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Pinguisane and dimeric pinguisane-type sesquiterpenoids from the Japanese liverwort *Porella acutifolia* subsp. *tosana*

Toshihiro Hashimoto, Hiroshi Irita, Masami Tanaka, Shigeru Takaoka, Yoshinori Asakawa*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

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Abstract

Two new pinguisane-type, three new Diels–Alder reaction-type dimeric pinguisane sesquiterpenoids and known sesqui and diterpenoids were isolated from the ether extract of the Japanese liverwort *Porella acutifolia* subsp. *tosana*. Their absolute stereostructures were established by a combination of extensive 2D-NMR, CD spectra, X-ray crystallographic analysis, modified Mosher's method and chemical correlation.

Keywords: *Porella acutifolia* subsp. *tosana*; Jungermanniales; Hepaticae; Acutifolones A and B; Bisacutifolones A–C; Pinguisane- and dimeric pinguisane-type sesquiterpenes; Diels–Alder reaction type; Biogenesis; Chemosystematics

1. Introduction

Liverworts contain oil bodies, which consist of lipophilic terpenoids and aromatic compounds (Asakawa, 1982a, 1995). *Porella* species belonging to the Jungermanniaceae are distributed in both northern and southern hemispheres. From Japan, 18 *Porella* species are described (Furuki & Mizutani, 1994). *Porella* species are known to produce terpenoids with interesting biological activities, for example, fish killing, antimicrobial, ornithine decarboxylase and cathepsin B inhibitory activity (Asakawa, 1990, 1995, 1998). The genus *Porella* comprises two groups: (a) species with pungent taste [(−)-polygodial] and (b) species without a pungent taste. *Porella acutifolia* subsp. *tosana* belongs to the latter group. Previously, we reported the isolation and the structure elucidation of germacrane- and guaiane-type sesquiterpene lactones and sacculatane-type diterpene dialdehyde from this liverwort (Toyota, Ueda & Asakawa, 1991). Further fractionation of the ether extract from a large amount of *P. acutifolia* subsp. tosana resulted in the isolation of two new pinguisane-type sesquiterpenoids, acutifolones A (**6**) and B (**7**), and three dimeric pinguisane-type sesquiterpenoids, bisacutifolones A (**13**), B (**18**) and C (**22**), along with three known terpenoids (**5-8**) and their structures were reported in preliminary publications (Hashimoto, Irita, Tanaka, Takaoka & Asakawa, 1998a; Hashimoto, Irita, Tanaka, Takaoka & Asakawa, 1998b). In this paper we report the absolute stereostructures of the newly isolated compounds, their biogenesis and describe some chemosystematic relationships among the *Porella* species. In addition, the structure of bisacutifolone B (**18**), previously reported has been revised.

2. Result and discussion

Air-dried and ground *P. acutifolia* subsp. tosana collected in Kochi, Japan was extracted with ether. The crude extract was checked by TLC and GC–MS to confirm the presence of β-elemene (**1**), valencene (**2**), bicyclgermacrene (**3**) and perrottetianal (**8**). The remaining extract was subjected to column chromatography on Silica gel and Sephadex LH-20 to afford two

* Corresponding author. Tel.: +81-88-622-9611; fax: +81-88-655-3051.

E-mail address: asakawa@ph.bunri-u.ac.jp (Y. Asakawa).

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new pinguisane-type sesquiterpenes named acutifolone A (**6**) and B (**7**) and three novel pinguisane-type sesquiterpene dimers named bisacutifolone A (**13**), B (**18**) and (**22**), together with six known terpenoids (**4**, **5**, **8**).

2.1. 7-Oxopinguisenol-12-methyl ester (5)

Compound (**5**) is the key pinguisane-type sesquiterpene keto ester for the new compounds, which was previously isolated from the title liverwort (Toyota et al., 1991). However, their absolute stereochemistry remained to be clarified. In order to establish its absolute configuration, CD spectrum was measured. It showed a positive Cotton effect at 287 nm, indicating that C-5 of **5** might have *S* configuration. This assumption was further confirmed by application of the modified Mosher's method for α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester (Ohtani, Kusumi, Kashman & Kakisawa, 1991) to the diol (**9**) obtained from **5**. Reduction of **5** with $NaBH_4$ gave two alcohols (**9**) and (**10**). The NOESY spectrum of **9** indicated the NOEs between (1) H-7 and H-14 and (2) H-7 and C_5-OH, whilst compound **10** showed the NOE between H-7 and H-1. On the basis of the NOEs, compound **9** possessed a 7α-hydroxyl group and **10** a 7β-hydroxyl group. Since the ^{1}H-NMR of **9** showed that the hydroxyl group at C-7 was equatorial, we applied the modified Mosher's method for MPTA ester of **9**. Compound **9** was esterified with (+)- and (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl) and dimethylaminopyridine (DMAP) in CH_2Cl_2 to afford (+)-MTPA ester (**11**) and (-)-MTPA ester (**12**), respectively. The values of difference of each chemical shift [$\Delta\delta$ values; $\delta(-) - \delta(+)$] for (+)-MTPA ester (**11**) and (−)-MTPA ester (**12**) are shown in Fig. 1. On the basis of the above results, the configuration at C-7 is *R*, consequently that of C-5 in **5** must have the *S*-configuration.

2.2. Acutifolone A (6)

Compound **6** was obtained as colorless prisms, mp 102–104°C. The molecular formula, $C_{16}H_{22}O_3$, was determined by high-resolution mass spectrometry (HR-MS) (*m*/*z* 262. 1552). The presence of an α,β,γ,δ-unsaturated ketone [269 nm (log ε 4.18), 1657 cm^{-1}] and esters (1732 cm^{-1}) was confirmed by IR and UV spectra. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **6** were similar to 7-oxopinguisenol-12-methyl ester (**5**) (Toyota et al., 1991), except for the absence of one hydroxyl group in place of a tetrasubstituted double bond, indicating that **6** was the dehydrated compound of **5**. The location of the tertiary olefin group at C-6 was established by HMBC and NOESY experiments (Table 2). In HMBC of **6**, the H-6 proton was correlated to C-4, C-5, C-7, C-8 and C-10 carbons. The NOESY spectrum showed the presence of NOEs between (1) H-6 and H-10, H-11 and (2) H-1 and H-4, indicating that **6**

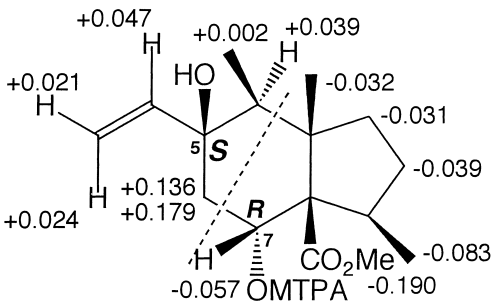

Fig. 1. Δδ values [δ(−)–δ(+)] for (+)-MTPA ester (**11**) and (−)-MTPA ester (**12**) of 7α-hydroxypinguisenol-12-methyl ester (**9**).

Table 1
^{1}H (600 MHz) and ^{13}C NMR spectral data (150 MHz) for **6** and **7** (in $CDCl_3$)[a,b]

	6		**7**	
	H	C	H	C
1	2.21 *m*	45.7	3.24 *m*	27.3
2	1.68 *m*	31.4	1.55 *m*	33.2
	1.73 *m*		2.20 *m*	
3	1.53 *m*	40.9	1.08 *m*	37.2
	1.65 *m*		1.77 *dd* (12.6, 8.0)	
4	2.62 *q* (7.1)	38.5	1.84 *dq* (7.1, 1.9)	45.5
5		160.3		84.6
6	5.99 *s*	124.3	2.49 *dd* (19.1, 1.9)	50.0
7		197.1	2.58 *d* (18.1)	203.5
8		67.3		72.3
9		49.9		52.3
10	6.46 *dd* (17.4, 11.0)	136.8	5.85 *dd* (17.4, 11.3)	134.4
11	5.45 *d* (11.0)	120.1	5.34 *dd* (11.3, 1.1)	115.9
	5.69 *d* (17.4)		5.48 *dd* (17.4,1.1)	
12		171.2		168.7
13	1.25 *d* (7.1)	15.6	1.41 *d* (7.7)	20.1
14	1.11 *s*	23.3	1.10 *s*	19.4
15	1.18 *d* (7.1)	18.0	1.03 *d* (7.1)	12.9
COO*Me*				
	3.67*s*	51.1		

[a] Coupling constants *J* (in Hz) in parentheses.
[b] Assignments are based on DEPT, COSY, HMQC and HMBC.

Table 2
HMBC and NOE correlations for **6** (600 MHz in $CDCl_3$)

H	HMBC correlation	NOE
1	C-2, C-7, C-8, C-9, C-13	H-3, H-4, H-13
2	C-1, C-3, C-8, C-9, C-13	
3	C-1, C-2, C-4, C-8, C-9, C-14	H-1, H-14
4	C-3, C-6, C-9, C-14, C-15	H-1, H-11, H-14, H-15
6	C-4, C-5, C-7, C-8, C-10	H-10, H-11
10	C-4, C-5, C-6	H-6, H-10, H-15
11	C-5, C-10	H-4, H-6, H-10, H-15
13	C-1, C-2, C-8	H-1
14	C-3, C-4, C-8, C-9	H-3, H-4, H-15
15	C-4, C-5, C-9	H-4, H-10, H-11, H-14

might be the 5,6-dehydroxylated compound of **5**. Conclusive evidence for the absolute structure for **6** was obtained from the chemical correlation. Dehydration of **5** with $POCl_3$ in pyridine gave a dehydrated product the physical and spectral data of which were identical to those of the natural product (**6**).

2.3. Acutifolone B (7)

Compound **7** was obtained as colorless prisms (mp 138–140°C). The molecular formula, $C_{15}H_{20}O_3$ was established by HR-MS (*m/z* 248.1406). The presence of a δ-lactone (1769 cm^{-1}; δ_C 168.7) and a ketone (1725 cm^{-1}; δ_C 203.5) was confirmed by IR and ^{13}C-NMR spectra. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of compound **7** were very similar to those of 7-oxopinguisenol-12-methyl ester (**5**), except for the presence of a lactone group in place of one hydroxyl group and one ester group, showing that **7** might be the C-12 and C-6 lactonized compound of **5**. The relative stereostructure of **7** was deduced from analysis of HMBC and NOESY spectra (Table 3) and finally established by its X-ray crystallographic analysis as shown in Fig. 2. The absolute configuration of **7** was suggested to be the same as **5** and **6**.

2.4. Bisacutifolone A (13)

Compound **13** was obtained as colorless prisms (mp 204–206°C). The molecular formula, $C_{32}H_{44}O_7$ was established by HR-MS (*m/z* 540.3058). The UV, IR, ^{1}H- and ^{13}C-NMR spectra (Tables 4 and and 5) of **13** showed the presence of two α,β-unsaturated ketones [251 nm (log ε 4.11); 1657 cm^{-1}; δ_C 195.1, 195.3] and two methyl esters (1732 cm^{-1}; δ_H 3.66 *s*, 6H, 2 × OMe; δ_C 171.2) and one allylic hydroxyl group (3513 cm^{-1}; δ_H 4.35, *bs*; δ_C 68.9) which was confirmed by the formation of an acetate (**14**) (δ_H 2.11, *s*, 3H; δ_H 5.50 *bs*, 1H). The ^{1}H- and ^{13}C-NMR spectra of **13** also contained the signals of two tertiary methyls, four secondary methyls and one tetrasubstituted olefinic group

Table 3
HMBC and NOE correlations for **7** (600 MHz in $CDCl_3$)

H	HMBC correlation	NOE
1	C-2, C-7, C-8, C-9, C-13	H-3
2	C-1, C-3, C-8, C-9, C-13	
3	C-1, C-2, C-4, C-8, C-9, C-14	H-1, H-14
4	C-3, C-6, C-9, C-14, C-15	H-6, H-14, H-15
6	C-4, C-5, C-7, C-8, C-10	H-4, H-10, H-11
10	C-5, C-6	H-6, H-11
11	C-5, C-10	H-6, H-10
13	C-1, C-2, C-8	H-1
14	C-3, C-4, C-8, C-9	H-3, H-4, H-15
15	C-4, C-5, C-9	H-4, H-15

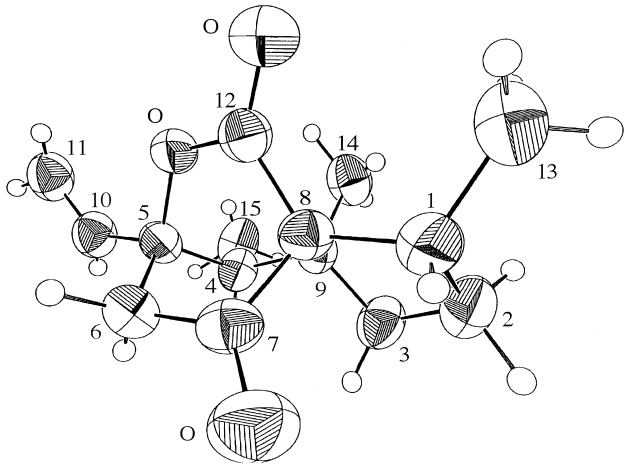

Fig. 2. ORTEP drawing of acutifolone B (**7**).

and one trisubstituted olefinic group which were confirmed by the presence of only one olefinic proton signal at δ_H 5.55 (*d*, *J* = 2.2 Hz). The location of the C-5–C-6 trisubstituted double bond was established by the HMBC (Table 6) in which the H-6 correlated to the C-4, C-5, C-8 and C-10 carbons and the H-10 correlated to the C-4, C-5, C-6. C-5′, C-6′, C-7′ and C-

Table 4
^{1}H NMR data for **13**, **18** and **22** (600 MHz in $CDCl_3$)[a,b]

H	13	18	22
1	2.18 *m*	2.17 *m*	2.19 *m*
2	1.75 *m*	1.75 *m*	1.77 *m*
	1.96 *m*	1.95 *m*	1.94 *m*
3	1.59 *m*	1.59 *m*	1.60 *m*
	1.87 *m*	1.86 *m*	1.89 *m*
4	2.91 *dq* (7.1, 2.2)	2.91 *dd* (7.1, 1.9)	2.91 *dq* (7.1, 1.9)
6	5.55 *d* (2.2)	5.82 *d* (2.2)	5.59 *d* (1.9)
10	3.58 *dd* (6.0, 5.9)	3.48 *dd* (5.9, 5.9)	3.55 *dd* (5.2, 4.7)
11	1.61 *m*	1.78 *m*	1.68 *m*
	2.13 *m*	1.91 *m*	1.83 *m*
13	1.06 *d* (6.9)	1.07 *d* (6.9)	1.06 *d* (6.9)
14	0.90 *s*	0.91 *s*	0.91 *s*
15	1.29 *d* (7.1)	1.28 *d* (7.1)	1.28 *d* (7.1)
1′	1.82 *m*	1.76 *m*	1.96 *m*
2′	1.67 *m*	1.64 *m*	1.68 *m*
	1.78 *m*	1.73 *m*	1.81 *m*
3′	1.56 *m*	1.53 *m*	1.55 *m*
	1.68 *m*	1.81 *m*	1.73 *m*
4′	2.58 *q* (6.9)	2.63 *d* (7.0)	2.31 *q* (7.1)
10′	4.35 *bs*	4.29 *bs*	2.16 *m*
			2.36 *m*
11′	1.72 *m*	1.84 *m*	1.65 *m*
	2.03 *m*	1.89 *m*	1.81 *m*
13′	0.97 *d* (6.6)	1.00 *d* (6.6)	0.98 *d* (6.9)
14′	1.01 *s*	1.02 *s*	0.94 *s*
15′	1.27 *d* (6.9)	1.16 *d* (7.1)	1.16 *d* (7.1)
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	3.66 *s*	3.66 *s*	3.66 *s*

[a] Coupling constants *J* (in Hz) in parentheses.
[b] Assignments are based on DEPT, COSY, HMQC and HMBC.

Table 5
^{13}C NMR spectral data for **13**, **18** and **22** (150 MHz, $CDCl_3$)[a]

C	13	18	22	C	13	18	22
1	40.5	40.8	41.1	1′	43.8	44.7	43.1
2	30.7	30.8	30.9	2′	31.4	31.6	31.3
3	35.9	36.2	36.3	3′	41.0	42.1	40.1
4	40.4	40.6	40.9	4′	40.8	40.0	42.8
5	168.5	169.3	169.5	5′	159.6	159.3	159.6
6	124.0	124.2	124.1	6′	133.1	133.2	132.1
7	195.1[b]	195.7[c]	195.5	7′	195.3[b]	195.4[e]	194.1
8	69.5	69.4	69.6	8′	67.3	66.9	67.9
9	52.5	52.4	52.4	9′	49.3	48.1	49.6
10	37.0	37.2	36.6	10′	68.9	65.6	30.0
11	24.8	25.0	27.7	11′	29.6	28.7	18.3
12	171.2[c]	171.2[f]	171.4[h]	12′	171.2[c]	171.3[f]	171.4[h]
13	14.5	14.7	14.5	13′	13.7	13.8	13.9
14	19.2	19.4	19.6	14′	23.3	24.1	22.1
15	13.0	13.2	13.3	15′	18.3	18.1	16.1
OMe	51.0[d]	51.0[g]	51.0[i]	16′	50.9[d]	51.0[g]	50.9[i]

[a] Assignments are based on DEPT, COSY, HMQC and HMBC.
[b–i] Assignments interchangeable.

11′ carbons. The NOESY spectrum (Table 6) of **13** indicated the presence of NOEs between (1) H-6 and H-1′, and H-10′, (2) H-10 and H-4, and H-15 and (3) H-10′ and H-6, H-1′, H-4′, and H-15′. The additional HMBC and NOESY spectral data are shown in Table 6. On the basis of the above spectral evidence, a pinguisane-type sesquiterpene dimer (**13**), which could be formed by Diels–Alder reaction of two pinguisane ester (**6**), was deduced. This presumption was confirmed by X-ray crystallographic analysis of **13** as shown in Fig. 3.

The absolute configuration of **13** was elucidated following two experimental results described below. The

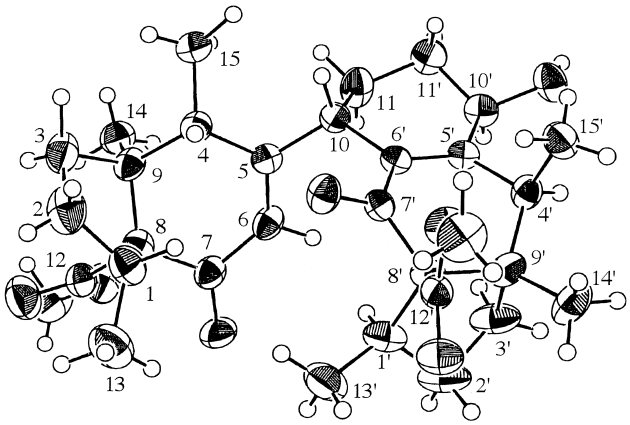


Fig. 3. ORTEP drawing of bisacutifolone A (**13**).

CD spectrum of the *p*-bromobenzoate (**15**) of **9** showed a positive first Cotton effect at 255 nm ($\Delta\varepsilon$ +30.10) and a negative second Cotton effect at 230 nm ($\Delta\varepsilon$ −3.21). The absolute configuration of C-10′ was presumed to be *R* by the application of an exciton chirality method (Koreeda, Harada & Nakanishi, 1974) to the conjugated enone system in CD spectrometry (Fig. 4). In order to obtain the more precise establishment of the absolute configuration of **13**, the modified Mosher's method was carried out as mentioned earlier. Compound **13** was esterified with (−)- and (+)-MTPA chloride in dicyclohexylcarbodiimide (DCC) and DMAP, to give (+)-MTPA ester (**16**) and (−)-MTPA ester (**17**), respectively. The $\Delta\delta$ values [$\delta(-) - \delta(+)$] (Fig. 5) revealed that the absolute configuration at C-10′ was represented as *R*. Thus, the structure of **13** must be that of a Diels–Alder reaction-type dimeric pinguisane sesquiterpenoid.

Table 6
HMBC and NOE correlation of **13** (600 MHz in $CDCl_3$)

	HMBC	NOE
H-1	C-2, C-8, C-13	H-4, H-13
H-2	C-1, C-3, C-8	
H-3	C-1, C-4, C-9, C-14	
H-4	C-3, C-5, C-6, C-7, C-8, C-9, C-14, C-15	H-1, H-10, H-15
H-6	C-4, C-5, C-8, C-10	H-1′, H-10′
H-10	C-4, C-5, C-6, C-11, C-5′, C-6′, C-7′, C-11′	H-4, H-15
H-11	C-5, C-6′, C-10′, C-11′	
H-13	C-1, C-2, C-8	H-1
H-14	C-3, C-4, C-8, C-9	H-15
H-15	C-4, C-5, C-9	H-4, H-10, H-14
H-1′	C-2′, C-7′, C-8′, C-12′	H-6, H-4′, H-10′, H-13′
H-2′	C-3′, C-8′, C-9′, C-13′	
H-3′	C-1′, C-2′, C-8′, C-9′, C-14′	
H-4′	C-5′, C-6′, C-8′, C-9′, C-10′, C-14′, C-15′	H-1′, H-10′, H-14′, H-15′
H-10′	C-5′, C-6′	H-6, H-1′, H-4′, H-15′
H-11′	C-10, C-11, C-5′, C-10′	
H-13′	C-1′, C-2′, C-8′	H-1′
H-14′	C-3′, C-4′, C-8′, C-9′	H-4′, H-15′
H-15′	C-4′, C-5′, C-9′	H-4′, H-10′, H-14′

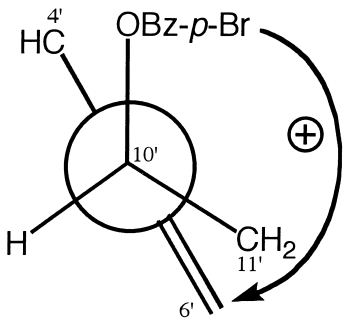


Fig. 4. A positive Cotton effect for allyl benzoate (**15**).

2.5. Bisacutifolone B (*18*)

Compound **18** has the same molecular formula, $C_{32}H_{44}O_7$ (HR-MS: *m*/*z* 540.3093) as that of **13**. The UV and IR absorption bands and ^{1}H- and ^{13}C-NMR spectra (Tables 4 and 5) quite resembled those of **13**. The ^{13}C-NMR spectrum of **18** was almost identical to **13**, except for the C-10′ and the presence of the positive first Cotton effect at 259 nm in CD spectrum of **18** resembled that of **13**. The above spectral data and ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY experiments (Table 7) suggested that **18** was the C-10′ epimer of **13**. To confirm this assumption, the CD spectrum of *p*-bromobenzoate (**19**) of **18** was measured. Contrary to expectation, a positive first Cotton effect at 259 nm was observed. This phenomenon could be explained by the fact that the absorption positions of Cotton effects of both *p*-bromobenzoate and dienone are very close. Thus, the allyl benzoate rule in CD spectrometry could not apply to **19**. To establish the absolute stereochemistry of **18**, the modified Mosher's method for MTPA ester of **18** was carried out. Compound **18** was esterified with (+)- and (−)-MTPA chloride in DCC and DMAP to give (+)-MTPA ester (**20**) and (−)-MTPA ester (**21**), respectively. On the basis of the results of the modified Mosher's method, C-10′ possessed the *S* configuration (Fig. 6), and thus, the absolute configuration of **18** was depicted as C-10′ epimer of **13**. Previously, the structure of **18** was represented as C-10 isomer of **13**, taking a strong hold of the result of the positive Cotton effect of the benzoate (**19**) (Hashimoto et al., 1998a, 1998b). But, this conclusion is ruled out by the further experiments described above.

Table 7
NOE correlation for **18** and **22** (600 MHz in $CDCl_3$)

	18	**22**
H-1	H-4, H-13	H-4, H-13
H-4	H-1, H-10, H-15	H-1, H-10, H-14, H-15
H-6	H-1′, H-10′	H-1′
H-10	H-4, H-15	H-4, H-15
H-13	H-1	H-1
H-14	H-15	H-4, H-15
H-15	H-4, H-10, H-14	H-4, H-10, H-14
H-1′	H-6, H-4′, H-10′, H-13′	H-6, H-4′, H-10′, H-13′
H-4′	H-1′, H-10′, H-14′, H-15′	H-1′, H-10′, H-14′, H-15′
H-10′	H-6, H-1′, H-4′, H-15′	H-1′, H-4′, H-15′
H-13′	H-1′	H-1′
H-14′	H-4′, H-15′	H-4′, H-15′
H-15′	H-4′, H-10′, H-14′	H-4′, H-10′, H-14′

2.6. Bisacutifolone C (*22*)

The molecular formula, $C_{32}H_{44}O_6$, has been established by HR-MS (*m*/*z* 524.3141). The presence of an α,β-unsaturated ketone [249 nm (log ε 4.09), 1657 cm^{-1}] and an ester (1732 cm^{-1}) was confirmed by IR and UV spectra. The ^{1}H-, ^{13}C- (Tables 4 and 5), and 2D-NMR spectra of compound **22** were almost identical to **13** and **18**, except for the absence of one hydroxyl group in place of a methylene group, indicating that **22** was the dehydroxylated compound of **13** and **18**. This assumption was also confirmed by ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectral data

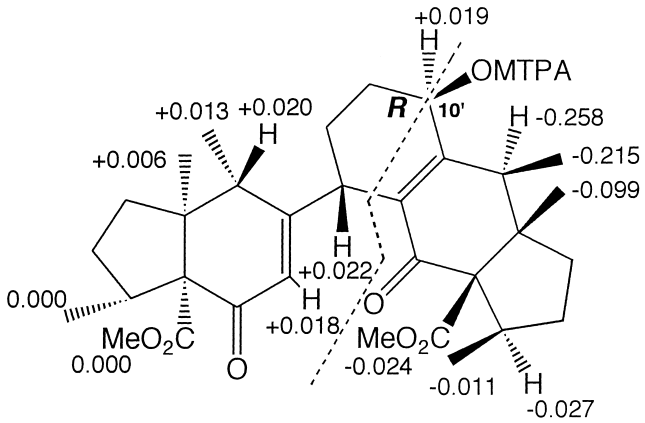


Fig. 5. Δδ values [δ(−)−δ(+)] for (+)-MTPA ester (**16**) and (−) MTPA ester (**17**) of bisacutifolone A (**13**).

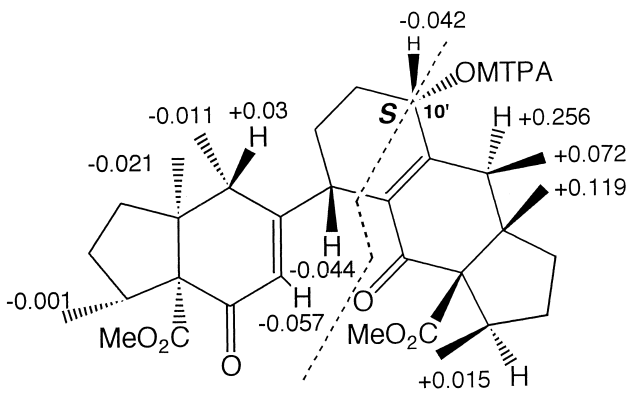


Fig. 6. Δδ values [δ(−)−δ(+)] for (+)-MTPA ester (**20**) and (−) MTPA ester (**21**) of bisacutifolone B (**18**).

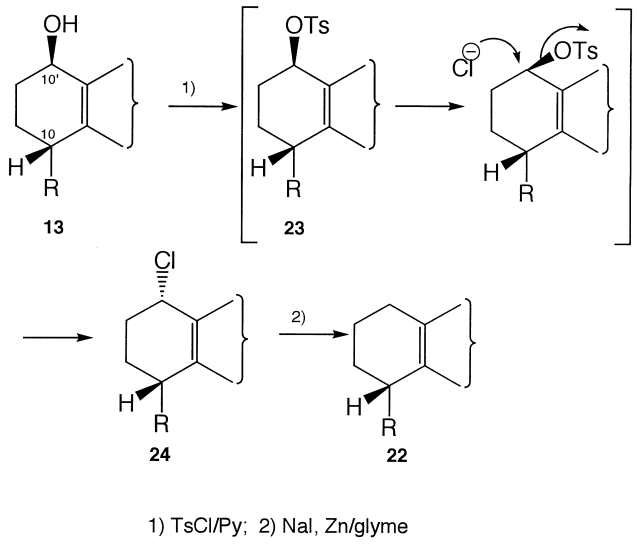


Fig. 7. Formation of bisacutifolone C (**22**) from bisacutifolone A (**13**).

(Table 7) and the chemical correlation between **22** and bisacutifolone A (**13**). Treatment of **13** with tosyl chloride in pyridine did not give 10′β-tosylate (**23**) but yielded 10′α-chlorinated compound (**24**) ($C_{32}H_{43}O_6Cl$; HR-MS m/z 558.2769). It is considered that compound **24** might be obtained from tosylate (**23**) by attacking of chloride anion as S_N2 reaction as shown in Fig. 7. Reduction with NaI and Zn in glyme (Fujimoto & Tatsuno, 1976) afforded a dechlorinated product the spectral data of which including the CD spectrum were identical to those of the natural **22**. Thus, the absolute structure of **22** was established to be dehydroxylated bisacutifolone A (**13**) or B (**22**).

The possible biogenetic pathways of the three new Diels–Alder reaction-type dimeric pinguisane sesquiterpenoids (**13**, **18**, and **22**) are shown in Fig. 8. Firstly, biological Diels–Alder cycloaddition reaction occurs between 6–11 diene of acutifolone A (**6**) and 10–11 dienophile of another molecule which attacks from low side. Then, 5′–10′ double bond is isomerized to 5′-6′

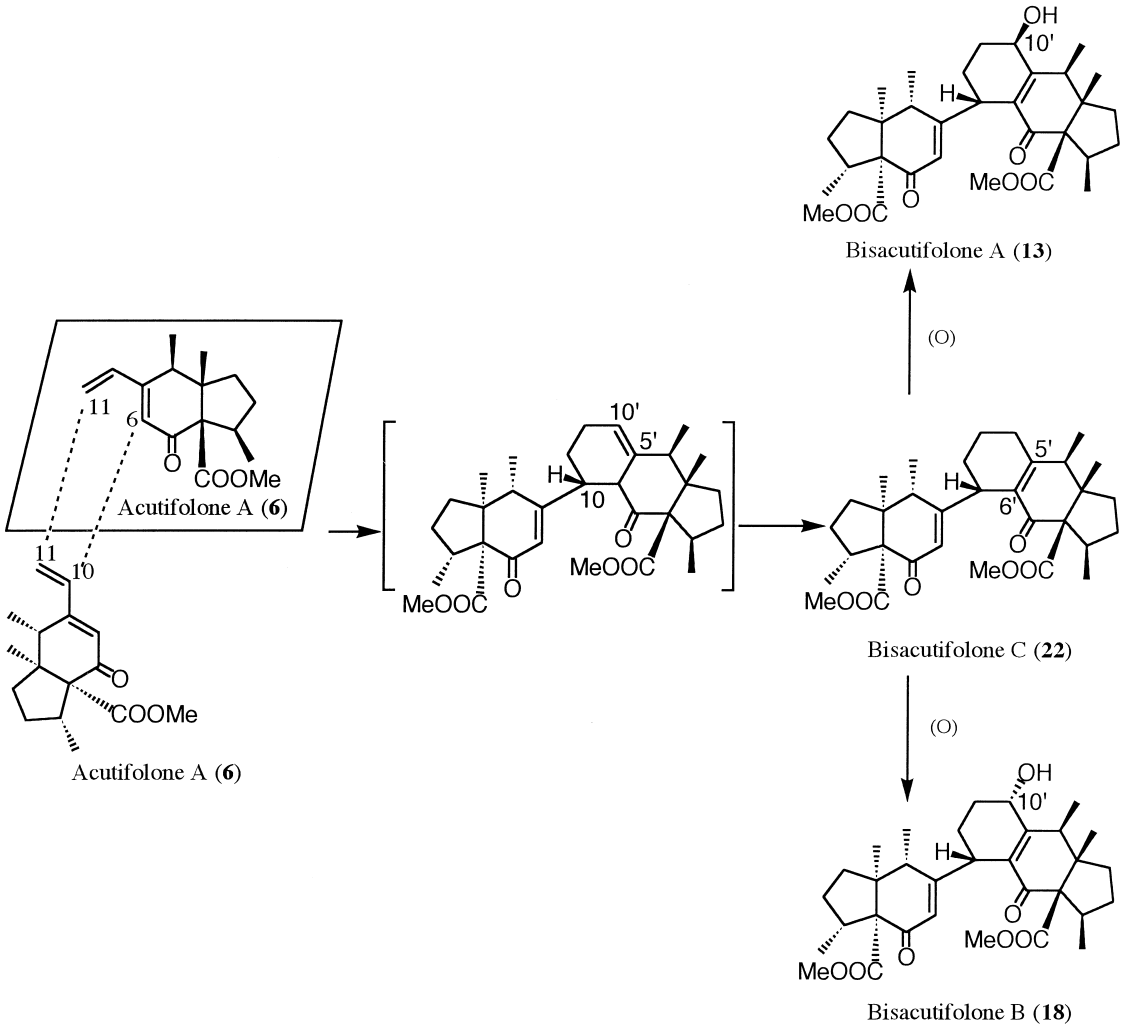
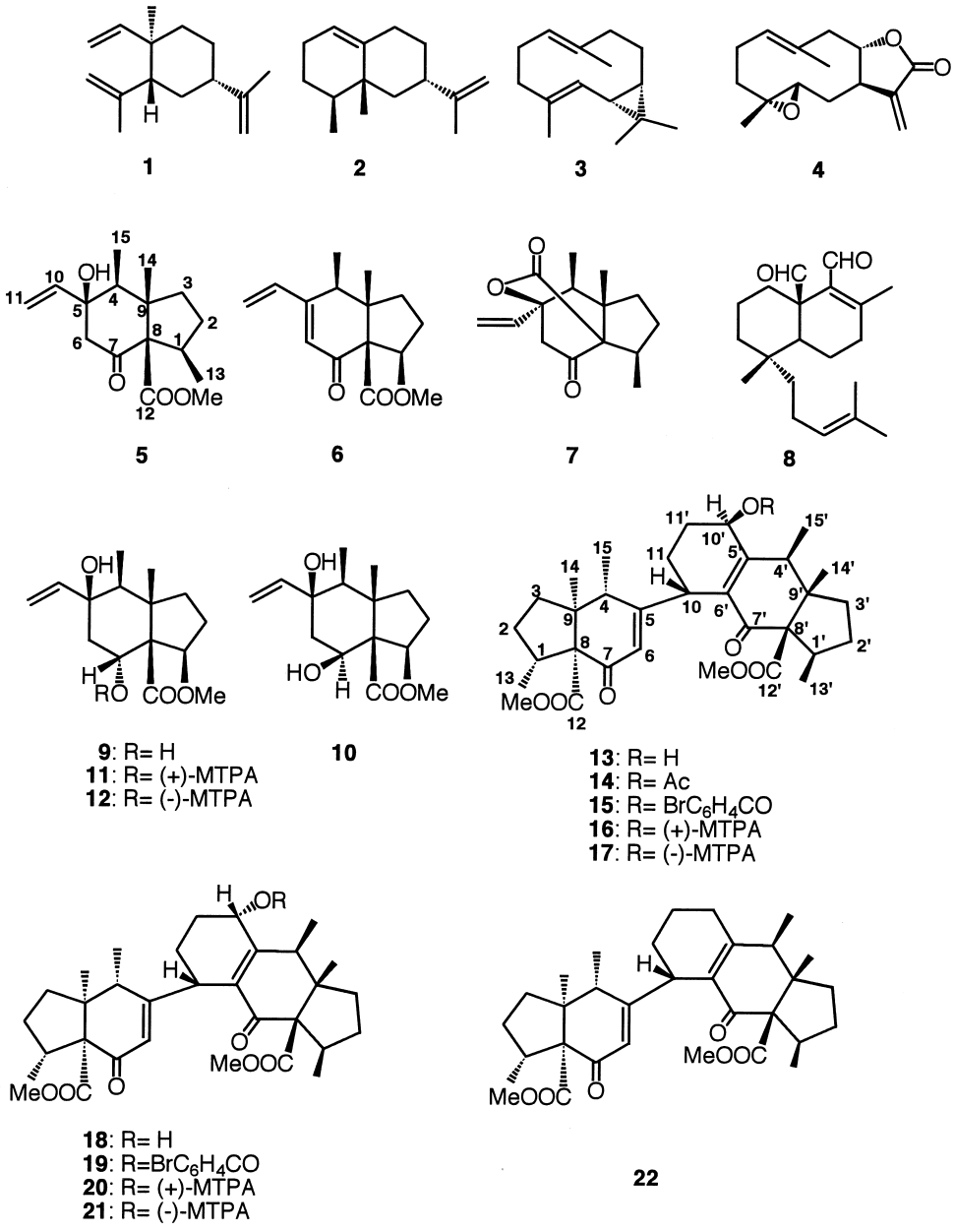


Fig. 8. Possible biogenetic pathway of bisacutifolone A–C (**13, 18** and **22**).

to give bisacutifolone C (**22**). Furthermore, C-10′ of **22** is hydroxylated to give two dimeric pinguisanes, bisacutifolone A (**13**) and bisacutifolone B (**18**). In order to confirm the above biogenetic Diels–Alder-type dimerization, compound **6** was treated with *p*-toluenesulphonic acid (*p*-TsOH) in toluene (110–120°C, over night) but only the starting material was recovered.

Many Diels–Alder-type dimeric cycloaddition compounds have been isolated from higher plants and fungi. Ichihara and Oikawa (1998) reported the isolation of phytotoxins, solanapyrone A and B from *Alternaria solani* which might be biosynthesized through Diels–Alder-type reaction and proposed the presence of solanapyrone synthase. Sporle, Becker, Gupta, Veith and Huch (1989, 1991) found that the liverwort *Plagiochila moritziana* produced plagiospirolides (A–D) which might be biosynthesized through Diels–Alder-type reaction from the sesquiterpene lactone, diplophyllolide and the diterpene, fusicoccadiene. The dimeric pinguisanes (**13**, **18**, **22**) might be synthesized through enzymatic Diels–Alder reactions.

Pinguisane-type sesquiterpenoids are significant chemical constituents of the *Porella* species (Asakawa, 1982b, 1995). However, dimeric pinguisanes have not been found in the other *Porella* species so far examined. It is considered that *P. acutifolia* subsp. *tosana* is chemically different from the other *Porella* species because it contains pinguisane dimers as the major



components. Previously, three guaianolides have been isolated from *P. acutifolia* subsp. *tosana* collected in different localities (Toyota et al., 1991); these lactones have not been detected in the present sample. This result implies that there are two chemo types of *P. acutifolia* subsp. *tosana* in Japan.

3. Experimental

3.1. General

Melting points were uncorrected. TLC was carried out on silica gel precoated glass plates (Kieselgel 60 F_{254}, Merck) with *n*-hexane–EtOAc (1:1, 2:1 and 4:1) and CH_2C_{12}–EtOAc (2:1 and 3:1). Detection was with Godin reagent (Godin, 1954). For normal phase CC, silica gel 60 (70–230 μm, Merck) and silica gel C-300 (230–400 μm, Wako) were used. The mixture of $CHCl_3$–MeOH (1:1) was used as solvent for CC on Sephadex LH-20. UV and CD spectra were measured in EtOH. $[\alpha]_D$ was measured in $CHCl_3$.

3.2. Spectral data

The ^{1}H- and ^{13}C-NMR spectra were obtained with a Varian Unity 200 (200 MHz) or a Varian Unity 600 (600 MHz) spectrometer. EI mass were measured at 70 eV. The temperature programming of GC–MS analysis was performed from 80°C, then 80–250°C at 15°C min^{-1} and finally isothermal at 250°C for 13 min. Injection temperature was 260°C. A fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) using He as carrier gas (1 ml min^{-1}).

3.3. Plant material

Porella acutifolia (Lehm. et Lindenb.) subsp. *tosana* (Steph.) Hatt. was collected in May 1996 at Umazi, Kochi, Japan and identified by YA and confirmed by Dr. M. Mizutani. A voucher specimen has been deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.4. Separation and isolation

Porella acutifolia subsp. *tosana* was dried for 1 day and ground mechanically. The ground material (1.24 kg) was extracted with ether for 1 week. Filtration and evaporation of the solvent gave a green oil (23.92 g). A small amount of the crude extract was analyzed by TLC and GC–MS to detect the presence of β-elemene (**1**), valencene (**2**), bicyclogermacrene (**3**) and perrottetianal (**8**). The remaining extract was chromatographed on silica gel using *n*-hexane–EtOAc gradient to give 37 fractions. Fr. 1 (1.220 g) contained a mixture of β-elemene (**1**), valencene (**2**) and bicyclogermacrene (**3**). Fr. 11–13 (3.469 g) was rechromatographed on silica gel using CH_2Cl_2–EtOAc gradient to give perrottetianal (**8**) (1.350 g) and acutifolone B (**7**) (65 mg). Fr. 20–21 (2.236 g) was rechromatographed on silica gel using the same solvent system described in Fr. 11–13 to divide into two fractions, Fr. 20–21A (145 mg) and Fr. 20–21B (248 mg). The former was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to afford acutifolone A (**6**) (60 mg). The latter was purified on silica gel CC (*n*-hexane–EtOAc gradient) to yield 7-oxopinguisenol-12-methyl ester (**5**) (177 mg), acutifolone A (**6**) (32 mg) and bisacutifolone C (22) (10 mg). Fr. 29 (1.026 g) was recrystallized from ether to give 4α,5β-epoxy-8-epiinnunolide (**4**) (664 mg). Fr. 35 (741 mg) was chromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1), followed by silica gel CC (CH_2Cl_2–EtOAc gradient) to give bisacutifolone A (**13**) (282 mg) and bisacutifolone B (**18**) (27 mg). Fr. 36, 37 (963 mg) was treated in the same manner described above to give bisacutifolone A (**13**) (198 mg).

3.5. Acutifolone A (*6*)

Colorless prisms; mp 102–104°C; $[\alpha]_D^{19}$ +2.10 (*c* 1.73, $CHCl_3$); HR-MS: *m/z* 262.1552, $C_{16}H_{22}O_3$ requires 262.1568; EI-MS: *m/z* 262 (M^+, 12%), 230 (3), 203 (4), 180 (10), 153 (3), 108 (100), 79 (9), 32 (15); FT-IR (KBr) cm^{-1}: 1732 (COO), 1657 (C=O); UV: λ_{max} nm (log ε): 269 (4.18); ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data: (Table 1).

3.6. Acutifolone B (*7*)

Colorless prisms; mp 138–140°C; $[\alpha]_D^{19}$ −94.9 (*c* 0.66, $CHCl_3$); HR-MS: *m/z* 248.1406, $C_{15}H_{20}O_3$ requires 248.1412; EI-MS: *m/z* 248 (M^+, 48%), 219 (28), 206 (63), 178 (97), 165 (71), 150 (57), 123 (100), 95 (29), 55 (43), 32 (21); FT-IR (KBr) cm^{-1}: 1769 (COO); 1725 (C=O); ^{1}H- (600 MHz) and ^{13}C-NMR data: (Table 1); CD: λ_{max} nm (Δε): 297 (−2.93), (*c* 8.1 × 10^{-3}); Crystal data: Crystal dimensions = 0.5 × 0.3 × 0.1 mm, monoclinic, space group $P2_1$ with $a = 11.343$ (0)Å, $b = 8.296$ (0)Å, $c = 7.288$ (0)Å, $\beta = 106.507$ (0)°, $V = 657.6$ (0)Å^3, $Z = 2$, Dx = 1.25 mg m^{-3}, Dm = 1.30 mg m^{-3}, and μ(Cu K_α) = 6.553 mm^{-1} by Mac Science MXC 18 instrument. Final *R* value was 0.058 for 936 reflections.

3.7. Bisacutifolone A (*13*)

Colorless prisms; mp 204–206°C; $[\alpha]_D^{21}$ +59.0 (*c* 0.51, $CHCl_3$); HR-MS: *m/z* 540.3058, $C_{32}H_{44}O_7$ requires 540.3087; EI-MS: *m/z* 540 (M^+, 100%), 480

(40), 465 (15), 405 (12), 357 (8), 203 (8), 176 (11), 123 (15), 32 (25); FT-IR (KBr) cm^{-1}: 3513 (OH), 1732 (COO), 1657 (C=O); UV: λ_{max} nm (log ε): 251 (4.11); ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) data: (Tables 4 and 5); Crystal data: Crystal dimensions = 0.5 × 0.2 × 0.2 mm, orthorhombic; space group $P2_12_12_1$ with $a = 13.720$ (5)Å, $b = 21.053$ (7)Å, $c = 9.954$ (5)Å, $V = 2875.3$ (2)Å^3, $Z = 4$, Dx = 1.25 mg m^{-3}, Dm = 1.30 mg m^{-3}, and μ(Cu K_α) = 6.64 mm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.046 for 2156 reflections. CD: λ_{max} nm ($\Delta\varepsilon$): 259 (+16.37), 226 (−3.75) (c 9.0 × 10^{-5}).

3.8. Biscutifolone B (**18**)

Colorless oil; $[\alpha]_D^{20}$ +18.1 (c 0.74, $CHCl_3$); HR-MS: m/z 540.3093, $C_{32}H_{44}O_7$ requires 540.3087; EI-MS: m/z 540 (100%), 522 (12), 480 (25), 462 (14), 402 (7), 368 (7), 203 (8), 176 (11), 123 (16), 95 (9), 32 (52); FT-IR (KBr) cm^{-1}: 3480 (OH), 1730 (COO), 1655 (C=O); UV: λ_{max} nm (log ε): 249 (4.15); ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) data: (Tables 4 and 5); CD: λ_{max} nm ($\Delta\varepsilon$): 259 (+9.68), 236 (−7.39) (c 8.6 × 10^{-5}).

3.9. Bisacutifolone C (**22**)

Colorless amorphous powder; $[\alpha]_D^{21}$ +69.5 (c 0.36, $CHCl_3$); HR-MS: m/z 524.3141, $C_{32}H_{44}O_6$ requires 524.3138; EI-MS: m/z 524 (M^+, 100%), 464 (44), 449 (18), 436 (12), 389 (12), 341 (16), 229 (12), 203 (10), 175 (9), 123 (13), 95 (7), 40 (9); FT-IR (KBr) cm^{-1}: 1732 (COO), 1657 (C=O), UV: λ_{max} nm (log ε): 249 (4.09); ^{1}H- (600 MHz) and ^{13}C-NMR data (150 MHz): (Tables 4 and 5); CD: λ_{max} nm ($\Delta\varepsilon$): 260 (+15.44), 238 (−7.82), (c 5.7 × 10^{-5}).

3.10. $NaBH_4$ reduction of **5**

To compound **5** (126 mg) in MeOH (10 ml) was added $NaBH_4$ (105.7 mg), and stirred at 0°C for 30 min. The reaction mixture was concentrated in vacuo. To the residue were added CH_2Cl_2 (100 ml) and HCl (100 ml) The CH_2Cl_2 layer was washed with sat. NaCl solution and 1 N HCl and the organic layer was dried over $MgSO_4$. After removal of the solvent gave the crude oil (122 mg) which was purified by silica gel CC (CH_2Cl_2/EtOAc, gradient) to give **9** (44.3 mg, Y. 35.0%) and **10** (55.8 mg, Y. 44.2%).

3.11. 7α-Hydroxypinguisenol-12-methyl ester (**9**)

Colorless amorphous powder; $[\alpha]_D^{22}$ −72.1 (c 0.51, $CHCl_3$); HR-MS: m/z-H_2O 264.1697, $C_{16}H_{24}O_3$ requires 264.1726; EI-MS: m/z 282 (M^+, 1%), 264 (18), 232 (56), 204 (34), 183 (89), 155 (49), 123 (100), 95 (28), 55 (26): FT-IR (KBr) cm^{-1}: 3534 (OH), 1705 (COO); ^{1}H-NMR (600 MHz): δ 0.85 (3H, d, J = 6.9 Hz, H-15), 0.96 (3H, s, H-14), 1.09 (3H, d, J = 6.9 Hz, H-13), 3.77 (3H, s, COO*Me*), 4.71 (1H, ddd, J = 2.5, 4.4, 12.5 Hz, H-7), 5.08 (1H, dd, J = 1.1, 10.7 Hz, H-11), 5.21 (1H, dd, J = 1.1, 17.3 Hz, H-11), 5.83 (1H, dd, J = 10.7, 17.3 Hz, H-10).

3.12. 7β-Hydroxypinguisenol-12-methyl ester (**10**)

Colorless amorphous powder, $[\alpha]_D^{22}$ −44.2 (c 0.52, $CHCl_3$); HR-MS: m/z 282.1816, $C_{16}H_{26}O_4$ requires 282.1831; EI-MS: m/z 282 (M^+, 3%), 246 (8), 194 (15), 184 (66), 155 (100), 135 (16), 123 (29), 95 (20), 55 (39); FT-IR (KBr) cm^{-1}: 3495 (OH), 1696 (COO); ^{1}H-NMR (600 MHz): δ 0.88 (3H, d, J = 6.9 Hz, H-13), 0.97 (3H, d, J = 6.9 Hz, H-15), 1.14 (3H, s, H-14), 3.79 (3H, s, COO*Me*), 4.38 (1H, dd, J = 3.0, 5.2 Hz, H-7), 5.06 (1H, dd, J = 1.9, 10.7 Hz, H-11), 5.34 (1H, dd, J = 1.9, 17.0 Hz, H-11), 5.66 (1H, ddd, J = 1.1, 10.7, 17.0 Hz, H-10).

3.13. Chlorination of (+)-MTPA

(+)-MTPA (501 mg) in $SOCl_2$ (1.5 ml) was refluxed with NaCl (300 mg) at 90°C for 50 h. The reaction mixture was dissolved in a small amount of anhydrous benzene. The reaction mixture was filtered through a small column packed with cotton and concentrated in vacuo to give (+)-MTPACl (438 mg) (Y. 80.8%).

3.14. Chlorination of (−)-MTPA

(−)-MTPA (501 mg) in $SOCl_2$ (1.5 ml) was refluxed with NaCl (301 mg) at 90°C for 25 h. The reaction mixture was treated in the same manner described above to give (−)-MTPA chloride (439.3 mg) (Y. 81.3%). (+)- and (−)-MTPA chlorides thus obtained were used for the esterification without further purification.

3.15. Preparation of (+)-MTPA ester of **9**

To compound **9** (8.1 mg) in pyridine (0.25 ml) was added (+)-MTPA chloride (0.05 ml), DMAP (24.4 mg) and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and then the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with sat. NaCl solution, 1 N HCl and 5% $NaHCO_3$ successively and dried over $MgSO_4$. Filtration and evaporation of solvent gave the crude oil (119.6 mg) which was purified by silica gel CC (*n*-hexane/EtOAc, gradient) to afford (+)-MTPA ester (**11**) (13.2 mg, Y. 92.3%).

3.16. (+)-MTPA ester (**11**)

Colorless oil; $[\alpha]_D^{21}$ +14.8 (*c* 0.70, $CHCl_3$); HR-MS: *m/z* 498.2194; $C_{26}H_{33}O_6F_3$ requires 498.2230; EI-MS: *m/z* 498 (M^+, 1%), 480 (6), 264 (17), 247 (87), 233 (25), 205 (25), 189 (81), 187 (100), 145 (32), 121 (36), 105 (28), 55 (31), 32 (75); FT-IR (KBr) cm^{-1}: 3536 (OH), 1728 (COO); ^{1}H-NMR (600 MHz): δ 0.85 (3H, *d*, *J* = 6.9 Hz, H-15), 0.94 (3H, *d*, *J* = 6.6 Hz, H-13), 0.99 (3H, *s*, H-14), 3.68 (3H, *s*, COO*Me*), 5.10 (1H, *dd*, *J* = 0.8, 10.7 Hz, H-11), 5.22 (1H, *dd*, *J* = 0.8, 17.3 Hz, H-11), 5.76 (1H, *dd*, *J* = 10.7, 17.3 Hz, H-10), 6.20 (1H, *dd*, *J* = 4.9, 12.2 Hz, H-7).

3.17. Preparation of (−)-MTPA ester of **9**

To compound **9** (7.3 mg) in pyridine (0.25 ml) was added (−)-MTPA chloride (0.05 ml), DMAP (16.5 mg) and stirred at room temperature for 2 h and the reaction mixture was treated in the same manner as described above to give the residue (110.2 mg), which was further purified by silica gel column CC (*n*-hexane/EtOAc, gradient) to give (−)-MTPA ester (**12**) (8.8 mg, Y. 68.3%).

3.18. (−)-MTPA ester (**12**)

Colorless oil; $[\alpha]_D^{21}$ −53.4 (*c* 0.81, $CHCl_3$); HR-MS: *m/z* 498.2229; $C_{26}H_{33}O_6F_3$ requires 498.2230; EI-MS: *m/z* 498 (M^+, 1%), 480 (6), 264 (15), 247 (78), 189 (90), 187 (100), 145 (38), 121 (32), 105 (33), 55 (29); FT-IR (KBr) cm^{-1}: 3530 (OH), 1728 (COO); UV λ_{max} nm (log ε): 201 (4.19); ^{1}H-NMR (600 MHz): δ 0.85 (6H, *d*, *J* = 7.1 Hz, H-13, H-15), 0.96 (3H, *s*, H-14), 3.49 (3H, *s*, COO*Me*), 5.12 (1H, *dd*, *J* = 0.8, 10.7 Hz, H-11), 5.24 (1H, *dd*, *J* = 0.8, 17.3 Hz, H-11), 5.80 (1H, *dd*, *J* = 10.7, 17.3 Hz, H-10), 6.15 (1H, *dd*, *J* = 4.9, 12.2 Hz, H-7).

3.19. Dehydration of **5**

To compound **5** (30.2 mg) in pyridine (2 ml) was added $POCl_3$ (0.22 ml), the mixture stirred for 1.5 h and refluxed for 2 days. The residue was partitioned between $CHCl_3$ and H_2O and the organic layer was washed with sat. NaCl solution, 1 N HCl, 5% $NaHCO_3$ and dried over $MgSO_4$. After filtration and evaporation of the solvent gave the residue (26 mg) which was purified by silica gel CC (*n*-hexane/EtOAc, gradient) to give **6** (6.1 mg, Y. 21.6%) the physical and spectroscopic data of which were identical to those of natural acutifolone A (**6**).

3.20. Acetylation of **13**

Compound **13** (52 mg) in pyridine (3 ml) was acetylatetd with acetic anhydride (3 ml) at room temperature for 48 h. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The organic layer was washed with sat. NaCl solution, 1 N HCl, 5% $NaHCO_3$ and dried over $MgSO_4$ and concentrated in vacuo to give the residue (48 mg) which was purified by prep. TLC (silica gel plate, *n*-hexane: EtOAc 2:1) to furnish a monoacetate (**14**) (21.1 mg, Y. 37.5%).

3.21. Bisacutifolone A mono acetate (**14**)

Colorless oil; $[\alpha]_D^{19}$ +71.0 (*c* 0.65, $CHCl_3$): HR-MS: *m/z* 582.3212, $C_{34}H_{46}O_8$ requires 582.3193; EI-MS: *m/z* 582 (M^+, 100%), 522 (35), 462 (17), 447 (10), 368 (10), 340 (11), 176 (9), 123 (15), 95 (7), 32 (10); FT-IR (KBr) cm^{-1}: 1734 (COO), 1663 (C=O); UV λ_{max} nm (log ε): 248 (4.30); ^{1}H-NMR (200 MHz): δ 0.90 (3H, *s*, H-14), 0.96 (3H, *d*, *J* = 6.7 Hz, H-13′), 0.99 (3H, *s*, H-14′), 1.06 (3H, *d*, *J* = 6.7 Hz, H-13), 1.17 (3H, *d*, *J* = 6.9 Hz, H-15′), 1.29 (3H, *d*, *J* = 7.2 Hz, H-15), 2.11 (3H, *s*, OAc), 3.66 (3H, *s*, COO*Me*), 3.67 (3H, *s*, COO*Me*), 5.50 (1H, *br.s*, H-10′), 5.54 (1H, *d*, *J* = 2.2 Hz, H-6). ^{13}C-NMR (50): δ 124.4 (*d*, C-6), 135.5 (*s*, C-6′), 155.1 (*s*, C-5′), 167.0 (*s*, C-5), 170.1, 171.0, 171.2 (*s*, C-12, 12′ or OAc), 194.7, 195.0 (*s*, C-7 or 7′).

3.22. Preparation of p-bromo benzoate of **13**

To compound **13** (11 mg) in pyridine (2 ml) was added *p*-bromobenzoyl chloride (104 mg), DMAP (58 mg) and the mixture stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and the residue partitioned between $CHCl_3$ and H_2O. The organic layer was washed with sat. NaCl solution, 1 N HCl, 5% $NaHCO_3$ and dried by $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the residue (71.6 mg), which was purified by silica gel column chromatography (*n*-hexane/EtOAc, gradient) to give the benzoate (**15**) (15.5 mg, Y. 100%).

3.23. Bisacutifolone A p-bromobenzoate (**15**)

Colorless oil; $[\alpha]_D^{23}$ +69.1 (*c* 0.55, $CHCl_3$); HR-MS: *m/z* 722.2432, $C_{39}H_{47}O_8Br$ requires 722.2454; EI-MS: *m/z* 724 100%), 722 (M^+, 93), 664 (18), 522 (33), 462 (40), 402 (16), 368 (19), 340 (23), 185 (35), 183 (36), 123 (25), 32(21); FT-IR (KBr) cm^{-1}: 1728 (COO), 1663 (C=O); UV λ_{max} nm (log ε): 203 (4.27), 251 (4.23); ^{1}H-NMR (200 MHz): δ 0.91 (3H, *s*, H-14), 0.97 (3H, *s*, H-14′), 0.99 (3H, *d*, *J* = 4.4 Hz, H-13′), 1.07 (3H, *d*, *J* = 6.7 Hz, H-13), 1.19 (3H, *d*, *J* = 7.0 Hz, H-15′), 1.31 (3H, *d*, *J* = 7.1 Hz, H-15), 3.67 (3H, *s*, COO*Me*), 3.68 (3H, *s*, COO*Me*), 3.68 (1H, *m*, H-10), 5.58 (1H, *d*, *J* = 1.9 Hz, H-6), 5.75 (1H, *br.s*, H-10′). CD: λ_{max} nm ($\Delta\varepsilon$): 255 (+30.10), 230 (−3.21), (*c* 1.5 × 10^{-3}).

3.24. Preparation of (+)-MTPA ester of **13**

Compound **13** (19 mg) in anhydrous CH_2Cl_2 (4 ml) was treated with (+)-MTPA (72 mg), DCC (90 mg), DMAP (59 mg). The reaction mixture was treated in the same manner as described in the preparation of the MTPA ester of **11** to afford (+)-MTPA ester (**16**) (18.9 mg, Y. 69%).

3.25. (+)-MTPA ester (**16**)

Colorless oil; $[\alpha]_D^{20}$ +45.8 (*c* 0.71, $CHCl_3$); HR-MS: *m/z* 756.3492, $C_{42}H_{51}O_9F_3$ requires 756.3486; EI-MS: *m/z* 756 (M^+, 100%), 696 (16), 522 (9), 462 (10), 368 (8), 189 (22), 123 (11), 32 (26); FT-IR (KBr) cm^{-1}: 1738 (COO), 1665 (C=O); UV λ_{max} nm (log ε): 204 (4.05) 250 (4.10); ^{1}H-NMR (600 Mz): δ 0.89 (6H, *s*, H-14, 14′), 0.92 (3H, *d*, *J* = 6.9 Hz, H-15′), 0.94 (3H, *d*, *J* = 6.6 Hz, H-13′), 1.05 (3H, *d*, *J* = 6.9 Hz, H-13), 1.26 (3H, *d*, *J* = 7.4 Hz, H-15), 3.64 (3H, *s*, COO*Me*), 3.66 (3H, *s*, COO*Me*), 5.51 (1H, *d*, *J* = 2.2 Hz, H-6), 5.62 (1H, *t*, *J* = 4.3, 4.4 Hz, H-10′).

3.26. Preparation of (−)-MTPA ester of **13**

To compound **13** (21 mg) in anhydrous CH_2Cl_2 (4 ml) was added (−)-MTPA (37.1 mg), DCC (48.8 mg), DMAP (24.3 mg) and stirred at room temperature for 1 day. To the reaction mixture was added (−)-MTPA (37.6 mg), DCC (48.6 mg), DMAP (25.3 mg) and further stirred for 1 day. The reaction mixture was treated in the same manner as described above to give the crude oil (128 mg) which was purified by silica gel column chromatography (*n*-hexane/EtOAc, gradient) to give (−)-MTPA ester (17) (22.7 mg, Y. 77%).

3.27. (−)-MTPA ester (**17**)

Colorless oil; $[\alpha]_D^{20}$ +14.5 (*c* 0.77, $CHCl_3$); HR-MS: *m/z* 756.3482, $C_{42}H_{51}O_9F_3$ requires 756.3485; EI-MS: *m/z* 756 M^+, 100%), 696 (14), 522 (9), 462 (10), 368 (11), 189 (29), 123 (15), 105 (10), 32 (13); FT-IR (KBr) cm^{-1}: 1738 (COO), 1665 (C=O); UV λ_{max} nm (log ε): 204 (4.16), 249 (4.20); ^{1}H-NMR (600 MHz): δ 0.71 (3H, *d*, *J* = 6.9 Hz, H-15′), 0.79 (3H, *s*, H-14′), 0.89 (3H, *s*, H-14), 0.93 (3H, *d*, *J* = 6.3 Hz, H-13′), 1.05 (3H, *d*, *J* = 6.9 Hz, H-13), 1.27 (3H, *d*, *J* = 7.1 Hz, H-15), 3.62 (3H, *s*, COO*Me*), 3.66 (3H, *s*, COO*Me*), 5.53 (1H, *d*, *J* = 2.2 Hz, H-6), 5.64 (1H, *t*, *J* = 4.4, 4.4 Hz, H-10′).

3.28. Preparation of p-bromo benzoate of **18**

Compound **18** (3.8 mg) in pyridine (2 ml) was esterified with *p*-bromobenzoyl chloride (14.2 mg) in the presence of DMAP (8.6 mg) at room temperature for 2 h. The reaction mixture was treated in the same manner as described above to furnish the benzoate (**19**) (3.4 mg, Y. 65.9%).

3.29. Bisacutifolone B p-bromobenzoate (**19**)

Colorless amorphous powder; $[\alpha]_D^{23}$ −3.0 (*c* 0.10, $CHCl_3$); HR-MS: *m/z* 722.2479, $C_{39}H_{47}O_8Br$ requires 722.2454; EI-MS: *m/z* 724 (77%), 722 (M^+, 73), 522 (84), 462 (100), 434 (27), 402 (45), 387 (33), 338 (31), 202 (39), 200 (39), 185 (46), 183 (46), 123 (43), 91 (27); FT-IR (KBr) cm^{-1}: 1728 (COO), 1663 (C=O); UV λ_{max} nm (log ε): 247 (4.64); ^{1}H-NMR (200 Mz): δ 0.91 (3H, *s*, H-14), 0.99 (3H, *s*, H-14′), 1.03 (3H, *d*, *J* = 6.0 Hz, H-13′), 1.09 (3H, *d*, *J* = 6.8 Hz, H-13), 1.18 (3H, *d*, *J* = 6.9 Hz, H-15′), 1.30 (3H, *d*, *J* = 7.1 Hz, H-15), 3.55 (1H, *t*, *J* = 6.9Hz, H-10), 3.66 (3H, *s*, COO*Me*), 3.67 (3H, *s*, COO*Me*), 5.74 (1H, *br.s*, H-10′), 5.83 (1H, *d*, *J* = 2.2 Hz, H-6). CD: λ_{max} nm ($\Delta\varepsilon$): 259 (+11.56), 241 (−10.77), (*c* 1.5×10^{-3}).

3.30. Preparation of (+)-MTPA reaction of **18**

Compound **18** (3.2 mg) in anhydrous CH_2Cl_2 was stirred with (+)-MTPA (21 mg), DCC (23 mg), DMAP (17 mg) (2 ml) at room temperature for 4 days. The reaction mixture was treated in the same manner as described above to give (+)-MTPA ester (**20**) (4.4 mg, Y. 99%).

3.31. (+)-MTPA ester (**20**)

Colorless oil; $[\alpha]_D^{20}$ +9.0 (*c* 0.13, $CHCl_3$); HR-MS: *m/z* 756.3505, $C_{42}H_{51}O_9F_3$ requires 756.3485; EI-MS: *m/z* 756 (M^+, 53%), 755 (100), 696 (7), 522 (43), 462 (39), 368 (20), 189 (55), 123 (22), 105 (22), 95 (12), 44 (14); FT-IR (KBr) cm^{-1}: 1738 (COO), 1665 (C=O); UV λ_{max} nm (log ε): 201 (4.38) 246 (4.31); ^{1}H-NMR (600 MHz): δ 0.83 (3H, *s*, H-14′), 0.89 (3H, *s*, H-14), 0.96 (3H, *d*, *J* = 6.3 Hz, H-13′), 1.06 (6H, *d*, *J* = 6.9 Hz, H-13, 15′), 1.26 (3H, *d*, *J* = 7.1 Hz, H-15), 3.62 (3H, *s*, COO*Me*), 3.68 (3H, *s*, COO*Me*), 5.61 (1H, *d*, *J* = 2.2 Hz, H-6), 5.62 (1H, *t*, *J* = 4.8 Hz, H-10′).

3.32. Preparation of (−)-MTPA ester of **18**

Compound **18** (2.0 mg) was stirred with (−)-MTPA (20 mg), DCC (30 mg), DMAP (17 mg) at room temperature for 5 days. Treatment of the residue (35 mg) in the same manner as described above afforded (−)-MTPA ester (**21**) (2.6 mg, Y. 93%).

3.33. (−)-MTPA ester (**21**)

Colorless oil; $[\alpha]_D^{20}$ −9.9 (*c* 0.13, $CHCl_3$); HR-MS: *m/z* 756.3466, $C_{42}H_{51}O_9F_3$ requires 756.3485; EI-MS:

m/z 756 (M^+, 52%), 755 (100), 695 (7), 522 (40), 462 (36), 368 (18), 189 (49), 123 (19), 105 (20), 95 (10), 44 (9); FT-IR (KBr) cm^{-1}: 1736 (COO), 1665 (C=O); UV λ_{max} nm (log ε): 202 (4.56), 245 (4.50); ^{1}H-NMR (600 Mz): δ 0.87(3H, *s*, H-14), 0.94 (3H, *s*, H-14′), 0.97 (3H, *d*, *J* = 6.3 Hz, H-13′), 1.06 (3H, *d*, *J* = 6.9 Hz, H-13), 1.13 (3H, *d*, *J* = 7.1 Hz, H-15′), 1.25 (3H, *d*, *J* = 7.1 Hz, H-15), 3.64 (3H, *s*, COO*Me*), 3.68 (3H, *s*, COO*Me*), 5.55 (1H, *d*, *J* = 2.2 Hz, H-6), 5.58 (1H, *t*, *J* = 4.1 Hz, H-10′).

3.34. Reaction of **13** with p-toluenesulfonyl chloride

To compound **13** (20.3 mg) in pyridine (1 ml) was added *p*-toluenesulfonyl chloride (148 mg) and the mixture was stirred at room temperature for 26 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O and the organic layer extracted sat. NaCl solution, 1 N HCl and 5% $NaHCO_3$ and dried over $MgSO_4$. After filtration and evaporation of solvent gave a residue (23.0 mg) which was purified by silica gel CC (CH_2Cl_2/AcOEt, gradient) to give **24** (18.6 mg, Y. 88.6%).

3.35. 10′α-Chlorobisacutifolone C (**24**)

Colorless oil; $[\alpha]_D^{25}$ +12.4 (*c* 0.83, $CHCl_3$); HR-MS: *m/z* 558.2769, $C_{32}H_{43}O_6Cl$ requires 558.2749; EI-MS: *m/z* 560 (M^+ +2, 5%), 558 (M^+, 12), 522 (98), 490 (22), 462 (100), 432 (23), 402 (40), 374 (18), 338 (65), 227 (20), 176 (21), 153 (26), 123 (44), 95 (20), 3 (44); FT-IR (KBr) cm^{-1}: 1734 (COO), 1661 (C=O); UV λ_{max} nm (log ε): 250 (4.21); ^{1}H-NMR (600 MHz): δ 0.90 (3H, *s*, H-14), 0.99 (3H, *d*, *J* = 6.6 Hz, H-13′), 1.02 (3H, *s*, H-14′), 1.07 (3H, *d*, *J* = 6.9 Hz, H-13), 1.15 (3H, *d*, *J* = 7.1 Hz, H-15′), 1.28 (3H, *d*, *J* = 7.1 Hz, H-15), 3.66, 3.66 (6H, *s*, 2 × COO*Me*), 3.66 (3H, *s*, COO*Me*), 4.60 (1H, *br.s*, H-10′), 5.81 (1H, *d*, *J* = 2.2, H-6). ^{13}C-NMR (150 MHz): δ 56.0 (*d*, C-10′), 124.4 (*d*, C-6), 134.3 (*s*, C-6′), 155.6 (*s*, C-5′), 169.5 (*s*, C-5), 171.1, 171.3 (*s*, C-12 or 12′), 195.2, 195.2 (*s*, C-7 or 7′).

3.36. Reduction of **24**

NaI (20.4 mg), Zn (15.8 mg) in glyme (2 ml) was added to compound **24** and stirred at 60–80°C for 1 h and then at room temperature for 16 h. The reaction mixture was filtered through a short column packed with celite and eluted with ether. The ether solution was washed with H_2O, sat. NaCl solution and dried over $MgSO_4$. Work-up as usual gave the residue (7.8 mg), which was purified by silica gel column chromatography (*n*-hexane/EtOAc, gradient) to give (**22**) (1.8 mg, Y. 18%): $[\alpha]_D^{20}$ +48.8 (*c* 0.05, $CHCl_3$); CD: λ_{max} nm ($\Delta\varepsilon$): 260 (+10.42), 238 (−5.71), (*c* 7.6×10^{-5}) whose HR-MS, EI-MS IR, UV, ^{1}H- and ^{13}C-NMR were identical to those of the natural bisacutifolone C (**22**).

3.37. Reaction of acutifolone A (**6**) with p-TsOH

To compound **6** (21.1 mg) in toluene (2 ml) was added *p*-TsOH (50 mg) and refluxed at 110–120°C for 24 h. The reaction mixture was extracted with $CHCl_3$ and washed with H_2O, Na_2CO_3 and the organic layer was dried over $MgSO_4$. Work-up as usual recovered the starting material (**6**) (10.7 mg, Y. 50.7%).

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