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Two Novel Diels-Alder Reaction-Type Dimeric Pinguisane Sesquiterpenoids and Related Compounds from the Liverwort *Porella acutifolia* subsp. *tosana*

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

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

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Abstract: From the ether extract of the liverwort *Porella acutifolia* subsp. *tosana*, two new pinguisane-type and two novel Diels-Alder reaction-type dimeric pinguisane sesquiterpenoids have been isolated. Their absolute structures were determined by a combination of 2D-NMR and CD spectra, X-ray crystallographic analysis, modified Mosher's method, and chemical degradation. © 1998 Elsevier Science Ltd. All rights reserved.

Liverworts (Hepaticae) contain both terpenoids and aromatic compounds which constitute the oil bodies, and occasionally produce their own peculiar dimeric compounds (e.g., bisbibenzyls, dimeric isocuparanes and dimeric labdanes) possessing interesting biological activities such as anti-microbial, cytotoxic, DNA polymerase β inhibitory and neuritic sprouting activities.[1,2] Previously, we reported the isolation and structure elucidation of four new germacranolides, two new guaianolides and a new pinguisane-type sesquiterpene alcohol, 7-oxo-pinguisenol-12-methyl ester (**1**) from the liverwort *Porella acutifolia* subsp. *tosana*.[3] In pursuit of pharmacologically interesting substances found in liverworts, we have reinvestigated the chemical constituents of the Et_2O extract of the same species and isolated two new pinguisane-type sesquiterpenoids named acutifolones A and B (**2**, **3**) and two novel Diels-Alder reaction-type dimeric pinguisane sesquiterpenoids named bisacutifolones A and B (**4**, **5**). Here we wish to report the isolation and structure elucidation of **2~5.**

The ether extract (23.9 g) of dry material (1.24 kg) of *P. acutifolia* subsp. *tosana* collected in Kouchi in 1996 was subjected repeatedly to column chromatography on Sephadex LH-20 ($CHCl_3$:MeOH=1:1) and silica gel (*n*-hexane-AcOEt, gradient) to afford acutifolones A (**2**)[5] (100 mg) and B (**3**)[6] (65 mg), and bisacutifolones A (**4**)[7] (268 mg) and B (**3**)[8] (65 mg) with three known compounds, 4α, 5β-epoxy-8-epi-inunolide[2] (1.331 g), perrottetianal A[2] (1.346 g) and 7-oxo-pinguisenol-12-methyl ester (**1**)(1.050 g).

The relative structure of **1** has previously been determined, but the absolute structure remained to be clarified.[4] We elucidated the absolute configuration of **1** as follows. Reduction of **1** with $LiAlH_4$ gave 7α-hydroxyl- (**6**) and 7β-hydroxyl derivatives (**7**) in 31 % and 25 % yields, respectively. The stereochemistry at C-7 of compounds **6** and **7** was determined by the NOESY spectra (**6**: NOE between H-7 and H-14; **7**: NOE between H-7 and H-1). Compound **6** was esterified with (-)- and (+)-MTPA-Cl and DMAP in pyridine to afford the (-)-MTPA ester (**8**) and the (+)-MTPA ester (**9**), respectively. The Δδ values [$\delta_{(-)}$-$\delta_{(+)}$] shown in Figure 1 indicated that the absolute configuration of C-7 of **6** was represented as *R* by modified Mosher's method.[8] The absolute configuration of 7-oxo-pinguisenol-12-methyl ester was thus determined as **1**.

The FT-IR and UV spectra of acutifolone A (**2**) ($C_{16}H_{22}O_3$) indicated the presence of a carbomethoxyl (1732 cm^{-1}) group and an α, β, γ, δ–conjugated carbonyl [1657 cm^{-1}; λ_{max}269 nm (log ε 4.18)] group. The ^1H

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and ^{13}C NMR spectra of **2** were quite similar to those of **1** except for the presence of a trisubstituted olefin [δ_H 5.99 (*s*, H-6); δ_C 124.3 (*d*, C-6), 160.3 (*s*, C-5)] in place of the methylene protons at H-6 of **1**, suggesting that **2** was the dehydrated compound of **1**. Dehydration [$POCl_3$ / Py] of **1** gave **2** in 93 % yield; hence, the absolute structure of acutifolone A (**2**) was determined as the dehydrated compound of 7-oxo-pinguisenol-12-methyl ester (**1**).

The FT-IR spectra of acutifolone B (**3**) ($C_{15}H_{20}O_3$) indicated the presence of a γ-lactone (1768 cm^{-1}) and a carbonyl (1724 cm^{-1}) group. The ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **1** except for the disappearance of a carbomethoxyl [δ_H 3.70 (*s*, -OMe); δ_C 51.2 (*s*, -OMe)] group of **1**, suggesting that **3** was a compound obtained by the demethanolysis compound of **1**. The stereostructure of **3** was deduced from careful analysis of HMBC and NOESY spectra of **3** and finally established by its X-ray crystallography[9] as shown in Figure 2.

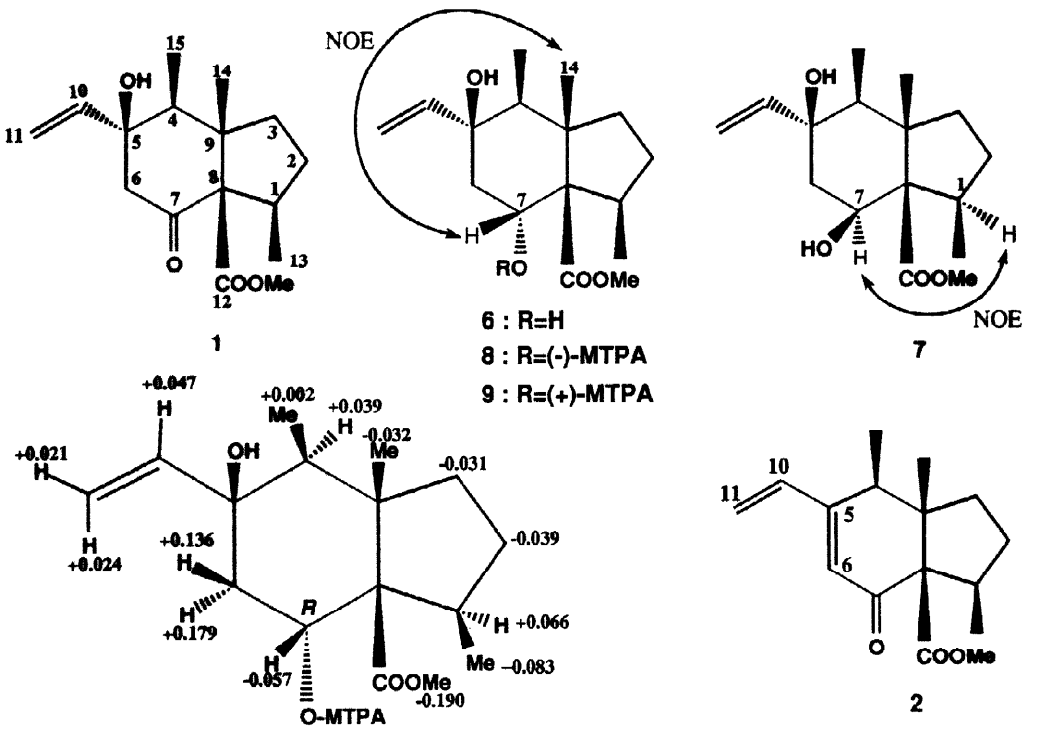
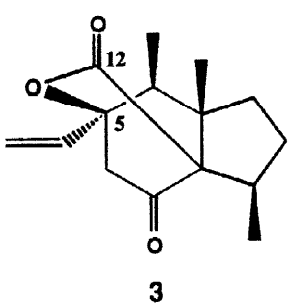


Figure 1. Δδ Values [$\delta_{(-)} - \delta_{(+)}$] for the MTPA esters (**8** and **9**)



3

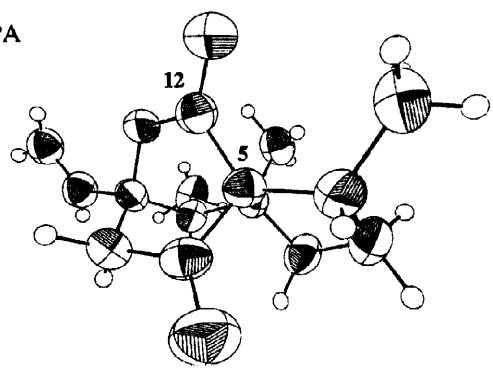
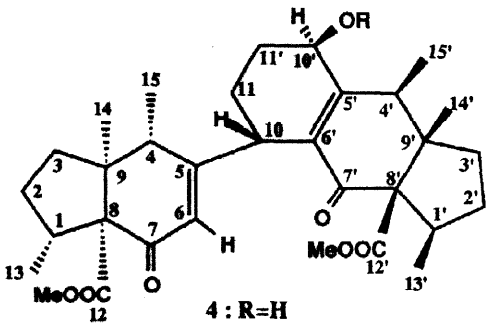


Figure 2. ORTEP Drawing of **3**

Bisacutifolone A (**4**), obtained as colorless prisms, has the molecular formula $C_{32}H_{44}O_7$ established by HR-MS (M^+: m/z 540.3058), suggesting that **4** was a dimeric compound of acutifolone A (**2**). The IR and UV spectrum of **4** indicated the presence of a hydroxyl (3513 cm^{-1}) group, an α, β-unsaturated carbonyl [1657 cm^{-1}; λ_{max} 251 nm (log ε 4.11)] group and a carbomethoxyl (1732 cm^{-1}) group. The ^1H and ^{13}C NMR spectra of

4 showed the presence of a trisubstituted α, β-unsaturated ketone [δ_H 5.55 (*d*, *J*=2.2 Hz); δ_C 124.0 (*d*, C-6), 168.5 (*s*, C-5), 195.1 (*s*, C-7)], a tetrasubstituted α, β-unsaturated ketone [δ_C 133.1 (*s*, C-6'), 159.6 (*s*, C-5'), 195.3 (*s*, C-7')], two carbomethoxyl [δ_H 3.66 (*s*); δ_C 171.2 (*s*)] and a secondary alcohol [δ_H 4.35 (*br.s*, H-10)], which was confirmed by the formation of a monoacetate (**10**) [δ 2.11 (3H, s)] on acetylation with Ac_2O and pyridine. The stereostructure of **4** was deduced from careful analysis of HMBC and NOESY spectra of **4** and finally established by X-ray crystallography[10] of 4 as shown in Figure 3. The absolute configuration of **4** was elucidated by two experimental results described below. The CD spectrum of *p*-bromobenzoate (**11**) of 4 showed a positive first Cotton effect at 255 nm (Δε +30.10) and a negative second Cotton effect at 230 nm (Δε -3.12), and the absolute confuguration of C-10' was presumed to be *R* configuration by an exciton chirality method[11] as applied to the conjugated enone. Compound **4** was esterified with (-)- and (+)-MTPA, DCC and DMAP in CH_2Cl_2 to afford the (-)-MTPA ester (**12**) and the (+)-MTPA ester (**13**), respectively. The Δδ values [$\delta_{(-)}$-$\delta_{(+)}$] shown in Figure 4 revealed that the absolute configuration of C-10' of **4** was represented as *R*. The absolute configuration of bisacutifolone A was thus determined as **4**.

Bisacutifolone B (**5**) has the same molecular formula $C_{32}H_{44}O_7$ (HR-MS (M^+: m/z 540.3058) as that of bisacutifolone A (**4**). The IR, UV, and ^1H and ^{13}C NMR spectra of **5** quite resembled those of **4**, except that the chemical shift [δ_H3.48 (*dd*, *J*=5.9, 5.9 Hz)] of a methine at C-10 in **5** appeared at higher field than that [δ_H3.58 (*dd*, *J*=5.9, 6.0 Hz)] of **4**. The absolute structure of bisacutifolone B (**5**) was determined from careful analysis of HMBC and NOESY spectra of **5**, which were smilar to those of **4**, and the CD spectrum [259 nm (Δε +11.56) and 241 nm (Δε -10.77)] of *p*-bromobenzoate (**14**) of **5** as the C-10 epimer of **4**.



4 : R=H
10 : R=Ac
11 : R=*p*-Br-Bz
12 : R=(-)-MTPA
13 : R=(+)-MTPA

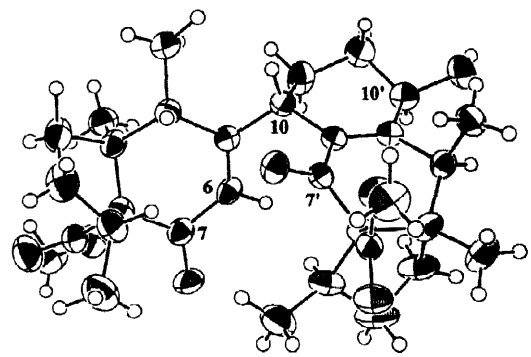
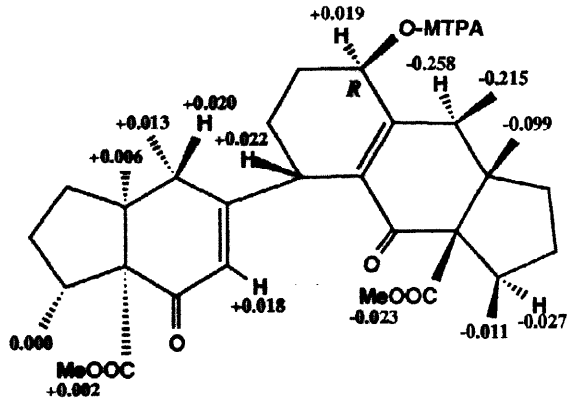
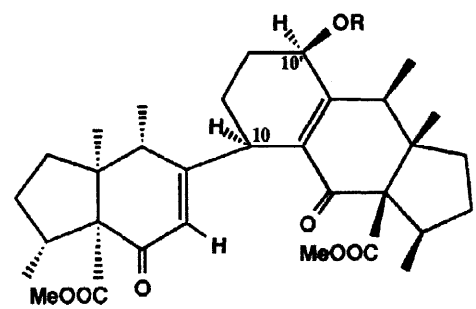


Figure 3. ORTEP Drawing of **4**





5 : R=H
14 : R=*p*-Br-Bz

Figure 4. Δδ Values [$\delta_{(-)}$ - $\delta_{(+)}$] for the MTPA esters (**12** and **13**)

Bisacutifolones A and B (**4**, **5**) from the liverwort *P. acutifolia* subsp. *tosana* might be biosynthesized through the enzymatical Diels-Alder reaction of acutifolone A (**2**) itself, because the dimeric products were not obtained by heating of **2** in toluene. This is the first report of dimeric pinguisane sesquiterpenoids from nature. Compounds **1-5** did not show cathepsins B and L inhibitory activities. The work on other bioassays of **1-5** is in progress.

References and Notes

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2. Y. Asakawa, Chemical Constituents of Bryophytes in "*Progress in the Chemistry of Organic Natural Products*" (W. Herz, W. G. Kirby, Moore, W.Steglich and Ch. Tamm eds.), Vol. **65**, P. 1. Wien-NewYork, Springer (1996).
3. M. Toyota, A. Ueda, and Y. Asakawa, *Phytochemistry*, **30**, 567 (1991).
4. **2** : colorless prisms; mp 102-104°; $[\alpha]_D^{19}$+2.08° (*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^+), 180, 108 (100), 79; FT-IR (KBr) cm^{-1}: 1732 (COO-), 1657 (C=O); UV (EtOH) λ_{max} nm(log ε): 269 (4.18); ^{1}H NMR($CDCl_3$): δ 1.11 (3H, *s*, H-14), 1.18 (3H, *d*, *J*=7.1 Hz, H-15), 1.25 (3H, *d*, *J*= 7.1 Hz, H-13), 3.67 (3H, *s*, OMe), 5.45 (1H, *d*, J=10.7 Hz, H-11), 5.69 (1H, *d*, J=17.3 Hz, H-11), 5.99 (1H, *s*, H-6), 6.46 (1H, *dd*, J=10.7, 17.3 Hz, H-10); ^{13}C NMR ($CDCl_3$): δ 120.1 (*t*, C-11), 124.3 (*d*, C-6), 136.8 (*d*, C-10), 160.3 (*s*, C-5), 171.2 (*s*, C-12), 197.1 (*s*, C-7).
5. **3** : colorless prisms; mp 138-140°; $[\alpha]_D^{19}$ -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^+), 178, 165, 123 (100); FT-IR (KBr) cm^{-1}: 1768 (COO-); 1724 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.03 (3H, *d*, *J*=7.1 Hz, H-15), 1.10 (3H, *s*, H-14), 1.41 (3H, *d*, *J*= 7.1 Hz, H-13), 1.43 (3H, *s*, H-18), 5.34 (1H, dd, J=1.1, 11.3 Hz, H-11), 5.48 (1H, dd, J=1.10, 17.4 Hz, H-11), 5.85 (1H, *dd*, J=11.3, 17.4 Hz, H-10); ^{13}C NMR ($CDCl_3$): δ 84.6 (*s*, C-5), 115.9 (*t*, C-11), 134.4 (*d*, C-10), 168.7 (*s*, C-12), 203.5 (*s*, C-7).
6. **4** : colorless prisms; mp 204-206°; $[\alpha]_D^{24}$ +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 (100), 480, 123, 32; FT-IR (KBr) cm^{-1}: 3513 (OH), 1732 (COO-), 1657 (C=O); UV (EtOH) λ_{max}nm (logε): 251 (4.11); ^{1}H NMR($CDCl_3$): δ 0.90 (3H, *s*, H-14), 0.97 (3H, *d*, *J*= 6.6 Hz, H-13'), 1.01 (3H, *s*, H-14'), 1.06 (3H, *d*, *J*=6.9 Hz, H-13), 1.27 (3H, *d*, *J*= 6.9 Hz, H-15'), 1.29 (3H, *d*, *J*= 7.1 Hz, H-15), 3.66 (6H, *s*, 2xCOOMe), 4.35 (1H, *br.s*, H-10'), 5.55 (1H, *d*, J=2.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 124.0 (*d*, C-6), 133.1 (*s*, C-6'), 159.6 (*s*, C-5'), 168.5 (*s*, C-5), 168.7 171.2 (*s*, C-12, 12'), 195.1 ((*s*, C-7), 195.3 (*s*, C-7').
7. **5** : 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), 480, 462, 123, 32; FT-IR (KBr)cm^{-1}: 3479, 1730, 1655 (C=O); ^{1}H NMR($CDCl_3$): δ 0.91 (3H, *s*, H-14), 1.00 (3H, *d*, *J*= 6.6 Hz, H-13'), 1.02 (3H, *s*, H-14'), ,1.07 (3H, *d*, *J*=6.9 Hz, H-13), 1.16 (3H, *d*, *J*= 7.1 Hz, H-15'), 1.28 (3H, *d*, *J*= 7.1 Hz, H-15), 3.66 (6H, *s*, 2x COOMe), 4.29 (1H, *br.s*, H-10'), 5.82 (1H, *d*, J=2.2 Hz, H-6), ^{13}C NMR ($CDCl_3$): δ 124.2 (*d*, C-6), 171.3 (*s*, C-12, 12'), 195.4 (*s*, C-7), 195.7 (*s*, C-7').
8. T. Kusumi, T. Hamada, M. O. Ishitsuka, I. Ohtani and H. Kakizawa, *J. Org. Chem.*, **57**, 1033 (1992).
9. The crystal data for **2** are as follows : monoclinic; space group $P2_1$ with a=11.343 (0), b=8.296 (0), c=7.288 (0) Å, β=106.507 (0)°, V=657.6 (0)Å^3, Z=2, and μ(Cu K-α)=6.553mm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.058 for 936 reflections.
10. The crystal data for **3** are as follows : Orthorhombic; space group $P2_12_12_1$ with a=13.720 (5), b=21.053 (7), c=9.954 (5)Å, V=2875.3 (2)Å^3, Z=4, and μ(Cu K-α)=6.64mm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.046 for 2156 reflections.
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