Indolizine Derivatives with Biological Activity II: *N'*-Substituted Hydrazides of 2-Methyl-3-indolizinecarboxylic Acid

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Abstract □ Synthesis and study of the antimonoamine oxidase activity of some *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid are reported. The activities were generally of about the same order as those of the corresponding hydrazides of 2-indolizinecarboxylic acid.

Keyphrases □ Indolizine derivatives—various *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid synthesized, evaluated for effect on monoamine oxidase activity □ Hydrazides, *N'*-substituted—of 2-methyl-3-indolizinecarboxylic acid synthesized, evaluated for effect on monoamine oxidase activity □ Monoamine oxidase activity—effect of various *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid □ Structure–activity relationships—various *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid synthesized, evaluated for effect on monoamine oxidase activity

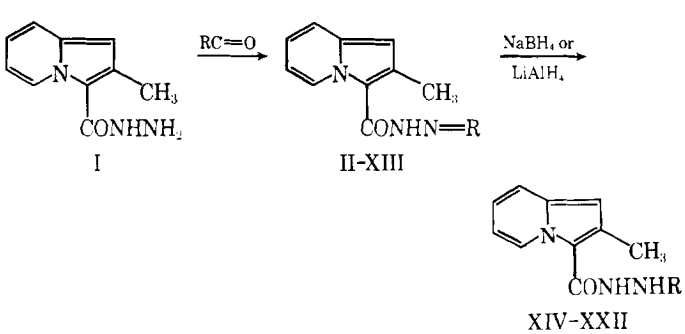
Previously (1), the synthesis and *in vitro* antimonoamine oxidase activity of a series of *N'*-substituted hydrazides of 2-indolizinecarboxylic acid, analogs of the well-known hydrazides of indolecarboxylic acids (2), were reported. The compounds were prepared for an investigation on analogs of biologically active indole derivatives in which indole was replaced by indolizine, which is related to it in structure and reactivity. To study structure–activity relationships in this new type of monoamine oxidase inhibitor, a series of *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid was prepared to determine the importance of the type of acyl radical.

In hydrazides of indolecarboxylic acids having the same alkyl radical on the *N'*-nitrogen, the activity is greater, at least within the limits in which such comparisons can be made, for derivatives in the 3-position (2).

EXPERIMENTAL

Chemistry—The *N'*-substituted hydrazides of 2-methyl-3-indolizinecarboxylic acid (XIV–XXII) were synthesized according to Scheme I. By reaction with aldehydes and ketones, 2-methyl-3-indolizinecarbohydrazide (I) provided the corresponding hydrazides (II–XIII) which were reduced with sodium borohydride.

It was not possible to reduce the *p*-methylbenzylidenehydrazide (X) by this method, and it was effected with lithium aluminum hydride. In spite of numerous attempts under various experimental conditions, it was not possible to reduce the benzylidenehydrazide (VIII), the 3,4-methylenedioxybenzylidenehydrazide (IX), and the α-methylbenzylidenehydrazide (XI) by the methods described. At the end of each reduction, the IR spectrum of the product obtained lacked the band characteristic of the C=N group of the initial hydrazides.



I II-XIII XIV-XXII

Scheme I

Synthesis[1]—*N'-Alkylidene- and N'-Arylalkylidenehydrazides (II–XIII)*—The appropriate aldehyde or ketone (0.006 mole) was added to a solution of 0.005 mole of I (3) in 65 ml of methanol. The mixture was stirred at room temperature or was heated for various times. With the 3,4-methylenedioxybenzaldehyde and ketones, the reaction was promoted by the addition of 0.005 mole of acetic acid. The products precipitated directly on cooling or by concentration of the solution and were crystallized from anhydrous ethanol (Table I).

N'-Alkyl- and N'-Arylalkyl-2-methyl-3-indolizinecarbohydrazides (XIV–XIX, XXI, and XXII)—Sodium borohydride (0.10–0.20 mole) was added slowly and with stirring to 0.021 mole of the appropriate hydrazide (II–VII, XII, or XIII) dissolved in the minimum amount of solvent. After the addition, stirring was continued for 5 hr. Then the solvent was driven off under vacuum, and the residue was treated with water and extracted with ether. The organic solution was dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was recrystallized from anhydrous ethanol (Table II).

N'-p-Methylbenzyl-2-methyl-3-indolizinecarbohydrazide (XX)—Compound X (0.0046 mole), in 50 ml of anhydrous tetrahydrofuran, was added to a suspension of 0.021 mole of lithium aluminum hydride in 20 ml of anhydrous tetrahydrofuran. The mixture was boiled under reflux for 5 hr and was stirred overnight at room temperature. Excess lithium aluminum hydride was destroyed with water-saturated ether. The mixture was filtered, the solvent was driven off from the filtrate under reduced pressure, and the residue was recrystallized from anhydrous ethanol, yielding 45%, mp 124–125°.

Antimonoamine Oxidase Activity *In Vitro*—The compounds (Table II) were tested *in vitro* as inhibitors of pig plasma monoamine oxidase at 37° and pH 6.98 using a spectrophotometer[2] at 253 nm. The enzyme preparation and the test procedure were described previously (1). The inhibitors were added as solutions in dioxane. The I_{50} values (I_{50} = molar concentration of the inhibitor producing 50% inhibition) were calculated graphically.

RESULTS AND DISCUSSION

All compounds tested were 3–7.5 times as active as iproniazid, used as a reference standard, in the inhibition of monoamine oxidase (Table II). In comparison to the corresponding hydrazides derived from 2-indolizinecarboxylic acid (1), I, XIV–XX, and XXII were less active, even if sometimes only slightly; the exception was XXI. The unsubstituted hydrazide (I) showed an activity one-tenth of that of the hydrazide of the preceding series (1). Apart from this last case, however, the position of the acyl radical in the indolizine system apparently did not have an excessively important role in the activity of the compound.

Concerning the type of substituent on the *N'*-nitrogen of the hydrazide chain, arylalkyl groups generally exercised a more favorable influence than simple alkyl groups. In the series having the alkyl group, the activity decreased with an increase in chain length (XIV–XVI); with the same number of carbons, branching appeared to have a positive effect (XVI and XVII).

[1] Melting points were determined with a Büchi apparatus and are not corrected. IR spectra were taken in mineral oil using a Perkin-Elmer 257 spectrophotometer.

[2] Cary 118.

Table I—*N'*-Alkylidene- and *N'*-Arylalkylidenehydrazides

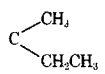
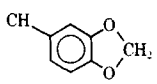
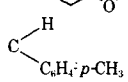
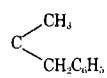
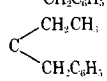
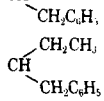
Compound	R	Reaction Time, hr/ Reaction Temperature	Melting Point	Yield, %	Molecular Formula	Analysis, % Calc.	Found
II	$CHCH_3$	3/20°	177–179°	73	$C_{12}H_{13}N_3O$	C 66.95 H 6.09 N 19.52	67.11 6.03 19.41
III	$CHCH_2CH_3$	12/20°	148–150°	65	$C_{13}H_{15}N_3O$	C 68.10 H 6.59 N 18.33	68.21 6.54 18.11
IV	$CHCH_2CH_2CH_3$	12/20°	158–159°	76	$C_{14}H_{17}N_3O$	C 69.11 H 7.04 N 17.27	68.97 7.13 17.04
V		3/40°	101–102°	65	$C_{14}H_{17}N_3O$	C 69.11 H 7.04 N 17.27	69.21 6.94 17.15
VI		2/40°	158–160°	75	$C_{15}H_{17}N_3O$	C 70.56 H 6.71 N 16.46	70.42 6.88 16.31
VII		3/40°	130–131°	70	$C_{16}H_{19}N_3O$	C 71.34 H 7.11 N 15.60	71.62 7.14 15.48
VIII		12/20°	190–192°	86	$C_{17}H_{15}N_3O$	C 73.63 H 5.45 N 15.15	73.70 5.71 15.02
IX		1/20°	202–204°	88	$C_{18}H_{15}N_3O_3$	C 67.28 H 4.71 N 13.08	67.12 4.72 13.20
X		1/40°	185–187°	80	$C_{18}H_{17}N_3O$	C 74.20 H 5.88 N 14.42	74.02 5.80 14.35
XI		24/50°	167–169°	74	$C_{18}H_{17}N_3O$	C 74.20 H 5.88 N 14.42	74.29 5.91 14.59
XII		2/40°	130–131°	81	$C_{19}H_{19}N_3O$	C 74.73 H 6.27 N 13.76	74.87 6.12 13.54
XIII		8/40°	118–119°	82	$C_{20}H_{21}N_3O$	C 75.21 H 6.63 N 13.16	75.37 6.71 13.40

Table II—*N'*-Alkyl- and *N'*-Arylalkyl-2-methyl-3-indolizinecarbohydrazides and Their Monoamine Oxidase Inhibitory Activity

Compound	R	Reaction Solvent[a]	Melting Point	Yield, %	I_{50}	X[b]	Molecular Formula	Analysis, % Calc.	Found
Iproniazid		—	—	—	9.42×10^{-4}	1	—	—	—
I	H	—	—	—	1.6×10^{-4}	5.89	—	—	—
XIV	CH_2CH_3	A	127–128°	75	2.15×10^{-4}	4.38	$C_{12}H_{15}N_3O$	C 66.34 H 6.96 N 19.34	66.50 7.01 19.52
XV	$CH_2CH_2CH_3$	A	113–114°	79	2.45×10^{-4}	3.84	$C_{13}H_{17}N_3O$	C 67.50 H 7.41 N 18.17	67.61 7.29 18.29
XVI	$CH_2CH_2CH_2CH_3$	A	95–97°	60	3.14×10^{-4}	3	$C_{14}H_{19}N_3O$	C 68.54 H 7.81 N 17.13	68.62 7.70 17.19
XVII		A	94–95°	72	2.08×10^{-4}	4.53	$C_{14}H_{19}N_3O$	C 68.54 H 7.81 N 17.13	68.71 7.92 16.97
XVIII		B	121–123°	63	2.25×10^{-4}	4.19	$C_{15}H_{19}N_3O$	C 70.00 H 7.44 N 16.33	70.13 7.35 16.22
XIX		B	121–122°	71	2.5×10^{-4}	3.77	$C_{16}H_{21}N_3O$	C 70.82 H 7.80 N 15.49	70.65 7.64 15.68
XX	$CH_2C_6H_4$-*p*-CH_3	—	124–125°	45	1.87×10^{-4}	5.04	$C_{18}H_{19}N_3O$	C 73.69 H 6.53 N 14.33	73.87 6.49 14.48
XXI		A	103–104°	83	1.26×10^{-4}	7.48	$C_{19}H_{21}N_3O$	C 74.24 H 6.89 N 13.67	74.45 6.81 13.72
XXII		B	97–98°	79	2.29×10^{-4}	4.11	$C_{20}H_{23}N_3O$	C 74.74 H 7.21 N 13.07	74.53 7.27 13.20

[a] Solvent A = methanol; Solvent B = ethanol–methanol (1:1). [b] X = (I_{50} iproniazid/I_{50} compound).

Among the cycloalkyl substituents, the cyclopentyl group had a more favorable action than the cyclohexyl group.

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COMMUNICATIONS

Correction to "Ionization Constants of Cephalosporin Zwitterionic Compounds"

Keyphrases □ Ionization constants—cephalosporin zwitterionic compounds, error in equations corrected □ Cephalosporin zwitterionic compounds—ionization constants, error in equations corrected □ Zwitterionic cephalosporin compounds—ionization constants, error in equations corrected

To the Editor:

In reviewing the derivation given in the paper "Ionization Constants of Cephalosporin Zwitterionic Compounds" (1), it was found that a factor was dropped in Eq. 12. This omission introduced an error into succeeding equations, and it is the purpose of this communication to correct this error.

The experimental results and calculations were made using the equations shown below rather than those in the paper and, therefore, do not need to be changed. In addition, the *Results and Discussion* section is correct in the paper.

The correct form of Eq. 12 is:

$$[A] = \left\{ \frac{[B] + [H^+] - [OH^-] - [A]}{K_3K_4 - \{H^+\}^2 \dfrac{Y_{NR^-}}{Y_{+HNRH}}} \right\} \left\{ K_3K_4 + K_3\{H^+\} \frac{Y_{NR^-}}{Y_{NRH}} \right\} + [Z]$$

The correct form of Eq. 13 is:

$$\{H^+\}^2 \frac{Y_{NR^-}}{Y_{+HNRH}} \{[Z] - [A]\} = K_3\{H^+\} \frac{Y_{NR^-}}{Y_{NRH}} \left\{ [B] + \frac{\{H^+\}}{Y_{H^+}} - \frac{K_\omega}{\{H^+\}Y_{OH^-}} - [A] \right\} + K_3K_4 \left\{ [B] + \frac{\{H^+\}}{Y_{H^+}} - \frac{K_\omega}{\{H^+\}Y_{OH^-}} - 2[A] + [Z] \right\}$$

The correct form of Eq. 14 is:

$$\delta = K_3\epsilon + K_3K_4\xi$$

A plot of δ/ξ *versus* ϵ/ξ will be linear with a slope of K_3 and δ/ξ will equal K_3K_4 at $\epsilon/\xi = 0$. Also, the value of ϵ/ξ at $\delta/\xi = 0$ will equal $-K_4$.

Equation 14 (as corrected here) may be solved using simultaneous equations by utilizing titrimetric and spectrophotometric data obtained at each of two pH values. By labeling the two sets of data as 1 and 2, K_3 and K_4 can be calculated according to:

$$K_3 = \frac{\dfrac{\delta_1}{\xi_1} - \dfrac{\delta_2}{\xi_2}}{\dfrac{\epsilon_1}{\xi_1} - \dfrac{\epsilon_2}{\xi_2}}$$

$$K_4 = \frac{\delta_1 - K_3\epsilon_1}{K_3\xi_1}$$

which are the corrected forms of Eqs. 15 and 16, respectively.

(1) W. H. Streng, H. E. Huber, J. L. DeYoung, and M. A. Zoglio, *J. Pharm. Sci.*, **65**, 1034 (1976).

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Nitrofurantoin Solubility in Aqueous Pyridoxine Hydrochloride Solutions

Keyphrases □ Nitrofurantoin—aqueous solubility, effect of pyridoxine hydrochloride ■ Solubility—nitrofurantoin in aqueous solutions, effect of pyridoxine hydrochloride □ Pyridoxine hydrochloride—effect on aqueous solubility of nitrofurantoin ■ Antibacterials, urinary—nitrofurantoin, aqueous solubility, effect of pyridoxine hydrochloride □ Vitamins—pyridoxine hydrochloride, effect on aqueous solubility of nitrofurantoin

To the Editor:

Excess nitrofurantoin (approximately 50 mg) was added to 40 ml of an appropriate test solution (0–20.0% pyridoxine hydrochloride in aqueous pH 3 or 5 buffer[1]) in a 45-ml screw-capped bottle. The tightly closed container was wrapped in aluminum foil to keep out light, placed in a constant-temperature water bath at 37 ± 0.1°, and rotated[2] for at least 20 hr. Experiments indicated that equilibrium was established within 10–16 hr. The test so-

[1] Citric acid–dibasic sodium phosphate buffer; ionic strength of 0.7.

[2] Menhold rotating apparatus, Lester, Pa.