

Synthesis and Absolute Configuration of Phyllanthurinolactone, the Leaf-closing Factor of a Nyctinastic Plant, *Phyllanthus urinaria* L.

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Abstract: Phyllanthurinolactone (**1**) and its diastereoisomer **19** were synthesized, only the former of which was bioactive as the leaf-closing factor of *Phyllanthus urinaria* L. X-ray analysis of the tetraacetylglucoside **18** was executed, and the absolute configuration of **1** was determined as 6*S*, 7*aR*. © 1997, Elsevier Science Ltd. All rights reserved.

The phenomenon of nyctinasty or "plant sleep" has been recorded since the ancient days of Alexander the Great¹. For example, the pinnate leaves of a large tamarind tree (*Tamarindus indica* L.) fold together at night as if the tree sleeps¹. In 1995 Yamamura and his coworkers isolated 3.1 mg of phyllanthurinolactone (**1**, Fig. 1) from 19.2 kg of the fresh nyctinastic plant *Phyllanthus urinaria* L. as its leaf-closing factor, which was bioactive² only for that plant in the daytime at a very low concentration of 1×10^{-7} M. They proposed structure **1** for phyllanthurinolactone, although the absolute configuration of the aglycone part remained unknown².

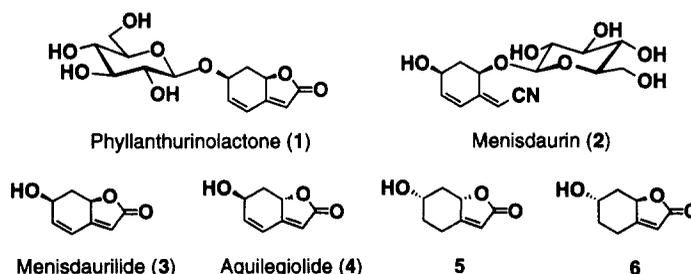


Figure 1. Structures of phyllanthurinolactone (**1**) and related compounds.

There are some reports on the isolation and identification of plant constituents with the structures (**2-4**) related to **1** (Fig. 1). Menisdaurin (**2**) was isolated by Takahashi et al. in 1978 from the vines of *Menispermum dauricum*, and its acid hydrolysis afforded menisdaurilide (**3**)³. Aquilegiolide (**4**) is a stereoisomer of **3**, and was isolated in 1984 by Guerriero and Pietra from roots of *Aquilegia strata*⁴. Both **3** and **4** were also isolated in 1993 from the rhizomes of *Sinomenium acutum* by Otsuka et al., who determined the absolute configuration of **3** as depicted in the formula by the X-ray analysis of its *p*-bromobenzoate⁵. The absolute configuration of **4** could be correlated with that of **3**^{4,5}. We speculated that the absolute configuration of the aglycone part of

phyllanthurinolactone might be *6S*, *7aR* like that of **3**. In order to prove or disprove this hypothesis, we decided to undertake the synthesis of (*6S*, *7aR*)-**1** and its diastereoisomer (*6R*, *7aS*)-**19**.

Although Majewski et al. had reported the enantioselective synthesis of *ent*-dihydromenisdaurilide (**5**) and *ent*-dihydroaquilegiolide (**6**)⁶, we chose a different way to the synthesis of **1** and **19** as summarized in Fig. 2. Our plan is to prepare and resolve (\pm)-menisdaurilide (**3**) by employing D-glucose as a resolving agent to separate the two diastereoisomeric glucosides **17** and **18**. Subsequent deprotection of **17** gives **1**, while that of **18** affords **19**, one of which must be the natural phyllanthurinolactone. The absolute configuration of the two diastereoisomers **17** and **18** is expected to be determined by some appropriate means.

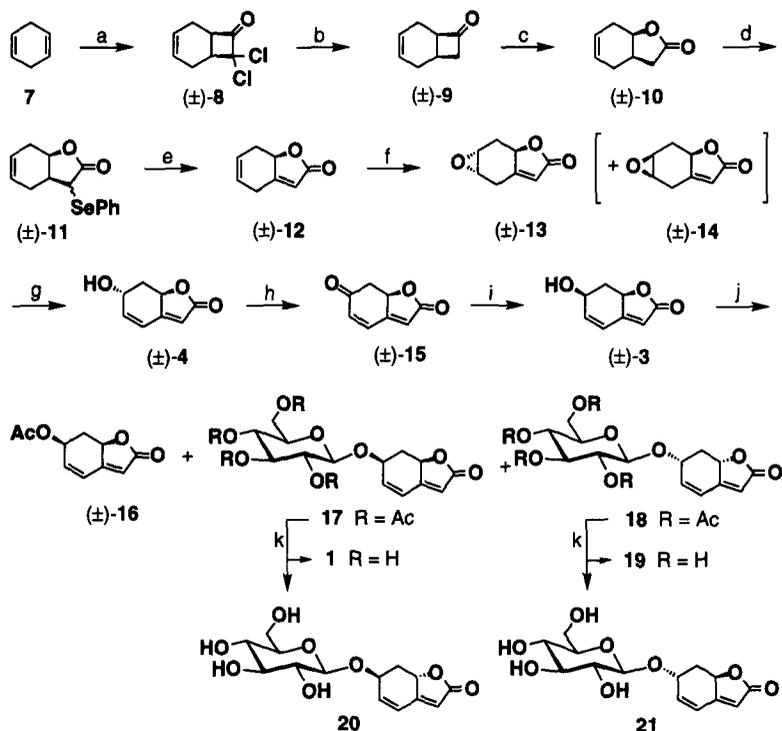


Figure 2. Synthesis of phyllanthurinolactone (**1**).

Reagents: (a) CCl_3COCl , Zn, Et_2O , ultrasound (70%). -(b) Zn, AcOH (85%). -(c) H_2O_2 , AcOH, H_2O (95%). -(d) 1) LDA, THF; 2) PhSeBr (90%). -(e) H_2O_2 , AcOH, THF (60%). -(f) MCPBA, CH_2Cl_2 (71% of (\pm)-**13**; 13% of (\pm)-**14**). -(g) 0.05 eq. K_2CO_3 , MeOH (78%). -(h) PCC, NaOAc, MS 4A, CH_2Cl_2 (81%). -(i) NaBH_4 , CeCl_3 , EtOH (79%). -(j) 1.5 eq. 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide, 2.1 eq. Ag_2CO_3 , 0.5 eq. AgOTf, CH_2Cl_2 ; then flash chromatog. over SiO_2 and gel filtration through Bio-beads S x 3 (65% of (\pm)-**16**; 15.0% of **17**; 15.6% of **18**). -(k) 0.1 eq. KCN, MeOH; then filtration through RP-18 and HPLC purification over Cosmosil 5C18AR by elution with MeOH- H_2O / 1:9 (52% each of **1** and **19** and 22% each of **20** and **21**).

The first step of our synthesis was the ultrasound-promoted cycloaddition⁷ of dichloroketene to **7**. The resulting dichloroketene (\pm)-**8** was reduced with zinc and acetic acid⁹ to give (\pm)-bicyclo[4.2.0]oct-3-en-7-one (**9**), whose Baeyer-Villiger oxidation⁹ afforded the lactone (\pm)-**10**. Phenylselenation of (\pm)-**10** to (\pm)-**11** was

followed by its oxidation with hydrogen peroxide to furnish (\pm)-**12**¹⁰. Oxidation of (\pm)-**12** with *m*-chloroperbenzoic acid (MCPBA) yielded a mixture of a crystalline and oily epoxides in 71 and 13% yield, respectively. The major and crystalline isomer was identified as (\pm)-**13**, because its treatment with potassium carbonate¹¹ gave (\pm)-aquilegionolide (**4**), whose ¹H- and ¹³C-NMR spectral data were in good accord with those reported for the natural **4**^{4,5}. In order to invert the configuration at C-6, the alcohol (\pm)-**4** was oxidized to (\pm)-**15**, which was reduced with sodium borohydride in the presence of cerium (III) chloride¹² to afford (\pm)-menisdaurilide (**3**), whose ¹H- and ¹³C-NMR spectral data were in accord with those reported for the natural **3**^{3,5}.

After some experimentation, Koenigs-Knorr glucosidation of (\pm)-**3** with 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide was achieved by using silver carbonate and silver triflate as the catalysts¹³. Although the major product of this reaction turned out to be (\pm)-**16** (65% yield)¹⁴, the two diastereoisomeric glucosides could be secured in 15.0 and 15.6% yield, respectively. Fortunately, one of the glucosides obtained in 15.6% yield was crystalline (m.p. 176.5-177.5 °C), and its structure could be solved by X-ray analysis¹⁵. Its computer-generated stereoview is shown in Fig. 3. This crystalline tetraacetate was thus (*6R*, *7aS*)-**18**.

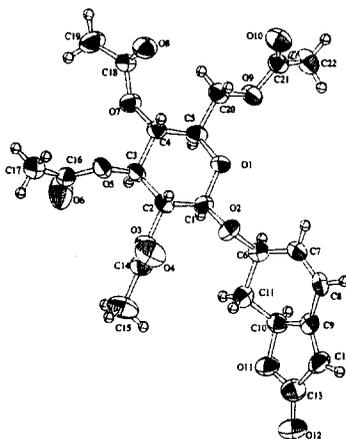


Figure 3. Perspective view of **18**.

The tetraacetates **17** and **18** were converted to the free glucosides **1** and **19**, respectively, by treatment with potassium cyanide in methanol^{16,17}. Although the removal of the acetyl protective groups of **17** was executed under such mild conditions, the yield of **1** was only 52% accompanied by 22% yield of the diastereoisomeric glucoside **20**. The ¹H- and ¹³C-NMR spectra of our synthetic **1**¹⁸ coincided with the authentic spectra of phyllanthurinolactone (**1**) sent to us by Prof. Yamamura. The overall yield of **1** was 0.84% based on **7** (11 steps). Similarly, **18** afforded 52% of **19**¹⁹ and 22% of **21**. It should be noted that the ease of epimerization of **3** to **4** was reported previously⁴. Both the glucosides **1** and **19** were kindly bioassayed by Prof. Yamamura. Only **1** was bioactive at the concentration of 10⁻⁴ g/l, while **19** was totally inactive even at 10⁻³ g/ml. Accordingly, phyllanthurinolactone must be (*6S*, *7aR*)-**1**. Full details of this work will appear in *Bull. Soc. Chim. France*.

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- X-ray analysis of **18**: Crystal size, 0.2 x 0.3 x 0.3 mm. All data were obtained on Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo $K\alpha$ radiation. Crystal data: $\text{C}_{22}\text{H}_{26}\text{O}_{12}$, $M_r = 482.44$, orthorhombic, space group $P2_12_12_1$, $a = 15.954(4)$ Å, $b = 20.133(4)$ Å, $c = 7.174$ (3) Å, $V = 2304(1)$ Å³, $Z = 4$, $D_x = 1.391$ g/cm³, $F(000) = 1016$, and $\mu(\text{Mo}K\alpha) = 1.142$ cm⁻¹. Of the 1789 independent reflections collected, 1071 reflections with $I > 3.0\sigma(I)$ were used for the structure determination. The final refinement converged with $R = 0.042$ and $R_w = 0.041$ for 307 parameters. Atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.
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- Properties of synthetic **1**: amorphous solid; $[\alpha]_D^{25} = -38.3$ ($c = 1.1$, H_2O) [ref.² $[\alpha]_D^{21} = -6.0$ ($c = 0.20$, H_2O)]; $^1\text{H-NMR}$ (270 MHz, D_2O): $\delta = 1.77$ (q, $J = 10.6$ Hz, 1H), 3.04 (dt, $J = 10.6$ and 5.3 Hz, 1H), 3.27 (t, $J = 8.2$ Hz, 1H), 3.34-3.50 (m, 2H), 3.50 (t, $J = 8.6$ Hz, 1H), 3.73 (dd, $J = 12.2$ and 5.6 Hz, 1H), 3.91 (d, $J = 12.2$ Hz, 1H), 4.69 (d, $J = 8.0$ Hz, 1H), 4.80-4.87 (m, 1H), 5.14 (dd, $J = 13.2$ and 4.6 Hz, 1H), 5.94 (s, 1H), 6.45 (d, $J = 9.9$ Hz, 1H), 6.74 (d, $J = 9.9$ Hz, 1H); $^{13}\text{C-NMR}$ (67.8 MHz, D_2O) $\delta = 39.2, 62.3, 71.1, 74.6, 76.1, 77.3, 77.6, 80.9, 103.5, 112.0, 122.3, 142.3, 167.0, 178.5$. HR FABMS (positive): $[\text{M}+\text{H}]^+$ Found m/z 315.107, $\text{C}_{14}\text{H}_{19}\text{O}_8$ requires m/z 315.108.
- Properties of synthetic **19**: amorphous solid; $[\alpha]_D^{25} = +9.3$ ($c = 0.8$, H_2O); $^1\text{H-NMR}$ (270 MHz, D_2O): $\delta = 1.74$ (q, $J = 10.6$ Hz, 1H), 3.04 (dt, $J = 10.6$ and 5.3 Hz, 1H), 3.27 (t, $J = 8.2$ Hz, 1H), 3.36-3.47 (m, 2H), 3.47 (t, $J = 9.2$ Hz, 1H), 3.71 (dd, $J = 12.2$ and 6.4 Hz, 1H), 3.91 (d, $J = 12.2$ Hz, 1H), 4.66 (d, $J = 8.2$ Hz, 1H), 4.83-4.89 (m, 1H), 5.13 (dd, $J = 13.5$ and 5.0 Hz, 1H), 5.95 (s, 1H), 6.46 (d, $J = 10.0$ Hz, 1H), 6.74 (d, $J = 10.0$ Hz, 1H); $^{13}\text{C-NMR}$ (67.8 MHz, D_2O) $\delta = 37.8, 62.2, 71.1, 74.5, 75.6, 77.2, 77.6, 80.9, 102.8, 112.0, 122.4, 143.4, 167.1, 178.5$. HR FABMS (positive): $[\text{M}+\text{H}]^+$ Found m/z 315.107, $\text{C}_{14}\text{H}_{19}\text{O}_8$ requires m/z 315.108.