Preparation of Conjugates. a) To a solution of 80 mg of the N-hydroxysuccinimide ester of succinylated BSA in 2 ml of DMFA were added 20 mg of a peptide and NEM to pH 8.0. The mixture was stirred for 20 h and, after the addition of a few drops of 25% ammonium solution, it was dialyzed against water and lyophilized.

b) A solution of 40 mg of a peptide in 10 ml of water and, slowly, dropwise, 0.6 ml of a 1% solution of glutaraldehyde in water were added to a solution of 60 mg of polytuftsin analogue in 10 ml of physiological solution. The mixture was stirred for 2 h and, after the addition of 20 mg of $NaBH_4$, it was dialyzed against water and lyophilized.

LITERATURE CITED

1. M. Redfern, New Sci., 121, No. 1654, 26 (1989).
2. P. Bratnagar, E. Papas, H. Blum, D. Milich, D. Nitecki, M. Karels, and G. Vyas, Proc. Natl. Aad. Sci. USA, 79, 4400-4404 (1982).
3. T. Hopp, Mol. Immunol., 21, 13-16 (1984).
4. S. Brown, C. Howard, A. Zuckerman, and M. Steward, J. Immunol. Methods, 72, 41-48 (1984).
5. G. Flouret, S. Terada, T. Kato, R. Gualtieri, and A. Lipkowski, Int. J. Peptide Protein Res., 13, No. 2, 137-141 (1979).
6. M. Bodanszky and Y. Z. Kwei, Int. J. Peptide Protein Res., 12, No. 1, 69-74 (1978).
7. S. M. Andreev, M. V. Sidorova, O. A. Rakova, D. G. Tsvetkov, and L. A. Fonina, Bioorg. Khim., 13, No. 5, 696-700 (1987).
8. Y. Trudelle, A. Brack, A. Delmas, S. Pedoussaut, and P. Rivaille, Int. J. Peptide Protein Res., 30, 54-60 (1987).

FORMATION OF PEROXIDE COMPOUNDS ON THE OXIDATION OF LIGNIN
BY OXYGEN IN AN ORGANIC SOLVENT

O. V. Ignatenko, A. S. Arkhangel'skaya and É. I. Chupka   UDC 630.813.11

The results are given of a determination of the steady-state concentrations of peroxide compounds on the oxidation of lignin by oxygen in dioxane. An iodometric method of determining peroxides was used, with spectrophotometric control of the amount of iodine formed. It was established that the achievable steady-state concentration of peroxide compounds on the oxidation of lignin is 0.28-0.44 wt. % of O_{act} (oxygen pressure 1 atm, temperature 50-80°C). With a rise in the temperature, the steady-state concentration of peroxide compounds decreased. The addition of water (30 vol. %) to the organic solvent led to an increase in the rate of accumulation of peroxide compounds. It has been shown that the presence of alkaline and acidic catalysts exerts no appreciable influence on the achievable steady-state concentration and the rate of accumulation of peroxide compounds during the oxidation of lignin.

It is known that the oxidation of lignin, as a phenolic compound, is accompanied by the formation of peroxide compounds (o- and p-hydroperoxides of cyclohexadienone, quinolide perixodes, and hydrogen peroxide) [1]. Because of their high chemical activity, these peroxide compounds (PCs) are the main intermediate oxidation products participating in the further initiation and development of the chain of oxidative transformations of lignin. The instability of the hydroperoxides and the quinolide peroxides of lignin does not permit their isolation and identification as individual compounds. As a rule, iodometric methods are used to determine the stead-state concentration of PCs. The concentration of peroxides in the course of soda-oxygen digestion has been determined by such a method [2].

Recently, great attention has been devoted to the development of new ecologically harmless methods of obtaining cellulose pulp. One of such methods is solvolytic digestion (the delignification of wood in organic solvents). In organic media, the o- and p-hydroperoxides are present in the molecular form; i.e., in comparison with alkaline aqueous solutions, the radical breakdown of the hydroperoxides with the formation of active alkoxy radicals participating in chain propagation reactions becomes more probable. There is no information in the literature on the amount of PCs formed in the initial stages of solvolytic delignification and their participation in the subsequent oxidative transformations of the lignin. It therefore appears of interest not only to show the presence of PCs in the initial stages of solvolytic delignification but also to obtain quantitative results permitting the determination of the achievable concentration of peroxides and an estimate of the importance of processes involving their decomposition under these conditions (at a temperature not higher than 80°C).

Dioxane was used as the organic solvent. This choice of solvent was determined by two factors. In the first place, lignin dissolves readily in dioxane, and, in the second place, a method has been proposed for obtaining cellulose pulp with the use of dioxane as the delignifying reagent.

The experimental results obtained by the use of iodometric methods of determining peroxides with spectrophotometric control of the amount of iodine formed are given in Fig. 1. The achievable steady-state concentration of PCs in the oxidation of deciduous lignin was low (0.28-0.44 wt. % of O_{act}). This was connected, in the first place, with the low rate of oxidation in organic media under the conditions of mild oxidative treatment (partial pressure of oxygen 1 atm., temperature 50-80°C). Furthermore, processes resulting in the decomposition of PCs take place in parallel with those leading to their formation. Thus, the steady-state concentrations of PCs measured experimentally are determined by the rates of their formation and decomposition.

The occurrence of PC decomposition processes was confirmed by a decrease in the maximum achievable steady-state concentration with a rise in the temperature (Fig. 1). This was connected with the fact that the activation energy of the decomposition of peroxide compounds is 2-3 times greater than the activation energy of the peroxide-forming process. According to the literature, the activation energy of the thermal decomposition of quinolide peroxides ranges between 120 and 130 kJ/mole (29-32 kcal/mole) [4, p. 133], while the activation energies of the radical reactions leading to the formation of PCs do not, as a rule, exceed 50 kJ/mole (12 kcal/mole) [5]. Thus, with a rise in the temperature, the processes of PC decomposition accelerate to a greater degree, leading to a fall in the steady-state concentrations of peroxides, as has been observed experimentally.

An analysis of the kinetic curves (Fig. 1) showed that the rate of accumulation of PCs during the oxidation of deciduous lignin increased with a rise in the temperature. This permitted an estimation of the effective activation energy of the accumulation fo PCs. Calculation by means of the Arrhenius equation gave a value of 11 kJ/mole.

The addition of water (30 vol. %) to the organic solvent led to increases both in the effective activation energy (20 kJ/mole, Fig. 2) and in the rate of accumulation of PCS during the oxidation of deciduous lignin (Table 1).

In the determination of peroxides in an aqueous dioxane medium containing 10% of water it was found that with a rise in the temperature the rate of accumulation of peroxides during the oxidation of lignin did not increase, but, on the contrary, decreased (Table 1). This is possibly connected with a marked increase in the rate of the radical decomposition of the lignin PCs in the dioxane—water (9:1, V:V) medium. This hypothesis has also permitted an explanation of the experimental results obtained on a micromanometric apparatus in the determination of the rate of consumption of oxygen by lignin in dioxane and in aqueous dioxane media of various compositions. According to these results, the maximum rate of oxidation of lignin is observed in aqueous dioxane containing 10 vol. -% of water. Thus, the (almost twofold) increase in the rate of oxidation of lignin found in this medium may be connected with the appearance of free active radicals in the system as a result of the accelerated radical decomposition of the PCS formed.

It was established that in the oxidation of coniferons lignin in dioxane the nature of the kinetic curves of the accumulation of PCs and the achievable concentrations were comparable with the results obtained in the investigation of the oxidation of deciduous lignin
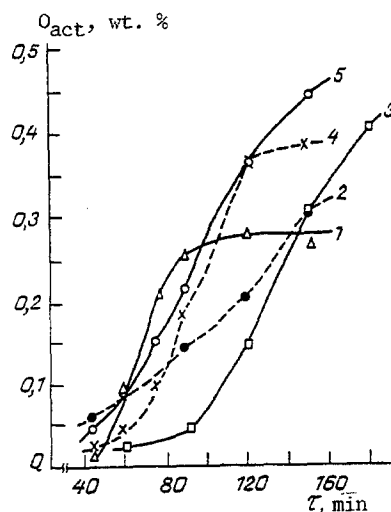
Fig. 1   Fig. 2

Fig. 1. Kientic curves of the accumulation of peroxide compounds during the oxidation of deciduous 1, 4, 5) and coniferous (2, 3) lignins by oxygen in dioxane: 1) 80°C; 2, 4) 70°C; 3, 5) 50°C. partial pressure of O_2 1 atm.
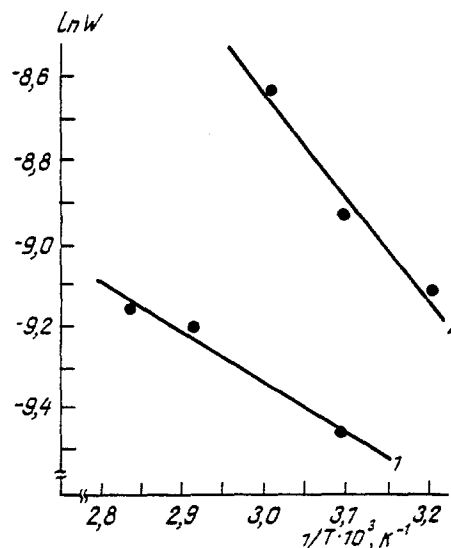
Fig. 2. Temperature dependence of the rate of accumulation of peroxide compounds in the oxidation of deciduous lignin by oxygen. 1) Dixoane; 2) dioxane-water (7:3) (V:V). Partial pressure of O_2 1 atm.

TABLE 1. Rate of Accumulation of Peroxide Compounds in the Oxidation of Deciduous Lignin

Temperature °C	$W \cdot 10^{-3}$, wt. % Oact/min		
	dioxane	dioxane-water (9:1, V:V)	dioxane-water (7:3, V:V)
40	—	5,3	—
50	4,7	3,4	8,0
60	5.8	—	10,7
70	—	1,2	—

(Fig. 1). However, in contrast to the oxidation of deciduous lignin in dioxane, in the oxidation of coniferous lignin a rise in the temperature led to a lowering of the rate of accumulation of peroxides: at 50°C it was $5.0 \cdot 10^{-3}$ wt. % O_{act}/min, and at 70°C $2.0 \cdot 10^{-3}$ wt. % O_{act}/min. In all probability, the results obtained show that in this temperature interval the rate of decompositon of PCs of the guaiacyl type exceeds the rate of composition of the corresponding PCs of the syringyl type.

In order to accelerate the solvolytic delignification of wood, it has been proposed to use acidic and alkaline catalysts [6], preference being given to acidic catalysts, the role of which consists in the hydrolystic cleavage of lignocarbohydrate bonds.

We have investigated the influence of catalysts (sodium hydroxide and hydrochloric acid) on the formation of PCs in the oxidation of deciduous lignin in dioxane. The experimental results are given in Table 2. It was found that the presence of either the alkaline or the acidic catalyst had no appreciable influence on the achievable steady-state concentration of PCs and the rate of their accumulation.

Thus, the investigations performed have permitted an estimation of the achievable steady-state concentrations of PCS during the oxidation of lignin in an organic medium (0.28-0.44 wt. % O_{act}) and the drawing of a conclusion concerning not only the PC decomposition processes taking place under the conditions of the mild oxidative treatment of lignin and but also

TABLE 2. Rate of Accumulation of Peroxide Compounds in the Oxidation of Deciduous Lignin in Dioxane in the Presence of Catalysts (temperature 60°C)

Catalyst (10 vol. %)	Maximum steady-state concentration (wt. % O_{act})	Rate of accumulation of PCs, $W \cdot 10^3$, wt. % O_{act}/min
—	0,42	5,8
0,1 N . NaOH	0,38	5,2
0,1 N . HCl	0,34	5,5
0,2 N . HCl	0,42	7,1
2,0 N . HCl	0,44	7,2

the influence of the composition of an aqueous dixoane medium on the rate of the processes involved in the formation and decomposition of PCS.

EXPERIMENTAL

Pepper pine dioxane lignin (coniferous lignin) and aspen (deciduous) lignin obtained by the procedure of [7] were investigated.

The procedure described in [8] was taken as a basis. The calibration curve (graph of the dependence of the optical density at 470 nm on the concentration of active oxygen, O_{act}, in μg/25 ml) was plotted in accordance with the recommendations of the same paper [8]. A 0.1 g sample of lignin was dissolved in 5 ml of absolute dioxane in a flask fitted with a condenser and placed in a thermostat while oxygen was bubbled through the solution continuously. The temperature of the experiment was varied from 50 to 80°c. After predetermined intervals of time, 0.5 ml aliquots were taken from the reaction mixture and each was transferred to a 25 ml measuring flask with acetic acid—chloroform (2:1). A capillary was inserted and the mixture was purged with argon for 1.5 min, 1 ml of a freshly prepared 50% solution of potassium iodide was added, and, after the passage of argon for another 1 min, the flask was stopped and was left in the thermostat for 1 h. The optical density of the solution was measured in a flask with an optical pathlength of 1 cm relative to water. The determination in a blank experiment (solution of unoxidized lignin) was carried out similarly. The optical density of the solution in the blank experiment was deducted from the optical density of the solution being analyzed.

LITERATURE CITED

1. L. M. Strugin, L. S. Vartanyan, and N. M. Émanuel', Usp. Khim., 37, No. 6, 969 (1968); K. Kratzl, P. Claus, W. Lonsky, and J. S. Gratzl, Wood Sci. Technol., 8, No. 1, 35 (1974).
2. I. P. Deineko, V. Z. Slonim, S. N. Nikol'skii, and M. Ya. Zarubin, Khim. Drev. No. 5, 25 (1983); I. P. Deineko, S. N. Nikol'skii, V. Z. Slonim, and M. Ya. Zarubin, Khim. Drev., No. 1, 39 (1984).
3. V. M. Reznikov, T. S. Anikeenko, and L. G. Matusevich, USSR Inventors' Certificate 926128; Byull Izobret., No. 17, 145 (1982).
4. V. A. Roginskii, Phenolic Antioxidants: Reactivity and Efficacy [in Russian], Nauka, Moscow (1988).
5. N. M. Émanuel', E. T. Denisov, and Z. K. Maizus, Chain Reactions of the Liquid-Phase Oxidation of Hydrocarbons [in Russian], Nauka, Moscow (1965).
6. B. D. Bogomolov and A. S. Groshev, Khim. Drev., No. 3, 3 (1980).
7. J. Pepper, P. Baylis, and E. Adler, Can. J. Chem., 39, No. 8, 1241 (1959).
8. V. L. Antonovskii and M. M. Buzlanova, The Analytical Chemistry of Organic Peroxide Compounds [in Russian], Khimiya, Moscow (1978).