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# The Structures of Thiolutin and Aureothricin, Antibiotics Containing a Unique Pyrrolinonodithiole Nucleus

BY WALTER D. CELMER AND I. A. SOLOMONS

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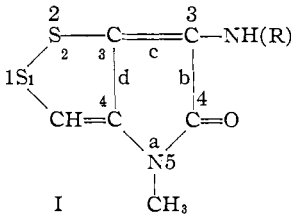
Thioulutin (Ia) and aureothricin (Ib) are shown to be acetamido and propionamido derivatives, respectively, of 3-amino-5-methylpyrrolin-4-ono-(4,3-d)-1,2-dithiole (Ic).

Thiolutin[1] and aureothricin[2] are similar yellow crystalline, sulfur-containing antibiotics isolated from among the elaboration products of different *Streptomyces* species. Both substances are of interest because of their high activity against a variety of fungi, ameboid parasites, Gram-positive, Gram-negative, and acid fast bacteria.[1-3]

Celmer, *et al.*,[4] previously characterized Thiolutin and aureothricin as acetamido and propionamido derivatives, respectively, of a common hydrolysis product, $C_6H_6N_2OS_2$.

The purpose of this paper is to report studies on the antibiotic Thiolutin (Ia) which led to the elucidation of its structure as the 3-acetamido derivative of 3-amino-5-methylpyrrolin-4-ono-(4,3-d)-1,2-dithiole (Ic). The related antibiotic aureothricin (Ib) is incidentally shown to be the 3-propionamido derivative of Ic.[5]



I

a, R = $CH_3CO$–
b, R = $CH_3CH_2CO$–
c, R = H

Thiolutin, $C_8H_8N_2O_2S_2$, is a neutral, optically inactive substance which is remarkably thermostable and sublimes without decomposition when heated *in vacuo*. Both of its oxygen and nitrogen atoms are associated with amide linkages, one characterized as acetamido and the other as part of a pyrrolinono ring. The infrared spectrum of Thiolutin (Fig. 1) exhibits carbonyl bands at 6.00 and 6.12 $\mu$, NH stretching and bending absorption at 3.12 and 6.45 $\mu$, respectively, and strong C=C stretching

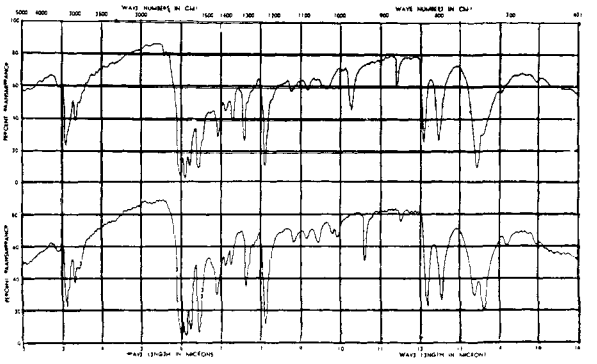


Fig. 1.—Infrared absorption spectra of Thiolutin (upper) and aureothricin (lower) determined in potassium bromide pellets.

absorption at 6.25 $\mu$ which are consistent with these functional assignments. Thiolutin exhibits an ultraviolet absorption spectrum (Fig. 2) characterized by a dominant maximum at 388 m$\mu$ ($E^{1\%}_{1\text{ cm.}}$ 480, $\epsilon$ 11,000); the base of this peak extends into the visible spectrum which accounts for the yellow color of this antibiotic.

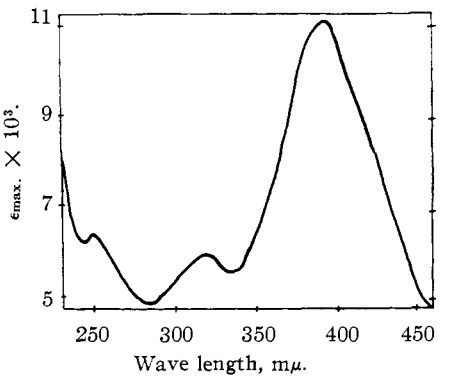



Fig. 2.—Ultraviolet absorption spectrum of Thiolutin in methanol solution.

As the result of a series of qualitative tests,[6] the sulfur in Thiolutin was suspected of existing as a disulfide bond attached to unsaturated carbon. The synthetic compound dithiobenzoyl (i),[7,9] which contains a disulfide group attached to unsaturated carbon, was prepared and its properties compared to those of Thiolutin. This model and Thiolutin failed to give Grote's cyanide-nitroprusside disul-

(1) F. W. Tanner, Jr., J. A. Means and J. W. Davisson, Abstracts 118th Meeting, American Chemical Society, September 7-8, 1950.

(2) H. Umezawa, K. Maeda and H. Kosaka, *Japanese Medical J.*, **1**, 512 (1948).

(3) H. Seneca, J. H. Kane and J. Rockenbach, *Antibodies and Chemotherapy*, **2**, 357 (1952).

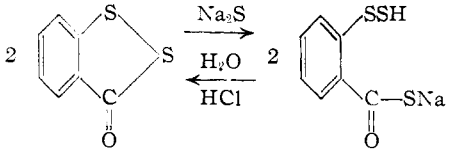
(4) (a) W. D. Celmer, F. W. Tanner, Jr., M. Harfenist, T. M. Lees and I. A. Solomons, THIS JOURNAL, **74**, 6304 (1952); (b) W. D. Celmer and I. A. Solomons, *Antibiotics Annual*, 622 (1953-1954).

(5) (a) We are indebted to Mr. H. Oatfield for suggesting this systematic name. The authors propose the generic name "pyrrothine" for the antibiotic nucleus Ic; terms such as acetopyrrothine and propiopyrrothine would then chemically differentiate Thiolutin and aureothricin. (b) It is of interest that Thiolutin and aureothricin are the first recognized examples of microbiologically active unsaturated lactams. Antibiotics containing unsaturated lactone structures have been previously characterized; *i.e.*, protoanemonin and patulin. *Cf.* Y. Asahina and A. Fujita, *Acta Phytochim. (Japan)*, **1**, 1 (1922); R. B. Woodward and G. Singh, THIS JOURNAL, **71**, 758 (1949).

(6) Thiolutin failed to give tests for reactive sulfur groups such as thiocarbonyl or thiol with nitroprusside (*cf.* Grote, *J. Biol. Chem.*, **93**, 25 (1931)) or selenious acid (*cf.* Werner, *Sci. Proc. Roy. Dublin Soc.*, **22**, 387 (1941)).

(7) S. Smiles and E. W. McClelland, *J. Chem. Soc.*, **121**, 89 (1922).

fide test.[8] Vigorous reducing agents, such as a zinc–hydrochloric acid, liberated hydrogen sulfide from both Thiolutin and dithiobenzoyl. Furthermore, both compounds displayed the unusual property of dissolving in aqueous sodium bisulfide or sodium sulfide solution and precipitating intact following acidification. Evidence that a chemical reaction actually occurred with Thiolutin was furnished by the feeble antibiotic activity and radically different ultraviolet absorption ($\lambda_{max.}$ 360 m$\mu$, $E^{1\%}_{1\ cm.}$ 1085) of its sulfide solution.[9]




i ii

The reaction of Thiolutin with refluxing 20% aqueous sodium hydroxide resulted in extensive degradation and methylamine, ammonia and acetic acid were isolated and identified from among the products. A Friederich N-alkyl determination on the intact antibiotic confirmed the presence of an N-methyl group as such. Hydrolysis of Thiolutin in a two phase dioxane–hydrochloric acid system gave a weak amine, "pyrothine," $C_6H_6N_2OS_2$ (Ic), isolated as the crystalline monohydrochloride salt ($pK_a$ 2.9). The same product was isolated following acid hydrolysis of aureothricin. Reaction of acetic or propionic anhydride with pyrrothine regenerated Thiolutin or aureothricin, respectively, which established the relationships of these two antibiotics and precluded the possibility of rearrangement during their acid hydrolysis.[4,10]

The properties of pyrrothine, such as low basicity, characteristic red color test with *glutaconic* aldehyde[11] and the formation of an acidic benzenesulfonamido derivative[4b] showed the presence of a primary vinyl amine, C=C—$NH_2$. Kuhn-Roth determinations on Thiolutin and pyrrothine disclosed one C-methyl group in the intact antibiotic but none in its deacetylated compound. The infrared spectrum of pyrrothine hydrochloride exhibited a single carbonyl absorption peak at 6.1 $\mu$ which was associated with a conjugated carboxamido group (Fig. 3). Efforts to characterize this remaining carboxamido function by hydrolytic means yielded greenish-brown intractable resins.

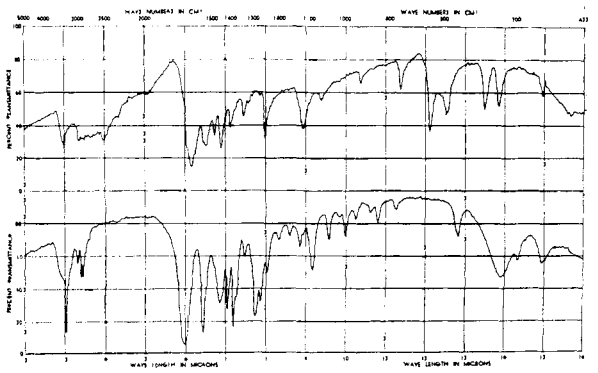


Fig. 3.—Infrared absorption spectra of pyrrothine hydrochloride hydrate (upper) and desthiolutin (DL-3-acetamido-1,5-dimethyl-2-pyrrolidone) (lower) determined in potassium bromide pellets.

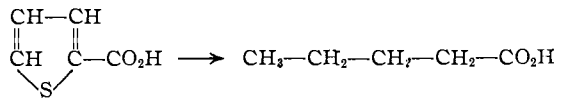
Desulfurization of Thiolutin with Raney nickel in ethanol solution afforded a white, homogeneous, crystalline product "desthiolutin," $C_6H_{14}N_2O_2$. The relative compositions of starting material and product indicated that in addition to replacement of the sulfur by hydrogen during the conversion of Thiolutin to desthiolutin, two olefinic bonds were saturated.[12]

Like Thiolutin, desthiolutin is a neutral, optically inactive substance whose infrared spectrum (Fig. 3) disclosed strong carboxamido absorption.[13] It also contains an N-methyl group. However, colorless desthiolutin exhibits no ultraviolet absorption maxima above 220 m$\mu$ and is devoid of antibiotic activity.

Hydrolysis of desthiolutin afforded the key to its structure. Cleavage of the acetamido linkage, accomplished in dioxane–hydrochloric acid, gave a sirupy hydrochloride salt of an amine, $C_6H_{12}N_2O$ (IIb), which was characterized as its crystalline picrate salt ($pK_a$ 7.4).

The free base of IIb was obtained as a colorless oil which was readily reconverted to crystalline desthiolutin by acylation with acetic anhydride. Compound IIb contained a saturated, primary amino group, evidenced by its relatively strong basicity and quantitative liberation of nitrogen by the Van Slyke nitrous acid reaction. Spectrographic evidence demonstrated the presence of a remaining carboxamido grouping in IIb and more vigorous hydrolytic means were sought to bring about its cleavage.

Treatment of desthiolutin or IIb with 20% hydrochloric acid in a sealed tube at 150° for several hours yielded a diamino acid, $C_6H_{14}N_2O_2$ (III), isolated as its crystalline dihydrochloride salt. Thus, a lactam structure was present in desthiolutin and its partially hydrolyzed compound IIb. The relative stability of this substance toward acid hydroly-




iii iv

(8) Grote (ref. 6) reported that ordinary disulfides were reduced by cyanide to substances which could then be detected with his nitroprusside reagent. He observed, however, that aryl disulfides, such as di-*p*-tolyl disulfide, failed to react.

(9) It appears possible that the disulfide linkage in Thiolutin opens in sulfide solution to form acid-unstable thiosulfenic acid derivatives in a manner similar to that postulated for dithiobenzoyl by Tarbell and Harnish (*Chem. Revs.*, **49**, 14 (1951)).

(10) Reduction of pyrrothine with zinc–hydrochloric acid liberated two moles of hydrogen sulfide much more rapidly than Thiolutin, presumably because of greater solubility in the reaction mixture.

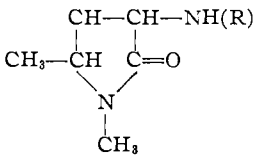
(11) F. Feigl, V. Anger and R. Zappert, *Mikrochem.*, **16**, 74 (1934)

(12) Reduction of thiophene-2-carboxylic acid (iii) to *n*-valeric acid (iv) by Raney nickel represents an analogous reaction (*cf.* F. F. Blicke and D. G. Sheets, THIS JOURNAL, **71**, 4010 (1949)):

(13) Comparison of the infrared spectra of Thiolutin and desthiolutin determined in potassium bromide suspension (Fig. 1 and 3 respectively) reveals that the 6.25 $\mu$ band assigned to C=C in Fig. 1 is absent in Fig. 3. Significantly, the 6.12 $\mu$ band associated with a conjugated carboxamido group in Fig. 1 is shifted to a lower wave length to give broad absorption at 6.0 $\mu$ in Fig. 3 which is actually two carbonyl bands, clearly resolvable by redetermining the spectrum in dilute dioxane solution.

sis indicated it to be a five- or six-membered lactam ring. However, the presence of a C-methyl group and an N-methyl group in the deacetylated product established a five-membered ring in desthiolutin.

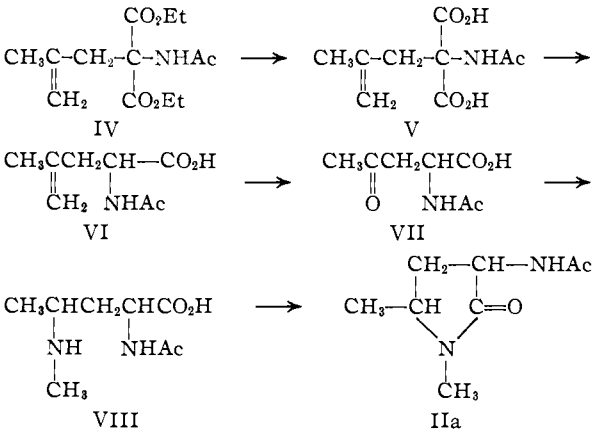
The diamino acid gave a positive ninhydrin $\alpha$-amino acid test. Several possible structures could be expressed for this compound: however, only DL-$\alpha$-amino-$\gamma$-N-methylaminovaleric acid, $CH_3$–$CHNHCH_3$–$CH_2$–$CHNH_2$–$CO_2H$ (III), received serious consideration. It was reasoned that the lactam precursor of the diamino acid would have to accommodate two double bonds in the original Thiolutin molecule; *cf.* moiety IX. This prerequisite was satisfied by only one possibility, illustrated in structure II, and led to the characterization of desthiolutin as 3-acetamido-1,5-dimethyl-2-pyrrolidone (IIa).



IIa, R = $CH_3CO$
b, R = H

The validity of these structural deductions was confirmed by actual synthesis of desthiolutin (IIa), by the route outlined in Chart I.

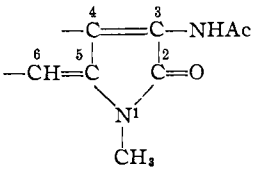
CHART I



Each step of the synthesis gave crystalline substances in good yield.[14] Synthetic IIa, after several recrystallizations from carbon tetrachloride, was obtained in a form (theoretically, two stereoisomers exist) identical with desthiolutin in respect to melting point, mixed melting point and infrared spectrum.

The proven structure of the Raney nickel reduction product desthiolutin (IIa) establishes the relative positions of all of the carbon, hydrogen and oxygen in Thiolutin. Placement of two double bonds and two sulfur atoms in IIa would then satisfy all of the compositional requirements of the antibiotic. The hypothetical moiety IX illustrates the only possible arrangement of a diene containing the same ring nucleus as IIa and serves to explain the lack of optical activity of Thiolutin and the racemic nature of desthiolutin.

(14) Compound VIII was the only intermediate which did not exhibit a sharp melting point, a fact which is probably due to the presence of a mixture of stereoisomers.



IX

Further consideration of IX dictates carbon atoms 4 and 6 as the only possible sites for the attachment of the two sulfur atoms. A disulfide bond bridging these positions is in agreement with the observed chemical and physical properties.[15] Thus, a unique pyrrolinono-1,2-dithiole structure is defined for Thiolutin (Ia).

## Experimental[16]

**Crystalline Thiolutin.**—Filtered fermentation broths of a strain of *Streptomyces albus* were extracted with *n*-butanol and the solvent fraction partially evaporated *in vacuo* until the crystallization of crude Thiolutin was induced. The crystals were recovered by filtration and recrystallized from *n*-butanol to yield a practically pure product.[1] For analytical purposes, the antibiotic was recrystallized from dimethylformamide and dried to constant weight at 100° *in vacuo*. The crystals were obtained as bright yellow felted needles which decomposed over a range of 260 to 270°. Thiolutin sublimed quantitatively at a bath temperature of 200° (0.1 mm.). The neutral character of Thiolutin was indicated by the ability of water-immiscible solvents to extract the antibiotic from aqueous solutions at *p*H values from 2 to 11. Group analyses indicated that Thiolutin contained one C-methyl group and one N-methyl group. Its ultraviolet spectrum exhibited the following absorption: $\lambda_{max.}$ 250 m$\mu$, $\epsilon$ 6300; $\lambda_{max.}$ 311 m$\mu$, $\epsilon$ 5700; $\lambda_{max.}$ 388 m$\mu$, $\epsilon$ 11,000.

*Anal.* Calcd. for $C_8H_8N_2O_2S_2$: C, 42.09; H, 3.53; N, 12.28; S, 28.07; C-$CH_3$(1), 6.58; N-$CH_3$(1), 6.58. Found: C, 42.12; H, 3.77; N, 12.19; S, 27.72; C-$CH_3$, 6.50; N-$CH_3$, 5.06.

Direct molecular weight determinations on Thiolutin by either the Rast or Signer methods were extremely variable. The molecular formula $C_8H_8N_2O_2S_2$ (mol. wt., 228) was established by the fact that acid hydrolysis yielded a $C_2$ (acetic acid) compound and a $C_6$ (pyrrothine) compound. Moreover, desulfurization of Thiolutin yielded desthiolutin, $C_8H_{14}N_2O_2$, whose constitution was confirmed by synthesis.

**Alkaline Degradation of Thiolutin.**[17]—A solution of sodium hydroxide (40 g.) in water (200 ml.) was placed in a 3-necked stainless steel flask and Thiolutin (2.001 g., 8.77 mmoles) was added. The solution was boiled while a slow stream of nitrogen was passed over the surface of the liquid and out of the apparatus through a reflux condenser sur-

(15) Notable efforts have been made attempting to relate the structure of organic sulfur compounds with their ultraviolet absorption spectra (*cf.* E. A. Fehnel and M. Carmack, THIS JOURNAL, **71**, 84, 231, 2889 (1949); **72**, 1292 (1950)). However, spectra-structure correlations in this field have not reached the degree of certainty desired for rigorous structure elucidation of natural products containing complex sulfur linkages. Several pertinent observations have been made, however, which have bearing on the subject on hand. Significantly, the disulfide bond is a potent chromophore in its own right. Compounds containing this function will absorb over a wide wave length range, depending on substitution. For example, dialkyl disulfides, tetramethylene disulfides and trimethylene disulfides (*i.e.*, lipoic acid) exhibit long wave length maxima at approximately 250, 286 and 340 m$\mu$, respectively (*cf.* M. Calvin and J. A. Barltrop, THIS JOURNAL, **74**, 6153 (1952)). The disulfide bond placed into conjugation with an existing chromophore exerts a pronounced bathochromic influence exemplified by the spectra of salicylic acid, thiosalicylic acid and dithiobenzoyl which exhibit long wave length maxima at 302, 310 and 355 m$\mu$, respectively. It is reasoned, therefore, that the observed ultraviolet absorption spectrum of Thiolutin is compatible with a structure which involves active chromophoric participation of a disulfide bond.

(16) Melting points determined on a Kofler apparatus. Light absorption determined in methanol solution on a Carey spectrophotometer.

(17) We wish to acknowledge the contributions of Dr. M. Harfenist in this experiment.

mounted by a spray trap. The gas was passed through two traps in series, each containing standardized aqueous hydrochloric acid. After 22 hours, titration of an aliquot revealed that 0.81 mole of volatile base per mole of antibiotic had been absorbed in the first trap and essentially none in the second. (Increasing the time of boiling to 42 hours gave 0.96 mole of volatile base per mole of antibiotic.)

The contents of the first trap was evaporated and a portion of the resulting white solid was triturated with acetic acid to give cubic, isotropic crystals of ammonium chloride, identified by elemental analyses and index of refraction.

*Anal.* Calcd. for $NH_4Cl$: N, 26.19; H, 7.43; Cl, 66.28; $n_D$, 1.639. Found: N, 25.70; H, 7.52; Cl, 65.45; C, 0.51; $n_D$, 1.638.

Another portion of the residue was dissolved in warm anhydrous ethanol and fractions were precipitated by the addition of increasing amounts of benzene. After essentially all of the ammonium chloride had precipitated, the remaining solution was evaporated, leaving a residue of methylammonium chloride, m.p. 226°, identified by its melting point, undepressed mixed melting point with a standard sample and conversion to N-methylbenzamide m.p. 80°, undepressed by admixture with a known sample.

An aliquot of the original alkaline solution was acidified and the liberated hydrogen sulfide measured in lead acetate solution amounted to only 0.03 mole per mole of antibiotic. Another portion of the alkaline solution was acidified with sulfuric acid and on distillation yielded a distillate containing 0.80 mole of volatile acid per mole of Thiolutin. The product was identified as acetic acid by preparation of the *p*-acetotoluidide m.p. 147° undepressed by admixture with a known sample.

**Acid Degradation of Thiolutin and Aureothricin; Isolation of Pyrrothine.**—The following procedure was scaled up a thousand-fold without appreciable effect on the yield of pyrrothine. A suspension of Thiolutin (0.020 g.) in purified dioxane (1 ml.) was heated to reflux temperature and then treated with concentrated hydrochloric acid (0.2 ml.). Two phases formed and within a few minutes the Thiolutin was completely in solution and the reaction mixture changed from clear orange to greenish-brown in color. After ten minutes, rust colored crystalline pyrrothine hydrochloride separated from the lower acid phase. After thirty minutes, the reaction mixture was cooled and 0.012 g. of the product was recovered by filtration. Recrystallization from dilute hydrochloric acid gave hydrated yellow prisms which decomposed with sintering at approximately 200°.

The above experiment repeated with a sample of aureothricin[18] yielded the same product in comparable yield. The ultraviolet spectrum of pyrrothine hydrochloride hydrate exhibited the following absorption: $\lambda_{infl.}$ 229 mμ, ε 5400; $\lambda_{max.}$ 309 mμ, ε 6100; $\lambda_{max.}$ 381 mμ, ε 11,000.

*Anal.* Calcd. for $C_6H_6N_2OS_2 \cdot HCl \cdot H_2O$: C, 29.94; H, 3.77; N, 11.64; S, 26.62; Cl, 14.72; $H_2O$, 7.49; N-$CH_3$(1), 6.58; neut. equiv., 240.7. Found: C, 30.22; H, 3.86; N, 11.67; S, 26.86; Cl, 15.06; $H_2O$, 7.62; N-$CH_3$, 5.06; C-$CH_3$, 0.0; neut. equiv., 239.5.

The free base of pyrrothine was prepared as an olive-colored precipitate, m.p. 191–195° dec., following addition of excess ammonia to an aqueous solution of the hydrochloride salt.

*Anal.* Calcd. for $C_6H_6N_2OS_2$: C, 38.70; H, 3.23. Found: C, 38.62; H, 3.76.

The molecular formula of pyrrothine was established as $C_6H_6N_2OS_2$ (mol. wt., 186) instead of some multiple value by conversion to derivatives which gave reproducible molecular weight values. For example, the ε-carbomethoxycaproamido derivative of pyrrothine was especially suitable for the Rast determinations since it melted sharply without decomposition and was very soluble in camphor.

**ε-Carbomethoxycaproamido Pyrrothine.**—ε-Carbomethoxycaproyl chloride (3 g.) was added to a suspension of pyrrothine hydrochloride hydrate (0.5 g.) in chloroform (25 ml.) containing pyridine (1 ml.). The reaction mixture was agitated until a clear solution resulted (10 minutes). Two volumes of hexane were added and the supernatant solution was rapidly decanted from a resulting sirupy pyridine hydrochloride layer. After standing a few minutes, the product crystallized from the decanted solution as bright yellow needles (0.5 g.), m.p. 162–163.5°. The product recrystallized from chloroform–hexane, m.p. 163.5–164.0°, was used for analyses.

*Anal.* Calcd. for $C_{14}H_{18}N_2O_4S_2$: C, 49.11; H, 5.30; N, 8.18; S, 18.72; mol. wt., 342. Found: C, 49.51; H, 5.45; N, 7.71; S, 18.99; mol. wt., 339, 344.

**Thiolutin from Pyrrothine.**—A stirred solution of pyrrothine hydrochloride hydrate (0.5 g.) in water (50 ml.) was treated with an excess of acetic anhydride (5 g.). Within a few moments a mass of yellow crystals separated from solution. After standing ten minutes, the crystals were recovered by filtration. The product (0.55 g.) was identified as Thiolutin by its melting point, infrared spectrum, papergram migration and antibiotic activity.

**Aureothricin from Pyrrothine.**—The above procedure was repeated using propionic anhydride as the acylating agent. The product obtained was identified as aureothricin by comparison with an authentic sample.[18] Light absorption: $\lambda_{max.}$ 248 mμ, ε 6100; $\lambda_{max.}$ 312 mμ, ε 3900; $\lambda_{max.}$ 388 mμ, ε 11,000.

*Anal.* Calcd. for $C_9H_{10}N_2O_2S_2$: C, 44.61; H, 4.16; N, 11.56; S, 26.40. Found: C, 44.97; H, 4.12; N, 11.54; S, 26.40.

Aureothricin and Thiolutin are most readily identified (and differentiated) by means of their infrared spectra. Thiolutin was characterized by two similar, sharp bands at 12.1 and 12.5 μ, a dominantly, strong band at 13.45 μ and a partially resolved band at 13.6 μ. Aureothricin exhibited two similar, sharp bands at 12.2 and 12.6 μ, a partially resolved band at 13.45 μ and a dominantly strong band at 13.6 μ (*cf.* Fig. 1).

**Zinc–Hydrochloric Acid Reduction of Pyrrothine.**—A solution of pyrrothine hydrochloride hydrate (1.200 g., 5 mequiv.) in water (25 ml.) was placed in a flask equipped with a sealed stirrer, a dropping funnel, a nitrogen inlet tube and a gas delivery tube leading into standard iodine solution. Zinc dust (2.5 g.) was added to the solution and stirred while concentrated hydrochloric acid (10 ml.) was added dropwise. A slow stream of nitrogen was passed over the reaction mixture to sweep out the hydrogen sulfide which was generated immediately after the addition of acid. After one hour, a total of 9.31 mequiv. of hydrogen sulfide had been trapped which represented 93.1% of the sulfur in pyrrothine.

**Thiolutin–Sodium Bisulfide Solution.**—Thiolutin (100 mg.) in aqueous sodium bisulfide reagent (10 cc., C.P. Baker, 9% available $H_2S$) formed a clear yellow solution which exhibited an ultraviolet absorption peak at 360 mμ, $E^{1\%}_{1cm.}$ 1085. This solution retained only 11% of the microbiological activity expected of its Thiolutin content. Acidification regenerated full antibiotic activity in the form of pure crystalline Thiolutin which precipitated in practically quantitative yield.

**Desthiolutin** (IIa).[17]—A stirred suspension of Thiolutin (50 g., 0.219 mole) in 2B ethanol (1 l.) was heated to boiling. An ethanolic Raney nickel suspension (900 ml.) containing 350 g. of the catalyst was added. A stream of hydrogen was then bubbled into the stirred, refluxing reaction solution. (The addition of hydrogen was not essential for the preparation of desthiolutin but appeared to improve the yield and quality of the product.) After three hours the catalyst was removed by filtration and the colorless filtrate evaporated to dryness *in vacuo*. The crystalline residue, 27.7 g. (0.154 mole, 70% yield) was recrystallized from carbon tetrachloride (1 l.); yield 20 g., m.p. 126–127.5°. Additional recrystallizations from carbon tetrachloride elevated the melting point to a constant value of 132.0–132.8°. Desthiolutin was optically inactive and exhibited no ultraviolet absorption maxima above 220 mμ. Potentiometric titration revealed no acidic or basic groups.

*Anal.* Calcd. for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46; N-$CH_3$(1), 8.96; mol. wt., 170. Found: C, 56.78; H, 8.24; N, 16.19; N-$CH_3$, 7.15; mol. wt. (Rast), 187, (Signer) 160.

**Hydrolysis of Desthiolutin.** (a) **DL-3-Amino-1,5-dimethyl-2-pyrrolidone** (IIb).—A solution of desthiolutin (2 g.) in hot dioxane (20 ml.) was treated with concentrated hydrochloric acid (5 ml.) and refluxed for 2 hours. After cooling, the lower phase was collected and evaporated to dryness *in vacuo*. A viscous, sirupy residue remained. Addition of cold 40% potassium hydroxide solution followed

(18) A sample of aureothricin was obtained through the courtesy of Dr. Umezawa.

by ether extraction gave a free amine as an oil which was purified by distillation, b.p. 68° at 0.2 mm., $n^{26}D$ 1.4835.

*Anal.* Calcd. for $C_6H_{12}N_2O$: C, 56.22; H, 9.44. Found: C, 55.94; H, 9.59.

Acylation of IIb with acetic anhydride gave desthiolutin, m.p. 131–132°, identified by melting point, mixed melting point and infrared spectrum. The amine IIb could be purified and characterized more conveniently by means of its crystalline picrate salt, m.p. 195–196 (darkened at 188°).

*Anal.* Calcd. for $C_6H_{12}N_2O \cdot C_6H_3N_3O_7$: C, 40.34; N, 4.23; N, 19.60; C–$CH_3$(1), 4.21; neut. equiv., 357.3. Found: C, 40.37; H, 4.27; N, 19.62; C–$CH_3$, 2.78; neut. equiv., 356; $pK_a$, 7.4.

(b) DL-α-Amino-γ-N-methylaminovaleric Acid.—A solution of desthiolutin (3 g.) in 20% hydrochloric acid (9 ml.) was placed in a heavy walled Pyrex tube which was then sealed. The tube was heated to 150–170° for 3 hours, cooled and opened. The contents were evaporated to dryness *in vacuo*. The residue, a hygroscopic solid, was dissolved in ethanol (50 ml.) and treated with acetone (100 ml.) to induce crystallization. After 24 hours well-formed colorless crystals were recovered (0.8 g.), m.p. 185–187° (decomposed with gas evolution).

*Anal.* Calcd. for $C_6H_{14}N_2O_2 \cdot 2HCl$: C, 32.89; H, 7.36; N, 12.79; Cl, 32.35; C–$CH_3$(1), 6.86. Found: C, 33.07; H, 7.28; N, 12.17; Cl, 32.36; C–$CH_3$(1), 2.02.

**Synthesis of Desthiolutin.** (a) **Ethyl Acetamidomethylallylmalonate (IV).**—A modified method of Albertson and Archer[19] was used. Ethyl acetamidomalonate (109 g.) was added to refluxing ethanolic sodium ethoxide (1 l., from 11.5 g. of sodium). Methallyl bromide (82.6 g.) was added over a period of 15 minutes and refluxing was continued for 2 hours longer. The reaction mixture was then evaporated under reduced pressure. The residue was extracted with chloroform (400 ml.) and filtered. The filtrate was evaporated to give IV as a colorless crystalline solid 128 g., m.p. 92–95°. Recrystallization of IV from water gave a product with a slightly higher melting point, 95–96°, lit.[19] m.p. 92–93°.

(b) **Acetamidomethallylmalonic Acid (V).**—A solution of IV (128 g.) in ethanol (300 ml.) was treated with 5 *N* aqueous sodium hydroxide (650 ml.) and heated under reflux for 30 minutes. The saponification mixture was then concentrated *in vacuo* to remove the ethanol, cooled (ice-water bath) and cautiously acidified with concentrated hydrochloric acid (250 ml.). The resulting crystals were collected and dissolved in acetone (400 ml.), filtered to remove a small amount of sodium chloride and evaporated *in vacuo* yielding a crystalline residue of V (73 g.), m.p. 127–128°. A sample of V spontaneously decarboxylated during 7 days standing at room temperature.

(c) DL-2-Acetamido-4-pentenoic Acid (VI).—A sample of V (72.6 g.) was heated at 155–158° (oil-bath) for 30 minutes, after which time carbon dioxide evolution had ceased. The solid, crystalline cake weighed 56.4 g. A chloroform (200 ml.) extraction removed an oily impurity and left 35.4 g. of VI, m.p. 150–155°. Another 11.7 g. of VI, m.p. 147–150°, was recovered from the chloroform by precipitation with hexane. For analytical purposes, a sample of VI was recrystallized from ethyl acetate, m.p. 158–159°.

*Anal.* Calcd. for $C_6H_{13}NO_3$: C, 56.13; H, 7.65. Found: C, 56.12; H, 7.72.

(d) DL-α-Acetamidolevulinic Acid (VII).—A cooled (ice-water bath) solution of VI (17.1 g.) in absolute ethanol (100 ml.) was treated with a stream of ozonized oxygen (0.78 mequiv. of ozone per minute) for 150 minutes. After aeration to remove excess ozone, the solution was placed into a standard Parr hydrogenation bottle containing palladium-on-charcoal catalyst (5 g.). The bomb was shaken for 75 minutes under hydrogen pressure (25 p.s.i.) at 25°. The reaction mixture was filtered to remove the catalyst and the filtrate was evaporated *in vacuo*. The sirupy residue was dissolved in ethyl acetate (100 ml.) and partially evaporated to induce crystallization of VII (9 g.), m.p. 124–125.5°, recrystallized from ethyl acetate, m.p. 125.5–126.5°.

*Anal.* Calcd. for $C_7H_{11}NO_4$: C, 48.55; H, 6.40. Found: C, 48.10; H, 6.46.

The 2,4-dinitrophenylhydrazone derivative of VII was obtained as yellow needles, m.p. 156–158° dec.

*Anal.* Calcd. for $C_{13}H_{15}N_5O_7$: C, 44.19; H, 4.28. Found: C, 44.22; H, 4.48.

A solution of VII (0.8 g.) in 20% hydrochloric acid (10 ml.) was heated at reflux temperature for 2 hours. The solution was evaporated and the residue was recrystallized from alcohol–acetone, m.p. 156–158°; lit.[20] m.p. 159–160° for DL-α-aminolevulinic acid hydrochloride (DL-β-acetylalanine hydrochloride).

(e) DL-α-Acetamido-γ-N-methylaminovaleric Acid (VIII).—A solution of VII (8.65 g.) in ethanol (50 ml.) in a cold (ice-water bath) stainless steel bomb (300-ml. capacity) was treated with 10.5 *N* ethanolic methylamine solution (100 ml.) at a rate which avoided a reaction temperature above 20°. Palladium-on-charcoal catalyst (3 g.) was added and the bomb was shaken in a standard autoclave for 3 hours at 75° under hydrogen pressure (1200 p.s.i.). The catalyst was removed by filtration and the solution evaporated *in vacuo*. The product VIII was obtained as a white waxy solid (8 g.) which melted over a wide range (80–100°), presumably because it consisted of a mixture of stereoisomers. Its infrared spectrum was consistent with structure VIII.

(f) DL-3-Acetamido-1,5-dimethyl-2-pyrrolidone (IIa).—A sample of VIII (1.0 g.) was placed in a short path distillation apparatus and heated *in vacuo*. A colorless distillate (0.5 g.) was obtained, b.p. 145° at 1.2 mm., which solidified on standing. Recrystallization from carbon tetrachloride (30 ml.) gave IIa (0.27 g.) m.p. 123–127°. Two additional recrystallizations from the same solvent gave the product, m.p. 131.2–132.4°, identical with desthiolutin (mixed melting point, 131.5–132.5°).

*Anal.* Calcd. for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46. Found: C, 56.59; H, 8.29; N, 16.27.

Synthetic IIa and desthiolutin exhibited indistinguishable infrared spectra (*cf.* Fig. 3).

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