

STUDIES ON THE MODE OF ACTION AND STRUCTURE OF GLUCONIMYCIN

Sir:

In 1966 an antibiotic was isolated from a streptomycete and named gluconimycin by SHIM and DEWADAR. This antibiotic was tentatively characterized as an iron-containing oligopeptide of the sideromycin type¹⁾. In the course of investigations on iron-containing metabolites (for a review see ref. 2) we resumed the work on this antibiotic. While studying the mode of action it became apparent that the active substance from *Streptomyces* AS 9 strongly inhibited macromolecular synthesis similar to the action of members of the actinomycin group.

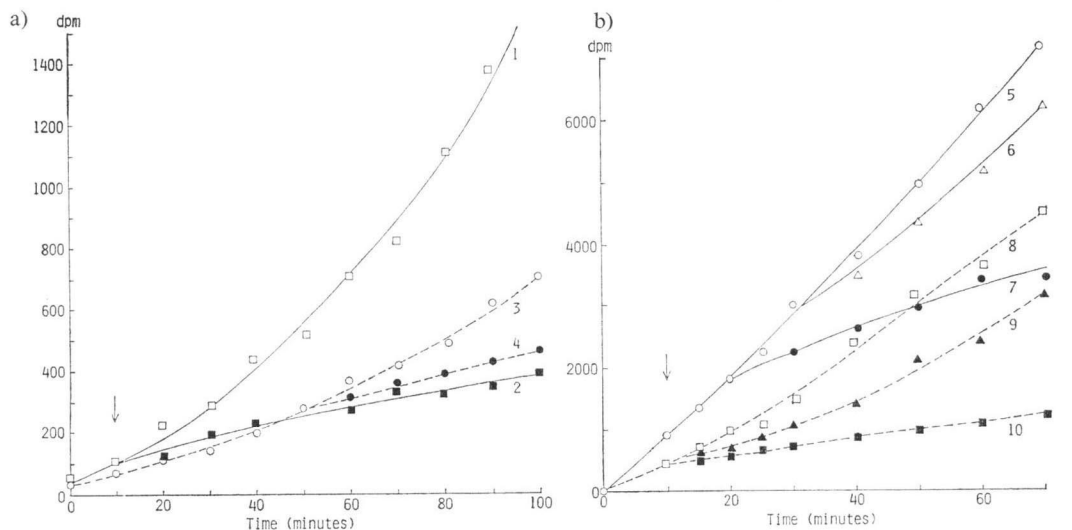
Fermentation was performed as described¹⁾. The antibiotic was extracted from the culture fluid by ethyl acetate, the extract was washed with sodium carbonate buffer at pH 10, several times with water and concentrated to dryness. The raw material was precipitated from a chloroform solution by petroleum ether. At this purification stage the product showed one spot in paper chromatography on Whatman No. 1¹⁾, but sever-

al spots of yellowish to red coloured substances and UV absorbing material in TLC on silica gel using chloroform - methanol - water = 65: 25: 4 as a solvent. Only three of them showed antibiotic activity against *Bacillus subtilis* in a bioautogram. Chromatography on Sephadex LH-20 (eluant; methanol), silica gel (eluant; chloroform with 0~5% ethanol), cellulose (eluant; methanol) and preparative TLC (solvent; ethylacetate - propanol-2 - water = 5: 2: 1) yielded an almost homogeneous preparation of the main product (A).

Biophotometer studies revealed that substance A inhibited growth of *Bacillus subtilis* ATCC 6051 and *Micrococcus luteus* ATCC 15932 effectively at 2 $\mu\text{g/ml}$ and 0.2 $\mu\text{g/ml}$ respectively. *Escherichia coli* was not inhibited at 1 mg/ml in the agar diffusion test. *In vivo* tests of macromolecular synthesis were done with *Micrococcus luteus*, as this microorganisms proved to incorporate ¹⁴C-thymidine for the measurement of DNA synthesis. Results are shown in Fig. 1.

To confirm these results, the inhibition of DNA and RNA polymerases was studied *in vitro*, and 50% inhibition of DNA polymerase I of *E. coli* and RNA polymerase of *E. coli* was observed at

Fig. 1. Inhibition of a) protein and DNA synthesis, b) RNA and DNA synthesis by gluconimycin in *Micrococcus luteus*. The arrows indicate the time of addition of the antibiotic.



	1	2	3	4	5	6	7	8	9	10
Labelled compound	T	T	Lys	Lys	U	U	U	T	T	T
Gluconimycin $\mu\text{g/ml}$	—	0.5	—	0.5	—	0.1	0.5	—	0.1	0.5

T: thymidine, Lys: lysine, U: uracil.

5 $\mu\text{g/ml}$. DNA polymerase of calf thymus was scarcely inhibited at 20 $\mu\text{g/ml}$.

Substance A was an orange-yellow powder, sparingly soluble in water, soluble in methanol, ethanol, acetone and chloroform, insoluble in petroleum ether. The UV spectrum showed a single absorption band at 442 nm ($E_{1\text{cm}}^{1\%}$ in methanol=100). Hydrolysis in 6 N hydrochloric acid for 24 hours at 110°C and separation of the amino acids by two-dimensional TLC on silica gel yielded Thr, Val, Pro, Sar, MeVal and MeAla (1. 1-butanol - acetic acid - water=4:1:1; 2. phenol - water = 75:25). The Thr-spot was broadened. Therefore, the occurrence of hydroxy-threonine or another unknown amino acid cannot be excluded.

Gluconimycin differs from the actinomycins of the Z-group by the presence of proline. The amino acid composition of gluconimycin is similar to that of actinomycins of the X-group. In circular paper chromatography (solvent; *n*-butyl acetate - di-*n*-butyl ether=3:1/10% aq. sodium-*m*-cresotinate) the R_{fC_2} -value was 0.48, which is between the reported values for X_0 and X_1 . TLC on silica gel (solvent: ethyl acetate - propanol-2 - water=5:2:1) gave an R_{fC_2} -value of 0.79 for gluconimycin and 0.97 for actinomycin X_1 . The NMR-spectra indicated non-identity of the two peptide chains and the absence of keto derivatives. 4-Hydroxy-L-proline, which is present in the actinomycin $X_{0\beta}$ and $X_{0\delta}$, is definitely absent. The low mobility in paper chromatography, the solubility in water and the NMR-spectra point to the presence of an unidentified hydroxycompound. Furthermore, N-methylalanine has not been found so far in actinomycins of the X-series³⁾. The low productivity of the strain and the obvious instability of the compound hindered further identification.

From our results it is concluded that the main active compound from *Streptomyces* AS 9 is an actinomycin which has hitherto not been described. The biological activity of the formerly iso-

lated product is—as far as it had been tested—identical to that compound described in this communication.

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HANS-JÜRGEN PLATTNER
BARBARA STENS
HANS DIEKMANN*

Lehrgebiet für Mikrobiologie der Universität
Hannover, Schneiderberg 50, D-3000 Hannover,
Federal Republic of Germany

IBRAHIM R. SHIMI

Department of Biochemistry,
The University of Ain Shams
Abbassiah, Cairo, Egypt Arabic Republic

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* To whom all correspondence should be addressed.

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