A Carbon-13 Nuclear Magnetic Resonance Study of Ristocetins A and B and their Derivatives

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A detailed assignment of the ^{13}C n.m.r. spectra of ristocetins A and B, and several derivatives, is given. The assignment not only defines hitherto doubtful configurations at anomeric carbons of the carbohydrate portion, but also paves the way for ^{13}C biosynthetic studies.

RISTOCETINS A and B are glycopeptide antibiotics elaborated by the micro-organism *Nocardia lurida* and are known to act by binding to bacterial cell-wall mucopeptides terminating in the sequence -D-Ala-D-Ala.[1] Cross-linking of the cell wall is thus inhibited during the process of biosynthesis, leading to a cessation of growth and the eventual destruction of the cell by lysis. The complete three-dimensional structure of ristocetin A has been determined by a combination of spectroscopic and chemical methods [2-4] and the stereochemistry of the complex with Ac-D-Ala-D-Ala has also been elucidated.[5]

We present here a detailed assignment of the ^{13}C n.m.r. spectra of ristocetin A (I), ristocetin B (II), and their derivatives di-*N*-acetylristocetin A (III), di-*N*-acetyltetra-*O*-methylristocetin A (IV), ψ-aglycone (V), di-*N*-acetyl-ψ-aglycone (VI), di-*N*-acetylhexa-*O*-methyl-ψ-aglycone (VII), and di-*N*-acetylhexa-*O*-methyl-di-*O*-acetyl-ψ-aglycone (VIII). This assignment confirms many of the structural details established mainly by ^1H n.m.r. spectroscopy,[2] allows the configuration of the carbohydrate portion to be correctly defined, refines the details of the complex with Ac-D-Ala-D-Ala, and paves the way for ^{13}C biosynthetic studies.

RESULTS AND DISCUSSION

Proton noise-decoupled spectra of ristocetins A and B and ψ-aglycone [6] were obtained in $(CD_3)_2SO$ at 80 °C and their chemical shifts, measured taking the chemical shift of dimethyl sulphoxide (DMSO) as 39.6 p.p.m. relative to internal $SiMe_4$, are presented in Table 1. Spectra

TABLE 1

Signal	Multiplicity †	Risto A DMSO	Risto A D_2O	Risto B DMSO	ψ-Aglycone DMSO	ψ-Aglycone D_2O	Risto A calc. D_2O	Assignment	Method of assignment §
1	s	171.9	172.2	173.4	172.7	175.4			
2	s	170.1	172.7	170.2	170.4	173.5			
3	s	170.1	172.0	170.1	170.2	172.7			
4	s	169.0	171.5	169.3	169.6	172.3			
5	s	168.8	170.9	168.8	169.1	171.7			
6	s	168.1	170.9	168.3	168.7	171.2			
7	s	167.6	170.5	167.9	168.6	171.2			
8	s	157.1	157.8	157.3	157.0	159.2	158.1	D5	*c,a*
9	s	155.9	158.5	156.0	157.2	158.5	159.2	D3	*c*
10	s	155.2	157.8	155.2	155.6	158.5	155.0	E6	*c*
11	s	155.1	157.2	155.2	155.9	157.7	157.1	F3	*c*
12	s	155.0	156.6	154.8	155.2	156.1	156.7	F5	*c*
13	s	154.5	155.6	154.6	155.3	157.3	151.0	A4	*c*
14	s	154.5	155.4	154.5	155.3	157.2	149.4	C4	*c*
15	s	153.5	155.1	153.4	149.3	151.3	153.6	B1	*b*
16	s	152.9	154.8	152.9	149.2	151.1	152.2	B3	*b*
17	s	146.6	149.8	146.0	147.4	149.3	149.0	G5	*b,a*
18	s	143.1	145.8	143.4	142.2	145.9	145.8	G4	*b,a*
19	s	137.0	136.4	137.2	137.0	137.4	139.9	C1	*b,a*
20	s	136.4	134.6	136.4	136.4	137.3	139.3	D1	*b,a*
21	d	135.4	135.9	135.4	135.8	137.2	136.5	E2	*b*
22	s	134.1	135.2	134.1	134.3	136.4	135.9	B5	*a*
23	s	133.3	134.0	133.4	133.3	136.4	131.5	F1	*a*
24	s	133.3	133.8	133.0	134.8	134.4	138.2	A1	*f*
25	s	131.7	133.0	131.3	130.6	132.6	133.4	B2	*a*
26	d	~128.0	130.4	~128.1	~128.4	130.9		A2	*f*
27	d	~128.0	130.4	~128.1	~128.4	130.3	~130	C2	*f*
28	d	~128.0	129.4	~128.1	~128.4	129.7		A6	*f*
29	d	~128.0	128.8	~128.1	~128.4	129.6		C6	*f*
30	s	~128.0	128.4	~128.1	127.5	128.9	128.1	E3	*b*
31	d	125.9	127.5	125.9	125.9	128.8	127.3	E4	*b,c*
32	s	125.3	126.3	125.3	125.9	127.7	125.6	G1	*f*
33	d	124.1	127.7	123.8	124.9	128.8	125.1	G2	*a*
34	d	123.1	124.8	123.1	122.8	125.2	124.0	A5	*c*
35	d	122.4	124.2	122.5	122.7	124.6	123.4	C5	*c*
36	d	121.8	123.8	121.8	122.1	124.6	121.3	A3	*c*
37	d	120.9	122.1	121.3	121.3	123.3	120.9	C3	*c*
38	s	120.2	122.1	120.4	120.9	122.3	122.2	E1	*b*

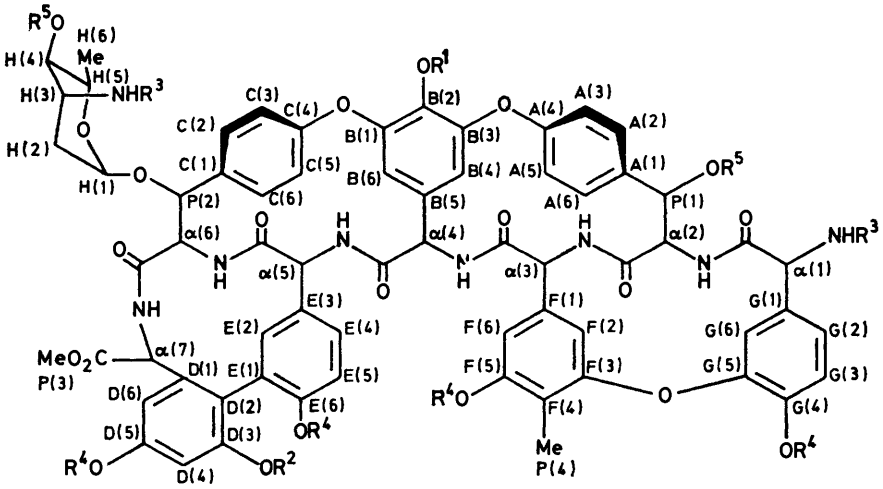
TABLE 1 (*continued*)

Signal	Multiplicity †	Risto A DMSO	Risto A D_2O	Risto B DMSO	ψ-Aglycone DMSO	ψ-Aglycone D_2O	Risto A calc. D_2O	Assignment	Method of assignment §
39	s	119.9	120.5	120.1	117.4	119.8	120.6	D2	*a*
40	d	117.5	119.4	117.5	118.1	120.3	118.7	G3	*f*
41	d	116.1	118.6	116.3 }	116.7	118.9	119.6	E5	*b,c*
42	d	116.1	121.1	115.7 }	117.5	122.3	118.9	G6	*a*
43	s	114.6	117.3	114.6	113.7	117.3	119.5	F4	*c*
44	d	110.2	110.7	110.5	109.5	110.9	111.6	F6	*a*
45	d	108.8	110.3				109.3	N1	*a,b*
46	d	107.5	109.8	107.5	103.8 ‡	105.8 ‡	110.8	F2	*b/a*
47	d	106.2	107.0	106.3	107.1	108.5	109.0	B4	*c*
48	d	105.2	107.0	105.4	105.8	109.2	109.7	D6	*c*
49	d	103.6	105.7	103.4	105.4	107.8	106.2	B6	*c*
50	d	102.0	104.8	102.0	103.4 ‡	105.6 ‡	104.3	D4	*b/a*
51	d	100.3	102.3	100.3			102.2	M1	*a,c,e*
52	d	100.3	102.3	100.3			101.3	L1	*f*
53	d	98.2	100.1	102.9			102.4	K1	*a,c,e*
54	d	97.1	98.2	97.1			99.3	J1	*c*
55	d	92.4	94.2	92.4	92.5	94.7		H1	*a*
56	d	84.9	85.3				84.9	N4	*b*
57	d	80.9	82.1				81.9	N2	*b*
58	d	77.2	78.6	73.9			79.2	K2	*b*
59	d	77.2	78.1	76.0			80.0	M2	*b*
60	d	76.3	77.8				77.5	N3	*b*
61	d	75.8	76.0				75.6	K5	*b*
62	d	74.9	76.0	76.6			75.8	K3	*b*
63	d	74.5	76.0	74.4	74.4	76.5		P2	*a,c*
64	d	73.5	74.3	73.5			74.3	J3	*b*
65	d	72.9	73.8				70.5	M5	*b*
66	d	72.0	73.2	72.0			72.8	L4	*b*
67	d	71.3	72.1	71.4	71.4	72.8	72.8	P1	*a,c*
68	d	70.9	71.6	70.9			71.6	J5	*b*
69	d		~71.3				70.5	K4	*b*
70	d			~70.2			71.1	J2	*b*
71	d	~70.2					71.1	L3	*b*
72	d						71.1	L2	*b*
73	d		~70.8				74.6	M3	*b*
74	d						69.5	M4	*b*
75	d	68.2	69.7	68.2			69.5	L5	*b*
76	d	67.6	68.7	68.2	67.9	69.3	69.5	H4	*b*
77	d	66.4	68.3	66.4			68.7	K6	*b*
78	t	66.4	67.5	66.4			67.5	J4	*b*
79	d	63.8	65.7	63.8	63.9	66.2	66.2	H5	*b*
80	t	61.2	62.5				62.4	N5	*b*
81	t	61.2	62.2	61.1			61.6	J6	*b*
82	t	61.2	62.1				63.8	M6	*b*
83	d	60.6	61.9	60.3	60.4	63.2	59.6	α2	*c*
84	d	60.4	61.9	60.2	60.1	62.7	61.1	α6	*c*
85	d	58.1	57.1	58.1	57.1	57.6		α1	*c,a*
86	d	57.1	59.0	57.9	57.9	59.4		α3	*c*
87	d	56.4	57.9	56.4	56.6	58.7	57.8	α7	*c*
88	d	54.7	56.2	54.6	54.6	56.6	56.0	α4	*c*
89	d	52.8	54.7	52.8	53.0	55.6	54.9	α5	*c*
90	q	51.9	54.6	51.7	51.8	55.0	51.0	P3	(*c*)
91	d	48.3	49.7	48.1	48.0	50.5	52.0	H3	*b*
92	t	30.9	31.8	30.9	31.0	32.1	28.7	H2	*b*
93	q	18.3	18.7	18.0	18.3	19.0	18.4	H6	*b,a*
94	q	17.7	18.1	17.5			17.4	L6	*b,a*
95	q	8.2	9.0	7.8	8.1	9.0	9.2	P4	*b*

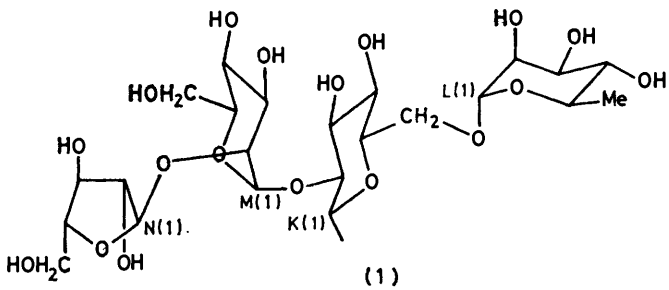
† s = singlet, d = doublet, t = triplet, q = quartet.
§ *a*, Comparison of derivatives; *b*, chemical shift comparison; *c*, off-resonance ^1H decoupling; *e*, relaxation; *f*, logical exclusion;
‡ The assignments of these pairs of signals may be reversed. A brace enclosing a group of signals in any column indicates that the assignments in this group are unknown. A brace in the assignment ' column indicates that relative assignments are unknown for all derivatives.

were also obtained in D_2O, and in mixtures of D_2O and DMSO, from which the D_2O and DMSO spectra can be correlated, allowing assignments in one solvent to be transferred to the other. The ^{13}C chemical shift data measured in D_2O (relative to DSS) are also included in Table 1. Figure 1 shows the 25.2 MHz ^{13}C spectrum of ristocetin A, 0.11M in DMSO at 80 °C; and Figure 2 shows the corresponding spectrum of ψ-aglycone. The lines of the ristocetin spectrum are much broader, due to the slower tumbling of the more polar, more aggregated molecule. Chemical-shift data for the derivatives of these compounds are presented later in the text, where appropriate. Significant shifts on addition of one equivalent of Ac-D-Ala-D-Ala to ristocetin A are given in Figure 4.

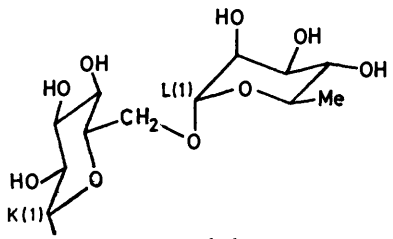
A number of ^1H selective decoupling experiments have been performed in DMSO. Assignments were made either by single specific irradiations or by graphical inter-



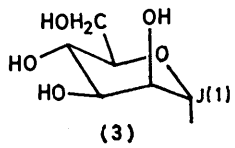
(I) R^1 = (1), R^2 = (3), R^3 = H_2^+, R^4 = R^5 = H
(II) R^1 = (2), R^2 = (3), R^3 = H_2^+, R^4 = R^5 = H
(III) R^1 = (1), R^2 = (3), R^3 = Ac, R^4 = R^5 = H
(IV) R^1 = (1), R^2 = (3), R^3 = Ac, R_4 = Me, R_5 = H
(V) R^1 = R^2 = R^4 = R^5 = H, R^3 = H_2^+
(VI) R^1 = R^2 = R^4 = R^5 = H, R^3 = Ac
(VII) R^1 = R^2 = R^4 = Me, R^3 = Ac, R^5 = H
(VIII) R^1 = R^2 = R^4 = Me, R^3 = R^5 = Ac



(1)



(2)



(3)

polation of a number of such irradiations. The ^1H assignments used are those reported in ref. 2 (ristocetin A) and ref. 7 (ψ-aglycone). The multiplicities of the signals in the ^{13}C-{^1H} off-resonance decoupled spectra indicate the number of protons attached to the corresponding carbon nuclei, and are included in Table 1.

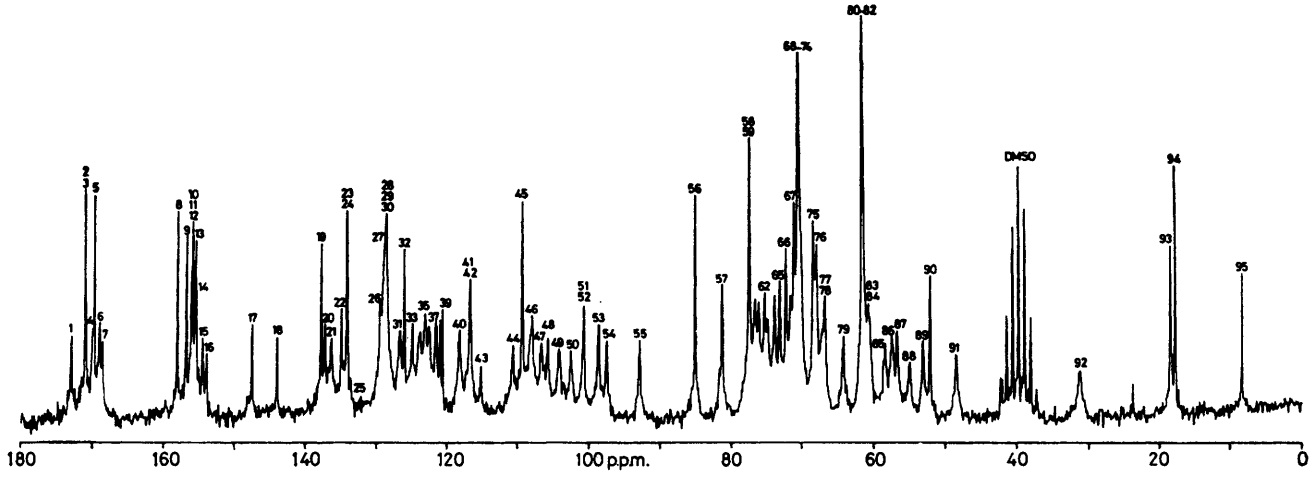


FIGURE 1 The 25.2 MHz ^{13}C spectrum of ristocetin A (0.11M in DMSO at 80 °C)

There exist chemical-shift data for a number of excellent model compounds. The most significant of these is vancomycin, whose ^{13}C spectrum has recently been assigned.[9] A large part of the molecule (rings A—E) is very similar to ψ-aglycone, except that the acid functionality is not esterified, and there is a chlorine atom at positions A(3) and C(3).[10] The amino-sugar ristosamine [H(1)–H(6)] is also absent. Approximate chemical shifts for rings F and G can be estimated from those for ristomycinic acid.[11] Those for the rest of the ψ-aglycone could be estimated using empirical rules.[12] Shift changes on derivatization were a crucial part of the assignment, which also relied on solvent titrations, selective ^1H decoupling experiments, and relaxation studies. Many of the assignments were first made on the ψ-aglycone spectrum, which is both simpler and sharper than that of ristocetin, and then transferred to ristocetin by chemical-shift comparisons. Details of the assignment are given below. Signals are numbered as in Table 1.

Assignment of the ^{13}C *Resonances.*—(a) *Carboxy and carbonyl carbons.* No attempt has been made to assign these signals. It is hoped that biosynthetic studies now in progress,[13] in conjunction with ^1H decoupling experiments and chemical-shift calculations, will lead to an assignment of this region.

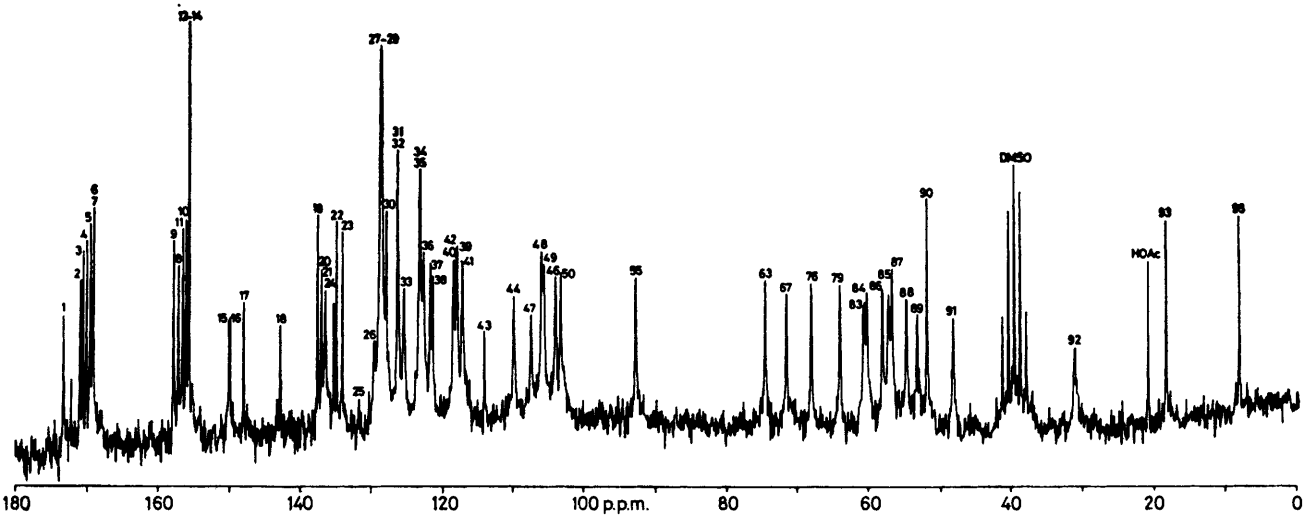


FIGURE 2 The 25.2 MHz ^{13}C spectrum of ψ-aglycone (0.1M in DMSO at 80 °C)

(b) *Aromatic carbons in the* 140—160 *p.p.m. region.* The eleven signals in this region originate from all the oxygen-bearing aromatic carbons[14] except for B(2), which resonates further upfield. Chemical-shift cal-

culations [11] lead us to predict that signals 17 and 18 should correspond to G(5) and G(4), respectively. These respective assignments are supported by the small shift of 17 and larger shift of 18 on methylation of the phenols. We would also expect [9] signals 15 and 16 to correspond to B(1) and B(3), and this is strikingly confirmed by an upfield shift of *ca.* 4.0 p.p.m. on removal of the tetrasaccharide from ristocetin A.

The remaining signals are grouped closely together, and have only been assigned for ψ-aglycone. The assignments were made using off-resonance decoupled spectra, in which protons coupled by long-range coupling were irradiated, thus removing the coupling and leaving narrower and taller ^{13}C resonances. The assignments of signals 8 and 9 in ψ-aglycone to D(5) and D(3) respectively are in accord [12] with downfield shifts of 3.9 and 4.2 p.p.m. on methylation. Signals 13 and 14 shift very little, as expected.

(c) *Aromatic carbons in the* 129—140 *p.p.m. region.* The seven signals in this region can be assigned to C(1), D(1), E(2), B(5), F(1), A(1), and B(2) by chemical-shift calculations. E(2) is the only doublet in off-resonance spectra (and is also much broader in noise-decoupled spectra) and is immediately assignable to 21. The only other obvious assignment is that of B(2) to signal 25, by its downfield shift of 1.4 p.p.m. on methylation of the phenols. This shift only occurs in ψ-aglycone: signal 25 is 1.1 p.p.m. further downfield in ristocetin A, and does not move on *O*-methylation, as predicted from the presence of the tetrasaccharide in this position.

By its chemical shift (in DMSO solution), D(1) is predicted to be 20 and C(1) 19, and this is confirmed by comparison of the ristocetin A and ψ-aglycone chemical shifts with those in the spectra of the *N*-Ac-*O*-Me derivatives [(IV) and (VII)] in which 20 moves slightly upfield but 19 does not. In these same spectra, signal 23 is shifted 0.4 p.p.m. upfield, while signal 22 shifts 0.3 p.p.m. upfield in the ψ-aglycone spectrum only. This strongly suggests that 22 is B(5) [B(2) is glycosylated in ristocetin and therefore is not affected by *O*-methylation] and 23 is F(1), leaving 24 as A(1). This is confirmed by off-resonance ^1H decoupling; for example, weak irradiation of ψ-glycone at δ 7.2 decouples the protons attached to A(3) and A(5) [7] and markedly sharpens signal 24, leaving the others relatively unchanged.

(d) *Aromatic carbons in the* 112—129 *p.p.m. region.* Off-resonance ^1H decoupling experiments allow us to differentiate between signals from quaternary carbons (singlets) and from proton-bearing carbons (multiplets), and it is simpler to consider these two groups separately.

There are only five quaternaries in this region, of which the highest field signal (43) is coupled to the protons of the aromatic methyl group and may be immediately assigned to F(4). Signal 39 is also readily assigned to D(2) by its upfield shift of 2.5 p.p.m. on removal of the mannose [attached *ortho* to D(2)]. It is possible to make a very good estimate of the expected chemical shifts of E(3) and E(1) using the model compound vancomycin, and on this basis we assign E(3) to signal 30 and E(1) to signal 38. The assignment of E(3) has been verified in ψ-aglycone by irradiation of the three-bond-coupled amide NH proton. Signal 32 is thus G(1), as expected from its chemical shift.

The remaining thirteen signals in this region are due to carbons bearing a hydrogen atom; four overlap and cannot be assigned individually. Signal 33 is assigned to G(2) by its upfield shift (0.7 p.p.m. in ristocetin and 0.6 p.p.m. in ψ-aglycone) on acetylation of the terminal NH_2 group. Signal 31 is assigned to E(4) on the basis of its chemical shift and off-resonance ^1H decouplings performed on ristocetin A. The four overlapping signals (26—29) must, therefore, correspond to A(2), C(2), A(6), and C(6). The remaining signals can be dealt with in two groups: A(5), C(5), A(3) and C(3) to low field and G(3), E(5) and G(6) to higher field. Unfortunately, the three to higher field shift so much on removal of the sugars that assignments must be made separately for ristocetin A and ψ-aglycone. By chemical-shift arguments we assign signal 41 to E(5): this assignment can also be made by off-resonance specific ^1H decoupling. On *N*-acetylation we observe a downfield shift of 0.5 p.p.m. for signal 42 in ristocetin, which allows this to be assigned to G(6), and therefore signal 40 to G(3). In ψ-aglycone, the shift on *N*-acetylation is much smaller, and it is less ambiguous to make the assignment by observation of the shifts on *O*-methylation (signal 42 moves 0.3 p.p.m. upfield). It is gratifying to find the same anomalous solvent titration effect for the signal assigned to G(6) in both compounds. These assignments may be confirmed by off-resonance ^1H decoupling experiments.

The four signals 34—37 can only be distinguished by off-resonance ^1H decoupling with difficulty. The most probable assignments are given in Table 1, and agree with those expected from chemical shift comparisons.

(e) *Aromatic and anomeric carbons in the* 90—112 *p.p.m. region.* Here once again many signals shift significantly on removal of the sugars, and assignments must be made separately for ristocetin and ψ-aglycone.

H(1) is the only anomeric carbon remaining in ψ-aglycone, and is thus readily assigned to signal 55. The only difference between ristocetins A and B is that ristocetin B lacks the 2-*O*-D-arabinosyl-D-mannosyl disaccharide [15] [compare (I) and (II)]. We therefore expect that in the ristocetin B spectrum the signals arising from M(1) and N(1) will be absent, and that due to K(1) will be shifted (model compounds [16] predict a downfield shift of *ca.* 0.5 p.p.m.: in this case the shift is 2.6 p.p.m. downfield). On this basis, and using chemical-shift arguments, we assign the high-field signal 45 to N(1) and signals 51 and 53 to K(1) and M(1). Relaxation data suggest that signal 53 is K(1), and this is confirmed by a report from Sztaricskai *et al.*,[4] based on $^1J_{CH}$ coupling constants, assigning K(1) to 53 and M(1) to 51. Irradiation at the frequency of the proton coupled to J(1) assigns J(1) to 54 and therefore L(1) to 52.

The remaining assignments of the aromatic nuclei were made, as far as possible, by specific ^1H decoupling. However, it is not possible to distinguish F(2) and F(6)

[and, in ψ-aglycone, D(4)] by decoupling, as the ^1H resonances are so close together. These assignments were, therefore, made by consideration of the shifts on methylation of the phenols. Signal 44 shifts 1.9 p.p.m. upfield, and is assigned to F(6). In ristocetin, chemical-shift arguments [9,11] lead to the ready assignment of signals 46 and 50 as F(2) and D(4) respectively. However, in ψ-aglycone, signal 46 is shifted a remarkable *ca.* 3.7 p.p.m. upfield, and the relative assignments of F(2) and D(4) are by no means obvious. On methylation the two signals, originally at 103.8 and 103.4 p.p.m., move to 103.0 [F(2)] and 99.8 [D(4)] p.p.m.,[17] but model compounds * could not provide a secure assignment.

(f) *The remaining sugar carbons.* Assignments in this region were made by chemical-shift calculations and a full list of calculated resonance positions is given in Table 2. Extensive use was made of model compounds listed by Sztaricskai *et al.*;[4] our assignments agree with those in ref. 4 in most cases. Four values are given for the arabinose moiety; these are for the α- and β-furanose and -pyranose forms. In Table 2, no account is taken of the temperature variations of sugar resonant frequencies, as these are likely to be small.[18] The assignments are all straightforward. Ristosamine signals [H(2)–H(5)] can be identified by comparison with ψ-aglycone, and arabinose [N(2)–N(5)] and mannose [M(2)–M(6)] by comparison with ristocetin B.

It is noteworthy that the signal at 84.8 p.p.m. is remarkably low field for a sugar signal, and can only reasonably be assigned to C(4) of an α-D-arabinofuranosyl moiety. This point is considered further below.

(g) *Backbone and high-field carbons.* The isolated signals P(1) and P(2) in ψ-aglycone are assigned to 67 and 63 respectively by selective ^1H decoupling. The assignment in ristocetin is then made by comparison of chemical shifts in both DMSO and D_2O. The assignment of signal 90 to P(3) is obvious from the multiplicity in off-resonance spectra: the assignment of signal 95 to P(4) is equally trivial.[11] The only signals remaining are due to the seven α-carbons. These have been assigned by specific ^1H irradiations on the ψ-aglycone spectrum, and transferred to ristocetin by chemical-shift analogy. The α(1) carbon resonance can also be assigned by its upfield shift of 2.0 p.p.m. on acetylation of the adjacent terminal amino-group. The shifts for α(4), α(5), and α(6) are in good agreement with those calculated from vancomycin.

Configuration of the Arabinose.—A preliminary report of the next two sections has already appeared.[19] Previous work carried out on the configuration and ring size of the arabinose moiety has been performed by a group of Hungarian workers, who arrived at two conflicting conclusions. In 1974, Sztaricskai *et al.*[15] carried out a permethylation of ristocetin A using methyl iodide in DMSO in the presence of sodium hydride (Hakomori's method [20]) and, after hydrolysis, isolated 2,3,5-tri-*O*-methyl-D-arabinose, indicating the presence of an arabinofuranoside. Then in 1978, Neszmélyi *et al.*[21] looked at the product derived from acetolysis and subsequent hydrolysis of ristocetin A (Ac_2O–conc. sulphuric acid, 0 °C, 14 days [15]) by ^{13}C n.m.r. spectroscopy and showed that the arabinose was present as the tri-*O*-acetylpyranoside. From the present study it is clear, both from the chemical shift of the arabinose anomeric carbon, and from the signal at 84.8 p.p.m., that in ristocetin A itself the arabinose is present as the α-D-furanosyl form, in agreement with similar recent experiments performed by Sztaricskai *et al.*[4]

The conclusion illustrates the dangers of using acetylation to determine the configuration of sugars: it is well known that many acetylation procedures lead to pyranoid rings regardless of the initial ring size.[22] On the other hand, the conditions used during the Hakomori permethylation allow far less opportunity for isomerisation.

Anomeric Configuration of the Mannose.—In the ^1H n.m.r. study of ristocetin A,[2] it was not possible to confirm the anomeric configuration of the isolated mannopyranose monosaccharide. However, it should be possible to determine this from ^{13}C n.m.r. spectroscopy, from both coupling constants and chemical shifts. Bock and Pedersen [23] have shown that α- and β-glycosides have characteristically different ^1J couplings from the anomeric carbon to the directly attached proton. In particular, for phenyl glycosides, the α-form has a ^1J of *ca.* 173 Hz, and the β-form of *ca.* 163 Hz. We have measured the ^1J for $J(1)$ to be 173 ± 2 Hz, indicating the α-form, in agreement with the findings of Sztaricskai *et al.*[4] and contradicting earlier conclusions.[24] We have also synthesized the α- and β-phenyl-D-mannosides, for which chemical-shift values are given in Table 2, confirming that the mannose is present in the α-form.

Attachment of the Sugars.—^1H N.m.r. studies [2,3] have previously shown that ristosamine is attached *via* the aliphatic hydroxy-group at P(2). This is confirmed by acetylation of the aliphatic alcohols of ψ-aglycone [comparing (VII) and (VIII)] which shifts the signal from P(1) 0.7 p.p.m. downfield, and leaves P(2) unchanged.

In an earlier work,[3] we cited ^{13}C evidence for the position of attachment of the tetrasaccharide. As vancomycin carries a sugar at the ring B phenol [B(2)], it is an ideal model compound to estimate the effect on the chemical shifts in ring B of removal of a sugar from this position. In Figure 3, we give details of the changes in chemical shift on removal of the tetrasaccharide plus mannose, and compare them with the changes on removal of the disaccharide from vancomycin;[9] although the shift differences at ring B in ristocetin are different from those in vancomycin, it is clear that a sugar is being removed from this position. The differences in ring D indicate that a sugar is also being removed from here: it has already been shown [2] that mannose is attached at D(3). The differences in rings F and G are thought to be due to conformational changes induced by the mannose monosaccharide, which is very close to these rings.

Binding of Ac-D-Ala-D-Ala.—A ^1H n.m.r. study of the

* Models used were *o*-cresol and 4-hydroxy-6-(2,4-dihydroxy-6-methylphenyl)pyran-2-one and their *O*-methyl derivatives.

TABLE 2

Calculation of sugar ^{13}C resonances (D_2O at 30 °C)

[a] Nucleus	δ/p.p.m.	Nucleus	[c] δ/p.p.m.	Nucleus	δ/p.p.m.	[d] Nucleus	[e] δ/p.p.m.	Nucleus	δ/p.p.m.	Nucleus	δ/p.p.m.	[f] Nucleus	[g] δ/p.p.m.	Nucleus	δ/p.p.m.	Nucleus	δ/p.p.m.	Nucleus	δ/p.p.m.	Nucleus	δ/p.p.m.
H1		J1α	99.2	J1β	95.0	K1	101.9	K1[β]	102.4	L1	101.3	M1 [i]	102.2	N1αf	109.3	N1βf	103.2	N1αp	105.1	N1βp	101.0
H2	~28.7	J2α	71.0	J2β	71.3	K2	74.7	K2[α]	79.2	L2	71.1	M2 [i]	80.0	N2αf	81.9	N2βf	77.5	N2αp	71.8	N2βp	69.4
H3[b]	52.0	J3α	74.2	J3β	77.0	K3	77.0	K3[α]	75.8	L3	71.1	M3	~74.6	N3αf	77.5	N3βf	75.7	N3αp	73.4	N3βp	69.9
H4	69.5	J4α	67.5	J4β	67.5	K4	70.5	K4	70.5	L4	72.8	M4	69.5	N4αf	84.9	N4βf	83.1	N4αp	69.4	N4βp	70.0
H5	66.2	J5α	71.5	J5β	73.3	K5	75.6	K5	75.6	L5	69.5	M5	~70.5	N5αf	62.4	N5βf	64.2	N5αp	67.3	N5βp	63.8
H6	18.4	J6α	61.7	J6β	62.0	K6	68.7	K6	68.7	L6	17.4	M6	63.8								
						Risto B		Risto A													

[a] T. T. Thang, G. Lukacs, S. Omura, P. Bartner, *et. al.*, *J. Am. Chem. Soc.*, 1978, **100**, 663. [b] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669. [c] Synthetic α- and β-phenyl-D-mannoside, D_2O, ambient temperature. [d] Shifts for β-phenyl-D-glucoside (Sigma London) at ambient temperature: D_2O solution C-1 101.9 C-2 74.7 C-3 77.4 C-4 71.3 C-5 77.7 C-6 62.4: DMSO solution C-1 100.4 C-2 74.5 C-3 77.0 C-4 68.8 C-5 76.7 C-6 60.9 [e] C. Laffitte, A. M. Nguyen Phuoc Du, F. Winternitz, R. Wylde, and F. Pratviel-Sosa, *Carbohydr. Res.*, 1978, **67**, 105. [f] J. O. Previato, L. Mendonca-Previato, and P. A. J. Gorin, *Carbohydr. Res.*, 1979, **70**, 172. [g] R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, 1979, **35**, 1427. [h] P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 1975, **53**, 1212. [i] P. A. J. Gorin, *Can. J. Chem.*, 1973, **51**, 2375.

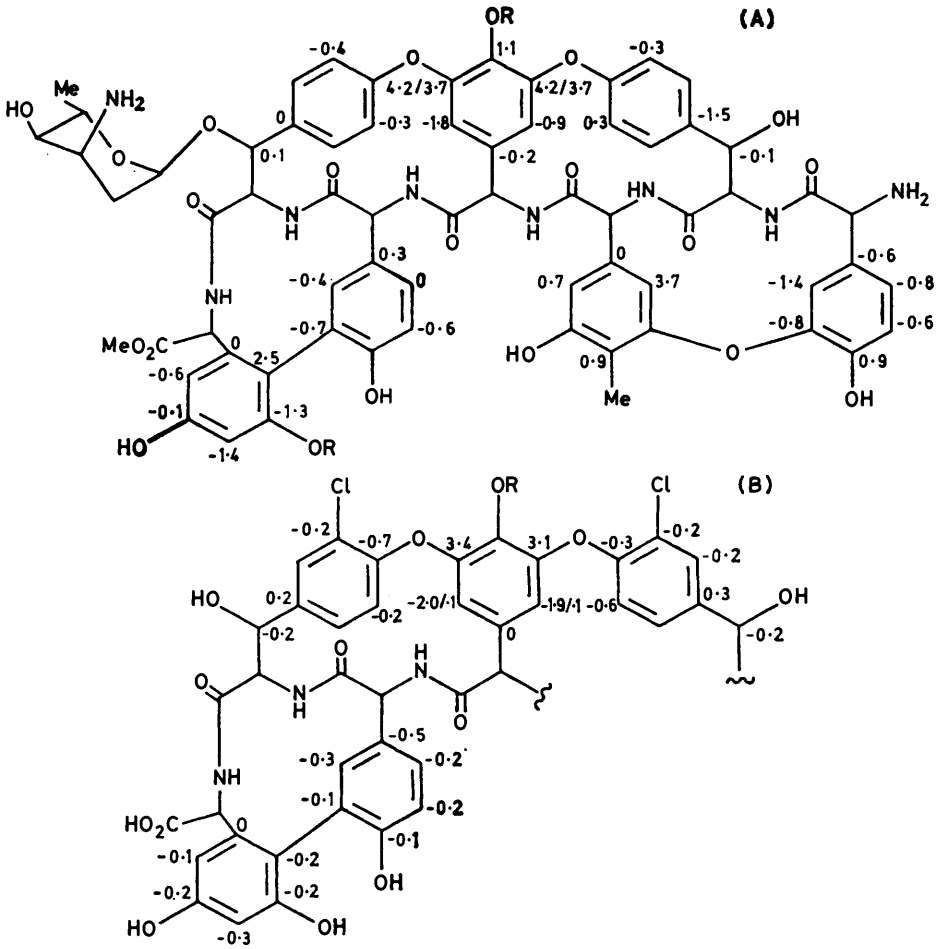


FIGURE 3 Changes in chemical shift on removal of the sugars from ristocetin A and vancomycin (a negative sign indicates an upfield shift). (A) Ristocetin A ⟶ ψ-aglycone. (B) Vancomycin ⟶ aglucovancomycin

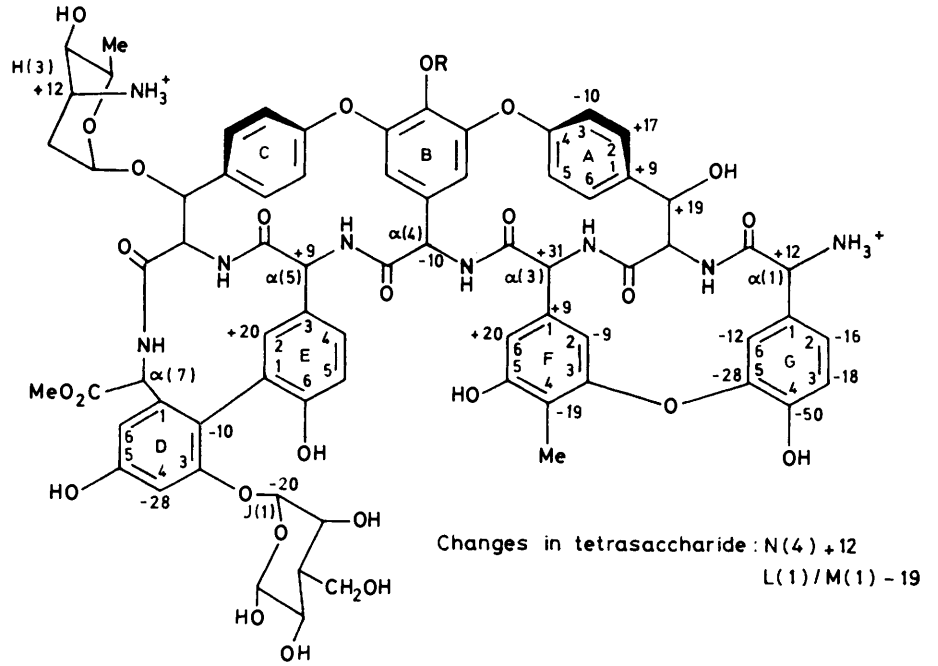


FIGURE 4 Chemical-shift changes on addition of 1 molar equivalent of Ac-D-Ala-D-Ala to ristocetin A. Changes are given in Hz, and a positive sign indicates a downfield shift. Spectra were recorded at 80 °C in 80% D_2O–20% DMSO, pH* 4.0—4.3. Only changes greater than 8 Hz are indicated

interaction of ristocetin A with Ac-D-Ala-D-Ala [5] has shown that the dipeptide binds to a cleft in the ' front ' face of ristocetin (see Figures 3 and 4 in ref. 5). The shifts in the ^{13}C spectrum on binding of the dipeptide (in Hz at 25.2 MHz) are shown in Figure 4. The data are consistent with the details worked out from ^1H n.m.r. results and lead to the following conclusions: (a) the sugars are not involved in the binding; (b) changes in the local environment of rings A, D, E, F, and G occur upon binding. These may be due to changes in solvent accessibility to these rings, or due to direct interactions with the peptide (or both); (c) the chemical-shift changes of ring G carbons are generally relatively large and to high field. These changes may be caused by ring G lying above a plane defined by the three atoms of the carboxylate anion of the peptide. A carboxy-group has a shielding effect for nuclei lying over such a plane.[25]

EXPERIMENTAL

Ristocetins A and B were supplied by Lundbeck & Co., Copenhagen, and used without further purification. The ristocetin A derivatives [3] and ψ-aglycone and its derivatives [6,7] were prepared by the published procedures. α- and β-phenyl-D-mannosides were prepared by standard methods [8] from penta-*O*-acetyl-D-mannose (Sigma London). Spectra were generally recorded at 80 °C and with concentrations approximately 0.1M for ψ-aglycone and 0.08M for ristocetin in tubes of 5 mm o.d. Additions of Ac-D-Ala-D-Ala were carried out at 80 °C in a solution of 80% D_2O and 20% DMSO. The pH was measured on a Corning 125 meter using a Corning combination glass electrode, and adjusted using 2M solutions of NaOH and HCl in D_2O.

The spectra were obtained at 25.2 MHz on a Varian X-L 100 A, operating in the Fourier Transform mode. Typical spectra had a spectral width of 5 000 Hz with an acquisition time of 0.6 s and were collected into 6K memory points. Specific ^1H decouplings were generally performed at a power level of 20—30% maximum decoupler power.

We wish to thank the S.R.C., the Royal Society, and Smith Kline and French (Welwyn, U.K.) for financial support.

[0/1592 *Received*, 20*th October*, 1980]

REFERENCES

[1] M. Nieto and H. R. Perkins, *Biochem. J.*, 1971, **123**, 789.
[2] J. R. Kalman and D. H. Williams, *J. Am. Chem. Soc.*, 1980, **102**, 897.
[3] D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.
[4] F. Sztaricskai, A. Neszmélyi, and R. Bognár, *Tetrahedron Lett.*, 1980, 2983.
[5] J. R. Kalman and D. H. Williams, *J. Am. Chem. Soc.*, 1980, **102**, 906.
[6] A. F. Norris, D. H. Williams, and V. Rajananda, *J. Chem. Soc. Pakistan*, 1979, **1**, 29.
[7] V. Rajananda, Thesis, University of Cambridge, 1979.
[8] M. A. Jermyn, *Aust. J. Chem.*, 1954, **7**, 202.
[9] A. Bongini, M. P. Williamson, J. Feeney, and D. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 201.
[10] G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams, and G. A. Smith, *Nature*, 1978, **271**, 223.
[11] T. M. Harris, C. M. Harris, J. R. Fehlner, R. Bognár, and F. Sztaricskai, *J. Org. Chem.*, 1979, **44**, 1009.
[12] F. W. Wehrli and T. Wirthlin, ' Interpretation of C-13 NMR Spectra ', Heyden, London, 1978.
[13] S. Hammond, M. P. Williamson, and D. H. Williams, unpublished work.
[14] D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans. 1*, 1979, 787.
[15] F. Sztaricskai, R. Bognár, and M. M. Puskás, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 75.
[16] T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2425.
[17] W. J. McGahren, J. H. Martin, G. O. Morton, R. T. Hargreaves, R. A. Leese, F. M. Lovell, G. A. Ellestad, E. O'Brien, and J. S. E. Holker, *J. Am. Chem. Soc.*, 1980, **102**, 1671.
[18] F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 1976, **51**, 179.
[19] M. P. Williamson and D. H. Williams, *Tetrahedron Lett.*, 1980, 4187.
[20] S. Hakomori, *J. Biochem.* (*Tokyo*), 1964, **55**, 205.
[21] A. Neszmélyi, F. Sztaricskai, A. Lipták, and R. Bognár, *J. Antibiotics*, 1978, **31**, 974.
[22] M. L. Wolfrom and W. A. Szarek in ' The Carbohydrates ' W. Pigman and D. Horton, eds., Academic Press, New York, 1972, vol. 1A, p. 221.
[23] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293.
[24] N. N. Lomakina, R. Bognár, M. G. Brazhnikova, F. Sztaricskai, and L. I. Muravyeva, Abstracts, 7th International Symposium on the Chemistry of Natural Products, Zinate, Riga, 1970, p. 625.
[25] L. M. Jackman and S. Sternhell, ' Applications of NMR Spectroscopy in Organic Chemistry ', Pergamon Press, Oxford, 1969, p. 88; L. Pogliani and M. Ellenberger, *J. Am. Chem. Soc.*, 1974, **96**, 1621.