ESTERS AND AMIDES OF EDEINE A

J. MAZERSKI, H. WOJCIECHOWSKA, W. ZGODA, B. WOYNAROWSKA and E. BOROWSKI

Department of Pharmaceutical Technology and Biochemistry,
Technical University, 80–952 Gdańsk, Poland

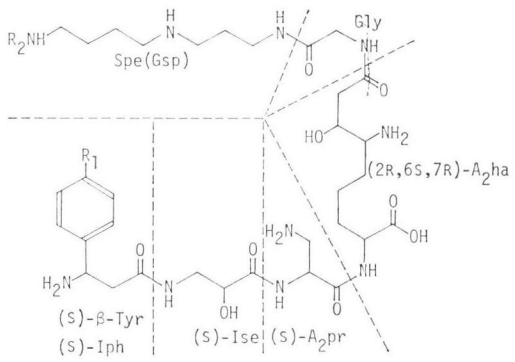
(Received for publication October 27, 1980)

Methods for the synthesis of esters and amides of edeine A were developed and a number of derivatives of this type was obtained and characterized. The antimicrobial activities of the derivatives are comparable to the activity of the native antibiotic and indicate that the presence of free carboxyl group in edeine is not essential for its biological activity.

Edeines are peptide antibiotics produced by *Bacillus brevis* Vm 4[1~3]. They are active against Gram-positive and Gram-negative bacteria and fungi[1,4] and in higher concentrations inhibit also the proliferation of animal cells[5]. Edeine antibiotics eliminate the plasmids determining bacterial antibiotic resistance[6] and in animals exhibit considerable immunosuppressive activity[7].

The organism produces four closely related biologically active compounds named edeines A, B, D and F[3,8,9]. The structures of edeines A and B have been established by HETTINGER *et al.*[10~12] and those of edeines D and F by our group[8,9]. The compounds are pentapeptides conjugated with polyamines (Fig. 1).

Fig. 1. Chemical structures of the edeine antibiotics.
Edeine A: R_1=OH, R_2=H; edeine B: R_1=OH, R_2=CNH_2(=NH); edeine D: R_1=H, R_2=H; edeine F: R_1=H, R_2=CNH_2(=NH).



Edeines contain in their molecules four non-protein amino acids: (S)-isophenylalanine (Iph) or (S)-β-tyrosine (β-Tyr), (S)-isoserine (Ise), (S)-2,3-diaminopropionic acid (A_2pr), and 2(R),6(S)-diamino-7(R)-hydroxyazelaic acid (A_2ha) and also glycine and polyamines spermidine or guanylspermidine[9,11].

Edeines are produced by *Bacillus brevis* Vm4 as a mixture of active and inactive isomers[12]. The structures of edeines of both series have been ultimately established by total synthesis[13,14].

Among the peptide antibiotics the edeines exhibit unique mechanisms of action. In bacteria at minimum inhibitory concentration they inhibit specifically and reversibly the synthesis of DNA[15]. At higher concentrations they inhibit irreversibly the biosynthesis of protein[16]. In eukaryotic microorganisms edeines inhibit only protein biosynthesis[17]. The ability to differentiate prokaryotic and eukaryotic microorganisms, based on differences in structure and functioning of the DNA replicating apparatus, is the most interesting biological property of edeines[17]. Nevertheless, the interaction of edeines with ribosomes, common to prokaryotic and eukaryotic organisms and resulting in the inhibition of protein biosynthesis, does not allow the practical use of these antibiotics as chemotherapeutic agents due to the high animal toxicity of these compounds (unpublished data).

In our studies on the structure-activity relationships of edeines, based on chemical modifications

and aimed at the dissociation of their inhibitory activities with respect to protein and DNA biosynthesis, we have focused our attention on the role of the carboxyl group in biological properties of these compounds.

A number of derivatives of edeine A with a modified carboxyl group, namely esters and amides, was synthesized and characterized.

The esters of edeine A were synthesized by two ways. One of them was the esterification of edeine A sulphate in the presence of thionyl chloride in the appropriate alcohol[18]. Another method was based on the esterification of a derivative with properly protected amino groups. By the first method only the methyl and ethyl esters could be obtained, whereas the latter method was suitable for the synthesis of esters with higher alcohols as well. In the latter case the benzyloxycarbonyl protection of amino groups was applied. Pentabenzyloxycarbonyl edeine A was obtained by the method described by RZESZOTARSKA *et al.*[10]. Esters of protected edeine A were synthesized by a classical method[20].

Using these methods the methyl, ethyl, butyl and pentyl esters have been obtained.

The amides of edeine A were synthesized from pentabenzyloxycarbonyl edeine A by the azide method using the diphenylphosphoryl azide (DPPA) reagent[21]. The following derivatives were obtained: ethyl, butyl, hexyl, 2-(N,N-dimethylamino)-ethyl, 4-aminobutyl*, and 5-(N,N-dimethylamino)-pentyl amides.

The amides of pentabenzyloxycarbonyl edeine A were purified by crystallization and characterized by IR spectroscopy, elementary analysis, and chromatography of products obtained on acid hydrolysis.

The benzyloxycarbonyl groups were removed by hydrogenation in the presence of palladium in 80% formic acid[22] and the *t*-butyloxycarbonyl group by the action of anhydrous formic acid[23].

Edeine A used for the syntheses of the above derivatives was obtained and isolated in our laboratory as described previously[2,3].

The esters and amides of edeine A were isolated and characterized as sulphates. Their antimicrobial

Table 1. The esters and the amides of edeine A.

No.	Substituent	Total yield %	Rf value*	Biological activity IC_{50} [mcg/ml]		
				B. subtilis	*E. coli*	*S. cerevisiae*
1	OCH_3	95; 49	0.23	35	22	3
2	OCH_2CH_3	95; 37	0.28	30	24	9
3	$O(CH_2)_3CH_3$	41	0.62	8	12	4
4	$O(CH_2)_4CH_3$	42	0.64	17	10	4
5	$NHCH_2CH_3$	56	0.25	20	30	5
6	$NH(CH_2)_3CH_3$	59	0.43	23	20	2, 5
7	$NH(CH_2)_5CH_3$	49	0.65	26	13	5
8	$NH(CH_2)_2\overset{+}{N}H(CH_3)_2$	45	0.12	55	20	14
9	$NH(CH_2)_4\overset{+}{N}H_3$	40	0.13	44	20	12
10	$NH(CH_2)_5\overset{+}{N}H(CH_3)_2$	36	0.15	135	37	29
11	OH (edeine A)	—	0.19	90	100	32

* Merck DC-Alufolien Kieselgel 60 GF254 in the solvent system: *n*-butanol - pyridine - acetic acid - water, 6 : 2 : 3 : 5.

* For the synthesis of this amide mono-*t*-butyloxycarbonyl-1,4-diaminobutane has been used[24].

activity against prokaryotic (bacteria) and eukaryotic (yeasts) microorganisms, as compared to edeine A, is presented in Table 1.

Experimental

Direct esterification. To the suspension of edeine A sulphate (0.98 g) in 70 ml of the appropriate alcohol (methanol or ethanol) 1.19 g of thionyl chloride were added with stirring and cooling. After refluxing for 40 hours the solution was evaporated under reduced pressure to a small volume and poured into a mixture of ethyl ether and acetone (1: 1, v/v). The resulting precipitate was centrifuged and dried under vacuum. The purity of the obtained esters was determined by TLC. The yield was 95%.

Pentabenzyloxycarbonyl edeine A. Edeine A sulphate (4.9 g) was dissolved in 80 ml of 5% (w/v) $NaHCO_3$ and treated with 100 ml of 7% (w/v) O-benzyloxycarbonyl-8-hydroxyquinoline in dimethylformamide. The mixture was stirred at room temperature for one week. The reaction mixture was poured into 2 liters of 2 N hydrochloric acid with vigorous stirring. The resulting precipitate was separated, washed with water, dried and crystallized from methanol. The yield was 78%. For analytical purposes the compound was recrystallized from methanol and dried over P_2O_5. M.p.: 199~200°C.

Anal.: Calcd.: C 61.56, H 6.18, N 9.84%. Found: C 61.4, H 6.3, N 9.7%.

Esters of pentabenzyloxycarbonyl edeine A. The pentabenzyloxycarbonyl edeine A (0.36 g) was suspended in 100 ml of the appropriate dry alcohol saturated with dry hydrogen chloride and stirred for 10 days. The esters were isolated by either of two ways. In case of esters of lower alcohols, the reaction mixture was poured into 1 liter of cold water. The reaction mixture with higher alcohols was poured into 750 ml of dry ethyl ether.

All precipitates obtained were washed with acetone and ethyl ether and crystallized from a mixture of acetone and methanol to give the following derivatives:

Methyl ester: m.p. 200~201°C, yield 85%. Anal.: Calcd.: C 61.80, H 6.26, N 9.74%. Found: C 61.7, H 6.1, N 9.8%.

Ethyl ester: m.p. 202~203°C, yield 83%. Anal.: Calcd.: C 61.98, H 6.34, N 9.64%. Found: C 62.1, H 6.4, N 9.8%.

n-Butyl ester: m.p. 180~181°C, yield 76%. Anal.: Calcd.: C 62.34, H 6.49, N 9.46%. Found: C 62.2, H 6.3, N 9.5%.

n-Pentyl ester: m.p. 190~191°C, yield 71%. Anal.: Calcd.: C 62.65, H 6.56, N 9.37% Found: C 62.8, H 6.8, N 9.2%.

Amides of pentabenzyloxycarbonyl edeine A. The pentabenzyloxycarbonyl edeine A (0.5 g) was dissolved in 20 ml of dimethylformamide (DMF) and 0.29 g of DPPA (diphenylphosphoryl azide), 100 mg of triethylamine and 1 mmole of the appropriate amine were added. The mixture was allowed to stand at room temperature overnight and dropped into 200 ml of cold methanol (0°C). The precipitate was separated, washed with methanol and crystallized from the following solvent mixture: butanol - methanol - water, 4: 1: 1. By this method the following derivatives were obtained.

Ethyl amide: m.p. 219°C, yield 75%. Anal.: Calcd.: C 62.03, H 6.41, N 10.61%. Found: C 62.0, H 6.2, N 10.7%.

Butyl amide: m.p. 215~216°C, yield 78%. Anal.: Calcd.: C 62.47, H 6.56, N 10.41%. Found: C 62.6, H 6.7, N 10.3%.

Hexyl amide: m.p. 215°C, yield 69%. Anal.: Calcd.: C 62.91, H 6.70, N 10.22%. Found: C 62.8, H 6.6, N 10.1%.

2-N,N-Dimethylaminoethyl amide: m.p. 200~201°C, yield 70%. Anal.: Calcd.: C 61.85, H 6.56, N 11.24%. Found: C 62.0, H 6.6, N 11.4%.

4-(N-*t*-Butyloxycarbonyl)-aminobutyl amide: m.p. 213~214°C, yield 57%. Anal.: Calcd.: C 61.73, H 6.65, N 10.54%. Found: C 62.0, H 6.6, N 10.3%.

5-N,N-Dimethylaminopentyl amide: m.p. 182~183°C, yield 64%. Anal.: Calcd.: C 62.50, H 6.77, N 10.94%. Found: C 62.2, H 6.9, N 11.1%.

Removal of N-protecting groups

(a) Benzyloxycarbonyl groups — 0.15 mmole of protected compound was dissolved in 100 ml of 80% HCOOH, 30 mg of 10% palladium on carbon catalyst was added, and the mixture was stirred at room temperature for 6 hours in a hydrogen atmosphere. The catalyst was then removed by filtration and the filtrate was evaporated under reduced pressure to a small volume. The stoichiometric quantity of 1 N H_2SO_4 was added, and the solution was dropped into 20 ml of acetone. The precipitate was isolated by centrifugation and dried.

(b) *t*-Butyloxycarbonyl group — 0.2 g of 4-Boc-aminobutyl amide of pentabenzyloxycarbonyl edeine A was dissolved in 10 ml of anhydrous HCOOH. After 30 minutes the solution was diluted with 80% HCOOH (90 ml) and the benzyloxycarbonyl groups were removed as described above.

Thin-layer Chromatography

TLC on silica gel (Merck DC-Alufolien Kieselgel 60 GF254) was used for control of the reactions and purity of the products. The chromatograms of unprotected compounds were developed in the solvent system: butanol - pyridine - acetic acid - water, 6: 2: 3: 5 and protected compounds in the system: ethyl acetate - methanol - water, 20: 4: 3. The substances were visualized by UV light (compounds with benzyloxycarbonyl groups) or by spraying with ninhydrin reagent (compounds with free amino groups).

Biological Activity

Prokaryotic microorganisms (*Escherichia coli* K-12, *Bacillus subtilis*) and eukaryotic microorganism (*Saccharomyces cerevisiae* ATCC 9763) were used as the test organisms for determining the biological activity of esters and amides of edeine A.

As measure for the activity we have used the concentration of compound causing 50% inhibition of microbial growth (IC_{50}) determined by the turbidimetric method. Cells were grown on liquid media: bacteria on Sensitive Test Medium (Difco) at 37°C, pH 7.2; yeast on medium containing 1% Bacto-Pepton (Difco), 2% glucose, and 0.5% NaCl at 29°C, pH 6.8. The density of cell suspensions was determined on Specol Zeiss-Jena at 660 nm after 18 hours incubation for bacteria and after 20 hours for yeast.

Conclusions

Presented in this paper are methods for the synthesis of esters and amides of edeine A providing the various derivatives in satisfactory yields.

As shown in Table 1, the esters and amides of edeine A exhibit antimicrobial activity similar to the activity of the parent antibiotic, indicating that the presence of a free carboxyl group in edeine A is not essential for its biological activity.

The polycationic character of the edeine A molecule would suggest that the biological activity of the antibiotic can be influenced by different agents due to the influence on the ionization state.

It was found that the IC_{50} for *Saccharomyces cerevisiae* ATCC 9763 when determined on a medium of pH 5.6~5.8 was 4~6 times higher than at pH 6.8[17].

A similar effect was observed for amides of edeine A which have additional functional ionizing groups (*e.g.* compounds No. 8, 9, 10, Table 1) in comparison with esters and other amides (compounds No. 1~7).

These results suggest that increased ionization of the edeine molecule impedes the penetration of the antibiotic across the cytoplasmatic membrane.

The synthesized esters and amides have been used in our studies on structure-activity relationships in edeines[25].

Acknowledgements

We thank Dr. R. ANDRUSZKIEWICZ for making available to us the mono-*t*-butyloxycarbonyl-1,4-diaminobutane and Dr. J. GRZYBOWSKA for creating a cordial atmosphere about this work. The authors also acknowledge the financial support by the Institute of Organic Chemistry, Polish Academy of Science.

References

1) KURYŁO-BOROWSKA, Z.: Antibiotical properties of the strain *Bacillus brevis* Vm_4. Bull. State Inst. Marine and Trop. Med., Gdańsk, Poland 10: 83~98, 1959
2) BOROWSKI, E.; H. CHMARA & E. JERECZEK-MORAWSKA: Antibiotic edeine. V. Isolation and purification of edeine A and B. Chemotherapia 12: 12~25, 1967
3) BOROWSKI, E.; H. CHMARA & E. JERECZEK-MORAWSKA: The antibiotic edeine. VI. Paper and thin-layer chromatography of components of the edeine complex. Biochim. Biophys. Acta 130: 560~563, 1966
4) CHMARA, H.: Antibiotic edeine. VII. Biological activity of edeine A and B. Acta Microbiol. Polon. 17: 59~66, 1968
5) KURYLO-BOROWSKA, Z. & J. HEANEY-KIERAS: The uptake and subcelluar localization of the peptide antibiotic edeine A in HeLa cells in suspension culture. Exp. Cell Res. 124: 371~379, 1979
6) BOROWSKI, J.; E. BOROWSKI, A. CIEPAT, D. DZIERŻANOWSKA, P. JAKUBICZ & A. SMOCZEWSKI: Elimination of plasmids determining bacterial antibiotic resistance by edeine. Drugs Exptl. Clin. Res. 3: 189~191, 1977
7) BOROWSKI, J.; P. JAKONIUK & E. BOROWSKI: Edeine as an immunosuppressive agent. Drugs Exptl. Clin. Res. 3: 183~188, 1977
8) WOJCIECHOWSKA, H.; J. CIARKOWSKI, H. CHMARA & E. BOROWSKI: The antibiotic edeine. IX. The isolation and the composition of edeine D. Experientia 28: 1423~1424, 1972
9) WOJCIECHOWSKA, H.; A. KONITZ, J. GUMIENIAK, W. ZGODA, J. MAZERSKI, K. DZIĘGIELEWSKI, R. ANDRUSZKIEWICZ, J. GRZYBOWSKA & E. BOROWSKI: Structures and some derivatives of edeine antibiotics. Vol. 1, pp. 243~246, Proc. 11th IUPAC Intern. Symp. on Chemistry of Natural Products. Bulgarian Academy of Sciences, 1978
10) HETTINGER, T. P. & L. C. CRAIG: Edeine. II. The composition of the antibiotic peptide edeine A. Biochemistry 7: 4147~4152, 1968
11) HETTINGER, T. P.; Z. KURYŁO-BOROWSKA & L. C. CRAIG: Edeine. III. The composition of the antibiotic peptide edeine B. Biochemistry 7: 4153~4159, 1968
12) HETTINGER, T. P. & L. C. CRAIG: Edeine. IV. Structures the antibiotic peptides edeines A_1 and B_1. Biochemistry 9: 1224~1232, 1970
13) WOJCIECHOWSKA, H.; R. ANDRUSZKIEWICZ, J. GRZYBOWSKA, A. CZERWINSKI, J. GUMIENIAK, J. MAŹERSKI, W. ZGODA & E. BOROWSKI: Total synthesis of edeine antibiotics and their analogues. Vol. 3, pp. 36~39, Proc. 11th IUPAC Intern. Symp. on Chemistry of Natural Products, Golden Sands, Bulgarian Academy of Science, 1978
14) BOROWSKI, E.; R. ANDRUSZKIEWICZ, A. CZERWIŃSKI, J. GRZYBOWSKA & H. WOJCIECHOWSKA: Some aspects of synthesis of edeine antibiotics. pp. 421~424, Proc. 6th Am. Peptide Symp., Pierce Chemical Company, 1979
15) KURYŁO-BOROWSKA, Z.: On the mode of action of edeine. Effect of edeine on the bacterial DNA. Biochim. Biophys. Acta 87: 305~313, 1964
16) KURYŁO-BOROWSKA, Z. & W. SZER: Inhibition of bacterial DNA synthesis by edeine. Effect on *Escherichia coli* mutants lacking DNA polymerase I. Biochim. Biophys. Acta 287: 236~245, 1972
17) WOYNAROWSKA, B.; H. CHMARA & E. BOROWSKI: Differential mechanism of action of the antibiotic edeine on prokaryotic and eukaryotic organisms point to new basis for selective toxity. Drugs Exptl. Clin. Res. 5: 181~186, 1979

18) KUPRYSZEWSKI, G. & T. SOKOŁOWSKA: Nowe metody estryfikacji aminokwasów. Acta Biochim. Polon. 4: 85~92, 1957
19) RZESZOTARSKA, B. & G. PAŁKA: A new method of introducing N-carbobenzoxy- and N-4-nitrocarbobenzoxy-protection in amino acids. Bull. Acad. Polon. Sci. 16: 23~27, 1968
20) SYNGE, R. D. M.: The synthesis of some dipeptides related to gramicidin S. Biochem. J. 42: 99~104, 1948
21) SHIOIRI, T.; K. NINOMIYA & S. YAMADA: Diphenylphosphoryl azide. A new convenient reagent for a modified CURTIUS reaction and for the peptide synthesis. J. Amer. Chem. Soc. 94: 6203~6205, 1972
22) HOFMANN, K.; E. STUTZ, G. SPÜHLER, H. YAJIMA & E. T. SCHWARTZ: Studies on polypeptides. XVI. The preparation of N^{ε}-formyl-L-lysine and its application to the synthesis of peptides. J. Amer. Chem. Soc. 82: 3727~3732, 1960
23) HALPERN, B. & D. E. NITECKI: The deblocking of *t*-butyloxycarbonyl-peptides with formic acid. Tetrahedron Lett. 1967: 3031~3033, 1967
24) ANDRUSZKIEWICZ, R.; H. WOJCIECHOWSKA & E. BOROWSKI: Syntheses of edeine antibiotics and their analogs. I. Synthesis of N-(3-aminopropyl)-N,N′-di-*t*-butyloxycarbonyl-1,4-diaminobutane. Polish J. Chem. 52: 1167~1177, 1978
25) MAZERSKI, J.; B. WOYNAROWSKA & E. BOROWSKI: Correlation between the chemical structure and biological activity of the edeine A derivatives. Peptides 1978, pp. 373~375, Proc. 15th Europ. Peptide Symp., Wrocław University Press, 1979