CHEMICAL STUDIES ON THE NYCTINASTIC LEAF-MOVEMENT REGULATED BY A BIOLOGICAL CLOCK

Minoru Ueda and Shosuke Yamamura*
Department of Chemistry, Faculty of Science and Technology,
Keio University, Hiyoshi, Yokohama 223-8522, Japan

Abstract-Most leguminosae plants close their leaves in the evening, as if to sleep, and open them early in the morning. This circadian rhythm is known to be controlled by the biological clock of such plants. Extensive studies on other nyctinastic plants led to the isolation of a variety of leaf-closing and leaf-opening substances. Based on our experiments on these bioactive substances, we found that the circadian rhythmic leaf-movement of these plants is controlled by a biological clock that regulates the balance of concentration between leaf-opening and -closing substances.

1. INTRODUCTION

In contrast with animals, plants are rooted and unable to move from one place to another by themselves. However, they are not static but sensitively respond to a variety of environmental factors such as light, temperature, and humidity, as well as to chemical substances represented by allelopathic compounds that result in visually detectable movement. This visual movement is classified into three main kinds: 1) tropistic, 2) nastic, and 3) taxic.

The present review focuses on a variety of bioactive compounds related to the leaf movement of nyctinastic plants, whose leaves close at night and open by day according to a circadian rhythm. This rhythm is regulated by a biological clock with a cycle of about 24 hours. This movement is called nyctinastic movement. Nyctinasty is usually observed in most leguminosae plants. In any event, such an exciting biological phenomenon has attracted much attention since the fourth century B.C.

Historically, the discovery of a biological clock was based on the observation of nyctinastic movement in *Mimosa pudica* L. In the 18th century, a French scientist discovered that the rhythm involved in nyctinastic leaf movement was maintained even under continuous darkness in a cave.[1] He hypothesized that the leaf movement is controlled by the intrinsic rhythm of the plant. Later, Bünning named this intrinsic rhythm the "circadian rhythm," and revealed that it was produced by a biological clock that exists in all living organisms on Earth.[2]

In 1880, Charles Darwin, well known for his biologically important book entitled "Origin of Species" also published an invaluable and voluminous book entitled "The Power of Movement in Plants" based on experiments conducted by himself and his son Francis, using more than three hundred different kinds of plants including nyctinastic ones represented by *M. pudica*.[3]

2. LEAF-MOVEMENT FACTORS IN NYCTINASTIC PLANTS

Nyctinastic movement has been believed to be controlled by Schildknecht's turgorins which induce leaf-closing movement of the plants.[4] Schildknecht said that all leaf-movements are controlled by turgorin, a new class of phytohormone which regulates the turgor of the plants. However, we revealed that turgorin is not a genuine leaf movement factor, and nyctinastic movement is regulated by a chemical substance that differs depending on the plant. Our present results are in accordance with Umrath *et al.*'s physiologically significant opinion that every family or subfamily of plants has its own leaf movement factor that is effective only for plants belonging to its own family, although they have not identified any leaf movement factor.[5] Also, we revealed that there exist leaf-opening substances together with leaf-closing ones in every nyctinastic plant. Leaf-opening substances differed in certain aspects from indole-3-acetic acid (IAA) that has been believed to induce the leaf-opening of nyctinastic plants[6]: 1) the bioactivities of the leaf-opening substances (*ca.* 1×10^{-6} M) were much stronger than that of IAA ($> 1 \times 10^{-4}$ M), and 2) the bioactivity of the leaf-opening substances were specific to the genus of the plant while that of IAA was nonspecific.

The presence of leaf-opening substances indicates that nyctinastic movement is controlled not only by the change in the concentration of the leaf-closing factor, but also by the competitive interaction between leaf–closing and leaf–opening substances. The idea of the co-existence of a leaf-opening substance with a leaf-closing substance is very reasonable in terms of the previously mentioned result that K^+ ions enter and leave plant cells *via* ion channels which are regulated differently.[7]

2.1 The establishment of a general procedure for the isolation of bioactive substances for nyctinasty.

The most difficult problem encountered in the chemical study of nyctinastic movement lies in the isolation of bioactive substances. The difficulty in the isolation comes from the weak biological activity observed by bioassay. The bioactivity gradually diminished and finally was lost in the course of the isolation procedure. We thought that this phenomenon is attributed to the co-existence of both the leaf-opening and -closing substances in the same separated fraction. When these two bioactive substances are mixed in the same fraction, the respective bioactivities should cancel each other, and no bioactivity can be monitored by bioassay. All of the previous attempts for the separation of the leaf-closing substance were carried out by using a combination of the gel filtration chromatography and HPLC. It is supposed that all such studies on the isolation failed because no attempt was made to separate the leaf-closing substance from the leaf-opening ones. Thus, it is crucial to separate these two inversely bioactive substances from each other in the first step of the isolation procedure.

We attempted to apply polymer gel chromatography for the separation of leaf-opening and -closing substances. After many separation conditions were examined, we found that only an Amberlite XAD-7 column chromatography could separate these two substances completely based on the difference in polarity. The result was very satisfactory. The highly polar leaf-closing substance was eluted by 100% H_2O eluent; on the other hand the comparatively low polar leaf-opening substance was eluted by the MeOH-containing eluent, and the bioassay gave completely distinct results in the full course of the isolation. The Amberlite XAD-7 was proved to be very effective for separation of the two leaf-

movement factors.

After the separation of the two bioactive fractions of leaf-closing and -opening activity, the separations using gel filtration chromatography, MPLC using ODS glass column, and preparative HPLC using ODS column were carried out. Especially, the HPLC using a combination of three columns showed very good separation to enable us to isolate the leaf-closing substance.

We have now established a fine procedure for the isolation of the leaf-movement factors for nyctinasty. Our procedure was applicable to other nyctinastic plants, and many bioactive substances for nyctinasty were isolated. By our introduction of an Amberlite XAD-7 column chromatography and HPLC using a combination of plural ODS columns, significant advances would be made in the chemical study on leaf-movements, leading to understanding of the leaf-movement at the molecular level.

2.2 Leaf-movement factors of Cassia mimosoides *L.*

In order to detect the leaf movement factors in *C. mimosoides* L., we developed a bioassay using its leaves. The movement of the leaves detached from each stem and immersed in water was also found to follow the circadian rhythm. The young leaves of *C. mimosoides* L. to be tested were immersed in distilled water and allowed to stand at room temperature overnight. The leaves that opened again the next morning were used for the bioassay.

The fresh whole leaves of *C. mimosoides* was extracted with MeOH in a short-step process and the extract was carefully separated according to the isolation procedure shown in Figure 1, to afford a small amount of the leaf-closing substance that was proven to be potassium chelidonate (**1**).[8] Potassium chelidonate was also found in *Cassia occidentalis* L., which belongs to the same species as *C. mimosoides*.[8]

The natural and synthetic **1** induced the closing of *Cassia* leaves at 10^{-6} ~ 10^{-7} M in the daytime. Interestingly, the corresponding sodium salt (**2**, R = Na) as well as the free acid (**3**, R = H) showed no bioactivity. Also, potassium acetate, potassium oxalate, and potassium salt of phloroglucinol did not show any leaf-closing activity in the plant. Therefore, these results indicate that both the heterocyclic structure and the potassium ion were indispensable for the bioactivity of **1**. Potassium chelidonate (**1**) as a "turgolin" plays an important role in the leaf movement of nyctinastic plants *Cassia mimosoides* and *C. occidentalis* L.

On the other hand, calcium 4-*O*-β-D-glucopyranosyl-*cis*-*p*-coumarate (**4**) was isolated as the leaf–opening substance of *Cassia mimosoides* L.[9] Compound (**4**) lost its bioactivity under strongly polar isolation conditions. This is because the counter cations that dissociated from the carboxylate of **4** were lost or exchanged with other cations during the separation using polar solvents. This substance effectively opened the leaves of *Cassia mimosoides* L. at 4×10^{-6} M at night; however, it exerted no effect on other nyctinastic plants even at a concentration of 1×10^{-4} M. The potassium salt of **4**, which was isolated together with **4**, exhibited no biological activity, and calcium acetate was effective only at 1×10^{-2} M. Thus, both calcium ions and their counter ions were essential for the bioactivity of **4**. Together with

1, two leaf-movement factors with contrasting activities were isolated from a nyctinastic plant.

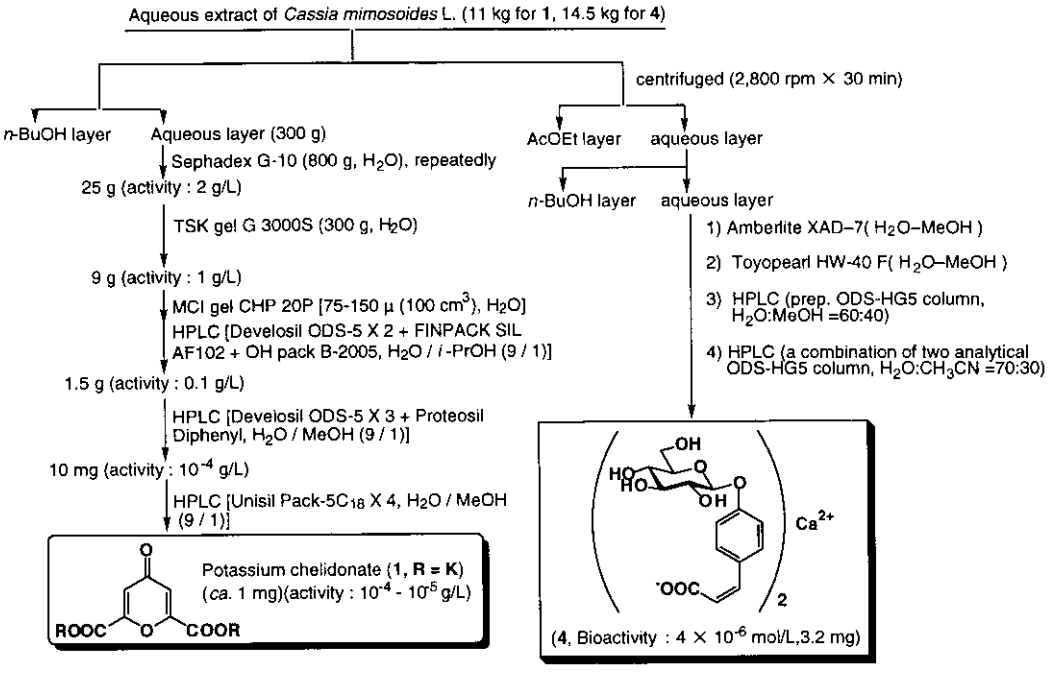


Figure 1. Isolation procedure for potassium chelidonate (**1**) as the leaf-closing substance, and calcium 4-*O*-β-D-glucopyranosyl-*cis*-*p*-coumarate (**4**), as the leaf-opening substance in *C. mimosoides* L.

2.3 Leaf-movement factors of Phyllanthus urinaria *L.*

Phyllanthurinolactone (**5**) (3.1 mg from 19.2 kg)[10] and phyllurine (**6**) (0.5 mg from 10.0 kg)[11] were isolated as the leaf-closing and -opening substances, respectively, from the nyctinastic plant *Phyllanthus urinaria* L., which belongs to the *Euphorbiaceae* family (Figure 2). Since other leaf-closing substances have been isolated from the *Fabaceae* family, it would be interesting to study the difference in the chemical structure of the leaf-movement factors between these two families. Because of this difference in species, the structure of bioactive substances greatly differs from those of other leguminosae plants. Phyllanthurinolactone (**5**) is the only glycoside-type leaf-closing substance ever isolated.

The structural determinations of **5** and **6** were carried out by means of 2D NMR experiments (Figure 3). NOE experiments and coupling constants revealed the stereochemical relationships.

The absolute configuration of **5** was determined from the total synthesis by Mori *et al.*[12] They also synthesized all the possible stereoisomers relative to the diol moiety of the aglycon. Bioactivity was only observed in the natural *syn*-(6*S*, 7a*R*) form.

Phyllanthurinolactone (**5**) was effective for the leaf–closing of *P. urinaria* at 1×10^{-7} M in the daytime, and phyllurine (**6**) was effective at 5×10^{-5} M; however, they exerted no effect on other nyctinastic plants even at 1×10^{-4} M. Likewise, the leaf–closing substances of other nyctinastic plants exerted no

effect on *P. urinaria* even at 1×10^{-2} M.

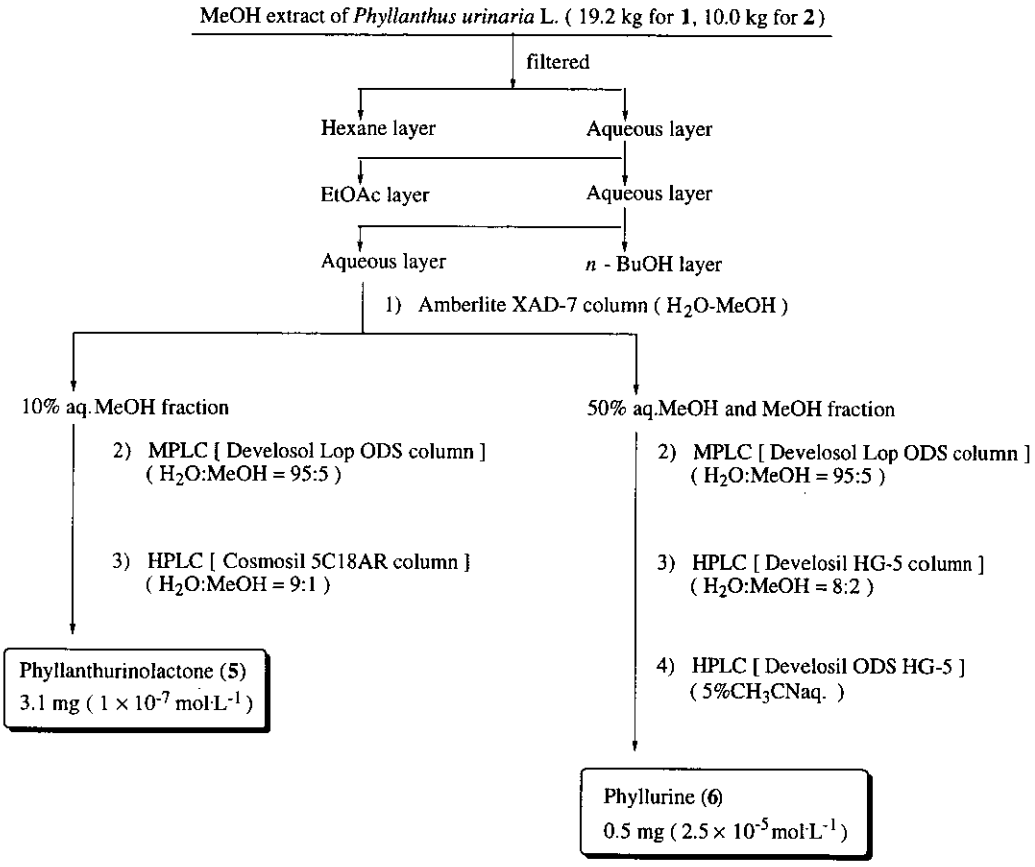


Figure 2. Isolation procedure for phyllanthurinolactone (**5**) and phyllurine (**6**).

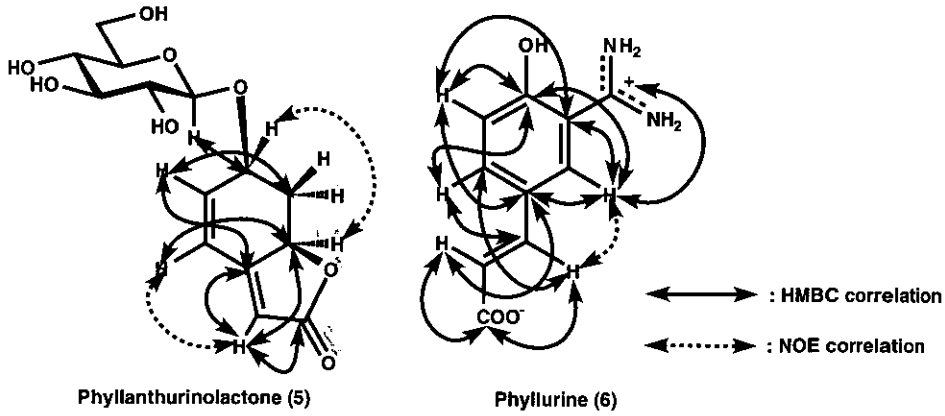


Figure 3. Important HMBC and NOE correlations in phyllanthurinolactone (**5**) and phyllurine (**6**).

2.4 Leaf-movement factors of Lespedeza cuneata G. Don

Potassium D–idarate (**7**) (1.8 mg) as a leaf-closing factor,[13] and potassium lespedezate (**8**) and potassium isolespedezate (**9**, geometrical isomer of **8**) as leaf-opening substances[14] were isolated from *Lespedeza cuneata* G. Don (10.1 kg), according to the bioassay using the leaves of *Cassia mimosoides* L. We have isolated two leaf-movement factors, **7** and **8** (or **9**), with contrasting activity from one plant.

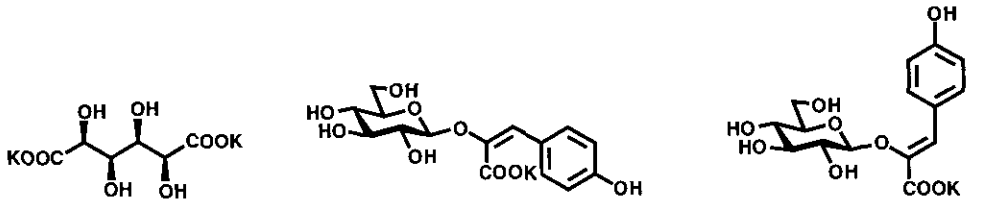


Figure 4 Structures of the leaf-movement factors in *L.cuneata*

Because of the stiffness of the stem, the leaves of *Lespedeza cuneata* G. Don transported the sample solution poorly, and it was insufficient for use in the bioassay. We used the leaves of *Cassia mimosoides* L. for the bioassay instead of the leaves of *Lespedeza cuneata* G. Don to overcome this experimental difficulty. The bioactivities of **7**, **8**, and **9** were quite specific for the leaves of *Cassia mimosoides* L. at 5 $\times 10^{-7}$ M in the daytime.

The stereochemistry of **7** was determined by comparing its spectroscopic data and bioactivity with those of various potassium tetrahydroxy dicarboxylates prepared from D–hexoses. All other diastereomers are biologically inactive or only very weakly active.[15] Thus, the bioactivity of **7** was specific with respect to its stereochemistry.

To investigate structure–activity relationships, various analogs of **8** were subjected to bioassay (Figure 5). The bioactivities of sodium salts and free acids were as strong as those of the natural products. Interestingly, *p*-hydroxyphenylpyruvic acid (**10**) and its salt (**11**) opened the leaves in 10^{-4} M even at night. From these results, it is obvious that one of the most important structures for leaf-opening activity is a *p*-hydroxyphenylpyruvate unit.[15]

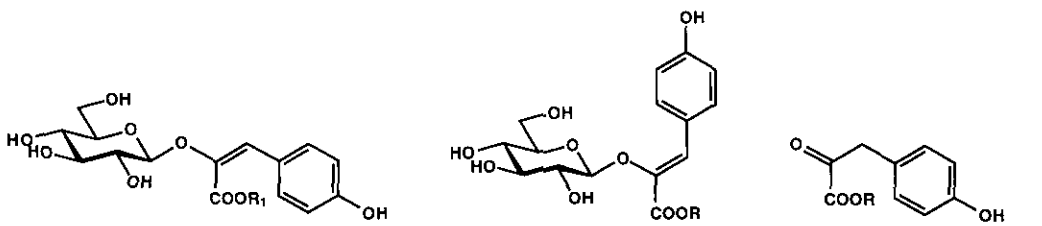
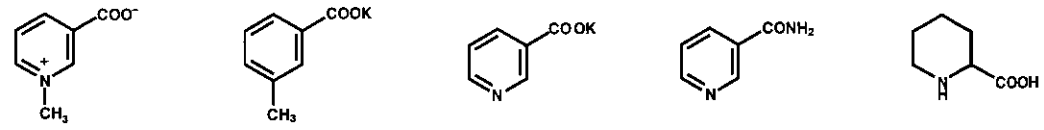


Figure 5. Bioactivities of potassium lespedezate (**8**) and its analogs.

2.5 Trigonelline from Aeschynomene indica

We isolated trigonelline (**12**) as a leaf–closing factor of the nyctinastic plant *Aeschynomene indica*.[16] The strongly polar leaf-closing substance was eluted from an Amberlite XAD-7 column with H_2O, while the weakly polar leaf-opening substance was adsorbed on the column. Thus, these two bioactive substances were completely separated.

Trigonelline (**12**) at 1×10^{-7} M was effective only for the leaf–closing of *A. indica*, and not for other nyctinastic plants, such as *C. mimosoides* and *M. pudica*. The heterocyclic structure of **12** was proved to be essential for its bioactivity from the bioassay using structurally related compounds (**13-16**) (figure 6).



Trigonelline (12) (1×10^{-7} M) | **(13)** Inactive | **(14)** Inactive | **(15)** Inactive | **(16)** Inactive

Figure 6. Structure-activity relationship in trigonelline (**12**)

Trigonelline (**12**) was also reported to be a G2 factor present in the cotyledons of garden peas that promotes cell arrest in the G2 stage (DNA synthetic stage) of both roots and shoots.[17] Trigonelline promotes cell arrest in the G2 stage in 40% of all root cells. In the absence of trigonelline, this cell population is arrested in the G1 stage. Tramontano *et al.* reported that trigonelline is the first chemically identified "hormone" that controls the cell cycle in plants or animals.[17c] These results are very interesting in terms of the relationship between the cell cycle and the biological clock.

2.6 cis–p–*Coumaroylagmatine, the Leaf–opening Substance in* Albizzia julibrissin *Durazz*

The nyctinastic movement of the leaves of the silk tree, *Albizzia julibrissin* Durazz is as well known as the rapid movement of *Mimosa pudica* L. leaves.

We isolated *cis–p*–coumaroylagmatine (**17**) (1.2 mg) from *A. julibrissin* (1.8 kg) based on a bioassay using the leaves of the original plant.[18] This leaf-opening substance kept the leaves open until 9:00 PM at 5×10^{-6} M. The bioactivity of **17** was specific to this plant.

The structural determination of **17** was carried out by means of 2D NMR experiments (Figure 7). It is interesting that *A. julibrissin* contains only the thermodynamically unstable *Z* isomer, and no *E* isomer. *cis–p*–Coumaroylagmatine (**17**) and its *trans* isomer were synthesized from agmatine and *cis–p*–coumaric acid.[19] Synthetic **17** shows the same level of activity as the authentic sample of natural *cis–p*–coumaroylagmatine, and the synthetic *trans* isomer of **17** opened the leaves of *A. julibrissin* at concentrations as low as 1×10^{-3} M.

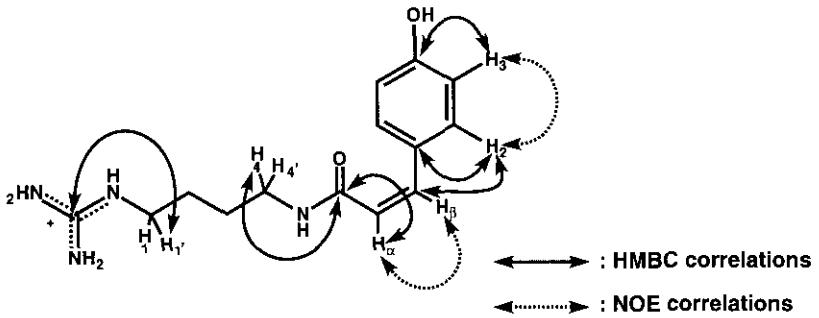


Figure 7. Leaf-opening substance (**17**) in *Albizzia julibrissin* Durazz

3. SUMMARY OF THE ISOLATION OF LEAF-MOVEMENT FACTORS

As already mentioned, several bioactive substances controlling the leaf movement of nyctinastic plants have been detected (Figure 8).

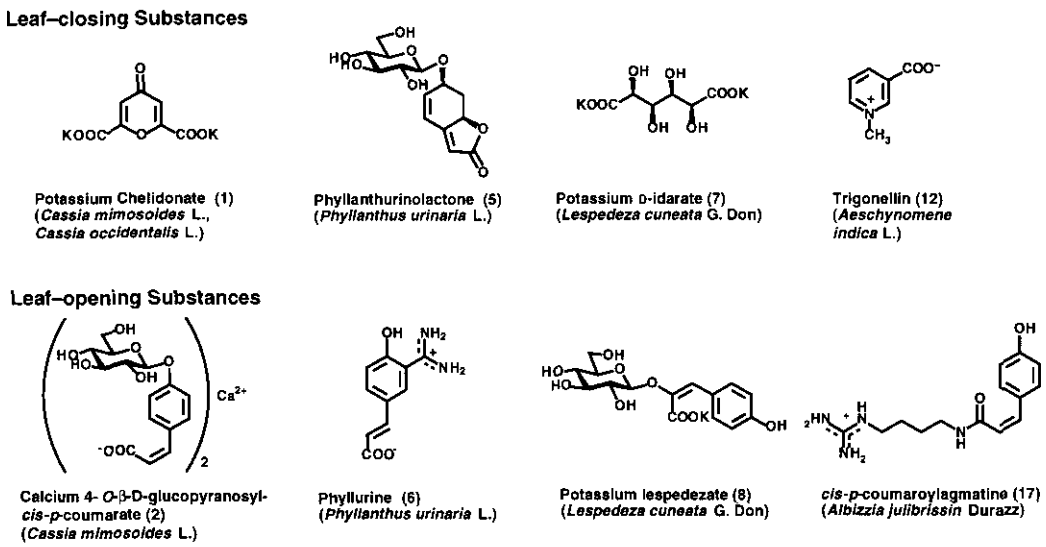


Figure 8. Bioactive substances controlling the leaf movement of nyctinastic plants

It is believed that nyctinastic movement is controlled by turgorin, a leaf-closing phytohormone common to all nyctinastic plants. However, the leaf-closing substances of nyctinastic plants were proven to be different from each other. There also exist leaf-opening substances that compete with leaf-closing ones in controlling the leaf movement of nyctinastic plants. It is important that both leaf-closing and -opening substances have been isolated from the same plant, as shown in the cases of *Cassia mimosoides*, *Lespedeza cuneata*, and *Phyllanthus urinaria*. Therefore, each nyctinastic plant has its own leaf-opening and leaf-closing substances, and nyctinastic movement is controlled by these compounds.

L-Trp was also isolated as a weak leaf-opening substance from several plants,[11] which was effective at as low as 1×10^{-4} M on the leaves of all nyctinastic plants similar to IAA. It is proposed that the bioactivity of L-Trp is attributed to IAA, which is known as an important metabolite of L-Trp. The bioassay was

carried out by the addition of the sample solution at 11:00 PM.; thus, this long period necessary for the bioassay to detect the leaf-opening activity is sufficient for the metabolism of L-Trp into IAA. Thus, L-Trp should not be a genuine leaf-opening substance.

Moreover, these leaf-movement factors have four properties *in common;*

1) At the concentration of approximately $10^{-6} - 10^{-7}$ M, all bioactive substances exert their effects only on the plants where they originate. This concentration is almost the same as that of known phytohormones, such as IAA and gibberellin.
2) The bioactivity of all leaf-movement factors is specific to the original plant from which it was isolated.
3) All leaf-closing substances have a common physiological property in that they compete with IAA at 10^{-6} M. However, much more IAA (*ca.* 10^{-4} M) is required to achieve a competitive state than the natural abundance of IAA in a plant body.
4) The bioactivity of these compounds is dependent on their stereochemistry. Optically active **5** and **7** were the only bioactive ones among their stereoisomers. This suggests that a specific receptor participates in the process of signal transduction by these substances.
5) The leaf-opening substance competes with the leaf-closing substance (Tables 1 and 2). When the concentration of the leaf-closing substance was higher than that of the leaf-opening substance, the leaves were closed during the day and *vice versa.*

Table 1. Competitive Interaction of **7** with **8**

7 [M]	**8** [M]	Day	Night
1×10^{-5}	1×10^{-5}	+−	−−
1×10^{-5}	0.5×10^{-5}	−−	−−
0.5×10^{-5}	1×10^{-5}	++	+−
1×10^{-6}	1×10^{-6}	+−	−−
1×10^{-6}	0.5×10^{-6}	+−	−−
0.5×10^{-6}	1×10^{-6}	++	−−

Leaf movement is represented by the following: ++ completely open; +− at random; −− completely closed

Table 2. Competitive Interaction of **1** and **2**

1 [M]	**2** [M]	Day	Night
1×10^{-5}	1×10^{-5}	++	+−
0.5×10^{-5}	2.5×10^{-5}	++	++
2.5×10^{-5}	0.5×10^{-5}	−−	−−
1×10^{-6}	1×10^{-6}	++	−−
0.5×10^{-6}	2.5×10^{-6}	++	++
2.5×10^{-6}	0.5×10^{-6}	−−	−−

Leaf movement is represented by the following: ++ completely open; +- at random; −− completely closed

4. CHEMICAL CONTROL OF LEAF MOVEMENT IN NYCTINASTIC PLANTS

From the viewpoint of chemical studies, the next important problem to be solved regarding the leaf movement of nyctinastic plants is how these compounds control the nyctinastic leaf movement.

We have isolated two leaf-movement factors of contrasting bioactivities from the same nyctinastic plants. This was unexpected, and is not compatible with the established theory proposed by Schildknecht.[2] The discovery of leaf-opening substances from nyctinastic plants prompted us to assume that nyctinastic leaf-movement is controlled by a competitive interaction between leaf-closing and leaf-opening substances. As described above, three sets of leaf–movement factors were isolated from three plants, *Lespedeza cuneata* G. Don, *Cassia mimosoides* L., and *Phyllanthus urinaria* L. These bioactive substances inhibited each other competitively. Then, we assumed that the concentration of these two substances changes through a day.

As shown in Table 2, potassium D-idarate (**7**), a leaf-closing substance of *L. cuneata*, interacts competitively with potassium lespedezate (**8**), a leaf-opening substance of the same plant. When the concentration of **7** was higher than that of **8**, the leaves were closed during the day, and *vice versa*. Furthermore, we have made an important discovery that plant extracts of all the nyctinastic plants collected during the day and at night exhibited opposite bioactivity to each other; the extract collected in the daytime exhibited leaf-opening activity, and kept the leaves open even at night, while the extract collected at night exhibited weak leaf-closing activity, and kept the leaves closed even during the daytime.[13] It is important that the bioactivity of the plant extract completely reflected the status of the collected leaves; the plant extract collected when the leaves were closed showed leaf-closing activity and *vice versa*.

Presumably, these bioactive substances are not stored, but are metabolized in the course of time. The enzymatic transformation during metabolism should be controlled by a biological clock. Thus, chemical studies on this transformation should give us an important clue to revealing the chemical control of leaf movement by a biological clock.

We demonstrate the chemical mechanism for nyctinasty of *Lespedeza cuneata* G. Don, as an example.[15, 20] The extracts of *L. cuneata* collected during the day (around 10:00 AM) and at night (around 7:00 PM) showed inverse bioactivity; in other words, the former showed leaf–opening activity but the latter showed leaf–closing activity. These results suggest that the balance of concentration between **7** and **8** (or **9**, *cis* isomer of **8**) is reversed in these two extracts.[24] HPLC analysis revealed that the extract collected during the day contained twice as much **8** and **9** as the extract collected at night, 10:00 PM (Table 3).

Table 3. Concentrations of **8**, **9** and **10** in *Lespedeza cuneata* G. Don.

Time of collection	Concentration (M)		
	8	**9**	**10**
Day	2.7×10^{-5}	4.1×10^{-5}	2.5×10^{-5}
Night	2.2×10^{-5}	1.8×10^{-5}	1.2×10^{-5}

Water content of the plant material is calculated to be 80% of total weight.

This twofold increase in the concentrations of **8** and **9** is sufficient to inverse the bioactivity of the extract from our previous research on the competition experiment between **7** and **8** (or **9**) (Table 1). Thus, both **8** and **9** are metabolized in the evening, and biosynthesized in the morning. Moreover, we discovered that the concentrations of **8** and **9** in the plant are inversely proportional to that of potassium 4-hydroxyphenylpyruvate (**10**).

The effectiveness of the pyruvate (**10**) (5×10^{-5} M) was one-hundredth of that of **8** (or **9**) (8×10^{-7} M) at inducing leaf-opening movement. The extract collected at night contained fivefold as much **10** as the extract collected during the day. This result strongly suggests that **8** is biosynthesized from **10** in the morning, and is metabolized (and deactivated) to **10** by enzymatic hydrolysis in the evening, 5:00 PM (Figure 15).

This new model of the regulation of leaf-movement was strongly supported by the measurement of β-glucosidase activities of the crude enzyme prepared from plants collected during the day and the evening.[15] The crude enzyme was prepared according to the method of Watanabe *et al.*[21] We collected the leaves of *L. cuneata* and prepared acetone powder. We used **8** and **9** as substrates of the enzyme, and quantitatively analyzed **10** produced by treatment of the acetone powders by HPLC. β-Glucosidase activity was observed only in the acetone powder prepared from the plant collected in the evening (Table 4).

On the other hand, the concentration of the leaf-closing substance was proved to be almost constant through a day. We propose a new model for the regulation of nyctinastic movement in *L. cuneata*: enzymatic transformation of leaf-movement factors (**9**) and (**10**) inversed the balance of concentration between **7** and **9**, wherein a biological clock should control leaf-movement by activation or expression of the enzyme concerned in this step.

Table 4. Enzymatic transformation of **8** to **10** with the acetone powder.

Time of collection	Concentration (M)	
	8	**10**
day	1.0×10^{-3}	ND
evening	1.0×10^{-3}	2.2×10^{-5}
blank	1.0×10^{-3}	ND

To 1.0 mL of 1.0 mM **8** solution in 0.1 M citrate buffer (pH 5.0) was added acetone powder (1.0 g). Then, the reaction mixture was incubated at 37 °C for two hours. After quenching the reaction, the reaction mixture was analyzed by HPLC.

Our study on the nyctinastic movement of *L. cuneata* revealed that nyctinastic leaf-movement is controlled by a biological clock through the regulation of the activity of β-glucosidase which hydrolyzes the leaf-opening substance of this plant. A similar model would be applicable in the cases of *Phyllanthus*

urinaria L. and *Cassia mimosoides* L. In the case of *P.urinaria*, the leaf-closing substance, which is a glucoside, would be hydrolyzed by the β-glucosidase whose activity is regulated by a biological clock. There is some possibility that the regulation of all nyctinastic leaf-movements can be explained by only one mechanism, namely, that either the leaf-closing or –opening substance is a glucoside in all nyctinastic plants. The biological clock regulates the activity of β-glucosidase which deactivates the glucoside-type leaf-movement factor to control the internal balance of concentration between leaf–closing and –opening substances.

5. CONCLUSION

Leaf-movement in nyctinastic plants has long been believed to be controlled by a common phytohormone. However, our result contradicts this theory. Indeed, we advance a new theory of the chemical control of nyctinastic leaf movement; nyctinastic leaf-movement is controlled by the balance of concentration between two bioactive substances, leaf-opening and -closing substances, which is inversed through the day according to the rhythm created by their biological clock. A biological clock regulates this balance through the control of the β-glucosidase activity (Figure 9).[22]

Our leaf-movement factors, whose concentrations have been proven to change according to a circadian rhythm, should be genuine chemical signals that control leaf-movement in nyctinastic plants from a physiological viewpoint. Our present result will develop the science of this field from plant physiology into bioorganic chemistry. And we expect that these results would bring us a clue in the investigation of the nature of the biological clock in highly organized plants.

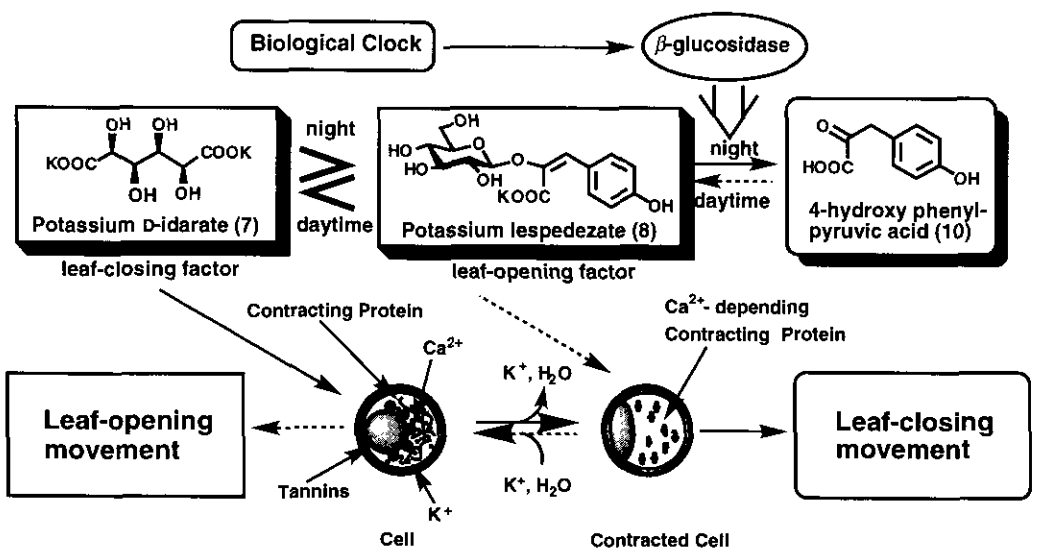


Figure 9. Chemical control of leaf movement in nyctinastic plants; solid arrows show the leaf-closing process; dotted arrows show the leaf-opening process.

ACKNOWLEDGMENTS

We are indebted to the Ministry of Education, Science, Sports and Culture (Japan) and Asahi Glass Foundation for financial support.

REFERENCE AND NOTES

1. Du Fay, Observation botanique. Histoire de L'Academie Royale des Sciences (Paris 1729), p.35.
2. E. Bünning, 'The Physiological Clock, Third Edition,' English Univ. Press, London, 1973.
3. C. Darwin, 'The Power of Movement in Plants' John Murray, London, 1880.
4. H. Schildcknecht, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 695.
5. W. Bielenberg, H. Esterbauer, M. Hayn, and K. Umrath, *Phyton*, 1984, **24**, 1.
6. S. Watanabe and K. Umrath, *Phyton*, 1983, **23**, 49.
7. Y. Lee, 'The Pulvinus in Motor Organ for Leaf Movement,' ed. by R. L. Satter, H. L. Gorton and T. C. Vogelmann, American Society of Plant Physiologists, Rockville, MD, 1990, pp. 130-141.
8. E. Miyoshi, Y. Shizuri, and S. Yamamura, *Chemistry Lett.*, **1987**, 511.
9. M. Ueda, T. Ohnuki, and S. Yamamura, *Phytochemistry*, 1998, **49**, 633.
10. M. Ueda, T. Shigemori-Suzuki, and S. Yamamura, *Tetrahedron Lett.*, 1995, **36**, 6267.
11. M. Ueda, M. Asano, and S. Yamamura, *Tetrahedron Lett.*, 1998, **39**, 9731.

12a. K. Mori, G. Audran, Y. Nakahara, M. Bando, and M. Kido, *Tetrahedron Lett.*, 1997, **38**, 575. b) G. Audran and K. Mori, *Eur. J. Org. Chem.*, 1998, 57.

13. M. Ueda, T. Ohnuki, and S. Yamamura, *Tetrahedron Lett.*, 1997, **38**, 2497.
14. H. Shigemori, N. Sakai, E. Miyoshi, Y. Shizuri, and S. Yamamura, *Tetrahedron Lett.*, 1989, **30**, 3991.
15. M. Ueda, T. Ohnuki, and S. Yamamura, *Tetrahedron*, 1998, **54**, 12173.
16. M. Ueda, M. Niwa, and S. Yamamura, *Phytochemistry*, 1995, **39**, 817.

17a. D. G. Lynn, K. Nakanishi, S. L. Patt, J. L. Occolowitz, S. Almeida, and L. S. Evans: *J. Am. Chem. Soc.*, 1978, **100**, 7759. b) L. S. Evans, M. S. Almeida, D. G. Lynn, and K. Nakanishi, *Science*, 1979, **203**, 1122. c) W. A. Tramontano, C. M. Hartnett, D. G. Lynn, and L. S. Evans, *Phytochemistry*, 1982, **21**, 1201. d) W. A. Tramontano, D. G. Lynn, and L. S. Evans, *Phytochemistry*, 1983, **22**, 673. e) D. G. Lynn, D. H. Lewis, W. A. Tramontano, and L. S. Evans, *Phyotchemistry*, 1984, **23**, 1225.

18. M. Ueda, C. Tashiro, and S. Yamamura, *Tetrahedron Lett.*, 1997, **38**, 3253.
19. M. Ueda, Y. Sawai, Y. Shibazaki, C. Tashiro, T. Ohnuki, and S. Yamamura, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 2133.
20. M. Ueda, T. Ohnuki, and S. Yamamura, *Chemistry Lett.*, **1998**, 179.

21. H. Watanabe, S. Watanabe, R. Nakajima, J.-H. Moon, K. Shimokihara, J. Inagaki, H. Etoh, T. Asai, K. Sakata, and K. Ina, *Biosci. Biotech. Biochem.*, 1993, **57**, 1101.
22. H. Toriyama and M. J. Jaffe, *Plant Physiol.*, 1972, **49**, 72.

Received, 10th December, 1998