BOLEGREVILOL, A NEW LIPID PEROXIDATION INHIBITOR FROM THE EDIBLE MUSHROOM *SUILLUS GREVILLEI*

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A lipid peroxidation inhibitor, bolegrevilol was isolated from the edible mushroom *Suillus grevillei*. The structure was determined as 1-acetoxy-6-geranylgeranyl-2,4-dihydroxybenzene.

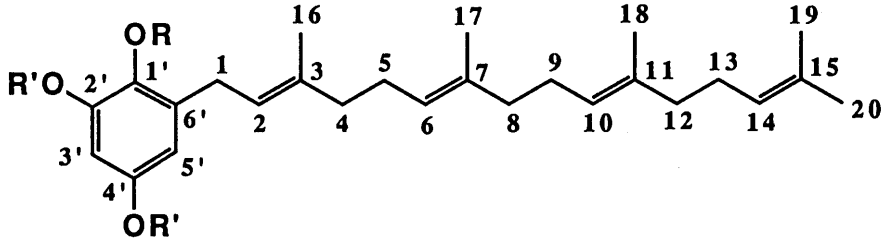
KEYWORDS lipid peroxidation inhibitor; Bolegrevilol; 1-acetoxy-6-geranylgeranyl-2,4-dihydroxybenzene; *Suillus grevillei*; Boletaceae

Accumulation of lipid peroxides in animal tissues is widely recognized to be responsible for various kinds of disease. Aging, radiation and some chemicals increase the lipid peroxides in tissues, and they causes deterioration of health and generation of cancer. Inhibiting the increase of the lipid peroxides in vivo should be extremely important in medical science and very beneficial to human welfare. We screened[1] natural inhibitors contained in fungi. The extracts of the edible mushrooms genus *Suillus (Boletus)* were found to have potent inhibitory effects. The present paper describes the structure of the inhibitor from *Suillus grevillei*.

Bolegrevilol(I),[2] obtained as a colorless oil, had a molecular formula $C_{28}H_{40}O_4$ (m/z M^+ Found 440.2918, Calcd. 440.2928). IR absorption (neat) established the presence of hydroxyl (ν_{O-H}=3350 cm^{-1}) and carbonyl ($\nu_{C=O}$=1740cm^{-1}) functionalities. In the UV spectrum (MeOH solution), absorption at 209 and 280nm (ε=30600 and 2500) indicated a hydroquinol chromophore.

The ^{1}H-NMR spectrum ($CDCl_3$) showed signals due to the two meta-coupled (J=2.9Hz) aromatic protons (δ 6.18 and 6.22), the methyl protons of acetate (δ 2.31,3H,s), which indicated 1,2,4,6-tetrasubstitution and acetoxy functionalities on the benzene ring, five vinylic methyl groups [δ 1.59(9H,s), 1.66(3H,s), 1.68(3H,s)], a benzyl methylene group [δ 3.14(2H,d,J=7)], four olefinic protons [δ 5.19(1H,bt,J=7), 5.05-5.15(3H,m)] and six vinyl methylene groups [δ 1.90-2.15(12H,m)], assignable to the geranylgeranyl group.

The ^{13}C-NMR data[3] also indicated the presence of a geranylgeranyl chain, an aromatic ring and an acetate. The INADEQUATE spectrum showed the twenty contour plots exhibited by all of the two-carbon pairs, (C-1',C-2'), (C-2',C-3'), (C-3',C-4'), (C-4',C-5'), (C-5',C-6'), (C-6',C-1'), (C-6',C-1), (C-1,C-2), (C-2,C-3),(C-3,C-4), [(C-4,C-5), (C-8,C-9), (C-12,C-13)],[4] [(C-5,C-6), (C-9,C-10), (C-13,C-14)],[4] [(C-6,C-7), (C-10,C-11)],[4] [(C-7,C-8), (C-11,C-12)],[4] (C-14,C-15), (C-3,C-16), [(C-7,C-17), (C-11,C-18)],[4] (C-15,C-19),



I : R=Ac, R'=H
II : R=Ac, R'=CH_3
III : R=H , R'=CH_3

(C-15,C-20) and [-C=O(Ac), $-CH_3$(Ac)], revealed the connectivities of all of the adjacent carbon atoms. These data indicated that (I) was 6-geranylgeranyl-1,2,4-oxygenated(OH,OH,OAc)benzene. Treatment of (I) with ethereal diazomethane yielded an oily O-dimethylated product(II, m/z M^+ 468). Hydrolysis of (II) with 5% KOH-MeOH afforded an oily hydrolyzate(III, m/z M^+ 426). The determination of the acetoxyl group in (I) was accomplished by the comparison of ^{13}C-NMR chemical shifts between (II) and (III).[5] The signal due to 1'-carbon was shifted downfield (5.5ppm) by the hydrolysis of the acetate, so the structure of (I) was represented by 1-acetoxy-6-geranylgeranyl-2,4-dihydroxybenzene. The ^{13}C-NMR chemical shifts of the vinyl methyl groups of (I) (δ 16.0, 16.0, 16.2) indicated that the double bonds in the geranylgeranyl chain were E-configurations.[6]

Polyisoprenylhydroquinones and polyisoprenylquinones have been found in members of the fungi, family Boletaceae.[7] These compounds may also have inhibitory effects on the lipid peroxidation.

REFERENCES AND NOTES

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Rat hepatocytes were isolated in a viable stage from male Wister rats and stored at -80°C. Lipid peroxidation was stimulated in the cell suspension by adenosine 5'-diphosphate(ADP)-nicotinamide adenine dinucleotide phosphate(NADPH) or ADP-ascorbic acid. The lipid peroxides were determined by the thiobarbituric acid method using malondialdehyde as a standard.

2)The acetone extract of the fresh fruit-body of *Suillus grevillei* was partitioned between Et_2O and water. The Et_2O fraction was chromatographed on a silica gel culumn. The active fraction was purified by preparative HPLC to give bolegrevilol in 0.005% yield, showed 50% inhibition at a concentration of 1.0 μg/ml(IC_{50}) of the rat hepatocyte lipid peroxidation induced by ADP-NADPH (enzymatic) or ADP-ascorbic acid (non-enzymatic). However, using rat hepatocyte previously boiled under nitrogen for 20 minutes, bolegrevilol at the same concentration showed no inhibition of the non-enzymatic lipid peroxidation induced by ADP-ascorbic acid.

3)^{13}C-NMR data for (I) in $CDCl_3$. δ 28.9(C-1), 121.1(C-2), 137.1(C-3), 39.7(3C,C-4,8,12), 26.6, 26.7, 26.8(C-5,9,13), 124.0, 124.3, 124.5(C-6,10,14), 135.0, 135.2(C-7,11), 131.3(C-15), 16.0(C-16), 16.0, 16.2(C-17,18), 17.7(C-19), 25.7(C-20), 130.8(C-1'), 147.9(C-2'), 102.4(C-3'), 153.9(C-4'), 108.4(C-5'), 135.6(C-6'), 20.6[CH_3(Ac)], 170.9[C=O(Ac)].

4)These two-carbon pairs showed one contour plot.

5)The assignments for aromatic carbons of (II) and (III) were based upon a comparison with values calculated by the method in J. B. Sthothers, Carbon-13 NMR Spectroscopy, Academic Press, New York, 1972. The ^{13}C-NMR data for the aromatic carbons of (II) and (III) in $CDCl_3$ were (II): δ 132.0(C-1'), 151.8(C-2'), 97.6(C-3'), 157.8(C-4'), 105.2(C-5'), 135.2(C-6'). (III) : δ 137.5(C-1'), 146.8(C-2'), 96.8(C-3'), 152.9(C-4'), 105.6(C-5'), 127.5(C-6').

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