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Inoscavin A, A New Free Radical Scavenger from the Mushroom *Inonotus xeranticus*

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Abstract: A new free radical scavenger named inoscavin A was isolated from the methanolic extract of the mushroom *Inonotus xeranticus*. The structure of inoscavin A was elucidated by NMR spectroscopic method.

It has been well known that free radicals are implicated in the pathogenesis of various diseases such as myocardial and cerebral ischemia, arteriosclerosis, inflammation, and cancer-initiation and aging processes.[1,2] Therefore, we have screened chemically novel free radical scavengers as therapeutic agents for these diseases from natural sources including mushrooms, microbial metabolites, and oriental medicines.[3] Recently, we found the mushroom *Inonotus xeranticus* producing a novel free radical scavenger named inoscavin A (Fig. 1). In this paper, we report the isolation, structural elucidation and antioxidative activity of inoscavin A.[4]

Methanolic extract of the fruiting body of *Inonotus xeranticus* (500 g) was partitioned between EtOAc and water. The organic layer was separated by silica gel, Sephadex LH-20, and ODS column chromatographies, consecutively, followed by ODS TLC developed with 67% aqueous MeOH. Purification by reversed-phase HPLC eluted with 60% MeOH afforded yellow powder of 10.3 mg inoscavin A.

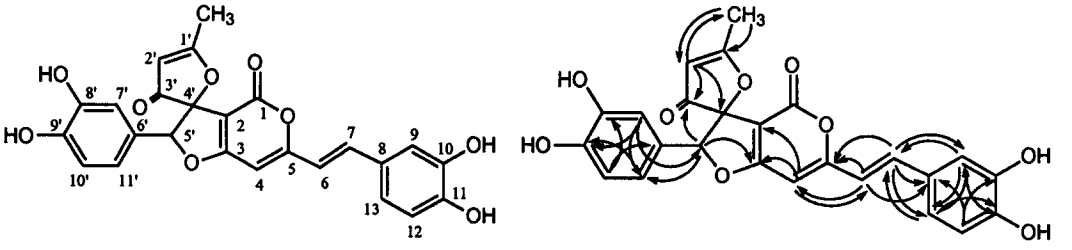

Fig. 1. Structure and HMBC data of inoscavin A.

The molecular formula of inoscavin A was determined to be $C_{25}H_{18}O_9$ on the basis of HRFAB-MS [$(M+H)^+$, *m/z* found 463.1045, calcd 463.1029] in combination with ^{13}C NMR data. The UV maximum at 389 nm indicated that inoscavin A has hispidin moiety.[5] The IR absorptions suggested the presence of hydroxyl (3430 cm^{-1}) and α,β-unsaturated carbonyl (1700 cm^{-1}) groups. The ^1H NMR spectrum exhibited twelve signals including two *AMX* spin systems (6.59, 6.75 and 6.71 ppm, and 7.00, 6.79 and 7.08 ppm) in the aromatic

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region originated from two 1,3,4-trisubstituted benzene rings and two *trans* conjugated olefinic methines at 6.72 and 7.44 ppm (J=15.9 Hz). The ^{13}C NMR spectrum contained twenty five signals including carbonyl and ester carbonyl carbons (203.1 and 160.6 ppm), oxygenated sp^2 quaternary carbons (167.0, 176.8 and 192.9 ppm) and four hydroxylated aromatic carbons (146.3, 147.0, 147.8 and 149.5 ppm) which were characterized by the aids of DEPT and HMQC spectral data.

To establish connectivities of carbons through two or three bonded long-range couplings, HMBC experiments were carried out. The HMBC correlations unambiguously revealed the presence of hispidine moiety and 3,4-dihydroxyphenyl group in inoscavin A, as shown in Fig. 1. 5-Methyl-3(2*H*)-furanone moiety was assigned by long-range correlations from methyl protons at 1.98 ppm to carbons at 192.9 (C-1') and 105.1 (C-2') ppm, and from methine proton at 5.57 ppm to carbons at 20.0 (Me-1'), 203.1 (C-3'), and 94.5 (C-4') ppm. The chemical shift values of these carbons are in good agreement with the corresponding carbons of 3(2*H*)-furanone. The combination of above data and the long-range couplings observed from the protons at 5.65 (H-5') and 6.50 (C-4) ppm revealed that inoscavin A had two five-membered rings bonded as a spiro type. The characteristic low field shifted chemical shift value of C-5' (95.9 ppm) suggested possibility of sp^2 carbon but small $^1J_{CH}$ value (153 Hz) in comparison with those of C-2' (180 Hz) and C-4 (178 Hz) established C-5' as oxygenated sp^3 carbon. Though some structurally related compounds[5,6] have been reported, inoscavin A is a new type hispidine compound possing a unique dihydroketofuranspirodihydrofuran moiety.

Inoscavin A inhibited rat liver microsomal lipid peroxidation with IC_{50} value of 0.3 μg/ml, which was five times as active as vitamin E (1.5 μg/ml). It also scavenged DPPH radical with IC_{50} value of 0.1 μg/ml.

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4. Inoscavin A: $[\alpha]_D$=0 (c=9.0, CH_3OH), UV λ_{max} nm (ε) in MeOH : 262 (11,822), 389 (11,568) ; IR ν_{max} (KBr) : 3430, 1700, 1695, 1593, 1549, 1384, 1276, 1127 cm^{-1} ; HRFAB-MS ; *m/z* found 463.1045, $C_{25}H_{18}O_9$ requires 463.1029 ; ^1H NMR (600MHz, CD_3OD): δ 1.98 (s, Me-1'), 5.57 (s, H-2'), 5.65 (s, H-5'), 6.50 (s, H-4), 6.59 (dd, J=8.1, 1.6, H-11'), 6.71 (d, J=1.6, H-7'), 6.72 (d, J=15.9, H-6), 6.75 (d, J=8.1, H-10'), 6.79 (d, J=8.2, H-12), 7.00 (dd, J=8.2, 1.8, H-13), 7.08 (d, J=1.8, H-9), 7.44 (d, J=15.9, H-7). ^{13}C NMR (150 MHz, CD_3OD): δ 20.0 (Me-1), 94.5 (C-4'), 95.5 (C-4), 95.9 (C-5'), 99.5 (C-2), 105.1 (C-2'), 115.0 (C-9), 115.1 (C-7'), 115.5 (C-10'), 115.8 (C-12), 116.6 (C-6), 120.2 (C-11'), 122.7 (C-13), 123.2 (C-6'), 128.5 (C-8), 139.9 (C-7), 146.3 (C-8'), 147.0 (C-10), 147.8 (C-9'), 149.5 (C-11), 160.6 (C-1), 167.0 (C-5), 176.8 (C-3), 192.9 (C-1'), 203.1 (C-3').
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