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Squamocin-O_1 and squamocin-O_2, new adjacent bis-tetrahydrofuran acetogenins from the seeds of *Annona squamosa*

Hiroshi Araya[a], Mahendra Sahai[b], Shubhra Singh[b], Ajit Kumar Singh[b], Mitsuzi Yoshida[c], Noriyuki Hara[d], Yoshinori Fujimoto[d,*]

[a]*National Institute for Agro-Environmental Sciences, Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan*
[b]*Department of Medicinal Chemistry, I.M.S., Banaras Hindu University, Varanasi 221005, India*
[c]*Pharmacology Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[d]*Department of Chemistry and Materials Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152-8551, Japan*

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Abstract

Two bis-tetrahydrofuran acetogenins, squamocin-O_1 (**1**) and squamocin-O_2 (**2**), were isolated from a MeOH extract of seeds of *Annona squamosa* L. Their structures were determined by spectral means including precursor-ion scanning mass spectral analysis for their aminal derivatives. The configurations at the oxymethine chiral centers were assigned as 12*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S* for **1** and 12*S*,15*R*,16*R*,19*R*,20*R*,23*R*,24*S*, 28*S*,36*S* for **2**, based on ^{1}H NMR analysis of their Mosher's ester derivatives and CD data.

Keywords: *Annona squamosa*; Annonaceae; Bis-tetrahydrofuran acetogenin; Squamocin

1. Introduction

Annonaceous tetrahydrofuran acetogenins have attracted much interest due to their broad range of biological activities (Alali et al., 1999). *Annona squamosa* L. (Annonaceae) is well known for its edible tropical fruits and as custard apple, and its seeds are reported to have insecticidal and abortifacient properties (Chopra et al., 1956). In a continuation of previous studies, two adjacent bis-tetrahydrofuran acetogenins named squamocin-O_1 (**1**) and squamocin-O_2 (**2**) (Fig. 1) were isolated from a fraction of the MeOH extract. Previously, the isolation and structure elucidation of more than twenty acetogenins from of the seeds of *Annona squamosa* L., among which squamocin (**3**) and squamostatin-A were two major constituents (Fujimoto et al., 1988, 1990, 1994; Sahai et al., 1994; Araya et al., 1994a,b).

2. Results and discussion

Compounds **1** and **2** were initially obtained as a mixture (planar structure of this mixture was briefly reported: Araya et al., 1994c) by reversed phase HPLC from a fraction which was more mobile than squamostatin-A. The separation of the mixture was achieved by reversed-phase HPLC with MeOH–CH_3CN–H_2O–iPrOH (120:40:30:1) as an eluting solvent, to afford the more mobile squamocin-O_1 (**1**) and the less mobile squamocin-O_2 (**2**).

Compounds **1** and **2** showed UV (210 mn) and IR (1750 cm^{-1}) absorptions typical of α,β-unsaturated-γ-lactone moiety of annonaceous acetogenins. The same molecular formula, $C_{37}H_{66}O_8$, was assigned to **1** and **2** on the basis of HR-FAB-MS data. The ^{1}H and ^{13}C NMR spectra of compounds **1** and **2** resembled those of **3**, but had additional oxymethine signal (δ_H 3.60/δ_C 71.5 for **1** and 3.58/71.7 for **2**). The spectral data suggested that compounds **1** and **2** were mono-hydroxylated analogs of **3**. The EI-MS spectra indicated that compounds **1** and **2** belong to an adjacent bis-tetrahydrofuran family and the bis-tetrahydrofuran moiety is located

* Corresponding author. Tel.: +81-3-5734-2241; fax: +81-3-5734-2241.
E-mail address: fujimoto@cms.titech.ac.jp (Y. Fujimoto).

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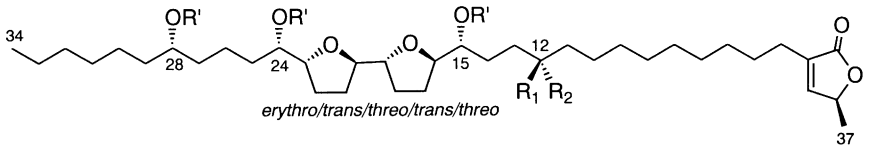


1: R_1 = OH, R_2 = R' = H
1r: R_1 = -O-(*R*)-MTPA, R' = -(*R*)-MTPA, R_2 = H
1s: R_1 = -O-(*S*)-MTPA, R' = -(*S*)-MTPA, R_2 = H
2: R_1 = R' = H, R_2 = OH
2r: R_1 = H, R_2 = -O-(*R*)-MTPA, R' = -(*R*)-MTPA
2s: R_1 = H, R_2 = -O-(*S*)-MTPA, R' = -(*S*)-MTPA
3: R_1 = R_2 = R' = H
3r: R_1 = R_2 = H, R' = -(*R*)-MTPA
3s: R_1 = R_2 = H, R' = -(*S*)-MTPA

Fig. 1. Structures of squamocin-O_1 (**1**), squamocin-O_2 (**2**) and squamocin (**3**).

from C-15 to C-24 (Fig. 2). Further, the fragmentation pattern suggested that the extra hydroxyl group should be located along the methylene chain between the lactone and bis-tetrahydrofuran moieties, although routine mass analysis failed to assign the position of the hydroxyl group.

We previously developed a precursor ion-scanning method for mass spectral analysis of acetogenin aminal derivatives (Hirayama et al., 1993; Araya et al., 1994c). Application of this method was found to be useful in establishing the position of the hydroxyl group. The *N*,*N*-dimethylethylenediamine derivative of **1** (**1a**, for the structure, see Fig. 3) clearly showed ions at *m*/*z* 293 and 323 due to the fission C11–C12 and C12–C13 followed by dehydration, respectively, in the precursor-ion spectrum from *m*/*z* 72 ion $[CH_2{=}CHN^+H(CH_3)_2]$ (Fig. 3). Thus, the hydroxyl group was unequivocally assigned to the C-12 position. The spectrum also confirmed the positions of the bis-tetrahydrofuran moiety and C-28 hydroxyl group. The precursor ion-scanning spectrum of the *N*,*N*-dimethylethylenediamine derivative of **2** was essentially same as that of **1a**, confirming that **1** and **2** have the same planar structure.

The configurations at the oxymethine centers were established as follows. The stereochemistry around the bis-tetrahydrofuran moiety of **1** and **2** was readily determined to be *threo*/*trans*/*threo*/*trans*/*erythro* (from C-15 to C-24) by comparing their ^1H and ^{13}C NMR spectral data (Table 1) with those of **3** and stereochemically defined bis-tetrahydrofuran acetogenins (Sahai et al., 1994). The absolute configuration at C-36 was established to be *S*, typical for annonaceous acetogenins, on the basis of negative Cotton effects at 239

Table 1
^{13}C NMR spectral data for squamocin-O_1 (**1**) and squamocin-O_2 (**2**)

C	**1**	**2**
1	173.9	173.9
2	134.3	134.3
3	25.1	25.1
4	27.3	27.4
5–9	[a]	[a]
10	25.8	25.8
11	37.5	37.7
12	71.5	71.7
13	33.5	34.3
14	[a]	[a]
15	74.3	74.6
16	83.1	83.2
17	28.4	28.4
18	28.9	29.0
19	82.2[b]	82.2[c]
20	82.5[b]	82.5[c]
21	28.9	29.0
22	24.8	24.8
23	82.8	82.9
24	71.3	71.2
25	32.4	32.5
26	22.1	22.2
27	37.3	37.4
28	71.7	71.9
29	37.5	37.7
30	25.6	25.7
31	[a]	[a]
32	31.8	31.9
33	22.6	22.6
34	14.0	14.1
35	148.9	148.8
36	77.4	77.4
37	19.2	19.2

[a] The signals appeared in the region of δ 29.0–30.0.
[b] Assignments may be interchanged within the column.
[c] Assignments may be interchanged within the column.

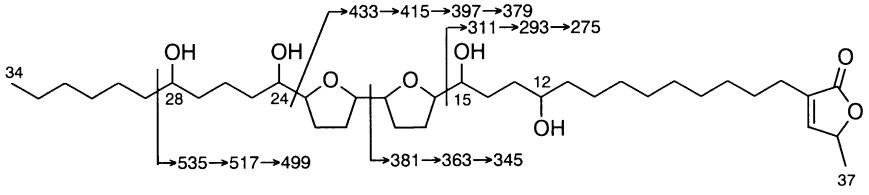
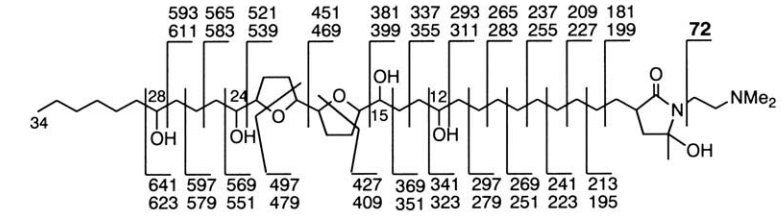


Fig. 2. EI-MS fragmentation pattern of squamocin-O_1 (**1**) and squamocin-O_2 (**2**).



1a

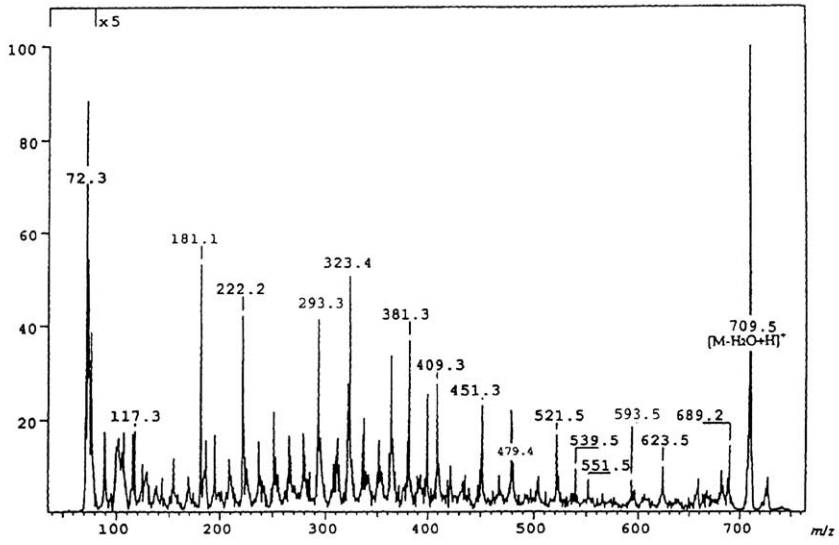


Fig. 3. Precursor ion-scanning spectrum of *N*,*N*-dimethylethylenediamine derivative (**1a**) of squamocin-O_1 (**1**). The accompanied *m*/*z* values are due to dehydration ions.

Table 2
^{1}H NMR spectral data for (*R*)- and (*S*)-MTPA esters (**1rs**–**3rs**)

No.	**1r**	**1s**	**2r**	**2s**	**3r**	**3s**
4	2.27 (*t*[a])	2.26 (*t*[a])	2.27 (*t*[a])	2.26 (*t*[a])	2.26 (*t*[a])	2.26 (*t*[a])
12	4.94 (*m*)	5.00 (*m*)	5.02 (*m*)	5.09 (*m*)	–	–
15	4.98 (*m*)	5.00 (*m*)	4.95 (*m*)	5.09 (*m*)	5.02 (*m*)	5.06 (*q*)
16	3.96 (*q*)	3.94 (*m*)	3.89 (*m*)	3.99 (*m*)	3.99 (*m*)	4.03 (*q*)
19	3.63 (*m*)	3.77 (*m*)	3.62 (*m*)	3.78 (*m*)	3.65 (*m*)	3.79 (*m*)
20	3.80 (*m*)	3.77 (*m*)	3.78 (*m*)	3.78 (*m*)	3.83 (*m*)	3.79 (*m*)
23	3.86 (*m*)	3.94 (*m*)	3.86 (*m*)	3.95 (*m*)	3.87 (*m*)	3.96 (*m*)
24	5.14 (*q*-like[b])	5.20 (*q*-like[b])	5.14 (*m*)	5.20 (*m*)	5.14 (*m*)	5.20 (*q*[b])
28	5.02 (*m*)	5.00 (*m*)	5.02 (*m*)	4.99 (*m*)	5.02 (*m*)	4.99 (*m*)
34	0.874 (*t*[c])	0.859 (*t*[c])	0.874 (*t*[c])	0.860 (*t*[c])	0.874 (*t*[c])	0.860 (*t*[c])
35	6.98 (*brs*)	6.98 (*brs*)	6.98 (*brs*)	6.98 (*brs*)	6.98 (*brs*)	6.98 (*brs*)
36	4.99 (*m*)	4.99 (*m*)	4.99 (*m*)	4.99 (*m*)	4.99 (*m*)	4.99 (*m*)
37	1.40 (*d*[d])	1.40 (*d*[d])	1.39 (*d*[d])	1.39 (*d*[d])	1.41 (*d*[d])	1.40 (*d*[d])
MeO	3.48	3.49	3.51	3.50	3.51	3.51
	3.51	3.51	3.51	3.50	3.53	3.53
	3.53	3.53	3.53	3.52	3.61	3.55
	3.60	3.53	3.57	3.53		

[a] $J = 6.9$ Hz.
[b] $J = 6.6$ Hz.
[c] $J = 7.2$ Hz.
[d] $J = 6.4$ Hz.

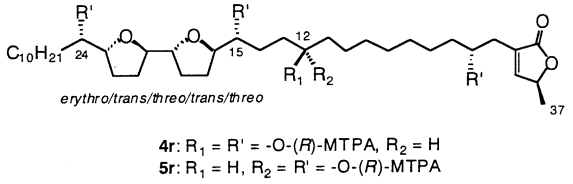
nm in their CD spectra. The C-28 configuration of **1** and **2** was deduced from the ^{1}H chemical shifts of the terminal methyl group (C-34) in their (*R*)- and (*S*)-tetra-MTPA ester derivatives (**1r**/**1s** and **2r**/**2s**) (Table 2) (Nishioka et al., 1994). The (*R*)-MTPA esters **1r**, **2r** and **3r** all displayed signals due to 34-H_3 at δ 0.874, while the (*S*)-MTPA esters **1s**, **2s** and **3s** consistently exhibited the corresponding signals at δ 0.860. Thus, 28*S* configuration was assigned to compounds **1** and **2**, since compound **3** is known to have 28*S*.

Absolute configuration of the bis-tetrahydrofuran moiety of **1** was also determined to be 15*R*,16*R*,19*R*,20*R*,23*R*,24*S*, the same as previously reported for **3** (Sahai et al., 1994). The alternative 15*S*,16*S*,19*S*,20*S*,23*S*,24*R* configuration was ruled out, since the chemical shifts of 28-H, 24-H and 23-H of **1rs** and **2rs** were essentially identical to those of the respective squamocin derivatives **3r** and **3s**. The whole data discussed above revealed that compounds **1** and **2** have 15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S* configuration, but are epimeric at the C-12 position. The chemical shifts of H-15 and H-16 of **1r** and **2r** were not in accord with those of **3r**, as expected from the occurrence of the additional MTPA group at C-12. Shi et al. proposed a novel application of Mosher's ester method for the determination of the absolute stereochemistry of epimeric carbinol centers (Shi et al., 1997). This method was successfully applied to compounds **1** and **2**. The chemical shifts for pertinent ^{1}H signals of the MTPA esters, **1r** and **2r**, are listed in Table 3. The Table also includes the corresponding data [(*R*)-MTPA esters (**4r** and **5r**)] of structurally related acetogenins, 12-hydroxybullatacins A (**4**) and B (**5**) (Shi et al., 1997). The sign and magnitude of the values of $\Delta\delta_H$ (**1r**–**2r**) are in excellent agreement with those of $\Delta\delta_H$ (**4r**–**5r**). These studies established that compound **1** has 12*R* configuration whereas compound **2** has 12*S*. The assignments were further corroborated by comparing the ^{13}C NMR data of **1**, **2**, **4** and **5**: $\delta_{C\text{-}12}$ and $\delta_{C\text{-}15}$; 71.5, 74.3 for **1**; 71.7, 74.6 for **2**; 71.5, 74.2 for **4** (12*R*) (Shi et al., 1997); 71.8, 74.4 for **5** (12*S*) (Shi et al., 1997). The stereochemical structure of **1** (12*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S*) and **2** (12*S*,15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S*) are shown in Fig. 1.

Table 3
Difference in ^{1}H chemical shifts (ppm) between MTPA esters, **1r**, **2r**, **4r** and **5r**

Esters	H-12	H-15	H-16	H-19	H-20
1r	4.94	4.98	3.96	3.63	3.80
2r	5.02	4.95	3.89	3.62	3.78
$\Delta\delta_H$ (**1r**–**2r**)	–	+0.03	+0.07	+0.01	+0.02
4r[a]	4.93	4.97	3.96	3.63	3.80
5r[a]	5.00	4.95	3.89	3.62	3.79
$\Delta\delta_H$ (**4r**–**5r**)[a]	–	+0.02	+0.07	+0.01	+0.01

[a] Adopted from Shi et al., 1997.



4r: R_1 = R' = -O-(*R*)-MTPA, R_2 = H
5r: R_1 = H, R_2 = R' = -O-(*R*)-MTPA

Squamocin-F (**6**) (Fig. 4), isolated by our group from the seeds of *A. squamosa*, is a related 12-hydroxylated acetogenin, and the configuration at C-12 of **6** remained to be elucidated (Sahai et al., 1994). With a set of accurate ^{13}C NMR spectral data for compounds **1** and **2**, the C-12 configuration of **6** was determined as *R* (therefore the absolute configuration 12*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,36*S* could be assigned), since the ^{13}C NMR values near the stereogenic center [37.6 (C-11), 71.7 (C-12), 33.5 (C-15)] are in good agreement with those of squamocin-O_1, but not with those of squamocin-O_2. It should be noted that the C-15 chemical shifts show diagnostic difference between the C-12 epimers (δ 33.5 for **1** vs 34.3 for **2**, see Table 1).

Salzmanin, recently isolated from *A. salzmanii* roots, has the same planar structure as compounds **1** and **2**. This closely related bis-tetrahydrofuran acetogenin is reported to have 12*R**,15*R**,16*R**,19*R**,20*S**,23*R**,24*S**,28*S** (*implies relative configuration) configuration (*threo*/*trans*/*erythro*/*cis*/*erythro*) (Queiroz et al., 1999).

The brine shrimp toxicity (Alkofahi et al., 1989) of **1** and **2** was much less than that of **3** (LD_{50} values: 1.0 ppm for **1**, 1.0 ppm for **2**, and 0.07 ppm for **3**). The cytotoxic activity of squamocin-O_1 (**1**) and squamocin-O_2 (**2**) against human K562 leukemia and HLE hepatoma cells were also investigated. Compounds **1** and **2** displayed much lower activity on the cancer cell lines, compared with squamocin (**1**: K562, $IC_{50} = 4.0\times10^{-4}$ μg/ml; HLE, $IC_{50} = 3.7\times10^{-3}$ μg/ml, **2**: K562, $IC_{50} = 4.3\times10^{-4}$ μg/ml; HLE, $IC_{50} = 3.5\times10^{-3}$ μg/ml, **3**: K562, $IC_{50} = 2.4\times10^{-5}$ μg/ml; HLE, $IC_{50} = 5.0\times10^{-5}$ μg/ml).

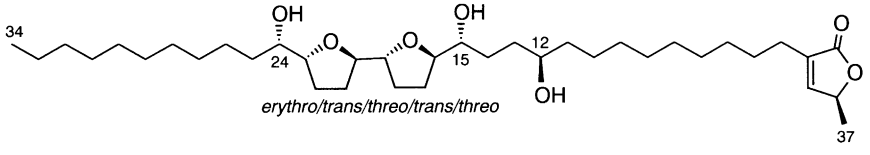


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Fig. 4. Structure of squamocin-F (**6**).

3. Experimental

3.1. General

EI- (70 eV) and FAB-MS spectra were obtained with a JEOL JMS-AX505HA spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GSX-500 spectrometer or LAMBDA-400 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. IR spectra were determined on a JASCO IR-810 spectrometer. UV spectra were obtained on a Shimadzu UV-200 spectrometer. The method and derivatization for the precursor ion scanning were detailed in our previous paper (Hirayama et al., 1993). HPLC was performed on a Shimadzu LC-6A apparatus equipped with a SPD-6A UV detector (220 nm).

3.2. Isolation

In the previous HPLC separation of the MeOH extract of *A. squamosa* seeds (1 kg) (Fujimoto et al., 1994), a more mobile fraction than squamostatin-A was collected and stored. This fraction showed a broad peak about 12.5 min (14.0 min for squamostatin-A) when analyzed by HPLC (column: Shimadzu Shim-Pack CLC-ODS (25 cm × 10 mm i.d.); solvent: MeOH–H_2O 10:1; flow rate 1.0 ml/min). The separation of the peak afforded a mixture of compounds **1** and **2** (46 mg). This was further separated by HPLC (column: STR PREP-ODS (25 cm × 10 mm i.d.); solvent, MeOH–CH_3CN–H_2O–iPrOH (120:40:30:1); flow rate 6.0 ml/min; typical retention times, 39 min for **1**, 43 min for **2**) to furnish compounds **1** and **2**. (*R*)- and (*S*)-tetra-MTPA esters were prepared from compounds **1** and **2** (1 mg each) as described previously (Sahai et al., 1994).

3.3. Squamocin-O_1 (**1**)

White wax (20 mg), $[\alpha]_D^{25}$ +17.7° (c = 0.6, MeOH), CD (MeOH) $\Delta\varepsilon$ (nm) −0.45 (239), UV λ_{max} (MeOH) nm (log ε): 210 (3.8). IR ν_{max} ($CHCl_3$) cm^{-1}: 3690, 3585, 3460, 1750. EI-MS m/z: 620, 602, 517, 505, 415, 397, 379, 363, 345, 293, 275, 97. HR-FAB-MS m/z: 639.4792 $[M+H]^+$ (calc. for $C_{37}H_{67}O_8$, 639.4835). ^{1}H NMR δ: 0.88 (3H, *t*, J = 6.4 Hz, H-34), 1.41 (3H, *d*, J = 6.4 Hz, H-37), 2.26 (2H, *t*, J = 7.8 Hz, H-3), 3.45 (1H, *br t*, J = 7.8 Hz, H-15), 3.60 (2H, *m*, H-12, H-28), 3.76–3.96 (5H, *m*, H-16, -19, -20, -23, -24), 5.00 (1H, *qq*, J = 6.8, 1.9 Hz, H-36), 6.99 (1H, *s*, H-35). ^{13}C NMR spectral data: Table 1. ^{1}H NMR spectral data of (*R*)- and (*S*)-MTPA esters: Table 2.

3.4. Squamocin-O_2 (**2**)

White wax (9 mg), $[\alpha]_D^{25}$ +17.4° (c = 1.0, MeOH), CD (MeOH) $\Delta\varepsilon$ (nm)−0.45 (239), UV λ_{max} (MeOH) nm (log ε): 210 (3.8). IR ν_{max} ($CHCl_3$) cm^{-1}: 3690, 3585, 3460, 1750. The EI-MS spectrum was essentially identical to that of **1**. HR-FAB-MS m/z: 639.4781 $[M+H]^+$ (calc. for $C_{37}H_{67}O_8$, 639.4835). ^{1}H NMR δ: 0.88 (3H, *t*, J = 6.9 Hz, H-34), 1.41 (3H, *d*, J = 6.4 Hz, H-37), 2.26 (2H, *t*, J = 7.8 Hz, H-3), 3.45 (1H, *br t*, J = 7.8 Hz, H-15), 3.58 (2H, *m*, H-12, H-28), 3.76–3.96 (5H, *m*, H-16, -19, -20, -23, -24), 5.00 (1H, *qq*, J = 7.0, 1.8 Hz, H-36), 6.99 (1H, *s*, H-35). ^{13}C NMR data: Table 1. ^{1}H NMR spectral data of (*R*)- and (*S*)-MTPA esters: Table 2.

3.5. Biological assays

Brine shrimp test was perfomed according to a published method (Alkofahi et al., 1989). Cytotoxic activities against K562 and HLE cells were determined according to the procedure described in our previous paper (Yoshida et al., 2001). The IC_{50} values showed in the text are the average of triplicate assays.

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