Absolute Configuration of Reveromycin A, an Inhibitor of the Signal Transduction of Epidermal Growth Factor

Makoto Ubukata,*[a] Hiroyuki Koshino,[a] Hiroyuki Osada[a] and Kiyoshi Isono[b]

[a] *The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan*
[b] *Department of Marine Science, School of Marine Science and Technology, Tokai University, Shimizu, Shizuoka 424, Japan*

The absolute configuration of reveromycin A, a new inhibitor of the signal transduction of epidermal growth factor (EGF), is determined on the basis of chemical degradation and spectroscopic evidence.

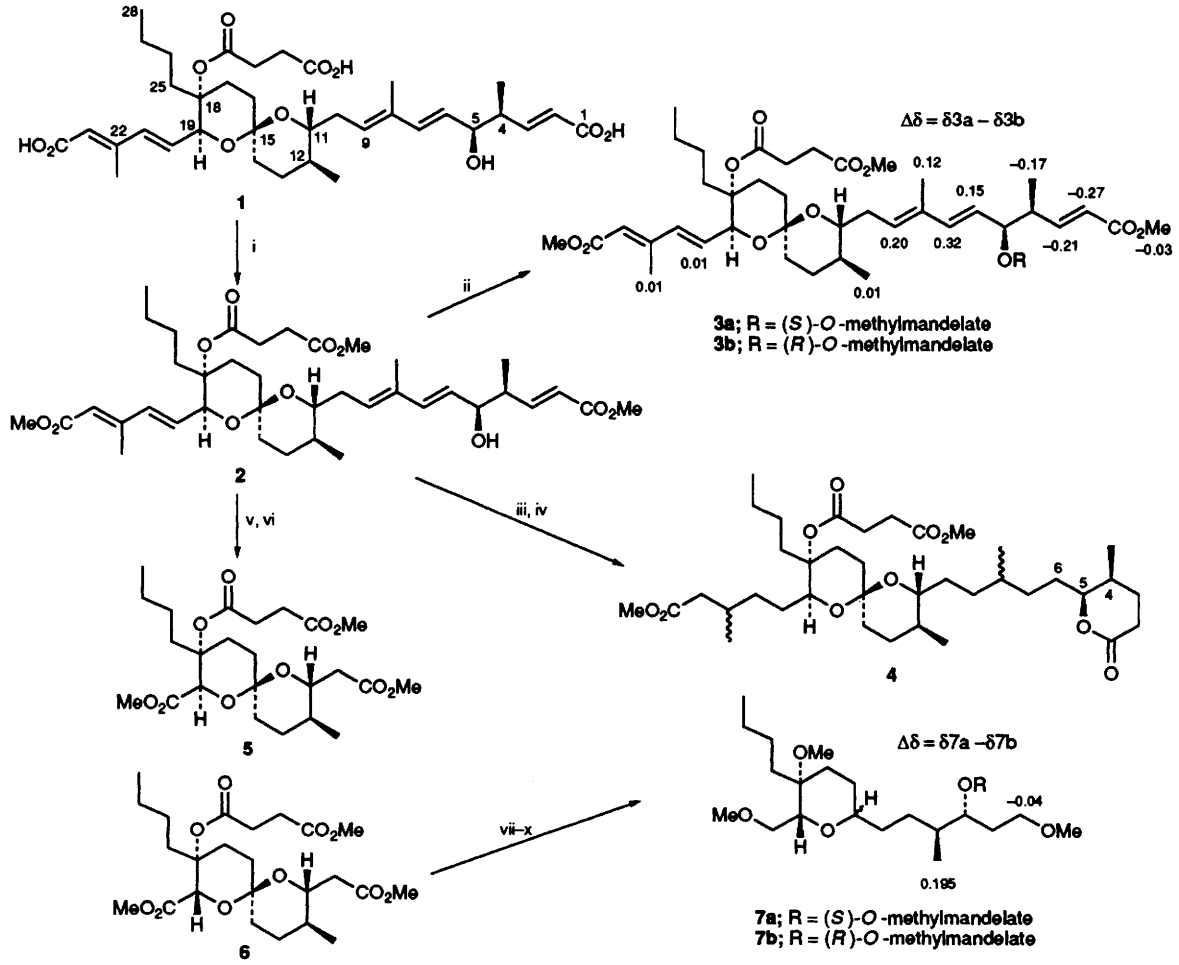
Reveromycin A has been isolated from a soil actinomycete as a novel inhibitor of mitogenic activity induced by epidermal growth factor (EGF) in a mouse epidermal keratinocyte.[1] Although the structure of reveromycin A has been reported,[2] its stereochemistry remains unknown. In this paper, we report the absolute configuration of reveromycin A **1** determined through chemical degradation and spectroscopic analyses.

The absolute configurations of **1** was fully assigned on the basis of an analysis of the derivatives **2–9** obtained as outlined in Scheme 1 and Fig. 1. With respect to the reveromycin numbering system, which is used throughout the discussion for clarity, the seven stereogenic centres are C-5 in structure **3**, C-4 in **4**, C-11, -12, -15, -18, -19 in **5**.

The absolute configuration at C-5 of trimethyl ester **2**, which was obtained by treatment of **1** with diazomethane, was determined by a variation of Trost's method.[3] The trimethyl ester **2** was converted into the corresponding (*S*)-*O*-methylmandelate **3a** and (*R*)-*O*-methylmandelate **3b** as shown in Scheme 1. From the chemical-shift differences ($\Delta\delta = \delta_{3a} - \delta_{3b}$) of diastereoisomers **3a** and **3b** shown in Scheme 1, the absolute configuration *R* at C-5 was deduced.

The trimethyl ester **2** was converted into lactone **4** to determine the relative configurations at C-4 and C-5 by the data of coupling constants in the ^{1}H NMR. Because of the bulkiness of the side chain of the lactone moiety, the lactone ring exists as a half-chair conformation and the side chain should be in a pseudoequatorial position.† The vicinal coupling constant (*J* 2.1 Hz) between 5-H and 4-H indicates that the C-4 Me exists in an pseudoaxial position and the relative configuration at C-4 and C-5 in **2** was assigned as *R*/*S* or *S*/*R*. Since the absolute configuration at C-5 in **2** is *R*, the absolute configuration at C-4 in **2** must be *S*.

The relative stereochemistry at C-11, -12, -15, -19 of **1** was established by analyses of the NOE data[2] of the trimethyl ester **2**. The large coupling constant (*J* 10.1 Hz) between 11-H and 12-H indicates that both the protons exist in *trans* diaxial. The



Scheme 1 *Reagents and conditions*: i, CH_2N_2; ii, *O*-methylmandelate, DCC, 4-PPY; iii, H_2, 10% Pd/C; vi, TsOH, benzene, reflux; v, $RuCl_3$, $NaIO_4$; vi, CH_2N_2; vii, $LiAlH_4$; viii, MeI, NaH; ix, Et_3SiH, $SnCl_4$; x, *O*-methylmandelate, DCC, DMAP

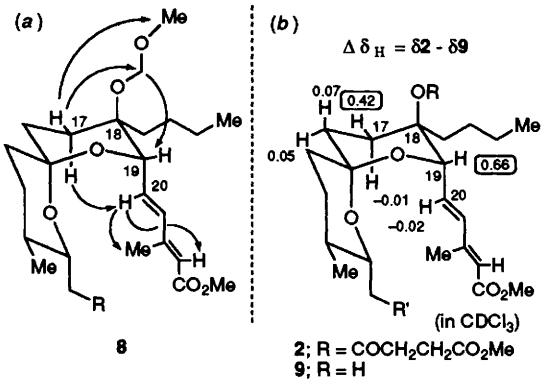


Fig. 1 Relative stereochemistry at C-18; (*a*), NOE data of 18-MOM ether **8**, (*b*), deshielding effect of succinate

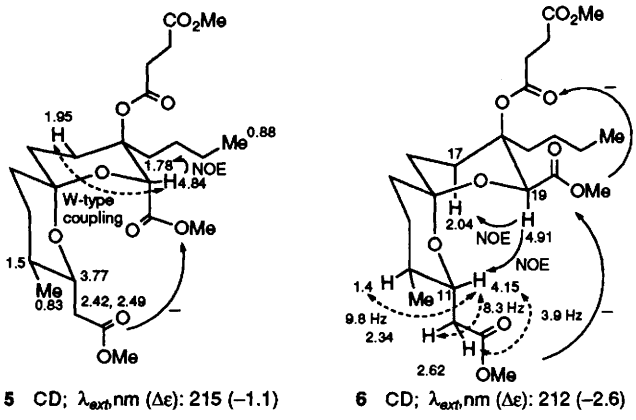


Fig. 2 Stereochemistry of **5** and **6**

NOEs between 11-H and 20-H, and 20-H and 17-H_{ax} show that the orientation of the side chain at C-19 is axial. To determine the relative configuration at C-18, reveromycin **1** was converted into 18-*O*-methoxymethyl (MOM) ether **8** in four steps: i, 1N LiOH; ii, CH_2N_2; iii, Ac_2O, Py; iv, MOMCl, Pr^i_2NEt. The NOEs between the equatorial proton at C-17 and both methylene protons and methoxy methyl protons of the MOM group, and the methylene protons and equatorial proton at C-19, showed that the oxygen atom at C-18 exists in an axial position as shown in Fig. 1(*a*). This conclusion was further confirmed by the data of the chemical shift differences ($\Delta\delta_H = \delta_2 - \delta_9$) between **2** and **9** as shown in Fig. 1(*b*). By the deshielding effects of the succinate carbonyl group which exists in the axial position, the equatorial proton at C-17 and 19-H, but not the axial proton at C-17 and 20-H in **2**, appear at lower field than the corresponding protons in **9** in ^{1}H NMR.

To establish the absolute configurations of the spiroketal moiety, the trimethyl ester **2** was degraded into the spiroketal core fragments **5** and **6**. The relative stereochemistry of **5** and **6** were deduced from the coupling constant data and NOE data as shown in Fig. 2. From the NOEs between 19-H–17-H_{ax} and 19-H–11-H in **6**, the orientation of the methoxy carbonyl group at C-19 was revealed to be equatorial. The CD of **5** showed a rather weak negative Cotton effect, $\Delta\varepsilon_{215}$ −1.1 in MeOH. The CD of **6** which is the epimer at C-19 showed a strong enough Cotton effect, $\Delta\varepsilon_{212}$ −2.6, to assign the absolute configuration, which demonstrates the additivity[4] of the two negative chiralities as shown in Fig. 2. The projection of the two chromophores on C-11 and C-19 should be counter-clockwise, and the two chromophores on C-19 and C-18 should be also counter-clockwise. Thus, the absolute configurations of **5** and **6** were determined as being those shown in Scheme 1.

To confirm the absolute configuration of the spiroketal moiety, reductive cleavage of the spiroketal[5] in **6** was executed. The trimethyl ester **6** was converted into trimethyl ether **7** under the reaction condition shown in Scheme 1. Reduction of the sterically hindered ketal **7** with Et_3SiH in the presence of $SnCl_4$ afforded a diastereoisomeric mixture of the alcohol which was converted into the (*S*)-*O*-methylmandelate **7a** and (*R*)-*O*-methylmandelate **7b**. Although the diastereoisomeric mixture at C-15 could not be separated, the chemical shifts of 12-Me and 9-H in **7a** and **7b** were determined by 1D HOHAHA by irradiating 11-H in the both derivatives. According to the Trost's method,[3] the chemical shift differences ($\Delta\delta_H = \delta_{7a} - \delta_{7b}$) of diastereoisomers **7a** and **7b** show that the absolute configuration at C-11 is *R* which indirectly fixed the configurations at C-12, -15, -18, -19 in **2** as *S*/*S*/*R*/*S*, respectively.

The CD spectrum of **1** showed split Cotton effects, $\Delta\varepsilon_{251}$ −12.1 and $\Delta\varepsilon_{230}$ + 5.6, indicating a negative chirality between two chromophores in the molecule. The result corroborates the absolute configuration of the spiroketal moiety in **1**.

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Footnote

† ^{1}H NMR data for the lactone moiety in **4**: ($CDCl_3$, 400 MHz) δ 2.53, 2-H; δ 1.67, 2.00, 3-H; δ 2.03, 4-H. δ 0.97, C-4-Me; δ 4.97, 5-H, $J_{4,5}$ 2.1, $J_{5,6a}$ 10.4, $J_{5,6b}$ 2.1 Hz; δ 1.40, H-6a; δ 1.49, H-6b. Stable conformations of a model [4, 5-(*S*,*S*)-4-methyl-5-isopentyl-5-hydroxypentan-5-olide] of **4** were obtained by the COSMIC force field calculation: conformer 1 (4-methyl pseudoaxial, 5-isopentyl pseudoequatorial), total energy = 5.021 kcal mol^{-1} (1 cal = 4.184 J); conformer 2 (4-methyl pseudoequatorial, 5-isopentyl pseudoaxial), total energy = 20.380 kcal mol^{-1}.

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