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# A new antheridiogen from the fern *Pityrogramma calomelanos* (L.) Link

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**Abstract.** A new antheridiogen from *Pityrogramma calomelanos* of the family Polypodiaceae (Sensu Bower) has been extracted and tested on *Pityrogramma calomelanos*, *Onychium siliculosum* (Desv) C. chr, and *Onychium japonicum* (Thbg) Kze. The antheridiogen, here designated as Apit, did not produce uniform effect on the species tested. It promotes dark germination in all, but induces antheridia only in *Pityrogramma calomelanos* and *Onychium siliculosum* under light and dark conditions. The antheridium-inducing capacity of Apit is markedly different on the two ferns, being more vigorous under light condition in *Pityrogramma calomelanos* and less in *Onychium siliculosum*. Contrary to this, more antheridium-bearing prothalli have been counted in the latter under darkness. Higher dilutions are less effective in both. Dark germination has been found most effective in case of *Onychium japonicum*. The results indicate that the intensity of the effect of antheridiogen varies under different conditions and between species of the same genus. Also, an antheridiogen is not specific in the induction of antheridia or dark germination of spores, but it may initiate any of the two or both.

**Keywords.** Antheridiogen; Apit; Apt.

## 1. Introduction

Since the discovery of antheridium-inducing substance in *Pteridium aquilinum* by Döpp (1950) several leptosporangiate ferns have been reported to contain such substances which are capable of inducing antheridia (Näf 1956, 1959, 1960, 1965, 1969; Pringle 1961; Näf *et al* 1969; Schedlbauer and Klekowski 1972). Of the several known antheridiogens one from *P. aquilinum* (abbr. Apt) has been extensively investigated and its high degree of specificity has been claimed by Näf *et al* (1975) on the basis of its activity remaining restricted within some polypodiaceous ferns (Sensu Bower) only. The degree of specificity would be sharper when the antheridiogen from the same species collected from two different sources shows different activity (Schedlbauer 1974).

The new antheridiogen hereafter called Apit, which constitutes the subject matter of the present communication has been proposed on the ground of its differential sensitivity as mentioned above and its ability to induce germination under dark condition.

## 2. Materials and methods

The spores of *P. calomelanos* and *Onychium siliculosum* were collected in October 1978 from the Pokhra Valley, Nepal and those of *O. japonicum* were gathered from the

vicinity of Royal Botanical Gardens, Kathmandu. After surface sterilization with 3% sodium hypochlorite solution the spores of *P. calomelanos* were inoculated in petriplates containing Parker's macronutrients and Thompson's micronutrient culture medium (Klekowski 1969) solidified with 1% agar and autoclaved at 15 lbs pressure for 15 min. After 27 days the entire population of the cordate prothalli having antheridia were removed carefully with forceps and the solidified medium allowed to stay upside down in the same petriplate. The process is known as inverted plate method (Voeller 1966). These plates were then inoculated afresh with surface sterilized spores of *P. calomelanos* to find out whether *P. calomelanos* produced any antheridiogen capable of inducing antheridia in the new gametophytes earlier than control.

After ascertaining that *P. calomelanos* was able to produce antheridiogen, its spores were inoculated in 50 ml flasks containing the solidified growth medium. On the 26th day, 10 ml of sterile double distilled water was added to each of the flasks and placed in the room temperature for 6 hr and then incubated in a freezer for 18 hr. After thawing, the liquid was filtered off and the filtrate diluted 10,000 times (1:10,000) (Endo *et al* 1972). Serial dilutions were prepared by the liquid nutrient medium, which were later autoclaved and after cooling inoculated with the surface sterilized spores of *P. calomelanos*, *O. siliculosum* and *O. japonicum*. One set of each was placed under continuous fluorescent light of 300 ft-c intensity and the others kept in darkness at $24 \pm 2°C$ in a culture room. After germination the dark set was daily exposed to light for 1 hr and observations recorded on every odd day.

## 3. Results

### 3.1 *Effect of Apit on germination*

3.1.1 *In light*: Two sets of different dilutions of Apit-mixed medium with the spores of *P. calomelanos, O. siliculosum* and *O. japonicum* were made and one set of each was placed under 300 ft-c intensity of fluorescent light, whereas the other incubated in dark. The above fern spores do not normally germinate in dark. It was observed on the third day that in light the action of antheridiogen was maximum on *O. siliculosum* where the germination percentage goes on increasing with the increase in dilution (table 1). Under such conditions increasing trend in germination was also observed for *P. calomelanos*, but it was found to be less effective when compared with *O. siliculosum*. The germination at 1:1 dilution was 50% in *O. siliculosum* and 23% in *P. calomelanos*; and 91% in the former and 70% in the latter at 1:10,000 dilution. In case of *O. japonicum* Apit activity was found to be somewhat different, where germination increase was recorded upto 1:100 dilution (76%) but higher dilutions decreased germination and it remains only 39% in 1:10,000 dilution.

3.1.2 *In dark*. When the spores are inoculated in antheridiogen-mixed medium, dark germination was induced. It occurs only in lower dilutions of antheridiogen and higher ones were not effective in case of *P. calomelanos* and *O. siliculosum*. The antheridiogen-induced dark germination was most effective in *O. japonicum* where dark germination was induced in higher dilutions also, though the percentage of germinated spores in them was less (table 1). In fact the most effective dilution of Apit for dark germination varied in all the three taxa: for *P. calomelanos* it was 1:1 for *O. siliculosum* 1:10 and for *O. japonicum* 1:10 and 1:100.

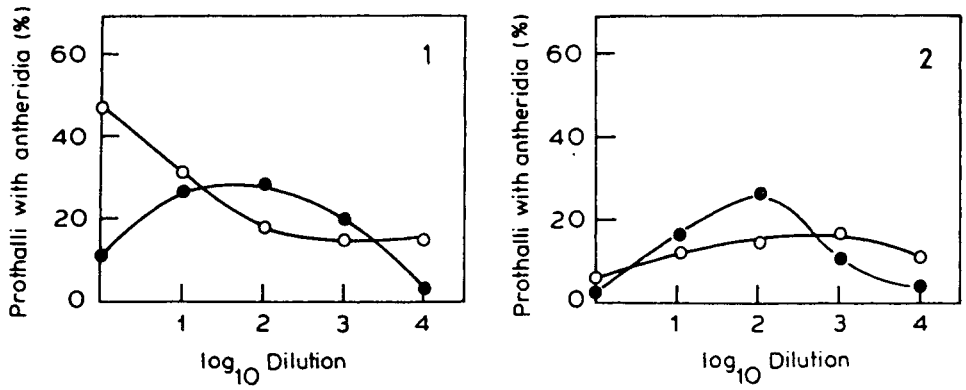
**Table 1.** Apit-induced germination in light and darkness (%) of spores of *P. calomelanos*, *O. siliculosum* and *O. japonicum* on the 6th day.

| Test | Apit dilution | Germination in 300 ft.c light (%) | 95% confidence limit | Germination in dark (%) | 95% confidence limit |
|---|---|---|---|---|---|
| *P. calomelanos* | 0 | 52* | 41·8–62·1 | 0* | 0–0 |
| | 1:1 | 23 | 17·4–29·5 | 7 | 2·9–13·9 |
| | 1:10 | 34 | 27·5–41·0 | 4 | 1·1–9·9 |
| | 1:100 | 43 | 32·2–53·2 | 1 | 0·0–5·5 |
| | 1:1000 | 57 | 46·6–66·9 | – | 0–0 |
| | 1:10000 | 70 | 63·1–76·3 | – | 0–0 |
| *O. siliculosum* | 0 | 43 | 33·2–53·3 | 0 | 0–0 |
| | 1:1 | 50 | 44–56 | 19 | 13–27 |
| | 1:10 | 65 | 60–70 | 33 | 26·42 |
| | 1:100 | 77 | 72–82 | 7 | 2·9–13·9 |
| | 1:1000 | 84 | 81–87 | 0 | 0–0 |
| | 1:10000 | 91 | 85–95 | 0 | 0–0 |
| *O. japonicum* | 0 | 36 | 26·7–46·2 | 0 | 0–0 |
| | 1:1 | 42 | 37–49 | 23 | 17·4–29·5 |
| | 1:10 | 61 | 53·9–69·2 | 39 | 35–44 |
| | 1:100 | 76 | 66·9–82·0 | 40 | 30–51 |
| | 1:1000 | 66 | 57·4–73·9 | 13 | 9–20 |
| | 1:10000 | 39 | 35–42 | 9 | 6–12 |

For each observation 100–200 spores were examined.
* Actual germination percentage.

### 3.2 *Antheridium initiation by Apit*

Antheridium formation takes about 32 days after inoculation of the spores of *P. calomelanos*; 30 days in *O. siliculosum* and 40 days in *O. japonicum* in control. Spores inoculations with different dilutions of Apit, mixed in growth medium, were observed after 9 days of germination. It is found that the antheridiogen has no effect whatsoever on *O. japonicum*. But in *P. calomelanos* and *O. siliculosum* some interesting results were obtained. Apit starts its activity as early as on the 9th day of germination in *P. calomelanos* when antheridium formation was recorded in lower dilutions of 1:1 and 1:10 (figure 3) but higher dilutions were not effective. The highest dilution tested (1:10,000) initiates antheridium only on the 15th day. Maximum activity of antheridiogen was observed on the 17th day in 1:1 dilution (figure 1) which gradually decreased with increase in dilution. Apit is also effective in inducing antheridia in *O. siliculosum*, but specificity of the antheridiogen is a little different from what was observed for *P. calomelanos* (table 2). In this case the most effective dilutions were 1:10 and 1:100. In 1:1 dilution prothalli with antheridium were observed on the 11th day while no antheridium bearing prothalli could be detected on the 9th day. Higher dilutions were less effective and on the 17th day only a few antheridium bearing prothalli were observed 1:10,000 dilution. Apit was found to be least effective in *O. siliculosum* so far as the antheridium inducing activity is concerned.




**Figures 1 and 2. 1.** Per cent prothalli with antheridium in the presence of Apit ($log_{10}$ dilution) in light. **2.** Per cent prothalli with antheridium in the presence of Apit ($log_{10}$ dilution) in dark; (○) *P. calomelanos;* (●) *O. siliculosum.*

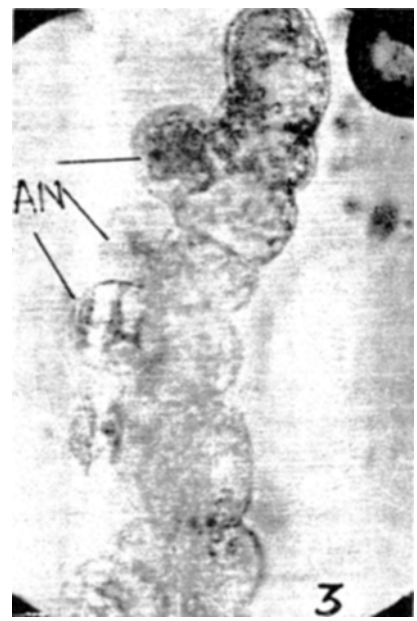



**Figure 3.** Initiation of antheridium at 1:1 concentration of Apit. AN, Antheridium

### 3.3 *Effect of Apit in light and dark in the initiation of antheridia*

The above results obtained under light condition in both the plants are plotted in figure 1 which indicates that the activity of Apit was maximum (c. 47%) in 1:1 dilution on the 17th day, and gradually decreased in higher dilutions. In *O. siliculosum*, however, it first increases and then goes on decreasing with the increase of dilution.

Under dark condition Apit is more effective in *O. siliculosum* although the antheridia

**Table 2.** Number of prothalli with antheridia after (days) inoculation in *P. calomelanos* and *O. siliculosum*.

| Apit dilution | *P. calomelanos* Days after germination 9 | 11 | 13 | 15 | 17 | *O. siliculosum* Days after germination 9 | 11 | 13 | 15 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1:1 | 5·00 | 11·00 | 17·50 | 21·50 | 47·18 | – | – | 4·50 | 10·00 | 11·50 |
| 1:10 | 4·50 | 8·60 | 15·45 | 20·00 | 32·00 | – | 5·28 | 12·75 | 15·40 | 27·25 |
| 1:100 | – | 4·25 | 6·80 | 13·40 | 19·00 | – | 4·60 | 10·25 | 11·50 | 29·00 |
| 1:1000 | – | – | 2·20 | 5·25 | 16·00 | – | – | 9·00 | 9·75 | 21·45 |
| 1:10000 | – | – | – | 5·15 | 10·50 | – | – | – | – | 3·00 |

bearing prothalli were less than what they were found in light in both the species. *P. calomelanos* was less sensitive under dark in the presence of Apit. The maximum activity that was observed in darkness was 1:100 and 1:1000 when 17 and 26·5% antheridium bearing prothalli were found to be present in *P. calomelanos* and *O. siliculosum*, respectively, and this was followed by the usual decline in the effectiveness. The decline is rapid in case of *O. siliculosum* and slow in *P. calomelanos* (figure 2).

## 4. Discussion

The present study provides information about Apit, a new antheridiogen extracted from *P. calomelanos*. The activity of Apit has been measured both at the intergeneric and interspecific levels. It is interesting to note that the activity of Apit is distinct from other antheridiogens so far reported by others (Näf 1979). Apit initiated dark germination in spores of all the three ferns tested. Earlier, it had been observed that antheridiogen which induced antheridium promoted dark germination of the spores of the same species (Näf 1966; Weinberg and Voeller 1969; Voeller 1971) with the exception of *Ceratopteris* whose antheridiogen could not induce dark germination in the same (Schedlbauer 1976). Similar activity of $GA_3$ was in *Anemia phyllitidis* where it promoted dark germination besides inducing antheridia (Schraudolf 1962), and this was later confirmed by Endo *et al* (1972).

The identical functioning of $GA_3$ and antheridiogen from *A. phyllitidis* has led to the establishment of structural similarity between the two (Nakanishi *et al* 1971). No report is available so far that antheridiogens can cancel light requirement for spore germination in others than its own.

The induction of dark germination by Apit in *P. calomelanos* was expected, but the same in *O. siliculosum* and *O. japonicum* led to the assumption of the presence of two distinct factors, one for dark germination of spores and other for inducing antheridium in Apit. The two factors are probably species-selective because the antheridiogen induced dark germination in all the three species tested but its antheridium-producing capacity was limited only to *P. calomelanos* and *O. siliculosum*, and was not extended to *O. japonicum*. It may be that the factor inducing dark germination is more active than the factor which induces antheridia. One thing, however, seems certain that the antheridiogen which induces dark germination need not necessarily induce antheridia

in the same species. The factor of light was of no help to Apit in causing antheridia formation. The inference of the observation may be taken as a point in supporting the hypothesis of the existence of independent factors responsible for inducing spore germination and antheridia.

It is expected that different antheridiogens will be different from one another as an antheridiogen is structurally different from $GA_3$ in having different $R_f$ values inspite of both possessing the property of antheridial initiation and dark germination. The difference is ascribable to the absence of intergeneric and interfamilial activity of the antheridiogens (Schdlbouer 1974). Like Apt (antheridiogen from *Pteridium aquilinum*) Apit is also found to be active within the alliance of Bower's Polypodiaceae. Yet, it can be clearly distinguished from other antheridiogens in that it is not only able to initiate antheridium but also initiate dark germination in other ferns of the family. The dilution of antheridiogen is another factor which controls germination as well as antheridium formation. Under light, germination percentage goes on increasing with the enhancement of dilution in *P. calomelanos* and *O. siliculosum* but the increase in germination in *O. japonicum* is limited at a certain dilution level after which inhibition started. The answer to why unlike *P. calomelanos* and *O. siliculosum*, the germination percentage in *O. japonicum* decreased with higher dilutions of Apit in light could, unfortunately, not be provided at this stage. Raghavan (1976, 1977) proposed that $GA_3$ activated mRNA of dry spores which is responsible for the synthesis of protein necessary for germination. The same factor may have played the role in dark germination of spores induced by Apit. The final conclusion can be drawn that Apit is species-selective for induction of antheridia and promotion of dark germination.

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