TABLE I

PHYSICAL CONSTANTS AND ELEMENTARY ANALYSIS OF GLYCINE PEPTIDE ESTERS

	M.P.[a]	Formula	R_f[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Trityldiglycine *t*-butyl ester	167–168	$C_{27}H_{30}N_2O_3$		75.31	75.56	7.02	6.99		
Trityldiglycine *t*-amyl ester	148–149	$C_{28}H_{32}N_2O_3$		75.64	75.78	7.25	7.12		
Diglycine *t*-butyl ester hydrochloride	172–173	$C_8H_{17}ClN_2O_3$	0.63	42.76	42.85	7.62	7.57	12.46	12.59
Diglycine *t*-amyl ester hydrochloride	160–161	$C_9H_{19}ClN_2O_3$	0.69	45.28	45.15	8.02	7.79	11.72	11.91
Diglycine isobutyl ester·HCl	139–140	$C_8H_{17}ClN_2O_3$	0.63	42.76	42.71	7.62	7.64	12.46	12.40
Trityltriglycine *t*-butyl ester	149–150	$C_{29}H_{33}N_3O_4$		71.43	71.71	6.82	6.74		
Trityltriglycine *t*-amyl ester	145–147	$C_{30}H_{35}N_3O_4$		71.84	72.20	7.03	7.45		
Triglycine *t*-butyl ester·HCl	174–175	$C_{10}H_{20}ClN_3O_4$	0.51	42.62	42.76	7.15	7.02	14.91	14.65
Triglycine *t*-amyl ester·HCl	170	$C_{11}H_{22}ClN_3O_4$	0.56	44.66	44.54	7.49	7.43	14.21	14.37
Triglycine isobutyl ester·HCl	154–155	$C_{10}H_{20}ClN_3O_4$	0.51	42.62	42.55	7.15	7.18	14.91	15.10
Tetraglycine *t*-butyl ester·HCl	179–180	$C_{12}H_{23}ClN_4O_5$	0.40	42.54	42.73	6.84	6.90	16.53	16.37
Tetraglycine isobutyl ester·HCl	162–163	$C_{12}H_{23}ClN_4O_5$	0.40	42.54	42.76	6.84	6.75	16.53	16.74

[a] All melting points were determined in capillary tubes. The temperatures were not corrected. [b] Average R_f values of the pure compounds in Solvents II. In a mixture of several peptide esters the respective R_f value of each peptide becomes smaller than the value cited.

checked by chromatography in order to exclude contamination with peptide.[7]

Paper chromatography. Descending chromatography was carried out with the solvent systems (I): *n*-butyl alcohol–acetic acid–water 4:1:1 (freshly prepared), and (II): *sec*-butyl alcohol–formic acid–water 75:13:12 (aged more than 14 days[8]) on Whatman No. 1 filter paper allowing the solvent front to migrate 52–53 cm. The detecting spray reagent consisted of 0.5 g. of ninhydrin dissolved in a mixture of 100 ml. of absolute ethanol and 10 ml. of acetic acid.

A second method to detect peptides, peptide esters, and diketopiperazine was that of chlorination and subsequent treatment of the chromatogram with tolidine-potassium iodine as described by Reindel and Hoppe.[9]

For the quantitative determination 5–20 $\times 10^{-18}$ moles of the esters were spotted in 5; 10; and 15-μl. aliquots of both the known and the unknown solutions in alternating order on Whatman No. 1 filter paper. The chromatograms were prepared with solvent II. The color was produced by carefully dipping the air-dried chromatogram into a solution of 1.0 g. of ninhydrin in 100 ml. of ethanol and 10 ml. of acetic acid. After drying the chromatograms at room temperature for 10 min. they were kept in an oven (saturated with water vapor[10]) for 25 min. at 60°. After one more hour at room temperature the spots were cut out and extracted with 10 ml. of 0.1% cadmium acetate in methanol.[11,12] The extinction was measured in a Beckman spectrophotometer, model B, at 505 mμ. The resulting values were reproducible within ± 5%.

Diketopiperazine in the polycondensate of isobutyl glycinate was estimated after hydrolyzing it to diglycine with 0.2*N* barium hydroxide for 30 min. at room temperature. The hydrolysate (after precipitating the barium ion with 0.2*N* sulfuric acid) was chromatographed with solvent II, and the readings were compared with those of a standard solution containing diglycine and tetraglycine.

Paper electrophoresis on Whatman No. 1 MM, 1300 volts at 30 volts/cm. and a buffer according to Michl[13] gave a good separation of *t*-butyl glycinate up to the heptaglycinate while diketopiperazine remained close to the origin where the sample had been spotted.

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(7) The glycine peptide isobutyl esters were prepared by direct esterification of the corresponding peptide with isobutyl alcohol and hydrochloric acid.

(8) R. P. Kendall and H. S. B. Marshall, *Chemistry & Industry*, 1353, (1959).

(9) F. Reindel and W. Hoppe, *Chem. Ber.*, **87**, 1103 (1954).

(10) M. Stefl, J. Tulach, and A. Sovova, *Collection Czechoslov. Chem. Commun.*, 25, 435 (1960).

(11) J. Heilmann, J. Barrollier, and E. Watzke, *Z. Physiol. Chem.*, 309, 219 (1957).

(12) Gl. N. Atfield and C. J. O. R. Morris, "Separation and quantitative determination of amino acids by high voltage paper electrophoresis," paper submitted at the 390th Meeting of the Biochemical Society, January 23, 1960.

(13) H. Michl, *J. Chromatog.*, **1**, 93 (1958).

Preparation of Aromatic Nitramines. Alkaline Nitration Using Phenyllithium as Base[1]

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A variety of methods exists for the preparation of aromatic nitramines such as the oxidation of diazotates,[2] the direct nitration of amines,[3] and the alkaline nitration of amines using an alkyl nitrate and a basic condensing agent.[4] The first of these methods suffers from the facts that large quantities of solution must be manipulated to obtain small amounts of nitramine and the yield of by-products is high. The direct nitration procedure

(1) This research was supported by a Frederick Gardner Cottrell grant from the Research Corporation.

(2) E. Bamberger and L. Storch, *Ber.*, 26, 471 (1893); E. Bamberger and K. Landsteiner, *Ber.*, 26, 482 (1893); and E. Bamberger, *Ber.*, 27, 359, 914, 1273 (1894).

(3) E. D. Hughes, C. Ingold, and R. B. Pearson, *J. Chem. Soc.*, 4357 (1958).

(4)(a) A. Angeli and M. V. Maragliano, *Atti. accad. naz. Lincei*, **14 II**, 127 (1905) and (b) E. Bamberger, *Ber.*, **53**, 2321 (1920).

is applicable only to compounds with heavily negatively substituted rings. The alkaline nitration method using ethyl nitrate and potassium ethoxide in ethanol and ether[4b] is fairly convenient.

We have developed an alkaline nitration preparation of aromatic nitramines using phenyllithium as the base and amyl nitrate as the nitrating agent.

$$ArNH_2 \xrightarrow{C_6H_5Li} ArNHLi \xrightarrow{AmONO_2} (ArNNO_2)^-Li^+ \xrightarrow{H^+} ArNHNO_2$$

There are several advantages to this approach. First, it is simple to carry out. The phenyllithium solutions are much easier to prepare than the highly concentrated potassium ethoxide solutions employed by Bamberger.[4b] Finally the stable, commercially available amyl nitrate is convenient to use.

The disadvantages of this procedure are that the yields are low (about 40%) and amines bearing substituents reacting with phenyllithium do not yield nitramines. However, the yields may be increased somewhat by alternate additions of phenyllithium and amyl nitrate and unchanged amine can be recovered from the reaction mixture.

Most of the nitramines were not isolated as such but were converted directly to the corresponding *N*-methyl-*N*-nitroanilines, a reaction which proceeds in practically quantitative yields. Thus, the

$$Ar{-}NHNO_2 \xrightarrow[(CH_3)_2SO_4]{KHCO_3} Ar{-}N(CH_3)(NO_2)$$

yield of the *N*-methyl derivative is an accurate measure of the yield of simple nitramine. The *N*-methyl-*N*-nitroanilines are more stable than the unmethylated nitramines. The compounds prepared in this way are listed in Table I.

TABLE I

PREPARATION OF *N*-METHYL-*N*-NITRO-*p*-X-ANILINES

X	Method[a]	Yield, %	M.P.	Tit. M.P.
CH_3	A	96	72.5–74.8	74.5–75.5[b]
CH_3	B	44	73.8–74.6	74.5–75.5[b]
CH_3	C	35	74.0–74.5	74.5–75.5[b]
H	B	34	36.8–37.5	38.5–39.5[c]
H	D	52	36.6–37.6	38.5–39.5[c]
F	B	39	68.6–69.1	[d]
Br	B	35	84.5–85.0	83.5–84.5[e]
Cl	B	38	52.5–53.5	48–49[e]
CH_3O	B	36	68.1–69.1	[f]

[a] A (from the barium salt of the nitramine), B (from the aromatic amine), C (from the *N*-methylarylamine), D (from the aromatic amine by three successive nitrations). [b] Ref. 7. [c] Ref. 8. [d] *Anal.* Calcd. for $C_7H_7N_2O_2F$: C, 49.41, H, 4.15, N, 16.47. Found: C, 49.18; H, 4.26, N, 61.50. [e] E. Bamberger, *Ber.*, 30, 1260, 1261 (1897). [f] *Anal.* Calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.57; H, 5.77; N, 15.53.

It was also found that these compounds could also be formed by direct nitration of *N*-methylanilines (method C).

EXPERIMENTAL

Barium salt of N-nitro-p-toluidine. To a solution of 5.36 g. (0.050 mole) of *p*-toluidine in 100 ml. of anhydrous ether was added rapidly 55 ml. (0.068 mole) of a 1.23*M* phenyllithium solution in ether.[5] After 30 seconds, 15 ml. (0.112 mole) of amyl nitrate[6] was added and the mixture was allowed to stand at 25° for 45 min. It was then washed with 40 ml. of water and two 15-ml. portions of 2*M* potassium hydroxide solution. The combined washes were extracted twice with 25-ml. portions of ether and after cooling to 0° were treated first with 10 ml. of 3.0*M* ammonium chloride solution and then with 50 ml. of 1.0*M* barium chloride solution. The precipitate was collected by filtration, washed with ice water, and dried in air. There was obtained 8.10 g. of a light tan powder which when dissolved in boiling water, filtered, and allowed to crystallize yielded 5.52 g. (38%) of small, almost white crystals which decomposed violently at about 250°.

Preparation of N-nitro-N-methyl-p-toluidine. Method A. From the barium salt of N-nitro-p-toluidine. A mixture of 2.00 g. (0.0091 mole) of the barium salt of *N*-nitro-*p*-toluidine, 22.7 g. (0.228 mole) of potassium bicarbonate, 100 ml. of water, and 4.3 ml. (0.046 mole) of methyl sulfate was shaken or stirred at 25° for 4 hr. Then another 4.3-ml. portion of methyl sulfate was added, and agitation was contained for 16 more hr. The resulting mixture was extracted with two 30-ml. portions of ether. The combined ether solutions were washed with 25 ml. of 5% potassium hydroxide solution and 25 ml. of saturated brine. After drying over magnesium sulfate, the ether was evaporated on a 50° water bath, and the residue was crystallized from petroleum ether (b.p. 60–68°) to give 1.10 g. (96%) of glistening plates, m.p. 72.5–74.8° (reported[7] m.p. 74.5–75.5°).

Method B. From p-toluidine. To a solution of 5.36 g. (0.05 mole) of *p*-toluidine in 100 ml. dry ether was added all at once 65 ml. (0.07 mole) of 1.07*M* phenyllithium solution in ether. After 30 seconds, 15 ml. (0.11 mole) of amyl nitrate was swirled in, and the mixture was allowed to stand at 25° for 45 min. The ether solution was washed with 40 ml. of water and two 15-ml. portions of 2*M* potassium hydroxide solution. The combined aqueous solutions were washed with two 25-ml. portions of ether and then saturated with carbon dioxide. To the resulting solution was added 100 g. (1.0 mole) of potassium bicarbonate and 46 ml. (0.5 mole) of methyl sulfate, and the mixture was shaken or stirred for 20 hr. It was then extracted with two 50-ml. portions of ether and the combined ether solutions were washed first with 35 ml. of 2*M* potassium hydroxide solution and then with 35 ml. saturated brine. After drying over magnesium sulfate, the ether solution was decolorized with Darco and evaporated on a 50° water bath. The residue was crystallized from petroleum ether to give 3.69 g. (44%) of very light tan plates, m.p. 73.8–74.6° (lit.[7] m.p. 74.5–75.5°).

Method C. From N-methyl-p-toluidine. To a solution of 6.06 g. (0.050 mole) of *N*-methyl-*p*-toluidine in 100 ml. anhydrous ether was added quickly 55 ml. (0.068 moles) of 1.23*M* phenyllithium solution in ether, and then, after 30 seconds, 15 ml. (0.112 mole) of amyl nitrate. The mixture was allowed to stand 45 min. at 25° and was then cooled to 0° and extracted first with 50 ml. of ice-cold 1% hydrochloric acid solution and immediately thereafter with 30 ml. of

(5) R. G. Jones and H. Gilman, *Org. Reactions*, VI, 353 (1951).

(6) Commercial amyl nitrate (Ignition Improver DB-36), kindly supplied by the Ethyl Corporation, was used directly.

(7) J. Pinnow, *Ber.*, 30, 835 (1897).

cold 10% potassium carbonate solution and 25 ml. of saturated brine. The ether solution was dried over magnesium sulfate and evaporated on a water bath at 50°. The residue was dissolved in 100 ml. of petroleum ether and crystallized at Dry Ice-acetone bath temperatures. The product was recrystallized from petroleum ether to give 2.90 g. (35%) of glistening plates, m.p. 74.0–74.5° (reported[7] m.p. 74.5–75.5°).

Method D. N-Nitro-N-methylaniline. To a stirred solution of 1.50 g. (0.016 mole) of aniline in 50 ml. dry ether at 0° was added alternatively every 60 seconds 20 ml. (0.019 mole) of 0.94*M* phenyllithium in ether and 2.20 ml. (0.016 mole) of amyl nitrate until 60 ml. in all of phenyllithium solution and 6.60 ml. of amyl nitrate (three portions of each) had been added. The mixture was stirred 15 min., washed once with 50 ml. of water and twice with 25 ml. of 2*M* potassium hydroxide solution. The combined aqueous solutions were saturated with carbon dioxide. Then 28 g. (0.33 mole) of sodium bicarbonate and 15 ml. (0.16 mole) of methyl sulfate was added, and the mixture was stirred 5 hr. after which another portion (5 ml.) of methyl sulfate was added, and stirring was continued for 12 hr. Sufficient water was added to dissolve the sodium bicarbonate, and the solution was extracted two times with 50-ml. portions of ether. The combined ether extracts were washed twice with 10-ml. portions of 2*M* potassium hydroxide solution, dried over magnesium sulfate, and evaporated on a 50° water bath. The residue was dissolved in 50 ml. of dry ether and chromatographed on a 1.5 × 15 cm. column of activity grade I alumina using ether as eluant. The product was rapidly eluted. The residue from evaporation of the ether was crystallized from petroleum ether to give 1.28 g. (52%) of glistening white plates, m.p. 36.6–37.6° (reported[8] m.p. 38.5–39.5°).

The solution in which the reaction was carried out was extracted three times with 20 ml. of 5% hydrochloric acid. The combined acid solutions were made just basic and shaken with 5 ml. of acetic anhydride until the acrid odor of the latter disappeared. The acetanilide (0.40 g. (18%), m.p. 112°) resulting was isolated by ether extraction.

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(8) E. Bamberger, *Ber.*, **27**, 367 (1894).

Reactions with Diazoalkanes. I. Action of Diazomethane on *N*-Phenylmaleimide, with a Special Note on the Pyrolysis Products of the Pyrazoline

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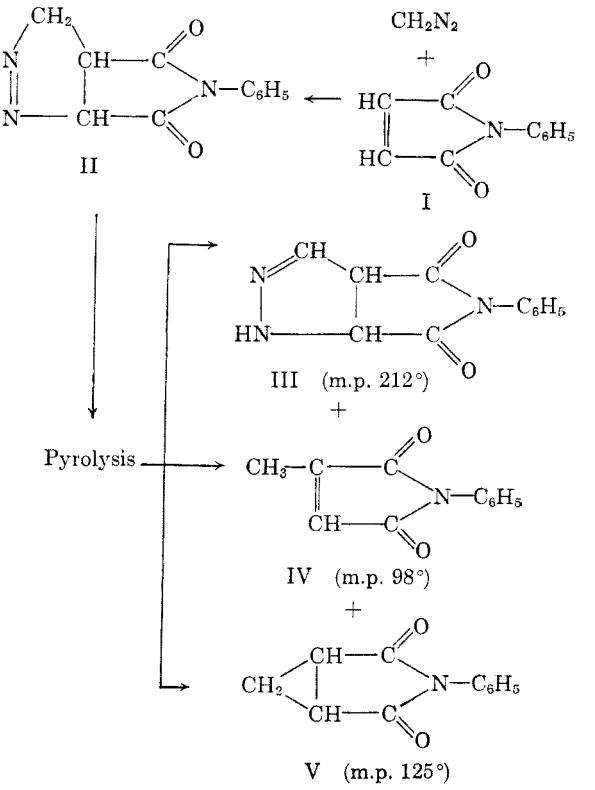
Recently Mustafa *et al.*[1] claimed that diazomethane reacts with *N*-phenylmaleimide (I) in the cold to yield directly Δ^2-pyrazoline (III). They also claimed that pyrolysis of III gave only one product, m.p. 98°, which they described as cyclopropane-2,3-(*N*-phenyl)dicarboximide from its identity with Perkin's product.[2]

(1) A Mustafa, S. M. A. Zayed, and S. Khattab, *J. Am. Chem. Soc.*, **78**, 145 (1956).

We have reinvestigated the action of diazomethane on *N*-phenylmaleimide and found that the product was a derivative of Δ^1-pyrazoline and not of Δ^2-pyrazoline (III) as claimed by Mustafa *et al.*[1]

This conclusion was based on the absence of any N—H stretching frequency in its infrared spectrum.[3]



Pyrolysis of the Δ^1-pyrazoline derivative (II). On heating the Δ^1-pyrazoline derivative (II) at 130° [*cf.* ref. (1)], it was recovered unchanged. However, when the substance was heated above its melting point in a vacuum for about six hours, it gave a mixture of three substances melting at 98°, 125°, and 212° (*cf.* Experimental). The main product, m.p. 98°, described by Mustafa *et al.*[1] to be a cyclopropane derivative (V), proved to be *N*-phenylcitraconimide by identity with an authentic specimen. Its ultraviolet spectrum[4] showed two bands at λ_{max} 223, 275 mμ, ϵ_{max} 18,300, 2600, respectively (Fig. 1), and was very similar to that of maleimide λ_{max} 220, 275 mμ, ϵ_{max} 9,300, 3,000, respectively (*cf.* Fig. 1). The slight bathochromic shift and the hyperchromic effect in the short wave-

(2) T. W. D. Gregory and W. H. Perkin, *J. Chem. Soc.*, **83**, 788 (1903).

(3) The infrared measurements were carried out by potassium bromide wafer technique using a Perkin-Elmer Infracord Model 137.

(4) Ultraviolet spectra were carried out using a Perkin-Elmer Spectracord Model 4000 in cyclohexane solutions.

(5) A. E. Gillam and E. S. Stern, *An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, London, Edward Arnold Ltd., 1955, p. 92.