III, b.p. 83.2–90.0° (750 mm.), 11.0 g., $n^{20.9}$D 1.4388, 99.6%; and IV, b.p. 90–98° (750 mm.), 1.0 g., $n^{20.9}$D 1.4365, 95.2%. Cut II was a colorless, mobile liquid, freezing at −11°, $n^{28.6}$D 1.4356, d^{24} 0.901 g./ml., net heat of combustion 710 kcal./mole,[10] miscible with water, ethanol, methylhydrazine, benzene and hexane, and immiscible with hydrazine, yield 11.1% (from 1-chloroaziridine). This cut was found to be extremely explosive when heated in an oxygen atmosphere.[11]

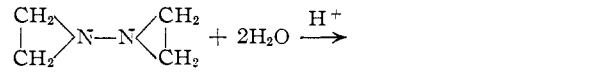
Anal. of Cut II: Calcd. for $C_4H_8N_2$: C, 57.14; H, 9.52; N, 33.33; mol. wt., 84.12; *MR*, 24.83. Found for $C_4H_8N_2$: C, 56.90; H, 9.96; N, 33.12; mol. wt., 84.2 (cryoscopic in benzene), 84.5 (potentiometric with potassium iodate); *MR*, 24.39.

1,1′-Biaziridine, in contrast to the simple alkyl hydrazines, is feebly basic, and could not be titrated with aqueous acid. However, a picric acid derivative of 1,1′-biaziridine was prepared in ether solution. After recrystallization from ethanol the compound slowly decomposed on heating without melting. An analysis with potassium iodate indicated that the oxidation of this compound involves a 6.12 electron change (theory 6.00).

Discussion

Proof of Structure of 1,1′-Biaziridine.—Several pieces of evidence have been obtained which indicate that the isolated product is 1,1′-biaziridine. Strong absorption in the infrared spectrum at 8.25 and 11.5 μ was interpreted to be convincing evidence that the compound contained at least one ethylenimine ring, since these frequencies have been assigned as the vibration frequencies of the ethylenimine ring itself.[12] The infrared spectrum also indicated the absence of N–H bonding (at 3.0 μ) and C=N bonding (at approximately 6.0 μ).[13]

When oxidized in 6 *N* hydrochloric acid solution with aqueous potassium iodate, the compound was found to undergo a six-electron change, which is characteristic of symmetrically disubstituted alkylhydrazines.[14] The observed electron change is explained readily in terms of the usual acid-catalyzed ring opening of ethylenimine derivatives, in this case to form *sym*-bis-β-hydroxyethylhydrazine



$$HOCH_2CH_2NHNHCH_2CH_2OH$$

The calculated molar refraction of the compound is also in keeping with the assigned structure. A nuclear magnetic resonance spectrum indicated that all of the hydrogen atoms are equivalent.[15] The method of preparation and these physical and chemical properties support the proposed 1,1′-biaziridine structure assigned to the compound.

Proof of Structure of the 1-Haloaziridines.—The compounds isolated from the reaction of sodium hypohalite with ethylenimine are considered to be the desired 1-haloaziridines on the basis of their method of preparation, immiscibility with water, infrared spectra (which show absorption at 8.25 and 11.5 μ), and the use of the chloro compound in the preparation of 1,1′-biaziridine.

(10) The authors are indebted to Mr. A. L. Parrette and to Mr. C. A. Leonard for heat of combustion data.

(11) Private communication from Dr. Adalbert Elek, Elek Microanalytical Laboratories, Los Angeles, Calif.

(12) H. T. Hoffman, Jr., THIS JOURNAL, **73**, 3028 (1951).

(13) Aliphatic Schiff bases of the type RN=CHR′ where R = CH_3, C_2H_5, C_3H_7, *i*-C_3H_7 and C_4H_9, and R′ = CH_3, C_2H_5 and C_3H_7 all absorb strongly in the region 1666 to 1674 cm.$^{-1}$ (approximately 6.0), which has been assigned as the characteristic absorption frequency for the C=N bond (L. Kahovec, *Acta Phys. Austria*, **1**, 307 (1958)). Acetaldazine, CH_3CH=NN=$CHCH_3$ (an isomer of 1,1′-biaziridine), absorbs strongly at 1627 cm.$^{-1}$ (W. West and R. B. Kellingsworth, *J. Chem. Phys.*, **6**, 1 (1938)).

(14) W. R. McBride, *et al.*, *Anal. Chem.*, **25**, 1042 (1953).

(15) J. D. Roberts, unpublished work.

AZUSA, CALIFORNIA

[CONTRIBUTION FROM PARKE, DAVIS & COMPANY'S MULTIPLE FELLOWSHIP IN MEDICINAL CHEMISTRY, MELLON INSTITUTE]

6-Diazo-5-oxo-L-norleucine, a New Tumor-inhibitory Substance.[1a] Preparation of L-, D- and DL-Forms[1b]

BY HORACE A. DEWALD[2] AND ALEXANDER M. MOORE[2]

RECEIVED DECEMBER 18, 1957

6-Diazo-5-oxonorleucine has been prepared by several methods. The synthesis was achieved by covering the amino and α-carboxyl functions of glutamic acid with appropriate protecting groups, converting the γ-carboxyl of glutamic acid to a diazo ketone and then removing the protecting groups by selective hydrolysis; and in crude form by the diazotization of the aminoketone, 5-oxolysine.

6-Diazo-5-oxo-L-norleucine has been prepared by several methods and has been submitted for biological tests which indicated it to be effective *in vitro* in inhibiting the growth of the bacterium *Escherichia coli*[3b] and the fungus *Torulopsis albida*[3a] and, in mice, in inhibiting the growth of the Crocker sarcoma-180 tumor.[4]

One method used to prepare 6-diazo-5-oxo-L-norleucine (VIII) involved using the 1-methyl ester of N,N-phthaloyl-L-glutamic acid (II) as the starting material. Sheehan and Bolhofer had prepared N,N-phthaloyl-L-glutamic anhydride (I) of unknown optical purity.[5] Subsequently, Tipson[6] showed Sheehan and Bolhofer's product to contain 88% of the L- and 12% of the DL-forms, based on the optical rotation reported by them. We found that treatment of the 88%–12% mixture with sodium methoxide at 5° gave a mixture of the 1-methyl esters II of N,N-phthaloyl-L(and DL)-

(1) (a) For paper II of this series, see H. W. Dion, S. A. Fusari, Z. L. Jakubowski, J. G. Zora and Q. R. Bartz, THIS JOURNAL, **78**, 3075 (1956). (b) Presented before the Division of Medicinal Chemistry at the 129th National Meeting of the American Chemical Society, Dallas, Texas, April, 1956.

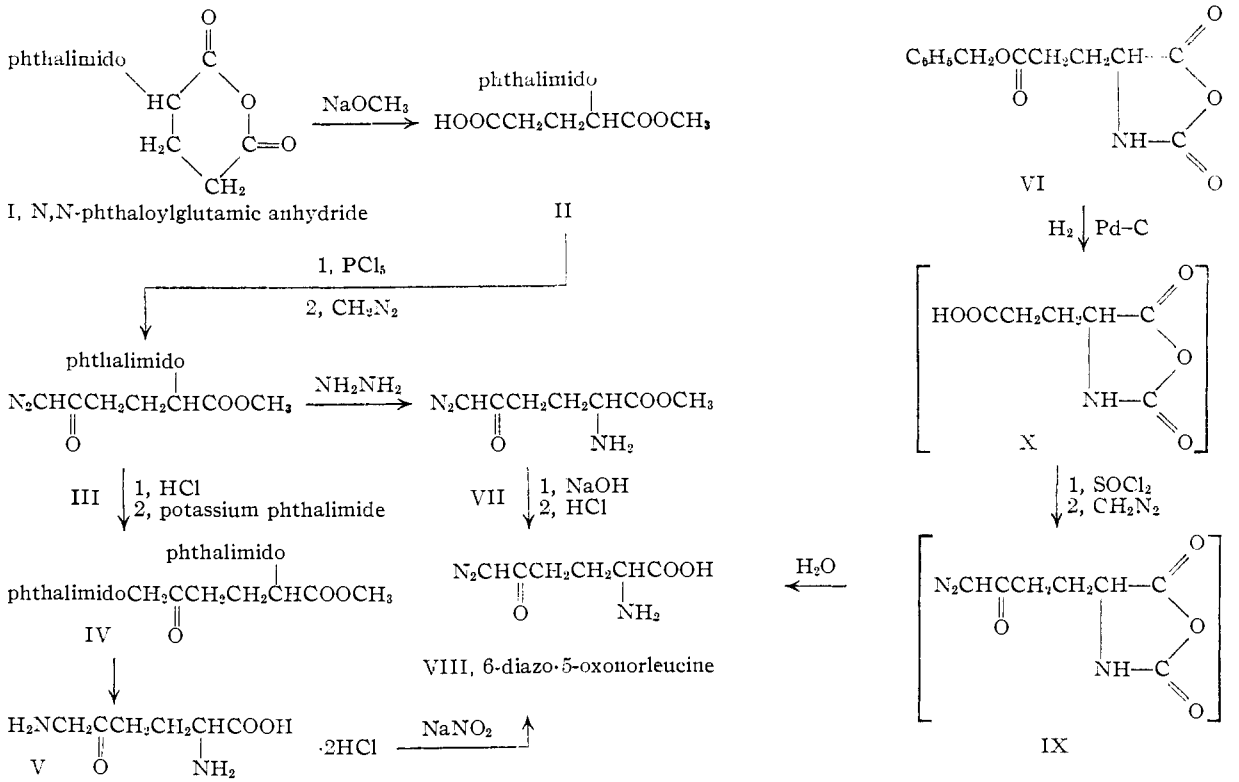
(2) Parke, Davis & Co., Detroit, Mich.

(3) (a) J. Ehrlich, G. L. Coffey, M. W. Fisher, A. B. Hillegas, D. L. Kohberger, H. E. Machamer, W. A. Rightsel and F. R. Roegner, *Antibiotics & Chemotherapy*, **6**, 487 (1956); (b) R. E. Maxwell and V. E. Nickel, *ibid.*, **7**, 81 (1957).

(4) D. A. Clarke, H. C. Reilly and C. C. Stock, Abstracts of Papers 129th Meeting, American Chemical Society, Dallas, Texas, April, 1956, p. 12-M.

(5) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2469 (1950).

(6) R. S. Tipson, *J. Org. Chem.*, **21**, 1353 (1956).



glutamic acids from which it was possible to obtain the L-form optically pure, m.p. 138–139°, $[\alpha]^{23}D$ −48°, in fair yield. The fact that some racemization occurred in the reaction with sodium methoxide was shown by isolating the 1-methyl ester of N,N-phthaloyl-DL-glutamic acid in an amount almost double the maximum amount which was theoretically obtainable if racemization had not occurred.

The 1-methyl ester II of N,N-phthaloyl-L-glutamic acid was converted to the acid chloride and thence to the corresponding diazoketone III. The phthaloyl group was removed from the 1-methyl ester III of N,N-phthaloyl-6-diazo-5-oxo-L-norleucine by refluxing with at least two equivalents of hydrazine in methylene dichloride to obtain the methyl ester VII of 6-diazo-5-oxo-L-norleucine. Compound VII was immediately converted to the sodium salt of the free acid by hydrolysis at 0° for 24 hours in aqueous methanol containing one equivalent of sodium hydroxide. Careful acidification of the reaction mixture to *p*H 6.5 yielded the desired 6-diazo-5-oxo-L-norleucine (VIII), which was obtained in the solid state by distilling the methanol, freezing the residual solution and subliming the ice from the frozen mass *in vacuo*. This product, which contained 30 to 40% 6-diazo-5-oxo-L-norleucine based on ultraviolet absorption spectra ($E^{1\%}_{1cm}$ 200 to 280 at 275 mμ), was purified by carbon chromatography to yield solids with $E^{1\%}_{1cm}$ values of 480 to 550. Recrystallization of the product from alcohol–water gave pale yellow crystals, m.p. 145–155° dec. This product was proved to be identical with the 6-diazo-5-oxo-L-norleucine prepared by the method of Westland, *et al.*,[7] and with that obtained from culture broths of an unidentified Streptomyces by Dion, *et al.*,[1] on the basis of the *Escherichia coli* growth inhibition test, infrared and ultraviolet absorption spectra and X-ray diffraction patterns.

In view of the instability of 6-diazo-5-oxo-L-norleucine in alkaline solutions, it is surprising that such vigorous reaction conditions could be used in the above method of preparation.

Another method used to prepare 6-diazo-5-oxo-L-norleucine involved starting with the benzyl ester of 2,5-dioxo-4-oxazolidinepropionic acid, L-form (VI).[8] Hydrogenolysis of VI using a palladium–carbon catalyst yielded the 2,5-dioxo-4-oxazolidinepropionic acid, L-form, (X). This compound was not purified but was converted directly into the acid chloride which was subsequently converted by diazomethane to the diazoketone IX. The oxazolidine ring of the diazoketone was opened by subjecting a dilute solution of the substance IX to the action of sodium hydroxide. The 6-diazo-5-oxo-L-norleucine was obtained in pure form using the purification method described above. The 6-diazo-5-oxo-L-norleucine obtained by this method was shown to be identical with that obtained by the previously described method on the basis of the *Escherichia coli* growth inhibition test.[3b]

The DL- and D-isomers of 6-diazo-5-oxo-L-norleucine were prepared by employing the first of the above methods starting with the 1-methyl esters II of N,N-phthaloyl-DL (and D)-glutamic acids, which were in turn obtained from N,N-phthaloyl-DL(and D)-glutamic anhydrides by the method of Sheehan.[5] 6-Diazo-5-oxo-DL-norleucine also was

(7) R. D. Westland, S. A. Fusari and H. M. Crooks, Jr., Abstracts of Papers, 129th Meeting, American Chemical Society, Dallas, Texas, April, 1956, p. 14-M.

(8) E. R. Blout, R. G. Karlson, P. Doty and B. Hargitay, THIS JOURNAL, **76**, 4492 (1954).

prepared in crude form from the methyl ester[5] IV of 5-oxo-DL-2,6-diphthalimidohexanoic acid by acid hydrolysis to 5-oxo-DL-lysine (V) followed by diazotization of the terminal amino group. The physical properties of the 6-diazo-5-oxo-D-norleucine were identical with those of the L-isomer[1] except that the optical rotation of the D-form was observed to be $[\alpha]^{25}D$ −16.1° (*c* 1.92, in water). The physical properties of the 6-diazo-5-oxo-DL-norleucine are: m.p. 145–155° dec., $E^{1\%}_{1cm}$ 683 at 274 mμ and 376 at 244 mμ. The X-ray diffraction patterns and also the infrared absorption curves of the optically active and racemic forms show marked differences. For comparison of the infrared curves see Fig. 1.

Acknowledgment.—The authors wish to thank Mr. C. E. Childs and associates, Parke, Davis & Co., and Dr. C. Tiedcke, Laboratory of Microchemistry, Teaneck, N. J., for the microanalyses reported. They also are indebted to Dr. J. M. Vandenbelt and Mr. R. B. Scott, Parke, Davis & Co. and Dr. F. A. Miller and associates, Mellon Institute, for infrared and ultraviolet absorption measurements. They also wish to express their appreciation to Dr. H. M. Crooks, Jr., for his interest and advice.

Experimental

5-Oxo-DL-lysine, Dihydrochloride, Monohydrate (V).—A mixture of 15.2 g. (0.035 mole) of 5-oxo-2,6-diphthalimidohexanoic acid, methyl ester[5] (IV) and 130 ml. of concentrated hydrochloric acid was heated under reflux for 16 hours. Three 20-ml. portions of acid were added during the reflux period. The mixture was cooled to 0° and 10.9 g. of phthalic acid was collected by filtering. The filtrate was extracted with ether and partially decolorized by treatment with Darco. The filtrate was adjusted to a *p*H of 8 with 4 *N* sodium hydroxide. This solution was warmed and was added rapidly to a hot mixture of 9 g. of picric acid in 100 ml. of water. The picrate of 5-oxolysine precipitated almost immediately as a voluminous yellow product. The mixture was allowed to stand for 20 hours and then the picrate was collected by filtration. The yellow solid weighed 8.5 g., corresponding to 63% yield. A small sample for analysis was recrystallized twice from water, m.p. 157–158° dec.

Anal. Calcd. for $C_{12}H_{15}N_5O_{10}$: C, 37.01; H, 3.88; N, 17.99. Found: C, 37.19; H, 3.78; N, 18.31.

The remainder of the picrate in 60 ml. of 6 *N* hydrochloric acid was warmed on a steam-bath for 30 minutes. The mixture was cooled and that portion of the picric acid which had crystallized was removed by filtration. The filtrate was extracted with ether to remove the remainder of the picric acid. The aqueous solution was decolorized and concentrated to a glass *in vacuo*. This glass was redissolved in water, shell-frozen and lyophilized. The colorless powder was crystallized from water by the addition of absolute ethanol; 2.6 g. of colorless solid, 53% yield, m.p. 114–115°.

Anal. Calcd. for $C_8H_{12}N_2O_3 \cdot 2HCl \cdot H_2O$: C, 29.36; H, 6.57; N, 11.42; Cl, 28.25. Found: C, 29.10; H, 6.57; N, 11.61; Cl, 28.15.

Crude 6-Diazo-5-oxo-DL-norleucine (VIII).—A solution of 0.25 g. (0.001 mole) of 5-oxolysine dihydrochloride in 10 ml. of water was cooled in an ice-bath to 5° and the *p*H was adjusted to 4 with sodium hydroxide. A solution of 0.14 g. (0.002 mole) of sodium nitrite in 2 ml. of water was added in one portion. After standing 10 minutes the yellow solution was shell-frozen and lyophilized. The hygroscopic yellow powder (0.35 g.) showed weak absorption in the ultraviolet, $E^{1\%}_{1cm}$ 38.2 at $\lambda^{H_2O}_{max}$ 275 mμ, corresponding to 5.8% 6-diazo-5-oxo-DL-norleucine, or 2.9% 6-diazo-5-oxo-L-norleucine. This powder was found to show the inhibitory properties of 6-diazo-5-oxo-L-norleucine (D.O.N.) against susceptible and resistant strains of the bacterium *Escherichia coli*.[9]

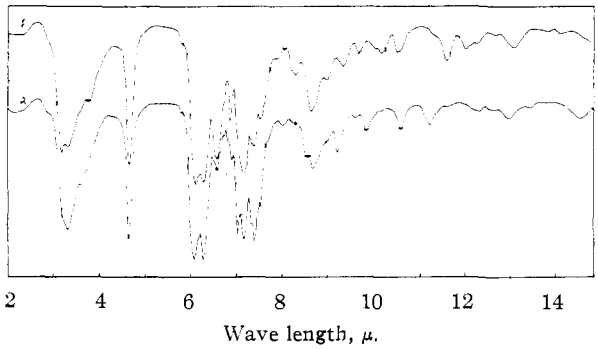


Fig. 1.—Infrared spectra of 6-diazo-5-oxo-L-norleucine (upper curve) and 6-diazo-5-oxo-DL-norleucine (lower curve) in pressed KBr pellets.

N,N-Phthaloyl-DL-glutamic anhydride (I) was prepared in 80% yield by the procedure of King[10] as a colorless powder, m.p. 193–197°.

N,N-Phthaloyl-DL-glutamic Acid, 1-Methyl Ester (II).—N,N-Phthaloyl-DL-glutamic anhydride was converted to the 1-methyl ester (m.p. 151–153°) in 56% yield by the method of Sheehan.[5]

6-Diazo-5-oxo-N,N-phthaloyl-DL-norleucine, Methyl Ester (III).—A suspension of 21.8 g. (0.075 mole) of N,N-phthaloyl-DL-glutamic acid, 1-methyl ester, and 15.6 g. (0.075 mole) of phosphorus pentachloride in 120 ml. of anhydrous ether was swirled occasionally at room temperature until a clear solution resulted. After two hours, the solvent was distilled *in vacuo*; more toluene was added and the distillation was repeated to remove all phosphorus oxychloride. The residue was redissolved in anhydrous ether and added dropwise at 5° to an ether solution of diazomethane (prepared from 30 g. of nitrosomethylurea). The excess diazomethane and ether were evaporated cautiously. The diazoketone weighed 25 g. The yellow oil was not crystallized; analyses for diazo nitrogen indicated 90–92% purity. Maxima in the ultraviolet spectrum were observed in water at λ 276, 240 and 220 mμ, with $E^{1\%}_{1cm}$ 248, 345 and 1140, respectively.

6-Diazo-5-oxo-DL-norleucine, Methyl Ester (VII).—The 6-diazo-5-oxo-N,N-phthaloyl-DL-norleucine, methyl ester, (25.0 g.) was dissolved in 200 ml. of methylene chloride, and 7.9 g. (two equivalents) of hydrazine hydrate was added. The mixture was stirred at room temperature for 24 hours and then stored at 0° overnight. A total of 14.4 g. of the hydrazine salt of 1,4-phthalazinediol was separated by filtering the mixture. The yellow filtrate was evaporated *in vacuo* to yield 11.0 g. of 6-diazo-5-oxo-DL-norleucine, methyl ester, as a yellow oil. The infrared spectrum of this oil showed that the protecting phthaloyl group had been largely removed, but the ultraviolet absorption spectrum exhibited maxima at λ 225 and 240 mμ ($E^{1\%}_{1cm}$ 330 and 180, respectively), as well as the expected band at 274 mμ ($E^{1\%}_{1cm}$ 200). The absorption at 225 mμ indicates the presence of some unchanged phthalimido compound.

6-Diazo-5-oxo-DL-norleucine (VIII).—Crude 6-diazo-5-oxo-DL-norleucine, methyl ester (11.0 g.) was dissolved in 300 ml. of methanol, and 70 ml. of 1.0 *N* sodium hydroxide was added at 0°. After storing overnight at 0°, the cold

(9) This yellow powder was assayed by R. E. Maxwell and Violet S. Nickel against sensitive and resistant strains of *E. coli* by procedures previously reported.[3b] Against a D.O.N.-sensitive strain, the yellow powder showed activity corresponding to 2.0 to 2.5% D.O.N., a result consistent with the ultraviolet data. Against a D.O.N.-resistant strain, it showed activity corresponding to only 0.1 to 0.2% D.O.N., suggesting the presence of salts or other substances slightly inhibitory to *E. coli*. Since acid is known to destroy D.O.N., a solution of the yellow powder was cooled to 0° and brought to *p*H 1.0 and after 20 minutes at this temperature was neutralized. Assay of the neutralized solution against the D.O.N.-sensitive strain gave results corresponding to a destruction of a content of about 2.5% D.O.N. Furthermore, the neutralized solution was assayed against the D.O.N.-resistant strain, and the activity of the solution was the same low value observed against this strain before acidification.

(10) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

red solution was adjusted to a *p*H of 6.5 with 2 *N* hydrochloric acid. Most of the methanol was removed *in vacuo*, the residual aqueous solution was shell-frozen and lyophilized to yield 12 g. of brown powder, $E^{1\%}_{1cm}$ 240 at $\lambda^{H_2O}_{max}$ 275 mμ.

The crude material was purified by passage through a carbon column as follows. A slurry of 15 g. of Darco G-60 and 15 g. of Celite 503 in 200 ml. of 1% aqueous acetone was poured into a glass column (22 mm. diameter). The holdup volume was 100 ml. A solution of 200–250 mg. of the crude 6-diazo-5-oxo-DL-norleucine in 10 ml. of 1% aqueous acetone was added to the column and as the charge approached the carbon surface, fresh 1% aqueous acetone was added. The eluate was collected in 10-ml. fractions and tested both with ninhydrin and spectrophotometrically at 275 mμ with the use of a Beckman model-DU spectrophotometer. The peak concentrations of product usually occurred at 1.5 to 1.9 holdup volumes. These fractions were shell-frozen and dried by ice-sublimation *in vacuo* to yield 35–40 mg. of pale-yellow powder. The solid from six such columns was crystallized by dissolving in 0.5 ml. of water and then adding 5 volumes of absolute ethanol. From six columns there was obtained 124 mg. of microcrystals, m.p. 145–55° dec., $E^{1\%}_{1cm}$ 660 at $\lambda^{H_2O}_{max}$ 274.5; and, in this manner, 4.25 g. of crude amino acid of $E^{1\%}_{1cm}$ 240 $\lambda^{H_2O}_{max}$ 275 mμ yielded 270 mg. of 6-diazo-5-oxo-DL-norleucine, or a yield of 6.8% based on N,N-phthaloyl-DL-glutamic acid, 1-methyl ester (II).

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.10; H, 5.30. Found: C, 41.98; H, 5.12.

A 35.5-mg. sample in 10 ml. of water was allowed to stand at room temperature for one week; it underwent 63% decomposition as measured by ultraviolet absorption spectra. Decomposition proceeds more rapidly in alkaline solution; dilute solutions of diazoöxonorleucine stored four days at room temperature in distilled water and in buffered solutions of *p*H 7.0, 8.0 and 9.0 decomposed 10, 31, 73 and 100%, respectively.

N,N-Phthaloyl-L-glutamic Anhydride (I).—An intimate mixture of 30 g. of L-glutamic acid and 30 g. of phthalic anhydride was heated in an oil-bath at 140–150° for 20 minutes. The melt was allowed to cool to 110° and 36 ml. of acetic anhydride was added. The temperature was maintained at 100–110° for 5 minutes and then 100 ml. of xylene was added. After cooling overnight, the solid was collected on a filter and washed with dry ether. The yield was 26.0–27.5 g., amounting to 50–53% of theory, m.p. 193–196° with preliminary softening. This anhydride was not optically pure, $[\alpha]^{23}D$ −36.5 to −38° (*c* 3, dioxane). Pure N,N-phthaloyl-L-glutamic anhydride has a rotation $[\alpha]^{21}D$ −43.1° (*c* 1.75 in dioxane).[6]

N,N-Phthaloyl-L-glutamic Acid, 1-Methyl Ester (II).—N,N-phthaloyl-L-glutamic anhydride (25.9 g., 0.1 mole), with $[\alpha]D$ −37°, was added portionwise to a stirred solution of sodium methoxide in methanol (175 ml., 0.57 *N*) at 5° during ten minutes. The solution was evaporated *in vacuo*. The residue was dissolved in 150 ml. of water, mixed with 150 ml. of chloroform and the *p*H was adjusted to 1.0 with concentrated hydrochloric acid. The layers were separated and the aqueous layer was extracted again with chloroform. The chloroform extracts were dried over magnesium sulfate and evaporated *in vacuo*. The hot residual oil was diluted with 50 ml. of dry ether. After standing at room temperature five hours, the crystalline product was collected on a filter. The colorless needles weighed 8.7 g. and melted at 138–140°, $[\alpha]^{22.5}D$ −44.5° (*c* 3 in methanol). An analytical sample was recrystallized from ethyl acetate–hexane, m.p. 138–139°, $[\alpha]^{23}D$ −48° (*c* 3 in methanol).

Anal. Calcd. for $C_{14}H_{13}NO_6$: C, 57.74; H, 4.50; N, 4.81. Found: C, 57.58; H, 4.44; N, 4.78.

The filtrate from above deposited another 6.5 g. of colorless solid, fine powder m.p. 149–152°. This is the racemic form of N,N-phthaloylglutamic acid, 1-methyl ester.[5] By concentrating the mother liquors, additional crops of impure methyl esters melting in the range 118–129° were obtained.

6-Diazo-5-oxo-N,N-phthaloyl-L-norleucine, Methyl Ester (III).—N,N-Phthaloyl-L-glutamic acid, 1-methyl ester, (7.3 g.) was converted to the acid chloride with phosphorus pentachloride and treated with excess diazomethane according to the procedure described above for the DL-form to yield impure 6-diazo-5-oxo-N,N-phthaloyl-L-norleucine, methyl ester, a yellow semi-solid weighing *ca.* 8.0 g.

6-Diazo-5-oxo-L-norleucine, Methyl Ester (VII).—The oily 6-diazo-5-oxo-N,N-phthaloyl-L-norleucine, methyl ester (8.0 g., *ca.* 0.025 mole) was dissolved in 150 ml. of methylene chloride and 1.92 g. (0.06 mole) of anhydrous hydrazine was added in two portions during an 8-hour period of gentle refluxing. The mixture was cooled and filtered to remove 4.2 g. of the hydrazine salt of 1,4-phthalazinediol. The filtrate was concentrated and after standing overnight at 0°, another 0.2 g. of the salt was collected. 6-Diazo-5-oxo-L-norleucine, methyl ester (4.2 g.) was obtained by evaporation of the solvent *in vacuo*, $E^{1\%}_{1cm}$ 310 at $\lambda^{H_2O}_{max}$ 275 mμ. A second strong absorption at 220 mμ with a shoulder at 240 mμ indicated incomplete hydrazinolysis of the protecting phthaloyl group.

6-Diazo-5-oxo-L-norleucine (VIII).—Crude 6-diazo-5-oxo-L-norleucine, methyl ester (3.6 g., 0.02 mole) was dissolved in 120 ml. of methanol and the yellow solution was cooled to 0°. To the cold solution was added 20 ml. of 1.0 *N* sodium hydroxide. After standing at 0° for 16 hours, the *p*H of the solution was adjusted to 6.5 with 2 *N* hydrochloric acid. The methanol was distilled *in vacuo*. The aqueous residue was frozen and lyophilized to yield 4.0 g. of crude 6-diazo-5-oxo-L-norleucine, $E^{1\%}_{1cm}$ 238 at $\lambda^{H_2O}_{max}$ 275 mμ. The yellow powder (3.6 g.) was chromatographed on activated carbon as described previously for the DL-form. The 250 mg. of amino acid thus obtained was recrystallized from water–ethanol to give pale yellow crystals, yield 140 mg., m.p. 145–155° dec., $E^{1\%}_{1cm}$ 660 at $\lambda^{H_2O}_{max}$ 275 mμ. This compound was identical with the compound described by Westland[7] and Dion[1] by comparison of their infrared spectrum and by microbiological assay *vs. E. coli* and *T. albida*.

N,N-Phthaloyl-D-glutamic Anhydride (I).—This compound, prepared from commercial D-glutamic acid in the same manner as the L-compound above, was obtained chemically pure in 47% yield, m.p. 195–200°, $[\alpha]^{23}D$ +37.0 ± 0.5° (*c* 3 in dioxane).

Anal. Calcd. for $C_{13}H_9NO_5$: C, 60.24; H, 3.50; N, 5.41. Found: C, 60.38; H, 3.65; N, 5.58.

N,N-Phthaloyl-D-glutamic acid, 1-methyl ester (II) was prepared in the same manner as the L-form described above, in 28% yield, colorless needles, m.p. 138–139°, $[\alpha]^{22}D$ +47° (*c* 3 in methanol).

Anal. Calcd. for $C_{14}H_{13}NO_6$: C, 57.74; H, 4.50; N, 4.81. Found: C, 57.59; H, 4.57; N, 4.92.

6-Diazo-5-oxo-N,N-phthaloyl-D-norleucine, 1-Methyl Ester (III).—N,N-Phthaloyl-D-glutamic acid, methyl ester, (7.4 g., 0.025 mole), was converted to the acid chloride and treated with excess diazomethane as described above for the DL-isomer to yield approximately 9.5 g. of semi-crystalline 6-diazo-5-oxo-N,N-phthaloyl-D-norleucine, methyl ester.

6-Diazo-5-oxo-D-norleucine, Methyl Ester (VII).—The crude 6-diazo-5-oxo-N,N-phthaloyl-D-norleucine, methyl ester, from above was dissolved in 100 ml. of methylene chloride. Anhydrous hydrazine (1.9 g., 0.06 mole) was added and the mixture was heated under reflux for 8 hours. The insoluble hydrazine salt (4.3 g.) was collected by filtration. The filtrate was evaporated *in vacuo*. The residue was triturated again in methylene chloride, treated with Filter-cel and filtered again. The yellow oil that remained after evaporating the solvent *in vacuo* weighed about 2.5 g. The ultraviolet absorption spectrum in water ($E^{1\%}_{1cm}$ 420, $\lambda^{H_2O}_{max}$ 275 mμ) showed no signs of the intensely absorbing phthalimido structure.

6-Diazo-5-oxo-D-norleucine (VIII).—Crude 6-diazo-5-oxo-D-norleucine, methyl ester, from above was dissolved in 30 ml. of methanol and treated with 20 ml. of 1.0 *N* sodium hydroxide at 0°. After 18 hours at 0°, the *p*H of the reaction mixture was adjusted to 6.8 with dilute hydrochloric acid. The methanol was distilled *in vacuo*. The residual aqueous solution was shell-frozen and dried *in vacuo* to yield 3 g. of brown powder ($E^{1\%}_{1cm}$ 275, $\lambda^{H_2O}_{max}$ 275 mμ). The crude product was purified by passage over carbon columns (250 g. of Darco G-60 and 250 g. of Celite 545) as described previously. The yellow powder (305 mg.) obtained from the column purification was crystallized from water–ethanol to yield 120 mg., $E^{1\%}_{1cm}$ 683, $\lambda^{H_2O}_{max}$ 274.5 mμ, $[\alpha]^{25}D$ −16.1 ± 0.5° (*c* 1.98 in water).

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.10; H, 5.30; N, 24.56. Found: C, 41.77; H, 5.25; N, 24.33.

2,5-Dioxo-4-oxazolidinepropionic Acid, Benzyl Ester (VI).—This Leuchs anhydride was prepared in 70% yield

by the reaction of phosgene with the 5-benzyl ester[11] of L-glutamic acid in tetrahydrofuran according to the procedure of Blout and co-workers.[8] After one recrystallization from ethyl acetate–hexane, the colorless solid melted at 91–93°.

4-(4-Diazo-3-oxobutyl)-2,5-oxazolidinedione, L-Form (IX).—A solution of 2.6 g. (0.01 mole) of the benzyl ester from above was hydrogenated in 70 ml. of anhydrous ethyl acetate over 0.6 g. of 10% Pd–C catalyst at 30 lb. hydrogen pressure for one hour. The catalyst was removed by filtration using Filter-Cel. The filtrate was evaporated *in vacuo* to yield a colorless crystalline solid that weighed 1.65 g. and was presumed to be X. This solid was treated immediately with 15 ml. of thionyl chloride. After 15 minutes of gentle warming (*ca.* 40°), a clear yellow solution resulted. The solution was evaporated *in vacuo*. The resulting yellow oil was redissolved in 20 ml. of dry tetrahydrofuran and the solution was added dropwise with stirring to an ice-cold tetrahydrofuran solution of diazomethane (prepared from 7 g. of nitrosomethylurea). The mixture was allowed to warm to room temperature, and after filtering, it was evaporated *in vacuo*. The orange-yellow gum appeared to be decomposing with the evolution of gas.

6-Diazo-5-oxo-L-norleucine (VIII).—Approximately 1 g. of the crude anhydride IX from above was treated with 15 ml. of water, and normal sodium hydroxide was added to a *p*H of 9. After 30 minutes, the *p*H was adjusted to 6.5 with hydrochloric acid and the solution was frozen and lyophilized to yield 1.2 g. of crude 6-diazo-5-oxo-L-norleucine, $E^{1\%}_{1cm}$ 347 at $\lambda^{H_2O}_{max}$ 275 mμ. The yellow solid was chromatographed on activated carbon as described previously. The amino acid was recrystallized from water–ethanol, yield 48 mg., m.p. 142–150° dec., $E^{1\%}_{1cm}$ 640 at $\lambda^{H_2O}_{max}$ 275 mμ. This product was identical with the 6-diazo-5-oxo-L-norleucine previously described according to the assay on the growth inhibition of *E. coli*.[3b]

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[CONTRIBUTION FROM THE CHEMICAL CORPS, CHEMICAL RESEARCH DIVISION, CHEMICAL WARFARE LABORATORIES]

Organic Phosphorus Compounds. III.[1] O,O'-Dialkyl Alkylphosphonothioates and O-Alkyl Alkylphosphonochloridothioates

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The lower members of the series of O,O'-dialkyl methyl- and ethylphosphonothioates were prepared from the appropriate alkylphosphonothioic dichlorides and the sodium alkoxides by the method of Razumov and co-workers.[3] Several O-alkyl methyl- and ethylphosphonochloridothioates were prepared from the corresponding alkylphosphonothioic dichlorides and also by a one-batch procedure in which the appropriate alkylphosphonous dichloride in an inert reaction medium was converted successively with one mole equivalent each of an alcohol and an organic base to the corresponding alkyl alkylphosphonochloridite and further by the addition of sulfur to the desired chloridothioate without isolation of the intermediate.

The recent resolution of O-ethyl ethylphosphonothioic acid, $C_2H_5P(O)(OC_2H_5)SH$ or $C_2H_5P(S)(OC_2H_5)OH$, into its optically active isomers[4] made a practical method for the preparation of O-alkyl methyl- and ethylphosphonothioic acids desirable. Dialkyl esters of the type $RP(S)(OR')_2$ (I) and O-alkyl alkylphosphonochloridothioate of the type $RP(S)(OR')Cl$ (II) in which R represents a methyl or ethyl group and R' is an alkyl group seemed to be suitable starting materials for the preparation of the desired O-alkyl alkylphosphonothioic acids, $RP(O)(OR')SH$ or $RP(S)(OR')OH$ (III). Several representatives of the series of the esters I have been prepared previously by Russian investigators according to the following methods: (1) addition of sulfur to the corresponding dialkyl alkylphosphonites[3,5]; (2) reaction of the appropriate alkylphosphonothioic dichloride, $RP(S)Cl_2$ (IV), with sodium alkoxide[3] or with the appropriate alcohol and a base[3,5]; (3) treatment of O,O'-dialkyl sodium phosphothioite, $(R'O)_2P(S)Na$, with an alkyl halide[6]; and (4) reaction of dialkyl alkylanilidophosphonates with carbon disulfide.[7] However, chloridothioates of type II apparently have not been prepared before.

Since the esters I and the chloridothioates II can be converted readily by treatment with one mole equivalent of alcoholic alkali hydroxide to the alkali salts of the corresponding thioic acids III,[8] the preparation of the lower members of both series of derivatives I and II from methyl- and ethylphosphonous dichloride was investigated for the purpose of working out reliable preparative methods which would make these valuable intermediates readily accessible.

According to the methods reported for the esters I, their preparation from the phosphonous dichlorides can be accomplished by two different methods in two steps each. Conversion of the phosphonous dichloride with sulfur to the corresponding IV followed by treatment of the IV with an alcohol in the presence of a suitable hydrogen chloride acceptor[3,5] results in the formation of the desired I. The same products also are obtained when the phosphonous dichloride is first treated with the alcohol and base and the resulting dialkyl alkylphosphonite then treated with one mole equivalent of sulfur.

The addition of sulfur to methyl- and ethylphosphonous dichloride proceeds readily in excellent yields by the method of McIvor, McCarthy and Grant[9] according to which the calculated amount

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(8) A paper on the preparation of the III by this method is in preparation.

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