Five New Phenolics from the Roots of *Ficus beecheyana*

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From the ethanolic extract of the roots of *Ficus beecheyana*, *threo*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**1**), *erythro*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (**2**), *trans*-4,5-bis(4-hydroxy-3-methoxyphenyl)-1,3-dioxacyclohexane (**3**), *threo*-3-(4-hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol (**4**), 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**5**), and 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**6**) were isolated. The structures of the new compounds **1**−**5** were elucidated by the analysis of their spectroscopic data.

Ficus beecheyana Hook. & Arn. (Moraceae) is a semideciduous tree with brown, tomentose branches and is widely distributed in east Asia, especially in mainland China, Hong Kong, Vietnam, and Taiwan.[1] Its rhizomes have long been used as a folk medicine to treat rheumatism and diabetes, and as a carminative,[2] but no phytochemical studies have been carried out on this plant part. The roots of *F. beecheyana* are closely related to the rhizomes and again have not been investigated phytochemically. A chemical investigation on an ethanolic extract of the roots of *F. beecheyana* was thus undertaken and has led to the isolation and characterization of five new phenolics (**1**−**5**) along with one known compound (**6**). This report describes the isolation and structural elucidation of the new compounds.

The ethanolic extract from the roots of *F. beecheyana* was suspended in H_2O and then partitioned sequentially using *n*-hexane, $CHCl_3$, and *n*-BuOH. The $CHCl_3$-soluble fraction was then subjected to silica gel flash column chromatography and HPLC to give five new phenolics (**1**−**5**), together with one known compound, 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**6**).[3]

Compound **1** was isolated as an amorphous powder. A pseudomolecular ion of *m*/*z* 272.1042, revealed by HREIMS, led to its elemental formula, $C_{16}H_{16}O_4$, consistent with the elimination of $-CH_2OH$ and $-OCH_2CH_3$ units from the molecule. Its IR absorption maxima at 3412, 1605, and 1516 cm^{-1} and a UV absorption peak at 280 nm suggested the presence of an oxygenated aromatic ring. The ^1H NMR spectrum (Table 1) of **1** exhibited two sets of ABX-type signals [δ_H 6.29 (1H, br s), 6.48 (1H, br d, J = 8.1 Hz); 6.71 (1H, d, J = 8.1 Hz); δ_H 6.51 (1H, br d, J = 8.2 Hz), 6.52 (1H, br s), and 6.69 (1H, d, J = 8.2 Hz)] and two methoxy signals [δ_H 3.68 and 3.72], indicating that **1** has two guaiacyl groups.[4] In the aliphatic region, ABMX-type and ethoxyl signals [δ_H 2.98 (1H, ddd, J = 8.8, 8.8, 3.8 Hz, H-2), 3.83 (1H, dd, J = 10.9, 3.8 Hz, H_a-1), 4.15 (1H, dd, J = 10.9, 8.8 Hz, H_b-1), and 4.39 (1H, d, J = 8.8 Hz, H-3); δ 1.19 (3H, dd, J = 7.0, 7.0 Hz), 3.34 (1H, m), and 3.40 (1H, m)] were observed. Among 19 ^{13}C NMR signals, 12 aromatic signals (six C and six CH; with four 1,2-dioxygenated carbons at δ_C 144.3, 144.9, 146.2, and 146.3) and two phenolic methyl signals (δ_C 55.8, 55.9) (Table 2) were present. The remaining five aliphatic signals included three oxygenated carbons [δ_C 64.3 (CH_2), 66.9 (CH_2), and 87.9 (CH)], one CH_3 (δ_C 15.3), and one CH (δ_C 54.8). The signal at δ_H 2.98 (C-2 resonated at δ_C 54.8) was assigned to a benzylic methine proton due to HBMC correlations with C-1, C-3, C-1′, C-1″, C-2″, and C-6″. The proton resonating at lower field at δ 4.39 (H-3) was considered as being linked with an ethoxyl group and a guaiacyl group. The H-3 (δ 4.39) resonance exhibited correlations with C-1, C-2, C-1′, C-2′, C-6′, C-1″, and C-1‴ in the HMBC spectrum and helped to confirm the assigned partial structure. The NOESY spectrum revealed mutual correlations between H_3-7′/H-2′ and H_3-7″/H-2″. These correlations permitted the structural assignment of **1** as 2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol. Comparison of the coupling constant between H-2 and H-3 ($J_{2,3}$ = 8.8 Hz) with published data of related phenolic isomers provided evidence for the determination of the *threo* configuration of the C-2 and C-3 substituents.[4,5] Additionally, the chemical shifts of C-1, C-2, C-3, H-2, and H-3 of **1** were also in accordance with those of *threo*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-methoxypropan-1-ol isolated from *Aralia bipinnata*.[5] Thus, **1** was concluded to be *threo*-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol. To our knowledge, compound **1** has been isolated from a natural source for the first time, although this compound has been synthesized by Gellerstedt and Agnemo, but the spectral data of **1** were not included in their report.[6]

Compounds **2** and **3** were isolated as a mixture. This mixture was acetylated using pyridine and Ac_2O, then the products were subjected to a normal-phase HPLC separation to yield their peracetylated derivatives **7** and **8**, respectively. Compound **7** was obtained as an amorphous powder, and its molecular formula was determined as $C_{25}H_{30}O_9$ by the HREIMS data and from its ^{13}C NMR spectrum. Its ^1H NMR spectrum (Table 1) exhibited two sets of acetyl guaiacyl groups [δ_H 6.58 (1H, d, J = 1.4 Hz), 6.63 (1H, dd, J = 8.1, 1.4 Hz), 6.85 (1H, d, J = 8.1 Hz), 3.67 (3H, s), and 2.26 (3H, s); δ_H 6.40 (1H, d, J = 1.4 Hz), 6.66 (1H, dd, J = 8.0, 1.4 Hz), 6.89 (1H, d, J = 8.0 Hz), 3.57 (3H, s), and 2.26 (3H, s)], a 1,2,3,3-tetrasubstituted propane group [δ_H 3.10 (1H, ddd, J = 7.5, 7.0, 4.3 Hz, H-2),

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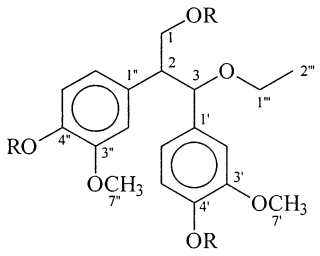

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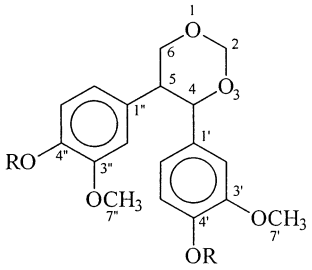
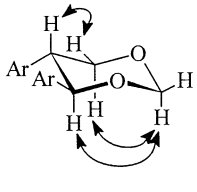
2 R = H, *erythro*
7 R = Ac, *erythro*





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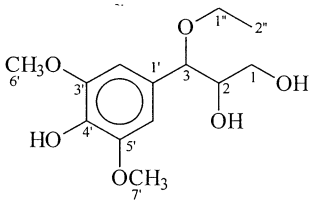
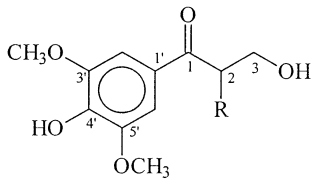
8 R = Ac



4



5 R = OH
6 R = H

4.30 (1H, dd, J = 11.0, 7.0 Hz, H_a-1), 4.53 (1H, dd, J = 11.0, 7.5 Hz, H_b-1), 4.56 (1H, d, J = 4.3 Hz, H-3), and 2.00 (3H,s); the consecutive protons were revealed from the COSY spectrum], an ethoxyl group [δ 1.19 (3H), 3.26 (1H, m), and 3.41 (1H, m)], and an acetyl group [δ_H 2.00 (s)]. It possessed spectroscopic data closely comparable with those of **1** except for having three acetoxyl groups instead of three hydroxyl groups. It showed a small coupling constant between H-2 and H-3 ($J_{2,3}$ = 4.3 Hz), revealing an *erythro* configuration in contrast to the *threo* configuration signals between H-2 and H-3 ($J_{2,3}$ = 8.8 Hz) observed for **1**.[7,8] Therefore, the structure of **7** was established as *erythro*-2,3-bis(4-acetoxy-3-methoxyphenyl)-3-ethoxypropan-1-ol acetate.

Analysis of the HREIMS, DEPT, and ^{13}C NMR spectra of **8** gave a molecular formula of $C_{22}H_{24}O_8$, indicating 11 indices of hydrogen deficiency. The IR absorption bands at 1605 and 1507 cm^{-1} suggested the presence of an aromatic functionality. The ^{1}H NMR spectrum (Table 1) revealed partial structural characteristics such as aromatic proton signals [δ 6.62 (br s), 6.64 (br d), and 6.83 (d); δ 6.34 (br s), 6.66 (br d), and 6.90 (d)], two phenolic acetyl groups (δ 2.24 and 2.25), and two phenolic methyl groups (δ 3.64 and 3.65). The NOESY spectrum of **8** showed correlations between δ 6.34 (br s)/3.65 (OCH_3) and δ 6.62 (br s)/3.64 (OCH_3), revealing the relative positions of the two substituents in the phenyl group which could be established as an acetyl guaiacyl unit. The residual structural units included two oxygenated methylenes (δ_C 94.1, and 71.2), two methines (δ_C 83.9, and 49.6), and two oxygen atoms. The remaining index of hydrogen deficiency was consistent with the presence of a 1,3-dioxacyclohexane unit, and the signal at δ_C 94.1 was assigned to a methylenedioxy carbon. The contiguous sequence of H-4, -5, and -6 (Table 1) was revealed from COSY correlations. In a HMBC experiment, correlations between H-4 and C-2, -5, -6, -1′, -2′, -6′, -1″ and H-5 and C-4, -6, -1′, -1″, -2″, -6″ were observed. Therefore, the structure of **8** was assigned as 4,5-bis(4-acetoxy-3-methoxyphenyl)-1,3-dioxacyclohexane. The stereochemistry of **8** was established by a combination of observed coupling constants and data from its NOESY spectrum. The H-4 signal was assigned to a benzylic proton with an axial orientation due to the large coupling constant (δ 4.59, d, J = 9.6 Hz) with H-5 (δ 3.05). The H-5 proton was linked to a methylene (H_2-6) group from its coupling constants (J = 11.2, 9.6, 4.5 Hz). The NOESY spectrum of **8** showed correlations between H-5 and H_{eq}-6, H_{ax}-6 and H_{ax}-2, and H-4 and H_{ax}-2 (as in the chair conformation of **8**). These data were supportive of having the two aryl groups both in the equatorial orientation. Therefore, compound **8** was assigned as *trans*-4,5-bis(4-acetoxy-3-methoxyphenyl)-1,3-dioxacyclohexane.

Compound **4**, obtained as an amorphous powder, was assigned in its ^{13}C NMR spectrum 13 carbon signals including two oxygenated methylenes (δ_C 62.4 and 64.3) (Table 2), two methoxyls (δ_C 56.3 × 2), one methyl (δ_C 15.3), two oxygenated methines (δ_C 75.4 and 82.6), and six aromatic carbons (δ_C 103.9 × 2, 129.4, 134.6, 147.2 × 2). In the ^{1}H NMR spectrum, two singlet phenyl protons (δ 6.54), two phenolic methyl groups (δ 3.87, 6H), and an exchangeable phenolic proton (δ 5.50) suggested the presence of a 4-hydroxy-3,5-dimethoxyphenyl moiety. A NOESY correlation between δ_H 6.54 and 3.87 indicated the presence of a syringyl group. An ethoxyl group attached to a chiral carbon was revealed from the ^{1}H NMR [δ_H 1.18 (3H, t, J = 7.0 Hz), 3.33, 3.44 (each 1H, m)] and ^{13}C NMR (δ_C 15.3 and 64.3) data. A doublet signal at δ_H 4.19 (1H, H-3) showed a HMBC correlation with δ_C 64.3 (−O*C*H$_2$CH$_3$) and NOESY correlations with signals at δ_H 3.33 and 3.44, and this was assigned as geminal to an ethoxy group. The contiguous protons to H-2 [δ 3.68 (1H, ddd, J = 8.2, 4.5, 3.2 Hz)] and then to H-1 [δ 3.35 (1H, dd, J = 11.8, 4.5 Hz) and 3.55 (1H, dd, J = 11.8, 3.2 Hz)] were established from the COSY spectrum. Furthermore, the large coupling constant between H-2 and H-3 (J = 8.2 Hz) revealed a *threo* configuration.[9] The aryl group in **4** was located at C-3 on the basis of the HMBC correlations of H-3/C-1′, -2′, and -6′. Hence, **4** was determined to be *threo*-3-(4-hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol.

Compound **5** was assigned a molecular formula of $C_{11}H_{14}O_6$ by HREIMS, two carbons and six hydrogen atoms less than **4**. Its IR spectrum indicated the presence of hydroxyl (3431 cm^{-1}), aromatic (1605 and 1516 cm^{-1}), and

Table 1. ^{1}H NMR Data for Compounds **1**, **4**, **5**, **7**, and **8** ($CDCl_3$, 500 MHz)[a]

position	1	4	5	7	8
1	3.83 dd (10.9, 3.8)	3.35 dd (11.8, 4.5)		4.30 dd (11.0, 7.0)	
	4.15 dd (10.9, 8.8)	3.55 dd (11.8, 3.2)		4.53 dd (11.0, 7.5)	
2	2.98 ddd (8.8, 8.8, 3.8)	3.68 ddd (8.2, 4.5, 3.2)	5.10 dd (5.4, 3.4)	3.10 ddd (7.5, 7.0, 4.3)	4.97 d (6.5)
					5.31 d (6.5)
3	4.39 d (8.8)	4.19 d (8.2)	3.70 dd (11.6, 5.4)	4.56 d (4.3)	
			3.99 dd (11.6, 3.4)		
4					4.59 d (9.6)
5					3.05 ddd (11.2, 9.6, 4.5)
6					3.98 dd (11.2, 11.2)
					4.24 dd (11.2, 4.5)
2′	6.52 br s	6.54 s	7.20 s	6.40 d (1.4)	6.62 br s
5′	6.69 d (8.2)			6.89 d (8.0)	6.83 d (8.0)
6′	6.51br d (8.2)	6.54 s	7.20 s	6.66 dd (8.0, 1.4)	6.64 br d (8.0)
7′	3.72 s	3.87 s	3.94 s	3.57 s	3.64 s
8′		3.87 s	3.94 s		
1″		3.33 m			
		3.44 m			
2″	6.29 br s	1.18 t (7.0)		6.58 d (1.4)	6.34 br s
5″	6.71 d (8.1)			6.85 d (8.1)	6.90 d (8.3)
6″	6.48 br d (8.1)			6.63 dd (8.1, 1.4)	6.66 br d (8.3)
7″	3.68 s			3.67 s	3.65 s
1‴	3.34 m			3.26 m	
	3.40 m			3.41 m	
2‴	1.19 dd (7.0, 7.0)			1.11 dd (7.0, 7.0)	
CH_3CO-				2.00 s	2.24 s
				2.26 s	2.25 s
				2.26 s	

[a] Signals without multiplicity were assigned from the COSY or HMQC spectrum.

Table 2. ^{13}C NMR Data for Compounds **1**, **4**, **5**, **7**, and **8** ($CDCl_3$, 125 MHz)[a]

position	1	4	5	7	8
1	66.9 t	62.4 t	197.4 s	65.0 t	
2	54.8 d	75.4 d	74.0 d	51.6 d	94.1 t
3	87.9 d	82.6 d	65.9 t	81.1 d	83.9d
4					
5					49.6d
6					71.2 t
1′	132.4 s	129.4 s	124.7 s	139.1 s	139.2 s
2′	109.4 d	103.9 d	106.0 d	111.0 d	110.8 d
3′	146.3 s	147.2 s	147.1 s	139.0 s	138.0 s
4′	144.9 s	134.6 s	140.9 s	150.8 s	150.6 s
5′	113.7 d	147.2 s	147.1 s	122.1 d	122.2 d
6′	120.3 d	103.9 d	106.0 d	119.1 d	118.8 d
7′	55.9 q	56.3 q	56.6 q	55.8 q	55.9 q
8′		56.3 q	56.6 q		
1″	131.2 s	64.3 t		136.2 s	135.9 s
2″	111.6 d	15.3 q		114.1 d	113.6 d
3″	146.2 s			138.9 s	138.9 s
4″	144.3 s			150.3 s	151.0 s
5″	114.2 d			121.9 d	122.7 d
6″	120.6 d			121.6 d	119.6 d
7″	55.8 q			55.8 q	55.9 q
1‴	64.3 t			64.7 t	
2‴	15.3 q			15.1 q	
CH_3CO-				20.6 q	20.6 q
				20.6 q	20.6 q
				20.9 q	
CH_3CO-				168.9 s	171.7 s
				168.9 s	171.7 s
				171.0 s	

[a] Multiplicities were obtained from DEPT experiments.

conjugated carbonyl (1667 cm^{-1}) groups. Besides signals for a 3,5-dimethoxy-4-hydroxyphenyl unit, the remaining three carbon signals were one conjugated carbonyl (δ_C 197.4) and two oxygenated carbons (δ_C 65.9 and 74.0). The UV peak at 287 nm also confirmed the presence of a 3,4,5-trioxygenated benzoyl group.[10] Comparison of the ^{13}C NMR data of **5** with those of **4** revealed that the ethoxyl group in **4** was replaced by an oxo function (δ_C 197.4) (Table 2). Therefore, **5** was an 1-oxo analogue of **4**. The HMBC spectrum of **5** displaying correlations between C-1/H_2-1, H-2, H-2′, and H-6′ was supportive of this conclusion. Consequently, **5** was established as 2,3-dihydroxy-3-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. The IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. The NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker DMX-500 SB spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EIMS and HREIMS were recorded on a Finnigan TSQ-700 and a JEOL SX-102A spectrometer, respectively. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). HPLC was performed using a Hichrosorb Si 60 (10 μm) column (250 × 10 mm).

Plant Material. The roots of *F. beecheyana* were collected at Nankang in north Taiwan in June 1999 and were identified by Mr. Chii-Cheng Liao of the Department of Botany, National Taiwan University. Voucher specimens (No. 19990615) have been deposited at Chung Hwai College of Medical Technology, Tainan, Taiwan.

Extraction and Isolation. The dried crude EtOH extract of the roots of *F. beecheyana* (12 kg) was a gift from Dr. An-Pang Lin, Jen-Ai Chinese Medical United Clinic, Taipei, Taiwan. The ethanolic extract (81 g) was suspended in H_2O (500 mL) and then partitioned sequentially using *n*-hexane, $CHCl_3$, and *n*-BuOH (500 mL × 3). The $CHCl_3$-soluble fraction was evaporated under a vacuum to give an oily residue (39 g), which was chromatographed over silica gel 60 (230-400 mesh) and eluted sequentially with *n*-hexane−EtOAc (5:3), *n*-hexane−EtOAc (5:7), and EtOAc to give subfractions I, II, and III, respectively. Subfraction I was purified by using the same column with *n*-hexane−EtOAc (1:5) as eluent to yield **1** (26 mg) and a mixture of **2** and **3** (4 mg). The mixture of **2** and **3** was dissolved in pyridine (3 mL) and Ac_2O (3 mL) and left overnight at room temperature. Then, the reaction mixture was poured into ice water (30 mL) and stirred for 1 h. The resultant suspension was extracted with ethyl acetate (30 mL

× 2). The ethyl acetate layer was washed with 1 N HCl, 3% aqueous $NaHCO_3$, and then brine water, sequentially. The organic layer was purified on silica gel chromatography with *n*-hexane−EtOAc (2:1) as eluent to yield the pure peracetylated derivatives **7** (3 mg) and **8** (2 mg). Subfraction II was purified by using the same chromatographic procedure with *n*-hexane−EtOAc−C_6H_6 (2:20:1) as eluent to give **6** (7 mg). Subfraction III was purified by using silica gel chromatography with *n*-hexane−EtOAc−Me_2CO (1:20:2) as eluent to afford **4** (3 mg) and **5** (10 mg).

***threo*-2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol (1):** amorphous white powder; $[\alpha]^{25}_D$ +16° (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 3412, 1605, 1516, 1377, 1264 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 280.2 (3.39) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 272 $[(M - C_3H_8O_2)]^+$ (16), 181 (100), 167 (17), 149 (43), 137 (9); HREIMS *m*/*z* $[M - (C_3H_8O_2)]^+$ 272.1042 (calcd for $C_{16}H_{16}O_4$ 272.1049).

***threo*-3-(4-Hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol (4):** amorphous white powder; $[\alpha]^{25}_D$ +28° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3420, 2930, 1612, 1518, 1460, 1329, 1215, 1115 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 271 (3.59) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 272 (M^+, 3), 226 (2), 211 (100), 196 (3), 183 (10), 167 (35), 123 (12); HREIMS *m*/*z* 272.1250 (calcd for $C_{13}H_{20}O_6$ 272.1260).

2,3-Dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (5): amorphous white powder; $[\alpha]^{25}_D$ 0° (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3431, 2928, 2855, 1667, 1605, 1516, 1464, 1423, 1271, 1119, 1034 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 286 (3.61) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 242 (M^+, 4), 212 (8), 196 (4), 181 (100), 153 (13), 137 (7); HREIMS *m*/*z* 242.0791 (calcd for $C_{11}H_{14}O_6$ 242.0790).

***erythro*-2,3-Bis(4-acetoxy-3-methoxyphenyl)-3-ethoxypropan-1-ol acetate (7):** amorphous powder; $[\alpha]^{25}_D$ −14° (*c* 0.14, $CHCl_3$); IR (KBr) ν_{max} 2973, 1767, 1740, 1605, 1510, 1370, 1198, 1034 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 279 (3.48) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 474 (M^+, 2), 272 (3), 232 (88), 181 (100), 153 (21), 135 (5), 93 (10); HREIMS *m*/*z* 474.1883 (calcd for $C_{25}H_{30}O_9$ 474.1890).

***trans*-4,5-Bis(4-acetoxy-3-methoxyphenyl)-1,3-dioxacyclohexane (8):** amorphous powder; $[\alpha]^{25}_D$ −46° (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 2924, 2853, 1761, 1605, 1507, 1372, 1198, 1028 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 273 (3.35) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 416 (M^+, 36), 374 (68), 253 (93), 239 (62), 225 (68), 211 (90), 197 (100); HREIMS *m*/*z* 416.1489 (calcd for $C_{22}H_{24}O_8$ 486.1471).

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