Plant Bioregulators, 1

# Synthesis of Phyllanthurinolactone, the Leaf-Closing Factor of *Phyllanthus urinaria* L., and Its Three Stereoisomers

**Gérard Audran and Kenji Mori***

Department of Chemistry, Faculty of Science, Science University of Tokyo,
Kagurazaka 1-3, Shinjuku-ku, Tokyo 162, Japan
Fax: (internat.) +81-3/3235-2214

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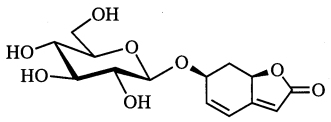
Phyllanthurinolactone (**1**) and its three stereoisomers **18**−**20** were synthesized, and only **1** was bioactive as the leaf-closing factor of a nyctinastic plant, *Phyllanthus urinaria* L. X-ray analysis of the tetraacetylglucoside **17** was executed, and the absolute configuration of **1** was determined as 6*S*,7a*R*.

The phenomenon of nyctinasty or "plant sleep" has been recorded since the ancient time of Alexander the Great[1]. For example, the pinnate leaves of a large tamarind tree (*Tamarindus indica* L.) fold together at night as if the tree sleeps[1]. In 1995 Yamamura and his coworkers isolated 3.1 mg of phyllanthurinolactone (**1**, Scheme 1) from 19.2 kg of the fresh nyctinastic plant *Phyllanthus urinaria* L. as its leaf-closing factor[2]. It was bioactive only for that plant in the daytime at a very low concentration of $1 \times 10^{-7}$ M. They proposed the structure **1** for phyllanthurinolactone, although the absolute configuration of the aglycone part remained unknown[2].

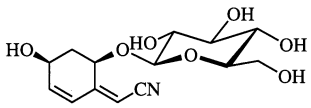
There are some reports on the isolation and identification of plant constituents with structures (**2**−**4**) related to **1** (Scheme 1). In 1978 Takahashi et al. isolated menisdaurin (**2**) from the vines of *Menispermum dauricum*, and acid hydrolysis of **2** afforded menisdaurilide (**3**)[3]. 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 X-ray analysis of its *p*-bromobenzoate[5]. 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 6*S*,7a*R* like that of **3**. In order to prove or disprove this hypothesis, we undertook the synthesis of (6*S*,7a*R*)-**1** and its stereoisomers. This paper describes the full details of the synthesis, which was reported as a preliminary communication[6].

Although Majewski et al. reported the enantioselective synthesis of *ent*-dihydromenisdaurilide (**5**) and *ent*-dihydroaquilegiolide (**6**)[7], we chose a different route to achieve the synthesis of **1** as summarized in Schemes 2, 3 and 4. Our plan was to prepare and resolve (±)-menisdaurilide (**3**) by employing D-glucose as a resolving agent to separate the two diastereoisomeric glucosides **16** and **17**. Subsequent deprotection of **16** would give **1**, while that of **17** would afford **19**. Either **1** or **19** must be the natural phyllanthurinolactone. The absolute configuration of the two diastereoisomers **16** and **17** was expected to be determined by some appropriate means. The known unsaturated ketone (±)-**7**, readily prepared from 1,4-cyclohexadiene[8][9], was subjected to the Baeyer-Villiger oxidation[10] to give the lactone (±)-**8**. Phenylselenation[11] of (±)-**8** to (±)-**9** was followed by its oxidation with hydrogen peroxide to furnish (±)-**10**. Oxidation of (±)-**10** with *m*-chloroperbenzoic acid

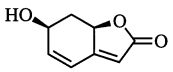
Scheme 1. Structures of phyllanthurinolactone (**1**) and related compounds



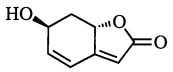
Phyllanthurinolactone (**1**)



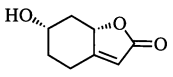
Menisdaurin (**2**)



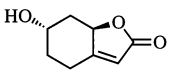
Menisdaurilide (**3**)



Aquilegiolide (**4**)



**5**



**6**

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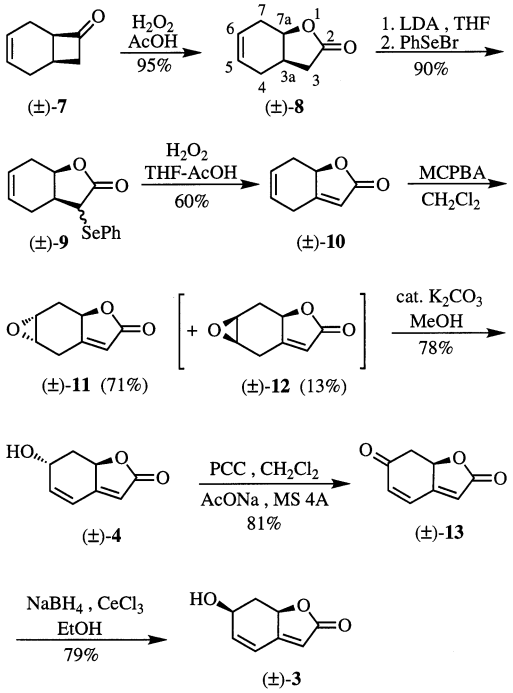
(MCPBA) yielded a mixture of a crystalline and an oily epoxides in 71 and 13% yield, respectively. The major and crystalline isomer was identified as (±)-**11**, because its treatment with potassium carbonate[12] gave (±)-aquilegiolide (**4**), whose $^1$H- and $^{13}$C-NMR spectral data were in good accord with those reported for the natural **4**[5]. In order to invert the configuration at C-6, the alcohol (±)-**4** was oxidized to (±)-**13**, which was reduced with sodium borohydride in the presence of cerium(III) chloride[13] to afford (±)-menisdaurilide (**3**), whose $^1$H- and $^{13}$C-NMR spectral data were in accord with those reported for the natural **3**[3][5]. Treatment of the unstable epoxide (±)-**12** with potassium carbonate in methanol afforded an additional amount of crude (±)-**3**.

Scheme 2. Synthesis of (±)-menisdaurilide (**3**), the aglycone part




After some experimentation, Koenigs-Knorr glucosidation of (±)-**3** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**14**) was achieved as shown in Scheme 3 by employing silver carbonate and silver triflate as the catalysts[14]. Although the major product of this reaction turned out to be (±)-**15** (65.0% yield), the two diastereoisomeric glucosides could be secured in 15.0 and 15.6% yield, respectively. In other words, (±)-**3** was resolved. It should be added that the similar acetylation of the sugar acceptor with acetobromo-D-glucose was noticed previously in the course of another silver triflate catalyzed Koenigs-Knorr reaction[15]. The imidate method of Schmidt[16] did not improve the yield of this glucosidation step.

Fortunately, one of the glucosides obtained in 15.6% yield was crystalline, and its structure could be solved by X-ray analysis. Its computer-generated perspective view is shown in Figure 1. This crystalline tetraacetate was thus (6*R*,7a*S*)-**17**.

Scheme 3. Glucosidation of (±)-**3**

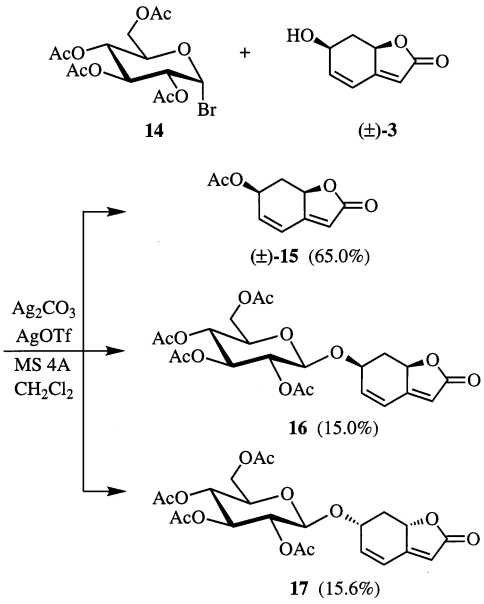
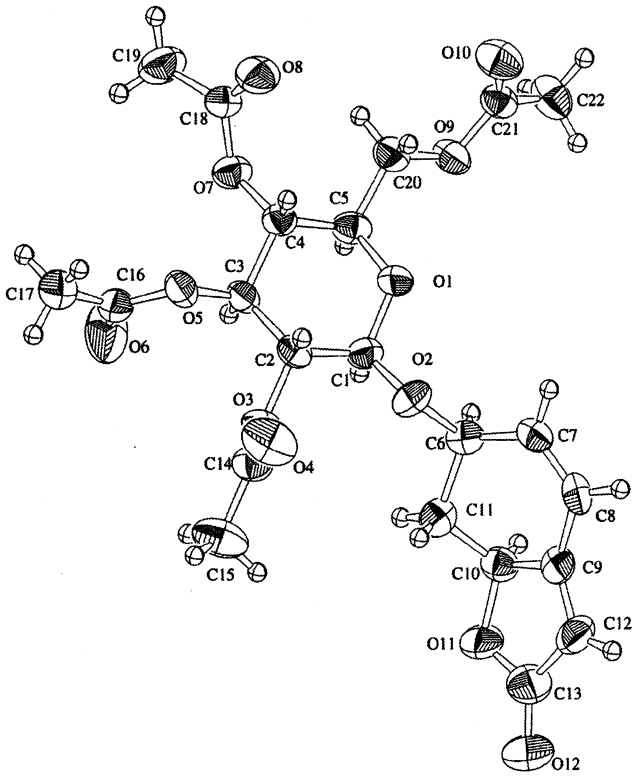


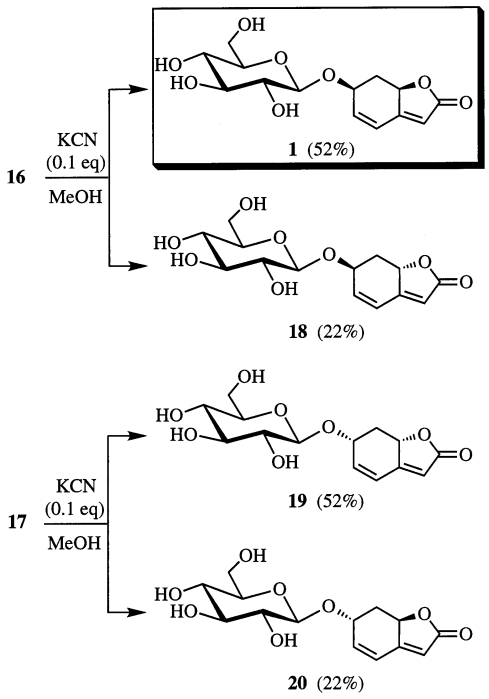

Figure 1. Perspective view of **17**; displacement elipsoids are drawn at the 50% probability level for non-H atoms




As summarized in Scheme 4, the tetraacetates **16** and **17** were converted to the free glucosides **1** and **19**, respectively, by treatment with potassium cyanide in methanol[17][18]. Conventional deacetylation procedures under more basic conditions resulted in decomposition of the deacetylation product. Even under such mild and weakly basic conditions for the removal of the acetyl groups of **16**, the yield of **1**

was only 52% accompanied by 22% yield of the diastereoisomeric glucoside **18**. Epimerization at C-7a of **16** or **1** under the weakly basic conditions was not unexpected because the ease of epimerization of menisdaurilide (**3**) to aquilegiolide (**4**) had been reported[4] and the cooccurrence of **3** and **4** in *Sinomenium acutum* had also been observed[5]. Indeed, the hydrogen atom at C-7a of **1** could be replaced with deuterium as shown by the disappearance of its $^1$H-NMR signal at $\delta = 5.14$ when **16** was treated with potassium cyanide in [$D_4$]methanol. Similarly, the tetraacetate **17** afforded 52% of **19** and 22% yield of **20**. The $^1$H- and $^{13}$C-NMR spectra of our synthetic **1** coincided with authentic spectra of phyllanthurinolactone (**1**). The overall yield of **1** was 0.84% based on 1,4-cyclohexadiene (11 steps) or 1.41% based on (±)-**9** (9 steps).

Scheme 4. Preparation of phyllanthurinolactone (**1**) and its stereoisomers **18**−**20**




The leaf-closing activity of our synthetic glucosides **1**, **18**, **19** and **20** was bioassayed employing the leaves of *Phyllanthus urinaria* L. Only phyllanthurinolactone (**1**) was bioactive at concentrations of $10^{-3}$, $10^{-4}$, and $10^{-5}$ g/l, while **18**, **19** and **20** were totally inactive even at concentrations of $10^{-3}-10^{-2}$ g/l.

In conclusion, phyllanthurinolactone, the leaf-closing factor of a nyctinastic plant *Phyllanthus urinaria* L., was synthesized from (±)-**7**, and its structure was establised as (6*S*,7a*R*)-**1**. The chemical signal for the leaf-closing movement of *Phyllanthus urinaria* L. is the lactonic glucoside **1**, and its stereoisomers so far examined are biologically inactive. The present study adds another example to illustrate the importance of chirality in biological recognition[19]. The racemates of menisdaurilide (**3**) and aquilegiolide (**4**) were synthesized as synthetic intermediates.

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## Experimental Section

*General:* For analytical thin-layer chromatography, Merck silica gel F-254 on glass was used. Column chromatography was performed with Merck silica gel 60 art 7734 (70−230 mesh). − All b.p.s and m.p.s are uncorrected values. − Infrared spectra were recorded with a Hitachi Perkin-Elmer 1600. − NMR spectra were recorded at 270 MHz for $^1$H and 67.8 MHz for $^{13}$C with a Jeol JNM-EX 270L spectrometer using $CDCl_3$ or $D_2O$ as solvents. Chemical shifts (δ) are relative to TMS ($\delta = 0.00$) in the case of $CDCl_3$ and to *t*BuOH ($^1$H: $\delta = 1.23$ and $^{13}$C: $\delta = 32.7$) in the case of $D_2O$ as internal standards. − Optical rotations were measured with a Jasco DIP-1000.

*(±)-3aα,4,7,7aα-Tetrahydrobenzofuran-2(3H)-one* (**8**): To a stirred solution of (±)-**7** (8.00 g, 65.5 mmol) in a mixture of 200 ml of acetic acid/water (9:1) at 5°C was added dropwise a 34% hydrogen peroxide solution (9.2 g, 92 mmol). The homogeneous mixture was stirred at 5°C for 14 h, then poured into water (200 ml) and extracted with $CH_2Cl_2$ (600 ml). The organic layer was washed several times with saturated $NaHCO_3$ solution (until the acid had been neutralized), and brine (100 ml), dried with $MgSO_4$ and concentrated. The residue was distilled to afford 8.55 g (95%) of (±)-**8**, yellow oil, b.p. 115°C/4 Torr (ref.[20] b.p. 80°C/0.2 Torr). − IR (neat): $\tilde{\nu}_{max} = 3040$ cm$^{-1}$ (C−H), 1775 (C=O), 1625 (C=C). − $^1$H NMR (270 MHz, $CDCl_3$): $\delta = 1.90-2.75$ (m, 7 H, 3-H, 3a-H, 4-H and 7-H), 4.71 (q, $J = 4.6$ Hz, 1 H, 7a-H), 5.65−5.77 (m, 2 H, 5-H and 6-H). − $^{13}$C NMR (67.8 MHz, $CDCl_3$): $\delta = 26.1$, 27.4 and 36.7 (3-C, 4-C and 7-C), 32.1 (3a-C), 77.7 (7a-C), 124.0 and 125.7 (5-C and 6-C), 177.0 (2-C). − $C_8H_{10}O_2$ (138.2): calcd. C 69.55, H 7.30; found C 69.22, H 7.31.

*(±)-7,7a-Dihydrobenzofuran-2(4H)-one* (**10**) *via (±)-3-(Phenylselenyl)-3aα,4,7,7aα-tetrahydrobenzofuran-2(3H)-one* (**9**): A solution of (±)-**8** (6.31 g, 45.5 mmol) in THF (20 ml) was added to a solution of LDA (1.3 eq.) in THF (100 ml) at −78°C. The mixture was stirred at −78°C for 1 h, then a solution of PhSeBr (13.4 g, 56.6 mmol) in THF (40 ml) was added. After 1 h, the reaction mixture was slowly allowed to reach room temp. and hydrolyzed with 1 N HCl solution (30 ml). The ethereal solution was washed with water, saturated $NaHCO_3$ solution, brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ether gradient) to give 12.0 g (90%) of (±)-**9** as a red oil. To an ice-cooled and stirred solution of (±)-**9** (12.0 g; 41.0 mmol) and AcOH (2 ml) in THF (300 ml), a 34% $H_2O_2$ solution (18.0 ml, 180 mmol) was added at 4°C. After 2 h, a saturated $NaHCO_3$ solution (330 ml) was added and the mixture was stirred at room temp. for 1 h. The solution was diluted with diethyl ether and the ethereal layer was washed with saturated $NaHCO_3$ solution, brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ether, 2:3) to give 3.37 g (60%) of (±)-**10**. − IR (neat): $\tilde{\nu}_{max} = 3090$

$cm^{-1}$ (C−H), 3024 (C−H), 1750 (C=O), 1641 (C=C). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 2.10−2.25 (m, 1 H, 7-H), 2.85−3.00 (m, 1 H, 7-H), 3.20 and 3.36 (AB, *J* = 20.5 Hz, 2 H, 4-H), 4.99 (t, *J* = 8.0 Hz, 1 H, 7a-H), 5.70−5.80 (m, 2 H, 5-H and 6-H), 5.85 (s, 1 H, 3-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 28.2 and 32.6 (4-C and 7-C), 78.4 (7a-C), 113.3 (3-C), 123.0 and 123.7 (5-C and 6-C), 168.2 (3a-C), 173.6 (2-C). − $C_8H_8O_2$ (136.2): calcd. C 70.58, H 5.92; found C 70.03, H 5.85. This compound did not give correct combustion analytical data due to its extreme instability.

*(±)-5α,6α-Epoxy-5,6,7,7aα-tetrahydrobenzofuran-2(4H)-one* (**11**) *and (±)-5β,6β-Epoxy-5,6,7,7aα-tetrahydrobenzofuran-2(4H)-one* (**12**): To a stirred solution of *m*-chloroperoxybenzoic acid (7.18 g, 33.2 mmol) in $CH_2Cl_2$ (80 ml) was added at 0°C the lactone (±)-**10** (3.00 g, 22.0 mmol) in $CH_2Cl_2$ (30 ml). The solution was stirred for 24 h at room temp., then diluted with $CH_2Cl_2$ (100 ml). The organic solution was washed with $Na_2SO_3$ solution (50 ml), saturated $NaHCO_3$ solution (50 ml) and brine (50 ml), then dried with $MgSO_4$ and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (hexane/ether gradient) to give 2.38 g (71%) of *trans*-epoxide (±)-**11** and 0.44 g (13%) of *cis*-epoxide (±)-**12**.

*trans-Epoxide* (±)-**11**: Recrystallization from MeOH afforded colorless needles, m.p. 128−130°C. − IR (KBr): $\tilde{\nu}_{max}$ = 3019 $cm^{-1}$ (C−H), 1745 (C=O), 1644 (C=C), 1295 (C−O), 1033 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.73 (dd, *J* = 14.2 and 10.5 Hz, 1 H, 7-H), 3.05 (ddd, *J* = 14.2, 6.7 and 2.7 Hz, 1 H, 7-H), 3.15−3.25 (m, 2 H, 4-H), 3.28−3.33 (m, 1 H, 5-H or 6-H), 3.35−3.45 (m, 1 H, 5-H or 6-H), 5.05 (dd, *J* = 9.9 and 7.9 Hz, 1 H, 7a-H), 5.81 (s, 1 H, 3-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 26.7 and 30.3 (4-C and 7-C), 50.4 and 52.9 (5-C and 6-C), 78.0 (7a-C), 114.8 (3-C), 165.7 (3a-C), 172.4 (2-C). − $C_8H_8O_3$ (152.1): calcd. C 63.15, H 5.30; found C 63.07, H 5.23.

*cis-Epoxide* (±)-**12**: IR (neat): $\tilde{\nu}_{max}$ = 3010 $cm^{-1}$ (C−H), 1750 (C=O), 1640 (C=C), 1020 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.96 (dd, *J* = 13.0 and 7.0 Hz, 1 H, 7-H), 2.80−2.90 (m, 2 H, 4-H and 7-H), 3.15−3.45 (m, 3 H, 4-H, 5-H and 6-H), 4.82 (t, *J* = 9.5 Hz, 1 H, 7a-H), 5.92 (s, 1 H, 3a-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 27.5 and 32.4 (4-C and 7-C), 49.5 and 53.3 (5-C and 6-C), 77.6 (7a-C), 117.1 (3-C), 166.7 (3a-C), 172.6 (2-C). − $C_8H_8O_3$ (152.1): calcd C 63.15, H 5.30; found C 62.27; H 5.43. − HR FAB-MS (positive); *m/z*: 153.0555 $[M + H]^+$ (calcd. 153.0552 for $C_8H_9O_3$). − This *cis*-epoxide was unstable and did not give correct combustion analytical data.

*(±)-6a-Hydroxy-7,7aα-dihydrobenzofuran-2(4H)-one* (**4**): To a solution of (±)-**11** (2.30 g, 15.1 mmol) in anhydrous MeOH (150 ml) was added at 4°C anhydrous $K_2CO_3$ (104 mg; 0.75 mmol). The solution was stirred at 4°C for 0.5 h, then an excess of $NH_4Cl$ was added. The solvent was removed under reduced pressure and the crude product was suspended in $CH_2Cl_2$ (50 ml), the mixture filtered and concentrated. The residue was subjected to column chromatography on silica gel (hexane/AcOEt, 1:2) to give 1.79 g (78%) of (±)-aquilegiolide **4**. Recrystallization from benzene afforded colorless needles, m.p. 85−87°C, (ref.[5] for the natural enantiomer m.p. 95−97°C). − IR (KBr): $\tilde{\nu}_{max}$ = 3406 $cm^{-1}$ (O−H), 3082 (C−H), 1725 (C=O), 1643 (C=C), 1030 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.80 (dt, *J* = 12.5 and 4.0 Hz, 1 H, 7-H), 1.92 (d, *J* = 4.3 Hz, 1 H, OH), 2.65 (dd, *J* = 12.5 and 4.7 Hz, 1 H, 7-H), 4.66 (m, 1 H, 6-H), 5.30 (ddd, *J* = 12.5, 4.7 and 1.6 Hz, 1 H, 7a-H), 5.83 (s, 1 H, 3-H), 6.31 (dd, *J* = 9.9 and 5.3 Hz, 1 H, 5-H), 6.62 (d, *J* = 9.9 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 37.1 (7-C), 64.2 (6-C), 76.5 (7a-C), 112.1 (4-C), 121.7 (5-C), 137.8 (3-C), 163.1 (3a-C), 173.8 (2-C). − The $^1H$- and $^{13}C$-NMR data taken of a $[D_4]$methanol solution were identical with those reported for natural **4**[5]. − $C_8H_8O_3$ (152.1): calcd. C 63.15, H 5.30; found C 62.99, H 5.22.

*(±)-6-Oxo-7,7a-dihydrobenzofuran-2(4H)-one* (**13**): To a mixture of anhydrous sodium acetate (1.73 g, 21.0 mmol), 4-Å molecular sieves (1.5 g), and (±)-**4** (1.60 g, 10.5 mmol) in $CH_2Cl_2$ (70 ml) was added portionwise pyridinium chlorochromate (2.91 g, 13.5 mmol). The mixture was stirred at room temp. for 2 h and filtered through a pad of florisil. The filtrate was washed with 1 N HCl (50 ml), saturated $NaHCO_3$ solution (50 ml) and brine (50 ml). The organic phase was dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt, 2:3) to afford 1.28 g (81%) of (±)-**13**. Recrystallization from MeOH afforded yellow plates, m.p. 104−106°C. − IR (KBr): $\tilde{\nu}_{max}$ = 1745 $cm^{-1}$ (C=O), 1679 (C=C), 1641 (C=C), 1020 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 2.61 (dd, *J* = 15.5 and 12.2 Hz, 1 H, 7-H), 3.39 (dd, *J* = 15.5 and 6.3 Hz, 1 H, 7-H), 5.30 (ddd, *J* = 12.2, 6.3 and 2.0 Hz, 1 H, 7a-H), 6.22 (s, 1 H, 3-H), 6.35 (d, *J* = 9.9 Hz, 1 H, 4-H or 5-H), 7.53 (d, *J* = 9.9 Hz, 1 H, 4-H or 5-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 46.0 (7-C), 78.6 (7a-C), 118.2 (3-C), 135.4 and 136.0 (4-C and 5-C), 159.7 (3a-C), 172.2 (2-C), 194.6 (6-C). − $C_8H_6O_3$ (150.1): calcd. C 64.00, H 4.03; found C 63.88, H 4.17.

*(±)-6β-Hydroxy-7,7aα-dihydrobenzofuran-2(4H)-one* (**3**): A solution of (±)-**13** (1.00 g, 6.67 mmol) and $CeCl_3 \cdot 7\ H_2O$ (2.50 g, 6.67 mmol) in absolute EtOH (70 ml) was stirred at room temp. for 1 h. The solution was cooled to 5°C and treated with $NaBH_4$ (268 mg, 7.1 mmol) in one portion. The reaction mixture was stirred for an additional 0.5 h before concentrating it under reduced pressure to a residue which was partitioned between 80 ml of pH = 7 phosphate buffer and 80 ml of $CH_2Cl_2$. The aqueous layer was extracted with $CH_2Cl_2$ (5 × 50 ml) and the combined extracts were dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt, 1:2) to afford 801 mg (79%) of (±)-menisdaurilide (**3**). Recrystallization from benzene afforded colorless needles, m.p. 105−107°C (ref.[5] for the natural **3** m.p. 109−111°C). − IR (KBr): $\tilde{\nu}_{max}$ = 3390 $cm^{-1}$ (O−H), 3092 (C−H), 1723 (C=O), 1636 (C=C), 1010 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.70 (dt, *J* = 13.4 and 11.0 Hz, 1 H, 7-H), 2.10 (d, *J* = 6.9 Hz, 1 H, OH), 2.88 (dt, *J* = 11.0 and 5.5 Hz, 1 H, 7-H), 4.60−4.70 (m, 1 H, 6-H), 4.90 (ddd, *J* = 13.4, 5.5 and 1.8 Hz, 1 H, 7a-H), 5.76 (s, 1 H, 3-H), 6.33 (d, *J* = 10.1 Hz, 1 H, 4-H), 6.55 (dd, *J* = 10.1 and 2.0 Hz, 1 H, 5-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 40.5 (7-C), 67.2 (6-C), 79.1 (7a-C), 111.7 (4-C), 120.3 (5-C), 145.0 (3-C), 164.3 (3a-C), 174.7 (2-C). − These spectral data were identical with those reported for the natural **3**[3][5]. − $C_8H_8O_3$ (152.1): calcd. C 63.15, H 5.30; found C 63.24, H 5.27.

The same procedure described for the synthesis of (±)-**4** has been conducted with (±)-**12** giving crude (±)-**3** in 75% yield. This sample contained ca. 20% of (±)-**4** and ca. 20% of an unidentified compound and could not be purified at this step.

*(±)-6β-Acetyloxy-7,7aα-dihydrobenzofuran-2(4H)-one* (**15**), *(−)-(6S,7aR)-6-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)oxy-7,7a-dihydrobenzofuran-2(4H)-one* (**16**) *and (+)-(6R,7aS)-6-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)oxy-7,7a-dihydrobenzofuran-2(4H)-one* (**17**): To a mixture of (±)-**3** (400 mg, 2.63 mmol), **14** (1.62 g, 3.95 mmol) and 4-Å molecular sieves (1.0 g) in $CH_2Cl_2$ (25 ml) were added in one portion $Ag_2CO_3$ (1.54 g, 5.59 mmol) and AgOTf (338 mg, 1.32 mmol). The mixture was stirred at room temp. for 2 h, then filtered through a pad of Celite.

The filtrate was extracted with saturated $NaHCO_3$ solution (20 ml) and brine (20 ml). The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography on silica gel (hexane/AcOEt gradient) permitted to separate the two diastereoisomeric glucosides (**16** and **17**) and 332 mg (65.0%) of the acetylated aglycone (±)-**15**. Final purification by gel filtration through a Bio-beads SX3 column for each glucoside (hexane/AcOEt,1:1) afforded 190 mg (15.0%) of **16** as a foam and 198 mg (15.6%) of **17** as a solid.

(±)-**15**: Recrystallization from a mixture of $iPr_2O/CH_2Cl_2$ (10:1) afforded colorless needles, m.p. 103−104°C. − IR (KBr): $\tilde{\nu}_{max}$ = 3104 $cm^{-1}$ (C−H), 1736 (C=O), 1639 (C=C), 1010 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.76 (dt, *J* = 13.5 and 10.8 Hz, 1 H, 7-H), 2.12 (s, 3 H, Me), 2.94 (dt, *J* = 5.4 Hz, 1 H, 7-H), 4.96 (ddd, *J* = 13.5, 5.4 and 2.0 Hz, 1 H, 7a-H), 5.67 (ddd, *J* = 10.2, 5.4 and 2.3 Hz, 1 H, 6-H), 5.88 (s, 1 H, 3-H), 6.19 (d, *J* = 9.9 Hz, 1 H, 5-H), 6.67 (dd, *J* = 9.9 and 2.3 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 21.6 (Me), 36.8 (7-C), 68.6 (6-C), 78.0 (7a-C), 112.9 (4-C), 122.1 (5-C), 139.4 (3-C), 162.6 (3a-C), 170.7 and 173.5 (CO). − $C_{10}H_{10}O_4$ (194.2): calcd. C 61.85, H 5.19; found C 61.67, H 5.11.

**16**: $[\alpha]_D^{25}$ = −14.2 (*c* = 0.80, $CHCl_3$). − IR (KBr): $\tilde{\nu}_{max}$ = 1746 $cm^{-1}$ (C=O), 1646 (C=C), 1190 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.79 (q, *J* = 11.0 Hz, 1 H, 7-H), 2.05 (s, 3 H, Me), 2.07 (s, 3 H, Me), 2.09 (s, 3 H, Me), 2.13 (s, 3 H, Me), 3.00 (dt, *J* = 11.0 and 5.5 Hz, 1 H, 7-H), 3.71−3.80 (m, 1 H, 5′-H), 4.18 and 4.29 (ABX, *J* = 12.2, 5.0 and 2.3 Hz, 2 H, 6′-H), 4.59−4.64 (m, 1 H, 6-H), 4.73 (d, *J* = 7.9 Hz, 1 H, 1′-H), 4.85 (ddd, *J* = 13.2, 4.6 and 1.0 Hz, 1 H, 7a-H), 5.00 (t, *J* = 7.9 Hz, 1 H, 2′-H), 5.09 (t, *J* = 9.9 Hz, 1 H, 4′-H), 5.22 (t, *J* = 9.6 Hz, 1 H, 3′-H), 5.85 (s, 1 H, 3-H), 6.21 (d, *J* = 9.9 Hz, 1 H, 5-H), 6.53 (dd, *J* = 9.9 and 2.3 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 20.4 (2 × Me), 20.5 (Me), 20.6 (Me), 38.1 (7-C), 61.7 (6′-C), 68.1 (4′-C), 71.0 (2′-C), 71.8 (5′-C), 72.4 (3′-C), 74.2 (6-C), 77.5 (7a-C), 100.0 (1′-C), 111.9 (3-C), 120.9 (4-C), 139.6 (5-C), 162.0, 169.0, 169.2, 170.0, 170.4 and 172.8 (3a-C and 5 × CO). − $C_{22}H_{26}O_{12}$ (482.4): calcd. C 54.77, H 5.43; found C 54.42, H 5.59.

Compound **17** was recrystallized from MeOH to afford colorless needles, and its structure was confirmed by X-ray analysis. − M.p. 176.5−177.5°C. − $[\alpha]_D^{25}$ = +9.4 (*c* = 1.0, $CHCl_3$). − IR (KBr): $\tilde{\nu}_{max}$ = 1746 $cm^{-1}$ (C=O), 1645 (C=C), 1220 (C−O). − $^1H$ NMR (270 MHz, $CDCl_3$): δ = 1.61 (dt, *J* = 13.2 and 10.6 Hz, 1 H, 7-H), 1.94 (s, 3 H, Me), 1.97 (s, 6 H, 2 × Me), 2.02 (s, 3 H, Me), 2.84 (dt, *J* = 5.3 Hz, 1 H, 7-H), 3.64-3.70 (m, 1 H, 5′-H), 4.10 and 4.19 (ABX, *J* = 12.4, 5.0 and 2.3 Hz, 2 H, 6′-H), 4.52−4.57 (m, 1 H, 6-H), 4.64 (d, *J* = 7.9 Hz, 1 H, 1′-H), 4.79 (ddd, *J* = 13.2, 4.6 and 1.3 Hz, 1 H, 7a-H), 4.92 (t, *J* = 9.2 Hz, 1 H, 2′-H), 5.03 (t, *J* = 9.6 Hz, 1 H, 4′-H), 5.15 (t, *J* = 9.6 Hz, 1 H, 3′-H), 5.77 (s, 1 H, 3-H), 6.29 (d, *J* = 9.9 Hz, 1 H, 5-H), 6.53 (dd, *J* = 9.9 and 2.3 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $CDCl_3$): δ = 20.5 (Me), 20.6 (3 × Me), 36.7 (7-C), 61.7 (6′-C), 68.1 (4′-C), 71.0 (2′-C), 71.9 (5′-C), 72.4 (3′-C), 73.8 (6-C), 77.1 (7a-C), 99.7 (1′-C), 111.7 (3-C), 120.7 (4-C), 141.3 (5-C), 162.2, 169.1, 169.3, 170.1, 170.5 and 172.8 (3a-C and 5 × CO). − $C_{22}H_{26}O_{12}$ (482.4): calcd. C 54.77, H 5.43; found C 54.69, H 5.57.

*(−)-(6S,7aR)-6-(β-D-Glucopyranosyl)-oxy-7,7a-dihydrobenzofuran-2(4H)-one (Phyllanthurinolactone 1) and (−)-(6S,7aS)-6-(β-D-Glucopyranosyloxy)-7,7a-dihydrobenzofuran-2(4H)-one* (**18**): To a stirred solution of **16** (50 mg, 0.10 mmol) in MeOH (2 ml) was added a 0.025 M solution of KCN in MeOH (400 ml, 0.01 mmol) and the mixture was stirred for 4 h at room temp. The homogeneous solution was filtered through an RP-18 glass beads column with $MeOH/H_2O$ (1:9). Final purification by HPLC using a Cosmosil $5C_{18}$-AR column with $MeOH/H_2O$ (1:9) gave 16.3 mg (52%) of **1** and 6.9 mg (22%) of **18**.

*Phyllanthurinolactone* **1**: $[\alpha]_D^{25}$ = −38.3 (*c* = 1.10, $H_2O$) ). − IR (KBr): $\tilde{\nu}_{max}$ = 3415 $cm^{-1}$ (OH), 1737 (C=O), 1639 (C=C), 1077 (C−O), 1039 (C−O). − $^1H$ NMR (270 MHz, $D_2O$): δ = 1.77 (q, *J* = 10.6 Hz, 1 H, 7-H), 3.04 (dt, *J* = 10.6 and 5.3 Hz, 1 H, 7-H), 3.27 (t, *J* = 8.2 Hz, 1 H, 2′-H), 3.34−3.50 (2 H, m, 4′-H and 5′-H), 3.50 (t, *J* = 8.6 Hz, 1 H, 3′-H), 3.73 and 3.91 (ABX, *J* = 12.2 and 5.6 Hz, 2 H, 6′-H), 4.69 (d, *J* = 8.0 Hz, 1 H, 1′-H), 4.80−4.87 (m, 1 H, 6-H), 5.14 (dd, *J* = 13.2 and 4.6 Hz, 1 H, 7a-H), 5.94 (s, 1 H, 3-H), 6.45 (d, *J* = 9.9 Hz, 1 H, 5-H), 6.74 (d, *J* = 9.9 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $D_2O$): δ = 40.8, 63.8, 72.6, 76.1, 77.6, 78.8, 79.1, 82.4, 105.0, 113.5, 123.8, 143.8, 168.6, 180.0. − HR FAB-MS (positive); *m/z*: 315.1066 $[M + H]^+$ (calcd. 315.1080 for $C_{14}H_{19}O_8$).

**18**: $[\alpha]_D^{25}$ = −314 (*c* = 0.20, $H_2O$). − IR (KBr): $\tilde{\nu}_{max}$ = 3441 $cm^{-1}$ (OH), 1743 (C=O), 1643 (C=C), 1078 (C−O), 1043 (C−O). − $^1H$ NMR (270 MHz, $D_2O$): δ = 1.90 (dt, *J* = 12.4 and 4.0 Hz, 1 H, 7-H), 2.81 (dd, *J* = 12.8 and 4.3 Hz, 1 H, 7-H), 3.26 (t, *J* = 8.5 Hz, 1 H, 2′-H), 3.42 (t, *J* = 9.2 Hz, 1 H, 4′-H), 3.46−3.51 (m, 1 H, 5′-H), 3.52 (t, *J* = 8.8 Hz, 1 H, 3-H), 3.73 and 3.93 (ABX, *J* = 12.6 and 5.3 Hz, 2 H, 6′-H), 4.65 (d, *J* = 7.6 Hz, 1 H, 1′-H), 4.77−4.84 (m, 1 H, 6-H), 5.41 (dd, *J* = 12.9 and 4.9 Hz, 1 H, 7a-H), 5.96 (s, 1 H, 3-H), 6.47 (dd, *J* = 9.6 and 5.4 Hz, 1 H, 5-H), 6.78 (d, *J* = 9.6 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $D_2O$): δ = 37.8, 63.8, 72.7, 75.4, 76.3, 78.8, 79.1, 81.0, 105.0, 114.8, 126.4, 138.3, 168.2, 180.0. − HR FAB-MS (positive); *m/z*: 315.1071 $[M + H]^+$ (calcd. 315.1080 for $C_{14}H_{19}O_8$).

The reaction performed in [$D_4$]methanol under the same conditions described above afforded two deuterated compounds at C-7a of **1** and **18**. The $^1H$-NMR spectra of deuterated **1** showed the disappearance of the signal attributed[2] to 7a-H at δ = 5.14 and the simplification of the signals due to the two methylene 7-H at δ = 1.77 and at δ = 3.04. The higher field signal became a triplet with a coupling constant of 10.6 Hz and the lower field signal became a doublet of doublets with coupling constants of 10.6 and 5.3 Hz.

*(+)-(6R,7aS)-6-(β-D-Glucopyranosyloxy)-7,7a-dihydrobenzofuran-2(4H)-one* (**19**) *and (+)-(6R,7aR)-6-(β-D-Glucopyranosyloxy)-7,7a-dihydrobenzofuran-2(4H)-one* (**20**): By using the same procedure as above with **17**, 52% of **19** and 22% of **20** were isolated.

**19**: $[\alpha]_D^{25}$ = +9.3 (*c* = 0.80, $H_2O$). − IR (KBr): $\tilde{\nu}_{max}$ = 3404 $cm^{-1}$ (OH), 1756 (C=O), 1640 (C=C), 1071 (C−O), 1046 (C−O). − $^1H$ NMR (270 MHz, $D_2O$): δ = 1.74 (q, *J* = 10,6 Hz, 1 H, 7-H), 3.04 (dt, *J* = 10.6 and 5.3 Hz, 1 H, 7-H), 3.27 (t, *J* = 8.2 Hz, 1 H, 2′-H), 3.36−3.47 (m, 2 H, 4′-H and 5′-H), 3.47 (t, *J* = 9.2 Hz, 1 H, 3′-H), 3.71 and 3.91 (ABX, *J* = 12.2 and 6.4 Hz, 2 H, 6′-H), 4.66 (d, 1 H, *J* = 8.2 Hz, 1′-H), 4.83−4.89 (m, 1 H, 6-H), 5.13 (dd, 1 H, *J* = 13.5 and 5.0 Hz, 7a-H), 5.95 (s, 1 H, 3-H), 6.46 (d, *J* = 10.0 Hz, 1 H, 5-H), 6.74 (d, *J* = 10.0 Hz, 1 H, 4-H). − $^{13}C$ NMR (67.8 MHz, $D_2O$): δ = 39.3, 63.8, 72.6, 76.1, 77.1, 78.7, 79.1, 82.4, 104.3, 113.5, 124.0, 144.9, 168.6, 180.1. − HR FAB-MS (positive); *m/z*: 315.1071 $[M + H]^+$ (calcd. 315.1080 for $C_{14}H_{19}O_8$).

**20**: $[\alpha]_D^{25}$ = +247 (*c* = 0.70, $H_2O$). − IR (KBr): $\tilde{\nu}_{max}$ = 3442 $cm^{-1}$ (OH), 1732 (C=O), 1634 (C=C), 1074 (C−O), 1046 (C−O). − $^1H$ NMR (270 MHz, $D_2O$): δ = 1.86 (dt, *J* = 12.8 and 4.3 Hz, 1 H, 7-H), 2.75 (dd, *J* = 12.8 and 5.9 Hz, 1 H, 7-H), 3.25 (t, *J* =

9.2 Hz, 1 H, 2′-H), 3.37 (t, $J$ = 9.2 Hz, 1 H, 4′-H), 3.46−3.51 (m, 1 H, 5′-H), 3.51 (t, $J$ = 8.9 Hz, 1 H, 3′-H), 3.70 and 3.91 (ABX, $J$ = 12.5 and 6.0 Hz, 2 H, 6′-H), 4.65 (d, $J$ = 7.9 Hz, 1 H, 1′-H), 4.70-4.78 (m, 1 H, 6-H), 5.40 (dd, $J$ = 12.5 and 4.3 Hz, 1 H, 7a-H), 5.96 (s, 1 H, 3-H), 6.41 (dd, $J$ = 9.9 and 5.6 Hz, 1 H, 5-H), 6.78 (d, $J$ = 9.9 Hz, 1 H, 4-H). − $^{13}$C NMR (67.8 MHz, $D_2O$): δ = 36.5, 63.8, 72.7, 75.2, 76.1, 78.8, 79.1, 80.9, 104.5, 114.8, 125.9, 139.4, 168.0, 180.0. − HR FAB-MS (positive); *m/z*: 315.1086 [M + H]$^+$ (calcd. 315.1080 for $C_{14}H_{19}O_8$).

*X-ray Crystal Structure of* (±)-**17**: Crystal size 0.2 × 0.3 × 0.3 mm. All data were obtained with a Rigaku AFC-5S automated four-circle diffractometer with graphite-monochromated Mo-$K_\alpha$ radiation. Final lattice parameters were obtained from a least-squares refinement using 25 reflections. Crystal data: $C_{22}H_{26}O_{12}$ (482.44); orthorhombic; space group $P2_12_12_1$; $a$ = 15.954(4), $b$ = 20.133(4), $c$ = 7.174 (3) Å; $V$ = 2304(1) Å$^3$; $Z$ = 4; $D_x$ = 1.391 g/cm$^3$; $F$(000) = 1016; μ(Mo-$K_\alpha$) = 1.142 cm$^{-1}$. The intensities were measured using $\omega/2\theta$ scans up to 45°. Three standard reflections were monitored every 150 measurements. The data were corrected for Lorentz and polarization factors. Decay and absorption correction were not applied. Of the 1789 independent reflections collected, 1071 reflections with $I > 3.0\sigma(I)$ were used for the structure determination and refinement. The structure was solved by direct methods using the TEXSAN crystallographic software package[21]. All non-H atoms were found in the Fourier map. All H atoms were calculated at geometrical positions and not refined. The refinement of atomic parameters was carried out by full-matrix least-squares refinement, using anisotropically temperature factors for all non-H atoms. The final refinement converged with $R$ = 0.042 and $Rw$ = 0.041 for 307 parameters. The minimum and maximum peaks in the final difference Fourrier map were −0.20 and 0.17 e Å$^{-3}$. Atomic scattering factors were taken from the International Tables for X-ray Crystallography[22]. The supplementary material includes the lists of atomic coordinates for the non-H atoms, the bond lengths and angles of **17** with their e.s.d.'s in parentheses[23].


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[23] Further details of the crystal structure investigation are deposited to the Cambridge Crystallographic Data Centre at the time of the publication of the preliminary communication[6] of this work as supplementary publication no. CCDC-100649. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: int. code + 44(1223)336-033, e-mail: deposit@ccdc.cam.ac.uk].


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