21. Akira Miyake, Akira Morimoto, and Tomoji Kinoshita: Studies on Antibiotics. I.* Acidomycin. (1). Isolation and Chemical Structure**.

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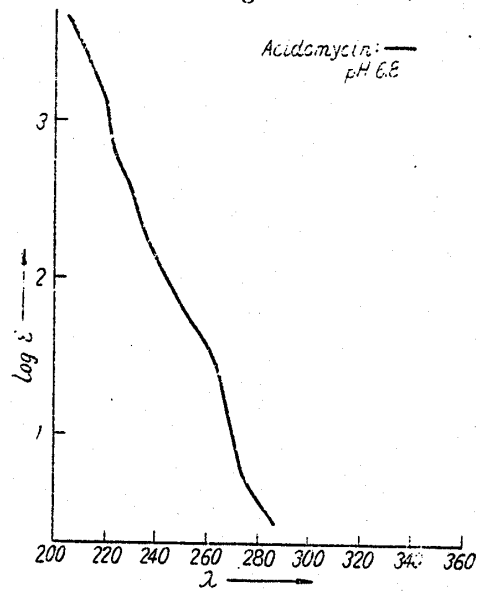
Acidomycin, an antibiotic active against *Mycobacterium tuberculosis*, was obtained from the culture of *Streptomyces acidomyceticus* and its variations which were first isolated and named by Koiti Ogata*****. The microbiological study of this Streptomyces was presented at the 17th Meeting of Japan Antibiotic Research Association held in Hiroshima on September 15, 1951, and at the 63rd Meeting of the same Association in Tokyo on September 24, 1952. Since last summer, studies on purification and chemical structure of this new antibiotic have been carried out. Prior to the completion of this work, we learned that this antibiotic has been independently isolated by the groups at Chas. Pfizer and Company[1], at Abbot Laboratories[2], at Lederle Laboratories[3], and at the National Institute of Health, Tokyo[4], and synthesized by the first two groups[1,2]. In this paper the study which was performed by us is presented.

Fig. 1



The culture broth containing acidomycin is filtered from the mycelium at pH 4.0. The acidic solution (pH 2.0) is extracted with an equal volume of butyl acetate. On concentrating the butyl acetate solution to one-eighth of the original volume, acidomycin begins to crystallize. Recrystallization from methanol gives long colorless needles, m.p. 137.5~138.5° (uncorr.). Data of elementary analysis are in agreement with $C_9H_{15}O_3NS$, $[\alpha]_D^{15}$: −46.2° (c=0.073 g. in 25 cc. of methanol). The antibiotic is easily soluble in methanol, ethanol, butanol, chloroform, acetone, ethyl acetate, butyl acetate, benzyl alcohol, and hot water, and less soluble in ether, petroleum ether, benzene, and cold water. As shown in Fig. 1, the ultraviolet spectrum of acidomycin has no characteristic absorption in the region from 200 to 360 mμ. Solutions of acidomycin in water or other organic solvents exhibit a blue fluorescence on exposure to ultraviolet light.

Acidomycin exhibits, *in vitro*, specific high activity against human and avian type tubercle bacilli. It is active against human type H2 strain at the concentration of 0.3 γ per cc. and against streptomycin-resistant strain of human type (Suita strain) at the concentration of 1.22 γ per cc.

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1) I.A. Solomons, *et al.*: J. Am. Chem. Soc., 74, 2946 (1952).
2) B.A. Sobin: *Ibid.*, 74, 1947 (1952); John C. Sylvester: Antibiotic & Chemotherapy, 2, 399 (1952); J.R. Schenck, A.F. Rose: Arch. Biochem. & Biophys., 40, 263 (1952); R.K. Clark, J.R. Schenck: *Ibid.*, 40, 270 (1952).
3) J.H. Williams, *et al.*: Antibiotic & Chemotherapy, 2, 333 (1952).
4) H. Umezawa, *et al.*: Paper presented at the 63rd Meeting of Japan Antibiotic Research Association, in Tokyo, September 24, 1952.

by the dilution of submerged culture, and against human type (Frankfurt) at the concentration of 0.5 γ per cc. by the assay of slide-culture method. Toxicity, LD_{50}, of acidomycin injected intravenously to mice is 35 mg./10 g., and subcutaneously, 200 mg./10 g. This toxicity is very weak compared with that of streptomycin. The results of alkali titration and esterification indicate the presence of a carboxyl group. In an excess sodium hydroxide solution or hot sodium carbonate solution acidomycin loses its optical activity and becomes a racemate of m.p. 117~118°.

Oxidation of acidomycin with potassium permanganate or chromic oxide gives pimelic acid (II), and oxidation with nitric acid gives adipic acid (III). These results of oxidation show that acidomycin has at least seven carbon atoms linked in a straight chain in its molecule.

In order to ascertain the type of sulfur linkage, acidomycin was desulfurized with Raney nickel. This procedure gives colorless crystals, m.p. 50~55°, and $b.p_{0.25}$ 200~202°, and the data of its elementary analysis agree with the formula $C_9H_{17}O_3N$ (IV). The desulfurization of acidomycin methyl ester (VIII) with Raney nickel gives, together with acetamide (V), a liquid of $b.p_2$ 172°, and agrees with the formula $C_{10}H_{19}O_3N$ (VI). Both (IV) and (VI) result from their starting materials by the removal of sulfur atom and addition of two hydrogen atoms. This indicates that the sulfur atom is one of ring members in acidomycin molecule. Hydrolysis of (IV) and (VI) with 20% hydrochloric acid gives an amino acid of m.p. 192~194°(decomp.), and has the formula $C_7H_5O_2N$ (VII).

Hydrolysis of acidomycin with N hydrochloric acid gives equivalent NH_4Cl and a water-insoluble acidic oil which gives positive sulfhydryl test. Fractional distillation at a reduced pressure of this oil and its methyl ester gives thioglycollic acid and an oil of $b.p_{0.3}$ 185~194°, respectively. The latter gives no sulfhydryl test and agrees with the formula $C_{11}H_{18}O_4S$ (IX). Desulfurization of (IX) with Raney nickel gives methyl oenanthate (X).

Reduction of acidomycin or its methyl ester with lithium aluminum hydride gives three reaction products, $C_9H_{17}O_2NS$ (XI), $C_9H_{19}ONS \cdot HCl$ (XII), and $C_9H_{21}ONS$ (XIII), respectively. From their chemical nature, it is learned that in (XI), m.p. 78~79°, the carboxyl group of acidomycin is reduced to a primary alcohol, and in (XII), m.p. 208~209°, the carbonyl group adjacent to imido group of (XI) is reduced to a methylene group, and in (XIII), m.p. 53~54°, the ring structure of (XII) is cleaved at the sulfur linkage and produces a sulfhydryl group. Desulfurization of (XI) and (XIII) with Raney nickel followed by urethane formation with phenyl isocyanate gives crystals having melting points of 112° and 275°, respectively.

After we obtained these data, we received the report of Solomons, *et al.*[1] On comparing both data, we found that acidomycin is identical with Solomons' 2-(5-carboxypentyl)-4-thiazolidone. The methyl ester of *dl*-2-(5-carboxypentyl)-4-thiazolidone was synthesized according to Solomons' method and compared with *dl*-acidomycin methyl ester. The mixture of the two compounds showed no depression of the melting point.

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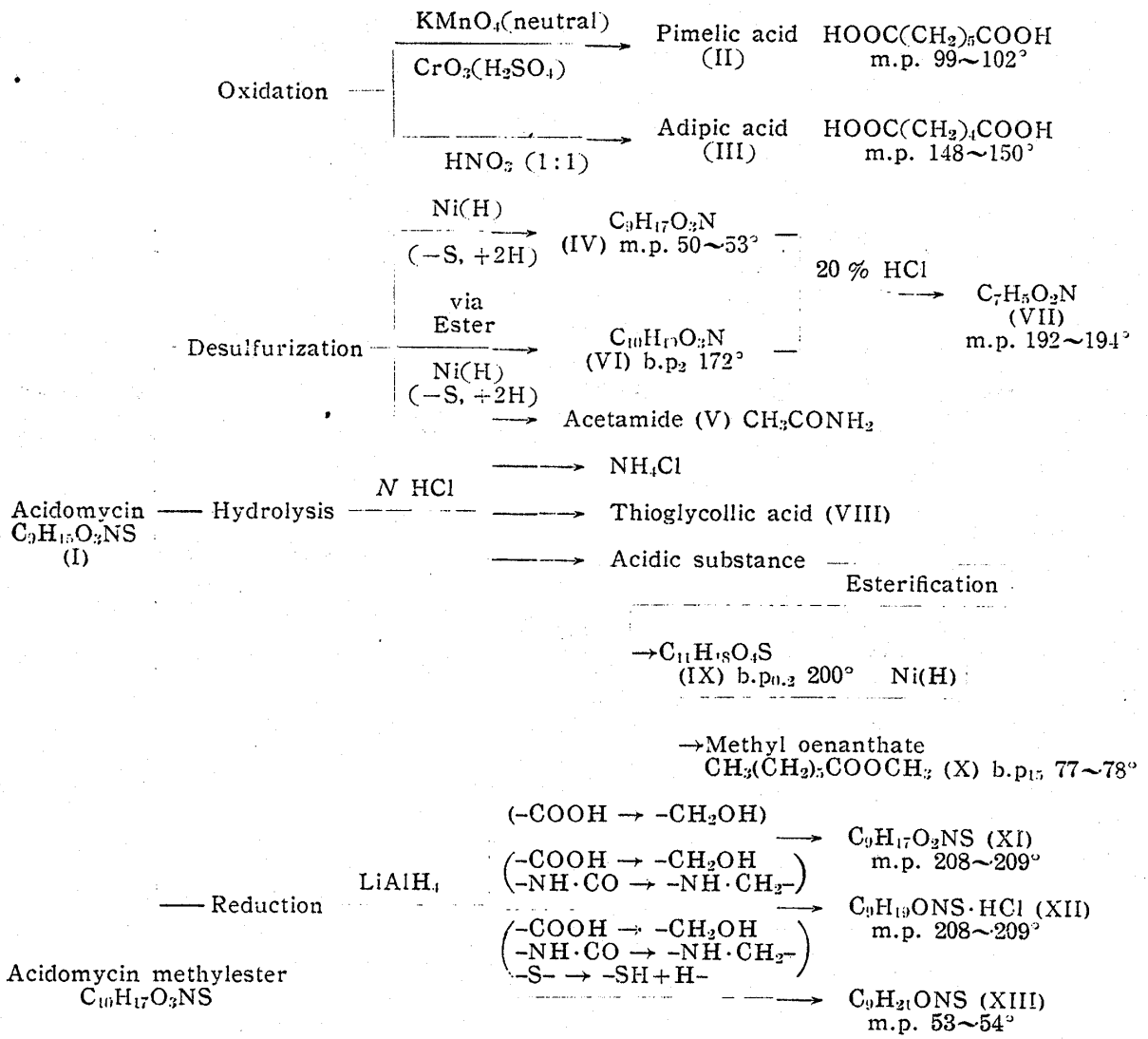
Experimental

Isolation of acidomycin—Three hundred L. of whole culture broth (pH 8.0~8.5, assayed 800 μ/cc.) is adjusted to pH 4.0 with 20% H_2SO_4 and filtered. The clarified beer is further adjusted to pH 2.0 with 20% H_2SO_4 and extracted with equal volume of butyl acetate. On concentrating the



butyl acetate extract to 4 L. at a reduced pressure and allowing to stand in a refrigerator, crude light brown acidomycin begins to crystallize. After leaving in refrigerator overnight the crystals are filtered, weighing 23 g. The crude crystals are dissolved in 300 cc. of methanol and decolorized with active carbon. On cooling, the long colorless needles crystallize out. Recrystallization from methanol gives 15.6 g. of pure acidomycin, m.p. 137.5~138.5°(uncorr.). The butyl acetate mother liquor of above preparation is extracted with 2% Na_2CO_3. The alkaline solution is adjusted to pH 4.0 and extracted four times with one-fifth its volume of butyl acetate. Another 0.8 g. of acidomycin is recovered from the butyl acetate extract. *Anal.* Calcd. for $C_9H_{15}O_3NS$: C, 49.75; H, 6.96; N, 6.45; S, 14.74. Found: C, 49.76; H, 6.90; N, 6.27; S, 14.34. Titration of carboxyl group with 0.01*N* NaOH: Sample 12.4 mg., NaOH consumed 5.83 cc., M.W. 212.7. Sample 6.8 mg., NaOH consumed 3.22 cc., M.W. 211.0 (blank test 0.1 cc. F=1.028).

Acidomycin methyl ester—i) Acidomycin is dissolved in dioxane and ice-cooled. To this solution, a slight excess of CH_2N_2 in cold ether is added and the mixture is allowed to stand for 2 hours. Evaporation of the solvents gives light yellow crystalline mass. Recrystallization from dilute methanol gives colorless needles, m.p. 54.5~55.5°; yield, 90~95%. *Anal.* Calcd. for $C_{10}H_{17}O_3NS$: C, 51.92; H, 7.41; N, 6.06; S, 13.86. Found: C, 51.69; H, 7.26; N, 5.63; S, 13.54.

ii) Acidomycin is dissolved in 15 volumes of absolute methanol. To the solution, dry HCl is introduced under cooling. Immediately after the gas is saturated, the gas and solvent are evaporated at reduced pressure. The residue is poured into ice water and extracted with ether. The ether solution is washed with 8% $NaHCO_3$ and dried with Na_2SO_4. Evaporation of the ether gives crude methyl ester. Recrystallization from dilute methanol gives pure crystals. Yield, 90~95%. Mixed melting point with the methyl ester obtained above shows no depression.

iii) Acidomycin is dissolved in 30 volumes of absolute methanol and one-half weight of acid-

treated ion exchange resin (Amberlite IR-120) is suspended in it. The methanol solution is warmed at 65° on a water bath for 4 hours. After cooling, the resin is filtered off and the solution is evaporated at a reduced pressure. The residue is extracted with ethyl acetate, followed by washing with $NaHCO_3$ and dried with Na_2SO_4. Evaporation of ethyl acetate gives the crude ester. Recrystaillization from ether gives pure methyl ester, m.p. 54.5~55.5°; yield, 90%. It is identical with the acidomycin methyl ester described above.

***dl*-Acidomycin**—Optically active acidomycin (m.p. 137.5~138.5°, $[\alpha]_D^{15}$: −46.2° (MeOH)) is dissolved in an excess NaOH or hot Na_2CO_3. Acidifying the solution with concentrated HCl gives rise to thin needles. These crystals are filtered, washed with water, and recrystallized from methanol. The pure crystals, m.p. 117~118° (uncorr.), show no optical activity. *Anal.* Calcd. for $C_9H_{15}O_3NS$: C, 49.75; H, 6.96; N, 6.45; S, 14.74. Found: C, 49.43; H, 6.89; N, 6.40; S, 14.79. Racemic acidomycin is more soluble in water and alcohols than the optically active acidomycin and the former shows one-half the antitubercular activity of the latter.

Oxidation of acidomycin i) **Pimelic acid (II)**— A) To a suspension of 10 g. of acidomycin in 500 cc. of water is added 19 cc. of 2*N* NaOH (mol. equiv.), and 20 g. of $KMnO_4$ is then added gradually. The violet color of the oxidizing agent disappears immediately. After 30 minutes' agitation and 2 hours' warming on a water bath, the precipitated MnO_2 is collected by filtration and washed with hot water. The filtrate and washing are concentrated to 200 cc. at a reduced pressure, acidified with HCl and extracted with 250 cc. of ethyl acetate. The extract is washed with water and dried with Na_2SO_4. On evaporation of the solvent, crude pimelic acid crystallizes out. Recrystallization from ethyl acetate-petroleum benzine or benzene gives colorless needles, m.p. 99~102°. *Anal.* Calcd. for $C_7H_{12}O_4$: C, 52.48; H, 7.55, M.W. 160. Found: C, 52.36; H, 7.56. M.W. 165±5 (Barger's method), 171 (alkali titration). This sample does not depress the m.p. of an authentic specimen of pimelic acid. Dimethyl ester, $b.p_{16}$ 123~125°; dihydrazide, m.p. 180~181.5°; picrate of dihydrazide, m.p. 154~155°; *p*-bromophenacyl ester, m.p. 134~135°. B) Ten g. of acidomycin is added to 200 cc. of 20% H_2SO_4 and 22 g. of CrO_3 is added, little by little. When all the oxidizing agent has been added the soultion is extracted with ether. From the ether solution 5.7 g. of pimelic acid are obtained by the usual procedure; yield, 77.5%. Phenacyl ester, m.p. 72~73°; dianilide, m.p. 150~152°.

ii) **Adipic acid (III)**—Three g. of acidomycin is oxidized by 10 cc. of HNO_3 (1:1) and then warmed on a water bath for 2 hours. On allowing to stand in a refrigerator, the reaction product crystallizes out. Recrystallization from warm ethyl acetate gives 0.8 g. of crystals, m.p. 148~150°. *Anal.* Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 48.73, 49.05; H, 6.77, 6.96. Mixed melting point of this sample with the authentic adipic acid shows no depression.

Desulfurization of acidomycin i) **$C_9H_{17}O_3N$ (IV)**—Thirty cc. of *N* NaOH and 5 g. of acidomycin are added to 470 cc. of water. In this solution 50 g. of Raney nickel is suspended, the mixture is refluxed at 160~170° in a metal bath for 3 hours, and allowed to cool. The nickel is filtered off, the filtrate is acidified with conc. HCl and evaporated to dryness at a reduced pressure. The residue is extracted with ethyl acetate and the extract is evaporated to dryness at a reduced pressure. The residue is reëxtracted with the same solvent. The solution is washed with equal volume of 0.1*N* HCl and then with *N* NaOH. The alkaline solution is acidified with HCl and extracted with ethyl acetate. Ethyl acetate is evaporated at a reduced pressure. Fractional distillation of the residue gives crystals of $b.p_{0.25}$ 200~202°, and m.p. 50~55°. *Anal.* Calcd. for $C_9H_{17}O_3N$: C, 57.72; H, 9.15; N, 7.48. Found: C, 57.49; H, 9.05; N, 7.34.

ii) **$C_{10}H_{19}O_3N$ (VI)**—Seventy-three g. of Raney nickel is added to a solution of 7.3 g. of acidomycin methyl ester in 500 cc. of methanol. The mixture is refluxed on a water bath for 5 hours. On cooling, nickel is filtered off and the filtrate is evaporated to dryness. The residue is extracted with ether. The ether is washed with water, dried with Na_2SO_4, and then evaporated at a reduced pressure. Fractionation of the residue gives acetamide, as well as a liquid (VI) of $b.p_2$ 172°. *Anal.* Calcd. for $C_{10}H_{19}O_3N$: C, 59.67; H, 8.90; N, 6.88. Found: C, 59.44, 59.14; H, 8.90, 8.92; N, 6.80.

Desulfurization of the ethyl ester gives a liquid of $b.p_{1.3}$ 178~180°. Hydrazide m.p. 129~130°. Benzaldehyde and *p*-nitrobenzaldehyde condensation product of the hydrazide m.p. 130~140°, and m.p. 199~201°, respectively.

iii) **$C_7H_{15}O_2N$ (VII)**—(IV) or (VI) is hydrolyzed with ten times its volume of 20% HCl under reflux for 4 hours. The acidic solution is evaporated to dryness. Neutralization with NH_3 followed by precipitation with dry acetone gives an amino acid. Recrystallization from methanol-acetone gives crystals of m.p. 192~194°(decomp.), which give positive ninhydrin test. *Anal.* Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.74; H, 10.74; N, 9.51.

Hydrolysis of acidomycin i) **Ammonium chloride**—Five g. of acidomycin is suspended in 5 cc. of *N* HCl and warmed at 100° in a boiling water bath for 5 hours. On cooling, a brown acidic oil separates out. Whole reaction product is extracted thoroughly with ethyl acetate. Evaporation of the aqueous layer, followed by crystallization from methanol, gives colorless crystals of ammonium chloride.

ii) **Thioglycollic acid**—The above ethyl acetate extract is washed with a small amount of water and dried with Na_2SO_4. Evaporation of the ethyl acetate, followed by fractionation of the residue gives 0.9 g. of colorless liquid, $b.p_2$ 77°. This liquid has a specific odor and gives positive sulfhydryl test. *Anal.* Calcd. for $C_2H_4O_2S$: C, 26.07; H, 4.37; S, 34.80. Found: C, 26.06, 26.17; H, 4.61, 4.37; S, 34.02. M.W. 97.3 (alkali titration).

iii) **Methyl oenanthate (X)**—Ten g. of the above acidic oil is esterified with CH_2N_2 or methanol and HCl. Fractionation of the ester gives 4 g. of light yellow liquid (IX), $b.p_{0.3}$ 185~195°. Refractionation raises the boiling point to 200°/0.1 mm Hg. (IX) gives negative sulfhydryl test and does not dissolve in alkaline solution. *Anal.* Calcd. for $C_{11}H_{18}O_4S$: C, 53.63; H, 7.37. Found: C, 53.06; H, 7.08.

Four g. of (IX) is refluxed in ethanol with 20 g. of Raney nickel for one hour. On cooling, nickel is filtered off and the ethanol is evaporated. There remains 2.5 g. of fragrant liquid. Fractionation of this liquid gives about 1 g. of a fraction, $b.p_{15}$ 73~74°. *Anal.* Calcd. for $C_8H_{16}O_2$: C, 66.63; H, 11.18. Found: C, 66.78; H, 10.98. Hydrazide m.p. 82~82.5°. *Anal.* Calcd. for $C_7H_{16}ON_2$: C, 58.30; H, 11.16; N, 19.43. Found: C, 58.06; H, 11.31; N, 19.98. The hydrazide is identical with the hydrazide of authentic oenanthic acid.

Reduction of acidomycin methyl ester with lithium aluminum hydride i) **$C_9H_{17}O_2NS$ (XI)**—To a solution of 10 g. of acidomycin methyl ester in 300 cc. of ether, 80 cc. of ether containing 5 g. of $LiAlH_4$ is added, dropwise, under stirring and cooling. White addition product crystallizes out immediately. After one hour's stirring, ether saturated with water is added to the reaction mixture and excess $LiAlH_4$ and the addition product are decomposed completely. Then the solution is acidified with 50 cc. HCl and extracted with ether. The aqueous layer is further extracted repeatedly with ethyl acetate. The extract is washed with water, then with $NaHCO_3$ solution and evaporated to dryness. Recrystallization of the residue from benzene gives 5.5 g. of colorless needles (XI), m.p. 78~79°; yield, 75 %. *Anal.* Calcd. for $C_9H_{17}O_2NS$: C, 53.17; H, 8.43; N, 6.89; S, 15.77. Found: C, 53.30; H, 8.21; N, 6.43; S, 15.20.

ii) **$C_9H_{19}ONS \cdot HCl$ (XII)**—The above aqueous layer is saturated with K_2CO_3 and extracted with ethyl acetate. The ethyl acetate solution is treated with dil. HCl and the acid solution is evaporated at a reduced pressure. Recrystallization of the residue from ethanol gives leaflet crystals (XII), m.p. 208~209°. *Anal.* Calcd. for $C_9H_{19}ONS \cdot HCl$: C, 47.88; H, 8.93; N, 6.20. Found: C, 47.88; H, 9.18; N, 5.82.

iii) **$C_9H_{21}ONS$ (XIII)**—The ethanolic mother liquor of (XII) is evaporated and the residue is dissolved in water. After saturation with K_2CO_3 the aqueous layer is extracted with ethyl acetate. The extract is dried with Na_2SO_4 and evaporated. Fractionation of the residue gives a colorless oil, $b.p_{0.5}$ 135~140°, which gives a positive sulfhydryl test. Recrystallization from benzene gives granular crystals (XIII), m.p. 53~54°. *Anal.* Calcd. for $C_9H_{21}ONS$: C, 56.50; H, 11.06; N, 7.32; S, 16.76. Found: C, 56.29; H, 10.67; N, 6.99; S, 17.19. Desulfurization of (XI) and (XIII) with Raney nickel, followed by condensation with phenyl isocyanate, give urethanes of m.p. 112°, and 275°, respectively.

Summary

Acidomycin, an antibiotic active against *Mycobacterium tuberculosis*, was obtained from the culture broth of *Streptomyces acidomyceticus* by extraction with butyl acetate, and its chemical structure was studied and confirmed to be identical with the thiazolidone antibiotic, 2-(5-carboxypentyl)-4-thiazolidone, isolated by the group at Chas. Pfizer and Company.

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