Pergamon

Tetrahedron Letters 41 (2000) 3433–3436

TETRAHEDRON
LETTERS

Syntheses of fluorescence-labeled artificial leaf-opening substances, fluorescent probe compounds useful for bioorganic studies of nyctinasty

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Received 9 February 2000; revised 28 February 2000; accepted 3 March 2000

Abstract

In a previous paper, the syntheses of potassium galactolespedezate (**1**) and potassium galactoisolespedezate (**2**), artificial leaf-opening substances useful for bioorganic studies of nyctinasty were reported. The fluorescent probe compounds, fluorescence-labeled galactoisolespedezates (**3**, **10**, **13**), designed on the basis of **1** and **2**, were prepared. In particular, compound **3** was bioactive at 5×10^{-5} M, one-fiftieth as strong as the natural leaf-opening substance. This fluorescent probe would be useful for bioorganic studies of nyctinasty.

Keywords: plants; natural products; biologically active compounds; fluorescent probe.

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.[3–16] These bioactive substances can be used for probe compounds which would be highly useful for the purification of their receptors to enable bioorganic studies of nyctinasty.

Investigation of the site where bioactive substances are perceived at the cellular level is an essential step towards the isolation and identification of the receptor molecule. A fluorescence-labeled bioactive substance is most suitable for the study of this problem, because the fluorescence-labeled compounds are widely used for the identification of receptor molecules.[17–19] In this paper, we report the syntheses of fluorescence-labeled leaf-movement factors designed on the leaf-opening substance of *Lespedeza cuneata* G. Don, potassium lespedezate (**4**) and potassium galactoisolespedezate (**5**).[4,5]

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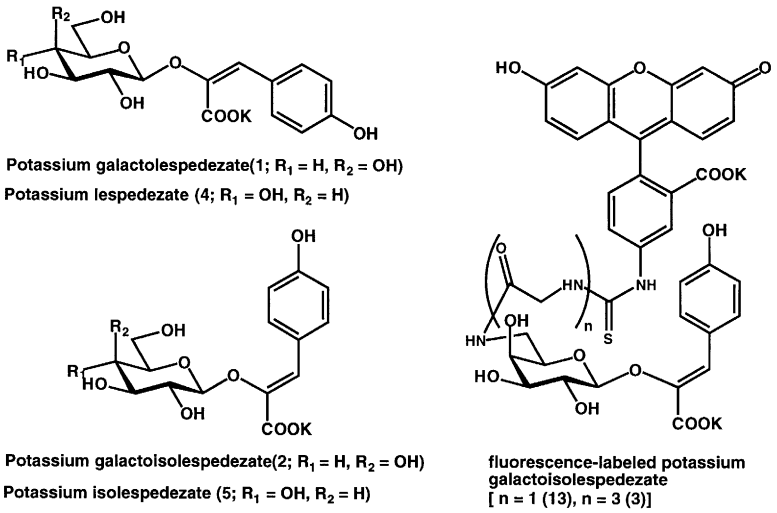
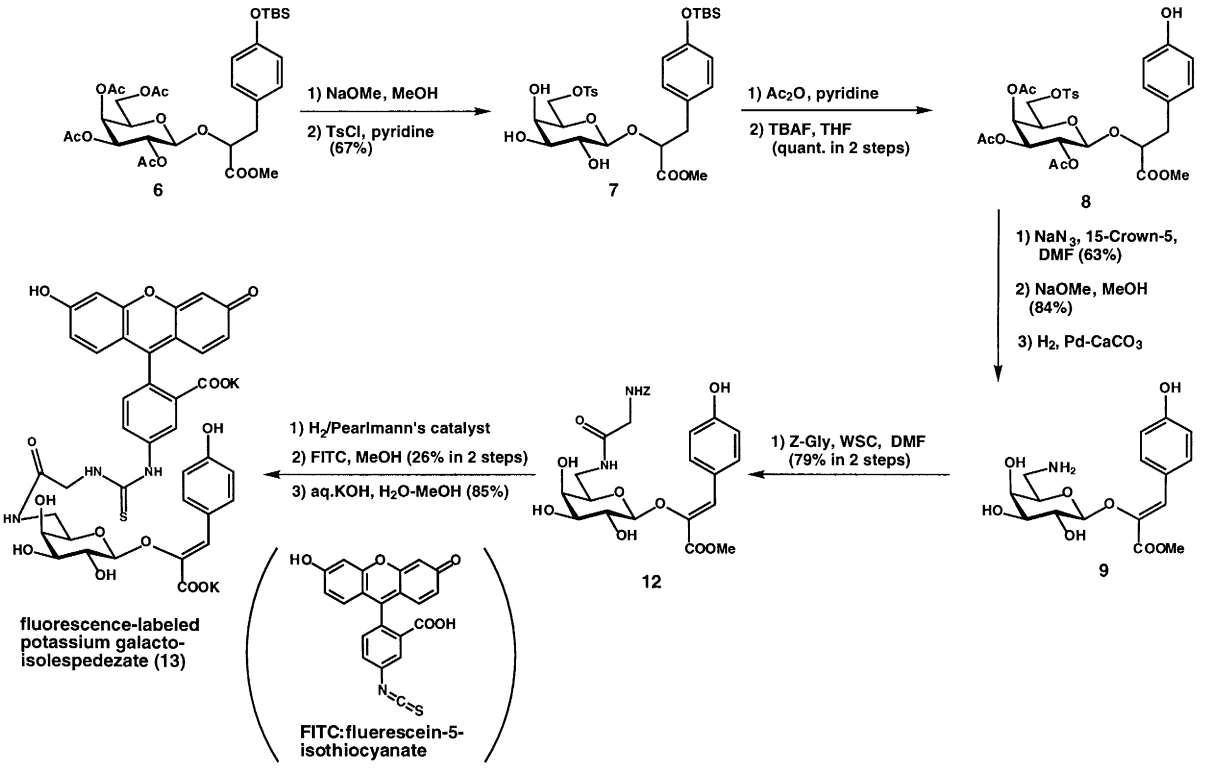
0040-4039/00/$ - see front matter
PII: S0040-4039(00)00394-4



The most important problem involved in the design of fluorescence-labeled leaf-movement factors is the unstability of **4** and **5** in the plant body, which are easily hydrolyzed by β-glucosidase. Thus, we designed a probe compound based on the structure of an artificial leaf-opening substance, potassium galactolespedezate (**1**) and potassium galactoisolespedezate (**2**), which could not be hydrolyzed in a plant body.[20,21]

The introduction of a large fluorescent functional group such as FITC (fluorescein-5-isothiocyanate) to artificial leaf-opening substances requires careful consideration of the structure–activity relationship in **4**



Scheme 1. Synthetic route of the fluorescence-labeled potassium galactoisolespedezate (**13**)

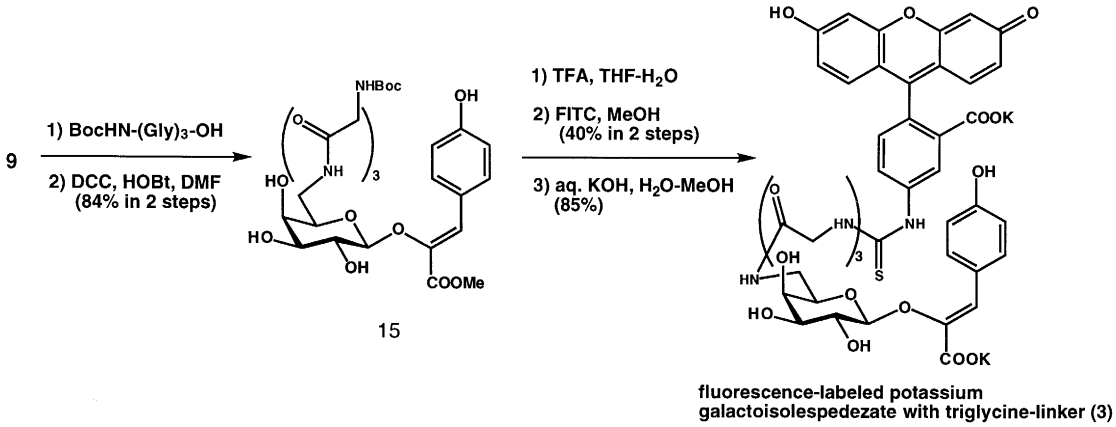
Table 1
Bioactivities of fluorescent probe compounds compared with **1**, **2**, **4** and **5**

compounds	Bioactivity (mol/L)
1 and **2**	1×10^{-6}
3	5×10^{-5}
4 and **5**	1×10^{-6}
10	5×10^{-4}
13	1×10^{-4}

and **5**. Previous studies showed that the substituents at the phenolic hydroxyl group of **4** and **5** as well as the carboxyl group led to a decrease of activity to 10^{-3} or 10^{-4} M.[5] However, considering the bioactivity of **1** and **2**, the substitution of the sugar moiety in **4** and **5**, from glucose to galactose, did not affect the bioactivity at all. From these results, it is expected that the introduction of a large fluorescent functional group in the hydroxyl group at the 6′-position of the galactose moiety would not weaken the bioactivity of **1** and **2** to any extent. Moreover, because of the resistance to the esterase in a plant body, an amide bond would be better than an ester bond to connect the FITC group with **1** and **2**. Thus, to introduce an FITC group at the 6′-position of **1** (or **2**), we should convert the hydroxy group on the 6′-position of **1** (or **2**) into an amino group.

Compound **6** was deprotected with sodium methoxide, converted into its tosylate **7**, and then acetylated to give **8** (Scheme 1). The resulting **8** was treated with sodium azide, and then reduced by catalytic hydrogenation, and the resulting amine **9** was coupled with FITC. After deprotection, the fluorescence-labeled potassium galactoisolespezate (**10**) was derived with a trace amount of potassium galactolespedezate (**11**). However, **10** showed leaf-opening activity at 5×10^{-4} M, which is one-five hundredth as strong as **1** and **2** (Table 1). The decrease of bioactivity could be due to the steric hindrance of the large fluorescent functional group; thus, the insertion of some linker such as glycine between the FITC group and the artificial leaf-opening substance would be effective for the improvement of bioactivity.

Scheme 1 shows the synthetic route of fluorescence-labeled potassium galactoisolespedezate (**13**). Coupling of amine **9** with Z-Gly was carried out using WSC in DMF to give **12**. Compound **12** was deprotected with Pearlmann's catalyst, coupled with FITC reagent, and treated with KOH to give **13**. The bioactivity of **13** was improved up to five times the strength of **10**, thus providing the effectiveness of the spacer (Table 1).



Scheme 2. Synthetic route of the fluorescence-labeled potassium galactoisolespedezate with triglycine-linker **3**

Finally, we synthesized the third fluorescent probe with a linker of glycylglycylglycine. According to the same procedure as that of **13**, using Z-Gly-Gly-Gly instead of Z-Gly, **3** could not be obtained because of the reduction of the enol double bond in catalytic hydrogenation. Thus, we changed the protective group of amine from a benzyloxycarbonyl to a *tert*-butoxycarbonyl group. Glycylglycylglycine (**14**) was coupled with $(Boc)_2O$ and the resulting protected tripeptide was coupled with **9** to give **15** (Scheme 2). Compound **15** was further coupled with FITC, then deprotected to give **3**.[22] The leaf-opening activity of fluorescent probe compound **3** was improved up to 5×10^{-5} M. The bioactivity of **3** was one-fiftieth as strong as that of the natural product; thus, **3** would be useful as a molecular probe for the bioorganic studies of nyctinasty.

Acknowledgements

We are indebted to the Ministry of Education, Science, Sports and Culture (Japan) for a Grant-in-Aid for Scientific Research on Special Promotion Research No. 09101001, the Asahi Glass Foundation, and a Pioneering Research Project in Biotechnology given by the Ministry of Agriculture, Forestry and Fisheries for financial support.

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22. Fluorescence-labeled galactoisolespedezate (**3**): ^{1}H NMR (400 MHz, CD_3OD, 30°C) δ 8.15 (1H, d, *J*=2.0 Hz), 7.75 (1H, dd, *J*=8.3, 2.0 Hz), 7.63 (2H, d, *J*=8.8 Hz), 7.02 (1H, d, *J*=8.3 Hz), 6.81 (1H, s), 6.77–6.70 (6H, m), 6.61 (2H, dt, *J*=8.7, 2.0 Hz), 4.85 (1H, d, *J*=8.8 Hz), 4.85 (1H, d, *J*=17.1 Hz), 3.86 (1H, d, *J*=17.1 Hz), 4.30 (1H, d, *J*=17.1 Hz), 3.92 (1H, d, *J*=17.1 Hz), 3.79–3.74 (4H, m), 3.59–3.55 (2H, m), 3.40 (1H, d, *J*=13.7, 5.4 Hz), 3.24 (1H, dd, *J*=13.7, 8.3 Hz) ppm; ^{13}C NMR (400 MHz, CD_3OD, 30°C)δ 184.9, 173.9, 173.3, 172.8, 172.1, 162.6, 159.6, 155.1, 150.2, 143.2, 133.9, 132.8, 131.2, 130.0, 127.7, 126.5, 123.4, 121.2, 116.8, 114.7, 112.4, 104.9, 104.4, 75.9, 75.5, 73.8, 71.2, 44.7, 44.2, 41.7, 33.8, 31.5, 24.5, 21.8, 15.2, 10.0; HR FAB MS (negative): $[M-2K+H]^-$ Found *m/z* 900.2019, $C_{42}H_{38}O_{16}N_5S$ requires *m/z*: 900.2034; IR (film) ν: 3294, 1736, 1661, 1639, 1606, 1586, 1546, 1513 cm^{-1}; $[\alpha]_D^{20}$ +58.0 (*c* 1.0, MeOH).