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"Mirror-Image" Manipulation of Curdione Stereoisomer Scaffolds by Chemical and Biological Approaches: Development of a Sesquiterpenoid Library

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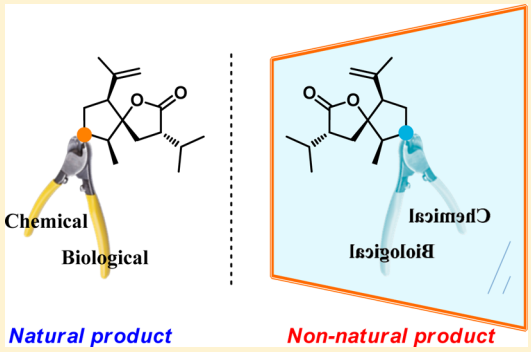
Supporting Information

ABSTRACT: The sesquiterpenoid curdione is one of the main bioactive components in the essential oil of *Rhizoma Curcumae* (*Curcuma wenyujin*, *Curcuma phaeocaulis*, and *Curcuma kwangsiensis*), which has been clinically used for the treatment of cancer in mainland China. Recently it was reported that natural curdione could be hydroxylated by *Aspergillus niger* and transferred to its corresponding curcumalactones under acidic conditions. Based on this study, the development of a sesquiterpenoid library through the "mirror-image" manipulation of bioactive (non)natural curdione scaffolds by chemical and biological approaches is presented herein. *A. niger* induced the hydroxylation of two pairs of curdione enantiomers, yielding the corresponding mirror-image hydroxylated curdiones. Simultaneously, the acid-mediated intramolecular "ene" rearrangements of these curdiones and hydroxylated curdione enantiomers yielded the corresponding mirror-image curcumalactones and hydroxylated curcumalactones. Among the 16 pairs of enantiomers obtained in this study, 23 compounds are new sesquiterpenoids. These curdione and curcumalactone derivatives are of particular interest, as they have the potential to be used as lead compounds and scaffolds in drug discovery.



Natural products and their derivatives historically have been a rich source of bioactive compounds for drug discovery and development.[1] Of the 1130 new drugs approved by the FDA from 1981 to 2010, approximately 50% are natural products or their derivatives.[2] However, in the past two decades, natural product-based drug discovery has declined, partly due to the difficulty in isolating or synthesizing natural products and their analogues.[3] Therefore, to retain their usefulness in the future, it is important to construct structurally diverse compounds from natural products.

Biotransformation is considered an efficient method of constructing complex and diverse compounds from natural products.[4,5] The biotransformation of bioactive natural products has the potential to produce regio- and stereoselective lead compounds for drug discovery under mild conditions.[6,7] For example, many commercial steroidal drugs are produced using biotransformations of steroid precursors by microbial whole cells.[8] It is not uncommon for natural products to have more than one stereogenic center with a significant influence on biological activity.[9] The biotransformation of optically active natural products may generate compounds with increased complexity and additional stereogenic centers. Furthermore, these compounds with particular molecular attributes might contribute to their success throughout the drug discovery process, including increased solubility and greater selectivity.[10] However, only a few studies with complete sets of stereoisomers of natural products as substrates for biotransformation have been reported.[11,12]

Curdione [(4*S*,7*S*)-Cd, **1a**, Figure 1] is one of the major sesquiterpenoids in the essential oil of *Rhizoma Curcumae* (*Curcuma wenyujin* Y.H. Chen et C. Ling, *Curcuma phaeocaulis* Valeton, and *Curcuma kwangsiensis* S.G. Lee et C.F. Liang) and has been used clinically as a remedy for the treatment of cervical cancer in mainland China for a long time.[13] This compound with a germacrane skeleton possesses multiple bioactive properties, such as anti-inflammatory and anticancer activities.[14] However, the lack of viable synthesis routes for the derivatization of curdione represents a fundamental obstacle to the continued discovery of new lead compounds. The curdiones possess two stereogenic centers and thus have four possible stereoisomers. Neocurdione [(4*S*,7*R*)-Cd, **1c**], a minor sesquiterpenoid in the essential oil of *Rhizoma Curcumae*, is the 7-epimer of curdione. The 4,7- (**1b**) and 4-epimers (**1d**) of curdione, non-natural sesquiterpenoids, are enantiomers of naturally occurring curdione and neocurdione, respectively.

Owing to the potential biological activities of curdione, both chemical[15] and biological procedures[16] have been developed for the preparation of curdione derivatives. For example, several

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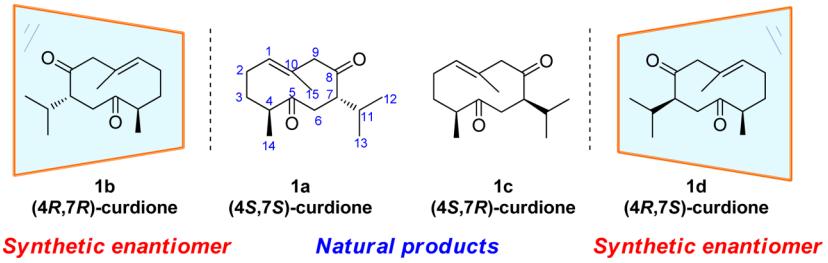

Figure 1. Structures of natural curdione (**1a**) and its stereoisomers. Curdione [(4*S*,7*S*)-curdione, **1a**] and neocurdione [(4*S*,7*R*)-curdione, **1c**] are natural products, while their enantiomers (**1b** and **1d**) are synthetic non-natural compounds.

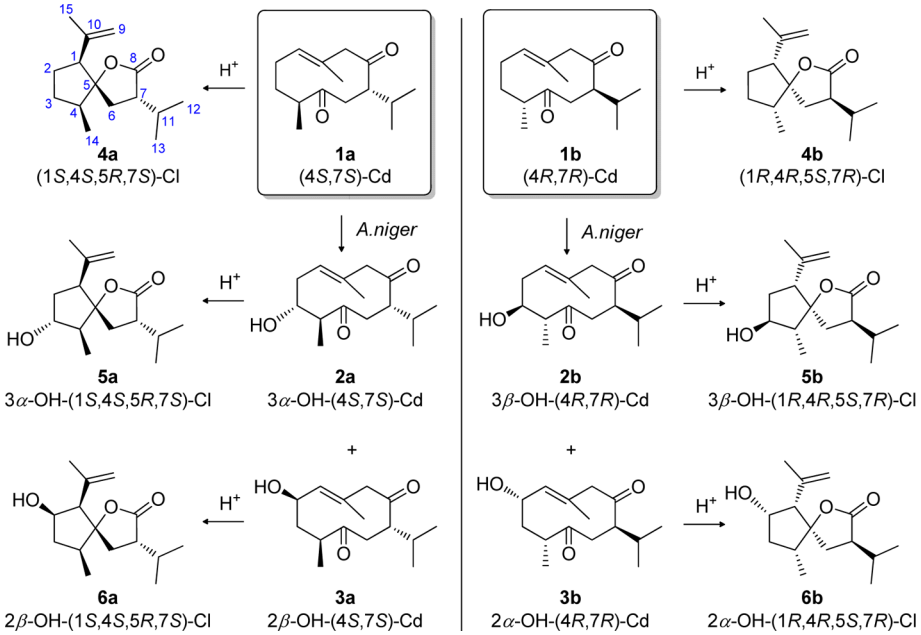

Figure 2. Mirror-image chemical and biological transformations of curdione enantiomers (**1a** and **1b**).

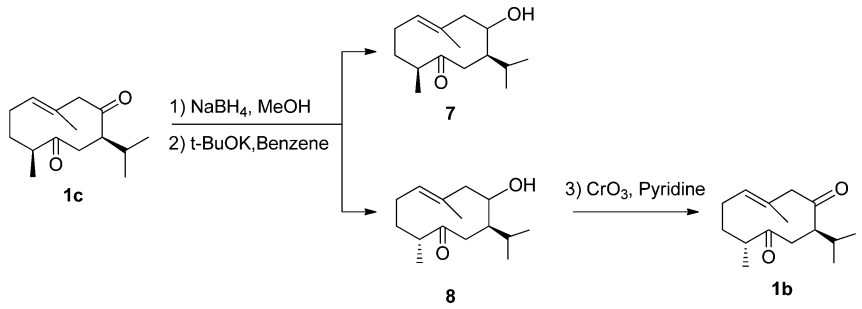

Figure 3. Synthesis of (4*R*,7*R*)-curdione (**1b**) using (4*S*,7*R*)-curdione (**1c**) as a substrate.

strains of filamentous fungi were used to convert curdione (**1a**) into more polar metabolites.[17,18] In previous papers, Asakawa and co-workers[19] and our group[20] have reported site-specific hydroxylation of curdione (**1a**) by the fungus *Aspergillus niger*, which yielded 3α-hydroxycurdione [3α-OH-(4*S*,7*S*)-Cd, **2a**] as the major metabolite (Figure 2, Figure S1, Supporting Information) and 2β-hydroxycurdione [2β-OH-(4*S*,7*S*)-Cd, **3a**] as the minor metabolite. Moreover, curdione (**1a**), 3α-hydroxycurdione (**2a**), and 2β-hydroxycurdione (**3a**) could be transformed into curcumalactone [(1*S*,4*S*,5*R*,7*S*)-Cl, **4a**], 3α-hydroxycurcumalactone [3α-OH-(1*S*,4*S*,5*R*,7*S*)-Cl, **5a**], and 2β-hydroxycurcumalactone [2β-OH-(1*S*,4*S*,5*R*,7*S*)-Cl, **6a**] possessing a spirolactone skeleton formed via transannular cyclization, upon acid treatment (Figure 2). Additionally, Kuroyanagi and co-workers also reported that the transannular cyclization of germacrene-type compounds might yield eudesmane-type sesquiterpenoids.[21] Although the spirolactone structure and potential bioactivity of curcumalactone (**4a**) have attracted significant interest, its challenging synthesis has been a major obstacle in exploring its promising chemistry and bioactivity. The first and only enantioselective total synthesis of curcumalactone was reported in 2012.[22]

In an attempt to obtain new pharmacologically active derivatives of curdione (**1a**) and curcumalactone (**4a**), herein the development of a sesquiterpenoid library by mirror-image

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Table 1. ^{13}C NMR Data (δ_C) for Curdiones and Hydroxycurdiones[a] ($CDCl_3$, 150 MHz)

position	1a	1c	2a	2c	2e	3a	3c	3e
1	131.5	131.2	127.0	126.6	126.8	133.8	134.6	133.7
2	26.3	25.5	36.4	34.6	34.0	68.1	67.1	67.6
3	34.0	32.7	73.6	74.4	74.7	42.4	41.0	41.6
4	46.7	45.7	53.3	50.9	48.7	44.2	40.0	44.1
5	211.1	210.3	210.9	210.3	209.7	215.1	208.6	211.9
6	44.2	42.0	44.0	43.0	43.0	44.4	43.6	42.1
7	53.6	52.5	53.1	52.5	53.4	54.1	52.5	52.7
8	214.3	212.6	211.6	212.8	213.5	210.5	212.3	209.4
9	55.8	55.2	55.3	55.8	55.1	56.2	57.5	53.8
10	129.8	129.1	133.0	131.7	131.9	130.8	128.7	131.4
11	29.9	30.9	29.8	30.5	30.7	29.9	31.6	30.9
12	19.8	20.2	21.0	20.0	21.0	19.7	20.9	21.0
13	21.1	21.0	19.8	21.0	20.3	21.1	20.3	20.4
14	18.5	18.2	15.1	17.8	14.1	18.5	18.9	18.7
15	16.50.	18.2	16.9	17.8	17.7	17.0	17.3	20.0

[a]The ^{13}C NMR data of **1b**, **1d**, **2b**, **2d**, **2f**, **3b**, **3d**, and **3f** are identical to those of **1a**, **1c**, **2a**, **2c**, **2e**, **3a**, **3c**, and **3e**, respectively.

Table 2. ^{13}C NMR Data (δ_C) for Curcumalactones and Hydroxycurcumalactones[a] ($CDCl_3$, 150 MHz)

position	4a	4c	5a	5c	5e	6a	6c	6e
1	53.5	56.2	51.3	53.9	55.0	58.9	61.5	65.5
2	23.7	27.3	34.9	36.7	38.7	69.8	71.9	74.6
3	26.8	31.3	73.9	76.4	74.8	40.0	40.9	41.4
4	41.7	43.4	51.1	50.8	47.6	39.8	40.4	41.8
5	92.4	94.6	91.2	92.7	94.7	93.1	94.3	93.0
6	22.4	28.0	22.8	28.6	28.0	24.6	28.9	29.0
7	46.4	46.7	46.0	46.2	46.0	46.6	46.5	46.5
8	178.4	178.1	177.7	177.9	176.9	178.2	177.6	177.4
9	113.3	114.3	113.5	115.2	115.2	116.4	117.1	115.4
10	143.3	145.7	142.1	144.7	144.7	141.8	141.8	142.9
11	28.2	27.8	28.0	27.8	27.7	28.3	27.7	27.8
12	18.0	20.6	17.7	18.0	20.7	20.6	20.6	20.6
13	20.5	18.0	20.2	20.5	18.0	18.1	18.1	18.1
14	13.7	12.8	11.3	10.3	7.4	14.1	13.0	12.8
15	24.0	21.6	23.7	21.6	21.1	27.2	24.8	22.9

[a]The ^{13}C NMR data of **4b**, **4d**, **5b**, **5d**, **5f**, **6b**, **6d**, and **6f** are identical to those of **4a**, **4c**, **5a**, **5c**, **5e**, **6a**, **6c**, and **6e**, respectively.

chemical and biological transformations of curdione and its three stereoisomers is described.

RESULTS AND DISCUSSION

(4*S*,7*S*)-Curdione (**1a**) and (4*S*,7*R*)-curdione (**1c**) can be isolated from the essential oil of *Curcuma wenyujin*. However, the non-natural (4*R*,7*R*)-curdione (**1b**) and (4*R*,7*S*)-curdione (**1d**) need to be synthesized. The synthesis of **1d** was performed following the reported approach by isomerization at C-4 of natural **1a** via a three-step reaction.[23] The reported method for the synthesis of **1b** is a multistep reaction (12 steps) and gave a low overall yield.[24] Inspired by the synthesis of **1d** from **1a**, we have developed a new synthesis method for non-natural (4*R*,7*R*)-curdione (**1b**) through isomerization at C-4 of the naturally occurring (4*S*,7*R*)-curdione (neocurdione, **1c**) via a similar three-step reaction, as described in Figure 3. Thus, (4*S*,7*R*)-curdione (**1c**) was reduced by treatment with $NaBH_4$ in MeOH, followed by *t*-BuOK-mediated isomerization at C-4 and subsequent oxidation, to yield (4*R*,7*R*)-curdione (**1b**). The 1D ^1H and ^{13}C NMR data of (4*R*,7*R*)-curdione (**1b**) and (4*R*,7*S*)-curdione (**1d**) matched those of natural (4*S*,7*S*)-curdione (**1a**) and (4*S*,7*R*)-curdione (**1c**) well, respectively (Tables 1 and 3, Figures S2 and S3, Supporting Information). However, their electronic circular dichroism (ECD) spectra (Figure S4, Supporting Information) were opposite of those of **1a** and **1c**, indicating that the synthetic compounds (4*R*,7*R*)-curdione (**1b**) and (4*R*,7*S*)-curdione (**1d**) are the enantiomers of (4*S*,7*S*)-curdione (**1a**) and (4*S*,7*R*)-curdione (**1c**), respectively.

With curdione and its three stereoisomers in hand, the compounds were subjected to microbial transformation. The synthetic (4*R*,7*R*)-curdione (**1b**), the enantiomer of **1a**, was selected as the substrate for biotransformation, where we anticipated it to behave similarly to **1a** in the presence of *A. niger* (As 3.739). The incubation of **1b** with *A. niger* for 3 days led to compounds **2b** and **3b** as major and minor metabolites, respectively (Figure 2 and Figure S1, Supporting Information). Compounds **2b** and **3b** were characterized from their HRMS, chiral HPLC analysis, and 1D NMR spectra. The ^{13}C NMR and HRMS data of **2b** and **3b** were in accordance with the molecular formula $C_{15}H_{24}O_3$. The 1D ^1H and ^{13}C NMR spectroscopic data of compound **2b** were identical to those of 3α-OH-(4*S*,7*S*)-curdione (**2a**) (Tables 1 and 3, Figures S5 and S6, Supporting Information), while the NMR data of **3b** were identical to those of **3a** (Tables 1 and 3, Figures S8 and S9, Supporting Information). Furthermore, compounds **2a** and **2b**,

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Table 3. ^{1}H NMR Data (δ_H) for Curdiones and Hydroxycurdiones[a] ($CDCl_3$, 600 MHz, J in Hz)

position	1a	1c	2a	2c	2e	3a	3c	3e
1	5.15, brs	5.13, brs	5.10, brs	5.27, brs	5.29, brs	5.10, d (10.2)	5.56, d (7.2)	5.00, d (10.0)
2	α, 2.12, m	α, 2.16, m	α, 2.09, m	α, 2.26, m	α, 2.32, dt (14.8, 8.1)	4.46, td (10.3, 5.8)	4.62, m	4.36, td (10.0, 4.7)
	β, 2.12, m	β, 2.07, m	β, 2.50, m	β, 2.51, m	β, 2.50, d (12.3)			
3	α,1.52, m	α,1.93, m	4.00, m	3.97, m	4.22, m	α, 2.21, m	α, 2.56, ddd (15.4, 10.2, 5.7)	α, 2.07, dt (13.3, 10.6)
	β, 2.06, m	β, 1.72, m				β, 1.86, m	β, 1.63, dt (14.6, 2.7)	β, 1.91, m
4	2.30, m	2.48, m	2.37, m	2.60, m	2.61, m	2.32, dd (11.6, 6.8)	2.97, ddd (9.8, 7.1, 2.3)	2.43, m
6	α, 2.67, m	α, 2.69, dd (15.0, 10.0)	α, 2.66, m	α, 2.70, dd (16.1, 9.2)	α, 2.42, m	α, 2.44, m	α, 2.41, dd (12.9, 11.0)	α, 2.75, m
	β, 2.38, dd (16.7, 2.3)	β, 2.38, dd (15.0, 2.8)	β, 2.41, m	β, 2.37, d (16.0)	β, 2.79, m	β, 2.44, m	β, 2.62, dd (12.9, 3.1)	β, 2.40, m
7	2.81, m	2.87, m	2.76, m	2.90, m	2.79, m	2.86, dd (11.4, 7.7)	3.12, ddd (11.3, 8.7, 3.0)	2.74, m
9	α, 3.05, d (10.9)	α, 2.86, m	α, 2.96, m	α, 3.06, m	α, 2.94, d (11.8)	α, 2.86, d (11.4)	α, 2.75, d (10.0)	α, 2.74, m
	β, 2.92, d (11.2)	β, 3.02, d (12.7)	β, 2.96, m	β, 3.06, m	β, 3.06, d (11.9)	β, 3.18, d (10.7) 7.7)	β, 3.17, d (10.0)	β, 3.20, d (13.7)
11	1.85, m	1.84, m	1.78, m	1.85, m	1.86, m	1.86 (m, H)	1.85, ddt (13.1, 8.9, 6.5)	1.86, dt (13.7, 7.3)
12	0.94, d (7.3)	0.95, d (6.7)	0.81, d (6.5)	0.89, d (6.6)	0.90, d (6.6)	0.95, d (6.6)	0.92, d (6.7)	0.95, d (6.8)
13	0.87, d (6.7)	0.90, d (6.6)	0.88, d (6.6)	0.95, d (6.6)	0.97, d (6.6)	0.87, d (6.5)	0.98, d (6.7)	0.90, d (6.7)
14	0.97, d (6.9)	1.03, d (7.2)	1.03, d (7.1)	1.16, d (7.1)	1.25, d (4.5)	1.01, d (7.0)	1.02, d (7.2)	1.07, d (7.2)
15	1.64, s	1.64, s	1.63, s	1.66, s	1.66, s	1.77, s	1.47, s	1.80, s

[a]The ^{1}H NMR data of **1b**, **1d**, **2b**, **2d**, **2f**, **3b**, **3d**, and **3f** are identical to those of **1a**, **1c**, **2a**, **2c**, **2e**, **3a**, **3c**, and **3e**, respectively.

Table 4. ^{1}H NMR Data (δ_H) for Curcumalactones and Hydroxycurcumalactones[a] ($CDCl_3$, 600 MHz, J in Hz)

position	4a	4c	5a	5c	5e	6a	6c	6e
1	2.75, dd (11.7, 8.7)	2.78, dd (8.8, 6.5)	3.05, m	2.77, t (8.4)	3.09−3.03, m	2.60, d (6.5)	2.85, d (6.8)	2.70, d (5.7)
2	α, 1.72, m	α, 1.99, m	α, 1.74, m	α, 1.60, m	α, 2.10, m	4.32, td (7.0, 4.3)	4.56, td (7.4, 5.0)	4.15, m
	β, 1.72, m	β, 1.60, m	β, 2.13, m	β, 2.35, m	β, 2.05, m			
3	α, 1.20, m	α, 1.49, m	3.78, m	4.00, q (8.0)	4.17, t (5.6)	α, 2.27, m	α, 1.94, m	α, 2.34, dt (13.8, 7.3)
	β, 1.93, m	β, 1.88, m				β, 1.29, m	β, 1.94, m	β, 1.57, m
4	2.41, ddq (11.0, 9.5, 6.8)	1.88, m	2.37, m	1.75 (m, 1H)	1.94, m	2.49, ddd (14.1, 9.1, 7.5)	2.35, ddt (16.7, 9.7, 6.9)	1.93, dq (11.5, 7.0)
6	α, 1.83, dd (10.6, 3.9)	α, 1.80, dd (12.9, 12.0)	α, 1.85, m	α, 2.10, dd (13.1, 9.2)	α, 2.05, m	α, 2.02, dd (13.5, 11.2)	α, 2.21, dd (13.2, 9.1)	α, 2.08, dd (13.1, 9.2)
	β, 1.83, dd (10.6, 3.9)	β, 2.04, m	β, 1.85, m	β, 1.83, t (12.5)	β, 1.88, t (12.7)	β, 2.37, dd (13.5, 9.8)	β, 1.87, m	β, 1.79, m
7	2.56, td (10.5, 4.8)	2.57, ddd (12.0, 9.2, 5.0)	2.56, m	2.56, ddd (11.9, 9.2, 4.9)	2.60, ddd (13.3, 9.1, 4.9)	2.75, ddd (11.2, 9.7, 5.0)	2.55, ddd (12.1, 9.1, 5.0)	2.57, ddd (11.9, 9.2, 4.9)
9	a, 4.99, m	a, 4.88, p (1.6)	a, 5.02, s	a, 4.94, s	a, 4.93, s	a, 5.20, s	a, 5.14, s	a, 5.03, s
	b, 4.91, m	b, 4.79, dd (2.0, 1.0)	b, 4.90, s	b, 4.88, s	b, 4.83, s	b, 5.15, s	b, 4.89, s	b, 4.84, s
11	2.20, m	2.16, pd (6.8, 5.0)	2.20, m	2.17, pd (6.8, 4.7)	2.19, dt (13.3, 6.5)	2.16, pd (6.9, 4.9)	2.17, m	2.18, pd (6.9, 4.9)
12	0.90, d (6.8)	0.99, d (6.9)	0.90, d (6.8)	0.90, d (6.8)	1.02, d (6.8)	1.00, d (6.9)	1.01, d (7.2),	1.01, d (6.9)
13	0.99, d (6.8)	0.90, d (6.8)	0.98, d (7.1)	1.00, d (6.9)	0.92, d (6.9)	0.91, d (6.8)	0.91, d (6.8)	0.91, d (6.8)
14	0.94, d (6.9)	0.98, d (6.4)	1.06, d (6.9)	1.05, d (6.8)	1.06, d (7.0)	1.03, d (6.9)	1.00, d (7.2)	1.05, d (6.8)
15	1.80, s	1.71, s	1.81, s	1.77, s	1.70, s	1.90, s	1.87, s	1.79, s

[a]The ^{1}H NMR data of **4b**, **4d**, **5b**, **5d**, **5f**, **6b**, **6d**, and **6f** are identical to those of **4a**, **4c**, **5a**, **5c**, **5e**, **6a**, **6c**, and **6e**, respectively.

as well as compounds **3a** and **3b**, were eluted at different retention times by chiral column chromatography, while each of the pairs exhibited mirror-image-like ECD spectra (Figures S7 and S10, Supporting Information). These results indicated that compounds **2b** and **2a** as well as **3b** and **3a** are pairs of enantiomers.

Following biotransformation, the synthetic (4*R*,7*R*)-curdione (**1b**) was used as a substrate for chemical transformation under acidic conditions. The observed results showed that the sesquiterpenoid **1b** could be readily converted to compound **4b** in a 91% yield by treatment with hydrochloric acid. Similarly, the isolated **4b** was found to be identical in all aspects to naturally occurring (1*S*,4*S*,5*R*,7*S*)-curcumalactone (**4a**)

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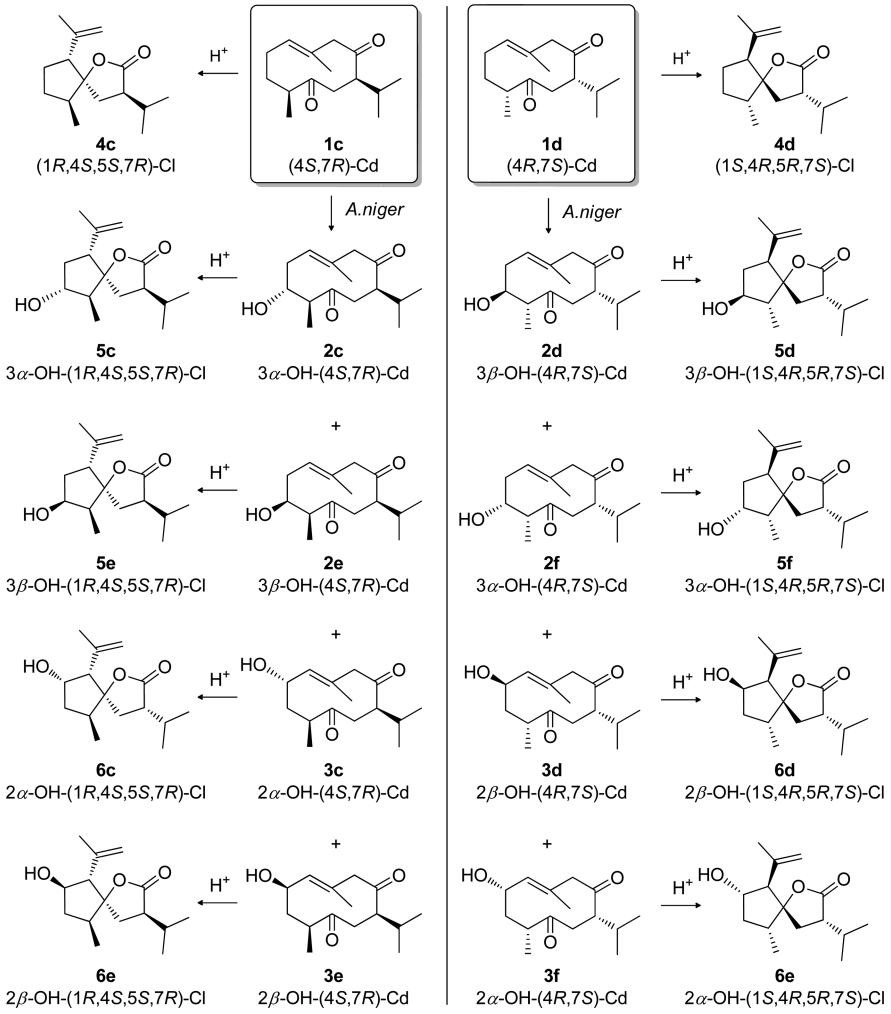

Figure 4. Mirror-image chemical and biological transformations of neocurdione enantiomers (**1c** and **1d**).

(Tables 2 and 4, Figures S11 and S12, Supporting Information), except that its ECD spectrum was opposite that of **4a** (Figure S13, Supporting Information). This evidence confirmed that the absolute stereochemistry of synthetic **4b** is opposite that of (1*S*,4*S*,5*R*,7*S*)-Cl (**4a**). Moreover, chemical transformation of 3β-OH-(4*R*,7*R*)-Cd (**2b**) and 2α-OH-(4*R*,7*R*)-Cd (**3b**) yielded the corresponding 3β-OH-(1*R*,4*R*,5*S*,7*R*)-Cl (**5b**) and 2α-OH-(1*R*,4*R*,5*S*,7*R*)-Cl (**6b**), which are the enantiomers of 3α-OH-(1*S*,4*S*,5*R*,7*S*)-Cl (**5a**) and 2β-OH-(1*S*,4*S*,5*R*,7*S*)-Cl (**6a**), respectively (Tables 2 and 4, Figures S14−19, Supporting Information). As mentioned above, it is interesting to observe that the chemical and biological transformations of one pair of curdione enantiomers (**1a** and **1b**) displayed mirror-image pathways (Figure 2). *A. niger* induced the hydroxylation of curdione enantiomers (**1a** and **1b**), yielding the corresponding oxidized mirror-image products (**2a** and **2b**, **3a** and **3b**), while the acid-mediated transannular cyclization of curdione enantiomers (**1a** and **1b**) and hydroxycurdiones (**2a** and **2b**, **3a** and **3b**) afforded the corresponding mirror-image products (**4a** and **4b**, **5a** and **5b**, **6a** and **6b**).

Inspired by the results of the mirror-image chemical and biological transformations of curdione enantiomers (**1a** and **1b**), we next focused on the corresponding transformations of the naturally occurring neocurdione [(4*S*,7*R*)-Cd, **1c**] and its synthetic enantiomer [(4*R*,7*S*)-Cd, **1d**]. Neocurdione (**1c**) was first fed into the culture of *A. niger*. After 3 days, four products were detected by TLC and HPLC analysis in the culture medium (Figure S1, Supporting Information). Further characterization by MS and NMR spectroscopic data (Tables 1 and 3) was used to identify the structures of the major metabolites **2e** and **3c** as 3β-hydroxyneocurdione [3β-OH-(4*S*,7*R*)-Cd, Figure 4] and 2α-hydroxyneocurdione [2α-OH-(4*S*,7*R*)-Cd], respectively. The minor metabolites **2c** and **3e** were identified as 3α-hydroxyneocurdione [3α-OH-(4*S*,7*R*)-Cd] and 2β-hydroxyneocurdione [2β-OH-(4*S*,7*R*)-Cd], respectively. Next, compounds **1c**, **2c**, **2e**, **3c**, and **3e** were subjected to chemical transformations under acidic conditions. The corresponding compounds **4c**, **5c**, **5e**, **6c**, and **6e** were produced. After purification, further analysis by NMR spectroscopy (Tables 2 and 4) confirmed the structures of **4c** and **5c**/**5e**/**6c**/**6e** to be (1*R*,4*S*,5*S*,7*R*)-curcumalactone and 3α/3β/2α/2β-hydroxy-(1*R*,4*S*,5*S*,7*R*)-curcumalactones (Figure 4), respectively. Therefore, *A. niger* induced hydroxylation of neocurdione (**1c**) to yield the corresponding hydroxylated derivatives, while the acid-mediated cyclization of **1c** and its hydroxylated derivatives produced the corresponding spirolactones (Figure 4), in a similar manner to the curdione enantiomers (**1a** and **1b**). Moreover, it is interesting to observe that the configuration at C-5 of **4c** (**5c**) was opposite that of **4a** (**5a**). These results indicated that the facile conversion of curdione stereoisomers to curcumalactone stereoisomers seems

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to involve different steric courses (Figure S20, Supporting Information). Since the configuration of **1a** and **1c** at C-7 is different, it can be determined that the isopropyl group might play an important role in the regio- and stereospecific spirolactone-forming reaction.

Furthermore, the chemical and biological transformations of (4*R*,7*S*)-curdione (**1d**), the enantiomer of neocurdione (**1c**), were carried out. In vivo biotransformation revealed that *A. niger* accepted **1d** as a substrate to generate four products (Figure S1, Supporting Information). The metabolites **2d**, **2f**, **3d**, and **3f** were found to have 1D ^{1}H and ^{13}C NMR spectroscopic data identical to those of **2c**, **2e**, **3c**, and **3e** (Tables 1 and 3, Figures S5, 6 and S8, 9, Supporting Information), while their ECD spectra were opposite those of **2c**, **2e**, **3c**, and **3e**, respectively (Figures S7 and S10, Supporting Information). This evidence confirmed that compounds **2d**, **2f**, **3d**, and **3f** are the corresponding enantiomers of **2c**, **2e**, **3c**, and **3e**. The chemical transformations of **1d** and **2d**/**2f**/**3d**/**3f** yielded compounds **4d** and **5d**/**5f**/**6d**/**6f**, respectively. After NMR and ECD analysis (Tables 2 and 4, Figures S11−S19, Supporting Information), the structures of **4d** and **5d**/**5f**/**6d**/**6f** were confirmed as (1*S*,4*R*,5*R*,7*S*)-curcumalactone and 3β/3α/2β/2α-hydroxy-(1*S*,4*R*,5*R*,7*S*)-curcumalactones, which are the corresponding enantiomers of **4c** and **5c**/**5e**/**6c**/**6e**. Therefore, the chemical and biological transformations of one pair of neocurdione enantiomers (**1c** and **1d**) also displayed mirror-image pathways (Figure 4), which are the same as the mirror-image chemical and microbial transformations of the curdione enantiomers (**1a** and **1b**).

In this report, the development of a sesquiterpenoid library through the mirror-image chemical and biological transformations of curdione stereoisomers is presented. *A. niger* induced the hydroxylation of two pairs of curdione enantiomers (**1a**:**1b** and **1c**:**1d**) and produced the corresponding mirror-image hydroxylated derivatives of curdione (**2a**−**f** and **3a**−**f**) via C−H activation. Through the biotransformation of three curdione stereoisomers (**1b**−**d**), 10 new hydroxylated curdiones (**2b**−**f** and **3b**−**f**) were obtained. Furthermore, the acid-mediated cyclization of these curdione enantiomers (**1a**:**1b** and **1c**:**1d**) and related hydroxylated curdione enantiomers (**2a**−**f** and **3a**−**f**) yielded the corresponding mirror-image curcumalactones (**4a**:**4b** and **4c**:**4d**) and hydroxylated curcumalactones (**5a**−**f** and **6a**−**f**) via transannular cyclization. Through the transannular cyclization of three curdione stereoisomers (**1b**−**d**) and 10 related hydroxylated curdiones (**2b**−**f** and **3b**−**f**), three new curcumalactones (**4b**−**d**) and 10 new hydroxylated curcumalactones (**5b**−**f** and **6b**−**f**) were obtained. Among the 32 compounds (16 pairs of enantiomers) purified in the course of our study, 23 are new sesquiterpenoids (enantiomers, **2b**−**f**, **3b**−**f**, **4a**−**d**, **5b**−**f**, and **6b**−**f**).

The compounds reported in this paper, especially the hydroxylated curdione and curcumalactones, which are rare natural products with a unique spirolactone ring system[25] and documented bioactivity,[26] were of particular interest. These compounds can provide valuable lead compounds or building blocks that are not accessible by currently available synthesis methods for drug discovery. To the best of our knowledge, no information regarding the biological activities of curcumalactone derivatives is currently available. In this regard, the potential anti-inflammatory and anticancer activities of curdiones, curcumalactones, and their derivatives will be further investigated. Moreover, by the target-oriented derivation of the sesquiterpenoid library, more diversified compounds may be obtained, which could be used for further evaluation of biological activities in vitro or in vivo.

EXPERIMENTAL SECTION

General Experimental Procedures. The electronic circular dichroism spectra were obtained by spectral scanning (220−420 nm) using a JASCO CD-2095 circular dichroism chiral detector. 1D and 2D NMR spectra were recorded using a Bruker AVANCE-600 in $CDCl_3$ with TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q spectrometer. High-performance liquid chromatography (HPLC) analysis was performed on a JASCO LC-2000 system using a Dikma Diamonsil C_{18} column (5 μm, 200 × 4.6 mm) with a JASCO UV-2075 detector.

Strain and Medium. *Aspergillus niger* AS 3.739, purchased from the China General Microbiological Culture Collection Center (Beijing, People's Republic of China), was cultured in the medium (15.0 g dextrose, 15.0 g sucrose, 5.0 g peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, and 1000 mL H_2O, pH 7.0) at 28 °C and 210 rpm for 48 h.

Plant Material. The dried rhizomes of *Curcuma wenyujin* were collected from Ruian, Zhejiang Province, China, in April 2010. The plant was identified by Dr. Y. H. Chen, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 4587) of the rhizomes is available from the plant collection in the Chinese Virtual Herbarium.

Preparation of Curdione (1a) and Neocurdione (1c). The essential oil was obtained by steam distillation from the rhizomes of *C. wenyujin*. Curdione (**1a**) and neocurdione (**1c**) were isolated from the essential oil of *C. wenyujin* by column chromatography with petroleum ether−EtOAc (50:1, v/v). The compounds were identified by NMR and MS techniques, and their ^{1}H and ^{13}C NMR and MS data were identical to those reported in the literature.[27,28]

Preparation of (4*R*,7*R*)-Curdione (1b) and (4*R*,7*S*)-Curdione (1d). The synthesis of (4*R*,7*S*)-curdione (**1d**) was performed based on the reported approach[23] by isomerization at C-4 of natural (4*S*,7*S*)-curdione (**1a**) via a three-step reaction. The synthesis of (4*R*,7*R*)-curdione (**1b**) was carried out using a similar method by isomerization at C-4 of natural (4*S*,7*R*)-curdione (neocurdione, **1c**). Step 1: Neocurdione (**1c**, 230 mg) was dissolved in 3 mL of methanol. $NaBH_4$ (50 mg, 1.2 equiv) was then added at 0 °C, and the mixture was stirred at 0 °C. The reaction was monitored by TLC. After the substrate had almost disappeared (5 h), the reaction solution was evaporated under reduced pressure. Water was added to the residue, and the solution was further acidified with several drops of HCl to reach pH 7. The mixture was extracted with EtOAc four times. The combined organic phase was evaporated under reduced pressure to yield the product compound **7** (Figure 2). Step 2: The crude oil of **7** was dissolved in 5 mL of benzene. Potassium *tert*-butoxide (30 mg, 0.3 equiv) was added to the solution. A reflux condenser was attached, and the reaction mixture was stirred at 83 °C for 7 h. After cooling to room temperature, the reaction solution was evaporated under reduced pressure to yield a crude oil. The crude oil was loaded on a silica gel column and eluted with a mixture of petroleum ether and EtOAc (30:1, v/v) to yield compound **8**. Step 3: Compound **8** was dissolved in 2 mL of pyridine. CrO_3 (100 mg, 2.8 equiv) was added to the solution. After stirring at room temperature for 5 h, the precipitate was removed by filtration with silica gel. The filtrate was concentrated in vacuo, and the residue was purified by chromatography [petroleum ether and EtOAc (30:1, v/v)] to obtain (4*R*,7*R*)-curdione (**1b**, 70 mg).

Biotransformation of Curdione Stereoisomers (1a−d) Using *A. niger*. The biotransformation of curdione stereoisomers was carried out according to the method of Asakawa et al.[19] Precultured mycelium (dry weight 5 g) was collected, washed, and incubated with 1000 mL of phosphate buffer (43.70 g $Na_2HPO_4{\cdot}12H_2O$, 12.17 g $NaH_2PO_4{\cdot}2H_2O$, and 1000 mL H_2O, pH 7.0). Concomitantly, the cells were supplemented with compounds **1a**−**d** (0.3 mg/mL) and incubated at 28 °C and 210 rpm. The biotransformation was monitored by TLC

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and HPLC. After 3 days, the reaction solution was centrifuged, and the supernatant was extracted with EtOAc. Then the organic phase was evaporated under reduced pressure. The crude product was passed through a silica column with a petroleum ether−EtOAc elution (30:1, v/v) to yield the related hydroxylated curdione stereoisomers (**2a−f** and **3a−f**).

Chemical Transformation of Curdione and Hydroxycurdione Stereoisomers (1a−d, 2a−f, and 3a−f). A total of 50 mg of curdione or hydroxycurdione stereoisomers dissolved in MeOH (2 mL) was added to 0.2 mL of HCl (1 M). The mixtures were stirred at 28 °C for 4 h. Water was then added to the mixture, and the solution was further neutralized with NaOH to reach pH 7. The mixture was extracted with CH_2Cl_2 four times. The combined organic phase was concentrated in vacuo, and the residue was purified by chromatography [petroleum ether and EtOAc (30:1, v/v)] to obtain related curcumalactones and hydroxycurcumalactones (**4a−d**, **5a−f**, and **6a−f**) in yields between 80% and 90%.

HPLC Analysis of the 16 Pairs of Sesquiterpenoid Enantiomers. High-performance liquid chromatography analysis was carried out using a JASCO LC-2000 system with a CD-2095 circular dichroism chiral detector. The chromatographic conditions were as follows: chromatographic column: Diamonsil C_{18} column (5 μm, 200 × 4.6 mm, Dikma Technologies Inc., Beijing, People's Republic of China), mobile phase: (A) CH_3CN−H_2O (60:40, v/v), (B) CH_3CN−H_2O (40:60, v/v), or (C) CH_3CN−H_2O (20:80, 0 min; 80:20, 30 min; 20:80, 35 min; v/v), flowing at a rate of 1.0 mL/min. Detection: 220 nm. For the curdione enantiomers, the elution times were 11.2 min for **1a** and **1b** using mobile phase A and 10.2 min for **1c** and **1d** using mobile phase A. For the 3-hydroxycurdione enantiomers, the elution times were 6.5 min for **2a** and **2b** using mobile phase B, 14.8 min for **2c** and **2d** using mobile phase C, and 14.4 min for **2e** and **2f** using mobile phase C. For the 2-hydroxylcurdione enantiomers, the elution times were 5.9 min for **3a** and **3b** using mobile phase B, 13.3 min for **3c** and **3d** using mobile phase C, and 12.8 min for **3e** and **3f** using mobile phase C. For the curcumalactone enantiomers, the elution times were 19.5 min for **4a** and **4b** using mobile phase A and 20.3 min for **4c** and **4d** using mobile phase A. For the 3-hydroxycurcumalactone enantiomers, the elution times were 10.4 min for **5a** and **5b** using mobile phase B, 16.1 min for **6c** and **6d** using mobile phase B, and 16.0 min for **6c** and **6d** using mobile phase B. For the 2-hydroxycurcumalactone enantiomers, the elution times were 18.2 min for **6a** and **6b** using mobile phase B, 12.0 min for **3c** and **3d** using mobile phase B, and 13.8 min for **3e** and **3f** using mobile phase B.

■ ASSOCIATED CONTENT

Supporting Information

1D NMR, UV, and CD spectra for related compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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