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綜 述

Taxonomy and Physiologically Active Compounds of Ganoderma——A Review

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INTRODUCTION

The traditional fungal Chinese herb, Lin-Chi(靈芝)played important role in the legends of folks to care many unspecified illness and such an image had kept in minds of oriental people for thousand years[1]. In recent years, an increasing at tempt had made to elucidate the linkage between the traditional herb and modern medicine for the reasons of searching new pharmaceutical resourece and new natural compounds, that over one hundred of reports had published under the name of *Ganoderma lucidum* to discuss the chemical constituents and physiologically active new compounds isolated from sexual stage of this fungus, by the development of methods in the fields of chromatography, spectrochemistry and bioassay systems.

On the other hand, the progress on the techniques of fungal taxonomy had allowed biologists to distinguish the very similar and complex species in the family Ganodermataceae(靈芝科). However, the traditional name, Lin-Chi, from mycologists' point of view was a general name referred to a large group of polyporous fungi(多孔菌)with hard basidiocarps(fruiting bodies) in red, white, yellow, purple or other colours[1] and *Ganoderma lucidum* is only one of about 40 species of Ganodermataceae[2]. Therefore, it is very possible that the name *Ganoderma lucidum* used in the published scientific papers might conteated many biological species, since the resemble species were difficult to delimit by macro-morphological characters that were used in higher plants. Then, in the first section of present review, the methods to treat the taxonomical status of *Ganoderma* species were extended and the name *Ganoderma lucidum* was considered as a group referred to that of all similar species.

Since there has been about equal number of reports concerning physiological activities of *Ganoderma* which crude extract was subjected for study. Due to the uncertainty of the material used, only those physiological activities with known chemical constituents were discussed in the present review. Because the chemical constituents of the material prepared from *Ganoderma* might be specific from species to species[3] and stage to stages[35].

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TAXONOMY OF GANODERMA

In general, the system stated by Corner (1983)[4] had been accepted by mycologists and the status is summarized as the followings:

Basidiomycotina(擔子菌亞門)
Hymenomycetes(帽菌綱)
Aphyllophorales(無菌褶菌目)
Ganodermataceae(靈芝科)
Ganoderma(靈芝屬)
Amauroderma(假靈芝屬)

Ganoderma was subdivided into sections[2]

Ganoderma(靈芝屬)
Ganoderma(靈芝組)
Ganoderma (靈芝亞組)
Trachyderma (粗皮靈芝亞組)
Phaeonema(紫芝組)
Phaeonema(紫芝亞組)
Elfvingia (樹舌亞組)

The subsection Ganoderma has characterized by the concept of morphology with the ovoid

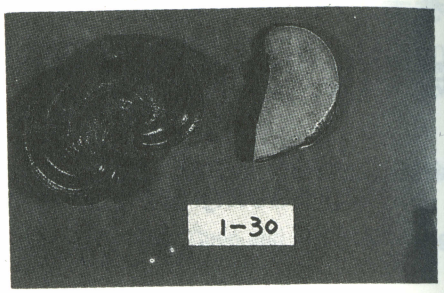


Fig. 1. A cultivated fruiting body of **Ganoderma** *lucidum*.

truncate(截頭型)double-walled basidiospores and laccate(假漆)crust(皮殼)(Fig. 1). However, these characters relied on naked eyes or light microscope were limited to the identification of the taxon up to subsection, and subdividing the species within the genus *Ganoderma* will depend on the methods stated below.

I, SCANNING ELECTRON MICROSCOPE(SEM)

The constant characteristics of fungi were sometime difficult to obtain since vegetative growth resulted in different morphology, but sexual spores were usually considered as an

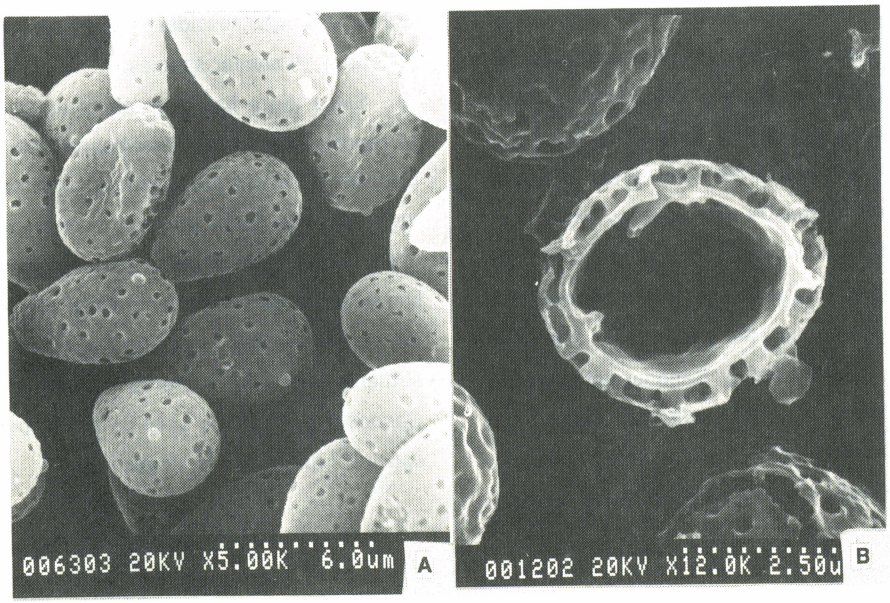


Fig. 2. Scanning electron microscopy of basidiospore of **Ganoderma tsugae**(A). The double layer strucutre is shown in broken spore(B).

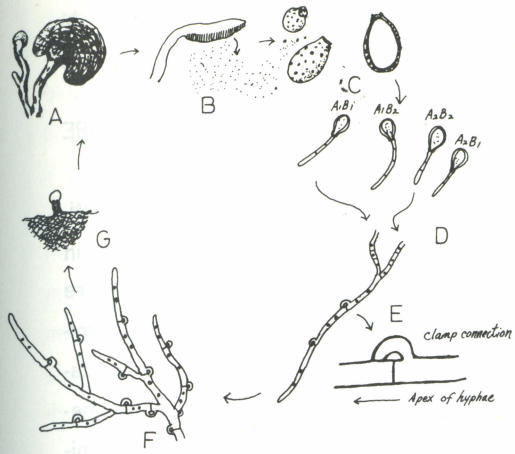


Fig. 3. The life cycle of **Ganoderma**, the matured fruiting body(A) discharges basidiospores from hymenial layer(B). The spore wall comprises two layers(C) and the spores with different mating-types germinate into monokaryon with single nuclei per cell(D). Mating of monokaryons only happens to those with different mating types and the biucleated dikaryon forms (E) with clamp connections. The dikaryon proliferates(F) to produce primodium of fruiting body(G) when the environment is feasible.

important organ to provide sufficient criteria among species. By the introduction of electron microscope, especially the scanning type, the detail structure of minute basidiospore(5-8 μM) was visualized (Fig. 2) and by this tool the two very similar species, *G. lucidum*and *G. tsugae* were distinguished[5-6]. This techniques had become an indispensable part of work in this field[7-9] and some other less characteristic parts of fruiting body such as crust and hymenial layer(子實層)were also observed[7-9].

2.INTER-FERTILITY TEST

Monokaryotic(單 核)strains germinated from single spore isolation demonstrated dirfferent mating types and the mating types were determined by a all possible mating combinations of selected monokaryotic strains(Fig. 3). Usually, clamp-connection(Fig. 3-E) formation of mycelium indicates different mating types pertained to the paired monokaryotic strains. The mode was to explain that if the isolates were originated from different species that there would be no chance to form dikaryon and clamp connection (Fig. 3-E), which was considered to be fertille. This time-consuming test provided precise determination of a biological species, since the monokaryotic strains from different fruiting bodies were inter-fertile, theoretically. However, the defect was obvious that it is laborious and it may become ambiguous when negative results were reached and these two sets of monokaryotic strain might be restrained by some unknown genetic barriers[8].

By using mating types of *Ganoderma*, four species were successfully separated in Taiwan early in 1985[10], and *G. australe* was identified which was to be mistreated as *G. austale*[8]. Similar works were also performed to distinguish *G. lucidum* and its allies[5-7,8-9].

3.CHEMOTAXONOMY

To overcome the diffculty in lacking obvious morphological characteristics, taxonomy based on chemical differences had been tried. Restriction fragment length polymorphism(RFLP) were carried out on delimiting 8 species of *Ganoderma*[7] and also on that patterns of *G. applanatum* and *G. australe*[8].

The application of RFLP on fungal material began in 1988 to distinguish the hybrid strains of the cultiated mushroom, *Agaricus brunnescens* (洋菇)[11]. The method comprised DNA purification of mycelial cell, restrictive hydrolysis, and then hybridization to specific probe. The radioautographic patterns derived from electrophoresis and southern blotting were compared.

This method required cultrues of *Ganoderma* and precise result was able to obtain if poper probe was used[8,9].

Electrophoretic patterns of isozymes were also used as a marker to classify the species of *Ganoderma* in which API-ZYM[9], lacase[9] esterase, lactate dehydrogenase, malate dehydrogenase and peroxidase[12] were analyzed and good correlation was foound in 50 cultures collected in Taiwan, but ambiguous result was obtained for those cultures from other areas[12].

Either RFLP or isozyme analysis required cultures of *Ganoderma* and purification process for DNA and protein. The tedious process and laborious assay method were not easy to perform, then, the alternative was to analyze secondary metabolites, especially for those bitter principles which were mainly composed by triterpenoids[13,14]. The triterpenoid patterns of HPLC[15] and TLC[16] of organic solvent extracts produced significant differences in fruiting bodies and mycelial met *Ganoderma* species.

4. CLASSIFICATION BASED ON CULTURE CHARACTERISTICS

Cultures of *Ganoderma* with different optimal growth temperatures, growth rates in media, pigment production and chlamydospore formation were also observed as a less determinative character for reference of characterization of species[4-8]. Basically, these parameters stated were not used alone and to determinate a species of *Ganoderma*, the combination of above mentioned methods were performed.

A tentative phylogenetic relationship derived from 50 *Ganoderma* strains collected in taiwan by Department plant Pathology, Talwan Agriculture Research Institute (農試所) based on the pattern of TLC proposed by the author

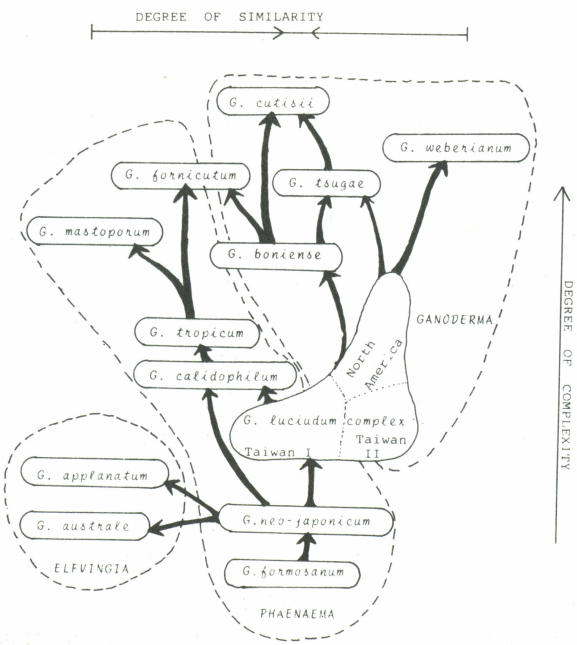


Fig. 4. A tentative phylogenetic relationship of **Ganoderma species based on the similarity and complexity of TLC pattern of acetone extract from fruiting bodies.**

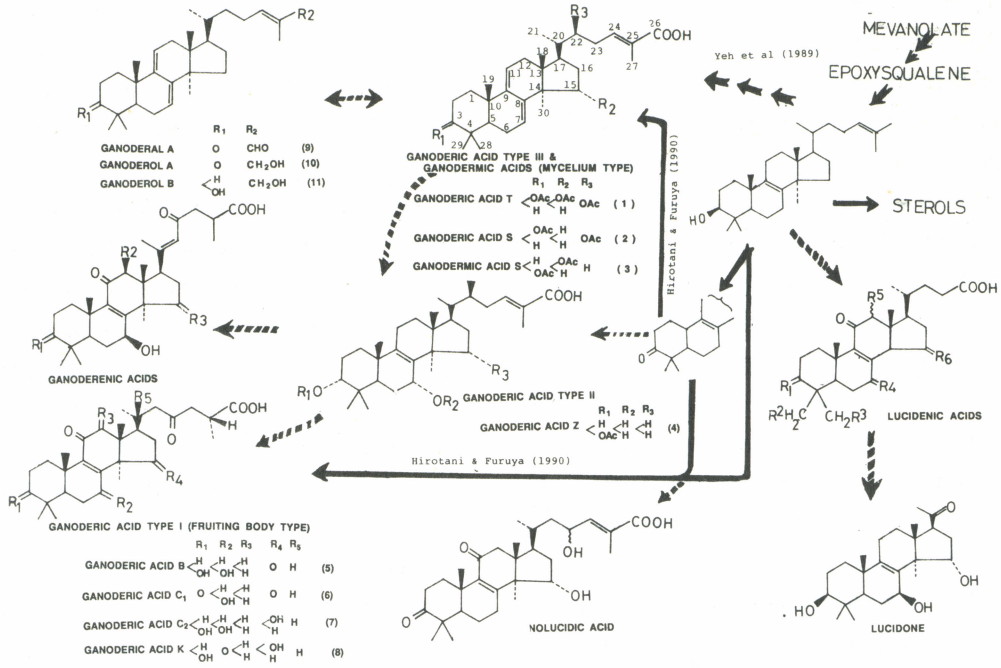


Fig. 5. Trierpenoids from **Ganoderma**.

was given in Fig.4.

TRITERPENOIDS

Over one hundred of highly oxygenated *Ganoderma lucidum* had reported in recent years. By the proliferation of trivial names some confusion had resulted[17]. However, these compounds were mainly ganodeic acids[18–19], ganodermic acids [30,31], ganoderenic acids[26,32], lucidenic acids[25,33–35], lucidone[24], ganoderal[22] and ganoderols[22]. The most abundant variety, ganoderic acids were subdivided into three types[17], The structure of these compounds and a possible biosynthetic pathway were summarized in Fig.5. These compounds were isolated from fruiting bodies or mycelial mat of static liquid culture and it seemed that the yield of triterpenes from mycelial pellets of shaking ilquid culture was very poor[36]. Different strains(or species) of *Ganoderma* produced different lucidenic and ganoderic acids[16] and the degree of oxygenation and number of acetoxy group in same strain was different in mycelial mat and fruiting body.[18–34]. Hirotani and Furuya (1990)[35] also found that ganoderic acids T, S and R (Type III) were the major triterpenoids in mycelium and by contract, ganoderic acids A, B, and H (Type I) were detected only in fruiting body. Therefore, it is obvious that these compounds are not existed in the same time in one single species.

The triterpenoids of *Ganoderma* were usually extracted by methanol[18], ethanol[19], acetone[24], chloroform[25], ether[26] or the mixture of these solvents[29–31]. The crude extracts of organic solvents composed about 3 to 6% of

dry fruiting body or 1 to 3% of mycelial mat of static liquid culture and only trace in mycelial pellets of shaking liquid culture. For acidic triterpenoids, ethanol extract was separated by alkaline aqueous layer from chloroform solution of the extract and precipitate was recovered by acidifying the aqueous layer to pH 3 with mineral acids[18-27]. Methylated derivatives and reverse phase chromatography was also reported[21]. The non-acidic triterpenoids remained in chloroform layer druing alkaline separation and similar process was used for purification[22,24]. Further purification by PLC and recrystalization was carried out for identification[29-31].

Structural analysis of the triterpenoids from *Ganoderma* was based on the information from IR, mass and NMR spectrometry, especially for the fast developing high resolution two -dimension NMR[18-35]. For analytic work, HPLC of reverse phase (C_{18} column, 2.5 mm i. d. x 30 cm) with a mobile phase of methanol gradient from 70% to 100% containing 1% acetic acid was reported[15]. TLC patterns produced valuable information for the classification of species of *Ganoderma*[16] but was less reliable for quantitative analysis.

PHYSIOLOGICAL ACTIVITY OF TRITERPENOIDS

Since Lin-Chi had known as a multiple effective herb in Chinese tradition and many reports based on crude extracts of *Ganoderma* also indicated therapeutic differences of various illness. Due to the varietal baundance of triterpenoids, it seemed be a clue to explain the multiple effect of this fungi, but the physiological activity of these newly isolated triterpenoids was still remained unclear and physiological activity of less than 20 of these compounds were reported.

In 1983, Toth et al[23] had proved the hepatoma cytotoxicity of triterpenes isolated from *Ganoderma lucidum* and these triterpenes were named ganoderic acid T and Z(Compound 3 and 4).

Kohda et al[19] in 1985 isolated ganoderic C_1 (Compound 6) and C_2(Compound 7) and an inhibitory effect against histamine release from rat mast cell was measured at a concentration of 0.4 mg/ml. The content of ganoderic acid C_2 in dry fruiting boodies from Hirostima was estimated around 0.21 to 0.47%(W/W).

Shiao et al (1986)[37] used mycelial mat of *Ganoderma lucidum* in the diet of rats and cholesterol and triacylglycerol contents in serum and liver had significantly reduced when that of control were compared. The hypocholesterolemic effect had attributed to those recently identified triterpenes, mainly ganodermic acids (Compound type III)[28-31], which exhibited structures analogous to those known sterols with similar effect. Komoda et al(1989) also suggested sterol with 7-oxo and 5- α-hydroxy group isolated from *Ganoderma lucidum* potently inhibited the synthesis of cholesterol from (24,25-3 H)-24,25-dihydrolanosterol at 18 μM level[39].

Angiotensin converting enzyme inhibitory effect of triterpenoids from *Ganoderma lucidum* was reported by the group of Morigiwa (1986)[22]. Five triterpenes, ganoderic acid K (8), S (2), ganoderal (9), ganoderol A (10) and B (11), isolated from 70% methanol extract and were found to be responsible for the inhibitory activity of the enzyme in vitro.

Triterpenes from *Ganoderma tsugae* exhibited reduction activity of anti-hepatotoxicity induced by CCl_4 were reported. SGTP and SGOT were reduced to normal value when CCl_4 induc-

tion of SGTP and SGOT reached 40 times of normal value in serum of mice. The polar fraction containing mainly Ganoderic acid B (Compound 5) was found to be effective at a level of 0.01 mg/kg of mouse[36].

However, the results from physiological assays of triterpenes from *Ganoderma* were not always promising. Ganodermic acid S (Compound 3) known as hypocholesterolemic agent[38] was found to enhance human platelets aggregation at a concentration at 50 μM in vitro. The morphology of platelets under SEM showed the formation of filopodia and pseudopod had greatly stimulated by ganodermic acid S[38].

Although the adverse effect of ganodermic acid S, it seems that the traditional way to prepare *Ganoderma* by cooking fruiting body with water might contain only trace of this compound[35], because ganodermic acid S was reported to be isolated from mycelial mat of static liquid culture[30] and the acetoxy groups at 3 and 15 positions limited its solubility in water.

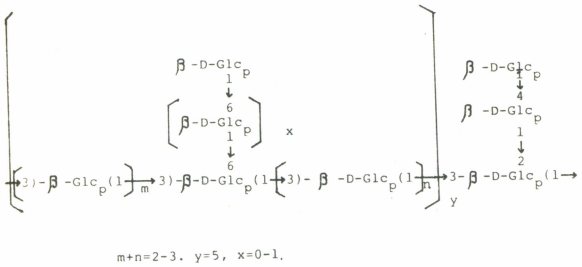
POLYSACCHERIDE OF GANODERMA

Polysaccharides of *Ganoderma* comprised one of the major source of physiologically active compounds in this fungi. These exocellular polysaccharides were either extracted from the dried fruiting body or separated from the broth of a shaking liquid culture.

Fractionation and purification of *Ganoderma* polysaccharide were usually performed by alcohol precipitation, ino-exchange chromatography, ultra filtration. size-exclusion chromatography (Gel permeation) and high voltage electrophoresis or the combination of these methods[40].

Structural analysis of purified polysaccharides was carried out by acid hydrolysis to determine monosaccharide constitution, enzyme hydrolysis to detect anomeric structure, methylation technique and Smith degradation to elucidate linkage type, and gas chromatography or combined mass spectrometry together with the information from NMR to clear up the structure of polysaccharide[41,55]. Recent methods were designed for the analytic work at a milligram level that greatly reduced the laborious purification loading[40]. However, due to the property of indefinite molecular weight of polysaccharide, the structure of polysaccharide was rather descriptive on the repeat unit than the concrete molecular details and the molecular weight of polysaccharide was expressed avaragely[40].

Biosynthesis of polysaccharide in fungi had suggested a complex intracellular enzyme system was operated to form the multiple layers of cell wall which composed of the skeletal elements of chitin and β-glucan, matrix components of α-glucan and glycoprotein, and other miscellaneous components such as mannan, galactan, chitosan, galactosamin polymer, polyuronides, melannis and lipids[56]. About 50% of fungi species produced exocellular soluble polysaccharide[57] which is believed to be released from cell wall and many of them with 1-3-β-linkage had stated to exhibited immunological activities[41-55]. *Ganoderma* contented approximately 1% (w/w) of neutral polysaccharidein dried fruting body and about 0.4% (w/w) in the broth of vigorous shaking liquid culture[58]. However, the structures of the antitumor polysaccharides isolated from *G. lucidum*, *G. Applanatum* and *G. iaponicum* had been elucidated by the groups of Mizuno[41,42,44,48,49,55] and Miyasaki[46,50]. The general structure of these polysaccharide was given in Fig.6. Bioassay system to detect



m+n=2-3. y=5, x=0-1.

Fig. 6. Structure of polysaccharide from ***Ganoderma***.

antitumor activity of polysaccharide was performed mainly by the sarcoma-180/mouse method[41-55], and a dose around 1-10 mg/kg of polysaccharide was found to be effective in reduction of tumor cell growth[53].

Neutral heteropolymers of glucose, xylose, mannose and galactose isolated from fruting body of *Ganoderma* had reported and alkali soluble polysaccharides usually contained approximately 10% of glucuronic acid[46]. These heteropolymer also exhibited antitumor activity against S-180 in mice[47]. However, only homopolymers of glucose was found in liquid culture and these may due to glucose was used as a sole carbon source in the medium of liquid culture, The molecular weight of antitumor polysaccharide was ranging from 4×10^5 to 1×10^6[41-55], but prolonged culture in liquid shaking culture may turn the broth into viscous gel and the polysaccharide in this stage was water insolube with a molecular weight over 2×10^6[55]. This phenomenon was explained by the recent study on schizophyllan that the polymer of primary structure in β-1-3-D-glucan with β-1, 6-D-glucose side chain had a property to form a rod like triple helix which was stable in water[59].

Generally, antitumor polysaccharides from *Ganoderma* are not too different from species to species and they share three common properties together with polysaccharides from other fungi:

(1) The molecular weight over 3×10^5 in primary structure[41-55].

(2) The polymers of linearly linked β-1-3-D residues as a main chain with β-1-6-D-glucose side chain residues. The degree of β-1-6-D-glucose branching is different in polysaccharides from various fungi. The primary structure of polysaccharide is around two β-1-6-D-glucose side residue for every five main-chain residues(2/5), 1/3 in schizophyllan isolated from *Schizophyllum commune* [60], 1/2 from *Cordyceps ophioglossoides*[61], 1/5 from *Peziza versiculosa*[62], 2/5 of lentinan from *Lentinus edodes*[63], and 2/7 from *Dictyophora indusiata*[64]. The linear 1-3-β-glucan without 1-6 β side chain showed no antitumor activity in curdlan[65].

(3) Polysaccharide in triple helix structure was responsible for its antitumor activity[59].

Immunological study on polysaccharides of *Ganoderma* demonstrated to induce natural kiling cell in human[66] and an anti-artificial-metastatic activity was observed[67] when the polysaccharides were administrated i.p.[68] or orally [66,67]. In order to explain how these macromolecular carbohydrates functioned in oral administration, ^{14}C-labelled polysaccharide from liquid culture of *G. tsugae* was fed orally to mice and radioactivity was detected 48.8% to 187.5% of sample in liver of mice[69]. But the absorptive mechanism in mammals is still unclear. Recent study on lentinan had revealed that the polysaccharide was acted as an immunomodulator and the possible mechanism had been proposed as in Fig.7[70].

For hypoglycemic activity, polysaccharides

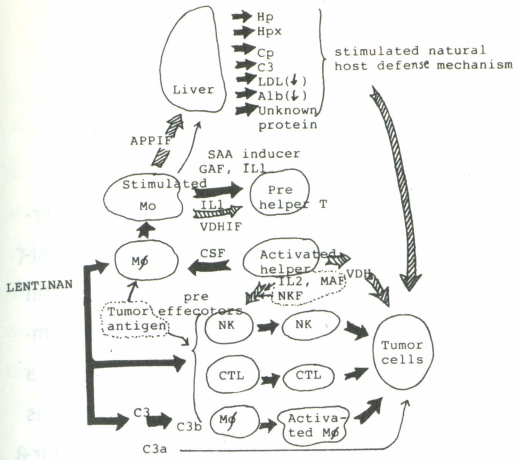
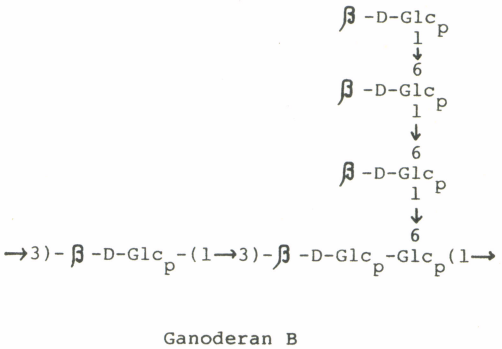


Fig. 7. Possible mode of action of lentinan proposed by Chihura et al (1987) to explain the action of structurally similar polysaccharide from **Ganoderma**.



Ganoderan B

Fig. 8. Structure of hypoglycemic polysaccharide from **Ganoderma lucidum**.

isolated from *Ganoderma lucidum* named ganoderan A,B, and C. (Fig.8) were reported to be with or without peptide binding[71,72]. Their molecular weight was renging from 2×10^4 to 7.4×10^3. The peptide binding ganoderan B was stated to increase the level of plasma insulin in normal and glucoseloaded mice. Administration of ganoderan B elicited Increases of activities of hepatic glucokinase, phosphofructokinase, glucose-6-phosphate and glycogen synthetase, decreased the hepatic glucose-6-phosphate dehydrogenase. The cholesterol and triglyceride levels in plasma and liver had unchanged during glycogen content was reduced[73].

Anti-inflammatory effect on carrageenan edema was also reported for polysaccharide isolated from *G. iaponicum*and *G. tsugae*[74].

Dietary fiber property of polysaccharide was suggensted[41] since α-amylase digestion reduced molecular weight of the polysaccharide from 2×10^6 to 1×10^6 in the broth of *G. tsugae* culture[75].

PHYSIOLOGICALLY ACTIVE PROTEIN

Tanaka et al(1989) isolated a new immunomodulatory protein from mycelial extract of *Ganoderma ludicum* and this protein was named LZ-8. The protein was purified by gel filtration and ionexchange chromatography, and electrophoresis using an in vitro bioassay measuring blast-formation atimulatory activity toward mouse spleen lymphocytes to monitor purification. LZ-8 was capable of hemagglutinating sheep red blood cells, but not active toward human red blood cells. In vivo, LZ-8 prevents the production of systemic anaphylaxis reaction in mice and reaction of antibody production[76]. The complete amino acid sequence of LZ-8 was determined to consisted of 110 amino acid with an acetylated amino acid and a molecular mass of 12,420 Da[77]. The LZ-8 chain showed considerable similarity to the variable region of immunoglobulin heavy chain in its sequence and its predicted secondary structure[77].

OTHER PHYSIOLOGICALLY ACTIVE COMPOUNDS

In addition to the histamine release inhibitory triterpenes[19], Oleic acid[78] and cyclooctasulfur[79] were isolated from mycelial broth of *G. lucidum* and proved to be anti-aler-

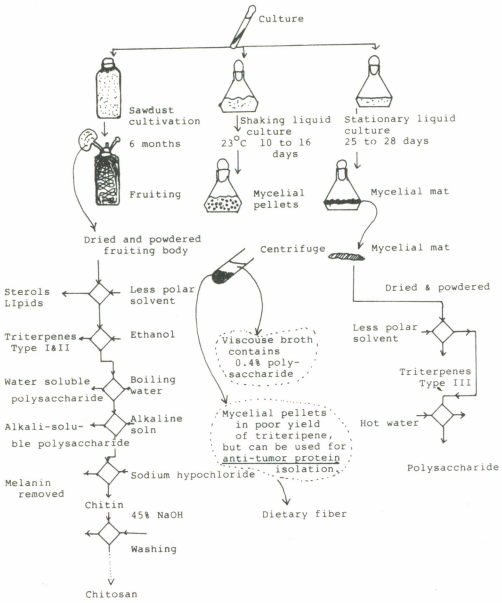


Fig. 9. A possible utilization for **Ganoderma** in different growth stages.

gic compounds[78,79] based on the assay system for peritoneal mast cells of rat. Cyclosulfur compounds were also known as major source of aroma in *Lentinus edodes*.

In contrast to the enhancing effect of platete aggregation by ganodermic acids, adenosine known as anti-platete aggregation agent was found to be rich in water extract from fruiting body of *G. lucidum* and it was measured at least 40 mg/100 g of water extract on dry basis[80].

The debatable germanium content in *Ganoderma* had once been reported as high as 800-2000 ppb in fruiting body on dry basis, but later analysis evidenced only 16-182 ppb on the same basis. Germanium content of this value is not exceeding that of other fungi or plants[81]. Therefore, physiological activity attributed to germanium in *Ganoderma* is skeptical.

FUTURE PROSPECT

In spite of the explosive finding of triterpenoid from *Ganoderma*, chemically, the physiological meaning of these compounds are still wating for further elucidation. For those compounds with known physiological activities, a proposal for more effective utilization of this fungi in different culture states was summarized in Fig.9.

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靈芝之分類學及生理活性物質

蘇慶華

摘　要

本文對近年來有關靈芝之分類地位，分類學之技術進展，百種以上三萜類物質之發現，多醣體以及相關生理活性物質作一回顧。由於靈芝之種類繁多，以傳統形態分類上極易混淆不易辨識，其代謝產物亦可能因生長階段而有不同，可能導致研究上之歧異，因此首先介紹最近包括以掃描式電顯(SEM)，DNA 限制酵素剪切片斷圖譜(RFLP)，同工酵素圖譜(Isozyme pattern)，交配反應及二次代謝產物分析，說明單一種(Species)之建立條件。本文並以丙酮萃取物之 TLC 圖譜進行靈芝種之區分及系統演化可能關係之探討。

三萜類之發現雖已近百種，但由於上述之原因，此近百種之成分並非同時存在於單一種中，故僅對具有生理活性之三萜類進行討論。其中包括抗組織氨釋放，抗血脂肪，Agiotensin converting 酵素抑制作用及肝臟之解毒活性。而其中一種三萜類則知有促進血小板凝聚之負面活性，但此種物質在靈芝子實體中並未測出。

另一方面以 β-1-3 鍵爲主體之葡萄多糖則構成靈芝之生理活性另一來源，包括抗腫瘤，降血糖及抗發炎等活性。其構造與抗腫瘤之關係亦有初步之結論。

由培養菌絲分離出之蛋白質 LZ-8，則亦顯示出免疫上之功能，其氨基酸順序亦已確認。此外，具有抗過敏性之油酸和八環硫，以及抗血小板凝聚之 Adenosine 則亦有報告。至於具爭議性之靈芝鍺含量則證明其含量與其他眞菌或高等植物並無差異，甚至更低。

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