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# COMPONENTS OF ANTARCTIC PLANTS WHICH INHIBIT SEED GERMINATION

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***Abstract:*** The inhibitions of seed germination and microbe growth by extracts of antarctic plants were examined. Assays for non-specific toxin activity of the extracts were also carried out using soybean leaves. Three kinds of mosses, three kinds of lichens and five kinds of algae were extracted with methanol.

The methanol extracts were evaporated, and the residues were extracted with chloroform. The chloroform layers and the aqueous layers were subjected to assays. Among them, chloroform extracts of two lichens inhibited seed germination and microbe growth. Purification of these extracts, and structure elucidation of the active components were discussed.

## 1. Introduction

Numerous compounds are known as the components of antarctic plants (NATIONAL INSTITUTE OF POLAR RESEARCH, 1982), but research of their biological activity against microbe and plants is rare. We examined eleven antarctic plants (alga, moss and lichen) by the inhibition effects against microbe and plants, and elucidated the structure of the active components.

## 2. Materials and Methods

### *2.1. Location and samples*

Points of sampling are shown in Fig. 1, and the data of the samples are shown in Table 1. Sample Nos. 1, 2, 6, 7, 8, 10 and 11 were collected in the area of Mt. Riiser-Larsen bordered on Amundsen Bay (*ca.* 67°S, 50°E). Number 3 was collected in Skarvsnes and Nos. 4 and 5 were collected in Langhovde near Syowa Station (*ca.* 69°S, 40°E). Number 9 was collected in the area of Mt. Vechernaya near Molodezhnaya Station (*ca.* 68°30′S, 46°E).

Habitats of the samples were as follows; Algae (Nos. 1, 5 and 9) were found in small meltwater streams, marine algae (Nos. 10 and 11) were found at a depth of 1–2 m in sea water. Three kinds of mosses were found in sorted polygons on the moraine (No. 2), in a lake at a depth of 3 m in fresh water (No. 3), and along the meltwater stream (No. 4). Lichens (Nos. 6, 7 and 8) were found on gravels in sorted polygons.

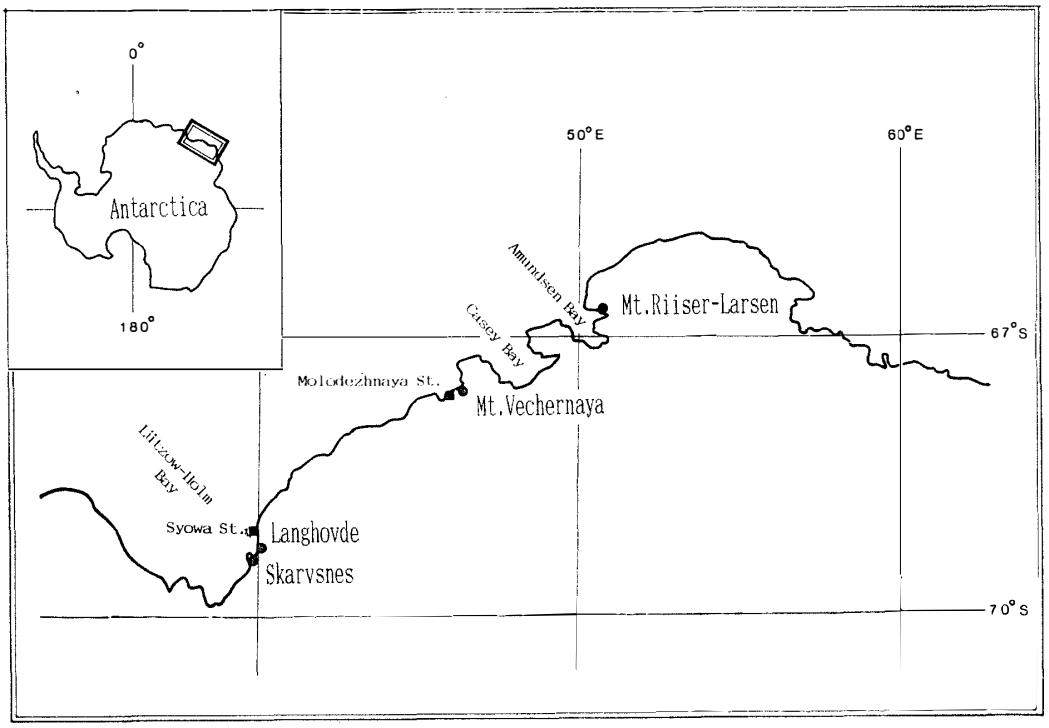


*Fig. 1. Index and location map.*

*Table 1. List of samples.*

| Samples No. | Sample | | Wet weight/ Dry weight (g) | Date | Location |
|---|---|---|---|---|---|
| 1 | *Prasiola crispa* | (alga) | 55/23.5 | 1988.02.20 | Mt. Riiser-Larsen |
| 2 | *Ceratodon purpureus* | (moss) | 575/91.6 | 1988.02.21 | Mt. Riiser-Larsen |
| 3 | *Bryum* sp. | (moss) | 540/18.1 | 1988.01.21 | Skarvsnes, M4-lake |
| 4 | *Bryum pseudotriquetrum* | (moss) | 2200/1700 | 1987.12.29 | Langhovde, Yotuike Valley |
| 5 | *Prasiola crispa* | (alga) | 150/131 | 1987.12.29 | Langhovde, Yotuike Valley |
| 6 | *Umbilicaria decussata* | (lichen) | 175/143.7 | 1988.02.20 | Mt. Riiser-Larsen |
| 7 | *Usnea sulphurea* | (lichen) | 80/70.3 | 1988.02.21 | Mt. Riiser-Larsen |
| 8 | *Physcia caesia* | (lichen) | 40/35.3 | 1988.02.21 | Mt. Riiser-Larsen |
| 9 | *Nostoc* sp. | (alga) | 195/168.4 | 1988.02.15 | Mt. Vechernaya |
| 10 | Unidentified | (marine alga) | 3500/69.4 | 1988.02.19 | Mt. Riiser-Larsen, Penguin Rookery |
| 11 | Unidentified | (marine alga) | 400/28.5 | 1988.02.19 | Mt. Riiser-Larsen, Penguin Rookery |

## 2.2. *Extraction*

Each sample was lyophilized, and 10 grams of each sample were extracted with 50 m*l* of cold methanol twice. The methanol extract was evaporated, and a small amount of water was added to the residue. It was extracted with chloroform (3 times). The chloroform phase was evaporated and 100 mg of the residue were dissolved in methanol to make standard solution (3% solution). The aqueous phase was also evaporated and the residue was dissolved in water to make standard solution.

*2.3. Seed germination assay*

A hundred $\mu l$ of each standard solution were added to 3 pieces of filter paper (diameter 20 mm). They were dried in a hood, and put into a tube. One m*l* water and seven sterilized seeds of barnyardgrass were added to the tube. The tube was kept at 25 °C for 3 days and lamina lengths were measured.

*2.4. Microbe assay*

Fifty $\mu l$ of each standard solution were added to a paper disk (diameter 5 mm) and the disk was dried in a hood. *Bacillus subtilis* or *Gliocladium virens* was suspended in sterilized water and sprayed onto a nutrient agar medium in Petri dishes. Dried disks were put onto the plates and the plates were kept at 27°C for 1 day in the dark. Then the size of the inhibition zones were measured.

*2.5. Leaf puncture assay*

This assay was carried out using leaves of soybean. The cotyledons were removed and placed into test tubes of water. A needle puncture was made, and the solution to be tested for biological activity was placed on the puncture. The leaves were placed at 25°C for 4 days, and the reaction was scored as positive or negative.

*2.6. Isolation of the active compound from No. 7*

Methanol extract of No. 7 was concentrated and stored at room temperature. After 1 day, yellow crystal (30 mg) was obtained from the extract. It was recrystalized from hot methanol. This crystal was insoluble in water, sparingly soluble in hot ethanol and easily soluble in chloroform and 1N potassium hydroxide solution. NMR spectra of the crystal were as follows. $^1$H-NMR($CDCl_3$): 1.76(3H, s), 2.11(3H, s), 2.66(3H, s), 2.68(3H, s), 5.98(1H, s), 11.04(1H, s), 13.33(1H, s) and 18.89 ppm(1H, s). $^{13}$C-NMR ($CDCl_3$): 7.5, 27.9, 31.3, 32.1, 59.1, 98.3, 101.5, 103.9, 105.2, 109.3, 155.2, 157.5, 163.9, 179.4, 191.7, 198.0, 200.3 and 201.8 ppm. $R_f$=0.47 (methanol: chloroform=7: 3). m.p. 201°C.

*2.7. Isolation of the active compound from No. 8*

Methanol extract of No. 8 (2.1 g) was subjected to silica gel column chromatography (ethyl acetate: hexane=7:3). Each fraction was subjected to the assays, and active fraction 3, was rechromatographed using the same solvent. Active fractions, 6 to 8, were combined and evaporated to dryness. The residue was dissolved in chloroform. Colorless crystal was obtained (35 mg). $^1$H-NMR($CDCl_3$): 2.10(3H, s), 2.46(3H, s), 3.92(3H, s), 5.16(1H, s), 6.21(1H, s) and 12.05 ppm (1H, s). $^{13}$C-NMR($CDCl_3$): 7.7, 24.1, 51.8, 105.2, 108.5, 110.5, 140.2, 158.0, 163.2 and 172.6 ppm. MS(CI): 197($M^+$+1), 165, 136 and 107. UV(methanol): 218.0, 270.0, and 304.2 nm. $R_f$=0.40 (ethyl acetate: hexane=3:7).

## 3. Results and Discussion

Among all samples, only the chloroform phase of Nos. 7 and 8 (lichens) had strong inhibition activity against the seeds of barnyardgrass. The same results were obtained

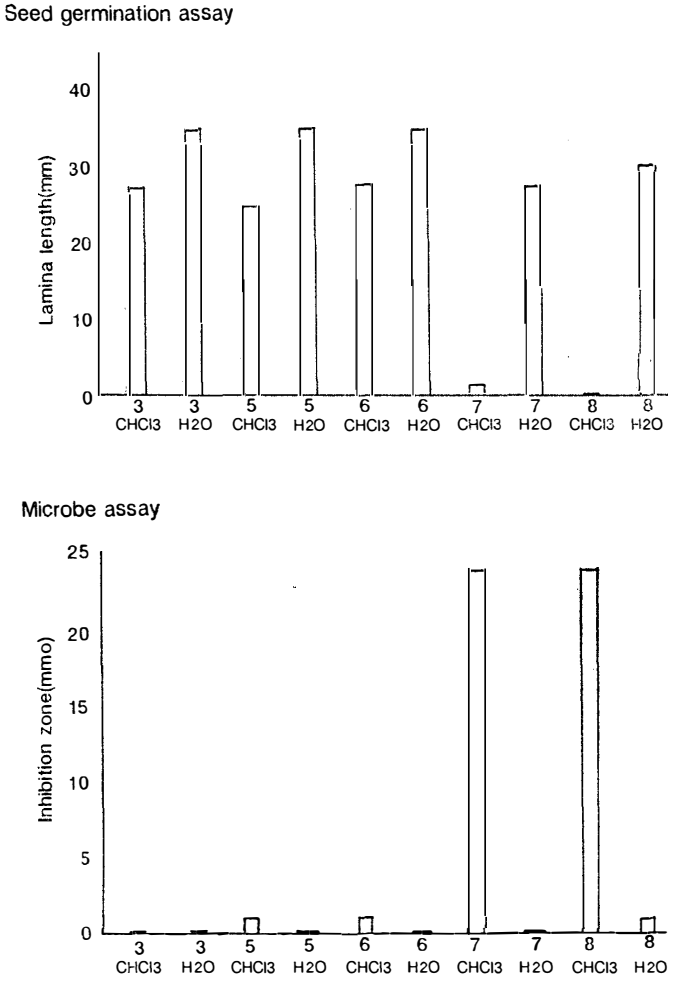


*Fig. 2. Results of assays of some samples.*

by the microbe assay. A 24-mm inhibition zone appeared around the disks of the chloroform phases of Nos. 7 and 8. Other samples showed no inhibition zone on the plate. All samples were negative by the leaf puncture assay. Figure 2 illustrates the results of these assays of some of the samples.

According to the results, we purified the active components from No. 7 and No. 8 as shown in experimental section.

In $^{1}$H-NMR spectrum of the crystal from No. 8, a signal at 12.0 ppm was assigned to a phenolic proton which had an intramolecular hydrogen bond. A signal at 5.2 ppm was also a phenolic proton, which had no intramolecular hydrogen bond. This compound also had one aromatic proton, one methoxy methyl group and two methyl groups.

The $^{13}$C-NMR spectrum shows that this compound has only 10 carbon atoms, which are carboxyl, aromatics, methoxy and two methyl carbons.

Mass spectrum shows a 197 peak which is assigned to $M^{+}+1$, so the molecular weight of the compound is 196, $C_{10}H_{12}O_{2}$. The results of the colored reactions of lichen components are K$-$, C$+$ (red) and $FeCl_{3}+$ (dark blue).

According to the data, the structure of this compound was determined as methyl

β-orcinol carboxylate (II) (Fig. 3). Chemical shifts of $^{13}$C-NMR of the compound entirely coincided with those of methyl β-orcinol carboxylate (SUNDHOLM and HUNECK, 1981). This compound is known as a component of lichens like "oakmoss" (BREHM *et al.*, 1983), but it is not known as a component of No. 8, *Physcia caesia*. However, as atranorin was reported to be the component of *Physcia caesia* (NATIONAL INSTITUTE OF POLER RESEARCH, 1982), and as atranorin is easily alcoholized to give methyl β-orcinol carboxylate (ASAHINA and SHIBATA, 1954), the crystal we obtained might be a degradation product of atranorin. The seed germination assay of this crystal showed inhibition activity against barnyardgrass. One hundred ppm (5 mmol) solution of β-orcinol carboxylate inhibited the germination of barnyardgrass.

The crystal of No. 7 was elucidated using NMR spectra and colored reactions. As expected, the data coincided with those of usnic acid (I) (Fig. 4) (ASAHINA and SHIBATA, 1954). Colored reactions also coincided with usnic acid. Less than 100 ppm (0.3 mmol) solution of usnic acid completely inhibited the germination of barnyardgrass.

IGARASHI and SASA (1988) reported that (−)-usnic acid isomethoxide was isolated from a culture medium of pathogenic fungi that infected persimon. It is very interesting that this compound shows a strong inhibitory effect on the seed germination test, and caused lesions on leaves by the leaf puncture method.

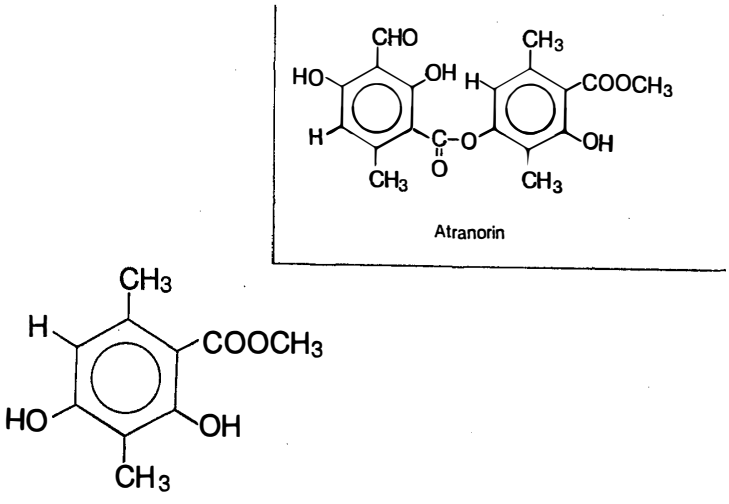


*Fig. 3. Structure of methyl β-orcinol carboxylate.*

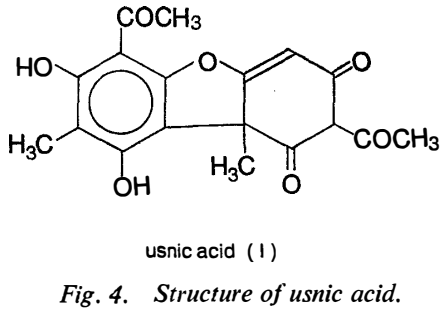


*Fig. 4. Structure of usnic acid.*

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