SYNTHESIS AND ANTIVIRAL ACTIVITY OF SPATIALLY-SCREENED PHENOLS: 1,3-BENZOXATHIOLAN-2-ONE DERIVATIVES

O. I. Shadyro,[1] V. A. Timoshchuk,[1] G. I. Polozov,[1] V. N. Povalishev,[2] O. T. Andreeva,[2] and V. E. Zhelobkovich[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 33, No. 7, pp. 25 – 27, July, 1999.

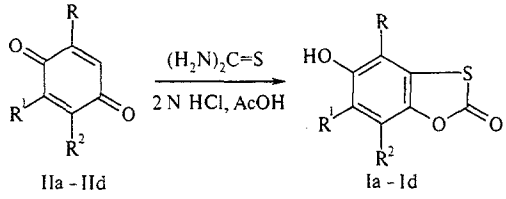
Original article submitted November 16, 1998.

At present, the chemistry of spatially screened phenols is being extensively developed. This fact is explained both by the practical value of these compounds (antioxidants, radiation protectors, drugs) and their role in investigations of the fundamental biochemical reactions in the cell, since these processes frequently involve reversible reactions of the oxidized and reduced forms of phenol derivatives.

In continuation of our previous works on the synthesis of spatially screened phenols and study of their biological activity [1 – 5], we have prepared a series of 1,3-benzoxathiolan-2-ones and characterized them with respect to antiviral activity. These compounds can be considered as structural analogs of the natural antioxidant α-tocopherol, which differ from the latter by the position of the phenol hydroxyl and by the presence of a five-member oxathiolane cycle instead of the six-member chroman cycle. According to the data of Burton et al. [6, 7], the change from six- to five-member cycle in the tocopherol structure leads to a significant increase in the antioxidant activity.

The synthesis of 5-hydroxy-1,3-benzoxathiolan-2-one (Ia) and related analogs from the corresponding *p*-benzoquinones and thiocyanic acid, as well as the ability of compounds Ia to suppress the activity of some pathogenic fungi, was reported by Fiedler [8]. Later, Lau and Kestner [9] developed a more convenient single-stage method for the synthesis of these compounds using thiourea reactions in the presence of mineral acids. It was demonstrated that intermediate products in these reactions are isothiuronium salts, which convert into 2-amino-6-hydroxybenzothiazole salts at room temperature and into 5-hydroxy-1,3-benzoxathiolan-2-one on heating [10].

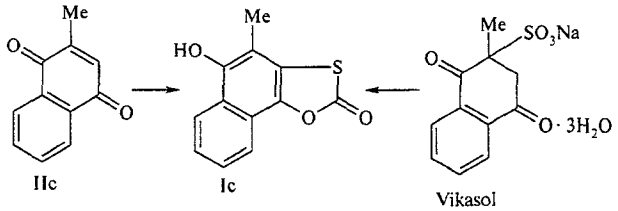
We have established that, under the conditions used for the synthesis of compound I, dialkyl-*p*-benzoquinones (IIb – IId) readily transform into the corresponding 5-hydroxy-1,3-benzoxathiolan-2-ones (Ib – Id) at a yield of 62 – 80%:



IIa - IId Ia - Id

At the same time, the attempts to alkylate Ia with isopropyl or *tert*-butyl alcohols in the presence of concentrated H_2SO_4 were unsuccessful both at room temperature and on heating. However, boiling a mixture of compound Ia with isopropyl alcohol in the presence of 60% $HClO_4$ leads, in the course of distilling off the alcohol, to the formation of compound Id with a final yield of 55%. The same process with *tert*-butyl alcohol yields no target compound.

In contrast to the apparent conclusion that the isothiuronium salt formed during the interaction of 2-methy-1,4-naphthoquinone (IIe) with thiourea cannot enter a cyclization reaction with the formation of an oxathiolane cycle [8], we have succeeded in obtaining 5-hydroxy-4-methyl-1,3-naphtho[1,2-d]oxathiolan-2-one (Ie) from IIe at a yield of 40%. Moreover, compound Ie was obtained from vicasol (a bisulfite derivative of IIe) and thiourea at an almost quantitative yield according to the following scheme:@@

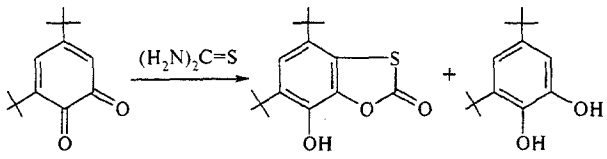


IIc Ie Vikasol

The reaction of 3,5-di-*tert*-butyl-1,2-benzoquinone (IIf) (a disubstituted *o*-benzoquinone) with thiourea leads to 7-hy-

[1] Belarus State University, Minsk, Belarus.
[2] Belarus Research Institute of Epidemiology and Microbiology, Minsk, Belarus.

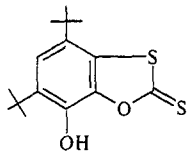
droxy-4,6-di-*tert*-butyl-1,3-benzoxathiolan-2-one (If) at a 62% yield:



IIf If

This process is accompanied by the formation of a considerable amount of 3,5-di-*tert*-butylpyrocatechol, which is a product of reduction of *o*-quinone IIf.

Previously [3], we have described the synthesis of 7-hydroxy-4,6-di-*tert*-butyl-1,3-benzoxathiolan-2-thione (Ig):



Ig

The proposed structures of the synthesized compounds were confirmed by the data of mass spectrometry and ^1H NMR spectroscopy. The physicochemical characteristics of compounds Ib – Ig are presented in Table 1.

EXPERIMENTAL CHEMICAL PART

The course of reactions was monitored and the purity of reaction products was checked by TLC on Silufol UV-254 plates eluted in the chloroform – methanol (9 : 1) or hexane – chloroform – ethyl acetate (5 : 3 : 2) systems. The melting temperatures of the synthesized substances were determined using a Boetius heating stage. The data of elemental analyses (C, H, S) agree with the results of analytical calculations. The ^1H NMR spectra were measured on Jeol PS-100 (100 MHz) and Bruker AC–200 (200 MHz) spectrometers using TMS as the internal standard. The mass spectra were obtained with a Shimadzu QP-5000 mass spectrometer using an ion source with direct sample injection, operated at a temperature of 200°C and an electron impact ionization energy of 70 eV.

For the synthesis of compounds reported previously, see the following: Ia [8], Ig [3], IIb [11], IId [12], and IIf [4].

General method for the synthesis of compounds Ia – If. A solution of 0.01 mole of the corresponding quinone in 10 ml of acetic acid is added dropwise to a solution of 0.015 mole thiourea in 20 ml of 2 N HCl, after which the mixture is heated for 1 h on a boiling water bath and cooled. The precipitated crystalline product is separated by filtration, washed with water, and crystallized from aqueous isopropyl alcohol or (upon drying) from hexane.

7-*tert*-Butyl-5-hydroxy-4-isopropyl-1,3-benzoxathiol an-2-one (Ic). To a suspension of 3.04 g (0.02 mole) of 2-isopropylhydroquinone [10] in 10 ml of *tert*-butyl alcohol was added with stirring and cooling on ice 3 ml of concentrated H_2SO_4, after which the reaction mixture was allowed to stand for 24 h at room temperature. Then 50 ml of chloroform was added and the reaction mass was washed sequentially with water, $NaHCO_3$ solution, and water again, dried over anhydrous Na_2SO_4, and evaporated in vacuum to dryness. The residue was crystallized from hexane to obtain 3.36 g (83%) of 5-*tert*-butyl-2-isopropylhydroquinone (III); m.p., 178°C; ^1H NMR spectrum in DMSO-d_6 (δ, ppm): 6.75, 6.65 (s, 2H, H_{arom}), 3.30 (q, 1H, CH), 1.40 (s, 9H, Me_3), 1.25 (d, 6H, Me_2).

To a solution of hydroquinone III in 2.1 g (0.01 mole) 20 ml of dioxane was added 1.5 ml of 1 N H_2SO_4 and 1.7 g of $KBrO_3$ in 15 ml of water and the mixture was heated with stirring for 15 min. Then the mixture was diluted with an equal volume of water and extracted with ether (3 × 25 ml). The ether extracts were combined, washed sequentially with water, $NaHCO_3$ solution, and water again, and evaporated to dryness. The residue, representing quinone IIc, was introduced without additional purification into the reaction with

TABLE 1. Physicochemical Characteristics of Compounds Ib – Ig

Compound	Yield, %	M.p., °C	Empirical formula	^1H NMR spectrum*: δ, ppm	Mass spectrum: m/z (I_{rel})
Ib	80	142 – 143	$C_{13}H_{16}O_3S$	6.85 (s, 1H, H_{arom}), 3.6 – 3.0 (m, 2H, CH), 1.40 (d, 6H, Me_2), 1.35 (d, 6H, Me_2)	252 (M^+, 67), 237 (100), 224 (7), 209 (17), 181 (24), 163 (37)
Ic	68	145 – 147	$C_{14}H_{18}O_3S$	6.90 (s, 1H, H_{arom}), 3.30 (q, 1H, CH), 1.40 (s, 9H, Me_3), 1.30 (d, 6H, Me_2)	
Id	78	108 – 109	$C_{13}H_{16}O_3S$	6.80 (s, 1H, H_{arom}), 5.02 (s, 1H, OH), 3.45 – 2.70 (m, 2H, CH), 1.28 (d, 6H, Me_2), 1.20 (d, 6H, Me_2)	252 (M^+, 70), 237 (100), 224 (5), 209 (15), 181 (22), 167 (35)
Ie	93	175 – 176	$C_{12}H_8O_3S$	8.25 (d, 1H, H_{arom}), 7.88 (d, 1H, H_{arom}), 7.62 (m, 2H, H_{arom}), 2.33 (s, 3H, Me)	232 (M^+, 92), 204 (100), 189 (1.5), 176 (34), 160 (13), 147 (23), 143 (35), 115 (47)
If	62	180 – 181	$C_{15}H_{20}O_3S$	7.15 (s, 1H, H_{arom}), 5.43 (s, 1H, OH), 1.42 (s, 9H, Me_3), 1.35 (s, 9H, Me_3)	280 (M^+, 17), 265 (100), 237 (4), 209 (3), 181 (5), 163, 147 (2)
Ig	85	135 – 136	$C_{15}H_{20}O_2S_2$	7.20 (s, 1H, H_{arom}), 5.6 – 5.5 (bs, 1H, OH), 1.44 (s, 9H, Me_3), 1.34 (s, 9H, Me_3)	296 (M^+, 35), 281 (100), 265 (3), 253 (6), 239 (3), 225 (11), 205 (5), 195 (3)

* ^1H NMR spectra of compounds Id, If, and Ig were measured in $CDCl_3$, otherwise – in $(CD_3)_2SO$.

thiourea (as described above) to obtain 1.97 g (78%) of compound Ic.

4,6-Diisopropyl-1,3-benzoxathiolan-2-one (Id). To a suspension of 1.68 g (0.01 mole) of compound Ia in 25 ml of isopropyl alcohol was added 1 ml of 60% $HClO_4$ and the reaction mixture was carefully heated in a flask with reflux until complete removal of the alcohol. The residue was crystallized from hexane to obtain 1.4 g (55%) of compound Id.

5-Hydroxy-4-methyl-1,3-naphtho[1,2-d]oxathiolan-2-one (Ie). To a solution of 10 g (0.03 mole) of vikasol in a mixture of 50 ml of 2 N HCl and 100 ml of acetic acid was added 15 g (0.2 mole) of thiourea and the reaction mixture was heated for 1 h on a boiling water bath. Then the mixture was treated as described above to obtain 6.5 g (93%) of compound Ie; m.p., 175°C.

EXPERIMENTAL BIOLOGICAL PART

The antiviral properties of the synthesized compounds were studied *in vivo* on a model of experimental lethal meningoencephalitis [13, 14]. The experiments were performed on a group of mice weighing 7 – 10 g inoculated with herpes simplex virus of type 1 (HSV-1) (Koptev strain) with an infection titer of 5.5log LD_{50}. The test animals were infected with the virus by intraperitoneal injection at a dose of 100LD_{50}. Both the compounds studied and placebo (control) were introduced into stomach at a dose of 1 – 200 mg/kg once per day over a period of 7 days. The samples and placebo were dissolved in a physiological 0.85% NaCl solution containing 10% of Tween 80.

The acute toxicity was determined by a conventional method [14] using data on the survival of animals. The antiviral activity of substances at the tolerated doses was estimated from the decrease in the loss of treated animals in comparison with the control group receiving placebo. The data were statistically processed by conventional methods [15, 16].

TABLE 2. Effect of the Synthesized Compounds on the Development of Meningoencephalitis in Mice Infected with HSV-1

Compound	Dose, mg/kg	Lethality ($M \pm m$), %	Protection level, %	P	CTR
Ionol	10	41.6 ± 14.86	52.15	< 0.01	10
	1	58.3 ± 14.86	38.45	< 0.05	
Ia	100	41.6 ± 14.86	52.15	< 0.01	100
	10	41.6 ± 14.86	52.15	< 0.01	
	1	58.3 ± 14.86	38.45	< 0.05	
Ib	200	37.5 ± 18.29	56.25	< 0.01	20
	100	41.6 ± 14.86	52.15	< 0.01	
	10	41.6 ± 14.86	52.15	< 0.01	
	1	66.6+14.21	27.15	> 0.05	
Ic	100	58.3 ± 14.86	38.45	< 0.05	10
	10	58.3 ± 14.86	38.45	< 0.05	
	1	66.6 ± 14.21	27.15	> 0.05	
Id	200	33.3 ± 14.21	60.45	< 0.01	200
	100	33.3 ± 14.21	60.45	< 0.01	
	10	41.6 ± 14.86	52.15	< 0.01	
	1	50.0 ± 15.07	43.75	< 0.01	
If	10	100.0 ± 7.66	0.0	> 0.05	0
	1	100.0 ± 7.66	0.0	> 0.05	
	0.1	100.0 ± 7.66	0.0	> 0.05	
Ig	10	100.0 ± 7.66	0.0	> 0.05	0
	1	100.0 ± 7.66	0.0	> 0.05	
	0.1	100.0 ± 7.66	0.0	> 0.05	
Placebo	—	93.75 ± 6.25	—	—	—

RESULTS AND DISCUSSION

The results of our investigation showed that compounds Ia – Id exhibit antiviral activity in the organism of experimental animals (Table 2). The antiviral effect was manifested in reducing the loss of animals from the lethal HSV-1 infection, although the degree of protection varied both relative to the placebo (control group) and in comparison to the reference drug ionol. A decrease in the level of lethality by 60.45 – 38.45% indicates a sufficiently high and reproducible protective action ($p < 0.001 – 0.05$). It was found that the substances tested, while differing rather insignificantly with respect to antiviral effect, exhibited a quite broad range of active concentrations, thus showing a considerable scatter as compared to ionol.

For example, the tolerated concentration of ionol was rather low (10 mg/kg) and provided a 52.15% decrease in the loss of animals against the control group. At a higher concentration (100 mg/kg), ionol led to premature loss of the animals tested (100% lethality), rather than producing any protective action. As the ionol concentration was reduced below 10 mg/kg, the level of protection decreased as well, while being still somewhat reliable even at a dose of 1 mg/kg. Thus, the chemotherapeutic ratio (CTR) of the reference drug ionol was 10. The same level was characteristic of the activity of compound Ic, and a close value (CTR = 20) was observed for compound Ib. At the same time, both these compounds showed a lower acute toxicity than ionol, their tolerated doses reaching 100 and 200 mg/kg, respectively.

Among the compounds tested, the most attractive antiviral properties were observed for compound Ia (CTR = 100) and, even more so, for compound Id (CTR = 200). The latter compound exhibited activity in the whole dose range of 1 – 200 mg/kg. As seen from Table 2, the minimum dose tested (1 mg/kg) ensured a pronounced and reliable protective antiviral effect, which suggests that the activity of this compound is retained even at still lower concentration. The value of CTR = 200 allows compound Id to be classified as a promising antiviral agent. Of the group of substances tested *in vivo*, no antiviral properties were observed for compound If and its thioanalog Ig.

Thus the results of our experiments do not contradict the assumption that there is a definite relationship between the antiviral activity of compounds in the series studied and their antioxidant properties.

REFERENCES

1. G. N. Shilov, A. I. Balakleevskii, O. I. Shadyro, et al., USSR Inventor's Certificate No. 182 759; *Byull. Izobret.*, No. 26 (1993).
2. D. K. Petrikevich, V. A. Timoshchuk, O. I. Shadyro, et al., *Khim.-Farm. Zh.*, **29**(12), 32 – 34 (1995).
3. L. A. Maslovskaya, D. K. Petrikevich, V. A. Timoshchuk, et al., *Zh. Obshch. Khim.*, **66**(11), 1893 – 1898 (1996).
4. L. A. Maslovskaya, D. K. Petrikevich, V. A. Timoshchuk, et al., *Zh. Obshch. Khim.*, **66**(11), 1899 – 1902 (1996).
5. O. I. Shadyro, G. N. Shilov, V. A. Timoshchuk, et al., *Vopr. Med. Khim.*, **43**(1), 41 – 57 (1997).
6. G. W. Burton and K. M. Ingold, *J. Am. Chem. Soc.*, **103**(21), 6472 – 6477 (1981).
7. G. W. Burton, T. Doba, E. J. Gabe, et al., *J. Am. Chem. Soc.*, **107**(24), 7053 – 7065 (1985).
8. H. Fiedler, *Chem. Ber.*, **95**(7), 1771 – 1785 (1962).
9. P. T. S. Lau and M. Kestner, *J. Org. Chem.*, **33**(12), 4426 – 4431 (1968).
10. P. T. S. Lau and T. E. Gompf, *J. Org. Chem.*, **35**(12), 4103 – 4108 (1970).
11. V. A. Bogolyubskii, *Zh. Obshch. Khim.*, **32**(3), 869 – 873 (1962).
12. C. I. Burmistrov and L. G. Romanovskaya, *Zh. Org. Khim.*, **1**(2), 321 – 323 (1965).
13. V. I. Votyakov, O. T. Andreeva, N. P. Mishaeva, et al., *Evaluation of the Specific Action of Antiviral Agents with Respect to Experimental Viral Encephalitis. Methodological Recommendations* [in Russian], Minsk (1986).
14. G. N. Pershin (ed.), *Methods of Experimental Chemotherapy* [in Russian], Meditsina, Moscow (1971).
15. P. F. Rokitskii, *Biological Statistics* [in Russian], Minsk (1967).
16. V. Yu. Urbakh, *Statistical Analysis in Biological Investigations for Medical Purposes* [in Russian], Moscow (1975).