Novel Long-range Isotope Effects in a Macrolide Antibiotic: Bafilomycin A_1

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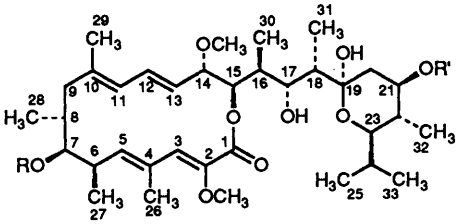
The ^1H and ^{13}C NMR spectra of bafilomycin A_1 (**1**), 21-*O*-trimethylsilylbafilomycin A_1 (**2**) and 7-*O*-acetyl-21-*O*-t-butyldimethylsilylbafilomycin A_1 (**3**) have been unambiguously solved in a variety of solvents by 1D and 2D NMR techniques. Partial deuteriation of the hydroxy groups of (**1**), (**2**), and (**3**) led to the observation of many novel and long-range isotope effects in the ^1H and ^{13}C spectra of (**1**), (**2**), and (**3**). These experiments also confirmed the existence in solution of the hydrogen-bonding network involving 19-OH, 17-OH, and C(1)=O for (**1**), (**2**), and (**3**), as was found in the crystalline state for (**1**). The possible mechanisms of the isotope effects are discussed.

The 16-membered diene macrolides known as the hygrolides possess bactericidal, fungicidal, antitumour, and antiparasitic activity.[1-12] The first hygrolide crystal structure—that of bafilomycin A_1 (**1**)—has recently been reported[13] and revealed



(**1**) R = R′ = H
(**2**) R = H; R′ = $Si(CH_3)_3$
(**3**) R = Ac; R′ = $SiBu^tMe_2$

the presence of a hydrogen bonding network involving 19-OH, 17-OH, and C(1)=O (Figure 1). The solution-state conformations of bafilomycin A_1 (**1**) were shown[14] to be very similar to the crystalline-state conformation and indirect evidence of the existence of the hydrogen-bonding network was obtained. Since the hydrogen-bonding may be important for the biological activity of (**1**), direct evidence of its existence in solution was sought.

The research groups of Lemieux[15] and Davies[16] have shown that the partial deuteriation of hydroxy groups involved in hydrogen-bonding leads to isotope effects ($^n\Delta$, n = number of bonds† over which Δ occurs) on the ^1H NMR resonances of the OH protons themselves (SIMPLE NMR). Similar effects have also been observed on the resonances of ^{13}C nuclei up to six bonds removed from the site of deuteriation.[17] This paper reports SIMPLE ^1H and ^{13}C NMR experiments on (**1**) and two derivatives (**2**) and (**3**), in a number of solvents. These studies not only confirmed the existence of the hydrogen-bonding network in (**1**), (**2**), and (**3**) in solution but also revealed a number of novel long-range NMR isotope effects. A preliminary report of the work on (**1**) has appeared.[18]

Experimental

Bafilomycin A_1 (**1**).—Compound (**1**) was obtained by fermentation as previously described.[14]

21-O-*Trimethylsilylbafilomycin* A_1 (**2**).—To a cooled solution of bafilomycin A_1 (50 mg, 0.08 mmol) in anhydrous pyridine (5 cm^3) at −20 °C was added chlorotrimethylsilane (60 mm^3, 0.64 mmol). The mixture was stirred for 15 min and evaporated to dryness. Anhydrous diethyl ether (10 cm^3) was added and the solution was filtered to remove pyridine hydrochloride. After evaporation the product was purified by preparative thin layer chromatography (alumina plates eluted with hexane–diethyl ether) to give the title compound (35 mg). $\delta_C[(CD_3)_2SO]$ see Table 1.

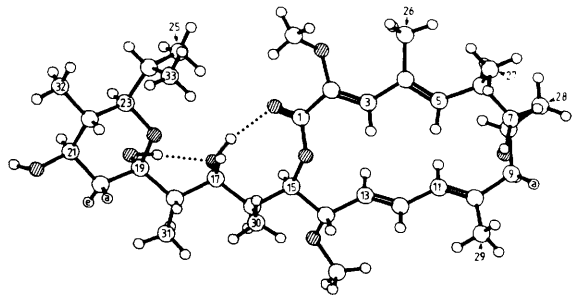


Figure 1. View of the crystal structure of bafilomycin A_1 (**1**). Oxygen atoms are shaded; the two hydrogen bonds connecting 19-OH, 17-OH, and 1-O are shown as dotted lines.

21-O-*t-Butyldimethylsilylbafilomycin* A_1.—To an ice-cooled solution of bafilomycin A_1 (400 mg, 0.64 mmol) and triethylamine (0.44 cm^3, 6.0 mmol) in dichloromethane (5 cm^3) was added dropwise t-butyl dimethylsilyl trifluoromethanesulphonate (0.3 cm^3, 1.0 mmol). The mixture was stirred at 0 °C for 10 min, after which an aqueous solution of sodium hydrogen carbonate (5 cm^3) was added. After separation, the aqueous layer was extracted with dichloromethane (2 × 10 cm^3) and the combined organic extracts were washed with water (25 cm^3), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (silica eluted with hexane–diethyl ether) to give the title compound (250 mg). *m/z* (FAB Na^+/NOBA) (relative intensity) 759 $[MNa]^+$ (63%); $\delta_C(CD_2Cl_2)$, 167.0, 142.9, 142.8, 141.0, 133.2, 132.9, 132.6, 126.6, 124.8, 98.5, 82.1, 80.7, 76.5, 75.6, 71.4, 70.4, 59.5, 55.1, 43.8, 41.8, 41.1, 41.0, 39.9, 37.0, 36.5, 27.8, 25.4, 21.1, 20.8, 19.6, 17.6, 16.7, 13.7, 13.4, 12.1, 9.3, 6.5, −4.6, and −5.2.

† For consistency of notation, n = number of formal bonds (excluding hydrogen bonds) between the two nuclei.

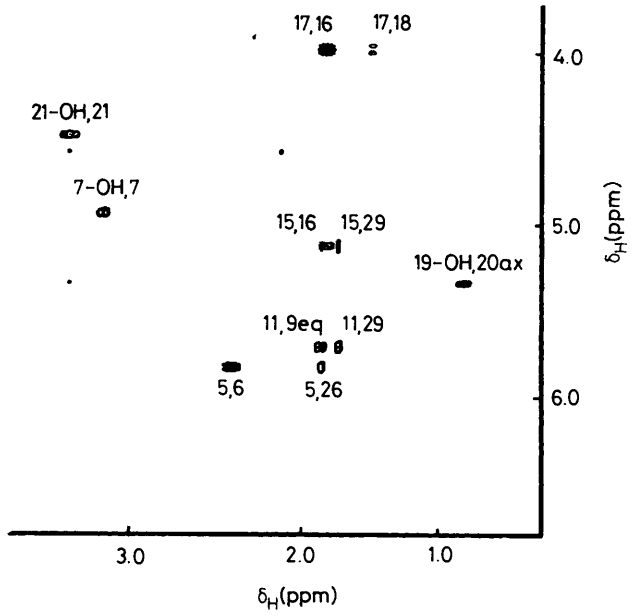


Figure 2. A contour plot of part of the 2D ^{1}H COSY-45 NMR spectrum of (**1**) in $(CD_3)_2SO$. The proton-to-proton scalar connectivity giving rise to the cross-peaks is indicated.

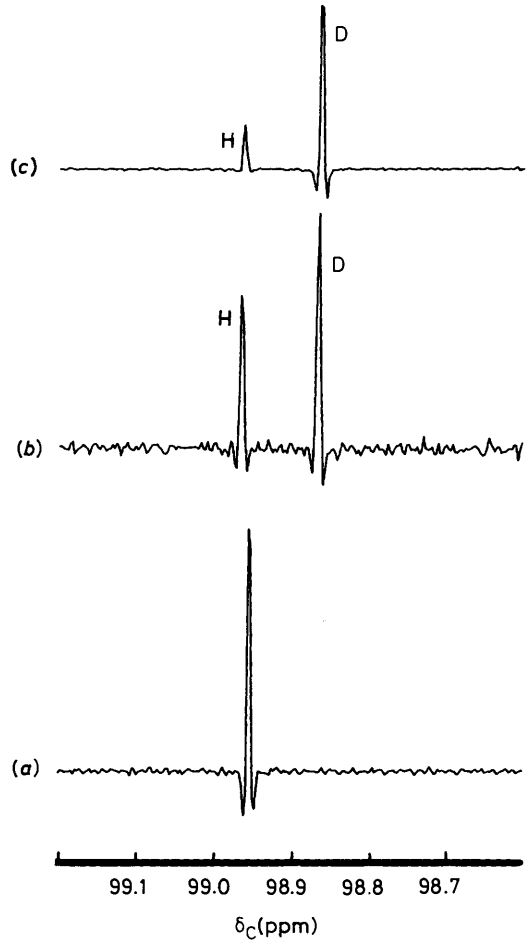


Figure 3. An expansion of the ^{13}C NMR spectrum of (**1**) in $CDCl_3$ in the region of the resonance of C-19 (*a*) control; (*b*) +7.5 mm^3 D_2O; (*c*) +17.5 mm^3 D_2O.

7-O-Acetyl-21-O-t-butyldimethylsilylbafilomycin A_1 (**3**).—To a solution of 21-t-butyldimethylsilylbafilomycin A_1 (50 mg, 0.068 mmol), triethylamine (250 mm^3, 3.4 mmol) and 4-dimethylaminopyridine (catalytic) in dichloromethane (10 cm^3) was added acetic anhydride (100 mm^3, 1.0 mmol). The reaction was stirred at 40 °C for two weeks, after which aqueous sodium hydrogen carbonate solution (10 cm^3) was added to the mixture and the two layers were separated. The aqueous layer was extracted with dichloromethane and the combined extracts were washed with water (25 cm^3), dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (silica eluted with hexane–diethyl ether) to give the title compound (42 mg). *m/z* (FAB Na^+/NOBA) (relative intensity) 801 $[M\text{Na}]^+$ (100%); $\delta_C(CD_2Cl_2)$ 171.4, 167.5, 143.0, 142.0, 141.8, 133.7, 133.5, 133.2, 127.6, 125.2, 99.2, 83.0, 81.7, 77.1, 76.3, 72.1, 71.1, 60.2, 55.7, 44.5, 42.5, 41.8, 41.6, 38.9, 37.7, 36.6, 28.5, 26.1, 22.2, 21.5, 21.5, 20.7, 18.3, 17.0, 14.5, 14.0, 12.7, 10.0, 7.2, −3.9, and −4.5.

NMR Spectra.—All NMR spectra were obtained at ambient temperature in the dual $^{13}C/^1H$ 5 mm probe of a Bruker AM400 equipped with a 'process controller'. The ^{1}H and ^{13}C NMR experiments on (**1**) used *ca.* 0.04 and *ca.* 0.09 to *ca.* 0.30 mol dm^{-3} solutions respectively. The ^{1}H and ^{13}C NMR experiments on (**2**) and (**3**) in $(CD_3)_2SO$ used 0.09 mol dm^{-3} solutions.

Partial deuteriation was achieved by the accurate addition of D_2O (1.0–7.5 mm^3) directly into the NMR sample. The magnetic field was carefully shimmed prior to data acquisition and care was also taken to ensure that the sample temperature had stabilised prior to data acquisition. The ^{13}C-free induction decays (FIDs) were zero-filled from 64 to 128 K prior to resolution enhancement by Gaussian multiplication and Fourier transformation. The ^{1}H FIDs were zero-filled from 16 to 32K prior to resolution enhancement and Fourier transformation. The final digital resolution in the ^{1}H and ^{13}C spectra was <0.6 and <3.0 ppb respectively. The standard convention of positive sign for downfield or high frequency shifts was used throughout this work. The sign of the Δ values was determined in SIMPLE spectra with unequal OH:OD ratios.

The ^{1}H and ^{13}C NMR spectra of (**1**) in $(CD_3)_2SO$ were assigned unambiguously using 2D ^{1}H COSY-45 (Figure 2), 2D ^{1}H, ^{13}C COSY and 2D ^{1}H, ^{13}C COLOC experiments as done previously for a $CHCl_3$ solution.[14] The ^{1}H NMR spectra of (**1**) in C_5D_5N and $(CD_3)_2CO$ were assigned by comparison with the $CDCl_3$ and $(CD_3)_2SO$ data. The ^{1}H and ^{13}C NMR spectra of (**2**) in $(CD_3)_2SO$ were assigned by comparison with those of (**1**). The ^{1}H and ^{13}C NMR spectra of (**3**) in $(CD_3)_2SO$ were assigned with the aid of 2D ^{1}H COSY-45 and 2D ^{1}H, ^{13}C COSY experiments as performed previously.[14] Tables 1 and 2 give the NMR chemical shifts and $^nJ_{H,H}$ values respectively for (**1**), (**2**), and (**3**) in $(CD_3)_2SO$. Table 3 compares the $^nJ_{H,H}$ values of (**1**) in four different solvents.

Results

SIMPLE ^{13}C *NMR Spectroscopy.*—(*a*) *Bafilomycin* A_1 (**1**). Partial deuteriation of the hydroxy groups of (**1**) in $CDCl_3$ led to the splitting of many resonances (Table 4). The C-19 resonance split into two lines due to a two bond isotope effect ($^2\Delta$) caused by the high-field shift of 19-OD *vs.* 19-OH (Figure 3). No indication of any further splitting was observed. By contrast, the C-17 resonance split into four lines due to both a $^2\Delta$ and a $^4\Delta$ isotope effect—the latter arising from the partial deuteriation of 19-OH (Figure 4). This result is analogous to those observed by Christofides and Davies for β-cyclodextrin and maltose.[17a] This result also confirms the presence in $CDCl_3$ solution of the hydrogen bond from 19-OH to 17-O observed in

Table 1. ^1H and ^{13}C NMR chemical shifts (δ in ppm) for (1), (2), and (3) in $(CD_3)_2SO$.

	δ_H			δ_C		
Atom	(1)	(2)	(3)	(1)	(2)	(3)
1	—	—	—	164.5	164.5	164.3
2	—	—	—	140.9	140.9	140.2
3	6.52	6.52	6.50	131.8	131.8	130.6
4	—	—	—	130.7	130.7	132.1
5	5.82	5.82	5.69	144.2	144.2	141.3
6	2.41	2.40	2.68	37.2	37.1	35.8
7	3.16	*ca.* 3.15	4.65	78.5	78.5	80.7
7-OH	4.93	4.91	—	—	—	—
8	1.72	*ca.* 1.71	*ca.* 1.90	39.5	*ca.* 40	37.4
9e	*ca.* 1.90	*ca.* 1.9	2.01	41.3	41.3	41.2
9a	2.02	2.02	1.72			
10	—	—	—	142.8	142.8	141.7
11	5.70	5.70	5.82	123.9	123.9	124.8
12	6.52	6.52	6.55	132.1	132.2	131.8
13	5.12	5.12	5.20	125.6	125.6	126.7
14	3.95	3.95	3.98	83.1	83.0	82.8
15	5.12	5.11	5.12	75.4	75.4	75.6
16	*ca.* 1.85	*ca.* 1.8–1.9	*ca.* 1.88	38.5	38.5	38.4
17	3.98	3.97	4.00	70.0	70.0	69.9
17-OH	4.54	4.54	4.53	—	—	—
18	1.59	1.61	1.61	42.5	42.4	42.4
19	—	—	—	98.7	98.6	98.5
19-OH	5.33	5.41	5.38	—	—	—
20a	1.05	1.13	1.12	42.9	43.0	43.1
20e	1.99	1.97	*ca.* 2.00			
21	*ca.* 3.35	3.59	3.60	68.7	71.0	71.2
21-OH	4.47	—	—	—	—	—
22	*ca.* 1.13	*ca.* 1.20	*ca.* 1.20	40.7	40.6	40.9
23	3.30	3.35	3.35	75.7	75.4	75.4
24	1.81	*ca.* 1.8–1.9	1.82	27.6	27.7	27.6
25	*ca.* 0.86	0.86	0.87	21.0	20.9	20.9
26	1.88	1.88	1.91	13.7	13.7	13.6
27	0.95	0.95	0.86	18.0	17.9	17.0
28	*ca.* 0.87	0.87	0.99	22.5	22.5	22.4
29	1.78	1.79	1.78	19.3	19.3	19.4
30	0.77	0.77	0.78	10.6	10.6	10.5
31	*ca.* 0.87	0.87	0.88	7.0	7.0	6.9
32	0.82	0.78	0.81	12.4	12.4	12.4
33	0.71	0.71	0.71	14.5	14.4	14.3
2-OCH_3	3.53	3.53	3.54	59.4	59.3	59.2
14-OCH_3	3.14	3.15	3.16	55.2	55.2	55.2
SiMe		0.07	0.04, 0.03		0.5	−4.1, −4.7
Bu^t			0.85			17.7, 25.8
$COCH_3$			2.13			20.9, 170.7

the crystal structure of (1) (Figure 1), since $^4\Delta$ effects are not normally observed in SIMPLE ^{13}C spectra of polyols in the absence of hydrogen bonding.[17] The resonance of C-1 was split into two by a $^6\Delta$ effect due to partial deuteriation of 17-OH, confirming the presence of the hydrogen bond from 17-OH to 1-O in $CDCl_3$ solution. The C-7 resonance broadened greatly upon partial deuteriation of 7-OH but then resharpened as more D_2O was added. This effect was caused by the rate of OH/OD exchange being of the same order as the $^2\Delta$ value on C-7. No splitting or broadening was observed for the C-21 resonance (Figure 4). Instead, fast exchange of hydrogen and deuterium on the C-21 oxygen caused the C-21 resonance to shift upfield gradually by up to $^2\Delta$ as the H/D ratio was decreased to zero. Thus, slow, intermediate, and fast exchange processes are all observed simultaneously for the hydroxy groups of (1) in SIMPLE ^{13}C NMR experiments in $CDCl_3$.

Partial deuteriation of the hydroxy groups of (1) in $(CD_3)_2SO$ led to more extensive splitting in the SIMPLE ^{13}C NMR spectrum and several novel effects were observed (Table 4), including $^6\Delta$ *ca.* +35, $^8\Delta$ *ca.* +19 ppb (C-1, Figure 5); $^8\Delta \sim {}^{10}\Delta$ *ca.* +20 ppb (C-3); $^{10}\Delta \sim {}^{12}\Delta$ *ca.* +20 ppb (C-5); $^9\Delta \sim {}^{11}\Delta$ *ca.* +14 ppb (C-10); $^7\Delta \sim {}^9\Delta$ *ca.* +12 ppb (C-12). The observation of two Δ effects at C-1 provides direct evidence, in $(CD_3)_2SO$ solution, of the existence of the 19-OH, 17-OH, O-1 hydrogen-bonding network observed in the crystal structure of (1).[14] The "Δ effects reported here include some of the longest-range isotope effects reported to date. In order to understand these effects more fully, the SIMPLE ^{13}C NMR spectra of two derivatives—21-*O*-trimethylsilylbafilomycin A_1 (2) and 7-*O*-acetyl-21-*O*-t-butyldimethylsilylbafilomycin A_1 (3)—were also studied. The aim of this additional work was to determine the effect on the SIMPLE NMR spectra of blocking the C-7 and C-21 hydroxy groups.

(*b*) 21-O-*Trimethylsilylbafilomycin* A_1 (2) *and* 7-O-*acetyl*-21-O-*t-butyldimethylsilylbafilomycin* A_1 (3).—Comparison of the δ_H, δ_C, and $^nJ_{H,H}$ values for (1), (2), and (3) (Tables 1 and 2) revealed that the solution conformations of these three molecules were quite similar. Partial deuteriation of the hydroxy groups of (2) and (3) in $(CD_3)_2SO$ led to SIMPLE ^{13}C NMR spectra very

Table 2. $^{n}J_{H,H}$ values (in Hz) for (1), (2), and (3) in $(CD_3)_2SO$.

$^{n}J_{H,H}$	(1)	(2)	(3)
$^{3}J_{5,6}$	8.9	*ca.* 9.0	9.1
$^{3}J_{6,7}$	1.8	*ca.* 2	2.4
$^{3}J_{7,7\text{-OH}}$	5.4	5.3	—
$^{3}J_{7,8}$	*a*	*a*	5.7
$^{3}J_{8,9ax}$	11.2	11.5	11.1
$^{3}J_{8,9eq}$	*a*	*a*	*a*
$^{2}J_{9ax,9eq}$	14.2	14.2	14.7
$^{3}J_{11,12}$	11.0	10.8	10.8
$^{3}J_{12,13}$	15.0	15.0	15.0
$^{3}J_{13,14}$	8.3	8.4	8.4
$^{3}J_{14,15}$	6.7	6.7	6.9
$^{3}J_{15,16}$	2.0	1.8	1.9
$^{3}J_{16,17}$	10.2	*ca.* 10	10.2
$^{3}J_{17,17\text{-OH}}$	5.7	5.6	5.6
$^{3}J_{17,18}$	1.5	*ca.* 1–2	1.5
$^{4}J_{17\text{-OH},18}$	*ca.* 0	*ca.* 0	*ca.* 0
$^{4}J_{19\text{-OH},20ax}$	1.6	2.1	1.5
$^{2}J_{20ax,20eq}$	12.6	12.3	12.3
$^{3}J_{20ax,21}$	10.8	10.1	10.3
$^{3}J_{20eq,21}$	4.8	4.6	4.7
$^{3}J_{21,22}$	*a*	10.1	10.3
$^{3}J_{22,23}$	10.3	*ca.* 10	10.3
$^{3}J_{23,24}$	2.1	1.9	2.0

[a] Obscured.

Table 3. $^{n}J_{H,H}$ values for bafilomycin A_1 (1) in various solvents.

$^{n}J_{H,H}$	$CDCl_3$	$(CD_3)_2SO$	$(CD_3)_2CO$	C_5D_5N
$^{3}J_{5,6}$	9.2	8.9	8.9	9.0
$^{3}J_{6,7}$	1.9	1.8	1.9	1.9
$^{3}J_{7,7\text{-OH}}$	*a*	5.4	*a*	5.8
$^{3}J_{7,8}$	*a*	*a*	6.9	6.6
$^{3}J_{8,9ax}$	11.5	11.2	*a*	11.3
$^{3}J_{8,9eq}$	*a*	*a*	*a*	*a*
$^{3}J_{9,9}$	14.0	14.2	*a*	14.0
$^{3}J_{11,12}$	10.7	11.0	10.9	10.8
$^{3}J_{12,13}$	15.0	15.0	15.1	15.0
$^{3}J_{13,14}$	9.4	8.3	9.4	8.9
$^{3}J_{14,15}$	8.9	6.7	*ca.* 8.5	7.7
$^{3}J_{15,16}$	1.4	2.0	1.4	1.6
$^{3}J_{16,17}$	10.8	10.2	10.8	10.4
$^{3}J_{17,17\text{-OH}}$	4.2	5.7	4.3	5.4
$^{4}J_{17\text{-OH},18}$	1.1	0	1.1	*a*
$^{3}J_{17,18}$	2.0	1.5	1.9	1.8
$^{4}J_{19\text{-OH},20ax}$	2.1	1.6	2.2	1.9
$^{2}J_{20,20}$	12.0	12.6	12.0	12.1
$^{3}J_{20ax,21}$	11.1	10.8	11.1	*a*
$^{3}J_{20eq,21}$	4.8	4.8	4.7	4.7
$^{3}J_{21,22}$	10.0	*a*	10.0	*a*
$^{3}J_{22,23}$	10.3	10.3	10.3	10.3
$^{3}J_{23,24}$	2.4	2.1	2.2	2.2

[a] Obscured or not observed.

similar to those observed for (1) (Table 5, Figure 6). It was concluded that the 7-OH and 21-OH groups made no contribution to the unusual isotope effects observed in the SIMPLE ^{13}C NMR spectra of (1).

SIMPLE ^{1}H *NMR Spectroscopy*—(*a*) *Bafilomycin* A_1 (1). Partial deuteriation of the hydroxy groups of (1) also led to isotopic splittings in the ^{1}H NMR spectrum in $CDCl_3$. The 17-OH resonance split into two, confirming the existence of the hydrogen bond *from* 19-OH. However, the $^{6}\Delta$ value was negative (Table 4) showing that the generalisation [16] of positive $^{n}\Delta$ for an acceptor OH does not always hold. No Δ effect was

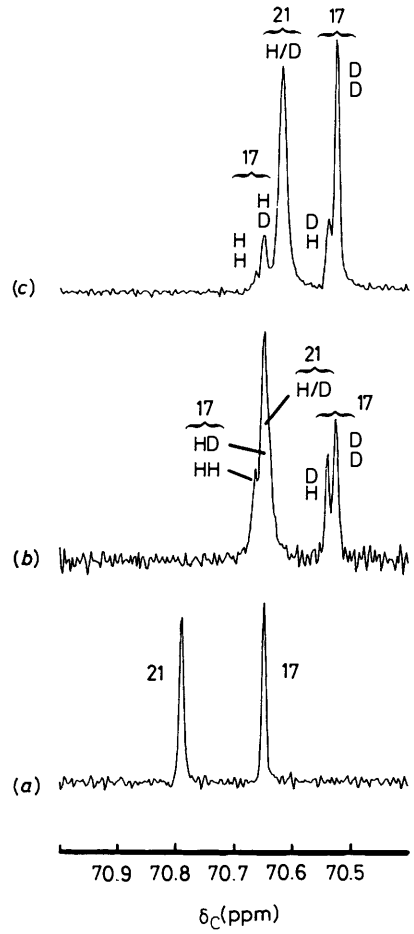


Figure 4. Corresponding expansions to Figure 3 but in the region of C-17 and C-21. The four lines labelled HH, HD, DH, and DD refer to the C-17 resonances of isotopomers of (1) with 17-OH, 19-OH; 17-OH, 19-OD; 17-OD, 19-OH, and 17-OD, 19-OD, respectively.

observed at 19-OH although a negative Δ would be predicted [15,16] for this donor OH. The resonance of 15-H also split into two (Figure 7). This is a novel $^{5}\Delta$ effect and the first reported example of a SIMPLE ^{1}H NMR effect on a proton other than a C*H*OH or OH proton.

SIMPLE ^{1}H NMR experiments were also carried out on (1) in $(CD_3)_2SO$, $(CD_3)_2CO$, and C_5D_5N (Table 4). Several interesting results were observed. The $^{4}\Delta$ on 17-OH changed sign and magnitude with a change of solvent (*vide infra*). No $^{4}\Delta$ was observed on 19-O*H* in $(CD_3)_2CO$ or $CDCl_3$ whereas negative $^{4}\Delta$ values (of different magnitude) were observed in $(CD_3)_2SO$ and C_5D_5N. A remarkable $^{7}\Delta$ was observed on 15-H, in addition to a $^{5}\Delta$, in C_5D_5N (Figure 8).

21-O-*Trimethylsilylbafilomycin* A_1 (2) *and* 7-O-*acetyl*-21-O-*t-butyldimethylsilylbafilomycin* A_1 (3).—Partial deuteriation of the hydroxy groups of (2) and (3) in $(CD_3)_2SO$ led to SIMPLE ^{1}H NMR spectra very similar to those observed for (1) (Table 5) although two Δ values were observed on 15-H in (3) rather than one Δ in (1) and (2). It was concluded that the 7-OH and 21-OH groups made no contribution to the isotope effects observed in the SIMPLE ^{1}H spectra of (1).

Discussion

The isotope effects observed in the SIMPLE NMR experiments on (1), (2), and (3) are some of the longest-range effects ever observed and the mechanisms by which these effects operate is

Table 4. Isotope effects[a] ("Δ) observed over *n* bonds upon partial deuteriation of the hydroxy protons of bafilomycin A_1 (**1**).

	^{13}C "Δ (ppb)		^{1}H "Δ (ppb)			
Atom	$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2CO$	$(CD_3)_2SO$	C_5D_5N
1	+16 (6)	+19 (8), +35 (6)				
2		*ca.* −9 (9)				
3		+20 (10), +20 (8)				
5		+20 (12), +21 (10)				
6		−32 (3)				
7	*ca.* −147 (2)	−121 (2)		*ca.* −9 (3)		*ca.* −8 (3)
10		+14 (11), +14 (9)				
12		+12 (9), +12 (7)				
14	*b*	−18 (7), −18 (5)				
15	+16 (4)	+20 (4)	−3.9 (5)	−4.6 (5)	−8.9 (5)	*ca.* −4 (7), *ca.* −11 (5)
16	−22 (3)	−60 (3)				
17	−14 (4), −125 (2)	−118 (2)	−8.4 (3)	−9.3 (3)	*b*, *c*	
17-OH			*ca.* −3 (6)	+2.2 (6)	+13.2 (6)	*ca.* −10 (3)
18	−18 (3), −29 (3)	−15 (3), −50 (3)				*ca.* +8 (6)
19	−99 (2)	−96 (2)				
19-OH			0 (6)	0 (6)	−4.7 (6)	*ca.* −14 (6)
20	−28 (3)		−4.4 (3)			
21		−112 (2)				
22		−47 (3)				
30		*ca.* −10				

[a] A negative sign implies an upfield (low frequency) shift; the number of bonds *n* over which the effect operates is given in parentheses. [b] Broadening observed. [c] Obscured—resonance overlap.

Table 5. Isotope effects[a] "Δ (in ppb) observed over *n* bonds upon partial deuteriation of the hydroxy groups of (**1**), (**2**), *and* (**3**) in $(CD_3)_2SO$.[d]

	^{13}C "Δ			^{1}H "Δ		
Atom	(**1**)	(**2**)	(**3**)	(**1**)	(**2**)	(**3**)
1	+19 (8), +35 (6)	+20 (8), +34 (6)	+20 (8), +34 (6)			
2	*ca.* (−)9	—	*b*			
3	+20 (10), +20 (8)	+20 (10), *ca.* +20 (8)	+22 (10), +22 (8)			
4	—	—	—			
5	+20 (12), +20 (10)	*ca.* 15–20 × 2 (12, 10)	+18 (12), +18 (10)			
6	−32 (3)	−29 (3)	—			
7	−121 (2)	−124 (2)	—			
8	*c*	*c*	—			
9	—	—	—			
10	+14 (11), +14 (9)	+13, +14 (11 and 9)	*b*			
11	—	—	—			
12	+12 (9), +12 (7)	*b*	*b*			
13	—	—	—			
14	−18 (7), −18 (5)	−17, −18 (7 and 5)	−14, −15 (7 and 5)			
15	+20 (4)	*c*	+18 (4)	−8.9 (5 and 7)	−8.7 (5 and 7)	−4.5 (5), −4.5(7)
16	−60 (3)	−60 (3)	−60 (3)			
17	−118 (2)	−119 (2)	−118 (2)	*b*, *c*	*c*	*c*
17-OH	—	—	—	+13.2 (6)	+13.2 (6)	+13.1 (6)
18	−15 (3), −50 (3)	−12 (3), −55 (3)	−12 (3), −53 (3)			
19	−96 (2)	−94 (2)	−95 (2)			
19-OH	—	—	—	−4.7 (6)	−5.3 (6)	−4.9 (6)
21	−112 (2)	—	—			
22	−47 (3)	—	—			
30	*ca.* 10	*b*	*c*, *b*			

[a] Positive Δ implies downfield shift; the numbers of bonds over which the Δ operates is given in parentheses. [b] Broadening observed. [c] Obscured. [d] Broadening was also observed for the ^{13}C resonances of C-20, C-28, and C-29 of all three compounds.

of considerable interest, but poorly understood.[19] In the SIMPLE NMR literature two schools of thought have arisen.[19] Reuben has argued[17b,c] that the $^{4}\Delta$ values observed in the SIMPLE ^{13}C NMR spectra of some polyols arise from isotopic perturbation of equilibria involving 'flip-flop' hydrogen-bonds of the type:

O1–H · · · O2–H H–O1 · · · H–O2

On the other hand Christofides and Davies and co-workers have argued that the effects are transmitted directly through the hydrogen-bond.[17a] For SIMPLE ^{1}H NMR spectroscopy this group has recently stated[16e] that the isotope effects are transmitted through the hydrogen-bonds but have magnitudes which reflect relative populations of molecular conformations.

For bafilomycin A_1 (**1**), the possibility of a 'flip-flop' hydrogen bond equilibrium involving 19-OH and 17-OH can be elimin-

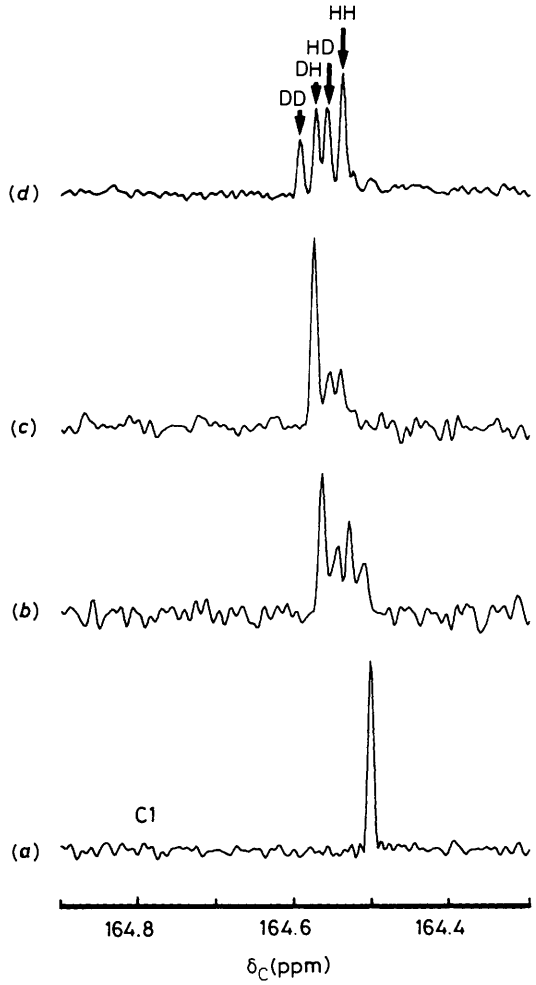


Figure 5. An expansion of the ^{13}C spectrum of (**1**) in $(CD_3)_2SO$ in the region of the resonance of C-1 (*a*) control; (*b*) +3.0 mm^3 D_2O; (*c*) +6.0 mm^3 D_2O; (*d*) as (*c*) but with additional 6.0 mm^3 H_2O. H/D labelling as for Figure 4.

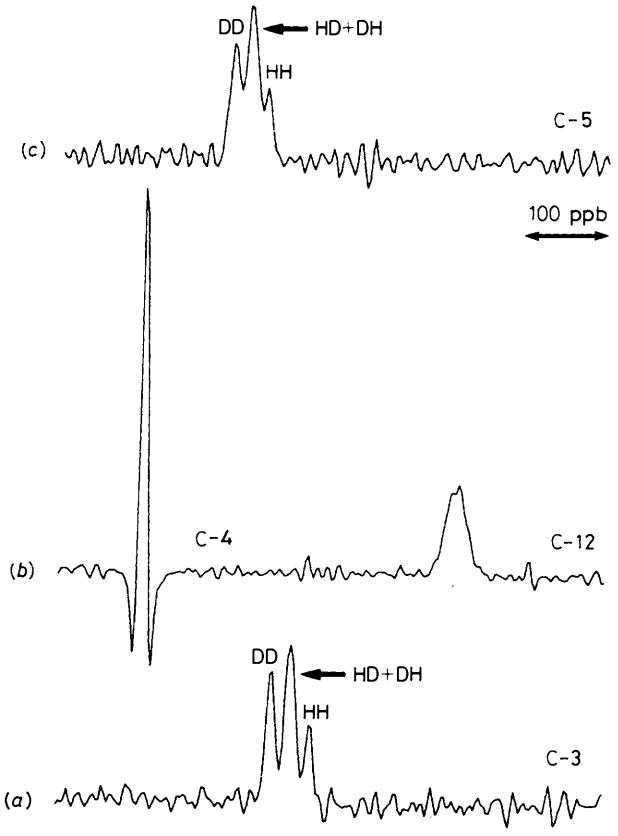


Figure 6. Expansions of the ^{13}C NMR spectra of (**3**) in $(CD_3)_2SO$ after the addition of D_2O (1.0 mm^3) in the region of the resonances of (*a*) C-3; (*b*) C-4 and C-12; (*c*) C-5.

ated. In all solvents studied (Table 3) $^4J_{19\text{-}OH.20ax}$ has a minimum value of *ca.* 1.6 Hz. This indicates that the 20-H_{ax}–C(20)–C(19)–19-O–19-OH unit adopts a W conformation with very little motional averaging about the C(19)–19-O bond. However, the 'flip-flop' equilibrium is only one of many conformational equilibrium processes which could contribute to the Δ values in (**1**), (**2**), and (**3**). The $^nJ_{H,H}$ values for (**1**) have been found to be slightly solvent dependent (Table 3) with the largest variations seen between $CDCl_3$ and $(CD_3)_2SO$ for $^3J_{13.14}$, $^3J_{14.15}$, and $^3J_{17.17\text{-}OH}$.[14] These new results indicate that limited conformational flexibility may exist in the macrolide ring and the C-16 to C-19 side chain.[14] Therefore conformational contributions to the observed Δ values in (**1**), (**2**), and (**3**) cannot be eliminated and the earlier statement in the communication on (**1**)[18] should be so modified.

Steric isotope effects are also possible in (**1**), (**2**), and (**3**).[20,21,22] Anet proposed that steric isotope effects would be observed on proton chemical shifts when the inter-proton distance is appreciably less than the sum of the van der Waals radii (2.4 Å), no matter how many chemical bonds separate the nuclei concerned.[20] This was borne out by the observation of *upfield* $^5\Delta$ values on the ^1H NMR chemical shifts of a 1,3-dioxane and a half-cage acetate—conformational effects being ruled out in both cases.[20] Ernst *et al.* later observed *downfield* steric $^7\Delta$ values on carbon atoms *pseudogeminal* to deuteriated methyl groups in the ^{13}C NMR spectra of some methylcyclophanes.[21] Conformational contributions were excluded and whilst the Δ values were ascribed as clearly through-space, the interatomic distances involved were reported to be larger than in the work by Anet but were not given.[21] The smaller effective 'size' of deuterium *vs.* hydrogen accounts for the upfield ^1H Δ values and the downfield ^{13}C Δ values in these sterically strained systems. Rappoport *et al.*[22] also reported steric ^1H NMR Δ values in a series of deuteriated trimesitylethenols.[22] In this case the steric isotope effects propagated conformational changes (and therefore caused further Δ effects) in the propeller conformations adopted by the molecules.[22]

In the crystal structure of (**1**) the interproton distance between 15-H and 17-OH is only 2.16 Å.[13] Irradiation of 15-H in ^1H NOE difference spectroscopy experiments led to a large (> 5%) NOE on 17-OH, whereas irradiation of 17-H led to only a medium-size (2–5%) NOE on 17-OH.[14] This result confirms the spatial proximity of 15-H and 17-OH in solution as well as in the crystal. Deuteriation of 17-OH could thus lead to a steric isotope effect which should be manifested as upfield and downfield Δ values at 15-H and C-15, as indeed was observed (Tables 4 and 5). Relief in the steric interaction between 15-H and 17-OH (by deuteriation) may then propagate itself directly, leading to a change in the electron distribution in the conjugated 1-O to C-5 system or indirectly *via* a redistribution of conformational populations leading to a similar electronic change.

Electron redistribution in the conjugated 1-O to C-5 system is consistent with the pattern of ^{13}C NMR isotope effects observed for (**1**), (**2**), and (**3**) in $(CD_3)_2SO$ *i.e.*, relatively strong positive isotope effects at C-1, C-3, and C-5 but only weak negative or zero effects at C-2 and C-4 (Tables 4 and 5). Similar alternating effects are also observed for the C(10)–C(14) diene system. These latter effects are more difficult to

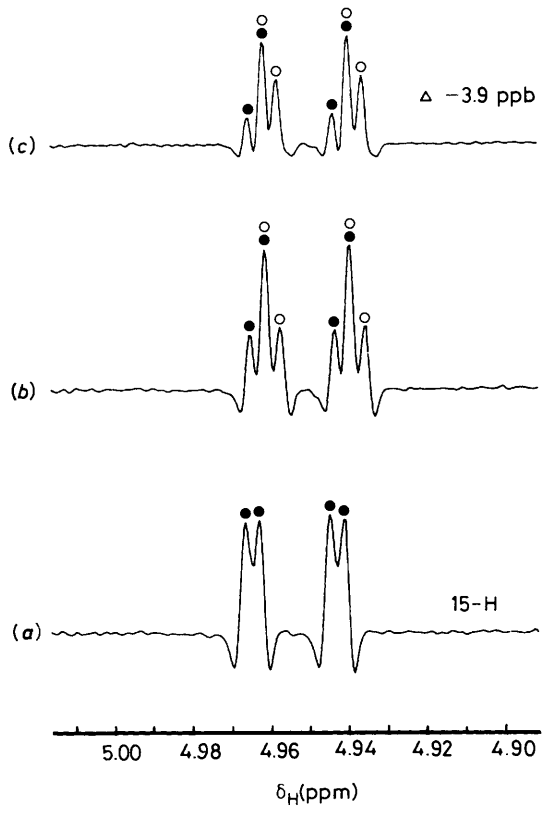


Figure 7. Expansion of the ^1H NMR spectrum of (**1**) in $CDCl_3$ in the region of the resonance of 15-H (*a*) control; (*b*) +1.0 mm^3 D_2O; (*c*) +1.5 mm^3 D_2O. The filled and unfilled circles represent the resonance of 15-H in protonated and deuteriated isotopomers, respectively.

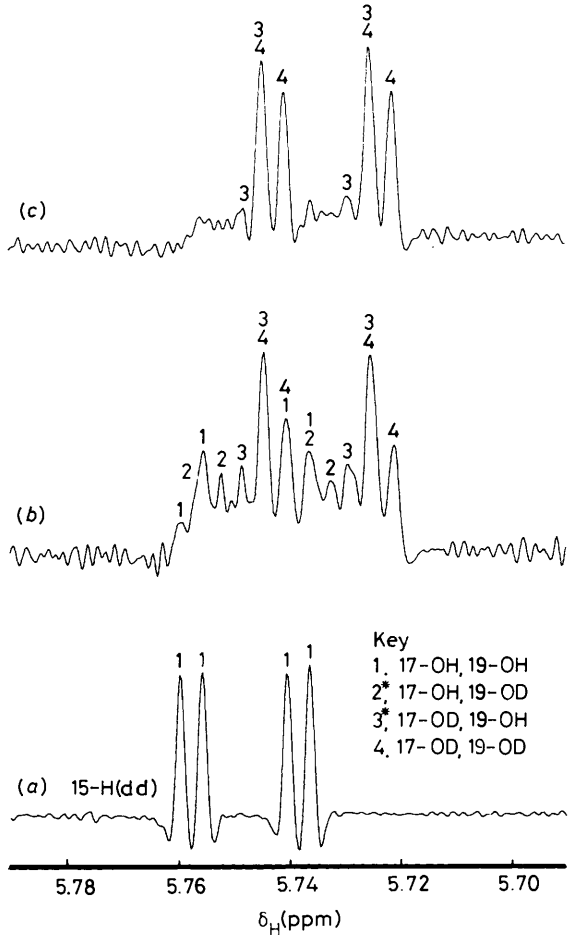


Figure 8. Expansion of the ^1H NMR spectrum of (**1**) in C_5D_5N in the region of the resonance of 15-H (*a*) control; (*b*) +1.0 mm^3 D_2O; (*c*) +2.0 mm^3.

explain and could be conformational in origin or more speculatively could be the result of the electrons in the C-10 to C-14 diene moiety sensing the electronic changes in the 1-O to C-5 system. Naturally, if the ^{13}C Δ effects are transmitted directly through the hydrogen-bonds then electronic redistribution in the 1-O to C-5 system would also be anticipated.

Conclusions

The extremely long-range isotope-effects observed for bafilomycin A_1 could have a variety of origins—direct, conformational, steric, or steric/conformational. This work has demonstrated that the C-7 and C-21 hydroxy groups play no part in these mechanisms. Furthermore, the possibility of 'flip-flop' hydrogen bonds has been eliminated. Further unravelling of the mechanism of the isotope effects awaits studies on analogous but conformationally rigid systems.

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