STRUCTURE OF A NEW MACROLIDE ANTIBIOTIC, X-14952B

Sir:

Recently, new macrolide antibiotics possessing various biological activities have been isolated from fermentation broths of streptomycetes.\(^1\) In the course of our search for antimicrobial substances from microorganisms, a new antibacterial antibiotic, X-14952B (1) was isolated from the fermentation broth of a *Streptomyces* sp. In this communication, we wish to report the structure elucidation of 1 by means of \(^1\)H and \(^{13}\)C NMR spectroscopic analyses.

Antibiotic 1 [mp 96~98°C; \([\alpha]_D^{25}\) +79.4° (c 1.0, CHCl\(_3\)); UV \(\lambda_{max}\) nm 230 sh, 280 sh; IR \(\nu_{max}\) cm\(^{-1}\) 3450, 2960, 2940, 2775, 1715, 1607, 1340, 1225, 1075] showed an (M+Na\(^+\)) ion peak at \(m/z\) 802 in the FAB-MS. The molecular formula, \(C_{42}H_{69}NO_{12}\) for 1 was deduced from the FAB-MS, elemental analysis and \(^{13}\)C NMR spectroscopic analyses. The \(^{13}\)C NMR spectrum of 1 demonstrated that 1 is structurally similar to irumamycin (2), the structure of which has been elucidated by ŌMURA et al.\(^2\) The \(^{13}\)C NMR analysis of 1 revealed the presence of nine methyls, nine methylenes, fourteen methines including nine carbons bonded to oxygen, and an anomeric carbon (\(\delta_c\) 98.4), a hemiketal carbon (\(\delta_c\) 94.3), six olefinic carbons, a carbamoyl carbon (\(\delta_c\) 157.7), an ester carbonyl (\(\delta_c\) 173.7) and a ketone carbonyl (\(\delta_c\) 217.5). The appearance of the anomeric and the carbamoyl carbons in addition to five carbons (\(\delta_c\) 37.0 t, 75.2 d, 74.7 d, 72.2 d, and a methyl carbon at \(\delta\) 17.7) arising from the sugar moiety involved in 2 indicated the existence of 3-O-carbamoyl-2-deoxy-\(\beta\)-D-rhamnose in 1. Furthermore, the chemical shift values of the twenty-three carbons arising from the aglycone moiety of 1 were coincident with those of 2. However, both characteristic signals due to the epoxy carbons at \(\delta_c\) 66.4 (d) and 64.6 (s) observed in 2 were not present in 1 but an additional signal due to a carbon bonded to oxygen and a methine were observed at \(\delta_c\) 77.0 (d) and 55.4 (d) respectively. This spectral evidence indicates that 1 possesses the same 20-membered lactone moiety as 2 but differs in the alkyl side chain. The structure of the C\(_{12}\) alkyl side chain of 1 involving four methyls, three methylenes, four methines and a ketone carbonyl, was deduced from 2D-NMR analysis and a retroaldol reaction. Contour plot of the 2D proton-proton shift correlation spectrum of 1 is shown in Fig. 1. As shown in the 2D-NMR spectrum, the newly observed proton signal (\(\delta\) 2.65), which overlaps with the methylene signals at the 2-position, is assignable to the methine proton at C-24 coupled to the methine proton (\(\delta\) 3.55, double doublet) at base of the hydroxyl group at C-23 and the methylene proton (\(\delta\) 1.58). The methine proton at \(\delta\) 3.55 also couples with the methine proton (\(\delta\) 1.53) at C-22 bearing a methyl group. Therefore, an ethyl group should be substituted to C-24 because the aforementioned methylene proton couples with the methyl proton at \(\delta\) 0.83. The location of an ethyl ketone to C-24 was evidenced from a downfield
Fig. 1. 2D Proton-proton shift correlation spectrum of X-14952B.

Table 1. $^{13}$C and $^1$H NMR chemical shift for X-14952B (1).

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$\delta_{\text{C}}^a$</th>
<th>$\delta_{\text{H}}^b$</th>
<th>Carbon No.</th>
<th>$\delta_{\text{C}}^a$</th>
<th>$\delta_{\text{H}}^b$</th>
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<td>1</td>
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<td></td>
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<td>5.7</td>
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<tr>
<td>2</td>
<td>43.6</td>
<td>2.57, 2.67</td>
<td>19</td>
<td>82.2</td>
<td>4.85</td>
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<tr>
<td>3</td>
<td>94.3</td>
<td></td>
<td>20</td>
<td>33.5</td>
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</tr>
<tr>
<td>4</td>
<td>35.3</td>
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<td>0.80</td>
</tr>
<tr>
<td>5</td>
<td>117.0</td>
<td>5.50</td>
<td>21</td>
<td>37.2</td>
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<tr>
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<td>22</td>
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<td>1.97</td>
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$^a$ Measured in CDCl$_3$ at 75 MHz with TMS as an internal standard.

$^b$ Measured in CDCl$_3$ at 400 MHz with TMS as an internal standard.
shift (Δ 6.0 ppm) due to intramolecular hydrogen bonding of the ketone carbonyl with the hydroxyl group at 23-position. The 1H and 13C NMR chemical shifts of 1 assigned from comparative analysis with those of 2 are shown in Table 1. To confirm the structure of the alkyl moiety a retroaldol reaction was carried out on 1. A solution of 1 dissolved in ethanol was heated to reflux with 10% aqueous sodium hydroxide and then partially distilled into a solution of 2,4-dinitrophenylhydrazine hydrochloride (DNPH • HCl). The resulting crystals were identified as 3-hexanone dinitrophenylhydrazone 3 by mp 126~129°C; microanalysis (C12H16N4O4); FAB-MS (MH+ at m/z 281); UV λmax nm (log ε) 228 (4.18), 260 sh, 362 (4.32) and 1H NMR in CDCl3 (H-1, δ 1.04; H-2, 2.42; H-4, 2.45; H-5, 1.70 and H-6, 1.02).

The combined evidence of the 1H, 13C NMR spectra and the retroaldol degradation to 3-hexanone support structure 1 for antibiotic X-14952B, a 20-membered macrolide lactone attached to a neutral sugar and a C12 alkyl side-chain. It is of interest to note that the slight structural difference in the side-chains of 1 and irumamycin (2) result in the former being primarily an antibacterial and the latter an antifungal agent. Similar structure-activity effects are seen in the concanamycins,3,4) virustomycin A,5) bafilomycin6) and L-681,110A,7)

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References