absorbance were plotted against temperature, and the curves obtained for 3 different absorbance levels are shown in the insert of Figure 2. The results obtained indicate that the required thrombin concentration is minimal and, therefore, that sensitivity is maximal, somewhere in the vicinity of 25–26 °C. It is probable that increased viscosity of the gelforming mixture at lower temperatures is responsible for making the particle sedimentation method more sensitive just above room temperature than it is at 37 °C. Thus, under suitable conditions of temperature and fibrinogen ccncentration, thrombin levels of less than 1 U/ml can be readily determined by means of this procedure. Even lower concentrations should be similarly measurable using polymer beads which settle more slowly, eg. Bio-Gel P-2 (minus 400 mesh).

In the 3rd experiment, a range of fibrinogen concentrations was studied at different thrombin levels, with temperature being maintained at 22 °C (room temperature). From the data shown in Figure 3 it is apparent that fibrinogen concentrations as low as 0.003% can still be determined quantitatively by means of this method. One reason for this marked sensitivity probably is the lack of agitation in the incubated samples. The possibility of adapting this procedure to the quantitative determination of extreme hypofibrinogenemia would appear to be indicated[3].

Zusammenfassung. Die photometrische Absorptionsmessung für Fibrin-Gerinsel mit zum Teil sedimentierten Polyacrylamid-Partikeln ermöglicht eine quantitative Bestimmung sehr niedriger Thrombin- und Fibrinogen-Konzentrationen.

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The Antibiotic Edeine IX: The Isolation and the Composition of Edeine D

Previously we reported that the antibiotic edeine, produced by *Bacillus brevis* Vm4, appeared to be a complex of 4 biologically active principles named edeines A, B, C and D which could be separated on paper or thin layer chromatography[1]. We have also reported the preparative isolation of edeines A and B on cation exchange resins[2], carboxymethyl cellulose[3] and Sephadex[4]. Both compounds can also be isolated by counter current distribution[5]. Preparations of edeines A and B thus obtained are usually contaminated by their very closely related inactive isomers which could be removed in high voltage electrophoresis[6].

The composition[5,7] and the structure[6] of edeines A and B have been established. Both compounds are oligopeptides and conjugates of polyamines. Amino acid composition of both antibotics is identical. They contain one residue of each: glycine, 2-hydroxy-3-amino-propionic acid (isoserine), β-tyrosine, 2, 3-diaminopropionic acid and 2, 6-diamino-7-hydroxy-azelaic acid. Edeines A and B differ in the nature of polyamine moiety which is spermidine or guanylspermidine, respectively. In the present communication the preparative isolation and the determination of composition of edeine D is reported.

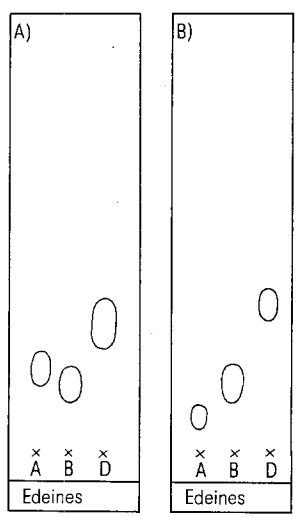

Fig. 1. Paper chromatography of edeine on Whatman No 1. A) Descending chromatography for 17 h in the solvent system: n-propanol: methanol:ammonia:water = 8:2:3:0,54. B) Ascending chromatography for 26 h on filter paper buffered with phsophate-citrate buffer (pH = 5) in the solvent system: phenol:buffer (phosphate-citrate pH = 5) = 1:1. Visualization with ninhydrin.

The crude edeine complex was obtained according to the previously described procedure[2] and further purified by counter current distribution[7] in solvent system prepared with one part of 80% aqueous phenol and one part of ammonium acetate buffer (0,15 M ammonium acetate and 0,3 M acetic acid), appliyng 500 transfers of upper phase.

The purified edeine complex obtained contained varying amounts of edeine D depending on the fermentation conditions and composition of fermentation medium. In some preparations substantial amounts of edeine D were present, although usually this component is produced as minor constituent of the complex.

The preparative isolation of edeine D from the complex could be effectively done by means of column chromatography in solvent system: isopropanol: ammonia: water = 60:35:5 and silicagel (particles below 0.08 mm) as absorbent or n-propanol: methanol: water: ammonia = 8:2:3:0,54 and cellulose. Using the former system, which is preferable, 70 mg of pure edeine D were obtained. Paper chromatography of edeine D as compared with edeines A and B is presented on Figure 1.

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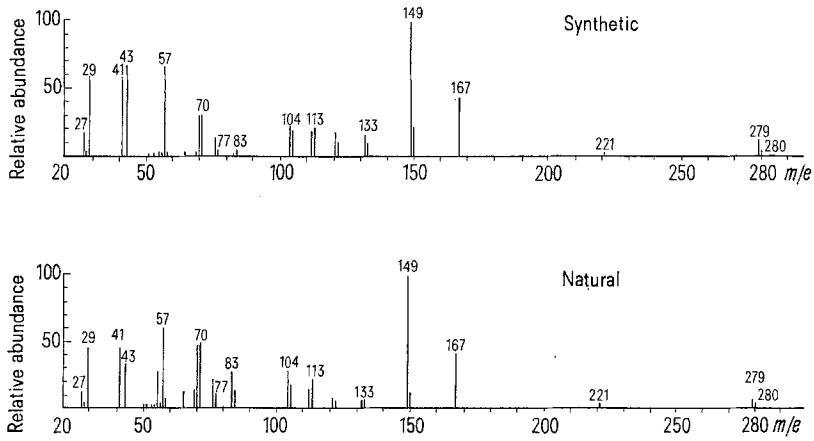

Fig. 2. Mass spectrum of Di-N-TFA-β-phenyl-β-alanine ethyl ester obtained with 70volt electrons.

Edeine D gives positive ninhydrin and sodium nitropruside reactions and negative Sakaguchi and Pauly reactions, indicating the presence of free primary and secondary amino groups and the lack of guanyl and phenol groups.

The composition of the antibiotic was determined after hydrolysis in 6 *N* HCl at 100 °C for 27 h. The hydrolysate was analyzed by paper and thin layer chromatography and high voltage electrophoresis. The following compounds were identified: glycine, 2-hydroxy-3-aminopropionic acid, 2,6-diamino-7-hydroxyazelaic acid and its dehydratation product 2,6-diamino-Δ^7-azelaic acid, 2,3-diaminopropionic acid, spermidine and an amino acid which could not be identified with any of the standards. All products, except the last one, were also found in the hydrolysate of edeine A[8] which also additionally contained β-tyrosine not found in edeine D. Instead of β-tyrosine edeine D contained the above-mentioned new amino acid residue.

For structural elucidation the non-identified amino acid was preparatively isolated from the hydrolysate by column chromatography on silicagel in the solvent system: n-propanol: water = 19:1. Its $[\alpha]_D^{23} = -10{,}3°$ (in water). Ethyl ester of N,N-di-trifluoracetyl derivative was prepared and analyzed by mass spectrometry on LKB instrument (Model 9000) with E-301 column. The mass spectrum was identical with that of the authentic sample ethyl ester of N,N-di-trifluoracetyl-β-phenyl-β-alanine obtained synthetically[9]. Both mass spectra are shown on Figure 2.

In both spectra parental ions (P) did not appear and the fragment ions of highest molecular weight were: *m/e* 280 = P-(C_6H_5 + C_2H_4) and *m/e* 279 = P-(C_6H_5 + C_2H_5). The base peak was *m/e* 149 = P-($C_4F_6NO_2$ + C_2H_4).

N,N-di-TFA-methyl esters of both natural and synthetic amino acids exhibited also identical retention time in gas chromatography in 2 columns (both 2 m): OV-17 (temp. 210°, retention time 9.9 min) and SE-30 (210°, ret. time 13.1 min).

Natural and synthetic amino acids in free form and as their ethyl esters were also identical in paper and thin layer chromatography and in high voltage electrophoresis.

The results obtained indicate that edeine D is a close analogue of edeine A in which the residue of β-tyrosine was replaced by β-phenyl β-alanine. The rotation data of the latter one indicate the L-configuration.

β-phenyl-β-alanine is an amino acid very rarely found in natural products. It was previously isolated from islandotoxin[10]. It exhibits an interesting property of inducing morphological changes in *Bacillus brevis* producing gramicidine[11].

Zusammenfassung. Edein D wurde aus dem komplexen Antibioticum Edein mit Hilfe von Verteilungs-Chromatographie abgetrennt. Als Bausteine von Edein D wurden 3-Amino-3-phenyl-propionsäure, 3-Amino-2-hydroxypropionsäure, 2,3-Diaminpropionsäure, 2,6-Diamin-7-hydroxy-azelainsäure, Glycin und Spermidin ermittelt.

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The Effect of *p*-Benzoquinone and Quinol on the IAA-Oxidase Activity

Phenolic compounds form a special group of the growth regulators – a group of natural growth inhibitors[1]. They effect the growth processes most frequently through the IAA-oxidase system[2]. This enzyme controls the endogenic concentration of IAA[3,4].

The free phenolic substances in the plant cells succumb to transformation reactions (glycosylation, esterification and methylation) or they are substrates of peroxidases and phenoloxidases. In the phenol-phenoloxidase system, quinones are products of the reaction. Quinones are compounds of wide occurence in nature, which importance in biochemistry is becoming constant more recognized[5]. Quinones effect a number of enzyme reactions owing to their oxido-reduction potential[6,7]. The electronic structures of quinones are in relation to their biological activity[8].