Diarylheptanoids from the Rhizomes of *Curcuma kwangsiensis*

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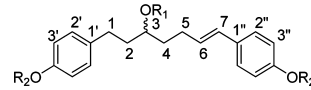
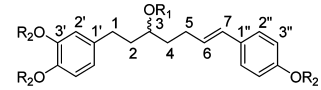
Twelve new diarylheptanoids and six known compounds were isolated from rhizomes of *Curcuma kwangsiensis*. Structures of the new compounds were elucidated by spectroscopic and chemical methods as (3*S*)- and (3*R*)-1,7-bis(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (**1a** and **1b**), (3*S*)- and (3*R*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (**2a** and **2b**), (3*S*)- and (3*R*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol (**3a** and **3b**), (3*R*)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**4b**), (3*S*)- and (3*R*)-3-acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-heptene (**5a** and **5b**), (3*S*)- and (3*R*)-3-acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptanes (**6a** and **6b**), and (*E*)-1,7-bis(4-hydroxyphenyl)-6-hepten-3-one (**7**). The absolute configurations were determined using the modified Mosher's method. Separation of the enantiomeric mixtures (**1a** and **1b**, **2a** and **2b**, **3a** and **3b**, **4a** and **4b**, **5a** and **5b**, **6a** and **6b**) was achieved on a chiral column using acetonitrile−water mixtures as eluents. The *S* enantiomers exhibited negative specific optical rotations in MeOH, and the *R* enantiomers were positive. Inhibitory effects of the compounds on nitric oxide production in lipopolysaccaride-activated macrophages were evaluated.

Curcuma kwangsiensis S.G.Lee *et* C.F.Ling (Zingiberaceae) is widely distributed in southwest regions of People's Republic of China including Guangxi, Sichuan, Guangdong, and Yunnan Provinces. Its roots are one of the most important crude drugs, frequently listed in prescriptions of traditional Chinese medicine for the treatment of stomach trouble and "Oketsu",[1] various syndromes caused by the obstruction of blood circulation, such as arthralgia, psychataxia, and dysmenorhea.

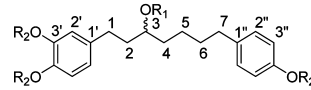
Zingiberaceae plants have been reported to be rich in diarylheptanoids,[2,3] sesquiterpenoids,[4,5] and monoterpenes,[6] some of these compounds possessing significant vasorelaxant,[7] anticancer,[8,9] antioxidant,[2,10] anti-inflammatory,[11] and heptoprotective activities[12,13] and nitric oxide (NO) production inhibitory activities.[14] During the course of our studies on bioactive constituents from 70% EtOH extracts of rhizomes of *C. kwangsiensis*, 12 new diarylheptanoids (**1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4b**, **5a**, **5b**, **6a**, **6b**, and **7**) were isolated along with six known ones (**4a** and **8**−**12**). Interestingly, by means of the modified Mosher's method, these diarylheptanoids with one asymmetric carbon at the 3-position were found to exist as enantiomeric mixtures (**1a** and **1b**, **2a** and **2b**, **3a** and **3b**, **4a** and **4b**, **5a** and **5b**, **6a** and **6b**). The *S* enantiomer predominated in those mixtures with different ratios of *S* and *R* enantiomers (2:1 for **1a** and **1b**, 6:1 for **2a** and **2b**, 3:1 for **3a** and **3b**, 3:1 for **4a** and **4b**, 3:1 for **5a** and **5b**, and 3:2 for **6a** and **6b**). HPLC separation of the six couples of diarylheptanoid isomers with one chiral center at C-3 was achieved for the first time. This paper describes the isolation and structural elucidation of these new diarylheptanoids and reports the inhibitory effect of the compounds on NO production in LPS-activated macrophages.



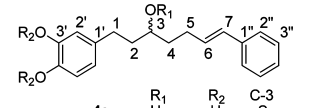
	R_1	R_2	C-3
1a	H	H	*S*
1b	H	H	*R*
1c	H	CH_3	*S*
1d	H	CH_3	*R*



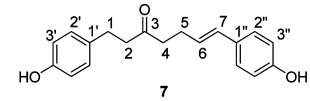
	R_1	R_2	C-3
2a	H	H	S
2b	H	H	R
2c	H	CH_3	S
2d	H	CH_3	R
5a	Ac	H	S
5b	Ac	H	R
5c	Ac	CH_3	S
5d	Ac	CH_3	R



	R_1	R_2	C-3
3a	H	H	S
3b	H	H	R
3c	H	CH_3	S
3d	H	CH_3	R
6a	Ac	H	S
6b	Ac	H	R



	R_1	R_2	C-3
4a	H	H	S
4b	H	H	R



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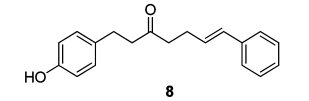

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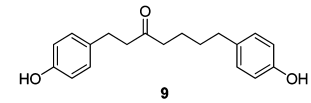

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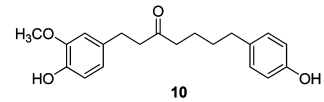

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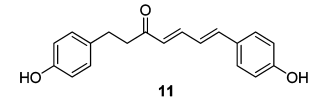

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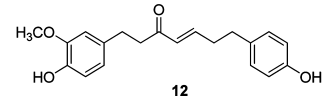

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Results and Discussion

Compound **1**, a yellow oil, was obtained as an enantiomeric mixture of **1a** and **1b**. The HRESIMS spectrum exhibited a unique $[M + NH_4]^+$ ion at *m/z* 316.1897 corresponding to the molecular formula $C_{19}H_{22}O_3$ (calcd for $C_{19}H_{26}NO_3$, 316.1907). The ^{1}H NMR spectrum of **1** revealed two *para*-substituted benzene rings [δ_H 6.64 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.97 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 6.66 (2H, d, J = 8.7 Hz, H-3″ and H-5″), and δ_H 7.11 (2H, d, J = 8.7 Hz, H-2″ and H-6″)] and *trans*-olefinic protons at δ_H 5.98 (dt, J = 16.0, 6.8 Hz, H-6) and δ_H 6.24 (d, J = 16.0 Hz, H-7). The ^{13}C NMR spectrum revealed signals of two oxygenated sp^2 carbons (δ 156.5 and 157.8), and treatment of **1** with trimethylsilyldiazomethane in MeOH gave a mixture of dimethyl derivatives **1c** and **1d**. Thus, the presence of two 4-hydroxyphenyl groups in **1** was evident. In addition, the ^{13}C NMR spectrum showed the presence of seven nonaromatic carbons in **1** [four methylenes (δ_C 30.4, 32.3, 38.5, and 40.8), two olefinic (δ_C 128.3 and 131.2), one hydroxymethine (δ_C 71.3)], suggesting a diarylheptanoid structure. HMBC correlations from H-6 to C-1″, H-7 to C-1″, C-2″, and C-6″, and H-2″/H-6″ to C-7 confirmed a double bond at C-6

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**4** in CD_3OD (δ in ppm, J in Hz)[a]

	1		**2**		**3**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.52 ddd (14.9, 9.6, 7.0)	32.3	2.48 ddd, (14.8, 9.8, 5.5)	32.5	2.48 ddd, (14.8, 9.9, 6.2)	32.5	2.52 ddd, (15.0, 9.1, 7.2)	32.6
1b	2.64 ddd (14.9, 9.7, 5.7)		2.60 ddd, (14.8, 9.6, 6.8)		2.61 ddd, (14.8, 9.8, 6.2)		2.63 ddd, (15.0, 8.8, 6.6)	
2	1.66 m	40.8	1.66 m	40.7	1.66 m[b]	40.7	1.72 m	40.7
3	3.49 m	71.3	3.56 m	71.4	3.52 m	71.9	3.60 m	71.4
4	1.55 m	38.5	1.56 m	38.5	1.47 m[b]	38.4	1.63 m	38.2
5a	2.17 m	30.4	2.18 m	30.4	1.35 m	26.5	2.31 m	30.4
5b	2.25 m		2.27 m		1.44 m[b]			
6	5.98 dt (16.0, 6.8)	128.3	6.00 dt (15.6, 7.3)	128.4	1.57 m[b]	33.2	6.26 dt (16.3, 6.6)	128.0
7	6.24 d (16.0)	131.2	6.26 d (15.6)	131.1	2.52 t (7.5)	36.2	6.39 d (16.3)	131.5
1′		134.6		135.5		135.6		135.4
2′	6.97 d (8.5)	130.5	6.62 d (1.9)	116.7	6.63 d (2.0)	116.7	6.65 d (1.9)	116.7
3′	6.64 d (8.5)	116.2		146.2		146.2		146.2
4′		156.5		144.3		144.3		144.3
5′	6.64 d (8.5)	116.2	6.63 d (8.0)	116.4	6.67 d (8.1)	116.4	6.66 d (7.6)	116.4[c]
6′	6.97 d (8.5)	130.5	6.49 dd (8.0,1.9)	120.8	6.50 dd (8.1,2.0)	120.8	6.52 dd (7.6, 1.9)	120.8
1″		131.1		131.1		135.0		139.4
2″	7.11 d (8.7)	128.3	7.13 d (8.6)	128.3	6.98 d (8.5)	130.4	7.32 m[b]	127.1
3″	6.66 d (8.7)	116.4	6.68 d (8.6)	116.4	6.69 d (8.5)	116.2	7.26 m[b]	129.6
4″		157.8		157.7		156.4	7.16 m	131.5
5″	6.66 d (8.7)	116.4	6.68 d (8.6)	116.4	6.69 d (8.5)	116.2	7.26 m[b]	129.6
6″	7.11 d (8.7)	128.3	7.13 d (8.6)	128.3	6.98 d (8.5)	130.4	7.32 m[b]	127.1

[a] The ^{1}H NMR data of **1**−**3** were obtained at 600 MHz, while the ^{1}H NMR data of **4** were obtained at 300 MHz. The ^{13}C NMR data of **2** and **3** were obtained at 150 MHz, while the ^{13}C NMR data of **1** and **4** were obtained at 75 MHz. [b] The proton resonances are overlapped.

(δ_C 128.3) and C-7 (δ_C 131.2), attached to C-1″. Placement of the double bond was supported further by the splitting patterns of the olefinic protons. The OH was placed at C-3, as determined by long-range correlations from H-1 to C-3, C-1′, C-2′, and C-6′, H-2 to C-1′, H-2′/H-6′ to C-1, and H-3 to C-1 in the HMBC spectrum.

The absolute configuration of the chiral center at C-3 and the enantiomeric ratio of **1a** and **1b** in **1** were determined by the modified Mosher's method.[15,16] Thus, the dimethyl derivatives **1c** and **1d** were converted to the corresponding (*S*)-MTPA esters **1e** and **1f** and (*R*)-MTPA esters **1g** and **1h**, respectively. Analysis of the ^{1}H NMR spectra of the two Mosher ester mixtures established the absolute configuration at C-3 of the enantiomeric diarylheptanoid mixture as *S* and *R* in a ratio of 2:1. Therefore, the diarylheptanoid **1** was concluded to be a 2:1 mixture of (3*S*)- and (3*R*)-1,7-bis(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (**1a** and **1b**).

Compound **2** was obtained as a mixture of enantiomers (**2a** and **2b**). The molecular formula of **2** was determined to be $C_{19}H_{22}O_4$ on the basis of HRESIMS (*m*/*z* 332.1845 $[M + NH_4]^+$) and NMR data. The ^{1}H NMR data of **2** were very similar to those of **1**, with the differences being that a set of *para*-substituted aromatic protons were absent and were replaced by a set of 1,3,4-trisubstituted aromatic protons [δ_H 6.63 (1H, d, J = 8.0 Hz), 6.62 (1H, d, J = 1.9 Hz), and 6.49 (1H, dd, J = 8.0, 1.9 Hz)]. The conclusion was supported by the ^{13}C NMR data. The COSY, HMQC, and HMBC NMR experiments of **2** indicated that it was 1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol,[17] which had been obtained previously from *Curcuma comosa* Roxb. (Zingiberaceae); however, the absolute configuration of this compound had not been determined. In order to determine the absolute configuration at C-3, the modified Mosher's method was applied to the trimethyl derivatives **2c** and **2d**, as a mixture prepared from **2**. On the basis of the differences of ^{1}H NMR data between (*S*)-MTPA esters **2e** and **2f** and (*R*)-MTPA esters **2g** and **2h**, compound **2** was determined to be a 6:1 mixture of (3*S*)- and (3*R*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (**2a** and **2b**).

Compound **3** was also obtained as a mixture of two enantiomers (**3a** and **3b**). The molecular formula, $C_{19}H_{24}O_4$ ($[M + NH_4]^+$ 334.2011, calcd for 334.2013), was determined by HRESIMS. The ^{1}H NMR data of **3** showed aromatic signals similar to those of **2**, including 1,3,4-trisubstituted aromatic protons [δ_H 6.67 (1H, d, J = 8.1 Hz), 6.63 (1H, d, J = 2.0 Hz), and 6.50 (1H, dd, J = 8.1, 2.0 Hz)] and 1,4-disubstituted aromatic protons [δ_H 6.69 (2H, d, J = 8.5 Hz) and 6.98 (2H, d, J = 8.5 Hz)]. However, in the ^{1}H NMR spectrum of **3**, the *trans*-olefinic proton signals were replaced by aliphatic protons [δ_H 1.57 (2H, m, H-6) and 2.52 (2H, t, J = 7.5 Hz, H-7)]. The structure indicated was supported by ^{13}C NMR, COSY, and HSQC data (Table 1). The OH group was shown to be at C-3 of the aliphatic chain by HMBC correlations of H-1 with C-3, C-1′, C-2′, and C-6′, H-2 with C-1′, H-2′/H-6′ with C-1, and H-3 with C-1. Compound **3** was therefore formulated as 1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol. The modified Mosher's method was also applied to determine the absolute configuration at C-3 of **3**, as well as the ratio of *S* and *R* enantiomers. Compound **3** was elucidated as a 3:1 mixture of **3a** and **3b**.

Compound **4** had the molecular formula $C_{19}H_{22}O_3$. The NMR data demonstrated that **4** and 1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol[3] should be the same compound. Determination of the absolute configuration at C-3 of this diarylheptanoid using the modified Mosher's method found it to be a 3:1 mixture of (3*S*)- and (3*R*)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**4a** and **4b**).

Compound **5** was also obtained as a mixture of enantiomers (**5a** and **5b**), as a yellowish oil. The HRESIMS analysis (*m*/*z* 374.1957 $[M + NH_4]^+$) in combination with the ^{1}H and ^{13}C NMR data (Table 2) indicated the molecular formula to be $C_{21}H_{24}O_5$. The ^{1}H and ^{13}C NMR data of **5** in the aromatic regions were very similar to those of **2**, suggesting that **5** also possessed a 1,3,4-trisubstituted benzene ring and a 1,4-disubstituted benzene ring. However, distinctive differences in the ^{13}C NMR spectrum were observed between these molecules. The chemical shifts of C-2 and C-4 of **5** were upfield 3.3 and 3.2 ppm, respectively, while that of C-3 was downfield 3.8 ppm; in addition, an acetyl carbon signal at δ_C 21.3 and 173.1 was present in the ^{13}C NMR spectrum of **5**. Notably, the HMBC correlations of a methyl proton at δ_H 2.00 with the ester carbonyl carbon (δ_C 173.1) and C-3 (δ_C 75.2) and of H-3 (δ_H 4.91) with the ester carbonyl carbon (δ_C 173.1) were observed. These facts suggested that **5** was the C-3-acetylated derivative of **2**.

In order to determine the absolute configuration of C-3 and the ratio of *S* and *R* enantiomers, compound **5** was methylated to protect the phenolic OH groups (**5c** and **5d**) and then deacetylated with K_2CO_3−MeOH to give the corresponding 3-OH derivatives (**2c** and **2d**). Mosher acylation of the enantiomeric mixture (**2c** and **2d**) with both (*R*)- and (*S*)-MTPA chloride yielded the C-3 (*S*)- (**2e** and **2f**)

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **5**, **6**, and **7** in CD_3OD (δ in ppm, J in Hz)[a]

	5		**6**		**7**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.47 m	32.2	2.46 m[b]	32.3	2.79 br d (6.2)	30.2
2	1.82 m	37.4	1.78 m	37.3	2.77 br d (6.2)	45.7
3	4.91 m	75.2	4.84 m	75.4		212.9
4	1.71 m	35.3	1.58 m[b]	35.1	2.59 t (7.4)	43.7
5	2.16 m	30.2	1.31 m	25.8	2.40 q (7.4)	28.3
6	5.98 dt (15.7, 7.3)	127.6	1.56 m[b]	32.7	5.99 dt (16.1, 7.1)	126.9
7	6.26 d (15.7)	131.5	2.50 m[b]	35.9	6.30 d (16.1)	131.7
1′		134.6		134.7		133.4
2′	6.60 d (2.0)	116.6	6.60 d (2.1)	116.6	7.02 d (8.3)	130.4
3′		146.3		146.4	6.70 d (8.3)	116.3[c]
4′		144.5		144.5		156.8
5′	6.65 d (8.1)	116.5	6.66 d (7.9)	116.5	6.70 d (8.3)	116.3[c]
6′	6.47 dd (8.1, 2.0)	120.7	6.47 dd (7.9, 2.1)	120.7	7.02 d (8.3)	130.4
1″		130.9		134.7		130.8
2″	7.15 d (8.7)	128.3	6.97 d (8.2)	130.4	7.17 d (8.3)	128.3
3″	6.69 d (8.7)	116.4	6.69 d (8.2)	116.2	6.73 d (8.3)	116.4[c]
4″		157.8		156.4		157.9
5″	6.69 d (8.7)	116.4	6.69 d (8.2)	116.2	6.73 d (8.3)	116.4[c]
6″	7.15 d (8.7)	128.3	6.97 d (8.2)	130.4	7.17 d (8.3)	128.3
3-OAc	2.00 s	21.3	2.00 s	21.3		
		173.1		173.1		

[a] The ^{1}H NMR data of **5**, **6**, and **7** were obtained at 600 MHz. The ^{13}C NMR data of **5** and **7** were obtained at 150 MHz, while the ^{13}C NMR data of **6** were obtained at 75 MHz. [b] The proton resonances are overlapped. [c] Assignments may be exchanged.

and (*R*)-α-methoxy-α-(trifluoromethyl) phenylacetyl (MTPA) esters (**2g** and **2h**). Consequently, compound **5** was elucidated as a 3:1 mixture of (3*S*)- and (3*R*)-3-acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-heptene (**5a** and **5b**).

Compound **6** was also obtained as a mixture of two enantiomers (**6a** and **6b**). The molecular formula, $C_{21}H_{26}O_5$, was determined by HRESIMS. The ^{1}H and ^{13}C NMR data of **6** were very close to those of **5**, with the *trans*-olefinic resonances of a double bond [C-6 (δ_C 127.6) and C-7 (δ_C 131.5)] being replaced by saturated aliphatic resonances. Compound **6** was identified as 3-acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane, a reduced derivative of **5**, on the basis of the 2D NMR (COSY, HSQC, and HMBC) data. In order to determine the absolute configuration of C-3 by the modified Mosher's method, **6** was deacetylated with HCl−MeOH to give the corresponding 3-deacetyl derivatives (**3a** and **3b**) and then converted to the corresponding *S* (**3e** and **3f**) and *R* (**3g** and **3h**) Mosher esters as performed for **3**. Consequently, compound **6** was identified as a 3:2 mixture of (3*S*)- and (3*R*)-3-acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptanes (**6a** and **6b**).

Compound **7** was obtained as a yellowish oil. The molecular formula of **7** was determined as $C_{19}H_{20}O_3$ by HRESIMS. The ^{1}H NMR spectrum of **7** showed the presence of two *para*-substituted benzene rings [δ_H 6.70 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 7.02 (2H, d, J = 8.3 Hz, H-2′ and H-6′), 6.73 (2H, d, J = 8.3 Hz, H-3″ and H-5″), and 7.17 (2H, d, J = 8.3 Hz, H-2″ and H-6″)] and two *trans*-olefinic protons at δ_H 5.99 (dt, J = 16.1, 7.1 Hz) and 6.30 (1H, d, J = 16.1 Hz). The ^{13}C NMR spectrum displayed 19 signals consistent with four methylene (δ_C 28.3, 30.2, 43.7, and 45.7), two olefinic (δ_C 126.9 and 131.7), one carbonyl (δ_C 212.9), and 12 aromatic ring carbons and suggesting a diarylheptanoid structure. The ^{1}H NMR spectrum exhibited some similarities between **7** and 1-(4-hydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one,[3] except that the H-4″ signal of aromatic ring was absent in **7**, being replaced by an OH group. Furthermore, the ^{13}C NMR spectrum showed a significant downfield shift for C-4″ (δ_C 157.9) compared to that of the known compound. This deduction was supported by HSQC and HMBC spectra. The attachment of 4-hydroxyphenyl moieties at C-1 and C-7, respectively, was confirmed by the HMBC correlations of H-1 with C-1′, C-2′, and C-6′, H-2 with C-1′, H-2′/H-6′ with C-1, H-7 with C-1″, C-2″, and C-6″, H-6 with C-1″, and H-2″/H-6″ with C-7. Therefore, compound **7** was identified as (*E*)-1,7-bis(4-hydroxyphenyl)-6-hepten-3-one.

Table 3. Inhibitory Effect of Compounds Isolated from *Curcuma kwangsiensis* on NO Production Induced by LPS in Macrophages[a]

compound	$IC_{50} \pm SD$ (μM)	compound	$IC_{50} \pm SD$ (μM)
1a	9.83 ± 0.79	**6a**	49.51 ± 3.32
1b	16.11 ± 2.18	**6b**	28.49 ± 2.24
2a	42.02 ± 1.88	**7**	8.93 ± 0.81
2b	20.56 ± 2.02	**8**	6.98 ± 0.57
3a	5.83 ± 0.52	**9**	5.58 ± 0.47
3b	9.66 ± 0.88	**10**	10.62 ± 0.93
4a	3.86 ± 0.65	**11**	4.79 ± 0.42
4b	7.34 ± 0.66	**12**	2.69 ± 0.21
5a	6.54 ± 0.58	indomethacin[b]	12.96 ± 1.16
5b	15.63 ± 1.15	hydrocortisone[b]	40.64 ± 3.22

[a] NO concentration of control group: 3.24 ± 0.21 μM. NO concentration of LPS-treated group: 33.46 ± 2.13 μM. [b] Positive control.

In addition to 12 new diarylheptanoids (**1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4b**, **5a**, **5b**, **6a**, **6b**, and **7**), six known ones, (3*S*)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**4a**),[3] 1-(4-hydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one (**8**),[3] 1,7-bis(4-hydroxyphenyl)-3-heptanone (**9**),[18] 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone (**10**),[19] 1,7-bis(4-hydroxyphenyl)hepta-4*E*-6*E*-dien-3-one (**11**),[8] and 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-(4*E*)-4-hepten-3-one (**12**),[20] were isolated and identified by comparison of their spectroscopic data with those reported in the literature.

Separation of the enantiomeric mixtures (**1a** and **1b**, **2a** and **2b**, **3a** and **3b**, **4a** and **4b**, **5a** and **5b**, **6a** and **6b**) was achieved by reversed-phase chiral HPLC using a Chiralpak AD-RH column (150 mm × 4.6 mm, 5 μm) with acetonitrile−water mixtures as eluents. The specific optical rotations of the isolated *S* enantiomers (**1a**, **2a**, **3a**, **4a**, **5a**, and **6a**) were negative, and the *R* enantiomers were opposite.

All compounds isolated were examined for their inhibitory effects on NO production induced by LPS in macrophages (Table 3). Cell viability was determined by the MTT method to find whether inhibition of NO production was due to cytotoxicity of the test compounds. As shown in Table 3, indomethacin (IC_{50} 12.96 ± 1.16) and hydrocortisone (IC_{50} 40.64 ± 3.22) were used as positive controls.[21−23] All of the isolated diarylheptanoid derivatives significantly suppressed NO production. Compounds **1a**, **3a**, **3b**, **4a**, **4b**, **5a**, **7**, **8**, **9**, **11**, and **12** showed strong inhibition of NO

production induced by LPS in macrophages with IC_{50} values of 9.83, 5.83, 9.66, 3.86, 7.34, 6.54, 8.93, 6.98, 5.58, 4.79, and 2.69 μM, respectively. Compounds **1b**, **5b**, and **10** exhibited moderate activities, which were close to that of indomethacin.

The diarylheptanoids with a carbonyl group at C-3 exhibited conspicuous inhibitory activity (e.g., **7**, **8**, **9**, **10**, **11**, **12**). For each pair of enantiomers, the inhibitory effect of the *S* enantiomer was not significantly different from that of the corresponding *R* enantiomer. Introduction of OH or OCH_3 groups to the phenyl groups and the presence of double bonds on the heptyl chain were not important for the activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Shimadzu UV 2201 spectrophotometer. IR spectra were conducted on a Bruker IFS 55 spectrometer. NMR experiments were performed on Bruker ARX-300 and AV-600 spectrometers. The chemical shifts are stated relative to TMS and expressed in δ values (ppm), with coupling constants reported in Hz. HRESIMS were obtained on a Bruker APEX-II mass spectrometer, and ESIMS were recorded on an Agilent 1100-LC/MSD TrapSL mass spectrometer. Silica gel GF_{254} prepared for TLC and silica gel (200−300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, People's Republic of China). Sephadex LH-20 was a product of Pharmacia. Octadecyl silica gel was purchased from Merck Chemical Company Ltd. RP-HPLC separations were conducted using a Waters 600 series pumping system equipped with a Waters 490 UV detector and performed with a C_{18} column (250 mm × 20 mm, 10 μm; GL Science Inc.). Separation of enantiomers was carried out on a Chiralpak AD-RH column (150 mm × 4.6 mm, 5 μm; Daicel Chemical Industries, Ltd.) using a Shimadzu LC-6A liquid chromatography instrument equipped with a Shimadzu SPD-6AV UV−vis detector. All reagents were HPLC or analytical grade and were purchased from Tianjin Damao Chemical Company. Spots were detected on TLC plates under UV light or by heating after spraying with anisaldehyde−H_2SO_4 reagent.

Plant Material. Rhizomes of *Curcuma kwangsiensis* were collected from Guangxi Province, China, and identified by Professor Qishi Sun, of the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (AG-20061015) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and Isolation. The rhizomes of *C. kwangsiensis* (10 kg) were cut into approximately 2 cm pieces and extracted with 70% EtOH (100 L) for 2 h. The resulting EtOH extract was concentrated in vacuo, suspended in H_2O, and partitioned successively with cyclohexane, EtOAc, and *n*-BuOH. The EtOAc extract (65 g) was subjected to silica gel CC with $CHCl_3$−MeOH (100:1 to 0:100) to obtain nine fractions (A−I), which were combined according to TLC analysis. Fraction C (6 g) was chromatographed on a Sephadex LH-20 column with $CHCl_3$−MeOH (1:1) to give three subfractions (FC.1−FC.3). Fraction C.3 (1.2 g) was purified by ODS open column chromatography [MeOH−H_2O (1:9 to 8:2)] and resolution of fraction C.34 by RP-HPLC with MeOH−H_2O (3:2) to afford compound **7** (23.2 mg, t_R 78.4 min) and compound **11** (20.5 mg, t_R 86.3 min). Fraction F (12.5 g), eluted with $CHCl_3$−MeOH (1:9), was chromatographed over silica gel, eluting with a gradient of increasing MeOH (1:9 to 8:2) in $CHCl_3$, to give five subfractions (FF.1−FF.5). Fraction F.3 (2.4 g) was purified by preparative TLC [$CHCl_3$−MeOH (1:9)] to obtain **1** (56.3 mg, R_f = 0.32) and **8** (15.1 mg, R_f = 0.43), and subfraction F.4 (540 mg) was separated by RP-HPLC with MeOH−H_2O (1:1) to afford compound **4** (18.2 mg, t_R 83.2 min), compound **5** (28.4 mg, t_R 70.4 min), and compound **6** (21.6 mg, t_R 93.5 min). Fraction G (8.6 g) was subjected to CC on Sephadex LH-20 [$CHCl_3$−MeOH (1:1)] to give four subfractions (FG.1−FG.4). Fraction G.2 (640 mg) was purified by RP-HPLC [MeOH−H_2O (11:9)] to obtain **12** (11.4 mg, t_R 36.9 min), **10** (21.2 mg, t_R 48.3 min), and **9** (19.2 mg, t_R 60.1 min). Fraction G.4 (320 mg) was purified by RP-HPLC [MeOH−H_2O (9:10)] to obtain compounds **2** (23.2 mg, t_R 50.2 min) and **3** (12.7 mg, t_R 55.9 min).

Separation of Enantiomeric Mixtures. In order to afford sufficient quantities of enantiopure compounds for biological assay, the isolated enantiomeric mixtures were separated using a Shimadzu LC-6A liquid chromatography instrument equipped with a Shimadzu SPD-6AV UV−vis spectrometric detector and by a Chiralpak AD-RH column [150 mm × 4.6 mm, 0.5 mL/min, detection at 220 nm, eluted with a mixture of A (acetonitrile) and B (water)]. Compound **1** (5.0 mg) was chromatographed, using acetonitrile−water (50:50), to provide compounds **1a** (3.1 mg, t_R 14.2 min) and **1b** (1.5 mg, t_R 10.3 min); compound **2** (4.0 mg), to provide compounds **2a** (3.0 mg, t_R 17.4 min) and **2b** (0.5 mg, t_R 14.0 min), A:B = 4:6; compound **3** (3.0 mg), to provide compounds **3a** (2.0 mg, t_R 20.0 min) and **3b** (0.7 mg, t_R 17.1 min), A:B = 3:7; compound **4** (2.5 mg), to provide compounds **4a** (1.5 mg, t_R 25.7 min) and **4b** (0.6 mg, t_R 23.5 min), A:B = 45:55; compound **5** (5.5 mg), to provide compounds **5a** (4.0 mg, t_R 8.0 min) and **5b** (1.2 mg, t_R 14.0 min), A:B = 1:1; compound **6** (2.3 mg), to provide compounds **6a** (1.1 mg, t_R 126.9 min) and **6b** (0.8 mg, t_R 117.2 min), A:B = 3:7.

(3*S*)-/(3*R*)-1,7-Bis(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (1a and 1b): yellowish oil; $[\alpha]^{25}_D$ −3.5 (*c* 0.1, MeOH, **1a**), $[\alpha]^{25}_D$ +3.5 (*c* 0.1, MeOH, **1b**); UV (MeOH) λ_{max} (log ε) 226 (4.05), 280 (3.47) nm; IR ν_{max} (KBr) 3375, 2933, 2856, 1610, 1515, 1448, 1365, 1238, 1113, 1023, 820 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 75 MHz), Table 1; HRESIMS *m*/*z* 316.1897 $[M + NH_4]^+$ (calcd for $C_{19}H_{26}NO_3$, 316.1907).

(3*S*)-/(3*R*)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-hepten-3-ol (2a and 2b): yellowish oil; $[\alpha]^{25}_D$ −3.7 (*c* 0.1, MeOH, **2a**), $[\alpha]^{25}_D$ +3.7 (*c* 0.05, MeOH, **2b**); UV (MeOH) λ_{max} (log ε) 223 (3.85), 278 (3.16) nm; IR ν_{max} (KBr) 3372, 2940, 1712, 1609, 1513, 1446, 1368, 1242, 1172, 1021, 967, 811 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), Table 1; HRESIMS *m*/*z* 332.1845 $[M + NH_4]^+$ (calcd for $C_{19}H_{26}NO_4$, 332.1856).

(3*S*)-/(3*R*)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol (3a and 3b): yellowish oil; $[\alpha]^{25}_D$ −2.9 (*c* 0.1, MeOH, **3a**), $[\alpha]^{25}_D$ +2.9 (*c* 0.05, MeOH, **3b**); UV (MeOH) λ_{max} (log ε) 219 (3.94), 282 (3.32) nm; IR ν_{max} (KBr) 3375, 2933, 2856, 1610, 1515, 1448, 1365, 1281, 1238, 1113, 957, 820 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), Table 1; HRESIMS *m*/*z* 334.2011 $[M + NH_4]^+$ (calcd for $C_{19}H_{28}NO_4$, 334.2013).

(3*R*)-1-(3,4-Dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (4b): yellowish oil; $[\alpha]^{25}_D$ +5.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.15), 283 (3.64) nm; IR ν_{max} (KBr) 3382, 2940, 2860, 1687, 1613, 1514, 1451, 1379, 1144, 1026, 830 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) and ^{13}C NMR (CD_3OD, 75 MHz), Table 1; HRESIMS *m*/*z* 316.1899 $[M + NH_4]^+$ (calcd for $C_{19}H_{26}NO_3$, 316.1907).

(3*S*)-/(3*R*)-3-Acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6*E*)-6-heptene (5a and 5b): yellowish oil; $[\alpha]^{25}_D$ −5.7 (*c* 0.1, MeOH, **5a**), $[\alpha]^{25}_D$ +5.7 (*c* 0.1, MeOH, **5b**); UV (MeOH) λ_{max} (log ε) 208 (4.26), 262 (3.04) nm; IR ν_{max} (KBr) 3397, 2938, 1709, 1654, 1611, 1516, 1445, 1379, 1263, 1200, 1028, 819 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), Table 2; HRESIMS *m*/*z* 374.1957 $[M + NH_4]^+$ (calcd for $C_{21}H_{28}NO_5$, 374.1962).

(3*S*)-/(3*R*)-3-Acetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptanes (6a and 6b): yellowish oil; $[\alpha]^{25}_D$ −5.3 (*c* 0.1, MeOH, **6a**), $[\alpha]^{25}_D$ +5.3 (*c* 0.05, MeOH, **6b**); UV (MeOH) λ_{max} (log ε) 209 (4.17), 262 (3.12) nm; IR ν_{max} (KBr) 3397, 2939, 2846, 1733, 1613, 1517, 1459, 1374, 1242, 1116, 1026, 959, 820 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 75 MHz), Table 2; HRESIMS *m*/*z* 376.2127 $[M + NH_4]^+$ (calcd for $C_{21}H_{30}NO_5$, 376.2118).

(*E*)-1,7-Bis(4-hydroxyphenyl)-6-hepten-3-one (7): yellowish oil; UV (MeOH) λ_{max} (log ε) 201 (4.86), 262 (3.05) nm; IR ν_{max} (KBr) 3362, 2939, 1701, 1611, 1514, 1447, 1372, 1234, 1031, 830 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), Table 2; HRESIMS *m*/*z* 314.1740 $[M + NH_4]^+$ (calcd for $C_{19}H_{24}NO_3$, 314.1751).

Methylation of 1, 2, 3, and 5. An enantiomeric mixture of **1** (**1a** and **1b**) (2 mg) and trimethylsilyldiazomethane (2.0 M solution in *n*-hexane) (0.5 mL) in MeOH (0.5 mL) was stirred at room temperature overnight. After the excess trimethylsilyldiazomethane was decomposed with AcOH, the reaction mixture was evaporated to dryness. The resulting residue was purified by preparative TLC (cyclohexane−acetone, 4:1, R_f = 0.46), yielding a mixture of **1c** and **1d** (1.8 mg). Similarly, methylation of **2** (**2a** and **2b**) (2.2 mg), **3** (**3a** and **3b**) (2.2 mg), and **5** (**5a** and **5b**) (3.6 mg) yielded a mixture of **2c** and **2d** (2.0 mg), **3c** and **3d** (1.8 mg), and **5c** and **5d** (3.4 mg), respectively.

Compounds 1c and 1d: yellowish oil; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (2H, d, J = 8.5 Hz, H-2″, 6″), 7.12 (2H, d, J = 8.4 Hz, H-2′, 6′), 6.84 (2H, d, J = 8.5 Hz, H-3″, 5″), 6.82 (2H, d, J = 8.4 Hz, H-3′,

5′), 6.35 (1H, d, J = 15.8 Hz, H-7), 6.07 (1H, dt, J = 15.8, 7.3 Hz, H-6), 3.80 (3H, s, OMe), 3.78 (3H, s, OMe), 3.70 (1H, m, H-3), 2.67 (2H, m, H-1), 2.31 (2H, m, H-5), 1.77 (2H, m, H-2), 1.65 (2H, m, H-4).

Compounds 2c and 2d: yellowish oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (2H, d, J = 8.8 Hz, H-2″, 6″), 6.84 (2H, d, J = 8.8 Hz, H-3″, 5″), 6.78 (1H, d, J = 8.7 Hz, H-5′), 6.74 (1H, dd, J = 8.7, 2.0 Hz, H-6′), 6.73 (1H, d, J = 2.0 Hz, H-2′), 6.36 (1H, d, J = 16.2 Hz, H-7), 6.07 (1H, dt, J = 16.2, 7.0 Hz, H-6), 3.86 (3H, s, OMe), 3.85 (3H, s, OMe), 3.80 (3H, s, OMe), 3.71 (1H, m, H-3), 2.69 (2H, m, H-1), 2.32 (2H, m, H-5), 1.79 (2H, m, H-2), 1.65 (2H, m, H-4).

Compounds 3c and 3d: yellowish oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (2H, d, J = 8.4 Hz, H-2″, 6″), 6.82 (2H, d, J = 8.4 Hz, H-3″, 5″), 6.78 (1H, d, J = 7.7 Hz, H-5′), 6.73 (1H, dd, J = 7.7, 1.8 Hz, H-6′), 6.72 (1H, d, J = 1.8 Hz, H-2′), 3.87 (3H, s, OMe), 3.86 (3H, s, OMe), 3.78 (3H, s, OMe), 3.62 (1H, m, H-3), 2.69 (2H, m, H-1), 2.32 (2H, m, H-5), 1.79 (2H, m, H-2), 1.65 (2H, m, H-4).

Compounds 5c and 5d: yellowish oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (2H, d, J = 8.6 Hz, H-2″, 6″), 6.83 (2H, d, J = 8.6 Hz, H-3″, 5″), 6.78 (1H, d, J = 8.9 Hz, H-5′), 6.71 (1H, dd, J = 8.9, 1.8 Hz, H-6′), 6.70 (1H, d, J = 2.0 Hz, H-2′), 6.32 (1H, d, J = 16.0 Hz, H-7), 6.03 (1H, dt, J = 16.0, 6.7 Hz, H-6), 5.00 (1H, m, H-3), 3.86 (3H, s, OMe), 3.85 (3H, s, OMe), 3.80 (3H, s, OMe), 2.57 (2H, m, H-1), 2.21 (2H, m, H-5), 2.05 (3H, s, 3-OAc), 1.87 (2H, m, H-2), 1.74 (2H, m, H-4).

Hydrolysis of Enantiomeric Mixtures of 5c, 5d, and 6 (6a and 6b). To a solution of a mixture of **5c** and **5d** (3.0 mg) in MeOH (1.5 mL) was added anhydrous K_2CO_3 (10 mg), and the mixture was stirred at 30 °C until deacetylation was complete (9 h). Then the reaction mixture was evaporated to dryness. The residue was purified by preparative TLC (cyclohexane−acetone, 4:1, R_f = 0.42) and yielded a mixture of **2c** and **2d** (2.4 mg).

To a solution of **6** (4.0 mg) in MeOH (1.5 mL) was added a drop of analytical grade HCl. The resulting mixture was stirred at room temperature overnight and dried under vacuum. The residue was purified by preparative TLC ($CHCl_3$−MeOH, 40:3, R_f = 0.23) and yielded a mixture of **3a** and **3b** (3.4 mg).

Preparation of Mosher Esters of Enantiomeric Mixtures of 1c and 1d, 2c and 2d, 3c and 3d, and 4a and 4b. To a solution of the mixture of **1c** and **1d** (0.9 mg) in anhydrous pyridine (400 μL) was added (*R*)-(−)-MTPA chloride (12 μL) at 10 °C. After having been stirred at room temperature for 12 h, the mixture was evaporated to dryness and purified by preparative TLC (cyclohexane−acetone, 6:1, R_f = 0.45) to provide a mixture of (*S*)-MTPA ester (**1e** and **1f**) (0.7 mg). Using the same method, (*S*)-(+)-MTPA chloride afforded a mixture of (*R*)-MTPA ester (**1g** and **1h**) in the same yield.

Following the above procedure, the corresponding (*S*)-MTPA ester and (*R*)-MTPA ester were prepared from the enantiomeric mixtures of **2c** and **2d**, **3c** and **3d**, and **4a** and **4b**, respectively. ^{1}H NMR spectra of all of the Mosher esters were recorded in $CDCl_3$, and the chemical shift differences of the proton resonances between the (*S*)-MTPA ester and the (*R*)-MTPA ester from the corresponding precursor were calculated; the results are summarized in Figure S1.

NO Production Bioassay. Mouse monocyte-macrophage RAW 264.7 cells (ATCC TIB-71) were purchased from the Chinese Academy of Science. RPMI 1640 medium, penicillin, streptomycin, and fetal bovine serum were purchased from Invitrogen (New York). Lipopolysaccharide (LPS), dimethylsufoxide (DMSO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), indomethacin, and hydrocortisone were obtained from Sigma Co. (St. Louis, MO). RAW 264.7 cells were suspended in RPMI 1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% heat-inactivated fetal bovine serum. The cells were harvested with trypsin and diluted to a suspension in fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 2 h at 37 °C in 5% CO_2 in air. Then, the cells were treated with 1 μg/mL of LPS for 24 h with or without various concentrations of test compounds. DMSO was used as a solvent for the test compounds, which were applied at a final concentration of 0.2% (v/v) in cell culture supernatants. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent.[24] Briefly, 100 μL of the supernatant from incubates was mixed with an equal volume of Griess reagent (0.1% *N*-[1-naphthyl]ethylenediamine and 1% sulfanilamide in 5% H_3PO_4). Cytotoxicity was determined by the MTT colorimetric assay, after 24 h incubation with test compounds. The concentration of NO_2^- was calculated by a working line from 0, 1, 2, 5, 10, 20, 50, and 100 μM sodium nitrite solutions, and the inhibitory rate on NO production induced by LPS was calculated by the NO_2^- levels as follows:

$$\text{Inhibitory rate (\%)} = 100 \times \frac{[NO_2^-]_{LPS} - [NO_2^-]_{LPS+sample}}{[NO_2^-]_{LPS} - [NO_2^-]_{untreated}}$$

Experiments were performed in triplicate, and data are expressed as the mean ± SD of three independent experiments.

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Supporting Information Available: HRESIMS and 1D and 2D NMR spectra of **1**−**7** and their derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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