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Structure and Synthesis of a New, Thiazolidone Antibiotic

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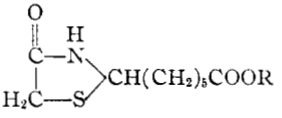
A new antibiotic, isolated from culture broths of a strain of *Streptomyces* and exhibiting *in vitro* activity against *Mycobacterium tuberculosis*, has been shown by degradation and synthesis to be (−)2-(5-carboxypentyl)-4-thiazolidone.

Two laboratories have recently reported the independent isolation,[1,2] characterization and synthesis[3,4] of a new *Streptomyces* antibiotic effective *in vitro* against *Mycobacterium tuberculosis*. Exchange of samples for comparison has substantiated the identity of the antibiotic from these two sources. Degradation and synthesis have shown this compound, which has been named actithiazic acid,[1] to be (−)2-(5-carboxypentyl)-4-thiazolidone (I).



I, R = H
Ia, R = CH_3

This paper is concerned with our characterization studies leading to elucidation of the structure and to synthesis of the compound.

I was isolated by solvent extraction of the fermentation broth and finally purified by recrystallization from methanol. The antibiotic was recrystallized from various solvents to constant melting point and optical rotation, and it was shown by solubility analysis[5] to be at least 98% pure. From elementary analyses, the formula $C_9H_{15}O_3NS$ was calculated. Potentiometric titration indicated an equivalent weight of 214 (pK_a 5.10 at 28°) in agreement with the analytical data. An active hydrogen[6] value of 1.8 was obtained. The absence of C–SH, C=S and C–S–S–C groupings was indicated by lack of color formation with sodium nitroprusside reagent.[7] Only end absorption was observed in the ultraviolet spectrum. Carbonyl bands in the infrared spectrum of I at 1640 and 1710 cm.$^{-1}$ are attributed to carboxamide and carboxyl groupings, respectively; broad absorption is present in the –OH and –NH stretching region. The absence of absorption in the –NH bending region at 1475–1600 cm.$^{-1}$ is in agreement with observations of previous investigators[8] that cyclic amides do not exhibit this absorption.

The reaction of I in methanol with ethereal diazomethane yields the microbiologically active methyl ester, $C_{10}H_{17}O_3NS$ (Ia). The infrared spectrum of this compound in chloroform exhibits two carbonyl absorption peaks, at 1680 and 1720 cm.$^{-1}$, and well-defined maxima in the N–H stretching region. Surprisingly, the methyl ester as well as a number of other esters[9] is considerably more active *in vitro* against *Mycobacterium tuberculosis* than the parent compound.

Optically active salts were obtained by carefully neutralizing aqueous suspensions of the acid with alkali metal hydroxides, freeze-drying the resulting solutions and crystallizing the salts from alcohols. However, on standing in dilute alkaline solution the salts racemized, presumably due to hydrolytic cleavage and reclosure of the thiazolidone ring. The product (m.p. 122–123°) isolated by acidification and recrystallization from water was optically inactive but could be resolved *via* the brucine salts. Recrystallization of this racemate from chloroform afforded another optically inactive material, melting at 116–117° and also resolvable. Either form could be obtained from a supersaturated aqueous solution by seeding with the appropriate type of crystal. The infrared spectrum (Nujol) of the lower melting form of the racemate is identical with that of I, whereas the spectrum of the higher melting form is different. However, since the spectra of all three are identical in dioxane, the two racemic forms are regarded merely as crystal modifications.

Oxidation of I with 6 *N* nitric acid converts the sulfur to sulfate, liberates the nitrogen as ammonia and yields a mixture of oxalic, adipic and pimelic acids. Carbon dioxide is evolved during the reaction. The extent of oxidation with potassium permanganate in alkaline solution is dependent on the alkalinity of the system. When the reaction was carried out in sodium hydroxide, slightly over three moles of potassium permanganate were consumed, and pimelic acid was isolated.

The behavior of I toward acid under various conditions proved to be very interesting. I could be dissolved in 80–100% sulfuric acid or refluxed in glacial acetic acid and recovered without apparent change, including retention of optical activity. Hydrolysis of I in aqueous dioxane containing mineral acid was evidenced by the liberation of nitrogen as ammonium salt, detection of free aldehyde as pimelaldehydic acid and the formation of a sulfur-containing, nitrogen-free dibasic acid (II) as the major degradation product. Purification of II was accomplished by conversion to the dimethyl ester (IIa) with diazomethane followed by fractional distillation. The empirical formula of

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(3) J. R. Schenck and A. F. DeRose, *Archiv. Biochem.*, in press; R. K. Clark, Jr., and J. R. Schenck, *ibid.*, in press.

(4) Communication to the Editor, W. M. McLamore, W. D. Celmer, V. V. Bogert, F. C. Pennington and I. A. Solomons, THIS JOURNAL, **74**, 2946 (1952).

(5) M. Kunitz and J. H. Northrop, *Cold Spring Harbor Symposia Quant. Biol.*, **6**, 325 (1938).

(6) F. A. Hochstein, THIS JOURNAL, **71**, 305 (1949).

(7) I. W. Grote, *J. Biol. Chem.*, **93**, 25 (1931).

(8) H. Lenormont, *Compt. rend.*, **224**, 1219 (1947).

(9) Frank C. Pennington, Walter D. Celmer, W. M. McLamore, V. V. Bogert and I. A. Solomons, THIS JOURNAL, **75**, 109 (1953).

IIa, $C_{11}H_{18}O_4S$, suggests a ring structure or an ethylenic linkage. The ultraviolet spectrum of IIa, $\lambda_{max}^{m\mu}$ 226, ϵ 4600, supports the substituted vinyl sulfide grouping,[10] as illustrated.

$$RO_2C-CH_2-S-CH=CH-(CH_2)_4-CO_2R$$

II, R = H
IIa, R = CH_3

Pimelaldehydic acid was obtained as the major product following mercuric chloride hydrolysis[11] of I.

I is readily desulfurized by Raney nickel. One of the products obtained when the reaction was carried out in boiling ethanol was identified as acetamide. Isolation of acetamide from this thiazolidone is in accord with observations made during comparable desulfurizations of penicillin and model compounds.[12] The cleavage of a carbon–nitrogen bond in penicillin, which also contains the grouping $-S-\overset{R}{\underset{|}{C}}H-\overset{|}{N}-\overset{O}{\overset{\|}{C}}-$, was postulated, with subsequent desulfurization.

The methyl ester of desthio-I was obtained by carrying out the reaction on Ia with Raney nickel in anhydrous dioxane under somewhat milder conditions. Analyses were in agreement with the expected formula, $C_{10}H_{19}O_3N$. Complete hydrolysis of the desthio ester in sodium hydroxide afforded the known ω-aminoheptanoic acid,[13] and the desthio ester itself was identified as methyl ω-acetamidoheptanoate by comparison with a synthetic sample.

Treatment of an acetic acid solution of I at room temperature with excess hydrogen peroxide resulted in a stepwise uptake of oxygen (one equivalent, 0.5 hr.; second equivalent, 40 hr.). Attempts to isolate a sulfoxide of I gave a levorotatory oil. The sulfone was obtained as a dextrorotatory, crystalline solid whose infrared spectrum exhibited a band at 1140 cm.$^{-1}$ characteristic of sulfones.[14,15] The behavior of this sulfone toward hydrolytic agents, however, differed from that of conventional sulfones. For example, sulfur dioxide was quantitatively evolved when this derivative was refluxed in very dilute hydrochloric acid for a short period of time. Nitrogen was completely accounted for as ammonium chloride, and free aldehyde liberated during the hydrolysis was identified as pimelaldehydic acid. Similarly, mercuric chloride hydrolysis liberated this aldehyde acid. Attempts to prepare alkali metal salts of the sulfone were unsuccessful due to hydrolytic destruction of the compound (liberation of sulfite) in alkaline solution. The hydrolytic behavior, reminiscent of the characteristic properties of aldehyde–bisulfite compounds, is consistent with the proposed sulfone formulation.

Racemic 2-(5-carbomethoxypentyl)-4-thiazolidone was prepared by refluxing mercaptoacetamide with the methyl ester of pimelaldehydic acid in benzene containing a catalytic amount of *p*-toluenesulfonic acid. Saponification of the resulting ester, followed by resolution of the racemic acid *via* the brucine salts, afforded (−)2-(5-carboxypentyl)-4-thiazolidone, which was identical in all respects with the antibiotic.

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Experimental

Isolation of (−)2-(5-Carboxypentyl)-4-thiazolidone (I) from Fermentation Broth.—Thirty liters of a *Streptomyces* fermentation broth containing about 3 g. (microbiological assay) of I was filtered. The filtrate was adjusted to *p*H 3.5 and extracted countercurrently with 10 l. of *n*-butanol. The solvent extract was re-extracted with 3 l. of aqueous sodium bicarbonate, and this solution was then adjusted to *p*H 4.0. The antibiotic (about 1.4 g.) was extracted countercurrently with 1.7 l. of butyl acetate, the solution treated with 2 g. of carbon and filtered. The filtrate, containing about 1.2 g. of the active compound, was evaporated under vacuum to 30 ml. When the solution was cooled, crystals of I (0.85 g.) precipitated. The crude product was dissolved in 50 ml. of methanol, the solution treated with a small amount of carbon and filtered. The filtrate was concentrated *in vacuo* to about 8 ml. and cooled overnight at 5°. The crystals were filtered, washed and dried *in vacuo* to give 0.73 g. of I, m.p. 139–140°, $[\alpha]^{25}D$ −54° (*c* 1, methanol), $[\alpha]^{25}D$ −60° (*c* 1, ethanol).

Anal. Calcd. for $C_9H_{15}O_3NS$: C, 49.74; H, 6.96; N, 6.45; S, 14.76; neut. equiv., 217.3. Found: C, 49.64; H, 7.14; N, 6.36; S, 14.71; neut. equiv., 214 (pK_a 5.10 at 28°).

Methoxyl and Kuhn–Roth C-methyl determinations were negative. Using the method of Hochstein[2] an active hydrogen value of 1.8 was obtained in spite of the low solubility of I under the conditions of the determination.

Preparation of (−)2-(5-Carbomethoxypentyl)-4-thiazolidone (Ia).—I (3.5 g.) was dissolved in 150 ml. of methanol and treated with an excess of ethereal diazomethane. The solvent was evaporated to dryness, and the residue was dissolved in 25 ml. of ether. The ether solution was washed with aqueous sodium bicarbonate, then with water, dried over anhydrous sodium sulfate and evaporated to 10 ml. Hexane (10 ml.) was added, and the mixture was cooled in an acetone–Dry Ice-bath. The crystalline precipitate was filtered, washed with 1:1 ether–hexane, dried (3.3 g.) and recrystallized from the same mixed solvent; yield 2.5 g., m.p. 53–54°, $[\alpha]^{25}D$ −50.9° (*c* 1, methanol).

Anal. Calcd. for $C_{10}H_{17}O_3NS$: C, 51.92; H, 7.41; N, 6.06; S, 13.86; CH_3O, 13.41. Found: C, 51.92; H, 7.43; N, 6.15; S, 13.62; CH_3O, 12.9.

Ia was also prepared by reaction of the acid chloride of I with methanol.[5]

Preparation of Racemic I.—To a mixture of 25 g. of I and 50 ml. of water, 121 ml. of 1.078 *N* sodium hydroxide was added slowly. After 6 hours the mixture was acidified with 114 ml. of 1.15 *N* hydrochloric acid, and the precipitate filtered and dried. The racemic I (23.4 g.) melted at 122–123°, $[\alpha]^{25}D$ 0° (*c* 1, methanol). The melting point was unchanged by recrystallization from water.

Anal. Calcd. for $C_9H_{15}O_3NS$: C, 49.74; H, 6.96; N, 6.45; S, 14.76. Found: C, 49.78; H, 6.95; N, 6.34; S, 14.95.

By recrystallization of this substance from chloroform a second crystalline modification of the racemate was obtained, m.p. 116–117°. Found: C, 49.90; H, 6.95; N, 6.36; S, 14.73.

Either modification can be obtained from a supersaturated aqueous solution by seeding with the crystals of that form.

Nitric Acid Oxidation of I.—Nitric acid (11 ml. of 6 *N*) and 1.1 g. (5.1 millimoles) of I were mixed and warmed gently. The evolved gases were flushed with nitrogen into 500 ml. of 0.1932 *N* barium hydroxide solution. After the initial reaction was complete, the mixture was heated on a steam-bath for 1 hour, and then nitrogen was passed through for an additional 2 hours. The barium carbonate (1.48 g., 7.52 millimoles) corresponded to 1.43 molar equivalents. Barium sulfate (0.96 molar equivalent) was precipitated from the reaction mixture by the addition of barium chloride. The filtrate was neutralized with concentrated ammonium hydroxide, and calcium oxalate (0.21 molar equivalent) was

precipitated by treatment with calcium chloride. Extraction of the aqueous filtrate, adjusted to *p*H 2, with ether yielded 0.6 g. of a crystalline product. Extraction of this material with benzene left a residue (0.057 g.) identified as adipic acid by m.p. (150–152°) and neut. equiv. (72.2). A mixed melting point with an authentic sample was undepressed. Crystals isolated from the benzene extract (0.24 g., m.p. 87–110°) were identified by infrared analysis as a mixture of adipic and pimelic acids. The raffinate from the ether extraction yielded 2.06 molar equivalents of ammonia, at least one of which must be derived from the nitric acid.

Potassium Permanganate Oxidation of I.—I (2.01 g., 9.3 millimoles) was dissolved in 100 ml. of 0.101 *N* sodium hydroxide. A solution of potassium permanganate (0.127 *M*) was added dropwise with stirring until a permanent faint pink color was obtained (225 ml.). The precipitated manganese dioxide was filtered, repulped in hot water and refiltered. The combined filtrates were distilled *in vacuo* to about 25 ml. Concentrated hydrochloric acid was then added to *p*H 1.3, and the solution was exhaustively extracted with small portions of ether. The solvent extracts were combined, dried over anhydrous sodium sulfate and then evaporated to dryness. The crystalline product (0.88 g.) was recrystallized from benzene–hexane and then from benzene; m.p. 102–103°, mixed m.p. with pimelic acid 104–105°.

Anal. Calcd. for $C_7H_{12}O_4$: C, 52.48; H, 7.55; neut. equiv., 80.1. Found: C, 52.58; H, 7.64; neut. equiv., 79.9.

The *p*-bromophenacyl ester, prepared according to Kelly and Kleff,[16] melted at 134–135°, and the melting point was not depressed by admixture with an authentic sample.

Mercuric Chloride Hydrolysis of I.—A solution of I (2.17 g.) in hot water was treated with 50 ml. of 0.02 *M* aqueous mercuric chloride solution and heated to reflux temperature. The reaction mixture became cloudy and gave a strongly positive Fuchsin-aldehyde test. After 1 hour the solution was allowed to cool, and the white mercuric salts which precipitated were removed by filtration. The filtrate was then extracted with an equal volume of ethyl acetate, the organic phase collected and washed with an equal volume of water. The ethyl acetate extract was dried (anhydrous sodium sulfate) and concentrated at reduced pressure to leave an oil (0.85 g.) identified as pimelaldehydic acid by means of its crystalline oxime, m.p. 110–111°,[17] and 2,4-dinitrophenylhydrazone, m.p. 136–138°.

Acid Hydrolysis of I.—A warm solution of I (8.68 g.) in 87 ml. of purified dioxane was treated with 16.7 ml. of 12 *N* aqueous hydrochloric acid and heated to reflux temperature. Within 15 minutes ammonium chloride began to precipitate from the reaction mixture, accompanied by separation of a lower aqueous layer. After 2 hours the reaction mixture was chilled in an ice–water-bath and filtered to remove some solid ammonium chloride. The organic layer was separated from the lower aqueous solution of the salt and treated with an excess of ethereal diazomethane. After being kept at room temperature for 1 hour, the reaction mixture was concentrated at reduced pressure to 200 ml. The solution was then washed successively with 50 ml. of 0.5% sodium bicarbonate and water. Fractional distillation at reduced pressure afforded a forerun of the solvents and the following fractions: (1) 0.9 g. of a colorless oil, b.p. 61–65° (0.4 mm.), identified as crude methyl ester of pimelaldehydic acid by means of its semicarbazone, m.p. 116–118° (see below); (2) 3.0 g. of a light yellow, odoriferous oil, b.p. 160–180° (0.4 mm.). A dark brown sirup (3.5 g.) remained in the distillation flask. Fraction 2 was redistilled to give 1.3 g. of an oil, b.p. 162–168° (0.4 mm.), $n^{25}D$ 1.4850, d^{25}_4 1.128; ultraviolet absorption, $\lambda^{m\mu}_{max}$ 226, ϵ 4600 in 2,2,4-trimethylpentane, believed to be IIa.

Anal. Calcd. for $C_{11}H_{18}O_4S$: C, 53.64; H, 7.36; S, 13.02; CH_3O, 25.2. Found: C, 53.60; H, 7.49; S, 13.30; CH_3O, 22.3.

Raney Nickel Desulfurization of I in Ethanol.—One gram of I was refluxed in 50 ml. of ethanol (3A) with 15 g. of Raney nickel for 17 hours. The nickel was filtered, the filtrate neutralized to a phenolphthalein end-point and concentrated to dryness under reduced pressure. Extraction of the residue with hot chloroform afforded 0.100 g. of a solid that sublimed readily, m.p. 75–78° (Kofler block). A mixed melting point with freshly sublimed acetamide (m.p. 77–79.5°) was not depressed. The substance was further identified as acetamide by infrared spectrum and by hydrolysis to acetic acid, which was characterized as its *p*-bromophenacyl ester, m.p. 82–85°, undepressed by admixture with an authentic sample.

The acidic fraction from the hydrogenolysis was a non-homogeneous, water-soluble oil that failed to crystallize or to yield pure derivatives. The nature of these acidic products was not completely elucidated.

Refluxing I in ethanol that had been standing over Raney nickel caused no change in the antibiotic; consequently, acetamide is formed only in the presence of the catalyst.

Desulfurization of Ia in Dioxane.—Ia (3.0 g.) and 45 g. of Raney nickel were stirred in 125 ml. of dry dioxane at 25° for 4 hours. The catalyst was centrifuged and washed thoroughly with dioxane. The dioxane solutions were combined and evaporated under vacuum, leaving a light yellow, sulfur-free oil (2.30 g.), which was crystallized from ether–petroleum ether to give colorless needles, m.p. 31–32°.

Anal. Calcd. for $C_{10}H_{19}O_3N$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.55; H, 9.58; N, 6.86.

The mixed melting point with authentic methyl ω-acetamidoheptanoate (m.p. 35–36°) was 33–36°; the infrared spectra were identical and very similar to that of Ia.

Crude desthio-Ia (1.85 g.) was heated on a steam-bath in 35 ml. of 15% aqueous sodium hydroxide for 18 hours. The resulting solution was passed through a column containing about 200 g. of Amberlite IR 120 resin on the acid cycle. The resin was thoroughly washed with water, removed from the column, eluted batchwise with 500 ml. of water containing 23 ml. of ammonia, filtered and washed. The filtrate was concentrated to dryness under vacuum to leave 0.84 g. of a crystalline solid, m.p. 183–188°. The material was recrystallized from methanol–petroleum ether, and then twice from acetone–water–methanol; yield 0.30 g., m.p. 193–194°. The reported m.p. of ω-aminoheptanoic acid is 186–187°.[9]

Anal. Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65. Found: C, 58.11; H, 10.34; N, 9.63.

Synthesis of Methyl ω-Acetamidoheptanoate.—Crude acid chloride of methyl hydrogen pimelate (130 g.) was added slowly with stirring to 550 ml. of cold ammonium hydroxide. The resulting colorless crystals were filtered, washed and dried; yield 78.1 g., m.p. 78–79.5°. Recrystallization from benzene–petroleum ether raised the m.p. to 79–80.5°. The filtrate and washings were extracted twice with 250-ml. portions of chloroform to give additional product, which was recrystallized from benzene–petroleum ether; weight 19.7 g., m.p. 79–80.5°.

Anal. Calcd. for $C_8H_{15}O_3N$: C, 55.47; H, 8.73. Found: C, 55.64; H, 8.62.

The amide (95 g.) was converted to **methyl 6-cyanocaproate** by treatment with 44.5 g. of phosphorus pentachloride at 100° for 1 hour. Ice and ether were added; the ether layer was washed with aqueous sodium carbonate and a saturated solution of sodium chloride, dried over anhydrous sodium sulfate and evaporated; the residue was distilled at 0.4 mm. Three fractions, b.p. 76–86°, 86–91° and 91–94° (total weight 71.8 g.), respectively, were taken. The infrared spectra were essentially identical.

Anal. (Center fraction) Calcd. for $C_8H_{13}O_2N$: C, 61.91; H, 8.44. Found: C, 61.79; H, 8.42.

A portion of the nitrile (3.10 g.) was hydrogenated in 25 ml. of acetic anhydride at atmospheric pressure over platinum oxide catalyst. The catalyst was filtered and washed with acetic acid, and the combined filtrates were concentrated under vacuum. The residue was dissolved in aqueous sodium bicarbonate and extracted three times with a total of 125 ml. of chloroform. The combined solvent extracts were dried over anhydrous sodium sulfate and the chloroform evaporated. The residue was recrystallized twice from ether–petroleum ether; yield 2.4 g., m.p. 32–34°. An additional recrystallization from the same solvents raised the m.p. to 35–36°.

Anal. Calcd. for $C_{10}H_{19}O_3N$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.68; H, 9.46; N, 7.16.

The synthetic methyl ω-acetamidoheptanoate was identical with desthio-Ia.

(16) T. L. Kelly and P. A. Kleff, THIS JOURNAL, **54**, 4444 (1932).

(17) M. Kershbaum, *Ber.*, **60**, 902 (1927).

I-Sulfone.—A solution of I (2.17 g.) in 80 ml. of glacial acetic acid was treated at room temperature with 10 ml. of 30% aqueous hydrogen peroxide and the total volume brought to 100 ml. with additional glacial acetic acid. After 40 hours the solution remained at a constant dextrorotation of +0.19° (0.2 dm.). The solution was then freeze-dried, and the colorless solid residue was washed with hot benzene and filtered. This residue (2.40 g.) was extracted with two 20-ml. portions of acetone, which were filtered and the filtrates combined. The acetone extracts were evaporated to dryness, yielding colorless crystals, m.p. 132–135°. Recrystallization from 15 ml. of water gave needles (1.01 g.), m.p. 142–143°, $[\alpha]^{25}D$ +43° (*c* 1, methanol).

Anal. Calcd. for $C_9H_{15}O_5NS$: C, 43.37; H, 6.08; N, 5.62; S, 12.85. Found: C, 43.65; H, 6.18; N, 5.85; S, 13.10.

Attempts to determine the neutral equivalent of I-sulfone in 50% aqueous alcohol solution gave a rapid uptake of one equivalent of 0.1 *N* alkali followed by a slow further uptake. Utilizing excess 0.1 *N* alkali and back-titrating with standard acid revealed an uptake of two equivalents of alkali. The acidified sample evolved approximately one equivalent of sulfur dioxide. A weakly acidic solution of I-sulfone at room temperature did not release detectable amounts of sulfur dioxide. Hydrolytic processes forming sulfite in alkaline solutions apparently explain the titration data.

A solution of one milliequivalent of I-sulfone (0.249 g.) in 95 ml. of 0.053 *N* hydrochloric acid was refluxed. The liberated sulfur dioxide was collected with the aid of a slow stream of nitrogen, and measured in a solution of standard 0.1 *N* iodine. At the end of 2.0 hours, 0.95 molar equivalent of sulfur dioxide had been evolved. An aldehyde, also liberated during the hydrolysis, was identified as pimelaldehydic acid by means of its crystalline 2,4-dinitrophenylhydrazone (0.371 g.), m.p. 135–137°.

Methyl Ester of Pimelaldehydic Acid.—Methyl hydrogen pimelate, prepared by the method of Swann, *et al.*,[18] was converted in the usual manner with thionyl chloride to the acid chloride (b.p. 76–78° (0.3 mm.), $n^{26}D$ 1.4455). Freshly distilled acid chloride (200 g.) was dissolved in 1 l. of dry mixed xylenes, b.p. 137–140°, containing a suspension of 20 g. of 5% palladium–charcoal catalyst.[19] The stirred mixture was heated at reflux temperature while a stream of hydrogen was bubbled through the solution. At the end of 2 hours, 90% of the theoretical amount of hydrogen chloride had been evolved, and its rate of liberation became slow. The catalyst was removed and the solvent distilled at reduced pressure. The residual oil was purified by fractional distillation to yield the aldehyde as a colorless liquid (101 g.), b.p. 70° (0.5 mm.), $n^{25}D$ 1.4310.

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.74; H, 8.86.

The semicarbazone crystallized from ethyl acetate as colorless plates, m.p. 117–118°.

Anal. Calcd. for $C_9H_{17}O_3N_3$: C, 50.23; H, 7.91. Found: C, 50.17; H, 7.71.

Pimelaldehydic Acid.—The methyl ester of pimelaldehydic acid (8.0 g.) was hydrolyzed by boiling in 1 *N* hydrochloric acid (80 ml.) for 12 min. After cooling, the clear solution was extracted with ethyl acetate. Evaporation of the solvent gave an oil (7.0 g.) characterized as pimelaldehydic acid by its crystalline oxime, m.p. 110–111°[13] and 2,4-dinitrophenylhydrazone, m.p. 138–139°.

Anal. Calcd. for $C_{13}H_{16}O_6N_4$: C, 48.15; H, 4.97. Found: C, 48.18; H, 4.75.

Synthesis of Racemic I.—A mixture of 39.2 g. of the methyl ester of pimelaldehydic acid and 68.0 g. (3 molar equivalents) of mercaptoacetamide[20] was heated with stirring on a steam-bath for 5 minutes under a nitrogen atmosphere. Benzene (1000 ml.) and *p*-toluenesulfonic acid (0.3 g.) were added, and the mixture stirred and boiled under reflux for 4.5 hours in an apparatus with provision for removal of the water formed during the condensation. The benzene solution was washed with water and the solvent removed *in vacuo*. Distillation of the residue yielded 28.1 g. of a slightly yellow solid, b.p. 180–210° (0.3 mm.). Recrystallization of this material from cold ether gave white crystals of racemic Ia, m.p. 60.5–62°.

Anal. Calcd. for $C_{10}H_{17}O_3NS$: C, 51.92; H, 7.41; N, 6.06; S, 13.86. Found: C, 51.92; H, 7.39; N, 6.03; S, 13.97.

The methyl ester (4.62 g.) was stirred with 200 ml. of 0.120 *N* sodium hydroxide solution for 4.5 hours. Unchanged ester was extracted with 200 ml. of ether. Acidification of the aqueous solution to *p*H 4 with 1 *N* hydrochloric acid precipitated the racemic acid. The product (2.95 g.), m.p. 121–122°, was collected and washed with water. Extraction of the mother liquors with five 100-ml. portions of ethyl acetate yielded an additional 0.5 g. Recrystallization of this material from water afforded white crystals of racemic I, m.p. 121–122°. The other crystalline modification of the racemic acid (m.p. 115–117°) was obtained by recrystallization from chloroform.

Resolution of Synthetic I.—A mixture of 5 g. of racemic I and 9.05 g. of brucine was dissolved in 50 ml. of ethanol and crystallized at 5°. The resulting brucine salt, weighing 6.2 g. (m.p. 55–56°), was recrystallized from ethanol to give 1.9 g. of material, m.p. 60–61°, which was dissolved in 14 ml. of water and acidified with 6 *N* hydrochloric acid to give 0.47 g. of I, m.p. 136–138°. After being recrystallized from water, the product (0.39 g.) had m.p. 139–140° and $[\alpha]^{25}D$ −51.4° (*c* 1, methanol); it was identical with I isolated from fermentation broth.

The original ethanolic filtrate of the brucine salts was concentrated *in vacuo* to a heavy sirup, which crystallized after being kept for 4 weeks; m.p. 85–90°. The crude product (weight 7.2 g.) was dissolved in 30 ml. of water and acidified with 6 *N* hydrochloric acid. The crude (+) I (weight 2.24 g., m.p. 114–124°) was dissolved in 90 ml. of hot water, the solution cooled to 45° and the resulting crystals filtered. The product (1.35 g.) had m.p. 128–132° and $[\alpha]^{25}D$ +31°. An additional recrystallization from water in the same manner yielded (+) I (0.62 g.) m.p. 138–139°, $[\alpha]^{25}D$ +57°. The microbiological activity of (+) I is one-fifth that of (−) I against *Mycobacterium tuberculosis*.

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(19) This Rosenmund reduction without special catalyst poisoning gave a 63% yield of aldehyde. The aldehyde was stored in sealed ampoules under nitrogen at Dry Ice temperature if not utilized immediately after distillaton. Compare G. B. Brown, M. D. Armstrong, A. W. Mayer, W. P. Anslow, Jr., B. R. Baker, M. V. Querry, S. Bernstein and S. R. Safir, *J. Org. Chem.*, **12**, 160 (1947).

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