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Bioxanthracenes from the Insect Pathogenic Fungus *Cordyceps pseudomilitaris* BCC 1620

II. Structure Elucidation

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Structures of eleven bioxanthracenes (**1**~**11**) and two monomers (**12** and **13**), isolated from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620, were elucidated. The structure, including the axial stereochemistry, of one of the major symmetrical dimers (**1**) was determined by X-ray crystallographic analysis, while the stereochemistries of the other isomers were deduced by chemical conversions and spectroscopic means.

In the accompanying paper,[1] we described the taxonomy of the producing strain, the fermentation, the isolation and the antimalarial activity of bioxanthracenes **1**~**11** and monomers **12**, **13** (Fig. 1), isolated from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. Major metabolites, **1**~**5**, and a minor isomer, **11**, were spectroscopically identical with ES-242s, previously isolated from *Verticillium* sp.[2,3] Recently, TATSUTA *et al.* synthesized ES-242-4 together with its atropisomer (compounds **1** and **7**) based on oxidative homo-dimerization of a corresponding enantiomerically pure monomer.[4] ES-242-5 and its atropisomer (compounds **4** and **8**) were synthesized by selective reduction of compounds **1** and **7**, respectively.[5] Thus, the (3*S*, 4*S*, 3′*S*, 4′*S*)-configuration has already been established for these four compounds. The axial stereochemistry of **1** and **7** has also been elucidated by the single crystal X-ray analysis of a synthetic analogue of **7** and chemical transformations.[6] However, the stereochemistry of none of the other ES-242s has ever been presented. Herein, we report the structure elucidation, including stereochemistries, of the new and known bioxanthracenes **1**~**11** and monomers **12**, **13**.

Results and Discussion

Planar structures of compounds **1**~**13**, isolated from *C. pseudomilitaris* BCC 1620, were elucidated mainly by NMR analyses (^{1}H, ^{13}C, DEPTs, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC), which were supported by MS, IR and UV data. The three dimensional structures lead to three pairs of stereoisomers: **1** and **7**; **4** and **8**; and **9** and **10**. Representative HMBC correlations of a naturally novel C-10–C-10′ dimer **8** and a new C-10–C-5′ dimer **10** are shown in Fig. 2, as a representative of the elucidation of basic skeletons. Physicochemical properties (mp, optical rotation, UV, IR, MS) of the six compounds **1**~**5** and **11** were identical with those of ES-242s (ES-242-4, -3, -2, -5, -1, and -8, respectively) reported in the literature.[2,3] ^{1}H- and ^{13}C-NMR chemical shifts data of corresponding ES-242s, isolated from *Verticillium* sp., were kindly provided by Dr. S. TOKI, Kyowa Hakko Kogyo Co. Ltd., and the data were consistent with those of the six compounds isolated from strain BCC 1620. The ^{1}H-NMR data of compounds **7** ($[\alpha]_D^{27}$ −103°, *c* 0.14, $CHCl_3$) and **8** ($[\alpha]_D^{25}$ −62°, *c* 0.11, $CHCl_3$) were consistent with those reported for the

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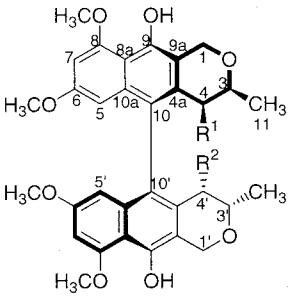
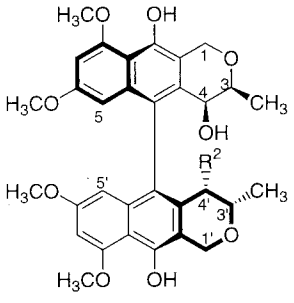
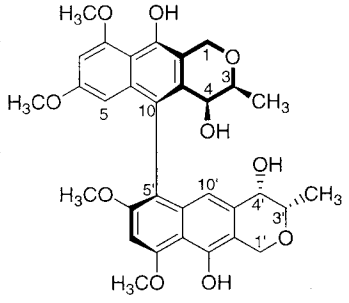
Fig. 1. Structures of compounds isolated from *Cordyceps pseudomilitaris* BCC 1620.



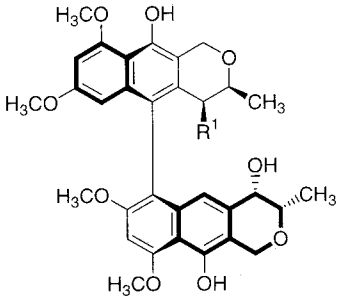
1 : R^1 = OH, R^2 = OH
2 : R^1 = OAc, R^2 = OH
3 : R^1 = OAc, R^2 = OAc
4 : R^1 = OH, R^2 = H
5 : R^1 = OAc, R^2 = H
6 : R^1 = H, R^2 = H



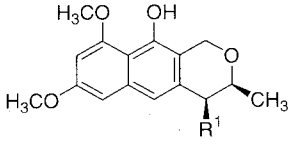
7 : R^2 = OH
8 : R^2 = H



9

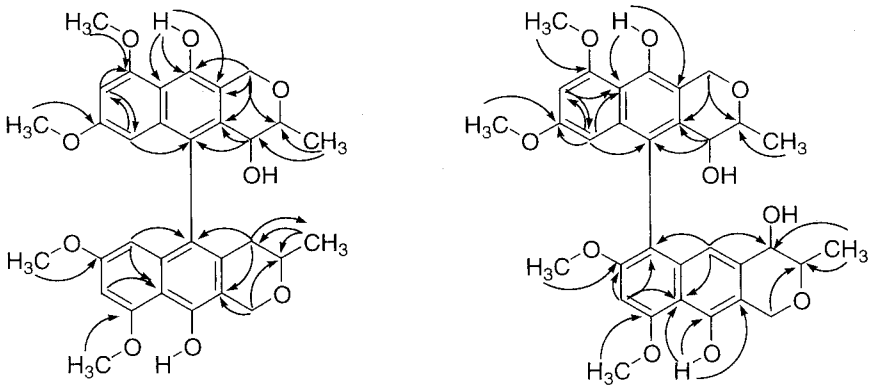


10 : R^1 = OH
11 : R^1 = H



12 : R^1 = OH
13 : R^1 = H

Fig. 2. HMBC correlations observed for **8** and **10**.



synthetic atropisomers of ES-242-4 (lit. $[\alpha]_D^{22}$ −86°, $CHCl_3$, concentration has not been recorded)[4] and ES-242-5 (lit. $[\alpha]_D$ −53°, *c* 0.97, $CHCl_3$),[5] respectively. Compound **12** ($[\alpha]_D^{24}$ −33°, *c* 0.036, $CHCl_3$) was identical with a synthetic monomer (lit. $[\alpha]_D$ −28°, *c* 0.55, $CHCl_3$).[7] The symmetrical dimer **6** is probably identical to ES-242-6[3] as judged by their similarity of UV spectra. However, we were unable to confirm that the two samples possess identical stereochemistry due to the lack of other physico-chemical data (mp., optical rotation, IR, MS, NMR) of ES-242-6 from *Verticillium* sp.

Assignment of neither ^{1}H- nor ^{13}C-NMR spectra of the reported ES-242s (corresponding to compounds **1**~**6** and **11**) and of synthetical compounds **7**, **8** and **12** has ever been

Table 1. ^{1}H-NMR data of the new compounds **9**, **10**, and **13**.[a]

position	**9**	**10**	**13**
1	5.26 d (15.7)	5.07 d (15.6)	5.12 d (15.4)
	4.83 d (15.7)	4.80 d (15.6)	4.76 d (15.4)
3	3.61 q (6.5)	3.72 qd (6.4, 1.0)	3.80 m
4	3.74 s	3.92 brd (ca. 6)	2.80 m
	-	-	2.77 m
5	5.98 d (2.0)	6.03 d (2.2)	6.60 d (2.2)
7	6.45 d (2.1)	6.45 d (2.2)	6.37 d (2.1)
10	-	-	6.93 s
11	1.27 d (6.5)	1.23 d (6.4)	1.37 d (6.1)
6-OC*H*$_3$	3.44 s	3.45 s	3.86 s
8-OC*H*$_3$	4.06 s	4.06 s	3.99 s
9-O*H*	9.57 s	9.47 s	9.19 s
4-O*H*	2.90 brs	1.94 brd (ca. 6)	-
1'	5.11 d (15.7)	5.07 d (15.6)	-
	4.73 d (15.7)	4.72 d (15.6)	-
3'	3.73 qd (6.5, 0.8)	3.69 qd (6.4, 1.4)	-
4'	4.03 brd (ca. 8)	4.05 brd (ca. 4)	-
7'	6.78 s	6.74 s	-
10'	6.49 s	6.61 s	-
11'	1.32 d (6.4)	1.30 d (6.5)	-
6'-OC*H*$_3$	3.76 s	3.71 s	-
8'-OC*H*$_3$	4.18 s	4.17 s	-
9'-O*H*	9.52 s	9.42 s	-
4'-O*H*	1.69 brd (ca.8)	2.08 brd	-

[a]Recorded in $CDCl_3$.

Table 2. ^{13}C-NMR data of symmetric dimers **1**, **3**, **6**, **7** and monomers **12**, **13**.[a]

position	**1**	**3**	**6**	**7**	**12**	**13**
1	65.3 (t)	65.1 (t)	64.7 (t)	64.6 (t)	65.2 (t)	64.8 (t)
3	73.9 (d)	73.3 (d)	70.5 (d)	73.4 (d)	73.8 (d)	70.4 (t)
4	66.7 (d)	66.9 (d)	34.3 (t)	65.5 (d)	68.5 (d)	36.1 (t)
4a	136.1 (s)[b]	131.1 (s)	134.8 (s)	137.4 (s)[b]	136.7 (s)	134.9 (s)
5	97.8 (d)	98.8 (d)	97.1 (d)	97.9 (d)	99.2 (d)	98.5 (d)
6	157.9 (s)[c]	157.0 (s)	157.3 (s)	157.6 (s)[c]	157.2 (s)	157.2 (s)
7	98.4 (d)	98.0 (d)	97.1 (d)	97.9 (d)	98.0 (d)	97.6 (d)
8	157.6 (s)[c]	157.2 (s)	157.3 (s)	157.4 (s)[c]	157.0 (d)	157.1 (s)
8a	110.4 (s)	110.5 (s)	109.2 (s)	110.5 (s)	110.1 (s)	108.9 (s)
9	150.1 (s)	149.7 (s)	148.7 (s)	149.7 (s)	149.2 (s)	149.2 (s)
9a	114.4 (s)	115.8 (s)	114.9 (s)	114.1 (s)	113.9 (s)	114.9 (s)
10	123.8 (s)	125.1 (s)	124.1 (s)	123.5 (s)	118.3 (d)	116.4 (d)
10a	135.6 (s)[b]	135.6 (s)	133.6 (s)	135.5 (s)[b]	135.9 (s)	135.5 (s)
11	17.1 (q)	17.1 (q)	21.5 (q)	17.1 (q)	16.9 (q)	21.6 (q)
6-OCH_3	55.4 (q)	55.3 (q)	55.2 (q)	55.1 (q)	55.4 (q)	55.3 (q)
8-OCH_3	56.4 (q)	56.3 (q)	56.1 (q)	56.4 (q)	56.2 (q)	56.0 (q)
4-OC(=O)CH_3	-	169.0 (s)	-	-	-	-
4-OC(=O)CH_3	-	19.3 (q)	-	-	-	-

[a]Recorded in $CDCl_3$. [b,c]Assignments are interchangeable for each compound.

Table 3. ^{13}C-NMR data of hetero-dimers **2**, **4**, **5**, **8**, **9**, **10** and **11**.[a]

position	**2**	**4**	**5**	**8**	**9**	**10**	**11**
1	65.2 (t)	65.3 (t)	65.2 (t)	64.6 (t)	65.1 (t)	65.1 (t)[b]	64.7 (t)
3	73.5 (d)	73.9 (d)	73.6 (d)	74.1 (d)	73.5 (d)	73.9 (d)	70.4 (d)
4	66.7 (d)	66.6 (d)	66.8 (d)	66.2 (d)	66.3 (d)	66.5 (d)	34.4 (t)
4a	131.2 (s)	135.3 (s)	130.9 (s)	136.4 (s)	137.5 (s)	135.4 (s)	137.4 (s)[b]
5	98.0 (d)	98.2 (d)	97.8 (d)	97.1 (d)	98.4 (d)	98.3 (d)	97.7 (d)
6	157.9 (s)	157.8 (s)[b]	157.6 (s)	157.5 (s)[b]	157.3 (s)	157.1 (s)	157.0 (s)[c]
7	98.0 (d)	97.5 (d)	98.5 (d)	97.9 (d)	97.9 (d)	97.7 (d)	96.7 (d)
8	157.2 (s)[b]	157.3 (s)	157.2 (s)	157.5 (s)[b]	157.3 (s)	157.4 (s)[c]	157.4 (s)
8a	110.6 (s)	110.5 (s)	110.6 (s)	110.3 (s)	110.5 (s)	110.4 (s)	109.2 (s)
9	149.8 (s)	149.4 (s)	149.2 (s)	149.1 (s)	149.6 (s)	149.3 (s)	149.0 (s)
9a	115.4 (s)	114.3 (s)	115.3 (s)	114.1 (s)	114.2 (s)	114.2 (s)[d]	115.2 (s)
10	125.3 (s)	125.4 (s)	126.9 (s)	125.8 (s)	122.2 (s)	123.1 (s)	121.4 (s)
10a	135.5 (s)[c]	135.8 (s)[c]	135.4 (s)[b]	135.1 (s)[c]	135.5 (s)[b]	136.2 (s)	135.2 (s)[b]
11	17.0 (q)	17.1 (q)	17.1 (q)	17.0 (q)	16.9 (q)	16.9 (q)	21.5 (q)
6-O*C*H$_3$	55.3 (q)	55.4 (q)[d]	55.3 (q)	55.2 (q)	55.2 (q)	55.1 (q)	55.0 (q)
8-O*C*H$_3$	56.3 (q)[d]	56.3 (q)	56.3 (q)	56.3 (q)[d]	56.3 (q)	56.3 (q)	56.2 (q)
4-OC(=O)CH$_3$	168.9 (s)	-	169.0 (s)	-	-	-	-
4-OC(=O)*C*H$_3$	19.3 (q)	-	19.4 (q)	-	-	-	-
1'	65.2 (t)	64.7 (t)	64.7 (t)	65.3 (t)	65.1 (t)	65.0 (t)[b]	65.1 (t)
3'	73.7 (d)	70.4 (d)	70.3 (d)	70.7 (d)	73.5 (d)	73.5 (d)	73.6 (d)
4'	66.8 (d)	34.4 (t)	34.5 (t)	34.7 (t)	68.1 (d)	68.0 (d)	68.0 (d)
4a'	135.7 (s)[c]	134.1 (s)	133.8 (s)	136.4 (s)	135.5 (s)[c]	137.1 (s)	135.0 (s)[b]
5'	98.8 (d)	97.6 (d)	98.4 (d)	96.8 (d)	114.8 (s)	114.2 (s)[d]	115.4 (s)
6'	157.0 (s)	157.7 (s)[b]	156.6 (s)	157.3 (s)[b]	154.7 (s)	153.8 (s)	153.9 (s)
7'	97.7 (d)	97.2 (d)	96.7 (d)	96.8 (d)	94.2 (d)	94.0 (d)	94.2 (d)
8'	157.4 (s)[b]	157.7 (s)[b]	157.3 (s)	157.3 (s)[b]	157.3 (s)	157.3 (s)[c]	156.9 (s)[c]
8a'	110.4 (s)	109.4 (s)	109.3 (s)	109.2 (s)	110.5 (s)	110.3 (s)	110.5 (s)
9'	149.9 (s)	149.5 (s)	149.9 (s)	149.1 (s)	149.4 (s)	149.2 (s)	149.3 (s)
9a'	114.6 (s)	115.5 (s)	115.5 (s)	115.3 (s)	114.2 (s)	114.0 (s)[d]	113.9 (s)
10'	123.7 (s)	122.6 (s)	122.7 (s)	122.8 (s)	116.5 (d)	117.3 (d)	116.5 (d)
10a'	136.1 (s)	135.2 (s)[c]	135.2 (s)[b]	135.0 (s)[c]	136.0 (s)[b]	136.2 (s)	134.7 (s)[b]
11'	17.1 (q)	21.5 (q)	21.5 (q)	21.5 (q)	16.7 (q)	16.6 (q)	16.7 (q)
6'-O*C*H$_3$	55.3 (q)	55.3 (q)[d]	55.2 (q)	55.1 (q)	57.0 (q)	56.8 (q)	56.9 (q)
8'-O*C*H$_3$	56.4 (q)[d]	56.3 (q)	56.2 (q)	56.2 (q)[d]	56.3 (q)	56.2 (q)	56.2 (q)

[a]Recorded in $CDCl_3$. [b-d]Assignments are interchangeable for each compound.

presented. Therefore, ^{1}H-NMR data of new compounds **9**, **10** and **13** are listed in Table 1, and in the experimental section for other compounds. ^{13}C-NMR data of all the compounds (**1**~**13**) isolated from *C. pseudomilitaris* BCC 1620 are listed in Tables 2 and 3.

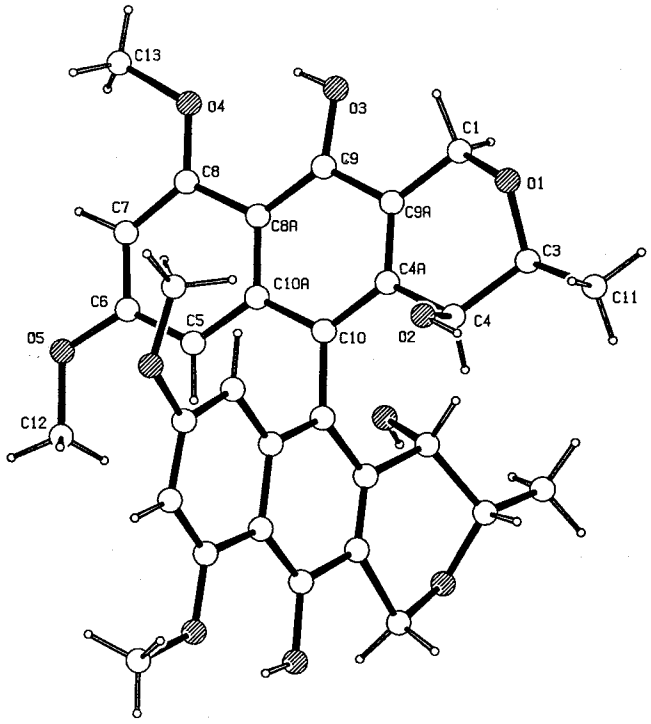
Absolute Structures of the C-10–C-10′ Dimers

A three-dimensional single crystal of **1** was obtained by recrystallization from acetone. Relative stereochemistries of **1**, including the axial stereochemistry, were determined by X-ray crystallographic analysis (Fig. 3). This result was consistent with TATSUTA's stereochemical elucidation.[6] In the crystal structure of **1**, the methyl group on C-3 occupies

a *pseudo* equatorial position, and H-3 is placed in the *pseudo* axial position of the pseudochair ring conformation. Hydroxyl group on C-4 occupies a *pseudo* axial orientation, hence, H-4 is located on *pseudo* equatorial. The small coupling constant of $J_{3,4}$=1.0 Hz, observed in ^{1}H-NMR spectrum of **1** (in $CDCl_3$), was in good agreement with the conformation observed in the crystal structure.

Absolute structures of compounds **2** (ES-242-3) and **3** (ES-242-2) were determined by their conversion into **1** by deacetylation. Initial trial of alkaline hydrolyses of **2** or **3** under various conditions failed, probably due to the high steric hindrance around the acetate moiety. However, treatment of **2** with excess $LiAlH_4$ in THF gave a major product ($[\alpha]_D^{29}$ −55°, *c* 0.18, $CHCl_3$) which is identical to the naturally occurring **1** ($[\alpha]_D^{26}$ −56°, *c* 0.18, $CHCl_3$) as compared by ^{1}H- and ^{13}C-NMR and analytical HPLC-UV (ODS column, MeCN-H_2O, co-injection). In the same fashion, compound **3** was converted into **1** (54% yield; $[\alpha]_D^{29}$ −53°, *c* 0.18, $CHCl_3$). Thus, compounds **2** and **3** are the mono- and diacetates of **1** respectively, possessing (3*S*, 4*S*, 3′*S*, 4′*S*)-configuration and same axial stereochemistry as **1**.

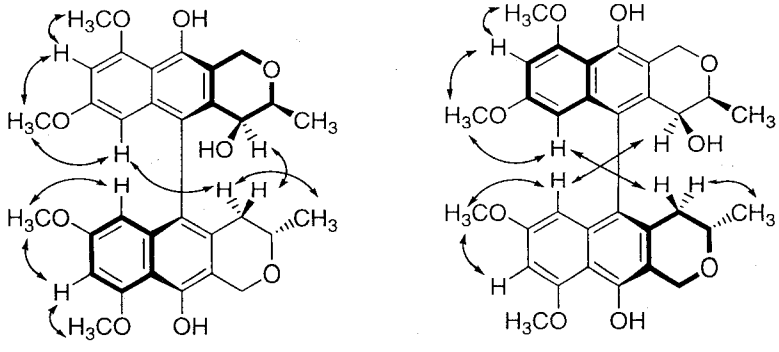
Fig. 3. Crystal structure of **1**.



$LiAlH_4$ reduction of the acetate **5** ($[\alpha]_D^{26}$ +12°, *c* 0.46, $CHCl_3$) gave **4** (48% yield; $[\alpha]_D^{29}$ +22°, *c* 0.23, $CHCl_3$) which is spectroscopically (^{1}H- and ^{13}C-NMR, MS, analytical HPLC-UV) consistent with the naturally occurring sample (**4**: $[\alpha]_D^{27}$ +22°, *c* 0.23, $CHCl_3$). This indicated that compound **5** is the acetate of **4**. Some 2D-NMR information were consistent with the stereochemistries established for compounds **4**, **5** and **8**. A NOESY spectrum (in methanol-d_4) of compound **4** showed a correlation between the *pseudo* axial H-4′ (δ 1.95, dd, J=17.1, 10.6 Hz) and H-5, and the *pseudo* equatorial H-4′ (δ 2.19, dd, J=17.1, 3.1 Hz) correlated with H-4 (δ 3.85, s) (Fig. 4). Similar NOESY correlations (in $CDCl_3$) were observed for compound **5** (ES-242-1). In addition, correlations of a high field shifted proton signal due to an acetyl group (δ 1.21, 3H, s) with H-5′ and H-7′ were also observed. On the other hand, compound **8** showed NOESY correlation (in $CDCl_3$) between the *pseudo* equatorial H-4′ (δ 2.10, dd, J=17.0, 2.8 Hz) and H-5, and a correlation between H-4 (δ 3.73, s) and H-5′.

In the NOESY spectrum of the symmetrical dimer **6** (in methanol-d_4), correlation between the *pseudo* axial H-4 (δ 2.11, dd, J=17.0, 10.2 Hz) and H-5′ was observed. This

Fig. 4. NOESY correlations observed for compounds **4** and **8**.



indicated that compound **6** possesses the same sense of axial stereochemistry as the major metabolites, **1**~**5**. By analogy, this compound should also have (3*S*, 3′*S*)-configuration, and the monomer **13** should have a (3*S*)-configuration.

Stereochemistries of the C-10–C-5′ Dimers **9**, **10** and **11**

In the ^{1}H-NMR spectrum ($CDCl_3$) of a C-10–C-5′ dimer **9**, H-4 appeared as a singlet (δ 3.74), and H-3 as a quartet, indicating a small $J_{3,4}$ value. The $J_{3',4'}$-value was estimated to be 0.8 Hz from the H-3′ signal (qd, J=6.5, 0.8 Hz). With the same logic for the stereochemical consideration of compound **1** and other C-10–C-10′ dimers, the *cis*-relationship between the C-3 methyl group and the C-4 hydroxyl group, as well as C-3′ methyl group and C-4′ hydroxyl group, were indicated. This compound should have the (3*S*, 4*S*, 3′*S*, 4′*S*)-configuration. Similarly, the $J_{3,4}$-value of 1.0 Hz and the $J_{3',4'}$-value of 1.4 Hz observed for compound **10** established the (3*S*, 4*S*, 3′*S*, 4′*S*)-configuration. This information indicates that the two compounds, **9** and **10**, are a pair of atropisomers. The NOESY spectrum of **9** showed a correlation between H-4 and H-10′, which suggested the axial stereochemistry as depicted in Fig. 1. This in turn established the stereostructure of **10** as shown. The NOESY spectrum of a dehydroxy derivative **11** (ES-242-8) (in $CDCl_3$) indicated a correlation between *pseudo* axial H-4 (*cis* to the C-3 methyl group) and H-10′. Compound **11** should, therefore, have the same sense of axial stereochemistry as **10**.

Experimental

Physico-chemical Properties of Compounds **1**~**13**

Compound **1** (ES-242-4): Pale yellow prisms (acetone); mp 185~186°C (dec.); $[\alpha]_D^{26}$ −56° (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (4.98), 296 (4.09), 308 (4.15), 337 (3.94) nm; IR (KBr) ν_{max} 3394, 2937, 1626, 1578, 1362, 1257, 1204, 1156, 1095, 1047, 979, 829, 730 cm^{-1}; MS (ESI-TOF) *m/z* 601 $[M+Na]^+$, 561, 543; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.53 (2H, s, 9-O*H*), 6.45 (2H, d, J=2.2 Hz, H-7), 5.98 (2H, d, J=2.1 Hz, H-5), 5.23 (2H, d, J=15.8 Hz, H-1a), 4.81 (2H, d, J=15.8 Hz, H-1b), 4.05 (6H, s, 8-OCH_3), 3.81 (2H, s, H-4), 3.68 (2H, qd, J=6.3, 1.0 Hz, H-3), 3.45 (6H, s, 6-OCH_3), 1.48 (2H, brs, 4-O*H*), 1.27 (6H, d, J=6.4 Hz, H-11); *Anal.* C 66.46%, H 5.92%, calcd for $C_{32}H_{34}O_{10}$, C 66.43%, H 5.92%.

Compound **2** (ES-242-3): Pale yellow crystals (AcOEt-hexane); mp 283~285°C (dec.); $[\alpha]_D^{26}$ +1° (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (5.07), 296 (4.18), 308 (4.25), 339 (4.04), 354 (4.12) nm; IR (KBr) ν_{max} 3392, 2940, 1739, 1625, 1578, 1362, 1155, 1095, 1047, 829 cm^{-1}; MS (ESI-TOF) *m/z* 643 $[M+Na]^+$, 561, 543; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.57 (1H, s, 9-O*H* or 9′-O*H*), 9.46 (1H, s, 9′-O*H* or 9-O*H*), 6.46 (1H, d, J=2.1 Hz, H-7), 6.42 (1H, d, J=2.2 Hz, H-7′), 5.96 (1H, d, J=2.1 Hz, H-5), 5.92 (1H, d, J=2.2 Hz, H-5′), 5.34 (1H, d, J=1.5 Hz, H-4), 5.32 (1H, d, J=15.7 Hz, H-1a), 5.22 (1H, d, J=15.7 Hz, H-1′a), 4.88 (1H, d, J=15.7 Hz, H-1′b), 4.80 (1H, d, J=15.7 Hz, H-1b), 4.06 (3H, s, 8-OCH_3 or 8′-OCH_3), 4.04 (3H, s, 8′-OCH_3 or 8-OCH_3), 3.87 (1H, s, H-4′), 3.86 (1H, m, H-3′), 3.79 (1H, qd, J=6.4, 1.6 Hz, H-3), 3.43 (3H, s, 6′-OCH_3), 3.42 (3H, s, 6-OCH_3), 1.28 (3H, d, J=6.3 Hz, H-11′), 1.26 (1H, s, 4′-O*H*), 1.14 (3H, s, 4-OAc), 1.10 (3H, d, J=6.4 Hz, H-11); *Anal.* C 65.79%, H 5.84%, calcd for $C_{34}H_{36}O_{11}$, C 65.80%, H 5.85%.

Compound **3** (ES-242-2): Pale yellow powder; mp 160~161°C; $[\alpha]_D^{26}$ +39° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (5.10), 296 (4.23), 309 (4.30), 340 (4.10), 355 (4.19) nm; IR (KBr) ν_{max} 3391, 2943, 1736, 1624, 1579, 1364, 1232, 1156, 1096, 1048, 831, 754 cm^{-1}; MS (ESI-TOF) *m/z* 685 $[M+Na]^+$, 603, 543; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.47 (2H, s, 9-O*H*), 6.42 (2H, d, J=2.2 Hz, H-7), 5.90 (2H, d, J=2.2 Hz, H-5), 5.42 (2H, d, J=1.6 Hz, H-4), 5.27 (2H, d, J=15.7 Hz, H-1a), 4.88 (2H, d, J=15.6 Hz, H-1b), 4.05 (6H, s, 8-OCH_3), 3.96 (2H, qd, J=6.5, 1.6 Hz, H-3), 3.41 (6H, s, 6-OCH_3), 1.13 (6H, s, 4-OAc), 1.12 (6H, d, J=*ca.* 6 Hz, H-11); *Anal.* C 65.27%, H 5.84%, calcd for $C_{36}H_{38}O_{12}$, C 65.25%, H 5.78%.

Compound **4** (ES-242-5): Pale yellow powder; mp 154~157°C; $[\alpha]_D^{27}$ +22° (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (5.07), 297 (4.23), 309 (4.29), 347 (4.10) nm; IR (KBr) ν_{max} 3393, 2936, 1625, 1577, 1361, 1154, 1092, 1047, 935, 826 cm^{-1}; MS (ESI-TOF) *m/z* 585 $[M+Na]^+$, 563 $[M+H]^+$, 545, 388; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.50 (1H, s, 9-O*H*), 9.47 (1H, s, 9′-O*H*), 6.47 (1H, d, J=2.2 Hz, H-7), 6.40 (1H, d, J=2.2 Hz, H-7′), 5.97 (1H, d, J=2.3 Hz, H-5), 5.96 (1H, d, J=2.3 Hz, H-5′), 5.25 (1H, d, J=15.8 Hz, H-1a), 5.20 (1H, d, J=15.4 Hz, H-1′a), 4.84 (1H, d, J=15.7 Hz, H-1b), 4.83 (1H, d, J=15.5 Hz, H-1′b), 4.07 (3H, s, 8-OCH_3), 4.04 (3H, s, 8′-OCH_3), 3.81 (1H, d, J=1.0 Hz, H-4), 3.74 (1H, m, H-3′), 3.68 (1H, qd, J=6.5, 1.0 Hz, H-3), 3.46 (3H, s, 6-OCH_3), 3.46 (3H, s, 6′-OCH_3), 2.10~2.00 (2H, m, H-4′), 1.28 (3H, d, J=6.5 Hz, H-11), 1.25 (1H, s, 4-O*H*), 1.16 (3H, d, J=6.1 Hz, H-11′); ^{1}H-NMR (methanol-d_4, 400 MHz) δ 6.59 (1H, d, J=2.2 Hz, H-7), 6.54 (1H, d, J=2.2 Hz, H-7′), 6.18 (1H, d, J=2.0 Hz, H-5′), 5.94 (1H, d, J=2.0 Hz, H-5), 5.19 (1H, d, J=15.7 Hz, H-1a), 5.17 (1H, d, J=15.3 Hz, H-1′a), 4.81 (1H, d, J=15.5

Hz, H-1b), 4.79 (1H, d, J=15.3 Hz, H-1′b), 4.10 (3H, s, 8-OCH_3), 4.08 (3H, s, 8′-OCH_3), 3.85 (1H, s, H-4), 3.80 (1H, m, H-3′), 3.73 (1H, q, J=6.4 Hz, H-3), 3.50 (3H, s, 6′-OCH_3), 3.42 (3H, s, 6-OCH_3), 2.19 (1H, dd, J=17.1, 3.1 Hz, H-4′a), 1.95 (1H, dd, J=17.1, 10.6 Hz, H-4′b), 1.22 (3H, d, J=6.4 Hz, H-11), 1.15 (3H, d, J=6.1 Hz, H-11′).

Compound **5** (ES-242-1): Pale yellow crystals (MeOH); mp 235~238°C (dec.); $[\alpha]_D^{26}$ +12° (c 0.46, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (5.13), 296 (4.31), 309 (4.37), 345 (4.15) nm; IR (KBr) ν_{max} 3398, 2939, 1739, 1625, 1578, 1363, 1153, 1097, 1048, 829 cm^{-1}; MS (ESI-TOF) m/z 627 $[M+Na]^+$, 605 $[M+H]^+$, 545; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.51 (1H, s, 9-OH), 9.39 (1H, s, 9′-OH), 6.48 (1H, d, J=2.0 Hz, H-7), 6.36 (1H, d, J=2.1 Hz, H-7′), 5.93 (1H, d, J=2.0 Hz, H-5), 5.89 (1H, d, J=2.0 Hz, H-5′), 5.35 (1H, s, H-4), 5.29 (1H, d, J=15.7 Hz, H-1a), 5.18 (1H, d, J=15.4 Hz, H-1′a), 4.88 (1H, d, J=15.5 Hz, H-1′b), 4.84 (1H, d, J=15.7 Hz, H-1b), 4.07 (3H, s, 8-OCH_3), 4.03 (3H, s, 8′-OCH_3), 3.88 (1H, m, H-3′), 3.79 (1H, qd, J=6.3, 1.0 Hz, H-3), 3.44 (3H, s, 6′-OCH_3), 3.42 (3H, s, 6-OCH_3), 2.15 (1H, dd, J=17.0, 3.2 Hz, H-4′a), 2.03 (1H, dd, J=17.0, 10.4 Hz, H-4′b), 1.21 (3H, s, 4-OAc), 1.17 (3H, d, J=6.1 Hz, H-11′), 1.10 (3H, d, J=6.4 Hz, H-11); *Anal.* C 67.44%, H 6.08%, calcd for $C_{34}H_{36}O_{10}$, C 67.54%, H 6.00%.

Compound **6**: Pale brown powder; mp 128~131°C (dec.); $[\alpha]_D^{28}$ +118° (c 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (4.92), 310 (4.07), 329 (3.87), 345 (3.89) nm; IR (KBr) ν_{max} 3401, 2968, 2934, 1624, 1578, 1361, 1154, 1050, 936, 829 cm^{-1}; MS (ESI-TOF) m/z 547 $[M+H]^+$, 391, 269; HRMS (ESI-TOF) m/z $[M+H]^+$ 547.2324 (calcd for $C_{32}H_{35}O_8$, 547.2332); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.44 (2H, s, 9-OH), 6.42 (2H, d, J=2.1 Hz, H-7), 5.98 (2H, d, J=2.1 Hz, H-5), 5.20 (2H, d, J=15.4 Hz, H-1a), 4.84 (2H, d, J=15.4 Hz, H-1b), 4.05 (6H, s, 8-OCH_3), 3.73 (2H, m, H-3), 3.48 (6H, s, 6-OCH_3), 2.13~2.10 (4H, m, H-4), 1.17 (6H, d, J=6.2 Hz, H-11); ^{1}H-NMR (methanol-d_4, 400 MHz) δ 6.56 (2H, d, J=2.2 Hz, H-7), 6.01 (2H, d, J=2.0 Hz, H-5), 5.17 (2H, d, J=15.4 Hz, H-1a), 4.81 (2H, d, J=15.5 Hz, H-1b), 4.10 (6H, s, 8-OCH_3), 3.78 (2H, m, H-3), 3.48 (6H, s, 6-OCH_3), 2.18 (2H, dd, J=17.0, 3.6 Hz, H-4a), 2.11 (2H, dd, J=17.0, 10.2 Hz, H-4b), 1.17 (6H, d, J=6.1 Hz, H-11).

Compound **7** (atropisomer of ES-242-4): Pale yellow powder; mp 187~190°C (dec.); $[\alpha]_D^{27}$ −103° (c 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (4.94), 309 (4.14), 336 (3.92), 351 (3.98) nm; IR (KBr) ν_{max} 3391, 2938, 1623, 1588, 1362, 1157, 1096, 1048, 939, 829 cm^{-1}; MS (ESI-TOF) m/z 601 $[M+Na]^+$, 543, 454, 413; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.52 (2H, s, 9-OH), 6.46 (2H, d, J=2.0 Hz, H-7), 5.90 (2H, d, J=2.1 Hz, H-5), 5.22 (2H, d, J=15.9 Hz, H-1a), 4.91 (2H, d, J=15.9 Hz, H-1b), 4.06 (6H, s, 8-OCH_3), 3.89 (2H, s, H-4), 3.65 (2H, q, J=6.3 Hz, H-3), 3.46 (6H, s, 6-OCH_3), 1.62 (2H, brs, 4-OH), 1.23 (6H, d, J=6.3 Hz, H-11); *Anal.* C 66.44%, H 5.95%, calcd for $C_{32}H_{34}O_{10}$, C 66.43%, H 5.92%.

Compound **8** (atropisomer of ES-242-5): Pale yellow powder; mp 267~270°C (dec.); $[\alpha]_D^{25}$ −62° (c 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (5.22), 310 (4.42), 333 (4.18), 347 (4.20) nm; IR (KBr) ν_{max} 3420, 2934, 1624, 1577, 1361, 1155, 1090, 1048, 827 cm^{-1}; MS (ESI-TOF) m/z 585 $[M+Na]^+$, 545, 413; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.48 (1H, s, 9-OH), 9.43 (1H, s, 9′-OH), 6.47 (1H, d, J=2.1 Hz, H-7), 6.40 (1H, d, J=2.1 Hz, H-7′), 6.01 (1H, d, J=2.0 Hz, H-5), 5.86 (1H, d, J=2.1 Hz, H-5′), 5.21 (1H, d, J=15.8 Hz, H-1a), 5.19 (1H, d, J=15.4 Hz, H-1′a), 4.86 (1H, d, J=15.5 Hz, H-1′b), 4.85 (1H, d, J=15.7 Hz, H-1b), 4.07 (3H, s, 8-OCH_3), 4.05 (3H, s, 8′-OCH_3), 3.73 (1H, s, H-4), 3.64 (1H, m, H-3′), 3.62 (1H, qd, J=6.4, 0.9 Hz, H-3), 3.47 (3H, s, 6-OCH_3), 3.45 (3H, s, 6′-OCH_3), 2.60 (1H, dd, J=17.0, 10.6 Hz, H-4′a), 2.10 (1H, dd, J=17.0, 2.8 Hz, H-4′b), 1.61 (1H, brs, 4-OH), 1.21 (3H, d, J=6.4 Hz, H-11), 1.16 (3H, d, J=6.1 Hz, H-11′); *Anal.* C 68.23%, H 5.96%, calcd for $C_{32}H_{34}O_9$, C 68.32%, H 6.09%.

Compound **9**: Pale yellow powder; $[\alpha]_D^{24}$ +23° (c 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (5.02), 309 (4.14), 351 (4.11) nm; IR (KBr) λ_{max} 3399, 2934, 1626, 1578, 1360, 1092, 979 cm^{-1}; MS (ESI-TOF) m/z 601 $[M+Na]^+$, 579 $[M+H]^+$, 561, 543, 487, 485, 457; HRMS (ESI-TOF) m/z $[M+H]^+$ 579.2236 (calcd for $C_{32}H_{35}O_{10}$, 579.2236).

Compound **10**: Pale yellow powder; mp 196~199°C (dec.); $[\alpha]_D^{25}$ −74° (c 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (5.09), 309 (4.21), 352 (4.15) nm; IR (KBr) ν_{max} 3394, 2938, 1626, 1604, 1585, 1360, 1206, 1093, 982, 830 cm^{-1}; MS (ESI-TOF) m/z 601 $[M+Na]^+$, 561, 543; *Anal.* C 66.45%, H 5.95%, calcd for $C_{32}H_{34}O_{10}$, C 66.43%, H 5.92%.

Compound **11** (ES-242-8): Pale yellow powder; mp 171~173°C (dec.); $[\alpha]_D^{27}$ +3° (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (4.96), 312 (4.07), 345 (4.00) nm; IR (KBr) ν_{max} 3397, 2935, 1626, 1605, 1580, 1361, 1204, 1090, 982, 829 cm^{-1}; MS (ESI-TOF) m/z 585 $[M+Na]^+$, 563 $[M+H]^+$, 545, 413, 347; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.46 (1H, s, 9′-OH), 9.42 (1H, s, 9-OH), 6.75 (1H, s, H-7′), 6.58 (1H, s, H-10′), 6.40 (1H, d, J=2.1 Hz, H-7), 5.99 (1H, d, J=2.1 Hz, H-5), 5.19 (1H, d, J=15.5 Hz, H-1a), 5.12 (1H, d, J=15.6 Hz, H-1′a), 4.84 (1H, d, J=15.5, H-1b), 4.73 (1H, d, J=15.7 Hz, 1′b), 4.18 (3H, s, 8′-OCH_3), 4.04 (3H, s, 8-OCH_3), 3.76 (1H, m, H-3), 3.74 (3H, s, 6′-OCH_3), 3.74 (1H, s, H-4′), 3.69 (1H, m, H-3′), 3.48 (3H, s,

6-OCH_3), 2.27 (1H, dd, J=16.7, 2.0 Hz, H-4a), 2.14 (1H, dd, J=16.7, 10.4 Hz, H-4b), 1.32 (3H, d, J=6.5 Hz, H-11'), 1.16 (3H, d, J=6.2 Hz, H-11).

Compound **12**: Pale yellow powder; $[\alpha]_D^{28}$ −33° (c 0.036, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 236 (4.83), 288 (4.08), 301 (4.06), 330 (3.90), 344 (3.92) nm; MS (ESI-TOF) m/z 313 $[M+Na]^+$, 291 $[M+H]^+$, 273; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.26 (1H, s, 9-O*H*), 7.27 (1H, s, H-10), 6.70 (1H, d, J=2.0 Hz, H-5), 6.45 (1H, d, J=2.0 Hz, H-7), 5.10 (1H, d, J=15.6 Hz, H-1a), 4.74 (1H, d, J=15.6 Hz, H-1b), 4.36 (1H, s, H-4), 4.02 (3H, s, 8-OCH_3), 3.88 (3H, s, 6-OCH_3), 3.82 (1H, q, J=6.4 Hz, H-3), 2.01 (1H, brs, 4-O*H*), 1.44 (3H, d, J=6.3 Hz, H-11).

Compound **13**: Pale yellow powder; mp 138~140°C; $[\alpha]_D^{28}$ +107° (c 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 237 (4.79), 290 (3.67), 302 (3.68), 325 (3.46), 340 (3.53) nm; IR (KBr) ν_{max} 3417, 2943, 2812, 1637, 1585, 1362, 1202, 1146, 1043, 936, 847, 811 cm^{-1}; MS (ESI-TOF) m/z 275 $[M+H]^+$; *Anal.* C 70.03%, H 6.69%, calcd for $C_{16}H_{18}O_4$, C 70.05%, H 6.61%.

Single Crystal X-Ray Diffraction Analysis of **1**

A yellow single crystal of dimensions 0.20×0.40×0.50 mm^3, crystallized from an acetone solution, was mounted in a sealed capillary for data collection. All measurements were made using an Enraf-Nonius CAD4 diffractometer with Mo-Kα radiation (λ=0.70183 Å) at room temperature (298 K) using variable scan speed in 2θ to a maximum 2θ value of 49.84°. The crystal belongs to the tetragonal space group, P4(3), with a=b=10.340 (1), c=34.242 (3) Å, and V=3661.0(3) $Å^3$. A total of 3284 unique reflections were measured of which 1785 reflections were observed I>2σ (I). The structure was solved by direct methods using SHELXS-97 and refined using SHELXL-97. The non-hydrogen atoms excepts those of solvent molecules were refined anisotropically. Full-matrix least-squares refinements on F^2 gave a final discrepancy index of 0.1386 and goodness-of-fit of 2.89. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.51 and −0.50 $e^-/Å^3$, respectively. All computations were carried out using the maXus suite.

$LiAlH_4$ Reduction of **2**, **3** and **5**

To a THF (1 ml) solution of compound **2** (20.0 mg) was added $LiAlH_4$ (20 mg) and the mixture was stirred for 2 days at room temperature. After usual aqueous workup, the crude product was purified by preparative HPLC (Nova-Pak HR C_{18}, 6 μm, 40×100 mm, MeCN/H_2O=50 : 50, 20 ml/minute), followed by silica gel column chromatography (MeOH/CH_2Cl_2=2 : 98) to obtain **1** (10.2 mg, 55% yield). In the same fashion, compound **3** (20.0 mg) was converted into **1** (9.5 mg, 54%), and compound **5** (20.0 mg) to **4** (9.0 mg, 48%).

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