Alanine Derivatives with Reactive Groups

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Abstract □ The synthesis of diazoketone analogs of amino acids and associated problems were investigated with *N*-phthaloyl-DL-alanine serving as a model. The carboxyl was activated by conversion to the acid chloride or, under mild conditions, to the mixed anhydride obtained with ethyl chloroformate or dicyclohexylcarbodiimide; the product was reacted with diazomethane. Deblocking the phthaloyl group with hydrazine gave 3-amino-1-diazo-2-butanone as a well-characterized solid salt and as a less stable oil. Further reactions of the blocked diazoketone of DL-alanine, such as conversion to α-haloketones, Wolff rearrangement, and deuterium exchange on C-1 were investigated. 3-Amino-1-diazo-2-butanone had moderate inhibitory activity against mouse mammary adenocarcinoma in cell culture.

Keyphrases □ Alanine derivatives, various—synthesized, evaluated for cytotoxic activity □ Cytotoxic activity—various alanine derivatives evaluated □ Amino acids—various alanine derivatives synthesized, evaluated for cytotoxic activity □ Structure-activity relationships—various alanine derivatives evaluated for cytotoxic activity

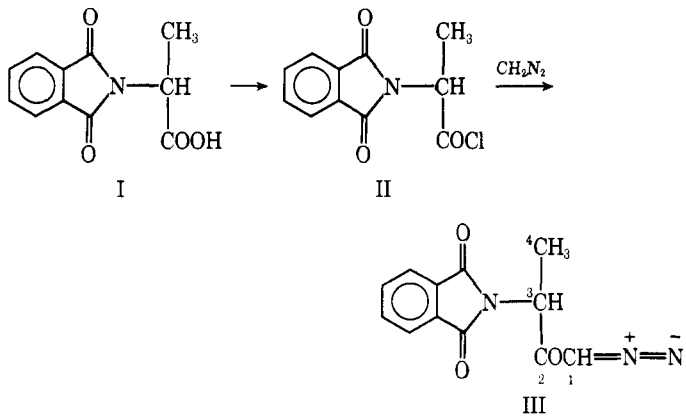
Diazoketone or diazoester analogs of amino acids include well-known antibiotics such as 6-diazo-5-oxo-L-norleucine and azaserine, which were found to have antitumor activity. Both of these antibiotics owe their biological activity to their inhibitory activity on various enzymes in which glutamine is one of the substrates. Enzymes in the purine nucleotide biosynthetic pathway appear to be most sensitive to these agents (1–4). These enzymes include phosphoribosylformylglycinamidine synthetase (EC 6.3.5.3), carbamoyl-phosphate synthase (ammonia) (EC 2.7.2.5), and amidophosphoribosyltransferase (EC 2.4.2.14). In addition, 6-diazo-5-oxo-L-norleucine inhibited the synthesis of cytidine triphosphate from uridine triphosphate, of guanosine 5′-phosphate from xanthosine 5′-phosphate, and of D-glucosamine from fructose 6-phosphate (5). Azaserine alkylates, by its diazomethyl group, the thiol group of a cysteine residue in the active site of EC 6.3.5.3 (6–8) and was considered (9) to be the first example of a classical-type antimetabolite causing specific active-site-directed irreversible inhibition.

DISCUSSION

Analogs of other amino acids having either the diazoketone group or the chloroketone group are of interest as potentially antiproliferative agents. Certain cancer cells have an absolute requirement for cysteine (10, 11) and serine (12–15).

The synthesis of amino acid analogs with reactive groups presents challenges in terms of the choice of a blocking group suitable for introducing the reactive group and removable under mild conditions in the presence of such a group. Protection of the amino group with phthaloyl has been used extensively in peptide synthesis (16) and in the synthesis of certain amino acid homologs by the Wolff rearrangement. It offers the advantage of mild deblocking with hydrazine, protection of the two hydrogens of the amino group, and considerable stability. In this study, *N*-phthaloyl-DL-alanine (I, Scheme I) was used initially and various methods of introducing the diazoketone group, as well as various deblocking procedures, were investigated.

One target compound in this study was 3-amino-1-diazo-2-butanone, which has the potentiality of being an irreversible inhibitor of pyridoxal phosphate-containing enzymes such as L-alanine-glutamic acid transaminases. The analog has the required functionality for the initial



Scheme I

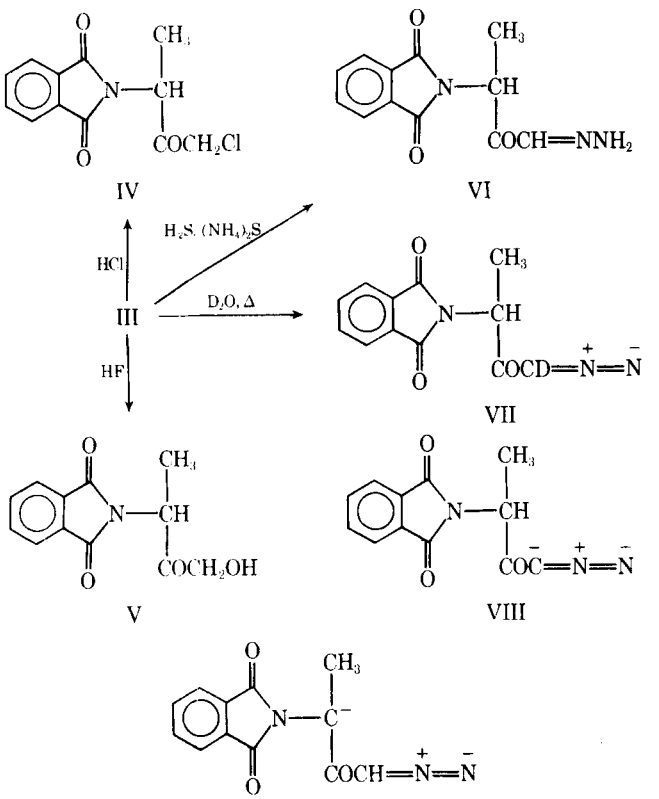
Schiff-base formation and the diazoketone group as a potential alkylating function. Some developed methods were applied to the synthesis of the corresponding cysteine derivatives (17). The diazoketone III was obtained either by interaction of diazomethane with the acid chloride II or directly from I by a mixed anhydride procedure with ethyl chloroformate or dicyclohexylcarbodiimide. The acid chloride II could be made either by the conventional method or by a milder method using thionyl chloride and dimethylformamide (18). These two procedures gave good yields of the diazoketone. The mixed anhydride method (19) gave good yields; but with the dicyclohexylcarbodiimide method, the yield was poor (27%), as might be expected (20), although this disadvantage is compensated for by the mildness of the method.

Some of the chemical properties and reactions of the blocked diazoketone were investigated before carrying out the deblocking experiments. Treatment of III with hydrochloric acid gave 1-chloro-3-phthalimido-2-butanone (IV, Scheme II). On treatment of III with hydrofluoric acid, however, 1-hydroxy-3-phthalimido-2-butanone (V) was isolated and not the expected fluoro compound. Reaction of III with hydrogen sulfide in the presence of ammonium sulfide gave 1-hydrazono-3-phthalimido-2-butanone (VI).

UV spectral studies on III led to the postulate that the most acidic hydrogen was in the 3-position, giving rise to the anion IX (21). To test this hypothesis, III was selectively deuterated with deuterium oxide in acetonitrile-d_3 in solution for 4 days at 55–60°. The ^{1}H-NMR spectrum of the product showed an exclusive incorporation of deuterium on C-1, as indicated in the structure shown for VII. This observation was confirmed by ^{13}C-NMR spectroscopy, which resulted in disappearance of the C-1 resonance in III on deuteration, permitting an unequivocal assignment of C-1 and C-3 resonances at 53.8 and 52.4 ppm, respectively. No deuterium exchange was observed in the related hydrazone derivative (VI) under similar conditions (see *Experimental*). Thus, the changes of UV absorption in III should be interpreted in terms of formation of the anion VIII, rather than the isomeric anion IX, which was assumed to be formed under these conditions (21).

Various methods for the preparation of homologs also were investigated. Application of silver oxide catalyzed the Wolff rearrangement of III in methanol, giving the higher homologous ester methyl 3-phthalimidobutyrate (X, Scheme III), which gave 3-aminobutyric acid (XI) on hydrolysis and 3-aminobutyrohydrazide (XII) on hydrazinolysis. Photochemical rearrangement of III in dry methanol also gave X, whereas pyrolysis in benzyl alcohol and γ-collidine at 175° in the absence of silver oxide gave the benzyl ester of the higher homologous acid (XIII).

Although cleavage of phthaloylalanine (I) with hydrazine gave rise to alanine and phthaloyl hydrazide exclusively, a similar reaction with III resulted in three products, which were separated by chromatography on a silica gel column (Scheme IV). In addition to the oily 3-amino-1-diazo-2-butanone (XIV), a solid phthaloyl hydrazide salt (XV) could also



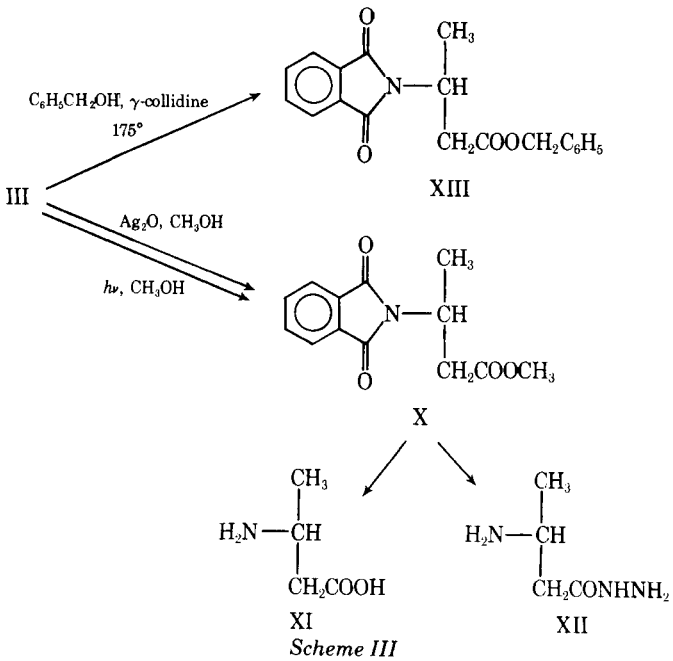
Scheme II

be isolated. Attempts to hydrazinolyze III with the unsymmetrical 1,1-dimethylhydrazine or the symmetrical 1,2-dimethylhydrazine at room temperature to avoid salt formation were not successful. The salt XV could not be dissociated by treating its ethyl acetate solution with aqueous sodium carbonate. The base XIV was unstable, probably because of self-condensation leading to a pyrazine derivative.

The compounds synthesized in this study were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA3) cells in culture[1]. Compound XIV at 1×10^{-4} *M* inhibited 20% of growth of these cells. The α-chloroacetyl derivative (IV), the substituted diazoketone derivative (III), and the phthaloylhydrazide salt (XV) were inactive at 10^{-4} *M*. The relative insolubility of VI precluded its being tested at a higher concentration than 3×10^{-5} *M*, at which it was inactive. In addition, higher homologous alanine derivatives such as XI and XII were inactive at 10^{-4} *M*.

EXPERIMENTAL[2]

1-Diazo-3-phthalimido-2-butanone (III)—*Method A: Acid Chloride Method*—A mixture of I (3.0 g, 13.7 mmoles) and excess thionyl chloride (10 ml, 0.14 mole) was heated at 60° with stirring for 1 hr, and a clear solution was obtained. The excess thionyl chloride was removed *in vacuo*. The residue was dissolved in dry benzene (3 × 25 ml), the solution was evaporated to remove traces of thionyl chloride, and the residue was dried *in vacuo* over potassium hydroxide.



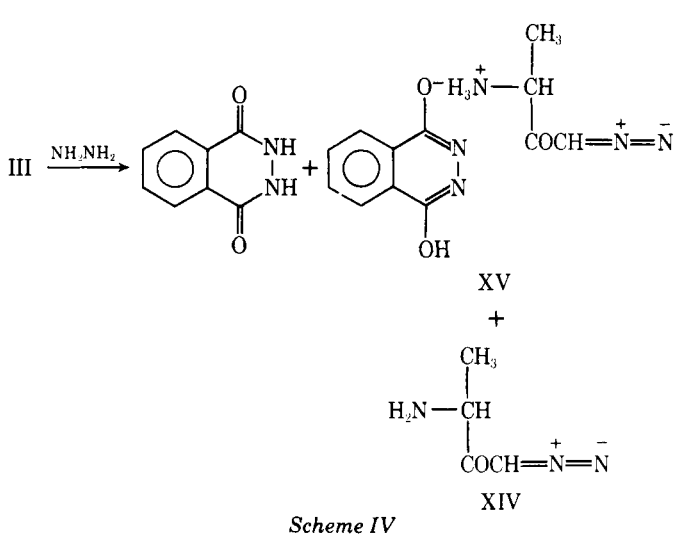
Scheme III

The crude product (3.25 g, 99%), mp 71–72°, was used for the next step without further purification. A solution of crude *N*-phthaloyl-DL-alanyl chloride (3.25 g, 13.6 mmoles) in dry benzene (25 ml) was added slowly, with stirring, to an ice-cold ethereal solution of diazomethane (3 g, 71.5 mmoles, from 21.5 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[3]). The reaction mixture was left at room temperature overnight, and excess diazomethane and solvent were removed by passing nitrogen through the reaction mixture.

After the yellowish oily residue was dried *in vacuo*, crystalline material separated out. The solid material was filtered out and recrystallized from ethyl acetate. The yield was 2.6 g (78%), mp 107°; IR: ν_{max}^{KBr} 2119 (diazo), 1720, 1702, and 1691 (keto) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.69 (d, CH_3, J = 7 Hz), 4.96 (q, 3-CH, J = 7 Hz), 5.51 (s, 1-CH), and 7.95 (aromatic H) ppm; ^{13}C-NMR ($CDCl_3$): 190.3 (2-C=O), 167.6 (phthaloyl C=O), 134.4, 131.9, 123.5 (aromatic C), 53.8 (1-CH), 52.4 (2-CH), and 14.6 (4-CH_3) ppm.

Anal.—Calc. for $C_{12}H_9N_3O_3$: C, 59.25; H, 3.73. Found: C, 59.13; H, 3.91.

Method B: Mixed Carboxylic-Carbonic Anhydride Method—A solution of I (2 g, 9.1 mmoles) in anhydrous tetrahydrofuran (25 ml) was cooled to −10° and treated with triethylamine (1.3 ml, 9.5 mmoles, dried over potassium hydroxide and distilled). Then freshly distilled ethyl chloroformate (0.9 ml, 9.3 mmoles) was added dropwise with stirring when a white precipitate of triethylamine hydrochloride separated out.



Scheme IV

[1] The mouse mammary adenocarcinoma cells (TA3) were grown in stationary tube cultures in RPMI 1640 medium containing 10% horse serum. An inoculum of 50,000 cells in 1 ml of medium was supplemented with 1 ml of medium containing the test compound. The tubes were incubated in an upright position for 3 days, and growth was estimated by protein assay. The growth in controls varied from six- to 10-fold. Every concentration (10^{-4}–10^{-6} *M*) was tested in five tubes each. For compounds found to be inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50% inhibitory concentration.

[2] Melting points (uncorrected) were determined by the capillary method. UV spectra were determined with a Cary model 14 spectrophotometer. IR spectra were determined with a Perkin-Elmer 457 spectrophotometer, ^{1}H-NMR spectra were obtained with a Varian A60A instrument, and ^{13}C-NMR spectra were obtained with a Varian XL-100A instrument. The positions of peaks are expressed in δ (parts per million) downfield from tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate for solutions in deuterium oxide. Thin-layer chromatograms were run on glass plates coated with Merck HF-254 silica gel; spots were detected by their UV absorption or by spraying with a ninhydrin reagent.

[3] Diazald, Aldrich Chemical Co., Milwaukee, Wis.

The reaction mixture was stirred at −10° for 0.5 hr and filtered, and the residue was washed with anhydrous ether (10 ml).

The filtrate and the washing were added slowly, with stirring, to an ice-cold ethereal solution of diazomethane (1.2 g, 28.6 mmoles, from 8.6 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[3] in 80 ml of ether). The reaction mixture was left at room temperature overnight, and excess diazomethane and solvent were removed with a current of dry nitrogen. The oily residue was evaporated *in vacuo*, dissolved in ether (50 ml), and washed with water (20 ml), followed by 5% $NaHCO_3$ (20 ml) and again with water (20 ml). The ether extract was evaporated *in vacuo*, and crystalline material separated out. This material was filtered, washed with petroleum ether, and dried; the yield was 1.67 g (76%), mp 106–107°. The product was identical with that obtained by Method A on the basis of mixed melting-point, IR, NMR, and TLC data.

Method C: Dimethylformamide and Thionyl Chloride Method—Thionyl chloride (0.17 ml, 0.23 mmole) was added slowly with stirring to a solution of I (0.5 g, 2.2 mmoles) in dry dimethylformamide (5 ml), and the mixture was cooled to −10°. The stirring was continued at from −5 to −10° for 1 hr, and the reaction mixture was evaporated *in vacuo*. The residue was dissolved in dry benzene (5 ml), added to an ice-cooled ethereal solution of diazomethane (1.2 g, 28.6 mmoles, from 8.6 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[3]), and left at room temperature overnight. Excess diazomethane and solvent were removed by passing dry nitrogen through the solution.

The residue was extracted with ether, and the ether was evaporated *in vacuo*. The residue was crystallized from a mixture of ether and petroleum ether; the yield was 0.33 g (60%), mp 106–107°. The compound was identical with the product obtained by Methods A and B.

Method D: Dicyclohexylcarbodiimide Method—Compound I (1 g, 4.56 mmoles) was added to a solution of dicyclohexylcarbodiimide (1 g, 4.8 mmoles) in dry tetrahydrofuran (15 ml); the solution turned turbid, and a white precipitate separated out. The suspension was stirred at room temperature for 4 hr, added slowly to an ice-cold ethereal solution of diazomethane (1.2 g, 28.6 mmoles, from 8.6 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[3]), and left overnight at room temperature. Excess diazomethane and solvent were removed by passing nitrogen through the solution.

The residue was dried *in vacuo* and then taken up in ether. The white precipitate of dicyclohexylurea that separated out was removed by filtration. The filtrate was evaporated *in vacuo*, and the residue was crystallized from ether; the yield was 0.3 g (27%), mp 106–107°. The compound was identical with the product obtained by Methods A, B, and C.

1-Chloro-3-phthalimido-2-butanone (IV)—An ethereal solution of hydrochloric acid (5 ml, 24%) was added slowly to an ice-cold ethereal solution of 1-diazo-3-phthalimido-2-butanone (100 mg in 15 ml). The reaction mixture was stirred at room temperature overnight (18 hr) and was then evaporated *in vacuo*. The crystalline residue was taken up in petroleum ether, and the mixture was filtered.

The crystals were washed with petroleum ether and dried; the yield was 95 mg (92%), mp 119–120°. The compound gave a positive Baker's test (22); IR: ν_{max}^{KBr} 1771, 1741, and 1705 (keto) cm^{-1} but no diazo peak at 2119 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.68 (d, CH_3, J = 7 Hz), 4.27 (s, 1-CH_2), 5.21 (q, 3-CH, J = 7 Hz), and 7.95 (aromatic protons) ppm.

Anal.—Calc. for $C_{12}H_{10}ClNO_3$: C, 57.26; H, 4.00; N, 5.56. Found: C, 57.22; H, 4.30; N, 5.81.

Attempted Synthesis of 1-Fluoro-3-phthalimido-2-butanone: Formation of 1-Hydroxy-3-phthalimido-2-butanone (V)—*Method A*—A solution of hydrofluoric acid (from 2 ml of aqueous 48% HF in 10 ml of anhydrous ether) was added dropwise to a stirring suspension of 1-diazo-3-phthalimido-2-butanone (100 mg) in anhydrous ether (25 ml) until a clear solution was obtained. The reaction mixture was stirred at room temperature for 1 hr and was then evaporated *in vacuo*; the resulting gum was dried *in vacuo*. TLC [benzene–ether (1:1)] of the product showed one major and two minor spots.

The product corresponding to the major spot was isolated by column chromatography on 200–325-mesh silica gel[4] with benzene–ether (1:1) as the eluent; the yield was 680 mg (71%), mp 81–82° (after crystallization from ether). IR, NMR, and elemental analyses indicated that the product was 1-hydroxy-3-phthalimido-2-butanone; IR: ν_{max}^{KBr} 3520, 3460 (OH), 1772, 1740, and 1705 (keto) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.68 (d, CH_3, J = 7 Hz), 3.24 (b, OH), 4.42 (s, α-CH_2), 4.98 (q, 3-CH, J = 7 Hz), and 7.82 (aromatic protons) ppm.

Anal.—Calc. for $C_{12}H_{11}NO_4$: C, 61.79; H, 4.75; N, 6.00. Found: C, 62.14; H, 4.89; N, 5.88.

Method B—1-Diazo-3-phthalimido-2-butanone (500 mg) was suspended in anhydrous ether (30 ml), and the suspension was cooled in ice. Anhydrous hydrogen fluoride gas was passed through the suspension for 6 hr, and a clear solution was obtained. The solution was left overnight at room temperature and was then evaporated by a nitrogen stream. The residue, on TLC, showed the presence of the starting material and two spots of lower R_f, one corresponding to that of 1-hydroxy-3-phthalimido-2-butanone.

The residue was dissolved again in anhydrous ether (30 ml), and a slow stream of hydrogen fluoride gas was passed through this solution for 24 hr. Examination of the reaction product at 6-hr intervals indicated the presence of the starting material and a major spot due to 1-hydroxy-3-phthalimido-2-butanone. The major fraction was isolated by chromatography on silica gel and was identified as 1-hydroxy-3-phthalimido-2-butanone as described for Method A.

1-Hydrazono-3-phthalimido-2-butanone (VI)—Ammonium sulfide solution (4%, 4 drops) was added to an ice-cold solution of 1-diazo-3-phthalimido-2-butanone (100 mg in 10 ml of alcohol). Hydrogen sulfide gas was passed through the solution for 2.5 hr, and then nitrogen was passed through it for 1 hr. The yellowish solution was evaporated *in vacuo*, and the residue was crystallized from an alcohol–ether mixture. The suspension was filtered, and the crystals were washed and dried. The yield was 66 mg (66%), mp 182–183° (with frothing); IR: ν_{max}^{KBr} 3360, 3285, 3210 (NH_2), 1768, 1750, and 1720–1680 (C=O) cm^{-1}; ^{1}H-NMR (CD_3CN): δ 1.63 (d, CH_3, J = 7 Hz), 5.40 (q, 3-CH, J = 7 Hz), 7.20 (s, 1-CH), 7.95 (s, aromatic protons), 2.23, and 7.03 (b, NH_2) ppm; ^{13}C-NMR (CD_3CN): 194.4 (2-C=O), 168.8 (phthaloyl C=O), 135.2, 133.0, 123.9 (aromatic), 134.6 (1-CH), 51.6 (3-CH), and 15.2 (4-CH_3) ppm.

Anal.—Calc. for $C_{12}H_{11}N_3O_3$: C, 58.76; H, 4.52; N, 17.13. Found: C, 58.76; H, 4.80; N, 16.32.

Hydrazinolysis of III: Formation of 3-Amino-1-diazo-2-butanone (XIV) and Its Phthaloylhydrazide Salt (XV)—Triethylamine (1.8 ml, 13 mmoles) and a methanolic hydrazine solution (50%; 2 ml, 31.25 mmoles) were added slowly to a stirred solution of III (2 g, 4.23 mmoles, in 75 ml of methanol). The mixture was cooled in ice and stirred in a cold room for 16 hr. TLC [ethanol–water (7:3)] of the reaction mixture showed the absence of the starting material and the presence of two UV-absorbing and ninhydrin-positive spots.

The reaction mixture was evaporated *in vacuo* to remove triethylamine and unreacted hydrazine, the residue was taken up in ethyl acetate, and the white solid material that separated (phthaloylhydrazide) was removed by filtration. When the filtrate was evaporated *in vacuo*, a crystalline material separated out. This material was filtered off, washed with cold ether, and dried; the yield was 650 mg (29%), mp 120–121° dec. The presence of a diazo peak in the IR spectrum and of aromatic protons in the NMR spectrum indicated that the compound was a phthaloyl hydrazide salt of XIV; IR: ν_{max}^{KBr} 3220, 3208, 3050 ($^+NH_3$, OH phenolic), 2108 (diazo), 1630, 1595, 1575, and 1535 (keto) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.66 (d, CH_3, J = 7 Hz), 4.86 (q, CH, J = 7 Hz), 5.63 (s, 2-CH), and 7.78 (aromatic protons) ppm.

Anal.—Calc. for $C_{12}H_{13}N_5O_3$: C, 52.35; H, 4.75; N, 25.44. Found: C, 52.40; H, 4.88; N, 25.67.

The mother liquor was concentrated, chromatographed on a column (55.8 × 1.9 cm) of 200–325-mesh silica gel, and eluted with methanol. The fraction corresponding to the lower R_f value was isolated as a brown gummy mass. It was dissolved in ethyl acetate, and the solution was filtered to remove traces of silica gel. After evaporation, the yield was 0.54 g (58%). The gum showed a diazo peak in its IR spectrum and the expected NMR spectrum. It decomposed slowly on standing, however, and the expected elemental analysis could not be obtained; ^{1}H-NMR ($CDCl_3$): 1.29 (d, CH_3, J = 7 Hz), 3.58 (q, 3-CH, J = 7 Hz), 5.67 (s, 1-CH), and 2.92 (NH_2) ppm; IR: ν_{max}^{KBr} 3340 (NH_2), 2108 (diazo), and 1630 (keto) cm^{-1}.

Methyl 3-Phthalimidobutyrate (X)—*Method A*—The DL-isomer was prepared by following the procedure described by Balenovic *et al.* (23) for the L-isomer, mp 60°; IR: ν_{max}^{KBr} 1771, 1773, 1706, and 1610 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.51 (d, CH_3, J = 7 Hz), 2.98 (m, 2-CH_2), 3.62 (s, ester CH_3), 4.82 (m, 3-CH), and 7.77 (m, aromatic protons) ppm.

Anal.—Calc. for $C_{13}H_{13}NO_4$: C, 63.14; H, 5.29; N, 5.66. Found: C, 63.24; H, 5.34; N, 5.59.

Method B (Photochemical Rearrangement)—A solution of III (0.5 g, 2.06 mmoles) in dry methanol (80 ml) was purged with dry nitrogen for 1 hr. Then it was cooled with circulating tap water and irradiated under nitrogen for 78 hr with a high-pressure mercury lamp[5] (100 w) in

[4] SilicAR CC7, Mallinckrodt, St. Louis, Mo.

[5] Hanovia Lamp Division, Newark, N.J.

an immersion well equipped with a Pyrex filter. The reaction mixture was evaporated *in vacuo*, and the residue was placed on a dry column (76 × 2.5 cm) containing 240 g of silica gel (activity grade III). Then the column was developed with ether–petroleum ether (1:1).

The fraction with R_f 0.40 was separated and extracted with ether. The ether solution was evaporated to dryness, and the residue was crystallized from petroleum ether; the yield was 148 mg (19%), mp 47–53°. The product was identical with that obtained by Method A on the basis of mixed melting-point, IR, NMR, and TLC data.

Benzyl 3-Phthalimidobutyrate (XIII)—Compound III (256 mg, 1.05 mmoles) was dissolved in benzyl alcohol [2 ml, purified as described by Wilds and Meader (24)] and redistilled γ-collidine (2 ml), and the solution was heated to 175° when vigorous nitrogen evolution started. The reaction was allowed to proceed until the evolution of nitrogen ceased (~15 min). After cooling, ether was added, and the resulting solution was extracted with dilute aqueous hydrochloric acid to remove γ-collidine. The ether layer was evaporated *in vacuo*.

The residue was placed on a dry column (102 × 2.5 cm) containing silica gel (activity grade III) and was developed with ether. The fraction with the highest R_f value was separated and extracted with chloroform. The chloroform extract was removed *in vacuo*, giving a yellow oil that could not be crystallized but gave the expected NMR spectrum; ^{1}H-NMR ($CDCl_3$): δ 1.52 (d, CH_3, J = 7 Hz), 3.07 (m, 2-CH_2), 4.58 (m, 3-CH), 5.05 (s, benzyl CH_2), 7.25 (s, benzyl aromatic protons), and 7.75 (m, phthaloyl aromatic protons) ppm.

3-Aminobutyric Acid (XI)—This compound was prepared by the method of Balenovic *et al.* (23) as described for the L-isomer. The yield was 141 mg (67%), mp 187–189°; IR: ν_{max}^{KBr} 3020, 2830, 2590 (NH_3), 1620, and 1580 (carboxylate C=O) cm^{-1}; ^{1}H-NMR (D_2O): δ 1.32 (d, CH_3, J = 7 Hz) and 3.58 (m, CH) ppm.

Anal.—Calc. for $C_4H_9NO_2$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.38; H, 8.64; N, 13.51.

Hydrazinolysis of X: Formation of 3-Aminobutyrohydrazide (XII)—Triethylamine (0.9 ml, 6.5 mmoles) and hydrazine (95%, 0.3 ml, 9.46 mmoles) were added to a solution of X (500 mg, 2.05 mmoles) in methanol (19 ml). The reaction mixture was stirred at room temperature for 67 hr, and a white precipitate of phthaloyl hydrazide separated out. This precipitate was filtered, and the filtrate was evaporated and dried completely *in vacuo*. TLC [methanol–chloroform (1:4)] showed two spots. The spot with the higher R_f was due to phthaloyl hydrazide (UV absorbing), and the one with the lower R_f was ninhydrin positive. By column chromatography, the fraction corresponding to the lower R_f was isolated and characterized as the hydrochloride. The yield was 80 mg (21%), mp 201–202°; IR: ν_{max}^{KBr} 2940 ($^+NH_3$) and 1689 (C=O) cm^{-1}; ^{1}H-NMR (D_2O): δ 1.32 (d, CH_3, J = 7 Hz), 2.70 (d, CH_2, J = 7 Hz), and 3.77 (q, CH, J = 7 Hz) ppm.

Anal.—Calc. for $C_4H_{11}N_3O{\cdot}2HCl$: C, 25.27; H, 6.89; N, 22.10. Found: C, 25.45; H, 6.67; N, 21.87.

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