Short Communication

A Novel Antioxidative Synergist
Isolated from *Osbeckia*
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In recent reports, several flavonoids, hydrolyzable tannins and related compounds (especially ellagic acid), which showed strong inhibitory effects on lipid peroxidation using both a food model system and *in vitro* systems, have been isolated from *Osbeckia chinensis* L.^{1~3)} During the isolation of antioxidative tannins, we also isolated a novel type of antioxidative synergist, which was subsequently named as osbeckic acid. In this communication, we report the structural determination of osbeckic acid and discuss its synergistic effect on the antioxidative activity of α -tocopherol and two polyphenols that were isolated from *O. chinensis* L.

An MeOH extract of *O. chinensis* L. was chromatographed on Amberlite XAD-2 and repeatedly purified on HPLC, using a Develosil ODS-L-5 column eluted with 0.03% AcOH. Osbeckic acid (**1**) was obtained as a colorless oil, $[\alpha]_D^{25} + 83.5^\circ$ ($c=0.2$, MeOH), and the UV spectrum $\lambda_{\max}$ (MeOH) nm (ϵ) 256 (12600) is consistent with a furoic ester. The mass spectrum of **1** could not be obtained, since the compound did not volatilize even under FAB conditions. The ¹H-NMR spectrum (200 MHz, D₂O) of **1** showed the presence of the furan structure, with two aryl doublets ($J=3.5$ Hz) centred at δ 7.13 (1H, d, H-4') and 6.44 (1H, d, H-3'), and one secondary hydroxyl group at 5.24 (1H, s, H-2).

The ¹³C-NMR spectrum (50 MHz, D₂O) was assigned as follows: δ 170.3 (C-1), 158.5 (carboxyl C at C-5'), 154.1 (C-2'), 144.4 (C-5'), 118.2 (C-4'), 109.8 (C-3') and 66.6 (C-2).

Treatment of **1** with diazomethane in anhydrous methanol gave the dimethyl ester (**2**). **2** was obtained as colorless needles, mp 134~135°C, $[\alpha]_D^{25} + 79.3^\circ$ ($c=0.2$, Me₂CO). High-resolution EIMS established its molecular formula as C₉H₁₀O₆ (obsd. m/z , 214.0458; calcd., 214.0477). UV $\lambda_{\max}$ (CHCl₃) nm (ϵ): 256 (16600); IR ν (CHCl₃) cm⁻¹: 3550 (OH), 1745, 1725 (C=O), 1600, 1510 (aromatic ring), 1305, 1140 (C-O). The MS fragment pattern of **2** was 214 [M]⁺, 183 [M-OMe]⁺, 155 [100, M-COOMe]⁺ and 124 [M-CHOHCOOMe-H]⁺. Treatment of **2** with acetic anhydride-pyridine and subsequently by HPLC gave the *O*-acetyl derivative (**3**). **3** was obtained as a colorless oil, UV $\lambda_{\max}$ (CHCl₃) nm (ϵ): 253 (15800); MS m/z : 256 [M]⁺; ¹H-NMR (200 MHz, CDCl₃) δ 7.15 (1H, d, $J=3.5$ Hz, H-4'), 6.55 (1H, d, $J=3.5$ Hz, H-3'), 6.12 (1H, s, H-2), 3.84, 3.80 (each 3H, s, OMe), 2.18 (3H, s, COMe). The H-2 proton at δ 5.24 in the ¹H-NMR spectrum of **2** was shifted downfield to δ 6.12 as shown in ¹H-NMR spectrum of **3**. In 1975, Barton *et al.*⁴⁾ obtained methyl 2-hydroxy-2-(5-methoxycarbonyl-2-furyl)acetate by treating daucic acid, a widely distributed sugar acid, with dry hydrogen chloride in anhydrous methanol. By comparing the physical and chemical data, the dimethyl ester of osbeckic acid (**2**) was completely identified as methyl 2-

- 1 $R^1 = H$, $R^2, R^3 = H, OH$
- 2 $R^1 = Me$, $R^2, R^3 = H, OH$
- 3 $R^1 = Me$, $R^2, R^3 = H, OAc$

FIG. 1. Structure of Osbeckic Acid.

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FIG. 2. Synergistic Antioxidative Action of Osbeckic Acid with α -Tocopherol, Methyl Gallate and Punicacortein A.

—●—, control; —▲—, $16 \mu\text{M}$ α -tocopherol; —△—, $16 \mu\text{M}$ osbeckic acid; —▲—, $4 \mu\text{M}$ α -tocopherol; —▲—, $4 \mu\text{M}$ α -tocopherol + $16 \mu\text{M}$ osbeckic acid; —●—, $4 \mu\text{M}$ methyl gallate; —●—, $4 \mu\text{M}$ methyl gallate + $16 \mu\text{M}$ osbeckic acid; —■—, $4 \mu\text{M}$ punicacortein A; —■—, $4 \mu\text{M}$ punicacortein A + $16 \mu\text{M}$ osbeckic acid.

hydroxy-2-(5-methoxycarbonyl-2-furyl)acetate. From these results, the chemical structure of osbeckic acid was confirmed as **1** [2-hydroxy-2-(5-carboxyl-2-furyl) acetic acid] (Fig. 1).

The synergistic effects of osbeckic acid on the antioxidative activity of α -tocopherol and two polyphenols isolated from *O. chinensis* L., methyl gallate and punicacortein A were in a food model system using the thiocyanate method.⁵⁾ The concentrations of α -tocopherol, methyl gallate and punicacortein A were fixed at $4 \mu\text{M}$, and then four different concentrations of osbeckic acid ($0 \sim 16 \mu\text{M}$) were added (data

not shown). The antioxidative activity of α -tocopherol was considerably increased on concurrent use with osbeckic acid. The addition of osbeckic acid at a concentration of $16 \mu\text{M}$ to α -tocopherol ($4 \mu\text{M}$) produced antioxidative activity that was stronger than that of standard α -tocopherol at a concentration of $16 \mu\text{M}$. The same synergistic effect was observed when osbeckic acid was added together with methyl gallate or punicacortein A, these synergistic effects being summarized in Fig. 2.

It is well recognized that a synergistic effect exists when phenolic antioxidants are used together with certain organic acids such as citric acid. This is attributed to the metal chelating function of the organic acids.⁶⁾ Osbeckic acid may involve the same mechanism for its synergism, although the synergistic mechanism for osbeckic acid is not clearly understood.

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