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## Note

# Differentiation of $N^{\alpha}$- and $N^{\beta}$-substituted α, β-diaminopropionic acids

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L-α,β-Diaminopropionic acid (L-$A_2$pr) is a constituent amino acid of a series of biologically active peptides, mainly peptide antibiotics. It has been found in hydrolysates of bleomycin[1], capreomycins[2], tuberactinomycins[3], viomycin[4], tallysomycins[5] and edeines[6,7]. A problem usually encountered in the structural analysis of such compounds containing α,β-diaminopropionic acid is the determination of the mode of linkage of the amino acid amino groups. The method usually employed for this determination involves dinitrophenylation followed by peptide hydrolysis and separation of Dnp-amino acids using paper electrophoresis[6,8].

We have developed a convenient and sensitive method, based on peptide dansylation[9], hydrolysis and one-dimensional thin-layer chromatographic separation of the isomeric Dns-α,β-diaminopropionic acids. We used this method for the isomeric purity analysis of synthesized edeine D isomers.

## EXPERIMENTAL

### *Reagents*

5-Dimethylamino-1-naphthalenesulphonyl chloride (Dns chloride) was purchased from Pierce (Rockford, IL, U.S.A.). $N^{\alpha}$-Boc-$A_2$pr (Boc = *tert.*-butoxycarbonyl) and $N^{\beta}$-Z-$A_2$pr (Z = benzyloxycarbonyl) were prepared according to known methods[10,11]. Synthetic edeine D isomers were obtained as described by Borowski *et al.*[12]. Chemicals and solvents were of analytical-reagent grade.

### *Solvent systems*

The following solvents (composition by volume) were used: (A) ethanol–water (25:4); (B) isopropanol–acetone–water (6:5:1); (C) *n*-butanol–isopropanol–water (6:4:1); (D) *n*-butanol–acetone–water (12:7:2); (E) ethyl acetate–methanol–28% ammonia (25:10:4); (F) isopropanol–28% ammonia (11:1).

### *Procedure*

Amounts of 1.0 μmole of $N^{\alpha}$-Boc-$A_2$pr, $N^{\beta}$-Z-$A_2$pr, edeine D α-isomer and edeine D β-isomer were separately dissolved in 0.2 ml of 0.1 *N* sodium hydrogen carbonate solution, then 0.2 ml of Dns chloride in acetone (8 mg/ml) was added. The mixture was vigorously stirred and allowed to stand at room temperature in the dark

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TABLE I

$R_F$ VALUES OF Dns-DIAMINOPROPIONIC ACIDS AND Dns-OH IN SOLVENT SYSTEMS A–F

| *Compound* | $R_F$ | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| α-Dns-$A_2$pr | 0.60 | 0.29 | 0.32 | 0.39 | 0.37 | 0.17 |
| β-Dns-$A_2$pr | 0.30 | 0.08 | 0.19 | 0.27 | 0.27 | 0.12 |
| Dns-OH | 0.81 | 0.78 | 0.58 | 0.86 | 0.55 | 0.62 |

for 3 h. The solvents were evaporated under reduced pressure and the residue was dissolved in 0.5 ml of 6 *N* hydrochloric acid and then heated at 105°C for 16 h in a sealed tube. The acid was removed by evaporation under reduced pressure at 40°C. The residue was dissolved in 0.3 ml of water and 3-μl aliquots of the solution were taken for TLC analysis using Kieselgel 60 $F_{254}$ plates (Merck, Darmstadt, G.F.R.). The substances were revealed by longwave UV radiation.

## RESULTS AND DISCUSSION

Both mono-Dns derivatives of α,β-diaminopropionic acid, similarly to mono-Dns derivatives of basic amino acids (*e.g.*, arginine, lysine, ornithine), are readily soluble in water and can therefore be readily separated from other Dns-amino acids by non-polar solvent extraction.

The chromatographic behaviour of Dns-α,β-diaminopropionic acids and 5-dimethylamino-1-naphthalenesulphonic acid (Dns-OH) in one-dimensional TLC on silica gel plates is shown in Table I. The results indicate that the compounds can be distinguished on the basis of their $R_F$ values. It is interesting that in all of the solvent systems tested α-Dns-$A_2$pr was more mobile than the corresponding β-derivative.

The procedure described was employed for the isomeric purity analysis of synthesized edeine D isomers. Edeine D, as well as other components of the edeine antibiotic complex, exists in form of two isomeric peptides differing in the mode of

| *Edeine* | $R_1$ | $R_2$ |
|---|---|---|
| A | –OH | –H |
| B | –OH | –C(=NH)–NH$_2$ |
| D | –H | –H |
| F | –H | –C(=NH)–NH$_2$ |

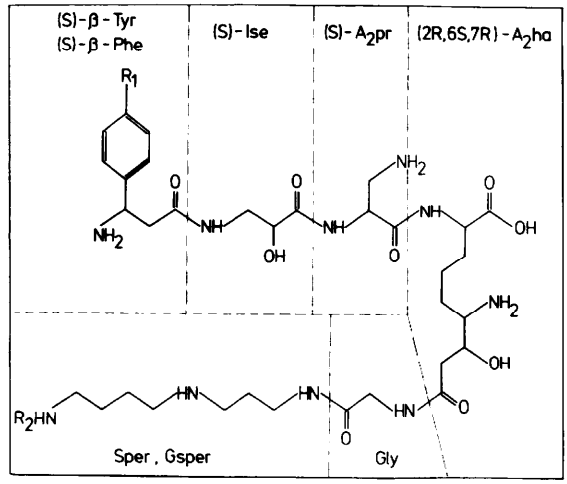


Fig. 1. Structures of edeine antibiotics. β-Tyr = β-tyrosine; β-Phe = β-phenyl-β-alanine; Ise = isoserine; $A_2$pr = α,β-diaminopropionic acid; $A_2$ha = 2,6-diamino-7-hydroxyazelaic acid; Gly = glycine; Sper = spermidine; Gsper = guanylspermidine.

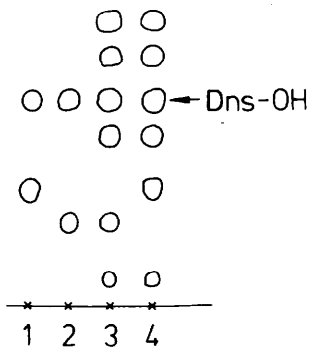


Fig. 2. Chromatogram of hydrolysate components of Dns-edeine D isomers. 1 = α-Dns-$A_2$pr; 2 = β-Dns-$A_2$pr; 3 = α-isomer of edeine D; 4 = β-isomer of edeine D. The chromatogram was developed twice in solvent system F.

linkage of the $A_2$pr amino groups with the isoserine residue. The peptides in which isoserine is linked with the α-amino group of $A_2$pr (α-isomers) have been described as active isomers[6,13]. The β-isomers have been shown to be biologically inactive. The structural formulae of the α-isomers are presented in Fig. 1.

It was necessary to establish whether isomerization of any of the synthetic edeine peptides occurred during the deprotection and isolation processes. Dansylation of edeine D isomers followed by acid hydrolysis and TLC separation of the resulting dansyl derivatives of α,β-diaminopropionic acid showed that neither isomer was contaminated by the other (Fig. 2).

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REFERENCES

1 T. Takita, Y. Muraoka, K. Maeda and H. Umezawa, *J. Antibiot.*, 21 (1968) 79.
2 B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson and T. Webb, *Nature (London)*, 231 (1971) 301.
3 H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba and T. Kaneko, *Tetrahedron Lett.*, (1971) 2043.
4 B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson and T. Webb, *Experientia*, 27 (1971) 501.
5 M. Konishi, K. Saito, K. Numata, T. Tsuno, K. Asama, H. Tsukiura, T. Naito and H. Kawaguchi, *J. Antibiot.*, 30 (1977) 789.
6 T. P. Hettinger and L. C. Craig, *Biochemistry*, 9 (1970) 1224.
7 H. Wojciechowska, J. Ciarkowski, H. Chmara and E. Borowski, *Experientia*, 28 (1972) 1423.
8 S. Nomoto, T. Teshima, T. Wakamiya and T. Shiba, *J. Antibiot.*, 30 (1977) 955.
9 W. R. Gray and B. S. Hartley, *Biochem. J.*, 89 (1963) 59P.
10 M. Waki, Y. Kitajima and N. Izumiya, *Synthesis*, (1981) 266.
11 J. Grzybowska, R. Andruszkiewicz and H. Wojciechowska, *Polish J. Chem.*, 53 (1979) 935.
12 E. Borowski, R. Andruszkiewicz, A. Czerwiński, J. Grzybowska and H. Wojciechowska. In E. Gross and J. Meienhofer (Editors), *Peptides, Proc. 6th American Peptide Symp.*, Pierce Chemical Company, Rockford, IL, 1979, p. 421.
13 H. Wojciechowska, A. Konitz, J. Gumieniak, W. Zgoda, J. Mazerski, K. Dzięgielewski, R. Andruszkiewicz, J. Grzybowska and E. Borowski, in *Proc. 11th Int. Symp. Chemistry of Natural Products, Golden Sands, September 17–23, 1978*, Vol. 1, Bulgarian Academy of Sciences, Sofia, 1978, p. 243.