tained. The product distilled at 93–96° (18 mm.),[11] n_D^{24} 1.4718 and solidified and melted at 59–61°.

4-Trifluoromethylaniline. A solution of 30 g. (0.157 mole) of 3-trifluoromethylnitrobenzene was hydrogenated under 2.5 atm. in the presence of Raney nickel. After hydrogen uptake was complete (1.5 hr.), the solution was filtered from the catalyst. After removal of solvent, the residues from this experiment and a second run were distilled at 84–86° (18 mm.), n_D^{25} 1.4775. The combined yield amounted to 48.0 g. (95%).[12]

In a similar manner, 4-trifluoromethylaniline b.p. 102–102.5° (30 mm.), n_D^{22} 1.4831 was obtained in 80% yield; lit. b.p. 117° (40 mm.).[12]

When palladized charcoal was used as catalyst in these reductions, residual poisons (possibly from sulfur tetrafluoride) appeared to retard hydrogenation. Several portions of catalyst had to be added in order for hydrogen uptake to be completed.

N,N-Bis(2-hydroxylethylated) aromatic amines. N,N-Bis(2-hydroxyethyl)-2-methoxy-1-naphthylamine. Two-tenths mole (34.6 g.) of 2-methoxy-1-naphthylamine[13] and 21 cc. (0.42 mole) of ethylene oxide were placed in a stainless steel rocker type bomb and heated and shaken for 5 hr. at 90–100°. After cooling, the viscous mass was treated with pentane after which it solidified. It was filtered and dried and weighted 46.4 g. (89% yield). After recrystallization from absolute alcohol and pentane it melted at 75–76°.

Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.14; H, 7.26; N, 5.30.

Acknowledgment. The authors are greatly indebted to Mr. D. C. Wimer of the Analytical Research unit of Abbott Laboratories for the results described in Table I.

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(11) F. Swarts, *Bull. acad. roy. sci. Belg.* [3] 35, 375 (1898) reports b.p. of 201.5° at atmospheric pressure.

(12) Prepared in 74% yield by N. L. Drake, *et al.*, *J. Am. Chem. Soc.*, 68, 1602 (1946) by high pressure reduction of the nitro compound with Raney nickel. B.p. 74–75° (10 mm.).

(13) Obtained from L. Light and Co. Ltd., Colnbrook, Bucks, England.

[FROM THE SCIENTIFIC DEPARTMENT, MINISTRY OF DEFENCE]

A New Amino Acid Reagent, 2,4-Dinitro-5-fluoroaniline

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2,4-Dinitro-5-fluoroaniline and its acetyl derivative give with amino acids well defined derivatives. In the former case, the presence of the aromatic amino group in these derivatives permits the preparation of characteristic azo-dyes.

Since the observation of Sanger[2] that 2,4-dinitrofluorobenzene can be used as a reagent for the amino group in amino acids and peptides, a number of similar substances have been proposed for the same purpose: 2,4-dinitro-1,5-difluorobenzene,[3] 2,4-dinitro-1-fluoro-5-chlorobenzene,[4] ethyl 3-nitro-4-fluorobenzoate,[5] fluorobenzene-2,4,-6-tricarboxylic acid[6] and 3-nitro-4-fluorobenzaldehyde.[7,8] In the present investigation, a variant of this method has been studied which permits the introduction of a new amino group together with the 2,4-dinitrophenyl radical. The reagent used was 2,4-dinitro-5-fluoroaniline (or its acetyl derivative). This reagent has two advantages, first that the amino group has a bathochromic effect (the maximum of 350 mμ in Sanger's dinitrophenyl derivatives being shifted to 400–410 mμ), and second that the aromatic amino group can be diazotized and coupled and thereby converted into an azo dye which lends itself to the detection of very small amounts, *e.g.*, on paper chromatograms.

2,4-Dinitro-5-fluoroacetanilide was prepared by the nitration of 3-fluoroacetanilide; it is deacylated easily by means of 50% sulfuric acid to the base which has been prepared before[9] by a different route.

For the preparation of 3-fluoroacetanilide the method developed previously[10] in this laboratory, is superior to the older method based on 3-nitroaniline.[11]

Both 2,4-dinitro-5-fluoroaniline and its *N*-acetyl derivative react with amino acids in the presence of sodium bicarbonate. The products crystallize easily and show characteristic crystal forms (See Tables II and III). The diazotization of the

(1) This paper forms part of a thesis submitted by M. Bentov to the Hebrew University in fulfilment of the requirements for the degree of Ph.D.

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(8) Similar derivatives, *viz.*, 4-diethylamino-3,5-dinitrophenyl derivatives, have been briefly described by H. Edmunds and W. S. Reith, *Biochem. J.*, 57, XVIII (1954) and by A. Drèze and W. S. Reith, *Biochem. J.*, 63, 21P (1956). *Cf.* also M. Justial, E. Scoffone, and P. de la Llosa, *Bull. Soc. chim. France*, 1551 (1959); E. Scoffone, P. de la Llosa, and M. Justial, *Bull. Soc. chim. France*, 1553 (1959); Z. Talik and E. Plażek, *Bull. Acad. Polon. Sci., Chem. Sci. Series*, 8, 227 (1960).

(9) F. Swarts, *Rec. Trav. chim.*, 35, 161 (1915).

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TABLE I

R_F VALUES FOR SOME AMINO ACIDS AND THEIR DERIVATIVES

Amino acid	Free Acid				Dinitrophenyl Deriv.				Dinitroaminophenyl Deriv.
	Phenol-water[a]	Collidine-lutidine[a]	Butanol-acetic acid[a]	Butanol + 3% NH_3[a]	Propanol-cyclohexane (3:7)[b]	*t*-Amyl alcohol[c]	Ethyl and benzyl alcohol (1:9)[c]	Butanol-water[d]	Butanol-water
Glutamic acid	0.31	0.20	0.37	0.01	0.05	0.04	0.07	0.14	0.26
Phenylalanine	0.85	0.48	0.66	0.46	0.74	0.74	0.63	0.71	0.56
Serine	0.36	0.28	0.31	0.05	0.05	0.21	0.18	0.32	0.15
Isoleucine	0.84	0.45	0.68	0.40	...	...	...	0.73	0.52
Methionine	0.81	0.42	0.57	0.05	...	...	...	0.65	0.76

[a] R. J. Block, R. LeStrange, and G. Sweig, *Paper Chromatography* (Academic Press Inc., New York, 1952), p. 67. [b] R. Consden, A. H. Gordon, and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944). [c] S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951). [d] E. F. Mellon, A. H. Korn, and S. R. Hoover, *J. Am. Chem. Soc.*, **75**, 1675 (1953).

TABLE II

2,4-DINITRO-5-ACETAMIDOPHENYL DERIVATIVES OF AMINO ACIDS

Amino acid	M.P.	Recryst. from	Product Yield, %	Carbon, %		Hydrogen, %		Nitrogen, %		Spectrum (in ethanol) [mμ (log ε)]
				Calcd.	Found	Calcd.	Found	Calcd.	Found	
DL-Phenylalanine	208	Dil. alcohol	96					14.4	14.6	220 (4.30); 302 (4.26); 405 (4.02)
DL-Methionine	252 dec.[a]	Glacial acetic acid	87	40.0	39.6	4.6	4.8	14.3	14.0	305 (4.31); 356 (4.12); inflexion at 402 (3.72)
DL-Serine	201	Alcohol	73	40.2	40.3	3.6	3.8	17.1	17.4	337 (4.31); 405 (4.03)

[a] Monohydrate.

TABLE III

2,4-DINITRO-5-AMINOPHENYL DERIVATIVES OF AMINO ACIDS

Amino acid	M.P.	Recryst. from	Product Yield, %	Carbon, %		Hydrogen, %		Nitrogen, %		Spectrum (in ethanol) [mμ (log ε)]
				Calcd.	Found	Calcd.	Found	Calcd.	Found	
DL-Isoleucine	204	Dil. alcohol	73	46.1	46.1	5.1	5.4	17.9	17.6	338 (4.30); 410 (3.96)
DL-Methionine	216	Methanol	97	40.0	39.7	4.2	4.4	16.9	16.8	336 (4.39); 410 (4.00)
DL-Serine	209	Methanol	97	37.7	37.5	3.5	3.8	19.6	19.2	335 (4.29); 410 (4.00)
DL-Glutamic acid	102	Water	91	40.2	39.7	3.7	4.4	17.1	16.8	338 (4.30); 410 (4.00)

condensation products and subsequent coupling with α-naphthol give intensely colored azo dyes. This reaction can be carried out both in solution and on paper; the intensity of the absorption is determined (in the latter case by means of a densitometer)[12] and compared with standard samples. The method has been applied both to paper strips and to circular filter paper.[13]

The following spectra were observed (in alkaline medium): glutamic acid 425 mμ (5.54); 640 mμ (3.78); isoleucine 425 mμ (4.30); 610 mμ (2.78); serine 425 mμ (6.90); 600 mμ (5.00); methionine 425 mμ (3.95); phenylalanine 425 mμ (5.70); 610 mμ (2.95).

In carrying out the chromatography of the products, the best results were obtained with water-saturated butanol as the moving phase. The R_F values so obtained are compared in Table I with those reported for the corresponding free amino acids and their 2,4-dinitrophenyl derivatives. The spread of the R_F values for the newly described derivatives is not unfavorable.

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EXPERIMENTAL

3-Fluoronitrobenzene. 3-Nitroaniline (34 g.; 0.4 mole) was dissolved in 110 ml. of 40% fluoboric acid (0.5 mole) and diazotized at 0° with a solution of 17 g. (0.4 mole) of sodium nitrite in 34 ml. of water. The stirring was continued at 0° for 30 min. and the precipitate filtered, washed with cold fluoboric acid, alcohol and ether, and dried; yield, 53 g. (89%). A mixture of 13 g. of the salt and 25 g. of high-boiling mineral oil was heated until decomposition set in. When the reaction subsided, the heating was continued for a short time and the product isolated by steam distillation. The distillate was extracted with ether and the extract washed with 25 ml. of 5% sodium hydroxide solution, dried, and distilled; b.p. 96–97° (23 mm.), yield, 4.3 g. (57%).

3-Fluoroaniline was prepared in 93% yield from 3-fluoronitrobenzene by catalytic hydrogenation in alcohol as solvent

and in the presence of palladium-charcoal as catalyst; b.p. 64–66° (4 mm.).

3-Fluoroacetanilide.[10] To the solution of 8.5 g. (0.75 mole) of 3-fluoroaniline in 50 ml. of benzene, a small excess of acetic anhydride was added. When the exothermic reaction had subsided, the mixture was kept at room temperature for 12 hr., and the volatile constituents were removed *in vacuo*. The residue crystallized spontaneously in form of colorless needles, m.p. 84°, yield, 11.5 g. (98%).

2,4-Dinitro-5-fluoroacetanilide. With stirring and cooling (ice-salt mixture), 11 g. (0.7 mole) of the foregoing substance was added in small quantities to a mixture of 30 g. of concd. nitric acid (d = 1.49) and 90 g. of concd. sulfuric acid. After 1 hr., the mixture was poured onto ice and the solid filtered, washed, and recrystallized from alcohol; m.p. 119°, yield, 7.2 g. (42%).

Anal. Calcd. for $C_8H_6FN_3O_5$: C, 39.5; H, 2.5; N, 17.3. Found: C, 39.4; H, 2.4; N, 17.4.

2,4-Dinitro-5-fluoroaniline. The mixture of 10 g. (0.05 mole) of the foregoing compound and 30 ml. of 50% sulfuric acid was refluxed for 2 hr. and poured onto ice. The solid product was filtered and recrystallized from alcohol; m.p. 186–187°; yield, 8 g. (96%).

Anal. Calcd. for $C_6H_4FN_3O_4$: N, 21.0; F, 9.5. Found: N, 21.0; F, 9.7.

Preparation of N-(2,4-dinitro-5-acetamidophenyl) glycine. To the solution of 0.17 g. (0.002 mole) of glycine and 0.5 g. of sodium bicarbonate in 10 ml. of water, there was added a solution of 1.2 g. (0.006 mole) of 2,4-dinitro-5-fluoroacetanilide in 50 ml. of alcohol. The mixture was stirred at room temperature for 2 hr., the alcohol removed *in vacuo* and water added. The excess of the reagent was then removed by filtration and the filtrate acidified with dilute hydrochloric acid. The yellow precipitate was recrystallized from alcohol; m.p. 243°; yield, 0.7 g. (87%). $\lambda_{max}^{C_2H_5OH}$ 334 mμ (4.32); 410 mμ (4.02).

Anal. Calcd. for $C_{10}H_{10}N_4O_7$: C, 40.3; H, 3.3; N, 18.71. Found: C, 40.7; H, 3.3; N, 18.2.

The analogous reactions with other amino acids are summarized in Table II.

*Preparation of N-(2,4-dinitro-5-aminophenyl)-*DL*-phenylalanine.* The mixture of 0.8 g. (0.05 mole) of DL-phenylalanine, 0.9 g. of sodium bicarbonate, 1.5 g. (0.14 mole) of 2,4-dinitro-5-fluoroaniline, and 30 ml. of alcohol was heated until a clear solution resulted. After 30 min. at room temperature, 20 ml. of water was added, and the filtered solution heated *in vacuo*, in order to remove the alcohol, and acidified with dilute hydrochloric acid; from methanol, m.p. 235°, yield, 1.6 g. (93%). $\lambda_{max}^{C_2H_5OH}$ 334 mμ (4.26); 410 (3.96).

Anal. Calcd. for $C_{15}H_{14}N_4O_6$: C, 52.0; H, 4.0; N, 16.2. Found: C, 52.2; H, 4.3; N, 16.1.

The analogous reactions with other amino acids are summarized in Table III; in these cases 50% alcohol was used as the reaction medium.

Diazotization and coupling with α-naphthol. To an ice-cold solution of 0.004 mole of the 2,4-dinitro-5-aminophenyl derivative in 5 ml. of 10% hydrochloric acid, 1.4 ml. of a 20% sodium nitrite solution was added with agitation, followed by 0.6 g. of α-naphthol, dissolved in 6 ml. of 10% sodium hydroxide solution.

Procedure for paper chromatography. The test solution was applied to the paper with a glass capillary until a round spot of about 1 cm. diameter formed. The spot was left to dry for a minute or two.

The filter paper used was Whatman's No. 1. The solvent was *n*-butyl alcohol, saturated with water. The developed chromatogram was sprayed successively with 10% hydrochloric acid, a 1% solution of sodium nitrite, and a solution of 1 g. of α-naphthol in 10 ml. of 10% sodium hydroxide. The chromatogram was then dried at 50–60°.

TEL-AVIV, ISRAEL

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

Synthesis of Selectively Protected Homoserine and α,γ-Diaminobutyric Acid Derivatives[1]

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The interaction between α-halogeno-γ-butyrolactone, *N*-acylated-α-amino-γ-butyrolactones, or α-benzamido-γ-halogenobutyric acid esters and between ammonia, benzylamine, and dibenzylamine was studied. α-Dibenzylamino-γ-butyrolactone was employed in the synthesis of a series of homoserine and α,γ-diaminobutyric acid derivatives, in which functional groups appeared selectivity masked or free for reaction.

The occurrence in nature of homoserine[3] and of α,γ-diaminobutyric acid[4] lends interest to the synthesis of these compounds and of such of their derivatives in which protection as well as activation of the functional groups is selectively provided for.

In this work, α-dibenzylamino-γ-butyrolactone, an intermediate in homoserine synthesis, was used as a fundamental substance for conversion to linear, lactonic, and lactamic homoserine and α,γ-diaminobutyric acid derivatives, with the three functional groups, in part or *in toto* selectively protected. The derivatives prepared in this manner are arrived at directly, obviating the preparation of the acids themselves.

The reaction between α-bromo-γ-butyrolactone[5] and dibenzylamine yielded α-dibenzylamino-γ-

(1) Presented in part before the XXVIth Scientific Meeting of the Israel Chemical Society, Jerusalem, April 1960, *cf.* M. Frankel, Y. Knobler, and T. Sheradsky, *Bull. Res. Counc. Israel*, **9A**, 56 (1960).

(2) Part of a Ph.D. Thesis to be submitted to the Hebrew University.

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