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# Synthesis of (±)-cyclic dehypoxanthine futalosine, the biosynthetic intermediate in an alternative biosynthetic pathway for menaquinones

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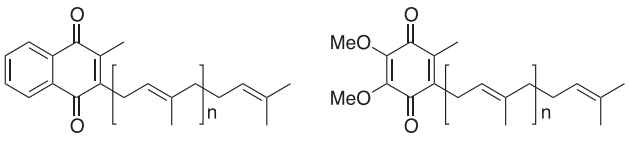
ABSTRACT

The first synthesis of (±)-cyclic dehypoxanthine futalosine (cyclic DHFL), a biosynthetic intermediate in the futalosine pathway for menaquinones operating in microorganisms, has been achieved. Efficient growth of the *Streptomyces coelicolor* mutant, which lacks the cyclic DHFL synthetase gene (*mqnC* gene) was observed in the presence of synthetic (±)-cyclic DHFL.



Many bacteria use menaquinone (MK) as carriers of electrons in their electron transport chains.[1,2] *Escherichia coli* uses ubiquinone (Q) under aerobic conditions, but MK under anaerobic conditions (Fig. 1). On the other hand, *Bacillus subtilis* uses only MK as a lipid-soluble molecule that shuttles electrons between membrane-bound protein complexes. In mammalian cells, MK plays multiple roles in the activation of a family of proteins involved in blood coagulation[3] and bone metabolism.[4] Thus, MK is a critical molecule for survival of both microorganisms and mammals and is classified as a vitamin.

The biosynthesis of MK has been extensively studied in *E. coli*. MK is derived from chorismic acid (or its anionic form, chorismate) by a series of transformations utilizing eight enzymes, *MenA* to *MenH*, and generating various intermediates, including *o*-succinylbenzoic acid and 1,4-dihydroxy-2-naphthoic acid (Scheme 1).[2] However, Borodina et al. reported that the whole-genome analysis of *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis* revealed the absence of the known menaquinone biosynthetic pathway genes, *menB* to *menF*.[5] Furthermore, some pathogenic microorganisms, such as *Helicobacter pylori* and *Campylobacter jejuni*, which are known to cause gastric carcinoma and diarrhea, respectively, have also been reported to lack *men* gene homologs, even though they synthesize MKs.[6,7] These results suggest that there is a novel pathway for the biosynthesis of MK in some microorganisms. Recently, Seto, Dairi, et al. reported evidence for the presence of a new biosynthetic pathway acquired by extensive tracer experiments with $^{13}$C-labeled glucose.[8] They succeeded in outlining the pathway by a combination of bioinformatics and biochemical experiments (Scheme 1).[9] Compared to the classical one, the new pathway involves unique transformations from chorismate, the common starting material. Namely, futalosine (**1**), which was previously isolated from the fermentation broth of *Streptomyces* sp. MK359-NF1,[10] was derived from chorismic acid and then was converted to dehypoxanthine futalosine (DHFL, **2**) by the release of hypoxanthine. Six-membered ring formation gave cyclic dehypoxanthine futalosine (cyclic DHFL, **3**), followed by the loss of a three-carbon unit to afford 1,4-dihydroxy-6-naphthoic acid (DHNA, **4**). It should be noted that this compound is very similar to an intermediate of the classical pathway in that they are




n = 5-8 : Menaquinone (MK)  n = 5-9 : Ubiquinone (Q)

**Figure 1.** Structures of menaquinones and ubiquinones.

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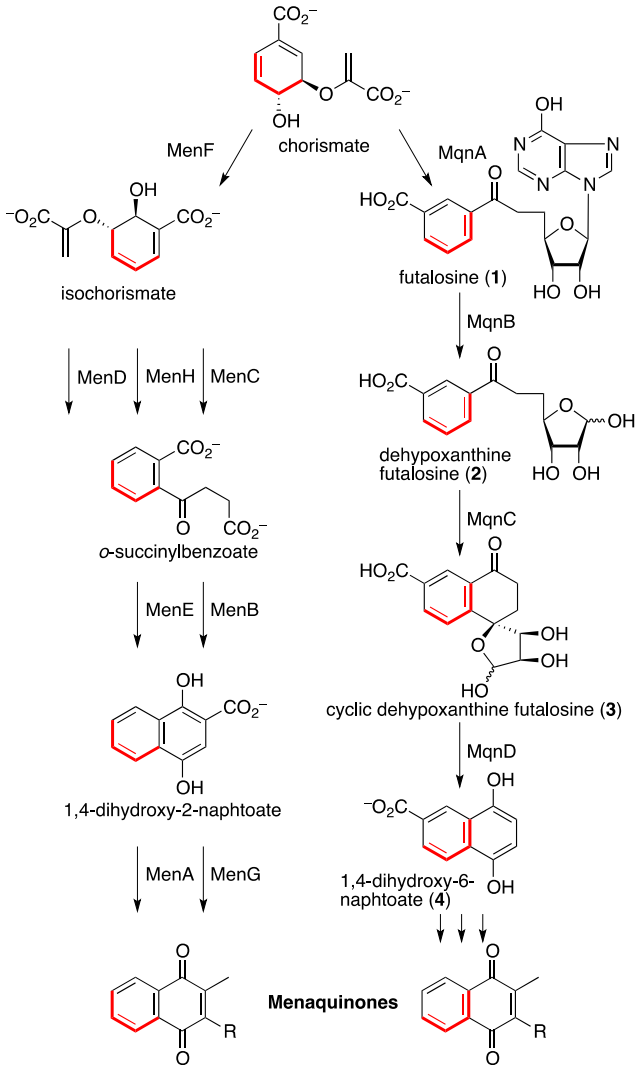
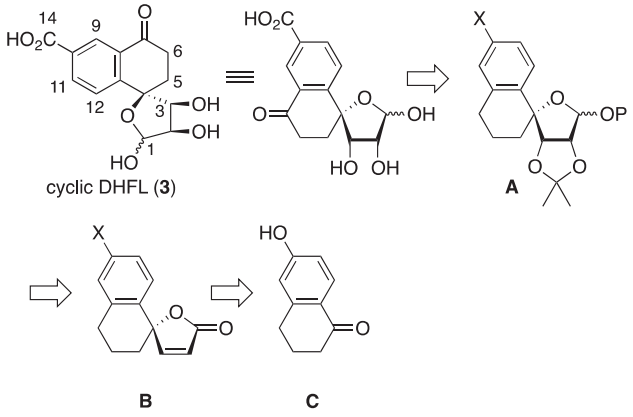



**Scheme 1.** Menaquinone biosynthetic pathways. The four carbon units highlighted by red lines were derived from erythrose-4-phosphate. The units were located at different positions in the final menaquinones.

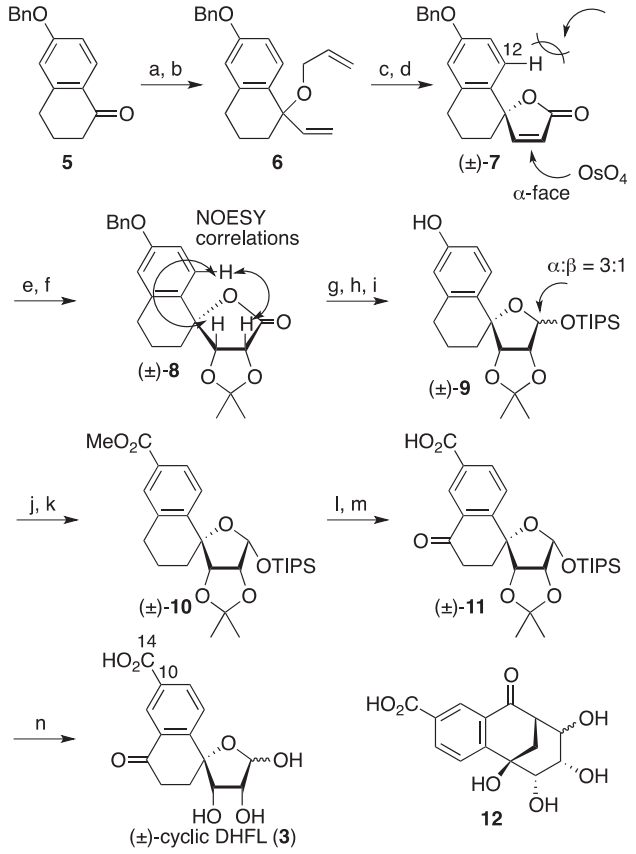
positional isomers. Dairi et al. named this new pathway the futalosine pathway.[9] Because mammals and some beneficial intestinal bacteria, such as lactobacilli, lack this futalosine pathway, it would be a promising target for the development of chemotherapeutics against *H. pylori* and *C. jejuni*.[11] Although enzymes for the conversion of DHFL to cyclic DHFL and cyclic DHFL to DHNA have been identified, detailed mechanisms of these unique transformations have not been disclosed. Recently, Yokoyama et al. reported the crystal structure of MqnD (TTHA1568) from *Thermus thermophilus* HB8 as a co-crystal with L-(+)-tartaric acid.[12] They proposed a binding site for the target compound of MqnD and a putative reaction mechanism for the transformation of cyclic DHFL to DHNA (vide infra). However, further studies are necessary to establish a detailed reaction mechanism for the development of effective chemotherapeutics. Although there is an urgent need to provide samples of cyclic DHFL for this purpose, it is very difficult to isolate cyclic DHFL from the bacterial culture broth because of its extreme scarcity and instability.[9] We became interested in synthesizing cyclic DHFL to confirm the structure of the natural product and provide samples for bioorganic experiments. Very recently, Dairi et al. and Tanner et al. independently reported an efficient synthesis of futalosine and aminodeoxyfutalosine, which has an adenine moiety instead of the hypoxanthine in **1** and confirmed the latter to be an intermediate in the futalosine pathway in *H. pylori* and *C. jejuni*. These methods enabled us to provide a sufficient amount of **1** and its derivatives for studies on menaquinone biosynthesis and the development of inhibition assays for MqnB.[13] This communication describes the synthesis and biological activity of (±)-cyclic DHFL.

Scheme 2 shows our retrosynthetic analysis of (±)-cyclic DHFL. Because cyclic DHFL has diverse functional groups which, in combination, include multiple oxidation states (i.e., hydroxyl, keto, and carboxylic groups and lactol) in a relatively small molecule, the key to the synthesis of **3** is in the order of introduction of the oxygen functional groups. In view of the instability of cyclic DHFL, the carboxyl and keto groups that might cause side reactions should be installed in the later stages of the synthesis. The two hydroxyl groups would be installed by dihydroxylation of the double bond of **B**, and spiro-γ-butenolide (**B**) would be synthesized from commercially available tetralone (**C**).

Scheme 3 summarizes our synthesis of (±)-**3**. According to Carda's protocol,[14] the known tetralone derivative **5**[15] was converted to spiro-γ-butenolide **7**. Namely, ring closing metathesis of diene **6** with Grubbs second-generation catalyst was followed by allylic oxidation to give spiro-γ-butenolide (±)-**7** in good yield. Next, $OsO_4$-catalyzed dihydroxylation of **7** gave the corresponding diol exclusively (>10:1) in moderate yield (ca. 40%). The relative stereochemistry of the major isomer was confirmed by NOESY experiments after derivatization into **8**. This stereoselectivity can be rationalized by the steric effect of H-12 shielding the β-face of the double bond of **7**. Attempts to improve the yield of this transformation were unsuccessful. The best result was 57% yield achieved using a relatively large amount (0.6 equiv) of $OsO_4$ with trimethylamine-*N*-oxide (TMNO) and methanesulfonamide. Lactone **8** was then carefully reduced by LAH at a low temperature to afford the corresponding lactol. Various reducing agents such as DIBAL were not effective in this reaction. Initially, lactol was protected as a simple methyl group, but we found that deprotection of methyl acetal was difficult in the final stage of the synthesis. We therefore selected silyl acetal (TIPS) for the lactol protecting group. Treatment of lactol with TIPSOTf gave the corresponding silyl acetal. The resulting anomeric isomers were separated after the hydrogenolysis of the benzyl group (**9**, $\alpha$:$\beta$ = 3:1). The stereochemistry of the separated major α-isomer was determined by a NOESY experiment on **9**, and the α-isomer was used for the subsequent transformations. The corresponding triflate of **9** was subjected to palladium-catalyzed methoxycarbonylation[16] to afford **10**. The benzylic position of **10** was oxidized with $PhI(OAc)_2$ and TBHP in the presence of $K_2CO_3$,[17] and then the methyl ester was hydrolyzed to give **11**. The deprotection of the acetonide and TIPS groups with-




**Scheme 2.** Retrosynthetic analysis of cyclic DHFL (**3**).

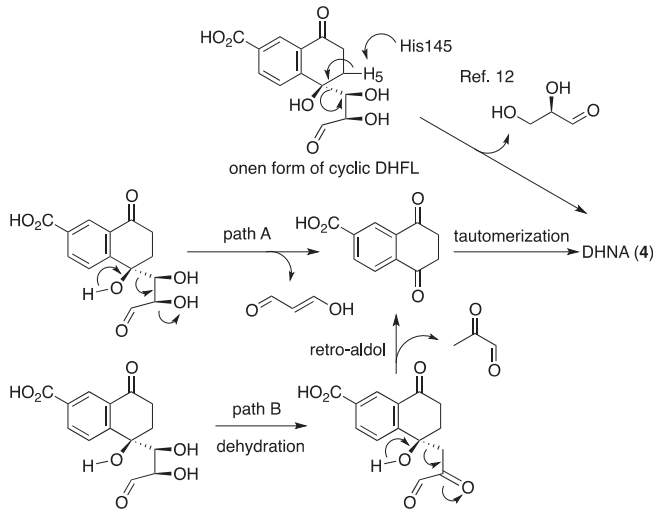



**Scheme 3.** Synthesis of (±)-cyclic DHFL (**3**). Reagents, conditions and yields: (a) vinylmagnesium chloride, THF, −78 °C (94%); (b) NaH, allyl bromide, DMF, 0 °C to rt (86%); (c) Grubbs' second gen. cat., $CH_2Cl_2$ (98%); (d) $CrO_3$, 3,5-DMP, $CH_2Cl_2$ −40 °C (99%); (e) $OsO_4$, TMAO, $MeSO_2NH_2$, *t*-BuOH, acetone, 0 °C (57%); (f) 2,2-dimethoxypropane, PPTS, reflux (73%); (g) $LiAlH_4$, THF, 0 °C (90%); (h) TIPSOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C (quant.); (i) Pd/C, $H_2$, MeOH, EtOAc (73%); (j) $Tf_2O$, pyridine 0 °C (96%); (k) $Pd(Ph_3P)_4$, $Et_3N$, CO, MeOH, DMSO, THF, 60 °C (quant.); (l) $PhI(OAc)_2$, TBHP, $K_2CO_3$, *n*-butyl butanoate −20 °C (66%); (m) LiOH, THF, $H_2O$ (83%); (n) $BiCl_3$, $H_2O$, $CH_3CN$, THF (20%).

out degradation of the resultant cyclic DHFL was very difficult under standard acidic conditions. We obtained **12** as a degradation product of cyclic DHFL, which was produced by an intramolecular aldol reaction under neutral, basic, and strongly acidic conditions. However, we ultimately found that $BiCl_3$-mediated hydrolysis[18] of the acetonide and TIPS groups of **11** gave (±)-cyclic DHFL (**3**). The HPLC retention time of the synthetic **3** was identical to that of natural cyclic DHFL, and the $^1H$ and $^{13}C$ NMR spectra were also in good accordance, except for the $^{13}C$ NMR chemical shifts of C-10 and C-14.[19] The small differences for these chemical shifts can be ascribed to differences in the pH or concentration of the samples.

Next, the biological activity of synthetic (±)-**3** was examined by the same method as described by Dairi et al.[9] Synthetic (±)-**3** was added to an agar plate, and the growth of the *S. coelicolor* mutant, which lacks the cyclic DHFL synthetase gene (*mqnC* gene)[9] was examined. Although, this mutant cannot grow in the absence of MK, efficient growth was observed in the presence of synthetic (±)-**3** (10 μg/ml).

In the final stage of our synthesis, the degradation products of cyclic DHFL (**12**) were obtained during the hydrolysis of the acetonide and lactol protecting groups (Scheme 3). These compounds were produced by an intramolecular aldol reaction. This indicates that the open form of **3** is easily formed in aqueous media. On the basis of this observation, we formulated potential enzymatic reaction mechanisms for the conversion of cyclic DHFL into DHNA as shown in Scheme 4. As described above, Yokoyama et al. sug-




**Scheme 4.** Putative reaction mechanisms for the conversion of cyclic DHFL to DHNA catalyzed by MqnD.

gested a putative mechanism of this reaction based on the deprotonation of H-5 by His-145 of *Tth*MqnD.[12] Indeed His-145 would be essential, but there is a reason to reconsider this mechanism. The precursor of DHNA would be the corresponding 1,4-diketone, because keto–enol tautomerism can easily give a hydroquinone moiety. The 1,4-diketone could be derived from either of the two different pathways (A or B in Scheme 4). Because the resulting side products, propanedial or pyruvaldehyde, are different in the two pathways, an analysis of the byproduct should reveal the enzymatic reaction mechanism.

In summary, we achieved the first synthesis of (±)-cyclic DHFL. Synthetic (±)-**3** enabled the survival of the mutant that lacks MqnC. Improvement of the synthesis and the establishment of the enzymatic reaction mechanisms using the synthetic sample are currently underway in our laboratory.

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## References and notes

1. Bentley, R.; Maganathan, R. *Microbiol. Rev.* **1982**, *46*, 241–280.
2. Maganathan, R. *Vitam. Horm.* **2001**, *61*, 173–218.
3. Dahlbäck, B.; Villoutreix, B. O. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 1311–1320.
4. Adams, J.; Pepping, J. *Am. J. Health Syst. Pharm.* **2005**, *62*, 1311–1320.
5. Borodina, I.; Krabben, P.; Nielsen, J. *Genome. Res.* **2005**, *15*, 802–827.
6. Marcelli, S. W.; Chang, H.-T.; Chapman, T.; Chalk, P. A.; Miles, R. J.; Poole, R. K. *FEMS Microbiol. Lett.* **1996**, *138*, 59–64.
7. Moss, C. W.; Lambert-Fair, M. A.; Nicholson, M. A.; Guerrant, G. O. *J. Clin. Microbiol.* **1990**, *28*, 395–397.
8. Seto, H.; Jinnai, Y.; Hiratsuka, T.; Fukawa, M.; Furihata, K.; Itoh, N.; Dairi, T. *J. Am. Chem. Soc.* **2008**, *130*, 5614–5615.
9. Hiratsuka, T.; Furihata, K.; Ishikawa, J.; Yamashita, H.; Itoh, N.; Seto, H.; Dairi, T. *Science* **2008**, *321*, 1670–1673.
10. Hosokawa, N.; Naganawa, H.; Kasahara, T.; Hattori, S.; Hamada, M.; Takeuchi, T.; Yamamoto, S.; Tsuchiya, K. S.; Hori, M. *Chem. Pharm. Bull.* **1999**, *47*, 1032–1034.
11. (a) Dairi, T. *J. Antibiot.* **2009**, *62*, 347–352; (b) Kurosu, M.; Begari, E. *Molecules* **2010**, *15*, 1531–1553.
12. Arai, R.; Murayama, K.; Uchikubo-Kamo, T.; Nishimoto, M.; Toyama, M.; Kuramitsu, S.; Terada, T.; Shirouzu, M.; Yokoyama, S. *J. Struct. Biol.* **2009**, *168*, 575–581.
13. (a) Arakawa, C.; Kuratsu, M.; Furihata, K.; Hiratsuka, T.; Itoh, N.; Seto, H.; Dairi, T. *Antimicrob. Agents Chemother.* **2011**, *55*, 913–916; (b) Li, X.; Tanner, E.

*Tetrahedron Lett.* **2010**, *51*, 6463–6465; (c) Li, X.; Apel, D.; Gaynor, E. C.; Tanner, M. E. *J. Biol. Chem.* **2011**, *286*, 19392–19398.
14. Carda, M.; Castillo, E.; Rodríguez, S.; Uriel, S.; Marco, J. A. *Synlett* **1999**, 1639–1641.
15. Durden, J. A., Jr. *J. Agric. Food Chem.* **1971**, *19*, 432–440.
16. Cacchi, S.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1985**, *26*, 1109–1112.
17. Zhao, Y.; Yeung, Y.-Y. *Org. Lett.* **2010**, *12*, 2128–2131.
18. Swamy, N. R.; Venkateswarlu, Y. *Tetrahedron Lett.* **2002**, *43*, 7549–7552.
19. Properties of the selected synthetic compounds (**8**): $^{1}$H NMR (400 MHz, $CDCl_3$):$\delta$ 1.39 (s, 3H), 1.54 (s, 3H), 1.95–2.05 (m, 2H), 2.10 (m, 1H), 2.46 (m, 1H), 2.76–2.92 (m, 2H), 4.63 (d, $J$ = 5.5 Hz, 1H), 5.05 (brs, 2H), 5.10 (d, $J$ = 5.5 Hz, 1H), 6.80 (d, $J$ = 10.5 Hz, 1H), 6.80 (d, $J$ = 10.5 Hz, 1H), 6.82 (s, 1H), 7.30–7.45 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$):$\delta$ 19.2, 26.0, 26.8, 29.1, 29.8, 69.9, 76.3, 81.8, 86.1, 113.4, 113.9, 115.2, 125.7, 127.3, 128.0, 128.5, 128.6, 136.5, 139.0, 158.6, 174.0. Anal. Calcd for $C_{23}H_{24}O_5$: C, 72.01, H, 6.36. Found: C, 72.08, H, 6.49. (**11**): $^{1}$H NMR (400 MHz, $CDCl_3$):$\delta$ 1.00–1.12 (m, 21H), 1.28 (brs, 1H), 1.35 (s, 3H), 1.60 (s, 3H), 2.46 (m, 2H), 2.69 (m, 2H), 4.81 (brs, 2H), 5.65 (s, 1H), 8.07 (d, $J$ = 8.2 Hz, 1H), 8.21 (dd, $J$ = 1.8, 8.2 Hz, 1H), 8.61 (d, $J$ = 1.8 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$):$\delta$ 11.8, 17.66, 17.69, 25.1, 26.6, 31.4, 35.3, 85.8, 86.5, 89.3, 104.3, 113.4, 128.2, 129.1, 129.3, 131.4, 134.4, 151.4, 169.5, 196.7; HR-MS-ESI ($m/z$): $[M+Na]^+$ calcd for $C_{26}H_{38}NaO_7Si$, 513.2285; found, 513.2302. Synthetic (±)-cyclic DHFL [anomeric mixture (ca. $\beta$:$\alpha$ = 6:4]: colorless powder; $^{1}$H NMR (400 MHz, $CD_3OD$):$\delta$ 2.18 (ddd, $J$ = 5.0, 13.3, 13.3 Hz, 0.4H), 2.27 (ddd, $J$ = 5.0, 13.3, 13.3 Hz, 0.6H), 2.66 (m, 0.4H), 2.70 (m, 0.6H), 2.83 (m, 0.6H), 2.86 (m, 0.4H), 3.07 (ddd, $J$ = 5.0, 13.7, 18.8 Hz, 0.6H), 3.15 (ddd, $J$ = 5.2, 13.5, 18.5 Hz, 0.4H), 3.99 (d, $J$ = 5.3 Hz, 0.6H), 4.06 (dd, $J$ = 3.3, 4.5 Hz, 0.4H), 4.18 (d, $J$ = 4.5 Hz, 0.4H), 4.32 (dd, $J$ = 4.6, 5.3 Hz, 0.6H), 5.43 (d, $J$ = 3.3 Hz, 0.4H), 5.56 (d, $J$ = 4.6 Hz, 0.6H), 7.55 (d, $J$ = 7.6 Hz, 0.6H), 8.07 (d, $J$ = 8.0 Hz, 0.4H), 8.21 (d, $J$ = 7.6 Hz, 0.6H), 8.22 (d, $J$ = 8.0 Hz, 0.4H), 8.54 (s, 0.4H), 8.56 (s, 0.6H); $^{13}$C NMR (100 MHz, $CD_3OD$):$\delta$ 32.7 (C-5, $\alpha$), 33.4 (C-5, $\beta$), 36.4 (C-6, $\alpha$), 36.6 (C-6, $\beta$), 71.7 (C-2, $\beta$), 77.7 (C-2, $\alpha$), 77.8 (C-3, $\beta$), 79.7 (C-3, $\alpha$), 84.8 (C-4, $\alpha$), 86.0 (C-4, $\beta$), 98.5 (C-1, $\beta$), 102.7 (C-1, $\alpha$), 127.0 (C-12, $\beta$), 128.8 (C-12, $\alpha$), 129.0 (C-9, $\alpha$), 129.5 (C-9, $\beta$), 132.3 (C-8, $\beta$), 132.8 (C-8, $\alpha$), 133.9 and 134.1 (C-10, $\alpha$ and $\beta$ lit.[9] $\delta$ 131.6 and 131.9), 135.4 (C-11, $\alpha$), 135.4 (C-11, $\beta$), 153.1 (C-13, $\beta$), 153.9 (C-13, $\alpha$), 167.3 and 169.0 (C-14, $\alpha$ and $\beta$, lit.[9] $\delta$ 168.8 and 168.8), 198.9 (C-7, $\beta$), 199.5 (C-7, $\alpha$); HR-MS-ESI ($m/z$): $[M-H]^+$ calcd for $C_{14}H_{13}O_7$, 293.06613; found, 293.06671. $^{1}$H and $^{13}$C NMR spectra are in good accordance with those of the reported natural product.