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7-ARYL-4a-HYDROXY-4a,6,7,7a-TETRAHYDRO-ISOXAZOLO[4,5-*b*]QUINUCLIDINES AS NO DONORS

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7-Aryl-4a-hydroxy-4a,6,7,7a-tetrahydroisoxazolo[4,5-b]quinuclidines have been prepared from 2-arylmethylene-3-quinuclidones and hydroxylamine and they are able to release NO upon mild oxidation with $K_3[Fe(CN_6)]$ in basic medium.

Keywords: arylmethylene quinuclidine, hydroxylamine, NO donors, quinuclidone oxime, hydrogenation.

Certain 2-arylmethylene- and 2-arylmethyl-3-quinuclidone oximes are able to generate NO upon mild oxidation and to stimulate soluble guanylate cyclase i.e. the enzyme catalyzing the synthesis of cyclic 5'-guanosine monophosphate which is finally responsible for a whole series of biological effects [1-4]. In this work we have carried out an investigation of the reaction of *Z*-2-arylmethylene-3-quinuclidones with hydroxylamine with the aim of preparing novel, potential NO donors.

Hydroxylamine and its derivatives can take part in reaction with α,β-unsaturated compounds to form the corresponding oximes or *via* addition of hydroxylamine at the electron deficient β-carbon atom and subsequent cyclization to isoxazoles [5, 6] or a compound of the 4a-hydroxytetrahydroisoxazole series for a derivative of 2-methylene-3-quinuclidone [7].

A study of the reaction mixtures obtained by treating the 3-quinuclidone derivatives **1a-d** with hydroxylamine hydrochloride has shown that, along with the corresponding oximes **2-4** there are formed in the reaction the hydrogenated isoxazolo[4,5-*b*]quinuclidines **5a-d** which could be separated and identified by spectroscopic methods (Tables 1-3).

Changing hydroxylamine hydrochloride for the base in the reaction with compound **1b** lowers the yield of the isomeric oximes **2/3b** from 39 to 23% and the bicyclic derivative **6b** becomes the main reaction product (yield 51%). It is likely that protonation activates the carbonyl group and accelerates the oximation and that β-addition predominates in its absence, (in agreement with reported data [6]). In fact, in the case of the reaction of the 2-pyridylmethylene ketone (**1e**) with hydroxylamine the dominating process is addition at the β-position of the α,β-unsaturated system to give the cyclic tautomer of the hydroxyamino derivative 4a-hydroxy-7-(2-pyridyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine (**5e**) as the principal product.

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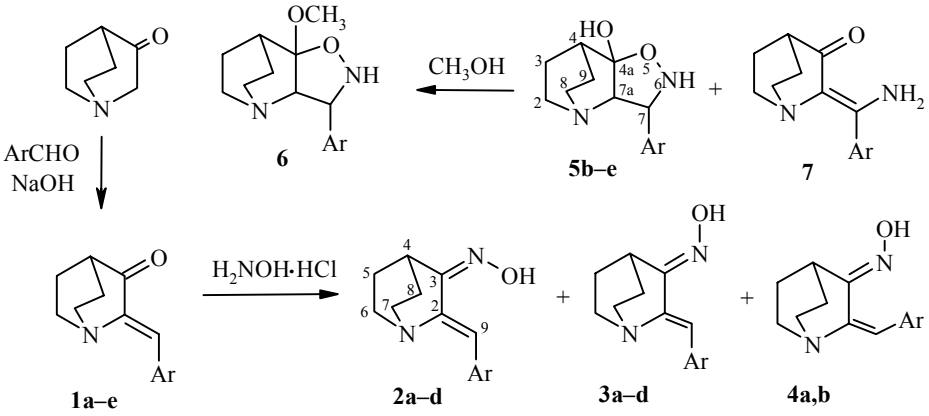
TABLE 1. Characteristics of Compounds Synthesized

Compound	Empirical formula	Found, % / Calculated, %			mp, °C, solvent	R_f*	Yield, %
		C	H	N			
1e	$C_{13}H_{14}N_2O$	73.0 / 72.9	6.9 / 6.6	13.1 / 13.1	112-114 (dec.), *i*-PrOH	0.66	42
2/3c HCl	$C_{15}H_{19}N_2O_2 \cdot HCl$	61.4 / 61.1	6.6 / 6.5	9.3 / 9.5	196-198, acetone	0.51	16
5b·2HCl	$C_{14}H_{18}N_2O_3 \cdot 2HCl \cdot 2H_2O$	44.6 / 45.0	7.0 / 7.0	7.5 / 7.5	148-150, water	0.33	4
5c·HCl	$C_{15}H_{20}N_2O_3 \cdot HCl \cdot H_2O$	55.1 / 54.6	6.6 / 7.0	8.4 / 8.5	181-183, *i*-PrOH	0.22	17
5d·2HCl	$C_{14}H_{18}N_2O_3 \cdot 2HCl \cdot H_2O$	47.5 / 47.3	5.9 / 6.2	7.5 / 7.9	176-178, *i*-PrOH	0.14	8
5e·HCl	$C_{13}H_{17}N_3O_2 \cdot HCl$	54.6 / 55.0	6.7 / 6.4	14.8 / 14.8	192 (dec.), MeOH	0.23	54
6	$C_{15}H_{20}N_2O_3 \cdot H_2O$	61.0 / 61.2	7.4 / 7.5	9.5 / 9.5	167-168, MeOH	*[2]	51
7·NH_2OH·2HCl	$C_{13}H_{15}N_3O \cdot 2HCl \cdot NH_2OH$	46.7 / 46.6	6.4 / 6.0	16.7 / 16.7	175-185 (dec.), *i*-PrOH	0.54	15
7	$C_{13}H_{15}N_3O \cdot 0.5H_2O$	65.8 / 65.5	6.7 / 6.7	17.0 / 17.0	145-147, (dec.), $CHCl_3$	0.54	87

* $CHCl_3$–MeOH, 50:3.
*[2] Hydrolyzed to **5b** upon chromatography.



1–7 a Ar = Ph, **b** Ar = 2-HOC_6H_4, **c** Ar = 2-$MeOC_6H_4$, **d** Ar = 4-HOC_6H_4, **e** Ar = 2-Py

Oximes **2-4** were not separated from the reaction mass. It is impossible to rule out an additional factor promoting the predominant β- addition in the reaction of ketone **1e** with $NH_2OH \cdot HCl$ which is the formation of a stable cation (**B**) to which the hydroxylamine base also adds. The actual basicity of the quinuclidine nitrogen in (*Z*)-2-arylmethylene-3-quinuclidones is low (according to L. S. Khabarova for the (*Z*)-2-(3'-chlorophenyl)-methylene-3-quinuclidone in 50% ethanol the pK_a is 4.54 ± 0.05).

TABLE 2. Chemical Shifts in the ^{1}H NMR Spectra of the Sythesized Compounds, δ, ppm

Compound	Solvent	4-H*	5,8-CH_2 (3,9-CH_2)*[2] (m)	6,7-CH_2 (2,8-CH_2)*[2] (m)	9-H (7-H)*[2]	3'-H (m)	4'-H (m)	5'-H (m)	6'-H (m)	7a-H
1b	$CDCl_3$	3.88	2.00-2.30	3.28, 3.57	7.19 (s)	6.88	7.33	6.84	7.35	—
1e	$CDCl_3$	2.62	2.01	2.90-3.15	7.14 (s)	8.56	7.66	7.16	8.60	—
3b·HCl	CD_3OD	3.95	2.04, 2.19	3.57, 3.74	7.25 (s)	6.99	7.32	6.96	7.29	—
4b·HCl	DMCO-d_6	3.88	1.90-2.10	3.46, 3.64	7.28 (s)	6.85	7.16	6.75	7.94	—
5b·2HCl	DMCO-d_6–D_2O, 1:1	2.63	1.90-2.20	3.30-3.70	5.13 (d)	7.0	7.35	7.0	7.35	4.26 (d)
5c·HCl*[3]	CD_3OD	2.21	1.50-2.00	2.65-3.25	4.72 (d)	7.02	7.34	6.99	7.46	3.41 (d)
5d*[4]	DMCO-d_6	2.05	1.30-1.75	2.45-3.00	4.11 (q)	6.72 (d)	—	6.72 (d)	7.20 (d)	2.96 (d)
5e·2HCl*[5]	DMCO-d_6	2.40	1.65-2.05	3.10-3.55	4.82	7.70 (d)	7.85 (t)	7.40 (q)	8.59 (d)	3.83 (d)
6*[6]	DMCO-d_6	2.05	1.30-1.80	2.50-3.15	4.50 (d)	6.78	7.10	6.75	7.20	3.02 (d)
7*[7]	$CDCl_3$	2.57	1.85	2.77, 2.99	—	8.81 (d)	7.69 (t)	7.26 (q)	8.57 (d)	—
7·2HCl·NH_2OH*[8]	DMCO-d_6	2.74	1.94, 2.14	3.32, 3.66	—	8.30 (d)	8.18 (t)	7.72 (q)	8.81 (d)	—

* The 4-H signal is a quintet; the remaining signals are multiplets unless indicated otherwise in the brackets.

*[2] Numbering in brackets relates to series **5** and to compound **6**.

*[3] OMe: 3.89 (s).

*[4] 6-H: 5.92 (d), $J_{6\text{--H, 7-H}} = 13.2$, $J_{7\text{-H, 7a-H}} = 6.2$.

*[5] 6-H: 6.95 (br. s), OH: 7.01 (br. s), $^+$NH: 11.4, $J_{6\text{-H, 7-H}} = 12.5$, $J_{7\text{-H, 7a-H}} = 5.2$.

*[6] OMe: 3.15 (s).

*[7] NH_2: 6.70 (br. s) and 9.33 (br. s).

*[8] NH_3^+: 10.47, NH^+: 12.3, NH, OH: 8.98, 9.61, and 10.16 (all br. s).

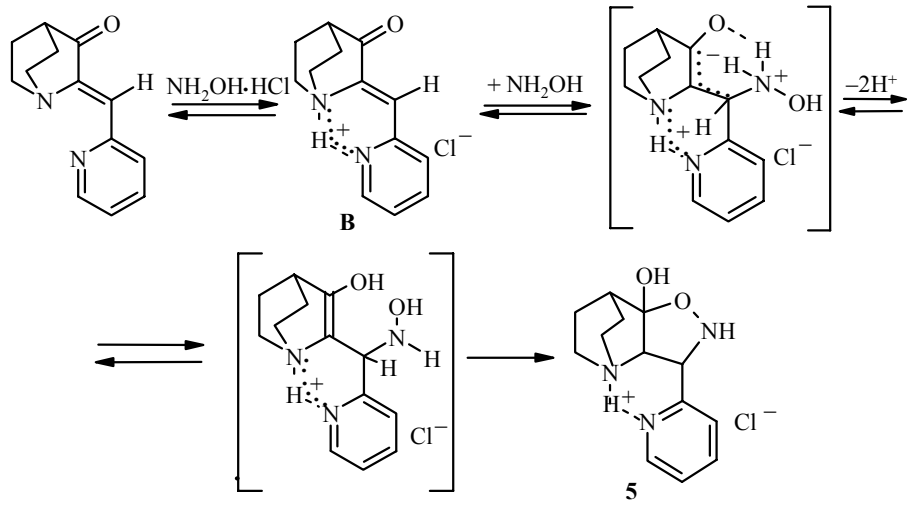
TABLE 3. Chemical Shifts in the ^{13}C NMR Spectra of the Synthesized Compounds, δ, ppm

Compound	Solvent	2-C (7a-C)*	3-C (4a-C)*	4-C	5,8-C (3,9-C)*	6, 7-C (2,8-C)*	9-C (7-C)*	1'-C	2'-C	3'-C	4'-C	5'-C	6'-C
3b·HCl	$(CD_3)_2CO–D_2O$, 1:1	129.9	153.0	22.8	20.6	49.7	117.6	117.4	152.7	115.6*[2]	132.0*[3]	120.8*[2]	132.6*[3]
4b·HCl	DMSO-d_6–CD_3OD, 1:1	133.2	152.7	24.7	22.3	51.1	122.5	120.4	157.3	116.4*[2]	132.0*[3]	119.9*[2]	132.4*[3]
5c·HCl*[4]	DMSO-d_6–CD_3OD, 1:2	62.6	107.0	32.7	23.1; 23.7	41.3; 49.7	78.4	124.8	159.5	112.3	130.9	122.1	130.8
5d·2HCl	DMSO-d_6	64.9	105.3	31.2	22.0; 22.3	40.3; 48.5	79.0	127.7	129.0	115.4	157.2	115.4	129.0
7	$CDCl_3$	118.5	205.3	40.5	26.5	49.4	147.4*[3]	—	150.6*[3]	127.4	136.0	124.3	148.3
7·2HCl ·NH_2OH	DMSO-d_6	110.2	195.0	38.3	21.2	51.2	148.7*[3]	—	149.4*[3]	124.0	139.3	126.8	149.8

* Numbering in brackets relates to series **5**

*[2], *[3] Possible reversed signal assignments are marked with the same characters.

*[4] OCH_3: 56.2.



It is indeed likely that the electron acceptor properties of the pyridine ring, increased by protonation and the presence of the stabilization due to the formation of hydrogen bonds, promotes the reductive fission of the N–O bond and that this leads to the formation of the enamino ketone **7** (initially separated as the $NH_2OH{\cdot}HCl$ complex) during reduction of **5e**·nHCl or its open form with excess hydroxylamine.

The cyclic structure of compounds **5a-e** is confirmed by the absence of absorption due to C=O or C=N groups in their IR spectra and the presence of several bands in the region 3100-3400 cm^{-1} for the absorption of the OH and NH groups. The ^1H NMR spectra show signals for the 7-H (4.1-4.8 ppm) and 7a-H protons (3.0-4.0 ppm), the vicinal situation of which follows from the presence of a spin-spin coupling $J_{7,7a} \sim 5.2$ Hz. The placement of the nitrogen at position 6 rather than 5 of the pentacyclic ring follows from the presence of a spin-spin coupling $J_{7,6} \sim 12.5$ Hz for the 7-H and 6-H protons in the spectrum of **5e**·HCl (Table 2). The ketal nature of atom $C_{(4a)}$ was confirmed by its greater shielding (^{13}C δ 105-107 ppm, Table 3) when compared with the carbonyl carbon (~ 200 ppm for ketones **1**). Analogous differences are seen in the ^{13}C NMR chemical shifts for 4a-hydroxy-4aH-chromeno[3,2-*b*]quinuclidines [2].

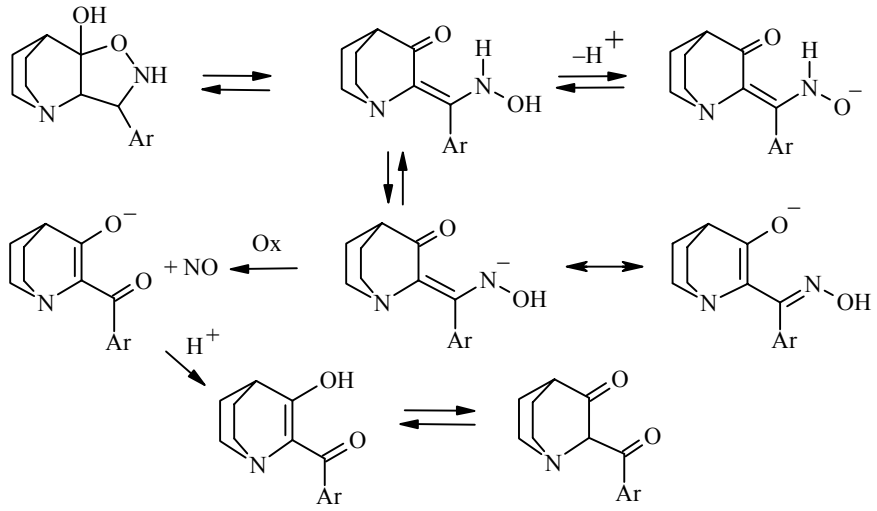
The enamino ketone structure of compound **7** follows from the ^1H and ^{13}C NMR spectra which show the presence in the molecule of a 2'-substituted pyridine ring and a quinuclidine system with an exocyclic double bond at atom $C_{(2)}$ and keto group at $C_{(3)}$. In addition, the ^1H NMR spectrum of compound **7** shows two broad singlets, corresponding to protons exchanging for deuterium in CD_3OD and assigned to the NH_2 group at the exocyclic double bond. The presence in the ^{13}C NMR spectrum of a signal at 205 ppm shows the presence of a keto group in compound **7**. The ^1H (in CD_3OD) and ^{13}C (in CD_3OD and DMSO-d_6) NMR spectra of compound **7**·NH_2OH·2HCl show the same signals as in the spectra of **7,** however the chemical shifts of the nuclei which correspond to these identical signals differ in themselves. The origins of these differences can be judged by the low field region of the ^1H NMR spectrum of **7**·NH_2OH·2HCl where (in addition to the broad signals due to the protons of the NH_2 group) there are observed three broad singlets at 10.16, 10.47 and 12.3 ppm with intensities of 1, 3, and 1 proton units. The lowest field of these signals can be assigned to the quinuclidine ring proton and the two remaining to the NH_3^+ and OH of the hydroxylamine hydrochloride. Hence compound **7** presents itself as a 1:1 complex of the hydrochloride of **7** with hydroxylamine hydrochloride.

As in previous work, testing of the ability of the novel 7-aryl-4a-hydroxy-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidines **5b-e** to behave as NO donors was carried out via their oxidation with $K_3[Fe(CN)_6]$ in NaOH (0.4 N) at 80°C and subsequent detection of the nitroprusside anion formed using a differential pulsed method [2]. The yield of NO (%) was calculated as a ratio of peaks for the second wave of sodium nitroprusside for a solution of the investigated oxime (h_{test}) and a standard solution of the same concentration (h_{ref}):

	$h_{test}/h_{ref}\cdot 100\%$
5b·2HCl	3-6
5c·HCl	3-10
5d·2HCl	1.5-1.7
5e·HCl	17-30
Sodium nitroprusside ($c = 4\cdot10^{-4}$ M, 4% EtOH–H_2O)	100

As is evident from the data given, all of the compounds **5b-e** can generate NO upon oxidation. In contrast to previously studied oximes [1, 2], in this case the degree of formation of the nitric oxide is not a consequence of the effect of the *o*-hydroxy group since both the isomeric hydroxy- (**5b,d**) and the 2-methoxy derivative (**5c**) form NO to an insignificant degree. The most powerful NO donor is the pyridine derivative **5e** and this exceeds in activity the o-hydroxy derivative **4b** from the previous series.

The probable mechanism for formation of the NO can be described by the following scheme:



Hence, in this study, we have investigated the methods of synthesis and certain reactions of 7-aryl-4a-hydroxy-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidines and shown that these cyclic tautomers of γ-hydroxyamino ketones can generate NO through mild oxidation.

EXPERIMENTAL

NMR spectra were taken on a Varian Unity Plus 400 instrument at 400 MHz for the ^1H nucleus and 100.6 MHz for the ^{13}C nucleus and mass spectra on a Finnigan MAT SSQ 710. The melting points were determined in a sealed capillary. TLC was carried out on Silufol UV-254 plates and revealed using UV light and the Dragendorff reagent. The parameters for the compounds obtained are given in Table 1.

(*Z*)-2-(2'-Pyridyl)methylene-3-quinuclidone (1e). The 3-quinuclidone hydrochloride (0.124 mol) and NaOH (0.124 mol) were refluxed in ethanol (96%, 150 ml) for 30 min and a further amount of NaOH (0.124 mol) and pyridine-2-aldehyde (0.124 mol) were then added. The product was refluxed for 10 h (until disappearance of the quinuclidone by TLC) and the ethanol distilled off. The residue was dissolved in water (100 ml), extracted with chloroform (3 × 150 ml), the extracts dried with K_2CO_3, and the chloroform distilled off. The residue was extracted with refluxing petroleum ether (60-70°C boiling range, 3 × 70 ml). After evaporation of the petroleum ether from the combined extracts, crystallization from 2-propanol (150 ml) gave the bright yellow ketone **1e**.

4a-Methoxy-7-(2'-hydroxyphenyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine (6b), 4a-Hydroxy-7-(2'-hydroxyphenyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine Dihydrochloride (5b·2HCl), and the Mixture of Bases (2/3b). A solution of NaOH (100 mmol) in water (9.4 ml) was added to **1b** (67 mmol) and $NH_2OH·HCl$ (100 mmol) in MeOH (300 ml) and the suspension was allowed to stand at room temperature overnight. The NaCl precipitate was separated and the filtrate was evaporated to a reduced volume (100 ml) and diluted with water (200 ml). The precipitate (13.4 g) was refluxed with chloroform (100 ml) to remove the mixture of bases **2/3b** (3.0 g). The residue after extraction was dissolved in refluxing MeOH (250 ml) and the solution then evaporated to 100 ml to give **6b** as light yellow crystals (9.4 g). When the methanol mother liquors of **6b** were evaporated to lower volume (50 ml) the product **4b** (0.7 g, 4.2%) was obtained. Attempted recrystallization of **6b** from refluxing water caused a rapid precipitation of yellow crystalline starting ketone **1b**. Solution of **6b** (1.6 g) in aqueous methanol (2:1, 30 ml) and acidification with an excess of concentrated hydrochloric acid to pH 3 caused the precipitation of the dihydrochloride **5b** (50%).

4a-Hydroxy-7-(2'-methoxyphenyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine Hydrochloride (5c·HCl) and 2-(2'-Methoxyphenyl)methylene-3-quinuclidone Oxime Hydrochlorides (2/3c·HCl). A. A solution of **1c** [2] (31 mmol) and $NH_2OH·HCl$ (34 mmol) in MeOH (170 ml) was allowed to stand overnight at room temperature, the solvent was evaporated, and the product was decomposed using a saturated K_2CO_3 solution, extracted with chloroform (3 × 50 ml), the extracts dried with K_2CO_3, and the chloroform was distilled off. The residue was refluxed with acetone (150 ml) and the insoluble precipitate which contained **5c** was recrystallized from MeOH to give **5c** (1.6 g). This was dissolved in MeOH (10 ml) and converted to the hydrochloride by the addition of the calculated amount of a solution of HCl in anhydrous ether. Evaporation to 1/3 volume and addition of acetone (20 ml) gave **5c·HCl** (1.6 g) which was separated. The acetone solution after removal of **5c** was evaporated and the residue was crystallized from methanol to give **2/3c** (1.5 g, 17%). It was dissolved in acetone and acidified with the calculated amount of ethereal HCl to give **2/3c·HCl** (1.5 g) (the isomeric ratio was 37: 63).

B. A solution of **1c** (2.5 mmol), $NH_2OH·HCl$ (2.7 mmol) and NaOH (1.35 mmol) in 96% EtOH (50 ml) was left at room temperature for one day. According to ^{1}H NMR data the reaction product contained **2/3/5c·HCl** in the ratio 73:8:22.

C. A solution of **1c** (2.5 mmol), NH_2OH (2.7 mmol), and AcOH (1.25 mmol) in 96% EtOH (50 ml) was left at room temperature for one day. According to ^{1}H NMR data the reaction product contained **2/3/5c·HCl** in the ratio 75:13:13.

4a-Hydroxy-7-(4'-hydroxyphenyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine Dihydrochloride (5d·2HCl) and 2-(4'-Hydroxyphenyl)methylene-3-quinuclidone Oxime Hydrochlorides (2/3d·HCl). A. $NH_2OH·HCl$ (20.5 mmol) was added to a solution of the sodium salt **1d** [2] (18.6 mmol) in MeOH (200 ml) and the suspension obtained was left overnight at room temperature. The MeOH was evaporated off, the residue was dissolved in water (100 ml), and the precipitate was separated to give the mixture **2/3d** (2.2 g). This was suspended in water (20 ml) and converted to the hydrochloride by the addition of the calculated amount of 0.5 N HCl to give **2/3d·HCl** (2.2 g, 44%) in the ratio 26: 74 and identical to that obtained previously [2]. After 2 days, a precipitate (0.4 g), containing **5d** mixed with **2d,** was obtained from the aqueous extract. Extraction of this mixture with refluxing chloroform gave **5d** (0.3 g) which was suspended in MeOH and converted to the hydrochloride by the addition of an excess of a solution of HCl in anhydrous ether.

B. A suspension of **1d** (10 mmol) and $NH_2OH·HCl$ (12 mmol) in MeOH (200 ml) was left overnight at room temperature. According to ^{1}H NMR, the clear solution obtained contained **2d** (57%), **5d** (20%), and side products (approximately 23%).

4a-Hydroxy-7-(2'-pyridyl)-4a,6,7,7a-tetrahydroisoxazolo[4,5-*b*]quinuclidine Hydrochloride (5e·HCl), the Dihydrochloride Complex of 2-Amino-(*Z*)-2-(2'-pyridyl)methylene-3-quinuclidone with NH_2OH (7e·NH_2OH·2HCl), and 2-Amino-(*Z*)-2-(2'-pyridyl)methylene-3-quinuclidone (7e). A solution of *Z*-2-(2'-pyridyl)methylene-3-quinuclidone (**1e**, 14 mmol) and $NH_2OH·HCl$ (16 mmol) in MeOH (60 ml) was left at room temperature overnight (the color of the reaction mixture changed from yellow to crimson) and the light

pink precipitate of **5e·HCl** separated. The mother liquor was evaporated, the residue was dissolved in 2-propanol (50 ml), and $NH_2OH·HCl$ (3 mmol) was added. After 10 days, the dihydrochloride of **7e** with NH_2OH was filtered off. The **7e** base was prepared by treatment of the complex with K_2CO_3 solution and extraction with chloroform. After drying with K_2CO_3 and distillation of the chloroform, the residue was transferred to a silica gel column and eluted with a mixture (100 ml) of MeOH and $CHCl_3$ (1:10).

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