# Cochinolide, a New γ-Alkylidene Bicyclic Butenolide with Antiviral Activity, and Its β-Glucopyranoside from *Homalium cochinchinensis*

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A new γ-alkylidene bicyclic butenolide designated as cochinolide (**1**) and its β-glucopyranoside (**3**) were isolated from the root bark of *Homalium cochinchinensis* (Flacoutiaceae). Their structures, except absolute stereochemistries, were determined by spectroscopic means. Cochinolide (**1**) showed moderate antiviral activities against HSV-1 and -2.

*Homalium cochinchinensis* (Lour.) Druce (Flacoutiaceae) has been used in Taiwan as a folk medicine for gonorrhea and as an astringent.[1] We investigated this plant as part of a screen to identify nonnucleosidic lead compounds with antiviral activity from natural sources.[2] There have been no reports of its chemical constituents. We present the isolation of a new antiviral-active γ-alkylidene bicyclic butenolide designated as cochinolide (**1**) and its glucopyranoside (**3**) from the root bark of this plant.

The root bark was separated into four fractions with a Soxhlet apparatus using hexane (fraction A), benzene (fraction B), chloroform (fraction C), and methanol (fraction D) as solvents. Purification of fraction B[3] by combination of column chromatography and flash chromatography yielded the new butenolide **1** designated as cochinolide. Cochinolide was isolated as an optically active yellow amorphous mass {$[\alpha]^{22}_{589}$ +109.4° (*c* 0.0576, MeOH)}. The molecular formula was determined as $C_{15}H_{14}O_4$ by the appearance of a peak at *m/z* 297.0535 (calcd for $C_{15}H_{14}O_4K$; 297.0520) in the HRFABMS and from its diacetate. The IR spectrum (in $CHCl_3$) showed characteristic bands due to hydroxyl groups (3604, 3450 $cm^{-1}$) and a carbonyl group (1763 $cm^{-1}$), which was assigned to a butenolide function because of absorption in the higher frequency region. This deduction was supported by the appearance of a signal at $\delta_C$ 169.50 attributable to an ester carbonyl carbon in the $^{13}C$ NMR spectrum ($CDCl_3$). The presence of a monosubstituted benzene ring was also indicated by the appearance of three sequential signals [$\delta_H$ 7.31 (1H, t, $J$ = 7.2 Hz), 7.36 (2H, t, $J$ = 7.2 Hz), 7.41 (2H, d, $J$ = 7.2 Hz)] in the $^1H$ NMR spectrum ($CDCl_3$) and an absorption maximum at 278 nm in the UV spectrum (MeOH).

Further examination of the $^1H$ NMR spectrum suggested the presence of an ethylene unit [$\delta_H$ 1.75−1.85, 1.95−2.05, 2.40−2.50, 2.80−2.87 (each 1H, m)], a secondary carbinol proton [$\delta_H$ 4.54−4.58 (m)], and an olefinic proton [$\delta_H$ 5.89 (1H, d, $J$ = 4.2 Hz)] (see Table 1). These units would be able to extend to the partial structure of (C)$CH_2CH_2CH(OH)CH$═(C) based on an $^1H$ decoupling experiment, in which the carbinol signal was found to be coupled with not only the olefinic proton but also the more shielded methylene protons ($\delta_H$ 1.75−1.85, 1.95−2.05) in the ethylene units. Furthermore, one more secondary carbinol proton assigned to a benzyl alcohol unit was observed at $\delta_H$ 5.70 as a singlet. These assignments mentioned above were supported by differential NOE experiments and the $^{13}C$ NMR spectrum (see Table 1).

The crosspeak between the benzylic proton ($\delta_H$ 5.70) and a lactone carbonyl carbon ($\delta_C$ 169.50) in the COLOC experiment (8 Hz) indicated the location of the benzyl alcohol function at the α position of the butenolide skeleton. The benzyl proton additionally showed crosspeaks between two quaternary carbons at $\delta_C$ 140.71 ($C_3$) and 148.21 ($C_{3a}$). On the other hand, the olefinic proton ($\delta_H$ 5.89) showed crosspeaks between two quaternary carbons at $\delta_C$ 148.21 ($C_{3a}$) and 158.19 ($C_{7a}$). These observations, especially the common bond connections of the quaternary carbon at $\delta_C$ 148.21 between these protons, indicated that cochinolide was a 3-hydroxybenzyl γ-alkylidene bicyclic butenolide like **1**. Treatment of **1** with acetic anhydride in pyridine gave diacetate **2** [HRFABMS *m/z* 381.0739 (calcd for $C_{19}H_{18}O_6K$: 381.0741); $\nu_{max}$ 1777, 1736 $cm^{-1}$ (CO); $\delta_H$ 2.07, 2.17 (each 3H, s, COC$H_3$), 5.54−5.56 (1H, m, C*H*OAc), 6.67 (1H, s, C*H*OAc)], supporting the proposed structure of **1** for cochinolide (see Table 2).

The second product, **3**, was obtained from fraction D[3] as an optically active yellow amorphous mass {$[\alpha]^{22}_{589}$ +40° (*c* 0.28, MeOH)} by a combination of column chromatography and preparative TLC. The molecular formula of **3** was deduced as $C_{21}H_{24}O_9$ from the appearance of a peak at *m/z* 459.1077 (calcd for $C_{21}H_{24}O_9K$; 459.1057) in the HRFABMS. The NMR data of **3** showed the presence of signals assignable to a hexose {$C_1H$ [$\delta_H$ 4.41 (d, $J$ = 7.5 Hz); $\delta_C$ 101.70], $C_2H$ [$\delta_H$ 3.24−3.28 (m); $\delta_C$ 73.24], $C_3H$ [$\delta_H$ 3.40−3.44 (m); $\delta_C$ 76.21], $C_4H$ [$\delta_H$ 3.40−3.44 (m); $\delta_C$ 69.88], $C_5H$ [$\delta_H$ 3.29−3.31 (m); $\delta_C$ 75.84], $C_6H_2$ [$\delta_H$ 3.76 (1H, dd, $J$ = 12.2, 4.8 Hz), 3.85 (1H, dd, $J$ = 12.2, 3.0 Hz); $\delta_C$ 61.57]} in addition to signals due to a cochinolide unit (see Table 1).

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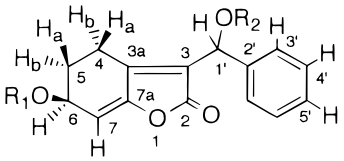
**Table 1.** NMR Data[a] of **1** and **3**

| C | $\delta_H$ | NOE | $\delta_C$ | COLOC (8Hz) |
|---|---|---|---|---|
| 2 | | | 169.50<br>[169.50] | 1′-H (3) |
| 3 | | | 140.71<br>[140.99] | 1′-H (2) |
| 3a | | | 148.21<br>[148.70] | 7-H (3), 1′-H(3) |
| 4 | $\begin{cases} H_b: 2.40-2.50\ (1H, m) \\ H_a: 2.80-2.87\ (1H, m) \end{cases}$<br>$\left[\begin{cases} H_b: 2.54\ (1H, dt, J = 18.1, 6.0) \\ H_a: 2.99\ (1H, dt, J = 18.1, 7.0) \end{cases}\right]$ | 4-$H_a$ (24), 6-H (2), 1′-H (2)<br>4-$H_b$ (22), 1′-H (1) | 19.53<br>[19.40] | |
| 5 | $\begin{cases} H_a: 1.75-1.85\ (1H, m) \\ H_b: 1.95-2.05\ (1H, m) \end{cases}$<br>[1.94−2.00 (2H, m)] | 4-$H_a$ (3), 6-H (5), 7-H (1), 1′-H (3)<br>4-$H_b$ (1), 6-H (6), 7-H (1), 1′-H (3) | 31.33<br>[27.65] | |
| 6 | 4.54−4.58 (m)<br>[4.59 (dd, $J$ = 9.8, 4.7)] | 4-$H_b$ (1), 5-$H_a$ (1), 5-$H_b$ (3), 7-H (4) | 64.83<br>[71.63] | |
| 7 | 5.89 (d, $J$ = 4.2)<br>[5.98 (d, $J$ = 4.7)] | 6-H (3) | 112.28<br>[110.48] | |
| 7a | | | 158.19<br>[150.38] | 6-H (3), 7-H (2) |
| 1′ | 5.70 (s)<br>[5.70 (s)] | 3′-H (4) | 68.88<br>[68.01] | 3′-H (3) |
| 2′ | | | 125.88<br>[126.50] | |
| 3′ | 7.41 (d, $J$ = 7.2)<br>[7.43 (d, $J$ = 7.3)] | | 125.96<br>[125.77] | |
| 4′ | 7.36 (t, $J$ = 7.2)<br>[7.36 (t, $J$ = 7.3)] | | 128.80<br>[127.77] | |
| 5′ | 7.31 (d, $J$ = 7.2)<br>[7.29 (t, $J$ = 7.3)] | | 128.27<br>[128.45] | |
| 1″ | [4.41 (d, $J$ = 7.5)] | | [101.70] | |
| 2″ | [3.24−3.28 (m)] | | [73.24] | |
| 3″ | [3.40−3.44 (m)] | | [76.21] | |
| 4″ | [3.40−3.44 (m)] | | [69.88] | |
| 5″ | [3.29−3.31 (m)] | | [75.84] | |
| 6″ | $\left[\begin{cases} 3.76\ (1H, dd, J = 12.2, 4.8) \\ 3.85\ (1H, dd, J = 12.2, 3.0) \end{cases}\right]$ | | [61.57] | |

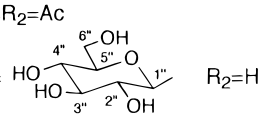
[a] $^1$H NMR (500 MHz in $CDCl_3$ for **1** or in $CDCl_3$+1 drop of $CD_3OD$ for **3**) are reported downfield from internal TMS at 0.00 ppm, and peak multiplicities are quoted in Hz. $^{13}$C NMR assignments are related to internal $CDCl_3$ at 77.00 ppm. $^1$H and $^{13}$C NMR assignments in **1** are based on decoupling, DEPT, differential NOE, C−H COSY or HMQC, and COLOC or HMBC experiments. For NOE and COLOC experiments on **1**, the numbers in parentheses denote the percent enhancement and the number of the bonds involved in the correlation, respectively. The numbers in square brackets denote the chemical shift in **3**.

Acetylation of **3** afforded the corresponding pentaacetate **4** [HRFABMS *m/z* 669.1579 (calcd for $C_{31}H_{34}O_{14}K$; 669.1586); $\delta_H$ 1.94, 1.96, 1.97, 2.03, 2.09 (each 3H, s, COC$H_3$)]. The hexose unit in **3** was determined to be a $\beta$-glucopyranoside substituted at the anomeric carbon based on a large coupling constant ($J$ = ca. 9 Hz) between the well-separated vicinal methine protons in the $^1$H NMR spectrum of the acetate **4** (see Table 2).

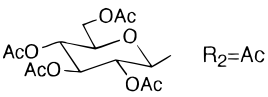


**1**: $R_1$=$R_2$=H (cochinolide)

**2**: $R_1$=$R_2$=Ac



**3**: $R_1$= $R_2$=H



**4**: $R_1$= $R_2$=Ac

The allylic methine carbon ($\delta_C$ 71.63) of the glucoside **3** was deshielded by 6.8 ppm compared to that of cochinolide (**1**) ($\delta_C$: 64.83) in the $^{13}$C NMR spectrum, whereas the benzylic proton ($\delta_H$ 6.58) of the acetylated glucoside **4** by 0.88 ppm compared to that of **1** ($\delta_H$ 5.70) in the $^1$H NMR spectrum. These observations indicated that the aglycon of **3** should be connected to glucose through the allylic alcohol functional group at the $C_6$ position, but not the benzylic one (at $C_3$). Thus, the second product was confirmed to be cochinolide $\beta$-glucopyranoside (**3**).

Using our previously reported procedure,[2] antiviral activities of both compounds against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) were tested. Interestingly, **1** showed moderate activities against HSV-1 ($EC_{50}$ 8.0 mg/mL) and HSV-2 ($EC_{50}$ 22.2 mg/mL),[4] but the glucopyranoside **3** was inactive.

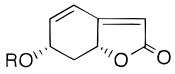
In conclusion, we confirmed the existence of a new 6-hydroxy-$\gamma$-alkylidene bicyclic butenolide with antiviral activity and its glucopyranoside in the root bark of *H. cochinchinensis*. Although it has been shown that menisdaurilide (**5**),[5] aquilegiolide (**6**),[5b,6] and phyllanthrinolactone (**7**)[7] appear in menispermaceous, ranunculaceous, and euphorbiaceous plants, respectively, as structurally related 6-hydroxy bicyclic butenolides, they have an isomeric double bond but lack a benzyl alcohol unit. Thus, this is the first example of the isolation of a 6-hydroxy $\gamma$-alkylidene bicyclic butenolide with a benzyl alcohol function from a plant source. The ninefold excess of **3** in isolated yields compared to **1** might

**Table 2.** NMR Data[a] of **2** and **4**

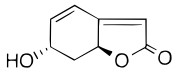
| C | $\delta_H$ | | $\delta_C$ | |
|---|---|---|---|---|
| 2 | | | 169.54 | [169.55] |
| 3 | | | 136.71 | [136.82] |
| 3a | | | 148.59 | [148.92] |
| 4 | 2.84 (t, $J$ = 6.4) | [$H_b$: 2.61 (1H, dt, $J$ = 17.3, 6.0); $H_a$: 2.74 (1H, ddd, $J$ = 17.3, 8.8, 4.7)] | 27.56 | [28.39] |
| 5 | 2.00−2.01 (2H, m) | [$H_a$: 1.80−1.90 (1H, m); $H_b$: 1.95−2.00 (1H, m)] | 19.47 | [19.40] |
| 6 | 5.54−5.56 (m) | [4.44 (dd, $J$ = 9.8, 4.6)] | 66.37 | [72.71] |
| 7 | 5.91 (t, $J$ = 5.3) | [5.86 (d, $J$ = 4.6)] | 107.39 | [109.89] |
| 7a | | | 151.47 | [150.25] |
| 1′ | 6.67 (s) | [6.58 (s)] | 69.80 | [69.52] |
| 2′ | | | 124.09 | [123.59] |
| 3′ | 7.43 (d, $J$ = 7.0) | [7.3 (d, $J$ = 7.2)] | 125.73 | [126.63] |
| 4′ | 7.38 (t, $J$ = 7.0) | [7.30 (t, $J$ = 7.2)] | 128.85 | [128.81] |
| 5′ | 7.34 (d, $J$ = 7.2) | [7.26 (t, $J$ = 7.2)] | 128.76 | [128.66] |
| 1″ | | [4.59 (d, $J$ = 8.1)] | | [100.36] |
| 2″ | | [4.90 (dd, $J$ = 9.5, 8.1)] | | [71.35] |
| 3″ | | [5.14 (t, $J$ = 9.5)] | | [72.65] |
| 4″ | | [4.99 (t, $J$ = 9.5)] | | [68.31] |
| 5″ | | [3.63−3.66 (m)] | | [71.98] |
| 6″ | | [4.08 (1H, dd, $J$ = 12.2, 4.9); 4.16 (1H, dd, $J$ = 12.2, 2.7)] | | [61.44] |
| CH3 | 2.07<br>2.17 (each s) | [1.94; 1.96; 1.97 (each s); 2.03; 2.09] | 20.87<br>21.03 | [20.57; 20.60; 20.63; 20.74; 20.88] |
| CO | | | 167.14<br>170.33 | [167.30; 169.09; 169.33; 170.30; 170.62] |

[a] $^1$H NMR (500 MHz in $CDCl_3$) are reported downfield from internal TMS at 0.00 ppm, and peak multiplicities are quoted in Hz. $^{13}$C NMR assignments are related to internal $CDCl_3$ at 77.00 ppm. $^1$H and $^{13}$C NMR assignments are based on decoupling, DEPT, C−H COSY or HMQC, and COLOC or HMBC experiments. The numbers in square brackets denote the chemical shift in **4**.

suggest that the glucoside **3** is a real natural product in this plant. The absolute stereochemistries on the two chiral centers of cochinolide (**1**) are currently under investigation in our laboratory.




**5**: R=H
**7**: R=β-D-glucose




**6**

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a JASCO IR-700 spectrophotometer. UV spectrum was measured on a Hitachi U-3400. ORD and CD spectra were recorded on JASCO J-20 and J-500 spectrophotometers, respectively. $^1$H and $^{13}$C NMR spectra were recorded with a JEOL JNM GSX-500a spectrometer with tetramethylsilane as internal reference. HRFABMS was recorded on a JEOL JMX-HX 110A spectrometer with a direct inlet system. For column chromatography and flash chromatography Si gel 60 (70−230 mesh ASTM; Merck) and Si gel 60 (230−400 mesh ASTM; Merck) were used, while for TLC and preparative TLC Si gel $GF_{254}$ (Merck) was used.

**Plant Material.** *H. cochinchinensis* was collected in southern Taiwan (San-ti, Men, Pintung Hsien) in August 1993, and divided into five parts of root bark, root wood, trunk bark, trunk wood, and leaves. The dried root bark (195 g) was finely chipped for extraction. A voucher sample (no. Chen 6061) has been deposited with the herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

**Extraction.** The root bark was successively extracted in a Soxhlet apparatus using hexane, $C_6H_6$, $CHCl_3$, and MeOH (1 L × 2 for each) for 8 h. Evaporation of each solvent gave the corresponding extract [hexane (fraction A: 0.64 g), $C_6H_6$ (fraction B: 2.74 g), $CHCl_3$ (fraction C: 2.61 g), and MeOH (fraction D: 16.84 g)].

**Separation of Fraction B.** Fraction B was separated by column chromatography using $CHCl_3$ and then a gradient solvent system of MeOH in $CHCl_3$ to give six major fractions. Further purification of the less polar second fraction by flash chromatography with hexane−EtOAc (1:1) yielded **1** (0.074 g, 0.038%), which was homogeneous on TLC and shown to be pure in the $^1$H NMR spectrum.

**Cochinolide (1):** a yellow amorphous mass; IR ($CHCl_3$) $\nu_{max}$ 3604, 3450, 1763 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 278 nm (3.41); $^1$H and $^{13}$C NMR, see Table 1; HRFABMS $m/z$ 297.0535 ($MK^+$), calcd for $C_{15}H_{14}O_4K$ 297.0529; ORD ($c$ 0.0576, MeOH) $[\alpha]^{22}$ +104° (600 nm), +193° (500), +444° (400); CD ($c$ 2.2 × 10$^{-3}$, MeOH) [$\theta$]: 0 (500 nm), +6270 (290), 0 (400).

**Acetylation of 1.** A mixture of **1** (0.010 g), $Ac_2O$ (0.1 mL), and pyridine (0.1 mL) was allowed to stand at room temperature overnight. After workup, purification of the residue by preparative TLC (EtOAc−hexane 1:5) gave **2** as a yellow amorphous mass (0.006 g, 42%): IR ($CHCl_3$) $\nu_{max}$ 1777, 1736 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 2; HRFABMS $m/z$ 381.0739 ($MK^+$), calcd for $C_{19}H_{18}O_6K$ 381.0741.

**Separation of Fraction D.** A portion (12.06 g) of fraction D was separated by column chromatography

($CHCl_3$−MeOH 1:1) to give three major fractions. Further purification of a portion (0.037 g) of the most polar fraction (1.518 g) by preparative TLC ($CHCl_3$−MeOH 10:1, triple developments) yielded **3** [0.011 g, calcd amount: 0.638 g (0.327%)], which was homogeneous on TLC and shown to be pure in the $^1$H NMR spectrum.

**Cochinolide-*β*-glucopyranoside (3):** a yellow amorphous mass; IR (Nujol) $\nu_{max}$ 3376 (broad), 1751 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; HRFABMS *m*/*z* 459.1077 ($MK^+$), calcd for $C_{21}H_{24}O_9K$ 459.1057; ORD (*c* 0.28, MeOH) $[\alpha]^{22}$ +40° (589 nm).

**Acetylation of 3.** A mixture of **3** (0.007 g), $Ac_2O$ (0.07 mL), and pyridine (0.07 mL) was allowed to stand at room temperature for 3 h. After workup, **4** was obtained as a yellow amorphous mass (0.010 g, quant.): IR (Nujol) $\nu_{max}$ 1751 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 2; HRFABMS *m*/*z* 669.1579 ($MK^+$), calcd for $C_{31}H_{34}O_{14}K$ 669.1586.

## References and Notes


(1) Gan, W. S. *Manual of Medicinal Plants in Taiwan*; National Research Institute of Chinese Medicine, Taiwan, 1965; Vol. 3, p 567.

(2) (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.

(3) We also isolated some salicin derivatives from fractions B, C, and D. The results will be reported elswhere.

(4) Acyclovir was used as a control [HSV-1 ($EC_{50}$ 0.24 mg/mL) and -2 ($EC_{50}$ 0.22 mg/mL)].

(5) (a) Takahashi, K.; Matsuzawa, S.; Takani, M. *Chem. Pharm. Bull.* **1978**, *26*, 1677−1681. (b) Otsuka, H.; Ito, A.; Fujioka, N.; Kawamata, K.-I.; Kasai, R.; Yamasaki, K.; Satoh, T. *Phytochemistry* **1993**, *33*, 389−392.

(6) Guerriero, A.; Pietra, F. *Phytochemistry* **1984**, *23*, 2394−2396.

(7) Ueda, M.; Shigemori-Suzuki, T.; Yamamura, S. *Tetrahedron Lett.* **1995**, *36*, 6267−6270. Interestingly, this product was isolated as the active principle of a nyctinatic plant showing a strong leaf-closing effect.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