SEARCH FOR NO DONORS. PART I. 3-QUINUCLIDONE OXIMES

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 31, No. 5, pp. 26 – 32, May, 1997.

Original article submitted June 24, 1996.

Identification of the "endothelial relaxation factor " as the endogenic NO formed from *L*-arginine in the vascular endothelium [1] has stimulated the search for new generation of cardiovascular drugs [2 – 5]. At present, it has been established that biochemical synthesis of NO proceeds by different pathways. Exogenic organic nitrates (nitroglycerin, nitrosorbid, and other classical vasodilators) are subject to reduction [6], as well as the new NO precursors such as N-nitropyrazoles [7] and furoxanes [8]. On the contrary, *L*-arginine – the main NO source in the organism – is oxidized by the enzyme NO-synthase (NOS) to NO and *L*-citrulline [2]. NOS is also capable of forming NO from β-mercaptoethylguanidine [9, 10]. Study of the well-known vasodilator molsidomin showed that this agent generates NO by reaction with atmospheric oxygen [3]. It was found that 1,2-diazetine-1,2-dioxides form NO upon thermolysis and/or hydrolysis [11]. Muller et al. [12] reported on the use of (±)-3-(*E*)-4ethyl-2[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK 409) as the NO donor in solutions at pH ~ 7.5, but failed t o establish a group (oxime, nitro, or both) acting as the source of NO [12]. Recently we have demonstrated that 3-quinuclidone oximes may serve as the source of NO and activate the soluble guanylate cyclase [13]. The purpose of this work was to extend the group of oximes and study their hypotensive activity.

The NO precursors were obtained on the basis of both well-known (Va/VIa, Vb/VIb, Ve/VIe, Vi/VIi) [14], (Vc/VIc, Vd/VId, VIIb] [13], and newly synthesized (Vf/VIf – Vh/VIh) 2-arylmethylene-3-quinuclidone oximes and their hydrogenated analogs IXb and IXc. The compact rigid framework structure of quinuclidine (1-azabicyclo[2.2.2]octane) provides spatial fixation of the substituents and sufficiently high solubility of hydrochlorides, while the double bond in position 2 sometimes allows us to obtain the (*Z*/*E*) isomer pairs and estimate the effect of spacing between aromatic and oxime group.

The initial (*Z*)-2-arylmethylene-3-quinuclidones (Ia – Ih) were obtained by a well-known method based on the condensation of 3-quinuclidone with aromatic aldehydes in the presence of NaOH [14 – 16]. The NaOH content was 1 mole per mole aldehyde for aldehydes containing no OH groups in the aromatic nucleus, and 2 mole per mole aldehyde for the hydroxyaldehydes. In order to avoid the self-condensation of quinuclidone [17] and disproportionation of aldehydes in a strongly alkaline medium, NaOH and aldehyde were simultaneously introduced into a boiling solution of quinuclidone in ethanol. At a temperature of 80°C, the reaction with benzaldehyde is completed within 2 h, while the process with 2- or 4-hydroxybenzaldehydes takes 30 h, with 4-dimethylaminobenzaldehyde – 18 h, 4-methoxybenzaldehydes – 8 h, and with 2-methoxybenzaldehydes – 30 min. The 4-hydroxy derivative Id is isolated from the reaction mixture in the form of a sodium salt, which is related to its comparatively low solubility, while the 2- and 3-isomers (Ib and If) are obtained under the same conditions in a neutral form. Because furfural exhibits gumming at 80°C, the reaction was performed at room temperature. The yields and properties of new ketones Ic, If, and Ih are presented in Tables 1 – 3.

Conversion of the (*Z*) isomers into an equilibrium mixture of (*Z*) and (*E*) isomers was previously performed in HCl-saturated chloroform [18]. Under these conditions, 2-hydroxyketone Ib is virtually completely (TLC) converted into a cyclic semiketal, chromeno[3,2-b]quinuclidine III. During the crystallization from MeOH, compound III readily transforms into IV, especially so in the presence of trace amounts of acid. The ketal structure of compound IV is confirmed by the strong screening of atom C-4a (as manifested by the small chemical shift δ = 96.7 ppm compared to ~ 200 ppm for ketones I and II [19]). Treatment of Ib or III with an HCl solution in *i*-PrOH or BuOH leads to the corresponding *iso*-propoxy and *n*-butoxy derivatives (X, XI), which can be readily separated by extraction with heptane from the more polar

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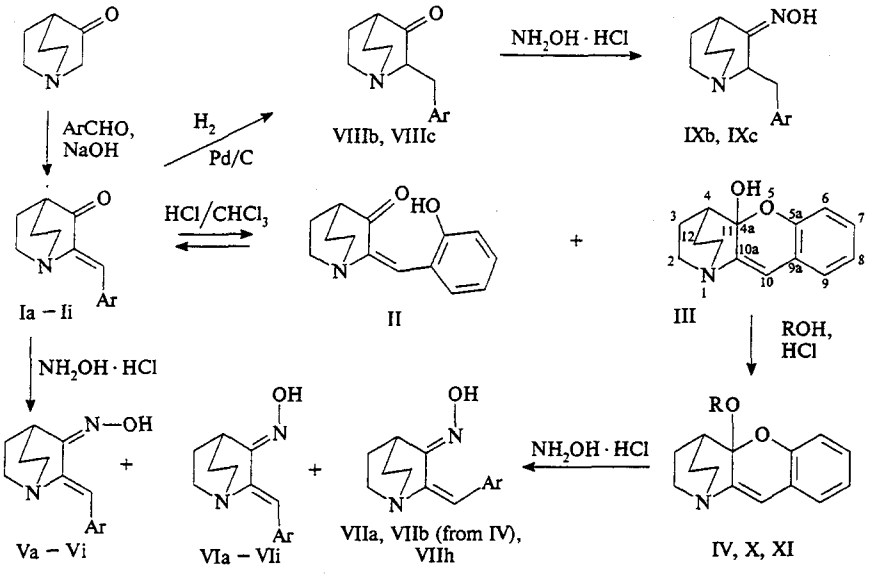
0091-150X/97/3105-0243$18.00



Ar = Ph (a), 2-HOC_6H_4 (b), 2-$MeOC_6H_4$ (c), 4-HOC_6H_4 (d), 4-$MeOC_6H_4$ (e), 3-HOC_6H_4 (f), 2-furyl (g), 4-$Me_2NC_6H_4$ (h), 4-$O_2NC_6H_4$ (i); R = Me (IV), *i*-Pr (X), *n*-Bu (XI).

compound III present in the mixture. The reaction is reversible: the room-temperature equilibrium in methanol is achieved after 2 days, and that in isopropanol and *n*-butanol, after about a month. We failed to obtain a reaction with benzyl alcohol.

Interaction of ketones Ib – Ii with $NH_2OH \cdot HCl$ is usually not accompanied by changes in the *Z*-configuration of C=C bonds and leads to hydrochlorides of the corresponding oximes, comprising a mixture of *anti* (Vb – Vi) and *syn* (VIb – VIi) isomers identified on the basis of NMR spectra reported in [18]. The ratio of V(Z/E)VI in the final hydrochlorides was as follows: 50(Z/E)50 (b); 60(Z/E)40 (c); 26(Z/E)74 (d); 10(Z/E)90 (e); 5(Z/E)95 (f); 4(Z/E)96 (g); 45(Z/E)55 (h); 2(Z/E)98 (i). We did not attempt to separate the *syn* and *anti* isomers. The initial ketones had a bright-yellow color. Some of the final oximes were colorless, while oximes Vb(Z/E)VIb, Ve(Z/E)VIe, Vh(Z/E)VIh, and Vi(Z/E)VIi exhibited a yellow color; as expected, compound VIIb was colorless because the aromatic ring remains unconjugated as a result of steric constraints.

In the preliminary publication [13], we have reported that the above reaction of compound Ia leads to a mixture of Va, VIa, and VIIa with the component ratio ~ 1 : 1 : 1, from which VIIa · HCl was isolated upon crystallization from MeOH. Subsequent investigations showed that interaction of Ia with a free hydroxylamine base yields VIa and traces of the two other isomers. A 2-h boiling of Ia with hydroxylamine hydrochloride in methanol led to a mixture of Va(Z/E)VIa with traces of VIIa, the content of VIIa increasing with the duration of boiling. Thus, a weakly acidic medium, formed upon the dissociation of hydroxylamine hydrochloride or oximes, catalyzes isomerization of the initially formed *syn*-(*Z*) isomer into *anti*-(*Z*), followed by its conversion into the *anti*-(*E*) conformation. Boiling hydrochlorides of Va(Z/E)VIa for 20 h in pure methanol or in the presence of 0.5 eq. hydroxylamine hydrochloride yields a mixture of all the three isomers. Note that the isomerization of Va(Z/E)VIa and Vc(Z/E)VIc bases is observed during their flash-chromatography on a column with silica gel eluted with a $CHCl_3$–MeOH system.

Monohydrochloride VIIh exhibits a rather rapid oxidation in air (involving the aromatic ring according to the ^1H NMR data), which is manifested by the conversion from orange to red color. At the same time, oxime dihydrochloride and other hydrochlorides in the crystalline state are stable during at least one year.

TABLE 1. Yields and Properties of Synthesized Compounds

Compound	Yield, %	M.p., °C	Solvent for crystallization	Empirical formula
Ic*	90	118 – 119	*i*-PrOH	$C_{15}H_{17}NO_2$
If	37	183 – 186	*i*-PrOH	$C_{14}H_{15}NO_2$
Ih	61	138 – 139	*i*-PrOH	$C_{16}H_{20}N_2O$
III*	30	186 – 188	heptane	$C_{14}H_{15}NO_2 \cdot H_2O$
IV*	72	75 – 76	heptane	$C_{15}H_{17}NO_2$
Vc · HCl + VIc · HCl	18	194 – 195	*i*-PrOH	$C_{15}H_{18}N_2O_2 \cdot HCl$
Vd · HCl + VId · HCl	44	176 (decomp.)	*i*-PrOH	$C_{14}H_{16}N_2O_2 \cdot HCl \cdot MeOH$
Vf · HCl	78	206	MeOH	$C_{14}H_{16}N_2O_2 \cdot HCl$
Vh · HCl + VIh · HCl	32	223 – 230	*i*-PrOH	$C_{16}H_{21}N_3O \cdot 0.5HCl$
VIIa · HCl	37	207 (decomp.)	MeOH	$C_{14}H_{16}N_2O \cdot HCl$
VIIb · HCl*	87	191 – 193	MeOH	$C_{14}H_{16}N_2O_2 \cdot HCl$
VIIh · HCl	8	209 (decomp.)	*i*-PrOH	$C_{16}H_{21}N_3O \cdot HCl \cdot 1.5H_2O$
VIIh · 2HCl	Quant	181 – 183 (decomp.)	–	$C_{16}H_{21}N_3O \cdot 2HCl \cdot 1.5H_2O$
IXb · HCl*	85	185 – 186	EtOH – acetone, 1 : 2	$C_{14}H_{18}N_2O_2 \cdot HCl \cdot 0.5EtOH$
IXc · HCl	57	185 (decomp.)	EtOH	$C_{15}H_{20}N_2O_2 \cdot HCl$
X	77	96 – 98	Heptane	$C_{17}H_{21}NO_2$
XI	54	oil	–	$C_{18}H_{23}NO_2$

* Replace incorrect melting temperatures reported previously [13].

The only product of reaction between compound IV and $NH_2OH \cdot HCl$ is VIIb · HCl, which yields 30% of a Vb(*Z*/*E*)VIb · HCl mixture upon a one-weak exposure in the form of methanol solution. At the same time, Va and VIIa bases exhibit no isomerization in methanol even after a 30-day period of time. Reaction of compound III with $NH_2OH \cdot HCl$ under similar conditions leads to a Vb(*Z*/*E*)VIb(*Z*/*E*)VIIb · HCl mixture.

Catalytic hydrogenation [15, 20] of compounds Ib and Ic smoothly leads to 2-arylmethyl-3-quinuclidones VIIIb and VIIIc, respectively, followed by conversion into oxime hydrochlorides IXb · HCl (68% *anti* isomer) and IXc · HCl (95% *syn* isomer). In the former case, the *anti* configuration is probably stabilized by an intramolecular hydrogen bond with an *ortho*-phenol hydroxyl.

In order to rapidly check the ability of the oximes described above and 3-quinuclidone oxime (XII) to serve as NO donors under biological conditions, we have developed a special indirect electrochemical method using $K_3[Fe(CN)_6]$ as the oxidant. For the complete conversion of NO^+ into stable nitroprusside anions $[Fe(CN)_5NO]^{2-}$, a 2×10^{-3} M solution of $K_4[Fe(CN)_6]$ in 0.2 M NaOH was added to a 2×10^{-4} M oxime solution in 4% aqueous EtOH and the mixture was heated for 5 min at 80°C. Then the reaction was terminated by adjusting the pH to 5.0 with a saturated citric acid solution at room temperature.

$$RR'C{=}NOH + [Fe(CH)_6]^{3-} + OH^- \rightarrow$$
$$\rightarrow RR'C{=}O + NO + [Fe(CN)_6]^{4-} + H_2O$$

$$[Fe(CN)_6]^{3-} + NO \rightarrow NO^+ + [Fe(CN)_6]^{4-}$$

$$NO^+ + [Fe(CN)_6]^{4-} \rightarrow [Fe(CN)_5NO]^{2-} + CN^-$$

The resulting nitroprusside is reduced at the mercury drop electrode, giving rise to two characteristic polarographic waves with $E_{1/2} = -0.30$ and -0.70 V. The selectivity of measurements was increased by using a differential-pulsed method. The NO yields (%) were calculated as the ratio of the second-wave peaks of the oxime studied (htest) to that of a standard sodium nitroprusside (SNP) solution with the same concentration (href). The results of calculation are listed in Table 4.

In order to study the oxidation pathway of the oximes, we have studied the process on a greater scale, with the reaction time increased to 30 min. Preliminary experiments with compound VIIb (TLC, Table 2) showed that the reaction mass contains, in addition to the initial (*E*) oxime VIIb, its (*Z*) isomer Vb and ketone Ib. Similar results were also obtained in the absence of $K_3[Fe(CN)_6]$, which excluded isomerization caused by intermediate radical particles. Later, it was found

TABLE 2. Chromatographic Mobilities (R_f) of the Synthesized Compounds on Silufol UV-254 Plates In the $CHCl_3$ – MeOH (10 : 0.6) System

Ar	I	V	VI	VII	VII	*anti*-IX	*syn*-IX
a	0.92	0.49	0.74	0.31	–	–	–
b*	0.93	0.67	0.79	0.48	0.46	0.29	0.29
c	0.88	0.39	0.54	–	0.51	0.22	0.34
d*	0.74	0.44	0.44	–	–	–	–
e	0.88	0.43	0.62	–	–	–	–
f	0.61	–	0.32	–	–	–	–
g	0.90	0.60	0.60	–	–	–	–
h	0.90	0.70	0.70	0.45	–	–	–
i	0.93	–	0.78	–	–	–	–

* $CHCl_3$ – MeOH (10 : 1), compound III, $R_f = 0.17$; compound IV, $R_f = 0.40$.

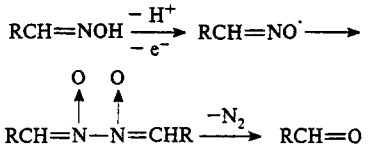
TABLE 3. Chemical Shifts in ^1H and ^{13}C NMR Spectra of Synthesized Compounds (δ, ppm)

Compound	Solvent	4-H (q)	5, 8-CH_2 (m)	6, 7-CH_2 (m)	=CH (s)	2'-H	3'-H (m)	4'-H (m)	5'-H (m)	6'-H (m)	MeX
Ic	$CDCl_3$	2.60	2.00	2.97, 3.12	7.54	–	6.85	7.28	6.94	8.48	3.83
If	$CDCl_3$	2.61	2.00	2.97, 3.12	6.94	7.75	–	6.82	7.21	7.40	–
Ih	CD_3OD	2.60	2.00	2.95, 3.15	6.99	6.67	8.01	–	8.01	6.67	3.03
Vb · HCl	D_2O	3.88	2.05 – 2.20	3.45 – 3.65	8.37	–	7.10 – 7.50				3.89
VIc · HCl	D_2O	3.03	2.05 – 2.20	3.45 – 3.65	7.27	–	7.10 – 7.50				3.88
Vd · HCl	D_2O	3.02	2.05 – 2.20	3.45 – 3.65	8.45	7.22	6.97	–	6.97	7.22	–
VIf	CD_3OD	3.59	1.70 – 1.80	2.90, 3.09	6.65	7.55	–	7.20	7.09	6.66	–
VIf · HCl	DMSO – H_2O, 10 : 1	3.69	1.82, 2.03	3.40 – 3.55	7.19	6.75 – 6.85	–	6.75 – 6.85	7.26	6.77	–
VId · HCl	D_2O	3.88	2.05 – 2.20	3.45 – 3.65	7.37	7.14	6.84	–	6.84	7.14	–
Vh · HCl	D_2O	2.60	1.80	2.80 – 3.10	7.72	7.83	6.66	–	6.66	7.83	2.97
VIh · HCl	D_2O	3.59	1.80	2.80 – 3.10	6.60	7.77	6.69	–	6.69	7.77	2.96
VIIh · HCl	D_2O	4.10	1.95 – 2.20	3.53, 3.70	6.78	8.01	6.69	–	6.69	8.01	3.02
VIIh · 2HCl	D_2O	4.05	2.00 – 2.20	3.60 – 3.80	7.00	8.08	7.48	–	7.48	8.08	3.25
VIIb	DMSO	3.83	1.90 – 2.05	3.40 – 3.65	7.39	–	7.39	7.39	7.39	7.79[2]	–
IXb · HCl	CD_3OD	2.89	2.00 – 2.20	3.05 – 4.05 [1]	4.95 (m)[3]	–	6.90	7.16	6.89	7.30	–
IXc · HCl	CD_3OD	3.79	1.95 – 2.15	3.15 – 3.95 [1]	4.56 (m)[3]	–	7.03	7.33	6.97	7.32	3.89

Notes. [1] +CH_2Ar; [2] +2'-OH; [3] 2-H.

that compound IIb may convert into Ib in an alkaline medium, which indicates that the (*Z*/*E*) isomer equilibrium in ketones is pH-dependent. The formation of ketones is apparently due to oxidation because oximes are not hydrolyzed in an alkaline medium. These results agree with the data presented in Table 4: the maximum amount of ketones is formed in the case of compounds VIIb and IXb, which are the best NO donors.

It must be noted that TLC characterizes the general susceptibility of oximes to oxidation, rather than their ability to produce NO, since it was reported that oximes may exhibit electrochemical oxidation with the evolution of nitrogen [21].



The proposed method is quite simple and can even be used for testing compound Vi, in which the presence of nitro group masks the polarographic waves of nitroprusside during the electrochemical NO determination.

EXPERIMENTAL CHEMICAL PART

The ^{1}H NMR spectra were measured on a Varian Unity Plus 400 spectrometer (USA), and the mass spectra were obtained with a Finnigan MAT SSQ 710 (Switzerland) instrument. The melting temperatures were determined in a sealed capillary. TLC patterns were obtained on Silufol UV-254 plates (Czech Republic) visualized under UV illumination and developed with Dragendorff reagent. Characteristics of the synthesized compounds are presented in Tables 1 – 3. The data of elemental analyses coincide with the results of analytical calculations.

(*Z*)-2-Arylmethylene-3-quinuclidones (Ia – Ih). To a mixture of 0.124 mole of 3-quinuclidone hydrochloride and 0.124 mole NaOH in 150 – 200 ml of 96% ethanol, boiled for 30 min, was added another 0.124 mole NaOH (0.25 mole in the case of hydroxyaldehydes) and 0.124 mole of the corresponding aldehyde. The mixture was boiled (kept for 24 h at 20°C for Ig) until disappearing of quinuclidone in the TLC pattern. Then the ethanol was evaporated and the residue washed with water, separated, and dried. The product was recrystallized from ethanol with activated charcoal to obtain bright-yellow ketones with melting temperatures coinciding with the published data.

(*Z*)-2-(2-Hydroxyphenyl)methylene-3-quinuclidone (Ib). The residue obtained upon ethanol evaporation was dissolved in 100 ml of water and extracted with chloroform (4 × 70 ml). The extract was dried with potassium carbonate, the solvent was evaporated, and the target product recrystallized from 100 ml of 2-propanol.

(*Z*)-2-(4-Hydroxyphenyl)methylene-3-quinuclidone (Id). Method A. The residue obtained upon ethanol evaporation was dissolved in 50 ml of water and cooled. The precipitate was separated, dried, and recrystallized from 100 ml of 2-propanol to obtain a sodium salt of ketone Id.

Method B. To the residue obtained upon ethanol evaporation was added with stirring 0.24 mole of 1% acetic acid. Then the reaction mass was continuously extracted with chloroform. The extract was dried with potassium carbonate, the solvent was evaporated, and the target product Id recrystallized from 100 ml of 2-propanol.

(*Z*)-2-(3-Hydroxyphenyl)methylene-3-quinuclidone (If). A mixture of 0.124 mole of 3-quinuclidone hydrochloride and 0.124 mole 3-hydroxybenzaldehyde and 0.38 mole

TABLE 4. Oxime Hydrochlorides as NO Donors and Soluble Guanylate Cyclase Activators

Compound	NO Yield upon oxidation		Guanylate cyclase activity against control (1.0) (% activation) for the test compound concentration		
	with $K_3[Fe(CN)_6]$ in the presence of NaOH	with atmospheric air in borate buffer (pH 9.2)	10^{-5} M	10^{-4} M	10^{-3} M
Vb / VIb	4	0	1.40 ± 0.19	2.30 ± 0.51 (30)	2.50 ± 0.84
Vc / VIc	6	0	–	–	–
Vd / VId	4	0	–	–	–
Ve / VIe	2	0	1.30 ± 0.06	1.50 ± 0.06 (19)	1.30 ± 0.06
VIIa	2	0	–	–	–
VIIb	17	4	3.60 ± 1.02	5.70 ± 1.05 (74)	3.00 ± 0.96
IXb	10	0.5	–	–	–
IXc	4	0	–	–	–
XII	2	0	–	–	–
XIII	6	–	–	–	
SNP	100	–	–	7.70 ± 0.69 (100)	–

NaOH in 100 ml of water was boiled for 10 h, cooled to room temperature, and mixed with 0.45 mole of AcOH. The brown precipitate was filtered, washed with water, dried, crystallized from 200 ml of 2-propanol, and recrystallized from 150 ml of 2-propanol with activated charcoal to obtain 8.35 g of compound If in the form of yellow crystals; m.p., 183 – 186°C. In addition, 2.2 g of pure ketone was obtained upon evaporation of the filtrate.

4a-Hydroxy-4aH-chromeno[3,2-b]quinuclidine (III) and 4a-methoxy-4a*H*-chromeno[3,2-b]quinuclidine (IV). Ketone Ib (0.026 mole) was introduced with stirring into 100 ml of HCl-saturated chloroform, which led to the appearance of a precipitate that rapidly transformed into an oil. Then the chloroform was distilled off and an attempt at recrystallizing the residue from 100 ml MeOH was made. Since no precipitation took place, the solution was evaporated, treated with a 50% aqueous solution of potassium carbonate, and extracted with chloroform (3 × 50 ml). The extracts were dried with K_2CO_3, evaporated, and treated with 50 ml of heptane to obtain 1.8 g (30%) of colorless compound III; ^{1}H NMR spectrum, DMSO (δ, ppm): 1.50 – 1.95 (m, 3-CH_2, 12-CH_2), 2.29 (qv, 4-H), 2.70 – 2.90 (m, 2-CH_2, 11-CH_2), 6.23 (s, 10-H), 6.90 – 7.20 (m, 6-H, 7-H, 8-H, 9-H).

Evaporation of the heptane solution yields 3.1 g (50%) of a methoxy derivative of compound IV; ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 1.50 – 1.90 (m, 3-CH_2, 12-CH_2), 2.58 (qv, 4-H), 2.90 – 3.05 (2-CH_2, 11-CH_2), 3.25 (s, CH_3), 6.35 (s, 10-H), 6.93 (m, 8-H), 6.99 (m, 6-H), 7.13 (m, 7-H), 7.14 (q, 9-H); ^{13}C NMR spectrum, $CDCl_3$ (δ, ppm): 22.2 and 22.7 (3-C, 12-C), 30.1 (4-C), 46.9 and 51.3 (2-C, 11-C), 49.7 (CH_3), 96.7 (4a-C), 114.5 (10-C), 116.0 (7-C), 121.6 (8-C), 122.6 (9a-C), 127.1 and 128.1 (6-C, 8-C).

Chromeno[3,2-b]quinuclidines (III, IV, IX, XI). A suspension of ketone Ib (0.13 mole) in 30 ml of the corresponding HCl-saturated alcohol was allowed to stand at room temperature for 2 days which suffices to attain equilibrium (as indicated by TLC). Then the alcohol was distilled off and the residue was poured into a 10% aqueous solution of potassium carbonate and extracted with chloroform (3 × 60 ml). The extracts were dried with K_2CO_3, the chloroform was evaporated, the residue extracted with 50 ml of heptane, and the insoluble precipitate of compound III separated to leave a virtually pure heptane solution of the corresponding 4a-alkoxy-4a*H*-chromeno[3,2-b]quinuclidine IV or X.

Compound XI was additionally purified on a column with silica gel Silpearl (Czech Republic) in a chloroform – MeOH gradient from 100 : 1 to 15 : 1. ^{1}H NMR spectrum of compound XI, $CDCl_3$ (δ, ppm): 0.74 (H_δ), 1.10 – 1.30 ($H_{\beta,\gamma}$), 1.50 – 1.90 (3-CH_2, 12-CH_2), 2.56 (4-H), 3.40 – 3.65 (H_α), 3.90 – 4.10 (2-CH_2, 11-CH_2), 6.37 (10-H), 6.90 – 7.15 (6-H, 7-H, 8-H, 9-H); ^{13}C NMR spectrum of compound XI, $CDCl_3$ (δ, ppm): 13.5 (C_δ), 19.0 (C_γ), 22.3 and 22.8 (3-C, 12-C), 30.9 (4-C), 31.5 (C_β), 46.9 and 51.4 (2-C, 11-C), 61.8 (C_α), 96.7 (4a-C), 114.5 (10-C), 115.9, 121.3, 127.0. 127.9 (6-C, 7-C, 8-C, 9-C), 122.5 (9a-C), 142.9 (10a-C), 150.8 (5a-C).

Hydrochlorides of *anti-* and *syn-(Z)*-2-phenylmethylene-3-quinuclidone oximes (Va / VIa · HCl). A mixture of 28 mmole of ketone Ia and 31 mmole of hydroxylamine hydrochloride in 90 ml of methanol was boiled for 5 h. Then the reaction mass was evaporated to half the initial volume to obtain 1.6 g (21%) of Va / VIa oxime hydrochloride mixture.

***Anti-* and *syn-(Z)*-2-phenylmethylene-3-quinuclidone oximes Va and VIa and their hydrochlorides.** The mother liquors obtained in the preceding experiment were evaporated to dryness, treated with a potassium carbonate solution and extracted with chloroform (3 × 50 ml). The isomers were separated on a column with silica gel Silpearl (15 × 1.5 cm). First, compound Va was eluted first chloroform, and then compound VIa was obtained with a chloroform – MeOH (100 : 1) system.

The corresponding hydrochlorides were obtained by slowly adding 0.98 mole of an HCl solution in anhydrous ether to a vigorously stirred suspension of oxime bases in ether. Each of the hydrochlorides of oximes Va and VIa contained about 5% of the other isomer (^{1}H NMR data). Attempts at further purification by crystallization from alcohols led to a mixture of Va, VIa, and VIIa oxime hydrochlorides.

***Syn-(E)*-2-phenylmethylene-3-quinuclidone oxime hydrochloride (VIIa · HCl).** A mixture of compound Ia and hydroxylamine hydrochloride in methanol was boiled for 4 h and left to stand for 1 day at room temperature to obtain 1.2 g of precipitated oxime VIIa hydrochloride. The mother liquor containing equal proportions of Va, VIa, and VIIa (^{1}H NMR

TABLE 5. Antihypertensive Activity of Synthesized Compounds

Compound	Decrease of arterial pressure (Torr) at a dose (mg / kg, i.p.)		
	0.1	1.0	10.0
Ib	2.0 ± 1.7[1)]	6.0 ± 1.7*	6.7 ± 0.8*[2)]
Va	2.0 ± 0.8	4.5 ± 0.5*	10.0 ± 1.2*
Vb	4.5 ± 0.5*	7.5 ± 1.3*	12.7 ± 0.7*
Vc	1.3 ± 0.7	5.3 ± 0.7*	12.0 ± 2.3*
Vd	3.3 ± 0.7*	7.3 ± 1.8*	10.0 ± 1.0*
Ve	4.0 ± 1.2	8.0 ± 0.8*	12.0 ± 2.0*
Vg	2.0 ± 1.0	8.0 ± 1.2*	11.3 ± 0.7*
Vh	2.0 ± 0.8	3.5 ± 1.7	11.0 ± 1.3*
VIIa	3.5 ± 1.7	4.8 ± 1.0*	16.0 ± 2.2*
VIIb	2.7 ± 1.8	6.0 ± 1.2*	10.0 ± 2.8*[3)]
IXb	1.3 ± 0.7	7.5 ± 1.0*	10.0 ± 1.0*
IXc	1.0 ± 0.9	7.3 ± 7.0*	11.3 ± 1.3*
XIII	1.0 ± 1.0	5.3 ± 0.7*	10.0 ± 1.0*
XIV	4.3 ± 0.3*	9.0 ± 1.0*	13.0 ± 1.0*
Nitroglycerin	16.4 ± 2.6*	29.6 ± 1.3*	...
SNP	24.8 ± 4.1*	42.8 ± 5.6*	...

* Statistically significant pressure reduction against the initial level ($p < 0.05$; $n = 3 – 6$); [1)] 0.5 mg / kg; [2)] 5.0 mg / kg; [3)] peroral.

data) was evaporated and recrystallized from 30 ml of hot water to obtain another 1.2 g of VIIa hydrochloride.

***Syn*-(*Z*)-2-phenylmethylene-3-quinuclidone oxime (Va).** To 16 mmole of ketone Ia in 200 ml of methanol was added 18 mmole of hydroxylamine hydrochloride and 18 mmole of a 10 N aqueous NaOH. After standing for 1 day at room temperature, the NaCl precipitate was separated and the filtrate was evaporated to a residual volume of 20 ml, containing 3.45 g (89.6%) of Va oxime.

(*Z*)-2-(3-Hydroxyphenyl)methylene-3-quinuclidone oxime hydrochloride (Vf / VIf · HCl). A mixture of 0.022 mole of ketone If and 0.024 mole of hydroxylamine hydrochloride in 100 ml of methanol was boiled for 5 h. The reaction mass was evaporated to about half the initial volume to obtain 2.4 g (39%) of the target oxime hydrochloride with m.p. 206°C. Then the mother liquor was evaporated to yield another 2.4 g of the product with m.p. 202°C.

(*Z*)-2-(3-Hydroxyphenyl)methylene-3-quinuclidone oxime (Vf / VIf). A mixture of 0.022 mole of ketone If and 0.03 mole of a methanol solution of hydroxylamine (obtained from 0.03 mole hydroxylamine hydrochloride and 0.03 mole of 2 N NaOMe) was allowed to stand for 1 day at room temperature. Then the reaction mass was evaporated until the onset of crystallization to obtain 2.8 g (52%) of the target oxime with m.p. 187°C. Then the mother liquor was evaporated to yield another 0.6 g of the product with m.p. 168°C.

Hydrochlorides of 2-(4-dimethylaminophenyl)methylene-3-quinuclidone oximes (Vh / VIh / VIIh). To a suspension of 20 mmole of ketone Ih in 180 ml of methanol was added with stirring 22 mmole of hydroxylamine hydrochloride. After 2 h the mixture turned into a homogeneous solution, which was allowed to stand for 1 day until no initial ketone was detected in the bulk (TLC). Then the solvent was distilled off and the residue triturated with 100 ml of ether, from which 2 g of a Vh / VIh · HCl (45 : 55) mixture was obtained. The ether-insoluble residue was boiled in 80 ml of an isopropanol – acetone (4 : 1) mixture, filtered hot, and cooled to obtain 2 g of a precipitate containing predominantly VIIh · HCl. Recrystallization of the precipitate from 30 ml of 2-propanol yielded 0.65 g of pure VIIh · HCl in the form of yellow crystals becoming reddish on exposure to air.

Duhydrochloride of oxime VIIh was obtained in the form of air-stable pink crystals on adding an equimolar amount of HCl-saturated ether with stirring to a suspension of VIIh · HCl in ether.

***Syn*-(*E*)-2-(2-Hydroxyphenyl)methylene-3-quinuclidone oxime hydrochloride (VIIIb · HCl).** To a solution of 11 mmole of compound IV in 30 ml of methanol was added 13 mmole of hydroxylamine hydrochloride and the reaction mass was boiled for 1 h to precipitate 2 g of VIIb · HCl (the reaction does not proceed at room temperature).

2-(2-Hydroxyphenylmethyl)-3-quinuclidone (VIIIb). A solution of 17.5 mmole of compound Ib in 250 ml of 96% ethanol was hydrogenated at atmospheric pressure and room temperature over 0.2 g of 10% Pd / C. Then the catalyst was separated and the solution boiled with with 0.1 g of activated charcoal, filtered, evaporated to a volume of 50 ml, and cooled to obtain 1.1 g of colorless crystals with m.p. 145 – 146°C (reported m.p., 137 – 139°C [17]).

2-(2-Methoxyphenylmethyl)-3-quinuclidone (VIIIc). Compound VIIIc was obtained similarly to VIIIb proceeding from a solution of 16.6 mmole of compound Ic in 300 ml of 96% ethanol, hydrogenated for 2 h.

2-(2-Hydroxyphenylmethyl)-3-quinuclidone oxime hydrochloride (IXb · HCl). A suspension of 11 mmole of compound VIIIb and 12 mmole of hydroxylamine hydrochloride in 200 ml of 96% ethanol was allowed to stand overnight at room temperature. Then the solution was evaporated, the oily residue mixed with 30 ml acetone, and 2.8 g of the target compound in the form of colorless crystals separated by filtration.

2-(2-Methoxyphenylmethyl)-3-quinuclidone oxime hydrochloride (IXc · HCl). Compound IXc · HCl was obtained similarly to IXb · HCl by evaporating the corresponding reaction mass to a volume of 15 ml.

Hydrochlorides of 3-quinuclidone oxime (XII) and ethoxycarbonyl-3-quinuclidone oxime (XIII). Compounds XII and XIII were synthesized from the corresponding ketones by the methods described in [22, 23].

Oxidation of oximes in air. The oxime (0.2 mmole) was added to 10 ml of an 0.2 N NaOH solution (2 mmole) and the mixture treated in an open vessel for 30 min at 80°C. Then the reaction mass was cooled to room temperature (adding 3.4 mmole of acetic acid in the case of hydroxyphenyl derivatives) and extracted with chloroform (3 × 5 ml). Aliquots of the extract were dried over potassium carbonate and analyzed by TLC (Table 2).

EXPERIMENTAL PHARMACOLOGICAL PART

The spasmolytic properties of the hydrochlorides of the synthesized compounds were studied *in vitro* using thoracic aorta preparations isolated from male rats weighing 320 – 360 g. The experiments were performed on an Ugo Basile (Italy) complex setup for the investigation of isolated organs. A sample chain of 6 – 8 aorta rings was placed in a temperature-controlled bath (volume, 20 ml; temperature, 37°C) filled with a Krebs – Henseleit buffer (mM): NaCl, 130; KCl, 4.6; $CaCl_2$, 2.5; $NaHPO_4$, 1.2; $NaHCO_3$, 12.7; $MgCl_2$, 0.1; glucose, 7.7. The vascular tone was monitored in the isotonic regime with a 2-g initial load. The spasmolytic effect was revealed by gradually adding increasing concentrations (10^{-12} to 10^{-4} M) of the test compounds to the bath against the background of developed aorta spasms induced by noradrenalin (10^{-6} M).

The effect of the synthesized compounds on the arterial pressure was studied in a group of male rats with renovascular hypertension, narcotized with urethane (1.5 g / kg, i.p.). The pressure in the common carotid artery was monitored by a setup based on the Pb 23 transducer (Statham, USA), an

HSE 550A measuring unit (Hugo Sachs Elektronik, Germany), and a Mark III recorder (Watanabe, Japan). The test compounds in the form of aqueous solutions were introduced via catheter into the jugular vein at a dose of 0.1, 1.0, or 10.0 mg/kg. The reference preparations *in vitro* and *in vivo* were represented by nitroglycerin and sodium nitroprusside.

RESULTS AND DISCUSSION

It was found that oximes of unsubstituted 3-quinuclidone (XII), 2-ethoxycarbonyl-3-quinuclidone (XIII), and unsaturated oximes VIIa (Ar = Ph) and Ve / VIe (Ar = 4-$MeOC_6H_4$) are weak NO donors. Hydroxyl substitution in the benzene ring increases the efficiency of oximes as NO precursors, with the maximum NO yield observed for the 2-OH derivatives VIIb and IXb. The significant distinction between the 2-HO-substituted (*Z*) (Vb / VIb) and (*E*) (VIIb) isomers and the moderate difference between VIIb and its unsaturated analog IXb indicate that the efficiency of the NO production is determined primarily by the proximity of HO and =NOH groups, rather than than by the transfer of the electronic effects of substituents via the conjugation chain.

The best NO donors, VIIb and IXb oximes, are subjected to oxidation even on exposure to atmospheric air in an alkaline solution (Table 4). In this case, the trapping and polarographic determination of NO was based on the following reactions [24]:

$$2NO + O_2 \rightarrow NO_2$$

$$NO + NO_2 + 2OH^- \rightarrow 2NO_2^- + H_2O$$

$$[Fe(CN)_6]^{4-} + ON_2^- + H_2O \rightarrow [Fe(CN)_6]_2^- + CN^- + 2OH^-$$

The conversion of oximes into ketones under these conditions was confirmed by the TLC data.

As is known, the biological action of NO is based on the activation of water-soluble guanylate cyclase, an enzyme catalyzing the synthesis of cyclic 5′-guanosine monophosphate responsible for vasodilatation and arterial pressure reduction. In this connection, we have studied the effect of oximes on the activity of soluble guanylate cyclase from human thrombocytes. The activity was determined with respect to SNP by a radioimmune method described previously [11]. The concentration dependence of the effect is described by a typical bell-shaped curve with a maximum at about 10^{-4} M. The degree of enzyme activation by the oximes studied correlated with the polarographic data: the best NO donors were the best guanylate cyclase activators (Table 4).

Experiments on urethane-narcotized rats with renovascular hypertension showed that intravenous injections of all the synthesized compounds produced a dose-dependent reduction in the arterial pressure: 0.1 mg/kg, by less that 4.5 Torr; 1.0 mg/kg, 4.5 – 9.0 Torr; 10.0 mg/kg, 10.0 – 16.0 Torr. It was also found that ketone Ib, which is a precursor of oxime Vb, exhibits a low hypotensive activity. The doses of reference preparations producing an equal effect in comparison with that of the maximum dose of the synthesized compounds (10.0 mg/kg), were lower by at least two orders of magnitude (0.1 mg.kg) (Table 5). The compounds differed but little from one another with respect to the activity, which did not allow us to make definite conclusions on the relationship of the chemical structure and biological action.

Experiments on isolated rat aorta preparations showed that the synthesized compounds possess no spasmolytic properties in the concentration range studied (10^{-12} to 10^{-4} M). Unlike this, both nitroglycerin and sodium nitroprusside induced relaxation of the noradrenalin-stimulated contractions of smooth vascular muscles; the medium-efficiency drug concentrations were 5×10^{-7} and 10^{-9} M, respectively.

The results of our experiments showed that, despite a clearly pronounced antihypertensive effect, the synthesized compounds produced no direct spasmolytic action. One of the possible explanations is the necessity of preliminary metabolism of these compounds to NO in the organism, as in the case of molsidomin [3].

Thus, all the 3-quinuclidone oximes studied are capable of forming NO during oxidation under soft conditions, while the most active compound (VIIb) is comparable with SNP with respect to the soluble guanylate cyclase activation *in vitro*. The effect increases in the presence of an aromatic substituent, especially that carrying a 2-HO group. The somewhat lower activity of IXb as compared to VIIb and the significant drop in the activity of (*Z*)-Vb / VIb isomer suggest that a phenol group occurring in the vicinity of the oxime fragment may participate in some oxidation stages, thus probably stabilizing reactive intermediates or forming the O-nitroso esters [25].

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