UDC 615.31:[547.78+547.772].012.1

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SUBSTITUTED 7-AZA-8-AZA(OXA)-BICYCLO[4.3.0]-6,9-NONADIENES

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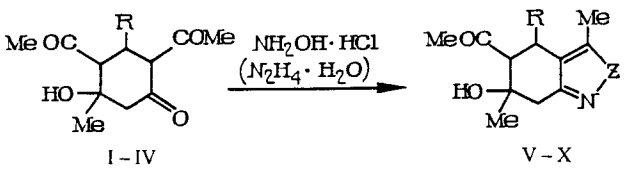
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 29, No. 1, pp. 44 – 46, January, 1995.

Original article submitted November 29, 1993.

Hydroxylamine- and hydrazine-induced heterocyclization of readily available 5-hydroxy-2,4-diacetyl-5-methyl-3R-cyclohexanones (R = methyl, phenyl, m-nitrophenyl, α-furyl) yielded 6-acetyl-5-hydroxy-5,9-dimethyl-7R-1-aza(oxa)-2-azabicyclo[4.3.0]-2,8-nonadienes (R = methyl, phenyl, *m*-nitrophenyl, α-furyl). The biological activity of the latter was tested using the *Escherichia coli* phage T_4, and was also tested on a stored lyophilized culture of *Yersinia pestis* EU.

Isoxazoles and pyrazoles are known to have a broad spectrum of biological action. In particular, they exhibit antituberculosis, antileprosy, hypolipidemic, and antitumor activity [1]. However, the biological effect of condensed pyrazoles and isoxazoles, especially of polyfunctional substituted ones, has been studied to a far lesser extent. The antimicrobial activity of condensed isoxazoles and pyrazoles and their use as intermediates in the synthesis of antihelminthic drugs was reported in the literature [1, 2]. Here, we present the results of a study of the synthesis and biological activity of bicyclic systems including the pyrazole and isoxazole rings: **3-acetyl-4-hydroxy-4,9-dimethyl-2-R-7-aza-8-aza(oxa)bicyclo[4.3.0]-6,9-nonadienes (V – X).** Compounds of this type were obtained in preparative yields upon heterocyclization of 5-hydroxy-2,4-diacetyl-5-methyl-3-R-cyclohexanones I – IV when treated with the binucleophilic reagents hydroxylamine and hydrazine. The starting β-diketones I – IV are readily available products of the aldol condensation of acetylacetone with aldehydes (acetaldehyde, furfural, benzaldehydes).



I – IV V – X

Z = O (V – VII), NH (VIII – X);

R = Me (I, V), Ph (II, VI, VIII), α-Fu (III, VII, IX), or $C_6H_4NO_2$-3 (IV, X).

The literature describes the synthesis of isoxazole VI without indicating the yield (when the reactants were boiled eight hours in ethanol) [1]. An attempt to reproduce the synthesis procedure did not give the desired results. We found conditions that enabled us to obtain products V – VII with yields up to 84% (holding the reaction mixture at 20°C during 12 – 16 h in a water and alcohol solution). The mild reaction conditions prevent possible degradation of the initial β-ketols I – IV [2]. The reaction proceeds successfully with the participitation of a 1,3-dicarbonyl fragment. The acetyl group at the fourth carbon atom remains unchanged because of steric factors. Absorption bands are noted in the IR spectra of C=N groups for isoxazoles V – VII at 1640 – 1630 cm^{-1}, for pyrazoles VIII – X in the region 1590 – 1580 cm^{-1}; stretching vibrations of the carbonyl group of the acetyl substituent ($\nu_{C=O}$ at 1710 – 1680 cm^{-1}) are present. The isoxazoles V – VII are characterized by ν_{OH} at 3350 cm^{-1}. The spectra of pyrazoles VIII – X have a broad band of associated NH- and OH- groups in the region 3400 – 3200 cm^{-1}. The physicochemical properties of compounds V – X are given in Table 1.

We studied the biological activity of azaheterocycles V – X on a model of the T_4 coliform (Table 2). The experiments

TABLE 1. Characteristics of Compounds V – X

Compound	Yield, %	M. p.,°C	Molecular formula
V	55	147 – 150	$C_{12}H_{17}NO_3$
VI*	84	138 – 140	$C_{17}H_{19}NO_3$
VII	71	151 – 153	$C_{15}H_{17}NO_4$
VIII*	88	204 – 208 (with decomp.)	$C_{17}H_{20}N_2O_2$
IX	79	198 – 200 (with decomp.)	$C_{15}H_{18}N_2O_3$
X	92	193 – 195	$C_{17}H_{19}N_3O_4$

* Described in Refs. [1, 3].

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TABLE 2. Influence of Substituted 7-Aza-8-aza(oxa)bicyclo[4.3.0]-6,9-nonadienes (V – X) on Storage Life of the Vaccine Strain of the Plague Microbe EU (Research Institute of Epidemiology and Hygiene) in the Lyophilied State with Retention of 50% Viability

Compound	Concentration of substance, μg / ml	Storage life, % of control	Compound	Concentration of substance, μg / ml	Storage life, % of control
V	2	90	VIII	2	93
	5	90		5	71
	10	104		10	88
	25	104		25	95
VI	2	69	IX	2	100
	5	98		5	98
	10	94		10	98
	25	130		25	64
VII	2	62	X	2	88
	5	64		55	86
	10	76		10	92
	25	79		25	122
			Control	25	100

showed that the tested compounds have a moderate inhibiting effect with respect to phage T_4: under the action of IX and X, the survival rate of the phage dropped to 53 and 58%, while for compounds V, VII, and VIII the survival rate was 61, 64, and 66%, respectively. Among the studied compunds, the lowest inhibiting effect on the T_4 bacteriophage was exhibited by compound VI, upon action of which the survival rate was 89%.

The moderate inhibiting effect of the tested compounds on the T_4 phage suggested testing them as potential antioxidants and cryoprotectors in experiments involving lyophilization of bacterial strains. For this purpose, compounds V – X were tested in experiments to determine how they affect the storage life of the live dry plague vaccine EU. The indicated substances were found to change the storage life of the vaccine strain of the plague microbe EU (Research Institute of Epidemiology and Hygiene) in the lyophilized state (see Table 2). Compounds VI and X in a concentration of 25 μg/ml increase it by 30 and 22%, respectively.

Consequently, the synthesized 7-aza-8-aza(oxa)bicyclo[4.3.0]-6,9-nondienes have a moderate inhibiting effect on the T_4 coliform bacteriophage, while two of the compounds (VI and X) are promising for use as antioxidants and cryoprotectors in the lyophilization of bacteria.

CHEMICAL EXPERIMENTAL PART

The infrared spectra were recorded in vaseline oil on a Specord M80 spectrometer (GDR). 5-Hydroxy-2,4-diacetyl-5-methyl-3-R-cyclohexanones (I – IV) and 3-acetyl-4-hydroxy-4,9-dimethyl-2-R-1,2-diazabicyclo[4,3,0]-6,9-nonadienes (VIII – X) were obtained by the familiar procedure in [3].

3-Acetyl-4-hydroxy-4,9-dimethyl-2-R-1-oxa-2-azabicyclo[4.3.0]-6,9-nonadienes (V – VIII). Typical procedure. 0.01 mole of cycloketol I – IV was dissolved with heating in 25 ml of ethanol and 5 ml of an aqueous solution of 0.02 mole of $H_2NOH \cdot HCl$ was added. The reaction mixture was held for 12 – 16 h at room temperature. We filtered off the solid precipitate, washed it with water, and recrystallized it from ethanol.

BIOLOGICAL EXPERIMENTAL PART

The biological activity of the synthesized chemical compounds was determined on a model of the T_4 coliform bacteriophage by the procedure proposed by Fonshtein, et al., [4] in an indicator culture of *Escherichia coli* B by the two-layer agar method. The antiphage activity was expressed in percent of inactivation by the formula $A = (C_{exp} / C_{con}) \times 100\%$, where C_{exp} is the number of surviving phage particles in an experiment, and C_{con} is the number surviving in the control group.

The influence of the heterocyclic compounds on the storage life of live plague vaccine EU in a lyophilized state was determined by the procedure indicated in [5]. Each substance was tested in concentrations of 2, 5, 10, and 25 μg / ml dissolved in 0.1% chemically pure ethanol.

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