Amino-acids and Peptides. Part 19.[1] Conformational Studies of the Monamycins, a Family of Cyclohexadepsipeptide Antibiotics

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Evidence derived largely from studies of monamycins D_1 and H_1 in solution using ^1H n.m.r., ^{13}C n.m.r., and i.r. spectroscopy supports a single conformation (Figure 6) for each congener. It incorporates a β-loop with hydrogen bonding of the NH groups of the Val residue to the carbonyl of the hydroxypiperazic acid residue.

PREVIOUS papers have described the production, isolation, and elucidation of the molecular structure of the monamycins (1), a family of fifteen cyclohexadepsipeptide congeners with antibacterial activity largely against Gram-positive bacteria.[2-7] This study is concerned with the application of spectroscopic procedures to the determination of the favoured conformations of monamycins in solution.

In the last few years it has been shown that the conformations of cyclic peptides and depsipeptides in solution can be elucidated by using a combination of physical methods, potential-energy calculations, and model building.[8-15] N.m.r. spectroscopy has been the most powerful of the physical techniques used for this purpose, and the advent of ^{13}C n.m.r. spectroscopy and lanthanoid shift reagents has increased further the potential of this method.

Various features of the structures of the monamycins have bearing on these studies of conformation. They have only one monosubstituted amide bond in the 18-membered ring. This limits the conformational information available from measurement of the vicinal coupling $^3J_{NHCH}$ which follows a $\cos^2\phi$ relationship for the dihedral angle ϕ between the coupling protons.[8,16] However, this relationship has been useful in various cases for determining the side-chain conformations of the amino acid residues, using $^3J_{CHCH}$ as the probe. In monamycins such measurements have been applied to piperazic acid residues, and in determining the conformation with respect to rotation about the C_α–C_β bond in the other amino-acids.

The presence of three cyclic imino-acid residues and one *N*-methyl-amino-acid in monamycins imparts considerable rigidity to the molecule. On the other hand, the *NN*-disubstituted amide bonds may be either *cis*- or *trans*-oriented (*Z* or *E*). Nevertheless, the all-*trans*-conformation is generally preferred, for cyclohexapeptides or depsipeptides.[11,17] Another feature of such peptides is the tendency to form intramolecular hydrogen bonds, with a typical N–H · · · O=C hydrogen bond enclosing a ten-membered ring, often termed the 'β-loop' (or 'β turn'). Various methods [8] have been employed to locate this hydrogen bond in peptides and cyclic peptides, where it is a common feature in the crystal and in solution. The bonded N–H stretching band in the i.r. absorption spectrum at high dilution,[8] the low temperature coefficient of the N–H chemical shift,[8,9,12] slow exchange of N–H with deuterium,[8,9,12] and the small low field shift of the N–H signal on addition of trifluoroethanol [14] have all been used to identify intramolecular N–H · · · O=C hydrogen bonds. ^{13}C N.m.r. spectroscopy has in recent years become a most

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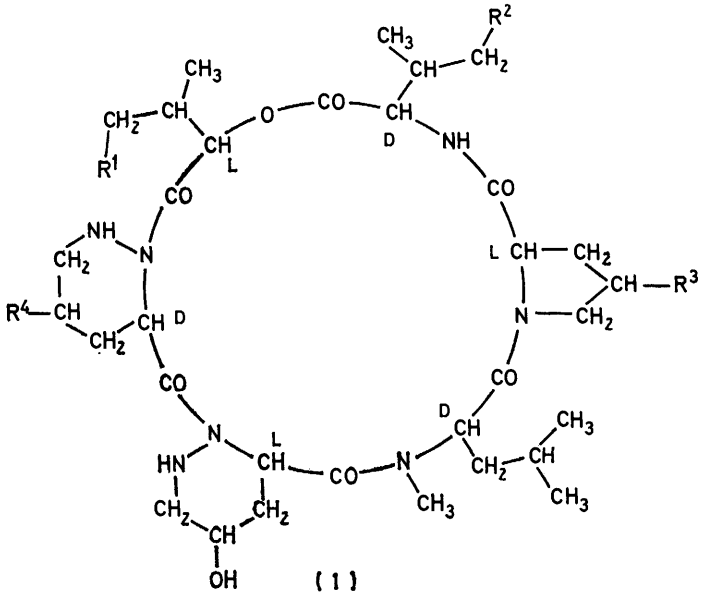
useful addition to the physical methods for elucidating peptide conformation, since the chemical shifts are very sensitive to conformational changes, particularly those



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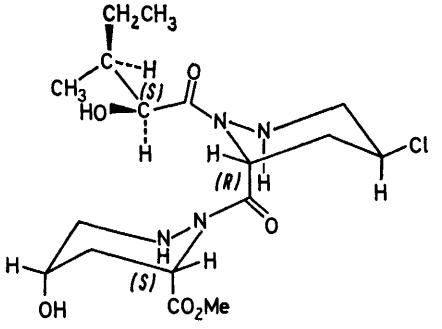

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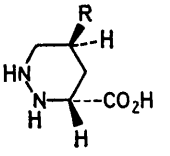
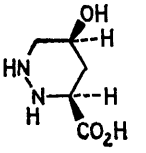


(3) R = H
(4) R = Cl



(5)

associated with *cis–trans* isomerism about the peptide bond.[9,11,13,15] We have investigated the application of these techniques to monamycin.

EXPERIMENTAL

The isolation, purification, and characterisation of the monamycins and of the products derived from hydrolysates have been described in earlier publications.[2-5]

N.m.r. spectra were recorded with Varian HA-100, XL-100/15, and HR-220 spectrometers, the XL-100 being used for both ^1H (100 MHz) and ^{13}C (25.2 MHz). C.d. spectra were determined with a Dichrograph Mark II–S instrument, by courtesy of Jobin Yvon Instruments Ltd. I.r. spectra at high dilution (3 cm cell; carbon tetrachloride or chloroform as solvent; $\leqslant 0.005$M) were measured with a Perkin-Elmer 257 instrument.

^1H N.m.r. studies were conducted on the recrystallised mixtures of chlorinated congeners, the corresponding non-chlorinated series, and the purified congeners, monamycins H_1 and D_1. ^{13}C N.m.r. studies, which required concentrated solutions (*ca.* 0.5M), were conducted on the mixtures of chlorinated congeners and on the corresponding non-chlorinated species, consisting largely of monamycins H_1 and D_1, respectively. The minor congeners in the mixtures

Monamycin	R^1	R^2	R^3	R^4
A	H	H	Me	H
B_1	H	H	Me	H
B_2	H	Me	H	H
B_3	Me	H	H	H
C	Me	H	Me	H
D_1	Me	H	Me	H
D_2	H	Me	Me	H
E	Me	Me	Me	H
F	Me	Me	Me	H
G_1	H	H	Me	Cl
G_2	H	Me	H	Cl
G_3	Me	H	H	Cl
H_1	Me	H	Me	Cl
H_2	H	Me	Me	Cl
I	Me	Me	Me	Cl

gave low intensity signals (*ca.* 20% of the main) which did not interfere with the assignments of the main carbon resonances.

TABLE 1

N.m.r. data and assignments for the tripeptide methyl ester (2) [a]

δ	No. of protons	J/Hz	δ (after D_2O exch.)	Assignment
6.04	1 bd) [b]	6	6.04	PipC$_\alpha$H [e]
5.34	1 (bd)	13		NH
5.04	1 (dd)	4, 11		NH
5.00	1 (bd)	7	4.99	PipC$_\alpha$H [e]
4.54	1 (d)	3	4.53	Acid C$_\alpha$H
4.08	1 (bm)	Σ 30 [c]	4.05	CHCl
3.95	1 (bs)	Σ 8 [c]	3.91	C*H*OH
3.71	3 (s)		3.70	OCH_3
3.5—1.1	13 (cm)		3.5—1.1 [d]	Not assigned
1.03	3 (d)	7	1.03	CH_3·CH
0.88	3 (t)	7	0.88	CH_3·CH_2

[a] $CDCl_3$, followed by D_2O exchange. [b] b = broad, d = doublet, m = multiplet, s = singlet, c = complex, t = triplet. [c] Sum of coupling constants, from the band-width at half height. [d] Less one H (OH ⟶ OD). [e] Chloro- and hydroxy-piperazic acid α-protons not differentiated. [f] Isoleucic acid.

RESULTS

The following amino-acids which occur as residues in the monamycins (1) have been well characterised: L-proline or *trans*-4-methyl-L-proline, D-valine or D-isoleucine, *N*-methyl-D-leucine, and novel cyclic imino-acids each possessing the hexahydropyridazine ring, *ester* (2). The tripeptide methyl ester (2) derived from the products of mild alkaline hydrolysis of chlorinated monamycin [4] provided valuable information for assignment purposes (Table 1). The α-proton multiplets, particularly after deuterium oxide exchange, were clearly resolved at δ 6.04, 4.99, and 4.53, the last appearing as a

TABLE 2

Partial assignments [a] in the H^1 spectra (δ values; *J*/Hz in parentheses) of chlorinated monamycin H_1

Assignment	$CDCl_3$	$(CD_3)_2CO$	CD_3CN	$(CD_3)SO$
Val NH	7.56 (d, 9.0)	7.52 (d, 9.2)	7.36 (d, 9)	7.10 (d, 9)
HyPip OH [c]	6.60 (d, 9.8)	6.38 (bs)		
ClPip CαH [b]	6.01 (bd, *ca.* 6)	6.07 (bd, 6.5)	5.88 (dd, 2, 6)	5.70 (bd, 6)
HyPip CαH [b]	5.60 (dd, 3, 5)	5.79 (bd, 6.5)	5.58 (2, 7)	*ca.* 5.3
Ile(OH) [d] CαH	5.52 (d, 6)	5.70 (d, 6.5)	5.52 (d, 6)	5.56 (d, 6)
MeLeu CαH [b]	5.48 (m)	5.56 (t, 7.5)	*ca.* 5.32 (t, 7.5)	*ca.* 5.3
ClPip NH [b]	*ca.* 5.5 (m)	5.08 (dd, 2, 12)	4.93 (t, 7.5)	*ca.* 5.3
HyPip NH [b]	4.72 (dd, 2, 12)		*ca.* 4.6	
MePro CαH	4.64 (bd, 8.5)	4.70 (d, 8)	4.54 (d, 8)	4.44 (d, 8)
Val CαH [c]	4.52 (dd, 7, 9)	4.54 (dd, 7, 9)	4.44 (dd, 6.5, 9)	4.26 (bm)
ClPip CHCl	4.23 (m, Σ*J* 33)	4.36 (m, Σ*J* 33)	4.26 (bm)	4.26 (bm)
HyPip C*H*OH [c]	3.75 (bd, 8, *ca.* 10)	3.72 (bs)	3.81 (bs)	3.50 (bs)
MeLeu NCH_3	3.23 (s)	3.16 (s)	3.29 (s)	3.04 (s)

[a] Other side-chain protons are not assigned. [b] Assigned by the effect of lanthanoid shift reagents. [c] Assigned by decoupling experiments. [d] Isoleucic acid.

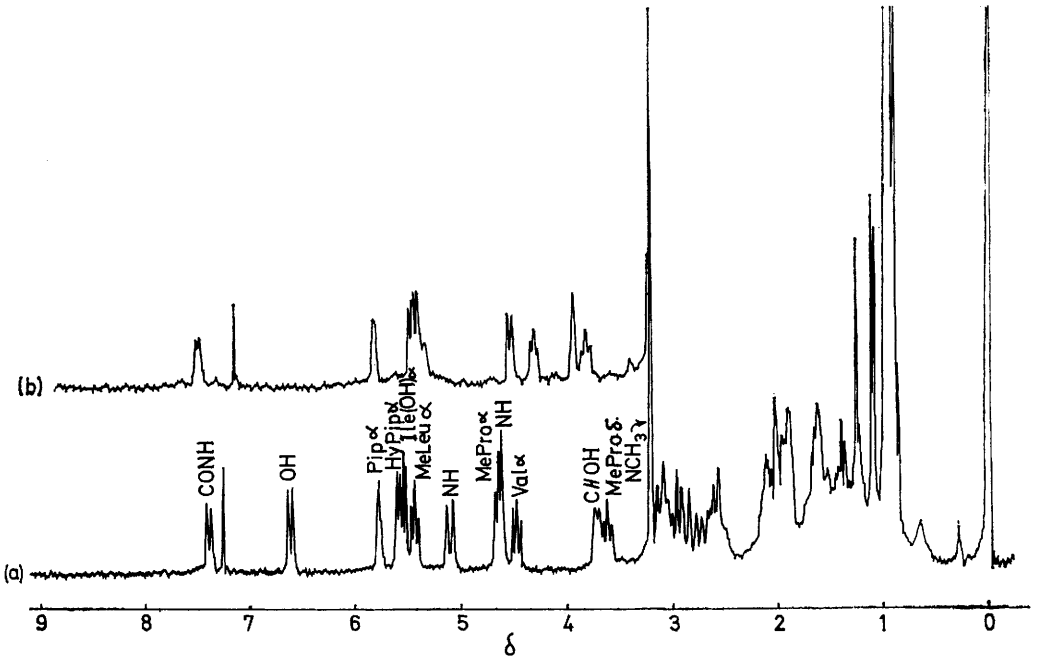


FIGURE 1 The ^1H spectrum at 220 MHz of monamycin H_1; (a) in $CDCl_3$ alone, (b) in $CDCl_3$–$CF_3{\cdot}CO_2D$ (trace)

namely (3*R*)-piperazic acid (3), (3*R*,5*S*)-5-chloropiperazic acid (4) and (3*S*,5*S*)-5-hydroxypiperazic acid (5); in addition there is an anhydro-derivative of (5), probably (3*S*)-Δ^4(or Δ^5)-piperazic acid.[2] The hydroxy-acid forming the depside link may be either 2-hydroxy-3-methyl-L-butanoic acid (6) or 2-hydroxy-3-methyl-L-pentanoic acid (7) (L-isoleucic acid). Monamycin D_1 (*M* 691) and monamycin H_1 (*M* 725), selected as typical congeners for the n.m.r. studies, were purified and characterised as in earlier investigations.[3]

^1H *N.m.r. Assignments.*—(a) *The tripeptide methyl* sharp doublet assigned to the 2-proton of the 2-hydroxy-3-methyl-L-pentanoic acid residue; the other two similar multiplets were attributed to the α-protons of the piperazic acid residues. The broad multiplet at δ 4.05 is similar in shape and position to a multiplet at δ 4.23 found in the ^1H n.m.r. spectrum of chlorinated but *not* non-chlorinated monamycins. The narrow multiplet at δ 3.91 in the spectrum of the tripeptide, which is similar to that in the spectra of both monamycins H_1 (δ 3.75) and D_1 (δ 3.73), was assigned to C*H*(OH). The coupling constants for protons in the piperazic acid residues are so

similar for the tripeptide and the monamycins that it can be assumed that the conformations of the hexahydropyridazine rings are the same.

(b) *Monamycin* H_1. The ^1H n.m.r. spectrum (220 MHz) of this congener in deuteriochloroform solution after addition of a trace of trifluoroacetic acid (to remove signals for the exchangeable protons) is shown in Figure 1. The presence of three rapidly exchanging (OH, 2 × doublet at δ 4.64 to the α-proton of the methylproline residue. The two remaining low field multiplets, at δ 6.01 and 5.60, which were very similar to corresponding signals in the spectrum of the tripeptide, were assigned to the α-protons of the chloro- and hydroxy-piperazic acid residues, respectively. The doublet for the α-proton of methylproline is unusual for C*H*·CH_2. However, it has been reported for proline residues in other

TABLE 3

Partial assignments [a] in the ^1H spectra (δ values; J/Hz in parentheses) of non-chlorinated monamycin D_1

Assignment	$CDCl_3$	$(CD_3)_2CO$	CD_3CN	CD_3OD	CCl_4
Val NH	7.37 (d, 9.0)	7.02 (d, 9)	7.18 (d, 9)	7.52 (d, 8)	7.11 (d, 9)
HyPip OH [c]	6.59 (d, 9.9)	6.12 (d, 9)	5.84 (d, 9)		6.13 (d, 8.5)
Pip C_αH [b]	5.79 (bm)	*ca.* 5.6	5.63 (bm)	*ca.* 5.6	5.61 (bm)
HyPip C_αH [b]	*ca.* 5.65	*ca.* 5.6	5.52 (bm)	*ca.* 5.6	5.49 (bm)
Ile(OH) [d] C_αH	*ca.* 5.6 (d, 6)	*ca.* 5.6 (d, 6)	5.58 (d, 6)	5.57 (d, 6)	*ca.* 5.3 (d, 6)
MeLeu C_αH	5.48 (t, 7.5)	5.45 (t, 7)	5.34 (t, 8)	*ca.* 5.6	5.26 (t, 8)
Pip NH [b]	5.13 (bd, 11)	5.14 (dd, 2, 12)	5.10 (dd, 3, 13)		
HyPip NH [b]	4.69 (bd, 10)	4.80 (t, 8)	4.80 (dd, 6, 8)		
MePro C_αH	4.65 (bd, 8)	4.58 (bd, 8)	4.54 (bd, 8)	4.59 (d, 8)	4.50 (d, 8)
Val C_αH [c]	4.49 (dd, 6.5, 9)	4.41 (dd, 7, 9)	4.35 (dd, 7, 9)	4.44 (dd, 5, 8)	4.33 (dd, 7, 9)
HyPip C*H*OH	3.73 (bd, *ca.* 10)	3.66 (bm)	3.57 (bm)	*ca.* 3.7 (bm)	3.59 (bm)
MeLeu NCH_3	3.22 (s)	3.10 (s)	3.07 (s)	3.25 (s)	2.90 (s)

[a] Other side-chain protons not assigned. [b] Assigned by lanthanoid-induced shifts. [c] Assigned by decoupling experiments. [d] Isoleucic acid.

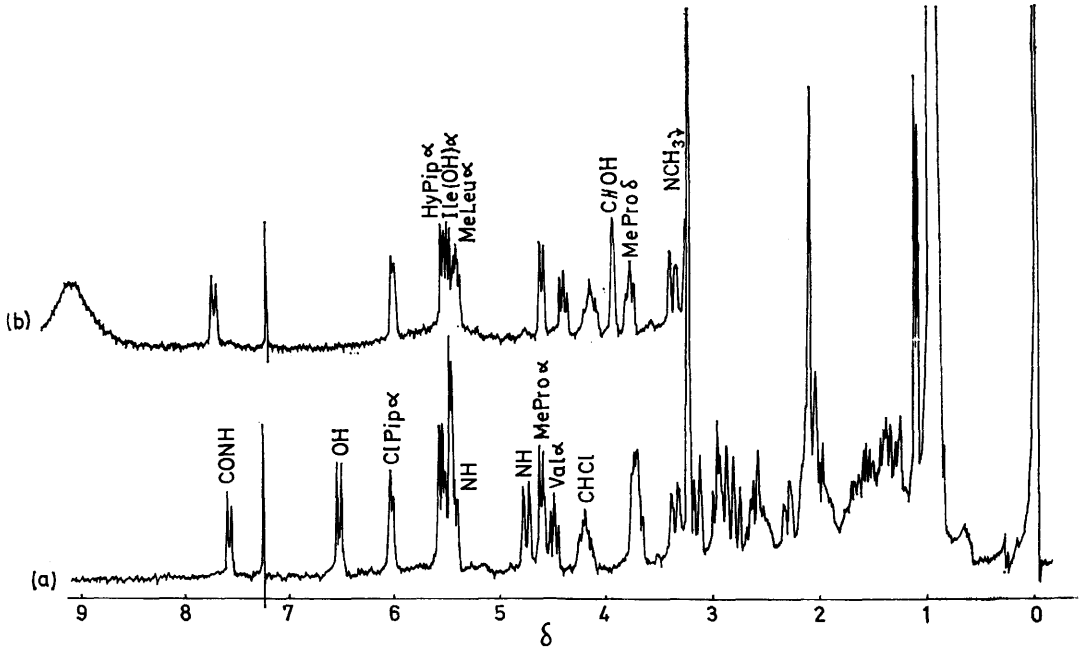


FIGURE 2 The ^1H spectrum at 220 MHz of monamycin D_1; (a) in $CDCl_3$ solution, (b) in $CDCl_3$–CF_3·CO_2H (trace)

NH in piperazic acid residues) and one slowly exchanging proton (CONH) is evident. Decoupling experiments established the positions of the signals due to CONH·CH in the valine residue and CH·OH in the hydroxy-piperazic acid; the two pairs had large vicinal coupling constants of 9.2 and 9.8 Hz, respectively. Lanthanoid-induced shifts (LIS), brought about by Eu([$^2H_9$]fod)$_3$, confirmed these assignments. The distorted triplet at δ 5.48 was attributed to the α-proton of the *N*-methylleucine, the sharp doublet at δ 5.52 to the O·CH·CO of the 2-hydroxy-3-methyl-L-pentanoic acid, and the broad

cyclic peptides.[18] The C_α–H and C_β–H bonds, with dihedral angles (φ) of *ca.* 90 and 30°, account for the coupling constants $^3J_{\alpha\beta}$ of 0 and 8.5 Hz, respectively. The broad multiplet at δ 4.21 (ΣJ *ca.* 33 Hz) was assigned to CHCl by analogy with the tripeptide (2). Apart from the sharp singlet at δ 3.23 (*N*-Me), the high-field part of the spectrum was too complex for further assignment.

(c) *Monamycin* D_1. The α-proton resonances are more clearly resolved and assigned in the spectrum of monamycin D_1 (Figure 2, Table 3). Assignments were

[18] D. J. Patel, *Biochemistry*, 1973, **12**, 667.

again confirmed by spin-decoupling experiments, lanthanoid-induced shifts, exchange with deuterium oxide, and comparison with the spectra of the tripeptide (2) and of monamycin H_1.

(d) *Lanthanoid-induced shifts.* It is well known that Eu([$^2H_9$]fod)$_3$ [(6,6,7,7,8,8,8-heptafluoro-2,2-di[$^2H_3$]-methyl[$^2H_3$]octane-2,4-dionato)europium(III)] binds strongly to hydroxy-groups in alcohols, phenols, or carboxylic acids, and to amines and carbonyl groups, but less strongly to amide groups in peptides.[19] Addition of small quantities to a solution of monamycin D_1 or H_1 in deuteriochloroform caused a large downfield shift of the OH doublet in the ^1H n.m.r. spectrum and a smaller shift of the C*H*·OH signal of the hydroxypiperazic acid residue, but there was scarcely any shift of the three NH signals. A more detailed analysis of the effect on the spectrum of monamycin H_1 (Figure 3) distinguished the signals of the α-protons, CH·OH and CHCl, the three N–H protons, and NC*H*$_3$. The relevance of these results to conclusions concerning conformations of the monamycins is discussed later. It is clear that

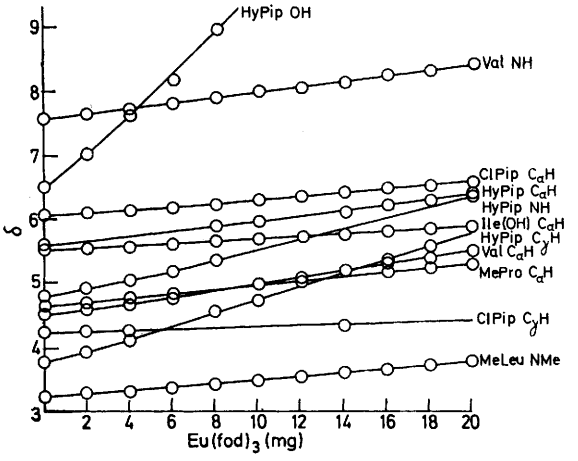


FIGURE 3 The effect of progressive addition of Eu(fod)$_3$ on the chemical shift of various protons in monamycin H_1, indicating pronounced binding to the hydroxy-group

binding of the shift reagent to monamycins occurs almost entirely on the hydroxy-group.

(e) ^{13}C *N.m.r. spectra.* The spectrum of a sample of non-chlorinated monamycin in deuteriochloroform solution (mainly monamycin D_1) is illustrated in Figure 4. Minor peaks are assigned to the other congeners present in the mixture. (For example the most abundant of the minor congeners, monamycin F, has valine replaced by isoleucine; peaks due to this congener are clearly visible throughout the spectrum.) There are 33 major carbon resonances resolved out of the expected total of 34, with two overlapping signals for carbon atoms at δ 24.85; they separate on addition of hexadeuteriobenzene to the solution. At low field there are six carbonyl signals as expected. The α-carbon signal of the isoleucic acid is found in midfield, δ 75.7; between δ 45.5 and 61.2 there are signals for nine carbon atoms, which include the α-carbons of the five amino acids, the CHOH and the three CH_2N groups. The remaining high-field region, δ 11.0—38.0, includes the signals for

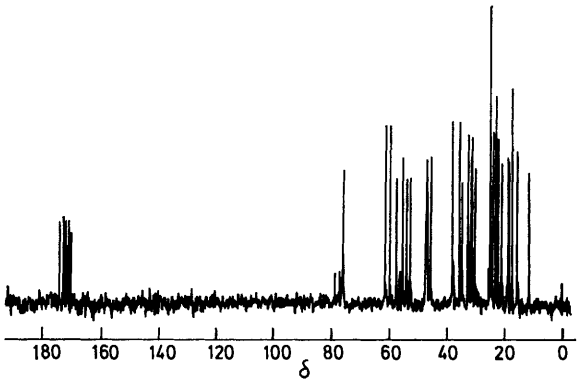


FIGURE 4 The ^{13}C spectrum at 25.2 MHz of non-chlorinated monamycin (primarily monamycin D_1). Spectral conditions: 150 mg monamycin in 0.4 ml $CDCl_3$, 5 mm tube, pulse width 75 μs, 3 033 transients. Minor congeners present appear as small peaks

the remaining side chain carbon atoms, and the NMe of the MeLeu residue.

The chlorinated monamycins (monamycin H_1) gave a similar ^{13}C spectrum, the only major differences being the shifts of C_α, C_β, C_γ, and C_δ in the chlorinated piperazic acid residue. Assignments in both compounds were made by using off-resonance and single-resonance decoupling, comparisons with model compounds (Table 4), and addition of Eu([$^2H_9$]fod)$_3$ and Gd([$^2H_9$]fod)$_3$ to solutions in deuteriochloroform. The latter shift reagent, which has a distance-dependent, line-broadening effect without affecting the chemical shifts, allowed immediate assignment of all the carbon signals of the hydroxypiperazic acid residue. Model compounds such as the free amino- or hydroxy-acids and derivatives were not useful for assignment except for the side-chain γ- and δ-carbon atoms. Comparison of the spectra with those of actinomycin K_{2t}, which also contains *trans*-4-methyl-L-proline,[20] enabled the assignment of the MePro carbon signals in monamycin.

Evidence for the Presence of an Intramolecular Hydrogen Bond.—I.r. spectra of monamycins at high dilution in carbon tetrachloride (0.002—0.0001M) provided important information relating to hydrogen bonding. To simplify the interpretation of the data, the protons in the hydroxy- and imino-groups of the piperazic acids were first exchanged with deuterium, by shaking solutions of the monamycins in carbon tetrachloride with deuterium oxide. The amide NH stretching band occurred at 3 300 cm^{-1}, as in the case of the intramolecularly-bound NH groups of valinomycin (3 309 cm^{-1}) and of gramicidin S (3 314 cm^{-1}),[8] but differed from that for the ' free ' NH

[19] A. F. Cockerill, G. L. O. Davies, R. C. Harden, and D. M. Rackham, *Chem. Rev.*, 1973, **73**, 553.
[20] A. B. Mauger and W. A. Thomas, in preparation.

frequency of serratamolide (3 430 cm^{-1}).[21] This indicates that the valine NH proton is intramolecularly bound, probably to the carbonyl group of the hydroxypiperazic acid residue.

Three ^1H n.m.r. procedures have been employed to demonstrate for peptides the presence or absence of

TABLE 4

^{13}C Chemical shifts in the monamycins

Monamycin D_1 [a]			Monamycin H_1 [b]		
δ	CH_n	Assignment	δ	CH_n	Assignment
11.54	CH_3	Ile(OH) δ	11.32	CH_3	Ile(OH) δ
15.58	CH_3	Ile(OH) γ	15.39	CH_3	Ile(OH) δ
17.28	CH_3	MePro (Me)	17.15	CH_3	MePro (Me)
18.45	CH_3	Val γ	18.30	CH_3	Val γ′
18.78	CH_3	Val γ′	18.66	CH_3	Val γ′
20.95	CH_2	Pip γ	22.02	CH_3	MeLeu δ
22.31	CH_3	MeLeu δ	23.01	CH_3	MeLeu δ′
23.06	CH_3	MeLeu δ′	24.14	(CH_2)	Ile(OH) γ
24.11	(CH_2) [c]	Pip β	24.94	(CH)	MeLeu γ
24.85	(CH_2)	Ile(OH) γ	30.08	CH_2	HyPip β
24.85	CH	MeLeu γ	31.35	(CH)	MePro γ
30.12	CH_2	HyPip β	31.43	CH_3	MeLeu (NMe)
31.19	(CH)	MePro γ	32.68	(CH)	Val β
31.49	CH_3	MeLeu (NMe)	34.51	(CH_2)	MeLeu β
32.59	(CH)	Val β	35.17	(CH_2)	ClPip β
34.74	CH_2	MePro β	35.57	CH	Ile(OH) β
35.57	(CH)	Ile(OH) β	37.79	(CH_2)	MePro β
37.95	CH_2	MeLeu β	45.54	CH	HyPip α
45.54	CH	HyPip α	48.41	CH	ClPip α
46.84	CH	Pip α	51.11	CH	ClPip γ
46.97	CH_2	Pip δ	52.22	CH	MeLeu α
52.58	CH	MeLeu α	54.10	(CH_2)	ClPip δ
53.93	CH_2	MePro δ	54.10	CH_2	HyPip δ
55.27	CH_2	HyPip δ	54.72	(CH_2)	MePro δ
57.35	CH	Val α	57.12	CH	Val α
59.57	CH	HyPip γ	59.37	CH	HyPip γ
61.21	CH	MePro α	60.69	CH	MePro α
75.70	CH	Ile(OH) α	75.36	CH	Ile(OH) α
170.04	CO	HyPip	169.88	CO	
170.79	CO		170.67	CO	
171.18	CO		170.91	CO	Not assigned
171.73	CO	Not assigned	171.37	CO	
172.67	CO		172.31	CO	
174.02	CO		171.71	CO	

[a] *ca.* 0.2M in $CDCl_3$ solution. [b] $CDCl_3$ + 1 drop CD_3OD. [c] Tentative assignment indicated by brackets. [d] Model compounds examined include isoleucic acid, isoleucine, piperazic acid, leucine, acetyl-leucine methyl ester, *N*-methyl-leucyl-proline t-butyl ester, and the 2,4-dinitrophenyl derivatives of hydroxypiperazic acid, chloropiperazic acid, and *trans*-4-methylproline. The cyclic peptides actinomycins C and K_{2t} and gramicidin S were also used as model compounds.

protons either intramolecularly bound or shielded from the solvent. The change in the CONH chemical shift with temperature [8,9,12] ($d\delta/dT$) is 0.006 6 p.p.m. deg^{-1} in [$^2H_6$]dimethyl sulphoxide and 0.002 5 in [$^2H_6$]acetone. In previous studies on evolidine,[22] for example, it was suggested that low coefficients (*ca.* 0.002) indicated protons shielded from solvent, whereas values $\geqslant$0.006 indicated exposed protons. The second method involves the rate of exchange of the CONH proton in deuteriated solvents.[8,9,12] For monamycin, in anhydrous [$^2H_4$]-methanol, the exchange of the amide proton was extremely slow ($t_{\frac{1}{2}}$ 2—3 days). In aqueous methanol (D_2O–CD_3OD), the rate of exchange was increased ($t_{\frac{1}{2}}$ ~ few hours), but it was still low enough to indicate an NH proton shielded from the solvent, or intramolecularly bound. Urry has demonstrated that the addition of 1,1,1-trifluoroethanol to solutions of cyclic peptides provides a sensitive test; signals due to protons shielded from the solvent shift slightly downfield, whereas protons exposed to the solvent experience large upfield shifts.[14] In monamycin there was a slight downfield shift, again indicating that the valine NH proton is either intramolecularly bound, or otherwise shielded from the solvent.

Temperature Effects on Monamycin (C.d. and N.m.r. Studies).—The presence of 34 resolved carbon signals in

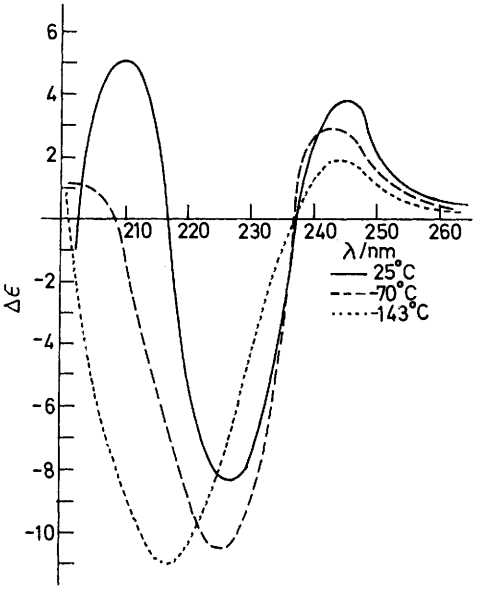


FIGURE 5 Schematic representation of the c.d. curve for monamycin at varying temperatures [7.4 mg of monamycin dissolved in methanol–ethanol (70 : 30; 200 ml)]

the ^{13}C n.m.r. spectra of the monamycins D_1 and H_1 indicates that a single species, or a mixture of several species rapidly interconverting to give a time-averaged spectrum, exists in solutions. When a solution of monamycin D_1 in [$^2H_4$]methanol was cooled to −90 °C, broadening of all the proton resonances occurred. This could arise through a reduction in rate of interconversion of conformers, or through aggregation of the single conformation at the lower temperature. A similar broadening effect is observed at normal temperatures in very concentrated solutions of monamycin D_1 in deuteriochloroform (>250 mg ml^{-1}). This suggests that aggregation is occurring rather than conformational changes. Aggregation effects have been observed with the cyclic decapeptide tyrocidine B,[23,24] and with the

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actinomycins [25-27] in aqueous solutions. As far as can be determined, the proton–proton coupling constants in the monamycins do not vary with temperature. Again this suggests that aggregation rather than conformational change is occurring. Similar changes are observed in the c.d. spectra of monamycin in methanol and methanol–ethanol in the temperature range −140 to 20 °C (Figure 5). The peak at 227 nm in the negative region increases in amplitude with decreasing temperature, while the positive peak at 210 nm decreases with decreasing temperature, disappearing at *ca.* −100 °C. This is in accord with either conformational changes *or* aggregation taking place at low temperatures.

DISCUSSION

The data obtained make it possible to reach certain conclusions concerning the conformations of the monamycins. The ^{13}C and ^{1}H n.m.r. studies indicate that there is no difference between the gross conformations of chlorinated and non-chlorinated monamycins. Detailed analysis of the ^{1}H spectrum of the tripeptide methyl ester (2), together with previous knowledge of the stereochemistry and structure of the individual hydroxy- and chloro-piperazic acids, indicated that these residues both adopted the chair conformation in the tripeptide. In each case the α-carboxamide group was axially-oriented, but for the chloropiperazic acid residue the γ-chlorine atom was equatorial, whereas in the hydroxypiperazic acid the hydroxy-group was axial. The preference of the α-carboxamide groups for the axial positions has been previously observed in *N*-acylated pipecolic acid derivatives,[28] and in a variety of 2-substituted 1-acylpiperidines.[29] This conformation of the piperazic acid residues is also found in monamycin H_1 since the multiplicities of the α- and γ-protons in these residues are identical in the two compounds. Similarly, in monamycin D_1, the piperazic acid residue lacking the γ-substituent has the standard chair conformation, again with the α-carboxamide group axial. The large coupling constants (9.8 Hz) for CH_γ and OH in monamycin indicate a *trans*-relationship.[30] Moreover the i.r. spectra of monamycins at high dilution suggest that the hydroxy-group is ' free ' ($\nu_{max.}$ 3 610 cm^{-1}) and not hydrogen bonded to the carbonyl of the hydroxypiperazic acid residue, which is probably hydrogen bonded to the valine NH. Coupling constants, one large (*ca.* 11 Hz) and one small (0—2 Hz), for NH of the two piperazic acid residues in both the tripeptide and monamycin indicate axial orientation for these two protons, coupled to the axial and equatorial CH_2. The partial conformation of the tripeptide methyl ester (2) incorporating these features is shown.

The presence of an intramolecular hydrogen bond in the monamycins, probably between the D-valine NH and the carbonyl of the hydroxypiperazic acid residue, to form the low energy Type I,4 ⟶ 1, β-loop (or β turn) is strongly indicated by the evidence of ^{1}H n.m.r. and i.r. spectroscopy. Additional information is provided by the NH·CH coupling constant in the valine residue (9.1 Hz); this is in accord with the *trans*-(*anti*-)orientation of the N–H and C–H bonds required in such a structure.[16]

The side-chains of the non-cyclic amino- or hydroxy-acids present appear to be relatively freely rotating about the C_α–C_β bonds. The vicinal coupling constants in each case lie between the values expected for *trans*- or *gauche*-arrangements of the side-chains. The unusual appearance of the methylproline residue α-proton

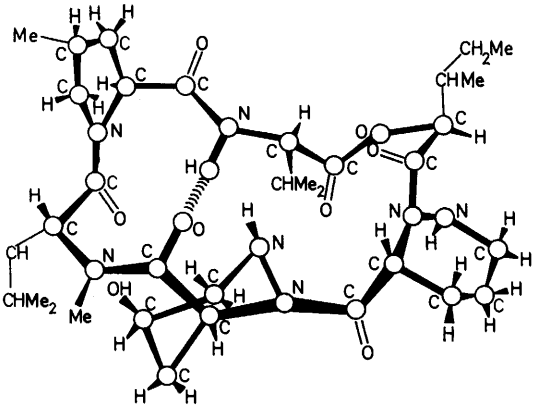

FIGURE 6 A conformation of monamycin D_1 which fits the experimental data, and including the 4 ⟶ 1 hydrogen bond, enclosing a β-loop

signal as a broad doublet indicates a ring conformation more buckled than in normal proline residues; this is attributed to the γ-methyl group and the position of the MePro residue in the ' corner ' of the β-loop.

At present, we are unable to prove that all six peptide or depside bonds are *trans*-oriented in the monamycins, although there is substantial evidence in support of this. The strong i.r. stretching frequency at 1 550 cm^{-1} is indicative of *trans*-peptide bonds. Although ^{13}C n.m.r. spectroscopy has provided convincing evidence in other cases relating to *cis–trans* amide isomerism, particularly for proline residues,[31-35] the absence of information relating to the ^{13}C n.m.r. spectra of *N*-acylated derivatives of methylproline, the three piperazic acids, and the methyl-leucine residue limited the application of the technique in this case. The presence of a 4 ⟶ 1

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intramolecular hydrogen bond in monamycin is not compatible with *cis*-peptide bonds for the HyPip-MeLeu and MeLeu-MePro linkages. However attempts to build models of monamycin with any of the peptide bonds *cis* resulted in highly crowded, high-energy forms which were rejected. We conclude, therefore, that the all-*trans*-conformation is that favoured for the monamycins.

Based on the arguments outlined above, a conformation which fits all the available information is shown in Figure 6. The evidence allows little variation, except perhaps in the arrangement of the side-chains of the non-cyclic amino- and hydroxy-acids. Alternative structures, with the D-Val NH hydrogen-bonded to other carbonyl groups, have been considered. The all-*trans*-arrangement of amide and ester bonds, and the steric requirements of the relatively rigid imino-acid residues, preclude other possible structures, such as that with a hydrogen bond between the D-Val NH and the D-Pip carbonyl. Apart from the fact that the β-loop is a common feature in the tertiary structures of peptides and proteins, supporting evidence stems from the finding that the axial hydroxy-group in the HyPip residue is not hydrogen bonded to the axial HyPip carbonyl group, which is therefore available for bonding to the Val NH.

Variable-temperature n.m.r. and c.d. studies indicate strongly that aggregation of monamycin molecules occurs in organic solvents (methanol and/or ethanol) at low temperatures, and to a lesser extent at high concentrations at ambient temperatures. This phenomenon has been reported previously for the cyclodecapeptide tyrocidine B,[23,24] and the actinomycins,[25-27] but only in aqueous solution. In the case of valinomycin, the involvement of aggregation in the mechanism of action has been discussed.[36]

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