Organic LETTERS

Letter

pubs.acs.org/OrgLett

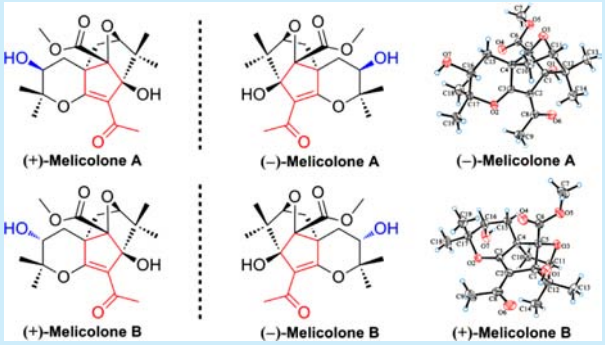
(±)-Melicolones A and B, Rearranged Prenylated Acetophenone Stereoisomers with an Unusual 9-Oxatricyclo[3.2.1.1^{3,8}]nonane Core from the Leaves of *Melicope ptelefolia*

Jin-Fang Xu, Hui-Jun Zhao, Xiao-Bing Wang, Zhong-Rui Li, Jun Luo, Ming-Hua Yang, Lei Yang, Wen-Ying Yu, He-Quan Yao, Jian-Guang Luo,* and Ling-Yi Kong*

State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

Supporting Information

ABSTRACT: Melicolones A (**1**) and B (**2**), a pair of rearranged prenylated acetophenone epimers with an unusual 9-oxatricyclo-[3.2.1.1^{3,8}]nonane core, were isolated from the leaves of *Melicope ptelefolia*. Further chiral high-performance liquid chromatography resolution gave enantiomers (+)- and (−)-**1**, as well as (+)- and (−)-**2**, respectively. The structures and absolute configurations of the pure enantiomers were determined by extensive spectroscopic data and single crystal X-ray diffraction. All the isolated enantiomers exhibited potent cell protecting activities against high glucose-induced oxidative stress in human vein endothelial cells.



The genus *Melicope* (Rutaceae) comprises about 233 species widely distributed in the tropical regions all over the world.[1] Many *Melicope* species have been used as folk medicines due to their excellent pharmacological activities.[2] Phytochemical studies on this genus reveal the presence of a number of constituents including alkaloids, flavonoids, benzopyrans, and acetophenones.[3] Among them, the prenylated acetophenones are considered to be the chemotaxonomic markers of *Melicope* species.[4] Although the acetophenones isolated from *Melicope* species are commonly substituted by prenyl or geranyl groups, the aromatic structure has been retained in most of the cases.[4,5] Only a few examples have been presented hitherto for nonaromatic prenylated acetophenones.[6]

Melicope ptelefolia (Champ. ex Benth.) T. Hartley is a deciduous shrub or arbor distributed in Southeast Asia. Previous chemical investigations on this plant mainly focused on its roots and stems resulting in the isolation of alkaloids and benzopyrans.[7,8] Recently, in our further chemical research of the leaves of *M. ptelefolia*, two diastereoisomeric pairs of enantiomers, (±)-melicolone A (**1**) and B (**2**), the rearranged prenylated acetophenones featuring an unprecedented 9-oxatricyclo[3.2.1.1^{3,8}]nonane core, were obtained (Figure 1). Herein, we report their structure elucidation, postulated biogenetic pathway, and biological activities.

Melicolone A (**1**)[9] was obtained as a colorless prism. Its molecular formula was determined as $C_{19}H_{26}O_7$ by HR-ESI-MS (m/z 367.1752 [M + H]$^+$, calcd for $C_{19}H_{27}O_7$, 367.1751), indicating 7 degrees of unsaturation. The IR spectrum exhibited obvious absorption bands for hydroxyl (3464 cm^{-1}), carbonyl (1735 cm^{-1}), and vinyl (1618 cm^{-1}) functionalities, respectively.

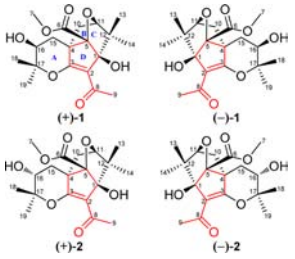


Figure 1. Structures of (±)-melicolones A (**1**) and B (**2**).

The ^{1}H and ^{13}C NMR data of **1** (Table 1) interpreted with the help of HSQC and HMBC spectrum, revealed the presence of signals attributable to one acetyl group [δ_H 2.37 (3H, s); δ_C 199.2, 30.4], one carbomethoxy group [δ_H 3.80 (3H, s); δ_C 169.1, 52.4], four tertiary methyls (δ_H 0.86, 1.11, 1.38, 1.54, each 3H, s), two methylenes, two oxygenated methines, seven quaternary carbons including three oxygenated (δ_C 85.9, 91.5, 102.1), and a pair of olefinic carbons (δ_C 117.7, 171.3). These above observations accounted for three out of the seven unsaturations, suggesting that **1** had four rings.

Two isolated −CH_2−CHO− fragments of C-10−C-11 and C-15−C-16 as drawn with the blue bond (Figure 2) were readily established by the ^{1}H−^{1}H COSY. Subsequently, these fragments with the quaternary carbons and oxygen atoms were connected to delineate the planar structure of **1** by the HMBC spectrum

Received: November 20, 2014
Published: December 16, 2014

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 1 and 2

	1				2	
no.	$\delta_H{}^a$ (multi, J in Hz)	$\delta_C{}^a$	$\delta_H{}^b$ (multi, J in Hz)	$\delta_C{}^b$	$\delta_H{}^b$ (multi, J in Hz)	$\delta_C{}^b$
1		89.9		91.5		91.1
2		116.2		117.7		117.4
3		169.1		171.3		173.3
4		51.4		53.3		49.2
5		100.2		102.1		103.5
6		167.0		169.1		169.3
7	3.72 (s)	51.4	3.80 (s)	52.4	3.79 (s)	52.3
8		196.1		199.2		199.2
9	2.32 (s)	30.0	2.37 (s)	30.4	2.38 (s)	30.5
10	α 2.30 (d, 12.8)	41.1	α 2.41 (d, 12.8)	42.6	α 2.52 (d, 13.0)	45.0
	β 1.57 (dd, 12.8, 5.2)		β 1.65 (dd, 12.8, 5.2)		β 1.83 (dd, 13.0, 5.2)	
11	4.10 (d, 5.2)	86.0	4.12 (d, 5.2)	88.5	4.07 (d, 5.2)	88.7
12		47.4		49.3		49.3
13	1.02 (s)	22.7	1.11 (s)	23.2	1.10 (s)	23.2
14	0.79 (s)	19.1	0.86 (s)	19.7	0.89 (s)	19.8
15	α 1.45 (dd, 12.2, 4.5)	33.3	α 1.56 (dd, 12.2, 4.5)	34.6	α 1.63 (dd, 13.9, 4.9)	34.0
	β 1.84 (dd, 12.2, 12.2)		β 1.98 (dd, 12.2, 12.2)		β 2.23 (dd, 13.9, 3.4)	
16	3.55 (ddd, 12.2, 4.5, 5.0)	67.6	3.65 (dd, 12.2, 4.5)	69.8	3.77 (dd, 4.9, 3.4)	69.8
17		84.1		85.9		85.6
18	1.30 (s)	20.0	1.38 (s)	20.8	1.43 (s)	24.3
19	1.48 (s)	26.8	1.54 (s)	27.3	1.50 (s)	26.9
1-OH	5.06 (br s)					
16-OH	5.38 (d, 5.0)					

^{a}Measured in DMSO-d_6. ^{b}Measured in CD_3OD.

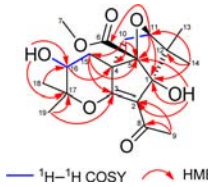


Figure 2. $^{1}H-^{1}H$ COSY and key HMBC correlations of melicolone A (**1**).

recorded in DMSO-d_6 (Supporting Information (SI), Figure S10). Two proton resonances that showed no correlation with any carbons in HSQC (SI, Figure S9) were assigned to 1-OH (δ_H 5.06, br s) and 16-OH (δ_H 5.38, d, J = 5.0 Hz), respectively, by the HMBC correlations of 1-OH to C-1 (δ_C 89.9) and 16-OH to C-16 (δ_C 67.6). In the HMBC spectrum (Figure 2), the correlations from CH_3-18 (19) to C-16 and C-17, from H_2-15 to C-3, C-4, and C-17 and from 16-OH to C-15, C-16, and C-17, as well as a weak but distinctive four-bond correlation from CH_3-19 to C-3 allowed the elaboration of the ring A in **1** to be a 2,2-dimethyl-3-hydroxypyran moiety.[10] The multiple HMBC correlations of H_2-10/C-4, C-5, and C-12; H-11/C-1, C-4, C-5, C-10, and C-12; CH_3-13 (14)/C-11, C-12, and C-1; and 1-OH/C-5 established a cyclohexane moiety consisting of C-1, C-5, C-4, and C-10 to C-12, which was divided to rings B and C by a C-5–O–C-11 oxygen bridge. A carbomethoxy was attached to C-5 based on the HMBC correlations of 6-OCH_3/C-6 and H-11/C-6 together with the oxygenated nature of C-5 (δ_C 102.1). In addition, the crucial HMBC correlations of 1-OH/C-2 and CH_3-9/C-8 and C-2 indicated the connectivity between C-1 and C-2, and C-2 and C-8, respectively. At this point, the molecule required an additional ring and an olefinic bond to satisfy the remaining two degrees of unsaturation, suggesting that the enolic carbon C-3 (δ_C 171.3) was bound to C-2 (δ_C 117.7) through a double bond to construct a five-membered ring D. Thus, the planar structure of **1** possessing a unique 9-oxatricyclo[3.2.1.1^{3,8}]nonane core was finally established as shown (Figure 2).

The relative configuration of compound **1** was determined on the basis of ROESY experiment recorded in DMSO-d_6 (Figure 3). The ROESY correlations of OH-16/CH_3-18, OH-16/Hβ-15,

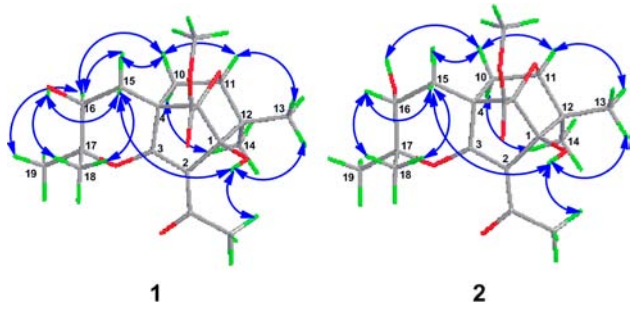


Figure 3. Key ROESY correlations (blue lines with double arrows) for melicolones A (**1**) and B (**2**).

CH_3-18/Hβ-15, Hβ-15/OH-1, CH_3-19/H-16, H-16/Hα-15, H-16/H_2-10, and Hα-15/Hβ-10 demonstrated that CH_3-18, OH-16, Hβ-15, and OH-1 were situated at the same side of the molecule with β-orientation, while C-10, C-12, Hα-15, H-16, and CH_3-19 were accordingly assigned to be α-configured. Subsequently, the ROESY correlations of OH-1/CH_3-13, H-11/CH_3-13, Hβ-10/H-11, and CH_3-14/Hα-10 indicated that 1-OH, CH_3-13, H-11, Hβ-10 were in a cofacial position of the cyclohexane ring, while CH_3-14 and Hα-10 were at the opposite side. Thus, the cyclohexane ring fused the rigid oxygen bridge and the five-membered ring (ring D) adopted a folder conformation as shown in Figure 3. In any case, the NOEs

dx.doi.org/10.1021/ol5033738 | Org. Lett. 2015, 17, 146–149

observed were in complete agreement with the structure and relative configuration of melicolone A (Figure 3).

The optical rotation of **1** was measured to be 0 and no Cotton Effect (CE) was observed on its ECD spectra, suggesting that **1** was a racemic mixture. Subsequent chiral HPLC resolution of **1** led to the separation of a pair of enantiomers, (−)-**1** and (+)-**1** (1:1, SI, Figure S17). As expected, (+)-**1** and (−)-**1** exhibit mirror-like CD curves (SI, Figures S18 and S19) and totally opposite optical rotations.[9] Fortunately, single crystals of (−)-**1** were obtained, and a Cu Kα X-ray crystallographic analysis was conducted, which not only corroborated the planar structure and the relative configuration of compound **1** as assigned above but also established the absolute configuration of (−)-**1** and (+)-**1** unambiguously. As shown in Figure 4, the absolute configuration

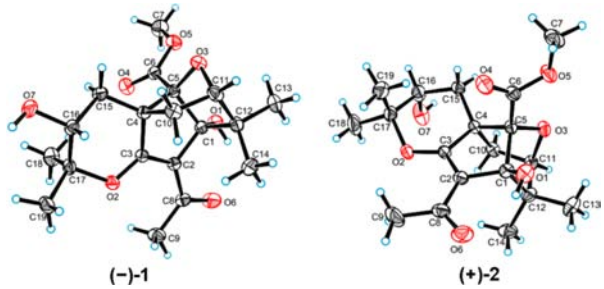


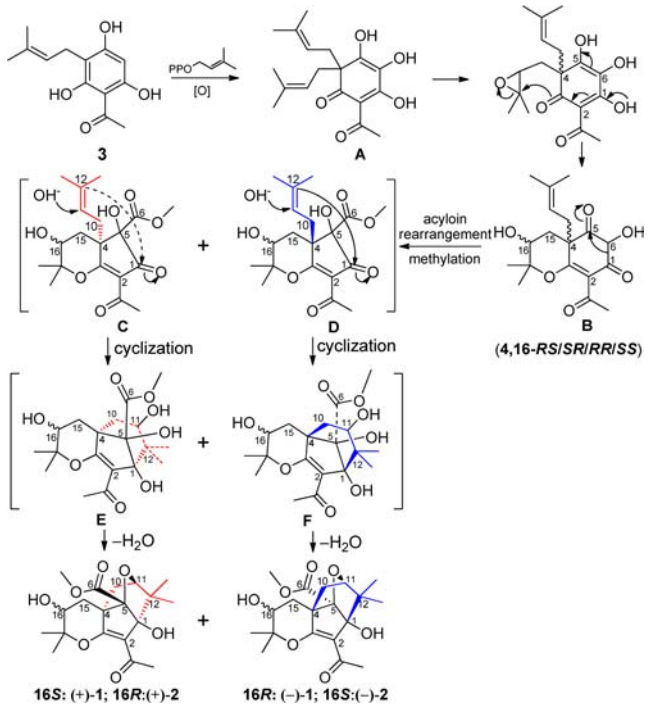
Figure 4. X-ray structures of (−)-melicolone A [(−)-**1**] and (+)-melicolone B [(+)-**2**].

of (−)-**1** was finally determined to be 1*R*, 4*R*, 5*R*, 11*S*, and 16*R* by Flack absolute structure parameter 0.03(5). Correspondingly, the absolute configuration of its enantiomer (+)-**1** was defined as 1*S*, 4*S*, 5*S*, 11*R*, and 16*S*.

Melicolone B (**2**)[11] was obtained as a colorless prism. The molecular formula of **2** was found to be the same as that of **1** ($C_{19}H_{26}O_7$), as deduced from HR-ESI-MS (*m*/*z* 389.1573 [M + Na]$^+$, calcd for $C_{19}H_{26}NaO_7$, 389.1571). The ^{1}H and ^{13}C NMR spectra of **2** recorded in CD_3OD were similar to those of **1**, except for obvious differences in H-16 [δ_H 3.65 (dd, J = 12.2, 4.5 Hz) for **1**; δ_H 3.77 (dd, J = 4.9, 3.4 Hz) for **2**] and the carbon shifts of C-4, C-5, C-10, and CH_3-18 (Table 1). Detailed analysis of its HSQC and HMBC correlations (SI, Figure S1) revealed that compound **2** had the same plane structure as that of **1**. The full assignments of protons and carbons were achieved by the interpretation of the 2D NMR (Table 1). In the ROESY spectrum of **2** recorded in DMSO-d_6 (Figure 3). The correlations of H-16/CH_3-18, H-16/Hβ-15, CH_3-18/Hβ-15, 16-OH/Hβ-10, and Hα-15/Hβ-10 allowed the assignments of H-16, CH_3-18, and Hβ-15 as β-orientation of the molecule and 16-OH as α-direction, which was further confirmed by the small coupling constant between H-16 and Hβ-15 ($J_{15\beta,16}$ = 3.4 Hz).[12] Other ROESY correlations were identical with those in **1**, suggesting that **1** and **2** had the same relative configuration in the 9-oxatricyclo[3.2.1.1^{3,8}]nonane core. Accordingly, **2** was concluded to be an epimer differing only in the stereochemistry at C-16 to **1** (Figure 3). Similarly, **2** was also a racemic mixture. Further chiral HPLC purification yielded (+)-**2** and (−)-**2** (1:1, SI, Figure S31), which displayed opposite CD Cotton effects (SI, Figures S32 and S33) and optical rotations.[11] Single crystals of (+)-**2** were also obtained and subjected to an X-ray diffraction experiment using mirror Cu Kα radiation. The absolute configuration of (+)-**2** was determined as 1*S*, 4*S*, 5*S*, 11*R*, and 16*R* by Flack absolute structure parameter 0.02(6), whereas (−)-**2**, the enantiomer of (+)-**2**, should be 1*R*, 4*R*, 5*R*, 11*S*, and 16*S* configured (Figure 4).

Structurally, compounds **1** and **2** represent the first examples of rearranged nonaromatic acetophenone derivatives with an unprecedented 9-oxatricyclo[3.2.1.1^{3,8}]nonane core. Although there are a total of five stereogenic centers in the scaffolds of **1** and **2**, the rigid oxygen bridge architecture of the molecule in essence restricts the number of stereochemical possibilities to two diastereoisomeric pairs of enantiomers, (±)-melicolones A (**1**) and B (**2**). Biogenetically, **1** and **2** should be derived from the precursor **3**, a normal acetophenone co-occurred in *M. ptelefolia*.[13] An intermediate (**A**) derived from the oxidation and prenylation of **3** was followed by nonface-selective epoxidation[14] of prenyl chain and subsequent cyclization to form **B** with four possible stereoisomers (*RS*, *SR*, *RR*, and *SS*). Then **B** underwent acyloin rearrangement[15] and methylation to produce stereoisomers **C** and **D**. Subsequently, the α- and β-prenyl chain in **C** and **D** intramolecularly cyclized with the ketone group (C-1) from below or above of the molecule plane to generate **E** and **F**, respectively.[16] Finally, **E** and **F** constructed the oxygen bridge by dehydration, forming two enantiomeric pairs of epimers (+)-**1**/**2** and (−)-**1**/**2**, respectively (Scheme 1).

Scheme 1. Plausible Biosynthesis of Compounds 1 and 2



Previous reports demonstrated that the leaves of *M. ptelefolia* possessed antioxidant activity.[17] High glucose increased the generation of reactive oxygen species (ROS), which could cause oxidative stress.[18] Therefore, the protective effects of the isolates against high glucose-induced oxidative stress were investigated using human umbilical vein endothelial cells (HUVECs) according to the reported protocol with modification.[18] After 35 mM glucose exposure, cell viability was markedly decreased to 67.6 ± 3.4% (Model). Then, pretreatment with the enantiomers (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** at 5 μM significantly inhibited cell damage and restored cell survival to 79.2 ± 2.3%, 85.5 ± 4.3%, 102.4 ± 3.2%, and 98.7 ± 3.8%, respectively (Figure 5), using resveratrol as a positive control[19] (cell viability of 76.9 ± 4.5% at

dx.doi.org/10.1021/ol5033738 | *Org. Lett.* 2015, 17, 146−149

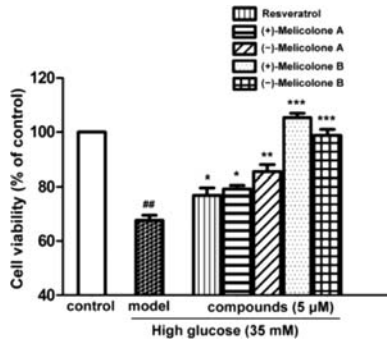


Figure 5. Effects of (±)-melicolones A and B on cell viability. The data were means ± SD expressed as percentage of control value. $^{\#\#}p < 0.01$ vs control group, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs high glucose group.

5 μM). The obtained results indicated the isolates could help prevent diabetic endothelial dysfunction and related complications.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, full spectroscopic data (NMR, MS, UV, IR, and CD) of compounds **1** and **2**, and crystallographic data files (CIF) for (−)-**1** and (+)-**2** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: luojg99@163.com.
*E-mail: cpu_lykong@126.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the New Century Excellent Talents in University (NCET-12-09-77), the Program for Changjiang Scholars and Innovative Research Team in University (IRT1193), and Huahai Graduate Innovation Fund (CX13B-007HH).

■ REFERENCES

(1) Zhang, D. X.; Hartley, T. G. *Flora of China (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, China, 2008; Vol. *11*, pp 70−73.
(2) Johnson, A. J.; Kumar, R. A.; Rasheed, S. A.; Chandrika, S. P.; Chandrasekhar, A.; Baby, S.; Subramoniam, A. *J. Ethnopharmacol.* **2010**, *130*, 267−271.
(3) (a) Sultana, N.; Hartley, T. G.; Waterman, P. G. *Phytochemistry* **1999**, *50*, 1249−1253. (b) Chen, I. S.; Chen, H. F.; Cheng, M. J.; Chang, Y. L.; Teng, C. M.; Tsutomu, I.; Chen, J. J.; Tsai, I. L. *J. Nat. Prod.* **2001**, *64*, 1143−1147. (c) Chen, J. J.; Cho, J. Y.; Hwang, T. L.; Chen, I. S. *J. Nat. Prod.* **2008**, *71*, 71−75. (d) Li, G. L.; Zhu, D. Y. *J. Nat. Prod.* **1998**, *61*, 390−391.
(4) Adsersen, A.; Smitt, U. W.; Simonsen, H. T.; Christensen, S. B.; Jaroszewski, J. W. *Biochem. Syst. Ecol.* **2007**, *35*, 447−453.
(5) Chan, J. A.; Shultis, E. A.; Carr, S. A.; DeBrosse, C. W.; Eggleston, D. S.; Francis, T. A.; Hyland, L. J.; Johnson, W. P.; Killmer, L. B.; Staiger, D. B.; Westley, J. W. *J. Org. Chem.* **1989**, *54*, 2098−2103.
(6) Simonsen, H. T. *Phytochem. Lett.* **2012**, *5*, 371−375.
(7) Li, G. L.; Zhu, D. Y.; Pandey, R. K. *Phytochemical and Biological Studies on Evodia lepta*; Ho, C., Ed.; ACS Symposium Series 859; American Chemical Society: Washington, DC, 2003.
(8) Li, S. G.; Tian, H. Y.; Ye, W. C.; Jiang, R. W. *Biochem. Syst. Ecol.* **2011**, *39*, 64−67.
(9) Melicolone A (**1**): colorless prism (CH_3OH/H_2O); UV (CH_3OH) λ_{max} (log ε) 197 (2.02), 277 (8.80) nm; IR (KBr) ν_{max} 3463, 2989, 2971, 1734, 1618, 1620, 1415, 1386, 1197 cm^{-1}; HR-ESI-MS *m*/*z* 367.1752 $[M + H]^+$ (calcd for $C_{19}H_{27}O_7$, 367.1751). Compound **1** was further separated into enantiomers (−)-**1** (4.8 mg, t_R 15.5 min) and (+)-**1** (4.7 mg, t_R 17.5 min) by chiral AD-H column (*n*-hexane/isopropanol, 80:20, *v*/*v*; flow rate, 2.3 mL/min). Compound (−)-**1**: colorless prism, mp 193−195 °C; $[\alpha]^{25}_D$ −30.8 (*c* 0.13, CH_3OH). Compound (+)-**1**: colorless prism, mp 193−195 °C, $[\alpha]^{25}_D$ +32.8 (*c* 0.11, CH_3OH).
(10) Zhang, H.; Tao, L.; Fu, W. W.; Liang, S.; Yang, Y. F.; Yuan, Q. H.; Yang, D. J.; Lu, A. P.; Xu, H. X. *J. Nat. Prod.* **2014**, *77*, 1037−1046.
(11) Melicolone B (**2**): colorless prism (CH_3OH/H_2O); UV (CH_3OH) λ_{max} (log ε) 197 (2.00), 279 (9.89) nm; IR (KBr) ν_{max} 3461, 2987, 2969, 1733, 1615, 1439, 1413, 1384, 1195 cm^{-1}; HR-ESI-MS *m*/*z* 389.1573 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_7$, 389.1571). Compound **2** was further separated into enantiomers (+)-**2** (4.6 mg, t_R 18.0 min) and (−)-**2** (4.0 mg, t_R 19.5 min) by chiral AD-H column (*n*-hexane/isopropanol, 80:20, *v*/*v*; flow rate, 2.3 mL/min). Compound (+)-**2**: colorless prism, mp 233−235 °C; $[\alpha]^{25}_D$ +21.0 (*c* 0.10, CH_3OH). Compound (−)-**2**: colorless prism, mp 233−235 °C; $[\alpha]^{25}_D$ −18.2 (*c* 0.11, CH_3OH).
(12) Han, M. L.; Zhang, H.; Yang, S. P.; Yue, J. M. *Org. Lett.* **2012**, *14*, 486−489.
(13) Abas, F.; Shaari, K.; Israf, D. A.; Syafri, S.; Zainal, Z.; Lajis, N. H. *J. Food Compos. Anal.* **2010**, *23*, 107−112.
(14) Lindsey, C. C.; Gomez-Diaz, C.; Villalba, J. M.; Pettus, T. R. R. *Tetrahedron* **2002**, *58*, 4559−4565.
(15) (a) Lavie, D.; Szinai, S. *J. Am. Chem. Soc.* **1958**, *80*, 707−710. (b) Majerski, Z.; Sarac Arneri, R.; Skare, D.; Loncar, B. *Synthesis* **1980**, *1*, 74−75.
(16) Tian, W. J.; Yu, Y.; Yao, X. J.; Chen, H. F.; Dai, Y.; Zhang, X. K.; Yao, X. S. *Org. Lett.* **2014**, *16*, 3448−3451.
(17) Abas, F.; Lajis, N. H.; Israf, D. A.; Khozirah, S.; Kalsom, Y. U. *Food Chem.* **2006**, *95*, 566−573.
(18) Cao, G.; Cai, H.; Cai, B. C.; Tu, S. C. *Food Chem.* **2013**, *140*, 273−279.
(19) Yun, J. M.; Chien, A.; Jialal, I.; Devaraj, S. *J. Nutr. Biochem.* **2012**, *23*, 699−705.