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CARBON-13 NMR SPECTRA OF RIFAMYCINS

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Abstract—The natural abundance ^{13}C Fourier transform magnetic resonance spectra of rifamycin S and some of its derivatives have been studied. A combination of five different approaches has made unambiguous assignments for most of the resonances possible: (1) comparative study of the non-decoupled and noise-decoupled spectra; (2) ^{13}C spectral characteristics; (3) spectral comparison between derivatives; (4) selective proton decoupling; (5) biogenetic evidence. Pulse and Fourier transform ^{13}C NMR spectroscopy provides a more complete picture of these complex molecules than was previously obtained by ^{1}H NMR spectroscopy.

INTRODUCTION

The rifamycins are a family of antibiotics obtained by fermentation and chemical modification. They have been used successfully in the therapy of diseases caused by gram-positive and gram-negative bacteria and especially in tubercular infections.[1-3] The basic structure of the rifamycins is shown in Fig 1. It fundamentally consists of two parts: a naphthoquinone chromophore which is spanned by an aliphatic bridge called the ansa. In particular, rifamycin B is the one produced by fermentation, on oxidation it gives rifamycin O, which can then be transformed into rifamycin S by hydrolysis with loss of glycolic acid. Rifamycin SV is obtained by the reversible reduction of the latter. The structure of rifamycins was determined independently by: (1) extended chemical degradation of rifamycin S, mainly followed by ^{1}H NMR spectroscopy;[4] (2) X-ray diffraction study of the *p*-iodoanilide of rifamycin B.[5]

The present study shows that ^{13}C NMR spectroscopy is an important technique, in addition to ^{1}H NMR spectroscopy,[6-8] for structure elucidation of new rifamycin derivatives. In fact, the ansa carries many H atoms and has been successfully studied by ^{1}H NMR. On the other hand, the chromophore carries few H atoms and ^{13}C NMR appears the more suitable tool. This work served as an essential prerequisite to the study of biogenesis, which has been reported elsewhere.[9] The use of ^{13}C NMR spectroscopy to detect ^{13}C enrichment at specific positions is a very elegant and powerful aid to the elucidation of biosynthetic pathways.[10-12]

RESULTS AND DISCUSSION

The natural abundance proton noise-decoupled ^{13}C Fourier transform spectrum of rifamycin S in $CDCl_3$ solution is shown in Fig 2. It can be seen that the signals of all 37 C atoms are well separated and spread over a range of about 200 ppm. The assignments reported were made essentially by five approaches:

(1) *Comparative study of the non-decoupled and noise-decoupled spectra.* This approach was possible in this favourable case because the overlap of the components of multiplets, arising from carbon 13-proton coupling, is not too severe. In fact, it allowed not only primary, secondary, tertiary and quaternary carbon atoms to be distinguished, but also the direct coupling constants (^{1}J) and some of the long range ones (^{2}J and ^{3}J) to be obtained. The use of proton off-resonance decoupling confirmed all these attributions.

(2) *^{13}C spectral characteristics.* Data on chemical shifts, coupling constants and steric effects available in the literature[13-15] were used. In particular it has been assumed that there are very significant steric effects in this case, as it is most likely that the rifamycin conformation in solution can be considered as fixed.

(3) *Spectral comparison with other rifamycin derivatives.* This approach proved necessary because of the complexity of the molecule under study.

(4) *Selective proton decoupling.* For the moment this approach has only been used to assign those carbons which had crucial biogenetic implications. The checking of the tentative assignments by this approach will be the subject of a future work.

(5) *Biogenetic evidence.* Examination of the spectra of samples of rifamycin S obtained by fermentation in the presence of ^{13}C enriched precursors made it possible to distinguish between alternative assignments.[9]

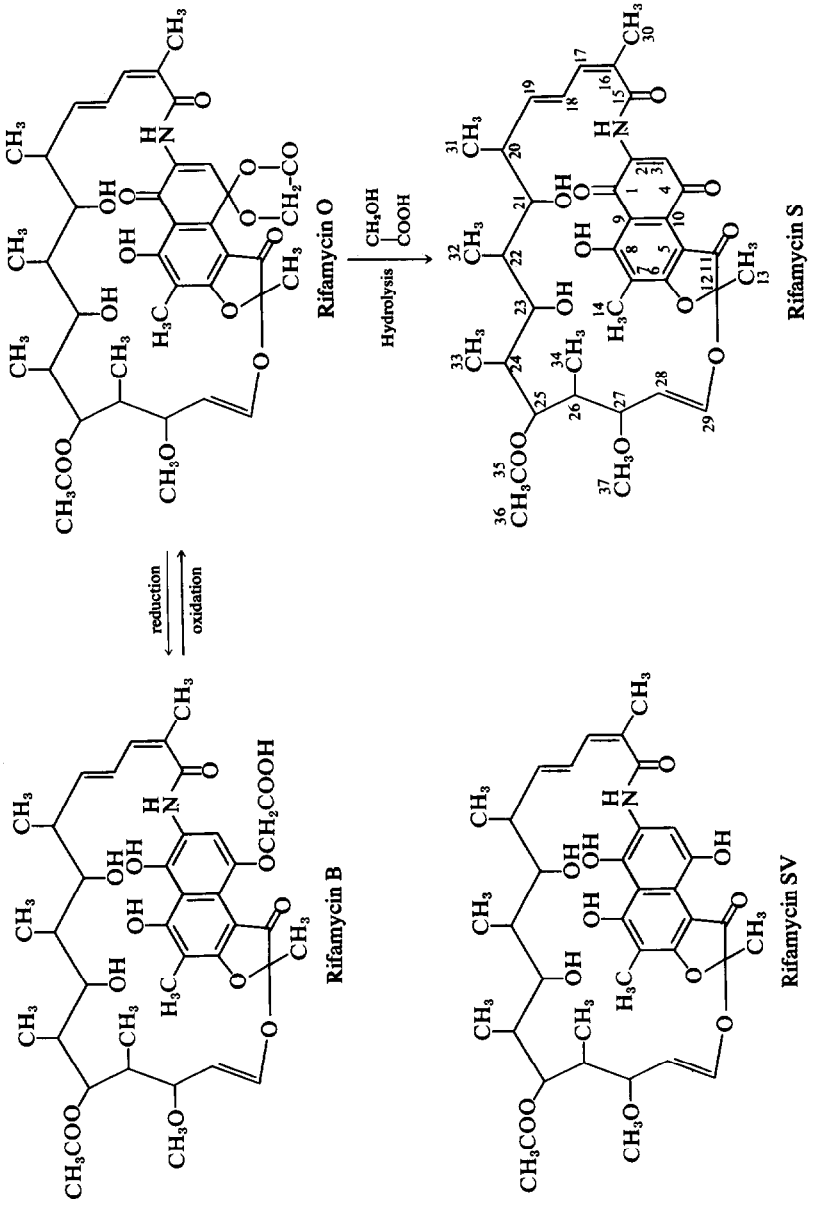

Fig 1. Basic structure of rifamycins.

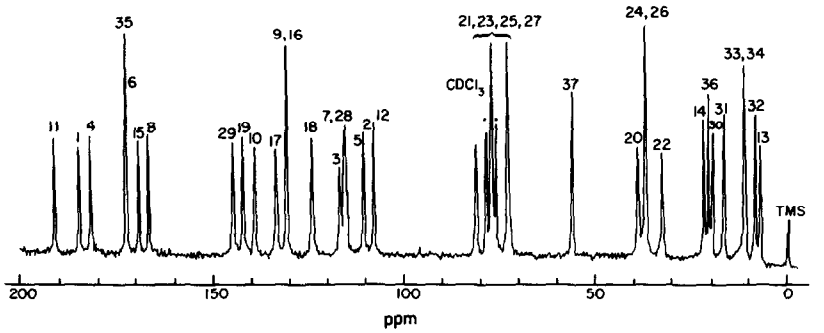

Fig 2. Rifamycin S-^{1}H noise-decoupled FT^{13}C NMR spectrum ($CDCl_3$-60°C-6·25 kHz range).

For discussion's sake the signals (Fig 2) can be divided into four groups characteristic of different types of C atoms. The first region, about 0–50 ppm, characteristic of sp^3 C atoms bonded to C atoms; the second region, about 50–100 ppm, characteristic of sp^3 C atoms bonded to heteroatoms; the third region, about 100–150 ppm, characteristic of sp^2 C atoms not bonded to heteroatoms; the fourth region, about 150–200 ppm, characteristic of CO carbons and aromatic carbons bound to oxygen.

Assignments in the first region (0–50 ppm). The high field 2 kHz range, FT ^{13}C spectrum of rifamycin S is shown in Fig 3. The upper trace refers to the non-decoupled spectrum, the lower trace to the noise-decoupled spectrum. By comparing the two spectra eight quartets could be identified, deriving from the eight Me groups linked to C atoms, namely, C-13, C-14, C-30, C-31, C-32, C-33, C-34 and C-36. It appears that five quartets show further multiplicities, while the remaining three show only ^1J (e.g. C-32 *vs* C-13). Thus, the five quartets correspond to Me groups bound to tertiary C atoms and are C-31, C-32, C-33, C-34 and also C-30 (see later), while the remaining three correspond to Me groups bound to quaternary C atoms and are C-13, C-14 and C-36.

Among the latter ones C-13 was assigned to the higher field signal because it is bound to an sp^3 C atom, C-14 and C-36 to the lower field signals, because they are bound to sp^2 C atoms. As far as

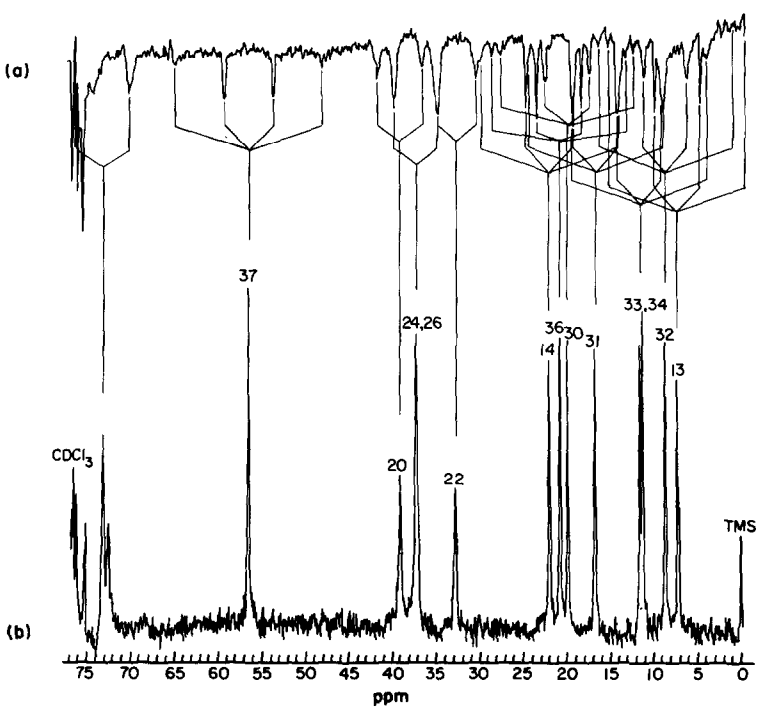

Fig 3. Rifamycin S-Fourier Transform ^{13}C NMR spectrum ($CDCl_3$-60°C-high field 2 kHz range) (a) ^{1}H non-decoupled (b) ^{1}H noise-decoupled.

the resonance of Me-13 is concerned, it has a β-CO group, whose effect relative to the corresponding cycloalkane is to shift its resonance significantly upfield.[18] The chemical shifts of C-14 and C-36 are in agreement with the values observed for the Me resonances of aromatic hydrocarbons[17] and acetyl groups.[18] The choice between them was possible upon examination of the spectrum of 25-desacetyl rifamycin S (5) (Fig. 4), where the signal of C-36 is absent.

Among the other five Me groups which show further multiplicities due to ^2J and ^3J, the signal at lowest field was assigned to C-30 because it is linked to an sp^2 C atom and shows only one further multiplicity, attributed to the *cis* three bond coupling with H-17 (about 5 Hz). The four remaining Me groups 31, 32, 33 and 34 which are spread over an interval of about 8 ppm, cannot be unambiguously attributed without ^1H selective decoupling, i.e. by irradiating the corresponding proton signals.[6] Nonetheless a tentative assignment is proposed. Such an assignment is based on consideration of the "γ effect," i.e. the steric polarization shift which has been observed whenever a carbon is close to a γ-carbon or a γ-oxygen.[14] On the assumption that the conformation of rifamycin S in solution[19] substantially corresponds to that, shown in Fig 5, derived by X-ray analysis,[5] the γ-effect is expected to operate on C-31, C-32, C-33 and C-34 and can be represented by the number and type of groups in γ-position as follows: C-31/C_{22}-H; C-32/C_{20}-H, C_{21}-H, C_{23}-H, C-33/C_{22}-H, C_{26}-H, C_{25}-OAc, C-34/C_{24}-H, C_{25}-H, C_{27}-OMe. The signal at highest field is then tentatively attributed to C-32, and that at the lowest field to C-31; C-33 and C-34 should then resonate at the same field.

Similarly, tentative assignments are proposed for the methine carbons C-20, C-22, C-24 and C-26, which are easily identified from the corresponding doublets in the non-decoupled spectrum. For these carbons the γ-effect can be represented as follows: C-20/Me 32; C-22/Me 31 and Me 33; C-24/Me 34; C-26/Me 33. The signal at highest field is then tentatively attributed to C-22 and that at the lowest to C-20 because it is α to an sp^2 carbon atom. C-24 and C-26 should then resonate at the same field.

Assignments in the second region (80–100 ppm)

The signal of C-37 is easily identified by its multiplicity in the non-decoupled spectrum and by its chemical shift.

The methine carbons linked to oxygen, C-21, C-23, C-25 and C-27, are easily identified being doublets with a large ^1J in the non-decoupled spectrum. Their attribution is not possible without the

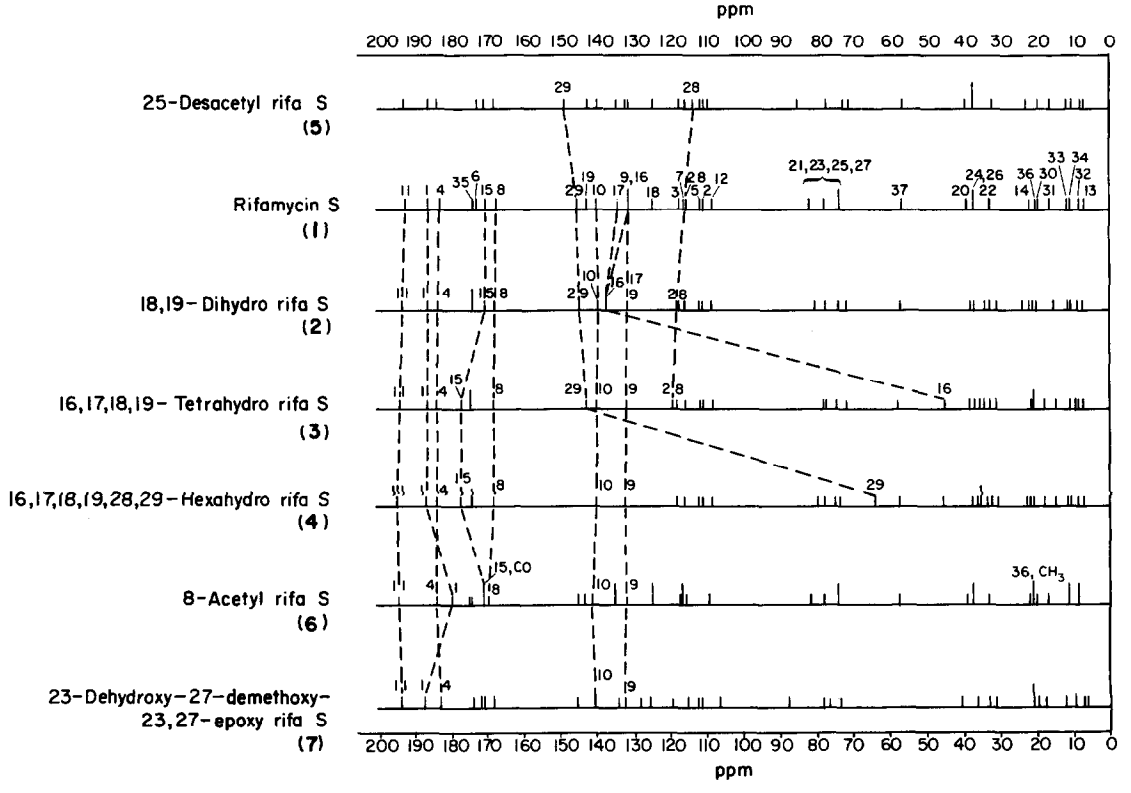

Fig 4. Correlation of ^{13}C chemical shifts for some rifamycin derivatives.

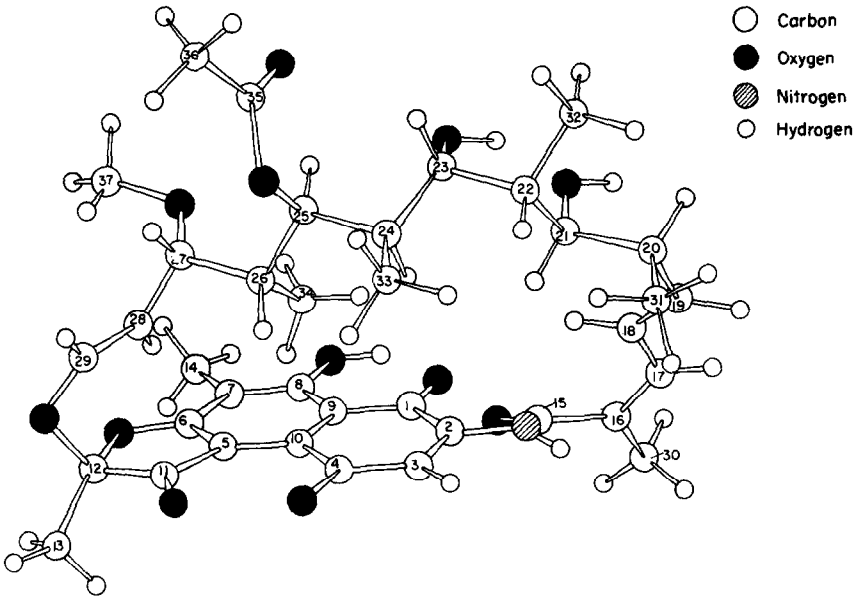

Fig 5. Stereomodel of rifamycin S.

appropriate ^1H selective decoupling which should be decisive as the corresponding proton signals are sufficiently separated.[6]

Assignments in the third region (100–150 ppm)

The low field region, 2·5 kHz range FT ^{13}C spectrum of rifamycin S is shown in Fig 6. The upper trace refers to the non-decoupled, the lower one to the noise-decoupled spectrum. By comparing the two spectra it can be seen that six signals are doublets and the others are fundamentally singlets. From inspection of the structure the

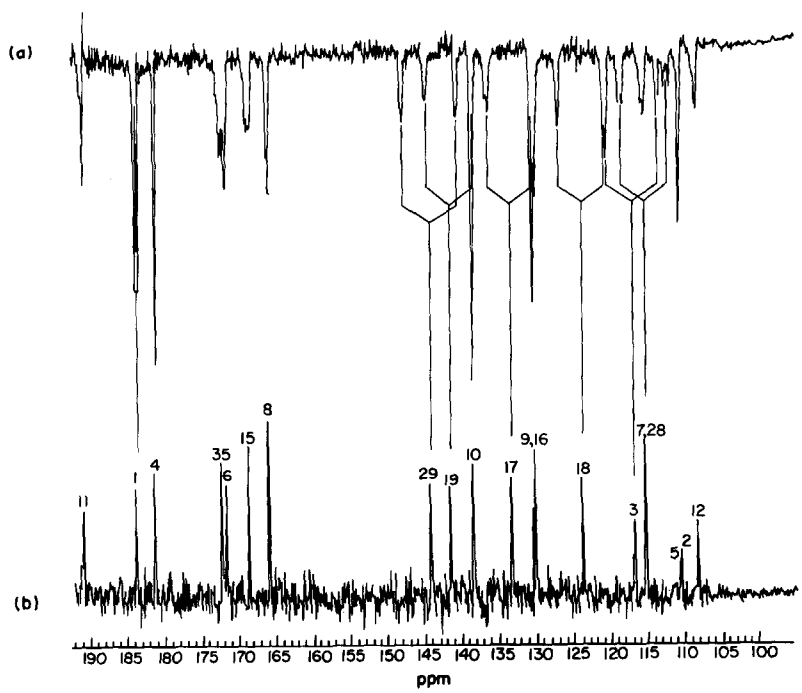

Fig 6. Rifamycin S-Fourier Transform ^{13}C NMR spectrum ($CDCl_3$-60°C-low field 25 kHz range) (a) ^1H non-decoupled (b) ^1H noise-decoupled.

carbons giving rise to doublets must be C-3, C-17, C-18, C-19, C-28 and C-29. The other signals must be C-2, C-5, C-9, C-10, C-16 and C-12.

Among the doublets, C-3 was assigned at 117·1 ppm because it is the only one in this region still present in the spectrum of hexahydro rifamycin S (4) (Fig 4), where the three double bonds of the ansa have been hydrogenated. By comparing this spectrum with that of tetrahydro rifamycin S (3) (Fig 4) two new signals are apparent in this region or the latter. C-29 was assigned to the low field signal and C-28 to the high field signal because the conjugation effect of the enolether group causes a negative charge on C-28, which is shielded.[20] The attribution of the three signals corresponding to carbons C-17, C-18 and C-19 was established as follows: C-17 and C-19 were assigned by ^1H selective decoupling; C-18 by exclusion. It is noteworthy that a comparison of the conjugated system (from C-15 to C-19) of rifamycin S[7] with similar model systems[21–23] indicated an inversion of the attributions for C-17 and C-19 and would have thus led to an incorrect assignment. Nevertheless the literature values for α, β unsaturated carbonyl compounds[24] are in agreement with the chemical shifts observed for carbons C-16 and C-17 in the spectrum of 18, 19-dihydrorifamycin S (2) (Fig 4).

The assignments of the singlets in this region were accomplished using data found in the literature. C-16 was assigned to the signal at 130·5 ppm as it occurs as a broad singlet in the non-decoupled spectrum and because it is shifted downfield in 18,19-dihydrorifamycin S (2) (Fig 4). C-12 is actually an sp^2 C atom and its appearance in this region can be rationalized considering that it has two α oxygens and one α carbonyl. Our assignment is in agreement with correlation data[13,16] and is

Table 1. ^{13}C Chemical shifts of the rifamycin derivatives **1**, **2**, **3**, **4**, **5**, **6** and **7**[a]

Carbon	1	2	3	4	5	6	7
1	184·6	184·7	184·8	185·6	184·6	177·8	185·6
2	110·6	110·6	110·4	110·9	110·5	114·6[g]	110·4[g]
3	117·1	116·9	117·0	117·4	116·6	116·3[g]	119·0
4	182·0	182·1	182·1	183·2	182·0	182·0	181·6
5	110·8	110·9	110·8	111·6	111·0	115·8[g]	110·9[g]
6	172·4	172·8	172·8	173·5	171·7	173·2	172·6
7	115·9	115·3	115·0	115·6	115·4	123·9	114·2
8	166·7	166·7	166·8	167·5	166·8	168·4	166·7
9	130·7	130·7	130·9	131·8	130·6	133·6	131·0
10	139·0	138·8	138·7	139·4	138·8	139·8	139·1
11	191·7	191·5	192·0	193·6	191·2	192·4	192·0
12	108·3	108·0	107·5	108·0	109·0	108·6	105·8
13	7·5	7·6	7·5	7·8	7·5	8·8	8·1
14	22·1	21·7	21·3[e]	20·9[e]	22·9	22·0	21·2[e]
15	169·5	169·6	175·5	176·5	169·3	169·4	169·8
16	130·5	136·6	45·0	45·2	131·2	130·7	127·1[f]
17	133·8	136·6	35·6[d]	30·4[d]	133·2	133·6	133·5[f]
18	124·0	23·5[d]	20·8[e]	21·6[e]	123·6	124·1	124·3[f]
19	142·1	31·0[d]	31·1[d]	32·3[d]	141·6	142·1	139·1
20	38·8	34·2	34·5[d]	33·4[d]	39·7	38·8	40·5[d]
21	73·3[b]	71·5[b]	71·3[b]	73·2[b]	71·1[b]	73·3[b]	73·4[b]
22	32·6	33·0	33·0[d]	35·1[d]	32·4	32·8	31·0[d]
23	73·3[b]	73·7[b]	73·9[b]	74·1[b]	72·3[b]	73·5[b]	76·0[b]
24	37·3	37·9	38·2[d]	35·1[d]	37·6	37·4	32·6[d]
25	77·1[b]	77·3[b]	77·3[b]	77·6[b]	77·1[b]	77·2[b]	77·7[b]
26	37·3	37·1	36·9[d]	35·8[d]	37·6	37·4	35·9[d]
27	81·5[b]	80·0[b]	77·8[b]	79·3[b]	85·1[b]	81·0[b]	87·2[b]
28	115·5	117·6	118·4	37·4[d]	112·8	115·8[g]	111·2[g]
29	144·8	143·8	141·6	63·8	147·7	144·2	144·4
30	20·1	19·9	17·9	18·2	20·1	20·0	20·3[e]
31	16·8	15·2	14·7[c]	15·2[c]	16·5	17·0	17·7[e]
32	8·8	8·6	8·7[c]	9·1[c]	8·2	8·8	7·5[c]
33	11·6	11·4[c]	9·3[c]	10·5[c]	12·4[c]	11·3	9·5[c]
34	11·6	10·7[c]	11·0[c]	11·0[c]	11·0[c]	11·3	12·0[c]
35	173·0	172·8	172·8	173·5	—	172·8	170·2
36	21·0	20·9	21·3[e]	22·3[e]	—	21·0	21·2[e]
37	56·7	56·9	57·5	57·4	56·1	57·0	—

[a]in ppm downfield from TMS as internal standard.
[b,c,d,e,f,g]assignments within each of these groups of signals not known.

corroborated by its appearance as a quartet with a small ^2J. Comparison with data for aromatic compounds[25] shows that C-9 and C-10 should resonate at lower fields than C-5 and C-7. Furthermore, C-9 and C-10 were assigned on the basis of the non-decoupled spectrum (Fig 6) where C-10 appears as a doublet with a small ^3J due to coupling with H-3. C-7 was assigned to the signal at 115·9 ppm because it appears as a quartet and on the basis of biogenetic evidence.[9] Of the two remaining signals in this region, which by exclusion must be C-2 and C-5, the lower field one was assigned to C-5 on the basis of biogenetic evidence.[9]

Assignments in the fourth region (150–200 ppm)

The signal at 173·0 ppm was assigned to C-35 because it is absent in the spectrum of 25-desacetyl rifamycin S (Fig 4) and owing to its multiplicity in the non-decoupled spectrum (Fig 6). The signal at 169·5 ppm was assigned to C-15 on the basis of its chemical shift[26] and considering that it is at the same field in the dihydro derivative and shifted downfield in the tetra and hexahydro derivatives (Fig 4). In fact, conjugation of a carbonyl group with a double bond increases the electron density at the carbonyl carbon with consequent shielding.[24,27]

The remaining five C atoms to be attributed can be divided into two groups: the two aromatic carbons bearing oxygen, which resonate at higher fields and the three carbonyls, which resonate at lower fields.[13] Comparison with data for phenolic compounds indicates that C-8 should resonate at a higher field than C-6 and this was confirmed by biogenetic studies.[9] The signal at 184·6 ppm was assigned to C-1 because it is shifted upfield in the spectrum of 8-acetyl rifamycin S (**6**) (Fig 4). This behavior is interpreted as due to the presence in rifamycin S of the strong intramolecular H-bond between C-8 OH and the acceptor C-1 CO,[28] that induces polarization of the C=O bond with deshielding of C-1. This attribution is corroborated by the multiplicity shown in the non-decoupled spectrum indicating a three bond *trans* coupling constant ^3J of 9 Hz. Consequently, the attribution of C-4 was made on the basis of its structural similarity to C-1 and was placed at 182·0 ppm. Finally, the signal at the lowest field in the spectrum was assigned to C-11, in agreement with the value expected for a cyclopentenone.[29]

The conformation of 23-dehydroxy-27-demethoxy-23,27-epoxy-rifamycin S (**7**) is quite different from that of rifamycin S and we can expect the signals of all the sp^3 carbons of the ansa to move with respect to rifamicyn S because they experience different γ-effects, whilst those of the chromophore do not. Examination of the spectrum clearly confirms such a trend (Fig 4).

On examination of the correlation chart of ^{13}C chemical shifts for the rifamycin derivatives studied one additional aspect is apparent, i.e. the chemical shift difference between C-29 and C-28 decreases on passing from rifamycin S to dihydro and tetrahydro rifamycin S. This can be considered as due to the fact that the more hydrogenated derivatives allow more flexibility to the chain and this is reflected by a decrease of the conjugation in the enolether.

Table 2. One bond (^1J) and some two bond (^2J) and three bond (^3J) coupling constants of rifamycin S[a]

Carbon	^1J	^2J	^3J	Carbon	^1J	^1J	^3J
1	—	—	9	20	130	[b]	[b]
2	—	[b]	—	21	140	[b]	[b]
3	183	—	—	22	122	[b]	[b]
4	—	—	—	23	140	[b]	[b]
5	—	—	—	24	122	[b]	[b]
6	—	—	[b]	25	140	[b]	[b]
7	—	6	—	26	122	[b]	[b]
8	—	—	[b]	27	140	[b]	[b]
9	—	—	—	28	150	6	[b]
10	—	—	4	29	192	6	[b]
11	—	—	[b]	30	128	—	5
12	—	3	—	31	128	[b]	[b]
13	132	—	—	32	128	[b]	[b]
14	131	—	—	33	131	[b]	[b]
15	—	—	[b]	34	131	[b]	[b]
16	—	[b]	[b]	35	—	6	—
17	157	[b]	[b]	36	130	—	—
18	153	[b]	[b]	37	140	—	—
19	159	[b]	[b]				

[a]Values in Hz.
[b]Not determined.

The ^{13}C chemical shifts of rifamycin S (**1**), dihydrorifamycin S (**2**), tetrahydro rifamycin S (**3**), hexahydrorifamycin S (**4**), 25-desacetyl rifamycin S (**5**), 8-acetyl rifamycin S (**6**), and 23-dehydroxy-27-demethoxy-23,27-epoxy rifamycin S (**7**), are reported in Table 1. The one bond coupling constants ^1J and some ^2J and ^3J for rifamycin S are reported in Table 2.

EXPERIMENTAL

The ^{13}C spectra were recorded at 25·15 MHz on a Jeol PS-100 instrument equipped with PFT 100 Fourier transform accessory. This includes deuterium lock and high power noise modulated heteronuclear decoupling, capable of at least a 50 ppm decoupling range. The pulse width for a 90° pulse was 18 μsec, though generally a much shorter pulse (approx. 30°) was employed. The number of accumulations used ranged from 2000 to 50,000, but was normally around 4000. The concentration of the rifamycin samples ranged from 0·3 to 0·6 M.

The rifamycins **1**, **3**, **4** and **7** were prepared according to the procedures of Oppolzer,[28] **5** according to Maggi,[30] while **2** and **6** according to Cricchio.[31]

Acknowledgments—We thank Dr. R. Cricchio for supplying the rifamycins and Mr. K. Eguchi for technical assistance.

Note added in proof. We have recently become aware of a paper by R. Hollenstein and W. von Philipsborn,

Helv. Chim. Acta, **55**, 2030 (1972) reporting ^{13}C NMR spectra of open-chain linear-conjugated dienones: our assignments for C-17 to C-19 are in agreement with those reported by these authors.

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