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Chemical Analysis of Antineoplastic Components of *Coriolus versicolor* (L. ex Fr.)Quél., *Pleuroteus ostreatus* (Fr.)Kummer, and *Lentinus edodes* (Berk.)Sing.

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Abstract ☐ The carpophores of three Korean mushrooms, *Coriolus versicolor, Pleurotus ostreatus,* and *Lentinus edodes* were respectively extracted with hot water and the extracts were dialyzed through Visking tube. They were found to contain an antitumor activity against sarcoma-180 implanted in mice. The components of these aqueous extracts were found to be polysaccharide and protein by color reactions including anthrone and Lowry-Folin tests. The hydrolysis of the polysaccharide with 3% HCl-MeOH and trimethylsilylation yielded four monosaccharides: glucose, mannose, galactose and xylose which were identified by G.L.C. After hydrolysis of protein with 6N HCl, fourteen to seventeen amino acids including aspartic and glutamic acids were detected by an amino acid analyzer.

Keywords ☐ Basidiomycetes—the family *Polyporceae—Coriolus versicolor, Pleurotus ostreatus,* and *Lentinus edodes.* Chemical analyses of the antineoplastic components—identification of four monosaccharides: glucose, mannose, galactose and xylose of the antitumor polysaccharides. Identification of 14~17 amino acids of the protein fractions of the antitumor extracts.

As previously reported[1], the aqueous extracts from three Korean mushrooms, *Coriolus versicolor* (L. ex Fr.) Quel., *Pleurotus ostreatus* (Fr.)Kummer and *Lentinus edodes* (Berk.)Sing. had very high antineoplastic activity against sarcoma-180 implanted in mice[1~7]. This paper reports that these extracts are protein-bound polysaccharides.

MATERIALS AND METHODS

Materials

Three mushrooms, *Coriolus versicolor, Pleurotus ostreatus* and *Lentinus edodes* were used as in the previous report[1].

Extraction and Separation[8~12]

These procedures are also the same as those of the previous report. Three aqueous extracts from three Korean mushrooms were prepared in the same manner as the report.

Color Reactions[13,14]

A). Molish test: The sample solution (2 ml) was thoroughly mixed with naphthol (5.0 % ethanol solution) and one ml of c-H_2SO_4 was added carefully to make two layers.

B). Anthrone test: Two milliliters of the sample soultion were thoroughly mixed with two ml of anthrone (0.2% c-H_2SO_4 solution).

C). Tryptophan test: 77% H_2SO_4 (7 ml) was added to one ml of the sample solution and the mixture was cooled at 10~15° C. One

milliliter of 1% fresh aqueous tryptophan solution was added and warmed on a boiling water bath for 20 minutes to be cooled to room temperature.

D). Iodine test: Two milliliters of sample solution were mixed with one drop of diluted hydrochloric acid and two ml of iodine solution were added to observe the change of reaction color.

E). Ninhydrin test: Two milliliters of sample solution were mixed with one per cent aqueous ninhydrin solution (2 ml) at pH 7 and warmed on a boiling water bath for two minutes.

F). Ninhydrin test after hydrolysis: Two hundred milligrams of the sample were hydrolyzed with 6N HCl at 110±5°C for 24 hours in an ampule filled with nitrogen gas and filtered to be tested with ninhydrin solution as the above.

G). Biuret test: One per cent $CuSO_4 \cdot 5H_2O$ solution (2–3 drops) was added to two ml of the sample solution to be mixed with two ml of lN-NaOH.

H). Lowry-Folin test: One milliliter of the sample solution was mixed with one ml of alkaline sodium carbonate-cupric sulfate solution to be settled for 10 minutes. Folin-Ciocalteau reagent (0.5 ml) was added to observe change of color reaction.

Analysis of Polysaccharides

The quantitative analysis of the sample was conducted by using anthrone solution, and glucose was used as control. The color density was measured at 625 nm with Spectronic 20 (Bauch & Lomb Co.). By a calibration curve, the content of polysaccharide of the samples was calculated. To determine the identity and content of monosaccharides, five mg of samples were dissolved in 3% HCl-MeOH and methanolyzed at 100±5°C for 20 hours in an ampule filled with nitrogen gas. The filtrate was evaporated in vacuum and dissolved in one ml of pyridine. Hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) were added to pyridine solution and stirred vigorously for 30 seconds. After trimethylsilylation, monosaccharide analysis was processed by GLC[15,16] as shown in Table I. Each monosaccharide standard was trimethylsilyllized as the above and the retention time was measured with GLC. The identification of monosaccharides was accomplished by comparing retention times with those of standard monosaccharides. To determine the content of monosaccharides, a weight ratio of monosaccharides was calculated on the basis of the peak area of GLC chromatogram with half width (HW) method and planimetry.

Protein Analysis[17]

For the quantitative analysis of protein, samples were subjected to Lowry-Folin test and the absorbance of color was measured at 720 nm. By a calibration curve prepared by

Table I: Measurement condition (G. C.)

Column	3 % OV-17(80–100 mesh shimalite) 3 mm ϕ × 2m boronsilicate glass column.
Temperature	Column : 130°C Detector: 170°C
Flow rate	N_2 : 50 ml/min H_2 : 60 ml/min (0.8 kg/cm^2) Air : 88 ml/min (1.2 kg/cm^2)
Attenuation	16 × 10^2 a. f. s. (ampere full scale)

Table II: Measurement condition (amino acid analyzer)

Column size	9 mm ID × 550 mm 9 mm ID × 100 mm
Ion exchange resin	Hitachi-custom ion exchange 2613 Hitachi-custom ion exchange 2611
Flow rate	Buffer solution : 60 ml/hr Ninhydrin : 30 ml/hr
Wavelength	15 mm tubular flow cell. 570 nm(red) 440 nm(green)
Buffer solution	pH 3.25, pH 4.25. pH 5.28 sodium citrate buffer solution
Column temperature	55°C
Reaction bath temperature	100°C

using albumin as control, the protein content of the sample was determined. To determine the identity and content of amino acids, 200 mg of the samples were dissolved in 50 ml of 6N HCl and hydrolyzed at 110 ± 5°C for 24 hours in an ampule filled with nirogen gas. After filtration, the filtrate was evaporated in vacuum and dissolved in 0.1N HCl. The solution was diluted with pH 2.2 citrate buffer solution and analyzed for amino acids by an amino acid analyzer (Hitachi amino acid autoanalyzer, Model KLA-5) as shown in Table II.

The standard solution was prepared by adjusting their concentration to 2.0 μmoles/ml with 0.1N HCl and diluting with the buffer. They were also chromatographed by the amino acid analyzer. Compared with the chromatogram of the standard amino acids, the amino acids of the samples were identified and the contents of the amino acids were calculated on the basis of the peak area by HW method.

RESULTS AND DISCUSSION

Color Reactions

The results of the color reactions of the samples were summarized in Table III. The results showed that the extracts of the mushrooms contained protein-bound polysaccharides. The extracts of *Pleurotus ostreatus, Lentinus edodes,* and *Coriolus versicolor* were, respectively, designated as No. 405, No. 412 and No. 419 as shown in the previous reports.

Table III: Results of various color reactions on the mushroom extracts

Color reaction	Mushroom extracts		
	No. 405	No. 412	No. 419
Molish test	++ purple	++	++
Anthrone test	++ dark green	++	++
Iodine test	−−	−−	−−
Tryptophan test	++ violet-brown	++	++
Ninhydrin test	+ blue-violet	−	−
Ninhydrin test after acid hydrolysis	++ violet	+	++
Biuret test	+ puple-blue	+	+
Lowry-Folin test	++ dark blue	++	++

The Contents of Polysaccharides

The polysaccharide contents of the samples were shown in Table IV. The major monosaccharide was found to be glucose. The retention times of the monosaccharides were listed in Table V.

Table IV: Contents of the sugars of the polysaccharide fraction in the mushroom extracts

	Mushroom extracts		
	No. 405	No. 412	No. 419
Polysaccharide Content(%)	40	80	55
Monosaccharide Contents (%)			
Glucose	61.2	85.9	64.9
Mannose	12.5	6.9	16.0
Galactose	9.4	5.6	2.5
Xylose	trace	1.4	16.5

Table V: Retention times of TMS-monosaccharides by GLC

Standard monosaccharide	Retention time
Glucose	14.5
Galactose	11
Fructose	7.5
Mannose	8
Sorbose	7.2
Arabinose	3.3
Xylose	4.6
Rhamnose	3.5

Table VI: The content of amino acids in the protein fraction of the mushroom extracts

	Mushroom extracts		
	No. 405	No. 412	No. 419
Protein Content(%)	32	20	39
Amino Acid Content (%)			
Lysine	5.78	3.98	3.61
Histidine	1.78	0.46	1.60
Arginine	1.88	0.37	none
Aspartic acid	13.87	16.11	13.51
Threonine	7.65	10.28	4.88
Serine	8.08	10.94	4.22
Glutamic acid	14.15	12.18	13.09
Proline	1.16	0.94	0.95
Glycine	11.67	13.26	12.28
Alanine	10.53	8.77	11.24
Cysteine	trace	none	none
Valine	6.85	8.86	10.83
Methionine	trace	none	none
Isoleucine	5.55	4.92	5.66
Leucine	6.70	7.27	10.73
Tyrosine	1.67	0.74	2.16
Phenylalanine	2.67	0.92	4.63

* Ammonia was also detected.

The Amino Acid Contents of Proteins

The protein contents of the samples were listed in Table VI. They contained a small amount of arginine and sulfur-containing amino acids but a large quantity of aspartic and glutamic acids. The acidic amino acids were much more abundant than the basic amino acids. The amino acid contents of the proteins were also shown in Table VI.

CONCLUSION

The aqueous extracts of three Korean mushrooms, *Pleurotus ostreatus, Lentinus edodes,* and *Coriolus versicolor,* which have previously shown very high antineoplastic activity against sarcoma-180 implanted in mice, were found to contain protein-bound polysaccharides. The polysaccharide consisted of four monosaccharides, glucose, mannose, galactose and xylose. In the protein portions of the three extracts, 17, 15, and 14 amino acids were respectively identified.

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