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Structure and biological activity of novel FN analogs as flowering inducers

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ABSTRACT

(12*Z*,15*Z*)-9-Hydroxy-10-oxooctadeca-12,15-dienoic acid (**1**) and norepinephrine (**2**) undergo cycloaddition to afford FN1 (**3**) and FN2 (**4**), both of which induce flowering in *Lemna paucicostata*. Although the derivatives of **1** were suggested to also yield FN-like compounds after reacting with **2**, their structures have not been elucidated. In this report, we investigated the structure and stereochemistry of seven novel FN analogs. These analogs were shown to be formed in the same regio- and stereocontrolled manner as FNs. Moreover, the activity of FN analogs on flowering induction was investigated, and we determined that all analogs, except for compound **8**, were effective flowering inducers for *L. paucicostata*.

1. Introduction

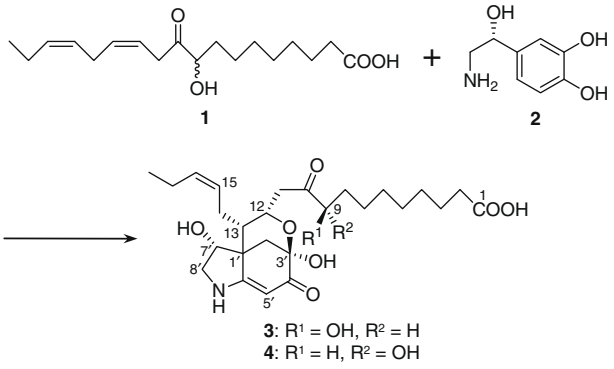
The controlling of the transition from vegetative growth to flowering is important in agriculture, horticulture, and plant breeding because this transition was the first step of sexual production in plants. FN1 (**3**) and FN2 (**4**), which were identified as artificial flowering inducers of *Lemna paucicostata*, were formed by cycloaddion of (12*Z*,15*Z*)-9-hydroxy-10-oxooctadeca-12,15-dienoic acid (**1**) with norepinephrine (**2**) (Scheme 1).[1,2] FNs strongly induce the flowering in *L. paucicostata* at quite low doses; however, their effect seems to be restricted to the several species of plants. To date, it is well known that the natural flowering signals in plants are the proteins encoded by *FLOWERING LOCUS T* (*FT*) and its orthologs.[3,4] Although the orthologs of *FT* have not been found in *Lemna* plants, most flowering-related components known in *Arabidopsis thaliana*, a model plant, have been suggested to also play roles in *Lemna* flowering.[5,6] Study on indentifying the FN's mode of action will allow developing chemicals that induce the flowering of many species of plants.

The structure–activity relationship (SAR) study of FN using the reaction products of **1** and its analogs with **2**/epinephrine (**5**) revealed that the tricyclic structure is essential for the biological activity and that the other structural moieties derived from **1** are modifiable without total loss of activity.[7] In that study, we tentatively identified the structures of the reaction products by LC–PDA/MS and HRMS analyses. Their fully-structural identities and absolute configurations are essential for future chemical and biological studies. We report here the structural and stereochemical determinants of the novel FN analogs **6**–**12**. Furthermore, we also describe the SAR study of FN for flowering induction in *L. paucicostata*.

2. Results and discussion

2.1. Synthesis and purification of FN analogs

The analogs of FN (**6**–**12**, Fig. 1) were prepared in accordance with the previous method.[7] Fatty acids **13**–**15** (Fig. 2) and **2** were



Scheme 1.

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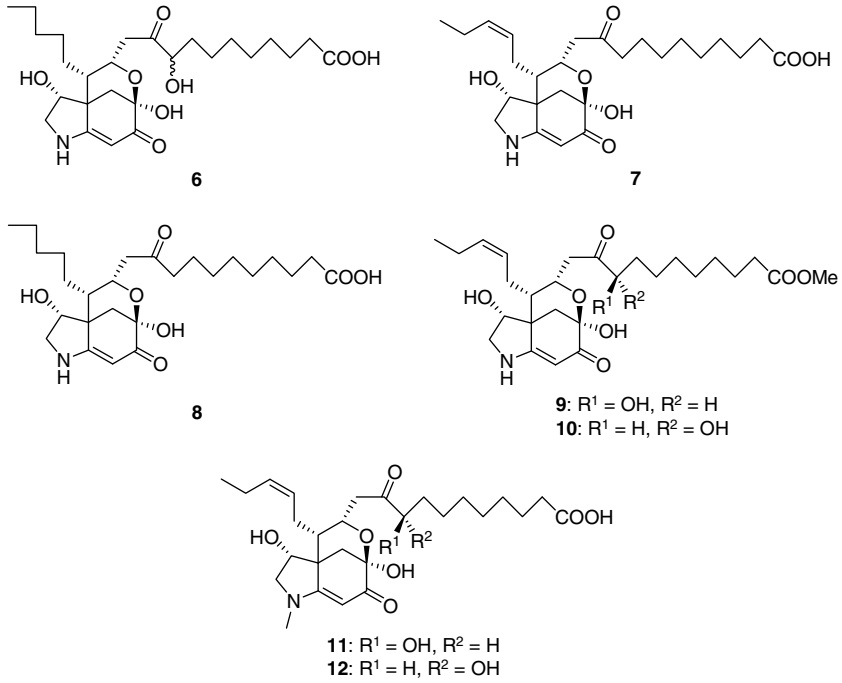

Figure 1. Structures of FN analogs **6**–**12**.

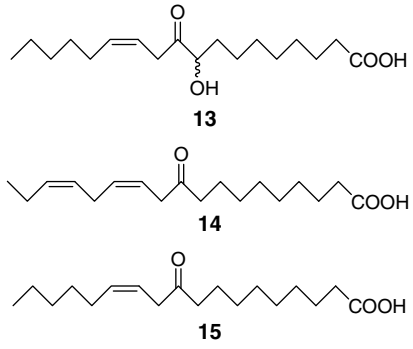

Figure 2. Structures of fatty acids **13**–**15**.

reacted at 25°C under O_2 atmosphere to give the reaction mixtures containing the desired analogs **6**–**8**, **11**, and **12**, which were purified by reverse-phase HPLC. C-9 Epimers of compound **6** could not be separated due to the difficulty in the HPLC resolution. *N*-Methylated analogs of FN, **11** and **12**, were obtained by cycloaddition of **1** with **5** and subsequent purification by reverse-phase HPLC. These preparation and isolation techniques afforded pure compounds **6**–**8**, **11**, and **12** in 2–18% yields. It should be noted that these yields do not reflect the real conversion yields because of an unavoidable loss derived from their decomposition in the purification steps. Compounds **9** and **10** were easily prepared from FN1 and FN2, respectively, by methylation with (trimethylsilyl)diazomethane.

2.2. Structure elucidation of FN analogs

Compound **6** exhibited a pseudo molecular ion at *m/z* 518.2728 $[M+Na]^+$ (calcd for $C_{26}H_{41}NNaO_8$, 518.2730). The ^1H and ^{13}C NMR data of **6** (Table 1), except for signals due to the saturation of 15-olefin, gave the almost same results as those of FN1/2,[2] which indicated that a similar structural relationship existed between **6** and FN1/2. The structure of **6** was deduced from detailed analysis of the ^1H and ^{13}C NMR data aided by 2D NMR experiments (^1H–^1H COSY, HSQC, and HMBC). The ^1H–^1H connectivities of C-11 to C-12 and C-13 to C-18 and HMBC correlations of H-2′ to C-3′ and C-13 suggested that a tricyclic moiety was formed in the same regiomanner as FN1/2. The NOESY correlations of H-7′ at (*S*)-oxymethine to H-13 and H-14 and H-2′ to H-11 revealed that the stereochemistry around the tricyclic system of **6** was identical to that of FN1/2 (Fig. 3). Therefore, the compound has the structure **6** shown in Figure 1.

The molecular formula of compound **7** was determined to be $C_{26}H_{39}NO_7$ by HRMS (*m/z* 500.26260 for $[M+Na]^+$), which indicated that **7** had one oxygen less than FN1/2. The ^1H and ^{13}C NMR spectra (Table 1) indicated that **7** possessed an FN-like structure, where the characteristic signals derived from the tricyclic moiety were observed. The different signals sets due to C-9 deoxygenation were observed in C-8, 9, 10, and 11. The ^1H–^1H COSY connectivities of C-11 to C-12 and C13 to C18 and HMBC correlations of H-2′ to C-3′ and C-13 and H-13 to C-1′ revealed that cycloaddition of **14** with **2** was performed in the same regiomanner as that of **1** with **2**. The stereochemistry of tricyclic moiety in **7** was deduced from the NOESY correlations of H-13, H-14, and H-17 to H-7′, H-8′ to H-13, H-12 to H-13, and H-2′ to H-11 to be identical to that of FN1/2 (Fig. 3). On the basis of the above evidence, the structure of **7** was identified for 9-deoxy analog of FN1/2.

Compound **8** has the molecular formula $C_{26}H_{41}NO_7$ by HRMS showing that **8** had two protons more than compound **7**. This was confirmed by the loss of C-15 olefine signals in the ^1H and ^{13}C NMR of **8** (Table 2). Other signals were almost identical to those of **7**. The NOESY correlations of H-13 and H-14 to H-7′, H-12 to H-13, and H-2′ to H-11 revealed that the stereochemistry of the tricyclic ring system at C-1′, C-3′, C-12, and C-13 is the same with that of FN1/2 (Fig. 3).

Compounds **9** and **10** were prepared from FN1 and FN2, respectively, by methyl esterification and their molecular formulas were both determined to be $C_{27}H_{41}NO_8$ from each HRMS result (*m/z* 530.27310 $[M+Na]^+$ for **9** and *m/z* 530.27316 $[M+Na]^+$ for **10**). Their ^1H and ^{13}C NMR spectra (Table 2) resembled those of FN1/2, except for the presence of one *O*-methyl group. The use of ^1H–^1H COSY,

Table 1
NMR data of compounds **6**–**8** (500 MHz, acetone-d_6).

No.	**6**[a]		**7**		**8**	
	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz
1	174. 8		174.7		175.1	
2	34.2	2.28, 2H, t,7.2	34.1	2.28, 2H, t, 7.3	34.2	2.27, 2H, t, 7.3
3	25.6, 25.7	1.59, 2H, m	25.6	1.59, 2H, t, 7.6	25.5	1.59, 2H, m
4						
5	30.0, 30.2, 30.5	1.30–1.40	29.9 (2), 30.0	1.20–1.30	29.9, 30.1, 30.2	1.25–1.40
6	30.5		30.2		30.5	
7						
8	34.1	1.50, 1H, m 1.73, 1H, m	24.0	1.49, 2H, m	24.0	1.50, 2H, m
9	77.3, 77.7	4.03, 1H, m	43.1	2.37, 2H, m	43.1	2.43, 2H, m
10	211.7		208.4		208.7	
11	41.4, 41.6	2.63, 1H, dd, 5.5, 16.5 2.97, 1H, dd, 7.6, 16.5	46.1	2.49, 1H, dd, 5.8, 15.8 2.64, 1H, m	46.1	2.53, 1H, dd, 5.2, 15.9 2.69, 1H, dd, 7.9, 15.9
12	70.7, 70.9	4.61, 1H, m	70.7	4.60, 1H, m	70.7	
13	39.8, 39.5	1.27, 1H, m	39.9	1.45, 1H, m	39.8	1.48, 1H, m
14	26.8, 26.9	1.40, 1H, m 1.81, 1H, m	24.5	2.16, 1H, m 2.64, 1H, m	26.8	1.39, 1H, m 1.79, 1H, m
15	32.0	1.30–1.40	130.5	5.26, 1H, m	32.1	1.25–1.40
16	32.8		132.4	5.33, 1H, m	32.8	
17	23.1, 23.2	1.25–1.45	21.5	2.16, 2H, m	23.2	
18	14.3	0.90, 3H, m	14.2	1.01, 3H, t, 7.6	14.3	0.90, 3H, t, 7.3
1′	57.5		58.7		57.4	
2′	32.4	1.96, 1H, d, 12.8 2.05, 1H, m	32.5	1.99, 1H, d, 12.5 2.08, 1H, dd, 1.6, 7.7	32.4	1.95, 1H, d, 13.1 2.05, 1H, m
3′	93.9		93.7		93.8	
4′	187.4		186.3		187.7	
5′	93.9	5.47, 1H, br	93.7	5.45, 1H, br	n.d.	5.38, 1H, br
6′	174.7		173.0		174.6	
7′	73.0	4.36, 1H, d, 3.0	71.8	4.40, 1H, d, 3.7	73.0	4.35, 1H, m
8′	54.9	3.52, 1H, d, 12.2 3.93, 1H, d, 3.7, 12.2	54.7	3.50, 1H, d, 12.2 3.91, 1H, dd, 3.7, 12.2	54.8	3.51, 1H, d, 11.0 3.91, 1H, m

[a] Diastereomeric mixture.

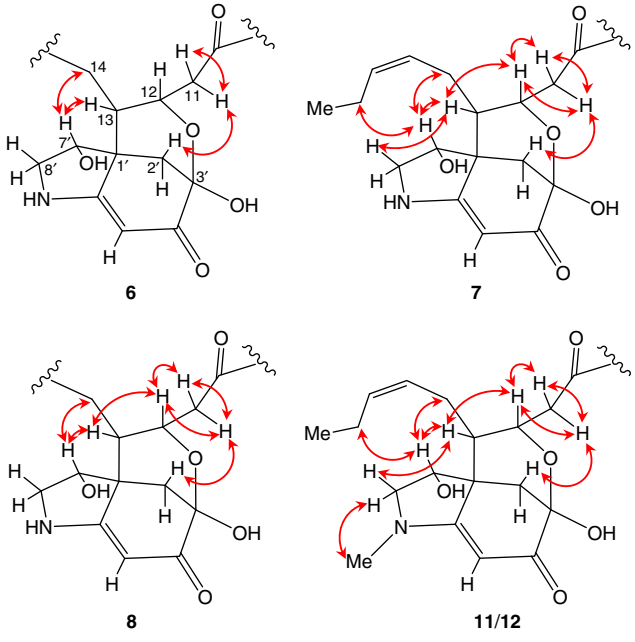

Figure 3. Selected NOESY correlations for **6**–**8**, **11**, and **12**.

HSQC, and HMBC spectra led to the full assignment of the ^{1}H and ^{13}C NMR chemical shifts as shown in Table 2. The key NOESY correlations showed the stereochemistry of **9** and **10** was the same as that of FN1 and FN2, respectively (data not shown). In the previous study,[7] the biological activity of **9** and **10** were tentatively evaluated by the reaction products derived from methyl ester of **1** and **2**. Here, we investigated whether their structures are identical to **9**/**10** by comparison of LC–PDA/MS data. The LC characteristics, UV spectra, and MS of analogs **9** and **10** were equal with those of the reaction products of the methyl ester **1** and **2** (data not shown), confirming that **9** and **10** are identical to the compounds described in the previous study.

Compounds **11** and **12** were determined to have the following molecular formula, $C_{27}H_{41}NO_8$, from HRMS. The ^{1}H and ^{13}C NMR data of **11** and **12** (Table 2), except for *N*-methyl group, gave the almost same results as those of FN1/2. The ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY experiments of **11** and **12** gave the same assignments of the respective signals and stereochemistry as those of FN1/2 (Table 2 and Fig. 3). These results indicated that they have structures **11** and **12** as depicted in Figure 1.

2.3. Biological activity of 6–12

The above analogs **6**–**12** were evaluated for their ability to induce the flowering in *L. paucicostata*. With the exception of **8**, these compounds proved to be active (Fig. 4). Compound **6**, in which 15-olefinic bond is saturated, is significantly less active compared to FN1/2. As we suggested in the previous study,[7] 15-olefinic bond in **6** is not essential for its activity, whereas the presence is favorable for high activity. The effect of the 9-hydroxy group on the flowering activity was investigated with analog **7**. The elimination of the hydroxy group in FN1/2 resulted in considerably decreasing activity. This result suggested that the 9-hydroxy group is also not essential for activity but is required to show high activity. Although the C-9 stereochemistry in FN1/2 seemed to be important for biological activity in the previous study,[2,7] we could not observe such difference in these isomers (**3** and **4**). This is consistent with the fact that the other pair of C-9 epimers (**9** and **10**; **11** and **12**) showed the almost identical effect on the induction of flowering (see below). Probably, the presence of a hydroxy group at C-9 is responsible for a specific interaction between ligand and protein. The character of this

Table 2
NMR data of compounds **9**–**12** (500 MHz, MeOH-d_4: **9** and **10**, acetone-d_6: **11** and **12**).

No.	**9**		**10**		**11**		**12**	
	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz
1	174.1		174.2		174.7		174.8	
2	34.3	2.29, 2H, t, 7.3	34.3	2.29, 2H, t, 7.3	34.1	2.28, 2H, t, 7.6	34.2	2.29, 2H, t, 7.4
3	25.6	1.59, 2H, m	25.6	1.59, 2H, m	25.6	1.60, 2H, m	25.6	1.60, 2H, m
4	30.4, 30.5, 29.3-30.5	1.25-1.42	30.4, 30.5 (2), 30.6	1.25-1.40	29.7 (2), 30.0 (2)	1.25-1.40	29.7 (2), 30.0 (2)	1.25-1.40
5								
6								
7								
8	34.0	1.45, 1H, m	34.1	1.50, 1H, m	33.9	1.49, 1H, m	34.1	1.49, 1H, m
		1.72, 1H, m		1.70, 1H, m		1.71, 1H, m		1.70, 1H, m
9	77.7	4.01, 1H, m	77.3	3.98, 1H, m	77.7	4.01, 1H, dd, 4.1, 7.6	77.3	4.00, 1H, br
10	211.4		210.2		211.3		211.4	
11	41.7	2.68, 1H, dd, 4.9, 15.9	41.6	2.59, 1H, dd, 4.9, 17.1	41.7	2.47, 1H, dd, 4.6, 16.4	41.5	2.52, 1H, dd, 4.0, 16.5
		2.92, 1H, dd, 8.5, 15.9		2.92, 1H, dd, 7.3, 17,1		2.92, 1H, dd, 8.2, 16.4		2.95, 1H, dd, 9.5, 16.5
12	70.9	4.62, 1H, m	70.7	4.65, 1H, m	70.9	4.56, 1H, m	70.8	4.59, 1H, br
13	40.3	1.48, 1H, m	39.9	1.48, 1H, m	40.3	1.49, 1H, m	40.0	1.49, 1H, m
14	24.6	2.21, 1H, m	24.5	2.20, 1H, m	24.6	2.20, 1H, m	24.5	2.19, 1H, m
		2.65, 1H, m		2.65, 1H, m		2.64, 1H, m		2.65, 1H, m
15	130.5	5.30, 1H, m	130.4	5.27, 1H, m	130.3	5.30, 1H, m	130.2	5.26, 1H, m
16	132.6	5.35, 1H, m	132.6	5.34, 1H, m	132.6	5.35, 1H, m	132.7	5.35, 1H, m
17	21.5	2.18, 2H, m	21.5	2.18, 2H, m	21.5	2.17, 2H, m	21.5	2.17, 2H, m
18	14.2	1.02, 3H, t, 7.3	14.2	1.02, 3H, t, 7.3	14.2	1.01, 3H, t, 7.3	14.2	1.01, 3H, t, 7.6
1′	57.3		56.6		58.7		58.9	
2′	32.3	2.01, 1H, d, 12.2	32.3	2.00-2.01, 2H, br	32.5	1.99, 1H, d, 12.8	32.6	2.05, 1H, d, 12.8
		2.08, 1H, m				2.07, 1H, m		2.13, 1H, m
3′	93.8		93.8		93.9		94.1	
4′	187.4		187.4		186.3		186.4	
5′	n.d.	n.d.	n.d.	n.d.	93.9	5.47, 1H, br	94.1	5.47, 1H, br
6′	174.4		174.3		173.0		173.5	
7′	73.0	4.42, 1H, d, 3.7	73.0	4.42, 1H, m	71.8	4.38, 1H, d, 3.7	71.8	4.39, 1H, br
8′	54.7	3.51, 1H, d, 12.2	54.7	3.51, 1H, d, 12.2	62.9	3.48, 1H, d, 12.5	63.0	3.50, 1H, br
		3.93, 1H, dd, 3.7, 12.2		3.92, 1H, m		4.18, 1H, dd, 3.7, 12.5		4.19, 1H, br
Me	51.4 (*O*-Me)	3.61, 3H, s	51.4 (*O*-Me)	3.61, 3H, s	33.8 (*N*-Me)	3.09, 3H, s	34.0 (*N*-Me)	3.11, 3H, s

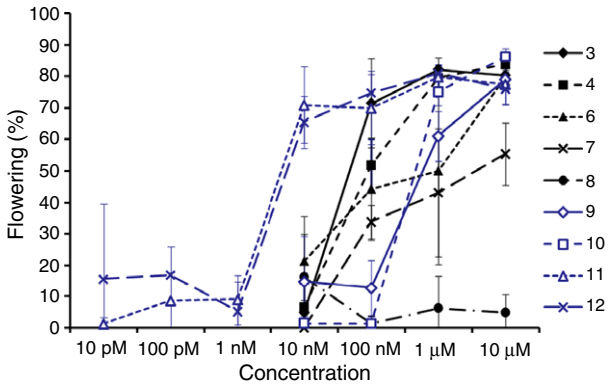

Figure 4. Flower-inducing activity of FN analogs. The error bars indicate the standard deviations of three replicates.

interaction is also illustrated by the absence of activity observed with the compound **8**. Analog **8**, which lacks of both, the 15-olefine and 9-hydroxy group, was almost inactive. The loss of the 9-hydroxy group and the saturation of 15-olefine no longer permits **8** to be correctly positioned in the binding side of the protein. This result is inconsistent with the previously reported suggestion that **8** retained the flowering activity. The absence of activity indicates that unknown active compounds were present in the reaction mixture. Introduction of methyl group at the terminal carboxy group (**9** and **10**) dramatically decreased activity at low concentrations compared to FN1/2. The carboxy group in FNs might work as a hydrogen bond donor to the target protein. The *N*-methylated derivatives (**11** and **12**) were considerably stronger than the parent compounds **3** and **4**. Although the primary effect of this portion would be an enhancement of the hydrophobicity of the molecule to bind the target protein, the other type of *N*-alkylated analogs should be synthesized to address the reason of this enhancement of biological activity.

3. Conclusions

In this report, we have elucidated the structure and stereochemistry of compounds **6**–**12** that were synthesized from fatty acids (**1** and **13**–**15**) and catecholamines (**2** and **5**), which provide access to synthesizing a variety of FN analogs. We observed that FN analogs, except for **8**, displayed significant activity with respect to flowering in *L. paucicostata*. The results of the SAR study of FN were summarized in Figure 5. The continuous studies to design novel analogs will be necessary to extent our knowledge of the structural factors governing the biological activity in FNs.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a JNM λ500A spectrometer (JEOL, Tokyo, Japan). High-resolution mass spectra were obtained with a JMS-T100LC AccuTOF mass spectrometer (JEOL). HPLC separation was performed with a JASCO (Tokyo, Japan) LC system. Solvents for HPLC were purchased from Kanto Chemical (Tokyo, Japan). A two-solvent system was used to generate the mobile phase for HPLC: solvent A, 0.05% aq TFA; solvent B, MeCN.

4.2. Preparation of FN analogs 6–12

4.2.1. Analog 6

To a solution of fatty acid **13** (40 mg, 128 μmol) in DMSO (1.2 mL), **2** (20 mM in water; 12.8 mL, 256 μmol), Tris–HCl buffer (1 M, pH 8.0, 6.4 mL), and water (38 mL) were added. The reaction

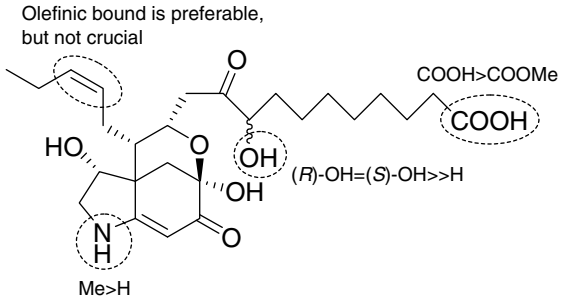

Figure 5. Summary of SAR study of FNs.

was carried out at 25 °C for 15 h under O_2 atmosphere. After acidification of reaction mixture with 1% aq HCOOH, the products were extracted with EtOAc (3 × 50 mL). EtOAc layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by HPLC [column, CAPCELL PAK UG120 20 × 250 mm (Shiseido, Tokyo, Japan); solvent, 35% B/(A+B); flow rate, 10 mL/min] to give **6** as a brown oil (4.0 mg, 8.1 μmol, 6%). HRMS (ESI$^+$) *m/z* 518.2728 [M+Na]$^+$ (calcd for $C_{26}H_{41}NNaO_8$). ^{1}H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-17/C-16, C-18, H-18/C-17, C-16, H-2′/C-13, C-3′, C-4′.

4.2.2. Analog 7

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20 × 250 mm; solvent, 40% B/(A+B); flow rate, 10 mL/min. Brown oil (2%). HRMS (ESI$^+$) *m/z* 500.2626 [M+Na]$^+$ (calcd for $C_{26}H_{39}NNaO_7$). ^{1}H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-9, C-10, H-9/C-8, C-10, H-11/C-10, C-12, H-14/C-15, C-16, H-17/C-18, H-18/C-16, C-17, H-2′/C-13, C-3′, C-4′, C-6′, H-7′/C-6′, H-8′/C-1′, C-6′, C-7′.

4.2.3. Analog 8

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20 × 250 mm; solvent, 35% B/(A+B); flow rate, 10 mL/min. Brown oil (2%). HRMS (ESI$^+$) *m/z* 502.2783 [M+Na]$^+$ (calcd for $C_{26}H_{41}NNaO_7$). ^{1}H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-10, H-9/C-8, C-10, H-11/C-10, C-12, C-13, H-13/C-1′, H-18/C-17, C-16, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, C-8′, H-8′/C-7′.

4.2.4. Analogs 9 and 10

To a solution of **3**/**4** (3 mg, 6.0 μmol) in MeOH (1 μL), a solution of (trimethylsilyl)diazomethane (2 M in hexane; 500 μL) was added dropwise and stirred for 5 min. After removing the solvent and reagent under vacuum, the resulting oil was **9**/**10**. HRMS (ESI$^+$) *m/z* 530.2731 [M+Na]$^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **9**, *m/z* 530.2732 [M+Na]$^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **10**. ^{1}H and ^{13}C NMR: Table 2. HMBC correlation peaks (**9**): H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-9, H-11/C-10, C-12, H-14/C-12, C-13, C-15, C-16, H-17/C-15, C-16, C-18, H-18/C-16, C-17, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, H-8′/C-6′,C-7′, OC*H*$_3$/C-1.

4.2.5. Analogs 11 and 12

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20 × 250 mm; solvent, 23% B/(A+B); flow rate, 10 mL/min. Brown oil (18% for **11**, 8.0% for **12**). HRMS (ESI$^+$) *m/z* 530.2732 [M+Na]$^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **11**, *m/z* 530.2732 [M+Na]$^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **12**. ^{1}H and ^{13}C NMR: Table 2. HMBC correlation peaks (**11**): H-2/C-1, C-3, H-3/C-1, C-2, H-9/C-8, C-7, H-11/C-10, C-12, C-13, H-13/C-11, C-12, C-14, C-1′, C-2′, H-14/C-13, C-15, C-16, H-15/C-14, C-17, H-16/C-14, C-17, H-17/C-15, C-16, C-18, H-18/C-16, C-17, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, C-8′, H-8′/C-1′, C-6′, C-7′, NC*H*$_3$/C-6′, C-8′.

4.3. Flower induction assay

The flower induction assays were performed according to the previous study.[7] A three-frond colony of *L. pucicostata* 151 (P151, a gift from Prof. O. Tanaka) was placed on E medium containing test sample and 6-benzylaminopurine, and incubated on for 10 days at 25 °C under continuous light. The percentage of fronds with flowers was determined. All experiments were performed with three replicates and reproducibility was checked on different days.

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