Research, University of Saskatchewan, Saskatoon, Saskatchewan, undertook the testing against the Ehrlich ascites carcinoma. The antimicrobial evaluation of certain of the compounds described was supervised by Dr. V. S. Gupta of the Department of Veterinary Physiology, University of Saskatchewan. Thanks are extended to Mrs. J. L. Brandvold who prepared several of the compounds and to Dr. D. G. K. Gorecki of the College of Pharmacy, University of Saskatchewan, who undertook the screening of IId and IIf as potential diuretic agents.

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Indolizines II: Search for Potential Oral Hypoglycemic Agents

A. U. DE [x] and B. P. SAHA

Abstract □ A few 1,2-bis(*N*-alkylaminomethyl)indolizines, simple indolizinecarboxylic acids, and several 6-alkoxyindolizine-2-carboxylic acids were synthesized and screened as possible oral hypoglycemic agents. The absence of any significant hypoglycemic activity excludes these compounds from the predicted structural lead provided by some hypoglycemic *Vinca* alkaloids, such as vincamine, vindoline, and vindolinine, having the indolizine ring as one structural component. But an extension of the rationale that indolizines are also the structural components of some carcinolytic *Vinca* alkaloids, such as vincristine and vinblastine, used in cancer chemotherapy provided encouraging results. One indolizine derivative showed significant antineoplastic activity in Ehrlich ascites carcinoma.

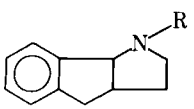
Keyphrases □ Indolizine derivatives—synthesized and screened as possible oral hypoglycemic agents □ Hypoglycemic agents, potential—synthesis and screening of indolizine derivatives □ Antineoplastic agents—screening of indolizine derivatives

The rationale for undertaking the synthesis of some *N*-alkyl 1,2,3,3a,4,8b-hexahydroindeno[1,2-*b*]pyrroles (I) and 2-(*N*-alkylaminomethyl)indolizines (II) as possible oral hypoglycemic agents was discussed previously (1, 2). Three additional compounds (I, R = *n*-pentyl, and II, R = *n*-pentyl or *n*-hexyl) were prepared according to the reported methods (1, 2) and biologically evaluated (Table I).

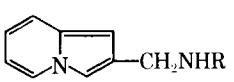
The failure of the compounds (II) to show any significant activity might be due to some undesirable biotransformation taking place through the very active 1- and 3-positions of II in any of the four intermediate steps of absorption, transport, barrier passage, and metabolism. Therefore, indolizine derivatives of type III, having only one active position free, were synthesized (Scheme I).

EXPERIMENTAL

Chemistry—Ethyl 2-pyridyl acetate (III*b*), prepared from 2-picolyllithium (III*a*) and ethyl carbonate according to the modification proposed by Goldberg *et al.* (3), was condensed with ethyl bromopyruvate to furnish indolizine 1,2-dicarboxylate (III*c*) (4). The diester was treated with various alkylamines to give 1,2-bis(*N*-alkylcarboxamido)indolizines (III*d*) and subsequently reduced with lithium aluminum hydride to 1,2-bis(*N*-alkylaminomethyl)-indolizines (III). The reaction between III*c* and an amine might also lead to an imide (III*e*) under the conditions followed, but the usual elemental analysis and IR and NMR spectra confirmed the diamide structure (III*d*).



I



II

Table I—Hypoglycemic Activity[a]

Compound	Compound Type	R	Maximum Blood Sugar Lowering, %
1	I	H	5
2	I	*n*-C_5H_{11}	7
3	II	*n*-C_5H_{11}	12
4	II	*n*-C_6H_{13}	10
5	III	CH_3	5
6	III	C_2H_5	Nil
7	III	*n*-C_3H_7	Nil
8	III	*n*-C_4H_9	Nil
9	III	*n*-C_5H_{11}	5
10	IV	H	5
11	IV	COOH	4
12	V	CH_3	3
13	V	C_2H_5	11
14	Tolbutamide		40

[a] Hypoglycemic tests were carried out by Central Drug Research Institute, Lucknow, India.

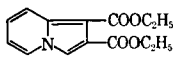
The synthesized compounds (III) were inactive. The introduction of two basic side chains with one active position free in III abolished the activity in comparison to II. It appeared that introducing an activating group and an acid function in the indolizine moiety while keeping positions 1 and 3 free might lead to better biological response. Moreover, various carboxylic acids and their derivatives, such as 5-methylpyrazole-3-carboxylic acid, 5-methylisoxazole-3-carboxylic acid (5), salicylic acid, and mesoxalic acid (6), have significant activity.

The simple indolizinecarboxylic acids (IV: R = H or COOH) were already known (4, 7) and prepared accordingly. 2-Methylpyridine-5-sulfonic acid (V*b*), obtained from 2-picoline (V*a*) by sulfonation with fuming sulfuric acid (8), was fused with potassium hydroxide to 5-hydroxy-2-methylpyridine (V*c*) and subsequently methylated with diazomethane to 5-methoxy-2-methylpyridine (V*d*: R = methyl) according to a modification of the method of Marion and Cockburn (9).

Attempts at methylation of V*c* with methyl iodide and dimethyl sulfate completely failed. However, ethylation of V*c* with diethyl sulfate led to 5-ethoxy-2-methylpyridine (V*d*: R = ethyl). The failure in alkylation with dimethyl sulfate and methyl iodide might be due to the weaker polarized character in C—O and C—I bonds of these molecules in comparison to diethyl sulfate, the ionic character being a necessary feature for this type of reaction.

Condensation of 5-alkoxy-2-methylpyridines (V*d*) with ethyl bromopyruvate and their subsequent cyclization with sodium bi-

Table II—Antineoplastic Activity[a] of Diethylindolizine-1,2-dicarboxylate (IIIc) in Ehrlich Ascites Carcinoma



Cells Injected into Each Animal	Average Number of Cells per Milliliter in Control (C)	Average Number of Cells per Milliliter in Test (T)	T/C of Cells	Percent Inhibition of Ascitic Cells, (1 − T/C) × 100	Average Weight of Ascitic Fluid in Control (C), g	Average Weight of Ascitic Fluid in Test (T), g	T/C Ascitic Fluid	Percent Inhibition of Ascitic Fluid, (1 − T/C) × 100
18.5×10^6	450.0×10^6	80.5×10^6	0.1788	82.12	0.8	0.2	0.25	75.00
Mitomycin (as standard), 18.5×10^6	450.0×10^6	0	0	100.00	0.8	0	0	100.00

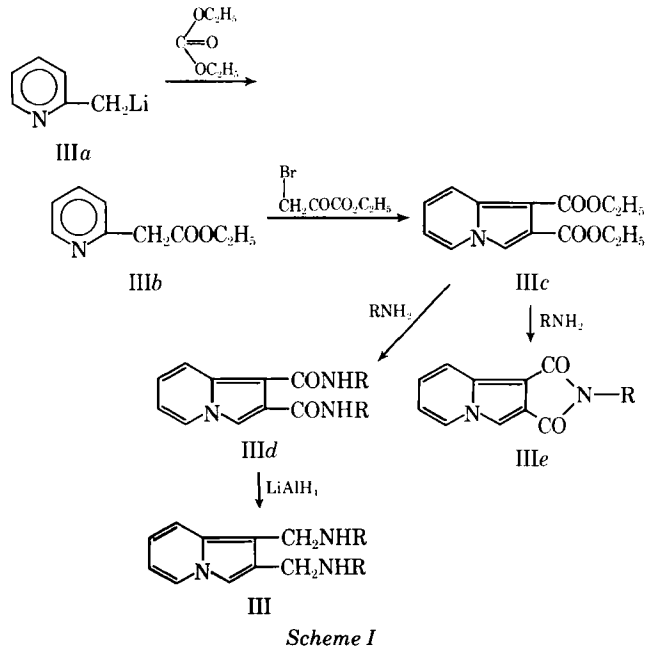
[a] Antineoplastic activity test was carried out by B.C. Panda (14).

carbonate (7) gave 6-alkoxyindolizine-2-carboxylic acids (V: R = methyl or ethyl), confirmed by the usual elemental analysis and IR and NMR spectra (Scheme II). Attempts to prepare 6-hydroxyindolizine-2-carboxylic acid (V: R = H) from Vc and ethyl bromopyruvate according to various methods (4, 7, 10) failed in the cyclization step, although the quaternary salt formation in the intermediate step took place. This failure in cyclization of the quaternary salt might be due to some deactivating influence of the hydroxy group in contrast to an alkoxy substituent at the same position.

Biological Activity—Hypoglycemic activity of the synthesized compounds are recorded in Table I. The absence of any significant activity in the indolizine derivatives (II–V) excludes at least this class of compounds (11) from the purview of Svoboda's (12) prediction on the structural lead provided by some hypoglycemic *Vinca* alkaloids. However, three types of structural variations on a prototype molecule were examined. A comparison of the relative activities of the compounds (II–V) indicates that the introduction of two basic side chains and the freeing of one active position as in III substantially decrease the activity. The other two variations, made by including an activating group and/or an acid function, retain less (type IV compounds) or about the same degree of activity (type V compounds) as the prototypes.

Hypoglycemic activity was determined orally at a dose level of 250 mg/kg body weight in each group of three overnight fasted albino rats, using tolbutamide as the standard. Blood sugar was estimated at 0, 2, and 4 hr according to the method of Nelson and Somogyi (13), and the maximum blood sugar lowering values were recorded.

The rationale for the synthesis of some indolizine derivatives,



Scheme I

being the constituents of some carcinolytic *Vinca* alkaloids such as vincristine and vinblastine, has been extended in the field of cancer chemotherapy also. With an exploratory objective, several indolizine derivatives were tested in Ehrlich ascites carcinoma, with ascitic live cell count and tumor weight being the activity parameters (14). Diethyl indolizine-1,2-dicarboxylate showed significant activity (Table II). Both the control and test groups contained five healthy Swiss albino mice of the same sex and approximately the same age and body weight (18–20 g).

The compounds were administered as suspensions in sterile buffered isotonic saline (pH 7.2) at a dose level of 50 mg/kg ip. Mitomycin, also in the same vehicle, was used as a standard at a dose level of 1 mg/kg. A fixed number of Ehrlich ascites carcinoma cells, collected from the donor mice, was transplanted into the intraperitoneal cavities of the control and test groups.

A day of incubation was allowed; then from the 2nd day seven consecutive doses were administered. Evaluation was made on the 9th day on the basis of the live cell counts and weight of the ascitic fluids collected from the intraperitoneal cavities of the control and test groups. Food and water were withheld 6 hr before the testing operation started.

Systematic investigations are being conducted with a wide variety of indolizines, and the results of investigations will be reported later.

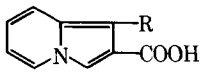
Syntheses[1]—N-n-*Pentyl-1,2,3,3a,4,8b-Hexahydroindeno[1,2-b]-pyrrole (I)*—Compound I was prepared according to the method described earlier (1) from 1,2,3,3a,4,8b-hexahydroindeno[1,2-*b*]pyrrole (2 g, 12.58 mmoles) and *n*-pentyl bromide (2.09 g, 13.84 mmoles). The product was distilled at 120°/0.6 mm to provide a colorless liquid. The yield was 0.7 g (24%), mp (picrate) 118–119°.

Anal.—Calc. for $C_{16}H_{23}N$: C, 83.85; H, 10.04; N, 6.11. Found: C, 83.90; H, 9.71; N, 5.93.

1,2-Bis(N-alkylcarboxamido)indolizines (IIId)—A mixture of diethyl indolizine-1,2-dicarboxylate (IIIc, 1 mole) and the appropriate alkylamine (2.2 moles) was heated in a sealed tube for 8 hr in a water bath. The diamide was purified by crystallization from dilute ethanol (Table III).

IIId (R = methyl): IR ν_{max}^{KBr} 3201 (NH) and 1652 (C=O) cm^{-1}. IIId (R = *n*- butyl): IR ν_{max}^{KBr} 3020 (NH) and 1650 (C=O) cm^{-1}. IIId (R = methyl): NMR ($CDCl_3$) τ 1.93–2.26 (3 H, 3-H, 5-H, and 8-H), 2.83–3.57 (2 H, 6-H and 7-H), 5.5–5.9 (2 H, 1-CON*H*CH_3 and 2-CON*H*CH_3), 7–7.16 (3 H, 2-CONHC*H*$_3$), and 8.53–8.77 (3 H, 1-CONHC*H*$_3$), a total of 13 protons. The ratio of aliphatic protons to aromatic protons was: calc., 1.6; found, 1.576.

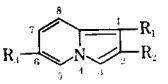
1,2-Bis(N-alkylaminomethyl)indolizines (III)—The appropriate diamide (IIId, 1 mole) was reduced with lithium aluminum hy-



IV

[1] All melting points are uncorrected. IR spectra were determined in a Perkin-Elmer spectrophotometer, and NMR spectra were determined in a Varian A60 spectrometer calibrated against tetramethylsilane at 60 MHz, 540-cps sweep width, and 300-cps sweep offset.

Table III—Characteristics of Substituted Indolizines



Compound	R_1	R_2	R_3	Melting Point	Boiling Point	Formula	Analysis, %	Calc.	Found
II[a]	H	—CONH-*n*-C_5H_{11}	H	158–160° dec.	120–125°/ 0.8 mm	$C_{14}H_{18}N_2O$	C	73.05	73.13
							H	7.83	7.90
							N	12.17	12.21
II[a]	H	—CONH-*n*-C_6H_{13}	H	—	120–125°/ 0.5–0.6 mm	$C_{15}H_{20}N_2O$	C	73.77	73.82
							H	8.20	8.12
							N	11.48	11.53
II[a]	H	—CH_2NH-*n*-C_5H_{11}	H	—	110–115°/ 0.9 mm	$C_{14}H_{20}N_2$	C	77.77	77.41
							H	9.26	9.62
							N	12.96	12.54
II[a]	H	—CH_2NH-*n*-C_6H_{13}	H	—	115–120°/ 0.5–0.7 mm	$C_{15}H_{22}N_2$	C	78.27	78.44
							H	9.57	9.41
							N	12.17	12.20
III*d*[b]	$R_1 = R_2$	—$CONHCH_3$	H	168–170°	—	$C_{12}H_{13}N_3O_2$	C	62.34	62.40
							H	5.63	5.61
							N	18.18	18.18
III*d*	$R_1 = R_2$	—$CONHC_2H_5$	H	184–186°	—	$C_{14}H_{17}N_3O_2$	C	64.86	65.00
							H	6.56	6.52
							N	16.21	16.11
III*d*	$R_1 = R_2$	—CONH-*n*-C_3H_7	H	110–112°	—	$C_{16}H_{21}N_3O_2$	C	66.89	66.81
							H	7.32	7.36
							N	14.64	14.90
III*d*	$R_1 = R_2$	—CONH-*n*-C_4H_9	H	108–110°	—	$C_{18}H_{25}N_3O_2$	C	68.58	68.50
							H	7.93	8.01
							N	13.33	13.42
III*d*	$R_1 = R_2$	—CONH-*n*-C_5H_{11}	H	113–115°	—	$C_{20}H_{29}N_3O_2$	C	69.97	70.11
							H	8.45	8.41
							N	12.25	12.30
III[c,d]	$R_1 = R_2$	—CH_2NHCH_3	H	103–104°	—	$C_{12}H_{17}N_3$	C	70.94	71.10
							H	8.37	8.29
							N	20.68	20.60
III[d]	$R_1 = R_2$	—$CH_2NHC_2H_5$	H	—	72–74°/ 0.8 mm	$C_{14}H_{21}N_3$	C	72.73	72.75
							H	9.09	9.10
							N	18.18	18.20
III[e]	$R_1 = R_2$	—CH_2NH-*n*-C_3H_7	H	—	105°/0.6 mm	$C_{16}H_{25}N_3$	C	74.13	74.32
							H	9.65	9.51
							N	16.21	16.15
III[e]	$R_1 = R_2$	—CH_2NH-*n*-C_4H_9	H	—	60–65°/1 mm	$C_{18}H_{29}N_3$	C	75.26	74.98
							H	10.10	10.31
							N	14.63	14.51
III[d]	$R_1 = R_2$	—CH_2NH-*n*-C_5H_{11}	H	—	65–67°/0.65 mm	$C_{20}H_{33}N_3$	C	76.19	76.10
							H	10.47	10.51
							N	13.33	13.41
V	H	COOH	OCH_3	170–172° dec.	—	$C_{10}H_9NO_3$	C	62.84	63.00
							H	4.71	4.52
							N	7.33	7.50
V	H	COOH	OC_2H_5	193–194° dec.	—	$C_{11}H_{11}NO_3$	C	64.39	64.50
							H	5.37	5.41
							N	6.82	6.72
V*e*	H	$COOCH_3$	OCH_3	96–97°	—	$C_{11}H_{11}NO_3$	C	64.39	64.51
							H	5.37	5.21
							N	6.82	6.84

[a] Methods described in Ref. 2. [b] Prepared from dry methylamine gas. [c] Crystallized from petroleum ether (bp 40–60°). [d] Reduction was done in tetrahydrofuran. [e] Reduction was done in ether.

dride (2 moles) in sodium-dried tetrahydrofuran or ether by refluxing for 16 hr. Excess lithium aluminum hydride was decomposed with water and filtered, and the filtrate was dried over anhydrous sodium sulfate. The solvent was removed *in vacuo*, and the residual crude amine was purified by crystallization from petroleum ether (bp 40–60°) or by distillation under reduced pressure to yield a colorless to pale-yellow product (Table III).

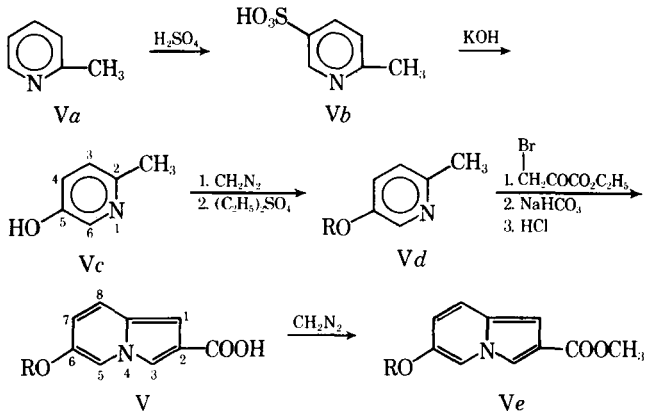
5-Hydroxy-2-methylpyridine (Vc)—2-Methylpyridine-5-sulfonic acid (V*b*, 28 g, 0.162 mole), potassium hydroxide (102 g, 1.82 moles), and water (5 ml) were thoroughly mixed in a wrought-iron pan. The mass was slowly heated to 200° when it melted and was maintained at this temperature for 10 min. The melt was cooled, dissolved in water (100 ml), partly neutralized with about 100 ml of concentrated hydrochloric acid, treated with charcoal, and filtered.

The filtrate was finally neutralized with carbon dioxide under cooling in an ice bath. A pale-yellow phenolic compound precipitated, and it was filtered, washed with two 5-ml portions of ice water, dried at 100°, and extracted with dry chloroform in a soxhlet apparatus. The solvent was removed to yield white and extremely light needles (12 g), mp 167–169°. A second crop of phenol (4 g) was obtained by evaporating the mother liquor in an enamel tray on a steam bath to dryness and then in a vacuum desiccator and finally extracting with chloroform as already described. The total yield was 16 g (90.9%).

5-Alkoxy-2-methylpyridines (Vd)—5-Methoxy-2-methylpyridine (V*d*:R = methyl) was prepared from 5-hydroxy-2-methylpyridine (Vc:0.10 mole) and diazomethane (0.26 mole) in the usual way (9), mp (picrate) 133–134°.

5-Ethoxy-2-methylpyridine (V*d*: R = ethyl) was prepared as follows. 5-Hydroxy-2-methylpyridine (Vc: 12 g) was dissolved in sodium hydroxide (10%, 44 ml), and diethyl sulfate (20.4 g) was added dropwise with stirring and heating on a water bath. The reaction mixture was kept distinctly alkaline to phenolphthalein by the simultaneous dropwise addition of sodium hydroxide (10%). After the addition was over, the reaction mixture was refluxed for 8 hr, cooled, and extracted with ether.

The ether layer was dried over anhydrous sodium sulfate. Ether was removed and the residual liquid was distilled at 50–55°/1 mm to yield 5-ethoxy-2-methylpyridine as a colorless liquid (4 g,



Scheme II

26.33%); the picrate crystallized from diluted ethanol, mp 142–144°. The alkaline mother liquor was neutralized with carbon dioxide, and 5-hydroxy-2-methylpyridine (1 g) was recovered.

Anal. —Calc. for $C_8H_{11}NO$: C, 70.08; H, 8.03; N, 10.22. Found: C, 70.1; H, 8.20; N, 9.91.

6-Alkoxyindolizine-2-carboxylic Acids (V) —A solution of 5-alkoxy-2-methylpyridine (V*d*: R = ethyl, 4 g, 19.5 mmoles) and ethyl bromopyruvate (5.7 g, 29.2 mmoles) in chilled absolute ethanol (22.8 ml) was heated under anhydrous conditions on a steam bath for 4 hr and kept at room temperature for 3 days. To reduce the reaction time, the method was modified by using cold, dry acetone (15 ml) in place of ethanol, and the mixture was heated on a steam bath for 1.5 hr only. The solvent was removed by distillation under reduced pressure, the cold residue was diluted with water (20 ml), and the solution was extracted with chloroform to remove the colored impurities.

Sodium bicarbonate was added until effervescence ceased, and the liberated 5-alkoxy-2-methylpyridine (1.8 g) was extracted with ether. Sodium bicarbonate (2.3 g) was added to the aqueous solution and the mixture was heated on a steam bath for 4 hr. While still warm the solution was acidified with 2 *N* hydrochloric acid to furnish a buff to chocolate-colored precipitate and was then recrystallized from ethanol (1 g). It gave intense blue fluorescence in ethanol and blue spots with Ehrlich's reagent (Table III).

6-Methoxyindolizine-2-carboxylic acid (V: R = methyl): IR ν_{max}^{KBr} 2950 (OH), 1670 (C=O), and 1252 (C—O) cm^{-1}. 6-Ethoxyindolizine-2-carboxylic acid (V: R = ethyl): IR ν_{max}^{KBr} 2980 (OH), 1671 (C=O), and 1255 (C—O) cm^{-1}.

Methyl-6-methoxyindolizine-2-carboxylate (Ve: R = Methyl)— To a suspension of 6-methoxyindolizine-2-carboxylic acid (V: R = methyl, 0.7 g) in dry ether (5 ml), a solution of diazomethane (0.3 g) in ether (15 ml) was added slowly to maintain a gentle evolution of nitrogen gas. The reaction was allowed to continue overnight and the resulting solution was filtered. The solvent was removed to furnish a crude product, which was crystallized from diluted ethanol as yellow crystals (0.1 g), mp 96–97° (Table III); IR: ν_{max}^{KBr} 1713 (C=O) and 1220 (C—O) cm^{-1}; NMR ($CDCl_3$): τ 6.23–6.33 (6 H, OCH_3 and $COOCH_3$) and 2.396–3.698 (5 H, 1-H, 3-H, 5-H, 7-H, and 8-H), a total of 11 protons. The ratio of aliphatic protons to aromatic protons was: calc., 1.2; found, 1.18.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 14, 1974, from the *Department of Pharmacy, Jadavpur University, Calcutta-700032, India.*

Accepted for publication August 15, 1974.

The authors thank the Central Drug Research Institute, Lucknow, India, for the hypoglycemic test report.

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