Isolation of Salicin Derivatives from *Homalium cochinchinensis* and Their Antiviral Activities[1]

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The chemical constituents of *Homalium cochinchinensis* were examined. From the root bark, in addition to the previously reported cochinolide and its β-glucopyranoside, cochinchiside A (**1**) and tremulacinol (**4**) were isolated together with three known compounds [benzoic acid, tremulacin (**2**), and tremuloidin (**3**)]. From the leaves, cochinchiside B (**5**) was isolated as new compound. The structures of the new compounds (**1**, **4**, **5**) were determined by spectroscopic and/or chemical methods. Antiviral testing of compounds **2**−**5** against HSV-1 and HSV-2 showed that tremulacin (**2**) and cochinchiside B (**5**) were weakly active. Tremulacin (**2**) was also weakly active against HIV-1.

Homalium cochinchinensis (Lour.) Druce (Flacourtiaceae) has been used in Taiwan as a folk medicine for gonorrhea and as an astringent.[2] We have investigated this plant previously to search for non-nucleoside lead compounds with antiviral activity from natural sources[3] and reported the isolation of cochinolide from the root bark as a new γ-alkylidene bicyclic butenolide with antiviral activity, along with its β-glucopyranoside.[1] In the present paper, we describe the isolation of further salicin derivatives from the root bark and the leaves of this plant, including three new compounds (**1**, **4**, **5**). Antiviral tests against herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) and human immunodeficiency virus type-1 (HIV-1) were performed on some of the isolated products.

Results and Discussion

The root bark, trunk bark, and leaves of *H. cochinchinensis* were subjected to separate extractions in a Soxhlet apparatus successively using hexane (Fr. A), either benzene (Fr. B) or ether (Fr. E), chloroform (Fr. C), and methanol (Fr. M). From the trunk bark, no additional components were obtained besides those found from the root bark and the leaves.

Initially, the root bark of *H. cochinchinensis* was examined and purified by a combination of column chromatography and preparative TLC. The extraction led to the isolation of seven components: cochinchiside A (**1**), tremulacin (**2**), tremuloidin (**3**), tremulacinol (**4**), benzoic acid, cochinolide, and cochinolide β-glucopyranoside.

Tremulacin (**2**) was isolated as a major constituent of the root bark of *H. cochinchinensis*. The IR spectrum showed absorption bands due to OH (3442 cm^{-1}) and C=O (1727 cm^{-1}) groups, and the HRFABMS gave a peak at *m*/*z* 567.1285, consistent with a molecular formula of $C_{27}H_{28}O_{11}$ (calcd for $C_{27}H_{28}O_{11}K$, 567.1269). The ^1H NMR spectrum (Table 1) indicated the presence of a monosubstituted benzene moiety [δ 7.36 (2H, t, J = 7.9 Hz), 7.50 (1H, t, J = 7.6 Hz), and 8.00 (2H, d, J = 7.0 Hz)], a salicyl alcohol unit [δ 4.95 and 5.09 (each 1H, d, J = 12.5 Hz), 6.98 (1H, t, J = 7.6 Hz), 7.02 (1H, d, J = 7.6 Hz), 7.16 (1H, d, J = 7.6 Hz), and 7.25 (1H, t, J = 7.6 Hz)], a C-2 acylated β-glucose unit [δ 3.51−3.53 (1H, m), 3.89−3.92 (4H, m), 5.16 (1H, t, J = 8.2 Hz), and 5.31 (1H, t, J = 8.2 Hz)], and a *cis*-1-butene sequence [δ 2.40−2.47 (1H, m), 2.58−2.65 (2H, m), 2.95 (1H, dt, J = 14.6, 8.1 Hz), 5.69 (1H, d, J = 9.9 Hz), and 6.03 (1H, dt, J = 9.9, 3.8 Hz)] in the molecule. These assignments were supported by the ^{13}C (Table 1) and 2D NMR spectra (H−H COSY and COLOC). In the ^{13}C NMR spectrum (Table 1) three C=O functions were observed, attributable to a ketone (δ 206.1) and two esters (δ 166.0, 169.6). The *cis*-1-butene sequence could be ex-

Table 1. NMR Data of Compound **1** and Tremulacin (**2**)[a]

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1		155.4		155.0
2		124.3		124.8
H-3	7.31 (dd, 7.7, 1.8)	130.8	7.16 (d, 7.6)	129.6
H-4	7.06 (dt, 7.7, 0.7)	123.0	6.98 (t, 7.6)	123.0
H-5	7.35 (t, 7.7)	131.0	7.25 (t, 7.6)	130.1
H-6	7.10 (dd, 7.7, 0.7)	115.2	7.02 (d, 7.6)	115.5
H_2-7	5.23 (d, 11.9) 5.37 (d, 11.9)	64.1	4.95 (d, 12.5) 5.09 (d, 12.5)	63.7
H-1′	5.10 (d, 7.6)	101.1	5.16 (d, 8.2)	99.4
H-2′	3.85−3.99 (m)	71.8	5.31 (t, 8.2)	73.9
H-3′	5.25−5.30 (m)	76.1	3.89−3.92 (m)	74.6
H-4′	3.85−3.99 (m)	69.2	3.89−3.92 (m)	69.4
H-5′	3.62−3.67 (m)	78.0	3.51−3.53 (m)	76.1
H_2-6′	3.85−3.99 (m)	61.9	3.89−3.92 (m)	60.9
1″		78.6		78.3
H-2″	5.77 (dt, 9.8, 1.9)	127.3	5.69 (d, 9.9)	127.4
H-3″	6.09 (dt, 9.8, 1.9)	132.2	6.03 (dt, 9.9, 3.8)	132.1
H_2-4″	2.47−2.54 (m) 2.62−2.68 (m)	26.7	2.40−2.47 (m) 2.58−2.65 (m)	26.5
H_2-5″	2.57−2.62 (m) 2.88 (dt, 14.4, 7.8)	35.2	2.58−2.65 (m) 2.95 (dt, 14.6, 8.1)	35.3
6″		205.9		206.1
7″		169.9		169.6
1‴		129.3		129.4
H-2‴ (6‴)	8.11 (2H, dd, 7.7, 1.4)	130.0	8.00 (2H, d, 7.6)	129.9
H-3‴	7.47 (2H, t, 7.7)	128.5	7.36 (2H, t, 7.6)	128.4
H-4‴ (5‴)	7.60 (1H, tt, 7.7, 1.4)	133.6	7.50 (1H, t, 7.6)	133.3
7‴		168.0		166.0

[a] ^1H NMR (500 MHz in $CDCl_3$) are reported relative to TMS at 0.00 ppm, and peak multiplicities (δ value) in parenthesis are stated in Hz. ^{13}C NMR assignments are relative to internal $CDCl_3$ at 77.00 ppm. ^1H and ^{13}C NMR assignments are based on decoupling, DEPT, differential NOE, C−H COSY or HMQC, and COLOC or HMBC experiments.

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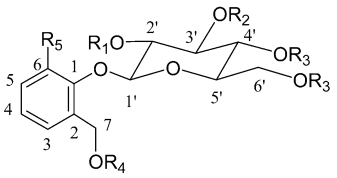
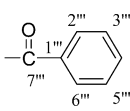
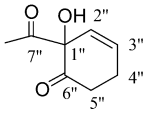
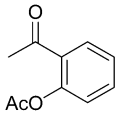
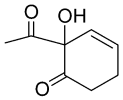
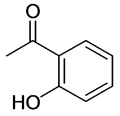


1 $R_1 = R_3 = R_5 = H$, $R_2 =$  , $R_4 =$ 

1a $R_1 = R_3 =$ Ac, $R_5 = H$, $R_2 =$ COPh, $R_4 =$ 

2 $R_1 =$ COPh, $R_2 = R_3 = R_5 = H$, $R_4 =$ 

2a $R_1 =$ COPh, $R_2 = R_3 = R_5 = H$, $R_4 =$ 

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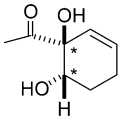
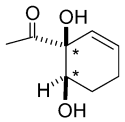
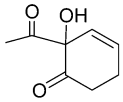
4 $R_1 =$ COPh, $R_2 = R_3 = R_5 = H$, $R_4 =$ 

4a $R_1 =$ COPh, $R_2 = R_3 = R_5 = H$, $R_4 =$ 

5 $R_1 =$ COPh, $R_2 = R_3 = H$, $R_4 =$  , $R_5 =$ OH

5a $R_1 = R_2 = R_3 = R_4 =$ Ac, $R_5 =$ OMe

* relative stereochemistry

tended to a (1-hydroxy-6-oxocyclohex-2-en-1-yl)carboxylate unit because of an easy aromatization of the *cis*-1-butene moiety when **2** was treated with 15% aqueous H_2SO_4, resulting in the formation of salicyl tremuloidin (**2a**). The ^{1}H NMR spectrum showed a new set of signals due to the salicylate function [δ 6.93 (1H, dt, J = 7.3, 1.0 Hz), 6.76 (1H, dt, J = 8.2, 1.0 Hz), 7.41 (1H, ddd, J = 8.2, 7.3, 1.7 Hz), and 7.68 (1H, dd, J = 8.2, 1.7 Hz)] in place of the *cis*-1-butene unit in **2**. The combination of each partial structure led to the conclusion that **2** was tremulacin,[4] which was first isolated from the bark of *Populus tremula*.[5] It was reported that tremulacin (**2**) includes a β-D-glucopyranoside unit;[5] however, no chemical evidence was given to corroborate this. The failure in the reduction of **2** by enzymatic hydrolysis forced us to examine a different approach. Thus, treatment of **2** with $LiAlH_4$ afforded the corresponding salicin derivative in 46% yield, leading to the establishment of the presence of a β-D-glucopyranoside unit.

Compound **1** was obtained as a light yellow amorphous mass. Its isomeric nature similar to tremulacin (**2**) was suggested by the presence of a peak at *m*/*z* 567.1276 (calcd for $C_{27}H_{28}O_{11}K$, 567.7268) in the HRFABMS and by absorption bands due to OH (3498 cm^{-1}) and C=O (1723 cm^{-1}) groups in the IR spectrum. Although a similar signal pattern was observed in the ^{1}H NMR spectrum (Table 1), H-2 and H-3 in the glucose unit of **1** resonated at δ 3.85−3.99 (m) and 5.25−5.30 (m), respectively, while the corresponding signals in **2** were observed at different regions [δ 5.31 (t, J = 8.2 Hz, H-2) and 3.89−3.92 (m, H-3)]. Acetylation of **1** with Ac_2O−pyridine resulted in the formation of a tetraacetate (**1a**) [FABMS *m*/*z* 619 [M − OAc]$^+$; IR 1753 cm^{-1}; ^{1}H NMR δ 1.92, 1.95, 2.10, 2.12 (each 3H, s, $COCH_3$)], in which the (1-hydroxy-6-oxocyclohex-2-en-1-yl)-carboxylate unit was aromatized to a salicylate during the acetylation reaction. In addition to these data, a ^{13}C (Table 1) and 2D NMR spectra (H−H COSY and HMBC) inspection indicates that **1** must be a C-3 position benzoyl-migrated derivative from the C-2 position of the glucose unit. The large coupling constant (J = 7.6 Hz in **1**; J = 7.8 Hz in **1a**) of the anomeric proton (H-1′) at δ 5.10 in **1** and δ 5.19 in **1a** allows us to deduce an axial configuration, suggesting the presence of a β-glucopyranoside unit in these molecules.

Spectral data examination of the third isolated compound (**3**), showing data analogous to tremulacin (**2**), led to its identification as the known deacylated salicin derivative, tremuloidin (**3**).[6]

The most polar compound (**4**) was isolated as a colorless amorphous mass. The IR spectrum showed absorption bands due to OH (3428 cm^{-1}) and C=O (1722 cm^{-1}) groups, and the HRFABMS gave a peak at *m*/*z* 553.1668, consistent with a molecular formula of $C_{27}H_{30}O_{11}$ (calcd for $C_{27}H_{30}O_{11}$-Na, 553.1686). The ^{1}H and ^{13}C NMR spectra gave a signal pattern similar to that of tremulacin (**2**) except for the (1-hydroxy-6-oxocyclohexe-2-en-1-yl)carboxylate unit. A new signal due to a carbinol methine unit [δ_H 3.81 (1H, dd, J = 10.6, 5.4 Hz); δ_C 74.5 (CH)] in place of the ketonic function of **2** was observed. These data as well as 2D NMR spectra suggest that compound **4** is a dihydro derivative of **2**. Accordingly, the reduction of **2** was investigated with various hydride reagents (Table 2).

While the formation of a complex mixture was observed with sodium borohydride (run 1), reduction with zinc borohydride (run 2) gave a homogeneous product on TLC, corresponding to compound **4**. However, its ^{1}H NMR spectrum showed that the product contained an epimeric alcohol (vide infra) in a 3:2 ratio. Reduction with a diborane solution gave a single product corresponding to the epimer (**4a**) (run 3). In the ^{1}H NMR spectrum, **4a** showed almost the same signal pattern as that of **4** except for a (1,6-dihydroxycyclohex-2-en-1-yl)carboxylate unit. The carbinol methine proton in the cyclohexene ring of **4** appeared at δ 3.81 (dd, J = 10.6, 5.4 Hz), whereas in **4a** it occurred at δ 4.03 (dd, J = 9.8, 5.9 Hz). The adjacent methylene signals appeared respectively at δ 1.85−1.89 and 2.10−2.21 (each 1H, m) in the former and at δ 1.73−1.76 (2H, m) in the latter. In the ^{13}C NMR spectra, the carbons carrying an oxygen atom were observed at δ 74.5 and 77.7 in **4**, whereas at δ 70.8 and 74.2 in **4a**, suggesting epimeric compounds.

In both alcohols, the large coupling constant of the carbinol methine proton (J = 10.6 Hz in **4**; J = 9.8 Hz in **4a**) indicates an axial conformation. In compound **4**, the methylene proton at the C-5 position appeared at lower field than in **4a**, whereas the carbinol methine proton was observed at lower field in **4a**. These downfield-shifted signals depend on a deshielding effect due to the ester carbonyl group, leading to a *trans*-relation between two hydroxy groups in the former and a *cis*-relation in the latter alcohol (Figure 1). It is known that diastereoselectivity can be controlled by a chelated intermediate during the hydride

Table 2. Hydride Reduction of Tremulacin (**2**)

run	reagent (mol equiv)	solvent	temp (°C)	time (h)	result
1	$NaBH_4$ (1)	dry EtOH	-5	0.25	cm[a]
2	$Zn(BH_4)_2$ (10)	dry THF/Et_2O	rt	3.5	quant. (**4**:**4a** = 3:2)
3	1M BH_3 in THF (1)	dry THF	rt	2.0	**4a**: quant.

[a] Complex mixture.

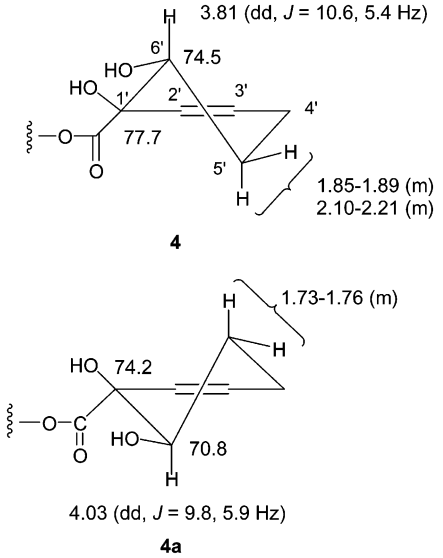


Figure 1. Selected NMR data and the relative configurations for the cyclohexenediol systems of **4** and **4a**.

reduction of α-hydroxyketone.[7] Thus, zinc borohydride gave a *trans*-diol by intramolecular hydride delivery via a chelated transition state, reasonably supporting the predominant formation of a *trans*-glycol system in the reduction of **2**. In contrast, the stereoselective formation of a *cis*-glycol system in the BH_3 reduction of **2** may depend on an intermolecular hydride attack from the opposite site of the OH group at the C-1 position in the possible five-membered cyclic structure chelated with the borane reagent (BH_3).

From the leaves of *H. cochinchinensis*, Fr. C was treated in the same manner as described above, giving compound **5** as a pale yellow amorphous mass. A molecular formula of $C_{27}H_{28}O_{12}$ was deduced by HRFABMS [*m*/*z* 567.1478 (calcd for $C_{27}H_{28}O_{12}Na$, 567.1479)]. The ^1H NMR spectrum showed a signal pattern almost identical with that of tremulacin (**2**). However, in the aromatic region three sequentially substituted benzene protons, assignable to an oxygen-substituted salicyl alcohol unit at either the C-6 or C-3 position, were observed at δ 6.68 (1H, dd, *J* = 7.8, 1.4 Hz), 6.84 (1H, dd, *J* = 7.8, 1.4 Hz), and 6.92 (1H, t, *J* = 7.8 Hz). Since no NOE enhancement was observed between the benzylic methylene protons [δ 4.90, 5.07 (each 1H, d, *J* = 12.2 Hz)] and an aromatic proton at either δ 6.68 or 6.84, it was suggested that 3-hydroxysalicyl alcohol may be the most probable structure for compound **5**. However, characteristic cross-peaks between both H-3 and H-5 and C-1 in addition to the cross-peak between benzylic methylene protons and C-3 in a COLOC experiment allowed us to exclude a 3-hydroxysalicyl alcohol system. Therefore, a 6-hydroxysalicyl alcohol system was apparent in compound **5**. The position of the free OH group in compound **5** was finally determined by a NOE experiment after methylation followed by acetylation. Although methylation with either diazomethane or dimethyl sulfate failed, treatment with trimethylsilyl diazomethane gave a methylated product, albeit in low yield (8.5%), in which the cyclohexene ring and the benzoyl group were lost. Acetylation of the methyl ether yielded a pentaacetate (**5a**) [HRFABMS *m*/*z* 565.1309 [M + K]$^+$, calcd for $C_{24}H_{24}O_{13}K$, 565.1324], in which a newly inserted methoxy group (δ 3.77) showed a NOE enhancement with an aromatic proton (δ 6.82). These facts indicated that the methoxy group was located at the C-6 position in the salicyl alcohol unit. While our study was in progress, Chou et al. reported the debenzoyl derivative of compound **5** from *Idesia polycarpa*.[8]

Table 3. Antiviral Activities of Some Isolated Products[a]

	EC_{50} (μM)		
compound	HSV-1	HSV-2	HIV-1
cochinolide[b]	31	86	nt[c]
2	87	86	52
3	>256	>256	nt[c]
4	>188	>188	nt[c]
5	76	76	>18
ACV[d]	1.1	1.0	nt[c]
DDI[e]	nt[c]	nt[c]	2.1
AZT[f]	nt[c]	nt[c]	0.02

[a] No toxicity was observed for all the compounds tested at concentrations of less than 10 g/mL. [b] The reported data in ref 1. [c] nt = not tested. [d] Acyclovir. [e] Dideoxyinosine. [f] Azidothymidine.

Using a previously reported procedure,[3] compounds **2**−**5** were subjected to antiviral testing against HSV-1 and HSV-2. Acyclovir (ACV) was used as a control (Table 3). Two components, **2** and **5**, showed weak activity against both types of HSV series, whereas **3** and **4** were inactive, suggesting that the presence of a (1-hydroxy-6-oxocyclohexe-2-en-1-yl)carboxylate unit in the molecule might be responsible for the activity. Tremulacin (**2**) and cochinchiside B (**5**) were further tested for antiviral activity against HIV-1[9] (Table 3). Dideoxyinosine (DDI) and azidothymidine (AZT) were used as controls. The test shows tremulacin (**2**) to be only weakly active.

It was found that *H. cochinchinensis* contains salicin glucosides such as tremulacin (**2**) as major secondary metabolites. Compounds **1**, **4**, and **5** were isolated as new salicin derivatives. Farnsworth et al. reported the presence of homaloside D, an isomer of cochinchiside B (**5**) in *H. ceylanicum*.[10] Accordingly, salicin glucosides are characteristic components in the genus *Homalium*. Although tremulacin (**2**) and its related components were isolated as optically active forms, their stereochemistries have remained unclear because of the facile aromatization of the (1-hydroxy-6-oxocyclohex-2-en-1-yl)carboxylate unit throughout acid and/or base treatment.

Experimental Section

General Experimental Procedures. Melting points were determined on a micro melting point hot-stage instrument (Yanagimoto) and are uncorrected. IR spectra were recorded on a JASCO IR-700 spectrophotometer. UV spectra were measured on a Hitachi U-3400. ORD and CD spectra were recorded on JASCO J-20 and J-500 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were recorded with JEOL JNM GSX-500α spectrometers with tetramethylsilane as internal reference. FABMS and HRFABMS were recorded on a JEOL JMX-HX 110A spectrometer with a direct inlet system. For column chromatography and flash chromatography silica gel 60 (70−230 mesh ASTM; Merck) and silica gel

60 (230−400 mesh ASTM; Merck) were used, while for TLC and preparative TLC silica gel GF254 (Merck) was used.

Extraction and Isolation. The root bark (195 g) of *H. cochinchinensis* was extracted in a Soxhlet apparatus using hexane (Fr. H: 0.64 g), benzene (Fr. B: 2.74 g), chloroform (Fr. C: 2.61 g), and methanol (Fr. M: 16.84 g) as previously described.[1] Similar treatment of the trunk bark (19.9 g) and the leaves (9.5 g) except in the use of Et_2O in place of benzene as a solvent afforded Fr. H (0.07 g from the trunk bark; 0.13 g from the leaves), Fr. E (0.09 g from the trunk bark; 0.10 g from the leaves), Fr. C (0.20 g from the trunk bark; 0.20 g from the leaves), and Fr. M (1.54 g from the trunk bark; 1.92 g from the leaves). Each fraction was subjected to repeated separation using column chromatography, flash chromatography, and preparative TLC. The root bark from Fr. B gave six components: benzoic acid (0.054 g, 0.028%), cochinolide[1] (0.074 g, 0.038%), cochinchiside A (**1**) (0.017 g, 0.009%), tremulacin (**2**) (0.412 g, 0.211%), tremuloidin (**3**) (0.003 g, 0.002%), and tremulacinol (**4**) (0.029 g, 0.015%). Among them **2** (0.442 g, 0.227%; total 0.854 g, 0.438%) and **4** (0.192 g, 0.099%; total 0.221 g, 0.114%) were additionally isolated from Fr. C. From Fr. D more polar cochinolide *β*-glucopyranoside[1] (0.637 g, 0.3265%) was isolated. On the other hand, from Fr. C of the leaves cochinchiside B (**5**) (0.067 g, 0.0919%) was obtained.

Compound 1 (cochinchiside A): pale yellow amorphous mass; IR ($CHCl_3$) ν_{max} 3498, 1723 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 222 (4.09), 273 nm (3.45); CD (*c* 1.9 × 10^{-5}, MeOH) [θ] −18958 (216 nm); ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 567.1276 $[M + K]^+$, calcd for $C_{27}H_{28}O_{11}K$, 567.1268.

Acetylation of 1. A mixture of **1** (0.005 g, 9.5 × 10^{-5} mol), Ac_2O (0.1 mL), and pyridine (0.1 mL) was stored at room temperature overnight and then at 60 °C overnight. After workup, the crude product was purified by preparative TLC to afford the tetraacetate (**1a**) as a yellow amorphous mass (0.006 g, 99%): IR ($CHCl_3$) ν_{max} 1753 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 228 (4.82), 273 nm (4.21); CD (*c* 2.15 × 10^{-5}, MeOH) [θ] 14814 (230 nm), −12012 (217); ^1H NMR ($CDCl_3$) δ 1.92, 1.95, 2.10, 2.12 (each 3H, s, Me), 3.95 (1H, m, H-5′), 4.21 (1H, dd, *J* = 12.2, 2.4 Hz, H-6′), 4.32 (1H, dd, *J* = 12.2, 5.4 Hz, H-6′), 5.19 (1H, d, *J* = 7.8 Hz, H-1′), 5.25, 5.38 (each 1H, d, *J* = 13.0 Hz, H_2-7), 5.35 (1H, t, *J* = 9.5 Hz, H-4′), 5.49 (1H, dd, *J* = 9.5, 7.8 Hz, H-2′), 5.55 (1H, t, *J* = 9.5 Hz, H-3′), 7.09 (1H, d, *J* = 7.8 Hz, H-3″), 7.13 (1H, t, *J* = 7.8 Hz, H-5), 7.14 (1H, d, *J* = 7.8 Hz, H-6), 7.32 (2H, t, *J* = 7.8 Hz, H-5, H-5″), 7.42 (1H, d, *J* = 7.8 Hz, H-3), 7.44 (2H, t, *J* = 7.4 Hz, H-3‴, H-5‴), 7.56 (1H, dt, *J* = 7.9, 1.7 Hz, H-4″), 7.57 (1H, t, *J* = 7.4 Hz, H-4‴), 7.99 (2H, dd, *J* = 7.4, 0.7 Hz, H-2‴, H-6‴), 8.07 (1H, dd, *J* = 7.9, 1.7 Hz, H-6″); ^{13}C NMR ($CDCl_3$) δ 20.4 (Me) 20.5 (Me), 20.7 (Me), 61.7 (C-7), 61.9 (C-6′), 68.3 (C-4′), 71.0 (C-2′), 72.1 (C-5′), 73.1 (C-3′), 99.5 (C-1′), 116.2 (C-6), 123.3 (C-1″), 123.7 (C-5″), 123.9 (C-4), 126.0 (C-3″), 128.6 (C-3‴, C-5‴), 129.6 (C-5, C-1‴), 129.7 (C-3), 129.8 (C-2), 129.9 (C-2‴, C-6‴), 132.0 (C-6″), 133.6 (C-4″), 133.9 (C-4‴), 150.6 (C-2″), 154.6 (C-1), 164.2 (C-7″), 165.8 (C-7‴), 169.3 (CO), 169.4 (CO), 169.6 (CO), 170.6 (CO); FABMS *m/z* 619 $[M - OAc]^+$.

Tremulacin (2): colorless prisms, mp 122−125 °C (lit.[5] mp 122−123 °C); IR (Nujol) ν_{max} 3442, 1727 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 220 (4.29), 272 nm (3.65); ORD (*c* 0.47, MeOH) [α] −94° (600 nm), −168° (500); CD (*c* 0.47, MeOH) [θ] −5303 (268 nm); ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 567.1285 $[M + K]^+$, calcd for $C_{27}H_{28}O_{11}K$, 567.1269.

Hydrolysis of 2. A mixture of **2** (0.050 g, 9.5 × 10^{-5} mol) and 15% aqueous H_2SO_4 (2.5 mL) was stirred at 100 °C for 1.5 h. After workup, the crude solid was washed with ethyl acetate to afford salicyl tremuloidin (**2a**) as a colorless powder (0.021 g, 43%): IR (KBr) ν_{max} 3428, 1706, 1673 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 231 (4.37), 274 (3.44), 306 nm (3.56); UV (MeOH + 1% NaOH) λ_{max} 234, 273, 314 nm; CD (*c* 1.9 × 10^{-5}, MeOH) [θ] −24717 (237 nm), 17698 (220); ^1H NMR ($CDCl_3$) δ 3.57 (1H, ddd, *J* = 9.5, 4.7, 3.0 Hz, H-5′), 3.72 (1H, t, *J* = 9.5 Hz, H-4′), 3.84 (1H, t, *J* = 9.5 Hz, H-3′), 3.87 (1H, dd, *J* = 12.2, 4.7 Hz, H-6′), 3.97 (1H, dd, *J* = 12.2, 3.0 Hz, H-6′), 5.23 (1H, d, *J* = 8.1 Hz, H-1′), 5.25, 5.29 (each 1H d, *J* = 13.1 Hz, H_2-7), 5.38 (1H, dd, *J* = 9.5, 8.1 Hz, H-2′), 6.76 (1H, dt, *J* = 8.2, 1.0 Hz, H-3″), 6.93 (1H, dd, *J* = 7.3, 1.0 Hz, H-5″), 7.06 (1H, dt, *J* = 7.5, 0.7 Hz, H-4), 7.13 (1H, d, *J* = 7.5 Hz, H-6), 7.32 (1H, dif t, *J* = 7.5 Hz, H-5), 7.35 (2H, t, *J* = 7.9 Hz, H-3‴, H-5‴), 7.36 (1H, d, *J* = 7.5 Hz, H-3), 7.41 (1H, ddd, *J* = 8.2, 7.3, 1.7 Hz, H-4″), 7.47 (1H, tt, *J* = 7.9, 1.4 Hz, H-4‴), 7.68 (1H, dd, *J* = 8.2, 1.7 Hz, H-2″), 8.02 (1H, dd, *J* = 7.9, 1.4 Hz, H-2‴); ^{13}C NMR ($CDCl_3$) δ 61.6 (C-7, C-6′), 70.2 (C-4′), 73.7 (C-2′), 74.8 (C-3′), 76.2 (C-5′), 99.4 (C-1′), 112.3 (C-1″), 115.5 (C-6), 117.3 (C-5″), 119.0 (C-3″), 123.0 (C-4), 125.1 (C-2), 128.3 (C-3‴), 129.2 (C-1‴), 129.4 (C-3), 129.66 (C-2‴), 129.72 (C-2″), 129.9 (C-2‴), 133.2 (C-4‴), 135.5 (C-4″), 154.7 (C-1), 166.0 (C-7‴), 170.0 (C-7″); FABMS *m/z* 511 $[M + H]^+$.

Reduction of 2 with $LiAlH_4$. A mixture of **2** (0.051 g, 9.7 × 10^{-5} mol) and $LiAlH_4$ (0.037 g, 9.7 × 10^{-4} mol) in THF (3 mL) was stirred at room temperature for 7 h. After workup, the crude product was purified by preparative TLC ($CHCl_3$−MeOH, 5:1) to give a colorless amorphous mass (0.013 g, 46%), mp 180−185 °C, which was compared with commercially available salicin: IR (Nujol) ν_{max} 3344 cm^{-1}; $[\alpha]_{589}^{20}$ −52.6° (*c* 2.9, H_2O); ^1H NMR (DMSO-d_6) δ 3.15−3.19 (1H, m, H-4′), 3.27 (1H, t, *J* = 3.1 Hz, H-6′), 3.28−3.33 (1H, m, H-5′), 3.48 (1H, dd, *J* = 11.9, 6.1 Hz, H-3′), 3.70 (1H, dd, *J* = 12.2, 2.1 Hz, H-2′), 4.46−4.63 (each 1H, d, *J* = 14.3 Hz, H_2-7), 4.77 (1H, d, *J* = 7.6 Hz, H-1′), 7.01 (1H, t, *J* = 7.3 Hz, H-6), 7.09 (1H, dd, *J* = 7.3 Hz, H-4), 7.21 (1H, dt, *J* = 8.2, 1.8 Hz, H-5), 7.36 (1H, dd, *J* = 7.6, 1.8 Hz, H-3).

Tremuloidin (3): colorless powder, mp 162−168 °C (lit.[6] mp 201−204 °C); ^1H NMR ($CDCl_3$) δ 3.50−3.52 (1H, m, H-5′), 3.69 (1H, t, *J* = 9.4 Hz, H-4‴), 3.83 (1H, d, *J* = 11.8 Hz, H-6′), 3.85 (1H, t, *J* = 9.4 Hz, H-3′), 3.93 (1H, dd, *J* = 11.8, 3.8 Hz, H-6′), 4.35, 4.65 (each 1H, d, *J* = 12.9 Hz, H_2-7), 5.20 (1H, d, *J* = 8.0 Hz, H-1′), 5.35 (1H, dd, *J* = 9.4, 8.0 Hz, H-2′), 7.01 (1H, d, *J* = 7.6 Hz, H-6), 7.03 (1H, t, *J* = 7.6 Hz, H-4), 7.24 (1H, t, *J* = 7.6 Hz, H-5), 7.27 (1H, d, *J* = 7.6 Hz, H-3), 7.46 (1H, t, *J* = 7.6 Hz, H-3″), 7.59 (1H, t, *J* = 7.6 Hz, H-4″), 8.09 (1H, d, *J* = 7.6 Hz, H-3″); HRFABMS *m/z* 413.1221 $[M + Na]^+$, calcd for $C_{20}H_{22}O_8Na$, 413.1212.

Compound 4 (tremulacinol): colorless amorphous mass; IR ($CHCl_3$) ν_{max} 3428, 1722 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 221 (4.04), 230 (4.01), 273 nm (3.32); ORD (*c* 0.149, MeOH) [α] −66.6° (600 nm), −109.7° (500); CD (*c* 2.8 × 10^{-5}, MeOH) [θ] −54 (283 nm), −304 (267), −42857 (224); ^1H NMR ($CDCl_3$) δ 1.85−1.89 (1H, m, H-5″), 2.10−2.21 (3H, m, H-5″, H_2-4″), 3.49−3.52 (1H, m, H-5′), 3.87−4.02 (4H, m, H-3′, H-4′, H_2-6′), 4.93, 5.11 (each 1H, d, *J* = 12.2 Hz, H_2-7), 5.20 (1H, d, *J* = 8.0 Hz, H-1′), 5.35 (1H, dd, *J* = 9.5, 8.0 Hz, H-2′), 5.44 (1H, d, *J* = 9.7 Hz, H-2″), 5.85 (1H, dt, *J* = 9.7, 3.5 Hz, H-3″), 7.00 (1H, t, *J* = 7.9 Hz, H-4), 7.03 (1H, d, *J* = 7.9 Hz, H-6), 7.22 (1H, dd, *J* = 7.9, 1.5 Hz, H-3), 7.26 (1H, td, *J* = 7.9, 1.5 Hz, H-5), 7.37 (2H, t, *J* = 7.6 Hz, H-3‴, H-5‴), 7.52 (1H, tt, *J* = 7.6, 1.2 Hz, H-4‴), 8.02 (2H, dd, *J* = 7.6, 1.2 Hz, H-2‴, H-6‴); ^{13}C NMR ($CDCl_3$) δ 24.2 (C-4″), 26.5 (C-5″), 60.6 (C-6′), 63.6 (C-7), 69.1 (C-4″), 74.2 (C-2′), 74.5 (C-3′, C-6″), 76.3 (C-5′), 77.7 (C-1″), 115.7 (C-3), 123.2 (C-5), 125.5 (C-1), 126.2 (C-2″), 128.4 (C-3‴, C-4‴), 130.0 (C-6), 130.2 (C-2‴, C-6‴), 131.7 (C-3″), 133.4 (C-4‴), 155.2 (C-2), 166.3 (C-7‴), 172.8 (C-7″); HRFABMS *m/z* 553.1668 $[M + Na]^+$, calcd for $C_{27}H_{30}O_{11}Na$, 553.1686.

Reduction of 2 with BH_3 (run 3 in Table 2). A 1 M solution of BH_3 in THF (0.02 mL, 2 × 10^{-5} mol) was added to an ice-cooled solution of **2** (0.009 g, 1.7 × 10^{-5} mol) in THF (0.5 mL), and then the whole was stirred under ice-cooling for 2 h. After workup, the epimer (**4a**) was obtained as a colorless amorphous mass (0.008 g, 95%): IR ($CHCl_3$) ν_{max} 3426, 1724 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 219 (4.06), 230 (3.99), 273 nm (3.26); ORD (*c* 6.57 × 10^{-2}, MeOH) [α] −30.4° (600 nm), −57.8° (500); CD (*c* 6.9 × 10^{-5}, MeOH) [θ] −129 (283 nm), −3554 (267), −30048 (226); ^1H NMR ($CDCl_3$) δ 1.73−1.76 (2H, m, H_2-5″), 2.11−2.15 (2H, m, H_2-4″), 3.44 (1H, ddd, *J* = 9.5, 3.9, 2.6 Hz, H-5′), 3.67 (1H, t, *J* = 9.3 Hz, H-4′), 3.76 (1H, t, *J* = 9.3 Hz, H-3′), 3.77 (1H, dd, *J* = 12.4, 3.9 Hz, H-6′), 3.86 (1H, dd, *J* = 12.4, 2.6 Hz, H-6′), 4.03 (1H, dd, *J* = 9.8, 5.9 Hz, H-6), 4.91, 5.07 (each 1H, d, *J* = 12.5, H_2-7), 5.14 (1H, d, *J* = 7.8 Hz, H-1′), 5.23 (1H, dd, *J* = 9.3, 7.8 Hz, H-2′), 6.95 (1H, t, *J* = 7.6 Hz, H-5), 6.98 (1H, d, *J* = 7.6 Hz, H-3), 7.16 (1H, d like, *J*

= 7.6 Hz, H-6), 7.21 (1H, t like, J = 7.6 Hz, H-4), 7.37 (1H, t, J = 7.6 Hz, H-3‴), 7.50 (1H, t, J = 7.6 Hz, H-4‴), 8.00 (1H, d, J = 7.6 Hz, H-2‴); ^{13}C NMR ($CDCl_3$) δ 24.9 (C-4″), 25.4 (C-5″), 60.9 (C-6″), 63.6 (C-7), 69.4 (C-4′), 70.8 (C-6″), 74.0 (C-2′), 74.2 (C-1″), 74.5 (C-3′), 76.4 (C-5′), 115.3 (C-3), 123.1 (C-5), 125.1 (C-2″), 125.6 (C-1), 128.4 (C-3‴, C-5‴), 129.8 (C-6), 130.1 (C-2‴, C-6‴), 130.2 (C-4), 132.8 (C-3″), 133.4 (C-4‴), 155.2 (C-2), 166.3 (C-7‴), 174.5 (C-7″); HRFABMS m/z 569.1432 $[M + K]^+$, calcd for $C_{27}H_{30}O_{11}K$, 569.1425.

Compound 5 (cochinchiside B): colorless amorphous mass; IR ($CHCl_3$) ν_{max} 3442, 1727 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 222 (4.02), 276 nm (3.45); ORD (c 1.01 × 10^{-2}, MeOH) [α] −74° (600 nm), −148° (500); CD (c 1.86 × 10^{-5}, MeOH) [θ] −13963 (218 nm); ^{1}H NMR ($CDCl_3$) δ 2.40−2.45 (1H, m, H-4″), 2.49−2.59 (2H, m, H-4″, H-5″), 2.75 (1H, dt, J = 14.7, 7.8 Hz, H-5″), 3.40−3.42 (1H, m, H-5′), 3.78−3.86 (2H, m, H_2-6′), 3.88−3.93 (2H, m, H-3′, H-4′), 4.90, 5.07 (each 1H, d, J = 12.2 Hz, H_2-7), 5.00 (1H, d, J = 8.2 Hz, H-1′), 5.39 (1H, t, J = 8.2 Hz, H-2′), 5.68 (1H, dd, J = 9.8, 1.8 Hz, H-2″), 6.02 (1H, dt, J = 9.8, 4.0 Hz, H-3″), 6.68 (1H, dd, J = 7.8, 1.4 Hz, H-5), 6.84 (1H, dd, J = 7.8, 1.4 Hz, H-4), 6.92 (1H, t, J = 7.8 Hz, H-5), 7.34 (2H, t, J = 7.6 Hz, H-3‴, H-5‴), 7.49 (1H, t, J = 7.6 Hz, H-4‴), 8.02 (2H, d, J = 7.6 Hz, H-2‴, H-6‴); ^{13}C NMR ($CDCl_3$) δ 26.5 (C-4″), 35.2 (C-5″), 60.7 (C-6′), 63.1 (C-7), 69.3 (C-4′), 74.1 (C-2′), 74.6 (C-3′), 76.3 (C-5′), 78.0 (C-1″), 103.1 (C-1′), 117.8 (C-6), 121.1 (C-4), 126.3 (C-5), 127.4 (C-2″), 128.5 (C-3‴, C-5‴), 128.6 (C-3), 129.0 (C-1‴), 130.0 (C-2‴, C-6‴), 132.2 (C-3″), 133.5 (C-4‴), 142.2 (C-2), 149.8 (C-1), 166.1 (C-7‴), 169.5 (C-7″), 205.9 (C-6″); HRFABMS m/z 567.1478 $[M + Na]^+$, calcd for $C_{27}H_{28}O_{12}Na$, 567.1479.

Methylation of 5 Followed by Acetylation. A solution of **5** (10 mg, 1.84 × 10^{-2} mol) in MeOH−MeCN (1:9, 0.4 mL) containing *N*,*N*-diisopropylethylamine (0.005 mL, 2.57 × 10^{-2} mmol) was stirred with a 10% solution of $TMSCH_2N_2$ in hexane (0.06 mL, 4.25 × 10^{-2} mmol) at room temperature overnight. After workup, the crude product was purified with preparative TLC (acetone−$CHCl_3$, 3:1) to give the methyl ether as a colorless amorphous mass (0.5 mg, 9%): ^{1}H NMR ($CDCl_3$ containing one drop of CD_3OD) δ 3.30 (1H, m, H-5″), 3.34−3.46 (5H, m, H-2′, H-3′, H-4′, H_2-6′), 3.81 (3H, s, OMe), 4.35, 4.77 (each 1H, d J = 11.9 Hz, H_2-7), 4.69 (1H, dd, J = 7.9, 1.4 Hz, H-1′), 6.85 (1H, d, J = 8.0 Hz, H-3), 6.91 (1H, d, J = 8.0 Hz, H-5), 7.06 (1H, t, J = 8.0 Hz, H-4); ^{13}C NMR ($CDCl_3$ containing one drop of CD_3OD) δ 55.8 (OMe), 59.8 (CH_2), 61.0 (C-6′), 69.5 (C-4′), 74.0 (C-2′), 76.2 (C-3′), 76.4 (C-5′), 103.5 (C-1′), 112.2 (C-5), 122.3 (C-3), 125.5 (C-4), 135.2 (C-2), 143.5 (C-1), 151.3 (C-6).

A solution of the methyl ether (0.5 mg, 1.6 × 10^{-3} mol) in pyridine (0.1 mL) and Ac_2O (0.1 mL) was stored at 60 °C for 1.3 h. After workup, the crude product was purified with preparative TLC (hexane−AcOEt, 3:2) to give the pentaacetate (**5a**) as a colorless amorphous mass (1.1 mg, quant): ^{1}H NMR ($CDCl_3$) δ 1.95, 1.97, 1.98, 2.03, 2.04 (each 3H, s, Me), 3.53 (1H, ddd, J = 9.5, 4.2, 2.8 Hz, H-5′), 3.77 (3H, s, OMe), 4.02 (1H, dd, J = 12.2, 2.8 Hz, H-6′), 4.11 (1H, dd, J = 12.2, 4.2 Hz, H-6′), 5.01 (1H, d, J = 7.6 Hz, H-1′), 5.09, 5.21 (each 1H, d, J = 13.5 Hz, H_2-7), 5.11 (1H, t, J = 9.5 Hz, H-4′), 5.19 (1H, t like, J = 9.5 Hz, H-2′), 5.23 (1H, t, J = 9.5 Hz, H-3′), 6.82 (1H, dd, J = 8.0, 1.5 Hz, H-5), 6.88 (1H, dd, J = 8.0, 1.5 Hz, H-3), 7.05 (1H, t, J = 8.0 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 20.6 (Me), 20.7 (Me), 20.8 (Me), 21.0 (Me), 22.7 (Me), 55.8 (OMe), 61.5 (C-7), 61.7 (C-6′), 68.4 (C-4′), 71.57 (C-2′), 71.62 (C-3′), 72.7 (C-5′), 100.9 (C-1′), 112.1 (C-5), 120.6 (C-3), 125.5 (C-4), 131.9 (C-2), 142.4 (C-1), 151.6 (C-6), 169.4 (CO), 169.5 (CO), 170.4 (CO), 170.8 (CO); HRFABMS m/z 565.1309 $[M]^+$, calcd for $C_{24}H_{30}O_{13}$, 565.1324.

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References and Notes

(1) Part 2 in the series Studies on the Chemical Constituents of *Homalium cochinchinensis*. For Part 1, see: Ishikawa, T.; Nishigaya, K.; Uchikoshi, H.; Chen, I.-S. *J. Nat. Prod*. **1998**, *61*, 534−537.
(2) Gan, W. S. *Manual of Medicinal Plants in Taiwan*; National Research Institute of Chinese Medicine: Taipei, 1970; Vol. 3, p 567.
(3) (a) Ishikawa, T.; Kotake, K.-I.; Ishii, H. *Chem. Pharm. Bull*. **1995**, *43*, 1039−1041. (b) Chansakaow, S.; Ruangrungsi, N.; Ishikawa, T. *Chem. Pharm. Bull*. **1996**, *44*, 1415−1417.
(4) (a) Pearl, I. A.; Darling, S. F. *Tetrahedron Lett*. **1970**, 3827−3830. (b) Pearl, I. A.; Darling, S. F. *Phytochemistry* **1971**, *10*, 3161−3166. (c) Dommisse, R. A.; van Hoof, L.; Vlietincke, A. J. *Phytochemistry* **1986**, *25*, 1201−1204.
(5) Thieme, H.; Richter, R. *Pharmazie* **1966**, *21*, 251−251.
(6) Pearl, S. I. A.; Darling, F. *J. Org. Chem*. **1959**, *24*, 731−735.
(7) Greenes, N. In *Comprehensive Organic Synthesis*, Trost, B. M., Fleming, I., Eds.; Pergamon Press: Oxford, UK, 1991; Vol. 8, p 1.
(8) Chou, C.-J.; Lin, L.-C.; Tsai, W.-J.; Hsu, S.-Y.; Ho, L.-K. *J. Nat. Prod*. **1997**, *60*, 375−377.
(9) Ikeda, R.; Haraguchi, Y.; Ikeda, Y.; Kondo, S.; Takeuchi, T.; Hoshino, H. *Antiviral Res*. **1996**, *29*, 163−173.
(10) Ekabo, O. A.; Farnsworth, N. R.; Santisuk, T.; Rentrakul, V. *J. Nat. Prod*. **1993**, *56*, 699−707.

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