NEW XENIA DITERPENOIDS FROM A SOFT CORAL
XENIA SPECIES

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Abstract - Six new xenia diterpenoids with an opened A-ring containing an
aliphatic acid have been isolated from a soft coral Xenia sp. The structures were
determined on the basis of the spectroscopy.

Bicyclic diterpenoids possessing a cyclononane skeleton which have been isolated from soft corals Xenia
sp., Nephtea sp. and Alcyonium sp., as well as from gorgonians,2 are called xenia diterpenoids.3 The
structures of the diterpenoids have been classified into three groups: xenicins, xeniolide, and
xeniaphyllanes.3

As part of our studies on soft corals Xenia species we have recently undertaken an investigation of an
unidentified Xenia sp., collected in the area of Bonotsu, Kagoshima prefecture.1 Previous reports have
described the structure elucidation of nine new xenia xeniolides and related compounds isolated from the
methanol extract4 and seven new xenia diterpenoids containing an opened A-ring, which were acylated with
a series of C_{16}-C_{20} saturated fatty acids, from the acetone extract of the same organism.5 Further
investigation of chemical constituents of the acetone extract has led to the isolation of six new xenia
diterpenoids, xeniaethers C (1), D (2), and E (3) and azamilides H (4), I (5), and J (6). In this report, we
describe their isolation and structure elucidation.

Xeniaether C (1), C_{20}O_{30}O_{4}, contained absorption bands corresponding to a hydroxyl group (3450 cm^{-1})
and a conjugated diene (1625 cm^{-1}) in the ir spectrum. The 1H nmr spectrum was similar to that of
xeniaether A (7),4 except for resonances due to two methine protons on an epoxide (δ 2.79; 1H, d, J=4.0
Hz, H-8; δ 3.02; 1H, ddd, J=4.0, 5.5, and 11.0 Hz, H-9) in place of olefinic protons at C-8 and C-9 in 7. The gross structure was elucidated as follows. Resonances due to two olefinic methyl protons (δ 1.77 and 1.78, 3H each, br s, H-16 and H-17) and three olefinic protons (δ 5.62; 1H, d, J=15.2 Hz, H-12, δ 5.86; 1H, br d, J=11.0 Hz, H-14, and δ 6.67; 1H, dd, J=11.0 and 15.2 Hz, H-13) were observed, suggesting a 4-methyl-1(E),3-pentadiene moiety. H-3 hydroxymethyl protons appeared at δ 3.57 (2H, d, J=6.2 Hz), which were coupled to a hydroxyl proton (δ 2.00; 1H, t, J=6.2 Hz, OH). H-1 oxymethylene protons (δ 3.40; 1H, dd, J=7.6 and 11.3 Hz, δ 3.82; 1H, t, J=7.6 Hz) were coupled to H-1α (δ: 2.96; 1H, dt, J=7.6 and 11.3 Hz), the latter of which was further coupled to H-4a (δ 3.12; 1H, dd, J=7.7 and 11.3 Hz). The H-8 epoxy proton was coupled to the H-9 another epoxy which in turn was coupled to H-10 (δ 2.71; 1H, br t, J=11.7 Hz, δ 2.72; 1H, overlapped). A broad singlet (δ 5.13; 2H) was due to exo methylene protons at C-19. The relative stereochemistry of all chiral centers was determined by nOe experiments in C6D6 (Figure 1). NOes from H-4a (δ 3.18; 1H, dd, J=8.1 and 11.4 Hz) to H-1α (δ 3.38; 1H, dd, J=8.0 and 11.9 Hz, 3.5%), as well as NOes from H-11a (δ 2.84; 1H, dt, J=8.0 and 11.9 Hz) to H-1β (δ 3.69; 1H, t, J=8.0 Hz, 5.4%), and to H-3 (δ 3.57; 1H, dd, J=6.6 and 11.9 Hz, δ 3.65; 1H, dd, J=5.9 and 11.4 Hz, 3.5%) were observed. These data suggested that in the major conformer H-4a and H-1α occurred on the same face of the ring system and H-11a, H-1β, and H-3 were on the opposite face to H-4a. The major conformer of the 9-membered ring was also elucidated by the observation of the NOes between H-4a and H-10α (δ 2.58; 1H, t, J=11.8 Hz, 11.5%) and H-6β (δ 1.41; 1H, br t, J=15.0 Hz, 2.7%), between H-6β and H-8 (δ 2.27; 1H, d, J=3.8 Hz, 3.1%) and H-9 (δ 2.72; 1H, dt, J=3.8 and 11.8 Hz, 1.1%), and between H-18 (δ 1.08; 3H, s) and H-5α (δ 1.24; 1H, br d, J=15.0 Hz, 1.2%) and H-6α (δ 1.80; 1H, dt, J=3.3 and 15.0 Hz, 1.4%). The stereochemistry of the epoxide and C-18 methyl group was therefore established to be α and β, respectively. Furthermore, the α-configuration of the epoxide was confirmed by the large coupling constant (J=11.8 Hz) between H-9 and one of methylene protons (H-10α), indicating a trans-diaxial relationship (Figure 2). Assuming a β-epoxide structure, the coupling constant between H-9 and H-10α would be predicted to be small by the torsion angle (ca.120°). The side chain moiety was deduced to be in the α-configuration from an nOe between H-4a and H-13 (4.3%). Therefore, the structure of 1 was deduced to be 8,9-α-epoxyxeniaether A.

The 1H nmr spectra of xeniaether D (2), C40H64O5, and xeniaether E (3), C38H60O5, were similar in many respects to those of 1, except that 2 and 3 displayed resonances due to an additional fatty acyl chain. The fatty acyl groups in 2 and 3 were determined to be stearoyl and palmitoyl respectively, as suggested
by the fragment ions at m/z 283 and 255 in the negative ion FAB mass spectra. Location of the acyl groups was determined to be at C-3 by the downfield chemical shifts of H-3 methylene protons (δ 4.11) compared to those of 1 (δ 3.57). Thus, xeniaethers D (2) and E (3) are 3-stearylxeniaether C and 3-palmitylxeniaether C, respectively.

![Figure 1. NOes (%)](image1)

The ¹H nmr spectra of azamilide H (4), C₄₀H₆₈O₇, and azamilide I (5), C₃₈H₆₄O₇, were indistinguishable, and resembled those of azamilide A (8), except for resonances due to additional epoxy protons at ca. 2.67 (1H, m, H-9) and δ 2.75 (1H, d, J=4.0 Hz, H-8). In addition, the olefinic protons at δ 5.25 (1H, d, J=11.7 Hz, H-8) and 5.85 (1H, m, H-9), observed in 8, were absent. The stereochemistry of the epoxide, which could not be unequivocally deduced by nOe experiments in 4 and 5, was tentatively assumed to be α as in the case of related compounds (2) and (3) on comparison of the chemical shift data. The value of the coupling constant (J=11.9 Hz) between H-4a (δ 3.80; 1H, dd, J=8.6 and 11.9 Hz) and H-11a (δ 2.55; 1H, ddd, J=3.8, 8.5, and 11.9 Hz) proved that the ring junction was trans. The geometry of the olefinic bond at C-12 was concluded to be E on the basis of an nOe from H-13 (δ 6.96; 1H, dd, J=11.0 and 15.4 Hz) to H-4a (δ 3.80; 1H, dd, J=8.6 and 11.9 Hz, 15.3%). The presence of a stearoyl group in 4 and a palmitoyl group in 5 was confirmed by fragment ions at m/z 283 and 255 respectively in the negative
ion FAB mass spectra. Location of the acyl groups was determined to be at C-1 by the observation of nOes between the acetyl protons and H-4a (0.5 %) and H-10a (0.6 %). Thus, azamilides H (4) and I (5) are deduced to be 8,9-α-epoxyazamilide A and 8,9-α-epoxyazamilide B, respectively.

Figure 4. NOes (%) observed for 6.

Azamilide J (6), C_{40}H_{68}O_{8}, has one more oxygen than 4, and the molecular formula indicated an additional degree of unsaturation. The $^1$H nmr spectrum was similar to that of 1, except that resonances due to methyl protons at C-7 in 4 were absent and instead resonances due to an epoxy protons were observed at δ 2.48 and 2.76 (1H each, d, J=5.3 Hz). The relative stereochemistry was determined by nOe measurements. The observed nOes could be interpreted only when the conformation as depicted in Figure 4 was assumed. Thus, the nOe correlation between one of the H-18 methylene protons (δ 2.48) and H-8 (δ 3.15; 1H, d, J=3.9 Hz) (4.0%) suggested the α-orientation of the 7,18 epoxide. The presence of a stearoyl group was confirmed by a fragment ion at m/z 283 in the negative ion FAB mass spectrum. Comparison of the chemical shifts of C-1 (65.6) and C-3 (64.4) with those of 4 tentatively suggested the positions of the acetyl group at C-1 and the acyl group at C-3. Thus, azamilide J (6) is 7,18-α-epoxyazamilide H.

EXPERIMENTAL

Extraction and Isolation. The organisms (collection No. 114; dry weight: 750 g) collected at Bonotsu, Kagoshima prefecture were chopped into small pieces and extracted twice with acetone (8 l x 2) for 3 days at r. t. The combined acetone solutions were concentrated to afford a dark reddish residue (22 g). The residue was suspended into H_{2}O (250 ml) and extracted with CH_{2}Cl_{2} (200 ml x 3). The CH_{2}Cl_{2} layer was dried over Na_{2}SO_{4}, filtered, and evaporated to dryness. A portion (7.5 g) of the CH_{2}Cl_{2} extract (15 g) was absorbed on silica gel and subjected to column chromatography of silica gel packed in hexane, frs (200 ml) being collected as follows: A: CH_{2}Cl_{2}-hexane, 1:9, B: CH_{2}Cl_{2}, C: EtOH-CH_{2}Cl_{2}, 1:49, D: EtOH-CH_{2}Cl_{2}, 1:19, E: EtOH-CH_{2}Cl_{2}, 1:9, F: EtOH-CH_{2}Cl_{2}, 1:16, G: EtOH-CH_{2}Cl_{2}, 1:1, H: EtOH. Xeniaethers D (2) (0.7 mg), E (3) (4.2 mg), and J (6) (2.3 mg) were isolated from the fr B using
Sephadex LH-20 with MeOH-CH2Cl2 (1:1), prep. tlc with hexane-ether (1:1), and hplc on ods with H2O-MeOH (3:2). Azamilides B (4.8 mg), C (1.3 mg), D (1.1 mg), H (4) (16.5 mg), I (5) (11.0 mg), and xeniaether C (1) (4.4 mg) were isolated from the fr D using Sephadex LH-20 with MeOH-CH2Cl2 (1:3 to 1:1), prep. tlc with hexane-ether (1:1) and ether-CH2Cl2 (1:3), and hplc on ods with H2O-MeOH (12:13 to 1:9). The fr E was further subjected to silica gel chromatography with ether-CH2Cl2 mixtures of increasing polarity (1:4 to 1:1) and then EtOH-CH2Cl2 (1:19 to 3:22), to a column of Sephadex LH-20 with MeOH-CH2Cl2 (1:1), and to hplc on ods with H2O-MeOH (19:1 to 3:17) to afford azamilides A (16 mg), G (1.4 mg), E (4.5 mg), and F (1.0 mg).

Xeniaether C (1). Oil, [α]D 37.5° (c 0.07, MeOH); uv (MeOH) λmax 239 nm (ε 12000); ir (film) v max 3450 and 1624 cm⁻¹; 1H nmr (400 MHz, CDCl3): δ 1.37 (3H, s, H-18), ca. 1.67 (2H, overlapped, H-6β and H-6α), 1.74 (1H, overlapped, H-5α), 1.77 and 1.78 (3H each, br s, H-16 and H-17), 1.94 (1H, dt, J=2.6 and 14.3 Hz, H-6β), 2.00 (1H, t, J=6.2 Hz, OH), 2.71 (1H, br t, J=11.7 Hz, H-10α), 2.72 (1H, overlapped, H-10β), 2.79 (1H, d, J=4.0 Hz, H-8), 2.96 (1H, dt, J=7.6 and 11.3 Hz, H-11α), 3.02 (1H, ddd, J=4.0, 5.5, and 11.0 Hz, H-9), 3.12 (1H, dd, J=7.7 and 11.3 Hz, H-4a), 3.40 (1H, dd, J=7.6 and 11.3 Hz, H-1α), 3.57 (2H, d, J=6.2 Hz, H-3), 3.82 (1H, t, J=7.6 Hz, H-1β), 5.13 (2H, br s, H-19), 5.62 (1H, d, J=15.2 Hz, H-12), 5.86 (1H, br d, J=11.0 Hz, H-14), and 6.67 (1H, dd, J=11.0 and 15.2 Hz, H-13); 1H nmr (C₅D₅): δ 1.08 (3H, s, H-18), 1.24 (1H, br d, J=15.0 Hz, H-5α), 1.41 (1H, br t, J=15.0 Hz, H-6β), 1.62 and 1.67 (3H each, br s, H-16 and H-17), ca. 1.65 (1H, overlapped, H-5β), 1.80 (1H, dt, J=3.3 and 15.0 Hz, H-6α), 2.27 (1H, d, J=3.8 Hz, H-8), 2.41 (1H, dd, J=3.8 and 11.8 Hz, H-10β), 2.58 (1H, t, J=11.8 Hz, H-10α), 2.72 (1H, dt, J=3.8 and 11.8 Hz, H-9), 2.84 (1H, dt, J=8.0 and 11.9 Hz, H-11α), 3.18 (1H, dd, J=8.1 and 11.9 Hz, H-4a), 3.38 (1H, dd, J=8.0 and 11.9 Hz, H-1α), 3.57 (1H, dd, J=6.6 and 11.4 Hz, H-3), 3.65 (1H, dd, J=5.9 and 11.4 Hz, H-3), 3.69 (1H, t, J=8.0 Hz, H-1β), 4.82 and 4.89 (1H each, br s, H-19), 5.84 (1H, d, J=15.4 Hz, H-12), 5.95 (1H, br d, J=11.0 Hz, H-14), and 6.99 (1H, dd, J=11.0 and 15.4 Hz, H-13); 13C nmr (100 MHz, CDCl3): δ 18.5 (C-17), 21.7 (C-5), 26.0 (C-16), 30.0 (C-6), 32.4 (C-18), 35.0 (C-10), 44.4 (C-4a), 53.0 (C-11a), 61.1 (C-9), 62.1 (C-8), 65.8 (C-3), 70.6 (C-1), 72.0 (C-7), 86.0 (C-4), 119.2 (C-19), 124.9 (C-14), 126.2 (C-13), 132.5 (C-12), 136.0 (C-15), and 141.5 (C-11); (+) FABms m/z 357 (M⁺+Na); HREIms m/z 334.2115 (M⁺, calcd for C₂₀H₉₀O₄, 334.2142).

Xeniaether D (2). Oil, [α]D -30.0° (c 0.14, MeOH); uv (MeOH) λmax 240 nm (ε 14000); ir (film) v max 3450, 1730 and 1620 cm⁻¹; 1H nmr (CDCl₃): δ 0.88 (3H, t, J=6.8 Hz, CH₃CH₂-). 1.25 [s, ...
Xeniaether E (3). Oil, [α]D -43.0° (c 0.03, MeOH); uv (MeOH) λmax 240 nm (ε 14000); ir (film) vmax 3450, 1735 and 1630 cm⁻¹. The ¹H and ¹³C nmr spectra were indistinguishable with those of 4. (+) FABms m/z 623 (M⁺+Na); (-) FABms m/z 599 (M⁻-H) and 283 (C₁₇H₃₅COO⁻).

Azamilde H (4). Oil, [α]D -62.0° (c 0.10, MeOH); uv (MeOH) λmax 240 nm (ε 12000); ir (film) vmax 3450, 1730 and 1620 cm⁻¹; ¹H nmr (CDCl₃): δ 0.88 (3H, t, J=7.1 Hz, CH₃CH₂-), 1.25 [s, (CH₂)n], 1.33 (3H, s, H-18), 1.34 (6H, s, H-16 and H-17), ca. 1.45 (2H, overlapped, H-5α and H-6α), ca. 1.80 (2H, overlapped, H-5β and H-6β), 1.98 (3H, s, AcO), 2.33 (2H, t, J=7.5 Hz, -CH₂CH₂COO), 2.55 (1H, ddd, J=3.8, 8.5, and 11.9 Hz, H-11a), ca. 2.67 (1H, overlapped, H-9), ca. 2.74 (1H, overlapped, H-10β), 2.75 (1H, d, J=4.0 Hz, H-8), 3.18 (1H, dt, J=4.6 and 10.6 Hz, H-10α), 3.80 (1H, dd, J=8.6 and 11.9 Hz, H-4a), 3.85 (1H, dd, J=3.7 and 11.9 Hz, H-1α), 3.91 (1H, dd, J=8.4 and 11.9 Hz, H-1β), 4.63 (2H, br s, H-3), 5.13 (2H, br s, H-19), 5.89 (1H, d, J=15.4 Hz, H-14), 6.26 (1H, br d, J=11.0 Hz, H-12), and 6.96 (1H, dd, J=11.0 and 15.4 Hz, H-13); ¹³C nmr (CDCl₃): δ 14.1 (CH₃CH₂-), 21.0 (CH₃COO⁻), 22.7-34.5 [-(-CH₂)n-], 27.6 (C-10), 29.3 and 30.5 (C-5 and C-6), 33.2 (C-6), 34.2 (C-4a), 50.0 (C-11a), 60.5 (C-8 or C-9), 61.4 (C-9 or C-8), 64.4 (C-3), 65.3 (C-1), 71.2 (C-15), 72.5 (C-7), 118.8 (C-19), 122.9 (C-13), 131.6 (C-12), 135.7 (C-4), 142.9 (C-14), 144.2 (C-11), 170.8 (CH₃COO⁻), and 173.6 (-CH₂COO⁻); (+) FABms m/z 683 (M⁺+Na); (-) FABms m/z 659 (M⁻-H) and 283 (C₁₇H₃₅COO⁻); HREIms m/z 642.4893 (M⁺+H₂O, calcd for C₄₀H₆₆O₆, 642.4858).

Azamilde I (5). Oil, [α]D -29.0° (c 0.07, MeOH); uv (MeOH) λmax 240 nm (ε 14000); ir (film) vmax 3450, 1735 and 1630 cm⁻¹. The ¹H and ¹³C nmr spectra were indistinguishable with those of 4. (+) FABms m/z 655 (M⁺+Na); (-) FABms m/z 631 (M⁻-H) and 255 (C₁₅H₃₁COO⁻).
Azamilide J (6). Oil, \([\alpha]_D^{20} -79.0^\circ\) (c 0.08, MeOH); uv (MeOH) \(\lambda_{\text{max}} 239\text{ nm} (\varepsilon 14000)\); ir (film) \(v_{\text{max}} 3450, 1735\) and 1630 cm\(^{-1}\); 1\(^{H}\) nmr (CDCl\(_3\)): \(\delta 0.88\) (3H, t, \(J=6.8\text{ Hz}, \text{CH}_3\text{CH}_2\)), 1.25 [s, -(CH\(_2\))\(_n\)], 1.34 and 1.35 (3H each, s, H-16 and H-17), 1.63 (2H, overlapped, two protons of H-5\(\alpha\), H-5\(\beta\), or H-6\(\alpha\)), 1.91 (1H, dt, \(J=2.5\) and 13.7 Hz, H-5\(\alpha\), H-5\(\beta\), or H-6\(\alpha\)), 1.99 (3H, s, OAc), 2.15 (1H, ddd, \(J=2.4, 4.2\) and 12.8 Hz, H-6\(\beta\)), 2.33 (2H, t, \(J=7.7\text{ Hz}, -\text{CH}_2\text{CH}_2\text{COO}^-\)), 2.39 (1H, br t, \(J=12.3\text{ Hz}, \text{H-10}\alpha\)), 2.48 (1H, d, \(J=5.3\text{ Hz}, \text{H-18}\)), 2.59 (1H, ddd, \(J=4.0, 7.4,\) and 12.0 Hz, H-11\(a\)), 2.76 (1H, d, \(J=5.3\text{ Hz}, \text{H-15}\)), 2.79 (1H, br dd, \(J=3.9\) and 12.3 Hz, H-10\(\beta\)), 3.15 (1H, d, \(J=7.4\) and 12.0 Hz, H-1\(\alpha\)), 3.94 (1H, dd, \(J=4.0\) and 12.0 Hz, H-1\(\beta\)), 4.64 (2H, br s, H-3), 5.17 (2H, br s, H-19), 5.89 (1H, d, \(J=15.2\text{ Hz}, \text{H-14}\)), 6.23 (1H, br d, \(J=10.8\text{ Hz}, \text{H-12}\)), and 6.84 (1H, dd, \(J=10.8\) and 15.2 Hz, H-13); 13\(^{C}\) nmr (CDCl\(_3\)): \(\delta 14.1\) (CH\(_3\text{CH}_2\)), 21.0 (CH\(_3\text{COO}^-\)), 22.7-34.4 [-CH\(_2\))\(_n\)], 27.3 (C-10), 29.2 (C-5), 29.5 and 29.8 (C-16 and C-17), 30.2 (C-6), 34.8 (C-4a), 46.3 (C-18), 49.7 (C-11a), 54.4 (C-9), 55.6 (C-7), 59.0 (C-8), 64.4 (C-3), 65.6 (C-1), 71.0 (C-15), 119.1 (C-19), 122.3 (C-13), 132.2 (C-12), 134.9 (C-4), 143.9 (C-14), 170.7 (CH\(_3\text{COO}^-\)), and 173.6 (-CH\(_2\text{COO}^-\)); (+) FABms \(m/z 681\) (M\(^{+}\)+Na); (-) FABms \(m/z 657\) (M\(^{+}\)-H) and 283 (C\(_{17}\)H\(_{35}\)COO\(^+\)).

ACKNOWLEDGMENTS

This work was partly supported by the Kagoshima Scholarship Foundation. We are grateful to Dr. Y. Minami (Taiho Yakugaku Co., Ltd.) for measuring the FABmass spectra, and to Drs. S. Moore (Massy University) and to Jeffrey L. C. Wright (NRC, Inst. Marine Bioscience, Canada) for valuable discussions.

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Received, 11th March, 1996