Synthesis of N^{δ}-Hydroxyornithine

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N^{δ}-Tosyl-N^{δ}-benzyloxy-DL-ornithine was synthesized starting from γ-(N-tosyl-N-benzyloxy)-aminopropyl bromide and diethyl acetamidomalonate and resolved enzymatically to give the L- and D-isomers. Removal of one or both of protecting groups gave N^{δ}-benzyloxyornithine or N^{δ}-hydroxyornithine. The reduction product of N^{δ}-hydroxy-L-ornithine was identical with authentic L-ornithine.

N^{δ}-Hydroxyornithine is one of the most commonly found constituents of naturally occurring peptides.[1] In these compounds the α-amino and the carboxyl group of a N^{δ}-hydroxyornithine residue are linked by amide bonds to form a cyclic hexapeptide and the hydroxyamino group is present in the form of a hydroxamic acid derivative. These peptides, for example, ferrichrome[2] and albomycin[3] have been found to have potent microbial growth factor or antibiotic activities.

Synthesis of N^{δ}-hydroxyornithine have been attempted by two group of workers, but the amino acid was not isolated.[4,5]

This paper presents the successful synthesis of N^{δ}-hydroxyornithine *via* optical resolution of N^{δ}-tosyl-N^{δ}-benzyloxy-DL-ornithine and subsequent removal of the tosyl and benzyl groups.

O-Benzylhydroxylamine(I) was first converted into the tosyl derivative(II), which was alkylated with 1,3-dibromopropane to γ-(N-tosyl-N-benzyloxy)-aminopropyl bromide(III). This was allowed to react with diethyl sodio-acetamidomalonate, and the product was heated with concentrated hydrochloric acid–acetic acid to yield racemic N^{δ}-tosyl-N^{δ}-benzyloxyornithine(DL-V).

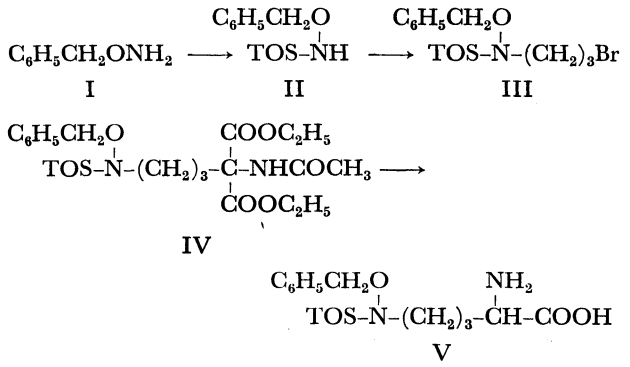

Fig. 1

Optically active N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine and its D-isomer were prepared by enzymatic resolution of the N^{α}-acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-DL-ornithine (DL-VI) with aniline in the presence of papain. The resulting N^{α}-acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine anilide (L-VII) and N^{α}-acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-D-ornithine (D-VI) showed $[\alpha]_D^{25}$ −53.4° and $[\alpha]_D^{25}$ −18.1° in chloroform solution. N^{δ}-Tosyl-N^{δ}-benzyloxy-L-ornithine and its D-isomer (L–V and D–V) were prepared by acid hydrolysis in hydrochloric acid–acetic acid solution. The amino acid (L-V and D-V) thus obtained exhibited optical rotation of $[\alpha]_D^{23.5}$ +20.7° and $[\alpha]_D^{25}$ −21.0°, respectively, in acetic acid solution.

DL-VI was also obtained by racemization of D-VI using acetic anhydride or acetic anhydide–acetic acid.

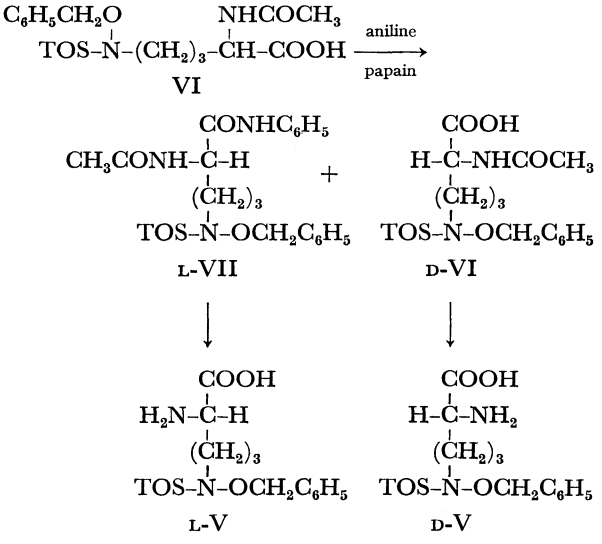

Fig. 2

As a mild method for the removal of a tosyl group in the synthesis of relatively unstable compounds, Weisblat *et al.*[6] used 30% hydrogen bromide in acetic acid in the presence of phenol at room temperature. They described detosylation of *N*-substituted sulfonamides, such as TOS–NRR′; R,R′=H, alkyl, aryl, acyl or alkoxycarbonyl. However similar condition has never been applied for detosylation of tosylhydroxamate series, TOS–N(OR)R′; R=$CH_2C_6H_5$, R′=alkyl. The removal of a tosyl group from the amino acid (V) was accomplished by the

1) W. Keller-Schierlein, V. Prelog, and H. Zähner, "Fortshritte der Chemie Organischer Naturstoffe XXII," Springer-Verlag, Wien (1964), p. 279—316.

2) J. B. Neilands, *J. Amer. Chem. Soc.*, **74**, 4846 (1952); J. A. Garibaldi and J. B. Neilands, *ibid.*, **77**, 2429 (1955); S. J. Rogers and J. B. Neilands, *Biochemistry*, **3**, 1850 (1964); S. J. Rogers, R. A. J. Warren, and J. B. Neilands, *Nature*, **200**, 167 (1963).

3) J. Turkova, O. Mikes, and F. Sorm, *Experientia*, **19**, 633 (1963).

4) S. J. Rogers, *Dissertation Abstr.* **25** (**10**), 5538 (1965); S. J. Rogers, University Microfilms, Inc., Ann Arbor, Michigan, order No 65-3073.

5) L. G. Makevnina and N. A. Poddubunaya, *Zh. Obsch. Khim.*, **36** (**10**), 1755 (1966).

6) D. I. Weisblat, B. J. Magerlein, and D. R. Myers, *J. Amer. Chem. Soc.*, **75**, 3630 (1953).

action of 36% hydrogen bromide in acetic acid in the presence of phenol at room temperature for 50 hr to give N^{δ}-benzyloxy-DL-ornithine monohydrobromide (DL-VIII·HBr). Similar treatment of the D- and L-amino acid (D- and L-V) with this reagent gave the corresponding D- and L-N^{δ}-benzyloxy derivatives (D- and L-VIII).

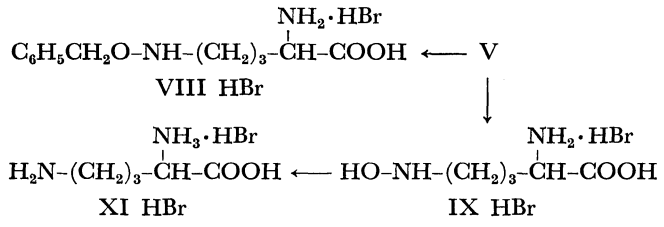

Fig. 3

Simultaneous cleavage of the tosyl and benzyl group from the amino acid (D-, L-, and DL-V) was carried out by the action of the above described reagent at room temperature for 10 days to give N^{δ}-hydroxyornithine monohydrobromide (D-, L-, and DL-IX), which showed blue color with ninhydrin and gave red color with the triphenyltetrazolium reagent.[7] The salt (X) of N^{δ}-hydroxy-DL-ornithine with one mole of 2-nitro-1,3-indanedione was obtained from DL-IX HBr by the procedure of Rogers.[4]

L- and D-IX were hydrogenated to give L- and D-ornithine monohydrobromide (L- and D-XI) in the presence of palladium charcoal.

It was demonstrated by measurements of the melting point, infrared spectrum, optical rotation, and elemental analysis that the L-isomer(XI) was identical with authentic L-ornithine monohydrobromide.

Experimental

Melting points were all determined with a Yanagimoto electric micromelting point apparatus and are uncorrected, and optical rotations were measured with a Yanagimoto automatic polarimeter OR-50.

O-Benzylhydroxylamine (I). It was prepared by the method described by Fuller and King.[8]

O-Benzyl-N-tosylhydroxylamine (II). To a chilled solution of *O*-benzylhydroxylamine (246 g, 2.0 mol) in pyridine (300 m*l*), a solution of *p*-toluenesulfonyl chloride (381 g, 2.0 mol) in pyridine was added slowly during 2 hr. The solution was stirred at room temperature for 2 hr and allowed to stand overnight. The material precipitated was filtered off and the filtrate was evaporated *in vacuo*. The residual oil was extracted with ethyl acetate (1.2 *l*) and the organic layer was washed with 2N hydrochloric acid and water, dried over sodium sulfate, and then evaporated *in vacuo*. The residual oil was solidified by adding petroleum ether. The product was recrystallized from ethyl acetate–petroleum ether; yield 454 g (82%), mp 93—97°C.

Found: C, 60.79; H, 5.45; N, 4.92%. Calcd for $C_{14}H_{15}NO_3S$: C, 60.64; H, 5.45; N, 5.05%.

γ-(N-Tosyl-N-benzyloxy)-aminopropyl Bromide (III). To a solution of sodium (23 g, 1.0 g-Atom) in ethanol (1.2 *l*) was added II (277 g, 1.0 mol) with vigorous stirring at 70°C. After a clear solution was obtained, trimethylene dibromide (404 g, 2.0 mol) was added. The mixture was refluxed for 8 hr, and evaporated *in vacuo* to give oily material, which was extracted with ethyl acetate and the organic layer was thoroughly washed with water and dried over sodium sulfate. Evaporation of the solvent afforded oily product which was crystallized from ethyl acetate–*n*-hexane; yield 350 g (88%), mp 73—77°C.

Found: C, 51.36; H, 5.11; N, 3.69%. Calcd for $C_{17}H_{20}O_3NSBr$: C, 51.26; H, 5.06; N, 3.51%.

Diethyl γ-(N-Tosyl-N-benzyloxy)-aminopropyl Acetamidomalonate (IV). To a solution of sodium (18.4 g, 0.8 g-Atom) in ethanol (1.5 *l*) was added diethyl acetamidomalonate (174 g, 0.8 mol) while stirring. After 5 min, III (319 g, 0.8 mol) was added and the mixture was refluxed for 8 hr. The solvent was then removed *in vacuo* and the residue was extracted with chloroform. The chloroform solution was successively washed with water, dilute hydrochloric acid and water, dried over magnesium sulfate, and evaporated *in vacuo*. The oily residue was crystallized from ethyl acetate–petroleum ether and then from ethanol; yield 299 g (70%), mp 135—138°C.

Found: C, 58.68; H, 6.12; N, 4.99%. Calcd for $C_{26}H_{34}N_2O_8S$: C, 58.42; H, 6.41; N, 5.24%.

N^{δ}-Tosyl-N^{δ}-benzyloxy-DL-ornithine (DL-V). A solution of IV (160 g, 0.3 mol) in a mixture of acetic acid (1.2 *l*) and concentrated hydrochloric acid (480 m*l*) was heated under gentle reflux for 8 hr. The solution was evaporated *in vacuo* and 14% aqueous ammonia was added to the residue. The resulting solid was collected by filtration, washed with water, and recrystallized from acetic acid–water; yield 106 g (90%), mp 207—210°C (decomp.).

Found: C, 57.84; H, 6.04; N, 6.89%. Calcd for $C_{19}H_{24}N_2O_5S$: C, 58.15; H, 6.17; N, 7.14%.

N^{α}-Acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-DL-ornithine (DL-VI). DL-V (70 g, 0.18 mol) was mixed with glacial acetic acid (420 m*l*) and warmed with gentle stirring to give a clear solution. The solution was cooled for several minutes and acetic anhydride (26 m*l*, 0.25 mol) was carefully added. The resulting solution was warmed to 80°C with stirring, held at this temperature for 5 hr and then evaporated *in vacuo*. The syrupy residue was taken up in a minimum quantity of ethyl acetate and the solution was chilled. After standing for 2 hr at 5°C, the crystals separated. Recrystallization from ethyl acetate gave colorless crystals; yield 58 g (75%), mp 142.5—144.0°C.

Found: C, 57.93; H, 6.01; N, 6.29%. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45%.

Resolution of N^{α}-Acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-DL-ornithine (DL-VI). a) N^{α}-Acetyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine Anilide (L-VII). DL-Acetylamino acid (DL-VI) (74 g, 0.171 mol) was dissolved in 0.75N sodium hydroxide (600 m*l*) and the solution adjusted to pH 6.05—6.20 with 0.5M citric aicd. The solution was diluted with distilled water to the final volume of 1.2 *l*. Papain (20 g) was added to the solution together with freshly distilled aniline (20 m*l*) and cysteine hydrochloride monohydrate (10 g). The mixture was incubated for 36 hr at 38°C; the anilide deposited was filtered off, washed with water, 7% aqueous ammonia and water.

Recrystallization from *n*-propyl alcohol gave colorless crystals; yield 40 g (92%), mp 203—205°C, $[\alpha]_D^{25}$ −53.4° (*c* 1, chloroform).

Found: C, 63.25; H, 6.07; N, 8.14%. Calcd for $C_{27}H_{31}N_3O_5S$: C, 63.64; H, 6.13; N, 8.25%.

7) G. A. Snow, *J. Chem. Soc.*, **1954**, 2588.
8) A. T. Fuller and H. King, *ibid.*, **1947**, 963.

b) N^{α}-*Acetyl*-N^{δ}-*tosyl*-N^{δ}-*benzyloxy*-D-*ornithine (*D-*VI).* The above filtrate, combined with the washings was adjusted to pH 2 with 5N hydrochloric acid. Then the precipitated material was filtered off, washed with water and dried. Recrystallization from ethyl acetate gave colorless crystals; yield 32.4 g (88%), mp 131.0—133.5°C, $[\alpha]_D^{25}$ −18.1° (*c* 1, chloroform).

Found: C, 58.00; H, 5.91; N, 6.06%. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45%.

N^{δ}-*Tosyl*-N^{δ}-*benzyloxy*-L-*ornithine (*L-*V).* L-VII (40 g, 78.7 mmol) was dissolved in glacial acetic acid (300 m*l*) while gently warming and concentrated hydrochloric acid (120 m*l*) was carefully added. After refluxing for 8 hr, the solution was evaporated *in vacuo* and aqueous ammonia was added to the residue. After standing for 2 hr at 5°C, the deposits were filtered off with suction, washed with water, and dried. Recrystallization from dilute acetic acid gave 25.8 g (83%), mp 222.5—224.8°C (decomp.), $[\alpha]_D^{23.5}$ +20.7° (*c* 3, acetic acid).

Found: C, 57.98; H, 6.46; N, 7.38%. Calcd for $C_{19}H_{24}N_2O_5S$: C, 58.15; H, 6.16; N, 7.14%.

N^{δ}-*Tosyl*-N^{δ}-*benzyloxy*-D-*ornithine (*D-*V).* This was obtained from D-VI by the method described above. Yield 86%, mp 220—223°C (decomp.) (from dilute acetic acid), $[\alpha]_D^{25}$ −21.0° (*c* 2, acetic acid).

Found: N, 7.25%. Calcd for $C_{19}H_{24}N_2O_5S$: N, 7.14%.

N^{δ}-*Benzyloxy*-L-*ornithine Monohydrobromide (*L-*VIII HBr).* To 18 m*l* of a solution of 36% hydrogen bromide-acetic acid and phenol (2.5 g) in a glass-stoppered bottle, L-V (3.92 g, 10 mmol) was added at room temperature. After stirring for 50 hr, the solution was evaporated to dryness *in vacuo* at 35°C. The residual oil was triturated several times with ether and dissolved in water (50 m*l*). The solution was washed repeatedly with ether and then kept in a refrigerator overnight, the crystals deposited was removed by filtration and the filtrate was evaporated *in vacuo* at 35°C. The above residue was dissolved in water (5 m*l*). The solution was adjusted to pH 4—5 with pyridine, and addition of ethanol (30 m*l*) and ether (50 m*l*) yielded crystals, which were purified by dissolving them in water and adding ethanol-ether. D- and DL-V were treated in the same manner to yield the corresponding D- and DL-VIII·HBr. The results are summarized in Table 1.

Table 1. Melting points, yields, optical rotations and elemental analyses of VIII

Compd.		L-VIII	D-VIII	DL-VIII
Mp (°C)		155—157 (decomp.)	155—156 (decomp.)	149—150 (decomp.)
Yield (%)		30	32	52
$[\alpha]_D$ (°)		+9.0 (*c* 1, H_2O, 27°C)	−8.4 (*c* 1, H_2O, 22°C)	—
Formula		$C_{12}H_{19}N_2O_3Br$	$C_{12}H_{19}N_2O_3Br$	$C_{12}H_{19}N_2O_3Br$
Calcd	C	45.15	45.15	45.15
	H	6.00	6.00	6.00
	N	8.78	8.78	8.78
Found	C	44.68	45.13	44.88
	H	6.08	6.06	6.12
	N	8.86	8.85	8.84

N^{δ}-*Benzyloxy*-DL-*ornithine (*DL-*VIII).* This was obtained from V by the method described above, except that the solution was adjusted to pH 8 by adding aqueous ammonia. The solution was evaporated to dryness again. The residue was crystallized from water-ethanol (Yield 53%), mp 195—203°C (decomp.). It gave positive ninhydrin test and negative Beilstein test. For the analysis, the product was recrystallized from water-ethanol.

Found: C, 60.43; H, 7.57; N, 11.58%. Calcd for $C_{12}H_{18}N_2O_3$: C, 60.48; H, 7.61; N, 11.76%.

N^{δ}-*Hydroxyornithine Monohydrobromide (IX·HBr).* To a mixture of 36% hydrogen bromide-acetic acid (18 m*l*) and phenol (2 g) in a glass-stoppered bottle, V (3.92 g, 10 mmol) was added at room temperature. After stirring for 10 days at room temperature, the solution was evaporated *in vacuo* at 50°C. The residual oil was triturated with ether. The residue was dissolved in water (50 m*l*) and the solution was washed repeatedly with ether and then evaporated *in vacuo* below 50°C. The residual oil was dissolved again in water (2 m*l*), the solution was adjusted to pH 4—5 with pyridine, and addition of ethanol deposited crystals. For analysis, the product was recrystallized from water-ethanol. Ninhydrin, triphenyltetrazolium, and Beilstein tests were all positive. The results are summarized in Table 2.

Table 2. Melting points, optical rotations, and elemental analyses of IX.

Compd.		L-IX	D-IX	DL-IX
Mp (°C)		174—176 (decomp.)	175—177 (decomp.)	199—201 (decomp.)
$[\alpha]_D^{25}$ (°)		+8.3 (*c* 2, H_2O)	−8.2 (*c* 2.5, H_2O)	—
Formula		$C_5H_{13}N_2O_3Br$	$C_5H_{13}N_2O_3Br$	$C_5H_{13}N_2O_3Br$
Calcd	C	26.21	26.21	26.21
	H	5.72	5.72	5.72
	N	12.23	12.23	12.23
Found	C	26.36	26.38	26.39
	H	5.69	5.70	5.88
	N	12.04	12.06	12.03

N^{δ}-*Hydroxy*-DL-*ornithine Mono-2-nitro-1,3-indanedione (X).* This compound was obtained from DL-IX by the procedure of Rogers.[4] Yellow needles, mp 212—219°C (decomp.). (lit,[4] mp 210—212°C (decomp.)).

Found: C, 49.60; H, 5.00; N, 12.38%. Calcd for $C_{14}H_{17}N_3O_7$: C, 49.56; H, 5.05; N, 12.38%.

Catalytic Reduction of L-*IX.* A solution of L-IX (0.5 g, 2.2 mmol) in water was hydrogenated in the presence of 5% palladium charcoal catalyst (0.5 g) at room temperature. After 20 hr, the catalyst was removed and the filtrate was evaporated *in vacuo*. The residual crystalline product was recrystallized from water-ethanol (Yield 74%). The corresponding D-isomer (D-XI·HBr) was obtained in the same

Table 3. Melting points, optical rotations and elemental analyses of XI.

Compd.		L-XI	D-XI	Authentic L-XI
Mp (°C)		243—246 (decomp.)	244—246 (decomp.)	245—247 (decomp.)
$[\alpha]_D$ (°)		+9.9 (*c* 2, H_2O, 23°C)	−9.5 (*c* 1.75, H_2O, 26°C)	+9.8 (*c* 2, H_2O, 23°C)
Formula		$C_5H_{13}N_2O_2Br$	$C_5H_{13}N_2O_2Br$	$C_5H_{13}N_2O_2Br$
Calcd	C	28.18	28.18	28.18
	H	6.15	6.15	6.15
	N	13.15	13.15	13.15
Found	C	28.13	28.21	28.41
	H	5.95	6.22	6.15
	N	12.91	13.34	13.23

manner with a 72% yield. This was positive to ninhydrin test and negative to triphenyltetrazolium test. The results are summarized in Table 3.

Racemization of N^{α}-*acetyl*-N^{δ}-*tosyl*-N^{δ}-*benzyloxy*-D-*ornithine (*D-*VI).* *a)*: A solution of D-VI (21.7 g, 0.05 mol) in acetic anhydride (40 m*l*) was heated at 120°C for 2 hr, and then evaporated *in vacuo*. The residual pale yellow syrup was dissolved in acetone (30 m*l*) and water (10 m*l*) was added. The mixture was stirred for 2 hr at room temperature and evaporated *in vacuo*. The residual syrup was dissolved in hot ethyl acetate (30 m*l*), and the racemic compound (VI), which precipitated on standing in a deep freezer, was collected, yield 16.4 g, mp 137—139°C. Recrystallization from ethyl acetate raised the melting point to 139.5—141°C. The product was optically inactive in chloroform solution.

b): A solution of D-VI (21.7 g) in a mixture of acetic acid (30 m*l*) and acetic anhydride (7 m*l*) was refluxed for 75 min, and then evaporated *in vacuo*. DL-VI was obtained with the same treatment as described above. Yield 16.1 g, mp 136°C. Recrystallization from ethyl acetate raised the melting point to 139—140°C. The product showed no optical rotation.