REEXAMINATION OF THE ^{1}H AND ^{13}C NMR SPECTRAL ASSIGNMENTS OF THIOSTREPTON

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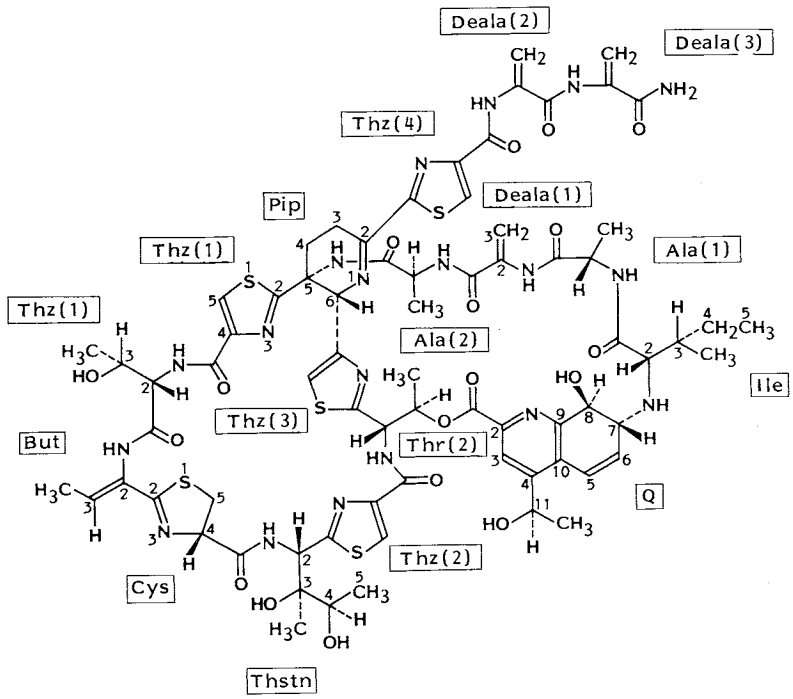
The use of 2D NMR techniques on unlabeled and biosynthetically multiple ^{13}C-labeled samples enabled us to refine the ^{1}H and ^{13}C NMR spectral assignments for thiostrepton.

Thiostrepton (**I**) was first isolated in 1954 from *Streptomyces azureus*[1~3] and is an inhibitor of Gram-positive bacteria[4]. It has so far not found clinical use, but is employed extensively as a tool in molecular biology. The complete structure of thiostrepton was elucidated by X-ray crystallography in conjunction with chemical methods[5,6]. Thiostrepton was the subject of earlier NMR investigation by TORI *et al.*[7] and subsequently by HENSENS and ALBERS-SCHÖNBERG[8,9].

For the purpose of establishing the biosynthetic pathway to **I** we needed to confirm unequivocally the assignments of all the ^{1}H and ^{13}C resonances. We reexamined the NMR data by employing a variety of 2D NMR techniques on unlabeled and biosynthetically multiple labeled samples of thiostrepton. In this paper we describe the results of this work, which include several revisions of the data previously



Thiostrepton (**I**)

reported in the literature[8,9].

Results and Discussion

The reexamination of the ^1H NMR data was necessary in part because we used $CDCl_3$-CD_3OD (4:1) not only as a solvent for ^{13}C NMR[9], but also for ^1H NMR spectroscopy. In this solvent mixture, thiostrepton was much more soluble, and resonance lines in the NMR spectra exhibited better dispersion. The more polar solvent caused shifts of most resonances relative to earlier data[8]. Although a deuterium-exchange of OH-signals took place, better resolution could be obtained and an exchange of NH-protons, as reported earlier[8], was not observed.

Only a few protons, such as the quartet of dehydrobutyrine (But) 3-H at 6.07 ppm (J=7.10 Hz), the triplet of Ile 5-H (CH_3) at 0.74 ppm (J=7.13 Hz), and the singlet of thiostreptine (Thstn) 3-CH_3 at 0.99 ppm were readily assigned from the 300 MHz ^1H NMR spectrum by direct inspection; due to overlap of many signals and similarities in chemical shift direct assignments for most other resonances could not be obtained with certainty.

All proton signals were assigned by 2D NMR techniques, especially, ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C long range correlation (COLOC).

Except for thiazole (Thz)(2) 5-H (8.13 ppm) which showed a cross correlation to Thstn 2-H (5.62 ppm), the other 3 Thz 5-H and quinaldic acid (Q) 3-H protons did not exhibit any correlations in the COSY spectrum. Their assignment was achieved by the COLOC spectra, in which Thz 5-H displayed two-bond couplings with the corresponding Thz C-4 as well as three-bond couplings with Thz C-2. The Q 3-H proton also showed a three-bond coupling to the carbonyl carbon of the quinaldic acid moiety.

The ^{13}C NMR spectrum revealed signals for all 72 carbons. Multiplicities (10 quartets, 7 triplets, 23 doublets and 32 singlets) were determined from an edited distortionless enhancement by polarization transfer (DEPT) analysis.

Our ^{13}C assignments largely confirmed earlier data[9], which were based entirely on 1D NMR spectroscopy. The use of 2D NMR experiments, such as ^1H-^{13}C COSY and COLOC on unlabeled samples and 2D-INADEQUATE on biosynthetically multiple labeled samples showed that 15 revisions to the ^{13}C assignments were necessary. Only the assignment of these 15 carbons will be discussed in the following. All chemical shifts differ from previous data, since our NMR spectra were measured in $CDCl_3$-CD_3OD (4:1) with $CDCl_3$ as an internal reference standard.

Based upon the ^1H-^{13}C COSY spectrum, proton signals were assigned to their respective carbons through their one-bond couplings.

The experiment identified differences in the assignments of Thstn 3-CH_3 (18.34 ppm), Thr(2) CH_3 (18.68), Ala(2) CH_3 (18.81), Ala(1) CH_3 (18.88), Thr(1) CH_3 (18.92), Q C-11 (64.35), Ile C-2 (65.60) and Q C-8 (67.34).

Most of the quaternary carbons demonstrated ^1H-^{13}C long range couplings in the COLOC experiments. The assignments of dehydroalanine (Deala)(2) CO (162.00 ppm) and Thz(2) C-2 (166.38 ppm) were confirmed by three-bond couplings to both Deala(2) 3-H protons (6.54 and 5.48 ppm) and to Thz(2) 5-H (8.13 ppm), respectively. Thz(3) C-2 (169.97 ppm) displayed a two bond coupling to Thr(2) 2-H (5.62 ppm).

A feeding experiment with L-[1,2-$^{13}C_2$]serine as a precursor resulted in incorporations of this amino acid into several moieties of thiostrepton[10]. The analysis of the 2D-INADEQUATE spectrum of the

Table 1. ^{13}C and ^{1}H NMR spectral data of thiostrepton (δ in ppm, J in Hz).

Ile CO	173.71 s		Deala(3) 3	104.27 t	6.37 *E* (d, 1.17), 5.52 *Z* (d, 1.17)
Ala(2) CO	173.31 s		Deala(2) 3	103.28 t	6.54 *E* (d, 1.93), 5.48 *Z* (d, 1.93)
Cys CO	171.97 s		Deala(1) 3	102.79 t	5.66 *E* (d, 1.92), 5.19 *Z* (s, br)
Cys 2	170.16 s		Cys 4	78.98 d	4.83 (dd, 9.60, 12.60)
Thz(3) 2	169.97 s		Thstn 3	77.19 s	
Thz(1) 2	169.73 s		Thr(2) 3	72.00 d	6.19 (m)
Ala(1) CO	168.84 s		Thstn 4	67.73 d	3.67 (d, 6.48)
Thz(4) 2	168.35 s		Q 8	67.34 d	4.32 (d, 1.75)
Thz(2) 2	166.38 s		Thr(1) 3[a]	66.46 d	1.35 (m)
Deala(3) CO	165.99 s		Ile 2	65.60 d	2.81 (d, 4.58)
Thr(1) CO	165.47 s		Q 11	64.35 d	5.16 (d, 6.43)
Deala(1) CO	162.92 s		Pip 6	64.21 d	5.16 (s, br)
Thz(2) CO	162.10 s		Q 7	59.02 d	3.46 (dd, 1.70, 5.50)
Deala(2) CO	162.00 s		Pip 5	57.57 s	
Pip 2	161.94 s		Thr(2) 2	55.83 d	5.62 (d, 7.62)
Thz(1) CO	161.67 s		Thr(1) 2	55.63 d	4.27 (dd, 3.25, 7.60)
Q CO	160.80 s		Thstn 2	53.06 d	5.62 (d, 9.90)
Thz(4) CO	159.56 s		Ala(2) 2	51.95 d	4.59 (pd, 6.48, 7.77)
Thz(3) 4	157.17 s		Ala(1) 2	49.35 d	3.67 (dq, 5.40, 6.81)
Q 9	154.55 s		Ile 3	38.49 d	1.69 (m)
Q 4	153.41 s		Cys 5	34.78 t	3.51 (dd, 9.00, 11.50)
Thz(2) 4	150.09 s		Pip 4[b]	29.16 t	3.92 (m), 2.16 (m)
Thz(4) 4	149.92 s		Ile 4	24.57 t	1.21 (m), 0.95 (m)
Thz(1) 4	146.40 s		Pip 3	24.57 t	3.32 (m), 2.76 (m)
Q 2	143.56 s		Q CH_3	22.53 q	1.21 (d, 6.61)
Deala(2) 2	134.20 s		Thr(1) CH_3	18.92 q	0.70 (d, 6.50)
Deala(3) 2	133.02 s		Ala(1) CH_3	18.88 q	1.03 (d, 6.80)
But 3	132.53 d	6.07 (q, 7.10)	Ala(2) CH_3	18.81 q	1.28 (d, 6.62)
Deala(1) 2	132.28 s		Thr(2) CH_3	18.68 q	1.56 (d, 6.47)
Q 6	129.99 d	6.23 (dd, 5.62, 9.90)	Thstn 3-CH_3	18.34 q	0.99 (s)
But 2	128.45 s		Thstn 5	15.88 q	1.15 (d, 6.63)
Thz(4) 5	127.55 d	8.14 (s)	Ile 3-CH_3	15.52 q	0.82 (d, 6.91)
Q 10	127.20 s		But CH_3	15.20 q	1.46 (d, 7.05)
Thz(2) 5	125.36 d	8.13 (s)	Ile 5	11.25 q	0.74 (t, 7.13)
Thz(1) 5	124.88 d	8.01 (s)			
Q 5	123.15 d	6.73 (d, 10.08)			
Q 3	122.26 d	7.13 (s)			
Thz(3) 5	118.16 d	7.40 (s)			

Deala(2) NH 9.82 (s, br); piperidine (Pip) 5-NH 9.72 (s, br); Deala(3) NH 8.96 (s, br); Thr(2) NH 8.62 (d, 8.80); But NH 8.47 (s, br); Deala(1) NH 7.82 (s, br); Ala(1) NH 7.62 (d, 5.33); Thstn NH 7.43 (d, 9.92); Ala(2) NH 6.99 (d, 7.78); Thr(1) NH 6.91 (d, 7.66).

[a] The unusually low frequency chemical shift of Thr(1) 3-H (1.35 ppm) results from a strong shielding interaction due to the proximity of the proton to the ring currents of the dihydroquinoline ring[8].

[b] The well-known deshielding of an equatorial proton in a six-membered ring explains the resonance of Pip 4-H_α at 3.92 ppm. Even greater downfield shifts for the corresponding protons were found for related compounds like the thiopeptins[8]. The deshielding is probably enhanced due to the orientation of the proton to the thiazole and an acylamino substituents at C-5[8].

biosynthetically labeled sample showed several ^{13}C-^{13}C correlations and allowed the assignment of Q C-2 (labeled from C-2 of serine), Thz(2) CO and Deala(3) CO (both labeled from carbon 1 of serine).

Deala(3) CO (165.99 ppm)↔(133.02 ppm) Deala(3) C-2

Thz(2) CO (162.10 ppm) ↔(150.09 ppm) Thz(2) C-4

Q CO (160.80 ppm) ↔(143.56 ppm) Q C-2

The other correlations were consistent with previously established assignments.

Experimental

^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$-CD_3OD (4:1) at a field strength of 7.1 T on an IBM AF-300 spectrometer. Samples were prepared in 5 mm tubes, and spectra were internally referenced to the solvent resonance ($CDCl_3$).

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