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LEAF–OPENING SUBSTANCE OF *MIMOSA PUDICA* L.; CHEMICAL STUDIES ON THE OTHER LEAF MOVEMENT OF MIMOSA

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Abstract : We have isolated mimopudine (**1**) as a leaf–opening substance of mimosa that keeps the leaves open even at night. **1** was quite effective for leaf–opening of *Mimosa pudica* L. at 2×10^{-5} M at night, but not effective for other nyctinastic plants even at 1×10^{-2} M. Interestingly, the leaves kept open at night with **1** were sensitive to stimulus by touch. This result suggests that **1** is effective only for the obstruction of slow nyctinastic leaf–closing movement, but not effective for the rapid thigmonastic movement. Therefore, the slow nyctinastic movement of mimosa is controlled by a different mechanism from that of the rapid thigmonastic movement.

The sensitive 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. It also shows very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock. : the leaves open in the daytime and close at night. Historically, the nyctinastic movement of mimosa was the beginning for the discovery of a biological clock. As early as the 18th century, a French scientist first discovered that nyctinastic movement continued even in complete darkness. [1] The former movement was proved to be controlled by some chemical substance early in the 20th century.[2] On the other hand, the latter has been almost neglected so far, because the slow nyctinastic movement is thought to be caused by the same chemical substance as that in the rapid movement.[3] For this reason, the two leaf–movements of mimosa have often been confused. However, we have isolated the leaf–opening substance of mimosa that keeps the leaves open even at night, but it did not disturb the rapid leaf-movement caused by physical stimulus. This paper discloses the first evidence to demonstrate that the rapid and slow movements of the leaves of mimosa are caused by different chemical substances, respectively.

The most important problem in the isolation of the leaf–openig substance from *Mimosa pudica* L. was the difficulty of bioassay. Because of the high sensitivity of the leaf, it closed easily in gentle wind through the long period of observation of leaf–opening. We used a mimosa leaf cut at the lamina and carried out the bioassay in a glass tube to overcome this difficulty. The bioactive fraction kept the leaf open even at 9:00 PM.

The fresh leaves of *Mimosa pudica* L. (12.2 kg) were immersed in methanol (ca. 58 L) for two weeks and concentrated *in vacuo*. Purification of the bioactive substance was carried out with monitoring the leaf–opening activity of the mimosa leaf. The concentrated aqueous extract was partitioned with *n*–

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hexane, ethyl acetate, then with *n*–butanol. The aqueous layer showed strong leaf-closing activity because of the leaf–closing substance that causes rapid leaf–movement of mimosa. It was supposed that this strong leaf-closing activity masked the bioactivity of the leaf-opening substance. Thus, the *n*–butanol layer was carefully separated by Amberlite XAD–4 column chromatography eluted with MeOH–H_2O(8 : 2, 9 : 1 and 10 :0), and the 80% MeOH eluate showed weak leaf–opening activity. Then, the 80% MeOH eluate was carefully separated by Amberlite XAD–7 column chromatography eluted with MeOH– H_2O (0 : 10, 1 : 9, 3 : 7, 4 : 6, 5 : 5, and 10 :0), and the 30% MeOH and 40% MeOH eluate showed weak leaf–opening activities. The strong leaf–closing activity,[4] which was observed in the H_2O eluate, was completely separated from the fraction of leaf–opening activity. The bioactive 30% MeOH aq. fraction was further purified by Toyopearl HW-40 Fine column chromatography with 35% MeOH aq., HPLC using preparative Develosil ODS HG–5 column with 40% MeOH aq. and purification by HPLC using analytical column (CAPCELL PAK UG80) with 40% MeOH aq. to give L-Tryptophane (6.1 mg) as a colorless powder. On the other hand, the other bioactive fraction, 40% MeOH eluate, was purified by Toyopearl HW-40 Fine column chromatography with 35% MeOH aq., HPLC using preparative Cosmosil 5C18AR column with 40% CH_3CN aq., and HPLC using a combination of two analytical columns (Develosil HG-5 and Develosil UG-5) with 20% CH_3CN aq. to give mimopudine (**1**, 0.5 mg) as a yellow powder.

Structural determination of **1** was carried out by means of NMR and ESI MS experiments. Positive ESI MS experiment gave the ions corresponding to $[M+Na]^+$, $[M+H]^+$, $[M+2Na]^{2+}$, $[M+2H]^{2+}$. HR ESI MS experiment was carried out against the peak of $[M+H]^+$ at *m/z* 338.1434 to give the formula of $C_{14}H_{20}O_5N_5$. Because the ^1H NMR spectrum of **1** was very simple, **1** was concluded to have a symmetric dimer structure. FG–HMQC, FG–HMBC experiments gave the structure of **1**.[5] **Figure 1** contains important correlations observed in the HMBC and HOHAHA experiments. Weak coupling between $H_{1'}$ and $H_{2'}$ was

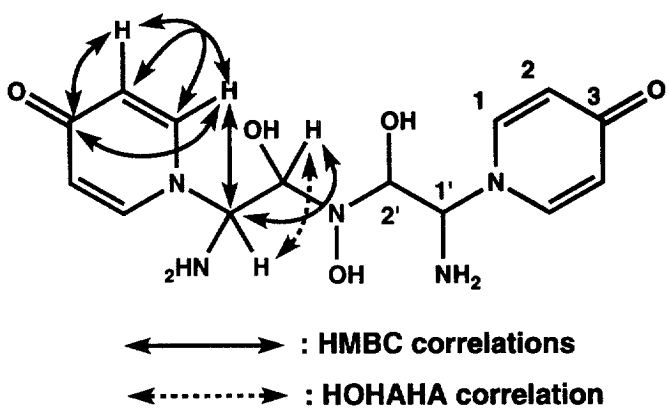

Fig.1. Important correlations in the HMBC and HOHAHA experiments of **1**.

observed only in the HOHAHA experiment. The chemical shifts of C_4 (90.5 ppm) and H_4 (5.30 ppm) position suggested that this carbon connects to both oxygen and nitrogen.[6] An ESI-linked scan experiment against the peak of $[M+Na]^+$ ion gave the fragment inos shown in **Fig. 2**. Compound **1** was unstable in aqueous solution, and was easily decomposed in D_2O during the over-weekend NMR experiment to give pyridone.

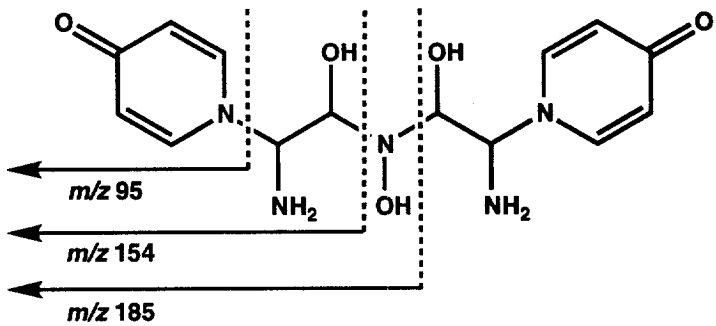

Fig. 2. Important fragmentations in the ESI-linked scan experiment of **1**.

Compound **1** was quite effective for leaf–opening of *Mimosa pudica* L. at 2×10^{-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^{-2} M. And the leaf–opening factors of other plants, potassium lespedezate from *Lespedeza cuniata* G. Don.[7] and *cis–p*–coumaroylagmatine from *Albizzia julibrissin* Durazz.[8] were not effective for the leaves of mimosa even at 1×10^{-2} M. On the other hand, L-Trp was also effective at as low as 5×10^{-4} M on mimosa leaves and the leaves of all nyctinastic plants as observed in indole-3-acetic acid (IAA).

It is proposed that the bioactivity of L-Trp is attributed to IAA, which is an important metabolite of L-Trp. IAA has been already reported to show weak leaf-opening activity to the leaves of all nyctinastic plants.[9] The long period needed for the bioassay of L-Trp to detect the leaf-opening activity is sufficient for the metabolism of L-Trp into IAA. All leaf-movement factors previously isolated by us showed specific bioactivity on each plant species;[10] thus, the genuine leaf-opening substance of mimosa should be **1**.

Interestingly, the leaves of *Mimosa pudica* L. kept open at night with **1** were sensitive to physical stimulus by touch, as observed in the daytime. This result suggests that **1** is effective only for the obstruction of the slow nyctinastic leaf–closing movement, but not effective for the rapid thigmonastic movement. We have now been able to separate these two leaf-movements at the molecular level, and demonstrate that the slow nyctinastic movement of mimosa has a different mechanism from that of the rapid thigmonastic movement.

Similar to other nyctinastic plants,[9] *Mimosa pudica* L. should have some leaf–closing factor that causes slow leaf–movement besides the excitatory substance that causes the rapid leaf–movement. In fact, Umrath *et al.*[11] reported the existence of two leaf–closing factors in the mimosa extract. The nyctinastic leaf-movement is assumed to be controlled by the competitive interaction between leaf–closing and opening factors.[12] We are continuing our search for the leaf–closing factor of this plant.

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4. Quite recently, a reliable excitatory substance has been successfully obtained from *Mimosa pudica* L. in our laboratory.; see Ueda, M and Yamamura, S. *Curr. Org. Chem.* **1998**, *2*, 437.
5. **1**: ^{1}H NMR (400 MHz, D_2O, rt) δ 7.90 (2 H, d, J = 7 Hz, H_1), 6.60 (2 H, d, J = 7 Hz, H_2), 5.30 (1 H, br.s, H_2'), 4.05 (1 H, d, br.s, H_1'); ^{13}C NMR (100 MHz, D_2O, 35 °C) δ 182.2 (C_5), 146.0 (C_1), 119.5 (C_2), 90.5 (C_2'), 65.0 (C_1') ppm.; ESI MS (positive): *m/z* 360 $[M+Na]^+$, 338 $[M+H]^+$, 191 $[M+2Na]^{2+}$, 169 $[M+2H]^{2+}$; HR ESI MS (positive): calcd. for $C_{14}H_{20}O_5N_5$ 338.1464, found *m/z* 338.1434; IR λ 1636, 1537, 1400 cm^{-1}.
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