Structural Elucidation of a Novel Erythromycin, 13-Cyclopentyl-13-desethyl-erythromycin B, from a Recombinant *Saccharopolyspora erythraea* Strain, NRRL 2338 pIG/1

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In a preceding paper,[1] we described the production and isolation of 13-cyclopentyl-13-desethyl-erythromycin B, **1**, from the genetically modified organism *Saccharopolyspora erythrae* NRRL 2338 pIG/1. This paper describes the NMR studies used to confirm the structure of **1**, and to establish the relative stereochemistry of the substituent at the C-13 position.

The structure of **1** was confirmed by analysis of NMR data, obtained at 500 MHz in $CDCl_3$. ^1H-^1H coupling systems within the molecule were revealed by TOCSY spectra, obtained with 30 ms and 80 ms mixing times. Direct (^1J) and long-range (^2J and ^3J) ^1H-^{13}C correlations were revealed by gradient-selected edited HSQC and gradient-enhanced HMBC experiments respectively. Key HMBC correlations, which enabled the presence of the macrolide ring in **1** to be confirmed, are shown in Figure 1.

The positions of attachment of the cladinose and desosamine sugars were confirmed by virtue of HMBC correlations from H-1′ to C-5 and from H-1″ to C-3. The chemical shifts of C-1′ and C-1″ identified these two carbons as anomeric. The ^1H-^1H coupling systems around these two sugars and the rest of the molecule were identified by TOCSY experiments.

A high resolution electrospray mass spectrum obtained for **1** revealed a sodiated molecular ion with formula $[C_{40}H_{71}NO_{12}Na]^+$. This corresponds to a molecular formula of $C_{40}H_{71}NO_{12}$, which is consistent with the identity of **1** as 13-cyclopentyl-13-desethyl-erythromycin B.

A double-quantum COSY experiment showed that H-13 was coupled to the H-14 signal at 2.15 ppm, and therefore confirmed the linkage of C-13 to C-14. H-14 was then identified as a methine hydrogen, from the edited HSQC experiment. A 1D TOCSY experiment showed that the H-15 ~ H-18 hydrogens also belonged to the coupling system which included H-13 and H-14, and the edited HSQC experiment confirmed that the four carbons C-15, 16, 17 and 18 were all methylenes. Attachment of these four methylenes *via* C-15 to C-14 accounted for all the atoms of the molecular formula of $C_{40}H_{71}NO_{12}$, and cyclisation of the C-18 methylene onto C-14 accounted for all the unfulfilled valencies of the molecule. The structure of **1** was therefore confirmed as 13-cyclopentyl-13-desethyl- erythromycin B. Full ^1H and ^{13}C NMR assignments for **1** are listed in Table 1. These are comparable with those reported in the literature[2] for erythromycin A.

The conformation and relative stereochemistry of **1** was investigated as follows. Erythromycin analogues crystallise in one of two preferred conformations,[3,4] which are also present in the solution state. The preferred solution conformation of a given derivative can be

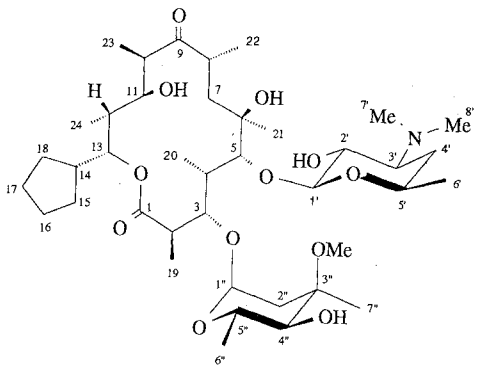

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Fig. 1. Key HMBC correlations of **1**.

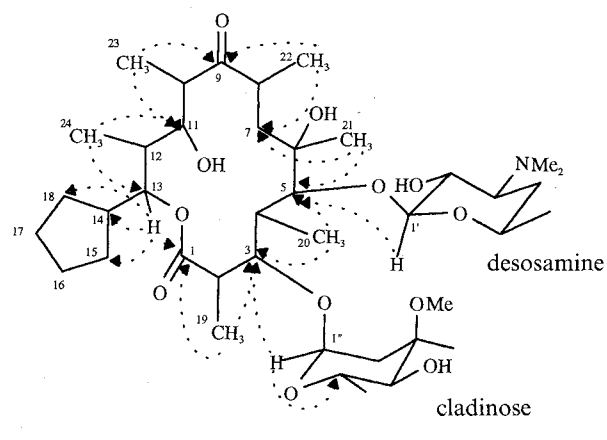
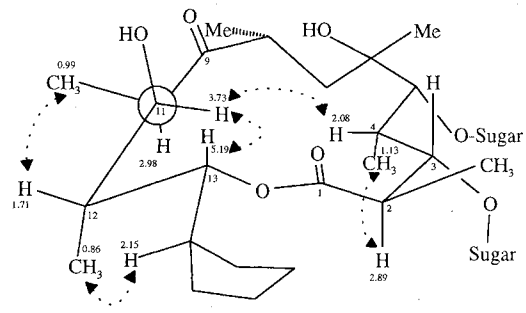


Table 1. ^{1}H and ^{13}C assignments for **1**.

Atom number	^{13}C chemical shift, from ^{13}C NMR spectrum	^{1}H chemical shift and multiplicity
1	176.0	—
2	45.0	2.89 1H dq J=9.4, 7.1
3	80.4	4.02 1H dd J=9.4, 1.7
4	39.2	2.08 1H multiplet
5	83.8	3.59 1H d J=7.4
6	75.4	—
7	38.0	2.00 1H dd J=14.7, 10.8 *ca.* 1.65 1H multiplet
8	45.0	2.71 1H dqd J=10.6, 6.8, 2.6
9	*ca.* 220.0	—
10	38.9[a]	2.98 1H qd J=6.8, 1.5
11	69.4	3.73 1H dd J=9.9, 1.2
12	38.8[a]	1.71 1H multiplet
13	78.3	5.19 1H dd J=10.5, 1.0
14	41.7	2.15 1H br sextet
15	30.4	*ca.* 1.69 1H multiplet *ca.* 1.21 1H multiplet
16	25.4	*ca.* 1.63 1H multiplet *ca.* 1.52 1H multiplet
17	25.1	*ca.* 1.63 1H multiplet *ca.* 1.52 1H multiplet
18	29.0	*ca.* 1.69 1H multiplet *ca.* 1.21 1H multiplet
19	15.8	1.18 3H d J=7.1
20	9.24	1.13 3H d J=7.0
21	27.4	1.46 3H s
22	18.5	1.14 3H d J=6.8
23	9.5	0.99 3H d J=6.8
24	9.16	0.86 3H d J=7.1
1′	103.2	4.43 1H d J=7.3
2′	70.9	3.24 1H dd J=10.3, 7.3
3′	65.4	2.51 1H ddd J=12.0, 10.6, 4.1
4′	29.0	*ca.* 1.68 1H multiplet *ca.* 1.24 1H multiplet
5′	68.8	3.50 1H br sextet
6′	21.5	1.22 3H d J=6.1
7′,8′	40.1	2.32 2×3H s
1″	96.5	4.90 1H d J=4.6
2″	35.1	2.36 1H d J=15.2 +small (<1 Hz) 1.58 1H multiplet
3″	72.6	—
4″	78.0	3.02 1H d J=9.1
5″	65.6	4.01 1H multiplet
6″	18.7	1.29 3H d J=6.3
7″	21.4	1.24 3H s
8″	49.5	3.31 3H s

[a] Assignments for signals with asterisks may be interchangeable.

Fig. 2. Approximate representation of conformation of **1**, showing how observed ROESY correlations are consistent with this conformation.



recognised by key NOE interactions,[5,6] and by analysis of ^{1}H-^{1}H chemical shifts and coupling constants.[7] Thus, in **1**, a ROESY correlation between H-4 and H-11 indicated that **1** adopted a similar molecular conformation to the 'folded out' conformation revealed by X-ray crystallography of erythromycin A hydroiodide dihydrate[3] (Figure 2). No evidence for the presence of the alternative erythromycin conformation[4] (*e.g.* an H-11-H-3 correlation) was seen in the ROESY spectrum. All other observed ROESY correlations and ^{1}H-^{1}H couplings were consistent with **1** existing in a similar conformation to that of erythromycin A hydroiodide dihydrate.[3] A ROESY correlation between H-11 and H-13 enabled the relative stereochemistry at C-13 to be identified as the same as that in the 13-ethyl-erythromycins, as shown Figure 2.

The ROESY and ^{1}H-^{1}H coupling data therefore indicate that the relative stereochemistry of **1** is as shown above.

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