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BIOACTIVE SUBSTANCES FROM LESPEDEZA CUNEATA L. G. DON AND THEIR BIOLOGICAL ACTIVITIES

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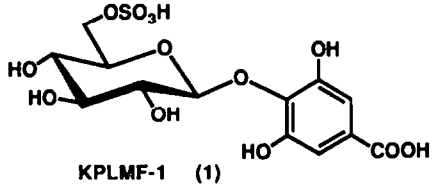
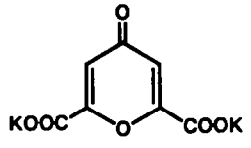
Abstract - *Potassium lespedezate (3) and potassium isolespedezate (4) were isolated from the nyctinastic plant Lespedeza cuneata L. G. Don. These compounds (3 and 4) were quite effective for leaf-opening of the plant Cassia mimosoides L. at* 8×10^{-7} *mol/l. The interaction between the leaf-closing substance (2) and leaf-opening substances (3 and 4) shall govern in circadian rhythm of Cassia plants.*

The plant Mimosa pudica (Ojigi So in Japanese) and related nyctinastic plants have been well known for their organisms having an internal clock, and great efforts have been made for understanding the mechanism of the thigmonastic and nyctinastic movements of Mimosa pudica.[1] In 1916, Ricca's ingeneous experiments strongly suggested that the movement of the plant must be controlled by some bioactive substances.[2] Since then, a number of scientists have attempted to search for these bioactive compounds. Recently, Schildknecht et al. have succeeded in isolation of some turgolins from the plants Mimosa pudica, Acacia karoo, Albizzia julibrissin, Oxalis strica, and others. In particular, they have reported that the leaf movement factor (K-PLMF1) (1)[1,3] is regarded as the truly bioactive substance controlling both thigmonastic and nyctinastic movements. In 1987, we have isolated potassium chelidonate (2) from the nyctinastic plants Cassia mimosoides L. (Kawaraketsumei in Japanese) and Cassia occidentalis L. (Habu So in Japanese) as a leaf-closing factor.[4]



KPLMF-1 (1)



Potassium chelidonate (2)

We report herein isolation of bioactive substances from the plant Lespedeza cuneata L. G. Don (Medohagi in Japanese) and their biological activities. In order to detect stimulants of nyctinastic plants, we have adopted two different methods using the leaves of Mimosa pudica and Cassia mimosoides L. In bioassay of leaf-closing factor, we have used the leaves

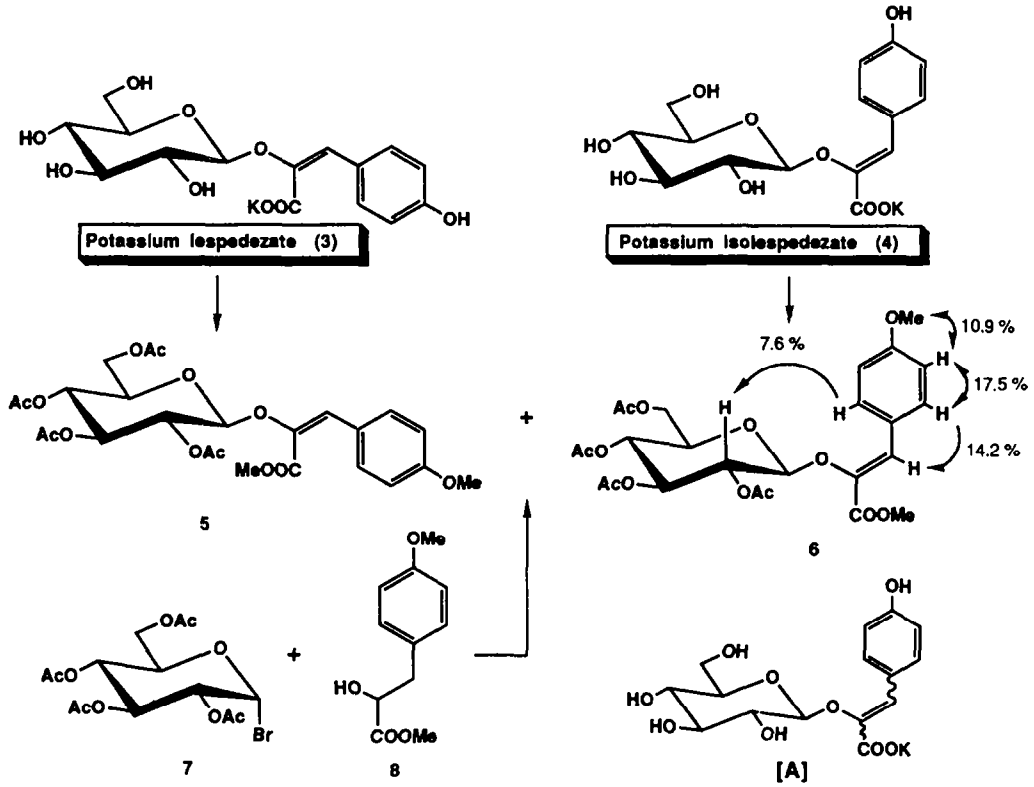
of Mimosa pudica. Our Mimosa test is quite similar to the Fitting-Hess-Schildknecht test using the leaves of the plant,[1,3] which shows a rapid response, but must be carefully carried out because it is quite sensitive to H^+ ion [the leaves were closed on addition of dil. H_2SO_4 (10^{-4} mol/l)], temperature, humidity, and others. In bioassay of leaf-opening factor, we have used the leaves of Cassia mimosoides L. The young leaves to be tested have been immersed in distilled water and allowed to stand at room temperature for several hours. As usual, the leaves closed at night. However, when the bioactive principles were contained in the test solution, the leaves opened again even at night. The Cassia test is quite reproducible, but must be carried out carefully, because the minimum active concentration varied with the conditions (temperature, humidity, and others).

Results and Discussion

The fresh whole herb of Lespedeza cuneata L. G. Don was extracted with methanol for a week, and then carefully separated according to the isolation procedure cited in Fig. 1 and 2. In each procedure, the fractions containing leaf-closing and leaf-opening substances could be obtained separately by column chromatography on TSK gel G3000SW with H_2O as an eluant. As shown in Fig. 1, a very small amount of the leaf-closing substance (<0.1 mg) was isolated from Lespedeza cuneata L. G. Don. The UV spectrum of this substance was quite similar to that of the leaf-closing substance isolated from Mimosa pudica by us.[5] However, the structure of this compound has not yet been determined and further study on this point is in progress.

In Fig. 2, two leaf-opening substances (**3** and **4**) were obtained as colorless powder having the following spectral data ; **3** : $[\alpha]_D^{25}$ -57.4° (c 0.65, H_2O) : FAB-MS (negative) 379$(M-H)^-$, 341$(M-K)^-$; UV (H_2O) 268 nm(ε, 13000); IR (KBr) 3400, 1630(sh.), 1600(sh.), 1580, 1510 cm^{-1}; ^1H NMR [D_2O, internal reference : dioxane (δ 3.71)] δ 3.41(1H, t, J=9.8Hz), 3.46(1H, dd, J=7.8, 9.3Hz), 3.50(1H, m), 3.54(1H, dd, J=9.3, 9.8Hz), 3.84(1H, dd, J=5.9, 12.7Hz), 3.89(1H,dd, J=2.0, 12.7Hz), 4.88(1H, d, J=7.8Hz), 6.12(1H, s), 6.79(2H, d, J=8.8Hz), 7.18(2H, d, J=8.8Hz); ^{13}C NMR [D_2O, internal reference : dioxane (δ 67.6)] δ 61.9(t), 70.7(d), 74.1(d), 76.7(d), 77.3(d), 101.5(d), 110.0(d), 116.4(dx2), 127.9(s), 130.6(dx2), 150.7(br.s), 155.6(s), 172.4(br. s). **4** : $[\alpha]_D^{25}$ +53.1° (c 1.0, H_2O) ; FAB-MS (positive) 381$(M+H)^+$, 343$(M-K+H_2)^+$; UV (H_2O) 286 nm(ε, 20000); IR (KBr) 3400, 1605, 1570, 1510 cm^{-1} ; ^1H NMR [D_2O, internal reference : dioxane (δ 3.71)] δ 3.33(1H, m), 3.42(1H, t, J=9.3Hz), 3.52(1H, t, J=9.3Hz), 3.55(1H, dd, J=7.3, 9.3Hz), 3.64(1H, dd, J=5.1, 12.5Hz), 3.75(1H, dd, J=1.7, 12.5Hz), 5.01(1H, d, J=7.3Hz), 6.73(1H, s), 6.87(2H, d, J=8.8Hz), 7.69(2H, d, J=8.8Hz) ; ^{13}C NMR [D_2O, internal reference : dioxane (δ 67.6)] δ 61.5(t), 70.4(d), 74.8(d), 76.9(d), 77.4(d), 102.2(d), 116.5(dx2), 121.4(d), 127.0(s), 132.8(dx2), 146.2(br.s), 156.9(s), 172.6(br.s).

The ^1H and ^{13}C NMR spectra of **4** indicate the presence of β-D-glucopyranoside (δ 3.33, 3.42, 3.52, 3.55, 3.64, 3.75, 5.01; 61.5, 70.4, 74.8, 76.9, 77.4, and 102.2), 1,4-disubstituted benzene ring (δ 6.87, 7.69; 116.5, 127.0, 132.8, and 156.9), a



trisubstituted double bond (δ 6.73; 121.4 and 146.2), and a carboxylate group (δ 172.6). The isomer **3** also has the corresponding signals. Furthermore, both potassium lespedezate (**3**) and potassium isolespedezate (**4**), having the same molecular formula ($C_{15}H_{17}O_9K$), afforded the mixture of these two compounds on standing at room temperature, suggesting that **3** and **4** were isomeric to each other. Thus, the structure of both potassium salts are represented by [A]. Finally, from the comparison of chemical shifts of olefinic protons (**3** : δ 6.12, **4** : δ 6.73) and aromatic protons (**3** : δ 6.79 and 7.18 , **4** : δ 6.87 and 7.69), it is obvious that the carboxylate group and the benzene ring are located in cis position in **3**, and trans one in **4**. To confirm the structures, both potassium lespedezate (**3**) and potassium isolespedezate (**4**) were converted into the acetates **5** and **6**, respectively [1. Amberlite IR-120B(H^+) / H_2O; 2, CH_2N_2 / MeOH; 3, Ac_2O / Pyr.]. The stereostructure of **4** was confirmed by NOE experiments of **6**. The acetates **5** and **6** were synthesized starting from D-acetobromo-glucose (**7**) and methyl 3-p-methoxyphenyl-2-hydroxy-propionate (**8**) (1, AgOTf, Molecular Sieves 4A / CH_2Cl_2; 2, DDQ / toluene at refluxing temperature). The physical properties of the synthetic acetates **5** and **6** were completely identical with those of the compounds obtained from natural bioactive salts (**3** and **4**) in all respects including the optical rotation.[6]

Although the leaf-closing substances have been found by Schildknecht et al. and by us, this is the first example to isolate the leaf-opening substances from plants. To

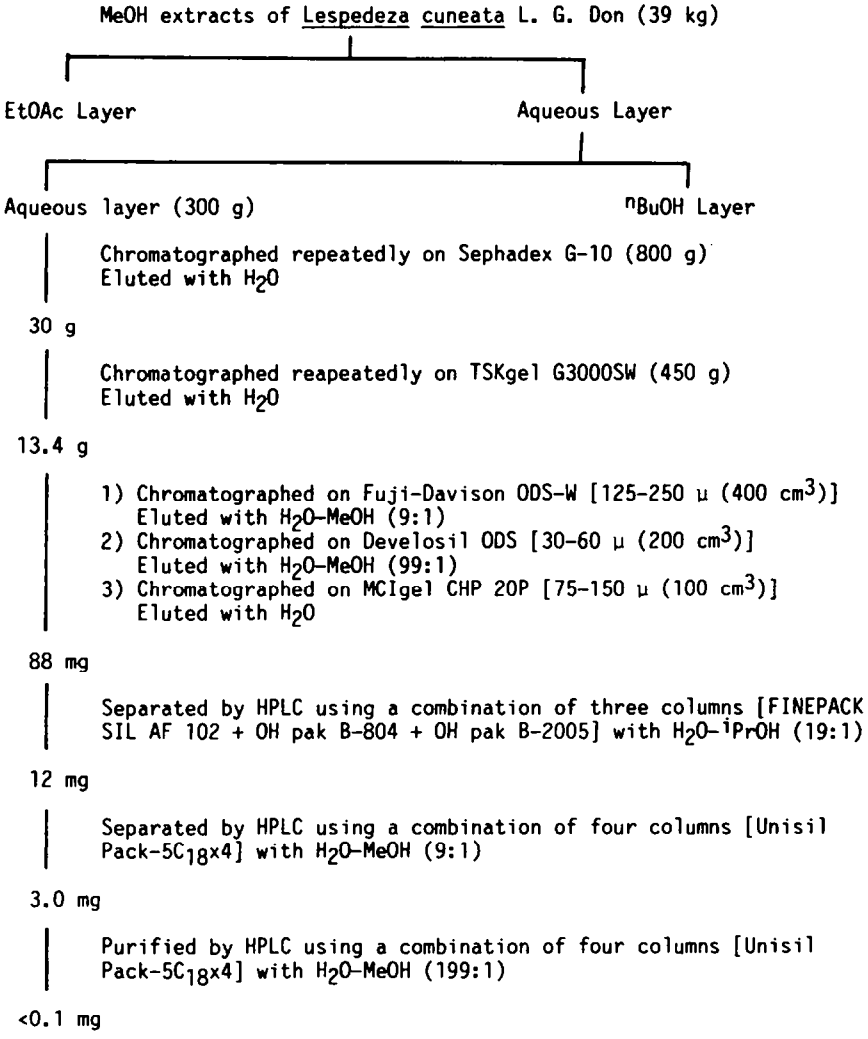


Fig. 1. Isolation procedure of the leaf-closing substance from the plant Lespedeza cuneata L. G. Don

investigate structure-activity relationships, various compounds shown in Fig. 3 were synthesized and the leaf-opening activities of these compounds were examined by Cassia test. Table 1 shows the comparison of leaf-opening activities of these compounds. Activities of the sodium salts (**9** and **11**) were indistinguishable from those of natural ones (**3** and **4**). The free acids (**10** and **12**) were slightly less active than natural ones. Protected compounds (**13** and **14**) and saturated compounds (**15** and **16**) were slightly active. Interestingly, p-hydroxy-phenylpyruvic acid (**18**) and its salt (**17**) were moderately active. From these results, it is obvious that substituents at phenolic hydroxy group as well as carboxy group led to decrease activity, and saturation of the double bond also led to decrease activity.

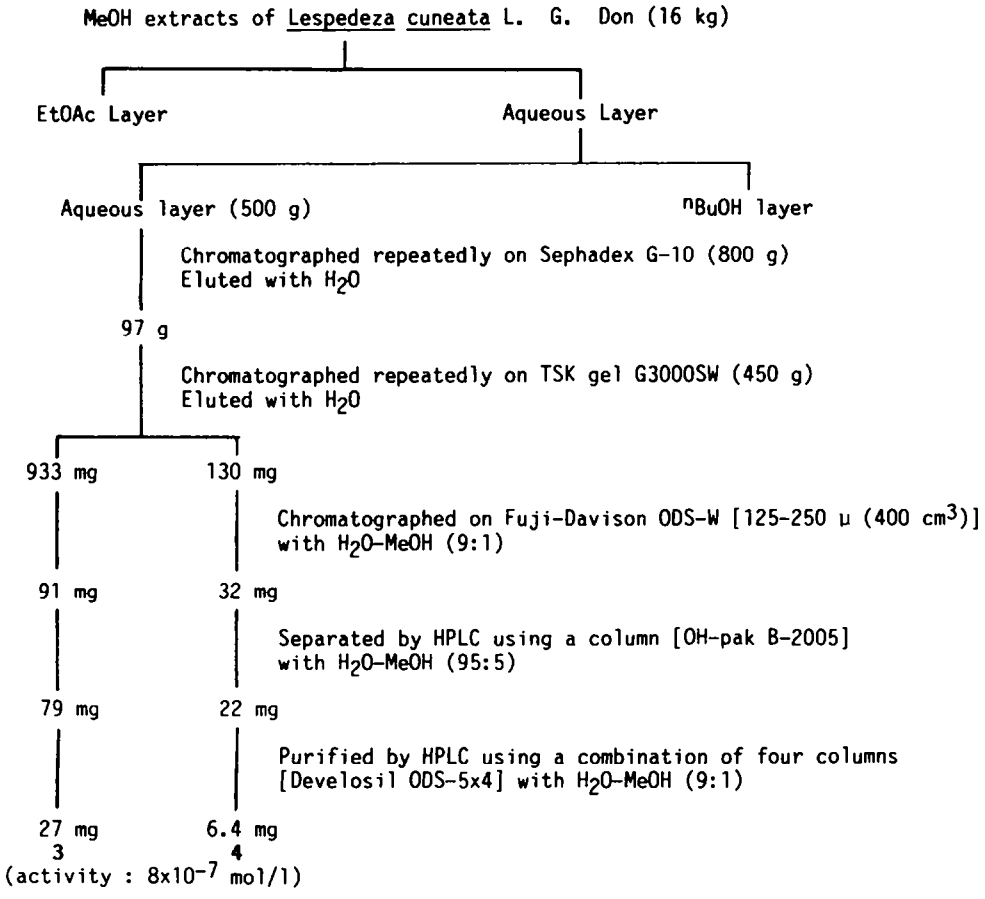
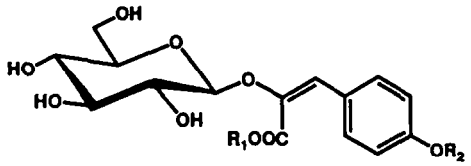


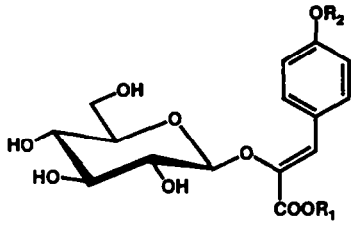
Fig. 2. Isolation procedure of the leaf-opening substances from the plant Lespedeza cuneata L. G. Don

Thus, the most important part for leaf-opening activity is a p-hydroxy-phenylpyruvate unit, and β-D-glucopyranose unit may also play significant roll for the activity.

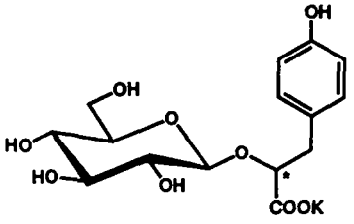
Table 2 shows leaf-opening effect of potassium lespedezate (**3**), potassium isolespedezate (**4**), and indole-3-acetic acid (IAA) known to induce the opening of the leaves of nyctinastic plants.[7] Natural ones (**3** and **4**) specifically induce leaf-opening of the plants, belong to the genus Cassia. Cassia mimosoides L. (at 8×10^{-7} mol/l), Cassia occidentalis L., and Cassia tora L. (at 1×10^{-5} mol/l). Furthermore, remarkable differences between IAA and **3** or **4** were obserbed as mentioned below. When the leaves of Cassia mimosoides L. were treated with IAA ($<10^{-4}$mol/l), they remained open toward evening, but closed at night (around 11 p.m.). The lower the IAA concentration was, the shorter was the period of opening. On the other hand, the leaves treated with **3** or **4** ($>10^{-5}$ mol/l) remained open all days, but upon



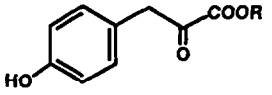
3 R_1=K, R_2=H
9 R_1=Na, R_2=H
10 R_1=H, R_2=H



4 R_1=K, R_2=H
11 R_1=Na, R_2=H
12 R_1=H, R_2=H
13 R_1=K, R_2=Me
14 R_1=Me, R_2=H



15 and 16 * isomers



17 R=K
18 R=H

Fig. 3

Table 1. Comparison of Leaf-Opening Bioactivity of Various Compounds

compounds	active concentration (mol/l)
3	8×10^{-7}
4	8×10^{-7}
9	8×10^{-7}
10	5×10^{-6}
11	8×10^{-7}
12	5×10^{-6}
13	1×10^{-3}
14	1×10^{-4}
15	1×10^{-3}
16	1×10^{-3}
17	5×10^{-5}
18	1×10^{-4}

the treatment with 3 or 4 ($<10^{-5}$ mol/l), the leaves were closed toward evening and gradually opened again at night. The lower the 3 or 4 concentration was, the later was the opening time. These phenomena may be very important for understanding the mechanism of action of IAA and natural substance 3 or 4. And it may be concluded that natural ones (3 and 4) cause leaf-opening on nyctinastic plants, but action of IAA is to reduce nyctinastic closure of such plants. Although IAA induced the opening of the leaves of all plants mentioned in

Table 2. Effect of Potassium Lespedezate (3), Potassium Isolespedezate (4), and Indole-3-Acetic Acid (IAA) on The Leaf-Opening of Various Plants

concentration (mol/l)		$1x10^{-3}$	$1x10^{-4}$	$1x10^{-5}$	$1x10^{-6}$	$8x10^{-7}$
Cassia mimosoides L.	3 or 4	++	++	++	+	+-
	IAA	++	+	-	--	--
Cassia occidentalis L.	3 or 4	++	++	+	-	--
	IAA	++	+	-	--	--
Cassia tora L.	3 or 4	++	++	+	-	--
	IAA	++	+	-	--	--
Mimosa pudica	3 or 4	-	--	--	--	--
	IAA	++	+	-	--	--
Albizzia julibrissin	3 or 4	-	--	--	--	--
	IAA	++	-	--	--	--
Arachis hypogaea L.	3 or 4	-	--	--	--	--
	IAA	++	-	--	--	--
Kummerowia striata	3 or 4	-	--	--	--	--
	IAA	++	++	-	--	--
Lespedeza cuneata L. G. Don	3 or 4	+-	+-	+-	+-	--
	IAA	++	+	-	--	--
Phaseolus angularis	3 or 4	-	--	--	--	--
	IAA	++	++	-	--	--

++ completely open; + nearly open; +- at random; - nearly closed; -- completely closed

Table 2, natural ones (3 and 4) induce the opening of the leaves of the plants belonging to the genus Cassia. Therefore, the activities of 3 and 4 were specific to the genus of plant and that of IAA was non-specific. We suggest that each leaf-opening substance exists in each plant.

Table 3 shows some interaction between potassium chelidonate (2) and potassium lespedezate (3) or potassium isolespedezate (4) in nyctinastic movement of Cassia mimosoides L. When

Table 3. Effect of Potassium Chelidonate (2) on The Potassium Lespedezate (3) or Potassium Isolespedezate (4) - Induced Leaf-Opening of The Plant Cassia mimosoides L.

concentration of 3 or 4 (mol/l)	ratio of 2 and 3 or 4							
	1:0	10:1	2:1	1:1	0.5:1	0.1:1	0:1	control(H_2O)
day time								
$1x10^{-4}$	--	--	-	+-	++	++	++	
$1x10^{-5}$	--	--	-	+-	++	++	++	++
$1x10^{-6}$	--	--	-	+-	++	++	++	
night								
$1x10^{-4}$	--	+-	+	++	++	++	++	
$1x10^{-5}$	--	-	+-	+	++	++	++	--
$1x10^{-6}$	--	--	-	+-	+	++	++	

++ completely open; + nearly open; +- at random; - nearly closed; -- completely closed

the concentration of **2** was higher than that of **3** or **4**, the leaves were closed in the day time. On the other hand, when the concentration of **3** or **4** was higher than that of **2**, the leaves were opened at night. When the concentration ($1x10^{-6}$ mol/l) of **2** was as low as that of **3** or **4**, the movement of the leaves was similar to that of the leaves immersed in distilled water (control test). They showed the same movement as if they moved in natural circadian rhythm. Therefore, the circadian rhythm of Cassia leaves is governed in the interaction between **2** and **3** or **4**. Thus, both **2** and **3** or **4** may be necessary for the leaves to manifest the effect of circadian rhythm or the action of the biological clock through control of osmotica as well as the maintenance of the permeability of motor cell membranes in Cassia mimosoides L..

We suggest that leaf-closing and leaf-opening substances exist individually in various nyctinastic plants. Isolation of these substances from various nyctinastic plants is further in progress. As we now possess the biologically active components (**2**, **3**, and **4**), we shall hopefully obtain additional data to clarify the mechanism for the circadian rhythm of the nyctinastic plants.

Experimental

General Procedures : Sephadex G-10 (40-120 μ in particle size, Pharmacia Fine Chemicals) and TSKgel G3000SW (Toyo Soda MFG. Co., Ltd.) were used for gel filtration chromatography. Fuji-Davison ODS-W (125-250 μ, Fuji-Davison Chemical Ltd.), Develosil ODS (30-60 μ, Nomura Chemical Co., Ltd.), and MCI gel CHP 20P (75-150 μ, Mitsubishi Kasei Co., Ltd.) were used for medium pressure column chromatography. Sizes of columns using for HPLC were φ10 mm x 500 mm (FINEPACK SIL AF 10Ø), φ10 mm x 250 mm (Develosil ODS), φ10 mm x 500 mm (OHpak B-804), φ22 mm x 500 mm (OHpak B-2005), and φ6 mm x 250 mm (Unisil pack-$5C_{18}$). Silica gel TLC and column chromatography were performed on Kieselgel 60 PF_{254} (Merck) and Silica Gel 60 K070 (Katayama Chemical). Melting points were uncorrected. Optical rotations were measured in 100 mm cell using a JASCO DI-4 polarimeter. Mass spectra were obtained with a Hitachi model M-80 instrument. IR spectra were recorded with a JASCO A202 spectrometer. ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz spectra were recorded with a JEOL JNM-GX 400FT NMR spectrometer in D_2O using dioxane as an internal reference [^1H NMR (δ 3.71 ppm) and ^{13}C NMR (δ 67.6 ppm)] or in other solvents using TMS as an internal standard.

Plant Materials : The plants used for the bioassay of leaf-closing as well as leaf-opening, Mimosa pudica (Ojigi So in Japanese), Cassia mimossoides L. (Kawaraketsumei), Cassia occidentalis L. (Habu So), Cassia Tora L. (Ebisu Gusa), Lespedeza cuneata L. G. Don (Medohagi), Arachis hypogaea L. (Nankinmame), Phaseolus angularis (Azuki), Kummerowia striata (Yahazu So), and Albizzia julibrissin (Nemunoki) were grown in a greenhouse of Keio University at 23 to 33 °C for several months.

The Mimosa Test : Mimosa pudica plants had six to eight expanded four-pinnae leaves which responded well to touch stimuli. The third or fourth leaf from the top was used, because it responded constantly. A four - pinnae leaf was detached from the stem with a sharp razor blade and immersed in distilled water (ca. 200 μl) in a 300 μl - polyethylene tube in the greenhouse around 11 a.m. to allow them to recover from cutting injury (for around one hour). Each test solution (ca. 100 μl) was carefully poured into the test tubes by a

micro-syringe. The reaction time depends on the concentration of active substances, the minimum amount of which was judged by leaf-closing in one hour.

The Cassia Test : The young leaves detached from the stem of the plant Cassia mimosoides L. with a sharp razor blade were used for the bioassay. Two leaves were immersed in distilled water (ca. 0.9 ml) in a 20-ml glass tube in the greenhouse around 11 a.m. to allow them to recover from cutting injury (for around one hour). Each test solution (ca. 100 µl) was carefully poured into the test tubes by a micro-syringe around 11 a.m.. In the bioassay of leaf-closing factor, the reaction time depends on the concentration of active substances, the minimum amount of which was judged by leaf-closing in a few hours. In the bioassay of leaf-opening factor, most leaves immersed in the test solution containing active substances (at $<1x10^{-5}$ mol/l) closed toward evening and then opened again. Other leaves immersed in test solution containing active substances (at $>1x10^{-5}$ mol/l) remained open toward evening. The reaction time depends on the concentration of active substances, the minimum amount of which is judged by leaf-opening around 11 p.m..

Isolation of The Leaf-Closing Substances from The Plant Lespedeza Cuneata L. G. Don : The fresh whole herb (39 kg) of the plant Lespedeza cuneata L. G. Don, which was grown in crowds on the Tama River in September, was extracted with methanol for a week. The extract was evaporated under reduced pressure. The residue was partitioned between EtOAc (2 l) and H_2O (2 l), and then aqueous layer was washed with ^nBuOH (1 l). The aqueous layer was evaporated under reduced pressure. The residue (300 g) was repeatedly chromatographed on Sephadex G-10 (800 g) using H_2O as an eluant. The fraction (30 g) having leaf-closing activity by Mimosa test was chromatographed on TSK gel G3000SW (450 g) using H_2O as an eluant. The effective fraction (13.4 g) was successively submitted to three medium pressure columns on Fuji-Davison ODS-W [125-250 µ (400 cm^3)], Develosil ODS [30-60 µ (200 cm^3)], and MCI gel CHP-20P [75-150 µ (100 cm^3)] with H_2O-MeOH (9:1), H_2O-MeOH (99:1), and H_2O respectively. The effective fraction (88 mg) was separated by HPLC using a combination of three columns [FINEPACK SIL AF 102 + OH pak B-804 + OH pak B-2005] with H_2O-^{i}PrOH (19:1). The effective fraction (12 mg) was further separated by HPLC using a combination of four columns [Unisil pack-5C_{18}x4] with H_2O-MeOH (9:1). The active oil (3 mg) so far obtained was purified by HPLC using a combination of four columns [Unisil pack-5C_{18}x4] with H_2O-MeOH (199:1) to give white powder (<0.1 mg).

Isolation of The Leaf-Opening Substances from The Plant Lespedeza Cuneata L. G. Don : The MeOH extract from the plant (16 kg) described above, was partitioned between EtOAc (1 l) and H_2O(1 l), and then aqueous layer was washed with ^nBuOH (1 l). The aqueous solution was evaporated under reduced pressure. The residue (500 g) was repeatedly chromatographed on Sephadex G-10 (800 g) using H_2O as an eluant. Two leaf-opening bioactive fractions (933 and 130 mg) were obtained by chromatography on TSK gel G3000SW (450 g). Each fraction was submitted to a medium pressure column on Fuji-Davison ODS-W [125-250 µ (400 cm^3)] with H_2O-MeOH (9:1) to give the corresponding bioactive fractions (91 and 32 mg). These two effective fractions were independently separated by HPLC using a column [OH pak B-2005] with H_2O-MeOH (95:5) to give two active fractions (79 and 22 mg) respectively. These two residues were independently purified by HPLC using a combination of four columns [Develosil ODS-5x4] with H_2O - MeOH to give two bioactive compounds as colorless powder (27 and 6.4 mg : activity 10^{-6} - 10^{-7} mol/l). The former compound is named potassium lespedezate (**3**) and the latter compound is named potassium isolespedezate (**4**). The spectrum data of these compounds (**3** and **4**) were described in the text.

Conversion of Potassium Lespedezate (3) into 5 : **3** (3.0 mg) was acidified through a column of Amberlite IR-120B(H^+) with H_2O as an eluant, and the solvent was evaporated. A solution of the residue in MeOH (1 ml) was treated with CH_2N_2/Et_2O (Ca. 0.2 ml) at room temperature for 30 min. and the solvent was removed in vacuo. The residue was treated with Ac_2O (0.2 ml) - Pyr. (0.2 ml) at room temperature for 1 h and then evaporated under reduced pressure. The residue was purified by TLC (hexane-EtOAc=1:1) to afford **5** (3.4 mg; 80% in 3steps) as a colorless oil : $[\alpha]_D^{25}$ -35.1° (c 0.70, $CHCl_3$) ; HRMS (EI) : calcd for $C_{25}H_{30}O_{13}$ 538.1683, found 538.1666 ; IR (film) : 1755, 1720, 1640, 1605, 1570, 1515cm^{-1} ; ^{1}H NMR (C_6D_6) : 1.65(3H, s), 1.68(3H,s), 1.71(3H, s), 1.91(3H, s), 3.13(1H, ddd, J=2.2, 5.4, 9.3Hz), 3.23(3H, s), 3.28(3H, s), 4.01(1H, dd, J=2.2, 12.2Hz), 4.17(1H, dd, J=5.4, 12.2Hz), 4.80(1H, d, J=7.8Hz), 5.25(1H, dd, J=9.3, 9.8Hz), 5.45(1H, t, J=9.3Hz), 5.55(1H, dd, J=7.8, 9.8Hz), 6.69(2H, d, J=8.8Hz), 6.88(1H, s), 7.30(2H, d, J=8.8Hz).

Conversion of Potassium Isolespedezate (4) into 6 : According to essentially the same procedure as described above, **4** (1.6 mg) afforded **6** (1.9 mg, 82% in 3steps) as a colorless oil ; **6** : $[\alpha]_D^{26}$ -10.0° (c 1.0, $CHCl_3$) ; HRMS (EI) calcd for $C_{25}H_{30}O_{13}$ 538.1683, found 538.1673 ; IR (film) : 1760, 1720, 1640, 1605, 1575, 1515cm^{-1} ; ^{1}H NMR (C_6D_6) : δ 1.59(3H, S), 1.62(3H, s), 1.70(3H, s), 1.80(3H,s), 3.08(1H, ddd, J=2.5, 4.4, 9.8Hz), 3.22(3H, S), 3.45(3H, s), 3.82(1H, dd, J=2.5, 12.2Hz), 4.04(1H, dd, J=4.4, 12.2Hz), 5.29(1H, t, J=9.8Hz), 5.48(1H, t, J=9.8Hz), 5.59(1H, d, J=7.8Hz), 5.69(1H, dd, J=7.8, 9.8Hz), 6.75(2H, d, J=9.3Hz), 7.20(1H, S), 7.79(2H, d, J=9.3Hz) ; ^{13}C NMR ($CDCl_3$) : 20.5(q), 20.6(q), 20.6(q), 20.7(q), 52.2(q), 55.3(q), 61.6(t), 68.4(d), 71.6(d), 71.9(d), 72.8(d), 99.3(d), 113.8 (dx2), 125.3(s), 126.9(d), 132.6(dx2), 138.1(s), 160.6(s), 164.2(s), 169.5(s), 170.0(s), 170.2(s), 170.5(s).

Synthesis of 6 from 7 and 8 : To a suspension of **7** (86 mg, 2eq.), **8** (22.4 mg), and dry powdered Molecular Sieves 4A (200 mg) in dry CH_2Cl_2 (2 ml) was added silver triflate (55 mg, 2eq.) at 0 °C under Ar for 30 min. The reaction mixture was filtered through a celite and the insoluble solid was washed with EtOAc(50 ml). The filtrate was washed with aq. $NaHCO_3$ (40 ml) and saturated aq. NaCl (50 mlx2) successively. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane-EtOAc =1:1) to give colorless oil (26.4 mg 46%), a solution of which in dry toluene (4 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (34.8 mg, 5eq.) and refluxed for 3 days under Ar atomosphere. The reaction mixture was filtered through a celite and the insoluble solid was washed with EtOAc (20 ml). The filtrate was evaporated under reduced pressure and the residue was purified by preparative TLC (hexane-EtOAc=3:1) to afford **6** (21.6 mg, 82%).

Conversion of 3 into 9 and 10 : **3** (41.5 mg) was acidified through a column of Amberlite IR-120B(H^+) with H_2O as an eluant to afford **10** (32.5 mg, 87%). To a stirred solution of **10** (25.0 mg) in H_2O (2.0 ml) was added Na_2CO_3 (3.9 mg). After being stirred at room temperature for 0.5 h, the reaction solution was evaporated under reduced pressure to afford **9** (23.5 mg, 89%) as a colorless powder. The spectral data of **9** were identical with those of the natural potassium salt (**3**). **10** : $[\alpha]_D^{26}$ -48.1° (c 0.93, H_2O) ; ^{1}H NMR (D_2O) δ 3.44(1H, dd, J=7.8, 9.8Hz), 3.46(1H, dd, J=7.8, 9.3Hz), 3.50(1H, m), 3.54(1H, dd, J=9.3, 9.8Hz), 3.71(1H, dd, J=5.9, 12.7Hz), 3.88(1H, dd, J=2.0, 12.7Hz), 4.90(1H, d, J=7.8Hz), 6.68(1H, S), 6.82(2H, d, J=8.8Hz), 7.22(2H, d, J=8.8Hz).

Conversion of 4 into 11 and 12 : **4** (51.0 mg) was acidified through a column of Amberlite IR-120B(H^+) with H_2O as an eluant to afford **12** (39.0 mg, 85%). To a stirred solution of **12** (30.0 mg) was added Na_2CO_3 (4.6 mg). After being stirred at room temperature for 0.5 h, the reaction mixture was evaporated under reduced pressure to afford **11** (29.0 mg, 92%) as colorless crystals. The spectral data of **11** were identical with the natural potassium salt (**4**). **12** : m.p. 192-193 °C ; $[\alpha]_D^{25}$ +60.6° (c 1.0, H_2O) ; IR (KBr) : 3400, 1675, 1640, 1600, 1580, 1515cm^{-1} ; ^{1}H NMR (D_2O) δ 3.28(1H, ddd, J=2.2, 4.4, 9.3Hz), 3.45(1H, t, J=9.3Hz), 3.50(1H, t, J=9.3Hz), 3.59(1H, dd, J=7.8, 9.3Hz), 3.62(1H, dd, J=4.4, 12.7Hz), 3.72(1H, dd, J=2.2, 12.7Hz), 4.94(1H, d, J=7.8Hz), 6.82(2H, d, J=8.8Hz), 7.00(1H, s), 7.65(2H, d, J=8.8Hz) ; ^{13}C NMR (D_2O) δ 61.5(t), 70.3(d), 74.7(d), 76.8(d), 77.2(d), 102.5(d), 116.5(dx2), 125.7(s), 128.0(d), 133.8(dx2), 139.9(s), 158.1(s), 168.8 (s).

Conversion of 6 into 13 : To a stirred solution of **6** (37.5 mg) in MeOH-H_2O(4:1, 2.0 ml) was added 1 mol/l aq. KOH (0.7 ml). After being stirred at room temperature for 1 h, the reaction mixture was acidified through a column of Amberlite IR-120B(H^+) with H_2O as an eluant and then evaporated under reduced pressure. To a stirred solution of the residue in H_2O (2.0 ml) was added K_2CO_3 (4.8 mg). After being stirred at room temperature for 0.5 h, the reaction mixture was evaporated to afford **13** (23.3 mg 84%) as a colorless powder : $[\alpha]_D^{26}$ +37.4° (c 1.0, H_2O) ; ^{1}H NMR (D_2O) δ 3.33(1H, m), 3.41(1H, t, J=9.3Hz), 3.50(1H, t, J=9.3Hz), 3.55(1H, dd, J=7.3, 9.3Hz), 3.63(1H, dd, J=5.4, 12.7Hz), 3.75(1H, dd, J=2.0, 12.7Hz), 3.81(3H, s), 5.01(1H, d, J=7.3Hz), 6.72(1H, s), 6.96(2H, d, J=8.8Hz), 7.75(2H, d, J=8.8Hz).

Conversion of 12 into 14 : A solution of **12** (24.4 mg) in MeOH (2.0 ml) was treated with CH_2N_2/Et_2O (0.5 ml) at -78 °C for 0.5 h. The reaction mixture was evaporated to afford **14** (21.1 mg, 80%) as a colorless oil : $[\alpha]_D^{23}$ +72.1° (c 1.0, MeOH), ^{1}H NMR (CD_3OD) δ 3.22(1H, m), 3.36(1H, dd, J=8.8, 9.3Hz), 3.43(1H, t, J=8.8Hz), 3.50(1H, dd, J=7.3, 8.8Hz), 3.60(1H, dd, J=5.4, 12.2Hz), 3.75(1H, dd, J=2.4, 12.2Hz), 3.81(3H, s), 5.10(1H, d, J=7.3Hz), 6.77(2H, d, J=8.8Hz), 6.99(1H, s), 7.75(2H, d, J=8.8Hz).

Conversion of 4 into 15 and 16 : Potassium isolespedezate (**4**) (11.1 mg) was hydrogenated over palladium-carbon (ca. 1 mg) in H_2O (1.5 ml) at room temperature for 5 h. After a reaction mixture was filtered through a celite, the filtrate was evaporated under reduced pressure. The residue was separated by HPLC using a combination of four columns [Develosil ODS-5x4] with H_2O-MeOH (95:5) to give **15** (5.1 mg, 46%) and **16** (4.6 mg, 41%) as colorless powders : **15** : $[\alpha]_D^{24}$ -15.9° (c 1.0, H_2O) ; ^{1}H NMR (D_2O) δ 2.93(1H, dd, J=9.1, 15.0Hz), 2.98(1H, dd, J=9.1, 15.0Hz), 3.27(1H, dd, J=7.8, 9.3Hz), 3.33(2H, complex), 3.43(1H, t, J=9.3Hz), 3.63(1H, dd, J=5.1, 12.4Hz), 3.81(1H, dd, J=3.0, 12.4Hz), 4.38(1H, d, J=7.8Hz), 4.42(1H, t, J=9.1Hz), 6.79(2H,d, J=8.8Hz), 7.16(2H, d, J=8.8Hz) ; **16** : $[\alpha]_D^{25}$ -21.8° (c 1.0, H_2O) ; ^{1}H NMR (D_2O) δ 2.90(1H, dd, J=7.1, 15.0Hz), 2.97(1H, dd, J=5.6, 15.0Hz), 3.25(3H, complex), 3.36(2H, complex), 3.78(1H, dd, J=2.2, 12.5Hz), 4.17(1H, dd, J=5.6, 7.1Hz), 4.29(1H, d, J=7.8Hz), 6.80(2H, d, J=8.8Hz), 7.17(2H, d, J=8.8Hz).

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References

1) H. Schildknecht, Angew. Chem., Int. Ed. Engl., **22**, 695 (1983) and many references cited therein.

2) U. Ricca, Nuov G. Bot. Ital. (Nuova Seria), **23**, 51 (1916).

3) H. Schildknecht and K. Schumacher, Pure Appl. Chem., **54**, 2501 (1982) and many references cited therein.

4) E. Miyoshi, Y. Shizuri, and S. Yamamura, Chem. Lett., **1987**, 511.

5) unpublished results

6) H. Shigemori, N. Sakai, E. Miyoshi, Y. Shizuri, and S. Yamamura, Tetrahedron Lett., **30**, 3991 (1989).

7) R. C. McEvoy and W. L. Koukkari, Physiol. Plant. **26**, 143 (1972).; R. L. Satter, P. Marinoff, and A. W. Galston, Plant Physiol., **50**, 235 (1972).; R. L. Satter and A. W. Galston, BioScience, **23**, 407 (1973).; C. N. Williams and V. Raghavan, J. Ext. Bot., **17**, 742 (1966).; S. Watanabe and T. Shibaoka, Plant Cell Physiol., **24**, 641 (1983).; S. Tsurumi, Y. Asahi, and S. Suda, Bot. Mag. Tokyo, **98**, 89 (1985).