Pergamon

Tetrahedron Letters 43 (2002) 7655–7658

TETRAHEDRON LETTERS

# A novel leaf-movement inhibitor of a nyctinastic weed, *Sesbania exaltata* Cory, designed on a naturally occurring leaf-opening substance and its application to a potential, highly selective herbicide

Noboru Takada,[a] Eisuke Kato,[a] Katsuhiro Ueda,[b] Shosuke Yamamura[a] and Minoru Ueda[a,*]

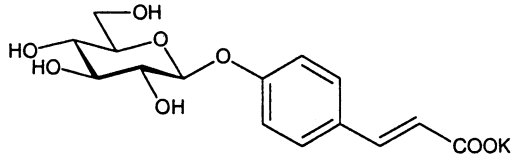
[a]*Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223-8522, Japan*
[b]*Department of Chemistry, Biology and Marine Science, University of The Ryukyus, Nishihara-cho, Okinawa 903-0213, Japan*

Received 7 August 2002; revised 29 August 2002; accepted 2 September 2002

**Abstract**—We isolated 4-*O*-β-D-glucopyranosyl-*trans*-*p*-coumarate (**1**), the leaf-opening substance of *Sesbania exaltata* Cory. The leaf-movement inhibitor, designed on the chemical mechanism of nyctinasty, could keep the leaves of *S. exaltata* open till the leaves withered and died. © 2002 Elsevier Science Ltd. All rights reserved.

We have isolated the biological substances controlling nyctinasty from several plants, and revealed the chemical mechanism of control of nyctinasty by a biological clock.[1] Nyctinasty is controlled by two endogenous leaf-movement factors of contrasting bioactivities: leaf-closing substance which makes the leaf close and leaf-opening substance which makes the leaf open. The bioactivity of these leaf-movement factors is extremely specific to the original plant from which they were isolated. In the plant body, the rhythm of nyctinasty is generated by change in balance of concentrations between the two leaf-movement factors according to a circadian rhythm.[2] This change in the balance can be attributed to hydrolysis of the glucoside-type leaf-movement factor into the corresponding aglycon by the action of β-glucosidase whose activity is controlled by a biological clock.[3] According to this mechanism, we have succeeded in the inhibition of leaf-movement by using a synthetic leaf-movement inhibitor designed on the structure of a naturally occurring leaf-opening substance.[4] And it was revealed that the nyctinastic plant withers and dies without nyctinastic leaf-closure. This is an important discovery to answer the historic question, 'Why does the leguminous plant sleep?'

Also, this result showed that we can inhibit the leaf-closure of a voluntary leguminous plant using a synthetic leaf-movement inhibitor designed on its leaf-opening substance. As an application, the leaf-movement inhibitor can be used as a potential environment-friendly herbicide of extremely high specificity to the target weed. The leaf-movement inhibitor would have complete selectivity to the target weed because of the extremely high specificity of the leaf-opening substance to the plant containing it. When we use the leaf-movement inhibitor as a herbicide, it would be effective only for the target leguminous weed and have no effect on vicinal plants, insects, birds, animals, and human beings. For example, genetically engineered soybean (Monsanto's Roundup Ready Soybeans) is widely cultivated in USA as the most important leguminous crop.




potassium 4-*O*- -D-glucopyranosyl-*trans*-*p*-coumarate (**1**)

Unfortunately, some leguminous weeds, such as *Sesbania exaltata* Cory and *Senna obtsusifolia*, are resistant to Roundup and can grow in the soybean fields after the treatment of it. No existing herbicide can remove these leguminous weeds without any damage to the soybean, which belongs to the same genus. However, leaf-movement inhibitor **5** for these weeds will be able to remove them without any damage to the soybean

* Corresponding author. Tel.: +81-45-566-1713; fax: +81-45-566-1697; e-mail: ueda@chem.keio.ac.jp

0040-4039/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(02)01848-8

and other vicinal organisms. In this paper, we describe the isolation of a naturally occurring leaf-opening substance from *Sesbania* plant, and development of the leaf-movement inhibitor of *S. exaltata* which is a potential ecological herbicide.

Based on the previous study that the plants belonging to the same genus have the same leaf-movement factors,[5] we carried out the isolation of the leaf-opening substance of *Sesbania speciosa* Taub belonging to the same genus as one of the target weeds, *S. exaltata*.

The fresh whole plant of *S. speciosa* (14.9 kg), which was collected in Okinawa, Japan, was extracted with MeOH (50 L) for a week and concentrated in vacuo. The concentrated extract was partitioned with *n*-hexane, ethyl acetate, and then *n*-BuOH. Isolation of the leaf-opening substances was carried out based on a bioassay using a leaf of *S. speciosa*. The bioactive fraction kept the leaf open till 8:00 pm. The aqueous layer was separated by Amberlite XAD-7 column chromatography eluted with MeOH–$H_2O$ (0:100, 10:90, 30:70, 50:50 and 100:0). The leaf-opening activity was observed in the 10% aqueous MeOH fraction. Thus, this fraction was separated by MPLC using TSK-G3000S gel with 30% aqueous EtOH. The bioactive fraction was further purified by HPLC using preparative Develosil ODS-SR-5 column with 30% aqueous MeOH, and then preparative Develosil ODS-HG-5 with 10% aqueous MeOH to give 4-*O*-β-D-glucopyranosyl-*trans*-*p*-coumarate (**1**, 21 mg) as a leaf-opening substance, whose structure was determined by NMR and HRFABMS.[6] Compound **1** showed leaf-opening activity at $1.4\times10^{-3}$ M against the leaves of *Sesbania* plants, such as *S. speciosa* and *S. exaltata*. On the other hand, **1** did not show any leaf-opening activity against the leaves of nyctinastic plants belonging to other genuses, such as *Albizzia julibrissn* Durazz., *Leucaena leucocephala* L., *Phyllantus urinaria* L. and *Desmanthus virgatus* (Fig. 1). Therefore, it was clarified that **1** was a genus-specific leaf-opening substance for *Sesbania* plants.

In the bioassay, natural leaf-opening substance **1** could not make the leaves of *S. exaltata* wither and die, because it may be easily metabolized by β-glucosidase in the plant body before the leaves withered to die. The artificial leaf-movement inhibitor, which is resistant to the hydrolysis by β-glucosidase, was necessary for the development of the herbicide. In the previous study, it was shown that a galactose derivative of glucoside-type leaf-opening substance kept the leaves open until the leaves withered and died because of its resistance to the hydrolysis by β-glucosidase.[7]

Therefore, we prepared several derivatives of **1** for the structure–activity relationship study (Scheme 1).

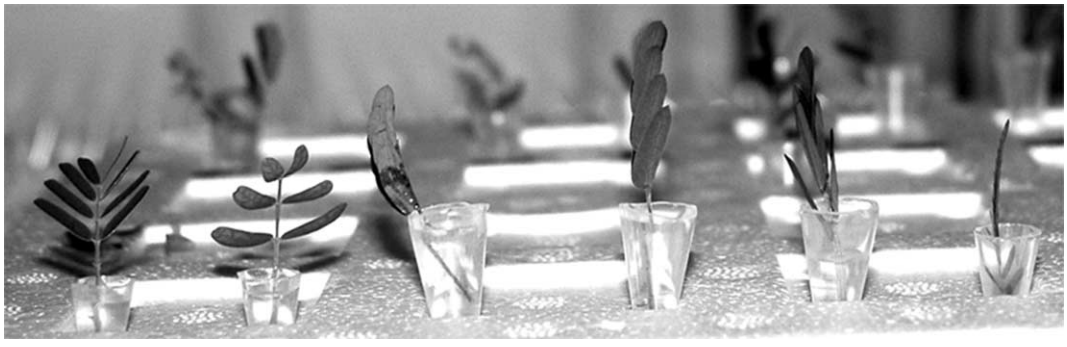
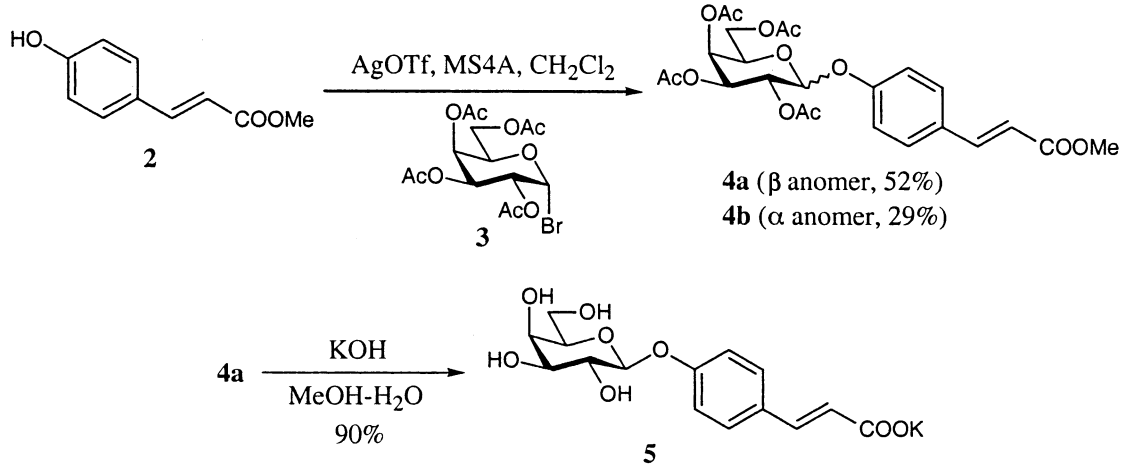


**Figure 1.** Genus-specific leaf-opening activity of **1**. (a) *S. speciosa* Taub., (b) *S. exaltata* Cory, (c) *A. julibrissn* Durazz., (d) *L. leucocephala* L.,(e) *P. urinaria* L., (f) *D. virgatus*. Every leaf was treated with $3\times10^{-3}$ M of **1**. This photo was taken at 8:00 pm.




**Scheme 1.** Synthesis of 4-*O*-D-galactopyranosyl-*trans*-*p*-coumarate (**5**).

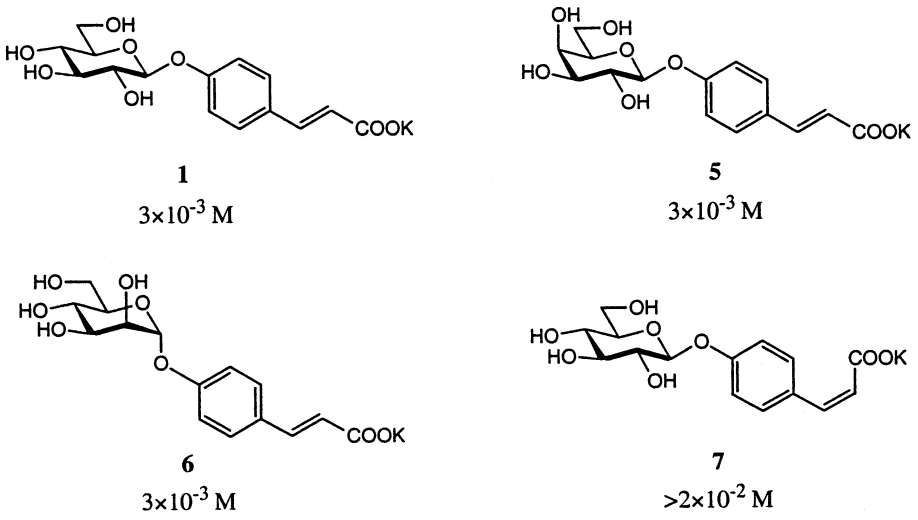



**Figure 2.** The structure–activity relationship study on the leaf-opening substance of *S. exaltata*.

Namely, a coupling reaction of methyl *trans-p*-coumarate (**2**) and D-acetobromogalactose **3** was carried out with AgOTf–MS 4A to give the desired β-galactoside **4a** (52%) and undesired α-galactoside **4b** (29%). Desired **4a** was deprotected using aqueous KOH to give **5** (90%). Other derivatives **6** and **7** were also prepared by similar procedure.[8] The sugar derivatives **5** and **6** were as effective as **1** against the leaves of *S. exaltata*, while **7**, which was the *cis* isomer of **1**, was not effective even at $1.4\times10^{-2}$ M in the bioassay. Based on the structure–activity relationship study using some derivatives of **1**, it was suggested that the structure of aglycon was essential for leaf-opening activity, while the structure of the sugar moiety was not important (Fig. 2). However, it was suggested that the presence of the sugar part was necessary for leaf-opening activity, since *trans-p*-coumarate did not show any leaf-opening activity. These results suggested that **5** and **6**, which would not be hydrolyzed by β-glucosidase, can be used as a potential herbicide for *S. exaltata*.

Then, we carried out the bioassay using **5** against the leaves of *S. exaltata*. Fortunately, **5** kept the leaves open till the leaves withered and died on the bioassay (Fig. 3). This result suggested that the leaf-movement inhibitor **5** would be a potential, highly selective herbicide of *S. exaltata*.

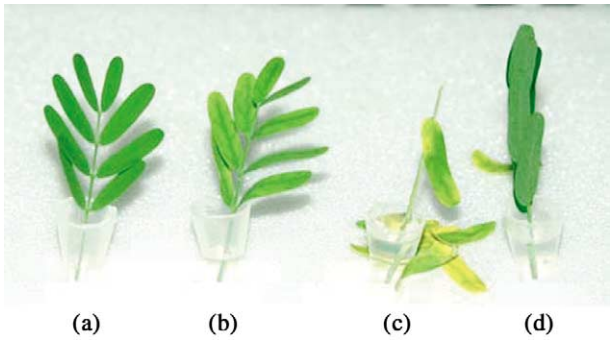



**Figure 3.** The status of leaves of *S. exaltata* at 8:00 pm by treatment with $4\times10^{-3}$ M of leaf-movement inhibitor **5**. (a) 1st day; (b) 2nd day; (c) 3rd day; (d) control.

In conclusion, we have succeeded in the isolation and structural determination of the leaf-opening substance, 4-*O*-β-D-glucopyranosyl-*trans-p*-coumarate (**1**), which is effective against *S. exaltata* Cory, a leguminous weed growing in a field of genetically engineered soybean after the treatment of Roundup. Based on our proposed mechanism of the leaf-movement in leguminous plants, we designed and prepared the leaf-movement inhibitor **5**. This synthetic inhibitor showed genus-specific activity against genus *Sesbania*, and was not metabolized by β-glucosidase to make the *Sesbania* leaves wither and die. This result shows that the leaf-movement inhibition designed on the leaf-opening substance can be used as a potential, highly selective herbicide without damage to the surrounding organisms.

## Acknowledgements

This work was supported by the Ministry of Education, Science, Sports and Culture (Japan) for Grant-in-Aid for Scientific Research (No. 1302467 and 12680598), Pioneering Research Project in Biotechnology given by the Ministry of Agriculture, Forestry and Fisheries, Kato Memorial Bioscience Foundation, Naito Foundation, Kurata Foundation and Moritani Foundation.

## References

1. Ueda, M.; Yamamura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1400–1414 and references cited therein.
2. Ueda, M.; Asano, M.; Sawai, Y.; Yamamura, S. *Tetrahedron* **1999**, *55*, 5781–5792.
3. Ohnuki, T.; Ueda, M.; Yamamura, S. *Tetrahedron* **1998**, *54*, 12173–12184.
4. Ueda, M.; Sugimoto, T.; Sawai, Y.; Yamamura, S. *Tetrahedron Lett.*, in press.

5. Ueda, M.; Okazaki, M.; Ueda, K.; Yamamura, S. *Tetrahedron* **2000**, *56*, 8101–8105.
6. 4-*O*-β-D-Glucopyranosyl-*trans*-*p*-coumarate (**1**): $^1$H NMR (400 MHz, $D_2O$): 7.41 (2H, d, $J=8.8$ Hz), 7.17 (1H, d, $J=15.6$ Hz), 6.95 (2H, d, $J=8.8$ Hz), 6.25 (1H, d, $J=15.6$ Hz), 4.98 (1H d, $J=7.6$ Hz), 3.75 (1H, dd, $J=2.0$, 12.4 Hz), 3.57 (1H, dd, $J=5.6$, 12.4 Hz), 3.47 (1H, ddd, $J=2.0$, 5.6, 9.0 Hz), 3.43 (1H, dd, $J=9.0$, 9.2 Hz), 3.39 (1H dd, $J=7.6$, 9.0 Hz), 3.31 (1H, dd, $J=9.0$, 9.2 Hz) ppm; $^{13}$C NMR (100 MHz, $D_2O$): 176.6, 158.3, 141.1, 131.0 130.2 (2C), 123.7, 117.5 (2C), 100.7, 77.0, 76.4, 73.7, 70.3, 61.3 ppm; IR (film) $\nu$ 3359, 1640, 1604, 1509 cm$^{-1}$; $[\alpha]_D^{21}$ −59.2° (*c* 1.00, $H_2O$); HR FAB MS (negative): $[M-H]^-$ found $m/z$ 325.0938, $C_{15}H_{17}O_8$ requires $m/z$ 325.0923.
7. Ueda, M.; Sawai, Y.; Yamamura, S. *Tetrahedron* **1999**, *55*, 10925–10936.
8. 4-*O*-β-D-Galactopyranoyl-*trans*-*p*-coumarate (**5**): $^1$H NMR (270 MHz, $D_2O$): 7.40 (2H, d, $J=8.5$ Hz), 7.17 (1H, d, $J=16.1$ Hz), 6.95 (2H, d, $J=8.5$ Hz), 6.23 (1H, d, $J=16.1$ Hz), 4.89 (1H d, $J=7.0$ Hz), 3.81 (1H, d, $J=2.8$ Hz), 3.72–2.55 (5H, m) ppm; $^{13}$C NMR (100 MHz, $D_2O$): 177.9, 158.5, 141.1, 130.9, 130.2 (2C), 123.6, 117.5 (2C), 101.3, 76.3, 73.4, 71.3, 69.3, 61.6 ppm; IR (film) $\nu$ 3316, 1637, 1604, 1508 cm$^{-1}$; $[\alpha]_D^{20}$ −46.7° (*c* 1.00, $H_2O$); HR FAB MS (negative): $[M-H]^-$ found $m/z$ 325.0938, $C_{15}H_{17}O_8$ requires $m/z$ 325.0923.

4-*O*-α-D-Mannnopyranoyl-*trans*-*p*-coumarate (**6**): $^1$H NMR (400 MHz, $D_2O$): 7.34 (2H, d, $J=8.6$ Hz), 7.13 (1H, d, $J=16.0$ Hz), 6.93 (2H, d, $J=8.6$ Hz), 6.20 (1H, d, $J=16.0$ Hz), 4.93 (1H, d, $J=2.0$ Hz), 3.96 (1H, dd, $J=2.0$, 3.4 Hz), 3.85 (1H, dd, $J=3.4$, 9.4 Hz), 3.56 (1H, dd, $J=3.7$, 6.5 Hz), 3.54 (1H, dd, $J=2.0$, 6.5 Hz), 3.49 (1H, dd, $J=3.7$, 5.4 Hz), 3.47 (1H, ddd, $J=2.0$, 5.4, 9.4 Hz) ppm; $^{13}$C NMR (100 MHz, $D_2O$): 176.7, 157.2, 141.1, 130.6, 130.1 (2C), 123.6, 117.9 (2C), 98.8, 74.3, 71.3, 70.8, 67.5, 61.6 ppm; IR (film) $\nu$ 3267, 1638, 1604, 1551, 1509 cm$^{-1}$; $[\alpha]_D^{20}$ +98.6° (*c* 1.00, $H_2O$); HR FAB MS (negative): $[M-H]^-$ found $m/z$ 325.0929, $C_{15}H_{17}O_8$ requires $m/z$ 325.0923.

4-*O*-β-D-Glucopyranoyl-*cis*-*p*-coumarate (**7**): $^1$H NMR (400 MHz, $D_2O$): 7.23 (2H, d, $J=9.0$ Hz), 6.89 (2H, d, $J=9.0$ Hz), 6.29 (1H, d, $J=12.8$ Hz), 5.81 (d, $J=12.8$ Hz), 4.96 (1H, d, $J=7.4$ Hz), 3.73 (1H, dd, $J=2.4$, 12.6 Hz), 3.56 (1H, dd, $J=5.5$, 12.8 Hz), 3.45 (1H, ddd, $J=2.4$, 5.5, 9.4 Hz), 3.42 (1H, dd, $J=9.1$, 9.3 Hz), 3.37 (1H, dd, $J=7.4$, 9.3 Hz), 3.30 (1H, dd, $J=9.1$, 9.4 Hz) ppm; $^{13}$C NMR (100 MHz, $D_2O$): 178.5, 157.8, 132.6, 132.4 (2C), 131.4, 126.8, 117.8 (2C), 101.6, 77.7, 77.1, 74.5, 71.0, 62.1 ppm; IR (film) $\nu$ 3400, 1700, 1560, 1400 cm$^{-1}$; $[\alpha]_D^{22}$ −40.2° (*c* 0.25, $H_2O$); HR FAB MS (negative): $[M-H]^-$ found $m/z$ 325.0936, $C_{15}H_{17}O_8$ requires $m/z$ 325.0923.