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The biological significance of leaf-movement, an approach using a synthetic inhibitor of leaf-closure

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Abstract—Nyctinastic leaf-movement, which is folding and opening movement of the leaves according to circadian rhythm, have been widely observed in leguminous plants. Although this movement has been known since the age of Alexander the Great, the question 'Why does leguminous plant sleep?' has always puzzled many scientists studying nyctinasty. We have revealed that nyctinastic leaf-movement is essential for the survival of leguminous plants by inhibiting leaf-movement using synthetic leaf movement inhibitor. Leguminous plant will wither and die without nyctinasty. Our result gives an important clue for the historic mystery, 'Why does the leguminous plant sleep?' © 2002 Elsevier Science Ltd. All rights reserved.

Plants are unable to move from one place to another. However, folding and opening movement of the leaves according to circadian rhythm have been widely observed in leguminous plants. This periodic leaf-movement is called nyctinasty and has been known since the age of Alexander the Great.[1] On the other hand, the question 'Why does the leguminous plant sleep?' has always puzzled many scientists studying nyctinasty. Darwin concluded that nyctinasty provided protection from chilling in addition to actual freezing,[2] whereas later studies made important objections against his hypothesis.[3,4] Bünning proposed that nyctinasty protected the photoperiodic time keeping system from moonlight, because moonlight falling on leaves during night might prevent accurate measurement of night length.[5] However, no experimental evidence to date has been reported that explains the biological significance of nyctinasty. Research has been hindered because we could not inhibit the leaf movement. Also, no mutant without nyctinasty has been reported so far. Thus, genetic approach to this issue will be difficult. Now we have succeeded in inhibiting leaf movement using synthetic inhibitor of leaf closure based on the substance that naturally induces leaf opening.[6] Our result provide the first experimental evidence to answer the question 'Why does the leguminous plant sleep?'

Schildknecht's turgorin had been widely believed to be a endogeneous factor controlling leaf movement,[1] but we revealed that it did not show any bioactivity under physiological conditions.[6,7] Nyctinasty is controled by two endogenous factors of contrasting bioactivities: leaf-closing substance which makes the leaf closed and leaf-opening substance which makes the leaf open.[6] The

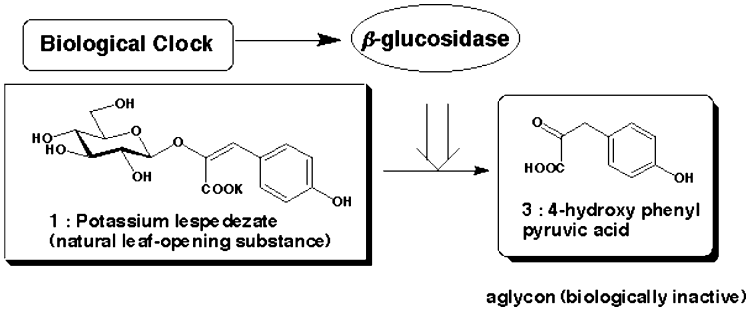

Figure 1. The control of internal concentration of **1** by a biological clock that causes the nyctinastic leaf-movement.

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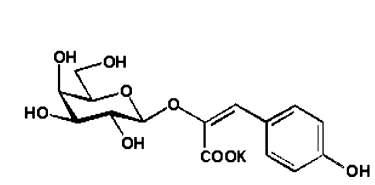
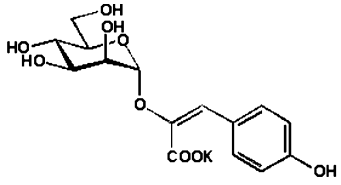
bioactivity of these factors is extremely specific to the original plant from which they were isolated. In the plant body, the rhythm of nyctinasty is generated by change in balance of concentrations between two leaf-movement factors according to a circadian rhythm.[6] This change in balance can be attributed to the hydrolysis of glucoside-type leaf-movement factor into the corresponding aglycon by β-glucosidase, whose activity is controlled by a biological clock (Fig. 1).[6]

According to the mechanism shown in Fig. 1, it was expected that structurally modified leaf-opening substance that cannot be hydrolyzed by β-glucosidase would keep the leaf open constantly, and, thus, inhibit the leaf-closure (causing 'insomnia'). Potassium lespedezate (**1**) was isolated as a glucoside-type leaf opening substance that is effective for the leaf of a leguminous plant, *Cassia mimosoides* L.[6] Structure–activity relationship studies had shown that structural modification on the sugar moiety of **1** caused no decrease in bioactivity.[6,8] Then, based on the structure of **1**, we designed and synthesized a potential leaf-movement inhibitors (**2**, **4**, and **5**) that are expected not to be hydrolyzed by β-glucosidase in the plant body (Fig. 1).[6,8] Leaf-movement inhibitors showed novel bioactivities in bioassay; the leaves detached from the stem of *C. mimosoides* and placed in H_2O kept doing the circadian rhythmic leaf movement (Fig. 2). Both of leaf-opening substance (**1**) and leaf-movement inhibitors (**2**, **4**, and **5**) can keep the leaves open even at night at 1×10^{-6} mol/l. When the leaves were treated with 3×10^{-6} mol/l of **1**, its leaf-opening activity lasted for only 2 days. After that, the leaves became closed at night again. This is because **1** is gradually hydrolyzed into **3** within a few days in the plant body (Fig. 1). On the other hand, leaf-opening activity of **2** lasted even after 1 week. The leaves treated with 3×10^{-6} mol/l of **2** remained open until it withered and died after 2 weeks (Fig. 2). Same results were obtained by using **4** and **5**. These results clearly showed that leaf closure is essential for survival of this plant.

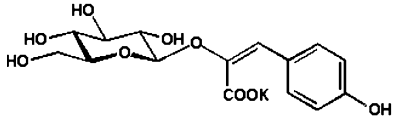
The death of leaf is also attributable to potential toxic feature of **2**, **4**, and **5**. However, we have some experimental proofs that they operate as leaf-movement inhibitors, and not as toxins in the plant body. Strong correlation was observed between leaf-opening activity and death of plant leaf. When the leaves were treated with analogs of **2** that did not show leaf-opening activity even at 1×10^{-3} mol/l, such as one with a reduced double bond (**6a** and **6b**)[9] and another with phenolic methyl ether (**7**)[10] that were prepared from **11** (Scheme



2 : Potassium galactolespedezate

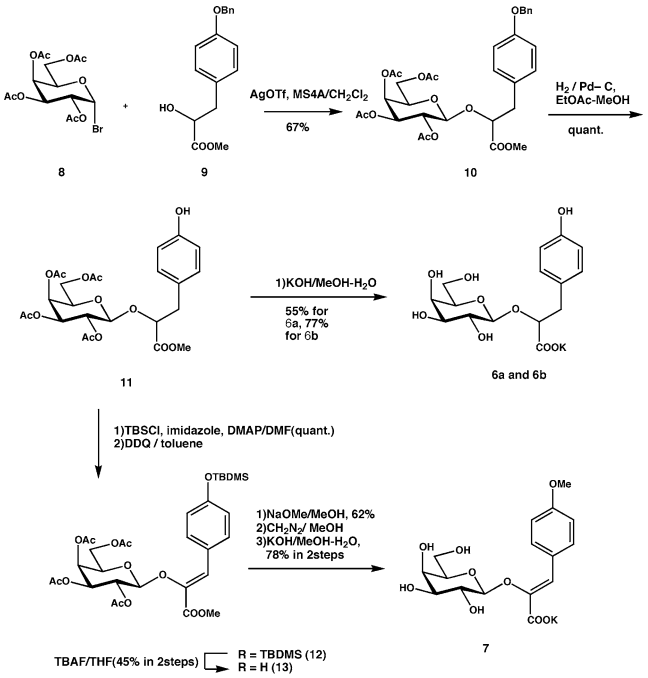


4 : Potassium mannolespedezate



5 : Potassium L-lespedezate

Figure 2. The effect of a leaf-movement inhibitor (**2**) on the leaf of *C. mimosoides*. Each leaf was soaked in a 3×10^{-6} mol/l aqueous solution of **2** or distilled water only for the control sample; from left, the leaves on the 1st, 4th, and 14th day and a control on 14th day at 9:00 pm. These experiments were carried out in a biotron under the following conditions: 12 h light/12 h dark, at 27°C, 50% humidity.



Scheme 1. Chemical synthesis of analogs of **2** without leaf-opening activity.

1), the leaves did not suffer any damage with them and never withered and died even after 2 weeks.

Moreover, **2**, **4**, and **5** showed extremely specific bioactivity to the leaf of *C. mimosoides*, that is a special feature also observed in **1**.[6] And **2**, **4**, and **5** showed no leaf-opening activity with leaves of other plants, such as *Mimosa pudica* L., *Albizzia julibrissin* Durazz, *Aeschynomene indica* L. and *Phyllanthus urinaria* L., even at 3×10^{-5} mol/l. Also, after 2 weeks, no death of the leaf was observed about these plants. This specific bioactivity cannot be interpreted if **2**, **4**, and **5** operated as some toxin in the plant body of *C. mimosoides*. These results strongly suggested that **2**, **4**, and **5** operated as leaf-movement inhibitors in the plant body and cause withering and death of *C. mimosoides* by inhibiting leaf closure.

And also, these results suggest an important application of the leaf-movement inhibitor as a potential environment-friendly herbicide of extremely high specificity. A herbicide based on leaf-movement inhibitor would enable complete selectivity to the target leguminous weed from which the substance was isolated, and have no effect on vicinal plants, insects, birds, animals, and human beings. For example, soybean [*Glycine max* (L.) Merr.] is the most important leguminous crop, and genetically engineered soybean (Monsanto's Roundup Ready Soybeans) is widely cultivated in the USA. Unfortunately, some leguminous weeds, such as *Sesbania exaltata* Cory and *Senna obtusifolia*, are resistant to Roundup and can grow in fields of genetically engineered soybean after the treatment of Roundup. These weeds cause serious trouble because no existing herbicide can remove these weeds without damaging the soybean, which belongs to the same genus as them. However, the use of a leaf-movement inhibitor as a herbicide could remove these weeds without damaging the soybean and other vicinal organisms.

Our result gives an important clue for the historic mystery, 'Why does the leguminous plant sleep?' We showed that nyctinastic leaf-movement is essential for the survival of leguminous plants by using synthetic inhibitor of nyctinasty.

Acknowledgements

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9. Compound **6a**: ^{1}H NMR (400 MHz, D_2O, 30°C): δ 7.25 (2H, d, $J=8.3$ Hz), 6.88 (2H, d, $J=8.3$ Hz), 4.43 (1H, t, $J=6.8$ Hz), 4.37 (1H, d, $J=7.3$ Hz), 3.91 (1H, d, $J=2.0$ Hz), 3.75 (1H, dd, $J=7.3$, 11.1 Hz), 3.71 (1H, dd, $J=4.8$, 11.1 Hz), 3.62–3.56 (3H, m), 3.08 (1H, dd, $J=5.8$, 13.6 Hz), 3.05 (1H, dd, $J=6.8$, 13.6 Hz); ^{13}C NMR (100 MHz, D_2O, 30°C): δ 180.0, 156.7, 133.4, 130.9, 117.8, 105.2, 82.4, 77.6, 75.1, 73.2, 72.3, 71.1, 63.3; IR (film): ν 3365, 1716, 1615, 1516 cm^{-1}; HRMS (negative FAB) calcd. for $C_{15}H_{19}O_9$ [M−K]$^-$: 343.1026, found: 343.1049; $[\alpha]_D^{22}=-10.9°$ (c 0.57 in H_2O). Compound **6b**: ^{1}H NMR (400 MHz, D_2O, 30°C): δ 7.24 (2H, d, $J=8.3$ Hz), 6.87 (2H, d, $J=8.3$ Hz), 4.65 (1H, t, $J=6.3$ Hz), 4.24 (1H, d, $J=7.8$ Hz), 3.92 (1H, d, $J=3.4$ Hz), 3.77 (1H, dd, $J=7.8$, 11.2 Hz), 3.74 (1H, dd, $J=4.3$, 11.2 Hz), 3.70–3.61 (2H, m), 3.57 (1H, dd, $J=7.8$, 9.8 Hz), 3.11 (1H, dd, $J=5.9$, 14.1 Hz), 3.06 (1H, dd, $J=6.3$, 14.1 Hz); ^{13}C NMR (100 MHz, D_2O, 30°C): δ 179.8, 156.7, 133.4, 131.1, 117.7, 104.6, 81.7, 77.7, 75.2, 73.4, 72.3, 71.0, 63.4; IR (film): ν 3365, 1721, 1597, 1516 cm^{-1}; HRMS (negative FAB) calcd. for $C_{15}H_{19}O_9$ [M−K]$^-$: 343.1026, found: 343.1026; $[\alpha]_D^{22}=+2.0°$ (c 0.70, H_2O).

10. Compound **7**: ^{1}H NMR (400 MHz, D_2O, 30°C): δ 7.65 (2H, d, $J=8.8$ Hz), 6.86 (2H, d, $J=8.8$ Hz), 6.60 (1H, s), 4.82 (1H, d, $J=7.6$ Hz), 3.75 (1H, d, $J=3.6$ Hz), 3.69 (3H, s), 3.64 (1H, dd, 8.0, 10.0 Hz), 3.57–3.43 (4H, m); ^{13}C NMR (100 MHz, D_2O, 30°C): δ 172.4, 160.6, 147.1, 133.3, 128.3, 121.7, 115.7, 103.3, 77.3, 74.6, 73.1, 71.5, 70.3, 62.6; IR (film): ν 3362, 1604, 1574, 1511 cm^{-1}; HRMS (negative FAB) calcd. for $C_{16}H_{19}O_9$ [M–K]$^-$: 355.1029, found: 355.1003; $[\alpha]_D^{22}=+56.2°$ (*c* 0.79, H_2O).