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180. Akira Miyake: δ-Hydroxy-γ-oxo-L-norvaline, a New Antitubercular Antibiotic. (2). Synthetic Studies.*2

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In the preceding paper, δ-hydroxy-γ-oxo-L-norvaline was assumed for the structure of a new antibiotic, HON, and now the assumption was confirmed by synthesis.

First, according to the usual method of amino acid synthesis, ethyl ester of N-acetyl-α-ethoxycarbonyl-γ-oxo-DL-norvaline (III) was obtained from diethyl N-acetamidomalonate and bromoacetone, and bromination of (III) gave (IV). The bromine atom in (IV) was exchanged with hydroxyl or acetoxyl group and hydrolysis of the product gave δ-hydroxy-γ-oxo-DL-norvaline (VII). The detail of this synthesis is shown in Chart 1. When N-formyl or N-benzoyl derivative of diethylaminomalonate was used as the starting material, a similar reaction also occurred and this reaction sequence is shown in Chart 2.

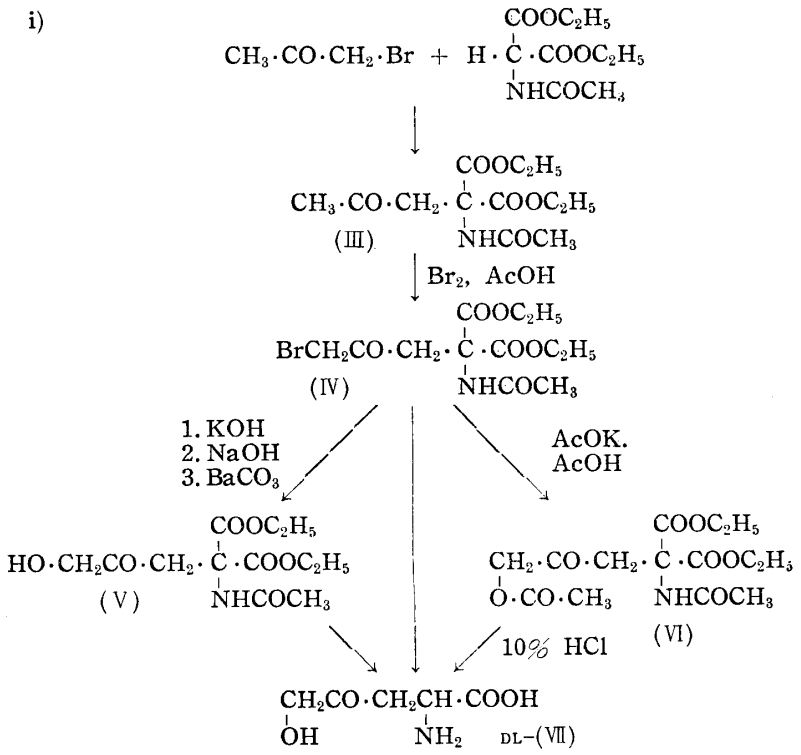

Chart 1.

Next, according to Harrington, *et al.*,[1] ethyl ester of N-acetyl-δ-chloro-γ-oxo-DL-norvaline (XVI) was obtained and hydrolysis of (XVI) gave DL-HON, whose details are shown in Chart 3. When tested *in vitro* against tubercle bacilli, DL-HON was half as active as L-HON obtained from the culture broth of *Streptomyces akiyoshiensis novo sp.* The infrared absorption spectrum of synthetic DL-HON was identical with that of the racemate derived from naturally occurring L-HON. As the optically active salt of DL-HON was difficult to crystallize, optical resolution was tried on the optically active salt of its intermediate.

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*2 This constitutes Part XXIX of a series entitled "Studies on Antibiotics" by S. Tatsuoka. Part XXVIII: This Bulletin, 8, 1071(1960).

1) C. R. Harrington, *et al.*: Biochem. J., **27**, 338(1933).

ii)

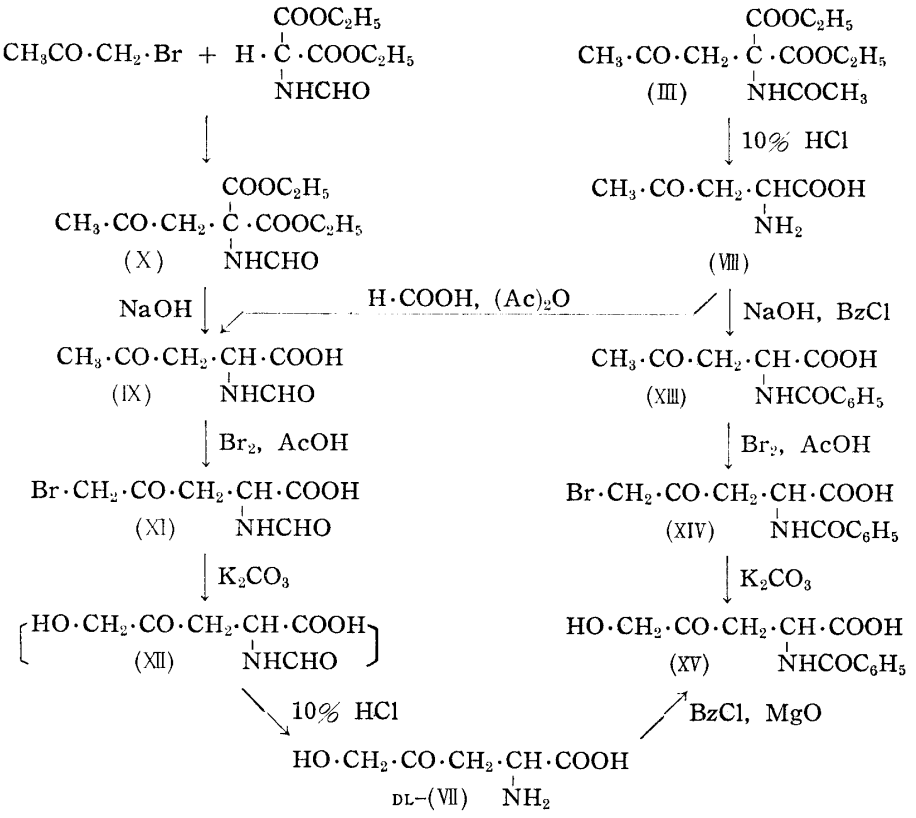

Chart 2.

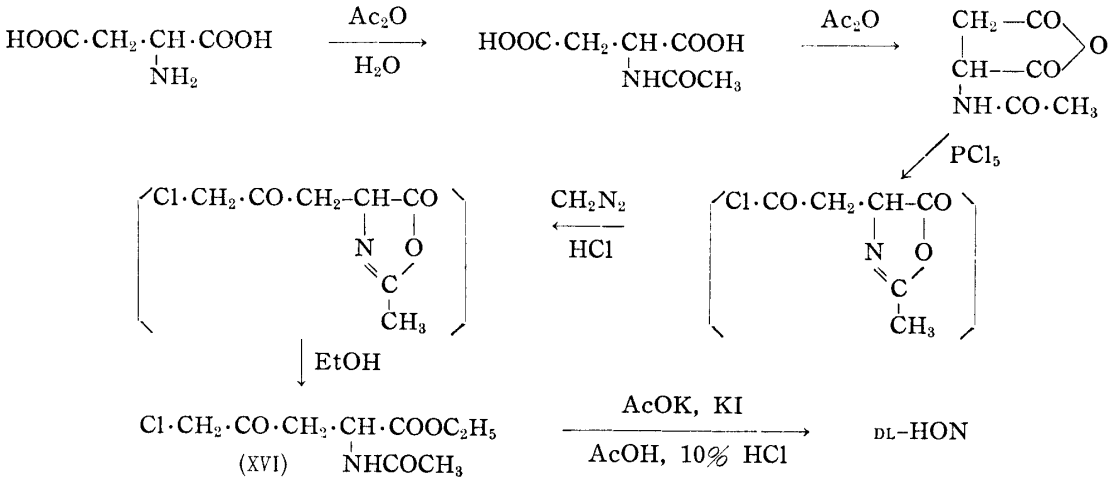

Chart 3.

N-Acetyl-δ-hydroxy-γ-oxo-DL-norvaline (XVII) was used as the intermediate. Of the brucine salts of D-(XVII) and L-(XVII), the brucine salt of L-(XVII) was more easily obtained because it is less soluble in ethanol. Brucine was removed from the L-(XVII) salt and hydrolyzed to give L-HON, $[\alpha]_D^{20}$ −6° (c=1, H_2O).

Infrared absorption spectrum and *in vitro* antitubercular activity of this compound were also identical with that of naturally occurring L-HON. DL-HON was obtained by deacetylation of N-acetyl-L-HON.

Experimental*3

Ethyl Ester of N-Acetyl-α-ethoxycarbonyl-γ-oxo-DL-norvaline (III)—To a solution of 4.8 g. of metallic Na in 960 cc. of EtOH, 434 g. of ethyl acetoamidomalonate was added and cooled to 10~15°. To the solution, 330 g. of bromoacetone in 660 cc. of EtOH was added dropwise, when the reaction proceeded exothermally. The reaction mixture was left standing overnight and then warmed for 0.5 hr. Crystals of NaBr separated in the solution were dissolved by addition of H_2O, EtOH was distilled off, and the resultant aqueous solution was extracted with AcOEt. The AcOEt solution was washed with H_2O and dried over Na_2SO_4. Distillation of the solvent and additon of Et_2O gave crystals which were recrystallized from EtOH to colorless plates, m.p. 103~105°. Yield, 273 g. (50%).

Ethyl Ester of N-Acetyl-α-ethoxycarbonyl-γ-oxo-δ-bromo-DL-norvaline (IV)—A solution of 37 g. of (III) in 685 cc. of AcOH was kept at 40~50° and 88 g. of Br_2 in 264 cc. of AcOH was added dropwise, whereby reaction proceeded with decolorization. AcOH was distilled off and the residue was extracted with AcOEt. The AcOEt solution was washed successively with H_2O, $NaHCO_3$ solution, and H_2O, and dried over Na_2SO_4. Distillation of AcOEt and addition of Et_2O gave crystals which were recrystallized from EtOH to colorless prisms, m.p. 95~96°. Yield, 102 g. (58%).

Ethyl Ester of N-Acetyl-α-ethoxycarbonyl-γ-oxo-δ-hydroxy-DL-norvaline (V)—A solution of 700 mg. of NaOH in 20 cc. of MeOH was cooled, 1 g. of ethyl formate was added, and heated for 2 hr. To the solution, 3.5 g. of the bromo derivative (IV) was added and refluxed for 16 hr. After cool, the separated NaBr was filtered off and the filtrate evaporated under a reduced pressure. The residue was extracted with 100 cc. of AcOEt, the AcOEt solution was washed with 20 cc. of H_2O, and dried over Na_2SO_4. Distillation of the solvent gave 1.5 g. of a yellow oil. Without further purification this oil was used as the starting material for the next reaction.

Ethyl Ester of N-Acetyl-α-ethoxycarbonyl-γ-oxo-δ-acetoxy-DL-norvaline (VI)—A solution of 12 g. of the ethyl ester (IV) and 4 g. of anhyd. AcOK dissolved in 36 cc. of AcOH was kept at 70~75° for 5 hr. with stirring, when KBr crystallized out. After cool, the reaction mixture was poured into 150 cc. of ice water, neutralized with $NaHCO_3$, and extracted with 300 cc. of AcOEt. The AcOEt solution was dried over Na_2SO_4 and evaporated. Addition of Et_2O to the resultant residue gave crystals which were recrystallized from EtOH to colorless needles, m.p. 105~106°. Yield, 9.5 g. (86%). *Anal.* Calcd. for $C_{14}H_{21}O_8N$: C, 50.75; H, 6.39; N, 4.24. Found : C, 50.49; H, 6.39; N, 4.14.

γ-Oxo-DL-norvaline (VIII)—A mixture of 137 g. of the ethyl ester (III) and 1400 cc. of 10% HCl was heated for 2 hr. in a water bath and HCl was distilled off *in vacuo*. Addition of Me_2CO to the resultant residue gave crystals of γ-oxo-DL-norvaline hydrochloride, m.p. 159~160° (decomp.).[2] Yield, 74 g. (93%). A solution of 55 g. of the crystals dissolved in a small amount of H_2O was neutralized with pyridine. Evaporation of H_2O and addition of EtOH gave crystals which were recrystallized from Me_2O–H_2O to colorless needles, m.p. 137° (decomp.). Yield, 34 g. (78%). *Anal.* Calcd. for $C_5H_9O_3N$: C, 45.79; H, 6.92; N, 10.68. Found : C, 45.76; H, 6.76; N, 10.84.

N-Formyl-γ-oxo-DL-norvaline (IX)—i) To a solution of 56.5 g. of (VIII) in 850 cc. of HCOOH, 282 cc. of Ac_2O was added dropwise. As the reaction proceeded exothermally slight cooling was continued so as to maintain the reaction mixture below 50°. After the addition, the reaction mixture was stirred for 1 hr. at 50°. Distillation of the solvent and addition of a small amount of EtOH gave crystals which were recrystallized from hot H_2O to colorless prisms, m.p. 141~142°. Yield, 77%. *Anal.* Calcd. for $C_6H_9O_4N$: C, 45.28; H, 5.70; N, 8.80. Found : C, 45.45; H, 5.82; N, 8.80.

ii) A solution of 7 g. of the ethyl ester (X) in 21 cc. of EtOH was cooled to 10~20°, a solution of 2 g. of NaOH in 4 cc. of H_2O and 20 cc. of EtOH was added dropwise, and kept standing for 2 hr. After dilution with H_2O, the reaction mixture was passed through a column packed with ion-exchange resin, Amberlite IR-120 (H), and Na^+ was adsorbed on the resin. The effluent was combined with the washing and concentrated to 50 cc. *in vacuo*. The concentrate was heated on a water bath, whereby decarboxylation occurred. After cool, the solution was concentrated to dryness and recrystallization of the residue from EtOH gave colorless prisms, m.p. 141~142°. Mixed fusion of (IX) produced via the two routes of (i) and (ii) did not show any depression in m.p.

Ethyl Ester of N-Formyl-α-ethoxycarbonyl-γ-oxo-DL-norvaline (X)—A mixture of 8.1 g. of metallic Na in 165 cc. of EtOH and 67.9 g. of ethyl formylamidomalonate was heated at 50~60° for 0.5 hr. under stirring, whereby the Na salt of ethyl formylamidomalonate crystallized out. While keeping the mixture at 10°~15°, 55 g. of bromoacetone in 110 cc. of EtOH was added dropwise. After standing overnight, the reaction mixture was refluxed for 0.5 hr., cooled to room temperature, and H_2O was added to dissolve the precipitated NaBr. After removing EtOH from the mixture, the aqueous solution was extracted with AcOEt, the AcOEt extract was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. Distillation of the residue gave 63 g. of an oil, b.$p_{0.2}$ 151~155°. Yield, 75%.

*3 All m.p.s are not corrected.

2) O. Wiss, *et al.* : Helv. Chim. Acta, **35**, 407(1952).

N-Formyl-δ-bromo-γ-oxo-DL-norvaline (XI)—A solution of 5 g. of (IX) in 25 cc. of AcOH was kept at 60~70° and 5.5 g. of Br_2 in 20 cc. of AcOH was added dropwise to the solution with stirring, when bromination occurred with decolorization. Stirring was continued for 2 hr. at 60°, AcOH was removed, and the residue was recrystallized from EtOH to give 2.3 g. of colorless crystals, m.p. 169°. Yield, 30%. *Anal.* Calcd. for $C_6H_8O_4NBr$: C, 30.27; H, 3.39; N, 5.88; Br, 33.57. Found : C, 30.57; H, 3.37; N, 5.98; Br, 32.62.

N-Formyl-δ-hydroxy-γ-oxo-DL-norvaline (XII)—A mixture of 7 g. of Na_2CO_3 added with stirring into 5.5 g. of (XI) in 550 cc. of H_2O was kept standing overnight. Cations in the reaction mixture were removed by treatment with Amberlite IR-120 (H) and the resultant HBr was removed with H_2O by distillation. Neutralization with pyridine and removal of the residual H_2O gave an amorphous product. Due to the difficulty of crystallization, this crude product was used as the starting material for the next reaction.

N-Benzoyl-γ-oxo-DL-norvaline (XIII)—Benzoylation of 50 g. of (VIII) in 500 cc. of H_2O with 500 cc. of 10% NaOH and 60 cc. of BzCl under Schotten-Baumann conditions gave 5.6 g. of colorless crystals, m.p. 137°. Yield, 80%. *Anal.* Calcd. for $C_{12}H_{13}O_4N$: C, 61.27; H, 5.57; N, 5.96. Found : C, 60.78; H, 5.67; N, 5.69.

N-Benzoyl-δ-bromo-γ-oxo-DL-norvaline (XIV)—A solution of 18 g. of Br_2 in 53 cc. of AcOH was added dropwise at 45~50° to 23.5 g. of (XIII) in 120 cc. of AcOH, when the reaction proceeded smoothly with decolorization. At the end of the reaction, AcOH was distilled off and Et_2O was added, whereupon colorless crystals separated out. Recrystallization from EtOH gave colorless plates, m.p. 174° (decomp.). Yield, 23 g. (73%). *Anal.* Calcd. for $C_{12}H_{12}O_4NBr$: C, 45.88; H, 3.83; N, 4.46. Found: C, 45.71; H, 3.83; N, 4.17.

N-Benzoyl-δ-hydroxy-γ-oxo-DL-norvaline (XV)—17 g. of Na_2CO_3 was added to 31.5 g. of (XIV) in 300 cc. of H_2O with stirring, and when the pH of reaction mixture reached 7, another 18 g. of Na_2CO_3 was added and the mixture was kept standing overnight. After being extracted with AcOEt the reaction mixture was acidified with HCl and extracted with AcOEt. This AcOEt solution was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The Et_2O-insoluble part of the residue was recrystallized from EtOH to colorless crystals, m.p. 130°. *Anal.* Calcd. for $C_{12}H_{13}O_4N$: C, 57.37; H, 5.22; N, 5.58. Found : C, 57.71; H, 5.30; N, 5.51.

δ-Hydroxy-γ-oxo-DL-norvaline (VII) (DL-HON)—i) A solution of 1.5 g. of the crude ethyl ester (V) dissolved in 20 cc. of 10% HCl was heated in a boiling water bath, when CO_2 evolved and the solution darkened. After 1 hr.'s heating, the mixture was evaporated to dryness *in vacuo* at a low temperature. The residue was dissolved in 10 cc. of H_2O, decolorized with activated charcoal, and neutralized with pyridine. Addition of 30 cc. of EtOH gave colorless needles. Three recrystallizations from Me_2CO-H_2O gave 300 mg. of colorless needles. Yield, 30% from crude (V). *Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found : C, 40.63; H, 6.20; N, 9.64. IR λ_{max}^{Nujol} μ: 3.10, 3.33, 3.45, 3.53, 3.82, 4.03, 4.30 (shoulder), 4.90, 5.80, 6.08, 6.22, 6.45, 6.60, 6.87 (shoulder), 6.98 (shoulder), 7.10, 7.30 (shoulder), 7.45, 7.88, 8.02, 8.25, 8.61, 8.78, 9.04, 9.27, 9.62, 10.12, 10.50, 10.78, 11.08, 11.65, 12.50, 14.30 (shoulder), 14.70.

ii) To a solution of 3.5 g. of the ethyl ester (IV) dissolved in 70 cc. of EtOH and stirred at room temperature, a solution of 0.88 g. of NaOH in 3.5 cc. of H_2O and 14 cc. of EtOH was added dropwise and the mixture was left standing overnight. The reaction mixture was heated for 8 hr. After cool, 70 cc. of H_2O was added to the mixture and the cation in the solution was removed by passing through a column of 20 cc. of Amberlite IR-120 (H). From the effluent an acid fraction was separated and concentrated to 20 cc. *in vacuo*. The concentrate was heated for 1 hr. with 10 cc. of conc. HCl in a boiling water bath and evaporated to dryness. The residue was treated as in (i) to give 140 mg. of needles. Yield, 10%.

iii) A solution of 3.5 g. of the ethyl ester (IV) and 12 g. of $BaCO_3$ in 30 cc. of H_2O was heated on a boiling water bath for 6 hr., during which CO_2 gas was introduced. The reaction mixture was filtered and then passed through a column of Amberlite IR-120 (H). The effluent and the washing of the column were combined and concentrated to 30 cc. *in vacuo*. A mixture of the concentrate and 10 cc. of 10*N* HCl was heated on a boiling water bath for 1 hr. and evaporated to dryness. Treatment of the residue as in (i) gave 150 mg. of colorless needles. Yield, 10%.

iv) A mixture of 3.5 g. of the ethyl ester (VI) and 33 cc. of 10% HCl was boiled in CO_2 atmosphere for 45 min. and evaporated *in vacuo* to dryness. Treatment of the residue in almost the same manner as in (i) gave 850 mg. of colorless needles. Yield, 54.5%.

v) A solution of 4 g. of (XII) dissolved in 30 cc. of 10% HCl was hydrolyzed as in (iv). Treatment of the reaction mixture as in (i) gave 200 mg. of colorless needles. Yield, 10%.

The colorless needles obtained from all above methods were compared with DL-HON derived from natural L-HON and their Rf values in paper partition chromatography (solvent system BuOH-AcOH-H_2O (4:2:1) 0.22; PrOH-AcOH-H_2O (50:5:45) 0.37), their behavior in paper electrophoresis (Theorell's buffer of pH 6.2, 350 v., 3.5 hr.), and their ultraviolet and infrared absorptions were identical.

Ethyl Ester of N-Acetyl-δ-chloro-γ-oxo-DL-norvaline (XVI)—This compound was synthesized according to the method of Harrington, *et al.*[1] DL-Aspartic acid was acetylated and then converted to aspartic anhydride with Ac_2O. The anhydride was changed to the acid chloride of azlactone with PCl_5. Action of CH_2N_2 and opening the azlactone ring with EtOH gave the expected product in the form of colorless needles, m.p. 121~122°. *Anal.* Calcd. for $C_9H_{14}O_4NCl$: C, 45.87; H, 5.99; N, 5.94; Cl, 15.05. Found : C, 46.16; H, 6.16; N, 5.67; Cl, 15.15.

DL-HON—A mixture of 1.2 g. of the ethyl ester (XVI) in 10 cc. of AcOH and 700 mg. of fresh AcOK was heated in a flask, equipped with a reflux condenser, in an oil bath for 2 hr. After cooling the mixture, the deposited KCl was filtered off and the filtrate was evaporated under a reduced pressure. The residue was extracted with AcOEt, the AcOEt solution was washed with $NaHCO_3$ solution, and dried over Na_2SO_4. Evaporation of the solvent gave 1.2 g. of a brown oil.

The oil was dissolved in 20 cc. of 10% HCl and heated for 1.5 hr. on a boiling water bath and then evaporated under a reduced pressure to dryness. Dissolution of the residue in 5 cc. of H_2O, decolorization with activated charcoal, neutralization with pyridine, and addition of 25 cc. of H_2O gave colorless needles. Recrystallization from Me_2CO-H_2O gave 200 mg. of purified needles. Yield, 27%. *Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found : C, 40.92; H, 6.21; N, 9.54. Infrared absorption, Rf value in paper partition chromatography, and behavior in paper electrophoresis (Theorell's buffer of pH 6.2, 350 v., 3.5 hr.) were identical with those of the DL-HON prepared through another route.

N-Acetyl-δ-hydroxy-γ-oxo-DL-norvaline (XVII)—A solution of 5 g. of the ethyl ester (V) in 20 cc. of H_2O was boiled in a flask equipped with a reflux condenser, when the evolution of CO_2 ceased in 10 min. After cool, the reaction mixture was decolorized with activated charcoal. Evaporation of the reaction mixture under a reduced pressure gave 4 g. of an oil (XVII). Crystallization of this oil was very difficult and it was identified as its brucine salt (XVIII).

Brucine Salt of N-Acetyl-δ-hydroxy-γ-oxo-L-norvaline Dihydrate (XVIII)—A solution of 4 g. of the above oil (XVII) in 10 cc. of EtOH and 10 g. of brucine base in 30 cc. of EtOH were mixed and heated. To the clear solution, 300 cc. of Et_2O was added, the precipitate that appeared was collected by decantation, and recrystallized from EtOH to 600 mg. of colorless plates, m.p. 118~119° (decomp.); $[\alpha]_D^{20}$ −5° (c=1, EtOH). Yield, 8.5%. *Anal.* Calcd. for $C_{30}H_{41}O_{11}N_3$: C, 58.15; H, 6.67; N, 6.78. Found: C, 59.06; H, 6.58; N, 6.84.

δ-Hydroxy-γ-oxo-L-norvaline (L-HON)—A solution of 600 mg. of the above brucine salt (XVIII) dissolved in 20 cc. of H_2O was passed through a column of 20 cc. of Amberlite IR-120 (H). The resin was washed with H_2O, the effluent and the washing were combined and concentrated to 20 cc. *in vacuo*. The concentrate and 1 cc. of conc. HCl were heated for 1 hr. on a boiling water bath. After cool, the solution was concentrated *in vacuo* to 3 cc., treated with activated charcoal, and neutralized with pyridine. Addition of 15 cc. of EtOH gave colorless needles. Recrystallization from Me_2CO-H_2O gave 30 mg. of needles, $[\alpha]_D^{20}$ −6° (c=1, H_2O). Yield, 10%. This crystal showed no definite m.p. Ultraviolet and infrared absorptions, Rf values in paper partition chromatography, and the behavior in paper electrophoresis of this compound were identical with those of naturally occurring L-HON.

Racemization of L-HON—A solution of 1 g. of L-HON in 5 cc. of AcOH and 5 cc. of Ac_2O was heated on a boiling water bath for 2 hr., whereby the Ninhydrin reaction became negative. After cool, the solution was evaporated *in vacuo* to dryness to give a dusky red oil. A mixture of this oil and 5 cc. of 10% HCl was heated for 1 hr. on a boiling water bath, cooled, 5 cc. of H_2O was added, and Cl^- was removed by 20 cc. of Amberlite IR-45 (OH). The resin was washed with 50 cc. of H_2O, the effluent and the washing were combined, and concentrated to 1 cc. *in vacuo*. The concentrate was decolorized with activated charcoal. Addition of a small amount of Me_2CO to the concentrate gave colorless needles. Recrystallization from Me_2CO-H_2O gave 100 mg. of purified needles, $[\alpha]_D^{22}$ 0° (c=3, H_2O). Yield, 10%. *Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found : C, 40.39; H, 6.28; N, 9.78. Infrared absorption, Rf value in paper partition chromatography, behavior in paper electrophoresis, and microbiological properties of this compound were identical with those of DL-HON synthesized through another route.

Summary

The structure of δ-hydroxy-γ-oxo-L-norvaline was proposed for the new antibiotic, HON. This compound was synthesized through two different routes. Optical resolution of the racemate was achieved by fractional crystallization of brucine salts of N-acetyl-δ-hydroxy-γ-oxo-DL-norvaline. Physicochemical and microbiological properties of the synthesized δ-hydroxy-γ-oxo-L-norvaline revealed that this compound was identical with naturally occurring L-HON.

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