aminophenyl)carbodiimide and heated for 2 hr. The dioxane was removed under reduced pressure. The residue was crystallized from dilute ethanol; yield 850 mg (38%), mp 242° dec.

Anal. Calcd for $C_{24}H_{27}N_7O_2S$: C, 60.35; H, 5.69. Found: C, 60.62; H, 5.95.

3-(3,4-Dicyclohexyliminosemicarbazido)-1,2-benzisothiazole 1,1-dioxide was prepared by the procedure above; yield 46%, mp 238°.

Anal. Calcd for $C_{20}H_{29}N_5O_2S$: C, 59.52; H, 7.21; N, 17.35. Found: C, 60.21; H, 7.71; N, 16.79.

Acknowledgment.—We wish to express our thanks to our colleagues in microanalysis, physical chemistry, and pharmacology, who obtained the data referred to in this paper.

Hypotensive 1,2-Benzisothiazole 1,1-Dioxides. III. 3-[2-(2-Methyl-2-butylidene)hydrazino]-1,2-benzisothiazole 1,1-Dioxide and Related Analogs

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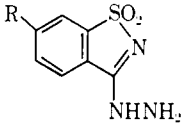
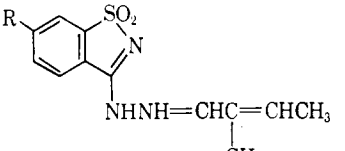
A series of 1,2-benzisothiazole 1,1-dioxides have been prepared, some of which produce significant hypotensive activity. One of the members of this series, 3-[2-(2-methyl-2-butylidene)hydrazino]-1,2-benzisothiazole 1,1-dioxide (III), produces significant hypotensive activity in unanesthetized animals. A consistent observation for active members of the series has been the erratic hypotensive activity in renal hypertensive rats. *In vivo* and *in vitro* activity indicates a mechanism of action other than a direct effect on the nervous systems or the vascular smooth muscle.

In the preceding papers a series of 1,2-benzisothiazole 1,1-dioxides has been reported.[1,2] Many of the compounds in the series exhibited significant hypotensive activity; however, 3-[2-(2-methyl-2-butylidene)hydrazino]-1,2-benzisothiazole 1,1-dioxide (III) was given preference for extensive pharmacological study primarily because of its potent and relatively reproducible activity in a large number of pharmacological test preparations.



I, R = H
II, R = Cl



III, R = H
IV, R = Cl

Experimental Section

Pharmacological Methods. Hypertensive Rats.—Compound III was administered orally (20 mg/kg) to groups of three renal[3] and three steroid (DOCA)[4] hypertensive rats. Blood pressures were determined during the day by the method of Friedman and Freed[5] before and at 1, 2, 3, 5, 6, and 7 hr following drug administration. Because of the increased activity of III with daily administration, the drug was given, and blood pressures were determined, on at least 5 consecutive days. The hypotensive response to III was also measured during 19 days of continuous administration of the drug. The blood pressures from the three rats in each group were averaged at each hour and over the 7-hr interval. The results were expressed as a per cent decrease from control at the hour of maximum decrease and for the 7-hr period. Other compounds from the series evaluated in the same animal preparation include 3-hydrazino-1,2-benzisothiazole 1,1-dioxide (I), which is the starting material for preparation of III, 6-chloro-3-hydrazino-1,2-benzisothiazole 1,1-dioxide (II), and 6-chloro-3-[2-(2-methyl-2-butylidene)hydrazino]-1,2-benzisothiazole 1,1-dioxide (IV).

Hypertensive Dogs.—Two dogs were selected from a colony of renal hypertensive dogs made hypertensive by a modification of the methods of Goldblatt, *et al.*,[6] and Page.[7] In dog ZH-114 the left kidney was removed and the right kidney was wrapped in silk. About six months later the right renal artery was constricted by a Goldblatt clamp. In dog ZH-124 both renal arteries were partially occluded with a Goldblatt clamp. These clamps were tightened eight months following the initial surgery. Following femoral arterial puncture blood pressure observations were made before and at 1, 3, and 6 hr after oral drug administration. Each dog received 10 mg/kg of III for 5 consecutive days followed by a rest period of 9 days at the end of which time medication was was resumed at a dose of 20 mg/kg for 5 days. Postmedication pressures were taken to measure the extent of delayed activity.

Hemodynamic Studies.—Two normotensive dogs were anesthetized with sodium phenobarbital (150 mg/kg) intravenously. The trachea was intubated for artificial respiration and the thoracic cavity was opened for exposure of the ascending aorta. A probe from an electromagnetic blood flow meter was positioned on the aorta for cardiac output measurement. The carotid artery was cannulated and pressure was determined by transducer measurement. Lead II of the electrocardiogram was monitored for heart rate. Stroke volume was calculated from the cardiac output and heart rate. Peripheral vascular resistance (PVR) was derived by the following formula. These values were

$$\text{Total PVR} = \frac{\text{arterial blood pressure (mm)} \times 100}{\text{cardiac output (ml/min)}}$$

measured and calculated before and at 30-min intervals following intravenous administration of 10 mg/kg of III.

Sympathetic Postganglionic Nerve Stimulation.—In three normotensive phenobarbital-anesthetized (150 mg/kg) dogs the thoracic cavity was opened and the cardiac-accelerator nerve originating from the stellate ganglion was dissected free. Control stimulation (14 v, 20 cps with a 0.5-msec duration for 30 sec) of the sympathetic postganglionic nerve fibers produced cardiac acceleration that was compared to postdrug neural-induced tachycardia.[8] In addition, the per cent change in heart rate was

(1) J. J. Traverso, C. W. Whitehead, J. F. Bell, H. Boaz, and P. W. Willard, *J. Med. Chem.*, **10**, 840 (1967).

(2) C. W. Whitehead, J. J. Traverso, J. F. Bell, and P. W. Willard, *ibid.*, **10**, 844 (1967).

(3) G. F. Kemph and I. H. Page, *J. Lab. Clin. Med.*, **27**, 1192 (1942).

(4) H. Selye, *J. Clin. Endocrinol.*, **6**, 117 (1946).

(5) M. Friedman and S. C. Freed, *Proc. Soc. Exptl. Biol. Med.*, **70**, 670 (1949).

(6) H. Goldblatt, J. Lynch, R. R. Hanzal, and W. W. Summerville, *J. Exptl. Med.*, **59**, 347 (1934).

(7) I. H. Page, *Science*, **89**, 273 (1939).

(8) D. C. Harrison, C. A. Chidsey, and E. Braunwald, *Proc. Soc. Exptl. Biol. Med.*, **112**, 37 (1963).

compared to the response obtained when guanethidine or saline was administered to groups of three animals each. All drugs were given at 5 mg/kg iv.

Sympathetic Preganglionic Nerve Stimulation.—Two normotensive cats were anesthetized with chloralose (50 mg/kg iv). The left nictitating membrane was attached to an ink-writing system for kymographic recording. The cervical sympathetic nerve was exposed for stimulation (10 v, 20 cps with a 0.5-msec duration for 30 sec) by a Grass stimulator. Contraction of the nictitating membrane and carotid blood pressure measurements were recorded before and at 30-min intervals following the intravenous administration of III (5 mg/kg).

Results and Discussion

Hypertensive Rats.—Both renal and steroid hypertensive rats responded to III when administered orally (20 mg/kg) (Figure 1). The renal hypertensive rats' response was better following chronic administration of the drug than was the steroid rats' response. In the renal rats, III produced a greater response with chronic administration. No tolerance has been observed with this compound.

Compound I had borderline activity when administered orally to renal hypertensive rats, 3.0–9.0% (Table I). Any hypotensive activity less than 5–6% is considered insignificant, since this is the magnitude of variation observed with saline controls.[9] Substituting chlorine in the 6 position of I (II) increased the activity considerably, 12–28%. Substituting chlorine in the 6 position of III (IV) did not increase the hypotensive activity significantly over the hypotensive activity of III. Thus, the addition of chlorine at position 6 of I appears to be more effective in increasing hypotensive activity, than it is with a similar change of III.

Table I

Hypotensive Activity of Three 1,2-Benzisothiazole 1,1-Dioxides[a]

	I		II		IV	
Days	X	M	X	M	X	M
1	3.7	11.2	12.2	17.9	9.9	15.7
2	8.1	14.5	15.8	22.0	15.2	20.7
3	*b*		20.4	25.1	16.3	23.0
4	*b*		21.6	28.5	19.9	24.1
5	9.0	15.9	27.8	32.4	20.2	25.0

[a] X = mean value for per cent decrease of blood pressure over a 7-hr period for three renal hypertensive rats. M = maximum depression of blood pressure during a 7-hr test period. [b] Animals were dosed, but no pressures were taken.

Hypertensive Dogs.—The results on dogs ZH-114 and ZH-124 are shown in Figures 2 and 3. During both treatment periods there was a fall in blood pressure following each daily dose with a gradual decrease in control blood pressure. With the 10-mg/kg dose there appeared to be an increasing hypotensive response with successive doses (Figure 2). The hypotensive response did not increase with the 20-mg/kg dose probably because the maximum dose range had been exceeded. An increase in heart rate did not accompany the blood pressure decrease with either dose of drug.

Hemodynamic Studies.—In phenobarbital-anesthetized dogs, when III was administered at 10 mg/kg iv, the heart rate increased at 3, 4, and 5 hr (Figure 4). The

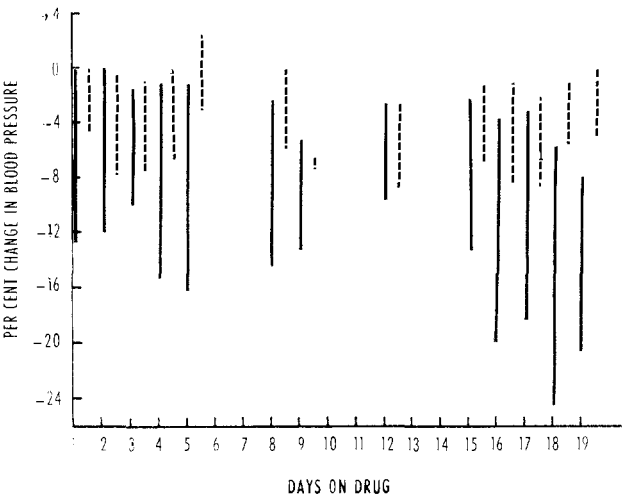


Figure 1.—The top of each solid and broken line represents daily control mean blood pressure determinations on groups of three renal (———) and three steroid (– – – –) hypertensive rats. Zero per cent change in blood pressure was established from the control data on the first day. The length of the lines represents the mean per cent blood pressure decrease from control during a 7-hr period following the daily oral administration of III at 20 mg/kg. The animals were dosed daily for 19 days regardless of whether blood pressures were determined.

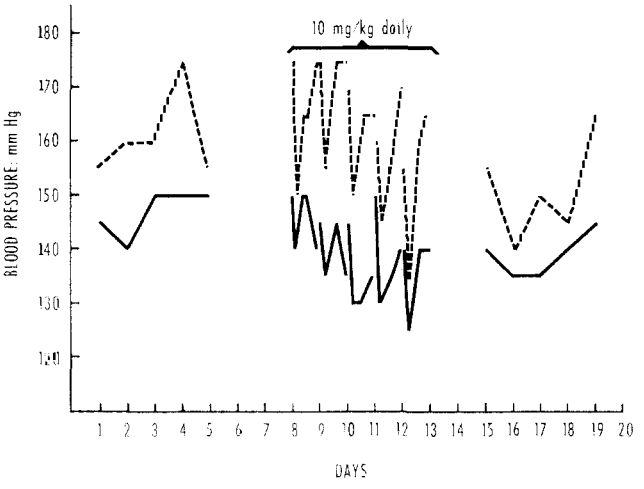


Figure 2.—Blood pressure response of two renal hypertensive dogs (dog ZH-114 and dog ZH-124) to the oral administration of III (for 5 days at 10 mg/kg). Blood pressures were determined for 5 days previous to and 5 days following drug administration.

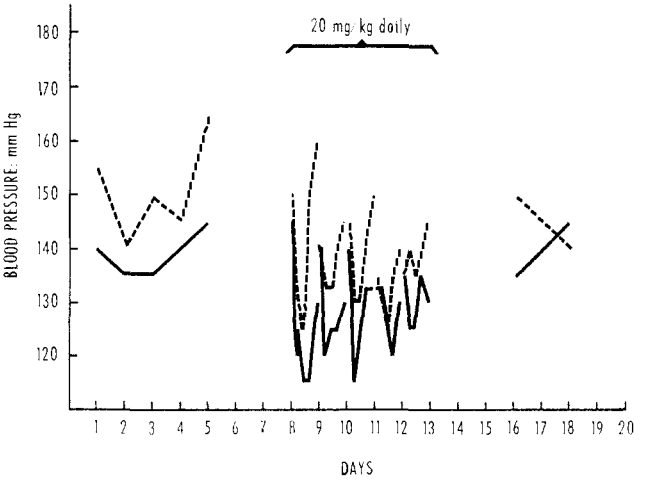


Figure 3.—Blood pressure activity using the same animals and procedure as in Figure 2 following the oral administration of III at 20 mg/kg.

(9) P. W. Willard, C. E. Powell, and F. G. Henderson, *Proc. Soc. Exptl. Biol. Med.*, **115**, 785 (1964).

depressor response was maximal at about 2 hr (30%) with a return to control values at 4 hr. The stroke volume (Figure 4) did not change from control values, which indicates that III probably did not lower blood pressure acutely by depressing cardiac activity. The cardiac output increased (Figure 5) as a result of the elevation in heart rate. The hypotensive effect was, therefore, associated with a decrease in peripheral vascular resistance and not as a result of any cardiac depressant activity.

Sympathetic Nerve Stimulation.—In the dog the cardioaccelerator nerve was not blocked when stimulated post-ganglionically (Figure 6). The saline control and III curves are parallel, which indicates no drug-induced block. Guanethidine does block the postganglionic sympathetic nerve endings and in less than 1 hr after drug administration. A hypotensive dose of III did not block the contraction of the cat nictitating membrane induced by stimulation of the preganglionic cervical sympathetic nerve (Figure 7). Thus, III is not a ganglionic or an adrenergic neurone blocking drug.

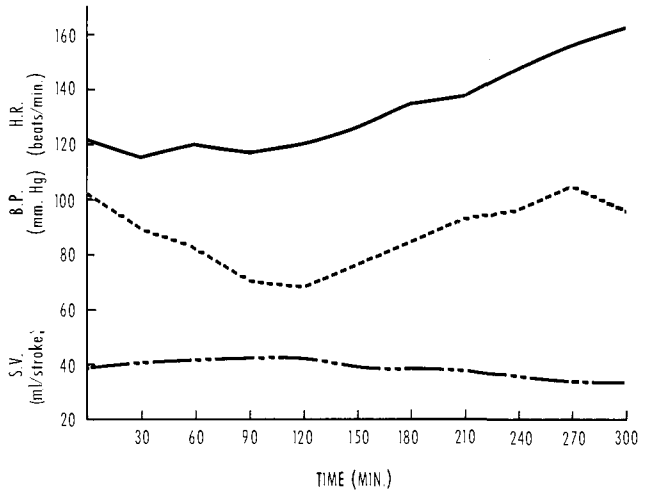


Figure 4.—Response of heart rate (HR), blood pressure (BP), and stroke volume (SV) following intravenous administration of 10 mg/kg of III to anesthetized dogs.

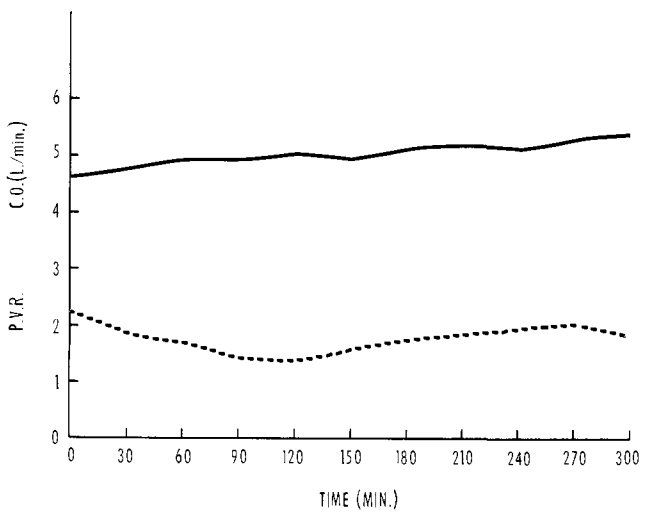


Figure 5.—Cardiac output (CO) and peripheral vascular resistance (PVR) after intravenous administration of III (10 mg/kg) to anesthetized dogs.

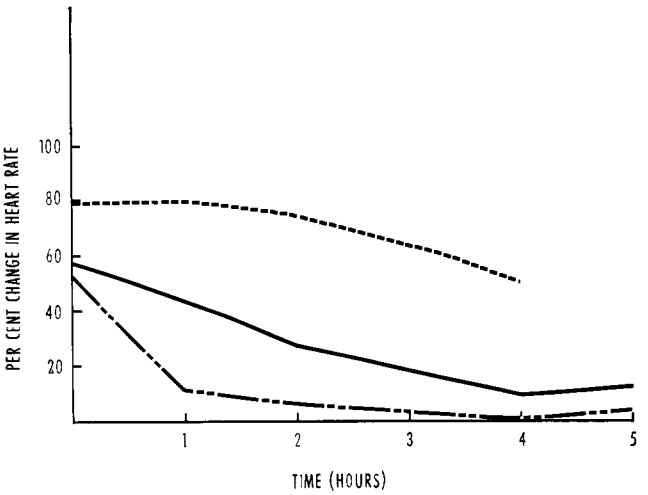


Figure 6.—A comparison of the effect of guanethidine (— — —), III (———), and saline control (– – – –) on change in heart rate following stimulation of the cardioaccelerator nerve in groups of three anesthetized dogs.

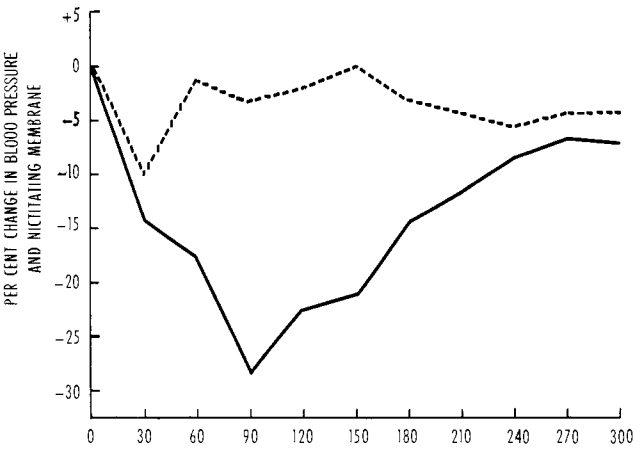


Figure 7.—Effect of III on blood pressure (———) and nictitating membrane response (– – – –) in chloralose–ether-anesthetized cats.

Other Pharmacological Evaluation.—Compound III did not have any direct action on strips of rabbit ileum suspended in Tyrode's solution.[10] Concentrations as large as 2.0 mg/10 ml did not alter the normal rhythm, amplitude, or tone of the isolated rabbit ileum. With doses of 1.0 μg/10 ml of epinephrine, III (80 μg/10 ml) blocked the contractile activity of the catecholamine 50% on the rabbit uterus.

Monoamine oxidase activity was determined spectrophotometrically[11] by measuring the absorbance decrease with kynuramine as substrate. Compound III did not inhibit monoamine oxidase in a concentration of $1 \times 10^{-4} M$. Using the same method, pargyline inhibits monoamine oxidase about 50% at a concentration of $1.7 \times 10^{-7} M$.

No diuretic activity or alteration in urinary Na/K ratios, as determined by the method of Lipschitz, *et al.*,[12] was observed when rats were dosed orally with 5, 50, or 100 mg/kg of III. Using the method of

(10) J. H. Gaddum, W. S. Peart, and M. Voight, *J. Physiol.* (London), **108**, 467 (1948).

(11) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

(12) W. L. Lipschitz, Z. Hadidian, and A. K. Kerpesar, *J. Pharmacol. Expl. Therap.*, **79**, 97 (1943).

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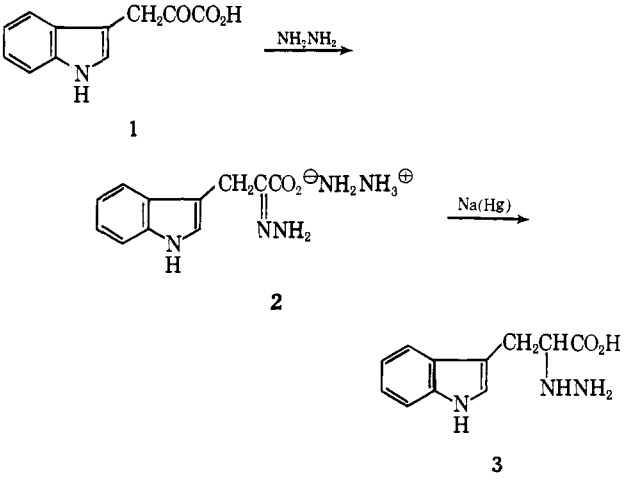
Three β-(3-indolyl)-α-hydrazinopropionic acids were synthesized and tested for *in vitro* and *in vivo* inhibition of DOPA decarboxylase. All were found to be highly active, and one of them, DL-β-(5-hydroxy-3-indolyl)-α-hydrazinopropionic acid, is among the most potent inhibitors of DOPA decarboxylase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