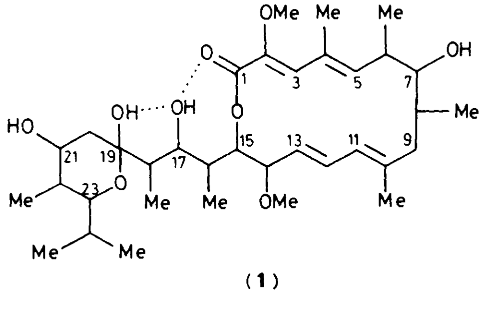
Novel Long-range ^{1}H and ^{13}C N.M.R. Isotope Effects Transmitted *via* Hydrogen Bonds in a Macrolide Antibiotic: Bafilomycin A$_1$

Jeremy R. Everett

Beecham Pharmaceuticals, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey RH3 7AJ, U.K.

Novel, long-range (up to 12 bonds) isotope effects transmitted *via* hydrogen bonds were observed in the ^{1}H and ^{13}C n.m.r. spectra of bafilomycin A$_1$ (**1**) upon partial deuteriation of the exchangeable protons.

Bafilomycin A$_1$ (**1**), is a member of the hygrolide class of macrolide antibiotics which are characterised by their 16-membered, diene lactone rings.[1—3] The crystal structure of bafilomycin A$_1$ has recently been solved[4] and reveals the presence of an intramolecular hydrogen-bonding network involving C(19)–OH, C(17)–OH, and O(1). Indirect evidence (^{1}H nuclear Overhauser enhancements, $^{n}J_{\mathrm{H,H}}$ values, and ^{13}C NT_1 values) shows that this hydrogen-bonding network, which may be important for the biological activity of bafilomycin A$_1$, is also present in solution.[4] It has recently been shown that the partial deuteriation of hydroxy groups involved in hydrogen bonding leads to isotope effects on the OH protons themselves (SIMPLE n.m.r.).[5,6] Similar effects, also involving hydrogen-bonded systems, have been observed on the resonances of ^{13}C nuclei up to six bonds removed from the site of deuteriation.[7] ^{1}H and ^{13}C N.m.r. experiments on (**1**) not only confirmed the presence of the hydrogen-bonding network involving C(19)–OH, C(17)–OH, and O(1) but also revealed novel, long-range n.m.r. isotope effects.



(**1**)

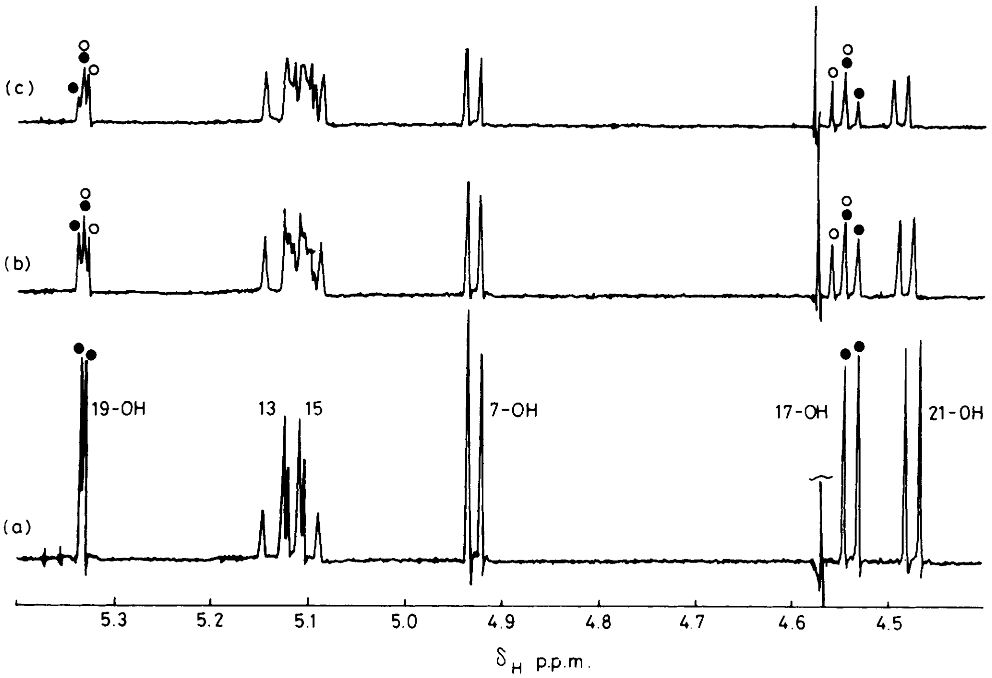


Figure 1. Expansion of the 400 MHz ^{1}H n.m.r. spectrum of (**1**) in $(CD_3)_2SO$ in the region of the hydroxy resonances; (a) control, (b) +1.0 μl D_2O, (c) +2.0 μl D_2O. The full circles (●) and open circles (○) label the ^{1}H resonances of hydroxy groups with OH and OD neighbours, respectively.

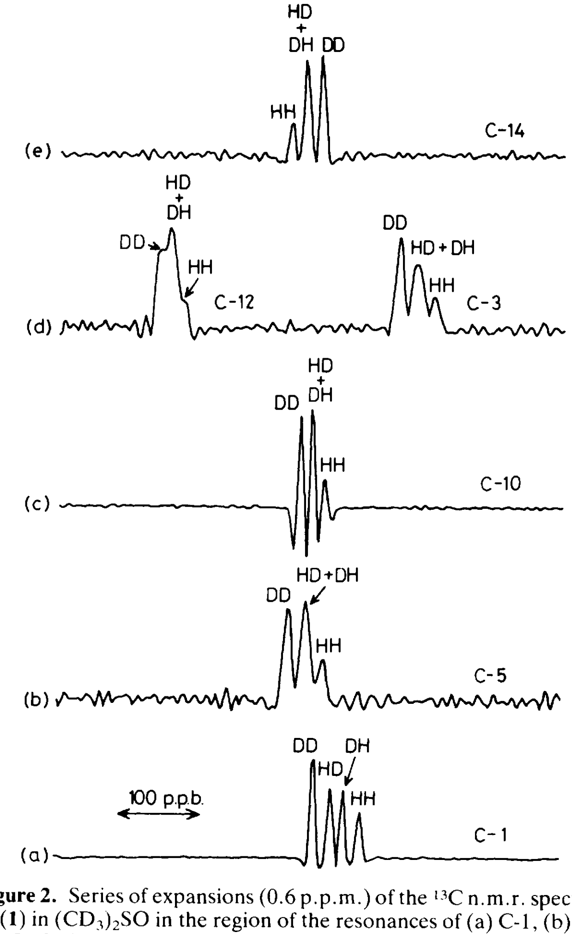


Figure 2. Series of expansions (0.6 p.p.m.) of the ^{13}C n.m.r. spectrum of (**1**) in $(CD_3)_2SO$ in the region of the resonances of (a) C-1, (b) C-5, (c) C-10, (d) C-12 and C-3, and (e) C-14. The hydroxy groups have been partially deuteriated by the addition of 3 μl of D_2O. The labels HH, HD, DH, and DD refer to the isotopomeric constitution of the C-19 and C-17 hydroxy groups, respectively.

The SIMPLE ^{1}H n.m.r. spectrum of (**1**) in $(CD_3)_2SO$ exhibited several isotope effects ($^{n}\Delta$, n = number of bonds† over which Δ operates), some of which are shown in Figure 1. The resonances of 19-O*H* and 17-O*H* split into two doublets ($^{6}\Delta \sim -4.7$ and $^{6}\Delta \sim +13.2$ p.p.b.,‡ respectively). Some previous work[6] implies that the signs of the Δ values indicate the direction of the hydrogen bond and on this basis 19-OH is a donor and 17-OH an acceptor group. However, the observation of $^{6}\Delta \sim -3$ p.p.b. at 17-OH and no effect at 19-OH in the SIMPLE ^{1}H n.m.r. spectrum of (**1**) in $CDCl_3$ solvent indicates that the interpretation of the sign of $^{n}\Delta$ must be made with caution.[6e] Novel $^{5}\Delta$ effects were observed at 15-H in both $CDCl_3$ (−3.9 p.p.b.) and $(CD_3)_2SO$ (−8.9 p.p.b., Figure 1) solvents. This is the first observation of a ^{1}H SIMPLE effect at a proton remote from the OH groups involved in the hydrogen bonding and demonstrates that the technique will be useful for molecules other than the polyols investigated[5,6] to date.

Extensive isotopic splitting occurred in the SIMPLE ^{13}C NMR spectrum of (**1**) in $(CD_3)_2SO$ and several novel long-range $^{n}\Delta$ effects were observed (Figure 2) including $^{6}\Delta \sim +35$, $^{8}\Delta \sim +19$ p.p.b. (C-1); $^{8}\Delta \sim {}^{10}\Delta \sim +20$ p.p.b. (C-3); $^{10}\Delta \sim {}^{12}\Delta \sim +20$ p.p.b. (C-5); $^{9}\Delta \sim {}^{11}\Delta \sim +14$ p.p.b. (C-10); $^{7}\Delta \sim 9\Delta \sim +10$ p.p.b. (C-12), and $^{5}\Delta \sim {}^{7}\Delta \sim -18$ p.p.b. (C-14). The observation of *two* Δ effects at C-1 provides direct evidence for the existence in $(CD_3)_2SO$ solution of the hydrogen-bonding network involving C(19)–OH, C(17)–OH, and O(1). These results include the longest-range isotope effects observed to date (to the best of our knowledge). All the ^{13}C $^{n}\Delta$ effects were also observed in a derivative of (**1**) with both the 7-OH and 21-OH groups blocked.[8] The $^{n}\Delta$ effects could arise either through conformational equilibrium isotope

---

† For SIMPLE ^{1}H n.m.r., it has been shown[5,6] that the Δ effects operate through the hydrogen bonds. For SIMPLE ^{13}C n.m.r. the mechanism is still somewhat uncertain (see text). For consistency of notation in this work, n is used to denote the number of formal chemical bonds (excluding hydrogen bonds) between the two nuclei involved.

‡ 1 P.p.b. ≡ 0.001 p.p.m.

effects (perturbation of hydrogen-bonding equilibria) or through direct isotope effects on hydrogen bonds of fixed disposition.[6,7] No ^{13}C $^{n}\Delta$ effects were observed at C-4, C-9, C-11, or C-13 in either solvent although in principle they would be expected at every carbon if conformational effects were postulated. It was therefore concluded that, in this case, the ^{13}C $^{n}\Delta$ effects were direct and operating through fixed hydrogen bonds.

Our thanks are due to Dr. R. J. Dorgan and Mr. G. Baker for the supply of (**1**) and much helpful advice.

Received, 13th August 1987; Com. 1193

References

1 G. Werner, Ph.D Thesis, University of Tübingen, 1982.

2 G. Werner, H. Hagenmaier, K. Albert, H. Kohlshorn, and H. Drautz, *Tetrahedron Lett.*, 1983, **24**, 5193.

3 G. Werner, H. Hagenmaier, H. Drautz, A. Baumgartner, and H. Zahner, *J. Antibiotics*, 1984, **37**, 110.

4 G. Baker, P. J. Brown, R. J. Dorgan, J. R. Everett, S. V. Ley, A. M. Z. Slawin, and D. J. Williams, *Tetrahedron Lett.*, 1987, **28**, 5565.

5 R. U. Lemieux and K. Bock, *J. Antibiotics*, 1979, **32** SUPPL., S-163; K. Bock and R. U. Lemieux, *Carbohydr. Res.*, 1982, **100**, 63.

6 J. C. Christofides and D. B. Davies, (a) *J. Chem. Soc., Chem. Commun.*, 1982, 560; (b) *ibid.*, 1985, 1533; (c) *Magn. Reson. Chem.*, 1985, **23**, 582; (d) *J. Chem. Soc., Perkin Trans. 2*, 1987, 97; (e) J. C. Christofides, D. B. Davies, J. A. Martin, and E. B. Rathbone, *J. Am. Chem. Soc.*, 1986, **108**, 5738.

7 (a) J. C. Christofides and D. B. Davies, *J. Am. Chem. Soc.*, 1983, **105**, 5099; J. Reuben, *ibid.*, (b) 1984, **106**, 6180; (c) 1985, **107**, 1747; (d) 1985, **107**, 1756; (e) 1986, **108**, 1735; (f) 1987, **109**, 316.

8 J. R. Everett, unpublished observations.