Microbial and Chemical Transformation Studies of the Bioactive Marine Sesquiterpenes (*S*)-(+)-Curcuphenol and -Curcudiol Isolated from a Deep Reef Collection of the Jamaican Sponge *Didiscus oxeata*

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Microbial and chemical transformation studies of the marine sesquiterpene phenols (*S*)-(+)-curcuphenol (**1**) and (*S*)-(+)-curcudiol (**2**), isolated from the Jamaican sponge *Didiscus oxeata*, were accomplished. Preparative-scale fermentation of **1** with *Kluyveromyces marxianus* var. *lactis* (ATCC 2628) has resulted in the isolation of six new metabolites: (*S*)-(+)-15-hydroxycurcuphenol (**3**), (*S*)-(+)-12-hydroxycurcuphenol (**4**), (*S*)-(+)-12,15-dihydroxycurcuphenol (**5**), (*S*)-(+)-15-hydroxycurcuphenol-12-al (**6**), (*S*)-(+)-12-carboxy-10,11-dihydrocurcuphenol (**7**), and (*S*)-(+)-12-hydroxy-10,11-dihydrocurcuphenol (**8**). Fourteen-days incubation of **1** *with Aspergillus alliaceus* (NRRL 315) afforded the new compounds (*S*)-(+)-10β-hydroxycurcudiol (**9**), (*S*)-(+)-curcudiol-10-one (**10**), and (*S*)-(+)-4-[1-(2-hydroxy-4-methyl)phenyl)]pentanoic acid (**11**). *Rhizopus arrhizus* (ATCC 11145) and *Rhodotorula glutinus* (ATCC 15125) afforded (*S*)-curcuphenol-1α-D-glucopyranoside (**12**) and (*S*)-curcudiol-1α-D-glucopyranoside (**13**) when incubated for 6 and 8 days with **1** and **2**, respectively. The absolute configuration of C(10) and C(11) of metabolites **7**−**9** was established by optical rotation computations. Reaction of **1** with NaNO$_2$ and HCl afforded (*S*)-(+)-4-nitrocurcuphenol (**14**) and (*S*)-(+)-2-nitrocurcuphenol (**15**) in a 2:1 ratio. Acylation of **1** and **2** with isonicotinoyl chloride afforded the expected esters (*S*)-(+)-curcuphenol-1-*O*-isonicotinate (**16**) and (*S*)-(+)-curcudiol-1-*O*-isonicotinate (**17**), respectively. Curcuphenol (**1**) shows potent antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans*, methicillin-resistant *Staphylococcus aureus*, and *S. aureus* with MIC and MFC/MBC ranges of 7.5−25 and 12.5−50 μg/mL, respectively. Compounds **1** and **3** also display in vitro antimalarial activity against *Palsmodium falciparium* (D6 clone) with MIC values of 3600 and 3800 ng/mL, respectively (selectivity index >1.3). Both compounds were also active against *P. falciparium* (W2 clone) with MIC values of 1800 (S.I. >2.6) and 2900 (S.I. >1.6) ng/mL, respectively. Compound **14** shows anti-hepatitis B virus activity with an EC$_{50}$ of 61 μg/mL.

(*S*)-(+)-Curcuphenol (**1**) and curcudiol (**2**) are bioactive sesquiterpene phenols isolated from the marine sponges *Didiscus flavus* and *Epipolasis species*.[1,2] Curcuphenols display potent antifungal activity against *Candida albicans* with an MIC of 8 μg/mL and antitumor activity against several human cancer cell lines with average MICs of 0.1−10 μg/mL.[1] Curcuphenol and its dehydro derivative also inhibit proton-potassium ATPase, and hence they were patent topics to treat stomach disorders and peptic ulcers.[2−4] (*R*)-(−)-Curcuphenol isolated from the gorgonian coral *Pseudopterogorgia rigida* is reported active against *Staphylococcus aureus* and *Vibrio anguillarum*.[5]

Microbial metabolism studies have been used successfully as model systems to predict metabolic pathways in humans or to increase the efficacy of drugs by metabolic activation. Fungi and mammals have similar enzyme systems since they are both eukaryotes.[6] Hence, many fungi have been used as in vitro models for predicting mammalian drug metabolism.[7] In addition, the application of biocatalysis to marine natural products has been shown

to be a powerful tool for the generation of new, active and less toxic derivatives for lead optimization and to establish structure−activity relationships (SAR).[8,9]

In an attempt to improve the activity and better understand the SAR of (*S*)-(+)-curcuphenol (**1**) and (*S*)-(+)-curcudiol (**2**), isolated from the Jamaican sponge *Didiscus oxeata* Hechtel, 1983, **1** and **2** were subjected to a series of microbial and chemical transformation studies. The present study represents the first report of a microbial metabolism for the curcuphenol class of marine sesquiterpenes.

Results and Discussion

Twenty-six growing cultures were screened for their ability to biotransform **1** and **2**. Few cultures were able to metabolize and transform both compounds to more polar metabolites. *Kluyveromyces marxianus* var. *lactis* (ATCC 2628), *Aspergillus alliaceus* (NRRL 315), and *Rhizopus arrhizus* (ATCC 11145) were selected for preparative-scale fermentation of **1** because they entirely depleted and converted **1** into 10 more polar metabolites, **3**−**12**. *Rhodotorula glutinus* ATCC 15125 was able to transform **2** to a single more polar metabolite, **13**.

The HRFTMS spectrum of **3** suggested the molecular formula C$_{15}$H$_{22}$O$_2$ and five degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **3** (Table 1) suggested a monohydroxylated derivative of **1**. The methylene proton singlet resonating at δ 4.61 and correlated with the methylene

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**Table 1.** ^{13}C and ^1H NMR Spectral Data of Metabolites **3–5**[a]

	3		4		5[b]	
postion	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	153.0, s		153.2, s		154.3, s	
2	114.3, d	6.82, s	116.4, d	6.58, s	113.9, d	6.72, s
3	137.0, s		136.7, s		139.3, s	
4	119.6, d	6.86, d (7.8)	121.8, d	6.72, d (7.9)	118.6, d	6.72, d (7.9)
5	127.5, d	7.13, d (7.8)	127.1, d	7.02, d (7.8)	127.0, d	7.02, d (7.9)
6	133.0, s		135.0, s		134.4, s	
7	31.8, d	3.04, m	31.1, d	3.04, m	31.1, d	3.07, m
8	37.1, t	1.61, 2H, m	36.8, t	1.74, m 1.62, m	36.6, t	1.65, m 1.55, m
9	26.3, t	1.93, 2H, m	25.8, t	1.98, 2H, m	25.6, t	1.93, 2H, m
10	124.3, d	5.12, dd (6.9, 6.8)	126.9, d	5.40, dd (6.8, 6.8)	126.6, d	5.31, dd (6.9, 6.8)
11	132.0, s		130.2, s		133.1, s	
12	25.9, q	1.68, 3H, s	69.3, t	3.97, 2H, s	68.7, t	3.85, 2H, s
13	17.9, q	1.53, 3H, s	13.9, q	1.55, 3H, s	13.5, q	1.47, 3H, s
14	21.2, q	1.22, 3H, d (6.9)	21.6, q	1.21, 3H, d (6.9)	21.2, q	1.13, 3H, d (6.9)
15	65.3, t	4.61, 2H, s	21.1, q	2.26, 3H, s	64.6, t	4.48, 2H, s

[a] In CDCl$_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. Coupling constants (J) are in Hz. Carbon multiplicities were determined by DEPT135 experiment: s = quaternary, d = methine, t = methylene, q = methyl carbons. [b] In CDCl$_3$–CD$_3$OD (9.5:0.5).
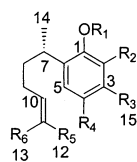

		R_1	R_2	R_3	R_4	R_5	R_6
S (+)-Curcuphenol	**(1)**	H	H	CH$_3$	H	CH$_3$	CH$_3$
S (+)-15-Hydroxycurcuphenol	**(3)**	H	H	CH$_2$OH	H	CH$_3$	CH$_3$
S (+)-12-Hydroxycurcuphenol	**(4)**	H	H	CH$_3$	H	CH$_2$OH	CH$_3$
S (+)-12,15-Dihydroxycurcu-phenol	**(5)**	H	H	CH$_2$OH	H	CH$_2$OH	CH$_3$
S (+)-15-Hydroxycurcuphenol-12al	**(6)**	H	H	CH$_2$OH	H	CHO	CH$_3$
S -Curcuphenol-1α-D-glu-copyranoside	**(12)**	α-D-glu	H	CH$_3$	H	CH$_3$	CH$_3$
S (+)-4-Nitrocurcuphenol	**(14)**	H	H	CH$_3$	NO$_2$	CH$_3$	CH$_3$
S (+)-2-Nitrocurcuphenol	**(15)**	H	NO$_2$	CH$_3$	H	CH$_3$	CH$_3$
S (+)-Curcuphenol-1-isonic-otinate	**(16)**	Isonico	H	CH$_3$	H	CH$_3$	CH$_3$

carbon at δ 65.3 was assigned as H$_2$-15 on the basis of ^3J-HMBC correlations with C-2 and C-4 and a ^2J-HMBC correlation with the quaternary carbon C-3. Hence, metabolite **3** was shown to be (S)-(+)-15-hydroxycurcuphenol.
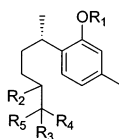
		R_1	R_2	R_3	R_4	R_5
S (+)-Curcudiol	**(2)**	H	H	OH	CH$_3$	CH$_3$
S (+)-12-Carboxy-10,11di-hydrocurcuphenol	**(7)**	H	H	H	β-COOH	β-CH$_3$
S (+)-12-Hydroxy-10,11-di-hydrocurcuphenol	**(8)**	H	H	H	β-CH$_2$OH	β-CH$_3$
S (+)-10β-Hydroxycurcudiol	**(9)**	H	β-OH	OH	CH$_3$	CH$_3$
S (+)-Curcudiol-10-one	**(10)**	H	=O	OH	CH$_3$	CH$_3$
S -Curcudiol-1α-D-glu-copyranose	**(13)**	α-D-glu	H	OH	CH$_3$	CH$_3$
S (+)-Curcudiol-1-isonico-tinate	**(17)**	Isonico	H	OH	CH$_3$	CH$_3$
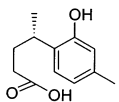
S (+)-4-[1-(2-Hydroxy-4-methyl)phenyl]pentanoic acid (**11**)

The HRMS and ^1H and ^{13}C NMR data of **4** (Table 1) suggested an analogue of **1** with an additional hydroxyla-

tion. The methylene proton singlet resonating at δ 3.97, which correlated with the methylene carbon at δ 69.3, was assigned as H$_2$-12 on the basis of its ^3J-HMBC correlation with the olefinic C-10 and the methyl C-13 and a ^2J-HMBC connection with the quaternary olefinic carbon at δ 130.2 (C-11). Analysis of NOESY data confirmed that the side chain C-7–C-13 is freely rotating, as indicated by NOESY correlations between the aromatic H-5 and both H$_2$-8 and H$_2$-9. NOESY correlation between H$_2$-12 and the olefinic H-10 indicated their E-orientation and supported hydroxylation at C-12 rather than at C-13. Hence, metabolite **4** was shown to be (S)-(+)-12-hydroxycurcuphenol.

The HRFTMS and ^1H and ^{13}C NMR data of **5** (Table 1) suggested that **5** is a dihydroxylated derivative of **1**. The methylene proton singlets resonating at δ 3.85 and 4.48 were assigned as H$_2$-12 and H$_2$-15, respectively. This was based on a ^3J-HMBC correlation between H$_2$-12 and the olefinic C-10 and the methyl C-13 and the ^2J-HMBC correlation with the quaternary olefinic carbon at δ 133.1 (C-11). Similarly, H$_2$-15 also shows a ^3J-HMBC correlation with C-2 and C-4 as well as a ^2J-HMBC correlation with the quaternary carbon C-3. Again, hydroxylation at C-12 rather than C-13 is confirmed with the NOESY correlation between H-10 and H$_2$-12, indicating the double bond is E in configuration. Hence, metabolite **5** was shown to be (S)-(+)-12,15-dihydroxycurcuphenol.

The HRFTMS spectrum of **6** suggested the molecular formula C$_{15}$H$_{20}$O$_3$ and six degrees of unsaturation. The IR spectrum of **6** displayed a strong absorption band at 1668 cm^{-1}, suggesting an α,β-unsaturated aldehyde functionality. The ^1H and ^{13}C NMR data of **6** (Table 2) suggested that **6** is a monohydroxylated and aldehyde-containing derivative of **1**. The methylene proton singlet resonating at δ 4.62 was assigned as H$_2$-15. This was based on its ^3J-HMBC correlation with C-2 and C-4 as well as a ^2J-HMBC correlation with the quaternary aromatic carbon C-3. The downfield proton singlet resonating at δ 9.30, which correlated with the methine carbon at δ 195.9, was assigned as a C-12 aldehyde functionality. This was based on its ^3J-HMBC correlation with the methyl C-13 and the olefinic methine C-10, which is shifted downfield due to its β-location to the aldehyde C-12. Hence, metabolite **6** was shown to be (S)-(+)-15-hydroxycurcuphenol-12-al.

A molecular formula of C$_{15}$H$_{22}$O$_3$ was proposed for **7** on the basis of its HRFTMS data. The IR spectrum of **7** displayed a strong absorption band at 1691 cm^{-1}, suggesting a carbonyl group. The ^1H and ^{13}C NMR data of **7** (Table 2) suggested the reduction of the $\Delta^{10,11}$ system with the

Table 2. ^{13}C and ^1H NMR Spectral Data of Metabolites **6–8**[a]

position	6		7		8[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	154.0, s		154.0, s		155.7, s	
2	114.2, d	6.80, s	116.1, d	6.55, s	117.7, d	6.27, s
3	140.0, s		136.6, s		137.8, s	
4	119.6, d	6.86, d (7.8)	121.8, d	6.69, d (7.9)	122.5, d	6.33, d (7.8)
5	127.7, d	7.12, d (7.8)	127.0, d	6.98, d (7.8)	128.6, d	6.68, d (7.8)
6	139.4, s		130.0, s		131.8, s	
7	32.2, d	3.16, m	31.5, d	3.02, m	33.4, d	2.73, m
8	35.7, t	1.88, m	37.0, t	1.74, m	38.9, t	1.23, m
		1.77, m		1.62, m		1.20, m
9	27.2, t	2.31, 2H, m	26.5, t	1.57, 2H, m	26.6, t	1.09, m
						0.94, m
10	155.7, d	6.44, dd (7.2, 7.0)	23.0, t	1.22, 2H, m	34.9, t	1.17, m
						1.15, m
11	132.1, s		30.0, d	2.05, m	37.4, d	1.63, m
12	195.9, d	9.30, s	170.0, s		68.9, t	3.01, dd (10.4, 5.7) 2.91, dd (10.5, 6.4)
13	9.3, q	1.64, 3H, s	13.5, q	1.67, 3H, d (4.4)	17.9, q	0.56, 3H, d (6.8)
14	21.2, q	1.26, 3H, d (6.9)	21.5, q	1.15, 3H, d (6.9)	22.1, q	0.82, 3H, d (6.9)
15	65.1, t	4.62, 2H, s	21.2, q	2.22, 3H, s	21.8, q	1.91, 3H, s

[a] In CDCl$_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. [b] In CD$_3$CN–CDCl$_3$ (9:1) at 50 °C. Coupling constants (J) are in Hz. Carbon multiplicities were determined by DEPT135 experiment: s = quaternary, d = methine, t = methylene, q = methyl carbons.

Table 3. ^{13}C and ^1H NMR Spectral Data of Metabolites **9–11**[a]

position	9		10		11	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	153.5, s		153.7, s		153.7, s	
2	116.7, d	6.61, s	116.8, d	6.65, s	116.8, d	6.64, s
3	136.6, s		137.4, s		137.2, s	
4	121.3, d	6.69, d (7.7)	121.7, d	6.71, d (7.7)	121.6, d	6.71, d (7.7)
5	126.9, d	7.02, d (7.8)	126.5, d	7.01, d (7.7)	126.5, d	7.02, d (7.7)
6	130.4, s		128.8, s		129.0, s	
7	31.6, d	3.12, m	31.2, d	3.00, m	30.9, d	3.08, m
8	34.4, t	1.84, m 1.58, m	31.7, t	1.94, m 1.76, m	32.7, t	2.34, 2H, m
9	29.4, t	1.47, m 1.27, m	33.7, t	2.56, 2H, m	31.9, t	1.88, m 1.79, m
10	78.9, d	3.44, brd (7.8)	216.5, s		180.0, s	
11	73.9, s		76.6, s			
12	26.3, q	1.13, 3H, s	27.0, q	1.35, 3H, s		
13	23.2, q	1.11, 3H, s	26.9, q	1.34, 3H, s		
14	21.1, q	1.22, 3H, d (6.8)	21.3, q	1.25, 3H, d (6.8)	21.1, q	1.26, 3H, d (6.9)
15	20.9, q	2.24, 3H, s	21.1, q	2.28, 3H, s	20.1, q	2.26, 3H, s

[a] In CDCl$_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. Coupling constants (J) are in Hz. Carbon multiplicities were determined by DEPT135 experiment: s = quaternary, d = methine, t = methylene, q = methyl carbons.

terminal oxidation of C-12 to a carboxyl group. The quaternary carbonyl carbon resonating at δ 170.0 was assigned as C-12. This was based on the ^3J-HMBC correlation between this carbon with the methyl doublet H$_3$-13. Hence, metabolite **7** was shown to be (*S*)-(+)-12-carboxy-10,11-dihydrocurcuphenol.

Compound **8** was proposed as a monohydroxylated derivative of **1** on the basis of its HRFTMS data. The ^1H and ^{13}C NMR data of **8** in CDCl$_3$ displayed a double set which turned to a sharp single set by running the spectra in CD$_3$CN–CDCl$_3$ (9:1) at 50 °C (Table 2). Two sets of signals may be a result of hydrogen bonding between the C-1 OH and C-12 OH. At higher temperature both signals merge into a single set. These data suggested the reduction of the $\Delta^{10,11}$ system with terminal oxidation of C-12 to a hydroxyl group. The methylene carbon resonating at δ 68.9 was assigned as C-12. This was based on the ^3J-HMBC correlation between this carbon and the methyl doublet H$_3$-13. This is further supported by the COSY coupling of both H$_2$-12 double doublets with H-11. Hence, metabolite **8** was shown to be (*S*)-(+)-12-hydroxy-10,11-dihydrocurcuphenol.

The MS data of **9** indicated that it is a dihydroxylated derivative of **1**. The ^1H and ^{13}C NMR data of **9** (Table 3) suggested the reduction of the $\Delta^{10,11}$ system with hydroxylation at C-10 and C-11. The quaternary oxygenated carbon resonating at δ 73.9 was assigned as C-11. This was based

on the ^2J-HMBC correlation between this carbon with both methyl singlets H$_3$-12 and H$_3$-13. The latter methyl groups also displayed ^3J-HMBC correlation with the oxygenated methine carbon at δ 78.9, which suggested the location of the other hydroxyl group at C-10. This is further confirmed by ^2J- and ^3J-HMBC correlation of C-10 with H$_2$-9 and H$_2$-8, respectively. COSY correlation between H$_2$-9 and H-10 further supported this assignment. Hence, metabolite **9** was shown to be (*S*)-(+)-10-hydroxycurcudiol.

(*S*)-(+)-Curcudiol (**2**) provided an excellent starting point for the assignment of the unknown configuration at C(10) and C(11) of metabolites **7**, **8**, and **9**. The presence of three freely rotating methylene groups between the C(7) stereocenter and the distant additional asymmetric carbon at C(11) of **7** and **8** minimizes any direct chiroptical perturbation between stereocenters and allows for an application of van't Hoff's principle of optical superposition. Previous studies have established the scope of van't Hoff analysis for the assignment of configuration of organic products.[10–15] The molar rotations ($[M]_D$) of curcudiol and metabolites **7–9** are shown in Table 6.[16] (*R*)-(−)-2-Methylpentanoic acid provided a structurally analogous and therefore suitable $[M]_D = -10.7$ increment for C(11) of carboxylic acid **7** (entry 2).[17] (*S*)-(−)-2-Methylpentanol was used as a C(11) increment for metabolite **8** and was found to have an $[M]_D = -13.3$ (entry 3).[18,19] For triol **9**, (*S*)-(−)-2-methylpentane-

Table 4. ^{13}C and ^1H NMR Spectral Data of Metabolites **12** and **13**[a]

	12		**13**	
position	δ_C	δ_H	δ_C	δ_H
1	154.8, s		154.6, s	
2	116.7, d	6.87, s	116.9, d	6.81, s
3	136.4, s		136.5, s	
4	123.9, d	6.84, d (7.9)	123.5, d	6.80, d (7.9)
5	126.6, d	7.08, d (7.9)	126.6, d	7.02, d (7.9)
6	134.2, s		134.8, s	
7	31.2, d	3.26, m	30.4, d	3.35, m
8	37.2, t	1.66, m 1.51, m	38.6, t	1.52, m 1.43, m
9	26.2, t	1.90, 2H, m	29.3, t	1.31, 2H, m
10	124.8, d	5.11, dd (6.9, 6.8)	43.4, t	1.55, 2H, m
11	131.2, s		74.1, s	
12	25.7, q	1.67, 3H, s	28.9, q	1.10, 3H, s
13	17.6, q	1.53, 3H, s	22.3, q	1.03, 3H, s
14	21.5, q	1.18, 3H, d (6.8)	21.5, q	1.13, 3H, d (6.8)
15	21.3, q	2.28, 3H, s	21.4, q	2.23, 3H, s
1′	101.9, d	4.87, d (5.3)	101.8, d	4.85, d (6.6)
2′	73.6, d	3.63, m	73.7, d	3.72, m
3′	75.7, d	3.42, m	76.1, d	3.35, m
4′	69.6, d	3.73, m	71.6, d	3.67, m
5′	76.6, d	3.72, m	76.7, d	3.67, m
6′	61.7, t	3.89, 2H, m	61.5, t	3.83, 2H, m

[a] In CDCl$_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. Coupling constants (J) are in Hz. Carbon multiplicities were determined by DEPT135 experiment: s = quaternary, d = methine, t = methylene, q = methyl carbons.

2,3-diol ($[M]_D = -37.2$) was selected as an increment in the analysis of the configuration at C(10) (entry 4) because the substitution pattern around the two stereocenters in these compounds is closely related.[20]

On the basis of these chiroptical reference data, and since the *S*-configuration at C(7) in the entire series of curcuphenol and curcudiol metabolites remained constant, the expected molar rotations for all possible diastereomers of **8** and **9** were readily derived (Table 7). Unambiguous stereochemical assignments were possible due to the considerable differences in observed and calculated molar rotations between the different stereoisomers. The 7*S*,11*R*-configuration of **7** and **8** and the 7*S*,10*R*-assignment for **9** provided the best agreement between the observed data and the values obtained by increment additions for the two stereocenters.

A molecular formula of C$_{15}$H$_{22}$O$_3$ was suggested by the HRFTMS spectrum of **10**. The IR and ^1H and ^{13}C NMR data of **10** (Table 3) suggested the close similarity with **9** with the replacement of the C-10 hydroxy with a ketone group. The IR spectrum of **10** displayed a strong absorption band at 1707 cm^{-1}, suggesting ketone functionality. The quaternary ketone carbon resonating at δ 216.5 was assigned as C-10. This was based on the ^3J-HMBC correlation between this carbon and both methyl singlets H$_3$-12 and H$_3$-13 and H$_2$-8, in addition to a ^2J-HMBC correlation with H$_2$-9. Hence, metabolite **10** was shown to be (*S*)-(+)-curcudiol-10-one.

The HRFTMS spectrum of **11** suggested the molecular formula C$_{12}$H$_{16}$O$_3$. The IR spectrum of **11** displayed a strong absorption band at 1714 cm^{-1}, suggesting a carboxylic functionality. The ^1H and ^{13}C NMR data of **11** (Table 3) indicated oxidative cleavage of the terminal isopropyl segment C11/C13 with terminal oxidation of C-10 to acid. The quaternary carbonyl carbon resonating at δ 180.0 was assigned as C-10. This was based on the ^2J- and ^3J-HMBC correlation of this carbon with both H$_2$-9 and H$_2$-8, respectively. Hence, metabolite **11** was shown to be (*S*)-(+)-4-[1-(2-hydroxy-4-methyl)phenyl)]pentanoic acid.

Glycosidation of **1** with the monosaccharide hexose was evident from the molecular formula C$_{21}$H$_{32}$O$_6$ deduced from the HRFTMS spectrum of **12**. The ^1H and ^{13}C NMR data of **12** (Table 4) suggested glucosidation at C-1. The downfield methine carbon resonating at δ 101.9 was assigned as the anomeric C-1′. Although the HMBC correlation between H-1′ and C-1 was not apparent, glycosidation must be at C-1, as it is the only hydroxylated carbon in the parent molecule. The NMR data of the sugar match those reported for glucose.[21] The α-orientation of the glycosidic linkage was deduced from the small coupling constant of H-1′ ($J = 5.3$).[21] Mild acid hydrolysis of **12** and TLC analysis of the aqueous aliquot revealed α-D-glucose. Metabolite **12** was then shown to be (*S*)-curcuphenol-1α-D-glucopyranoside.

The HRFTMS spectrum of **13** suggested the molecular formula C$_{21}$H$_{34}$O$_7$ and indicated possible glycosidation of **2**. The ^1H and ^{13}C NMR data of **13** (Table 4) showed close similarity to that of **12** with curcudiol as the aglycone

Table 5. ^{13}C and ^1H NMR Spectral Data of Metabolites **14−17**[a]

	14		**15**		**16**		**17**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	158.2, s		153.3, s		148.2, s		148.0, s	
2	118.9, d	6.71, s	136.1, s		122.7, d	6.94, s	122.8, d	6.93, s
3	134.7, s		135.9, s		137.0, s		137.0, s	
4	142.0, s		123.4, d	6.78, d (7.8)	123.4, d	7.09, d (7.9)	123.4, d	7.09, d (7.9)
5	125.5, d	7.98, s	132.9, d	7.27, d (7.8)	127.4, d	7.22, d (7.9)	127.4, d	7.21, d (7.9)
6	132.7, s		133.9, s		136.1, s		136.0, s	
7	31.5, d	3.06, m	31.9, d	3.26, m	32.0, d	2.86, m	32.8, d	2.84, m
8	36.9, t	1.63, 2H, m	36.8, t	1.66, m 1.57, m	37.7, t	1.63, 2H, m	38.1, t	1.64, 2H, m
9	25.9, t	1.94, 2H, m	26.2, t	1.94, 2H, m	26.2, t	1.88, 2H, m	29.4, t	1.27, 2H, m
10	124.1, d	5.11, dd (7.1, 7.0)	124.4, d	5.09, dd (7.1, 7.0)	124.3, d	5.00, dd (6.9, 6.8)	44.0, t	1.48, m 1.39, m
11	132.5, s		131.8, s		131.9, s		71.1, s	
12	25.7, q	1.67, 3H, s	25.9, q	1.66, 3H, s	25.8, q	1.55, 3H, s	29.5, q	1.21, 3H, s
13	17.6, q	1.53, 3H, s	18.0, q	1.53, 3H, s	17.8, q	1.48, 3H, s	22.5, q	1.19, 3H, s
14	20.6, q	1.25, 3H, d (6.7)	21.0, q	1.21, 3H, d (6.7)	21.1, q	1.19, 3H, d (6.9)	21.1, q	1.12, 3H, d (6.6)
15	21.2, q	2.57, 3H, s	22.7, q	2.58, 3H, s	22.4, q	2.35, 3H, s	21.7, q	2.35, 3H, s
1′					164.1, s		164.0, s	
2′					137.1, s		137.1, s	
3′					127.9, d	8.01, d (5.7)	127.9, d	8.01, d (5.6)
4′					151.0, d	8.87, d (5.1)	151.1, d	8.87, d (5.1)
5′					151.0, d	8.87, d (5.1)	151.1, d	8.87, d (5.1)
6′					127.9, d	8.01, d (5.7)	127.9, d	8.01, d (5.6)

[a] In CDCl$_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C NMR. Coupling constants (J) are in Hz. Carbon multiplicities were determined by DEPT135 experiment: s = quaternary, d = methine, t = methylene, q = methyl carbons.

Table 6. Molar Rotations ($[M]_D$) of Curcudiol (**2**), Metabolites **7**−**9**, and Chiroptical Reference Materials

entry	compound	$[M]_D$
1	(*S*)-(+)-curcudiol (**2**)	+28.4 (*c* = 0.1, CHCl₃)
2	(*R*)-(−)-2-methylpentanoic acid	−10.7 (*c* = 0.1, CHCl₃)
3	(*S*)-(−)-2-methylpentanol	−13.3 (*c* = 0.1, CHCl₃)
4	(*S*)-(−)-2-methylpentane-2,3-diol	−37.2 (*c* = 0.6, Et₂O)
5	(*S*)-(+)-12-carboxy-10,11-dihydrocurcuphenol (**7**)	+20.0 (*c* = 0.1, CHCl₃)
6	(*S*)-(+)-12-hydroxy-10,11-dihydrocurcuphenol (**8**)	+34.3 (*c* = 0.2, CHCl₃)
7	(*S*)-(+)-10β-hydroxycurcudiol (**9**)	+61.8 (*c* = 0.2, CHCl₃)

Table 7. Calculated Molar Rotations ($[M]_D$) for Metabolites **7**−**9** and Differences between Observed and Calculated Values

entry	metabolite	configuration	calc $[M]_D$	$\|\Delta\|$ (obs $[M]_D$ − calc $[M]_D$)
1	**7**	(7*S*,11*S*)	+39.1	19.1
2	**7**	(7*S*,11*R*)	+17.7	2.3
3	**8**	(7*S*,11*S*)	+15.1	19.2
4	**8**	(7*S*,11*R*)	+41.7	7.4
5	**9**	(7*S*,10*S*)	−8.8	70.6
6	**9**	(7*S*,10*R*)	+65.6	3.8

instead of curcuphenol. Hence, metabolite **13** was then shown to be (*S*)-curcudiol-1α-D-glucopyranoside.

Reaction of **1** with NaNO₂ and concentrated HCl afforded **14** and **15** in a 2:1 ratio. The HRFTMS spectrum of **14** suggested the molecular formula $C_{15}H_{21}O_3N$ and proved successful nitration of **1**. The ¹H and ¹³C NMR data of **14** (Table 5) indicated nitration of aromatic ring. The quaternary carbon resonating at δ 142.0 was assigned to the nitrated C-4. This was based on the ³*J*-HMBC correlation between this carbon and H₃-15 and H-2 singlets. The downfield proton singlet H-5 at δ 7.98 also shows ³*J*-HMBC correlation with the aliphatic C-7 and aromatic C-1 and C-3. H-2 also shows ³*J*-HMBC correlation with the quaternary C-6 and C-15 methyl group, which supported nitration at C-4. Hence, compound **14** was then shown to be (*S*)-(+)-4-nitrocurcuphenol.

HRFTMS data of **15** proposed a molecular formula identical to that of **14**. The ¹H and ¹³C NMR data of **15** (Table 5) indicated close similarity to that of **14** with nitration of the aromatic ring at C-2 instead of C-4. The quaternary carbon resonating at δ 136.1 was assigned to the nitrated C-2. This was based on its ³*J*-HMBC correlation with the H₃-15 singlet and H-4 doublet (δ 6.78). The exchangeable proton singlet at δ 10.64 is assigned as a C-1 phenolic functionality, which is downfield shifted due to hydrogen bonding with the C-2 NO₂ group. The quaternary carbon C-1 is also upfield shifted (−4.9 ppm) by α-nitration, as compared with the same carbon in **14**. Hence, compound **15** was then shown to be (*S*)-(+)-2-nitrocurcuphenol.

Reaction of **1** and **2** with isonicotinic acid chloride afforded **16** and **17**, respectively. The HRFTMS spectra of **16** and **17** indicated successful esterification. The IR spectrum of **16** and **17** displayed strong absorption bands at 1747 and 1746 cm⁻¹, respectively, suggesting an ester functionality. The ¹H and ¹³C NMR data of **16** and **17** (Table 5) showed typical data sets for the 1-*O*-isonicotinate moiety; hence compounds **16** and **17** were proved to be (*S*)-(+)-curcuphenol-1-*O*-isonicotinate and (*S*)-(+)-curcudiol-1-*O*-isonicotinate, respectively.

All compounds were submitted for antimalarial and antituberculosis assays. None of the compounds reported showed activity against tuberculosis, while only **1** and **3** were active against malaria. Curcuphenol (**1**) shows potent antimicrobial activity against *Candida albicans*, *Crypto-*

coccus neoformans, methcillin-resistant *Staphylococcus aureus*, and *S. aureus* with MIC and MFC/MBC values in the range 7.5−25 and 12.5−50 μg/mL, respectively. Curcuphenol (**1**) and 15-hydroxycurcuphenol (**3**) also display in vitro antimalarial activity against *Palsmodium falciparium* (D6 clone) with MIC values of 3600 and 3800 ng/mL, respectively (selectivity index >1.3). Both compounds were also active against *P. falciparium* (W2 clone) with MIC values of 1800 (S.I. >2.6) and 2900 (S.I. >1.6) ng/mL, respectively.

Of all the compounds, (*S*)-(+)-4-nitrocurcuphenol (**14**) shows moderate inhibitory activity against hepatitis B virus (HBV) replication in cultures of 2.2.15 cells with EC₅₀ and EC₉₀ values of 61 and 203 μg/mL, respectively. The cytotoxicity of **14** (CC₅₀ = 39 μg/mL) reduced its selectivity index and hence its therapeutic value. Further SAR and lead optimization studies would be required to improve the bioactivity for this class of natural products.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured at room temperature with a JASCO DIP-370 digital polarimeter. UV analyses were run using a Perkin-Elmer Lambda 3B UV/vis spectrophotometer. The IR spectra were recorded on a ATI Mattson Genesis Series FTIR spectrophotometer. The ¹H and ¹³C NMR spectra were recorded in CDCl₃, on a Bruker DRX NMR spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C NMR. The HRMS spectra were measured on a Bioapex FTMS with electrospray ionization. TLC analyses were carried out on precoated Si gel G₂₅₄ 500 μm (E-Merck) plates, with the following developing system: EtOAc−cyclohexane (44:56) or CHCl₃−CH₃CN (75:25). For column chromatography, Si gel 60, 40 μm, was used. Compound medium α consists of (per liter of distilled water) glucose, 20 g; NaCl, 5 g; K₂HPO₄, 5 g; yeast extract (BBL, Cockeysville, MD), 5 g; peptone (Difco, Detroit, MI), 5 g.

Animal Material. The sponge was collected using closed circuit rebreathers from caves and vertical coral walls (−80 m) at Rio Bueno, Jamaica, on July 12, 2000. The sponge forms a massive semispherical mass with a brain-like grooved surface. The texture is pulpy, and the sponge is easily crushed. The color in life is orange; the interior is lighter in color. The sponge produces copious amounts of mucus and has filamentous algal epibionts on the surface. The skeleton consists of fine, widely spaced tracts of oxea in two size categories, with a palisade of smaller oxea on the surface. Curved "anisocaliculate" microrhabds are common throughout the sponge. The sponge is *Didiscus oxeata* Hechtel, 1983 (order Halichondrida, family Desmoxyiidae). A voucher specimen has been deposited at the Natural History Museum, London, U.K. (BMNH 2001.7.20.1).

Chemicals. (*S*)-(+)-Curcuphenol (**1**) and (*S*)-(+)-curcudiol (**2**) were isolated from the Jamaican sponge *Didiscus oxeata*. Both compounds were authenticated by comparing their physical and spectral data with the literature.[1,2]

Organisms. Microbial metabolism studies were conducted as previously reported.[7,8] Twenty-six microbial cultures, obtained from the University of Mississippi, Department of Pharmacognosy culture collection, were used for screening. These microbes include *Absidia glauca* ATCC 22752, *Aspergillus alliaceus* NRRL 315, *Aspergillus fumigatus* ATCC 26934, *Aureobasidium pullulans* ATCC 9348, *Chaetomium cochliodes* NRRL 2320, *Cunninghamella blakesleeana* SC-2138, *Cunninghamella echinulata* NRRL 3655, *Cunninghamella* species NRRL 5695, *Cytophaga heparina* ATCC 13125, *Debaryomyces polymorphus* ATCC 20280, *Fusarium oxysporum* f. *cepae* ATCC 11711, *Kluyveromyces marxianus* var. *lactis* ATCC 2628, *Lipomyces lipofer* ATCC 10742, *Micrococcus roseus* ATCC 416, *Microsporum gypseum* ATCC 14683, *Mucor griseocynus* ATCC

1207a, *Mucor ramannianus* ATCC 9628, *Nocardia* species ATCC 21145, *Penicillium brevicompactum* ATCC 10418, *Penicillium claviforme* MR 376, *Rhizopus arrhizus* ATCC 11145, *Rhodotorula glutinus* ATCC 15125, *Rhodotorula rubra* ATCC 20129, *Streptomyces argillaceus* ATCC 12956, *Streptomyces flocculus* ATCC 25453, and *Streptomyces rutgersensisa* B-1256.

Microbial Metabolism of Curcuphenol (1). Each of *Kluyveromyces marxianus* var. *lactis* ATCC 2628, *Aspergillus alliaceus* NRRL 315, and *Rhizopus arrhizus* ATCC 11145 were separately grown in five 1 L culture flasks, each containing 250 mL of compound medium α. A total of 300.0 mg of **1** was mixed with 1 mL of ethanol and evenly distributed among the stage II (24 h) cultures at a concentration of 60 mg/1 L flask. After 6 days in *R. arrhizus* and 14 days in other organisms, the incubation mixtures for each organism were pooled and filtered. Each filtrate (1.2 L) was extracted with EtOAc (4 × 2 L) and were then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Each residue (560, 520, and 480 mg, respectively) was flash chromatographed over 50 g of Si gel by gradient elution starting with 100% $CHCl_3$ and ending with 100% MeOH.

The combined less polar fractions (160 mg) from *K. marxianus* column were subjected to column chromatography on 20 g of sephadex LH20, using isocratic $CHCl_3$−MeOH (50:50), and the combined similar subfractions were further fractionated on 15 g of 10% (w/w) $AgNO_3$-impregnated Si gel using an isocratic 3% CH_3CN−$CHCl_3$ solvent system. Preparative TLC on Si gel G (CH_3CN−$CHCl_3$, 25:75) twice afforded **3** (4.7 mg, R_f 0.47), **4** (4.2 mg, R_f 0.56), and **8** (10.7 mg, R_f 0.58). The combined polar fractions (110 mg) from *K. marxianus* column were further chromatographed on 20 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (50:50), and then preparative TLC on Si gel G (CH_3CN−$CHCl_3$, 25:75, 2×) to afford **5** (4.5 mg, R_f 0.09), **6** (2.9 mg, R_f 0.23), and **7** (2.6 mg, R_f 0.34).

The least polar fractions of the *A. alliaceus* column were subjected to preparative TLC on Si gel G (CH_3CN−$CHCl_3$, 8:92) to afford **10** (2.8 mg, R_f 0.39). The polar fractions of the same extract were subjected to column chromatography on 20 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (50:50), to afford **9** (21.8 mg, R_f 0.57) and **11** (6.6 mg, R_f 0.35) (Si gel G, CH_3CN−$CHCl_3$, 35:65).

The polar fractions of *R. arrhizus* extract (190 mg) were subfractionated on 30 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (50:50), to afford **12** (136.8 mg, R_f 0.26, Si gel G, MeOH−$CHCl_3$, 10:90).

Microbial Metabolism of Curcudiol (2). *Rhodotorula glutinus* ATCC 15125 was grown in five 1 L culture flasks, each containing 250 mL of compound medium α. A total of 300.0 mg of **2** was mixed with 1 mL of ethanol and evenly distributed among the stage II (24 h) cultures at a concentration of 60 mg per 1 L flask. After 8 days the incubation mixtures were pooled, filtered, and treated as previously described to afford 485 mg of residue, which was flash chromatographed over 50 g of Si gel 60 using $CHCl_3$, gradient elution with increasing proportions of MeOH, and finally MeOH. The polar fractions (70 mg) were subfractionated on 20 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (50:50), to afford **13** (8.7 mg, R_f 0.17, Si gel G, MeOH−$CHCl_3$, 10:90).

Nitration of Curcuphenol (1). About 335 mg of **1** were dissolved in 1.5 mL of concentrated HCl and immediately kept in an ice bath. About 200 mg of $NaNO_2$ was gradually added, and the reaction mixture was stirred for 30 min followed by addition of a 20 mL brine solution. The solution was then extracted with $CHCl_3$ (3 × 10 mL). The organic layer was washed with 5% $NaHCO_3$ (20 mL) solution and H_2O (20 mL) and finally dried over anhydrous Na_2SO_4. The residue (300 mg) was fractionated over 20 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (1:1), and then preparative TLC on Si gel G (CH_3CN−$CHCl_3$, 2.5:97.5) to afford **14** (18.3 mg, R_f 0.53) and **15** (6.4 mg, R_f 0.84).

Esterification of 1 and 2 with Isonicotinoyl Chloride. About 45 mg each of **1** and **2** were dissolved in 1 mL of pyridine. After addition of 50 mg of isonicotinoyl chloride, each reaction mixture was stirred at room temperature for 20 h. Each reaction mixture was treated with 10 mL of saturated

NaCl solution and extracted with $CHCl_3$ (3 × 5 mL). The organic solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Each residue was separately chromatographed over 20 g of Sephadex LH20, using isocratic $CHCl_3$−MeOH (1:1). Preparative TLC of the reaction mixture of **1** on Si gel G using *n*-hexane−EtOAc (70−30) afforded **16** (4.7 mg, R_f 0.57). Preparative TLC of the reaction mixture of **2** on Si gel G (MeOH−$CHCl_3$, 8:92) afforded **17** (5.7 mg, R_f 0.46).

Mild Acid Hydrolysis of 12 and 13. A solution of 7 mg of **12** or 2 mg of **13** in 1.5 mL of MeOH is treated with 1 mL of 0.1 M H_2SO_4. Each solution is stirred for 1 h at 60 °C and then diluted with 5 mL of H_2O. The whole solution was concentrated under reduced pressure to about 4 mL and then extracted with $CHCl_3$ (2 × 4 mL). The aqueous aliquot was neutralized with $BaCO_3$, filtered, and evaporated under vacuum. The residue was dissolved in 0.2 mL of pyridine and TLC-analyzed along with authentic α-D-glucose on Si gel G$_{254}$ using $CHCl_3$−MeOH−H_2O (18:3:1, lower phase).

(S)-(+)-Curcuphenol (1): colorless oil, $[\alpha]_D^{25}$ +26.6° (*c* 0.35, $CHCl_3$), reported +24.6 ± 2°;[1] UV, IR, ^1H, ^{13}C NMR, and MS data were identical to previously reported data.[1,2]

(S)-(+)-Curcudiol (2): colorless oil, $[\alpha]_D^{25}$ +9.0° (*c* 0.78, $CHCl_3$), literature value +9.2°;[1] UV, IR, ^1H, ^{13}C NMR, and MS data were similar to literature.[1,2]

(S)-(+)-15 Hydroxycurcuphenol (3): colorless oil, $[\alpha]_D^{25}$ +10.6° (*c* 0.57, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 252 (3.48), 244 (3.52), 235 (3.52), 226 (3.47) nm; IR ν_{max} (neat) 3348, 3100−2800, 1616, 1586, 1428, 1376, 1243, 820 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m/z* 233.1547 (calcd for $C_{15}H_{21}O_2$, 233.1542 (M − H)$^-$).

(S)-(+)-12 Hydroxycurcuphenol (4): colorless oil, $[\alpha]_D^{25}$ +50.3° (*c* 1.06, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 251 (3.24), 244 (3.23), 238 (3.22), 229 (3.21) nm; IR ν_{max} (neat) 3367, 2957−2856, 1616, 1453, 1422, 1288, 946 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m/z* 269.1327 (calcd for $C_{15}H_{22}O_2Cl$, 269.1308 (M + Cl)$^-$).

(S)-(+)-12,15-Dihydroxycurcuphenol (5): colorless oil, $[\alpha]_D^{25}$ +20.4° (*c* 1.17, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 251 (3.20), 244 (3.19), 239 (3.24), 230 (3.18) nm; IR ν_{max} (neat) 3328, 2957−2855, 1616, 1586, 1455, 1428, 1288, 1239, 1010, 818 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m/z* 249.1507 (calcd for $C_{15}H_{21}O_3$, 249.1491 (M − H)$^-$).

(S)-(+)-15-Hydroxycurcuphenol-12al (6): colorless oil, $[\alpha]_D^{25}$ +10.9° (*c* 0.23, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 252 (3.92), 245 (3.95), 236 (3.93), 229 (3.89) nm; IR ν_{max} (neat) 3348, 2957−2853, 1668 (C=O), 1640, 1453, 1428, 1237, 751 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFTMS *m/z* 247.1331 (calcd for $C_{15}H_{19}O_3$, 247.1334 (M − H)$^-$).

(S)-(+)-12-Carboxy-10,11-dihydrocurcuphenol (7): colorless oil, $[\alpha]_D^{25}$ +20.0° (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 252 (3.48), 244 (3.52), 235 (3.52), 226 (3.47) nm; IR ν_{max} (neat) 3384, 2950−2856, 1691 (C=O), 1619, 1418, 1288, 946, 757 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFTMS *m/z* 249.1628 (calcd for $C_{15}H_{21}O_3$, 249.1491 (M − H)$^-$).

(S)-(+)-12-Hydroxy-10,11-dihydrocurcuphenol (8): colorless oil, $[\alpha]_D^{25}$ +34.3° (*c* 0.20, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 250 (3.28), 244 (3.22), 236 (3.12), 226 (3.47) nm; IR ν_{max} (neat) 3340, 2950−2855, 1428, 1238, 946, 757 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFTMS *m/z* 237.1848 (calcd for $C_{15}H_{25}O_2$, 237.1855 (M + H)$^+$).

(S)-(+)-10β-Hydroxycurcudiol (9): colorless oil, $[\alpha]_D^{25}$ +61.8° (*c* 0.20, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 244 (2.46), 238 (2.46), 224 (2.41) nm; IR ν_{max} (neat) 3335, 3005−2868, 1618, 1586, 1421, 1217, 810, 753 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRFTMS *m/z* 287.1419 (calcd for $C_{15}H_{24}O_3Cl$, 287.1414 (M + Cl)$^-$).

(S)-(+)-Curcudiol-10-one (10): colorless oil, $[\alpha]_D^{25}$ +40.5° (*c* 0.60, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 251 (3.51), 246 (3.52), 231 (3.50), 229 (3.48) nm; IR ν_{max} (neat) 3399, 2959−2858, 1617, 1586, 1454, 1421, 1289, 809 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRFTMS *m/z* 249.1487 (calcd for $C_{15}H_{21}O_3$, 249.1496 (M − H)$^-$).

(S)-(+)-4-[1-(2-Hydroxy-4-methyl)phenyl)]pentanoic acid (11): colorless oil, $[\alpha]_D^{25}$ +20.0° (*c* 2.20, $CHCl_3$); UV λ_{max}

(log ε) (MeOH) 250 (2.86), 244 (3.89), 231 (2.86) nm; IR ν_{max} (neat) 3323, 3016−2877, 1714 (C=O), 1617, 1586, 1416, 1288, 946, 811, 756 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRFTMS *m/z* 207.1017 (calcd for $C_{12}H_{15}O_3$, 207.1021 (M − H)$^-$).

(*S*)-Curcuphenol-1α-D-glucopyranoside (12): colorless oil, $[\alpha]_D^{25}$ −20.3° (*c* 43.30, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 252 (2.09), 245 (2.12), 239 (2.16), 226 (2.10) nm; IR ν_{max} (neat) 3373, 3049−2869, 1613, 1577, 1504, 1453, 1376, 1256, 1087, 815, 756 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 4; HRFTMS *m/z* 415.1902 (calcd for $C_{21}H_{32}O_6Cl$, 415.1893 (M + Cl)$^-$).

(*S*)-Curcudiol-1α-D-glucopyranoside (13): colorless oil, $[\alpha]_D^{25}$ −10.7° (*c* 2.40, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 252 (3.48), 244 (3.52), 235 (3.52), 226 (3.47) nm; IR ν_{max} (neat) 3348, 3100−2800, 1616, 1586, 1428, 1376, 1243, 820 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 4; HRFTMS *m/z* 433.2012 (calcd for $C_{21}H_{34}O_7Cl$, 433.1993 (M + Cl)$^-$).

(*S*)-(+)-4-Nitrocurcuphenol (14): yellow powder, $[\alpha]_D^{25}$ +20.3° (*c* 5.76, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 258 (2.52), 252 (2.53), 240 (2.54), 234 (2.52) nm; IR ν_{max} (neat) 3343, 2965−2872, 1618, 1573, 1522, 1309, 1266, 1217, 907, 757 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5; HRFTMS *m/z* 262.1430 (calcd for $C_{15}H_{20}O_3N$, 262.1443 (M − H)$^-$).

(*S*)-(+)-2-Nitrocurcuphenol (15): yellow powder, $[\alpha]_D^{25}$ +3.9° (*c* 1.80, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 251 (2.98), 246 (3.01), 237 (3.03), 231 (3.02) nm; IR ν_{max} (neat) 3448, 2967−2870, 1606, 1587, 1547, 1453, 1276, 1231, 1054, 823 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5; HRFTMS *m/z* 262.1480 (calcd for $C_{15}H_{20}O_3N$, 262.1443 (M − H)$^-$).

(*S*)-(+)-Curcuphenol-1-*O*-isonicotinate (16): colorless oil, $[\alpha]_D^{25}$ +11.1° (*c* 0.23, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 252 (3.98), 244 (4.05), 235 (4.03), 225 (3.99) nm; IR ν_{max} (neat) 2958−2852, 1747 (C=O), 1616, 1504, 1453, 1269, 1238, 1119, 815 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5; HRFTMS *m/z* 324.1933 (calcd for $C_{21}H_{26}O_2N$, 324.1964 (M + H)$^-$).

(*S*)-(+)-Curcudiol-1-*O*-isonicotinate (17): colorless oil, $[\alpha]_D^{25}$ +2.1° (*c* 0.90, CHCl$_3$); UV λ_{max} (log ε) (MeOH) 250 (3.41), 244 (3.42), 238 (3.42), 226 (3.44) nm; IR ν_{max} (neat) 3416, 2964−2857, 1746 (C=O), 1616, 1505, 1409, 1376, 1269, 1238, 1112, 818 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5; HRFTMS *m/z* 342.2091 (calcd for $C_{21}H_{28}O_3N$, 342.2063 (M + H)$^-$).

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