HYBRID BIOSYNTHESIS OF A NEW MACROLIDE ANTIBIOTIC BY A DAUNOMYCIN-PRODUCING MICROORGANISM*

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In previous papers[1,2] we described the derivation of new macrolide antibiotics using the technique of hybrid biosynthesis. In a series of further trials, we obtained two new compounds by incubating a daunomycin producer *Streptomyces* sp. KA-464 in the presence of cerulenin with the 16-membered lactone protylonolide, which is a precursor of tylosin biosynthesis and possesses a structure corresponding to the aglycone moiety. This paper describes the isolation and structures of a new macrolide aglycone and a macrolide antibiotic.

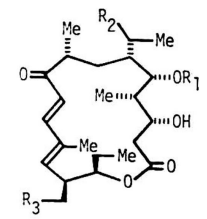
Streptomyces sp. strain KA-464 (KCC U-0202) was cultured in a daunomycin production medium (4% glucose, 1.5% dried yeast, 0.2% NaCl, 0.1% KH_2PO_4, 0.1% $CaCO_3$, 0.01% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.001% $ZnSO_4 \cdot 7H_2O$, 0.001% $CuSO_4 \cdot 5H_2O$, pH 7.0). To the culture medium 80 μg/ml of cerulenine was added initially and at a 24-hour interval to prevent the *de novo* synthesis of the aglycone moiety, the daunomycinones. After 72 hours of cultivation, 100 μg/ml of protylonolide was added and the cultivation was continued for a further 48 hours. The cultured broth (5 liters) was centrifuged to separate cells. The cells were extracted with one liter of acetone, and the extract was concentrated *in vacuo*. The residue was combined with the supernatant, which was then extracted with ethyl acetate. The combined organic extracts were evaporated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$ - MeOH - conc. NH_4OH, 15: 1: 0.05) followed by preparative TLC on silica gel developed with ethyl acetate - acetone (8: 1). Seventy-three mg of 19-hydroxyprotylonolide (**2**) and 82 mg of 23-hydroxyprotylonolide (**3**) were obtained as white powders.

Compounds **2** and **3** showed UV λ_{max}^{MeOH} at 283 nm (log ε 4.41 and 4.21, respectively) based on $\alpha,\beta,\gamma,\delta$-unsaturated ketone. The elemental analyses and the mass spectra (**2**, **3** M^+ *m/z* 410) suggested that compounds **2** and **3** were oxidized derivatives of **1**.

^{1}H NMR spectrum of **2** indicated the presence of resonances at δ 7.25 (d, J=16.0 Hz, H-11), 6.28 (d, 16.0, H-10), 5.60 (d, 10.0, H-13), 4.66 (dt, 3.8, 9.8, H-15), 4.24 (dq, 1.8, 7.5, H-19), 4.08 (d, 10.2, H-5) and 3.60 (d, 9.8, H-3). ^{1}H NMR spectrum of **3** was similar to that of **2**, but the signal at δ 4.24 which is observed in **2** was not seen, while downfield shifts of the signals at H-13 (δ 5.85) and H-15 (δ 4.91) and an upfield shift of the signal at H-5 (δ 3.7) were observed. A signal due to methylene proton at C-23 were observed at δ 3.7 ppm, around which H-3 and H-5 were also assigned.

Comparison of ^{13}C NMR spectra of **1**, **2** and **3** presented in Table 1 indicates the presence of a hydroxyl group at C-19 in **2** and at C-23 in **3**. Based upon the above spectroscopic data, the structures of **2** and **3** were assigned as shown in Fig. 1. 19-Hydroxyprotylonolide (**2**) is a new compound. 23-Hydroxyprotylonolide (**3**) has been obtained by chemical transformation of tylo-

Fig. 1. Structures of protylonolide (**1**), 19-hydroxyprotylonolide (**2**), 23-hydroxyprotylonolide (**3**) and 23-hydroxy-5-*O*-desosaminylprotylonolide (**4**).



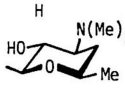
	R_1	R_2	R_3
1	H	H	H
2	H	OH	H
3	H	H	OH
4		H	OH

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Table 1. ^{13}C NMR chemical shifts for protylonolide (1), 19-hydroxyprotylonolide (2) and 23-hydroxyprotylonolide (3).

Carbon No.	1	2	3
1	174.6	174.7	174.5
2	39.4	39.2	39.5
3	66.9	66.7	67.0
4	45.1	44.7	45.0
5	72.7	71.3	72.8
6	38.6	39.6	38.3
7	32.8	29.2	32.8
8	40.0	40.5	40.0
9	203.9	204.1	204.6
10	118.6	118.3	118.8
11	147.7	148.2	147.9
12	133.5	133.6	135.9
13	145.3	145.9	141.7
14	38.7	38.7	47.1
15	78.8	78.7	75.2
16	24.7	24.7	25.4
17	9.5	8.9	9.4
18	9.6	9.6	9.7
19	22.9	67.5	22.8
20	11.8	22.1	11.8
21	17.8	17.7	17.8
22	13.0	13.0	13.1
23	16.2	16.1	62.2

Chemical shifts are given in ppm relative to TMS as internal standard.

sin.[3] Compound **3** and chemically obtained 23-hydroxyprotylonolide were identical in mass and NMR spectroscopy as well as thin-layer chromatography. The hydroxylations of protylonolide at 19 and 23 positions by *Streptomyces* sp. KA-464 are of interest because the former one is not observed in tylosin biosynthesis by *S. fradiae*, while the latter one is an important reaction occurring after, but not before, the mycaminosylation of protylonolide.[4]

This experiment was attempted with the hope of obtaining new daunosaminylated derivatives of protylonolide. However, the desired compounds were not found. It is possible that the daunosamine-binding enzyme is highly specific for the chromophoric aglycone of daunomycin in *Streptomyces* sp. KA-464.

Compound **3** was further desosaminylated into 23-hydroxy-5-*O*-desosaminylprotylonolide (**4**) by a pikromycin-producing strain, *Streptomyces* sp. AM-7762, in the presence of cerulenin, as was the case with protylonolide converted to 5-*O*-desosaminylprotylonolide.[1] The structure of compound **4** was deduced from the mass, UV and ^1H NMR spectral data. The ^1H NMR spectrum of **4** showed the appearance of the resonances at δ 1.81 (s, $N(CH_3)_2$) and 4.23 (d, J=7.5, H-1′) by comparison with those of compound **3**. Moreover, the EI-mass spectrum of **4** showing the molecular ion peak at m/z 567, the aglycone peak at m/z 393, and the sugar peak at m/z 174 (desosamine) strongly indicate that **4** is 23-hydroxy-5-*O*-desosaminylprotylonolide as shown in Fig. 1. Compound **4** is a new macrolide antibiotic.

In contrast, the corresponding 19-hydroxyl derivative compound **2** was not converted by strain AM-7762. It is suggested that the attachment of desosamine to the C-5 position is inhibited by the presence of a hydroxyl group at C-19.

Compounds **2** and **3** have no antibiotic activity, but compound **4** was as active *in vitro* as 5-*O*-desosaminylprotylonolide (M-4365 G_1[5]).

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