On the Chemical Nature of the Antibiotic Edeine*

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ABSTRACT: The antibiotic "edeine" has been isolated in pure form and characterized by hydrolytic studies. On complete hydrolysis it has been found to yield five amino acid fragments and the base spermidine. The amino acids glycine, isoserine, isotyrosine, α,β-diaminopropionic acid, and a more complex amino acid of unknown structure are present in equimolar amounts. Evidence from a thin-film dialysis study indicates two of each residue to be present, but with the possibility of dissociation to half this molecular size.

A number of years ago one of us (Kurylo-Borowska, 1959) published data showing that a certain strain of *Bacillus brevis* named Vm4 produced a new antibiotic principle when grown on an appropriate yeast broth. This principle appeared to be of strong hydrophylic character and could be successfully extracted in good yield from the culture medium only by liquefied phenol. It could be adsorbed on activated charcoal and eluted by acid methanol, however with considerable loss of activity.

The activity was freely dialyzable through collodion membranes but all attempts to get good fractionation on a chromatographic column were unsuccessful. Countercurrent distribution (CCD)[1] seemed to offer the most promise when aqueous solutions and liquefied phenol were used to form the two-phase system. The apparatus available at the time permitted only a small number of transfers to be applied, not enough to achieve a purity comparable to that reached with the other antibiotics from *B. brevis* after a thousand or more transfers had been applied. Nonetheless, sufficient purification was achieved to indicate that the antibiotic principle was indeed different from any known antibiotic hitherto isolated from *B. brevis* and probably from any other microorganism as well. The principle was named "edeine."

Further studies (Kurylo-Borowska, 1959) of the active principle by electrophoresis showed that it was a strongly basic material. Acid hydrolysis gave six spots by two-dimensional chromatography in various solvent systems when ninhydrin was used to locate the spots. Four of these were thought to occur in the correct positions for glycine, arginine, tyrosine, and glutamic acid. Sufficient material for conclusive identification was not available and the tentative interpretation of three of the spots now requires modification as will be seen from the results presented here.

Several years later it was found that this antibiotic interfered with protein synthesis in a rather interesting way, namely, by inhibiting deoxyribonucleic acid (DNA) synthesis (Kurylo-Borowska, 1962). It therefore seemed well worthwhile to attempt isolation and further purification of sufficient material to make a study of the amino acid composition and structure. The progress to date is reported in this paper. Although the investigation is not yet complete, the building blocks of the antibiotic are so unique in the field of polypeptide antibiotics as to justify publication of the work as far as we have gone.

Materials and Methods

The culture used for the fermentation was the same as that isolated earlier (Kurylo-Borowska, 1959). For production of the larger amounts required, a number of 6-l. flasks was employed as described by Kurylo-Borowska (1959) using the yeast broth media.

When the cultures had reached activities of the order of 20–30 units/ml (Kurylo-Borowska, 1959) they were centrifuged for 20 min at 3000 rpm in order to remove the bacteria. The supernatant was shaken with 1.4 volumes of liquefied phenol (Mallinckrodt, about 88%) and the phenol layer was removed in a separatory funnel. It was extracted with double the volume of 5% acetic acid and the phenol layer was then discarded. The aqueous layer was extracted several times with ethyl ether to remove the residual phenol and evaporated to dryness in a rotatory evaporatory at 30°. From 1 l. of culture approximately 2 g of residue was obtained.

A preliminary fractionation of the crude extract was made by gel filtration on Sephadex G-25. The column used was 60 cm long and 4 cm in diameter. Approximately 2 g of the residue, placed initially in 8 ml of water, could be fractionated in a single run. The elution pattern obtained at a flow rate of 4.6 ml/5 min is shown in Figure 1. The cut extending from fractions 116–137, which contained all the antibiotic activity, was taken for further fractionation by CCD. It usually weighed about 350 mg after lyophylization.

The distribution was carried out in a 500-tube CCD

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[1] Abbreviations used: CCD, countercurrent distribution; nmr, nuclear magnetic resonance; FDNB, fluorodinitrobenzene.

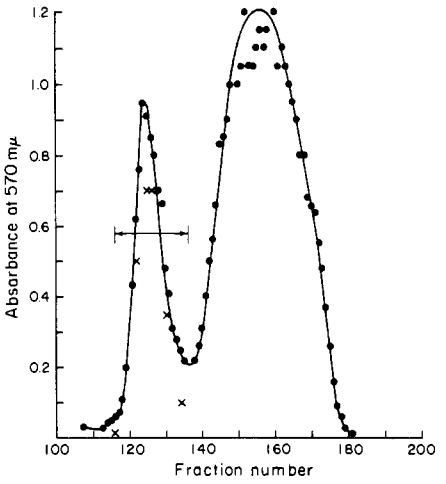

FIGURE 1: Preliminary fractionation of edeine extract on Sephadex. Analysis ●—● ninhydrin method (Moore and Stein, 1954). × — × = relative antibiotic activity.

train of the automatic type described by Craig *et al.* (1951). The phase volumes were 5/5 ml. A total of 2.8 g of the gel filtration cuts from a number of runs was loaded into tubes 0–21 in the system made by equilibrating equal volumes of the buffer and liquid phenol. The buffer solution was 0.15 M ammonium acetate–0.30 M acetic acid. A settling time of 2 min was required. The room temperature was 25°.

After 600 transfers, residue weight analysis (Craig, 1960) gave the pattern shown in Figure 2A. Bioassay (Kurylo-Borowska, 1959) after careful removal of the phenol by ether extraction indicated antibiotic activity to be present only in the well-separated band at tubes 300–375. The material in all the tubes except 310–365 was removed and replaced by fresh system. The distribution was then continued to 1900 transfers by the recycling procedure. Weight analysis now gave the single band shown in Figure 2B. No residue was found in the other tubes of the train. The active antibiotic was recovered by removal of the phenol by extraction with ether, concentration of the aqueous phase in a rotatory evaporator at 30°, and lyophilization. This gave 713 mg of residue.

Hydrolysis of Edeine and Isolation of the Products. Investigation of various hydrolysis times by amino acid analysis with the automatic amino acid analyzer according to the method of Spackman *et al.* (1958) indicated that the peptide was difficult to hydrolyze. A hydrolysis time of 36 hr in a sealed evacuated tube with 6 N HCl at a temperature at 108° was found to be optimum. The amino acid pattern given by the buffers and conditions recommended for the known amino acids was first determined.

The hydrolysate was also studied by thin layer chromatography with silica gel G as the adsorbent and with the solvent system: *n*-butyl alcohol–acetic acid–water (60:20:30). R_F values for this system are given in Table I. The system *n*-butyl alcohol–water–acetic acid–pyridine (60:50:30:20) was used for the second direction. Its eparated spots III and IV (as shown in Table I) which were not separated in the first system but spots I, II, and VI moved practically with the front. Six distinct spots were obtained with the combined systems.

An attempt was made to isolate the fragments resulting from the hydrolysis by CCD in the system *n*-butyl alcohol–1.5 N HCl. The distribution was performed in a 1000 tube train with 3/3 volumes. At 1000 transfers the patterns shown in Figure 3 were obtained. The single adsorbing band in tubes 390–400 was re-

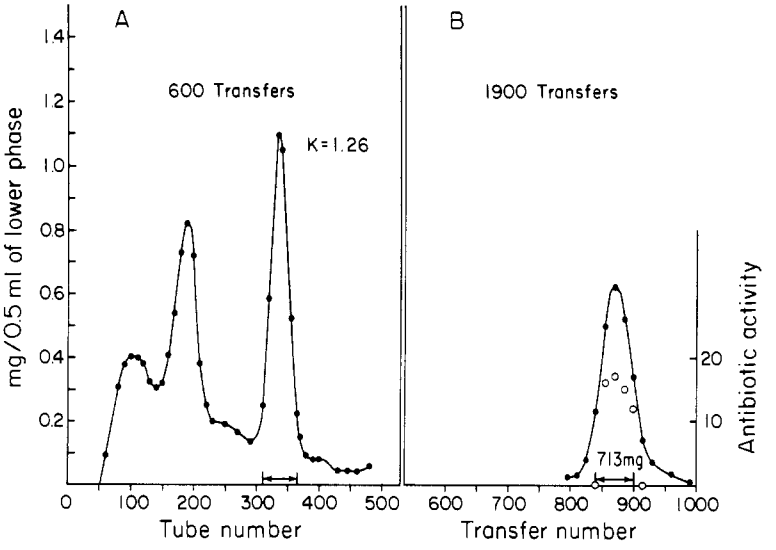

FIGURE 2: Countercurrent distribution patterns of the active cut from Figure 1. The antibiotic activity (○) is given as width of the inhibition zone in millimeters given by 10 μl of solution. System = buffer (0.15 M NH_4Ac–0.3 M HAc)–liquid phenol.

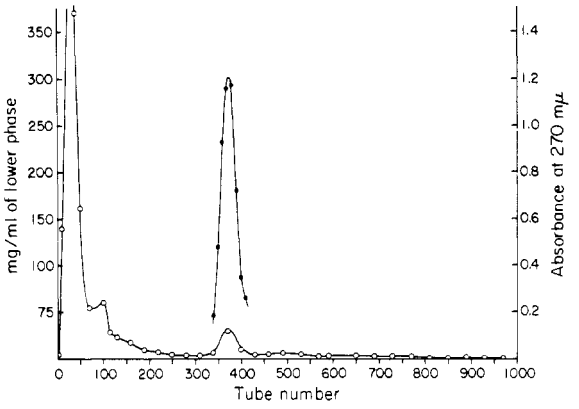

FIGURE 3: Countercurrent distribution patterns of the hydrolysis products of edeine. O—O = weight; ●—●— = absorbance. System = *n*-butyl alcohol–1.5 N HCl.

TABLE I: Thin Layer Chromatography Behavior of Fragments.

System	Peak	R_F	Ninhydrin Color
1[a]	I	0.29	Orange
1	II	0.35	Light brown
1	III	0.25	Dark violet
1	IV	0.23	Red
1	V	0.05	Violet
1	VI	0.62	Yellow
2[b]	III	0.61	Dark violet
2	IV	0.53	Red
2	V	0.50	Violet

[a] System 1 = *n*-butyl alcohol–acetic acid–water (60:20:30). [b] System 2 = *n*-butyl alcohol–water–acetic acid–pyridine (60:50:30:20).

covered by evaporation of the solvent in the rotatory evaporator. It gave only a single spot by thin layer chromatography corresponding to VI of Table I.

In subsequent experiments the single absorbing band was removed from the hydrolysate by a short CCD run (50 transfers) with phase volumes of 5/3 and the same system. The combined fractions of lower *K* were then recovered and separated by chromatography on Bio-Rad AG 50-X2, 200–400 mesh, hydrogen form, in a column 60 × 2 cm. The sample (from 300 mg of edeine) was put on the column in 5 ml of 0.5 M HCl. It was eluted with 0.5 N HCl at a flow rate of 25.2 ml/hr until 120 effluents of 4.2 ml had been collected. The acid strength of the eluent was then increased to 1 M HCl and 3.9 ml/hr. Finally, the acid strength of the eluent was increased to 3 M HCl and 11-ml fractions were collected until 500 fractions had been collected at a flow rate of 66 ml/hr.

Analysis by ninhydrin (Moore and Stein, 1954) of 0.050-ml aliquots gave the pattern shown in Figure 4. Those fractions omitted from the chart contained no ninhydrin-positive material. The pure fragments were recovered as the hydrochlorides by evaporation on the rotary evaporator. The residue from each peak gave only a single spot when checked by thin layer chromatography in the system used for the data of Table I.

The hydrochlorides of bands 1 and 2 were converted to the free amino acids by chromatography on a short column (3 × 0.9 cm) of Bio-Rad AG 50-X2 in the hydrogen form. The column was washed with water until the effluent was neutral and the amino acid then eluted from the column with dilute ammonia. Evaporation of the effluent gave the free amino acid.

Isoserine. The free amino acid from band I, Figure 4, crystallized from water–ethanol. The crystals were

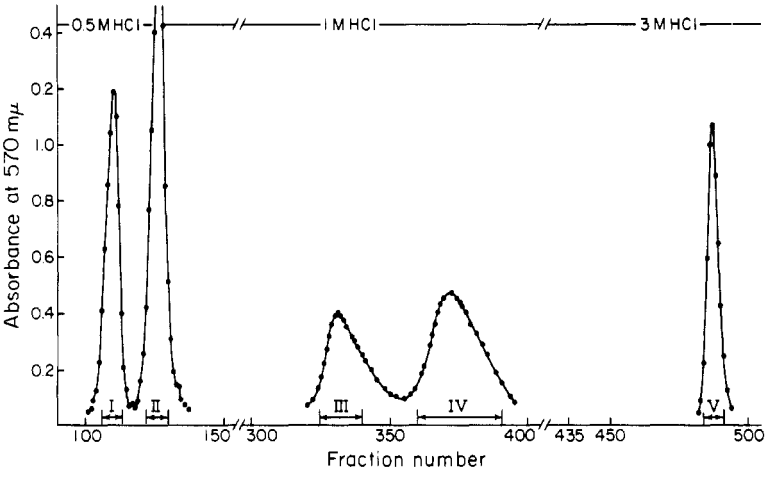

FIGURE 4: Effluent pattern of the chromatographic separation of the hydrolysis products of edeine with Bio-Rad AG-50X2.

dried at 78° over P_2O_5 for 3 hr under a high vacuum; $[\alpha]_D^{25}$ −24° (*c* 0.75%, H_2O).

Anal. Calcd for $C_3H_7NO_3$: C, 34.28; H, 6.71; N, 13.33. Found: C, 34.15; H, 6.50; N, 13.20.

Isoserine (DNP) Methyl Ester. The amino acid (30 mg) was converted to the DNP derivative by treatment for 2 hr with 100% excess of fluoro-2,4-dinitrobenzene in 50% ethanol containing 2.5% sodium bicarbonate. The excess reagent was extracted with ether and the derivative extracted with ethyl acetate after acidification with HCl. The ethyl acetate was dried over sodium sulfate and evaporated to dryness. The residue was freed from dinitrophenol by filtration of an ethyl acetate solution through a silica gel column (0.9 × 30 cm). The DNP derivative was removed from the column by changing the eluting solvent to methanol. The residue after evaporation gave a single spot on the chromatoplate (silica gel G) with an R_F of 0.04.

The DNP-isoserine was converted to the methyl ester by standing at room temperature overnight in 10% anhydrous methanolic HCl. After evaporation the small amount of unreacted acid was removed by passing a solution of it in ethyl acetate through a small column of silica gel. The ester was not adsorbed. The eluent on evaporation gave the desired product. On a chromatoplate with silica gel G it gave a single spot with an R_F of 0.57 in the solvent system chloroform–ethyl acetate (1:1).

Glycine. The material in band II of Figure 4 was converted to the free amino acid as previously described. It crystallized readily from ethanol–water.

Anal. Calcd for $C_2H_5NO_2$: C, 32.00; H, 6.72; N, 18.66. Found: C, 32.22; H, 6.58; N, 18.9.

2,3-Diaminopropionic Acid Monohydrochloride. The residue from band III of Figure 4 crystallized readily from ethanol–water as fine needles. It was dried under high vacuum at 100° for analysis; $[\alpha]_D^{25}$ +11.1° (*c* 1.47%, 5% HCl).

Anal. Calcd for $C_3H_9ClN_2O_2$: C, 25.63; H, 6.45; N, 19.93. Found: C, 25.90; H, 6.41; N, 19.47.

Spermidine Trihydrochloride. The residue from band V of Figure 4 crystallized readily from hot ethanol when ethyl acetate was added. After collection the crystals were dried at 78° for analysis.

Anal. Calcd for $C_7H_{21}Cl_3N_3$: C, 33.10; H, 8.33; N, 17.2. Found: C, 33.05; H, 8.55; N, 17.15.

Isotyrosine Hydrochloride. The residue obtained from the ultraviolet absorbing band of Figure 3, or from a band of similar *K* after a distribution of 50 transfers, crystallized readily in needles from a small volume of ethanol when ethyl acetate was added. The crystalline material was dried for analysis at 78° over P_2O_5 under a high vacuum; $[\alpha]_D^{25}$ +7.8° (*c* 1.67%, H_2O).

Anal. Calcd for $C_9H_{12}ClO_3$: C, 49.62; H, 5.55; N, 6.43. Found: C, 49.95; H, 5.61; N, 6.58.

The DNP methyl ester was prepared by the same procedure described for DNP-isoserine methyl ester. The DNP derivative gave a spot on the chromatoplate with silica gel in the ethyl acetate–chloroform system (1:1) with an R_F of 0.22. The methyl ester gave a spot with an R_F of 0.93.

Paper Electrophoresis Data. Table II gives comparative data for edeine and the fragments.

TABLE II: Paper Electrophoresis Behavior of Edeine and Fragments.

Compd	pH 6.4 Buffer,[b] 2300 v, 19 ma, 45 min (Distance Migrated in cm)	pH 1.9 Buffer,[c] 2500 v, 20 ma, 45 min (Distance Migrated in cm)
Edeine	...	11.2
Histidine (ref)	3.2	...
Glycine	0	7.1
Isoserine	0	7.5
α,β-Diaminopropionic acid	5.6	11.2
Isotyrosine	0	6.6
Spermidine	9.1	21.5

[a] Paper = MN chromatography paper 214. [b] pH 6.4 buffer = pyridine–acetic acid (200:8) diluted to 2000 ml. [c] pH 1.9 buffer = formic acid–acetic acid (60:240) diluted to 2000 ml.

Discussion

In the preliminary investigations designed to find the best and most convenient way of isolating edeine in pure form all attempts to find a satisfactory column chromatographic method failed. Although adsorption on activated charcoal gave purification only part of the activity was recoverable. Gel filtration with G-25 Sephadex was found to remove much inactive material as shown in Figure 1, but alone was not capable of giving the pure antibiotic.

Countercurrent distribution in a buffered phenol–water system did not give a pure band of the antibiotic directly from the crude extract but appeared to do so after the preliminary fractionation with gel filtration as shown in the patterns of Figure 1.

The band from Figure 2 with antibiotic activity gave a single spot when tested by thin layer chromatography on silica gel G in various systems. It gave a single spot in paper electrophoresis experiments whose position indicated that it was strongly basic.

When a trial amino acid analysis was made under the hydrolysis and chromatographic conditions usually used for proteins, three distinct bands aside from a large ammonia band were obtained. In position these corresponded to those expected for glycine, valine, and histidine. But thin layer chromatography failed to confirm the presence of either valine or histidine as

evidenced by the position of the spot or its color with ninhydrin. Only the presence of glycine was confirmed. In any case, the hydrolysis data at this stage could not be used as an indication of the purity of the preparation. Hydrolysis on a small preparative scale was therefore undertaken in order to isolate sufficient of each of the components to obtain elementary analysis and to determine the structure of the individual building blocks.

Countercurrent distribution of the hydrochlorides from a hydrolysate in a system containing HCl gave the pattern shown in Figure 3. Although the weight pattern was not promising, the absorbance analysis at 270 mμ indicated the fragment with the absorption band in the ultraviolet to be entirely localized in a well-separated band. On isolation it crystallized as the hydrochloride.

The elementary analysis and the absorption spectra, Figure 5, indicated the substance to be tyrosine hydrochloride. But the absence of a band in the position of tyrosine in the amino acid analysis experiment with the automatic analyzer together with the chromatoplate behavior of the hydrolysate excluded tyrosine. A study of nmr spectra of the substance in D_2O gave a very strong suggestion that it was isotyrosine. A signal at $\delta = 3.08$ ppm showed a double doublet ($J = 7$ and 1.5 cycles per second) indicating a methylene group. The data could be interpreted on the basis of a spin–spin splitting, $J = 7$ cycles per second, caused by an adjacent single proton. As the two methylene protons are not identical, each branch of the doublet could be further split into two lines found with $J = 1.5$ cycles per second. A signal at $\delta = 4.7$ ppm could be interpreted as due to a methene proton partially hidden under the chloroform peak. Finally, the signals from $\delta = 6.83$–7.50 ppm could be assigned as aromatic.

For a comparison racemic isotyrosine was synthesized by the method of Possner (1912) from a *p*-hydroxycinnamic acid obtained commercially. The synthetic material gave an R_F value on the chromatoplate which was identical with that from edeine and also gave the same yellow spot on spraying with ninhydrin. When the infrared spectra (KBr pellet) were compared a difference was found as expected since the synthetic product was racemic. To avoid this difficulty both were converted to the DNP methyl ester derivatives and compared by infrared spectrometry in chloroform solution. In this medium the spectra were identical. The ultraviolet spectra, Figure 5, of the amino acid hydrochlorides were identical and the same was true for the nmr spectra in D_2O.

Thin layer chromatography of solute from every 20th tube of the distribution of Figure 3 from tube 0–350 showed all to contain more than one solute except for the tubes 0–25. These gave only one spot. When the solvent was evaporated from a cut of 0–25 the residue was crystallized from ethanol–ethyl acetate. The striking characteristic of this compound was its strong basicity as shown by paper electrophoresis, a basicity exceeding that of any natural amino acid. As there was no infrared absorption in the carbonyl

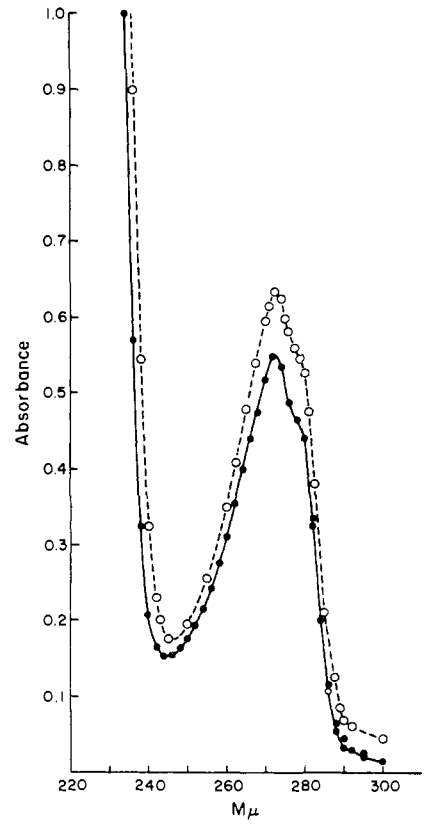

FIGURE 5: Ultraviolet absorption spectra of isotyrosine and edeine. ●—● = isotyrosine, 0.086 mg/ml of H_2O; ⊙—⊙ = edeine.

region it was reasonable to assume that this compound was an amine. A comparison of the elementary composition of a series of strongly basic amines occurring in biological fluids with the substance isolated from the distribution showed agreement only with spermidine. The identity was fully confirmed by comparison with a commercial sample of spermidine in the infrared spectrum, on the chromatoplate, and by paper electrophoresis. The compound from edeine and commerically obtained spermidine were converted to picrates. This derivative of both substances gave the same melting point of 209° and showed no depression in a mixture melting point.

In order to resolve the overlapping bands between tubes 25 and 325 of Figure 3, experiments were made with ion-exchange chromatography using Bio-Rad AG50 in the hydrogen form. Very clear-cut separation was obtained by a prolonged elution schedule as shown in Figure 4, with all components clearly resolved including the spermidine band.

The crystalline material from band I gave the same elementary analysis as serine but this possibility could be definitely excluded by the absence of a serine band in the pattern from the amino acid analyzer. Also, thin layer chromatography and the distinct orange color which developed on spraying with ninhydrin

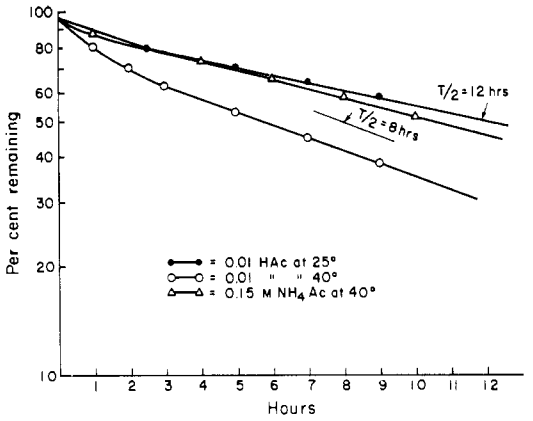

FIGURE 6: Thin-film dialysis patterns of edeine in an acetylated membrane calibrated with bacitracin (mol wt 1420) $T/2$ at 25° = 13.7 hr, $T/2$ at 40° = 8 hr.

indicated it to be different. The nmr spectrum in D_2O of the free amino acid was of little help. A better nmr spectrum was given when the material was converted to the DNP methyl ester. At δ = 3.36 ppm (1 H) there was a broad band indicating a hydroxyl proton. At δ = 3.85 ppm signals for five protons were found with a large peak for the methoxyl of the ester and probably a double doublet, only one side of which was visible because of partial overlapping with the large peak. The visible doublet had a coupling constant of J = 1.5 cycles per second.

A sample of authentic racemic isoserine was obtained from Nutritional Biochemicals but although it was identical by thin layer chromatography a difference was found in the infrared pattern with the KBr pellet. Accordingly, the 2,4-dinitrophenyl methyl esters of both the unknown and the synthetic sample were prepared. These were identical both by thin layer chromatography and by infrared. The optical rotation obtained indicated the *levo* form (Freudenberg, 1914).

The crystalline material from band II had the same elementary composition as glycine and the hydrolysate of edeine showed a band in the pattern from the amino acid analyzer which was in the glycine position. The identity was confirmed by isolation of adequate material for comparison by elementary analysis, infrared spectroscopy, and thin layer chromatography.

The crystalline material from band III of Figure 4 was shown by paper electrophoresis to be a basic compound. With the amino acid analyzer it gave a band in the histidine position but this amino acid was excluded by the behavior of the substance on thin layer chromatography. At the suggestion of Dr. S. Moore that it could be α,β-diaminopropionic acid judging from its position on the analyzer pattern, authentic material was obtained from Calbiochem and compared. Elementary analysis and comparison by paper electrophoresis, thin layer chromatography, and infrared spectroscopy (KBr) confirmed the identity.

The solute in band IV of Figure 4 has not as yet been identified. The weight recovered indicated it to be of a molecular weight of the same order as isotyrosine. It has not thus far crystallized from any solvent and has not been brought to a form suitable for elementary analysis. Paper electrophoresis indicated it to be a neutral amino acid but its behavior on thin layer chromatography and the nmr spectrum of its DNP methyl ester indicated it to be different from any of the known amino acids.

The DNP derivative prepared by FDNB as described for isoserine in the Materials and Methods section proved to be very water soluble but was slowly extracted by several portions of ethyl acetate. The residue on evaporation of the ethyl acetate and examination by thin layer chromatography in the ethyl acetate–acetic acid system (10:7) showed two streaking, rather poorly defined spots. In order to get them into a more easily separable, less polar form, the mixture was treated with 10% dry HCl in methanol. The resulting mixture of esters was then separated on a column of silica gel from which one compound could be eluted by ethyl acetate and the other with ethyl acetate containing 20% methanol. The first compound eluted showed one well-defined spot of R_F 0.43 by thin layer chromatography in the system ethyl acetate–chloroform (1:1). When the second compound to be eluted was treated with FDNB it yielded a product with the same R_F value on thin layer chromatography and the same solubility properties as the first. Its behavior on paper electrophoresis at various pH values indicated it to have the same number of acid and basic groups. These data together with the nmr spectra indicated a dicarboxyl-diamino compound.

The molecular size of intact edeine was estimated by the method of thin film dialysis (Craig and Konigsberg, 1961) in a cellophane membrane whose porosity had been adjusted by acetylation so as to provide a 50% escape time for bacitracin, mol wt 1420, of 8 hr at 40° or 13.7 hr at 25°. Edeine gave the escape patterns shown in Figure 6 at these temperatures when 0.01 N acetic acid was the solvent and at 40° when 0.15 M ammonium acetate was used as the solvent. This behavior is not that expected from a single pure solute behaving ideally nor is it that expected from a solute showing reversible association.

However, the recoveries of the fragments obtained on hydrolysis and fractionation, together with the absorbance of the intact edeine as compared with isotyrosine, may be informative in this connection. Table III shows the weights of the fragments obtained from a total of 1 g of edeine and the molecular weights of the hydrochlorides, assuming a molecular weight of 200 for the unknown fragment. These data suggest equimolar amounts of each of the six building blocks, although the yield of glycine is a little high. This was supported by determination of the ninhydrin color yield from the new amino acids and recalculation of the amounts of each component from the amino acid analysis pattern when the sample was run on a medium column and the elution schedule on the amino acid

TABLE III: Molecular Weights of Hydrochlorides of Fragments and Recoveries from 1 g of Edeine.

Fragment	Mol Wt	Yield (mg)
Glycine	111.5	146
Isoserine	140.6	147
Diaminopropionic acid	140.5	166
Isotyrosine	217.6	222
Spermidine	254	313
Unknown	200 (assumed)	207

analyzer was pH 3.28 buffer for 3.5 hr and then pH 5.28 buffer for 5 hr.

The absorption spectra curves of edeine and isotyrosine are shown in Figure 5. An aqueous solution of isotyrosine containing 0.086 mg/ml gives an absorbance of 0.518 at 270 mμ. Edeine is a noncrystalline, highly hygroscopic material but a solution with an optical density of 0.525 at 270 mμ was found to contain 0.343 mg/ml by residue weight determination (Craig, 1960). Because of the similarity of the spectra the isotyrosine band must be the only absorbing group in edeine and it is permissable to calculate a minimal molecular weight of 860 for edeine assuming 1 mole of isotyrosine. However, such a small size is not consistent with the rate of escape of the major component in Figure 6. Twice this would be comparable with the molecular weight of bacitracin and is suggested by the diffusion data.

The escape patterns of Figure 6 indicate the presence of 10–15% of a smaller component, and is a result found also with another preparation of edeine. The behavior suggests the possibility that some kind of monomer–dimer relationship exists which is not readily reversible under the conditions of the dialysis. The fractionation data together with the hydrolysis and subsequent fractionation speak against the likelihood of 10–15% of an entirely different peptide being present.

The ammonia band found in the pattern from the amino acid analyzer corresponded to approximately 1 mole/residue of the other hydrolysis fragments isolated. It can be calculated from the analytical data that a total of 10 basic groups and only six carboxyl groups are present in the monomer. Thus, five carboxyl groups would be required if amide linkages join the six hydrolysis fragments in linear linkage. Only one would remain to join ammonia and give a primary amide but the peptide is possibly cyclic. The ammonia in this case could arise from decomposition.

It probably is of some interest that two new isoamino acids have been found for the first time in one antibiotic polypeptide. Diaminopropionic acid has been found before but it can be considered as one of the iso types. This little known series may have biogenetic significance.

It was of interest to see if spermidine would be incorporated directly into the antibiotic if it were in the culture medium. This was proven to be the case by addition of ^{14}C-labeled spermidine to the culture medium and isolation of the edeine by paper chromatographic procedures. Hydrolysis of the spot having antibiotic activity and separation of the products on a column of Dowex 50-X8 gave a band corresponding to spermidine which contained the ^{14}C isotope. When a similar experiment was performed using ^{14}C-labeled methionine the same result was obtained. This was as expected considering the known biosynthetic pathway of spermidine (Greene, 1957). When the experiment was repeated with tyrosine but not methionine labeled, only one zone corresponding to an amino acid with ^{14}C was obtained. This corresponded to the isotyrosine of edeine. Isomerization of the tyrosine obviously had occurred. These results have been reported in part (Kurylo-Borowska and Tatum, 1966) and will be reported in full in future studies by one of us (Z. Kurylo-Borowska, unpublished data).

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