[Chem. Pharm. Bull. 19(12)2631—2634(1971)]

UDC 547.466.46.04 : 546.56.04

Studies on the β-Lysine Peptide. I. Formation of Copper Complex[1]

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(Received April 26, 1971)

In a recent paper[3] we described the chromatographic separation of the hydrolysis products derived from racemomycin produced by a mutant strain of *Streptomyces racemochromogenus*.[4] Racemomycin on hydrolysis yields L-β-lysine(L-β, ε-diaminocaproic acid) which has been also isolated from streptothricin group antibiotics, viomycin,[5] capreomycin-I,[6] tuberactinomycin-B and -O,[7] and as an intermediate in L-lysine fermentation.[8] The absolute structure of β-lysine(L-configuration) has been established by van Tamelen, *et al.*[9] by synthesis.

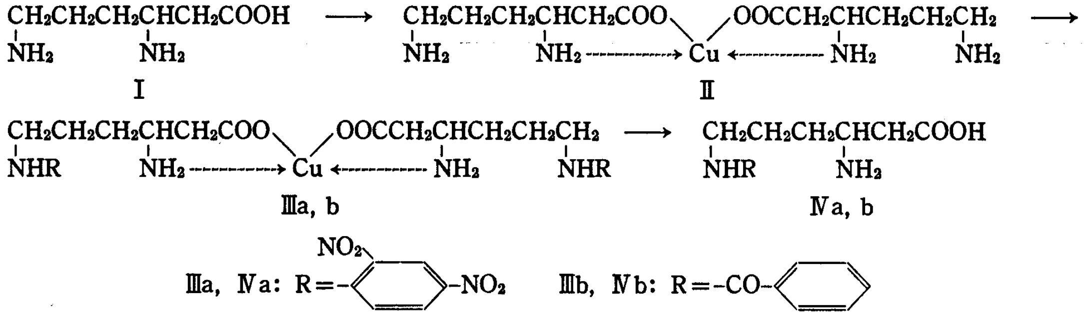

Chart 1

The physical and chemical properties (Table I), infrared (IR) spectrum,[10] specific rotation,[8] and pK'_a values[8] of 3.30, 9.25 and 10.65 of the β-lysine isolated from racemomycin were identical with those of L-β-lysine.[9]

Further elucidation of the structure was carried out by mass spectrum of ethyl dibenzoyl-β-lysinate[11] in which eliminations of -OEt, $-COC_6H_5$ from the molecular ion and cleavages of C_β-C_γ, C_δ-C_ε and negligible fission between the C_α and the carbonyl-carbon were characterized.

The copper complex (II) of L-β-lysine was prepared from copper hydroxide.[12] The copper complex (V) of L-α-lysine was also prepared for comparison. The IR spectrum of II suggested that II is non-crystalline and the spectrum was differentiated from that of the complex (V) as shown in Fig. 1. The ultraviolet (UV) spectrum of II in aqueous solution

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1) This work was presented at the 91th Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *1-14, Bunkyo-machi Nagasaki-shi.*
3) H. Taniyama Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1627 (1971).
4) T. Sugai, *J. Antibiotics Ser. B* **9**, 170 (1956).
5) T.H. Haskell, S.A. Fusari, R.P. Frohardt, and Q.R. Bartz, *J. Am. Chem. Soc.*, **74**, 599 (1952).
6) E.B. Herr, *Antimicrob. Agents and Chemoth.*, 1962, 201.
7) It was presented at the 24th Annual Meeting of Chemical Society of Japan, Osaka, April 1971 by K. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, S. Noda, S. Taie and G. Abe,
8) R.N. Costilow, O.M. Rochovansky, and H.A. Barker, *J. Biol. Chem.*, **241**, 1573 (1966).
9) E.E. van Tamelen, and E.E. Smissman, *J. Am. Chem. Soc.*, **75**, 2031 (1953).
10) W.F. Phillips, and H.S. Ragheb, *J. Chromatog.*, **19**, 147 (1965).
11) The parent peak was not detected in either L-β-lysine ethyl ester or L-β-lysine benzyl ester.
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Table I. Physico-chemical Constants of L-β-Lysine and Its Derivatives

	Compounds			
	Hydrochloride (I)	Dibenzoate	DiDNphenylate	Ethyl dibenzoyl-β-lysinate
Appearance	white needle under dryness	white powder (AcOEt–acetone)	yellowish powder (AcOEt)	white needle (acetone–AcOEt)
mp (uncorr.)	153—155°	140—141°	198—202°	132—135°
Optical rotation	+19° (c=1, H_2O, 25°)	undefined	−125° (c=0.5, acetone, 25°)	−27° (c=0.5, $CHCl_3$, 25°)
IR $\nu_{C=O}^{KBr}$ (cm^{-1})	1715	1760	1710	1725
UV_{max} (mμ)	end absorption (H_2O)	237, ε 5.75×10^3 (EtOH)	364, ε 3.3×10^4 (DMFA)	226, ε 2.03×10^4 (EtOH)
Elemental *Anal.*	Calcd. for $C_6H_{14}O_2N_2\cdot2HCl\cdot2H_2O$: C, 28.23; H, 7.84; N, 10.98; Cl, 27.84% Found: C, 28.54; H, 7.98; N, 10.63; Cl, 27.44%.	Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.91% Found: C, 67.76; H, 6.12; N, 7.64%.	Calcd. for $2C_9H_9O_5N_3$: C, 45.19; H, 3.76; N, 17.57% Found: C, 45.34; H, 3.83; N, 17.28%.	Calcd. for $C_{22}H_{16}O_4N_2$: C, 68.92; H, 7.04; N, 7.28% Found: C, 68.64; H, 6.95; N, 7.05%.

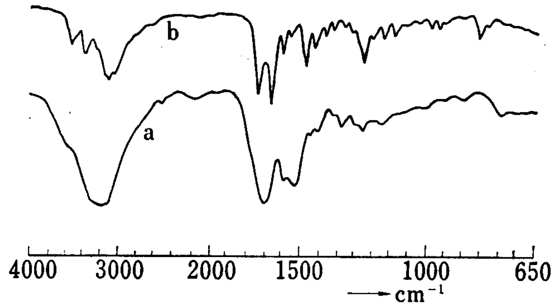

Fig. 1. IR Spectrum of the Copper Complexes in KBr

a: β-lysine copper complex
b: α-lysine copper complex

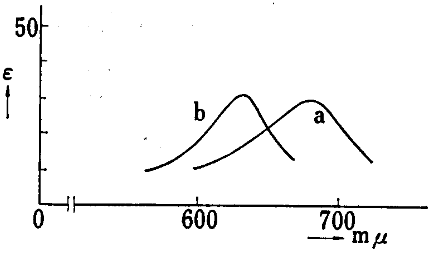

Fig. 2. UV Spectrum of the Copper Complexes in Water

a: β-lysine copper complex
b: α-lysine copper complex

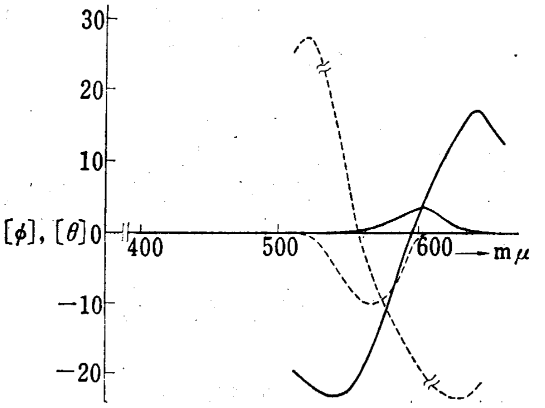

Fig. 3. ORD and CD Curves of the Copper Complexes in Water

———: β-lysine copper complex
-------: α-lysine copper complex

was characterized by comparison with that of the complex (V) as shown in Fig. 2. Optical rotatory dispersion (ORD) and circular dichroism (CD) measurements of II in aqueous solution showed a positive Cotton effect in the region of the visible d→d absorption band, but the spectra of V had a negative Cotton effect[13] (Fig. 3).

In comparison of the ORD and CD curves of II with those of V, it was clearly observed that not only the former had Cotton effect opposite that of the latter, but also there was a significant difference of the amplitude between them. These results may be interpreted in terms of the six-membered ring conformation in the copper complex of the optical active β-amino acid, though the consideration is not necessarily drawn only on the basis of the above ORD and CD data.

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[13] T. Yasui, *Bull. Chem. Soc. Japan*, **38**, 1746 (1965).

Table II. Physico-chemical Constants of L-β-Lysine ε-Derivatives

	Compounds	
	IVa	IVb
Appearance (crystallized)	yellowish powder (n-BuOH–H_2O)	white powder (EtOH–ether)
Ninhydrin test	positive	positive
mp (uncorr.)	222—225°	216—218°
Optical rotation	$+15\pm4°$ ($c=0.05$, 0.5N HCl, 20°)	$+20\pm2°$ ($c=1$, H_2O, 15°)
Elemental *Anal.*	Calcd. for $C_{12}H_{16}O_6N_4$: C, 46.15; H, 5.13, N, 17.95% Found: C, 45.88; H, 5.30; N, 17.68%	Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.40; H, 7.20; N, 11.20% Found: C, 61.57; H, 7.41; N, 11.04%
IR $\nu_{C=O}^{KBr}$ (cm^{-1})	1705 (hydrochloride)	1715 (hydrochloride)
UV_{max} (mμ)	363 ε 1.28×10^4 (0.5N HCl)	229 ε 1.12×10^4 (0.5N HCl)

The following two derivatives, ε-DNP-β-lysine (IVa) and ε-benzoyl-β-lysine (IVb), were derived from the complex (II). Their physico-chemical properties are summarized in Table II.

In the nuclear magnetic resonance (NMR) spectra of these derivatives (in hydrochloride form) in deuterium water, the signals due to the ε-methylene protons(triplet) shifted downfield by about δ 0.67 for the compound IVa and δ 0.42 for the compound IVb, respectively, compared with that of β-lysine hydochloride. In the course of the preparation of IVa and IVb, di-DNP-β-lysine and dibenzoyl-β-lysine were not detected in the reaction mixtures by thin-layer chromatography (TLC) (silica gel, Merck brand).

From these results, it is suggested that, when L-β-lysine is coordinated to copper ion in aqueous solution, the nitrogen atoms are activated by the configurational stereochemistry.

Experimental

Dibenzoyl-β-lysine——Dibenzoyl-β-lysine was prepared with benzoyl chloride (2.5 fold mole) in 20% NaOH aqueous solution, following successive reprecipitation from acetone–AcOEt. Yield: 80%. IR ν_{max}^{KBr} (cm^{-1}): 3000, 2790, 2610, 2500, 1895, 1760, 1660, 1590, 1570, 1440, 1410, 1320, 1280, 1170, 1120, 1090, 1065, 1020, 990, 925, 810—800, 700.

Bis(dinitrophenyl)-β-lysine——The compound was synthesized with DNFB (3 fold mole) in 5% $NaHCO_3$ aqueous solution. Precipitate was chromatographed on silica gel with ethyl acetate (*Rf* 0.4). IR ν_{max}^{KBr} (cm^{-1}): 3380, 3100, 2930, 1710, 1620, 1590, 1520, 1420, 1340, 1315, 1145, 925, 835, 745.

Ethyl Dibenzoyl-β-lysinate——To a suspention of β-lysine hydrochloride (*Rf* 0.32, Toyo-Roshi No. 51 UH using solvent system of n-BuOH–pyridine–HOAc–H_2O–t-BuOH (15: 10: 3: 12: 4)) in abs. EtOH was passed hydrogen chloride and then the reaction mixture was evaporated to dryness. The procedure was repeated three times (*Rf* 0.58 on paper, IR ν_{max}^{KBr} (cm^{-1}): 3160, 3040, 1710, 1600, 1470, 1400, 1210, 1020. The product was benzoylated with benzoyl chloride up to negative ninhydrin test. The precipitate was reprecipitated with acetone–ether and then recrystallized from acetone–AcOEt. IR ν_{max}^{KBr} (cm^{-1}): 3340, 3100, 2940, 1725, 1640, 1583, 1540, 1450, 1318, 1250, 1240, 1170, 1025, 805, 700. NMR ($CDCl_3$) δ: 1.25 t. $J=7$ Hz 3H, 1.7 m. 4H, 2.58 d. $J=6$ Hz 2H, 3.46 2H, 4.10 q. $J=7$ Hz 2H, 4.35 m. 1H, 6.87 m. 2H, 7.43, 7.70 m. 10H (TMS as internal standard). Mass Spectrum m/e: 382 (M^+), 337 (M^+-OEt), 277 (M^+-C_6H_5CO), 260, 248, 231, 220, 174, 162, 156, 134, 122, 105.

ORD and CD Measurements——The ORD and CD curves in the region from 700 to 200 mμ were obtained by a JASCO ORD/UV-5 spectrometer with CD attachment (J-5) using c, 1.0—3.3% in a 5 mm cell at 28—30°.

Bis(L-β-lysinate) Copper (II)——The copper complex corresponding to II has physical properties as follows. IR ν_{max}^{KBr} (cm^{-1}): 3400, 3000, 1600, 1470, 1400, 1280, 1175, 1120, 750. UV $\lambda_{max}^{H_2O}$ (mμ) 680 (ε: 30) (peak), 500 (ε: 4) (trough). ORD ($c=3.3$, H_2O): $[M]^{30}$ (mμ) $+50°$ (635) (peak), $-23°$ (540) (trough), $-12°$ (460) (peak), $-26°$ (420). CD ($c=3.3$, H_2O): $[\theta]^{30}$ (mμ) $+1.8°$ (650), $+3°$ (600) (peak), $+1.5°$ (550). The complex was very hygroscopic and unable to be analyzed.

Synthesis of IVa——The complex (II), 0.2 g, in 4 ml of water was treated with DNFB (0.4 g) in EtOH for 4 hr at room temperature. The precipitate was filtered and washed with water, ethanol, and ether, and then purified by reprecipitation from water. The dried powder corresponding to IIIa, $[\alpha]_D^{23}$: $+23°$ ($c=1$, HOAc), is yellowgreenish substance with mp (decomp.) of about 180°. Compound IIIa (150 mg) in HOAc (5 ml) was desalted with H_2S for 2 min. The filtrate was dried up to a crude powder melting above 183°. Yield: 43 mg. Positive to ninhydrin test. *Rf* 0.71 on paper. Purification of the compound (IVa) was carried out by cellulose column (2.6 × 55 cm) using *n*-BuOH-saturated water (*Rf* 0.56 on paper). Yellow precipitate corresponding to IVa was obtained during the concentration. IR ν_{max}^{KBr} (cm^{-1}): 3400, 2980, 1705, 1615, 1590, 1490, 1420, 1330, 1180, 1125, 1050, 920, 830, 740 in its hydrochloride. NMR (D_2O) δ: 1.9 m. 4H, 2.90 d. $J=6$ Hz 2H, 3.65 t. $J=6$ Hz 2H, 3.76 m. 1H, 7.18 d. $J=11$ Hz 1H, 8.35 d. $J=11$ Hz 1H, 9.12 s. 1H in hydrochloride.

Synthesis of IVb——The complex (II) was benzoylated in 5% $NaHCO_3$ with cooling to precipitate a greenish powder corresponding to IIIb (*Rf* 0.59 on paper). The precipitate was filtered and washed with water and ether. The intermediate IIIb was dissolved in HOAc, and desalted with H_2S. The filtrate was evaporated to a crude powder, TLC on silica gel: *Rf* 0.30 (MeOH). Further purification was achieved with cellulose column (2.5 × 60 cm). Fractions showing one spot (by ninhydrin test) were collected and evaporated to a small volume, and precipitated with EtOH, ether, and pet. ether. IR ν_{max}^{KBr} (cm^{-1}): 2940, 1715, 1630, 1570, 1540, 1490, 1410, 1310, 1215, 1180, 1160, 715 in hydrochloride. NMR (D_2O) δ: 1.75 m. 4H, 2.79 d. $J=6$ Hz 2H, 3.40 t. $J=6$ Hz 2H, 3.65 m. 1H, 7.5—7.86 m. 5H in hydrochloride. NMR of L-β-lysine hydrochloride as reference δ: 1.70 m. 4H, 2.72 d. $J=6$ Hz 2H, 2.98 t. $J=5.5$ Hz, 3.57 m. 1H.

Acknowledgement The authors are grateful to Dr. M. Suzuki, Tanabe Seiyaku Co., Ltd., for his advice and discussions relating to this work.

Chem. Pharm. Bull.
19(12)2634—2636(1971)

UDC 547.728.2.09 : 615.212.076.9

Mode of Action of Analgesic Effect of 2-Methylaminomethyl-2,3-dihydrobenzofuran (EPS-4032)

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(Received May 25, 1971)

Through our intensive pharmacological screening of benzofuran derivatives, we found that 2-monoalkylaminomethyl-2,3-dihydrobenzofuran analogues, especialy, 2-methylaminomethyl-2,3-dihydrobenzofuran (EPS-4032) revealed relatively potent analgesic effect.[2]

The purpose of our present work was to investigate the mode of action of analgesic effect of EPS-4032 in mice comparing with morphine.

Method

The analgesic effect of these drugs were tested by means of the electrical stimulation method (40 volt, 20 msec, 1 Hz) in male mice weighing 14—16 g.[3]

Result and Discussion

As shown in Table I and Fig. 1, analgesic effect of EPS-4032 as well as morphine was apparently antagonized by pretreatment of reserpine or tetrabenazine. Several studies suggest that the analgesic effect of morphine result from an interaction with brain mono-

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[1] Location: *4-6-10, Koishikawa, Bunkyo-ku, Tokyo.*

[2] S. Toyoshima, N. Hirose, T. Ohgoh and A. Sugii, *Yakugaku Zasshi*, **88**, 503 (1968).

[3] T. Ohgoh, A. Kitahara and T. Takahashi, *Oyo Yakuri*, **4**, 195 (1970).