were 0.9 kcal., this isomer was found to contain an appreciable amount of the twist conformation.

Experimental

2,6-Dimethyl- and 2,6-Diisopropylcyclohexanone.—Hydrogenation (2000–2500 p.s.i., 170–230°, Raney nickel catalyst) of the symmetrical dialkylphenols gave isomeric mixtures of the corresponding cyclohexanols.[21,22] The mixed *cis*- and *trans*-ketones were obtained in high yield by chromic acid oxidation.[23]

2,6-Di-*t*-butylcyclohexanone.—Catalytic reduction of 2,6-di-*t*-butylphenol at 200°, 1700 p.s.i., 10% by weight Raney nickel, gave 2,6-di-*t*-butylcyclohexanone directly.[22] The mixture of ketones contained about 90% of the *cis* isomer, which was purified by recrystallization from aqueous methanol. The *trans* isomer, b.p. 144–145.5° (30 mm.), was obtained by fractional distillation of the residue.

2,6-Diethylcyclohexanone.—A mixture of *cis*- and *trans*-2,6-diethylcyclohexanone was prepared by pyrolysis of the barium salt of α,α'-diethylpimelic acid, following the procedure outlined by Newman, *et al.*[24]

Carvomenthone.—Hydrogenation (3 atm., 5% rhodium-on-alumina, rm. temp.) of carvone, $\alpha^{25}D$ 58.0° (1 dm., neat), gave a mixture of carvomenthone and isocarvomenthone, $\alpha^{25}D$ −25.1° (1 dm., neat).[4]

Geometry of the Isomeric Ketones.—The relative configurations of the 2,6-dialkylcyclohexanones were established by separation and collection of small amounts of each isomer by vapor phase chromatography followed by lithium aluminum hydride reduction. The *cis*-ketone in each case gave two isomeric alcohols, while only one was obtained from the *trans*-ketone, as determined by vapor phase chromatography.

Equilibration.—Equilibration of the ketones was in general effected by dilute sodium methoxide in methanol. Sealed test-tubes containing 2 ml. of the basic solution and about 0.1 g. of ketone were immersed in a constant temperature bath, removed at intervals, and the contents poured into a separatory funnel containing water and pentane. The pentane solution was evaporated and the residue was analyzed by vapor phase chromatography.

Potassium *t*-butoxide in *t*-butyl alcohol at 50° and 89° was used to equilibrate 2,6-di-*t*-butylcyclohexanone. These conditions were required because of the extreme stability of this system toward base; a 0.1 *M* solution of potassium *t*-butoxide in *t*-butyl alcohol at 25° caused isomerization at an inconveniently slow rate. A rough calculation[25] indicates that this compound is approximately 10^6 times less "acidic" than the dimethyl analog.

The diisopropyl ketone was examined in both solvents to assure no solvent dependency; no difference in the equilibrium constant was observed.

Analysis of Isomer Distribution.—A 10-foot 15% tricyanoethoxypropane-on-firebrick column was used at 100–140° for analysis of the ketones. Very clean separation of the *cis* and *trans* isomers was obtained in each case except for the carvomenthone–isocarvomenthone mixture. A planimeter was used to determine the areas of the chromatographic curves; at least forty of these curves were obtained for each equilibrium. The attainment of equilibrium was shown by the constancy of successive determinations. Each isomer was collected and recycled to show that no isomerization occurred during this process.

Lithium aluminum hydride reduction of mixtures of carvomenthone and isocarvomenthone having different rotations (and, hence, known relative isomer content)[4,26] gave mixtures of the four isomeric carvomenthols. These alcohols were separable by vapor phase chromatography, and, by using the different ketone mixtures, it was possible to relate each alcohol to the ketone from which it was derived. The equilibrium mixture (25°) of carvomenthone and isocarvomenthone gave 70.0% + 21% = 91 ± 1% carvomenthols and 7.6% + 1.4% = 9 ± 1% isocarvomenthols.

(21) R. B. Carlin, *J. Am. Chem. Soc.*, **67**, 928 (1945).

(22) T. H. Coffield, A. H. Filbey, G. G. Ecke and A. J. Kolka, *ibid.*, **79**, 5019 (1957).

(23) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(24) M. S. Newman, I. Waltcher and H. F. Ginsberg, *J. Org. Chem.*, **17**, 962 (1952).

(25) D. J. Cram, B. Rickborn, C. A. Kingsbury and P. Haberfield, *J. Am. Chem. Soc.*, **83**, 3678 (1961).

(26) These mixtures were prepared by treatment with base at different temperatures; they differ only in the relative amounts of the 2-position isomers, being stereochemically equivalent at the 5-position.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

The N-(2-Hydroxyarylidene) Protecting Group in Peptide Synthesis

BY JOHN C. SHEEHAN AND VICTOR J. GRENDA[1]

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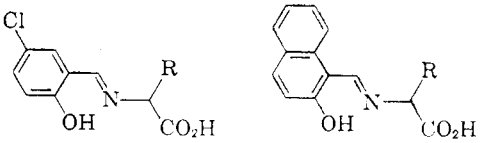
5-Chlorosalicylaldehyde and 2-hydroxyl-1-naphthaldehyde have been used as reagents for the protection of amino functions during the synthesis of peptides. Thus, N-(5-chlorosalicylidene)-L-valine and N-(2-hydroxy-1-naphthal)-L-valine were coupled with ethyl glycinate and methyl L-phenylalaninate, employing N,N'-dicyclohexylcarbodiimide, to afford the corresponding N-(2-hydroxyarylidene) derivatives of L-valylglycine ethyl ester and L-valyl-L-phenylalanine methyl ester in good yield. Removal of the N-arylidene residues was accomplished under extraordinarily mild conditions of acid hydrolysis. No racemization of the peptide derivatives was observed.

Although benzaldehyde condenses readily with salts and esters of amino acids to afford Schiff bases,[2] analogous products with free amino acids have not been isolated,[2–4] with the exception of the ε-N-benzylidene derivative of the basic amino acid, L-lysine.[5,6] McIntire[7] investigated the reaction of a number of aromatic aldehydes with free amino acids at room temperature and reported the formation of crystalline Schiff bases (I and II) prepared in good yields from 5-chlorosalicylaldehyde and 2-hydroxy-1-naphthaldehyde, respectively.



The increased stability of these N-(2-hydroxyarylidene)-amino acids (as compared to the corresponding benzaldehyde analogs), which is ex-

(1) Bristol Laboratories Fellow 1958; U. S. Public Health Fellow 1959.

(2) M. Bergmann, H. Ensslin and L. Zervas, *Ber.*, **58**, 1034 (1925); O. Gerngross and E. Zuhlke, *ibid.*, **57**, 1482 (1924).

(3) H. Dakin, *J. Biol. Chem.*, **82**, 439 (1929).

(4) J. M. Gulland and T. H. Mead, *J. Chem. Soc.*, 210 (1935).

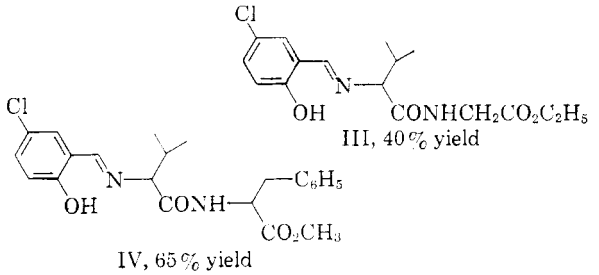
(5) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

(6) B. Witkop and T. W. Beiler, *J. Am. Chem. Soc.*, **76**, 5589 (1954).

(7) F. C. McIntire, *ibid.*, **69**, 1377 (1947).

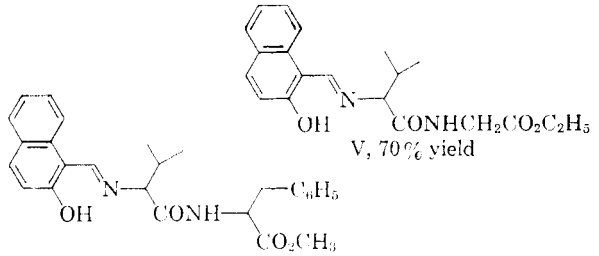
plained by strong intramolecular hydrogen bonding[8] between the arylidene nitrogen and the phenolic proton, suggested the utilization of the 2-hydroxyarylidene residue as an amine-protecting[9] function during peptide synthesis. Racemization of the optically active amino acids protected by the N-(2-hydroxyarylidene) system cannot be operative through the classical azlactonization mechanism generally described for N-acylated amino acids,[10] although other mechanisms for racemization may be postulated. Thus, N-(5-chlorosalicylidene)-L-valine (I, R is isopropyl) and N-(2-hydroxy-1-naphthal)-L-valine (II, R is isopropyl) were prepared according to the procedure of McIntire,[7] who described preparations with amino acids of unstated optical purity. Both of the yellow N-arylidenevaline derivatives were readily crystallized in a pure state. Recrystallization of I (R is isopropyl), although unnecessary, was usually accompanied by some cleavage to valine. Compound II (R is isopropyl) was stable in all anhydrous solvents tried and was recrystallized easily from ethanol in good yield. Both derivatives were cleaved, using mild conditions, to L-valine of optical purity similar to that of the starting L-valine.

Utilizing the two arylidene derivatives of L-valine, the following four amino-protected peptides were synthesized by means of the carbodiimide method for carboxyl activation.[11] Best results



III, 40% yield

IV, 65% yield

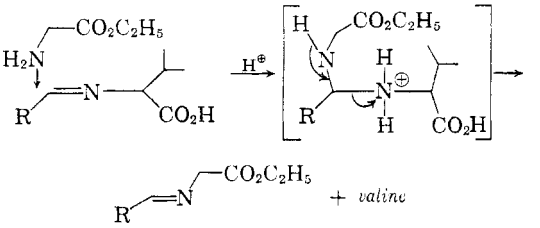


V, 70% yield

VI, 83% yield

were obtained by combining the N,N′-dicyclohexylcarbodiimide with a suspension of N-arylidene-L-valine in methylene chloride at room temperature and immediately adding the free amino ester.[12]

Using this procedure, the preparation of N-(5-chlorosalicylidene)-L-valyl-L-phenylalanine methyl ester (IV) and N-(5-chlorosalicylidene)-L-valylglycine ethyl ester (III) was accompanied by 10 and 18% cleavage, respectively, of the protected valine. On the other hand, N-(2-hydroxy-1-naphthal)-L-valylglycine ethyl ester (V) and N-(2-hydroxy-1-naphthal)-L-valyl-L-phenylalanine methyl ester (VI) were obtained without complications. A possible explanation of this side reaction may be represented by the scheme



Although methylene chloride solutions of the crude peptides were reasonably stable to dilute acid and bicarbonate washings, these operations were not essential for obtaining a pure product. All four peptides were obtained as crystalline yellow solids after removal of the reaction solvent; further purification was usually limited to washing the product with ether followed by recrystallization.

Hydrolysis of the N-(2-hydroxy-1-naphthal) protecting group was accomplished readily at room temperature with slightly more than one equivalent of dilute hydrochloric acid (peptide V) or with two equivalents of acid at 40° (peptide VI). Conditions for the removal of the N-(5-chlorosalicylidene) group were exceptionally mild. Both peptide derivatives III and IV could be titrated in aqueous dioxane at ice temperature with one equivalent of hydrochloric acid; *i.e.*, the protecting group was displaced by a proton at 0°. Completion of reaction was determined by disappearance of the yellow color of the N-(2-hydroxyarylidene) system and in each case occurred within 3 hours. The liberated aldehydes were removed by ether extraction. In this manner compounds III and V were cleaved in high yields to L-valylglycine ethyl ester hydrochloride. In both cases identity was established by the preparation of N-formyl derivatives and comparison of melting points, infrared spectra (potassium bromide) and optical rotations with a sample of N-formyl-L-valylglycine ethyl ester obtained *via* N-formyl-L-valine.[13] Cleavage of compounds IV and VI produced the expected L-valyl-L-phenylalanine methyl ester hydrochloride which was shown to be identical with that compound obtained from the acidic hydrolysis of N-formyl-L-valyl-L-phenylalanine methyl ester[13] by comparison of melting points, optical rotations and infrared spectra (potassium bromide). In no case could racemization be detected.

Preliminary experiments designed to remove the Schiff base protecting groups from peptide deriva-

(8) L. Pauling, "Nature of the Chemical Bond," 2nd Ed., Cornell University Press, Ithaca, N. Y., 1945, p. 319.

(9) T. Wieland and W. Schafer, *Ann.*, **576**, 104 (1952), attempted to synthesize aminoacetylthiophenol using a benzylidene residue for the protection of the amino group.

(10) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **99**, 143 (1932).

(11) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(12) Although there appeared to be competition between the amino ester and the N-arylidene-L-valine for reaction with the activated carboxyl species, as observed by the precipitation of N,N′-dicyclohexylurea after carbodiimide was added, extensive cleavage with the precipitation of valine occurred when the addition order was altered.

(13) J. C. Sheehan and D. D. H. Yang, *J. Am. Chem. Soc.*, **80**, 1154 (1958).

tives IV and V by means of sodium bisulfite were not promising. In some cases, a mixed anhydride procedure might be used at the coupling stage.

This study has demonstrated that 5-chlorosalicylaldehyde and 2-hydroxy-1-naphthaldehyde are promising reagents for the protection of amino functions during the synthesis of peptides. The peptide derivatives formed are highly colored and easily crystallized and therefore should be of considerable advantage for the separation and isolation of more complex synthetic peptides. Hydrolytic cleavage under extraordinarily mild conditions, especially in the case of the N-(5-chlorosalicylidene) derivatives, should distinguish the N-(2-hydroxyarylidene) function selectively from the protecting groups currently used in peptide synthesis. Further, the removal of the protecting group can be followed by the disappearance of the characteristic yellow color of the N-(2-hydroxyarylidene) system.

Experimental[14]

N-(2-Hydroxy-1-naphthal)-L-valine.[8]—To a suspension of 2 g. (0.017 mole) of finely ground L-valine[15] in 480 ml. of ethanol and 35 ml. of methanol, there was added 4.41 g. (0.026 mole) of 2-hydroxy-1-naphthaldehyde. After 14 hours of stirring, the valine had dissolved and the solvent was evaporated under reduced pressure to yield a yellow solid. The crystals were collected by centrifugation and washed well with anhydrous ether to remove the excess aldehyde. The crystalline residue weighed 4.27 g. (92%), m.p. 181.5–185° dec., sint. 177° dec., $[\alpha]^{25}D$ −92.4° (*c* 1 in ethanol).

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.00; H, 6.35; N, 5.14.

Acid Hydrolysis of N-(2-Hydroxy-1-naphthal)-L-valine.—To a suspension of 150 mg. (0.55 millimole) of N-(2-hydroxy-1-naphthal)-L-valine in 3 ml. of 50% methanol, there was added with stirring dilute hydrochloric acid sufficient to maintain the *p*H at approximately 1.5 for 1 hour. During this period decolorization of the Schiff base and precipitation of the aldehyde occurred. The methanol was removed under reduced pressure and the precipitated aldehyde extracted with ether. Lyophilization of the colorless aqueous solution produced L-valine hydrochloride as a white solid. The hydrochloride was dissolved in 2 ml. of ethanol and 57 mg. of crude L-valine was precipitated by addition of 0.20 ml. (0.84 millimole) of tri-*n*-butylamine. Recrystallization from water–ethanol produced 55 mg. (84%) of plates melting above 300° with sublimation; $[\alpha]^{30}D$ +45.7° (*c* 1 in glacial acetic acid).

N-(2-Hydroxy-1-naphthal)-L-valylglycine Ethyl Ester (V).—To a suspension of 0.50 g. (1.84 millimoles) of N-(2-hydroxy-1-naphthal)-L-valine in 15 ml. of methylene chloride there was added, with stirring, 0.38 g. (1.84 millimoles) of N,N′-dicyclohexylcarbodiimide followed by 0.19 g. (1.84 millimoles) of freshly prepared ethyl glycinate.[16] After stirring 16 hours at room temperature, the precipitated urea was removed by filtration and the solution evaporated under reduced pressure to a crystalline yellow solid. The crude product was washed with ether and recrystallized from methylene chloride–ether to yield 0.44 g. (70%) of yellow needles, m.p. 180–184°. An analytical sample was obtained by recrystallization from ethanol; m.p. 183–184°, $[\alpha]^{25}D$ −38.9° (*c* 1 in ethanol).

Anal. Calcd. for $C_{20}H_{24}N_2O_4$: C, 67.39; H, 6.79; N, 7.86. Found: C, 67.38; H, 6.81; N, 7.79.

N-(2-Hydroxy-1-naphthal)-L-valyl-L-phenylalanine Methyl Ester (VI).—To a suspension of 0.30 g. (1.11 millimoles) of N-(2-hydroxy-1-naphthal)-L-valine in 10 ml. of methylene chloride, there was added 0.23 g. (1.11 millimoles) of N,N′-dicyclohexylcarbodiimide followed by 0.20 g. (1.10 millimoles) of freshly prepared L-phenylalanine methyl ester.[17] After 24 hours of stirring at room temperature, the precipitated urea was removed and the solution concentrated to a crystalline solid. The crude product was washed with ether and recrystallized from methylene chloride–ether to afford yellow needles, 0.40 g. (83%), m.p. 215–218°. Recrystallization from methanol produced an analytical sample, m.p. 217–219°, $[\alpha]^{26}D$ − 25.6° (*c* 1 in dioxane).

Anal. Calcd. for $C_{26}H_{28}N_2O_4$: C, 72.20; H, 6.53; N, 6.48. Found: C, 72.10; H, 6.34; N, 6.30.

N-(5-Chlorosalicylidene)-L-valine.[8]—A suspension of 3 g. (0.026 mole) of L-valine[15] and 6 g. (0.038 mole) of 5-chlorosalicylaldehyde in 720 ml. of absolute ethanol and 50 ml. of methanol was stirred until solution was complete (12 hours). The product (yellow needles) was washed with 30–60° petroleum ether (to remove excess aldehyde); 5.2 g. (79%), m.p. 145–148°.

Anal. Calcd. for $C_{12}H_{14}NO_3Cl$: C, 56.35; H, 5.52; N, 5.48. Found: C, 56.14; H, 5.68; N, 5.43.

Hydrolysis of N-(5-Chlorosalicylidene)-L-valine.—A solution of 75 mg. (0.29 millimole) of N-(5-chlorosalicylidene)-L-valine in 4 ml. of 25% methanol–water rapidly decolorized with the precipitation of 5-chlorosalicylaldehyde. The aldehyde was extracted with ether and the aqueous phase was evaporated under reduced pressure. Recrystallization from water–ethanol produced 30 mg. (88%) of L-valine, melting above 280° with sublimation; $[\alpha]^{29}D$ +45.7° (*c* 1 in glacial acetic acid).

N-(5-Chlorosalicylidene)-L-valylglycine Ethyl Ester (III).—To a suspension of 0.5 g. (1.95 millimoles) of N-(5-chlorosalicylidene)-L-valine in 15 ml. of methylene chloride, there was added 0.4 g. (1.95 millimoles) of N,N′-dicyclohexylcarbodiimide followed rapidly by the addition of 0.20 g. (1.95 millimoles) of freshly prepared ethyl glycinate.[16] After 24 hours of stirring at room temperature the precipitate was removed by filtration and the filtrate washed successively with *N* hydrochloric acid, *N* sodium bicarbonate and water. The dried (sodium sulfate) methylene chloride solution was concentrated to a small volume, and the yellow peptide was induced to crystallize by addition of ether; yield 0.27 g. (40%), m.p. 117–121°. Recrystallization from methylene chloride–ether produced an analytical sample, m.p. 124.5–127°, $[\alpha]^{25}D$ +31.8° (*c* 0.6 in dioxane).

Anal. Calcd. for $C_{16}H_{21}N_2O_4Cl$: C, 56.38; H, 6.21; N, 8.22. Found: C, 56.30; H, 6.20; N, 8.30.

Extraction of the crude N,N′-dicyclohexylurea with hot water and evaporation of the extract produced 40 mg. of valine. This corresponds to 18% cleavage of the Schiff base acid.

N-(5-Chlorosalicylidene)-L-valyl-L-phenylalanine Methyl Ester (IV).—To a suspension of 0.5 g. (1.95 millimoles) of N-(5-chlorosalicylidene)-L-valine in 15 ml. of methylene chloride, there was added 0.4 g. (1.95 millimoles) of N,N′-dicyclohexylcarbodiimide followed by the rapid addition of 0.35 g. (1.95 millimoles) of freshly prepared L-phenylalanine methyl ester.[17] After 15 hours at room temperature, the heavy white precipitate was removed by filtration and the solution was evaporated to a crystalline mass. The ether-washed product was recrystallized from methylene chloride–ether to produce 0.53 g. (65%) of yellow needles, m.p. 148–150.5°. An analytical sample was obtained by recrystallization from methanol, m.p. 150–151°, $[\alpha]^{25}D$ +32.4° (*c* 2.4 in methylene chloride).

Anal. Calcd. for $C_{22}H_{25}N_2O_4Cl$: C, 63.38; H, 6.04; N, 6.72. Found: C, 63.17; H, 6.07; N, 6.86.

Extraction of the crude urea with hot water and evaporation of the extract afforded 23 mg. of valine (10% cleavage of the Schiff base amino acid).

L-Valyl-L-phenylalanine Methyl Ester Hydrochloride. A. From N-(2-Hydroxy-1-naphthal)-L-valyl-L-phenylalanine Methyl Ester (VI).—A suspension of 0.16 g. (0.36 millimole) of VI in 43 ml. of 40% methanol was dissolved

(14) All melting points were determined on a Kofler block and are corrected. The microanalyses were performed by Dr. S. M. Nagy and his associates.

(15) $[\alpha]^{29}D$ +47.2° (*c* 1 in glacial acetic acid); $[\alpha]^{26}D$ +27.0° (*c* 4 in 6 *N* hydrochloric acid).

(16) R. W. Chambers and F. H. Carpenter, *J. Am. Chem. Soc.*, **77**, 1522 (1955).

(17) Prepared by neutralizing an aqueous solution of L-phenylalanine methyl ester hydrochloride to *p*H 9 with potassium carbonate and subsequently extracting into ether. The hydrochloride was prepared by Fischer esterification.

during the addition of 7.3 ml. of 0.1 *N* hydrochloric acid. Periodic gentle warming (40°) decolorized the Schiff base with precipitation of the aldehyde. Methanol was removed under reduced pressure and the precipitated aldehyde was extracted with ether. Lyophilization of the colorless aqueous solution produced a solid which was crystallized from methanol–ether; yield 0.10 g. (87%), m.p. 193–198° dec. Recrystallization from methanol–ether yielded an analytical sample, m.p. 196.5–199° dec., $[\alpha]^{28}D$ +19.3° (*c* 2 in water).

Anal. Calcd. for $C_{15}H_{23}N_2O_3Cl$: C, 57.23; H, 7.37; N, 8.89. Found: C, 57.29; H, 7.56; N, 9.14.

B. From N-(5-Chlorosalicylidene)-L-valyl-L-phenylalanine Methyl Ester (IV).—A solution of 0.1 g. (0.24 millimole) of IV in 10 ml. of 70% purified dioxane–water was decolorized at ice-bath temperature by the addition of 2.5 ml. of 0.1 *N* hydrochloric acid over a period of 2 hours. The *p*H was controlled between 4 and 5 (*p*H meter) during 90% of the addition. After complete addition the *p*H was 3.3. The dioxane was removed under reduced pressure and the precipitated aldehyde was extracted with ether. Lyophilization of the colorless aqueous solution produced a white solid which crystallized from methanol–ether; 63 mg. (83%), m.p. 190–197° dec. Recrystallization from methanol–ether gave an analytical sample, m.p. 198–200° dec., $[\alpha]^{25}D$ +19.2° (*c* 2 in water). The infrared spectrum (potassium bromide) was identical with that of the hydrochloride obtained from part A.

Anal. Calcd. for $C_{15}H_{23}N_2O_3Cl$: C, 57.23; H, 7.37; Found: C, 57.31; H, 7.41.

The hydrochloride was shown to be identical to that obtained from the acidic hydrolysis of N-formyl-L-valyl-L-phenylalanine methyl ester[13] by comparison of melting points, optical rotations and infrared spectra (potassium bromide).

L-Valylglycine Ethyl Ester Hydrochloride. A. From N-(2-Hydroxy-1-naphthal)-L-valylglycine Ethyl Ester (V).—To a solution of 0.14 g. (0.39 millimole) of V in 7 ml. of 60% ethanol at room temperature, there was added 0.43 ml. of *N* hydrochloric acid. Decolorization of the solution and precipitation of the aldehyde occurred within 30 minutes. The ethanol was removed under reduced pressure and the aldehyde was extracted with ether from an aqueous solution of the peptide hydrochloride. Lyophilization of the aqueous layer produced 91 mg. of a colorless oil which crystallized from purified dioxane to give 78 mg. (85%) of hygroscopic plates, softening above 70°. Further purification as the hydrochloride proved difficult; for identification purposes, the N-formyl derivative was prepared (general procedure described below).

Formyl derivative: 81% yield (analytical sample), m.p. 157–158.5°, $[\alpha]^{25}D$ −61.1 (*c* 1 in ethanol).

Anal. Calcd. for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.33; H, 8.06; N, 12.36.

B. From N-(5-Chlorosalicylidene)-L-valylglycine Ethyl Ester (III).—A solution of 0.13 g. (0.38 millimole) of III in 4 ml. of 75% purified dioxane–water was decolorized at ice-bath temperature by the addition of 3.9 ml. of 0.1 *N* hydrochloric acid over 2.5 hours. During the addition, 3 ml. of dioxane was added in order to avoid precipitation of the Schiff base. The *p*H was controlled between 4 and 5 (*p*H meter) during 80% of the addition of acid. After complete addition the *p*H was 3.0. The dioxane was evaporated under reduced pressure and the precipitated aldehyde was extracted with ether from an aqueous solution of the hydrochloride. Lyophilization of the colorless aqueous layer produced a colorless oil which crystallized from methylene chloride–dioxane to yield hygroscopic white plates, 81 mg. (90%), softening above 70°.

The infrared spectra (potassium bromide, chloroform) of this hydrochloride were identical to that of the hydrochloride obtained from part A.

Formyl derivative: 82% yield (analytical sample), m.p. 157–158°, $[\alpha]^{25}D$ −61.1° (*c* 1 in ethanol).

Anal. Calcd. for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88. Found: C, 52.28; H, 7.77.

Identity with N-formyl-L-valylglycine ethyl ester, as prepared *via* N-formyl-L-valine,[13] was established by comparison of melting points, optical rotations and infrared spectra (potassium bromide).

N-Formyl-L-valylglycine Ethyl Ester.—The formyl derivative was prepared from L-valylglycine ethyl ester hydrochloride using a modification of the procedure for the formylation of L-4-carboxy-2,2-dimethylthiazolidine hydrochloride.[18]

To a solution of 50 mg. (0.21 millimole) of L-valylglycine ethyl ester hydrochloride in 0.68 ml. of 98% formic acid containing 14 mg. (0.21 millimole) of sodium formate, there was added 0.34 ml. of acetic anhydride. After stirring for 4 hours the excess anhydride was decomposed with a few drops of cold water and the acidic constituents were neutralized with saturated sodium bicarbonate solution. The product was extracted into methylene chloride, which was then washed with water and dried over sodium sulfate. Concentration of the methylene chloride solution produced 39 mg. (81%) of white needles, m.p. 152–157°. Two recrystallizations from ethyl acetate produced an analytical sample, m.p. 157–158.5° (reported[13] 156–157° uncor.), $[\alpha]^{25}D$ − 61.1° (*c* 1 in ethanol).

Anal. Calcd. for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.33; H, 8.06; N, 12.36.

(18) J. C. Sheehan and D. D. H. Yang, *J. Am. Chem. Soc.*, **80**, 1158 (1958).