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Direct observation of the target cell for jasmonate-type leaf-closing factor: genus-specific binding of leaf-movement factors to the plant motor cell

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Abstract—We report the synthesis of the novel fluorescence-labeled jasmonate glycoside **2** based on β-D-glucopyranosyl 12-hydroxyjasmonate **1**, which is a leaf-closing substance of *Albizzia julibrissin* Durazz. The fluorescence study using **2** revealed that the target cell for **1** is a motor cell. Probe **2** bound to the motor cells of two plants belonging to genus *Albizzia*. This result suggested that a receptor for **2**, which is common among genus *Albizzia* would be involved in the nyctinastic leaf movement.

Most leguminous plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm controlled by a biological clock. Charles Darwin, well known for his theory of evolution, carried out the pioneering work on this field.[1] And in the 1970s, physiological studies, especially those using *Albizzia saman*, revealed that nyctinastic leaf movement is induced by the swelling and shrinking of motor cells in the pulvini, a small organ located in the joint of the leaf to the stem.[2] Flux of potassium ions across the plasma membranes of the motor cells is followed by massive water flux, which results in swelling and shrinking of these cells. We revealed that nyctinasty is controlled by a pair of leaf-movement factors: leaf-opening and leaf-closing substances.[3] And, we have also revealed that the target cell of the leaf-opening substance of the *Cassia* plant is a motor cell.[4]

However, most of the physiological studies on nyctinasty were carried out using plants belonging to genus *Albizzia*. Considering that each nyctinastic plant has a pair of leaf-movement factors whose bioactivities are specific to the plant genus,[5] bioorganic studies of nyctinasty using *Albizzia* plants would be important for the connection of results between bioorganic and physiological studies. We have already revealed that the target cell of the leaf-opening factor of genus *Albizzia* is a motor cell by using fluorescence-labeled leaf-opening factor.[6] And we isolated potassium β-D-glucopyranosyl 12-hydroxyjasmonate (**1**) as a leaf-closing substance among genus *Albizzia*.[7] Bioactivity of **1** was not effective for plants belonging to other genus, such as *Cassia mimosoides*, *Phyllnathus urinaria*, and *Mimosa pudica*. However, no bioorganic study was carried out using **1**. Thus, it will be important to clarify whether the genus-specific bioactivity of the leaf-movement factors could be due to the involvement of a genus-specific receptor. In this paper, we synthesized probe **2** to identify the target cell of **1**, and carried out fluorescence studies using **2** to address this issue.

We developed a novel fluorescent probe (**2**) based on the structure of **1**, a leaf-closing substance of genus *Albizzia*. From our previous study, it was already shown that potassium β-D-glucopyranosyl tuberonate (**3**),[8] a cis-isomer of **1**, had no leaf-closing activity for *Albizzia* plant. This result strongly suggested that an aglycon moiety of **1** would be important for leaf-closing activity. Then, we synthesized potassium β-D-galactopyranosyl 12-hydroxyjasmonate (**4**) for a structure–activity relationship study of **1** (Scheme 1). Aglycon (**5**) was synthesized from commercially available (±)-methyl

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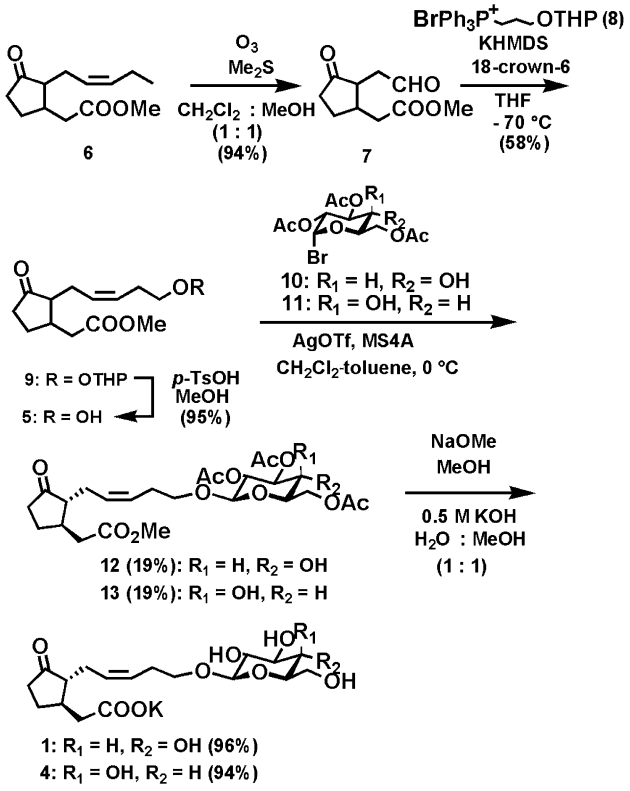
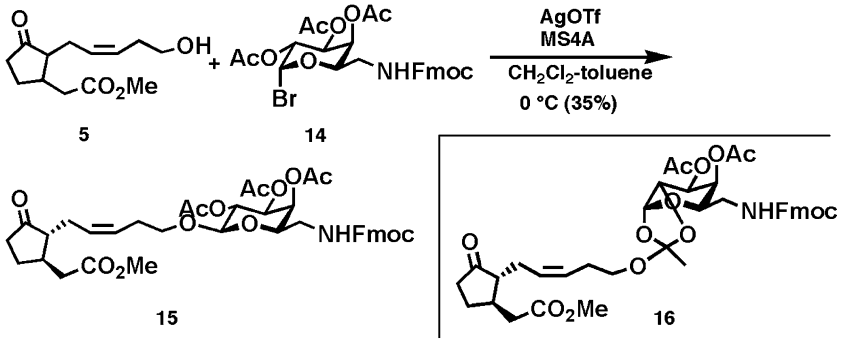


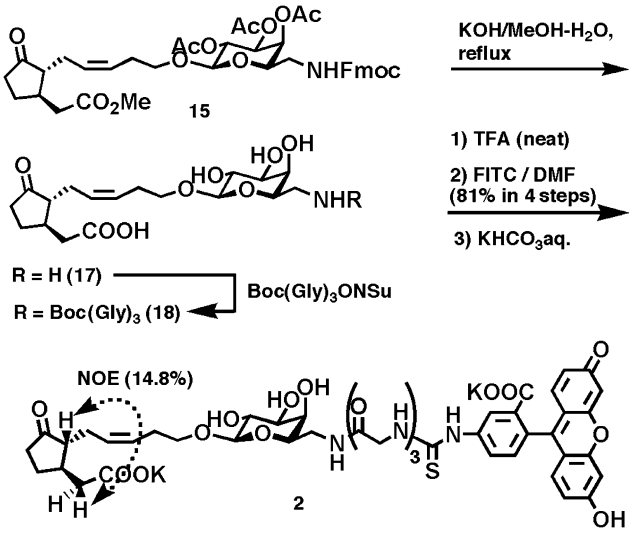
Scheme 1. Synthesis of the glycosides of 12-hydroxyjasmonate.

jasmonate (**6**).[9] Ozonolysis of **6** gave aldehyde **7**. Then, Wittig reaction of **7** with **8** gave THP-protected aglycon of (±)-**2** (**9**). The reaction was carried out with excess amount of 18-crown-6 to give the (*Z*)-isomer predominantly. The resulting **9** was deprotected by *p*-TsOH to give (±)-methyl 12-hydroxyjasmonate (**5**), which was used in the glycosidation reaction with **10** or **11**. Glycosidation of **5** with **11** and successive deprotection gave β-D-galactopyranosyl 12-hydroxyjasmonate (**4**)[10] as a mixture of diastereomers in 19% yield with 39% of acetylated **5**. And glycosidation conditions recently developed by Schmidt and co-workers[11] and Suzuki et al.[12] gave almost no coupling product. The resulting **4** showed leaf-closing activity for *Albizzia julibrissin* at 1×10^{-5} M. Similar to other glycoside-type leaf-movement factors, these results showed that the leaf-closing activity of **1** was not affected by the structure modification in the sugar moiety. Thus, probe **2** was designed according to the molecular design of a previously developed probe.[13] FITC was introduced on the 6′-position of the sugar moiety with a glycylglycylglycyl linker connecting **4** and FITC by an amide linkage. AMCA, which was a fluorescent dye used in our preceding fluorescence study, cannot be used because of intrinsic blue auto fluorescence in the section of *Albizzia* plants.

Probe **2** was synthesized from **5** and Fmoc-protected 6′-aminogalactosyl bromide (**14**) as shown in Schemes 2 and 3. Under normal Königs–Knorr condition, coupling product **15** was obtained in 35% yield (Scheme 2) with 28% of acetylated aglycon. The low yield would be due to the decomposition of the substrate under acidic reaction condition. However, the addition of 2,6-lutidine to keep the reaction media neutral resulted in no improvement in the yield of glycosidation product: 59% of the corresponding orthoester (**16**)[14] was obtained



Scheme 2. Glycosidation of **5**.



Scheme 3. Synthesis of probe **2**.

with 9% of **15**. Then, all protective groups in **15** were deprotected simultaneously by the treatment with KOH to give **17**, which was coupled with *O*-Boc-glycyl-glycylglycyl *N*-hydroxysuccinimide. The resulting **18** was deprotected by TFA and coupled with FITC to give fluorescence labeled probe (**2**).[15] trans-Relationship of the two side chains in **2** was determined by NOE experiment shown in Scheme 3. Probe **2** was effective for the leaf-closing of *A. julibrissin* at 1×10^{-4} M.

We used probe **2** to seek the target cell for **1**. For this purpose, a binding experiment using plant sections was carried out. A leaf of *A. julibrissin* was cut by a microslicer (Dousaka EM Co., Ltd) to a thickness of 30 μm. Then the sections containing a motor cell were incubated for 4.5 h in a 0.1 M phosphate buffer (pH 7) containing 1×10^{-4} M of **2**. After staining, the stained sections were twice incubated for 10 min with water to remove excess fluorescence probes. Then, the stained sections were monitored by a fluorescent microscope (ECLIPSE E800, Nicon Co., Ltd) with an appropriate filter (B-2A, Nicon Co., Ltd). Figure 1 shows photographs of plant pulvini, which contains a motor cell, under a fluorescence microscope. The staining pattern for the yellowish-green fluorescence of probe **2** was observed on the surface of the motor cell (Fig. 1). No staining was observed in the control section, which was treated with an aqueous solution containing no **2** (Fig. 1). These results strongly suggested that the target cell for leaf-closing substance **2** is a motor cell. This and previous[6] results showed that a motor cell of *A. julibrissin* has a set of receptors for leaf-opening and leaf-closing substances.

Next, we examined genus specificity in binding of **2** to the plant section. We revealed that **1** is a common leaf-closing substance among three *Albizzia* plants, containing *A. julibrissin* or *Albizzia saman*.[7] And **1** was not effective for the plants belonging to other plant genus. These results strongly suggested the existence of some specific receptor for **2** that is common among the same genus. First, we examined the specificity in the bioactivity of **2**. Probe **2** did not show leaf-closing activity against leaves of *C. mimosoides* L., *P. urinaria*, and *Leucaena leucocephara* at 1×10^{-4} M, whereas it was effective at the same concentration for the leaf-closing of two *Albizzia* plants: *A. julibrissin* and *A. saman*. From these results, the binding of **2** is expected to be specific to the section of plants belonging to genus *Albizzia*, and no binding would be observed in the experiment using the section of other plants. Then, we used probe **2** for the binding experiment with the sections of *C. mimosoides*, *P. urinaria*, and *L. leucocephara* together with that of *A. saman*. The binding experiments were carried out according to the same procedure used in the case of *A. julibrissin*. Thus, it was revealed that the sections of *A. julibrissin* and *A. saman* gave a fluorescence image resulting from **2**, *L. leucocephara* gave weak fluorescence,and no other sections gave the image (Fig. 2). Genus *Luecaena* to which *L. leucocephara* belongs is thought to be in a close relationship to a genus *Albizzia*. Weak fluorescence seen in *L. leucocephara* suggested the structural similarity in leaf-closing substance between them. Red stains seen in the fluorescence images are due to the porphyrine in the plant tissue. These results showed that the binding of probe **2** with a motor cell is specific to genus *Albizzia* and suggested that a genus-specific receptor molecule for the genus-specific leaf-movement factor on a motor cell would be involved in nyctinasty.

From these results, we have shown that *Albizza* plants have a receptor for **1** in their motor cells. Together with

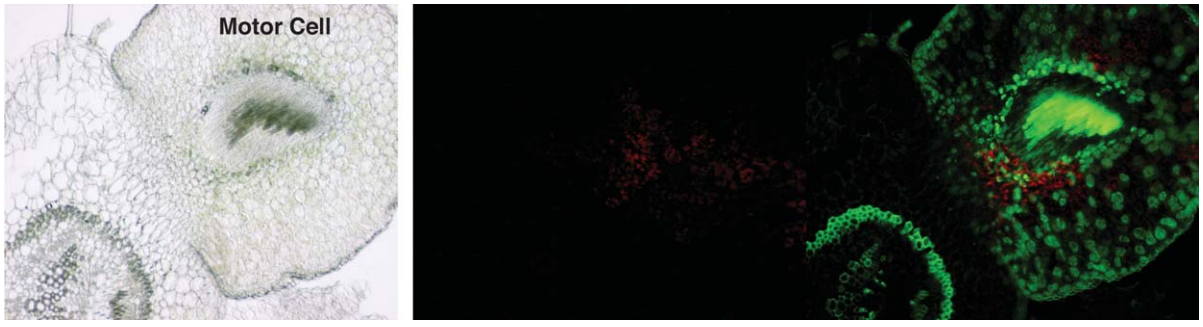


Figure 1. Binding experiment using **2** with plant section of *A. saman* containing motor cell (left: Nomarskii image of the plant section containing motor cell, center: fluorescence image of blank section, right: Fluorescence image of plant section treated with 1×10^{-4} M of **2** [excitation: 450–490 nm]).

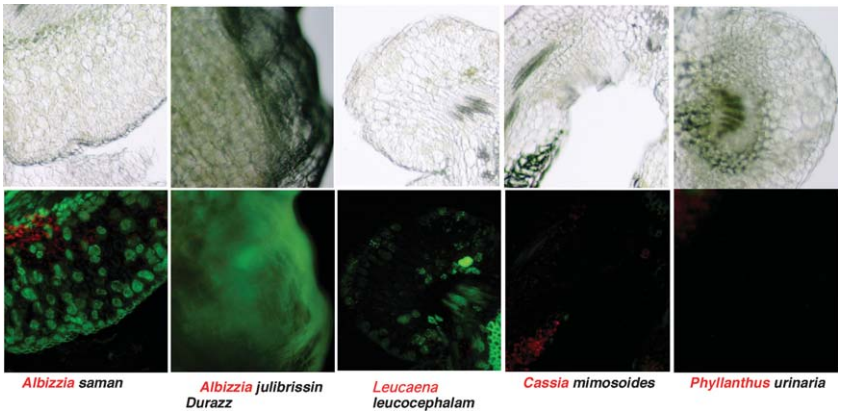


Figure 2. Photographs of plant sections in the binding experiments, which show specific binding of probe **1** with the motor cell of *Albizzia* plants (from the left, *A. saman*, *A. julibrissin*, *Leucaena leucocephala*, *Cassia mimosoides*, and *Phyllanthus urinaria*; upper: Nomarskii image of plant section, lower: fluorescent image of plant section after treatment with 1×10^{-4} M of probe **2** [excitation: 450–490 nm]).

the former result of the specific binding of a leaf-opening substance to the motor cell of genus *Albizzia*,[6] it was strongly suggested that the *Albizzia* plant would have a pair of receptors corresponding to leaf-opening and -closing substances, and both of them are common among the same genus. This result showed that the genus-specific receptor for the leaf-movement factor would be involved in the nyctinasty. And, it was estimated that each plant genus would have a genus-specific combination of leaf-movement factors and receptor molecules for them. Genus-specific recognition of the ligand by a specific receptor of the leaf-movement factor strongly suggests that the membrane receptor concerning nyctinasty would be differentiated in the comparatively latter process of the evolution in the plant kingdom when the leguminous plants differentiated to various genuses.

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10. Compound **4**: ^{1}H NMR (300 MHz, D_2O, 21 °C): 5.51 (2H, dt, $J = 6.6$, 11.7 Hz), 4.40 (1H, d, $J = 7.8$ Hz), 3.97–3.86 (2H, m), 3.82–3.61 (5H, m), 3.49 (1H, t, $J = 7.8$ Hz), 2.56 (1H, dd, $J = 4.8$, 14.4 Hz), 2.45–1.99 (10H, m), 1.53 (1H, m); ^{13}C NMR (150 MHz, D_2O, 22 °C): 229.4, 183.4, 130.0, 129.3, 104.4, 76.7, 74.4, 72.4, 70.3, 62.5, 56.0, 44.2, 40.0, 39.7, 28.9, 28.4, 26.5; HR ESI MS (negative): $[M-K]^-$ found m/z 387.1661, $C_{18}H_{27}O_9$ requires m/z 387.1661; IR (film) v: 3360, 2920, 2870, 1730, 1565, 1400, 1070, 660 cm^{-1}.
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13. Ueda, M.; Sawai, Y.; Wada, Y.; Yamamura, S. *Tetrahedron* **2000**, *56*, 5123–5130.
14. Compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C): 7.76 (2H, d, $J = 7.5$ Hz), 7.58 (2H, d, $J = 7.5$ Hz), 7.40 (2H, t, $J = 7.5$ Hz), 7.31 (2H, t, $J = 7.5$ Hz), 5.79 (1H, d, $J = 6.0$ Hz), 5.43 (2H, dt, $J = 8.7$, 11.4 Hz), 5.36 (1H, br s), 5.15 (1H, m), 5.05 (1H, m), 4.45–4.35 (2H, m), 4.30 (1H, t, $J = 6.0$ Hz), 4.22 (1H, m), 3.69 (3H, s), 3.50 (2H, m), 2.66 (1H, dd, $J = 6.0$, 11.4 Hz), 2.38–2.04 (17H, m), 1.95 (1H, m), 1.65 (3H, s), 1.50 (1H, m); ^{13}C NMR (150 MHz, $CDCl_3$, 23 °C): 218.9, 172.6, 170.4, 170.1, 156.5, 144.0, 143.9, 140.7, 129.4, 128.8, 128.1, 128.0, 127.8, 127.2, 125.2, 124.7, 120.9, 120.1, 97.7, 73.4, 71.4, 70.0, 67.1, 54.0, 47.3, 38.7, 38.1, 37.8, 27.9, 27.2, 25.7, 23.5, 21.3, 21.0, 20.7; HR ESI MS (positive): $[M+Na]^+$ found m/z 772.2943, $C_{39}H_{45}O_{13}NNa$ requires m/z 772.2940; IR (film) v: 3373, 3017, 2951, 1740, 1533, 1437, 1371, 1232, 1074, 1047, 760 cm^{-1}.
15. Compound **2**: ^{1}H NMR (600 MHz, CD_3OD, 27 °C): 8.28 (1H, d, $J = 1.8$ Hz), 8.02 (1H, br s), 7.90 (1H, dd, $J = 1.8$,

8.4 Hz), 7.24 (1H, d, $J = 8.4$ Hz), 6.79 (2H, d, $J = 8.4$ Hz), 6.78 (1H, d, $J = 1.8$ Hz), 6.64 (2H, dd, $J = 1.8$, 8.4 Hz), 5.53 (1H, td, $J = 7.2$, 11.4 Hz), 5.46 (1H, td, $J = 7.2$, 11.4 Hz), 4.37 (3H, m), 4.04–3.85 (6H, m), 3.69 (1H, br s), 3.59 (1H, t, $J = 6.6$ Hz), 3.58 (1H, t, $J = 6.6$ Hz), 3.33 (1H, m), 2.71 (1H, dd, $J = 10$,19.8 Hz), 2.43 (2H, t, $J = 6.6$ Hz), 2.40–2.24 (4H, m), 2.17–2.10 (4H, m), 1.53 (1H, m); ^{13}C NMR (150 MHz, CD_3OD, 27 ° C): 221.7, 213.2, 205.1, 184.1, 172.3, 163.1, 154.2, 146.0, 142.2, 133.8, 132.3, 130.5, 130.3, 130.0, 129.1, 127.4, 125.8, 124.4, 123.0, 122.2, 120.3, 117.1, 114.8, 113.6, 113.5, 111.4, 113.6, 113.5, 111.4, 103.5, 64.4, 63.9, 62.8, 62.6, 56.4, 55.1, 43.3, 39.7, 39.2, 38.6, 36.9, 31.8, 30.8, 28.2, 26.4, 1 signal in methanol; HR FAB MS (negative): $[M+H]^+$ found m/z 946.2863, $C_{45}H_{48}O_{16}N_5S$ requires m/z 946.2817; IR (film) v: 3566, 2931, 2526, 2331, 1683, 1635, 1608, 1558, 1541, 1508, 1458, 1437, 1396, 1205, 1136, 1036, 839, 800, 723 cm^{-1}.