CHAPTER 5: EXTRACTION, DEVELOPMENT AND CHEMISTRY OF ANTI-CANCER COMPOUNDS FROM MEDICINAL MUSHROOMS

Synopsis

The main antitumour compounds presently isolated from mushroom fruit-bodies, submerged cultural mycelial biomass or liquid culture broth have been identified as either water soluble β-D-glucans with heterosaccharide chains of xylose, mannose, galactose or uronic acid or β-D-glucan-protein complexes – proteoglycans. Methods of extraction and purification are outlined. Levels of anti-cancer activity are related to molecular weight, degree of branching and solubility in water of the respective molecules. The main medically important polysaccharide compounds to have achieved clinical relevance, viz. Lentinan, Schizophyllan, PSK and PSP, and Grifron-D are discussed.

Hot water extracts of many mushrooms used in traditional Chinese medicine and other folk medicines have long been said to be efficacious in the treatment of various diseases including many forms of cancer. The use of medicinal mushroom extracts in the fight against cancer is well known and documented in China, Japan, Korea, Russia and now increasingly in the USA (Mizuno *et al*., 1995). However, it is only within the last three decades that chemical technology has been able to isolate the relevant compounds and use them in controlled experiments. They have been extensively screened for medical properties especially for anticancer application (Mizuno, 1999). Many species of mushrooms have been found to be highly potent immune system enhancers, potentiating animal and human immunity against cancer (Wasser and Weis, 1999a, Borchers *et al*., 1999, Kidd, 2000; Ikekawa, 2000; Feng *et al*., 2001). While at least 30 mushroom species have yielded compounds with pronounced anticancer actions in xenographs only a small number have taken the next step, viz. objective clinical assessment for anticancer potential in humans.

Polysaccharides

Polysaccharides are a structurally diverse group of biological macromolecules of widespread occurrence in nature. They are composed of repetitive structural features that are polymers of monosaccharide residues joined to each other by glycosidic linkages. In this way they differ structurally from proteins and nucleic acids. Polysaccharides present the highest capacity for carrying biological information since they have the greatest potential for structural variability. The amino acids in proteins and the nucleotides in nucleic acids can interconnect in only one way while the monosaccharide units in oligosaccharides and polysaccharides can interconnect at several points to form a wide variety of branched or linear structures (Sharon and Lis, 1993). As a consequence, this enormous potential variability in polysaccharide structure allows for the flexibility necessary for the precise regulatory mechanisms of various cell-cell interaction in higher organisms such as man.

Many, if not all, Basidiomycete mushrooms have been shown to contain biologically active antitumour and immunostimulative polysaccharides. In a recent review Reshetnikov *et al*. (2001) have listed 650 species and 7 intraspecific taxa from 182 genera of higher Hetero- and Homo-basidiomycetes that contain pharmacologicaly active polysaccharides that can be derived from fruit-bodies, culture mycelium and culture broths. In general, there is normally a higher level and number of different polysaccharides extracted from fruit-bodies than from the other cultural sources. As discussed in Chapter 9 an important direction for future studies on mushroom polysaccharides will be by submerged fermenter culture to produce reliable, consistent and safe products.

The first definitive studies on these anticancer substances came in the late 1960s with the reports by Ikekawa *et al.* (1968, 1969) and Chihara *et al*. (1969, 1970). They demonstrated that extracts of several different mushroom species exhibited remarkable host-mediating antitumour activities against xenographs, e.g. Sarcoma 180. These observations brought immediate public attention. In both studies the compounds were easily extracted with hot water, and shown to be various types of polysaccharides. The polysaccharides are non-toxic and appear to affect tumours indirectly following administration, suggesting that the anticancer action is mainly host-mediated. The species xenograph, suitable dosage and schedule, are essential to achieve the anti-tumour effects (Jong and Donovick, 1989, Jong *et al*., 1991).

Antitumour polysaccharides isolated from mushrooms (fruit-body, submerged cultured mycelial biomass or liquid culture broth) are either water-soluble β-D-glucans, β-D glucans with heterosaccharide chains of xylose, mannose, galactose, and uronic acid or β-D-glucan-protein complexes – proteoglycans (Table 1). As a general rule the protein-linked glucans have a greater immunopotential activity than the corresponding glucans. Polysaccharide antitumour agents that have been developed commercial in Japan are shown in Table 2 and Fig. 1 (Mizuno, 1999).

Table 1 Antitumour active polysaccharides isolated from medicinal higher Basidiomycete mushrooms (from Wasser and Weis, 1999b).

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Phragmobasidiomycetes			
Auriculariales			
Auriculariacea			
Auricularia auricula-judae (Bull.) Wettst.	(1-3)- β-glucan	-	-
Tremellales			
Tremellaceae			
Tremella fuciformis Berk.	Glucuronoxylomannan, T-7, T-19 (exopolysaccharides), mannose, xylose, glucuronic acid	Glucuronoxylomannan	Xylose, glucuronic acid, mannose
T. mesenterica Ritz.:Fr.	β-D-glucuronosyl (epitope)	-	
Homobasidiomycetes			
Aphyllophoromycetideae			
Ganodermatales			
Ganodermataceae			
Ganoderma lucidum (Curt.:Fr.) P. Karst.	FI-1a (β-glucan), FIII-2b (hetero-β-glucan), acidic heteroglucan, chitin xyloglucan	-	β-glucan
G. applanatum (Pers.) Pat.	FI-1-B-1 (β-glucan)	F-1a-1-b (β-glucan), heteroglucans, peptidoglucans	-
G. tsugae Murr.	Heteroglucan, heterogalactan, β-glucan, glucan	Heteroglucan, α-glucan	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Polyporales			
Schizophyllaceae			
Schizophyllum commune Fr.:Fr	-	-	Sonifilan, SPG or Schizophyllan (β-glucan)
Polyporaceae			
Dendropolyporus umbelliatus (Pers.:Fr.) Jül.	GU-2, GU-3, GU-4, AP (β-glucan)	-	β-glucan
Grifola frondosa (Dick.:Fr.) S.F. Gray	Grifolan (β-glucan), Fa-1a-β (acidic β-glucan), FIII-2c (hetero-β-glucan), xyloglucan, mannoglucan, fucomannoglucan	Heteroglucan protein, manogalactofucan, heteroxylan, fucoxylan, galactomannoglucan	-
Fommes fomentarius (L.:Fr.) Fr.	β-glucan	β-glucan	-
Fomitopsis pinicola (Schw.:Fr.) P.Karst	F-1a-2-β (β-glucan) α-(1-6)-linked	α- and β-glucan	-
Albatrellus confluens (Alb. et Schw.:Fr.) Kotl. et Pouz.	(1-3)- β-D-glucan	(1-3)- β-D-glucan	-
Trametes versicolor (L.:Fr.) Lloyd	β-glucan	Coriolan, PSK, Krestin (β-glucan -protein)	-
Lenzites betulinus (L.:Fr.) Fr.	β-glucan	-	-
Wolfiporia cocos (Schw.) Ryv. et Gilbn.	Pachymaran (β-glucan)	-	-
Hericium erinaceus (Bull.:Fr.) Pers.	β-glucoxylan, glucoxylan protein, galactoxyloglucan protein	-	-
Ionotus obliquus (Pers.:Fr.) Bound.et Sing.	Polysaccharide fraction in the Allium-test	-	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Gasteromycetideae, Phallaceae			
Dictyophora indusiata Fisch.	T-2 HN (O-acetylated-(1-3)- β-D-mannan), T-3-M[1] (α-(1-3) linked D-mannan) , T3-G, T-4-N, T-5-N (three kinds of β-D-glucans), T-3 Ad (Neutral heterogalactan)	-	-
Phallus impudicus L.:Pers.	Pl-2 (glucomannan)	PI-2 (glucomannan)	-
Lentinus edodes (Berk.) Sing.	Lentinan (β-D-glucans)	KS-2-a-mannan-peptide, LEM, LAP (heteroglucan-protein), EP3	LEM, LAP (heteroglucan-protein), EP3
Pleurotus ostreatus (Jacq.:Fr.) Kumm.	Acidic polysaccharide fraction, HA (β-glucan)	-	β-glucan, heteroglucan
P. chitrinopileatus Sing.	Heteroglucan, β-glucan-protein, glycoprotein (FI, FII, FIII)	-	-
P. pulmonarius (Fr.:Fr.) Quél. (=P.sajor-caju Fr.:Fr.) Tricholomataceae	Xyloglucan, xylanprotein	-	-
Panellus serotimus (Pers.:Fr.) Kühn.	Heteroglucan, (1-6)- β-d-glucosyl-branched (1(2-3)- β-D-glucans	-	-
Omphalina epichysium (Pers.:Fr.) Quél	EL-2 (β-glucan)	-	-
Flammulina velutipes (Curt.:Fr.) P.Karst.	EA_6, EA_6-PII (β-glucan-protein)	Proflamin (glycoprotein)	-

Taxa	Fruiting body	Submerged cultured mycelial biomass	Liquid cultured broth
1	2	3	4
Leucopaxillus giganteus (Fr.)Sing.	Mannoxyloglucan, heteroglucan, glucan, xyloglucan, xylogalacetoglucan, galactoxyloglucan	-	-
Hypsizygus marmoreus (Peck) Bigel.	β-(1-3)-D-glucan	-	-
Agaricaceae			
Agaricus blazei Murr.	FI_1-a-β (β-glucan), FIII2-β (β-glucan-protein), FA-1a-β (hetero-β-glucan), FA-2b-β (RNA), FV-1 (insoluble β-glucan)	ATOM (glucomannan-protein)	AB-FP (mannan-protein)
A. bisporus (J.Lge) Imbach	β-glucan	-	-
Pluteaceae			
Volvariella volvacea (bull.:Fr.) Sing.	VVG (β-1-3)-D-glucans, α-manno-β-glucan	-	-
Strophariaceae			
Pholiota nameko (T.Ito) S.Ito et Imai	Galacto-β-glucan	-	-
Crepidotaceae			
Crepidotus mollis (Schaeff.:Fr.) Kumm.	CPS (β-glucan)	-	-
Bolbitiaceae			
Agrocybe aegerita (Brit.) Sing.	α-(1-3)- β-glucans	-	-

Table 2 Polysaccharide antitumor agents developed in Japan (immunotherapeutical drugs as biological response modifiers, BRM) (Mizuno, 1999)

Name of drug	Krestin	Lentinan	Sonifilan
Abbreviation	PSK	-	SPG
Common Name	Krestin	Lentinan	Schizophyllan
Company	Sankyo, Kureha	Ajinomoto, Yamanouchi, Morishita	Taito, Kaken
Marketed date	May 1977	December 1985	April 1986
Fungus (origin)	*Trametes versicolor* (mycelium)	*Lentinus edodes* (fruit body)	*Schizopyllum commune* (medium product)
Polysaccharide Structure	β-glucan-protein -1,6- branching -1,3: 1,4-main chain	β-glucan -1,6-branching -1,3-main chain	β-glucan β-1,6-branching β-1,3-main chain
MW	100,000	500,000	450,000
Specific rotation	-	+ 14-22^{o} (NaOH)	+ 18-24^{o} (water)_
Pharmaceutical	1-g sack	1-mg vial	20-mg ampoule (2 ml)
Price	¥ 1,000	¥ 9,500	¥ 9,500
Dose route	p.o.	i.p., i.v.	i.p., i.v.
Cancer treated	Cancer of digestive organ, lung and breast	Cancer of stomach	Cervical cancer

Exopolysaccharides in culture media can be extracted by simply adding 96% ethanol (volume ratio 1:1), the precipitate collected by centrifugation, dissolved in distilled water and dialysed against distilled water for 2 days. The homogeneity of the exopolysaccharides can then be analysed by gel filtration through Sephadex G-200 (Babitskaya *et al*., 2000).

Fig. 1 Three mushrooms from which the antitumour polysaccharide agents have been developed in Japan and China. A: Krestin (PSK) from *Trametes versicolor* (mycelium); B: Lentinan from *Lentinus edodes* (fruit body); and C. Schizophyllan from *Schizophyllum commune* (medium product) (Mizuno, 1999).

Extraction, fractionation, purification and chemical modification

There is a broad similarity in the various methods that have been developed to extract the anti-cancer polysaccharides from mushroom fruit-bodies, mycelium and liquid media (Mizuno, 1999).

In the initial step dried mushroom powder or mycelium is repeatedly heated in 80% ethanol to extract and eliminate low molecular weight substances. Crude fractions 1, 11 and 111 are obtained from the remaining ethanol extract residue by extraction with water (100^{o}C, 3h), 1% ammonium oxalate (100^{o}C, 6h) and 5% sodium hydroxide (80^{o}C, 6h) in that order (Fig. 2). Further purification of the polysaccharides are achieved by a combination of techniques including ethanol concentration, fractional precipitation, acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration and affinity chromatography (Fig. 3).

There is a growing interest in increasing the activity of medicinal mushroom polysaccharides by various chemical modifications and perhaps creating a range of semi-synthetic compounds not unlike the penicillin story. Chemical modification can be achieved by oxido-reductohydrolysis (Smith degradation) and also by formolysis. Some positive improvements in activity have been recorded but it is still at a very early stage (Mizuno, 1999).

A recent study by Yap and Ng (2001) has established a more efficient procedure for the extraction of β-D-glucans from *Lentinus edodes* (Fig. 4). The β-D-glucan was isolated through ethanol precipitation and freeze-drying in liquid nitrogen. Purity testing, using a carbohydrate analysis column, gave 87.5% purity. From a commercial aspect this method is less time-consuming, more efficient and of relatively low cost when compared to the original Chihara *et al*. (1970) and Mizuno (1999) methods (Table 3).

Fig. 2 Fractional preparation of polysaccharides from mushrooms (Mizuno, 1999).

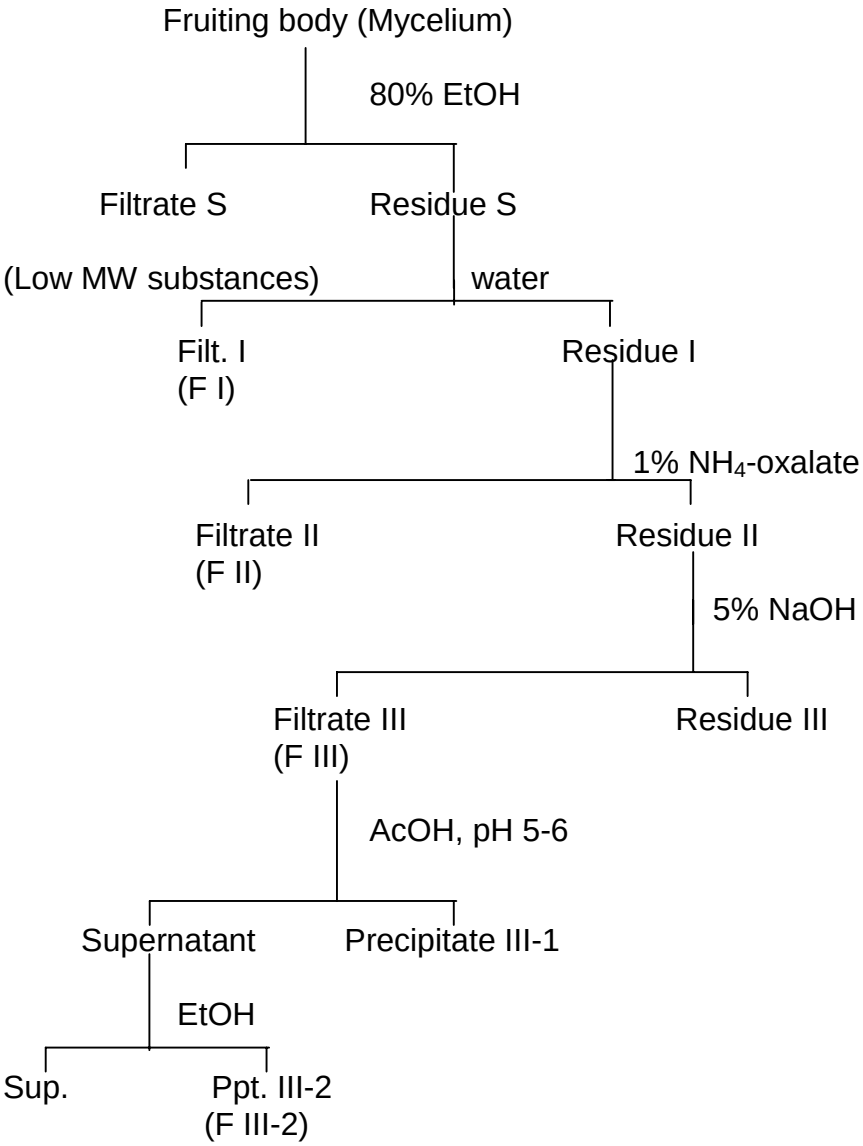


Fig. 3. Fraction purification of polysaccharides by chromatography (Mizuno 1999).

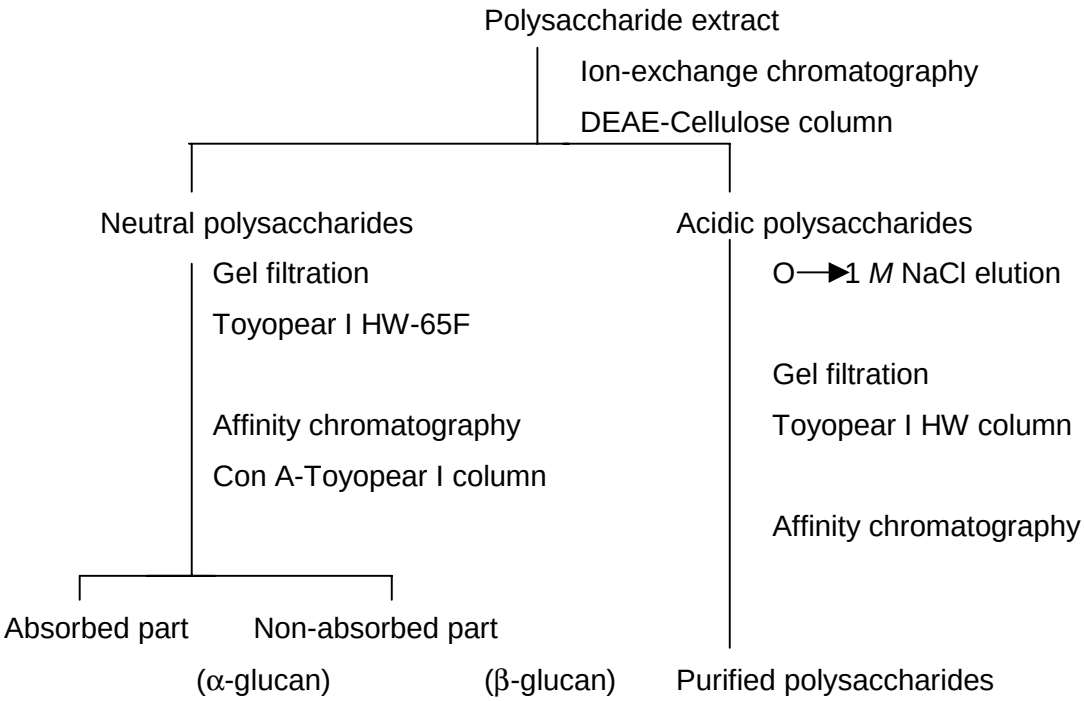


Fig. 4 New method for extracting lentinan from *Lentinus edodes* (Yap and Ng, 2001).

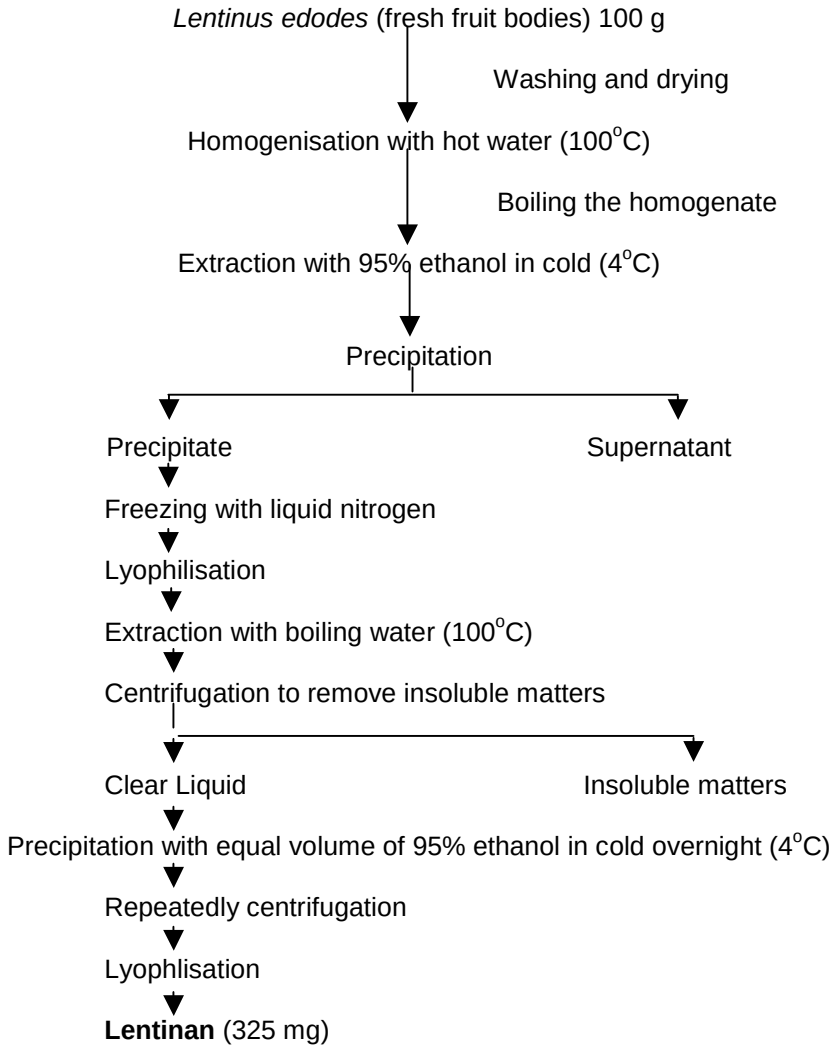


Table 3 Comparison of two methods of preparation of β-D glucan from *Lentinus edodes* (adapted from Yap and Ng, 2001)

Characteristics of methods	Method of extracting lentinan	
	Chihara's method	**New biochemical method**
Number of days taken to prepare extract	14	5
Requirement of sophisticated equipment or rarely used chemicals	Many	None except liquid nitrogen
Cost of preparation	High	Low
Total yields from 100g of fresh mushrooms	4 mg	325 mg
Percentage concentration of lentinan in extract produced (%)	96.03	87.50
Purity obtained	99.23	87.65

β-D-glucans

The basic β-D-glucan is a repeating structure with the D-glucose units joined together in linear chains by beta-bonds (β). These can extend from carbon 1 of one saccharide ring to carbon 3 of the next (β1-3), from carbon 1 to carbon 4 (β1-4) or from carbon 1 to carbon 6 (β1-6). Mostly there is a main chain which is either β1-3, β1-4 or mixed β1-3, β1-4 with β1-6 side chains. The basic repeating structure of a β1-3 glucan with β1-6 side chains is shown in Figs, 5 and 6. Levels of anticancer activity are related to their molecular weight, branching and solubility in water. The study of their steric structures by NMR analyses and X-ray diffractions clarified that active β-D-glucan shows a triple-stranded right-winding helix structure (Bluhm and Sarco, 1977). Not all β-D-glucans contained in fungi exhibit antitumour activity. The extent of occurrence of this activity seems to be influenced by solubility in water, size of molecules, and the β-(1-6)-bonding system in the β-(1-3) major chain. Some of the water insoluble β-glucans are soluble in dilute alkali and then can show marked antitumour activity (Bohn and BeMillar, 1995).

Lentinan from *L. edodes* and Schizophyllan from *S. commune* are the two best studied and commercially available β-D-glucans and have been shown to have strong immunomodulating and anticancer properties (see Chapters 6 and 7). They consist of a main chain of (1->3)-linked β-D-glucopyranosyl units with β-D-glucopyranosyl branch units linked 1->6 at, on average, an interval of three main chain units, degree of branching (DB 0.33), and have average molecular weights of 500,000 and 450,000 respectively (Sasaki and Takasuka, 1976). Within each batch of these β-D-glucans there can be considerable variation in molecular size. It has been suggested that immune response to β-D-glucans could be in part non-specific and determined by size rather than by chemical structure (Bohn and BeMillar, 1995).

Individual species-derived β-D-glucans have unique molecular structures (Ohno *et al*., 1988) and it has been surmised that the higher ordered structures (triple helices) of high molecular weight β-D-glucans could be responsible for the considerable immunomodulatory activity (Maeda *et al*., 1988). Only higher molecular weight molecules apparently form triple helical structures which are stabilised by the β-D-glucopyranosyl branch units (Saito *et al*., 1991). There is good evidence to propose that both Lentinan and Schizophyllam are active only when they exist in a single helical structure (Saits *et al*., 1991).

Clinical use of Lentinan and Schizophyllan as immunotherapeutic agents for cancer treatment will be discussed in Chapter 7. From a structure-activity concept it has been suggested that the antitumour activity of (1->3)- β-glucans resides in the helical conformation of the glucan backbone, possibly triple-stranded, but perhaps, even more important, is the presence of hydrophyllic groups located on the outside surface of the helix. Furthermore, increased water solubility favours enhanced

antitumour activity while the location of substituent groups would also be important (Bohn and BeMillar, 1995).

Recent studies have demonstrated that the concentration of polysaccharides in certain medicinal mushroom species can be related to the stage of development of the mushroom fruitbody and also to the time after harvest and subsequent storage conditions (Minato *et al*., 1999, 2001). Immunomodulating activities of extracts from *L. edodes* decreased rapidly when the mushrooms had been stored at 20^{o}C for 7 days while no decrease occurred at low temperature storage (1^{o} and 5^{o}C). The decrease in activity was related to the decrease in concentration of Lentinan which was degraded by internal β-glucanase activity (Minato *et al*., 1999). A similar series of experiments on the immunomodulating activity of extracts from *L. edodes* and *G. frondosa* showed, in each case, an increase in activity during growth and development of the fruitbody followed by a decrease at the final stages of maturation. These activities were paralleled by similar concentration changes in Lentinan and Grifron, the respective β-glucans (Minato *et al*., 2001).

These observations are highly significant both from a pharmaceutical and functional food point of view. It becomes imperative that medicinal mushrooms should be harvested at the optimum β-glucan concentration in the fruitbody and also that the harvested fruitbodies should be stored at correct temperature conditions before processing or consumption. Such results must surely compromise the use of medicinal mushrooms derived for distant parts which must involve inadequate environmental conditions and subsequent loss of β-glucans. As a result of these studies it is obvious that the pattern of polysaccharide formation in other medicinal mushrooms should be examined. Where polysaccharides are produced by

fermentation processes it is much easier to then harvest at optimum production points as is already practised in other fermentations such as with antibiotics.

Heteropolysaccharides and Glycoproteins

While water-soluble β-D-glucans are widely distributed in mushroom species, many species also contain β-D-glucans with heterosaccharide chains of xylose, mannose, galactose and uronic acid which can be extracted by salt and alkali treatments. Other species can contain polysaccharide-peptides or glycoproteins which are polypeptide chains or small proteins to which polysaccharide β-D-glucan chains are stably attached (Boldizsar *et al*., 1998) (Fig. 7).

Hot water extracts from *Grifola frondosa*, the Maitake mushroom, contain the D-Fraction which appears to be a highly active anticancer agent for both animals and humans (Jones, 1998; Maitake Products Inc., 1998). The D-Fraction is obtained from the hot water crude extract by deproteination. Maitake D-Fraction contains mainly β-D-glucan with 1-6 main chains and 1-4 branchings together with the more common 1-3 main chains and 1-6 branching.

Ganoderma lucidum, the Reishi mushroom, contains β-D-glucan in hot water extracts together with glucuronoglucan, xyloglucan, unannoglucan, xylomannoglucan and other active heteroglucans and protein complexes. Purifications involve using salts, alkali and DMSO (Mizuno *et al*., 1984).

Fig. 5 Primary molecular diagram of mushroom beta-D-glucan (Kidd, 2000)

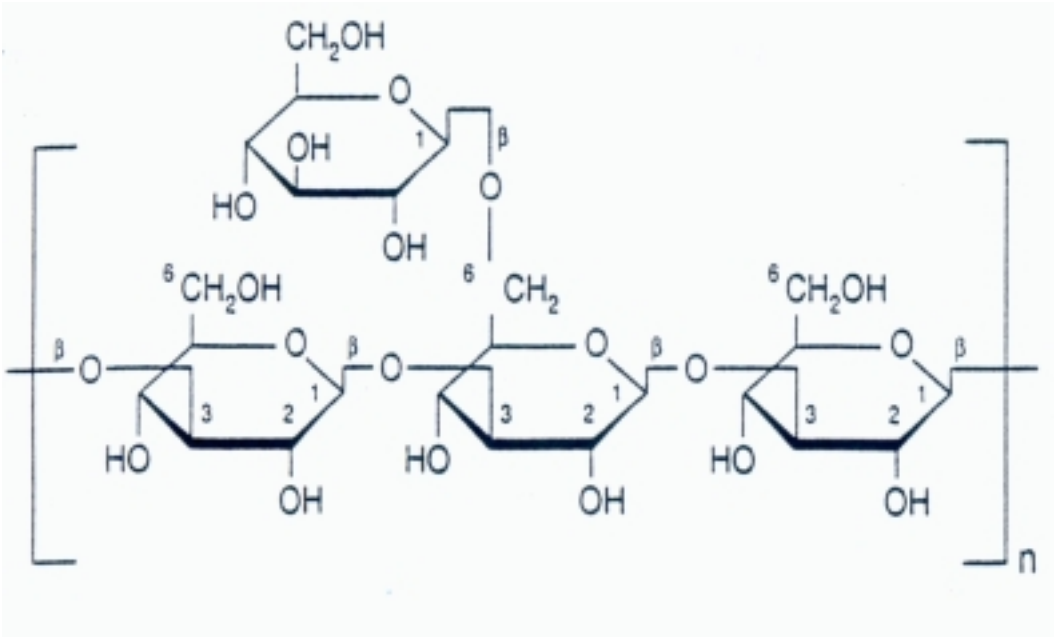


Fig. 6 Molecular model of the right-handed triple spiral helix of antitumour-active-beta-D-glucan (Schizophyllan) (Mizuno, 1999).

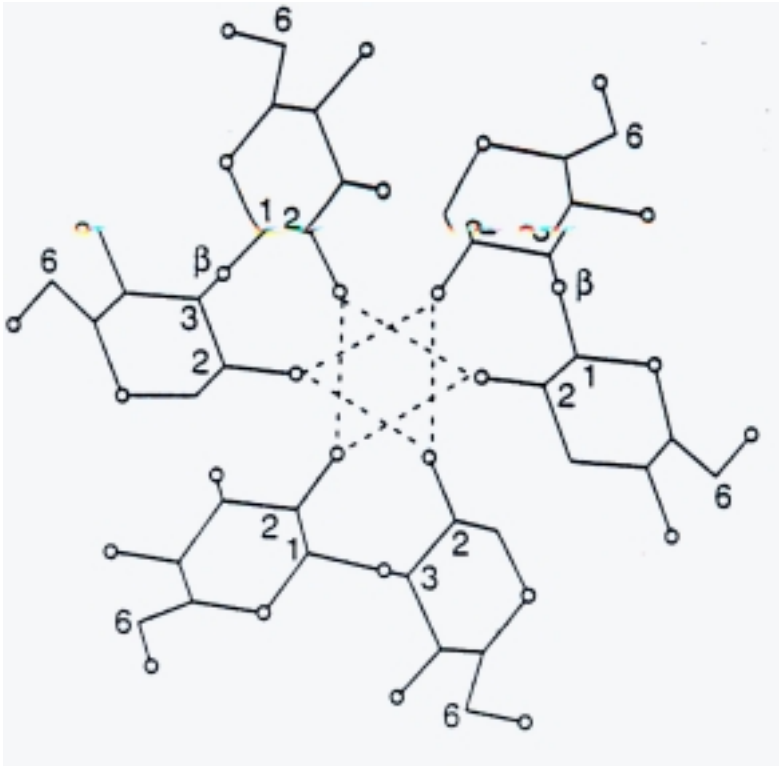
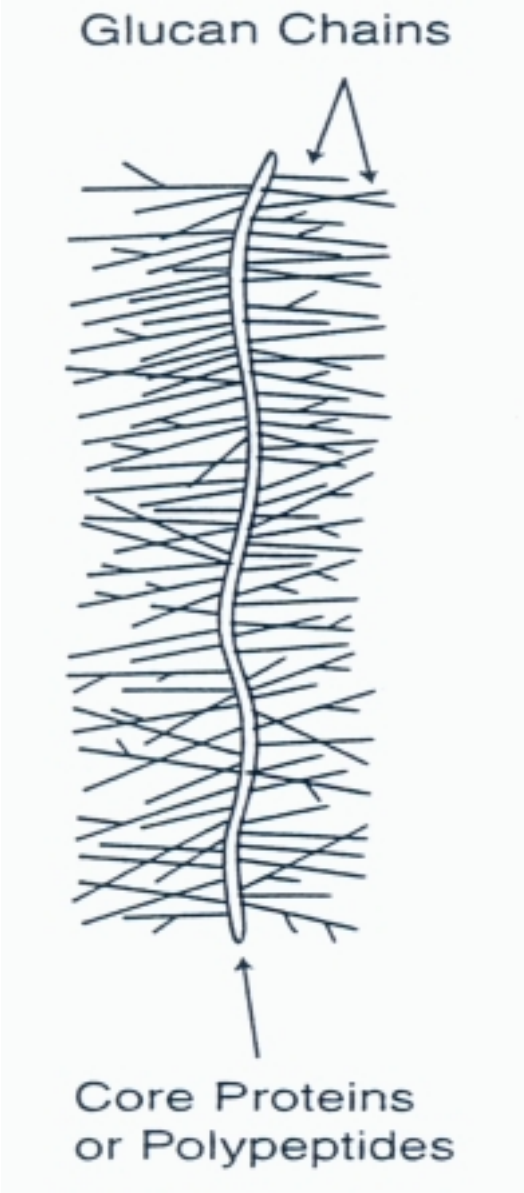


Fig. 7 The molecular plan of a mushroom proteoglycan



Hot water extracts from cultured mycelium of *Lentinus edodes* contain polysaccharide KS-2, an α-mannan peptide containing the amino acids serine, threonine, alanine and proline.

LEM and LAP extracts are derived from *L. edodes* mushroom mycelium and culture media respectively and are glycoproteins containing glucose, galactose, xylose, arabinose, mannose and fructose. LEM also contains nucleic acid derivatives, vitamin B compounds and ergosterol. LEM and LAP both demonstrate

strong antitumour activity by i.p., and p.o. in animals and humans. LEM is prepared from a hot water extract of powdered mycelia, incubated for 50-60 h at 40-50^{o}C and partially hydrolysed by endogenous enzymes. The residue was extracted with water, 60^{o}C, and the filtrate freeze dried. The final light brown powder was LEM. The yield of LEM is about 6-7 g/kg medium. LAP is obtained as the filtrate of a water solution of LEM by adding 4 volumes of ethanol. The yield of LAP is approximately 0.3 g/g LEM. An immunoactive substance EP3 has been obtained by further fractionation of LEM. The active substance is considered to be a water soluble lignin containing numerous carboxyl groups (Susuki *et al*., 1990). LEM and LAP are, therefore, complex mixtures of compounds which are now being further purified (Hobbs, 2000).

An antitumour active β-glucan-protein (EA_6) has been isolated from the fruit-body of *Flammulina velutipes* while a new antitumour glycoprotein has been isolated from cultured mycelium. This glycoprotein, “Proflamin” (mw = 16,000) is water soluble and contains 90% protein and 10% saccharide and has activity against allogeneic and syngeneic tumours (Zhang *et al*., 1999).

PSK (polysaccharide-K) and PSP (polysaccharide-peptide) have been derived from *Trametes* (*Coriolus*) *versicolor*. PSK is extracted from a mycelial strain CM-101 and is approximately 62% polysaccharide and 38% protein. The glucan portion of PSK consists of a β1-4 main chain and β1-3 side chain, with β1-6 side chains that bond to a polypeptide moiety through O-N-glycosidic bonds. The polypeptide portion is rich in aspartic, glutamic and other amino acids and has a molecular weight ranging from 94,000-100,000 daltons and is orally bioavailable (Sakagami and Aoki, 1991). This compound has been systematically tested against a wide range of human cancers with some considerable success (Ikuzawa *et al*., 1988, Kidd 2000).

PSP was first isolated from cultured deep-layer mycelium of the COU-1 strain of *Trametes versicolor* in 1983 (Yang, 1999). PSP may contain at least four discrete molecules, all of which are true proteoglycans. PSP differs from PSK in its saccharide makeup, lacking fucose and containing arabinose and rhamnose. The polysaccharide chains are true β-glucans; mainly 1-4, 1-2 and 1-3 glucose linkages together with small amounts of 1-3, 1-4 and 1-6 galactose, 1-3 and 1-6 mannose, and 1-3 and 1-4 arabinose linkages. The molecular weight of PSP is approximately 100,000 daltons and can be easily delivered by oral route. PSP is rapidly gaining recognition with many successful human cancer trials (Jong and Yang, 1999) (Chapter 7). Although the molecular weights of PSK and PSP are approximately 100,000 daltons, PSP does not contain fucose and PSK lacks arabinose and rhamnose (Yang and Ying, 1993). Saphadex gel chromatography, DEAE-cellulose column chromatography and HPLC reveal that the polysaccharides and peptides of PSP are clearly bound and not separated. Where there is polysaccharide there is polypeptide. PSP polysaccharide is connected with a small molecular weight protein. Up to now at least 10 kinds of 'protein bound' polysaccharides have been isolated, e.g. coriolan I and II - most are covered by US and Japanese patents. However, only PSK and PSP have been used in clinical trials. It should be noted that Japanese and Chinese scientists still prefer to use the *Coriolus* generic name instead of *Trametes*.

Active Hexose Correlated Compounds (AHCC)

This is a proprietary extract prepared from the co-cultivation of several Basidiomycete mushrooms including *Lentinus edodes, Trametes versicolor* and *Schizophyllum commune* grown on rice (Ghoneum *et al*., 1995). However, there is no data available on the exact species complement or on methods of preparation. It

is apparently a hot water extract following enzyme treatment, and the extract contains polysaccharides, amino acids and minerals and is orally bioavailable. The glucans present are stated to have low molecular weight, c. 5,000 daltons and are α-1-3 type. These details are surprising since typically low molecular weight material is normally inactive and α-glucans have minimal immuno-potentiating activity. However, there have been limited studies and reports suggesting an interesting level of efficacy against hepatocellular carcinoma (Kamiyama, 1999). Ghoneum (1998) found that a derivative, arabinoxylane, derived from this fermentation increased human NK activity by a factor of 5 over two months.

Dietary Fibre

High molecular weight compounds excreted without digestion and absorption by humans are called dietary fibres. Mushrooms contain dietary fibres belonging to β-glucans, chitin and heteropolysaccharides (pectinous substances, hemicellulose, polyuronides etc), making up as much as 10-50% in the dry matter. Much of the active polysaccharides, water soluble or insoluble, isolated from mushrooms, can be classified as dietary fibres (i.e. β-glucan, xyloglucan, heteroglucan, chitinous substance) and their protein complexes. Many of these compounds have carcinostatic activity and by physicochemical interactions they will absorb possible carcinogenic substances and hasten their excretion from the intestine. Thus, mushrooms in general may have an important preventative action for colorectal carcinoma (Mizuno, 1996).

In summary - while a variety of polysaccharides from various sources have been shown to enhance the immune system the most active appear to be branched (1-3)-β-D-glucans. All have a common structure, a main chain consisting of (1-3)-

linked β-D-glucopyranosyl units along which are randomly dispersed single β-D-glucanopyranosyl units attached by 1-6 linkages giving a comb-like structure with various conformations. The (1-3)- β-D-glucan backbone is essential and the most active immune stimulating polymers have degrees of branching between 0.20 and 0.33. Information has been accumulating both that triple helical structures formed from high molecular weight polymers are possibly important for immunopotentiating activity and that activity is independent of any specific ordered structure. Immunopotentiating activity depends mainly on a helical conformation and on the presence of hydrophilic groups located on the outside surface of the helix. Most of the active (1-3)- β-D-glucans have been isolated from Basidiomycetes (Bohn and BeMiller, 1995).

While most attention has been given to studies demonstrating the medicinal effects of the polysaccharides from single mushroom species, several studies are suggesting that the human and murine immune systems can be given greater stimulation by using mixtures of polysaccharides from several proven medicinal mushrooms (Ghoneum *et al*., 1995; Wedam and Haynes, 1997; Sawai *et al*., 2002). A complementary effect of each mushroom component on enhancing immunological function can be expected from mixed medicinal mushroom extracts (see also Chapters 6 and 7).

Terpenoids

Certain terpenoids and their derivatives have been isolated from mushroom species from the Polyporales and Ganodermatales and have been shown to be cytotoxic. At least 100 different triterpenoids have been identified from fruiting bodies and mycelium of *Ganoderma lucidum* and *G. applanatum* and include

ganoderic, ganoderenic, lucidenic acids- and several ganoderals (for references see Wasser and Weis, 1999b). A cytotoxic tricyclic sesquiterpene, illudin, isolated from *Omphalotus olearius* and *Lampterimyces japanicus* shows interesting anticancer properties. Furthermore, the semisynthetic illudin analog, 6-hydroxy-methylcylfulvene (HMAF) has inensity profiles of a tumour growth inhibitor. HMAF is undergoing phase I human clinical trials and could well be a promising new anticancer drug (Weis, 1996).

References

Babitskatya, U.G., Sherba, V.V. , Mitropolskaya, N.Y. and Bisko, N.A. 2000. Expolysaccharides of some medicinal mushrooms: production and composition. *International Journal of Medicinal Mushrooms* **2**, 51-54.

Bluhm, T.L. and Sarco, A. 1977. The triple helical structure of lentinan, a β-(1-3)-D-glucan. *Canadian Journal of Chemistry* **55**, 293-299.

Boldizsar, I.,Horvath, K., Szedlay, G. and Molnar-Perl, I. 1998. Simultaneous GC-MS quantitation of acids and sugars in the hydrolyzates of immunostimulants, water soluble polysaccharides of Basidiomycetes. *Chromatographia* (Germany) **47**, 413-419.

Bohn, J.A. and BeMiller, J.N. 1995. (1-3)- β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers* **28**, 3-14.

Borchers, A.T., Stern, J.S. Hackman, R.M., Keen, C.L. and Gershwin, H.E. 1999. Mushrooms, tumors and immunity. *Proceedings of the Society for Experimental Biology and Medicine* **221**, 281-293.

Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T. and Fukuoka, F. 1969 . Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. *Nature* **222,** 687-688.

Chihara, G., Hamuro, J., Maeda, Y., Arai, Y. and Fukuoka, F. 1970. Fractionation and purification of the polysaccharides with marked antitumour activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing., an edible mushroom. *Cancer Research* **30**, 2776-2781.

Feng, W., Nagai, J. and Ikekawa, T. 2001. A clinical pilot study of EEM for advanced cancer treatment with EEM for improvement of cachexia and immune function compared with MPA. *Biotherapy* **15**, 691-696.

Ghoneum, M. 1998. Enhancement of human natural killer cell activity by modified arabinoxylane from rice bran (MGM-3). *International Journal of Immunotherapy* 14, 88-89.

Ghoneum, M., Wimbley, M., Salem, F., McKlain, A., Attalan, N. and Gill, G. 1995. Immunomodulatory and anticancer effects of active hemicellulose compound (AHCC). *International Journal of Immunotherapy* 11, 23-28.

Hobbs, C.R. 2000. Medicinal value of *Lentinus edodes* (Berk.) Sing (Agaricomycetideae). A literature review. *International Journal of Medicinal Mushrooms* **2**, 187-302.

Ikekawa, T., Nakanishi, M., Uehara, N., Chihara, G. and Fukuoka, F. 1968. Antitumour action of some Basidiomycetes, especially *Phellinus linteus*. GANN, **59**, 155-157.

Ikekawa, T., Uehara, N., Maeda, Y., Nankinishi, M. and Fukoka, F. 1969. Antitumour activity of aqueous extracts of edible mushrooms. *Cancer Research* **29**, 734-735.

Ikikawa, T. 2000. On biological activity of mushrooms. *Biotherapy* **14**, 945-951.

Ikuzawa, M., Matsunaga, K. *et al*. 1988. Fate and distribution of an antitumour protein-bound polysaccharide PSK (Krestin). *International Journal of Immunopharmacology* **10**, 415-423.

Jones K. 1998. Maitake: a potent medicinal food. *Alternative Comparative Therapy*, Dec. 420-429.

Jong, S. and Yang, X. 1999. PSP – a powerful biological response modifier from the mushroom *Coriolus versicolor*. In *Advanced Research in PSP*. (ed. Yang, Q.) Hong Kong Association for Health Care Ltd., 16-28.

Jong, S.C. and Donovick, R. 1989. Antitumour and antiviral substances from fungi. *Advances in Applied Microbiology* **34**, 183-261.

Jong, S.C., Birmingham, J.M. and Pai, S.H. 1991. Immunomodulatory substances of fungal origin. *Journal of Immunology and Immunopharmacology* **11**, 115-122.

Kamiyama, Y. 1999. Improving effect of active hexose correlated compounds (AHCC) on the prognosis of postoperative hepatocellular carcinoma patients. *European Surgical Research*, **31**, 216.

Kidd, P.M. 2000. The use of mushroom glucans and proteoglycans in cancer therapy. *Alternative Medicine Review* **5**, 4-27.

Maeda, Y.Y., Watanabe, S.T., Chihara, C. and Rokutanda, M. 1988. Denaturation and renaturation of a β-1,6:1,3-glucan, lentinan associated with expression of T-cell mediated responses. *Cancer Research* **48**, 671-675.

Maitake Products Inc. 1999. Maitake D-fraction technical support document. Paramus, New Jersey, Maitake Products Inc.

Minato, K., Mizuno, M., Ashida, H., Hashimoto, T., Terai, H. and Tsuchida, H. 1999. Influence of storage conditions on immunomodulating activities of *Lentinus edodes*. *International Journal of Medicinal Mushrooms* **1**, 243-250.

Minato, K., Mizuno, M., Kawakami, S., Tatsuoka, S., Denpo, Y., Tokimato, K. and Tsuchida, H. 2001. Changes in immunomodulating activities and content of antitumour polysaccharides during growth of two mushrooms, *Lentinus edodes* (Berk.) Sing and *Grifola frondosa* (Dicks:Fr.) S.F. Gray. *International Journal of Medicinal Mushrooms* **3** 1-7.

Mizuno, T. 1996. A development of antitumour polysaccharides from mushroom fungi. *Food Ingredients Journal (Japan)*, **167**, 69-85.

Mizuno, T. 1999. The extraction and development of antitumour-active polysaccharides from medicinal mushrooms in Japan. *International Journal of Medicinal Mushrooms* **1**, 9-29.

Mizuno, T., Kato, N. *et al.*. 1984. Fractionation, structural features and antitumour activity of water-soluble polysaccharides from "Reishi" the fruit body of *Ganoderma lucidum*. *Nippon Negeckagahu Kaishi* **58**, 871-880.

Mizuno, T., Sakai, T. and Chihara, G. 1995. Health foods and medicinal usages of mushrooms. *Food Reviews International* **11**, 69-81.

Ohno, N., Adachi, Y. and Yadomae, T. 1988. Conformations of fungal β-D-glucans in the fruit body of edible fungi assessed by cross polarisation-magic angle spinning carbon-13 nuclear magnetic resonance spectroscopy. *Chemistry and Pharmaceutical Bulletin* **36**, 1198-1204.

Reshetnikov, S.V., Wasser, S.P. and Tan, K.K. 2001. Higher Basidiomycetes as a source of antitumour and immunostimulating polysaccharides (Review). *International Journal of Medicinal Mushrooms* **3**, 361-394.

Saito, H., Yoshioka, Y., Uchara, N., Aketagawa, J., Tanaka, S. and Shibata, Y. 1991. Relationship between conformation and biological response for (1->3)- β-D-glucans in the activation of coagulation Factor G from limulus amebocyte lysate and host-mediated antitumour activity. Demonstraiton of single-helix conformation as a stimulant. *Carbohydrate Research* **217**, 181-190.

Sakagami, H. and Aoki, T. 1991. Induction of immunopotentiation activity by a protein-bound polysaccharide. PSK (review). *Anticancer Research* **11**, 993-1000.

Sasaki, T. and Takasuka, N. 1976. Further study of the structure of lentinan, an antitumour polysaccharide from *Lentinus edodes*. *Carbohydrate Research* **47**, 99-104.

Sawai, M., Adachi, Y., Kanai, M., Matsui, S. and Yadomae, T. 2002. Extraction of conformationally stable (1-6)-branched (1-3)- β-glucans from premixed edible mushroom powders by cold-alkaline solution. *International Journal of Medicinal Mushrooms* (in press).

Sharon, N. and Lis, H. 1993. Carbohydrates in cell recognition. *Scientific American Journal*, 74-81.

Susuki, H. *et al.* 1990. Structural characterisation of the immunoactive and antiviral water-soluble lignin in an extract of the culture medium of *Lentinus edodes* mycelium (LEM). *Agricultural and Biological Chemistry* **254**, 479-487.

Wasser, S.P. and Weis, A.L. 1999a. Therapeutic effects of substances occurring in higher Basidiomycete mushrooms: a modern perspective. *Critical Reviews in Immunology* **19**, 65-96.

Wasser, S.P. and Weis, A.L. 1999b. Medicinal properties of substances occurring in higher Basidiomycete mushrooms: current perspectives (review). *International Journal Medicinal Mushrooms* **1**, 31-62.

Wedam, W. and Hayes, A. 1997. Breast cancer. *Journal of Naturopathic Medicine* 7, 86-87.

Weis, A. 1996. Semisynthetic drugs of fungal origin. Challenges of preclinical anticancer drug development. *5th International Conference on Chemical Synthesis of Antibiotics and Related Microbial Products Abstracts*. Debrecan. Hungarian Academy of Science, p5.

Yang, Q-Y. 1999. Yun Zhi polysaccharopeptide (PSP) and the general aspects of its research. In *Advanced Research in PSP* (ed. Quing-yao Yang). The Hong Kong Assocation for Health Care Ltd., Hong Kong, 29-38.

Yang, X-T. and Ying, Z. 1993. The electrophoretical analysis of the components of polysaccharide-peptide. In *PSP International Symposium Anthology of Theses and Abstracts*. (ed. Quing-yao Yang). Fudan University Press, 35-40.

Yap, A-T. and Ng, M-L.M. 2001. An improved method for the isolation of lentinan from the edible and medicinal shiitake mushroom, *Lentinus edodes* (Berk.) Sing. (Agaricomycetideae). *International Journal of Medicinal Mushrooms* **3**, 6-19.

Zhang, H., Gong, F., Feng, Y. and Zhang, C. 1999. Flammulin purified from the fruit bodies of *Flammulina velutipes* (Curt. Fr) P. Karst. *International Journal of Medicinal Mushrooms* **1**, 89-92.