REVISED NMR ASSIGNMENTS FOR RAPAMYCIN

Sir:

Recently, considerable interest has been given to the antifungal antibiotic, rapamycin,[1,2] because of its immunosuppressant properties[3] and its clear structural relationship to FK-506. FK-506 and rapamycin strongly inhibit the peptidyl-prolyl isomerase, FK-506-binding protein,[4] and this may be the crux of the mechanism of their immunosuppressive properties. Despite the strong similarity in the structures of the presumed pharmacophores of these two entities, differences exist in their biological effects. Whereas FK-506 inhibits production of IL-2 and the IL-2 receptor, rapamycin appears to inhibit neither.[5]

The structure of rapamycin was deduced by X-ray analysis[6] and a detailed NMR analysis has been published.[7] In $CDCl_3$, rapamycin exists as a mixture of conformers in a ratio of about 3 : 1. This further complicates already complex spectra, particularly the proton spectrum. An investigation of proton and carbon spectra of rapamycin indicated that many of the assignments made in 1980 (without the benefit of the modern 2D methods) were incorrect. With the aid of COSY, HETCOR and DEPT experiments we have reassigned the proton and carbon spectra from basic principles. The results are presented in Table 1.

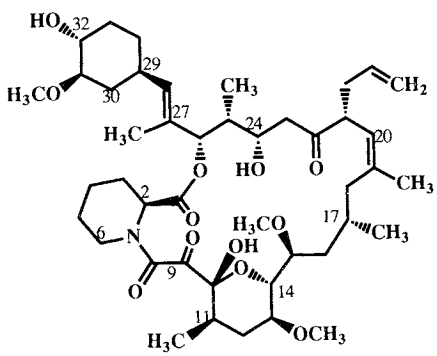
The numbering system used here is that commonly used for macrolides and which has been used for FK-506.[8] It is different from that used previously for rapamycin but the authors believe it is preferable. Assignments listed in Table 1 take account of the difference in numbering systems. Although many of the reassignments, especially in the carbon spectra, are trite, for example, the interchange in the assignment of peaks at 27.0 and 27.3 ppm to C-3 and C-12, others are of considerable significance such as the resonances assigned to C-19, C-20, C-22 and C-30 and the *C*- and *O*-methyl carbons.

These data will be of considerable significance to future work on the biosynthesis and derivatization of rapamycin.

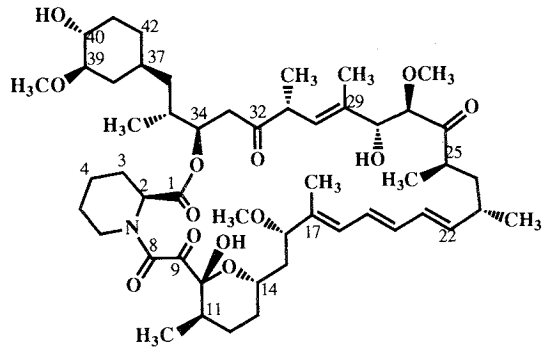
Experimental

Rapamycin was produced by fermentation of *Streptomyces hygroscopicus* ATCC 29253 using the media and conditions previously described.[9] Antibiotic was isolated from the fermentation broth by addition of polystyrene resin, Amberlite XAD-16, at 10%. The mixture was stirred for 2 hours, centrifuged and decanted to obtain a mixed paste of resin and mycelia. This was extracted twice with two volumes of acetone. The combined acetone extracts were concentrated under reduced pressure to an aqueous slurry which was further extracted twice with toluene. Emulsions were broken by the addition of acetone (*ca.* 5%) to the mixture. The toluene layer was concentrated and the oily residue was chromatographed over silica gel developed with a gradient from hexane and acetone 1 : 1 to 100% acetone. Rapamycin-containing fractions were pooled, concentrated and rechromatographed over Sephadex LH-20 in methanol. Fractions containing rapamycin were combined and rechromatographed on Sephadex LH-20 in chloroform - heptane - ethanol (10 : 10 : 1). Fractions containing only rapamycin, as analysed by TLC (Merck Kieselgel 60 HF_{254} developed in hexane - acetone, 1 : 1) were combined and concentrated to a solid residue which was used for these NMR experiments.

NMR experiments were carried out on a General Electric GN500 spectrometer in $CDCl_3$ with TMS



FK-506



Rapamycin

Table 1. Reassigned chemical shifts for the carbons and protons of rapamycin.

Carbon No.	Carbon type	Major carbon	Minor carbon	Major proton	Minor proton	Previous major carbon	Assignments[7] major proton
1	C=O	169.2				169.2	
2	CH	51.3	56.2	5.29	4.29	51.4	5.26
3	CH_2	27.0		2.34, 1.76	?, 1.84	27.3	1.92, 1.60
4	CH_2	20.6		1.78, 1.47		20.6	
5	CH_2	25.3		1.75, 1.48		25.3	
6	CH_2	44.2	39.8	3.59, 3.44	4.44, 3.2	44.2	3.56, 3.40
8	C=O	166.8				166.8	
9	C=O	192.5				192.5	
10	O-C-OH	98.5	98.8			98.5	
11	CH	33.7		1.98		33.2	1.95
12	CH_2	27.3		1.60, 1.60		27.0	2.32, 1.45
13	CH_2	31.3		1.62, 1.33	1.53, ?	38.4	
14	CH-OC	67.2	67.8	3.86	3.79	67.2	3.85
15	CH_2	38.8	38.8	1.85, 1.52	1.87, 1.58	38.8	1.73
16	$CH-OCH_3$	84.4	84.4	3.67	3.61	84.4	3.64
17	C=C	135.5				135.5	
18	CH=C	129.6	129.2	5.97	5.89	129.6	5.94
19	CH=C	126.4		6.39		133.6	6.28
20	CH=C	133.6	133.6	6.32	6.25	126.4	6.36
21	CH=C	130.1	129.9	6.15	6.15	130.2	6.12
22	CH=C	140.2	140.9	5.54	5.51	126.7	5.51
23	CH	35.2	35.6	2.32	2.30	35.2	2.29
24	CH_2	40.2	40.2	1.50, 1.20	1.47, 1.20	31.6	1.83, 1.68
25	CH	41.4	41.0	2.74	2.87	41.4	2.72
26	C=O	215.6				215.5	
27	$CH-OCH_3$	84.9	86.3	3.71	3.67	84.9	3.70
28	CH-OH	77.3	77.2	4.17	4.20	77.2	4.15
29	C=C	136.1				136.1	
30	CH=C	126.8	126.8	5.42	5.49	140.1	5.38
31	CH	46.6		3.33		46.6	3.30
32	C=O	208.2				208.1	
33	CH_2	40.7	41.0	2.74, 2.60	2.87, 2.74	40.7	2.72, 2.56
34	CH-OCO	75.7		5.17	5.12	75.7	5.15
35	CH	33.1		1.98	2.01	33.7	
36	CH_2	38.4		1.22, 1.12		40.2	
37	CH	33.2		1.39	1.38	33.2	1.50
38	CH_2	34.2	34.2	2.10, 0.68	2.09, 0.64	34.2	2.08, 0.64
39	$CH-OCH_3$	84.4	84.4	2.93	2.90	84.4	2.91
40	CH-OH	73.9	73.9	3.37	3.35	73.9	3.35
41	CH_2	31.3		1.99, 1.33	1.97, 1.30	31.3	
42	CH_2	31.7		1.70, 1.00		31.3	
43	11-CH_3	16.2		0.95		13.8	0.92
44	17-CH_3	10.2		1.65	1.74	13.0	1.62
45	23-CH_3	21.5		1.05		15.9	1.02
46	25-CH_3	13.8		1.00		21.5	0.96
47	29-CH_3	13.0		1.74	1.65	10.1	1.72
48	31-CH_3	16.0		1.11		15.9	1.06
49	35-CH_3	15.9		0.92		16.2	0.89
50	16-OCH_3	55.8		3.13		59.4	3.32
51	27-OCH_3	59.5		3.34	3.40	56.5	3.39
52	39-OCH_3	56.5		3.41	3.36	55.8	3.11

as external reference. ^{1}H NMR spectra, COSY, HETCOR and LRHETCOR were measured at 499.96 MHz. ^{13}C NMR spectra and DEPT experiments were performed at 125.64 MHz.

Addendum in Proof

After this note had been accepted, a paper on the biosynthesis of rapamycin appeared. (N. L. PAIVA, A. L. DEMAIN and M. F. ROBERTS: J. Nat. Products. 54: 167~177, 1991) It included several reassignments for the ^{13}C NMR signals of the major rotamer of rapamycin. We agree with all of the reassignments made but our results indicate that seven more carbons still require revision. These include interchanges between the three methoxyl carbons, and interchanges between the pairs of carbons 11 and 35, 3 and 12. This last interchange is particularly significant to the interpretation of the results of the biosynthetic experiments. The authors had difficulty in finding a precursor to carbons C-12, C-13, postulated as part of a polyketide chain. These new assignments indicate that 1-[^{13}C]-acetate led to significant enrichment (to 2.4%) in C-12. Enrichment into C-13 is more difficult to ascertain because signals for this carbon and C-41 are coincident in this solvent.

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JAMES B. MCALPINE
SUSAN J. SWANSON
MARIANNA JACKSON
DAVID N. WHITTERN

Bioactive Microbial Metabolites Project,
Pharmaceutical Products Division,
AP-9A Abbott Laboratories,
Abbott Park, Illinois 60064-3500, U.S.A.

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