Cytotoxic Flavonoids and α-Pyrones from *Cryptocarya obovata*

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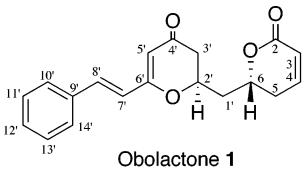
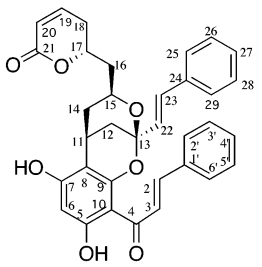
One α-pyrone, obolactone (**1**), two chalcones, kurzichalcolactone B (**2**) and obochalcolactone (**3**), and two flavanones, oboflavanones A (**4**) and B (**5**), have been isolated from the fruits and the trunk bark of *Cryptocarya obovata*. The structures of the new compounds were elucidated by spectroscopic interpretations. The absolute configuration of obolactone (**1**) was established by circular dichroism. Obolactone (**1**) and obochalcolactone (**3**) display significant activity in in vitro cytotoxic assays against the KB cell line. Biosynthetic pathways for oboflavanones and obochalcolactone are suggested.

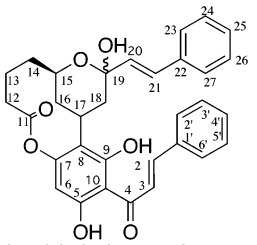
In continuation of our ongoing search for antitumor compounds from Vietnamese plants,[1] we investigated *Cryptocarya obovata* R. Br. (Lauraceae), a plant collected at Yen Chau, Son La Province in the north of Hanoi, Vietnam.[2] This plant is a tropical tree of the cinnamon family, which we found to possess a medium cytotoxicity on human nasopharyngeal carcinoma KB cells (56% and 23% inhibition at 10 μg mL^{-1} for ethanolic extracts of the fruit and trunk bark, respectively). In this paper, we report the structural elucidation and cytotoxicity evaluation of five new compounds: α-pyrone **1**, chalcones **2** and **3**, and flavanones **4** and **5**. We also describe the isolation of the known compounds kurzichalcolactone A,[3] kurziflavolactones A−D,[3] pinocembrin,[4] pinosylvin,[5] 3,5,7-trihydroxychalcone,[6] cryptofolione,[7] (−)-epicatechin,[8] procyanidin B_2,[9] and cinnamtannin B_1,[10] which were identified by comparison of their physical and spectral data with values already reported.



Obolactone **1**

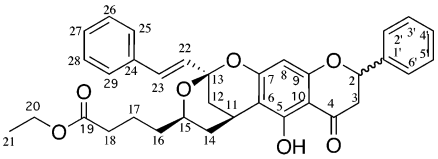


obochalcolactone **3**



kurzichalcolactone A : 19α- or 19β-OH **6**

kurzichalcolactone B : 19β-or 19α-OH **2**



oboflavanone A (2*S*) **4**

oboflavanone B (2*R*) **5**

Results and Discussion

An ethanolic extract of the dried trunk bark of *C. obovata* was purified by chromatography on silica gel and then by HPLC or preparative TLC to afford obolactone (**1**), kurzichalcolactone B (**2**), and obochalcolactone (**3**) as well as the known compounds kurzichalcolactone A (**6**), kurziflavolactones A−D, 5,7,9-trihydroxychalcone, pinocembrin, pinosylvin, and three tannins, (−)-epicatechin, procyanidin B_2, and cinnamtannin B_1. Similar treatment of the ethanolic extract of the dried fruits afforded oboflavanones A (**4**) and B (**5**) and the known cryptofolione.

Obolactone (**1**) was obtained as pale yellow crystals from ethyl acetate and exhibited in its mass spectrum a molecular ion peak at *m*/*z* 310. The IR spectrum showed two absorption bands at 1724 and 1626 cm^{-1} corresponding to an unsaturated δ-lactone and a conjugated ketone, respectively. The ^{1}H NMR spectrum of **1** displayed five aromatic protons at δ 7.37 and 7.53, a set of olefinic protons at δ 6.55 and 7.39 (J = 15.9 Hz) with an *E* configuration, one isolated olefinic proton at δ 5.54, and two other olefinic protons at δ 6.08 and 6.93 with a *Z* configuration (J = 9.9 Hz). HMBC correlations allowed the proposed structure of obolactone as shown in **1**. The relative configuration of obolactone (2′*R*,6*R* or 2′*S*,6*S*) was deduced from X-ray crystallographic analysis (Figure 1). There are four different molecules in the asymmetric unit that adopt nearly the same conformation in which the two lactone planes are practically perpendicular, except for the orientation of their phenyl group (Figure 2). Finally, the positive signal of the Cotton effect in the CD curve of **1** at 262 nm, corresponding to the n → π* transition, suggested a C-6*R* configuration, and comparison with the CD values of other α-pyrones such

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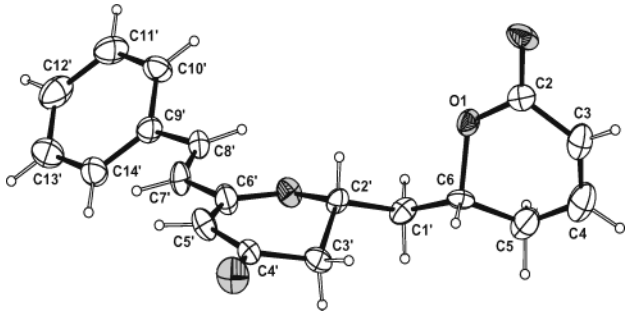

Figure 1. ORTEP drawing of obolactone **1** (molecule B).

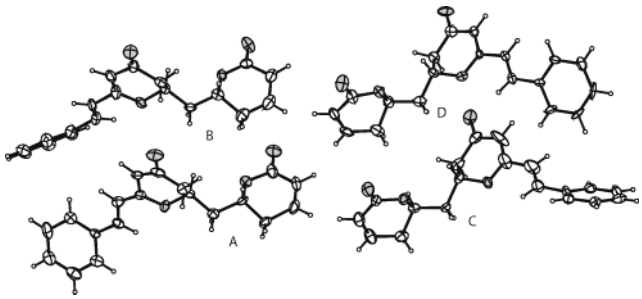

Figure 2. Molecules A, B, C, and D in the asymmetric unit of obolactone **1**.

as cryptopyranomoschatones[11] and cryptocaryalactone[12] allowed us to assign a 2′*R*,6*R* configuration for obolactone (**1**). Figure 1 shows the ORTEP drawing of one molecule (B) possessing the 2′*R*,6*R* absolute configuration.

Compound **2** showed a molecular ion peak at *m*/*z* 526 and a mass spectrum fragmentation pattern similar to those of many chalcones and flavanones. The IR absorptions of **2** at 3579, 1712, and 1628 cm^{-1} indicated the presence of hydroxyl, lactone, and conjugated ketone functions. A comparison of the NMR spectra of **2** with those of kurzichalcolactone A (**6**), isolated from this plant and previously from *Cryptocarya kurzii*,[3] revealed the strong structural similarity of these two compounds (Table 1) and indicated that compound **2** was a stereoisomer of **6**. Analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectra permitted the assignment of all proton and carbon signals for **2**. There are three asymmetric carbons in compounds **2** and **6**, at the 15, 17, and 19 positions. The signals for H-15 and H-17 in the ^1H NMR spectrum of both **2** and **6** are strictly identical, suggesting that the two isomers possess the same configuration at these positions and only differ by the α or *β* position at the C-19 position. Moreover, the small coupling constants observed for H-17 in compounds **2** and **6** indicated an equatorial position for H-17 in both compounds, whereas the H-15 peak appearance, which ressembled that of kurziflavolactones,[3] revealed an axial position. As described previously for **6**,[3] **2** could be formed by a coupling of 5,7,9-trihydroxychalcone (**7**) to kurzilactone (**8**) also isolated from *C. kurzii* [3,13] (Scheme 1 in Supporting Information). Phenol coupling would lead to a linear intermediate, which would cyclize twice to form the C8−C17 bond as well as the pyran ring. The recent stereoselective synthesis of both (5*S*,7*S*)- and (5*R*,7*S*)-isomers of **8** led to the revision of the structure of natural **8** and determination of its 5*R*,7*S* absolute configuration.[14] If we assume that natural (5*R*,7*S*)-kurzilactone (**8**) is the common precursor of **2** and **6**, the configuration at C-15 of the two kurzichalcolactones would be fixed. Unfortunately, suitable X-ray analysis could not be obtained to define the complete stereochemistry of **2** and **6**.

Table 1. NMR Data for Kurzichalcolactones B (**2**) and A (**6**) in $CDCl_3$

	2		**6**	
number	δC	δH	δC	δH
2	143.1	7.72 d (15.6)	143.3	7.72 d (16)
3	128.6	8.31 d (15.6)	128.9	8.22 d (16)
4	193.8		193.4	
5	163.5		162.6	
6	94.9	6.09 s	97.0	6.11s
7	164.5		167.4	
8	106.2		105.5	
9	161.0		159.6	
10	106.1		106.2	
11	174.7		175.1	
12	34.7	2.30 tl (7.0)	34.6	2.22 tl (7)
13	23.9	1.50-1.80 m	22.1	1.50-1.80 m
14	35.9	1.50-1.80 m	36.1	1.50-1.80 m
15	71.0	3.70 m	71.4	3.78 m
16	36.1	1.50-1.80 m	36.5	1.50-1.80 m
17	21.9	3.55 m	24.6	3.58 dd (3; 2.5)
18	34.2	2.17 dd (12.9; 2.7)	34.4	2.12 dd (13; 2.5)
		1.91 dd (12.9; 3.1)		2.20 dd (13; 3)
19	99.4		100.0	
20	131.4	6.94 d (16.1)	132.4	7.12 d (16)
21	131.2	6.42 d (16.1)	131.0	6.68 d (16)
22	137.3		137.5	
23	127.8	7.54 dl (7.2)	128.3	7.53 m
24	129.3	7.37 tl (7.5)	129.5	7.36 m
25	129.0	7.29 tl (7.3)	129.5	7.36 m
26	129.3	7.37 tl (7.5)	129.5	7.36 m
27	127.8	7.54 m	128.3	7.53m
1′	136.6		136.7	
2′	129.6	7.71 m	130.0	7.62 m
3′	130.0	7.46 m	130.0	7.46 m
4′	130.9	7.46 m	130.0	7.46 m
5′	130.0	7.46 m	130.0	7.46 m
6′	129.6	7.71 m	130.0	7.62 m
OH-5		14.50 s		14.10 s

Obochalcolactone (**3**) was obtained as a yellow powder and exhibited a molecular ion at *m*/*z* 550 in its mass spectrum and a fragmentation pattern also similar to that of chalcones and flavanones. The IR absorptions at 3580, 1717, and 1628 cm^{-1} indicated the presence of a hydroxyl group, an unsaturated *δ*-lactone, and a conjugated ketone, respectively. ^{13}C and ^1H NMR spectra (Table 2) revealed a structural similarity with obolactone (**1**) added to that of 5,7,9-trihydroxychalcone **7**. Comparison with the spectra of **7** suggested a substitution at C-8. Structural differences between obolactone (**1**) and compound **3** were found for the pyran part. The COSY spectrum of **3** indicated the presence of a set of coupling relations between H-11 resonating at *δ* 3.61 and signals at *δ* 2.10 and 2.23 corresponding to the geminal H-12 protons. The connectivity of segment C-12−C-13−C-22 and the linkage between C-8 and C-11 were established by HMBC correlations. The presence of an acetal unit in **3** was deduced from a signal at *δ* 99.2 in its ^{13}C spectrum. Moreover, the location of the cyclic acetal at the 9 position, as an alternative to the 7-acetal substitution, was confirmed by unequivocal NOESY correlations between H-6 and the two hydroxyl groups at C-5 and C-7. The NOESY correlations also revealed the relative configurations of C-11, C-13, and C-15; in particular the cross-peaks from H-15 to H-12-α allowed assignment of the stereochemistry of the two rings linked at C-11 and C-13. Finally, the 17*R* configuration could be assigned, as for **1**, from the positive Cotton effects at 257 nm. Thus, the structure of obochalcolactone was concluded to be **3** with the absolute configuration at C-11, C-13, and C-15 undetermined. Biogenetically, **3** could come from the coupling of obolactone **1** and 3,5,7-trihydroxychalcone **7** (Scheme 2 in Supporting Information).

Table 2. NMR Data for Obochalcolactone (**3**) and Oboflavanones A (**4**) and B (**5**)

	3		**4**		**5**	
number	δC	δH	δC	δH	δC	δH
2	142.2	7.72 d (15.6)	79.2	5.38 dd (13.1; 2.8)	79.2	5.45 dd (12.6; 3.0)
3	128.2	8.19 d (15.6)	43.7	2.82 dd (17.2; 2.8)	43.4	3.09 dd (17.1; 12.6)
				3.08 dd (17.2; 13.1)		2.84 dd (17.1; 3.0)
4	192.6		196.2		196.1	
5	166.6		164.1		164.1	
6	96.3	6.10 m	106.6		106.6	
7	161.6		161.0		161.0	
8	104.7		95.2	6.12 s	95.2	6.13 s
9	159.0		160.0		159.9	
10	105.1		103.0		103.1	
11	23.6	3.61 m	22.8	3.56 m	22.7	3.56 m
12	33.4	2.23 dd (12.8; 2.7);	33.9	2.12 dd (12.9; 2.7)	33.9	2.12 dd (12.9; 2.7)
		2.10 dd (12.8; 3.5)		1.87 dd (12.9; 2.1)		1.86 dd (12.9; 2.2)
13	99.2		98.8		98.8	
14	35.6	1.86 m	35.2	1.67 m; 1.79 m	35.2	1.71 m; 1.79 m
15	67.5	4.03 m	70.0	3.72 m	70.1	3.72 m
16	40.8	2.14 ddd (14.2; 8.1; 6.3)	35.3	1.52 m; 1.68 m	35.3	1.52 m; 1.66 m
		1.91 ddd (14.2; 11.1; 6.5)				
17	75.2	4.73 m	21.0	1.64 m; 1.77 m	21.0	1.63 m; 1.78 m
18	29.4	2.49 ddl (18.4; 6.0)	34.3	2.29 dd (7.3; 7.0)	34.3	2.12 dd (7.2; 7.0)
		2.35 ddt (18.4; 11.4; 2.5)				
19	146.1	6.89 ddd (9.7; 6.0; 2.4)	173.6		173.6	
20	121.0	5.85 dl (9.7; 2.1)	60.3	4.10 q (7.1)	60.3	4.10 q (7.1)
21	163.7		14.4	1.23 tl (7.1)	14.3	1.23 tl (7.1)
22	129.7	6.67 d (16.1)	129.3	6.30 d (16.1)	129.2	6.30 d (16.1)
23	131.8	7.14 d (16.1)	131.2	6.88 d (16.1)	131.2	6.88 d (16.1)
24	136.6		136.1		136.1	
25	127.5	7.56 m	127.0	7.53 m	127.0	7.42 m
26	129.1	7.34 m	128.7	7.34 m	128.7	7.33 m
27	129.1	7.34 m	128.3	7.34 m	128.3	7.33 m
28	129.1	7.34 m	128.7	7.34 m	128.7	7.33 m
29	127.5	7.56 m	127.0	7.53	127.0	7.42 m
1'	135.8		138.6		138.6	
2'	128.6	6.98 m	126.2	7.53 m	126.3	7.42 m
3'	129.2	7.53 m	129.0	7.53 m	129.0	7.42 m
4'	130.3	7.21 m	129.0	7.34m	128.9	7.33 m
5'	129.2	7.53 m	129.0	7.53 m	129.0	7.42 m
6'	128.6	6.98 m	126.2	7.53 m	126.3	7.42 m
OH-5		14.09 s		11.85 s		12.35 s
OH-7		9.85 s				

Oboflavanones A (**4**) and B (**5**) had similar mass spectra with molecular ions at *m*/*z* 554 and fragments at *m*/*z* 131, 104, and 103. The ^{1}H and ^{13}C NMR spectra of these two compounds (Table 2) were nearly the same, indicating that they were stereoisomers. Examination of ^{1}H and ^{13}C NMR spectral data of **4** indicated a close structural relationship with obochalcolactone (**3**) (presence of a bicyclic hemiacetal group and two monosubstituted benzene rings). In addition the ^{1}H NMR confirmed the presence of a trisubstituted flavanone (δ 5.38, dd, 1H, J = 13.1 and 2.8 Hz, H-2, δ 2.82, dd, 1H, J = 17.2 and 2.8 Hz, H-3α, and δ 3.08, dd, 1H, J = 17.2 and 13.1 Hz, H-3β) instead of a chalcone unit in **3**. A triplet and a quadruplet at δ 1.23 and 4.10, respectively, suggested the presence of an ethyl ester group, and the HMQC and HMBC spectra supported linkage of the bicyclic hemiacetal to the flavanone unit at C-6 and C-7. The chelated hydroxyl group at C-5 (δ 11.85) showed NOESY correlation with H-11. This confirmed the substitution of the 5-hydroxyflavanone at positions 7 and 6. Finally, the presence of an aliphatic side chain at C-15 was made on the basis of long-range H−C couplings. These combined data were consistent with structure **4** for oboflavanone A. The relative configuration at C-11, C-13, and C-15 was deduced from NOE associations between 8- and 15-H. The absolute configuration at carbon 2 was assigned as 2*S* from the positive and negative Cotton effects in the CD spectra at 334 nm (n → π* transition) and 290 nm (π → π* transition).[15] With the structure of **4** assigned, **5** was readily identified as the 2*R* epimer of **4** from the strong similarity of their ^{1}H and ^{13}C NMR spectra and the negative and positive Cotton effects at 334 and 290 nm, respectively. Thus, the structures of oboflavanones A and B were assigned to be **4** and **5** with the absolute configuration at C-11, C-13, and C-15 undetermined. Oboflavanones **4** and **5** could derive from condensation of pinocembrin (**9**) and a compound such as **10** (Scheme 3 in Supporting Information). However, the presence of an ethyl ester side chain in **4** and **5** may be artifactual since the plant was extracted with ethanol.

Compounds **1**−**6** were screened for cytotoxicity against the KB cell line.[16] Obolactone (**1**) and obochalcolactone (**3**) showed cytotoxic activities with IC_{50} values of 3 and 5 μM, respectively. Kurzichalcolactones A (**6**) and B (**2**) were about 10 times less cytotoxic than **1**, whereas oboflavanones A (**4**) and B (**5**) had no inhibitory effect on the growth of KB cells.

Experimental Section

General Experimental Procedures. Melting points were measured using a Buchi B-540 melting-point apparatus and are uncorrected. Optical rotations and CD spectra at 20 °C were measured on a Perkin-Elmer 241 polarimeter and a Jobin Yvon CD6 dichrograph. UV spectra were recorded on a Varian Cary 100 spectrometer and IR on a Perkin-Elmer Spectrum BX FT-IR spectrometer; HRCI and EI mass spectra were recorded on a Kratos MS 80 or MS 50, respectively. The NMR

spectra were recorded in $CDCl_3$ on a Bruker AMX 400 spectrometer. Chemical shifts (relative to TMS) are in ppm (δ), and coupling constants (in parentheses) in Hz. Column chromatography (CC) was performed using silica gel Merck H60. Preparative plates (PLC) [silica gel 60 F_{254}] were also used for purification. Preparative HPLC was performed on a Waters PrepPak cartridge (Porasil 15−20 μm 125 Å, 57 × 300 mm) at 50 mL/min using a Waters Delta prep 3000 apparatus. Semipreparative HPLC was carried out on Waters RCM (Prep Nova-Pak HR silica 6 μm 60 Å, 25 × 100 mm) at 10 mL/min.

Plant Material. The trunk bark of *Cryptocarya obovata* R. Br. was collected at Yen Chau, Son La Province, 200 km west of Hanoi, North Vietnam, in February 1996. The plant was identified by one of us (V.D.). Voucher specimens (VN 0055) are deposited at the Herbarium of the Institute of Ecology (NCST), Hanoi, Vietnam.

Extraction and Isolation. The dried ground trunk bark of *C. obovata* (1.7 kg) was extracted with EtOH at 30 °C, and the solvent was evaporated under vacuum to give a crude extract (200 g). An aliquot (60.1 g) of this residue was subjected to column chromatography (silica gel) using a step gradient of heptane−acetone (9:1 to 0:100) to give 15 fractions, of which fractions 10 and 12 were found to be cytotoxic. Fraction 7 (3.18 g) was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 95.5:0.5) to provide pinocembrin (1.5 g). Fraction 8 (307 mg) was separated by column chromatography (CH_2Cl_2−MeOH, 95.5:0.5−90:10) and preparative TLC (CH_2Cl_2−MeOH, 95:5) to give pinosylvin (10 mg). Fraction 10 (4.4 g) was separated by silica gel column chromatography using a step gradient of CH_2Cl_2−MeOH (98:2 to 95:5) as eluent to provide obochalcolactone (**3**) (88 mg), trihydroxychalcone (36 mg), kurzichalcolactone A (**6**) (393 mg), and kurziflavolactones A (20 mg), B (15 mg), C (10 mg), and D (23 mg). Kurzichalcolactone B (**2**) (81 mg) was also obtained from this fraction after HPLC purification (heptane−AcOEt−CH_3CO_2H, 85:15:0.5, v/v). Fraction 12 (5.76 g) was separated by silica gel column chromatography (CH_2Cl_2−MeOH, 98:2−95−5) to give obolactone (**1**) (3.3 g), which was recrystallized in AcOEt. Five grams of the most polar fraction 13 (11.8 g) was separated by reversed-phase silica gel column chromatography (MeOH−H_2O, 3:7−100:0) and preparative TLC (EtOH) to give cinnamtanin B_1 (11 mg) and procyanidin B_2 (33 mg).

Air-dried ground fruits (1.8 kg) of *C. obovata* were extracted with EtOH at 30 °C, resulting in 178 g of extract. CH_2Cl_2 was added to 30 g of this extract. Fractionation on silica gel column chromatography of the CH_2Cl_2 extract (8.7 g) with CH_2Cl_2−MeOH (100:0−50:50) and further chromatography of one of the fractions on silica gel (CH_2Cl_2−MeOH, 97:3) yielded cryptofolione (125 mg). The EtOH crude extract (100 g) was also diluted in heptane−CH_2Cl_2 (50:50), and the soluble part (24.4 g after evaporation of the solvent) was fractionated by silica gel chromatography (CH_2Cl_2−MeOH, 100:0−95:5). One fraction was purified by HPLC (heptane−AcOEt−CH_3CO_2H−2-propanol, 97:3:0.5:0.05) to yield oboflavanone A (**4**) (60 mg) and oboflavanone B (**5**) (51 mg).

Obolactone (1): pale yellow needles; mp 116° C (AcOEt); $[\alpha]_D^{25}$ +286° (*c* 1.12, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 330 (21010), 385 (22973), 200 (27940) nm; CD (EtOH) λ_{ext} ($\Delta\epsilon$) 202 (+15.5), 241 (+1.5), 262 (+1.5), 351 (+3.45); IR ($CHCl_3$) ν_{max} 1724, 1655, 1626, 1581, 1567, 1397, 1342,1249, 1235, 1030, 967, 814 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.09 (1H, dt, J = 14.7 and 5.1 Hz, H-1′α), 2.50 (1H, m, H-1′β), 2.51 (2H, m, H-5), 2.55 (1H, dd, J = 16.7 and 4.5 Hz, H-3′α), 2.62 (1H, dd, J = 16,7 and 12.2 Hz, H-3′β), 4.74 (1H, m, H-6), 4.77 (1H, m, H-2′), 5.54 (1H, s, H-5′), 6.08 (1H, dt, J = 9.9 and 1.8 Hz, H-3),; 6.55 (1H, d, J = 15.9 Hz, H-7′); 6.93 (1H, dt, J = 9.9 and 3.9 Hz, H-4); 7.37 (3H, m, H-11′, H-12′, H-13′);7.39 (1H, d, J = 15.9 Hz, H-8′); 7.53 (2H, m, H-10′, H-14′); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 39.4 (C-1′); 41.3 (C-3′); 74.6 (C-6); 75.7 (C-2′); 106.3 (C-5′); 121.3 (C-7′); 121.5 (C-3); 127.8 (C-10′, C-14′); 128.9 (C-11′, C-13′); 129.8 (C-12′); 135.0 (C-9′); 137.6 (C-8′); 144.8 (C-4); 163.7 (C-2); 168.0 (C-6′); 192.3 (C-4′); EIMS *m/z* 310 $[M]^{+\bullet}$ (100), 242 (11), 197 (67), 171 (36), 144 (44), 131 (100), 116 (22), 103 (22), 97 (10), 77 (12); HRMS *m/z* 311.1293 $[M + H]^+$ (calcd for $C_{19}H_{19}O_4$ 311.1283).

X-ray Crystal Structure Analysis of Obolactone (1).[17] A very thin pale yellow crystal of 0.03 × 0.30 × 0.40 mm, was obtained from ethyl acetate. The compound crystallized in the monoclinic system, space group *C*2, Z = 16, a = 117.98(9) Å, b = 7.368(3) Å, c = 7.352(3) Å, β = 92.04(3)°, V = 6387 $Å^3$, d_c = 1.291 g cm^{-3}, $F(000)$ = 2624, λ(Cu Kα) = 1.5418 Å, μ = 0.735 mm^{-1}. The four different molecules of the asymmetric unit explain the surprisingly long parameter a of the unit cell. Intensity data were measured with a CAD4-Nonius diffractometer, using graphite-monochromated Cu Kα radiation and the θ−2θ scan technique up to θ = 60° (−132 ≤ h ≤ 129, −6 ≤ k ≤ 8, l: 0 to 8); 7782 reflections were collected, of which 6705 were unique, and 4721 of them considered as observed having $I \geq 2\sigma(I)$. The structure was solved by direct methods using the SHELXS86[18] program and refined by full-matrix least-squares, based upon unique F^2 with the SHELXL93[19] program. This analysis showed that the four molecules (A, B, C, D) of the asymmetric unit had to be associated in pairs: A and B, C and D. The atomic coordinates of molecules A and B are thus strongly linked together by the relations xA = xB, yA = 0.5 + yB, zA = 0.5 + zB, except for that of the phenyl group atoms. Molecules A and B are effectively nearly the same, but their phenyl groups are tilted by 103°. Identical relations exist between coordinates of molecules C and D, but in addition, their phenyl groups appeared disordered, existing in two alternative perpendicular positions (86°) around the pivot atoms C9′and C12′ (occupancy factors: 0.56/0.44), the respective phenyl tilts being 85° for the major C and D positions and 87.3° for the minor ones. Thus, for the four molecules, the phenyl groups were treated as rigid bodies and atoms of weight 0.44 were only refined isotropically. All hydrogen atoms were calculated at theoretical positions and assigned an isotropic displacement parameter equivalent to 1.2 that of the bonded atom. In the final stages of refinement, Friedel reflections were merged. Finally, refinement of 795 parameters converged to R1(F) = 0.0892 for the 3351 observed reflections and wR2(F^2) = 0.1978 for all the 5178 data with a goodness-of-fit S factor of 1.166. The residual electron density was found between 0.32 and −0.42 e $Å^{-3}$. In the crystal packing, the pairs of molecules are lengthened along the a axis, the homologous lactone rings being stacked parallel, while the phenyl rings are oriented perpendicularly to each other.

Kurzichalcolactone B (2): yellow powder; $[\alpha]_D^{25}$ −96.0° (*c* 0.85; $CHCl_3$); UV (EtOH) λ_{max} 346 (31623), 237 (25011), 201 (59052); (EtOH + NaOH 0.1 N) λ_{max} 358 (21902), 292 (16538), 247 (32702), 202 (114894); IR ($CHCl_3$) ν_{max} 3579, 3220, 1709, 1628, 1594, 1563, 1497, 1432, 1337, 1228, 1140, 972, 935, 873, 829 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 62.5 MHz), see Table 1; CIMS *m/z* 527 ($[M + H]^+$).

Obochalcolactone (3): yellow powder; $[\alpha]_D^{25}$ −52.7° (*c* 1.21; $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 348 (19596), 248 (22819), 203 (62276) nm; (EtOH + NaOH 0.1 N) λ_{max} (ϵ) 401 (16676), 293 (13491), 249 (26059), 205 (124289) nm; CD (EtOH) λ_{ext} ($\Delta\epsilon$) 202 (+1.4), 204 (−3.9), 224 (+2.6), 232 (+1.8), 257 (+10.4); 307 (−3); IR ($CHCl_3$) ν_{max} 3580, 3225, 1717, 1628, 1553, 1498, 1449, 1420, 1344, 1229, 1153, 1082, 1042, 1020, 971, 874, 818 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 62.5 MHz), see Table 2; CIMS *m/z* 550 ($[M]^{+\bullet}$) (100), 437 (87), 367 (10), 131 (57), 104 (5), 103 (21), 91 (5), 77 (8); HRMS *m/z* 551.2059 ($[M + H]^+$) (calcd for $C_{34}H_{31}O_7$, 551.2070).

Oboflavanone A (4): white powder; $[\alpha]_D^{25}$ −64.7° (*c* 0.87; $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 293 (46815), 247 (18572), 230 (23505), 209 (45959), 205 (46814) nm; (EtOH + NaOH 0.1 N) λ_{max} (ϵ) 363 (7228), 293 (20491), 248 (28293), 204 (109558) nm; CD (EtOH) λ_{ext} ($\Delta\epsilon$) 219 (+5.1), 247 (−6.7), 270 (−3.3), 289 (−7.5), 313 (+2.3); IR ($CHCl_3$) ν_{max} 3229, 1727, 1642, 1580, 1449, 1375, 1349, 1297, 1153, 1097, 1061, 1002, 935, 882, 831 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 62.5 MHz), see Table 2; EIMS *m/z* 554 ($[M]^{+\bullet}$) (86), 546 (6), 509 (8), 439 (18), 395 (14), 359 (8), 335 (10), 307 (11), 291 (10), 281 (7), 269 (7), 256 (5), 179 (4), 177 (7), 165 (8), 131

(100), 104 (10), 103 (21), 91 (7), 77 (4); HRMS *m*/*z* 555.2392 ([M + H]$^+$) (calcd for $C_{34}H_{35}O_7$, 555.2383).

Oboflavanone B (5): white powder; $[\alpha]_D^{25}$ −57.9° (*c* 0.91; $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 293 (21198), 247 (16802), 230 (21130), 205 (42156) nm; (EtOH + NaOH 0.1 N) λ_{max} (ϵ) 363 (5637), 293 (17062), 248 (23839), 204 (99252) nm; CD (EtOH) λ_{ext} ($\Delta\epsilon$) 221 (−27.6), 235 (−1.0), 247 (−14.9), 291 (+21.6), 334 (−6.0); IR ($CHCl_3$) ν_{max} 3229, 1727, 1642, 1580, 1449, 1375, 1349, 1297, 1153, 1097, 1061, 1029, 935, 882, 831 cm^{-1};^{1}H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 62.5 MHz), see Table 2; CIMS *m*/*z* 577 ([M + Na]$^+$), 555 ([M + H]$^+$); HRMS *m*/*z* 555.2401 ([M + H]$^+$) (calcd for $C_{34}H_{35}O_7$, 555.2383).

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Supporting Information Available: Proposed biosynthetic pathways, Schemes 1−3. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Crystallographic data for obolactone (**1**) reported in this paper have been deposited with Cambridge Crystallographic Center (No. CCDC 222244). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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