The Formation and Rearrangement of Isocyano Peptides

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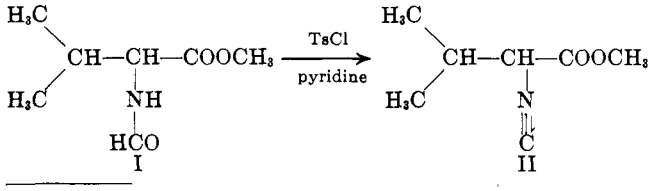
While dehydration of gramicidin A (a linear N-formyl pentadecapeptide ethanolamide) with a large excess (~40 moles) of tosyl chloride in pyridine led to the unstable isocyanide, comparatively stable isocyanides were obtained by dehydration of N-formyl-DL-valine methyl ester (I) and ethyl N-formyl-DL-valylglycinate (VIIa). By contrast N-formyl-DL-valinamide (III) on dehydration gave α-formylaminoisovaleronitrile (IV), and N-formyl-DL-valylglycinamide (VIIb) gave N-formyl-DL-valinecyanomethylamide (IX) *via* the initially formed unstable isocyanide VIII by a rearrangement which is pictured as an intramolecular dehydration.

Gramicidin A is a *linear* peptide antibiotic whose terminal amino group is substituted by formyl and whose terminal carboxyl group is linked to ethanolamine.[2,3] The only functional group is the hydroxyl of the terminal aminoethanol whose substitution modifies the action of gramicidin.[4] In an analogous fashion, modification of the formylvaline terminal of gramicidin would yield information on its influence on the antibiotic properties.

The reaction of gramicidin A with tosyl chloride[5] affects both ends of the gramicidin molecule. Depending on conditions both esterification of the free hydroxyl group and dehydration of the formamino terminal are observed.

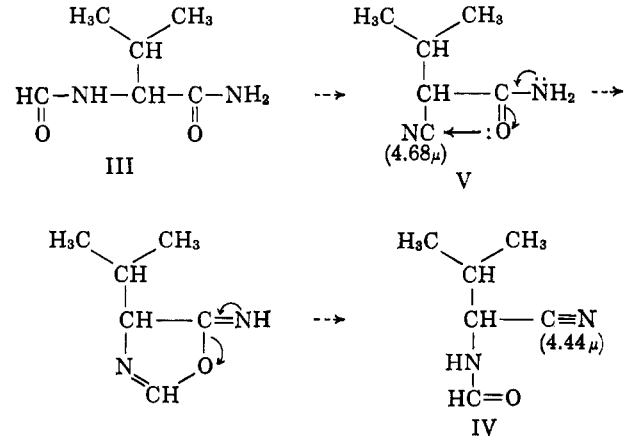
When gramicidin was treated with a moderate excess (2:1) of tosyl chloride in pyridine, no isocyanide was formed, regardless of temperature and time of reaction. When a large excess of tosyl chloride (40:1) was used, however, the isocyanide derivative of gramicidin was detected by its characteristic infrared absorption peak at 4.70 μ. The isocyanide is very reactive, and the 4.70-μ peak disappeared in contact with glacial acetic acid at 20°. During the formation of the isocyanide of gramicidin the extinction of the four tryptophan units at 282 mμ decreased by 10%. In addition prolonged reaction with tosyl chloride led to the gradual disappearance of isocyanide and the formation of colored products. The interaction of isocyanide groups[6] with indoles is well known.[7]

In order to study the range of such interactions the behavior of simple formylvaline model derivatives on dehydration was studied. N-Formyl-DL-valine methyl ester (I) was dehydrated to give methyl α-isocyanoisovalerate (II). Tosyl chloride, as well as phosphorus oxychloride and phosgene, effected this dehydration



I II

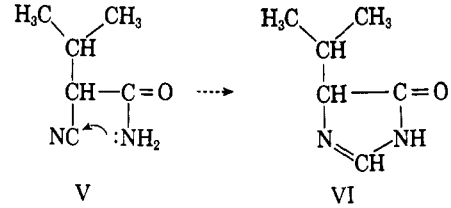
smoothly.[8] The dehydration of N-formyl-DL-valinamide (III) or N-formyl-DL-valine by tosyl chloride led to red-brown pigments, in addition to ninhydrin-reac-



III V IV

tive products. Colored products are generally observed when peptides react with tosyl chloride in pyridine and do not necessarily arise from an isocyanide intermediate. N-Formyl-DL-valinamide (III) was dehydrated to its nitrile IV and not the isonitrile V. Even at the early stage of the dehydration of III only the peak of the nitrile at 4.44 μ was observed in the infrared spectrum of the reaction mixture. The question arises whether the nitrile IV is formed directly by dehydration of the amide III, by preferential hydration of an isocyano–cyano derivative, or by an intramolecular dehydration mechanism as pictured in III → V → IV.

Cyclization of the initially formed isocyanide V to the 4-imidazolone VI is another possibility. Indeed, one of the fractions obtained by paper chromatography showed an absorption maximum characteristic of 4-



V VI

imidazolone[9] at 259 mμ in 0.1 *N* hydrochloric acid. However, there is considerable doubt about the nature of this product, since it does not have the lability normally associated with 4-imidazolones.

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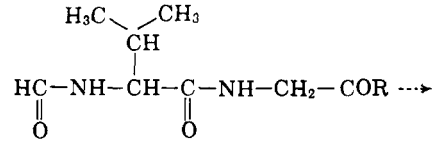
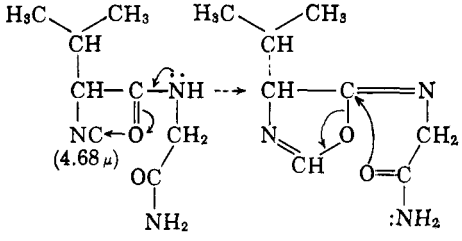
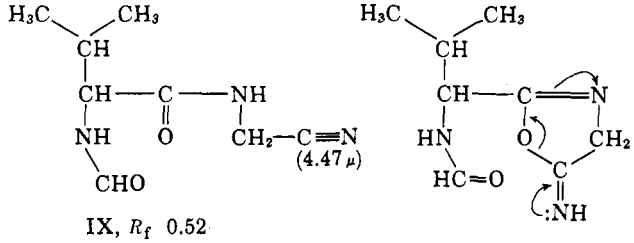


VIIa, R = OEt
b, R = NH_2; R_f 0.20



VIII, R_f 0.68



IX, R_f 0.52

N-Formyl-DL-valylglycine ethyl ester (VIIa) and its amide VIIb were also dehydrated to isocyanides. The purified isocyanide of the peptide ester was quite stable at room temperature. In acid solution it reverted rapidly to the formylamino derivative VIIa.

The dehydration of formylvalylglycinamide (VIIb, R_f 0.20) was different. There an intermediate (R_f 0.68) was obtained which showed absorption peaks of both the isocyano and cyano group. Repeated thin layer chromatography (silicic acid, EtOA–CH_3OH 5:1) led to separation of the isocyanide from the nitrile. The initial isocyanide VIII (R_f 0.68) is unstable and, on standing in polar solvents, rearranges to formyl-DL-valinecyanomethylamide (IX, R_f 0.52), a crystalline compound. This rearrangement is best pictured as an intramolecular process (VIII → IX) in analogy to the dehydration of other amino acid amides,[10,11] where the use of O^{18} lent support to an intramolecular mechanism.

Experimental

Dehydration of Gramicidin A with Tosyl Chloride.—Commercial gramicidin A (206 mg., Nutritional Biochemicals Corp., lot no. 1848) was dissolved in 3 ml. of pyridine and tosyl chloride (751 mg., purified) was added. Since dehydration under cooling with ice–water resulted only in O-tosylation (infrared band at 7.3 μ), the pyridine solution was stirred at room temperature for 3 hr. and poured into 100 ml. of ice-cold water. The brown precipitate was collected by filtration and washed thoroughly with cold water and ether. The residue was dissolved in 20 ml. of chloroform and washed successively with ice-cold 0.1 *N* hydrochloric acid, 5% sodium bicarbonate, and water. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to leave 184 mg. of a tan glass: $\epsilon_{282m\mu}$ (g./l.) 11.2 (ethanol), 4.70 (isonitrile group), and 7.30 μ (–O–SO_2– group). The isocyanide of gramicidin in methanol when passed through Dowex 1X8 (HO^- form) gave almost colorless material which in the infrared still showed the presence of the isonitrile peak. This isonitrile fraction was dissolved in 0.5 ml. of glacial acetic acid and kept for 3 hr. When the acetic acid was evaporated *in vacuo*, the residue had no more isonitrile absorption at 4.70 μ.

Formyl-DL-valinamide (III).—Formyl-DL-valine was esterified with diazomethane and the noncrystalline viscous ester was converted to the amide in methanol solution saturated with ammonia on standing for 3 days at 20°. The amide, yield 60% from formylvaline, was recrystallized from methanol, m.p. 197.5–198.5°. The analytical sample, recrystallized from water, had m.p. 199.5–201°.

Anal. Calcd. for $C_6H_{12}N_2O_2$: C, 49.99; H, 8.39; N, 19.44. Found: C, 50.23; H, 8.17; N, 19.16.

Formyl-DL-valylglycine Ethyl Ester (VIIa).—Formyl-DL-valine (4.35 g.) was suspended in 50 ml. of methylene chloride and 3 g. of dicyclohexylcarbodiimide was added. After 0.5 hr. at 20° an ethereal solution (30 ml.) of glycine ethyl ester, prepared from its hydrochloride (5.6 g.) and triethylamine (6 ml.), was added. The reaction mixture was kept at 20° for 1 hr. An additional 3 g. of carbodiimide was added and the ester in methylene chloride was added after 1 hr. After 6 hr. dicyclohexylurea (6.3 g.) was removed by filtration and the filtrate was concentrated until crystallization started. The crystals (4.5 g.) were collected and washed with ether. From the mother liquor an additional crop (1.1 g.) was obtained. Recrystallization from ethyl acetate and subsequently from 75% aqueous ethanol gave analytically pure material, m.p. 136–137.5°.

Anal. Calcd. for $C_{10}H_{18}N_2O_4$: C, 52.17; H, 7.88; N, 12.17. Found: C, 52.09; H, 7.86; N, 12.03.

Formyl-DL-valylglycinamide (VIIb).—Formyl-DL-valylglycine ethyl ester (1.8 g.) was dissolved in methanol (70 ml.) and saturated with anhydrous ammonia under cooling with ice–water. The methanol solution was allowed to stand in a stoppered bottle for 3 days at 20°. After removal of the solvent, crystalline formyl-DL-valylglycinamide (1.1 g.) was obtained and recrystallized from methanol, m.p. 182.5–183.5°.

Anal. Calcd. for $C_8H_{15}N_3O_3$: C, 47.75; H, 7.51; N, 20.88. Found: C, 48.03; H, 7.46; N, 20.76.

Dehydration of Formyl-DL-valine Methyl Ester (I).—Formyl-DL-valine methyl ester (5.2 g.) was dissolved in pyridine (15 ml.) and tosyl chloride (6.4 g.) was added. The pyridine solution was stirred in an ice–water bath until, after 2 hr., the starting material had disappeared. After the addition of 200 ml. of water the reaction mixture was extracted five times with ether (40 ml.); the combined extracts were washed with 10% tartaric acid, and water, and dried. When the ether was removed under reduced pressure, 3.3 g. of methyl α-isocyanoisovalerate (odor!) was obtained. On thin layer chromatography on silica (benzene–ethyl acetate, 4:1 v./v.) this fraction showed one major spot (R_f 0.55). Vapor phase chromatography [4% NGS on Chromosorb W, column 0.4 cm. × 170 cm., 82°, carrier gas (N_2) 14 cc./min.] showed one single peak, retention time 4.8 min.; infrared absorption ($CHCl_3$) 4.62 (isocyanide) and 5.68 μ (ester).

The isocyano ester was characterized by its condensation reaction with dinitrophenylglycine (DNP-glycine), acetaldehyde, and benzylamine[12] to yield DNP-glycyl-N-benzyl-DL-alanyl-DL-valine methyl ester, m.p. 162–164°.

Anal. Calcd. for $C_{24}H_{29}N_5O_8$: C, 55.91; H, 5.67; N, 13.59. Found: C, 56.11; H, 5.79; N, 13.28.

The chloroform solution of this isocyano ester rapidly decolorized a solution of 10% bromine in chloroform. Thin layer chromatography (benzene–ethyl acetate, 4:1) showed the formation of an unstable bromination product which on standing overnight in chloroform decomposed or rearranged to a ninhydrin-positive (R_f 0) and ninhydrin-negative material.

Dehydration of Formyl-DL-valinamide (III).—Formyl-DL-valinamide (532 mg.) was suspended in pyridine (3 ml.) and tosyl chloride (579 mg.) was added. The suspension changed to a solution within a few minutes. After 3 hr. at 20° the pyridine was removed *in vacuo* and the viscous residue was extracted with ethyl acetate. The ethyl acetate extract was evaporated and the residue was dissolved in methylene chloride. Insoluble starting material (40 mg., m.p. 189–193°) was filtered off. The methylene chloride was evaporated and the residue was taken up in methanol. The methanol solution was passed through a column of Dowex 50 × 8. Purification by thin layer

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chromatography in benzene–ethyl acetate (4:1) led to crystalline α-formylaminoisovaleronitrile (IV, 43.2 mg.), m.p. 39.5–40°, R_f 0.07.

Anal. Calcd. for $C_6H_{10}N_2O$: N, 22.23. Found: N, 22.24.

This compound showed the typical nitrile absorption at 4.44 μ. The mother liquor of IV contained material with λ_{max} 259 mμ. This material was stable against acid and alkali, and, even after treatment with 0.1 *N* acid or alkali overnight, no change in the spectrum was observed.

Dehydration of Formyl-DL-valylglycine Ethyl Ester (VIIb).—Formyl-DL-valylglycine ethyl ester (230 mg.) and tosyl chloride (700 mg.) were dissolved in pyridine (2 ml.) and the mixture was stirred for 3 hr. at 20°. Within a few minutes the solution took on a wine red color. The pyridine solution was diluted with cold petroleum ether (100 ml.). The precipitated oily material was washed with cold petroleum ether, then extracted ten times with cold ether (25 ml.). The combined ethereal extracts were evaporated and the residue was purified by preparative thin layer chromatography (silicic acid, benzene–ethyl acetate, 4:1 v./v.). A band (R_f 0.45), which showed positive ninhydrin reaction after treatment with ethanol–6.0 *N* hydrochloric acid (1:1 v./v.) and subsequent heating, was eluted with methanol. The viscous isocyanide of VIIb had R_f 0.45 (benzene–ethyl acetate, 1:4) and 0.70 (ethyl acetate–methanol, 5:1); 4.68 (isonitrile) and 5.74 μ (ester).

The purified isocyanide was stable on standing at 20° but reverted partly to decomposition products which gave a yellow coloration with ninhydrin reagent. When ethyl acetate was used in place of ether for the extraction of the isocyanide, there was obtained a decomposition product, R_f 0.28 (ethyl acetate–methanol, 5:1), which after treatment with hot mineral acid gave a positive ninhydrin reaction.

Dehydration of Formyl-DL-valylglycinamide (VIIc).—Formyl-DL-valylglycinamide (194 mg.) and tosyl chloride (900 mg.) in pyridine (3 ml.) were stirred at 20° for 3 hr. and poured into cold ether (100 ml.). The precipitate was washed with cold ether and extracted with cold ethyl acetate (25 ml.) which was quickly concentrated to a sirup under reduced pressure. This extract was purified by thin layer chromatography in ethyl acetate–methanol (5:1, v./v.). A band having R_f 0.67–0.68 was first eluted with chloroform, then with methanol. Both extracts were separately evaporated to dryness and examined. The infrared spectrum of both samples had the characteristic absorption of the isocyanide group at 4.70 μ. In addition the sample from the methanol extract also contained the 4.47-μ absorption peak characteristic of nitriles. When samples of the isocyanide fraction were left standing in chloroform (20°, 1 hr.), thin layer chromatography showed formation of a new compound with the characteristic nitrile absorption.

N-Formyl-DL-valinecyanomethylamide (IX).—From the ether-washed dehydration product of formylvalylglycinamide (216 mg.) and tosyl chloride (992 mg.) in pyridine, the nitrile IX was extracted into ethyl acetate. The residue (242 mg.) was purified by preparative thin layer chromatography. The major band, which was eluted with methanol, after removal of the methanol and trituration in ethyl acetate, yielded colorless crystals, m.p. 137–138.5°.

Anal. Calcd. for $C_8H_{13}N_3O_2$: N, 22.99. Found: N, 22.69.

The Reduction of 1-Benzylmethylpyrazinium Salts with Sodium Borohydride

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The reaction of three 1-benzylalkylpyrazinium salts with sodium borohydride gave the corresponding 1-benzylalkylpiperazines.

The reaction of sodium borohydride with substituted pyridinium salts has been shown to yield di- and tetrahydropyridines as the major products. The enamine system formed by the initial attack of the hydride on the pyridinium ion is attacked by a proton from the solvent and then a second attack by hydride ion to form the tetrahydropyridine.[2] A pyrazinium salt could form only enamine or imine double bonds by hydride ion reaction, and thus it was anticipated that sodium borohydride reduction of pyrazinium salts in protic solvents would form piperazines and not partially reduced pyrazines. No example of the reaction of pyrazinium salts with sodium borohydride has been reported. The benzo derivatives, 1-methyl- and 1-ethylquinoxalinium salts, are reported to form 1-substituted 1,2,3,4-tetrahydroquinoxalines.[3] The reactions of 1 benzyl-3-methyl-, 1-benzyl-3,5-dimethyl-, and 1-benzyl-2,5-dimethylpyrazinium bromides with sodium borohydride now have been shown to give the corresponding piperazines and are the subject of this paper.

The reaction of benzyl bromide with the methyl pyrazines gave a single salt in each instance. It was assumed on the basis of steric grounds that the less hindered nitrogen had undergone salt formation giving IIa, b, and c. Proton magnetic resonance spectroscopy did not assist in the characterization, for the difference in chemical shifts of the methyl resonance peaks was not great.[4]

The reaction of 1-benzyl-3-methylpyrazinium bromide (IIa) and 1-benzyl-3-5-dimethylpyrazinium bromide (IIb) with sodium borohydride in water gave products homogenous to gas chromatography. Elemental analyses and infrared and proton magnetic resonance spectra were consistent with complete saturation of the heterocyclic ring to the piperazine. The single isomer of 1-benzyl-3,5-dimethylpiperazine which formed had the methyl groups *cis* and equatorial, for only this conformation was consistent with the proton magnetic resonance spectrum. In common with IIIa the methyl protons appeared as a doublet (0.91 p.p.m., J = 7 c.p.s.), the benzyl methylene hydrogen as a singlet (3.39 p.p.m.), the aromatic hydrogens as a singlet (7.22 p.p.m.), and the amino hydrogen at *ca.* 1.2 p.p.m. The axial and equatorial hydrogens at positions 2 or 6 of IIIb are nonidentical. The axial hydrogens appear as a triplet at 1.49 p.p.m.; the coupling constants of the axial hydrogens with the geminal equatorial hydrogens and with the adjacent axial hydrogens at positions 3 (or 5) are approximately equal (11 c.p.s.). The 2,6-equatorial hydrogens appear as a quartet at about 2.66 p.p.m. and the 3,5-diaxial

(1) This work is a portion of the research of J. J. T. to be presented to the Graduate Faculty of the University of New Hampshire in partial fulfillment of the Doctor of Philosophy degree.

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