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Selective Reduction of Peptide-ester Groups in Aqueous Solution[1]

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For the selective reduction of carboxyl groups in peptides, diborane and sodium borohydride were examined. Because of accompanying reductive side reaction, the reduction of carboxyl groups with diborane in tetrahydrofuran was unsatisfactory. On the other hand, by the use of sodium borohydride in aqueous solution, certain model peptide esters were easily reduced to corresponding alcohols without any appreciable side reaction.

The selective reduction of carboxyl groups in peptides and proteins to corresponding alcohols directly or *via* esters, in connection with the structural and enzymatic studies of proteins, has been tried by many groups of organic chemists.[3] The interpretation of such studies, however, is still hindered by the fact that few reagents have absolute specificity for carboxylic groups in peptides and proteins, whereas many powerful reducing agents are available. In the present paper, some model peptides and peptide-esters were subjected to reaction for the trial on selective reduction of carboxylic ester groups.

Reduction of Peptides with Diborane

Carboxylic acids are generally considered to be relatively resistant to reducing agents. Since Brown, *et al*, however, demonstrated that diborane is particularly effective for the reduction of carboxyl groups to corresponding alcohols even in the presence of other functional groups,[4] it was thought advisable to apply the diborane reduction to peptide chemistry.

As a preliminary work, N-acetyl-DL-phenylalanine (Ia) and N-benzoyl-DL-alanine (Ib) were treated with 1.1 equivalents of diborane in tetrahydrofuran at room temperature for 2 hr. Although the expected reduction of carboxyl groups was occurred without any appreciable side reaction, the yields of N-acetyl-DL-phenylalaninol (IIa) and N-benzoyl-DL-alaninol (IIb) were only 38% and 38.5% respectively, and about one half of starting materials was recovered. In the reaction with 3 equivalents of diborane, as shown in Table I, considerable amounts of the fully reduced product (IIIa or IIIb) and the aminoborane compound (IVa or IVb), in addition to the N-acyl amino alcohol (IIa or IIb) were isolated.

Table I. Yields of Reduction Products[a] (%)

From Ia			From Ib		
IIa	IIIa	IVa	IIb	IIIb	IVb
49	20	17.5	33.5	28	36.5

a) reagent, 3 moles (BH_3); time, 2 hr

On the basis of its chemical and spectral properties the aminoborane compound (IVb) from Ib is formulated as N-borylbenzyl-DL-alaninol. Compound IVb was treated with hydro-

1) The preliminary communication of this paper appeared in *Tetrahedron Letters*, **1968**, 3575.
2) Location: *Kita-12, Nishi-6, Sapporo.*
3) J.P. Greenstein and M. Winitz, "Chem. of Amino Acids," J. Wiley, N.Y., 1960, p. 1586.
4) H.C. Brown and W. Korytnyk, *J. Am. Chem. Soc.*, **82**, 3866 (1960).

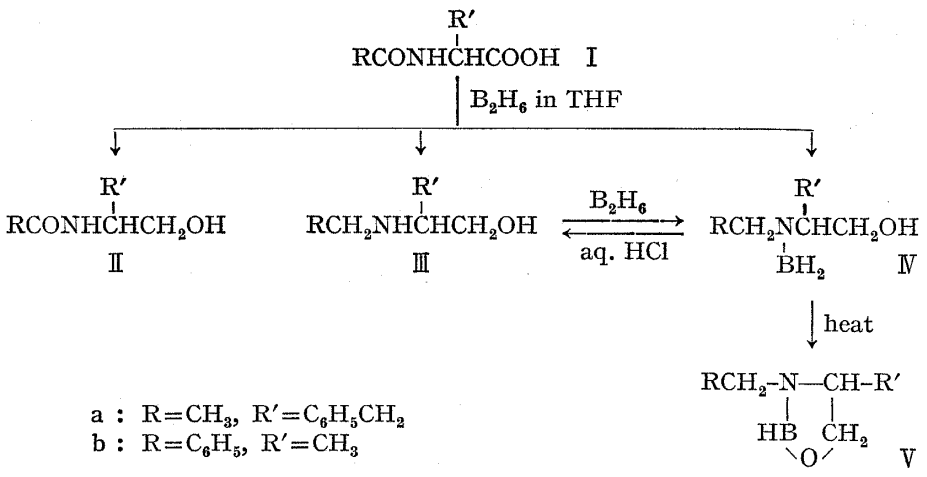

Chart 1

chloric acid to yield IIIb, which was readily reconverted to IVb by the treatment with diborane in tetrahydrofuran at room temperature. The hydroxyl group, in the infrared spectrum, is easily assignable and very strong bands of BH_2 appear at 2382 and 2362 cm^{-1}. In the mass spectrum, the parent peak, however, does not appear, while the highest molecular ion peak at 175 arises by loss of H_2 from the parent molecule (Fig. 1). It seemed reasonable to assume that IVb was decomposed to yield 3-benzyl-4-methyl-1,3,2-oxazaborolidine (Vb) on heating under highly reduced pressure. Further work on the chemistry of V are in progress. The structure of IVa was determined in a same manner. Because of these accompanying reductive side reaction, yield of the reducton of carboxyl group was unsatisfactory.

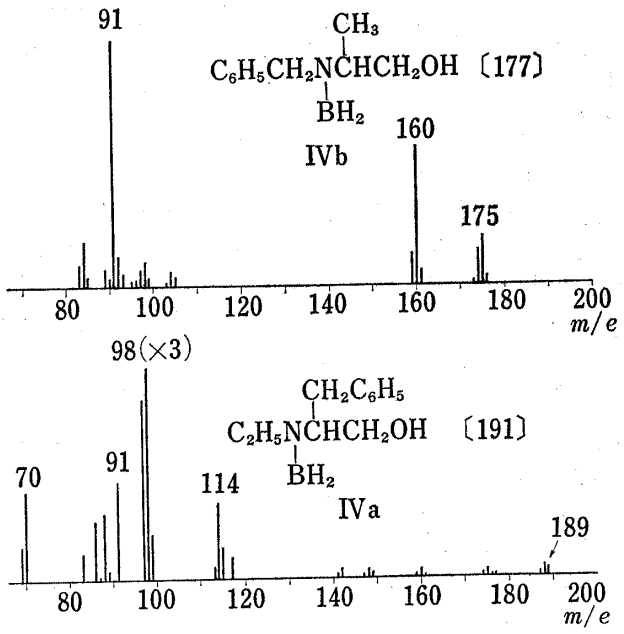

Fig. 1. Mass Spectra of N-Borylalkyl Amino Alcohols

Further, N-benzoylglycyl-DL-alanine (VI) was subjected to the reduction with diborane, followed by acid hydrolysis with 6N hydrochloric acid, and the resultant products were converted to a mixture of N-trifluoroacetyl amino acid butyl ester (VII), which was analyzed by gas chromatography in the manner described by Gehrke, *et al.*[5] The recovery of amino acids which were calculated from comparison with the internal standard of N-trifluoroacetyl isoleucine butyl ester was shown in Table II.

As amount of the reagent increases, not only the recovery of the C-terminal alanine, but that of non-terminal glycine decreases apparently as a result of non-specific reduction.

Atassi and Rosenthal reported on the specific reduction of carboxyl groups in some dipeptides[6] and proteins[7] with diborane in tetrahydrofuran. However, in view of the above

5) W.M. Lamkin and C.W. Gehrke, *Anal. Chem.*, 37, 383 (1965); D.L. Stalling, G. Gille and C.W. Gehrke, *Anal. Biochem.*, 18, 118 (1967).
6) A.F. Rosenthal and M.Z. Atassi, *Biochem. Biophys. Acta*, 147, 410 (1967).
7) M.Z. Atassi and A.F. Rosenthal, 7th Intern. Cong. of Biochem., Abs., 1967, p. 614.

Table II. Recovery of Amino Acids

Mole (BH_3)	Gly (%)	Ala (%)	Time (min)	Gly (%)	Ala (%)
1	93	66	10	83	64
2	54	15	30	76	50
3	50	14	60	67	43
	time, 2 hr			reagent, 3 moles	

data as well as the fact that amide groups can be easily reduced with diborane,[8] and that such an organic solvent as tetrahydrofuran is usually used for the reaction,[9] it may be suggested that careful selection of reaction condition is required for specific reduction of carboxyl groups of peptides[5] and proteins[6] with diborane.

Selective Reduction of Peptide Esters with Sodium Borohydride

A number of attempts were made to develop the reduction of ester groups in protein esters with lithium aluminium hydride or lithium borohydride in organic solvents[3] and few in aqueous methanol,[10] more or less accompanied by simultaneous reduction of peptide bonds. A possible means for obtaining the selective reduction at protein ester groups might be the use of a reagent which have more proper activity for the reduction of ester groups in aqueous medium.

It has been generally accepted that carboxylic esters are unaffected by sodium borohydride. However, Rapoport, *et al.* showed that large excess of reagent can bring about the reduction,[11] while Yamada, *et al.* reported that amino acid esters can be reduced in water-containing media.[12] Since we also occasioned to have related observation in the course of our studies on the reaction of peptide in aqueous solution, a study was undertaken to explore the desired selective reaction.

As a preliminary experiment, N-benzoylglycyl-DL-alanine ethyl ester (VIII) or N-benzoyl-DL-alanylglycine ethyl ester (IX) was treated with ten fold excess of sodium borohydride in aqueous solution at room temperature for 1.5 hr. The recovered amino acids was examined by paper chromatography. The color yield of Ninhydrin reaction indicated that about 70%

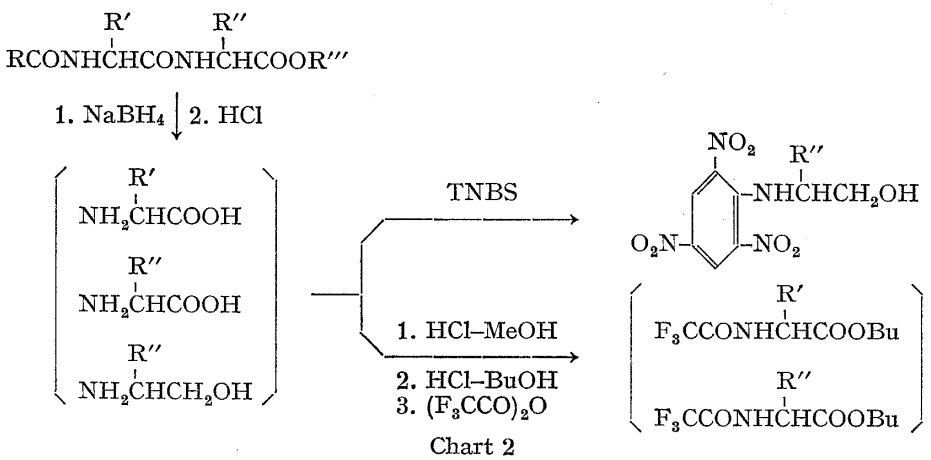

Chart 2

8) H.C. Brown and P. Heim, *J. Am. Chem. Soc.*, **86**, 3566 (1964); M.J. Kornet, P.A. Thio and S.I. Tan, *J. Org. Chem.*, **33**, 3637 (1968).
9) H.C. Brown, "Hydroboration," Benjamin, N.Y., 1962.
10) J.C. Crawhall and D.F. Elliott, *Biochem. J.*, **61**, 264 (1955).
11) M.S. Brown and H. Rapoport, *J. Org. Chem.*, **28**, 3261(1963).
12) H. Seki, K. Koga, H. Matsuo, S. Ohki, I. Matsuo and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 995 (1965).

of C-terminal amino acid, alanine or glycine, disappeared, whereas non-terminal one, glycine or alanine, stayed almost unchanged.

In order to confirm this selective reaction, various acyl dipeptide esters were subjected to the reaction followed by quantitative analysis. Two methods for the analysis of the reaction rate were applied. One is the detection of amino alcohols by trinitrophenylation which was developed by Satake, *et al.* for the purpose of the analysis of amino acids,[13] and the other, as already described above, the detection of recovered amino acids as trifluroacetyl amino acid *n*-butyl esters.[5]

One mmole/liter solution of N-benzoyl dipeptide esters (IX,XI,XII,XIII,XIV) treated with ten fold excess of sodium borohydride in aqueous solution at room temperature. The reaction mixture was heated with 6N hydrochloric acid and the resultant amino alcohol was treated with sodium 2,4,6-trinitrobenzene-1-sulfonate to convert to the corresponding N-(2,4,6-trinitrophenyl)-amino alcohol (X), which was separated from 2,4,6-trinitrophenyl amino acids by the extraction with ether from alkaline solution and analyzed quantitatively by its optical density at 350 mμ on a spectrophotometer. The N-(2,4,6-trinitrophenyl)-amino alcohols (X) thus obtained from C-terminal amino acids were almost in pure state and identified by the comparison of thin-layer chromatography with the authentic samples prepared by independent synthesis. As shown in Fig. 2, the yield of N-(2,4,6-trinitrophenyl)-amino alcohols increased as a function of reaction time, and the satisfactory result were given by the reaction for several hours, except in case of valine ester.

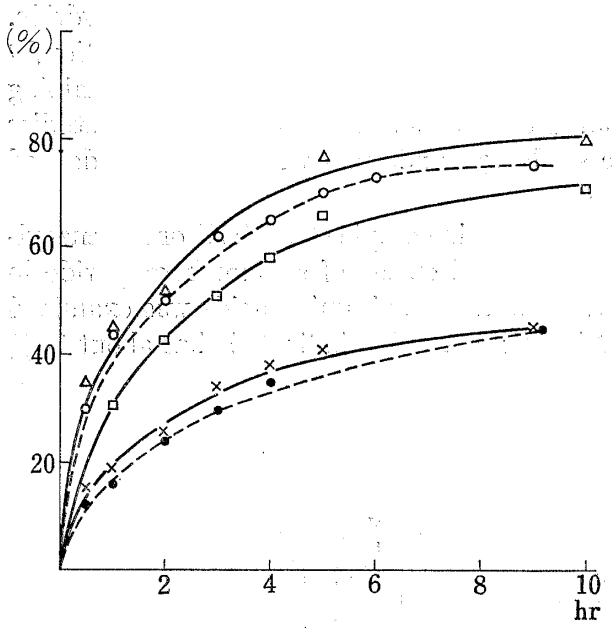

Fig. 2. Yields of TNP-amino Alcohols

—△—: Bz. DL-Ala-GlyOEt (IX)
—×—: Bz. Gly-L-ValOEt (XIII)
—□—: Bz.Gly-L-IleOMe (XII)
—○—: Bz.Gly-L-PheOEt (XI)
—●—: Bz.Gly-L-ValOMe (XIV)

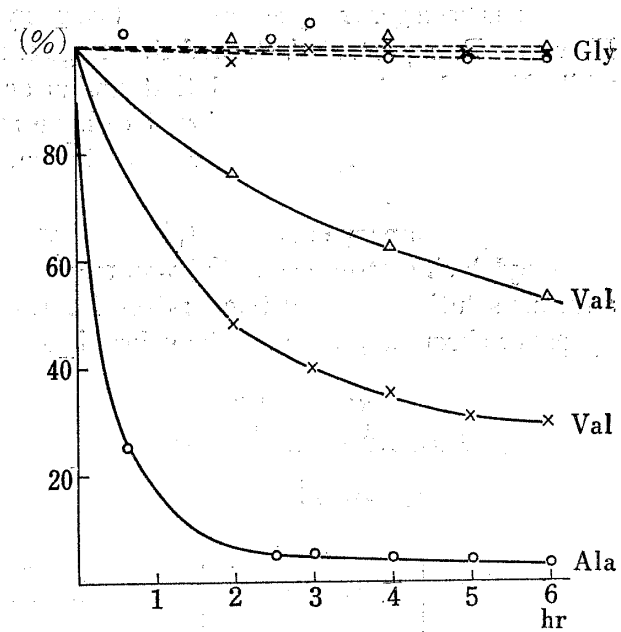

Fig. 3. Recovery of Amino Acids

—○—: Ac.Gly-DL-AlaOEt
—×—: Ac.Gly-L-ValOMe
—△—: Ac.Gly-L-ValOMe at 0°

N-Acetyldipeptide esters, N-acetylglycyl-DL-alanine ethyl ester (XV) and N-acetylglycyl-L-valine methyl ester (XVI), which are more soluble in water than N-benzoyldipeptide esters, were also subjected to the reaction and the recovery of amino acids as determined gas chromatographically is shown in Fig. 3. C-Terminal alanine and valine decreased as the reaction

13) T. Okuyama and K. Satake, *J. Biochem.* (Tokyo), **47**, 454 (1960); K. Satake, T. Okuyama, M. Ohashi and T. Shinoda, *ibid.*, **47**, 654 (1960).

proceeds though the rate for the latter is rather slow, whereas non-terminal glycine was invariably recovered in nearly quantitative yield.

Table III. 2,4,6-Trinitrophenyl Amino Alcohols

Trinitrophenyl-	Yield (%)	mp (°C)	UV(1N HCl) $\lambda_{max}m\mu$ (ε)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
DL-Alaninol[17]	75	111—112	350 (14300)	$C_9H_{10}O_7N_4$	37.77	3.52	19.58	37.73	3.43	19.34
L-Phenylalaninol[17]	90	104—106	352 (12400)	$C_{15}H_{14}O_7N_4$	49.73	3.90	15.47	49.76	4.01	15.72
L-Isoleucinol	84	94—95	352 (14000)	$C_{12}H_{16}O_7N_4$	43.90	4.91	17.07	43.95	5.17	17.07
L-Valinol[18]	73	109.5—110.5	352 (14900)	$C_{11}H_{14}O_7N_4$	42.04	4.49	17.83	42.09	4.50	17.68

Recently, sodium borohydride has been used for the reduction of disulfide groups in proteins,[14] and in some cases, it was reported that some peptide bonds were reduced by the reagent,[15] though Crestfield, *et al.* showed that this was minimized in the presence of EDTA.[14b]

The results presented in this work demonstrate that by the use of sodium borohydride in aqueous solution under simple conditions, certain model peptide esters can be reduced to corresponding alcohols without any appreciable side-reaction. A study of the application of this method to larger peptides and proteins is in progress.

Experimental

N-Benzoylglycyl-L-phenylalanine Ethyl Ester (XI)——A solution of 1.79 g of N-benzoylglycine and 1.01 g of triethylamine in 20 ml of acetonitrile was chilled to −5° with stirring, treated with 1.08 g of ethyl chloroformate. After 15 min a cold mixture of 2.3 g of L-phenylalanine ethyl ester hydrochloride and 1.01 g of triethylamine in 30 ml of tetrahydrofuran was added. The mixture was stirred for 1 hr at 0° and then allowed to stand overnight at room temperature. The precipitate was removed, and the solvent was replaced by 50 ml of ethyl acetate. The ethyl acetate solution was washed with 10% hydrochloric acid, water, aqueous sodium bicarbonate and water. The dried solution was evaporated to give 3.6 g of crude N-benzoylglycyl-L-phenylalanine ethyl ester (XI). Recrystallization from ethyl acetate–hexane gave 2.5 g (71%) of colorless fine needles, mp 100—102°. *Anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.91. Found: C, 68.20; H, 6.37; N, 7.78.

N-Benzoyl-DL-alanylglycine Ethyl Ester (IX)[16]——N-Benzoyl-DL-alanylglycine ethyl ester was synthesized from 1.93 g of N-benzoyl-DL-alanine and 1.4 g of glycine ethyl ester hydrochloride as in the foregoing experiment. Recrystallization from benzene–hexane gave 2.1 g of colorless needles, mp 106—107°. *Anal.* Calcd. for $C_{14}H_{18}O_4N_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.43; H, 6.47; N, 10.11.

N-Benzoylglycyl-L-isoleucine Methyl Ester (XII)——To a cold solution of 1.79 g of N-benzoylglycine in 5 ml of dimethylformamide at −5° to 0° was added 2.06 g of N,N′-dicyclohexylcarbodiimide in 5 ml of tetrahydrofuran with stirring, and then added a mixture of 1.81 g of L-isoleucine methyl ester hydrochloride and 1.01 g of triethylamine in 50 ml of tetrahydrofuran. The mixture was allowed to stand at room temperature for 2 hr, and the precipitated dicyclohexylurea and triethylamine hydrochloride was removed by filtration and the solvent was replaced by ethyl acetate. The ethyl acetate solution was washed with 10% sodium carbonate, sodium chloride saturated water, and 10% hydrochloric acid, and the dried over anhydrous sodium sulfate. Evaporation of the solvent gave 2.8 g (93%) of crude N-benzoylglycyl-L-isoleucine methyl ester (XII), which was recrystallized from benzene–hexane to give 2.3 g (75%) of a colorless solid, 110—112°. *Anal.* Calcd. for $C_{16}H_{22}O_4N_2$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.69; H, 7.17; N, 9.04.

14) *a*) S. Moore, R.P. Cole, H.G. Gundlach and W.H. Stein, Proc. Intern. Cong. Biochem. 4th, Vienna 1958, Pergamon, N.Y., 1960; *b*) A.M. Crestfield, J. Skupin, S. Moore and W.H. Stein, *Fed. Proc.*, **19**, 341 (1960); *c*) W.D. Brown, *Biochem. Biophys. Acta*, **44**, 365 (1960).

15) J.R. Kimmel and A.J. Parcells, *Fed. Proc.*, **19**, 341 (1960); J.M. Gillespie, I.J. O'Donnell, E.O.P. Thompson and E.F. Woods, *J. Textile Inst.*, **51**, T703 (1960).

16) T. Curtius and C.F. van der Linden, *J. Prak. Chem.*, **70**, 153 (1904).

N-Benzoylglycyl-L-valine Methyl Ester (XIV)——XIV was obtained in a manner similar to the synthesis of XII. Recrystallization from ethyl acetate–hexane gave colorless fine needles in yield of 79%, mp 143—144°. *Anal.* Calcd. for $C_{15}H_{20}O_4N_2$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.37; H, 6.83; N, 9.41.

N-Benzoylglycyl-L-valine Ethyl Ester (XIII)——To a solution of 584 mg of N-benzoylglycyl-L-valine methyl ester (XIV) in 5 ml of ethanol, 5 ml of 6N sodium hydroxide was added and allowed to stand at room temperature for 30 min. After evaporation of ethanol *in vacuo*, the solution was acidified by the addition of hydrochloric acid. The precipitate was collected by filtration, washed with water and recrystallized from water to give 420 mg (76%) of colorless fine needles of N-benzoylglycyl-L-valine, mp 176—178°. *Anal.* Calcd. for $C_{14}H_{18}O_4N_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.53; H, 6.73; N, 10.17.

A solution of 2 g of N-benzoylglycyl-L-valine in 30 ml of ethanol was saturated with dry hydrogen chloride. The solvent was evaporated *in vacuo*, and the residue was taken up in dichloromethane and washed with 10% sodium carbonate and water. Evaporation of the solvent gave 2.15 g (89%) of a colorless solid of N-benzoylglycyl-L-valine ethyl ester (XIII), which was recrystallized from benzene–hexane to give 1.95 g (81%) of colorless fine needles, mp 111.5°. *Anal.* Calcd. for $C_{16}H_{22}O_4N_2$: C, 62.74; H, 7.24; N, 9.14. Found: C, 62.63; H, 7.23; N, 8.86.

N-Acetylglycyl-DL-alanine Ethyl Ester (XV)——A solution of 1.17 g of acetylglycine and 1.01 g of triethylamine in 40 ml of acetonitrile was chilled to −5° and treated with 1.09 g of ethyl chloroformate. After 10 min a cold solution of DL-alanine ethyl ester (prepared from 1.53 g of DL-alanine ethyl ester hydrochloride and 1.01 g of triethylamine) in 20 ml of acetonitrile was added. The mixture was stirred for 1 hr at −5° to 0° and then for 1 hr at room temperature. The precipitated triethylamine hydrochloride was removed by filtration and the solvent was replaced by 50 ml of hot tetrahydrofuran. The tetrahydrofuran solution was allowed to stand for 3 hr in a refrigerator. The precipitate was removed again and the filtrate was evaporated *in vacuo* to yield 2.1 g (100%) of crude N-acetylglycyl-DL-alanine ethyl ester (XV), mp 95—110°. The crude XV was chromatographed on a column of 25 g of silicagel. Elution with ethyl acetate gave 1.8 g (83%) of XV, which was recrystallized from benzene–hexane to give colorless fine needles, mp 113—115°. *Anal.* Calcd. for $C_9H_{16}O_4N_2$: C, 49.99; H, 7.46; N, 12.96. Found: C, 50.00; H, 7.31; N, 13.23.

N-Acetylglycyl-L-valine Methyl Ester (XVI)——XVI was synthesized from 590 mg of acetylglycine and 837 mg of valine methyl ester hydrochloride as in the foregoing experiment. Recrystallization from benzene–hexane gave 867 mg (75%) of colorless needles, mp 91—93°. *Anal.* Calcd. for $C_{10}H_{18}O_4N_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.09; H, 7.77; N, 12.11.

L-Isoleucinol——To a solution of 550 mg of lithium aluminum hydride in 15 ml of anhydrous ether, 1.518 g of L-isoleucine methyl ester in 15 ml of ether was added dropwise with stirring. After 1 hr, 25 ml of ether and a small volume of water was added for decomposition of excess of lithium aluminium hydride. The precipitate was filtered off and the filtrate was dried over sodium sulfate and evaporated to yield 1.134 g (81%) of a colorless oil, bp 97° (14mmHg).

2,4,6-Trinitrophenylation of Amino Alcohols——The following N-(2,4,6-trinitrophenyl)-amino alcohols was prepared in a manner to the experiment described by Satake, *et al.* for the 2,4,6-trinitrophenylation of amino acids.[13] The general experiment was carried out as follows: 1 mmole of an amino alcohol (DL-alaninol,[17] L-phenylalaninol,[17] L-isoleucinol, L-valinol[18]), 1 mmole of sodium 2,4,6-trinitrobenzene-1-sulfonate dihydrate and 200 mg of sodium bicarbonate were dissolved in 10 ml of water, and the solution was kept in dark place at room temperature. After 2 hr, the solution was acidified to pH 1 by the addition of hydrochloric acid. The precipitated yellow crystals were collected by filtration and recrystallized from aqueous methanol. The results and some properties are shown in Table III.

Reduction of N-Acetyl-DL-phenylalanine (Ia) with Diborane——a) With 1.1 Equivalents of BH_3: To a stirred solution of 1.04 g (5 mmole) of N-acetyl-DL-phenylalanine (Ia)[19] in 15 ml of anhydrous tetrahydrofuran, 3 ml (5.4 mmole) of a 1.82N diborane solution (based on BH_3) in tetrahydrofuran was added at room temperature in a stream of nitrogen. Stirring was continued for 2 hr. The reaction mixture was evaporated to dryness *in vacuo*. Ten ml of 10% sodium carbonate was added to the residue, the mixture was extracted with ethyl acetate several times. The combined extracts (50 ml) were washed with 10% sodium carbonate then with water, dried over anhydrous sodium sulfate, and evaporated to leave 362 mg (38%) of a colorless solid of N-acetyl-DL-phenylalaninol (IIa), mp 96—100°. Recrystallization from dichloromethane–hexane gave colorless neeldes, mp 101—102°. IR ν_{max}^{Nujol} cm^{-1}: 3300 (broad, OH), 1640 (amide). *Anal.* Calcd. for $C_{11}H_{15}O_2N$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.17; H, 7.96; N, 7.10.

The sodium carbonate layer was acidified by the addition of hydrochloric acid and the precipitated solid filtered to yield 569 mg (55%) of unchanged starting material.

b) With 3 Equivalents of BH_3: The reaction of 1.04 g (5 mmole) of Ia with 8.3 ml (15 mmole) of a 1.82N diborane solution in tetrahydrofuran was carried out as described above. The residual oil left on evaporation of ethyl acetate was chromatographed on silicagel to give three products.

17) P. Karrer, P. Portmann and M. Suter, *Helv. Chim. Acta*, **31**, 1617 (1948).
18) P. Karrer, P. Portmann and M. Suter, *Helv. Chim. Acta*, **32**, 1156 (1949).
19) H. Hellmann and F. Lingens, *Z. Physiol. Chem.*, **297**, 283 (1954).

The first fraction which was eluted with benzene–ethyl acetate (4:1) was 155 mg (17.5%) of an oil of N-borylethyl-DL-phenylalaninol (IVa) which was crystallized on standing at room temperature. Recrystallization from benzene–hexane to give colorless fine needles, mp 77° (decomp.). IR ν_{max}^{Nujol} cm^{-1}: 3560 (broad, OH), 2400 (BH_2). Mass spectrum *m/e*: 189 (M-2), 188, 114, 98, 91. *Anal.* Calcd. for $C_{11}H_{18}ONB$: C, 69.16; H, 9.42; N, 7.33. Found: C, 69.06; H, 9.67; N, 7.51.

The second fraction which was eluted with ethyl acetate was 474 mg (49%) of colorless solid of N-acetyl-DL-phenylalaninol (IIa). Recrystallization from dichloromethane–hexane gave colorless fine needles, mp 102°. It was identical with an authentic sample prepared by acetylation of phenylalaninol with acetyl chloride in aqueous sodium hydroxide solution, with regard to mixture melting point, thin-layer chromatography and infrared spectra.

The final fraction which was eluted with ethyl acetate–ethanol (2:1) was 174 mg (20%) of N-ethylphenylalaninol (IIIa).[20] Recrystallization from water gave colorless neeldes, mp 87°. *Anal.* Calcd. for $C_{11}H_{17}ON$: C, 73.70; H, 9.56; N, 7.81. Found: C, 73.60; H, 9.49; N, 7.84.

Reduction of N-Benzoyl-DL-alanine with Diborane——a) With 1.1 Equivalents of BH_3: N-Benzoyl-DL-alanine (0.965 g, 5 mmole) (Ib)[21] was reduced with 1.1 equivalents of diborane in tetrahydrofuran in a manner similar to the reaction on N-acetyl-DL-phenylalanine (Ia). The ethyl acetate extracts was evaporated to yield 343 mg (38.5%) of a colorless solid. Recrystallization from benzene–hexane gave fine needles of N-benzoyl-DL-alaninol (IIb), mp 103—105°. IR ν_{max}^{Nujol} cm^{-1}: 3340 (OH), 1630 (amide). *Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.03; H, 7.41; N, 7.59.

The sodium carbonate layer on acidification with hydrochloric acid gave 480 mg (50%) of a recovered starting material.

b) With 3 Equivalents of BH_3: The reaction was carried out as described above. The residual oil left on evaporation of ethyl acetate was chromatographed on silcagel to give three products.

The first fraction which was eluted with benzene–ethyl acetate (4:1) was 322 mg (36.4%) of a colorless solid of N-borylbenzyl-DL-alaninol (IVb). Recrystallization from benzene–hexane gave colorless needles, mp 95° (decomp). IR ν_{max}^{Nujol} cm^{-1}: 3520 (OH), 2382, 2362 (BH_2). Mass spectrum *m/e*: 175 (M-2), 174, 160, 91. *Anal.* Calcd. for $C_{10}H_{16}ONB$: C, 67.85; H, 9.11; N, 7.91. Found: C, 67.86; H, 9.22; N, 7.81.

The second fraction which was eluted with ethyl acetate was 300 mg (33.5%) of a colorless solid of N-benzoyl-DL-alaninol (IIb). Recrystallization from benzene–hexane gave colorless fine needles, mp 103—105°. It was identical with an authentic sample prepared by another route, with regard to mixture melting point, thin layer chromatography and infrared spectra.

The third fraction which was eluted with ethanol–ethyl acetate (1:2) was 230 mg (28%) of a colorless solid. Recrystallization from hexane or cyclohexane gave colorless fine needles of N-benzyl-DL-alaninol (IIIb), mp 67°.[22] IR ν_{max}^{Nujol} cm^{-1}: 3200 (broad, OH, NH). Picrate, mp 135—137°. *Anal.* Calcd. for $C_{16}H_{18}O_8N_4$: C, 48.73: H, 4.60; N, 14.21. Found: C, 48.63; H, 4.55; N, 14.16.

N-Borylbenzyl-DL-alaninol (IVb)——To a solution of 187 mg of N-benzylalaninol (IIIb) in 30 ml of tetrahydrofuran in nitrogen atmosphere, 3 ml of 1.2N diborane solution in tetrahydrofuran was added and allowed to stand at room temperature for 4 hr. The solvent was evaporated to dryness. The residue was dissolved in 5 ml of methanol, and methanol was evaporated to removed boric acid three times. The residual oil was chromatographed on silicagel column. Elution with ethyl acetate–benzene (5:1) gave 108 mg (54%) of a colorless solid of IVb, which was recrystallized from benzene–hexane to give colorless needles, mp 95° (decomp.).

N-Borylethyl-DL-phenylalaninol (IVa)——IVa was synthesized from N-ethyl-DL-phenylalaninol (IIIa) as in the foregoing experiment in yield of 74%, mp 77° (decomp.).

Acid Hydrolysis of IVb——A solution of 60 mg of IVb in 10 ml of 6N hydrochloric acid was refluxed for 2 hr. After evaporation of hydrochloric acid, the residue was dissolved in 5 ml of methanol, and methanol was distilled off five times. The residual solid was recrystallized from ethanol-ether to give 51 mg (75%) of colorless needles of N-benzyl-DL-alaninol hydrochloride (IIIb-HCl), mp 98—99°.[22] *Anal.* Calcd. for $C_{10}H_{16}ONCl$: C, 59.55; H, 8.00; N, 6.94. Found: C, 59.53; H, 8.14; N, 6.97.

Acid Hydrolysis of IVa——N-Ethyl-DL-phenylalaninol hydrochloride (IIIa-HCl) was obtained from IVa as described above, mp 139—141°.[20]

Reduction of N-Benzoylglycyl-DL-alanine (VI) with Diborane——N-Benzoylglycyl-DL-alanine (VI)[23] (25 mg, 0.1 mmole) in 5 ml of tetrahydrofuran was reduced with diborane followed by acid hydrolysis with 6N hydrochloric acid under reflux for 5 hr. The mixture was evaporated to dryness *in vacuo* and the dry residue was stirred in 1.2N hydrochloric acid in methanol at room temperature for 30 min. The methanol was removed by vacuum distillation and the mixture of methyl ester hydrochlorides was converted to the mixture of *n*-butyl ester hydrochlorides by the treatment with 10 ml of 1.2N hydrochloric acid in *n*-butanol

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21) E. Fischer, *Chem. Ber.*, **32**, 2451 (1899).
22) A. Stoll, J. Peyer and A. Hoffmann, *Helv. Chim. Acta*, **26**, 929 (1943).
23) T. Curtius and E. Lambotte, *J. Prak. Chem.*, **70**, 114 (1904).

at 100° for 1 hr. After evaporation of *n*-butanol, the residue was treated with 0.5 ml of trifluoroacetic anhydride in 5 ml of dichloromethane for 2 hr at room temperature. The anhydride and solvent were distilled off *in vacuo* to give the mixture of N-trifluoroacetyl amino acid *n*-butyl esters (VII), to which 6.07 mg (0.0215 mmole) of N-trifluoroacetyl isoleucine *n*-butyl ester as an internal standard in 1 ml of acetone was added and the whole mixture was analyzed by gas chromatography. Shimazu Gas Chromatograph GC-1 having 1.5% NGS column (3 m) was used.

Preliminary Experiment for the Reduction of Peptide Esters with Sodium Borohydride——To a solution of 2.5 mg of N-benzoylglycyl-DL-alanine ethyl ester (VIII)[24] in 10 ml of water, 10 mg of sodium borohydride was added and allowed to stand at room temperature. After 1.5 hr 4 drops of 6N hydrochloric acid was added and water was evaporated *in vacuo*. The residue was dissolved in 0.7 ml of 6N hydrochloric acid and heated at 95° to 105° for 5 hr. The hydrochloric acid was evaporated to dryness. The recovery of amino acids was examined by paper chromatography (BuOH–AcOH–H_2O, 4:1:1 and PhOH–H_2O, 5:1), which indicated that about 70% of C-terminal alanine disappeared, but non-terminal glycine remained almost unchanged.

Reduction of Dipeptide Esters with Sodium Borohydride——a) Spectrophotometric Determination of N-(2,4,6-Trinitrophenyl) Amino Alcohol: To 1 ml of 0.01 mole/liter ethanol solution of N-benzoyl dipeptide esters (IX, XI, XII, XIII, XIV), 8 ml of water and 1 ml of 0.1 mole/liter aqueous solution of sodium borohydride was added, and the whole volume was adjusted to 10 ml. The clear solution was allowed to stand at room temperature. One ml aliquot of this solution was placed in an another flask, excess of sodium borohydride was decomposed by the addition of 1 drop of 6N hydrochloric acid, and water was evaporated *in vacuo*. The residue was dissolved in 1 ml of 6N hydrochloric acid and transferred into a test tube, which was sealed and heated at 120° for 5 hr. After evaporation of hydrochloric acid, the residual mixture of an amino alcohol and amino acids was dissolved in 2 ml of 2% aqueous sodium bicarbonate and 1 ml of 0.1 mole/liter aqueous solution of sodium 2,4,6-trinitrophenyl-1-sulfonate and allowed to stand at room temperature in a dark place for 3 hr. The reaction mixture was extracted with ether several times. After evaporation of ether, the residual N-(2,4,6-trinitrophenyl) amino alcohol was dissolved in 10 ml of 1N hydrochloric acid and assayed by its absorbance at 350 mμ on a Hitachi Recording Spectrophotometer EPS-3T, as shown in Fig. 2. One ml of distilled water, in place of a sample solution, was used as a blank.

b) Gas Chromatographic Determination of the Recovered Amino Acids: Acetyl dipeptide esters (XV, XVI) (0.1 mmole) and sodium borohydride (1 mmole) were dissolved in 10 ml of water and allowed to stand at room temperature or at 0°. One ml aliquot of this solution was subjected to acid hydrolysis. The hydrochloric acid was evaporated to dryness *in vacuo*, and the residue was converted to the mixture of N-trifluoroacetyl amino acid *n*-butyl esters and analyzed by gas chromatography as described above.

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