cedure. A precipitate was obtained upon acidification after amination of the intermediate for 5 days at 37° with 15 parts of concd. ammonium hydroxide in a stoppered flask. The leucylvaline and valylglycylphenylalanine were similarly prepared. Since none of these compounds were found in the literature, their constants and those of some intermediates are presented in Table I. Some of the results in Tables II and IV further confirm their identity. Four of the peptides are capable of existence as D–D, L–L racemate or D–L, L–D racemate (*cf.* refs. 14, 17). The proportion of unblocked L-residue is however, theoretically the same with either racemate. The valylvalines were a gift from Dr. J. W. Hinman of the Upjohn Laboratories; their preparation has been described.[14] These peptides were received as the hydrochlorides, for which theoretical neutral equivalents had been found in the Kalamazoo Laboratories. Since these substances are quite hygroscopic, samples were weighed by difference.

Papergrams.—The paper-strip work was carried out with use of details recommended by Gage, Douglass and Wender.[18] The solutions employed were the same as those used for microbiological determinations, except that it was necessary to concentrate the solutions in order to obtain visible color in the spots.

Culture.—*Lactobacillus arabinosus* 17-5 *and Streptococcus faecalis* R were organisms that were originally obtained from the American Type Culture Collection and had been maintained in this Laboratory for some time. Since each of these has been frequently used here in routine bioassay, and their observable cultural requirements have remained unchanged, their identity is relatively assured. *L. brevis* was obtained shortly before use, as ATTC 4510.

The cultural procedure was as described in an earlier paper[19] with, however, 400 mg. of norleucine, 400 mg. of glycine, 10 g. of succinic acid, 20 mg. of xanthine, and 500 mg. of urea added per l. of medium.

Procedure for Sequence Determination.—The following is typical of the runs on the five dipeptides in Table II and the two in Table III. Peptide to an amount of 1.0 mg. was dissolved in 1.0 ml. of water (in some cases aliquots of a larger amount of solution were employed). To this was added 1 ml. of dry pyridine containing 20 mg. of phenyl isothiocyanate (or of phenyl isocyanate) and the clear solution was placed at 37° for 3–4 hr. The solution was then evaporated in a vacuum desiccator over sulfuric acid, and the residue was hydrolyzed with 2 ml. of 6 *N* hydrochloric acid for 18 hr. in the autoclave at 15 lb. pressure.

Excess hydrolytic acid was removed by heating over steam and finally evaporation in a desiccator over sodium hydroxide flakes. The residue obtained was made up into solution, the *p*H adjusted to 6.8, and appropriate amounts of clear liquid taken for either paper analysis or for microbiological assay. Each recovery value used in the final calculations was an average of four points obtained at different assay levels.

The second run reported for glycyl-DL-valine involved the addition of a drop of aqueous brom thymol blue indicator solution to the reaction flask, with adjustment of *p*H to 7.2–7.4 at the start of reaction, and after two hours, by addition of 0.2 *N* sodium hydroxide solution.

The cold partial hydrolysis of the third treatment of Table IV employed 1 ml. of dioxane saturated with HCl per mg. of original tripeptide at 25° for 6 hr. After reaction, the dioxane–HCl was removed in a vacuum desiccator over sodium hydroxide.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE PURDUE RESEARCH FOUNDATION, PURDUE UNIVERSITY]

The Reaction of 1,3-Dichloro-2,4,6-trinitrobenzene with Amino Acids[1,2]

BY H. FEUER, G. B. BACHMAN AND J. P. KISPERSKY[3]

The reaction of 1,3-dichloro-2,4,6-trinitrobenzene with amino acids results in the formation of dibasic acids. The preparations of N,N′-bis-(carboxymethyl)-2,4,6-trinitrophenylenediamine (styphnylbisglycine) and N,N′-bis-(carboxyethyl)-2,4,6-trinitrophenylenediamine (styphnylbis-β-alanine) are described. Nitration of these acids leads to N,N′-dinitro substituted compounds which may be converted to the corresponding acid chlorides. These react readily with alcohols to give esters which also are obtained directly by nitrating the esters of styphnylbisglycine and styphnylbis-β-alanine.

1,3-Dichloro-2,4,6-trinitrobenzene (styphynyl chloride) has recently become readily available by a new synthesis which was developed in this Laboratory.[4] The reaction of styphnyl chloride (I) with amino acids has now been investigated (see Fig. 1). A literature search revealed that the only reference to this type of compound is reported by Hirayama[5] who prepared the trinitrophenyl derivatives of several amino acids from picryl chloride. His reaction conditions, a two-phase system (water–toluene) in the presence of sodium hydroxide at room temperature, have now been employed successfully for the preparation of the esters of two diamino dibasic acids from styphnyl chloride (Va,b), but have given negative results in the preparation of the corresponding free acids. When the reaction with the free amino acids was carried out at room temperacture (*ca.* 25°), 87% of the styphnyl chloride was recovered. At higher temperatures (*ca.* 90–95°) a very small yield of a dark red solid which still contained chlorine was obtained. A good yield of styphnylbisglycine, however, was obtained at 50° in the presence of aqueous ethanol and a sufficient quantity of sodium carbonate to form the disodium salt of the acid. The latter crystallized from the reaction mixture upon cooling. Styphnyl chloride was also condensed with β-alanine in a similar manner.

Nitration of the above acids and their esters to the N,N′-dinitro derivatives (IIIa,b and VIa,b) was successfully accomplished with a mixture of equal volumes of concd. sulfuric acid and fuming nitric acid. No good crystallizing medium could be found for the N,N′-dinitrostyphnylbisglycine. Attempts to recrystallize it from hot water caused hydrolysis of the side-chain with the formation of styphnic acid (1,3-dihydroxy-2,4,6-trinitrobenzene). This hydrolysis was not observed with N,N′-dini-

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(3) Aerojet Engineering Corporation, Azusa, California.

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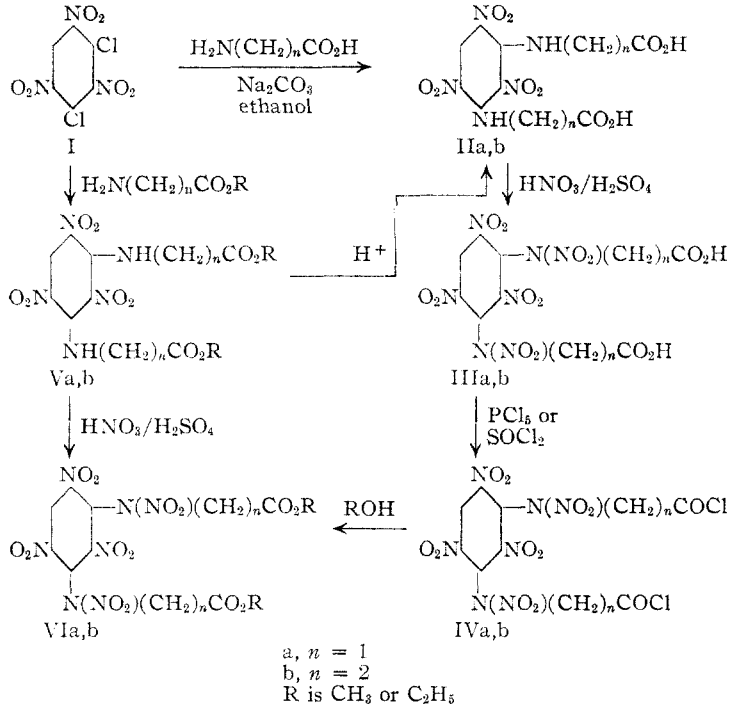

Fig. 1.—Preparation of styphnyl derivatives of amino acids.

trostyphnylbis-β-alanine (IIIb), which could be recrystallized satisfactorily from hot water.

In the preparation of the acid chlorides a remarkable difference in reactivity of N,N′-dinitrostyphnylbis-β-alanine and N,N′-dinitrostyphnylbisglycine was noticed. While the acid chloride of the former (IVb) was obtained by refluxing with excess thionyl chloride, the latter acid was recovered unchanged from such treatment and also from treatment with phosphorus oxychloride, phosphorus trichloride or oxalyl chloride. The acid chloride (IVa) of IIIa was finally obtained by reaction with phosphorus pentachloride.

The acid chlorides reacted rapidly with alcohols at 25° to give the corresponding esters. The methyl, ethyl and *n*-propyl esters were prepared in this manner.

The esters as well as the free acids are sensitive to shock and decompose with evolution of oxides of nitrogen at or about their melting points.

Experimental[6]

Styphnylbisglycine (IIa).—Styphnyl chloride,[4] 8.43 g. (0.03 mole), 6.36 g. (0.06 mole) of anhydrous sodium carbonate and 4.50 g. (0.06 mole) of glycine were added to 200 ml. of 50% ethanol. The stirred mixture was heated to 50° for 30 minutes and then to 60° for ten minutes. All of the material dissolved leaving a clear amber-colored solution. The reaction mixture was cooled to 10°. The yellow solid was separated and slurried with 100 ml. of absolute ethanol, again separated and dried in vacuum. The yield of disodium styphnylbisglycine was 11.31 g. (93.5%). The explosion point of a sample was found to be 204°.

Acidification of a solution of the above salt in 200 ml. of water with 13 ml. of 5 *N* hydrochloric acid gave styphnylbisglycine, m.p. 200–205° dec., as a yellow powder in 69% yield. It is soluble in acetone, dioxane, methanol and ethanol but insoluble in water, benzene, chloroform, carbon tetrachloride, petroleum ether and ether.

Anal. Calcd. for $C_{10}H_9O_{10}N_5$ (dibasic acid): C, 33.4; H, 2.51; N, 19.5; neut. equiv., 179.5. Found: C, 33.2; H, 2.60; N, 19.5; neut. equiv., 176.

Styphnylbisglycine Ethyl Ester (Va).—Glycine ethyl ester hydrochloride,[7] 8.37 g. (0.06 mole), 8.46 g. (0.03 mole) of styphnyl chloride and 6.36 g. (0.06 mole) of anhydrous sodium carbonate were added to a mixture of 60 ml. of water and 45 ml. of toluene. The mixture was heated to 50° and after five minutes a yellow crystalline solid began to precipitate. Heating was discontinued after 15 minutes and the mixture was stirred for an additional 75 minutes. The solid was then filtered and recrystallized from hot benzene. A yield of 8.4 g. (67%) of the expected product, m.p. 165–166° was obtained.

Anal. Calcd. for $C_{14}H_{17}O_{10}N_5$: C, 40.5; H, 4.10; N, 16.8. Found: C, 40.3; H, 4.10; N, 17.0.

Acid Hydrolysis of Styphnylbisglycine Ethyl Ester (Va).—A 1.5-g. (0.0036 mole) sample of styphnylbisglycine ethyl ester was suspended in a mixture of 5 ml. of water, 10 ml. of concd. hydrochloric acid and 2 ml. of dioxane. The slurry was heated to reflux for eight hours, then cooled and filtered. The dry solid was extracted with 30 ml. of boiling benzene. The benzene-soluble fraction, which was unchanged ester, weighed 0.63 g. Styphnylbisglycine (IIa), 0.31 g. (24%), was obtained as the benzene-insoluble fraction and melted with decomposition at 189°. Recrystallization from water raised the melting point to 198° dec. A mixed melting point with a sample of styphnylbisglycine obtained from the previously described preparation was found to be 200° dec.

N,N′-Dinitrostyphnylbisglycine (IIIa).—In a 100-ml. flask equipped with stirrer and thermometer, were placed 30 ml. of concd. sulfuric acid and 30 ml. of fuming nitric acid. To this mixture, 8.30 g. (0.023 mole) of styphnylbisglycine was added portionwise, and the resulting solution was heated to 40° for 30 minutes. It was then cooled to 25° and poured onto ice. The white solid which precipitated was filtered and dried. A total of 9.1 g. (88%) of IIIa, m.p. 168.8° dec., was obtained. It was soluble in acetone, dioxane and methanol, but insoluble in benzene, carbon tetrachloride, chloroform, methylene chloride, nitromethane and cold water.

In an attempt to recrystallize a sample from hot water decomposition was observed. Nitrogen dioxide was evolved and the solution turned yellow. Yellow needle-like crystals deposited, m.p. 171°. Recrystallization from absolute ethanol raised the melting point to 176°, and a mixed melting point with an authentic sample of styphnic acid (1,3-dihydroxy-2,4,6-trinitrobenzene) gave no depression.

Since the acid (IIIa) could not be recrystallized satisfactorily from any of the solvents tried, it was submitted for analysis without further purification.

Anal. Calcd. for $C_{10}H_7O_{14}N_7$: C, 26.7; H, 1.56; N, 21.8. Found: C, 26.7; H, 1.63; N, 21.7.

N,N′-Dinitrostyphnylbisglycine Ethyl Ester (VIa).—Styphnylbisglycine ethyl ester, 2.07 g. (0.005 mole) was added in small portions to 10 ml. of concd. sulfuric acid and 10 ml. of fuming nitric acid. The mixture was heated to 40° for one-half hour, then cooled and poured with stirring onto 500 g. of ice. A white precipitate formed immediately. After drying, 2.5 g. (98%) of VIa was obtained. Recrystallization from ethanol gave white platelets, m.p. 130–131° dec.

Anal. Calcd. for $C_{14}H_{15}O_{14}N_7$: C, 33.3; H, 2.97; N, 19.4. Found: C, 33.4; H, 2.90; N, 19.6.

N,N′-Dinitrostyphnylbisglycyl Dichloride (IVa).—In a small round-bottom flask provided with a drying tube were placed 5.0 g. (0.011 mole) of N,N′-dinitrostyphnylbisglycine and 4.65 g. of powdered phosphorus pentachloride. The solids were mixed by swirling the flask, and the mixture was allowed to stand for 72 hours at 25°. Twenty ml. of benzene was then added and the mixture was filtered. After drying, 3.5 g. (65%) of solid product, m.p. 138° dec., was secured. An additional 0.65 g., m.p. 137° dec., was ob-

(6) All melting points are uncorrected.

(7) C. Harries and M. Weiss, *Ann.*, **327**, 365 (1903).

tained after removal of the benzene from the filtrate under reduced pressure.

Anal. Calcd. for $C_{10}H_5O_{12}N_7Cl_2$: C, 24.7; H, 1.03; N, 20.2. Found: C, 24.7; H, 1.06; N, 20.2.

Several esters were prepared by adding the acid chloride at 25° to the minimum amount of anhydrous alcohol necessary for solution. The esters crystallized from the solution upon cooling.

TABLE I

ESTERS OF N,N'-DINITROSTYPHNYLBISGLYCINE

R	M.p. of product, °C. (dec.)	Crystallizing medium	Formula	Analyses, % Calcd.	Found
Methyl	161	EtOH	$C_{12}H_{11}O_{14}N_7$	C, 30.18	30.30
				H, 2.30	2.39
				N, 20.54	20.44
Ethyl[a]	130–131	EtOH	$C_{14}H_{15}O_{14}N_7$	C, 33.30	33.40
				H, 2.97	2.90
				N, 19.40	19.60
n-Propyl	145	MeOH	$C_{16}H_{19}O_{14}N_7$	C, 36.02	36.22
				H, 3.56	3.50
				N, 18.38	18.32

[a] A mixed melting point with an authentic specimen prepared by nitration of ester Va gave no depression.

Styphnylbis-β-alanine (IIb).—Styphnyl chloride, 2.82 g. (0.01 mole), 2.51 g. (0.02 mole) of β-alanine hydrochloride, 33.3 ml. of 95% ethanol and 20 ml. of water were placed in a 300-ml. three-neck flask equipped with stirrer, thermometer and dropping funnel. The mixture was heated to 50° and 3.18 g. (0.03 mole) of sodium carbonate dissolved in 13 ml. of water was added dropwise over a period of 40 minutes. Heating was continued for ten minutes at 60°; then the flask was cooled to 1° and the salt which precipitated was filtered. An orange colored salt (3.35 g.) was obtained. It was dissolved in 200 ml. of water and 3.12 ml. of 5 *N* hydrochloric acid was added. A total of 2.14 g. (55%) of product was obtained, m.p. 198° dec. The acid was soluble in acetone, dioxane, methanol and ethanol, but insoluble in benzene, chloroform, carbon tetrachloride and ether.

Anal. Calcd. for $C_{12}H_{13}O_{10}N_5$: C, 37.2; H, 3.36; N, 18.1. Found: C, 36.9; H, 3.18; N, 18.1.

Styphnylbis-β-alanine Methyl Ester (Vb).—Styphnyl chloride, 2.82 g. (0.01 mole), 2.79 g. (0.02 mole) of β-alanine methyl ester hydrochloride and 2.12 g. (0.02 mole) of sodium carbonate were added to 15 ml. of toluene and 20 ml. of water. The mixture was heated to 50° for four hours and after cooling, the precipitate was filtered. There was obtained 1.33 g. (31%) of product which melted at 149–153°, and at 151–155° after repeated recrystallization from acetone. The ester was soluble in acetone, dioxane, benzene and ethanol, but was insoluble in water.

Anal. Calcd. for $C_{14}H_{17}O_{10}N_5$: C, 40.5; H, 4.10; N, 16.8. Found: C, 40.9; H, 4.36; N, 16.6.

N,N'-Dinitrostyphnylbis-β-alanine (IIIb).—A 7.05-g. (0.018 mole) sample of styphnylbis-β-alanine was dissolved in a mixture of 15 ml. of concd. sulfuric acid and 15 ml. of fuming nitric acid. The solution was heated with stirring to 40–45° for 30 minutes and after cooling to 25° was poured onto 300 g. of ice. The white solid, which precipitated was filtered off and recrystallized from hot water. An 80% yield of IIIb was obtained, m.p. 170° dec. It was soluble in acetone, dioxane and methanol, but insoluble in benzene, chloroform, carbon tetrachloride and petroleum ether.

Anal. Calcd. for $C_{12}H_{11}O_{14}N_7$: C, 30.2; H, 2.31; N, 20.5. Found: C, 30.2; H, 2.38; N, 20.3.

N,N'-Dinitrostyphnylbis-β-alanine Methyl Ester (VIb).—Ester (Vb), 0.2 g. (0.48 mmole) was added to a mixture of 2 ml. of concd. sulfuric acid and 2 ml. of fuming nitric acid. The solution was heated to 40° for 30 minutes and after cooling to 25° was poured onto ice. A sticky, white solid settled out, which, after recrystallization from absolute ethanol was obtained in the form of white needles, m.p. 119–120° dec. (yield 70%).

Anal. Calcd. for $C_{14}H_{15}O_{14}N_7$: C, 33.3; H, 2.97; N, 19.4. Found: C, 33.6; H, 3.29; N, 19.3.

N,N'-Dinitrostyphnylbis-β-alanyl Dichloride (IVb).—N,N'-Dinitrostyphnylbis-β-alanine, 0.5 g. (0.001 mole), was added to 5 ml. of thionyl chloride, in a 50-ml. flask. A condenser topped with a drying tube was affixed to the flask and the mixture was heated to reflux. Dissolution of the acid had occurred after two hours, but heating was continued for four hours. The excess thionyl chloride was removed under reduced pressure, and 10 ml. of petroleum ether (60–70°) was added to the residue. The mixture was filtered and a total of 0.45 g. (90%) of product was obtained, m.p. 106° dec. It was soluble in benzene, acetone, dioxane, ethyl acetate, nitromethane and ethylene chloride, but was insoluble in carbon tetrachloride, chloroform and petroleum ether.

Anal. Calcd. for $C_{12}H_9O_{12}N_7Cl_2$: C, 28.0; H, 1.75; N, 19.1. Found: C, 28.18; H, 1.83; N, 19.1.

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[CONTRIBUTION FROM THE WHITMORE LABORATORIES OF THE PENNSYLVANIA STATE COLLEGE]

Methyl β-(m-Chloroanilino)-acrylate

BY F. W. GRAY,[1,2] HARRY S. MOSHER,[3] F. C. WHITMORE[4] AND T. S. OAKWOOD

m-Chloroaniline readily adds in good yield to methyl propiolate to give the known methyl β-(*m*-chloroanilino)-acrylate which can be readily cyclized directly to 4-hydroxy-7-chloroquinoline. This constitutes another approach to this important quinoline intermediate for the synthesis of antimalarials of the chloroquin type.

A direct synthesis of 4-hydroxy-7-chloroquinoline has been achieved by Price, Leonard and Reitsema,[5] and Northey and Dreisbach[6] by the cyclization of methyl β-(*m*-chloroanilino)-acrylate (I, R, R' = H).

This intermediate, however, was obtained from *m*-chloroaniline and methyl formylacetate in only 31% yield.

We have undertaken a reinvestigation of this approach with the purpose of preparing the β-(*m*-chloroanilino)-acrylate by an alternate method. Efforts to prepare β-(*m*-chloroanilino)-acrylates (I, R, R' = H) by the condensation of *m*-chloroaniline with methyl or ethyl β-alkoxyacrylates failed to give any crystalline products; the products were oils which gave *sym*-bis-*m*-chlorophenylurea upon attempted cyclization. Methyl β,β-dimethoxypropionate reacted with *m*-chloroaniline hydrochloride to give a crystalline compound, m.p. 125–126°, which gave the correct analysis for methyl β-(*m*-chloroanilino)-acrylate (I, R, R' = H). However, this product gave only a poor yield of *sym*-bis-*m*-chlorophenylurea[5] on attempted cyclization.

(1) Taken in part from the Ph.D. thesis of F. W. Gray, August, 1949. Colgate-Palmolive-Peet Co., Jersey City, N. J.

(2) Parke, Davis and Co. Fellow 1947–1948.

(3) Department of Chemistry, Stanford University, Stanford, Calif.

(4) Deceased.

(5) Price, Leonard and Reitsema, THIS JOURNAL, **68**, 1256 (1946).

(6) Northey and Dreisbach, U. S. Patent 2,478,125, Aug. 2 (1949).