Phenyl Ethers from Cultured Lichen Mycobionts of *Graphis scripta* var. *serpentina* and *G. rikuzensis*

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Spore-derived mycobionts of the lichen *Graphis scripta* var. *serpentina* and *G. rikuzensis* were cultivated on a malt-yeast extract medium supplemented with 10% sucrose and their metabolites were investigated. 3,3′-Dihydroxy-5,5′-dimethyldiphenyl ether was isolated from the cultures of the mycobionts of *G. scripta* var. *serpentina*, while a new phenyl ether, rikuzenol, along with two known diphenyl ethers, violaceol-I and violaceol-II, were isolated from those of *G. rikuzensis*. The structure of the new compound was determined by spectroscopic methods. Violaceol-I was chemically synthesized and interconversion between violaceol-I and violaceol-II was proven.

Key words *Graphis scripta* var. *serpentina*; *Graphis rikuzensis*; lichen; isolated mycobiont; phenyl ether

Lichens, symbiotic associations of mycobiont and photobiont partners, produce a variety of characteristic secondary metabolites.[1] Some lichen substances have been found to exhibit a wide range of potentially useful biological activities, *e.g.*, antibiotic and anticancer activities, and a monoamine oxidase inhibitory effect.[2] On the other hand, our recent studies have demonstrated that cultures of spore-derived lichen mycobionts are capable of producing certain lichen substances or novel metabolites under osmotically stressed conditions.[3,4] It was pointed out that cultures of lichen mycobionts could be new sources of bioactive compounds. In a continuation of our studies on cultured lichen mycobionts,[5] we cultivated spore-derived mycobionts of *Graphis scripta* (L.) ACH. var. *serpentina* MEYER and *G. rikuzensis* (VAIN.) NAK. and isolated four phenolics from their cultures. In this paper, we describe the isolation and characterization of these compounds.

Polyspore-derived mycobionts of *Graphis scripta* var. *serpentina* and *G. rikuzensis* collected in Hidakokufu, Gifu Prefecture, Japan and Aizuwakamatsu, Fukushima Prefecture, Japan, respectively, were cultured on conventional malt-yeast extract medium supplemented with 10% sucrose at 18 °C in the dark. After 8—10 months, the cultivated colonies were harvested and extracted with acetone.

Subsequent purification of the extract from the cultured mycobionts of *G. scripta* var. *serpentina* by preparative TLC and preparative HPLC afforded compound **1**. The ^{1}H- and ^{13}C-NMR spectra of **1**, together with its structural formula $C_{14}H_{14}O_3$, suggested a symmetrical diphenyl ether structure for **1**. Compound **1** was identified as 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether, which has been isolated from *Emericella falconensis*.[6] This is the first instance of isolation of this compound from cultured mycobionts of lichens.

Fractionation of the extract from cultured mycobionts of *G. rikuzensis* by a combination of chromatographic procedures afforded three compounds, **2**, **3** and **4**.

Compounds **2** and **3** were found to be isomers with a molecular formula $C_{14}H_{14}O_5$. The ^{1}H- and ^{13}C-NMR spectral features suggested **2** and **3** to be violaceol-I and violaceol-II, respectively, both of which are dimeric ethers consisting of 3,4,5-trihydroxytoluene units. Further structural confirmation was given by detailed 2D-NMR spectral analyses of their

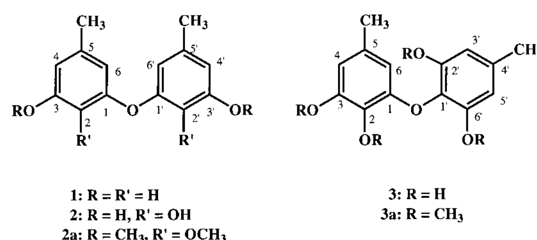

Chart 1

methylated compounds, **2a** and **3a**. Compounds **2** and **3** have already been obtained as an inseparable mixture from *Emericella violacea*.[7] It was, however, found in our study that **2** and **3** could be separated by HPLC, but isomerized after standing in MeOH. This surprising finding prompted us to synthesize violaceol-I in order to confirm the unexpected phenomenon.

Compound **5** prepared from methyl gallate[8] was reduced with $LiAlH_4$ to afford **6**. Subsequent hydrogenolysis of **6** gave **7**. On the other hand, bromoveratraldehyde was treated with $NaBH_4$ and the resulting alcohol **8** was protected as a tetrahydropyranyl (THP) ether to yield **9**. Ullmann reaction[9] of **7** and **9** afforded **10**, which was hydrogenated over Pd–C to **2a**. The methyl ether **2a** was deprotected with BBr_3 (Chart 2). The phenolic product, which should exclusively be **2**, was analyzed by HPLC to be found to be a mixture of **2** and **3** in the ratio of 18 : 11. This result confirmed that diphenyl ether **2** could be isomerized to **3**.

A new compound **4**, named rikuzenol, was obtained as colorless oil. The HR-EI mass spectrum of **4** exhibited a peak at *m/z* 382.1058 $[M]^+$, indicating a molecular formula of $C_{21}H_{18}O_7$. It showed UV maxima at 208, 230sh, 253sh, and 280 nm. Its ^{1}H-NMR spectrum exhibited signals for three methyl groups at δ 2.01, 2.06 and 2.21 (each s), two aromatic protons at δ 6.59 and 6.60 (each br s), and a pair of *meta*-coupled aromatic protons at δ 6.47 and 6.75 (each m). The ^{13}C-NMR spectrum of **4** showed three methyl signals and eighteen aromatic carbon signals, of which four were CH carbons and fourteen quaternary sp^2 carbons. These spectral features, together with its molecular formula and co-occurrence of violaceols-I and II, suggested that the compound

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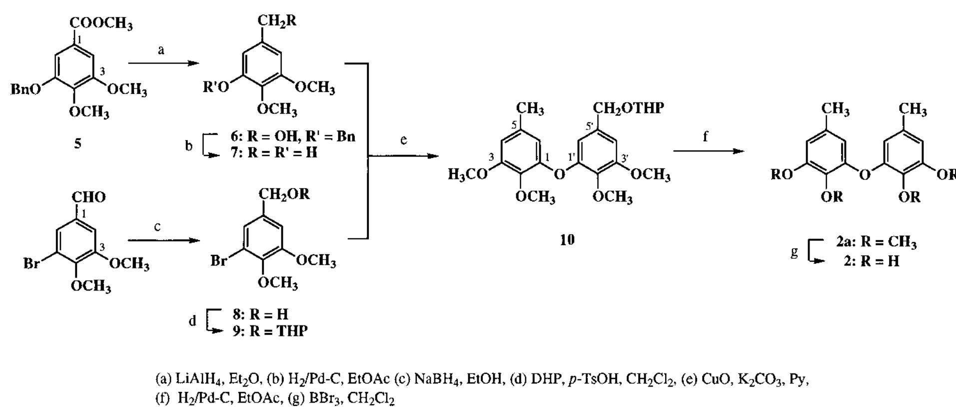

Chart 2

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **2**, **3** and **4** in CD_3OD

C	**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		$146.6^{b,y)}$		$148.3^{b,y)}$		$143.9^{d)}$
2		134.9		132.8		$136.3^{e,x)}$
3		$147.5^{b,x)}$		$147.1^{b,x)}$		$147.3^{f,x)}$
4	$6.40^{a,x)}$ m	$112.5^{c,x)}$	$6.30^{a,x)}$ m	$111.3^{c,x)}$	$6.59^{a,x)}$ br s	$114.8^{g,x)}$
5		129.0		129.8		$128.0^{h,x)}$
6	$6.15^{a,y)}$ m	$111.6^{c,y)}$	$5.90^{a,y)}$ m	$107.9^{c,y)}$		$124.2^{i,x)}$
Me	2.12 br s	21.0	2.05 br s	21.2	$2.06^{b,x)}$ br s	$19.18^{j)}$
1′		$146.6^{b,y)}$		130.1		$143.4^{d)}$
2′		134.9		151.6		$137.4^{e,y)}$
3′		$147.5^{b,x)}$	6.27 br s	109.7		$147.5^{f,y)}$
4′	$6.40^{a,x)}$ m	$112.5^{c,x)}$		136.7	$6.60^{a,y)}$ br s	$114.5^{g,y)}$
5′		129.0	6.27 br s	109.7		$128.3^{h,y)}$
6′	$6.15^{a,y)}$ m	$111.6^{c,y)}$		151.6		$124.0^{i,y)}$
Me	2.12 br s	21.0	2.21 br s	21.4	$2.01^{b,y)}$ br s	$19.21^{j)}$
1″						$150.1^{k,w)}$
2″						136.3
3″						$151.0^{k,z)}$
4″					$6.47^{c,z)}$ m	$112.8^{l,z)}$
5″						135.0
6″					$6.75^{c,w)}$ m	$115.7^{l,w)}$
Me					2.21 br s	21.2

a—l) Values with the same superscript may be interchangeable. *x—w*) The carbon signals are correlated to the proton signals with the same superscript by means of HMBC and HMQC.

consisted of three units of 3,4,5-trihydroxytoluene, which were connected to each other through a carbon–carbon bond as well as two ether linkages. The heteronuclear multiple-bond correlation (HMBC) experiments (Fig. 1) and ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) of **4** allowed us to assign all of the ^{1}H- and ^{13}C-NMR signals in each unit of 3,4,5-trihydroxytoluene (Table 1). The corresponding signals (C-1—C-6, 5-Me to C-1′—C-6′, 5′-Me) due to two units with a single aromatic proton resonated at nearly identical frequencies. The signals for C-6 and C-6′ were observed as quaternary carbons at δ 124.2 and 124.0 in the ^{13}C-NMR spectrum of **4**, while that for C-6 of violaceol-I appeared at δ 111.6. These findings suggested that two units of 3,4,5-trihydroxytoluene were linked through a carbon–carbon bond between C-6 and C-6′. On the other hand, the ^{13}C-NMR spectral features of the residual unit (C-1″—C-6″, 5″-Me) with two aromatic protons differed remarkably from those of the former units and showed an asymmetrical character. Accordingly, **4** could be assumed to have a structure in which diphenyl derivative **A** or dibenzofuran **B** (Fig. 2) was connected with another unit of trihydroxytoluene through an ether linkage(s).

The structure was finally confirmed by detailed 2D-NMR studies on a methylated compound of **4** (Fig. 1). Methylation of **4** with Me_2SO_4 gave pentamethyl ether **4a**, indicating the presence of five hydroxyl groups in **4**. Duplicated HMBC interactions from an aromatic proton (H-4/H-4′) to two carbon signals attached to a methoxyl group (C-2 and C-3/C-2′ and C-3′) and to a signal for a methyl group (5-Me/5′-Me) indicated a partial structure **p-1**, and further HMBC cross peaks between OMe (δ 3.89) and C-3″, between H-4″ and C-2″, C-3″, and between H-6″ and C-1″, C-2″ revealed a partial structure **p-2** (Fig. 2). Two partial structures could be depicted as a single structure **4a**. The proposed structure was further supported by nuclear Overhauser enhancement spectroscopy (NOESY) experiments (Fig. 1). Consequently, the structure of the new compound rikuzenol was represented by **4**.

Phenyl ether derivatives represented by violaceols-I and II

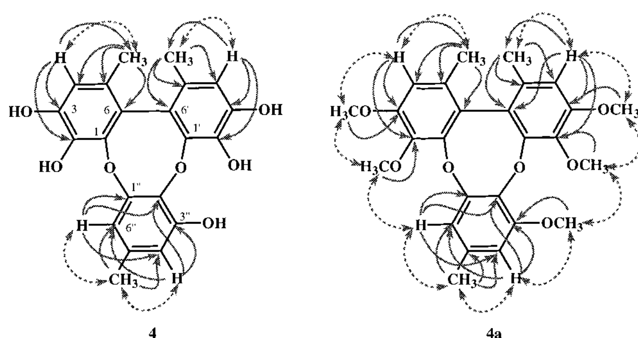

Fig. 1. HMBC Correlations (Bold Arrows) and NOEs (Dotted Arrows) Observed for Compounds **4** and **4a**

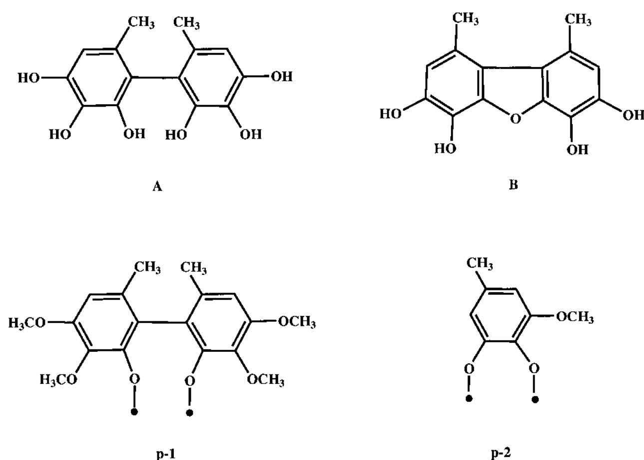

Fig. 2

have so far been isolated as fungal metabolites, but not from natural thalli of lichens, although orcinol, a unit of 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether, is known as a lichen substance. This is the first instance of the isolation of phenyl ethers from lichen mycobionts. It is of great interest from the viewpoint of their physiological and biological significances that **1**, **2** and **3** were found to exhibit antimicrobial activity. It might be postulated that the metabolic pathways of the diphenyl ether derivatives could be expressed for survival of the mycobiont in pre-lichenized conditions.

Experimental

Melting points were measured on a Yanaco micro melting point apparatus and are reported uncorrected. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and the IR spectra on a Shimadzu FTIR-8200 infrared spectrophotometer. HR-EI-MS were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with Varian VXR-500, Varian Gemini-300 and Varian Gemini-200 spectrometers, with tetramethylsilane as internal standard. HPLC was performed using a Waters system (600E Multisolvent Delivery System, 486 Tunable Absorbance Detector). Thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck) and spots were visualized under UV light.

Plant Material Specimens of *Graphis scripta* var. *serpentina* and *G. rikuzensis* were collected from the bark of trees in Hidakokufu, Gifu Prefecture, Japan (500 m alt.) and Aizuwakamatsu, Fukushima Prefecture, Japan (400 m alt.), respectively. The voucher specimens were identified by Prof. M. Nakanishi of Hiroshima University, Japan and were deposited at Osaka City Institute of Public Health and Environmental Sciences with the registration No. NH9881341 (*G. scripta* var. *serpentina*) and No. NH9810144 (*G. rikuzensis*). Mycobionts of *Graphis* spp. were obtained from the spores discharged from apothecia of a thallus, and were cultivated in test tubes containing modified MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g, H_2O 1 l, pH 7) at 18 °C in the dark. After cultivation for 8—10 months, the colonies and slants were harvested and freeze-dried.

Isolation of Compounds

Graphis scripta var. *serpentina*: The harvested colonies (56 test tubes, dry weight 14.11 g) were extracted with acetone at room temperature, and the combined extracts were concentrated under reduced pressure to give a residue (446 mg). The extracts were repeatedly subjected to preparative TLC (toluene–acetone 4 : 1) and preparative HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O, 9 : 11), giving rise to **1** (2.9 mg). **1**: UV λ_{max} nm: 207, 232sh, 278. UV maxima were measured by HPLC equipped with a photodiode array detector, using MeCN–H_2O (1 : 1) as a solvent. ^{1}H-NMR: as in ref 6. ^{13}C-NMR (CD_3OD): δ 21.6 (5-Me), 104.3 (C-2), 111.8, 112.0 (C-4, C-6), 141.6 (C-5), 159.6, 159.7 (C-1, C-3). HR-EI-MS *m/z* 230.0938 [M]$^+$ (Calcd for $C_{14}H_{14}O_3$, 230.0944).

G. rikuzensis: The harvested colonies (81 test tubes, dry weight 11.34 g) were extracted with acetone at room temperature, and the combined extracts were concentrated under reduced pressure to give a residue (377 mg). The slants were collected and extracted in the same way to give a residue (401 mg). The extracts were repeatedly purified by preparative TLC (toluene–acetone 4 : 1) and preparative HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O, 2 : 3 or 7 : 13) to give **2** (8.4 mg), **3** (9.4 mg), and **4** (3.4 mg). Compounds **2** and **3** were separated as single compounds, but isomerized to give a mixture of **2** and **3** after standing in MeOH. **2** (violaceol-I): colorless oil. UV λ_{max} nm: 207, 231sh, 280.* ^{1}H- and ^{13}C-NMR: Table 1. **3** (violaceol-II): colorless oil. UV λ_{max} nm: 207, 251sh, 274.* ^{1}H- and ^{13}C-NMR: Table 1. *UV maxima were measured by an HPLC equipped with a photodiode array detector, using MeCN–H_2O (2 : 3) as a solvent. HR-EI-MS *m/z* 262.0843 [M]$^+$ (Calcd for $C_{14}H_{14}O_5$, 262.0842).

4: Colorless oil. UV λ_{max} nm: 208, 230sh, 253sh, 280. UV maxima were measured by an HPLC equipped with a photodiode array detector, using MeCN–H_2O (2 : 3) as a solvent. ^{1}H- and ^{13}C-NMR: Table 1. HR-EI-MS *m/z* 382.1058 [M]$^+$ (Calcd for $C_{21}H_{18}O_7$, 382.1053).

Methylation of 2 and 3 K_2CO_3 (110 mg) and Me_2SO_4 (0.1 ml) were added to a mixture of **2** and **3** (10.3 mg) in acetone (10 ml) and the resulting solution was heated under reflux for 6.5 h. After removal of K_2CO_3 by filtration, the reaction mixture was diluted with aq. $NaHCO_3$ solution and extracted with $CHCl_3$. The washed and dried organic layer was concentrated *in vacuo* and the residue was purified by preparative TLC (Et_2O) and preparative HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O, 11 : 9) to yield **2a** (4.6 mg) and **3a** (3.3 mg).

2a: Colorless oil. UV λ_{max}^{EtOH} nm (log ε): 206.5 (4.73), 232sh (4.29), 271 (3.27). IR ν_{max}^{KBr} cm^{-1}: 1578, 1506, 820. ^{1}H-NMR ($CDCl_3$): δ: 2.24 (6H, br s, 5-Me, 5′-Me), 3.85 (6H, s, 2-OMe, 2′-OMe), 3.87 (6H, s, 3-OMe, 3′-OMe), 6.31 (2H, m, H-6, H-6′), 6.50 (2H, m, H-4, H-4′). NOE: 3-OMe↔H-4. HR-EI-MS *m/z* 318.1459 [M]$^+$ (Calcd for $C_{18}H_{22}O_5$, 318.1468).

3a: Colorless crystals, mp. 92 °C (MeOH). UV λ_{max}^{EtOH} nm (log ε): 206.5 (4.81), 234sh (4.01), 269 (3.29). IR ν_{max}^{KBr} cm^{-1}: 1601, 1506, 818. ^{1}H-NMR ($CDCl_3$): δ: 2.16 (3H, br s, 5-Me), 2.37 (3H, br s, 4′-Me), 3.75 (6H, s, 2′-OMe, 6′-OMe), 3.85 (3H, s, 3-OMe), 3.97 (3H, s, 2-OMe), 5.96 (1H, m, H-6), 6.36 (1H, m, H-4), 6.45 (2H, br s, H-3′, H-5′). NOE: 3-OMe↔H-4; 5-Me↔H-4, H-6; H-3′, H-5′↔4′-Me, 2′-OMe, 6′-OMe. HR-EI-MS *m/z* 318.1459 [M]$^+$ (Calcd for $C_{18}H_{22}O_5$, 318.1468).

Synthesis of Violaceol-I (2) Compound **5** was prepared from methyl gallate according to ref. 8. $LiAlH_4$ (280 mg) was added to a solution of **5** (2 g) in anhydrous ether (40 ml) and the mixture was stirred at 60 °C for 15 min and at room temperature for 4 h. The reaction mixture was poured into water and then acidified with diluted HCl. The aqueous solution was extracted with ether and the ethereal solution was washed with water and saturated brine, dried, and evaporated to give **6** as quantitative yield. **6**: ^{1}H-NMR ($CDCl_3$): δ: 1.83 (1H, br s, $CH_2O\underline{H}$), 3.85, 3.86 (each 3H, s, OMe×2), 4.57 (2H, br s, 1-$C\underline{H}_2$OH), 5.12 (2H, br s, 5-O$C\underline{H}_2$Ar), 6.60, 6.62 (each 1H, br s, H-2, H-6), 7.3—7.4 (5H, m, ArH). EI-MS *m/z*: 274 [M]$^+$.

A solution of **6** (1.87 g) in MeOH (30 ml) was hydrogenated over 5% palladium-charcoal (2.2 g) at room temperature and atmospheric pressure and then the filtered solution was evaporated. The residue was purified on silica gel column chromatography ($CHCl_3$) to afford **7** (1.09 g, 95%). **7**: ^{1}H-NMR ($CDCl_3$): δ: 2.24 (3H, s, 1-Me), 3.81, 3.83 (each 3H, s, OMe×2), 6.00 (1H, br s, OH) 6.27, 6.42 (each 1H, br s, H-2, H-6). EI-MS *m/z*: 168 [M]$^+$, 153 [M−Me]$^+$.

$NaBH_4$ (37 mg) was added to a solution of 5-bromoveratraldehyde (500 mg) in ethanol (5 ml). After standing at room temperature for 30 min, the solution was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to give **8** (474 mg, 94%). **8**: ^{1}H-NMR ($CDCl_3$): δ 2.40 (1H, br s, $CH_2O\underline{H}$), 3.82, 3.85 (each 3H, s, OMe×2), 4.57 (2H, br s, 1-

C$\underline{H}_2$OH), 6.85, 7.08 (each 1H, br s, H-2, 6) HR-EI-MS *m/z* 245.9908 $[M]^+$ (Calcd for $C_9H_{11}{}^{79}BrO_3$, 245.9892), 247.9877 $[M]^+$ (Calcd for $C_9H_{11}{}^{81}BrO_3$, 247.9872).

p-TsOH·H_2O (*p*-toluenesulfonic acid monohydrate) (1.9 g) was added to a solution of **8** (474 mg) and DHP (3,4-dihydro-2*H*-pyran) (420 mg) in dry CH_2Cl_2 (5 ml), and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with $CHCl_3$, and then washed with saturated $NaHCO_3$ solution and saturated brine. The organic layer was dried and evaporated, and then the residue was subjected to prep. TLC ($CHCl_3$) to give **9** (376 mg, 59%). **9**: ^{1}H-NMR ($CDCl_3$): δ: 1.5—2.0 (6H, m, THP-$CH_2\times3$), 3.70, 3.90 (each 1H, m, THP-OCH_2), 3.84, 3.87 (each 3H, s, OMe×2) 4.41, 4.70 (each 1H, d, J=12.0 Hz, 1-CH_2O), 4.69 (1H, br t, J=5.0 Hz, THP-OCH), 6.85, 7.08 (each 1H, br s, H-2, H-6) HR-EI-MS *m/z* 330.0471 $[M]^+$ (Calcd for $C_{14}H_{19}{}^{79}BrO_4$, 330.0468), 332.0454 $[M]^+$ (Calcd for $C_{14}H_{19}{}^{81}BrO_4$, 332.0447).

Finely powdered anhydrous K_2CO_3 (250 mg) and CuO (50 mg) were added with stirring to a solution of **7** (185 mg) and **9** (221 mg) in anhydrous pyridine (5 ml) and then the mixture was heated for 6 h on an oil bath under N_2 atmosphere. The temperature of the bath was kept at 150 °C during the reaction. After the reaction ended, the mixture was poured into ether. This ethereal solution was washed successively with 3% NaOH, 5% citric acid solution, and saturated brine, dried over anhydrous $MgSO_4$, and then the solvent was evaporated off. The residual substance was purified by prep. TLC ($CHCl_3$) to give an oily product **10** (47.5 mg, 17% yield from **9**). **10**: ^{1}H-NMR ($CDCl_3$): δ: 1.5—1.9 (6H, m, THP-$CH_2\times3$), 2.23 (3H, br s, 5-Me), 3.5, 3.9 (each 1H, m, THP-OCH_2), 3.85, 3.87×2, 3.90 (each 3H, s, OMe×4) 4.38, 4.66 (each 1H, d, J=12.0 Hz, 5′-CH_2O), 4.65 (1H, br t, J=5.0 Hz, THP-OCH), 6.30, 6.49, 6.51, 6.72 (each 1H, br s, H-4, H-4′, H-6, H-6′). HR-EI-MS *m/z* 418.1981 $[M]^+$ (Calcd for $C_{23}H_{30}O_7$, 418.1993).

A solution of **10** (47.5 mg) in EtOAc was hydrogenated over 5% palladium-charcoal (0.6 g) at room temperature and atmospheric pressure and then the filtered solution was evaporated. The residue was subjected to prep. TLC (Et_2O) to afford **2a** (13 mg, 36%). ^{1}H-NMR ($CDCl_3$): δ: 2.24 (6H, br s, 5-Me, 5′-Me), 3.85, 3.87 (each 6H, s, OMe×4), 6.30, 6.49 (each 2H, br s, H-4, H-4′, H-6, H-6′). EI-MS *m/z*: 318 $[M]^+$.

BBr_3 (2 drops) was added to a solution of **2a** (13 mg) in anhydrous CH_2Cl_2 and the mixture stirred at room temperature for 1.5 h under a N_2 atmosphere. The reaction mixture was evaporated to give **2** (11 mg). The product was unchanged in MeOH or MeCN–H_2O for 12 h. After separation by prep. HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O), the product was dissolved in MeOH and set aside for 6 h to yield a mixture of **2** and **3** at a ratio of 18 : 11. The separation process with an HPLC column seemed to be requisite for the rearrangement of **2** and **3**.

Methylation of 4 K_2CO_3 (55 mg) and Me_2SO_4 (0.05 ml) were added to a solution of **4** (3.4 mg) in acetone (10 ml) and the resulting solution was heated under reflux for 6.5 h. The reaction mixture was worked up in the same way as for **2a** and **3a** and the crude product was purified by prep. TLC (Et_2O) and prep. HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O, 7 : 3) to yield **4a** (2.4 mg). **4a**: Colorless crystals, mp. 171—172 °C (MeOH). $[\alpha]_D^{28}$ +19° (c=0.21, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 207 (4.83), 250.5sh (4.20), 285sh (3.54). IR ν_{max}^{KBr} cm^{-1}: 1605, 1589, 826. ^{1}H-NMR ($CDCl_3$): δ: 2.16 (3H, s, 5-Me), 2.19 (3H, br s, 5′-Me), 2.28 (3H, br s, 5″-Me), 3.68 (3H, s, 2-OMe), 3.72 (3H, br s, 2′-OMe), 3.84 (3H, br s, 3-OMe), 3.85 (3H, br s, 3′-OMe), 3.89 (3H, br s, 3″-OMe), 6.48 (1H, d, J=2.0 Hz, H-4″), 6.66 (1H, br s, H-4), 6.68 (1H, br s, H-4′), 6.75 (1H, m, H-6″). ^{13}C-NMR ($CDCl_3$): δ: 19.7, 19.8 (5-Me, 5′-Me), 21.4 (5″-Me), 56.0 (3″-OMe), 56.1×2 (3-OMe, 3′-OMe), 61.1 (2-OMe), 61.3 (2′-OMe), 108.3 (C-4″), 110.68 (C-4), 110.73 (C-4′), 115.2 (C-6″), 124.66 (C-6), 124.71 (C-6′), 131.3 (C-5′), 131.6 (C-5), 132.8 (C-5″), 137.5 (C-2″), 140.1 (C-2′), 140.3 (C-2), 147.5, 148.3, 149.9 (C-1, 1′, 1″), 151.8 (C-3″), 153.1 (C-3), 153.3 (C-3′). HR-EI-MS *m/z* 452.1817 $[M]^+$ (Calcd for $C_{26}H_{28}O_7$, 452.1836).

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