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# NEW PHENYLPROPANOID-SUBSTITUTED FLAVAN-3-OLS FROM THE LEAVES OF *CASTANOPSIS SCLEROPHYLLA*

**Yong-Lin Huang,[a,b] Yosuke Matsuo,[a] Takashi Tanaka,[*,a] Isao Kouno,[a] Dian-Peng Li,[b] and Gen-ichiro Nonaka[c]**

[a]Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852-8521, Japan; t-tanaka@nagasaki-u.ac.jp
[b]Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Guilin 541006, China
[c] Usaien Pharmaceutical Company, Ltd., 1-4-6 Zaimoku, Saga 840-0055, Japan

**Abstract** – In the course of a chemotaxonomical study of Chinese Castanopsis species, two previously unknown flavan-3-ols, named sclerophynins A and B, were isolated from the leaves of *C. sclerophylla*, together with five related known compounds. On the basis of spectroscopic analysis, the new compounds were characterized to be (4*S*,8*R*,9*S*)-4-(3,4-dihydroxyphenyl)-8-(3,4,5-trihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-2-one and (4*S*,7*S*,8*R*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,6,7-tetrahydro-5-glucopyranosyloxy-7-hydroxy-2*H*,8*H*-benzo[1,2-*b*:5,4-*b*']dipyran-2-one, respectively.

The genus Castanopsis belongs to the family Fagaceae and contains approximately 120 species.[1] Over 50 species have been recorded in East Asia, many of which are important as ornamental trees and for use as construction materials. Seeds of some species are edible and the leaves and bark have been used medicinally. Despite the large number of species, however, few chemotaxonomical studies have been performed. Previously, characteristic triterpene hexahydroxydiphenoyl (HHDP) esters were isolated from the leaves of *C. cuspidata* var. *sieboldii* and *C. hystrix*.[2,3] The triterpene HHDP esters have been found only in these Castanopsis species and have been shown to have chemotaxonomical significance. Thus, we began a phytochemical study of the phenolic constituents of the Chinese Castanopsis species.

*C. sclerophylla* is an evergreen tree distributed throughout Southern China.[4] The leaves and seeds are rich in tannins and are used for the treatment of hemorrhage, chronic ulcers and diarrhea. Our preliminary

analysis, using HPLC and TLC, indicated that the leaves do not contain triterpene HHDP esters. It did, however, reveal the presence of unusual flavan-3-ols. This paper presents the structure determination of two previously unknown compounds.

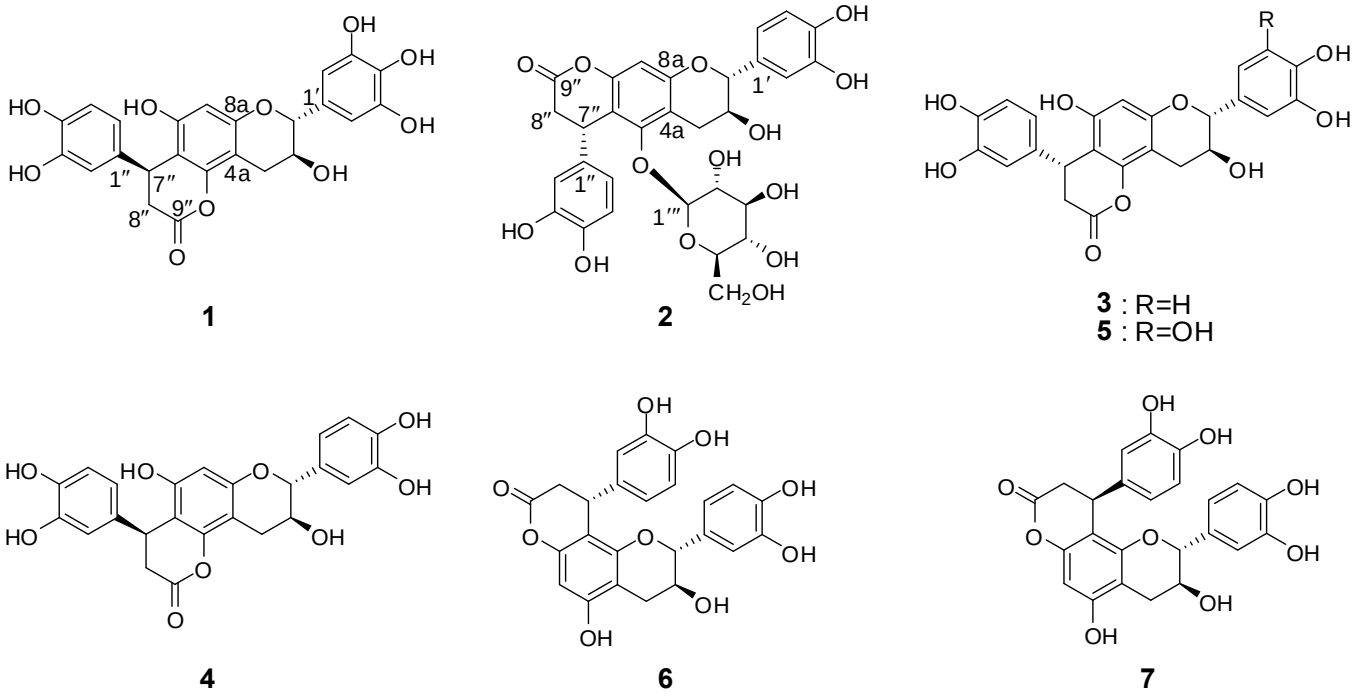


**Figure 1.** Structures of compounds **1**-**7**

The fresh leaves were extracted with 80% aqueous acetone, and the extract was separated by a combination of Sephadex LH-20, Diaion HP20SS, MCI gel CHP20P and Toyopearl HW40F column chromatography. TLC analysis of the fractions, with detection by anisaldehyde-$H_2SO_4$ reagent, revealed the presence of catechin-related compounds that gave characteristic red colorations. Seven flavan-3-ols were isolated. Five of these were identified, by comparison with spectroscopic data reported in the literature. They were: (4*R*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-2-one (**3**),[5] (4*S*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-2-one (**4**),[5] (4*R*,8*R*,9*S*)-4-(3,4-dihydroxyphenyl)-8-(3,4,5-trihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-2-one (apocynin D) (**5**),[6] (2*R*,3*S*,10*R*)-2,10-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-2*H*,8*H*-benzo-[1,2-*b*:3,4-*b*']dipyran-8-one (rhinchoin Ia) (**6**),[5] (2*R*,3*S*,10*S*)-2,10-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-8-one (**7**) (Figure 1).[5] Interestingly, (+)-catechin was not obtained from this plant, despite the fact that the core flavan-3-ol of these compounds is (+)-catechin.

**Table 1.** $^{1}$H-(500 MHz) and $^{13}$C-(125 MHz) NMR data for **1** and **2** (in acetone-$d_6$)[a]

| | **1** | | | **2** | |
|---|---|---|---|---|---|
| Position | $^{1}$H | $^{13}$C | Position | $^{1}$H | $^{13}$C |
| 2 | 4.62 (d, 6.3) | 82.9 | 2 | 4.80 (d, 6.4) | 82.3 |
| 3 | 4.05 (ddd, 7.0, 6.3, 5.6) | 67.5 | 3 | 4.03, m | 67.6 |
| 4 | 2.86 (dd, 16.4, 5.6) | 27.3 | 4 | 3.19, m | 28.3 |
| | 2.67 (dd, 16.4, 7.0) | | | 2.89 (dd, 16.6, 4.5) | |
| 5 | | 151.7 | 5 | | 152.2 |
| 6 | | 106.5 | 6 | | 114.6 |
| 7 | | 154.1 | 7 | | 154.1 |
| 8 | 6.24 (s) | 99.2 | 8 | 6.42 (s) | 101.4 |
| 4a | | 101.6 | 4a | | 115.2 |
| 8a | | 155.5 | 8a | | 155.2 |
| 1' | | 131.1 | 1' | | 131.6 |
| 2', 6' | 6.45 (s) | 107.5 | 2 ' | 6.84 (d, 1.7) | 114.6 |
| 3', 5' | | 146.3 | 3 ' | | 145.6 |
| 4' | | 133.3 | 4' | | 145.6 |
| 1" | | 134.9 | 5' | 6.76 (d, 8.3) | 115.7 |
| 2" | 6.64 (d, 2.0) | 114.8 | 6' | 6.68 (dd, 8.3, 1.7) | 119.1 |
| 3" | | 114.8 | 1" | | 134.5 |
| 4" | | 145.8 | 2" | 6.69 (d, 2.0) | 115.2 |
| 5" | 6.71 (d, 8.0) | 116.1 | 3" | | 145.8 |
| 6" | 6.48 (dd, 8.0, 2.0) | 119.1 | 4" | | 144.6 |
| 7" | 4.49 (dd, 6.7, 1.8) | 34.9 | 5" | 6.66 (d, 8.0) | 116.0 |
| 8" | 3.06 (dd, 15.9, 6.7) | 38.1 | 6" | 6.64 (dd, 8.0, 2.0) | 118.6 |
| | 2.83 (dd, 15.9, 1.8) | | 7" | 4.97 (dd, 6.4, 1.8) | 34.3 |
| 9" | | 168.0 | 8" | 3.12 (dd, 16.0, 6.4) | 37.9 |
| | | | | 2.84 (dd, 16.0, 1.8) | |
| | | | 9" | | 168.5 |
| | | | 1'" | 4.76 (d, 7.3) | 106.4 |
| | | | 2'" | 3.43, m | 75.0 |
| | | | 3'" | 3.41, m | 77.7 |
| | | | 4'" | 3.33 (t, 9.2) | 70.9 |
| | | | 5'" | 3.20, m | 77.6 |
| | | | 6'" | 3.80 (dd, 12.1, 2.5) | 62.4 |
| | | | | 3.62 (dd, 12.1, 6.2) | |

[a] Chemical shifts are given in δ values; multiplicities and coupling constants ($J$ in Hz) in parentheses.

Two unidentified compounds remained, compounds **1** and **2**. Compound **1** was characterized to be a flavan-3-ol, based on its dark blue coloration with ferric chloride reagent and reddish orange coloration with the anisaldehyde-$H_2SO_4$ reagent.[7] HR FAB-MS analysis confirmed the molecular formula as $C_{24}H_{20}O_{10}$, which is the same as that of apocynin D (**5**). The $^{1}$H and $^{13}$C NMR spectra (Table 1) were also

related to those of **5**. The $^{13}$C NMR spectrum, in particular, was almost superimposable, indicating that **1** is an isomer of **5**. The location of a pyrogallol ring at C-2 and a catechol ring at C-7" were confirmed by HMBC correlations of the aromatic protons with the methine carbons (Figure 2). The chemical shift of the A-ring methine (δ 99.2) were in agreement with those of C-6 substituted flavan-3-ols [**3**: δ 99.2, **4**: δ 99.3, and **5**: δ 99.7] rather than those of C-8 substituted isomers [**6**: δ 96.0 and **7**: δ 96.1].[5,6] This was confirmed by HMBC correlations of C-8a with H-8 and H-2. The presence of a free hydroxyl group at C-7, and the formation of a cyclic ester with the C-5 hydroxyl group, was confirmed by the deuterium induced differential isotope shift of the A-ring carbons. This is caused by the substitution of the hydroxyl proton with deuterium (Figure 3). The $^{13}$C NMR spectra of **1** were measured in acetone-$d_6$+$H_2O$ (95:5, v/v) and acetone-$d_6$+$D_2O$ (95:5, v/v), and the chemical shifts were carefully compared. The C-7 signal showed a relatively large chemical shift difference ($\Delta\delta_{OH\text{-}OD}$ 0.107) compared to those of C-5 ($\Delta\delta_{OH\text{-}OD}$ -0.008) and C-8a ($\Delta\delta_{OH\text{-}OD}$ -0.008). These results demonstrated the presence of a hydroxyl group at the C-7 position.

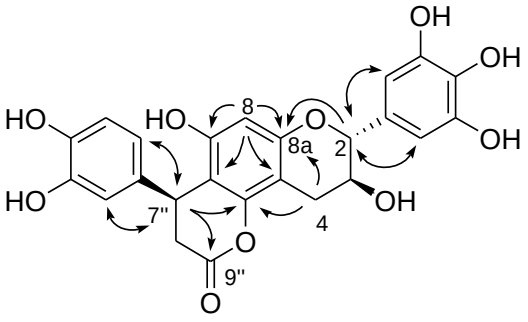


**Figure 2.** Selected HMBC correlations (H to C) of **1**

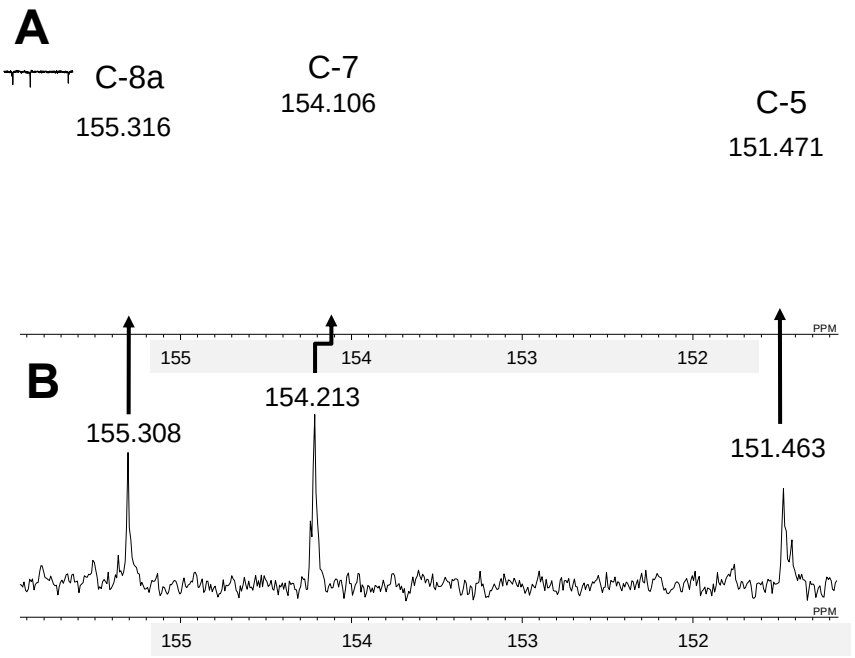


**Figure 3.** Deuterium induced differential isotope shift of the A-ring carbons of **1**.
A: in acetone-$d_6$ + $D_2O$ (95:5), B: acetone-$d_6$ + $H_2O$ (95:5)

Regarding the stereochemistry of compound **1**, a 2,3-*trans* configuration of the flavan-3-ol C-ring was apparent from the large coupling constant between H-2 and H-3 (6.3 Hz). Absolute configuration, including that of C-7", was determined by comparison of the CD spectrum with those of known compounds.[8] The negative sign of the Cotton effects, at 278 nm ($[\theta]_{278}$ $-1.1\times10^5$) and 231 nm ($[\theta]_{231}$ $-3.3\times10^4$), was similar to those of **4,** with 2-*R*, 7"-*S* configuration. Compounds **3** and **5**, with 7"-*R* configuration, showed a positive Cotton effect at 230 nm. Based on these spectroscopic results, the structure of **1** was determined to be as shown in Figure 1 and was named sclerophynin A. The systematic name, according to the IUPAC system, is (4*S*,8*R*,9*S*)-4-(3,4-dihydroxyphenyl)-8-(3,4,5-trihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-2-one.

Compound **2** produced $^1$H and $^{13}$C NMR signals similar to those of **1** (Table 1), indicating that **2** is also a 2,3-*trans*-flavan-3-ol. Two major differences were observed, however. The first was observed in the aromatic regions, where two sets of ABX-type signals, arising from two catechol rings, were apparent instead of the signals of the pyrogallol and catechol rings of **1**. The second difference was the appearance of signals attributable to a sugar moiety, the chemical shifts and $^1$H-$^1$H coupling constants of which suggested that **2** is a β-glucopyranoside. Acid hydrolysis and subsequent HPLC analysis confirmed that the sugar was D-glucose.[9] The HMBC correlations of C-5 (δ 152.2), with the glucose anomeric proton (δ 4.76) and C-ring H-4 (δ 3.19 and 2.89), indicated that the glucose was attached to the C-5 hydroxyl group through a glycosidic linkage. The substitution of the phenylpropanoid unit at the C-6 position was indicated by the appearance of HMBC correlations of C-8a with H-8 and H-2. The molecular formula $C_{30}H_{30}O_{14}$ was shown by the HR FAB-MS (*m/z* 615.5579 $[M+H]^+$, Calcd for $C_{30}H_{31}O_{14}$ 615.5598), and this indicated lactone formation at the C-7 hydroxyl group. Furthermore, the CD spectrum showed a negative Cotton effect at 228 nm ($[\theta]_{228}$ $-4.9\times10^4$) indicating 7"-*S* configuration.[8] The Cotton effect related to the C-2 configuration at 278 nm was not observed in the spectrum, however, it was deduced to be *R* from its biogenetic relationship to the coexisting compounds **3**–**7**. Based on these results, **2** was characterized to be (4*S*,7*S*,8*R*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,6,7-tetrahydro-5-glucopyranosyloxy-7-hydroxy-2*H*,8*H*-benzo-[1,2-*b*:5,4-*b*']dipyran-2-one and was named sclerophynin B. Compound **2** is a rare example where the phenylpropanoid unit, at the C-6 position, has formed a lactone ring with the C-7 hydroxyl group. To the best of our knowledge, the compound vaccinin A, isolated from blueberry leaves, is the only precedent

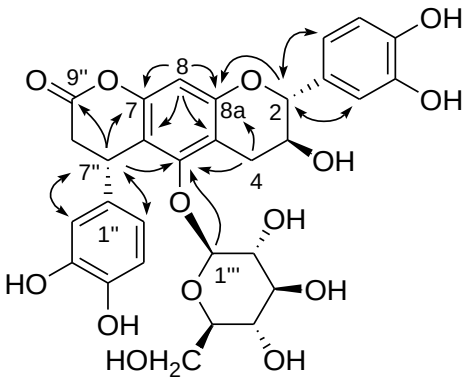


**Figure 4.** Selected HMBC correlations (H to C) of **2**

with a related structure.[10] In other related compounds, such as **3**–**5**, the lactone ring was formed with the C-5 hydroxyl group.

Phenylpropanoid-substituted flavan-3-ols have also been isolated from *C. hystrix* and *C. cuspidata* var. *sieboldii*.[5,11] In other plants, such as blueberry,[10] chinchona bark,[12,13] and *Kandelia candel*,[14] phenylpropanoid-substituted flavan-3-ols are accompanied by the free flavan-3-ols and related procyanidins. TLC analysis, with anisaldehyde-$H_2SO_4$ detection, indicated the presence of dimeric and oligomeric procyanidins in the leaves of *C. screrophylla*. In contrast, catechin and gallocatechin, which are structural cores of compounds **1**–**7**, were not detected in the leaves of this plant. Further studies on the phenolic constituents of *C. sclerophylla* and other Chinese Castanopsis species are currently in progress.

## EXPERIMENTAL

IR spectra were obtained with a JASCO FT/IR-410 spectrophotometer (JASCO Co., Tokyo, Japan). UV and CD spectra were measured with JASCO V-560 UV/VIS and JASCO J-725N spectrophotometers, respectively. $^1$H and $^{13}$C NMR spectra were recorded in acetone-$d_6$ with Varian Unity plus 500 (Varian, Palo Alto, CA) and JEOL JNM-AL400 (JEOL Ltd., Tokyo, Japan) spectrometers operating at 500 and 400 MHz for $^1$H, and 125 and 100 MHz for $^{13}$C, respectively. Coupling constants were expressed in Hz, and chemical shifts were given on a δ (ppm) scale. MS was recorded on a Voyager-DE PRO (Applied Biosystems, Foster City, CA) and a JEOL JMS-700N spectrometer. 2,5-dihydroxybenzoic acid and m-nitrobenzyl alcohol were used as the matrix for MALDI-TOF-MS and FAB-MS measurements, respectively. Column chromatography was performed with Diaion HP20SS (Mitsubishi Chemical Co. Tokyo, Japan), MCI gel CHP 20P (75-150 μm; Mitsubishi Chemical), Sephadex LH-20 (25-100 μm; GE Healthcare Bio-Science AB, Uppsala) and Toyopearl HW40F (75 μm; Tosoh Bioscience Japan, Tokyo, Japan). TLC was performed on precoated Kieselgel 60 F254 plates (0.2 mm thick; Merck, Darmstadt, Germany) with toluene-ethyl formate-formic acid (1:7:1, v/v/v) or chloroform-methanol-water (7:3:0.5 or 8:2:0.2, v/v/v). Spots were detected by UV illumination (254 nm) and by spraying with 2% ethanolic $FeCl_3$, anisaldehyde-$H_2SO_4$, and 10% sulfuric acid reagents, followed by heating.

### Plant material

The fresh leaves of *C. sclerophylla* were collected at Guangxi Institute of Botany, Guilin, China, and identified by Prof. Yan Liu. The voucher specimen (CS20090720) was deposit in the Herbarium of Guangxi Institute of Botany.

**Extraction and separation**

The fresh leaves (8.60 kg) were extracted with 80% acetone (40 L) at room temperature for 24 h and evaporated by rotary evaporation. The extraction was repeated 3 times. The extract (570 g) was partitioned between $H_2O$ (3 L) and $Et_2O$ (1 L) 3 times. The aqueous layer was fractionated by Sephadex LH-20 column chromatography (10 cm i.d. × 40 cm) with water containing increasing proportions of MeOH (10–100%, 10% stepwise, each 2 L) and finally with 60% acetone, to yield 9 fractions (Fr. 1–9). Fraction 5 (73.2 g), which was positive to anisaldehyde-$H_2SO_4$ reagent, was subjected to Diaion HP20SS column chromatography (4 cm i.d. × 40 cm) with MeOH-$H_2O$ (10:90-100:0), to give eight subfractions (fr.5-1–fr.5-8). Fraction 5-3 (29.8 g) was separated by a combination of column chromatography over Sephadex LH-20 (6 cm i.d. × 40 cm) with 10–90% MeOH (10% stepwise elution), MCI gel CHP20P (3 cm i.d. × 40 cm) with 10–90% MeOH, and Toyopearl HW40F (3 cm i.d. × 30 cm) with 10–90% MeOH, to afford **1** (42 mg), **6** (7 mg), and **7** (32 mg). Similarly, chromatography of fraction 5-5 (20.1 g) yielded **2** (12 mg), **3** (156 mg), **4** (164 mg), and **5** (22 mg).

**Sclerophynin A (1).** Brown amorphous powder, $[\alpha]_D^{26}$ +22.6 (*c* 0.11, MeOH); HR-FAB-MS *m/z*: 469.4237 $[M+H]^+$ (Calcd for $C_{24}H_{21}O_{10}$: 469.4247); IR $\nu_{max}$ $cm^{-1}$: 3234, 2402, 1753, 1698, 1449; UV $\lambda_{max}$ (MeOH) nm (log ε): 284 (3.92), 207 (4.91); CD (MeOH) [θ] (nm): $+1.7\times10^5$ (223), 0 (227), $-3.3\times10^4$ (231), 0 (235), $+1.2\times10^5$ (245), 0 (273), $-1.1\times10^5$ (278); MALDI-TOF-MS *m/z*: 491 $[M+Na]^+$; $^1$H-NMR (500 MHz, acetone-$d_6$) and $^{13}$C-NMR (125 MHz, acetone-$d_6$). See Table 1.

**Sclerophynin B (2).** Brown amorphous powder, $[\alpha]_D^{20}$ -24.0 (*c* 0.11, MeOH); HR-FAB-MS *m/z*: 615.5579 $[M+H]^+$ (Calcd for $C_{30}H_{31}O_{14}$: 615.5598); IR $\nu_{max}$ $cm^{-1}$: 3369, 1739, 1518, 1270; UV $\lambda_{max}$ (MeOH) nm (logε): 282 (4.03), 207 (4.39); CD (MeOH) [θ] (nm): $+5.8\times10^5$ (206), 0 (215), $-4.9\times10^5$ (228), 0 (249); MALDI-TOF-MS *m/z*: 637 $[M+Na]^+$; $^1$H-NMR (500 MHz, acetone-$d_6$) and $^{13}$C-NMR (125 MHz, acetone-$d_6$). See Table 1.

**Determination of aldose configuration**

A solution of **2** (0.5 mg) in 0.5 M HCl (0.3 mL) was heated at 90 °C in a screw-capped vial for 2 h. The mixture was neutralized by addition of Amberlite IRA400 ($OH^-$) and filtered. The filtrate was dried *in vacuo*, dissolved in pyridine (50 μL) containing L-cysteine methyl ester (10 mg/mL) and heated at 60 °C for 1 h. A solution (100 μL) of *o*-tolyl isothiocyanate in pyridine (10 mg/mL) was added to the mixture, which was then heated at 60 °C for 1 h. The final solution was analyzed directly by HPLC [Cosmosil 5$C_{18}$ AR II (250×4.6 mm i.d., Nacalai Tesque Inc.); 25% MeCN in 50 mM $H_3PO_4$; flow rate, 0.8 mL/min;

detection, 250 nm]. The $t_R$ of the peak at 18.16 min coincided with that of the derivative prepared from D-glucose ($t_R$ of L-form: 16.48 min).

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