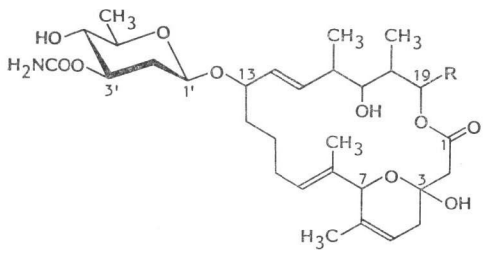
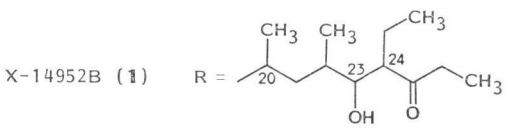
STRUCTURE OF A NEW MACROLIDE ANTIBIOTIC, X-14952B

Sir:

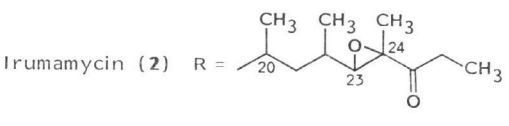
Recently, new macrolide antibiotics possessing various biological activities have been isolated from fermentation broths of streptomycetes.[1] In the course of our search for antimicrobial substances from microorganisms, a new antibacterial antibiotic, X-14952B (**1**) was isolated from the fermentation 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 λ_{max}^{EtOH} nm 230 sh, 280 sh; IR ν_{max}^{KBr} 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. 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 (δ_C 98.4), a hemiketal carbon (δ_C 94.3), six olefinic carbons, a carbamoyl carbon (δ_C 157.7), an ester carbonyl (δ_C 173.7) and a ketone carbonyl (δ_C 217.5). The appearance of the anomeric and the carbamoyl carbons in addition to five carbons (δ_C 37.0 t, 75.2 d, 74.7 d, 72.2 d, and a methyl carbon at δ 17.7) arising from the sugar moiety involved in **2** indicated the existence of 3-*O*-carbamoyl-2-deoxy-β-D-rhamnoside 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 δ_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 δ_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 (δ 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 (δ 3.55, double doublet) at base of the hydroxyl group at C-23 and the methylene proton (δ 1.58). The methine proton at δ 3.55 also couples with the methine proton (δ 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 δ 0.83. The location of an ethyl ketone to C-24 was evidenced from a downfield





X-14952B (**1**)



Irumamycin (**2**)

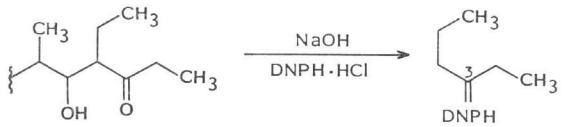


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Fig. 1. 2D Proton-proton shift correlation spectrum of X-14952B.

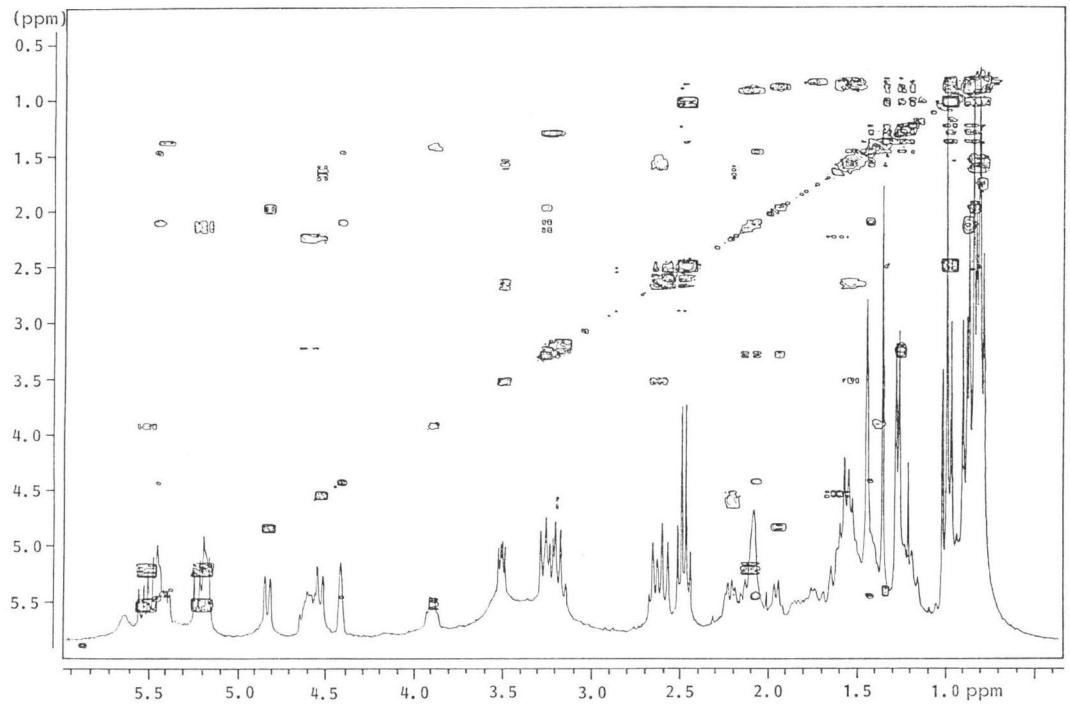

Table 1. ^{13}C and ^{1}H NMR chemical shift for X-14952B (**1**).

Carbon No.	δ_C[a)]	δ_H[b)]	Carbon No.	δ_C[a)]	δ_H[b)]
1	173.7	—	18-CH_3	5.7	0.85
2	43.6	2.57, 2.67	19	82.2	4.85
3	94.3	—	20	33.5	1.75
4	35.3	2.1~2.2	20-CH_3	16.0	0.80
5	117.0	5.50	21	37.2	0.95, 1.20
6	133.7	—	22	32.8	1.53
6-CH_3	19.1	1.49	22-CH_3	12.9	0.83
7	80.3	4.46	23	77.0	3.55
8	135.4	—	24	55.4	2.67
8-CH_3	11.0	1.39	24-$CH_2$$CH_3$	22.9	1.58
9	129.7	5.44	24-CH_2CH_3	12.0	0.84
10	27.3	1.80, 2.10	25	217.5	—
11	26.2	1.2~1.3	26	37.4	2.50
12	35.5	1.49, 1.68	27	7.6	1.00
13	82.6	3.93	1′	98.4	4.57
14	134.8	5.56	2′	37.0	1.68, 2.28
15	134.6	5.24	3′	75.2	4.65
16	42.3	2.13	3′-$OCONH_2$	157.7	
16-CH_3	17.4	0.88	4′	74.7	3.25
17	78.2	3.26	5′	72.2	3.30
18	34.9	1.97	5′-CH_3	17.7	1.32

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 ^{1}H and ^{13}C 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 ($C_{12}H_{16}N_4O_4$); FAB-MS (MH^+ at m/z 281); UV λ_{max}^{EtOH} nm (log ε) 228 (4.18), 260 sh, 362 (4.32) and ^{1}H NMR in $CDCl_3$ (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 ^{1}H, ^{13}C 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 C_{12} 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] bafilomycin[6] and L-681,110A.[7]

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