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STRUCTURE ELUCIDATION OF RP 63834 A NEW MACROCYCLIC LACTONE ANTIBIOTIC

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ABSTRACT

The structure of RP 63834, a new macrocyclic lactone antibiotic, was determined using homo and heteronuclear 2D NMR spectroscopy. The molecular formula $C_{63}H_{111}O_{20}N_3$ agrees with IR, FAB-MS and NMR measurements. The skeletal structure was inferred from the concerted compilation of the results of COSY, HETCOR, HMBC and HOHAHA experiments using a matrix approach. This approach allows consideration of all possible correlations consistent with the experimental data.

INTRODUCTION

RP 63834 is a new macrocyclic lactone antibiotic which was recently isolated in our laboratory from *Streptomyces* strain n° S-13361. A preliminary investigation of its physico-chemical properties indicated that its structure was related to those of azalomycin[1-3], copiamycin[4,5], scopafungin[2,6], niphymycin[7-9] and guanidylfungin[10,11]. The structures of these antibiotics were previously derived by combining substructures assigned to degradation products by ^1H and ^{13}C NMR and by mass spectrometry.

A few years ago, Bax et al [12] used modern two-dimensional NMR techniques to determine the structure of desertomycin, a related antibiotic. These techniques greatly facilitate the structure elucidation of natural compounds. However, in our case, the strong degeneracy of the resonances prevents the unambiguous interpretation of the results of single experiments. In order to overcome this problem, we used a strategy allowing the concerted compilation of the results of homo and heteronuclear 2D NMR experiments. We were thus able to completely elucidate the skeletal structure of RP 63834 by means of four different types of 2D experiments : HETCOR[13], COSY[14], HMBC[15] and HOHAHA[16]. This structure was confirmed by the heteronuclear relay experiment [17].

RESULTS

In a first step, we investigated the physicochemical properties (infrared, mass, ^1H and ^{13}C NMR) of RP 63834 in order to determine its chemical family and its molecular formula.

The infrared spectrum of RP 63834 revealed the presence of a guanidine function (1625 cm^{-1}), of a carboxylic acid salt (1600 and 1380 cm^{-1}) and of ester or lactone groups (1715 cm^{-1} and 1260 cm^{-1}). It showed similarities with those recorded for scopafungin[2,6] and guanidylfungin [10,11] and oriented our research towards this family of compounds.

By FAB as well as D/CI mass spectrometry, RP 63834 gave a MH^+ ion at m/z = 1230. Negative FAB also showed peaks for ions at m/z = 1186, 1144 and 1126, which, by comparision with other compounds of this family were interpreted as : $M^{+\cdot}$-COOH, $M^{+\cdot}$- $COCH_2COOH$ and $M^{+\cdot}$-$OCOCH_2COOH$.

Moreover, the elemental composition has been determined by liquid secondary ion mass spectrometry (LSIMS) at high resolution. The experimental value for $[MH]^+$ (1280.7835) allowed to obtain the molecular formula: $C_{63}\ H_{112}\ O_{20}\ N_3$ ($[MH]^+$). The difference between the experimental and theoretical mass value is of 0.4 mDa. ($[MH]^+$ expected: 1230.7839).

Its ^{13}C spectrum contained peaks for 63 carbons (table I), including three carboxylates, one guanidyl, eight olefinic, one hemiketal, fifteen oxy-methines, seven alkyl-methines, seventeen methylenes, nine methyls and two equivalent N-methyls. The distinction between methines and methylenes was effected by means of the attached proton test[18]. This test also allowed to detect signal superimpositions (for example methines and methylenes at 44.6 and 43.3 ppm.).

The ^1H spectrum revealed the presence of six resolved olefinic protons as well as several signals which are characteristic of this class of compounds. Among others, 2 methylene protons appearing as a singlet at 3.12 ppm were easily deuterated in CD_3OD and thus identified as the methylene protons of the malonyl monoester. As described above, the presence of the malonyl group is confirmed by the observed fragmentation in negative FAB MS spectrum. The multiplet at 5.15 ppm was readily assigned to the proton linked to the carbon bearing the malonyl group by comparison with previously published work [11]. For the same reason the doublet observed at 3.2 ppm was ascribed to the proton on the carbon vicinal to the hemiketal, and the double doublet at 4.62 ppm to the proton on the ester carbon closing the macrocyclic lactone ring. These assignments were confirmed by subsequent experiments. Two equivalent N-methyls at 2.72 ppm, one N-methylene at 3.08 ppm and two methyls linked to unsaturated carbons (1.46 and 1.5 ppm) were also observed. Most CHOH as well as CH_2 and $CHCH_3$ are extensively overlapped.

Because of the strong degeneracy of the ^1H spectrum and of the close proximity of many carbon resonances, it was necessary to combine the results obtained by several different homo and heteronuclear 2D experiments to complete the structure elucidation of this molecule. In a first step, the chemical shifts of the protons directly linked to the carbons were determined by the standard heteronuclear correlation pulse sequence. To facilitate the compilation of the data, each resonance was numbered in order of decreasing ^{13}C chemical shift. Residue numbers, types, as well as their ^{13}C and ^1H chemical shifts are listed in table I. The same labeling was used in the analysis of all subsequent 2D NMR experiments. The results of each experiment were summarized on a matrix in which all the unambiguous correlations were indicated by the letter "U" whereas those which were possible but not certain were indicated by the letter "P". This approach allows consideration of all possible assignents consistent with the available data. New results are used to narrow the range of possible assignments in the previous matrix.

Starting from well resolved ^1H resonances, unambiguous correlations in a COSY[14] experiment allowed the identication of several molecular substructures (I to VI). Substructure I, starting from the N-methylene at 3.08 ppm (n° 38), must include the guanidine residue, since the molecule contains only 3 nitrogens. The presence of 2 N-methyls indicates that this function is dimethylated. In order to determine the position of these methyls another COSY experiment had to be performed in DMSO-d_6. In this solvent, three NH protons, were observed. The NH at 7.4 ppm was correlated with the N-methylene (n° 38) while the signal at 7.5 ppm, integrating for two protons, was correlated with the N-methyls (n° 53). These results suggest that the two methyls are on different nitrogens and that the guanidine is positively charged, most probably forming an internal salt with the malonic acid residue. This interpretation is supported by the observation of signals corresponding to COO^- vibrations at 1600 and 1380 cm^{-1} in the IR spectrum. The coupling network of I is interrupted by a quaternary olefinic carbon (n° 5 or 8).

The structure of II was determined by following the coupling network starting from the double doublet at 4.62 ppm (n° 15), identified as the proton on the ester carbon closing the macrocyclic lactone ring. This coupling network is terminated on one end by the methylene n° 33 which, considering its proton and carbon chemical shifts (1.75 - 1.98 ppm and 45.2 ppm), could be linked to an olefinic carbon (n° 5 or 8). This fragment also includes two conjugated trans-double bonds (J 6-9 = J 10-7 = 14 Hz). At the other end, the degeneracy of the alkyl methine protons (n° 31 or 35) prevents the elucidation of the remainder of this partial structure.

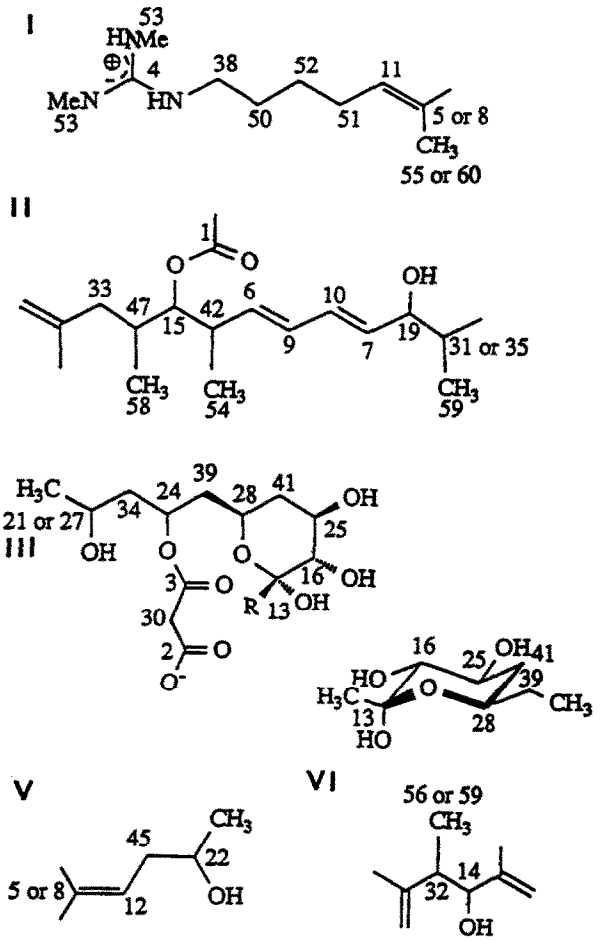
Table I: ^{1}H and ^{13}C Chemical shifts of RP 63834

($\delta\ ^{13}C$ CD_3OD = 49.9 ; $\delta\ ^{1}H$ CD_3OD = 3.2)

^{13}C signal number	^{13}C δ (ppm) & multiplicity		Corresponding ^{1}H δ (ppm) and J (Hz)	Final assignment standard n°
1	177.1	(s)		1
2	173.9	(s)		malonyl
3	171.6	(s)		malonyl
4	157.3	(s)		50
5	137.5	(s)		4
6	136.9	(d)	5.38 (d,d) 14,10	38
7	135.2	(d)	5.58 (d,d) 14,6	35
8	133.9	(s)		43
9	131.9	(d)	5.95 (d,d) 14,10	37
10	131.5	(d)	6.05 (d,d) 14,10	36
11	128.1	(d)	5.08 (t) 6	44
12	127.6	(d)	5.30 (t) 6	5
13	99.7	(s)		20
14	81.5	(d)	3.94 (m)	3
15	79.7	(d)	4.62 (m)	40
16	77.2	(d)	3.20 (d) 10	21
17	76.0	(d)	3.70 (m)	9
18	75.9	(d)	3.69 (m)	11
19	73.6	(d)	4.33 (d,d) 3,6	34
20	72.2	(d)	3.74 (m)	18
21	72.2	(d)	3.81 (m)	13
22	71.8	(d)	3.91 (m)	7
23	71.6	(d)	3.72 (m)	32
24	70.7	(d)	5.15 (m)	26
25	69.7	(d)	3.77 (m)	22
26	66.1	(d)	4.00 (m)	30
27	65.5	(d)	3.80 (m)	28
28	65.4	(d)	3.97 (m)	24
29	46.6	(t)	1.38 (m)	29
30	46.1	(t)	3.12 (s)	malonyl
31	45.8	(d)	1.45 (m)	33
32	45.3	(d)	2.42 (m)	2
33	45.2	(t)	1.76 & 1.98 (m)	42
34	44.6	(t)	1.58 (m)	27
35	44.6	(d)	1.45 (m)	12
36	43.3	(t)	1.38 & 1.5 (m)	31
37	43.3	(d)	1.42 (m)	8
38	42.6	(t)	3.08 (t) 8	48
39	42.0	(t)	1.55 & 1.65 (m)	25
40	41.5	(t)	1.67 & 1.73 (m)	19
41	41.2	(t)	1.20 & 1.80	23
42	41.2	(d)	2.41 (m)	39
43	40.8	(d)	1.50 (m)	17
44	39.2	(t)	1.4 & 1.80 (m)	10
45	34.2	(t)	2.15 (m)	6
46	33.8	(t)	1.25 & 1.48 (m)	14
47	33.1	(d)	1.9 (m)	41
48	30.6	(t)	1.25 (m)	16
49	30.4	(t)	1.25 & 1.32 (m)	15
50	29.9	(t)	1.50 (m)	47
51	28.7	(t)	1.94 (m)	45
52	27.3	(t)	1.28 (m)	46
53	28.4	(q)	2.75 (s)	2 equiv. N methyls
54	17.9	(q)	0.9 (d) 7	39 Me
55	16.0	(q)	1.46 (s)	43 Me
56	15.3	(q)	0.74 (d) 7	2 Me
57	14.7	(q)	0.79 (d) 7	17 Me
58	13.8	(q)	0.78 (d) 7	41 Me
59	11.1	(q)	0.74 (d) 7	33 Me
60	10.7	(q)	1.50 (s)	4 Me
61	10.4	(q)	0.80(d) 7	12 Me
62	10.0	(q)	0.82 (d) 7	8 Me

Substructure III, starting from the doublet at 3.2 ppm (n° 16), ascribed to the methine vicinal to the hemiketal carbon (n° 13), includes the hemiketal ring and the malonic acid residue. The closure of the ring is analogous to what was observed in other molecules of the same family[5-12]. The two vicinal oxymethine protons (n° 16 and 25) are diaxial (J 16-25 = 10 Hz). The chemical shift of proton n° 24 (5.15 ppm) indicates that it is bound to the carbon bearing the malonic acid residue. Once more, the elucidation of the structure beyond methylene n° 34 is prevented by the degeneracy of oxymethine resonances (n° 21 or 27).

Substructure VI is bound on both sides to quaternary carbons, while V is interrupted on one side by a quaternary olefinic carbon (n° 5 or 8) and on the other side, its coupling pattern is highly degenerate. It was therefore necessary to have recourse to heteronuclear experiments in order to complete this structure determination. For sensitivity reasons, we performed long-range heteronuclear correlation experiments in the "reverse" mode using the HMBC sequence[15]. The observed correlations allowed us to confirm the identification of the previously determined fragments and tofurther elucidate the structure (fig. 1, structure fragments inside dashed boxes).



Starting with substructure I, we know from previous experiments that the terminal olefinic CH n° 11 had to be linked to a quaternary carbon (either 5 or 8) bearing a methyl group (55 or 60). Methyl 55 shows correlations with both olefinic car bons 8 and 11 as well as with methylene 33, which is one of the terminal residues of fragment II, we are now able to link I to II. II is terminated at the oxymethine n° 19 (4.43 ppm) which is linked to an alkylmethine at 1.45 ppm i.e. n° 31 or 35. The long-range HMBC shows unambiguous correlations between 19, methine 31 and methyl 59. This methyl also has correlations with oxymethine 23. However it is not possible to progress any further in the coupling network in that direction.

Substructure II also includes residue n° 15, which has been assigned to the ester closing the macrocyclic lactone ring. This interpretation is confirmed by the effective correlation between carbonyl 1 and proton 15. Carbonyl 1 is also correlated to CH n° 32, methyl 56 and CHOH n° 14, thereby also linking fragment VI to fragment II. At the other end of fragment VI, CHOH n° 14 correlates with methyl 60 and olefinic CH n° 12. Methyl 60 itself correlates with C 5 and with CH 12, which is the terminal residue of V. Fragment V is therefore linked to fragment VI. At the other end of V, oxymethine n° 22 shows a correlation with methyl 62. The latter also correlates with CH 37 and either CH 17 or 18.

In substructure III, we were able to link methylene 40 to the hemiketal carbon 13 and to an oxymethine (either 20 or 21). Using only unambiguous correlations, we were not able to complete the structure elucidation. At this point, the matrix approach proved to be especially helpful. Table II shows a fragment of the COSY matrix, including all residues not yet assigned as well as terminal residues of already known fragments, and table III shows the HMBC matrix corresponding to the same residues. The COSY experiment shows correlations only between geminal and vicinal protons, whereas in the case of the HMBC, two bonds and three bonds correlations are in many cases equally intense. Weak longer range correlations can also be observed the in conjugated olefinic systems. Taking into account the logic of each experiment, as well as chemical shift considerations, we underlined in the COSY matrix the results which are also compatible with the HMBC observations.

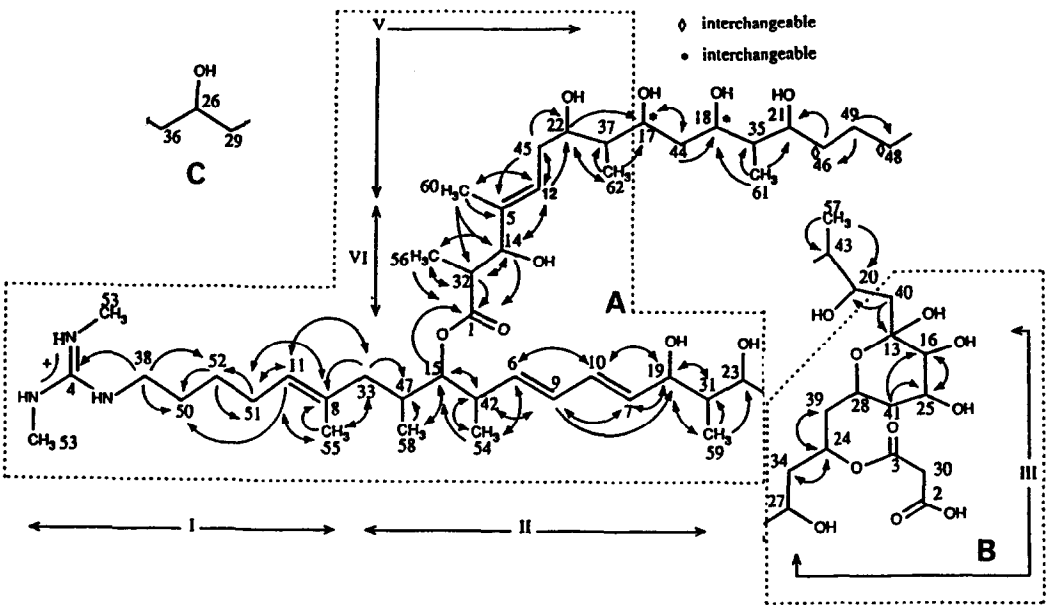


Figure I: Structural fragments of RP 63834 determined using the HMBC experiment. Correlations are indicated by arrows. The regions within dashed boxes were determined using only unambiguous correlations, whereas the remainder of the structure was determined using the matrix approach. The numbering of the atoms refers to table I.

At the loose end of fragment II, we saw that methyl 62, linked to methine 37, had an HMBC correlation to either CHOH 17 or 18. CHOH 17 and 18 are almost equivalent (δ ^{1}H = 3.70 and 3.69 ppm, δ ^{13}C = 76.0 and 75.9 ppm) and therefore should have a very similar structural neighborhood. Looking at our matrix, we can see that these two CHOH could only be linked to methines 35 and/or 37 and/or to methylene 44. Moreover, methylene 44 does not show correlations to any other residues than 17 and/or 18 and has a fairly low field ^{13}C chemical shift (39.2 ppm) suggesting two β effects from both CHOH 17 and 18.

Table II : Portion of the COSY matrix (correlations compatible with both the COSY and HMBC - table III - are underlined).

	17	18	19	20	21	22	23	26	27	29	31	34	35	36	37	40	43	44	46	48	49	57	59	61	62
17	.	.	.	.	.	.	.	.	.	P	P	.	P	P	P	.	.	P	.	.	.	.	.	.	.
18	.	.	.	.	.	.	.	.	.	P	P	.	P	P	P	.	.	P	.	.	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	P	.	P	.	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	.	.	.	.	.	P	P	.	P	P	P	P	P	.	.	.	.	.	.	.	.
21	.	.	.	.	.	.	.	.	.	P	P	P	P	P	P	.	P	.	P	P	P	.	.	.	.
22	.	.	.	.	.	.	.	.	.	P	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.
23	.	.	.	.	.	.	.	.	.	P	P	.	P	P	P	P	P	.	.	.	.	.	.	.	.
26	.	.	.	.	.	.	.	.	.	P	.	.	.	P	.	.	.	.	.	.	.	.	.	.	.
27	.	.	.	.	.	.	.	.	.	P	.	P	.	P	.	.	P	.	P	P	P	.	.	.	.
29	P	P	.	P	P	P	.	P	P	.	.	.	.	.	.	.	P	.	.	.	.	.	.	.	.
31	P	P	P	.	P	.	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	P	.
34	.	.	.	.	P	.	.	.	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
35	P	P	.	.	P	.	.	.	P	.	.	.	.	.	.	.	.	.	.	.	.	.	P	P	.
36	P	P	.	P	P	P	P	P	P	.	.	.	.	.	.	.	P	.	.	.	.	.	.	.	.
37	P	P	.	P	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	U
40	.	.	.	P	.	.	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
43	.	.	.	P	P	.	P	.	P	P	.	.	.	P	.	.	.	.	P	P	P	U	.	.	.
44	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	.	.	.	.	P	.	.	.	P	.	.	.	.	.	.	.	P	.	.	P	P	.	.	.	.
48	.	.	.	.	P	.	.	.	P	.	.	.	.	.	.	.	P	.	P	.	P	.	.	.	.
49	.	.	.	.	P	.	.	.	P	.	.	.	.	.	.	.	P	.	P	P	.	.	.	.	.
57	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.
59	.	.	.	.	.	.	.	.	.	.	P	.	P	.	.	.	.	.	.	.	.	.	.	.	.
61	.	.	.	.	.	.	.	.	.	.	P	.	P	.	.	.	.	.	.	.	.	.	.	.	.
62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.	.	.
	17	18	19	20	21	22	23	26	27	29	31	34	35	36	37	40	43	44	46	48	49	57	59	61	62

Table III : Portion of the HMBC matrix

^{1}H \ ^{13}C	17	18	19	20	21	22	23	26	27	29	31	34	35	36	37	40	43	44	46	48	49	57	59	61	62
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.	.	.	.	U	.	.
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	.
22	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	U
23	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	.
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
44	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	P	.	.	.	.
48	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	P	.	P	.	.	.	.
49	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	.	.	.	.	.
57	.	.	.	P	P	.	.	.	.	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.
59	.	.	U	.	.	.	U	.	.	.	U	.	.	.	.	.	.	.	.	.	.	.	.	.	.
61	P	P	.	P	P	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.	.	.	.	.
62	P	P	.	.	.	P	.	.	.	.	.	.	.	.	U	.	.	.	.	.	.	.	.	.	.
	17	18	19	20	21	22	23	26	27	29	31	34	35	36	37	40	43	44	46	48	49	57	59	61	62

Therefore, methylene 44 is between oxymethines 17 and 18, and the terminal CHOH is itself linked to a $CHCH_3$ (35 and 61). The linkage to 21 is obvious from the matrix. 21 could be linked to either 43, 46, 48 or 49. The fairly high field chemical shifts of 46, 48 and 49 (33.8, 30.6 and 30.4 ppm respectively) suggest weak positive β effects and/or strong negative γ effects. The 46-49-48 substructure is supported by the observation of long-range heteronuclear correlations between one of the geminal protons of 49 (1.32 ppm) and carbons 46 (33.8 ppm) and 48 (30.4 ppm). The only other correlation exhibited by these residues, satisfying both COSY and HMBC conditions, is to 21. The sequence 46-49-48 should therefore be linked on one side to CHOH 21, and on the other, to either 43 or 27.

Substructure III, was terminated on one side on methylene 40, bound to the hemiketal ring. The previous discussion indicates that on the other side, 40 is also linked to CHOH 20, itself bound to $CH43\text{-}CH_3$57. CH43 could be bound to either the 46-49-48 sequence, methylene 29 or 36 or to CHOH 23 or 27. In order to be able to eliminate some of these possibilities, we will pursue the structure determination at the other end of fragment III. Methylene 34 could only be bound to CHOH 21 or 27. As 21 is already positioned, 27 is the only possible solution. From residue 27, further assignement becomes ambiguous. The problem is the same in the case of residue 23 which terminates fragment II. Residues 26, 29 and 36 remain to be positioned. Moreover, if we look at their possible COSY correlations, we see that 26 can only be between 29 and 36.

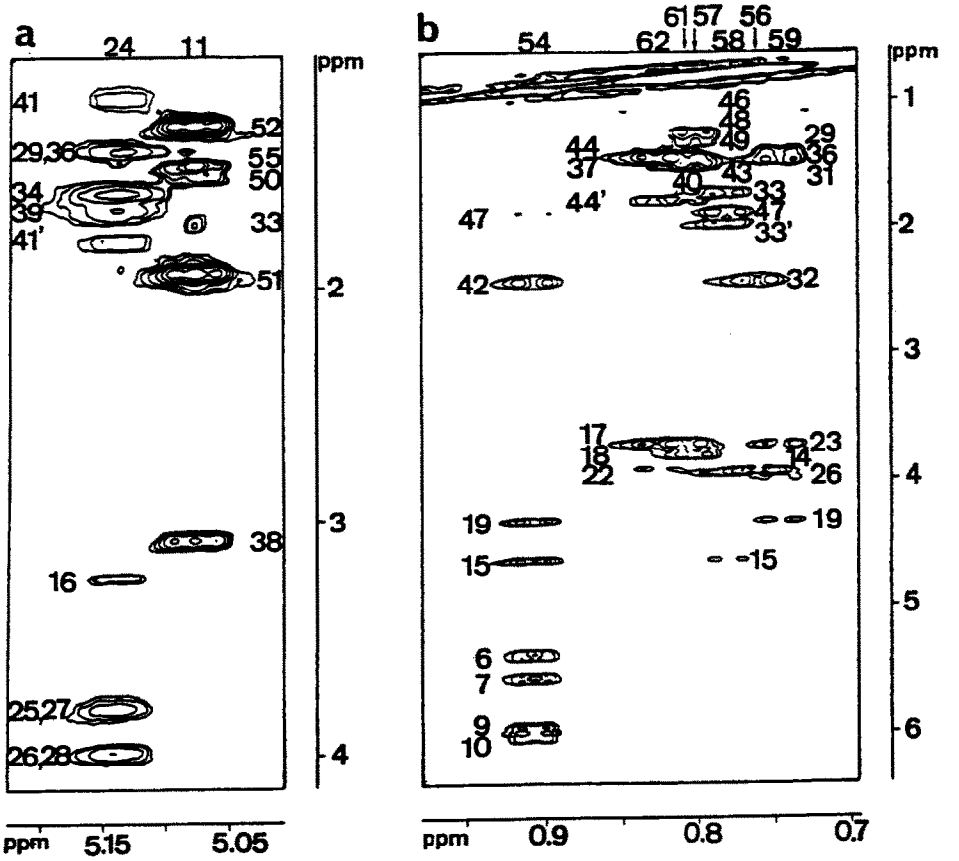


Figure 2: Enlargements of the HOHAHA contour plot (tm = 250 msec), a) region corresponding to residues 24 and 11. b) region corresponding to the methyl resonances.

The above evidence leads to three structural fragments (Fig. 1) : fragment A is terminated on one end by either 46 or 48 and on the other by 23, B is terminated by 43 and 27 and C by 29 and 36. Connection of the three structural fragments, completing the structure elucidation, was achieved by a HOHAHA experiment. Fig. 2B shows an enlargement of the region corresponding to methyl resonances, which is particularly informative, since signals corresponding to these resonances are well resolved. We can see that methyl 57 (which is bound to 43), has relay correlations to 46, 48 and 49, thus establishing the linkage between residues 43 of B and 48 of A. Methyl 59 (on methine 31, vicinal to CHOH 23) has correlations to 23, 29,26 and 36 whereas oxymethine 24 (Fig. 2 A) has correlations to 34, 27 and 29, thus confirming that C is positioned between the two loose ends of A and B, completing the determination of the skeletal structure of RP 63834.

A NOESY [19] experiment confirmed the Z-stereochemistry about the two methylated double bonds. Given the flexibility of the molecule, the determination of the stereochemistry of the remaining chiral centers would require extensive molecular modelling and is beyond the scope of this work. Heteronuclear relays [17], indicated by arrows on fig. 3, are in agreement with the proposed structure.

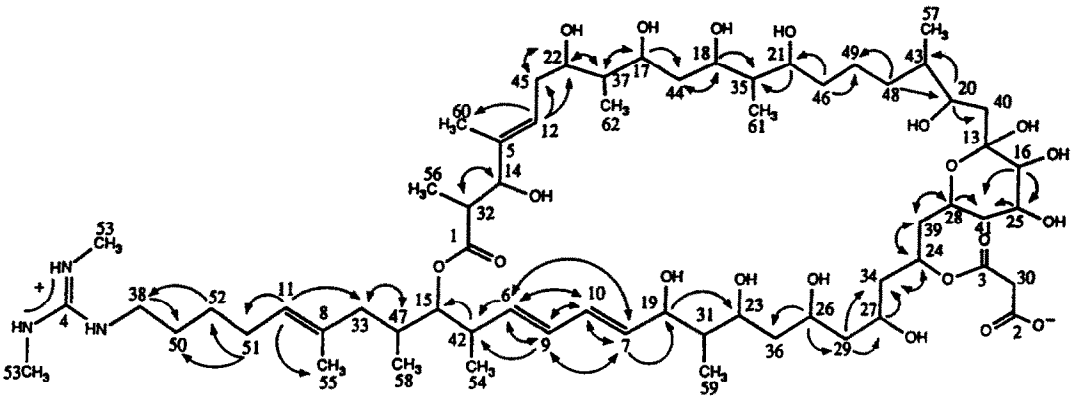


Figure 3: Complete structure of RP 63834. Heteronuclear relays are indicated by arrows.

CONCLUSION

The structure of RP 63834 is closely related to those of other antibiotics of the same family. Its 41 membered macrocyclic polyhydroxyl lactone ring is larger than those of scopafungin, azalomycin and guanidylfungin (36 res.) and copiamycin (32 res.). Similarly to those antibiotics, it has a malonyl monoester, an intramolecular hemiketal ring and a side chain with a terminal guanidine. In the case of RP 63834, contrary to other antibiotics of this class, the guanidyl function is dimethylated.

Unlike the structure determination of other antibiotics in this serie, the structure elucidation of RP 63834 utilized the combination of several 2D NMR experiments. The analysis of the results of these experiments was facilitated by the matrix presentation described above, allowing the consideration of all possible correlations consistent with the experimental data. In a first step, the COSY provides the unambiguous determination of several structural fragments, however, the strong degeneracy of the proton resonances prevents a complete structural elucidation by homonuclear experiments alone. The heteronuclear short-range correlation experiment provides the correspondance between ^{1}H and ^{13}C resonances and allows the interactive compilation of the results from the COSY and HMBC. Indeed, the results from the HMBC allow the elimination of assignment possibilities inconsistent with this new data set and the progressive extension of the structural fragments. Constant interaction between the results of the COSY and the HMBC is necessary in order to be able to distinguish between sequential and longer range correlations in the HMBC experiment and to make decisions when results from the HMBC are ambiguous. These three experiments were sufficient to elucidate the major part of the structure. However, a few linkages remained unresolved. The relays observed using the HOHAHA experiment starting from well resolved resonances allowed a choice to be made between the limited number of possibilities involved at that stage, thus completing the structure determination. This structure is the only one consistent with all the experimental data.

The heteronuclear relay experiment could be used as a final test. It should be noted that this latter experiment was however less informative than the HMBC experiment, since it does not show correlations across quaternary carbons and long-range relays from methyl resonances could not be observed. Instead we had to rely on correlations among often overlapping methines and methylenes. Nevertheless, this experiment confirmed the previous results, since all observed correlations were consistent with the proposed structure (fig. 3). The assignment procedure described above only involves well-defined logical steps and could be automated. It is analogous to the scheme developed by Billeter [21] and al. for semi-automatic sequential resonance assignments in proteins.

MATERIALS & METHODS

NMR. A sample of 80 mg of RP 63834 dissolved in 0.5 ml of $^{12}CD_3OD$ was used unless otherwise stated. All NMR experiments were performed at 30°C on a Bruker AM 400 spectrometer using a $^{1}H/^{13}C$ 5 mm dual probe. ^{1}H homonuclear connectivity networks were determined by COSY [14] and HOHAHA experiments [16]. Several HOHAHA were performed with mixing time ranging between 30 and 400 msec. The ^{13}C spectrum was acquired with WALTZ composite pulse decoupling. ^{13}C resonance multiplicities were determined by the attached proton test[18]. Proton-carbon one bond connectivities were established with the standard heteronuclear 2-dimensional experiment with ^{1}H decoupling in the f_1 domain [13]. The mixing intervals before and after the mixing pulses were set to 3.3 and 1.65 msec respectively. Long range ^{1}H-^{13}C connectivities were detected in the "reverse" mode using the HMBC sequence [15]. Two experiments were performed using mixing delays of 50 and 70 msec. These two experiments were summed. Heteronuclear relayed coherence transfer experiments were also performed in the reverse mode using the sequence developed by LERNER and BAX[17]. The magnetization transfer between coupled protons was effected by the HARTMAN-HAHN mechanism. ^{13}C decoupling was done by composite pulse decoupling using the GARP1 sequence[20] during the entire acquisition period. Mixing times of 30 and 70 msec were used and the two experiments were summed. The NOESY experiment [19] was performed with a mixing time of 250 msec.

MASS SPECTROMETRY. FAB (Xe, 8 Kv) spectra were obtained in a glycerol-thioglycerol matrix on a Kratos AEI MS 50. D/CI (reactant gas NH_3) spectra were recorded on a Finnigan triple stage quadrupole TSQ 46. Mass determination by high resolution was performed on a VG Autospec (LSIMS, resolution 10,000, matrix: meta-benzyl alcohol, V-scan mode). Nosiheptide ($[MH]^+$ = 1222.1556) and ranatensin ($[MH]^+$ = 1281.6202) were used as references.

IR SPECTROSCOPY. IR spectra were obtained in methanol (FMIR) on a FT spectrometer Nicolet 60 SXR.

REFERENCES

(1) Iwasaki, S.; Namikoshi, M.; Sasaki, K.; Fukushima, K. and Okuda S.; Chem. Pharm. Bull, 1982, 30, 4006-4014.

(2) Iwasaki, S.; Namikoshi, M.; Sasaki, K.; Yano, M.; Fukushima, K.; Nozoe, S. and Okuda,S.; Chem. Pharm. Bull.,1982, 30, 1669-1673.

(3) Takesako, K.; Nakamura, T.; Obayashi, A.; Iwasaki, S.; Namikoshi, M.; Okuda, S.; Beppu, T.; J. Antibiotics, 1986, 39, 713-716.

(4) Fukushima, K.; Arai, T.; Iwasaki, S.; Namikoshi, M.; and Okuda S.; J.Antibiotics 1982, 35, 1480-1494.

(5) Arai, T.; Uno, J.; Horimi, I.; Fukushima, K.; J. Antibiotics, 1984, 37, 103-109.

(6) Samain, D.; Cook, J.C. Jr.; Rinehart, K.L. Jr.; J. Am. Chem. Soc., 1982, 104, 4129-4141.

(7) Bassi, L.; Joos, B.; Gassmann, P.; Kaiser, H.P.; Leuenberger, H. and Keller-Schierlein W.; Helv. Chem. Acta, 1983, 66, 92-117.

(8) Keller-Schierlein W.; Joos, B.; Kaiser, H.P. and Gassmann, P.; Helv. Chem. Acta, 1983, 66, 226-258.

(9) Gassmann, P.; Hagmann, L.; Keller-Schierlein W. and Samain, D.; Helv.Chem.Acta, 1984, 67, 696-705.

(10) Takesako, K. and Beppu, T.; J. Antibiotics, 1984, 37, 1161-1169.

(11) Takesako, K. and Beppu, T.; J. Antibiotics, 1984, 37, 1170-1186.

(12) Bax, A.; Aszalos, A.; Dinya, Z.; Sudo, K.; J. Am. Chem. Soc., 1986, 108, 8056-8063.

(13) Bax, A.; J. Magn. Reson. 1983, 53, 517-520.

(14) Marion, D.; Wüthrich, K.; Biochem. Biophys. Res. Comm., 1983, 113, 967-974.

(15) Bax, A. and Summers, M.F.; J. Am. Chem. Soc., 1986, 108, 2093-2094.

(16) Bax, A.; Davis, D.G.; J. Magn. Reson. 1985, 65, 355-360.

(17) Lerner, L. and Bax, A.; J. Magn. Reson., 1986, 69, 375-380.

(18) Patt, S.L. and Shoolery, J.N.; J. Magn. Reson., 1985, 46, 535-539.

(19) Meier, B.H. and Ernst, R.R.; J. Am. Chem. Soc., 1979, 101, 6441-6442.

(20) Shaka, A.J.; Barker, P.B.; Freeman, R.; J. Magn. Reson., 1985, 64, 547-552.

(21) Billeter, M.; Basus, V.J.; Kuntz, I.D.; J. Magn. Reson., 1988, 76, 400-415.