Zusammenfassung. In Gegenwart des exfolierten Tonminerals Vermikulit wird die Zitronensäurebildung durch *Aspergillus niger* besonders stimuliert. Dabei wird die Induktionsphase verkürzt sowie die gesamte Gärgeschwindigkeit im messbaren Bereich der Zitronensäurebildungsphase erhöht. Die Stimulierung scheint einzig auf verbesserten Haushalt der Spurenelemente zurückzuführen zu sein.

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A Spindle Analogue in *Saccharomyces cerevisiae* Hansen

In a previous communication[1], an illustration was presented of a tubular spindle-like structure around which the chromatin was disposed as a coiled element in vegetatively dividing cells of *Saccharomyces cerevisiae* (cf. Figure 5 of this report). The explanation was offered of the alignment of the daughter complements of chromosomes on this structure as a prelude to the subsequent distribution between the mother cell and the bud, with the aid of centrosomes. In the present report evidence is presented for what appears to us as stages in the formation of such a spindle-like element.

S. cerevisiae, Hansen (CBS 1171), obtained from the yeast division of the Centraalbureau voor Schimmelcultures at Delft, in Holland, was the yeast used in these investigations. Cells were from a 24 h growth on barley-wort-agar medium which was in turn obtained by inoculating cells from a 16-hour-old synchronized liquid culture, as detailed in the earlier communication[1]. 1 batch of smears was fixed in Carnoy's fluid for 2 h (Figures 1–7) and the other in Helly's (Figure 8) for 10 min[2]. The fixed cells were stained with Giemsa with prior extraction in NaCl at 60 °C for $1^1/_2$ h and hydrolysed in NHCl at 60 °C for $8^1/_2$ min[1,3].

In Figure 1 can be seen the centrally-located deeply-stained chromatin through which has emerged a fine unstained tubular structure with a stained knob, giving the appearance of a germinating seed. The cell at (a) in Figure 2 reveals the gradual elongation of the tube at one pole of the chromatin mass. A stained spherical dot is also seen. The chromatin in the cell in Figure 3 looks expanded. In Figure 4, the central unstained tube terminates in 2 divided stained entities at 1 pole, and the chromatin is wrapped round the central tube in 2 densely stained coils. The cell in Figure 5 reveals the spindle-like element in a better perspective with the chromatin tightly coiled round. Figure 6 is suggestive of loosely coiled chromatin, and at close examination reveals 3 dumbbell-shaped and 2 discrete chromosomes at the top (arrow). 2 parallel complements of chromosomes are seen in Figure 7. Each of these could be seen terminating in a thin drawn-out thread, converging at the pole in a stained dot. A second stained dot is seen at the other pole. Figure 8 is presented to show that comparable pictures were obtained with Helly-fixed cells also. Here again, at (a) a very fine unstained tube is seen piercing the deeply-stained chromatin. The anchoring of the ends could also be made out (arrow). In cell (b) a similar thin unstained element is seen attached to the chromatin (arrow). It may be of interest here to recall the centriolar structures connected to an unstained furrow, with a centrally-situated stained mass, demonstrated by the hematoxylin technique of SUBRAMANIAM[4].

The structure, as seen in these photographs, is very suggestive of a spindle element. The individual and divided stained entities at the poles, as seen in these cells, confirm the earlier demonstration of centrosomes

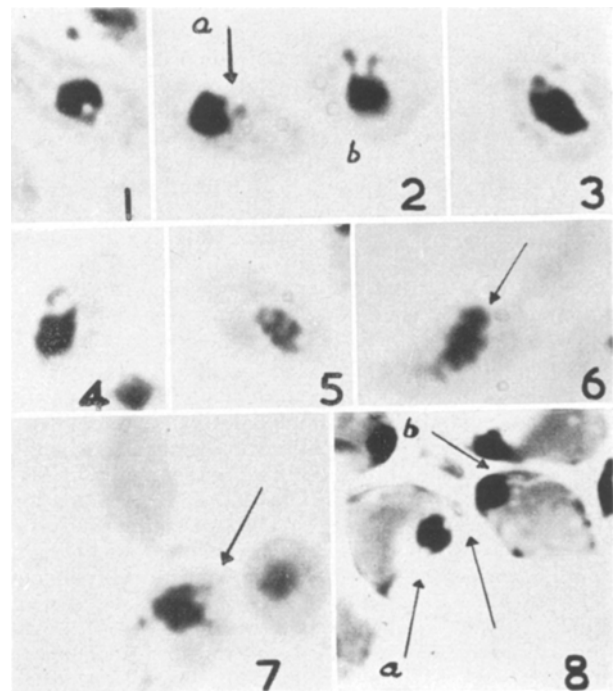

Fig. 1. Early stage in the appearance of the central tube. Ca. × 2800.

Fig. 2. Elongation of the tube with a centrosome in cell (a). Dividing centrosomes are seen in cell (b). Ca. × 2800.

Fig. 3. Chromatin expanded on the tube. Centrosome seen. Ca. × 2800.

Fig. 4. Tube more clear. Dividing centrosomes at one pole. Ca. × 2800.

Fig. 5. Chromatin coiled round the tube. Ca. × 2800.

Fig. 6. Coiled appearance of chromatin. Chromosomes arranged in tiers. Ca. × 3000.

Fig. 7. Parallel arrangement of chromosomes. Note the 2 thin threads converging in a centrosome. Ca. × 3000.

Fig. 8. Slender tube piercing through the chromatin (Helly-Giemsa preparation). Ca. × 3000.

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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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Genetisch Instituut der Rijks-Universiteit Groningen, Haren (Gr., Netherlands), July 25, 1966.

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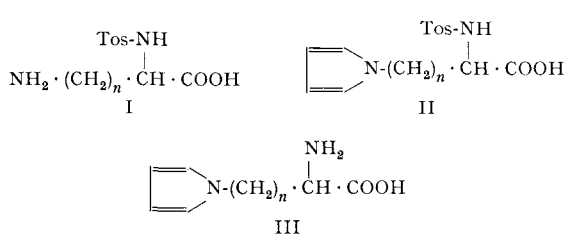
β-Pyrrolo-L-Alanine, a New Antimetabolite of Phenylalanine and Tyrosine in *Escherichia coli*

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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