paper sprayed with ninhydrin reagent (0.2% in 95 vol. of BuOH and 5 vol. of $2N$ AcOH) was dried rapidly in oven at 110°, β-aspartylglycopeptide always stained a specific blue color, otherwise, if at 80°, it was brown as reported by Marks, *et al.*[2]

The authors are very grateful to Prof. Izumiya, Faculty of Sciences, Kyushu University and his colleagues who gave us kindly pure sample of N-benzyloxycarbonyl-L-asparatic acid α-benzyl ester and N-benzyloxycarbonyl-L-aspartic acid β-benzyl ester and to Chugai Seiyaku Co., Ltd. for the supplying of glucosamine HCl. Thanks are due to the members of the Analysis Room of this Faculty for elemental and spectral analysis.

Summary

N-(L-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine has been prepared by the condensation of N-benzyloxycarbonyl-L-aspartic acid α-benzyl ester with 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucosylamine in the presence of dicyclohexylcarbodiimide followed by hydrogenolysis and de-O-acetylation on the resulting 1-(α-benzyl N-benzyloxycarbonyl-L-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose to remove the protecting groups.

N-(L-α-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine and N-(L-γ-glutamyl)-2-acetamido-2-deoxy-β-D-glucosylamine have been prepared by the same method as for β-aspartylglycopeptide.

(Received January 28, 1965)

(Chem. Pharm. Bull.
13(9)1046~1052(1965))

UDC 547.457.1.05

137. **Akira Yamamoto and Hisao Tsukamoto**: Studies on Amino Sugars. III.[*1] Isolation and Identification of Crystalline N-Acetylglucosamine-asparagine Compound from Ovalbumin.[*2]

(*Faculty of Pharmaceutical Sciences, Kyushu University*[*3])

In recent years some evidence has accumulated that the polysaccharide moiety of ovalbumin glycopeptide is attached to an aspartyl residue of peptide chain through N-acetylglucosamine.[1~6]

Concerning the chemical nature of the aspartic acid-glucosamine linkage, there are following three possibilities: (1) iminoether type,[3] the amide group of asparagine linked to C-1 of the glucosamine not through C-N-C but C-O-C bonds; (2) glucosaminylamine[1,2,5] type, an N-glucosaminide, involving the amide group of asparagine, analo-

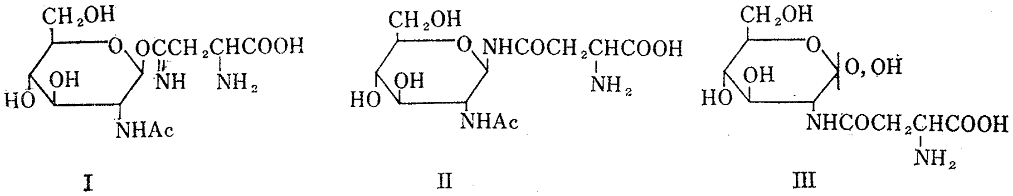
---

[*1] Part II: This Bulletin, 13, 1041 (1965).

[*2] Communicated in Biochem. Biophys. Res. Com., 15, 151 (1964).

[*3] Katakasu, Fukuoka (山本 陽, 塚元久雄).

1) F.R. Jevons: Nature, **181**, 1345 (1958).
2) R.H. Nuenke, L.W. Cunningham: J. Biol. Chem., **236**, 2452 (1961).
3) I. Yamashina, M. Makino: J. Biochem. (Tokyo), **51**, 359 (1962).
4) J.R. Clamp, L. Hough: Chem. & Ind. (London), 82 (1963).
5) G.S. Marks, R.D. Marshall, A. Neuberger: Biochem. J., **87**, 274 (1963).
6) F. Micheel, E.-A. Ostmann, G. Pielmeier: Tetrahedron Letters, No. 2, 115 (1963).



I II III

Chart 1. Proposed Structure of Carbohydrate-Protein Linkage in Ovalbumin

gous to glycinamide ribotide;[7] (3) N-acylglucosamine type,[6] peptide linkage of the β-carboxyl group of aspartic acid and the amino group of glucosamine.

During the cource of this study, Marks, *et al.*[5] have succeeded in the synthesis of N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (I) and also in the isolation of a substance behaving electrophoretically and chromatographically like compound (I) from partial hydrolysate of ovalbumin glycopeptide. After present work has been outlined in the previous communication,[*2] the reports of Yamashina, *et al.*[8] and Bogdanov,[9] *et al.* who obtained the same results, became available here. However, they have not isolated the product in pure, crystalline form.

In this paper we present the evidence of identity of chemically synthesized compound (I)[*1] with the crystalline compound resulting from the partial acid hydrolysis of ovalbumin glycopeptide, which was obtained by enzymatic degradation of ovalbumin.

Methods and Results

N-(L-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (I), N-(D-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (II) and N-(L-α-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (III) were synthesized by the method described in the previous papers in this series.[*1] Ovalbumin from fresh hens' eggs was prepared by the procedure of Warner[10] and recrystallized three times, dialyzed and lyophilized. The preparation was found to contain 2.01% of mannose and 1.29% of glucosamine as estimated by Johansen's modification[11] of the procedure of Winzler[12] and by the method of Rondle and Morgan,[13] respectively.

For the ascending paper chromatography on Toyo-Roshi No. 51 paper, a number of solvents were used. Solvent 1 : butanol-acetic acid-water=4:1:5; Solvent 2 : butanol-pyridine-water=3:1:1;[14] Solvent 3 : water-saturated phenol in an atmosphere of ammonia;[15] Solvent 4 : butanol-acetic acid-water=12:3:5.[5] The paper electrophoresis on Toyo-Roshi No. 51 paper (24×12 cm.) was accomplished using 5*N* AcOH[16] as solvent at 800 volts for 30 min. Amino acids and dinitrophenyl derivatives were estimated by means of two dimensional thin-layer chromatography using solvent system 5 and 6, butanol-acetic acid-water=4:1:1, phenol-0.08% ammonia=4:1 and solvent system 7 and 8, chloroform-methanol-acetic acid[17]=95:5:1, butanol-ethanol-water[18]=4:1:5,

---

7) R.A. Peabody, D.A. Goldthwait, G.R. Greenberg : J. Biol. Chem., **221**, 1071 (1956).
8) I. Yamashina, K. Ban-I, M. Makino : Biochim. et Biophys. Acta, **78**, 382 (1963).
9) V.P. Bogdanov, E.D. Kaverzneva, A.P. Andreyeva : *Ibid.*, **83**, 69 (1964).
10) R.C. Warner : The Proteins (Academic Press, Inc., New York), **2**, 435 (1952).
11) P.G. Johansen, R.D. Marshall, A. Neuberger : Biochem. J., **77**, 239 (1960).
12) R.J. Winzler : Meth. Biochem. Anal., **2**, 279 (1955).
13) C.J.M. Rondle, W.T.J. Morgan : Biochem. J., **61**, 586 (1955).
14) L. Hough, J.K.N. Jones, W.H. Wadman : J. Chem. Soc., **1950**, 1702.
15) S.M. Partridge : Biochem. J., **42**, 238 (1948).
16) H. Tsukamoto, A. Yamamoto : This Bulletin, **7**, 434 (1959).
17) M. Brenner, A. Niederwieser, G. Pataki : Experientia, **17**, 145 (1961).
18) P.W. Kent, G. Lawson, A. Senior : Science, **113**, 354 (1951).

respectively. Four spray reagents were used: ninhydrine; chlorine-iodide-starch;[19] periodate-benzidine; 3,6-dinitrophthalic acid.[20] Dinitrophenylation of amino acids and glycopeptide were done according to the method of Sanger, *et al.*[21]

Preparation of Glycopeptide

Ovalbumin, 94 g. was dissolved in 2 L. of water and adjusted to pH 7.5 by *N* NaOH. The protein was denatured by heating in a water bath at 100° for 5 min. with stirring. After cooling, to the suspension was added 2 L. of water containing 200 ml. of EtOH, 5.88 g. of calcium chloride and 0.4 g. of Pronase-P (Kaken Kagaku Co., Ltd., Tokyo) and the pH was adjusted to 8.0. The mixture was incubated at 37° for 2 days and *N* NaOH was added to maintain the pH at 8.0 at intervals. The reaction mixture was filtered and the filtrate was passed through a Dowex 50-X8 (H^+) column (3.2× 82 cm.) and then, eluted with desalted water until the orcinol test was negative. This was easily carried out by spot-testing. Thus, a portion of each fraction was spotted on a thin-layer plate of sillica gel G prepared by spray technique and orcinol reagent (1.6% orcinol-60% $H_2SO_4=1:7.5$) was sprayed. Heating of the plate at 110° for 10 min. in an oven revealed a spot of violet-red color.

By the paper chromatography in Solvent 1 of the eluate, it was confirmed that the removing of free amino acids and small peptides were complete. No spot was observed above Rf 0.03 on paper. Orcinol-positive fractions were collected and followed by immediate neutralization with Amberlite IRA-410 (HCO_3^-). The filtrate and washings were combined and evaporated under reduced pressure to 150 ml. at the temperature below 35°. After addition of nine volume of ethanol to this concentrate, it was allowed to stand overnight at 4° and the precipitates were collected by centrifugation and washed with ethanol and acetone. This material was submitted to above entire procedure three more times. Orcinol-positive fractions of the final procedure were applied to a Sephadex G-25 column (3.2×55 cm.) to remove further impurities, and eluted with desalted water at the rate of 25 ml. per hr. The eluate was collected in 10 ml. portions with the fraction collector and determined for mannose. The elution diagram showed a single, but broad peak. The fractions were collected and evaporated to dryness under reduced pressure to yield colorless clear syrup. By the addition of ethanol, it was solidified and filtered on the glass filter and washed with ethanol and acetone. The drying over phosphorus pentoxide yielded 2.14 g. of glycopeptide (Complex-P).

Characterization and Further Purification of Glycopeptide (Complex-P)

The mannose content of this glycopeptide was 55.1% and overall recovery was 62.7% of original mannose contained in ovalbumin. After hydrolysis in 1.5*N* HCl at 98° for 3 hr., it revealed to contain 2.8 moles of glucosamine per 5 moles of mannose. Hydrolysis in constant boiling HCl at 100° for 16 hr. in a sealed tube and successive dinitrophenylation of hydrolysate of Complex-P revealed the presence of 1.1 moles of aspartic acid, 0.3 moles of leucine per 5 moles of mannose and very traces of threonine by two-dimensional thin-layer chromatography in Solvent 5 and 6.

Complex-P was chromatographed on paper with Solvent 1, 2, and 3, but only one spot that did not move at the origin was observed. The glycopeptide, however, was separated into two bands by paper electrophoresis in 5*N* AcOH. A slower-moving, main band stained a dark blue color similar to asparagine, but the other one a purple

---

[19] H. N. Rydon, P. W. G. Smith: Nature, **169**, 922 (1952).
[20] T. Momose, U. Nakamura: Talanta, **10**, 115 (1963).
[21] F. Sanger, E. O. P. Thompson: Biochem. J., **53**, 353 (1953).

color by ninhydrine reagent. In this case, paper was rapidly heated in an oven at 110° for 5 min.

Then, aqueous eluate of both bands from paper was subjected to amino acid analysis after hydrolysis in constant boiling HCl at 100° for 16 hr. in a sealed tube. The thin-layer chromatography in Solvent 5 and 6 revealed that the main band contained aspartic acid as only amino acid and the other one aspartic acid, leucine and trace of threonine. The pronase digestion of the latter indicated to liberate a small amount of leucine to give a substance having the same mobilities and the same color as the main band by ninhydrin coloration.

Therefore, the problem arose whether a mixture of glycopeptide would be able to separate preparatively into above two components. The chromatography by Sephadex G-25 using 0.1*N* AcOH as elution solvent, according to the method of Marks, *et al.*,[22] charcoal-celite chromatography and DEAE-cellulose chromatography using sodium phosphate buffer, were therefore carried out, but all attempts were unsuccessful. By zone electrophoresis in starch using 5*N* AcOH, Complex-P was extensively decomposed.

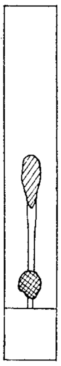


Fig. 1. Thin-layer Chromatogram of Complex-P

Methyl cellosolve-$CHCl_3$-H_2O (7.5:4:2.5)

Finally, the behavior of the glycopeptide in a number of solvents was examined in thin-layer chromatography. As a result, methyl cellosolve-chloroform-water=75:40:25 was found to be the most useful solvent, and Complex-P was found to separate into two spots although accompanied by some streaking. The lower and upper spot correspond to the slower and faster band in paper electrophoresis, respectively. This solvent system was also applied to column chromatography in silica gel. Complex-P, 30 mg., was applied to a column prepared from 6 g. of silica gel equilibrated thoroughly with above solvent and developed by the same solvent with an aid of air-compressor. Separation of the mixture occurred incompletely, but the hydrolysate of the slower eluate contained aspartic acid as only amino acids. The homogenity was confirmed by paper electrophoresis and thin-layer chromatography and hydrolysis revealed the presence of 0.93 moles of aspartic acid, and 2.9 moles of glucosamine per five moles of mannose, giving the same result as Marks, *et al.*[5] and Yamashina, *et al.*[8] This gave positive test for ninhydrin, orcinol and periodate-benzidine reagent, but negative for 3,6-dinitrophthalic acid,[20] which gives red-brown color with reducing sugar on paper.

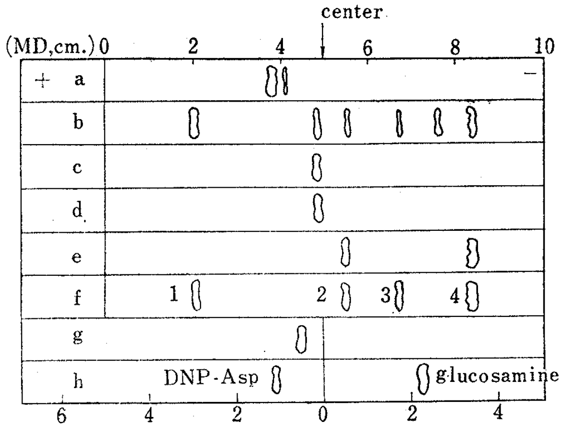


Fig. 2. Paper Electrophoresis

Solvent: 5*N* AcOH, 800 v., 30 min.
- a: Complex-P
- b: Hydrolysate of complex-P
- c: Natural compound (I)
- d: Synthetic compound (I)
- e: Hydrolysate of I
- f: Authentic compounds
  1. N-Acetylglucosamine and mannose
  2. Aspartic acid
  3. Leucine
  4. Glucosamine HCl

Solvent: Pyridine-AcOH buffer, pH 3.6, 19 mA., 1 hr.
- g: DNP-derivative of compound (I)
- h: Hydrolysate of DNP-(I)

Isolation and Identification of N-Acetylglucosamine-asparagine compound

---

[22] G. S. Marks, R. D. Marshall, A. Neuberger, H. Papkoff: Biochim. et Biophys. Acta, 63, 340 (1962).

This compound was isolated from partial hydrolysate of Complex-P. The condition of hydrolysis chosen was based on results of paper electrophoresis of hydrolysates, and spots colored with ninhydrin were estimated by densitometer. As a result of several experiments in acids and alkali, it was concluded that the substance corresponding to synthetic compound (I) was liberated in maximum yield by hydrolysis in $2N$ HCl at 100° for 25 min.

A solution of 1.9 g. of Complex-P in 114 ml. of $2N$ HCl was heated accurately for 25 min. in a boiling water bath. After ice-cooling the mixture was diluted with 400 ml. of ice-water, neutralized to pH 5.6 with 950 ml. of Amberlite IR-4B(OH). The filtrate and washings, 1.5 L., were reduced to 50 ml. under reduced pressure and passed through Dowex 50-X8 (H^+) column (0.9×12.5 cm.). The column was thoroughly washed with water to remove neutral sugars and followed by the displacement elution with $0.15N$ NH_3 and fractionated into 10 ml. portions.

The desired material colored to a characteristic blue color similar to synthetic compound (I) on paper which was developed with ninhydrin by heating at 110° for 5 min. This color was similar to that of asparagine and N-acetylglucosamine-L- and -D-asparagine compound, but quite distinguishable from N-acetylglucosamine-L-isoasparagine compound, the other amino acids and glucosamine. This distinction has been confirmed for α- and β-aspartyl-peptides by other workers.[23]

The desired material was mainly contained in neutral fractions, but it was partly in ammonia alkaline fractions. They were neutralized by addition of Amberlite IRC-50 (H^+) resin, which can not adsorb compound (I). The neutral fractions were combined and placed on an Amberlite IR-45 (Cl^-) column (1.6×15 cm.) and eluted with water to remove acidic substances. Finally, the aqueous eluate was passed through Amberlite IRC-50 (equilibrated by acetate buffer, pH 4.7,[24] 1.6×10 cm.) to remove basic substances. The fractions gave characteristic ninhydrin color were collected and evaporated under reduced pressure to a colorless clear syrup which crystallized from aqueous ethanol in a refrigerater to yield 75 mg. of colorless plates, m.p. 209~212° (decomp.). After two recrystallization from cold aqueous ethanol gave colorless plates, m.p. 215~222° (decomp.) as hydrate. It did not sharply melt, but turned brown at 205° and to caramel at 222°. Recrystallization from hot aqueous ethanol, analogous to synthetic compound*1 (I), raised the m.p. to 263~266° (decomp.) as anhydrous compound, $[\alpha]_D^{24}$ +24.6° (c=1.9, H_2O). *Anal.* Calcd. for $C_{12}H_{21}O_8N_3$: C, 42.98; H, 6.31; N, 12.53. Found : C, 42.66; H, 6.31; N, 12.05. Natural compound showed no depression of mixed melting point with synthetic one, but the mixed melting point with compounds (II) and (III) showed a depression. X-Ray powder diffraction patterns, infrared absorption spectra*1 were completely superimpossible. Paper chromatography and paper electrophoresis also indicated the identity of both compounds. 2,4-Dinitrophenyl derivatives of both compounds behaved chromatographically similar on paper and was hydrolysed to DNP-aspartic acid and free glucosamine in $2N$ HCl at 100° for 4 hr.

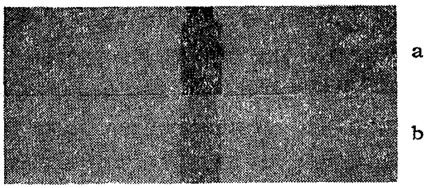


Fig. 3. X-Ray Diffraction Powder Patterns of N-(L-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (Trihydrate)

Powder method : Cu Kα radiation, Ni filter, Camera diameter 5.027×2 cm.
a) Natural compound
b) Synthetic compound

---

[23] W. J. LeQuesne, G. T. Young : J. Chem. Soc., 24 (1952); P. M. Bryant, R. H. Moore, P. J. Pimlott, G. T. Young : *Ibid.*, 3868 (1959).

[24] J. C. Winters, R. Kunin : Ind. Eng. Chem., **41**, 460 (1949).

From these observations it was concluded that N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine can be given for the structure of an isolated natural compound. Therefore, the present investigation leaves no doubt about the fact that an asparaginyl residues, which is a part of peptide chains, is attached through its amide group to reducing end of N-acetylglucosamine, which is a part of the oligosaccharide chain in ovalbumin glycopeptide.

Discussion

Present investigation was carried out in order to determine the nature of carbohydrate-protein bond in ovalbumin. The glycopeptide isolated from pronase digestion of ovalbumin was separated into two glycopeptides by paper eletrophoresis, thin-layer chromatography and silica gel chromatography. Analyses of these glycopeptides revealed the following molar composition : glucosamine, 3; aspartic acid, 1; mannose, 5 and glucosamine, 3; aspartic acid, 1; leucine, 1; mannose, 5, respectively. The fact that pronase digestion of the latter libelates a small amount of leucine to give the former glycopeptide indicates that the amino acid sequence around the oligosaccharide is aspartic acid-leucine or leucine-aspartic acid. Other workers also obtained the same results.5,8,9)

Although the structure of N-acetylglucosamine-asparagine compound obtained from partial hydrolysis of the glycopeptides was established for N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine by comparison of the properties with synthetic compound (1), Micheel, *et al.*6) found that the glycopeptides mixture obtained by hydrolysis with diethylaminoethanol was hydrolysed with $6N$ hydrochloric acid at 100° for 6 hr. and the suitably processed hydrolysate submitted to elution analysis showed the presence of the same elution peak as synthetic N-(L-β-aspartyl)-β-D-glucosamine. Furthermore, since hydrolysates of glycopeptides from serum γ-globulin and gelatin gave the same peaks in the elution effluent curve, it was surmised that such a linkage as N-acylglucosamine type is of general occurrence in glycoproteins.

Under the same condition with Micheel, *et al.*,6) our glycopeptides were extensively decomposed till the component of carbohydrates and amino acids and have no reducing power. Furthermore, in paper chromatography and paper electrophoresis of the hydrolysate, no spots gave a typical blue color, which was specific for a β-aspartamido linkage and was quite distinguishable from that of N-acetylglucosamine-isoasparagine compound, most amino acids and glucosamine when paper was developed with ninhydrin spray reagent. These observations ruled out the presence of such a structure as N-acylglucosamine type, stable in acid hydrolysis, in our glycopeptides.

Thus, the only possibility of the presence of such linkage in original ovalbumin may be explained by a surmise that glycopeptides having N-(L-β-aspartyl)-β-D-glucosamine-structure can be split by pronase at carbohydrate-protain linkage.

N-Acetylglucosamine-asparagine compound isolated in the present work was found to be quite resistant to Pronase-P, although Eylar25) and Kamiyama, *et al.*26) reported that pronase may split the carbohydrate-protein linkage in α_1-acid glycoprotein. No activity was observed in liver, kidney and testis homogenate of rabbit.

The authors are very grateful to the members of the Analysis Room of this Faculty for elemental and spectral analysis.

---

25) E.H. Eylar : Biochem. Biophys. Res. Com., 8, 195 (1962).
26) S. Kamiyama, K. Schmid : Biochim. et Biophys. Acta, 58, 80 (1962).

Summary

Ovalbumin was hydrolysed with Pronase-P, and the carbohydrate-containing fragments were isolated as two glycopeptides. From partial hydrolysate of glycopeptides in 2*N* hydrochloric acid, N-acetylglucosamine-asparagine compound was isolated in pure, crystalline state, and its structure was established for N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine.

(Received January 28, 1965)

Chem. Pharm. Bull.
13(9)1052~1064(1965)

UDC 615.782-011

138. **Yoshio Hamashima, Kazuo Tori, and Akira Takamizawa** : Camphane Derivatives. VII.[*1] Syntheses and Structure of 3-Methyl-3*a*β, 7*a*β-bornano[3,2-*d*]oxazolidin-2-one and its Derivatives.

(*Shionogi Research Laboratory, Shionogi & Co., Ltd.*[*2])

In the previous paper[1] the reaction of 3α-methylaminocamphor (I) with phosgene was reported to give two products, Ⅲ and Ⅳ, besides the main product N-methyl-3-camphorcarbamoyl chloride (Ⅱ). When the reaction mixture was left for a few days, Ⅱ could not be isolated, but Ⅲ was obtained as colorless needles, m.p. 82°, together with a small amount of Ⅳ. In the latter case, the product initially formed seems to be Ⅱ which is subsequently converted into Ⅲ by hydrochloric acid produced during the reaction. Actually, Ⅱ was converted into Ⅲ on gentle warming for a while in methanolic hydrochloric acid or in thionyl chloride.

The compound (Ⅲ), $C_{12}H_{18}O_2NCl$, is an isomer of Ⅱ. Since, in contrast to Ⅱ, Ⅲ does not react readily with amines, the character of the chlorine atom in Ⅲ is not of a carbamoyl chloride type. While the optical rotatory dispersion (ORD) spectrum of Ⅱ exhibits a positive Cotton effect, Ⅲ gives only a negative plain curve, although a strong carbonyl band at 1760 cm^{-1} still appears in the infrared spectrum of Ⅲ. The proton magnetic resonance (NMR) spectrum of Ⅲ shows a slightly doubling doublet at 5.74 τ ($J=4.2$ and 1.0 c.p.s.) due to the proton on the nitrogen- or oxygen-bearing carbon atom and a triplet-like signal at 7.12 τ ascribable to the proton on the bridgehead C-4 in a bornane skeleton. This implies that the proton giving the doublet is attached at the C-3 position and has an *exo* configuration (*exo* C-3). If this proton is postulated to be either *endo* C-3, *exo* C-2, or *endo* C-2, its signal should appear as a broad singlet, a sharp doublet, or a sharp singlet, respectively.[2~5] Thus Ⅲ may be formulated as a bornane derivative *cis*-fused with an oxazolidinone ring, that is, 3-methyl-7*a*-chloro-3*a*β,7*a*β-bornano[3,2-*d*]oxazolidin-2-one. The *cis* ring juncture is probably preferable

---

[*1] Part Ⅵ. T. Takahashi, H. Fujimura, Y. Hamashima : Yakugaku Zasshi, **84**, 918 (1964).

[*2] Sagisu, Fukushima-ku, Osaka (浜島好男, 通 和夫, 高見沢 映).

1) T. Takahashi, H. Fujimura, Y. Hamashima : Yakugaku Zasshi, **84**, 579 (1964).

2) W.D. Kumler, J.N. Shoolery, F.V. Brutcher, Jr. : J. Am. Chem. Soc., **80**, 2533 (1958); see also, J.D. Connolly, R. McCrindle : Chem. & Ind. (London), **1965**, 379.

3) F.A.L. Anet : Can. J. Chem., **39**, 789 (1961).

4) T.J. Flautt, W.F. Erman : J. Am. Chem. Soc., **85**, 3212 (1963).

5) For a review, see S. Sternbell : Rev. Pure and Appl. Chem., **14**, 15 (1964).