ties to the effect of the meningeal cell layer on nerve cell differentiation which were described previously[15]. It was also observed that some of the isolated neurons can survive in this condition for a longer period of time in culture. Therefore, the meningeal CM seemed to contain active factors which influenced the maintenance of some neurons. However, the most pronounced stimulatory effect was observed with the glial CM both on the neurons in the mixed population and on those which were well isolated. Suggestions that there are exchanges between neurons and glial cells have been made for a long time. Our results show that substances released by glial cells had a favorable effect upon neuronal maturation and survival. A similar fact has been described by Monard et al.[10], who observed that CM obtained from tumor glial cells stimulated the differentiation of neuroblastoma cells in culture. Luduena[12] showed that spinal ganglionic neuron differentiation is stimulated equally by heart fibroblast and by glial cell conditioned media. Our observations indicate that neurons from the central nervous system are more dependent on the specific presence of glial cell conditioned medium in the culture.

Fig. 2. Isolated neurons from 7-day-old chick embryo brains in culture. Phase contrast micrographs. *a* Control culture after 5 days; *b* 5-day-old culture exposed to glial CM. × 190.

1 Acknowledgment. We acknowledge the expert technical assistance of Mrs M.F. Knoetgen.
2 N.C. Dulak and H.M. Temin, J. Cell Physiol. *81,* 153 (1973).
3 N.C. Dulak and H.M. Temin, J. Cell Physiol. *81,* 161 (1973).
4 S.W. Melbye and M.A. Karasek, Exp. Cell Res. *79,* 279 (1973).
5 H.P. Gordon and M.C. Brice, Exp. Cell Res. *85,* 303 (1974).
6 V.L.P. Schacter, Exp. Cell Res. *63,* 19 (1970).
7 M. Solursh and S. Meier, Devl Biol. *30,* 279 (1973).
8 K. Watanabe, Devs. Growth Differentiation *13,* 107 (1971).
9 N.K. White and S.D. Hauschka, Exp. Cell Res. *67,* 479 (1971).
10 D. Monard, E. Solomon, M. Rentsch and R. Gysin, Proc. natl Acad. Sci. *70,* 1894 (1973).
11 Y. Schurch-Rathgeb and D. Monard, Nature *273,* 308 (1978).
12 M.A. Luduena, Devl Biol. *33,* 268 (1973).
13 M. Sensenbrenner, N. Springer, J. Booher and P. Mandel, Neurobiology *2,* 49 (1972).
14 P. Athias, M. Sensenbrenner and P. Mandel, Differentiation *2,* 99 (1974).
15 M. Sensenbrenner and P. Mandel, Exp. Cell Res. *87,* 159 (1974).
16 M. Touzet and M. Sensenbrenner, Devl Neurosci. *1,* 159 (1978).
17 J. Booher and M. Sensenbrenner, Neurobiology *2,* 97 (1972).
18 M. Sensenbrenner, G.G. Jaros, G. Moonen and P. Mandel, Neurobiology *5,* 207 (1975).

Germination inhibition activity of a naturally occurring lignan from *Aegilops ovata* L. in green and infrared light

Y. Gutterman, M. Evenari, R. Cooper, E.C. Levy and D. Lavie

Institute for Desert Research, Sede Boqer, Department of Biology, Ben-Gurion University of the Negev, and Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot (Israel), 8 August 1979

Summary. The naturally occurring lignan, monoepoxylignanolide (MEL) from *Aegilops ovata* L., inhibits germination of lettuce achenes (seeds) in incandescent light but not in the dark. The 'action spectrum' after preincubation of MEL in darkness shows inhibition in the regions of 399 nm and 712–804 nm, but after pretreatment with incandescent light inhibition is seen at 500–577 nm and 712–804 nm. The infrared inhibition by MEL is not reversible by red light. The pretreatment of MEL with incandescent light gives rise to a photoproduct which, together with MEL itself, inhibits the germination of lettuce achenes in the dark.

We have previously reported[1,2] the occurrence of a naturally occurring lignan from *Aegilops ovata* L. which inhibits the germination of lettuce achenes in incandescent light but not in darkness. So far, the known naturally occurring germination inhibitors[3,4] have been found to be more effective in darkness than in light; this report was the first describing such a compound (a monoepoxylignanolide (MEL)) possessing a reverse activity. This novel phenomenon has now been investigated further. The activity of MEL was tested at various concentration levels and the effect of variations of wavelength of light on MEL during the inhibition of germination was examined. Irradiation of MEL using incandescent light shows that this lignan is photolabile and gives rise to a photoproduct which, when tested together with MEL, inhibits the germination of lettuce achenes in the dark, although neither compound alone shows such an effect.

The solubility of MEL in water was initially determined using UV calibration on standard solutions in ethanol. The maximum solubility was found to be 160 ppm. From this saturated stock solution of MEL in H_2O different dilutions were prepared, and tested by placing a filter paper with 50 lettuce achenes, *Lactuca sativa* cv. 'Great Lakes' in a Petri dish containing 1.5 ml of solution, and germination tests were carried out in darkness and under light. Although not effective in darkness, MEL inhibits germination in light even at concentrations of 20 ppm (figure 1).

In a separate set of experiments using materials and methods already described[1], filter papers were impregnated with 1 mg quantities of pure MEL and put into Petri dishes. Each of these papers was covered with a 2nd untreated filter paper, and distilled water (1.5 ml) was added. The dishes were then left for about 22 h for equilibration. These pretreatment conditions were necessary since MEL, as

stated above, is sparingly soluble in water. The Petri dishes were kept in a germinator at 26 °C either in darkness or under continuous incandescent light for various periods of time, and then 50 lettuce achenes, *Lactuca sativa* cv. 'Great Lakes', were placed on the upper filter paper. The sides and the bottom of every Petri dish were wrapped with aluminium foil for protection, and only the top was irradiated. The light was filtered through a number of glass filters (Balzers, Liechtenstein) known to transmit only definite wavelengths with an accuracy of $\lambda \pm 5$ nm, so that every test batch received appropriate light continuously for 24 h through the selected glass filter. The incandescent light source had an intensity of 2.2×10^4 erg cm^{-2} sec^{-1}. After every treatment the number of germinating seeds was counted and the 'action spectrum' recorded. This spectrum for the inhibitory effect of MEL after its pretreatment in darkness shows that in the region of 399 nm and 712–804 nm inhibition is observed, whereas from 448–702 nm there is no inhibiting effect. In the 712–804 nm range there is clearly a minimum germination at 725 nm (figure 2). This differs from the pretreatment of MEL with incandescent

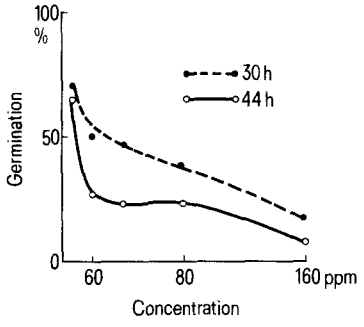

Fig. 1. The percent germination of 'Great Lakes' lettuce achenes at 26 °C under incandescent light after 30 and 44 h, using varying concentrations of MEL in H_2O.

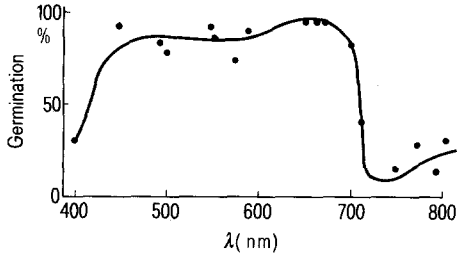

Fig. 2. The action spectrum of MEL (1 mg) after preconditioning for 48 h in darkness and subsequent germination under different light conditions over 24 h at 26 °C.

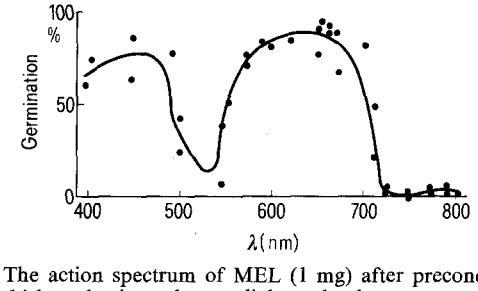

Fig. 3. The action spectrum of MEL (1 mg) after preconditioning for 12–14 h under incandescent light and subsequent germination under different light conditions over 24 h at 26 °C.

Table 1. Germination of *Lactuca sativa* 'Great Lakes' achenes at 26 °C after 24 and 48 h under illumination by far-red (735 nm), red (660 nm) and incandescent light, or darkness. The achenes were soaked on filter papers impregnated with 1 mg MEL

Light conditions	Germination (%)	
	H_2O	Monoepoxylignanolide
Incandescent light 24 h	93	0
Dark 24 h	96	94
Red 24 h	95	93
Far-red 24 h	0	0
Far-red 24 h → red 24 h	57	0

Table 2. Germination of *Lactuca sativa* cv. 'Great Lakes' achenes at 26 °C, after 24 h of inhibition under conditions of light and darkness, soaked on filter papers impregnated with 1 mg monoepoxylignanolide (MEL) and the photoproduct (0.5 mg)

Germination conditions	24 h
Dark + H_2O	81.0*
Dark + photoproduct 0.5 mg	78.5*
Dark + photoproduct + MEL 1 mg	16.5
Dark + H_2O	74.0
Dark + MEL 1 mg	61.5
Incandescent Light + H_2O	54.5
Incandescent Light + MEL 1 mg	5.5

* Pretreatment of filter paper for 45 min in darkness before addition of achenes. For all other tests, pre-treatment by soaking of filter papers for 22 h before addition of achenes.

light for 12–14 h, where the inhibitory effect is much larger between 749–804 nm (about 100%), and there is also an effect between 500–557 nm (figure 3). MEL inhibits also at the wave length of far-red (735 nm) but this inhibition is not reversible by red light (table 1).

These results prompted us to investigate whether MEL itself underwent any chemical change under similar light conditions. Irradiation of MEL in tert-BuOH with incandescent light through a pyrex tube for 14 h led to the formation of a different compound, as detected by TLC. Subsequent isolation of this photoproduct indicated a 10% conversion of the starting material. When a similar solution was kept in darkness, no observable changes could be detected. The spectroscopic data for the photoproduct, compared with those already established for MEL, indicate that the product is an isomer of MEL.

In experiments carried out by impregnating a filter paper with this photoproduct alone, no germination inhibition activity in darkness was observed. However, the addition to this impregnated filter paper of 1 paper containing MEL (1 mg), led to a pronounced inhibition under the same conditions of darkness (table 2).

The influence of various wavelengths of light on germination in the presence of MEL and its photoproduct shows that the activation spectrum of this mixture is definitely different from that of phytochrome, since there is inhibition between 500–557 nm and 749–804 nm. It appears to us that the specific activity of the photoproduct; the inhibition of germination in the dark in the presence of MEL, is a characteristic which should be further investigated.

1 D. Lavie, E.C. Levy, A. Cohen, M. Evenari and Y. Gutterman, Nature *249*, 388 (1974).
2 R. Cooper, E.C. Levy and D. Lavie, J. Chem. Soc. chem. Commun. *1977*, 794.
3 D. Gross, Phytochemistry *14*, 2105 (1975).
4 C.F. Van Sumere, J. Cottonie, J. de Greef and J. Kint, Rec. Adv. Phytochem. *4*, 165 (1972).