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cis–p–COUMAROYLAGMATINE, THE GENUINE LEAF–OPENING SUBSTANCE OF A NYCTINASTIC PLANT, *ALBIZZIA JULIBRISSIN* DURAZZ

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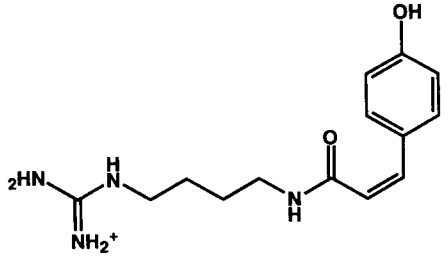
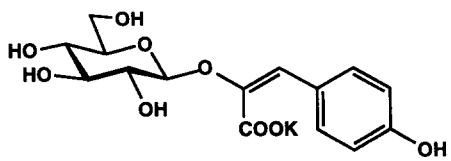
Abstract : *cis–p*–Coumaroylagmatine (**1**) was isolated from *Albizzia julibrissin* Durazz as a bioactive substance for nyctinasty. The compound was quite effective for leaf–opening of the plant at 1×10^{-5} M at night. Nyctinastic movement of *Albizzia julibrissin* Durazz is assumed to be controlled by the balance of concentration between **1** and an unknown leaf–closing substance. © 1997 Elsevier Science Ltd.

Nyctinastic movement of the silk tree, *Albizzia julibrissin* Durazz (nemu–no–ki in Japanese), is one of the most well–known plant movements, along with the rapid movement of *Mimosa pudica* L. Hitherto, we have isolated several chemical substances that control the leaf-movement of the nyctinastic plants, e.g., potassium chelidonate,[1] trigonelline,[2] and phyllanthurinolactone[3] as a leaf-closing factor, and the results strongly suggested that different leaf–closing substances exist in each nyctinastic plant.[4] Furthermore, 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.[5] indicates that the nyctinastic movement is not controlled by the concentration of leaf-closing factor, but the balance of concentration between leaf–closing and leaf–opening substances.[6] Nevertheless, attempts at isolation of the leaf–opening substance using bioassay with the leaf of the original plant from which the substance was extracted have been unsuccessful because of the difficulty of bioassay. We report here the identification of *cis–p*–coumaroylagmatine (**1**) as a leaf-opening factor of *Albizzia julibrissin* Durazz by bioassay using the leaf of the original plant. This is the first example of the identification of a genuine leaf–opening factor of a nyctinastic plant.



***cis–p*–coumaroylagmatine (1)**



potassium lespedezate (2)

The most important problem in the isolation of the leaf–movement factor from *Albizzia julibrissin* Durazz was the difficulty of bioassay using the original plant leaf. Because of the stiffness of the leaf stem of the grown plant (or small tree), it is difficult for the leaf to pump up the sample solution through the vessel. For this reason, previous studies of the leaf–movement factor of this plant were based on the bioassay using the leaf of mimosa.[7] As already mentioned in the previous papers, [2,3,4] however, most of the nyctinastic plants have their own different leaf–movement factors. Therefore, the previously isolated leaf-movement factors of this plant are not considered to be valid in respect of *Albizzia julibrissin* Durazz. Thus, it was required to develop a new bioassay using the leaf of the original plant for the identification of genuine leaf–movement factors. We used the young first or second leaf of the plant which is cultivated from the seed to circumvent this difficulty. Fortunately, the soft stem of the young leaf pumped up the sample very well, and was satisfactory for the bioassay.

The fresh leaf of *Albizzia julibrissin* Durazz (1.8 kg) was immersed in methanol (13.0 L) for two weeks and concentrated *in vacuo*. Purification of the bioactive substance was carried out with monitoring the leaf–opening activity of the leaf of *Albizzia julibrissin* Durazz. The bioactive fraction kept the leaf open even at 9:00 PM. The concentrated aqueous extract was partitioned with ethyl acetate, then with *n*–butanol. The strongly bioactive *n*–butanol layer was then partitioned with H_2O. This aqueous layer showed strong leaf–opening activity to the leaf of *Albizzia julibrissin* Durazz, and then back–partition of the BuOH layer with H_2O was very effective for the removal of most impurities. This 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). Bioactive 10% MeOH eluate was further chromatographed on a Sephadex G–25 column, and then divided into several fractions by using Lop-ODS glass column with 30% MeOH aq. The Bioactive fraction was further purified by HPLC using preparative Cosmosil 5C18AR column with 30% MeOH aq. and, finally, repeated purification by HPLC using the combination of three analytical Develosil ODS-HG5 columns with 40% acetonitrile aq. gave **1** (1.2 mg) as a colorless powder. Leaf-opening activity of the bioactive fractions was concentrared into **1**, and other fractions not containing **1** were biologically inactive on this bioassay.

Structural determination of **1** was carried out by means of 2D–NMR spectroscopy. FG–HMQC, FG–HMBC, COSY, and NOESY experiments gave the structure of **1**.[8] Figure **1** contains important correlations observed in the HMBC and NOE experiments. The signal at δ 157.5 ppm, observed only as a cross peak in the HMBC experiment, suggests the presence of a guanidino function.

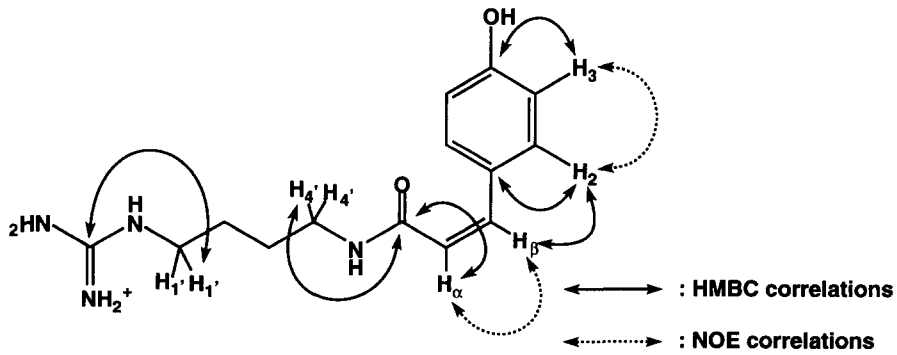


Figure 1. Structure determination of *cis–p*–coumaroylagmatine.

From the coupling constant and observed NOE correlation between olefinic protons, **1** was determined to have *Z*–configuration (J = 12 Hz). The *E*-isomer of **1** was reported to be isolated from nature,[9] but the *Z*–isomer has not been reported. It is interesting that *Albizzia julibrissin* Durazz contains the thermodynamically unstable *Z*–isomer solely, and no *E*–isomer was contained. *cis–p*–Coumaroylagmatine (**1**) was quite effective for leaf–opening of *Albizzia julibrissin* Durazz at 1×10^{-5} M at night, but not effective on other nyctinastic plants, *Aeschynomene indica*, *Phyllanthus urinaria* L. and *Mimosa pudica* L. even at 1×10^{-2} M. Interestingly, **1** was effective only for the leaf of*Cassia mimosoides* L. at 1×10^{-2} M.

cis–p–Coumaroylagmatine (**1**) was synthesized from agmatine and *cis–p*–coumaric acid.[10] *cis–p*–Coumaric acid was prepared by means of photo–isomerization of the commercially available *trans*-isomer. It was derivatized to the hydroxysuccineimide ester, and then coupled with agmatine using DCC to give **1**. The *trans*–isomer of **1** was also synthesized according to the same procedure. Synthetic **1** was as effective as the authentic sample of natural *cis–p*–coumaroylagmatine, and the synthetic *trans*-isomer of **1** was effective only at 1×10^{-3} M for the leaf of *Albizzia julibrissin* Durazz.

On the other hand, indole-3-acetic acid (IAA), which is known to be effective for the leaf–opening of *Mimosa pudica* L.,[11] was also effective for the leaf–opening of *Albizzia julibrissin* Durazz at 5×10^{-4} M. And *cis*–cinnamic acid, an analog of the partial structure of **1**, is known to have the same bioactivity as IAA. Accordingly, the *cis–p*–coumaroyl moiety is assumed to be an effective moiety, and the agmatine moiety to be a some carrier. The *cis–p*–coumaroyl–like moiety is also found in another leaf–opening substance **2**,[5] suggesting that it is an important structure for the induction of leaf–opening movement.

Similar to other nyctinastic plants, *Albizzia julibrissin* Durazz should have some leaf–closing factor, and the nyctinastic leaf-movement is assumed to be controlled by the competitive interaction between leaf–closing and opening factors. We are continuing our search for the leaf–closing factor of this plant.

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