Communications to the Editors

ENDURACIDIN, A NEW ANTIBIOTIC. V

STRUCTURES OF NEW BASIC AMINO ACIDS, ENDURACIDIDINE AND ALLOENDURACIDIDINE

Sir:

The acid hydrolysis of enduracidin[1], an antibiotic isolated from *Streptomyces fungicidicus* No. 5477, yielded glycine, aspartic acid, alanine, serine, threonine, allothreonine*, ornithine, citrulline, α-amino-4-hydroxyphenylacetic acid, α-amino-3, 5-dichloro-4-hydroxyphenylacetic acid and two new basic amino acids, designated enduracididine (I) and alloenduracididine (II).

The hydrolysate was purified by ion-exchange chromatography on Dowex 50W×8 using a hydrochloric acid gradient. I was eluted before II in this system. Rechromatography on Dowex 50 W×8 using pyridine-acetic acid buffer gave pure I and II as crystalline salts and their elemental analyses indicated the same empirical formula $C_6H_{12}N_4O_2$ for both I and II (Table 1).

I and II can be resolved on the paper chromatogram with *n*-propyl alcohol - ammonium hydroxide - water, 7 : 1 : 2 (I, Rf 0.27; II, Rf 0.34) and by means of an amino acid autoanalyzer (I, retention volume 117 ml; II, retention volume 124 ml)**.

The optical rotation of I mono oxalate was less positive in alkaline solution than in acid solution ($[\alpha]_D^{22}$ +63.3° in H_2O+1 M HCl***; +57.6°, H_2O+1 M NaOH; +55.7°, H_2O+2 M NaOH; +56.4°, H_2O+3 M NaOH). II mono oxalate increased under similar condition ($[\alpha]_D^{23}$+8.7°, H_2O+1 M HCl; +13.3°, H_2O+1 M NaOH; +16.0°, H_2O+2 M NaOH; +14.0°, H_2O+3 M NaOH).

These data suggest an L-α-amino acid configuration for I and D-α-amino acid configuration for II (CLOUGH-LUTZ-JIRGENSONS

Table 1. Properties of enduracididine (I) and alloenduracididine (II) salts

	Formula	M. P. (decomp.)	Elemental analysis %	
			Calcd.	Found
I Mono-oxalate	$C_6H_{12}N_4O_2 \cdot C_2H_2O_4$	201~203°	C, 36.64; H, 5.38; N, 21.37	C, 36.75; H, 5.22; N, 21.28
II Mono-oxalate	$C_6H_{12}N_4O_2 \cdot C_2H_2O_4$	197~198°	C, 36.64; H, 5.38; N, 21.37	C, 36.36; H, 5.30; N, 21.14
I Di-flavianate	$C_6H_{12}N_4O_2 \cdot 2C_{10}H_6N_2O_8S \cdot H_2O$	220~225°	C, 38.14; H, 3.20; N, 13.69; S, 7.83	C, 38.18; H, 3.15; N, 13.22; S, 7.90
II Di-flavianate	$C_6H_{12}N_4O_2 \cdot 2C_{10}H_6N_2O_8S \cdot H_2O$	218~222°	C, 38.14; H, 3.20; N, 13.69; S, 7.83 mol. wt., 818.7	C, 38.45; H, 3.15; N, 13.55; S, 8.10; mol. wt., 814.8 (X-ray diffraction method)
I Mono-hydrobromide	$C_6H_{12}N_4O_2 \cdot HBr$	235~236°	C, 28.47; H, 5.18; N, 22.14; Br, 31.57	C, 28.18; H, 5.06; N, 21.89; Br, 31.12
II Mono-hydrobromide	$C_6H_{12}N_4O_2 \cdot HBr$	249~252°	C, 28.47; H, 5.18; N, 22.14; Br, 31.57	C, 28.70; H, 5.24; N, 22.16; Br, 31.98

* Threonine and allothreonine were resolved by paper chromatography with the upper phase of a system composed of *n*-butyl alcohol - methyl ethyl ketone - ammonium hydroxide - water (5 : 3 : 1 : 1).

** Autoanalyzer determinations were performed with Beckman amino acid analyzer model 120B (pH 5.28 citrate buffer, 15 cm column, flow rate 30 ml/hr) and α(2-iminohexahydro-4-pyrimidyl) glycine isolated from the hydrolysate of capreomycin[5] showed retention volume 111.5 ml.

*** These represent the number of moles of hydrochloric acid or sodium hydroxide per mole of I or II in water.

rule)[2]. Enzymatic evidence also agreed with this assignment (Table 2).

I and II gave almost equivalent color yield of the ninhydrin complex and had HW values between histidine and lysine at 570 mμ by means of an amino acid autoanalyzer, but gave negative copper-ninhydrin test[3]. The VAN SLYKE analysis indicated one primary amino function (I, mono oxalate 5.53 %; II, mono oxalate 5.36 % after 7 min) and mono-N-acetyl I and II (acetic anhydride in water) gave negative ninhydrin test.

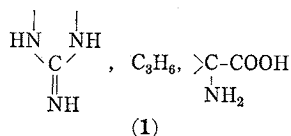
When the primary amino function of methyl ester of I and II was tritylated, the protons of the carbomethoxyl group were shifted up-field 70~73 cps in the NMR spectra at 100 Mc.[4]

Titration of the mono-acetic acid salt in aqueous solution gave pKa′ values of *ca.* 2.5, 4.7 (acetic acid), 8.3 (neut. equiv. 240 ±10) and *ca.* 12 in both I and II. The pKa′ value 8.3 disappeared in mono-N-acetyl I and II. The pKa′ value *ca.* 2.5 disappeared and pKa′ 8.3 was shifted to pKa′ 6.4 in methyl esters of I and II. These data suggest the α-amino acid structure for I and II.

In addition with the high nitrogen contents and the strong basic function which corresponds to a pKa′ *ca.* 12, the low ultraviolet maximum [I and II mono oxalate: $\lambda_{max}^{H_2O}$ 255~260 mμ (ϵ about 12)] and the IR spectra (I di-hydrochloride: ν_{max}^{KBr} 1960 and 1594 cm^{-1}, II di-hydrochloride: ν_{max}^{KBr} 1680 and 1580 cm^{-1}) suggest the existence of guanidine group. Permanganate oxidation of I and II afforded guanidine, which was characterized as the picrate, m. p. 330°C (decomp.). The IR spectrum of this picrate agreed with authentic guanidine picrate and established the nature of three nitrogens.

Alkaline degradation of I and II by 0.3 N barium hydroxide solution gave the neutral amino acid $C_6H_{11}N_3O_3$, indicating the presence of a ureido function, with the formation of ammonia.

The negative Sakaguchi reaction of I and II indicate that the guanidine group is not a mono-substituted one. These results may be summarized in the expression (1).



(1)

Table 2. Enzymatic oxidation with D-amino acid oxidase†

	Enduracididine	Alloenduracididine
Recovery of amino acid††	71.8%	0%

† A preparation from hog kidney purchased from Worthington Biochemical Corporation.
†† After 14 hours of incubation at 37°C.

Fig. 1. NMR spectrum of enduracididine mono oxalate*

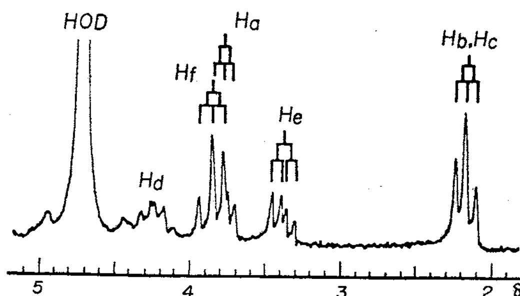
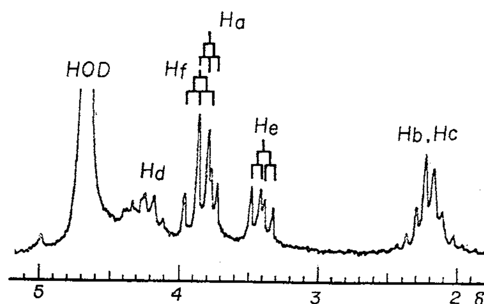
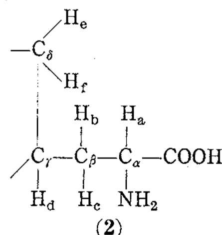


Fig. 2. NMR spectrum of alloenduracididine mono oxalate*



* NMR spectra were obtained in deuterium oxide with an external tetramethylsilane reference on a Varian HA-100 spectrometer.

The NMR spectra (Figs. 1 and 2) and the double resonance technique indicate that both I and II have the following partial structure (2).



(2)

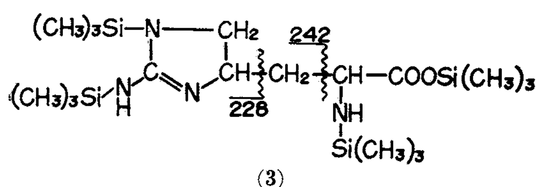
The H_a proton in I and II was shifted down field about 0.4 ppm in mono-N-acetyl

derivatives and assigned to a methine group attached to both carboxyl and amino group.

The difference between the NMR spectra of the H_b and H_c protons of I and II suggests a configurational difference at C_α atom in these two amino acids.

These evidences suggest that the disubstituted guanidine group must be linked to partial structure (2) at C_γ and C_δ atoms.

This assignment was further supported by their mass spectra. I and II were subjected to silylation with N,O-*bis*(trimethyl silyl) acetamide and the resulting tetra-(trimethyl silyl) derivatives of I and II exhibited peaks at m/e 460 (M^+) and m/e 445 (M^+-CH_3). Two intense peaks* are also present at m/e 242 and m/e 228 which could be attributed to fragmentation which are illustrated in structure (3).



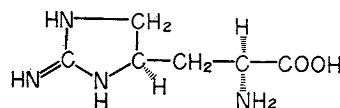
(3)

More conclusively, the high-resolution mass spectra of tetra-(trimethyl silyl) I and II showed a fragment of mass 228 at m/e 228.1357 (from tetra-TMS-I) and m/e 228.1361 (from tetra-TMS-II) (Calcd. for $C_9H_{22}N_3Si_2$: 228.1352).

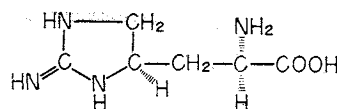
The absolute stereochemistry R at C_γ position of II mono-perchlorate, $C_6H_{12}N_4O_2 \cdot HClO_4$, has been determined by X-ray crystallography and will be reported in detail in a later paper by K. KAMIYA *et al.*

The ORD curves [I mono-oxalate ORD (c=0.335, 1 N HCl) $[\phi]^{24}\times 10^{-2}$ (mμ): +14.9° (280), +11.2° (300), +6.3° (350), +4.2° (400), +3.1° (450), +2.4° (500); II mono-oxalate ORD (c=0.342, 1 N HCl) $[\phi]^{24}\times 10^{-1}$ (mμ): +13.0° (280), +10.7° (300), +6.9° (350), +4.6° (400), +3.5° (450), +2.7° (500)] and the different retention volumes of I and II in an amino acid autoanalyzer show that the absolute stereochemistry at C_γ of I is also defined as R.

The structures are thus α(S)-amino-β-4(R)-(2-iminoimidazolidinyl)-propionic acid for I and α(R)-amino-β-4(R)-(2-iminoimidazolidinyl)-propionic acid for II.



(I)



(II)

Acknowledgement

The authors are grateful to Dr. R. TAKEDA for his interest throughout this work, to Messrs. H. KAWASHIMA and S. YAGI for the provision of starting materials, to Mr. T. HONGO for amino acid analysis.

Thanks are also due to Dr. Y. ASAHI, Mr. M. KAN and their staff for measurements of NMR spectra, mass spectra, optical rotation and elemental analysis.

SATOSHI HORII
YUKIHIKO KAMEDA

Microbiological Research Laboratories,
Research and Development Division,
Takeda Chemical Industries, Ltd.
Higashiyodogawa-ku, Osaka, Japan

(Received August 15, 1968)

References

1) ASAI, M.; M. MUROI, N. SUGITA, H. KAWASHIMA, K. MIZUNO & A. MIYAKE: Enduracidin, a new antibiotic. II. Isolation and characterization. J. Antibiotics 21 : 138~146, 1968.
2) DJERASSI, C.: Optical rotatory dispersion applications to organic chemistry. p. 212, McGraw-Hill Book Co. Inc., New York, 1960.
3) LARSEN, P. O. & A. KJAER: Paper chromatographic differentiation between α-monoamino acids and other ninhydrin-positive substances. Biochim. Biophys. Acta 38 : 148~150, 1960.
4) STAMMER, C.H. & R.G. WEBB: A new method for distinguishing α-amino acids from their β- and γ-isomers. Tetrahedron Letters No. 40 : 4895~4899, 1966.
5) HERR, E. B., Jr.; W. B. SUTTON & W. M. STARK: Chemical and biological studies of capreomycin. Transactions of the 21st research conference in pulmonary disease. pp. 367~369, 1962.

* There are no stronger peaks than m/e 228 and m/e 242 in all peaks from mass 150 to the molecular ion.