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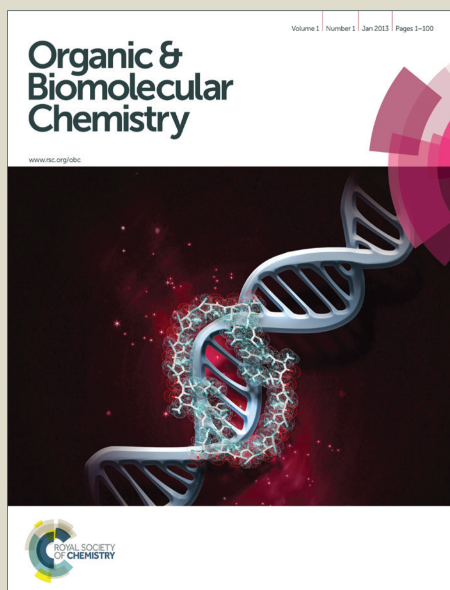

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Natural Nitric Oxide (NO) inhibitors from the rhizomes of *Curcuma phaeocaulis*†

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An exploration for Nitric Oxide (NO) inhibitors from the rhizomes of *Curcuma phaeocaulis* afforded one new salvialane-type sesquiterpene, phasalvione (**1**), two novel nor-sesquiterpenes, phaeocaudione (**2**) and phaeocauone (**3**), one aromatic acid 3-methyl-4-(3-oxo-butyl)-benzoic acid (**4**), two *γ*-elemene-type sesquiterpenes, 8*β*(H)-elema-1,3,7(11)-trien-8,12-lactam (**5**) and 8*β*-methoxy-isogermafurenolide (**6**), one eudesmane-type sesquiterpene, phaeusmane I (**7**), and one cyclic diarylheptanoid, phaeoheptanoxide (**8**). Their structures were established based on extensive spectroscopic analysis. The absolute configurations of compounds **1** and **2** were assigned using the circular dichroism data of the $[Rh_2(OCOCF_3)_4]$ complex, and the absolute configuration of **1** was further established by single crystal X-ray crystallography. It is noteworthy that compounds **5–7** were racemates analyzed by chiral HPLC. Furthermore, inhibitory effects of the isolated compounds on nitric oxide production in LPS-activated macrophages were evaluated. Compounds **1**, **3** and **4** showed strong inhibitory activities on NO production with IC_{50} values of 7.46 ± 0.69, 2.35 ± 0.17 and 3.49 ± 0.31 μM, respectively. A plausible biosynthetic pathway for **1–4** in *C. phaeocaulis* was also discussed.

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Introduction

Curcuma belongs to the Zingiberaceae family and includes about 70 species of rhizomatous herbs in the natural world. Approximately 20 *Curcuma* species are distributed in China, a few of which are being used as herbal medicine and food. Rhizoma Curcumae, (*Ezhu* in Chinese), is a common traditional Chinese medicine (TCM) that has been used for the treatment of Blood Stasis Syndrome (BSS) which is caused by the obstruction of blood circulation, such as arthralgia, psychataxia, and dysmenorrhea.[1] Recent pharmacological studies of this plant have shown a variety of activities, including anti-inflammatory,[2,3] antitumor[4,5] and platelet aggregation inhibitory[6] effects.

The Chinese Pharmacopoeia states that Rhizoma Curcumae should be the dry rhizomes derived from *Curcuma wenyujin* Y.H. Chen et C. Ling, *Curcuma phaeocaulis* Valeton, or *Curcuma kwangsiensis* S.G. Lee et C.F. Liang.[7] Though their pharmacological activities and chemical characteristics are obviously different, it is difficult to distinguish the origins of these raw materials in clinic due to the similarities in morphological characters. The literatures report that phenolic pigments[8] and essential oils[9-11] are the main constituents in *Curcuma* plants. Recently, our research group has been examining the constituents of *C. kwangsiensis*, *C. phaeocaulis* and *C. wenyujin* as well as their inhibitory activities against LPS-induced nitric oxide production, and has hitherto reported the isolation and identification of several new sesquiterpenoids and diarylheptanoids.[12-18] We found that sesquiterpenes were mainly isolated from *C. wenyujin* and *C. phaeocaulis*, while diarylheptanoids were the major compounds from *C. kwangsiensis*. Moreover, guaiane-type and germacrane-type sesquiterpenes were mainly isolated from *C. wenyujin*, while guaiane-type and eudesmane-type sesquiterpenes were the major compounds from *C. phaeocaulis.* Although obvious chemical differences were found among these three species, most of the isolated compounds exhibited remarkable inhibitory activities against nitric oxide production.

As part of our ongoing research for biologically active sesquiterpenoids from the genus *Curcuma*, and in order to provide a potential explanation for usage of these three species as Chinese herbal medicine in the treatment of inflammatory diseases, materials in the remaining

fractions were further fractionated to afford four new sesquiterpenes (**1**, **5–7**), two novel nor-sesquiterpenes (**2** and **3**), one new cyclic diarylheptanoid (**8**) and one new aromatic acid (**4**) from the rhizomes of *Curcuma phaeocaulis* (Figure 1). Herein, we describe the isolation and structural elucidation of these compounds and their *in vitro* anti-inflammatory evaluation on inhibitory activities against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophages. Furthermore, the biogenetic pathway for compounds **1–4** is proposed (Schemes 1 and 2).

Results and discussion

Phasalvione (**1**) was isolated as colorless crystals and the HRESIMS of the quasi-molecular $[M+Na]^+$ ion at *m/z* 273.1466 established the molecular formula $C_{15}H_{22}O_3$ for **1**, indicating five indices of hydrogen deficiency. The IR spectrum showed the absorptions of hydroxyl at 3442 cm^{-1} and conjugated carbonyl at 1687 cm^{-1}. The ^1H NMR spectrum (Table 1) showed signals corresponding to protons of three quaternary methyl groups (δ_H 1.56 and 0.79, each s; 2.28, brd, J = 1.9 Hz) and one tertiary methyl (δ_H 1.19, d, J = 7.4 Hz), and an oxygenated methine proton (δ_H 3.84, dd, J = 11.1, 3.9 Hz). The ^{13}C NMR data (Table 1) indicated the presence of a conjugated carbonyl carbon (δ_C 203.3), one carbonyl carbon (δ_C 211.3), four methyl carbons (δ_C 16.7, 20.9, 21.9, 26.4), one oxygenated carbon (δ_C 81.0), and two olefinic carbons (δ_C 128.5, 153.9). Detailed comparison of the ^{13}C NMR spectrum of **1** with those of the known compound 3 (CAS registration number 1582279-41-1) isolated from the diversity-enhanced extracts of *Curcuma zedoaria*[19] together with the characteristic ultraviolet absorption at 250 (3.78) nm of **1** suggested that the α,β-unsaturated ketone could be located between C-7(11) and C-8 in **1**. This deduction was further supported by the HMBC correlations from Me-12(13) to C-7, C-8 and C-11, and from H-6 to C-1, C-5, C-7, C-10, C-11 and C-15 (Figure 2). The C-1−C-2−C-3−C-4−C-14 moiety was indicated by the $^1H-^1H$ COSY data (Figure 2). Furthermore, the NOESY correlations of H-6 with Me-14, H-1 and H-3β; Me-15 with H-2α, and H-4 with H-2α and H-3α, suggested that Me-14, H-6 and H-1 were β-oriented, and Me-15 was α-oriented (Figure 3). In order to unambiguously assign the absolute configuration of **1**, we resorted to single crystal X-ray crystallography (Figure 4),

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which established the absolute configuration of **1** as 1*S*, 4*S*, 6*R*, 10*S*. The absolute configurations at C-1 in **1** was further supported by using the bulkiness rule for the $Rh_2(OCOCF_3)_4$-induced circular dichroism data, wherein the E band (around 350 nm) was demonstrated to be useful for determining the absolute configuration of chiral secondary and tertiary alcohols.[20] The $Rh_2(OCOCF_3)_4$-induced CD spectrum **1** displayed a positive Cotton effect at 350 nm (the E band), which confirmed the 1*S*-configuration by applying the bulkiness rule[21]. Thus, compound **1** was identified as (1*S*, 4*S*, 6*R*, 10*S*)-1-hydroxysalvial-7(11)-ene-5,8-dione, for which we propose the trivial name, phasalvione. Compound **1** has an isodaucane or salvialane skeleton, which is the rare group of sesquiterpenoids.

Phaeocaudione (**2**) was deduced to have the molecular formula $C_{14}H_{20}O_3$, as indicated by the observed ion at *m/z* 259.1304 $[M + Na]^+$ in its HRESIMS. The IR spectrum showed the absorption bands of hydroxy (3443 cm^{-1}), α,β-unsaturated ketone (1689, 1624 and 1457 cm^{-1}), and saturated carbonyl (1711 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) displayed signals corresponding to four quaternary methyl singlets (δ_H 1.31, 1.87, 2.21, 2.29). The ^{13}C NMR data (Table 1) indicated the presence of two carbonyl carbons (δ_C 206.6, 204.2), four methyl carbons (δ_C 23.4, 21.2, 25.1, 27.4), one oxygenated carbon (δ_C 82.5), and two olefinic carbons (δ_C 130.9, 151.0). C-1−C-2 and C-4−C-5 moieties were indicated by the ^1H−^1H COSY spectrum (Figure 2). The HMBC correlations from Me-13 to C-2, C-3, C-4 and from Me-12 to C-6, C-7, C-10, C-11, as well as analysis of the proton chemical shifts, implied the existence of two partial structures, i.e., Me-13−C-3(C-4)−C-2 and Me-11(Me-12)−C-10−C-6−C-7 fragments. In addition, the HMBC spectrum showed correlations from H-4 to C-1, C-2, C-3, C-5, C-6, C-7, C-8 and C-9, and Me-14 to C-8 and C-9. Analysis of the above HMBC correlations and indices of hydrogen deficiency indicated the presence of two cyclic moieties linked at C-4 and C-8, and the linkage of an acetyl group to C-8 (Figure 2). Furthermore, the NOESY correlations of H-4 with Me-13, Me-14; H-5β with H-1β and H-2β, and H-1α and H-2α suggested that H-4 and acetyl group at C-8 had a *cis*-configuration supported by the molecular modeling. In addition, the correlations of Me-13 with H-5β and H-5α, no correlation between H-4 and H-5β further confirmed that H-4 and

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Me-13 were co-facial, and were randomly assigned as α-orientation (Figure 3). Finally, *S*-configuration of C-3 was supported by a positive Cotton effect at 350 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum based on the empirical bulkiness rule proposed by Snatzke.[20,21] Therefore, the absolute configuration of compound **2** was determined to be 3*S*, 4*R*, 8*R*. On the basis of these findings, the structure of **2** was determined, and it was given the trivial name phaeocaudione, having a unique skeleton or ring system.

Compound **3** was determined to have the molecular formula $C_{13}H_{20}O_4$ on the basis of the HRESIMS *m/z* 263.1253 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1259) and NMR spectra. The IR spectrum showed bands corresponding to hydroxy (3431 cm^{-1}) and conjugated carbonyl (1630 cm^{-1}). The ^1H NMR spectrum (Table 1) showed three quaternary methyl singlets (δ_H 1.37, 1.38, 2.40) and one olefinic proton (δ_H 6.64, s). The ^{13}C NMR data (Table 1) indicated the presence of a conjugated carbonyl carbon (δ_C 202.6), three methyl carbons (δ_C 23.0, 26.1, 27.6), three oxygenated aliphatic carbons (δ_C 72.5, 80.6, 84.2), and two olefinic carbons (δ_C 141.0, 145.2). The above NMR spectroscopic data were similar to wenyujinin L[22], a nor-sesquiterpene instead of monoterpenoid isolated from *C. wenyujin*, except for the appearance of two oxygenated quaternary carbons and a quaternary methyl instead of a methine. The location of the functional groups and NMR data assignments of **3** were determined by HSQC and HMBC spectroscopic analysis. The long-range correlations from Me-15 to C-1, C-9 and C-10; from Me-14 to C-3, C-4 and C-5, and from H-5 to C-1, C-4, C-6 and C-14 (Figure 2), indicated three hydroxyl groups attached to the quaternary carbons C-1, C-4 and C-10, respectively. The acetyl group was attached to C-7 due to the long-range correlations (Figure 2) from Me-12 to C-11, C-7 and C-9, and from H-9 to C-1, C-6, C-10 and C-11, together with the characteristic ultraviolet absorption at 227 (3.64) nm. Furthermore, the NOESY correlations (Figure 3) of H-5 with H-6α, H-3α and H-2α, and Me-14 with Me-15, H-6β, H-3β and H-2β suggested that Me-15 and Me-14 were β-oriented, while H-5 and 1-OH were α-oriented, which means that the two cyclic moieties are in *cis*-form. Thus, the structure of **3** was characterized, and it was named phaeocauone.

Compound **4** had the molecular formula $C_{12}H_{14}O_3$, required six degrees of unsaturation, as

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indicated by HRESIMS *m/z* 229.0844 $[M+Na]^+$ (calcd for $C_{12}H_{14}O_3Na$, 229.0841) and 207.1024 $[M+H]^+$ (calcd for $C_{12}H_{15}O_3$, 207.1021). The IR spectrum indicated the presence of carbonyl (1694 cm^{-1}) functions and an aromatic ring (1611, 1575 cm^{-1}). The ^1H NMR spectrum (Table 1) displayed two quaternary methyl groups (δ_H 2.16, 2.35, each s), two isolated methylene groups (δ_H 2.72, t, J = 7.5 Hz; 2.92, t, J = 7.5 Hz), and three olefinic protons (δ_H 7.87, d, J = 1.8 Hz; 7.85, dd, J = 8.0, 1.8 Hz; 7.20, d, J = 8.0 Hz) indicating an AMX system. The ^{13}C NMR spectrum (Table 1) exhibited 12 carbon resonances corresponding to two carbonyl carbons (δ_C 207.7, 172.3) including a carboxyl carbon, two methyl carbons (δ_C 19.5, 30.3), six olefinic carbons (δ_C 145.9, 136.6, 132.1, 128.9, 128.3, 127.5) and two methylene carbons at (δ_C 43.4, 27.2). The above NMR spectroscopic data were similar to 3,7-methylindan-5-carboxylic acid,[23] a nor-sesquiterpene having an indan skeleton isolated from *C. wenyujin*, except for the appearance of a carbonyl carbon, a quaternary methyl group instead of a tertiary methyl group, and the disappearance of a methine. The location of the functional groups and NMR data assignments of **4** were determined by HSQC and HMBC spectroscopic analysis. The HMBC correlations of H-2 with C-1, C-3, C-4, C-5 and C-10, and Me-14 with C-3 and C-4 established that the 4-oxo-normal-butyl was located at C-1. The HMBC correlations from H-6 to C-1, C-8 and C-9, and H-9 to C-1, C-6, C-8 and C-15 together with the AMX system confirmed the presence of a carboxyl group at C-7 (Figure 2). Thus, the structure of **4** was determined as a 4,5-*seco*-indan skeleton, and it was named 3-methyl-4-(3-oxo-butyl)-benzoic acid.

Compound **5** was obtained as a colorless amorphous powder. The molecular formula, $C_{15}H_{21}NO$, consistent with six degrees of unsaturation, was determined by HRESIMS (*m/z* 232.1704 $[M+H]^+$, calcd for 232.1701) and NMR data. The IR spectrum indicated the presence of a lactam (3417, 1694 cm^{-1}) group and olefinic bonds (1454 cm^{-1}). The ^1H NMR spectrum of **5** exhibited the signals for three methyl groups including two olefinic methyl singlets (δ_H 1.80, 1.76), and an aliphatic methyl singlet (δ_H 1.16), while the ^{13}C NMR spectrum displayed 15 carbon signals including a carbonyl carbon (δ_C 175.7) and six olefinic carbons (δ_C 111.6, 113.6, 125.2, 145.8, 147.5, 155.3) (Table 2), suggesting **5** was a sesquiterpene of high functionality. Three olefinic proton signals (δ_H 5.72, dd, J = 17.5, 10.8 Hz; 4.97, d, J = 10.8 Hz; 4.96, d, J = 17.5 Hz) were attributed to a monosubstituted terminal

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double bond, and two olefinic proton signals (δ_H 4.95, 4.72, each s) indicated the presence of a disubstituted terminal double bond in the HSQC spectrum. The above NMR spectroscopic data were similar to elema-1,3,7(11),8-tetraen-8,12-lactam,[24] a sesquiterpene lactam possessing an elemane-type skeleton isolated from *C. wenyujin*, except for the appearance of a methine and a methylene instead of two olefinic carbons and one olefinic proton, together with the characteristic ultraviolet absorption at 207 (3.61) nm of **5**, suggesting that the double bond between C-8 and C-9 was reduced in **5**. This deduction was further supported by the HMBC correlations from Me-15 to C-1, C-5, C-9 and C-10; from H-9β to C-7, C-8 and C-10, and from H_2-6 to C-7, C-8, C-10 and C-11 (Figure 2). Furthermore, the NOESY correlations (Figure 3) of H-8 with H-6β, H-9β and Me-15; H-5 with H-6α, and Me-15 with H-6β and H-9β suggested that Me-15 and H-8 were β-oriented, and H-5 was α-oriented. Thus, the structure of **5** was characterized, and it was named 8β(H)-elema-1,3,7(11)-trien-8,12-lactam.

The optical rotation value of **5** was almost zero, which suggested it should be a racemate. Further chiral HPLC analysis was performed on a Chiralpak AD-RH liquid chromatography column (150 mm × 4.6 mm, 5 μm; Daicel) with 45% MeCN/H_2O at a rate of 1.0 ml/min. The retention times (t_R) of the enantiomers of **5**, namely **5a** and **5b**, were 10.5 and 17.7 min, respectively. Their relative abundance was ca. 1:1 according to their relative peak areas (51.9% for **5a** and 48.1% for **5b**) in the HPLC chromatogram (see ESI Figure S52a †). Further separation of the enantiomers of **5** was not carried out due to paucity of sample.

Compound **6** was obtained as a colorless oil. The molecular formula, $C_{16}H_{22}O_3$, consistent with six degrees of unsaturation, was determined by HRESIMS (*m/z* 263.1647 $[M+H]^+$, calcd for 263.1647) and NMR data. The IR spectrum indicated the presence of an ester carbonyl (1767 cm^{-1}) group and olefinic bands (1638, 1455 cm^{-1}). The ^1H NMR spectrum of **6** exhibited the signals for three methyl groups including two olefinic methyl singlets (δ_H 1.84, 1.77), an aliphatic methyl singlet (δ_H 1.20), and a methoxy group (δ_H 3.14, s), while the ^{13}C NMR spectrum displayed 16 carbon signals including a carbonyl carbon (δ_C 173.8) and six olefinic carbons (δ_C 112.3, 114.6, 125.4, 146.6, 149.5, 160.8) (Table 2), suggesting **6** was an elemane-type sesquiterpene possessing a lactone moiety. The above NMR spectroscopic data were similar to 8β-hydroxy-isogermafurenolide,[25] except for the appearance of a methoxy group, supposing that the methoxy group was located at C-8 in **6**. This deduction was further

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supported by the HMBC correlations from 8-OMe to C-8; from H-9*β* to C-1, C-8, C-10 and C-15, and from H_2-6 to C-5, C-7, C-8, C-10 and C-11 (Figure 2). Furthermore, the NOESY correlations (Figure 3) of 8-OMe with H-9*β* and Me-15; H-5 with H-9*α* and H-1, and H-9*β* with Me-15 and Me-14 suggested that Me-15 and 8-OMe were *β*-oriented, and H-5 was *α*-oriented. Compound **6** was a new compound and named 8*β*-methoxy-isogermafurenolide.

The specific rotation value of **6** was -2.1 (*c*= 0.06, MeOH), which suggested that it was also a racemate. In view of the racemate of **5**, compound **6** was also subjected to Chiralpak AD-RH liquid chromatography analysis using 45% MeCN/H_2O at 1.0 ml/min. Two enantiomers of **6**, namely **6a** and **6b**, at t_R 9.6 and 12.5 min, respectively (Figure S52b), and their relative contents were completely identical according to their relative peak areas in the HPLC chromatogram (50.0% for **6a** and 50.0% for **6b**). Unfortunately, the enantiomers of **6** were not obtained on account of the small amount of **6**.

Compound **7** had the molecular formula $C_{15}H_{23}NO_3$, as indicated by the observed ion at *m/z* 266.1757 $[M+H]^+$ in its HRESIMS. The IR spectrum exhibited absorption at 1668 cm^{-1} typical for an unsaturated lactam group.[15] The ^1H NMR spectrum (Table 2) exhibited signals corresponding to three quaternary methyl groups (δ_H 1.00, 1.13, 1.70, each s) and one oxygenated methine proton (δ_H 3.15, dd, *J*=12.6, 3.2 Hz). The ^{13}C NMR data (Table 2) indicated the presence of a carbonyl carbon (δ_C 177.4), two olefinic carbons at (δ_C 125.2, 158.9), and two oxygenated carbons (δ_C 72.4, 79.2). The ^1H and ^{13}C NMR spectra of **7** were similar to those of phaeusmane H.[17] The major difference between **7** and phaeusmane H was the absence of two double bond carbons and one olefinic proton, while one methine and one methylene group were detected in **7**. The degrees of unsaturation and chemical shifts suggested that a double bond between C-8 and C-9 was reduced in **7**. This was confirmed by the 2D NMR data of **7**, especially by the key HMBC correlations from Me-15 to C-1, C-5, C-9 and C-10; from H-6*β* to C-8; from H-6*α* to C-7, C-8, C-10 and C-11, and from H-9*α* to C-1, C-8 and C-15 (Figure 2). Furthermore, NOESY correlations of H-8 with Me-15 and H-9*β*; H-1 with H-5 and H-9*α*, and Me-15 with Me-14 (Figure S44) suggested *α*-orientation for H-5 and H-1, and *β*-orientation for Me-15, Me-14 and H-8. Thus, the structure of compound **7** was determined and named phaeusmane I.

The specific rotation value of 7 was -11.4 (*c*= 0.11, MeOH). Further chiral HPLC

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(DIONEX; Ultimate 3000) analysis was performed on a Lux Cellulose-2 chiral liquid chromatography column (Phenomenex, 250×4.6 mm, 3μm) with 90% CH_3CN/H_2O at a rate of 0.7 ml/min. The retention times (t_R) of the enantiomers of **7**, namely **7a** and **7b**, were 9.8 and 12.1 min, respectively abundance was *ca.* 1:1 according to their relative peak areas (50.5% for **7a** and 49.5% for **7b**) in the HPLC chromatogram (Figure S53).

Compound **8**, a yellowish amorphous powder, had the molecular formula $C_{19}H_{22}O_5$ as indicated by HRESIMS *m/z* 353.1360 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_5Na$, 353.1365). The IR spectrum indicated the presence of hydroxyl groups (3442 cm^{-1}) and aromatic rings (1634 cm^{-1}). The ^1H NMR spectrum (Table 2) displayed a characteristic ABX system of a benzene ring (δ_H 6.62, dd, *J*= 8.1, 1.5 Hz; 6.65, d, *J*= 8.1 Hz; 6.78, d, *J*= 1.5 Hz) and a typical AA′BB′system (δ_H 6.60, d, *J*= 8.3 Hz; 6.92, d, *J*= 8.3 Hz, each 2H) in **8**. The ^{13}C NMR spectrum (Table 2) exhibited 19 carbon resonances corresponding to three oxygenated carbons (δ_C 65.7, 72.7, 75.0), three oxygenated olefinic carbons (δ_C 145.8, 146.3, 156.4). The above NMR spectroscopic data were similar to engelheptanoxide C,[26] a cyclic diarylheptanoid isolated from the stems of *Engelhardia roxburghiana*, except for the disappearance of a methoxy group. The location of the functional groups and NMR data assignments of **8** were determined by HSQC and HMBC spectroscopic analysis. The relative stereochemistry of **8** was determined by its NOESY spectrum (Figure 3), the same as that of engelheptanoxide C. Thus, the structure of **8** was named phaeoheptanoxide.

The discovery of compounds **1–4** in the genus of *Curcuma* is rather unusual from the view point of chemotaxonomy. Compounds **1** and **2** could be produced from dehydrocurdione, one of the characteristic constituents of *C. phaeocaulis* (Scheme 1). Epoxidation of dehydrocurdione followed by attack of the enol from C-10 opens the epoxide to generate C-6−C-10 bond in compound **1**. In biogenetic process of compound **2**, the important intermediate iii, which was a structural analogue of the known compound 3 (CAS registration number 1582279-41-1) isolated from the diversity-enhanced extracts of *C. zedoaria*[19], was presumed to be synthesized via the enolization of dehydrocurdione at C-9 and aldol condensation between two carbonyl groups. The key intermediate iv, which could be produced from iii by rearrangement and ring cleavage, produced intermediate v through enzyme catalysis of dioxygenases. Under hydroxylation and reduction reactions, intermediate

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v could yield compound **2** with an unprecedented skeleton. Compounds **3** and **4** were assumed to be produced by sequential reactions depicted in Scheme 2. Curcumenol, a major component of *C. phaeocaulis*, was presumed to be synthesized by monooxygenase to produce a 7,11-epoxy derivative. Then, quasi-Favorskii-type rearrangement associated with opening of the epoxide afforded a ring-contraction product, curcumolide, isolated from the *C. wenyujin*[27]. Intermediate curcumolide undergoes hydrolysis, decarboxylation, dehydration and oxidization reactions to produce intermediate x. There is a possible process in the enzymatic formation of compound **3** from hydration, hydroxylation and reduction reactions. Compound **3** was a key biosynthetic precursor of wenyujinin L[22], a nor-sesquiterpene isolated from *C. wenyujin*. Compound **4** was afforded by ring-opening of 3,7-methylindan-5-carboxylic acid[23], a nor-sesquiterpene having an indan skeleton isolated from *C. wenyujin*, which was derived from vii by oxidization reaction.

Nitric oxide (NO) plays an important role in the inflammatory process, and an inhibitor of NO production may be considered as a potential anti-inflammatory agent[28-30]. In the present study, all isolated compounds were tested for their inhibitory effects on NO production induced by LPS in macrophages. Cell viability in the present experiment was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method to find out whether inhibition of NO production was due to cytotoxicity of the test compounds (data not shown). As shown in Table 3, hydrocortisone (58.79±3.32 μM) was used as a positive control. Compounds **1**, **3**, **4** and **8** exhibited potent inhibitory activities against NO production with IC_{50} of 7.46±0.69, 2.35±0.17, 3.49±0.31 and 16.49±1.16 μM, respectively. Analysis of the inhibitory effects of these constituents, together with the early bioactivity data of similar sesquiterpenes, revealed that the α,β-unsaturated γ-lactone in sesquiterpenoids conferred stronger inhibitory effects compared to the α,β-unsaturated γ-lactam in sesquiterpenoid alkaloids. For example, isogermafurenolide[14], isolated from *C. wenyujin* by our research group, showed stronger inhibitory activity on NO production than compound **5** for γ-elemene-type sesquiterpenes. A similar phenomenon was observed for eudesmane-type sesquiterpenes, (7*Z*)-1β,4β-dihydroxy-5α,8β(*H*)-eudesm-7(11)-en-8,12-olide[17] showed strong inhibitory activity, while compound **7** didn't show inhibitory activity up to 100 μM. Comparison of inhibitory effects of the γ-elemenolides revealed that the H atom at C-8 was of

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pivotal importance. Compound **6** and *β*-hydroxy-isogermafurenolide[16] showed weak inhibitory effects since the H-8 were substituted by methoxy and hydroxy groups, respectively. Furthermore, phaeusmane H[17] showed potent inhibition due to the double bond between C-8 and C-9 for eudesmane-type sesquiterpene alkaloids. The cytotoxic activities of compounds **1–8** against RAW 264.7 macrophages were also tested by the MTT assay, and none of the compounds exhibited significant cytotoxicity at their effective concentration for the inhibition of NO production. Further bioactive studies of the isolated compounds are warranted, and additional studies are currently underway in our laboratory.

Conclusions

In summary, four previously unreported sesquiterpenes, two novel nor-sesquiterpenes, one new cyclic diarylheptanoid and one new aromatic acid were isolated from the rhizomes of *C. phaeocaulis*. The common skeletons of the sesquiterpenes identified from this genus comprise germacrane, eudesmane, and guaiane types. To our knowledge, the presence of salvialane skeleton sesquiterpenes which has only been found in *C. zedoaria*[19] is unusual in the genus *Curcuma*. Therefore, our results could be helpful in chemotaxonomical classifications of the genus *Curcuma*. By analyzing the phytochemical constituents of this genus, compound **2** was found to possess a new carbon skeleton. Compound **3** has a ring system of indan, which has only been found in *C. wenyujin*[22] and *C. zedoaria*[19,23]. Compound **4**, an aromatic acid, was identified as a rare 4,5-*seco*-indan skeleton. Moreover, it was presumed to be synthesized via the mevalonic acid pathway in biogenetic process. Meanwhile, compounds **5** and **7** were identified as rare nitrogen-containing *γ*-elemene-type and eudesmane-type sesquiterpenes, respectively. Interestingly, compounds **5–7** existed as racemates, which were analyzed by chiral liquid chromatography. A combination of X-ray crystal diffraction and methods of $Rh_2(OCOCF_3)_4$-induced CD spectrum based on the empirical bulkiness rule was employed to resolve the absolute configurations of the isolated compounds **1** and **2**. Furthermore, inhibitory effects of the isolated compounds on nitric oxide production in LPS-activated macrophages were evaluated. Compounds **1**, **3**, **4** and **8** exhibited more potent inhibition than the positive control, hydrocortisone, indicating their potential as promising compounds for the further research and development of anti-inflammatory agents. A plausible biogenetic

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pathway for new compounds **1–4** is proposed as shown in Schemes 1 and 2. Carbonyl enolization, epoxidation and rearrangement reactions could be the key enzyme-catalysed reactions in the biosynthetic diversification of sesquiterpenoids.

Experimental section

General experimental procedures

Melting points were determined on an X-4 digital display micromelting point apparatus and are uncorrected. 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. CD spectra were determined on a Bio-Logic Science MOS-450 spectrometer. NMR experiments were performed on Bruker ARX-300 and AV-600 spectrometers. The chemical shifts were stated relative to TMS and expressed in δ values (ppm), with coupling constants reported in Hz. HRESIMS were obtained on an Agilent 6210 TOF mass spectrometer. Silica gel GF254 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 LC-6AD liquid chromatograph with a YMC Pack ODS-A column (250×20 mm, 5 μm, 120 Å) and SPD-10A VP UV/VIS detector. Analysis of racemates of **5** and **6** was carried out on a Chiralpak AD-RH column (150 mm × 4.6 mm, 5 μm; Daicel Chemical Industries, Ltd.) using an Agilent 1260 liquid chromatography instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler and a G4212B UV detector. Analysis of racemates of **7** was carried out on a Chiralpak Lux Cellulose-2 column (250×4.6 mm, 3µm; Phenomenex.) using an DIONEX liquid chromatography instrument (DIONEX, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler and a Ultimate 3000 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

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Rhizomes of *C. phaeocaulis* were collected from Chengdu, Sichuan Province, China, and identified by Professor Qishi Sun, Department of Pharmaceutical Botany, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (**CP-20100715**) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and Isolation

Dry rhizomes of *C. phaeocaulis* (10 kg) were cut into approximately 2 cm pieces and extracted with 95% ethanol (100 L×2 h×2). The resulting concentrated extract (0.6 kg) in vacuo, was suspended in H_2O (3 L), and partitioned successively with cyclohexane, EtOAc, and *n*-BuOH (3 L×3). The EtOAc extract (105 g) was subjected to silica gel column chromatography (10×80 cm) and eluted with cyclohexane/acetone (100:1, 40:1, 20:1, 10:1, 4:1, 2:1, 1:1 and 0:1 v/v) to obtain six fractions (EA-EF), which were combined according to TLC analysis. Fraction EA (23 g) was subjected to silica gel column chromatography (6×80 cm) and eluted with CH_2Cl_2/EtOAc (from 40:1 to 0:1) to produce seven fractions (EA1-EA7). Fraction EA3 (290 mg) was chromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1; 1.5×30 cm) to give compounds **5** (4.8 mg) and **6** (3.5 mg). Fraction EC (15 g) was subjected to silica gel column chromatography (6×80 cm) and eluted with a gradient of increasing acetone (0-100%) in *n*-hexane, to afford fractions EC1-EC7. EC3 (4.8 g) was chromatographed over reversed-phase C_{18} silica gel column (2.5×30 cm) chromatography eluted with MeOH/H_2O (30:70, 50:50, 70:30 and 100:0 v/v) to give five fractions EC3-1 to EC3-4, and subfraction EC3-2 (180 mg) was separated by preparative HPLC (55% MeOH/H_2O, 6 mL/min) to afford compounds **1** (8.7 mg, t_R = 49 min) and **2** (13.8 mg, t_R = 55 min). Fraction EF (28 g) was subjected to a silica gel column (6 × 80 cm), with elution using a gradient of increasing MeOH (0-100%) in CH_2Cl_2, to give five fractions EF1-EF5. EF3 (4.8 g) was chromatographed over reversed-phase C_{18} silica gel column (2.5×30 cm) chromatography eluted with MeOH/H_2O (30:70, 50:50, 70:30 and 100:0 v/v) to give six fractions EF3-1 to EF3-6, and subfraction EF3-4 (128 mg) was separated by preparative HPLC (30% MeOH/H_2O, 6 mL/min) to afford compounds **3** (14.2 mg, t_R = 43 min) and **7** (13.7 mg, t_R = 53 min). Fr. EF3-5 (251 mg) was separated further by Sephadex LH-20 (MeOH/H_2O, 1:1; 1.5×30 cm) to give compounds **4** (61.1 mg) and **8** (10.5 mg).

Organic & Biomolecular Chemistry Accepted Manuscript

Phasalvione (1): Colorless crystals (MeOH); mp 186−187 °C; $[\alpha]_D^{25}$ +85.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 250 (3.78) nm; $Rh_2(OCOCF_3)_4$-induced CD ($CDCl_3$) nm ($\Delta\varepsilon$) 350 (+0.25); IR (KBr) ν_{max} 3442, 2925, 1687, 1626, 1384, 1116 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 273.1466 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1467).

Phaeocaudione (2): Yellowish oil (MeOH); $[\alpha]_D^{25}$ +78.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.55) nm; $Rh_2(OCOCF_3)_4$-induced CD ($CDCl_3$) nm ($\Delta\varepsilon$) 348 (+0.08); IR (KBr) ν_{max} 3443, 2968, 2933, 1711, 1689, 1624, 1457, 1377, 1359, 1190, 1171 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 259.1304 $[M + Na]^+$ (calcd for $C_{14}H_{20}O_3Na$, 259.1310).

Phaeocauone (3): Colorless oil (MeOH); $[\alpha]_D^{25}$ +57.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.64) nm; IR (KBr) ν_{max} 3431, 2924, 1630, 1384, 1116 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 263.1253 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1259).

3-methyl-4-(3-oxo-butyl)-benzoic acid (4): Colorless amorphous powder (MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.00) nm; IR (KBr) ν_{max} 3395, 2962, 1694, 1611, 1575, 1428, 1308, 1168 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 229.0844 $[M + Na]^+$ (calcd for $C_{12}H_{14}O_3Na$, 229.0841); *m/z* 207.1024 $[M + H]^+$ (calcd for $C_{12}H_{15}O_3$, 207.1021).

8*β*(H)-elema-1,3,7(11)-trien-8,12-lactam (5): Colorless amorphous powder (MeOH); $[\alpha]_D^{25}$ -4.2 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.61) nm; IR (KBr) ν_{max} 3417, 2926, 2854, 1694, 1454, 1382, 1030 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 232.1704 $[M + H]^+$ (calcd for $C_{15}H_{22}NO$, 232.1701).

8*β*-methoxy-elema-1,3,7(11)-trien-8,12-lactone (6): Colorless oil (MeOH); $[\alpha]_D^{25}$ -2.1 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.82) nm; IR (KBr) ν_{max} 2925, 2853, 1767, 1697, 1638, 1455, 1383, 1275, 1091 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 263.1647 $[M + H]^+$ (calcd for $C_{16}H_{23}O_3$, 263.1647).

Phaeusmane I (7): Colorless amorphous powder (MeOH); $[\alpha]_D^{25}$ -11.4 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.84) nm; IR (KBr) ν_{max} 3394, 2935, 2867, 1668, 1450, 1385, 1276, 1098 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 266.1757

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$[M + H]^+$ (calcd for $C_{15}H_{24}NO_3$, 266.1756).

Phaeoheptanoxide (8): Yellowish amorphous powder (MeOH); $[\alpha]_D^{25}$ -64.8 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.82) nm; IR (KBr) ν_{max} 3442, 2923, 2853, 1634, 1384, 1116 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 353.1360 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_5Na$, 353.1365).

$Rh_2(OCOCF_3)_4$-induced CD method of 1 and 2

According to a published procedure[20,21], 0.5 mg of compound tested (**1** and **2**) was dissolved in a dry solution of the stock $[Rh_2(OCOCF_3)_4]$ complex (1.5 mg) in $CHCl_3$ (200 μl) and was subjected to CD measurements at a concentration of 2.5 mg/ml. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the alcohol moiety.

X-ray diffraction analysis of 1

Phasalvione (**1**) was crystallized in MeOH using the vapor diffusion method, colorless flaky crystal of **1** were obtained. Data were collected using a Sapphire CCD with a graphite monochromated Cu Kα radiation, λ = 1.54184 Å at 173.0 (3) K. Crystal data: $C_{15}H_{22}O_3$, M = 250.33, space group orthorhombic, $P\ 2_1\ 2_1\ 2_1$; unit cell dimensions were determined to be a = 7.73264 (17) Å, b = 8.92045 (17) Å, c = 20.1079 (4) Å, α = 90.00 °, β = 90.00 °, γ = 90.00 °, V = 1387.02 (5) $Å^3$, Z = 4, Dx = 1.199 mg/m^3, F (000) = 544, μ (Cu Kα) = 0.656 mm^{-1}. 10900 reflections were collected to θ_{max} = 62.63 °, in which 2171 reflections were observed $[F^2 > 4\sigma(F^2)]$. The structure was solved by direct methods using the SHELXS-97 program (Sheldrick 2008), and refined by the program SHELXL-97 and full-matrix least-squares calculations.[31] In the structure refinements, non-hydrogen atoms were placed on the geometrically ideal positions by the “ride on” method. Hydrogen atoms bonded to oxygen were located by the structure factors with isotropic temperature factors. The final refinement gave R = 0.0290, R_W = 0.0781, S = 1.046 and Flack = 0.12 (19). Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (deposition no.: CCDC 1400424).

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NO production bioassay

Mouse monocyte-macrophage RAW 264.7 cells (ATCC TIB-71) were purchased from the Chinese Academy of Sciences. RPMI 1640 medium, penicillin, streptomycin, and fetal bovine serum were purchased from Invitrogen (New York, NY, USA). LPS, dimethylsufoxide (DMSO), MTT, and hydrocortisone were obtained from Sigma Co. RAW 264.7 cells were suspended in RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and 10% heat-inactivated fetal bovine serum. The cells were harvested with trypsin and diluted to a suspension in fresh medium. 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. The nitrite concentration in the medium was measured as an indicator of NO production according to the Griess reaction. Briefly, RAW 264.7 cells were seeded into 96-well tissue culture plates at a density of 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 in the presence or absence of test compounds. After incubation at 37 ˚C for 24 h, 100 μl of cell-free supernatant was mixed with 100 μl of Griess reagent (mixture of equal volumes of reagent A and reagent B, A: 1% (w/v) sulfanilamide in 5% (w/v) phosphoric acid, B: 0.1% (w/v) of N-(1-naphthyl)ethylenediamine). Cytotoxicity was determined by the MTT colorimetric assay, after 24 h incubation with test compounds. Nitrite concentrations and the inhibitory rates were calculated by a calibration curve prepared with sodium nitrite standards.[12,32]

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† Electronic supplementary information (ESI) available: Copies of HRESIMS and original NMR spectra for all the new compounds, CD spectra for compounds **1** and **2**, and chiral HPLC analytical chromatograms for compounds **5**, **6** and **7** are provided.

‡ These two authors contributed equally to this work.

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**-**4**.[a]

Position	**1**		**2**		**3**		**4**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	81.0	3.84, dd (11.1, 3.9)			84.2		145.9	
2	33.3	1.66, m	33.3	2.11, m	30.1	2.28, dt (12.8, 10.8)	27.2	2.92 2H, t (7.5)
		2.04, m		2.46, m		1.63, dd (12.8, 8.2)		
3	29.4	1.88, m	39.3	1.64, m	41.2	1.90, m	43.4	2.72 2H, t (7.5)
		1.74, m		1.73, m		2.04, dt (13.4, 9.4)		
4	48.5	2.41, m	82.5		80.6		207.7	
5	211.3		51.5	2.88, dd (5.8, 5.6)	51.1	2.18, dd (12.4, 5.0)	128.9	7.20, d (8.0)
6	55.3	4.01, s	29.8	2.81, m	21.7	2.47, dd (17.1, 5.0)	128.3	7.85, dd (8.0, 1.8)
				2.22, m		2.11, dd (17.1, 12.4)		
7	128.5		130.9		141.0		127.5	
8	203.3		204.2				172.3	
9	54.8	2.40 2H, brd (1.9)	76.9		145.2	6.64, s	132.1	7.87, d (1.8)
10	42.4		206.6		72.5		136.6	
11	153.9		151.0		202.6			
12	26.4	1.56, s	25.1	1.87, s	26.1	2.40, s		
13	21.9	2.28, brd (1.9)	21.2	2.21, s				
14	20.9	1.19, d (7.4)	23.4	1.31, s	27.6	1.37, s	30.3	2.16, s
15	16.7	0.79, s	27.4	2.29, s	23.0	1.38, s	19.5	2.35, s

[a] ^{1}H NMR spectra measured at 600 MHz, ^{13}C NMR spectra measured at 75 MHz; spectrum of compound **3** was obtained in CD_3OD, and spectra of **1**, **2**, **4** were obtained in $CDCl_3$.

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **5**-**8**.[a]

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	5			**6**		**7**			**8**	
Position	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
1	147.5	5.72, dd		149.5	5.76, dd	79.2	3.15, dd	1	75.0	4.57, dd
		(17.5, 10.8)			(17.6, 10.7)		(12.6, 3.2)			(11.5, 1.4)
2	111.6	4.97, d		112.3	4.96, d	29.3	1.60, m	2	41.1	1.75, dq
		(10.8)			(10.7)					(13.6, 2.1)
										1.66, m
		4.96, d			4.95, d		1.62, ddd	3	65.7	4.13, m
		(17.5)			(17.6)		(12.6, 11.5, 3.1)			
3	113.6	4.95, s		114.6	4.97, s	41.6	1.44, ddd	4	39.4	1.60, m
							(12.2, 9.2, 3.1)			1.44, ddd
		4.72, s			4.76, s		1.73, m			(14.1, 12.0, 2.6)
								5	72.7	3.81, m
4	145.8			146.6		72.4		6	39.7	1.69, m
										1.56, m
5	53.5	2.00, dd		55.4	2.13, dd	55.0	1.18, dd	7	31.9	2.58, m
		(14.2, 3.9)			(13.4, 4.1)		(13.4, 3.4)			2.53, m
6	28.5	2.63, dd		28.5	2.63, dd	23.1	2.96, dd	1′	136.1	
		(14.2, 3.9)			(13.4, 4.1)		(13.4, 3.4)			
		2.49, t			2.58, t		2.19, t			
		(14.2)			(13.4)		(13.4)			
7	155.3			160.8		158.9		2′	114.8	6.78, d (1.5)
8	54.5	4.00, dd		107.7		56.0	3.90, dd	3′	146.3	
		(12.2, 5.6)					(11.8, 5.7)			
9	46.9	1.22, dd		49.8	1.66, d	49.4	0.68, t	4′	145.8	
		(12.5, 12.2)			(13.7)		(11.8)			
		2.01, dd			2.10, d		2.47, dd			
		(12.5, 5.6)			(13.7)		(11.8, 5.7)			
10	40.6			42.0		40.8		5′	116.2	6.65, d (8.1)
11	125.2			125.4		125.2		6′	118.9	6.62, dd (8.1,1.5)
12	175.7			173.8		177.4		1″	134.6	
13	8.2	1.80, s		8.2	1.84, s	8.0	1.70, s	2″, 6″	130.5	6.92, d (8.3)
14	24.9	1.76, s		25.0	1.77, s	22.3	1.13, s	3″, 5″	116.2	6.60, d (8.3)
15	17.0	1.16, s		18.1	1.20, s	13.7	1.00, s	4″	156.4	
NH		6.48, brs	OMe	50.8	3.14, s					

[a] ^{1}H NMR spectra measured at 600 MHz, ^{13}C NMR spectra measured at 75 MHz; spectrum of compound **5** was obtained in $CDCl_3$, and spectra of **6**, **7**, **8** were obtained in CD_3OD.

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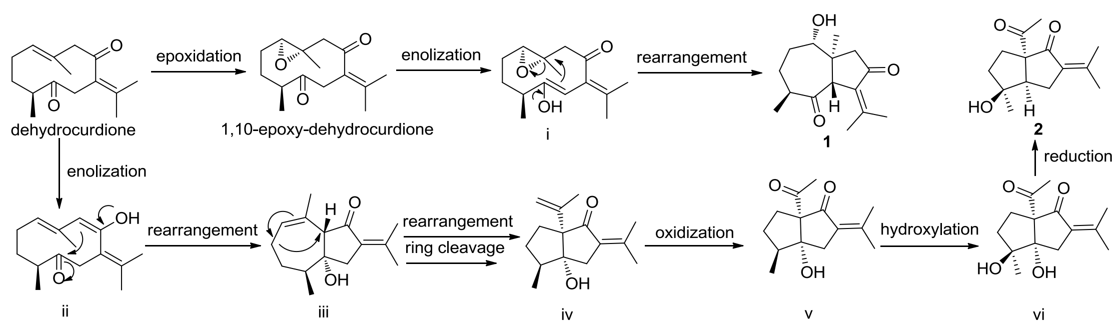
Table 3. Inhibitory Effect of Compounds Isolated from *Curcuma Phaeocaulis* on NO Production Induced by LPS in Macrophages

Compound	IC_{50} (μM)[a]
1	7.46±0.69
2	76.34±5.38
3	2.35±0.17
4	3.49±0.31
5	81.11±9.07
6	69.98±6.21
7	>100
8	16.49±1.16
hydrocortisone[a]	58.79±3.32

[a] Positive control

Scheme 1. Plausible biogenetic pathway for compounds **1** and **2**.



Scheme 2. Plausible biogenetic pathway for compounds **3** and **4**.

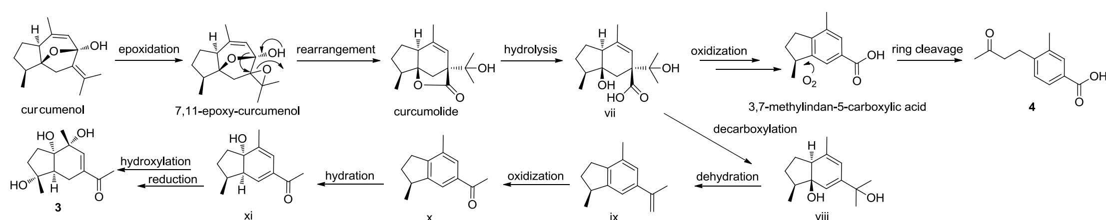


Figure 1. Structures of compounds **1-8** isolated from *Curcuma phaeocaulis* Valeton.

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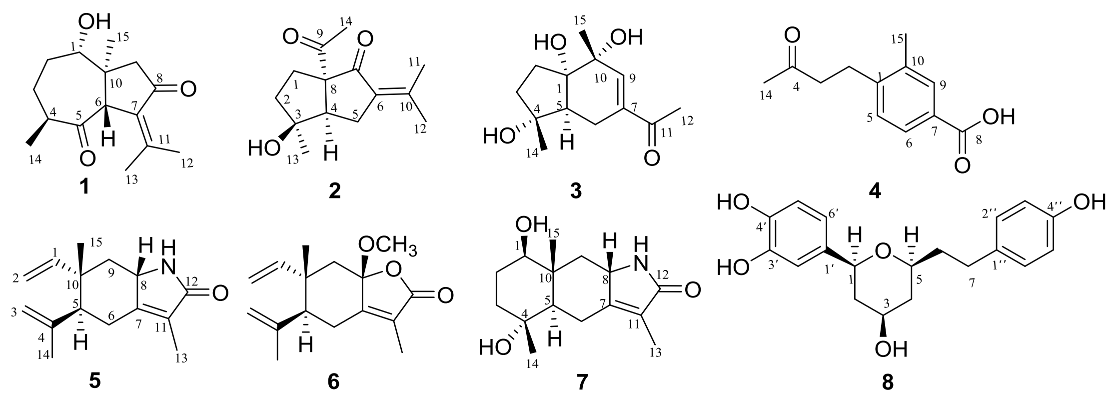

Figure 2. Selected HMBC correlations of compounds **1-8**.

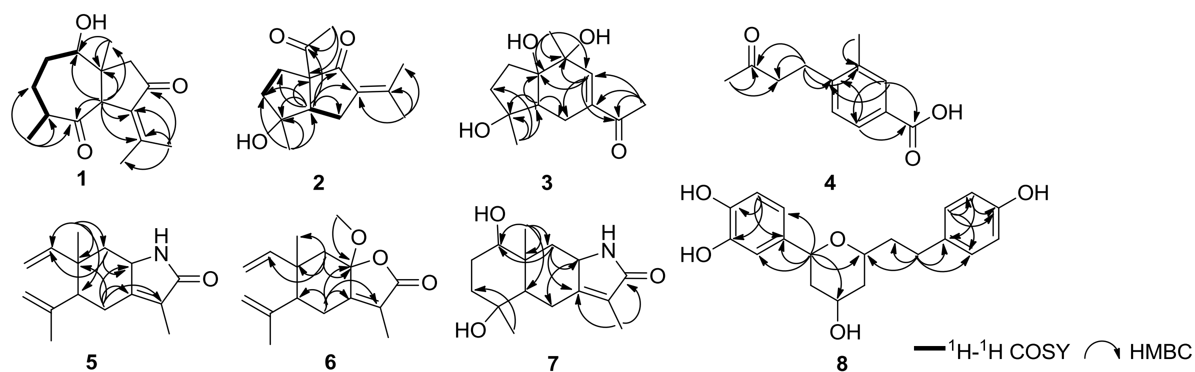

Figure 3. Key NOESY correlations of compounds **1-3**, **5**, **6** and **8**.

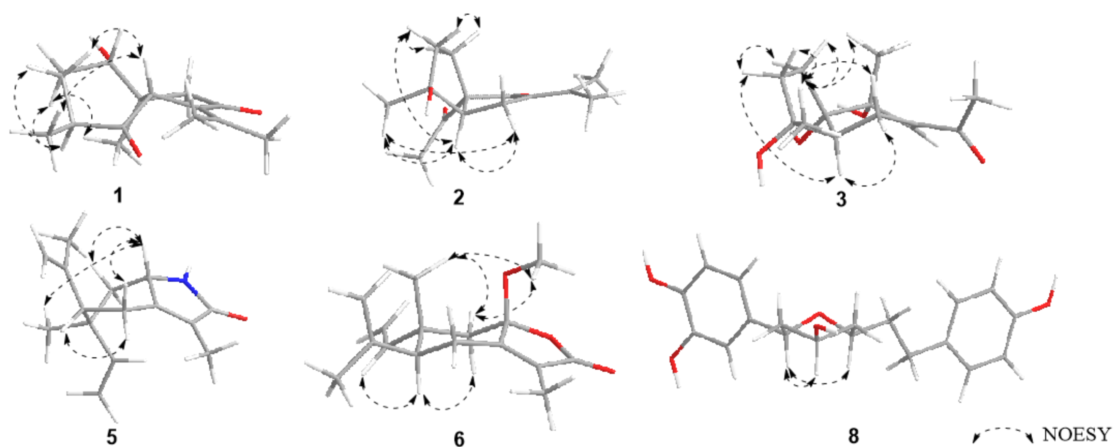

Figure 4 X-ray crystal structure of **1**.

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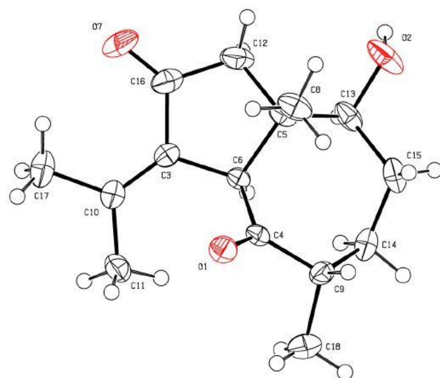

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