

## PEPTIDE-TYPE ANTIBIOTICS

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The antibiotic Telomycin, isolated from a culture produced by an unidentified *Streptomyces*, was first reported<sup>1</sup> in 1958. The relatively low toxicity and high activity against Gram-positive organisms<sup>2</sup> mark Telomycin as a potentially interesting antibiotic from a medical standpoint.

The sample<sup>3</sup> employed for structural studies appeared to be essentially homogeneous on the basis of counter-current distribution studies, paper chromatography and electrophoresis. Telomycin is easily detected on paper chromatography by a characteristic blue fluorescence in the ultra-violet, or by ninhydrin, or by the t-butyl hypochlorite starch-iodide reagent. Determination of molecular weight by various methods indicates an approximate value of 1100–1200.

Acidic hydrolysis of Telomycin, followed by ion-exchange chromatography or by electrophoresis, revealed glycine, alanine, serine, aspartic acid, threonine, allo-threonine, erythro- $\beta$ -hydroxyleucine<sup>4</sup> and two new amino-acids which we have identified as 3-hydroxyprolines.

The structure of erythro- $\beta$ -hydroxyleucine was established on the pure, isolated amino-acid by periodate degradation to isobutyraldehyde and by hydriodic acid reduction to leucine as illustrated in *Figure 1*. The configuration was established as erythro by comparison with synthetic samples; the

m.p. 211–212° (dec.),  $[\alpha]_D^{28}$  24.95 (1% in water)

$[\alpha]_D^{28}$  34.8 (0.4% in N HCl)

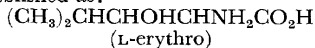
$C_6H_{13}NO_3$  molecular formula

Ninhydrin positive. One mole  $CO_2$  released.

Consumes one mole of periodate to give isobutyraldehyde.

Reduces to leucine with hydriodic acid.

Formula established as:



*Figure 1.* A  $\beta$ -hydroxyleucine from Telomycin

amino-acid was assigned the L-configuration by application of Clough-Lutz-Jirgensons rule and enzymatic evidence. Synthetic *N*-acetyl-erythro- $\beta$ -hydroxy-DL-leucine was resolved enzymatically to produce synthetic erythro- $\beta$ -hydroxy-L-leucine, which was shown to be identical in chemical and physical properties to the amino-acid isolated from Telomycin.

The structures of the two new hydroxyprolines were deduced on the basis of elemental analyses and reduction to proline by hydriodic acid (*Figure 2*). The structural assignment has been fully confirmed by comparison with the racemates of 3-hydroxyproline synthesized by an unambiguous route, as outlined in *Figure 3*.

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- (i) Two isomeric amino-acids isolated by ion-exchange chromatography.  $C_5H_9NO_3$ ; ninhydrin gives yellow colour.
- (ii) Hydriodic acid and red phosphorus produces proline, but they are different (electrophoresis, paper chromatography, optical rotation) from the known 4-hydroxyprolines.
- (iii) These hydroxyprolines are designated "slow" and "fast" moving, which relates to behaviour upon electrophoresis.

Figure 2. Hydroxyprolines from Telomycin

5-Phthalimido-2-pentenoic acid was converted to 2-bromo-3-methoxy-5-phthalimido pentanoic acid by the general procedure used by Carter and West in their synthesis of threonine. After separation by crystallization, the racemates were individually converted into 3-methoxyprolines. The phthalimido group was removed by one equivalent of base followed by hydrolysis in *n* hydrochloric acid. Basification of the amine hydrochloride brought about cyclization to the 3-methoxyproline structure. The methoxy group was cleaved to afford the individual racemic 3-hydroxyprolines. The hydroxyprolines from Telomycin were shown to correspond to the synthetic products by electrophoresis, paper chromatography and colour reactions.

From the alkaline hydrolysis (barium hydroxide) of Telomycin the amino-acids glycine, alanine, aspartic acid, the two 3-hydroxyprolines,

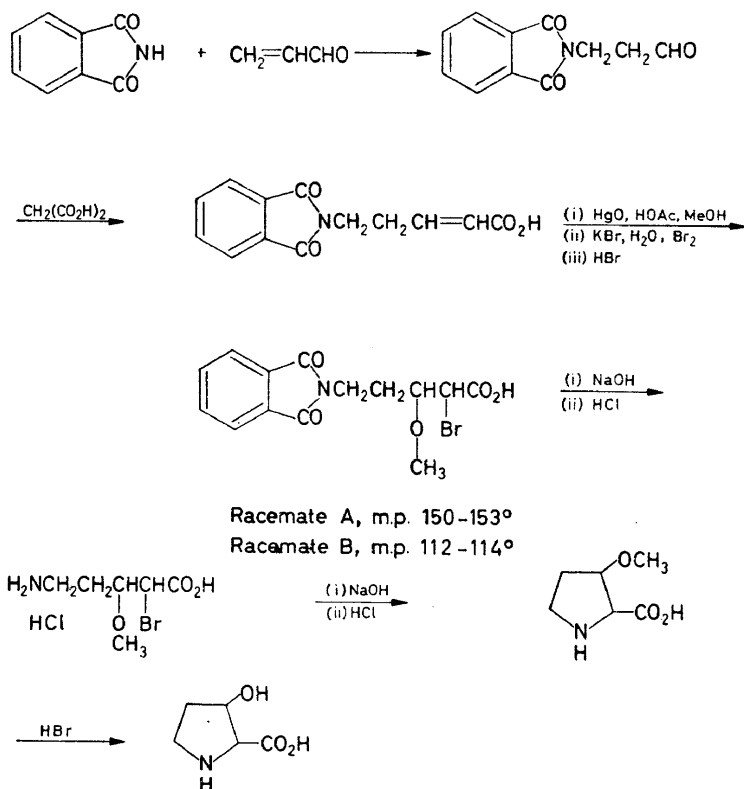


Figure 3. Synthesis of 3-hydroxyprolines

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tryptophan and a tryptophan analogue were separated. As anticipated, serine, threonine, and hydroxyleucine were largely destroyed in the alkaline medium. The identification of tryptophan was made on the basis of comparison of the infra-red spectra of the isolated natural product and the corresponding dinitrophenyl derivative with authentic tryptophan and DNP-tryptophan samples. The "tryptophan analogue" was identified as  $\beta$ -methyltryptophan on the basis of mass spectrograph data (courtesy of Professor K. Biemann, MIT) and comparison with a synthetic sample kindly provided by Professor H. R. Snyder of the University of Illinois (Figure 4).



Figure 4. Identification of two "tryptophans" in Telomycin

Certainly the best known of the peptide-type antibiotics are the penicillins. Classification of the penicillins as peptide in nature is justified by the production of amino-acids on total hydrolysis and also by the fact that biogenetically the penicillins have been shown to arise from L-cysteinyl-L-valine.

Our announcement<sup>5</sup> in March 1958 that "... we have prepared this compound [6-amino-penicillanic acid (6APA)] via a totally synthetic route ... We have shown that one can acylate with various acid chlorides and obtain the corresponding penicillins," together with the development of industrially feasible biochemical routes to 6APA has been followed by the phenomenal growth of the "new penicillins". Although the enzymatic removal of the natural penicillin side-chain is reported to be an efficient process, it seemed worthwhile to investigate a purely chemical means for converting penicillin G to 6APA derivatives bearing other side chains.

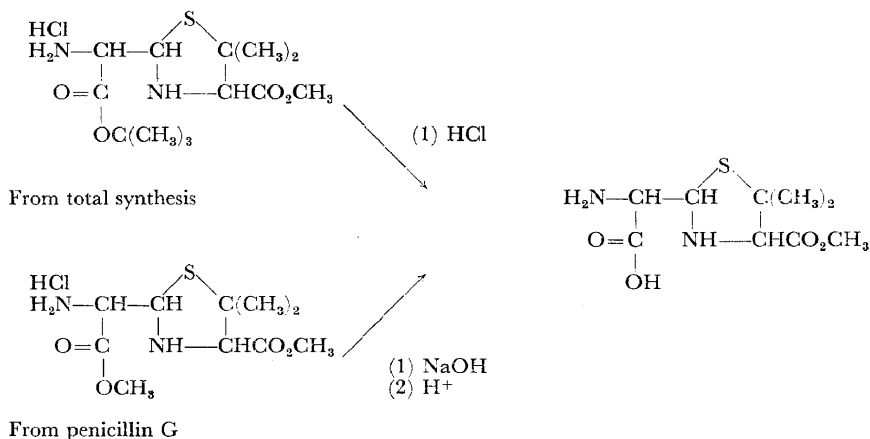


Figure 5. Relation of the partial and total synthetic series

Since the phenylacetamido group has the usual low reactivity of a typical amide toward acidic or basic hydrolyses and the  $\beta$ -lactam amide function is extremely susceptible to solvolyses, the chemical replacement of the side chain without opening the  $\beta$ -lactam ring appears to be a formidable task. Our laboratory has previously reported<sup>6</sup> the removal of the penicillin G side chain and concomitant ring opening and closing to effect a "partial synthesis" of 6APA from penicillin G. This involves degradation to an intermediate in the totally synthetic scheme for 6APA and the penicillins devised in this laboratory; *Figures 5, 6, 7 and 8* illustrate this approach.

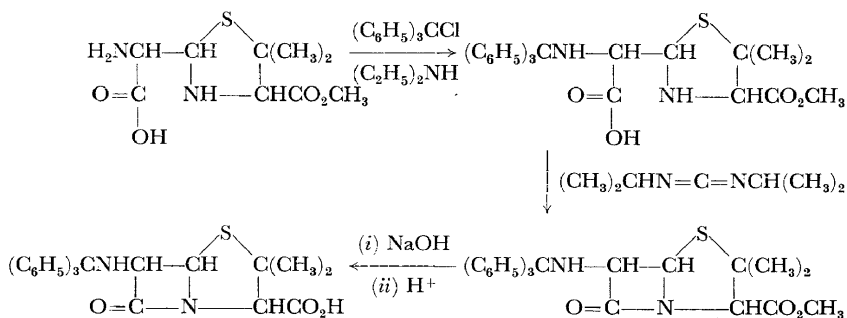


Figure 6. Synthesis of 6-tritylamino-penicillanic acid



Figure 7. Partial (from penicillin G) and total synthesis of 6-aminopenicillanic acid

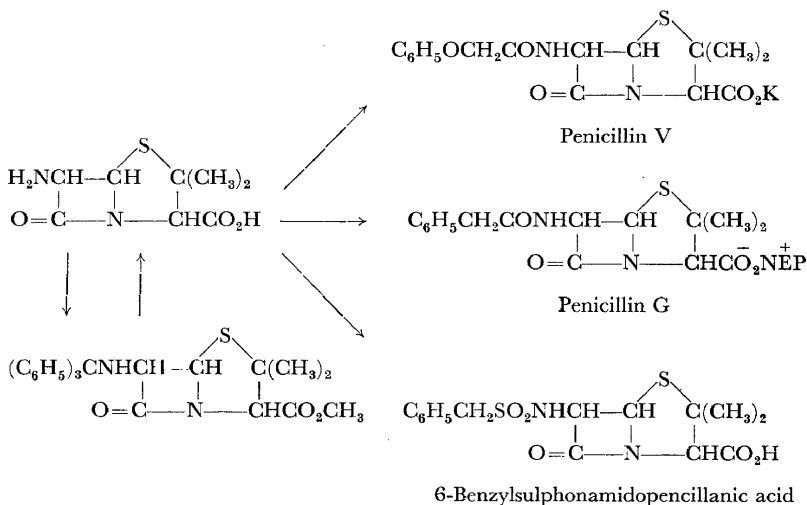


Figure 8. Partial and total general synthesis of penicillins



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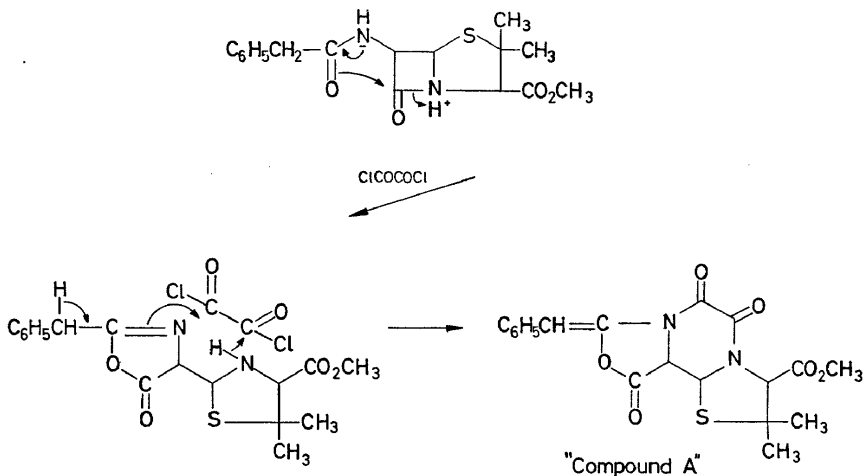


Figure 12. A possible mechanism for the formation of "Compound A"

suggested in *Figure 12*. Since this mechanism presumes an acid catalysis the reaction was repeated in the presence of pyridine and using the oxalyl chloride-dioxan complex, as illustrated in *Figure 13*. The crystalline product, obtained in 50–60 per cent yield, showed the typical ultra-violet fluorescence of oxazolidine-4,5-diones. The spectral evidence again

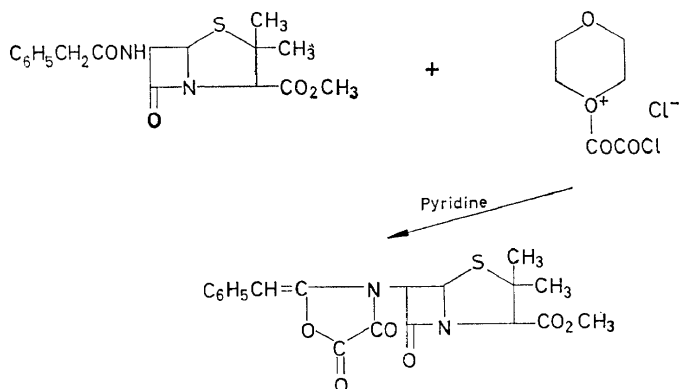


Figure 13. Reaction of methyl benzylpenicillinate with oxalyl chloride-dioxan complex and pyridine

confirmed the assigned structure. A parallel reaction took place with benzyl benzylpenicillinate and the crystalline product was hydrogenolyzed, as shown in *Figure 14*.

Benzyl benzylpenicillinate oxazolidine-4,5-dione can be reduced to the corresponding saturated structure with zinc and buffered acetic acid (*Figure 15*). Of particular interest is the fact that the reduced product was

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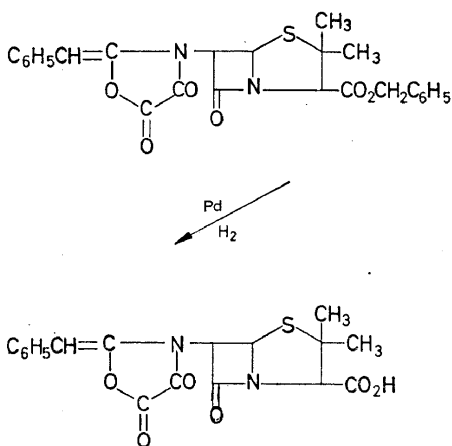


Figure 14. Hydrogenolysis of benzyl ester

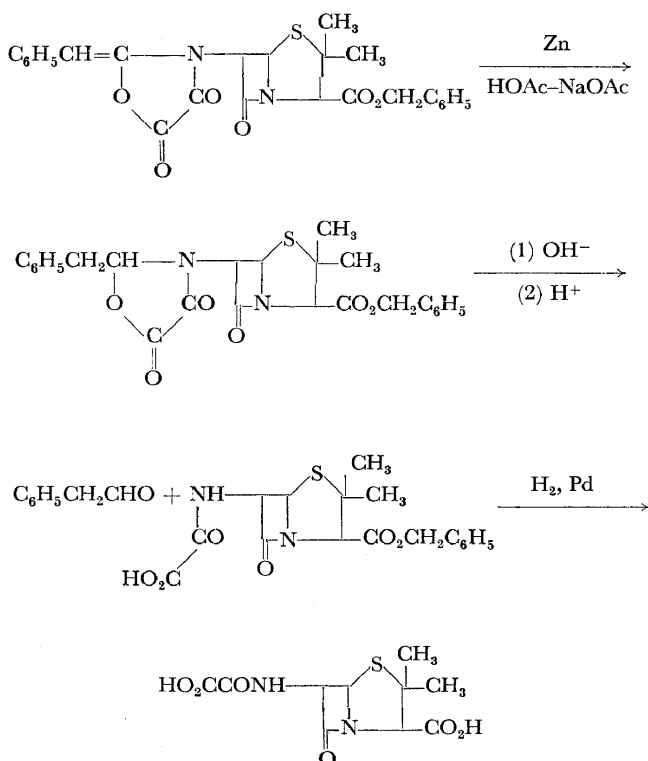


Figure 15. Reduction of benzyl benzylpenicillinate oxazolidine-4,5-dione and preparation of 6-oxamidopenicillanic acid

readily hydrolyzed in nearly neutral solution to produce almost quantitatively phenylacetaldehyde (isolated as the 2,4-dinitrophenylhydrazone) and 6-oxamidopenicillanic acid as the benzyl ester. Hydrogenolysis gave 6-oxamidopenicillanic acid, thereby completing the first interchange of functions on the intact 6-aminopenicillanic acid nucleus by purely chemical means. Experiments designed to produce 6APA itself are continuing.

## References

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- <sup>4</sup> J. C. Sheehan, K. Macda, A. K. Sen, and J. A. Stock. *J. Am. Chem. Soc.* **84**, 1303 (1962).
- <sup>5</sup> J. C. Sheehan in *Amino Acids and Peptides with Antimetabolic Activity*, (Eds. G. E. W. Wolstenholme and C. M. O'Connor), p. 258 J. A. Churchill Ltd., London (1958); *See also* J. C. Sheehan, *Canadian Patent* 610,096 (Dec. 6, 1960); *Canadian Patent* 619,205 (April 25, 1961); Patent Application in the United States, March 1, 1957.
- <sup>6</sup> J. C. Sheehan and K. R. Henery-Logan. *J. Am. Chem. Soc.* **81**, 5838 (1959).
- <sup>7</sup> J. C. Sheehan and E. J. Corey. *J. Am. Chem. Soc.* **74** 360 (1952); *cf.* R. Stolle and M. Luther. *Ber.* **53**, 314 (1920).
- <sup>8</sup> *Chemistry of Penicillin*, (Eds. H. T. Clarke, J. R. Johnson, and R. Robinson, pp. 239-242, Princeton University Press, New Jersey (1949).