

Antitumour Activities of Lichen Polysaccharides

S. SHIBATA, Y. NISHIKAWA and M. TANAKA

Faculty of Pharmaceutical Sciences, University of Tokyo

F. FUKUOKA and M. NAKANISHI*

National Cancer Center Research Institute, Tokyo

Received January 17, 1968

Antitumor-Wirkung einiger Polysaccharide aus Flechten

Zusammenfassung. Einige Polysaccharide, die aus Flechten isoliert wurden, hemmen das Wachstum des Sarkoms-180 in Mäusen und verursachen in vielen Fällen vollständige Regression der Tumoren.

Summary. Some polysaccharides isolated from lichens significantly inhibited the growth of implanted sarcoma-180 in mice and caused complete tumour regression in many cases.

Previously we reported (SHIBATA et al., 1968) antitumour activities of macromolecular components of some Basidiomycetes against implanted sarcoma-180 in mice. The inhibiting effect was suggested to be host-mediated. The present study deals with the antitumour activities of the water-soluble macromolecular components of lichens. We observed first a remarkable inhibiting activity of aqueous extract of *Gyrophora esculenta* MIYOSHI (an edible lichen named in Japanese "Iwatake") against implanted sarcoma-180 by the testing method as follows: The growth of subcutaneously implanted sarcoma-180 in Swiss albino mice was observed for 5 weeks under administration of testing material by intraperitoneal injection daily for 10 days.

The tumour inhibiting activity of lichen was shown by the precipitates prepared by adding ethanol to the aqueous extracts. Some other samples prepared from the lichens of different species were tested under the same conditions. All the samples so far tested showed very high inhibiting effects against the growth of sarcoma-180, and resulted in complete regression of the tumours in many cases (Table). The repeated examinations gave no fluctuation of the results. The ethanol-precipitates contain no nitrogen. The absence of polyphenolic substances is shown by the negative ferric chloride reaction and by infrared spectra. All physical and chemical evidences suggested that the anti-tumour active substance is polysaccharide.

As the lichen polysaccharides, lichenin [$\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked glucan], (CUNNINGHAM and MANNERS, 1964; PERLIN and SUZUKI, 1962), isolichenin [$\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 4)$ linked glucan] (CHANDA, HIRST and MANNERS, 1957; PEAT, WHELAN,

* We express our deep thanks to Dr. W. NAKAHARA, the director of the National Cancer Center Research Institute, for his kind advice, Prof. D. MIZUNO for the test against Ehrlich tumour, Prof. B. LINDBERG and Prof. S. PEAT for the samples of lichen polysaccharides, and to Dr. S. KUROKAWA for his kind co-operation in lichenology. We are indebted to Mr. T. KOMIYA, Mr. L. XAVIER FILHO and Miss M. AI for their co-operations in experiments. Thanks are also due to Ministry of Health and Welfare of Japan for grant.

Table. Antitumour activities of ethanol-precipitates separated from aqueous extracts of some lichens on sarcoma-180

Samples	No. of mice	Complete regression	Average tumour weight (g)	Inhibit. ratio (%)
<i>Gyrophora esculenta</i> MIYOSHI				
Aq. extract	10	0/10	3.6	65.9
Control	10	0/10	10.5	
Ethanol ppt.	10	0/10	1.0	90.0
Control	10	0/10	10.5	
Ethanol ppt.	10	4/10	0.5	93.7
Control	10	0/10	7.4	
Commercial sample				
Ethanol ppt. ^a	6	1/6	0.2	95.9
Control	10	0/10	4.4	
<i>Cetraria islandica</i> (L.) ACH. var. <i>orientalis</i> ASAHINA				
Ethanol ppt.	9	2/9	1.2	89.3
Control	9	0/9	11.5	
<i>Usnea baylei</i> (STIRT.) ZAHLBR.				
Ethanol ppt.	9	7/9	0.3	96.4
Control	10	0/10	9.5	
<i>Cladonia mitis</i> SANDST.				
Ethanol ppt.	9	8/9	0.06	99.4
Control	10	0/10	9.5	
<i>Parmelia caperata</i> (L.) ACH.				
Ethanol ppt.	10	5/10	1.6	82.7
Control	10	0/10	9.5	

Prior to water extraction, lichens were extracted with organic solvents. — Dose: 200 mg/kg/day for 10 days.

^a 200 mg/kg × 6 and 100 mg/kg × 4.

TURVEY and MORGAN, 1961) and pustulan [$\beta(1 \rightarrow 6)$ linked glucan] (DRAKE, 1943; LINDBERG and MCPHERSON, 1954) are known, and the occurrence of some heteropolysaccharides (ASPINALL, HIRST and WARBUSTER, 1955; BUSTON and CHAMBERS, 1933) in lichens has also been reported. Almost all the lichen materials employed in the present study have not been investigated on their polysaccharides, except *Cetraria islandica* which was known to contain both lichenin and iso-lichenin. A survey work on the distribution of tumour inhibiting polysaccharides in lichens and the chemical studies on their structures are now in progress. The purified polysaccharide of *Gyrophora esculenta* has almost been established to be identical with pustulan isolated from *Umbilicaria pustulata* (L.) HOFFM. and *U. hirsuta* (Sw.) ACH., by the comparison of the infrared spectra, sedimentation patterns and optical rotatory dispersion curves with the authentic sample kindly supplied by Prof. B. LINDBERG. The polysaccharide fraction of *Gyrophora esculenta* was inactive against sarcoma-180 (ascites) and solid Ehrlich tumour by the survival test.

References

- ASPINALL, G. O., E. L. HIRST, and M. WARBUSTER: The alkali-soluble polysaccharide of the lichen, *Cladonia alpestris* (Reindeer moss). *J. chem. Soc.* **1955**, 651.
- BUSTON, H. W., and V. H. CHAMBERS: Some cell-wall constituents of *Cetraria islandica* ("Iceland moss"). *Biochem. J.* **27**, 1691 (1933).
- CUNNINGHAM, W. L., and D. J. MANNERS: Carbohydrate-metabolizing enzymes. XI. Hydrolysis of lichenin by enzyme preparations from malted barley and *Rhizopus arrhizus*. *Biochem. J.* **90**, 596 (1964).
- DRAKE, B.: Untersuchungen über einige Polysaccharide der Flechten, vornehmlich das Lichenin und das neuentdeckte Pustulin. *Biochem. Z.* **313**, 388 (1943).
- LINDBERG, B., and J. MCPHERSON: Studies on the chemistry of lichens. VI. The structure of pustulan. *Acta chem. scand.* **8**, 985 (1954).
- PEAT, S., W. J. WHELAN, J. R. TURVEY, and K. MORGAN: The structure of isolichenin. *J. chem. Soc.* **1961**, 623.
- PERLIN, S. A., and S. SUZUKI: The structure of lichenin. Selective enzymolysis studies. *Canad. J. Chem.* **40**, 50 (1962).
- SHIBATA, S., Y. NISHIKAWA, F. M. CHENG, F. FUKUOKA, and M. NAKANISHI: Antitumour studies on some extracts of Basidiomycetes. *Gann* (in press) (1968).

Prof. S. SHIBATA
Faculty of Pharmaceutical Sciences
University of Tokyo, Bunkyo-Ku
Tokyo (Japan)