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Note

A polysaccharide produced by laboratory cultivation of *Poria cocos* Wolf

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Poria cocos Wolf (Polyporaceae), a fungus that grows under the ground on the roots of pine trees, has been used in Chinese medicine as a diuretic, under the name of Hoelen or Fu-Ling (Bukuryo in Japanese). A polysaccharide named pachyman (mol. wt. ~370,000) was isolated therefrom, and shown[1] to be a linear, (1→3)-β-D-glucan possessing 9–10 branches of (1→6)-linked β-D-glucopyranosyl groups, and an internal, (1→6)-β-D-linkage.

Recently, host-mediated, antitumor activities of fungal polysaccharides were reported, and pachyman was also studied in that connection. Pachyman itself showed no antitumor activity, whereas, after treatment with periodate or urea, or carboxymethylation, host-mediated, antitumor activity was shown against implanted Sarcoma 180 in mice[2].

The present study is concerned with laboratory cultivation of *Poria cocos* in order to isolate its polysaccharide for comparison with the pachyman of naturally occurring Fu-Ling.

EXPERIMENTAL

Inoculation. — A cut piece of naturally growing sclerotium of *Poria cocos* Wolf, collected in the Chiba Prefecture, was bored with a cork borer under sterile conditions, in order to obtain a small fragment of the inner portion of sclerotium, which was then incubated at 25° in the dark on a potato–dextrose–agar medium, to form well developed hyphae. The hyphae were inoculated into liquid, Hamada's Matsutake medium [D-glucose (20 g), ammonium tartrate (1 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7\ H_2O$ (0.5 g), ferric citrate (5 mg), $ZnSO_4 \cdot 7\ H_2O$ (4.4 mg), $MnSO_4 \cdot 4$–$6\ H_2O$ (5 mg), $CaCl_2 \cdot 6\ H_2O$ (55.5 mg), nicotinic acid (0.5 mg), folic acid (0.5 mg), and dist. H_2O (1.000 L) for stationary cultivation. After growth for 2 months at 25° in the dark, the hyphae were harvested, washed with cold water, and lyophilized.

Extraction. — The lyophilized hyphae (~12 g) were extracted successively with petroleum ether, ether, acetone, 80% methanol, and water, to remove the variously soluble components. The residual hyphae were extracted with a 6M aqueous

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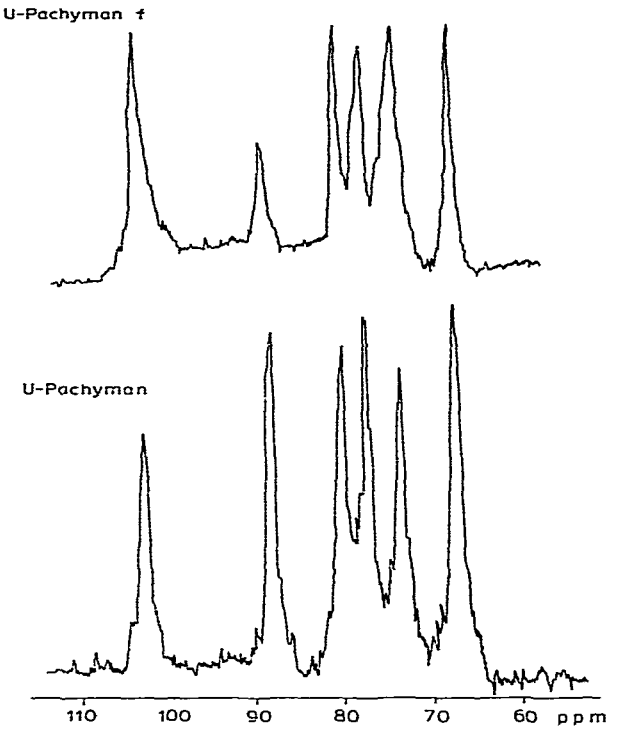

Fig. 1 ^{13}C-N.m.r. spectra of U-Pachyman f and U-Pachyman.

solution of urea on a boiling-water bath, the extract was filtered, the filtrate was dialyzed, and ethanol was added to the inner solution to afford a precipitate that was collected by centrifugation, successively washed with ethanol and ether, and dried, giving crude polysaccharide (0.4 g). The crude polysaccharide thus isolated from the laboratory-cultivated *Poria cocos* was purified by the process of freezing and thawing; yield, ~3.3% of the dry weight of the hyphae. The polysaccharide thus prepared is tentatively named U-pachyman f*.

Spectral determination of U-pachyman f. — The i.r. spectrum was recorded with a Jasco Model DS-701G spectrometer (KBr pellet); the presence of β-anomeric linkages was proved by absorption maxima at 885 and 800 cm^{-1}, which were also given by U-pachyman obtained from a natural preparation of Fu-Ling. The ^{13}C-n.m.r. spectrum was recorded with a JEOL-FX 60 spectrometer, for a solution of U-pachyman f (60 mg) in Me_2SO-d_6 (2 mL) in a tube (10 mm o.d.).

The ^{13}C-n.m.r.-spectral data for U-pachyman f, recorded under complete proton decoupling at 15 MHz, were compared with those given by pachyman and U-pachyman, and proved their identity and the presence of (1→3)-β-D-glucosyl linkages.

*U: Treated with urea; f: produced by a fermentation process–laboratory cultivation.

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Pachyman	102.8	72.8	**85.9**	68.4	76.2	61.1
U-pachyman	102.9	72.8	**86.2**	68.5	76.4	60.9
U-pachyman f	103.0	73.1	**86.4**	68.6	76.2	60.8
Methyl β-D-glucoside[3]	104.2	74.1	76.9	70.7	76.8	61.9
3-*O*-Methyl-β-D-glucose[3]	97.0	74.7	**86.2**	69.9	76.7	61.7

Determination of sugar components. — U-Pachyman f was hydrolyzed with M H_2SO_4 for 6 h at 100°. The hydrolyzate was made neutral with Amberlite IRA-47 (OH^-) resin, and the solution was analyzed with a JEOL-JLC-6AH Sugar Analyzer by the orcinol–H_2SO_4 method; absorbances at 510 and 440 nm; the results, automatically recorded, showed that the major sugar component of U-pachyman f is glucose, with a trace of mannose.

Methylation analysis of U-pachyman f. — U-Pachyman f (40 mg) was first methylated by the Hakomori method (4 times), and then twice by the Kuhn method, to afford a fully methylated derivative that gave no OH absorption in the i.r. spectrum. This product was hydrolyzed with M H_2SO_4 (3 mL), and the hydrolyzate was made neutral with Amberlite IRA-47 (OH^-) resin, evaporated to dryness, and the residue treated with $NaBH_4$ for 12 h. After treatment with Amberlite IR-120 (H^+) resin, the boric acid was removed by distillation with methanol, and the product was acetylated with 1:1 acetic anhydride–pyridine for 12 h at room temperature. The acetylated product was submitted to g.l.c. in a column (2 m × 2 mm) of 2.0% of OV 225 at 220°. The g.l.c.–mass-spectral data were recorded with a GC-MS JEOL D-300 instrument equipped with a JEOL JMA 2000 disc system under the following conditions: acceleration voltage 3.00 kV, ionization energy 70 eV, ion-source temperature 190°, and vacuum $<10^{-7}$ torr.

The g.l.c.–mass spectrum of the alditol acetate derivatives derived from the hydrolyzate of permethylated U-pachyman f showed that 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucitol was the major product; its m.s. fragment-peaks agreed well with those given by the corresponding product derived from naturally occurring pachyman.

All of the evidence obtained in the present study showed that the major polysaccharide, U-pachyman f, produced by the laboratory cultivation of *Poria cocos* is almost identical with the U-pachyman prepared from naturally occurring Fu-Ling, the sclerotium of *Poria cocos*.

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