Mycinamicin Biosynthesis: Isolation and Structural Elucidation of Novel Macrolactones and a Seco Acid Produced by a Mutant of *Micromonospora griseorubida*

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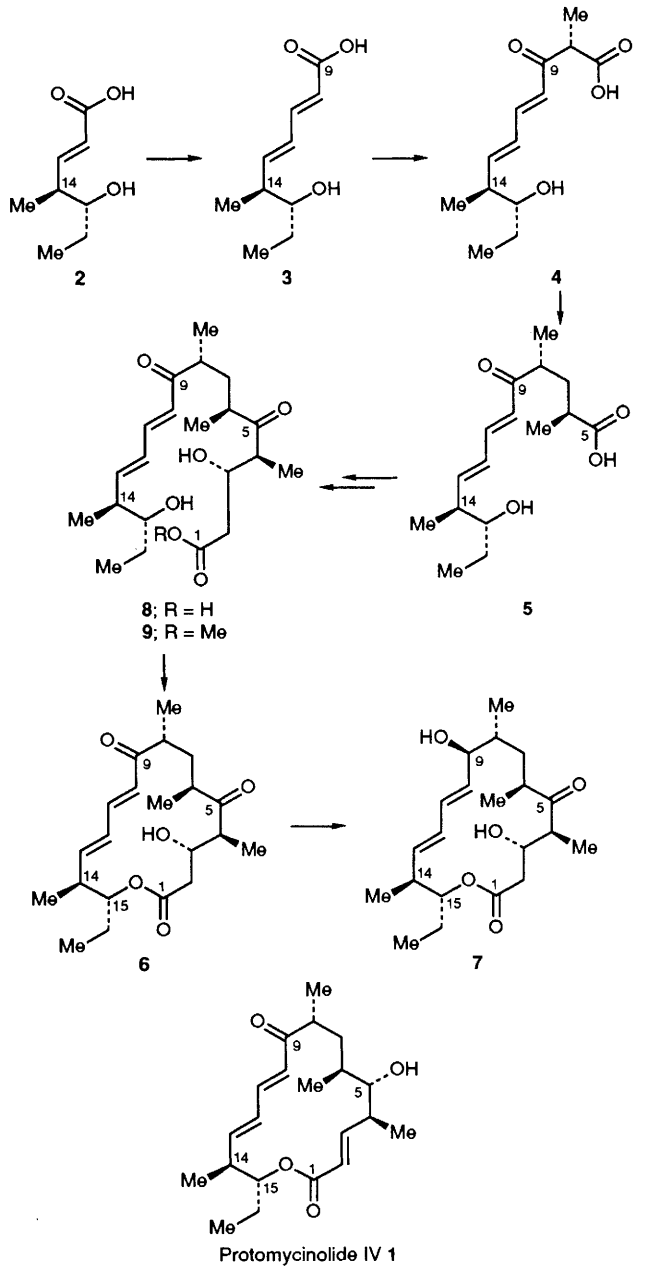
Novel macrolide compounds and their biosynthetic intermediate, a seco acid, were isolated from the culture filtrate of a mutant strain of *Micromonospora griseorubida*, and their structures were determined on the basis of their spectroscopic data and X-ray diffraction analysis.

Mycinamicins are 16-membered macrolide antibiotics produced by *Micromonospora griseorubida*.[1–4] The first intermediate in the biosynthesis of the mycinamicin aglycone is protomycinolide IV **1**.[5,6] Recently, we reported on the isolation and structural studies of mycinonic acids I **2**, II **3**, III **4** and IV **5**, which were considered to be intermediates for formation of the mycinamicins at an early stage in the biosynthetic pathway.[7,8] These results strongly suggest that the stereochemistry of the polyketide chain-elongation process must proceed systematically at each condensation stage as shown in erythromycin[9] and tylosin.[10] In our mutagenic studies on *M. griseorubida*, we found that it produced 5-deoxy-2,3-dihydro-3-hydroxy-5-oxoprotomycinolide IV **6** and its 9-deoxo-9-hydroxy compound **7** and their biosynthetic intermediate, the seco acid **8**. In this paper, we describe the isolation of these novel metabolites, and elucidation of their structure and absolute configuration. This is the first successful isolation of the seco acid as a biosynthetic intermediate of macrolide compounds from a fermentation broth.

The mutant strain C-3-14 was isolated after treatment of mycinamicin-producing strains of *M. griseorubida* with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Culture conditions and production media were as described previously.[8] The fermentation broth of this mutant strain contained mycinonic acids I **2**, II **3**, III **4** and IV **5**, macrolide compounds **6** and **7**, and their seco acid **8**, but it did not produce mycinamicins. The culture filtrate of a mutant strain was extracted at pH 9.0 with an equal volume of ethyl acetate. The organic layer contained compounds **6** and **7**. Their biosynthetic intermediate **2**, **3**, **4** and **5** and the seco acid **8** were contained in the aqueous layer. Compounds **6** and **7** were purified by reversed-phase preparative HPLC. Colourless single crystals of **7** were grown from acetone–hexane (m.p. 132–134 °C). Extraction of the aqueous layer with diethyl ether at pH 3.0 and treatment of the crude mycinonic acids and the seco acid **8** obtained, with a solution of (trimethylsilyl)diazomethane yielded their methyl esters. Compound **9**, the methyl ester of the seco acid **8**, was purified by reversed-phase preparative HPLC.

The molecular formula of **6** was determined to be $C_{21}H_{32}O_5$ from high-resolution fast-atom-bombardment mass spectrometry (HR-FABMS) [$(M + H)^+$, *m/z* 365.2356; calc. 365.2328]. The presence of an α,β,γ,δ-unsaturated ketone was suggested by the UV absorption maximum at 277 nm. The structure was further elucidated by comparing the ^1H and ^{13}C NMR spectral data of **1** and **6**. The data of **6** are similar to those for compound **1**, except that the ^1H NMR signals of 2,3-double bond and 5-CH(OH) observed at δ 5.77 (1H, d, 2-H), 6.59 (1H, d, 3-H) and 3.29 (1H, d, 5-H) in **1** disappeared, while new methylene and hydroxymethine signals appeared at δ 2.34 (1H, dd, 2-H_a), 2.55 (1H, dd, 2-H_b)



Scheme 1 Proposed biosynthetic pathway

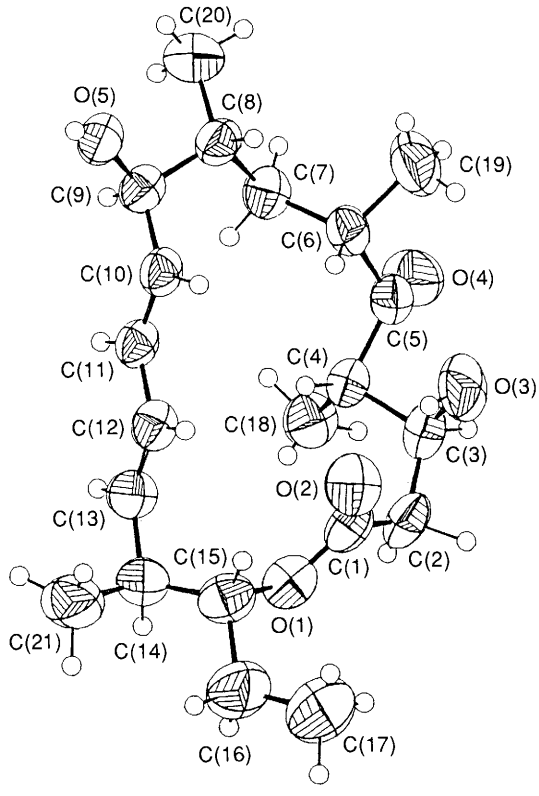


Fig. 1 Molecular structure of compound **7**

and 3.82 (1H, m, 3-H) in **6**. Accordingly, compound **6** was identified as 5-deoxy-2,3-dihydro-3-hydroxy-5-oxoprotomycinolide IV. The ^{13}C NMR spectrum supported this conclusion.

The molecular formula of **7** was determined to be $C_{21}H_{34}O_5$ from HR-FABMS [$(M + Na)^+$, m/z 389.2293; calc. 389.2304]. The UV absorption maximum at 233 nm suggested the presence of an α, β, γ, δ-unsaturated diene. The ^1H and ^{13}C NMR data of **7** are similar to those for compound **6** except for the absence of the ketone carbonyl carbon of **6** at δ_C 204.9 (s, C-9) and the presence of a hydroxymethine signal upfield at δ_C 77.8 (d, C-9) in **7**. Therefore the compound was identified as 9-deoxo-5-deoxy-2,3-dihydro-3,9-dihydroxy-5-oxoprotomycinolide IV. Furthermore, in order to investigate the stereochemistry, the relative configuration of **7** was determined by X-ray crystallography. The absolute configuration was considered to be (3*S*, 4*R*, 6*S*, 8*R*, 9*S*, 14*S*, 15*R*)-9-deoxo-5-deoxy-2,3-dihydro-3,9-dihydroxy-5-oxoprotomycinolide IV by comparing the stereochemistry of **7** and mycinamicin IV[11] (Fig. 1).†

The molecular formula of **9** was determined to be $C_{22}H_{36}O_6$ from HR-FABMS data [$(M + H)^+$, m/z 397.2613; calc. 397.2590]. The presence of an α, β, γ, δ-unsaturated ketone was suggested by a UV maximum at 280 nm. This was also evident from the ^1H NMR spectrum which showed characteristic signals of an unsaturated ketone at δ_H 6.19 (1H, d, 10-H), 7.24 (1H, dd, 11-H), 6.26 (1H, dd, 12-H) and 6.23 (1H, dd, 13-H). In the ^{13}C NMR spectrum there were no substantial differences between compounds **9** and **6**, with the chemical shifts of carbon atoms 1–15 corresponding (depending on substituent). The signals of the unsaturated ketone and ester carbonyl groups were observed at δ_C 203.4 (s, C-9) and 172.6 (s, C-1). From these results, compound **9** was concluded to be the methyl ester of the seco acid corresponding to compound **6**, methyl 3,15-dihydroxy-5,9-dioxo-4,6,8,14-tetramethylheptadeca-10,12-dienoate.

Under the mycinamicin-producing fermentation conditions, fermentation of the mutant strain C-3-14 did not produce mycinamicins, but instead compound **6**, the 9-deoxo-9-hydroxy compound **7** and their biosynthetic intermediate, the seco acid **8**, and mycinonic acids I **2**, II **3**, III **4** and IV **5** accumulated in the culture filtrate. Protomycinolide IV **1** and other mycinamicin biosynthetic intermediates were converted into mycinamicin II more efficiently by this mutant.[12] Accordingly, these results suggest that these secondary metabolites

† *Crystal data*: $C_{21}H_{34}O_5$, M_r = 366.49, trigonal, space group *R*3, $a = b = 28.73(1)$, $c = 6.918(1)$ Å, $V = 4947.1$ Å^3, $Z = 9$, $D_c = 1.11$ g cm^{-3}. Data were measured on a Mac Science MXC18 automatic four-circle diffractometer using graphite-monochromated Cu-Kα radiation with the ω–2θ scan technique. The structure was solved by direct methods and full-matrix least-squares refinement gave R = 0.054, for 1369 unique reflections. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

produced by the mutant of *M. griserubida* are blocked at the stage of the dehydration of the C-3 position and the reduction of the C-5 position in the chain assembly for protomycinolide IV **1** formation as shown in Scheme 1.

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