Imidazolecarboxhydrazides. I. Chemistry and Biological Evaluation[1]

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Received April 19, 1966

Interaction of dimethyl imidazole-4,5-dicarboxylate with hydrazine and some alkyl- and aryl-substituted hydrazines provided a series of imidazole acid hydrazides. The molecular structure of these compounds was elucidated by chemical and/or spectroscopic methods. Most of these compounds together with some intermediates were screened for monoamine oxidase (MAO) inhibitory activity. Under our test conditions, two of these compounds were found to be significantly active for inhibiting the enzyme. They were imidazole-4,5-dicarboxylic acid 1-methylhydrazide 2-methylhydrazide (IV) and 1-methylimidazole-4,5-dicarboxylic acid dihydrazide (XII).

Since the discovery of biological activity of isonicotinic acid hydrazide[2,3] and its therapeutic use as an antituberculous and antidepressant agent, numerous carboxylic acid hydrazides[4-6] have been synthesized for biological evaluation. At the present, there are a few heterocyclic acid hydrazides that are potential monoamine oxidase (MAO) inhibitors and are being utilized in medicine.

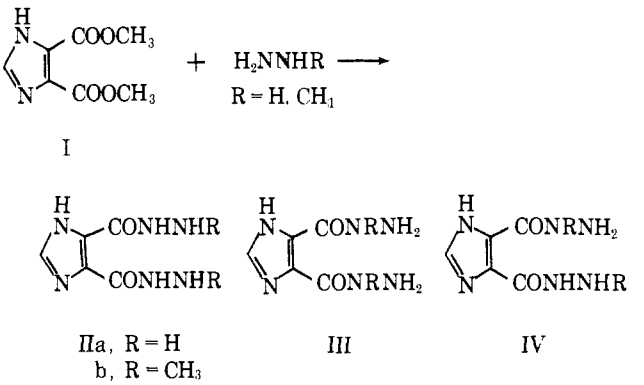
Our interest in the synthesis of imidazole-4,5-dicarboxylic acid hydrazides was for their value as possible intermediates in the synthesis of bicyclic pyridazine- and imidazole-containing systems. However, also of chief interest was a study on the chemistry of these hydrazides and their biological properties. The present paper is a report on the research carried out in the chemistry of these acid hydrazides and screening methods for determining their MAO inhibitory properties. Attempts were also made to relate the variation in the biological activity as a function of chemical structure.

Chemistry.—A quantitative yield of imidazole-4,5-dicarboxylic acid dihydrazide[7] (IIa), obtained at room temperature as a result of the interaction of dimethyl imidazole-4,5-dicarboxylate (I) and hydrazine, suggested that possibly I could be employed for the preparation of a series of substituted acid hydrazides desirable for our study.

The reaction of monosubstituted hydrazine with I, where the formation of positional isomers was possible, advanced the idea for studying the factors influencing the ratio of these isomers in a given reaction and their relative physical and biological properties.

The interaction of methylhydrazine with I in methanol has been attempted[8] and assumed to give only IIb in a low yield without proof of the structural formula, which also could conceivably be III or IV (Chart I). Our investigation, however, revealed that the reaction product was one compound in the absence of a solvent and two in the presence of a solvent such as 1-butanol. Preliminary study of these products by elemental analysis and infrared spectroscopy showed that they were dihydrazides of type II, III, or IV, and that the high-melting isomer, which was obtained in the presence of a solvent, was identical with the product obtained in the absence of a solvent. Attempts were made to identify these compounds by converting them to the corresponding amides by cleaving the nitrogen-nitrogen bond with Raney nickel, by the method reported by Ainsworth,[9] and comparing these amides with the authentic samples obtained as a result of reacting I with methylamine or ammonia. This method, however, was not successful; neither a product nor the starting compound could be isolated from the Raney nickel–ethanol slurry. This failure might be due to the formation of an imidazole acid hydrazide–nickel complex. Nmr spectroscopy was employed next for the elucidation of the structure of these isomers. The structure imidazole-4,5-dicarboxylic acid 1-methylhydrazide 2-methylhydrazide (IV) was assigned to the low-melting, water-soluble isomer because its nmr spectrum revealed the presence of two resonance peaks for methyl protons at δ 3.6 and 2.9. Due to the deshielding influence of the carbonyl group, these resonance peaks were assigned to methyl groups next to and away from the carbonyl group, respectively. Comparison of the position of these resonance peaks with the single resonance peak at δ 3.0 for the methyl protons of the high-melting isomer established the structure of this compound to be imidazole-4,5-dicarboxylic acid bis(2-methylhydrazide) (IIb) rather than III. A further confirmation of the molecular structure of IIb was accomplished by treating it with acetone. Unlike the reaction of IIa, which with acetone yields a dihydrazone, only the starting

CHART I



(1) This investigation was supported in part by research Grant CA-06120 from the National Cancer Institute, Bethesda, Md.

(2) E. A. Zeller, J. Barsky, J. R. Fouts, W. F. Kirchheimer, and L. S. Van Orden, *Experienta*, **8**, 349 (1952).

(3) B. B. Brodie, A. Pletscher, and P. A. Shore, *J. Pharmacol. Exptl. Therap.*, **116**, 9 (1956).

(4) H. L. Yale, K. Losee, J. Martins, M. Holsing, F. M. Perry, and J. Bernstein, *J. Am. Chem. Soc.*, **75**, 1933 (1953).

(5) P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedus, and O. Straub, *Ann. N. Y. Acad. Sci.*, **80**, 555 (1959).

(6) T. S. Gardner, E. Wenis, and J. Lee, *J. Med. Pharm. Chem.*, **2**, 133 (1960).

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

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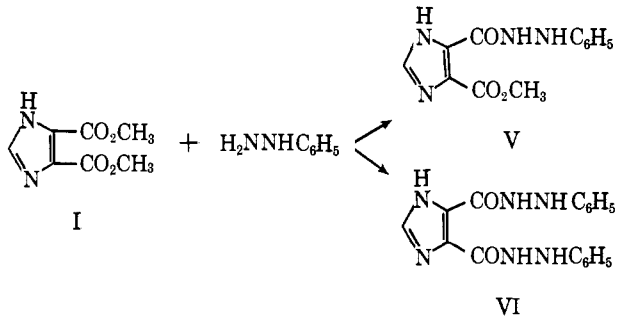
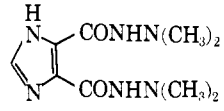
compound was obtained after heating IIb with acetone for 48 hr.

In a like manner, the reaction of I with phenylhydrazine was examined and found to be inconsistent with the literature report.[8] The reaction product in the absence of a solvent was not analogous to the methyl derivatives IIb, III, or IV, but rather a high yield of a half-ester, which by chemical and physical methods, discussed for VI, proved to be V (Chart II). However, when the reaction was carried out in the presence of 1-butanol, the major product was imidazole-4,5-dicarboxylic acid bis(2-phenylhydrazide) (VI) although a very small quantity of V, together with another substance which was not identified, was also isolated. The assignment of the structure VI, to the major product of the reaction was made first on the basis of its infrared and nmr spectra. A single resonance peak for phenyl protons at δ 6.9 indicated the existence of equivalent magnetic environments for phenyl rings. This assignment was further substantiated by treating the product with acetone analogous to the treatment given to methyl derivative IIb. In this case, likewise, no hydrazone was formed and only the starting compound was isolated. Similarly, the molecular structure of V was proved.

CHART II



The reaction of 1,1-dimethylhydrazine with I occurred under a somewhat strenuous condition to give imidazole-4,5-dicarboxylic acid bis(2,2-dimethylhydrazide) (VII). The low rate of formation of VII could

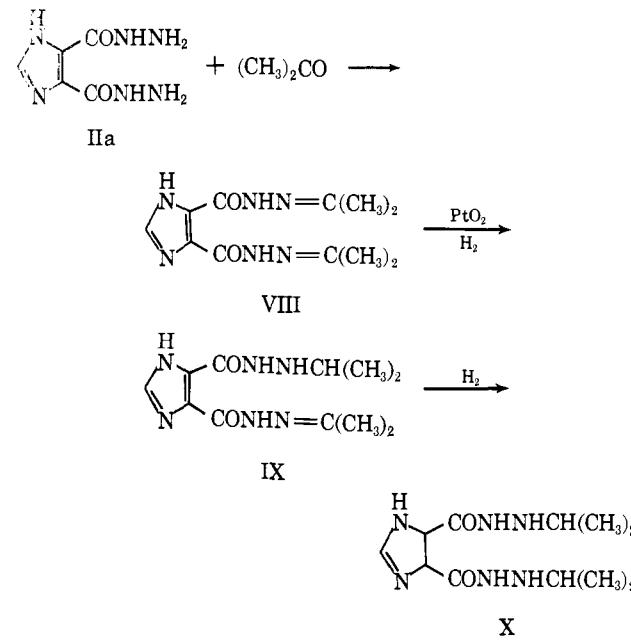


VII

possibly be ascribed to the influence of the steric hindrance. The steric factors together with a relative lessening of hydrogen bonding in the molecule might be the elements which contribute to the relative water solubility and low melting point of VII as compared with the other structures in the series.

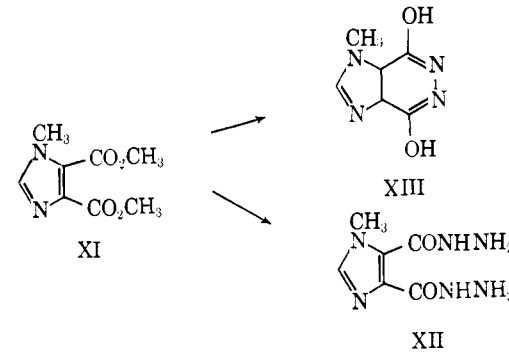
Because of pronounced biological properties manifested by some aliphatic and heterocyclic acid isopropylhydrazides,[6,10] it was also of interest to prepare imidazole-4,5-dicarboxylic acid bis(2-isopropylhydrazide) (X). Due to the difficulty in obtaining isopropylhydrazine, synthesis of the intermediate imidazole-4,5-dicarboxylic acid bis(2-isopropylidenehydrazide) (VIII) as a possible route for the synthesis of X was explored. Prolonged heating of IIa in acetone and methanol afforded VIII, and catalytic hydrogenation of VIII at high temperatures afforded X. The isolation of a half-hydrogenated product, imidazole-4,5-dicarboxylic acid 2-isopropylidenehydrazide 2-isopropylhydrazide (IX), after a short period of hydrogenation, suggested that this reduction occurs stepwise, according to Chart III.

CHART III



It was desirable to synthesize 1-methylimidazole-4,5-dicarboxylic acid dihydrazide (XII) for its value as an intermediate in the synthesis of some heterocyclics and also for the purpose of comparison of its physical and biological properties with IIa. Interaction of hydrazine with diethyl 1-methylimidazole-4,5-dicarboxylate has been reported by Jones[7] to yield only 1,4-dihydroxy-5-methylpyridazino[4,5-*d*]imidazole (XIII). Exploration of the possibility of synthesizing XII revealed that, by adding hydrazine to dimethyl 1-methylimidazole-4,5-dicarboxylate[11] (XI), XII could be obtained without any difficulty (Chart IV). Unlike IIa, XII is a relatively low-melting, water-soluble compound and manifests pronounced biological activity.

CHART IV



(10) F. H. McMillan, F. Leonard, R. I. Meltzer, and J. A. King, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 457 (1953).

(11) R. A. Baxter and F. S. Spring, *J. Chem. Soc.*, 232 (1945).

The interaction of nitrous acid with most of these acid hydrazides yielded 1-nitroso derivative. Although no product was obtained when XII was treated with nitrous acid, the interaction of IIa and nitrous acid afforded a product whose infrared spectrum indicated the formation of an azide. Due to extreme explosiveness of this substance, the elucidation of its molecular structure has been deferred to a later date.

Experimental Section

Melting points were determined in capillary tubes on a Thomas–Hoover capillary apparatus and those below 250° were corrected. All evaporations were made *in vacuo* from rotatory evaporators. Infrared spectra were determined in KBr disks with a Beckman IR5 and Perkin-Elmer 337 spectrophotometer, and nmr spectra with Varian A-60 spectrophotometer at ambient temperature. Chemical shifts are reported in parts per million, δ, downfield from tetramethylsilane. Thin layer chromatography (tlc) was run on Brinkmann silica gel HF 254 and HF 254 + 366 with various mixtures of solvents; spots were located by visual examination under a short wave ultraviolet light. Elemental analyses were done by the Microanalytical Laboratory, Department of Chemistry, University of Texas, Austin, Texas, and in part by Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

Imidazole-4,5-dicarboxylic Acid Dihydrazide (IIa).—To a solution of 2.75 g (0.015 mole) of dimethyl imidazole-4,5-dicarboxylate in 30 ml of methanol was added 1.0 g (0.03 mole) of 95% hydrazine. The mixture was heated under reflux for 1 hr during which time a white precipitate was formed. After cooling to room temperature the precipitate was filtered, washed three times with methanol, and air dried to yield 2.6 g (95%) of IIa: mp >300°; ν_{max} 3320, 3250 (NH), 1680, 1655 cm^{-1} (C=O).

Anal. Calcd for $C_5H_8N_6O_2$: C, 32.61; H, 4.38; N, 45.64. Found: C, 32.89; H, 4.47; N, 45.69.

Imidazole-4,5-dicarboxylic Acid Bis(2-methylhydrazide) (IIb) and Imidazole-4,5-dicarboxylic Acid 1-Methylhydrazide 2-Methylhydrazide (IV). Method A.—To a solution of 2.75 g (0.015 mole) of dimethyl imidazole-4,5-dicarboxylate in 55 ml of 1-butanol was added 3.7 g (0.08 mole) of methylhydrazine. The mixture was heated under reflux. A crystalline substance started to separate in the reaction mixture after 2 hr. Heating was continued for an additional 10 hr. The crystals were filtered hot to give 0.6 g (19%) of IV: mp 195–197°; ν_{max} 3300 (NH), 1645 cm^{-1} (C=O); nmr peaks (D_2O), δ 8.12 (imidazole CH), 3.6 (N^1CH_3), 2.9 (N^2CH_3).

Anal. Calcd for $C_7H_{12}N_6O_2$: C, 39.62; H, 5.66; N, 39.62. Found: C, 39.68; H, 5.62; N, 39.64.[12]

The filtrate was evaporated and the residue was crystallized from water to give 1.7 g (53%) of IIb: mp 210–212°; ν_{max} 3280, 3225 (NH), 1660, 1640 cm^{-1} (C=O); nmr peaks (TFA), δ 8.2 (imidazole CH), 3.0 (CH_3); tlc (methanol–ether–water, 1:1:0.05), R_f (IIb) 0.5, R_f (IV) 0.03.

Anal. Calcd for $C_7H_{12}N_6O_2$: C, 39.62; H, 5.66; N, 39.62. Found: C, 39.34; H, 5.76; N, 39.76.

Method B.—The same quantity of reagents as in method A was employed in this method but no solvent was used. The mixture was heated under reflux for 15 min and then left at room temperature for 6 hr. The excess methylhydrazine was evaporated, and the residue was crystallized from water to give 1.9 g (60%) of IIb, mp 210–212°. No melting point depression was observed when this product was mixed with the high-melting isomer under method A, and their infrared spectra were identical.

Imidazole-4,5-dicarboxylic Acid Methyl Ester 2-Phenylhydrazide (V).—To 2.75 g (0.015 mole) of dimethyl imidazole-4,5-dicarboxylate was added 5.4 g (0.050 mole) of phenylhydrazine. The mixture was heated under reflux for 24 hr and then cooled to room temperature. The yellowish solid was washed several times with ether and a small amount of methanol and finally crystallized from ethanol to give 3.0 g (76%) of V: mp 247–250°; ν_{max} 3315 (NH), 1700, 1660 cm^{-1} (C=O).

Anal. Calcd for $C_{12}H_{12}N_4O_3$: C, 55.38; H, 4.61; N, 21.53. Found: C, 55.27; H, 4.62; N, 21.57.

Imidazole-4,5-dicarboxylic Acid Bis(2-phenylhydrazide) (VI).—To a solution of 2.75 g (0.02 mole) of dimethyl imidazole-4,5-dicarboxylate in 60 ml of dry 1-butanol was added 9.3 g (0.08 mole) of 95% phenylhydrazine. The mixture was heated under reflux for 20 hr. White crystals which had fallen off in the reaction flask were separated to give 0.8 g of an unknown side product, whose identification was not pursued beyond its melting point (223–225°). Heating the filtrate for another 20 hr yielded 0.25 g more of the same side product. Continuation of heating for an additional 20 hr did not yield any more of this substance. The reaction mixture was cooled to room temperature and then left in a refrigerator for 6 hr. The white precipitate was filtered and crystallized from methanol to give 1.5 g (23%) of VI: mp 219–220°; ν_{max} 3330 (NH), 1695, 1665, 1640 cm^{-1} (C=O); nmr peaks (TFA), δ 6.9 (C_6H_5); tlc (isoamyl alcohol–H_2O, 40:1), R_f (VI) 0.85, R_f (side product) 0.05.

Anal. Calcd for $C_{17}H_{16}N_6O_2$: C, 60.71; H, 4.76; N, 25.00. Found: C, 61.07; H, 4.95; N, 24.50.

Imidazole-4,5-dicarboxylic Acid Bis(2,2-dimethylhydrazide) (VII).—To 3.70 g (0.02 mole) of dimethyl imidazole-4,5-dicarboxylate was added 6 g (0.1 mole) of 1,1-dimethylhydrazine. The mixture was heated under reflux for 40 hr at which time a white solid was precipitated. The excess 1,1-dimethylhydrazine was aspirated *in vacuo*. The residue of imidazole-4,5-dicarboxylic acid bis(2,2-dimethylhydrazide) was washed with ether and dried in a vacuum desiccator to give 3.6 g (78%) of VII: mp 179–183°;[13] ν_{max} 3350 (OH, NH), 1650 cm^{-1} (C=O), broad.

Anal. Calcd for $C_9H_{16}N_6O_2 \cdot H_2O$: C, 41.84; H, 7.02; N, 32.54. Found: C, 42.09; H, 7.1; N, 32.7.

Imidazole-4,5-dicarboxylic Acid Bis(2-isopropylidenehydrazide) (VIII).—A suspension of 3 g (0.016 mole) of imidazole-4,5-dicarboxylic acid dihydrazide in a mixture of 150 ml of acetone and 40 ml of methanol was heated under reflux for 24 hr. The cloudy solution was filtered and evaporated. The residue crystallized from benzene containing 5% methanol to give 3.5 g (80%) of VIII with 0.5 mole of water: mp 245–250°;[14] ν_{max} 3330, 3240 (NH), 1690, 1660, 1635 cm^{-1} (C=O); nmr peaks ($CDCl_3$), δ 7.9 (imidazole CH), 2.2 (CH_3).

Anal. Calcd for $C_{10}H_{16}N_6O_2 \cdot 0.5H_2O$: C, 48.35; H, 6.22; N, 30.77. Found: C, 48.13; H, 6.44; N, 31.18.

Imidazole-4,5-dicarboxylic Acid 2-Isopropylidenehydrazide 2-Isopropylhydrazide (IX) and Imidazole-4,5-dicarboxylic Acid Bis(2-isopropylhydrazide) (X).—To a solution of 1.0 g (0.0038 mole) of imidazole-4,5-dicarboxylic acid bis(2-isopropylidenehydrazide) in 60 ml of dry methanol was added 0.1 g of PtO_2. The mixture was hydrogenated at 2.9 kg/cm^2 (40 psi) and 50° for 0.5 hr by a low-pressure Parr hydrogenation apparatus. After this time, the inlet valve for hydrogen was shut off. Heating and shaking was continued for an additional 3.5 hr. The mixture was cooled to room temperature. The crystals were filtered. Crystallization of this residue from ethanol gave 0.6 g (59%) of IX: mp 253–256°; ν_{max} 3240, 3275 (NH), 1680, 1630 cm^{-1} (C=O).

Anal. Calcd for $C_{13}H_{18}N_6O_2$: C, 49.62; H, 6.76; N, 31.57. Found: C, 49.50; H, 6.85; N, 31.46.

When the foregoing procedure was repeated at 4.2 kg/cm^2 (60 psi) of hydrogen at 70° for a period of 36 hr, evaporation of the solution *in vacuo* gave 0.7 g (58%) of X: mp 210–212°; ν_{max} 3290, 3230 (NH), 1660, 1650 cm^{-1} (C=O); nmr peaks (TFA), δ 8.3 (imidazole CH), 3.5–4.0 (CH), 1.11–1.23 (CH_3).

Anal. Calcd for $C_{13}H_{20}N_6O_2$: C, 49.25; H, 7.46; N, 31.34. Found: C, 49.36; H, 7.31; N, 30.45.

1-Methylimidazole-4,5-dicarboxylic Acid Dihydrazide (XII).—To a solution of 1.84 g (0.01 mole) of dimethyl imidazole-4,5-dicarboxylate in 70 ml of dry methanol was added 1.1 g (0.02 mole) of sodium methoxide. After cooling the mixture in an ice bath, 17 g (0.12 mole) of methyl iodide was added. The mixture was heated under reflux for 4 hr and then its volume was reduced *in vacuo* to 10 ml. This was assumed to be a solution of dimethyl 1-methylimidazole-4,5-dicarboxylate. To this solution was added 1.0 g of (0.03 mole) of 95% hydrazine and left at room temperature for 3 hr at which time a white crystalline compound

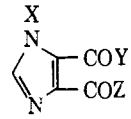
(12) Average of duplicated nitrogen analysis.

(13) Due to difficulties encountered in crystallizing VII, the elemental analysis was done prior to its crystallization. After crystallizing VII from acetone containing a small quantity of methanol, its melting point increased to 187–189°.

(14) During the process of melting, VIII underwent a change of state at about 170° and finally melted at 248°. When a suspension of VIII in dry acetone was heated under reflux for a long period of time a crystalline solid was obtained that melted at 223–226°. This compound was assumed to be VIII without waver of crystallization. The product of catalytic hydrogenation of this compound was X.

TABLE I

IMIDAZOLECARBOXYLIC ACID HYDRAZIDES



No.	X	Y	Z	Mp, °C[a]	Formula	Ptosis inhibition: Oral dose, mmoles/kg	Ptosis inhibition: Activity prior to reserpine	Ptosis inhibition: Mean ptotic score
IIa	H	$HNNH_2$	$HNNH_2$	>300	$C_5H_8N_6O_2$	1.1	Normal	1.0
IIb	H	$HNNHCH_3$	$HNNHCH_3$	210–212	$C_7H_{12}N_6O_2$	1.1	Normal	0.5
IV	H	$HNNHCH_3$	CH_3NNH_2	195–197	$C_7H_{12}N_6O_2$	0.275	Increased	2.6
V	H	$HNNHC_6H_5$	OCH_3	247–250	$C_{12}H_{12}N_4O_3$	1.1	Increased	0.8
VI	H	$HNNHC_6H_5$	$HNNHC_6H_5$	219–220	$C_{17}H_{16}N_6O_2$	1.1	Increased	1.0
VII	H	$HNN(CH_3)_2$	$HNN(CH_3)_2$	179–183[b]	$C_9H_{16}N_6O_2 \cdot H_2O$	1.1	Reduced	0.5
VIII	H	$HNN{=}C(CH_3)_2$	$HNN{=}C(CH_3)_2$	245–250[c]	$C_{11}H_{16}N_6O_2 \cdot 0.5H_2O$	1.1	Reduced	0.5
IX	H	$HNN{=}C(CH_3)_2$	$HNNHCH(CH_3)_2$	253–256	$C_{11}H_{18}N_6O_2$	...	...	...
X	H	$HNNHCH(CH_3)_2$	$HNNHCH(CH_3)_2$	210–212	$C_{11}H_{20}N_6O_2$	1.1	Increased	0.8
XII	CH_3	$NHNH_2$	$HNNH_2$	232–235[d]	$C_6H_{10}N_6O_2$	0.525	Increased	2.2
Isocarboxazid			...	...	...	0.275	Increased	3.8

[a] Melting points vary somewhat with the rate of heating. [b] Melting point before crystallization. [c] See ref 14. [d] When the melting point was determined at a rapid rate of heating.

was separated. This compound was crystallized from methanol and water to give 0.9 g (50%) of XII: mp 232–235°; ν_{max} 3345, 3285 (NH), 1665, 1645 cm^{-1} (C=O).

Anal. Calcd for $C_6H_{10}N_6O_2$: C, 36.36; H, 5.09; N, 42.41. Found: C, 36.40; H, 5.29; N, 42.54.

Pharmacological Methods.—The compounds listed in Table I were screened for possible monoamine oxidase (MAO) inhibitory activity by the method of Aceto and Harris.[15] In addition to this test, a study was made to determine the degree of sleeping time potentiation by some of the compounds in mice. Finally, the effect of the series on rabbit blood pressure, respiration, EKG, and EEG, following intravenous administration, was investigated.

MAO Inhibition.—A dose of 1.1 mmoles/kg was administered by the oral route to mice. Since some of the compounds were relatively insoluble in water, a 3% suspension of gum acacia in distilled water was used as a vehicle. Accordingly, mice used in the experiment were removed from food for 24 hr prior to oral intubation of the test compounds, while water was available *ad libitum*. Five mice in the weight range of 20 to 28 g were intubated with the calculated dose and observed for general signs of activity over a 2-hr period. Reserpine was then administered at a dose level of 2.5 mg/kg by the intraperitoneal route and allowed to remain undisturbed for the next 3 hr. At this time, each mouse was evaluated as to the degree of ptosis by at least two individuals. The results of the ratings were compared with two sets of control animals, one receiving the vehicle and reserpine only, the other receiving only aqueous reserpine. The dose of IV and XII had to be reduced due to extreme symptoms of toxicity demonstrated at the 1.1-mmoles/kg level. Isocarboxazid, a therapeutically employed MAO inhibitor, was administered in analogous manner for purposes of comparison of activity (see Table I).

Sleeping Time Potentiation.—Most MAO inhibitors potentiate the sleeping time of mice under hexobarbital narcosis. Consequently, 10 mice each for IV and XII, which manifested significant inhibitory activity in the ptosis test, were orally administered at a dose level of 0.275 mmole/kg and 0.525 mmole/kg, respectively. Two hours later the animals were given 60 mg/kg of hexobarbital sodium intraperitoneally. Ten mice, receiving only the vehicle orally and hexobarbital intraperitoneally were used as controls. 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.

Effect on Rabbit Blood Pressure, Respiration, EKG, and EEG.—Rabbits were anesthetized with 30 mg/kg of pentobarbital prior to intravenous administration of the compound. The animals were then surgically prepared for direct cannulation into the right common carotid artery to record blood pressure while the left external jugular vein was cannulated for administration of the compounds. The diffuse cortical electroencephalographic effects were monitored through subdermally implanted electrodes. Electrocardiographic responses were recorded from needle electrodes in the legs and chest. Graded doses of each compound were administered at various time intervals and the results were evaluated before the next administration.

Results and Discussion

A summary of the results obtained in the ptosis study is recorded in Table I. Compounds IV and XII manifest significant activity as is shown by its ability to prevent reserpine-induced ptosis. If the activity of these compounds is compared on a mole to mole basis, it may be seen that isocarboxazid is approximately 1.5 times as active as IV and 3 times as active as XII in ptosis inhibition. These two compounds, together with VII, are soluble in water. Although water solubility seems to be a criterion for an enhanced biological activity in this series, molecular structure plays a more important role. In VII both hydrogens in the N^2 position of the chains are substituted by methyl groups, which in a sense confirms the postulate[16] that, in order for an acid hydrazide to have a MAO inhibitory property, the presence of at least one hydrogen on N^2 is necessary. Except for IV and XII, the remaining compounds under our test conditions did not demonstrate MAO inhibitory property. Therefore, one may postulate that the hydrazide linkage in the less soluble compounds in this series is too stable to release the active hydrazine[17] at the target site. However, VII is a soluble compound, but does not possess activity analogous to IV and XII. Compounds VII and VIII both demonstrated some degree of toxicity which was manifested by reduced activity in mice before reserpine administration. A similar reaction was observed when isocarboxazid was administered. Compounds V, VI, and X caused the mice to show an increased activity 2 hr after administration, but were not able to inhibit the

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(16) J. H. Biel, A. Horita, and A. E. Drukker, "Psychopharmacological Agents," Maxwell Gordon, Ed., Academic Press Inc., New York, N. Y., 1964, p 359.

(17) J. H. Biel, P. A. Nuhfer, and A. C. Conway, *Ann. N. Y. Acad. Sci.*, **80**, 568 (1959).

reserpine-induced ptosis. In these instances, apparently the stimulation mechanism is not of MAO inhibition origin.

Compounds IV and XII show significant ($P > 0.05$) increased sleeping times in mice. The control group had an average sleeping time of 11.7 ± 8.0 min. The animals receiving IV showed a sleeping time of 24.4 ± 5.0 min and those receiving XII showed 28.3 ± 2.4 min. These data correlated well with the data obtained in the ptosis-inhibition study, verifying the ptosis test that these two compounds have MAO inhibitory activity. Had the activity of these compounds been merely stimulatory, then a decreased sleeping time would have been observed.

In view of the mode and rate of administration of aqueous solutions of IV and XII, the effect on rabbit blood pressure and respiration was somewhat delayed in becoming apparent. After a 5-hr time lapse from the initial administration of IV at a dose level of 0.85 mmole/kg iv, there was an acute rise in blood pressure to a systolic level of 150 mm from a previous level of 110 mm. This pressure remained fairly steady for 2 hr after which time toxic manifestations were noted.

The respiratory rate showed a gradual increase over the entire period of observation from a level of 30/min after pentobarbital anesthesia to approximately 70/min after 3.5 hr even with additional pentobarbital. These data suggest central stimulatory activity sufficient to reverse pentobarbital depression. Indeed, once the stimulatory effects become apparent, the animal required an additional total dose of 46 mg/kg of pentobarbital in order to maintain the anesthetized state.

The results of the EEG study showed that at the dose level of 180 mg/kg ($T + 5$ hr) bundled spiking could be observed. At this time, the animal was tested for response to pain and deep tendon reflexes. The negative results indicated that the faster EEG activity was probably not due to light anesthesia.

Changes observed in the EKG were not uncommon for animals under anesthesia for this period of time and showed no myocardial damage or cardiotropic activity.

Similar intravenous administration of XII at 1.35 mmoles/kg did not show the type of response analogous to IV. Further research on this is in progress.

Acknowledgment.—The authors wish to thank Mrs. J. Rim for her technical assistance and Mr. G. Johnson, Mr. D. Calley, and Miss M. Golaz for their help in the biological evaluation of the compounds.

3-Phenylcinnolines. I. Some Reactions and Derivatives of 3-Phenylcinnoline-4-carboxylic Acids

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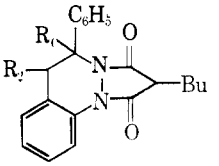
Received February 24, 1966

A series of amide, hydrazide, and ester derivatives of the title acids and two phenylbutazone analogs of 3-phenylcinnoline were prepared. These were examined for pharmacological activity.

In a continued search for less toxic analogs of phenylbutazone, the report of lower toxicity of its benzyl analog[1] and the ready availability of 3-phenylcinnoline-4-carboxylic acid[2] led initially to the preparation of the "benzyl-bridged"[3] analogs,[4] Ia and Ib, and of derivatives of this acid for general pharmacological testing. Later, the development of methods[5] for converting this acid to the 4-chloro analog from which cinnolines vaguely analogous to chloroquine might be prepared, and the discovery of the hypotensive activity of certain 4-aminocinnolines encouraged an expansion of the series.



Ia, $R_1 + R_2 =$ —
b, $R_1 = R_2 = H$

The starting materials (Chart I and Table I) were prepared by a slight modification of the procedures of Baumgarten and Furnas[2] (see Experimental Section). Attempts to extend this synthesis were only partially successful; under the conditions used, a reasonable variation of substituents appears to be feasible on the phenyl of the aldehyde portion of the hydrazones II, but several attempts to use other aldehydes such as acetaldehyde, phenylacetaldehyde, or isonicotinaldehyde were unsuccessful. Further, substituents in the phenylhydrazine ring of II appear to be limited to those which allow mild conditions for the Friedel–Crafts reaction: methyl (IIf and IIg) went well as was reported by Baumgarten and Furnas for IIf,[2] but fluoro (II, R′ = F; R = H) would not allow cyclization to the isatin.[6] In a single experiment with the methoxyhydrazone IIh a low yield of slightly impure acid (IVh) was obtained after rearrangement. Since

(1) J. Büchi, J. Aminann, R. Lieberherr, and E. Eichenberger, *Helv. Chim. Acta*, **36**, 75 (1953).

(2) H. E. Baumgarten and J. L. Furnas, *J. Org. Chem.*, **26**, 1536 (1961).

(3) The phenyl-bridged analogs have been prepared in several laboratories: H. S. Lowrie, *J. Med. Pharm. Chem.*, **5**, 1362 (1962), and references therein.

(4) The 4-phenyl isomer of Ia has recently been reported by (a) D. E. Ames, R. F. Chapman, and H. Z. Kucharska, *J. Chem. Soc.*, 5659 (1964); and (b) T. Wagner-Jauregg, F. Schatz, and U. Jahn [U. S. Patent 3,222,366 (1965); French Patent 1,393,596 (1965)] who prepared a series of related derivatives.

(5) Paper II: H. S. Lowrie, *J. Med. Chem.*, **9**, 670 (1966).

(6) The sequence was reported[2] unsuccessful with the corresponding chloro compound.