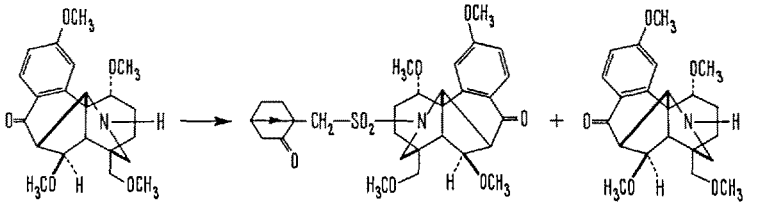
The Total Synthesis of Delphinine: Resolution of the Racemic Relay Compound into Optical Antipodes by an Asymmetric Reaction

We have described recently the total synthesis of the racemate **1a** and its identification with the readily available optically active delphinine degradation product **1b**[1]. Since we wished to use the naturally derived material as a relay compound for the synthesis of various delphinium alkaloids, it was imperative to achieve the resolution of the totally synthetic racemate into optical antipodes. After numerous unsuccessful attempts at resolution by conventional methods, we have noticed that if the racemate was allowed to react with D-camphorsulphonyl chloride in pyridine, the reaction stopped when approximately 50% of the secondary amine has reacted. Isolation of the unreacted free base yielded material, which was optically active and showed an optical rotatory dispersion curve (ORD) antipodal to the ORD recorded for the natural degradation product.



Consequently, we have achieved the preparation of the totally synthetic optically active antipode **1b** in the following manner: The racemate **1a** was dissolved in dry pyridine and treated with 1 mole of L-camphorsulphonyl chloride at 0 °C. The temperature was allowed to rise to 20 °C and the mixture was kept overnight. The unreacted free base was purified by preparative TLC and converted to the acid oxalate which was recrystallized to a constant melting point of 195–196 °C from methanol-ether. This melting point was identical with the melting point of the 'natural' degradation product oxalate and the 2 materials did not show melting point depression. The ORD's of both the synthetic and 'natural' oxalates were superimposable. Both materials gave superimposable IR-spectra (KBr and $CHCl_3$). Synthetic oxalate – Found: C, 60.38; H, 6.57; N, 2.82%. Calculated for $C_{24}H_{31}O_9N$: C, 60.36; H, 6.54; N, 2.93%. The totally synthetic optically active free base **1b** was liberated from the oxalate and recrystallized to a constant melting point of 144.5 °C from ether. This melting point was undepressed by admixture of the 'natural' free base **1b** which had an identical melting point. The 2 materials showed also identical IR- (KBr and $CHCl_3$), mass- and NMR-spectra, identical ORD's and identical behaviour in several TLC systems. The totally synthetic **1b** gave a molecular ion at $m/e = 387.2046$ (calculated for $C_{22}H_{29}O_5N$: 387.2040).

The resolution was very efficient and ca. 120 mg of the totally synthetic optically active base **1b** were prepared. The application of similar methods to the resolution of other racemic secondary amines is being studied.

Zusammenfassung. Das totalsynthetische Razemat DL **1a** gab bei Reaktion mit L-Camphorsulfonylchlorid das Sulfonamid **2** und die optisch-aktive Base **1b**. Die Identität dieser totalsynthetischen, optisch aktiven Base **1b** mit dem entsprechenden Abbauprodukt von Delfinin wurde bewiesen.

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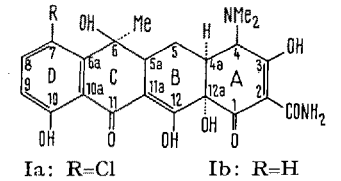
Opposite Chirality of Pillaromycin A to Tetracyclines: The X-Ray Analysis of Achromycin[1] Hydrochloride

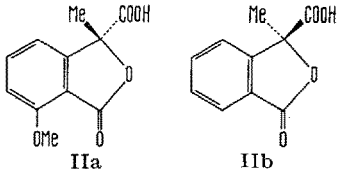
Crystal structures of tetracyclines have been reported previously by some authors. In 1959 Hirokawa et al.[2] analyzed the structure of Aureomycin[1] hydrochloride and a refinement of the structure was performed by Donohue et al.[3]. The structure of Terramycin[1] hydrochloride was studied by Takeuchi and Buerger[4], and their work was extended by Cid-Dresdner[5]. Though these investigators have established the relative configuration of naturally occurring tetracyclines, the configuration in an absolute sense has not been reported. Meanwhile a determination of the absolute configuration of tetracyclines has been reported by Dobrynin et al.[6]. They derived (+)-7-methoxy-methylphthalide-3-carboxylic acid (IIa) from Aureomycin, and spectropolarimetrically compared IIa with (−)-3S-methylphthalide-3-carboxylic acid (IIb), which was derived from L-*o*-acetylaminoatrolactinic acid, and it was revealed that the former has 3*R*-configuration. Since their work, the formula I has generally been accepted also in an absolute sense.

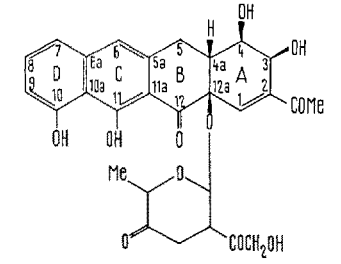
Recently, Asai et al.[7] determined the structure of pillaromycin A (III), a new antibiotic isolated from the culture of *Streptomyces flavovirens* No. 65786[8], and it was found that its structure is closely related to that of tetracyclines. However, the absolute configuration of this antibiotic, established by us from the X-ray analysis of pillaronone monobromoacetate[9] (IV), which is a deriva-

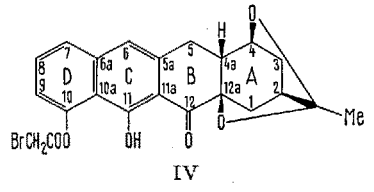
tive of the aglycone obtained from pillaromycin A, was revealed to be opposite to that of tetracyclines at C-4, C-4a and C-12a. From the viewpoint of their biosyntheses, it seems unusual, because pillaromycin A and tetracyclines are both produced by bacteria belonging to the same genus. Therefore the X-ray analysis of Achromycin hydrochloride, which is one of the representative tetracyclines, has now been carried out for the confirmation of this structural difference.

Slow evaporation from a methanol-*n*-butanol solution of Achromycin hydrochloride gave prismatic crystals which, on X-ray examination, proved to be orthorhombic, $a = 10.95$, $b = 12.78$, $c = 15.83$ A. The systematic absences show that the space group is $P2_12_12_1$. The intensity data were measured on a Hilger & Watts linear diffractometer with MoKα radiation. Lorentz and polarization corrections were made and a total of 1732 unique reflexions were collected. The chlorine atom position was determined from a three-dimensional Patterson function and it was found to be analogous to that of previously published tetracycline hydrochloride crystals. A trial calculation of structure factors was made based on the coordinates and temperature factors of Aureomycin hydrochloride reported by HIROKAWA et al.[2], excepting Cl (7) which is replaced by hydrogen atom in Achromycin. The discrepancy index (27%)









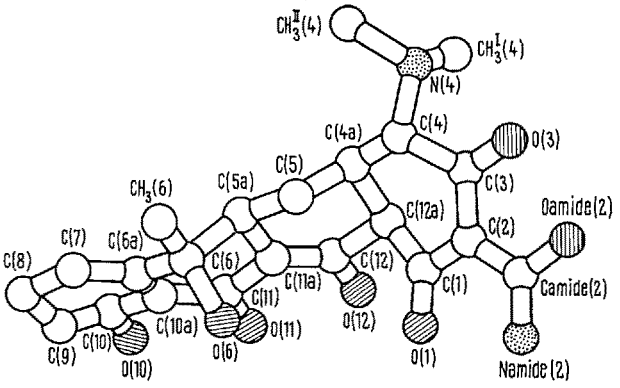


Fig. 1. The Achromycin cation, projected down the *c* axis.

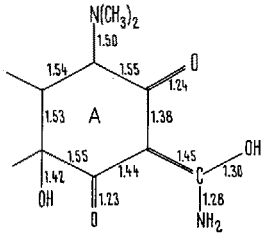


Fig. 2. Bond distances relating to ring A.

[1] Achromycin, Aureomycin and Terramycin are the registered trade names of the American Cyanamid Co. and Chas. Pfizer and Co. for the compounds having generic names tetracycline, 7-chlorotetracycline and 5-hydroxytetracycline, respectively.

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indicated that these crystals were isomorphous. A three-dimensional electron density map revealed the presence of all C, N and O atoms in the molecule as clearly defined peaks at or close to the positions deduced from those of Aureomycins and no other significant peaks were contained. The refinement of positional parameters and isotropic thermal parameters for the non-hydrogen atoms was carried out using the block-diagonal least-squares calculation. The final value of discrepancy index was 11.5% for 1732 observed data. For the determination of the absolute configuration, structure factors were calculated for all observed hkl and $\bar{h}\bar{k}\bar{l}$ reflexions, with the anomalous dispersion corrections included. The differences between $I_o(hkl)$ and $I_o(\bar{h}\bar{k}\bar{l})$ were measured visually from Weissenberg photographs taken with CrKα radiation. Of those 84 Bijvoet pairs, 82 showed differ-

ences in the same direction, and the parameters were found to represent the left-hand coordinate system. The absolute configuration of Achromycin thus obtained (Ib) was consistent with that determined by DOBRYNIN et al.[6]. A projection of the structure down the *c* axis is given in Figure 1.

At this stage we re-examined the absolute configuration of pillaronone monobromoacetate by using the same instrument and the same computer program. 39 Bijvoet pairs out of 40 supported the previous result. The opposite configuration at 4, 4a and 12a of pillaronone to tetracyclines was thus confirmed. Though it might require further investigations to apply the absolute configuration obtained with pillaronone directly to pillaromycin A, it seems to be improbable that the configurations of the 3 asymmetric carbon atoms were simultaneously reversed in the chemical processes from the latter to the former. In this way the production by bacteria of the same genus of structurally related antibiotics with opposite chirality at A ring was demonstrated.

The close similarity among hydrochlorides of Achromycin, Aureomycin and Terramycin was recognized in their conformations. The 4 statements made by DONOHUE et al.[3] for Aureomycin hydrochloride, verified by CID-DRESDNER[5] for Terramycin hydrochloride, have proved to be true also for Achromycin hydrochloride. For instance, an abnormal amide group, with the C-N bond length shorter than the C-O bond was also found in the present analysis (Figure 2). Bond distances in Figure 2 suggest that double conjugation is maintained with 2 carbonyl groups and the amide group.

Similarities were also found in their hydrogen bonding. The molecule is held together through a three-dimensional net of hydrogen bonds to the chlorine ions, each chlorine being attached to 4 different molecules. It was clarified that the hydroxyl group (proton donor) in Terramycin and the chlorine atom (proton accceptor) in Aureomycin have no significant influence on intermolecular interactions and, therefore, on crystal structures of their hydrochlorides.

Zusammenfassung. Durch Röntgen-Strukturanalyse wurde die absolute Konfiguration des Antibiotikums Achromycin ermittelt. Sie ist zu derjenigen des verwandten, aus Kulturen von *Streptomyces flavovirens* stammenden Pillaromycins A entgegengesetzt.

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Incorporation of C^{14}-Leucine in vivo by the Meal Moth *Plodia interpunctella* During Development

Amino acid incorporation is used as a criterion of protein synthesis. No one would disagree with the statement that protein synthesis is a measure of nucleic acid metabolism. Thus, during the metamorphosis of several insect species, the ratio of RNA/DNA increases just prior to adult emergence or during the early phases of adult development[1,2]. This event is correlated with similar increases in amino acid activation[3] and transamination[4] both of which are related to protein synthesis. Several workers have used labelled amino acids in studies of protein synthesis in various insects. Most studied the biosynthetic capacity of different tissues at different developmental stages. SHIGEMATSU[5], working with the fat body of the last larval instar of *Bombyx mori*, showed that marked biosynthetic activity coincided with an increased haemolymph protein content. PRICE[6,7] showed that fat body from 4-day-old *Calliphora erythrocephala* larvae had the highest rate of incorporation, while a marked decline followed at later stages and by the sixth day very little incorporation was found. CHIPPENDALE and KILBY[8] examined the relationship between the proteins of the haemolymph and the fat body during metamorphosis of the large white butterfly, *Pieris brassicae*. They also noted[9] that the fat body and midgut, but not the haemolymph, in mid-fifth instar larvae of *Pieris* are active sites of protein synthesis. The purpose of this study is to investigate the rate of protein synthesis at different developmental stages of the insect *Plodia interpunctella*.

Material and methods. The insects were cultured in our laboratory in cylindrical glass tubes 10×20 cm. As rearing medium, a mixture of chicken mash, glycerine and honey in a 6:1:1 proportion was used. The room temperature was between 28–30 °C and the relative humidity 40–50%. Under the above conditions the total life cycle of the insect, from the day of hatching untill the day of adult emergence was at least 28 days. The ages used here were: Larvae 15-day-old (L_{15}); 18-day-old (L_{18}); Spinning Larvae [(SL) last instar, which developed a green hue]; Pre pupae [(PP) immobile spinning larvae]; Pupae 4-day-old (P_4); 6-day-old (P_6); and 8-day-old (P_8); and newly emerged adults males and females. 8×10^{-3} μCi of radioactive solution were injected into each individual with an 'Agla' micrometer syringe. The animals were homogenized with a *Tris* buffer solution of pH 7.5 at 2 and 4 h after injection. The homogenate was boiled for 10 min and placed in a water-bath with mechanical shaking at 37 °C for 2 h, after adding an equal volume of 1 *N* NaOH. The mixture was then centrifuged in a 'Sorvall RC 2B' centrifuge for 20 min at 1510 *g*. The precipitate was discarded and the supernatant was used for further study. The proteins were precipitated by adding 3 *M* trichloroacetic acid. Each etherdried sample of precipitated proteins was taken in acetone and dried on the tared planchet before being introduced in the

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