Hagedorn and Jensen,[13] which was modified for autoanalyzer determination, oral doses of 100 mg/kg did not change rat blood glucose.

The 1,2-benzisothiazole 1,1-dioxide series produced hypotensive activity that increased in potency with continuous administration of each active drug (Figure 1) (Table I). An acute hypotensive activity was produced in anesthetized animal preparations (Figures 4 and 7); however, this acute response may not have been the same as the activity with chronic administration (Figures 1–3). The acute and the chronic responses may indeed have been responses from two different mechanisms. The responses observed indicate that III probably has a mechanism other than blockade of the central or peripheral nervous systems.

(13) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923).

A New Class of Potent Decarboxylase Inhibitors. β-(3-Indolyl)-α-hydrazinopropionic Acids

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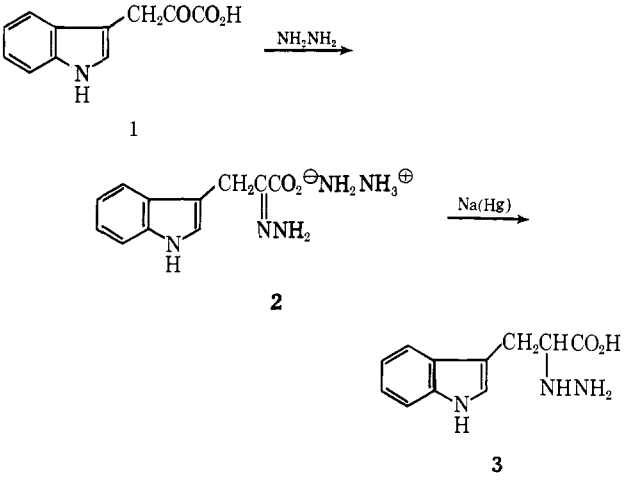
Three β-(3-indolyl)-α-hydrazinopropionic acids were synthesized and tested for *in vitro* and *in vivo* inhibition of DOPA decarboxylase. All were found to be highly active, and one of them, DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid, is among the most potent inhibitors of DOPA decarboxylase known.

The biological activity of α-hydrazino acids has only recently been the subject of investigation in spite of the close structural relationship of this class of compounds to the naturally occurring amino acids. In 1960, Carmi and co-workers[1] prepared a large number of aliphatic α-hydrazino acids as potential antimetabolites, especially in cancer therapy. These compounds, however, exhibited only a slight nonreproducible activity in Sarcoma 180 tests.[2] On the other hand, it has been found that certain aliphatic and particularly aromatic α-hydrazino acids are inhibitors of DOPA decarboxylase *in vivo* and *in vitro*.[3] Indeed, DL-α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid (HMD) exhibited a potency 1000 times that of α-methyldopa, its parent compound, and was the most potent DOPA decarboxylase inhibitor available at that time.[3d] In addition, HMD inhibited formation of serotonin in the kidneys of mice given 5-hydroxytryptophan.[3i] These results prompted us to synthesize and screen several new α-hydrazino acids in the indole series, namely, DL-β-(3-indolyl)-α-hydrazinopropionic acid, DL-β-(3-indolyl)-α-methyl-α-hydrazinopropionic acid, and DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid.

Chemistry.—The synthesis of DL-β-(3-indolyl)-α-hydrazinopropionic acid (see Scheme I) started with 3-indolylpyruvic acid (**1**), prepared according to the procedure described in a Japanese patent.[4] Treatment of **1** with slightly more than 2 equiv of hydrazine hydrate resulted in the formation of the hydrazine salt of 3-indolylpyruvic acid hydrazone (**2**). The hydrazone moiety was then reduced with sodium amalgam to afford DL-β-(3-indolyl)-α-hydrazinopropionic acid (**3**).

SCHEME I



The synthesis of DL-β-(3-indolyl)-α-methyl-α-hydrazinopropionic acid (see Scheme II) required as starting material 3-indolylacetone (**4**). This compound was prepared from indole acetic acid *via* the acetylative decarboxylation method described by Brown, *et al.*[5] Ketone **4** reacted smoothly with acetic acid hydrazide to give the acetylhydrazone **5**. When hydrazine itself was used, the product was found to be a mixture of hydrazone and ketazine. Condensation of intermediate **5** with HCN in DMSO as solvent gave the α-methyl-α-acetylhydrazinonitrile **6**. This adduct was then hydrolyzed in two steps. The nitrile group was first transformed to an amide by fortified HCl at 0°. The resulting product was isolated as the

(1) A. Carmi, G. Pollak, and H. Yellin, *J. Org. Chem.*, **25**, 44 (1960).

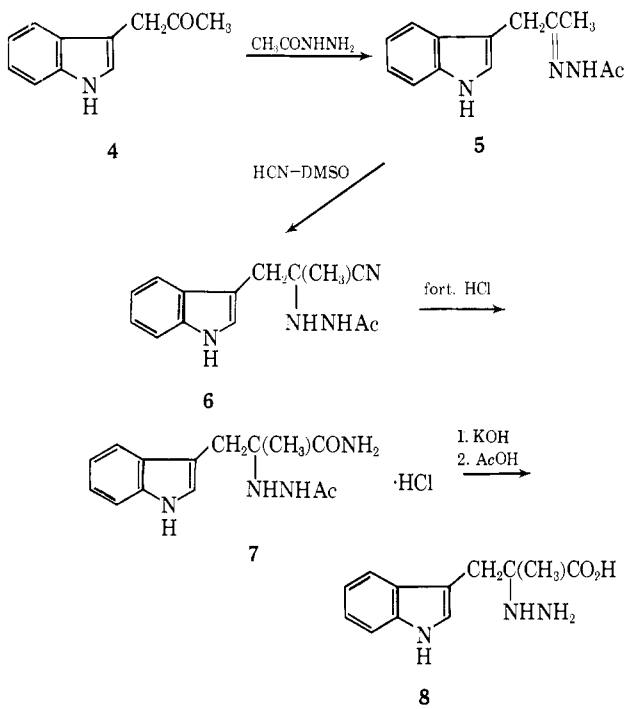
(2) G. Pollak, H. Yellin, and A. Carmi, *J. Med. Chem.*, **7**, 220 (1964).

(3) (a) S. Udenfriend, R. Connamacher, and S. M. Hess, *Biochem. Pharmacol.*, **8**, 419 (1961); (b) S. Udenfriend and P. Zaltzman-Nirenberg, *J. Pharmacol. Exptl. Therap.*, **138**, 194 (1962); (c) E. Hansson and W. G. Clark, *Proc. Soc. Exptl. Biol. Med.*, **111**, 793 (1962); (d) C. C. Porter, L. S. Watson, D. C. Titus, J. A. Totaro, and S. S. Byer, *Biochem. Pharmacol.*, **11**, 1067 (1962); (e) C. R. Creveling, J. W. Daly, and B. Witkop, *J. Med. Chem.*, **9**, 284 (1966).

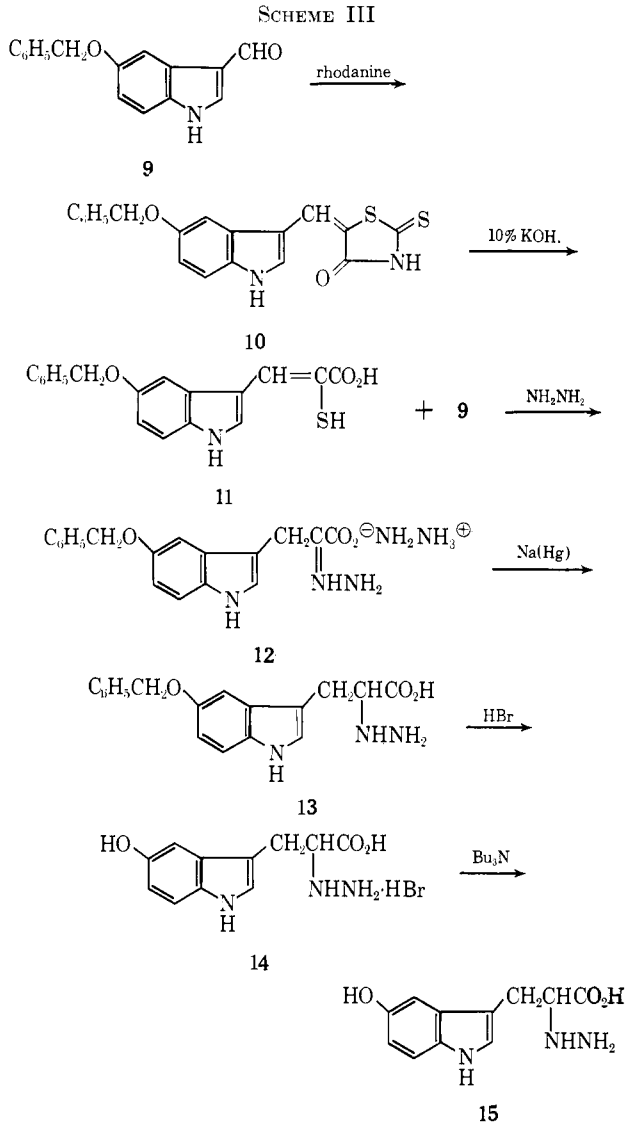
(4) S. Akabori, S. Sakurai, and T. Ito, Japanese Patent 4274 (1959); *Chem. Abstr.*, **54**, 13146 (1960).

(5) J. B. Brown, H. B. Henbest, and E. R. H. Jones, *J. Chem. Soc.*, 3172 (1952).

Scheme II



Scheme III



hydrochloride salt **7**. On refluxing **7** in ethanolic KOH overnight, the amide was hydrolyzed to a carboxylic acid and the acetyl group was removed from the hydrazino moiety. This gave DL-β-(3-indolyl)-α-methyl-α-hydrazinopropionic acid (**8**).

The final analog in this series, DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid, was prepared as shown in Scheme III. 5-Benzyloxy-3-indolecarboxaldehyde (**9**) was condensed with rhodanine by heating in pyridine solution. Refluxing the resulting rhodanine derivative (**10**) with aqueous KOH afforded a 63% yield of the desired β-(5-benzyloxy-3-indolyl)-α-sulfhydrylacrylic acid (**11**). The starting material, 5-benzyloxy-3-indolecarboxaldehyde (**9**) was also produced in the reaction (33% yield) but was easily separated from **11**. Next, the α-sulfhydrylacrylic acid side chain of **11** was converted to an α-hydrazonopropionic acid moiety by heating in ethanol solution with excess hydrazine. The product **12** was then reduced to DL-β-(5-benzyloxy-3-indolyl)-α-hydrazinopropionic acid (**13**) with 3% sodium amalgam. The removal of the benzyl protecting group was accomplished by treatment with liquid HBr at −75°. The resulting salt **14**, upon addition of tri-*n*-butylamine to pH 5.5, liberated the zwitterionic final product, DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid (**15**). The analytical data of these compounds can be found in Table I.

Biological Activity.—In Table II the new α-hydrazino acids in the indole series and two highly active compounds of the DOPA family are compared for their ability to inhibit DOPA decarboxylase *in vitro* and *in vivo*. It will be seen that the indole-α-hydrazino acids are all effective inhibitors of DOPA decarboxylase. In particular, DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid was found to be almost twice as active *in vitro* and *in vivo* as DL-α-hydrazino-α-methyldopa (HMD), and is therefore one of the most potent inhibitors of DOPA decarboxylase known.

Experimental Section[6]

Hydrazine Salt of 3-Indolylpyruvic Acid Hydrazone (2).—To a stirred mixture of 3-indolylpyruvic acid[4] (1, 12.2 g, 0.06 mole) in ethanol (75 ml) was added slowly 99–100% hydrazine hydrate (7.50 g, 0.15 mole). The temperature rose from 24 to 30°, and within a few minutes a clear red solution resulted. The flask was refrigerated overnight, and then the crystalline product was filtered, washed with cold ethanol, and dried to give 9.70 g (7.28 g)[7] of **2**.

DL-β-(3-Indolyl)-α-hydrazinopropionic Acid (3).—To a stirred solution of **2** (8.60 g, 0.0345 mole) in water (100 ml) was added 2.3% NaHg (200 g), and the system was purged and stirred under N_2 for 3 days. The aqueous phase was then separated from the pool of mercury, extracted once with ether, stirred with charcoal (0.2 g), and filtered. The pH of the filtrate was adjusted to 5.0 by addition of acetic acid, and the product which precipitated was filtered off, washed with water (three 10-ml portions), and purified as follows. The moist cake was suspended in water (50 ml) and 10% HCl was added to pH 1.2. The solution was then charcoaled (0.5 g), filtered, and treated with a sodium acetate

(6) Melting points were determined with a Thomas–Hoover Uni-Melt apparatus in unsealed capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord in Nujol mulls. We are indebted to Mr. A. Kalowsky for ultraviolet spectra, and to Mr. R. N. Boos and associates for microanalyses. Solvents were concentrated on a rotary evaporator. All products were dried to constant weight *in vacuo* at 50° unless otherwise stated; in addition, P_2O_5 was used to expedite drying those solids obtained from aqueous media.

(7) Weight yield of pure product after recrystallization; *cf.* Table I for solvent used and physical properties of the analytical sample.

TABLE I

β-(3-INDOLYL)-α-HYDRAZINOPROPIONIC ACIDS AND THEIR INTERMEDIATES

Compd[a]	Mp, °C	Yield, % Crude	Yield, % Pure[b]	Crystn solvent[c]	Formula[d]	Infrared[e] max, μ	Ultraviolet[r] λmax, mμ (ε)
2	156[l]	67	50	A	$C_{11}H_{15}N_3O_2$	2.90 (m), 3.00 (m), 3.6–3.8 (m), 4.60 (w), 6.10 (w), 6.30 (m)	270 (7790), 287 (5780)[f]
3	260[l]	77	69	C	$C_{11}H_{13}N_3O_2$	2.85 (s), 3.00 (m), 3.6–3.9 (m), 4.50 (m), 6.10 (m), 6.20 (s), 6.35 (s)	275 (5390), 281 (5720), 288 (4950)[g]
5	218–220		94	D	$C_{13}H_{15}N_3O$	2.96 (s), 3.08 (m), 6.08 (s), 6.18 (s), 6.45 (s)	273 (7040), 281 (7060), 291 (5920)[h]
6	134–136	43	34	C	$C_{14}H_{16}N_4O$	3.00 (s), 3.08 (m), 3.15 (s), 4.52 (w), 6.12 (s), 6.20 (s), 6.36 (w), 6.50 (w)	274 (5890), 282 (6330), 290 (5490)[h]
7	207–208		59	D	$C_{14}H_{18}N_4O_2 \cdot HCl \cdot 0.5H_2O$[n]	3.00 (m), 3.15 (m), 4.12 (w), 4.32 (m), 5.85 (s), 6.20 (w), 6.40 (m)	276 (5720), 283 (6140), 291 (5310)[h]
8	200–202[l]	79	59	C	$C_{12}H_{15}N_3O_2$	3.02 (s), 3.7–4.7 (w), 6.12 (s), 6.28 (s), 6.50 (m)	273 (5410), 279 (5670), 288 (4640)[i]
10	287–288[l]	93	74	B	$C_{19}H_{14}N_2O_2S_2$	3.05 (w), 5.98 (s), 6.20 (w), 6.35 (s), 13.7 (m), 13.9 (m)	284 (16,350), 300 (16,170), 436 (38,900)[j]
11	176–178[l]	63			$C_{18}H_{15}NO_3S$[o]	2.87 (m), 3.7–4.3 (w), 6.05 (s), 6.20 (w), 6.35 (m)	283 (11,500), 365 (9530)[k]
12	200–201[l,m]		73	D	$C_{18}H_{21}N_5O_3$	2.87 (m), 3.00 (s), 3.7–4.3 (w), 6.10 (m), 6.3–6.4 (s)	233 (18,000), 250 (10,400), 293 (3770), 308 (2740)[j]
13	217–218[l]	77	51	C	$C_{18}H_{19}N_3O_3$[p]	2.90 (m), 3.7–4.3 (w), 6.12 (s), 6.32 (s)	278 (6180), 290 (5370)[j]
15	240–242[l]	67	55	C	$C_{11}H_{13}N_3O_3$[q]	2.85 (s), 2.98–3.02 (w), 3.7–4.4 (w), 6.12–6.28 (s)	277 (5600), 293 (4630)[j]

[a] Compounds 1, 4, and 9 are known; 14 was too unstable to determine its physical properties. [b] Only a single crystallization was required to purify the crude products. The yield (pure) is based on starting material. [c] A, 80% aqueous methanol; B, tetrahydrofuran–ethanol; C, *cf.* Experimental Section; D, this product crystallized directly from the reaction medium in an analytically pure state. [d] All pure compounds have C, H, N (Cl and S, where appropriate) analyses within ±0.30 of the calculated values, except where indicated. [e] All infrared spectra were taken in Nujol mulls; the intensity of the absorption band is designated as w = weak, m = medium, s = strong. [f] In water. [g] In 1 *N* NaOH. [h] In ethanol. [i] In 1 *N* HCl. [j] In dioxane. [k] In 95% ethanol. [l] Decomposition. [m] When immersed in the oil bath at 100° followed by a slow rise in temperature. [n] Neut equiv 319.0, mol wt 319.8. The analytical sample was dried *in vacuo* at 25°. [o] A satisfactory analytical sample could not be obtained for this intermediate. [p] The analytical sample was dried *in vacuo* at 100°. [q] The H analysis found was 0.33 over the calculated value. [r] Maxima below 222 mμ are not reported.

TABLE II

INHIBITION OF DOPA DECARBOXYLASE

Compd	*In vitro*[a] Amt in flask,[d] μmole	*In vitro*[a] Inhib, %	*In vitro*[a] Approx I_{50},[e] μmole	*In vitro*[a] Rel molar potency[f]	*In vivo*[b] No. of groups	*In vivo*[b] ED_{50},[g] mg/kg	*In vivo*[b] 95% conf lim	*In vivo*[b] Rel molar potency[f]
DL-α-Methyldopa[c]	0.20	25	0.9	1	10	4.7	3.73, 5.92	1
	2.00	64						
	20.00	82						
DL-α-Hydrazino-α-methyldopa[c] (HMD)	0.0002	17	0.0006	1500	10	0.088	0.080, 0.096	57
	0.002	85						
	0.02	97						
DL-β-(3-Indolyl)-α-hydrazinopropionic acid	0.0002	27	0.00067	1340	3	0.88	0.78, 0.99	5.5
	0.002	71						
	0.02	92						
	0.2	100						
DL-β-(3-Indolyl)-α-methyl-α-hydrazino-propionic acid	...	...	...	...	3	0.64	0.57, 0.72	8.1
DL-β-(5-Hydroxy-3-indolyl)-α-hydrazino-propionic acid	0.0002	40	0.00035	2570	3	0.054	0.048, 0.061	96.9
	0.002	79						
	0.02	97						
	0.2	100						

[a] The source of L-DOPA decarboxylase was a lyophilized aqueous extract of hog kidneys, and the standard anaerobic manometric procedure described previously[3d] was followed. [b] The mouse kidney serotonin assay was employed exactly as described previously.[3d] [c] The testing data for this compound was taken from Table I, footnote 3d. [d] Micromole of inhibitor in the 2.8-ml reaction mixtures. [e] Estimated amount of inhibitor, micromole in the 2.8-ml reaction mixture, required to inhibit the reaction rate 50%. [f] Potency of DL-α-methyldopa taken as 1. [g] With ten groups of five, or three groups of three mice per drug, regression lines were calculated by the method of least squares, and ED_{50}'s and 95% confidence limits were computed. Regression lines were linear and parallel within the limits of experimental error.

solution (4 g in 10 ml of water). The white precipitate was filtered, washed with water (four 8-ml portions), ethanol (four 8-ml portions), and ether (two 10-ml portions), then dried to give 5.80 g of **3**, mp 258° dec. Repeating this precipitation once more afforded 5.20 g of pure **3**.

Acetylhydrazone of 3-Indolylacetone (5).—To a stirred slurry of 3-indolylacetone[5] (**4**, 6.92 g, 0.040 mole) in methanol (15 ml) was added a solution of acetic acid hydrazide[8] (5.92 g, 0.080 mole) in methanol (10 ml). The mixture was refluxed for 10 min, then allowed to cool to room temperature. After an additional 30 min at 0°, the voluminous microcrystalline product was filtered, washed with cold methanol (8 ml) and ether (8 ml), then dried to afford 8.65 g of **5** which was analytically pure.

β-(3-Indolyl)-α-methyl-α-acetylhydrazinopropionamide (6).—A mixture of pulverized **5** (8.33 g, 0.0363 mole), sieve-dried dimethyl sulfoxide (70 ml), a few crystals of KCN and anhydrous HCN (5.00 ml, 0.126 mole) was stirred magnetically in a tightly stoppered flask for 24 hr. The solution which resulted was then concentrated *in vacuo* (2 days) to a thick honey. This was triturated well with ether (two 30-ml portions), then dissolved in dioxane (total volume: 30 ml) and passed through a column of Merck acid-washed alumina (100 g, made up in benzene), using dioxane as eluent. The early fractions (250 ml) were combined and concentrated *in vacuo*. The residual syrup was dissolved in 2-propanol (10 ml), then diluted with ether (12 ml) to incipient turbidity. Crystallization was induced by scratching. After 3 hr at room temperature and 3 hr at 0°, the white crystalline solid was filtered, washed with ether (two 7-ml portions), then dried to yield 3.17 g of pure **6**.

From the first-crop filtrate was obtained an additional 0.83 g of product, mp 132–134°. The combined yield then was 4.00 g (43%).

β-(3-Indolyl)-α-methyl-α-acetylhydrazinopropionamide Hydrochloride (7).—To a chilled flask containing **6** (1.024 g, 0.004 mole) was added fortified HCl (15 ml of concentrated HCl saturated at 0° with gaseous HCl). After stirring under N_2 for 5 min, a solution resulted and this was kept overnight at 0° in a dewar flask. A stream of N_2 was then passed through the cold solution for 1 hr to remove the excess gaseous HCl, and then the solution was concentrated (without heating) to about one-third volume when the product crystallized out voluminously. The resulting paste was diluted slowly with acetone (10 ml), and the crystals were filtered and washed with acetone (two 5-ml portions) and ether (two 5-ml portions). After drying *in vacuo* at room temperature, 0.75 g of amide was obtained which analyzed correctly for the hemihydrate of the HCl salt.

DL-β-(3-Indolyl)-α-methyl-α-hydrazinopropionic Acid (8).—A mixture of **7** (4.00 g, 0.0125 mole), 40% aqueous KOH (16 ml), and ethanol (8 ml) was stirred for 10 min when a solution resulted. The system was purged well with N_2 and then heated at reflux overnight (18 hr). The hot solution was then concentrated to one-half the original volume and the small quantity of solid which had separated was removed by filtration. The filtrate was cooled to 0° and the pH was adjusted to 5.4 by slow addition of acetic acid. The precipitate was collected, washed with water (two 10-ml portions), and dried to afford 2.40 g having mp 185–195°. For purification, it was dissolved in 2.5 *N* HCl (5 ml), charcoaled, then treated with a 50% aqueous sodium acetate solution (3 ml) in small portions. The voluminous crystallizate was kept at 0° for 1 hr, then filtered, washed with water (two 5-ml portions), and dried to afford 1.65 g of pure **8**.

5-(5-Benzyloxyindole-3-methylene)rhodanine (10).—A stirred mixture of 5-benzyloxy-3-indolecarboxaldehyde[9] (**9**, 21.00 g, 0.0836 mole), rhodanine (12.60 g, 0.0946 mole), and pyridine (150 ml) was heated under N_2. Solution occurred at 100°, and the reaction was kept at that temperature for 4 hr. After the heating period, the product began to separate voluminously as the flask was allowed to cool to room temperature. The stirred mass of orange crystals was treated with water (250 ml) in small portions, then filtered, washed with water (two 250-ml portions), and dried to yield 28.5 g (22.8 g)[7] of **10**, mp 285–288° dec.

β-(5-Benzyloxy-3-indolyl)-α-sulfhydrylacrylic Acid (11). A stirred mixture of **10** (3.67 g, 0.010 mole) and 10% aqueous KOH (112 ml) was refluxed under N_2 for 1 hr. The reaction was cooled to room temperature and 0.82 g (33%) of 5-benzyloxy-3-indolecarboxaldehyde (**9**) was removed by filtration. The stirred filtrate was cooled to 0° and acidified with 2.5 *N* HCl. The yellow product which separated was filtered, washed with water (two 20-ml portions), and then dried to afford 2.05 g. A dilute ammoniacal solution of **11** when treated with aqueous $FeCl_3$ gave the dark green color characteristic of a sulfhydryl group.[10]

A satisfactory analytical sample could not be obtained for this intermediate and so it was used as is in the next reaction.

Hydrazine Salt of β-(5-Benzyloxy-3-indolyl)pyruvic Acid Hydrazone (12).—A stirred slurry of **11** (2.93 g, 0.009 mole) in absolute ethanol (10 ml) was treated with 99–100% hydrazine hydrate (2.40 ml, 0.048 mole), and the mixture was heated under N_2. Solution occurred at 60° accompanied by vigorous evolution of H_2S. After 2 hr at reflux, the liberation of H_2S had ceased (no black precipitate when a stream of N_2 was passed through the reaction solution and exited into aqueous lead acetate) and the flask was allowed to cool to room temperature. The crystalline product which separated was filtered, washed with cold ethanol (three 8-ml portions), then dried. It weighed 2.35 g and was found to be analytically pure.

β-(5-Benzyloxy-3-indolyl)-α-hydrazinopropionic Acid (13).—To a well-stirred suspension of **12** (1.07 g, 0.003 mole) in water (15 ml) was added pulverized 3% NaHg (14 g). The system was purged and stirred under N_2 for 2 days. The aqueous phase was then decanted from the pool of mercury, filtered through Supercel, and treated dropwise with acetic acid (1.5 ml). The zwitterion precipitated voluminously and was filtered, washed with water (two 4-ml portions), then dried to give 0.75 g of product, mp 217° dec. For purification, the crude product was stirred with ethanol (4 ml) and solution was brought about by the addition of 2.5 *N* HCl (1.25 ml). After decolorization with charcoal, the solution (pH 1.8) was treated dropwise with tri-*n*-butylamine until a pH of 5.5 was attained. The microcrystalline product was collected, washed with ethanol (2 ml), and then dried *in vacuo* at 100° to afford 0.49 g of pure **13**.

DL-β-(5-Hydroxy-3-indolyl)-α-hydrazinopropionic Acid (15).—A dry flask containing **13** (7.50 g, 0.023 mole) was cooled to −75° in a Dry Ice–acetone bath, and anhydrous HBr was passed in until about 50 ml had condensed. The mixture was stirred at −75° for 1 hr, then all volatile liquid was evaporated *via* a stream of dry N_2 (1 hr) after the cold bath had been removed. When the flask had warmed to room temperature, the HBr salt **14** was stirred with dry ether (50 ml), then filtered and washed well with ether (five 10-ml portions) to remove traces of HBr and benzyl bromide. The hygroscopic tan powder (**14**) so obtained was dissolved in grain alcohol (50 ml) and filtered, and the pH of the filtrate was adjusted to 5.5 by the addition of tri-*n*-butylamine (7–8 ml). The precipitated zwitterion was collected, washed with cold ethanol (5 ml), and then dried to yield 3.60 g of product, mp 227–231° dec. This off-white powder was purified by dissolving it in 2.5 *N* HCl (15 ml) and diluting the solution with ethanol (25 ml). After stirring with charcoal (0.36 g), the mixture was filtered, and the pH of the filtrate was adjusted to 5.5 by slow addition of tri-*n*-butylamine. The white microcrystalline product was filtered, washed with ethanol (7 ml), and then dried to give 3.00 g of pure **15**.

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(8) T. Curtius and T. S. Hofman, *J. Prakt. Chem.*, [2] **53**, 524 (1896).
(9) From Regis Chemical Co., Chicago, Ill.
(10) C. Gränacher, M. Gerö, and V. Schelling, *Helv. Chim. Acta*, **7**, 577 (1924).