Biochemistry

 Volume 7, Number 12 December 11, 1968

Edeine. II. The Composition of the Antibiotic Peptide Edeine A*

Thomas P. Hettinger† and Lyman C. Craig

ABSTRACT: All of the components of the antibiotic peptide edeine A have now been identified. Edeine A contains one residue each of glycine, isoserine, β-tyrosine, α,β-diaminopropionic acid, spermidine, and 2,6-diamino-7-hydroxyazelaic acid. The last compound is a new amino acid which is reversibly transformed in part under hydrolytic conditions to 2,6-diamino-7-nonenedioic acid.

In the first paper of this series (Roncari *et al.*, 1966) we reported results with the isolation and structural studies of edeine A, an antibiotic peptide produced by *Bacillus brevis* Vm4. This substance is unusual among naturally occurring peptides in that, of the six major fragments obtained on acid hydrolysis, only two, glycine and α,β-diaminopropionic acid, are simple α-amino acids. Two β-amino acid components, isoserine and β-tyrosine, and the base spermidine have not yet been found in other antibiotic peptides.

In addition there is one further amino acid constituent which was partially characterized in our initial report. It appeared to be a diaminodicarboxylic acid with a molecular weight of at least 200, but insufficient information was available at that time for complete characterization. We have since carried out a number of experiments with this amino acid and, by various chemical and spectroscopic methods, have been able to arrive at a unique chemical structure. The elucidation of the structure was greatly facilitated by the isolation and characterization of an acid transformation product.

Experimental Section

Isolation of Edeine A. Edeine A was isolated from cultures of *B. brevis* Vm4 by the procedure previously described (Roncari *et al.*, 1966) with certain modifications. The changes, which will be described in the accompanying paper (Hettinger *et al.*, 1968), permit the isolation also of a second antibiotic, edeine B.

Hydrolysis of Edeine A and Isolation of the Products. Edeine A acetate (0.98 g) was refluxed in 200 ml of 6 N HCl for 30 hr. The solution was then evaporated under reduced pressure to a glassy residue. The removal of β-tyrosine from this residue was carried out by countercurrent distribution as before (Roncari *et al.*, 1966), and the remaining fragments were purified by chromatography on Bio-Rad AG 50-X2 (Figure 1). The major bands were collected and evaporated to dryness. The glycine band was contaminated by NH_4Cl and it was necessary to convert this amino acid into its isoelectric form to determine the yield. This was accomplished by adsorbing the hydrochloride on a small column (0.9 × 10 cm) of Bio-Rad AG 50-X2 H^+, washing with water until the effluent was neutral, and eluting with 1 N NH_4OH. Evaporation gave the free amino acid. Unknowns I and II were converted into the isoelectric forms in the same way and traces of ammonia were finally removed by repeated lyophilization from water.

Unknown I. This compound was a hygroscopic resin which could not be crystallized from any of the solvents tested, either as a salt or in the form of the free amino acid. However, it appeared homogeneous by paper chromatography and paper electrophoresis. It gave a single band with the amino acid analyzer. For analysis, the free amino acid was dried over P_2O_5 at room temperature for 1 month. *Anal.* Calcd for $C_9H_{18}N_2O_5 \cdot 0.5H_2O$: C, 44.43; H, 7.87; N, 11.52. Found: C, 44.16; H, 7.80; N, 11.19. A preparation

* Contribution from The Rockefeller University, New York, New York. *Received July 12, 1968.* This work was supported in part by U. S. Public Health Service Grant AM 02493.

† Postdoctoral fellow of the U. S. Public Health Service (Grant 1-F2-GM-35,426-01).

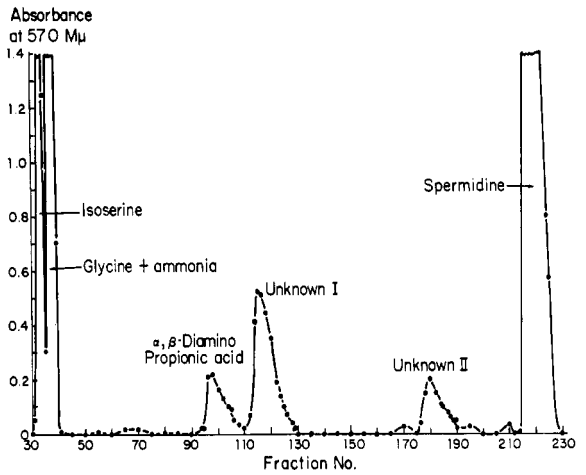


FIGURE 1: Chromatography of edeine A fragments. The hydrolysate of edeine A acetate (0.98 g) from which β-tyrosine had been removed by countercurrent distribution was dissolved in 10 ml of 0.5 N HCl and applied to a 2.2 × 55 cm column of Bio-Rad AG 50-X2 H^+ (200–400 mesh) equilibrated with 1 N HCl. Chromatography was carried out at 24° with a flow rate of 48 ml/hr in 12-ml fractions. The column was eluted with 1 N HCl up to fraction 150 and then with 3 N HCl for 80 fractions. For analysis, 50-μl samples were treated with 1 ml of ninhydrin reagent (Moore and Stein, 1954) and, after color development at 100° for 15 min, the solutions were diluted with 10 ml of 50% ethanol for absorbance determination at 570 mμ.

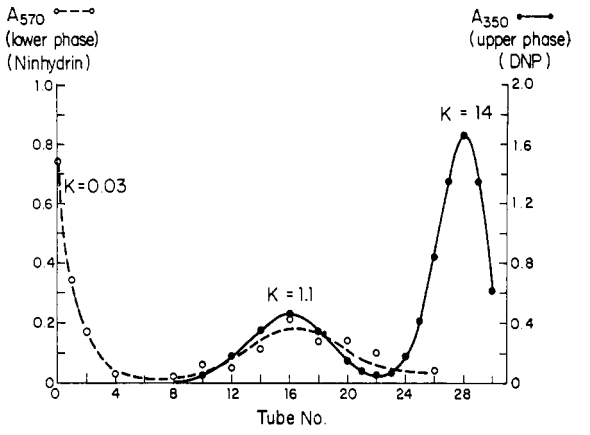


FIGURE 2: Countercurrent distribution of partially dinitrophenylated unknown I. Right ordinate, absorbance at 350 mμ (0.3 ml of upper phase plus 0.9 ml of methanol); left ordinate, absorbance at 570 mμ (0.3 ml of lower phase plus 1 ml of ninhydrin reagent plus 5 ml of diluent).

dried *in vacuo* at 95° for 2 hr gave similar results. Drying of the compound under more vigorous conditions was not attempted because of the possibility of loss of water by β elimination or lactam formation.

Unknown II. This compound, which appeared homogeneous by paper chromatography and electrophoresis, also could not be obtained in crystalline form. The data from the amino acid analyzer indicated that the substance was approximately 98% pure. For analysis it was dried in the same way as unknown I. *Anal.* Calcd for $C_9H_{16}N_2O_4 \cdot 0.5H_2O$: C, 47.99; H, 7.61; N, 12.44. Found: C, 47.65; H, 7.50; N, 12.25.

Partial Substitution of Unknown I. Unknown I · 2HCl (10 mg) was dissolved in 1 ml of 4% $NaHCO_3$ and treated with 2 ml of 0.2% v/v 2,4-dinitrofluorobenzene in ethanol for 1 hr with occasional shaking at room temperature. Excess reagent was extracted with ether and the aqueous phase was acidified with 1 ml of 1 N HCl and evaporated to dryness. The residue was put into tube 0 of a countercurrent distribution machine and distributed for 30 transfers in the system 1-butanol–0.1 N HCl (1:1), 3/3 ml volumes at 24° (Figure 2). The three major components which separated were judged to be the unchanged amino acid (K = 0.03), the mono-DNP derivative(s) (K = 1.1), and the bis-DNP derivative (K = 14) on the basis of their absorbance at 350 mμ and their reaction with ninhydrin. The electrophoretic behavior of these substances was in agreement with this interpretation. Determination of the weight–extinction ratios of the mono- and bis-DNP derivatives gave 203 and 254 g per mole, respectively, as the estimated molecular weight of the original amino acid, assuming a molar absorbancy index of 15,000/DNP group. The molecular weight calculated from the formula $C_9H_{18}N_2O_5$ is 234.

Preparation of the Acetyl Methyl Ester Derivative of Unknown I. Unknown I · 2HCl (30 mg) was dissolved in 3 ml of 2 M pyridine and cooled to 0°. Three 100-μl portions of cold acetic anhydride were added with stirring over 30 min. After another 60 min at 0°, the solution was evaporated to dryness several times with the addition of water. The residue was dissolved in water and passed through a 0.9 × 10 cm column of Bio-Rad AG 50-X2 H^+ equilibrated with water. The acidic effluent obtained on elution with water was evaporated to dryness to give the free acid, which was ninhydrin negative and free of pyridine. The acetylated compound was dissolved in 2 ml of methanol and esterified by treatment with a slight excess of diazomethane in ether. Evaporation of the solvent gave 25 mg of the acetyl methyl ester derivative.

A study of this derivative by nuclear magnetic resonance showed that only one acetyl and one methoxyl group were present. This suggested that one amino and one carboxyl group had formed a lactam during the acetylation procedure. In the infrared, the two amide carbonyl groups appeared as a split band at 1650 and 1680 cm^{-1}, and an absorption band at 1730 cm^{-1} was ascribed to the methyl ester carbonyl group. A hydroxyl group was suggested by a broad band at 3280 cm^{-1}. Methoxyl analysis was consistent with the presence of only one methyl ester group. *Anal.* Calcd for $C_{11}H_{17}N_2O_4OCH_3$: OCH_3, 11.4. Found: 10.9.

Periodate Oxidation. Tests for vicinal hydroxyl or amino groups were performed by the spectrophotometric periodate-consumption method of Dixon and Lipkin (1954). Reactions were carried out in 1 N NH_4OH at 25° with 10^{-4} M KIO_4. Periodate consumption was followed at 225 mμ after amino acid addition (final concentration 5 × 10^{-5} M) until completion of the reaction (0.2–2 hr). The absorbance difference per micromole of periodate consumed was determined by addition of excess of amino acid. Under these conditions, serine, isoserine, α,β-diaminopropionic acid,

TABLE I: Chromatographic and Electrophoretic Properties of Edeine A Fragments.

Fragment	R_F in Paper Chromatography[a]	Mobility in Paper Electrophoresis (mm)		Elution Time on the Amino Acid Analyzer (min)	
		pH 6.4[b]	pH 3.5[c]	12-cm Column[d]	55-cm Column[e]
Glycine	0.26	7	28		122
Isoserine	0.26	7	40		154
I	0.15	7	73	14	320
II	0.18	7	60	14	334
β-Tyrosine	0.57	7	60	27	>400
α,β-Diaminopropionic acid	0.21	102	167	74	
Spermidine	0.27	192	289		

[a] Whatman No. 3MM paper; butanol–acetic acid–pyridine–water (6:3:2:3). [b] Pyridine–acetic acid–water (100:4:896); 60 min at 40 V/cm. [c] Pyridine–acetic acid–water (10:100:890); 105 min at 35 V/cm. [d] pH 5.28 buffer; reference compounds norleucine, ammonia, and β-guanidinoalanine were eluted at 16, 100, and 123 min, respectively. [e] pH 3.25 for 140 min, then pH 4.25; norleucine was eluted at 225 min.

δ-hydroxylysine, β-hydroxy-γ-aminobutyric acid, and unknown I each consumed 1 mole of periodate. Homoserine, α,γ-diaminobutyric acid, aspartic acid, β-alanine, malic acid, and unknown II did not react with periodate. The spectra of the ultraviolet-absorbing products of the reaction of periodate with excess of unknown I and β-hydroxy-γ-aminobutyric acid were obtained by subtraction of the spectrum of the products of the serine–periodate reaction.

Permanganate Oxidation of Unknown II. The acetyl derivative of unknown II was prepared in the same way as described for unknown I, except that the product was not passed through the cation exchanger. The derivative prepared from 20 mg of unknown II·2HCl (69 μmoles) was dissolved in 3 ml of water and 2 ml of 0.1 M $KMnO_4$ was added. After 1 day at room temperature, the excess permanganate was discharged by adding a few drops of isopropyl alcohol. The MnO_2 was removed by centrifugation and filtration, and the filtrate was treated with Bio-Rad AG 50-X2 H^+ until a pH of 3 was reached. The solution was then passed through a 0.9 × 10 cm column of the same resin, the column was washed with water, and the acidic effluent was collected. Evaporation yielded 15 mg of residue. This was hydrolyzed in 4 ml of 6 N HCl for 2 hr at 100°. The major component in the hydrolysate was eluted at the methionine position on the amino acid analyzer. This was the position also found for authentic 2,6-diaminopimelic acid. The oxidation product was likewise indistinguishable from diaminopimelic acid by paper chromatography and electrophoresis. On paper chromatograms (Whatman No. 3MM paper; 1-butanol–acetic acid–pyridine–water, 6:3:2:3) both diaminopimelic acid and the product had an R_F of 0.12 and, on reaction with ninhydrin, gave blue-green colors which faded to a permanent yellow. Both remained at the origin on electrophoresis at pH 6.4 and 3.5, and migrated at the same rate toward the cathode at pH 1.9. The yield of diaminopimelic acid from the oxidation was 26 μmoles.

Results and Discussion

When edeine A hydrolysates were subjected to paper chromatography and electrophoresis, seven major ninhydrin-positive compounds were found to be present (Table I). Six of these components corresponded with those which had been previously isolated by Roncari *et al.* (1966), using countercurrent distribution and ion-exchange chromatography. These were glycine, isoserine, β-tyrosine, α,β-diaminopropionic acid, spermidine, and the diaminodicarboxylic acid which we have termed unknown I. We have now obtained in pure form all seven of the compounds, including the additional fragment, unknown II, by countercurrent distribution and chromatography on Bio-Rad AG 50-X2 H^+ (Figure 1).

The elemental analysis of unknown I suggested $C_9H_{18}N_2O_5$ as the probable empirical formula of the compound. However, the hygroscopic nature of the resin and the fact that a fractional mole of water appeared to be strongly bound required us to accept the formulation with some reservation. Nevertheless, support for this empirical formula was deduced from partial substitution experiments and thin-film dialysis. Thus, unknown I formed mono- and bis-DNP derivatives from whose spectra and extinction coefficient a molecular weight of about 200–250 for the free amino acid could be calculated. The theoretical value of the proposed formula is 234. The absorption spectra of the DNP derivatives further showed (Dubin, 1960) that both amino groups of unknown I were primary (λ_{max} 345 mμ in 80% methanol). The electrophoretic behavior of unknown I and the infrared spectrum of the acetyl

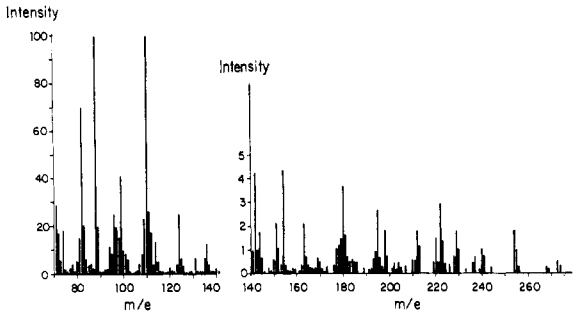


FIGURE 3: Mass spectrum of the acetyl methyl ester derivative of I.

methyl ester derivative indicated that the remaining functional groups in the molecule were two carboxyl groups and probably one hydroxyl group.

With a selective membrane (Craig, 1964) it was also possible to show by thin-film dialysis that the molecular weight of unknown I could not be much greater than 200. In fact, unknown I dialyzed even more rapidly than 2,6-diaminopimelic acid (molecular weight 190). Their $t_{1/2}$ values in 0.01 N acetic acid were 41 and 53 min, respectively. This unexpected result suggests that the two amino acids may exist in quite different conformations in aqueous solution.

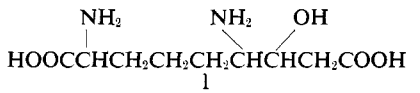
Unknown II was recovered from hydrolysates of edeine A in a yield even less than that of glycine. This suggested that it was (a) an impurity in the antibiotic preparation, (b) a peptide, (c) an amino acid which was extensively destroyed by acid hydrolysis, or (d) a transformation product of some other amino acid. The behavior of unknown II on chromatography and electrophoresis suggested that it might be in some way related to unknown I. Treatment of either unknown I or unknown II under the conditions used for acid hydrolysis of edeine A resulted in the same mixture of unknowns I and II.[1]

The fact that unknown II was isoelectric at pH 6.4 eliminated the possibility that it was a lactone of unknown I. Partial substitution of unknown II gave mono- and bis-DNP derivatives, indicating that it could not be a lactam. While unknown II could conceivably be a diastereoisomer of unknown I, the ultraviolet absorption spectra of the two amino acids revealed that the difference was more substantial than this. Thus, unknown II had intense end absorption (200 mμ (ε 12,000)), in contrast to unknown I, which had almost negligible absorption in the ultraviolet (200 mμ (ε 400)). These results, along with the established interconversion of unknowns I and II, suggested that unknown II was an acrylic acid derivative resulting from the dehydration of the corresponding β-hydroxy acid portion of unknown I. This was supported by elemental analysis of unknown II, which suggested the formula $C_9H_{16}N_2O_4$ or at least one having 1 mole of water less than unknown I.

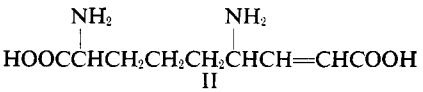
[1] In addition to having different R_F values, unknowns I and II are readily distinguished by their ninhydrin colors. Unknown I gives a stable blue color, while unknown II appears at first blue-grey and then fades to yellow-brown.

Unknown I, when oxidized with periodate under spectrophotometric conditions, consumed 1 mole of periodate and produced an ultraviolet-absorbing chromophore having the same spectral characteristics as malonic semialdehyde (260 mμ (ε 5000) in 1 N NH_4OH).[2] Unknown II did not react with periodate. This indicated that one amino group of unknown I was vicinal to the hydroxyl group (*i.e.*, in the γ position), *viz.*, $CH(NH_2)CH(OH)CH_2COOH$. The corresponding portion of unknown II would then have the aminocrotonic acid structure $CH(NH_2)CH{=}CHCOOH$. The carboxyl group in the latter structure would be expected to be more acidic than that in the β-hydroxy acid, but neither of these would be expected to be as acidic as an α-amino acid. The order of cathodic mobility, unknown I > unknown II > glycine, at pH 3.5 is therefore in agreement with these structures. The more hydrophilic character of the β-hydroxy acid likewise tends to explain the differences in the chromatographic behavior of unknowns I and II.

The remainder of the structure of unknown II was established by the oxidation of the diacetyl derivative with potassium permanganate. This gave 2,6-diaminopimelic acid in 37% yield and hence indicated that the amino groups of unknowns I and II were also in the 2,6 positions. With this information, the structure of unknown I was therefore established as 2,6-diamino-7-hydroxyazelaic acid (I) and that of unknown II as 2,6-diamino-7-nonenedioic acid (II).



I



II

Structures I and II could also be derived independently by nuclear magnetic resonance spectroscopy. The nuclear magnetic resonance spectra of I in D_2O (tetramethylsilane = 0 ppm) showed no methyl or other terminal groups, except for the $CH(ND_2)COOD$ (1 H, δ = 3.7 ppm, triplet) and CH_2COOD (2 H, δ = 2.4 ppm, multiplet) groups, requiring I to be an unbranched dicarboxylic acid. The other assignments were $(CH_2)_3$ (6 H, δ = 1.7 ppm, envelope), $CH(ND_2)$ (1 H, δ = 3.3 ppm, multiplet), and CH(OD) (1 H, δ = 4.1 ppm, multiplet).

In the nuclear magnetic resonance spectra of II, the protons $CH(ND_2)COOD$ (1 H, δ = 3.7 ppm, triplet) and $(CH_2)_3$ (6 H, δ = 1.7 ppm, envelope) were deemed to be homologous with those of I. The $CH(ND_2)$ proton was shifted downfield (1 H, δ = 3.9 ppm, multiplet, decoupled to a triplet by irradiation at δ = 6.4 ppm) and the CH(OD) and CH_2COOD protons were absent. Two vinylic protons, $=CHCOOD$ (1 H, δ = 6.0 ppm, doublet) and CH= (1 H, δ = 6.4

[2] Malonic semialdehyde was prepared *in situ* by periodate oxidation of β-hydroxy-γ-aminobutyric acid.

TABLE II: Major Fragments in the Mass Spectrum of the Acetyl Methyl Ester Derivative of I.

Fragment	Formula	*m/e* Found	*m/e* Calcd	Rel Intensity
M + 1	$C_{12}H_{21}N_2O_5$	273.1454	273.1445	0.3
M	$C_{12}H_{20}N_2O_5$	272.1371	272.1367	0.5
M − H_2O	$C_{12}H_{18}N_2O_4$	254.1254	254.1262	1.8
M − CH_3OH	$C_{11}H_{16}N_2O_4$	240.1121	240.1106	1.0
254 − CH_3OH	$C_{11}H_{14}N_2O_3$	222.1012	222.1001	3.0
M − $COOCH_3$	$C_{10}H_{17}N_2O_3$	213.1251	213.1235	0.8
254 − CH_2CO	$C_{10}H_{16}N_2O_3$	212.1168	212.1157	0.8
254 − CH_3CONH_2	$C_{10}H_{13}NO_3$	195.0876	195.0892	1.9
222 − CH_2CO	$C_9H_{12}N_2O_2$	180.0902	180.0896	1.8
222 − CH_3CONH_2	$C_9H_9NO_2$	163.0627	163.0631	2.0
213 − CH_3CONH_2 (Ia)	$C_8H_{12}NO_2$	154.0868	154.0865	4.4
Ib	$C_7H_{12}NO_2$	142.0862	142.0865	3.9
IIa (Ia − H_2O)	$C_8H_{10}NO$	136.0739	136.0760	12.0
III	$C_5H_9NO_3$	131.0563	131.0580	6.0
Ic	$C_6H_{10}NO_2$	128.0707	128.0709	0.5
IIb (Ib − H_2O)	$C_7H_{10}NO$	124.0756	124.0760	15.8
Id	$C_5H_8NO_2$	114.0573	114.0553	4.1
IIc (Ic − H_2O)	C_6H_8NO	110.0602	110.0604	100.0
Ie	$C_4H_6NO_2$	100.0380	100.0397	7.4
IV (III − CH_3OH)	$C_4H_5NO_2$	99.0314	99.0319	35.5
IId (Id − H_2O)	C_5H_6NO	96.0445	96.0448	8.1
V (III − $COCH_3$)	$C_3H_6NO_2$	88.0410	88.0397	100.0
VI	C_5H_8N	82.0661	82.0655	50.1
IIe (Ie − H_2O)	C_4H_4NO	82.0306	82.0292	5.6

ppm, doublet of doublets), were strongly coupled (J = 16 Hz), and the latter was further coupled (J = 8 Hz) to another proton. Irradiation at δ = 3.9 ppm caused the two vinylic protons to revert to a simple AB system. This established $\overset{\text{HOOC}}{\text{H}}\!>\!\text{C}\!=\!\text{C}\!<\!\overset{\text{H}}{\text{CH(NH}_2\text{)-}}$ as a partial structure for II. Incorporation of the remaining α-amino acid function and the methylene groups gives structure II as the only possible result. This leads unequivocally to structure I for the other amino acid.

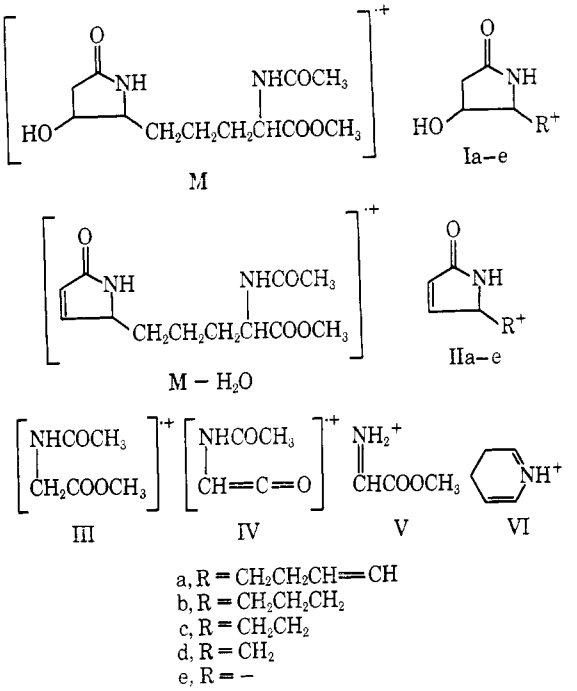
For structural analysis of I by mass spectroscopy, a number of derivatives were prepared, but none was wholly satisfactory for this purpose. The diethyl ester of I gave peaks at values of *m/e* higher than expected for the molecular ion, which indicated that the sample had probably polymerized in the process of vaporization. The bis(DNP) dimethyl ester derivative was too extensively fragmented to be useful (G. Roncari, unpublished results). The best results were obtained with the acetyl methyl ester, although, even in this case, the fragmentation was quite extensive and heterogeneous. The complex spectrum could be interpreted properly only after the major features of the structure had been established by other methods.

The highest mass peak was found at *m/e* 273 and corresponded to the M + 1 peak (Figure 3 and Table II). The peak at *m/e* 272 was only slightly more intense (0.5% of the base peak) and had the formula $C_{12}H_{20}N_2O_5$, which corresponded to the molecular ion of the monoacetyl monomethyl ester lactam of I. Peaks at *m/e* 254 and 240 resulted from loss of water or methanol, respectively, from the molecular ion. The loss of a molecule of water was a characteristic feature of the spectrum, and several of the fragments appeared to arise from the 254 peak. Thus, peaks at 222, 212, and 195 could be assigned to the 254 − CH_3OH, 254 − CH_2CO, and 254 − CH_3CONH_2 ions, respectively. The fragment Ia (*m/e* 154) (Chart I) could be derived from the molecular ion by loss of $COOCH_3$ and CH_3CONH_2, and the more intense IIa (*m/e* 136) could result from the dehydration of Ia. Fragments Ib–e could be obtained by cleavage of the main carbon chain and the corresponding dehydration products IIb–e were usually more intense. The fragments III–V arise from the α-amino acid portion of the molecule through proton rearrangement.

Finally, the fragment VI (C_5H_8N) could arise from the C-2 through C-6 portion of the molecule through a series of cleavage reactions. This peak is reminiscent of a similar ion ($C_5H_{10}N$) which appears in the mass spectra of lysine derivatives (Biemann *et al.*, 1961). That fragment VI has one more double bond is probably due to the fact that both amino groups of I are attached to secondary carbon atoms.

Because I and II are interconvertible under the condi-

CHART I



tions of acid hydrolysis, either of them could, in principle, be the amino acid present in intact edeine A. In order to decide, edeine A was hydrolyzed for a short time (2 hr) in 6 N HCl at 100°, and the hydrolysate was subjected to paper chromatography and electrophoresis. Under these conditions, I could be detected readily in the hydrolysate, but little or no II was found. This indicates that I is the natural amino acid residue and that II is an artifact of the hydrolytic procedure. The dehydration of β-hydroxy acids under hydrolytic conditions appears to be a general phenomenon (Lederer, 1964). The proportion of II increases during longer hydrolysis, although it appears that an equilibrium mixture is established after about 10 hr at 110°, with a ratio of I to II of about 3:1 (Hettinger *et al.*, 1968).

Since all of the major fragments derived from edeine A have now been identified, it is of interest to compare the yields of the various components with the theoretical values which can be calculated on the basis of several assumptions. These are that edeine A consists of one residue of each of the six fragments which are connected by means of five peptide bonds, and that the isolated salt is the tetraacetate. On this basis, the molecular weight of the free base is 755, and that of the acetate, 995. The yields given in Table III are in general somewhat low, as may be expected, since the recoveries of the fragments on countercurrent distribution and chromatography are not entirely quantitative. However, the values for glycine, isoserine, α,β-diaminopropionic acid, and spermidine are all fairly close to one residue per mole. The yields of I and II together amount to only about 0.5 mole/mole of edeine A. These amino acids, aside from being converted to one another,

TABLE III: Yields of Edeine A Fragments.[a]

Fragment	Formula Wt	mg/0.98 g of Edeine A Acetate	Moles/995 g of Edeine A Acetate
Glycine	75	65	0.88
Isoserine	105	87	0.84
I·2HCl	307	113	0.37
II·2HCl	289	43	0.15
β-Tyrosine·HCl	218	95[b]	0.44[b]
α,β-Diaminopropionic acid·HCl	141	156	1.12
Spermidine·3HCl	254	241	0.96

[a] Edeine A acetate (0.98 g) hydrolyzed for 30 hr in refluxing 6 N HCl. [b] After crystallization from ethanol–ethyl acetate.

appear to be transformed on acid hydrolysis to several other products which have not been identified. Kinetic studies indicate that the original content of I is more nearly equal to 1 residue (Hettinger *et al.*, 1968). The same is true for β-tyrosine, which is also destroyed to a considerable extent on acid hydrolysis.

The composition of edeine A alone serves to distinguish it from other naturally occurring polypeptide antibiotics. It bears no resemblance to the tyrocidines and gramicidins, which are also produced by strains of *Bacillus brevis*. Of the six fragments of edeine A, only glycine and diaminopropionic acid are constituents of other antibiotic peptides, and the amino acids isoserine, β-tyrosine, and 2,6-diamino-7-hydroxyazelaic acid have not previously been found in living organisms. The triamine spermidine, although of wide natural occurrence in the free state, rarely occurs as a component of peptides (Tabor and Tabor, 1964). A novel example of amide-linked spermidine is given by the alkaloid lunarine (Tamura *et al.*, 1965).

Several of the edeine A fragments are interesting from the functional and biosynthetic point of view. The isomerization of α-tyrosine to β-tyrosine (Kurylo-Borowska and Abramsky, 1968) has few analogies in biosynthetic pathways and the rare occurrence of β peptides raises questions concerning the function of these compounds. Such peptides will be resistant to hydrolysis by the majority of the common peptidases. Edeine A is unaffected by trypsin, chymotrypsin, pepsin, Nagarse, and aminopeptidase M. Resistance to the action of these enzymes probably has functional significance, since hydrolytic stability would seem to be required for an antibiotic to be effective.

Spermidine is largely responsible for the basic character of edeine A and is perhaps directly involved in its activity, since spermidine itself acts as an antibiotic at relatively higher concentrations (Tabor and Tabor, 1964).

The novel 2,6-diamino-7-hydroxyazelaic acid is in certain respects homologous with lysine and 2,6-diaminopimelic acid. This suggests several possible biosynthetic and functional relationships between these compounds. Considered in a different way, the new amino acid is also a β-hydroxy acid. By analogy with well-known pathways for fatty acid biosynthesis, it may be conjectured that I could be synthesized from a derivative of diaminopimelic acid and a two-carbon fragment.

As I has three asymmetric carbon atoms, the natural compound could exist as any one of eight different optical isomers. There is a suggestion that the material isolated can be a mixture of stereoisomers. While it emerges as a single band on the amino acid analyzer, the band is often asymmetrical. The nuclear magnetic resonance spectra of I show resonances assigned to the C-6, C-7, and C-8 protons which appear too complex for one isomer only. The epimerization of I can be expected to occur in more than one way, the most apparent of which is due to the reversible dehydration reaction. The directive influence of C-6 of II on the stereospecificity of hydration of the double bond would be expected to be small, and would lead to a mixture of *threo* and *erythro* modifications of I. Moreover, inversions at the other optical centers cannot be ruled out. Conditions which cause minimum steric alterations of I during its isolation are presently being sought.

Acknowledgment

We thank Mr. James Duke for the 60-MHz nuclear magnetic resonance spectra, Mr. William C. Jankowski of the Varian Co. for the 100-MHz spectra and for carrying out the decoupling experiments, and Dr. Floie Vane of the Hoffmann-LaRoche Co. for the mass spectra data. We also thank them and Drs. William C. Agosta and William I. Taylor for their interest and helpful discussions. We thank Mr. T. Bella for the microanalyses.

References

Biemann, K., Seibl, J., and Gapp, F. (1961), *J. Am. Chem. Soc. 83*, 3795.

Craig, L. C. (1964), *Science 144*, 1093.

Dixon, J. S., and Lipkin, D. (1954), *Anal. Chem. 26*, 1092.

Dubin, D. T. (1960), *J. Biol. Chem. 235*, 783.

Hettinger, T. P., Kurylo-Borowska, Z., and Craig, L. C. (1968), *Biochemistry 7*, 4153.

Kurylo-Borowska, Z., and Abramsky, T. (1968), *Fed. Proc. 27*, 788.

Lederer, E. (1964), *Proc. 6th Intern. Congr. Biochem. New York, 33*, 63.

Moore, S., and Stein, W. H. (1954), *J. Biol. Chem. 211*, 907.

Roncari, G., Kurylo-Borowska, Z., and Craig, L. C. (1966), *Biochemistry 5*, 2153.

Tabor, H., and Tabor, C. W. (1964), *Pharmacol. Rev. 16*, 245.

Tamura, C., Sim, G. A., Jeffreys, J. A. D., Bladon, P., and Ferguson, G. (1965), *Chem. Commun.*, 485.

Edeine. III. The Composition of the Antibiotic Peptide Edeine B*

Thomas P. Hettinger,† Zofia Kurylo-Borowska, and Lyman C. Craig

ABSTRACT: A new antibiotic peptide, edeine B, has been isolated from strain Vm4 of *Bacillus brevis*. Edeine B is composed of one residue each of glycine, isoserine, β-tyrosine, α,β-diaminopropionic acid, 2,6-diamino-7-hydroxyazelaic acid, and a novel base, *N*-guanyl-*N'*-(3-aminopropyl)-1,4-diaminobutane (guanylspermidine). Edeine B differs from edeine A in the replacement of an amino group on the spermidine residue by a guanidino group. Both antibiotics contain five free basic groups and one free carboxyl group.

Cultures of *Bacillus brevis* Vm4 have been shown to produce at least two distinct antibiotic substances (Borowski *et al.*, 1966; Kurylo-Borowska, 1967). The first of these, edeine A, has been partially characterized and found to contain equimolar amounts of glycine, isoserine, α,β-diaminopropionic acid, β-tyrosine, 2,6-diamino-7-hydroxyazelaic acid, and spermidine (Roncari *et al.*, 1966; Hettinger and Craig, 1968). A second antibiotic peptide, edeine B, whose presence had been indicated by paper chromatography (Borowski *et al.*, 1966), has now been isolated and its composition determined.

The previous isolation method, although capable of giving pure edeine A (Roncari *et al.*, 1966), is not suit-

* Contribution from The Rockefeller University, New York, New York. *Received July 12, 1968.* This work was supported in part by U. S. Public Health Service Grants AM 02493 and CA 03610-09.

† Postdoctoral fellow of the U. S. Public Health Service (Grant 1-F2-GM-35,426-01).