Synthesis and Pharmacological Evaluation of Some Pyrrolo[2,1-a]isoquinolines

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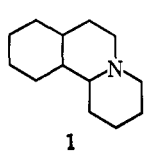
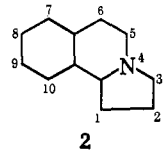
A series of compounds with the pyrrolo[2,1-a]isoquinoline ring system was synthesized by Tschitschibabin cyclization and subsequent transformations. The pharmacological activity of the new compounds was studied.

Organic and medicinal chemists have carried out numerous investigations of the benzo[a]quinolizine ring system (**1**) to elucidate the structures and biosynthetic pathways of the emetine group of alkaloids, and to synthesize amebicidal and psychotherapeutic[1] agents.



1



2

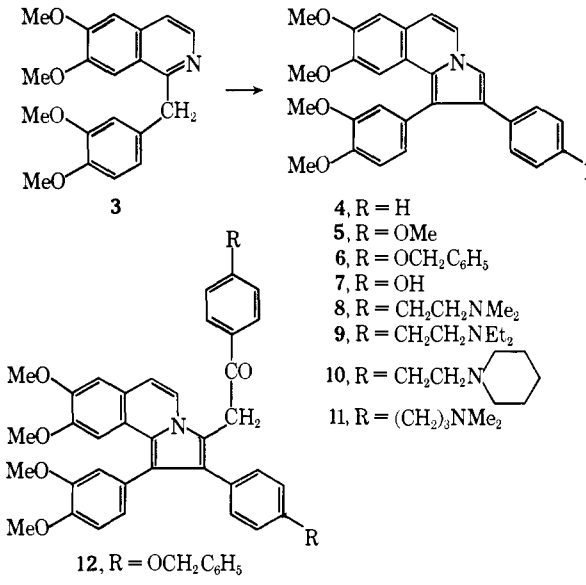
Comparatively few derivatives of the closely related pyrrolo[2,1-a]isoquinoline ring system (**2**) have been synthesized[2] and none of these has been examined for biological activity. The purpose of this study was to synthesize a series of compounds with this nucleus and to investigate their pharmacological activity.

Owing to the lack of information about biological activity, it seemed useful to have a variety of structural types available. We preferred to maintain the 6,7-dimethoxyisoquinoline moiety throughout the series, since this moiety, common to emetine, papaverine, tetrahydropalmatine, metofoline,[3] and related active substances,[1] seems compatible with, if not essential for, strong biological activity.

An extension of the Tschitschibabin synthesis of pyrrocolines[4] to isoquinolines and 3,4-dihydroisoquinolines provided a direct approach to ring system **2** and also allowed the preparation of compounds with the desired structural variety by using appropriate halocarbonyl and isoquinoline starting materials. A few examples of the Tschitschibabin reaction applied to 1-methylisoquinolines had previously been reported.[5]

We prepared three groups of compounds, starting with papaverine (Scheme I), 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline (Scheme II), and ethyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinidene-1-acetate (Scheme III). All cyclizations were carried out in one stage without isolating the quaternary intermediates; equimolecular quantities of reagents and excess sodium bicarbonate were used. A careful choice of conditions was necessary for good, or even reasonable, yields; usually phenacyl bromide reactions provided good yields, but in the case of haloketo esters, chloro derivatives were preferable to the corresponding bromo compounds.

SCHEME I



The reaction of papaverine (**3**, Scheme I) with phenacyl bromide, *p*-methoxy-, and *p*-benzyloxyphenacyl bromide gave **4**, **5**, and **6**, respectively. In preparing **6** on a larger scale, the mother liquors yielded a small amount of a yellow compound, analyzing for $C_{50}H_{43}NO_7$. This compound showed an aromatic ketone band in the ir and gave a dinitrophenylhydrazone. Structure **12** was considered to be highly probable on the basis of the strong nucleophilic character of position 3 of pyrrocoline.[6] The benzyloxy derivative **6** was debenzylated with palladium-on-charcoal catalyst in moist acetone to **7**. Diazomethane methylation of **7** to **5** proved that the nucleus was not hydrogenated under these conditions. Four basic ethers (**8**–**11**) were prepared from **7** sodium salt and *t*-aminoalkyl chlorides.

Reaction of 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline (**23**, Scheme II) and phenacyl bromide gave the 2-phenyl derivative **13**.[7] Hydrogenation of **13**

(1) (a) A. Brossi, H. Lindlar, M. Walter, and O. Schnider, *Helv. Chim. Acta*, **41**, 119 (1958); (b) A. Scriabine, A. Weissman, K. F. Finger, C. S. Delahunt, J. W. Constantine, and J. A. Schneider, *J. Am. Med. Assoc.*, **184**, 276 (1963).

(2) A review has been published by W. L. Mosby, "Heterocyclic systems with bridgehead nitrogen atoms," Interscience Publishers, Inc., New York, N. Y., 1961, p 344. Other recent contributions include V. Prelog, A. Langenmann, O. Rodig, and M. Terubah, *Helv. Chim. Acta*, **42**, 1301 (1959); V. Carelli, F. Liberatore, and F. Morlacchi, *Ann. Chim.* (Rome), **51**, 467 (1961); R. M. Acheson and F. Hole, *J. Chem. Soc.*, 748 (1962); W. Schneider and R. Menzel, *Arch. Pharm.*, **295**, 911 (1962); R. Huisgen, R. Grashey, and E. Steingruber, *Tetrahedron Letters*, 1441 (1963); R. Huisgen and H. Seidl, *ibid.*, 2019, 2023 (1963); A. Mondon, *Tetrahedron*, **19**, 911 (1963); T. Kametani, R. Yanase, and S. Takamo, *Yakugaku Kenkyu*, **37**, 23 (1966); *Chem. Abstr.*, **65**, 15320*b* (1966); F. Zymalkosky and F. Schmidt, *Arch. Pharm.*, **300**, 230 (1967).

(3) A. Brossi, H. Besendorf, B. Pellmont, M. Walter, and O. Schnider, *Helv. Chim. Acta*, **43**, 1459 (1960).

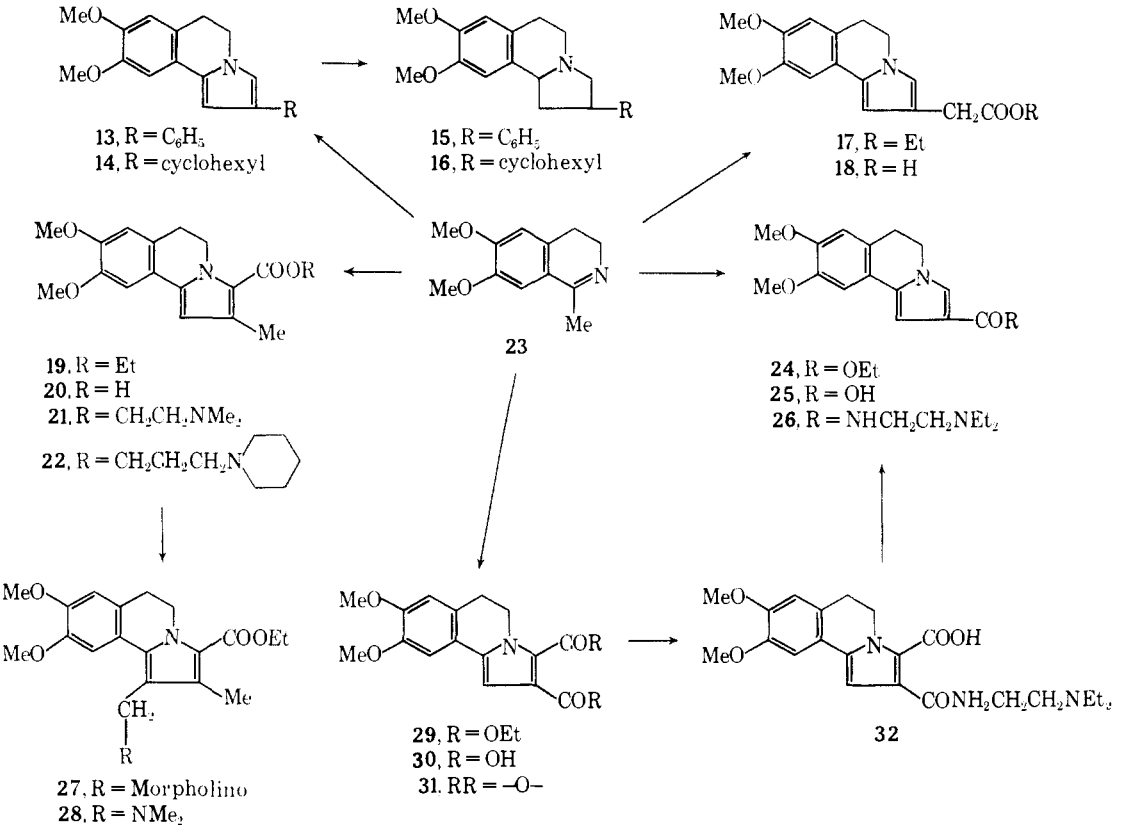
(4) (a) A. E. Tschitschibabin, *Ber.*, **60**, 1607 (1927); (b) W. L. Mosby, ref 2, p 239.

(5) (a) R. H. Sprague, U. S. Patent 2,622,082 (1952); *Chem. Abstr.*, **47**, 3159*a* (1953); (b) J. Thesinger and F. H. Funk, *Chem. Ber.*, **91**, 1546 (1958); (c) Y. Ban and M. Terashima, *Tetrahedron Letters*, 796 (1961).

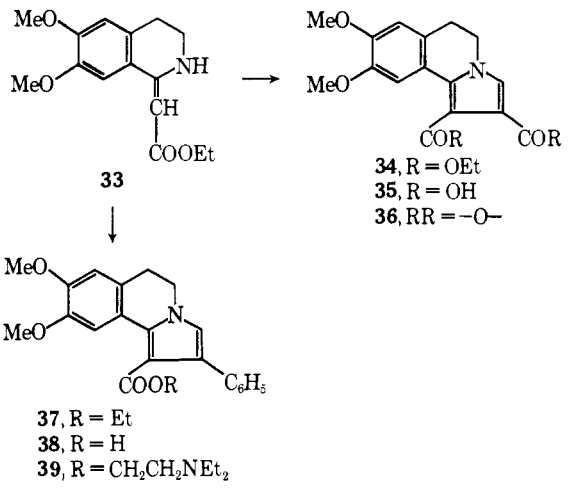
(6) See ref 4b and references cited therein.

(7) Since this work was completed, compound **14** has been reported by S. Sakai, A. Kubo, M. Inaba, M. Katagiri, and K. Tanno, *Yakugaku Zasshi*, **86**, 856 (1966); *Chem. Abstr.*, **65**, 18558*g* (1966).

SCHEME II



SCHEME III



with Raney nickel catalyst at 95–100° and 130 atm afforded the hexahydro (**14**) and decahydro (**16**) derivatives. Structure **14** was confirmed by synthesis from **23** with bromomethyl cyclohexyl ketone. Compound **16** (mol wt 315 by mass spectometry) showed only the characteristic veratrole chromophore in the uv, compared to the more complex strong absorption of compounds with the aromatic pyrrole nucleus (Table I). It did not show NH bands in the ir and was not acetylated by acetic anhydride in pyridine, thus excluding the possibility of hydrogenolysis of the benzylic 4–10b C–N bond. We also obtained **16** by further hydrogenation of **14** under the same conditions. When **13** was hydrogenated using platinum instead of nickel catalyst at 25° and 5 atm, a partial transformation to its tetrahydro derivative **15** took place. Compound **15** behaved like **16** toward acetylation and in the uv, but showed evidence of an unsubstituted phenyl group in the nmr spectrum. Data from ir and nmr spectra were inadequate to clarify the conformations of **15** and **16**.

Reaction of 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline (**23**) with haloketo esters gave carbethoxy-substituted pyrrolo[2,1-*a*]isoquinolines **17**, **24**, and **29** (Scheme II). Alkaline saponification furnished the corresponding acids **18**, **25**, and **30**. The dicarboxylic acid **30** gave the cyclic anhydride **31**, which reacted with N,N-diethylethylenediamine to give the "phthalamic" acid **32**, readily decarboxylated to **26**. The direction of attack on the cyclic anhydride was demonstrated by preparing **26** from **24** with diethylethylenediamine. Some water-soluble derivatives of the already known[5c] 2-methyl-3-carboxylic acid (**20**) were also prepared because ethyl ester **19**, included in the pharmacological screening, had shown hypotensive activity despite its insolubility (see Pharmacology). Owing to the sensitivity of **20** to acids, a common characteristic of this series, preparation of the acid chloride failed under a variety of conditions; however, basic esters **21** and **22** were obtained by transesterification with amino alcohols and sodium methoxide in toluene. Mannich bases **27** and **28** were also prepared and the expected structure was confirmed by the C-1 proton resonance disappearing in the nmr spectrum (τ 3.64 in the parent compound spectrum), while the C-10 proton signal was shifted downfield from τ 2.90 to 1.79. The dicarbethoxy compound **22** did not participate in the Mannich reaction under the same conditions.

A group of compounds (**34**–**39**) (Scheme III) was also prepared from ethyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinidene-1-acetate (**33**) by similar methods.

TABLE I
DERIVATIVES OF PYRROLO[2,1-*a*]ISOQUINOLINES

Compd	Starting material	Method	Yield, %	Recrystn solvent[a]	Mp, °C	Formula	Analyses	Uv spectra[b] λ_{max}, mμ (log ε)
4	3, phenacyl bromide	A	58	EA	183–184	$C_{28}H_{25}NO_4$	C, H, N	332 (3.98), 277 (4.67), 238 (4.48)
5	3, *p*-methoxyphenacyl bromide[c]	A	60	EA	177–179	$C_{29}H_{27}NO_5$	C, H, N	
6	3, *p*-benzyloxyphenacyl bromide[d]	A	48	B	174–176	$C_{35}H_{31}NO_5$	H, N; C[e]	
7	6	...	89	E	195–197	$C_{28}H_{25}NO_5$	C, H, N	
8	7	B	80	E	146–147	$C_{32}H_{34}N_2O_5$	C, H, N	
9	7	B	88	E	148–150	$C_{34}H_{38}N_2O_5$	C, H, N	
10	7	B	71	E–B	170–171	$C_{35}H_{38}N_2O_5$	C, H, N	
11	7	B	75	EA	163–165	$C_{33}H_{36}N_2O_5$	C, H, N	
12	3, *p*-benzyloxyphenacyl bromide[d]	A	5	B	188–189	$C_{50}H_{43}NO_7$	C, H, N	
13	23,[f] phenacyl bromide	A	79	EA	138–140	$C_{20}H_{19}NO_2$	C, H, N	319 (4.29), 286 (4.24), 252 (4.40)
14	23,[f] bromomethyl cyclohexyl ketone[g]	A	40	EA	120–122	$C_{20}H_{25}NO_2$	C, H, N	312[h] (4.19), 298 (4.23)
15	13	...	31	LP	121–123	$C_{20}H_{23}NO_2$	C, H, N	286 (3.54)[i]
16	13	...	47	PE	92–93	$C_{20}H_{29}NO_2$	C, H, N	286 (3.52)[i]
17	23,[f] ethyl γ-chloroacetoacetate[j]	A	40	LP	91–93	$C_{18}H_{21}NO_4$	C, H, N	308[h] (4.15), 296 (4.22)
18	17	C	83.5	E	159–160	$C_{16}H_{17}NO_4$	C, H, N	
21	19[k]	F	70	LP	98–99	$C_{20}H_{26}N_2O_4$	C, H, N	
22	19	F	73	EA	102–104	$C_{24}H_{32}N_2O_4$	N	307[h] (4.13), 286 (4.29)
24	23,[f] ethyl chloropyruvate[l]	A	28	E	111–113	$C_{17}H_{19}NO_4$	C, H, N	
25	24	C	91	E	232–234 dec	$C_{15}H_{15}NO_4$	C, H, N	
26	31	...	58	T	144–146	$C_{21}H_{29}N_3O_3$	C, H, N	
27	19	G	45	LP	154–155	$C_{23}H_{30}N_2O_5$	N	
28	19	G	38	LP	111–112.5	$C_{21}H_{28}N_2O_4$	N	
29	23, ethyl chlorooxalacetate[m]	A	75	E–W	90–92	$C_{20}H_{23}NO_6$	C, H, N	318 (4.20), 282 (4.06), 246 (4.29)
30	29	D	95	DMF–E	228–230 dec	$C_{16}H_{15}NO_6$	N	
31[n]	30	E	65	D	239–240	$C_{16}H_{13}NO_5$	C, H, N	
32	31	...	73.5	B	170–172	$C_{22}H_{29}N_3O_5$	N, COOH[o]	
34	33,[p] ethyl chloropyruvate[l]	A	25	E–LP	138–139	$C_{20}H_{23}NO_6$	C, H, N	317 (4.21), 290 (4.15)
35	34	D	61	E	228–230	$C_{16}H_{15}NO_6$	N	
36	35	E	74	DMF	286–287 dec	$C_{16}H_{13}NO_5$	C, H, N	
37	33, phenacyl bromide	A	75	EA	172–174	$C_{23}H_{23}NO_4$	C, H, N	330 (4.32), 219 (4.48)
38	37	C	97	DMF–E	209–211	$C_{21}H_{19}NO_4$	N	
39	37	F	72	EA	137–139	$C_{25}H_{28}N_2O_4$	N	

[a] B = benzene, D = dioxane, DMF = dimethylformamide, E = ethanol, EA = ethyl acetate, LP = petroleum ether (bp 80–120°), PE = petroleum ether (bp 40–70°), T = toluene, W = water. [b] In absolute EtOH. [c] F. Kröhnke and K. Ellegast, *Chem. Ber.*, **86**, 1556 (1953). [d] H. M. Priestley and E. Moness, *J. Org. Chem.*, **5**, 355 (1940). [e] C: calcd, 77.0; found, 76.3. [f] E. Späth and N. Polgar, *Monatsh. Chem.*, **51**, 197 (1929). [g] F. Asinger, M. Thiel, G. Peschel, and K. H. Meinicke, *Ann. Chem.*, **619**, 145 (1958). [h] Shoulder. [i] Homoveratrilamine 280 (3.41), 229 (3.89); salsolidine 286 (3.54). [j] J. F. Hamel, *Bull. Soc. Chim. France*, **29**, 396 (1921). [k] See ref 8. [l] J. Parrod, *Compt. Rend.*, **218**, 600 (1944). [m] P. Bouvier and H. Gault, *Bull. Soc. Chim. France*, 711 (1963). [n] Ir (KBr disk), 1845–1825 and 1775 cm^{-1}. [o] Titration with $LiOCH_3$ in C_6H_6–MeOH. [p] A. R. Battersby, H. T. Openshaw, and H. C. S. Wood, *J. Chem. Soc.*, 2465 (1953); N. A. Nelson, K. O. Gelotte, Y. Tamura, H. B. Sinclair, J. M. Schuck, W. J. Bauer, and R. W. White, *J. Org. Chem.*, **26**, 2599 (1961).

Both 1,2- and 2,3-dicarboxylic acids (**30**, **35**) were readily decarboxylated to mono-2-carboxylic acid **25** near their melting points. The reactions outlined in Schemes II and III with the correlations and physical data described demonstrate the normal course of Tschitschibabin reaction between isoquinolines and haloketo esters in the cases investigated.[8]

Pharmacology.—All compounds soluble as such, or as salts, were submitted to a group of pharmacological tests *in vitro* and *in vivo*. Insoluble compounds were tested only *in vivo* as carboxymethylcellulose suspensions. Compounds showing significant activity and their pertinent tests are listed in Table II. No compound had analgetic[9] or anesthetic[10] effects in the tail-clip tests or effects on the rats reactive motility in the open-field test.[11] Compound **15** showed α-adrenergic blocking activity. Compounds **10**, **15**, **21**, and **22** compared favorably with papaverine as smooth muscle relaxants. Compounds **16** and **32** were considered worthy of a further investigation. At a dose of 2–4 mg/kg iv **16** inhibited atrial fibrillation induced by local application of acetylcholine in the dog heart *in situ*[12]

(8) Reaction between ethyl 2-pyridylacetate and ethyl chloropyruvate had raised some doubts: D. R. Bragg and D. G. Wibberley, *J. Chem. Soc., C*, 2120 (1966).

(9) C. Bianchi and G. Franceschini, *Brit. J. Pharmacol.*, **9**, 280 (1954).

(10) C. Bianchi, *ibid.*, **11**, 104 (1956).

(11) P. L. Broadhurst in "Experiments in Personality," Vol. 1, H. J. Eysenck, Ed., Routlegde and Kegan, London, 1960, p 30.

(12) G. Marchetti, L. Merlo, L. Lombardi, and M. Cicardi, *Arch. Ital. Sci. Farmacol.*, **14**, 33 (1964).

TABLE II
PHARMACOLOGICAL ACTIVITY OF SOME PYRROLO[2,1-*a*]ISOQUINOLINES

Compd	Rabbit atrium: Neg inotropic effect[a]	Rabbit atrium: Epinephrine inhib[b]	Guinea pig ileum: Anticholinergic effect[c]	Guinea pig ileum: Spasmolytic effect[d]	Rabbit uterus[e]	Blood pressure[f]: mg/kg	Blood pressure[f]: % change	Spontaneous motility[g]: mg/kg po	Spontaneous motility[g]: reaction	LD_{50}[h]: mg/kg iv
8	25	10	7.4	>10	>10	10	0	50	0	20
9	20	>10	8	10	>10	10	0	10–50	+	12
10	25	>10	7.4	5	>10	10	0	50	0	18
11	...	5	14	>10	>10	10	0	50	0	25
15	20	>10	20	8	0.01	...	...	25	++	...
16	7	>10	0.1	>10	3.5	5	−30	25	+++	20
19	...	...	...	...	...	30[i]	−37	50	+	...
21	>50	>10	13	8	5	50	0	...	...	13
22	30	>10	25	7	10	10	−40	50	+	11
27	5	>10	40	20	>10	40	0	50	0	30
28	35	>10	20	10	...	10	0	...	...	35
30	>50	...	>50	...	...	10	−23	...	...	400
32	>50	>10	>50	>10	>10	5	−25	50	+	600
						20	−40			
Pronethalol	16	0.5	...	...	...	...	...			
Atropine sulfate	...	...	0.001	...	...	...	...			
Papaverine HCl	...	...	...	8	...	...	...			
Phentolamine HCl	...	...	...	...	0.01	...	...			

[a] Concentrations (μg/ml) reducing spontaneous atrial contraction to 50%. [b–e] Approximate ED_{50} values (μg/ml) against epinephrine, 0.5 μg/ml; acetylcholine bromide, 0.1 μg/ml; $BaCl_2$, 100 μg/ml; and epinephrine, 1 μg/ml, respectively. [f] Doses (iv) and effects on femoral arterial pressure of anesthetized dogs. [g] Intraduodenal administration in 0.5% carboxymethylcellulose suspension. [h] Reduction of spontaneous motility of mice in Jaquet oscillating cages: +, slight; ++, moderate; +++, strong. [i] Approximate values of acute toxicity in mice.

and protected rats from $CaCl_2$-induced ventricular arrhythmia.[13] This compound also blocked hypertensive response to epinephrine (0.1 μg/kg iv) in pithed rats (ED_{50} 3 mg/kg iv) and protected aggregated mice against amphetamine toxicity (ED_{50} 30 mg/kg ip). An oral dose of 50 mg/kg decreased charcoal propulsion[14] in the rat intestine to 40% of the control value. The hypotensive effect in dogs induced by **32** (see Table II) lasted 1–2 hr. In rabbits, doses of 50–60 mg/kg iv lowered arterial pressure to 60–50% of control values for 1.5–2 hr. Compound **32** showed very low toxicity in laboratory animals. The acute LD_{50} in rats and dogs was greater than 1 g/kg *po*. Subacute studies in rats at a dose of 100 mg/kg *po* daily for 30 days showed no toxic effects on weight, behavior, and major organs.

In order to investigate its mechanisms of action, **32** was administered to anesthetized dogs at 5–20 mg/kg iv. The contractile force of the heart and the rate of increase in ventricular pressure (dp/dt) were measured according to the techniques described by Bergamaschi[15] and Veragut and Krayenbühl.[16] Cardiac output and coronary flow were measured using electromagnetic flowmeters (Biotronex BL 610) implanted around the aorta and circumflex branch of the left coronary artery.[17] In all the experiments there was an increase in contractile force, dp/dt, cardiac output, and coronary flow, while peripheral and coronary resistances were reduced.[18]

(13) M. R. Malinow, F. F. Battle, and B. Malamud, *Arch. Intern. Pharmacodyn.*, **102**, 226 (1955).
(14) D. I. Macht and J. Barba-Gose, *J. Am. Pharm. Assoc.*, **20**, 558 (1931).
(15) M. Bergamaschi, A. H. Glässer, and L. Valentini, *Farmaco, Ed. Prat.*, **18**, 269 (1963).
(16) V. P. Veragut and H. P. Krayenbühl, *Cardiologia*, **47**, 96 (1965).
(17) G. V. Marchetti, L. Merlo, and V. Noseda, *Arch. Ges. Physiol.*, **298**, 200 (1968).

TABLE III
REACTIONS BETWEEN ISOQUINOLINES AND HALO KETONES

Compd	Time, hr	Temp, °C	Isolation procedure
4	2	r[a]	a
5	2	r	a
6	2	r	a
13	3	r	a
14	2	45	b
17	3.5	55	a
24	5	35	b
29	2.5	55	a
34	2.5	r	b
37	2	r	a

[a] Refluxing EtOH.

On the basis of the above findings, further chemical and pharmacological investigations are in progress on compound **32** and its analog, as well as on the analogs of **15** and **16**.

Experimental Section[19]

Melting points were taken in capillaries and are uncorrected. Uv, ir, nmr spectra and the mass spectrum were obtained, respectively, with a Hitachi-Perkin-Elmer spectrophotometer, a Perkin-Elmer Model 237 spectrophotometer, a Perkin-Elmer Model R 10 instrument, and a LKB gas chromatograph–mass spectrometer.

Reactions between Isoquinolines and Halo Ketones (Method A).—The appropriate phenacyl bromide (0.05 mole) or chloroketo ester (0.055 mole) was added to 0.05 mole of the appropriate isoquinoline and 0.15 mole of $NaHCO_3$ in 130 ml of absolute EtOH with continuous stirring. The mixture was stirred under the conditions indicated in Table III and the product was isolated as follows: (a) after cooling, the precipitate was filtered,

(18) A complete paper on these hemodynamic experiments is to be published elsewhere.
(19) Where analyses are indicated only by symbols of the elements or functions, analytical results were within 0.4% of the theoretical value.

washed (EtOH, water) to dissolve the salts, and then dried and recrystallized; (b) the EtOH was evaporated under reduced pressure to half volume and H_2O (60 ml) was added. After 3–5 hr at 0°, the precipitate was filtered, washed (EtOH–H_2O, H_2O), then dried, and recrystallized.

In a preparation of **6** on a 0.5-mole scale a yellow precipitate, mp 158–163°, was obtained from the C_6H_6 mother liquors of the recrystallization, by concentrating to half-volume and diluting with EtOH. This product, when recrystallized twice from C_6H_6 gave 9.35 g of **1-(3,4-dimethoxyphenyl)-2-(*p*-benzyloxyphenyl)-3-(*p*-benzyloxyphenacyl)-8,9-dimethoxypyrrolo[2,1-*a*]isoquinoline** (**12**): mp 188–189°; ir spectrum (KBr disk), 1685 cm^{-1} (C=O). *Anal.* ($C_{50}H_{43}NO_7$) C, H, N.

The 2,4-dinitrophenylhydrazone had mp 139–140° dec (EtOH). *Anal.* ($C_{56}H_{47}N_5O_{10}$) N.

When **23** and diethyl chlorooxalacetate were mixed in absolute EtOH, a crystalline addition compound was immediately formed, mp 93–94°. *Anal.* ($C_{12}H_{15}NO_2 \cdot C_8H_{11}ClO_5$) C, H, Cl, N. This compound did not contain Cl^-. Hydrogenation in 50% aqueous EtOH with PtO_2 at room temperature afforded salsolidine hydrochloride (6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline) and ethyl malate, both identical with authentic samples (ir comparison).

1-(3,4-Dimethoxyphenyl)-2-(*p*-hydroxyphenyl)-8,9-dimethoxypyrrolo[2,1-*a*]isoquinoline (**7**).—Two grams of 10% Pd–C moistened with 4 ml of H_2O was added to a solution of 10 g of benzyloxy compound **6** in 350 ml of Me_2CO. The mixture was hydrogenated in a Parr apparatus under 5 atm. The absorption of H_2 was completed in 6 hr. The solution was shaken under H_2 for another hour, then filtered and slightly acidified with an Et_2O solution of dry HCl giving 8 g (89%) of yellow **7**·HCl, mp 209–212°. An analytical sample had mp 220–223° (DMF–Me_2CO). *Anal.* ($C_{28}H_{25}NO_5 \cdot HCl$) N, Cl.

The hydrogenation did not affect the ring system; a sample of **7** in MeOH with ethereal CH_2N_2 gave the methoxy compound **5** (mixture melting point and ir comparison). The hydrochloride with Na_2CO_3 solution yielded free **7**, mp 195–197° (EtOH). *Anal.* ($C_{28}H_{25}NO_5$) C, H, N.

1-(3,4-Dimethoxyphenyl)-2-(*p*-dialkylaminoalkoxyphenyl)-8,9-dimethoxypyrrolo[2,1-*a*]isoquinolines (Method B).—The dialkylaminoalkyl ethers (**8–11**) were obtained as follows. Finely powdered **7**·HCl (9.82 g, 0.02 mole), suspended in 200 ml of dry PhMe, was treated with 0.06 mole of NaOMe (46 ml of 7% solution in dry MeOH). After stirring for 10 min, 0.02 mole of dialkylaminoalkyl chloride hydrochloride was added. The MeOH was distilled under reduced pressure and the mixture was heated at 100° with stirring for 3 hr, then cooled and extracted with 10% AcOH. The acid extracts were made alkaline with NaOH and extracted with $CHCl_3$. The extracts were washed (H_2O), dried (Na_2SO_4), and then evaporated. The residue was purified by crystallizing as indicated.

1,2,3,5,6,10b-Hexahydro-2-cyclohexyl-8,9-dimethoxypyrrolo[2,1-*a*]isoquinoline (**16**).—Compound **13** (80 g) in 400 ml of absolute EtOH and 400 ml of dioxane was hydrogenated in an autoclave with stirring at 95–100° under 130 atm of pressure for 80 hr with 25 g of Raney Ni. The catalyst was filtered and the solvent was evaporated under reduced pressure. The residue was taken up with 10% AcOH and filtered. The insoluble fraction consisted of 5,6-dihydro-8,9-dimethoxy-2-cyclohexylpyrrolo[2,1-*a*]isoquinoline (**14**) (see below). The acidic filtrate was slightly alkalinized with NH_4OH and extracted with $CHCl_3$. The extracts were dried (Na_2SO_4) and evaporated. The residue was distilled at 160–185° (0.002 mm). Crystallizing from petroleum ether (bp 30–70°) gave 39 g (47%) of **16**, mp 79–81°. Further crystallization did not increase the melting point; purification was achieved through the hydrogen sulfate or the hydrobromide perbromide. The hydrogen sulfate salt precipitated from EtOAc and recrystallized (EtOH–Me_2CO); mp 170–171°. *Anal.* ($C_{20}H_{29}NO_2 \cdot H_2SO_4$) N.

The hydrobromide perbromide, a yellow powder, was obtained from AcOH with Br_2 and anhydrous HBr and washed by trituration with CH_3OH; mp 144–146° dec. *Anal.* ($C_{20}H_{29}NO_2 \cdot HBr_3$) N, Br.

The pure base **16** was obtained from these salts and crystallized from petroleum ether (bp 30–70°); mp 92–93°. In the ir, the compound in $CHCl_3$ solution did not show Bohlmann bands[20] in the 2700–2800-cm^{-1} region. Resonance of the angular 10b proton could be located at τ 6.0, although partly masked by the strong CH_3O peak.[21] The compound was not acetylated by Ac_2O–pyridine; it gave a methiodide, mp 237–239°. *Anal.* ($C_{21}H_{32}INO_2$) C, H, N; I: calcd, 27.7; found, 27.1.

5,6-Dihydro-2-cyclohexyl-8,9-dimethoxypyrrolo[2,1-*a*]isoquinoline (**14**).—The AcOH-insoluble product, obtained in the preparation of **16**, was dried and recrystallized (EtOAc), yielding 24.5 g (30%) of **14**; mp 122–123.5°. This was identical with the product obtained by cyclization (method A) (mixture melting point, ir). Further hydrogenation of pure **14** under the same conditions gave **16**. However, application of more drastic conditions in the hydrogenation of **13** did not improve the yield of **16**, owing to the formation of a large amount of an oily product, perhaps hydrogenolyzed, which was not further investigated.

1,2,3,5,6,10b-Hexahydro-2-phenyl-8,9-dimethoxypyrrolo[2,1-*a*]isoquinoline (**15**).—Compound **13** (5 g) in 400 ml of AcOH was hydrogenated with 0.8 g of PtO_2 in a Parr apparatus for 25 hr at 25° under 5 atm. After filtering the catalyst, the solution was evaporated to a small volume at 50° under reduced pressure and then diluted with water. An insoluble fraction, separated by filtration, consisted mainly of unreacted **13**. The acid filtrate was alkalinized with NH_4OH and extracted with $CHCl_3$. The extracts were washed (H_2O), dried, and then evaporated. The residue was crystallized from petroleum ether (bp 80–120°), yielding 1.6 g (31.6%) of **15**, mp 115–117°. Further recrystallization from the same solvent gave an analytical sample, mp 121–123°. The ir and nmr spectra of **15** showed features similar to those of **16** as regards Bohlmann bands[20] and 10b proton resonance.[21] The ir spectrum (KBr disk) showed a band at 705 cm^{-1} which was present in the spectrum of phenyl derivative **13**, but not in those of cyclohexyl derivatives **14** and **16**. The nmr spectrum showed a peak at τ 2.67 (5 H). When this hydrogenation was carried out at 45–50° (80 atm), **15** underwent further hydrogenation and **16** was formed. Compound **15** was unaffected by Ac_2O in pyridine at room temperature.

8,9-Dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-acetic Acid (**18**) **(Method C).**—Compound **18** was obtained by refluxing a solution of 5 g of ester **17** and 5 g of KOH in 100 ml of EtOH for 2.5 hr, then diluting with H_2O to a clear solution, acidifying to pH 4.5 with AcOH, filtering, and recrystallizing (absolute EtOH); yield 3.8 g (83.5%), mp 159–160° dec.

8,9-Dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2,3-dicarboxylic Acid (**30**) **(Method D).**—A solution of 37.3 g (0.1 mole) of **29** in 1500 ml of 5% ethanolic NaOH was refluxed for 2.5 hr. The product precipitated as a disodium salt. Most of the solvent was evaporated under reduced pressure and the residue was dissolved in H_2O. The solution was filtered and acidified at 10° with dilute HCl to pH 2. The precipitated product was collected and crystallized (DMF–EtOH) giving 30 g (95%) of **30**, mp 228–230° dec.

When **30** was heated at 200° for 20 min, the monocarboxylic acid **25** was formed. This was identical with an authentic sample both by mixture melting point and ir comparison. The isomeric diacid **35** was decarboxylated to **25** in the same way.

8,9-Dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2,3-dicarboxylic Anhydride (**31**) **(Method E).**—Compound **30** (31.7 g) in 100 ml of Ac_2O and 600 ml of dry PhMe was heated to reflux with stirring. The acid dissolved and the anhydride began to separate as yellow plates within 1 hr. After 3 hr the mixture was cooled. The precipitate was filtered and recrystallized from dry dioxane giving 20.6 g (65%) of **31**: mp 239–240°; ir (KBr disk), 1860, 1770 cm^{-1}.

8,9-Dimethoxy-5,6-dihydro-2-(N-diethylaminoethylcarbamoyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylic Acid (**32**).—A mixture of 15 g (0.05 mole) of **31** and 5.8 g (0.05 mole) of N,N-diethylethylenediamine in 300 ml of dry C_6H_6 was refluxed with continuous stirring for 5 hr. The yellow anhydride color disappeared and a white crystalline precipitate was formed. This was filtered and washed with hot EtOAc, giving 15.3 g (73.5%) of **32**, mp 166–168°. An analytical sample, crystallized from C_6H_6 and EtOH–Et_2O, showed mp 170–172°, ir (KBr disk) bands between 1640 and 1550 cm^{-1}.

N-Diethylaminoethyl-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carboxamide (**26**). (a) Compound **32** (5 g) was decarboxylated in an oil bath at 180° in 1.5 hr. The dark residue was triturated with H_2O, dried, and crystallized from EtOH–Et_2O (charcoal) and PhMe, giving 2.6 g (58%) of **26**, mp

(20) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).

(21) M. Uskokovic, H. Bruderer, C. von Planta, T. Williams, and A. Brossi, *J. Am. Chem. Soc.*, **86**, 3364 (1964).

144–146°. An analytical sample, recrystallized twice from PhMe, had mp 147–148°.

(**b**) Compound **24** (5 g) was added to a solution of 0.4 g of Na in 10 g of N,N-diethylethylenediamine. The mixture was heated at 125–130° for 7 hr. A little H_2O was added and most of the amine was distilled under reduced pressure. The residue was taken up in 10% AcOH. After filtering, the filtrate was alkalinized with NH_4OH and extracted with $CHCl_3$. The extracts were washed (H_2O), dried (Na_2SO_4), and evaporated; the residue was triturated with Et_2O and crystallized (PhMe) giving **26**, mp 142–143°. This was identical with the compound obtained by decarboxylation (mixture melting point, ir).

N,N-Dimethylaminoethyl 8,9-Dimethoxy-5,6-dihydro-2-methylpyrrolo[2,1-*a*]isoquinoline-3-carboxylate (21) (Method F).—A solution of 15.7 g (0.05 mole) of ethyl 8,9-dimethoxy-5,6-dihydro-2-methylpyrrolo[2,1-*a*]isoquinoline-3-carboxylate (**19**)[5c] and 7 g (0.078 mole) of dimethylaminoethanol in 230 ml of PhMe was placed in the flask at the bottom of a Fenske-Todd column and made anhydrous by distilling until the boiling point reached 110°. Then 7.8 mmoles of NaOEt (4 ml of a 13% solution in anhydrous EtOH) was added and the solution was refluxed while distilling the EtOH (4 hr). The solution was cooled, washed (H_2O), and extracted with 10% AcOH. The acid extracts were alkalinized with NH_4OH and extracted with $CHCl_3$, and the extracts were dried and evaporated. The residue was crystallized from petroleum ether (bp 80–120°) giving 12.6 g (70%) of **21**, mp 98–99°. The hydrochloride had mp 252–255° (EtOH–Et_2O). *Anal.* ($C_{20}H_{26}N_2O_4 \cdot HCl$) Cl, N.

Ethyl 1-Morpholinomethyl-2-methyl-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (27) (Method G).—A solution of 6.3 g (0.02 mole) of **19**[5c] and 1.9 g (0.022 mole) of a 30% (w/v) solution of formaldehyde in 125 ml of AcOH was kept at 60° for 3 hr, then cooled, diluted (H_2O) to 600 ml, filtered, made basic with NH_4OH, and extracted with Et_2O. The extracts were dried (Na_2SO_4) and evaporated; the residue was crystallized from petroleum ether (bp 80–120°) giving 3.8 g (45%) of **27**: mp 154–155°; nmr, τ 1.79, 3.17, 7.62; **19**: nmr, τ 2.90, 3.21, 3.64, 7.59.

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Urinary Metabolites of 7-Chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one Dihydrochloride

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Metabolites of the title compound (Ia) extracted from human and dog urine were characterized by a combination of tlc and high-resolution mass spectrometry and were compared with authentic compounds prepared as described in the immediately following publication.[1] Only one metabolite, 7-chloro-1-(2-hydroxyethyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one (IV), was detected as a glucosuronic acid and/or sulfate conjugate in urine of two human subjects given 60 mg of Ia orally. It was estimated that one subject excreted roughly 25% of the administered drug in the first day as conjugated IV. Chronic oral administration of 40 mg/kg of Ia to a dog resulted in the urinary excretion of nonconjugated I, 7-chloro-1,3-dihydro-1-(2-ethylaminoethyl)-5-(2-fluorophenyl)-2H-1,4-benzodiazepin-2-one (II), and 1-(2-aminoethyl)-7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzodiazepin-2-one (III), and the excretion of conjugated II–IV, 7-chloro-5-(2-fluorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one (VI), and a phenolic derivative of 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one whose structure was not definitively established. In addition, 8-chloro-6-(2-fluorophenyl)-1,2-dihydro-4H-imidazo[1,2-*a*][1,4]benzodiazepine (VII) was shown to be an artifact resulting from tlc of III.

The synthesis and a comparison of the pharmacology of 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride (Ia) with that of other 1,4-benzodiazepines has been reported[2] as well as the clinical use of this compound as a hypnotic.[3]

The combination of thin layer chromatography (tlc) for metabolite isolation and purification and high-resolution mass spectrometry for characterization has been used to identify the metabolites in the rat of ^{3}H-labeled diazepam[4] and ^{14}C-labeled chlordiazepoxide.[5] In the present study these combined techniques were utilized to identify one metabolite of unlabeled Ia in human urine and five metabolites in dog urine.

Experimental Section

Urine Specimens.—The human urine was obtained from two female subjects who each received a 60-mg oral dose of Ia. Two collections were made; one consisted of the 24-hr urine excreted prior to drug administration (control urine) and the other was the urine excreted during the first day after dosing.

The dog urine was obtained from one animal which had received an oral dose of 40 mg/kg of Ia (in a gelatin capsule) daily for 6 months and from another which had not received the drug. Both of these samples were approximately 100 ml.

Isolation Procedures.—Each urine sample was first fractionated by solvent extraction. The urine adjusted to pH 9.0 with 1 *N* NaOH was extracted twice with equal volumes of ether. The combined ether extract which contained any I present plus neutral and basic nonpolar metabolites was concentrated to an oil and brought to 1 ml with EtOH. The aqueous phase was then adjusted to pH 7.0 with 1 *N* HCl and extracted twice with equal volumes of EtOAc to remove neutral but more polar metabolites. The combined EtOAc extract was dried (Na_2SO_4) and after evaporation of the solvent was brought to 1 ml with EtOH and designated "EtAc B.G." Now the aqueous phase was adjusted to pH 5.5, incubated for 3 hr at 37° with Glusulase[6]

(1) J. V. Earley, R. I. Fryer, D. Winter, and L. H. Sternbach, *J. Med. Chem.*, **11**, 774 (1968).

(2) (a) L. H. Sternbach, G. A. Archer, J. V. Earley, R. I. Fryer, E. Reeder, N. Wasyliw, L. O. Randall, and R. Banziger, *ibid.*, **8**, 815 (1965), designated as **12**. (b) See G. Zbinden and L. O. Randall, *Advan. Pharmacol.*, **5**, 254, 258 (1967), for animal pharmacology and clinical pharmacology of this compound which is designated Ro 5-6901.

(3) H. Jick, *Curr. Therap. Res.*, **9**, 355 (1967). Compound is referred to as Ro 5-6901.

(4) M. A. Schwartz, P. Bommer, and F. M. Vane, *Arch. Biochem. Biophys.*, **121**, 508 (1967).

(5) M. A. Schwartz, F. M. Vane, and E. Postma, *Biochem. Pharmacol.*, in press.

(6) Product of Endo Labs Inc., Garden City, N. Y., which contains 100,000 units of β-glucoronidase and 50,000 units of sulfatase/ml.