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DEPENDENCE OF PHENOL TOXICITY ON KIND AND POSITION OF SUBSTITUTES

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Abstract. Toxicity of 28 phenol derivatives with different alkyl substitutes in 2-, 6-positions and NH_2 , CN, OH functional groups in 4-position was studied. The least toxicity has phenols with two *tert*-butyl substitutes in *o*-position. The toxicity increases with functional *p*-substitutes removal from benzene nuclei. The obtained results can help to synthesize new nontoxic bioactive phenols.

Keywords: antioxidant, substituted phenols, toxicity.

1. Introduction

Contrary to the general point of view, peroxide oxidation of lipids is a physiological process of normal metabolizing tissues and follows the same laws as lipids oxidation *in vitro* in liquid phase. Fine regulation of lipid oxidation in cell takes place both by chemical ways and with the help of enzymes. The rate of lipid oxidation is supported by a few systems, each of which is working within strict limits on relating stages of the process, having their own limiting reactions [1].

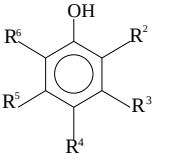
The lipid oxidation rate depends mainly on concentration changes of the substances, breaking the oxidation chains. Only natural antioxidants (basically phenols) have the ability to react with peroxide radicals. There is no interaction with pyroxile radicals of lipids in any other system of defense. None of the enzymes with antioxidant action break oxidation chain. Only natural antioxidants (AO) are inhibitors of radical processes. They can destroy redundant pyroxile radicals of lipids. Lipid oxidation rate is significantly affected by AO more than by other systems. It defines the unique role of natural AO in regulation of the intensity of lipid oxidation [2].

The low level quantity of lipids peroxide in normal tissues is explained by well-balanced processes of peroxide formation and expenses. Breaking this balance results in changing the antioxidant status of the organism and may be the cause of some pathologies [2, 3]. In some studies it was established that AO therapy was necessary for treating many diseases, and free radical processes do play a certain role.

For inhibition reactions of chain oxidation *in vivo* as substances, phenol derivatives are widely used .They possess physiological activity in mammals. There appeared Biologically Active Supplements and medicines of the antioxidant effect, which are of great help to treat many diseases, for example: dibunoli, mexidol, emoxipin, trimexidini, and probucoli [4-10]. In Russia the substances group of the antioxidant effect was chosen from Biologically Active Supplements [11].

For many substances toxicity was carefully studied; clinical, preclinical tests and experiments were carried out. However, in literature there is little information on toxicity of numerous synthesized phenols. They are joined into different groups and have various phenols, having substituents in position 2,4,6 to the OH group. For example, in [12], pulmonary toxicity of 24 phenols was studied on mice, which have various lipophil alkyl substituents in positions 2,4,6 and injected I.P in mammals.

In this study systematic investigations were carried out for estimation toxicity of 28 phenol compounds, having in p- position substituents with different heteroatoms. They have the same formula:



where R^2, R^4, R^6 – various substituents; $R^3 = R^5 = H$

2. Experimental

Synthesis of the studied phenols was described in [4, 13, 14].Toxicity of all compounds was estimated by a size of a lethal dose for 50 % of mice (LD_{50}), measured in mg/kg weight of animal. For some compounds, sizes as much as possible transferable dose (MTD) and a lethal

dose for 100 % of mice (LD_{100}) were additionally determined. Toxicity was determined on mice (males) of the line *Bulb* (mass 18–22 g) with a single I.P. These mammals were housed in standard conditions of vivarium. Water-soluble preparations were injected as solutions of distilled water; liposoluble – 10 % in solution Twin-80, used as solubilisator. Each doze of these preparations was tested on not less than 4 mammals. For calculation, Beren's method was used (the method of "frequency accumulation"), because it was simple and rather reliable [15].

3. Results and Discussions

All investigated substances are resulted in Table 1. It was necessary to estimate toxicity of the substituted phenols depending on:

i) the type of *p*-substituents under identical 2,6-di-*tert*-butyl substituents ;

ii) spacer length $–(CH_2)_n–$ between phenol ring and *p*-substituents under identical 2,6-di-*tert*-butyl substituents;

iii) the type of both *o*-substituents under identical *p*-substituents.

First, phenol toxicity was compared for various *p*-substituents and identical 2,6-di-*tert*-butyl substituents, possessing maximum screening effect. Toxicity estimated by the value LD_{50} was found to decrease in a row (in brackets values LD_{50} were given) of 2,6-di-*tert*-butylphenols having next *n*- substituents.

$$–NH_2\ (80) \approx –CH_2NH_2\ (70) > –CH_3\ (375) > –OH\ (390) \approx \approx –C(CH_3)_3\ (400) > –CN\ (450)$$

Toxicity decreasing of some phenol compounds from this row was confirmed with increasing the value of another parameter toxicity-MTD (in brackets the value MTD is given).

$$–NH_2\ (40) \approx –CH_2NH_2\ (30) >> –CH_3\ (250) = =–C(CH_3)_3\ (250) > –CN\ (300)$$

The same decrease in toxicity was observed for parameter LD_{100} in a row.

$$–NH_2\ (110) > –CH_2NH_2\ (130) > > –CN\ (525) > > –CH_3\ (650)$$

For phenols, having identical 2,6-di-*tert*-butyl substituents, dependence of their toxicity on the distance of the functional *p*-substituents from benzene ring was investigated (Table 2). The tendency was found that increase of phenol toxicity is connected with the spacer length $–(CH_2)_n–$ for all investigated types of substituents ($–NH_2$, $–NHCOCH_3$, –OH, –CN) both electronodonors and electronoacceptors (Table 2).

Table 1

Toxicity of the phenols containing various substituents. $R^3 = R^5 = H$

No. patt.	Substituents			MTD, mg/kg	LD_{50}, mg/kg	LD_{100}, mg/kg
	R^2	R^4	R^6			
1	$C(CH_3)_3$	CH_3	$C(CH_3)_3$	250	375	650
2	$C(CH_3)_3$	NH_2	$C(CH_3)_3$	40	80	100
3	$C(CH_3)_3$	CH_2NH_2	$C(CH_3)_3$	30	70	130
4	$C(CH_3)_3$	$(CH_2)_2NH_2$	$C(CH_3)_3$	60	75	80
5	$C(CH_3)_3$	$(CH_2)_3NH_2$	$C(CH_3)_3$	50	60	-
6	$C(CH_3)_3$	$(CH_2)_4NH_2$	$C(CH_3)_3$	-	50	-
7	$C(CH_3)_3$	$CH(CH_3)NH_2$	$C(CH_3)_3$	80	105	130
8	$CH(CH_3)_2$	$CH(CH_3)NH_2$	$C(CH_3)_3$	30	80	100
9	$CH(CH_3)_2$	$CH(CH_3)NH_2$	$CH(CH_3)_2$	27	35	45
10	CH_3	$CH(CH_3)NH_2$	$C(CH_3)_3$	-	50	100
11	$C(CH_3)_3$	$CH(C_2H_5)NH_2$	$C(CH_3)_3$	40	70	110
12	H	$(CH_2)_3NH_2$	$C(CH_3)_3$	100	165	-
13	$C(CH_3)_3$	$CH_2NHCOCH_3$	$C(CH_3)_3$	350	425	-
14	$C(CH_3)_3$	$(CH_2)_2NHCOCH_3$	$C(CH_3)_3$	-	175	-
15	$C(CH_3)_3$	$(CH_2)_3NHCOCH_3$	$C(CH_3)_3$	75	125	-
16	$C(CH_3)_3$	CN	$C(CH_3)_3$	300	450	525
17	$C(CH_3)_3$	CH_2CN	$C(CH_3)_3$	50	95	180
18	$C(CH_3)_3$	$(CH_2)_2CN$	$C(CH_3)_3$	200	360	475
19	H	$(CH_2)_2CN$	$C(CH_3)_3$	250	285	350
20	H	$(CH_2)_2CN$	H	200	302	400
21	CH_3	CH_2CN	$C(CH_3)_3$	50	152	250
22	CH_3	CN	$C(CH_3)_3$	50	150	-
23	H	CN	$C(CH_3)_3$	250	185	-
24	H	CN	H	200	300	-
25	$C(CH_3)_3$	OH	$C(CH_3)_3$	-	390	-
26	$C(CH_3)_3$	$(CH_2)_2OH$	$C(CH_3)_3$	-	300	-
27	$C(CH_3)_3$	$(CH_2)_3OH$	$C(CH_3)_3$	-	225	-
28	$C(CH_3)_3$	$C(CH_3)_3$	$C(CH_3)_3$	250	400	-

Table 2

Dependence of phenols toxicity on remoteness of *p*-substituents on a benzene ring. $R^4 = (CH_2)_nX$; $R^2 = R^6 = C(CH_3)_3$; $R^3 = R^5 = H$

n	X = CN			X = NH_2			X = $NHCOCH_3$			X = OH
	MTD, mg/kg	LD_{50}, mg/kg	LD_{100}, mg/kg	MTD, mg/kg	LD_{50}, mg/kg	LD_{100}, mg/kg	MTD, mg/kg	LD_{50}, mg/kg	LD_{100}, mg/kg	LD_{100}, mg/kg
0	300	450	525	40	80	100	-	-	-	390
1	50	95	180	30	70	130	350	425	500	-
2	200	360	475	60	75	80	-	175	-	300
3	-	-	-	50	60	-	75	125	200	225
4	-	-	-	-	50	-	-	-	-	-

However, for 2,6-di-*tert*-butylphenol with *p*-substituent – CN, separated only by one group $–CH_2–$ (*n* = 1) from benzene ring, a sharp toxicity increase, as compared with phenol toxicity, having n = 0, was observed. Further, for substances with the increasing bridges $–(CH)_n–$ toxicity sharply diminishes. But it remained greater than the phenol toxicity, having *p*-substituent – just near benzene ring (*n* = 0). It is interesting that the observed extreme dependence was repeating for all values, characterizing the toxicity of these compounds (MTD, LD_{50}, LD_{100}).

The observed occurrence of extreme phenol toxicity, having methylene (or methyl) group in *p*-position, could possibly be explained by greater lability of these phenols in comparison with other phenols. Thus there can be reactions of emergence of phenol radicals with the following reaction of demerization or reaction of disproportionation with formation of cyclohexadienone-2,5 [4, 16, 17].

For 2,6-di-*tert*-butylphenol with *p*-substituent – NH_2 the same tendency of toxicity increase, according to increase of spacer length $–(CH_2)_n–$ has been observed (Table 2). Toxicity increase was observed for this phenol (*n* = 1) with the help of values MTD and LD_{50}. It was interesting that sharper toxicity increase has been observed for similar phenol, but with CN substituent.

Identical dependence of sufficient toxicity increase with the spacer length $–(CH_2)_n–$ was observed for 2,6-di-*tert*-butylphenol having *p*-substituent $–(CH_2)_nNHCOCH_3$ (Table 2).

Symmetric 2,6-di-*tert*-butylphenol (as an example), containing group $–CH_2NH_2$ in *p*-position, toxicity dependence on the type of α-substituent in *p*-methylene group was investigated (Table 1, samples 3, 7, 11). It was found phenol toxicity to decrease after introduction CH_3 group in α-position of the substitute CH_2NH_2. The introduction of C_2H_5 group returns the substituted phenol toxicity to its initial value.

It was noted that introduction of acetyl group to *N*-position of symmetrical 4-aminomethyl-2,6-di-*tert*-butylphenol has decreased the substance toxicity according to the value LD_{50} 6 times (Table 1, samples 3, 13).

It was interesting to study the dependence of phenol toxicity on the type of substituents in *o*-positions.

For phenols (Table 1, samples 7-10), having identical *p*-substituent $–CH(CH_3)NH_2$, phenol toxicity influenced by the substituents of the types R_2 and R_6 (*tert*-butyl, *iso*-propyl, methyl) was investigated. Over all the values, describing toxicity (MTD, LD_{50}, LD_{100}), phenol toxicity increases with the quantity decrease of *tert*-butyl groups, which are in both o-positions of these phenol in a row:

tert-butyl + *tert*-butyl < *tert*-butyl +

+*iso*-propyl < *tert*-butyl + methyl < *iso*-propyl + *iso*-propyl

Such toxicity increase can be explained both by decrease of steric hindrance, created to electronodonor group –OH in terms of *tert*-butyl substituents size, and by hyper conjugation effect of *o*-substituents in OH-group [18].

For *n*-CN of the substituted phenols (Table 1, samples 16, 22-24) after decreasing the number of *tert*-butyl substituents in *o*-position the sharp toxicity increase with the following decrease thereof was found. Nevertheless the toxicity of CN-substituted 2,6-di-*tert*-butylphenol is less than the toxicity of other CN-substituted phenol derivatives.

The analogous dependence has been observed under investigation according to values MTD, LD_{50} and LD_{100} of three phenols, having $–CH_2CH_2CN$ group in *p*-position. They differ in quantity of *tert*-butyl substituents in *o*-position (Table 1, samples 18-20). Toxicity increasing was found when the quantity of *tert*-butyl substituents decrease. However, extreme dependence of mono-*tert*-butyl-substituted phenol toxicity has been observed.

So, the least toxicity of sterically hindred 2,6-di-*tert*-butilphenols has been observed for a few phenol groups with different *p*-substitutes. It can be explained by their worst bioaccessibility [4].

However, only for 2,6-di-*tert*-butyl-4-cyanophenol and 2-*tert*-butyl-6-methyl-4-cyanophenol (Table 1, samples 17, 21) reverse dependence was observed: the change of one *tert*-butyl group to methyl group resulted in essential toxicity decrease. This was proved by the growth of both measured values LD_{50} and LD_{100}.

The observed property dependence on the structure for various o-substituted phenols with polar substitutes in *p*-position does not correlate with the same dependence observed for those with no polar alkyl substitutes [12]. Perhaps after introducing the substituents of various types to phenols, the competition of a few tendencies is being

observed, each of them influencing selected toxicity of the investigated substances [19].

First, the change of lipophility takes place, which results in the change of phenol transport through lipid membrane layers to the following receptors. For example, in [20] the influence of AO on the activity of lipodependent proteinkinase was noted not only through lipid membrane changes, but through direct interaction with enzyme. As the substituted phenols are accumulated in lipids, lipophility dropping of substituted phenol should promote it to supply those fields of lipid membranes which are enriched with oxidized lipids and the products of their metabolism [19].

Second, the structure change results in the reaction ability change in substituted phenol groups, responsible for metabolizing with these phenols. OH-groups, particularly, are responsible for conjugation reactions with glucuronic acid and sulfates [21, 22]. In turn heteroatom groups in p-position take place participate in the processes on the first stage of metabolism with the following stage of metabolism by conjugation with possible metabolites – 2,5-cyclohexadienones formation [4, 12].

Third, the structure change of substituted phenols results in constant of ionization change of OH-group. This fact sufficiently influences the selective toxicity, connected with substrate ionization [19].

So, the above stated factors make it difficult to find the true phenol toxicity dependence on structure. Besides it is difficult to compare these results with those obtained for phenols with lipophilic alkyl substituents [12]. Indeed, from these results in [12] it follows that 2,6-di-*tert*-butyl-4-methylphenol is one of the strongest toxicants. Similar principal result difference may be explained by the fact that strong phenol toxicity has been studied on mice in this study, and in [12] specific mice organs (lungs) have been.

Hence, it is desirable to investigate specific effect of these phenols on various targets in some organs with the help of pharmakokinetics. Nevertheless, the obtained results in this study may be of help in pharmacological investigations and planning studies connected with synthesis of new, nontoxical, biologically active phenol compounds.

4. Conclusions

1. Phenols having two sterically hindered *tert*-butyl substitutes in *o*-position have the least toxicity.

2. Toxicity of 2,6-di-*tert*-butylphenol derivatives increases with removing of the functional *p*-substituents of benzene ring.

3. The declinations from the law of nature of toxicity changes were observed for *p*-substituted phenols, in which functional group is selected from benzene ring only by one methylene group.

4. The obtained results may be of help in pharmacological investigations and planning studies connected with synthesis of new, nontoxic, biologically active phenol compounds.

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ЗАЛЕЖНІСТЬ ТОКСИЧНОСТІ ФЕНОЛІВ ВІД ТИПУ І ПОЛОЖЕННЯ ЗАМІСНИКІВ

Анотація. *Вивчено токсичність 28 похідних фенолу, які мають алкільні замісники в 2- і 6-положенні і функційні групи NH_2 , CN, OH в 4-положенні. Показано, що найменшу токсичність мають феноли з двома трет-бутиловими замісниками в о-положенні. Токсичність зростає при віддаленні функційних п-замісників від бензольного ядра. Отримані результати вказують на можливість синтезу нових, нетоксичних, біологічно активних фенольних сполук.*

Ключові слова: *антиоксидант, заміщені феноли, токсичність.*