Minimum Molecular Weight of Crystalline Pancreatic Amylase from Swine.—The minimum molecular weights for swine pancreatic amylase given in Table IV were calculated according to Brand, *et al.*,[36] from the data given in Table III for the best values so far available for the amino acid make-up of the protein. Although the molar ratio for tyrosine was not the lowest found in the protein, this amino acid was chosen as the standard for these calculations because the determinations of tyrosine in the protein appear to be particularly satisfactory. Tyrosine had been determined in the amylase by three independent methods. The value used as the standard and considered as probably the most accurate was obtained by spectrophotometric analysis of the intact protein.[29] However, it also was close to the average of the values obtained for tyrosine by the other two methods of analysis, Table II. As shown in Table IV, the minimum molecular weight values for crystalline pancreatic amylase from swine, calculated from its amino acid make-up, averaged 51,300 ± 450. This value corresponds reasonably well with the value of 45,000 reported by Danielsson[37] for the molecular weight of crystalline pancreatic amylase from swine[38] as calculated from sedimentation and diffusion measurements.[37] The correspondence between the value obtained here for the minimum molecular weight of the protein from its amino acid composition and the value derived from sedimentation and diffusion data leads to the conclusion that pancreatic amylase from swine exists as a single molecular unit in solution rather than as a polymer of several units as has been reported for insulin.[39-41] This conclusion appears justified. However, at present, values for minimum molecular weights of a protein, calculated from analytical data for amino acids, must be interpreted with caution. Relatively small differences in the values for the amino acids, especially those present in low concentrations, materially change the values calculated for the average minimum molecular weight of the protein.

Side Chain Groups.—The data given in Table V for the side chain groups of crystalline pancreatic amylase were obtained from the data given in Tables I, III and IV by calculations according to Brand[36] and to Gordon[42] and their co-workers. These data must be considered tentative. Their satisfactory evaluation and interpretation must await the results of titration and of other studies of the properties of the protein. However, it is interesting to note that the presence of one terminal α-amino group and of one terminal carboxyl group suggests the presence of only one polypeptide chain. The relatively high number of glycine residues would permit flexibility of the polypeptide chain. On the other hand, the part played by five residues of cystine in a protein consisting of a single polypeptide chain is not clear. Information about the arrangement of the amino acids in the protein molecule is being sought.

(37) C. E. Danielsson, *Nature*, **160**, 899 (1947).

(38) K. H. Meyer, Ed. H. Fischer and P. Bernfeld, (a) *Helv. Chim. Acta*, **30**, 64 (1947); *Arch. Biochem.*, **14**, 149 (1947); *Experientia*, **3**, 106 (1947); (b) *Helv. Chim. Acta*, **31**, 1831 (1948).

(39) F. Sanger, *Nature*, **160**, 295 (1947).

(40) F. Sanger, *Biochem. J.*, **44**, 126 (1949).

(41) H. Gutfreund, *ibid.*, **42**, 544 (1948).

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Synthesis of N-(2-Benzyl-4-Δ²-oxazolinoyl)-valine[1]

BY HOMER ADKINS,[2] ROBERT M. ROSS,[3] DOROTHY CHYNOWETH SCHROEDER,[4,5] C. LYNN MAHONEY[6] AND WALTER W. GILBERT[4]

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Methyl N-[2-benzyl-4(L)-(Δ²-oxazolinoyl)]-D-valinate and its D,D-(Ib)-isomer were prepared in good over-all yields starting with carbobenzyloxyserine. The key step in this synthesis was the condensation of methyl serylvalinate with methyl phenyliminoacetate hydrochloride to give the desired oxazoline. N-(2-Benzyl-4(L)-Δ²-oxazolinoyl)-D-valine (Ia) was obtained in 14% yield when L-seryl-D-valine was condensed with the iminoester, but D-seryl-D-valine underwent negligible reaction. The structures of these compounds were confirmed by elemental analysis, infrared spectra and identification of hydrolysis products.

The synthesis of the Δ²-oxazoline with a peptide linkage at the 4-position (Ia or b) has been carried out as an extension of the recently reported preparation of the corresponding oxazole II.[7] Starting with carbobenzyloxyserine (III), the preparative route necessitated the synthesis of the hitherto unknown methyl serylvalinate (VI) or serylvaline (VIII) as key intermediates. This dipeptide (or the corresponding ester) was condensed with methyl (or ethyl) phenyliminoacetate to yield the desired oxazoline I (a or b).

Yields of the intermediates IV and V were excellent. The conversion of V to the oxazoline could best be done through methyl serylvalinate by hydrogenolysis of the carbobenzyloxy derivative V and allowing the latter material to condense with methyl phenyliminoacetate hydrochloride (Route B). In this way, both methyl N-[2-benzyl-4(L)-

(1) Abstracted in part from theses presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree.

(2) Deceased August 10, 1949.

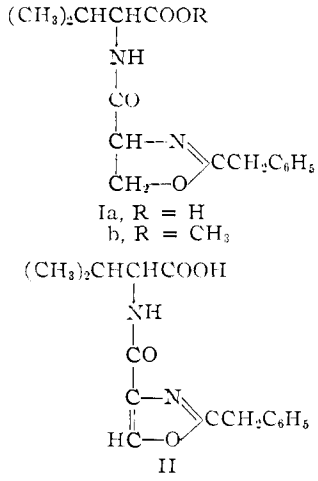
(3) Allied Chemical and Dye Corporation Fellow, 1947–1948.

(4) Supported in part by the research committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(5) Ciba Pharmaceutical Products, Inc., Summit, New Jersey. To whom inquiries regarding this manuscript should be sent.

(6) Chas. Pfizer and Co., Inc., Fellow, 1948.

(7) H. Adkins, R. M. Ross and D. C. Schroeder, THIS JOURNAL, **72**, 5401 (1950).



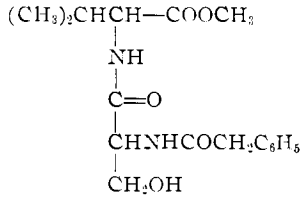
Ia, R = H
b, R = CH_3

II

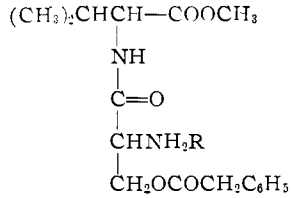
(Δ^2-oxazolinoyl)]-D valinate (Ib)[8] and the D-D isomer were isolated as oils in good over-all yields.

It is to be noted, however, that saponification of V followed by hydrogenolysis gave serylvaline in nearly quantitative yields, but the final condensation with the iminoester produced only small amounts of Ia (Route A). Actually, with L-seryl-D-valine, a 14% yield of the oxazoline was obtained, whereas D-seryl-D-valine underwent negligible reaction with the iminoester.

The structure of the oxazoline Ib was established by identification of the hydrolysis products. In alkaline medium, or upon prolonged standing, the L-D-isomer yielded crystalline methyl N-(N-phenylacetyl-L-seryl)-D-valinate (IX). Methyl N-(O-phenylacetyl-D-seryl)-D-valinate salts (Xa and Xb) were isolated when the D-D-isomer was hydrolyzed in acid solution. Further treatment of Xa with alkali and subsequent acidification gave phenylacetic acid.

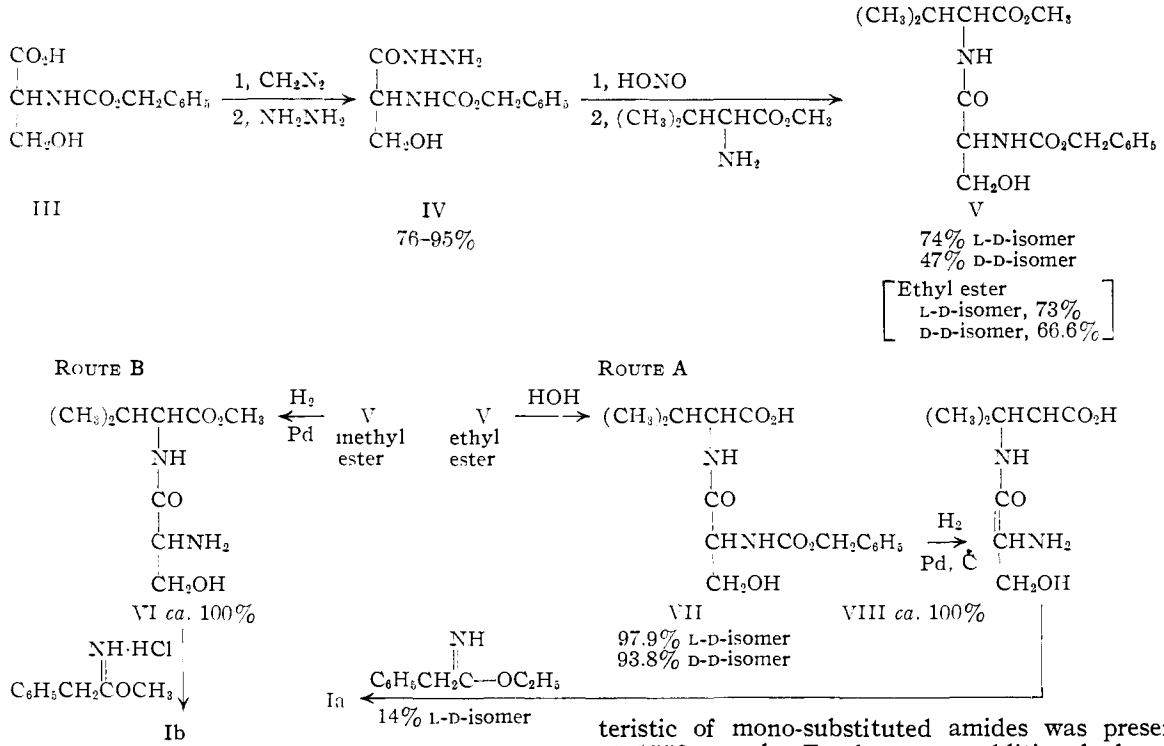


IX



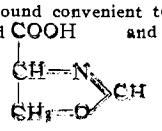
X a, R = HCl
b, R = $C_6H_3N_3O_7$

The infrared spectra of IX and of the corresponding oxazolines Ia and Ib were consistent with the proposed structures. With regard to the spectrum of the oxazoline acid (Ia), the >N—H, >C=O (carbonyl) and >C=O (amide) stretching frequencies were evident at 3301, 1716 and 1619 cm.$^{-1}$, respectively. A secondary band characteristic of mono-substituted amides was present at 1550 cm.$^{-1}$. Furthermore, additional absorption in the double bond region of the spectrum was found at 1667 cm.$^{-1}$, which is within the range of the unconjugated >C=N— frequencies.

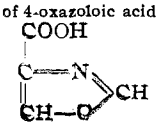


Selective reduction of the oxazole II was also considered as a possible means of synthesizing Ia. However, reduction of either the model compound N-(2-benzyl-4-oxazoloyl)-glycine or II with Raney

(8) It was found convenient to name I as a derivative of 4-Δ^2-oxazolinoic acid

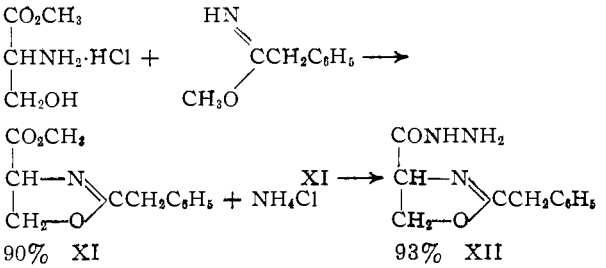


and II as a derivative of 4-oxazoloic acid



nickel–aluminum alloy and alkali[9] led to complete reduction, rupture of the ring or recovery of unchanged starting material.

In another unsuccessful approach to Ia or b, methyl serinate hydrochloride was condensed with methyl phenyliminoacetate to give 2-benzyl-4-carbomethoxy-2-oxazoline (XI).



Attempts to convert XI to the azide (through the hydrazide) or to the acid chloride for subsequent condensation with value or the valine ester were unsuccessful, even though the hydrazide XII was obtained readily and in excellent yield. Direct aminolysis of XI with the amino acid or the corresponding ester also failed.

We are indebted to earlier investigators for some of the general methods employed in this work. Preparation of serylvaline followed the Bergmann peptide procedure as modified by Fruton.[10] Sheehan[11] had previously reported the synthesis of the oxazoline XI *via* the same method used here. The preparation of oxazolines from iminoesters and amino alcohols, however, appears to have been first used by Bockmühl and Knoll.[12]

Experimental

NOTE: In some instances DL-serine and DL-valine were used in exploratory work, but the final compounds were prepared from the optically active amino acids.

N-Carbobenzyloxy-α-amino Acids.—Serine and valine were converted to their N-carbobenzyloxy derivatives in the customary fashion. "Alkacid" paper was found to be a convenient tool for checking the *p*H during the course of this reaction. Carbobenzyloxy chloride was prepared in the manner described by Carter, *et al.*[13] **N-Carbobenzyloxy-DL-serine**: yield 65%, m.p. 123–124° (from ethyl acetate–chloroform).[14] **N-Carbobenzyloxy-L-serine**[10]: yield 71%, m.p. 115–117° (from chloroform). The L-serine used here was obtained by the resolution of DL-serine or from raw silk hydrolysate.[15] **N-Carbobenzyloxy-DL-valine**: yield 98%, m.p. 68–70° (from chloroform); neut. equiv.: calcd. 251, found 259. **N-Carbobenzyloxy-D-valine**: yield 85%, m.p. 62–63.5° [from chloroform–petroleum ether (b.p. 60–68°) mixture], $[\alpha]^{23}D$ −6.1° (88.3 mg. in 2 ml. chloroform solution).[16] The D-valine used in preparing this compound was supplied through the courtesy of Merck and Co.

Resolution of Carbobenzyloxy-DL-serine.—Resolution was accomplished using the metho d of Doherty and Popenoe.[1] N-Carbobenzyloxy-DL-serine in the presence of papain was converted to N-[N-carbobenzyloxy-L-seryl]-anilide in 87.5% yield; m.p. 159–160° (from absolute ethanol), $[\alpha]^{27}D$ −17.9° (5% in glacial acetic acid). N-Carbobenzyloxy-D-serine was recovered from this reaction in 57% yield; m.p. 116–117°, $[\alpha]^{28}D$ −5.3° (in glacial acetic acid). L-Serine, $[\alpha]^{27.5}D$ +14.1° (186.9 mg. in 2 ml. of 1 *N* hydrochloric acid),[15] was obtained in 79% yield from the anilide.

Methyl N-Carbobenzyloxy-L-serinate.[10]—An ether suspension of N-carbobenzyloxy-L-serine was treated with a cold ether solution of diazomethane to give a quantitative yield of oily methyl N-carbobenzyloxy-L-serinate, $[\alpha]^{23}D$ +7.2° (127.2 mg. in 2 ml. of chloroform solution). The corresponding methyl N-carbobenzyloxy-D-serinate was prepared in the same manner.

Methyl N-Carbobenzyloxy-D-valinate.—N-Carbobenzyloxy-D-valine was esterified as above. The methyl ester, m.p. 57–59°, $[\alpha]^{23}D$ −5.5° (86.7 mg. in a 2 ml. of chloroform solution), was recovered in 98–100% yield.[18]

N-Carbobenzyloxyserylhydrazide (IV).[10]—The methyl esters of N-carbobenzyloxy-L- (and D-) serine were converted to the corresponding hydrazides, m.p. 179–180°, in 76–95% yield by the action of anhydrous hydrazine in methanol solution.

Methyl N-Carbobenzyloxy-L-seryl-D-valinate (V).—With cooling (0–5°) 7.13 g. (0.0282 mole) of N-carbobenzyloxy-L-serylhydrazide was dissolved in a mixture of 65 ml. of distilled water, 4.9 ml. of glacial acetic acid and 2.4 ml. of concentrated hydrochloric acid (sp. gr. 1.19). To this was added slowly with vigorous shaking a cold solution containing 2.5 g. of sodium nitrite in 16.3 ml. of water. The white oily azide which precipitated was extracted immediately with three 40-ml. portions of ethyl acetate. After washing with 33 ml. of cold water, two 33-ml. portions of cold 5% sodium bicarbonate solution and again with 33 ml. of cold water, the combined ethyl acetate extracts were dried over sodium sulfate. This dry solution was then added to an ethyl acetate solution of methyl D-valinate, prepared as described below.

Methyl N-carbobenzyloxy-D-valinate (10.12 g., 0.0382 mole) was subjected to hydrogenolysis in methanol solution over 5% palladium on activated charcoal catalyst[19] at one atmosphere hydrogen pressure and room temperature. The observed hydrogen absorption was 67% of theory. After removal of catalyst by centrifugation followed by filtration, the solution was concentrated under reduced pressure (water pump at 30°). Final methanol removal was completed using a vacuum pump for 15 minutes. The residue of methyl D-valinate weighed 5.45 g.

The reacting solution of azide and amino ester was allowed to stand at room temperature for 22 hours. At the end of this time the ethyl acetate solution was washed thoroughly with successive 60-ml. portions of 0.5 *N* hydrochloric acid, 5% sodium bicarbonate solution and water. It was then dried over anhydrous sodium sulfate, filtered and concentrated to dryness under reduced pressure at a temperature no higher than 30°. The crude crystalline residue was dried overnight at 0.1 mm. pressure. The yield was 7.4 g. (74% of theory), m.p. 126–130°. The crude peptide was recrystallized from ethyl acetate by the dropwise addition of petroleum ether (b.p. 60–68°) at room temperature; m.p. 143–144°, $[\alpha]^{26}D$ −35° (17.2 mg. in 1 ml. of chloroform solution).

Anal. Calcd. for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.87. Found: C, 58.07; H, 6.60.

Methyl N-Carbobenzyloxy-D-seryl-D-valinate (V).—This compound, m.p. 74–76°, $[\alpha]^{25}D$ +15.1° (104.6 mg. in 2.5 ml. of chloroform solution), was prepared by the same procedure as the L-D-isomer in 47% yield.

Anal. Calcd. for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.87. Found: C, 58.07; H, 6.75.

The corresponding ethyl esters were prepared by coupling the L and D moieties of N-carbobenzyloxyserylazide with ethyl

(9) (a) E. Schwenk, D. Papa, B. Whitman and H. Ginsberg, *J. Org. Chem.*, **9**, 175 (1944); (b) E. Schwenk, D. Papa, H. Hankin and H. Ginsberg, *Org. Syntheses*, **27**, 68 (1947).

(10) J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).

(11) Dr. John C. Sheehan, private communication to H. A.

(12) M. Bockmühl and R. Knoll, U. S. Patent 1,958,529 (1929).

(13) H. E. Carter, R. L. Frank and H. W. Johnston, *Org. Syntheses*, **23**, 13 (1943).

(14) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932), reported N-carbobenzyloxy-DL-serine; m.p. 125°.

(15) (a) D. G. Doherty, W. H. Stein and M. Bergmann, *J. Biol. Chem.*, **135**, 493 (1940); (b) W. H. Stein, S. Moore and M. Bergmann *ibid.*, **139**, 481 (1941); (c) W. H. Stein, S. Moore, G. Stamm, C. Chow and M. Bergmann, *ibid.*, **143**, 121 (1942).

(16) M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, **15**, 108 (1950), reported N-carbobenzyloxy-L-valine to be an oil.

(17) D. G. Doherty and E. A. Popenoe, Jr., *J. Biol. Chem.*, **189**, 447 (1951). The authors acknowledge the kind assistance of Mrs. Alva Rice in the resolution of serine.

(18) M. A. Nyman and R. M. Herbst, ref. 16, reported methyl N-carbobenzyloxy-L-valinate, m.p. 55–55.5°, $[\alpha]^{30}D$ +16.4° in ethanol.

(19) R. Mozingo, *Org. Syntheses*, **26**, 78, Method "C" (1946).

D-valinate (prepared from the hydrochloride in ether solution).

Ethyl N-carbobenzyloxy-L-seryl-D-valinate: yield 73.1%; m.p. 110.5–111°, $[\alpha]^{29}D$ −25° (79.3 mg. in 2 ml. of chloroform solution).

Anal. Calcd. for $C_{18}H_{26}N_2O_6$: C, 58.99; H, 7.15. Found: C, 59.14; H, 7.00.

Ethyl N-carbobenzyloxy-D-seryl-D-valinate: yield 66.6%, m.p. 68–69°.

Anal. Calcd. for $C_{18}H_{26}N_2O_6$: C, 58.99; H, 7.15. Found: C, 58.98; H, 6.98.

N-Carbobenzyloxyserylvaline (VII).—To 4.29 g. (0.0117 mole) of ethyl N-carbobenzyloxy-L-seryl-D-valinate in 39 ml. of methanol was added 11.70 ml. of 1 *N* sodium hydroxide solution. The saponification mixture was allowed to stand at 25° for 35 minutes. At the end of this time 12.35 ml. of 1 *N* hydrochloric acid was added, and the solution was concentrated to a thick sirup at water pump pressure while heating in a water-bath to 40–50°. The sirup was dissolved in 65 ml. of chloroform. Sodium chloride was removed by filtration, and chloroform was removed under reduced pressure. The residue was dried overnight under reduced pressure and weighed 3.87 g. (97.9% of theory). The N-carbobenzyloxy-L-seryl-D-valine was induced to crystallize only with difficulty by addition of water to a dioxane solution, followed by gradual removal of volatiles under reduced pressure. Crystals were collected after cooling the solution to 9° overnight, m.p. 141–142°.

Anal. Calcd. for $C_{16}H_{22}N_2O_6$: C, 56.79; H, 6.55. Found: C, 56.51; H, 6.56.

A later preparation of the compound gave a glossy material which failed to crystallize; $[\alpha]^{29}D$ −28° (24.2 mg. in 2 ml. of chloroform solution), neut. equiv.: calcd. 338, found 320.

N-Carbobenzyloxy-D-seryl-D-valine was prepared similarly, but was not obtained in crystalline form (98.8% of theory).

Serylvaline (VIII).—The hydrogenolysis of 3.87 g. (0.0114 mole) of N-carbobenzyloxy-L-seryl-D-valine was carried out in 125 ml. of absolute methanol over 3 g. of 5% palladium on activated charcoal catalyst[19] using an atmospheric hydrogenation apparatus. The hydrogen absorption was 148 ml. (50% of theory). The catalyst was collected on a Filter-Cel mat on a fritted glass funnel, and was washed with 75 ml. of absolute methanol. The methanol solutions were evaporated to dryness at 30–45° at water pump pressure, and the puffy white residue was dried 12 hours at 0.1 mm. and room temperature. Even after drying the L-seryl-D-valine remained a white amorphous solid rather than crystalline (*ca.* 100% of theory); $[\alpha]^{29.5}D$ 0.0° (14.9 mg. in 2 ml. of methanol solution), neut. equiv.: calcd. 204, found 201 (by formol titration).

D-Seryl-D-valine was also obtained as an amorphous solid (*ca.* 100% of theory). This extremely hygroscopic powder melted at 75–80°.

Anal. Calcd. for $C_8H_{16}N_2O_4$: C, 47.02; H, 7.90. Found: C, 47.74; H, 7.21.

N-[2-Benzyl-4(L)-4-(Δ^2-oxazolinoyl)]-D-valine (Ia).—In a 125-ml. round-bottom flask, 0.60 g. (0.0029 mole) of L-seryl-D-valine was suspended in 40 ml. of absolute ethanol. To the suspension was added 0.65 g. (0.0039 mole) of freshly distilled ethyl phenyliminoacetate.[20] The reaction mixture was stirred at room temperature for 24 hours. At the end of this time ethanol was removed under reduced pressure, and the non-volatile residue was heated for two additional hours at 50° and 0.1 mm.

The residue was washed thoroughly with anhydrous ether to remove phenylacetic acid and ethyl phenyliminoacetate. To the washed material was added 0.1 *N* hydrochloric acid until a *p*H of 2–3 (alkacid paper) was attained. The acidic suspension was extracted with chloroform, and the extract was washed with water and dried over anhydrous sodium sulfate. Chloroform was removed from the solution under reduced pressure (room temperature (15–40 mm.)). The residue was dissolved in 2 ml. of chloroform, and a solid product was crystallized by the dropwise addition of petroleum ether (b.p. 60–68°). Crystals of N-(2-benzyl-4(2)-oxazolinoyl)-D-valine were removed by filtration. The weight of this material was 0.12 g. and two additional recrystallizations of the product in this same manner were necessary to produce a product of reasonable purity, m.p. 146–148° (soft).

Anal. Calcd. for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.63; neut. equiv., 304. Found: C, 62.33; H, 6.74; neut. equiv., 318.

When the above experiment was repeated using D-seryl-D-valine and tripling the reaction time, none of the desired N-[2-benzyl-4(D)-4-(Δ^2-oxazolinoyl)]-D-valine could be isolated. The bulk of the material was found in the aqueous hydrochloric acid solution rather than the chloroform extract and was identified tentatively as a mixture of the dipeptide hydrochloride and some partially condensed material. This mixture, isolated by evaporating the aqueous hydrochloric acid solution to dryness (15–30 min. at 40°), was treated further with another equivalent of ethyl phenyliminoacetate in dry ethylene dichloride for 48 hours at *ca.* 25°. The reaction mixture was worked up in the same manner as before and again most of the material was found in the aqueous hydrochloric acid solution. The chloroform extract this time, however, yielded 100 mg. of crystalline material which after recrystallization from ethanol melted at 133–134°, and may have consisted mainly of the desired N-[2-benzyl-4(D)-4-(Δ^2-oxazolinoyl)]-D-valine.

Anal. Calcd. for $C_{16}H_{20}N_2O_4$: C, 63.15; H, 6.62. Found: C, 62.84; H, 7.16.

The aqueous acid residue was again recovered and after taking it up in anhydrous ethanol and filtering to remove inorganic salts, the ethanol was removed leaving a thick sirup. The latter was then subjected to a temperature of 100° (0.5 mm.) for one hour, but none of the desired oxazoline could be isolated after this treatment.

Methyl Serylvalinate (VI).—Methyl N-carbobenzyloxy-L-seryl-D-valinate (3.24 g., 0.00921 mole) in 150 ml. of absolute methanol was subjected to hydrogenolysis in the presence of 1.0 g. of 5% palladium on activated charcoal catalyst.[20] Within 20 minutes, 180 ml. (theory 227 ml.) of hydrogen had been absorbed (79%). Concentration of the filtered methanol solution under the diminished pressure of the water pump at 30–35°, and finally at a pressure of 0.1 mm. for 90 minutes, yielded 2.06 g. (*ca.* 100%) of sirupy methyl L-seryl-D-valinate. The free peptide ester was used immediately without purification; $[\alpha]^{23}D$ −1.7° (46.7 mg. in 2 ml. of chloroform solution).

Similarly, methyl D-seryl-D-valinate was prepared and used immediately to prevent conversion to the substituted diketopiperazine. The optical rotation of the crude methyl D-seryl-D-valinate was $[\alpha]^{24}D$ +6.23° (100 mg. in 2.5 ml. of chloroform solution).

Methyl N-[2-Benzyl-4(L)-4-(Δ^2-oxazolinoyl)]-D-valinate (Ib).—In a 125-ml. round-bottom flask fitted with a rubber-sealed Hershberg stirrer, a solution of 1.24 g. (0.00568 mole) of methyl L-seryl-D-valinate in 50 ml. of ethylene dichloride was prepared. Then 0.085 g. (0.00057 mole) of freshly distilled methyl phenyliminoacetate and 1.05 g. (0.00568 mole) of freshly precipitated methyl phenyliminoacetate hydrochloride were added. Stirring was started, and for a few seconds the solution was homogeneous. However, within one minute, the solution became turbid, and ammonium chloride began to precipitate. The reaction mixture was stirred at room temperature for 27 hours in the dark.

At the end of the stirring period the ammonium chloride was removed by filtration and the filtrate concentrated to dryness at 0.1 mm. and 35°. The residue, believed to be the crude oxazoline, weighed 1.68 g. (93.0% of theory); $[\alpha]^{23.2}D$ +21.5° (46.6 mg. in 2 ml. of chloroform solution). All attempts to crystallize this product proved futile and so purification was effected by distillation through a short path apparatus. This was accomplished using a bath temperature of 150–180° and pressure of 0.07 mm. with no apparent signs of decomposition. After redistillation, the oxazoline was still an oil; $[\alpha]^{23}D$ +29° (33.3 mg. in 2 ml. of chloroform solution), $[\alpha]^{23}D$ +60° (88.7 mg. in 2.5 ml. of dioxane solution).

Anal. Calcd. for $C_{17}H_{22}O_4N_2$: C, 64.13; H, 6.97; CH_3O, 9.74. Found: C, 63.32; H, 7.00; CH_3O, 10.75.

Another similar experiment also gave an oil; $n^{25}D$ 1.5173,

(20) S. M. McElvain and C. L. Stevens, THIS JOURNAL, **68**, 1917 (1946). Ethyl phenyliminoacetate, b.p. 96–98.5° (2 mm.), and methyl phenyliminoacetate, m.p. 47–49°, b.p. 62° (0.2 mm.), $n^{25}D$ 1.5210, were prepared from their respective hydrochlorides by treating an ether suspension of the hydrochloride with potassium carbonate solution.

$[\alpha]^{25}D$ +26° (76.8 mg. in 2 ml. chloroform solution). The infrared absorption spectrum of this material was in agreement with that expected for the methyl ester of N-[2-benzyl-4(L)-4-(Δ²-oxazolinoyl)]-D-valine.

Methyl N-[2-Benzyl-4(D)-4-(Δ²-oxazolinoyl)]-D-valinate.—Methyl D-seryl-D-valinate (1.21 g.) was condensed with methyl phenyliminoacetate hydrochloride (1.025 g.) by the above procedure. The crude oily product was distilled at a bath temperature of 175° at 0.2 mm., $[\alpha]D$ −50.6° (105 mg. in 2.5 ml. solution of chloroform). Again it was not possible to obtain the oxazoline in crystalline form. However, the yield of distilled product was 73%.

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; CH_3O, 9.74. Found: C, 63.88; H, 6.86; CH_3O, 9.78.

Methyl N-(N-Phenylacetyl-L-seryl)-D-valinate (IX).—A chloroform solution of 1.23 g. (0.00387 mole) of crude methyl N-[2-benzyl-4(L)-4-(Δ²-oxazolinoyl)]-D-valinate was washed with 5 ml. of 5% sodium bicarbonate solution. The chloroform solution was dried over sodium sulfate, filtered and concentrated under reduced pressure. The oily residue weighed 0.94 g. and became semi-solid after standing for two weeks. A small volume of ether was added and the crystalline material which separated was collected by centrifugation, washed twice with ether and dried. The mother liquor was concentrated, and trituration was repeated until six crystalline fractions, melting between 145–165° had been collected. These were combined and recrystallized from chloroform by dropwise addition of petroleum ether. This material weighed 340 mg. and melted 165–166° with softening at 160°. Further purification was effected by recrystallization from methanol–water; m.p. 166.5–167.5°, $[\alpha]^{22.8}D$ −41° (29.2 mg. in 2 ml. of chloroform solution). In view of previous observations concerning the opening of oxazoline rings[21] and on the basis of the melting point[22] this product was believed to be methyl N-(N-phenylacetyl-L-seryl)-D-valinate. This was further substantiated by the infrared spectrum and elemental analysis.

Anal. Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33; CH_3O, 9.27; sapn. equiv., 336.5. Found: C, 60.55; H, 7.14; N, 8.52; CH_3O, 9.32; sapn. equiv., 333.[23]

Methyl N-(O-Phenylacetyl-D-seryl)-D-valinate Hydrochloride (Xa).—Methyl N-[2-benzyl-4(D)-4-(Δ²-oxazolinoyl)]-D-valinate (Ib) (0.843 g.) was added to a dioxane solution containing two equivalents of concentrated hydrochloric acid. Hydrolysis was complete in 30 minutes as shown by a change in specific rotation from −70 to +9.0°. Removal of the solvent gave an oily residue which crystallized as fine white needles from dioxane, 0.678 g., m.p. 77–79°. Because no crystalline product could be isolated using any of the other common solvents, and on the basis of the elemental analysis, it was concluded that the hydrolysis product, methyl N-(O-phenylacetyl-D-seryl)-D-valinate hydrochloride had crystallized with one-half mole of dioxane.

Anal. Calcd. for $C_{17}H_{25}ClN_2O_5 \cdot {}^1/_2C_4H_8O_2$: C, 54.74; H, 7.01; Cl, 8.51; neut. equiv., 417. Found: C, 54.76; H, 6.72; Cl, 8.55; neut. equiv., 401.

Removal of the dioxane of crystallization by heating was not feasible because the compound tended to decompose when the temperature was raised to 90°. This was evinced by the formation of water insoluble materials. The removal of the dioxane was accomplished by dissolving 48.7 mg. of the crystalline product in methanol (0.1 ml.) and removing all of the volatile products under reduced pressure at room temperature. A white spongy solid Xa, 44.3 mg., remained which was completely water soluble.

Anal. Calcd. for $C_{17}H_{25}ClN_2O_5$: Cl, 9.51. Found: Cl, 9.29.

A sample of Xa (103 mg.) was further hydrolyzed in 1 *N* sodium hydroxide at room temperature. The reaction mixture was extracted with chloroform and 6.6 mg. of oil was removed but not identified. After acidification the aqueous solution was again extracted with chloroform and 19.9 mg. (60% of theory) of phenylacetic acid was isolated. Melting point (76.4°) and mixture melting point determinations served to identify this compound.

When a sample of the L-D-isomer Ib was subjected to the conditions of acid hydrolysis given above a change in specific rotation from +60 to +16° was noted within 15 minutes. No effort was made to isolate the product, presumably methyl N-(O-phenylacetyl-L-seryl)-D-valinate.

Methyl N-(O-Phenylacetyl-D-seryl)-D-valinate Picrate (Xb).—Partial hydrolysis also resulted when methyl N-[2-benzyl-4(D)-4-(Δ²-oxazolinoyl)]-D-valinate (Ib) was warmed (50°) with picric acid in 95% ethanol. The methyl N-(O-phenylacetyl-D-seryl)-D-valinate picrate (Xb) which formed gave yellow needles from ethanol; m.p. 157.5–158°.

Anal. Calcd. for $C_{23}H_{27}N_5O_{12}$: C, 48.85; H, 4.81. Found: C, 48.54; H, 4.46.

N-(2-Benzyl-4-oxazoloyl)-glycine.—To 5.0 g. of 2-benzyl-4-carboxyhydrazideoxazole[8] dissolved in 33 ml. of 1 *N* hydrochloric acid at 0° was added 1.75 g. of sodium nitrite in 20 ml. of water. The crystalline azide which precipitated was rapidly collected on a filter and added to a cold solution of 2.27 g. of glycine, 1.22 g. of sodium carbonate, and 1.93 g. of sodium bicarbonate in 47 ml. of water. The mixture was stirred four hours at 0° and overnight while warming to room temperature. The solution was filtered and acidified with 15% hydrochloric acid. An oily liquid separated and was taken up in ether. The crude N-(2-benzyl-4-oxazoloyl)-glycine recovered from the ether, was recrystallized from hot chloroform, yield 4.90 g. (82%), m.p. 126–127.5°.

Anal. Calcd. for $C_{13}H_{12}N_2O_4$: C, 59.99; H, 4.65; neut. equiv., 260. Found: C, 59.64; H, 4.57; neut. equiv., 260.

N-(2-Benzyl-4-tetrahydroöxazoloyl)-glycine.—In a 150-ml. beaker immersed in an ice-bath and fitted with a thermometer and a Hershberg stirrer, a solution of 1.40 g. of sodium hydroxide in 40 ml. of water was added to 1.40 g. of N-(2-benzyl-4-oxazoloyl)-glycine. To the cold (10°) solution was added 4.2 g. of Raney nickel–aluminum alloy in small increments over a period of three hours. The reduction mixture was maintained at 10° for 30 minutes after the last addition of alloy, then cooled to 0°, and filtered through a mat of Filter-Cel on a fritted glass funnel. The nickel residue was washed with 25 ml. of cold distilled water. The filtrate and wash water were then added in a thin stream to 9 ml. of concentrated hydrochloric acid in 4 ml. of water cooled to 0° and efficiently stirred. The acid solution containing a suspension of aluminum salts was extracted immediately with seven portions (25 ml. each) of chloroform. After the aqueous acid layer containing sodium and aluminum salts had been concentrated to dryness under reduced pressure, the salt residue was extracted repeatedly with hot chloroform. In this way 0.70 g. of white solid melting from 138–163° was obtained. This solid, N-(2-benzyl-4-tetrahydroöxazoloyl)-glycine, was recrystallized from ethyl acetate; m.p. 161–163°.

Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C, 58.96; H, 6.09; neut. equiv., 264. Found: C, 59.18; H, 5.92; neut. equiv., 280.

Reduction of N-(2-Benzyl-4-oxazoloyl)-D-valine (II).—The Schwenk reduction of the oxazole II was carried out as above except that the product was extracted from the aqueous acid solution with chloroform. To remove alumina carried down in the first extract, the chloroform solution was extracted with seven 15-ml. portions of 1% sodium hydroxide. The alkaline solution was then washed with 15 ml. of chloroform and made acid to a *p*H of 2 with 3 *N* hydrochloric acid. An oily white precipitate formed which was extracted with seven 25-ml. portions of chloroform. After drying over sodium sulfate the chloroform solution was concentrated to 7 ml. (15–30 mm. and 25°) and petroleum ether (60–68°) was added slowly; the inner walls of the flask were scratched to induce precipitation. This product (0.47 g.) was dissolved in a 1% solution of alkali and reprecipitated with hydrochloric acid for purification purposes. In this way a product believed to be N-(N-phenylacetyl-DL-alanyl)-D-valine,[24] m.p. 181–185°, was isolated. (*Anal.* Calcd. for $C_{10}H_{22}N_2O_4$: C, 62.72; H, 7.24,

(21) R. H. Wiley and L. L. Bennett, Jr., *Chem. Revs.*, **44**, 447 (1949).

(22) W. Baker and W. D. Ollis, *J. Chem. Soc.*, 556 (1951), reported methyl N-(N-phenylacetyl-L-seryl)-D-valinate, m.p. 164.5° (from methanol–water, $[\alpha]^{24}D$ −6.7° (*c* 1.0 in methanol). They prepared the compound by the Raney nickel desulfurization of methyl N-(N-phenylacetyl-L-seryl)-penicillamine.

(23) The saponification equivalent was determined by the method of M. L. Wolfrom, M. Konigsberg and S. Soltzberg, THIS JOURNAL, **58**, 490 (1936).

(24) "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 260.

Found: C, 62.91; H, 7.22). The filtrate was concentrated to dryness (reduced pressure and *ca.* 25°) and a residue of 0.17 g., m.p. 130–149°, isolated. No effective separation of this mixture was accomplished.

2-Benzyl-4-carbomethoxy-2-oxazoline (XI).—To 10.0 g. (0.0643 mole) of methyl DL-serinate hydrochloride was added 10.5 g. (0.0707 mole) of freshly distilled methyl phenyliminoacetate. The suspension was stirred overnight during which time the contents of the flask were protected from light. After 20 hours of stirring the suspension was filtered. The residue collected on the filter weighed 4.80 g. (3.44 g. theoretical for ammonium chloride). The filtrate was concentrated and distilled; b.p. 120–121° (0.2 mm.), $n^{25}D$ 1.5288. The yield of 2-benzyl-4-carbomethoxy-2-oxazoline (X) was 10.78 g. (76.5% of theory).

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98. Found: C, 65.62; H, 5.93.

O-Phenylacetyl-DL-serine.—The saponification of 4.00 g. (0.0183 mole) of methyl 2-benzyl-4-Δ^2-oxazolinoate with 18.25 ml. of 1 *N* sodium hydroxide in 100 ml. of methanol was carried out in four hours at room temperature. Methanol was then removed under reduced pressure at 30° and after filtration the solution was acidified with 19 ml. of 1 *N* hydrochloric acid. The white solid which precipitated was filtered and, after drying in a vacuum desiccator, weighed 3.3 g. (80.5% of theory); m.p. 159–160°. A positive ninhydrin test indicated that O-phenylacetyl-DL-serine, rather than the desired 2-benzyl-4-carboxy-2-oxazoline, was obtained.

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.18; H, 5.87; neut. equiv., 223. Found: C, 59.14; H, 5.68; neut. equiv., 219.

All attempts to prepare 2-benzyl-4-Δ^2-oxazolinoic acid or its sodium or potassium salt failed.

Attempted Preparation of Ia *via* 2-Benzyl-4-Δ^2-oxazolinoyl Chloride.—Saponification of 2-benzyl-4-carbomethoxy-2-oxazoline in absolute methanolic potassium hydroxide gave a glassy residue which was treated with oxalyl chloride.[25] After removal of excess oxalyl chloride, the residue was dissolved in acetone and treated with L-valine in the presence of a few drops of pyridine. The product was an unidentified golden yellow solid; m.p. 238–242° dec., neut. equiv. 516, sublimed at 180° bath temperature (0.1 mm.). None of the desired product was isolated.

Methyl O-Phenylacetyl-DL-serinate Hydrochloride.—To a solution of 1.00 g. (0.00456 mole) of 2-benzyl-4-carbomethoxy-2-oxazoline in 50 ml. of purified dioxane was added 0.76 ml. (0.00912 mole) of concentrated (12 *N*) hydrochloric acid. After 90 minutes at room temperature, the solution was concentrated to dryness at reduced pressure and 30°. Two recrystallizations from dioxane–absolute ether yielded methyl O-phenylacetyl-DL-serinate hydrochloride, m.p. 142–144°.

Anal. Calcd. for $C_{12}H_{16}ClNO_4$: C, 52.65; H, 5.89; Cl, 12.95; neut. equiv., 273. Found: C, 52.66; H, 5.85; Cl, 12.9; neut. equiv., 260.

Attempted Preparation of Ia *via* 2-Benzyl-4-carboxhydrazide-2-oxazoline (XII).—By the action of anhydrous hydrazine upon 2-benzyl-4-carbomethoxy-2-oxazoline (XI) in methanol a nearly quantitative yield of a compound believed to be 2-benzyl-4-carboxhydrazide-2-oxazoline (XII), m.p. 207–208° dec., was obtained.

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98. Found: C, 60.23; H, 6.09.

Attempts to form the azide in aqueous hydrochloric acid with sodium nitrite or in ethanol using amyl nitrite and sodium ethoxide were unsuccessful. However, by dissolving the hydrazide in cold glacial acetic acid and treating with sodium nitrite, there was some indication that the azide had formed. Even so, further treatment of the "azide" with DL-valine gave no evidence of a coupling product.

Attempted Aminolysis of 2-Benzyl-4-carbomethoxy-2-oxazoline.—To 2.60 g. (0.0119 mole) of 2-benzyl-4-carbomethoxy-2-oxazoline was added a chloroform solution of methyl DL-valinate (from 2.00 g. (0.0120 mole) of methyl DL-valinate hydrochloride). The solution was subjected to 100,000 p.s.i.[26] at room temperature for 16 hours. When the pressure was released the solution was light yellow in color with a trace of suspended matter. Concentration gave a viscous, clear yellow liquid which became glassy on drying at reduced pressure. This product may have been impure methyl N-(2-benzyl-4-Δ^2-oxazolinoyl)-valinate (Ib).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: CH_3O, 9.74. Found: CH_3O, 9.17.

Distillation of a small amount of this material at 225–250° (bath temperature) and 0.1 mm. was accomplished with difficulty. The distillate was a yellow gum and all attempts to obtain a crystalline material failed.

At atmospheric pressure an ether solution of 2-benzyl-4-carbethoxy-2-oxazoline and ethyl D-valinate showed no reaction after 22 hours at 0° and 24 hours at room temperature.

(23) A. L. Wilds and C. H. Shunk, THIS JOURNAL, **70**, 2427 (1948).

(26) High pressure accelerates the aminolysis reaction as will be shown in a subsequent communication from this Laboratory.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

Desethyllycoramine

BY W. C. WILDMAN[1] AND W. T. NORTON[2]

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Two synthetic routes to hydrophenanthridines related to the *Lycoris* alkaloids have been investigated. A synthesis of desethyllycoramine (IVa) from 4-(3-methoxyphenyl)-5-nitrocyclohexene is described.

Lycoramine, an alkaloid isolated from *Lycoris radiata* by Kondo, Tomimura and Ishiwata,[3] is considered to possess the structure represented by IVb.[4,5] In view of several anomalies in the proof of structure of lycoramine and other closely related compounds, it seemed desirable to find suitable synthetic routes to these compounds as a means of final verification of structure. This paper describes a synthesis of desethyllycoramine (IVa).

For this synthesis, 4-(3-methoxyphenyl)-5-nitrocyclohexene proved to be an ideal starting material. The double bond was in the proper position for the introduction of the vicinal glycol function and the nitro group could be converted readily to the heterocyclic nitrogen atom. Two alternate routes using this general plan were investigated to determine the point at which the hydroxyl groups could be introduced most successfully. Hydroxylation of VII with performic acid was not satisfac-

(1) National Heart Institute, National Institutes of Health, Bethesda, Maryland.

(2) Abstracted from a thesis submitted by W. T. Norton in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Princeton University, September, 1953.

(3) H. Kondo, K. Tomimura and S. Ishiwata, *J. Pharm. Soc. Japan*, **52**, 51 (1932).

(4) H. Kondo and S. Ishiwata, *Ber.*, **70**, 2427 (1937).

(5) An excellent review of the alkaloids of the *Amaryllidaceae* may be found in R. H. F. Manske, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 331.