TOTAL SYNTHESIS OF EDEINE D

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(Received for publication March 8, 1983)

Syntheses of the peptides with sequences postulated for active and inactive isomer of edeine D were carried out. The peptides obtained were identical with natural product in regard to chromatographic and electrophoretic properties. Biological data for synthesized compounds confirmed that in active and inactive isomer isoserine is linked with the α- or β-amino group of α,β-diaminopropionic acid, respectively.

Cultures of *Bacillus brevis* Vm_4 have been shown to produce a group of closely related antibiotic oligopeptides, named edeines[1,2], exhibiting a broad spectrum of antimicrobial activity[3,4]. These compounds are specific, reversible inhibitors of DNA synthesis in intact prokaryotic microbial cells[5] and specific inhibitors of protein synthesis in isolated ribosomes of prokaryotic and eukaryotic origin[6,7].

Edeines have been applied as biochemical reagents in bacterial genetic investigations[8,9] and have also been found to be a useful tool for studying various aspects of ribosome function and protein synthesis[7,10~12].

The ability of edeines to differentiate prokaryotic and eukaryotic microorganisms with regard to the inhibitory action on the biosynthesis of DNA[13] points to the possibility of exploitation of differences in structure and functioning of DNA replicating apparatus in Prokaryota and Eukaryota as a molecular basis of selective toxicity in the search for chemotherapeutical agents.

The antibiotic complex formed during biosynthesis contains mainly four active compounds, edeines A, B, D and F. These antibiotics are pentapeptide amides composed of glycine, polyamine and four non-protein amino acids: α,β-diaminopropionic acid, β-phenyl-β-alanine or β-tyrosine, isoserine and 2,6-diamino-7-hydroxyazelaic acid[2,14~16]. Last three compounds have not previously been found to be components of naturally occurring peptides.

Edeines, similarly to a series of peptides and derivatives of α,β-diaminopropionic acid[17,18], undergo reversible intramolecular aminolysis in water solutions[19]. Such a reaction occurs during isolation and storage of the antibiotics. Thus, each of the edeines exists as a mixture of two isomeric peptides differing from one another by the mode of linkage of the α,β-diaminopropionic acid functional groups. The compounds in which isoserine is linked with the α-amino group of α,β-diaminopropionic acid (α-isomers) were described as active isomers[19]. The β-isomers have been shown to be biologically inactive. The structural formulae of the α-isomers are presented in Fig. 1.

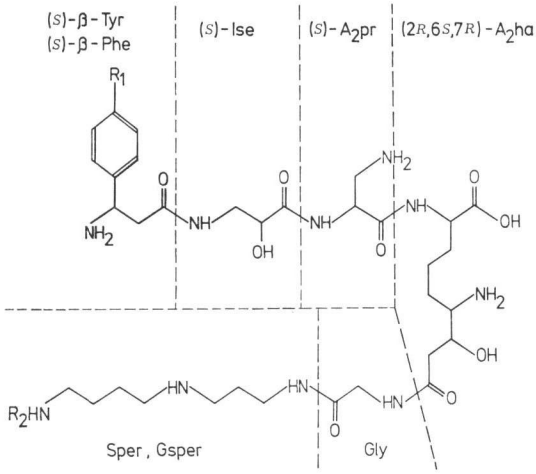
We undertook the synthesis of the both edeine D isomers to demonstrate which is the active one. Moreover, the synthesis was a confirmation for the postulated structure[20].

Results

The general concept of the synthesis was based on the coupling of tripeptides corresponding to

Fig. 1. Structures of edeine antibiotics.

β-Tyr=β-tyrosine, β-Phe=β-phenyl-β-alanine, Ise=isoserine, A_2pr=α,β-diaminopropionic acid, A_2ha=2,6-diamino-7-hydroxyazelaic acid, Gly=glycine, Sper=spermidine, Gsper=guanylspermidine



Edeine	R_1	R_2
A	-OH	-H
B	-OH	$-C(=NH)-NH_2$
D	-H	-H
F	-H	$-C(=NH)-NH_2$

N-terminal fragments of edeine D isomers with the dipeptide amide, as shown in Fig. 2.

2,6-Diamino-7-hydroxyazelaic acid, isolated from the acid hydrolysate of antibiotic complex, was converted into the α-methyl ester of the N^2-trityl, N^6-*t*-butoxycarbonyl derivative[21] for incorporation into a peptide chain of edeine. This compound was coupled with protected glycylspermidine[22] by the azide method in the presence of DPPA[23] yielding the protected *C*-terminal moiety of edeine D (**1**). After cleavage of the trityl group, the compound **2** so obtained was coupled with both protected isomeric tripeptides[24]. As a result of this reaction, protected edeine D isomers (**3, 4**) were obtained. Alkaline hydrolysis of the above compounds **3** and **4** followed by catalytic hydrogenolysis in formic acid afforded the final products: α- and β-isomer of edeine D (**7, 8**). The two peptides as the sulfates were precipitated with methanol, dried and stored for further investigations.

The properties of synthetic edeine D isomers and natural edeine D are shown in Table 1.

As it is evident from Table 1, the two peptides do not differ in their chromatographic and elec-

Fig. 2. The synthetic scheme for protected edeine D isomers.

See Fig. 1 for amino acid abbreviations; Boc, *t*-butoxycarbonyl; DPPA, diphenylphosphorazidate; MA, mixed anhydride; OMe, methyl ester; OBu^t, *t*-butyl ester; Trt, trityl; Z, benzyloxycarbonyl.

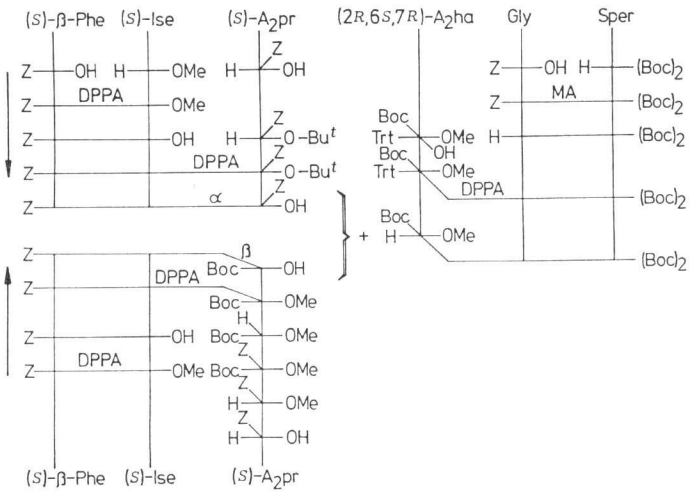


Table 1. Properties of synthetic edeine D isomers and natural edeine D.

Compound	Chromatography* (Rf)			Paper electrophoresis**
	1	2	3	Migration distance toward cathode (cm)
α-Isomer	0.24	0.37	0.54	16.3
β-Isomer	0.24	0.37	0.54	16.3
Natural edeine D***	0.24	0.37	0.54	16.3

* Chromatography on silica gel GF_{254} plates (Merck) in the solvent systems:
1. 1-Butanol - pyridine - acetic acid - water, 6: 2: 3: 5.
2. 1-Propanol - 25% ammonia - chloroform, 12: 8: 1.
3. 2-Propanol - 25% ammonia - water, 6: 4: 3.

** A buffer of pyridine - acetic acid - water, 10: 100: 890, pH 3.5 was used at 43 V/cm for 40 minutes. The substances were visualized with ninhydrin reagent.

*** Mixture of isomers.

Table 2. The antimicrobial activity of synthetic edeine D isomers.

Compound	IC_{50}* (μg/ml)			
	Saccharomyces cerevisiae ATCC 9763	*Escherichia coli* K-12	*Bacillus subtilis* BS-1060	*Serratia marcescens* ATCC 13880
α-Isomer	12	36	41	3
β-Isomer	>200	>200	>200	>200

* IC_{50}: concentration of compound causing 50% inhibition of microbial growth.

trophoretic properties from a natural sample of edeine D nor from one another. Attempts to differentiate them in other solvent systems also failed.

The chromatographic and electrophoretic patterns of the hydrolysate components of the natural and synthetic edeine D peptides have been shown to be identical.

It was necessary to find out whether isomerization of the synthetic peptides occurred during the deprotection and isolation processes. Dansylation of edeine D isomers followed by acid hydrolysis and TLC separation of the dansyl derivatives of α,β-diaminopropionic acid showed that the isomers were not contaminated by one another[25].

The biological activity of synthetic isomers of edeine D tested on model prokaryotic and eukaryotic organisms (Table 2) revealed that only the α-isomer was active. This conclusion is in agreement with the structural analysis of natural edeine D[20].

Experimental

FD-mass spectra were taken with a Varian MAT-711 instrument. Melting points were determined with a heated microscope (HMK Dresden, GDR). Microbiological assays were carried out as described by MAZERSKI *et al.*[26]. Paper electrophoresis was performed on Whatman No. 3. Optical rotations were measured with a Hilger Watts (London) polarimeter.

Boc
|
Trt-A_2ha-OMe **1**
└—Gly-Sper(Boc)$_2$

610 mg (1.03 mmol) of α-methyl ester of N^2-Trt,N^6-Boc-2,6-diamino-7-hydroxyazelaic acid was dissolved in 9 ml of DMF and cooled to −5°C. Then 442 mg (1.10 mmol) of amine component (GlySper(Boc)$_2$) was added and stirred vigorously. Next 0.275 ml of DPPA and 0.180 ml of triethylamine were added. The reaction mixture was left at room temperature for 24 hours, diluted with

40 ml of water and twice extracted with 100 ml of ethyl ether. The extracts were pooled, washed with water to remove DMF, concentrated and introduced into a chromatographic column (silica gel, benzene - acetone, 5: 2). The product was crystallized from ethyl ether - hexane yielding the compound **1** (815 mg, 81.1%), mp 75~76°C, $[\alpha]_D^{20}$ −47.5° (*c* 0.8, MeOH); FD-MS 975 $(M+1)^+$, 974(M^+).

Anal. Calcd: for $C_{53}H_{78}N_6O_{11}$: C 65.27, H 8.06, N 8.62.
Found: C 65.01, H 7.90, N 8.80.

Boc
|
A_2ha-OMe·HCl **2**
└—Gly-Sper(Boc)$_2$

715 mg (0.73 mmol) of compound **1** was detritylated with 20 ml of 75% aqueous acetic acid for 25 minutes. The reaction by-products were removed by extraction with hexane. Then, 7.3 ml of 0.1 N HCl was added to the water layer to form the corresponding hydrochloride. The solvent was evaporated *in vacuo* and the residue crystallized from an ethyl acetate - ether mixture to give 512 mg (89%) of compound **2**; mp 79~81°C; $[\alpha]_D^{20}$ −10° (*c* 1, MeOH); FD-MS 733 $(M+1)^+$, 732 (M^+).

Anal. Calcd: for $C_{34}H_{64}N_6O_{11}\cdot HCl\cdot H_2O$: C 51.86, H 8.58, N 10.67.
Found: C 51.99, H 8.70, N 10.61.

Boc
|
Z-β-Phe-Ise-A_2pr-A_2ha-OMe **3**
| └—Gly-Sper(Boc)$_2$
Z

To a solution of **2** (150 mg, 0.19 mmol) in DMF (4 ml) protected tripeptide corresponding to the *N*-terminal fragment of α-isomer of edeine D (115.5 mg, 0.19 mmol) was added at −5°C, then DPPA (0.060 ml) and triethylamine (0.065 ml) were added dropwise. After 24 hours the reaction mixture was diluted with 50 ml of ethyl acetate. The solution was washed with 0.2 N Na_2CO_3, 0.1 N HCl and water to pH 7. The solvent was evaporated *in vacuo* and the residue dissolved in a mixture of MeOH (2 ml) and DMF (0.5 ml). Then the crude protected peptide was purified by gel filtration on Sephadex LH-20 in MeOH (2×115 cm) and crystallized from ethyl acetate. Thus, 192.4 mg (75.6%) of compound **3** was obtained; mp 186~188°C; FD-MS 1,321 $(M+1)^+$, 1,320 (M^+).

Anal. Calcd: for $C_{65}H_{96}N_{10}O_{19}\cdot H_2O$: C 58.28, H 7.37, N 10.46.
Found: C 58.03, H 7.45, N 10.56.

Boc
|
Boc-A_2pr-A_2ha-OMe **4**
Z-β-Phe-Ise—┘ └—Gly-Sper(Boc)$_2$

Compound **4** was obtained from **2** (150 mg, 0.19 mmol) and the protected *N*-terminal tripeptide corresponding to β-isomer of edeine D (108.8 mg, 0.19 mmol) by the procedure described for the preparation of **3**. Yield 193 mg (78.0%); mp 156°C; FD-MS 1,287 $(M+1)^+$, 1,286 (M^+).

Anal. Calcd: for $C_{62}H_{98}N_{10}O_{19}\cdot H_2O$: C 57.04, H 7.72, N 10.73.
Found: C 56.90, H 7.50, N 10.54.

Boc
|
Z-β-Phe-Ise-A_2pr-A_2ha **5**
| └—Gly-Sper(Boc)$_2$
Z

The protected α-isomer of edeine D (**3**, 175 mg, 0.13 mmol) was dissolved in a mixture of MeOH (1.6 ml) and 4 N NaOH (0.4 ml) to hydrolyze the methyl ester. After 30 minutes the solution was added dropwise with cooling to 20 ml of 0.1 N HCl. The precipitate formed was filtered, washed with water, dried over P_2O_5 and crystallized from methanol - ethyl acetate mixture yielding 137.9 mg (79.6%) of the compound **5**; mp 178~180°C; FD-MS 1,307 $(M+1)^+$, 1,306 (M^+).

Anal. Calcd: for $C_{64}H_{94}N_{10}O_{19}\cdot H_2O$: C 57.99, H 7.30, N 10.57.
Found: C 57.74, H 7.36, N 10.68.

Boc
|
Boc-A_2pr-A_2ha **6**
Z-β-Phe-Ise—┘ └—Gly-Sper(Boc)$_2$

The alkaline hydrolysis of the methyl ester of compound **4** (170 mg, 0.13 mmol) was performed as described for the preparation of **5**. Yield 139 mg (82.5%); mp 134~135°C; FD-MS 1,273 $(M+1)^+$, 1,272 (M^+).

Anal. Calcd: for $C_{61}H_{96}N_{10}O_{19}\cdot H_2O$: C 56.73, H 7.65, N 10.85.
Found: C 56.85, H 7.82, N 10.70.

β-Phe-Ise-A_2pr-A_2ha$\cdot 2H_2SO_4$
└—Gly-Sper **7**

The compound **5** (125 mg, 0.09 mmol) was dissolved in 98% HCOOH (5 ml) and hydrogenolysis was performed in the presence of palladium black. After 5 hours the catalyst was filtered and washed with H_2O. Next 0.1 N H_2SO_4 (3.6 ml) was added to the filtrate and the solvent evaporated to a small volume (1 ml). The concentrated solution was added dropwise to a mixture of MeOH (20 ml) and EtOH (5 ml) with stirring and centrifuged. The precipitate obtained was suspended in acetone - hexane (1: 1) solvent mixture and centrifuged. The operation was repeated in hexane and the product dried in vacuum yielding 79 mg (84.7%) of the α-isomer of edeine D in the form of a white amorphous powder without defined melting point.

Anal. Calcd: for $C_{33}H_{58}N_{10}O_9\cdot 2H_2SO_4\cdot 3H_2O$: C 40.07, H 6.93, N 14.16.
Found: C 40.32, H 6.81, N 14.20.

A_2pr-A_2ha$\cdot 2H_2SO_4$
β-Phe-Ise—┘ └—Gly-Sper **8**

Compound **8** was prepared by removal of the Z- and Boc-groups from **6** (127 mg, 0.10 mmol) as was described above for **7**. Thus, 76.2 mg (78.3%) of an amorphous white powder without defined melting point was obtained,

Anal. Calcd. for $C_{33}H_{58}N_{10}O_9\cdot 2H_2SO_4\cdot 3H_2O$: C 40.07, H 6.93, N 14.16.
Found: C 40.25, H 6.90, N 14.22.

Acknowledgments

The authors acknowledge financial support of these studies by the Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw.

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