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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), -I (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 acetoxy, isobutyroyloxy and isovaleroyloxy groups, can be determined by the chemical shifts of their signals and the H-1 and/H-9 in the ^{1}H-NMR spectra. The cytotoxicity was also discussed.

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

Petasites formosanus Kitamura (Compositae) is a perennial herb and wildly 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 antiplatelet aggregation activity against arachidonic acid (AA) and collagen.[2] In our continued search for bioactive compounds, the constituents of the root of *P. formosanus* were investigated. Hot methanol extract was partitioned between H_2O and $CHCl_3$, 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**),[3] -D (**34**),[4] -G (**35**),[2] -H (**36**),[2] -Uc (**37**)[2] and -II (**38**),[5] two steroids; β-sitosterol (**39**)[6] and β-sitosterol-β-D-glucopyranoside (**40**),[7] two glycerol esters; phosphoric acid I (**41**)[8] and glycerol ester II (**42**),[8] fifteen benzenoids; *p*-hydroxybenzaldehyde (**43**),[9] 3,4-dihydroxybenzaldehyde (**44**),[10] *p*-hydroxybenzoic acid (**45**),[11] vanillin (**46**),[12] vanillic acid (**47**),[13] methyl protocatechuate (**48**),[14] methyl paraben (**49**),[15] protocatechuic acid (**50**),[16] *p*-methoxyphenylpropanoic acid (**51**),[17] *cis*-caffeic acid (**52**),[18] methyl caffeate (**53**),[19] ferulic acid (**54**),[20] caffeic acid (**55**),[21] chlorogenic acid (**56**)[22] and *N*-*p*-coumaroyltyramine (**57**),[23] one sulfoxide; 3-methylthio-2-propenoic acid methyl ester (**58**),[23] one flavonoid; morin (**59**),[24] one inositol; *scyllo*-inositol (**60**),[25] one β-lactone; 3-methyl-β-lactone (**61**),[25] and one triterpene; lupeol (**62**).[26] 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 ^{1}H-NMR spectra (Tables 1—4) as well as by ^{1}H–^{1}H homonuclear correlation spectroscopy (^{1}H–^{1}H COSY) spectra, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectra, the ^{1}H-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 ^{1}H- and ^{13}C-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**)[4] was further confirmed by a single crystal X-ray analysis and the result will be published elsewhere.

Bakkenolide-Db (**1**) exhibited the molecular formula $C_{21}H_{28}O_7S$ with the aid of high resolution electron impact mass spectrometry (HR-EI-MS). Except for the bakkenolide signals in the ^{1}H-NMR spectrum, the remaining peaks showed an acetoxy group at δ 2.00, and a *cis*-3-methylsulfinylacryloyloxy group ($OCOCH=CHSOCH_3$) 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 acetoxy methyl (δ 2.00) and H-9 (δ 5.76), indicating the acetoxy group was attached on C-9. Therefore, the *cis*-3-methylsulfinylacryloyloxy group should be on C-1. Compound **1** had UV absorption at 286 nm which corresponded to the *cis*-3-methylsulfinylacryloyloxy moiety. The circular dichroism (CD) spectrum of **1** showed a positive Cotton effect at 291 nm ($\Delta\varepsilon$+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 ($\Delta\varepsilon$−0.74) by the *cis*-3-methylsulfinylacryloyloxy 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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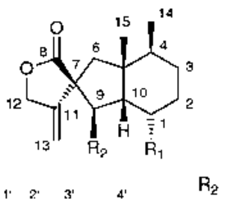
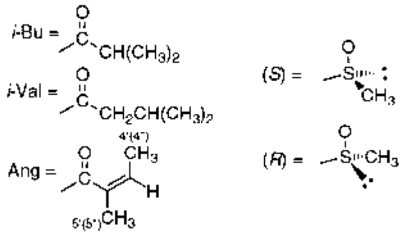


	R_1	R_2
1:	(*R*)-*cis*-OCOCH=CHSOCH$_3$	OAc
2:	(*S*)-*cis*-OCOCH=CHSOCH$_3$	OAc
3:	OAc	*cis*-OCOCH=CHSCH$_3$
4:	OAc	(*S*)-*trans*-OCOCH=CHSOCH$_3$
5:	(*S*)-*trans*-OCOCH=CHSOCH$_3$	OAc
6:	OAc	(*R*)-*cis*-OCOCH=CHSOCH$_3$
7:	OAc	(*S*)-*cis*-OCOCH=CHSOCH$_3$
8:	OAng	O-*i*-Val
9:	O-*i*-Val	OAng
10:	H	O-*i*-Bu
11:	H	O-*i*-Val
12:	O-*i*-Bu	OAng
13:	OAc	OAc
14:	OCOCH(CH$_3$)CH$_2$CH$_3$	O-*i*-Bu
15:	O-*i*-Val	O-*i*-Bu
16:	O-*i*-Bu	O-*i*-Val
17:	OCOCH(CH$_3$)CH$_2$CH$_3$	O-*i*-Val
18:	OCOCH(CH$_3$)CH$_2$CH$_3$	OAng
19:	O-*i*-Val	O-*i*-Val
20:	OH	OAng
21:	H	OH
22:	(*R*)-*cis*-OCOCH=CHSOCH$_3$	O-*i*-Val
23:	OH	O-*i*-Bu
24:	O-*i*-Bu	OH
25:	O-*i*-Val	OH
26:	OCOC(=CH$_2$)CH(OH)(CH$_3$)	OAc
27:	OAc	OH
28:	OH	*cis*-OCOCH=CHSOCH$_3$
29:	*cis*-OCOCH=CHSOCH$_3$	OH
30:	O-*i*-Bu	OCOCH=C(CH$_3$)$_2$
31:	OCOCH=C(CH$_3$)$_2$	O-*i*-Bu
32:	OH	OH
33:	OAng	OAc
34:	*cis*-OCOCH=CHSCH$_3$	OAc
35:	OAc	O-*i*-Val
36:	O-*i*-Bu	O-*i*-Bu
37:	H	O-*i*-Bu



Bakkenolide-Dd (**3**), with the molecular formula $C_{21}H_{28}O_6S$, is a regio-isomer of bakkenolide-D (**34**). In **3**, an acetoxy group (δ 1.83) was located on C-1 and a *cis*-methylthioacryloyloxy 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 $C_{21}H_{28}O_7S$ and the same substituents, one is acetoxy group, the other is 3-methylsulfinylacryloyloxy 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-methylsulfinylacryloyloxy 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-methylsulfinylacryloyloxy 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-methylsulfinylacryloyloxy group was at 268 in **4** and 266 nm in **5**, and the negative Cotton effect at 281 nm for **4** ($\Delta\varepsilon$ −0.87) and 274 nm for **5** ($\Delta\varepsilon$−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-methylsulfinylacryloyloxy substituent on C-9 which were proved by the ^{1}H–^{13}C 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 ($\Delta\varepsilon$+1.27) at 296 nm found in **6** and negative Cotton effect ($\Delta\varepsilon$−3.13) at 297 nm in **7**.[27,28]

Bakkenolides-Fa (**8**) and -Fb (**9**), $C_{25}H_{36}O_6$, are regio-isomers with each other. Two substituents, isovaleroyloxy and angeloyloxy (*trans*-OCOC(CH$_3$)=CHCH$_3$) groups, were observed from the ^{1}H-NMR spectra. The *trans* geometry of two methyl groups in the angeloyloxy group was proved by the chemical shift of vinyl H-3′ (or H-3″) at δ *ca.* 5.00[29] 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 isovaleroyloxy 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 isovaleroyloxy methylene (δ 1.94) and H-1 (δ 5.06), and C-1″ (166.0) with the angeloyloxy methyl (δ 1.80, H-5″) and H-9 (δ 5.90) in **9**. This result led us to conclude that the isovaleroyloxy group is on C-9 for compound **8** and on C-1 for compound **9**.

Bakkenolide-I (**10**), $C_{19}H_{28}O_4$, showed a very upfield shift of H-1 from δ *ca.* 5.00 to δ 1.56 with two proton integration in the ^{1}H-NMR spectrum by comparison with those of **1**—**9**. This upfield shift indicated that there was no acyloxy 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 isobutyroyloxy group on C-9.

Bakkenolide-J (**11**) possessed the molecular formula as $C_{20}H_{30}O_4$, one CH$_2$ unit more than that of **10**. An isovaleroyloxy group [δ 0.93 (6H, d, J=6.7 Hz, 2×CH$_3$), 2.07 (1H, m, CH), 2.20 (2H, d, J=7.4 Hz, CH$_2$)] in **11** was in place of an isobutyroyloxy group in **10**.

Bakkenolide-K (**12**) was determined to have the molecular formula $C_{24}H_{34}O_6$, one CH$_2$ unit less than that of **9**. Comparing the spectral data including 2D NMR data with those of **9**, an isobutyroyloxy group [δ 0.98 (6H, d, J=6.8 Hz, 2×CH$_3$), 2.15 (1H, sept, J=6.8 Hz, CH)] was substituted on C-1 instead of the isovaleroyloxy group.

Bakkenolide-L (**13**), $C_{19}H_{26}O_6$, was shown to have two acetoxy signals at δ 1.93 and 2.05 on C-1 and C-9, respec-

Table 1. ^{1}H-NMR Spectral Data for **1**—**8** (CDCl$_3$, δ, Multiplicity, J, Hz)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
H-1	5.10 dt (11.3, 5.1)	5.11 dt (11.2, 4.8)	5.12 dt (11.6, 5.2)	5.06 dt (12.0, 5.0)	5.18 m	5.04 dt (9.8, 5.0)	5.05 dt (9.6, 4.8)	5.13 m
H-2	1.83 m	1.76, 1.85 m	1.75, 1.80 m	1.73, 1.82 m	1.70, 1.82 m	1.70, 1.82 m	1.70, 1.72 m	1.85 m
H-3	1.36, 1.71 m	1.36, 1.74 m	1.37, 1.66 m	1.42, 1.69 m	1.40, 1.65 m	1.31, 1.68 m	1.35, 1.68 m	1.38, 1.69 m
H-4	1.59 m	1.62 m	1.50 m	1.60 m	1.60 m	1.54 m	1.55 m	1.59 m
H-6	1.95, 2.22 d (14.4)	1.95, 2.24 d (14.4)	1.94, 2.22 d (14.0)	1.98, 2.26 d (14.3)	1.96, 2.24 d (14.8)	1.88, 2.21 d (14.4)	1.96, 2.24 d (14.3)	1.95, 2.26 d (14.2)
H-9	5.76 d (11.3)	5.25 d (11.2)	5.83 d (11.2)	5.86 d (11.2)	5.89 d (11.6)	5.80 d (11.3)	5.81 d (11.3)	5.76 d (11.2)
H-10	2.80 dd (11.3, 5.1)	2.77 dd (11.2, 4.8)	2.71 dd (11.6, 5.2)	2.78 dd (11.2, 5.0)	2.76 dd (11.6, 5.0)	2.76 dd (11.3, 5.0)	2.78 dd (11.3, 4.8)	2.84 dd (11.2, 4.9)
H-12	4.65, 4.70 dt (11.2, 1.8)	4.64, 4.70 dt (11.2, 2.0)	4.65 br s	4.63, 4.71 d (13.0)	4.62, 4.70 d (12.2)	4.61, 4.70 dt (13.2, 1.8)	4.63, 4.73 dt (13.1, 1.8)	4.66 t (1.1)
H-13	5.18, 5.21 t (1.8)	5.19, 5.21 t (2.0)	5.15, 5.20 s	5.22, 5.26 s	5.19, 5.22 s	5.20, 5.22 t (1.8)	5.22, 5.25 t (1.8)	5.17, 5.21 t (1.1)
H-14	0.88 d (6.6)	0.91 d (7.2)	0.89 d (6.8)	0.92 d (6.6)	0.89 d (6.6)	0.86 d (7.2)	0.90 d (6.6)	0.90 d (6.4)
H-15	1.10 s	1.11 s	1.09 s	1.12 s	1.13 s	1.08 s	1.10 s	1.13 s
R$_1$	2.84 s (CH$_3$), 6.01, 6.98 d (10.3, CH=CH)	2.82 s (CH$_3$), 6.04, 6.97 d (10.4, CH=CH)	1.83 s (CH$_3$),	1.85 s (CH$_3$)	2.68 s (CH$_3$), 6.50, 7.60 d (14.8, CH=CH)	1.84 s (CH$_3$)	1.87 s (CH$_3$)	1.80 d (1.8, CH$_3$), 1.86 d (7.3, CH$_3$), 5.90 qq (7.3, 1.8)
R$_2$	2.00 s (CH$_3$)	2.02 s (CH$_3$)	2.39 s (CH$_3$), 5.84, 7.11 d (10.4, CH=CH)	2.70 s (CH$_3$), 6.67, 7.65 d (14.9, CH=CH)	2.05 s (CH$_3$)	2.79 s (CH$_3$), 6.24, 7.03 d (10.4, CH=CH)	2.81 s (CH$_3$), 6.28, 7.04 d (10.4, CH=CH)	0.91 d (6.6, 2×CH$_3$), 2.00 m (CH), 2.08 d (6.2, CH$_2$)

Table 2. ^{1}H-NMR Spectral Data for **9**—**16** (CDCl$_3$, δ, Multiplicity, J, Hz)

	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
H-1	5.06 m	1.56 m	1.52 m	5.00 dt (11.6, 5.2)	5.06 dt (12.4, 4.8)	5.06 dt (12.0, 4.3)	5.07 dt (11.6, 4.8)	5.05 dt (12.0, 4.8)
H-2	1.70, 1.78 m	1.40, 1.56 m	1.40, 1.52 m	1.73, 1.78 m	1.60 m	1.72, 1.83 m	1.70, 1.82 m	1.75, 1.80 m
H-3	1.37, 1.66 m	1.20, 1.56 m	1.15, 1.52 m	1.33, 1.64 m	1.60 m	1.38, 1.66 m	1.35, 1.67 m	1.36, 1.71 m
H-4	1.56 m	1.56 m	1.52 m	1.57 m	1.60 m	1.59 m	1.58 m	1.59 m
H-6	1.95, 2.24 d (14.3)	1.96, 2.19 d (14.8)	1.96, 2.19 d (14.3)	1.93, 2.22 d (14.8)	1.95, 2.22 d (14.8)	1.96, 2.26 d (14.4)	1.94, 2.25 d (14.8)	1.95, 2.23 d (14.4)
H-9	5.90 d (11.2)	5.43 d (11.6)	5.47 d (11.7)	5.89 d (11.2)	5.77 d (11.6)	5.79 d (11.2)	5.78 d (11.2)	5.80 d (11.2)
H-10	2.72 dd (11.2, 4.9)	2.34 d (11.6)	2.36 dt (11.7, 4.2)	2.71 dd (11.2, 5.2)	2.68 dd (11.6, 4.8)	2.65 dd (11.2, 4.3)	2.64 dd (11.2, 4.8)	2.67 dd (11.2, 4.8)
H-12	4.64, 4.68 dt (11.1, 1.8)	4.52, 4.69 dt (12.3, 1.8)	4.69 t (2.2)	4.62, 4.67 dt (12.8, 2.0)	4.65, 4.70 dt (12.8, 1.8)	4.63, 4.69 d (12.9)	4.62, 4.68 d (12.8)	4.66, 4.70 dt (11.2, 1.2)
H-13	5.16, 5.21 t (1.8)	5.13, 5.19 t (1.8)	5.14, 5.21 t (2.2)	5.15, 5.19 t (2.0)	5.18, 5.21 t (1.8)	5.17, 5.21 br s	5.17, 5.20 br s)	5.18, 5.22 t (1.2)
H-14	0.90 d (6.6)	0.89 d (7.2)	0.88 d (6.6)	0.88 d (6.8)	0.89 d (6.4)	0.90 d (6.6)	0.89 d (6.8)	0.87 d (6.4)
H-15	1.10 s	1.04 s	1.04 s	1.08 s	1.09 s	1.10 s	1.09 s	1.09 s
R$_1$	0.84 d (6.5, 2×CH$_3$), 1.90 m (CH), 1.94 d (5.0, CH$_2$)	—	—	0.98 d (6.8, 2×CH$_3$) 2.15 sept (6.8, CH)	1.93 s (CH$_3$)	0.86 t (7.0, CH$_3$), 1.06 d (7.0, CH$_3$), 1.40, 1.68 m (CH$_2$), 2.09 sex (7.0, CH)	0.89 d (6.8, 2×CH$_3$), 1.60 m (CH), 2.00 m (CH$_2$)	1.08, 1.10 d (6.8, 2×CH$_3$), 2.32 sept (6.8, CH)
R$_2$	1.80 d (1.8, CH$_3$), 1.86 d (7.3, CH$_3$), 5.90 qq (7.3, 1.8)	1.14, 1.15 d (7.2, 2×CH$_3$), 2.55 sept (7.2, CH)	0.93 d (6.7, 2×CH$_3$), 2.07 m (CH), 2.20 d (7.4, CH$_2$)	1.80 d (1.2, CH$_3$), 1.99 d (7.2, CH$_3$), 6.17 qq (7.2, 1.2)	2.05 s (CH$_3$)	1.15, 1.20 d (7.0, 2 x CH$_3$), 2.50 sept (7.0, CH)	1.15, 1.19 d (7.2, 2×CH$_3$), 2.49 sept) (7.2, CH)	0.89, 0.95 d (6.8, 2×CH$_3$), 2.06 m (CH), 2.14 m(CH$_2$)

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 $C_{24}H_{36}O_6$. Except for an isobutyroyloxy 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 was a 2-methylbutyroyloxy 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$_2$) and 2.09 (1H, sextet, J=7.0 Hz, CH). The HMBC experiment determined the isobutyroyloxy group was located on C-9 on the basis of the ^{1}H–^{13}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 $C_{24}H_{36}O_6$. Examining the ^{1}H-NMR and ^{1}H–^{1}H COSY spectra, an isobutyroyloxy and an isovaleroyloxy group were found to exist in the molecules. Comparing the chemical shifts of the isobutyroyloxy 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 isobutyroyloxy group was placed on C-9. On the other hand, the isobutyroyloxy 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 $C_{25}H_{38}O_6$ and $C_{25}H_{36}O_6$, respectively. They possessed 2-methylbutyroyloxy group shown by ^{1}H-NMR and ^{1}H–^{1}H COSY spectra. Compound **17** has another substituent, isovaleroyloxy 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″), 2.18 (2H, m, H-2″)] 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, qq, 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-

Table 3. ^{1}H Spectral Data for **17**—**24** (CDCl$_3$, δ, Multiplicity, *J*, Hz)

	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**
H-1	5.05 dt (11.2, 4.4)	4.98 dt (11.2, 4.8)	5.07 dt (11.2, 4.8)	3.94 dt (11.6, 4.4)	1.40 m	5.10 m	3.95 dt (11.6, 5.2)	5.17 dt (11.6. 5.6)
H-2	1.70, 1.82 m	1.70, 1.80 m	1.70, 1.81 m	1.55 m	1.40 m	1.74, 1.82 m	1.76, 1.82 m	1.64, 1.93 m
H-3	1.37, 1.65 m	1.24, 1.65 m	1.35, 1.65 m	1.55 m	1.40 m	1.38, 1.68 m	1.35, 1.70 m	1.36, 1.58 m
H-4	1.55 m	1.50 m	1.55 m	1.55 m	1.40 m	1.60 m	1.63 m	1.49 m
H-6	1.95, 2.23 d (14.0)	1.89, 2.19 d (14.2)	1.94, 2.22 d (14.0)	1.96, 2.23 d (14.4)	1.90, 2.08 d (14.0)	1.95, 2.23 d (14.4)	1.94, 2.23 d (14.4)	1.78, 2.16 d (14.4)
H-9	5.81 d (11.2)	5.87 d (11.2)	5.78 d (11.6)	5.70 d (11.2)	4.20 d (11.2)	5.78 d (11.2)	5.62 d (11.2)	4.53 d (11.2)
H-10	2.66 dd (11.2, 4.8)	2.65 dd (11.2, 4.8)	2.66 dd (11.6, 4.8)	2.74 dd (11.2, 4.4)	1.40 m	2.78 m	2.66 dd (11.2, 5.2)	2.53 dd (11.2, 5.6)
H-12	4.66, 4.71 dt (10.8, 1.2)	4.58, 4.63 dt (10.9, 1.7)	4.65, 4.70 d (12.8)	4.66, 4.70 d (13.6)	4.77, 4.90 d (12.8)	4.67, 4.71 dt (13.2, 1.2)	4.63, 4.69 d (13.2)	4.75, 4.92 d (12.4)
H-13	5.18, 5.22 t (1.2)	5.11, 5.15 t (1.7)	5.16, 5.21 s	5.16, 5.20 br s	5.11 br s	5.20, 5.23 t (1.2)	5.16, 5.19 br s	5.12, 5.13 br s
H-14	0.88 d (6.4)	0.84 d (6.6)	0.88 d (6.4)	0.89 d (6.8)	0.85 d (6.4)	0.90 d (6.8)	0.82 d (d, 6.6)	0.88 d (d, 6.4)
H-15	1.09 s	1.05 s	1.09 s	1.06 s	1.01 s	1.10 s	1.05 s	1.08 s
R$_1$	0.87 t (7.2, CH$_3$), 1.06 d (6.8, CH$_3$), 1.35,1.66 m (CH$_2$), 2.32 m (CH))	0.74 t (7.4, CH$_3$), 0.93 d (7.0, CH$_3$), 1.22,1.60 m (CH$_2$), 1.93 m (CH)	0.89 d (6.4, 2×CH$_3$), 2.01 m (CH), 2.02 m (CH$_2$)	—	—	2.86 s (CH$_3$), 6.01, 6.98 d (10.4, CH=CH)	—	1.13 d (6.8, 2×CH$_3$), 2.47 sept (6.8, CH)
R$_2$	0.95 d (6.8, 2×CH$_3$), 2.06 m (CH), 2.18 m (CH$_2$)	1.81 d (1.4, CH$_3$), 1.95 d (7.3, CH$_3$), 6.12 qq (7.3, 1.4)	0.94 d (6.8, 2×CH$_3$), 2.10 m (CH), 2.18 m (CH$_2$)	1.85 d (1.2, CH$_3$), 1.99 d (7.2, CH$_3$), 6.11 qq (7.2, 1.2)	—	0.95 d (6.4, 2×CH$_3$), 2.03 m (CH), 2.09 m (CH$_2$)	1.12, 1.14 d (6.8, 2×CH$_3$), 2.50 sept (6.8, CH)	—

Table 4. ^{1}H-NMR Spectral Data for **25**—**32** (CDCl$_3$, δ, Multiplicity, *J*, Hz)

	25	**26**	**27**	**28**	**29**	**30**	**31**	**32**
H-1	5.20 m	5.12 m	5.18 m	4.02 dt (11.3, 5.6)	5.10 m	5.04 dt (11.2, 4.8)	5.08 dt (11.2, 5.2)	4.13 dt (12.4, 4.8)
H-2	1.66, 1.96 m	1.72, 1.83 m	1.45 m	1.50 m	1.50 m	1.71, 1.77 m	1.71, 1.80 m	1.50 m
H-3	1.34, 1.58 m	1.42, 1.72 m	1.45 m	1.50 m	1.50 m	1.38, 1.64 m	1.32, 1.71 m	1.50 m
H-4	1.49 m	1.58 m	1.45 m	1.50 m	1.50 m	1.55 m	1.55 m	1.50 m
H-6	1.89, 2.16 d (14.4)	1.96, 2.25 d (14.0)	1.88, 2.15 d (14.8)	1.96, 2.24 d (14.4)	1.96, 2.24 d (14.4)	1.95, 2.23 d (14.4)	1.96, 2.26 d (14.4)	1.85, 2.12 d (14.4)
H-9	4.53 d (11.2)	5.74 d (11.2)	4.52 d (11.2)	5.75 d (11.3)	4.55 d (11.1)	5.85 d (11.2)	5.74 d (11.6)	4.48 d (11.2)
H-10	2.54 dd (11.2, 4.8)	2.83 dd (11.2, 5.2)	2.53 dd (11.2, 5.6)	2.77 dd (11.3, 5.6)	2.62 dd (11.1, 5.4)	2.69 dd (11.2, 4.8)	2.72 dd (11.2, 5.2)	2.58 dd (11.2, 4.8)
H-12	4.75, 4.92 d (12.8)	4.64, 4.68 d (12.8)	4.74, 4.91 d (12.8)	4.69 m	4.74, 4.91 d (12.7)	4.66 s	4.62, 4.67 d (12.8)	4.73, 4.91 dt (12.8, 2.4)
H-13	5.14 s	5.17, 5.20 br s	5.14, 5.16 br s	5.21, 5.23 t (1.1)	5.12, 5.15 s	5.16, 5.20 s	5.16, 5.19 s	5.10 t (2.4)
H-14	0.88 d (6.4)	0.91 d (6.4)	0.87 d (6.4)	0.89 d (6.3)	0.89 d (6.3)	0.90 d (6.4)	0.90 d (6.4)	0.86 d (6.6)
H-15	1.09 s	1.13 s	1.07 s	1.07 s	1.09 s	1.12 s	1.11 s	1.03 s
R$_1$	0.93 d (6.8, 2×CH$_3$), 2.02 m (CH), 2.14 d (8.2, CH$_2$)	1.35 d (6.8, CH$_3$), 4.53 q (6.8, CH), 5.78, 6.00 s (C=CH$_2$)	2.01 s (CH$_3$)	—	2.83 s (CH$_3$), 6.23, 6.98 d (10.3, (CH=CH)	1.01, 1.03 d (6.4, 2×CH$_3$), 2.24 sept (6.4, CH)	1.82, 2.13 s (2×CH$_3$) 5.43 s (CH=C)	—
R$_2$	—	1.95 s (CH$_3$)	—	2.80 s (CH$_3$) 6.24, 6.98 d (10.3, CH=CH)	—	1.89, 2.16 s (2×CH$_3$) 5.68 s (CH=C)	1.14, 1.16 d (6.8, 2×CH$_3$) 2.47 sept (6.8, CH)	—

methylbutyroyloxy group in **17** and **18** should be located at C-1.

Bakkenolide-Q (**19**), $C_{25}H_{38}O_6$, was found to contain two isovaleroyloxy groups, one [δ 0.89 (6H, d, *J*=6.4 Hz, 2×CH$_3$), 2.01 (3H, m, CH, CH$_2$)] on C-1 and the other [δ 0.94 (6H, d, *J*=6.8 Hz, 2×CH$_3$), 2.10 (1H, m, CH), 2.18 (2H, m, CH$_2$)] on C-9, by the comparison of these chemical shifts with those in **8** and **9**.

Bakkenolide-R (**20**) has the molecular formula $C_{20}H_{28}O_5$. The IR spectrum exhibited a broad absorption peak at 3655 cm^{-1} and the ^{1}H-NMR spectrum showed an upfield shifted H-1 signal at δ 3.94, indicating a hydroxyl group attached on C-1. The remaining signals constructed an angeloyloxy group at δ 1.85 (3H, d, *J*=1.2 Hz, H-4″), 1.99 (3H, d, *J*=7.2 Hz, H-5″) and 6.11 (1H, qq, *J*=7.2, 1.2 Hz, H-3″) on C-9 apparently.

Bakkenolide-S (**21**), $C_{15}H_{22}O_3$, was observed to have a broad absorption peak at 3566 cm^{-1} in the IR spectrum and an upfield shifted H-9 at δ 4.20 in the ^{1}H-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.

Bakkenolide-T (**22**) has the molecular formula $C_{24}H_{34}O_7S$. An isovaleroyloxy group [δ 0.95 (6H, d, *J*=6.4 Hz, 2×CH$_3$), 2.03 (1H, m, CH), 2.09 (2H, m, CH$_2$)] 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-methylsufinylacryloyloxy group [δ 2.86 (3H, s, SOCH$_3$) 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 ($\Delta\varepsilon$+1.25) in the CD spectrum of **22**.[27,28]

Bakkenolides-Ua (**23**) and -Ub (**24**), $C_{19}H_{28}O_5$, were also regio-isomers. For compound **23**, a peak at 3517 cm^{-1} in the

Table 5. The Chemical Shifts of Acetoxy, Isobutyroyloxy and Isovaleroyloxy Substituents on C-1 and C-9 in the ^{1}H-NMR Spectrum

		C-1	C-9
Acetoxy:	CH_3	δ 1.8—2.0	δ 2.0—2.1
Isobutyroyloxy:	CH	δ 2.1—2.5	δ 2.5—2.6
	CH_3	δ 1.0—1.1	δ 1.1—1.2
Isovaleroyloxy:	CH	δ 1.6—2.0	δ 2.0—2.1
	CH_2	δ 1.9—2.1	δ 2.1—2.2
	CH_3	δ 0.8—0.9	δ 0.9—1.0

Table 6. Cytotoxic Activities of **34**—**37** from *P. formosanus*

Cell line Compound	Cytotoxicity (IC_{50}, μM)				
	Hep G2	Hep G2,2,15	KB	CCM2	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}	0.664	36.4	42.56	2.90×10^{-2}

IR spectrum and a H-1 signal at δ 3.95 in the ^{1}H-NMR spectrum, compared with **20**, suggested the presence of a hydroxyl group on C-1. The other substituent on C-9 was an isobutyroyloxy group [δ 1.12, 1.14 (each 3H, d, J=6.8 Hz, 2×CH_3), 2.50 (1H, sept, J=6.8 Hz, CH)]. In addition, an IR hydroxyl band at 3500 cm^{-1} and the ^{1}H-NMR of H-9 at δ 4.53 in **24**, compared with **21**, suggested a hydroxyl group on C-9. The isobutyroyloxy group [δ 1.13 (6H, d, J=6.8 Hz, 2×CH_3), 2.47 (1H, sept, J=6.8 Hz, CH)] should be located on C-1.

Bakkenolide-V (**25**) exhibited the molecular formula $C_{20}H_{30}O_5$. 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 CH_2 unit, suggesting an isovaleroyloxy group on C-1 which also exhibited the ^{1}H-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 $C_{22}H_{30}O_7$. The ^{1}H-NMR spectrum showed the signal of an acetoxy group at δ 1.95. Two extra broad singlets at δ 5.78 and 6.00 for vinylidine 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 hydroxy functionality (IR 3651 cm^{-1}) constructed a 3-hydroxy-2-methylenebutyroyloxy [$OCOC(=CH_2)CH(OH)CH_3$] 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-methylenebutyroyloxy group should be attached on C-1. Hence, the acetoxy group would be on C-9.

Bakkenolide-X (**27**), $C_{17}H_{24}O_5$, exhibited two substituents, a hydroxyl at 3566 cm^{-1} from the IR spectrum and an acetoxy group at δ 2.01 from the ^{1}H-NMR spectrum. The signal of H-9 shifted upfield to δ 4.52 as that in **21**, **24** and **25**, indicating that the location of the hydroxyl group is on C-9. The acetoxy 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, $C_{19}H_{26}O_6S$. The ^{1}H-NMR spectrum showed two sets of the bakkenolide type signals bearing a *cis*-3-methylsufinylacryloyloxy 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 $C_{24}H_{34}O_6$. Two substituents were found from ^{1}H-NMR spectra: one is isobutyroyloxy group, the other is 3-methyl-2-butenoyloxy group. In **30**, the isobutyroyloxy group [δ 1.01, 1.03 (each 3H, d, J=6.4 Hz, 2×CH_3), 2.24 (1H, sept, J=6.4 Hz, CH)) should be attached on C-1 and 3-methyl-2-butenoyloxy group (δ 1.89, 2.16 (each 3H, s, 2×$=C(CH_3)_2$), 5.68 (1H, s, $CH=C$)] would be on C-9 by comparison of the chemical shifts of the isobutyroyloxy group with those in **12** and **24**. Using the same comparison with **10** and **23**, then, compound **31** possessed an isobutyroyloxy group [δ 1.14, 1.16 (each 3H, d, J=6.8 Hz, 2×CH_3), 2.47 (1H, sept, J=6.8 Hz, CH)] on C-9 and 3-methyl-2-butenoyloxy group [δ 1.82, 2.13 (each 3H, s, 2×$=C(CH_3)_2$), 5.43 (1H, s, $CH=C$)] on C-1.

Bakkenolide-III (**32**) was determined to have molecular formula $C_{15}H_{22}O_4$. The IR spectrum revealed a hydroxyl absorption at 3566 cm^{-1}, the ^{1}H-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 hydrolyzed product of bakkenolide-D (**34**) by Abe *et al.*,[4)] 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 acetoxy, isobutyroyloxy and isovaleroyloxy groups, in the ^{1}H-NMR spectra, it was found that these substituents on C-1 appeared to have more upfield ^{1}H-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-S3 micromelting 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 Report-100 spectrophotometer as KBr discs. The ^{1}H- and ^{13}C-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, Taiwan, in August 1992 and verified by Prof. C. S. Kuoh. 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 H_2O and $CHCl_3$, and then *n*-BuOH. The $CHCl_3$ extract (60 g) was subjected to chromatography on silica gel and eluted with C_6H_6–Me_2CO (25 : 1) to give nine fractions. The first fraction was further chromatographed with silica gel column (C_6H_{14} : EtOAc =19 : 1), preparative TLC and HPLC (C-18 column, MeOH : H_2O=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 chromatographed as before to obtain **34** (1.2 g), **1** (20 mg), **2** (5.0 mg), **3** (5.4 mg), **13** (0.8 mg) and **39** (0.6 g). 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 (C_6H_{14} : EtOAc=5 : 1) followed by HPLC (C-18 column, MeOH : H_2O=8 : 2) and HPLC (C-8 column, MeOH : H_2O=7 : 3) to yield **4** (4.2 mg), **5** (1.9 mg), **6** (29.0 mg), **7** (4.2 mg), **35** (536 mg), **36** (843 mg), **33** (53.6 mg), **20** (6.9 mg), **21** (3.7 mg), **22** (5.8 mg), **23** (5.6 mg), **24** (7.0 mg), **25** (4.2 mg), **32** (42.3 mg), **26** (5.5 mg), **27** (8.7 mg), **28** (1.0 mg), **29** (1.0 mg), **30** (1.3 mg), **31** (1.1 mg), **62** (1.1 g), **40** (3.2 g), **42** (12.1 mg), **43** (7.2 mg), **47** (21.4 mg) and **58** (0.7 mg). The ninth fraction was also chromatographed to give **37** (1.24 g), **44** (1.0 mg), **46** (0.8 mg), **48** (1.4 mg), **53** (5.6 mg) and **61** (45 mg). The *n*-BuOH layer (115 g) was subjected to chromatography on dianion HP-20 eluting with a gradient solvent of H_2O 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-LH-20 column and eluted with gradient solvent of H_2O and MeOH to yield **45** (9.7 mg), **50** (12.4 mg), **55** (44.8 mg), **56** (34.2 mg) and **59** (4.2 mg). The combined following four fractions were chromatographed repeatedly to afford **51** (14.2 mg), **52** (3.2 mg) and **57** (11.3 mg). The thirteenth to the sixteenth fractions were combined and rechromatographed on silica gel eluting with $CHCl_3$–MeOH (10 : 1) to give **49** (1.0 mg) and **54** (22.7 mg).

Bakkenolide-Db (**1**): Colorless oil. $[\alpha]_D$ $-72°$ ($c=0.20$, MeOH). HR-MS *m/z*: 424.1552 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 286 (3.09). IR ν_{max} cm^{-1}: 1770, 1747, 1716. MS (rel. int.) *m/z*: 424 (M^+, 25), 382 (62), 248 (22), 230 (18), 186 (32), 138 (31), 117 (100), 110 (25). ^{13}C-NMR ($CDCl_3$) δ: 15.4 (C-14), 19.6 (C-15), 21.1 (C-2″), 26.5 (C-2), 29.6 (C-3), 35.4 (C-4), 40.7 (C-4′), 43.2 (C-5), 46.2 (C-6), 51.0 (C-10), 54.7 (C-7), 70.6 (C-12), 72.8 (C-1), 80.6 (C-9), 108.5 (C-13), 123.6 (C-2′), 147.3 (C-11), 160.6 (C-3′), 163.4 (C-1′), 169.5 (C-1″), 177.7 (C-8). CD (2.4×10^{-4} M, MeOH) $\Delta\varepsilon_{212}$ -20.80, $\Delta\varepsilon_{240}$ -2.95, $\Delta\varepsilon_{269}$ 0, $\Delta\varepsilon_{291}$ 1.39. ORD (2.4×10^{-4} M, MeOH): $[\phi]_{204}$ -7356, $[\phi]_{218}$ -27580, $[\phi]_{247}$ -13490, $[\phi]_{269}$ -12980, $[\phi]$322 0.

Bakkenolide-Dc (**2**): Colorless oil. $[\alpha]_D$ $-150°$ ($c=0.04$, MeOH). HR-MS *m/z*: 424.1555 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 280 (3.82). IR ν_{max} cm^{-1}: 1770, 1737, 1717. MS (rel. int.) *m/z*: 424 (M^+, 26), 382 (52), 248 (20), 230 (21), 186 (30), 138 (21), 117 (100), 110 (22). CD (2.0×10^{-4} M, MeOH): $\Delta\varepsilon_{202}$ 0, $\Delta\varepsilon_{209}$ -18.02, $\Delta\varepsilon_{246}$ 0, $\Delta\varepsilon_{254}$ 0.35, $\Delta\varepsilon_{264}$ 0, $\Delta\varepsilon_{288}$ -0.74. ORD (2.0×10^{-4} M, MeOH): $[\phi]_{230}$ -19980, $[\phi]_{268}$ -498, $[\phi]_{285}$ 0, $[\phi]_{287}$ 332, $[\phi]_{299}$ -1264, $[\phi]_{324}$ 0.

Bakkenolide-Dd (**3**): Colorless oil. $[\alpha]_D$ $-103°$ ($c=0.031$, MeOH). HR-MS *m/z*: 408.1602 $[M]^+$ (Calcd for $C_{21}H_{28}O_6S$: 408.1606). UV λ_{max} nm (log ε): 290 (3.68). IR ν_{max} cm^{-1}: 1770, 1733, 1716. MS (rel. int.) *m/z*: 408 (M^+, 6), 248 (5), 230 (13), 186 (7), 110 (5), 101 (100). CD (7.6×10^{-5} M, MeOH): $\Delta\varepsilon_{206}$ -17.81, $\Delta\varepsilon_{250}$ -0.16, $\Delta\varepsilon_{293}$ -2.39, $\Delta\varepsilon_{323}$ 0. ORD (7.6×10^{-5} M, MeOH): $[\phi]_{204}$ 46890, $[\phi]_{213}$ 9625, $[\phi]_{216}$ 10394, $[\phi]_{222}$ 0, $[\phi]_{225}$ -2997, $[\phi]_{235}$ 0, $[\phi]_{268}$ 13120, $[\phi]_{313}$ 3131.

Bakkenolide-De (**4**): Colorless oil. $[\alpha]_D$ $-116°$ ($c=0.054$, MeOH). HR-MS *m/z*: 424.1553 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 268 (3.68). IR ν_{max} cm^{-1}: 1770, 1733, 1716. MS (rel. int.) *m/z*: 424 (M^+, 17), 382 (91), 248 (10), 230 (19), 186 (41), 138 (21), 117 (100), 110 (20). ^{13}C-NMR ($CDCl_3$) δ: 15.5 (C-14), 19.7 (C-15), 21.3 (C-2′), 26.6 (C-2), 29.5 (C-3), 35.7 (C-4), 39.6 (C-4″), 43.4 (C-5), 46.0 (C-6), 51.7 (C-10), 54.6 (C-7), 70.6 (C-12), 70.7 (C-1), 81.5 (C-9), 108.7 (C-13), 125.1 (C-2″), 147.3 (C-11), 152.4 (C-3″), 162.0 (C-1″), 169.8 (C-1′), 177.0 (C-8). CD (1.3×10^{-4} M, MeOH): $\Delta\varepsilon_{208}$ -13.86, $\Delta\varepsilon_{249}$ 0, $\Delta\varepsilon_{253}$ 0.14, $\Delta\varepsilon_{259}$ 0, $\Delta\varepsilon_{281}$ -0.87, $\Delta\varepsilon_{314}$ 0. ORD (1.3×10^{-4} M, MeOH): $[\phi]_{204}$ -18590, $[\phi]_{210}$ 0, $[\phi]_{217}$ -7979, $[\phi]_{232}$ -20340, $[\phi]_{253}$ -7865, $[\phi]_{269}$ -4014.

Bakkenolide-Df (**5**): Colorless oil. $[\alpha]_D$ $-74°$ ($c=0.019$, MeOH). HR-MS *m/z*: 424.1555 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 266 (3.26). IR ν_{max} cm^{-1}: 1770, 1737, 1716. MS (rel. int.) *m/z*: 424 (M^+, 32), 382 (77), 248 (24), 230 (26), 186 (30), 138 (60), 117 (100), 110 (30). CD (1.8×10^{-4} M, MeOH): $\Delta\varepsilon_{205}$ -9.70, $\Delta\varepsilon_{250}$ -0.57, $\Delta\varepsilon_{274}$ -1.00, $\Delta\varepsilon_{309}$ 0. ORD (1.8×10^{-4} M, MeOH): $[\phi]_{205}$ 43333, $[\phi]_{227}$ -8067, $[\phi]_{254}$ -1090, $[\phi]_{269}$ -131, $[\phi]_{287}$ -1851, $[\phi]_{333}$ 0.

Bakkenolide-Dg (**6**): Colorless oil. $[\alpha]_D$ $-65°$ ($c=0.29$, MeOH). HR-MS *m/z*: 424.1555 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 286 (3.12). IR ν_{max} cm^{-1}: 1770, 1747. MS (rel. int.) *m/z*: 424 (M^+, 8), 382 (2), 248 (9), 230 (11), 186 (12), 138 (5), 117 (100), 110 (5). ^{13}C-NMR ($CDCl_3$) δ: 15.4 (C-14), 19.6 (C-15), 21.2 (C-2″), 26.5 (C-2), 29.4 (C-3), 35.6 (C-4), 40.5 (C-4″), 43.2 (C-5), 46.2 (C-6), 51.5 (C-10), 54.4 (C-7), 70.5 (C-1), 70.7 (C-12), 82.2 (C-9), 108.1 (C-13), 123.4 (C-2″), 147.0 (C-11), 160.3 (C-3″), 163.0 (C-1″), 169.5 (C-1′), 177.5 (C-8). CD (1.4×10^{-4} M, MeOH): $\Delta\varepsilon_{210}$ -23.54, $\Delta\varepsilon_{239}$ -0.43, $\Delta\varepsilon_{272}$ 0, $\Delta\varepsilon_{296}$ 1.27, $\Delta\varepsilon_{370}$ 0. ORD (1.4×10^{-4} M, MeOH): $[\phi]_{204}$ 36250, $[\phi]_{211}$ 0, $[\phi]_{221}$ -20110, $[\phi]_{228}$ -15690, $[\phi]_{243}$ -11940, $[\phi]_{262}$ -14310.

Bakkenolide-Dh (**7**): Colorless oil. $[\alpha]_D$ $-31°$ ($c=0.042$, MeOH). HR-MS *m/z*: 424.1553 $[M]^+$ (Calcd for $C_{21}H_{28}O_7S$: 424.1556). UV λ_{max} nm (log ε): 287 (3.28). IR ν_{max} cm^{-1}: 1770, 1732, 1716. MS (rel. int.) *m/z*: 424 (M^+, 9), 382 (8), 248 (10), 230 (20), 186 (25), 138 (14), 117 (100), 110 (11). ^{13}C-NMR ($CDCl_3$) δ: 15.5 (C-14), 19.5 (C-15), 21.2 (C-2″), 26.7 (C-2), 29.6 (C-3), 35.4 (C-4), 40.6 (C-4″), 43.3 (C-5), 46.7 (C-6), 51.5 (C-10), 54.5 (C-7), 70.5 (C-1), 70.6 (C-12), 82.0 (C-9), 107.9 (C-13), 123.7 (C-2″), 147.1 (C-11), 160.3 (C-3″), 163.8 (C-1″), 169.8 (C-1′), 177.2 (C-8). CD (5.2×10^{-5} M, MeOH): $\Delta\varepsilon_{208}$ -66.64, $\Delta\varepsilon_{245}$ 0, $\Delta\varepsilon_{255}$ 2.50, $\Delta\varepsilon_{268}$ 0, $\Delta\varepsilon_{297}$ -3.13. ORD (5.2×10^{-5} M, MeOH): $[\phi]_{204}$ 0, $[\phi]_{232}$ -101600, $[\phi]_{268}$ -21140, $[\phi]_{278}$ -21220.

Bakkenolide-Fa (**8**): Colorless oil. $[\alpha]_D$ $-122°$ ($c=0.069$, MeOH). HR-MS *m/z*: 432.2512 $[M]^+$ (Calcd for $C_{25}H_{36}O_6$: 432.2511). IR ν_{max} cm^{-1}: 1770, 1745, 1712. MS (rel. int.) *m/z*: 432 (M^+, 14), 348 (21), 333 (48), 248 (73), 230 (67), 186 (45), 138 (31), 110 (29), 83 (100). ^{13}C-NMR ($CDCl_3$) δ: 15.2, (C-4′), 15.6 (C-14), 19.6 (C-15), 20.4 (C-5′), 22.3, 22.6 (C-4″, -5″), 25.0 (C-3″), 26.9 (C-2), 29.6 (C-3), 35.3 (C-4), 43.1 (C-2″), 43.4 (C-5), 46.5 (C-6), 51.7 (C-10), 54.9 (C-7), 70.5 (C-12), 70.6 (C-1), 82.6 (C-9), 108.3 (C-13), 128.6 (C-2′), 136.0 (C-3′), 148.1 (C-11), 167.5 (C-1′), 172.0 (C-1″), 177.0 (C-8). CD (2.2×10^{-4} M, MeOH): $\Delta\varepsilon_{208}$ -24.38, $\Delta\varepsilon_{238}$ 0, $\Delta\varepsilon_{248}$ 1.55, $\Delta\varepsilon_{278}$ 0. ORD (2.2×10^{-4} M, MeOH): $[\phi]_{205}$ 0, $[\phi]_{226}$ -38110, $[\phi]_{279}$ -5148.

Bakkenolide-Fb (**9**): Colorless oil. $[\alpha]_D$ $-123°$ ($c=0.13$, MeOH). HR-MS *m/z*: 432.2509 $[M]^+$ (Calcd for $C_{25}H_{36}O_6$: 432.2511). IR ν_{max} cm^{-1}: 1791, 1733, 1716. MS (rel. int.) *m/z*: 432 (M^+, 9), 331 (13), 248 (7), 230 (37), 186 (12), 138 (6), 110 (6), 83 (100). ^{13}C-NMR ($CDCl_3$) δ: 15.6 (C-14), 16.0, (C-4″), 19.7 (C-15), 20.4 (C-5″), 22.2 (C-4′, -5′), 24.5 (C-3′), 26.8 (C-2), 29.5 (C-3), 35.5 (C-4), 43.2 (C-2′), 43.3 (C-5), 46.8 (C-6), 52.0 (C-10), 55.0 (C-7), 70.5 (C-12), 70.6 (C-1), 82.6 (C-9), 108.1 (C-13), 127.1 (C-2″), 140.9 (C-3″), 147.9 (C-11), 166.0 (C-1″), 172.2 (C-1′), 178.1 (C-8). CD (3.0×10^{-4} M, MeOH): $\Delta\varepsilon_{212}$ -16.88, $\Delta\varepsilon_{246}$ 0, $\Delta\varepsilon_{255}$ 0.30, $\Delta\varepsilon_{304}$ 0. ORD (3.0×10^{-4} M, MeOH): $[\phi]_{202}$ 0, $[\phi]_{203}$ 646, $[\phi]_{205}$ 0, $[\phi]_{231}$ -28800, $[\phi]_{265}$ -6595, $[\phi]_{390}$ 0.

Bakkenolide-I (**10**): Colorless oil. $[\alpha]_D$ $-32°$ ($c=0.079$, MeOH). HR-MS *m/z*: 320.1985 $[M]^+$ (Calcd for $C_{19}H_{28}O_4$: 320.1987). IR ν_{max} cm^{-1}: 1780, 1737. MS (rel. int.) *m/z*: 320 (M^+, 27), 250 (29), 232 (33), 188 (66), 140 (20), 122 (100). CD (3.0×10^{-4} M, MeOH): $\Delta\varepsilon_{208}$ -13.58, $\Delta\varepsilon_{246}$ 0, $\Delta\varepsilon_{253}$ 0.25, $\Delta\varepsilon_{272}$ 0. ORD (3.0×10^{-4} M, MeOH): $[\phi]_{203}$ 7990, $[\phi]_{213}$ 0, $[\phi]_{227}$ -13880, $[\phi]_{274}$ 01763.

Bakkenolide-J (**11**): Colorless oil. $[\alpha]_D$ $-140°$ ($c=0.013$, MeOH). HR-MS *m/z*: 334.2143 $[M]^+$ (Calcd for $C_{20}H_{30}O_4$: 334.2144). IR ν_{max} cm^{-1}: 1789, 1737. MS (rel. int.) *m/z*: 334 (M^+, 20), 250 (30), 232 (23), 188 (20), 140 (30), 122 (100). ^{13}C-NMR ($CDCl_3$) δ: 16.3 (C-14), 19.2 (C-15), 20.9 (C-1), 21.2 (C-2), 22.3 (C-4″, -5″), 25.5 (C-3″), 30.7 (C-3), 36.3 (C-4), 43.4 (C-2″), 46.2 (C-5), 46.2 (C-6), 49.7 (C-10), 54.0 (C-7), 70.5 (C-12), 82.8 (C-9), 107.7 (C-13), 148.4 (C-11), 169.0 (C-1″), 173.0 (C-8). CD (1.9×10^{-4} M, MeOH): $\Delta\varepsilon_{205}$ 0, $\Delta\varepsilon_{212}$ -32.58, $\Delta\varepsilon_{261}$ 0. ORD (1.9×10^{-4} M, MeOH): $[\phi]_{206}$ -512, $[\phi]_{228}$ -6059, $[\phi]_{287}$ 0.

Bakkenolide-K (**12**): Colorless needles (MeOH), mp 139—141 °C. $[\alpha]_D$ $-148°$ ($c=0.010$, MeOH). HR-MS *m/z*: 418.2354 $[M]^+$ (Calcd for $C_{24}H_{34}O_6$: 418.2355). IR ν_{max} cm^{-1}: 1770, 1732, 1716. MS (rel. int.) *m/z*: 418 (M^+, 8), 348 (9), 248 (20), 230 (34), 186 (50), 138 (15), 110 (100). ^{13}C-NMR ($CDCl_3$) δ: 15.5 (C-14), 15.9 (C-4″), 18.5, 19.2 (C-3′,-4′), 19.6 (C-15), 20.3 (C-5″), 26.5 (C-2), 29.5 (C-3), 33.8 (C-2′), 35.4 (C-4), 43.3 (C-5), 45.7 (C-6), 52.0 (C-10), 55.0 (C-7), 70.5 (C-12), 70.6 (C-1), 80.6 (C-9), 108.1 (C-13), 127.0 (C-2″), 141.0 (C-3″), 147.8 (C-11), 166.0 (C-1″), 176.4 (C-1′), 178.0 (C-8). CD (2.5×10^{-4} M, MeOH): $\Delta\varepsilon_{213}$ -14.23, $\Delta\varepsilon_{246}$ 0, $\Delta\varepsilon_{256}$ 0.14. ORD (2.5×10^{-4} M, MeOH): $[\phi]_{213}$ 0, $[\phi]_{230}$ -25140, $[\phi]_{256}$ -7098.

Bakkenolide-L (**13**): Colorless oil. HR-MS *m/z*: 350.1277 $[M]^+$ (Calcd for $C_{19}H_{26}O_6$: 350.1279). IR ν_{max} cm^{-1}: 1772, 1733, 1716. MS (rel. int.) *m/z*: 350 (M^+, 4), 382 (8), 308 (58), 248 (79), 230 (45), 186 (58), 138 (66), 110 (58), 109 (100).

Bakkenolide-M (**14**): Colorless oil. $[\alpha]_D$ $-81°$ ($c=0.033$, MeOH). HR-MS *m/z*: 420.2511 $[M]^+$ (Calcd for $C_{24}H_{36}O_6$: 420.2512). IR ν_{max} cm^{-1}: 1789, 1737, 1732. MS (rel. int.) *m/z*: 420 (M^+, 9), 350 (19), 248 (100), 230 (92), 186 (84), 138 (51), 110 (43). ^{13}C-NMR ($CDCl_3$) δ: 11.6 (C-4′), 15.5 (C-14), 16.8 (C-5′),18.2 and 19.1 (C-3″, -4″), 19.7 (C-15), 26.3 (C-3′), 26.5 (C-2), 29.6 (C-3), 33.9 (C-2″), 35.2 (C-4), 41.1 (C-2′), 43.5 (C-5), 46.3 (C-6), 52.3 (C-10), 54.9 (C-7), 70.5 (C-12), 70.6 (C-1), 80.6 (C-9), 108.3 (C-13), 148.0 (C-11), 175.8 (C-1″), 176.1 (C-1′), 178.0 (C-8). CD (2.6×10^{-4} M, MeOH): $\Delta\varepsilon_{206}$ -16.26, $\Delta\varepsilon_{264}$ 0. ORD (2.6×10^{-4} M, MeOH): $[\phi]_{201}$ 1908, $[\phi]_{204}$ 0, $[\phi]_{227}$ -27840, $[\phi]_{305}$ -9417.

Bakkenolide-Na (**15**): Colorless oil. $[\alpha]_D$ $-60°$ ($c=0.010$, MeOH). HR-

MS *m/z*: 420.2511 $[M]^+$ (Calcd for $C_{24}H_{36}O_6$: 420.2512). IR ν_{max} cm^{-1}: 1791, 1733, 1716. MS (rel. int.) *m/z*: 420 (M^+, 9), 350 (20), 336 (6), 248 (83), 230 (84), 186 (74), 138 (50), 110 (45), 85 (54). CD (2.4×10^{-4} M, MeOH):$\Delta\varepsilon_{206}$ -14.45, $\Delta\varepsilon_{264}$ 0. ORD (2.4×10^{-4} M, MeOH): $[\phi]_{202}$ 0, $[\phi]_{227}$ -21940, $[\phi]_{281}$$-7743$.

Bakkenolide-Nb (**16**): Colorless oil. $[\alpha]_D$ $-81°$ (c=0.033, MeOH). HR-MS *m/z*: 420.2510 $[M]^+$ (Calcd for $C_{24}H_{36}O_6$: 420.2512). IR ν_{max} cm^{-1}: 1789, 1737, 1732. MS (rel. int.) *m/z*: 420 (M^+, 7), 336 (45), 248 (100), 230 (74), 186 (67), 138 (47), 110 (41), 85 (92). CD (2.3×10^{-4} M, MeOH): $\Delta\varepsilon_{206}$ -20.09, $\Delta\varepsilon_{302}$ 0. ORD (2.3×10^{-4} M, MeOH): $[\phi]_{205}$ -39770, $[\phi]_{218}$ -49350, $[\phi]_{267}$ -28350.

Bakkenolide-O (**17**): Colorless oil. $[\alpha]_D$ $-78°$ (c=0.017, MeOH). HR-MS *m/z*: 434.2665 $[M]^+$ (Calcd for $C_{25}H_{38}O_6$: 434.2668). IR ν_{max} cm^{-1}: 1784, 1737, 1732. MS (rel. int.) *m/z*: 434 (M^+, 7), 350 (27), 333 (12), 248 (66), 230 (50), 186 (47), 138 (30), 110 (27), 85 (73). CD (2.0×10^{-4} M, MeOH): $\Delta\varepsilon_{206}$ -11.96, $\Delta\varepsilon_{266}$ 0. ORD (2.0×10^{-4} M, MeOH): $[\phi]_{204}$ -3332, $[\phi]_{222}$ -12610, $[\phi]_{226}$ -12360, $[\phi]_{230}$ 12660, $[\phi]_{250}$ -5808, $[\phi]_{264}$ -3463.

Bakkenolide-P (**18**): Colorless oil. $[\alpha]_D$ $-78°$ (c=0.017, MeOH). HR-MS *m/z*: 432.2517 $[M]^+$ (Calcd for $C_{25}H_{36}O_6$: 432.2512). IR ν_{max} cm^{-1}: 1772, 1732, 1716. MS (rel. int.) *m/z*: 432 (M^+, 20), 330 (8), 248 (7), 230 (14), 186 (10), 110 (4), 83 (100). CD (2.0×10^{-4} M, MeOH): $\Delta\varepsilon_{206}$ -0.27, $\Delta\varepsilon_{214}$ -0.67, $\Delta\varepsilon_{219}$ -0.57. ORD (2.0×10^{-4} M, MeOH): $[\phi]_{202}$ 0, $[\phi]_{206}$ 4093, $[\phi]_{216}$ 2245, $[\phi]_{226}$ 2751, $[\phi]_{236}$ 1019, $[\phi]_{246}$ 2328, $[\phi]_{252}$ 1666, $[\phi]_{264}$ 2339, $[\phi]_{269}$ 1818,$[\phi]_{279}$ 2606, $[\phi]_{285}$ 2140, $[\phi]_{292}$ 2859,$[\phi]_{314}$ 2366, $[\phi]_{323}$ 3213.

Bakkenolide-Q (**19**): Colorless oil. $[\alpha]_D$ $-44°$ (c=0.010, MeOH). HR-MS *m/z*: 434.2669 $[M]^+$ (Calcd for $C_{25}H_{38}O_6$: 434.2668). IR ν_{max} cm^{-1}: 1772, 1733, 1716. MS (rel. int.) *m/z*: 434 (M^+, 27), 350 (22), 248 (36), 230 (52), 186 (32), 138 (27), 110 (25), 85 (82), 57 (100). CD (2.3×10^{-4} M, MeOH): $\Delta\varepsilon_{205}$ -0.39, $\Delta\varepsilon_{249}$ 0. ORD (2.3×10^{-4} M, MeOH): $[\phi]_{223}$ 1293, $[\phi]_{269}$ 12580.

Bakkenolide-R (**20**): Colorless oil. $[\alpha]_D$ $-87°$ (c=0.069, MeOH). HR-MS m/z: 348.1936 $[M]^+$ (Calcd for $C_{20}H_{28}O_5$: 348.1937). UV λ_{max} nm (log ε): 276 (2.64). IR ν_{max} cm^{-1}: 3566, 1770, 1732. MS (rel. int.) *m/z*: 348 (M^+, 10), 249 (13), 248 (30), 230 (10), 186 (25), 138 (34), 110 (38), 83 (100). CD (3.0×10^{-4} M, MeOH): $\Delta\varepsilon_{202}$ 0, $\Delta\varepsilon_{210}$ -16.00, $\Delta\varepsilon_{243}$ 0, $\Delta\varepsilon_{252}$ 0.47. ORD (3.0×10^{-4} M, MeOH): $[\phi]_{204}$ 26720, $[\phi]_{217}$ -191, $[\phi]_{231}$ -10910, $[\phi]_{246}$ 0.

Bakkenolide-S (**21**): Colorless oil. $[\alpha]_D$ $-87°$ (c=0.037, MeOH). HR-MS *m/z*: 250.1572 $[M]^+$ (Calcd for $C_{15}H_{22}O_3$: 250.1569). IR ν_{max} cm^{-1}: 3566, 1770. MS (rel. int.) *m/z*: 250 (M^+, 18), 233 (14), 188 (24), 140 (30), 112 (100). CD (3.0×10^{-4} M, MeOH): $\Delta\varepsilon_{203}$ 0, $\Delta\varepsilon_{211}$ -13.92, $\Delta\varepsilon_{238}$ 0, $\Delta\varepsilon_{249}$ 0.27. ORD (3.0×10^{-4} M, MeOH): $[\phi]_{206}$ 15440, $[\phi]_{217}$ 0, $[\phi]_{227}$ -4733, $[\phi]_{238}$ 0, $[\phi]_{286}$ 9967.

Bakkenolide-T (**22**): Colorless oil. $[\alpha]_D$ $-62°$ (c=0.058, MeOH). HR-MS *m/z*: 466.2021 $[M]^+$ (Calcd for $C_{24}H_{34}O_7S$: 466.2025). IR ν_{max} cm^{-1}: 1770, 1736, 1716. MS (rel. int.) *m/z*: 466 (M^+, 18), 382 (70), 248 (25), 230 (12), 186 (30), 138 (20), 135 (100), 110 (16). CD (1.3×10^{-4} M, MeOH): $\Delta\varepsilon_{207}$ -21.01, $\Delta\varepsilon_{233}$ -3.10, $\Delta\varepsilon_{269}$ 0, $\Delta\varepsilon_{291}$ 1.25. ORD (1.3×10^{-4} M, MeOH): $[\phi]_{202}$ 0, $[\phi]_{205}$ 15230, $[\phi]_{211}$ 0, $[\phi]_{222}$ -11050, $[\phi]_{238}$ -3367, $[\phi]_{249}$ -1788, $[\phi]_{257}$ -3631, $[\phi]_{281}$ 0.

Bakkenolide-Ua (**23**): Colorless oil. $[\alpha]_D$ $-84°$ (c=0.056, MeOH). HR-MS *m/z*: 336.1935 $[M]^+$ (Calcd for $C_{19}H_{28}O_5$: 336.1937). IR ν_{max} cm^{-1}: 3517, 1778, 1737. MS (rel. int.) *m/z*: 336 (M^+, 15), 249 (62), 248 (16), 230 (40), 186 (59), 138 (68), 110 (55), 109 (100). CD (2.5×10^{-4} M, MeOH): $\Delta\varepsilon_{200}$ 0, $\Delta\varepsilon_{207}$ -18.22, $\Delta\varepsilon_{251}$ 0. ORD (2.5×10^{-4} M, MeOH): $[\phi]_{204}$ 11010, $[\phi]_{210}$ 0, $[\phi]_{228}$ -21360, $[\phi]_{265}$ -6273.

Bakkenolide-Ub (**24**): Colorless oil. $[\alpha]_D$ $-84°$ (c=0.056, MeOH). HR-MS *m/z*: 336.1936 $[M]^+$ (Calcd for $C_{19}H_{28}O_5$: 336.1937). IR ν_{max} cm^{-1}: 3500, 1778, 1732. MS (rel. int.) *m/z*: 336 (M^+, 15), 249 (56), 248 (26), 230 (12), 186 (43), 138 (65), 110 (55), 109 (100). CD (3.1×10^{-4} M, MeOH): $\Delta\varepsilon_{207}$ -20.07, $\Delta\varepsilon_{213}$ -19.56, $\Delta\varepsilon_{257}$ 0. ORD (3.1×10^{-4} M, MeOH): $[\phi]_{211}$ 0, $[\phi]_{225}$ -33450, $[\phi]_{298}$ -4460.

Bakkenolide-V (**25**): Colorless oil. $[\alpha]_D$ $-76°$ (c=0.042, MeOH). HR-MS *m/z*: 350.2091 $[M]^+$ (Calcd for $C_{20}H_{30}O_5$: 350.2093). IR ν_{max} cm^{-1}: 3566, 1770, 1737. MS (rel. int.) *m/z*: 350 (M^+, 14), 248 (36), 230 (13), 186 (40), 138 (62), 110 (58), 109 (100). CD (2.4×10^{-4} M, MeOH): $\Delta\varepsilon_{208}$ -14.90 $\Delta\varepsilon_{266}$ 0. ORD (2.4×10^{-4} M, MeOH): $[\phi]_{210}$ 0, $[\phi]_{227}$ -20140.

Bakkenolide-W (**26**): Colorless oil. $[\alpha]_D$ $-62°$ (c=0.055, MeOH). HR-MS *m/z*: 406.1990 $[M]^+$ (Calcd for $C_{22}H_{30}O_7$: 406.1992). IR ν_{max} cm^{-1}: 3651, 1770, 1743, 1714. MS (rel. int.) *m/z*: 406 (M^+, 20), 346 (20), 291 (30), 248 (56), 230 (13), 186 (53), 138 (66), 110 (61), 109 (100). CD (2.7×10^{-4} M, MeOH): $\Delta\varepsilon_{203}$ 0, $\Delta\varepsilon_{211}$ -12.39, $\Delta\varepsilon_{243}$ 0, $\Delta\varepsilon_{253}$ 0.56, $\Delta\varepsilon_{297}$ 0. ORD (2.7×10^{-4} M, MeOH): $[\phi]_{206}$ 0, $[\phi]_{207}$ 796, $[\phi]_{208}$ 0, $[\phi]_{222}$ -16570, $[\phi]_{231}$ -17330, $[\phi]_{259}$ -4319.

Bakkenolide-X (**27**): Colorless oil. $[\alpha]_D$ $-69°$ (c=0.087, MeOH). HR-MS *m/z*: 308.1622 $[M]^+$ (Calcd for $C_{17}H_{24}O_5$: 308.1624). IR ν_{max} cm^{-1}: 3566, 1770, 1716. MS (rel. int.) *m/z*: 308 (M^+, 14), 266 (5), 248 (27), 230 (8), 186 (37), 138 (60), 110 (54), 109 (100). CD (2.8×10^{-4} M, MeOH): $\Delta\varepsilon_{209}$ -12.47, $\Delta\varepsilon_{245}$ 0. ORD (2.8×10^{-4} M, MeOH): $[\phi]_{205}$ 6670, $[\phi]_{211}$ 0, $[\phi]_{225}$ -13650, $[\phi]_{287}$ 0.

Bakkenolide-Ya (**28**) and Bakkenolide-Yb (**29**): Colorless oil. $[\alpha]_D$ $-62°$ (c=0.055, MeOH). UV λ_{max} nm: 282. IR ν_{max} cm^{-1}: 3465, 1766, 1708. MS (rel. int.) *m/z*: 382 (M^+ for $C_{19}H_{26}O_6S$, 14), 248 (10), 230 (8), 186 (20), 138 (60), 137 (100), 110 (30).

Bakkenolide-Za (**30**): Colorless oil. $[\alpha]_D$ $-124°$ (c=0.013, MeOH). HR-MS *m/z*: 418.2351 $[M]^+$ (Calcd for $C_{24}H_{34}O_6$: 418.2355). IR ν_{max} cm^{-1}: 1780, 1732, 1716. MS (rel. int.) *m/z*: 418 (M^+, 3), 330 (5), 248 (10), 230 (16), 186 (10), 138 (5), 110 (4), 83 (100). CD (3.2×10^{-4} M, MeOH): $\Delta\varepsilon_{202}$ 0, $\Delta\varepsilon_{216}$ -15.04, $\Delta\varepsilon_{247}$ 0, $\Delta\varepsilon_{256}$ 0.37. ORD (3.2×10^{-4} M, MeOH): $[\phi]_{205}$ 0, $[\phi]_{209}$ 7675, $[\phi]_{212}$ 0, $[\phi]_{220}$ -19720, $[\phi]_{228}$ -28710, $[\phi]_{232}$ -27880.

Bakkenolide-Zb (**31**): Colorless oil. $[\alpha]_D$ $-145°$ (c=0.010, MeOH). HR-MS *m/z*: 418.2354 $[M]^+$ (Calcd for $C_{24}H_{34}O_6$: 418.2355). IR ν_{max} cm^{-1}: 1780, 1736, 1716. MS (rel. int.) *m/z*: 418 (M^+, 13), 348 (7), 248 (29), 230 (34), 186 (29), 138 (17), 110 (13), 83 (100). CD (2.7×10^{-4} M, MeOH): $\Delta\varepsilon_{201}$ 0, $\Delta\varepsilon_{216}$ -19.31, $\Delta\varepsilon_{247}$ 0, $\Delta\varepsilon_{254}$ 0.37, $\Delta\varepsilon_{282}$ 0. ORD (2.7×10^{-4} M, MeOH): $[\phi]_{205}$ 0, $[\phi]_{207}$ 2362, $[\phi]_{209}$ 0, $[\phi]_{227}$ -37730, $[\phi]_{257}$ -13200.

Bakkenolide-III (**32**): Colorless oil. $[\alpha]_D$ $-33°$ (c=0.018, MeOH). HR-MS *m/z*: 266.1516 $[M]^+$ (Calcd for $C_{15}H_{22}O_4$: 266.1518). IR ν_{max} cm^{-1}: 3566, 1770. MS (rel. int.) *m/z*: 266 (M^+, 17), 248 (26), 230 (13), 186 (36), 138 (52), 110 (55), 109 (100). CD (3.2×10^{-4} M, MeOH): $\Delta\varepsilon_{206}$ -14.13, $\Delta\varepsilon_{262}$ 0. ORD (3.2×10^{-4} M, MeOH): $[\phi]_{212}$ 0, $[\phi]_{230}$ -11030, $[\phi]_{250}$ -3622, $[\phi]_{291}$ 0.

Cytotoxicity Assay The *in vitro* KB cytotoxicity assay was carried out according to the procedures by Geran *et al.*[30] and Ferguson *et al.*[31] The assay against P-388, CCM2, Hep G2 and Hep G2,2,15 tumor cells was based on a method reported by Lee *et al.*[32] and Cheng and his colleagues.[33]

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