ISSN 1070-4280 Russian Journal of Organic Chemistry, 2006, Vol. 42, No. 5, pp. 735–738. © Pleiades Publishing, Inc. 2006.
Original Russian Text © K. Rubina, E. Abele, S. Belyakov, I. Shestakova, Yu. Popelis, 2006, published in Zhurnal Organicheskoi Khimii, 2006, Vol. 42, No. 5, pp. 751–754.

Synthesis, Structure, and Cytotoxicity of *O*-[3-(5-Tetrazolyl)propyl]oximes

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Received February 14, 2005

Abstract—A two-stage method was developed for the conversion of phenyl- and pyridylaldoximes and ketoximes into the corresponding *O*-[3-(5-tetrazolyl)propyl]oximes. The structure of the sodium salt of 2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime was established X-ray diffraction analysis. This compound was shown to possess a high cytotoxic activity in vitro.

DOI: 10.1134/S1070428006050149

Tetrazole derivatives containing oxygen and nitrogen [1–4] are of interest as biologically active compounds [5]. Recently their action was extensively investigated with respect to the cardiovascular system [6–11]. Besides the tetrazole derivatives are used as antihistaminic [12], antiphlogistic [13], and antiulcer [14] preparations. Thiobis(alkylene-5-tetrazoles)[15] and 1-(2-ethylthio-6-aminomethylbenzothiazolyl)-2-phenyl-1,3,4,5-tetrazole [16] were found to possess antitumor activity. However the oxime tetrazole derivatives are poorly understood. It was only established that carbamates of tetrazolyl-(alkylene)oximes exhibited the pesticide activity [17]. As far as we know the cytotoxicity of tetrazole oxime derivatives has not been formerly investigated.

The goal of this study was the synthesis, structure identification, and the study of the cytotoxic activity of new *O*-[3-(5-tetrazolyl)propyl]oximes.

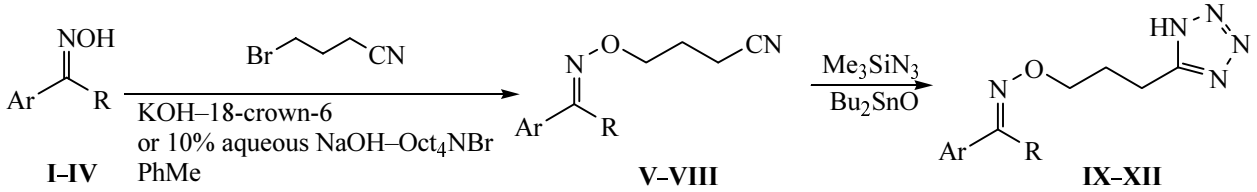
A method for preparation of these oximes **IX–XII** from aldoximes and ketoximes **I–IV** was developed (see the scheme).

Initial oximes **I–IV** in the phase-transfer catalytic systems $NCCH_2CH_2CH_2Br$–solid KOH–18-crown-6–toluene or $NCCH_2CH_2CH_2Br$–10% aqueous NaOH–Oct_4NBr–toluene afforded *O*-(3-cyanopropyl)oximes **V–VIII** in 30–97% yields. The subsequent reaction of oximes **V–VIII** with azidotrimethylsilane in the presence of Bu_2SnO [18] resulted in the formation of tetrazoles **IX–XII** in 39–65% yields.

2-Acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime (**XII**) was isolated as a sodium salt crystal hydrate. According to X-ray diffraction data (Fig. 1) three water molecules are involved in the coordination bonds with the sodium ion. The values of the principal bond lengths and bond angles in the anion of salt **XII** are given in Table 1; there are also listed the lengths of the coordination bonds formed by Na^+ ion.

In the crystal structure of compound **XII** an intricate system exists of intermolecular hydrogen bonds. As seen from Table 2, these bonds are somewhat longer than the statistical average values which for intermolecular

Scheme.



Ar = Ph (**I, II, V, VI, IX, X**), 2-pyridyl (**III, IV, VII, VIII, XI, XII**); R = H (**I, III, V, VII, IX, XI**), Me (**II, IV, VI, VIII, X, XII**).

Table 1. Interatomic distances (l) and bond angles (ω) in the structure of 2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime sodium salt crystal hydrate (**XII**)

Bond	*l*, A	Angle	ω, deg
N^3–N^4	1.349(3)	$N^4N^3N^2$	109.1(2)
N^3–N^2	1.307(3)	$N^{10}O^9C^8$	109.2(2)
O^9–N^{10}	1.399(3)	$C^6C^5N^4$	125.2(2)
O^9–C^8	1.438(3)	$C^6C^5N^1$	123.7(2)
C^5–C^6	1.490(3)	$N^4C^5N^1$	111.1(2)
C^5–N^4	1.332(3)	$C^5C^6C^7$	114.2(2)
C^5–N^1	1.331(3)	$O^9N^{10}C^{11}$	111.6(2)
C^6–C^7	1.514(3)	$N^3N^4C^5$	105.2(2)
N^{10}–C^{11}	1.276(3)	$C^5N^1N^2$	105.2(2)
N^1–N^2	1.344(3)	$C^{12}N^{13}C^{14}$	117.8(3)
N^{13}–C^{12}	1.352(4)	$N^3N^2N^1$	109.5(2)
N^{13}–C^{14}	1.335(4)	$C^6C^7C^8$	112.5(2)
C^7–C^8	1.514(3)	$O^9C^8C^7$	106.8(2)
C^{11}–C^{12}	1.487(4)	$N^{10}C^{11}C^{12}$	116.1(2)
C^{11}–C^{18}	1.499(4)	$N^{10}C^{11}C^{18}$	123.1(3)
C^{12}–C^{17}	1.386(4)	$C^{12}C^{11}C^{18}$	120.8(3)
C^{14}–C^{15}	1.397(5)	$N^{13}C^{12}C^{11}$	117.0(2)
C^{17}–C^{16}	1.368(6)	$N^{13}C^{12}C^{17}$	121.5(3)
C^{16}–C^{15}	1.355(7)	$C^{11}C^{12}C^{17}$	121.5(3)
Na^1···O^1	2.405(2)	$N^{13}C^{14}C^{15}$	122.6(4)
Na^1···O^2	2.318(2)	$C^{12}C^{17}C^{16}$	120.1(4)
Na^1···O^3	2.298(2)	$C^{17}C^{16}C^{15}$	118.9(3)
Na^1···N^{10}	2.506(3)	$C^{14}C^{15}C^{16}$	119.2(4)
Na^1···N^{13}	2.471(3)		

hydrogen bonds of OH···N and OH···O types equal 2.79 and 2.72 A respectively [19]. The projection of the crystal structure is given on Fig. 2.

We investigated some of compounds obtained for their cytotoxic activity in vitro. The toxicity evaluation of

Table 2. Parameters of hydrogen bonds in the crystal structure of 2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime sodium salt crystal hydrate (**XII**)

Bond D–H···A	Length of H-bond D···A, Å	Distance D···A, A	Angle, DH···Å, deg	Position of atom A
O^1–H^{1A}···N^3	2.893(3)	2.08(3)	175(3)	1–*x*, –*y*, 1–*z*
O^1–H^{1B}···N^4	2.804(3)	1.91(4)	174(3)	–1 + *x*, *y*, *z*
O^2–H^{2A}···O^1	2.836(3)	2.04(4)	167(3)	–*x*, –1–*y*, 1–*z*
O^2–H^{2B}···N^1	2.821(3)	2.00(5)	167(4)	*x*, –1+*y*, *z*
O^3–H^{3A}···N^2	2.792(3)	1.89(4)	174(3)	–1+*x*, –1+*y*, *z*
O^3–H^{3B}···O^2	2.883(3)	2.15(5)	153(4)	–*x*, –1–*y*, 1–*z*

compounds **VI, IX, X**, and **XII** on a cell line NIH 3T3 (mice embryos fibroblasts) showed that acetophenone *O*-(3-cyanopropyl)oxime (**VI**) is nontoxic (LD_{50} > 2000 mg/kg), and the toxicity grew in the tetrazoles serried **XII** < **X** < **IX** (LD_{50} in the range 191–717 mg/kg). The concentration of compounds ensuring the 50% cell loss *in vitro* (IC_{50}) was evaluated on five cell lines: HT-1080 (human fibrosarcoma), MG-22A (hepatoma of mice), B16 (melanoma of mice), and Neuro 2A (neuroblastoma of mice), and also on normal cell lines BHK-21 (kidney cells of golden hamster) and NIH 3T3 (mice embryos fibroblasts). The experiments demonstrated that acetophenone cyanooxime **VI** did not exhibit the cytotoxic activity. The raising of the compound hydrophilism by introducing a tetrazole ring in acetophenone *O*-[3-(5-tetrazolyl)propyl]oxime (**X**) led to the appearance of cytotoxicity: IC_{50} was from 3.5 (HT-1080) to 28 μg/ml (Neuro 2A). Consequently, sodium salt **XII** soluble in water possesses high cytotoxic activity for all cell lines, both cancerous and normal [for instance, IC_{50} 1.4 (HT-1080) and 0.9 μg/ml (BHK-21)]. Certain selectivity of cytotoxic action was observed for benzaldehyde *O*-[3-

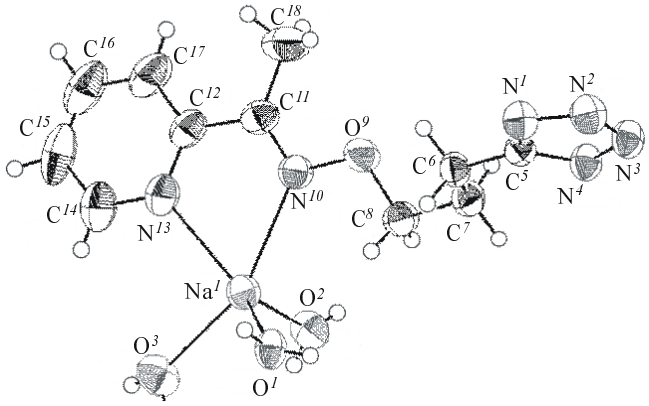


Fig. 1. Spatial model of the complex of 2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime (**XII**).

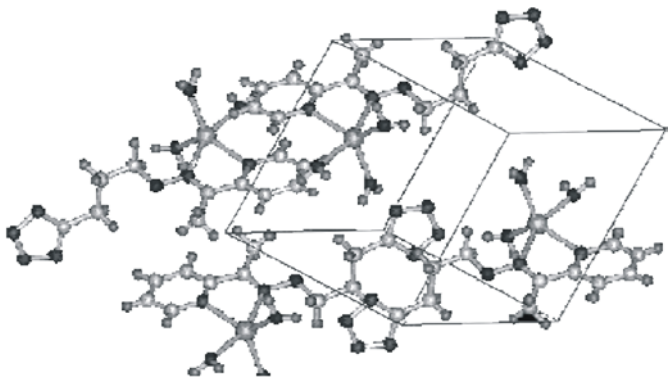


Fig. 2. Projection of the crystalline structure of the complex of 2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime (**XII**).

(5-tetrazolyl)propyl]oxime (**IX**). Its cytotoxicity for the line HT-1080 was 3.5 μg/ml, for NIH 3T3, 114 μg/ml.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were registered on a spectrometer Varian 200 Mercury at operating frequencies 200.06 and 50.31 MHz in $CDCl_3$, internal reference HMDS. Mass spectra were registered on a GC-MS HP 6890 instrument with ionizing electrons energy 70 eV. GLC was performed on a chromatograph Chrom-5 equipped with a flame ionization detector, a glass column (1200×3 mm), stationary phase 5% OV-101 on Chromosorb W-HP (80–100 mesh), carrier gas nitrogen, flow rate 60 cm^3/min. Oven temperature was varied in the range 180–250°C depending on the reaction mixture composition. Commercial dibutyltin oxide, 1-bromo-3-cyanopropane, azidotrimethylsilane, and 18-crown-6 (Acros) were used without additional purification. Oximes **I–IV** were obtained from the corresponding aldehydes and ketones [20].

Benzaldehyde *O*-(3-cyanopropyl)oxime (V). To a suspension of 1.35 g (10 mmol) of benzaldehyde oxime, 1.68 g (30 mmol) of solid, finely dispersed KOH, and 264 mg (1 mmol) of 18-crown-6 in 10 ml of toluene was added at vigorous stirring 1.48 g (10 mmol) of 1-bromo-3-cyanopropane. The reaction continued for 5 h at room temperature (GLC monitoring). Then the reaction mixture was filtered and evaporated. The reaction product was isolated by column chromatography on silica gel, eluent toluene (R_f 0.4). Yield 1.8 g (96%). ^{1}H NMR spectrum, δ, ppm: 2.10 m (2H, CH_2), 2.48 t (2H, OCH_2, *J* 7.2 Hz), 4.25 t (2H, CH_2CN, *J* 6.0 Hz), 7.36 m (3H, H$^{3–5}$), 7.56 m (2H, H^{2,6}), 8.07 s (1H, CH). ^{13}C NMR spectrum, δ, ppm: 14.1 (CH_2), 25.4 (OCH_2), 71.4 ($\underline{C}H_2C$), 119.3, 127.0, 128.7, 131.9, 149.2. Mass spectrum, *m/z* (I_{rel}, %): 188 (22) M^+, 187 (28), 157 (26), 131 (15), 120 (25), 105 (41), 104 (88), 94 (18), 89 (21), 78 (26), 77 (100), 65 (27), 51 (58). Found, %: C 70.39; H 6.43; N 14.78. $C_{11}H_{12}N_2O$. Calculated, %: C 70.19; H 6.43; N 14.88.

Acetophenone *O*-(3-cyanopropyl)oxime (VI) was similarly obtained within 4 h. The reaction mixture was filtered and used in the next stage of the synthesis without isolation of the product. Mass spectrum, *m/z* (I_{rel}, %): 202 (31) M^+, 20 (28), 171 (58), 134 (32), 117 (30), 118 (43), 106 (17), 104 (73), 94 (10), 78 (26), 77 (100), 51 (25).

2-Pyridinealdehyde *O*-(3-cyanopropyl)oxime (VII). To a suspension of 0.84 g (6.7 mmol)of 2-pyridinealdehyde oxime (**III**), 0.183 g (0.34 mmol) of tetraoctylammonium bromide, 5.4 ml of 10% aqueous NaOH in 7 ml of toluene was added 1.02 g (6.7 mmol) of 1-bromo-3-cyanopropane, the mixture was stirred for 5 h at 100°C, then filtered, the filtrate was evaporated on the rotary evaporator. The residue was purified by column chromatography (eluent ethyl acetate, R_f 0.45). Yield 0.38 g (30%). ^{1}H NMR spectrum, δ, ppm: 2.10 m (2H, CH_2), 2.50 t (2H, OCH_2, *J* 7.6 Hz), 4.32 t (2H, CH_2CN, *J* 5.8 Hz), 7.27 m (1H, H^5), 7.73 m (2H, H^{3,4}), 8.17 s (1H, CH), 8.61 m (1H, H^6). ^{13}C NMR spectrum, δ, ppm: 14.0 (CH_2), 25.3 (OCH_2), 71.9 ($\underline{C}H_2C$), 119.2, 121.1, 124.1, 136.5, 149.7, 149.9, 151.3. Mass spectrum, *m/z* (I_{rel}, %): 189 (4) M^+, 122 (12), 106 (19), 92 (17), 79 (100), 78 (62), 65 (10), 51 (30).

2-Acetylpyridine *O*-(3-cyanopropyl)oxime (VIII) was prepared as oxime **V** in 3 h. The residue was purified by column chromatography (eluent ethyl acetate, R_f 0.75). Yield 1.96 g (97%). ^{1}H NMR spectrum, δ, ppm: 2.10 m (2H, CH_2), 2.33 s (3H, Me), 2.50 t (2H, OCH_2, *J* 7.2 Hz), 4.33 t (2H, CH_2CN, *J* 5.6 Hz), 7.23 m (1H, H^5), 7.66 m (1H, H^4), 7.88 m (1H, H^3), 8.59 m (1H, H^6). ^{13}C NMR spectrum, δ, ppm: 11.2 (Me), 14.2 (CH_2), 25.5 (OCH_2), 71.7 ($\underline{C}H_2C$), 119.4, 120.5, 123.7, 136.2, 148.8, 154.0, 156.5. Mass spectrum, *m/z* (I_{rel}, %): 203 (17) M^+, 119 (28), 105 (52), 79 (100), 78 (70), 52 (20), 51 (37).

Benzaldehyde *O*-[3-(5-tetrazolyl)propyl]oxime (IX). To a suspension of 1.06 g (5.6 mmol) of aldehyde oxime **V** and 0.14 g (0.56 mmol) of Bu_2SnO in 2 ml of anhydrous toluene was added 1.3 g (11.3 mmol) of azidotrimethylsilane. The reaction mixture was stirred for 30 h at 100°C till disappearance of compound **V** (GLC monitoring). Then the reaction mixture was neutralized with a saturated solution of $NaHCO_3$, the precipitate was filtered off and dried in air, and then recrystallized from ethyl acetate. Yield 1 g (43%), colorless crystals, mp 90–92°C. ^{1}H NMR spectrum, δ, ppm: 2.10 m (2H, CH_2), 2.97 t (2H, OCH_2, *J* 8.0 Hz), 4.17 t (2H, CH_2CN, *J* 6.4 Hz), 7.41 m (3H, H$^{3–5}$), 7.61 m (2H, H^{2,6}), 8.22 s (1H, CH). ^{13}C NMR spectrum, δ, ppm: 19.9 (CH_2), 27.0 (OCH_2), 72.5 ($\underline{C}H_2C$), 126.8, 128.8, 129.9, 132.0, 148.8, 156.5. Found, %: C 56.81; H 5.62; N 30.01. C-$_{11}H_{13}N_5O$. Calculated, %: C 57.13; H 5.67; N 30.28.

Acetophenone *O*-[3-(5-tetrazolyl)propyl]oxime (X) was prepared in the same way in a 39% yield. On recrystallization from ethyl ether mp 68–70°C. ^{1}H NMR spectrum, δ, ppm: 2.17 s (3H, Me), 2.24 m (2H, CH_2), 3.18 t (2H, OCH_2, *J* 7.2 Hz), 4.26 t (2H, CH_2CN,

J 5.6 Hz), 7.34 m (3H, H^{3-5}), 7.56 m (2H, H^{2-6}). ^{13}C NMR spectrum, δ, ppm: 13.0 (Me), 20.3 (CH_2), 27.4 (OCH_2), 72.1 ($\underline{C}H_2C$), 126.0, 128.6, 129.4, 136.2, 156.3. Found, %: C 58.68; H 6.15; N 28.51. $C_{12}H_{15}N_5O$. Calculated, %: C 58.76; H 6.16; N 28.55.

2-Pyridinealdehyde *O*-[3-(5-tetrazolyl)propyl]-oxime (XI) was prepared in the same way in a 43% yield. The precipitate was recrystallized from a mixture of ethyl acetate and ethanol, mp ~40°C. The compound is unstable and decomposes in air. ^1H NMR spectrum, δ, ppm: 1.99 m (2H, CH_2), 2.71 t (2H, OCH_2, *J* 7.6 Hz), 4.21 t (2H, CH_2CN, *J* 6.6 Hz), 7.42 m (1H, H^5), 7.84 m (2H, $H^{3,4}$), 8.16 s (1H, CH), 8.31 s (1H, NH), 8.59 m (1H, H^6). ^{13}C NMR spectrum, δ, ppm: 21.7 (CH_2), 28.5 (OCH_2), 74.0 ($\underline{C}H_2C$), 120.4, 124.3, 136.9, 148.9, 149.6, 161.7. Found, %: C 51.61; H 5.18; N 36.10. $C_{10}H_{12}N_6O$. Calculated, %: C 51.72; H 5.21; N 36.19.

2-acetylpyridine *O*-[3-(5-tetrazolyl)propyl]oxime sodium salt (XII) was similarly prepared. The precipitate was recrystallized from a mixture of ethyl acetate and ethanol. Yield 0.86 g (65%), colorless crystals, mp 101–102°C. ^1H NMR spectrum, δ, ppm: 2.01 m (2H, CH_2), 2.25 s (3H, Me), 2.73 t (2H, OCH_2, *J* 7.6 Hz), 4.23 t (2H, CH_2CN, *J* 6.7 Hz), 7.39 m (1H, H^5), 7.84 m (2H, $H^{3,4}$), 8.59 m (1H, H^6). ^{13}C NMR spectrum, δ, ppm: 10.9 (Me), 21.8 (CH_2), 28.7 (OCH_2), 73.8 ($\underline{C}H_2C$), 120.0, 124.0, 136.6, 148.8, 153.5, 154.9, 160.3. Found, %: C 40.81; H 6.18; N 26.10. $C_{11}H_{20}N_6NaO_4$. Calculated, %: C 40.86; H 6.24; N 25.99.

Single crystals of compound **XII** triclinic, parameters of the unit cell: *a* 7.8108(3), *b* 9.6847(4), *c* 11.6714(6) Å, α 76.096(2), β 83.396(2), γ 77.316(2) deg, *V* 834.29(6) $Å^3$, *F*(000) 340, μ 0.12 mm^{-1}, d_{calc} 1.283 g/cm^3, *Z* 2, space group $P\bar{1}$.

Intensities of 6590 reflections (4087 among them independent) were measured on an automatic diffractometer Nonius KappaCCD (molybdenum radiation, λ 0.71073, graphite monochromator) till $2\theta_{max}$ 55°. In calculations were used 2620 reflections with $I > 3\sigma(I)$. The structure was solved by procedure [21]. The refinement was performed by least-squares procedure in the full-matrix anisotropic approximation using software *maxus* [22]. All hydrogen atoms were localized by difference synthesis and refined in isotropic approximation. The final value of the divergence factor is *R* 0.043.

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