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Bioluminescence as the Basis for the Detection of Trichothecenes

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The marine dinoflagellate, *Pyrocystis lunula*, luminesces strongly when subjected to a shear stress such as in stirring. The intensity of the luminescence reaches a maximum after approximately 2 hours of darkness. It was found that certain trichothecenes (popularly known as "Yellow Rain" compounds) inhibit the luminescence of *Pyrocystis lunula*; e.g. in the assay developed during this study, 2 micrograms of T-2 quench the luminescence of a test culture by 40%. The assay consists in preparing a suspension containing 100 cells/ml, storing it in the dark for 2 hours, then stirring mechanically for 1-1/2 minutes during which the output of a photometer absorbing the light produced is plotted on a strip chart recorder. Comparisons are then made of the displacement of the recorder pen with control cultures vs those containing a toxin.

Because an epoxide group is a necessary component of the trichothecenes, there was a possibility that the assay method was simply responding to the epoxide moiety. This is not the case, however, since a series of epoxide-containing compounds failed to inhibit the luminescence of the organism.

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19. ABSTRACT (Continued)

It is recommended that further studies be conducted to determine the feasibility of adapting this assay to field use. Each assay requires less than two minutes, and no special workup of field samples is required. Keywords:

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BIOLUMINESCENCE AS THE BASIS FOR THE DETECTION OF TRICHOTHECENES

INTRODUCTION

Reports of casualties among villagers in Southeast Asia and Afghanistan, as the result of Yellow Rain attacks, have been common occurrences in recent years. Mirocha et al (1983) suggested that the effects on victims matched those of the syndrome known as alimentary toxic aleukia (ATA) which results from the ingestion of grains infected with any of several genera of fungi. Fusarium and Stachybotrys produce chemical compounds known collectively as trichothecenes, which are recognized as the cause of ATA.

Claims made by the U.S. that trichothecenes have been used as chemical warfare (C.W.) agents in southeast Asia have been disputed by reputable scientists from both the Western and Eastern bloc nations. Several factors, however, have contributed to a lack of consensus, including the following:

1. The preferred, most sensitive, analytical method of detection of trichothecenes is the combined gas chromotography/mass spectrometry method. Even with the sophisticated equipment required, however, considerable experience in the analysis of trichothecenes must be amassed before accurate results can be obtained.

2. Pre-testing of field samples suspected of containing trichothecenes has not been done. Thus, samples devoid of any toxicants have probably been distributed to researchers in various countries and their negative findings cast doubt on the assertion that C.W. agents were used.

3. Diverse opinions abound as to the natural presence of toxic fungi in areas tested. Also, personnel collecting samples have not known of the need for samples from control sites as well as from targeted areas.

4. Trichothecenes are known to be unstable in the presence of soil microorganisms, therefore samples of soil taken in the field would give negative results unless they were analyzed soon after retrieval.

5. The literature on the effect of weathering and the effect of photosensitizing agents is sparse to non-existent.

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These factors could have tremendous effect on trichothecene instability during exposure to sunlight and/or rainfall.

The need for a sensitive and simple method for the detection of trichothecenes in the field is obvious. Unfortunately there is no functional group in these compounds that might provide a convenient means of identification. Chemically the trichothecenes are known as sesquiterpenoids and have been divided into four classes (Ueno, 1977) as shown in Fig. 1. When isolated, they are quite stable, crystalline solids having relatively high melting points, some exceeding 300°C.

All of these compounds have an epoxide ring, the destruction of which renders them biologically inactive. Mirocha et al. (1980) found that when trichothecenes are boiled in water at pH 3 the epoxide ring could be broken. Jarvis and Mazzola (1982) reported that the ring can also be broken by treatment with lithium aluminum hydride. The most sensitive analytical procedure so far has been the GC/MS procedure described and reviewed by Pathre and Mirocha (1977); Mirocha et al. (1976) and (1980); and Mirocha et al. (1983). However, the sensitivity of the method can be adversely affected by large concentrations of interfering substances, such as vegetation, to which the compounds might be bound under conditions in the field. The limit of detection has been stated to be on the order of 1 nanogram per injection under ideal conditions.

Tests designed for the detection of trichothecenes generally require times ranging from hours to days and, therefore, are not suitable for field use. Burmeister and Hesseltine (1970) studied the inhibitions of fungal growths with the use of impregnated discs. They also tried a pea germination test which responded to 0.5 microgram of trichothecene T-2* ml^{-1} in a 24 hour test. Other bioassays, based on the use of whole animals, extend from lethality in mice and rats to dermatitic response in rats, rabbits, and guinea pigs (Ueno, 1977; Pathre and Mirocha, 1977). These same teams of investigators developed a highly specific bioassay for trichothecene compounds based on the inhibition of protein synthesis in eucaryotic cells. Also, in an overnight test Resnick (Dugway Proving Ground, personal communication) could detect 1 ng of T-2 ml^{-1} with cell cultures. Immunochemical assays for trichothecenes would be desirable because they would provide specific identifications provided antibodies of each type could be made available. The problem has been that, because the trichothecenes are immunosuppressants, it has been difficult to form and isolate suitable antibodies. Chu and his associates at the University of Wisconsin (1979) and Fontelo's group at USAMRIID, Fort Detrick, MD (1982) coupled T-2 toxin to bovine serum albumin and

* The structure of T-2 is included in Fig. 8. It is defined as [4,15-diacetoxy-8-(3-methyl butyryloxy)-12,13-epoxy-Δ^9-trichothene-3-ol]

developed a radioimmunoassay which was sensitive to 10 ng T-2 ml^{-1}. Because of the relative complexities of the tests cited, and the times required, none of the above approaches are suitable for field use at present.

The Naval Research Laboratory has been conducting research on bioluminescence for several years and a quantitative method for determining the quenching of bioluminescence by chemical means has been developed. Because of its availability and the speed with which accurate assays could be obtained, it was decided to conduct several experiments with trichothecenes to determine whether they had any effect on the luminescence reaction.

The results indicated that, indeed, some of the trichothecenes inhibited bioluminescence considerably. This report describes the results obtained thus far and points out two potential applications for the method in future studies.

MATERIALS AND METHODS

Organism: The dinoflagellate, Pyrocystis lunula, was the bioluminescent source used in this study. It contains many cysts which emit light when the organism is subjected to a shear stress, such as in stirring. The organism, pictured in Fig. 2, is a lunate structure having a long axis of approximately 100 microns. While it is properly identified as a dinoflagellate, its flagella are present during only a small part of the life cycle so that the organism is mainly immotile. Because of its slow growth rate (the doubling time is approximately 4 days) tranfers need only be done monthly. The organism requires no special handling and, in fact, benefits from very static conditions. Stock cultures contain approximately 1000 cells/ml.

Culture conditions: Pyrocystis lunula was maintained in culture on f/2 medium described by Guillard and Ryther (1962). This medium was modified by the omission of silicate and the addition of THAM buffer to increase the final pH to 8.5. Artificial sea water was the base for this medium and was prepared with C.P. salts and distilled water from the formula of Lyman and Fleming (1940) modified by the addition of 0.144g $NaHCO_3$ per liter. Temperature of incubation was 20° ± 1°C. Illumination was provided by cool white fluorescent lamps, shaded to obtain a light intensity of 17 micro einsteins/cm^2. Illumination was on a cycle of 12 hours light and 12 hours dark.

Cell Number

In order to minimize clumping of the cells and permit accurate counting, the enzyme papain was added to the cell suspension at a concentration of 0.1% by weight toward the end

of the 12 hour light cycle. Cells were then counted with the aid of a Sedgwick Rafter chamber and their concentration adjusted to 100 cells per ml.

Assembly of an Assay

Three ml aliquots of the cell suspension at 100 cells/ml were dispensed into glass vials, 22 x 50 mm. Compounds to be tested were added as methanol solutions of 10 microliters total. Control suspensions contained 10 microliters of methanol. The vials containing the test cultures were placed in a carousel and kept motionless in the dark for two hours prior to the assay.

Stirring of the Organisms and Measurement of Bioluminescence

To be certain that the dinoflagellate culture emits the maximum amount of light, it is necessary that the culture be stirred vigorously. Stirring was accomplished with an acrylic rod equipped on one end with a thin strip of acrylic plastic. The other end of the rod was fitted into the chuck of a variable speed electric motor set at about 100 rpm. The rod was then inserted approximately 2/3 of the way into the sample vial. With the onset of stirring and the consequent shear force imposed on the organisms, luminescence lasted for an appreciable time. Stirring was continued for 1 1/2 minutes.

Bioluminescence was measured with a solid state photomultiplier described by Stiffey, Blank and Loeb (1985). A multirange stripchart recorder (Hewlett Packard, Model 7101B) with a chart speed of 5 cm/minute was connected to the photomultiplier; the circuitry was such that the recorder registered the cumulative light flux as a function of time.

A picture of the equipment used for the bioluminescence measurements is shown in Fig. 3.

The intensity of bioluminescence was linear between the limits of cell densities tested, i.e. between 10- and 100 cells/ml (Fig. 4). A concentration of 100 cells/ml was arbitrarily chosen for this study.

Calculation of Bioluminescent Quenching

Percent quenching was calculated with the equation:

$$\% \text{ quenching} = \frac{C - E}{C} \times 100$$

where C = displacement of the recorder pen, in mm, during stirring of the control culture, and E = the displacement of the pen during stirring of the doped test suspension.

Trichothecenes:

Samples were obtained from the following sources:

Sample	Source
T-2 Deoxynivalenol	Dr. Lou Carson, Food and Drug Administration; T-2 was also purchased from Calbiochem-Behring, San Diego, CA
Verrucarin A Roridin A-1 Trichoverrin A	Dr. Bruce Jarvis; preparations described in J. Organic Chem., 47, 1117, 1982
Roridin L-2	Dr. J. French, Warner Lambert Co., Ann Arbor, Michigan. See Tetrahedron Letters, 24, 249, 1983
Diacetoxyscirpenol	Dr. T.W. Doyle, Bristol Laboratories, Syracuse, N.Y. See J. Med. Chem., 25, 579, 1982.
Baccharin B-4 Baccharin B-5	Dr. Bruce Jarvis; preparations described in J. Org. Chem. 42, 4221, 1977.

RESULTS

Because the first flash of a luminescent organism is the most intense, the integrated light flux was high with the start of stirring of the suspension but its rate of gain decreased with time. Figure 5 represents a plot of the recorder pen vs. time for a control culture and for one treated with 8.3 micrograms T-2 (2.77 micrograms/ml). Note that most of the light had been emitted before the first minute had elapsed. In succeeding tests the practice was to measure the light emission over a period of 1 1/2 minutes to ensure that the assay would include all the stimulable bioluminescence.

In terms of a military threat, T-2 is considered one of the most important trichothecenes, therefore its effect on Pyrocystis was of particular interest. Figure 6A represents the average recorder readings, with the error bars, for triplicate samples of cultures treated with T-2 as supplied by Calbiochem*. Similar results were obtained with T-2 supplied by Dr. Jarvis (Univ. of Maryland). Figure 6B shows the average percent quenching of bioluminescence obtained with the two samples of T-2. At the lowest concentration tested, 1.0 microgram/ml, the difference between the control and the treated culture was significant at the 1% level.

In another experiment the study was extended by determining the response of Pyrocystis to T-2 at concentrations of 0.25-, 0.50-, and 1.0-microgram/ml as shown in Figure 7. Under the

*Calbiochem. Behring, Los Angeles, CA.

conditions of the test there was a highly significant effect at the 0.50 microgram/ml concentration (0.001 probability) and at the 0.25 microgram/ml concentration the effect was significant at the 0.15 probability level.

In addition to T-2, eight other trichothecenes were assayed by the test described. As shown in Fig. 8, those with bioluminescent quenching characteristics in addition to T-2 were diacetoxyscirpenol (DAS), roridin A, and verrucarin A. The first two named are Class A compounds and the latter are Class D. Only one Class B was tested (deoxynivalenol), also known as vomitoxin) and it had no quenching ability. No Class C compounds were tested. The remaining inactive compounds were trichoverrin A, a representative of Class A, and baccharin, baccharinol, and roridin L-2 from the macrocylic Class D group. All of these compounds were assayed at concentrations up to 10 micrograms/ml; the results of the active ones are summarized in Fig. 9, with the exception of T-2 whose activity was shown previously. An additional experiment performed with roridin A indicated that at a concentration of only 0.75 micrograms/ml it quenched the luminescence of Pyrocystis by 20%.

The favorable results obtained with T-2, verrucarin A, roridin A, and diacetoxyscirpenol indicated that the method might be useful in detecting the presence of trichothecenes in field situations, a factor of major importance if the method could be adapted to field use in those areas which might be subjected to Yellow Rain attack. It became a matter of interest, therefore, to determine: 1) whether a particular moiety of the trichothecenes might be responsible for the activity against bioluminescence, e.g. the epoxide ring, and 2) whether other toxins or poisons might show the same type of effect. To explore the significance of compounds containing epoxide rings, a group of representative compounds was obtained from Dr. James Griffith of the Polymeric Materials Branch, Chemistry Division, at NRL. The formulae for these compounds are shown in Fig. 10.

All of these compounds were tested at the single concentration of 2 micrograms/ml and the only one showing activity was the furan derivative, (C). It inhibited luminescence to the extent of 17% which was significant at the 0.10 probability level. These results indicated that the epoxide ring per se was not the only consideration in the quenching of bioluminescence, a fact which was borne out by the negative results with some of the other trichothecenes.

Prior to this particular study there had been research at NRL on the effects of heavy metals and detergents on bioluminescence. The conditions of those tests were considerably different in that luminescence was measured immediately after exposure to the toxicants but the concentrations were high (100 micrograms/ml). It was thought worthwhile to explore the effects of these same compounds at a much lower concentration,

but with the same 2 hour exposure time used with the trichothecenes. The concentration chosen was 1.0 microgram/ml and the results are summarized in Table 1.

Table 1. Effect of Selected Heavy Metals, T-2, and a Detergent, on Bioluminescence When Tested at 1.0 microgram/ml.

Compound or ion	Percent change	Probability level
T-2	-35	.01
SDS*	No significant effect	-
Zn^{++}	" "	-
Ni^{++}	" "	-
Hg^{++}	-100	.001

*Sodium dodecyl sulfate

It was not surprising that Hg^{++} had such a pronounced effect but it had also been thought that SDS would have some effect since, at the 100 microgram/ml concentration but essentially zero exposure time, it had been a potent inhibitor. At the 1.0 microgram/ml used here, there was no effect. Whether the detergency of SDS was a factor at the 100 microgram/ml concentration is unknown; its critical micelle concentration is approximately 720 micrograms/ml (Schick, 1967).

Additional compounds screened for their ability to quench bioluminescence were obtained through the courtesy of Dr. Lou Carson, of the Toxicology Division of the Food and Drug Administration. They were high purity compounds and identified as aldrin FDA 2, dieldrin EPA/FDA 34, heptachlor epoxide Ref. Std. 99.3%, and octachlor epoxide EPA/FDA 407 (70-155). The structural formulae shown in Fig. 11 indicate that three had the epoxide grouping and the fourth, aldrin, differed from dieldrin only in that it had no epoxide ring. The test concentration chosen was 10 micrograms/ml and the results are summarized in Table 2. Aldrin had no activity and the quenching of bioluminescence provided by the others was slight. The lack of quenching by dieldrin was less than that anticipated because a previous test here had shown it to be highly active. In retrospect, the inhibition associated with the earlier test may have been caused by a surfactant in the wettable powder formulation which was the source of dieldrin used at that time.

Table 2. Effect of Aldrin and Three Epoxide Compounds on the Quenching of Bioluminescence; concentration = 10 Micrograms/ml.

Compound	Percent Quenching	Probability Level
Aldrin	None	.01
Dieldrin	15	.05
Heptachlor Epoxide	17	.10
Octachlor Epoxide	17	.02

DISCUSSION

It is interesting to note that the quenching of bioluminescence is found in trichothecenes of different classes. For the sake of convenience Fig. 12 is shown below to indicate the numbering scheme assigned to trichothecenes; the macrocyclic ring shown here is that of a Class D compound but the carbon numbering is the same for all types.

There are interesting similarities among the four active compounds: a) T-2 and diacetoxyscirpenol have the same structure except for the substituent on C-8, b) neither verrucarin A nor roridin A has a substituent on C-8 and both are active; both have in common a macrocyclic ring and the only difference between them is the addition of a two carbon alcohol group in roridin A.

The addition of an epoxide group on the macrocyclic ring to give baccharin and baccharinol, reduces the bioluminescence quenching. Roridin L-2 and trichoverrin A also lack the tendency to quench bioluminescence and neither contains a closed macrocyclic ring although both have large substituent groups. Deoxynivalenol has fewer substituents than any of the above and it also is inactive. At this point there is no hypothesis offered for the ability of some compounds to quench bioluminescence, but it appears that epoxide groups per se are not responsible.

These studies have concerned the quenching of bioluminescence, and not toxicity, but there is a parallel to toxicity studies performed with other systems. We found bioluminescence quenching with T-2 and diacetoxyscirpenol, but not with deoxynivalenol, roridin L-2, and trichoverrin A. These three inactive compounds were shown to be two orders of magnitude less toxic than T-2 and diacetoxyscirpenol both in vivo (LD_{50}'s approximately 100 mg/kg in mice) and in vitro (against L-1210 cell line)(Jarvis and Mazzola, 1982; Jarvis et al., 1982).

For the sake of comparison, the table below shows a ranking of trichothecenes in terms of LD_{50} values for mice when injected intraperitoneally.

Table 3. LD_{50} Values (mg/kg) of Trichothecenes Concerning Mice (Uenc, 1977)

Compound	Mg/kg
Verrucarin A	0.5
Roridin A	1.0 (intravenous injection)
T-2	5.2
Baccharin B-4	5.0
Baccharinol B-5	9.0
Diacetoxyscirpenol	23.0
Deoxynivalenol	70.0

Diacetoxyscirpenol is ranked lower here than one would anticipate based on the bioluminescence quenching data, but the data with verrucarin A, roridin A, T-2, and deoxynivalenol parallel our data. Trichothecenes are considered potent phytotoxins, yet baccharins occur naturally in the Brazilian shrub Baccharis megapotamica (Jarvis and Mazzola, 1982; Kupchan, 1974). Furthermore this shrub can absorb thousands of parts per million of roridin A which is toxic to common agricultural plants such as corn, wheat, beans, and tobacco at concentrations of a few parts per million (Jarvis, Cutler, and Mandava).

There is a substantive difference between the response of Pyrocystis lunula, and bacteria, to trichothecenes. As stated previously, T-2 inhibits the bicluminescene of Pyrocystis lunula at concentrations below 1.0 microgram/ml. In contrast, when T-2 was added at 10 micrograms/ml to luminescent bacteria (from the Gulf of Mexico) it had no effect on their luminescence.

Because of its sensitivity to T-2, the quenching of luminescence of Pyrocystis is being considered as the basis for a field test. Such a detection system should be compact to the extent that it can be hand held, and work toward this end is planned. We are considering the installation of the photomultiplier tube and the amplification system in a phototube housing of a surplus Beckman DU spectrophotometer. The proposed readout device is an LED voltmeter which can be mounted such that it can operate in either an AC or DC mode.

In addition to the need for a hand-held device for use in the field, there is also a need for a method to monitor the rate at which trichothecenes leave the environment as the result of reaction with vegetation, or actinic degradation, or leaching by rainfall. The present method described in this report is already being used in our laboratory at NRL to study the rate at which T-2 disappears from Ficus leaves as the result of exposure to either UV-B radiation or sunlight. This will be the subject of a later report but the results indicate that roridin A reacts with leaf material such that, in less than 5 days, most of the trichothecene has disappeared. These results do not depend on actinic degradation or leaching by rainfall, since samples kept in the dark at room temperature lose their toxicity also. The utility of the method is apparent from the fact that as many as 55 assays have been run in a single afternoon; with the GC/MS method such a number would be out of the question.

Several improvements over the current method for determining the quenching of bioluminescence are being evaluated. For example, we have recognized that the Pyrocystis cells develop an enhanced luminosity with time in the dark which is contrary to the popularly held view that their peak is reached after only an

hour; perhaps the regimen involved in setting up the assays affects the performance of the cells. Regardless of the cause there will be a set of controls tested at the start of the luminescence tests and another set of controls at the end. Because a typical test requires several hours, the dark time of the cells tested initially is considerably different from those tested at the end, and if there has been an appreciable increase in luminescence during that period a correction factor can be applied to all the results obtained in between.

The ratio of toxicant/cell is of great importance in bioassays. Therefore, by reducing the Pyrocystis cell count from 100- to 10/ml the sensitivity of the assay would be increased. The increase would probably not be linear because the adsorption of toxicant to the walls of the test vessel becomes proportionately greater as the concentration is reduced. This raises the point, also, of the role played by exoskeletal material in acting as an adsorbent for the compound being tested. Two cultures can be identical in their concentrations of live Pyrocystis cells but differ considerably in their concentrations of exoskeletal material. In such a case the greatest effect of the test compound would be on the culture having the least exoskeletal material, if the assumption is made that the exoskeletal material has the same adsorptive capacity for the toxicant as the live cells. To address the problem of the varying exoskeletal material, it is necessary to include in each assay a reference standard to validate each test. As the result of research performed after much of the work described here, the use of 0.05 micrograms of Hg (as $HgCl_2$) per ml is recommended. Considerable experience emphasizing the importance of adsorption in bioassays has been accumulated in this laboratory (Hannan et al. 1973; Hannan and Thompson, 1977; Hannan and Patouillet, 1979).

A final word should be said regarding the chemical and biological approaches to the detection of trichothecenes. For an absolute identification and quantitation of the trichothecenes, the preferred method is that of GC/MS. The drawback is that a considerable workup of the sample is required and the sensitivity is decreased by such factors as vegetation on which the samples might be found. Also GC/MS is not suited for field use. The biological approach based on the quenching of bioluminescence lacks the specificity of the chemical method. The advantages are that the sample (such as a leaf) requires no workup; the sample can be placed directly in the culture and comparisons can be made to another culture containing a control leaf. This procedure has been used to study the rate of weathering of roridin A from the surface of Ficus leaves in this laboratory. In the presence of leaf material, a given concentration of toxicant has less effect than if it were added directly to a Pyrocystis culture but the gradual decline of toxicity can be determined in this way. Also the method should be easily adaptable for field use. There are many situations in

the field in which a premium must be placed on a "Go - No Go" judgment and the approach described here is pertinent in that regard.

For studies of the rate of disappearance of trichothecenes from vegetation, the bioluminescence quenching method in its present form is very satisfactory and should be exploited.

RECOMMENDATIONS

1. Develop a portable, battery-powered unit for assays of trichothecenes in the field.

2. Determine the increase in sensitivity resulting from a decrease of Pyrocystis lunula cell density from 100- to 10-cells/ml.

3. Extract luciferin and luciferase from Pyrocystis cells, and determine the quenching effect of trichothecenes added to a mixture of these extracts compared with their effect on the luminescence of intact cells.

4. Determine the synergistic effects, if any, of combinations of trichothecenes on the quenching of bioluminescence.

5. Test other naturally occurring chemicals for quenching ability.

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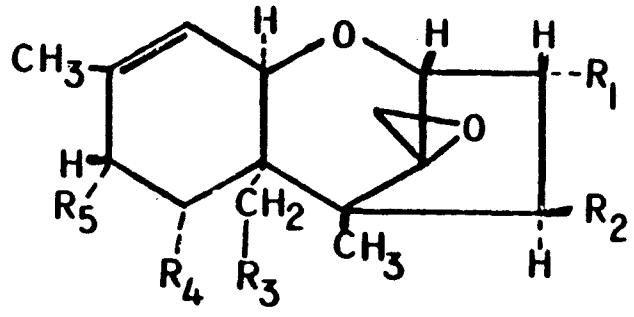
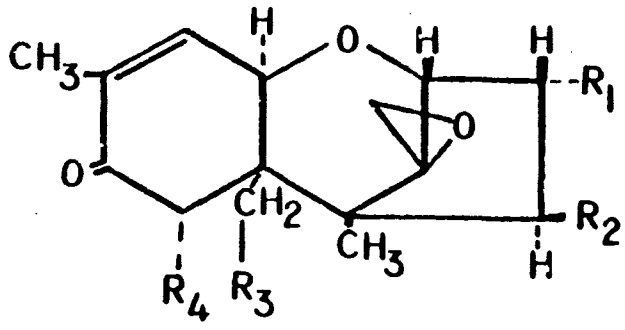


A



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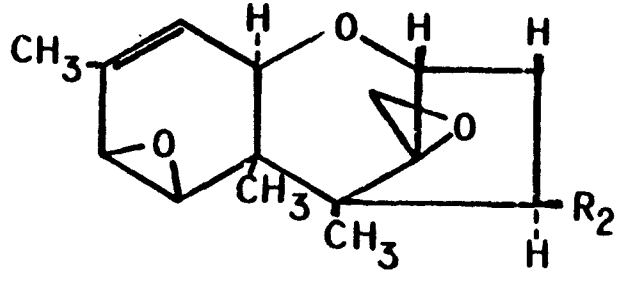

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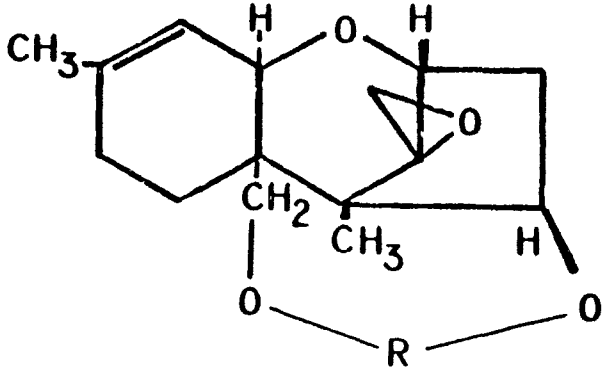


D

Fig. 1 — Classification of trichothecenes as devised by Ueno (1977)

BIOLUMINESCENCE INHIBITION

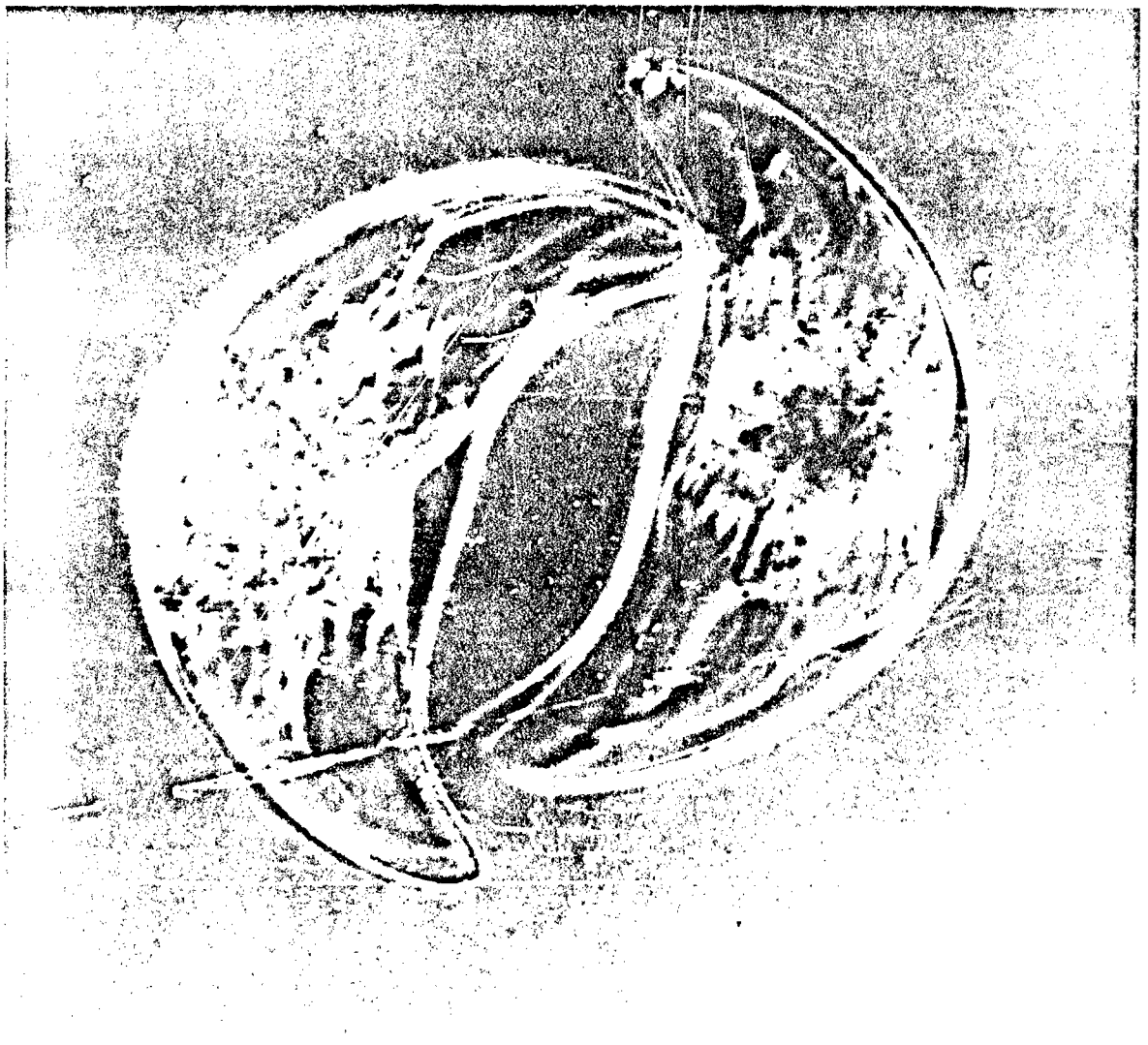


Fig. 2 — Picture of *Pyrocystis lunula* cells; the long axis is approximately 100 microns

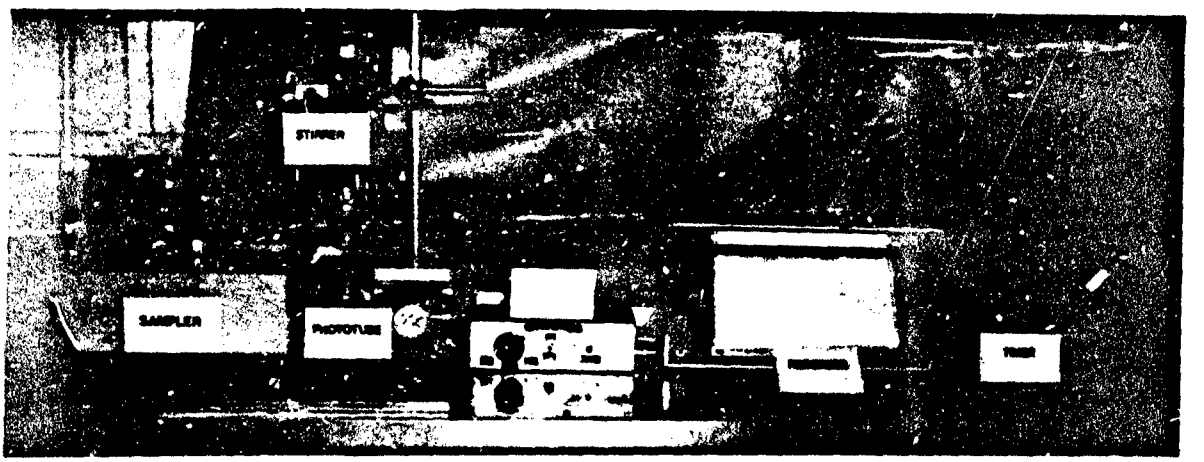


Fig. 3 — Equipment used for the measurement of bioluminescence intensity

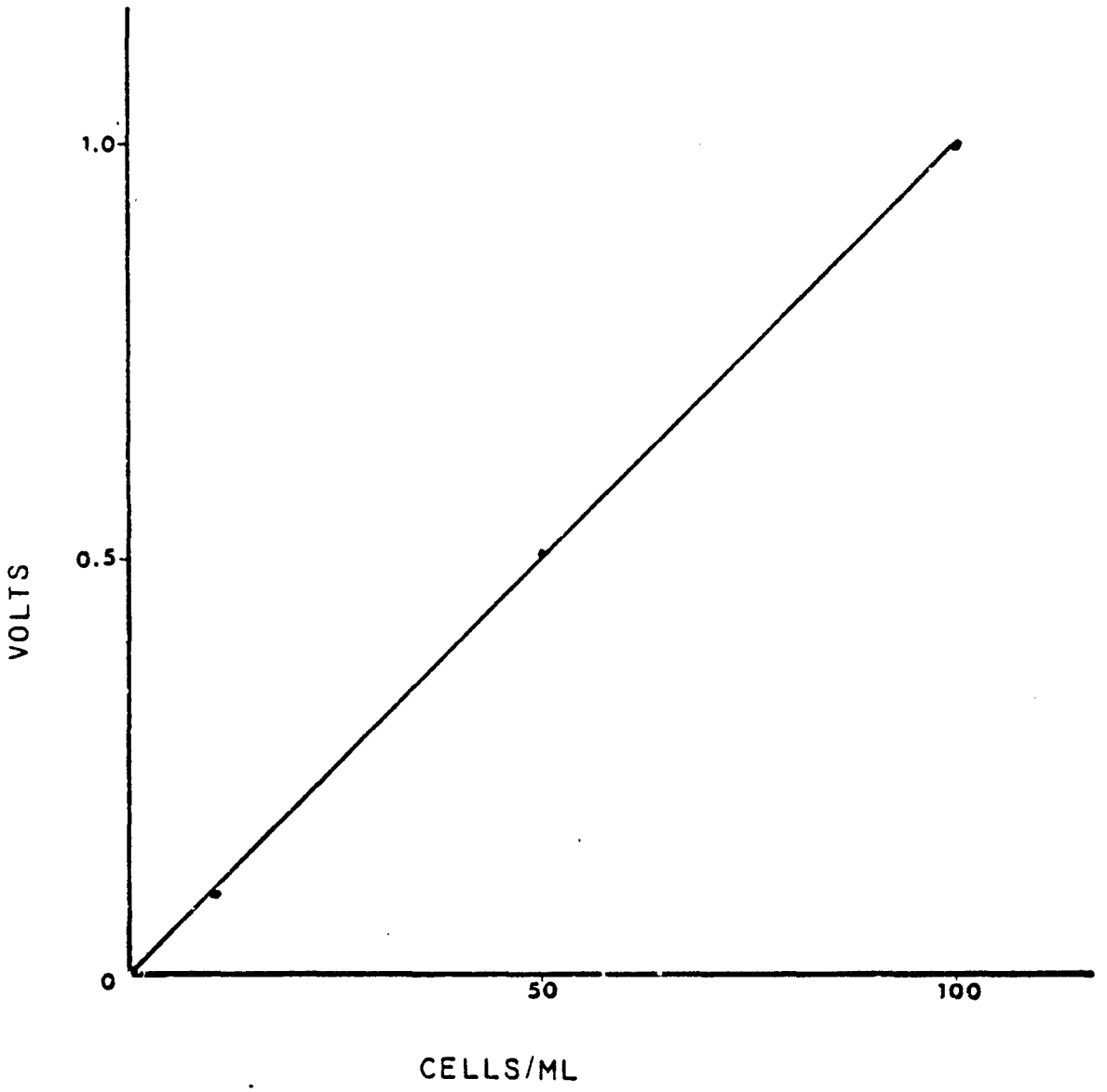


Fig. 4 — Bioluminescence intensity, as measured in volts on the recorder as a function of cell density

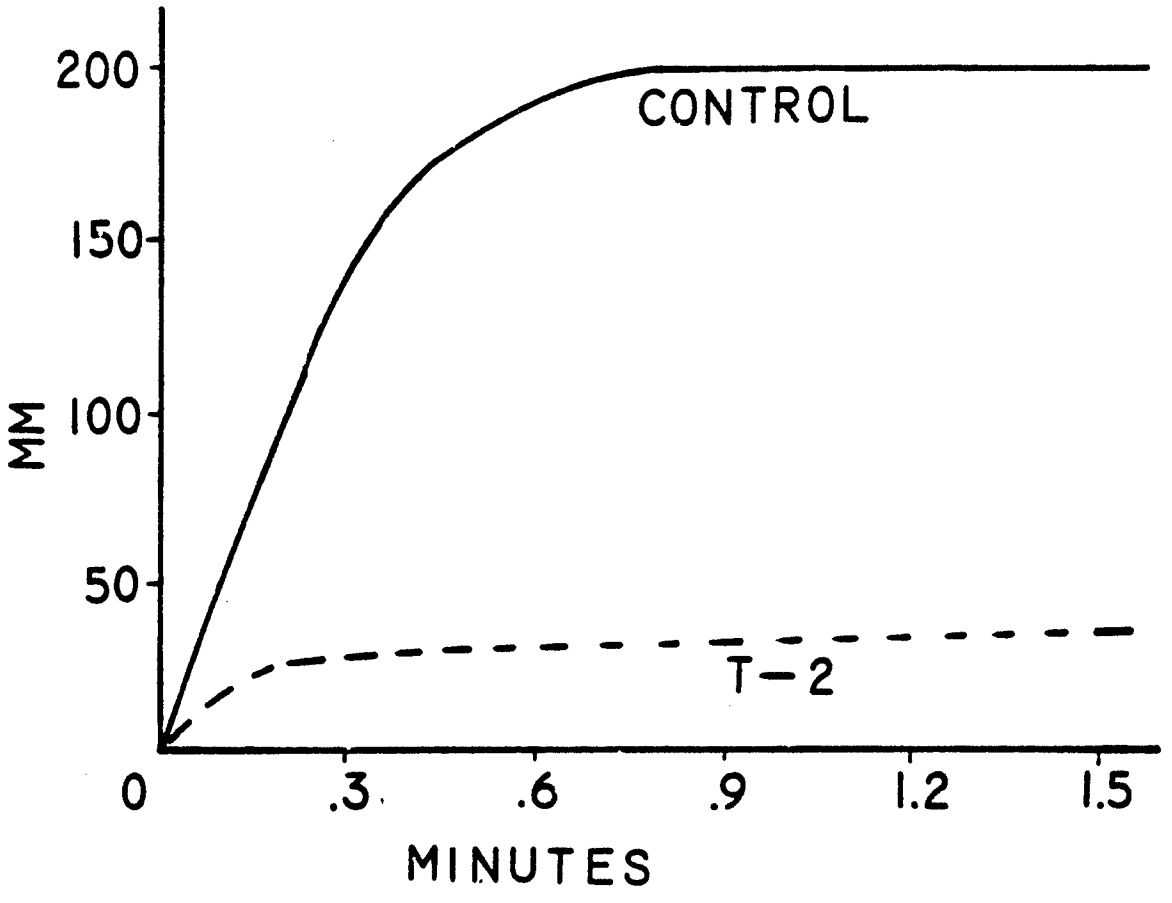


Fig. 5 — Comparison of recorder plots for a control culture and one containing 2.77 micrograms of T-2/ml

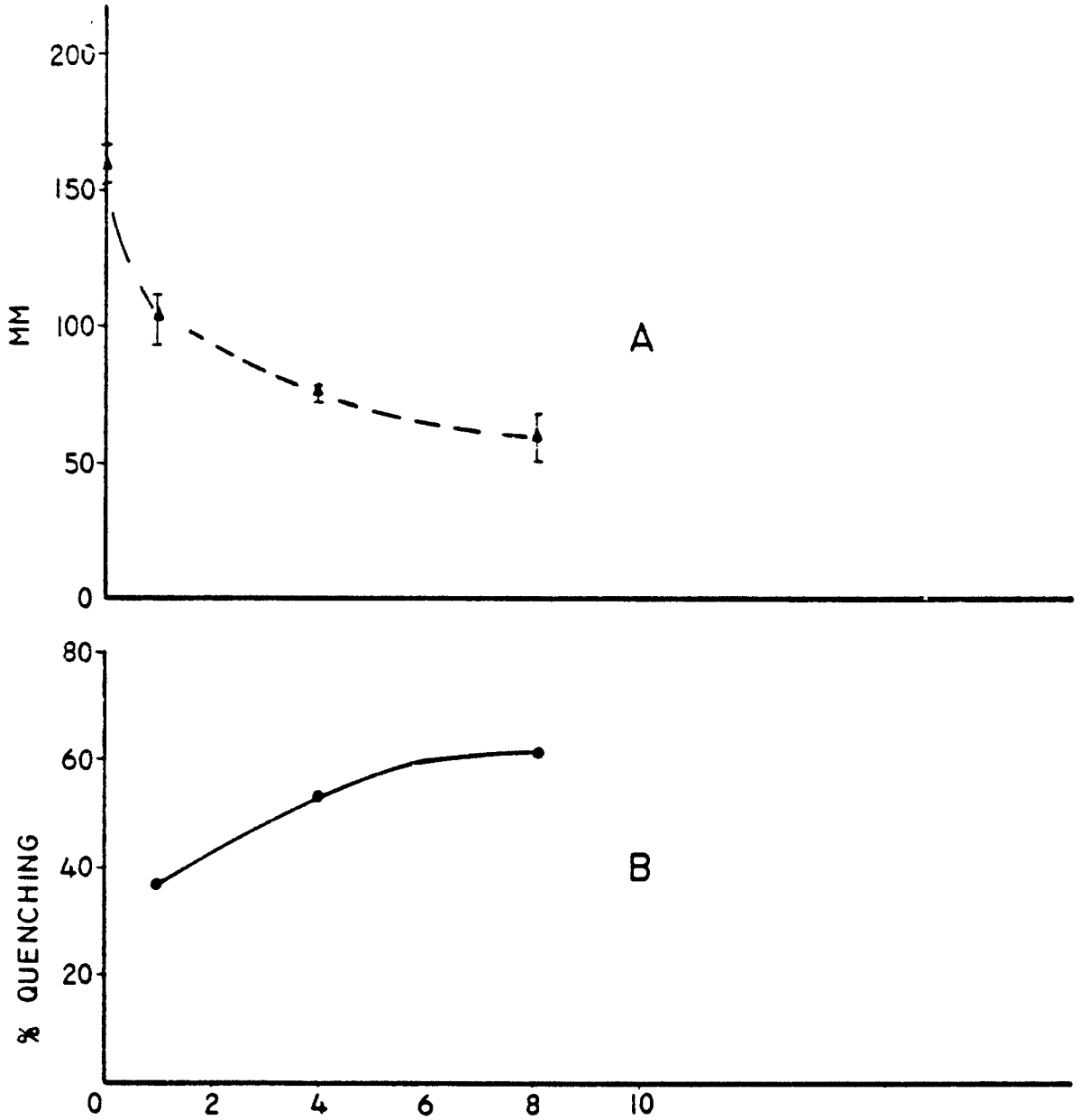


Fig. 6 — (A) Average recorder readings, and error bars, for triplicate cultures treated with various concentrations of T-2. (B) Percent quenching of bioluminescence by various concentrations of T-2.

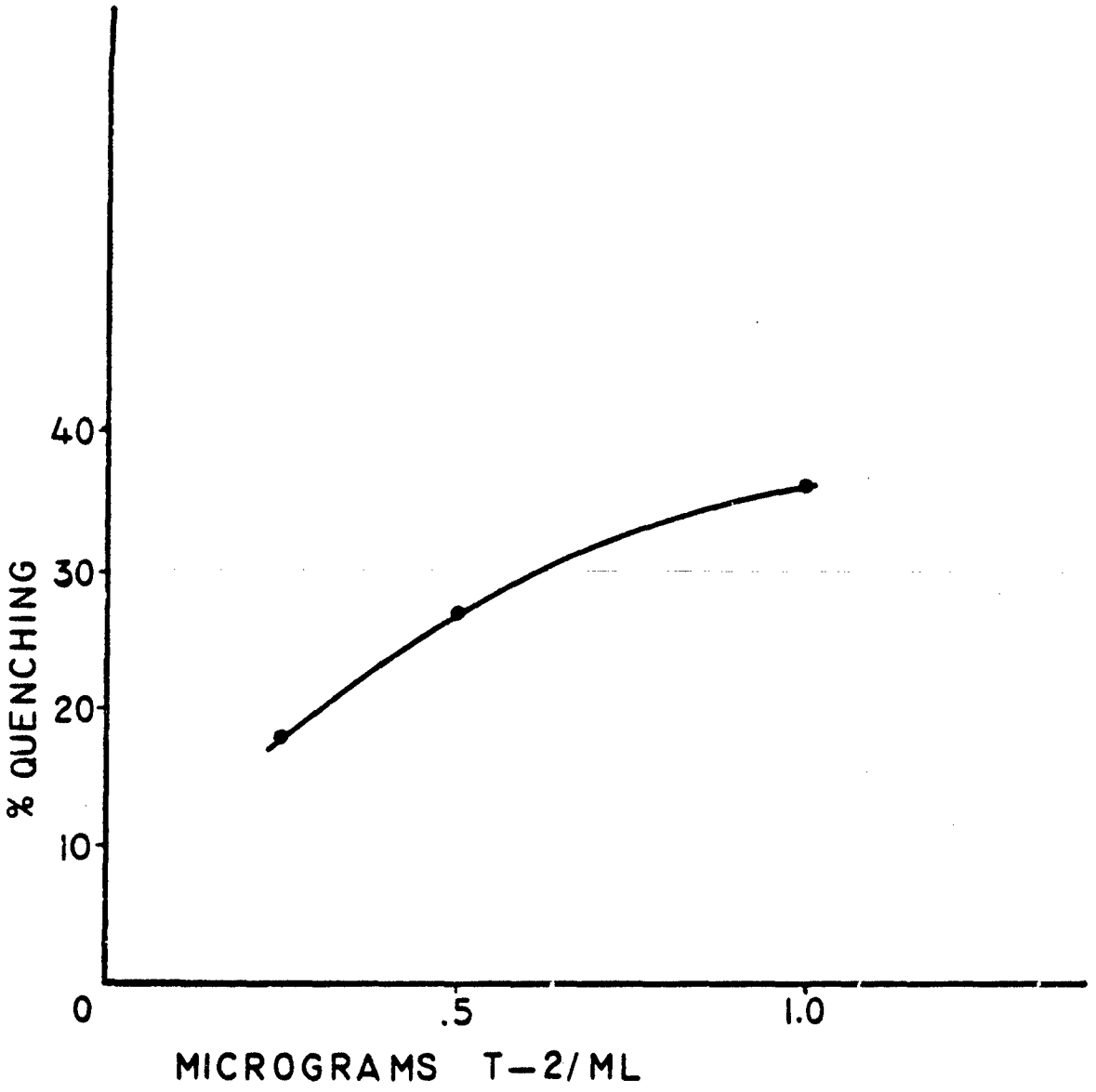
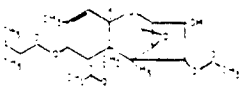


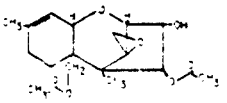
Fig. 7 — Percent quenching of bioluminescence by low concentrations of T-2

ACTIVE COMPOUNDS

CLASS | STRUCTURE

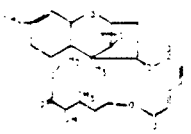


T-2

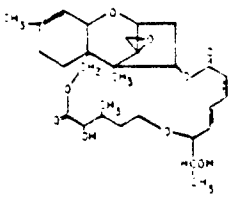


DIACETOXYSCIRPENOL

A



VERRUCARIN A

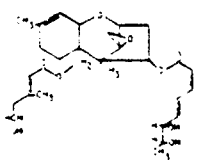


RORIDIN A

INACTIVE COMPOUNDS

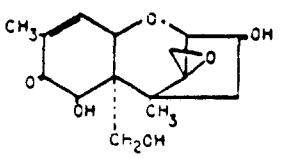
CLASS | STRUCTURE

A



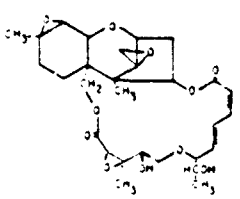
TRICHOVERRIN A

B

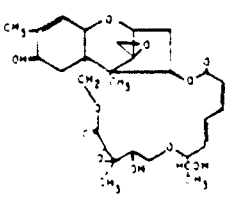


DEOXYNIVALENOL

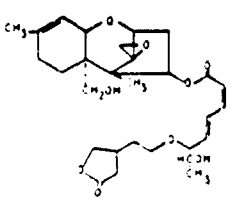
D



BACCHARIN



BACCHARINOL



RORIDIN L-2

Fig. 8 — Summary of trichothecenes assayed

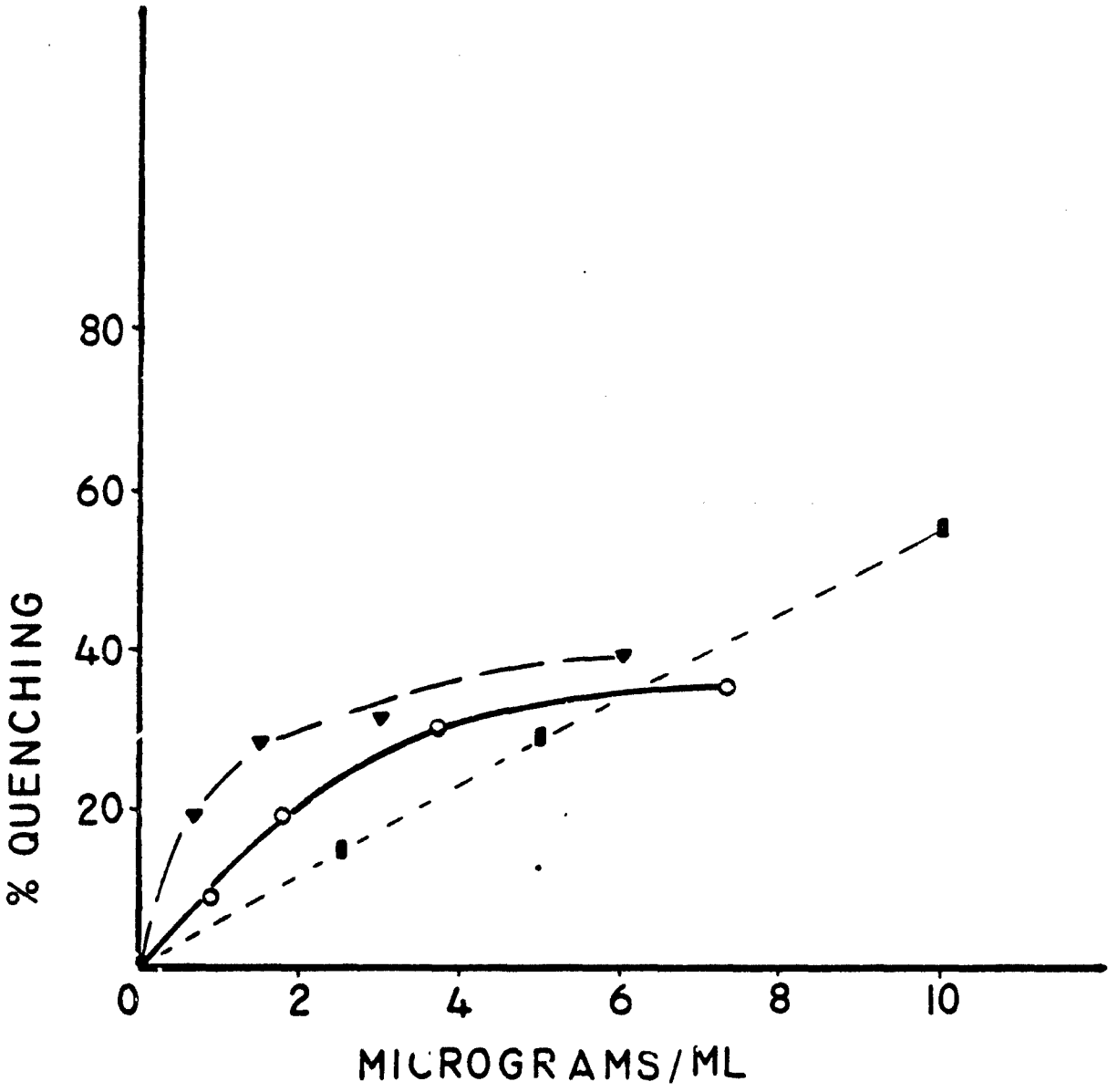
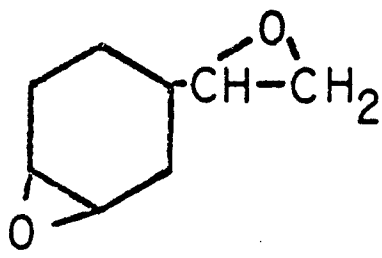
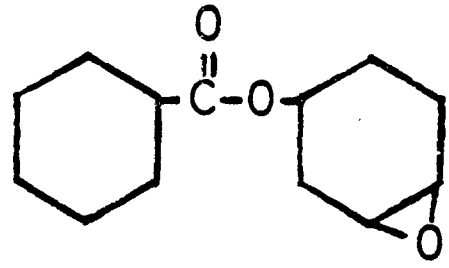


Fig. 9 — Percent quenching obtained with diacetoxyscirpenol (■), roridin A (▼), and verrucarin A (○)



(A)



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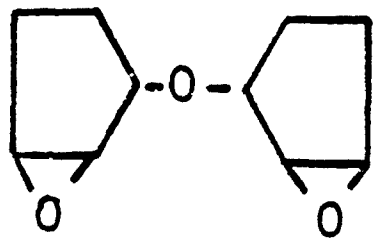

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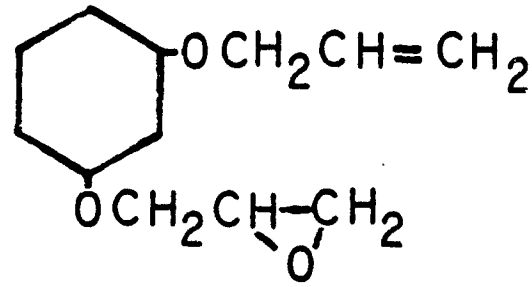
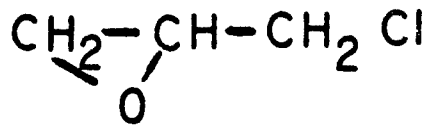
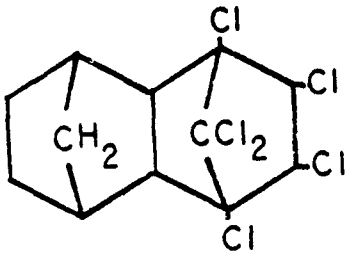


(D)

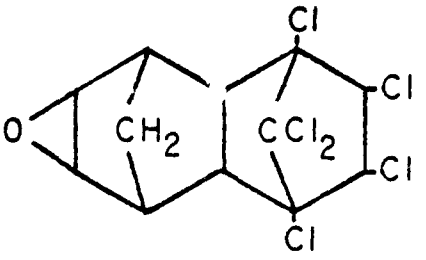


(E)

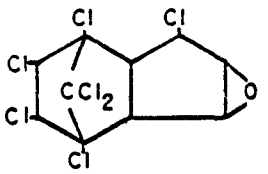
Fig. 10 — Compounds containing epoxide groups which were tested for quenching bioluminescence



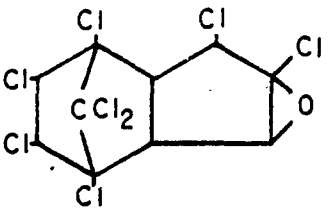
ALDRIN



DIELDRIN



HEPTACHLOR EPOXIDE



OCTACHLOR EPOXIDE

Fig. 11 — Pesticides screened for ability to quench bioluminescence

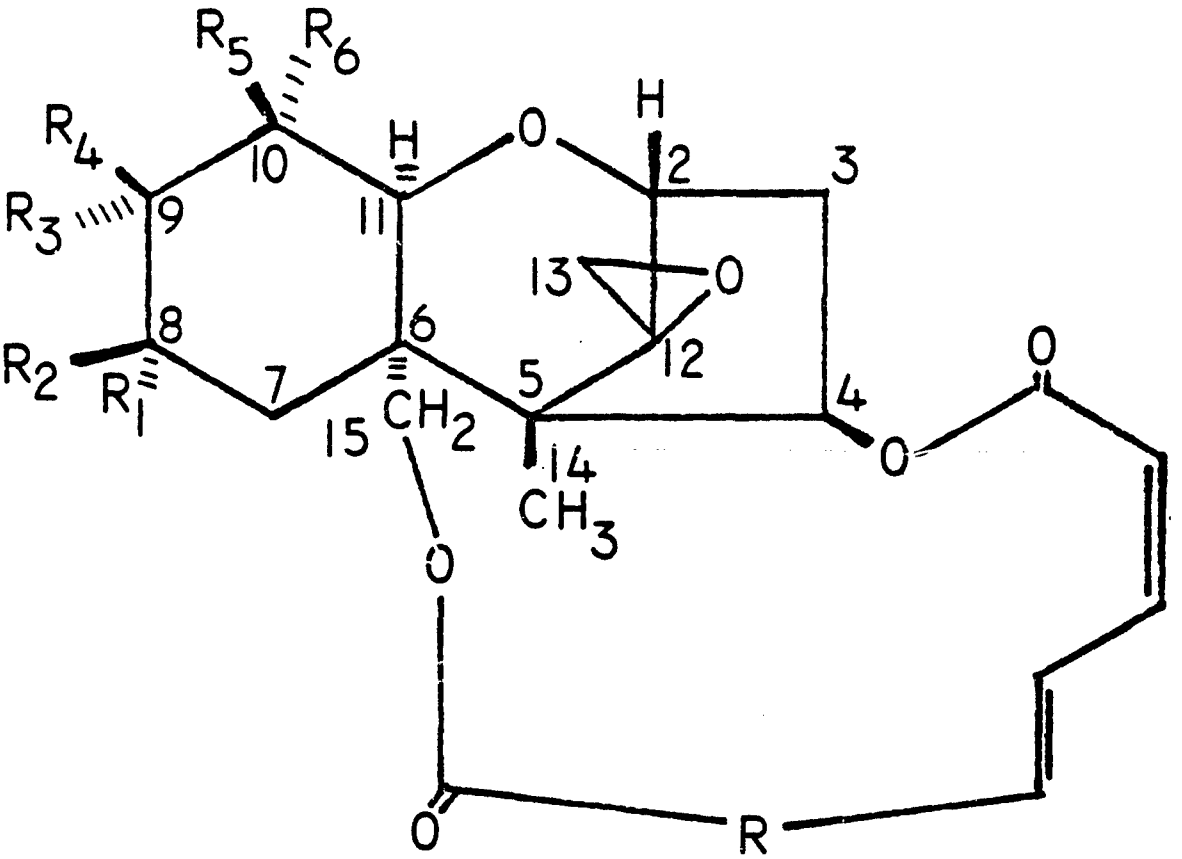


Fig. 12 — Carbon numbering scheme of trichothecenes; the example shown is that of a macrocyclic ring compound

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