The Bakkenolides from the Root of *Petasites formosanus* and Their Cytotoxicity

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Thirty-two new bakkenolides, bakkenolides-Db (1) -- Dh (7), - Fa (8), - Fb (9), - J (10) -- M (14), - Na (15), - Nb (16), - O (17) -- T (22), - Ua (23), - Ub (24), - V (25) -- X (27), - Ya (28), - Yb (29), - Za (30), - Zb (31) and - III (32), from the roots of *Petasites formosanus* together with thirty known compounds were isolated. The structures were characterized by spectral analysis. The locations, C-1 and/or C-9 of bakkenolide skeleton, of the substituents, such as acetoxyl, isobutyryloxy and isovaleroyloxy groups, can be determined by the chemical shifts of their signals and the H-1 and/or H-9 in the 1H-NMR spectra. The cytotoxicity was also discussed.

**Key words** Petasites formosanus; Compositae; bakkenolide; cytotoxicity

*Petasites formosanus* Kitamura (Compositae) is a perennial herb and widely distributed in Taiwan on high altitude mountains. It has been utilized as a folk medicine for antidote, analgesic, expectorant and for the treatment of hypertension and snake-bite.1 We have previously described the isolation of some bakkenolide type compounds from the same plant and their antiplalet aggregation activity against arachidonic acid (AA) and collagen.2 In our continued search for bioactive compounds, the constituents of the root of *P. formosanus* was investigated. Hot methanol was partitioned between H2O and CHCl3, and then n-BuOH. After repeated chromatography, the two organic layers gave thirty-two new bakkenolide type compounds, bakkenolides-Db (1), - Dc (2), - Dd (3), - De (4), - Df (5), - Dg (6), - Dh (7), - Fa (8), - Fb (9), - I (10), - J (11), - K (12), - L (13), - M (14), - Na (15), - Nb (16), - O (17), - P (18), - Q (19), - R (20), - S (21), - T (22), - Ua (23), - Ub (24), - V (25), - W (26), - X (27), - Ya (28), - Yb (29), - Za (30), - Zb (31) and - III (32), together with thirty known compounds: six bakkenolides; bakkenolides-B (33), - D (34), - G (35), - H (36), - Lc (37) and - II (38), two steroids; ß-sitosterol (39) and 3ß-sitosterol-ß-p-glucopyranoside (40), two glycerol esters; phosphoric acid I (41) and glycerol ester II (42), fifteen benzenoids; p-hydroxybenzaldehyde (43), 3,4-dihydroxybenzaldehyde (44), p-hydroxybenzoic acid (45), vanillin (46), vanillic acid (47), methyl protocatechuate (48), methyl paraben (49), protocatechuic acid (50), p-methoxyphenylpropanoic acid (51), cis-cafeic acid (52), methyl caffeate (53), ferulic acid (54), caffeic acid (55), chlorogenic acid (56), N-p-coumaroyltryamine (57), one sulfoxide; methyl-3-propenoic acid ester (58), one flavonoid; morin (59), one isostilbene; scyllo-inositol (60), one betaine; 3-methyl-ß-lactone (61), and one triterpene; lupeol (62). The known compounds were characterized by the comparison of their spectroscopic data with those reported.

All the new compounds were determined to be optically active and proved to consist of a bakkenolide-type skeleton bearing two substituents on C-1 and C-9, except 10, 11 and 21 which have one substituent only on C-9, by comparison of their 1H-NMR spectra (Tables 1—4) as well as by 1H-1H homonuclear correlation spectroscopy (1H-1H COSY) spectra, 1H-detected heteronuclear multiple quantum coherence (HMOC) spectra, the 1H-detected heteronuclear multiple bond connectivity (HMBC) spectra and the nuclear Overhauser and exchange spectroscopy (NOESY) spectra with those of bakkenolides-B (33) and - D (34) which were also found in this plant. The full assignments of the 1H- and 13C-NMR signals were also achieved by two dimensional (2D) NMR spectrometry. According to the NOESY spectra, the stereochemistry of the substituent on C-1 was deduced to be in the α direction because of the presence of nuclear Overhauser effect (NOE) between H-1 and H-10, H-15, whereas the stereochemistry of the substituent on C-9 was suggested to be in the β direction due to the NOE between H-9 and H-4. The complete structure and absolute stereochemistry of bakkenolide-D (34) was further confirmed by a single crystal X-ray analysis and the result will be published elsewhere.

Bakkenolide-Db (1) exhibited the molecular formula C21H25O3S with the aid of high resolution electron impact mass spectrometry (HR-EI-MS). Except for the bakkenolide signals in the 1H-NMR spectrum, the remaining peaks showed an acetoxyl group at δ 2.00, and a cis-3-methyl-sulfinylacryloxyloxy group (OCOCH=CHSOCH3) at δ 2.84 (3H, s) for sulfoxide methyl, and 6.01, 6.98 (each 1H, d, J = 10.3 Hz) for cis double bond protons. The regiochemistry of these two groups was determined by an HMBC experiment. The carbonyl carbon at δ 169.5 (C-1') presented the HMBC correlations with the acetoxyl methyl (δ 2.00) and H-9 (δ 5.76), indicating the acetoxyl group was attached on C-9. Therefore, the cis-3-methylsulfinylacrylicloxy group should be on C-1. Compound 1 had UV absorption at 286 nm which corresponded to the cis-3-methylsulfinylacrylicloxy moiety. The circular dichroism (CD) spectrum of 1 showed a positive Cotton effect at 291 nm (Δε = +1.39), indicating the absolute configuration of sulfoxide group was R.27,28 From the above data, the structure of bakkenolide-Db was suggested as 1.

Bakkenolide-Dc (2), an isomer of 1, showed almost the same spectral data as those of 1. The significant difference was the CD spectrum of 2 which exhibited a negative Cotton effect at 288 nm (Δε = -0.74) by the cis-3-methylsulfinylacrylicloxy group. This result indicated that the absolute configuration of the sulfoxide group should be S.27,28 Thus, the structure of bakkenolide-Dc was determined to be the epimer of 1 at the sulfur atom.

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Bakkenolide-Dd (3), with the molecular formula C_{31}H_{52}O_{5}S, is a regio-isomer of bakkenolide-D (34). In 3, an acetoxy group (δ 1.83) was located on C-1 and a cis-methylthioacryloxy group [δ 2.39 (3H, s, H-4′)], 5.84 (1H, d, J=10.4 Hz, H-2′), 7.11 (1H, d, J=10.4 Hz, H-3′)] on C-9 by comparison of the chemical shift of acetoxy methyl (δ 1.83) with that in 35 (δ 1.91).

Bakkenolides-De (4) and -Df (5) have the same molecular formula C_{31}H_{52}O_{5}S and the same substituents, one is acetoxy group, the other is 3-methylsulfanylacryloxy group with trans geometry which was indicated by the large spin–spin coupling constant between two vinyl protons. In 4, the acetoxy group (δ 1.85) presented on C-1 and the trans-3-methylsulfanylacryloxy group [δ 2.70 (3H, s, H-4′)], 6.67 (1H, d, J=14.9 Hz, H-2′), 7.65 (1H, d, J=14.9 Hz, H-3′)] on C-9 by the HMBC experiment which showed the existence of cross peaks between the carbonyl carbon at δ 169.8 (C-1′) and acetoxy methyl (δ 1.85) and H-1 (δ 5.06) as well as the carbonyl carbon at δ 162.0 (C-1′) and vinyl H-3′ (δ 7.65) and H-9 (δ 5.86). On the other hand, in 5, an acetoxy group (δ 2.05, H-2′) presented on C-9 and the trans-3-methylsulfanylacryloxy group [δ 2.68 (3H, s, H-4′)], 6.50 (1H, d, J=14.8 Hz, H-2′), 7.60 (1H, d, J=14.8 Hz, H-3′)] on C-1 by comparison of the chemical shift of acetoxy methyl (δ 2.05) with that in 1 or 2. The UV absorption for the trans-3-methylsulfanylacryloxy group was at 268 in 4 and 266 nm in 5, and the negative Cotton effect at 281 nm for 4 (Δε −0.87) and 274 nm for 5 (Δε −1.00) inferred the absolute configuration of sulfur to be S.27,28

Bakkenolides-Dg (6) and -Dh (7), the regio-isomers of 1 and 2, respectively, showed acetoxy substituent on C-1 and cis-3-methylsulfanylacryloxy substituent on C-9 which were proved by the 1H−13C long range correlation of the carbonyl carbon of C-1′ with H-1 and acetoxy methyl. Bakkenolide-Dg (6) exhibited R configuration on the sulfur atom whereas bakkenolide-Dh (7) exhibited S configuration due to the positive Cotton effect (Δε+1.27) at 296 nm found in 6 and negative Cotton effect (Δε−3.13) at 297 nm in 7.27,28

Bakkenolides-Fa (8) and -Fb (9), C_{31}H_{52}O_{5}S, are regio-isomers with each other. Two substituents, isovaleroloyx and angelyloxy (trans-OCOC(CH_{3})=CHCH_{3}) groups, were observed from the 1H-NMR spectra. The trans geometry of two methyl groups in the angelyloxy group was proved by the chemical shift of vinyl H-3′ (or H-3′) at δ ca. 5.0029 and the presence of NOE between H-3′ (or H-3′) and C-2′ (or C-2″) methyl. From the HMBC spectra, the C-1″ carbonyl (δ 172.0) showed the cross peaks with isovaleroloyx/methylene (δ 2.00, H-2′) and H-9 (δ 5.76) in 8, whereas the C-1′ carbonyl (δ 172.2) exhibited the relationship with the isovaleroloyx/methylene (δ 1.94) and H-1 (δ 5.06), and C-1″ (166.0) with the angelyloxy methyl (δ 1.80, H-5′) in 9. This result led us to conclude that the isovaleroloyx group is on C-9 for compound 8 and on C-1 for compound 9.

Bakkenolide-I (10), C_{31}H_{52}O_{5}S, showed a very upfield shift of H-1 from δ ca. 5.00 to δ 1.56 with two proton integration in the 1H-NMR spectrum by comparison with those of 1−9. This upfield shift indicated that there was no acetylxy substituent on C-1. The remaining signals at δ 1.14, 1.15 (each 3H, d, J=7.2 Hz, 2×CH_{3}), 2.55 (1H, sept, J=7.2 Hz, CH) suggested an isobutryroxy group on C-9.

Bakkenolide-J (11) possessed the molecular formula as C_{31}H_{52}O_{5}S, one CH_{3} unit more than that of 10. An isovaleroloyx group [δ 0.93 (6H, d, J=6.7 Hz, 2×CH_{2})], 2.07 (1H, m, CH), 2.20 (2H, d, J=7.4 Hz, CH_{2}) in 11 was in place of an isobutryroxy group in 10.

Bakkenolide-K (12) was determined to have the molecular formula C_{31}H_{52}O_{5}S, one CH_{3} unit less than that of 9. Comparing the spectral data including 2D NMR data with those of 9, an isovaleroloyx group [δ 0.98 (6H, d, J=6.8 Hz, 2×CH_{2}), 2.15 (1H, sept, J=6.8 Hz, CH)] in 12 was substituted on C-1 instead of the isovaleroloyx group.

Bakkenolide-L (13), C_{31}H_{52}O_{5}S, was shown to have two acetoxy signals at δ 1.93 and 2.05 on C-1 and C-9, respec-
tively, from the $^1$H-NMR spectrum. The assignment was made by comparison of the chemical shifts with those of 1—7.

Bakkenolide-M (14) was found to have the molecular formula $C_{30}H_48O_8$. Except for an isobutyroxy group [δ 1.15, 1.20 (each 3H, d, J=7.0 Hz, 2×CH$_3$)], 2.50 (1H, sept, J=7.0 Hz), there were 2-methylbutyryoxy group at δ 0.86 (3H, t, J=7.0 Hz, CH$_3$), 1.06 (3H, d, J=7.0 Hz, CH$_3$), 1.40, 1.68 (each 1H, m, CH$_3$) and 2.09 (1H, sextet, J=7.0 Hz, CH$_3$). The HMBC experiment determined the isobutyroxy group was located on C-9 on the basis of the $^1$H-$^1^3$C long range correlations between C-1" (δ 175.8) and H-9 (δ 5.79), H-2" (δ 2.09).

Bakkenolides-Na (15) and -Nb (16) were regio-isomers with the molecular formula $C_{31}H_48O_8$. Examining the $^1$H-NMR and $^1$H-$^1^3$C COSY spectra, an isobutyroxy group and an isovaleroxy group were found to exist in the molecule. 

Comparing the chemical shifts of the isovaleroxy group (δ 1.15, 1.19 (each 3H, d, J=7.2 Hz, 2×CH$_3$), 2.49 (1H, sept, J=7.2 Hz, CH) in 15 with those of 10 and 14, we decided that the isobutyroxy group was placed on C-9. On the other hand, the isobutyroxy group (δ 1.08, 1.10 (each 3H, d, J=6.8 Hz, 2×CH$_3$), 2.32 (1H, sept, J=6.8 Hz, CH) in 16 was placed on C-1 by similar comparison with that of 12.

Bakkenolides-O (17) and -P (18) were decided to have the molecular formulas $C_{31}H_48O_8$ and $C_{32}H_48O_8$, respectively. They possessed 2-methylbutyroxy group shown by $^1$H-NMR and $^1$H-$^1^3$C COSY spectra. Compound 17 has another substituent, isovaleroxy group on C-9 shown by the comparison of the chemical shifts of this group [δ 0.95 (6H, d, J=6.8 Hz, H-4" -5")], 2.06 (1H, m, H-3") with those in 11 and 16. Compound 18 has another substituent, angeloyloxy group [δ 1.81 (3H, d, J=1.4 Hz, H-4"), 1.95 (3H, d, J=7.3 Hz, H-5")], 6.12 (1H, qu, J=7.3, 1.4 Hz, H-3") on C-9 which was shown by the presence of NOE between H-5" (δ 1.95) and H-6β (δ 2.19). Consequently, the 2-
methylbutyroloxy group in 17 and 18 should be located at C-1.

Bakkenolene-Q (19), C_{25}H_{35}O_6, was found to contain two isovaleroyloxy groups, one at δ 0.89 (6H, d, J=6.4 Hz, 2×CH₃), 2.07 (3H, m, CH₃CH₂) on C-1 and the other at δ 0.94 (6H, d, J=6.8 Hz, 2×CH₃), 2.10 (1H, m, CH), 2.18 (2H, m, CH₂) on C-9, by the comparison of these chemical shifts with those in 8 and 9.

Bakkenolene-R (20) has the molecular formula C_{29}H_{43}O_5. The IR spectrum exhibited a broad absorption peak at 3655 cm⁻¹ and the ¹H-NMR spectrum showed an upfield shifted H-9 signal at δ 4.20 in the ¹H-NMR spectrum which were responsible for a hydroxyl group on C-9. A very upfield shifted H-1 signal at δ 1.40 (2H) indicated no substitution on C-1.

Bakkenolene-T (22) has the molecular formula C_{31}H_{45}O_5S. An isovaleroyloxy group at δ 0.95 (6H, d, J=6.4 Hz, 2×CH₃), 2.03 (1H, m, CH), 2.09 (2H, m, CH₂) was attached on C-9 which was determined by the comparison of its chemical shifts with those in 16, 17, and 19. A cis-3-methylsulfynilacryloxy group [δ 2.86 (3H, s, SOCH₃) and 6.01, 6.98 (each 1H, d, J=10.4 Hz, CH=CH)] was found on C-1 as in 1 and 2. The absolute configuration of sulfoxide group would be R shown by the positive Cotton effect at 291 nm (Δε+1.25) in the CD spectrum of 22.

Bakkenolides-Ua (23) and -Ub (24), C_{19}H_{32}O_5, were also regio-isomers. For compound 23, a peak at 3517 cm⁻¹ in the
IR spectrum and a H-1 signal at δ 3.95 in the 1H-NMR spectrum, compared with 20, suggested the presence of a hydroxyl group on C-1. The other substituent on C-9 was an isoxytroxyylo group [δ 1.12, 1.14 (each 3H, d, J=6.8 Hz, 2×CH₃)], 2.50 (1H, sept, J=6.8 Hz, CHI)]. In addition, an IR hydroxyl band at 3500 cm⁻¹ and the 1H-NMR of H-9 at δ 4.53 in 24, compared with 21, suggested a hydroxyl group on C-9. The isoxytroxyylo group [δ 1.13 (6H, d, J=6.8 Hz, 2×CH₃), 2.47 (1H, sept, J=6.8 Hz, CHI)] should be located on C-1.

Bakkenolide-V (25) exhibited the molecular formula C₁₅H₁₄O₃. A hydroxyl group on C-9 was inferred by the presence of an upfield shifted signal of H-9 at δ 4.53 as in 24. The molecular formula of 25 differed from that of 24 by the increment of a CH₂ unit, suggesting an isoxytroxyylo group on C-1 which also exhibited the 1H-NMR signals at δ 0.93 for two methyls, 2.02 for a methine and 2.14 for a methylene.

Bakkenolide-W (26) was shown to have molecular formula C₁₅H₁₄O₃. The 1H-NMR spectrum showed the signal of an acetox group at δ 1.95. Two extra broad singlets at δ 5.78 and 6.00 for vinylidene protons and a doublet methyl at δ 1.35 (J=6.8 Hz) coupled with a methine proton at δ 4.53 (q, J=6.8 Hz) bearing a hydroyl functionality (IR 3651 cm⁻¹) constructed a 3-hydroxy-2-methylbenzylidencylo [OCOC(=CH₂)CH(OH)CH₃] group. The presence of NOE between the signal at δ 1.83 (H-2) and the signal at δ 5.78 (one of the terminal double bond protons) indicated the 3-hydroxy-2-methylbenzylidencylo group should be attached on C-1. Hence, the acetox group would be on C-9.

Bakkenolide-X (27), C₁₅H₂₁O₃, exhibited two substituents, a hydroxyl at 3566 cm⁻¹ from the IR spectrum and an acetox group at δ 2.01 from the 1H-NMR spectrum. The signal of H-9 shifted upfield to δ 4.52 as in 21, 24 and 25, indicating that the location of the hydroxyl group is on C-9. The acetox group, apparently, should be on C-1.

Bakkenolides-Ya (28) and -Yb (29) were an unseparable regio-isomeric mixture (3:2) with the same molecular formula, C₁₅H₁₄O₃S. The 1H-NMR spectrum showed two sets of the bakkenolide type signals bearing a cis-3-methylsulfonylacycloyoxy group. One set assignable to 28 has the upfield shifted H-1 signal at δ 4.02 and the other set assignable to 29 has the upfield shifted H-9 at δ 4.55 indicating the presence of a hydroxyl group on C-1 and C-9, respectively.

Bakkenolides-Za (30) and -Zb (31) were regio-isomers with molecular formula C₁₅H₁₄O₃. Two substituents were found from 1H-NMR spectra: one is isoxytroxyylo group, the other is 3-methyl-2-butenoyloxy group. In 30, the isoxytroxyylo group [δ 1.01, 1.03 (each 3H, d, J=6.4 Hz, 2×CH₃), 2.24 (1H, sep, J=6.4 Hz, CHI)] should be attached on C-1 and 3-methyl-2-butenoyloxy group (δ 1.89, 2.16 (each 3H, s, 2×=C(CH₃)₂), 5.68 (1H, s, CH=C=)] would be on C-9 by comparison of the chemical shifts of the isoxytroxyylo group with those in 12 and 24. Using the same comparison with 10 and 23, then, compound 31 possessed an isoxytroxyylo group [δ 1.14, 1.16 (each 3H, d, J=6.8 Hz, 2×CH₃), 2.47 (1H, sept, J=6.8 Hz, CHI)] on C-9 and 3-methyl-2-butenoyloxy group [δ 1.82, 2.13 (each 3H, s, 2×=C(CH₃)₂), 5.43 (1H, s, CH=C=)] on C-1.

Bakkenolide-III (32) was determined to have molecular formula C₁₅H₁₄O₅. The IR spectrum revealed a hydroxyl absorption at 3566 cm⁻¹, the 1H-NMR spectrum showed two upfield shifted H-1 and H-9 signals at δ 4.13 and 4.48, respectively, which indicated the presence of two hydroxyl groups on C-1 and C-9. That is a diol of deacylated bakkenolide type compound. Although 32 has been reported as a hydroxylated product of bakkenolide-D (34) by Abe et al.,30 this is the first time 32 has been isolated naturally.

From the above results, it is shown that P. formosanus is a rich source of bakkenolide type sesquiterpenoids. Examining the chemical shifts of the C-1 and C-9 substituents, such as acetox, isoxytroxyylo and isoxytroxyylo groups, in the 1H-NMR spectra, it was found that these substituents on C-1 appeared to have more upfield 1H-NMR signals than those on C-9 (Table 5). This conclusion would be useful for structural elucidation of bakkenolide type compounds.

The isolated compounds were subjected to cytotoxicity evaluation. Among them, compounds 34—37 exhibited significant cytotoxicity in Hep G2, Hep G2,2,15, and P-338 test system (Table 6).

**Experimental**

Melting points were measured on a Yanagimoto MP-S1 melting point apparatus and not corrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH solution. The IR spectra were recorded on a Jasco IR-100 spectrophotometer as KBr discs. The 1H- and 13C-NMR spectra were recorded on Bruker AC-200, AMX-400 and Varian-400 Unity Plus spectrometers. Chemical shifts are shown in δ values with tetramethylsilane as internal reference. The mass spectra were performed in the EI or FAB (matrix: glycerol) mode on a VG 70-250 S spectrometer. Specific rotations were recorded on a Jasco DIP-370 polarimeter.

**Plant Material**

Petasites formosanus was collected at Al Li mountain, in Taiwan, in August 1992 and verified by Prof. C. S. Kooh. The specimen of this plant was deposited in the herbarium of National Cheng Kung University, Tainan, Taiwan.

**Extraction and Isolation**

Dry roots (4.1 kg) of P. formosanus were extracted with hot MeOH (×8) and concentrated to give a deep brown syrup (280 g). This syrup was partitioned between H₂O and CHCl₃, and then n-BuOH. The CHCl₃ extract (60 g) was subjected to chromatography on silica gel and eluted with CH₃OH-Me₂CO (25:1) to give nine fractions. The first fraction was further chromatographed with silica gel column (CH₃OH-EtOAc =9:1), preparative TLC and HPLC (C-18 column, MeOH: H₂O=8:2) to yield 8 (7.2 mg), 9 (12.9 mg), 10 (7.6 mg), 11 (1.3 mg), 12 (8.7 mg), 38 (3.9 mg), 14 (3.4 mg), 15 (1.0 mg), 16 (4.8 mg), 17 (1.7 mg), 18 (2.9 mg), 19 (1.0 mg) and 41 (3.4 mg). The third fraction was filtered and the crystal was

<p>| Table 5. The Chemical Shifts of Acetox, Isoxytroxyylo and Isoxytroxyoylo Substituents on C-1 and C-9 in the 1H-NMR Spectrum |</p>
<table>
<thead>
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<th>C-1</th>
<th>C-9</th>
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<tr>
<td>Acetox:</td>
<td>CH₃</td>
<td>δ 1.8—2.0</td>
</tr>
<tr>
<td>Isoxytroxyylo:</td>
<td>CH₃</td>
<td>δ 2.1—2.5</td>
</tr>
<tr>
<td>Isoxytroxyylo:</td>
<td>CH₃</td>
<td>δ 1.9—1.1</td>
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<td>δ 1.9—2.1</td>
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<td></td>
<td>CH₃</td>
<td>δ 0.8—0.9</td>
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| Table 6. Cytotoxic Activities of 34—37 from P. formosanus |
|---|---|---|---|---|---|
| Cell line Compound | Hep G2 | Hep G2,2,15 | KB | CC2M | P338 |
| 34 | 2.32×10⁻⁴ | 1.40×10⁻² | 26.9 | 40.97 | 1.74×10⁻² |
| 35 | 2.25×10⁻⁴ | 1.95×10⁻² | 31.4 | 24.64 | 6.84 |
| 36 | 2.10×10⁻⁴ | 0.245 | 9.85 | 22.21 | 2.20×10⁻² |
| 37 | 2.08×10⁻⁴ | 0.264 | 36.4 | 42.56 | 2.90×10⁻² |
chromatographed as before to obtain 34 (1.2 g), 2 (20 mg), 5 (5.0 mg), 3 (5.4 mg), 13 (0.8 mg) and 39 (0.6 mg). Combining the second fraction, the filtrate of the third fraction and the fourth to the eighth fractions, the mixture was repeatedly chromatographed over silica gel (CH2Cl2-EtOAc=5:1) followed by (CH2Cl2-5%MeOH, 1:10). IR (KBr, cm–1): 3428, 2928, 1510, 1450, 1400, 1375, 1240, 1110, 1050. The CH2Cl2 fraction (53 mg) was subjected to chromatography on diatom-H20 eluting with a gradient solvent of H2O and MeOH to give twenty-one fractions. The first two fractions afforded crystalline 60 (6.2 mg). The third to the eighth fractions were rechromatographed over Sephadex-LH20 column and eluted with gradient solvent of CH3OH and MeOH to yield 45 (7.9 mg), 50 (12.4 mg), 55 (44.8 mg), 56 (34.2 mg) and 59 (42.4 mg). The combined following four fractions were rechromatographed repeatedly over silica gel (61 (14.2 mg), 62 (3.2 mg) and 57 (11.3 mg). The thirteenth to the sixteenth fractions were combined and rechromatographed on silica gel eluting with CHCl3-MeOH (10:1) to give 49 (10 mg) and 54 (1 mg).

Bakkenolide-D (1): Colorless oil. [α]D 72º (c=0.20, MeOH). HRMS m/z 424.1552 [M]+ (Calcd for C24H38O4S: 424.1556). UV λmax nm (log ε): 286 (0.39). IR νmax cm–1: 1770, 1747, 1716. MS (rel. int. m/z) 424 (M)+, 306 (100), 288 (12), 272 (18), 259 (13), 242 (21), 237 (11), 221 (32), 205 (100), 197 (100), 181 (100), 165 (100), 151 (100), 139 (100), 121 (100), 107 (100), 93 (100), 81 (100), 67 (100). The C24H38O4S molecule was assigned on the basis of 1H NMR, 13C NMR, and HRMS data. The absolute configuration was determined by comparison of observed CD spectra with the literature.

Bakkenolide-D (2): Colorless oil. [α]D 150º (c=0.04, MeOH). HRMS m/z 424.1555 [M]+ (Calcd for C24H38O4S: 424.1556). UV λmax nm (log ε): 280 (3.82). IR νmax cm–1: 1770, 1737, 1717. MS (rel. int. m/z) 424 (M)+, 306 (12), 288 (26), 248 (20), 230 (21), 186 (30), 138 (21), 117 (100), 80 (100), 76 (100), 80 (100), 64 (100), 52 (100), 42 (100), 32 (100), 22 (100), 16 (100), 14 (100). The C24H38O4S molecule was assigned on the basis of 1H NMR, 13C NMR, and HRMS data. The absolute configuration was determined by comparison of observed CD spectra with the literature.

Bakkenolides-I (11): Colorless oil. [α]D 15º (c=0.31, MeOH). HRMS m/z 408.1602 [M]+ (Calcd for C24H38O4S: 408.1606). UV λmax nm (log ε): 290 (3.68). IR νmax cm–1: 1770, 1737, 1716. MS (rel. int. m/z) 408 (M)+, 290 (100), 250 (100), 208 (100), 160 (100), 112 (100), 90 (100), 82 (100), 74 (100), 66 (100), 58 (100), 50 (100), 42 (100), 34 (100), 26 (100), 18 (100), 12 (100). The C24H38O4S molecule was assigned on the basis of 1H NMR, 13C NMR, and HRMS data. The absolute configuration was determined by comparison of observed CD spectra with the literature.

Bakkenolides-J (5): Colorless oil. [α]D 74º (c=0.09, MeOH). HRMS m/z 424.1555 [M]+ (Calcd for C24H38O4S: 424.1556). UV λmax nm (log ε): 266 (3.26). IR νmax cm–1: 1770, 1737, 1716. MS (rel. int. m/z) 424 (M)+, 322 (12), 382 (24), 248 (20), 160 (30), 138 (30), 117 (100), 80 (100), 76 (100). The CH2Cl2 fraction (53 mg) was subjected to chromatography on diatom-H20 eluting with a gradient solvent of H2O and MeOH to yield twenty-one fractions. The first two fractions afforded crystalline 60 (6.2 mg). The third to the eighth fractions were rechromatographed over Sephadex-LH20 column and eluted with gradient solvent of CH3OH and MeOH to yield 45 (7.9 mg), 50 (12.4 mg), 55 (44.8 mg), 56 (34.2 mg) and 59 (42.4 mg). The combined following four fractions were rechromatographed repeatedly over silica gel (61 (14.2 mg), 62 (3.2 mg) and 57 (11.3 mg). The thirteenth to the sixteenth fractions were combined and rechromatographed on silica gel eluting with CHCl3-MeOH (10:1) to give 49 (10 mg) and 54 (1 mg).

Bakkenolide-D (1): Colorless oil. [α]D 72º (c=0.20, MeOH). HRMS m/z 424.1552 [M]+ (Calcd for C24H38O4S: 424.1556). UV λmax nm (log ε): 286 (0.39). IR νmax cm–1: 1770, 1747, 1716. MS (rel. int. m/z) 424 (M)+, 306 (100), 288 (12), 272 (18), 259 (13), 242 (21), 237 (11), 221 (32), 205 (100), 197 (100), 181 (100), 165 (100), 151 (100), 139 (100), 121 (100), 107 (100), 93 (100), 81 (100), 67 (100). The C24H38O4S molecule was assigned on the basis of 1H NMR, 13C NMR, and HRMS data. The absolute configuration was determined by comparison of observed CD spectra with the literature.

Bakkenolide-D (2): Colorless oil. [α]D 150º (c=0.04, MeOH). HRMS m/z 424.1555 [M]+ (Calcd for C24H38O4S: 424.1556). UV λmax nm (log ε): 280 (3.82). IR νmax cm–1: 1770, 1737, 1717. MS (rel. int. m/z) 424 (M)+, 306 (12), 288 (26), 248 (20), 230 (21), 186 (30), 138 (21), 117 (100), 80 (100), 76 (100), 80 (100), 64 (100), 52 (100), 42 (100), 32 (100), 22 (100), 16 (100), 14 (100). The C24H38O4S molecule was assigned on the basis of 1H NMR, 13C NMR, and HRMS data. The absolute configuration was determined by comparison of observed CD spectra with the literature.