The Synthesis of Four Optical Isomers of β-Hydroxyaspartic Acid

By Takeo KANEKO and Hakuji KATSURA

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In 1904 Skraup[1] suggested that he had found β-hydroxyaspartic acid in the hydrolyzate of casein, but in 1921 Dakin[2] reported that he found none of this amino acid in the hydrolyzate of casein, as compared with the *threo-* and *erythro*-β-hydroxy-DL-aspartic acid synthesized by him. Recently, *erythro*-β-hydroxy-L-aspartic acid ($[\alpha]_D+51°$)[3] has been obtained by the action of a transaminase on dihydroxyfumaric acid and L-glutamic acid. β-Hydroxyaspartic acid has been found in a culture solution of a certain sort of Azotobactor[4], and in a hydrolyzate of Phallicidine[5], a toxic substance isolated from *Amanita phalloides*. Some methods of synthesizing β-hydroxyaspartic acid[6] were reported a few years ago, but the absolute configurations of the optical isomers of this amino acid have never been determined. The present paper deals with the chemical determination of the absolute configurations of these optical isomers by the establishment of the configurational correlation between these isomers and glyceric acid.

Dakin[2] reported that *erythro-* and *threo*-β-hydroxy-DL-aspartic acids (DL-VI and II) were prepared by the ammonolysis of *erythro*-β-chloro-DL-malic acid (DL-IV)*. To determine the stereochemical behavior of this reaction, *cis*-[7] and DL-*trans*-epoxysuccinic acid[8] (I and DL-V) were treated with aqueous ammonia to obtain *threo-* and *erythro*-β-hydroxy-DL-aspartic acids (DL-II and VI) respectively[9]. From D_s-(−)-*trans*-epoxysuccinic acid (D-V)[8] and (−)-*erythro*-β-chloro-D_s-malic acid (D-IV)[8], which were derived from D_s-tartaric acid (III), the same *erythro*-β-hydroxy-L_s-aspartic acid ($[\alpha]_D$ +53°) (L-VI) was obtained. This acid was identical with that given by the enzymatic

1) Zd. H. Skraup, *Ber.*, **37**, 1596 (1904); *Z. physiol. Chem.*, **42**, 274 (1904); *Monatsh.*, **25**, 633 (1904).

2) H. D. Dakin, *J. Biol. Chem.*, **48**, 273 (1921); ibid., **50**, 410 (1922); cf. also E. Erlenmeyer, *Ann.*, **337**, 218 (1904); W. Lossen, *Ann.*, **348**, 306 (1906).

3) a) H. J. Sallach, *Federation Proc.*, **15**, 344 (1956); b) H. J. Sallach and T. H. Peterson, *J. Biol. Chem.*, **223**, 629 (1956); c) H. J. Sallach, ibid., **229**, 437 (1957); d) H. J. Sallach and M. L. Kornguth, *Biochim. Biophys. Acta*, **34**, 582 (1959).

4) N. F. Saris and A. I. Virtanen, *Acta Chem. Scand.*, **11**, 1440 (1957).

5) Th. Wieland, *Helv. Chim. Acta*, **44**, 919 (1961).

6) a) D. E. Metzler, J. B. Longenecker and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 639 (1954); b) M. L. Kornguth and H. J. Sallach, *Arch. Biochem. Biophys.*, **91**, 39 (1960); c) C. S. Franklin, *J. Chem. Soc.*, **1960**, 4709.

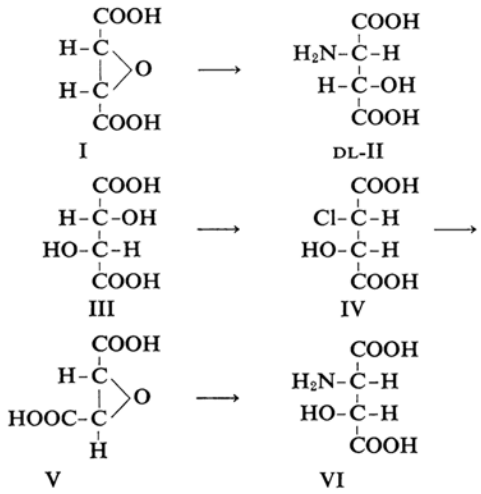
* This acid has been named "Chloromalic acid I" by Kuhn[8].

7) E. Weitz, H. Schobbert and H. Seibert, *Ber.*, **68**, 1163 (1935); O. Gawron, A. J. Glaid III, A. Lomont and S. Gray, *J. Am. Chem. Soc.*, **80**, 5856 (1958).

8) R. Kuhn and F. Ebel, *Ber.*, **58**, 919 (1925); R. Kuhn and R. Zell, ibid., **59**, 2514 (1926).

9) Cf. Y. Liwschitz, Y. Rabinsohn and A. Haber, *J. Chem. Soc.*, **1962**, 3589.

method[3]. These results show that epoxy acid (V) is the intermediate in the ammonolysis of chloromalic acid (IV) to amino acid (VI).

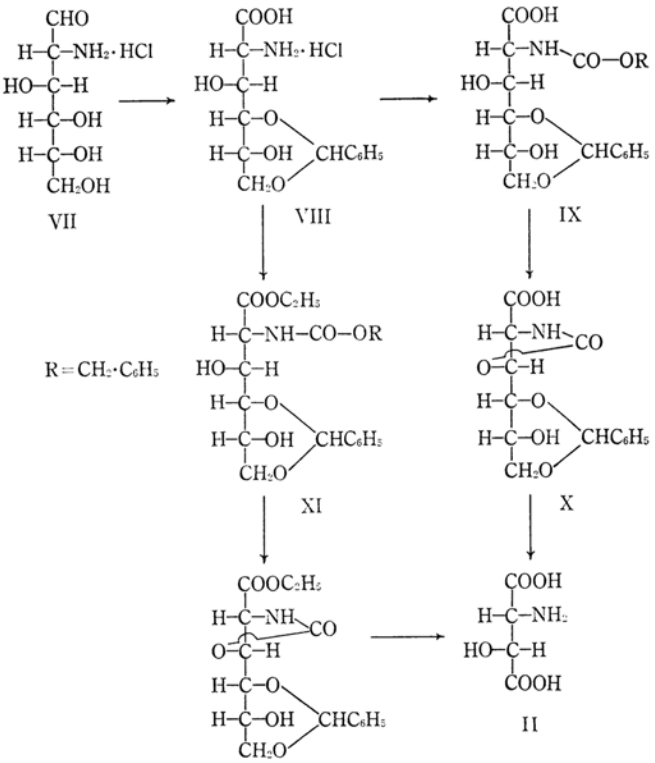


threo-β-Hydroxy-D$_s$-aspartic acid ($[\alpha]_D$ −1.2°) (D-II) was obtained from the oxazolidone derivative (X)[10] of 4,6-benzylidene-D$_g$-glucosaminic acid hydrochloride (VIII)[11], which was readily derived from D$_g$-glucosamine hydrochloride (VII), by hydrolysis of the benzylidene group and oxidation with periodic acid followed by bromine, and then by the hydrolysis of the oxazolidone ring. From ethyl 4,6-benzylidene-2-benzyloxycarbonylamido-2-deoxy-D$_g$-gluconate (XI)[11,12], which was derived from VIII, the *threo*-D$_s$-amino acid was also obtained by cyclization to the oxazolidone derivative followed by the oxidation and hydrolysis described above.

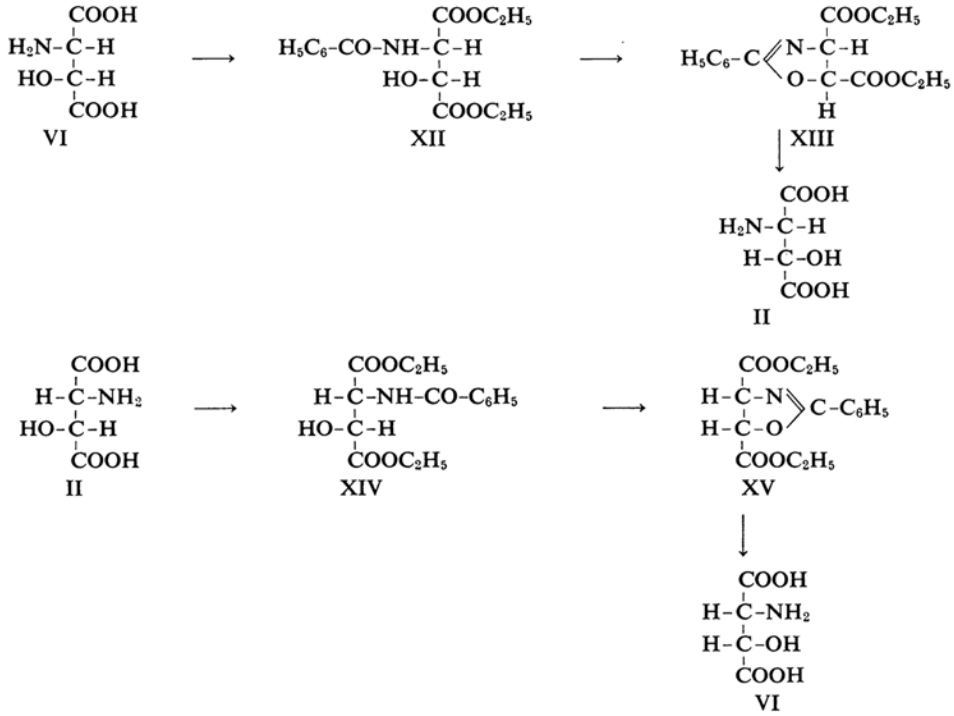
TABLE I. THE PHYSICAL AND PHYSIOLOGICAL NATURES OF β-HYDROXYASPARTIC ACID

Configuration	Taste (salty)	Specific rotation $[\alpha]_D$ in N-HCl	Specific rotation $[\alpha]_D$ in water
L-Erythro	Weak	+53.0°	+41.4°
D-Erythro	Very slightly	−49.2°	—
L-Threo	Slightly	+ 1.3°	− 8.9°
D-Threo	Tasteless	− 1.2°	+ 8.9°

threo-β-Hydroxy-L$_s$- and *erythro*-β-hydroxy-D$_s$-aspartic acids (L-II and D-VI) were obtained from *erythro*-β-hydroxy-L$_s$- and *threo*-β-hydroxy-D$_s$-aspartic acids (L-VI and D-II) via their



10) T. Kaneko and T. Inui, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 1075 (1961).
11) D. B. Hope and P. W. Kent, *J. Chem. Soc.*, **1962**, 3589.
12) P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, **21**, 345 (1915).



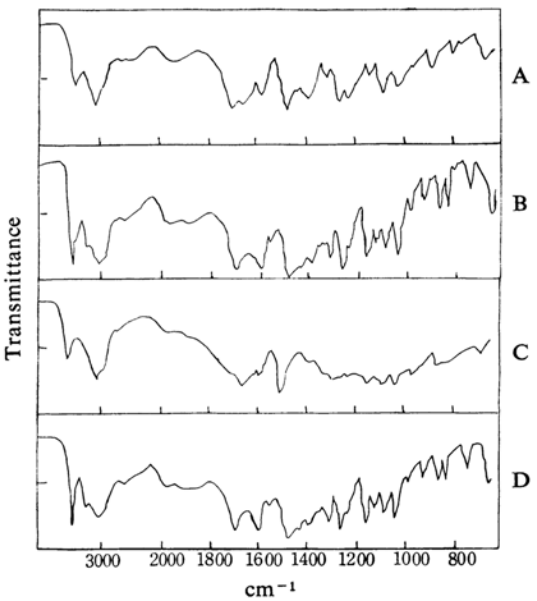


Fig. 1. Infrared spectra of (A) *threo*-β-hydroxy-DL-aspartic acid, (B) *erythro*-β-hydroxy-DL-aspartic acid, (C) *threo*-β-hydroxy-D- and L-aspartic acid and (D) *erythro*-β-hydroxy-D- and L-aspartic acid (in KBr disks).

oxazoline derivatives, XII and XV, respectively[13].

13) D. F. Elliott, *J. Chem. Soc.*, **1949**, 589; ibid., **1950**, 62; K. Pfister 3rd., C. A. Robinson, A. C. Shabica and M. Tishler, *J. Am. Chem. Soc.*, **71**, 1101 (1949).

The physical and physiological natures of optically-active β-hydroxyaspartic acids are summarized in Table I. In this table, it is shown that Lutz-Jirgensons' displacement rule[14] is applicable to these isomers.

The optically active and inactive isomers of β-hydroxyaspartic acid can not be identified by paper chromatography in various solvent systems. The infrared spectra of the isomers prepared in this paper are shown in Fig. 1.

Experimental

***threo*-β-Hydroxy-DL-aspartic Acid (DL-II) from *cis*-Epoxysuccinic Acid (I).**—A solution of 7.0 g. of I[7] in 70 ml. concentrated aqueous ammonia was heated in a sealed tube at 130°C for 18 hr. Crude DL-II was separated from the reaction mixture by concentration and then acidification with acetic acid, and recrystallization from water gave pure amino acid; yield, 8.6 g., 75%.

Found: C, 32.17; H, 4.76; N, 9.66. Calcd. for $C_4H_7O_5N$: C, 32.22; H, 4.73; N, 9.40%.

***erythro*-β-Hydroxy-DL-aspartic acid (DL-VI) from DL-*trans*-Epoxysuccinic Acid (DL-V).**—From 8 g. of DL-V[8], DL-VI was obtained in a similar manner; yield, 5.0 g., 55%.

Found: C, 32.11; H, 4.88; N, 9.03%.

***erythro*-β-Hydroxy-L-aspartic Acid (L-VI).**—*From D-(−)-trans-Epoxysuccinic Acid (D-V).*—From 6.0 g. of D-V[8], L-VI was obtained in a similar manner; yield, 4.9 g., 72%: $[\alpha]_D^{21}+53.2°$ (*c* 2.46,

14) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); ibid., **64**, 1221 (1931); ibid., **65**, 784 (1932).

1 N HCl); Found: C, 32.26; H, 4.83; N, 9.46%.

From erythro-β-Chloro-D$_8$-malic Acid (D-IV).—D-Chloromalic acid (D-IV)* was derived from dimethyl *erythro*-β-chloro-D$_8$-malate[15] by hydrolysis with 2.5N hydrochloric acid. A mixture of 10 g. of this acid and aqueous ammonia was heated in a sealed tube for 20 hr. and then concentrated in vacuo. The aqueous solution of this concentrate was treated through a Dowex 2 (OH-form) column and eluted with 2 N acetic acid. Crude L-VI was separated from the concentrate of this eluate and was then recrystallized from water to obtain the pure substance; yield, 5.5 g., 62%: $[\alpha]_D^{21}$ +53.0° (*c* 2.66, 1 N HCl); $[\alpha]_D^{21}$ +41.4° (*c* 2.43, water). Found: C, 32.23; H, 4.73; N, 9.17%. No pure *threo*-amino acid was separated by fractional crystallization from water or by partition chromatography through a Dowex 1 column by elution with 0.03 N formic acid[6b].

4, 6-Benzylidene-2-benzyloxycarbonylamido-2-deoxy-D$_8$-gluconic Acid (IX).—From 3.7 g. of VIII[11], IX was prepared in the usual manner; m. p., 167°C, $[\alpha]_D^{14}$ −51.0° (*c* 3.39, 99% ethanol); yield, 3.4 g., 83%. For analysis, pure substance obtained by recrystallization from ethyl acetate and petroleum ether and dried at 110°C for 22 hr. was used.

Found: C, 60.59; H, 5.52; N, 3.31. Calcd. for $C_{21}H_{23}O_8N$: C, 60.42; H, 5.52; N, 3.36%.

The Oxazolidone Derivative (X) of VIII.—*From IX.*—A mixture of 2.6 g. of IX and 1 g. of sodium hydroxide in 150 ml. of water was stirred continuously below 5°C for 6 hr. After the reaction mixture had been extracted with ether and the aqueous layer acidified with hydrochloric acid, the solution was extracted with ethyl acetate. Crude X (1.2 g., 62%) was separated by concentration of this extract and then recrystallized from ethyl acetate and petroleum ether to obtain the pure substance; m. p. 152°C, $[\alpha]_D^{14}$ −102.9° (*c* 3.15, methanol).

Found: C, 54.25; H, 4.92; N, 4.54. Calcd. for $C_{14}H_{15}O_7N$: C, 54.37; H, 4.89; N, 4.53%.

From VIII.—After a mixture of 25 g. of VIII, 25.5 g. of benzyloxycarbonyl chloride and 25 g. of sodium hydrogen carbonate in 400 ml. of water had been stirred at room temperature for 1 hr., a solution of 5 g. of sodium hydroxide in 20 ml. of water was added to this solution at 5°C and two solutions were stirred continuously together for 6 hr. Crude X (16.4 g., 78%) was obtained from the reaction mixture in a method similar to that described above.

***threo*-β-Hydroxy-D$_8$-aspartic Acid (D-II).**—*From X.*—A mixture of 3.0 g. of X and 30 ml. of 10% aqueous acetic acid was warmed in a boiling water bath for 1 hr.[16], and, on cooling, the reaction mixture was extracted with ether to remove the benzaldehyde. The aqueous layer was evaporated to dryness in vacuo, and the residue was dissolved in water. This solution was treated with 7 g. of a periodic acid dihydrate in 50 ml. of water and allowed to stand overnight at room temperature. The mixture was neutralized with 2 N sodium hydroxide, again set aside overnight, acidified to pH 4 by the addition of hydrochloric acid, and then treated with an aqueous barium chloride solution until no more salts were precipitated. A solution of 10 ml. of bromine in 90 ml. of a 2 N aqueous sodium hydroxide solution was added to the filtered solution. After standing overnight, the reaction mixture was acidified with hydrochloric acid and evaporated to dryness in vacuo. The residue was extracted with 99% ethanol, and the alcoholic extract was concentrated in vacuo. The residual oil was refluxed with 60 ml. of 6 N hydrochloric acid for 6 hr., and the solution was concentrated to dryness in vacuo. When the aqueous solution of the residual oil was treated with a Dowex 2 X8 (OH-form) column, elution with 2 N aqueous acetic acid and subsequent evaporation in vacuo gave crude D-II, yield, 1.1 g., 76%.

From XI.—An ethanolic solution (90 ml.) of 7 g. of XI[11,12] was added dropwise to a solution of 8 g. of sodium hydroxide in 100 ml. of 50% aqueous ethanol in an ice bath, and the mixture was stirred continuously for 7 hr. The reaction mixture was acidified with hydrochloric acid and concentrated to dryness in vacuo, and the residue was extracted with 99% ethanol. The extracts were concentrated in vacuo to a glassy solid (about 5 g.), and this solid was treated with 10% acetic acid, oxidized first with periodic acid, and then with bromine, and then hydrolyzed with hydrochloric acid. The product was isolated by the aid of a Dowex 2 column in the manner described above. The crude D-II weighed 1.5 g. (64%). Recrystallization from water gave the pure substance; $[\alpha]_D^{24}$ −1.2° (*c* 5.16, 1 N HCl), $[\alpha]_D^{28}$ +8.9° (*c* 0.91, water).

Found; C, 31.91; H, 4.71; N, 9.17. Calcd. for $C_4H_7O_5N$; C, 32.22; H, 4.73; N, 9.40%.

Diethyl *erythro*-β-Hydroxy-DL-aspartate Hydrochloride.—A suspension of 4.0 g. of DL-VI in 80 ml. of absolute ethanol was saturated with dry hydrogen chloride, and 125 ml. of dry ether was added. The crude crystals which separated were purified by recrystallization from absolute ethanol; yield, 3.3 g., 51%; m. p., 157~157.5°C (lit.[17]; m. p., 154~156°C).

Found; C, 39.54; H, 6.77; N, 5.52; Cl, 15.14. Calcd. for $C_8H_{16}O_5NCl$; C, 39.76; H, 6.67; N, 5.58; Cl, 14.70%.

Diethyl *N*-Benzoyl-*erythro*-β-hydroxy-DL-aspartate (DL-XII).—From 3.3 g. of the avove-mentioned ester hydrochloride, DL-XII was prepared in the usual manner and recrystallized from benzene and petroleum ether; yield, 3.0 g., 71%; m. p., 84°C.

Found; C, 58.29; H, 6.12; N, 4.46. Calcd. for $C_{15}H_{19}O_6N$: C, 58.24; H, 6.19; N, 4.53%.

Diethyl *N*-Benzoyl-*erythro*-β-hydroxy-L$_8$-aspartate (L-XII).—After 4.1 g. of L-VI had been esterified with absolute ethanol and dry hydrogen chloride, L-XII was obtained from the oily ester in the usual manner and recrystallized from benzene; yield, 5.4 g., 64%; m. p., 99~100°C; $[\alpha]_D^{16}$ +27.9° (*c* 4.52, 99% ethanol). Found: C, 58.52; H, 6.16; N, 4.45%.

Diethyl *N*-Benzoyl-*threo*-β-hydroxy-DL-aspartate (DL-XIV).—From 7.1 g. of DL-II, DL-XIV was

* M. p., 159°C, $[\alpha]_D^{21}$ −28.3° (*c* 3.19, ethyl acetate): literature[8]; m. p., 165~166°C, $[\alpha]_D$ −31°.

15) T. Kaneko and H. Katsura, *Chem. & Ind.*, **1960**, 1188; H. Katsura, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 98 (1961).

16) Cf. L. V. Vargha, *Ber.*, **68**, 18 (1935).

obtained in a similar manner and was recrystallized from benzene and petroleum ether; yield, 8.3 g., 56%; m. p., 101~102°C. Found: C, 58.55; H, 6.29; N, 4.40%.

Diethyl *N*-Benzoyl-*threo*-β-hydroxy-D_8-aspartate (D-XIV).—From 4.8 g. of D-II, D-XIV was obtained in a similar manner and was recrystallized from benzene; yield, 6.6 g., 62%; m. p., 121°C; $[\alpha]_D^{25}$ −21.2° (*c* 3.54, 94% ethanol). Found: C, 58.43; H, 6.17; N, 4.55%.

***threo*-β-Hydroxy-DL-aspartic Acid** (DL-II).—An oily oxazoline derivative[13] (DL-XIII) was obtained from 3.0 g. of DL-XII by the aid of 20 ml. of thionyl chloride. The crude oily DL-XIII was hydrolyzed by refluxing it with 6 N hydrochloric acid for 7 hr. The pure DL-II was obtained by a concentration of the hydrolyzate, followed by recrystallization from water; yield, 0.7 g., 48%.

Found: C, 32.05; H, 4.79; N, 9.00. Calcd. for $C_4H_7O_5N$: C, 32.22; H, 4.73; N, 9.40%.

***threo*-β-Hydroxy-L_8-aspartic Acid** (L-II).—From 9.0 g. of L-XIII, L-II was obtained in a similar manner via a crude oily oxazoline derivative (L-XIII) and purified by passing it through a Dowex 2 column, followed by recrystallization from water; yield, 3.6 g., 83%: $[\alpha]_D^{27}$ +1.3° (*c* 3.12, 1 N HCl); $[\alpha]_D^{27}$ −8.5° (*c* 1.76, water). Found: C, 32.33; H, 4.87; N, 9.43%.

***erythro*-β-Hydroxy-DL-aspartic Acid** (DL-VI).—A crystalline oxazoline derivative (DL-XV) was obtained from 3.0 g. of DL-XIV by the action of 20 ml. of thionyl chloride; it was purified by the same methods as have been described by Hauptmann[17]; m. p., 99°C (lit.; m. p., 97.5~99°C).

Found: C, 62.00; H, 5.97; N, 4.48. Calcd. for $C_{15}H_{17}O_5N$: C, 61.84; H, 5.88; N, 4.81%.

From a crude DL-XV derived from 8.3 g. of DL-XIV, DL-VI was obtained in a similar manner and was recrystallized from water; yield, 1.9 g., 39%.

Found: C, 32.26; H, 4.84; N, 9.63. Calcd. for $C_4H_7O_5N$: C, 32.22; H, 4.73; N, 9.40%.

***erythro*-β-Hydroxy-D_8-aspartic Acid** (D-VI).—From 6.4 g. of D-XIV, D-VI was obtained in a similar manner via an oily oxazoline derivative (D-XV) and was recrystallized from water; yield 0.7 g., 23%: $[\alpha]_D^{27}$ −49.2° (*c* 1.85, 1 N HCl). Found: C, 32.25; H, 4.70; N, 9.45%.

Department of Chemistry
Faculty of Science
Osaka University
Nakanoshima, Osaka

17) H. Hauptmann and H. Berl, *J. Am. Chem. Soc.*, **77**, 704 (1955).