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Stelliferins J–N, isomalabaricane-type triterpenoids from Okinawan marine sponge *Rhabdastrella* cf. *globostellata*

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ABSTRACT

Five new isomalabaricane-type triterpenoids, stelliferins J–N (**1**–**5**), were isolated from Okinawan marine sponge *Rhabdastrella* cf. *globostellata*. The structures of **1**–**5** were elucidated from the spectroscopic data and chemical means including application of a modified Mosher's method and an exciton chirality method.

1. Introduction

The sponges belonging to the genera *Rhabdastrella*, *Jaspis*, *Stelletta*, and *Geodia* (order Astrophorida) are known to a rich source of isomalabaricanes, which are a class of *trans-syn-trans* 6,6,5-tricyclic terpenoids possessing a side chain to be positioned at C-13.[1–3] We previously isolated isomalabaricane-type terpenoids, stelliferins A–F[4] and jaspiferals A–G,[5] from the Okinawan marine sponge *Jaspis stellifera*. During our search for structurally unique metabolites from Okinawan marine sponges, five new isomalabaricane-type triterpenoids, stelliferins J–N (**1**–**5**), were isolated from a marine sponge *Rhabdastrella* cf. *globostellata*. In this paper, we describe the isolation and structure elucidation of **1**–**5**.

2. Results and discussion

The sponge *R.* cf. *globostellata* (SS-201) collected off Ishigaki island, Okinawa, was extracted with MeOH and then $CHCl_3$. The combined extracts were partitioned between EtOAc and H_2O. The EtOAc-soluble portions were subjected to a silica gel and C_{18} column chromatographies, and then purified by C_{18} HPLC to yield stelliferins J (**1**, 0.00023%, wet weight), K (**2**, 0.00013%), L (**3**, 0.00040%), M (**4**, 0.00016%), and N (**5**, 0.00014%) and known triterpenoids rhabdasins D, E, and F.[2]

Stelliferin J (**1**) was isolated as an optically active colorless amorphous solid {$[\alpha]_D^{24}$ −56.9 (*c* 0.15, MeOH)}, and showed the pseudomolecular ion peak at *m*/*z* 551 $[M+Na]^+$ in the ESIMS. The HRESIMS analysis revealed the molecular formula to be $C_{32}H_{48}O_6$ (*m*/*z* 551.3338 $[M+Na]^+$, Δ −0.5 mmu). IR absorptions at 3387 and 1726 cm^{-1} implied the presence of hydroxy and carbonyl functionalities. The ^1H and ^{13}C NMR spectra of **1** (Table 1) were similar to those of stelliferin A[4] (Fig. 1) except for the signals of a side chain at C-13, implying that **1** was a triterpenoid possessing isomalabaricane-type skeleton with a different side chain at C-13 from that of stelliferin A.

The structure of the side chain (C-14 to C-27) was assigned as follows. The ^1H–^1H COSY spectrum revealed connections of C-16 to C-17 and C-22 to C-24 (Fig. 2). HMBC cross-peaks of H_3-18 to C-13, C-14, and C-15 suggested connectivities among C-13, C-15, and C-18 through C-14. The connection of C-16 to a carbonyl carbon (C-15) was indicated by an HMBC correlation for H-17 to C-15. Similarly, the connections of C-17 to C-21 and C-22 via C-20, and of C-24 to C-26 and C-27 via C-25 were disclosed by the analysis of HMBC correlations (Fig. 2). The geometry of disubstituted olefin (C-16–C-17) was assigned as *E* due to the *J* value of H-16/H-17 (16.1 Hz), while the 13*Z* configuration was disclosed by a NOESY correlation for H_3-30/H_3-18. Thus, the gross structure of stelliferin J (**1**) was elucidated as shown.

The *trans-syn-trans* junction of the tricyclic moiety and the β-orientation of the acetoxy group at C-3 of **1** were assigned by the analysis of the NOESY spectrum (Fig. 3) as well as comparison of

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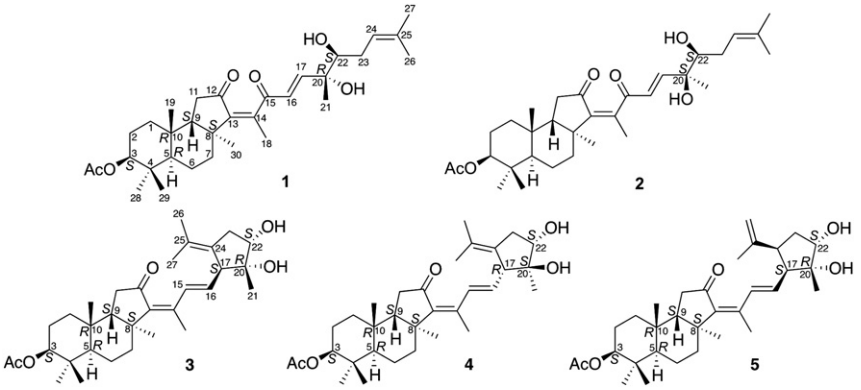

Table 1
^{1}H and ^{13}C NMR data for stelliferins J (**1**) and K (**2**) in $CDCl_3$

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	33.0	1.58, 1.37 (1H each, m)	33.0	1.58, 1.38 (1H each, m)
2	25.0	1.83, 1.69 (1H each, m)	25.0	1.83, 1.68 (1H each, m)
3	80.6	4.54 (1H, dd, *J*=11.6, 5.0 Hz)	80.6	4.55 (1H, dd, *J*=11.6, 5.0 Hz)
4	38.2	—	38.2	—
5	46.6	1.77 (1H, m)	46.6	1.77 (1H, m)
6	17.8	1.74, 1.50 (1H each, m)	17.8	1.73, 1.50 (1H each, m)
7	36.3	2.17, 2.02 (1H each, m)	36.3	2.17, 2.01 (1H each, m)
8	42.9	—	42.9	—
9	51.1	1.86 (1H, m)	51.1	1.86 (1H, m)
10	35.5	—	35.5	—
11	34.5	2.12 (2H, m)	34.5	2.12 (2H, m)
12	204.2	—	204.4	—
13	146.7	—	146.8	—
14	142.7	—	142.8	—
15	200.2	—	200.3	—
16	127.4	6.30 (1H, d, *J*=16.1 Hz)	127.2	6.32 (1H, d, *J*=16.0 Hz)
17	148.4	6.62 (1H, d, *J*=16.1 Hz)	150.2	6.62 (1H, d, *J*=16.0 Hz)
18	16.8	1.91 (3H, s)	16.9	1.92 (3H, s)
19	22.4	1.01 (3H, s)	22.4	1.02 (3H, s)
20	75.0	—	75.1	—
21	24.2	1.34 (3H, s)	22.4	1.30 (3H, s)
22	77.4	3.46 (1H, dd, *J*=10.0, 3.1 Hz)	76.2	3.47 (1H, dd, *J*=9.8, 3.1 Hz)
23	30.8	2.13, 2.09 (1H each, m)	29.7	2.19, 2.13 (1H each, m)
24	120.1	5.16 (1H, t, *J*=7.9 Hz)	120.1	5.17 (1H, t, *J*=6.7 Hz)
25	135.6	—	135.7	—
26	18.0	1.61 (3H, s)	18.0	1.62 (3H, s)
27	25.8	1.72 (3H, s)	25.9	1.73 (3H, s)
28	29.0	0.91 (3H, s)	29.0	0.92 (3H, s)
29	16.9	0.89 (3H, s)	16.9	0.89 (3H, s)
30	24.8	1.39 (3H, s)	24.9	1.38 (3H, s)
3-OAc	171.0	2.05 (3H, s)	171.0	2.06 (3H, s)
	21.2		21.2	

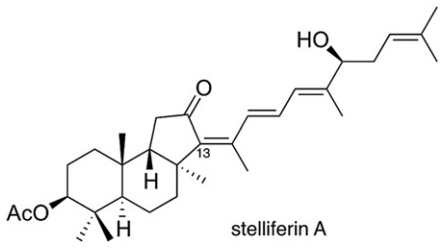

Fig. 1. Structure of stelliferin A.

the ^{1}H and ^{13}C NMR data for the tricyclic moiety of **1** with those of stelliferin A.[4]

The relative relationship for C-20/C-22 of **1** was assigned as follows. Acetonization of **1** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate in CH_2Cl_2 gave the 20,22-*O*-isopropyllidene derivative (**1a**). In the NOESY spectrum of **1a** (Fig. 4), H-17 was correlated to H_2-23 and one of the acetonide methyl (Me-A), while H-22 was correlated to H_3-21 and the other acetonide methyl (Me-B), indicating the cis configuration for C-20/C-22 of **1a**. Therefore, the *erythro* relationship for C-20/C-22 of **1** was established.

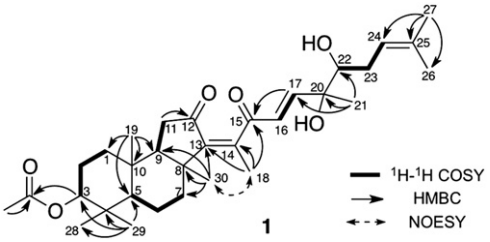

Fig. 2. Selected 2D NMR correlations for stelliferin J (**1**).

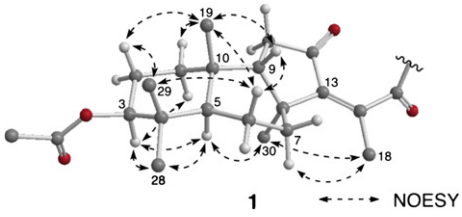

Fig. 3. Selected NOESY correlations and relative stereochemistry for the tricyclic moiety of stelliferin J (**1**) (protons of methyl groups were omitted).

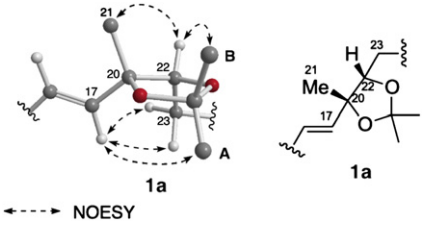

Fig. 4. Selected NOESY correlations and relative stereochemistry for C-20/C-22 of the 20,22-*O*-isopropyllidene derivative (**1a**) of stelliferin J (**1**) (protons of methyl groups were omitted).

To assign the absolute configuration for C-22 of **1**, a modified Mosher's method[6] was applied as follows. Treatment of **1** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoro-2-phenylacetyl chloride (MTPACl) gave the 22-(*S*)- and 22-(*R*)-MTPA esters (**1b** and **1c**, respectively). The $\Delta\delta$ values ($\Delta\delta=\delta_S-\delta_R$) obtained from the ^{1}H NMR data for **1b** and **1c** indicated that the absolute configuration of C-22 was *S* (Fig. 5).

The absolute configuration for C-3 of **1** was assigned as follows. Treatment of the 20,22-*O*-isopropyllidene derivative (**1a**) with K_2CO_3 in MeOH gave the deacetyl derivative (**1d**). The $\Delta\delta$ values obtained for the 3-(*S*)- and 3-(*R*)-MTPA esters (**1e** and **1f**,

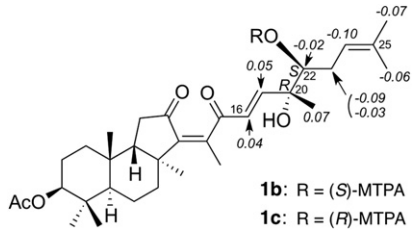

Fig. 5. $\Delta\delta$ values [$\Delta\delta$ (in ppm)$=\delta_S-\delta_R$] obtained for the 22-(*S*)- and 22-(*R*)-MTPA esters (**1b** and **1c**, respectively) of stelliferin J (**1**).

respectively) of **1d** suggested that the absolute configuration of C-3 was *S* (Fig. 6). Consequently, the absolute configurations at seven chiral centers in stelliferin J (**1**) were found to be 3*S*, 5*R*, 8*S*, 9*S*, 10*R*, 20*R*, and 22*S*.

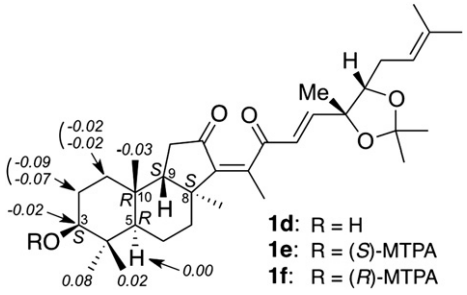

Fig. 6. Structures for the deacetyl derivative (**1d**) of the 20,22-*O*-isopropyllidene derivative (**1a**) of stelliferin J (**1**) and the 3-(*S*)- and 3-(*R*)-MTPA esters (**1e** and **1f**, respectively) derived from **1d**. $\Delta\delta$ values [$\Delta\delta$ (in ppm)$=\delta_S-\delta_R$] obtained for **1e** and **1f** were shown.

Stelliferin K (**2**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{24}$ −33.5 (*c* 0.08, MeOH)}. The HRESIMS analysis indicated that **2** had the same molecular formula, $C_{32}H_{48}O_6$ (*m*/*z* 551.3342 $[M+Na]^+$, Δ −0.1 mmu), as that of **1**. The ^1H and ^{13}C NMR data of **2** were similar to those of **1**, while differences were observed for the chemical shifts of CH-17, CH_3-21, CH-22, and CH_2-23 (Table 1). From these facts, **2** was deduced to be a stereoisomer of **1** at C-20 or C-22. To assign the relative relationship for C-20/C-22, the 20,22-*O*-isopropyllidene derivative (**2a**) was prepared from **2** by the same procedure as described for **1**. NOESY correlations for H-17/H-22, H-22/Me-B, and H_3-21/H_2-23 in **2a** indicated the *trans* configuration for C-20/C-22 in **2a**, thereby implying the *threo* relationship for C-20/C-22 of **2** (Fig. 7). The 20*S* and 22*S* configurations of **2** were assigned by the application of a modified Mosher's method (Fig. 8). The absolute stereochemistry for the tricyclic moiety of **2** was not elucidated due to a small isolated amount of **2**. Thus, the structure of stelliferin I was assigned to be **2**.

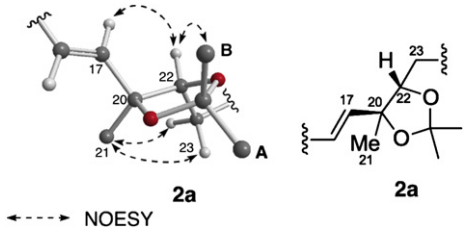

Fig. 7. Selected NOESY correlations and relative stereochemistry for C-20/C-22 of the 20,22-*O*-isopropyllidene derivative (**2a**) of stelliferin K (**2**) (protons of methyl groups were omitted).

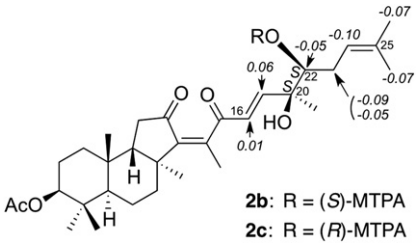

Fig. 8. $\Delta\delta$ values [$\Delta\delta$ (in ppm)$=\delta_S-\delta_R$] obtained for the 22-(*S*)- and 22-(*R*)-MTPA esters (**2b** and **2c**, respectively) of stelliferin K (**2**).

Stelliferin L (**3**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{24}$ +7.8 (*c* 0.55, MeOH)}. The HRESIMS analysis revealed the molecular formula to be $C_{32}H_{48}O_5$ (*m*/*z* 535.3386 $[M+Na]^+$, Δ−0.8 mmu). The UV absorption at 306 nm (log ε 4.2) implied the presence of a conjugated enone. The ^1H and ^{13}C NMR spectra (Table 2) showed the presence of a tricyclic moiety of isomalabaricane-type terpenoid with 3β-acetoxy group, which corresponded to that of **1**, as well as the signals due to a side chain at C-13. The structure of the side chain was assigned as follows. The $^1H-^1H$ COSY spectrum disclosed connectivities of C-15 to C-17 and C-22 to C-23 (Fig. 9). HMBC cross-peaks of H_3-18 to C-13, C-14, and C-15 suggested the presence of a diene moiety (C-13—C-16). HMBC correlations were observed for H_3-26 to C-25, C-27, and C-24, last of which was also correlated to H-17 and H_2-23, suggesting the connectivities of C-26 to C-24 and C-27 via C-25, C-17 to C-24, and C-23 to C-24. In addition, the connectivities among C-17, C-21, and C-22 through C-20 were implied by HMBC correlations for H_3-21 to C-17, C-20, and C-22. The geometries for olefins at C-13 and C-15 were assigned to be *Z* and *E*, respectively, due to NOESY correlations for H_3-30/H_3-18 and H_3-18/H-16 as well as the *J* value of H-15/H-16 (16.6 Hz). Thus, the gross structure of **3** was elucidated as shown in Fig. 9.

NOESY cross-peaks of H_3-21/H-16, H_3-21/H-22, and H-16/H-22 suggested that H-17, 20-OH, and 22-OH were all located on the α-side (Fig. 10). Thus, the relative stereochemistry of the cyclopentane moiety (C-17, C-20, and C-22 to C-24) of **3** was assigned as shown.

The absolute stereochemistry of stelliferin L (**3**) was assigned as follows. Treatment of **3** with K_2CO_3 in MeOH gave the deacetyl derivative (**3a**), which was treated with (*R*)-(−)- and (*S*)-(+)-MTPACl to afford the 3,22-bis-(*S*)- and 3,22-bis-(*R*)-MTPA esters (**3b** and **3c**, respectively). The $\Delta\delta$ values obtained for **3b** and **3c** indicated that the absolute configurations of C-3 and C-22 were both *S* (Fig. 11).

The absolute stereochemistry of the cyclopentane moiety (C-17, C-20, and C-22 to C-24) was supported by the application of an exciton chirality method.[7] For application of the exciton chirality method, the *O*-*p*-dimethylaminobenzoyl group was chosen as an exciton chromophore of **3**, since the UV spectrum of **3** showed a strong absorption due to a dienone chromophore at 306 nm. Treatment of **3** with *p*-dimethylaminobenzoyl chloride afforded the 22-*O*-dimethylaminobenzoate (**3d**) of **3**. Since the sign of the first Cotton effect at 319 nm was positive ($\Delta\varepsilon$ +8.1), the chirality between the dienone moiety and *p*-dimethylaminobenzoyl group of **3d** was assigned as shown in Fig. 12, suggesting that the absolute configurations of C-17, C-20, and C-22 were *S*, *R*, and *S*, respectively. Thus, the absolute configurations at eight chiral centers in stelliferin L (**3**) were elucidated to be 3*S*, 5*R*, 8*S*, 9*S*, 10*R*, 17*S*, 20*R*, and 22*S*.

Stelliferin M (**4**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{24}$ −187 (*c* 0.30, MeOH)}, and the HRESIMS revealed the molecular formula to be $C_{32}H_{48}O_5$, which was identical to that of **3**. The analysis of the NMR data (Table 2) implied **4** to be a stereoisomer of **3** on the cyclopentane moiety (C-17, C-20, C-22 to

Table 2
^{1}H and ^{13}C NMR data for stelliferins L–N (**3**–**5**) in $CDCl_3$

Position	**3**		**4**		**5**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	32.9	1.59, 1.37 (1H each, m)	33.0	1.58, 1.38 (1H each, m)	33.0	1.58, 1.38 (1H each, m)
2	25.0	1.81, 1.70 (1H each, m)	25.1	1.81, 1.67 (1H each, m)	25.1	1.83, 1.70 (1H each, m)
3	80.7	4.55 (1H, dd, J=11.5, 4.6 Hz)	80.9	4.55 (1H, dd, J=11.6, 5.0 Hz)	80.8	4.54 (1H, dd, J=11.4, 5.0 Hz)
4	38.0	—	38.2	—	38.1	—
5	46.4	1.76 (1H, br d, J=12.0 Hz)	46.6	1.76 (1H, br d, J=12.2 Hz)	46.5	1.75 (1H, br d, J=12.1 Hz)
6	18.1	1.68, 1.46 (1H each, m)	18.2	1.67, 1.47 (1H each, m)	18.2	1.67, 1.47 (1H each, m)
7	38.0	2.09, 2.03 (1H each, m)	38.0	2.10, 2.03 (1H each, m)	38.1	2.10, 2.04 (1H each, m)
8	44.2	—	44.2	—	44.3	—
9	50.1	1.82 (1H, m)	50.4	1.81 (1H, m)	50.2	1.82 (1H, m)
10	35.3	—	35.4	—	35.4	—
11	36.5	2.15 (2H, m)	36.6	2.16 (2H, m)	36.7	2.16 (2H, m)
12	206.5	—	206.7	—	206.7	—
13	145.3	—	145.2	—	145.3	—
14	141.9	—	142.6	—	141.7	—
15	131.0	7.71 (1H, d, J=16.6 Hz)	131.1	7.62 (1H, d, J=16.0 Hz)	132.5	7.73 (1H, d, J=15.6 Hz)
16	134.4	5.81 (1H, dd, J=16.6, 8.6 Hz)	135.9	6.05 (1H, dd, J=16.0, 8.2 Hz)	132.9	5.64 (1H, dd, J=15.6, 10.7 Hz)
17	56.2	3.32 (1H, br d, J=8.6 Hz)	57.4	3.17 (1H, br d, J=8.2 Hz)	56.6	2.86 (1H, dd, J=10.7, 7.5 Hz)
18	16.2	1.94 (3H, s)	16.7	1.94 (3H, s)	16.3	1.88 (3H, s)
19	22.2	1.01 (3H, s)	22.3	1.01 (3H, s)	22.3	1.00 (3H, s)
20	81.0	—	83.3	—	81.4	—
21	21.8	1.19 (3H, s)	19.5	1.24 (3H, s)	23.9	1.18 (3H, s)
22	76.4	3.90 (1H, t, J=6.9 Hz)	79.7	3.92 (1H, dd, J=6.2, 3.5 Hz)	77.2	3.96 (1H, dd, J=8.2, 3.7 Hz)
23	36.3	2.71 (1H, dd, J=18.2, 7.7 Hz) 2.29 (1H, br d, J=18.2 Hz)	37.7	2.89 (1H, dd, J=17.5, 5.6 Hz) 2.33 (1H, br d, J=17.5 Hz)	34.6	2.28, 1.74 (1H each, m)
24	130.5	—	131.8	—	45.5	3.20 (1H, m)
25	128.1	—	128.6	—	145.6	—
26	21.2	1.66 (3H, s)	21.4	1.65 (3H, s)	109.4	4.75, 4.66 (1H each, br s)
27	20.4	1.59 (3H, s)	21.1	1.61 (3H, s)	23.4	1.61 (3H, s)
28	28.8	0.91 (3H, s)	29.0	0.90 (3H, s)	29.0	0.90 (3H, s)
29	16.8	0.88 (3H, s)	16.9	0.88 (3H, s)	16.9	0.88 (3H, s)
30	24.6	1.36 (3H, s)	24.8	1.35 (3H, s)	24.8	1.34 (3H, s)
3-OAc	170.9	2.06 (3H, s)	171.0	2.05 (3H, s)	171.0	2.06 (3H, s)
	21.1		21.2		21.2	

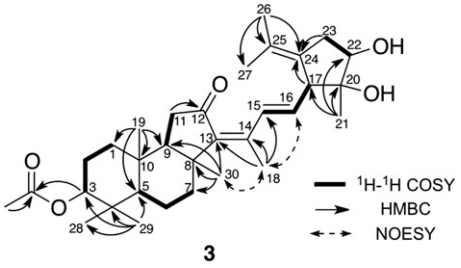

Fig. 9. Selected 2D NMR correlations for stelliferin L (**3**).

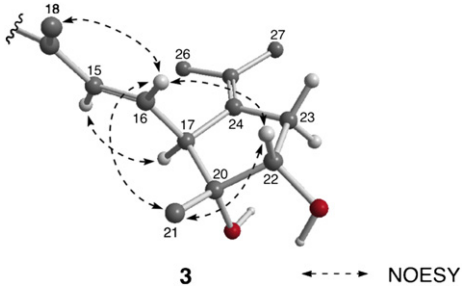

Fig. 10. Selected NOESY correlations and relative stereochemistry for the cyclopentane moiety (C-17, C-20, and C-22 to C-24) of stelliferin L (**3**) (protons of methyl groups were omitted).

C-24). The relative stereochemistry of the cyclopentane moiety was assigned by the analysis of NOESY data. NOESY correlations for H_3-21/H-23a, H_3-21/H-16, and H-22/H-17 suggested the α-orientation for 21-Me and β-orientations for H-17 and H-22 (Fig. 13).

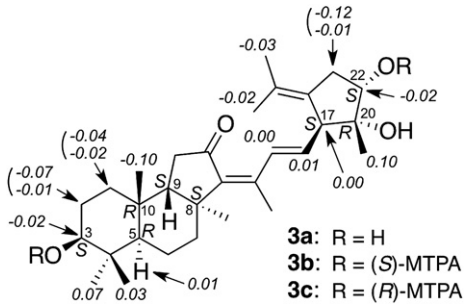

Fig. 11. Structures for the deacetyl derivative (**3a**) of stelliferin L (**3**), and the 3,22-bis-(*S*)- and 3,22-bis-(*R*)-MTPA esters (**3b** and **3c**, respectively) derived from **3a**. Δδ values [$\Delta\delta$ (in ppm)=$\delta_S-\delta_R$] obtained for **3b** and **3c** were shown.

A modified Mosher's method was applied to **4** in the same manner as described for **3**, indicating that the absolute configuration of C-3 was *S*. However, the absolute stereochemistry of the cyclopentane moiety (C-17, C-20, and C-22 to C-24) was not assigned owing to irregular distribution of δΔ value for H-16 (Fig. 14). Therefore, the absolute stereochemistry of the cyclopentane moiety was analyzed by the application of an exciton chirality method after introduction of a *p*-dimethylaminobenzoyl chromophore into the hydroxy group at C-22. The 22-*O*-*p*-dimethylaminobenzoate (**4d**) of **4** showed the negative first Cotton effect at 308 nm ($\Delta\varepsilon$ −13.3). From this observation, the chirality between the dienone moiety and the *p*-dimethylaminobenzoyl group at C-22 of **4d** was assigned as shown in Fig. 12, suggesting that the absolute configurations of C-17, C-20, and C-22 were *R*, *S*, and *S*,

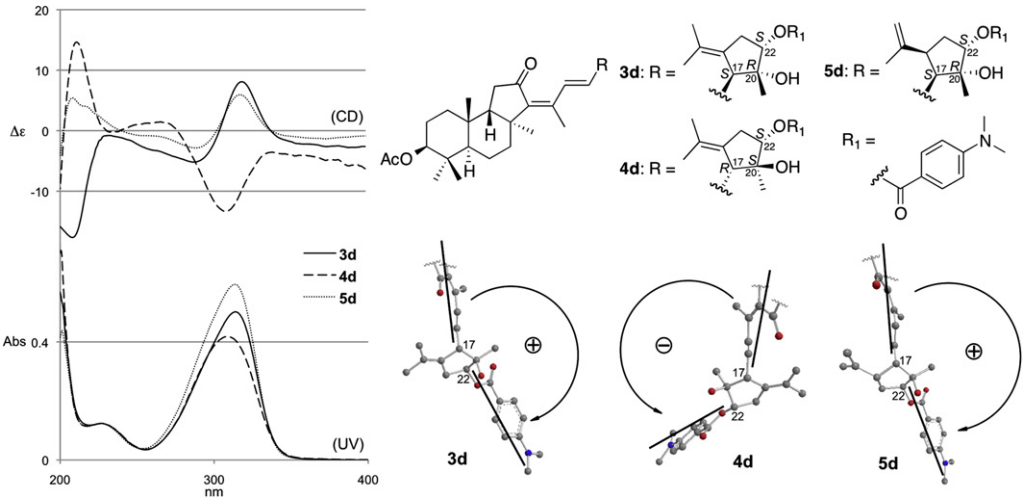

Fig. 12. CD and UV spectra and stereostructues of the *p*-dimethylaminobenzoates (**3d**–**5d**) of stelliferins L–N (**3**–**5**).

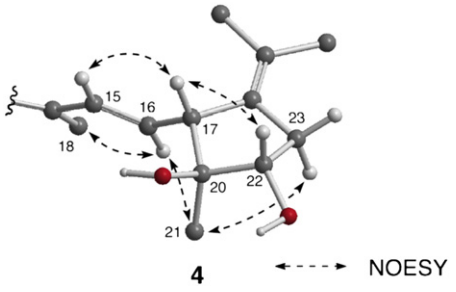

Fig. 13. Selected NOESY correlations and relative stereochemistry for the cyclopentane moiety (C-17, C-20, and C-22 to C-24) of stelliferin M (**4**) (protons of methyl groups were omitted).

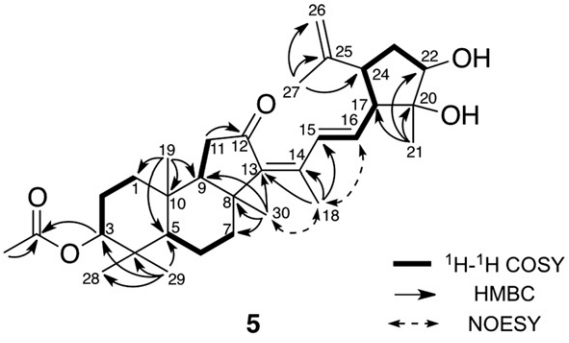

Fig. 15. Selected 2D NMR correlations for stelliferin N (**5**).

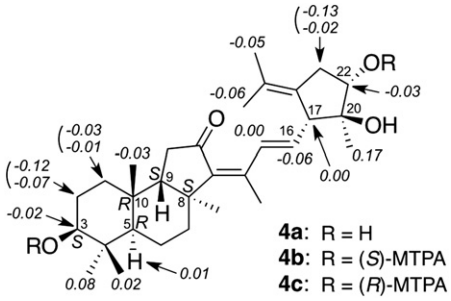

Fig. 14. Structures for the deacetyl derivative (**4a**) of stelliferin M (**4**), and the 3,22-bis-(*S*)- and 3,22-bis-(*R*)-MTPA esters (**4b** and **4c**, respectively) derived from **4a**. $\Delta\delta$ values [$\Delta\delta$ (in ppm)=$\delta_S-\delta_R$] obtained for **4b** and **4c** were shown.

respectively. Thus, the absolute configurations at eight chiral centers in stelliferin M (**4**) were concluded to be 3*S*, 5*R*, 8*S*, 9*S*, 10*R*, 17*R*, 20*S*, and 22*S*.

Stelliferin N (**5**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{24}$ +24.2 (*c* 0.27, MeOH)}. The HRESIMS analysis revealed the molecular formula to be $C_{32}H_{48}O_5$ (*m*/*z* 535.3389 $[M+Na]^+$, Δ −0.5 mmu). The ^{13}C NMR data of **5** was similar to that of **3**, except for the signals due to C-24 to C-27 (Table 2). In the ^1H NMR spectrum of **5**, the resonances of one 1,1-disubstituted olefin [δ_H 4.75 and 4.66 (each 1H, br s)], one sp^3 methine [δ_H 3.20 (1H, m)], and one methyl group attached to double bond [δ_H 1.61 (3H, s)] were observed, implying that **5** had an isopropenyl group at C-24 in place of an isopropylidene group of **3**. It was confirmed by the analyses of the 2D NMR spectra (Fig. 15). Thus, the gross structure of stelliferin N was assigned as shown in Fig. 15.

The relative stereochemistry for the cyclopentane moiety of **5** was deduced by the analysis of the NOESY spectrum (Fig. 16). NOESY cross-peaks of H-17/H-24 and H-24/H-23a indicated that these protons were α-oriented, while the β-orientations of Me-21 and H-22 were assigned by cross-peaks of H-16/H_3-21, H-16/H-22, and H_3-21/H-22.

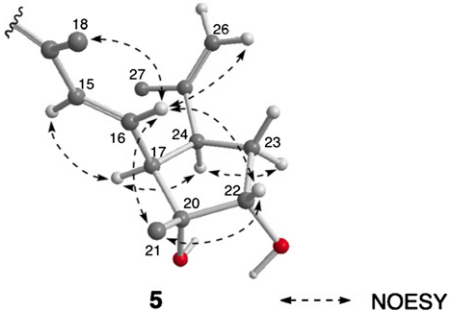

Fig. 16. Selected NOESY correlations and relative stereochemistry for the cyclopentane moiety (C-17, C-20, and C-22 to C-24) of stelliferin N (**5**) (protons of methyl groups were omitted).

The absolute stereochemistry of **5** was elucidated based on the application of a modified Mosher's method by the same manner as described in **3** (Fig. 17). The application of an exciton chirality method to the 22-*p*-dimethylaminobenzoate (**5d**) of **5** supported the assignment (Fig. 12). Thus, the absolute configurations at nine chiral centers in **5** were assigned as 3*S*, 5*R*, 8*S*, 9*S*, 10*R*, 17*S*, 20*R*, 22*S*, and 24*S*. Compound **5** is a 24-epimer of isomalabaricane-type triterpenoid, rhabdasin D.[2]

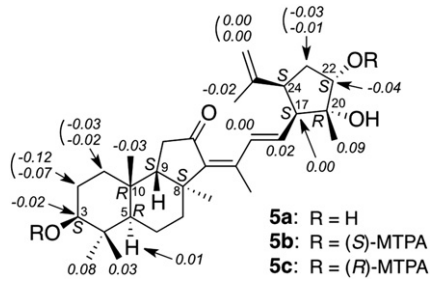

Fig. 17. Structures for the deacetyl derivative (**5a**) of stelliferin N (**5**), and the 3,22-bis-(*S*)- and 3,22-bis-(*R*)-MTPA esters (**5b** and **5c**, respectively) derived from **5a**. $\Delta\delta$ values [$\Delta\delta$ (in ppm)=$\delta_S-\delta_R$] obtained for **5b** and **5c** were shown.

Stelliferins J–N (**1**–**5**) are new isomalabaricane-type triterpenoids with a 3β-acetoxy group. Stelliferins J (**1**) and K (**2**) had a 15-keto and 20,22-diol groups, and may be biogenetically generated from stelliferin A,[4] while stelliferins L–N (**3**–**5**) seem to be biosynthesized from stelliferin A through similar biogenetic path as described for related isomalabaricane-type triterpenoids, rhabdastins D–F,[2] isolated from marine sponge *R. globostellata*. Stelliferins L (**3**) and N (**5**) exhibited antimicrobial activity against *Bacillus subtilis* (IC_{50} 8 μg/mL each), while stelliferins J–N (**1**–**5**) did not show cytotoxicity against L1210 murine leukemia cells (IC_{50}>10 μg/mL) in vitro.

3. Experimental section

3.1. General procedures

Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR, UV, and CD spectra were recorded on a JASCO FT/IR-230, a Shimadzu UV-1600PC, and a JASCO J-720 spectrophotometers, respectively. NMR spectra were measured by a Bruker AMX-600 NMR spectrometer and a JEOL ECA 500 spectrometer. The 7.26 and 77.0 ppm resonances of residual $CHCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. ESI and HRESI mass spectra were recorded on a Thermo Scientific Exactive spectrometer.

3.2. Sponge description

The yellowish-brown sponge *R.* cf. *globostellata* (SS-201) collected off Ishigaki, Okinawa, was kept frozen until used. The sponge oozes yellow coloration into ethanol, and is firm, compressible, springy sponge with a smooth surface. The sponge has a finely porous interior, barnacles in surface layer, a superficial layer of oxysphaerasters, orthotriaenes radially. Spicules are oxeas, long, thin, 900×~10 μm, orthotriaenes 650×10 μm, large and small oxysphaerasters 45 and 20 μm wide, and oxyasters 20 μm wide. The voucher specimen was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

3.3. Isolation of stelliferins J–N

The sponge *R.* cf. *globostellata* (SS-201, 1.0 kg wet weight) was extracted with MeOH and then $CHCl_3$. The combined extracts (69 g) were partitioned between EtOAc and H_2O. EtOAc-soluble portions (6.3 g) were subjected to a silica gel column ($CHCl_3$/MeOH) and C_{18} column (MeOH/H_2O) chromatographies to afford a mixture of triterpenoids, which was purified by C_{18} HPLC (YMC-Pack Pro C18, YMC Co. Ltd., 10×250 mm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent MeOH/H_2O) to yield stelliferins J (**1**, 0.00023%), K (**2**, 0.00013%), L (**3**, 0.00040%), M (**4**, 0.00016%), and N (**5**, 0.00014%).

*3.3.1. Stelliferin J (**1**).* Colorless amorphous solid; $[\alpha]_D^{24}$ −56.9 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 227 (log ε 4.0) and 301 (3.0, sh) nm; IR (film) ν_{max} 3387 and 1726 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 551 $[M+Na]^+$; HRESIMS: *m*/*z* 551.3338 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_6Na$, 551.3343).

*3.3.2. Stelliferin K (**2**).* Colorless amorphous solid; $[\alpha]_D^{24}$ −33.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} 227 (log ε 4.2) and 301 (3.4, sh) nm; IR (film) ν_{max} 3397, 1732, and 1716 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 551 $[M+Na]^+$; HRESIMS: *m*/*z* 551.3342 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_6Na$, 551.3343).

*3.3.3. Stelliferin L (**3**).* Colorless amorphous solid; $[\alpha]_D^{24}$ +7.8 (*c* 0.55, MeOH); UV (MeOH) λ_{max} 306 (log ε 4.2) nm; IR (film) ν_{max} 3417, 1730, and 1695 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m*/*z* 535 $[M+Na]^+$; HRESIMS: *m*/*z* 535.3386 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3394).

*3.3.4. Stelliferin M (**4**).* Colorless amorphous solid; $[\alpha]_D^{24}$ −187 (*c* 0.30, MeOH); UV (MeOH) λ_{max} 309 (log ε 4.3) nm; IR (film) ν_{max} 3421, 1732, and 1695 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m*/*z* 535 $[M+Na]^+$; HRESIMS: *m*/*z* 535.3393 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3394).

*3.3.5. Stelliferin N (**5**).* Colorless amorphous solid; $[\alpha]_D^{24}$ +24.2 (*c* 0.27, MeOH); UV (MeOH) λ_{max} 303 (log ε 4.2) nm; IR (film) ν_{max} 3345, 1732, and 1696 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m*/*z* 535 $[M+Na]^+$; HRESIMS: *m*/*z* 535.3389 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3394).

3.3.6. 20,22-O-Isopropyllidene derivatives of stelliferins J and K. To a CH_2Cl_2 solution (80 μL) of stelliferin J (**1**, 0.2 mg) were added 2,2-dimethoxypropane (20 μL) and pyridinium *p*-toluensulfonate (1.0 mg) at room temperature, and stirring was continued for 6 h. After evaporation of solvent, the residue was passed through a silica gel column ($CHCl_3$/MeOH 99:1) to afford a 20,22-*O*-isopropyllidene derivative (**1a**) of **1**. The 20,22-*O*-isopropyllidene derivative (**2a**) of stelliferin K (**2**) was prepared according to the same procedure as described above.

*3.3.6.1. 20,22-O-Isopropyllidene derivative (**1a**) of stelliferin J (**1**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 6.48 (1H, d, *J*=16.2 Hz, H-17), 6.24 (1H, d, *J*=16.2 Hz, H-16), 5.11 (1H, t, *J*=6.9 Hz, H-24), 4.55 (1H, dd, *J*=11.6, 5.0 Hz, H-3), 3.86 (1H, dd, *J*=7.9, 5.7 Hz, H-22), 2.19 (1H, m, H-7a), 2.17 (1H, m, H-23a), 2.12 (2H, m, H_2-11), 2.07 (3H, s, 3-OAc), 2.06 (1H, m, H-7b), 2.05 (1H, m, H-23b), 1.93 (3H, s, H_3-18), 1.86 (1H, m, H-9), 1.83 (1H, m, H-2a), 1.77 (1H, br d, *J*=12.5 Hz, H-5), 1.74 (1H, m, H-6a), 1.72 (3H, s, H_3-27), 1.69 (1H, m, H-2b), 1.62 (3H, s, H_3-26), 1.58 (1H, m, H-1a), 1.50 (1H, m, H-6b), 1.46 (3H, s, acetonide-Me), 1.40 (3H, s, acetonide-Me), 1.39 (1H, m, H-1b), 1.37 (3H, s, H_3-21), 1.30 (3H, s, H_3-30), 1.00 (3H, s, H_3-19), 0.92 (3H, s, H_3-28), and 0.89 (3H, s, H_3-29); ESIMS *m*/*z* 591 $[M+Na]^+$; HRESIMS: *m*/*z* 591.3660 $[M+Na]^+$ (calcd for $C_{35}H_{52}O_6Na$, 591.3656).

*3.3.6.2. 20,22-O-Isopropyllidene derivative (**2a**) of stelliferin K (**2**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 6.46 (1H, d, *J*=16.0 Hz, H-17), 6.31 (1H, d, *J*=16.0 Hz, H-16), 5.07 (1H, t, *J*=6.2 Hz, H-24), 4.56 (1H, dd, *J*=11.6, 5.0 Hz, H-3), 3.81 (1H, t, *J*=6.6 Hz, H-22), 2.33 (1H, m, H-23a), 2.21 (1H, m, H-23b), 2.18 (1H, m, H-7a), 2.13 (2H, m, H_2-11), 2.07 (3H, s, 3-OAc), 2.04 (1H, m, H-7b), 1.91 (3H, s, H_3-18), 1.87 (1H, m, H-9), 1.83 (1H, m, H-2a), 1.78 (1H, m, H-5), 1.73 (1H, m, H-6a), 1.71 (3H, s, H_3-27), 1.70 (1H, m, H-2b), 1.63 (3H, s, H_3-26), 1.58 (1H, m, H-1a), 1.52 (1H, m, H-6b), 1.46 (3H, s, acetonide-Me), 1.39 (1H, m, H-1b), 1.39 (3H, s, H_3-30), 1.36 (3H, s, acetonide-Me), 1.22 (3H, s, H_3-21), 1.03 (3H, s, H_3-19), 0.92 (3H, s, H_3-28), and 0.90 (3H, s, H_3-29); ESIMS *m*/*z* 591 $[M+Na]^+$; HRESIMS: *m*/*z* 591.3664 $[M+Na]^+$ (calcd for $C_{35}H_{52}O_6Na$, 591.3656).

3.3.7. 22-(S)- and 22-(R)-MTPA esters of stelliferin J. To a pyridine solution (60 μL) of stelliferin J (**1**, 0.2 mg) were added (*R*)-MTPACl

(10 μL) at room temperature, and stirring was continued for 2 h. After addition of MeOH (60 μL) and evaporation of solvent, the residue was applied to a silica gel column ($CHCl_3$/MeOH. 99:1), and then purified by C_{18} HPLC (YMC-Pack Pro C18, 10×250 mm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 9:1) to afford the 22-(*S*)-MTPA ester (**1b**) of stelliferin J (**1**). The 22-(*R*)-MTPA ester (**1c**) of stelliferin J (**1**) was prepared according to the same procedure as described above.

*3.3.7.1. 22-(S)-MTPA ester (**1b**) of stelliferin J (**1**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.56 (2H, m, Ph), 7.40 (3H, m, Ph), 6.53 (1H, d, *J*=16.1 Hz, H-17), 6.31 (1H, d, *J*=16.1 Hz, H-16), 5.16 (1H, dd, *J*=9.4, 3.9 Hz, H-22), 4.97 (1H, t, *J*=6.2 Hz, H-24), 4.56 (1H, dd, *J*=11.6, 5.0 Hz, H-3), 3.48 (3H, s, OMe), 2.30 (1H, m, H-23a), 2.26 (1H, m, H-23b), 2.14 (1H, m, H-7a), 2.11 (2H, m, H_2-11), 2.07 (3H, s, 3-OAc), 2.01 (1H, m, H-7b), 1.87 (3H, s, H_3-18), 1.85 (1H, m, H-9), 1.84 (1H, m, H-2a), 1.77 (1H, m, H-5), 1.73 (1H, m, H-6a), 1.71 (1H, m, H-2b), 1.61 (3H, s, H_3-27), 1.50 (3H, s, H_3-26), 1.47 (1H, m, H-6b), 1.36 (3H, s, H_3-30), 1.35 (3H, s, H_3-21), 1.01 (3H, s, H_3-19), 0.92 (3H, s, H_3-28), and 0.90 (3H, s, H_3-29); ESIMS *m*/*z* 767 $[M+Na]^+$; HRESIMS: *m*/*z* 767.3759 $[M+Na]^+$ (calcd for $C_{42}H_{55}O_8F_3Na$, 767.3741).

*3.3.7.2. 22-(R)-MTPA (**1c**) ester of stelliferin J (**1**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.53 (2H, m, Ph), 7.41 (3H, m, Ph), 6.48 (1H, d, *J*=16.1 Hz, H-17), 6.28 (1H, d, *J*=16.1 Hz, H-16), 5.18 (1H, dd, *J*=9.7, 3.3 Hz, H-22), 5.07 (1H, t, *J*=7.2 Hz, H-24), 4.56 (1H, dd, *J*=11.6, 5.0 Hz, H-3), 3.52 (3H, s, OMe), 2.39 (1H, m, H-23a), 2.29 (1H, br d, *J*=14.1 Hz, H-23b), 2.17 (1H, m, H-7a), 2.11 (2H, m, H_2-11), 2.07 (3H, s, 3-OAc), 2.02 (1H, m, H-7b), 1.89 (1H, m, H-9), 1.88 (3H, s, H_3-18), 1.84 (1H, m, H-2a), 1.77 (1H, m, H-5), 1.75 (1H, m, H-6a), 1.71 (1H, m, H-2b), 1.68 (3H, s, H_3-27), 1.56 (3H, s, H_3-26), 1.52 (1H, m, H-6b), 1.37 (3H, s, H_3-30), 1.28 (3H, s, H_3-21), 1.03 (3H, s, H_3-19), 0.92 (3H, s, H_3-28), and 0.90 (3H, s, H_3-29); ESIMS *m*/*z* 767 $[M+Na]^+$; HRESIMS: *m*/*z* 767.3754 $[M+Na]^+$ (calcd for $C_{42}H_{55}O_8F_3Na$, 767.3741).

*3.3.8. 3-(S)- and 3-(R)-MTPA esters of **1a**.* A solution of the 20,22-*O*-isopropyllidene derivative (**1a**, 0.2 mg) of stelliferin J (**1**) in MeOH (300 μL) was treated with K_2CO_3 (0.6 mg) at 45 °C, and stirring was continued for 13 h. The reaction mixture was diluted with H_2O (1 mL), and extracted with $CHCl_3$ (1 mL×3). The $CHCl_3$ layer was concentrated under reduced pressure to give a deacetyl derivative (**1d**) of **1a**. The 3-(*S*)- and 3-(*R*)-MTPA esters (**1e** and **1f**, respectively) of **1d** were prepared according to the same procedure as described in Section 3.3.7.

*3.3.8.1. 3-(S)-MTPA ester (**1e**) of **1a**.* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.55 (2H, m, Ph), 7.41 (3H, m, Ph), 6.48 (1H, d, *J*=16.1 Hz, H-17), 6.24 (1H, d, *J*=16.1 Hz, H-16), 5.11 (1H, t, *J*=7.5 Hz, H-24), 4.80 (1H, dd, *J*=11.6, 5.3 Hz, H-3), 3.86 (1H, dd, *J*=8.0, 5.5 Hz, H-22), 3.57 (3H, s, OMe), 2.00 (1H, m, H-2a), 1.93 (3H, s, H_3-18), 1.85 (1H, m, H-2b), 1.79 (1H, d, *J*=12.9 Hz, H-5), 1.72 (3H, s, H_3-27), 1.63 (3H, s, H_3-26), 1.62 (1H, m, H-1a), 1.46 (3H, s, acetonide-Me), 1.45 (1H, m, H-1b), 1.41 (3H, s, acetonide-Me), 1.40 (3H, s, H_3-30), 1.37 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.89 (3H, s, H_3-28), and 0.83 (3H, s, H_3-29); ESIMS *m*/*z* 765 $[M+Na]^+$; HRESIMS: *m*/*z* 765.3972 $[M+Na]^+$ (calcd for $C_{43}H_{57}O_7F_3Na$, 765.3949).

*3.3.8.2. 3-(R)-MTPA ester (**1f**) of **1a**.* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.55 (2H, m, Ph), 7.41 (3H, m, Ph), 6.48 (1H, d, *J*=16.4 Hz, H-17), 6.24 (1H, d, *J*=16.4 Hz, H-16), 5.12 (1H, t, *J*=6.1 Hz, H-24), 4.80 (1H, dd, *J*=11.6, 5.3 Hz, H-3), 3.86 (1H, dd, *J*=8.0, 5.8 Hz, H-22), 3.57 (3H, s, OMe), 2.22 (1H, m, H-2a), 1.93 (3H, s, H_3-18), 1.85 (1H, m, H-2b), 1.79 (1H, d, *J*=12.9 Hz, H-5), 1.72 (3H, s, H_3-27), 1.63 (3H, s, H_3-26), 1.62 (1H, m, H-1a), 1.46 (3H, s, acetonide-Me), 1.45 (1H, m, H-1b), 1.41 (3H, s, acetonide-Me), 1.40 (3H, s, H_3-30), 1.38 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.89 (3H, s, H_3-28), and 0.83 (3H, s, H_3-29); ESIMS *m*/*z* 765 $[M+Na]^+$; HRESIMS: *m*/*z* 765.3968 $[M+Na]^+$ (calcd for $C_{43}H_{57}O_7F_3Na$, 765.3949).

3.3.9. 22-(S)- and 22-(R)-MTPA esters of stelliferin K. The 22-(*S*)- and 22-(*R*)-MTPA esters (**2b** and **2c**, respectively) of stelliferin K (**2**) were prepared according to the same procedure as described in Section 3.3.7.

*3.3.9.1. 22-(S)-MTPA ester (**2b**) of stelliferin K (**2**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.51 (2H, m, Ph), 7.40 (3H, m, Ph), 6.59 (1H, d, *J*=16.0 Hz, H-17), 6.36 (1H, d, *J*=16.0 Hz, H-16), 5.12 (1H, dd, *J*=9.5, 4.0 Hz, H-22), 5.00 (1H, m, H-24), 4.56 (1H, dd, *J*=11.6, 5.3 Hz, H-3), 3.42 (3H, s, OMe), 2.33 (1H, m, H-23a), 2.24 (1H, m, H-23b), 2.07 (3H, s, 3-OAc), 1.89 (3H, s, H_3-18), 1.62 (3H, s, H_3-27), 1.52 (3H, s, H_3-26), 1.39 (3H, s, H_3-30), and 1.02 (3H, s, H_3-19); ESIMS *m*/*z* 767 $[M+Na]^+$; HRESIMS: *m*/*z* 767.3771 $[M+Na]^+$ (calcd for $C_{42}H_{55}O_8F_3Na$, 767.3741).

*3.3.9.2. 22-(R)-MTPA ester (**2c**) of stelliferin K (**2**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.51 (2H, m, Ph), 7.40 (3H, m, Ph), 6.54 (1H, d, *J*=16.3 Hz, H-17), 6.35 (1H, d, *J*=16.3 Hz, H-16), 5.17 (1H, dd, *J*=9.2, 3.9 Hz, H-22), 5.10 (1H, m, H-24), 4.55 (1H, dd, *J*=11.6, 4.9 Hz, H-3), 3.51 (3H, s, OMe), 2.41 (1H, m, H-23b), 2.29 (1H, dd, *J*=16.7, 8.8 Hz, H-23a), 2.07 (3H, s, 3-OAc), 1.88 (3H, s, H_3-18), 1.69 (3H, s, H_3-27), 1.59 (3H, s, H_3-26), 1.34 (3H, s, H_3-30), 1.03 (3H, s, H_3-19), 0.92 (3H, s, H_3-28), and 0.90 (3H, s, H_3-29); ESIMS *m*/*z* 767 $[M+Na]^+$; HRESIMS: *m*/*z* 767.3763 $[M+Na]^+$ (calcd for $C_{42}H_{55}O_8F_3Na$, 767.3741).

3.3.10. 3,22-Bis-(S)- and 3,22-bis-(R)-MTPA esters of stelliferins L–N. Stelliferins L–N (**3**–**5**, 0.2 mg each) were treated with K_2CO_3 (0.6 mg) in MeOH (300 μL) at 45 °C for 20 h, individually. The reaction mixture was diluted with H_2O (1 mL), and extracted with $CHCl_3$ (1 mL×3). The $CHCl_3$ layer was concentrated under reduced pressure to give deacetyl derivatives (**3a**–**5a**) of **3**–**5**. To a CH_2Cl_2 solution (200 mL) of **3a** (0.2 mg) were added 4-(dimethylamino)pyridine (0.15 mg), triethylamine (0.8 mL), and (*R*)-MTPACl (0.8 mL) at room temperature, and stirring was continued for 20 h. After addition of MeOH (60 μL) and evaporation of solvent, the residue was purified by a silica gel column ($CHCl_3$) and C_{18} HPLC (YMC-Pack Pro C18, 10×250 mm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 97:3) to afford the 3,22-bis-(*S*)-MTPA ester (**3b**). Similarly, the 3,22-bis-(*R*)-MTPA ester (**3c**) of **3** was prepared. Deacetyl derivatives of stelliferins M (**4a**) and N (**5a**) were converted into the 3,22-bis-(*S*)- and 3,22-bis-(*R*)-MTPA esters (**4b**/**5b** and **4c**/**5c**), individually, according to the same procedure as described in Section 3.3.7.

*3.3.10.1. 3,22-Bis-(S)-MTPA ester (**3b**) of stelliferin L (**3**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.77 (1H, d, *J*=14.8 Hz, H-15), 7.54 (4H, m, Ph), 7.41 (6H, m, Ph), 5.79 (1H, dd, *J*=14.8, 8.2 Hz, H-16), 5.03 (1H, t, *J*=8.1 Hz, H-22), 4.77 (1H, dd, *J*=11.6, 5.0 Hz, H-3), 3.64 (3H, s, OMe), 3.55 (3H, s, OMe), 3.29 (1H, d, *J*=8.2 Hz, H-17), 2.95 (1H, dd, *J*=16.6, 8.1 Hz, H-23a), 2.36 (1H, dd, *J*=16.6, 8.1 Hz, H-23b), 1.95 (3H, s, H_3-18), 1.92 (1H, m, H-2a), 1.79 (1H, d, *J*=12.0 Hz, H-5), 1.71 (1H, m, H-2b), 1.66 (3H, s, H_3-26), 1.62 (1H, m, H-1a), 1.58 (3H, s, H_3-27), 1.41 (1H, m, H-1b), 1.37 (3H, s, H_3-30), 1.23 (3H, s, H_3-21), 1.00 (3H, s, H_3-19), 0.96 (3H, s, H_3-28), and 0.85 (3H, s, H_3-29); ESIMS *m*/*z* 925 $[M+Na]^+$; HRESIMS: *m*/*z* 925.4108 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

*3.3.10.2. 3,22-Bis-(R)-MTPA ester (**3c**) of stelliferin L (**3**).* Colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ_H 7.77 (1H, d, *J*=15.7 Hz, H-15), 7.55 (4H, m, Ph), 7.41 (6H, m, Ph), 5.78 (1H, dd, *J*=15.7, 8.2 Hz, H-16), 5.05 (3H, t, *J*=8.2 Hz, H-22), 4.80 (1H, dd, *J*=11.9, 5.1 Hz, H-3), 3.57 (3H, s, OMe), 3.56 (3H, s, OMe), 3.29 (1H, d, *J*=7.7 Hz, H-17), 2.96 (1H, dd, *J*=15.1, 6.1 Hz, H-23a), 2.49 (1H, dd, *J*=15.1, 6.1 Hz, H-23b), 1.99 (1H, m, H-2a), 1.94 (3H, s, H_3-18), 1.84 (1H, m, H-2b), 1.78 (1H,

d, J=12.2 Hz, H-5), 1.69 (3H, s, H_3-26), 1.63 (1H, m, H-1a), 1.60 (3H, s, H_3-27), 1.45 (1H, m, H-1b), 1.37 (3H, s, H_3-30), 1.14 (3H, s, H_3-21), 1.03 (3H, s, H_3-19), 0.88 (3H, s, H_3-28), and 0.82 (3H, s, H_3-29); ESIMS m/z 925 $[M+Na]^+$; HRESIMS: m/z 925.4105 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

*3.3.10.3. 3,22-Bis-(S)-MTPA ester (**4b**) of stelliferin M (**4**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.76 (1H, d, J=16.0 Hz, H-15), 7.54 (2H, m, Ph), 7.50 (2H, m, Ph), 7.41 (6H, m, Ph), 5.71 (1H, dd, J=16.0, 10.0 Hz, H-16), 5.11 (1H, d, J=5.6 Hz, H-22), 4.77 (1H, dd, J=11.3, 4.7 Hz, H-3), 3.53 (3H, s, OMe), 3.52 (3H, s, OMe), 3.19 (1H, d, J=10.0 Hz, H-17), 3.04 (1H, br d, J=19.0 Hz, H-23a), 2.46 (1H, br d, J=19.0 Hz, H-23b), 1.92 (1H, m, H-2b), 1.79 (1H, d, J=12.6 Hz, H-5), 1.76 (3H, s, H_3-18), 1.71 (1H, m, H-2a), 1.61(1H, m, H-1a), 1.61 (6H, s, H_3-26 and H_3-27), 1.41 (1H, m, H-1b), 1.35 (3H, s, H_3-30), 1.20 (3H, s, H_3-21), 0.99 (3H, s, H_3-19), 0.96 (3H, s, H_3-28), and 0.85 (3H, s, H_3-29); ESIMS m/z 925 $[M+Na]^+$; HRESIMS: m/z 925.4113 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

*3.3.10.4. 3,22-Bis-(R)-MTPA ester (**4c**) of stelliferin M (**4**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.76 (1H, d, J=15.8 Hz, H-15), 7.56 (2H, m, Ph), 7.49 (2H, m, Ph), 7.40 (6H, m, Ph), 5.78 (1H, dd, J=15.8, 9.6 Hz, H-16), 5.14 (1H, d, J=6.0 Hz, H-24), 4.79 (1H, dd, J=11.8, 5.0 Hz, H-3), 3.57 (3H, s, OMe), 3.53 (3H, s, OMe), 3.19 (1H, d, J=9.6 Hz, H-17), 3.06 (1H, br d, J=19.0 Hz, H-23a), 2.49 (1H, br d, J=19.0 Hz, H-23b), 1.99 (1H, m, H-2b), 1.82 (1H, m, H-2a), 1.77 (1H, d, J=12.6 Hz, H-5), 1.71 (3H, s, H_3-18), 1.66 (6H, s, H_3-26 and H_3-27), 1.63 (1H, m, H-1b), 1.44 (1H, m, H-1a), 1.34 (3H, s, H_3-30), 1.03 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.88 (3H, s, H_3-28), and 0.82 (3H, s, H_3-29); ESIMS m/z 925 $[M+Na]^+$; HRESIMS: m/z 925.4111 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

*3.3.10.5. 3,22-Bis-(S)-MTPA ester (**5b**) of stelliferin N (**5**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.78 (1H, d, J=15.5 Hz, H-15), 7.53 (4H, m, Ph), 7.42 (6H, m, Ph), 5.62 (1H, dd, J=15.5, 10.9 Hz, H-16), 5.08 (1H, dd, J=9.5, 5.2 Hz, H-22), 4.78 (1H, br s, H-26a), 4.77 (1H, dd, J=11.7, 4.7 Hz, H-3), 4.72 (1H, br s, H-26b), 3.56 (3H, s, OMe), 3.53 (3H, s, OMe), 3.26 (1H, m, H-24), 2.85 (1H, d, J=10.9, 7.1 Hz, H-17), 2.54 (1H, m, H-23a), 1.92 (1H, m, H-2a), 1.89 (3H, s, H_3-18), 1.78 (1H, d, J=13.5 Hz, H-5), 1.70 (1H, m, H-2a), 1.61 (1H, m, H-1a), 1.60 (3H, s, H_3-28), 1.41 (1H, m, H-1b), 1.36 (3H, s, H_3-30), 1.21 (3H, s, H_3-21), 0.99 (3H, s, H_3-19), 0.95 (3H, s, H_3-28), and 0.84 (3H, s, H_3-29); ESIMS m/z 925 $[M+Na]^+$; HRESIMS: m/z 925.4112 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

*3.3.10.6. 3,22-Bis-(R)-MTPA ester (**5c**) of stelliferin N (**5**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.78 (1H, d, J=15.6 Hz, H-15), 7.54 (4H, m, Ph), 7.41 (6H, m, Ph), 5.61 (1H, dd, J=15.6, 10.7 Hz, H-16), 5.12 (1H, dd, J=8.9, 5.0 Hz, H-22), 4.79 (1H, m, H-3), 4.78 (1H, br s, H-26a), 4.73 (1H, br s, H-26b), 3.57 (6H, s, OMe), 3.34 (1H, m, H-24), 2.84 (1H, dd, J=10.7, 6.6 Hz, H-17), 2.57 (1H, m, H-23a), 1.99 (1H, m, H-2a), 1.89 (3H, s, H_3-18), 1.82 (1H, m, H-2b), 1.78 (1H, d, J=12.9 Hz, H-5), 1.63 (1H, m, H-1a), 1.62 (3H, s, H_3-27), 1.44 (1H, m, H-1b), 1.36 (3H, s, H_3-30), 1.12 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.88 (3H, s, H_3-28), and 0.82 (3H, s, H_3-29); ESIMS m/z 925 $[M+Na]^+$; HRESIMS: m/z 925.4107 $[M+Na]^+$ (calcd for $C_{50}H_{60}O_8F_6Na$, 925.4085).

3.3.11. 22-p-Dimethylaminobenzoates of stelliferins L–N. To a CH_2Cl_2 solution (250 μL) of stelliferin L (**3**, 0.2 mg) were added 4-(dimethylamino)pyridine (2.0 mg), triethylamine (1.7 μL), and *p*-dimethylaminobenzoyl chloride (1.8 mg) at room temperature, and stirring was continued for 21 h. After addition of MeOH (250 μL) and evaporation of solvent, the residue was subjected to a silica gel column (hexane/EtOAc. 3:1) and a reverse phase HPLC (Luna 5 μ Phenyl–Hexyl, 10×250 mm; flow rate, 2.5 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 95:5) to afford the 22-*p*-dimethylaminobenzoate (**3d**) of **3**. Stelliferins M (**4**) and N (**5**) were converted to 22-*p*-dimethylaminobenzoate (**4d** and **5d**), individually, according to the same procedure as described above.

*3.3.11.1. 22-p-Dimethylaminobenzoate (**3d**) of stelliferin L (**3**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.91 (2H, d, J=8.9 Hz, Bz), 7.81 (1H, d, J=15.5 Hz, H-15), 6.66 (2H, d, J=8.9 Hz, Bz), 5.83 (1H, dd, J=15.5, 8.9 Hz, H-16), 5.11 (1H, t, J=8.9 Hz, H-22), 4.56 (1H, dd, J=12.2, 5.5 Hz, H-3), 3.36 (1H, d, J=8.9 Hz, H-17), 3.05 (6H, s, NMe_2), 2.95 (1H, dd, J=17.8, 8.9 Hz, H-23a), 2.48 (1H, m, H-23b), 2.06 (3H, s, OAc), 1.94 (3H, s, H_3-18), 1.67 (3H, s, H_3-26), 1.62 (3H, s, H_3-27), 1.36 (3H, s, H_3-30), 1.26 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.91 (3H, s, H_3-28), and 0.89 (3H, s, H_3-29); ESIMS m/z 682 $[M+Na]^+$; HRESIMS: m/z 682.4101 $[M+Na]^+$ (calcd for $C_{41}H_{57}O_6NNa$, 682.4078); CD (MeOH) $\Delta\varepsilon$ (nm) +8.1 (319), −5.2 (288), and −17.7 (208); UV (MeOH) λ_{max} 314 (log ε 4.2) and 227 (3.6) nm.

*3.3.11.2. 22-p-Dimethylaminobenzoate (**4d**) of stelliferin M (**4**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.86 (2H, d, J=9.2 Hz, Bz), 7.86 (1H, d, J=15.6 Hz, H-15), 6.61 (2H, d, J=9.2 Hz, Bz), 6.14 (1H, dd, J=15.5, 9.2 Hz, H-16), 5.17 (1H, br d, J=6.9 Hz, H-22), 4.57 (1H, dd, J=11.6, 5.0 Hz, H-3), 3.29 (1H, d, J=9.2 Hz, H-17), 3.05 (6H, s, NMe_2), 2.07 (3H, s, OAc), 2.00 (3H, s, H_3-18), 1.67 (3H, s, H_3-26), 1.66 (3H, s, H_3-27), 1.39 (3H, s, H_3-30), 1.26 (3H, s, H_3-21), 1.02 (3H, s, H_3-19), 0.91 (3H, s, H_3-28), and 0.89 (3H, s, H_3-29); ESIMS m/z 682 $[M+Na]^+$; HRESIMS: m/z 682.4105 $[M+Na]^+$ (calcd for $C_{41}H_{57}O_6NNa$, 682.4078); CD (MeOH) $\Delta\varepsilon$ (nm) −13.3 (308), +1.4 (266), and +14.6 (211); UV (MeOH) λ_{max} 309 (log ε 4.1) and 227 (3.6) nm.

*3.3.11.3. 22-p-Dimethylaminobenzoate (**5d**) of stelliferin N (**5**).* Colorless amorphous solid; ^1H NMR ($CDCl_3$) δ_H 7.91 (2H, d, J=8.8 Hz, Bz), 7.82 (1H, d, J=15.5 Hz, H-15), 6.66 (2H, d, J=8.8 Hz, Bz), 5.70 (1H, dd, J=15.5, 10.7 Hz, H-16), 5.14 (1H, dd, J=9.3, 5.2 Hz, H-22), 4.76 (1H, br s, H-26a), 4.74 (1H, br s, H-26b), 4.55 (1H, dd, J=11.8, 5.2 Hz, H-3), 3.38 (1H, m, H-24), 3.06 (6H, s, NMe_2), 2.92 (1H, dd, J=10.7, 6.8 Hz, H-17), 2.56 (1H, m, H-23), 2.06 (3H, s, OAc), 1.90 (3H, s, H_3-18), 1.64 (3H, s, H_3-27), 1.36 (3H, s, H_3-30), 1.24 (3H, s, H_3-21), 1.01 (3H, s, H_3-19), 0.91 (3H, s, H_3-28), and 0.89 (3H, s, H_3-29); ESIMS m/z 682 $[M+Na]^+$; HRESIMS: m/z 682.4089 $[M+Na]^+$ (calcd for $C_{41}H_{57}O_6NNa$, 682.4078); CD (MeOH) $\Delta\varepsilon$ (nm) +5.9 (318), −2.9 (289), and+5.4 (208); UV (MeOH) λ_{max} 313 (log ε 4.3) and 227 (3.6) nm.

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