Antioxidant Properties of Natural *p*-Terphenyl Derivatives from the Mushroom *Thelephora ganbajun*

Wei-Min Yang[a,b], Ji-Kai Liu[a,*], Lin Hu[a], Ze-Jun Dong[a], Wan-Lin Wu[b], and Zhi-He Chen[b]

[a] Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, P. R. China. Fax: +86 871 5150227. E-mail: jkliu@mail.kib.ac.cn
[b] Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming 650031, P. R. China

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 359–362 (2004); received September 16/November 20, 2003

The antioxidant activity *in vitro* of three poly(phenylacetyloxy)-substituted 1,1′:4′,1″-terphenyl compounds from the edible mushroom *Thelephora ganbajun* were investigated. The IC_{50} values of compounds **1**–**3** for lipid peroxidation in rat liver homogenate were 400, 48, 54 μM, respectively. Compounds **1**–**3** increased superoxide dismutase (SOD) activity with EC_{50} values of 182, 74, 204 μM. They were also assessed on the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity with EC_{50} values of 49, 1233, 55 μM.

Key words: Thelephora ganbajun, Terphenyl Derivatives, Antioxidant Activity

Introduction

Free radicals such as active oxygen species are involved in the pathogenesis of various diseases such as myocardial and cerebral ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, cancer-initiation and the aging process (Hammond *et al.*, 1985; Halliwell and Gutteridge, 1990; Coyle and Puttfarcken, 1993). Consequently, antioxidants are now known to be prospects as 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 (Buxiang and Fukuhara, 1997). The importance of exploiting natural antioxidants from various sources and replacing synthetic antioxidants with natural ingredients has received increasing attention. At present, most of 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 basidiomycete *Thelephora ganbajun* locally known as 'Gan-ba-jun', is one of the most favorite edible mushrooms distributed in Yunnan province, in the Southwest of China (Mao, 1998). It grows in symbiosis with pine trees and has gastronomic interest due to its unique flavor. Despite the analysis of its essential oil (Lu *et al.*, 2000), *T. ganbajun* was poorly investigated for non-volatile constituents. Recently some new poly(phenylacetyloxy)-substituted 1,1′:4′,1″-terphenyl compounds including 3′,4,4″-trihydroxy-6′-methoxy[1,1′:4′,1″-terphenyl]-2″,5″-dione (**1**), tris[benzeneacetic acid]5′-methoxy-3′,6′-dioxo[1,1′:4′,1″-terphenyl]-2′,4,4″-triyl ester (**2**), and tris[benzeneacetic acid]7,8-dihydroxy-3-(4-hydroxyphenyl)dibenzofuran-1,2,4-triyl ester (**3**) were isolated from *T. ganbajun* (Hu and Liu, 2001; Hu *et al.*, 2001). Compound **1** was reported previously as an artificial product. As we demonstrated it is also a natural metabolite from higher fungi, but compounds **2** and **3** are novel *p*-terphenyls. In recent years, it has been reported that several 1,1′:4′,1″-terphenyl compounds exhibit considerable bioactivities: they are active toward HeLa and KB cells and potent IgE-antibody suppressants, and they have antiinsect and antibacterial and specific 5-lipoxygenase inhibitory activities (Yonezawa *et al.*, 1998; Belofsky *et al.*, 1998; Takahashi *et al.*, 1992). Because of their promising biological activities, they have generated strongly increasing research interest. The antioxidant activities of these three compounds *in vitro*, the rat liver homogenate lipid peroxidation inhibitory activity, the superoxide dismutase (SOD) activity and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity were investigated in the present study.

0939–5075/2004/0500–0359 $ 06.00

Results and Discussion

The antioxidant activity of compounds **1**–**3** (Fig. 1) was investigated and compared with that of well-known free radical scavengers such as VE (vitamin E) and BHA (butylated hydroxyanisole). Compounds **1**–**3** inhibited lipid peroxidation induced by non-enzymic Fe(II) ascorbic acid system in rat liver homogenate with IC_{50} values of 400, 48, 54 μM in a dose-dependent fashion, respectively, and the controls VE and BHA with IC_{50} values of 295 and 222 μM (Fig. 2A).

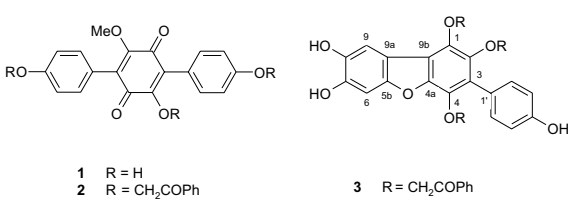
Compounds **1**–**3** scavenged superoxide radicals generated by the xanthine/xanthine oxidase system with EC_{50} values of 182, 74, 204 μM, respectively, and BHA (EC_{50} 424 μM) as control (Fig. 2B), and they also scavenged DPPH radicals with EC_{50} values of 49, 1233, 55 μM, respectively, and BHA (EC_{50} 110 μM) as control (Fig. 2C).

The present investigation demonstrated that three *p*-terphenyls isolated from the edible mushroom *Thelephora ganbajun* possess a potent lipid peroxidation inhibitory activity, SOD activity in rat liver homogenate, and DPPH radical scavenging activity. This result means that compounds **1**–**3** scavenge free radicals not only ascribed to their effects on the antioxidant enzymes but also via proton-donating action.

Experimental

Animals

Male SD rats, weighing 200–250 g, were used for *in vitro* studies in accordance with the Ethics Committee of Kunming Medical College (Grade II, Certificate No. 000208). Animals were obtained from the Animal Department, Yunnan Pharmacological Laboratories of Natural Products.



1 R = H
2 R = CH_2COPh
3 R = CH_2COPh

Fig. 1. Structures of compounds 3′,4,4″-trihydroxy-6′-methoxy[1,1′:4′,1″-terphenyl]-2″,5″-dione (**1**), tris[benzeneacetic acid]5′-methoxy-3′,6′-dioxo[1,1′:4′,1″-terphenyl]-2′,4,4″-triyl ester (**2**), and tris[benzeneatic acid]7,8-dihydroxy-3-(4-hydroxyphenyl)dibenzofuran-1,2,4-triyl ester (**3**).

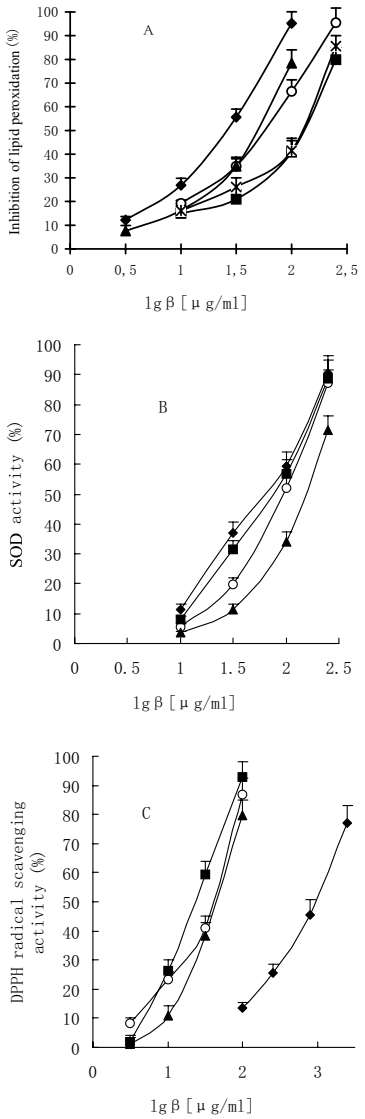


Fig. 2. Antioxidant activity for compounds **1** (■), **2** (◆), and **3** (▲), compared with VE (x) and BHA (O).
A. Lipid peroxidation inhibitory activity in rat liver homogenate. IC_{50} values for compounds **1**, **2**, **3**, VE and BHA were 135.2, 33.0, 37.4, 127.2, 40.4 μg/ml, respectively, and the activity rate of the vehicle (100% control) was 3 nmol MDA/mg protein.
B. SOD activity in rat liver homogenate. EC_{50} values for compounds **1**, **2**, **3** and BHA were 61.6, 51.5, 141.3, 76.4 μg/ml, respectively.
C. DPPH radical scavenging activity. EC_{50} values for compounds **1**, **2**, **3** and BHA were 16.7, 853.4, 37.9, 19.9 μg/ml, respectively. The data are given as mean ± S. E. M. n = 6 for each group. VE, BHA, MDA, SOD, and DPPH represented vitamine E, butylated hydroxyanisole, methane diacarboxylic aldehyde, superoxide dismutase, and 1,1-diphenyl-2-picrylhydrazyl, respectively.

Reagents

Compounds **1** (> 99%), **2** (> 99%), **3** (> 99%) were isolated from the fruit bodies of *Thelephora ganbajun* collected in Yunnan, and the structures were established by spectroscopic methods, including one- and two-dimensional NMR (COSY, HMQC and HMBC) (Hu *et al.*, 2001). The voucher specimen (HMAS 52851) was deposited at the herbarium of the Kunming Institute of Botany. Thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), xanthine, xanthine oxidase, were obtained from Sigma Chemical Co. Other chemicals and solvents used in the study were of analytical grade. For *in vitro* assays, compounds **1**–**3** were dissolved in DMSO.

Preparation of rat liver homogenate

This was carried out after Yue *et al.* (1995). The livers were chilled immediately after removal by immersion in ice-cold normal saline. The liver was perfused with ice-cold normal saline via the portal vein before homogenization. The liver homogenate was prepared in a ratio of 1 g of wet tissue to 9 ml of 0.86% NaCl by use of a glass homogenizer. The mixture was centrifuged for 10 min at 3000 × *g* and the supernatant was transferred and used as 10% rat liver homogenate.

Inhibitory activity against lipid peroxidation

This was assessed by measuring methane diacarboxylic aldehyde (MDA) in rat liver homogenate applying a modified thiobarbituric acid (TBA) method (Yue *et al.*, 1995; Ohkawa *et al.*, 1979). Reaction was initiated by the addition of 4 mM $FeCl_2$ (30 μl) into a mixture of 6 mM ascorbic acid (60 μl), 5% liver homogenate (4.0 mg protein/ml, 600 μl) and 30 μl of sample solution. The reaction mixture was incubated at 37 °C for 30 min. After incubation, the reaction was stopped by the addition of 20% trifluoroacetic acid (TFA, 500 μl) and then centrifuged at 3500 × *g* for 10 min. The reaction supernatant (1 ml) was mixed with 0.67% (w/v) TBA (500 μl), and then heated in boiling water bath for 10 min. After cooling down, the absorbance of the solution was determined at 532 nm on an UV spectrometer. Lipid peroxidation inhibitory activity was calculated as follows: [1−(T−B)/(C−B)] × 100%, in which T, C and B are absorbance values at 532 nm of the sample (T), the vehicle control (C) and the reaction zero time control (B), respectively.

SOD activity assay

It was assayed in rat liver homogenate by a nitrite method (Oyanagui, 1984; Shen and Chen, 2002) with minor modification. The xanthine/xanthine oxidase system was utilized to generate superoxide flux which oxidizes hydroxylamine to nitrite, and the nitrite was measured by the color developing reagent. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8, 250 μl), purified water (100 μl), 10 mM hydroxylamine hydrochloride (25 μl), 7.5 mM xanthine (25 μl), 23.4 mU/ml xanthine oxidase (25 μl), 1% liver homogenate (0.8 mg protein/ml, 10 μl) and 25 μl of sample solution. After incubation at 37 °C for 30 min, 3.3 g/l sulfanilic acid (250 μl) and 10 g/l naphthylamine (250 μl) were added to the mixture. SOD activity was assessed by measurement of the absorbance of the reaction solution at 550 nm. The inhibitory activity of the formation of nitrite was calculated as follows: [1−(T−B)/(C−B)] × 100%, in which T, C and B are absorbance values at 550 nm of the sample (T), the vehicle control (C) and the reaction zero time control (B), respectively.

Measurement of DPPH radical scavenging activity

The method of Blois (1958) and Yun *et al.* (2000) was applied. Each concentration of the sample solution in DMSO (20 μl) was added to 980 μl of 150 μM ethanolic DPPH solution. After vortex mixing, the mixture was incubated for 30 min at room temperature and the absorbance at 517 nm was measured. The DPPH radical scavenging activity of each compound was calculated as follows: [1−(Ai−Aj)/Ac] × 100%, in which Ai, Aj and Ac are absorbance values at 517 nm of the sample (Ai), the sample control (Aj, without DPPH, containing sample and vehicle ethanol) and the DPPH control (Ac, without sample, containing DPPH and vehicle DMSO), respectively.

Statistical analysis

All data were expressed as means ± S. E. M. IC_{50} and EC_{50} values were calculated by nonlinear regression analysis after logarithmic transformation of the sample concentrations.

Acknowledgement

We wish to acknowledge the financial support from the National Natural Science Foundation of China (30225048).

Belofsky G. N., Gloer K. B., Gloer J. B., Wicklow D. T., and Dowd P. F. (1998), New *p*-terphenyl and polyketide metabolites from the sclerotia of *Penicillium raistrickii*. J. Nat. Prod. **61**, 1115–1119.

Blois M. S. (1958), Antioxidant determination by the use of a stable free radical. Nature **181**, 1199–1200.

Buxiang S. and Fukuhara M. (1997), Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-metabolizing enzymes in mice. Toxicology **122**, 61–72.

Coyle J. T. and Puttfarcken P. (1993), Oxidative stress, glutamate, and neurodegenerative disorders. Science **262**, 689–695.

Halliwell B. and Gutteridge J. M. C. (1990), Role of free radicals and catalytic metal ions in human disease. Meth. Enzymol. **186**, 1–85.

Hammond B., Kontos H. A., and Hess M. L. (1985), Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfussion injury, and in cerebral vascular damages. Can. J. Physiol. Pharmacol. **63**, 173–187.

Hu L. and Liu J. K. (2001), Two novel phenylacetoxylated *p*-terphenyl derivatives from *Thelephora ganbajun* Zang. Z. Naturforsch. **56c**, 983–987.

Hu L., Gao J. M., and Liu J. K. (2001), Unusual poly-(phenyl-acetoxy)-substituted 1,1′:4′,1″-terphenyl derivatives from fruiting bodies of the basidiomycete *Thelephora ganbajun*. Helv. Chim. Acta **84**, 3342–3349.

Lu P. Y., Wen J., and Ju W. M. (2000), Chemical constituents from the oil of *Thelephora ganbajun* Zang. Nat. Prod. Res. Dev. **13**, 39–41.

Mao X. L. (1998), Economic Fungi of China, 1st ed. Science Press, Beijing, p. 458.

Ohkawa H., Ohishi N., and Yagi K. (1979), Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. **95**, 351–358.

Oyanagui Y. (1984), Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal. Biochem. **142**, 290–301.

Shen N. and Chen X. (2002), Assay methods for superoxide dismutase activity. In: Pharmacological Methodology (Xu S. Y., Bian R. L., and Chen X., ed.). The People's Public Health Publishing House, Beijing, pp. 529–537.

Takahashi A., Kudo R., Kusano G., and Nozoe S. (1992), 5-Lipoxygenase inhibitors isolated from the mushroom *Boletopsis leucomelas*. Chem. Pharm. Bull. **40**, 3194–3196.

Yonezawa S., Komurasake T., Kwada K., Tsuri T., Fuji M., Kugimiya A., Haga N., Mitsumori S., Inagaki M., Nakatani T., Tamura Y., Takechi S., Taishi T., and Ohtani M. (1998), Total synthesis of terprenin, a novel immunosuppressive *p*-terphenyl derivative. J. Org. Chem. **63**, 5831–5837.

Yue T. L., Glu J. L., Lysko P. G., Sponer G., Cheng H. Y., and Ruffolo R. R. J. (1995), Antioxidant activity of a β-adrenoceptor antagonist, carazolol, comparison with propranol and carvedilol. Pharmacol. Commun. **7**, 11–19.

Yun B. S., Lee I. K., Kim J. P., and Yoo I. D. (2000), Curtisians A~D, new free radical scavengers from the mushroom *Paxillus curtislii*. J. Antibiot. **53**, 114–122.