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SUGAR-DERIVATIVES OF POTASSIUM LESPEDEZATE, ARTIFICIAL LEAF-OPENING SUBSTANCES OF *LESPEDEZA CUNEATA* G. DON, DESIGNED FOR THE BIOORGANIC STUDIES OF NYCTINASTY

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Abstract : Potassium lespedezate (**1**) is the leaf-opening substance of a nyctinastic plant, *Lespedeza cuneata* G. Don. We synthesized two sugar-derivatives of **1**, potassium galactolespedezate (**2**) and mannoisolespedezate (**3**). Both **2** and **3** were quite effective for the leaf–opening of *L. cuneata.* at 8×10^{-7} M, as strong as **1**. However, **1** kept the leaf open only for a few days; on the other hand, **2** and **3** kept the leaf open after a week at that concentration. Thus, it is assumed that **2** and **3** could not be hydrolyzed by β-glucosidase in the plant body, and are therefore potentially useful molecular probes for the studies of the control of the nyctinastic leaf-movement by a biological clock.

Keywords: plants; natural products; biologically active compounds; glycoside; glycosidation

Most leguminosae plants close their leaves in the evening, as if to sleep, and open them in the morning.[1] This is called nyctinasty, and such a circadian rhythmic movement has been known to be controlled by their biological clocks.[2] Recently, we have identified several bioactive substances that regulate this leaf-movement,[3-15] and revealed that nyctinastic movement of the plants is controlled by the interaction between leaf-closing and -opening substances.[7, 11-15]

For a nyctinastic plant, *Lespedeza cuneata* G. Don,[8, 13, 14] we have shown that a change in the balance between leaf-opening and -closing substances controls the nyctinasty, and the balance is controlled by a biological clock through the regulation of the activity of β-glucosidase which hydrolyzes the glycosidic bond of potassium lespedezate (**1**), the leaf-opening substance of this plant. It is obvious that bioorganic studies to understand the molecular mechanism of the action and the activation of this β-glucosidase are very important. For this reason, **1**, the substrate of this enzyme, could become a useful molecular probe for these studies. However, a crucial point is that **1** is converted into its aglcon, potassium 4-hydroxyphenylpyruvate (**4**),[13, 14] which shows only weak bioactivity, by the action of β-glucosidase, and sufficient availability of **1** into the plant tissue could not be expected. Therefore, we tried to prepare the leaf-opening substance which could not be hydrolyzed by this enzyme.

It has already been reported that the most important part of **1** for the leaf-opening activity is the *p*-hydroxyphenylpyruvate unit.[5] Even the α-anomer of **1** was as effectively strong as **1** in the bioassay. Thus,

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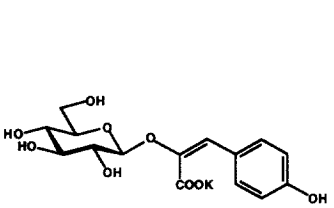
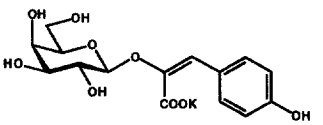
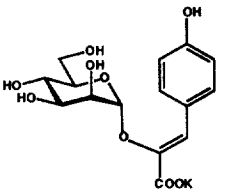
the glucose unit would serve for fixation of the enol double bond and the increase of solubility. From these results, the probe compounds were designed based on the idea that the derivatization of the sugar moiety of **1** would have little effect on the leaf-opening activity. We have, accordingly, synthesized two sugar-derivatives of **1**, that is, potassium galactolespedezate (**2**) and mannoisolepedezate (**3**).



Potassium lespedezate (1)

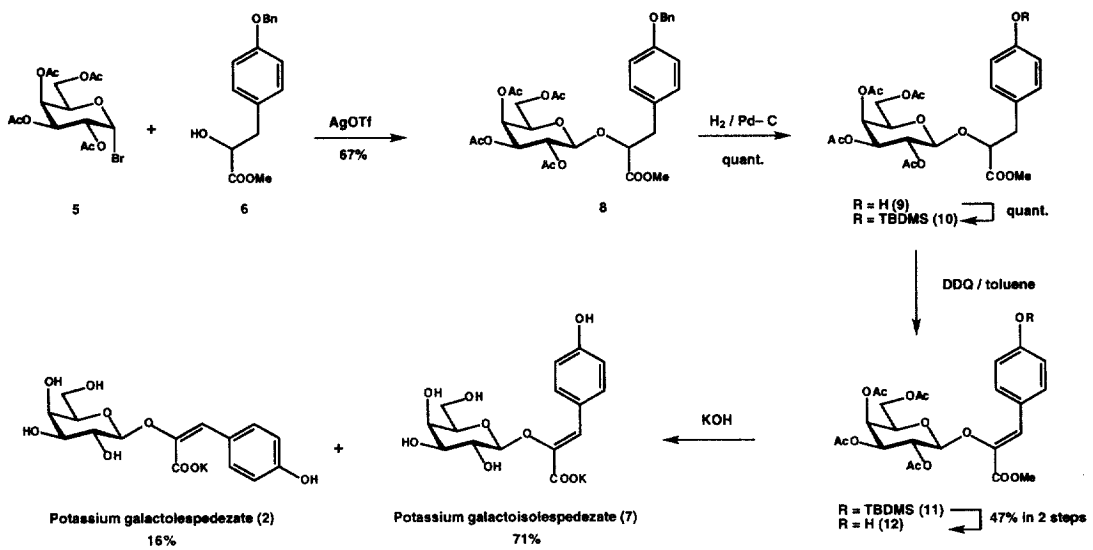


Potassium galactolespedezate (2)



Potassium mannoisolespedezate (3)

The syntheses of **2** and **3** essentially followed the synthetic route of **1** reported by Shigemori *et al.*[16] A coupling of D-acetobromogalactose (**5**) and methyl 3-*p*-hydroxyphenyl-2-hydroxypropionate (**6**) with AgOTf-Molecular Sieves 4A, and the following deprotection, DDQ oxidation, and hydrolysis with KOH gave potassium galactolespezedate (**2**) and potassium isogalactolespezedate (**7**) as shown in **Scheme 1**.[17]



Scheme 1. Synthesis of potassium galactolespedezate (**2**) and potassium galactoisolespedezate (**7**).

A racemic mixture of compound **6** was used for the glycosidation reaction, and the following reactions were carried out using both diastereomers without separation. DDQ oxidation using 6 eq. of DDQ gave mainly one isomer, **11**, which was deprotected with nBu_4NF in THF at 0°C to give phenol (**12**). Finally, potassium galactolespedezate (**2**) and galactoisolespedezate (**7**) were obtained from **12** on treatment with KOH in MeOH at 0°C in 16 and 71% yields, respectively. Compounds **2** and **7** were separated by HPLC using a Cosmosil 5C18AR column with 35% MeOHaq. Potassium α-mannoisolespezedate (**3**) was

prepared according to the same synthetic route using D-acetobromomannose.[18] The glycosidation reaction gave mainly the α-anomer, which was confirmed by the coupling constant between $H_{1'}$ and $C_{1'}$ ($^1J_{C\text{-}H}$ = 178.8 Hz).[19]

Both **2** and **3** were effective at 8×10^{-7} M, as strong as **1**, in the bioassay.[5] The leaf-opening activity of **1** lasted for only two days; after that, the leaf closed at night again. On the other hand, the bioactivity of **2** and **3** lasted even after a week. The leaves treated with 3×10^{-6} M of **2** or **3** kept opening until the leaves were withered and died after a week (**Table 1**). This result suggested that **1** would be completely hydrolyzed into **4** by β-glucosidase; however, the enzyme could not hydrolyze **2** and **3**. These results also suggested the importance of β-glucosidase in the regulation of nyctinasty. Thus, we have succeeded in the preparation of the probe compounds for the bioorganic study of β-glucosidase which plays the central role in the regulation of the nyctinastic leaf-movement by a biological clock.

Table 1. Time-course change of the status of the leaves treated with 3×10^{-6} M of **1**, **2**, and **3**

	Status of the leaves						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
1	++	++	+–	––	––	––	––
2	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++

Movement of the leaf was represented by following marks: ++ completely open; + nearly open; +– at random; – nearly closed; –– completely closed

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17. Potassium galactolespedezate (**2**): ^{1}H NMR (400 MHz, D_2O, 30 °C): 7.24 (2 H, d, J = 8 Hz), 6.84 (2 H, d, J = 8 Hz), 6.20 (1 H, s), 4.89 (1 H, t, J = 4 Hz), 3.99 (1 H, d, J = 2 Hz), 3.88-3.70 (5H, m) ppm.; ^{13}C NMR (100 MHz, D_2O, 30 °C): 172.6, 150.0, 143.4, 130.2, 127.1, 116.2, 109.1, 101.4, 76.3, 73.3, 71.3, 69.3, 61.7 ppm.; IR ν: 3260, 1590, 1514 cm^{-1}; $[\alpha]_D^{28}$ –24.2 (c 0.170, H_2O); HR FAB MS (negative): $[M–K]^-$ Found *m/z* 341.0823, $C_{15}H_{17}O_9$ requires *m/z* 341.0873.; Potassium galactoisolespedezate (**7**): ^{1}H NMR (400 MHz, D_2O, 30 °C): 7.78 (2 H, d, J = 9 Hz), 6.94 (2 H, d, J = 9 Hz), 6.75 (1 H, s), 4.99 (1 H, t, J = 8 Hz), 3.94 (1 H, d, J = 3 Hz), 3.83 (1 H, dd, J = 10, 8 Hz), 3.74 (1 H, dd, J = 12, 5 Hz), 3.71 (1 H, dd, J = 12, 4 Hz), 3.70 (1 H, dd, J = 10, 3 Hz), 3.64 (1 H, dd, J = 5, 4 Hz), ppm.; ^{13}C NMR (100 MHz, D_2O, 30 °C): 172.6, 150.0, 143.4, 130.2, 127.1, 116.2, 109.1, 101.4, 76.3, 73.3, 71.3, 69.3, 61.7 ppm.; IR ν: 3234, 1575, 1512 cm^{-1}; $[\alpha]_D^{28}$ +49.5 (c 0.715, H_2O); HR FAB MS (negative): $[M–K]^-$ Found *m/z* 341.0854, $C_{15}H_{17}O_9$ requires *m/z* 341.0873.
18. Potassium mannoisolespedezate (**3**): ^{1}H NMR (400 MHz, D_2O, 30 °C): 7.59 (2 H, d, J = 9 Hz), 6.96 (2 H, d, J = 9 Hz), 6.75 (1 H, s), 5.52 (1 H, t, J = 2 Hz), 4.23 (1 H, dd, J = 3, 2 Hz), 3.99 (1 H, dd, J = 10, 3 Hz), 3.70 (1 H, t, J = 10 Hz), 3.60 (1 H, dd, J = 13, 4 Hz), 3.56 (1 H, dd, J = 13, 2 Hz), 3.36 (1 H, dd, J = 13, 2 Hz), 3.36 (1 H, ddd, J = 10, 4, 2 Hz), ppm.; ^{13}C NMR (100 MHz, D_2O, 30 °C): 171.7, 156.1, 146.0, 131.9, 126.9, 120.1, 116.2, 100.1, 75.2, 71.4, 70.5, 66.8, 61.3 ppm.; IR ν: 3336, 1620, 1569, 1512 cm^{-1}; $[\alpha]_D^{28}$ +68.7 (c 1.26, H_2O); HR FAB MS (negative): $[M–K]^-$ Found *m/z* 341.0854, $C_{15}H_{17}O_9$ requires *m/z* 341.0873.
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