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The Synthesis of the 2-Amino-3-(3-indolyl)-butyric Acids (β-Methyltryptophans)

BY H. R. SNYDER AND DONALD S. MATTESON[1]

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Indole adds to ethylideneisopropylamine to form 3-(isopropylaminoethylidene)-indole, a substance similar to a Mannich base but having an ethylidene group in place of the methylene residue. Dibenzyl acetamidomalonate is prepared from diethyl acetamidomalonate and alkylated with the new base. Hydrogenolysis of the alkylation product yields the substituted malonic acid, which is converted to 2-amino-3-(3-indolyl)-butyric acid upon decarboxylation followed by hydrolysis of the acetamido group. The amino acid is separated into its two diastereoisomeric *dl*-pairs, and it is shown that these are not structural isomers nor merely different crystalline forms of one compound.

A convenient synthesis of *dl*-tryptophan[2,3] begins with gramine (the Mannich base from indole, formaldehyde and dimethylamine). It appeared likely that β-methyltryptophan (V) could be synthesized *via* a similar route if a way could be found to condense indole, acetaldehyde and an amine to form the base I.

Attempts to employ aldehydes other than formaldehyde in reactions of the Mannich type have met with only limited success; perhaps the best examples of such reactions are those which lead to cyclic products.[4] However, simple bases of the Mannich type have been obtained by the addition of active methylene compounds to aldimines. Benzalaniline has been condensed with acetoacetic ester,[5] malonic ester[6] and related compounds,[7] indoles,[8] pyrazolones,[9] β-naphthol[6] and, in the presence of boron fluoride, with methyl ketones.[10] Recently, indole has been added to Δ^1-piperideine (stable as the trimer) and to 3,4-dihydroquinoline.[11] Indole also has been condensed with the cyanohydrins of acetaldehyde and of acetone and with acetone and chloroform.[12]

The recent observation by Hurwitz[13] that *t*-butylamine forms stable aldimines with aliphatic aldehydes suggested the possibility of adding indole to ethylidene-*t*-butylamine to prepare 3-(*t*-butylaminoethylidene)-indole (Ia).

Before an attempt was made to condense indole with ethylidene-*t*-butylamine, experiments were performed to determine whether *t*-butylamine would participate in normal Mannich reactions. Under typical Mannich conditions,[4] *t*-butylamine hydrochloride, paraformaldehyde and indole give a moderate yield of 3-(*t*-butylaminomethyl)-indole; this secondary amine is the only product. When simpler primary amines are used in Mannich reactions under these conditions, the secondary amine formed condenses with an additional mole of indole and formaldehyde to form a tertiary amine[4]; the formation of a mixture of products severely limits the usefulness of primary amines as reagents for Mannich reactions. In the present case, it appears that the bulky *t*-butyl group shields the amino nitrogen from the second reaction.

To gather preliminary data that might point to a suitable method for condensing ethylidene-*t*-butylamine with indole, methylene-*t*-butylamine and indole were allowed to react under a variety of conditions, most of which resembled those for usual Mannich reactions. Moderate yields of 3-(*t*-butylaminomethyl)-indole generally were obtained.

However, only one method was successful for the reaction of ethylidene-*t*-butylamine with indole. When the reaction was allowed to take place at 0° in a solvent mixture of 75% glacial acetic acid and 25% benzene, 15% yields of the base Ia were obtained. Numerous attempts were made to improve the procedure. The acetic acid was dried by distillation from acetyl borate,[14] but without effect upon the yield. With other variations the reaction failed entirely; an apparently trivial change, the substitution of ether for benzene in the solvent mixture, was one of these.

At this point, it appeared likely that the steric hindrance caused by the *t*-butyl group was so great as to severely repress the condensation. Examination of a Fisher–Taylor–Hirschfelder model indicated that the base Ia has very few unstrained configurations, thus a low entropy of internal rotation both in the molecule itself and in the transition state leading to its formation. An isopropyl group (Ib) allows more rotational freedom in the side chain. The steric properties of isopropyl and *t*-butyl groups are similar enough so that ethylideneisopropylamine should be a fairly stable aldimine; this stability has been observed experimentally by Tiollais.[15]

When ethylideneisopropylamine and indole were allowed to react in acetic acid–benzene at 0°, the yield of 3-(isopropylaminoethylidene)-indole (Ib) was 60%. In addition, direct condensation of indole, acetaldehyde and isopropylamine gave a 40% yield of the base (Ib) under similar conditions.

The structure of the base Ib was confirmed by hydrogenation to 3-ethylindole.

The second step in the *dl*-tryptophan synthesis is the alkylation of diethyl acetamidomalonate by gramine.[2] The properties of 3-(*t*-butylaminomethyl)-, 3-(*t*-butylaminoethylidene)- and 3-(iso-

(1) National Science Foundation Fellow, 1954–1956.

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(3) H. R. Snyder and C. W. Smith, *ibid.*, **66**, 350 (1944).

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(5) R. Schiff, *Ber.*, **31**, 205 (1898).

(6) M. Betti, *Gazz. chim. ital.*, **30**, II, 301 (1900).

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(13) M. D. Hurwitz, U. S. Patent 2,582,128, Jan 8. 1952.

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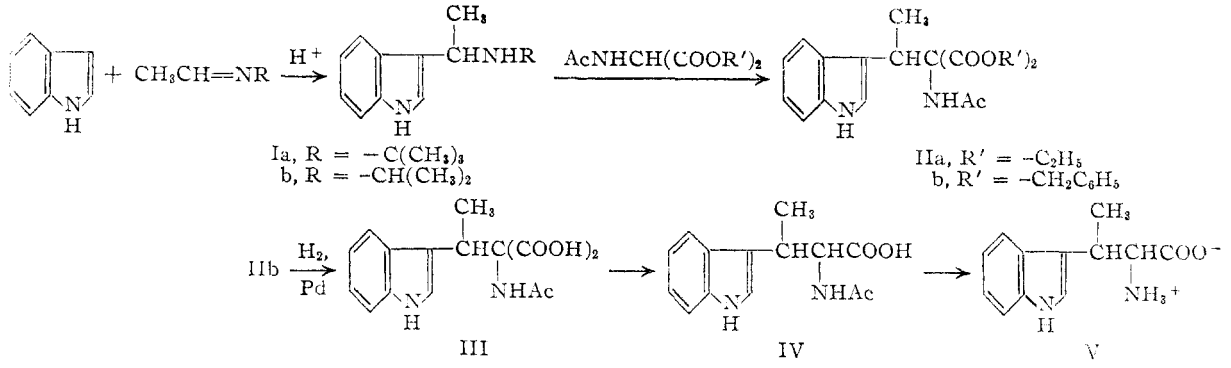

propylaminoethylidene)-indole as alkylating agents were investigated. The reaction rates were easily followed semi-quantitatively by titration of the volatile amine evolved. The most rapid alkylation occurred with 3-(*t*-butylaminoethylidene)-indole; the other two reagents gave approximately equal rates. Each requires a lower temperature or shorter time than reported[2] for gramine. Yields are excellent.

The next step, the alkaline hydrolysis of diethyl (3-indolylethylidene)-acetamidomalonate (IIa), took an unexpected course. Instead of the desired acid III, acetylglycine was obtained. Further investigation of this anomalous hydrolysis is being carried out, and, it is hoped, will be reported in a separate article.

This difficulty with the hydrolysis step was eliminated by the use of the dibenzyl ester IIb. Dibenzyl acetamidomalonate was prepared from diethyl acetamidomalonate by transesterification with benzyl alcohol and was alkylated by the base Ib. Dibenzyl (3-indolylethylidene)-acetamidomalonate was converted by hydrogen over a palladium–charcoal catalyst to (3-indolylethylidene)-acetamidomalonic acid (III), isolated and characterized as the ammonium salt.

Decarboxylation of the malonic acid III would be expected to yield two diastereoisomeric *dl*-pairs of 2-acetamido-3-(3-indolyl)-butyric acid (acetyl-β-methyltryptophan) (IV). Only one *dl*-pair was obtained in crystalline form; the other failed to crystallize upon treatment with various solvents, sublimation or partition chromatography (chloroform–water) on silicic acid. The crystalline *dl*-pair was produced in about 30% yields from decarboxylation of either the malonic acid III or its ammonium salt in several different solvents. Since the free acid III was difficult to crystallize, it seemed desirable to decarboxylate the ammonium salt directly. A minimum of discoloration occurred when a pyridine–water solvent was employed.

The crystalline and non-crystalline isomers of acetyl-β-methyltryptophan (IV) were separated and hydrolyzed with 4 *N* sulfuric acid, to avoid the likelihood of racemization encountered with alkaline hydrolysis. Each *dl*-pair of diastereoisomers of 2-amino-3-(3-indolyl)-butyric acid (β-methyltryptophan) (V) was obtained. The first *dl*-pair of V, hereinafter referred to as isomer A, was obtained upon hydrolysis of the crystalline acetylamino acid IV; the other *dl*-pair of V, referred to below as isomer B, came from the amorphous material IV. Both isomers had nearly the same decomposition point, but a mixture of the two melted considerably lower. The infrared spectra (Nujol) showed marked differences, and the gross crystalline forms were different.

Two approaches were used to prove that the isomers A and B were not merely different crystalline forms. The α-naphthylurea derivatives were prepared. Although the derivatives had the same decomposition point and gave no depression upon mixing, the infrared spectra (Nujol) were distinctly different. Other evidence was gained from a semi-quantitative determination of the solubilities of the amino acids (V) in 95% ethanol. Isomer A is soluble to the extent of at least 20 mg. per ml., while isomer B has a maximum solubility of not more than 5.5 mg. per ml. at room temperature. In addition, isomer B dissolves to the same extent in a solution of 20 mg. of isomer A per ml. as it does in pure 95% ethanol.

These solubility properties are important in the separation of the isomers, since isomer B is prepared from an impure acetylamino acid IV, but due to its lower solubility in water it is easily separated from any isomer A present by recrystallization. The isomer A, prepared from the crystalline acetylamino acid IV, is presumably free of isomer B to begin with.

To show that the isomers A and B are diastereoisomers, not structural isomers, advantage was taken of the fact that acetylation of tryptophan[16] and other amino acids[17] with excess acetic anhydride leads to racemization at the α-carbon atom; an azlactone is a probable intermediate.[15] Acetylation of isomer B gave the crystalline acetyl derivative of isomer A (28% yield).

Experimental[19]

Aldimines.—Ethylideneisopropylamine was made from acetaldehyde and isopropylamine by the general method for preparing aliphatic aldimines.[20] The most important modifications of the procedure were a final drying of the aldimine over a small amount of barium oxide and distillation at reduced pressure and room temperature with

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(17) V. du Vigneaud and C. E. Meyer, *ibid.*, **98**, 295 (1932).

(18) V. du Vigneaud and C. E. Meyer, *ibid.*, **99**, 143 (1932).

(19) All melting points are corrected. Infrared spectra were determined by Mr. James Brader. Analyses were performed by Mr. Joseph Nemeth and his associates.

(20) K. N. Campbell, A. H. Sommers and B. K. Campbell, THIS JOURNAL, **66**, 82 (1944).

condensation of the aldimine by a Dry Ice–acetone-bath. The yield of ethylidene-isopropylamine (1-mole scale) was 86%, $n^{19.9}$D 1.3877, reported[15] n^{14}D 1.3963; infrared C=N, 1677 cm.$^{-1}$, reported[21] 1672 cm.$^{-1}$. After storage for two months at 0° in an alkali-washed bottle, the refractive index was essentially unchanged, $n^{25.4}$D 1.3862.

A similar procedure with acetaldehyde and *t*-butylamine furnished an 82% yield of ethylidene-*t*-butylamine, $n^{19.2}$D 1.4005. No physical constants were reported by Hurwitz.[13]

Methylene-*t*-butylamine was prepared by the method of Hurwitz.[13] Freshly distilled material was used in all experiments. Contrary to the reported stability of this aldimine,[13] it was observed that the density and refractive index increased considerably after a few days storage at 0°. A sample which had been stored several months at room temperature showed no infrared absorption between 2500 and 1500 cm.$^{-1}$.

3-(*t*-Butylaminomethyl)-indole.—A hot solution of 27.4 g. (0.25 mole) of *t*-butylamine hydrochloride in 175 ml. of abs. ethanol was neutralized (*p*H 6–7) with 0.5 ml. of *t*-butylamine, and 7.51 g. (0.25 mole) of paraformaldehyde was added. The mixture was stirred and refluxed while a solution of 29.3 g. (0.25 mole) of indole in 75 ml. of abs. ethanol was added during 1 hr. Refluxing was continued for 1.5 hr., and the mixture was stored overnight in the refrigerator. The crystalline amine hydrochloride was shaken with 200 ml. of ether and 250 ml. of 1 *N* sodium hydroxide until it dissolved. The ether layer was dried over magnesium sulfate, and the ether was distilled (water-pump). The solid residue was recrystallized from ethanol–water. The yield of 3-(*t*-butylaminomethyl)-indole, m.p. 113–120°, was 19.8 g. (39%). An analytical sample was prepared by two recrystallizations from methylcyclohexane and three from methanol, m.p. 115.5–119°.

Anal. Calcd. for $C_{13}H_{18}N_2$: C, 77.20; H, 8.97; N, 13.85. Found: C, 77.37; H, 9.23, N, 14.03.

Reaction of Methylene-*t*-butylamine and Indole.—In each experiment, 0.05 mole of indole and 0.055 to 0.06 mole of methylene-*t*-butylamine were used; the product, 3-(*t*-butylaminomethyl)-indole, was isolated by extraction of basic material from aqueous solution with ether, evaporation of the ether, and recrystallization from methylcyclohexane or ethanol–water. The solvents, catalysts, reaction times, temperatures and yields are summarized as follows: 30 ml. abs. ethanol, 20 hr., reflux, 47%; 30 ml. glacial acetic acid, 10 ml. benzene, 0.5 hr., 50°, 33%; 50 ml. glacial acetic acid, 10 ml. benzene, 42 hr., 25°, 26%; 25 ml. abs. ethanol, 0.1 ml. concd. sulfuric acid, 4 hr. reflux, 31%; 25 ml. abs. ethanol, 0.2 ml. glacial acetic acid, 2 hr. reflux, 31%; 30 ml. abs. ethanol, 2 hr., reflux, 6%; 25 ml. methylcyclohexane, 16 hr., reflux, 12%.

3-(Isopropylaminoethylidene)-indole (Ib).—A solution of 29.3 g. (0.25 mole) of indole in 150 ml. of glacial acetic acid was stirred and kept below 15° while a mixture of 23.4 g. (0.275 mole) of ethylideneisopropylamine and 50 ml. of benzene was added dropwise during about 10 min. The solution was then allowed to stand at 0° for 2 to 4 days. The reaction mixture was poured into 500 ml. of ice-water and 50 ml. of ether. The ether layer was separated and extracted with two 100-ml. portions of 1 *M* potassium bisulfate. The combined aqueous solutions were washed with two 50-ml. portions of ether, then made basic with 10 *N* sodium hydroxide; during addition of the alkali, the temperature was kept below 25°. At this point, an oily layer formed and the base began to crystallize from it. To promote crystallization, 100 ml. of methylcyclohexane was added, and the mixture was allowed to stand at 5° for 3 hr. The crystals were collected and dried at room temperature. The yield of 3-(isopropylaminoethylidene)-indole (Ib), m.p. 108–114°, was 30.6 g. (60%). An analytical sample was prepared by two recrystallizations from toluene and one from acetone–toluene, m.p. 113–117.5°.

Anal. Calcd. for $C_{13}H_{18}N_2$: C, 77.18; H, 8.97; N, 13.85. Found: C, 77.39, H, 9.11; N, 13.39.

3-(*t*-Butylaminoethylidene)-indole (Ia).—The procedure used, starting with indole and ethylidene-*t*-butylamine, was similar to that described above for the preparation of 3-(isopropylaminoethylidene)-indole (Ib), except that a different method of isolating the product had to be used. After the acidic solution of the amine Ia was made basic with sodium hydroxide, the product did not crystallize, but was extracted with ether, the solution was dried with magnesium sulfate and the ether was distilled (water-pump). The oily residue was taken up in methylcyclohexane; crystallization required several days at 0°. The yields of Ia, m.p. 68–72°, were 10 to 18%. An analytical sample was prepared by five recrystallizations from methylcyclohexane, m.p. 69.5–72°.

Anal. Calcd. for $C_{14}H_{20}N_2$: C, 77.7; H, 9.31; N, 12.97. Found: C, 77.83; H, 9.53; N, 13.20.

Reaction of Indole, Acetaldehyde and Isopropylamine.—A solution of 5.86 g. (0.05 mole) of indole in 30 ml. of glacial acetic acid was chilled and stirred while 3.3 g. (0.055 mole) of isopropylamine was added. To this cold solution was added a solution of 2.3 g. (0.052 mole) of acetaldehyde in 10 ml. of benzene. The mixture was allowed to stand 2 days at 0°. The product was isolated in the manner described above for 3-(*t*-butylaminoethylidene)-indole (Ia). The yield of 3-(isopropylaminoethylidene)-indole (Ib), m.p. 110–117°, was 3.94 g., 39%.

Reduction of the Base (Ib) to 3-Ethylindole.—A solution of 3-(isopropylaminoethylidene)-indole (Ib) (2 g.) in 95% ethanol (25 ml.) was hydrogenated at 3 atm. over 0.06 g. of 30% Pd/C catalyst at 60–80° for 48 hr. (The apparatus used could not measure the small hydrogen absorption.) The 3-ethylindole was purified by vacuum distillation.

An authentic sample of 3-ethylindole was prepared by a modification of the method of Plancher and Carrasco,[22] a Fischer indole synthesis with phenylhydrazine and butyraldehyde. The reaction was run at about 190° in diethylene glycol until ammonia evolution ceased. Purified by distillation, the 3-ethylindole melted just above room temperature, reported[22] m.p. 43°.

The infrared spectra of the two samples indicated that they were identical.

Dibenzyl Acetamidomalonate.—A solution of 21.7 g. (0.1 mole) of diethyl acetamidomalonate[23] in 60 ml. of benzyl alcohol was heated with an oil-bath to 190–210°. A slow stream of nitrogen was bubbled through the solution, and ethanol was distilled out through a short Vigreux column. After 2 to 3 hr., 11 ml. of ethanol had distilled (theoretical: 0.2 mole, 11.5 ml.). The benzyl alcohol was then distilled at 20 mm.; the bath temperature was raised gradually to 185–190°, where distillation became slow. The residue was crystallized twice from 75 ml. of 2-propanol. The yield of dibenzyl acetamidomalonate, m.p. 110–113°, was 22.1 g. (65%). An analytical sample was prepared by one recrystallization from 2-propanol and two from toluene, m.p. 112–113°.

Anal. Calcd. for $C_{19}H_{19}NO_5$: C, 66.9; H, 5.61; N, 4.10. Found: C, 67.11; H, 5.64; N, 4.06.

Dibenzyl (3-Indolylethylidene)-acetamidomalonate (IIb).—A mixture of 10.11 g. (0.05 mole) of 3-(isopropylaminoethylidene)-indole, 17.07 g. (0.05 mole) of dibenzyl acetamidomalonate, 65 ml. of toluene and approx. 0.01 g. of sodium methoxide was heated at 85–95° with an oil-bath. A slow stream of nitrogen bubbled through the reaction mixture swept out the isopropylamine generated, and the rate of its evolution was followed by titration. The reaction was 95% complete in 6 to 8 hr. Crystallization was induced by scratching and allowed to proceed overnight. The yield of dibenzyl (3-indolylethylidene)-acetamidomalonate was 23.4–23.6 g. (97%), m.p. 120–125°, solidified, second m.p. 161–163°. An analytical sample was prepared by recrystallizations from 2-propanol, acetone, toluene–acetone and toluene, m.p. 166–178°.

Anal. Calcd. for $C_{29}H_{28}N_2O_5$: C, 71.9; H, 5.83; N, 5.79. Found: C, 72.22; H, 5.76; N, 5.82.

Other Alkylations.—The same general procedure described above for the preparation of dibenzyl (3-indolylethylidene)-acetamidomalonate (IIb) was used in all cases with only slight modifications.

Diethyl skatylacetamidomalonate was prepared from diethyl acetamidomalonate and 3-(*t*-butylaminomethyl)-indole at 105° in 71% yield. A low melting form, m.p. 144–145°, was obtained. When recrystallized and seeded

(21) L. Kahovec, *Acta Phys. Austriaca*, **1**, 307 (1948); *C. A.*, **42**, 6665 (1948).

(22) Beilstein, "Handbuch der Organischen Chemie," Vol. XX, 4th Ed., Julius Springer, Berlin, 1935, p. 319.

(23) The Dow Chemical Co., Midland, Michigan.

with an authentic sample[2] of m.p. 159°, the m.p. rose to 160–161°, not depressed by mixing with the known sample.

Diethyl (3-indolylethylidene)-acetamidomalonate (IIa), m.p. 163–167°, was prepared in 97% yield from diethyl acetamidomalonate and 3-(*t*-butylaminoethylidene)-indole (Ia) at 72–75°. An analytical sample was prepared by three recrystallizations from ethanol, m.p. 176.5–178°.

Anal. Calcd. for $C_{19}H_{24}N_2O_5$: C, 63.3; H, 6.71; N, 7.76. Found: C, 63.18; H, 6.58; N, 7.94.

Dibenzyl (3-indolylethylidene)-acetamidomalonate (IIb) (see preparation above) was also prepared from 3-(*t*-butylaminoethylidene)-indole (Ia) and dibenzyl acetamidomalonate in 91% yield.

Ammonium (3-Indolylethylidene)-acetamidomalonate (Ammonium Salt of III).—Dibenzyl (3-indolylethylidene)-acetamidomalonate (35.7 g., 0.074 mole) (free from lumps) was placed in a heavy-walled bottle with 125 ml. of 95% ethanol and 0.75 g. of 30% Pd/C catalyst. The mixture was shaken under 3–4 atm. of hydrogen until the theoretical amount of hydrogen was absorbed (1 hr.). The catalyst was removed and 15 ml. of ammonium hydroxide was added. Crystallization was allowed to proceed at 0° overnight. The crystals were collected, washed with acetone and dried to constant weight at room temperature. The yield of ammonium (3-indolylethylidene)-acetamidomalonate, m.p. 160–165° dec., was 21.9 g. (87%). An analytical sample was prepared by dissolving the salt in a small amount of water (25°) and recrystallizing it by adding ethanol, m.p. 159–162° dec.

Anal. Calcd. for $C_{15}H_{22}N_4O_6$: C, 53.24; H, 6.56; N, 16.56. Found: C, 53.16; H, 6.81; N, 16.40.

2-Acetamido-3-(3-indolyl)-butyric Acids (Acetyl-β-methyltryptophans) (IV).—A solution of 92.9 g. (0.279 mole) of ammonium (3-indolylethylidene)-acetamidomalonate in 93 ml. of water and 93 ml. of pyridine was refluxed for 5 hr. in a nitrogen atmosphere. Ammonium carbonate deposited in the reflux condenser. The solution was diluted with 465 ml. of water and filtered, then acidified with 46.5 ml. of sulfuric acid dissolved in 465 ml. of water. During acidification, the solution was heated sufficiently to prevent the product from separating as an oil, and crystallization was induced by scratching when about 60% of the acid had been added (*p*H 3). Crystallization was allowed to proceed at 0° overnight.

The yield of crystalline acetyl-β-methyltryptophan, isomer A, m.p. 211–215°, was 32.2 g. (44%). This crude material did not give satisfactory yields of the amino acid on hydrolysis. It was dissolved in 64 ml. of warm dimethylformamide and recrystallized by addition of 96 ml. of water. The yield of purified material, m.p. 214–218°, was 25.0 g. (34%). An analytical sample was prepared by dissolving the crude material in ethanol and crystallizing it by adding ethyl acetate, m.p. 216–218°.

Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.6; H, 6.20; N, 10.78. Found: C, 64.77; H, 6.12; N, 10.63.

The mother liquor, which contained isomer B, was extracted with four 230-ml. portions of ethyl acetate. The ethyl acetate solution was washed with two 115-ml. portions of water and extracted with two 230-ml. portions of 10% sodium bicarbonate solution. The aqueous solution was acidified with 4 *N* sulfuric acid and extracted with three 115-ml. portions of ethyl acetate. The ethyl acetate solution was washed with two 55-ml. portions of water and was concentrated under reduced pressure until a foamy gum, which weighed about 40 g., remained. This crude acetyl-β-methyltryptophan, isomer B, was satisfactory for hydrolysis to the amino acid.

2-Amino-3-(3-indolyl)-butyric Acids (β-Methyltryptophans) (V).—The purified acetyl-β-methyltryptophan (IV), isomer A described above (25.0 g.), was ground in a mortar, then refluxed under nitrogen with 150 ml. of 4 *N* sulfuric acid and 1.5 ml. of 1-octanol until it completely dissolved (about 20 hr.). The crude isomer B of IV described above (approx. 40 g.) was refluxed under nitrogen with 210 ml. of 4 *N* sulfuric acid for 5 hr. In each case, after the reflux period was complete, the sulfuric acid was neutralized (*p*H 8) with 0.4 *N* barium hydroxide. A little 1-octanol was added to prevent foaming, Dry Ice was added to precipitate excess barium ion, and the mixture was heated to boiling. The barium sulfate was filtered from the hot solution and the filtrate was concentrated to approx. 300 ml. under reduced pressure; foaming was controlled with 1-octanol. The warm solution was treated with charcoal and filtered, then concentrated to about 150 ml., at which point the amino acid began to crystallize. The product was allowed to crystallize at 0° overnight, then collected and dried under 0.1 mm. at room temperature. The yield of β-methyltryptophan (V), isomer A, from the first crop, m.p. 245–251° dec. (loses water of crystallization approx. 190°), was 14.3 g. It was recrystallized from 110 ml. of water, yield 11.2 g., m.p. 248–250° dec. An additional 1.7 g. of recrystallized isomer A, m.p. 246–250° dec., was obtained from the second crop. The yield of isomer B, m.p. 236–242° dec., was 20.7 g. It was recrystallized from 300 ml. of water, yield 13.8 g., m.p. 247–251° dec. It appeared that water of crystallization was lost at 0.5 mm. at room temperature.

Treatment of the combined aqueous mother liquors with acetic anhydride and sodium acetate yielded 4.6 g. of acetyl β-methyltryptophan (IV), isomer A, plus some of the noncrystalline isomer B. Much barium was found in these mother liquors by precipitation with sulfuric acid.

Analytical samples were prepared by dissolving the amino acid in glacial acetic acid, precipitating the acetate salt with benzene and converting the salt to the free amino acid by crystallization from water at *p*H 5. The amino acid was then recrystallized one or two times from water and dried 24 hr. at 100° (0.1 mm.) over phosphorus pentoxide; isomer A, m.p. 232–235° dec., 238–242° dec., isomer B, m.p. 237–240° dec., 246–248° dec., mixture m.p. 218–225° dec.

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 66.0; H, 6.45; N, 12.85. Isomer A, found: C, 66.20; H, 6.73; N, 12.58, 12.75. Isomer B, found: C, 66.31; H, 6.48; N, 12.57, 12.96.

α-Naphthylurea Derivatives of the β-Methyltryptophans.—The customary procedure[24] was followed. The sodium salt of the α-naphthylurea derivative of isomer B was only slightly soluble in water, but dissolved upon warming. Both α-naphthylurea derivatives were first obtained as amorphous solids but crystallized when warmed with ethylene dichloride. Analytical samples were recrystallized twice from 2-propanol–water: isomer A, m.p. 191–193° dec., isomer B, m.p. 192–194° dec.

Anal. Calcd. for $C_{23}H_{21}N_3O_3$: C, 71.3; H, 5.46; N, 10.88. Isomer A, found: C, 71.22; H, 5.61; N, 10.61. Isomer B, found: C, 71.62; H, 5.74; N, 10.77.

The melting point was not significantly depressed upon mixing the two isomers, but the infrared spectra (Nujol) showed large differences.

Racemization of β-Methyltryptophan by Acetylation.—To a solution of 0.10 g. of isomer B of β-methyltryptophan (V) (m.p. 244–247° dec.) and 0.5 g. of sodium bicarbonate in 5 ml. of water was added 1.0 ml. of acetic anhydride, and the mixture was agitated and warmed until a clear solution resulted. Sulfuric acid (4 *N*) was added until the solution was strongly acidic, and crystallization was induced by scratching. The yield of isomer A of acetyl β-methyltryptophan (IV), m.p. 214–218° (not depressed upon mixing with an authentic sample), was 33 mg. (28%). The infrared spectrum (potassium bromide pellet) was identical to that of a known sample. When isomer B of β-methyltryptophan was acetylated with the theoretical amount of acetic anhydride in the presence of excess sodium hydroxide, conditions which do not lead to racemization,[16] no solid acetyl derivative was obtained.

Solubility of β-Methyltryptophan Isomers.—A 20-mg. sample of the amino acid was weighed into a small vial and 1.00 ml. of 95% ethanol was added. After 2 days at room temperature (occasional shaking), the ethanol was withdrawn with a hypodermic syringe; the volume withdrawn was used in calculating solubility. The vial was dried in the oven and weighed. The solubility of isomer A was at least 20 mg./ml. The solubility of isomer B was 5.5 mg./ml.; this value was checked by dissolving the solid in hot ethanol (solubility apparently less than 15 mg./ml.) and allowing it to crystallize. The solubility of isomer B was the same in a solution of 20 mg./ml. of isomer A as in pure 95% ethanol.

(3-Indolylethylidene)-acetamidomalonic Acid (III).—An aqueous solution of the ammonium salt of III was acidified

(24) N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," Thomas Y. Crowell Co., New York, N. Y., 1947, p. 274.

and extracted with ethyl acetate. A small amount of chloroform was added and crystallization was induced by scratching. An analytical sample was not obtained.

The free acid III was decarboxylated by heating in several solvents, including methyl ethyl ketone, 2-propanol, acetonitrile and pyridine. The yield of crystalline acetyl β-methyltryptophan was always near 30%. Decarboxylation in water or toluene, which did not dissolve the acid III, led to red tar.

URBANA, ILL.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, RESEARCH DIVISION, LEDERLE LABORATORIES, AMERICAN CYANAMID COMPANY]

Vasodilator and Adrenergic Blocking Agents. I. 1,4-Disubstituted Piperazines and Related N-Phenylethylenediamine Derivatives

BY F. L. BACH, JR., H. J. BRABANDER AND S. KUSHNER

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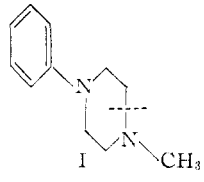
The syntheses and physiological properties of a series of 1,4-disubstituted piperazines and N-phenylethylenediamine derivatives are described. Also a discussion dealing with the importance of the phenyl and pyridylpiperazine nuclei in developing strong adrenergic blocking agents of this type is included.

In recent years a considerable number of compounds exhibiting strong vasodilator or adrenergic blocking action have been described in the literature.[1] However, because of undesirable side effects and poor oral activity, very few of these agents have found therapeutic application in the treatment of peripheral vascular disease. Two classes of compounds which have received considerable attention for their ability to inhibit responses to epinephrine are the β-diaralkylaminoethyl chlorides and the aralkylimidazolines. In our laboratories, pharmacological testing indicates that 1-methyl-4-phenylpiperazine (I) is also an effective vasodilator and adrenergic blocking agent.

To elucidate the structural requirements necessary for activity in this compound, we prepared a series of N-phenylethylenediamine derivatives. These diamines are related to products which would be formed by a scission of the piperazine ring of the active structure[2] I. The diamines were



synthesized by the interaction of one molecular equivalent of β-(N-ethyl-N-phenylamino)-ethyl chloride monohydrochloride with two molecular equivalents of the appropriate primary or secondary amine in a refluxing water–ethanol solution. Derivatives of a similar nature have been tested as antihistamines.[3,4] The fact that structural similarities exist between adrenergic blocking agents and compounds which antagonize spasmogenic substances, such as acetylcholine and histamine, has been discussed by Burger.[5] As shown in Table I, the simple isosteres derived from the parent compound I where one amino group is substituted by lower alkyls were relatively inactive[6] when compared to Priscoline[7] which was used as a standard. However, a marked increase in vasodilator activity was observed when these substituents were replaced by di-*n*-propyl or benzyl groups.

An extension of this work was the reconstitution of a piperazine ring to include one of the terminal N-atoms of the N-phenylethylenediamine structure. This led to the synthesis of a series of 1-[β-(N-ethyl-N-phenylamino)-ethyl]-4-substituted piperazines which are described in Table II. Preparation of these compounds involved a condensation between the β-(N-ethyl-N-phenylamino)-ethyl chloride monohydrochloride and 1-substituted piperazines in refluxing ethanol using sodium bicarbonate as an acid acceptor. The biphenylpiperazine analog was synthesized stepwise by condensing the appropriate haloalkylamine with diethanolamine to yield N-ethyl-N′,N′-di-(β-hydroxyethyl)-N-phenylethylenediamine. In the next step, reductive cyclization of the di-(β-hydroxyethyl)-amino portion of the molecule with 4-aminobiphenyl was accomplished in poor yield by subjecting a solution of the two reactants in dioxane over copper chromite to approximately 93 atmospheres of hydrogen for 7 hr. at 250°. An inspection of the activity ratings listed in Table II reveals a sharp change in biological response when the N-methyl, acetyl and carbethoxy groups in the piperazine moiety are replaced by phenyl, 2-pyridyl or 2-(6-methyl)-pyridyl radicals. Based on these findings a study of the scission products of I has by a circuitous route established the importance of not only the phenyl piperazine but also the pyridyl piperazine nucleus as a center of activity. It is also apparent that strong vasodilatation and adrenergic blocking action[8] are obtained when the simple alkyl substituent in I is replaced by a β-(N-ethyl-N-phenylamino)-ethyl chain II. Preliminary studies indicate that compound II is orally active in the test animal.

(1) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," 2nd Ed., The Macmillan Co., New York, N. Y., 1955, (Bibliography), pp. 592–595.

(2) W. T. Forsee and C. B. Pollard, THIS JOURNAL, **57**, 1788 (1935).

(3) A. M. Staub, *Ann. Inst. Pasteur*, **63**, 400, 420, 485 (1939).

(4) B. N. Halpern, *Arch. Intern. Pharmacodynamie*, **68**, 339 (1942).

(5) A. Burger, "Medicinal Chemistry," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1951, p. 359.

(6) See footnote (a), Table I, for explanation of vasodilator activity.

(7) 2-Benzyl-4,5-imidazoline monohydrochloride.

(8) See footntoes *b* and *c*, Table I, for explanation of adrenergic blocking action.