

AN ESR STUDY OF UV-IRRADIATED SOLUTIONS
OF TETRAMETHYLAMMONIUM MERCAPTOUNDECAHYDRO-*closo*-
-DODECABORATE⁽²⁻⁾ AT 77 K

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Received May 3, 1990

Accepted August 10, 1990

The superoxide anion-radicals $O_2^{\bullet -}$ are generated by the ultraviolet irradiation of air- or oxygen-saturated aqueous solutions of tetramethylammonium mercaptoundecahydro-*closo*-dodecaborate⁽²⁻⁾ at 77 K. The superoxide anion-radicals are formed from the oxygen molecule coordinated on the mercaptoundecahydro-*closo*-dodecaborate dianion. In the absence of this dianion no $O_2^{\bullet -}$ are generated. By the irradiation in the presence of hydrogen peroxide the formation of the superoxide anion-radicals is more intensive than in the presence of oxygen only. Simultaneously with $O_2^{\bullet -}$ generation the corresponding thiol radicals are formed. These radicals give the disulfide compound $[B_{12}H_{11}S]_2^{4-}$. A possible role of sulfur-centered radicals in the autoxidation transformations of the mercaptoundecahydro-*closo*-dodecaborate dianion is discussed.

Mercaptoundecahydro-*closo*-dodecaborate⁽²⁻⁾* $[B_{12}H_{11}SH]^{2-}$ (*I*) has been introduced in clinical practice for neutron capture therapy (NCT) of malignant brain tumours by Hatanaka¹ as a boron-10 enriched form of its disodium salt $Na_2B_{12}H_{11}SH$ (*Ia*). This polyhedral dianion *I* is preferentially accumulated in cancerous tissues in comparison with the surrounding ones. The mechanism of the prolonged accumulation of mercaptoborate in the former tissues has not been clear as yet, but a possible role of the sulfhydryl group present in this dianion should be accounted for². Unfortunately, the presence of the sulfhydryl group in the mercaptoborate leads to its easy autoxidation and thus to a significant instability of drug containing this dianion.

In this note we examine the ESR spectrum of mercaptoborate in the form of its di(tetramethylammonium) salt *Ib*, mercaptoborate-TMA, because this salt — in contrast to the disodium one — is a crystalline and nonhygroscopic compound. The goal of this study is to obtain basic information on radicals formed in aqueous solutions of *Ib*, as these radicals could play a crucial role in the reactivity of the mercaptoborate dianion. The compound *Ib* has been studied under both, anaerobic and aerobic conditions. In the latter case, the formation of the biologically important superoxide radicals $O_2^{\bullet -}$ (ref.³) has been found.

* Throughout this paper the name mercaptoborate is used.

EXPERIMENTAL

Materials

Mercaptoborate-TMA was synthesized and characterized at the Institute of Inorganic Chemistry (Czechoslovak Academy of Sciences), Prague. Aqueous solutions of hydrogen peroxide were prepared from analytical grade hydrogen peroxide Lachema (Czechoslovakia).

Instruments and Experimental Arrangement

ESR spectra were taken on an ERS-220 Instrument (Academy of Sciences of the G.D.R.) operating in the X-band with a magnetic field modulation of 100 kHz. The magnetic field was measured with a ^1H NMR magnetometer (Radiopan, Poland), and the microwave frequency was measured using a C3-54 frequency counter (U.S.S.R). The calibration was made with the aid of DPPH ($g = 2.0037$) and Mn(II)/ZnS ($g = 2.0024$, $A = 64.00 \cdot 10^{-4} \text{ cm}^{-1}$) standards. The samples were irradiated in the resonator by an HBO-200 high-pressure mercury lamp (Osram, F.R.G.).

Procedures

The solutions were prepared by mixing and stirring of the components within about 10 s. The solutions placed in cells were frozen to 77 K by immersing the cells in liquid nitrogen in a Dewar flask. This cooling was completed in about 15 s.

RESULTS

Anaerobic Conditions

The frozen oxygen-free solutions of mercaptoborate-TMA show no ESR signals. When irradiated by the UV light, the spectrum (see Fig. 2a) exhibits triplet signal of the nitroxide radicals with parameters $g = 2.0037$ and splitting constant $A_N = 2 \cdot 10 \text{ mT}$. This spectrum is more intensive than those measured under aerobic conditions, see Table I. The nitroxide radical spectrum vanishes by the warming of sample to the room temperature. Moreover, a weak signal of a sulfur centered radical was detected at 77 K, (isotropic singlet $g = 2.0263$), which grows by the decay of UV irradiation. The superoxide and methyl radicals were not proved.

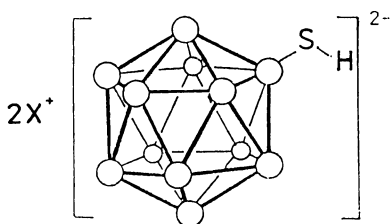


FIG. 1
Mercaptoundecyhydro-*closo*-dodecaborate
(mercaptoborate)

Ia, disodium salt (mercaptoborate-S),
 $X = \text{Na}^+$

Ib, di(tetramethylammonium) salt
(mercaptoborate-TMA), $X = \text{TMA}^+$

Aerobic Conditions

Air saturated solutions. The frozen air saturated solutions of mercaptoborate-TMA show no ESR signals. Under the UV irradiation, the anisotropic spectrum of superoxide anion radical $\text{O}_2^{\cdot-}$ with parameters $g_{\parallel} = 2.0339$ and $g_{\perp} = 2.0039$

TABLE I
Spin numbers of superoxide and nitroxide radicals generated from mercaptoborate-TMA

Mercaptoborate-TMA	$10^{-14}N$ [spins]	
	superoxide radicals	nitroxide radicals
Air saturated sol.	8.23	1.79
Oxygen saturated sol.	9.02	4.74
Addition of H_2O_2		
$[\text{H}_2\text{O}_2] = 0.1 \text{ mol dm}^{-3}$	33.30	0
$[\text{H}_2\text{O}_2] = 1.0 \text{ mol dm}^{-3}$	154.00	0
Oxygen free solution	0	15.1

$c_0 = 2.00 \cdot 10^{-3} \text{ mol dm}^{-3}$ of *Ib*; irradiation for 60 min at 77 K.

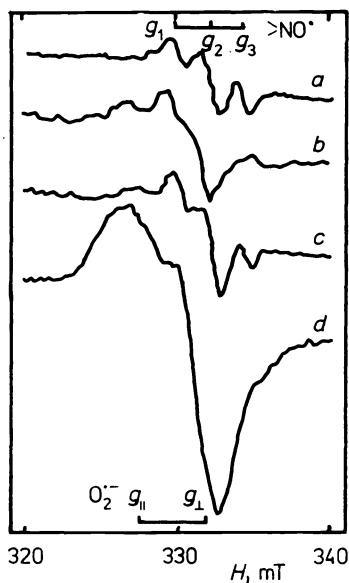


FIG. 2

ESR spectra of the radicals generated by the UV irradiation of frozen solution of mercaptoborate-TMA (*Ib*) at 77 K (initial conc. of *Ib* was $2 \cdot 10^{-3} \text{ mol dm}^{-3}$, microwave frequency 9.308 GHz, microwave power 20 mW, modulation amplitude 1.0 mT, modulation frequency 100 kHz, mercury high-pressure discharge lamp); solutions: *a* oxygen free, *b* air saturated, *c* oxygen saturated, *d* air saturated with H_2O_2 ($c = 0.1 \text{ mol dm}^{-3}$)

was found (Fig. 2b). The intensity of this spectrum is linearly growing by the time of irradiation. Also spectra of radicals formed from the cationic counterpart are generated (see Fig. 2): *a* triplet spectrum with parameters $g = 2.0039$ and splitting constant $A_N = 2.05$ mT which corresponds to the nitroxide radicals; *b* a weak quartet signal with parameters $g = 2.0057$ and $A_H = 2.60$ mT which corresponds to the methyl radicals. The formation of $O_2^{\cdot-}$ anion radicals depends on the presence of *Ib* because in its absence under the same experimental conditions no superoxide radicals were found.

Oxygen-saturated solutions. The frozen solution of mercaptoborate-TMA saturated with oxygen shows a weak isotropic signal ($g = 2.0733$, $\Delta H_{pp} = 4.0$ mT), which probably corresponds to the thiol radical arising from mercaptoborate-TMA/oxygen adduct *II*. Under the irradiation, the anisotropic spectrum with the parameters $g_{\parallel} = 2.0335$ and $g_{\perp} = 2.0041$ is generated which corresponds to the superoxide anion-radicals formation. The spectrum of nitroxide radical (triplet, $g = 2.0041$, $A_N = 2.08$ mT) is superposed to the spectrum of superoxide radical, see Fig. 2c.

Air-saturated solutions with hydrogen peroxide. The frozen air-saturated solutions of mercaptoborate-TMA in the presence of H_2O_2 show an intensive spectrum of the superoxide anion-radicals when irradiated by UV light (see Table I and Fig 2d). The spectrum is more intensive in comparison with the spectrum without hydrogen peroxide, because part of $O_2^{\cdot-}$ could arise directly from H_2O_2 .

DISCUSSION

The easy autoxidation of the sulfhydryl group in mercaptoborate dianion in its aqueous solutions seriously complicates application of the drug *Ia* in neutron capture therapy. Two principal products are formed by the action of oxygen: μ -disulfido-bis(undecahydro-*closo*-dodecaborate) $^{(4-)}$ $[B_{12}H_{11}S-SB_{12}H_{11}]^{4-}$ (*IV*) and the corresponding disulfoxide $[B_{12}H_{11}S(O)SB_{12}H_{11}]^{4-}$ (*VI*). These compounds can be obtained preparatively by rather different oxidation reactions: the disulfide compound *IV* can be prepared by the action of iodosobenzoic acid⁴ and the disulfoxide derivative *VI* by the treatment with hydrogen peroxide⁵ in alkaline aqueous medium.

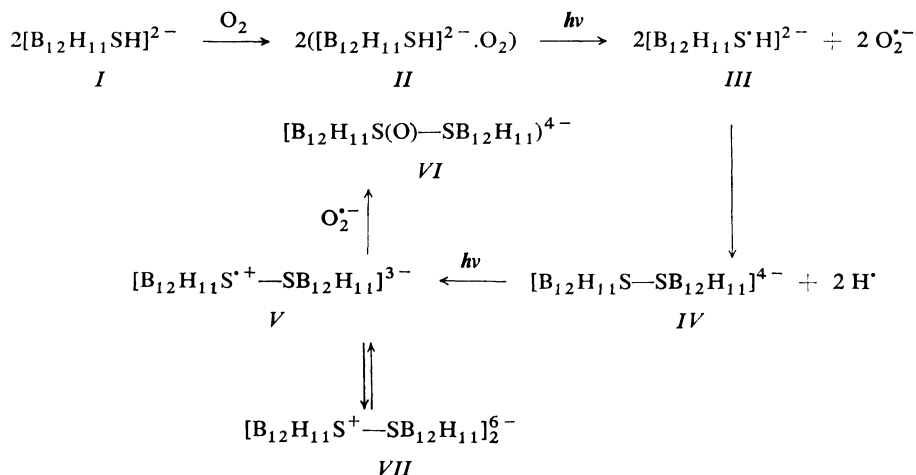
None global idea about the course of the autoxidation of mercaptoborate has been proved as yet. Therefore, the interaction of mercaptoborate-TMA with oxygen under the irradiation by ultraviolet light been examined by means of ESR spectra to assess chemical stability of mercaptoborate in aqueous solutions.

In the first step, the interaction of mercaptoborate with oxygen yields probably an adduct mercaptoborate. O_2 (*II*) which irradiated by the ultraviolet light gives radical with $g_{\parallel} = 2.0339$ and $g_{\perp} = 2.0039$ (Fig. 2b). On the basis of these values any unambiguous decision between superoxide anion-radical and hydroperoxyl radical cannot be made because of the known large range of g_{\parallel} -factor value in the former

case⁶. Nevertheless, under the experimental $\text{pH} = 7$ the superoxide anion-radicals prevail over the hydroperoxyl radicals with $\text{p}K_a = 4.8$ (ref.⁷) by a factor about 160. Moreover, the results of spin trapping of $\text{O}_2^{\cdot-}$ by Tiron (disodium salt of 1,2-dihydroxybenzene-3,5-disulfonic acid) confirm the presence of the superoxide anion-radical⁸.

The possible formation of *II* is supported by the following arguments: *a*) a change of electron density on the sulfur atom of the sulfhydryl group in *I* due to the adduct formation leads to the radical generation (weak signal in singlet spectrum, Fig. 2). The situation is very similar to that of the Co(II) -complexes interaction with oxygen⁹; *b*) an increase of oxygen concentration leads to the increase of the intensity of spectra of superoxide anion-radicals, (see Table I) and; *c*) in the absence of mercaptoborate, oxygen dissolved in water does not form $\text{O}_2^{\cdot-}$ radicals by the irradiation.

The tetramethylammonium cation decomposes simultaneously to give nitroxide radicals and, in some cases, methyl radicals.



SCHEME 1

Scheme 1 represents a possible course of autoxidative process which occurs in aqueous solutions of *I*. First, the unstable thiol radicals *III* and superoxide radicals are formed in the irradiated solutions of the adduct *II*. Two thiol radicals undergo the oxidation to the disulfide *IV*. Under UV irradiation, this disulfide compound can give the radical *V*. The formation of an anionic radical from *IV* has been described⁴ for acidified anhydrous media; this radical absorbs at 630 nm (CF_3COOH in dimethylformamide) and in its ESR spectrum a signal at $g = 2.019$ ($\Delta H_{\text{pp}} = 1.93$ mT) has been found. In aqueous solutions the unstable radical derivative *V* is in an equilibrium with a hypothetical double disulfide derivative¹⁰ *VII*. This compound prob-

ably corresponds to a non-specified product of the autoxidation of *I* detected by capillary isotachopheresis¹⁰ and thin layer chromatography¹¹.

The disulfide radical *V* gives with superoxide anion-radical the disulfoxide derivative *VI* as a final product of the autoxidation. This suggestion is supported by known chemical resistance of *VI* towards further oxidation¹². The formation of sulfur-centered radicals from *I* affords a principal key to a solution of autoxidation mechanism with possible consequences for stability and bioreactivity of drugs containing this dianion.

In conclusion it should be stressed that only a few chemicals can cause the formation of superoxide anion-radicals under the experimental conditions used here. The easy generation of $O_2^{\cdot-}$ in the presence of mercaptoborate can be explained by formation of the adduct *II* followed by the splitting of this adduct to the thiol radical *III* and to the superoxide anion-radical.

We are most grateful to Drs J. Plešek, T. Jelinek and B. Štibr for supplying the sample of mercaptoborate-TMA.

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Translated by the author (O.Š.).