered an integral part of the outer segment disc membrane, this finding supports the hypothesis (*1*) that the outer segment of the rod is renewed by continual synthesis of new discs.

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Vernolepin: A New, Reversible Plant Growth Inhibitor

Abstract. *Vernolepin (5 to 50 micrograms per milliliter), a novel sesquiterpenoid dilactone obtained from* Vernonia hymenolepis, *inhibits extension growth (from 20 to 80 percent) of wheat coleoptile sections. Inhibited tissues appear normal and their respiration is unaffected. If the inhibited sections are washed and subsequently treated with indole-3-acetic acid, the tissues respond to the auxin, but the degree of elongation is determined by the length of prior treatment with vernolepin. Administered simultaneously, increasing concentrations of auxin will significantly reduce the inhibitory effect of vernolepin, but there is no evidence for a competitive interaction between the two substances.*

A number of sesquiterpenoid plant growth inhibitors have been isolated from higher plants (*1*). The recent discovery of the widespread occurrence of (+)-abscisic acid in plants (*2*) and the strong inhibitory effects of this substance at very low concentrations suggest that it may have a regulatory function. Another sesquiterpene, heliangine (*3, 4*), has been found to inhibit extension growth of *Avena* coleoptile sections.

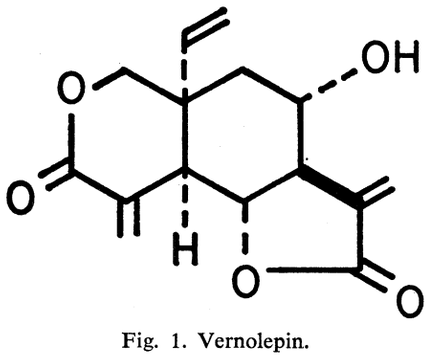

Fig. 1. Vernolepin.

When applied in combination with indole-3-acetic acid (IAA), however, activity curves ran parallel over a wide range of concentrations, which suggests a constant, independent physiological action of this inhibitor. Similar results have been reported for the so-called inhibitor-β from sycamore (*5*), now thought to consist mainly of abscisic acid (*2*).

During an evaluation of the effects on plant growth of several tumor-inhibitory compounds derived from plants, it was noted that several sesquiterpenoid dilactones, specifically elephantin (*6*), elephantopin (*6*), and vernolepin (*7*), were strong inhibitors of extension growth of wheat coleoptile sections. The biological activity of these compounds was determined by means of the straight-growth technique described by Sequeira and Kelman (*8*). Briefly, the technique consists of exposing 4-mm portions of wheat (var. Atlas 66) coleoptiles, removed 2 mm below the apex and soaked in $MnSO_4$ solution (1 μg/ml) for 2 to 3 hours, to various concentrations of the inhibitors. The inhibitors were dissolved in 2 percent sucrose and dispensed in vials, each containing three coleoptile sections. For controls, coleoptile sections were placed in vials containing 2 percent sucrose only. The vials were placed horizontally on a wheel rotating at 1 rev/min, and, after incubation in the dark (20 hours), the sections were removed and measured under a dissecting microscope. Inhibition or promotion of growth of treated coleoptiles was calculated as percentage of control growth.

Of the sesquiterpenoids tested, vernolepin (Fig. 1), isolated from *Vernonia hymenolepis* A. Rich. (*7*), was the most effective inhibitor. Extension growth was significantly inhibited by vernolepin at 5.0 μg/ml ($1.8 \times 10^{-5}M$) (Fig. 2). Above 50 μg/ml ($1.8 \times 10^{-4}M$), vernolepin almost completely inhibited extension growth.

Tests were then made to determine whether coleoptile sections that had been inhibited approximately 50 percent by vernolepin (25 μg/ml) could resume active growth after they had simply been washed with distilled water and transferred to solutions containing IAA at different concentrations. The degree of reversibility of inhibition would indicate whether the substance was directly toxic to the cells or had growth-regulatory properties. For this purpose, 4-mm wheat coleoptile sections were exposed to vernolepin at 25 μg/ml ($9.0 \times 10^{-5}M$) for 4, 12, and 18 hours. After thorough washing with distilled water, the sections were incubated with IAA for 14, 12, and 18 hours, respectively. The concentrations

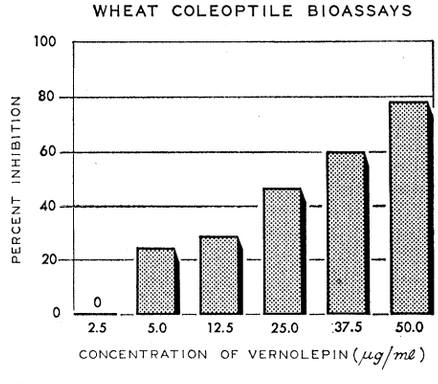

Fig. 2. Wheat coleoptile bioassays. Effect of various concentrations of vernolepin on growth of wheat coleoptile sections after 20-hour incubation in the dark. Methods as described in the text.

Table 1. Effect of simultaneous application of IAA and vernolepin on growth promotion (+) or growth inhibition (−) of wheat coleoptile sections.

Concentration of vernolepin (μg/ml)	Growth (percentage of control) with IAA (μg/ml):				
	0.00	0.03	0.05	0.25	0.50
0.0	0.0	+ 12.5	+ 59.3	+ 88.8	+ 98.4
12.5	− 29.1	− 15.2	− 0.2	+ 20.1	+ 50.7
25.0	− 53.0	− 36.6	− 21.7	− 5.2	− 10.4
37.5	− 66.1	− 56.7	− 45.2	− 42.6	− 36.0

of IAA were 0.05, 0.5, and 5.0 μg/ml for each of the three incubation periods. The degree of inhibition was the same, whether the coleoptiles were treated with vernolepin for 4, 12, or 18 hours, indicating that the effect of the inhibitor is very rapid. In the absence of IAA, no recovery of the inhibited coleoptiles occurred. Since endogenous auxin was probably exhausted in coleoptiles during the presoaking and treatment periods (7 to 21 hours), growth could not resume once the inhibitor was removed by washing. In the presence of exogenous IAA, the extent to which growth was resumed depended to a considerable extent on the length of time the coleoptiles had been exposed to vernolepin (Fig. 3). The inhibitory effect of vernolepin was reversed at all concentrations of IAA used, and the reversal was proportional to the amount of IAA supplied in almost every instance. However, reversal to the same level of growth response as was obtained in the presence of IAA alone was not observed.

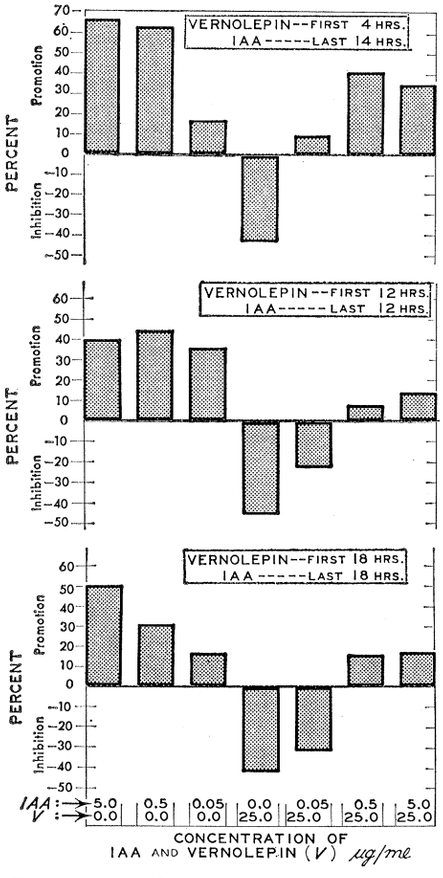

Fig. 3. Reduction of the inhibitory effect of vernolepin (25 μg/ml) on wheat coleoptile growth by various concentrations of IAA. Vernolepin was applied for 4, 12, and 18 hours and washed off; then IAA was applied for various periods as indicated.

Since vernolepin inhibited coleoptile elongation very rapidly, the reduction in reversibility apparent after the longer periods of exposure to vernolepin could be due to a low level of toxicity of this compound. Alternatively, the longer periods of exposure to vernolepin may speed up "aging" of coleoptile tissues, making them less responsive to IAA, or there may be increased nonspecific binding to cell walls which would make removal of the inhibitor by washing more difficult.

Reversibility of the inhibitory effect of vernolepin by IAA, as described above, suggested that the two substances might affect the same process in extension growth. To test this possibility, various amounts of vernolepin and IAA were administered simultaneously to wheat coleoptile sections, and, after a 20-hour incubation period, the length of the coleoptiles was compared with that of untreated controls. Vernolepin at 12.5, 25.0, and 37.5 μg/ml increasingly inhibited coleoptile growth, but in the presence of IAA at 0.03, 0.05, 0.25, and 0.50 μg/ml there was a significant reduction in the level of inhibition at each of the three concentrations of inhibitor, this effect being more pronounced at the highest concentration of IAA (Table 1). The curves obtained after simultaneous application of auxin and inhibitor were parallel over the range of concentrations used, indicating an independent effect of both substances. Similar results have been obtained with the inhibitor-β from sycamore (*5*) and with heliangine (*3*).

The lack of any immediate toxic effect of vernolepin was also demonstrated by comparing the respiratory rate of treated wheat coleoptile sections with that of untreated ones. At concentrations of vernolepin ranging from $1.14 \times 10^{-5}M$ to $1.14 \times 10^{-4}M$, the respiratory rate of treated coleoptile sections was identical to that of untreated sections, as measured with a Warburg respirometer. However, at the higher concentrations of vernolepin, there was considerable growth inhibition. Other natural growth inhibitors depress the respiration of plant tissues after an initial period of stimulation (*9*).

The ready response of wheat coleoptile tissues to IAA after vernolepin is washed off, and the lack of any pronounced effect of high concentrations of the inhibitor on the respiratory metabolism of similar tissues suggest that this substance affects an essential step in cell-wall synthesis or extensibility, and is not merely a toxic principle. Certain of the characteristics described here for vernolepin are similar to those described by Robinson and Wareing (*5*) for the inhibitor from sycamore, which is now thought to be abscisic acid. Vernolepin, however, does not have the abscision-promoting properties of abscisic acid. When the cotton petiole assay (*10*) was used, vernolepin did not accelerate abscision at concentrations ranging from 100 to 0.001 μg/ml.

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