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LEAF-OPENING SUBSTANCE OF A NYCTINASTIC PLANT, *CASSIA MIMOSOIDES*

MINORU UEDA, TAKASHI OHNUKI and SHOSUKE YAMAMURA*

Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku Yokohama 223, Japan

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Key Word Index—*Cassia mimosoides*; Leguminosae; nyctinasty; leaf-opening substance; calcium 4-*O*-β-D-glucopyranosyl-(Z)-*p*-coumarate; leaf-closing substance; potassium chelidonate.

Abstract—Calcium 4-*O*-β-D-glucopyranosyl-(Z)-*p*-coumarate, isolated from *Cassia mimosoides*, was effective for leaf-opening of the plant 4×10^{-6} M at night. This leaf opening substance and potassium chelidonate, which is a leaf-closing factor isolated previously, were inversely effective for the leaf of *Cassia mimosoides*.

INTRODUCTION

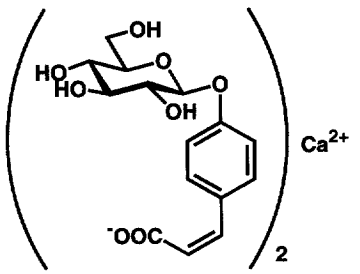
Some plants, such as *Mimosa pudica* L. and *Cassia mimosoides* L., "sleep" at night with their leaves closed and "wake" in the daytime with their leaves open. This is called nyctinastic movement and is known to be controlled by the biological clock. Schildknecht *et al.* have isolated turgorins as bioactive substances for the leaf-closing movement from several nyctinastic plants, e.g., *Mimosa pudica* L., *Acacia karoo*, etc [1, 2]. Thus, they assumed that this leaf-closing substance is common to all nyctinastic plants and the movement is controlled by change in the internal concentration of turgorin. However, these compounds were isolated by using an acidic solvent in the separation, and the sulfonyl group in the turgorin molecule turned out to be a free acid. The bioactivity of turgorin seems to be due to this free acid, because the potassium salt of turgorin isolated from plants had almost no bioactivity (unpublished result). Hitherto, we have identified several leaf-closing factors by using a neutral solvent in the separation to circumvent the effect of acid, and demonstrated that genuine leaf-closing and -opening substances differ in all nyctinastic plants [3–8]. Moveover, we have identified two leaf-movement factors of inverse bioactivity, leaf-closing and -opening substances, in the same nyctinastic plant, *Lespedeza cuneata* G. Don, and showed that inversion of the bioactivity is observed between plant extracts collected at night and in the daytime [6]. From this result, we concluded that the nyctinastic leaf-movement is controlled by the balance of concentration between the leaf-opening and leaf-closing substances. However, these two compounds were isolated with a bioassay using the leaf of *Cassia mimosoides* L., a plant different from that used for the extraction, *L. cuneata*. That is because the leaf of *L. cuneata* took up the sample solution poorly, and was insufficient for use in bioassay. Thus, to confirm our proposed mechanism of nyctinastic leaf-movement, we should identify several sets of inversely effective leaf-movement factors of a nyctinastic plant through bioassay using the leaves of the same plant from which they were extracted. Previously, we have isolated potassium chelidonate (**2**) as the leaf-closing substance of a nyctinastic plant, *C. mimosoides*, by bioassay using the leaves of the same plant [3]. In this paper, we report the identification of the leaf-opening substance (**1**) of *C. mimosoides*, isolated by bioassay using the leaves of the same plant, and this is the first case of the identification of two inversely effective leaf-movement factors in a nyctinastic plant through bioassay using the leaves of the same plant as plant material.

RESULTS AND DISCUSSION

The fresh whole plant of *Cassia mimosoides* L. was extracted with methanol. The extract was separated by monitoring the leaf-opening activity. The isolated bioactive substance was identified as **1** and ^{1}H and ^{13}C NMR. The *trans*-isomer of **1** is well known, but only the *cis*-isomer was obtained from this plant. Structural determination of **1** was by means of 2D-NMR experiments. FG-HMQC and HMBC experiments gave the structure of **1** (Fig. 1). ^{13}C NMR signal at δ 178.5 ppm

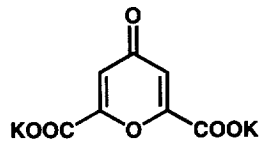
* Author to whom correspondence should be addressed.

Leaf-Opening Substance



Calcium 4-*O*-β-D-glucopyranosyl -*cis*-*p*-coumarate (1)

Leaf-Closing Substance



Potassium Chelidonate (2)

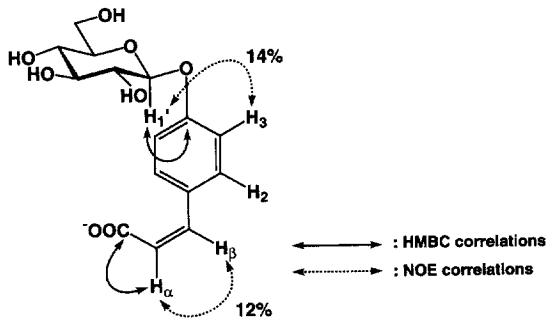


Fig. 1. Important correlations in the structure determination of **1**.

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

1 [M]	**2** [m]	Daytime	Night
1.0×10^{-5}	1.0×10^{-5}	++	+−
0.5×10^{-5}	2.5×10^{-5}	−−	−−
2.5×10^{-5}	0.5×10^{-5}	++	++
1.0×10^{-6}	1.0×10^{-6}	++	−−
0.5×10^{-6}	2.5×10^{-6}	−−	−−
2.5×10^{-6}	0.5×10^{-6}	++	++

Movement of the leaf was represented by following marks.: ++ completely open (degree of replication; 180°); +- at random; −− completely closed (degree of replication; 0°)

suggested the presence of a carboxylate function. *Cis*-configuration of the double bond was determined by the coupling constant (12 Hz) and large NOE (12%) between H_α and H_β.

1 was quite effective for leaf-opening of *C. mimosoides* at 4×10^{-6} M at night, but was not effective on other nyctinastic plants, *Aeschynomene indica* and *Mimosa pudica* L. even at 1×10^{-4} M. 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.

1 lost its bioactivity under isolation conditions with more polar solvent. Thus, it is supposed that the counter cation dissociated from the carboxylate of **1** was lost in the separation procedures using polar solvents. Analysis of the metal ion content in **1** by XPS showed the existence of a calcium ion as the counter ion of **1**. The potassium salt of **1**, which was isolated together with **1**, did not show any biological activity. Thus, a calcium ion was essential for the bioactivity of **1**. Calcium acetate was effective only at 1×10^{-2} M, thus, the organic counter anion of **1** is essential for the strong bioactivity of **1**.

As described above, two leaf-movement factors isolated from the same plant were inversely effective for the leaf of *Cassia mimosoides* L. indicating that the nyctinastic leaf-movement is controlled by the competitive interaction of these compounds as shown in Table 1. Now, we can identify a new set of inversely effective leaf-movement factors of a nyctinastic plant through bioassay using the leaves of the same plants. This result is complementary to our previous result on *Lespedeza cuniata* G. Don in which the plant used for bioassay was different from the source of the extracted plant material. Though it has been believed that the nyctinastic movement is controlled only by a leaf-closing factor [1, 2], results from these two plants, *L. cuneata* and *C. mimosoides*, strongly support our new model of the mechanism for chemical control of leaf-movement (Fig. 2): in nyctinastic plants, the balance of concentration between leaf-opening and leaf-closing substances is inverted through the day [6].

EXPERIMENTAL

General procedure

HPLC experiments were carried out on a JASCO 880PU and 875UV system equipped with preparative column and a Shimadzu LC-6A and LPD-6A system, respectively, monitoring *A* at 260 nm. Sizes of the columns used for HPLC were 20×250 mm, and 4.6×250 mm (Develosil ODS HG5, Nomura Chemicals Co. Ltd).

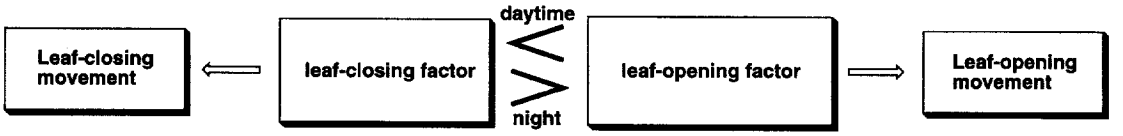


Fig. 2. New model for the chemical control of leaf-movement.

Plant material

The fresh whole plant of *Cassia mimosoides* L. (14.5 kg) collected on the shore of the Tama River (Tokyo) in August 1989 was immersed in MeOH (91:1) for 2 weeks and concd *in vacuo*.

Isolation

The concd aq. extract was partitioned with EtOAc, then with *n*–BuOH. The bioactive aq. 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 30% aq. MeOH eluate was further separated by stepwise elution using Toyopearl HW-40 Fine with 10% aq. MeOH and they 50% aq. MeOH. The bioactive substance was concd into 50% aq. MeOH fraction, and it was further divided into several fractions by HPLC using prep. Develosil ODS HG-5 column with 40% aq. MeOH. Final purification by HPLC using a combination of two analytical Develosil ODS-HG5 columns with 30% aq. CH_3CN gave **1** (3.2 mg) as a colorless syrup.

Calcium 4-O-*β*-D-*glucopyranosyl*-(*Z*)-p-*coumarate* (**1**). ^{1}H NMR (400 MHz, D_2O) δ 7.45 (2H, *d J* = 8 Hz, H_2), 7.11 (2H, *d J* = 8 Hz, H_3), 6.51 (1H, *d J* = 12 Hz, H_α), 6.03 (1H, *d J* = 12 Hz, H_β), 5.16 (1H, *d J* = 7 Hz, $H_{1'}$), 3.94 (1H, *dd J* = 3, 12 Hz, $H_{6'a}$), 3.77 (1H, *dd J* = 6, 12 Hz, $H_{6'b}$), 3.63 (1H, *ddd J* = 3, 6, 9 Hz, $H_{5'}$), 3.61 (1H, *t J* = 9 Hz, $H_{3'}$), 3.56 (1H, *dd J* = 7, 9 Hz, $H_{2'}$), 3.49 (1H, *d J* = 9 Hz, $H_{4'}$) ppm.; ^{13}C NMR (100 Mhz, D_2O, 35°) δ 178.5 (carbonyl), 157.8 (C_4), 132.6 (C_α), 132.4 (C_2), 131.4 (C_1), 126.8 (C_b), 117.8 (C_3), 101.6 ($C_{1'}$), 77.7 ($C_{5'}$), 77.1 ($C_{3'}$), 74.5 ($C_{2'}$), 71.0 ($C_{4'}$), 62.1 ($C_{6'}$) ppm.; IR 3400, 1700, 1560, 1400 cm^{-1}; UV-VIS l(e) 263 (8640) nm; [a]^{D}22 −40.2° (*c* = 0.25, H_2O).

Bioassay

The young leaves detached from the stem of the plant *Cassia mimosoides* L. with a sharp razor blade were used for the bioassay. One leaf was placed in H_2O (ca 1 ml) using a 20 ml glass tube in the greenhouse at 30°, and allowed to stand overnight. The leaves which opened again next morning (around 10:00 h) were used for the bioassay. Each test soln was carefully placed into the test tubes by a microsyringe around 10:00 h. The bioactive fraction was judged by the leaf-opening up to 21:00 h.

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