[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

THE SYNTHESIS OF "ISOTRYPTOPHAN," α-AMINO-β-(2-INDOLE)-PROPIONIC ACID

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Analogs and isosteres of tryptophan have been the object of several recent publications. Interest in these compounds stems from the fact that their close structural relationship to the natural amino acid suggests a possible biological activity as tryptophan antagonists. Derivatives containing methyl groups (1, 2) or fluorine (3) on the indole nucleus have been reported, and compounds containing the benzofuran (4), thianaphthene[1] (5), naphthalene (4, 6), and benzimidazole[1] (7) nuclei in place of indole have been prepared. In view of the marked tryptophan antagonism exhibited by several of these amino acids it was deemed of interest to prepare the isomer of tryptophan containing the alanine side chain attached to the two rather than the three position of the indole nucleus. Such a compound would be one of the isotryptophans.

In light of the elegant syntheses of tryptophan from gramine (8) the obvious intermediate needed for the synthesis of α-amino-β-(2-indole)propionic acid (VII) would be the gramine isomer, 2-dimethylaminomethylindole (IV). This amine has been prepared by Euler and Erdtman (9) in low yield by the cyclization of the readily available dimethylaminoacet-*o*-toluidide (III), using sodium ethoxide as catalyst. In the present work a study was made of this reaction, and it was found that the choice of catalyst was most critical. Sodium amide was found to be by far the best reagent, giving a 66% yield of the substituted indole, while potassium *tert*-butoxide, potassium amide, or lithium amide all gave less than 20% conversion. An alternative, though less convenient method of preparing the 2-dimethylaminomethylindole (IV) was developed starting with indole-2-carboxylic acid (I). This acid was converted to the acid chloride which, when treated with dimethylamine, gave indole-2-dimethylcarboxamide (II). The amide was reduced in good yield with lithium aluminum hydride to the gramine isomer (IV). Alkylation of diethylacetaminomalonate using the methosulfate of 2-dimethylaminomethylindole (IV) proceeded in good yield to form ethyl α-acetamino-α-carbethoxy-β-(2-indole)propionate (V). Hydrolysis of this ester to the isotryptophan (VII) was conveniently effected in two stages, first with sodium carbonate to form acetylisotryptophan (VI), and then with sodium hydroxide to give the desired amino acid (VII). The over-all yield of α-amino-β-(2-indole)-propionic acid (VII) from the easily accessible amide (III) was 31%.

In preliminary tests isotryptophan showed no inhibition of the growth of *Escherichia coli* at a dilution of 1 to 1000. With *Streptococcus viridans* complete inhibition was produced at a dilution of 1 to 1000 in the presence of 1 to 40,000

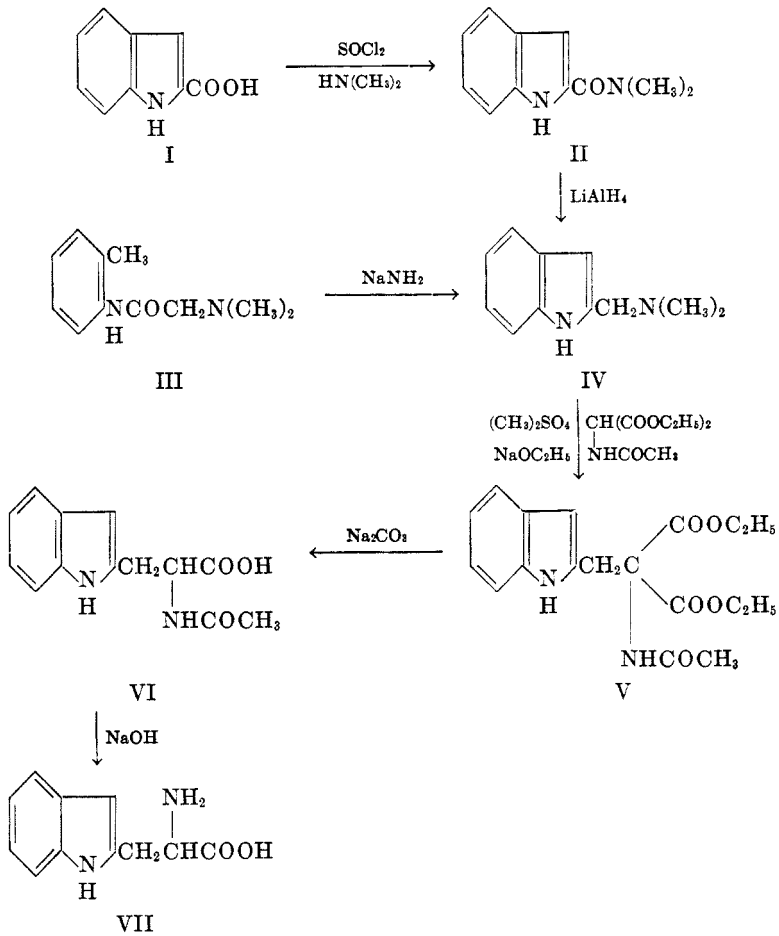
[1] α-Amino-β-(3-thianaphthene)propionic acid and α-amino-β-(2-benzimidazole)propionic acid were prepared in this laboratory before the appearance of these publications. Details of this work do not differ sufficiently to warrant separate publication.

dilution of tryptophan. A 1:10,000 dilution of tryptophan did not reverse this inhibition.

EXPERIMENTAL

Melting points are uncorrected.

2-Dimethylaminomethylindole (IV). Sodium (13.8 g., 0.6 mole) was converted to sodium amide by solution in 500 ml. of liquid ammonia using a trace of ferric chloride as catalyst.



Dimethylaminoacet-*o*-toluidide (48.1 g., 0.25 mole) (9) was added, and the excess ammonia was evaporated while stirring and passing in a stream of dry nitrogen. The flask was then immersed in a metal bath at 150°, and the bath was heated to 310° during 22 min. The reaction mixture was cooled under nitrogen, and to it was added 50 ml. of ethanol cautiously, followed by 400 ml. of water. About 250 ml. of the mixture was distilled to remove a little *o*-toluidine, and the residue was cooled and extracted with 200 ml. of ether. The extracts were washed with water and then extracted with dilute hydrochloric acid containing 40 ml. of the concentrated acid. The acid extract was treated with excess sodium carbonate, the product was extracted with ether, and the extract dried over potassium hydroxide and

fractionated. 2-Dimethylaminomethylindole was obtained, b.p. 143–145°/6 mm.; yield, 28.7 g. (66%).

Anal. Calc'd for $C_{11}H_{14}N_2$: N, 16.08. Found: N, 15.49.

The *picrate* was prepared and crystallized from ethanol, m.p. 182–184° [Euler and Erdtman (9) report m.p. 184–185°].

Anal. Calc'd for $C_{17}H_{17}N_5O_7$: C, 50.62; H, 4.25; N, 17.36.
Found: C, 51.30; H, 4.44; N, 18.06.

Cyclization experiments run under similar conditions using other catalysts gave the following yields: (a) potassium *tert*-butoxide, 16%; (b) potassium amide, 8%; (c) lithium amide, less than 20%. The 66% yield using sodium amide was replicated several times.

Indole-2-dimethylcarboxamide (II). Ethyl-2-indolecarboxylate (10), 44.6 g., was saponified with 14.2 g. of sodium hydroxide in dilute ethanol by boiling the solution for one-half hour. Ethanol was removed *in vacuo*, and the solution was diluted with 200 ml. of water and acidified with a slight excess of hydrochloric acid. The indole-2-carboxylic acid was filtered, washed well with water, and dried *in vacuo;* yield, 35.7 g. (94%). The acid was mixed with 100 ml. of absolute ether and 75 ml. of thionyl chloride. The dark mixture was allowed to stand for 16 hours after which it was evaporated to dryness *in vacuo* below 30°. The residue was taken up in 300 ml. of absolute ether, filtered, and the resulting solution was added slowly to a solution of 60 g. of dimethylamine in 75 ml. of dry ether. The ether was removed, and the product was purified by digestion with methanol; yield, 25 g. (56%). A sample was recrystallized from methanol for analysis, m.p. 173–176°.

Anal. Calc'd for $C_{11}H_{12}N_2O$: N, 14.89. Found: N, 14.28.

Lithium aluminum hydride reduction of indole-2-dimethylcarboxamide. Lithium aluminum hydride, 15 g., was added to 1 l. of absolute ether in a 2-l. flask attached to a Sohxlet extractor. Indole-2-dimethylcarboxamide, 24.5 g., was placed in the extraction thimble of the Sohxlet extractor and was continuously extracted with ether for 24 hours. Four grams of the amide remained unextracted at this time. The reduction mixture was decomposed carefully using 50 ml. of acetone, 100 ml. of water, and 50 ml. of 12 *N* sodium hydroxide. The ether solution was decanted, and the residue was extracted with 100 ml. of ether. The combined ether solution was dried over magnesium sulfate and fractionated. 2-Dimethylaminomethylindole was obtained, b.p. 145–150°/7 mm.; yield, 13.5 g. (71%). The picrate was prepared and recrystallized from ethanol, m.p. 182–184°; mixed with the picrate of the amine prepared by the cyclization reaction above the m.p. showed no depression.

Ethyl α-acetamino-α-carbethoxy-β-(2-indole)propionate (V). 2-Dimethylaminomethylindole (85 g., 0.488 mole) was dissolved in 500 ml. of absolute ethanol freshly distilled from sodium. Methyl sulfate, 63 g., was then added dropwise with stirring, keeping the temperature below 35° by cooling in ice. The solution was stirred for an additional 10 minutes after which 11.5 g. (0.5 mole) of sodium and 106 g. of diethylacetaminomalonate (0.488 mole) in 1 l. of absolute ethanol was added (temp. below 35°). The reaction mixture was stirred for 6 hours and was then allowed to stand at room temperature for 12 days (1–2 days may be adequate). The solvent was distilled *in vacuo*, and the residue was taken up in 800 ml. of chloroform. The chloroform solution was washed twice with 400-ml. portions of water, once with cold dilute hydrochloric acid containing 60 ml. of the concentrated acid (this extract on basification, extraction, and distillation gave 2.5 g. of unreacted 2-dimethylaminomethylindole), once with 300 ml. of water, and finally with 300 ml. of cold 10% sodium carbonate solution. The washed solution was dried over magnesium sulfate, and the chloroform was removed *in vacuo*. The crystalline residue was recrystallized from benzene-petroleum ether; yield, 96.4 g. (59%). A sample for analysis was crystallized from benzene, m.p. 159–160°.

Anal. Calc'd for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09.
Found: C, 61.81; H, 6.56; N, 7.86.

α-Acetamino-β-(2-indole)propionic acid (VI). The crude ethyl α-acetamino-α-carbethoxy-β-(2-indole)propionate above (93.3 g.) was mixed with an equal weight of anhydrous sodium carbonate and 950 ml. of water, and the mixture was refluxed for 17 hours. The solution was

cooled and acidified with 150 ml. of concentrated hydrochloric acid. The product was extracted with 1200 ml. of ethyl acetate in three portions, and the extracts were dried over magnesium sulfate. The product partially crystallized from the solution, so the solid was filtered and extracted with two 750-ml. portions of hot acetone. The acetone and ethyl acetate extracts were concentrated to a small volume, and petroleum ether was added. The product was recrystallized from 1 l. of 50% ethanol; yield, 65 g. (98%); m.p. 188–190°. A sample of the acid prepared for analysis had a m.p. 190–191°.

Anal. Calc'd for $C_{13}H_{14}N_2O_3$: N, 11.38. Found: N, 11.45.

α-Amino-β-(2-indole)propionic acid (Isotryptophan) (VII). The above acetylisotryptophan (68 g.) was dissolved in a solution containing 115 g. of sodium hydroxide in 800 ml. of water, and the mixture was refluxed for 20 hours. The solution was treated with carbon and neutralized in the cold to *p*H 6–7 with 220 ml. of concentrated hydrochloric acid. The mixture was stirred in ice for 1 hour after which the product was filtered and washed with water. It was redissolved in 500 ml. of water containing 25 ml. of 12 *N* sodium hydroxide and reprecipitated hot with 25 ml. of glacial acetic acid; yield, 45.1 g. (80%); m.p. 220–222° dec. A sample for analysis was recrystallized from water.

Anal. Calc'd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72.
Found: C, 64.02; H, 6.21; N, 13.68.

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SUMMARY

α-Amino-β-(2-indole)propionic acid, an isomer of tryptophan, has been prepared and has shown some antibacterial action.

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