Myrsinoic Acid E, an Anti-inflammatory Compound from *Myrsine seguinii*

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The methanolic extract of *Myrsine seguinii* yielded the novel anti-inflammatory compound, myrsinoic acid E (1), whose structure was elucidated to be 3,5-digeranyl-4-hydroxy benzoic acid. We synthesized 1- and its 3,5-diprenyl (2) and 3,5-difarnesyl analogues (3). Compounds 1–3 suppressed 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation of mouse ears by 59%, 14%, and 69% at a dose of 1.4 μmol.

**Key words:** anti-inflammatory; *Myrsine seguinii*; 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced edema; Pd-catalyzed carbonylation

Skin tumor promoters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) induce edema on the skin with cell proliferation.1,2) Inhibitors of TPA-induced edema can be expected to suppress various physiological responses involving cell proliferation as well as inflammation. In a search for new types of anti-inflammatory compounds, we found that the methanolic extract of *Myrsine seguinii* showed potent anti-inflammatory activity. We have already reported the isolation and identification of four novel related compounds, myrsinoic acid A (4),3) B, C and F.4) In our continuing search for other anti-inflammatory compounds from this plant, we isolated the novel terpeno-benzoic acid, myrsinoic acid E (1). However, we could not evaluate its anti-inflammatory activity because of its small amount. This prompted us to synthesize compound 1, as well as several analogues for further anti-inflammatory activity studies.

The methanolic extract of *M. seguinii* yielded myrsinoic acid E (1). The HR-EIMS analysis of this compound gave the molecular formula of C_{27}H_{38}O_{3} (found 410.2836) with nine unsaturations. The IR spectrum of 1 showed the presence of hydroxyl (3435 cm\(^{-1}\)) and carboxyl (1683 cm\(^{-1}\) and 3200–2400 cm\(^{-1}\)) groups. The \(^{1}H\)-NMR and \(^{13}C\)-NMR spectra of 1 showed close similarity with those of myrsinoic acid A (4). In the \(^{1}H\)-NMR spectrum, the only differences were the presence in 1 of an olefinic methine triplet (H-6′, -6′′) at δ 5.08 (t, J = 6.7 Hz, 2H) and a symmetrical three-methyl singlet at 1.76, 1.68, and 1.60 (H-9′, -9′′, -8′, -8′′, -10′, -10′′). These data indicate that compound 1 possessed two symmetric geranyl group at the 3, 5 position of 4-hydroxy benzoic acid. The \(^{13}C\)-NMR spectrum of 1 was in complete accordance with the proposed structure.

The synthesis of compound 1 and its analogues 2 and 3 is shown in Scheme 1. 4-Iodophenol (5) was used as a starting material. The introduction of a 3,5-digeranyl group was performed by using Hori’s procedure to give 6. Pd-catalyzed carbonylation afforded ester 9 in a 91% yield. Finally, hydrolysis of the methyl ester gave 1 in an 88% yield. The spectral data for synthetic 1 are in good accordance with those of natural 1. To examine the importance of the C-3 and C-5 side chain length on the anti-inflammatory activity, we designed target molecule 2 with a prenyl group and 3 with a farnesy group. The synthesis of 2 and 3 was performed as just described.

The anti-inflammatory activities of compounds 1–4, and 4-hydroxybenzoic acid are summarized in Table 1. Compounds 1, 3 and 4 showed strong anti-inflammatory activity, while compound 2 and 4-hydroxybenzoic acid exhibited low activity. The long terpene moieties of terpeno-benzoic acid-type compounds seem to have been essential for the anti-inflammatory activity.

**Experimental**

**Instruments.** The following instruments were used: a Bruker DR 500 FT-NMR spectrometer for \(^{1}H\) and \(^{13}C\)-NMR spectra, a Jeol JMS 700 mass spectrometer for mass spectra, and a Jasco FT-IR-480 Plus for IR spectra.

**Anti-inflammatory test.** Mouse inflammatory tests were conducted by Gshwendt’s method. TPA was...
found in the hexane-soluble portion (69 g), which and the extract was concentrated
(1.2 kg) we were extract with methanol, University.
animals of the Faculty of Agriculture at Shinshu
purchased from Sigma Chemical Co. This experiment
complied with the regulations concerning animal experimentation and the care of experimental
animals of the Faculty of Agriculture at Shinshu
University.

**Isolation of compound I.** Fresh leaves and twigs of *M. seguinii* (12 kg) were extracted with methanol, and the extract was concentrated *in vacuo*. The aqueous solution was successively partitioned with hexane /water and ethyl acetate/water. Strong activity was found in the hexane-soluble portion (69 g), which was chromatographed in a silica gel column with hexane/ethyl acetate by 10% stepwise elution. The 20% ethyl acetate/hexane eluate (13.8 g) was chromatographed on ODS with gradient elution from 70% methanol/water to methanol. The active fraction was further purified by HPLC (95% methanol/water) to give compound I (1.6 mg). HREIMS m/z (M\(^+\)): calcd: for C\(_{27}H_{36}O_8\), 410.2821; found, 410.2836. \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta\): 7.74 (s, 2H, H-2, -6), 5.36 (s, 1H, -OH), 5.32 (t, \(J = 6.6\) Hz, 2H, H-2’, -2’’), 5.08 (t, \(J = 6.7\) Hz, 2H, H-6’, -6’’), 3.38 (d, \(J = 6.8\) Hz, 4H, H-1’, -1’’), 2.09 (m, 8H, H-4’, -4’’, -5’, -5’’), 1.76 (s, 6H, H-9’, -9’’), 1.68 (s, 6H, H-8’, -8’’), 1.60 (s, 6H, H-10’, -10’’). \(^13\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta\): 170.44 (C-7), 158.01 (C-4), 138.84 (C-3’, -3’’), 131.94 (C-7’, -7’’), 130.46 (C-2, -6), 127.25 (C-3, -5), 123.86 (C-6’, -6’’), 121.26 (C-2’, -2’’), 120.92 (C-1), 39.73 (C-4’, -4’’), 29.48 (C-1’, -1’’), 26.46 (C-5’, -5’’), 25.68 (C-8’, -8’’), 17.72 (C-10’, -10’’), 16.24 (C-9’, -9’’). IR \(\nu_{\text{max}}\) (film) cm\(^{-1}\): 3435 (OH), 3200–2400 (COOH), 1683 (C = O), 1601 (C = C), 1436, 1260, 800.

**Dialkylation of 4-Iodophenols.** 3,5-Dialkyl-4-iodophenols were prepared according to the reported procedure.\(^3\)

\[ (2'E, 2'E)-4-Iodo-2-(3', 7'-dimethylocta-2', 6'-diene)-6-(3', 7'-dimethylocta-2', 6'-diene)phenol \]
\( (6) \)

IR (film) \(\nu_{\text{max}}\) cm\(^{-1}\): 3456, 2968, 2924, 1668, 1458, 1198. \(^1\)H-NMR (500 MHz, CDCl\(_3\)) \(\delta\): 7.26 (s, 2H), 5.36 (s, 1H, -OH), 5.27 (t, \(J = 7.1\) Hz, 2H), 5.08 (t, \(J = 6.6\) Hz, 2H), 3.29 (d, \(J = 7.1\) Hz, 4H), 2.00–2.20 (m, 8H), 1.74 (s, 6H), 1.69 (s, 6H), 1.60 (s, 6H). \(^13\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta\): 152.84, 138.64, 136.28, 131.94, 129.97, 123.85, 121.21, 82.69, 39.70, 29.15, 26.42, 25.74, 17.73, 16.19. HREIMS m/z (M\(^+\)): calcd. for C\(_{30}\)H\(_{37}\)O\(_4\)I, 492.1889; found, 492.1901.

\[ (2'E, 2'E, 6'E, 2'E)-4-Iodo-2-(2',6', 10'-trimethylocta-2', 6', 10'-tri-enyl)-6-(2', 6', 10'-trimethylocta-2', 6', 10'-tri-enyl)phenol \]
\( (7) \)

\(^1\)H-NMR data were identified with those reported.\(^5\)

\[ (2'E, 6'E, 2'E, 6'E)-4-Iodo-2-(2', 6', 10'-trimethylocta-2', 6', 10'-tri-enyl)-6-(2', 6', 10'-trimethyloct- \]

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1.67 (s, 6H), 1.60 (s, 12H). 13C-NMR (125 MHz, CDCl3) δ: 152.82, 138.69, 136.28, 135.53, 131.27, 129.92, 125.91, 124.40, 123.74, 121.18, 82.70, 29.15, 26.76, 26.44, 26.38, 25.69, 17.71, 16.25, 16.06. HREIMS m/z (M+) calcd. for C36H53O1, 628.3141; found, 628.3131.

**Carbonylation of 2,6-dialkylated 4-iodophenols.**

To a solution of 2,6-dialkylated 4-iodophenol (0.5 mmol) in 1 mL of N,N-dimethylformamide (DMF) were successively added NEt3 (0.14 mL, 1 mmol), MeOH (64 mg, 2.0 mmol), and Cl2Pd(PPh3)2 (0.025 mmol, 17.6 mg). The mixture was charged with CO (1 atm), heated to 60°C, and stirred for 6 h. The usual work up gave a pure methyl ester after column chromatography (hexane/ethyl acetate = 8:1).

(2′E,2″E)-Methyl-4-hydroxy-3-(3′,7′-dimethylocta-2′,6′-dienyl)-5-(3′-7′-dimethylocta-2′,6′-dienyl)benzoate (9). IR (film) νmax cm⁻¹: 3437, 2966, 2919, 2855, 1718, 1766, 1603, 1434, 1317, 1280, 1199, 771. 1H-NMR (500 MHz, CDCl3) δ: 7.70 (s, 2H), 5.84 (s, 1H), 5.31 (t, J = 7.0 Hz, 2H), 5.06 (t, J = 6.6 Hz, 2H), 3.88 (s, 3H), 3.93 (d, J = 7.0 Hz, 4H), 2.05–2.15 (m, 8H), 1.76 (s, 6H), 1.68 (s, 6H), 1.60 (s, 6H), 1.59 (s, 6H). 13C-NMR (125 MHz, CDCl3) δ: 167.27, 157.25, 138.65, 131.90, 129.76, 123.13, 123.89, 122.01, 121.42, 51.76, 39.74, 29.44, 26.48, 25.69, 17.71, 16.24. HREIMS m/z (M+): calcd. for C28H42O2, 424.2977; found, 424.2971.

Methyl 4-hydroxy-3-(3′-methyl-2′-butenyl)-5-(3′-methyl-2′-butenyl)benzoate (10). IR (film) νmax cm⁻¹: 3439, 2966, 2921, 2853, 1718, 1697, 1602, 1434, 1317, 1279, 1197, 771. 1H-NMR (500 MHz, CDCl3) δ: 7.69 (s, 2H), 5.81 (s, 1H), 5.31 (t, J = 7.2 Hz, 2H), 3.36 (d, J = 7.2 Hz, 4H), 1.95–2.20 (m, 16H), 1.78 (s, 12H). 13C-NMR (125 MHz, CDCl3) δ: 167.26, 157.09, 135.02, 129.77, 127.04, 122.08, 121.45, 51.77, 29.61, 25.82, 17.92. HREIMS m/z (M+): calcd. for C18H24O3, 288.1725; found, 288.1736.

**Hydrolysis of methyl ester.** 4-Hydroxy-3,5-dialkylated benzoic acids were prepared according to the reported procedure apart from using NaOH.

(2′E,2″E)-4-hydroxy-3-(3′,7′-dimethylocta-2′,6′-dienyl)-5-(3′-7′-dimethylocta-2′,6′-dienyl)benzoic acid (I). IR, 1H-NMR, 13C-NMR and HREIMS were identified with those of myrsinonic acid E (1).

4-Hydroxy-3-(3′-methyl-2′-butenyl)-5-(3′-methyl-2′-butenyl)benzoic acid (2). IR (film) νmax cm⁻¹: 3437, 3200–2800, 2960, 2926, 2856, 1687, 1603, 1434, 1287, 713. 1H-NMR (500 MHz, CDCl3) δ: 7.76 (s, 2H), 5.90 (br., 1H), 5.32 (t, J = 7.2 Hz, 2H), 3.37 (d, J = 7.2 Hz, 4H), 1.78 (s, 6H), 1.79 (s, 6H). 13C-NMR (125 MHz, CDCl3) δ: 171.57, 157.86, 135.21, 130.47, 127.16, 121.30, 121.08, 51.77, 29.55, 25.82, 17.92. HREIMS m/z (M+): calcd. for C17H22O2, 274.1569; found, 274.1597.

(2′E,6′E,2″E,6″E)-4-hydroxy-3-(3′,7′,11′-trimethylocta-2′,6′,10′-triienyl)benzoic acid (11). IR (film) νmax cm⁻¹: 3437, 2966, 2924, 2919, 1719, 1697, 1603, 1435, 1317, 1281, 1198, 771. 1H-NMR (500 MHz, CDCl3) δ: 7.72 (s, 2H), 5.80 (s, 1H), 5.32 (t, J = 6.9 Hz, 2H), 5.09 (s, 4H), 3.88 (s, 3H), 3.34 (s, 7H). 13C-NMR (125 MHz, CDCl3) δ: 167.24, 157.20, 143.8, 141.2, 138.6, 131.00, 129.79, 124.40, 124.14, 123.79, 122.05, 121.37, 51.53, 39.76, 39.71, 29.62, 29.50, 26.76, 26.50, 25.68, 17.68, 16.30, 16.04. HREIMS m/z (M+): calcd. for C32H38O3, 546.4073; found, 546.4084.

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**References**

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