Imidazolecarboxyhydrazides. II. Chemistry and Biological Evaluation[1]

JAY NEMATOLLAHI AND J. R. NULU

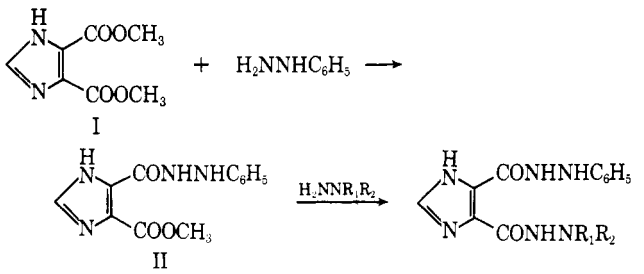
College of Pharmacy, University of Texas, Austin, Texas 78712

Received August 14, 1968

A series of imidazolecarboxyhydrazides, with two different hydrazide groups in the 4 and 5 positions of the imidazole ring, has been synthesized and evaluated biologically for their potential as possible psychopharmacological agents. As compared with isocarboxazid, the compounds were found to manifest MAO-inhibitory activity. The biological tests were performed on mice and consisted of reversal of reserpine-induced ptosis and hypothermia, and hexobarbital sleeping-time prolongation.

In an earlier paper[2] we have reported the synthesis and biological evaluation of some imidazolecarboxyhydrazides containing two identical hydrazide groups. This paper comprises the synthesis of analogous compounds with two different hydrazide groups in the 4 and 5 positions of the imidazole ring. A general route of synthesis of these compounds is depicted in Scheme I.

SCHEME I

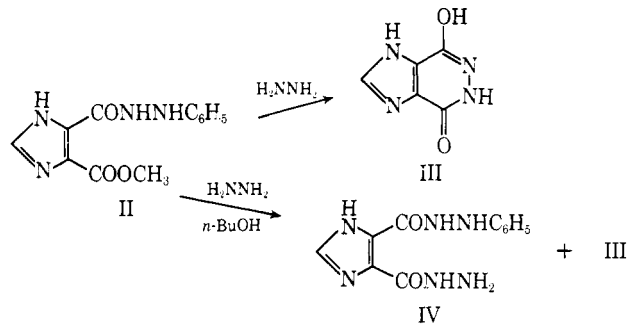


$R_1 = H, CH_3; R_2 = H, CH_3, CH_2C_6H_5, CH(CH_3)_2, =C(CH_3)_2, =CHC_6H_5$

The monoamine oxidase (MAO) inhibitory activity of these compounds was evaulated in mice by the three methods described under Pharmacology.

Chemistry.—The interaction of II with hydrazine, with no solvent present, gave a 1,4-dihydroxypyridazino[4,5-*d*]imidazole[3] (III) (Scheme II) whose structure

SCHEME II



was proved by comparing its ir spectrum with an authentic sample,[4] obtained by heating imidazole-4,5-dicarboxylic acid bis(hydrazide) with an excess of hydrazine. When II was allowed to react with hydrazine using *n*-BuOH as a solvent, two products were obtained, whose ratio was found to be dependent on the temperature at which the reaction occurs. One of the two products, a high-melting and less soluble substance, was identical with III. The identity of the second compound was determined by elemental analysis, ir, and nmr to be imidazole-4,5-dicarboxylic acid 2-phenylhydrazide hydrazide (IV). At an oil bath temperature of 105°, this reaction provided III and IV with an approximate ratio of 1:3. At 80°, only a small quantity of III was formed and IV was the major product. As a preliminary effort toward elucidation of the mechanism of formation of III, a solution of IV in *n*-BuOH was heated at 105° for 12 hr. A total isolation of IV from the reaction mixture indicated that IV was not an intermediate in the formation of III, but rather III was formed as a result of a one-step interaction between II and hydrazine.

The interaction of IV with acetone and benzaldehyde, individually, afforded imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2-isopropylidenehydrazide (V) and imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2-benzylidenehydrazide (VI), respectively. The presence of two CH_3 peaks, separated by 2.7 cps, in the nmr spectrum of V was ascribed to the *syn* and *anti* form of the hydrazone. According to a recent report,[5] the peak at δ 1.99 was assigned to *syn* and δ 1.94 to *anti*.

Catalytic hydrogenation of V provided imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2-isopropylhydrazide (VII). Compound VI, however, could not be reduced by this method. Imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2-benzylhydrazide (VIII) was synthesized by treating II with benzylhydrazine.

The reaction of methylhydrazine and II, directly or in the presence of a solvent, provided imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2-methylhydrazide (IX). The position of the methyl group was determined by comparison of the nmr spectrum of IX with an authentic sample[2] of imidazole-4,5-dicarboxylic acid bis(2-methylhydrazide) and imidazole-4,5-dicarboxylic acid bis(methylamide). The methyl peak for the latter compound was at about δ 2.9 as compared with δ 2.6 for IX, hence revealing the correctness of the assignment of structure IX to the reaction product. This result was further confirmed by the lack of reactivity of the compound with acetone, thus indicating the absence of an NH_2 group.

The reaction of II with 1,1-dimethylhydrazine provided imidazole-4,5-dicarboxylic acid 2-phenylhydrazide 2,2-dimethylhydrazide (X). Unlike the formation of the other hydrazides in the series, the rate of

(1) This investigation was supported in part by the University of Texas Research Institute Grant R-313.

(2) J. Nematollahi, W. Guess, and J. Autian, *J. Med. Chem.*, **9**, 660 (1966).

(3) Due to the presence of both OH and C=O bands in the ir spectrum of III, the molecule must exist partly in the keto and partly in the enol form.

(4) R. G. Jones, *J. Am. Chem. Soc.*, **78**, 159 (1956).

(5) G. Karabatsos and R. Taller, *Tetrahedron*, **24**, 3557 (1968).

formation of X was very slow. Steric effect, probably, is the major influencing factor in the rate of the formation of this compound.

In an attempt to synthesize carbamates and diazinoimidazoles, IV was treated with HNO_2 to provide imidazole-4,5-dicarboxylic acid 2-phenylhydrazide azide (XI). Due to the explosiveness of XI, no elemental analysis was performed, but its ir spectrum (2140 cm^{-1}) was indicative of an azide peak. This proof was further confirmed by the reactivity of XI with MeOH, providing a carbamate with a characteristic carbamate ir peak for C=O at 1700 cm^{-1} and nmr peak for CH_3 at δ 3.6. Further work on this part is under investigation.

Experimental Section

Melting points were determined in open capillary tubes in a Thomas–Hoover melting point apparatus and those below 230° were corrected. All evaporations were made *in vacuo* from rotatory evaporators. Ir spectra were determined in KBr disks with a Beckman IR 8 and nmr spectra with a Varian A-60 spectrophotometer at ambient temperature (TMS as reference). Elemental analyses were done by the Microanalytical Laboratory, Department of Chemistry, University of Texas at Austin, and in part by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide Hydrazide (IV).—To a suspension of 2 g (0.007 mole) of II in 100 ml of dry *n*-BuOH was added 2 g (0.07 mole) of dry $(H_2N)_2$. With the provision for exclusion of H_2O, the mixture was heated under reflux in an 80° oil bath. The heating was stopped after 10–12 hr. A small quantity of residue was filtered. The filtrate was concentrated to about 20 ml and then left at room temperature for 2 hr at which time a solid was separated. The solid was filtered and crystallized from 70% MeOH to give 1.2 g (60%) of IV, mp 214–216°. *Anal.* ($C_{11}H_{12}N_6O_2$) C, H, N.

Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide 2-Isopropylidenehydrazide (V).—A suspension of 1.3 g (0.005 mole) of IV in a mixture of 100 ml of Me_2CO and 30 ml of MeOH was heated under reflux for 8 hr. The suspension became a clear solution in the course of heating. The solution was evaporated to about 25 ml. A solid which had been precipitated was isolated and crystallized from 30% MeOH in H_2O to give 1.2 g (80%) of V, mp 254–256°. *Anal.* ($C_{14}H_{16}N_6O_2$) C, H, N.

Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide 2-Benzylidenehydrazide (VI).—To 0.65 g (0.0025 mole) of IV was added 10 ml of benzaldehyde. The mixture was heated under N_2 for 10 hr under reflux, with a condenser connected to a Hg bubbler. Upon completion of the reaction, excess benzaldehyde was distilled off at 0.5 mm. The crude product was washed three times with Et_2O and then crystallized from 50% MeOH in H_2O to give 0.65 g (75%) of VI, mp 258–261°. *Anal.* ($C_{18}H_{16}N_6O_2$) C, N, H: calcd, 4.63; found, 5.17.

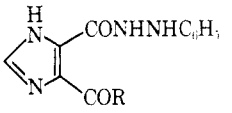
Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide 2-Benzylhydrazide (VIII).—To 25 ml of dry *n*-BuOH was added 0.8 g (0.02 g-atom) of potassium. After dissolving K by heating, 1.95 g (0.01 mole) of benzylhydrazine dihydrochloride was added. The mixture was heated for about 5 min, filtered into a flask containing 1.3 g (0.005 mole) of II, and then heated under reflux for 24 hr. The solid which had been separated was filtered and crystallized (MeOH) to give 0.7 g (40%) of VIII,[6] mp 191–194°. *Anal.* ($C_{18}H_{18}N_6O_2 \cdot H_2O$) C, H, N: calcd, 22.81; found, 23.67.

Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide 2-Methylhydrazide (IX).—A mixture of 1.04 g (0.004 mole) of II and 0.94 g (0.02 mole) of methylhydrazine was heated under reflux in an 85° bath. After cooling to room temperature, the mixture was washed twice (Et_2O). To the residue was added 5 ml of MeOH followed by C_6H_6 (30 ml). A solid which separated after 1 hr was crystallized from 30% MeOH–H_2O to give 0.65 g (60%) of IX, mp 212–214°. *Anal.* ($C_{12}H_{14}N_6O_2$) C, H, N.

Imidazole-4,5-dicarboxylic Acid 2-Phenylhydrazide 2,2-Dimethylhydrazide (X).—To 1.56 g (0.006 moles) of II was added 1.16 g (1.02 moles) of 1,1-dimethylhydrazine. The mixture was heated under reflux in an 85° oil bath for 55 hr. The resulting solid was washed three times with Et_2O and then dried in a vacuum desiccator to give 1.6 g (92%) of X.[7] Part of X was crystallized from Me_2CO containing a small quantity of MeOH for elemental analysis, mp 233–236°. *Anal.* ($C_{13}H_{16}N_6O_2$) C, H.

Pharmacology. MAO Inhibition.—The compounds listed in Table I were evaluated for MAO-inhibitory activity in mice by the method of Aceto and Harris.[8] The effect of the test compounds on reserpine-induced hypothermia was carried out according to the method of Garattini, *et al.*[9]

TABLE I



Compd	R	Oral dose, mmole/kg	Act. prior to reserpine	Mean ptotic score	Total sleep time, min	Effect of compounds on reserpine-induced hypothermia, body temp, °C ± SE, Hr after administrn 0	4	6	24
Control				3.75	8.7 ± 0.8	35.8 ± 0.8	28.7 ± 1.2	26.3 ± 0.2	26.6 ± 1.4
IV	$NHNH_2$	0.275	Increased	1.25	14.5 ± 1.6	36.1 ± 0.1	32.5 ± 0.1	30.3 ± 1.2	32.8 ± 2.1
V	$NHN{=}C(CH_3)_2$	0.275	Increased	1.40	12.2 ± 2.0	34.8 ± 0.4	32.8 ± 0.3	31.6 ± 0.8	32.7 ± 1.5
VI	$NHN{=}CHC_6H_5$	0.275	Increased	1.25	11.4 ± 1.8	34.7 ± 1.0	32.9 ± 0.6	32.6 ± 0.7	34.2 ± 1.3
VII	$NHNHCH(CH_3)_2$	0.275	Increased	1.25	8.9 ± 1.0	35.9 ± 0.7	33.4 ± 0.8	32.0 ± 0.8	33.2 ± 0.3
VIII	$NHNHCH_2C_6H_5$	0.275	Increased	1.12	19.2 ± 2.9	35.3 ± 0.5	32.8 ± 1.6	31.5 ± 0.7	31.3 ± 1.6
IX	$NHNHCH_3$	0.275	Increased	1.50	19.9 ± 0.7	36.0 ± 0.9	32.5 ± 1.5	29.8 ± 2.1	30.7 ± 0.4
X	$NHN(CH_3)_2$	0.275	Increased	1.12	20.1 ± 2.0	35.4 ± 1.2	32.8 ± 0.5	33.1 ± 0.9	32.2 ± 1.5
Isocarboxazid		0.1375	Increased	0.50	10.8 ± 1.5	35.6 ± 1.1	34.2 ± 0.2	34.7 ± 0.9	35.2 ± 0.9

Male albino mice weighing 20–35 g were used for the experiment. Animals were divided into groups of six. Due to the lack of solubility of the test compounds, they were administered orally as suspensions in 3% gum acacia in H_2O. For each experiment, mice were withdrawn from food for 24 hr prior to oral intubation, while H_2O was available *ad libitum*. All test compounds were given at a dose of 0.275 mmole/kg. The mice were observed for general signs of activity for 2 hr after the administration of the compounds. Reserpine was administered at a dose level of 2.0 mg/kg ip and allowed to remain undisturbed for the next 3 hr. Each mouse was then evaluated as to the degree of ptosis by two individuals. A scoring system adopted by Rubin, *et al.*,[10] was used. Scores of 4, 3, 2, and 1 were adopted for complete, three-quarters, one-half, and one-quarter closure of the eyelids, respectively. A nonptotic response or normal opening was scored zero. The results of the ratings were compared

(6) Some hydrazides are notorious for giving erroneous elemental analyses. Spectroscopic analysis, in particular nmr, indicates the correctness of structure VIII.

(7) Because of difficulty in crystallization of X, only C and H analyses were performed.

(8) M. D. Aceto and L. S. Harris, *J. Toxicol. Appl. Pharmacol.*, **7**, 329 (1965).

(9) S. Garattini, A. Giachetti, A. Jori, L. Pieri, and L. Valzelli, *J. Pharm. Pharmacol.*, **14**, 509 (1962).

(10) B. Rubin, M. H. Malone, M. H. Waugh, and J. C. Burke, *J. Pharmacol. Exptl. Therap.*, **120**, 125 (1957).

with control animals receiving only reserpine and the vehicle. Isocarboxazid, a therapeutically known MAO inhibitor, was administered for comparison of activity. Similarly, reversal of reserpine-induced hypothermia was conducted. The temperature of the animals was observed at 0, 4, 6, and 24 hr after the administration of reserpine.

Sleeping Time Potentiation.—Most of the MAO inhibitor drugs prolong sleeping time induced by hexobarbital. Groups of six animals were chosen for testing of each compound. Each animal was given 0.275 mmole/kg of each compound except isocarboxazid which was given 0.137 mmole/kg. Two hours after the administration of the compound, hexobarbital was administered (55 mg/kg ip) and sleeping time was recorded as the time from administration of hexobarbital until the mice regained the ability to return to a righted position three times within 20 sec.

Results and Discussion

The results of three pharmacological tests for biological evaluation of compounds listed in Table I indicate that MAO-inhibitory activity of these hydrazides is significantly greater than was reported in the earlier paper.[2] The increase in activity probably could be ascribed to the less symmetric nature of these molecules which render them more soluble. It is also possible that two different sites of action may exist for two different types of hydrazine moieties, thus giving rise to potentiation of biological activity.

A direct relationship of MAO-inhibitory activity of a hydrazide, as tested by reversal of reserpine-induced ptosis or hypothermia, to hexobarbital sleeping time prolongation has been postulated in the past. Such correlation, however, could not be substantiated by the results of our experiments; in the ptosis and hypothermia tests isocarboxazid was revealed to be more active than the compounds listed in Table I, but it did not cause a significant prolongation of hexobarbital-induced sleeping time.

As shown in Table I, the hydrazides with methyl or benzyl substituents manifest greater prolongation of hexobarbital-induced sleeping time than their unsubstituted analogs. Such higher activity, however, is not observed when mean ptotic scores or hypothermia test results were compared. At the present, no concrete reason can be given for such results.

Apparently more analogs in the series are needed to arrive at a proper conclusion regarding the relationship of these compounds to their MAO-inhibitory activity and antidepressant property.

Compounds with Potential Enzyme Inhibitory Activity. Hydroxylamine Analogs of 2-Propynylamine[1a]

DAVID H. MOORE, JOSEPH G. CANNON,[1b]

Division of Medicinal Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52240

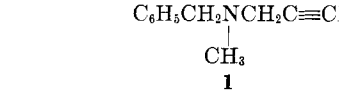
WILLIAM M. MCISAAC, AND BENG T. HO

Biological Research Division, Texas Research Institute of Mental Sciences, Houston, Texas 77025

Received July 5, 1968

As a part of a study of structure–activity relationships of compounds possessing monoamine oxidase inhibitory and/or 5-hydroxytryptophan decarboxylase inhibitory activity, a series of O- or N-2-propynylhydroxylamines structurally related to pargyline has been prepared. Substituents on the hydroxylamine systems were chosen from those which have been shown to be significant in the amine series. Biological test data indicate that certain of the compounds are active enzyme inhibitors.

Interest in organic hydroxylamines and in acetylenic amines has increased in recent years as a result of numerous reports of potent biological activity ascribable to these functional groups. Specifically, compounds of these types have been found to be inhibitors of dopamine β-oxidase,[2] 5-hydroxytryptophan decarboxylase (5-HTP decarboxylase),[3] and of monoamine oxidases (MAO).[4-9] Swett, *et al.*,[6] described structure–activity relationships in the pargyline (N-benzyl-N-methyl-2-propynylamine, **1**) series, indicating the necessity of the N-2-propynyl group for MAO inhibitory activity. Reports[7] of MAO inhibition by N-alkyl-N-methyl-2-propynylamines suggest that the aromatic ring of pargyline may not be essential for activity.



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In the present work, efforts were directed toward synthesis of the three isomeric tertiary hydroxylamines (**4**, **5**, **8**) which contain the benzyl, methyl, and 2-propynyl groups as in pargyline; the isomeric secondary hydroxylamines (**3**, **7**) which bear the benzyl and 2-propynyl groups; **9** and **10** which contain methyl and 2-propynyl groups; **2** and **6** which contain benzyl and

(1) (a) This investigation was supported in part by Grant GM-10753 from the National Institute of General Medical Sciences, by Grants MH-11168 and MH-12959 from the National Institute of Mental Health, and by the Britton Fund. Abstracted in part from a thesis submitted by D. H. M. (N.D.E.A. Title IV Predoctoral Fellow, 1963–1966) in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Iowa, 1968. (b) To whom all correspondence should be addressed.

(2) J. B. Van der Schoot, C. R. Creveling, T. Nagatsu, and S. Udenfriend, *J. Pharmacol. Exp. Ther.*, **141**, 74 (1963).

(3) E. L. Schumann, R. V. Heinzelman, M. E. Greig, and W. Veldkamp, *J. Med. Chem.*, **7**, 329 (1964).

(4) F. Benington, R. D. Morin, and L. C. Clark, Jr., *ibid.*, **8**, 100 (1965).

(5) A. L. Green, *Biochem. Pharmacol.*, **13**, 249 (1964).

(6) L. R. Swett, W. B. Martin, J. D. Taylor, G. M. Everett, A. A. Wykes, and Y. C. Gladish, *Ann. N. Y. Acad. Sci.*, **107**, 891 (1963).

(7) J. R. Boissier, R. Ratouis, C. Dumont, and J. Lesbros, *Chim. Ther.*, 320 (1966).

(8) C. F. Huebner, E. M. Donoghue, A. J. Plumner, and P. A. Furness, *J. Med. Chem.*, **9**, 830 (1966).

(9) J. Knoll, Z. Esceri, K. Keleman, J. Nievel, and B. Knoll, *Arch. Int. Pharmacodyn. Ther.*, **155**, 154 (1965).