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Leaf-closing Substance of *Mimosa pudica* L.; Chemical Studies on Another Leaf-movement of Mimosa II.

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Abstract : A plant, *Mimosa pudica* L. (Ojigi–so in Japanese), is known by the very rapid movement of the leaves when it is stimulated by touch, heating, etc. However, it is well known that there is another movement of the leaves in *M. pudica*, that is a very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock. We have isolated the chemical substance inducing the slow movement of the leaf of *M. pudica*. This chemical substance induced only the slow movement of the leaf and could not induce the rapid movement. The slow and rapid movements of mimosa would be regulated by different chemical substances.

Keywords: plants; natural products; biologically active compounds.

A plant, *Mimosa pudica* L. (Ojigi–so in Japanese) is known by the very rapid movement of the leaves when it is stimulated by touch, heating, etc. However, it is also well known that there is another movement of the mimosa leaf, that is a very slow, periodical movement of the leaves called nyctinastic movement: the leaves open in the daytime and close at night. Nyctinastic movement is known to be controlled by a biological clock, which was originally discovered from the careful studies on the leaf-movement of *M. pudica*. In the 18th century, a French scientist discovered that nyctinastic movement of *M. pudica* continued even in complete darkness in a cave.[1] This fascinating behavior of *M. pudica* prompted many scientists to study this plant, and now, it is generally recognized that all living organisms, from prokaryote to human being, have their own inherent rhythm.

The nyctinastic leaf-movement of *M. pudica* has been believed to be controlled by turgorins, which were isolated by Schildknecht in 1983 as the leaf-movement factors inducing the rapid movement of mimosa leaves.[2, 3] Schildknecht announced that turgolins are a new class of phytohormones that control both nyctinastic and thigmonastic movements. In 1984, however, Umrath *et al.* obtained two bioactive fractions (substances E and G) from *M. pudica*, and reported that they would be the genuine leaf-closing factor of this plant.[4] The percentage activities were 80% for E and 20% for G. We thought that Umrath's substance E induces the rapid movement; on the

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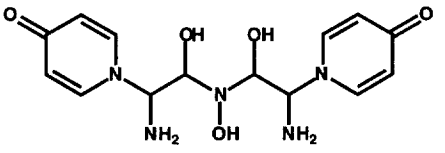
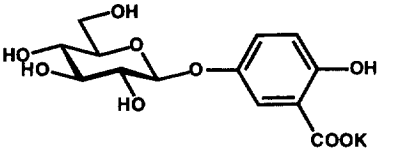
other hand, the substance G would induce the slow movement. The chemical substance that induces the slow and periodical leaf-movement of mimosa (nyctinastic movement), has been mostly neglected so far, because this movement is thought to be caused by the same chemical substance as that in the rapid movement. For this reason, the two leaf–movements of *M. pudica* have often been confused. However, we have reported the isolation of the leaf-opening substance of *M. pudica*, mimopudine (**1**), which kept the mimosa leaves open even at night.[5] Additionally, the leaves kept open by **1** responded to the stimulus by touch. This result showed that different chemical substances respectively regulate the rapid and slow movements of *M. pudica*. In this paper, we describe the isolation and structure determination of the leaf-closing substance (**2**) that induces nyctinastic leaf-closure of mimosa leaves.



Mimopudine (1)



Potassium 5-*O*-β-D-glucopyranosylgentisate (2)

The isolation of the leaf-closing substance was carried out based on the bioassay used in the isolation of **1**.[5] The leaf-closing activity was judged by the leaf-closure within two hours after the addition of the sample. The fresh leaves of *M. pudica* (11.2 kg) were immersed in methanol (60 L) for two weeks and concentrated *in vacuo*. After partition with organic solvents, the concentrated aqueous layer, which showed strong leaf-closing activity, was separated by using the Amberlite XAD-7 column chromatography. However, all the resulting fractions of leaf-closing activity were the ones inducing only rapid leaf-movement. The most important problem in the isolation of the leaf–closing substance which induces slow leaf-closure from *M. pudica* was the separation of the leaf-closing substance from the excitatory substance which induces the rapid movement of the mimosa leaves. We assumed that the nyctinastic activity of the leaf-closing substance could not be monitored because of the interference from the strong activity of the excitatory substance. Based on the report by Umrath,[4] we examined the application of the gel filtration chromatography for the separation of the excitatory and leaf-closing substances. After separation by Sephadex G-10, these bioactive substances were completely separated by Toyopearl HW-40S with H_2O.[6] At this stage, we have succeeded in the differentiation of the two leaf-closing movements of *M. pudica* at the molecular level. Further purification using HPLC with Cosmosil

5C18AR gave potassium 5-O-β-D-glucopyranosylgentisate (**2**, 1.1 mg) as a yellow syrup.

The structure of **2** was determined from the NMR experiments and negative mode ESI MS analysis.[7] The NOE spectrum gave a strong correlation between the anomeric proton ($H_{1'}$) and H_6, and the HMBC experiment gave a cross peak between $H_{1'}$ and C_5; thus, the glucose unit connected with the hydroxyl group of C_5 position (**Figure**). The chemical shifts in **2** corresponded with those previously reported.[8]

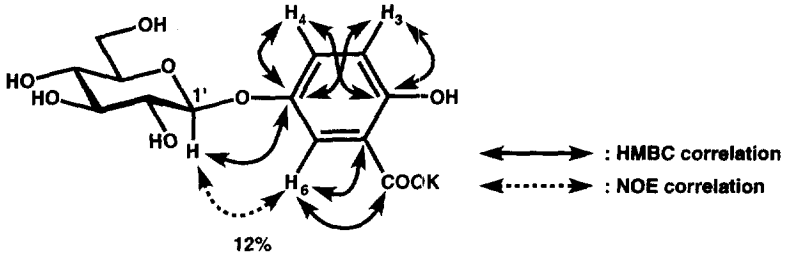


Figure. The NOE experiment in the 5-O-β-D-glucopyranosylgentisate (**2**)

Compound **2** was quite effective for leaf-closing of *M. pudica*. at 5×10^{-5} M at night, but not effective for other nyctinastic plants, *Aeschynomene indica*, *Phyllanthus urinaria* L., *Cassia mimosoides* L and *Albizzia julibrissin* Durazz. even at 1×10^{-4} M. Compound **2** induced only the slow leaf-closure of the mimosa leaf. Interestingly, all the leaf-closing and –opening substances of other plants were not effective for the leaves of *M. pudica* even at 1×10^{-4} M. And potassium gentisate (**3**), an aglycon of **2** was not effective even at 1×10^{-4} M against mimosa leaves.

In a previous paper,[9,10] we advanced a common mechanism for the regulation of nyctinastic leaf-movement: either the leaf-closing or -opening substance is a glycoside, and the glycoside is deactivated by a β-glucosidase whose activity is controlled by a biological clock. It is worthy of notice that all the nyctinastic plants, whose leaf-movement factors we have isolated so far, always have the glucoside-type leaf-closing or -opening substance. Our common mechanism is confirmed by the isolation of **2**. It is assumed that **2** would be converted into its biologically inactive aglycon, **4**, with β-glucosidase in the morning, similar to the case of *Phyllanthus urinaria* and *Lespedeza cuneata*.[11-13]

From these results, we have shown that the nyctinastic leaf-movement is induced by different chemical signals from the rapid movement of the plant. These two leaf-movements are regulated by different chemical substances and by different mechanisms. The nyctinastic leaf-movement is assumed to be controlled by the competitive interaction between leaf–closing and -opening substances.[9-13] Chemical studies on the mechanism of the nyctinastic leaf-movement of *M. pudica* is now in progress.

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6. The excitatory substance will be reported as a full paper.
7. Potassium 5-O-β-D-glucopyranosylgentisate (**2**): ^{1}H NMR (400 MHz, D_2O) δ 7.50 (1H, d, J = 3 Hz, H_6), 7.24 (1H, dd, J = 3 and 9 Hz, H_4), 6.86 (1H, d, J = 9 Hz, H_3), 4.95 (1H, d, J = 8 Hz, $H_{1'}$), 3.89 (1H, dd, J = 2.0, 12.5 Hz, $H_{6'a}$), 3.70 (1H, dd, J = 5.5, 12.5 Hz, $H_{6'b}$), 3.56 (1H, ddd, J = 2.2, 5.5, 9.0 Hz, $H_{5'}$), 3.53 (1H, t, J = 9 Hz, $H_{3'}$), 3.46 (1H, br.t, J = 9.0 Hz, $H_{2'}$), 3.44 (1H, d, J = 8.0, 9.0 Hz, $H_{4'}$). ^{13}C NMR (400 MHz, D_2O, 35 °C) δ 173.3 (carboxyl), 158.5 (C_2), 151.2 (C_5), 126.7 (C_4), 119.1 (C_3), 118.7 (C_6), 113.5 (C_1), 103.8 ($C_{1'}$), 78.2 ($C_{3'}$), 77.9 ($C_{5'}$), 74.8 ($C_{2'}$), 71.6 ($C_{4'}$), 62.6 ($C_{6'}$).; ESI MS (negative): $[M-K]^-$ *m/z* 315.
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