After 7 weeks of culture, small drops of exudates appear to be present on the surface of callus grown on the benzyladenine medium but not on the kinetin one. The exudates were taken off with a micropipet, eluted with 2 drops of water and essayed for two dimensional paper chromatography (PC). The solvent benzene-acetic acid-water BzAW (6:7:3 upper layer) was used for the first dimension and 2% acetic acid (2% HOAc) for the second. The chromatograms were sprayed with diazotized *p*-nitroaniline to visualize phenolics. Standard compounds were used for comparison, and the identity of coniferyl alcohol was confirmed by UV spectral analysis in the presence of diagnostic reagents.

Results and discussion. Two dimensional PC of crude exudates showed the presence of 4 major phenols. They were successively purified by PC and one of them showed identical Rf values in 5 solvents when co-chromatographed with standard coniferyl alcohol. The Rf values are: BzAW: 0.60. HOAc: 0.67. Isopropanol-ammonia-water (10:1:1): 0.77. Butanol-acetic acid-water (4:1:5 upper layer): 0.82. Propanol-ethyl acetate-water (7:1:2): 0.93.

Also both standard and natural substances absorb at short UV lamp and give the same UV spectrum: a maximum at 265 nm in methanol; and in alkaline medium, a maximum at 290 nm and a shoulder at 315 nm. The compound was identified as coniferyl alcohol.

Sections of the tissues grown on kinetin medium contain a large number of nodules of tracheids as revealed by safranin-fast green and polarized light microscopy. Tissues grown on benzyladenine were much less lignified. These results seem to be in accordance with those of SARGENT and SKOOG[6] who reported an increase of lignin biosynthesis in tobacco tissue culture when kinetin was added to the medium.

The occurrence of coniferin in the sap of many gymnosperms helped to establish a theory that lignin is formed from its aglycon coniferyl alcohol and it is apparent that the oxidase responsible for the dehydrogenative polymerization of lignin precursors is exclusively peroxidase[7]. Numerous studies on lignification have demonstrated a direct relation between peroxidase levels and lignification[8]. On the other hand, in experiments with tissue cultures grown with growth regulators it can be seen that the medium on which the cultures are grown can change the pattern of enzymes responsibles of lignification[9,10].

On the basis of our results, the known role of peroxidase in lignification as well as the role of growth regulators on enzymatic activity, a mechanism may be present for which, in the callus of *C. sativa* grown on benzyladenine medium, lower production of peroxidase must occur than in those grown on kinetin, because the polymerization of coniferyl alcohol with benzyladenine treatment appears to be retarded.

The next step of our work will be the study of perodixase activity in the callus of *C. sativa* when they are grown on both kinetine and benzyladenine.

Resumen. Alcohol coniferílico fue identificado de los exudados producidos por callos de *Castanea sativa* Mill. cultivados in vitro, originados de cotiledón.

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Parthenin: A Growth Inhibitor Behaviour in Different Organisms

Parthenin, a sesquiterpene lactone of the pseudoguaianolide class isolated from *Parthenium hysterophorus* Linn., (Asteraceae), is an inhibitor of seedling growth in a crop plant *Eleusine coracana* (Linn.) Gaertn. Var. Poorna[1]. The present study deals with its behaviour in certain phases of two fungi such as – sporangial germination and zoospore motility in *Sclerospora graminicola* (Sacc.) Shroet. and conidial development in – *Aspergillus flavus* Link., in order to find out whether this inhibitor does exercise the same property in others. – Parthenin samples were obtained from KANCHAN[1] who has isolated them in her recent work. Parthenin was dissolved in 0.1% ethyl alcohol which was not toxic to test organisms. Appropriate media for test organisms and concentration of parthenin adopted in the experiment were used as given in the Table. Leaves of *Pennisetum typhoides* S. and H., infected by the downy mildew fungus *S. graminicola* were collected, surface sterilized with 0.2% chlorine water and washed in sterilized distilled water. The leaves were cut into pieces, floated on water with their abaxial surface upwards in a petridish lined with moist filter paper and kept overnight. Next morning, sporangia were scraped from the abaxial surface of leaf bits while zoospores were obtained by sowing the sporangia in distilled water for $^1/_2$ h. Sporangia and zoospores were seperately transferred to a distilled water drop on micro glass slides kept for control and experiment. In the experiment, they were tested against parthenin. For culturing *A. flavus*, methods cited by GARBUTT and BARTLETT[2] were followed. Medium with parthenin was sterilized before inoculation of the fungus. Room temperature was between 23 and 30 °C while conducting these experiments. In control *S. graminicola* zoospores were released from the sporangia after 30 min. The zoospore swam for 20–30 min and subsequently gave rise to germ tubes. Zoospores were released from the sporangia, swam for 2–3 min but eventually disintegrated. The results reveal that parthenin at the concentration of 500 mg in 1 ml of ethyl alcohol + 1,000 ml of distilled water inhibits sporangial germination in $^1/_2$ h and zoospore motility in 2–3 min. At the same concentration and even more (i.e., upto 1,000 mg), it never produced any visible inhibitory response in the conidial development of *A. flavus*. The results were further confirmed by

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Parthenin behaviour in different organisms

Organism tested	Phase	Parthenin concentration	Duration	Result
Sclerospora graminicola	Sporangia and Zoospores[a]	500 mg/1 ml 95% ethyl alcohol/ 1000 ml distilled water	1 h	After 30 min, control zoospores were released from the sporangia, swam for 20–30 min and subsequently gave rise to germ tubes, while experimental, zoospores were released from the sporangia, swam for 2–3 min but eventually disintegrated.
Aspergillus flavus	Conidia	500 mg/1000 mg/1 ml 95% ethyl alcohol/ 30 g maltextract/5 g peptone/15 g agar/ 1000 ml distilled water	1 week	No visual difference in growth and sporulation from the control

[a] The term sporangial germination means the release of zoospores and their subsequent germination.

repeating the experiment. The situation clearly implies that an inhibitor to an organism does not exercise the same activity in other. In *S. graminicola*, the rate of inhibition of sporangial germination by parthenin is highly significant ($p < 0.001$).

Moreover, slight antibacterial and absence of antileukemic activity of parthenin have already been noticed[3]. At the same time, response to the chemical in different organisms is seen. In this context, caffeic acid and *p*-coumaric acid, the other constituents of growth inhibitors reported in *P. hysterophorus*, have to be checked for their inhibiting potential[1].

Summary. Differential behavior of a growth inhibitor parthenin, has been observed. It inhibits sporangial germination and zoospore motility in *Sclerospora graminicola* and does not exercise the same activity in the conidial development of *Aspergillus flavus* at the same or greater concentration.

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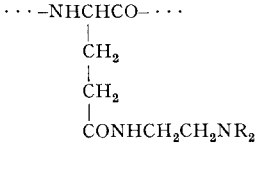
Polycationic Modified Polypeptides Enhancing Poly I:C Induced Viral Resistance

It is known that viral resistance of living cells and tissues provoked by several synthetic inducers can be enhanced to a great extent by polycationic substances. DEAE-dextran was the first[1] and one of the most frequently studied and most widely used polycations. Polycationic synthetic polyamino acids like poly-L-lysine and poly-L-ornithine, as well as some other polycationic substances, have similar effects[2,3].

The investigation of the effect of some polycationic modified derivatives of polyglutamic acid[4] seemed to be interesting, since some related derivatives belonging to the same group of compounds had been found to have antibacterial properties[5] similar to poly-L-lysine, and some possibility of using them therapeutically[6] had also been reported.

The compounds used in the present investigations were prepared from poly-methyl-α-poly-L-glutamate with 2-dialkylamino-ethylamines as described previously[4]. These polycationic macromolecules, with the following characteristic structural units as main constituents, are called poly-DMAE-glutamine if R = CH_3, and poly-DEAE-glutamine if R = C_2H_5:



The experiments were carried out in mouse L-929 fibroblast cells grown in 5% CO_2 atmosphere in Parker-199 medium supplemented with 10% of calf serum, adjusted with sodium hydrocarbonate to pH 7.2. Vesicular stomatitis virus, Indiana serotype (VSV) propagated in this culture was used for challenge. Virus assay was carried out on primary chick embryo cells by plaque titration.

The extent of the influence of polycations are expressed in 'minimal protective doses' of poly I:C (μg/ml), necessary for the complete protection of 10^6 L-cells against VSV. To determine this, 1-day-old cells were incubated at 37 °C for 12 h in 1 ml serum free media containing polycation in a concentration of 20 μg/ml and poly I:C in decreasing concentrations. After washing, the cells were challenged with 5×10^3 plaque forming units of VSV and incubated again at 37 °C for an additional 48 h. The results are shown in the Table.

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