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Enantioselective synthesis of phyllanthurinolactone, a leaf-closing substance of *Phyllanthus urinaria* L., and its analogs toward the development of molecular probes

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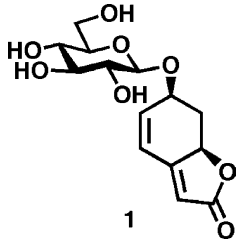
Abstract—We report enantioselective synthesis of phyllanthurinolactone (**1**), a leaf-closing substance of *Phyllanthus urinaria* L., and its analogs with sugars other than D-glucose. Structure–activity relationship study using them revealed that the structure of the sugar moiety did not affect their bioactivity at all. This result is very important for the development of molecular probes based on the structure of **1**.

Most leguminous plants close their leaves in the evening, as if to sleep, and open them early in the morning according to the circadian rhythm controlled by the biological clock.[1] Charles Darwin, well-known for his theory of evolution, was the first to establish the basis of this field in the 19th century.[2] Nyctinastic leaf movement is induced by the swelling and shrinking of motor cells in the pulvini, a small organ located in the joint of the leaf to the stem. Motor cells play a key role in plant leaf-movement. Flux of potassium ions across the plasma membranes of the motor cells is followed by massive water flux, which results in swelling and shrinking of these cells.[3] An issue of great interest is the regulation of the opening and closing of the potassium channels involved in nyctinastic leaf movement. We have revealed that nyctinasty is controlled by a pair of leaf-movement factors: leaf-opening and leaf-closing substances.[4] Recently, we revealed that the target cell of the leaf-opening substance is motor cells,[5] which play a key role in nyctinastic leaf-movement.[3] On the other hand, no attempt was carried out to clarify the target cell of the leaf-closing substances because the structure of most leaf-closing substance is so simple that structural modification toward a molecular probe, such as a fluorescence-labeled one, seems to be difficult because large fluorescence dye or a photoaffinity unit would decrease the bioactivity of the synthetic probe seriously.

Phyllanthurinolactone (**1**), a leaf-closing substance of *Phyllanthus urinaria* L., is a glycoside-type leaf-closing substance.[6] The structure of **1** is comparatively large enough for the structure modification essential for a synthetic probe. The synthesis of **1** was completed by Audran and Mori in 1998.[7] They synthesized all stereoisomers on the aglycon moiety of **1** and determined its absolute stereochemistry. By using these isomers, they also showed that the stereochemistry of natural **1** is essential for its bioactivity: No stereoisomer of **1** was biologically active. However, structure–activity relationship study on the sugar moiety of **1** was not undertaken. Thus, we synthesized the analogs of **1** with sugars other than D-glucose and studied the structure–activity relationship, which is an essential data for the development of biologically active molecular probes, such as fluorescence-labeled or photoaffinity labeling probes.



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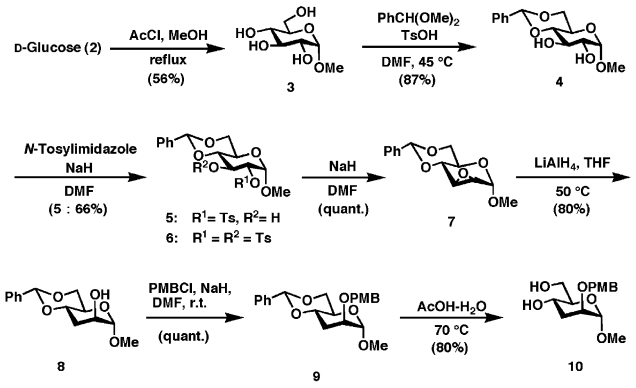


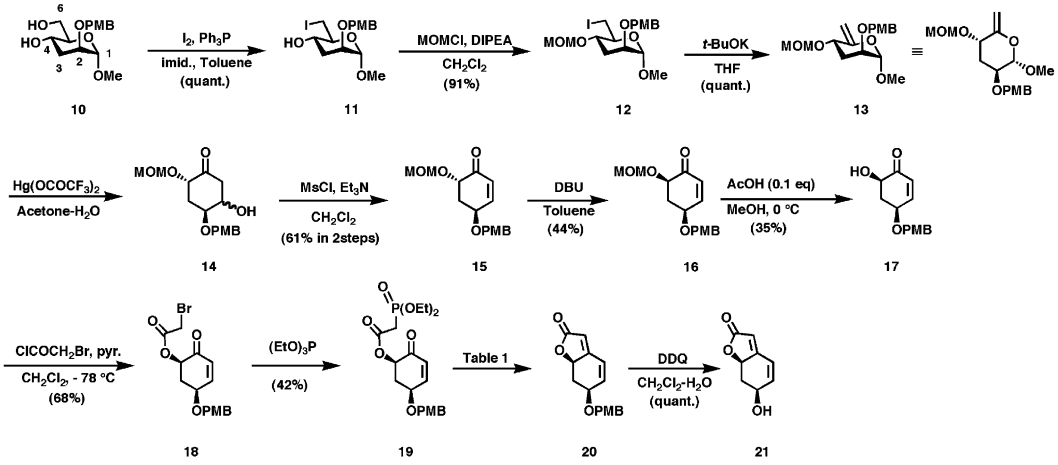
Scheme 1. Synthesis of compound **10**.

We planned the asymmetric synthesis of **1** using D-glucose as a chiral starting material for the aglycon moiety. Ferrier's carbocyclization reaction and intramolecular Horner–Emmons reaction would give the aglycon of **1** with natural absolute stereochemistry.

Methyl α-D-glucopyranoside (**3**) was protected with a benzylidene group, and then treated with *N*-tosylimidazole (1.0 equiv) and NaH (1.2 equiv) to give tosylate (**5**) (Scheme 1). When this reaction was carried out with *p*-toluenesulfonyl chloride (1.2 equiv) and 2 equiv of NaH, ditosylate (**6**) was obtained.

Compound **5** was treated with NaH to give epoxide (**7**).[8] Epoxide **7** was then reduced by $LiAlH_4$ according to the method by Taillefumier and Chapleur,[9] and the inversed 2′-hydroxyl group was protected by a *p*-methoxybenzyl group to give **9**.

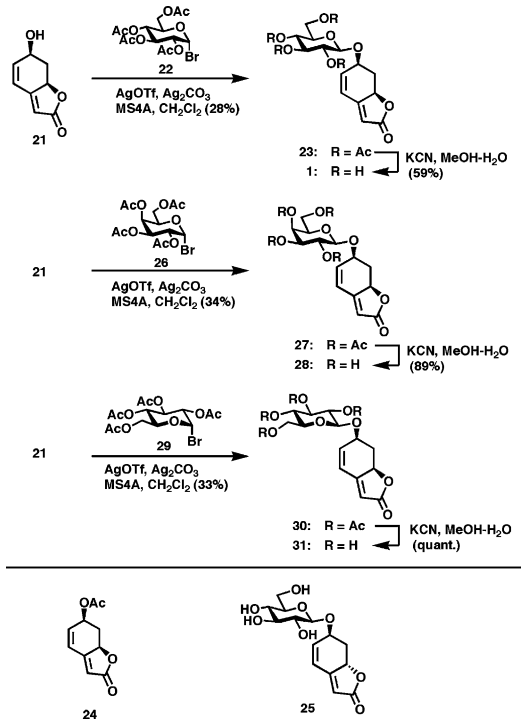
After deprotection of the benzylidene group, iodine was introduced to the 6-position of resulting **10** (Scheme 2). After protection of the 4-position by MOM, the product was treated with *t*-BuOK to give olefin **13**. Ferrier cyclization of **13** by catalytic $Hg(OCOCF_3)_2$ gave **14**.[10] Compound **14** was treated by MsCl to give α,β-unsaturated ketone **15**. α-Hydroxyl group of **15** was epimerized with DBU. Subsequent deprotection of MOM by using various acids, such as TsOH, TFA, ended in the recovery of **16**. However, 0.1 equiv of AcCl in MeOH at 0 °C gave **17** in 35% yield with recovered **16** in high yield (64%). When the amount of AcCl was increased to 0.5 equiv, the reaction gave a mixture of decomposed products. Then, we synthesized **17** by repeated deprotection of recovered **16**. Next, we examined the acylation of the resulting free hydroxyl group in **17** for the following intramolecular Hornor–Emmons reaction. But all attempts to introduce diethyl phosphonoacetic acid to α-hydroxyl group of **17** ended in failure. Thus, we introduced bromoacetyl chloride to the hydroxyl group of **17**, and then the resulting **18** was treated with triethyl phosphite to give **19**. Intramolecular Hornor–Emmons reaction of **19** was examined using various conditions (Table 1). Mild conditions using DBU gave a deacylated product (**17**) or a complex mixture. However, NaH gave the best result to give **20** in 43% yield. After deprotection by DDQ, agalycon with naturally occurring stereochemistry (**21**) was obtained. Glycosidation of **21** was carried out according to the method reported by Audran and Mori.[7] Coupling of **21** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**22**) was carried out with Ag_2CO_3 and AgOTf to give **23** with recovered **21** (Scheme 3). Acetylated aglycon **24**, resulted by nucleophilic attack of the hydroxyl group in **21** to the orthoester



Scheme 2. Synthesis of aglycon **21**.

Table 1. Reaction conditions for intramolecular Horner–Emmons reaction of **19**

Entry	**19** (equiv)	Conditions	Product (Yield %)
1[11]	1.0	LiBr (2.0 equiv), DBU (1.0 equiv), THF, −78 to −30 °C	**17** (59%)
2	1.0	DBU (1.0 equiv), THF, rt	Complex mixture
3	1.0	NaHMDS (1.0 equiv), THF, −78 °C	Complex mixture
4	1.0	K_2CO_3 (1.2 equiv), THF, rt	**20** (29%)
5	1.0	NaH (0.5 equiv), THF, 0 °C	**20** (42%)

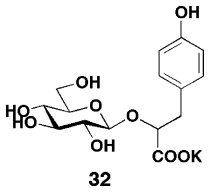


Scheme 3. Synthesis of phyllanthurinolactone (**1**) and its analogs.

intermediate from **22**, was also obtained in 52% yield as the main product of this reaction. Resulting **23** was deprotected with KCN to give **1** (59%) together with its epimer **25**[7] (37%) as a by-product. The α-proton in **23** would be easily abstracted by KCN and resulting stable furan-type intermediate gave a mixture of **1** and **25** by protonation. Physical data of synthetic **1** were completely identical to that of the naturally occurring **1**.[12] Fortunately, **24** can also be deacetylated by using KCN to give aglycon **1**, which can be used for coupling reaction with the sugar unit.

Similarly, we synthesized analogs of **1** with sugars other than D-glucose (Scheme 3). Aglycon **21** was coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**26**) or 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide (**27**). The coupling products were then deprotected with KCN to give galacto-**1** (**28**)[13] or L-**1** (**31**),[14] respectively.

Bioassay of synthetic **1**, **28**, and **31** using leaves of *P. urinaria* was carried out. Despite the variety in the structure of the sugar moiety, all of these analogs were effective at 1×10^{-7} M. This result showed that the structure of the sugar moiety did not affect the bioactivity of **1**. Similar result[15] was obtained in the case of potassium isolespedezate (**32**), a leaf-opening substance of *Cassia mimosoides* L.[16] In the case of **32**, we developed biologically active molecular probes based on **32** such as a fluorescence-labeled probe or a photoaffinity labeling probe by the introduction of large fluorescence dye[5] or a large photoaffinity labeling unit[17] into the sugar moiety. Similar design of a molecular probe would make it possible to develop biologically active molecular



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probes based on **1**. The syntheses of molecular probes based on **1** are now in progress.

Acknowledgements

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12. **1**: ^{1}H NMR (400 MHz, D_2O, rt): 6.75 (1H, dd, $J = 10.0$, 2.4 Hz), 6.47 (1H, br d, $J = 10.0$ Hz), 5.95 (1H, br s,), 5.15 (1H, ddd, $J = 13.6$, 5.2, 2.0 Hz), 4.85 (1H, dddd, $J = 10.4$, 5.2, 2.4, 2.0 Hz), 4.69 (1H, d, $J = 8.0$ Hz), 3.91 (1H, dd, $J = 12.4$, 2.4 Hz), 3.72 (1H, dd, $J = 12.4$, 5.6 Hz), 3.50 (1H, t, $J = 8.8$ Hz), 3.48 (1H, ddd, $J = 8.8$, 5.6, 2.4 Hz), 3.39 (1H, t, $J = 8.8$ Hz), 3.27 (1H, dd, $J = 8.8$, 8.0 Hz), 3.03 (1H, dt, $J = 10.4$, 5.2, Hz), 1.78 (1H, dt, $J = 13.6$, 10.4 Hz); ^{13}C NMR (100 MHz, D_2O, rt) 178.3, 167.1, 142.1, 122.1, 112.1, 103.5, 80.9, 77.5, 77.1, 75.7, 74.9, 71.1, 62.6, 39.5 ppm.; HR FAB MS (positive): $[M+H]^+$ Found m/z 315.1054, $C_{14}H_{19}O_8$ requires m/z 315.1080; IR (film) ν: 3388, 1735, 1637, 1585, 1387 cm^{-1}; $[\alpha]_D^{25}$ −30.0 (c 0.11, H_2O); $[\alpha]_D^{21}$ −6.0 (c 0.20, H_2O) in naturally occurring **1**.
13. **28**: ^{1}H NMR (400 MHz, D_2O, 40 °C): 6.75 (1H, dd, $J = 2.2$, 10.3 Hz), 6.47 (1H, d, $J = 10.3$ Hz), 5.95 (1H, s), 5.14 (1H, ddd, $J = 1.8$, 4.8, 13.2 Hz), 4.87–4.83 (1H, m),

4.63 (1H, d, $J = 7.9$ Hz), 3.93 (1H, d, $J = 3.3$ Hz), 3.80–3.69 (3H, m), 3.66 (1H, dd, $J = 3.3$, 9.9 Hz), 3.52 (1H, dd, $J = 7.9$, 9.9 Hz), 3.04 (1H, dt, $J = 4.8$, 10.6 Hz), 1.78 (1H, dt, $J = 10.6$, 13.2 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, 40 °C): 178.5, 167.1, 142.4, 122.2, 112.0, 103.9, 80.9, 76.8, 75.9, 74.3, 72.2, 70.1, 62.4, 39.3 ppm; HR FAB MS (positive): $[M+H]^+$ found m/z 315.1086, $C_{14}H_{19}O_8$ requires m/z 315.1080; IR (film) v: 3365, 1738, 1591 cm^{-1}; $[\alpha]_D^{25}$ −34.5 (c 0.11, H_2O).

14. **31**: ^{1}H NMR (400 MHz, D_2O, 40 °C): 6.75 (1H, dd, $J = 2.5$, 9.8 Hz), 6.45 (1H, d, $J = 9.8$ Hz), 5.95 (1H, s), 5.13 (1H, ddd, $J = 1.3$, 4.6, 13.2 Hz), 4.90–4.84 (1H, m), 4.71 (1H, d, $J = 7.8$ Hz), 3.85 (1H, dd, $J = 2.5$, 12.4 Hz), 3.66 (1H, dd, $J = 6.4$, 12.4 Hz), 3.43 (1H, m), 3.41 (1H, m), 3.33 (1H, t, $J = 9.3$ Hz), 3.21 (1H, dd, $J = 7.8$, 9.3 Hz), 3.05 (1H, dt, $J = 4.6$, 10.9 Hz), 1.74 (1H, dt, $J = 10.9$, 13.2 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, 40 °C): 178.6, 166.6, 143.3, 122.5, 112.0, 102.7, 80.9, 78.6, 77.3, 75.5, 75.4, 71.0, 62.3, 38.0 ppm; HR FAB MS (positive): $[M+H]^+$ found m/z 315.1070, $C_{14}H_{19}O_8$ requires m/z 315.1080; IR (film) v: 3354, 1739, 1593 cm^{-1}; $[\alpha]_D^{25}$ −9.3 (c 1.1, H_2O).
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