[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF COLORADO]

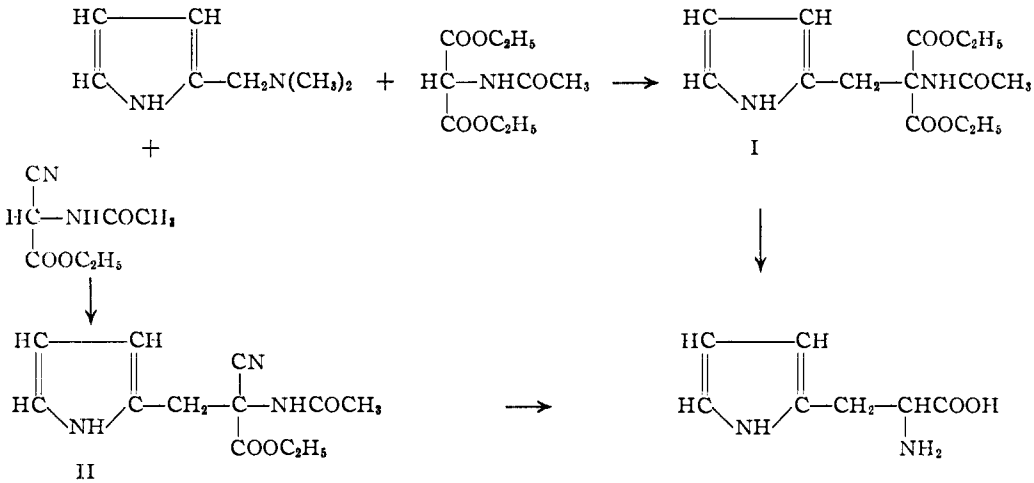
Alkylation of Substituted Malonic and Cyanoacetic Esters with 2-Diamethylaminomethylpyrrole. A Proposed Synthesis of β-2-Pyrrolealanine[1]

BY WERNER HERZ,[2] KARL DITTMER AND STANLEY J. CRISTOL

β-2-Thienylalanine[3,4,5] and β-2-furylalanine[6] have been shown to inhibit microbial growth, presumably due to their structural similarity to phenylalanine. The corresponding nitrogen analog, β-2-pyrrolealanine, is not described in the literature. As part of a program to correlate the structure of amino acids with their biological activity, it seemed desirable to prepare this substance so that it might be available for microbiological study.

Pure β-2-pyrrolealanine could not be isolated, but fractions containing it were obtained. The amino acid was characterized by its phenylurea derivative. These fractions showed strong inhibition of growth of certain microörganisms, and this inhibition was found to be reversed by phenylalanine.

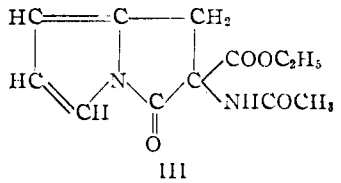
Alkylation with Dimethylaminomethylpyrrole.—The scheme outlined for the preparation of β-2-pyrrolealanine was



Attempts to synthesize β-2-pyrrolealanine by condensation of 2-pyrrolealdehyde with compounds containing active methylene groups[7] were abandoned when adaptation of recently-developed methods for the synthesis of tryptophan[8,9,10] appeared more promising.

The present paper describes the use of 2-dimethylaminomethylpyrrole[11] in alkylation reactions with ethyl malonate, ethyl cyanoacetate, ethyl acetamidomalonate, ethyl acetamidocyanoacetate, and ethyl phthalimidomalonate and hydrolysis experiments of such of these products as were intermediates for the projected synthesis.

Howe and co-workers[10] alkylated ethyl acetamidomalonate with gramine (dimethylaminomethylindole) by heating these reagents in refluxing toluene or xylene in the presence of catalytic amounts of solid sodium hydroxide. Substitution of dimethylaminomethylpyrrole for gramine, however, did not lead to the expected product (I). On cooling the reaction mixture, there was obtained a substance in 70–80% yield, which has the correct molecular weight and analysis for structure (III).



III

Lactams of this type from substituted pyrroleacrylic and pyrrolepropionic acids have been reported.[12,13]

Under the same conditions, attempted alkylation of ethyl cyanoacetate and ethyl cyanoacetamidoacetate produced much decomposition and yielded dark oils and tars on evaporation of the

(1) This work was supported in part under a contract from the Office of Naval Research.

(2) Present address: Department of Chemistry, University of Illinois, Urbana, Illinois.

(3) du Vigneaud, McKennis, Simmonds, Dittmer and Brown, *J. Biol. Chem.*, **159**, 385 (1945).

(4) Dittmer, Ellis, McKennis and du Vigneaud, *J. Biol. Chem.*, **164**, 761 (1946).

(5) Beerstecher and Shive, *ibid.*, **164**, 53 (1946).

(6) Clark and Dittmer, unpublished work.

(7) Herz and Dittmer, THIS JOURNAL, **69**, 503 (1947).

(8) Snyder and Smith, *ibid.*, **66**, 350 (1944).

(9) Albertson, Archer and Suter, *ibid.*, **66**, 500 (1944); **67**, 36 (1945).

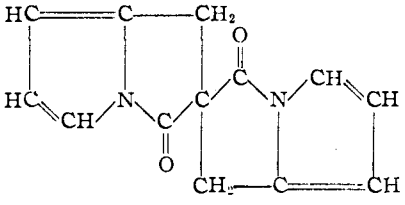
(10) Howe, Zambito, Snyder and Tishler, *ibid.*, **67**, 38 (1945).

(11) Herz, Dittmer and Cristol, *ibid.*, **69**, 1698 (1947).

(12) Fischer and Neber, *Ann.*, **496**, 1 (1932).

(13) Küster, Brudi and Koppenhöfer, *Ber.*, **58**, 1014 (1925).

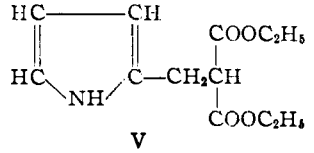
solvents which probably contained some of the condensation products but were not investigated due to the difficulties of purification. The alkylation of malonic ester produced, in addition to some oil, a small amount of white solid for which structure IV is suggested, such a compound arising by di-alkylation of malonic ester and lactam formation.



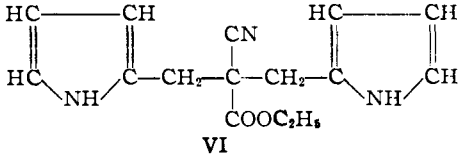
IV

In an effort to avoid lactam formation with ethyl acetamidomalonate and to obtain purer products with the other esters, use was made of an earlier procedure[8,14] which employed a quaternary salt of the tertiary amine, usually the methiodide, as alkylating agent and an inert solvent such as dioxane or dibutyl ether. Under these conditions, dimethylaminomethylpyrrole methiodide and ethyl acetamidomalonate also gave the lactam (III), though in lower yield, while malonic ester and ethyl cyanoacetamidoacetate gave dark uncharacterized oils.

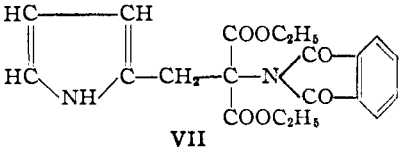
Albertson and co-workers[9] obtained the expected intermediate in the synthesis of tryptophan by the use of cold absolute ethanol as solvent for gramine and the malonate through the slow addition of dimethyl sulfate which served to quaternize the amine. Under similar conditions (I) was obtained from dimethylaminomethylpyrrole and ethyl acetamidomalonate in 85–95% yield. In the same fashion, using ethyl cyanoacetamidoacetate, (II) was formed in 90% yield. Alkylation of malonic ester produced an oil which was identified as (V) by conversion to the diamide. Ethyl cyanoacetate and ethyl phthalimidomalonate also could be alkylated; from the former the



V



VI



VII

(14) Snyder, Smith and Stewart, THIS JOURNAL, 66, 200 (1944).

product (VI) in which both active hydrogen atoms were replaced by 2-pyrrole-methyl radicals was isolated and identified as the amide, while the yield of pure crystalline material (VII) from the phthalimidomalonic ester condensation was very low. An attempt to alkylate hydantoin by this method failed.

Hydrolysis to β-2-Pyrrolealanine.—Early attempts to prepare β-2-pyrrolealanine by the basic hydrolysis of some of the compounds described in the preceding paragraphs made use of the lactam (III) before its structure had been recognized. These efforts were not successful. On the other hand, sodium hydroxide hydrolysis of (I), followed by hydrolysis in neutral and again in basic solution, produced a mixture which on neutralization gave a strong ninhydrin test, although neither of the two possible intermediates, 2-pyrrolemethylacetoamidomalonic acid and the acetyl derivative of pyrrolealanine, separated in the course of the hydrolysis. Evaporation *in vacuo*, extraction with absolute ethanol and dilution with acetone or anhydrous ether gave fractions which were identical with the material obtained by the hydrolysis of (II).

The latter compound, because of greater convenience, was employed in preference to the acetamidomalonate. Hydrolysis with sodium hydroxide ranging in concentration from 10–25%, neutralization and evaporation *in vacuo* produced partially organic material which gave a positive Ehrlich and ninhydrin test. It inhibited the growth of *S. cerevisiae* and *E. coli* and was reversed by phenylalanine.

By the action of phenyl isocyanate on this material there was obtained a well-defined crystalline compound whose neutral equivalent, analysis and chemical properties agreed with those expected of the phenylurea derivative of β-2-pyrrolealanine. This derivative showed little inhibitory activity, but hydrolysis with barium hydroxide in an autoclave at 120° regenerated the activity. Attempts to obtain pure pyrrolealanine by evaporation of the phenylurea hydrolysate *in vacuo* yielded colored material which quickly decomposed but still produced some inhibition.

These results left little doubt that the original crude material contained some pyrrolealanine contaminated primarily with inorganic salts. So far, however, we have failed in all our attempts to isolate pure pyrrolealanine by precipitation from water with organic solvents and salts, or by the formation of derivatives such as the benzoyl, naphthylurea, naphthalenesulfonamide and the carbobenzoxy derivatives.

Experimental

Dimethylaminomethylpyrrole Methiodide.—Eleven and one-half grams of dimethylaminomethylpyrrole[11] was dissolved in 75 ml. of absolute ethanol and treated with 15 g. of methyl iodide in the course of fifteen minutes. Heat was developed and a precipitate appeared. Since discoloration occurred when the flask was allowed to remain in the light, it was kept in the dark at room tem-

perature for two hours and then placed in the refrigerator. The yield of pink dimethylaminomethylpyrrole methiodide, which decomposed in air and became black above 160°, averaged 17–18 g. (63–68%).

Reaction of Dimethylaminomethylpyrrole with Ethyl Acetamidomalonate. (A) In Toluene.—Sixty ml. of toluene or xylene containing 1 g. of powdered sodium hydroxide was heated to boiling in a flask fitted with nitrogen inlet, stirrer and condenser. Then 9.3 g. of dimethylaminomethylpyrrole and 16.2 g. of ethyl acetamidomalonate were added and the mixture was refluxed in an atmosphere of nitrogen with stirring for two hours. During this time the color of the solution became dark red. The mixture was filtered by means of a preheated funnel and cooled. The yield of beige-colored material was 13–15 g. (70–80%). Recrystallization from hot water gave compound (III) as white needles, m. p. 151°, which gave a positive Ehrlich test and was soluble in ethanol, hot water, methanol and chloroform, partially soluble in ether, and insoluble in cold water.

Anal.[15] Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19; mol. wt., 250. Found: C, 57.59; H, 5.57; N, 11.05; mol. wt. (Rast), 259.

(B) In Dioxane, Using the Methiodide.—By following the procedure of Snyder and Smith,[8] 6.6 g. of dark-colored product was obtained from 11.3 g. of ethyl acetamidomalonate and 13.2 g. of dimethylaminomethylpyrrole methiodide after partial removal of the solvent and dilution with water. It was purified by recrystallizing from an ethanol–water mixture and again from 15% ethanol. M. p. of the white crystals was 147°, mixed m. p. with the material from (A), 151°.

(C) In Absolute Ethanol with Methyl Sulfate.—In a flask fitted with a stirrer, condenser and a dropping funnel were placed 100 ml. of absolute ethanol and 1.72 g. of clean sodium. After all the sodium had dissolved, 16.2 g. of ethyl acetamidomalonate and then 9.3 g. of dimethylaminomethylpyrrole were added. While the flask was cooled in an ice-bath and the mixture was stirred, 15.8 g. of methyl sulfate was added dropwise at such a rate that the temperature did not exceed 35°. After completion of the addition, the mixture was stirred for one hour and allowed to stand at room temperature overnight. The alcohol was evaporated *in vacuo;* the residue was diluted with 200 ml. of water and chilled. 21 g. (94%) of slightly-colored product separated. It was purified by dissolving in a little acetone, treating with charcoal, filtering, chilling, diluting with ice water and keeping in the refrigerator overnight. The shiny white platelets of compound (I) melted at 91° and were soluble in ethanol, acetone and hot water.

Anal. Calcd. for $C_{14}H_{20}N_2O_5$: N, 9.46. Found: N, 9.45.

Reaction of Dimethylaminomethylpyrrole with Ethyl Malonate.—Following procedure (A), 8.2 g. of malonic ester was allowed to react with 6.2 g. of dimethylaminomethylpyrrole in boiling toluene. No crystals separated from the cooled reaction mixture. Removal of the solvent gave a red oil which did not crystallize. Trituration with 50 ml. of ethanol caused most of the oil to dissolve; 0.5 g. of white solid remained undissolved. It gave a positive Ehrlich test and was recrystallized from a very small amount of benzene. The white crystals of (IV), m. p. 157°, were soluble in chloroform, methanol, hot benzene and hot alkali, and sparingly soluble in water and cold benzene.

Anal. Calcd. for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.43; N, 12.38; mol. wt., 226. Found: C, 68.85; H, 4.74; N, 12.08; mol. wt. (Rast), 228.

Hydrolysis of the alcohol solution of the oil with ethanolic potassium hydroxide gave a very impure organic acid which decomposed rapidly.

Following procedure (C), 12 g. of ethyl malonate was alkylated with 9.3 g. of dimethylaminomethylpyrrole. The oil obtained on dilution of the alcohol residue with water was extracted with two 25-ml. portions of ether. The dried ether extracts were distilled in a vacuum. After some unreacted malonic ester and dimethylaminomethylpyrrole had been collected, the temperature rapidly rose to 180°; 6 g. (33%) of (V), boiling at 184–187° (17 mm.) and somewhat colored, was obtained as a viscous oil.

The product was treated with ten times its volume of concentrated ammonium hydroxide, shaken and allowed to stand for a few days. The diamide derivative of (V) separated in 60% yield in long needles and was recrystallized from a little ethanol. It melted at 202° with previous darkening above 195°, gave a positive Ehrlich test and was soluble in water, less soluble in alcohol and acetone. On boiling with sodium hydroxide, the odor of ammonia was noted.

Anal. Calcd. for $C_8H_{11}N_3O_2$: N, 23.19. Found: N, 23.05.

Ethyl α-Acetamido-α-cyano-β-(2-pyrrole)-propionate (II).—Following procedure (C), 9.3 g. of dimethylaminomethylpyrrole was used to alkylate 12.7 g. of ethyl cyanoacetamidoacetate. On evaporation of the alcohol *in vacuo*, dilution with water and cooling, 17 g. (90%) of nearly white crystals separated. They were purified by dissolving in acetone, treating with charcoal, filtering, diluting with water and chilling for several hours. The white plates of (II) melted at 122° and were soluble in ethanol, acetone, benzene and hot water, insoluble in petroleum ether.

Anal. Calcd. for $C_{12}H_{15}N_3O_3$: N, 16.86. Found: N, 16.92.

Ethyl bis-(2-Pyrrolemethyl)-cyanoacetate (VI).—The alkylation of 11.3 g. of ethyl cyanoacetate with 9.3 g. of dimethylaminomethylpyrrole by procedure (C) produced a colored oil which could not be crystallized. It was taken up in ether, dried, and distilled *in vacuo*. After some unreacted ester had come over, 3 g. (30%) of a heavy yellow oil, boiling at 169–170° (6 mm.), was collected. It gave a positive Ehrlich test and became glassy on chilling.

The amide of (VI) was prepared by shaking the oil with ten times its volume of concentrated ammonium hydroxide, allowing the mixture to stand at room temperature for several days and finally chilling it for two days. The needles obtained in 45% yield were recrystallized from hot water and gave a positive Ehrlich test. They were melted at 153° and were soluble in ethanol, acetone and methanol, insoluble in hydrocarbon solvents.

Anal. Calcd. for $C_{13}H_{14}N_4O$: N, 23.13; mol. wt., 242. Found: N, 23.20; mol. wt. (Rast), 242.

Diethyl 2-Pyrrolemethylphthalimidomalonate (VII).—Only a viscous yellow oil was obtained by the alkylation of 23.5 g. of diethyl phthalimidomalonate with 9.3 g. of dimethylaminomethylpyrrole in the usual manner. Extraction with ether and removal of the ether *in vacuo*, and similar treatment with ethanol, produced a dark semi-solid mass which had not crystallized after two months. Part of this material was dissolved in much acetone and passed through an activated alumina column. The acetone was evaporated to small volume, cooled and diluted with water. On chilling, slightly colored crystals separated in very poor yield. An acetone solution of this product was treated with charcoal, filtered, diluted with water and chilled. The white plates melted at 133.5° and gave a positive Ehrlich test.

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: N, 7.29. Found: N, 7.15.

Hydrolysis of Ethyl α-Acetamido-α-cyano-β-(2-pyrrole)-propionate (II).—Twenty grams of (II) was refluxed with a solution of 25 g. of sodium hydroxide in 200 ml. of water for twenty-one hours. The red solution was neutralized carefully (vigorous evolution of carbon dioxide was noted, filtered from silica, decolorized with charcoal) and cooled. It was then acidified to *p*H 3 with

(15) This carbon–hydrogen analysis was determined by E. W. D. Huffman, Denver, Colorado.

concentrated hydrochloric acid, quickly extracted with four 200-ml. portions of ether to remove acetic acid, neutralized to *p*H 6–7, and evaporated to dryness *in vacuo*. After addition of absolute ethanol, the residue was again evaporated to dryness to remove the last traces of water. The residue (10 g.) gave a positive ninhydrin test.

Extraction with 100 ml. of hot absolute ethanol, treatment with norite, filtration, evaporation to small volume, dilution with anhydrous ether and chilling gave a small amount of brown solid. Further dilutions with ether, followed each time by cooling for a day, produced a total of 1.75 g. of impure amino acid-salt mixture.

Pure pyrrolealanine has so far not been obtained from these fractions by various techniques including selective extraction, fractional precipitation, insoluble salt formation, crystallization, electrophoresis and ion exchange. Analyses for carbon, hydrogen and nitrogen were consistently about two-thirds of the required amounts.

However, when treated with phenyl isocyanate, the crude salt-amino acid mixture gave a 22% yield of a phenylurea derivative analyzing for that of pyrrolealanine. Likewise, all of the crude fractions obtained from the basic hydrolysis of (II) inhibited the growth of *S. cerevisiae* (Fleischmann strain 139) and *E. coli* (ATCC 9723) when tested by the techniques previously described.[4] For half-normal inhibition a concentration of 28–32 γ per tube of crude material was required (each tube contained 6.5 ml. of medium and 1 ml. of addenda). The toxicity of the crude pyrrolealanine was counteracted by phenylalanine. The phenylurea derivative showed only slight inhibition at levels of 200 γ per tube, but hydrolysis with barium hydroxide restored the original high degree of activity. Further attempts are underway to obtain the pyrrolealanine in pure form.

Hydrolysis of Ethyl 2-Pyrrolemethylacetamidomalonate (I).—Ten grams of the ester was refluxed with 80 ml. of 15% sodium hydroxide for seven hours, treated with charcoal, filtered, neutralized to *p*H 5–6 and cooled. The ninhydrin test was negative. Concentration to small volume at this stage yielded no organic precipitate. The slightly acid solution was refluxed for two hours, treated with 12 g. of solid sodium hydroxide and boiled for an additional eleven hours. It was neutralized carefully, filtered, acidified to *p*H 3 and quickly extracted with ether, neutralized again, treated with charcoal, filtered and evaporated to dryness *in vacuo*. The residue gave a strong ninhydrin test and was extracted as described in the preceding paragraph. The yield of crude product, representing the sum of the various fractions, averaged 3–5 g. This crude material was identical in behavior with the material obtained from the hydrolysis of II.

Phenylurea Derivative of β-2-Pyrrolealanine.—Two grams of crude salt-amino acid product was dissolved in 10 ml. of water. To the well-cooled solution there were added 6 ml. of 2 *N* sodium hydroxide in 1-ml. portions and 1.2 ml. of phenyl isocyanate in 0.2-ml. portions with vigorous shaking. When the odor of phenyl isocyanate could no longer be noticed, the mixture was filtered from the slight precipitate of diphenylurea, decolorized with charcoal, made barely acid with dilute hydrochloric acid and cooled overnight. The yield of pink solid was 0.8 g. An additional 0.22 g. was obtained by further acidification of the filtrate. Recrystallization from aqueous ethanol gave 0.78 g. (22%) of white crystals. After drying *in vacuo*, the product melted at 182–183° (capillary) and at 206° on a Dennis melting point bar within three seconds. It decomposed in air and was stored in a vacuum desiccator.

Anal. Calcd. for $C_{14}H_{15}N_3O_3$: C, 61.51; H, 5.53; N, 15.38; neut. equiv., 273. Found: C, 61.6; H, 6.04; N, 15.19; neut. equiv., 275.

Hydrolysis of the Phenylurea.—Preliminary studies indicated that the phenylurea of phenylalanine could be hydrolyzed by 5% barium hydroxide. Fifty milligrams of the phenylurea of pyrrolealanine was placed in a test-tube together with 0.5 g. of barium hydroxide monohydrate and 9.5 ml. of water and heated at 120° in a pressure autoclave for six hours. The mixture was neutralized to *p*H 7 with 1 *N* sulfuric acid, filtered, and the volume adjusted to 20 ml. Of this neutralized filtrate 0.32 ml. of a 1 to 10 dilution inhibited the growth of *E. coli* to 50% of normal. Evaporation *in vacuo* gave 18 mg. of a brown residue, which decomposed very quickly, left a black residue on burning, and inhibited the growth of *E. coli*, but not as strongly as the solution.

Acknowledgments.—The authors wish to express their appreciation to Mrs. Charmion McMillan for the microbiological tests, and to Mr. Frank Rainwater for some of the carbon and hydrogen analyses; also to Professor Irving Goodman for the many hours spent in consultation during this work.

Summary

Dimethylaminomethylpyrrole was used to alkylate ethyl malonate, ethyl cyanoacetate, ethyl acetamidomalonate, ethyl cyanoacetamidoacetate, and ethyl phthalimidomalonate. The structures of the products have been given.

The product of the reaction between dimethylaminomethylpyrrole and ethyl cyanoacetamidoacetate on hydrolysis gave impure fractions of β-2-pyrrolealanine which was characterized as the phenylurea derivative.

These fractions inhibited the growth of *S. cerevisiae* and *E. coli*. The inhibition was reversed by phenylalanine.

BOULDER, COLORADO RECEIVED AUGUST 25, 1947