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Synthesis of the proposed structure of plakevulin A: revised structure of plakevulin A

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Abstract—A total synthesis of the proposed structure of plakevulin A was accomplished. However, the NMR spectral data of the synthetic plakevulin A were not identical of those of the reported compound. We next converted the synthetic plakevulin A into 1-dihydrountenone A. The ^{1}H and ^{13}C NMR spectral data of 1-dihydrountenone A were identical with those of reported plakevulin A except for the peaks derived from levulinic acid. Thus, we repurified sample of the natural product and confirmed that the natural sample contained 1-dihydrountenone A and levulinic acid in the ratio of one to one. We also found that not plakevulin A but 1-dihydountenone A possessed the inhibitory activity against mammalian DNA polymerases α and β.

During the course of our continuing exploration for a specific inhibitor of DNA polymerase,[1] we have recently reported that untenone A (**1**)[2] inhibits mammalian DNA polymerase α and β, and human terminal deoxynucleotidyl transferase (TdT).[3] Untenone A is structurally related to plakevulin A (**2**) isolated by one of our groups (Hokkaido University group),[4] and we started the synthesis of plakevulin A (Fig. 1). In this paper we describe the synthesis of the proposed structure for plakevulin A (**2**) and revision of the structure. Inhibitory activities against DNA polymerases are also presented.

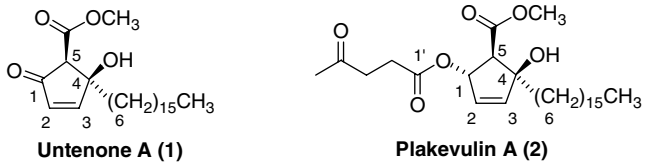

Figure 1. Structure of untenone A (**1**) and proposed structure of plakevulin A (**2**).

Our synthetic approach toward plakevulin A is based on the proposed biosynthetic pathway.[4] Thus, plakevulin A would be synthesized by the reduction of untenone A followed by the esterification of the resulting alcohol with levulinic acid. This approach is considered to be quite useful for systematic study of structure–activity relationships of this class of compounds.

Treatment of (±)-**3**, the key intermediate for the synthesis of untenone A,[2c,3,5] with TMSOTf and *i*Pr_2NEt in CH_2Cl_2 gave the TMS ether **4**. Methoxycarbonylation of (±)-**4** with LDA and $NCCO_2CH_3$[6] gave β-ketoesters **5** in 63% yield as a 5:1 inseparable diastereomeric mixture. Reduction of (±)-**5** with DIBAL-H (2.0 equiv) in CH_2Cl_2 gave (±)-**6** in 49% yield as an identified single isomer. The stereochemistry of **6** was established as shown in Scheme 1 by assigning its NOESY spectrum. NOEs between H-1 and H-5 indicated the *syn* relation

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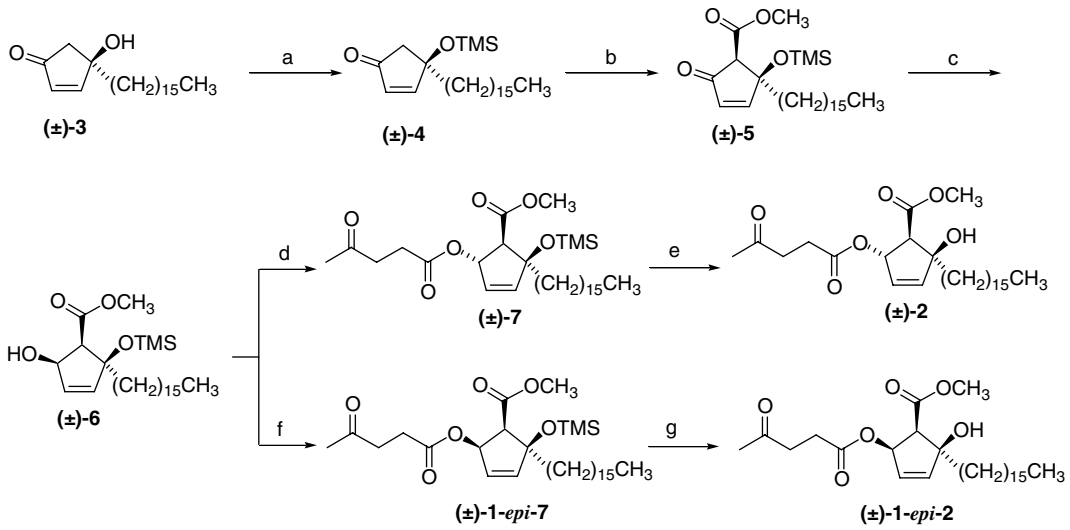


Scheme 1. Synthesis of the proposed structure of (±)-plakevulin A (**2**) and (±)-1-*epi*-**2**. Reagents and conditions: (a) TMSOFTf, *i*Pr_2NEt, CH_2Cl_2, 0 °C, 90%; (b) LDA, THF/HMPA, then $NCCO_2Me$, −42 °C, 63% (dr = 5:1); (c) DIBAL-H, CH_2Cl_2, −78 °C, 49%; (d) levulinic acid, DIAD, PPh_3, toluene, 52%; (e) TBAF, THF, 0 °C, 94%; (f) levulinic acid, EDCI, DMAP, 1,4-dioxane, 83%; (g) TBAF, THF, 0 °C, 49%.

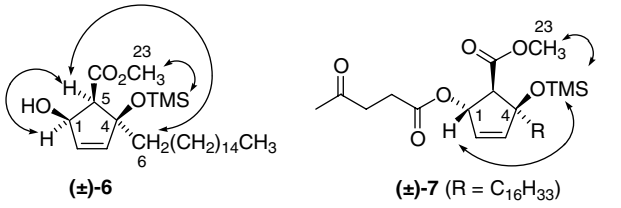


Figure 2. NOESY correlations expressed with arrow lines in (±)-**6** and (±)-**7**.

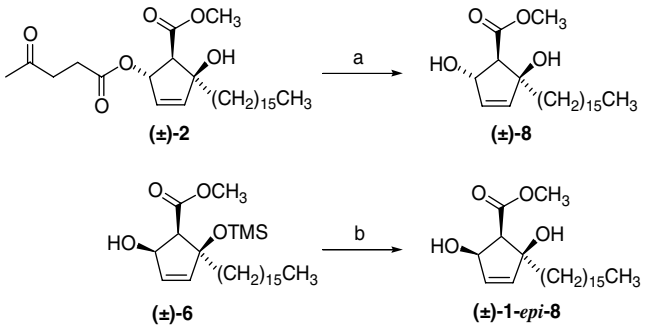
for H-1/H-5. The *anti* relation for 4-OTMS/H-5 was determined by NOEs between H-5 and H-6, and NOEs between H-23 and 4-OTMS (Fig. 2).

We next carried out the esterification of the alcohol (±)-**6**. The esterification was attempted by both inversion and retention of the configuration at C-1. Thus, (±)-**7** was obtained in 52% yield by the treatment of **6** with levulinic acid, DIAD, and PPh_3 in toluene.[7] The presence of NOEs between 4-OTMS and each of H-1 and H-23 indicated that the configuration of **7** was 1*S**,4*S**,5*R** (Fig. 2).

On the other hand, the esterification of **6** with levulinic acid and EDCI in the presence of DMAP afforded the epimeric (±)-1-*epi*-**7** in 83% yield.

Finally, TMS ethers of (±)-**7** and (±)-1-*epi* -**7** were desilylated with TBAF in THF to obtain (±)-**2** and (±)-1-*epi*-**2**, respectively.[8] However, the ^1H and ^{13}C spectral data of the synthetic **2** and 1-*epi*-**2** were different from those of the natural product as follows. In the ^{13}C NMR data of synthetic compound **2**, carbon signals due to C-1, C-2, C-3, C-5, and C-1′ appeared at δ_C 80.9, 131.5, 139.8, 57.7, and 172.4, respectively, while the corresponding carbons for the natural specimen were resonated at δ_C 78.2, 135.7, 136.9, 60.6, and 177.6, respectively. The proton signal (δ_H 5.34) of C-1 for the natural specimen was in higher field than that (δ_H 6.04) of the synthetic compound **2**. These observations indicated that C-1 of natural plakevulin A may be not the levulinyl ester but a free hydroxyl group. Based on these considerations, we next conducted the hydrolysis of the levulinyl ester moiety of (±)-**2**. Treatment of (±)-**2** with hydrazine in pyridine/AcOH[9] afforded the alcohol (±)-**8**[8] in 92% yield. The desilylation of alcohol (±)-**6** gave the epimeric (±)-1-*epi*-**8**[8] (Scheme 2). The ^1H and ^{13}C spectral data of synthetic (±)-**8** were identical with those of natural plakevulin A except for the peaks derived from levulinic acid. Therefore, the sample of natural plakevulin A could be estimated to be a 1:1 mixture of 1-dihydro form (**8**) of untenone A (**1**) and levulinic acid. On the other hand, the fraction (1.5 mg) previously reported as plakevulin A was subjected to an amino SiO_2 column chromatography ($CHCl_3$) to afford pure compound **8** as a white solid.[10] The previous incorrect conclusion seems to be attributed to the following misassignments of spectroscopic data: (1) the molecular formula of plakevulin A was determined by the molecular ion peak observed in FDMS (*m*/*z* 480.3427, M^+, Δ −2.4 mmu), although the peak intensity was weak, and (2) a weak peak was assigned for HMBC correlation between δ_H 5.34 (H-1) and δ_C 177.6 (ester carbonyl carbon of levulinic acid). These observations indicated that the levulinic acid ester previously proposed as plakevulin A existed as a trace constituent in the nearly 1:1 mixture of 1-dihydrountenone A and levulinic acid, although it might be an artifact.

Synthetic derivatives were tested for an enzymatic inhibition assay of mammalian DNA polymerases α (pol. α) and β (pol. β). The method was previously described by Mizushina et al.,[3] and the results are shown in Table 1. We found that synthetic (±)-1-*epi*-**2** and (±)-**8** moderately inhibited pol. α (IC_{50} 61 μM for (±)-1-*epi*-**2**; IC_{50} 66 μM for (±)-**8**) and weakly inhibit pol. β (IC_{50} 132 μM for (±)-1-*epi*-**2**; IC_{50} 179 μM for (±)-**8**). Interestingly, synthetic (±)-**2** did not influence pol. α and pol. β.



Scheme 2. Synthesis of the alcohol (±)-**8** and (±)-1-*epi*-**8**. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, pyridine/AcOH, 92%; (b) TBAF, THF, 64%.

Table 1. IC_{50} values of enzymatic inhibition against pol. α and pol. β

Compounds	IC_{50} (μM)	
	Pol. α	Pol. β
(±)-**2**	>200	>200
(±)-1-*epi*-**2**	61	132
(±)-**8**	66	179
(±)-1-*epi*-**8**	>200	>200

In conclusion, the structure of plakevulin A was revised to be the 1-dihydro form (**8**) of untenone A (**1**) by both a synthetic approach and repurification of the natural product. We also examined the structure–activity relationships of this class of compounds on pol. α and β. We found that not the proposed structure of plakevulin A but **8** possessed the inhibitory activity against mammalian pol. α and β. Synthetic materials presented here are limited to racemic compounds, and the synthesis and evaluation of biological activities of the enantiomerically pure material will be reported in due course.

Acknowledgements

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8. Compound (±)-**2**: white soild, mp 45–47 °C. IR (film) 3482, 3018, 2925, 2854, 1739, 1462, 1438, 1363, 1265, 1201, 1160, 1020, 759, 667 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.8 Hz, H-21), 1.26–1.34 (28H, br m, H-7–H-20), 1.80 (2H, m, H-6), 2.18 (3H, s, H-5′), 2.30 (1H, s, OH), 2.56 (2H, m, H-2′), 2.75 (2H, m, H-3′), 2.96 (1H, d, *J* = 4.4 Hz, H-5), 3.76 (3H, s, H-23), 5.91 (1H, dd, *J* = 5.4 Hz, 1.5 Hz, H-2), 5.94 (1H, dd, *J* = 5.4 Hz, 0.8 Hz, H-3), 6.04 (1H, ddd, *J* = 4.4 Hz, 1.5 Hz, 0.8 Hz, H-1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.2, 27.9, 29.4, 29.6, 29.6, 29.7 (×2), 29.7, 29.7 (×4), 29.8, 29.9, 31.9, 37.8, 40.8, 52.2, 57.7, 80.9, 85.3, 131.5, 139.8, 171.8, 172.4, 206.3. HRMS calcd for $C_{28}H_{48}O_6Na$ (M+Na^+) 503.3343, found 503.3307. Compound (±)-1-*epi*-**2**: white soild, mp 79–81 °C. IR (film) 3505, 3020, 2926, 2854, 1722, 1519, 1465, 1439, 1408, 1359, 1216, 1158, 1076, 1030, 929, 757, 669 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.7 Hz, H-21), 1.24–1.33 (28H, br m, H-7–H-20), 1.65 (2H, m, H-6), 2.19 (3H, s, H-5′), 2.55 (2H, t, *J* = 6.5 Hz, H-2′), 2.72 (2H, m, H-3′), 3.22 (1H, dd, *J* = 6.9 Hz, 1.0 Hz, H-5), 3.75 (3H, s, H-23), 4.06 (1H, s, OH), 5.82 (1H, br d, *J* = 6.9 Hz, 1.5 Hz, H-1), 5.90 (1H, dd, *J* = 5.6 Hz, 2.1 Hz, H-2), 6.08 (1H, d, *J* = 5.6 Hz, H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.1, 28.0, 29.4, 29.5, 29.6, 29.6, 29.6 (×2), 29.7 (×4), 29.8, 29.9, 31.9, 37.7, 40.4, 52.1, 53.9, 76.8, 83.4, 129.4, 142.2, 171.5, 172.0, 206.3. HRMS calcd for $C_{28}H_{48}O_6Na$ (M+Na^+) 503.3343, found 503.3311. Compound (±)-**8**: white soild, mp 80–82 °C. IR (film) 3445, 3019, 2927, 2855, 1724, 1520, 1465, 1439, 1374, 1041, 928, 759, 669 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.9 Hz, H-21), 1.22–1.37 (28H, br m, H-7–H-20), 1.81 (2H, m, H-6), 2.00 (1H, br s, 1-OH), 2.45 (1H, s, 4-OH), 2.83 (1H, d, *J* = 5.3 Hz, H-5), 3.79 (3H, s, H-23), 5.34 (1H, m, H-1), 5.84 (1H, dd, *J* = 5.7 Hz, 1.5 Hz, H-3), 5.94 (1H, dd, *J* = 5.7 Hz, 1.8 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.5, 29.4, 29.5, 29.6, 29.7 (×2), 29.7 (×5), 29.9, 31.9, 40.6, 52.1, 60.5, 78.2, 84.8, 135.7, 137.0, 172.6. HRMS calcd for $C_{23}H_{42}O_4Na$ (M+Na^+) 405.2975, found 405.2972. (±)-1-*epi*-**8**: IR (film) 3440, 3014, 2925, 2854, 1718, 1464, 1440, 1357, 1214, 1176, 1064, 931, 759, 667 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.8 Hz, H-21), 1.25–1.30 (28H, br m, H-7–H-20), 1.73 (2H, m, H-6), 2.99 (1H, d, *J* = 6.1 Hz, H-5), 3.04 (1H, br d, *J* = 8.0 Hz, 1-OH), 3.50 (1H, s, 4-OH), 3.80 (3H, s, H-23), 4.82 (1H, m, H-1), 6.03 (1H, d, *J* = 5.6 Hz, H-3), 6.09 (1H, dd, *J* = 5.6 Hz, 2.4 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.4, 29.3, 29.5, 29.6, 29.6, 29.6 (×2), 29.7 (×4), 29.9, 31.9, 39.3, 52.0, 55.0, 75.8, 83.9, 134.7, 140.0, 173.0. HRMS calcd for $C_{23}H_{42}O_4Na$ (M+Na^+) 405.2975, found 405.2989.
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10. $[\alpha]_D^{22}$ − 25 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3450 (br), 2921, and 1727 cm^{-1}.