PEPTIDE-TYPE ANTIBIOTICS

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

The sample 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-β-hydroxy-leucine and two new amino-acids which we have identified as 3-hydroxyprolines.

The structure of erythro-β-hydroxy-leucine 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

\[
\text{m.p. 211–212° (dec.), } [\alpha]_{D}^{28} 24-95 \text{ (1% in water)} \\
[a]_{D}^{28} 34-8 \text{ (0-4% in } \text{HCl)}
\]

C_{9}H_{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:

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(\text{CH}_{3})_{2}\text{CHCHOHCHNH}_{2}\text{CO}_{2}\text{H}
\]

(*l*-erythro)

*Figure 1.* A β-hydroxy-leucine from Telomycin

amino-acid was assigned the *l*-configuration by application of Clough-Lutz-Jirgensons rule and enzymatic evidence. Synthetic *N*-acetyl-erythro-β-hydroxy-*l*-leucine was resolved enzymatically to produce synthetic erythro-β-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*. 297
(i) Two isomeric amino-acids isolated by ion-exchange chromatography. C₅H₆NO₃; 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-Pthalimido-2-pentenoic acid was converted to 2-bromo-3-methoxy-5-pthalimido 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,
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](image)

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 $\alpha$-cysteinyl-$\alpha$-valine.

Our announcement in March 1958 that "... we have prepared this compound [6-amino-panicillanic 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](image)
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Since the phenylacetamido group has the usual low reactivity of a typical amide toward acidic or basic hydrolyses and the β-lactam amide function is extremely susceptible to solvolyses, the chemical replacement of the side chain without opening the β-lactam ring appears to be a formidable task. Our laboratory has previously reported\(^6\) 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.

![Chemical structures](image)

**Figure 6.** Synthesis of 6-tritylamopenicillanic acid

![Chemical structures](image)

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

![Chemical structures](image)

**Figure 8.** Partial and total general synthesis of penicillins

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The phenylacetamido side chain of penicillin G is normally far less reactive than is the $\beta$-lactam function. However, advantage might be taken of the fact that the side chain is a mono-substituted amide whereas the $\beta$-lactam is di-substituted. For example, one reaction which might differentiate between these two functions is interaction with oxalyl chloride, as illustrated in Figure 9. This reaction had been investigated briefly in the World War II

$$C_6H_5CH_2CONHCH_2CO_2CH_2C_6H_5 + ClCOCOCl \rightarrow C_6H_5CH=CC\overset{O}{\nrightarrow}NCH_2CO_2CH_2C_6H_5$$

Figure 9. Condensation products of oxalyl chloride with monosubstituted amides.

co-operative penicillin project as part of the structural studies. The reaction, as carried out in ether, with methyl benzylpenicillinate gave an unidentified crystalline product which apparently did not possess the $\beta$-lactam structure and was biologically inactive. These previously reported results are summarized in Figure 10.

$$C_6H_5CH_2CONHCH(CH_3)_2 \overset{S}{\nrightarrow}N\overset{\text{COCOCl}}{\nrightarrow}CHCO_2CH_3$$

Compound A: m.p. 170° "felted crystals".
Analysis corresponds to $C_{19}H_{18}O_5N_2S$
(Methyl penicillinate + oxalyl chloride minus two equivalents of hydrogen chloride)
u.v. 262 m$\mu$ ($E_m = 15,600$).
Compund B: Impure, m.p. 96–102°, apparently isomeric.

Figure 10. Reaction of methyl benzylpenicillinate with oxalyl chloride (ether)*

With Dr W. von Philipsborn the reaction was re-investigated. Our observations are outlined in Figure 11. On the basis of infra-red and ultra-violet spectra data, together with mechanistic considerations, we propose the structure shown in Figure 12 for the product obtained by interaction of methyl benzylpenicillinate with oxalyl chloride in ether. A mechanism is also

Compound A: m.p. 172–173°, pale yellow needles
Analysis corresponds to $C_{19}H_{18}O_5N_2S$
u.v.—262 m$\mu$ (log $\epsilon = 4.19$)
i.r.—($\text{CHCl}_3$)—Strong 1835 cm$^{-1}$, 1745 cm$^{-1}$
Broad at 1720–1680 cm$^{-1}$
No NH, OH, $\beta$-lactam or amide II
M.W. (Rast) 350
No u.v. fluorescence

Figure 11. Re-investigation of reaction between methyl benzylpenicillinate and oxalyl chloride (ether)

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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 ultraviolet fluorescence of oxazolidine-4,5-diones. The spectral evidence again 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

3. Obtained from Bristol Laboratories, Syracuse, New York, Division of Bristol-Myers Company, New York City, New York, USA.
7. J. C. Sheehan and E. J. Corey. J. Am. Chem. Soc. 74, 360 (1952);