reflux for 4.5 hr. gave a product which was filtered through alumina to yield a mixture of the hydrocarbon **36** and biphenyl. Steam distillation removed biphenyl and the residue was crystallized from methanol–ether to yield colorless crystals (2.85 g., 9.1 mmoles, 63% yield), m.p. 83–84°; ν (10% in CCl_4) 3050, 3015, 2925, 2855, 1650, 1598 cm.$^{-1}$; (10% in CS_2) 802, 774, 763, 732, 701 cm.$^{-1}$; $\lambda_{max}^{CHCl_3}$ 258 mμ (ϵ 15,300); n.m.r. (30% in CCl_4) 2.7–3.0 (10 H), 4.35 (2H, doublet over broadened singlet), 7.47 (1H, very broad multiplet), 7.80 (1H, multiplet), 8.13 (3H, doublet, J = 1.5 c./sec.), 8.2–9.2 (6H, unresolved), 8.93 τ (3H, singlet).

Anal. Calcd. for $C_{24}H_{26}$: C, 91.67; H, 8.33. Found: C, 91.89; H, 8.38.

Dehydrogenation of the Hydrocarbon 36.—Hydrocarbon **36** (2.00 g., 6.4 mmoles) and 30% palladium-on-charcoal (250 mg.) were heated together in a slow stream of nitrogen which was passed into a Dry Ice trap. The mixture was boiled (approximately 325°) for 1.5 hr. (until evolution of volatile materials ceased). *m*-Xylene (**38**) was isolated from the volatile products in the trap by gas chromatography and short-path distillation (109 mg., 16% yield). Its gas chromatographic retention time and its infrared and n.m.r. spectra were identical with those of authentic *m*-xylene.

Chromatography of the nonvolatile residue from the reaction gave a hydrocarbon mixture which was distilled (short path), b.p. 90–200° (3 mm.). Gas chromatography of the distillate yielded a small amount of 1-phenylnaphthalene (**39**) which was crystallized three times from pentane–ether at −70° and distilled to give a colorless, viscous oil (33 mg., 0.16 mmole, 2.5% yield). Its infrared spectrum was identical with that of authentic 1-phenylnaphthalene (Aldrich Chemical Co.). Nitration[25] of these samples gave 4-nitro-1-phenylnaphthalene, m.p. 129–130° and 129–130°, respectively, m.m.p. 129–130° (lit.[25] m.p. 132°).

Synthesis of 1-Phenylnaphthalene (39) from 1,1-Diphenylbutadiene (37).—A solution of allyl chloride (1.53 g., 20 mmoles) in ether (5 ml.) was added slowly to a mixture of magnesium (0.97 g., 40 mg.-atoms) in ether (5 ml.) with cooling in an ice bath. When addition was complete (15 min.) stirring was continued at 0° for 15 min. To this suspension of allylmagnesium chloride was added a solution of benzophenone (1.82 g., 10 mmoles) in ether (5 ml.) and the reaction mixture was allowed to come to room temperature. After another 2.5 hr. of stirring, work-up yielded crude allyldiphenylcarbinol, which was dissolved in a mixture of phosphorus oxychloride (5 ml.) and pyridine (10 ml.) and stored at room temperature overnight. Work-up with ice gave a product which was chromatographed on Florisil to give a colorless hydrocarbon, probably 1,1-diphenylbutadiene (**37**) which had a marked tendency to polymerize. Dehydrogenation of this product under the same conditions used for the dehydrogenation of the hydrocarbon **36** gave a mixture in which 1-phenylnaphthalene (**39**) was detected by gas chromatography. Nitration of the entire product gave 4-nitro-1-phenylnaphthalene, m.p. 128–129°, undepressed by admixture with authentic material.

Dihydropyran (41).—Tricycloionone (**26**, 1.00 g.) was cooled to 0° and treated with cold concentrated sulfuric acid (1 ml.). The mixture was stirred for 1 min. at 0°; then water (10 ml.) was added. Extraction with pentane yielded an oil which was distilled (short path), b.p. 30–35° (3 mm.). Redistillation, b.p. 70–75° (3 mm.), gave a colorless oil (**41**, 455 mg., 45% yield), ν (10% in CCl_4) 3045, 2920, 1680, 1605 cm.$^{-1}$; $\lambda_{max?}^{heptane}$ 199 mμ (ϵ 4680); n.m.r. (pure liquid) 5.46 (1H, doublet, J = 6 c./sec.), 7.9 (1H, multiplet), 8.1–9.2 (9H, unresolved), 8.38 (3H, broadened singlet), 8.73 (3H, singlet), 9.22 τ (3H, singlet). The spectra of this material indicated the presence of a single compound, but it decomposed on thin-layer or on column chromatography and on gas chromatography, and it resinified easily unless stored under nitrogen in a refrigerator. It reacted slowly with 2,4-dinitrophenylhydrazine reagent, but gave a mixture of derivatives which was not separated.

Anal. Calcd. for $C_{13}H_{20}O$: C, 81.20; H, 10.48. Found: C, 80.62; H, 10.44.

Acknowledgment.—We are indebted to the National Science Foundation Grant G22547 for financial support.

(25) R. Weiss and K. Woidich, *Monatsh.*, **46**, 453 (1925).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASSACHUSETTS]

A Total Synthesis of Holomycin

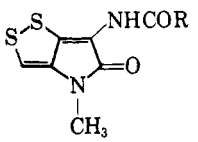
BY G. BÜCHI AND GEORGE LUKAS[1]

RECEIVED AUGUST 7, 1964

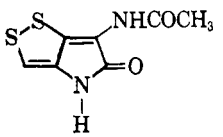
S-Benzyl-L-cysteine ethyl ester was acylated with diketene to form the N-acetoacetyl derivative which was cyclized with sodium ethoxide to racemic 3-acetyl-4-hydroxy-5-(S-benzylthio)methyl-3-pyrrolin-2-one. A novel dehydrogenation reaction using thionyl chloride gave 3-acetyl-4-hydroxy-5-(S-benzylthio)methylene-3-pyrrolin-2-one. This yellow ketone was converted to an oxime which on treatment with *p*-toluenesulfonyl chloride and sodium hydroxide rearranged to 3-acetamido-4-hydroxy-5-(S-benzylthio)methylene-3-pyrrolin-2-one. Tosylation followed by exposure to sodium benzylmercaptide furnished 3-acetamido-4-S-benzylthio-5-(S-benzylthio)methylene-3-pyrrolin-2-one. Removal of the two benzyl groups was accomplished with lithium in liquid ammonia. Air oxidation of crude 3-acetamido-4-sulfhydryl-5-sulfhydrylmethylene-3-pyrrolin-2-one in methanol solution containing hydrochloric acid gave synthetic holomycin identical in every respect with the natural antibiotic.

Certain *Streptomyces* species elaborate yellow-colored metabolites containing the pyrrolinonodithiole nucleus. The four substances thiolutin (**1**),[2] aureothricin (**2**),[2] isobutyropyrrothine (**3**),[3] and holomycin (**4**)[4] are of some interest because of their pronounced activities against a variety of Gram-positive and Gram-negative bacteria, amoeboid parasites, and fungi.

The synthesis of holomycin (**4**) described in this paper is based on the principle of building the two five-membered rings present in the structure at different



1, R = CH_3
2, R = CH_2CH_3
3, R = $CH(CH_3)_2$



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stages. A reasonable possibility was first to construct an appropriately substituted pyrrolone and to close the disulfide ring in a final stage. Treatment of

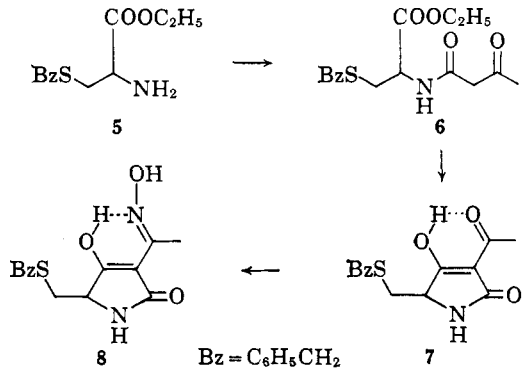
(1) National Institutes of Health Predoctoral Fellow 1961–1963.

(2) W. D. Celmer and I. A. Solomons, *J. Am. Chem. Soc.*, **77**, 2861 (1955), and earlier papers cited.

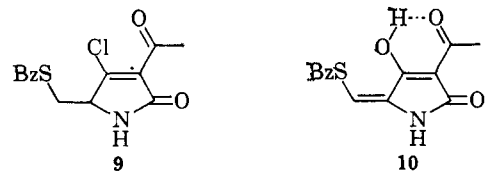
(3) D. S. Bhate, R. K. Hulyalkar, and S. K. Menon, *Experientia*, **16**, 504 (1960).

(4) L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, and H. Zähner, *Helv. Chim. Acta*, **42**, 563 (1959).

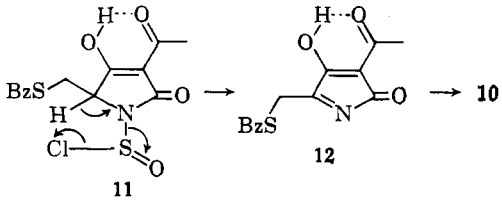
S-benzyl-L-cysteine ethyl ester (**5**)[5] with diketene[6] furnished the oily N-acetoacetyl derivative **6** which was cyclized with sodium ethoxide[6] to the racemic lactam **7**. Unusually intense infrared absorptions at 1710 (γ-lactam), 1665 (carbonyl), and 1610 cm.$^{-1}$ (double bond), remarkable acidity, and ultraviolet absorption at 277 mμ (ϵ 12,700) all indicated a completely enolic structure stabilized by an internal hydrogen bond. An n.m.r. spectrum[7] of the cyclization product **7** with signals at -3.1 (1H, singlet), 2.7 (5H, singlet), 3.2 (1H, singlet), 6.15 (1H, multiplet), 6.25 (2H, singlet), 7.2 (2H, multiplet), and 7.55 τ (3H, singlet) also was in accord with the structure assigned. As anticipated, condensation with hydroxylamine furnished a monooxime (**8**)[8] which combined with ferric chloride to give a blue complex.



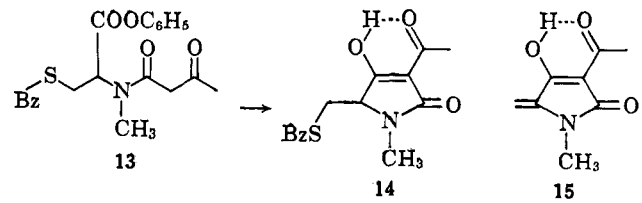
The next stage of the synthesis was initially concerned with the introduction of a protected sulfhydryl group at the C_4-position of the tetramic acid **7**. Attempts to prepare the benzylthioether by condensation with benzyl mercaptan[9] failed, and tosylation produced an ill-defined mixture which on treatment with sodium benzylmercaptide did not yield the desired product either. Next, it was hoped that thionyl chloride would react with the enolic hydroxyl group and that the halogen atom in the resulting chloride (**9**) could subsequently be replaced by a sulfur-containing function. Although the inertness of simple vinyl halides toward nucleophilic substitution is established, β-chloro-α,β-unsaturated carbonyl compounds do undergo this type of change, undoubtedly following an addition–elimination path. When the pyrrolinone **7** was allowed to react with thionyl chloride in benzene at room temperature, a yellow crystalline product separated from the solution. The spectroscopic properties of this substance indicated that it was not the anticipated chloride **9**. The appearance of a second maximum in the ultraviolet spectrum at 350 mμ suggested considerable extension of the original chromophore, and n.m.r. signals attributed to the $-S-CH_2-CH-$ grouping in the precursor **7** were replaced by a new singlet corresponding to one vinylic hydrogen (3.7 τ). These findings agree with structure **10**, but the geometric configuration of the side chain is left in doubt by the evidence available. Presumably it was the amino group rather than the hydrogen-bonded hydroxyl function which served as



nucleophile, and the product actually observed may have originated from the hypothetical chlorosulfite **11**. Decomposition, for example in the manner indicated (arrows in **11**), would lead to a further intermediate (**12**) by extrusion of sulfur monoxide. The latter is known to be in equilibrium with sulfur dioxide and elemental sulfur.[10] If a chlorosulfite is indeed an intermediate



in this oxidation, N-substituted lactams should not undergo this transformation; to test this hypothesis experimentally the N-methyl lactam **14** was prepared. Brief treatment of N-methyl-S-benzyl-L-cysteine ethyl ester[11] with diketene[6] resulted in formation of the N-methyl-N-acetoacetyl derivative **13** which was cyclized with sodium ethoxide at room temperature. The resulting N-methyltetramic acid **14** was also completely racemized, but was much more sensitive to base than its lower homolog **7**. When cyclizations were performed at elevated temperatures benzyl mercaptan was eliminated and the dienelactam **15** was the only isolable product. The spectroscopic properties of the two lactams **14** and **15** were in accord with the structures proposed; additional transformation products of the N-methyllactam **14** are described in the Experimental section. All attempts to dehydrogenate the substituted lactam **14** with thionyl chloride were fruitless. To our knowledge dehydrogenations of secondary

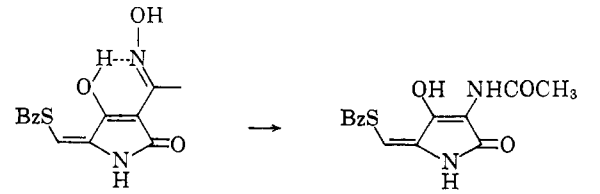


amides by thionyl chloride were unknown, but the literature does describe a few transformations in which this reagent acts as an oxidant. Thus, treatment of pyridine with thionyl chloride yields N-pyridyl-(4)-pyridinium chloride[12] and sulfur. Similarly, benzoin is oxidized to benzil.[13] It is further worthy to note that thionyl chloride was not the only agent capable ofoxidizing the pyrrolinone **7** and a somewhat less efficient conversion to **10** was brought about by isopropyl nitrite in the presence of hydrochloric acid.

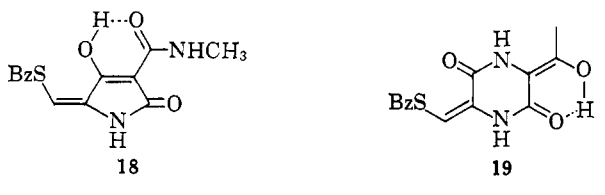
The transformations just described made the lactam

(5) C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, **38**, 417 (1944).
(6) Method of R. N. Lacey, *J. Chem. Soc.*, 850 (1954).
(7) Chemical shifts are recorded in τ-values: G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).
(8) 3-Acetyltetramic acids in general yield only monooximes: C. E. Stickings, *Biochem. J.*, **72**, 332 (1959).
(9) R. E. Ireland and J. A. Marshall, *J. Am. Chem. Soc.*, **81**, 6336 (1959).
(10) H. Zeise, *Z. physik. Chem.*, **B51**, 120 (1942).
(11) M. W. Kies, H. M. Dyer, J. L. Wood, and V. du Vigneaud, *J. Biol. Chem.*, **128**, 207 (1939); M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1114 (1953).
(12) E. Koenigs and H. Greiner, *Ber.*, **64**, 1049 (1931); K. Thomas and D. Jerchel, *Angew. Chem.*, **70**, 719 (1958).
(13) Y. Okumura, *J. Org. Chem.*, **28**, 1075 (1963).

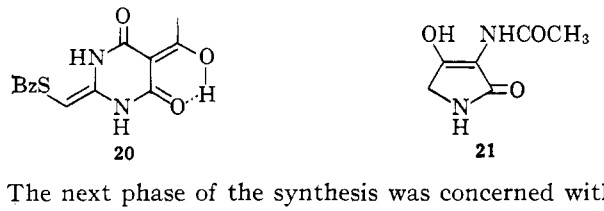
10 a readily available substance and it promised to be a key intermediate in the synthesis because it already contains both carbon–carbon double bonds present in the antibiotic. There was no reason to assume that the chelated hydroxyl group would exhibit greater reactivity in the dienelactam **10** than in its precursor **7** and it was decided to proceed with the conversion of the acetyl to the acetamido side chain. Condensation of the ketone **10** with hydroxylamine produced a single oxime (**16**). The product was suspected to exist entirely in the hydrogen-bonded enol form and this view was strengthened by preparation of an intensely blue ferric complex. It was now possible to take advantage of this particular configuration of the oxime **16**; in practice the oxime underwent a Beckmann rearrangement on treatment with tosyl chloride and sodium hydroxide in aqueous tetrahydrofuran solution. Attempted rearrangement of the oxime **16** directly, under acidic conditions, led to no useful result. Formally, there are three alternative structures for this oxime, each leading to a different product on rearrangement. An n.m.r. spectrum of this new intermediate exhibited a three-proton singlet at 7.85 τ which is characteristic of acetamides, whereas the N-methylamide **18** should give rise to a signal at 7.1–7.2 τ.[14] Further, infrared absorption at 1690 cm.$^{-1}$ is compatible only with the presence of a five-membered lactam, thus excluding structures **19** and **20** from further consideration. Finally, both the rearrangement product **17** and 3-acetamido-2,4-diketopyrrolidine (**21**)[15] gave a blue ferric test, while all enolic β-diketones encountered in this study developed red complexes with this reagent.



16 **17**

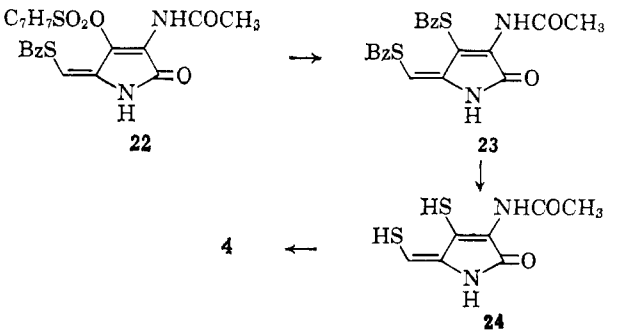


18 **19**



20 **21**

The next phase of the synthesis was concerned with replacement of the enolic hydroxyl group by a sulfur-containing function. It was hoped that destruction of the resonance-stabilized six-membered chelate ring would have greatly enhanced the reactivity of the hydroxyl group and, indeed, tosylation of the enol **17** proceeded smoothly. The resulting tosylate **22** combined readily with sodium benzylmercaptide[16] to yield the desired benzylthioether **23**. Both benzyl groups were removed reductively in liquid ammonia solution



22 **23** **24** **4**

using approximately five equivalents of lithium.[17] Oxidation of the crude dithiol **24** with air in strongly acidic aqueous methanol gave holomycin (**4**) whose melting point, infrared and ultraviolet spectra, and paper chromatographic behavior were identical with the corresponding properties of the natural antibiotic. The melting point of a synthetic sample was undepressed on admixture of natural material.[18] Holomycin was produced only when the dithiol **24** was oxidized in acidic solution. Thin-layer chromatography indicated a sharp decrease in yield at pH 4 and complete absence of the wanted product at pH 9. The reason for this behavior is not entirely clear, but it is not unreasonable to assume that the dithiol **24** and its precursors actually do not have the configurations at the exocyclic double bond indicated in **24** and that geometrical isomerization has to precede oxidation. Such an isomerization would undoubtedly occur through a cationic intermediate whose generation would be accelerated in acidic media. Before our synthesis was completed and announced in preliminary form,[19] Schmidt and Geiger[20] disclosed a synthesis of holomycin (**4**) proceeding along different lines but through the same dithiol intermediate **24**.

Experimental

Microanalyses were performed by Dr. S. M. Nagy and associates, M. I. T., or by the Scandinavian Microanalytical Laboratory, Herlev, Denmark. Melting points determined on a Kofler hot-stage microscope are corrected. Infrared spectra were measured by Mrs. N. F. Alvord on a Baird Model B recording spectrophotometer. High intensity bands and others relevant to structural assignments are listed textually. Ultraviolet spectra were measured in ethanol on a Cary Model 14 recording spectrophotometer. A Varian Associates A-60 instrument was used for recording nuclear magnetic resonance (n.m.r.) spectra. Peak positions are given in τ-values. Optical rotations were determined in a Zeiss polarimeter using a 1-dm. tube. Unless otherwise indicated, organic solutions were dried over anhydrous sodium sulfate. Solvents were evaporated under reduced pressure.

3-Acetyl-4-hydroxy-5-(S-benzylthio)methyl-3-pyrrolin-2-one (7).—Ether (300 ml.) and triethylamine (6 ml.) were added to a solution of S-benzyl-L-cysteine ethyl ester hydrochloride[5] (9.66 g., 0.035 mole) in water (50 ml.). Evaporation of the dried organic phase left the free amino ester (**5**) as a sirup which was dissolved in ethanol (15 ml.). Freshly distilled diketene (3.06 g.) was added at 5° during 10 min. to this stirred solution. The mixture was stirred for another 10 min. at 5° and for 1 hr. at room temperature. Evaporation yielded a pale yellow oil (**6**, 10.7 g.) which gave a purple color reaction with ferric chloride.

(14) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1959, pp. 56, 57.

(15) J. W. Cornforth, "The Chemistry of the Penicillin," Vol. VII, H. T. Clarke, Ed., John Wiley and Sons, Inc., New York, N. Y., 1953, p. 770.

(16) R. B. Woodward, M. P. Cava, W. D. Ollis, A. Hunger, H. V. Daeniker, and K. Schenker, *J. Am. Chem. Soc.*, **76**, 4749 (1954); *Tetrahedron*, **19**, 247 (1963).

(17) K.-D. Gundermann and G. Pape, *Chem. Ber.*, **95**, 2076 (1962).

(18) We wish to thank Professor V. Prelog and Dr. W. Keller-Schierlein, ETH, Zürich, for having performed this comparison.

(19) G. Büchi and G. Lukas, *J. Am. Chem. Soc.*, **85**, 647 (1963).

(20) U. Schmidt and F. Geiger, *Angew. Chem.*, **74**, 328 (1962); *Ann.*, **664**, 168 (1963).

It was dissolved in benzene (20 ml.) and heated to boiling for 3 hr. with a solution of sodium ethoxide (prepared from 0.81 g. of sodium) in 15 ml. of ethanol. After 12 hr. at room temperature the yellow solution was extracted with water (3 × 10 ml.). Extraction of the acidified aqueous solution with ether and removal of the solvent from the dried extract left a solid residue. Recrystallizations from carbon tetrachloride and from ethanol yielded colorless long needles (4 g., 42%), m.p. 114°, bright red color reaction with ferric chloride; ν_{max}^{KBr} 3400–2500, 1710, 1665, 1610 cm.$^{-1}$; λ_{max}^{EtOH} 244, 277 mμ (ε 5100, 12,700); n.m.r. ($CDCl_3$) −3.1 (1H, singlet), 2.7 (5H, singlet), 3.2 (1H, singlet), 6.15 (1H, multiplet), 6.25 (2H, singlet), 7.2 (2H, multiplet), and 7.55 τ (3H, singlet).

Anal. Calcd. for $C_{14}H_{15}NO_3S$: C, 60.64; H, 5.45; N, 5.05; S, 11.54. Found: C, 60.44; H, 5.52; N, 5.00; S, 11.45.

Oxime 8.—A mixture of **7** (0.277 g., 1 mmole), hydroxylamine hydrochloride (0.21 g.), and sodium acetate (0.25 g.) in 66% aqueous ethanol (6 ml.) was heated to 60–70° for 3 min. when a clear solution was obtained. It was cooled to room temperature and allowed to react for 2 days. A colorless monooxime (0.23 g., 79%) deposited from the solution and recrystallization from 66% aqueous ethanol afforded colorless needles, m.p. 160°; blue color reaction with ferric chloride; ν_{max}^{KBr} 3500–2600, 1680, 1635, 1585, 1525, 1490, 700 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{16}N_2O_3S$: C, 57.53; H, 5.52; N, 9.59; S, 10.95. Found: C, 57.40; H, 5.62; N, 9.65; S, 10.90.

3-Acetyl-4-hydroxy-5-(S-benzylthio)methylene-3-pyrrolin-2-one (10).—Thionyl chloride (0.24 g., 2 mmoles) was added to a solution of (**7**, 0.277 g., 1 mmole) in benzene (5 ml.). Yellow crystals (0.22 g., 84%) deposited from the reaction mixture after 24 hr. at room temperature. Recrystallization from ethanol afforded an analytical sample, m.p. 170° (sublimed at 140° (0.05 mm.)); dark brown color reaction with ferric chloride; ν_{max}^{KBr} 3500–2500 1710, 1690, 1660, 1600 cm.$^{-1}$; λ_{max}^{EtOH} 288, 350 mμ (ε 18,500, 11,600); n.m.r. (d_7-DMF) 0.85 (1H, singlet), 2.9 (5H, singlet), 3.7 (1H, singlet), 6.05 (2H, singlet), 7.85 τ (3H, singlet).

Anal. Calcd. for $C_{14}H_{13}NO_3S$: C, 61.07; H, 4.76; N, 5.08; S, 11.64. Found: C, 60.81; H, 4.82; N, 5.04; S, 11.71.

Reaction of 7 with Isopropyl Nitrite.—Isopropyl nitrite (0.12 g., 1.25 mmoles) and 1 drop of concentrated hydrochloric acid were added to a solution of **7** (0.277 g., 1 mmole) in tetrahydrofuran (2 ml.). The orange solution was allowed to stand overnight in a refrigerator and finally for 5 hr. at room temperature. Evaporation left a semisolid residue. Trituration with ether gave a yellow solid (0.09 g., 35%), m.p. 165–168°, which on recrystallization from ethanol afforded yellow plates, m.p. 170°. Infrared and ultraviolet spectra, melting point, mixture melting point, sublimation temperature and ferric chloride test established identity of this compound with the lactam **10**.

Anal. Calcd. for $C_{14}H_{13}NO_3S$: C, 61.07; H, 4.76; S, 11.64. Found: C, 60.98; H, 4.74; S, 11.68.

Oxime 16.—A solution of hydroxylamine hydrochloride (0.76 g., 11 mmoles) and sodium acetate (0.90 g., 11 mmoles) in water (40 ml.) was added to **10** (0.95 g., 3.45 mmoles) in tetrahydrofuran (42 ml.). After 2 days at room temperature the tetrahydrofuran was evaporated to yield a yellow solid. Recrystallization from ethanol gave pale yellow prisms (0.71 g., 71%), m.p. 158–160°; dark blue color reaction with ferric chloride; ν_{max}^{KBr} 3400–2600, 1680–1620, 1600 cm.$^{-1}$; λ_{max}^{EtOH} 297, 334 mμ (ε 15,600, 18,700).

Anal. Calcd. for $C_{14}H_{14}N_2O_3S$: C, 57.93; H, 4.86; N, 9.65; S, 11.02. Found: C, 57.74; H, 4.97; N, 9.70; S, 11.06.

3-Acetamido-4-hydroxy-5-(S-benzylthio)methylene-3-pyrrolin-2-one (17).—Sodium hydroxide (0.06 g., 1.4 mmoles) in water (6 ml.) was added during 30 min. to a stirred solution of the oxime **16** (0.154 g., 0.5 mmole) and tosyl chloride (0.095 g., 0.5 mmole) in tetrahydrofuran (5 ml.) at 50–55°. The dark red solution was allowed to react at 60–65° for 30 min., then it was cooled to room temperature and acidified with dilute hydrochloric acid. Evaporation of the tetrahydrofuran was followed by extraction of the residual mixture with benzene. The organic layer was washed with water, dried, and evaporated to leave a brown solid. This residue was washed with a small amount of warm ethanol, and the insoluble, tan solid was collected (0.045 g., 28%). It had m.p. 199–200° with partial sublimation beginning at 185°. An analytical sample was obtained by filtration of a chloroform solution of the crude product through a column of alumina (Woelm, activity IV) and subsequent recrystallization from ethanol. The pale yellow prisms gave a faint blue color reaction with ferric chloride; $\nu_{max}^{CHCl_3}$ 3440, 3390, 3250, 3000, 1690, 1650, 1600, 1525 cm.$^{-1}$; λ_{max}^{EtOH} 235, 330, mμ (ε 10,000, 23,700); n.m.r. ($CDCl_3$) −1.33 (1H, singlet), 2.7 (5H, singlet), 3.88 (1H, singlet), 6.0 (2H, singlet), 7.85 τ (3H, singlet).

Anal. Calcd. for $C_{14}H_{14}N_2O_3S$: C, 57.93; H, 4.86; N, 9.65; S, 11.02. Found: C, 57.92; H, 4.89; N, 9.64; S, 11.11.

Tosylate 22.—Tosyl chloride (1.8 g., 9.5 mmoles), triethylamine (3,3 ml., 2.4 mmoles), and **17** (2.3 g., 7.93 mmoles) were allowed to react in tetrahydrofuran (50 ml.) overnight at room temperature. Evaporation of the filtered reaction mixture left a brown residue which was dissolved in warm benzene. The solution was washed with water, dried, and evaporated. Recrystallization of the residue from ethanol afforded 2.8 g. (80%) of pale yellow prisms, m.p. 200–204° dec.; $\nu_{max}^{CHCl_3}$ 3400, 3200, 1700, 1650, 1625, 1600, 1510, 1390, 1190, 1170 cm.$^{-1}$; λ_{max}^{EtOH} 218, 356 mμ (ε 18,000, 21,000).

Anal. Calcd. for $C_{21}H_{20}N_2O_5S_2$: C, 56.75; H, 4.54; N, 6.30; S, 14.40. Found: C, 56.80; H, 4.57; N, 6.41; S, 14.53.

3-Acetamido-4-S-benzylthio-5-(S-benzylthio)methylene-3-pyrrolin-2-one (23).—Powdered sodium methoxide (0.68 g., 0.0125 mole) and freshly distilled benzyl mercaptan (1.5 ml., 0.0125 mole) were dissolved in ethanol (60 ml.). To this solution was added **22** (4.44 g., 0.01 mole) in tetrahydrofuran (80 ml.) and the mixture was heated to boiling for 8 hr. under an atmosphere of nitrogen. Evaporation of the solvents left a residue which was partitioned between chloroform and water. Removal of the solvent from the dried organic phase gave a brown solid, which upon recrystallization from ethanol yielded 1.59 g. (40%) of yellow prisms, m.p. 180°; ν_{max}^{KBr} 3400, 3250, 1690, 1660, 1625, 1600, 1525 cm.$^{-1}$; λ_{max}^{EtOH} 360 mμ (ε 23,400); n.m.r. ($CDCl_3$) 2.6–2.7 (10H, broad), 3.8 (1H, singlet), 6.05 (2H, singlet), 6.15 (2H, singlet), 7.9 τ (3H, singlet).

Anal. Calcd. for $C_{21}H_{20}N_2O_2S_2$: C, 63.63; H, 5.09; N, 7.07; S, 16.16. Found: C, 63.73; H, 5.12; N, 6.90; S, 16.09.

Holomycin (4).—Dry liquid ammonia (20 ml.) was collected in a 50-ml. flask fitted with a gas inlet tube, a magnetic stirrer, a Dry Ice condenser, and a soda-lime drying tube. A solution of the benzylthioether **23** (0.22 g., 0.5 mmole) in tetrahydrofuran (5 ml.) was introduced, followed by a piece of lithium wire (0.018 g., 2.5 mg.-atoms). The lithium was allowed to react with the vigorously stirred solution during 6–8 min., and a turbid, orange-colored mixture resulted. Evaporation left a solid which was dissolved in methanol (25 ml.). Hydrochloric acid (20%) was added dropwise until pH 2, and air was bubbled through the solution for 4 hr. while constant volume was maintained. Preparative thin-layer chromatography (benzene–methanol [9:1] on silica gel G) separated a bright yellow product (R_f 0.3) from more polar and inorganic materials. Removal of the yellow regions from the plates, extraction of the silica gel with acetone, and evaporation of the filtered extract left a crystalline yellow solid (18 mg., 15%). One recrystallization from methanol–ethyl acetate yielded shiny, orange, diamond-shaped plates, m.p. 265–271° dec. (lit. m.p. 264–271° dec.); ν_{max}^{KBr} 3400, 3200, 3000, 1660, 1630, 1595, 1540 cm.$^{-1}$. The spectrum was superimposable on the published[4] curve; λ_{max}^{EtOH} 246, 302, 385 mμ (ε 5400, 3000, 10,800), identical with the published[4] curve. A mixture melting point with an authentic sample showed no depression. Chromatography on a circular filter paper (Whatman No. 1) using a *n*-butyl acetate–*n*-butyl ether–10% aqueous sodium cresotinate (3:1:4) system could not distinguish between synthetic and a natural holomycin.

Experiments in the N-Methyl Series. 1-Methyl-3-acetyl-4-hydroxy-5-(S-benzylthio)methyl-3-pyrrolin-2-one (14).—N-Methyl-S-benzyl-L-cysteine[11] was esterified in 84% yield according to the thionyl chloride technique of Brenner and Huber.[11] The colorless prisms of the ethyl ester hydrochloride had m.p. 101°.

Anal. Calcd. for $C_{13}H_{20}ClNO_2S$: C, 53.87; H, 6.93; N, 4.83; S, 11.06. Found: C, 53.75; H, 6.95; N, 4.76; S, 11.20.

The free amino ester was liberated from the hydrochloride (85 g., 0.29 mole) and subsequently treated with diketene as described for the preparation of **6**. Cyclization of the crude N-acetoacetyl derivative **13** with an equimolar amount of sodium ethoxide at room temperature overnight gave a yellow sirup which solidified after a few hours. Recrystallization from cyclohexane yielded prisms (56 g., 65.5%), m.p. 62–63°, bright red color reaction with ferric chloride. An analytical sample was obtained upon recrystallization from ethanol; $\nu_{max}^{CHCl_3}$ 3400, 2950, 1705, 1650–1600, 1495, 1250, 700 cm.$^{-1}$; λ_{max}^{EtOH} 283 mμ (ε 10,800); n.m.r. ($CDCl_3$) −3.8 (1H, singlet), 2.7 (5H, singlet), 6.15 (1H,

triplet, J = 4 c./sec.) 6.3 (2H, singlet), 7.05 (2H, doublet, J = 4 c./sec.), 7.1 (3H, singlet), 7.55 τ (3H, singlet).

Anal. Calcd. for $C_{15}H_{17}NO_3S$: C, 61.85; H, 5.88; N, 4.81; S, 11.00. Found: C, 61.88; H, 5.97; N, 4.87; S, 10.91.

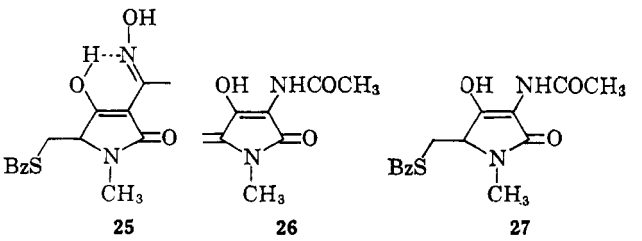
Oxime 25 was prepared from 29.1 g. (0.1 mole) of **14** by the technique described previously. However, the product did not separate from the reaction mixture. Evaporation of the ethanol left an oil–water mixture which was extracted with benzene. Removal of the solvent from the dried organic phase gave a sirup which upon slow crystallization from benzene yielded colorless crystals (27 g., 88%), m.p. 60–62°, blue color reaction with ferric chloride; $\nu_{max}^{CHCl_3}$ 3400–2500, 1670, 1610, 1580 cm.$^{-1}$; λ_{max}^{EtOH} 248, 311 mμ (ϵ 6350, 14,300).

Anal. Calcd. for $C_{15}H_{18}N_2O_3S$: C, 58.81; H, 5.92; N, 9.15; S, 10.45. Found: C, 59.20; H, 6.01; N, 8.96; S, 10.31.

1-Methyl-3-acetyl-4-hydroxy-5-methylene-3-pyrrolin-2-one (15).—When the cyclization of the N-acetoacetyl derivative **13** was performed at 80° for 3 hr., a yellow sirup was obtained which could not be induced to crystallize. Sublimation under high vacuum produced colorless plates of the diene lactam **15**, 15%, m.p. 80°; ν_{max}^{KBr} 1710, 1672, 1630, 1280, 1180, 940, 860 cm.$^{-1}$; λ_{max}^{EtOH} 228, 267 mμ (ϵ 8350, 23,400); n.m.r. ($CDCl_3$) −2.5 (1H, broad singlet), 4.66 (1H, triplet $J \sim 1$ c./sec.), 5.3 (1H, multiplet), 6.90 (3H, doublet $J \sim 1$ c./sec.), 7.45 τ (3H, narrowly spaced doublet).

Beckmann Rearrangement of Oxime 25.—Sodium hydroxide (0.24 g., 6 mmoles) in water (24 ml.) was added to a stirred solution of oxime **25** (0.61 g., 2 mmoles) and tosyl chloride (0.38 g., 2 mmoles) in 20 ml. of acetone at 50° during 20 min. After 12 hr. at 65° the solution was cooled, acidified with dilute hydrochloric acid, and concentrated. Extraction of the residue with benzene and removal of the solvent from the dried extract left a mixture of an oil and a small amount of light yellow crystals. Ether was added to dissolve the oil and the crystals were collected. In this manner light yellow cubes (0.066 g., 19%), m.p. *ca.* 190° (with sublimation), were obtained. Recrystallization from ethanol afforded an analytical sample of the substituted acetamide **26**. The pure compound gave a faint blue color reaction with ferric chloride; ν_{max}^{KBr} 3400–2600, 1690, 1670, 1640, 1540, 880, 840 cm.$^{-1}$; λ_{max}^{EtOH} 268, 342 mμ (ϵ 20,600, 1740); n.m.r. ($CDCl_3$) −1.00 (1H, singlet), 4.83 (1H, doublet, J = 2 c./sec.), 5.18 (1H, doublet, J = 2 c./sec.), 6.92 (3H, singlet), 7.75 τ (3H, singlet).

Anal. Calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.96; H, 5.62; N, 15.72.



Slow evaporation of the ethereal filtrate left an oil which solidified partially after several days. Repeated trituration with an ether–cyclohexane mixture resulted in the separation of a solid which was recrystallized from ethanol to yield colorless needles of **27** (0.091 g., 13%), m.p. 124°. Several recrystallizations raised the melting point to 154°; $\nu_{max}^{CHCl_3}$ 3400, 3200, 3000, 1690–1660, 1630, 1540, 700 cm.$^{-1}$; λ_{max}^{EtOH} 210, 270 mμ (ϵ 20,000, 5400); blue color with ferric chloride; n.m.r. ($CDCl_3$) −1.66 (1H, singlet), 1.33 (1H, broad), 2.7 (5H, singlet), 6.05 (1H, triplet, J = 4 c./sec.), 6.28 (2H, singlet), 7.1 (2H, doublet, J = 4 c./sec.), 7.16 (3H, singlet), 7.8 τ (3H, singlet).

Anal. Calcd. for $C_{15}H_{18}N_2O_3S$: C, 58.81; H, 5.92; N, 9.15; S, 10.45. Found: C, 58.84; H, 6.05; N, 9.13; S, 10.48.

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Synthesis of 4-Thio-D- and -L-ribofuranose and the Corresponding Adenine Nucleosides[1,2]

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The synthesis of 4-thio-D-ribose derivatives from L-lyxose is described. Methyl 2,3-O-isopropylidene-4-(*p*-tolylsulfonyl)-α-L-lyxopyranoside (Xa) when treated with sodium thiolbenzoate in N,N-dimethylformamide gave methyl 4-S-benzoyl-2,3-O-isopropylidene-4-thio-β-D-ribopyranoside (XIa). Deacetonation of this product followed by acetolysis gave 1,2,3,5-tetra-O-acetyl-4-thio-D-ribofuranose (XIVa) which was converted directly to 4′-thio-D-adenosine (XXVIIIa). In a similar fashion 1,2,3,5-tetra-O-acetyl-4-thio-L-ribofuranose (XIVb) and 4′-thio-L-adenosine (XXVIIIb) were prepared from D-lyxose. Deacetylation of the XIVb gave 4-thio-L-ribofuranose (IVb) as a sirup. Spectroscopic evidence indicated that IVb exists primarily in the thiofuranose form. Reactions such as acylation or glycoside formation also occur to give the thiofuranose derivatives. The sulfur atom of compound IVb easily reacts as a thiol, however, as indicated by easy disulfide formation and rapid iodine uptake, thus indicating a facile equilibrium between furanose (IV) and pyranose (VI) forms, a situation quite different from that reported for 5-thio-D-ribose.

There has been a great deal of activity in recent years in the synthesis of 5-thio sugars in which the pyranose ring oxygen has been replaced by sulfur. Thus, 5-thio-D-xylose,[3] 5-thio-D-ribose,[4] and 5-thio-D-glucose[5] among others have been synthesized. In all cases, the sulfur atom assumed the ring position to give a thiopyranose (III) configuration rather than the isomeric furanose (I) involving oxygen ring closure. A few 6-thiohexoses have been prepared[6]; however, no information was given concerning the ability of the sulfur atom to form a seven-membered ring.

The widespread occurrence in biological systems of D-ribose in its furanose form and the knowledge that substitution of sulfur for oxygen in compounds of biological importance has resulted in analogs of chemotherapeutic value made it of interest to investigate the synthesis of 4-thio-D-ribose and its derivatives.

It seemed reasonable to expect that the driving force which caused sulfur to assume the ring position of a thiopyranose might carry through to the 4-thio sugars

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