Synthesis of Rhodotorulic Acid

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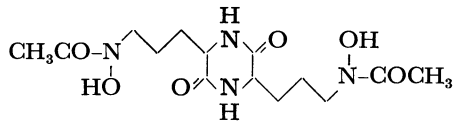
Rhodotorulic acid (I) was synthesized by a series of reaction starting from N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine. Detosylation and acetylation of intermediate, cyclo-di-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl (LL-IX), afforded cyclo-di-N^{δ}-acetyl-N^{δ}-benzyloxy-L-ornithyl (LL-XI), which, on reductive debenzylation, gave rhodotorulic acid.

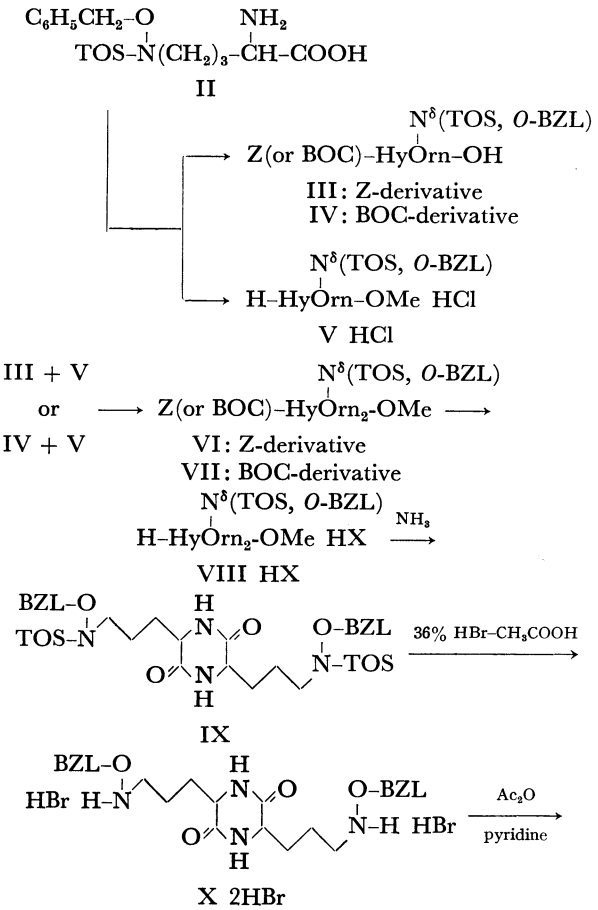
Rhodotorulic acid was first obtained by Atkin and Neilands[1] from supernatants of iron-deficient culture of a red yeast, *Rhodotorula pilimanae*. They found a potent growth-factor activity in this compound in assays with Arthrobacter species and determined its structure to be cyclo-di-N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithyl (LL-I). We have succeeded in synthesizing rhodotorulic acid (LL-I); the DD-isomer was also prepared.

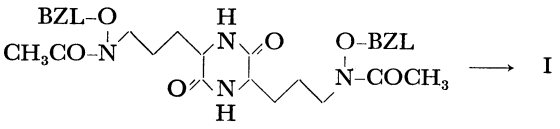


LL-I

Fig. 1

The synthetic route is shown in Fig. 2.





XI

$Z = C_6H_5CH_2OCO-$, $BOC = (CH_3)_3COCO-$,
$TOS = p\text{-}CH_3C_6H_4SO_2-$, $BZL = C_6H_5CH_2-$

Fig. 2. The abbreviations which are not listed in *Biochemistry* **5**, 1445 (1966), are H-HyOrn-OH for N^{δ}-hydroxyornithine and H-HyOrn-OH for N^{δ}-tosyl-N^{δ}-benzyloxyornithine.
N^{δ}-(TOS, *O*-BZL)

N^{α}-Benzyloxycarbonyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine (L-III) was obtained by benzyloxycarbonylation of N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine (L-II).[2] A *t*-butyloxycarbonyl derivative (L-IV) was prepared by the reaction of L-II with *t*-butyl 2,4,5-trichlorophenyl carbonate in the presence of benzyltrimethylammonium hydroxide. By the reaction of the methyl ester (L-V) with the carbobenzoxy or *t*-butyloxycarbonyl amino acid (L-III or L-V) *via* the mixed anhydride, benzyloxycarbonyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine methyl ester (LL-VI) or the corresponding N^{α}-*t*-butyloxycarbonyl-dipeptide methyl ester (LL-VII) was obtained. Treatment of LL-VI or LL-VII with an excess of hydrogen bromide - acetic acid or hydrogen chloride - ethyl acetate, N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine methyl ester hydrobromide or hydrochloride (LL-VIII HX) was obtained. Cyclo-di-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl (LL-IX) was formed by the action of excess of ammonia on LL-VIII HX in methanol at

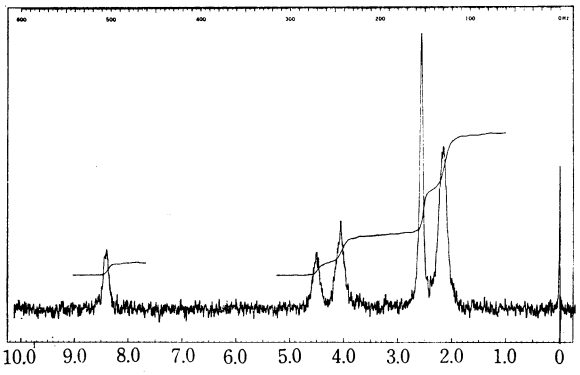


Fig. 3. NMR spectrum of synthetic rhodotorulic acid in trifluoroacetic acid.

1) C. L. Atkin and J. B. Neilands, *Biochemistry*, **7**, 3734 (1968).
2) Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori, This Bulletin, **45**, 1461 (1972).

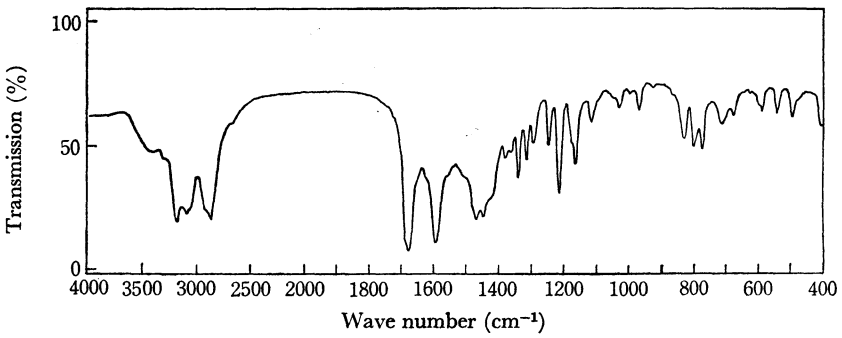
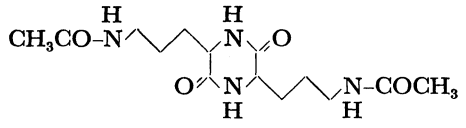


Fig. 4. IR spectrum of synthetic rhodotorulic acid.

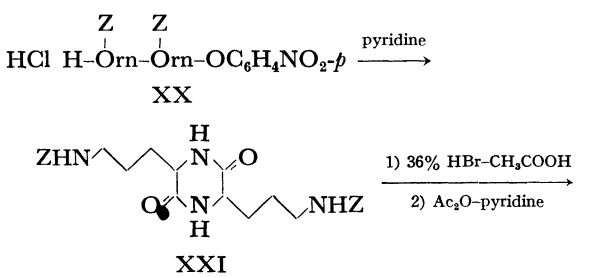
room temperature.[3] The tosyl group of LL-IX was removed by treatment with 36% hydrogen bromide-acetic acid and phenol for 50 hr at room tempeature to yield cyclo-di-N^{δ}-benzyloxy-L-ornithyl dihydrobromide (LL-X 2HBr). LL-X 2HBr was converted into the acetyl derivative (LL-XI) with an excess of acetic anhydride in pyridine. Hydrogenation of LL-XI in methyl alcohol in the presence of palladium charcoal at room temperatue gave cyclo-di-N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithyl; rhodotorulic acid (LL-I). The DD-isomer of rhodotorulic acid, DD-I, was also prepared in a similar manner. The synthetic rhodotorulic acid and its DD-isomer, LL-I and DD-I, exhibited specific rotations of −28.5° (*c* 1, water, at 25°C) and +28.0° (*c* 0.6, water, at 24°C) respectively. These products gave a positive ferric chloride test. The identity of the synthetic rhodotorulic acid with natural rhodotorulic acid[1] was substantiated through spectral comparison (IR and NMR) (Fig. 3 and 4).

The synthetic rhodotorulic acid was hydrogenated in the presence of Raney nickel catalyst to cyclo-di-N^{δ}-acetyl-L-ornithyl (LL-XII), which was identified by comparison of the melting points, optical rotations, IR and NMR spectra with authentic sample (XXIII) prepared from L-ornithine (Fig. 6).



LL-XII

Fig. 5



cyclo-di-N^{δ}-acetyl-L-ornithyl

XXIII

Fig. 6

3) E. Fischer and K. Raske, *Ber.*, **39**, 3981 (1906); N. Izumiya, T. Kato, Y. Fujita, M. Ohno, and M. Kondo, This Bulletin, **37**, 1809 (1964).

Experimental

Melting points of all the synthetic compounds were determined with a Yanagimoto electric micromelting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto automatic polarimeter OR-50. Nuclear magnetic resonance spectra were run on a Hitachi Perkin-Elmer R-20 High Resolution NMR spectrometer, using tetramethylsilane as an internal standard. Molecular weight was determined with a Hitachi Perkin-Elmer 115 molecular weight apparatus.

N^{δ}-*Tosyl*-N^{δ}-*benzyloxyornithine (*L-*II and* D-*II).* The compound was prepared by the procedure described in the previous paper.[2]

L-II: mp 222.5—224.8°C (decomp.), $[\alpha]_D^{23.5}$ +20.7° (*c* 3, acetic acid);

D-II: mp 217—223°C (decomp.), $[\alpha]_D^{25}$ −21.0° (*c* 2, acetic acid).

N^{α}-*Benzyloxycarbonyl*-N^{δ}-*tosyl*-N^{δ}-*benzyloxy*-L-*ornithine (*L-*III).* It was obtained from L-II by the usual procedure, using a mixture of tetrahydrofuran and water as the solvent. The colorless oily product solidified to a glass. Yield 93%, $[\alpha]_D^{23}$ −2.0° (*c* 2, acetic acid).

Found: N, 5.24%. Calcd for $C_{27}H_{30}N_2O_7S$: N, 5.32%.

N^{α}-t-*Butyloxycarbonyl*-N^{δ}-*tosyl*-N^{δ}-*benzyloxy*-L-*ornithine (*L-*IV).* To a suspension of L-II (15.7 g, 40 mmol) in methanol (80 m*l*), 40% benzyltrimethylammonium hydroxide (Triton B) (18.8 m*l*) was added. After stirring for 2 hr at room temperature, the solution was evaporated *in vacuo* at 50°C. The residue was then evaporated twice more with dimethylformamide *in vacuo* at 50°C in order to remove traces of water. The resulting syrup was dissolved in *t*-butanol (80 m*l*) and *t*-butyl 2,4,5-trichlorophenyl carbonate (11.9 g, 40 mmol) was added. After gentle stirring for 20 hr at 50—55°C, the reaction mixture was evaporated *in vacuo* and the residue taken up in water (70 m*l*). The solution was washed repeatedly with ether and acidified with M citric acid to about pH 3. The oily material thus deposited was extracted with ethyl acetate (600 m*l*). The extract was washed with water, dried over magnesium sulfate and evaporated *in vacuo*. The residue was crystallized from ethyl acetate - petroleum ether. Yield 18.5 g (94%), mp 58—66°C, $[\alpha]_D^{25}$ +11.2° (*c* 1, ethanol).

Found: C, 58.74; H, 6.54; N, 5.40%. Calcd for $C_{24}H_{32}N_2O_7S$: C, 58.52; H, 6.55; N, 5.69%.

N^{α}-t-*Butyloxycarbonyl*-N^{δ}-*tosyl*-N^{δ}-*benzyloxy*-D-*ornithine (*D-*IV).* It was prepared in the same manner as described for the preparation of L-IV. Yield, 97.5%, mp 59—65°C and $[\alpha]_D^{25}$ −9.0° (*c* 2, ethanol).

Found: C, 58.59; H, 6.75; N, 5.68%. Calcd for $C_{24}H_{32}N_2O_7S$: C, 58.52; H, 6.55; N, 5.69%.

N^{δ}-*Tosyl*-N^{δ}-*benzyloxy*-L-*ornithine Methyl Ester Hydrochloride (*L-*V HCl).* It was prepared from L-II by the usual

procedure. Recrystallization was effected from methanol - ether. Yield 83%, mp 151—152.5°C, $[\alpha]_D^{25}$ +18.9° (*c* 2, methanol).

Found: C, 54.18; H, 6.46; N, 6.14%. Calcd for $C_{20}H_{27}N_2O_5SCl$: C, 54.23; H, 6.14; N, 6.33%.

N^{δ}-Tosyl-N^{δ}-benzyloxy-D-ornithine Methyl Ester Hydrochloride (D-V HCl). D-V HCl was obtained through the same method as described above. Yield 96%, mp 149—150°C and $[\alpha]_D^{24.5}$ −19.3° (*c* 1, methanol).

N^{α}-Benzyloxycarbonyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine Methyl Ester (LL-VI). To a chilled solution of L-III (4.90 g, 9.3 mmol) and triethylamine (1.31 m*l*) in tetrahydrofuran (45 m*l*), isobutyl chloroformate (1.26 m*l*, 9.3 mmol) was added at −10°C. After 20 min, a mixture of V HCl (4.24 g, 9.3 mmol) and triethylamine (1.31 m*l*) in *N,N*-dimethylformamide (25 m*l*) was added to the above solution. The mixture was stirred overnight at room temperature. After the mixture had been evaporated *in vacuo*, the product was dissolved in ethyl acetate (50 m*l*). The solution was washed successively with N hydrochloric acid, water, 0.5M sodium bicarbonate, and water, and dried over anhydrous sodium sulfate. The filtered solution was evaporated to dryness *in vacuo*. The residual colorless oil resisted to attempt to crystallization. Yield 8.4 g, (98%).

Found: C, 61.59; H, 6.12; N, 6.25%. Calcd for $C_{47}H_{54}N_4O_{11}S_2$: C, 61.70; H, 5.95; N, 6.13%.

N^{δ}-Tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine Methyl Ester Hydrobromide (VII HBr). To a solution of LL-VI (8.3 g, 9.1 mmol) in acetic acid (6.5 ml), 36% hydrogen bromide - acetic acid (8.2 m*l*) was added. After 90 min, the solution was evaporated *in vacuo*. The reisdual oil was triturated with ether. Yield of the oily product, 6.3 g (79%).

N^{α}-t-Butyloxycarbonyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine Methyl Ester (LL-VII). This compound was prepared by coupling L-IV with L-V by the mixed anhydride method as described above; yield 96.7%, mp 63—69°C, $[\alpha]_D^{25}$ +1.1° (*c* 1, ethyl acetate).

Found: C, 60.09; H, 6.65; N, 6.10%. Calcd for $C_{44}H_{56}N_4O_{11}S_2$: C, 59.98; H, 6.41; N, 6.37%.

N^{δ}-Tosyl-N^{δ}-benzyloxy-L-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine Methyl Ester Hydrochloride (LL-VIII HCl). To a solution of LL-VII (18.76 g, 21.3 mmol) in ethyl acetate (30 m*l*), 3N hydrogen chloride in ethyl acetate (80 m*l*) was added. The solution, being allowed to stand at room temperature for 2 hr, was evaporated *in vacuo* and the oily residue crystallized by addition of ether. Yield 16.10 g (92.5%), mp 100—105°C, $[\alpha]_D^{25}$ +12.10° (*c* 1, methanol).

Found: C, 56.98; H, 6.24; N, 6.58%. Calcd for $C_{39}H_{49}N_4O_9S_2Cl$: C, 57.30; H, 6.04; N, 6.85%.

N^{α}-t-Butyloxycarbonyl-N^{δ}-tosyl-N^{δ}-benzyloxy-D-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-D-ornithine Methyl Ester (DD-VII). DD-VII was obtained by the same procedure as described above. Yield 90.3%, mp 63—69°C and $[\alpha]_D^{25}$ −1.4° (*c* 1, ethyl acetate).

Found: C, 60.01; H, 6.69; N, 6.03%. Calcd for $C_{44}H_{56}N_4O_{11}S_2$: C, 59.98; H, 6.41; N, 6.37%.

N^{δ}-Tosyl-N^{δ}-benzyloxy-D-ornithyl-N^{δ}-tosyl-N^{δ}-benzyloxy-D-ornithine Methyl Ester Hydrochloride (DD-VIII HCl). This compound was prepared from DD-VII by the same method as that described for the corresponding LL-dipeptide ester hydrochloride LL-VIII HCl.

Cyclo-di-N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithyl (LL-IX). This compound was prepared according to the general procedure.[3]

(a) From LL-VIII HBr: A solution of LL-VIII HBr (3.95 g, 4.59 mmol) in methanol (50 m*l*), previously saturated with dry ammonia at 0°C, was stored in a glass-stoppered bottle at room temperature for 2 days. A mass of crystals deposited was collected. The mother liquor was concentrated to dryness and crystalline residue was filtered with the aid of water. Recrystallization from methanol gave colorless crystals. Yield 1.9 g (54%), mp 197—199°C, $[\alpha]_D^{25.5}$ −31.5° (*c* 2, dimethylformamide).

Found: C, 61.15; H, 5.85; N, 7.48; S, 8.59%. Calcd for $C_{38}H_{44}N_4O_8S_2$: C, 60.94; H, 5.92; N, 7.48; S, 8.56%.

(b) From LL-VIII HCl: Similar treatment of LL-VIII HCl with ammonia gave LL-IX. Yield 47.7%, mp 174—175°C, $[\alpha]_D^{25.5}$ −31.3° (*c* 2, dimethylformamide).

Cyclo-di-N^{δ}-benzyloxy-L-ornithyl Dihydrobromide (LL-X 2HBr). To a solution of 36% hydrogen bromide - acetic acid (20 m*l*) and phenol (5 g) in a glass-stoppered bottle, LL-IX (1.88 g, 2.5 mmol) was added at room temperature. After stirring for 50 hr at room temperature, the solution was evaporated *in vacuo* at 30°C. The residual oil was crystallized by addition of ether. Crystals were filtered off and washed with ethanol and ether. Yield 0.78 g (52%). The product was recrystallized from water - ethanol, mp 183—186°C, $[\alpha]_D^{22}$ −12.0° (*c* 0.5, methanol).

Found: C, 47.51; H, 5.81; N, 9.10%. Calcd for $C_{24}H_{32}N_4O_4$ 2HBr: C, 47.85; H, 5.69; N, 9.30%.

Cyclo-di-N^{δ}-acetyl-N^{δ}-benzyloxy-L-ornithyl (LL-XI). LL-X 2HBr (0.78 g, 1.3 mmol) was mixed with pyridine (6.5 m*l*) and acetic anhydride (6.5 m*l*). After stirring for 20 hr at room temperature, the reaction mixture was evaporated to a syrup *in vacuo*. The residue was evaporated twice with water in order to decompose any acetic anhydride present. The final residue was taken up in ethyl acetate (30 m*l*) and the solution was repeatedly washed with water. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The residue was triturated with ether to yield a crude crystalline product, which was purified from ethanol - ether. Yield 68%, mp 97—99°C, $[\alpha]_D^{20}$ −16.4° (*c* 1, ethanol).

Found: C, 63.97; H, 6.86; N, 10.62%. Calcd for $C_{28}H_{36}N_4O_6$: C, 64.10; H, 6.92; N, 10.68%.

Cyclo-di-N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithyl; Rhodotorulic Acid (LL-I). A solution of LL-XI (0.46 g, 0.88 mmol) in methanol (25 m*l*) was hydrogenated in the presence of 5% palladium charcoal (0.75 g) for 20 hr at room temperature. After the addition of methanol (25 m*l*), the reaction mixture was warmed to 70°C and the catalyst was filtered off while hot. The filtrate was evaporated *in vacuo* at room temperature. The residual crystals were recrystallized from boiling water. Yield 0.2 g (66%), mp 217—218.5°C, (decomp.), $[\alpha]_D^{25}$ −28.8° (*c* 1, water).

Found: C, 48.81; H, 7.07; N, 16.18%. Calcd for $C_{14}H_{24}N_4O_6$: C, 48.83; H, 7.03; N, 16.27%.

The substance gave negative ninhydrin- and triphenyltetrazolium tests and positive ferric chloride test. NMR spectrum; Fig. 3, IR spectrum; Fig. 4.

DD-IX, DD-X 2HBr, DD-XI, and DD-I were prepared by the same methods as described for the preparation of the corresponding LL-isomers.

Cyclo-di-N^{δ}-tosyl-N^{δ}-benzyloxy-D-ornithyl (DD-IX). Yield 38.8%, mp 173—175°C, $[\alpha]_D^{25.5}$ +32.1° (*c* 2, dimethylformamide).

Found: C, 60.75; H, 5.90; N, 7.27%. Calcd for $C_{38}H_{44}N_4O_8S_2$: C, 60.94; H, 5.92; N, 7.48%.

Cyclo-di-N^{δ}-benzyloxy-D-ornithyl Dihydrobromide (DD-X 2HBr). Yield 33.4%, mp 183—185°C (decomp.), $[\alpha]_D^{22}$ +12.8° (*c* 0.5, methanol).

Found: C, 47.88; H, 5.64; N, 9.35%. Calcd for $C_{24}H_{34}N_4O_4Br_2$: C, 47.85; H, 5.69; N, 9.30%.

Cyclo-di-N^{δ}-acetyl-N^{δ}-benzyloxy-D-ornithyl (DD-XI). Yield

20.7%, mp 97—99°C, $[\alpha]_D^{20}$ −16.7° (*c* 0.3, ethanol).

Found: C, 63.93; H, 6.86; N, 10.74%. Calcd for $C_{28}H_{36}N_4O_6$: C, 64.10; H, 6.92; N, 10.68%.

*Cyclo-di-*N$^{\delta}$*-acetyl-*N$^{\delta}$*-hydroxy-*D*-ornithyl (*DD*-I)*; DD*-Isomer of Rhodotorulic Acid.* Yield 35%, mp 216—218.5°C (decomp.), $[\alpha]_D^{24}$ +28.0° (*c* 0.6, water).

Found: C, 48.72; H, 6.88; N, 16.05%. Calcd for $C_{14}H_{24}N_4O_6$: C, 48.83; H, 7.03; N, 16.27%.

*Catalytic Reduction of Rhodotorulic Acid (*LL*-I) to Cyclo-di-*N$^{\delta}$*-acetyl-*L*-ornithyl (*LL*-XII).* A suspension of LL-I (0.15 g, 0.44 mmol) in methanol (100 m*l*) was hydrogenated in the presence of Raney nickel (1 spatula) under atmospheric pressure at room temperature. After 6 days, the reaction mixture was warmed to 70°C in order to dissolve the precipitated product and filtered. The filtrate was evaporated *in vacuo* and the remaining crystals were recrystallized from boiling methanol. Yield 65 mg (48%), mp 270—275°C (decomp.), (lit,[1] mp *ca.* 265°C) and $[\alpha]_D^{25}$ −34.8° (*c* 0.5, water).

NMR (CF_3COOH): δ; 8.75 ppm (2H, side-chain amide protons), 8.33 (2H, ring-amide protons), 4.42 (2H, ring-carbon protons), 3.67 (4H, side-chain methylene protons adjacent to nitrogen), 2.52 (6H, acetyl methyl protons), 2.09 (8H, ethylene protons).

Found: C, 53.86; H, 7.95; N, 17.89%. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.83; H, 7.74; N, 17.94%.

N$^{\alpha}$*-t-Butyloxycarbonyl-*N$^{\delta}$*-benzyloxycarbonyl-*L*-ornithine (XIII).*

a): XIII was obtained as a syrup according to the same procedure for the D-isomer[4] from N^{δ}-benzyloxycarbonyl-L-ornithine using *t*-butyloxycarbonyl azide as *t*-butyloxycarbonylating reagent, except that magnesium oxide was used instead of sodium bicarbonate. Yield 41%, $[\alpha]_D^{25}$ +10.1° (*c* 1, ethyl acetate), (lit,[5] BOC-L-Orn(δ-Z)-OH, $[\alpha]_{578}^{18-25}$ −8.9° (*c* 1, acetic acid)).

b): XIII was also prepared according to the method of Broadbent *et al.*,[6] using *t*-butyl 2,4,5-trichlorophenyl carbonate. Yield 84%.

N$^{\alpha}$*-t-Butyloxycarbonyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine* p*-Nitrophenyl Ester (XIV).* This compound was obtained from XIII and *p*-nitrophenol according to the same procedure for the D-isomer,[4] except that ethyl acetate was used as the solvent. Yield 86%, mp 113—114.5°C (from ethyl acetate-petroleum ether), $[\alpha]_D^{25}$ −20.4° (*c* 1, ethyl acetate).

Found: C, 59.31; H, 6.29; N, 8.50%. Calcd for $C_{24}H_{29}N_3O_8$: C, 59.13; H, 6.00; N, 8.62%.

N$^{\delta}$*-Benzyloxycarbonyl-L-ornithine* p*-Nitrophenyl Ester Hydrochloride (XV).* To a suspension of XIV (9.74 g, 0.02 mol) in ethyl acetate (80 m*l*) was added 5N hydrogen chloride in ethyl acetate (40 m*l*). After 1 hr, crystals were collected with the aid of dry ether. Yield 8.3 g (98%), mp 164.5—167.5°C, $[\alpha]_D^{25}$ +27.3° (*c* 1, methanol).

Found: C, 53.74; H, 5.44; N, 10.05%. Calcd for $C_{19}H_{22}N_3O_6Cl$: C, 53.84; H, 5.23; N, 9.92%.

N$^{\alpha}$*-t-Butyloxycarbonyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine Methyl Ester (XVI).* *a)*: To a stirred mixture of XIII (9.86 g, 26.9 mmol) and Woodward reagent K (7.08 g, 28 mmol) in dimethylformamide (70 m*l*) was added triethylamine (4.1 m*l*, 30 mmol) at 0°C. After 2 hr, N^{δ}-benzyloxycarbonyl-L-ornithine methyl ester hydrochloride (8.51 g, 26.9 mmol) and triethylamine (4.1 m*l*) were added. The reaction mixture was stirred for 20 hr and then evaporated. The residual semi-solid was dissolved in ethyl acetate, and the solution was washed successively with water (three times), M sodium bicarbonate, water, 10% citric acid and water (three times), dried and evaporated. The oily residue was solidified by addition of petroleum ether. Recrystallization from ethyl acetate-petroleum ether gave XVI. Yield 10.9 g (67%), mp 73.0—74.5°C, $[\alpha]_D^{25}$ −10.7° (*c* 1, methanol).

Found: C, 60.98; H, 7.31; N, 8.89%. Calcd for $C_{32}H_{44}N_4O_9$: C, 61.13; H, 7.05; N, 8.91%.

b): To a stirred suspension of N^{δ}-benzyloxycarbonyl-L-ornithine methyl ester hydrochloride (7.03 g, 22.2 mmol) in chloroform (100 m*l*) was added triethylamine (4.48 g, 44.4 mmol) at 0°C. After 10 min, XIV (10.8 g, 22.2 mmol) was added. The mixture was stirred at 0°C for 15 min and at room temperature for 20 hr. Chloroform (100 m*l*) was added and the mixture was treated as described above except that the chloroform layer was washed with M sodium bicarbonate for the removal of the resultant *p*-nitrophenol. Yield 10 g (74%), mp 70—74°C, $[\alpha]_D^{25}$ −11.5° (*c* 1, methanol).

Found: C, 60.96; H, 7.20; N, 9.16%. Calcd for $C_{32}H_{44}N_4O_9$: C, 61.13; H, 7.05; N, 8.91%.

N$^{\delta}$*-Benzyloxycarbonyl-L-ornithyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine Methyl Ester Hydrochloride (XVII).* This compound was obtained through the same method as described above.

a): *From XVI-a*, mp 102—117°C, $[\alpha]_D^{25}$ +8.4° (*c* 1, methanol).

Found: N, 10.13%. Calcd for $C_{27}H_{37}N_4O_7Cl$: N, 9.92%.

b): *From XVI-b*, $[\alpha]_D^{25}$ +8.2° (*c* 1, methanol).

N$^{\alpha}$*-t-Butyloxycarbonyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine (XVIII).* To a stirred solution of XVI (6.28 g, 0.01 mol) in dioxane was added N sodium hydroxide (12 m*l*). The mixture was stirred overnight and acidified with 2N hydrochloric acid. The separated oil was extracted with ethyl acetate. Ethyl acetate layer was dried and evaporated to an oil. Yield 5.7 g (93%), $[\alpha]_D^{25}$ −3.5° (*c* 1, methanol).

Found: C, 60.08; H, 6.78; N, 8.86%. Calcd for $C_{31}H_{42}N_4O_9$: C, 60.57; H, 6.89; N, 9.12%.

N$^{\alpha}$*-t-Butyloxycarbonyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine* p*-Nitrophenyl Ester (XIX).* *a)*: A mixture of XVIII (3.15 g, 5.13 mmol) and di-*p*-nitrophenyl sulfite (1.07 g, 5.5 mmol) in pyridine was stirred at 0°C for 12 hr and at room temperature for 2 days. Resultant pale yellow solution was poured into N hydrochloric acid (150 m*l*). Semisolid precipitated was extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried and evaporated to give crystals. Yield 1.58 g (42%), mp 113.5—116°C, $[\alpha]_D^{25}$ −23.1° (*c* 1, methanol). Recrystallization from methanol-ether gave an analytical sample.

Found: C, 60.40; H, 6.28; N, 9.66%. Calcd for $C_{37}H_{45}N_5O_{11}$: C, 60.41; H, 6.12; N, 9.52%.

b): To a mechanically stirred mixture of XIII (7.7 g, 21 mmol) and XV (8.92 g, 21 mmol) in acetonitrile (200 m*l*), was added triethylamine (2.9 m*l*, 21 mmol) at 0°C. After 40 min, dicyclohexylcarbodiimide (4.4 g, 22 mmol) in a small portion of acetonitrile was added and the mixture was stirred overnight at room temperature. The deposits were collected and extracted with hot tetrahydrofuran (350 m*l*). The tetrahydrofuran was evaporated and the remaining crystals were collected with the aid of dry ether. Yield 7.21 g (47%), mp 116.5—118.5°C, $[\alpha]_D^{25}$ −26.2° (*c* 1, methanol).

N$^{\delta}$*-Benzyloxycarbonyl-L-ornithyl-*N$^{\delta}$*-benzyloxycarbonyl-L-ornithine* p*-Nitrophenyl Ester Hydrochloride (XX).* To a suspension of XIX (2.94 g, 4 mmol) in ethyl acetate (10 m*l*) was added 5N hydrogen chloride in ethyl acetate (28 m*l*). After 2 hr, the ethyl acetate was evaporated and the remaining crystals were collected with the aid of dry ether. Yield 2.36 g (88%),

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5) E. Schnabel, *Ann. Chem.*, **702**, 188 (1967).
6) W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc., C*, **1967**, 2632.

mp 124—128°C, $[\alpha]_D^{25}$ +27.3° (*c* 1, methanol).

Found: N, 10.53%. Calcd for $C_{32}H_{38}N_5O_9Cl$: N, 10.42%.

*Cyclo-di-*N$^{\delta}$*-benzyloxycarbonyl-*L*-ornithyl (XXI).* XX (2 g, 2.98 mmol) in the mixture of dimethylformamide (40 m*l*) and acetic acid (10 m*l*) was dropped over a period of 3 hr in the preheated pyridine (500 m*l*) at 70°C under vigorous stirring. Stirring was continued overnight at room temperature and the pyridine was evaporated. To the residue was added water and crystals were collected, 1.0 g (68%), mp 236—239°C (from dimethylformamide), $[\alpha]_D^{25}$ −19.5° (*c* 1, dimethylformamide). IR (KBr): 3325, 3175, 1690, 1550 cm^{-1}.

Found: C, 62.98; H, 6.32; N, 11.16; mol wt 470. Calcd for $C_{26}H_{32}N_4O_6$: C, 62.90; H, 6.50; N, 11.29%; mol wt 496.

*Cyclo-di-*L*-ornithyl Dihydrobromide (XXII).* XXI (10 g, 20.4 mmol) was treated with 20% hydrogen bromide in acetic acid (100 m*l*) with occasional shaking. A clear solution resulted and soon after a copious mass precipitated. After 6 hr, 20% hydrogen bromide in acetic acid (100 m*l*) was added again. After standing overnight, dry ether was added. The precipitate was collected and washed with ether. Yield 7.41 g (95%), mp 240—250°C (decomp.), $[\alpha]_D^{25}$ −28.1° (*c* 1, water).

Found: C, 30.36; H, 5.92; N, 13.80%. Calcd for $C_{10}H_{22}N_4O_2Br_2$: C, 30.78; H, 5.68; N, 14.36%.

*Cyclo-di-*N$^{\delta}$*-acetyl-*L*-ornithyl (XXIII).* *a)*: XXII (390 mg, 1 mmol) in pyridine (5 m*l*) was stirred at 50°C for 25 min. Thereafter acetic anhydride was added and stirring was continued for 5 hr at 50°C. The crystals were collected and washed successively with methanol, water and again methanol. Yield 250 mg (80%), mp 277—279°C (lit,[1] mp *ca.* 265°C), $[\alpha]_D^{21}$ −33.5° (*c* 1, water). IR (KBr): 3290, 3195, 3090, 1680, 1630, 1550 cm^{-1}.

Found: C, 53.89; H, 7.99; N, 17.75%. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.83; H, 7.74; N, 17.94%.

b): To a stirred suspension of XXII (390 mg) in pyridine (5 m*l*) was added triethylamine (0.28 m*l*). After 30 min, *p*-nitrophenyl acetate (724 mg, 4 mmol) was added at room temperature. After one day, the crystals were collected. Yield 190 mg (61%), mp 278—279°C (from methanol), $[\alpha]_D^{21.5}$ −34.6° (*c* 1, water).

c): To a stirred solution of sodium bicarbonate (168 mg, 2 mmol) in water (1 m*l*) was added XXII (390 mg). After 10 min, acetic anhydride (0.3 m*l*) was added at 0°C. The homogeneous solution was stirred for 10 min at 0°C and then for 90 min at room temperature. The deposited crystals were collected. Yield 120 mg (38%), mp 278—281°C (from methanol), $[\alpha]_D^{22}$ −36.2° (*c* 1, water).