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Inoscavin E, a Free Radical Scavenger from the Fruiting Bodies of *Inonotus xeranticus*

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Abstract A new free radical scavenger named inoscavin E was isolated from the fruiting bodies of *Inonotus xeranticus*. Its structure was determined as 2-(3,4-dihydroxyphenyl)-6-[(1*E*)-2-(3,4-dihydroxyphenyl)ethenyl]-4*H*-furo[3,2-*c*]pyran-4-one on the basis of spectroscopic analysis. Inoscavin E exhibited significant scavenging activity against the superoxide radical anion and ABTS radical cation.

Keywords inoscavin E, *Inonotus xeranticus*, free radical scavenger

Inonotus xeranticus (Berk.) Imaz. Et Aoshi. (Hymenochaetaceae) is a saprophytic fungus preferably living on deciduous trees and widely distributes in East Asia [1]. In previous investigation on antioxidant constituents from this mushroom, we isolated several free radical scavengers, inoscavins A∼D [2] and interfungins A∼C [3]. Our continuing search for new antioxidant principles from this material, a new free radical scavenger, designated as inoscavin E, has been isolated. In this paper, the isolation, structure determination, and free radical scavenging activity of inoscavin E are described.

The fresh fruiting bodies of *Inonotus xeranticus* were crushed and extracted with methanol at room temperature. The methanolic extract was concentrated under reduced pressure and the aqueous resultant was partitioned with hexane. The hexane layer was discarded, and EtOAc was added to the water phase and shaken. The EtOAc layer was concentrated and subjected to a column of Sephadex LH-20 eluting with a mixture of $CHCl_3$: MeOH (1 : 1, v/v). The antioxidant fraction was rechromatographed on a column of Sephadex LH-20 eluting with MeOH only, followed by reversed-phase column chromatography eluting with 70% aqueous MeOH. Finally, 4.0 mg of inoscavin E was purified by preparative reversed-phase TLC developed with 80% aqueous MeOH.

The molecular weight of inoscavin E was determined to

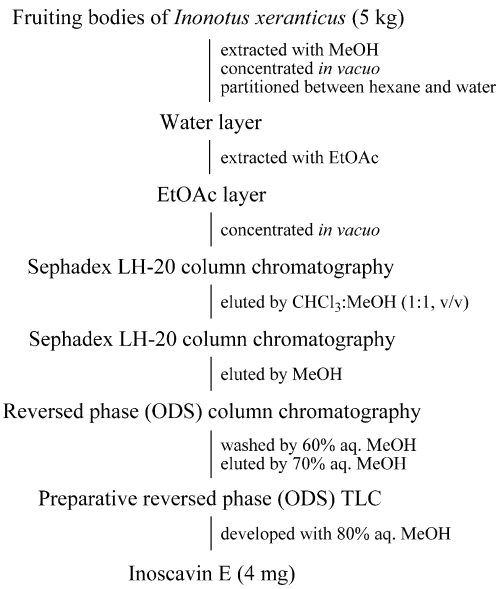

Fig. 1 Isolation procedures of inoscavin E.

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Table 1 ^{1}H- and ^{13}C-NMR data of inoscavin E in CD_3OD

No.	δ_C	δ_H
2	158.3	
3	100.3	6.96 (1H, s)[a]
3a	112.3	
4	161.3	
6	158.5	
7	96.5	6.83 (1H, s)
7a	162.7	
1′	122.3	
2′	112.6	7.20 (1H, d, *J*=2.0 Hz)
3′	146.9	
4′	148.0	
5′	116.8	6.83 (1H, d, *J*=8.4 Hz)
6′	117.9	7.16 (1H, dd, *J*=8.4, 2.0 Hz)
1″	117.4	6.70 (1H, d, *J*=16.0 Hz)
2″	135.5	7.30 (1H, d, *J*=16.0 Hz)
3″	129.3	
4″	114.6	7.04 (1H, d, *J*=2.0 Hz)
5″	146.8	
6″	148.3	
7″	116.6	6.77 (1H, d, *J*=8.0 Hz)
8″	121.7	6.95 (1H, dd, *J*=8.0, 2.0 Hz)

[a] Integral, multiplicity and coupling constants in parentheses.

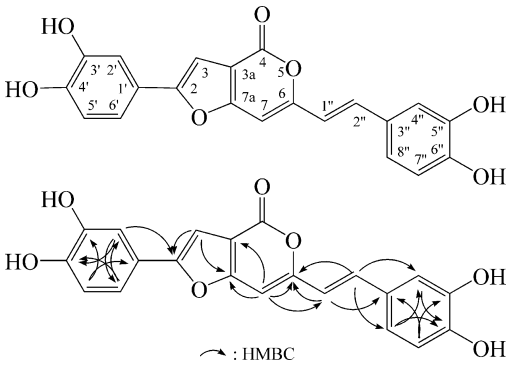

Fig. 2 Structure and HMBC correlations of inoscavin E.

be 378 by the ESI-mass measurements providing a quasi-molecular ion peaks at *m/z* 401 $[M+Na]^+$ in positive mode and at *m/z* 377 $[M-H]^-$ in negative mode. Its molecular formula was established as $C_{21}H_{14}O_7$ by the high-resolution ESI-mass measurement (*m/z* 379.0 $[M+H]^+$, Δ+3.7 mmu) in combination with ^{1}H- and ^{13}C-NMR data. This molecular formula requires 15 degrees of unsaturation. The ^{1}H-NMR spectrum of inoscavin E showed two AMX spin systems assignable to two 1,2,4-trisubstituted benzene moieties at δ 7.20 (1H, d, *J*=2.0 Hz), 7.16 (1H, dd, *J*=8.4, 2.0 Hz) and 6.83 (1H, d, *J*=8.4 Hz), and δ 7.04 (1H, d, *J*=2.0 Hz), 6.95 (1H, dd, *J*=8.0, 2.0 Hz) and 6.77 (1H, d, *J*=8.0 Hz), two sp^2 methine singlets at δ 6.96 and 6.83 and two olefinic methine doublets attributable to a *trans*-1,2-disubstituted double bond at δ 7.30 (1H, d, *J*=16.0 Hz) and 6.70 (1H, d, *J*=16.0 Hz). The ^{13}C-NMR spectrum exhibited 21 carbons, which were established as ten sp^2 methines and eleven sp^2 quaternary carbons by the aid of the HMQC spectrum. Eight signals of quaternary carbons were proposed to be ester carbonyl or oxygenated sp^2 carbons from their chemical shift values between δ 146 to 163 (Table 1). The structure of inoscavin E was determined by the HMBC spectrum, as shown in Fig. 2. The proton NMR spectrum showing a methine singlet at δ 6.83, AMX spin system in aromatic region and two olefinic methine doublets, and the UV absorption bands at λ_{max} (MeOH) (log ε) 411 (4.23), 325 (3.96) and 316 (3.92) nm suggested the presence of conjugated hispidin moiety, which was ubiquitous structure in mushroom polyphenols [4, 5]. This was also supported by the long-range correlations of H-7 to C-3a, C-7a, C-6, and C-1″, H-1″ to C-6 and C-3″, H-2″ to C-6, C-4″ and C-8″, H-4″ to C-6″ and C-8″, H-7″ to C-3″ and C-5″, and H-8″ to C-4″ and C-6″, and these chemical shift values were consistent with the corresponding protons and carbons of hispidin moiety [3, 5]. Additional long-range correlations from H-3 to C-2 and C-7a revealed that a furan moiety was fused with hispidin. A remaining 1,2,4-trisubstituted benzene group was connected to C-2 by the HMBC correlation from H-2′ to C-2. By the process of elimination, a remaining ester carbonyl carbon at δ 161.3 should be positioned to C-4. Therefore, the structure of inoscavin E was unambiguously determined as 3-deacetyl inoscavin C, a new antioxidant polyphenol with hispidin moiety.

Antioxidant activity of inoscavin E was evaluated by measuring free radical scavenging activity using three different radical species, the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) radical cation, superoxide radical anion and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. ABTS radical scavenging activity was carried out by using ABTS radical cation decolorization assay with minor modifications [6]. Inoscavin E exhibited potent ABTS radical cation scavenging activity with an IC_{50} value of 22 μM, which was higher activity than trolox (IC_{50}, 30 μM), a well-known antioxidant used as control, and less activity than caffeic acid (IC_{50}, 10 μM). Superoxide radical anion scavenging activity was evaluated by the xanthine/xanthine oxidase method with minor modifications [7]. Although it was three-times less active than caffeic acid, a well-known superoxide radical

scavenger, inoscavin E displayed significant superoxide radical anion scavenging activity with an IC_{50} value of 49 μM. However, inoscavin E showed no DPPH radical scavenging activity up to 100 μM.

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