

**179. Akira Miyake :**  $\delta$ -Hydroxy- $\gamma$ -oxo-L-norvaline, a New  
Antitubercular Antibiotic. (1). Structural Studies.\*<sup>2</sup>

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$\delta$ -Hydroxy- $\gamma$ -oxo-L-norvaline was first found in the culture broth of *Streptomyces akiyoshiensis novo sp.*<sup>1)</sup> and abbreviated as HON. It was isolated by strongly acidic ion exchanger chromatography and purified by recrystallization from acetone-water.<sup>2)</sup> HON inhibits the growth of pathogenic human tubercle bacilli, including streptomycin-resistant strains, but does not inhibit common gram-negative and -positive bacteria and fungi *in vitro*.<sup>3)</sup> It darkens at 80°, but does not show any definite melting or decomposition point. Qualitative tests show that HON has nitrogen but no halogen or sulfur. Elementary analysis and molecular weight measurement reveal that HON has a molecular formula of  $C_5H_9O_4N$ . The result of Van Slyke nitrogen determination, 10.02%, suggests that the nitrogen in the molecule is present as a primary amine,  $[\alpha]_D^{25} -8.2^\circ$  ( $c=3.4$ ,  $H_2O$ ).  $pK_a'$  2, 9.1. It colors yellowish brown with the Ninhydrin reagent. The  $R_f$  value in paper partition chromatography is 0.22 using butanol-acetone-water (4:2:1) as a solvent system. The ultraviolet absorption spectrum, as shown in Fig. 1, has a maximum at 271  $m\mu$  ( $\epsilon$  24). This datum suggests that HON has a non-conjugated carbonyl structure. The infrared absorptions at 5.05, 5.82, 6.12, 6.32, 6.75, and 7.12  $\mu$  suggest that HON is a kind of amino acid. No amino acid having a non-conjugated carbonyl group has been isolated from natural source and elucidation of the structure of HON was made as follows :

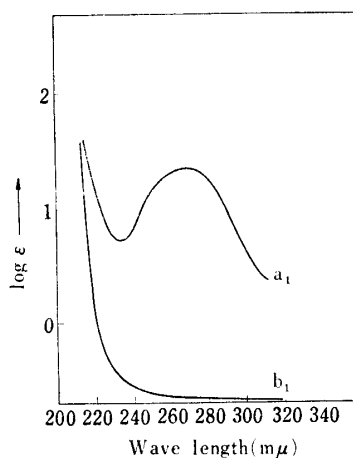


Fig. 1. Ultraviolet Absorption Spectra  
(in  $H_2O$ )

a : L-HON  
b : dihydro-L-HON

Hydrogenation of HON with platinum oxide and palladium charcoal resulted in absorption of one mole of hydrogen and a neutral amino acid,  $C_5H_{11}O_4N$ , was obtained. The absorption maxima at 271  $m\mu$  in ultraviolet spectrum and at 5.82  $\mu$  in infrared spectrum originating from carbonyl structure were not observed in the spectra of the hydrogenated compound. The yellowish brown color produced with the Ninhydrin reagent changed to violet by this hydrogenation. This color change also supports hydrogenation of the carbonyl group. Reaction of HON with sodium metaperiodate gave one mole each of L-

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\*<sup>2</sup> This constitutes Part XXVIII of a series entitled "Studies on Antibiotics" by S. Tatsuoka.

1) K. Kanazawa, *et al.* : Japan. Pat. Appl. No. 13461/1958.

2) *Idem* : Japan. Pat. Appl. No. 13462/1958.

3) *Idem* : Am. Rev. Respiratory Diseases, **81**, 924(1960).

aspartic acid and formaldehyde. The tentative structures (I or I') and (II or II') were forwarded for HON and dihydro-HON, respectively. To select the appropriate structure of dihydro-HON from the assigned formulae (II and II'), it was oxidized with sodium metaperiodate. From the compound of formula (II),  $\beta$ -formylalanine and formaldehyde are expected as the reaction products, while ammonia, formylacetic acid, and formaldehyde would be expected from formula (II'). Actually, formaldehyde and Ninhydrin-positive substance were obtained as the oxidation products. Although the latter product could not be isolated in a pure state, it was observed to behave as a neutral amino acid in paper ionophoresis (Theorell buffer at pH 6.25, 350 v, 4 hr.). This result supports the formulae (I) and (II) rather than (I') and (II'), and they were proved by syntheses as will be described in the next paper. As L-aspartic acid was obtained from oxidation products of HON, the configuration of optically asymmetric carbon in HON was found to be identical with that in naturally occurring L-aspartic acid.

N-Benzoyl-HON, m.p. 135~136°,  $\gamma$ -lactone of dihydro-HON hydrochloride, m.p. 211~214°, and  $\gamma$ -lactone of N-benzoyl-dihydro-HON, m.p. 172~173°, were prepared as derivatives of HON. The lactone configuration of these compounds was confirmed by elementary analyses and absorption at 5.63  $\mu$  in their infrared spectra.

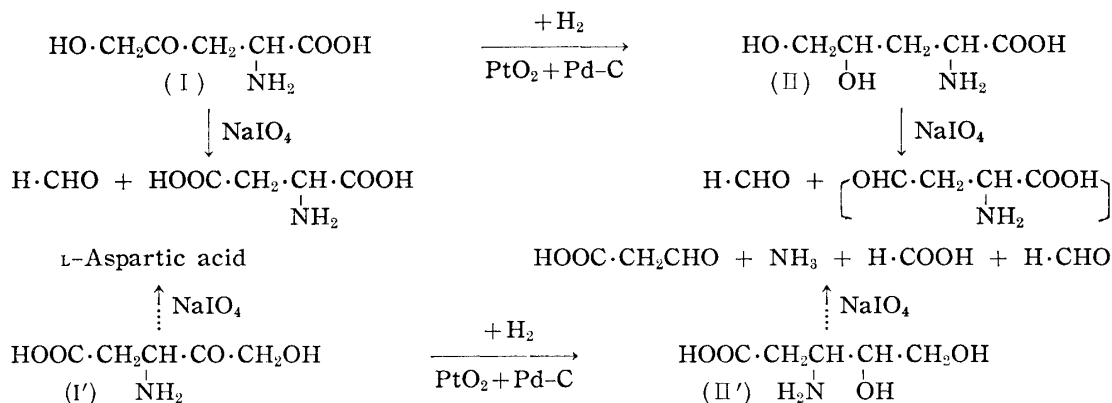


Chart 1.

### Experimental<sup>\*3</sup>

**Isolation of HON (I)**—*Streptomyces akiyoshiensis novo sp.* was cultivated at 28° for 4 days under aerated submerged conditions. A mixture of 25 L. of the cultured broth, 2.5 L. of saturated aqueous oxalic acid solution, and 20 g. of activated carbon was stirred for 1 hr. and filtered. The filtrate (pH 3.5) was passed through a column of 2 L. of Amberlite IR-120 (H<sup>+</sup>) at a flow rate of 1 L./hr., whereby the active principle was completely adsorbed on the resin. After washing with H<sub>2</sub>O, the column was eluted with 0.3N NH<sub>4</sub>OH at a flow rate of 150 cc./hr. The first 2 L. of the eluate contained no active principle, so it was discarded. On continuing elution, the pH of the eluate lowered to 3.5 and gradually became alkaline (pH 11). The second 2 L. of the eluate contained most of the active principle. The eluate was adjusted to pH 3.5 with HCl and concentrated *in vacuo* to 200 cc. The concentrate was decolorized with 2 g. of activated charcoal and diluted with 500 cc. of Me<sub>2</sub>CO, whereby the active principle crystallized out as light brownish needles. Recrystallization from Me<sub>2</sub>CO-H<sub>2</sub>O gave 10 g. of colorless needles. IR  $\lambda_{\text{max}}^{\text{Nujol}}$   $\mu$ : 3.13, 3.50, 3.73, 3.80, 4.08, 4.30 (shoulder), 5.05, 5.82, 6.12, 6.24, 6.32, 6.75 (shoulder), 6.88, 7.12, 7.22 (shoulder), 7.30, 7.50, 7.92, 8.17 (shoulder), 8.63, 8.78, 9.01, 9.23, 9.52, 10.28, 10.45 (shoulder), 10.89, 12.53 (shoulder), 12.68, 12.83, 14.02. Anal. Calcd. for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N: C, 40.81; H, 6.17; N, 9.52; mol. wt., 147.12. Found: C, 40.72; H, 5.91; N, 9.71; mol. wt. (titration), 144~150; (Barger's method), 175  $\pm$  25; (X-ray goniometry and density determination), 166.8  $\pm$  1.4.

**$\gamma,\delta$ -Dihydroxy-L-norvaline (II)**—Pd-C, prepared from 10 cc. of 1% PdCl<sub>2</sub> solution and 50.0 mg. of Norit A, and 200 mg. of PtO<sub>2</sub> were suspended in 25 cc. of H<sub>2</sub>O and hydrogenated with stirring until the absorption of H<sub>2</sub> had subsided. Then a solution of 300 mg. of HON (I) in 15 cc. of H<sub>2</sub>O was added

<sup>\*3</sup> All m.p.s are not corrected.

to the suspension and hydrogenation was continued for about 0.5 hr., whereby 60 cc. of  $H_2$  was absorbed. After filtration of the catalyst, the solution was evaporated *in vacuo*. Recrystallization of the residue from MeOH gave slightly hygroscopic colorless needles (II) (showing no definite m.p.). IR  $\lambda_{\text{max}}^{\text{KBr}}$   $\mu$ : 3.00, 3.28, 3.43, 4.28 (shoulder), 6.15, 6.63 (shoulder), 6.68, 7.14, 7.43, 8.10 (shoulder), 8.75 (shoulder), 9.08, 9.40, 9.75, 10.40, 11.50. *Anal.* Calcd. for  $C_5H_{11}O_4N$ : C, 40.26; H, 7.43; N, 9.39. Found: C, 39.54; H, 7.48; N, 9.14.

(II) and a small amount of conc. HCl were heated on a water bath for 0.5 hr. and concentrated *in vacuo*. Recrystallization of the residue from MeOH gave colorless crystals of the hydrochloride of  $\gamma$ -lactone of (II), m.p. 211~214° (decomp.). *Anal.* Calcd. for  $C_5H_{10}O_3NCl$ : C, 35.83; H, 6.01; N, 8.36; Cl, 21.16. Found: C, 36.03; H, 6.27; N, 8.33; Cl, 20.94.

**NaIO<sub>4</sub> Oxidation of HON (I)**—To a solution of 150 mg. of HON (I) in 5 cc. of  $H_2O$ , 500 mg. of NaIO<sub>4</sub> was added with stirring and the mixture was left standing overnight. On the following day, the reaction mixture was distilled *in vacuo* and the distillate was collected in a freezing trap. A mixture of 350 mg. of dimedone in 20 cc. of EtOH was warmed with the distillate on a water bath, when colorless crystals separated out. Recrystallization from EtOH gave needles, m.p. 186~187°. Yield, 185 mg. A mixture of this compound and an authentic sample of dimedone derivative of formaldehyde showed no m.p. depression.

To an aqueous solution of the distillation residue, an aqueous solution of  $(AcO)_2Cu$  was added, whereby a Cu salt precipitated out. The precipitate was collected by filtration and suspended in  $H_2O$ , and  $H_2S$  gas was introduced. After removal of CuS, the solution was concentrated and EtOH was added to the concentrate, whereby colorless plates crystallized out. *Anal.* Calcd. for  $C_4H_7O_4N$ : C, 36.09; H, 5.30; N, 10.52. Found: C, 35.98; H, 5.40; N, 10.61.  $[\alpha]_D^{20} + 23^\circ$  (c=1,  $NHCl$ ),  $+5^\circ$  (c=1,  $H_2O$ ).

The identity of this compound with L-aspartic acid was confirmed by comparing their IR spectrum and Rf values in paper partition chromatography.

**N-Benzoyl- $\delta$ -hydroxy- $\gamma$ -oxo-L-norvaline**—A mixture of 400 mg. of L-HON (I), 0.33 cc. of BzCl, 400 mg. of MgO, and 8 cc. of  $H_2O$  was stirred for 3 hr. with ice cooling. MgO was filtered off and the excess BzCl was removed by  $Et_2O$  extraction. The resultant solution was extracted with three 30-cc. portions of AcOEt. The AcOEt solution was washed with  $H_2O$ , dried over  $Na_2SO_4$ , and concentrated to 2 cc. *in vacuo*. 20 cc. of  $Et_2O$  was added to the concentrate, whereby a gummy substance separated out, which, after keeping in an ice box for 2~3 days, crystallized into colorless needles. Recrystallization from AcOEt-benzene gave crystals, m.p. 135~136°;  $[\alpha]_D^{20} - 15^\circ$  (c=1, EtOH). Yield, 100 mg. *Anal.* Calcd. for  $C_{12}H_{13}O_5N$ : C, 57.37; H, 5.22; N, 5.58. Found: C, 57.91; H, 5.24; N, 5.46.

**N-Benzoyl- $\delta$ , $\gamma$ -dihydroxy-L-norvaline  $\gamma$ -Lactone**—Benzoylation of (II) was carried out by the Schotten-Baumann method or with  $NaHCO_3$  and BzCl, and the reaction mixture was acidified and shaken with  $Et_2O$ . Concentration of the resultant aqueous solution gave colorless needles, m.p. 172~173°. *Anal.* Calcd. for  $C_{12}H_{13}O_4N$ : C, 61.27; H, 5.57; N, 5.96. Found: C, 61.08; H, 5.76; N, 5.90.

### Summary

*Streptomyces akiyoshiensis novo sp.*, which was isolated from a soil sample collected at Akiyoshido, Yamaguchi Prefecture, produced a new antibiotic,  $C_5H_9O_4N$ , active solely against pathogenic tubercle bacilli including strains resistant against streptomycin, INAH, and other hitherto known antituberculosis antibiotics and chemotherapeutics. This antibiotic was assumed to be a new neutral amino acid having a non-conjugated carbonyl group in the molecule and the structure of  $\delta$ -hydroxy- $\gamma$ -oxo-L-norvaline was proposed. From the chemical structure, the name HON was given to this new antibiotic.

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