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THE CHEMICAL CONTROL OF LEAF-MOVEMENT IN A NYCTINASTIC PLANT, *LESPEDEZA CUNEATA* G. Don.

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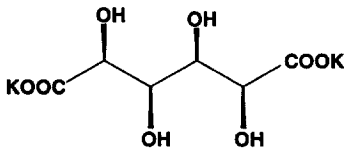
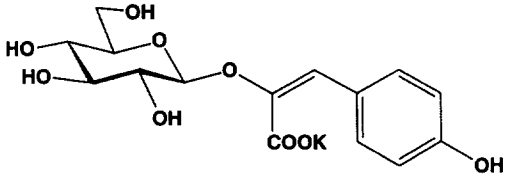
Abstract : Potassium D–idarate (**1**) was isolated from *Lespedeza cuneata* G. Don as a bioactive substance for nyctinasty. The compound was quite effective for leaf–closing of the plant at 1×10^{-6} M in the daytime and caused leaf–closing movement on the leaf of *Cassia mimosoides* L. Inversely effective two leaf-movement factors, **1** and potassium lespedezate (**2**), were isolated from the same plant. © 1997 Elsevier Science Ltd.

Nyctinastic plants, such as *Mimosa pudica* L. and *Cassia mimosoides* L., are well known for the movement of their leaves according to the circadian rhythm. Schildknecht *et al.* have isolated turgorins as bioactive substances for the leaf–closing movement from several nyctinastic plants, *e. g. Mimosa pudica* L., *Acacia karoo etc.*; [1,2] thus, they insisted that all nyctinastic movement is controlled by these compounds. Recently, however, we have isolated potassium chelidonate,[3] trigonelline,[4] and phyllanthurinolactone[5] as a leaf-closing factor, and the results strongly suggested that different leaf–closing substances exist in each nyctinastic plant.[4-7] Moreover, identification of potassium lespedezate (**2**) and potassium isolespedezate (geometrical isomer of **2**) from *Lespedeza cuneata* G. Don as leaf–opening substances by bioassay using the leaf of *Cassia mimosoides* L.[6] indicates that the nyctinastic movement is not controlled by the concentration of the leaf-closing factor, but by the balance of concentration between leaf–closing and leaf–opening substances. Nevertheless, no leaf-closing factor of the plant, *Lespedeza cuneata* G. Don, had hitherto been found. We report here the identification of potassium D–idarate (**1**) as a leaf-closing factor of *Lespedeza cuneata* G. Don by the bioassay using the leaf of *Cassia mimosoides* L. This is the first example of the identification of two leaf-movement factors of inverse bioactivity in the same nyctinastic plant. Thus, a new mechanism for the leaf-movement in a nyctinastic plant is proposed.



Potassium D–Idarate (1)



Potassium lespedezate (2)

The fresh whole plant of *Lespedeza cuneata* G. Don (10.1 kg) was immersed in methanol (60 L) for 3 weeks and concentrated *in vacuo*. Purification of the bioactive substance was carried out with monitoring the leaf–closing activity for the leaf of *Cassia mimosoides* L. Because of the stiffness of the stem of *Lespedeza cuneata* G. Don, this leaf pumped up the sample solution poorly, and it was insufficient to use for bioassay. For this experimental difficulty, we used the leaf of *Cassia mimosoides* L. for bioassay instead of the leaf of *Lespedeza cuneata* G. Don. The concentrated aqueous extract was partitioned with ethyl acetate, then with *n*–butanol. The bioactive aqueous layer was carefully separated by Amberlite XAD–7 column chromatography eluted with MeOH–H_2O (0 : 10, 1 : 9, 3 : 7, 5 : 5, and 10 :0). The bioactive H_2O eluate was further separated by using several gel filtration column chromatographies, such as Sephadex G-10 , G–25, Cellulofine GC–15m, and Toyopearl HW–40 Fine, and then divided into several fractions by HPLC using preparative Cosmosil 5C18AR column with H_2O. Purification by HPLC using a combination of two analytical Develosil ODS-HG5 columns is quite effective for the removal of most impurities. Final purification by HPLC using a combination of three analytical Develosil ODS-HG5 columns with H_2O gave **1** (1.8 mg) as a colorless powder.[8]

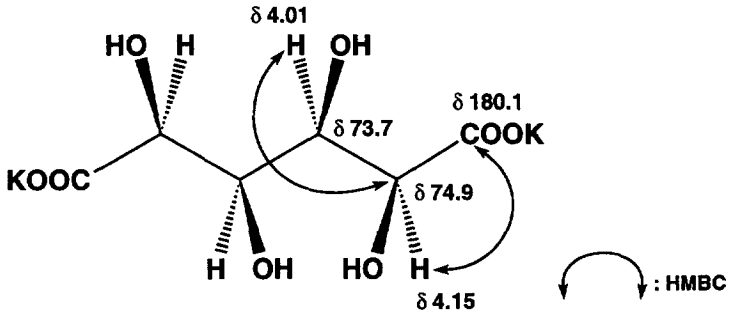


Figure 1. Structure determination of **1**.

Structural determination of **1** was carried out by means of 2D–NMR and negative ESI MS spectroscopy. HMQC and HMBC experiments gave the structure of **1** (Figure 1). ^{13}C NMR signal at δ 180.1 ppm suggests the presence of a carboxylate function. Negative mode ESI MS measurement gave the molecular ion $[M-H]^-$ at *m/z* 208.7, and the observation of $[M-2H]^{2-}$ at *m/z* 104.0 suggested that **1** was a dicarboxylic acid. **1** was quite effective for leaf–closing of *Cassia mimosoides* L. at 5×10^{-7} M in the daytime, but not effective on other nyctinastic plants, *Aeschynomene indica* and *Mimosa pudica* L. even at 1×10^{-4} M. Stereochemistry of **1** was determined by the comparison of spectroscopic data and bioactivity between **1** and various potassium tetrahydroxy dicarboxylates prepared from D–hexoses according to the known method.[9] Potassium D–idarate alone showed the same spectroscopic properties and bioactivity as the isolated natural **1**.

Table 1 shows the competitive interaction between **1** and **2**. When the concentration of **1** was higher than that of **2**, the leaves were closed in the daytime and vice versa.

Table 1. Interaction of **1** and **2** against the leaf of *Cassia mimosoides* L.

1 [M]	2 [M]	Daytime	Night
1×10^{-4}	1×10^{-4}	−−	+−
1×10^{-4}	0.5×10^{-4}	−−	−−
0.5×10^{-4}	1×10^{-4}	++	+−
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}	++	−−

Movement of the leaf was represented by following marks.: ++ completely open; +- at random; −− completely closed

As described above, two leaf–movement factors isolated from the same plant *Lespedeza cuneata* G. Don was inversely effective for the leaf of *Cassia mimosoides* L. This result indicates that nyctinastic leaf-movement is controlled by the competitive interaction of these compounds as shown in Table 1. For instance, the extracts of *Lespedeza cuneata* G. Don collected in the daytime (around AM 10:00) and at night (around PM 7:00) showed inverse bioactivity, in other words, the former showed leaf–opening activity but the latter showed leaf–closing activity. Thus, the balance of concentration between **1** and **2** is actually reversed in *Lespedeza cuneata* G. Don. Though it has been believed that nyctinastic movement is controlled only by the leaf-closing factor,[1, 2] our result introduces a new model of the mechanism for chemical control of leaf-movement (Fig. 2). Both **1** and **2** are able to control the leaf–movement of *Cassia mimosoides* L. Probably, these two compounds (**1** and **2**) must be the leaf–movement factor of the plant *Lespedeza cuneata* G. Don from which they have been isolated, because of the following experimental results.; 1) The leaf-movement factors, **1** and **2**, are effective at the concentration of $10^{-6} - 10^{-7}$ M,[10] almost the same level as other leaf-movement factors isolated by us.[3, 4, 5] 2) Both **1** and **2** are intrinsic in *Lespedeza cuneata* G. Don, and not contained in *Cassia mimosoides* L. 3) Inversion of concentration between **1** and **2** is really observed through a day.

We are further searching for sets of inversely effective leaf-movement factors of *Cassia mimosoides* L., *Phyllanthus urinaria* L., and *Albizzia julibrissin* Durazz through bioassay using the leaves of the same plants as extracted.

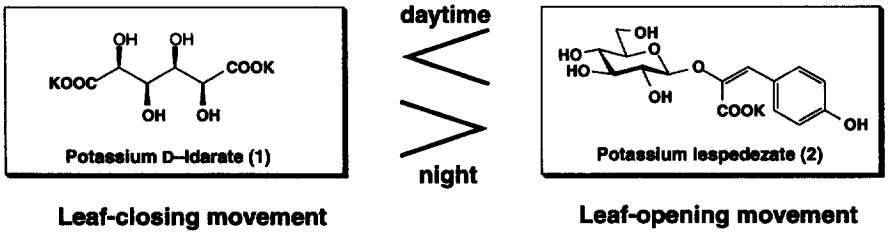


Figure 2. Model of leaf-movement in a nyctinastic plant, *Lespedeza cuneata* G. Don.

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8. Potassium Idarate (**1**): ^{1}H NMR (400 MHz, D_2O) δ 4.15 (2 H, br.s, H_1 & H_4), 4.01(2 H, br.s, H_2 & H_3) ppm.; ^{13}C NMR (100 MHz, D_2O, 35 °C) δ 180.1, 74.9, 73.7 ppm.; ESI MS (negative): $[M- H]^-$ *m/z* 208.7, $[M- 2H]^{2-}$ *m/z* 104.0. $[\alpha]^{D}_{22}$ + 5.3 ° (natural, c = 0.1, H_2O); $[\alpha]^{D}_{22}$ + 6.0° (synthetic, c = 0.1, H_2O).
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10. Fortunately, the leaf of the plant *Cassia mimosoides* L. exhibits a remarkable response for these two compounds in the same level of concenration as seen in potassium chelidonate. And purification of the leaf-closing factor from other plant extracts, such as *Albizzia julibrissin* Durazz, based on the bioassay using the leaf of *Cassia mimosoides* L. gave the bioactive substance effective only at 10^{-3} – 10^{-4} M.: Sata, N.; Miyoshi, E.; Shizuri, Y.; Yamamura, S. unpublished result.

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