in the yeast cell[3,5]. That the division of the centrosomes is timed with the division of the chromatin, is indicated in Figures 3, 4 and 7. While there is sufficient evidence for the participation of centrosomes in the movement of the chromosomes[3], it is not quite clear whether the centrosomes aid in the proper alignment of the chromosomes on the spindle-like element besides taking part in the subsequent distribution between the mother cell and the bud. Though the point is evident that the chromosomes align themselves on such a structure for an orderly distribution between the mother cell and the bud (Figures 4, 5, 6 and 7), it is rather difficult at this stage to state with precision that the fine unstained element, as seen in these photographs, could be the fibre apparatus demonstrated in electron micrographs of the yeast cell[2]. The existence of such a structure might possibly explain too the palisade or stacking arrangement of chromosomes at metaphase in *Lipomyces lipofer*, reported by ROBINOW[6]. Do we see, in such a spindle-like element of the yeast cell, the beginnings of the more elaborate spindle apparatus of the higher organisms?[7]

Zusammenfassung. Es gelang der photographische Nachweis einer spindelapparatähnlichen Struktur in *Saccharomyces cerevisiae* Hansen. Die regelmässige Chromosomenverteilung zwischen Mutterzelle und Knospe wird durch diese Struktur zusammen mit Zentrosomen ermöglicht.

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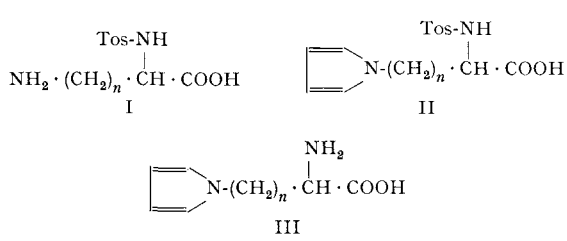
β-Pyrrolo-L-Alanine, a New Antimetabolite of Phenylalanine and Tyrosine in *Escherichia coli*

Many heterocyclic analogues of L-phenylalanine have proved to be of interest as antagonists of this amino acid[1], including β-2-pyrrylalanine[2]. Also, several amino acids with a heterocyclic substituent (e.g. 3-hydroxy-4-pyridone, uracil, pyrazole) attached through nitrogen to the β carbon atom of an alanine moiety occur in nature[3]. In both these contexts, a synthesis of β-pyrrolo-L-alanine (IIIa) appeared to be of interest.

A synthetic route to this compound was opened up by the finding[4] that 2,5-diethoxytetrahydrofuran and related compounds react with amino acids or their derivatives to give the appropriate N-substituted pyrrole derivatives. A suitable starting material was available in N^{α}-tosyl-L-α,β-diaminopropionic acid[5] (Ia). Condensation of this amino acid derivative with 2,5-diethoxytetrahydrofuran did, in fact, proceed smoothly in boiling acetic acid in the presence of sodium acetate to afford N-tosyl-β-pyrrolo-L-alanine (IIa, m.p. 196–197°)[6]. The tosyl derivative IIa was reduced with calcium in liquid ammonia to the desired β-pyrrolo-L-alanine (IIIa), m.p. 238–240° (decomp.), $[\alpha]_D$ − 49°, homogeneous by high-voltage paper electrophoresis (positive reactions with ninhydrin and Ehrlich's reagent). The 2 higher homologues, γ-pyrrolo-L-α-aminobutyric acid (IIIb) (m.p. 245°, decomp.; $[\alpha]_D$ + 20.7°) and δ-pyrrolo-L-norvaline (IIIc) (m.p. 249°, decomp.; $[\alpha]_D$ ± 0°) were obtained by the same route from N^{α}-tosyl-L-α,γ-diaminobutyric acid[5] (Ib) and N^{α}-tosyl-L-ornithine[7] (Ic), respectively.



a, $n = 1$; b, $n = 2$; c, $n = 3$.

When added to a mineral medium containing glucose[8], β-pyrrolo-L-alanine caused complete inhibition of the growth of *Escherichia coli* strain B at a concentration of $2.5 \cdot 10^{-5} M$, and 50% inhibition at a concentration of $5 \cdot 10^{-6} M$. The homologous compounds, IIIb and IIIc, had no antibacterial action under the same conditions at concentrations up to $10^{-2} M$. The effect of β-pyrrolo-L-alanine ($10^{-4} M$) was completely reversed by $5 \cdot 10^{-6} M$ L-phenylalanine or by $2 \cdot 10^{-5} M$ L-tyrosine; L-tryptophan ($10^{-5} M$) reversed the inhibition to the extent of 50%, and no greater effect could be achieved by increasing the concentration of tryptophan. Histidine was inactive.

The action of pyrrolo-alanine was found to be bacteriostatic and it could be shown that the analogue inhibits the formation of β-galactosidase by *E. coli* B.

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In all these features the action of β-pyrrolo-alanine resembles that of *p*-fluorophenylalanine; cross resistance to these 2 antagonists was accordingly tested for. Strains of *E. coli* B with a 100-fold increased resistance to *p*-fluorophenyl-DL-alanine and a 2000-fold increased resistance to β-pyrrolo-L-alanine, respectively, were obtained by 7 passages through synthetic media containing increasing concentrations of the appropriate antimetabolite.

Cross resistance to β-pyrrolo-L-alanine and *p*-fluorophenyl-DL-alanine. Bacterial growth was determined nephelometrically at 575 nm and is expressed as a % of the growth of control cultures in the absence of inhibitors. Growth of the parent strain was completely inhibited by $2.5 \cdot 10^{-5} M$ β-pyrrolo-L-alanine or $10^{-4} M$ *p*-fluorophenyl-DL-alanine

Inhibitor added	Concentration	Growth of strain resistant to Pyrrolo-alanine	*p*-Fluoro-phenylalanine
β-Pyrrolo-L alanine	$10^{-3} M$	125	0.1
	$10^{-4} M$	95	100
p-Fluorophenyl-DL-alanine	$10^{-3} M$	70	75
	$10^{-4} M$	112	115

The results given in the Table show that cross resistance to the 2 antagonists does, indeed, exist. It therefore appears likely that the mechanism of action of pyrrolo-alanine is closely related to that of *p*-fluorophenylalanine.

If this conclusion is correct, we should expect the heterocyclic analogue to be incorporated into bacterial protein.

Zusammenfassung. β-Pyrrolo-L-alanin, dessen Synthese beschrieben ist, hemmt das Wachstum von *E. coli* B auf synthetischen Nährböden. Die Hemmung wird von L-Phenylalanin und L-Tyrosin vollständig, von L-Tryptophan teilweise, nicht aber von Histidin aufgehoben. Stämme von *E. coli*, die gegen β-Pyrrolo-L-alanin bzw. *p*-Fluorophenyl-DL-alanin resistent sind, zeigen gekreuzte Resistenz.

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The Alkaline Ribonuclease of Rat Serum During Administration of 4-Dimethylaminoazobenzene and Copper Oxyacetate

ZYTKO and CANTERO[1] found that the 'alkaline ribonuclease' of rat serum was somewhat decreased after the animals had been fed for 2 weeks on a diet containing N-dimethylaminoazobenzene (DAB) and fell to 40% of normal when primary liver tumours had been induced. There was, however, a slight increase over normal value in rats bearing Novikoff ascites hepatomas. Elevated values had also been observed in the serum of human cancer patients compared with healthy subjects (ZYTKO and CANTERO[2]). This serum enzyme resembles rat liver alkaline ribonuclease and bovine ribonuclease in having an optimum pH of 7.8 but differs from these in that it is thermolabile. It does not split purine or pyrimidine mononucleotides as does acid ribonuclease. The change in absorption produced by its interaction with nucleic acid is ascribed to a hyperchromic effect due to hindrance of resonance of guanine rings in the polymer molecules (DE LAMIRANDE et al.[3]).

During the past 5 years we have carried out studies on various enzymes and other parameters during the dietary administration of DAB to rats, alone and together with copper oxyacetate. The addition of the copper salt greatly inhibits tumour induction by the azo dye (HOWELL[4], FARE[5]) and modifies various parameters, e.g. decrease in liver succinoxidase and cytochrome oxidase, liver copper storage (FARE and WOODHOUSE[6]), and dye binding in the liver protein (FARE[7]). Also the serum phenylenediamine oxidase activity (copper oxidase) was much diminished by DAB feeding but to a lesser degree when copper oxyacetate was also fed (WOODHOUSE[8]). From his measurements on azo dye binding FARE[7] suggested that the biochemical changes and the partial protection against the carcinogenic activity of the azo dye could be explained as resulting from a competition between the dye and copper for binding sites on protein. It was thought, therefore, that the estimation of the serum alkalinase ribonuclease described by ZYTKO and CANTERO[1] at intervals during the administration of the dye, alone, and together with the copper salt, might give further information.

Materials and methods. 4 groups of 6 male rats were fed, respectively, on the control maize diet, maize plus 0.09% DAB, maize plus 0.5% copper oxyacetate plus 0.09% DAB, and maize plus 0.5% copper oxyacetate. In all cases a diet of proprietary cube was substituted on Saturday and Sunday. Initially the animals were 200–250 g in weight and consumed about 10 g of mixed or basic diet each day, and the weights of the animals were recorded at fortnightly intervals. The enzyme content of blood from the tail vein was determined at intervals of 2–4 weeks over a period of 29 weeks, at which time those which received the DAB only had localized liver tumours, but those also given copper oxyacetate still presented essentially normal livers (FARE[5]). Blood specimens were always taken mid-week in the morning.

The tests were conducted as described by ZYTKO and CANTERO[1], the diluted serum being incubated with the

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