^{13}C NMR STUDY OF THIOSTREPTON AND THIOPEPTIN COMPONENTS

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A detailed ^{13}C NMR study of thiostrepton and two series of thiopeptin components is consistent with their proposed structures and allows many unequivocal assignments to be made.

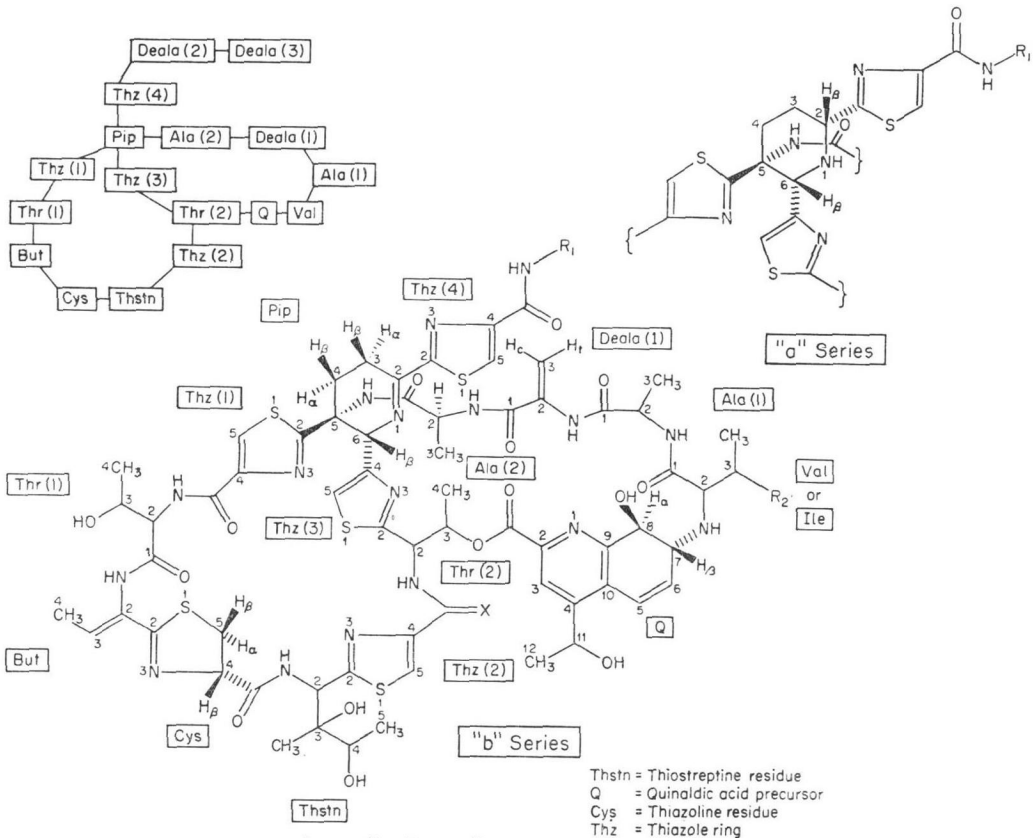
In the preceding paper, total structures for the **a** and **b** series of thiopeptin components isolated from *Streptomyces tateyamensis* were proposed (see Fig. 1), mainly on the basis of ^1H NMR comparison with thiostrepton. Comparison of the ^{13}C NMR spectra at 75 MHz of the various components with that of thiostrepton are consistent with these conclusions and has allowed us to extend and in some cases modify the tentative assignments made by TORI *et al.*[1] for thiostrepton. Spectra were obtained under a variety of conditions which, combined with different assignment techniques, has led to assignment of most of the 72 carbon atoms. One of the most useful involved the measurement of direct ^{13}C-^1H coupling constants from fully coupled spectra by "gated" decoupling which, even in the case of large molecules like the thiopeptins, provided sufficient resolution at 75 MHz to enable most of the constants to be determined. Use was also made of two- and three-bond ^{13}C-^1H coupling constants but involving sp^2 carbons only as those for sp^3 carbons were not sufficiently resolved.

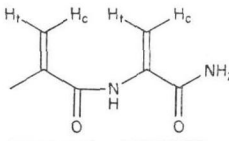
As the **a** series of thiopeptins contain a saturated piperidine ring, protonation with trifluoroacetic acid-*d* (TFA-*d*) was found to be very useful in characterizing the ring carbons as well as those of the three thiazole rings attached to the piperidine ring. Addition of TFA-*d* as in the case of CD_3OD leads to conformational changes as evidenced by ^1H NMR in $CDCl_3$ and was used to assign resonances of the Thr (1) and Q residues (see Fig. 1). Protonation of the Val nitrogen was not observed. Furthermore model studies and in particular the effect of substituting an amide by a thioamide group has, in the case of thiazole-4-carboxamide, allowed assignment of the Thz (2) ring carbons and those of Thr (2).

As reported earlier[2], we noted many of the resonances in CD_3OD - $CDCl_3$ (1 : 4) to give rise to splittings of the order of 0.1 ppm and that the intensities of the two peaks showed a time dependence. ^1H NMR spectra in the same solvent mixture were observed to display slow peptide NH exchange behavior which suggested deuterium-isotope induced shifts of those carbons adjacent to a slowly exchanging peptide NH group[3,4]. This was confirmed by measurement in CD_3OH - $CDCl_3$ (1 : 4) which gave spectra containing only singlets. This technique therefore allowed all carbons adjacent to a peptide NH group to be readily identified.

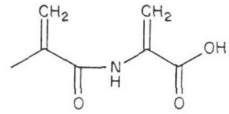
During the preparation of this manuscript TORI, *et al.*[5] have reported on the ^{13}C NMR assignments of thiopeptin B_a by comparison with those of thiostrepton and three siomycin components. Acetylation shifts were effectively used to assign resonances of the Thr (1) and Thstn residues and our studies concur in large part with their assignments in the $0 \sim 80$ ppm range. We differ significantly however with their assignments in the $130 \sim 190$ ppm region.

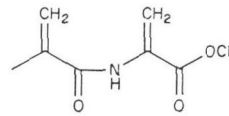
Fig. 1. Structure of thiopeptins.

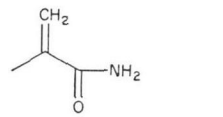


Thiostrepton A_1 : X = O; R_1 =  $R_2 = CH_2CH_3$

Thiostrepton A_2 : X = O; R_1 = H $R_2 = CH_2CH_3$

Thiopeptin B_o/B_b: X = S; R_1 =  $R_2 = CH_3$

Thiopeptin A_{1o}/A_{1b}: X = S; R_1 =  $R_2 = CH_3$

Thiopeptin A_{4o}/A_{4b}: X = S; R_1 =  $R_2 = CH_3$

Thiopeptin A_{3o}/A_{3b}: X = S R_1 = H $R_2 = CH_3$

Results and Discussion

For the purpose of discussion the spectra may be conveniently divided into four distinct zones, only the last two of which overlap in the aromatic carbons. A breakdown of the number and type of carbons in each region obtained from "gated" decoupled spectra is given in Table 2 and the data are consistent with the proposed structures as depicted in Fig. 1. Spectra of thiopeptin components, recorded in CD_3OD - $CDCl_3$ (1 : 4) and CD_3OH - $CDCl_3$ (1 : 4) at 40°C gave sharp spectra as illustrated for the methyl esters A_{1a} and A_{1b} of both series as well as thiostrepton A_1 in Fig. 2. The chemical shifts and ^{13}C-^1H coupling constants of all components are summarized in Table 1. For thiopeptins A_{1a} and A_{1b} the first column contains the chemical shift in ppm downfield of TMS and the one-bond ^{13}C-^1H coupling constant ($^1J_{CH}$) in brackets. Column two provides details of the fine structure obtained on "gated" decoupling with two-($^2J_{CCH}$) and three-bond ($^3J_{CCCH}$) couplings in brackets. The Table also includes the effect of different concentrations of TFA-*d* on the chemical shifts of thiopeptins B_a and A_{3a} in CD_3OH - $CDCl_3$ (1 : 4) and identifies all ^2H-isotope shifted carbons by an asterisk. The chemical shifts for thiostrepton and thiopeptin B_a are to higher field by *ca.* 1.8 ppm of those reported by TORI *et al.*[1,5] and may be attributed to differences in the referencing procedure (see footnote[a], Table 1). Unless where otherwise stated, the quoted chemical shift values in the text will refer to those of thiopeptin A_{1a}.

Identification of Peptide ^2H-Isotope Shifted Carbons

The ^{13}C NMR spectrum of thiostrepton in CD_3OD - $CDCl_3$ (1 : 4) displays twenty "split" resonances of which six occur in the peptide α-carbon region, four in the olefinic and ten in the amide carbonyl region. This implies the presence of ten slowly exchanging peptide NH groups in agreement with the structure for thiostrepton[1,6]. Similarly the spectra of members of both **a** and **b** series for thiopeptins

Fig. 2. ^{13}C NMR spectra of thiostrepton and thiopeptins A_{1a} and A_{1b} in CD_3OH - $CDCl_3$ (1 : 4) at 40°C.

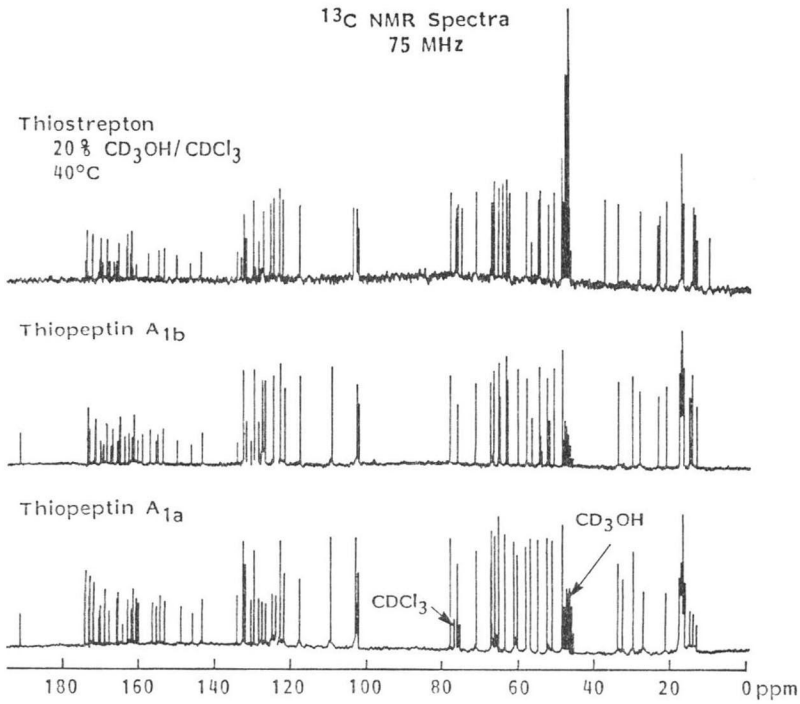


Table 1. ^{13}C NMR assignments of thiostrepton and thiopeptin components.[a]

Assignment	Thiopeptin B_a		Thiopeptin A_{1a}		Thiopeptin A_{1b}		Thiostrepton	Thiopeptin A_{4a}	Thiopeptin A_{3a}	
	δ ppm	+TFA-d[b]	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm	δ ppm	δ ppm	+TFA-d[c]
But Me	13.7	13.8	13.9 q (129)	br.m	13.7 q (130)	br.m	13.8	13.7	13.7	13.7 q (128)
Thstn 4-Me	14.6	14.7	14.5 q (127)	br.m	14.5 q (128)	br.m	14.5	14.5	14.6	14.6 q (127)
Val Me	15.0	15.8	15.1 q (129)	br.m	15.0 q (128)	br.m	14.2 Ile 3-Me	15.0	15.0	15.3 q (128±2)
Val Me	17.3	17.2	17.0 q (128)	br.m	17.2 q	br.m	9.9 Ile 5-Me	17.3	17.2	17.1 q
Ala Me (1)[d]	17.3	16.9	17.3 q	br.m	17.3 q	br.m	17.0	17.3	17.3	17.2 q
Thr Me (1)[d]	17.3	17.2	17.3 q	br.m	17.3 q	br.m	17.36	17.3	17.3	17.3 q
Thr Me (2)[d]	17.3	17.6	17.3 q	br.m	17.3 q	br.m	17.43	17.4	17.3	17.4 q
Ala Me (2)[d]	17.7	17.6	17.7 q (130±3)	br.m	17.7 q	br.m	17.53	17.7	17.7	17.7 q
Thstn 3-Me[d]	17.5	17.6	17.5 q (131)	d (4)	17.4 q	br.m	17.48	17.5	17.5	17.5 q
Q Me	21.2	21.3	21.3 q (128)	br.m	21.2 q (128)	br.m	21.2	21.3	21.2	21.3 q (128)
Pip C3	27.2	26.1 v.br	27.1 t (132)	br.m	23.4 t (132)	br.m	23.4	27.3	27.3	26.9 br.t (132±2)
Val C3	29.8	30.1	29.7 d (131)	br.m	29.7 d (131)	br.m	37.1 Ile C3	29.8	29.8	29.9 d (132±2)
Pip C4	32.5	32.3 br	32.4 t (131)	br.m	28.1 t (133)	br.m	27.9	32.4	32.5	32.4 t (134±2)
Cys C5	33.5	33.7	33.5 t (147)	br.m	35.5 t (148)	br.m	33.5	33.5	33.5	33.5 t (148)
Ala C2 (1)	48.3*	48.2	48.1 d (143±3)	br.s	48.2*d (141)	br.s	48.3*	48.2	48.3*	48.2 d (140)
Ala C2 (2)	50.9*	51.0	50.8 d (141)	br.s	50.6*d (141)	br.s	50.6*	50.9	50.8*	50.9 d (142)
$-CO_2CH_3$	—	—	51.6 q (147)	s	51.5 q (148)	s	—	—	—	—
Thstn C2	52.3*	52.5	52.1 d (137)	br.s	52.2*d (136)	br.s	51.8*	52.2	52.2*	52.3 d (137)
Thr C2 (1)	54.6*	54.9	54.5 d (143)	br.s	54.6*d (142)	br.s	54.4*	54.6	54.6*	54.7 d (140±3)
Pip C2	56.9	56.4 v.br	56.8 d (136)	br.m	160.9 s	v.br.m	161.0	56.9	56.9	56.8 d (138±3)
Pip C5	57.6	57.7 br	57.5 s	br.m	56.4* s	br.m	56.3*	57.6	57.7*	57.7 s
Q C7	57.6	57.9	57.7 d (140)	br.m	57.6 d (141)	br.m	57.7	57.7	57.7	57.8 d (142±2)
Thr C2 (2)	60.2*	60.3	60.2 d (139)	br.m	60.0* d (140)	br.m	54.6*	60.2	60.2*	60.3 d (138±2)
Pip C6	60.8	60.3 v.br	60.7 d (138)	br.m	62.9 d (140±2)	br.m	62.9	60.8	60.8	60.6 d (138±2)
Val C2	63.2	63.3	63.2 d (140±4)	br.s	63.2 d (141±2)	br.s	63.2	63.2	63.2	63.2 d (142)
Q C8	64.8	65.3	65.1 d	br.s	64.8 d (141±2)	br.s	64.2 Ile C2	64.9	64.9	65.2 d (138±3)
Thr C3 (1)	65.1	65.7	65.1 d	br.m	65.1 d (150±2)	br.m	65.3	65.1	65.1	65.2 d (146±5)
Q C11	66.5	66.3	66.2 d (144)	br.s	66.3 d (143±2)	br.s	66.3	66.4	66.4	66.4 d

Table 1. (Continued).

Assignment	Thiopeptin B_a		Thiopeptin A_{1a}		Thiopeptin A_{1b}		Thiostrepton	Thiopeptin A_{4a}	Thiopeptin A_{3a}	
	δ ppm	+TFA-d[b]	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm	δ ppm	δ ppm	+TFA-d[c]
Thstn C4	66.9	67.0	66.7 d (142±4)	br.m	66.8 d (146±2)	br.m	66.7	66.8	66.9	66.9 d (150±2)
Thr C3 (2)	70.8	71.0	70.8 d (154)	br.m	71.0 d (153±1)	br.m	70.8	70.8	70.8	70.9 d (152)
Thstn C3	75.8	76.0	75.8 s	s	75.8 s	s	76.0	75.8	75.8	75.9 s
Cys C4	77.7	77.9	77.6 d (140)	br.s	77.7 d (141)	br.s	77.7	77.7	77.7	77.8 d (140±2)
Deala C3 (1)	101.9	101.7	101.9 dd (161, 169)	d (~4)	101.9 br.t (164)	d (~4)	101.9	101.9	101.9	101.8 br.t (166±2)
Deala C3 (2)	101.7	102.7 br	102.3 dd (159, 169)	d (~4)	102.1 br.t (164)	d (~4)	102.1	102.6	—	—
Deals C3 (3)	107.3	108.8 br	109.0 dd (164, 168)	d (~4)	108.9 br.t (166)	br.s	103.1	—	—	—
Thz C5 (3)	117.1	119.1 v.br	117.0 d (190)	s	117.1 d (190)	s	117.1	117.1	117.1	117.9 d (190±4)
Q C3	121.3	121.4	121.2 d (171)	s	121.3 d (170)	s	121.2	121.3	121.3	121.3 d (170)
Q C5	122.2	122.3	122.1 d (162)	d (~4)	122.1 d (162)	d (4)	122.1	122.2	122.2	122.2 d (162)
Thz C5 (4)	123.1	124.2 br	123.2 d (192)	s	126.5 d (191)	s	126.4	122.9	123.0	123.4 d (192±3)
Thz C5 (1)	123.9	124.3	123.9 d (192)	s	123.8 d (193)	s	123.8	123.9	123.9	124.0 d (194±2)
Q C10	126.1	126.4	125.9 s	br.m	126.1 s	br.m	126.1	126.1	126.1	126.1 s
Thz C5 (2)	126.7	126.8	126.7 d (194)	s	126.8 d (195)	s	124.4	126.7	126.7	126.7 d (194)
But C2	127.5*	127.7	127.3 s	br.m	127.4* s	br.m	127.5	127.4	127.5	127.5 s
Q C6	128.8	129.1	128.8 d (166)	br.m	128.8 d (166)	br.m	128.9	128.8	128.8	128.9 d (166)
Deala C2 (3)	130.8*	130.1	129.5 s	d (4)	129.5* s	d (4)	132.0*	—	—	—
Deala C2 (1)	131.3*	131.5	131.1 s	d (~4)	131.1* s	d (~4)	131.2*	131.3	131.3*	131.4 s
But C3	131.5	131.8	131.6 d (155)	br.m	131.5 d (155)	br.m	131.5	131.5	131.5	131.6 d (152±2)
Deala C2 (2)	133.4*	133.4	133.1 s	d (4)	133.1* s	d (~4)	133.3*	132.4	—	—
Q C4	142.5	142.5	142.4 s	s	142.4 s	s	142.5	142.5	142.4	142.5 s
Thz C4 (1)	144.9	145.3	144.8 s	d (4)	145.1 s	d (4)	145.3	144.9	144.9	145.0 s
Thz C4 (4)	148.1	148.4	147.9 s	d (4)	148.8 s	d (4)	148.9	148.2	148.1	147.9 s
Q C2	152.5	152.5	152.4 s	br.s	152.5 s	br.s	152.5	152.5	152.5	152.5 s
Q C9	153.6	153.8	153.5 s	br.m	153.6 s	br.m	153.6	153.6	153.6	153.8 s
Thz C4 (2)	154.1	154.3	154.1 s	br.s	154.1 s	br.s	149.1	154.1	154.1	154.2 s
Thz C4 (3)	155.2	[d]	155.2 s	br.m	155.9 s	dd (4, 6)	156.1	155.2	155.2	154.2 br.s
Thz CO (4)	158.8*	158.5 br	158.8 s	br.s	158.5* s	d (~1)	158.6*	158.8	162.6*	162.6 s

Table 1. (Continued).

Assignment	Thiopeptin B_a		Thiopeptin A_{1a}		Thiopeptin A_{1b}		Thiostrepton	Thiopeptin A_{4a}	Thiopeptin A_{3a}	
	δ ppm	+TFA-d[b]	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm ($^1J_{CH}$)	$^{2,3}J_{CH}$	δ ppm	δ ppm	δ ppm	+TFA-d[c]
Q CO	159.7	160.0	159.6 s	br.s	159.8 s	br.s	159.8	159.7	159.7	159.9 s
Thz CO (1)	160.6*	160.5	160.6 s	br.s	160.5*	d (~1)	160.7*	160.7	160.6*	160.6 s
Deala CO (2)	160.9*	161.3	161.1 s	v.br.m	161.1* s	v.br.m	161.2*	165.3	—	—
Deala CO (1)	161.9*	162.2	161.9 s	v.br.m	161.7* s	v.br.m	161.8*	161.9	161.9*	162.0 s
Deala CO (3)	165.0	164.6	163.2 s	v.br.m	163.1	v.br.m	169.1	—	—	—
Thr CO (1)	164.5*	164.4	164.5 s	br.m	164.5* s	br.m	164.6*	164.5	164.6*	164.6 s
Thz C2 (2)	164.8	165.0	164.7 s	t (~7)	164.8 s	t (7)	165.2	164.8	164.9	165.0 s
Thz C2 (3)	166.6	167.4 br	166.5 s	t (7)	166.6 s	t (8)	165.4	166.6	166.7	167.0 s
Ala CO (1)	168.0*	167.8	167.8 s	br.m	168.0* s	br.m	168.1*	168.0	168.0*	168.0 s
Cys C2	169.2	169.4	169.2 s	br.m	169.2 s	dt (5, 5, 7)	169.2	169.2	169.1	169.2 s
Thz C2 (1)	169.4	168.4 v.br	169.3 s	br.m	168.8 s	br.d (7)	168.8	169.4	169.3	169.0 br.s
Cys CO	170.9*	171.1	170.9 s	br.m	170.9* s	br.m	171.0*	170.9	170.8*	170.9 s
Thz C2 (4)	171.7	[d]	171.6 s	br.m	167.3 s	br.d (7)	167.3	171.7	171.3	170.1 br.s
Ala CO (2)	171.8*	172.2	171.7 s	br.m	172.3* s	br.m	172.4	171.7	171.7*	171.8 s
Val CO	172.6*	173.4	172.9 s	br.m	172.5* s	br.m	172.5	172.7	172.5*	172.9 s
Thz C-S (2)	189.9*	190.1	189.9 s	d (~4)	189.9* s	br.s	161.1*	189.9	190.0*	190.1 s

[a] Chemical shifts are in ppm downfield of TMS in CD_3OH - $CDCl_3$ (1: 4) at 40°C using CD_3OH as internal standard found at 47.4 ppm downfield of TMS. Coupling constants given in brackets are $\pm$1.0 Hz unless otherwise indicated. Resonances marked with an asterisk are peptide ^{2}H-isotope shifted carbons in CD_3OD - $CDCl_3$ (1: 4) (see text).

Abbreviations: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; v=very; br=broad.

[b,c] Spectrum recorded after addition of 6% and 2% TFA-d, respectively.

[d] Resonance not observed because of exchange broadening.

Table 2. Distribution of ^{13}C NMR resonances in thiostrepton and thiopeptins.[a]

	A[b] (10~35)	B (45~80)	C (100~135)	D (140~190)	Total number of carbons
Thiostrepton A_1	15	16	16	25	72
Thiopeptin B_a	10 q, 3 t, 1 d } 14	2 s, 15 d } 17	5 s, 8 d, 3 t } 16	24 s	71
Thiopeptin A_{1a}	10 q, 3 t, 1 d } 14	2 s, 15 d, 1 q } 18	5 s, 8 d, 3 t } 16	24 s	72
Thiopeptin A_{1b}	10 q, 3 t, 1 d } 14	2 s, 14 d, 1 q } 17	5 s, 8 d, 3 t } 16	25 s	72
Thiopeptin A_{4a}	10 q, 3 t, 1 s } 14	2 s, 15 d } 17	4 s, 8 d, 2 t } 14	23 s	68
Thiopeptin A_{3a}	10 q, 3 t, 1 d } 14	2 s, 15 d } 17	3 s, 8 d, 1 t } 12	22 s	65

[a] Abbreviations: s=singlet, d=doublet, t=triplet, q=quartet.
[b] Spectral regions in brackets are in ppm.

have the same number of "split" resonances in the same region of their spectra as for thiostrepton with the exception of the characteristic resonance at 189.9 ppm occurring at 161.1 ppm in thiostrepton which has been attributed (see preceding paper) to the substitution of the Thz (2) amide carbonyl by a thioamide group. By contrast, the spectrum of thiopeptin A_{3a} lacks two of the "split" resonances in the olefinic region and two in the amide carbonyl region consistent with the structure proposed for A_{3a} which lacks both side chain Deala residues. This allowed the peptide carbonyls at 161.9, 161.1 and 163.2 ppm to be readily assigned to the Deala residues (1), (2) and (3), respectively. Similarly, that for Thz CO (4) would be expected to differ in thiopeptin A_{3a} and was assigned to the resonance at 158.8 ppm. This was confirmed on addition of TFA-*d* which resulted in an upfield shift and concomitant broadening of this resonance due to protonation of the piperidine ring nitrogen (see below). The other Thz CO (1) was assigned to the resonance at 160.6 ppm on the basis of their similar doublet nature (J=1 Hz) on "gated" decoupling. The singlet at 168.1 ppm in thiostrepton was assigned to the Ala CO (1) by TORI *et al.*[1,5] by comparison of thiostrepton with siomycin which has a Deala residue at this position. This leaves the four singlets at 164.5, 170.9, 171.7 and 172.9 ppm to be assigned to the remaining peptide carbonyls. The resonance at 171.7 ppm in either thiopeptin A_{1a}, A_{3a}, A_{4a} or B_a is observed at 172.4 in thiostrepton and 172.3 ppm in thiopeptin A_{1b} implicating the effect of the imine double bond in the latter and was, therefore, assigned to Ala CO (2). The resonances at 164.5 and 170.9 ppm were assigned to Thr CO (1) and Cys CO respectively on the basis of acetylation shifts[5] which leaves the resonance at 172.9 ppm to be assigned to Val CO. This resonance appears to be implicated in a conformational change involving the Val residue on addition of TFA-*d* (see below).

^{13}C NMR Assignments from Protonation Studies with TFA-*d*

Protonation studies of the **a** series of thiopeptins with TFA-*d* in CD_3OD - $CDCl_3$ (1: 4) as followed by ^1H NMR, indicate the Val nitrogen to be appreciably more hindered than the piperidine nitrogen. Changes in the vicinity of the Val residue do occur but do not appear to be a result of protonation of the Val nitrogen. This is also manifested in the ^{13}C NMR spectra of thiopeptin B_a and A_{3a} (see Table 1). Resonances affected by protonation of the piperidine ring nitrogen are selectively broadened and can therefore be readily distinguished from those involved in conformational changes on the basis that they

remain sharp. In addition, shifts of the terminal Deala resonances were observed but these could be readily identified so as not to interfere with the above assignments. We have observed that both side chain Deala residues are lost on treatment of B_a or $A_{1a/1b}$ with TFA and it is therefore of interest to note that protonation of the Deala residues in the NMR experiment appears to occur at nitrogen and not of the double bond since no exchange of the methylene protons for deuterium is evident by ^1H NMR or by ^{13}C NMR through loss of NOE. Steric hindrance of the ring Deala nitrogen may thus satisfactorily account for its lack of protonation in keeping with the slower rate of exchange of the NH proton in thiostrepton A_1 compared to those of the side chain Deala resonances (see companion paper). Individual assignments of resonances affected by the addition of TFA-*d* are discussed in the appropriate sections.

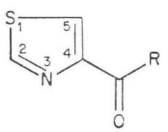
Model Compounds for Thiazole-4-carboxamide, Deala and Dihydroquinoline Substructures

Thiazole-4-carboxamides

In order to confirm the presence of a thioamide grouping in the thiopeptins, ^{13}C NMR spectra of a number of amides and their thio-analogs were measured. KALINOWSKI and KESSLER[7] have proposed a linear relationship between the chemical shift of the two types of carbons, namely

$$\delta_{C=S}=1.45\ \delta_{C=O}-46.5 \text{ ppm}$$

and we were interested whether the predictive value of the correlation could be extended to aromatic amides, in particular thiazole-4-carboxamides. Moreover, we wished to study the effect of substituting an amide for a thioamide group ($^1J_{CH}$), two-($^2J_{CHH}$) and three-bond ($^3J_{CCCH}$) carbon-proton couplings. The calculated $\delta_{C=S}$ values were found to be in good agreement with those observed for the amides listed in Table 3. In particular, $\delta_{C=S}$ of 189.7 ppm in thiazole-4-thiocarboxamide is in very good agreement with the calculated value of 189.6 ppm and that assigned to the Thz C=S (2) in the thiopeptins at 189.9 ppm.



6 R=NH_2
7 R=NHPh

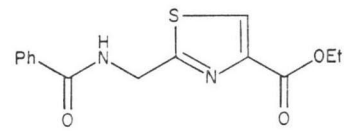


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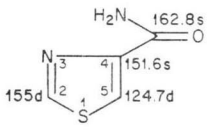
Table 3. Observed (δ_{obs}) and predicted (δ_{calcd}) chemical shifts of some thioamide carbons[a].

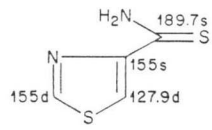
Amide/thioamide	$\delta^{C=O}_{obs}$	$\delta^{C=S}_{obs}$	$\delta^{C=S}_{calcd}$ [b]
Acetamide (**1**)	172.9	206.5	204.2
Benzamide (**2**)	168.9	202.5	198.5
Benzanilide (**3**)	166.0	198.2	194.2
α-Picolinamide (**4**)	166.8	195.3	195.4
3-Methyl-4-nitro-imidazole-2-*N*-methyl carboxamide (**5**)	160.8	185.4	186.7
Thiazole-4-carboxamide (**6**)	162.8	189.7	189.6

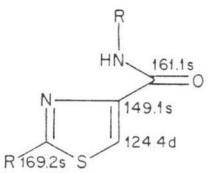
[a] Chemical shifts in ppm downfield of TMS in DMSO-d_6 at 40°C.
[b] See text.

Substitution by the thioamide group in thiazole-4-carboxamide (**6**) leads to downfield shifts of C4 and C5 whereas C2 remains unchanged (see Fig. 3). This allowed C4 and C5 of Thz (2) to be readily distinguished from the corresponding carbons of the other three thiazole

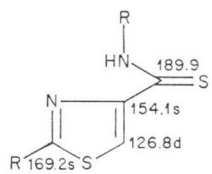
Fig. 3. ^{13}C NMR assignments of Thz (2) in thiostrepton and thiopeptin A_{1a} by comparison with thiazole-4-carboxamide and 4-thiocarboxamide.







Thz (2) of thiostrepton



Thz (2) of thiopeptin A_{1b}

Table 4. ^{13}C NMR data for ring carbons of thiazole derivatives **6**, **7** and **8**.

Compound	δ_{C2}[a]	δ_{C4}[a]	δ_{C5}[a]
6	155.0 dd ($^1J_{CH}$=216.5, $^3J_{CSCH5}$=7.5 Hz)	151.6 dd ($^2J_{CCH5}$=5, $^3J_{CNCH2}$=15 Hz)	124.7 d ($^1J_{CH}$=194.5 Hz)
7	155.2 dd ($^1J_{CH}$=216.5, $^3J_{CSCH5}$=7.5 Hz)	151.2 dd ($^2J_{CCH5}$=5, $^3J_{CNCH2}$=14 Hz)	125.6 d ($^1J_{CH}$=194.5 Hz)
8	170.8 dt ($^2J_{CCH}$=6, $^3J_{CSCH5}$=7.0 Hz)	146.1 d ($^2J_{CCH5}$= 4.5 Hz)	129.3 d ($^1J_{CH}$=194.5 Hz)

[a] Chemical shifts in ppm downfield of TMS in DMSO-d_6 at 40°C.

rings by comparison of thiostrepton with thiopeptin A_{1b} but not A_{1a} where otherwise the effect of the imine double bond would also be manifested. Confirmation of these assignments could be made on the basis of one-, two- and three-bond ^{13}C-^1H coupling constants which are summarized for the model thiazole derivatives **6**, **7** and **8** in Table 4. Assignments were made on the basis of previous work[8] and multiplicity of signals obtained from the coupled spectra. All spin systems were treated as first order and coupling constants were read directly from the spectra. All four thiazole rings in the thiopeptins are substituted at C2 and hence C5 will only show a diagnostic $^1J_{CH}$ value near 195 Hz and could thus be readily assigned to the doublets at 117.0, 123.2, 123.9 and 126.7 ppm. Individual C5 assignments could also be made. The resonance at 126.7 ppm has already been assigned to Thz C5 (2) on the basis of the amide→thioamide substitution. Only one C5 resonance, that of Thz (4), would be expected to be different for A_{1a} and A_{1b} as the imine double bond in the latter is conjugated with the thiazole ring and leads to a 3.3 ppm downfield shift of the resonance at 123.2 in A_{1a}. Titration of B_a with TFA-*d* enabled a clear distinction between C5 of Thz (1) and Thz (3) to be made on the basis of their respective distances from the piperidine nitrogen. A much larger downfield shift accompanied by broadening was observed for the resonance at 117.0 ppm which was therefore assigned to C5 of Thz (3) and that at 123.9 ppm to C5 of Thz (1) (see Table 5).

In the 2-substituted thiazole derivative **8**, H5 is coupled to both C4 ($^2J_{CCH}$=4.5 Hz) and C2 ($^3J_{CSCH}$= 7 Hz) and the latter is further split by the methylene protons of the side chain ($^2J_{CCH}$=6 Hz) (Table 4). These couplings are similar to those in **6** and **7** where additional coupling of H2 to C4 ($^3J_{CNCH}$=14~15 Hz) but not to C5 is observed. On this basis the C2 and C4 resonances of the thiazole rings in the thiopeptins were readily identified as shown in Table 5. Individual assignments of C4 could be made in a manner similar to those employed for C5, in particular using the titration results with TFA-*d*. As expected, C4 of Thz (2), assigned on the basis of the amide→thioamide substitution, was not affected whereas the resonance at 155.2 ppm was completely exchange-broadened in CD_3OH - $CDCl_3$ (1:4) containing 6% TFA-*d*. The resonance can however be observed at 1.0 ppm to higher field in the presence of 2% TFA-*d*. It was therefore assigned to C4 of Thz(3) being closest to the piperidine nitrogen. The assignment is in agreement with the additional observed coupling in Pip H6 ($^2J_{CCH}$=6.0 Hz) typical for $^2J_{CCH}$ couplings of this kind[9], and a significant difference in chemical shift from those of Thz (1) and Thz (4) at 144.9 and 148.1 ppm respectively, which both carry carboxamide groups at this position.

Assignment of the C2 resonances was not as straightforward. These should occur in the 160~175 ppm region in which there are fifteen resonances, nine of which are assigned to ^2H-shifted amide carbonyl carbons, leaving six to be assigned. The resonance at 163.2 ppm in Ala (CO_2CH_3), absent in thiopeptins A_{3a} and A_{4a} and at different positions in thiostrepton ($CONH_2$) and thiopeptin B_a (CO_2H) but the same in A_{1a} and A_{1b}, was assigned to the terminal Deala carbonyl group of the side chain. The

Table 5. ^{13}C NMR protonation shifts (TFA-*d*) and assignments of thiazole ring carbons in thiopeptin B_a.

Assignment		δ ppm[a]	δ (+6% TFA-*d*)	$\Delta\delta$[b] (ppm)	Comments
Thz (1)	C2	169.4 s	168.4 v.br	+1.0	168.8 ppm in thiostrepton and A_{1b}; +0.3 ppm shift with 2% TFA-*d* (A_{3a}); $^3J_{CSCH5}$=7.0 Hz (A_{1b}).
	C4	144.9 s	145.3	−0.4	$^2J_{CCH5}$=4.0 Hz (A_{1a} and A_{1b}).
	C5	123.9 d	124.3	−0.4	$^1J_{CH}$=192 Hz (A_{1a}), 193 Hz (A_{1b}).
Thz (2)	C2	164.8 s	165.0	−0.2	$^3J_{CSCH5}$=7.0 Hz, $^2J_{CCH}$=7.0 Hz (A_{1a} and A_{1b}).
	C4	154.1 s	154.3	−0.2	
	C5	126.7 d	126.8	−0.1	$^1J_{CH}$=194 (A_{1a}), 195 Hz (A_{1b})
Thz (3)	C2	166.6 s	167.4 br	−0.8	165.4 ppm in thiostrepton; $^3J_{CSCH5}$=7.0 Hz (A_{1a}), 8.0 Hz (A_{1b}); $^2J_{CCH}$=7.0 Hz (A_{1a}), 8.0 Hz (A_{1b})
	C4	155.2 s	[c]		+1.0 ppm shift with 2% TFA-d (A_{3a}); 156.0 ppm in thiostrepton and A_{1b}; $^2J_{CCH5}$=4.0 Hz, $^2J_{CCH}$= 6.0 Hz (A_{1b}).
	C5	117.0 d	119.1 v.br	−2.0	$^1J_{CH}$=190 Hz (A_{1a} and A_{1b}).
Thz (4)	C2	171.7 s	[c]		+1.2 ppm shift with 2% TFA-*d* (A_{3a}); 167.3 ppm in thiostrepton and A_{1b}; $^3J_{CSCH5}$=7.0 Hz (A_{1b}).
	C4	148.1 s	148.4	−0.3	148.8 ppm in thiostrepton and A_{1b}; $^2J_{CCH5}$=4.0 Hz (A_{1a} and A_{1b}).
	C5	123.1 d	124.2 br	−1.1	126.5 ppm in thiostrepton and A_{1b}; $^1J_{CH}$=192 Hz (A_{1a}), $^1J_{CH}$=191 Hz (A_{1b}).

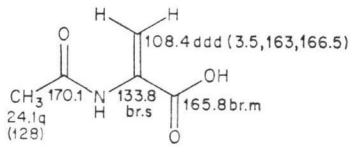
[a] Chemical shifts in ppm downfield of TMS in CD_3OH - $CDCl_3$ (1:4) at 40°C.
[b] Protonation shift in ppm, a negative sign denotes a downfield shift.
[c] Resonance not observed because of exchange broadening.

remaining five resonances are therefore attributed to C2 of the thiazoline (Cys) and four thiazole rings. Two of these would not be expected to be influenced by the addition of TFA-*d* and were therefore assigned to C2 of Thz (2) and Cys at 164.7 and 169.2 ppm which can be distinguished on the basis of their fine splitting pattern. C2 of Thz (2) at 164.7 ppm is a triplet (J=7 Hz) coupled to H5 as well as Thstn H2, whereas the signal for C2 of Cys appears as a doublet of triplets (J=5, 5, 7 Hz) through three-bond couplings to But H3, Cys H4 and most probably to one of the Cys H5 protons. A moderate coupling constant of 5 or 7 Hz between But H3 and Cys C2 is in keeping with that expected for a *cis* relationship[10]. Coupling to Cys H4 would be expected to be in the range 7~12 Hz on the basis of earlier work on substituted thiazolines[11]. Coupling to H5 was observed to be small (3 Hz) and may be due to averaging between two envelope forms as three-bonded carbon-proton couplings, like their proton-proton counterpart, obey a Karplus-type relationship[12]. In a frozen conformation of the thiazoline ring as observed in thiostrepton and the thiopeptins (see companion paper) where the dihedral angles between C2 and the H5 protons are *ca.* 95° and 145°, at least one of the couplings would be expected to be >3 Hz. Of the remaining C2 resonances only C2 of Thz (3) in A_{1b} is expected to be a triplet and is therefore assigned to the resonance at 166.6 ppm being coupled to both H5 and Thr H2 (2) ($^2J_{CCH}$=8 Hz)[9]. The other C2 resonances in A_{1a} overlap with other resonances but appear as broad doublets (J=7 Hz) at 168.8 and 167.3 ppm in A_{1b} which were assigned to Thz (1) and Thz (4), respectively, on the basis of the effect of the imine double bond. These assignments were confirmed by the protonation shifts with TFA-*d*. C2 of Thz (4) was completely exchange broadened in the presence of 6% TFA-*d* but can be seen 1.2 ppm to higher field of its usual position in 2% TFA-*d*, whereas C2 of Thz (1) undergoes a much smaller upfield shift.

2-Acetamido Acrylic Acid

Assignment of the Deala resonances in the thiopeptins was made by comparison with 2-acetamido acrylic acid whose spectrum was taken in DMSO-d_6 at 25°C (see Fig. 4). Magnetic non-equivalence of the methylene protons, in addition to the ^{1}H NMR chemical shift parameter, is also reflected in the $^1J_{CH}$ values of 163 and 166.5 Hz. A three-bond coupling to the peptide NH proton ($^3J_{CCNH}$=3.5 Hz) is also observed. Three resonances at 101.9, 102.3 and 109.0 ppm in the coupled spectrum of thiopeptin A_{1a} (see Table 1) give similar multiplets, the latter having almost identical coupling constants ($^1J_{CH}$=164, 168 Hz, $^3J_{CCNH}$=4 Hz) and was therefore assigned to C3 of the terminal Deala (3) residue. Comparison with the de-Deala products A_{3a} and A_{4a} shows that this resonance is missing in both components. Moreover, the resonance at 102.3 ppm is missing in component A_{3a} and was therefore assigned to C3 of Deala (2) and that at 101.9 ppm to C3 of the ring Deala residue. Both resonances display a correspondingly larger magnetic non-equivalence for the methylene protons as manifested in the $^1J_{CH}$ values of *ca.* 160 and 169 Hz which is also in evidence in the geminal ^{1}H-^{1}H coupling constant (see companion paper).

Fig. 4. ^{13}C NMR assignments of 2-acetamido acrylic acid in DMSO-d_6 at 25°C.



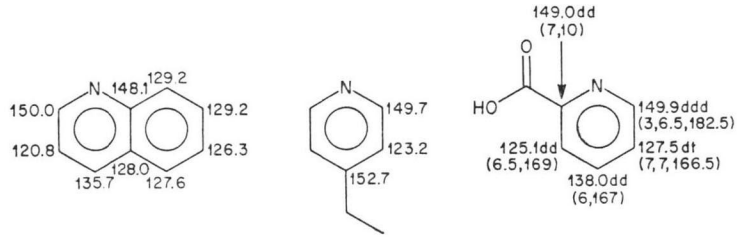
The resonance for C3 of the dehydrobutyrine (But) residue would be expected to occur downfield of this region and can be assigned on the basis of its multiplet structure (doublet of quartets) expected on the basis of two- and three-bond couplings to methyl (~6 Hz) and NH (~4 Hz) protons respectively. Consideration of the lower $^1J_{CH}$ value of 155 Hz for But C3 than for either of the couplings for C3 in the Deala residues as well as the effect of methyl substitution at the β-carbon on $^1J_{CH}^{cis}$ as seen by comparison of acrolein with crotonaldehyde (156.6→152.7 Hz) or α-methacrolein with tiglaldehyde (157.4→153.6 Hz)[13], suggests the smaller coupling for Deala C3 to be associated with the proton *cis* to the carbonyl function.

In the 125~140 ppm region of thiopeptin A_{1a}, the Deala C2 resonances at 129.5, 131.1 and 133.1 ppm were readily distinguishable on the basis of their ^{2}H-isotope shifted peaks as well as their doublet nature ($^2J_{CCH}$=4 Hz) on "gated" decoupling. The latter coupling is also observed for the α-carbon in acrylonitrile[14] and involves the proton *trans* to the cyano group. Comparison with thiopeptins A_{3a} and A_{4a} allowed individual assignments to be readily made (Table 1). By analogy, the remaining ^{2}H-isotope shifted carbon resonance in this region at 127.3 ppm appearing as a broad multiplet on "gated" decoupling, was assigned to But C2. The Deala carbonyl resonances were similarly assigned.

Dihydroquinoline Analogs

Only resonances belonging to the dihydroquinoline moiety remain to be assigned in the 100~160 ppm region. This was aided by the reported chemical shift data on quinoline and 4-ethylpyridine in $CDCl_3$[15] and our own data on α-picolinic acid in DMSO-d_6 as summarized in Fig. 5. Comparison of the chemical shift and ^{13}C-^{1}H coupling constant data of the latter with that reported for 2-cyanopyridine[16], allowed unequivocal assignments of the ring carbons to be made. It readily follows that the singlets at 125.9, 142.4 and 159.6 in thiopeptin A_{1a} can be assigned to Q C10, Q C4 and the ester carbonyl Q CO, respectively, leaving the singlets at 152.4 and 153.5 to be assigned to either Q C2 or Q C9. In α-picolinic acid C2 is coupled to H4 ($^3J_{CCCH}$=7 Hz) and H6 ($^3J_{CNCH}$=10 Hz) and the coupling to H3

Fig. 5. ^{13}C NMR assignments of some pyridine analogs.



$CDCl_3$ $CDCl_3$ DMSO-d_6

is unresolved (<1 Hz). Only coupling of H3 to C2 can occur in the dihydroquinoline moiety Q whereas C9 would be expected to be a broader resonance through possible two-bond coupling to H8 and three-bond coupling to H5 and H7. The slightly broadened singlet at 152.4 ppm was therefore assigned to C2 and the broader multiplet at 153.5 ppm to C9.

Of the three remaining doublets at 121.2 ($^1J_{CH}$=171 Hz), 122.1 ($^1J_{CH}$=162 Hz) and 128.8 ppm ($^1J_{CH}$=166 Hz) in this region, the sharpest resonance at 121.2 ppm can be readily assigned to Q C3 on the basis of its comparable $^1J_{CH}$ value (171 Hz) in α-picolinic acid (169 Hz). Similarly the resonance at 122.1 ppm which shows broad doublet components of the doublet on "gated" decoupling (dd, $J\simeq4$, 162 Hz) because of a two-bond coupling to Q H6 was assigned to Q C5 and that at 128.8 ppm showing broader doublet components approaching a triplet $J\simeq4$ Hz to Q C6, because of coupling to both Q H5 and H7.

^{13}C NMR Assignments of High Field Region A

The methyl resonances in thiostrepton were completely resolved under our conditions at 75 MHz in CD_3OD - $CDCl_3$ (1 : 4) at 40°C and only in the thiopeptin series in the case of thiopeptin A_{3a} in the presence of TFA-*d* (see Table 1). Thiostrepton has 15 resonances in the 10~35 ppm region whereas all thiopeptins have one resonance less comprising ten methyl quartets, three methylene triplets and one methine doublet confirming substitution of the Ile residue for Val. All resolved methyl quartets displayed similar single bond ^{13}C-^1H coupling constants and fine structure under "gated" decoupling conditions and hence no further assignments to those of TORI *et al.*[1,5] could be made.

The piperidine methylene triplets were readily assigned to the resonances at 27.1 (C3) and 32.4 (C4) ppm on the basis of their protonation shifts with TFA-*d* (see below) as well as the upfield shifts observed upon introduction of the imine double bond into the ring by comparing A_{1a}, A_{3a}, A_{4a} or B_a with either A_{1b} or thiostrepton. These correspond to 23.4 and 27.9 ppm in thiostrepton respectively, assigned on the basis that the former resonance slowly disappears due to proton-deuterium exchange at Pip C3 in CD_3OD - $CDCl_3$ (1 : 4), opposite to that assigned by TORI *et al.*[1,5] This acid-catalyzed exchange which proceeds *via* imine-enamine equilibrium was observed initially in the ^1H NMR spectrum of thiostrepton and is also observed in the **b** series of thiopeptins[2]. The remaining triplet at 33.5 ppm of appreciably larger $^1J_{CH}$=147 Hz was assigned to Cys C5 by TORI *et al.*[1,5] by comparison with althiomycin. The only doublet in this region at 29.7 ppm was unequivocally assigned to Val C3.

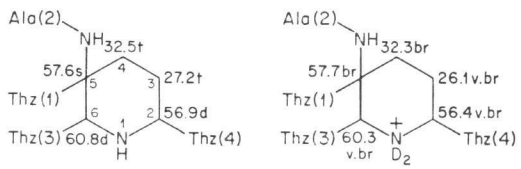
^{13}C NMR Assignments in 45~80 ppm Region B

The assignments of the two singlets at 75.8 and 57.5 ppm and quartet at 51.6 ppm in the "gated" decoupled spectrum of thiopeptin A_{1a} are readily made to Thstn C3, Pip C5 and the ester methyl group

of the terminal Deala (3) residue, respectively, the latter having $^1J_{CH}$=148 Hz characteristic of a methyl ester. The singlet at 57.5 ppm is ^{2}H-isotope shifted in CD_3OD - $CDCl_3$ (1 : 4) being adjacent to a slowly exchanging peptide NH group linked to Ala (2). The remaining signals are all doublets most of which can be assigned unequivocally. Of the six ^{2}H-isotope shifted carbons the signal at 48.1 ppm missing in siomycin was assigned to Ala C2 (1) by TORI *et al.*[1,5] and similarly the ^{2}H-isotope shifted resonance at 50.8 ppm was assigned to the other alanine α-carbon Ala C2 (2) having the same $^1J_{CH}$=141 Hz. Substitution of the amide by a thioamide group in **5** (Table 3) results in a downfield shift of the α-methyl carbon (26.2→32.6 ppm) and was used to assign the resonance at 60.2 ppm to Thr C2 (2) which occurs at 54.6 ppm in thiostrepton. Only two of the six ^{2}H-isotope shifted resonances at 52.1 and 54.5 ppm remain unassigned and must be either Thr C2 (1) or Thstn C2. On the basis of the smaller single bond coupling constant $^1J_{CH}$=137 Hz for the doublet at 52.1 ppm, the latter is assigned to Thstn C2 and that at 54.5 ppm ($^1J_{CH}$=143 Hz) to Thr C2 (1). The smaller coupling constant is consistent with a decrease in electro-negativity[17] expected on substitution of an iminothiol for a peptide group and the effect of increased steric crowding at Thstn C3[18].

By analogy with althiomycin, TORI *et al.*[1,5] assigned the resonance at 77.7 ppm to Cys C4 in thiostrepton which also occurs at this position in the thiopeptins. The resonance at 56.8 ppm in thiopeptin A_{1a} which broadens and moves upfield on addition of TFA-*d*, is missing in A_{1b} and thiostrepton and was assigned to Pip C2. The other carbon α- to the piperidine nitrogen (Pip C6) can be readily assigned to the resonance at 60.7 ppm which experiences a similar upfield protonation shift (see Fig. 6) and occurs upfield of the position of thiostrepton and thiopeptin A_{1b} (62.9 ppm) due to the effect of the imine double bond. Protonation of the piperidine nitrogen leads to upfield shifts of all ring carbons and the larger shift for Pip C3 is in keeping with a general trend of larger protonation shifts for β than α-carbons in amines whereas the almost negligible shift observed for the other β-carbon Pip C5 may be attributed to a lack of protons at this center[19,20]. Protonation of only the piperidine nitrogen occurs in the presence of 2% TFA-*d* but shifts of other resonances associated with conformational changes are evident on further addition of TFA-*d*. The two carbon resonance at 56.1 ppm in A_{1a} is resolved at 64.8 and 65.1 ppm in A_{1b} showing distinctly different $^1J_{CH}$ values of 141 and 150 Hz respectively and both of which experience the largest shifts with TFA-*d*. From the corresponding ^{1}H NMR shifts, Q C8 and Thr C3 (1) are assigned to these resonances. The large $^1J_{CH}$ value of the 146 Hz of the carbinol carbon in *N*-benzoyl L-threonine methyl ester allowed the resonance at 65.1 ppm with the larger $^1J_{CH}$ value to be assigned to Thr C3 (1). Esterification of an alcohol results in appreciably larger $^1J_{CH}$ values for the carbinol carbon and on this basis the resonance at 70.8 ppm with $^1J_{CH}$=154 Hz was assigned to C3 of the esterified Thr (2) residue. Consistent with the lower electro-negativity of an amine *versus* an alcohol functionality, two of the four remaining doublets occurring to higher field at 57.7 and 63.2 ppm having the smaller $^1J_{CH}$ values, were assigned to Q C7 and Val C2 respectively. The resonance at 66.5 ppm with a $^1J_{CH}$ value similar to that in 1-phenylethanol ($^1J_{CH}$=143 Hz) was assigned to Q C11. The remaining doublet at 66.9 ppm was therefore assigned to Thstn C4 in agreement with TORI *et al.*'s[1,5] firm assignment on the basis of acetylation shifts.

Fig. 6. ^{13}C NMR assignments of piperidine ring carbons in thiopeptin B_a in (a) the absence and (b) presence of 6% TFA-*d*.



Experimental

^{13}C NMR spectra were obtained in the FT mode using a Varian SC 300 NMR spectrometer. Spectra of thiostrepton and thiopeptin components were recorded in CD_3OH - $CDCl_3$ (1: 4) and CD_3OD - $CDCl_3$ (1: 4) at 40°C with concentrations in the range of 30~60 mg/0.4 ml in 5 mm tubes. The spectra were obtained under the following FT conditions: spectral width (SW) 15,000 Hz; acquisition time (AT) 0.4~0.6 second; pulse flipping angle *ca* 50°; number of transients (NT) 30,000~50,000. "Gated" decoupled spectra were similarly obtained using a pulse delay of 0.2 second with NT=70,000. Spectra of the model compounds were measured in DMSO-d_6 at 40°C with concentrations in the range 100~250 mg/0.4 ml using SW=15,000 Hz, AT=1 second, pulse flipping angle *ca.* 50° and NT 10~30.

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