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

Preparation of Some Benzimidazolylamino Acids. Reactions of Amino Acids with *o*-Phenylenediamines

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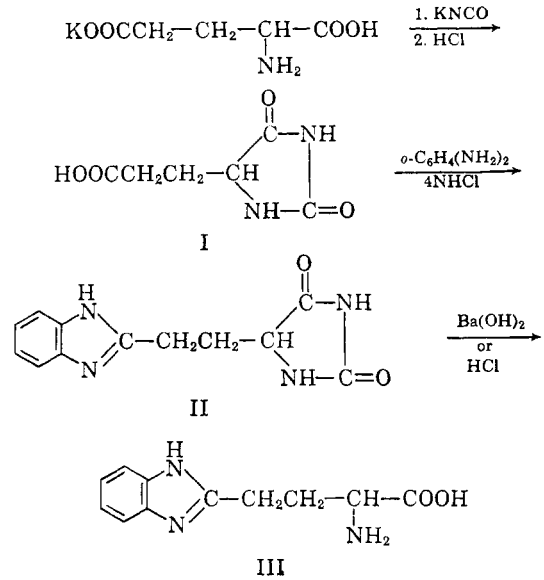
A paper chromatography method has been developed to evaluate Phillips' reactions, for preparing benzimidazoles,[1] while in progress. The reaction of L-(+)-glutamic acid with *o*-phenylenediamine in 5.5*N* hydrochloric acid has been shown to give good yields of only one product, namely, α-amino-γ-(2-benzimidazolyl)butyric acid with retention of configuration. The same acid has been obtained from *o*-phenylenediamine and 5-(β-carboxyethyl)hydantoin in hydrochloric acid solution. Under similar conditions, aspartic acid and *o*-phenylenediamine has been shown to give both β-(2-benzimidazolyl)-α-alanine and β-(2-benzimidazolyl)-β-alanine, as well as some vinylenebis-2,2'-benzimidazole. It has also been demonstrated that the simple α-aminomonobasic acids react normally with *o*-phenylenediamine under Phillips' conditions.

The incorporation of a benzimidazole moiety, into α-amino acid molecules appeared to be of interest since the benzimidazole grouping has known biological activity, and its attachment to a carrier group such as an amino acid could facilitate this activity. β-(2-Benzimidazolyl)alanine has been prepared and has shown sufficient activity to warrant further investigation of related compounds.[2]

A further modification that has attracted some interest is the inclusion in the α-amino acid molecule of an α-methyl group.[3] For example, α-methylmethionine has been reported to be a methionine antagonist[4]; the α-methyl derivative of 3,4-dihydroxyphenylalanine is a potent inhibitor of mammalian dihydroxyphenylalanine decarboxylase[5]; and α-methylglutamic acid has been reported to be a glutamic acid antagonist.[4]

The present investigation started with a study of the preparation of α-amino-γ-(2-benzimidazolyl)-butyric acid from *o*-phenylenediamine and glutamic acid. This method had been reported earlier to be unsuccessful.[2a] The first successful synthesis, in the present study, involved the preparation of 5 - (2 - β - benzimidazolylethyl)hydantoin (II), followed by the hydrolysis of the hydantoin.

Although earlier workers had reported the method of Phillips[1] to be of no value for preparing benzimidazoles from α-amino acids and *o*-phenylenediamine, we have found the method to be successful if the refluxing period is greatly extended. The reaction between natural glutamic acid and *o*-phenylenediamine in 4*N* hydrochloric acid was followed through the use of paper chromatography. The Phillips procedure gave an optically active amino acid (III) when L-(+)-glutamic



acid was the starting material whereas the method involving intermediate hydantoins gave a racemic product.

Since only one spot for a benzimidazoleamino acid was observed in these experiments, it must be concluded that only one amino acid was formed. It was shown to be the α-amino acid by converting it, almost quantitatively, to the corresponding hydantoin (II). The amino acids prepared during this investigation were isolated initially as hydrates. In general, they were obtained in anhydrous form by "azeotropic drying", *i.e.*, distilling with dry ethanol followed by dry benzene. While this work was in progress, Behringer, Hauser, and Kohl reported the synthesis of racemic α-amino-γ-(2-benzimidazolyl)butyric acid by a completely different method.[6]

The method was extended to 4-methyl-*o*-phenylenediamine and 4-nitro-*o*-phenylenediamine. The inclusion of a methyl group in the benzene ring increased the solubility in water of the cor-

(1) M. A. Phillips, *J. Chem. Soc.*, 172, 2393 (1928).

(2) (a) P. Mammalis, V. Petrow, and B. Sturgeon, *J. Chem. Soc.* 1600 (1950); (b) H. Lettré, W. Fritsch, and J. Porath, *Ber.* 84, 719 (1951); (c) S. Tatsuoka and H. Hitomi, *J. Pharm. Soc. Japan*, 71, 871 (1951).

(3) W. W. Umbreit, *Symposium on Amino Acid Metabolism*, McCollum-Pratt Institute, Baltimore, Md., (1954).

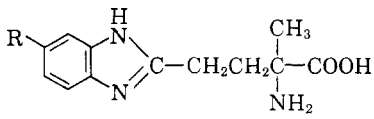
(4) G. A. Stein *et al.*, *J. Am. Chem. Soc.* 77, 697 (1955).

(5) K. Pfister 3rd *et al.*, *J. Am. Chem. Soc.* 77, 700 (1955).

(6) H. Behringer, L. Hauser, and K. Kohl, *Ber.* 92, 910 (1959).

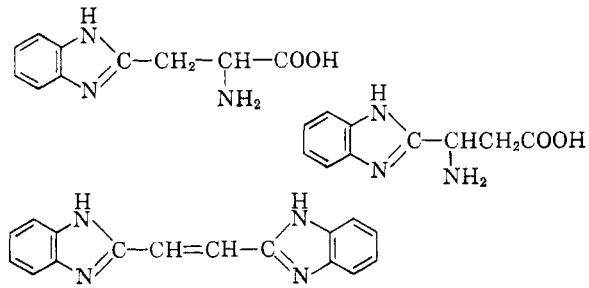
responding α-amino acid. The α-amino acid was the only product isolated. It was readily converted to the corresponding hydantoin. 4-Nitro-*o*-phenylenediamine and glutamic acid yielded only the corresponding α-amino acid also.

Benzimidazolylamino acids were also prepared from α-methylglutamic acid and *o*-phenylenediamine and 3,4-diaminotoluene by Phillips' method, as well as from the corresponding hydantoins. Only the α-amino acids were isolated.



R = H or CH_3

The reaction of *o*-phenylenediamine with aspartic acid, by Phillips' method, yielded both β-(2-benzimidazolyl)alanine and β-(2-benzimidazolyl)-β-alanine, as well as some vinylenebis 2,2′-benzimidazole.



A survey of the literature showed that the α-amino acid had been prepared by a number of workers, all employing essentially the same synthesis involving the use of diethyl aminomalonate.[2] This reaction was also followed by paper chromatography. Vinylenebisbenzimidazole dihydrochloride precipitated from the reaction mixture as the reaction progressed. It proved to be identical with a sample obtained from the American Cyanamid Co. The monohydrochloride of the α-amino acid was subsequently precipitated at *p*H 1, and the monohydrochloride of the β-amino acid was precipitated at *p*H 3. A sample of the α-amino acid prepared by an unambiguous method by R. L. Rogers was identical in all respects.[7] The α-amino acid was readily converted to a hydantoin but the β-amino acid, as expected, did not form a hydantoin. The α-amino acid was also prepared by converting potassium hydrogen aspartate to 5-carboxymethylhydantoin and refluxing a solution of the latter with *o*-phenylenediamine and hydrochloric acid for a long time.

In view of the results with glutamic and aspartic acids and in view of the existing discrepancies in the literature regarding the reactions of monobasic amino acids with *o*-phenylenediamine under Phillips' conditions,[1] an investigation of these latter reactions was undertaken.

Hughes and Lions reported that glycine and *o*-phenylenediamine do not condense when refluxed in 4*N* hydrochloric acid solution.[8] They succeeded in effecting the condensation by fusing hippuric acid with *o*-phenylenediamine. Crawford and Edward reported a different method.[9] They heated the *N*-benzoylated amine with two equivalents of *o*-phenylenediamine, two moles of hydrochloric acid, and two moles of 75% phosphoric acid in a sealed tube at 100–137° for ten hours. Yields were not reported for the free bases or the hydrochlorides of the 2-aminomethylbenzimidazoles.

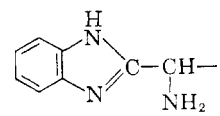
Lane obtained a 58% yield of 2-aminomethylbenzimidazole dihydrochloride by fusing ethyl aminoacetate hydrochloride with *o*-phenylenediamine in a nitrogen atmosphere.[10]

Sorm and Urban evaporated a solution of β-alanine and *o*-phenylenediamine in 15% hydrochloric acid to dryness and heated the residue for two hours at 160°. They reported a 35% yield of 2-(β-aminoethyl)benzimidazole dihydrochloride.[11]

Gump and Nikawitz prepared 2-[di(β-hydroxyethyl)aminomethyl]benzimidazole by refluxing a 4*N* hydrochloric acid solution of *N*,*N*-di(2-hydroxyethyl)glycine and *o*-phenylenediamine for ten hours.[12]

In the present investigation, a 5.5*N* hydrochloric acid solution of glycine and *o*-phenylenediamine was refluxed, and the progress of the reaction was followed with paper chromatography. The reaction progressed very slowly but after thirty hours of refluxing 50–60% yields of pure 2-aminomethylbenzimidazole dihydrochloride may be obtained. Even after thirty hours some *o*-phenylenediamine was still present. The dihydrochloride of 2-aminomethylbenzimidazole loses hydrogen chloride very readily.

L-(+)-Alanine and *o*-phenylenediamine in 5.5*N* hydrochloric acid behaved similarly, but the reaction was much slower. After seventy-two hours of refluxing, a 42% yield of 2-α-aminoethylbenzimidazole was obtained. The latter fluoresced blue in the ultraviolet, and with ninhydrin it gave first a golden yellow color which changed to brown and finally to violet. This color change appears to be characteristic of the structure,



(7) R. L. Rogers, dissertation, University of Pennsylvania, 1956.

(8) G. K. Hughes and K. D. Lions, *J. Proc. Roy. Soc. N. S. Wales*, **71**, 209 (1938).

(9) R. Crawford and J. T. Edward, *J. Chem. Soc.*, 673 (1956).

(10) E. S. Lane, *J. Chem. Soc.*, 3313 (1957).

(11) F. Sorm and J. Urban, *Collection Czechoslov. Chem. Communs.*, **15**, 196 (1950).

(12) W. S. Gump and E. J. Nikawitz, *J. Org. Chem.* **24**, 712 (1959).

The product, 2-α-aminoethylbenzimidazole, was found to be optically active but it displayed a change in the sign of rotation.

β-Alanine reacted more readily with *o*-phenylenediamine in 5.5*N* hydrochloric acid than either glycine or alanine. After twenty-four hours of refluxing, the *o*-phenylenediamine was almost completely used up and a 68% yield of pure 2-β-aminoethylbenzimidazole dihydrochloride was obtained.

α-Aminoisobutyric acid reacted extremely slowly with *o*-phenylenediamine in 5.5*N* hydrochloric acid. After one hundred and fifteen hours of refluxing, the paper chromatogram showed a trace of the desired product but only starting materials were isolated from the reaction mixture.

From these data for the monobasic amino acids, it may be concluded that when the amino group is in the β-position, rather than the α-position, the reactivity is enhanced. For the α-amino acids, increased substitution on the α-carbon atom reduces the reactivity, glycine > alanine > α-aminoisobutyric acid. With glutamic acid the reaction is almost exclusively with the γ- or free carboxyl group. With aspartic acids both of the carboxyl groups reacted but the β- or free carboxyl group accounted for the largest amount of product.

EXPERIMENTAL

The melting point values are uncorrected. They were determined in the apparatus described by Wagner and Meyer.[13]

Paper chromatography. The reactions which involved refluxing dilute hydrochloric acid solutions of *o*-phenylenediamines and amino acids were followed by paper chromatography. Samples (0.0005 to 0.001 ml.) were applied to Whatman No. 1 paper, and the chromatograms were run by the ascending method. The solvent system was 1-butanol (40 parts by vol.), acetic acid (10 parts by vol.), and water (50 parts by vol.).[14] The water layer was discarded after allowing the two phases to come to equilibrium. In a few cases the solvent system was pyridine–water (80:20).

The paper strips were 15 cm. by 5 cm. The lower end of the strip was immersed in the solvent to a depth of 1.5 cm., and the application line was 2 cm. from the bottom of the strip. A movement of the solvent front of 7 cm. from the application line was usually sufficient to effect a separation. The products, being benzimidazoles and hence sensitive to ultraviolet light, appeared under the 2537 Å. light either as spots of absorption or more frequently as spots of blue to white fluorescence. Since the products were also amino acids or 2-aminoalkylbenzimidazoles, the spots gave golden yellow or violet colorations when treated with 0.1% ninhydrin–acetone solution. Unchanged *o*-phenylenediamine dihydrochloride was detected, after the treatment with ninhydrin, by heating the paper strip for 1 min. at 120° whereupon a yellow-green color developed.

5-(β-Carboxyethyl)hydantoin (I). Potassium hydrogen glutamate was converted to the corresponding hydantoic acid by treatment with potassium cyanate in aqueous solution. The hydantoic acid was converted to the hydantoin (I) by heating with 6*N* hydrochloric acid.[15] The yield was 74%, m.p. 165.5–167.5°.

5-(2-β-Benzimidazolylethyl)hydantoin (II). Compound II was prepared from *o*-phenylenediamine and 5-(β-carboxyethyl)hydantoin by refluxing in 4*N* hydrochloric acid.[2a] The procedure was that of Mamalis *et al.*, except that a longer refluxing period was used. The yield of hydrochloride was 44%, m.p. 258–259° dec. The free base melted at 242.5–243.5° dec.

d,l-α-Amino-γ-(2-benzimidazolyl)butyric acid (III) *from 5-(2-β-benzimidazolylethyl)hydantoin.* (a) *Base hydrolysis of compound* II. A suspension of 5-(2-β-benzimidazolylethyl)-hydantoin (5.21 g., 0.0213 mole) and 26.6 g. (0.0844 mole) of barium hydroxide octahydrate in 24 ml. of distilled water was refluxed for 10 hr. The excess barium hydroxide was destroyed by passing carbon dioxide through the boiling mixture. The mixed barium salts were extracted with distilled water in a Soxhlet apparatus for 22 hr. Dilute sulfuric acid was added to the extract until no further precipitation occurred (*p*H 7). After filtration, the solution was evaporated under reduced pressure. This left a residue of the amino acid as a yellow powder. Repeated recrystallization from water, with the aid of decolorizing carbon, gave a very low yield (3%) of the colorless, crystalline amino acid. This compound decomposed over a wide range, 233–300°. After drying for 48 hr. at 61° and 2 mm., the product appeared to be a hemihydrate.

Anal. Calcd. for $C_{11}H_{13}N_3O_2 \cdot 1/2H_2O$: C, 57.87; H, 6.18; N, 18.42. Found: C, 57.62; H, 6.55; N, 18.77.

(b) *Acid hydrolysis of compound* II. The hydantoin (3.55 g., 0.0416 mole) in 26 ml. of 6*N* hydrochloric acid was refluxed for 51 hr. The progress of the reaction was followed by paper chromatography. Three spots were observed under ultraviolet light: R_f 0.55, weakly fluorescent; R_f 0.43, weakly absorbant; and R_f 0.30, blue fluorescence. Only spot R_f 0.30 gave a violet color with ninhydrin. When the area of the amino acid spot (R_f 0.30) no longer increased, the solution was adjusted to *p*H 5.5 with sodium carbonate, but no precipitation occurred at this *p*H (the hydantoin II precipitates at *p*H 5.5). When the *p*H was adjusted to *p*H 7.5, a white precipitate of the α-amino acid formed. It was obtained in 67% yield and a paper chromatogram showed it to be the pure α-amino acid. The water of hydration was removed from the product by several evaporations with ethanol and benzene. The compound was then dried in an Abderhalden apparatus over phosphorus pentoxide at 2 mm. to constant weight. The melting range was 233–300° dec.

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.06; H, 5.95; N, 18.99.

L-(+)-α-Amino-γ-(2-benzimidazolyl)butyric acid (III) *from o-phenylenediamine and* L-(+)-*glutamic acid.* L-(+)-Glutamic acid (22 g., 0.15 mole) and 10.8 g. (0.1 mole) of *o*-phenylenediamine were dissolved in 100 ml. of 5.5*N* hydrochloric acid, and the solution was refluxed for 30 hr. at which time paper chromatography showed the reaction was nearly completed. The solution was adjusted to *p*H 9 by the addition of sodium hydroxide and extracted with ether in a liquid-liquid extractor until the extract was colorless. The aqueous layer was adjusted to *p*H 7.5 with concd. hydrochloric acid, at which point the L-(+)-α-amino acid separated; yield 86%. The product was slightly pink in color although a paper chromatogram showed it to be pure. It was recrystallized from 90% ethyl alcohol and obtained as colorless crystals; melting point range 233–300°, yield 65%, α_D^{23} +55.1° (in 1*N* hydrochloric acid). The product was dried by several evaporations with ethanol and then dried over phosphorus pentoxide at 2 mm. to constant weight. The analytical results were almost identical with the analytical data shown above.

(13) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1939).

(14) K. V. Giri, *Nature*, **171**, 1159 (1953); **173**, 1194 (1954).

(15) H. D. Dakin, *Am. Chem. J.*, **44**, 48 (1910); H. D. Dakin, *Biochem. J.*, **13**, 398 (1919); J. L. Szabo and J. V. Karabinos, *J. Am. Chem. Soc.*, **66**, 650 (1944).

The infrared spectra of all of the samples of α-amino-γ-(2-benzimidazolyl)butyric acid prepared were identical. The spectra displayed the following peaks in cm.$^{-1}$: 1660 (s), 1640 (s), 1590 (s), 1530 (s), 743 (s).

The ultraviolet spectra for α-amino-γ-(2-benzimidazolyl)-butyric acid, run in water, along with similar data for benzimidazole[16] are listed below:

TABLE I

α-Amino-γ-(2-benzimidazolyl)-butyric Acid		Benzimidazole	
mμ	log ε	mμ	log ε
242	3.77	245	3.72
272	3.84	271	3.79
279	3.78	278	3.78

L-(+)-*α-Amino-γ-[2-(5 or 6-methylbenzimidazolyl)]butyric acid* (IV). This compound was prepared from 3,4-diaminotoluene and L-(+)-glutamic acid by the procedure used for preparing L-(+)-α-amino-γ-(2-benzimidazolyl)-butyric acid. The solution was refluxed for 48 hr., cooled, and neutralized to *p*H 7 at which point the reaction mixture solidified. The solid was broken up with 150 ml. of cold water and the precipitate removed by filtration. The solid was dissolved in 150 ml. of water, which contained enough sodium hydroxide to give a *p*H of 9. This solution was extracted with ether in a liquid-liquid extractor until the extract was colorless (16 hr.). The basic solution was cooled and carefully neutralized with concd. hydrochloric acid. At *p*H 8 a colorless precipitate formed and continued to form until *p*H 7 was reached. The yield was 66% and a paper chromatogram showed it to be pure. The R_f 0.37 spot absorbed in the ultraviolet and gave a strong violet color with ninhydrin.

The product was recrystallized from 90% ethyl alcohol and then from water, m.p. 229.5–230.5° dec., α_D^{24} +56° (in 1*N* hydrochloric acid). Water of hydration was removed by "azeotropic drying" with ethanol and benzene and then drying the product to constant weight over phosphorus pentoxide at 2 mm.

Anal. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.78; H, 6.48; N, 18.02. Found: C, 61.54; H, 6.48; N, 17.89.

Infrared: 1628 (s), 1585 (s), 1450 (m), 1415 (m), 1350 (m), 805 (m). Ultraviolet: 234 mμ (log ε 3.77), 278 mμ (3.89), 284 mμ (3.86).

That compound IV was an α-amino acid was shown by the fact that it was readily converted to a hydantoin. The amino acid in water solution was treated with a slight excess of potassium cyanate. After standing overnight, 1 ml. of concd. hydrochloric acid was added, and the solution was refluxed for 0.5 hr. The solution was neutralized to *p*H 7, but no precipitate formed. An oil was obtained by evaporating the solution. The oil was taken up in ethanol and the solution filtered. The addition of a few milliliters of concd. hydrochloric acid followed by the addition of a little benzene caused the precipitation of the hydrochloride of the desired hydantoin. It was recrystallized from ethanol and dried over phosphorus pentoxide at 61° and 2 mm. The melting point was 247–248°.

Anal. Calcd. for $C_{13}H_{14}N_4O_2 \cdot HCl$: C, 53.15; H, 4.84; N, 19.08; Cl, 12.07. Found: C, 53.11; H, 5.08; N, 18.86; Cl, 11.85.

This hydantoin was identical in all respects with the hydantoin prepared from 3,4-diaminotoluene and 5-(β-carboxyethyl)hydantoin by Phillips' procedure.

L-(+)-*α-Amino-γ-2-(5 or 6-nitrobenzimidazolyl)butyric acid* (V). This amino acid was prepared from 4-nitro-*o*-phenylenediamine and L-(+)-glutamic acid by refluxing in 5.5*N* hydrochloric acid for 50 hr. In this case, it was necessary to run the paper chromatograms on the neutralized reaction mixture which gave better defined spots: R_f 0.86 absorbing in the ultraviolet and due to the starting diamine; R_f 0.68 fluorescing white in the ultraviolet and unassigned; R_f 0.50 absorbing in the ultraviolet and giving a strong violet color with ninhydrin due to the desired amino acid; and R_f 0.14 which did not absorb in the ultraviolet but gave a violet coloration with ninhydrin due to glutamic acid.

After cooling, the reaction mixture was filtered to remove a small amount of tarry material, and the filtrate was adjusted with solid sodium bicarbonate to *p*H 6 and then with sodium hydroxide to *p*H 10. The basic solution was extracted with ether for 48 hr. and the aqueous solution then acidified with 12*N* hydrochloric acid to *p*H 4. At this point the amino acid precipitated, yield 81%, m.p. 195–200° dec. This product was contaminated with a little glutamic acid and a substance that fluoresced pink in the ultraviolet.

One recrystallization from water was sufficient to remove the glutamic acid. To remove the material that caused the pink fluorescence, the product was dissolved in 67% ethyl alcohol and ether added until turbidity occurred. On standing in the cold, a gelatinous precipitate formed which was removed by filtration through a sintered glass funnel of maximum porosity. This precipitate contained the major part of the impurity. The filtrate was evaporated under reduced pressure and the procedure repeated with 95% ethyl alcohol and ether. Again the precipitate contained some of the purity. The solid obtained from the filtrate was recrystallized three times from water to obtain a chromatographically pure amino acid; yield 30%, m.p. 195–198° dec. α_D^{24} +47° (in 1*N* hydrochloric acid). The hydrated sample was tan in color. After drying at 110° and 2 mm. for 24 hr., it became yellow in color.

Anal. Calcd. for $C_{11}H_{13}N_4O_4$: C, 49.99; H, 4.58; N, 21.20. Found: C, 49.75; H, 4.74; N, 20.99.

L-(+)-α-Amino-γ-[2-(5- or 6-nitrobenzimidazolyl)]butyric acid was converted to the corresponding hydantoin by the usual procedure, thus indicating it to be an α-amino acid. It was recrystallized from ethanol–benzene and obtained as a pale yellow solid, yield 58% m.p. 256–257° dec.

Anal. Calcd. for $C_{12}H_{11}N_5O_4$: C, 49.82; H, 3.83; N, 24.21. Found: C, 49.76; H, 4.02; N, 23.97.

This hydantoin proved to be identical with one obtained from 4-nitro-*o*-phenylenediamine and 5-(β-carboxyethyl)-hydantoin by Phillips' procedure. The yield was only 19% by the latter method. The hydantoin prepared by this method was refluxed in 6*N* hydrochloric acid solution for 72 hr. *d,l-α-*Amino-γ-[2-(5 or 6-nitrobenzimidazolyl)]butyric acid was isolated in 60% yield from this solution by the same procedure used for isolating the L-(+)-amino acid. The infrared and ultraviolet spectra of the two amino acids were identical.

Infrared: 1630 (s), 1600 (s), 1510 (s), 1470 (s), 1450 (s), 1410 (s), 1340 (s), 838 (m), Ultraviolet: 235 mμ (log ε 4.42), 306 (4.11).

d,l-α-Amino-α-methyl-γ-(2-benzimidazolyl)butyric acid VI. Preparation from 5-methyl-5-β-carboxyethylhydantoin. 5-Methyl-5-β-carboxyethylhydantoin is the hydantoin of α-methylglutamic acid and was prepared from levulinic acid by the procedure by G. L. Stein *et al.*[4] *o*-Phenylenediamine (10.8 g., 0.1 mole) and 5-methyl-5-β-carboxyethylhydantoin (27.9 g., 0.15 mole) were dissolved in 100 ml. of 4*N* hydrochloric acid, and the solution was refluxed for 6.5 hr. The product was precipitated by adding ammonium hydroxide to a *p*H of 5 to 6. The precipitate was treated with 50 ml. of water and enough concd. hydrochloric acid added to dissolve the material. The solution was decolorized with decolorizing carbon and the filtrate adjusted to *p*H 5–6 as before. The yield was 41%. The product was finally recrystallized from 40% ethyl alcohol, m.p. 295–296° dec. It was dried at 110° at 2 mm. over phosphorus pentoxide for 24 hr.

Anal. Calcd. for $C_{13}H_{14}N_4O_2$: C, 60.50; H, 5.46; N, 21.71. Found: C, 60.30; H, 5.31; N, 21.64.

(16) E. C. Fisher and M. M. Joullié, *J. Org. Chem.*, **23**, 1944 (1958).

The 5-methyl-5-(2-β-benzimidazolylethyl)hydantoin (10.8 g., 0.04444 mole) so obtained was treated with a solution of 19 g. (0.474 mole) of sodium hydroxide and the solution refluxed for 22 hr. The solution was cooled and neutralized with 6*N* hydrochloric acid. The product which slowly separated was extracted with dilute hydrochloric acid and the solution neutralized with sodium carbonate, yield 42%, m.p. 230–232° dec.

This amino acid (VI) was obtained in better yield as follows: *o*-Phenylenediamine (5.4 g., 0.05 mole) and 14 g. (0.075 mole) of 5-methyl-5-β-carboxyethylhydantoin were treated with 50 ml. of 6*N* hydrochloric acid and the solution refluxed for 96 hr. The reaction was followed with paper chromatography. The solution was cooled and the *p*H adjusted to 10 by the addition of sodium hydroxide. The solution was extracted with ether for 18 hr. and then neutralized with hydrochloric acid to *p*H 7. On standing a tan precipitate formed. It was recrystallized from 90% ethyl alcohol with the aid of decolorizing carbon and obtained as colorless needles, R_f 0.43, yield 47%, m.p. 230–232° dec. This amino acid gives a strong ninhydrin test and is almost quantitatively converted to the hydantoin with cyanic acid and hydrochloric acid, m.p. 295–296° dec. After "azeotropic drying" with ethanol and benzene, the product was dried at 80° at 2 mm. over phosphorus pentoxide to constant weight.

Anal. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.80; H, 6.43; N, 18.02. Found: C, 61.61; H, 6.64; N, 17.94.

Infrared: 1640 (s), 1615 (s), 1515 (s), 1465 (s), 1405 (s), 1350 (s), 755 (s), 740 (s). Ultraviolet: 241 mμ (log ε 3.88), 271 (3.95), 278 (3.90).

d,l-α-Amino-α-methyl-γ-[2-(5-methylbenzimidazolyl)] butyric acid dihydrochloride (VII). 3,4-Diaminotoluene (12.2 g., 0.1 mole) and 28 g. (0.15 mole) of 5-methyl-5-β-carboxyethylhydantoin were treated with 100 ml. of 6*N* hydrochloric acid and the solution refluxed for 74 hr. The solution was adjusted to *p*H 10 and extracted with ether for 20 hr. After neutralizing the solution, it was evaporated *in vacuo* to a sirup. As the solution was concentrated, inorganic salts precipitated and were removed by filtration. The oil was dissolved in ethanol and concd. hydrochloric acid. Addition of ether caused the formation of a white precipitate. The latter was dissolved in 110 ml. of 80% isopropyl alcohol, decolorized with charcoal, and ether then added to the point of turbidity. Cooling gave a 48% yield, m.p. 243–245° dec. Although paper chromatograms showed this sample to contain no other organic material, it could not be obtained analytically pure. Consequently it was converted to a dihydrochloride. A sample was dissolved in 2-propanol (33%)–ethanol (67%) and the solution saturated with hydrogen chloride. The product was dried overnight in an evacuated desiccator over sulfuric acid and then at 25° and 2 mm. over potassium hydroxide to constant weight (4 days).

Anal. Calcd. for $C_{13}H_{17}N_3O_2 \cdot 2HCl$: C, 48.76; H, 5.98; N, 13.12; Cl, 22.15. Found: C, 48.71; H, 5.97; N, 13.10; Cl, 22.09.

The R_f value was 0.41. Infrared: 1725 (v.s.), 1625 (m), 1580 (m), 1500 (v.s.), 1220 (v.s.), 1140 (s), 815 (s). This amino acid was converted to the corresponding hydantoin by the usual procedure. The hydantoin was recrystallized from 40% ethyl alcohol. The yield was 73%, m.p. 256–258° dec.

Anal. Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.70; H, 6.01; N, 20.45.

Reaction of o-phenylenediamine with d,l-aspartic acid. Preparation of vinylenebis 2,2'-benzimidazole (VIII), *β-(2-benzimidazolyl)alanine* (IX), *and β-(2-benzimidazolyl)-β-alanine* (X). A solution of 5.4 g. (0.05 mole) of *o*-phenylenediamine and 10 g. (0.075 mole) of *d,l*-aspartic acid in 50 ml. of 5.5*N* hydrochloric acid was refluxed for 70 hr. until the starting diamine was no longer detectable. At this time paper chromatography showed the following spots: R_f 0.36, fluorescing blue in the ultraviolet and with ninhydrin giving first a golden yellow color which changed to violet (X); R_f 0.28, fluorescing blue in the ultraviolet and giving a golden yellow color with ninhydrin (IX); R_f 0.18, no absorption in the ultraviolet and giving a violet color with ninhyrin due to aspartic acid; and R_f 0.75 and R_f 0.51 which fluoresced very strongly in the ultraviolet and may be attributed to VIII and its precursor 1-amino-1,2-bis(2-benzimidazolyl)ethane.

After 70 hr., the mixture was cooled and the solid VIII removed. The impure green solid appeared to be a hydrochloride of the vinylenebisbenzimidazole. The yield was 2.42 g., but the product was very impure. An analytical sample was prepared by repeated recrystallization from dilute hydrochloric acid (0.4*N*) with the aid of decolorizing carbon. This gave a yellow hydrochloride. The latter was treated with excess 10% sodium bicarbonate solution and washed thoroughly with water to obtain a yellow powder. It was dried at 25° and 2 mm. over phosphorus pentoxide to constant weight.

Anal. Calcd. for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.53. Found: C, 73.69; H, 4.80; N, 21.45.

Infrared: 1454 (m), 1435 (v.s.), 1318 (s), 1218 (s), 1028 (s), 970 (s), 746 (v.s.). Ultraviolet: 361 (shoulder) mμ (log ε 7.7), 377 mμ (9.1), 395 mμ (6.3).

The samples were dissolved in 50 ml. of ethanol to which had been added 1 ml. of concd. hydrochloric acid. The infrared and ultraviolet spectra were practically identical with those for a sample of vinylenebis-2,2'-benzimidazole obtained from the American Cyanamid Co.

The filtrate from compound VIII was carefully treated with sodium carbonate. As the *p*H approached one, a yellow precipitate formed (4.97 g.), which proved to be the monohydrochloride of β-(2-benzimidazolyl)-α-alanine (IX). This product was removed by filtration and recrystallized from 67% ethyl alcohol giving 2.74 g. (23%) of colorless β-(2-benzimidazolyl)-α-alanine hydrochloride, m.p. 242–244° dec. It was quantitatively converted to the free base by dissolving in a minimum amount of water and adding the calculated amount of sodium carbonate. A sample for analysis was prepared by recrystallization from water, followed by repeated "azeotropic drying" with benzene and then drying at 25° and 2 mm. over phosphorus pentoxide to constant weight (120 hr.).

Anal. Calcd. for $C_{10}H_{11}N_3O_2$: C, 58.54; H, 5.40; N, 20.48. Found: C, 58.35; H, 5.61; N, 20.31.

Infrared: 1540 (s), 1460 (s), 1450 (v.s.), 1400 (v.s.), 1280 (m), 1055 (m), 750 (v.s.). Ultraviolet (in water): 243 mμ (log ε 3.78), 271 mμ (3.88), 278 mμ (3.83).

The filtrate from β-(2-benzimidazolyl)-α-alanine hydrochloride (IX) was adjusted to *p*H 3–4 with sodium bicarbonate. At this point the hydrochloride of β-(2-benzimidazolyl)-β-alanine (X) precipitated (1.44 g.). Further adjustment of the filtrate from X to *p*H 6.4 gave 0.4 g. of the free base X. The monohydrochloride was obtained in colorless form by recrystallization from water with the aid of charcoal, yield 7%, m.p. 226–227°. The hydrochloride was converted to the free base by dissolving in a minimum amount of water and adding the calculated amount of sodium carbonate. A sample of the free base was prepared for analysis by recrystallization from water, followed by "azeotropic drying" with benzene and then drying at 25° and 2 mm. over phosphorus pentoxide to constant weight (120 hr.).

Anal. Calcd. for $C_{10}H_{11}N_3O_2$: C, 58.54; H, 5.40; N, 20.48. Found: C, 58.38; H, 5.27; N, 20.21.

Infrared: 1640 (v.s.), 1540–1520 (v.s.), 1425 (v.s.), 1400 (v.s.), 775 (s), 768 (s), 755 (s), 740 (s). Ultraviolet (in water): 245 mμ (log ε 3.79), 272 mμ (3.85), 279 mμ (3.79).

β-(2-Benzimidazolyl)-α-alanine was converted to the corresponding hydantoin, 5-(2-benzimidazolylmethyl)hydantoin, by heating with potassium cyanate in water solution for 0.5 hr., cooling, adding 2 ml. of 6*N* hydrochloric acid and again refluxing for 0.5 hr. Neutralization with sodium carbonate caused the precipitation of the hydantoin; yield 72%. Recrystallization from ethanol gave a pure, colorless product, m.p. 235–236°; yield 37%. It was "azeotropically dried" with benzene and then dried at 25° and 2 mm. over phosphorus pentoxide for 24 hr.

Anal. Calcd. for $C_{11}H_{10}N_4O_2$: C, 57.38; H, 4.38; N, 24.34. Found: C, 57.21; H, 4.45; N, 24.33.

β-(2-Benzimidazolyl)-β-alanine did not form a hydantoin under similar conditions.

Reactions of monobasic α-amino acids with o-phenylenediamine. 2-Aminomethylbenzimidazole dihydrochloride (XI). A solution of 10.8 g. (0.1 mole) of *o*-phenylenediamine and 11.25 g. (0.15 mole) of glycine in 100 ml. of 5.5*N* hydrochloric acid was refluxed for 30 hr. At this point paper chromatography indicated that little *o*-phenylenediamine remained. The solution was allowed to stand in the cold overnight and the hydrochloride then removed by filtration. It was recrystallized from ethanol with the aid of decolorizing carbon; yield 12.1 g. (56%), m.p. 263° dec. Two melting point values are reported in the literature, 263°[9] and 267°.[11] The sample for analysis was dried for 6 hr. over potassium hydroxide at 110° and 2 mm. Longer drying at 110° causes the loss of hydrogen chloride.

Anal. Calcd. for $C_8H_9N_3 \cdot 2HCl$: C, 43.65; H, 5.04; N, 19.09; Cl, 32.22. Found: C, 43.77; H, 5.11; N, 18.92; Cl, 31.94.

Infrared: 1630 (s), 1488 (s), 1430 (s), 1220 (s), 900 (s), 878 (s), 770 (s). Ultraviolet: 270 mμ (log ε 4.14), 277 mμ (4.09).

2-(β-Aminoethyl)benzimidazole dihydrochloride (XII). A solution of 10.8 g. (0.1 mole) of *o*-phenylenediamine and 13.4 g. (0.15 mole) of β-alanine in 100 ml. of 5.5*N* hydrochloric acid was refluxed for 24 hr. At this time the diamine was barely detectable by paper chromatography. The solution was cooled overnight and the hydrochloride removed by filtration. It was recrystallized from 90% ethyl alcohol with the aid of decolorizing carbon; yield 15.8 g. (68%), m.p. 268–309° dec. Sorb and Urban[12] reported 270–325°. The observed melting point is in reality a mixed m.p. due to a mixture of mono- and dihydrochlorides. Paper chromatography showed two spots: R_f 0.49 fluorescing weakly in the ultraviolet and giving a golden yellow color with ninhydrin (due to the monohydrochloride); and R_f 0.28 fluorescing strongly blue in the ultraviolet and giving a golden yellow color with ninhydrin (due to the dihydrochloride). R_f 0.49 disappeared when an ethanol solution of XII was saturated with hydrogen chloride before chromatograming.

A sample for analysis was recrystallized from ethanol saturated with hydrogen chloride and dried over phosphorus pentoxide for 18 hr. at 76° and 2 mm.

Anal. Calcd. for $C_9H_{11}N_3 \cdot 2HCl$: C, 46.17; H, 5.60; N, 17.95; Cl, 30.29. Found: C, 46.22; H, 5.70; N, 18.17; Cl, 29.95.

Infrared: 1640 (m), 1570 (m), 1525 (m), 1480 (m), 1470 (m), 1225 (m), 1160 (m), 965 (m), 895 (m), 745 (v.s.). Ultraviolet: 269 mμ (log ε 3.99), 276 mμ (4.00).

2-(α-Aminoethyl)benzimidazole dihydrochloride (XIII). A solution of 5.4 g. (0.05 mole) of *o*-phenylenediamine and 6.68 g. (0.075 mole) of L-(+)-alanine in 50 ml. of 5.5*N* hydrochloric acid was refluxed for 72 hr. At this time only a very small amount of diamine was detectable. The solution was evaporated and the solid residue taken up in a minimum amount of 4*N* hydrochloric acid. After cooling overnight, the solid was removed by filtration. It was recrystallized from ethanol with the aid of decolorizing carbon, yield 4.95 g. (42%), m.p. 132–138°. Paper chromatography showed the presence of a small amount of monohydrochloride (R_f 0.62) with the dihydrochloride (R_f 0.39). The R_f 0.62 spot disappeared when an ethanol solution of the product, saturated with hydrogen chloride, was chromatogramed.

A sample for analysis was recrystallized from ethanol saturated with hydrogen chloride. It was dried over potassium hydroxide for 6 hr. at 110° and 2 mm., α_D^{24} −6.3° in 1*N* HCl.

Anal. Calcd. for $C_9H_{11}N_3 \cdot 2HCl \cdot 1H_2O$: C, 42.87; H, 6.00; N, 16.67; Cl, 28.12. Found: C, 42.78; H, 6.19; N, 16.47; Cl, 27.98.

Infrared: 1615 (m), 1480 (s), 1460 (m), 1228 (m), 1144 (m), 758 (s). Ultraviolet: 270 mμ (log ε 4.03), 277 mμ (3.97).

The benzoyl derivative prepared from equivalent quantities of XIII and benzoyl chloride in pyridine solution was recrystallized from ethanol–ether, m.p. 254–256.5°.

Anal. Calcd. for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.32; H, 5.85; N, 15.82.

PHILADELPHIA 4, PA.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

Benzofuro[3,2-*b*]indoles

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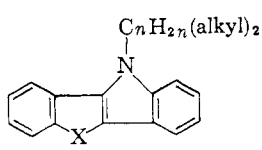
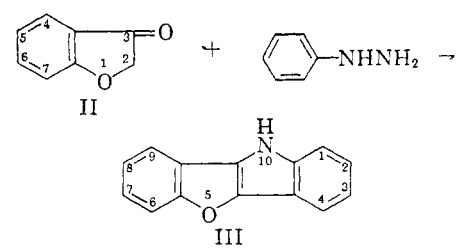
Several benzofuro[3,2-*b*]indoles have been synthesized and some of the intermediates involved were investigated.

The interesting pharmacological activity of certain 10-substituted thianaphtheno[3,2-*b*]indoles (Ia)[1] led us to investigate the synthesis of the corresponding benzofuro[3,2-*b*]indoles (Ib).



Ia. X = S; Ib. X = O

A search of the literature revealed only three references to this class of compounds[2–4] and in all



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