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Acylation of N^{δ}-Benzyloxyornithine

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Acetylation of N^{δ}-benzyloxyornithine monohydrobromide (I·HBr) with acetic anhydride gave the N^{δ}-acetylated product, which yielded N^{δ}-acetyl-N^{δ}-hydroxyornithine by catalytic reduction. On the other hand, acetylation or alkoxycarbonylation of N^{δ}-benzyloxyornithine(I) proceeded through N^{α}-acetylation, followed by spontaneous cyclization to form lactams, which yielded cyclic hydroxamic acids on catalytic reduction. These hydroxamic acid and N^{δ}-acetyl-N^{δ}-hydroxyornithine were readily hydrolyzed to give N^{δ}-hydroxyornithine.

In the preceding paper,[1] it was shown that N^{δ}-tosyl-N^{δ}-benzyloxyornithine gave N^{δ}-benzyloxyornithine(I) by detosylation under mild conditions. Since its benzyloxyamino-group may be converted into a hydroxamic group, I is expected to be used as a starting material for the synthesis of amino acid derivatives and peptides such as N^{δ}-acetyl-N^{δ}-hydroxyornithine,[2] rhodotorulic acid[3] and ferrichrome.[4] The present paper reports the behavior of I toward acylation.

Treatment of optically active or racemic N^{δ}-benzyloxyornithine monohydrobromide (L-, D-, and DL-I·HBr) with acetic anhydride in water yielded a viscous oily product (II), which gave, on catalytic hydrogenation with palladium on charcoal, optically active or racemic N^{δ}-acetyl-N^{δ}-hydroxyornithine (L-, D-, and DL-III). The results are summarized in Table 1.

Table 1. N^{δ}-Acetyl-N^{δ}-hydroxyornithine (III)

		L	D	DL
Mp (°C)		194—197 (decomp.)	195—197.5 (decomp.)	193—195.5 (decomp.)
Yield (%)		64	69	79
Found	C	44.01	44.20	44.16
	H	7.62	7.62	7.70
	N	14.51	14.62	14.46
Calcd for $C_7H_{14}N_2O_4$	C	44.20	44.20	44.20
	H	7.41	7.41	7.41
	N	14.73	14.73	14.73

NMR (D_2O) for DL-III: δ; 3.70 ppm (3H, δ-methylene and α-methine), 2.13 (3H, acetyl methyl protons), 1.80 (4H, β, γ-ethylene protons).

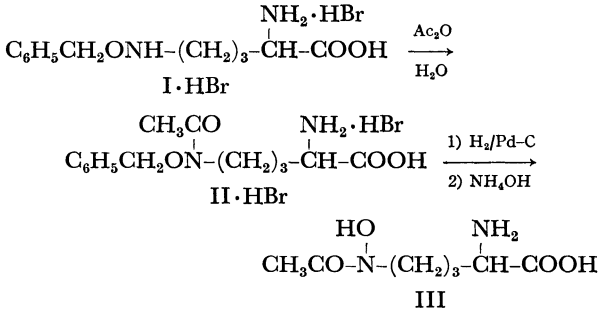

Fig. 1

Since these products gave positive ninhydrin- and ferric-test and negative triphenyltetrazolium reaction, their structure is established as formulated III in Fig. 1. Emery considered N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine (L-III) as a precursor in ferrichrome biosynthesis and prepared it from N^{δ}-hydroxyornithine obtained by the hydrolysis of fusarinine.[2] The melting point of our synthetic L-III was the same as that of Emery's product. No racemization occurred during the above mentioned synthesis of III, because hydrolysis and subsequent catalytic reduction of D-III gave D-ornithine monohydrochloride (D-V·HCl), $[\alpha]_D^{22}$ −12.2° (c 1, water), the optical activity of authentic L-isomer monohydrochloride obtained commercially being $[\alpha]_D^{22}$ +12.2° (c 1, water).

1) Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori, This Bulletin, **45**, 1461 (1972).
2) T. Emery, *Biochemistry*, **5**, 3694 (1966).
3) C. L. Atkin and J. B. Neilands, *Biochemistry*, **7**, 3734 (1968).
4) J. B. Neilands. *J. Amer. Chem. Soc.*, **74**, 4846 (1952).

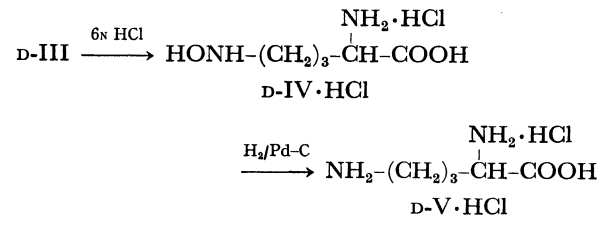

Fig. 2

It should be mentioned that N^{δ}-hydroxyornithine monohydrochloride (L-, and D-IV·HCl) has not been obtained previously, although isolation of the corresponding monohydrobromide was described in the preceding paper.[1] Further, L- and D-IV gave salts with 2-nitroindane-1,3-dione (L- and D-VI) by the procedure of Rogers.[5] Treatment of free I with two and a half equivalents of acetic anhydride in acetic acid afforded 1-benzyloxy-3-acetaminopiperidone (VII), instead of the expected product, N^{α}-acetyl-N^{δ}-acetyl-N^{δ}-benzyloxyornithine (VIII). The lactam (VII) was also obtained from I by treatment with one equivalent of *p*-nitrophenyl acetate in the presence of benzyltrimethylammonium hydroxide (Triton B) in dioxane followed by neutralization. Analogous lactam deivatives (IX and X) were obtained from free I. Attempted N^{α}-acylation of N^{δ}-benzyloxyornithine always resulted in spontaneous cyclization to give lactams.

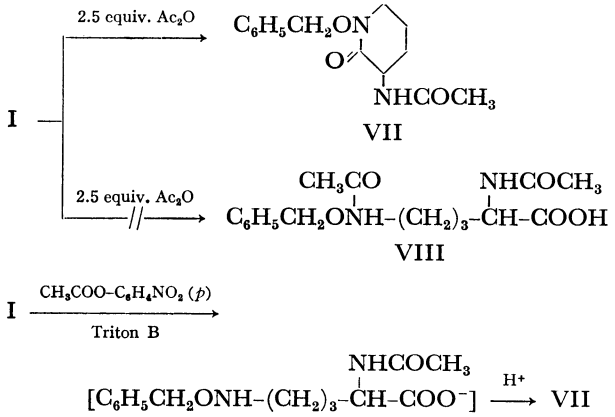

Fig. 3

It was found that the lactam (VII) reacts with hydrazine monohydrate to give N^{α}-acetyl-N^{δ}-benzyloxyornithine hydrazide (XI). Catalytic hydrogenation of VII and IX with palladium on charcoal yielded 1-hydroxy-3-acylaminopiperidones, XII and XIII. Treatment of D-XIII with 2N hydrochloric acid in dioxane afforded D-1-hydroxy-3-amino-2-piperidone monohydrochloride (XIV·HCl). It melted at 177—179°C. This is the same melting point as that of the monohydrochloride obtained from fusarinine,[4] although the latter is probably the optical enantiomorph (L-form). Finally, hydrolysis of D-XIV·HCl with 6N hydrochloric acid yielded N^{δ}-hydroxy-D-ornithine monohydrochloride.

5) S. J. Rogers, Univ. Microfilm (Ann Arbor, Mich.) Order No. 65—3073, Dissertation (1964).

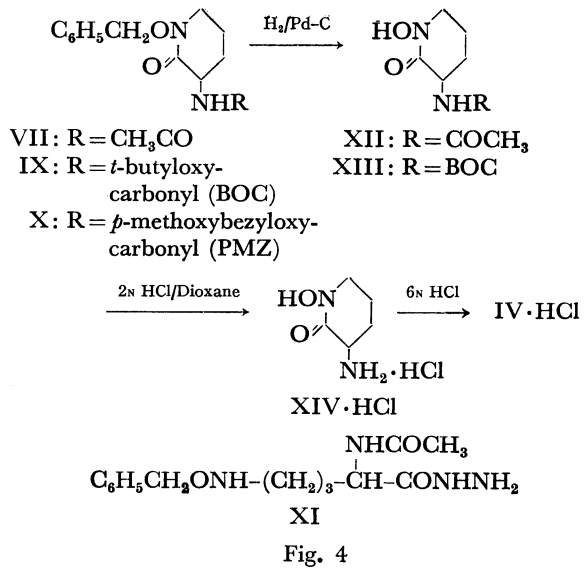

Fig. 4

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. Nuclear magnetic resonance spectra were taken on a Hitachi Perkin-Elmer R-20 High Resolution NMR spectrometer using tetramethylsilane as an internal standard.

N^{δ}-*Benzyloxyornithine (I).* This compound was prepared as monohydrobromide by the procedure described in the preceding paper.[1]

L-I·HBr: mp 155—157°C (decomp.), $[\alpha]_D^{27}$ +9.0° (*c* 1, water)

D-I·HBr: mp 155—157°C (decomp.), $[\alpha]_D^{22}$ −8.4° (*c* 1, water)

Free amino acid was obtained from monohydrobromide by treatment with aqueous ammonia. L-I: mp 198—202°C (decomp.), D-I: mp 198—205°C (decomp.).

N^{δ}-*Acetyl*-N^{δ}-*benzyloxy*-D-*ornithine Monohydrobromide (*D-*II·HBr).* To a solution of D-I·HBr (0.957 g, 3 mmol) in water (5 m*l*), acetic anhydride (0.375 m*l*) was added at room temperature. After it had been stirred for 1 hr at 50°C, the reaction mixture was evaporated *in vacuo*. The residual oil was used for subsequent operation without further purification.

The DL- and L-isomer were obtained as oily products in the same manner.

N^{δ}-*Acetyl*-N^{δ}-*hydroxy*-D-*ornithine (*D-*III).* A solution of D-II in methanol (15 m*l*) was hydrogenated in the presence of 5% palladium charcoal (0.15 g) at room temperature for 17 hr. The filtrate obtained from the reduction mixture was evaporated *in vacuo*. The residual oil was dissolved in water (0.15 m*l*), the solution was adjusted to pH 8—9 with aqueous ammonia, and addition of ethanol to the solution yielded crystals. Recrystallization from water-ethanol gave colorless crystals. The DL- and L-isomer were obtained in the same manner. The results are summarized in Table 1. Ninhydrin- and ferric chloride tests were positive and triphenyltetrazolium test was negative with these products.

N^{δ}-*Hydroxy*-D-*ornithine Monohydrochloride (*D-*IV·HCl).* D-III (0.37 g, 1.95 mmol) was hydrolyzed with 6N hydrochloric acid (15 m*l*) at 100°C, until ferric test became negative. The reaction mixture was evaporated to dryness *in vacuo* and the treatment was repeated after the addition of water. The residue was dissolved in water, the solution was adjusted to pH 4—5 with pyridine and evaporated to dryness *in vacuo*.

The residue was crystallized from water-ethanol-ether. Yield 78%, mp 179—181°C (decomp.), $[\alpha]_D^{19}-11.6°$ (*c* 1, water), positive triphenyltetrazolium, ninhydrin, and negative ferric chloride tests.

Found: C, 32.98; H, 6.78; N, 15.20%. Calcd for $C_5H_{13}N_2O_3Cl$: C, 32.52; H, 7.10; N, 15.18%.

L-Isomer (L-IV·HCl) was obtained from L-III bv the same procedure. Mp 178—180°C (decomp.), $[\alpha]_D^{19}+11.4°$ (*c* 0.5, water).

Found: C, 32.62; H, 7.29; N, 15.05%. Calcd for $C_5H_{13}N_2O_3Cl$: C, 32.52; H, 7.10; N, 15.18%.

N^{δ}-Hydroxy-D-ornithine Mono-2-nitro-1,3-indanedione (D-VI). This was obtained from D-IV by the procedure of Rogers.[5] Bright yellow crystals, mp 220—227°C (decomp.).

Found: C, 49.38; H, 5.11; N, 12.14%. Calcd for $C_{14}H_{17}N_3O_7$: C, 49.56; H, 5.05; N, 12.38%.

The L-isomer (L-VI) was obtained from L-IV by the same procedure. Bright yellow crystals, mp 221—227°C (decomp.).

D-Ornithine Monohydrochloride (D-V·HCl). A solution of D-IV (0.239 g, 1.3 mmol) in water (5 m*l*) was hydrogenated in the presence of 10% palladium charcoal (0.1 g) at room temperature, until the tetrazolium test became negative. The filtrate from the reduction mixture was evaporated *in vacuo* to give a crystalline product. Recrystallization from water-ethanol gave the desired product. Yield 92%, mp 242—245°C (decomp.), $[\alpha]_D^{22}-12.2°$ (*c* 1, water). (L-Ornithine monohydrochloride (obtained commercially). Mp 240—245°C (decomp.), $[\alpha]_D^{22}+12.2°$ (*c* 1, water)).

Found: C, 35.48; H, 7.84; N, 16.33%. Calcd for $C_5H_{13}N_2O_2Cl$: C, 35.61; H, 7.77; N, 16.62%.

DL-1-Benzyloxy-3-acetylaminopiperidone (DL-VII). DL-I (2.23 g, 0.01 mol) was dissolved in acetic acid (10 m*l*) by boiling and after the solution was allowed to cool for 1—2 min acetic anhydride (2.5 m*l*) was added in portions. The resulting solution was heated under reflux for 2 min and allowed to cool to room temperature. The reaction mixture was evaporated *in vacuo* and the evaporation was repeated after addition of water to the residue. The resulting crystals were taken up in ethyl acetate and the solution was washed repeatedly with water. The organic layer was dried over sodium sulfate and evaporated to dryness. The resulting crystalline residue was purified by recrystallization from ethyl acetate. Yield 46%, mp 151—152°C, ninhydrin, tetrazolium, and ferric chloride tests negative.

NMR (DMSO-d_6): δ; 8.10 ppm (1H, *N*-proton), 7.40 (5H, phenyl protons), 4.87 (2H, *O*-methylene protons), 4.30 (1H, C_3-proton), 3.45 (2H, C_6-protons), 1.86 (7H, C_4, C_5, and acetyl protons).

Found: C, 63.98; H, 6.77; N, 10.62%. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68%.

DL-1-Benzyloxy-3-t-butyloxycarbonylaminopiperidone (IX). To a suspension of DL-I (0.476 g, 2 mmol) in methanol (5 m*l*), 40% benzyltrimethylammonium hydroxide (0.94 m*l*) was added and the mixture was stirred until the suspension went into a clear solution. The resulting solution was evaporated *in vacuo* and the residue was evaporated successively with ethanol and benzene. To a solution of the residue in *t*-buthanol (4 m*l*), *t*-butyl 2,4,5-trichlorophenyl carbonate (0.601 g, 2.02 mmol) was added and stirred for 15 hr at 50—55°C. The reaction mixture was evaporated *in vacuo* and the residue was taken up in water. The solution was washed repeatedly with ether and was adjusted to pH 3.0—3.5 with solid citric acid. The deposit was extracted with ethyl acetate and the extract was washed with water, dried over sodium sulfate and evaporated *in vacuo*. The residue was crystallized from ethyl acetate-*n*-hexane; yield 50%. For analysis, the product was recrystallized from *n*-hexane, mp 116—117°C, ninhydrin, tetrazolium, and ferric chloride tests negative.

Found: C, 63.75; H, 8.10; N, 8.55%. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.72; H, 7.55; N, 8.75%.

*D-1-Benzyloxy-3-*p*-methoxybenzyloxycarbonylaminopiperidone (D-X).* The product was obtained from D-I by the method described above, except that *p*-methoxybenzyl 2,4,5-trichlorophenyl carbonate and dioxane as solvent were used. The product was recrystallized from ethyl acetate-*n*-hexane. Yield 61%, mp 112—114°C, $[\alpha]_D^{24}-43.2°$ (*c* 1, ethyl acetate).

Found: C, 65.58; H, 6.33; N, 7.23%. Calcd for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29%.

N^{α}-Acetyl-N^{δ}-benzyloxy-DL-ornithine Hydrazide (DL-XI). A solution of DL-VII (0.262 g, 1 mmol) and hydrazine monohydrate (0.1 m*l*) in dioxane (10 m*l*) was warmed for 2 hr at 100—110°C and evaporated *in vacuo*. The residue was crystallized from ethanol-ether. Yield 83%. For analysis, the product was recrystallized from ethanol-ether, mp 101—105°C, ninhydrin, tetrazolium, and ferric chloride tests negative.

Found: C, 57.37; H, 7.26; N, 18.90%. Calcd for $C_{14}H_{22}N_4O_3$: C, 57.12; H, 7.54; N, 19.04%.

DL-1-Hydroxy-3-acetylaminopiperidone (DL-XII). A solution of DL-VII (0.262 g, 1 mmol) in methanol (5 m*l*) was hydrogenated in the presence of 5% palladium charcoal (0.05 g) at room temperature for 24 hr. The filtrate from the reduction mixture was evaporated *in vacuo*. The residual crystalline product was recrystallized from methanol. Yield 76%, mp 203—205°C, negative ninhydrin, and tetrazolium tests, and positive ferric chloride test.

Found: C, 48.92; H, 7.22; N, 16.17%. Calcd for $C_7H_{12}N_2O_3$: C, 48.82; H, 7.03; N, 16.27%.

D-1-Hydroxy-3-amino-2-piperidone Hydrochloride (D-XIV·HCl). A solution of D-IX (1.46 g, 4.55 mmol) in methanol (20 m*l*) was hydrogenated in the presence of 5% palladium charcoal (0.25 g) at room temperature for 14 hr. The filtrate from the mixture was evaporated *in vacuo* (ferric positive and ninhydrin test negative). The resulting oily residue was treated with 2N hydrogen chloride-dioxane (44 m*l*) for 2 hr at room temperature. The reaction mixture was evaporated *in vacuo* and the residual oil crystallized from ethanol. Recrystallization from methanol-ethanol gave colorless crystals. Yield 51%, mp 208—215°C (decomp.), $[\alpha]_D^{24}-0.8°$ (*c* 0.83, water), ferric positive and tetrazolium test negative. The ninhydrin test gave yellow color.

Found: C, 36.08; H, 6.44; N, 16.56; Cl, 21.48%. Calcd for $C_5H_{11}N_2O_2Cl$: C, 36.04; H, 6.66; N, 16.82; Cl, 21.28%.

N^{δ}-Hydroxy-D-ornithine Monohydrochloride (D-IV·HCl). D-XIV (0.25 g, 1.5 mmol) was hydrolyzed with 6N hydrochloric acid (15 m*l*) at 100°C for 30 min, when the mixture gave negative ferric test. The reaction mixture was evaporated to dryness *in vacuo* and the evaporation was repeated after the addition of ethanol. The residue was crystallized from water-ethanol. The crystals were collected by filtration and recrystallized from water-ethanol. Yield 54%, mp 177—179.5°C, $[\alpha]_D^{24}-12.0°$ (*c* 1, water), tetrazolium and ninhydrin tests positive and ferric chloride test negative.

Found: C, 32.63; H, 6.92; N, 15.08%. Calcd for $C_5H_{13}N_2O_3Cl$: C, 32.52; H, 7.10; N, 15.18%.