OCH_3), 6.70 (2 H, s, NH_2), 7.33 (2 H, d, 3',5'-Ar H), 7.60 (2 H, m, NH_2), 7.83 (2 H, d, 2',6'-Ar H), 8.37 (1 H, s, C-7 H). Anal. $C_{18}H_{20}N_6O_2 \cdot {}^1/_2H_2O$ (C, H, N).

The 10-methyl compound **12a** was similarly obtained from **11a** in 87% yield. Anal. $C_{18}H_{20}N_6O_2 \cdot {}^3/_4H_2O$ (C, H, N).

Amino-4-deoxy-10-ethyl-10-deazapteroic Acid (13b). A solution of 1.17 g of the diamino ester **12b** in 36 mL of 2-methoxyethanol was warmed to 100 °C and 2.71 mL of 10% NaOH was added. Heating was continued for 15 min and the solvent was evaporated in vacuo. The residue was dissolved in 35 mL of H_2O and adjusted to pH 5–6 with concentrated HCl. The precipitate was collected, washed with H_2O and EtOH, and dried to leave 0.90 g (80%) of pale yellow crystals; HPLC (C_{18} Bondapak reverse phase, MeOH–0.1 M KH_2PO_4 (pH 6.7), 1:3) 98% pure; UV max at pH 13 235 nm, 255, 370; NMR (Me_2SO-d_6) δ 0.74 (3 H, t, CH_3), 1.70 (2 H, m, CH_2CH_3), 3.10 (3 H, m, C-9,10 H's), 6.56 (2 H, s, NH_2), 7.31 (2 H, d, 3',5'-Ar H), 7.50 (2 H, br s, NH_2), 7.80 (2 H, d, 2',6'-Ar H), 8.34 (1 H, s, C-7 H). The HPLC, UV, and NMR spectra were identical with those measured for **13b** previously reported.[1]

The 10-methyl analogue **13a** was similarly prepared from the ester **12a** in 84% yield. The HPLC, UV, and NMR spectra were also equal to the previously reported material.

Coupling of the acids **13a,b** with diethyl L-glutamate and saponification of the intermediate esters **14a,b** to yield the resolved diastereomeric acids **15a,b** was carried out via procedures reported in ref 1.

Acknowledgment. This work was supported by NIH Grants CA-28783 (J.I.D.), CA-18856 (F.M.S.), CA-22764 (F.M.S.), CA-10914 (R.L.K.), and the Elsa U. Pardee Foundation (F.M.S.).

Synthesis and Dopaminergic Activity of *trans*-6-Methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline and *trans*-1,2,3,4,4a,5,6,10b-Octahydro-4,7-phenanthroline Derivatives

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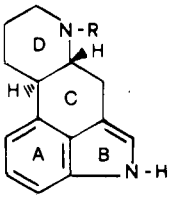
Received November 19, 1984

The synthesis and dopamine agonist activity of some derivatives of *trans*-6-methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (**6a–c**) are reported. These compounds can be regarded as analogues of ergoline derivatives with the indole nucleus replaced by indolizine. These congeners have been evaluated as inhibitors of prolactin release in vivo. *trans*-6-Methyl-8-ethyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (**6b**) proved to produce a dose-dependent inhibition of serum prolactin that was almost complete at the highest dose employed. Although effective, this compound was far less potent than bromocriptine. The 8-propyl derivative **6c** was weakly active only at very high doses, and the 8-methyl derivative **6a** proved to be completely ineffective. *trans*-4-Propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (**7**), a molecular simplification of hexahydropyrrolo-4,7-phenanthroline, proved to be the most potent among the newly synthesized compounds. These results, taken together with those of previous studies, suggest that the presence of the nitrogen of the indolizine nucleus and the N-7 in the octahydro-4,7-phenanthroline **7** are significant for the interaction with the dopamine receptor involved in the control of prolactin release.

A large variety of ergoline (1) derivatives, such as bromocriptine,[1] lisuride,[2] and pergolide,[3] have been extensively investigated over the last 10 years, and some of them are now used in the treatment of Parkinson's disease and prolactin-dependent diseases. The antiimplantative, antilactation, and tumor-regression effects reported for the various ergoline derivatives can be attributed to the inhibition of prolactin release by virtue of their dopamine (DA) agonist properties. Recently, some 9-oxaergolines have been found to possess potent dopaminergic activity in vivo.[4,5] The structure–activity relationships for central dopaminergic activity of ergoline derivatives and related compounds indicate that the presence of an aromatic ring system replacing either the indole or the pyrrole ring is required.[6] Also the presence of a tertiary and basic nitrogen atom, separated from the aromatic nucleus by two carbon atoms in a given conformation, is essential. The junction of the rings C and D must be trans, and the *N*-*n*-propyl group frequently enhances dopamine agonist effects.[7]

Kornfeld et al.[6] have suggested and confirmed that the dopaminergic activity of ergoline derivatives is attributable to the moiety of the rigid pyrroloethylamine included in the ergoline structure as shown in 1.



1

In previous papers[8] we reported the biological activities of some indolizinylalkylamines and hydrazides of indol-

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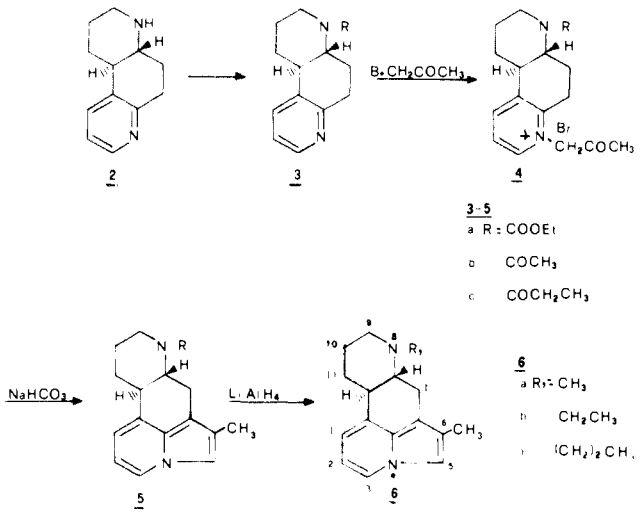
Scheme I



izinecarboxylic acids. These compounds have shown pharmacological activities similar to those of indole analogues. These results suggest that the NH group of indole is not critical for pharmacological activity, and the indolizine nucleus may be considered as a useful system for biologically active compounds.

In light of these observations we have synthesized and tested as dopaminergic inhibitors of prolactin release some *trans*-6-methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo-[3,2,1-*gh*]-4,7-phenanthrolines, analogues of the ergoline derivatives in which the indole nucleus is replaced by the indolizine ring and the rigid pyrroloethylamine moiety is retained. We have tested also some 1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline derivatives as models of molecular simplification of the hexahydropyrrolo-4,7-phenanthrolines.

Chemistry. The synthesis of indolizine analogues of ergoline was achieved from *trans*-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (**2**), described in an earlier report[9] (Scheme I).

Compound **2**, by treatment with ethyl chloroformate, acetic anhydride, and propionic anhydride, afforded the *N*-ethoxycarbonyl (**3a**), *N*-acetyl (**3b**), and *N*-propionyl (**3c**) derivatives, respectively. The reaction of **3a–c** with bromoacetone and the cyclization with sodium bicarbonate of the corresponding *N*-acetonyl bromide **4a–c** gave the *trans*-6-methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo-[3,2,1-*gh*]-4,7-phenanthrolines **5a–c**, which, by reduction with $LiAlH_4$, afforded the *N*-alkyl derivatives **6a–c**.

trans-4-Propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (**7**) was obtained by reduction of **3c** with $LiAlH_4$ (Scheme II). *cis*-4-Propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (**10**) was prepared by reduction with $LiAlH_4$ of propionyl derivative **9**, obtained by acylation of the *cis*-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline[9] (**8**) with propionic anhydride. Compounds **6a–c**, **7**, **10**, and **11** were transformed into maleates. The NMR spectrum of **9** shows two multiplets for each of 2eq- and 4a-H, centered at δ 3.77, 4.63 and at δ 4.21, 5.10; each signal has an integral of half proton. The two sets of multiplets correspond to different but equally populated conformations with respect to rotation about the N—C=O bond, according to the literature data for the *cis*-1-

Scheme II

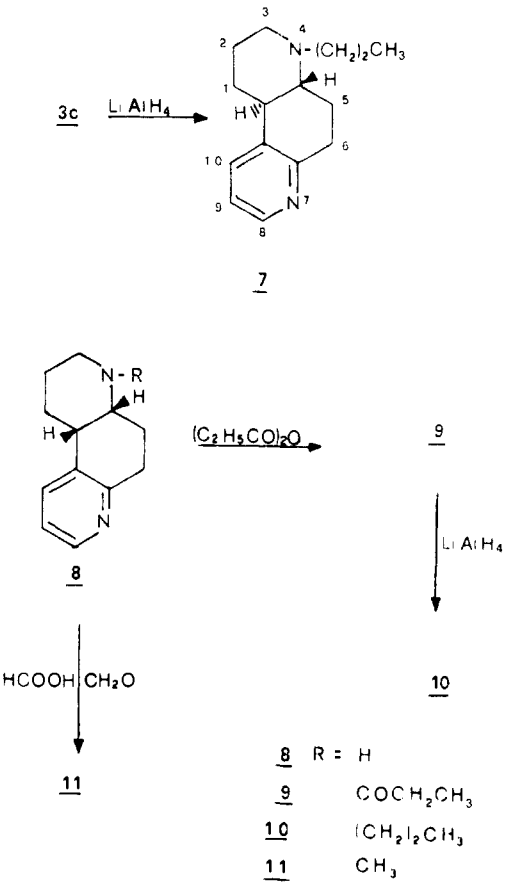


Table I. Acute Toxicity

no.	LD_{50}, mg/kg	CL[a] (95%), mg/kg	no.	LD_{50}, mg/kg	CL[a] (95%), mg/kg
6a	45	66.5 – 30	**7**	182.6	208.2 – 160.2
6b	227.9	409 – 127	**10**	139	151.8 – 127
6c	413.2	456.4 – 374.1	11	200	212 – 188.7

[a] Confidence limits.

acetyldecahydroquinoline.[10]

Pharmacology. The new derivatives have been evaluated for their ability to lower serum prolactin levels in reserpinized male rats, in comparison to bromocriptine chosen as a reference substance. In a preliminary study, the acute toxicity of the same compounds was studied in the rat, and the median lethal doses (LD_{50}), as well as their confidence limits (95%), were determined.

Methodological details concerning the evaluation both of the prolactin inhibiting activity and of the acute toxicity are given in the Experimental Section.

Results and Discussion

The LD_{50} values and their confidence limits for compounds **6a–c**, **7**, **10**, and **11** are reported in Table I. Lethal doses of all the compounds tested induced, immediately after intraperitoneal (ip) administration, muscular rigidity and spinal convulsions followed shortly by death. Muscular rigidity, in the absence of convulsions, was a common finding when the compounds were injected at doses slightly inferior to the lethal ones.

The results concerning the ability of these compounds to lower serum prolactin levels after ip administration are summarized in Table II. Control rats, which received single isotonic saline 18 h after reserpine pretreatment,

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Table II. Prolactin Inhibiting Activity

compd	dose, mg/kg ip	no. of animals	serum[a] prolactin, ng/mL	inhibn, %	signif level (p)
saline (control)		10	16.7 ± 1.83		
bromocriptine	0.001	6	12.7 ± 3.37	23.9	NS[b]
	0.01	6	6.6 ± 0.33	60.4	<0.001
	0.1	7	1.6 ± 0.61	90.4	<0.001
6a	11.2	5	18.9 ± 3.67		NS
6b	0.833	6	10.9 ± 0.59	34.7	<0.05
	4.165	7	4.7 ± 0.37	71.3	<0.001
	8.33	7	3.3 ± 1.20	80.2	<0.001
6c	103.3	6	10.2 ± 1.39	38.9	<0.05
7	0.0456	7	12.1 ± 0.89	27.5	NS
	0.456	6	8.8 ± 1.62	47.3	<0.05
	4.56	6	2.5 ± 0.66	85.0	<0.001
10	34.7	6	19.3 ± 4.13		NS
11	50.0	7	14.3 ± 4.61	14.3	NS

[a] Values are means ± standard error. [b] NS = not statistically different from controls.

Table III. Time Course of the Inhibitory Effect of Compound 7 on Prolactin Release

time (h) after drug administration	ip dose, mg/kg	no. of animals	serum[a] prolactin, ng/mL	inhibn, %	signif level (p)
1	0.0	10	16.7 ± 1.83		
1	4.56	6	2.5 ± 0.66	85.0	<0.001
3	0.0	7	15.6 ± 2.00		
3	4.56	7	4.6 ± 0.50	70.0	<0.001
6	0.0	9	16.5 ± 2.85		
6	4.56	9	9.6 ± 2.85	41.8	NS[b]

[a] Values are means ± standard error. [b] NS = not statistically different from controls.

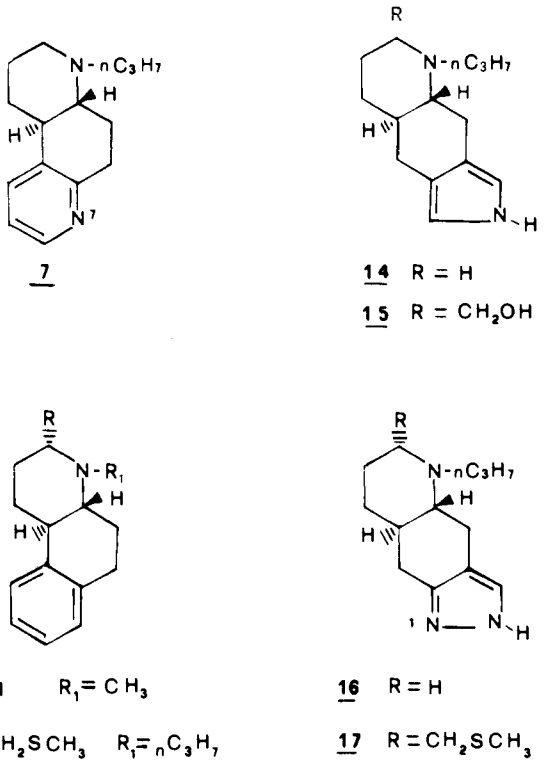
showed levels of serum prolactin ranging from 8.0 to 30.0 ng/mL, the mean value being 16.7 ± 1.83 ng/mL. In response to the maximum dose employed ($^1/_4$ of the LD_{50} for each substance), three compounds, **6a**, **10**, and **11**, proved to be completely ineffective; in fact, prolactin levels following their administration were not significantly different from those of controls. On the other hand, at the same level of dosage, **6c** produced a low (38.9%) but significant ($P < 0.05$) inhibition, while **6b** and **7** exerted a marked and dose-dependent inhibition of prolactin release, although being clearly less potent than bromocriptine. In fact, an inhibitory effect of about 50%, which was elicited by ca. 0.0059 mg/kg of bromocriptine, was obtained in response to 1.678 mg/kg of compound **6b** and 0.334 mg/kg of compound **7**, that is, at doses about 280 and 60 times larger, respectively, than that of bromocriptine itself on a weight to weight basis, or 580 and 130 times larger on a molar basis.

The regression lines for the inhibitory effect of bromocriptine ($y = 12.57 - 5.566 \log x$), of **7** ($y = 20.51 - 4.776 \log x$) and of **6b** ($y = 33.32 - 7.73 \log x$) had slopes not significantly different from each other, thus suggesting that these compounds activate the same receptors with a similar or common mechanism of action.

For compound **7**, which proved to be the most potent prolactin inhibitor among the newly synthesized compounds, the time course of its inhibitory effect was also evaluated. As shown in Table III, the effect, which was pronounced 1 h after the ip injection, was statistically highly significant even 3 h after drug administration. On the other hand, 6 h following injection, prolactin values of treated rats, although lower, were not significantly different from those of controls.

Present findings clearly indicate that indolizine analogues of ergoline retain inhibitory activity on prolactin release, thus suggesting the importance of the indolizine and pyridine aromatic nuclei for this biological activity. The low activity of compounds **6a–c** and the higher activity of the *N*-ethyl **6b** vs. the *N*-propyl derivative **6c** probably can be ascribed to the low stability of the indolizine nucleus; in fact, they are unstable when dissolved in aqueous solution for administration and are also air sensitive, as

Scheme III



reported in detail in the Experimental Section. Compound **7**, a molecular simplification of indolizine derivatives, proves to be still active: in fact, it is a fully effective dopaminergic agonist, being able to produce up to an almost complete inhibition of prolactin release. However, it is weakly potent being about 130 times less potent than bromocriptine on molar basis.

It is not possible only on the basis of the compounds synthesized in the present study to reach conclusions concerning the structure–activity relationship (SAR) in this series; however, when data obtained in this study are compared with those reported by previous studies, it seems possible to put forward that the nitrogen atom at the

position 7 in **7** is probably important for prolactin inhibiting activity. In support of this view is the fact that the octahydrobenzo[*f*]quinolines **12**[7] and **13**[6] (the deaza analogues of **7** reported in Scheme III) are almost devoid of activity. Further evidence in support of the importance of this nitrogen atom is given by the fact that the inhibitory activity on prolactin release of octahydropyrrolo[3,4-*g*]quinolines[6] **14** and **15** is intermediate, while that of octahydropyrazolo[3,5-*g*]quinolines[6] **16** and **17** is very high. The nitrogen at the position 1 in the derivatives **16** and **17** and the N-7 in the derivative **7** are in the same relationship with respect to the octahydroquinoline portion, as can be seen from Dreiding models of these molecules. In compounds **14** and **15** the absence of this nitrogen may be responsible for their lower activity. The nitrogen of the pyridine nucleus in **7** plays a crucial role, probably by interacting with its lone pair on the sp^2 orbital at the DA receptor responsible for the inhibition of prolactin release.

The inactivity of compounds **10** and **11** is in agreement with the hypothesis that the C and D rings must have a trans-junction.[7]

Experimental Section

Chemistry. Melting points (uncorrected) were determined with a Büchi apparatus. The ^1H NMR spectra were taken with a Varian EM-390 90-MHz spectrometer with Me_4Si as internal standard and $CDCl_3$ as solvent. The IR spectra were run on a Perkin-Elmer Model 297 spectrometer as Nujol mulls or liquid films. The elemental analyses for the new substances were within ±0.3% of the theoretical values and were carried out with a Perkin-Elmer 240 autoanalyzer. Mass spectra were recorded on a Hewlett Packard 5980A low-resolution mass spectrometer.

The ***trans*- and *cis*-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthrolines** (**2** and **8**) and the following derivatives: ***trans*-4-(ethoxycarbonyl)- (3a)**, ***trans*-4-acetyl- (3b)**, ***trans*-4-(ethoxycarbonyl)-7-acetonyl- (4a)**, ***trans*-4-acetyl-7-acetonyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (4b)**, and ***cis*-4-methyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenathroline** (11); ***trans*-6-methyl-8-(ethoxycarbonyl)- (5a)**, ***trans*-6-methyl-8-acetyl- (5b)**, and ***trans*-6,8-dimethyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenathroline (6a)** were prepared as previously described.[9]

***trans*- and *cis*-4-Propionyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (3c and 9).** A solution of **2** or **8** (1 g, 5.3 mmol) in propionic anhydride (3 mL, 23 mmol) was stirred at 100 °C for 5 h, cooled, diluted with water, neutralized with strong NaOH, and extracted with chloroform. The organic layer, dried over Na_2SO_4, after evaporation of the solvent, gave a residue, which was recrystallized.

Trans isomer 3c: yield 98%: mp 130–133 °C (from ethyl acetate); IR ν_{max} 1655 (C=O); NMR δ 1.12 (t, 3 H, CH_3, J = 7.5 Hz), 1.50 (m, 1 H), 1.88 (m, 2 H), 2.24 (m, 3 H), 2.40 (q, 2 H, $COCH_2$, J = 7.5 Hz), 3.06 (m, 3 H), 3.37 (m, 1 H), 3.52 (t, 2 H, 3-CH_2, J = 6 Hz), 7.08 (m, 1 H, 9-H), 7.48 (m, 1 H, 10- H), 8.38 (m, 1 H, 8-H). Anal. ($C_{15}H_{20}N_2O$) C, H, N.

Cis isomer 9: yield 98%; mp 118–120 °C (from cyclohexane); IR ν_{max} 1645 (C=O); NMR δ 1.12 (t, 3 H, CH_3, J = 7.5 Hz), 1.75 (m, 5 H), 2.12 (m, 1 H), 2.43 (q, 2 H, $COCH_2$, J = 7.5 Hz), 2.78 (m, 2 H), 3.11 (m, 2 H), 3.77 and 4.63 (2 m, 1 H, 2 eq-H), 4.21 and 5.10 (2 m, 1 H, 4a-H), 7.08 (m, 1 H, 9-H), 7.39 (m, 1 H, 10-H), 8.40 (m, 1 H, 8-H). Anal. ($C_{15}H_{20}N_2O$) C, H, N.

***trans*-4-Propionyl-7-acetonyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthrolinium Bromide (4c).** To a stirred solution of **3c** (1 g, 4.1 mmol) in hot ethyl acetate (15 mL) was added dropwise under N_2 a solution of freshly distilled bromoacetone (3 mL) in ethyl acetate (15 mL). The solution was refluxed for 8 h under N_2 and then stirred at room temperature overnight. The solid was filtered, washed with ethyl acetate, and dried; yield 88%; mp 172–173 °C; IR ν_{max} 1735 (C-CO-C), 1620 (N-CO-C). Anal. ($C_{18}H_{25}N_2O_2Br$) C, H, N.

***trans*-6-Methyl-8-propionyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (5c).** A suspension of $NaHCO_3$ (2.5 g) in absolute ethanol (30 mL) containing bromide **4c** (1.4 g, 3.6 mmol) was refluxed under N_2 for 9 h. After cooling, the solution was filtered and the filtrate was evaporated in vacuo. The residue, suspended in water, was extracted with chloroform. The extracts, filtered through basic alumina, were evaporated. Charcoal treatment and recrystallization of the residue from ethyl acetate gave **5c**; yield 78%; mp 189–190 °C; IR ν_{max} 1628 (C=O); NMR δ 1.18 (t, 3 H, CH_3, J = 7.5 Hz), 1.52 (m, 2 H), 1.88 (m, 2 H), 2.20 (s, 3 H, 6-CH_3), 2.34 (q, 2 H, $COCH_2$, J = 7.5 Hz), 2.90 (m, 2 H), 3.15 (m, 2 H), 3.98 (m, 2 H), 6.22 (m, 2 H, 1,2-H), 7.0 (s, 1 H, 5-H), 7.58 (m, 1 H, 3-H). Anal. ($C_{18}H_{22}N_2O$) C, H, N.

***trans*-6-Methyl-8-ethyl- and *trans*-6-Methyl-8-propyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (6b and 6c) and *trans*- and *cis*-4-Propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (7 and 10).** A solution of 3 mmol of **5b** (0.8 g) or **5c** (0.85 g) or **3c** (0.73 g) or **9** (0.73 g) in dry tetrahydrofuran (15 mL) was added dropwise under N_2 to a stirred suspension of $LiAlH_4$ (0.3 g, 8 mmol) in dry tetrahydrofuran. The reaction mixture was refluxed for 7 h (**6c**) or stirred at room temperature for 15 h (**6b**, **7**, and **10**). The excess of $LiAlH_4$ was destroyed with ethanol and 2 N NaOH, and the suspension was filtered. The solvent was evaporated and the crude residue, dissolved in chloroform, was filtered through a short column of neutral alumina. The solution, dried on Na_2SO_4, after evaporation of the solvent in vacuo gave an oily residue.

Compound 6b: yield 61%; NMR δ 1.05 (t, 3 H, CH_3, J = 7.5 Hz), 1.78 (m, 2 H), 2.25 (s, 3 H, 6-CH_3), 2.32–3.38 (m, 10 H), 6.28 (m, 2 H, 1, 2-H), 6.98 (s, 1 H, 5-H), 7.53 (m, 1 H, 3-H). Anal. ($C_{17}H_{22}N_2$) C, H, N.

Compound 6c: yield 72%; NMR δ 0.93 (t, 3 H, CH_3, J = 7.5 Hz), 1.25–2 (m, 6 H), 2.25 (s, 3 H, 6-CH_3), 2.45–3.48 (m, 8 H), 6.28 (m, 2 H, 1, 2-H), 7.0 (s, 1 H, 5-H), 7.58 (m, 1 H, 3-H). Anal. ($C_{18}H_{24}N_2$) C, H, N.

Compound 7: yield 94%; NMR δ 0.88 (t, 3 H, CH_3, J = 7.5 Hz), 1.10–2.92 (m, 13 H), 3.05 (m, 3 H), 7.12 (m, 1 H, 9-H), 7.58 (m, 1 H, H-10), 8.36 (m, 1 H, 8-H); MS, m/e 230 (M^+). Anal. ($C_{15}H_{22}N_2$) C, H, N.

Compound 10: yield 92%; NMR δ 0.9 (t, 3 H, CH_3, J = 7.5 Hz), 1.22–2.12 (m, 10 H), 2.50 (m, 3 H), 3.02 (m, 3 H), 7.0 (m, 1 H, H-9), 7.38 (m, 1 H, H-10), 8.35 (m, 1 H, H-8); MS, m/e 230 (M^+). Anal. ($C_{15}H_{22}N_2$) C, H, N.

Maleates of Compounds 6a–c, 7, 10, and 11. The suitable base in anhydrous ether was added slowly to a stirred equimolar solution of maleic acid in anhydrous ether. The maleates are highly hygroscopic, and it was not possible to crystallize them; they were used in the pharmacological tests after drying in vacuum. The bases **6a–c** and their maleates are unstable to light and air, but they can be kept for several months in an inert atmosphere in a refrigerator. Analytical results obtained for maleates were within ±0.4% of their theoretical values for C, H, N. The salts are monomaleates.

Pharmacology. Acute Toxicity. Fifty male Wistar rats weighing between 200 and 250 g were employed for each substance, which was always tested at five dose levels in groups of 10 animals per dose. The LD_{50} was estimated by evaluating the effect of single ip doses up to 14 days after the administration of drugs. The LD_{50} was determined according to the method of Weil.[11]

Prolactin Inhibiting Activity. Adult male Wistar rats weighing between 250 and 300 g were employed. They were kept in individual metabolic cages and housed in an air-conditioned room. Food in pellets and tap water were always available ad libitum. Eighteen hours before adminstration of the compounds, each rat received an ip pretreatment with reserpine (Sigma, St. Louis, MO), 2.5 mg/rat, in order to keep prolactin levels uniformly high.[12] Reserpine solution was made up according to Leyden et al.[13] at a final concentration of 2.5 mg/mL. The compounds tested as well as the reference substance, bromocriptine mesylate, were dissolved in distilled water so as to have the required dose per kilogram of body weight in 1 mL of water. Controls received, after reserpine pretreatment, 1 mL of distilled water/kg of body weight. One hour after treatment rats were killed by decapitation, and serum was collected. To determine the time course of the

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effect of compound 7 some animals were sacrificed 3 or 6 h after drug administration. Aliquots of 100 μL of serum were assayed in duplicate for prolactin by radioimmunoassay using the NIADDK kit. The bound fraction was separated from the free one by means of a 24-h incubation at room temperature with precipitating serum (Donkey anti-rabbit globulin, Wellcome RD17). The sensitivity of the assay was ca. 0.1 ng/tube. Results were expressed as nanograms of NIADDK rat prolactin RP3/milliliter of serum. The reference prolactin employed was 2.8 times more potent than NIADDK rat prolactin RP1. Taking into account the potency of the different reference rat prolactins, the values of serum prolactin obtained in our experiments are well into the range of values reported by other authors.[14-16] Statistical analysis of data was performed by means of the Student's t test.

Acknowledgment. We thank Professor M. Cardellini for a helpful discussion and E. Caraffa for skillful technical assistance in the pharmacological tests. We also thank NIADDK for the generous gift of radioimmunoassay materials. This research was supported by the Consiglio Nazionale delle Ricerche (CNR), Roma.

Registry No. 2, 80028-95-1; 3c, 101225-36-9; 4c·Br, 101225-38-1; 5b, 101225-44-9; 5c, 101225-39-2; 6a, 80029-03-4; 6a·maleate, 101225-51-8; 6b, 101225-40-5; 6b·maleate, 101225-45-0; 6c, 101225-41-6; 6c·maleate, 101225-46-1; 7, 101225-42-7; 7·maleate, 101225-47-2; 8, 80028-96-2; 9, 101225-37-0; 10, 101225-43-8; 10·maleate, 101225-49-4; 11, 80028-98-4; 11·maleate, 101225-50-7; prolactin, 9002-62-4.

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β_1-Selective Adrenoceptor Antagonists: Examples of the 2-[4-[3-(Substituted amino)-2-hydroxypropoxy]phenyl]imidazole Class. 2

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An attempt to develop a highly cardioselective β-adrenoceptor antagonist devoid of intrinsic sympathomimetic activity (ISA) focused on exploring structure–activity relationships around (*S*)-[*p*-[3-[[2-(3,4-dimethoxyphenyl)ethyl]-amino]-2-hydroxypropoxy]phenyl]-4-(2-thienyl)imidazole (1). Strategies to reduce or eliminate ISA centered on structural changes that could influence activation of the receptor by the drug itself or by a metabolite. The approaches involved (a) eliminating the acidic imidazole N–H proton, (b) incorporating substituents ortho to the β-adrenergic blocking side chain, (c) increasing steric bulk around the N–H moiety, (d) decreasing lipophilicity, (e) introducing intramolecular hydrogen bonding involving the imidazole N–H, and (f) displacing the imidazole ring from an activating position by the incorporation of a spacer element. The compounds were investigated in vitro for β-adrenoceptor antagonism and in vivo for ISA. From these studies, the most successful variation involved the insertion of a spacer between the imidazole and aryl rings. (*S*)-4-Acetyl-2-[[4-[3-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-2-hydroxypropoxy]phenyl]methyl]imidazole (**S-51**) was demonstrated to be highly cardioselective (dose ratio $\beta_2/\beta_1 > 9333$) and devoid of ISA.

Recent advances toward defining the structure of mammalian β_1 and β_2 adrenoceptors[1] have added further support to Lands' original subclassification of this receptor type.[2] The structural differences between these β receptors, as shown by peptide maps, must define, at least in part, the features that characterize selective adrenoceptor agents and provide a basis for the molecular rationalization of relative subreceptor affinities.

Of the two β receptor subtypes, the β_1 has received the greater attention in terms of defining the structural parameters that impart selectivity to an antagonist. It has been found in the (aminohydroxypropoxy)aryl class that receptor affinity is influenced by the aryl substituent and its position on the aromatic ring[3] and by the group attached to the side chain amino moiety.[4]

In a recent communication, we described a β_1-selective adrenoceptor antagonist (*S*)-2-[*p*-[3-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-2-hydroxypropoxy]phenyl]-4-(2-thienyl)imidazole (1), which exhibited an extraordinary degree of cardioselectivity as measured in guinea pig tissues ($\beta_2/\beta_1 = 8700$).[5] As with other recently reported highly β_1 selective agents such as ICI 89,406[6a] and RO 31-1118,[6b] 1 exhibited intrinsic sympathomimetic activity (ISA) in the in vivo reserpinized rat model.[7a] Since the possibility exists that the partial agonism of 1 could provide misleading pA_2 values in vitro, with a resulting error in the

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0022-2623/86/1829-1065$01.50/0