An Unusual Picoline Derivative from the Trifluoroacetolysis of Thiostrepton

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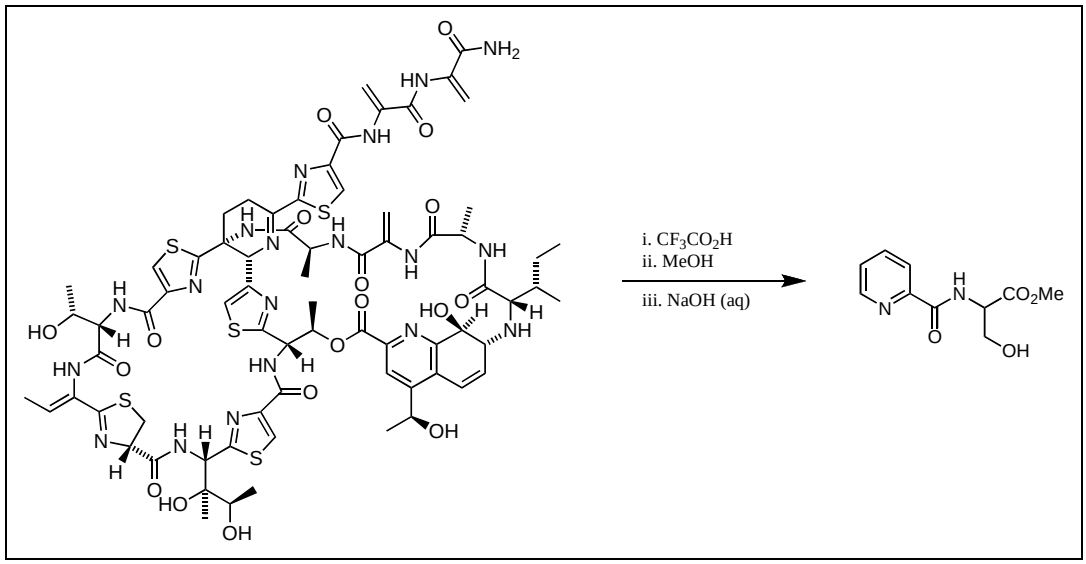

Trifluoroacetolysis of thiostrepton followed by treatment with methanol and aqueous sodium hydroxide led to the formation of *N*-(2-picolinoyl)serine methyl ester, the first pyridine-containing compound isolated from the chemical degradation of thiostrepton.

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INTRODUCTION

Thiostrepton (**1a**), the parent compound of the thiopeptide antibiotics [1], was first isolated in 1954 from *Streptomyces azureus* [2-4] and *S. hawaiiensis* [5], and later from *S. laurentii* (1977) [6]. The substance isolated from *S. hawaiiensis* was originally named bryamycin but was later shown to be identical to thiostrepton [7].

The majority of the structure of thiostrepton was determined by Dorothy Crowfoot Hodgkin through X-ray crystallography in 1970 [8], although several degradation studies were conducted in the years between the isolation of the natural product and Hodgkin's study in an attempt to elucidate the structure. Since that date, further X-ray crystallographic studies on tetragonal crystals using the anomalous dispersive signal from sulphur collected at the Cu Kα wavelength enabled Hunter and co-workers to solve the structure of the macrocycle of thiostrepton [9] and the landmark total synthesis of **1a** by Nicolaou *et al.* has verified its structural identity [10].

In recent years, few studies on the chemical degradation of thiopeptide antibiotics have been conducted, as the advent of sophisticated spectroscopic techniques such as COSY, HSQC and HMBC NMR spectroscopy, which can be performed routinely, has diminished the need to use chemical degradation as a means of structure determination. Also, the limited availability of the majority of thiopeptide antibiotics limits the use of degradation studies to only a fraction of the many compounds in this class. However, the study of the fragments produced by chemical degradation of thiopeptides can still provide valuable information, unavailable by conventional spectroscopic techniques,

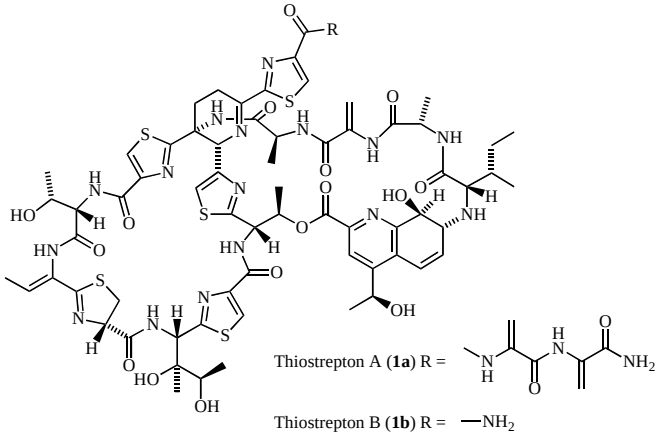


Figure 1. Thiostrepton, the parent thiopeptide antibiotic.

e.g. determination of the absolute stereochemistry of any stereogenic centres in the natural product. Degradation studies on thiopeptide antibiotics have also yielded an array of smaller compounds that have provided a synthetic challenge to chemists [11-15].

Thiostrepton is the most well studied thiopeptide antibiotic, and has been the subject of several degradation studies, mainly conducted during the 1960s. The first of these was by Bodanszky and co-workers in 1960 [16] who investigated the acidic hydrolysis of thiostrepton. Hydrolysis in a mixture of concentrated hydrochloric and formic acids yielded thiostreptoic acid (**2**) and three thiazolecarboxylic acids **3**, **4** and **5** (Figure 2).

Basic hydrolysis of thiostrepton in 5 *M* sodium hydroxide at 100 °C for 45 hours was found to yield thiazoles **3** and **4**, along with alanine-derived thiazole **6** on acidic hydrolysis (5 *M* HCl, 100 °C, 24 h) of the residue [17].

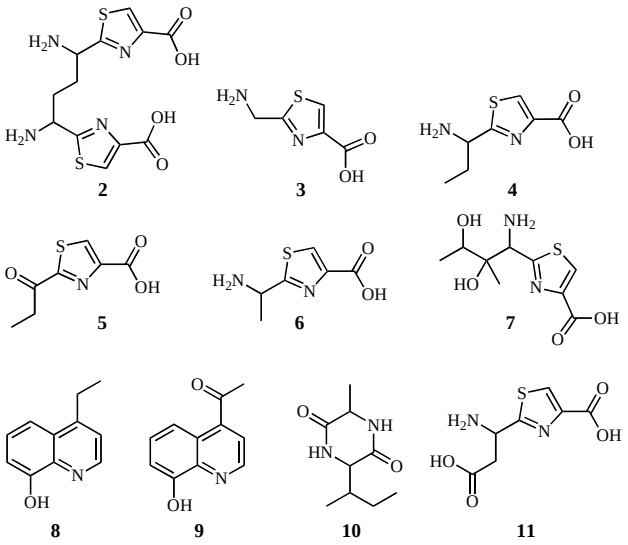


Figure 2. Degradation products previously isolated from thiostrepton.

Bodanszky *et al*. conducted further studies on the acidic hydrolysis of thiostrepton, finding that heating the natural product for 5 hours at 110 °C in a 1:1 mixture of concentrated hydrochloric and formic acids afforded thiostreptine (**7**) [18]. Pyrolysis of thiostrepton at 250-350 °C under 0.2 mm pressure yielded two quinolines, **8** and **9** from the quinaldic acid residue.[19]

A review of the above studies, together with some further investigations was published in 1964 [20]. It was reported that heating a solution of thiostrepton in glacial acetic acid gave diketopiperazine **10**, apparently derived from alanine and isoleucine.

Only one study on the oxidation of thiostrepton has been performed to date. In this investigation, thiostrepton was stirred in a mixture of 98% formic acid and 30% hydrogen peroxide for 15 minutes and the resulting residue subjected to acidic hydrolysis in boiling 11 *M* hydrochloric acid for 22 hours to give thiazolecarboxylic acid **11** [21].

To date, all of the thiostrepton degradation studies have been conducted under harsh conditions and yielded small fragments and amino acids, useful in the determination of absolute stereochemistry of the stereogenic centres in the thiopeptide, and to establish the presence of various residues (quinaldic acid, thiostreptine, *etc*.). The intact dehydropiperidine core of the natural product has yet to be isolated from chemical degradation experiments.

RESULTS AND DISCUSSION

In the light of the above degradation studies, a milder approach to the degradation of thiostrepton was sought in order to isolate thiostrepton B (**1b**) (Figure 1), a less biologically active thiopeptide antibiotic lacking the polydehydroalanine residues in the side chain of the natural product. Studies on related thiopeptides provided precedent for the selective cleavage of the dehydroalanine motif. For example, the single dehydroalanine residue in the side chain of promothiocin A (**12**) was successfully cleaved *via* acidic methanolysis using Amberlyte 15 ion exchange resin to give a mixture of promothiocin MO (**13**) and promothiocin MN (**14**) (Fig. 3) [22].

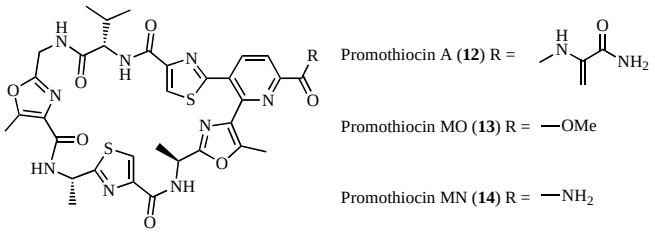
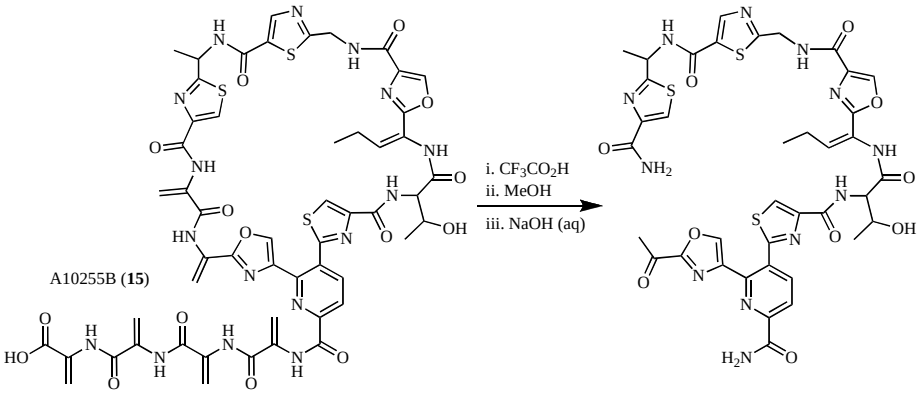


Figure 3. Promothiocin A and the products of acidic methanolysis.

Based on the success of this approach for the cleavage of the promothiocin A side chain, a similar reaction was carried out using thiostrepton. However, no reaction was observed on heating thiostrepton in methanol with Amberlyst 15 ion exchange resin, the product being spectroscopically identical to **1a** (Figure 4a) so another method was sought. It had previously been demonstrated by Debono *et al*. that thiopeptide A10255B (**15**), isolated from *Streptomyces gardneri*, could be selectively cleaved at the dehydroalanine residues by trifluoroacetolysis (Scheme 1) [23].

The trifluoroacetolysis of thiostrepton was carried out by stirring the natural product in neat TFA under nitrogen for 18 h, following the conditions employed successfully in the dehydroalanine cleavage of A10255B. However, instead of isolating thiostrepton B (**1b**), pyridine **16** (Figure 4b) was obtained in 75% yield after purification on silica (Figure 4c).

Scheme 1



Trifluoroacetolysis of A10255B

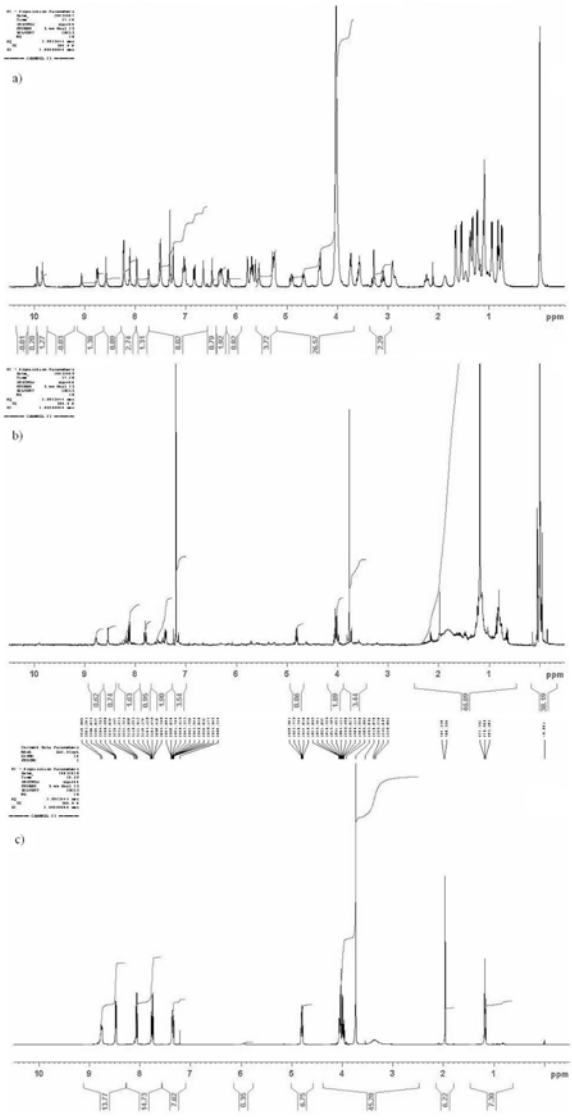
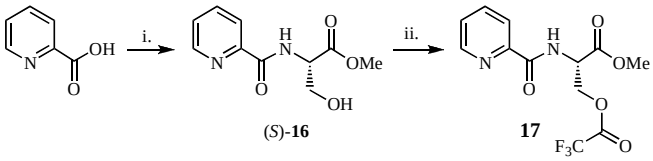


Figure 4. ^{1}H-NMR spectra of a) thiostrepton; b) crude pyridine **16**; c) pyridine **16** after purification on silica. Spectra a) and b) recorded in $CDCl_3$–CD_3OD (4:1), as used by Floss *et al.* to improve the solubility of thiostrepton; spectrum c) recorded in $CDCl_3$.[24]

The mechanism by which pyridine **16** was formed from thiostrepton is as yet unknown, although the likely sources are the dehydropiperidine core, the dehydroalanine-containing residues in the side chain or the terminal thiazole which on hydrolysis could react with a neighbouring dehydroalanine residue. The absence of any unmodified serine residues in the natural product suggests its formation by either thiazole hydrolysis or hydration of a dehydroalanine residue, possibly during the work-up. It is likely that the observed methyl ester was also formed during the work-up from an activated derivative of the corresponding carboxylic acid or amide.

Scheme 2



Synthesis of picoline derivative **16**.

Reagents and conditions. i. NMM, *i*BuOCOCl, THF, 0 °C, 30 min then (*S*)-Ser-OMe.HCl, 0 °C, 1 h, 75%; ii. TFAA, r.t., 72 h, 50%.

The divergent behaviour of thiostrepton under trifluoroacetolysis was attributed to the increased instability of the dehydropiperidine domain as compared to the series *d* domain of pyridine-containing thiopeptide antibiotics under the reaction conditions.

To confirm the identity of degradation product **16**, synthetic (*S*)-**16** was prepared from picolinic acid and (*S*)-serine methyl ester hydrochloride in a single step (Scheme 2). This product was found to be chromatographically and spectroscopically identical to the material isolated from the thiostrepton trifluoroacetolysis experiment [25]. *O*-Trifluoroacetate picoline derivative **17** was then synthesized to confirm that **16** contained a free hydroxyl group and was not the corresponding trifluoroacetate

ester. This was indeed shown to be the case, as **16** clearly lacks the ^{13}C-^{19}F coupling in the ^{13}C-NMR spectrum.

In conclusion, picoline derivative **16** has been isolated from the trifluoroacetolysis of thiostrepton, and is the first pyridine-containing degradation product isolated from this source.

EXPERIMENTAL

Acidic methanolysis of thiostrepton. To a stirred solution of thiostrepton (100 mg, 60.0 μmol) in methanol (3 ml), dried Amberlyst 15 ion exchange resin (300 mg) was added and the solution heated at reflux for 24 hours. The solution was filtered and the filtrate evaporated *in vacuo* to afford an off-white solid, spectroscopically and chromatographically identical to the starting material.

Trifluoroacetolysis of thiostrepton. Thiostrepton (100 mg, 60.0 μmol) was dissolved in trifluoroacetic acid (3 ml) and the solution stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the residue triturated with diethyl ether (3 x 5 ml). The solid was dissolved in methanol (10 ml), the solution filtered and the filtrate concentrated to half volume. Water (2.5 ml) and 0.1 *M* sodium hydroxide (0.5 ml) were added and the resulting solution concentrated to ¾ volume and filtered. The filtrate was extracted with ethyl acetate (3 x 10 ml) and the combined organic extracts washed with sat. aq. $NaHCO_3$ (15 ml), dried (Na_2SO_4) and evaporated *in vacuo* to afford a pale yellow solid. Purification on silica eluting with ethyl acetate:petroleum ether (2:1) gave *N*-(2-picolinoyl)serine methyl ester (10 mg, 75%) as an off-white solid, mp 56-58 °C ($CHCl_3$); R_f 0.20; $[\alpha]_D^{21}$ -3.0 (*c* 1.3, $CHCl_3$); ir (CH_2Cl_2): NH 3374, OH 3400-3200 br, CO 1740, 1672 cm^{-1}; ^1H-nmr (400 MHz; $CDCl_3$): δ 3.35 (br s, 1H, exch. D_2O, OH), 3.77 (s, 3H, OMe), 4.01 (dd, 1H, C*H*H, J = 3.6, 11.4 Hz), 4.04 (dd, 1 H, CH*H*, J = 4.1, 11.4 Hz), 4.82 (ddd, 1 H, α-H, J = 3.6, 4.1, 8.8 Hz), 7.40 (m, 1H, pyr-5), 7.80 (dt, 1H, pyr-4, J = 1.6, 7.3 Hz), 8.11 (dd, 1H, pyr-3, J = 0.8, 7.3 Hz), 8.53 (dd, 1H, pyr-6, J 1.6, 6.2 Hz), 8.79 (br d, 1H, exch. D_2O, NH, J = 8.8 Hz); ^{13}C nmr (100 MHz; $CDCl_3$): δ 52.8 (CH_3), 54.9 (CH), 63.4 (CH_2), 122.4 (CH), 126.6 (CH), 137.4 (CH), 148.3 (CH), 149.1 (C), 164.7 (C), 170.8 (C); lrms (ES+): m/z 225 (MH^+), 207, 165, 115; hrms (ES+): m/z [MH^+] calcd. for $C_{10}H_{13}N_2O_4$ 225.0870; found 225.0870 [MH^+].

***N*-(2-Picolinoyl)-(*S*)-serine methyl ester (16)**. *N*-methylmorpholine (4.18 ml, 37.3 mmol, 2.3 equiv.) and isobutyl chloroformate (2.33 ml, 17.8 mmol, 1.1 equiv.) were added sequentially to a stirred solution of picolinic acid (2.00 g, 16.2 mmol) in dry THF (30 ml) at 0 °C. The solution was stirred for 30 minutes and (*S*)-Serine methyl ester hydrochloride (2.77 g, 17.8 mmol, 1.1 equiv.) was added. The mixture was stirred for a further hour at 0 °C then partitioned between water (20 ml) and ethyl acetate (20 ml). The aqueous layer was further extracted with ethyl acetate (2 x 10 ml) and the combined organic extracts washed with brine (20 ml), dried (Na_2SO_4) and evaporated *in vacuo* to yield the product. Purification on silica eluting with ethyl acetate:petroleum ether (2:1) afforded the title compound (2.72 g, 75%) as an off-white solid, mp 59 °C ($CHCl_3$); R_f 0.20; $[\alpha]_D^{21}$ +39.4 (*c* 2.5, $CHCl_3$); ir (CH_2Cl_2): NH 3374, OH 3400-3200 br, CO 1740, 1672 cm^{-1}; ^1H-nmr (400 MHz; $CDCl_3$): δ 3.19 (br s, 1H, exch. D_2O, OH), 3.77 (s, 3H, OMe), 4.01 (dd, 1H, C*H*H, J = 3.6, 11.4 Hz), 4.04 (dd, 1 H, CH*H*, J = 4.1, 11.4 Hz), 4.82 (ddd, 1 H, α-H, J = 3.6, 4.1, 8.8 Hz), 7.40 (m, 1H, pyr-5), 7.80 (dt, 1H, pyr-4, J = 1.6, 7.3 Hz), 8.11 (dd, 1H, pyr-3, J = 0.8, 7.3 Hz), 8.53 (dd, 1H, pyr-6, J 1.6, 6.2 Hz), 8.79 (br d, 1H, exch. D_2O, NH, J = 8.8 Hz); ^{13}C nmr (100 MHz; $CDCl_3$): δ 52.8 (CH_3), 54.9 (CH), 63.4 (CH_2), 122.4 (CH), 126.6 (CH), 137.4 (CH), 148.3 (CH), 149.1 (C), 164.7 (C), 170.8 (C); lrms (ES+): m/z 225 (MH^+), 207, 165, 115; hrms (ES+): m/z [MH^+] calcd. for $C_{10}H_{13}N_2O_4$ 225.0870; found 225.0870 [MH^+]. *Anal*. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.51; H, 5.41; N, 12.33.

***N*-(2-Picolinoyl)-*O*-trifluoroacetyl-(*S*)-serine methyl ester (17)**. *N*-(2-Picolinoyl)-(*S*)-serine methyl ester (0.224 g, 1.00 mmol) was dissolved in trifluoroacetic anhydride (5 ml) and the solution stirred at room temperature for 72 hours. The solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (10 ml). The solution was washed sequentially with sat. aq. $NaHCO_3$ (10 ml), water (10 ml) and brine (10 ml), dried (Na_2SO_4) and evaporated *in vacuo* to yield the crude product as a yellow-brown oil. Purification on silica eluting with ethyl acetate:petroleum ether (2:1) gave the title compound (0.16 g, 50%) as a yellow oil; R_f 0.45; $[\alpha]_D^{21}$ +28.8 (*c* 2.5, $CHCl_3$); ir (film) NH 3101, CO 1730, 1672 cm^{-1}; ^1H-nmr (400 MHz; $CDCl_3$) 3.77 (s, 3H, OMe), 4.72 (dd, 1H, C*H*H, J = 3.9, 11.3 Hz), 4.77 (dd, 1H, CH*H*, J = 4.2, 11.3 MHz), 5.09 (m, 1H, α-CH), 7.43 (m, 1H, pyr-5), 7.82 (dt, 1H, pyr-4, J = 1.6, 7.7 Hz), 8.11 (d, 1H, pyr-3, J = 7.7 Hz), 8.55 (d, 1H, pyr-6, J = 4.5 Hz), 8.74 (br d, 1H, exch. D_2O, NH, J = 7.5 Hz); ^{13}C-nmr (100 MHz; $CDCl_3$) 51.83 (CH_3), 53.3 (CH), 66.8 (CH_2), 114.3 (q, C, $^1J_{C\text{-}F}$ = 283 Hz), 122.6 (CH), 127.0 (CH), 137.7 (CH), 148.0 (C), 148.4 (CH), 157.0 (q, C, $^2J_{C\text{-}F}$ = 42.9 Hz), 164.7 (C), 168.6 (C); lrms (CI+): m/z 320 (M^+), 207, 195, 177, 149, 127, 99, 75, 61.

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[25] Spectroscopically identical with the exception of the optical rotation. Picoline **16** isolated from thiostrepton had an optical rotation of -3.0 compared to +39.4 for the synthetic material prepared from (*S*)-serine methyl ester hydrochloride.