The Conformational Analysis of Bafilomycin A_1

Geoffrey H. Baker, Paul J. Brown, and Roderick J. J. Dorgan
Beecham Pharmaceuticals, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ
Jeremy R. Everett*
Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ

The ^1H and ^{13}C n.m.r. spectra of bafilomycin A_1, (**1a**), have been unambiguously solved by two-dimensional (2D) n.m.r. methods. Comparison of this spectral data with that reported for two other hygrolide antibiotics L-681, 110A_1 (**1b**) and L-155, 175 (**1c**), shows that the (*Z*)-C(4)–C(5) double bonds assigned to the latter two compounds are in fact *E*. The solution-state conformations of bafilomycin A_1 have been determined by the use of proton–proton coupling constants, ^1H nuclear Overhauser enhancements (n.O.e.s), and ^{13}C spin–lattice relaxation times (T_1s). The solution-state conformations have been compared with the known crystalline-state conformation of bafilomycin A_1 (with the aid of molecular modelling techniques) and are found to be very similar. In particular, it can be concluded that the hydrogen-bonding network involving 19-OH, 17-OH, and C(1)=O is intact in $CDCl_3$ solution.

During the past six years the structures of a number of naturally occurring 16-membered diene macrolide antibiotics have been published including L-681, 110,[1] the bafilomycins,[2–6] the hygrolidins,[7,8] leucanicidin,[9] L-155, 175,[10] and PD 118, 576.[11] This new class of macrolides which have bactericidal, fungicidal, antitumour, and antiparasitic effects have been termed the hygrolides.[12] The hygrolides share a common nucleus, (**1**), but have different substituents at C-2 and C-18.

All hygrolides except L-681, 110[1] and L-155, 175[10] have been reported to have an (*E*)-C(4)–C(5) bond. Werner *et al.*[4] stated that L-681, 110 A_1 and L-681, 110 A_2 were identical with the bafilomycins C_1 and C_2 although the latter two had (*E*)-C(4)–C(5) bonds.

In the course of our screening program for novel, biologically active, microbial metabolites we isolated a crystalline substance which was shown by ^1H and ^{13}C n.m.r. spectroscopy to be identical with bafilomycin A_1, (**1a**). *X*-Ray analysis of (**1a**) and its 21-(2′,2′,2′-trichloroethylcarbonate) derivative provided the first crystal-structure of any hygrolide and the absolute configuration at each chiral centre, respectively. A preliminary communication has appeared on this work.[13] The crystal structure of bafilomycin A_1 showed a hydrogen-bonding network involving 19-OH, 17-OH, and C(1)=O similar both to that found in the related 16-membered dilactone elaiophylin[14,15] and to that proposed to exist in hygrolidin.[12] It was considered important to determine whether or not this hydrogen-bonding network remains intact in solution for bafilomycin A_1, as has been proposed[14] for elaiophylin, since the hydrogen-bonding could be a crucial determinant of biological activity. In this paper we report the full solution-state conformational analysis of bafilomycin A_1 and a reassignment of the C(4)–C(5) double bond stereochemistry of L-681, 110[1] and L-155, 175[10] by comparison of their n.m.r. spectral data with the unambiguous data obtained on bafilomycin A_1.

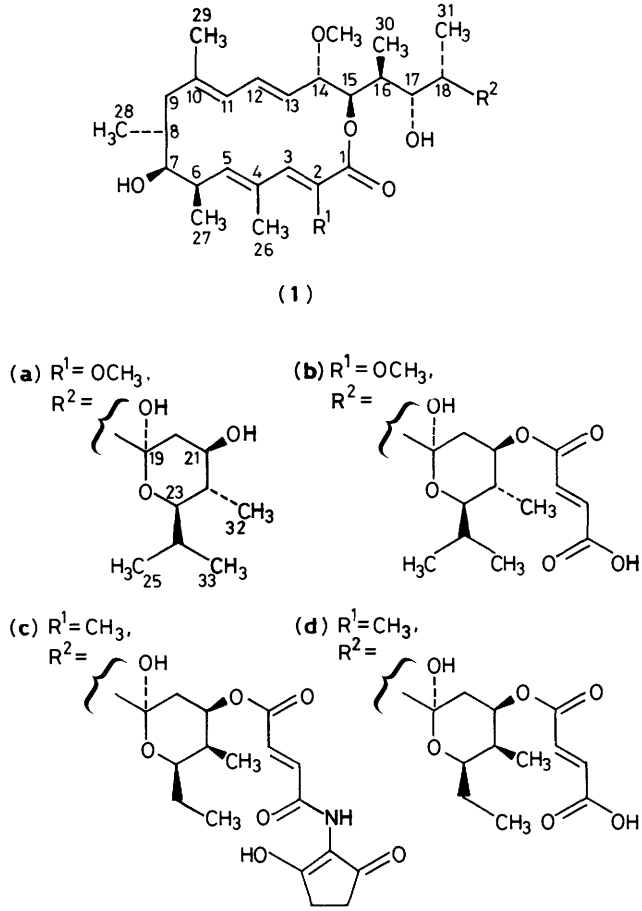

Figure 1. The structures of bafilomycin A_1 (**1a**), L-681, 110A_1 (**1b**), L-155, 175 (**1c**), and hygrolidin (**1d**).

Materials and Methods.—Bafilomycin A_1 was obtained from the fermentation broth of streptomycete RB143 (deposited in the Beecham culture collection at Brockham Park, Surrey). After fermentation for 6 days the broth (10 dm^3) was filtered through Celite to separate the mycelium. The mycelial cake was stirred in acetone (2.5 dm^3) for 16 h, filtered through Celite, and evaporated to a small volume. The concentrate was extracted with chloroform (1 dm^3), washed with water (3 × 1 dm^3), dried ($MgSO_4$), and evaporated to give the crude extract (4.9 g). This extract was purified by silica gel column chromatography with diethyl ether as the eluant to give pure bafilomycin A_1 (1.6 g) as a white foam. T.l.c. (SiO_2/Et_2O), R_F = 0.4; *m/z* (FAB Na^+/NOBA) (relative intensity) 645 $[M Na]^+$ (100%); $\lambda_{max.}$(EtOH), 247, 286 nm. Crystallisation from diethyl ether–

Table 1. Main parameters of the 2D n.m.r. experiments.

	2D H COSY	2D H,C COSY[a]	2D H,H,C RELAY[b]	2D H,C COLOC[c]
Number of scans per FID	32	32	64	512
FID matrix size	2 048 × 512	8 192 × 512	8 192 × 512	8 192 × 512
Spectrum matrix size	1 024 × 1 024	4 096 × 1 024	4 096 × 1 024	4 096 × 2 048

[a] Experiment tuned for $^1H_{C,H}$ 140 Hz; using variant with ^1H decoupling in both dimensions (ref. 19). [b] Experiment tuned for $^1J_{C,H}$ 140 Hz and $J_{H,H}$ 5.8 Hz. [c] Experiment tuned for $J_{C,H}$ 9.5 Hz.

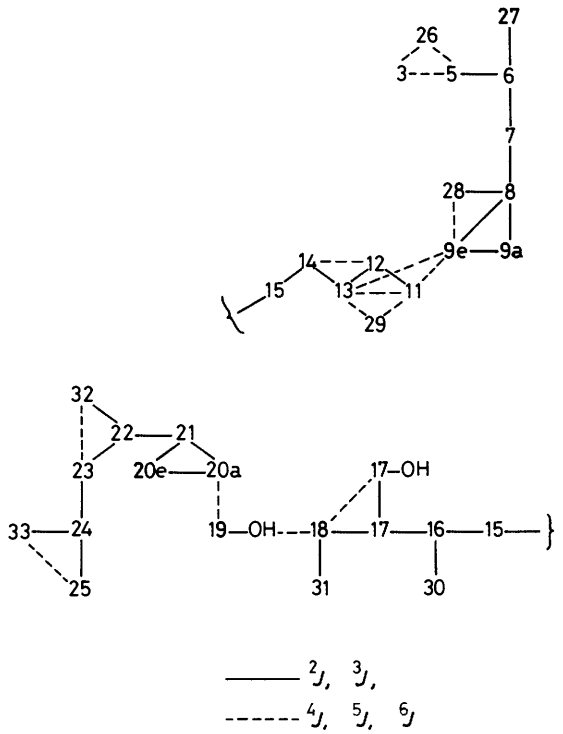

Figure 2. A proton-to-proton connectivity map for (**1a**) in $CDCl_3$/TMS from an analysis of the 2D ^1H COSY-45 n.m.r. spectrum.

light petroleum (b.p. 40–60 °C) afforded colourless needles, m.p. 149–151 °C.

^1H and ^{13}C n.m.r. experiments were conducted on a Bruker AM400 n.m.r. spectrometer, in a 5 mm $^1H/^{13}C$ dual probe using standard Bruker software and undegassed 0.03 and 0.3 mol dm^{-3} $CDCl_3$/TMS solutions, respectively. The ^1H nuclear Overhauser enhancement (n.O.e.) difference experiments were conducted using a modification of the method of Hall and Sanders[16] as described previously.[17] The ^1H spin–lattice relaxation time (T_1) experiment was conducted using a standard inversion-recovery pulse sequence and 19 values of the variable delay τ (range 0.001–1.0 s) exactly as previously described.[17] Values of T_1 were determined by the null-point method.[16] The ^{13}C T_1 experiment also used the inversion-recovery pulse sequence with a relaxation delay of 3 s, averaging 600 scans into 64K data points for each of 13 values of τ (range 0.001–0.70 s). The ^{13}C T_1 values were calculated using a non-linear, three-parameter fit to the experimental intensity data (Bruker software). The sweep widths of all 2D n.m.r. experiments were optimised prior to acquisition. The main parameters of the 2D n.m.r. experiments are detailed in Table 1.

Results and Discussion

(a) *Assignment of the* ^1H *and* ^{13}C *N.M.R. Spectra of Bafilomycin* A_1, (**1a**).—The 400 MHz ^1H n.m.r. spectrum of (**1a**) is complex and overlapped, especially in the high-field region, and an unambiguous assignment by one-dimensional n.m.r. experiments was not possible. A 2D ^1H correlation spectroscopy (2D ^1H COSY-45) experiment[18] was therefore used to establish the connectivity between all the pairs of mutually *J*-coupled protons in the molecule (Figure 2). As can be clearly seen from Figure 2, coupling connectivities are present right across the molecule, allowing an unambiguous assignment of all the proton resonances except those of the two methoxy groups which were assigned on the basis of n.O.e. experiments and chemical shifts. A remarkable feature of the COSY-45 spectrum is the presence of long-range connectivities between some of the protons, especially the ^6J between 13-H and the low-field 9-H.

Having established unambiguous assignments of the ^1H n.m.r. spectrum, 2D proton, carbon-13 chemical shift correlation experiments were used to obtain unambiguous assignments of the ^{13}C n.m.r. spectrum of (**1a**). A 2D ^1H, ^{13}C COSY[19] experiment was used to correlate (*via* $^1J_{CH}$) the chemical shifts of all directly bonded proton, carbon pairs. This spectrum allowed the unambiguous assignment of most of the ^{13}C resonances of protonated carbons. However problems occurred because of the overlap of resonances in the proton dimension, especially in the high-field methyl region. In order to overcome this problem a 2D ^1H, ^1H, ^{13}C RELAY[19] experiment was performed. The RELAY experiment establishes connectivities between ^{13}C nuclei and directly bonded ^1H nuclei (via $^1J_{CH}$) as well as more remote ^1H nuclei linked to the directly bonded ^1H nuclei *via* homonuclear coupling. The RELAY experiment thus contains elements of both the 2D ^1H COSY and 2D ^1H, ^{13}C COSY experiments (Figure 3). The RELAY spectrum overcame all the ambiguities in the assignment of the resonances of the protonated carbon-13 nuclei. The quarternary carbon resonances of (**1a**) were unambiguously assigned using a 2D ^1H, ^{13}C COLOC[19] experiment which establishes the connectivity between carbon-13 nuclei and protons *via* $^2J_{CH}$ or $^3J_{CH}$ (Figure 4). The analysis for all carbon types showed that there were eight errors in the previous ^{13}C assignment[3] of (**1a**).

(b) *A Comparison of the* ^{13}C *N.M.R. Data of Bafilomycin* A_1 *with* L-681, $110A_1$, (**1b**) *and* L-155, 175, (**1c**).—A comparison of the ^{13}C data of bafilomycin A_1 with that reported[1,10] for L-681, $110A_1$, (**1b**), and L-155, 175, (**1c**) immediately showed that the reported assignments[1,10] of C-8, C-11, C-12, C-14, C-16, C-17, C-22, C-26, C-27, C-28, C-29, C-30, and C-31 are scrambled. Unscrambling the assignments for L-681, $110A_1$ resulted in an agreement of ^{13}C chemical shifts to within 0.5 ppm for C-1 to C-10 as well as C-26, C-27, and C-28 (Table 2). The reported *Z*-configuration for the C(4)–C(5) bond is thus erroneous and should be *E*. The 2-CH_3 substitution of L-155, 175 makes a direct comparison with bafilomycin A_1 more difficult but on the basis of the similarity of the ^{13}C chemical shifts of C-6, C-26, and C-27 in the two compounds it is again concluded that the reported[10] (*Z*)-C(4)–C(5) double bond configuration of L-155, 175 is erroneous. This was confirmed by the close agreement between the unscrambled assignments of L-155, 175 and those reported by Seto *et al.*[7] for the closely related compound hygrolidin, (**1d**). For PD 118, 576 the ^{13}C assignments[11] of C-4

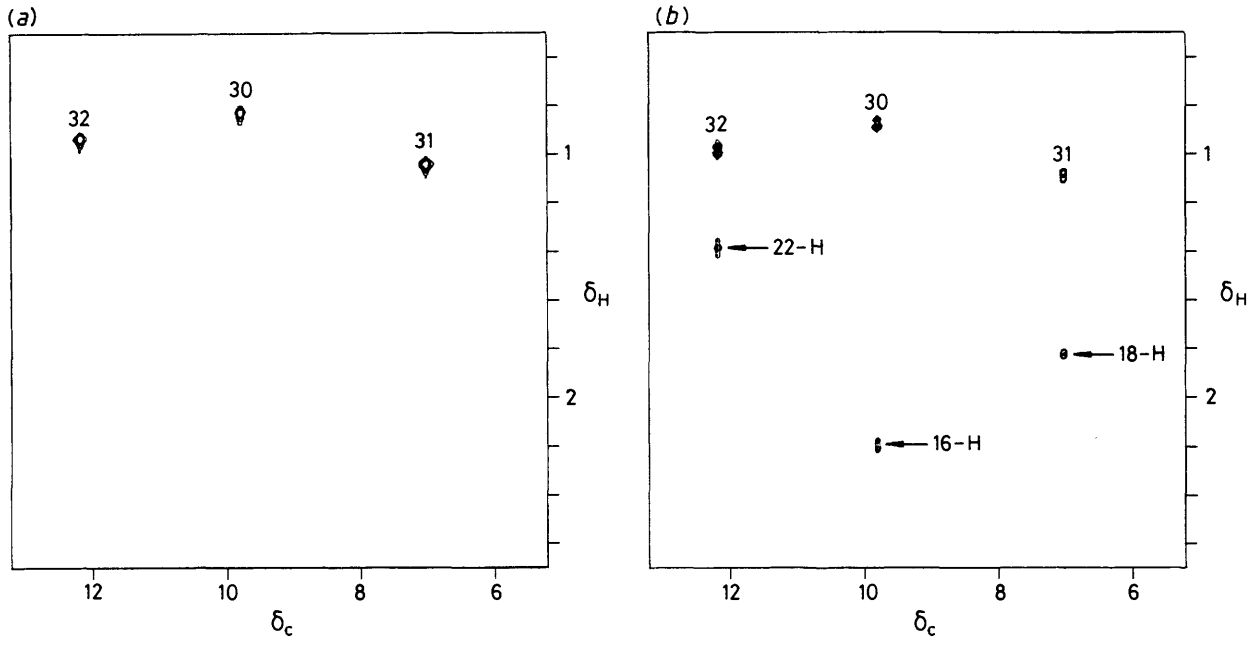

Figure 3. (*a*) A contour plot of the highest-field region of the 2D ^1H, ^{13}C COSY n.m.r. spectrum of (**1a**) in $CDCl_3$/TMS. (*b*) A contour plot of the same region of the 2D ^1H, ^1H, ^{13}C RELAY n.m.r. spectrum. The relay peaks to 22-H, 16-H, and 18-H are indicated by arrows. Note that in contrast to the 2D ^1H, ^{13}C COSY experiment there is no ^1H decoupling in the proton dimension.

and C-10 are switched and those of C-28, C-30, and C-31 are scrambled. A recent publication[20] on setamycin (found to be identical[20] to bafilomycin B_1) shows the C(4)–C(5) bond as *Z* whereas it in fact is *E*. The ^{13}C resonance assignments for this compound are also scrambled, since they are based on those of L-681, 110 A_1 and L-155, 175.

The Conformational Analysis of Bafilomycin A_1

The conformational analysis of bafilomycin A_1, (**1a**), will be discussed in terms of a comparison between the solution-state structure as determined by n.m.r. spectroscopy and the crystalline-state structure (Figure 5) as determined by *X*-ray crystallography. A preliminary account of the n.m.r. work has already appeared.[13]

(a) *Tetrahydropyran Ring.*—The solution state n.m.r. data was in complete agreement with the crystalline state conformation. The magnitudes of $^3J_{23,22}$, $^3J_{22,21}$, $^3J_{21,20eq}$, and $^3J_{21,20ax}$ allowed these couplings to be assigned as ax ax, ax ax, ax eq, and ax ax respectively. Thus the tetrahydropyran (THP) ring was in the same chair conformation as in the crystalline state with little or no interconversion to the ring-inverted conformation, which is expected to be very unstable. The low value of $^3J_{23,24}$ (~2.4 Hz) is consistent with the same staggered relationship between the two protons as in the crystalline-state. This low value and the equality of the ^{13}C NT_1 values (Table 3) for C-23 and C-24 indicate little motional freedom around the C(23)–C(24) bond. The large value of $^4J_{19\text{-}OH,20ax}$ ~ 2.2 Hz implies a *trans*-coplanar orientation of the 19-OH proton with respect to C(20), in very good agreement with the crystal structure, which has a value of −159.4° for the torsion angle OH(19)–O(19)–C(19)–C(20). These conformational conclusions, based on the *J*-couplings and ^{13}C T_1 values were corroborated by ^1H n.O.e. data. In particular the existence of the n.O.e. from Me-33 to H-22 *i.e.* n.O.e.[33-Me]22 and n.O.e.[22]20ax established the *syn*-1,3-diaxial disposition of 33-Me, 22-H, and 20-Hax as expected. No n.O.e.[25-Me]22 was observed, again, as expected.

In summary, the solution state conformation of the THP ring of bafilomycin A_1, (**1a**) is indistinguishable from the crystalline-state conformation.

(b) C(16)–C(17)–C(18) *Side Chain.*—The solution-state n.m.r. data was again found to be in agreement with the crystalline-state structure. The averaged ^{13}C NT_1 values (Table 3) of the protonated carbons of the lactone ring, sidechain, and THP ring were 0.29 ± 0.03, 0.29 ± 0.00, and 0.31 ± 0.01 s (range) respectively. Thus, the ^{13}C NT_1 values for the two rings and the sidechain are equal within experimental error. It was shown (by an inverse gated decoupling experiment) that the ^{13}C relaxation of the protonated carbon atoms of (**1a**) was dominated by dipolar interactions from their directly attached protons. The near-equality of the ^{13}C NT_1 values then leads to the conclusion[21] that bafilomycin A_1 tumbles approximately isotropically in solution with little or no motional freedom from the lactone ring out to C-18 on the sidechain, nor from the sidechain to the THP ring. This result is consistent with the presence of hydrogen-bonding from 19-OH to O-17 and 17-OH to O-1 holding the sidechain and THP ring in one preferred conformation in $CDCl_3$ solution.

The ^3J and n.O.e. values indicate that this preferred solution conformation is very similar to that in the crystal. The values of $^3J_{15,16}$, $^3J_{16,17}$, $^3J_{17,17OH}$, and $^3J_{17,18}$ (Table 3) are all consistent with the torsion angles between the respective protons in solution being very similar to those found in the crystalline state. Corey and Ponder[12] calculated three of these ^3J values on the basis of their MM2 derived hygrolidin structure, which has sidechain proton–proton torsion angles within ±4° of those found in the crystal structure of (**1a**). The calculated ^3J values[12] are within 1.3 Hz of the experimental values (Table 3). In addition the extreme values of $^3J_{15,16}$, $^3J_{16,17}$, and $^3J_{17,18}$ imply that little motional freedom is present around the sidechain single bonds. The n.O.e.[18]20ax establishes the *syn*-1,3-diaxial disposition of these two protons. This places the THP ring in the same conformation with respect to the sidechain as in the crystal structure *i.e.* with 19-OH hydrogen-bonded to O-17. The presence of n.O.e.[15]17-OH (large) and $^4J_{18,17\text{-}OH}$ ~ 1.1 Hz indicate that the 17-OH proton is fixed in a *W* conformation

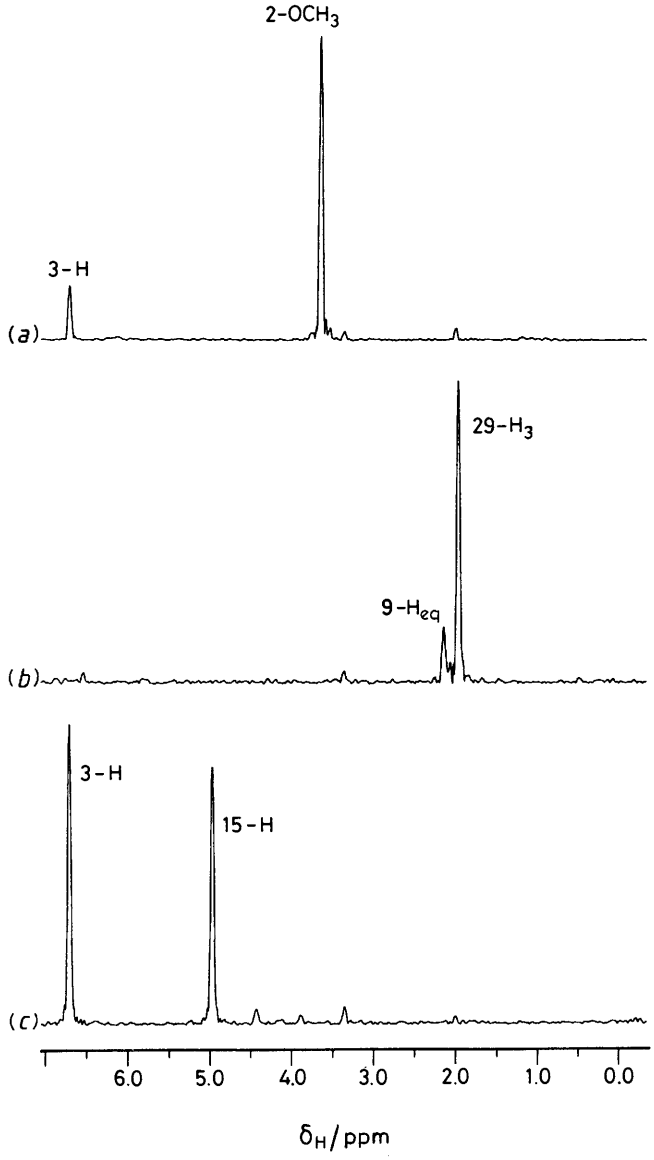

Figure 4. Three slices across the proton dimension of the 2D ^{1}H, ^{13}C COLOC n.m.r. spectrum of (**1a**) in $CDCl_3$/TMS at the ^{13}C chemical shifts of (*a*) C-2, 141.0; (*b*) C-10, 143.4; and (*c*) C-1, 167.3 ppm. The peak assignments show which protons have ^{2}J and ^{3}J connectivities to the respective carbons.

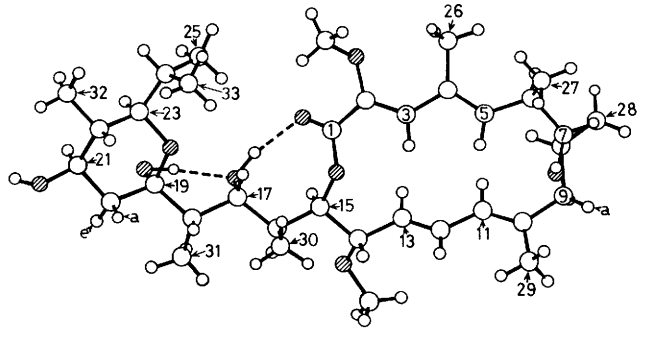

Figure 5. A view of the crystal structure of bafilomycin A_1, (**1a**). Oxygen atoms are shaded; the two hydrogen bonds connecting 19-OH, 17-OH and O-1 are shown as dotted lines.

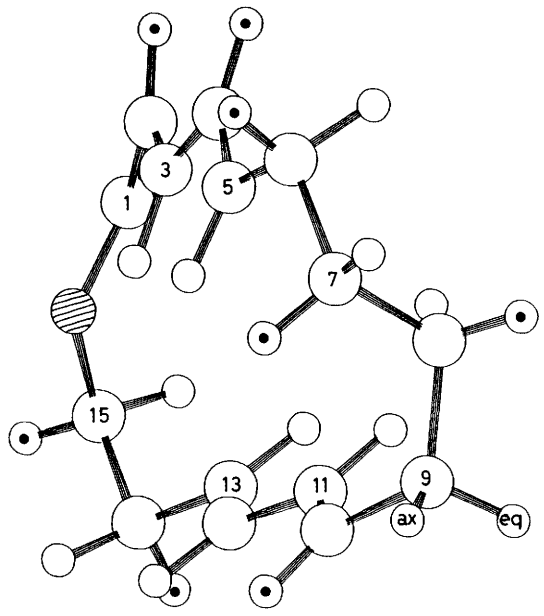

Figure 6. A side view of the 16-membered lactone ring of (**1a**) in the crystal structure with all substituents removed. The view is from C-8, C-9 looking towards O-15, C-1. Dummy atoms with dots indicate the position of substituents.

with respect to 18-H, close to 15-H, as in the crystal structure ($\angle$C(18)–C(17)–O(17)–OH(17) = $-178.6°$, $r_{15,17\text{-OH}} = 2.16$Å).

In summary, the solution-state n.m.r. data for the sidechain of (**1a**) is consistent with a conformation which is stabilised by two hydrogen bonds and indistinguishable from that present in the crystalline state.

(c) 16-*Membered Lactone Ring*.—In solution the large $^{3}J_{11,12}$ value of 10.7 Hz and the large n.O.e.[3]5 indicates that both the single bonds [C(3)–C(4) and C(11)–C(12)] linking the pairs of double bonds are in the same *trans*-conformation as in the crystal structure and that there is little or no motional averaging around C(11)–C(12). The magnitudes of the six vicinal coupling constants $^{3}J_{6,7}$, $^{3}J_{7,8}$, $^{3}J_{8,9eq}$, $^{3}J_{8,9ax}$, $^{3}J_{13,14}$, and $^{3}J_{14,15}$ are all consistent with the dihedral angles found about the corresponding bonds in the crystal structure. However, the value of $^{3}J_{5,6} \sim 9.2$ Hz would seem to be rather too large for a dihedral angle of $+145.3°$. It is of interest to note that MM2 modelling of the 16-membered lactone ring in hygrolidin [12] calculated this torsion angle to be $-175°$, which would be a better fit to the experimental value of $^{3}J_{5,6}$ found here.

In $CDCl_3$ solution, 9-H_{eq} suffers a ^{4}J to 11-H and a ^{6}J to 13-H whilst 9-H_{ax} exhibits no such coupling. Since it is well known [22] that allylic couplings are maximal when the aliphatic proton is out of the plane of the double bond, it was concluded that in solution, as in the crystal, 9-H_{eq} and 9-H_{ax} are out-of-plane and in-plane respectively with the C(10)=C(11) double bond (Figure 6).

A more rigorous comparison between the solution-state and crystalline-state conformations of bafilomycin A_1 was obtained with the aid of computer graphics. Distance-geometry calculations in the molecular modelling program suite MODEL [23] were used to obtain a list of all those pairs of protons separated by < 3 Å in the crystal structure. From this list, a matrix of proton-to-proton spatial proximity was constructed for the crystal structure. This was then compared with a corresponding matrix of proton-to-proton n.O.e.s, which is in effect a matrix of spatial proximity for the solution-state structure. If the solution state

Table 2. ^{13}C N.m.r. chemical shifts[a] for bafilomycin A_1, L-681, 110, L-155, 175, and hygrolidin.

Atom	Bafilomycin A_1 (**1a**)	L-681, 110A_1 (**1b**)[b]	L-155, 175, (**1c**)[b]	Hygrolidin, (**1d**)[c]
1	167.3	167.8	172.6	172.0
2	141.1	141.1	122.6	122.1
3	133.7	134.0	147.1[d]	146.6
4	132.8	133.3	134.9	134.3
5	143.3	143.4	145.2[d]	144.6
6	36.8	36.9	36.8[d]	36.7
7	80.8	81.3	81.5	81.2
8	40.2	40.2[d]	39.9[d]	39.7
9	41.3	41.4	41.3	41.2
10	143.4	143.6	143.1	142.7
11	125.0	125.6[d]	132.9[d]	125.3
12	133.2	133.5[d]	243.9[d]	132.5
13	126.8	127.4	127.7	127.4
14	82.3	82.5[d]	82.8[d]	82.6
15	76.8	77.1	76.3	76.0
16	37.2	37.3[d]	35.5[d]	35.3
17	70.6	70.9[d]	70.5[d]	70.3
18	42.1	42.1	41.7	41.6
19	99.0	99.2	99.6	99.3
20	43.5	40.0	34.4	34.1
21	70.7	76.1	73.3	73.1
22	40.9	38.2[d]	38.0[d]	37.8
23	75.9	75.4	71.3	71.2
24	27.9	28.0	25.4	25.2
25	21.2	21.2	10.8	10.7
26	14.0	14.0[d]	15.3[d]	15.1
27	17.3	17.3[d]	17.6[d]	17.4
28	21.7	21.8[d]	21.6[d]	21.5
29	20.2	20.2[d]	20.2[d]	20.0
30	9.8	9.9[d]	9.8[d]	9.6
31	7.1	7.1[d]	7.0[d]	6.9
32	12.2	12.3[d]	5.0[d]	4.8
33	14.3	14.4[d]	—	—
2-CH_3	—	—	13.8[d]	13.6
2-OCH_3	59.9	60.1	—	—
14-OCH_3	55.5	55.7	55.7	55.5

[a] δ_C Values ($\delta_{TMS} = 0$) given only for the 'molecular nucleus' and not the C-21 sidechain. [b] Reassigned with respect to the original work[1,10] by comparison with bafilomycin A_1 (this work) and hygrolidin.[7] [c] Assignment of Seto *et al.*[7] [d] Resonances reassigned.

structure is identical with the crystalline-state structure and if all n.O.e.s could be observed with equal facility then the spatial proximity maps for the crystal structure and solution-state structure should match. In reality this will not occur as several difficulties including resonance overlap, saturation transfer, differential relaxation times, and complex multi-spin interactions[16] prevent the observation of many n.O.e.s even if the protons are less than 3 Å apart. Conversely in some situations n.O.e.s will be observed beyond the arbitrary 3 Å cut-off distance. Figure 7 shows a typical series of ^1H n.O.e. difference spectra for bafilomycin A_1 underneath a control ^1H n.m.r. spectrum. Large n.O.e.s are seen for short-range interactions such as n.O.e.[3]5 11.5% ($r_{3,5}$ 2.2 Å in the crystal structure) in difference spectrum (*a*), which results from the irradiation of 3-H. Smaller n.O.e.s are seen for longer range interactions across the 16-membered ring, such as n.O.e.[3]13 and n.O.e.[3]11. 25-H_3 and the 2-OMe groups are situated on carbon atoms ten bonds apart. However, the crystal structure indicates a close spatial contact between the protons of the two methyl groups ($r_{min.}$ < 2.39 Å). In remarkable agreement with this, irradiation of the 2-OMe resonance results in a small n.O.e. to 25-H_3 [Figure 7(*d*)], thus demonstrating the spatial proximity of the two groups in solution as well as in the crystalline state.

114 n.O.e.s were observed in all (Table 1). Of these, 20 were not expected on the basis of the arbitrary 3 Å H,H cut-off distance set for the crystal structure. Twelve of the twenty n.O.e. contacts were accounted for when the cut-off distance was increased to 3.2 Å. The other eight unexpected n.O.e.s were small [1.2, 0.7, 0.5, 0.4, 0.3, and 0.2% (×3)] and were attributed either to longer range interactions (up to 3.7 Å) or to the result of slight conformational changes in solution relative to the crystal structure. On changing solvent from $CDCl_3$ to [$^2H_6$]DMSO the values of $^3J_{13,14}$, $^3J_{14,15}$, $^3J_{15,16}$, $^3J_{16,17}$, and $^3J_{17,17\text{-OH}}$ changed from 9.2, 8.9, 1.4, 10.8, and 4.2 Hz respectively to 8.3, 6.7, 2.0, 10.2, and 5.7 Hz respectively, with all other couplings changing by <0.5 Hz. These coupling constant changes indicate that the solution conformations of bafilomycin A_1 are slightly different in the two solvents. It is therefore possible that there is some limited conformational flexibility in the solution structure, which may also account for discrepancies between expected and observed n.O.e.s.

In addition, 28 n.O.e. contacts which would be expected on the basis of the crystal structure were not observed. In 13 cases, whilst a contact n.O.e.[*x*]*y* was not found, the corresponding contact n.O.e.[*y*]*x* was present. In 8 of these 13 cases, the missing n.O.e.s were to methylene or methyl protons which are expected[16] to be weak. In all of the remaining 'missing' cases observation of the n.O.e. was not technically possible due to resonance overlap.

The overall conclusion arrived at on the basis of the observation of more than 90 n.O.e.s between protons less than 3 Å apart in the crystal structure, is that the solution-state structure is qualitatively indistinguishable from the crystal structure.

Table 3. ^{1}H and ^{13}C n.m.r. chemical shifts (ppm), coupling constants (Hz), ^{1}H and ^{13}C T_1 values (s) and proton n.O.e.s for bafilomycin A_1 in $CDCl_3$/TMS.

					$^nJ_{HH}$			n.O.e.[i]			
Atom	δ_H[a]	δ_C[c]	$^{13}CNT_1$[d]	m[f]	^2J	^3J	^4J, ^5J, ^6J	Large	Medium	Small	$^1HT_1$ null
1	—	167.3		—							
2	—	141.1		—							
3	6.68	133.7	0.29	d			$^4J_{3,5} \sim 0.8$	5	13	11,12,26	0.72
4	—	132.8		—							
5	5.77	143.3	0.29	d,d,q		$^3J_{5,6} \sim 9.2$	$^4J_{5,3} \sim 1.0$, $^4J_{5,26} \sim 1.0$		3		0.48
6	2.54	36.8	0.31	d,d,q		$^3J_{6,5} \sim 9.1$, $^3J_{6,27} \sim 7.1$, $^3J_{6,7} \sim 1.9$			7	5,26,27	0.48
7	3.29	80.8	0.26	d,d					6	7-OH	0.53
7-OH	1.62	—	—								
8	1.90[b]	40.2	0.26						11	5,6,7,28	
9_{eq}	2.13[b]	41.3	0.32		$^2J \sim 14.0$[h]						~0.3
9_{ax}	1.95[b]				$^2J \sim 14.0$[h]	$^3J_{9ax,8} \sim 11.5$[h]					
10	—	143.4		—							
11	5.81	125.0	0.30	d,br		$^3J_{11,12} \sim 10.7$			13		0.49
12	6.51	133.2	0.28	d,d		$^3J_{12,11} \sim 10.7$, $^3J_{12,13} \sim 15.0$			14	3,11,13,14-OCH_3,29	0.63
13	5.16	126.8	0.29	d,d,br		$^3J_{13,14} \sim 9.4$, $^3J_{13,12} \sim 15.0$			11	3,12,14,15,14-OCH_3	0.87
14	3.89	82.3	0.31	d,d		$^3J_{14,13} \sim 9.0$, $^3J_{14,15} \sim 9.0$		12		13,14-OCH_3,15,16,30	0.50
15	4.95	76.8	*e*	d,d		$^3J_{15,16} \sim 1.4$, $^3J_{15,14} \sim 8.7$		17-OH	13,16	3,14	0.48
16	2.15[b]	37.2	0.29	d,d,q		$^3J_{16,30} \sim 6.8$, $^3J_{16,15} \sim 1.4$, $^3J_{16,17} \sim 10.8$			15	14,14-OCH_3,17,30,31	0.48
17	4.13	70.6	0.29	d,d,d		$^3J_{17,17\text{-OH}} \sim 4.1$, $^3J_{17,16} \sim 10.8$, $^3J_{17,18} \sim 2.0$			17-OH,18	19-OH,25,30	0.48
17-OH	4.66	—	—	d,d		$^3J_{17,OH,17} \sim 4.2$	$^4J_{17\text{-OH},18} \sim 1.1$		15,17		0.42
18	1.77	42.1	0.29			$^3J_{18,31} \sim 7.0$, $^3J_{18,17} \sim 2.1$	$^4J_{18,17} \sim 1.3$		17,20_{ax}	30,31	0.48
19	—	99.0	—	—							
19-OH	5.54			d			$^4J_{19\text{-OH},20} \sim 2.1$			23,31	0.82
20_{eq}	2.30	43.5	0.32	d,d	$^2J \sim 11.9$	$^3J_{20,21} \sim 4.8$			20_{ax},21		0.29
20_{ax}	1.16			d,d,d	$^2J \sim 12.1$	$^3J_{20,21} \sim 11.1$	$^3J_{20,19\text{-OH}} \sim 2.2$	20_{eq}	18	22	0.30
21	3.70	70.7	0.30	d,d,d		$^3J_{21,20} \sim 11.2$, $^3J_{21,20} \sim 4.7$, $^3J_{21,22} \sim 9.9$			23	20_{eq},22	0.66
21-OH		—	—								
22	1.33	40.9	0.31	d,d,q		$^3J_{22,32} \sim 6.5$, $^3J_{22,21} \sim 10.1$, $^3J_{22,23} \sim 10.1$				20_{ax},21,23,32,33	0.59
23	3.49	75.9	0.31	d,d		$^3J_{23,22} \sim 10.4$, $^3J_{23,24} \sim 2.4$			21	24	0.59
24	1.88	27.9	0.31	d,q,q		$^3J_{24,25} \sim 6.8$, $^3J_{24,33} \sim 6.8$, $^3J_{24,23} \sim 2.3$					0.48
25	0.90	21.2	1.35	d		$^3J_{25,24} \sim 6.8$			23,24	17,2-OCH_3	0.38
26	1.99	14.0	3.2	d			$^4J_{26,5} \sim 1.2$	6		3,2-OCH_3,27	0.66
27	1.07	17.3	1.6	d		$^3J_{27,6} \sim 7.1$			6.7	5,26,7-OH	0.42
28	0.935	21.7	0.98	d[g]		$^3J_{28,8} \sim 6.3$		7	6,8,9,9		0.30
29	1.94	20.2	2.9	d,d			$^4J_{29,11} \sim 1.1$, $^6J_{29,13} \sim 1.1$	12			0.66
30	0.83	9.8	2.6	d		$^3J_{30,16} \sim 6.9$			14,16, 17,18		0.48
31	1.05	7.1	2.4	d		$^3J_{31,18} \sim 7.3$			16,18,20e, 19-OH		0.48
32	0.941	12.2	1.8	d		$^3J_{32,22} \sim 6.5$			22,23,24	21	0.42
33	0.77	14.3	1.6	d		$^3J_{33,24} \sim 6.8$			22	24	0.43
2-OCH_3	3.64	59.9	~4.5	s						3,25,26	0.94
14-OCH_3	3.25	55.5	~4.1	s				14	12,13		0.79

[a] δ(TMS) = 0. [b] ex 2D ^{1}H, ^{13}C COSY. [c] δ(TMS) = 0. The ^{13}C resonances were edited into CH_3, CH_2, CH, and C subgroups using BB, DEPT-135 and DEPT-90 experiments. [d] Values for protonated carbons only; experiment valid for T_1 up to 1 s, NT_1 up to ~3 s for CH_3. For CH 2σ av ~ ±0.02, CH_3 2σ av ±0.01s. [e] Obscured by solvent $CDCl_3$ resonances. [f] Multiplicity of proton resonance in resolution-enhanced spectrum. [g] Low intensity, broad. [h] Ref. 3. [i] Large (≥5%), medium (2–5%), small n.O.e. (≤2%).

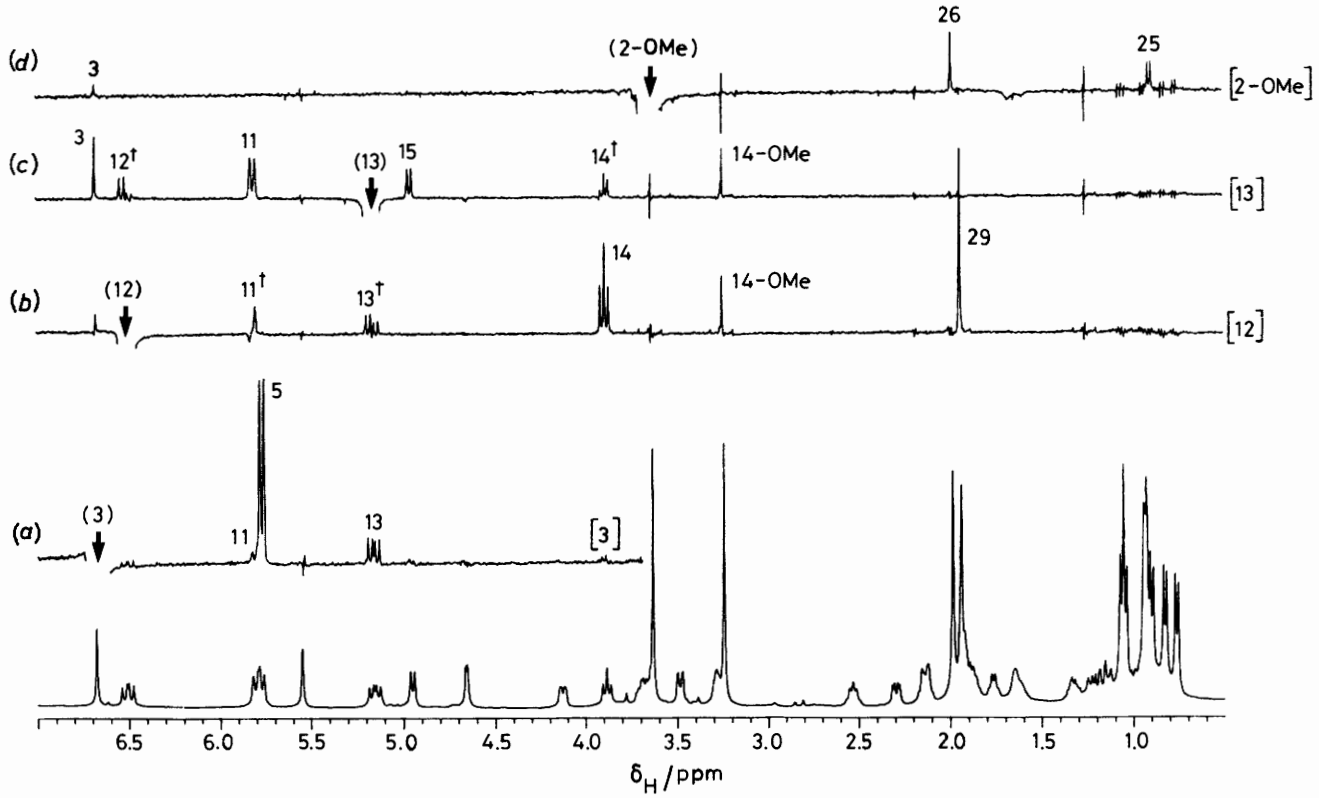

Figure 7. A series of 400 MHz ^{1}H n.O.e. difference spectra of (**1a**) in $CDCl_3$/TMS plotted above a control spectrum. The difference spectra result from the irradiation of (*a*) 3-H, (*b*) 12-H, (*c*) 13-H, and (*d*) 2-OMe. The dagger (†) indicates a mixed n.O.e./INDOR effect.

The Conformational Analysis of Bafilomycin A_1: *A Summary.*—(*a*) The THP ring is fixed in the same chair conformation in solution as in the crystal with the same orientation of the isopropyl sidechain.

(*b*) The C(16)–C(17)–C(18) sidechain occupies a conformation in solution indistinguishable from that found in the crystal.

(*c*) The 16-membered macrolide ring occupies a conformation in solution indistinguishable from that adopted in the crystal with the exception that the torsion angle between 5-H and 6-H may be larger in solution than in the crystal.

(*d*) Carbon-13 NT_1 values, proton coupling constants, and n.O.e.s all indicate that the hydrogen-bonding present in the crystal, involving 19-OH, 17-OH, and O-1 is also present in solution and is responsible for maintaining the THP ring and C(16)–C(17)–C(18) sidechain in the same orientation with respect to the 16-membered lactone ring as that found in the crystal structure. This conclusion has recently been confirmed by the observation of novel ^{1}H and ^{13}C n.m.r. isotope effects, transmitted *via* the hydrogen-bond network in the solution state.[24]

(*e*) The changes observed in some coupling constants between $CDCl_3$ and [$^2H_6$]DMSO solvents indicate that the conformations of bafilomycin A_1 are slightly different in the two solvents. Some limited conformational flexibility of bafilomycin A_1, in the macrolide ring and C(16)–C(18) sidechain is also possible.

Acknowledgements

Our thanks are due to Mr. A. E. Bird for helpful comments on the manuscript and to Mrs. A. Cullen for careful typing.

References

1 O. D. Hensens, R. L. Monaghan, L. Huang, and G. Albers-Schönberg, *J. Am. Chem. Soc.*, 1983, **105**, 3672.
2 G. Werner, PhD Thesis, University of Tübingen, 1982.
3 G. Werner, H. Hagenmaier, K. Albert, H. Kohlshorn, and H. Drautz, *Tetrahedron Lett.*, 1983, **24**, 5193.
4 G. Werner, H. Hagenmaier, H. Drautz, A. Baumgartner, and H. Zahner, *J. Antibiot.*, 1984, **37**, 110.
5 M. Meyer, W. Keller-Schierlein, H. Drautz, W. Blank, and H. Zahner, *Helv. Chim. Acta*, 1985, **68**, 83.
6 A. Kretschmer, M. Dorgerloh, M. Deeg, and H. Hagenmaier, *Agric. Biol. Chem.*, 1985, **49**, 2509.
7 H. Seto, H. Akao, K. Furihata, and N. Ōtake, *Tetrahedron Lett.*, 1982, **23**, 2667.
8 H. Seto, I. Tajima, H. Akao, K. Furihata, and N. Ōtake, *J. Antibiot.*, 1984, **37**, 610.
9 A. Isogai, S. Sakuda, S. Matsumoto, M. Ogura, K. Furihata, H. Seto, and A. Suzuki, *Agric. Biol. Chem.*, 1984, **48**, 1379.
10 M. A. Goetz, P. A. McCormick, R. L. Monaghan, D. A. Ostlind, O. D. Hensens, J. M. Liesch, and G. Albers-Schonberg, *J. Antibiot.*, 1985, **38**, 161.
11 J. H. Wilton, G. C. Hokanson, and J. C. French, *J. Antibiot.*, 1985, **38**, 1449.
12 E. J. Corey and J. W. Ponder, *Tetrahedron Lett.*, 1984, **25**, 4325.
13 G. H. Baker, P. J. Brown, R. J. J. Dorgan, J. R. Everett, S. V. Ley, A. M. Z. Slain, and D. J. Williams, *Tetrahedron Lett.*, 1987, **28**, 5565.
14 S. V. Ley, D. Neuhaus, and D. J. Williams, *Tetrahedron Lett.*, 1982, **23**, 1207.
15 K. Neupert-Laves and M. Dobler, *Helv. Chim. Acta*, 1982, **65**, 262.
16 J. K. M. Sanders and B. K. Hunter, 'Modern NMR Spectroscopy. A Guide for Chemists,' Oxford University Press, Oxford, 1987.
17 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1659.
18 A. Bax, 'Two-dimensional Nuclear Magnetic Resonance in Liquids,' D. Reidel, Dordrecht, 1984.
19 A. Derome, 'Modern NMR Techniques for Chemistry Research,' Pergamon Press, Oxford, 1987.
20 K. Otaguro, A. Nakagawa, and S. Ōmura, *J. Antibiot.*, 1988, **41**, 250.
21 G. C. Levy, *Acc. Chem. Res.*, 1973, **6**, 161.
22 S. Sternhell, *Rev. Pure Appl. Chem.*, 1964, **14**, 15.
23 E. K. Davies, CHEMGRAF, Program for Molecular Modelling, Chemical Crystallography Laboratory, Oxford University, developed and distributed by Chemical Design Ltd., Oxford, 1985.
24 J. R. Everett, *J. Chem. Soc., Chem. Commun.*, 1987, 1878.

Received 20*th September* 1988; *Paper* 8/03649G