Chemistry and Biological Activity of *N*-Substituted Hydroxylamines

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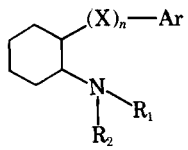
Abstract □ The synthesis and pharmacological properties of a novel series of *N*-substituted hydroxylamines are described. No useful CNS or cardiovascular activity was observed.

Keyphrases □ Hydroxylamines, *N*-substituted—synthesis and biological activity □ CNS activity—synthesis and screening of *N*-substituted hydroxylamines □ Cardiovascular activity—synthesis and screening of *N*-substituted hydroxylamines

Pharmacological activity was found in a series of cyclohexylamines (I) having either an aryl group or an aryl group linked through an oxygen or a carbon atom in the 2-position. 2-Phenyl-1-*N*-pyrrolidinocyclohexane (I*a*) is a member of a series of cholinesterase inhibitors (1) for which locomotor activity depression in mice has also been claimed (2). The aryloxycyclohexylamine series exemplified by I*b* displays antihistaminic, spasmolytic, and anesthetic properties (3, 4). The primary activity of the arylhydroxymethyl series (I*c*) is diuresis (5), whereas acetylated derivatives of I*c* show central nervous system (CNS) depressant activity (6).

The 2-substituted cyclohexylamines have now been extended by the synthesis of a series of arylhydroxylamine derivatives (I*d*) isosteric with I*c*. Since the Ar—N(OH)—C—C—N grouping was previously unknown, some open chain analogs of I*d* were also prepared for pharmacological screening.

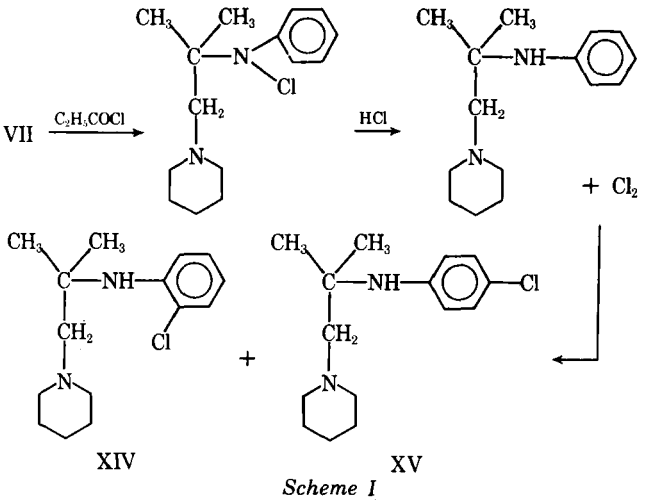


I*a*: Ar = C_6H_5; $(X)_n$ = O; $R_1 + R_2$ = —$(CH_2)_4$—
I*b*: Ar = C_6H_5; $(X)_n$ = O; $R_1 = R_2 = C_2H_5$
I*c*: Ar = 4-$OCH_3C_6H_4$; X_n = CH(OH); $R_1 + R_2$ = —$(CH_2)_5$—
I*d*: $(X)_n$ = N(OH)

CHEMISTRY

The *N*-substituted hydroxylamines (Table I) were prepared by condensation of nitrosobenzene or 2,6-dichloronitrosobenzene with the appropriate enamine, followed by reduction of this crude reaction product with sodium borohydride as previously described (7).

Attempts to convert some hydroxylamines to their *N*-acyloxy derivatives with the appropriate acid chloride or anhydride generally resulted in the isolation of a complex mixture of products. In the case of hydroxylamine (VII), reaction with propionyl chloride in refluxing chloroform gave the diamines XIV and XV. Formation of XIV and XV must occur by an analogous mechanism to that encountered in the Orton rearrangement (8) as shown in Scheme I. The trace of hydrochloric acid necessary to catalyze the reaction is presumably derived from partial hydrolysis of the acid chloride.



Scheme I

With methyl isocyanate, VIII gave the expected product (XVI), but the instability of this compound and related analogs prepared by the same method rendered them unsuitable derivatives for pharmacological screening.

The 2-arylhydroxylaminocyclohexylamines are believed to have the stereochemistry shown in XVII. This follows from consideration of the NMR spectrum of the crystalline diamine (XVIII) derived by hydrogenation of IV. This spectrum shows a narrow signal ($W_{1/2}$ = 10 Hz) for H_2, which eliminates the possibility of any 1,2-axial–axial interactions with adjacent protons and infers an axial configuration for the phenylhydroxylamino grouping. If it is assumed that the pyrrolidine substituent will have attained the more stable equatorial configuration in the formation of IV and hence, XVIII, the only possible arrangement of the two substituents is *cis*. An analogous synthesis of 1-*N*-pyrrolidino-2-phenylcyclohexane *via* the enamine derived from 2-phenylcyclohexanone has been shown[1] to give the *cis*-product by comparision with authentic samples of both the *cis*- and *trans*-isomers prepared by unequivocal routes.

DISCUSSION

All compounds were examined for CNS activity (9–15) and some for antihypertensive (16) and anticholinesterase (17) activity (Table II). Weak CNS depressant action was shown by II and VII, which potentiated barbiturate sleeping times at ED_{50}'s of 18 and 23 mg/kg, respectively, on oral administration in mice. Several hydroxylamines, at doses of 100 mg/kg, caused appreciable falls in blood pressure on intraperitoneal administration to rats in which hypertension had been induced by subcutaneous injections of desoxycorticosterone acetate (16). However, concurrent falls in heart rate were always observed and were sufficiently high in most cases to induce death in the animal subject.

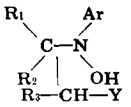
These results suggest that these substances exert their hypotensive action by a nonspecific smooth muscle and nervous depressant effect. Their anticholinesterase activity compared unfavorably with that of physostigmine, which produced 50% inhibition at 3.6×10^{-7} *M* under the same screening conditions (17).

EXPERIMENTAL

Melting points were determined with a hot-stage apparatus[2] and

[1] M. J. Readhead, Reckitt and Colman Pharmaceutical Division, 1973, personal communication.
[2] Kofler.

Table I—*N*-Arylhydroxylamines

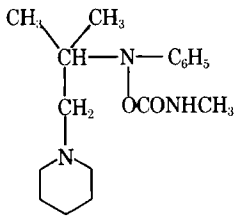


Compound[a]	R_1	R_2	R_3	Ar	Y	Method	Yield, %	Recrystallization Solvent	Melting Points (dec.)	Analysis, %	
										Calc.	Found
II	H	$-(CH_2)_4-$		C_6H_5	Morpholine	A	45	Ethanol–ether	218–221°	C 61.42	61.29
										H 8.05	7.68
										N 8.95	8.90
III	H	$-(CH_2)_4-$		C_6H_5	Piperidine	B	76	Ethanol	201–203°	C 65.68	65.45
										H 8.75	9.09
										N 9.01	8.63
IV	H	$-(CH_2)_4-$		C_6H_5	Pyrrolidine	B	17	Ethanol	219–220°	C 64.74	64.52
										H 8.48	8.61
										N 9.43	9.23
V	H	$-(CH_2)_4-$		C_6H_5	1-Methyl-piperazine	A	25	Methanol–ether	~230° (var.)	C 54.94[b]	55.22
										H 8.15	8.15
										N 11.32	11.57
VI	H	$-(CH_2)_4-$		$2,6\text{-}Cl_2C_6H_3$	Pyrrolidine	A	23	Ethanol–ether	~196° (var.)	C 52.55	52.46
										H 6.34	6.23
										N 7.66	7.83
VII	CH_3	CH_3	H	C_6H_5	Piperidine	B	92	Ethanol–ether	134.5–135.5°	C 72.54	72.77
										H 9.74	9.84
										N 11.28	11.28
VIII	CH_3	CH_3	H	C_6H_5	Pyrrolidine	B	86	Ether–light petroleum	112.5–113°	C 71.75	71.64
										H 9.46	9.65
										N 11.96	12.24
IX	CH_3	CH_3	H	$2,6\text{-}Cl_2C_6H_3$	Piperidine	A	17	Ethanol–ether	~265° (var.)	C 50.92	51.11
										H 6.56	6.52
										N 7.92	8.19
X	CH_3	CH_3	H	$2,6\text{-}Cl_2C_6H_3$	Pyrrolidine	A	37	Ethanol–ether	152–152°	C 49.50	49.32
										H 6.23	6.25
										N 8.25	8.25
XI	CH_3	H	H	C_6H_5	Piperidine	B	12	Ether–light petroleum	81–82°	C 71.75	71.71
										H 9.46	9.55
										N 11.96	12.05
XII	$-(CH_2)_5-$		H	C_6H_5	Morpholine	A	21	Ethanol–ether	185.5–187°	C 62.46	62.63
										H 8.32	8.30
										N 8.57	8.69
XIII	$-(CH_2)_5-$		H	C_6H_5	Piperidine	A	58	Ethanol–ether	174–175°	C 66.52	66.51
										H 8.99	8.92
										N 8.62	8.50

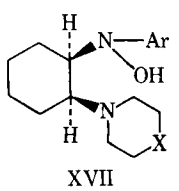
[a] All compounds are in the form of monohydrochloride salts except VII, VIII, and XI (free bases) and V (dihydrochloride salt). [b] Analyzed as hemihydrate.

are uncorrected. The structures of all compounds were assigned on the basis of compatible IR and NMR spectra.

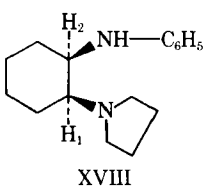
Hydroxylamines (Table I)—*Procedure A:* N-*(2-Morpholinocyclohexyl)phenylhydroxylamine (II)*—1-Morpholin-1-ylcyclohexene (16.7 g, 0.1 mole) in dry benzene (50 ml) was added over 20 min to a stirred suspension of nitrosobenzene (10.7 g, 0.1 mole) in dry benzene at 0–5°. The mixture was stirred at this temperature for a further 10 min and then allowed to warm up to room temperature. A color change from green to brown was observed, and the nitrosobenzene gradually dissolved as reaction took place. Stirring was continued for 10 min after all the nitrosobenzene had dissolved, and the solution was poured into an ice-cold mixture of sodium borohydride (3.8 g, 0.1 mole) in ethanol (50 ml). After standing for 15 hr, water (200 ml) followed by 2 *N* HCl was added. The solution was made alkaline with concentrated ammonium hydroxide and extracted with ether (2 × 100 ml). The extracts were combined, washed with water, dried (sodium sulfate), and evaporated; the residual oil was redissolved in ether and treated with ethereal dry hydrochloric acid until no further precipitation occurred. The precipitate was dried *in vacuo* and crystallized from ethanol–ether to give 9.0 g (45%) of II, mp 218–221°; IR ν_{max} (KBr): 3250 (N—OH) and 1595 (ArC=C) cm^{-1}.



XVI



XVII



XVIII

Procedure B: N-*[2-(2-Methyl-1-pyrrolidinopropyl)]phenylhydroxylamine (VIII)*—1-Pyrrolidin-1-ylisobutene (12.5 g, 100 mmoles) in dry toluene (50 ml) was added to a stirred suspension of nitrosobenzene (10.7 g, 0.1 mole) in dry toluene (50 ml) at −60°. The mixture was allowed to warm slowly in the cooling bath until all nitrosobenzene had reacted, and it was then poured into a suspension of sodium borohydride (3.8 g, 0.1 mole) in ethanol (50 ml) at −20°. Isolation (as in Procedure A) gave a white solid, which was recrystallized from ether–light petroleum, bp 40–60°, to give 20.4 g (86%) of VIII, mp 112.5–113°; IR ν_{max} (KBr): 3240 (N—OH) and 1596 (ArC=C) cm^{-1}.

2′ - Chloro - *N* - [2 - (2 - methyl - 1 - piperidinopropyl)]aniline (XIV) and Its 4′-Chloro Isomer (XV)—The hydroxylamine (VII) (5.7 g) and propionyl chloride (3.2 g) in chloroform (40 ml) were heated under reflux for 2.5 hr. The chloroform solution was washed with sodium bicarbonate and water and dried (sodium sulfate). Evaporation gave an oil which was chromatographed on alumina (Grade I). Elution with light petroleum (1:19) gave the diamine (1.01 g); NMR ($CDCl_3$): δ 1.29 (s, 6, $2CH_3$), 6.3–7.3 [m, 4, ArH, including a dd (1, J = 7 Hz, Ar 3H)]. The hydrochloride salt was recrystallized from ethanol–ether to give 0.7 g of XIV, mp

Table II—Comparative Pharmacological Data

Compound	CNS Tests, ED_{50}, mg/kg po Administration							Mean Fall in Blood Pressure[a], Desoxycorticosterone Acetate Intraperitoneal Administration	Anticholinesterase[b], % Inhibition
	Phenylquinone-Induced Writhing (Ref. 9)	Ataxia (Ref. 10)	Reserpinized Hypothermia (Ref. 11)	Strychnine Antagonism (Ref. 12)	Pentylenetetrazol Antagonism (Ref. 13)	Maximal Electroshock (Ref. 14)	Barbiturate-Induced Sleeping (Ref. 15)		
II	>300	>300	>100	>300	>300	>300	18	31% (100 mg/kg) cyanosis	9 (10^{-4} *M*)
III	>300	>300	>100	>300	>300	>300	~300	T[c] (100 mg/kg) 20% (10 mg/kg)	
IV	>100	>300	>100	>100	>100	>100	100		22 (10^{-4} *M*)
V	>100	>100	>100	>100	>100	>100	—	32% (100 mg/kg) cyanosis	
VI	>200	>200	>100	>200	>200	115	—		Inactive (10^{-4} *M*)
VII	>200	>200	>200	>200	>200	>200	23	Inactive (100 mg/kg)	13 (10^{-4} *M*)
VIII	>150	>150	>150	>150	>150	>150	—	T (100 mg/kg) 17% (10 mg/kg)	14 (10^{-4} *M*)
IX	>300	>300	>100	>300	>300	>300	—		
X	>100	>100	>100	>100	>100	>100	—	T (100 mg/kg)	15 (10^{-4} *M*)
XI	148	>200	>200	>200	>200	>200	>100		
XII	~300	>300	>300	>300	>300	>300	~100		9 (10^{-4} *M*)
XIII	86	>300	>300	>300	>300	>300	>100		

[a] Modified procedure to that described in Ref. 16. [b] Reference 17; concentration of acetylcholine is 0.01 *M*. [c] T = toxic.

166–166.5°. Elution with ether–light petroleum (2:3) gave the diamine (1.68 g); NMR ($CDCl_3$): δ 1.20 (s, 6, $2CH_3$) and 7.0 (A_2B_2q, 4, ArH). The hydrochloride salt (XV) was recrystallized from ethanol–ether (0.96 g), mp 167–168.5°.

***O*-Methylcarbamate of *N*-[2-(2-Methyl-1-pyrrolidinopropyl)]phenylhydroxylamine (XVI)**—The hydroxylamine (VIII) (1.5 g) and methyl isocyanate (0.7 g) in dry benzene (20 ml) were stirred at room temperature for 30 min. The residue, after removal of solvent, was crystallized from ether–light petroleum, bp 40–60°, to give XVI (1.38 g), mp varied dec. (Found: C, 65.85; H, 8.68; N, 14.71. $C_{16}H_{25}N_3O_2$ requires C, 65.95; H, 8.65; N, 14.42); IR ν_{max} (KBr): 3375 (NH), 1728 (C=O), and 1600 (ArC=C) cm^{-1}.

1-Anilino-2-pyrrolidinocyclohexane (XVIII)—The free base derived from the hydroxylamine (IV) (4.36 g, 0.016 mole) was hydrogenated in ethanol solution, using 10% palladium-on-charcoal (0.87 g) as the catalyst (hydrogen uptake 1.05 moles). After filtering and washing the catalyst with ethanol, the combined filtrates were evaporated under reduced pressure. The residue was crystallized from ethanol–water to yield 2.9 g (67%) of XVIII, mp 88–89° (Found: C, 78.71; H, 9.82; N, 1169. $C_{16}H_{24}N_2$ requires C, 78.63; H, 9.90; N, 11.46); IR ν_{max} (KBr): 3350 (NH) and 1602 (ArC=C) cm^{-1}.

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