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Stereochemical Interrelationship between Maridomycin and Leucomycin

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Epoxidation of 18-dihydroleucomycin A_3 (**5**) with *m*-chloroperbenzoic acid afforded 18-dihydromaridomycin II (**6**). Conversion of leucomycin A_3 (**1**) into maridomycin II (**2**) was also accomplished in a similar process. This evidence together with the result of X-ray analysis of 9-propionylmaridomycin III has finally confirmed the absolute stereochemistry of leucomycin A_3. In addition, Δ^2-9-epi-18-dihydromaridomycin III (**12**) was prepared by the $NaBH_4$ reduction of 9-dehydromaridomycin III (**11**) and the effect of the C-9 hydroxyl group on optical rotations was discussed.

Keywords——16-membered macrolide; maridomycin; leucomycin; epoxidation; C-9 epimer; Mills' rule

Recently, the total stereochemistry of maridomycins has been elucidated by chemical method and X-ray analysis.[2,3] Until then, the stereochemistry of 16-membered macrolide had been deduced on the basis of chemical studies and X-ray analyses of degradation products.[4,5] This is the first case in which the absolute stereochemistry of intact 16-membered macrolide has been determined. The configuration of C-9 hydroxyl group was found to be epimeric to that of leucomycins previously assigned by the application of the Benzoate or Mills' rule[6] and identical with that assigned by chemical method.[7] We tried to clarify the discrepancy of the configuration between the intact leucomycin and maridomycin.[5]

The coupling constants of $J_{8,9}$ (3.5 Hz) and $J_{9,10}$ (8.5—9.0 Hz) in natural maridomycins and their acyl derivatives were similar to those of leucomycin A_3.[8] In addition, $\Delta[M]_D$ between maridomycin III (MDM III) (**3**) and its 9-*p*-nitrobenzoate (**4**), $C_{48}H_{70}N_2O_{19}$, was −392°, showing the same tendency as in the case of 3,5-dinitrobenzoate of leucomycin A_3.

In order to interrelate leucomycin A_3 (LM A_3) (**1**) with maridomycin II (MDM II) (**2**), 18-dihydroleucomycin A_3 (**5**),[9] $C_{42}H_{71}NO_{15}$, which was obtained by $NaBH_4$ reduction of **1**, was epoxidized with an excess of *m*-chloroperbenzoic acid in $CHCl_3$. The products composed of some mixtures after selective reduction of N-oxide function with $Na_2S_2O_4$ were purified by silica gel column chromatography, mainly giving a pair of isomer **a** and **b**. A major isomer **a**, obtained in *ca.* 30% yield was found to be identical with 18-dihydromaridomycin II (**6**), $C_{42}H_{71}NO_{16}\cdot H_2O$, $[\alpha]_D^{25}$ −72.6° (EtOH), in infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectra, optical rotations, and melting point. There-

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5) After our studies on the structure of maridomycins had been completed, a report on X-ray analysis of the aglycone of 3,6-bicyclo-leucomycin A_3 was published and the configuration of C-9 hydroxyl group was found to be the same with maridomycin: A. Ducruix, C. Pascard, A. Nakagawa, and S. Omura, *Chem. Commun.*, **1976**, 947.
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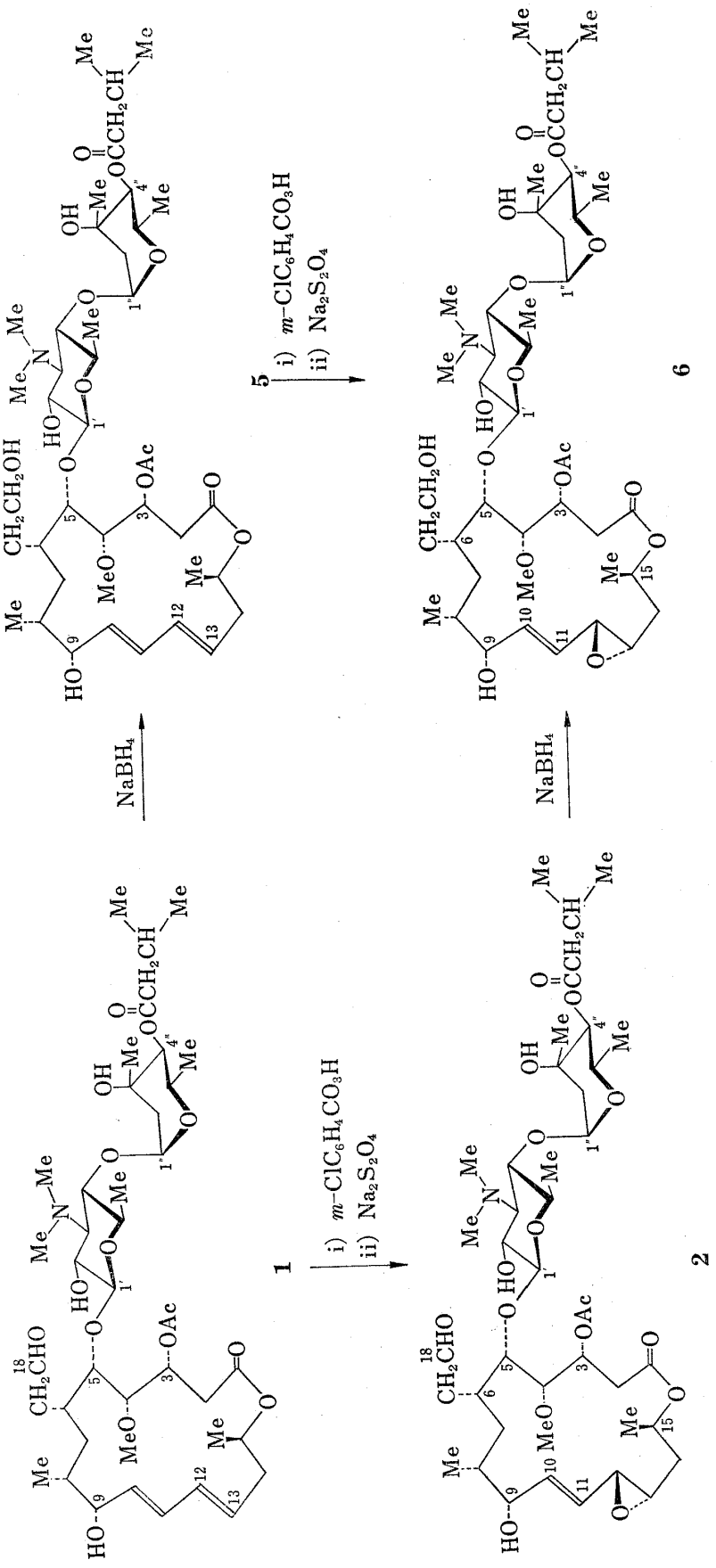

Chart 1

fore, stereochemistry in the portion, C-9 to C-13 of natural LM A_3 (**1**) has been established as shown in Chart 1, *i.e.*, the configuration at the C-9 is *R*, the geometries of both double bonds are *trans* (*E*), and the diene portion is nearly in *s-trans* conformation, furthermore the C-9 hydroxyl is directed to the outside of the macroring.

The minor isomer **b**, (**7**), $C_{42}H_{71}NO_{16}\cdot H_2O$, which was obtained in low yield, as a pure state, is assumed to be a positional isomer of 18-dihydro MDM II (**6**), *i.e.*, an epoxidized product at the C_{10}–C_{11} double bond from NMR and mass spectra. Although mass spectra of isomer **b** showed no molecular ion peak, fragment ion peak [M^+-AcOH-$(Me)_2CHCH_2COO\cdot$] was observed at *m/e* 684 and NMR spectrum of the isomer **b** differed from **6** in the chemical shifts and splitting patterns of H-9 and olefinic protons. In this way, epoxidation of the diene system (C_{10}–C_{13}) of the 16-membered ring with *m*-chloroperbenzoic acid was found to occur stereoselectively at C_{12}–C_{13}.

Then, direct conversion of LM A_3 (**1**) into MDM II (**2**) was accomplished in a similar manner in *ca.* 10% yield, as follows. LM A_3 (**1**) was epoxidized with *m*-chloroperbenzoic acid (2.5—3.5 equiv.) in $CHCl_3$. N-oxide of epoxidized product was successively reduced by $Na_2S_2O_4$ without purification. Then, a mixture of reduction products at the N-oxide function was chromatographed on a silica gel column. A major isomer **a** thus obtained was identified with MDM II (**2**) by IR, UV, NMR and mass spectra, optical rotations, and mixed melting point. Although the yield was low due to the formation of complicated mixtures of by-products, success of direct conversion gives further evidence that the absolute configuration of C-9 hydroxyl group in LM A_3 (**1**) and MDM II (**2**) is the same.

In this way, the configuration of C-9 has been determined to be epimeric to the previously assigned one from Mills' rule. Accordingly, the Mills' rule does not appear to be generally applicable to a allylic alcohol of these 16-membered rings.

Opposite shift in optical rotations was observed in the maridomycins depending on the configuration of C-9 hydroxyl group. Δ^2-18-dihydromaridomycin III (**9**), $C_{38}H_{63}NO_{14}\cdot H_2O$ which was prepared by the method in Chart 2, showed $[\alpha]_D^{23}$ −80.7° (EtOH), while Δ^2-9-*epi*-18-dihydromaridomycin III (**12**), preferentially produced by the $NaBH_4$ reduction of 9-dehydromaridomycin III (**11**) in MeOH–EtOH mixture, had $[\alpha]_D^{25}$ −52.9° (EtOH).

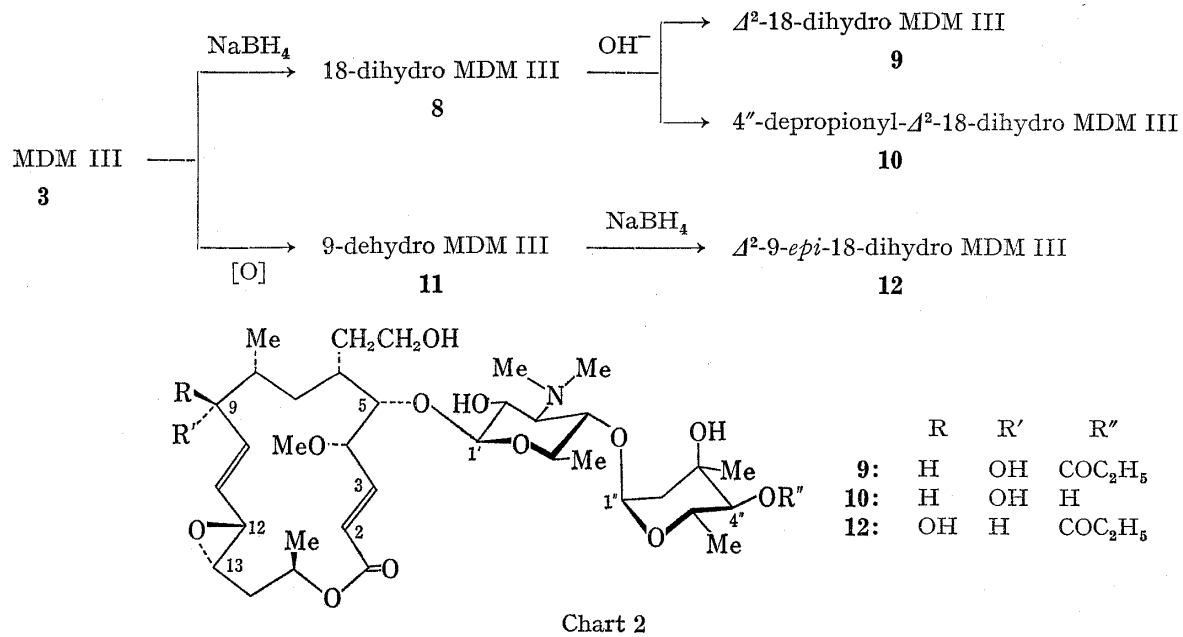

Chart 2

On the contrary to terpenoid allylic alcohol, the natural compound with the configuration represented by *R* is more levorotary than its epimer. The same tendency in optical rotations was observed also in the leucomycins.[7]

The values of $\Delta [M]_D$ between the compounds with natural configurations and their epimers at C-9 in Δ^2-18-dihydromaridomycin III (**9**), leucomycin A_3 (**1**) and leucomycin A_3 dimethylacetal are −215.7°, −251.4°, and −244.1°, respectively.[7] The effects of the structural changes in the C_2–C_3, C_{12}–C_{13}, and C_6 substituent are found to be negligibly small.

Consequently, in 16-membered ring such as maridomycins and leucomycins, the contribution of C-9 allylic alcohol to optical rotations is considered to be generally opposite to Mills' rule.[10]

The result now revealed is compatible with that assigned from NMR and IR data, on the premise that the plane of diene system is perpendicular to the mean plane of 16-membered ring.[7]

Since leucomycin A_3 (**1**) was chemically correlated with maridomycin II (**2**) and with propionylmaridomycin III whose stereochemistry was established by X-ray analysis, the absolute stereochemistry of natural leucomycin A_3 (**1**) now has been determined.

In addition, it is concluded that the naturally occurring 16-membered macrolide antibiotics of carbomycin-leucomycin group[11] have the same absolute stereochemistry with maridomycin revealed by X-ray analysis of propionylmaridomycin III,[3] and establishment of the stereochemistry will be much useful for the study on the structure-activity relationships in the 16-membered macrolide antibiotics.

Experimental[12]

Epoxidation of 18-Dihydroleucomycin A_3 (5)——To a solution of 18-dihydroleucomycin A_3 with $NaBH_4$ was dropwise added a solution of *m*-chloroperbenzoic acid (purity 85%, 720 mg) in $CHCl_3$ (15 ml) and the mixture was allowed to stand in the dark overnight at room temperature. Then, EtOH (30 ml) and aq. solution of $Na_2S_2O_4$ (870 mg; 5 mM) were added and the mixture was stirred under ice-cooling for 15 min. After concentration of the reaction mixture, it was poured into cold aq. $NaHCO_3$ solution (120 ml) and extracted twice with 150 ml of $CHCl_3$. The $CHCl_3$ extract, after successive washing with aq. $NaHCO_3$ and H_2O, was concentrated to give 1.02 g of crude substance. The crude material (968 mg) was subjected to a column chromatography of silica gel (70 g) and the column was developed with mixtures of benzene–acetone (3: 1→2: 1→1: 1), successively. Elution with benzene–acetone (2: 1) afforded an isomer **b** (35 mg), a mixture of isomer **a** and **b** (102 mg), and isomer **a** (331 mg), respectively. Isomer **a** was crystallized from isopropylether. Isomer **a**: mp 131—132°. $[\alpha]_D^{25}$ −72.6° (c=1.0, EtOH). UV (MeOH): end absorption. *Anal.* Calcd. for $C_{42}H_{71}NO_{16}\cdot H_2O$: C, 58.38; H, 8.52; N, 1.62. Found: C, 58.97; H, 8.57; N, 1.62. The isomer **a** was identified with 18-dihydromaridomycin II (**6**) from comparison of IR, NMR and mass spectra and behaviors on TLC. Isomer **b** (**7**): UV (MeOH) end absorption. Mass Spectrum *m/e*: 785 (M^+—AcOH), 684 (M^+—AcOH–$(CH_3)_2CHCH_2COO\cdot$), 367 (aglycone-AcOH), 402 (isovaleryl mycarosyl mycaminose). IR spectrum (KBr) of b slightly differed from that of **a** in the finger print region. NMR spectrum ($CDCl_3$) showed two olefinic protons from δ 5.0 to 6.0, but was not identical with that of isomer **a**.

Epoxidation of Leucomycin A_3 (1)——To a solution of leucomycin A_3 (1.8 g) in $CHCl_3$ (30 ml) was dropwise added a solution of *m*-chloroperbenzoic acid (1.08 g) in 20 ml of $CHCl_3$, and the mixture was allowed to stand at room temperature in the dark overnight. Then, work-up in a similar manner as described above afforded 1.33 g of crude material which was chromatographed on a silica gel column (60 g). Elution with benzene–acetone (3: 1) furnished a mixture of isomer **a** and **b** (129 mg) and isomer **a** (125 mg). Isomer **a** was crystallized from benzene to give colorless needles. mp 134—136°. $[\alpha]_D^{22}$ −76.4° (c=1.0, EtOH). UV (MeOH): end absorption. Isomer **a** was identical with maridomycin II (**2**) in all respects (IR, NMR and mass spectra, *Rf* values on TLC, and mixed mp.). Although isomer **b** was not obtained in a pure state, a mixture of isomer **a** and **b** (*ca.* 1: 1) showed only end absorption in UV spectrum. In comparison of NMR, IR and mass spectra of this mixture with isomer **a**, isomer **b** was assumed to be a positional isomer, *i.e.*, an epoxidized compound at the C-10, C-11 position.

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12) Melting points were determined with a Mettler FP 5 apparatus. NMR spectra were recorded on a Varian HA-100 spectrometer with tetramethylsilane as the internal standard. Chemical shifts were reported on the δ scale. Mass spectra were obtained with a JEOL JMS-01SG mass spectrometer using a direct inlet system. Thin-layer chromatography (TLC) was performed on Silica gel spotfilm (Tokyo Kasei Co.). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

MDM III-9-*p*-nitrobenzoate (4)——To a solution of MDM III (450 mg) in 4.5 ml of dry pyridine was added slowly *p*-nitrobenzoyl chloride (300 mg) under ice-cooling and the mixture was left to stand at room temperature for 5 hr. Then, it was poured into cold aqueous $NaHCO_3$ and the precipitate was extracted with AcOEt. The washed and dried extract was evaporated to give 597 mg of crude substance which was purified on a silica gel column (20 g). Elution with benzene–acetone (5: 1) furnished pure 9-*p*-nitrobenzoate (**4**) (292 mg) as a white powder. mp 141—144°. $[\alpha]_D^{20}$ −104.0° (c=1.0, EtOH). *Anal.* Calcd. for $C_{48}H_{70}N_2O_{19}\cdot H_2O$: C, 57.81; H, 7.28; N, 2.81. Found: C, 57.99; H, 7.08; N, 2.84. NMR ($CDCl_3$) δ: 2.54 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 4.0 (1H, dd, H-5), 4.45 (1H, d, H-1′), 4.64 (1H, d, H-4″), 5.08 (1H, dd, H-1″), 5.19 (1H, H-3), 5.37 (1H, dd, H-9), 5.92 (1H, dd, H-11), 6.18 (1H, dd, H-10), 8.14 (2H, d, aromatic H), 8.28 (2H, d, aromatic H), 9.71 (1H, s, –CHO).

18-Dihydro MDM III (8)——MDM III (3) (1.7 g) in 40 ml of 50% aqueous MeOH was reduced with 75.6 mg of $NaBH_4$ for 1 hr at room temperature. Dilution of the reaction mixture and extraction with AcOEt followed by usual work-up afforded 1.61 g of 18-dihydro MDM III (**8**) which was crystallized from isopropyl ether. mp 133—134°. $[\alpha]_D^{23}$ −77.2° (c=1.0, EtOH). *Anal.* Calcd. for $C_{41}H_{69}NO_{16}\cdot 1/2H_2O$: C, 58.55; H, 8.39; N, 1.67. Found: C, 58.57; H, 8.49; N, 1.66. NMR ($CDCl_3$): disappearance of aldehyde signal.

Δ^2-18-Dihydro MDM III (9)——18-Dihydro MDM III (**8**) (12.75 g) in 225 ml of MeOH was treated with 27 ml of 1 N methanolic KOH at 5°. After 20 hr, the reaction mixture was poured into ice-water and neutralized with 1 N AcOH, and concentrated to remove MeOH. The concentrate was extracted with AcOEt and the washed and dried extract was evaporated to give crude substance (8.52 g) which was chromatographed on a silica gel column (440 g). Elution with benzene–acetone (3: 1) afforded 1.24 g of Δ^2-18-dihydro MDM III (**9**), and further elution with benzene–acetone (2: 1) afforded 5.6 g of 4″-depropionyl-Δ^2-18-dihydro MDM III (**10**). Δ^2-18-Dihydro MDM III (**9**): mp 114—116°. $[\alpha]_D^{23}$ −80.7° (c=1.0, EtOH). *Anal.* Calcd. for $C_{38}H_{63}NO_{14}$: C, 60.22; H, 8.38; N, 1.85. Found: C, 59.90; H, 8.41; N, 1.55. NMR ($CDCl_3$) δ: 0.98 (3H, d, CH_3-8), 2.50 (6H, s, $-N(CH_3)_2$), 3.26 (3H, s, $-OCH_3$), 4.10 (1H, dd, H-9), 4.44 (1H, d, H-1′), 4.61 (1H, d, H-4″), 5.07 (1H, dd, H-1″), 5.37 (1H, dd, H-11), 5.98 (1H, d, H-2), 5.99 (1H, dd, H-10), 6.54 (1H, dd, H-3). Mass Spectrum m/e: 757 (M^+), 684 ($M^+-C_2H_5COO\cdot$), 374 (propionyl mycarosyl mycaminose), 367 (aglycone). 4″-Depropionyl-Δ^2-18-dihydro MDM III (**10**): mp 148—150° (from acetone). $[\alpha]_D^{24}$ −77.5° (c=1.0, EtOH). *Anal.* Calcd. for $C_{35}H_{59}NO_{13}\cdot H_2O$: C, 58.39; H, 8.54; N, 1.94. Found: C, 58.36; H, 8.47; N, 1.87. IR ν_{max}^{KBr} cm^{-1}: 1720 (α,β-unsaturated lactone), 1660 (C=C). NMR ($CDCl_3$) δ: 0.97 (3H, d, CH_3-8), 2.48 (6H, s, $-N(CH_3)_2$), 2.93 (1H, d, H-4″), 3.24 (3H, s, $-OCH_3$), 4.44 (1H, d, H-1′), 5.06 (1H, dd, H-1″), 5.38 (1H, dd, H-11), 5.98 (1H, d, H-2), 5.98 (1H, dd, H-10). Mass Spectrum m/e: 701 (M^+), 557 (mycaminosyl aglycone), 367 (aglycone), 318 (mycarosyl mycaminose).

Δ^2-9-Epi-18-dihydro MDM III (12)——9-Dehydro MDM III (**11**) (210 mg) in 8 ml of EtOH–MeOH (4: 1) was reduced with 18.9 mg of $NaBH_4$ overnight at room temperature. The reaction mixture was diluted with 1/15 M phosphate buffer (pH 7.7) and extracted with AcOEt. The washed and dried extract was concentrated to yield crude substance (172 mg), which was subjected to a silica gel column (25 g). Elution with benzene–acetone ((3: 1)→(2: 1)) gave 114 mg of Δ^2-9-*epi*-18-dihydro MDM III (**12**) showing one spot on TLC. mp 120—121°. $[\alpha]_D^{25}$ −52.9° (c=1.0, EtOH). *Anal.* Calcd. for $C_{38}H_{63}NO_{14}\cdot 1/2H_2O$: C, 59.51; H, 8.41; N, 1.83. Found: C, 59.32; H, 8.66; N, 1.67. UV (MeOH): end absorption. IR ν_{max}^{KBr} cm^{-1}: 1725 (α,β-unsaturated lactone), 1660 (C=C). NMR ($CDCl_3$) δ: 1.0 (3H, d, CH_3–8), 2.52 (6H, s, $-N(CH_3)_2$), 3.28 (3H, s, $-OCH_3$), 4.05 (1H, m, H-9), 4.44 (1H, d, H-1′), 4.63 (1H, d, H-4″), 5.08 (1H, dd, H-1″), 5.46 (1H, dd like, H-11), 6.02 (1H, d, H-2), 6.04 (1H, dd, H-10), 6.60 (1H, dd, H-3). No aldehyde signal. Mass Spectrum m/e: 757 (M^+), 684 ($M^+-C_2H_5COO\cdot$).

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