**p-Thyrozole: Synthesis and Properties**

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**Abstract**

p-Thyrozole is characterized as a prophylactic and medicinal agent for veterinary and medicine. Natural sources and methods of obtaining synthetic p-thyrozole are considered. Data on its toxicity, pharmacological activity and physicochemical properties are reported.

**Key words:** p-thyrozole, synthesis, toxicity, pharmacological properties, prophylactics of socially dangerous diseases

**INTRODUCTION**

Plant para-substituted hydroxyalkylphenols – lignins, flavonoids, tannin and their monomers – possess polyfunctional chemical and biological properties. Many representatives of this group have been used in medicine as bioantioxidants for a long time. Growing attention to these phenol compounds is connected with the problem of health prophylactics and a decrease of the action of unfavourable ecological factors and stress of any ethiology [1]. The most important representative of para-substituted hydroxyalkylphenols is p-thyrozole (2-(4-hydroxyphenyl)ethanol) [2] on the basis of which Auro preparation was developed in Russia [3]. A large contribution into the investigation of the pharmacological activity and development of synthetic methods to obtain p-thyrozole (Auro) was made by the scientists from Tomsk, Novosibirsk and Irkutsk. It was established that this compound possesses pronounced stress-protective effect, central stimulating action, anti-abstinence properties, immunomodulating and cardioprotective activity, noticeable anticancer and anti-inflammatory action [4, 5]. In general, this product may be considered as one of the efficient means of prophylactics and treatment of socially significant diseases with the high current level in Russia [6]. On the other hand, p-thyrozole can be used by healthy people to enhance mnemonic functions and the ability to concentrate attention, for example to improve advertence of drivers and their response time.

**NATURAL SOURCES OF p-THYROZOLE**

p-Thyrozole is widely represented in plants growing in different climatic zones and enters human food with the vegetable products. For example, this compound is present in many kinds of grape and therefore in cognac and in wine [7]; in olives and in olive oil [8], in blackcurrant [9]. Mainly p-thyrozole **1** is present in plants in the form of glycosides, most frequently as salidroside **2**.

Plants containing p-thyrozole, salidroside and other derivatives of p-thyrozole (bongardol, hydroxythyrrozole and many flavonoids) have been used since old times as universal remedies in Chinese, Tibetan, Japanese medicine. The extract of **Rhodiola rosea** L has found wide application in the folk medicine of the Altay. The major active substances of the extract,
p-thyrozole and salidroside, were isolated for the first time and characterized at the Novosibirsk Institute of Organic Chemistry (NIOCh), SB RAS [10]. The liquid extract of rhodiola (Extractum Rhodiolae fluidim) is widely used as a stimulant to treat asthenic state, undue fatigability, neneurasthenic exhaustion, vegetovascular dystonia, after somatic diseases or infections, in case of disorders of the nervous system, akinetic hypotonic syndrome, schizophrenia [11, 12]. Since 1969 this extract is included into the list of official remedies in Russia. A review on the physiological properties and data on the composition of the extracts of the roots of *Rhodiola rosea* and *Rhodiola quadrifida* was published [13].

Another drug form of the preparation is the tablets with the extract of *Rhodiola rosea* L.; this form turned out to be more stable in composition and during storage. The production of these tablets is more technological than the production of the liquid extract [14].

Besides the preparations mentioned above, the industrial production of the extract of biomass of *Rhodiola rosea* L. containing p-thyrozole was developed in Russia. It is used as a biologically active additive in cosmetics (for example in Zolotoy Koren cream etc. [15].

In addition to the native substance, the methods to obtain synthetic p-thyrozole were developed; synthetic p-thyrozole has successfully passes clinical tests [16]. Injection form of the preparation as a 1 % aqueous solution is under development. The injection form of the extract of rhodiola rosea containing p-thyrozole was patented in China; it is used to treat cardiovascular diseases [17].

**PHARMACOLOGICAL PROPERTIES**

The working capacity stimulating action of p-thyrozole and extracts of *Rhodiola rosea* L. containing this compound was proved in the experiments with animals and humans. The action was expressed as suppression of fatigability under increased amount of physical efforts. For mental work, p-thyrozole caused optimization of attention and increased the volume of short-term memory. Comparative tests showed that the individual compound 1 in a number of cases acts more efficiently than the extract [5]. The dose of p-thyrozole equal to 15 mg enhances the memorizing ability by a factor of 1.5–2 and has a general favourable effect on the physical state of a person [6, 11]. Anticipatory intake of p-thyrozole by drivers allows them to overcome extremal situations successfully, promoting increased attention, acceleration of the response, and decrease in sleepiness during long routes. p-Thyrozone exhibits anxiolytic action, which is pronounced as more adequate behaviour during stress situations and an increase in working capacity for hard physical work of tense metal work (for example, for miners, mountain climbers, sportsmen, teachers and students during sessions etc.). The above-listed properties of p-thyrozole point to its psychotropic activity.

Immunomodulating activity of p-thyrozole in experiments with animals was exhibited as an increase in the functional activity of lymphocytes, phagocytic activity of neutrophils and natural killers [5].

p-Thyrozone as antioxidant prevents enhancement of the peroxide oxidation of lipids in cell membranes [18].

p-Thyrozone is used abroad as a nutrition additive with corroborant and tonic effect. In Japan it is added into drinks, Japanese vodka sake, national meals, flour etc. [19].

An example of medical compositions is the joint application of iodine-containing preparations and p-thyrozone for prophylactics of the diseases of thyroid gland. In this case p-thyrozone acts as the initial product for the synthesis of iodine-containing hormone in the organism [19].

It was established that p-thyrozone slows down the growth of spontaneous and transplantable tumours, decreases the risk of cancer. In addition, when introduced in combination with cytotoxic agents, it weakens their toxicity and enhances their antitumour and antimetastatic action [5]. Course introduction of the compound for 6–10 days intramuscularly or with food into mice and rats with transplantable tumours decelerates the growth of tumours by 40–75 %, and slows down the pathological change of blood characteristics. In addition, p-thyrozone has a stress-regulating action on animals susceptible to spontaneous tumours, which generally results in an increase in the life span of these animals [5].
It is known that thyrozole-containing plant extracts possess antitoxic action against medicaments and other toxic substances [21], increase the resistivity of organisms to infections [21, 22]. With a number of strains of pathogenic bacteria, the antimicrobial action of $p$-thyrozole was discovered [23].

High anti-inflammatory activity of Aurol was demonstrated using the model of the respiratory explosion of phagocytes [24].

$p$-Thyrozole also possesses high adaptogenic activity, which does not have damaging action. A broad range of doses was revealed (0.1–5 mg/kg of body mass) within which adaptation processes can be regulated for a long time. This effect of $p$-thyrozole can be successfully used to enhance the nonspecific human tolerance and protection from unfavourable environmental factors [5].

The effect of the high doses of *Rhodiola rosea* L. preparations containing $p$-thyrozole is known to suppress the central nervous system [20]. It was established in the National Research Centre of Narcology of the Ministry of Public Health of RF (Moscow) that a single application of $p$-thyrozole in the doses 20 to 100 mg alleviates the abstinence syndrome in the case of opio-mania. After the introduction of the agent under these conditions, elimination of somatic disorders is observed; in the opinion of authors, this is connected with the effect of the agent on the activity of enzymes in blood plasma and on nitrergic mediator system of brain in the case of the syndrome of morphine cessation [25]. As a side action, an increase in blood pressure was observed in some patients with coronary pathology at the end of course treatment [5].

Two levels of the use of $p$-thyrozole [25] and the extracts of *Rhodiola rosea* [26] were revealed: low-dose stimulating and high-dose sedative. In the latter case it is necessary to take $p$-thyrozole in doses up to 200 mg/kg, which is equivalent to 100 mL of the extract of *Rhodiola rosea*. Of course, in this case the only acceptable method is the use of $p$-thyrozole but not the extract containing this compound. Below we consider other examples of the use of high thyrozole doses in medical practice.

Two methods are known to be the basis of the efficient therapy of the acute period of myocardial infarction: thrombolytic therapy and steentation leading to the removal of a thrombus. This is accompanied by blood inflow to the ischameric regions in which toxic products of metabolism are accumulated. Their arrival into organism is accompanied by recirculation, oxygenation, oxidative stress and arrhythmia. $p$-Thyrozole allows one to achieve a noticeable decrease in the above-listed unfavourable manifestation kinds as it possesses antioxidiant, antithrombocytotic, hemorheologic and membrane stabilizing activity. In particular, a decrease in the frequency of severe arthythmia in comparison with the reference group was detected in patients [27]. The ability of $p$-thyrozole to decrease the aggregative activity of thrombocytes and improve deformability of erythrocytes leads to an increase of blood flow and stimulates the contractile function of heart, which is likely to be the determinant factor in rehabilitation treatment of ischemia [28].

Later on it was established in the studies of the properties of $p$-thyrozole as a hemorheologic and antithrombotic means that $p$-thyrozole introduced intragastrically once in a dose of 100 mg/kg causes the effect comparable with that of the known preparation pentoxyphylline which is used in a dose of 400 mg/kg to improve blood rheology [29]. This broadens the application area for the injection form of $p$-thyrozole in the cases of unstable stenocardia, acute myocardial infarction, for prophylactics of repeated infarction, thrombosis and embolism after vascular operations.

**TOXICITY OF $p$-THYROZOLE**

It was shown in the chronic experiment with rats that synthetic $p$-thyrozole (Aurol) in the dose of 20–200 mg/kg does not cause any toxic action on animals. According to the data of Tomsk Institute of Pharmacology, SB RAMS, Aurol ($p$-thyrozole) in case of chronic introduction does not affect the general state, dynamics of body mass, hemogram, cardiovascular and central nervous systems, functional state of liver and kidneys of rats, and also body mass, parameters of peripheral blood and morphology of visceral organs of dogs.

It was established in the studies of the acute toxicity of thyrozole, carried out at the NIOCh,
SB RAS, that LD$_{50}$ for mice is 1750 mg/kg, for rats 7079 mg/kg; LD$_{100}$ for mice is 5000 mg/kg, for rats 7500 mg/kg. On the basis of these results, thyrozole was related to low-hazard substances.

A comparison of the basic results on the toxicity of synthetic 99 % $p$-thyrozole with the data on the toxicity of the extracts of rhodiola rosea where $p$-thyrozole is the active principle was carried out. The data obtained allow us to conclude that the presence of admixtures related to thyrozole in structure, in the total amount of 1 %, does not increase its toxicity and does not change the effect of the extract.

APPLICATION IN ANIMAL HUSBANDRY AND IN POULTRY FARMING

Adaptogens are known to be used in veterinary and in animal husbandry for the purpose of prophylactics and treatment of stress in animals. Preparations of eleutherococcus, gingseng and extracts of rhodiola rosea are used in practice. These remedies are assigned to correct technological stressed for 2–3 weeks [31]. The introduction of Aurol preparation into the practice of animal husbandry and poultry farming allows one to achieve more clearly pronounced immune response of the organism to stresses caused by technological reasons and finally to decrease the expenses for stockkeeping and growing young animals. The use of Aurol as a 1 % aqueous solution in the dose of 10 mL/day before and after regrouping of animals (or in the dose of 5 mL subcutaneously at the 14th and 7th day before regrouping) causes an increase in the parameters of nonspecific resistance which was observed in piglets and young cattle [32].

A positive effect of the application of small doses of Aurol was also detected for young poultry [32, 33]. It was established that weight increments in the experimental group of goslings start on the 3rd day after regrouping while in the reference group they start later: on the 4th day. During the whole fattening period, weight increments reach a 2-fold excess in the experimental group over the reference group. Reliable differences are detected in survival between the birds of the experimental and reference groups, while before regrouping these groups are usually characterized by identical survival. In the experiment, during the 1st day after regrouping the survival of birds in both groups decreased by 16.1 %; to the 6th day bird survival in the experimental group was recovered to the technologically admissible standard, while in the reference group survival continued to decrease and then stabilized only by the 10th day.

The level of the immune response of birds to vaccination was studied with hens in which the level of antihemagglutinin (AHA) was determined before vaccination. The vaccine contained the antigens for the virus of Newcastle disease (ND), infectious bronchitis, Hamboro and Reovius disease. The level of the immune response was controlled in the reaction of hemagglutination detention (HAD) on the basis of the AHA titre 14 days after vaccination.

It was established as a result of investigation that the geometrical mean titre of AHA in blood serum of birds of the experimental and reference groups was $(2.4\pm0.6)\log_2$ before vaccination, while in 14 days after vaccination it was $(7.9\pm0.8)\log_2$ in the experimental group and $(5.2\pm0.4)\log_2$ in the reference group. So, after intramuscular introduction of vaccine, Aurol introduced preliminarily into the birds intended for vaccination allows one to obtain a better pronounced subsequent immune response, which decreases the probability of diseases and increases the period before revaccination almost by a factor of 2 thus decreasing the expenses for bird vaccination.

COMMERCIAL $p$-THYROZOLE

Experimental lots of the product are manufactured at the Experimental Chemical Works of the NIOCh, SB RAS, according to the technological schedule adopted in accordance with established procedure. The product is the powder with the mass concentration of the major component not less than 99.0 % and melting temperature not lower than 90 °C [3]. $p$-Thyrozole corresponds to the technical specifications in appearance, colour, temperature of the start of melting, and ash content, as well as in the concentrations of toxic elements: lead, arsenic, cadmium, mercury,
pesticides and radionuclides. Additional requirements concerning eight microbiological parameters are also applied to the preparation for medical destination.

Commercial p-thyrozone, 2-(4-hydroxyphenyl)ethanol, contains 98 % major substance, T_m = 89–92 °C [34]. This reagent may be used for technical purposes. In the known methods of obtaining p-thyrozone, high purity with the major component content of 99 % and higher is achieved either by purification or through an increase in the selectivity of separate stages of its synthesis.

**PHYSICOCHEMICAL CHARACTERISTICS**

The empirical formula is C_8H_10O_2, molecular mass is 138.17; appearance: colourless needles or crystal powder with the odour of rhodiol rosea (of rose), taste is bitter; boiling temperature is 163–173 °C/4 mm Hg. The melting point of the product containing 99.7 % major substance is 92–93 °C.

Photostability: stable under storage for 6 months under natural illumination. UV spectrum (ethanol): \( \lambda_{\text{max}} = 278 \text{ nm}, \log \varepsilon = 3.23 \).

PMR spectrum in CD_3OD, \( \delta \): 2.74 (t, \( J = 7.5 \text{ Hz} \)) 2H ArCH\_2CH\_2OH; 3.71 (t, \( J = 7.5 \text{ Hz} \)) 2H ArCH\_2CH\_2OH; 6.7–7.1 (system AA’BB’) 4H.

Solubility at 20 °C (g/mL): readily soluble in isopropanol (0.12) and 95 % ethanol (0.16); soluble in water (0.044), acetone (0.047) and diethyl ether (0.048); hydrochloric acid (0.05), hardly soluble in benzene (0.012), toluene (0.015) and chloroform (0.01), extremely poorly soluble in hexane (0.0002). Aurol is combustible. Approximate maximal permissible concentration in the working area is 0.05 mg/m³ [17].

**METHODS OF OBTAINING**

*Extraction from plants*

Total content of p-thyrozone and its glucosides in the roots of rhodiola rosea is about 1%; it is substantially dependent on a number of factors of plant growth. It is possible to obtain one tenth of the indicated amount through extraction [10]. It was proposed to extract compound 1 from the distillery dregs of cognac production [35]. The indicated methods of obtaining the compound are characterized by low productivity.

**Synthesis methods**

Several methods were developed to synthesize p-thyrozone. A known multistage method implies nitration of phenyl acetate (p-nitro isomer dominating in the product), then it is reduced into amine, which is diazotized, and the resulting compound is hydrolyzed [36]. The yield of p-thyrozone is 34 %. Another low-selective method of synthesis involves oxyethylation of anisole with ethylene oxide in the presence of AlCl_3, with the formation of compound 1 in mixture with its m- and o-isomers [37].

More efficient methods are based on ethers of p-halogenated phemols. For instance, p-bromoanizole is used to obtain organomagnesium compound; then it is treated with ethylene oxide at a temperature of −10 °C. After hydrolysis, methyl ether of compound 1 is obtained. It is hydrolyzed, and product 1 is isolated with the yield of 31 %. Such a low yield is explained by the complexity of the hydrolysis of methoxyl group [38].

A known method to synthesize p-thyrozone from p-bromophenol is benzylation, alkylation of the organomagnesium compound with ethylene oxide, and debenzylation of the product of oxyethylation. One of the disadvantages of this method is low yield of the product (54.5 %) [39].

This disadvantage was eliminated in the Japanese patent [40] in which the initial compound is 4-tert-butoxychlorobenzene. Organomagnesium compound is obtained on this basis, with subsequent oxyethylation and hydrolysis. The yield of p-thyrozone is 90 %. Authors stress high purity of thus synthesized product.

This method was improved by using not ethers but phenols themselves. For instance, after the treatment of the ether solution of p-bromophenol with an excess of butyl lithium, dilithium compound was obtained. Through its interaction with ethylene oxide, followed by hydrolysis, it gives thyrozole with the yield of 80 % [41]. By improving this procedure, p-thyrozone 1 was obtained from phenol by its reaction with ethylene oxide in the presence of BuLi and Al(Bu-iso)_3 in toluene at 5 °C for 1 h; p-thyrozone was obtained with the yield of 95 %.
The indicated approach is very efficient but it is difficult to implement it practically due to the fire risk of production, so other approaches using available reagents are also urgent.

One more known method is the synthesis of p-thyrozole through the oxidation of 4-HOC₆H₄COCH₃ by alkyl nitrite at a temperature of –5 °C in the presence of the corresponding alkanol and HCl solution; the product is 4-HOC₆H₄COCH(OAlk)₂. p-Thyrozole is obtained from this product in two stages with the yield of 50–56 % [43].

Researchers from China proposed [44] the method of obtaining thyrozole on the basis of p-nitrotoluene. This compound interacts with formaldehyde to form 4-nitrophenethyl alcohol 5, which is then reduced; the amino product is diazotized and hydrolyzed to obtain compound 1.

Rather complicated synthetic approach to obtain compound 1 using 4-acetoxy styrene is also known [45].

In our opinion, the most acceptable technological solution to obtain high-purity product is the use of available 2,6-di-tert-butylphenol 6 to give 4-(2-hydroxyethyl)-2,6-di-tert-butylphenol 7 and its subsequent de-tert-butylation. Compound 6 is oxyethylated in the presence of butyl lithium and triisobutyl aluminium in toluene at 5 °C giving compound 7 with the yield of 95 % and purity 99 %; then it is dealkylated using p-toluene sulphonic acid at 220 °C. The removal of impurities is carried out by extraction with ethyl acetate and recrystallization giving compound 1 of 95 % purity with the yield of 95 % [46], however, we did not succeed in reproducing the stage of de-tert-butylation of compound 7 according to this patent. Technologically more acceptable operation seems to be thermolysis of compound 6 giving p-thyrozole with the yield of 50–60 % [47]. By present, the problems connected with the transformation of by-products formed during thermolysis of compound 6 into p-thyrozole have been solved, which increases the yield of the purified compound 1 to 70 % [48]. Smaller amount of side products is formed during preliminary transformation of compound 6 into bromide 7 which gives p-thyrozole in two stages with the yield of 75 % and purity 99.5 %. Product 1 obtained using this method turned out to be most suitable for manufacturing its injection form [49].

REFERENCES
