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Potassium aeshynomate, a leaf-opening substance of *Aeshynomene indica* L., containing a novel γ-amino acid

Minoru Ueda,[a] Takahito Hiraoka,[a] Masatake Niwa[b] and Shosuke Yamamura[a,*]

[a]*Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223-8522, Japan*
[b]*Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya 468, Japan*

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Abstract

Potassium aeshynomate (**1**) was isolated as an leaf-opening substance of the nyctinastic plant, *Aeshynomene indica* L. Compound **1** was quite effective for the leaf-opening of *A. indica* at 1×10^{-3} M, and was found to be a new type of leaf-movement factor containing a novel γ-amino acid moiety.

Keywords: plants; natural products; biologically active compounds; amino acids; amino acid derivatives.

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] and revealed that nyctinastic movement of the plants is controlled by the interaction between leaf-closing and -opening substances.[11–14] For some nyctinastic plants, we have already identified both the leaf-closing and -opening substances, and shown that a change in the balance between these two substances controls the nyctinasty.

From *Aeschynomene indica* L., a nyctinastic plant, trigonelline (**2**) was isolated as a leaf-closing substance. However, the counterpart of **2**, a leaf-opening substance, has been unidentified. We have now isolated potassium aeshynomate (**1**), which contained a novel γ-amino acid moiety in the molecule, as a leaf-opening substance of *A. indica*.

* Corresponding author. Fax: 81 45 563 5967; e-mail: yamamura@chem.keio.ac.jp

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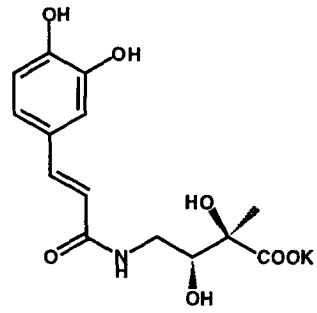
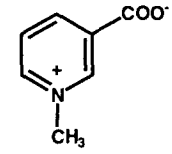


Potassium aeshynomate(1)



Trigonelline (2)

Isolation of the leaf-opening substance was carried out based on a bioassay using a leaf of *A. indica*. The bioactive fraction kept the leaves open until 8:00 pm. The fresh whole plant of *A. indica* (7.7 kg) was extracted with methanol for two weeks and concentrated in vacuo. The concentrated extract was partitioned with ethyl acetate, then with *n*-butanol. The bioactive aqueous layer was carefully separated by Amberlite XAD-7 column chromatography eluted with MeOH:H_2O (0:100, 10:90, 20:80, 50:50, and 100:0), and the 10% MeOH aq. fraction showed weak leaf-opening activity. The 10% MeOH aq. fraction was further purified by gel filtration column chromatography using Toyopearl HW-40S with 40% MeOH aq., and then HPLC using preparative Cosmosil 5C18AR column with 30% MeOH aq. repeatedly to give potassium aeshynomate (**1**, 8.7 mg).

Structural determination of **1** was carried out by means of NMR and FABMS experiments. A strong molecular ion corresponding to **1** was observed in the FABMS spectrum suggested that **1** exists as a potassium salt. HMQC and HMBC experiments gave the planar structure of **1**.[17] The aromatic region of the ^1H NMR spectrum of **1** showed that **1** has a 1,3,4-trisubstituted aromatic ring. There was also observed a conjugated carboxylic acid moiety in this region. Correlations observed between these two parts gave the structure of a coffeyl group (Fig. 1). On the other hand, the structure of the aliphatic region was determined to be α,β-dihydroxy-γ-amino acid from the correlations between the methyl proton ($H_{5'}$) and $C_{1'}$, $C_{2'}$, and $C_{3'}$ in the HMBC spectrum. The carbonyl group in the coffeyl moiety of **1** showed strong correlation with $H_{4'}$ in the HMBC spectrum, together with the IR spectrum, indicating that these two parts are connected with the nitrogen atom.

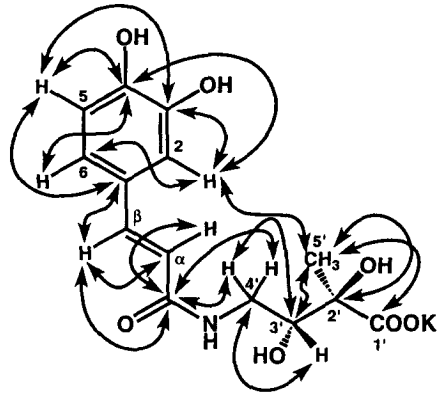
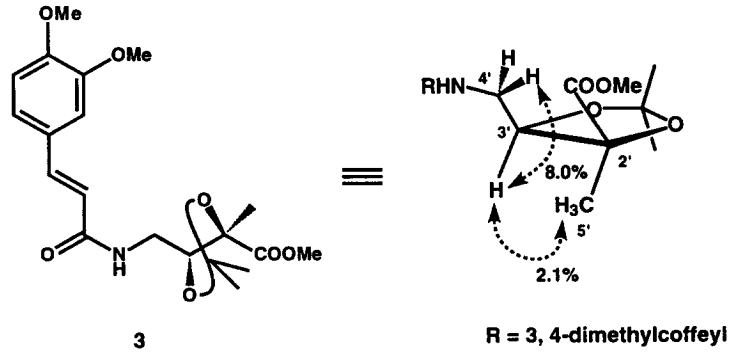


Figure 1. HMBC correlations in potassium aeshynomate (**1**)

The stereochemistry of **1** was determined by an NOE experiment using **3**. Compound **3** was prepared as follows: compound **1** (2.0 mg) was treated with Amberlite IR-120 (H^+), and then diazomethane in 50% MeOH aq. to give the corresponding methyl ester with three methoxy groups. The methyl ester and *p*-

toluenesulfonic acid were dissolved in 2,2-dimethoxypropane and stirred at room temperature overnight. The resulting **3** (1.8 mg)[18] was used for the NOE experiment. Small NOE (2.1%) was observed between $H_{5'}$ and $H_{3'}$ in **3** (Fig. 2). This result showed that the stereochemical relationship between the $C_{2'}$- and $C_{3'}$-hydroxy groups is *anti*, however, the absolute stereochemistry of **1** is to be studied. From a structural point of view, interestingly, **1** has a 2,3-dihydroxy-γ-amino acid moiety in the molecule.



3 **R = 3, 4-dimethylcoffeyl**

Figure 2. NOE correlations observed in **3**

Potassium aeshynomate (**1**) was effective at as high as 1×10^{-3} M only for the leaves of *A. indica*, and not effective for other nyctinastic plants, such as *Phyllanthus urinaria* L., *Mimosa pudica* L., and *Albizzia julibrissin* Durazz. even at 1×10^{-3} M. All of the leaf-movement factors previously isolated by us showed specific bioactivity on the corresponding plant species at 1×10^{-5}–10^{-6} M.[3–16] The bioactivity of **1** was one-hundredth as low as the leaf-opening substances found in other nyctinastic plants,[4,9–11,15] suggesting a possibility that much more effective leaf-opening substance would exist in *A. indica*. We are now searching for more effective leaf-opening substances from the extract of *A. indica*.

Acknowledgements

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17. Potassium aeshynomate (**1**): ^{1}H NMR (400 MHz, CD_3OD:D_2O=1:1, rt): 7.60 (1H, d, J=16 Hz, H_β), 7.18 (1H, d, J=2 Hz, H_2), 7.02 (1H, dd, J=2 and 8 Hz, H_6), 6.85 (1H, d, J=8 Hz, H_5), 6.35 (1H, d, J=16 Hz, H_α), 4.25 (2H, m, $H_{4'}$), 4.03 (1H, dd, J=7 and 5 Hz, $H_{3'}$), 1.40 (3H, s, $H_{5'}$) ppm.; ^{13}C NMR (100 MHz, D_2O, 35°C): 181.3 ($C_{1'}$), 170.3 ($C_{carbonyl}$), 148.3 (C_4), 147.0 (C_β), 145.4 (C_3), 127.9 (C_1), 123.5 (C_6), 117.1 (C_5), 115.9 (C_2), 115.3 (C_α), 77.6 ($C_{2'}$), 74.5 ($C_{3'}$), 66.5 ($C_{4'}$), 23.3 ($C_{5'}$) ppm.; IR ν: 1692, 1605, 1281, 1182 cm^{-1}; HR FABMS (positive): $[M-K+2H]^+$ Found *m/z* 310.0902, $C_{14}H_{17}O_7N$ requires *m/z* 310.0927; $[\alpha]_D^{22}$ −3.39 (*c* 0.24, 50% MeOH aq.)
18. Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$, 35°C): 7.60 (1H, d, J=16 Hz, H_β), 7.02 (1H, dd, J=2 and 8 Hz, H_6), 6.95 (1H, d, J=2 Hz, H_2), 6.80 (1H, d, J=8 Hz, H_5), 6.25 (1H, d, J=16 Hz, H_α), 4.55 (1H, dd, J=3 and 12 Hz, $H_{4a'}$), 4.15 (1H, dd, J=3 and 7 Hz, $H_{3'}$), 4.05 (1H, dd, J=7 and 12 Hz, $H_{4b'}$), 3.85 (6H, s, phenolic methoxy), 3.65 (3H, s, methoxy), 1.55 (6H, s, methyl), 1.40 (3H, s, $H_{5'}$) ppm. HR FABMS (positive): $[M+H]^+$ Found *m/z* 394.1855, $C_{20}H_{28}O_7N$ requires *m/z* 394.1865.