Rapid Paper

Evidence on the Strain-specific Terpenoid Pattern of *Ganoderma lucidum*

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Using the fruiting bodies obtained from different strains of *Ganoderma lucidum*, several terpenoids were analyzed, and the constituents showed a pattern peculiar to the strains. The fruiting body from Strain I contained C$_{27}$ lucidenic acids, and that from Strain II contained C$_{30}$ ganoderic acids as major products. A sample different from these strains showed a terpenoid pattern similar to that of Strain II. The results indicate that the terpenoid pattern can be useful to distinguish the strains between the two groups.

The fruiting body of the fungus *Ganoderma lucidum* (Polyporaceae) has been used as a folk medicine in China and Japan. It is said that some of its physiological effects are distinct depending upon the strains and the cultivating conditions.\(^1\)\(^-\)\(^3\) Of the fruiting body, several terpenoids\(^4\)\(^-\)\(^14\) and polysaccharides\(^15\)\(^-\)\(^18\) have been investigated in relation to their physiological effects. However, particular components characterizing individual strains have not been determined.

Khoda *et al.*\(^10\) have analyzed the terpenoid pattern of some strains of *G. lucidum* and showed the difference of contents of ganoderic acids B and C$_{2}$.\(^19\) Recently, we have cultivated two strains of *G. lucidum* (Strain I and Strain II) to make fruiting bodies by the bottle method using sawdust and rice bran. Both these strains formed fruiting bodies in an antlered shape,\(^15\) of which the terpenoid patterns were analyzed by high-performance liquid chromatography (HPLC). From the analysis, it was revealed that Strain I contained lucidenic acids (C$_{27}$ triterpenoids) and Strain II ganoderic acids (C$_{30}$ triterpenoids) as major products. On the other hand, the terpenoid pattern of the fruiting body (ordinary mushroom form) of *G. lucidum* (Nagano), which was cultivated in Nagano prefecture, was also analyzed to be similar to that of Strain II. This report clearly reveals the difference in terpenoid pattern between the strains of *G. lucidum*.

We also mention here the pattern change of lucidenic acids, which were induced in the early period and accumulated during the fruiting of Strain I.

**MATERIALS AND METHODS**

*Materials.* Two strains of *G. lucidum* were kindly provided by Mr. A. Honda (Nihon Joyaku Co., Ltd.) and are designated as Strains I and II, respectively. Both the strains were harvested in Saitama prefecture (Strain I in September 1981 at Hasuta, and Strain II in October 1980 at Ageo) and formed ordinary fruiting bodies with a cap at the optimum conditions for both the log-bed and sawdust-bottle cultivating methods. Strain I is popularly called “Saegusa” and its stem branches into three, but the stem of Strain II, which is called “Normal,” does not.

The fruiting bodies (ordinary mushroom form) of another strain of *G. lucidum*, which were cultivated in Nagano prefecture and are designated as “Nagano,” were also obligingly supplied by the Nagano-ken Nouson
Cultivation of Strains I and II. Strain I of *G. lucidum* was shake-cultured at 28°C in a liquid medium (ca. 150 ml) containing 5% glucose, 1% malt extract, 0.2% yeast extract, 0.2% peptone, 0.5% KH₂PO₄ and 0.25% MgSO₄·7H₂O for 2 weeks in 500 ml shaker flask. The grown mycelium was pipetted and inoculated into a sawdust-rice brain (4 : 1, v/v) solid medium with 60~65% moisture in a tall petri dish (8.00 x 15 cm), which was kept in dark conditions at 23°C and 80% humidity. After 2 weeks, the mycelium overgrowing medium was raked out and inoculated freshly into the same solid medium already mentioned, which had been pre-packed in the fruiting body-forming bottle (ca. 800 ml, made of polypropylene). The culture medium was again kept in the same dark conditions, and 2 weeks later, when the fungus overgrew and formed a mycelial mat (ca. 1 cm thickness) on the medium, it was moved into light conditions or fruiting-inducing conditions (white fluorescent light, 28°C, 90~95% humidity). Fruiting bodies were gradually induced and grew to an antlered form, and samples for analysis were harvested with the mycelial mat from the two bottles at 0 ~ 8 weeks after moving into the fruiting-inducing conditions.

Strain II was cultivated by the same procedure as that for Strain I; however, Strain II didn’t form a distinct mycelial mat as Strain I did. Strain II also formed antlered fruiting bodies, which were harvested at 9 weeks after moving into the fruiting-inducing conditions.

Preparation of the terpenoids. The fruiting bodies of *G. lucidum* (Strains I, II and Nagano) were extracted with EtOH, although in the case of Strain I, the fruiting bodies and mycelial mat on the medium were extracted every week. Each extract was partitioned between CHCl₃ and water. The CHCl₃ layer was dried with Na₂SO₄ and concentrated to give a residue, which contained particular terpenoids and was applied to the following HPLC analysis.

HPLC analysis. High-performance liquid chromatography was performed on a Hitachi 635 liquid chromatograph. The analytical procedure practically followed Kohda’s method. The CHCl₃ extract, which contained terpenoids, was dissolved in MeOH and passed through a Sep-pak C₁₈ cartridge (Waters Assoc.). The eluate was concentrated and dissolved again in MeOH, which contained thymol (0.3 mg/ml) as an internal standard, to give a sample solution of about 3 mg of eluate/ml. Ten μl (Strain I) or 4 μl (Strain II and Nagano) of each sample solution was subjected to the HPLC analysis. The analysis was done with 2% AcOH-CH₃CN (2.7 : 1) at a flow rate of 0.8 ml/min with a reverse-phase column (size: 3.9 mmφ x 15 cm, RESOLVE C₁₈, Waters Assoc.). The detector was set at 254 nm.

RESULTS AND DISCUSSION

*G. lucidum* (Strain I) was cultivated by the bottle cultivating method and formed antlered fruiting bodies. Its development and the yield of the CHCl₃ extract during fruiting are shown in Fig. 1. Fruiting bodies were induced within a week and then vigorously grew for 6 weeks after moving into the fruiting-inducing conditions. Thereafter, the water content decreased to harden the fruiting bodies. The yield of the CHCl₃ extract, which reflects the amount of the terpenoids, varied as the weight of the fungus changed. The terpenoid pattern of the CHCl₃ extract was analyzed by reverse-phase HPLC and its time-dependent change is shown in Fig. 2. At zero weeks, when the culture medium was moving into the fruiting-inducing conditions, terpenoids such as lucidenic acids and ganoderic acids had not been produced, but one week

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**FIG. 1.** Development of *G. lucidum* (Strain I) and the CHCl₃ Extract during Fruiting. Fruiting bodies and a mycelial mat of *G. lucidum* (Strain I) were harvested every week per two bottles. The solid line shows the weight of the harvested samples. The broken line shows the yield of the CHCl₃ extracts, which were prepared from EtOH extract of the samples. The values at 0 week were due to only the mycelial mat, because no fruiting bodies had been induced then.
Terpenoid Pattern of *G. lucidum*

Fig. 2. Reverse-phase HPLC of CHCl₃ Extracts from *G. lucidum* (Strain I) during Fruiting.

0, 1, 4 and 8 weeks' data are shown. Fruiting bodies had not been induced at 0 week, so only the mycelial mat on the medium was subjected to the analysis. LA-A, lucidenic acid A; LA-B, lucidenic acid B; LA-C, lucidenic acid C; LA-D₁, lucidenic acid D₁; LA-E₁, lucidenic acid E₁. Thymol was used as an internal standard. Lucidenic acid D₁ and thymol were eluted at the same retention time. The vertical axes are drawn to the same scale.

Fig. 3. Reverse-phase HPLC of CHCl₃ Extracts from *G. lucidum* (Strain II) (A) and (Nagano) (B).

GA-A, ganoderic acid A; GA-₆, ganoderic acid B; GA-C₁, ganoderic acid C₁; GA-C₂, ganoderic acid C₂. Thymol was used as an internal standard.

The terpenoids of the fruiting bodies of *G. lucidum* (Strain II, antlered form), which was cultivated in our laboratory, and *G. lucidum* (Nagano, mushroom form) were analyzed in the same way as that for *G. lucidum* (Strain I). The results are shown in Fig. 3. In contrast to Strain I, both Strain II and Nagano contained ganoderic acids as highly oxidized terpenoids, although the content ratio was different between Strain II and Nagano. Ganoderic acid C₂ was a major product in Strain II and ganoderic acid A was in Nagano. No definitive peaks due to lucidenic acids were observed in either fungus.

It is not clear whether the different content ratio of ganoderic acids between Strain II and Nagano was due to the difference of strains, cultivating conditions or fruiting body's forms. But, from this experiment, it is suggested that *G. lucidum* can be classified into at least two types, the lucidenic acid (C₂₇) type and the ganoderic acid (C₃₀) type, on the basis of its terpenoid pattern. This result is interesting when related to the different physiological effect of *G. lucidum* that depend on the strains or the cultivating conditions.¹⁻³

Although we have reported the isolation of C₂₇ lucidenic acids and C₃₀ ganoderic acids from a sample of *G. lucidum*,⁴⁻⁸ there is no discrepancy with the result in this paper, because the previous experiment was carried out later (at 1 week) lucidenic acids C, E₁ and an unidentified compound were detected as major products. The composition of the terpenoids gradually changed with the development of the fungus. Lucidenic acids A, B and D₁ were obviously observed as major components at 8 weeks in addition to the above compounds, although the content ratios of lucidenic acid E₁ and the unidentified compound slightly diminished. On the other hand, any clear peaks due to C₃₀ ganoderic acids were not observed in this fungus. From these observation, it was concluded that *G. lucidum* (Strain I) produced C₂₇ lucidenic acids as highly oxidized terpenoids during fruiting, which were induced in the early stage and accumulated with its pattern change.
by using a mixture of the fruiting bodies of two *G. lucidum*, “Saegusa” (Strain I) and “Normal” (Strain II), which was usually prepared for commercial availability.

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**REFERENCES AND NOTE**

19) Ganoderic acid C2 is called C in ref. 10, D in ref. 11 and D2 in ref. 7.