Anti-inflammatory Cyathane Diterpenoids from *Sarcodon scabrosus*

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Four novel diterpenoids were isolated from the fruiting bodies of *Sarcodon scabrosus* (Fr.) Karst. (Boraginaceae) together with neosarcodonin A. One of the novel compounds was elucidated to be a cyathane diterpenoid, namely neosarcodonin O, by its spectral data. The others were characterized as 19-O-linoleoyl, 19-O-oleoyl and 19-O-stearoyl derivatives of sarcodonin A, after comparison with the authentic samples synthetically prepared from sarcodonin A. These compounds, together with the five 19-O-acyl derivatives synthesized from sarcodonin A, each exhibited inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation on mouse ears by topical application.

Key words: *Sarcodon scabrosus*; sarcodonin; diterpenoid; anti-inflammation; 12-O-tetradecanoylphorbol-13-acetate (TPA)

We have already reported the presence and structural elucidation of the bitter principles, sarcodonins A (1) to H, in an extract of the fruiting bodies of *S. scabrosus* (Fr.) Karst. (Boraginaceae).¹² These compounds possessing a cyathane skeleton showed antibacterial and anti-inflammatory activities.²³ Oshima et al. have isolated cyathane diterpenoids, scabronines A–F, from this fungus and revealed their stimulation activity of nerve growth factor-synthesis.⁴⁵ In the present paper, we describe the isolation and characterization of four novel compounds (2–5), and their anti-inflammatory activities.

Purification of the methanolic extract of the fruiting bodies of *S. scabrosus* yielded 2 and 6. The¹H-NMR spectrum of 2 was similar to that of neosarcodonin A (7),³ except for the presence of a doublet assignable to a methyl group (δ 1.02, 3H), instead of the signal assignable to an oxygen-bearing methylene group (δ 3.47, 2H) found in the¹H-NMR spectrum of 7. This suggested that 2 was the 19-deoxy derivative of 7, this being confirmed by the HREIMS data (C₂₁H₃₂O₃; m/z: calcd., 332.2351; found, 332.2385). The stereochemistry of 2 was identical with that of 7, this being proven by the NOESY spectra of 2 and 7. We named this compound (2) neosarcodonin O. Compound 6 was identified as allocyathin B₂ based on the¹H-NMR and mass spectra.⁶

The CH₂Cl₂ extract of the fruiting bodies of *S. scabrosus* was purified chromatographically to yield three novel cyathane diterpenoids (3–5). The¹H-NMR spectrum of 3 was similar to that of 1, except for the presence of additional signals at δ 0.89 (3H), δ 1.34–1.26 (14H), δ 1.64 (2H), δ 2.04 (4H), δ 2.31 (2H), δ 2.77 (2H) and δ 3.31–5.39 (4H). The signals assignable to a 19-methylene group, which were observed at δ 3.58 (2H) in 1, were present at δ 3.93 and 4.15 (each 1H) in 3. These¹H-NMR spectral data suggest that 3 was a 19-O-acyl derivative of 1. The HREIMS data of 3 indicated that the molecular formula of 3 was C₃₈H₅₈O₄ (m/z: calcd., 578.4335; found, 578.4305), showing that the acyl group was a C₁₈-chain with three unsaturations (C₁₈H₂₁O). We prepared the 19-O-linoleoyl derivative of 1 on the presumption that the C₁₈-acyl group was a linoleic group. All the spectral data for the synthesized 19-O-
linoleoyl derivative of 1 were coincident with those for 3; thus 3 was identified to be 19-O-linoleoylsarcodonin A. In the same manner, 4 and 5 were elucidated to be 19-O-oleylsarcodonin A and 19-O-stearoylsarcodonin A, respectively, this being confirmed by a comparison of the spectral data with those of authentic samples derived from 1.

Five sarcodonin derivatives, 19-O-oleanoyl, 19-O-butyryl, 19-O-acetyl, 19-O-benzoyl and 19-O-pivaloyl derivatives (8–12) of 1 were prepared from 1. The inhibitory activities of 1–12 against mouse-ear inflammation induced by topical application (0.63 μmol/ear) of 12-O-tetradecanoylphorbol-13-acetate (TPA) are listed in Table 1. Compounds 3–5 and 8–12 showed inhibitory activities of 43–78%. These activities are similar to or lower than that of 1, indicating that the anti-inflammatory activity was not affected by the length or bulkiness of the acyl groups at C-19. The activities of 2 and 6 were lower than 40%, suggesting that oxidative substitution at C-19 might be necessary for exhibiting high anti-inflammatory activity.

**Experimental**

**General.** The following instruments were used: a Bruker DRX-500 FT-NMR spectrometer operating at 500.1 MHz for protons and at 125.8 MHz for carbons in CDCl₃, with TMS used as an internal standard; a Jeol JMS 700 mass spectrometers for mass spectra; a Jasco 1000 polarimeter for optical rotation.

**Isolation of 2 and 6.** The dry fruiting bodies (1.4 kg) of *S. scabrosus*, which had been collected in Nagano, were extracted with MeOH. The extract was evaporated in vacuo and partitioned between EtOAc and H₂O. The EtOAc layer was concentrated and then subjected to silica gel (Wakogel C-300, 360 g; Wako Pure Chemical Industries) column chromatography, using mixtures of n-hexane–EtOAc as the eluent. The eluate with n-hexane–EtOAc (1:1) was subjected to silica gel (C-300, 140 g) column chromatography, using mixtures of CHCl₃–acetone as the eluent. The eluate with CHCl₃–acetone (19:1) was again subjected to silica gel (C-300, 100 g) column chromatography, using mixtures of n-hexane–EtOAc as the eluent. The eluate with n-hexane–EtOAc (4:1) was subjected to silica gel (C-300, 8 g) column chromatography, using mixtures of n-hexane–EtOAc–AcOH (94:6:1) as the eluent (10 ml/fr). Frs. no. 18–27 were purified by HPLC with an ODS column (AQ-324, 10 × 300 mm; YMC), eluting with MeOH–H₂O (4:1) at a flow rate of 3.0 ml/min with detection at 254 nm. Concentration of the peaks with tR 33.7 min and 34.2 min yielded 2 (3.5 mg) and 6 (7.3 mg), respectively.

**Neosarcodonin O (2).** A colorless resin; [α]D 23° = -45° (c 0.31, MeOH); EIMS m/z (rel. int.): 332 [M]+ (3), 300 (61), 282 (75), 267 (64), 239 (100); IR νmax (film) cm⁻¹: 3462 (OH), 2958, 2867, 2814, 1689 (C=O), 1645, 1112, 1064; NMR δH: 0.96 (3H, s), 1.00 (3H, d, J = 6.7 Hz), 1.02 (3H, d, J = 6.7 Hz), 1.07 (3H, s), 1.40 (1H, m), 1.43 (1H, m), 1.51 (1H, m), 1.57 (2H, m), 2.30 (2H, m), 2.33 (1H, m), 2.80 (2H, m), 2.86 (1H, q, J = 6.7 Hz), 3.16 (1H, m), 3.28 (1H, m), 3.45 (3H, s), 4.61 (1H, s), 7.01 (1H, t, J = 5.0 Hz), 9.46 (1H, s); NMR δC: 18.2 (CH₃), 21.4 (CH₃), 22.2 (CH₃), 24.7 (CH₃), 27.3 (CH₃), 28.4 (CH₃), 29.1 (CH₂), 31.4 (CH₂), 36.4 (CH₂), 37.3 (CH₃), 40.2 (CH), 44.8 (C), 50.1 (C), 59.3 (CH₃), 79.5 (CH), 80.3 (CH), 138.2 (C), 138.7 (C), 142.1 (C), 160.0 (CH), 194.0 (CH). **Isolation of 3–5.** The dry fruiting bodies (150 g) of *S. scabrosus*, which had been collected in Nagano in 1985, were extracted with CH₂Cl₂ at 40 °C. The extract was evaporated in vacuo, giving residual materials (5.48 g). These materials were subjected to silica gel (C-300, 100 g) column chromatography, using mixtures of n-hexane–EtOAc as the eluent. The eluate with n-hexane–EtOAc (4:1) was subjected to silica gel (H-60, 100 g; Kanto Kagaku) column chromatography, using mixtures of n-hexane–acetone as the eluent. The eluate with n-hexane–acetone (9:1) was purified by HPLC with an ODS column (Develosil ODS-UG-5, 8.0 × 250 mm; Nomura Chemical), eluting with MeOH at a flow rate of 3.0 ml/min with detection at 350 nm. Concentration of the peaks with tR 9.3 min, 11.4 min and 15.3 min yielded 3 (8.8 mg), 4 (22.4 mg) and 5 (3.3 mg), respectively.

**19-O-Linoleoylsarcodonin A (3).** A colorless resin; [α]D 23° +348° (c 0.66, CHCl₃); EIMS m/z (rel. int.): 578 [M]+ (82), 298 (93), 280 (100), 265 (75), 251 (49), 199 (87); IR νmax (film) cm⁻¹: 3488 (OH), 2927, 2855, 1735 (C=O), 1675 (CHO), 1575, 1167; NMR δH: 0.89 (3H, t, J = 6.9 Hz, 9.69 (3H, s), 0.99 (3H, d, J = 6.9 Hz), 1.00 (3H, s), 1.26–1.36 (15H, m), 1.64 (2H, m), 1.68 (1H, m), 1.69 (2H, m), 1.76 (1H, m), 2.04 (4H, m), 2.31 (2H, t, J = 7.6 Hz), 2.39 (1H, m), 2.40 (1H, m), 2.53 (1H, m), 2.55 (1H, m), 2.77 (2H, t, J = 6.6 Hz), 3.08 (1H, m), 3.17 (1H, dd, J = 5.8 and 18.4 Hz), 3.73 (1H, m), 3.93 (1H, dd, J = 10.7 and 7.0 Hz), 4.15 (1H, dd, J = 10.7 and 7.0 Hz), 5.31–5.39 (4H, m), 6.12 (1H, d, 140 g) column chromatography, using mixtures of CHCl₃–acetone as the eluent. The eluate with CHCl₃–acetone (19:1) was again subjected to silica gel (C-300, 100 g) column chromatography, using mixtures of n-hexane–EtOAc as the eluent. The eluate with n-hexane–EtOAc (4:1) was subjected to silica gel (C-300, 8 g) column chromatography, using mixtures of n-hexane–EtOAc–AcOH (94:6:1) as the eluent (10 ml/frac). Frs. no. 18–27 were purified by HPLC with an ODS column (AQ-324, 10 × 300 mm; YMC), eluting with MeOH–H₂O (4:1) at a flow rate of 3.0 ml/min with detection at 254 nm. Concentration of the peaks with tR 33.7 min and 34.2 min yielded 2 (3.5 mg) and 6 (7.3 mg), respectively.

Table 1. Anti-inflammatory Activities of 1–12 in the Mouse Ear Inflammatory Test

<table>
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<th>Test compound</th>
<th>Inhibitory effect (%)</th>
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<td>2</td>
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<td>12</td>
<td>43</td>
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A sample (0.63 μmol) was applied to one of the mouse ears and, after 30 min, TPA (0.5 μg) was applied to both ears of the mouse. The edema was evaluated after 7 hr. The inhibitory effect is expressed as a percentage ratio of the edema. Five mice were used for each experiment.
To a solution of 19-O-Stearoylsarcodonin A (42.6 mg) was subjected to silica gel (C-300, 5 g) column chromatography using mixtures of n-hexane–EtOAc as the eluent. The eluate with n-hexane–EtOAc (4:1) was concentrated to dryness, giving 9 (25.3 mg).

In a similar manner, 3–5, 8 and 10–12 were prepared from 1. Their structures were confirmed from their NMR and mass spectral data.

19-O-Octanoylsarcodonin A (8). A colorless resin; [α]D22 +370° (c 0.42, CHCl3); HREIMS: calcd. for C28H43O4, 442.3108; found, 442.3038; EIMS m/z (rel. int.): 442 [M+] (72), 298 (34), 280 (95), 265 (100), 199 (68).

19-O-Butyrylsarcodonin A (9). A colorless resin; [α]D22 +1039° (c 1.74, CHCl3); HREIMS: calcd. for C23H39O4, 386.2470; found, 386.2457; EIMS m/z (rel. int.): 386 [M+] (80), 298 (27), 280 (75), 265 (92), 199 (65), 59 (100).

19-O-Acetyltsarcodonin A (10). A colorless resin; [α]D22 +705° (c 0.79, CHCl3); HREIMS: calcd. for C22H39O4, 358.2123; found, 358.2144; EIMS m/z (rel. int.): 358 [M+] (69), 298 (25), 280 (65), 265 (88), 199 (74), 59 (100).

19-O-Benzoylsarcodonin A (11). A colorless resin; [α]D24 +1540° (c 0.05, CHCl3); HREIMS: calcd. for C22H37O4, 420.2301; found, 420.2289; EIMS m/z (rel. int.): 420 [M+] (83), 298 (30), 283 (31), 280 (56), 265 (67), 199 (51), 105 (100).

19-O-Pivaloylsarcodonin A (12). A colorless resin; [α]D24 +1600° (c 0.10, CHCl3); HREIMS: calcd. for C25H36O4, 400.2614; found, 400.2631; EIMS m/z (rel. int.): 400 [M+] (63), 298 (31), 283 (43), 280 (87), 265 (100), 199 (79).

Anti-inflammatory Test. The mouse ear inflammatory test was conducted according to the Gschwendt method.71 The experiment complied with regulations concerning animal experimentation and the care of experimental animals of the Faculty of Agriculture at Shinshu University.

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References


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