Pyrrolo[4,3,2-*de*]isoquinolines with Central Nervous System and Antihypertensive Activities

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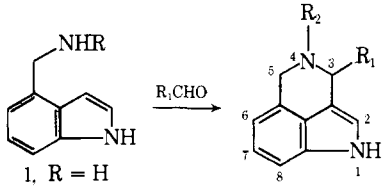
Two synthetic pathways are described leading to a novel series of pyrrolo[4,3,2-*de*]isoquinolines. Some of these derivatives show high activity in preventing reserpine-induced ptosis and in lowering the blood pressure of spontaneously hypertensive rats.

In a continuation of investigations of novel peri-fused tricyclic heterocyclic systems,[1] two synthetic routes to the pyrrolo[4,3,2-*de*]isoquinoline system (*e.g.*, **3**–**11**, see Scheme I) have been developed and various reactions of this system have been studied. This report describes the use of these routes for the preparation of a variety of pyrrolo[4,3,2-*de*]isoquinolines bearing substituents at one or more of positions 1, 3, 4, and 5, as well as a derivative containing a ring fused at the 4 and 5 positions. Some of these derivatives have been evaluated pharmacologically and the results are discussed.

Scheme I

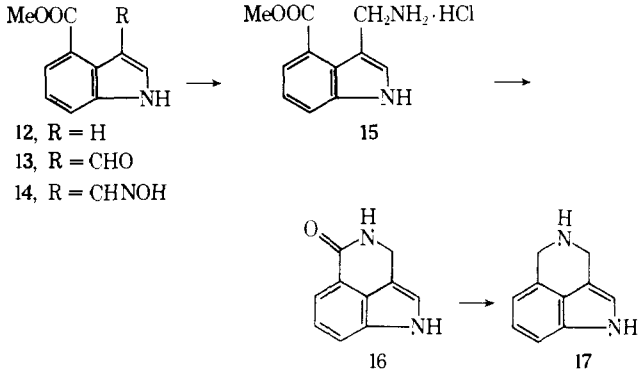


1, R = H
2, R = Me
3–11

Chemistry. The first synthetic route to the pyrrolo[4,3,2-*de*]isoquinoline system (Scheme I) involved an internal Mannich reaction of 4-aminomethylindole (**1**)[2] or 4-methylaminomethylindole (**2**)[3] with aliphatic or aromatic aldehydes to afford a series of 3-substituted and 3,4-disubstituted 1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinolines **3**–**11**. Their structures and physical data are collected in Table I.†

The second synthetic pathway (Scheme II) consisted of the conversion of methyl indole-4-carboxylate[5] (**12**) *via* a Vilsmeier reaction to the 3-formyl derivative **13**, oximation to the aldoxime **14**, and reduction of **14** and reduction of **14** catalytically with palladium in the presence of hydrochloric acid to afford the key intermediate, methyl 3-aminomethylindole-4-carboxylate hydrochloride (**15**) which could be isolated and characterized by nmr spectroscopy but for which an analysis was not obtained because of the ease with which it converts to **16**. Thus, treatment of **15** with sodium methoxide in ethanol at room temperature gave the 3,4-dihydropyrrolo[4,3,2-*de*]isoquinolin-5(1*H*)-one (**16**) in 84% yield. Alternatively, when the aldoxime **14** was reduced in acetic acid using platinum as the catalyst and the reaction mixture made alkaline during the work-up procedure, **16** was obtained directly but in only 22% yield.‡

Scheme II



12, R = H
13, R = CHO
14, R = CHNOH
15
16
17

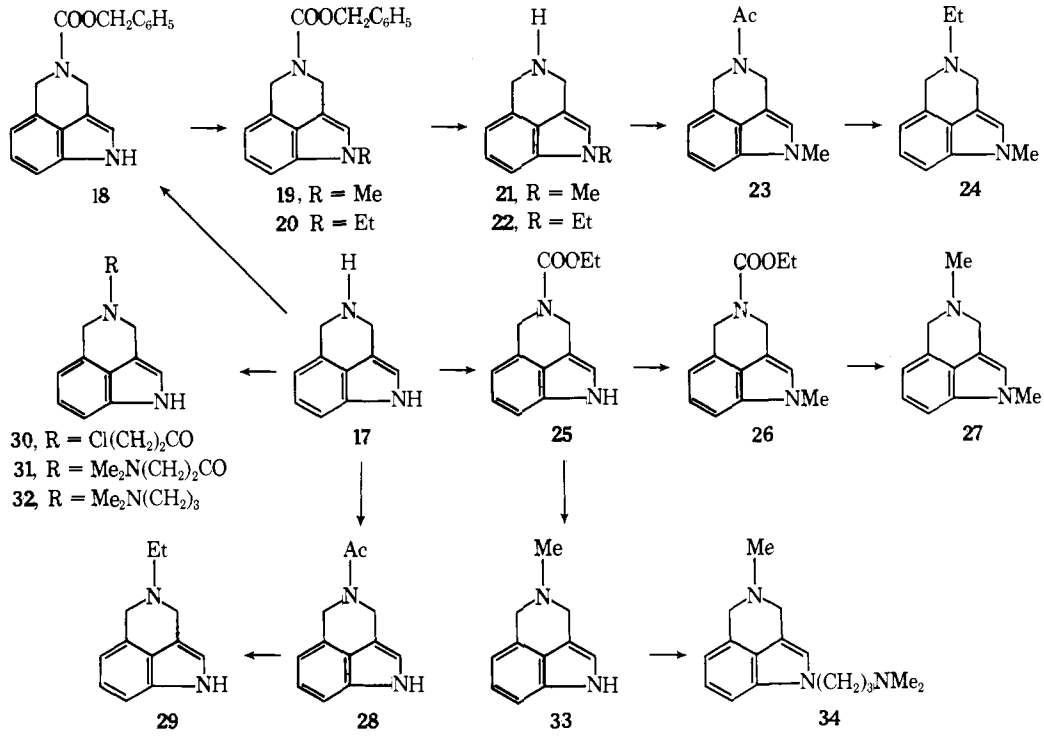
Reduction of lactam **16** with diborane afforded the unsubstituted form of the ring system, **17**, which made accessible for pharmacological screening a series of mono- and disubstituted derivatives bearing alkyl and dialkylaminoalkyl groups on the nitrogen atoms. These transformations of **17** are shown in Scheme III and involved the use of conventional series of reactions. Thus, the 1-alkyl compounds **21** and **22** were obtained from **17** *via* the benzyloxycarbonyl derivatives **18**–**20**. The 1,4-dialkyl derivative **24** was prepared from **21** *via* acylation and reduction. Alternatively, the 1,4-dimethyl analog **27** could be obtained from **17** by 1-alkylation of the carbamate **25**, followed by reduction. The 4-ethyl substituent of **29** was introduced *via* reduction of the 4-acetyl precursor **28**, and the 4-dimethylaminopropyl group in **32** was attached *via* the sequence **17** → **30** → **31** → **32**. The 4-methyl-1-dimethylaminopropyl derivative **34** was prepared by reduction of the carbamate **25** followed by alkylation with the basic halide.

The lactam **16** proved to be a useful starting material for the synthesis of pyrrolo[4,3,2-*de*]isoquinolines bearing substituents at position 5 and for derivatives having a ring fused to the 4 and 5 positions (see Scheme IV). Thus **16** was converted to the lactim ether **35** which was allowed to react, either as the free base or as a salt, with ammonium hydroxide, dimethylaminopropylamine, or hydrazine to afford respectively the 5-substituted derivatives **36**, **37**, and **38**. The 5-hydrazino derivative **38** was transformed to the isopropylidine derivative **40** and to the tetracyclic pyrrolo[4,3,2-*de*][1,2,4]triazolo derivative **39** by reaction with

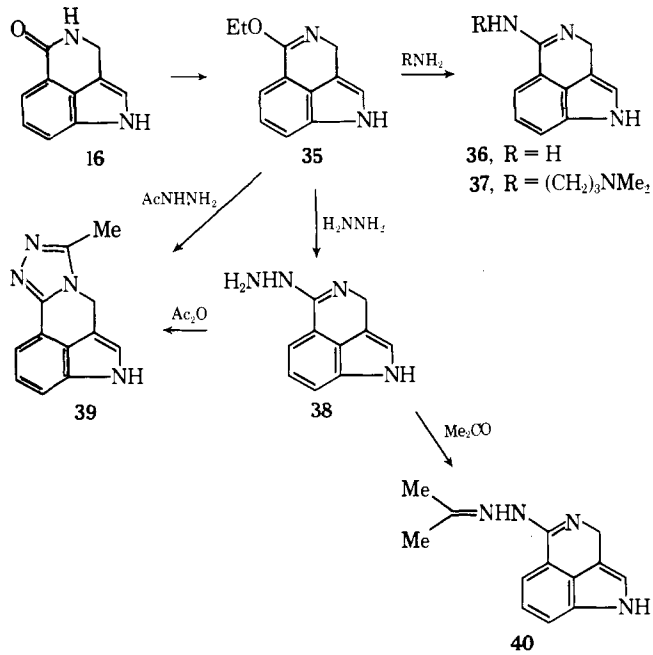
† After this work had been completed, the preparation, by a similar route, of **3** and **11** was reported in the patent literature.[4]

‡ During the course of this work, the synthesis of a lactam of type **16**, the corresponding 2,4-dimethyl derivative, was reported *via* the reductive ring closure of 4-acetyl-*N*-methyl-5-nitroisocarbostyril.[6]

Scheme III



Scheme IV



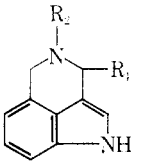
acetic anhydride. Compound **39** was also obtained directly from **35** by reaction with acetylhydrazine.

Pharmacology. Eighteen compounds were tested in mice and rats for their effects on the cardiovascular and central nervous system. The results are collected in Tables II and III along with the activities of some standard antidepressant and antihypertensive agents. Acute toxicity was investigated ip in albino mice. Graded doses of the compounds were administered to groups of five animals each. The approximate LD_{50} was determined from the 5-day mortality data. The effect of the compounds on the maximal electroshock seizure (MES) was investigated in albino mice after ip administration. The stimulation was carried out by means of corneal electrodes at 30 mA for 0.2 sec. The percentage of mice protected from the tonic phase of the seizure was recorded. As a measure of possible antidepressant activity, prevention of reserpine-induced ptosis was estimated by an adaptation of the method of Petersen, *et al.*[7] The percentage of mice in which ptosis was prevented was recorded. In both of these tests the ED_{50} was determined according to the method of Finney.[8]

The 3-phenylpyrrolo[4,3,2-*de*]isoquinolines **3**, **5**, and **7** were found to have weak activity in preventing the tonic phase of the MES seizure in mice. All the other compounds tested were inactive at dose levels up to 0.25 of the LD_{50}. Fourteen compounds were investigated by means of the antireserpine test. Three of these (**21**, **24**, and **40**) were more active than amitriptyline (ED_{50} 4.7 mg/kg); three others (**22**, **27**, and **38**) also showed an interesting level of activity (ED_{50} 7–9.5 mg/kg). The compounds active in preventing reserpine ptosis form two classes: the 5-hydrazino-1,3-dihydropyrrolo[4,3,2-*de*]isoquinolines **38** and **40** and the 1-alkyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinolines **21**, **22**, **24**, and **27**. It is worthy of note that the substitution patterns of the four latter compounds suggest that while a lower alkyl group at position 1 is mandatory for high activity, lower alkyl substitution is optional at position 4. The inactivity of **17**, **29**, and **33**, all bearing a hydrogen atom at position 1, is consistent with this observation.

Antihypertensive activity was determined in spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain. The basal systolic blood pressure of the rats was higher than 160 mm. Each compound was tested in four rats (two males and two females), 3–6 months old. The systolic blood pressure (BP) was measured by a tail-cuff technique[9] before oral administration of the test compounds and 1.5 and 4 hr thereafter; in the groups that had exhibited a BP decrease, a 24-hr reading was also taken. The effects on normal BP and interactions with several pressor agonists (epinephrine, tyramine, DMPP, angiotensin II, and vertical tilt) were studied in conscious cannulated normotensive rats according to the method of Cummings, *et al.*[10] Each compound was tested in at least four

Table I. 3-Substituted and 3,4-Disubstituted 1,3,4,5-Tetrahydropyrrolo[4,3,2-*de*]isoquinolines



No.	R_1	R_2	Mp, °C	Recrystn solvent	Yield, %	Formula	Analyses[a]
3	C_6H_5	H	190–193	EtOH	66	$C_{16}H_{14}N_2 \cdot C_4H_4O_4$[b,c]	C, H, N
4	4-$CH_3C_6H_4$	H	200–208	EtOH	15	$C_{17}H_{16}N_2$	C, H, N
5	4-$CH_3OC_6H_4$	H	192–195	EtOH	20	$C_{17}H_{16}N_2O \cdot C_4H_4O_4$[b]	C, H, N
6	3-$CH_3OC_6H_4$	H	183–185	Me_2CO	26	$C_{17}H_{16}N_2O$	C, H, N
7	4-ClC_6H_4	H	142–145	Me_2CO	54	$C_{16}H_{13}ClN_2 \cdot C_4H_4O_4$[b]	C, H, N, Cl
8	2-C_4H_3S[d]	H	158–159	C_6H_6	5	$C_{14}H_{12}N_2S$	C, H
9	$(CH_3)_2CH$	H	176–179	2-PrOH	35	$C_{13}H_{16}N_2$[e]	C, H, N
10	C_6H_5	CH_3	209–212	Et_2O	28	$C_{17}H_{16}N_2$	C, H, N
11	2-C_4H_3S[d]	CH_3	168–170	C_6H_6–pet. ether	34	$C_{15}H_{14}N_2S$	C, H, N, S

[a] Analyses were within ±0.4% for the elements indicated. [b] $C_4H_4O_4$ = maleic acid. [c] The free base of this compound was recently reported in ref 4. [d] C_4H_3S = thienyl. [e] This compound was recently reported in ref 4 to have mp 177–179°.

Table II. Pharmacological Activities

No.	Approx LD_{50}, mg/kg ip	MES,[a] ED_{50}, mg/kg ip	Prevn of reserpine ptosis, ED_{50}, mg/kg ip	BP lowering in SHR,[b] mg/kg po
3[c]	275	52	*d*	50 (+)
5[c]	125	29	*e*	25 (0)
7[c]	88	34	*d*	*e*
8	175	*d*	*e*	50 (++)
16	450	*d*	*e*	*e*
17[f]	325	*d*	*d*	25 (++)
21[f]	125	*d*	2.9	25 (++)
22[f]	112	*d*	9.5	25 (++)
24[f]	62	*e*	2.4	15 (++)
27[f]	88	*d*	7.2	25 (+)
29[f]	90	*d*	*d*	25 (+)
32[g]	275	*d*	*d*	50 (0)
33[f]	125	*d*	*d*	25 (++)
34[h]	325	*d*	*d*	75 (+)
37[g]	125	*d*	*d*	25 (0)
38[f]	175	*d*	7	12.5 (++)
39	>1200	>400	>200	300 (++)
40	225	*d*	2.3	50 (++)
Amitriptyline	94	11.3	4.7	*e*
Pargyline	370[i]	*e*	*e*	50 (++)
Guanethidine	125	*e*	*e*	12.5 (++)
Diazoxide	2200[j]	*e*	*e*	50 (++)

[a] Maximal electroshock seizure. [b] Blood pressure decrease (1.5 or 4 hr after dose) by 13–18%, ++; by 7–13%, +; less than 7%, 0. [c] Tested as the maleate salt. [d] Inactive at 0.25 of the LD_{50}. [e] Not tested. [f] Tested as the HCl salt. [g] Tested as the 2·HCl salt. [h] Tested as the 2·HBr salt. [i] G. M. Everett, R. G. Wiegand, and F. U. Rivaldi, *Ann. N. Y. Acad. Sci.*, **107**, 1068 (1963). [j] A. A. Rubin, F. E. Roth, R. M. Taylor, and H. Rosenkilde, *J. Pharmacol. Exp. Ther.*, 136, 344 (1962).

rats (Sprague-Dawley, males, 280–330 g) and the results were compared to a normally distributed control population of 70 rats. The doses were given by gavage 2 hr before the onset of recording.

In vitro, the effect on MAO was determined fluorometrically using rat brain as the enzyme source and kynuramine as the substrate.[11] The *in vivo* MAO inhibition was determined in a similar fashion, by measuring MAO activity in the brain and heart of male rats (body weight 145–155 g) treated orally with 50 mg/kg of the test compounds. The rats were killed by decapitation 2 hr after dosing. The MAO activity was expressed as the per cent of the activity found in saline-treated controls.

For the determination of antihypertensive activity, the compounds were tested at oral doses of 25–300 mg/kg, depending on the range of toxicity as estimated in mice ip.

Table III. Adrenergic Interactions and Inhibition of MAO

	Normotensive rat test			MAO inhibition			
				In vitro		*In vivo*	
No.	Dose, mg/kg po	Epinephrine reversal or suppression[a]	Tyramine potentiation[b]	Concn, mol	% inhibn	% inhibn, brain	% inhibn, heart
17	50	+	++	10^{-5}	34	42 ± 3	39 ± 1
21	25	+	++	10^{-5}	57		
22	25	+	+	10^{-5}	35		
24	15	0	+	10^{-5}	44		
				10^{-6}	15		
27	25	0	++	10^{-5}	38		
				10^{-6}	0		
29	25	+	0	10^{-5}	45		
38[c]				10^{-5}	51	2 ± 2	53 ± 5
				10^{-6}	41		
39	300	0	0	10^{-4}	25		
Pargyline	50	0[d]	+++	10^{-6}	69	62 ± 5	65 ± 2
Hydralazine	1	0	0	10^{-4}	45		
				10^{-5}	0		

[a] Diminished, biphasic, or reversed response, +; no effect on the epinephrine response, 0. [b] Tyramine response >60 mm, +++; 45–60 mm, ++; 30–45 mm, + [control response = 20.4 ± 8.9 (S.D.) mm]. [c] Not tested in normotensive rats. [d] Insignificant potentiation.

The compounds which had elicited a "marked" BP decrease of more than 13% were retested at lower doses to find the minimum effective dose. Almost all compounds tested had at least a modest BP lowering effect in SHR (Table II). At the maximum doses used, only **5**, **32** and **37**, all compounds with large substituents (*p*-methoxyphenyl or dimethylaminopropyl), failed to produce at least a "borderline" antihypertensive effect, defined as a BP decrease of 7–13%. The compounds with "marked" activity usually produced the peak effect 4 hr after the dose; only **21** and **33** were short acting, with the maximum effect at 90 min. The changes in BP observed 24 hr after the doses listed in Table II were not substantial; for example, compounds **8**, **22**, **34**, and **38** elicited 24-hr decreases of only 8–10%. The antihypertensive effects of guanethidine, diazoxide, and pargyline were comparable under identical conditions (Table II).

The compounds listed in Table III were tested in conscious normotensive rats and/or for MAO inhibition. A decrease in normal BP was observed only with the unsubstituted parent compound **17** (50 mg/kg po); the mean BP of 11 treated animals was 16 mm (13%) lower than the average of the normally distributed control population (n = 70), this difference being just less than two standard deviations of the control mean and therefore considered statistically insignificant. Four out of seven compounds tested were shown to reverse or inhibit to various degrees the pressor responses to epinephrine, whereas the responses to tyramine were usually potentiated (except by **29** and **39**). The responses to angiotensin were generally unchanged. Thus, the compounds tested appear to block α-adrenergic receptors; on the other hand, the potentiation of tyramine was suggestive of an inhibition of MAO. This combination of two activities with mutually opposing effects on the BP responses to sympathomimetic amines is difficult to analyze *in vivo*.

A mild MAO inhibiting activity was found *in vitro* for most compounds screened at 1×10^{-5} *M*. The hydrazino derivative **38** was about ten times more active than the other compounds *in vitro;* however, no substantial difference was found between **38** and the unsubstituted **17** in the inhibitory action on the myocardial MAO *in vivo*. The former did not inhibit the brain MAO, probably due to a poor penetration through the blood–brain barrier. Both were less potent inhibitors than pargyline. Hydralazine did not affect MAO at 1×10^{-5} *M in vitro* and no tyramine potentiation was seen in rats treated with the effective antihypertensive dose of hydralazine at 1 mg/kg po.

In conclusion, though significant pharmacological activities on the central nervous system and the cardiovascular system were found with compounds containing the pyrrolo[4,3,2-*de*]isoquinoline ring system, the overall pharmacological profiles detected in these studies are not suggestive of potential clinical usefulness of these agents in the antihypertensive or antidepressant therapy.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and need no correction. Nmr spectra were determined using a Varian A-60A spectrometer and the chemical shifts (δ) are reported in parts per million downfield from TMS. Analyses indicated by elemental symbols were within ±0.4% of the theoretical values and were done by Mr. W. Turnbull and staff of our laboratories.

3-Substituted and 3,4-Disubstituted 1,3,4,5-Tetrahydropyrrolo[4,3,2-*de*]isoquinolines (3–11, Table I). To a solution of **1** or **2** (0.05 mol) in glacial HOAc (70 ml) was added the appropriate aldehyde (0.05 mol). The resulting solution was kept at 22° for 16 hr and then heated on a steam bath for 10 min. Most of the HOAc was removed *in vacuo* and 10% aqueous NaOH solution was added until the mixture was basic. The resulting precipitate was isolated and purified by crystallization from the appropriate solvent.

Methyl 3-Formylindole-4-carboxylate (13). A solution of **12** (17.5 g, 0.1 mol) in ethylene dichloride (75 g) was added to a stirring mixture of *N*-methylformanilide (15.6 g, 0.115 mol) and $POCl_3$ (17.7 g, 0.155 mol). Stirring was continued at 22° for 1.5 hr and then at 50° for 0.5 hr and the mixture was poured into a solution of NaOAc (75 g) in cold water (150 ml). The organic phase afforded an oil which was purified by eluting from a silica gel column with 3% MeOH in $CHCl_3$. The yield was 18.2 g (90%) of a solid. The analytical sample was crystallized from C_6H_6 and had mp 135°. *Anal.* ($C_{11}H_9NO_3$) C, H, N.

Methyl 3-Formylindole-4-carboxylate Oxime (14). A mixture of $NH_2OH \cdot HCl$ (3.47 g, 0.05 mol), NaOAc (4.1 g, 0.05 mol), and **13** (2.03 g, 0.01 mol) dissolved in H_2O (20 ml) and MeOH (20 ml) was heated at 45–55° for 1 hr. The usual work-up procedure af-

forded the product (2.1 g, 96%): mp 178–179° (MeOH–H_2O). *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

Methyl 3-Aminomethylindole-4-carboxylate Hydrochloride (15). A mixture of oxime **14** (1.0 g, 0.0046 mol), 5% Pd/C (0.1 g), and methanolic HCl (55 ml) was hydrogenated at 22° and at atmospheric pressure. The catalyst was removed and the filtrate evaporated. The residue was crystallized from EtOH–Et_2O to give the product (0.846 g, 80%) which decomposes between 220 and 300°. Good analyses could not be obtained for this compound: nmr (D_2O) δ 4.05 (s, 3, $COOCH_3$), 4.28 (s, 2, CH_2NH_2·HCl).

3,4-Dihydropyrrolo[4,3,2-*de*]isoquinolin-5(1*H*)-one (16). (a) Compound **15** (102.7 g, 0.427 mol) in absolute EtOH (1000 ml) was added to a methanolic solution (1000 ml) of NaOMe (from 19.2 g of Na, 0.83 g-atom) and stirred at 22° for 1.5 hr. The solvents were removed *in vacuo* and the residue was treated with cold H_2O. The product was isolated by filtration, washed with H_2O, and dried to afford the crude product (6.2 g, 84%). It was crystallized from EtOH to give material with mp 232–234°: nmr (DMSO-d_6) δ 4.85 (d, 2, J = 1.0 Hz, CH_2), 7.00–7.85 (m, 5, aromatic H's and CONH), 11.2 (s, 1, indolic NH). *Anal.* ($C_{10}H_8N_2O$) H, N; C: calcd, 60.54; found, 57.43.

(b) The oxime **14** (1.6 g, 0.0075 mol) was hydrogenated at 22° and 1 atm in HOAc (60 ml) with PtO_2 (0.12 g). The catalyst was removed and the filtrate evaporated to dryness. The residue was triturated with 10% aqueous NaOH and the resulting solid collected, washed with H_2O, dried, and crystallized from EtOH to give the product (290 mg, 22%).

1,3,4,5-Tetrahydropyrrolo[4,3,2-*de*]isoquinoline (17). The lactam **17** (40 g, 0.23 mol) was added portionwise to diborane in THF (700 ml of a 1 *M* solution) at −5°. The temperature was allowed to rise to 22° and after 3 hr at this temperature, the mixture was refluxed for 3.5 hr. EtOH (360 ml) was added dropwise with cooling and then HCl was bubbled into the mixture while the solvents were removed by distillation. When the volume had been reduced to 200 ml, hot water (2000 ml) was added to give a homogenous solution which was poured into an excess of aqueous NaOH solution. The resultant precipitate was collected, washed with H_2O, and dried to afford the solid product (29.5 g, 80%): mp 240–241°. The HCl salt was obtained crystalline from MeOH and had mp >250°. *Anal.* ($C_{10}H_{12}ClN_2$) C, H, N.

4-Benzyloxycarbonyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (18). Carbobenzoxy chloride (19 g, 0.11 mol) and **17** (7.9 g, 0.05 mol) were combined with Et_3N (23 ml, 0.167 mol) in THF (60 ml). After 16 hr at 22° the usual work-up procedure gave the product, mp 142–144° ($CHCl_3$–Et_2O–hexane), in 64% yield. *Anal.* ($C_{18}H_{16}N_2O_2$) C, H, N.

1-Methyl-4-benzyloxycarbonyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (19). Compound **18** (9.2 g, 0.0314 mol) in THF (100 ml) was added to NaH (5.4 g of a 57% suspension in mineral oil, 0.13 mol) in THF. After 1.5 hr at 22°, methyl iodide (3.2 ml, 0.051 mol) in THF (40 ml) was added during 1 hr. After 1 hr at 22° the usual work-up procedure gave the product as an oil in 96% yield, which was used without further purification.

1-Ethyl-4-benzyloxycarbonyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (20). Compound **18** (7.8 g, 0.0265 mol) was allowed to react with EtI (6.4 ml, 0.08 mol) and NaH (3.6 g of a 57% suspension in mineral oil, 0.085 mol) in THF as described above to give the product: mp 112–114° (85%); nmr ($CDCl_3$) δ 1.4 (t, 3, J = 7 Hz, CH_3), 4.15 (q, 2, J = 7 Hz, CH_2), 4.92 (s, 4, CH_2N), 5.17 (s, 2, $CH_2C_6H_5$), 6.8–7.4 (m, 9, aromatic), which was used without further purification.

1-Methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (21). Compound **19** (9.5 g, 0.031 mol) in MeOH (100 ml) containing 900 mg of 5% Pd/C was treated with hydrogen for 2 hr at 22°. After the usual work-up procedure the product was converted to the HCl salt. It had mp >280° dec (C_6H_6–MeOH–Et_2O) (69% yield). *Anal.* ($C_{11}H_{13}ClN_2$) C, H, N.

1-Ethyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (22). This product was prepared from **20** in 59% yield by the same method used above for **19** and had mp 60–61° (MeOH–H_2O). The HCl salt of **22** had mp 275–276° (MeOH–Et_2O) (59% yield). *Anal.* ($C_{12}H_{15}ClN_2$) C, H, N.

4-Acetyl-1-methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (23). Compound **21** (2.0 g, 0.011 mol) was treated with Ac_2O (2 ml, 0.021 mol) in C_5H_4N (8 ml) at 22° for 16 hr to afford the solid product in 49% yield, mp 125–126°, which was used without further purification: nmr ($CDCl_3$) δ 3.75 (3, s, $COCH_3$), 2.2 (3, s, NCH_3), 4.8 (2, s, NCH_2), 5.0 (2, s, NCH_2); ir ($CHCl_3$) 1640 cm^{-1} (CON).

4-Ethyl-1-methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (24). Compound **23** (1.0 g, 0.0047 mol) was allowed to react with $LiAlH_4$ (0.456 g, 0.012 mol) in THF (25 ml) at 22° for 1 hr. The usual work-up procedure gave the product which was converted directly to the HCl salt. It had mp 249–250° (MeOH–Et_2O) and was obtained in 62% yield. *Anal.* ($C_{13}H_{17}ClN_2$) C, H, N.

4-Carbethoxy-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (25). A mixture of **17** (5.0 g, 0.029 mol), Et_3N (13.2 ml, 0.096 mol), ClCOOEt (5.8 ml, 0.06 mol), and THF (130 ml) was kept at 22° for 2 hr. The crude product, obtained by the usual work-up procedure, was chromatographed on silica gel. Elution with C_6H_6–EtOAc (1:1) gave the product (82.5% yield) as an oil: ir ($CHCl_3$) 1675 cm^{-1} (NCO); nmr ($CDCl_3$) δ 1.3 (3, t, J = 7.5 Hz, CH_2CH_3), 4.2 (2, q, J = 7.5 Hz, CH_2CH_3), 4.9 [4, s, $N(CH_2)_2$].

1-Methyl-4-carbethoxy-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (26). Compound **25** (6.0 g, 0.027 mol) was alkylated with MeI (4.9 ml, 0.078 mol) and NaH (3.3 g of a 57% suspension in mineral oil, 0.078 mol) in THF (60 ml) as described for the preparation of **19**, above. The product obtained was purified by elution from a silica gel column with C_6H_6–EtOAc (9:1) to afford the product (5.0 g, 78.5%): mp 76–78°; nmr ($CDCl_3$) δ 1.25 (3, t, J = 7 Hz, CH_2CH_3), 3.70 (3, s, NCH_3), 4.17 (2, q, J = 7 Hz, CH_2CH_3), 4.88 [4, s, $N(CH_2)_2$]; ir ($CHCl_3$) 1680 cm^{-1} (CONH).

1,4-Dimethyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (27). Compound **26** (5.0 g, 0.02 mol) was reduced in THF (100 ml) with $LiAlH_4$ (2.45 g, 0.066 mol) during 1 hr at 22°. The product was obtained as an oil by elution from a silica gel column with $CHCl_3$–MeOH (9:1). It was converted directly to the HCl salt which had mp 250–251° (MeOH–Et_2O) (50% yield). *Anal.* ($C_{12}H_{15}ClN_2$) C, H, N.

4-Acetyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (28). Acetylation of **17** (9.7 g, 0.056 mol) was carried out with Ac_2O (10 ml, 0.106 mol) in C_5H_4N (30 ml) during 2 hr at 50° to afford the product in 73% yield: mp 205–206° (MeOH). *Anal.* ($C_{12}H_{12}N_2O$) C, H, N.

4-Ethyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (29). Compound **28** (6.6 g, 0.033 mol) was treated with $LiAlH_4$ (6.5 g, 0.172 mol) in THF (400 ml) during 1 hr at 22° to afford the product: mp 183–186° (MeOH–H_2O). It was converted to the HCl salt which had mp 265–266° dec (MeOH–Et_2O) (80% yield). *Anal.* ($C_{12}H_{15}ClN_2$) C, H, N, Cl.

4-(3′-Chloropropionyl)-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (30). 3-Chloropropionyl chloride (4.2 ml, 0.043 mol), **17** (3.24 g, 0.0188 mol), and Et_3N (6.0 ml, 0.043 mol) were allowed to react in $CHCl_3$ during 2 hr at 22° to give, after the usual work-up procedure, the product, mp 158–161° ($CHCl_3$), in 82% yield. *Anal.* ($C_{13}H_{13}ClN_2O$) Cl, N.

4-(3′-Dimethylaminopropionyl)-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (31). A mixture of **30** (4.21 g, 0.0169 mol), Me_2NH·HCl (5.51 g, 0.0676 mol), and KOH (5.1 g, 0.092 mol) in EtOH (300 ml) was stirred at 22° for 16 hr. The resulting precipitate was isolated by conventional means to give the product: 3.92 g (90%); mp 106–107° ($CHCl_3$–Me_2CO–Et_2O). *Anal.* ($C_{15}H_{19}N_3O$) C, H, N.

4-(3′-Dimethylaminopropyl)-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (32). Compound **31** (4.3 g, 0.0167 mol) was reduced with $LiAlH_4$ (3.1 g, 0.082 mol) in THF (200 ml) during 30 min at 22°. A conventional work-up procedure afforded the product which was converted directly to the **2**·HCl salt: mp 260° dec (3.96 g, 74%). *Anal.* ($C_{15}H_{23}Cl_2N_3$) C, H, N.

4-Methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (33). The 4-carbethoxy derivative **25** (0.230 g, 0.001 mol) was added to $LiAlH_4$ (0.152 g, 0.004 mol) in THF (10 ml) at 0°. After 2 hr at 22° a conventional work-up procedure gave the product (0.150 g, 87%): mp 185–186° (Et_2O–hexane). The HCl salt had mp 243–244° (MeOH–Et_2O). *Anal.* ($C_{11}H_{13}ClN_2$) C, H, N.

1-(3′-Dimethylaminopropyl)-4-methyl-1,3,4,5-tetrahydropyrrolo[4,3,2-*de*]isoquinoline (34). Compound **33** (5.9 g, 0.034 mol) and NaH (1.5 g of a 57% suspension in mineral oil, 0.0356 mol) in DMF (100 ml) were warmed at 40° for 1.5 hr. 3-Dimethylaminopropyl chloride (19 g, 0.155 mol) was added with cooling, followed by warming at 40° for 1.5 hr. The usual work-up procedure afforded the product which was converted directly to the 2·HBr salt (7.8 g, 54%) which had mp 255–258° (C_6H_6–MeOH–Et_2O). *Anal.* ($C_{16}H_{25}Br_2N_3$) C, H, N.

5-Ethoxy-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (35). The lactam **16** (8.6 g, 0.05 mol) was added in one portion to a solution of triethyloxonium fluoroborate (12 g, 0.07 mol) in CH_2Cl_2 (500 ml) and the mixture stirred at 22° for 24 hr. The resulting solid was isolated to afford the HBF_4 salt of the product (12.5 g, 87%):

mp 233–234°. The salt (7.7 g) was suspended in MeOH–H_2O (1:1, 200 ml) and saturated aqueous $NaHCO_3$ solution was added at 0° until the mixture was alkaline. The resulting base **35** was collected, washed with H_2O, and dried to give 5.0 g (93.5%) of product: mp 138–140° (MeOH–H_2O). *Anal.* ($C_{12}H_{12}N_2O$) C, H.

5-Amino-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (36). To a suspension of **35** (0.2 g, 0.001 mol) in EtOH (2.0 ml) was added 25% NH_4OH (0.5 ml, 0.0014 mol) followed by 2.3 *N* HCl (0.5 ml, 0.01 mol). The mixture was refluxed for 0.5 hr, ether was added, and the resulting precipitate (200 mg, 98%) of the HCl salt was isolated. It had mp 233–235° (EtOH–EtOAc): nmr (DMSO) δ 5.03 (2, s, CH_2), 7.70 (4, m, aromatic H's), 10.0 (3, m, NH and NH_2). *Anal.* ($C_{10}H_{10}ClN_3$) H, Cl; C: calcd, 57.84; found, 57.43.

5-Hydrazino-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (38). A mixture of $H_2NNH_2 \cdot H_2O$ (3.0 g, 0.06 mol) and **35** (10.0 g, 0.05 mol) in MeOH (100 ml) was refluxed for 16 hr. Et_2O was added and the product (8.1 g, 87%) was obtained as a solid, mp 188–189°, after crystallization from an EtOH–$CHCl_3$–hexane mixture. The HCl salt had mp 310–312° (MeOH). *Anal.* ($C_{10}H_{11}ClN_4$) C, H, Cl.

5-(3′-Dimethylaminopropyl)-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (37). A mixture of **35** (5.0 g, 0.025 mol), 3-dimethylaminopropylamine (2.7 g, 0.026 mol), 2.3 *N* ethanolic HCl (12.5 ml, 0.029 mol), and EtOH (50 ml) was refluxed for 7 hr. Et_2O and excess ethanolic HCl were added to afford a precipitate of the 2·HCl salt of the product (6.9 g, 84%). It had mp 289–290° after crystallization from EtOH–EtOAc. *Anal.* ($C_{15}H_{22}Cl_2N_4$) C, H, N.

4,6-Dihydro-8-methylpyrrolo[4,3,2-*de*][1,2,4]triazolo[3,4-*a*]isoquinoline (39). A mixture of acetylhydrazine (5.0 g, 0.0674 mol) and **35** (9.0 g, 0.0448 mol) was refluxed in anhydrous EtOH (140 ml) for 48 hr under N_2. The EtOH was partially removed and an ether–hexane mixture was added. The resultant solid (9.3 g) was crystallized from MeOH to afford the product (7.3 g, 77%), mp 314–315°. *Anal.* ($C_{12}H_{10}N_4$) C, H, N. By refluxing a mixture of **38**·HCl and Ac_2O for 2 hr and then pouring it into aqueous NaOH solution, compound **39** is also obtained.

5-(Isopropylidinehydrazino)-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (40). The hydrazino derivative **38** (4.5 g, 0.0242 mol) was refluxed in Me_2CO (100 ml) for 10 min. Removal of Me_2CO and crystallization of the residue from a C_6H_6–MeOH–Et_2O mixture gave the product (3.0 g, 55%), mp 200–201°. *Anal.* ($C_{13}H_{14}N_4$) C, H, N.

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Potential Antitumor Agents. 10. Synthesis and Biochemical Properties of 5-*N*-Alkylamino-, *N,N*-Dialkylamino-, and *N*-Alkylacetamido-1-formylisoquinoline Thiosemicarbazones†

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In an attempt to exploit a postulated hydrophobic bonding region at the inhibitor binding site of ribonucleoside diphosphate reductase, several 5-substituted monoalkylamino, dialkylamino, and *N*-alkylacetamido derivatives of 1-formylisoquinoline thiosemicarbazone were prepared. Two of the derivatives demonstrated impressive antitumor activity against Sarcoma 180 ascites cells and several were potent inhibitors of the target enzyme, requiring concentrations in the range of 10^{-6}–10^{-8} *M* for 50% inhibition. 5-Methylamino-1-formylisoquinoline thiosemicarbazone, which was the most effective of the newly synthesized compounds, required a concentration of 3×10^{-8} *M* for 50% inhibition of reductase activity and increased the life span of tumor-bearing mice over untreated animals by a factor of 2.5 at an optimal daily dose of 40 mg/kg. This agent, at a therapeutic dosage level, caused almost complete inhibition of the incorporation of thymidine-^3H into the DNA of Sarcoma 180 cells *in vivo* which was maintained for up to 24 hr after exposure of the cells to the drug; slight but prolonged inhibition of RNA synthesis was also produced as measured by incorporation of uridine-^3H.

Several α-(N)-heterocyclic carboxaldehyde thiosemicarbazones have demonstrated inhibitory activity against transplanted rodent neoplasms,[1-9] spontaneous lymphomas of dogs,[10] and DNA viruses of the Herpes family.[11] The activity of these compounds is apparently due to inhibition of the biosynthesis of DNA[12-17] with the metabolic lesion occurring at the level of reduction of ribonucleotides to deoxyribonucleotides by the enzyme ribonucleoside diphosphate reductase.[11,13,15] From studies on the mechanism by which members of this class inhibit the activity of ribonucleoside diphosphate reductase, it has been postulated that inhibition is due to the coordination of iron by these compounds either by a preformed iron complex binding to the enzyme or by the free ligand complexing with the iron-charged enzyme.[18]

5-Hydroxy-2-formylpyridine thiosemicarbazone (5-HP) was selected as the first representative of this class of compounds for human trial as an antineoplastic agent,[19] because of the water solubility of its sodium salt, as well as its relatively great therapeutic index against animal tumors.[9,10,14] Unfortunately, 5-HP failed to achieve the impressive antineoplastic activity in man that it exhibited in laboratory animals. The reasons for this inactivity in man appear to be in part (a) the relatively low inhibitory potency for the target reductase enzyme [5-HP is approximately 100 times less active at the enzymatic level than

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