DPPH Radical Scavenging Activity of Ten Natural *p*-Terphenyl Derivatives Obtained from Three Edible Mushrooms Indigenous to China

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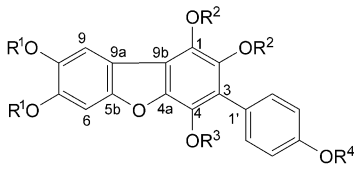
The antioxidant activities of ten natural *p*-terphenyl derivatives, **1** – **10**, obtained from the fruiting bodies of three edible mushrooms (*Thelephora ganbajun*, *Thelephora aurantiotincta, Boletopsis grisea*) indigenous to China were evaluated in comparison with BHA ('butylated hydroxyanisole' = (1,1-dimethylethyl)-4-methoxyphenol) and α-tocopherol by the DPPH ('1,1-diphenyl-2-picrylhydrazyl' = 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radical-scavenging method. The compounds **1** – **3** showed significant antioxidant activity. The antioxidant activities of compounds **1** – **10** and reference compounds followed the order: **2** > BHA > **1** > **3** > α-tocopherol > **10** > **9** > **6** > **5** > **8** > **7** > **4**. The compound **2** exhibited the strongest radical-scavenging activity with an EC_{50} value of 0.07 (EC_{50}(BHA) 0.09; EC_{50}(α-tocopherol) 0.25).

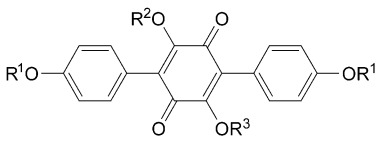
Introduction. – Active oxygen and, in particular, free radicals are considered to induce oxidative damage in biomolecules and to play an important role in aging, cardiovascular diseases, cancer, and inflammatory diseases [1 – 3]. In addition, they are also well known to be major causers of material degradation and food deterioration [4]. Consequently, antioxidants are now known to be prospective protective or therapeutic agents. In the past few years, addition of synthetic antioxidants has begun to be restricted because of their health risks and toxicity [5]. The importance of exploiting natural antioxidants from various sources and replacing synthetic antioxidants with natural ingredients has attracted increasing attention. At present, most of the natural antioxidants such as traditional nutrients, polyphenols, and flavonoids are obtained from plants. Few are reported to be from mushrooms, which are also abundant in secondary metabolites. The study of known and new natural derivatives in higher fungi might also support the development of new drugs, as well as health-promoting substances.

Natural *p*-terphenyl (= 1,1′:4,4″-terphenyl) compounds have been found so far only in lichens and fungi. These compounds differ mainly in the number and arrangement of OH groups, as well as in the extent of oxygenation of these groups. Despite various biological activities, *p*-terphenyls are reported to have attractive antioxidant activities. Curtisians A – D isolated from *Paxillus curtissii* showed strong antioxidant activities against lipid peroxidation, *ca.* 10 – 20 times that of vitamin E [6]. It is also reported that betulinan A and B isolated from *Lenzites betulina* showed potent antioxidant activities against lipid peroxidation [7]. These findings motivated us to measure the free-radical-scavenging activities of a series of *p*-terphenyl derivatives isolated [8 – 11] from three edible and delicious mushrooms (*Thelephora ganbajun*, *Thelephora aurantiotincta*, and *Boletopsis grisea*) indigenous to China. Here, we report the DPPH ('1,1-diphenyl-2-

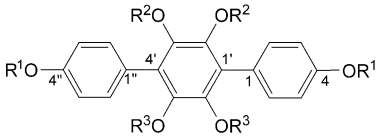
picrylhydrazyl' = 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radical-scavenging activities of ten compounds, **1** – **10**, isolated from the three mushrooms mentioned above.



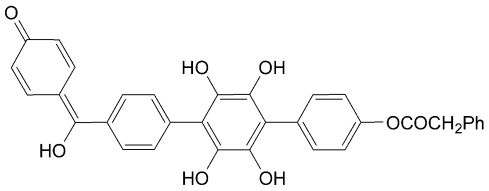
1 R^1=R^2=Ac, R^3=R^4=H
2 R^1=R^3=R^4=H, R^2=Ac
3 R^1=R^4=H, R^2=R^3=CH_2COPh
8 R^1=R^2=R^3=R^4=Ac



4 R^1=R^3=CH_2COPh, R^2=Me
5 R^1=R^3=H, R^2=Me
7 R^1=R^2=R^3=H



6 R^1=CH_2COPh, R^2=R^3=H
9 R^1=R^2=Ac, R^3=H



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Results and Discussion. – The method with DPPH as a stable free radical to measure radical-scavenging activity has been widely used. Antioxidants react with DPPH, which is a stable free radical, and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds. This method with the molar ratio of samples to DPPH radical indicating the activities of compounds seemed to reflect the activities of samples directly, for values obtained therefrom did not vary with the primary concentration of DPPH radical for test.

The radical-scavenging activity, measured by the molar ratio of antioxidant to DPPH radical required for 50% reduction in DPPH radical concentration at 30 min, is shown in the *Table*. The radical-scavenging activity decreased in the following order: **2** > BHA > **1** > **3** > α-tocopherol > **10** > **9** > **6** > **5** > **8** > **7** > **4**. The time-response curve for radical-scavenging activity of α-tocopherol, BHA ('butylated hydroxyanisole' = (1,1-dimethylethyl)-4-methoxyphenol), and compounds **1** – **3** was shown in the *Figure*. The results of our experiments demonstrated that all of the ten compounds tested possess radical-scavenging activity. It was also found that the free-radical-scavenging activity of **2** ($EC_{50} = 0.07$) was stronger than that of BHA ($EC_{50} = 0.09$) and α-tocopherol ($EC_{50} = 0.25$), and the activities of **1** ($EC_{50} = 0.12$) and **3** ($EC_{50} = 0.13$) were similar to that of BHA and stronger than that of α-tocopherol. BHA and α-tocopherol are well-known strong agents of DPPH radical-scavenging activity. Hence, compounds **1** – **3** are highly active. Compounds **4** – **10** had DPPH activities weaker than BHA and α-tocopherol. The DPPH radical-scavenging activity, which decreased in the order **2** > **3**, **1** > **8**, **6**, **9**, **10**, **7** > **4**, indicated that the number of free phenolic OH groups makes a statistically significant contribution to the free-radical-scavenging activities. In the case

Table 1. *DPPH Radical-Scavenging Effects of α-Tocophenol, BHA, and Compounds* **1**–**10**[a]) [b]) [c])

Compound	DPPH Activity EC_{50}
α-Tocopherol[d])	0.25 (± 0.01)
BHA[d])	0.09 (± 0.01)
1,2,4-Triacetoxy-3-(4-acetoxyphenyl)-7,8-dihydroxydibenzo[*b*,*d*]furan (**1**)	0.12 (± 0.02)
1,2-Diacetoxy-4,7,8-trihydroxy-3-(4-hydroxyphenyl)dibenzo[*b*,*d*]furan (**2**)	0.07 (± 0.02)
Ganbajunin B (**3**)	0.13 (± 0.01)
Ganbajunin A (**4**)	0.78 (± 0.03)
3-*O*-Methylatromentin (**5**)	0.44 (± 0.02)
Ganbajunin C (**6**)	0.33 (± 0.02)
Atromentin (**7**)	0.66 (± 0.03)
1,2,4,7,8-Pentacetoxy-3-(4-acetoxyphenyl)dibenzo[*b*,*d*]furan (**8**)	0.58 (± 0.02)
2′,3′-Diacetoxy-4,4″,5′,6′-tetrahydroxy[1,1′: 4′,1″-terphenyl] (**9**)	0.30 (± 0.01)
Aurantiotinin A (**10**)	0.27 (± 0.01)

[a]) Mean (standard deviation in parentheses) of three determinations. Differences at $P < 0.05$ were considered to be significant. [b]) Results were based on the values measured at 30 min. [c]) EC_{50} expressed as mol of antioxidant/mol of DPPH radical. [d]) α-Tocopherol and BHA were used as positive controls.

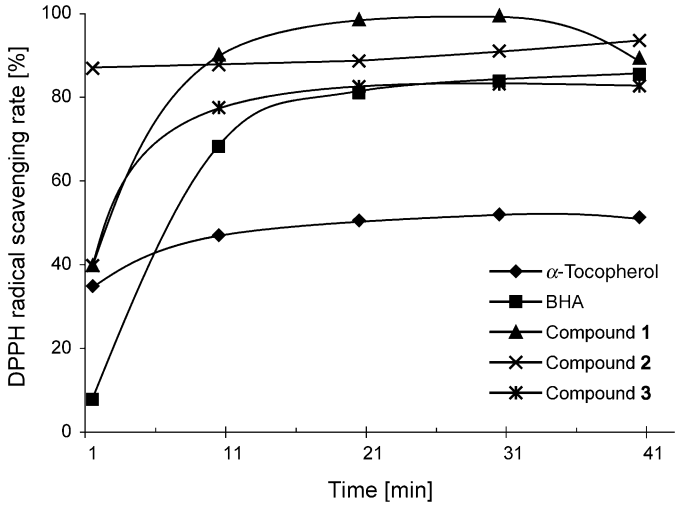

Figure. *Time-response curve for the radical-scavenging activity of α-tocopherol, BHA (positive control), and compounds* **1**–**3**. DPPH, Radical-scavenging rate (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$; 0.1 ml 0.525 mM solution of compounds **1**–**3**, α-tocopherol, and BHA in MeOH were added to 3.5 ml of DPPH radical (0.06 mM) in MeOH.

of compounds containing the same number of OH groups, compounds **6**, **9**, and **10** with two pairs of OH groups in *para*-positions with respect to each other showed stronger DPPH radical-scavenging activity than **7** with four isolated OH groups. Based on this comparison, it is suggested that the existence of OH groups in *para*-positions might enhance radical-scavenging activity of *p*-terphenyls.

According to the *Table*, compounds **1**–**3** with furan rings in their structures have stronger activities than other *p*-terphenyls without furan rings. In the case of **8** and **4**, both without OH groups, **8** exhibited stronger activity than **4**. All of these findings indicated that, for *p*-terphenyls, the formation of furan rings by two OH groups improves the free-radical-scavenging activity. It might be due to aromatic rings that form a conjugated system through furan rings and thereby facilitate the transport of the electron of the free radical.

Experimental Part

General. Ganbajunin A (**4**; >99%), *B* (**3**; >99%), *C* (**6**; >99%), and *3*-O-*methylatromentin* (**5**; >99%) were isolated from the fruiting bodies of the basidiomycete *Thelephora ganbajun* [10]; *aurantiotinin A* (**10**; >99%) and *atromentin* (**7**; >99%) were obtained from the fruiting bodies of the basidiomycete *Thelephora aurantiotincta* [11]; *1,2,4-triacetoxy-3-(4-acetoxyphenyl)-7,8-dihydroxydibenzo[*b,d*]furan* (**1**; >99%), *1,2-diacetoxy-4,7,8-trihydroxy-3-(4-hydroxyphenyl)dibenzo[*b,d*]furan* (**2**; >99%), *1,2,4,7,8-pentacetoxy-3-(4-acetoxyphenyl)dibenzo[*b, d*]furan* (**8;** >99%), and *2′,3′-diacetoxy-4,4″,5′,6′-tetrahydroxy[1,1′:4′,1″-terphenyl]* (**9**; >99%) were isolated from the fruiting bodies of the basidiomycete *Boletopsis grisea* [8]. The purity of all these ten compounds was measured by HPLC. The structures of **1**–**10** were established by spectroscopic and chemical means. α-Tocopherol, BHA ('butylated hydroxyanisole' = (1,1-dimethylethyl)-4-methoxyphenol), and DPPH ('1,1-diphenyl-2-picrylhydrazyl' = 2,2-diphenyl-1(2,4,6-trinitrophenyl)hydrazyl) radical were purchased from *Sigma Chemical Co.* All other reagents were of anal. grade or purer.

Measurement of DPPH Radical-Scavenging Activity. The DPPH radical-scavenging activity was tested according to the method of *Gordon et al.* [12] of compounds **1**–**10**, α-tocopherol, and BHA as 1.05-, 0.525-, and 0.21-mM solns. in MeOH. Of each of these solns., 0.1 ml was added to 3.5 ml of DPPH radical (0.06 mM) in MeOH. After further mixing, the decrease in absorbance was recorded at 515 nm with a UV/VIS spectrophotometer (*UV-2500 PC*, *Shimadzu*, Kyoto, Japan) at different time intervals (1, 10, 20, 30, 40 min), until the reaction reached a plateau against MeOH without DPPH radical as the blank reference. Absorbances were converted to the DPPH radical-scavenging rate according to the equation:

$$\text{DPPH radical scavenging rate } (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Each compound was assayed in triplicate, mean values for compounds **1**–**3** at different times were plotted in the *Figure*, and the EC_{50} values for **1**–**10** were given in the *Table*. Antiradical activity was defined as the relative concentration of antioxidant required to lower the initial DPPH concentration by 50% (EC_{50} (mol/l *p*-terphenyl compound per unit DPPH concentration)). 3.5 ml of 0.06 mM DPPH soln. plus 0.1 ml of MeOH was used as control, and 3.5 ml of MeOH plus 0.1 ml of sample soln. was added as blank. All experiments were carried out at r.t. (20°).

The method with DPPH as a stable free radical to measure radical scavenging activity has been widely used. This method with the molar ratio of samples to DPPH radical to indicate the activities of compounds seemed to reflect the activities of samples directly, for values obtained therefrom did not vary with the primary test conc. of the DPPH radical.

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REFERENCES

[1] T. Finkel, N. J. Holbrook, *Nature* **2000**, *408*, 239.
[2] K. Bauerova, A. Bezek, *Gen. Physiol. Biophys.* **1999**, *18*, 15.
[3] B. Halliwell, *Lancet* **1994**, *322*, 721.
[4] D. D. Kuthie, *Eur. J. Clin. Nutr.* **1993**, *45*, 759.
[5] S. Buxiang, M. Fukuhara, *Toxicology* **1997**, *122*, 61.
[6] B. S. Yun, I. K. Lee, J. P. Kim, I. D. Yoo, *J. Antibiot.* **2000**, *53*, 114.

[7] I. K. Lee, B. S. Yun, S. M. Cho, *J. Nat. Prod.* **1996**, *59*, 1090.
[8] L. Hu, Z. J. Dong, J. K. Liu, *Chin. Chem. Lett.* **2001**, *12*, 335.
[9] L. Hu, Z. J. Dong, J. K. Liu, *Acta Bot. Yunnan.* **2002**, *24,* 667.
[10] L. Hu, J. M. Gao, J. K. Liu, *Helv. Chim. Acta* **2001**, *84*, 3342.
[11] L. Hu, J. K. Liu, *Z. Naturforsch., C* **2003**, *58*, 452.
[12] M. H. Gordon, F. Paiva-Martins, M. Almeida, *J. Agric. Food Chem.* **2001**, *49*, 2480.

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