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SYNTHESIS OF ENDURACIDIDINE, A COMPONENT AMINO ACID OF ANTIBIOTIC ENDURACIDIN [1)]

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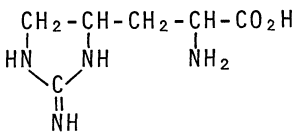
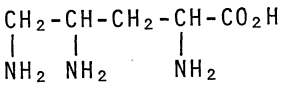
Enduracididine (1), a component amino acid of the antibiotic enduracidin, was synthesized starting from L-histidine. Bamberger cleavage of methyl L-histidinate followed by catalytic hydrogenation afforded a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-2,4,5-triaminopentanoic acid derivatives. Deprotection and guanidination of them yielded 1 of the natural configuration and its diastereoisomer, *i.e.*, the enantiomer of natural alloenduracididine respectively.

Two guanidino amino acids, enduracididine (1) and alloenduracididine (2), are both components of the peptide antibiotic enduracidin produced by *Streptomyces fungicidicus*.[2)] Chemical and spectroscopic studies indicated that they are diastereoisomers of 2-amino-3-(2-iminoimidazolidin-4-yl)-propionic acid differing only at the C-2 configuration. X-Ray analysis of 2 monoperchlorate determined its absolute configuration to be (2*R*,4*R*). Consequently, 1 was concluded to possess (2*S*,4*R*) configuration.[3)] Recently, 1 was also identified as a component of another antibiotic minosaminomycin.[4)]



1 2S,4R
2 2R,4R



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In this investigation, we describe the syntheses of 1 with the natural configuration and the enantiomer of 2 *via* guanidination of the intermediate 2,4,5-triaminopentanoic acid (3). Methyl L-histidinate was treated with *n*-butyryl chloride and Na_2CO_3 in a mixture of water and CH_2Cl_2 to afford 2,4,5-tributyrylamino-4-pentenoic acid methyl ester (4) in 42 % yield[5)]: Found: C, 58.30; H, 8.49; N, 11.23 %, mp 113-114°C, λ_{max}^{EtOH} (log ε): 206 (3.72), 210 (3.80), 251 (4.16)nm. The olefinic bond in 4 was hydrogenated in the presence of platinum oxide in acetic acid giving tributyrylaminopentanoic acid ester (5) in a quantitative yield[6)]: Found: C, 58.04; H, 9.00; N, 11.26 %, mp 108-114°C. Since the hydrogenation of this double bond can not be expected to proceed stereospecifically, the product (5) of this reaction could be a mixture of two diastereoisomers, *i.e.*, (2*S*,4*R*) and (2*S*,4*S*). Deprotection of the former would lead to the triamino acid with the desired stereochemistry for the

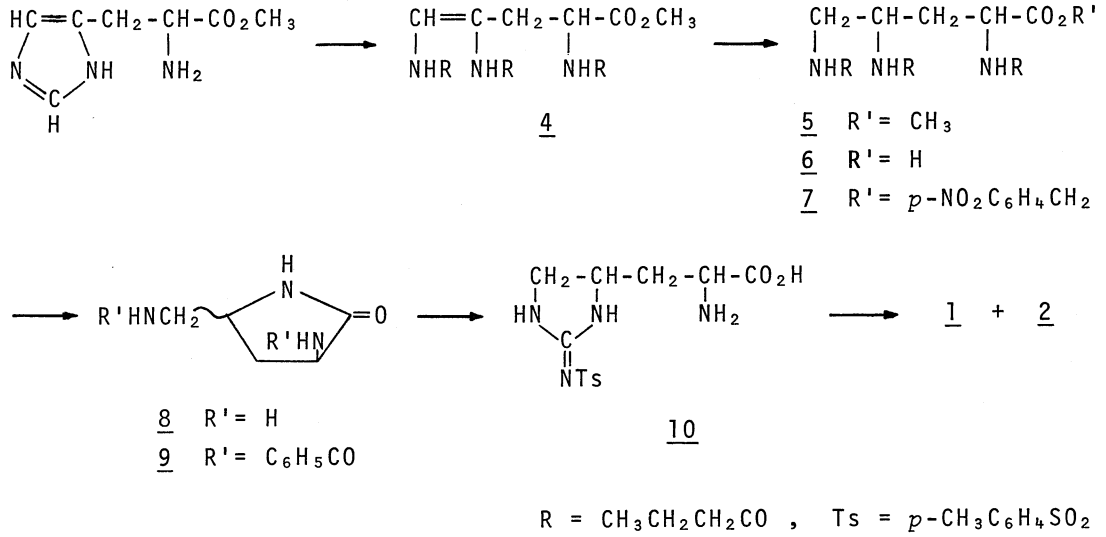
synthesis of 1, while the latter could be converted into the enantiomer of natural alloenduracididine.

The compound 5 was hydrolyzed by refluxing it in 2M HCl for 4 hr. The hydrolysis product was isolated as picrate: mp 219-222°C (dec.), whose elemental analysis (Found: C, 34.73; H, 2.95; N, 21.52 %) and IR spectrum (1730 cm^{-1}) indicated the γ-lactam (8) dipicrate structure rather than the triamino acid (3) di- or tripicrate. The lactam structure was also supported by the formation of lactam dibenzoate (9) on benzoylation with benzoyl chloride and triethylamine: Found: C, 67.39; H, 5.66; N, 12.15 %, mp 222-226°C.

For the guanidination, a form of triaminopentanoic acid with free amino groups on C-4 and C-5 is required. Thus, either hydrolysis or methanolysis of 5, 8, tributyrylaminopentanoic acid (6) and its *p*-nitrobenzyl ester (7)[7] was examined under various acidic conditions, *e.g.*, refluxing in 2M or 6M HCl, in 2M HBr or in 10 % dry HCl in methanol. However, the isolated product was the lactam (8) dipicrate in all cases.

Therefore, an alkaline hydrolysis of 8 was next investigated by refluxing it in 1M NaOH for 4 hr. Amino acid analysis of the resultant mixture indicated a formation of two new compounds with very close elution times (in a ratio of about 1 : 1) in place of 8 which disappeared completely. When this reaction mixture was allowed to stand at room temperature, the pH of the solution fell down to 8.5 by absorbing atmospheric CO_2 and a considerable amount of 8 was regenerated simultaneously. These facts showed that the lactam ring was cleaved by alkali to give sodium salt of 3 which partly recyclized even at pH 8.5. The two new peaks found in amino acid analysis might be assigned to the two diastereoisomers of 3.[8]

Without isolation of 3 in order to prevent the recyclization, the product of the alkaline hydrolysis was directly guanidinated. Thus, after ethanolic solution of 8 dihydrochloride had been heated under reflux with 4 equivalents of NaOH for 2 hr, 1 equivalent of *S*,*S*'-dimethyl-*N*-tosyliminodithiocarbonimidate[9] was added and the mixture was again refluxed for 2 hr. This reaction condition was chosen from the results of preliminary experiments using various amounts of alkali, where the best



4

5 R'= CH_3
6 R'= H
7 R'= *p*-$NO_2C_6H_4CH_2$

8 R'= H
9 R'= C_6H_5CO

10

R = $CH_3CH_2CH_2CO$, Ts = *p*-$CH_3C_6H_4SO_2$

yield of the tosylguanidino derivative (10)[10] of the highest optical rotatory power was obtained with use of 4 equivalents of NaOH. With theoretical amount, *i.e.*, 3 equivalents, the desired product 10 could not be obtained.

Since the compound 10 was rather unstable on standing, the reaction product containing 10 was directly treated with anhydrous HF at room temperature for 5 hr for detosylation.[9] Amino acid analysis of the detosylated mixture indicated that two main products with comparable yields were identical with 1 and 2, accompanying with one minor product of a smaller elution time.[11] The products 1 and 2 were separated by repeated column chromatography on Dowex 50 x 8 with 4M hydrochloric acid. The sum of the yields of 1 and 2 was 44 % form 5.

Synthetic 1 was isolated as bis-4-hydroxyazobenzene-4'-sulfonate hemihydrate: Found: C, 48.87; H, 4.46; N, 14.85; S, 8.82 %, mp 224-226°C (dec.), $[\alpha]_D^{17}$ +15.6° (c 0.5, CH_3OH), natural salt: mp 226-228°C (dec.), $[\alpha]_D^{17}$ +23.9°. Synthetic oxalate, mp 191-193°C (dec.), $[\alpha]_D^{21}$ +44.3° (c 0.23, H_2O), natural salt: mp 197-199°C (dec.), $[\alpha]_D^{21}$ +58.8°. The IR spectra of the both salts as well as the NMR spectrum of the oxalate of synthetic 1 were identical with those of the natural specimen. However, the mps and the $[\alpha]_D$ values were somewhat lower than those of the corresponding natural material presumably owing to the slight racemization at C-2 which occurred inevitably during the alkaline hydrolysis of 8, though care had been taken to avoid the use of excess alkali.

Synthetic 2 was also isolated as bis-4-hydroxyazobenzene-4'-sulfonate hydrate: Found: C, 48.06; H, 4.50; N, 14.79; S, 8.60 %, mp 232-234°C (dec.), and oxalate: mp 194-196°C (dec.). This product was identified with the natural alloenduracididine by means of amino acid analysis, paper chromatography and paper electrophoresis. Direct spectroscopic comparison was not possible because neither of the pure salts of natural 2 was available. ORD curve of the synthetic oxalate (500-280 nm) had the opposite sign to that of the natural specimen described in the literature,[3] thus indicating the enantiomeric relation between natural and synthetic 2, although the $[\alpha]_D$ values of the synthetic salts were too small for precise discussion on optical purity.

From the fact that the guanidination of a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-2,4,5-triaminopentanoic acid certainly afforded the enantiomer of natural alloenduracididine, whose absolute configuration (2*R*,4*R*) had been already established by X-ray analysis, as one of the products, the another product identified with natural enduracididine including optical property must have (2*S*,4*R*) configuration. Conclusively, the structure of enduracididine could be now determined to be (2*S*,4*R*)-2-amino-3-(2-iminoimidazolidin-4-yl)-propionic acid by the present synthetic study, in accordance with the assumption by Horii *et al.*[3]

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References and Footnotes

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1) This work was presented at the 32th Annual Meeting of The Chemical Society of Japan, Tokyo, April 1975.

2) K. Mizuno, M. Asai, S. Horii, M. Hori, H. Iwasaki, and J. Ueyanagi, *Antimicrobial Agents and Chemotherapy*, 1970, 6.

3) S. Horii and Y. Kameda, *J. Antibiot.*, 21, 665 (1968).

4) K. Iinuma, S. Kondo, K. Maeda, and H. Umezawa, *ibid.*, 28, 613 (1975).

5) The cleavage reaction of imidazole ring by means of acyl chloride has been long known as Bamberger cleavage; E. Bamberger and B. Berlé, *Justus Liebigs Ann. Chem.*, 273, 342 (1893).

6) The original Bamberger cleavage was modified by use of butyryl chloride instead of benzoyl chloride, since the double bond in 2,4,5-tribenzoylaminopentenoic acid methyl ester, prepared from methyl L-histidinate and benzoyl chloride according to Heath *et al.* (H. Heath, A. Lawson and C. Rimington, *J. Chem. Soc.*, 1951, 2215), could not be hydrogenated at all under the same condition. This might be attributed to the resonance stabilization of the olefinic bond by conjugation of two benzamido groups: λ_{max}^{EtOH} (log ε): 207 (4.26), 211 (4.38), 228 (4.48), 275 (4.11), 282 (4.11), 294 (4.06)nm.

7) Hydrolysis of 5 with NaOH in methanol afforded 6 as hygroscopic solid, which was converted into 7 with *p*-nitrobenzyl bromide and triethylamine in 69 % yield from 5: Found: C, 57.29; H, 7.45; N, 11.18 % in agreement with the calculated values for the hemihydrate, mp 185-187°C.

8) Contrary to our initial expectation, difficulties were encountered on separation of these diastereomers at any stages (3 — 10) until the final procedure in this synthesis.

9) J. V. Rodricks and H. Rapoport, *J. Org. Chem.*, 36, 46 (1971).

10) This product (10) was isolated either by preparative paper chromatography or by thin-layer chromatography (*n*-butanol-AcOH-H_2O 4 : 1 : 2). The structure was confirmed by its NMR spectrum; δ 2.27 (2H, t, *J*=6, H-3), 2.50 (3H, s, $\underline{CH_3}$-C_6H_4), 3.64-3.90 (2H, m, H-5), 4.08-4.60 (2H, m, H-2,4) and 7.60-8.20 ppm (4H, aromatic H) in CD_3OD.

11) This product is supposed to be 6-aminomethyl-2-iminotetrahydropyrimidine-4-carboxylic acid from its relative mobility in paper electrophoresis (1.0) and relative elution time in amino acid analysis (0.63) compared to 1, as well as IR spectrum of its bis-4-hydroxyazobenzene-4'-sulfonate; ν_{max} 1740, 1680, 1600 cm^{-1}.

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