# Quantitative Determination of Bitter Principles in Specimens of *Ganoderma lucidum* Using High-Performance Liquid Chromatography and Its Application to the Evaluation of Ganoderma Products

Jiang-Jing Gao,[a] Norio Nakamura,[a] Byung-Sun Min,[a,b] Akiko Hirakawa,[a] Feng Zuo,[a] and Masao Hattori*,[a]

[a] *Institute of Natural Medicine, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930–0194, Japan: and* [b] *Immunomodulator Laboratory, Korea Research Institute of Bioscience & Biotechnology; 305–600 Taejon, Korea.*
Received December 22, 2003; accepted March 8, 2004

**For quantitative determination of 19 triterpene constituents, including six ganoderma alcohols (1—6) and 13 ganoderma acids (7—19), in the products of *Ganoderma lucidum*, an analytical system was developed using high-performance liquid chromatography with an ODS column. The mobile phase was a linear gradient of 1% AcOH/$H_2O$–$CH_3CN$ and 2% AcOH/$H_2O$–$CH_3CN$, and the elution profile was monitored at 243 and 250 nm for ganoderma alcohols and acids, respectively. The relative standard deviations of this method were less than 2.35% and 2.18% ($n$=5) for intraday and interday assays, and the recoveries were 90.9—100.8% and 93.4—103.9% for constituents of alcohol and acid groups, respectively. This system was applied to a quantitative determination of the constituents in 10 different products of *G. lucidum*: six usual umbrella forms of the fruiting bodies, three antlered forms of the fruiting bodies and spores, and eight specimens from the same *G. lucidum* strain, which was parasitized on logs from different plants or different fungus beds. The analytical results indicated that the quantity and composition of these triterpenes differed appreciably among various specimens, but the relative ratio of the alcohols and acids was not significantly different when the same strain of *G. lucidum* was used.**

**Key words** *Ganoderma lucidum*; fruiting body; antlered form; spore; HPLC; triterpene

*Ganoderma lucidum* Karst (reishi in Japanese) is a well known Chinese crude drug, which has long been used widely in China, Japan, and other East Asian countries as both a tonic and a sedative. It is considered to be a natural medicine that promotes longevity and maintains the vitality of human beings.[1] Its beneficial clinical effects in patients with hepatitis, hyperglycemia, chronic bronchitis, cancer, muscular dystrophy, arteriosclerosis, hypertension, hypercholesterolemia, and leukopenia have been confirmed in pharmacologic studies in recent years.[2—9] The fruiting bodies, mycelia, and spores have recently received more and more attention not only as home remedies[10] but also as new drug sources.

In the past two decades, over 130 highly oxygenated triterpenes and related compounds have been isolated from the fruiting bodies, mycelia, and spores of *G. lucidum*. Some of these compounds show anti-HIV-1 (ganoderiol F and ganodermanontriol),[11] anticholesterol (ganoderic acids B and C),[12,13] antinociceptive (ganoderic acids A, B, and G and compound C6),[14] antihistamine (ganoderic acids C1 and C2),[15] anticomplement (ganoderiol F, ganodermanondiol, and ganodermanontriol),[16] and anti-HIV-1 protease (lucidumol B and ganodermanontriol)[17] activities, and cytotoxic effects against Meth-A (sarcoma) (ganodermanondiol and lucidunols A and B),[18] Lewis lung carcinoma (LLC) (lucidumols A and B and ganoderic acid $\theta$),[18] and T-47D (human breast cancer) (ganodermanondiol),[19] as well as inhibition of the glucosyltransferase from the cariogenic bacterium *Streptococcus mutans* (ganoderic acids S1 and C).[20]

With the successful artificial cultivation of *G. lucidum*, this mushroom and related products have been widely used as health foods and drugs at present. However, quantitative analysis of the constituents in the products of *G. lucidum* has rarely been conducted.[21—25] Because of the structural similarity of a number of constituents and the difficulty in obtaining sufficient amounts of standard compounds, no adequate method for a simultaneous analysis of multiple constituents has been reported. Recently, Ma *et al.* have analyzed four constituents in some strains.[26] In our previous studies, we reported the quantitative determination of several major constituents using HPLC[17] and an enzyme immunoassay of ganoderic acid A,[27] which is abundantly present in this mushroom. However, they were insufficient for a detailed evaluation of the products of *G. lucidum*. Furthermore, the quality evaluation of chemical components in specimens prepared on different plant logs parasitized with a single strain of *G. lucidum* have not yet been reported.

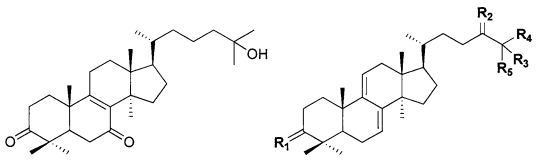
In the present paper, we report an HPLC method for the quantitative determination of 19 triterpene constituents, including six ganoderma alcohols with a hydroxy group in the side chain (compounds **1**—**6**, lucidumol A, ganodemanontriol, ganodematriol, lucidumol B, ganoderiol F, and ganodermanondiol, respectively) and 13 ganoderma acids with a carboxy group in the side chain (compounds **7**—**19**, ganoderic acids $\theta$, $\eta$, $\varepsilon$, and C2, compound C6, ganoderic acids G, $\gamma$, B, A, $\alpha$, C1, and H, and ganolucidic acid A, respectively), in specimens of *G. lucidum*. Moreover, using this HPLC method, various ganoderma products were evaluated chemically.

## Experimental

**Materials** Specimens of the fruiting bodies and spores were kindly provided by Total Technological Consultant Co. Ltd. (Tokyo, Japan), Suntory Co. Ltd. (Osaka, Japan), and Ochi Product Cooperative (Ochi-cho, Shimane, Japan). Specimens A—H and VI were artificially cultivated with the same strain of *G. lucidum* at Ochi-cho, Ochi-gun, Shimane prefecture, Japan. Specimens A—F were parasitized on six different trees: chestnut, plum, mulberry, cherry, buckeye, and Japanese oak trees. Specimens G and H were grown on fungus beds filled with sawdust from buckeye and bamboo in bottles, respectively.

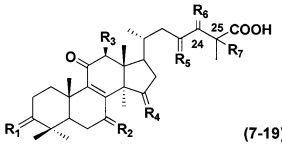
The standard compounds (**1**—**19**) used in this experiment were isolated

* To whom correspondence should be addressed. e-mail: saibo421@ms.toyama-mpu.ac.jp




Lucidumol A (**1**) (**2**—**6**)

| Constituent | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|
| Ganodermanontriol | (2) | O | 3-OH | OH | $CH_2OH$ | $CH_3$ |
| Ganodermatriol | (3) | 3-OH | $\Delta^{24(25)}$ | | $CH_2OH$ | $CH_2OH$ |
| Lucidumol B | (4) | 3-OH | 3-OH | OH | $CH_3$ | $CH_3$ |
| Ganoderiol F | (5) | O | $\Delta^{24(25)}$ | | $CH_2OH$ | $CH_2OH$ |
| Ganodermanondiol | (6) | O | 3-OH | OH | $CH_3$ | $CH_3$ |




(**7-19**)

| Constituent | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ |
|---|---|---|---|---|---|---|---|---|
| Ganoderic acid $\theta$ | (7) | 3-OH | O | 3-OH | O | 7-OH | $\Delta^{24(25)}$ | |
| Ganoderic acid $\eta$ | (8) | 3-OH | 2-OH | 3-OH | O | 7-OH | $\Delta^{24(25)}$ | |
| Ganoderic acid $\varepsilon$ | (9) | 3-OH | 2-OH | H | O | 7-OH | $\Delta^{24(25)}$ | |
| Ganoderic acid C2 | (10) | 3-OH | 2-OH | H | H | O | $H_2$ | H |
| Compound C6 | (11) | 3-OH | O | O | OH | O | $H_2$ | H |
| Ganoderic asid G | (12) | 3-OH | 2-OH | O | OH | O | $H_2$ | H |
| Ganoderic acid $\gamma$ | (13) | O | 2-OH | H | H | 7-OH | $\Delta^{24(25)}$ | |
| Ganoderic acid B | (14) | 3-OH | 2-OH | O | H | O | $H_2$ | H |
| Ganoderic acid A | (15) | O | 2-OH | H | H | O | $H_2$ | H |
| Ganoderic acid $\alpha$ | (16) | 3-OH | O | 2-OAc | H | O | $H_2$ | H |
| Ganoderic acid C1 | (17) | O | 2-OH | $H_2$ | O | O | $H_2$ | H |
| Ganoderic acid H | (18) | 3-OH | O | 2-OAc | O | O | $H_2$ | H |
| Ganolucidic acid A | (19) | O | $H_2$ | H | H | O | $H_2$ | H |

Fig. 1. Structures of Triterpene Constituents of *G. lucidum*

from the chloroform-soluble fractions of the MeOH extracts obtained from the spores and fruiting bodies of *G. lucidum*, as reported previously (Fig. 1).[11,16—19,28] The structures and purities of these compounds were confirmed with spectroscopic and chromatographic methods. Acetonitrile of analytical HPLC grade was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and acetic acid of analytical reagent grade was from Nacalai Tesque (Kyoto, Japan). The organic solvents and other chemical reagents were of analytical reagent grade and supplied by Wako Pure Chemical Industries Ltd. Column chromatography was carried out on Florisil (100—200 mesh, Nacalai Tesque). Thin-layer chromatography (TLC) was carried out on pre-coated silica-gel 60 $F_{254}$ plates (0.25 mm, Merck Co., Darmstadt, Germany), and spots were detected under a UV light and by spraying with 10% $H_2SO_4$ followed by heating. Millipore syringe filters (Millex-GP, 0.45-$\mu$m pore size) were purchased from Nihon Millipore (Tokyo, Japan).

**Instrumentation and HPLC Analysis** Analytical HPLC was carried out on a CCP 8020 system (Tosoh Co., Tokyo, Japan) consisting of a multi-solvent delivery pump (CCPM-II), an autoinjector, and a single-wavelength UV detector (UV-8020). The signals from the detector were recorded and analyzed on a C-R8A recorder (Shimadzu Co., Kyoto, Japan). A TSK gel ODS-80 Ts (Tosoh) column (150×4.6 mm i.d.) was used for analysis. The mobile phase was composed of 1% AcOH/$H_2O$–$CH_3CN$ (0 min, 45 : 55; 40 min, 40 : 60, for ganoderma alcohols) and 2% AcOH/$H_2O$–$CH_3CN$ (0 min, 75 : 25; 50 min, 70 : 30; 70 min, 60 : 40, for ganoderma acids). The flow rate was 1.0 ml/min, and the detecting wavelengths were set at 243 and 250 nm for ganoderma alcohols and acids, respectively. The operating temperature was maintained at 25 °C.

**Preparation of Standard Curves** Every standard compound was accurately weighed and dissolved in methanol to prepare a concentration of 1 mg/ml. Standard solutions of ganoderma alcohols and acids were prepared, followed by continuous dilution to give the seven respective concentrations. Linearities of two calibrations were determined with three injections for each concentration and plotted using linear regression of the peak-area ratio of each standard compound.

**Preparation of Samples for HPLC** The same amounts of pulverized fruiting bodies or spores were weighed and extracted three times with 20 volumes of $CHCl_3$ by refluxing in a boiling water bath for 3 h. After filtration, the combined solutions were evaporated to dryness *in vacuo*. These $CHCl_3$ extracts were chromatographed on a Florisil column eluted with hexane–acetone (9 : 1, 7 : 3) and $CHCl_3$–MeOH (1 : 2) to give three fractions, with monitoring by TLC. The following two fractions, corresponding to ganoderma alcohols and acids, were evaporated *in vacuo*, and each fraction was weighed accurately.

**Quantitative Determination of Triterpenes in Various Specimens of *G. lucidum*** The dried fractions were dissolved in suitable amount of methanol and filtered through a 0.45-$\mu$m Millipore filter unit. Then 10 $\mu$l of each sample solution was analyzed using HPLC. The contents of constituents in the test samples were calculated using the regression parameters obtained from standard curves.

**Recovery Tests** An appropriate amount of a certain specimen of *G. lucidum* was weighed accurately and extracted three times with 20 volumes of $CHCl_3$ for 3 h in a boiling water bath. After filtration and evaporation of the solvent, the redissolved extract was subjected to column chromatography on Florisil as described above. Both alcohol and acid fractions were dissolved in appropriate amounts of methanol and divided into four portions (with one as a control). Three portions (excluding the control) from each group were added to appropriate volumes of a mixture solution containing various amounts of standard compounds **2**—**6** or **7**—**15**, and then all four portions were evaporated to dryness and dissolved again in the same volume of methanol to give four concentrations of ganoderma alcohols and acids, respectively. All samples were filtered and subjected to HPLC analysis to calculate the recovery rates.

## Results and Discussion

**Quantitative Determination of 19 Triterpenens in *G. lucidum* Specimens Using HPLC** Although several HPLC methods have been reported for the determination of the constituents of *G. lucidum* specimens,[17,21—25] an isocratic elution system adopted by most researchers resulted in poor separation of a number of constituents in this mushroom. Therefore we employed the gradient elution system described above, which resulted in good separation of six ganoderma alcohols or 13 ganoderma acids, expect for ganoderic acids C1 and H (**17**, **18**). Figures 2 and 3 show typical chromatograms obtained from standard mixtures and different specimens of *G. lucidum*.

The standard compounds used in this experiment were isolated from chloroform-soluble fractions of methanol extracts of the fruiting bodies and spores of *G. lucidum*, according to the methods described in previous papers, and were identified by comparison of their spectroscopic 1D- and 2D-NMR, UV, IR and HR-EI-MS data with those reported.[16—19,28,29]

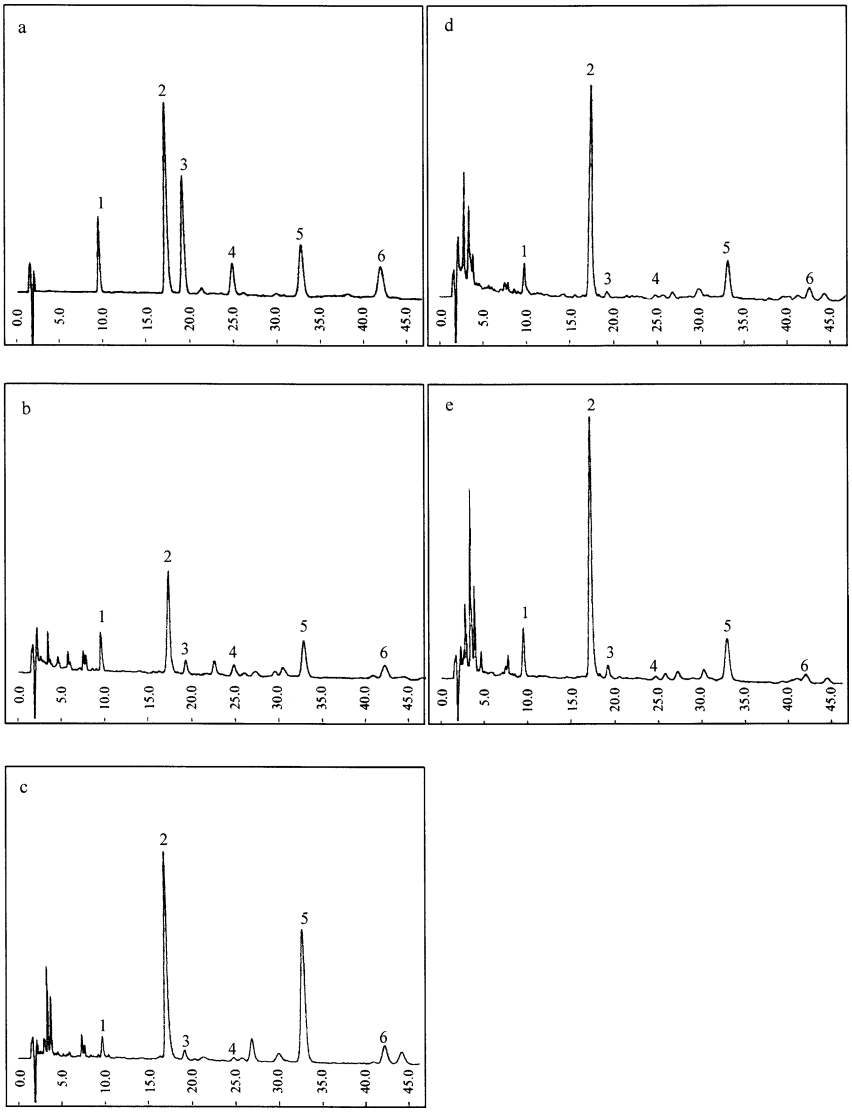



Fig. 2. HPLC Chromatograms of Ganoderma Alcohols in Various Specimens of *G. lucidum*

a. Standard mixture; b. fruiting body (specimen V); c. fruiting body (specimen VI); d. antlered form of the fruiting body (specimen VII); e. spore. **1**, Lucidumol A; **2**, ganodermanontriol; **3**, ganodermatriol; **4**, lucidumol B; **5**, ganoderiol F; **6**, ganodermanondiol.

The purities of all these standard compounds were more than 97% as estimated with a chromatographic method. The retention times are shown in Table 1. These peaks were distinguished by comparison with those of authentic samples by inspection of the retention times.

Calibration graphs were obtained, where $Y$ is the peak area and $X$ is the quantity of the same component (Table 1). Linear regression of the standard compounds showed good linearity, and the method permitted the determination of these constituents in *G. lucidum* specimens over a wide range of concentrations.

To verify the precision of this method, we analyzed standard mixtures of both groups at five concentrations with five repetitions on the same day and on 5 consecutive days. As shown in Table 2, the relative standard deviations (RSDs) of the intraday assay were 0.47—1.84% for the alcohol group and 0.58—2.02% for the acid group, while the RSDs of the interday assay obtained for a 5-d period were 0.90—2.35% and 0.46—2.18%, respectively.

The recovery rates were examined at three concentrations, and the constituents tested showed recoveries of 90.9—100.8% and 93.4—103.9% for ganoderma alcohols and acids, respectively (Table 3). These findings indicate that the method was satisfactory in terms of linearity, accuracy, and reproducibility. Due to the difficulty in obtaining sufficient amounts of standard compounds, the recovery rates were determined for 15 compounds only, and the quality of ganoderic acid $\alpha$ (**16**) was a relative value.

**Comparisons of Contents and Compositions of 19 Constituents among Various *G. lucidum* Specimens** Applying the above HPLC method, we performed quantitative analysis of the 19 triterpene constituents in 10 specimens consisting of nine fruiting bodies including three antlered forms and a spore (Table 4). Typical chromatograms of these samples are shown in Figs. 2 and 3. The total contents of alcohols and acids in the spore and antlered forms of the fruiting bodies (5549.2—7034.2 $\mu$g/g) were markedly higher than in the umbrella forms of fruiting bodies (2443.1—4441.2 $\mu$g/g). However, the spore, in which it was previously reported that the total triterpene content was 5—20 times that

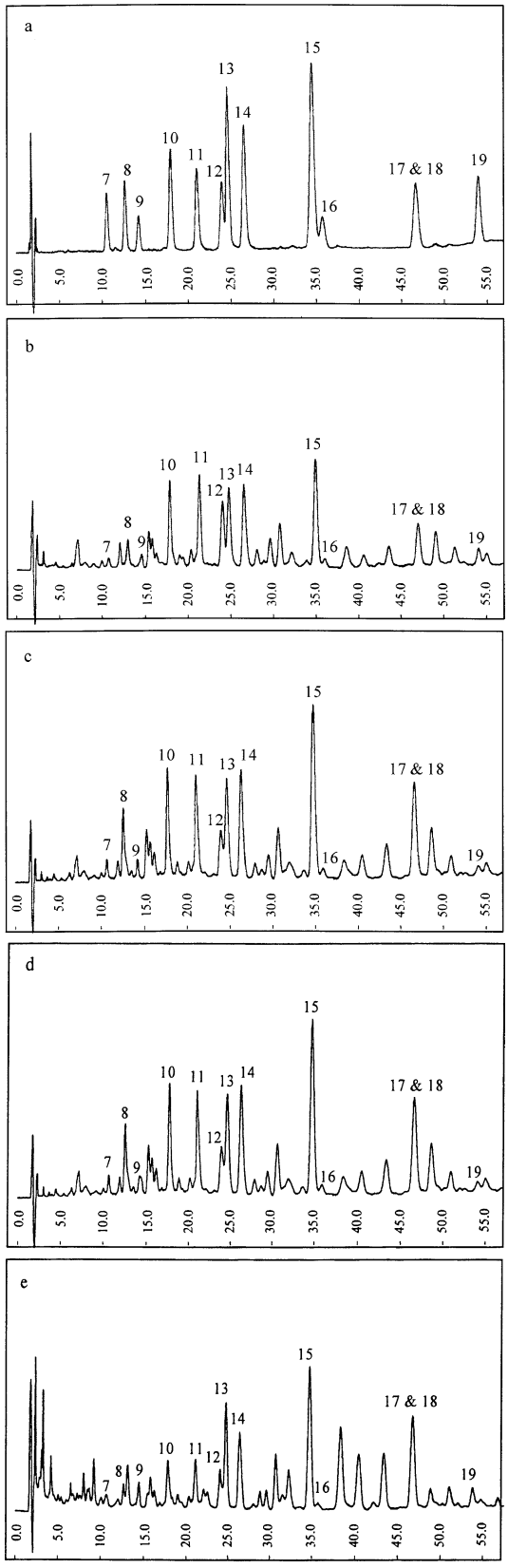



Fig. 3. HPLC Chromatograms of Ganoderma Acids in Various Specimens of *G. lucidum*

a. Standard mixture; b. fruiting body (specimen V); c. fruiting body (specimen VI); d. antlered form of the fruiting body (specimen VIII); e. spore. **7**, Ganoderic acid $\theta$; **8**, ganoderic acid $\eta$; **9**, ganoderic acid $\varepsilon$; **10**, ganoderic acid C2; **11**, compound C6; **12**, ganoderic acid G; **13**, ganoderic acid $\gamma$; **14**, ganoderic acid B; **15**, ganoderic acid A; **16**, ganoderic acid $\alpha$; **17**, ganoderic acid C1; **18**, ganoderic acid H; **19**, ganolucidic acid A.

Table 2. Intraday and Interday Relative Standard Deviations (min, $n=5$) of Constituents of *G. lucidum*

| Constituent | Intraday | Interday |
|---|---|---|
| Alcohol | | |
| Lucidumol A | 0.47 | 2.16 |
| Ganondermanontriol | 1.60 | 1.51 |
| Ganodermatriol | 0.97 | 0.90 |
| Lucidumol B | 1.84 | 1.49 |
| Ganoderiol F | 1.05 | 1.71 |
| Ganodermanondiol | 1.09 | 2.35 |
| Acid | | |
| Ganoderic acid $\theta$ | 1.15 | 2.06 |
| Ganoderic acid $\eta$ | 1.47 | 1.23 |
| Ganoderic acid $\varepsilon$ | 0.91 | 1.44 |
| Ganoderic acid C2 | 0.92 | 0.90 |
| Compound C6 | 1.66 | 1.85 |
| Ganoderic acid G | 1.38 | 1.99 |
| Ganoderic acid $\gamma$ | 1.79 | 1.40 |
| Ganoderic acid B | 1.19 | 1.70 |
| Ganoderic acid A | 0.58 | 0.46 |
| Ganoderic acid C1 & H | 1.70 | 0.98 |
| Ganolucidic acid A | 1.34 | 2.18 |

of the fruiting bodies,[17] only contained 5549.2 $\mu$g/g of triterpenes, which is less than that in the antlered forms. We assume that this is mainly due to the difference in harvesting time, manufacturing process, or genetic source. The HPLC chromatograms showed that most of the umbrella and antlered forms of the fruiting bodies and spores was similar in the relative ratio of acids and alcohols, where the relative acid contents were appreciably higher than the alcohol contents, except in specimen VI, which, inversely, showed an alcohol content two-fold higher than the acid content. The total alcohol content in this specimen was 2.6—8.3 times higher than that in other specimens of the fruiting bodies (Fig. 4). It is not clear whether the difference in the relative ratio of the alcohol and acid constituents between specimen VI and other specimens was attributable to the difference in genetic source, cultivating conditions, or manufacturing process. Since we have reported that ganoderma alcohols tend to be more potent in cytotoxic and anti-HIV activities,[11,17—19] it is of interest to select this characteristic strain of *G. lucidum* for medicinal purposes.

The triterpene compositions among 10 specimens differed greatly; the principal and secondary alcohols were ganodermanontriol (**2**) and ganoderiol F (**5**) in most specimens (I, II, IV, VI—VIII), while ganodermanondiol (**6**) was the major alcohol in specimens III and IX, and lucidumol A (**1**) was the secondary alcohol in specimens III, V, and X. On the other hand, the major acid was ganoderic acid A (**15**) in specimens I, III, IV, and VII—X, ganoderic acid C1 and H (**17**, **18**) in specimen II, compound C6 (**11**) in specimen V, and ganoderic acid $\gamma$ (**13**) in specimen VI. Ganoderic acid C2 (**10**) and compound C6 (**11**), which were contained in large amounts in most specimens, were present only in small amounts in specimen VI. These results indicate that the compositions and contents of triterpenes vary appreciably among different specimens.

To evaluate the triterpene content and composition among the fruiting bodies cultivated on different mushroom-growing logs, a single strain of *G. lucidum* was cultivated on chestnut, plum, mulberry, cherry, buckeye, and Japanese oak logs

Table 1. Retention Time Reproducibilities, Regression Equations, Correlation Coefficients ($\gamma$), and Linearity Ranges of Constituents of *G. lucidum*

| Constituent | Retention time reproducibility (min, $n$=5) | Regression equation | $\gamma$ | Linearity range ($\mu$g) |
|---|---|---|---|---|
| Alcohol | | | | |
| Lucidumol A | 9.62±0.134 | $y$=3504.2$x$+30.706 | 0.9998 | 0.1000—2.5000 |
| Ganondermanontriol | 17.38±0.268 | $y$=6986.7$x$+431.63 | 0.9998 | 0.2113—3.3796 |
| Ganodermatriol | 19.36±0.309 | $y$=6726.9$x$+130.38 | 0.9999 | 0.1703—4.2556 |
| Lucidumol B | 24.94±0.423 | $y$=6400.3$x$+78.675 | 0.9996 | 0.0500—3.2000 |
| Ganoderiol F | 33.06±0.597 | $y$=5271.5$x$+302.68 | 0.9997 | 0.1180—3.7736 |
| Ganodermanondiol | 42.40±0.744 | $y$=3902.1$x$+37.786 | 0.9996 | 0.1100—3.5200 |
| Acid | | | | |
| Ganoderic acid $\theta$ | 10.76±0.279 | $y$=973.3$x$+37.602 | 0.9995 | 0.0625—2.0000 |
| Ganoderic acid $\eta$ | 12.84±0.115 | $y$=1816.4$x$+94.572 | 0.9993 | 0.0625—2.0000 |
| Ganoderic acid $\varepsilon$ | 14.39±0.135 | $y$=1435$x$+64.269 | 0.9992 | 0.0625—2.0000 |
| Ganoderic acid C2 | 17.89±0.158 | $y$=1620.8$x$+110.53 | 0.9990 | 0.0625—4.0000 |
| Compound C6 | 21.29±0.147 | $y$=1826.7$x$+195.44 | 0.9993 | 0.1563—4.6890 |
| Ganoderic acid G | 23.96±0.169 | $y$=2331.8$x$+110.33 | 0.9985 | 0.0625—2.0000 |
| Ganoderic acid $\gamma$ | 24.62±0.188 | $y$= 2859.2$x$+343.11 | 0.9990 | 0.1250—4.0000 |
| Ganoderic acid B | 26.29±0.188 | $y$=2579.6$x$+271.56 | 0.9993 | 0.1250—4.0000 |
| Ganoderic acid A | 34.76±0.257 | $y$=2781.5$x$+332.81 | 0.9988 | 0.0886—5.6700 |
| Ganoderic acid $\alpha$ | 35.86±0.255 | — | — | — |
| Ganoderic acid C1 & H | 46.79±0.330 | $y$=2099.5$x$+279.79 | 0.9993 | 0.1772—2.8350 |
| Ganolucidic acid A | 53.84±0.291 | $y$=3295.1$x$+268.5 | 0.9995 | 0.1250—2.0000 |

Table 3. Recovery Rates of Constituents of *G. lucidum*

| Constituent | Added ($\mu$g) | Found ($\mu$g) | Recovery (%) | Mean±S.D. | RSD (%) |
|---|---|---|---|---|---|
| Alcohol | | | | | |
| Ganodermanontriol | 60.2 | 59.4 | 98.7 | 99.8±2.72 | 2.73 |
| | 30.1 | 30.9 | 102.9 | | |
| | 15.0 | 14.7 | 97.8 | | |
| Ganodermatriol | 40.0 | 36.7 | 91.7 | 97.9±5.63 | 5.76 |
| | 20.0 | 19.8 | 99.2 | | |
| | 10.0 | 10.3 | 102.7 | | |
| Lucidumol B | 16.0 | 15.1 | 94.4 | 96.7±2.72 | 2.82 |
| | 8.0 | 8.0 | 99.7 | | |
| | 4.0 | 3.8 | 95.9 | | |
| Ganoderiol F | 24.0 | 21.9 | 91.5 | 90.9±4.40 | 0.84 |
| | 12.0 | 11.0 | 91.2 | | |
| | 6.0 | 5.4 | 90.0 | | |
| Ganodermanondiol | 16.0 | 15.9 | 99.1 | 100.8±3.48 | 3.45 |
| | 8.0 | 8.4 | 104.8 | | |
| | 4.0 | 3.9 | 98.4 | | |
| Acid | | | | | |
| Ganoderic acid $\theta$ | 20.0 | 19.8 | 98.8 | 99.1±0.81 | 0.81 |
| | 10.0 | 10.0 | 100.0 | | |
| | 5.0 | 4.9 | 98.4 | | |
| Ganoderic acid $\eta$ | 20 | 20.3 | 101.6 | 102.7±0.92 | 0.89 |
| | 10 | 10.3 | 103.3 | | |
| | 5 | 5.2 | 103.1 | | |
| Ganoderic acid $\varepsilon$ | 20 | 19.9 | 99.4 | 97.8±3.46 | 3.54 |
| | 10 | 10.01 | 100.1 | | |
| | 5 | 4.7 | 93.8 | | |
| Ganoderic acid C2 | 40 | 41.8 | 104.5 | 103.9±4.81 | 4.63 |
| | 20 | 19.8 | 98.8 | | |
| | 10 | 10.4 | 108.4 | | |
| Compound C6 | 50 | 48.9 | 97.8 | 98.4±0.89 | 0.90 |
| | 25 | 24.5 | 98.0 | | |
| | 12.5 | 12.4 | 99.4 | | |
| Ganoderic acid G | 20 | 18.6 | 92.8 | 93.4±2.89 | 3.09 |
| | 10 | 9.7 | 96.5 | | |
| | 5 | 4.5 | 90.9 | | |
| Ganoderic acid $\gamma$ | 40 | 41.9 | 104.6 | 100.2±3.85 | 3.84 |
| | 20 | 19.5 | 97.4 | | |
| | 10 | 9.9 | 98.7 | | |
| Ganoderic acid B | 40 | 42.3 | 105.8 | 103.1±4.29 | 4.16 |
| | 20 | 21.1 | 105.4 | | |
| | 10 | 8.8 | 98.2 | | |
| Ganoderic acid A | 60 | 61.2 | 102.1 | 102.3±0.94 | 0.92 |
| | 30 | 31.0 | 103.3 | | |
| | 15 | 14.0 | 101.5 | | |

Table 4. Contents of Ganoderma Alcohols and Acids in Various *G. lucidum* Specimens

| Constituent | Fruiting body | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Alcohol | | | | | |
| Lucidumol A | 72.8[a)]±9.1[b)] | 69.6±17.2 | 50.0±10.1 | 71.5±9.7 | 106.9±21.5 |
| Ganondermanontriol | 235.3±50.7 | 196.7±19.5 | 19.2±2.3 | 153.3±21.4 | 120.9±12.2 |
| Ganodermatriol | 20.0±7.4 | 19.7±5.6 | —[c)] | 18.7±3.8 | 11.0±0.1 |
| Lucidumol B | 13.3±5.6 | 12.4±2.3 | 9.9±0.1 | 17.0±2.9 | 9.4±0.3 |
| Ganoderiol F | 156.5±30.1 | 131.2±15.6 | 18.9±1.5 | 103.9±15.5 | 42.2±2.5 |
| Ganodermanondiol | 67.9±10.6 | 54.3±3.0 | 123.7±3.6 | 64.7±4.2 | 29.6±2.2 |
| Total alcohols | 565.7±65.8 | 483.9±14.8 | 221.8±5.8 | 430.0±54.0 | 319.9±22.5 |
| Acid | | | | | |
| Ganoderic acid $\theta$ | — | — | 77.9±5.5 | — | 44.5±6.9 |
| Ganoderic acid $\eta$ | 88.2±9.1 | — | — | — | 258.7±16.0 |
| Ganoderic acid $\varepsilon$ | 44.3±5.2 | 40.2±1.4 | 81.8±8.5 | — | 85.2±14.6 |
| Ganoderic acid C2 | 598.5±35.1 | 144.6±3.8 | 175.3±16.8 | 219.9±20.8 | 514.7±14.6 |
| Compound C6 | 325.6±28.3 | 223.8±59.5 | 175.8±12.2 | 134.8±17.9 | 543.2±4.1 |
| Ganoderic acid G | 338.7±23.0 | 197.6±58.2 | 222.4±21.7 | 261.3±17.3 | 248.2±31.5 |
| Ganoderic acid $\gamma$ | 372.6±28.2 | 104.9±8.2 | 280.4±7.5 | 74.2±11.9 | 379.5±84.2 |
| Ganoderic acid B | 472.4±34.9 | 199.1±8.9 | 124.7±31.4 | 232.6±26.7 | 457.4±35.4 |
| Ganoderic acid A | 865.9±72.7 | 491.6±38.9 | 591.1±13.7 | 688.7±59.4 | 514.1±32.7 |
| Ganoderic acid $\alpha$ | 75.4±9.5 | 72.4±7.1 | 78.1±7.3 | 54.9±5.2 | 55.3±6.3 |
| Ganoderic acid C1 & H | 597.2±53.4 | 517.8±36.5 | 388.6±31.6 | 555.8±28.8 | 294.3±14.7 |
| Ganolucidic acid A | 96.6±12.3 | 38.3±1.9 | 25.0±3.9 | 43.3±5.1 | 57.5±10.0 |
| Total acids | 3875.5±274.5 | 2030.2±88.5 | 2221.2±53.0 | 2265.6±107.0 | 3452.6±204.8 |
| Total triterpenes | 4441.2±328.4 | 2514.1±94.0 | 2443.1±45.6 | 2695.6±180.1 | 3772.5±181.2 |

| Constituent | Fruiting body | | | | Spore |
|---|---|---|---|---|---|
| | VI | Antlered form | | | X |
| | | VII | VIII | IX | |
| Alcohol | | | | | |
| Lucidumol A | 281.7±65.9 | 143.1±6.8 | 74.0±14.6 | 198.5±37.3 | 317.6±24.5 |
| Ganondermanontriol | 1209.1±199.4 | 971.7±58.0 | 305.0±25.7 | 258.0±34.5 | 875.4±69.2 |
| Ganodermatriol | 41.2±8.0 | 38.1±2.2 | 31.9±7.5 | 18.4±3.7 | 27.7±2.9 |
| Lucidumol B | 13.9±4.7 | 13.8±2.4 | 17.3±3.2 | 22.3±6.5 | 19.1±1.4 |
| Ganoderiol F | 416.5±80.2 | 1232.0±62.9 | 266.4±34.2 | 84.2±18.0 | 186.2±22.9 |
| Ganodermanondiol | 110.9±17.7 | 343.8±19.8 | 63.8±5.6 | 284.5±25.9 | 76.4±8.6 |
| Total alcohols | 2073.3±357.8 | 2742.9±117.3 | 758.3±84.5 | 865.9±122.2 | 1502.4±119.3 |
| Acid | | | | | |
| Ganoderic acid $\theta$ | 29.5±4.2 | — | 215.1±13.4 | 283.9±27.1 | 63.9±9.0 |
| Ganoderic acid $\eta$ | 82.9±13.0 | — | 460.3±52.9 | 333.4±16.9 | 172.5±11.41 |
| Ganoderic acid $\varepsilon$ | 89.2±20.6 | 166.1±23.7 | 198.7±22.1 | 109.4±39.0 | 160.3±12.0 |
| Ganoderic acid C2 | — | 389.3±59.9 | 935.8±43.7 | 359.2±22.4 | 754.7±73.2 |
| Compound C6 | 76.8±8.1 | 65.7±17.4 | 828.6±26.8 | 618.1±42.3 | 122.3±42.5 |
| Ganoderic acid G | 95.8±18.9 | 366.3±42.1 | 294.6±15.3 | 451.7±55.9 | 257.3±13.9 |
| Ganoderic acid $\gamma$ | 448.7±33.4 | 252.6±22.4 | 523.8±28.4 | 488.2±41.4 | 549.7±38.3 |
| Ganoderic acid B | 56.2±21.3 | 160.5±4.3 | 660.5±56.2 | 366.1±12.1 | 309.2±26.1 |
| Ganoderic acid A | 99.5±26.9 | 1513.1±165.8 | 1098.1±41.5 | 943.6±36.6 | 801.4±46.8 |
| Ganoderic acid $\alpha$ | — | 92.0±2.0 | 90.0±5.6 | 97.5±13.4 | 64.4±6.4 |
| Ganoderic acid C1 & H | 117.9±19.9 | 807.5±91.0 | 944.1±65.7 | 923.9±24.8 | 701.2±15.0 |
| Ganolucidic acid A | 46.5±5.4 | 194.8±31.6 | 26.4±1.4 | 34.9±5.6 | 90.0±4.9 |
| Total acids | 1143.1±110.2 | 4007.9±457.3 | 6276.0±346.2 | 5009.8±188.0 | 4046.8±299.4 |
| Total triterpenes | 3216.4±472.4 | 6773.7±306.4 | 7034.2±274.8 | 5875.8±80.0 | 5549.2±317.3 |

*a*) $\mu$g/g (dry weight of sample). *b*) Standard deviation ($n$=3). *c*) Not detected.

(specimens A—F). Specimens G and H were obtained by cultivation in bottles filled with buckeye and bamboo sawdust, respectively. The HPLC chromatograms of both the alcohol and acid fractions of each sample were quite similar to those of specimen VI. The major alcohol was ganodermanontriol (**2**), while lucidumol B (**4**) was the minor one. For ganoderma acids, the quantity of ganoderic acid A (**15**), which was reported to be the major acid in fruiting bodies of *G. lucidum*,[17] was minor, and ganoderic acids C2 and $\alpha$ were not present, which was the same as in specimen VI in all eight specimens. These findings suggest that the composition of these triterpenes was not significantly influenced by the logs used for cultivation.

Comparing the total triterpenen contents (Fig. 5), both the ganoderma alcohols and ganoderma acids in these specimens were less than in specimen VI. In comparing the ratios of the

Table 5. Contents of Ganoderma Alcohols and Acids in Various Specimens of Ochi Reishi

| Constituent | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| Alcohol | | | | | | | | |
| Lucidumol A | 110.9[a)]±11.9[b)] | 130.2±10.5 | 80.3±8.2 | 168.7±16.1 | 91.8±10.1 | 97.2±5.3 | 154.7±15.5 | 121.1±11.9 |
| Ganondermanontriol | 360.1±33.3 | 402.9±30.9 | 311.2±23.7 | 403.7±22.2 | 351.6±36.0 | 317.2±20.1 | 506.0±28.3 | 479.9±24.3 |
| Ganodermatriol | 12.3±1.3 | 12.9±1.6 | 6.8±1.2 | 24.5±1.1 | 7.0±0.8 | 10.3±1.9 | 18.1±2.8 | 7.2±0.4 |
| Lucidumol B | 5.7±0.4 | 7.6±0.5 | 6.0±0.5 | 10.3±1.4 | 6.1±0.8 | 6.0±1.0 | 8.0±0.5 | 6.8±0.5 |
| Ganoderiol F | 91.6±9.3 | 83.0±2.3 | 52.7±4.3 | 147.8±10.8 | 65.6±10.6 | 72.2±6.6 | 145.0±12.2 | 172.7±11.0 |
| Ganodermanondiol | 26.3±2.0 | 28.5±1.5 | 26.6±4.9 | 25.0±0.8 | 36.0±2.7 | 21.5±2.5 | 34.5±3.4 | 60.6±7.1 |
| Total alcohols | 607.0±37.9 | 665.1±53.7 | 483.6±34.2 | 780.0±58.7 | 558.0±43.3 | 524.5±34.0 | 866.3±69.9 | 848.2±70.5 |
| Acid | | | | | | | | |
| Ganoderic acid $\theta$ | —[c)] | — | — | 21.0±0.4 | — | — | — | — |
| Ganoderic acid $\eta$ | 23.7±1.9 | 7.3±0.4 | — | 139.6±5.0 | 25.9±1.5 | 38.9±3.6 | 15.1±1.7 | 12.6±0.8 |
| Ganoderic acid $\varepsilon$ | 16.0±1.2 | — | — | 30.0±1.9 | 14.7±3.0 | 15.9±1.1 | 25.1±1.8 | 64.0±1.7 |
| Ganoderic acid C2 | — | — | — | — | — | — | — | — |
| Compound C6 | 99.3±8.7 | 29.4±1.5 | 7.8±1.4 | 294.0±3.0 | 153.6±13.3 | 109.3±10.0 | 47.3±4.1 | 47.2±1.8 |
| Ganoderic acid G | 19.4±2.1 | 19.2±1.3 | 7.2±0.9 | 53.3±0.5 | 20.0±1.9 | 12.4±1.1 | 19.1±2.5 | 5.0±0.4 |
| Ganoderic acid $\gamma$ | 140.5±10.7 | 112.2±9.8 | 44.4±8.8 | 359.6±6.1 | 50.4±4.2 | 79.0±6.7 | 247.3±2.7 | 129.5±7.6 |
| Ganoderic acid B | — | 13.3±0.9 | 7.5±1.9 | — | — | — | — | — |
| Ganoderic acid A | 30.0±2.7 | 43.9±5.4 | 17.8±1.3 | 62.0±1.4 | 2.3±0.4 | 11.6±0.8 | 39.6±3.3 | 5.7±0.9 |
| Ganoderic acid $\alpha$ | — | — | — | — | — | — | — | — |
| Ganoderic acid C1 & H | — | — | — | — | 21.9±1.7 | 21.5±2.2 | 7.4±0.8 | 90.6±7.2 |
| Ganolucidic acid A | — | — | 2.8±0.6 | 19.6±0.8 | — | — | 1.3±0.2 | — |
| Total acids | 329.9±23.5 | 225.4±18.6 | 87.3±14.0 | 979.2±44.5 | 288.6±29.5 | 288.5±28.0 | 402.1±43.0 | 354.6±15.2 |
| Total triterpenes | 935.8±44.4 | 890.5±69.2 | 590.4±58.2 | 1759.2±98.4 | 846.6±57.2 | 813.0±48.5 | 1268.4±107.1 | 1202.8±72.9 |

*a*) $\mu$g/g (dry weight of sample). *b*) Standard deviation ($n$=3). *c*) Not detected. A—F, parasitized on: A, chestnut, B, plum, C, mulberry; D, cherry, E, buckeye, F, Japanese oak. G and H, grown in bottles filled with buckeye and bamboo sawdust, respectively.

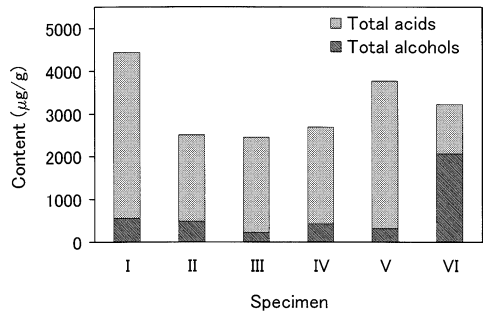



Fig. 4. Contents of Total Alcohols and Acids in Various Specimens of Umbrella Fruiting Body of *G. lucidum*

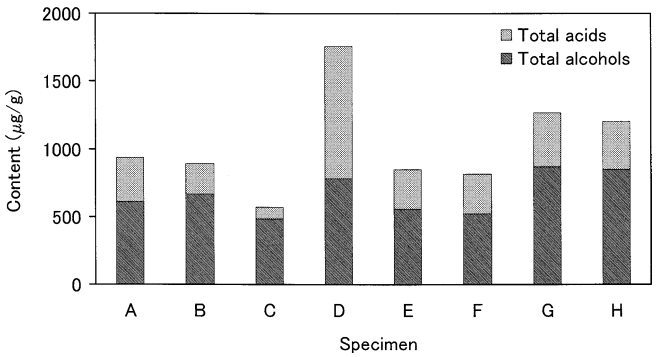



Fig. 5. Contents of Total Alcohols and Acids in Various Specimens of Ochi Reishi

A—F, Parasitized on six kinds of trees: A, chestnut, B, plum, C, mulberry, D, cherry, E, buckeye, F, Japanese oak. G and H, grown in bottles filled with buckeye and bamboo sawdust, respectyively.

alcohols and acids, the alcohols were present in 1.8-fold or more amounts than the acids in almost all samples (C was 5.5-fold greater), while in specimen VI the amount was 1.8-fold greater. Whereas in sample D (cherry log), the alcohol content was only 70% of the acids, and the total acid content was almost the same level as in specimen VI. The cherry tree may promote impudence of ganoderma acids when used as a culture medium for *G. lucidum*. There is no considerable variation was observed between specimens G and H.

It was reported that some physiological activities of this mushroom are dependent on the content and composition of triterpenes which vary with the strain and cultivating conditions.[25,29,30] On the basis of these experiments, we concluded that among various ganoderma products, the content and composition of triterpene constituents differed significantly in different specimens, but of a same strain, when the cultivation was carried out on different plants, the relative ratio of acid and alcohol contents was similar. Subsequently, this accurate, precise, and reliable analytical system for the determination of bitter principles in *G. lucidum* specimens may provide a useful means of chemical evaluation not only for scientific purposes but also for industrial applications.

**References**

1) Lieu C. W., Lee S. S., Wang S. Y., *Anticancer Res.*, **12**, 1211—1216 (1992).
2) Miyazaki T., Nishijima M., *Chem. Pharm. Bull.*, **29**, 3611—3616 (1981).
3) Franz G., *Planta Med.*, **55**, 493—497 (1989).
4) Furusawa E., Chou S. C., Furusawa S., Hirazami A., Dang Y., *Phytother. Res.*, **6**, 300—304 (1992).
5) Wang S. Y., Hsu M. L., Hsu H. H., Tzeng C. H., Lee S. S., Shiao M. S., Ho C. K., *Int. J. Cancer*, **70**, 699—705 (1997).
6) Kim H. W., Shim M. J., Kim B. K., *Seoul National University, Seoul*, **1998**, 1—3 (1998).
7) Kim D. H., Shim S. B., Kim N. J., Jang I. S., *Biol. Pharm. Bull.*, **22**, 162—164 (1999).
8) Lin Z. B., "Recent Advances in Chinese Herbal Drugs–Action and Uses," Beijing Scientific Publishing House, Beijing, 1991, pp. 133—

140.
9) Lin Z. B., "Modern Research of Ganoderma lucidum," 2nd ed., Beijing Medical University Press, Beijing, 2001, pp. 302—304.
10) Jiangsu New Medical College (ed.), "Zhongyao Dazidian," Shanghai Renmin Press, Shanghai, 1978, p. 1181.
11) El-Mekkawy S., Meselhy M. R., Nakamura N., Tezuka Y., Hattori M., Kakiuchi N., Shimotohno K., Kawahata T., Otake T., *Phytochemistry*, **49**, 1651—1657 (1998).
12) Sonoda Y., Sekigawa Y., Sato Y., *Chem. Pharm. Bull.*, **36**, 966—973 (1988).
13) Komoda Y., Shimizu M., Sonoda Y., Sato Y., *Chem. Pharm. Bull.*, **37**, 531—533 (1989).
14) Koyama K., Imaizumi T., Akiba M., Kinoshita K., Takahashi K., Suzuki A., Yano S., Horie S., Watanabe K., Naoi Y., *Planta Med.*, **63**, 224—227 (1997).
15) Kohoda H., Tokumoto W., Sakamoto K., Fuji M., Hirai Y., Yamasaki K., Komoda Y., Nakamura H., Ishihara S., Uchida M., *Chem. Pharm. Bull.*, **33**, 1367—1374 (1985).
16) Min B. S., Gao J. J., Hattori M., Lee H. K., Kim Y. H., *Planta Med.*, **67**, 811—814 (2001).
17) Min B. S., Nakamura N., Miyashiro H., Bae K. H., Hattori M., *Chem. Pharm. Bull.*, **46**, 1607—1612 (1998).
18) Min B. S., Gao J. J., Nakamura N., Hattori M., *Chem. Pharm. Bull.*, **48**, 1026—1033 (2000).
19) Gao J. J., Min B. S., Ahn E. M., Nakamura N., Lee H. K., Hattori M., *Chem. Pharm. Bull.*, **50**, 837—840 (2002).
20) Hada S., Hattori M., Namba T., *J. Med. Pharm. Soc. Wakan-Yaku*, **6**, 100—107 (1989).
21) Kohda H., Tokumoto W., Sakamoto K., Fujii M., Hirai Y., Yamasaki K., Komoda Y., Nakamura H., Ishihara S., Uchida M., *Chem. Pharm. Bull.*, **33**, 1367—1374 (1985).
22) Nishitoba T., Sato H., Shirasu S., Sakamura S., *Agric. Biol. Chem.*, **50**, 2125—2154 (1986).
23) Ding P., Zhang D. Y., Xu H. H., *Chin. Trad. Herb Drugs*, **32**, 310—312 (2001).
24) Ma L. G., Yao K. H., *Edib. Fungi China*, **17**, 3—4 (1998).
25) Min B. S., Gao J. J., You H. L., Nakamura N., Hattori M., *Nat. Med.*, in press (2004).
26) Ma L., Wu F., Chen R. Y., *Acta Pharm. Sin.*, **38**, 50—52 (2003).
27) Gao J. J., Min B. S., Akao T., Meselhy M. R., Nakamura N., Hattori M., *J. Trad. Med.*, **18**, 154—160 (2001).
28) Kikuchi T., Kanomi S., Kadota S., Murai Y., Tsubono K., Ogita Z., *Chem. Pharm. Bull.*, **34**, 3695—3721 (1986).
29) Kubo M., Matsuda H., Nogami M., Arichi S., Takahashi T., *Yakugaku Zasshi*, **103**, 871—877 (1983).
30) Hirotani M., Ino C., Furuya T., *Phytochemistry*, **33**, 379—382 (1993).