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ACTION OF ULTRASOUND ON DEOXYGENATED AQUEOUS SOLUTIONS OF D-GLUCOSE

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ABSTRACT

The action of ultrasound on deoxygenated aqueous solutions of D-glucose yielded D-gluconic acid, D-*arabino*-hexos-2-ulose, D-*ribo*-hexos-3-ulose, D-*xylo*-hexos-4- and -5-ulose, D-*gluco*-hexodialdose, 2-deoxy-D-*arabino*-hexonic acid, 5-deoxy-D-*xylo*-hexonic acid, 6-deoxy-L-*threo*-hexos-4,5-diulose, and 6-deoxy-D-*threo*-hexos-2,5-diulose. There was little cleavage of the carbon skeleton. The ratio of the deoxy compounds to the sum of the hexosuloses and the hexodialdose was smaller on sonolysis than on γ-irradiation.

INTRODUCTION

X-Rays and ultrasound are applied widely for medical diagnosis. X-Rays can cause chemical reactions in biological materials, which can lead to deleterious effects. Thus, the OH radicals generated can react with RNA and DNA and result in strand breaks[1]. The application of ultrasound is increasing because it is believed that, due to the low energy of the radiation, no risk exists. However, ultrasound can generate OH and H radicals under the brief high temperatures in the nearly adiabatic compression phase of oscillating or collapsing cavitation bubbles[2–4]. A comparison of the action of ultrasound and ionizing radiation on aerated aqueous solutions of D-glucose demonstrated that the same products were formed[5,6]. Glucose-peroxy radicals formed by the addition of oxygen to the primary glucosyl radicals are the intermediates responsible for the observed oxidation and degradation products. In the radiolysis of deoxygenated aqueous solutions of D-glucose, deoxy sugars are formed[7] in addition to oxidation products. The purpose of this work was to determine if deoxy compounds are products of the sonolysis of glucose.

EXPERIMENTAL

A solution of α-D-glucose (60mM) in twice-distilled water was deoxygenated using a stream of argon for 30 min. A portion (10 mL) of the solution was then irradiated at 5° using an 800-kHz ultrasound generator[8] (diameter of the transducer,

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2.5 cm; energy output, 10 W). γ-Irradiation was performed at 25° in a ^{60}Co source at 0.66 J/g.h. Aliquots (1 mL) of the irradiated solution were concentrated using a vacuum centrifuge. Methoximation, silylation, and reduction ($NaBH_4$ and $NaBD_4$) of the products were performed by the methods of Laine *et al.*[9] and Dizdaroglu *et al.*[10], and the derivatives were identified by g.l.c.–m.s. G.l.c. was performed at 210° on a Varian 3700 instrument fitted with a capillary column (110 m, 0.32 mm i.d.) having a cross-linked and surface-bonded silicone rubber phase (Durabond 1), and coupled directly to a Finnigan 212 mass spectrometer. Mass spectra were recorded on a cycle time of 3.5 s.

RESULTS

The products of irradiation of argon-saturated aqueous solutions of α-D-glucose (60mM) with ultrasound were identified by g.l.c.–m.s. after methoximation and trimethylsilylation or reduction with $NaBH_4$ or $NaBD_4$ followed by trimethylsilylation.

Methoximes exist as *syn*- and *anti*-isomers which, in general[9], have similar retention times in g.l.c. A comparison of the *m/z* values of the fragments obtained from derivatives obtained after reduction with $NaBH_4$ and $NaBD_4$ provides information on the number and position of the carbonyl and carboxyl groups. In agreement with Kawakishi *et al.*[11] it was found that lactones were not always completely reduced to alcohols and that the surviving substrates were converted into the methoxycarbonyl derivatives during removal of the boric acid with methanol.

A comparison of the gas chromatograms of the deoxygenated with those of the aerated[6] solutions of D-glucose irradiated by ultrasound shows, that, in the former, only minute amounts of products were formed by cleavage of the carbon skeleton. In the gas chromatogram of the methoximated and silylated samples, the derivatives in peaks 957 and 1022 corresponded to glucono-1,5-lactone and

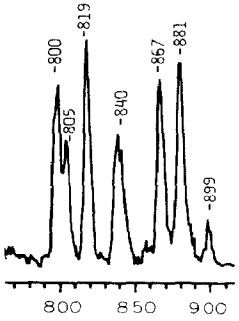


Fig. 1. Section of the gas chromatogram containing peaks for the methoximated and silylated hexosuloses and the hexodialdose obtained from the products of ultrasound irradiation of an argon-saturated 60mM D-glucose solution for 48 h at 5°. The peaks contained derivatives of D-*arabino*-hexos-2-ulose (800), D-*xylo*-hexos-5-ulose (805), D-*gluco*-hexodialdose (819), D-*xylo*-hexos-4-ulose (840), and D-*ribo*-hexos-3-ulose (867 and 881).

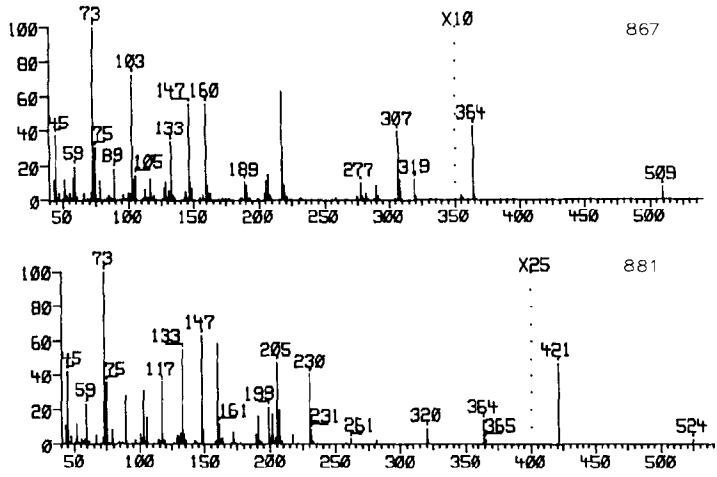
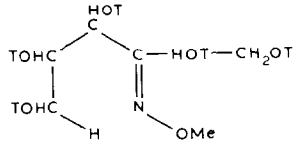


Fig. 2. Mass spectra of the derivative peaks 867 and 881 in Fig. 1.

glucono-1,4-lactone, respectively. The section of the gas chromatogram containing the hexosulose and the hexodialdose derivatives is given in Fig. 1, and derivatives of the following compounds were identified by comparison of the mass spectra with those of authentic samples[12]: D-*arabino*-hexos-2-ulose, D-*xylo*-hexos-5-ulose, D-*gluco*-hexodialdose, and D-*xylo*-hexos-4-ulose. Peak 840 also contained a small amount of a second component which could not be identified. The mass spectra (Fig. 2) of the derivatives in peaks 867 and 881 could be assigned to methoximes obtained from D-*ribo*-hexos-3-ulose. In the mass spectrum of the latter, which corresponds to that reported[12], a McLafferty rearrangement involving the transfer of H-6 to the N of the methoxime in position 3, which leads to *m/z* 320→230, is characteristic. In the other isomer, only cleavage of the carbon skeleton takes place, leading to fragment ions with *m/z* 103, 205, 307, 364 and 160, 217, 319. Only in the methoxime **1**, corresponding to the derivative in peak 881, is a McLafferty rearrangement possible.



1

T = Me_3Si

G.l.c.–m.s. of the products obtained by reduction ($NaBH_4$, $NaBD_4$) of the hexosuloses and the dialdose followed by trimethylsilylation and comparison with the data for authentic compounds led to the identification of derivatives of mannitol

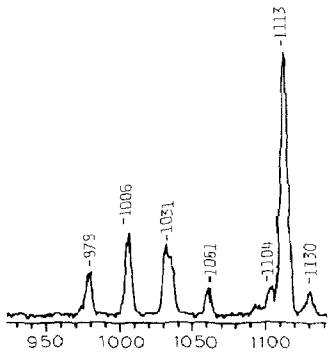


Fig. 3. Section of the gas chromatogram containing the deoxy compounds (reduction with $NaBH_4$).

(1285), allitol (1291), galactitol (1340), and iditol (1332) (only as a shoulder of the main peak at 1325 for the glucitol derivative), and hence to D-*arabino*-hexos-2-ulose, D-*ribo*-hexos-3-ulose, D-*xylo*-hexos-4-ulose, and D-*xylo*-hexos-5-ulose. The results were confirmed by the isotopic composition of mannitol, allitol, and galactitol obtained by reduction of the products by $NaBD_4$. Due to the overlapping with the glucitol peak, the isotopic composition of the fragments of iditol could not be evaluated.

In the gas chromatograms of the methoximated and silylated derivatives, peak 691 corresponded to 2-deoxy-D-*arabino*-hexono-1,5-lactone (Finnigan-MAT, NIH-NBS library No. 32510). Peak 717 could be attributed to 5-deoxy-D-*xylo*-hexono-1,4-lactone [fragments: 363 (10%) $\xrightarrow{-90}$ 273 (18%), 217 (44%), 117 (40%)]. In the gas chromatograms of the derivatives obtained by reduction with $NaBH_4$ or

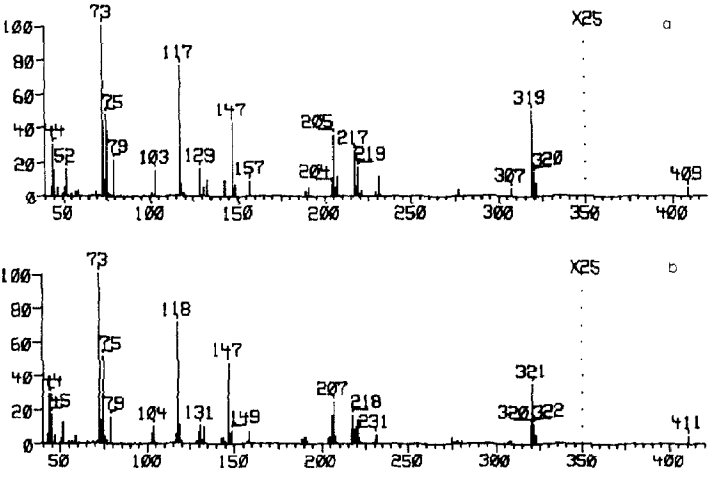
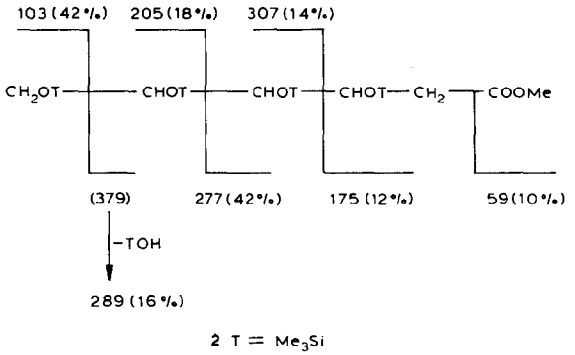


Fig. 4. Mass spectra of the derivative peak 1006 in Fig. 3: (*a*) reduction with $NaBD_4$ and (*b*) reduction with $NaBH_4$.

$NaBD_4$ followed by trimethylsilylation, a series of peaks appeared originating from deoxyhexitols (Fig. 3).

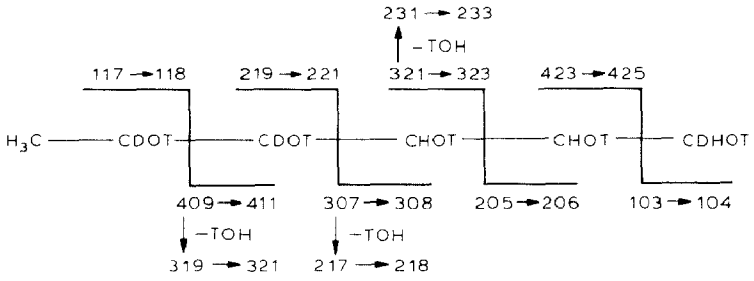
The mass spectrum of the derivative in peak 1113 of the protium form corresponded to a 2-deoxyhexitol. The spectrum of the deuterated samples showed, besides the fragment *m/z* 103 (44%) for $[CH_2OT]^+$ (T = $SiMe_3$), a strong fragment with *m/z* 105 (74%) for $[CD_2OT]^+$; hence, the original compound contained a COOH group. However, the deuterium content of the other fragments showed the peak to be a mixture of derivatives of a 2-deoxyhexitol-*1,1-*d_2 and 2-deoxyhexitol-*6,6-*d_2 which originated from 2-deoxy-D-*arabino*-hexonic acid and 5-deoxy-D-*xylo*-hexonic acid in agreement with the results obtained above.

The fragments of the derivative in peak 979 showed no mass shift due to deuteration and the spectrum corresponded to the trimethylsilylated methyl ester of 2-deoxy-D-*arabino*-hexonate (**2**).

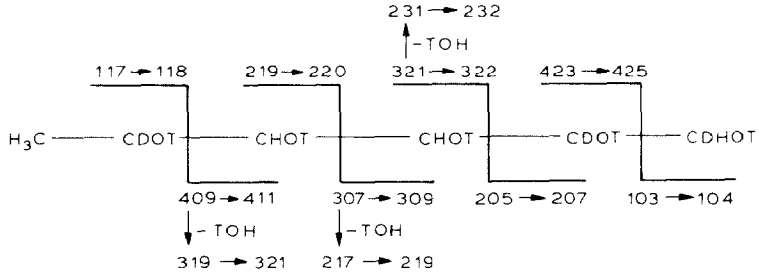


The spectra of the dervatives in peaks 1006 and 1031 (reduction with $NaBH_4$) are identical and can be attributed to a 6-deoxyhexitol. According to the mass shifts of the fragments in the deuterated samples, the product must contain 3 deuterium atoms, but their positions cannot be determined unambiguously. Each probably contains mixtures of a 6-deoxyhexitol-*1,4,5-*d_3 (**3**) and a 6-deoxyhexitol-*1,2,5-*d_3 (**4**). The spectra of the derivative in peak 1006 of the protium and deuterium forms are shown in Fig. 4 and the expected fragmentations are depicted in **3** and **4**. The prevailing deuterium shifts 217→218 and 219→221 correspond to a 6-deoxyhexitol-*1,4,5-*d_3 but, due to the observed shifts 205→207 and 321→322, a 6-deoxyhexitol-*1,2,5-*d_3 cannot be excluded.

The structures of the original molecules should be 6-deoxy-L-*threo*-hexos-4,5-diulose and 6-deoxy-D-*threo*-hexos-2,5-diulose, reduction of which gives 8 stereoisomers with two pairs being identical (6-deoxy-D-glucitol, 6-deoxy-L-iditol). In the gas chromatograms of the methoximated and silylated sample, the derivative in peak 450 could correspond to 6-deoxy-L-*threo*-hexos-4,5-diulose, the characteristic fragments of which are depicted in **5**. There are two peaks (410 and 422) the derivatives in which gave an intense ion with *m/z* 174 $[CH_3–C(=NOCH_3)–CHOT]$



3 T = Me_3Si



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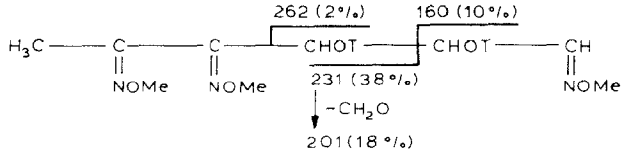


5 T = Me_3Si

which should be a main fragment of 6-deoxy-D-*threo*-hexos-2,5-diulose, but no additional characteristic ions were present.

The formation of hexosdiuloses on radiolysis of D-glucose has not been reported. However, when argon-saturated solutions of D-glucose were irradiated by ^{60}Co γ-rays, a dose of 32 J/g resulted in approximately the same total yield of products as did irradiation by ultrasound for 48 h. In the gas chromatograms of the products reduced by $NaBH_4$ and $NaBD_4$, these trimethylsilylated peaks 1006 and 1031 contained derivatives corresponding to hexosdiuloses.

In order to determine the yields of products, the peak areas in the gas chromatograms were compared with those of known amounts of authentic compounds. Reduction of the hexosuloses and the hexodialdose each gave two stereoisomers, one of which was glucitol. The peak of the derivative of galactitol (1340) and the sum of the peaks of the derivatives of mannitol (1285) and allitol

TABLE I

YIELD OF PRODUCTS

Product	*Ultrasound*[a]	*γ-Radiation*[b]
D-Gluconic acid	1.11mM	0.15
Hexosuloses + hexodialdose	4mM	0.73
2-Deoxy-D-*arabino*-hexonic acid	1.83mM	0.95
5-Deoxy-D-*xylo*-hexonic acid		0.08[c]
Ratio: Deoxyhexonic acids–Hexosuloses + hexodialdose	0.46	1.41

[a]60mM D-Glucose saturated with argon irradiated with ultrasound for 48 h (see Experimental). [b]*G*-values[7] for γ-radiolysis of evacuated, N_2O-saturated 60mM D-glucose. [c]In the literature, only the sum of the *G*-values of 5-deoxy-L-*threo*-hexos-4-ulose, 2-deoxy-D-*erythro*-hexos-3-ulose, 5-deoxy-D-*xylo*-hexodialdose, and 5-deoxy-D-*xylo*-hexonic acid are reported, but the last product, which is the only one detected in sonolysis, should be the main component.

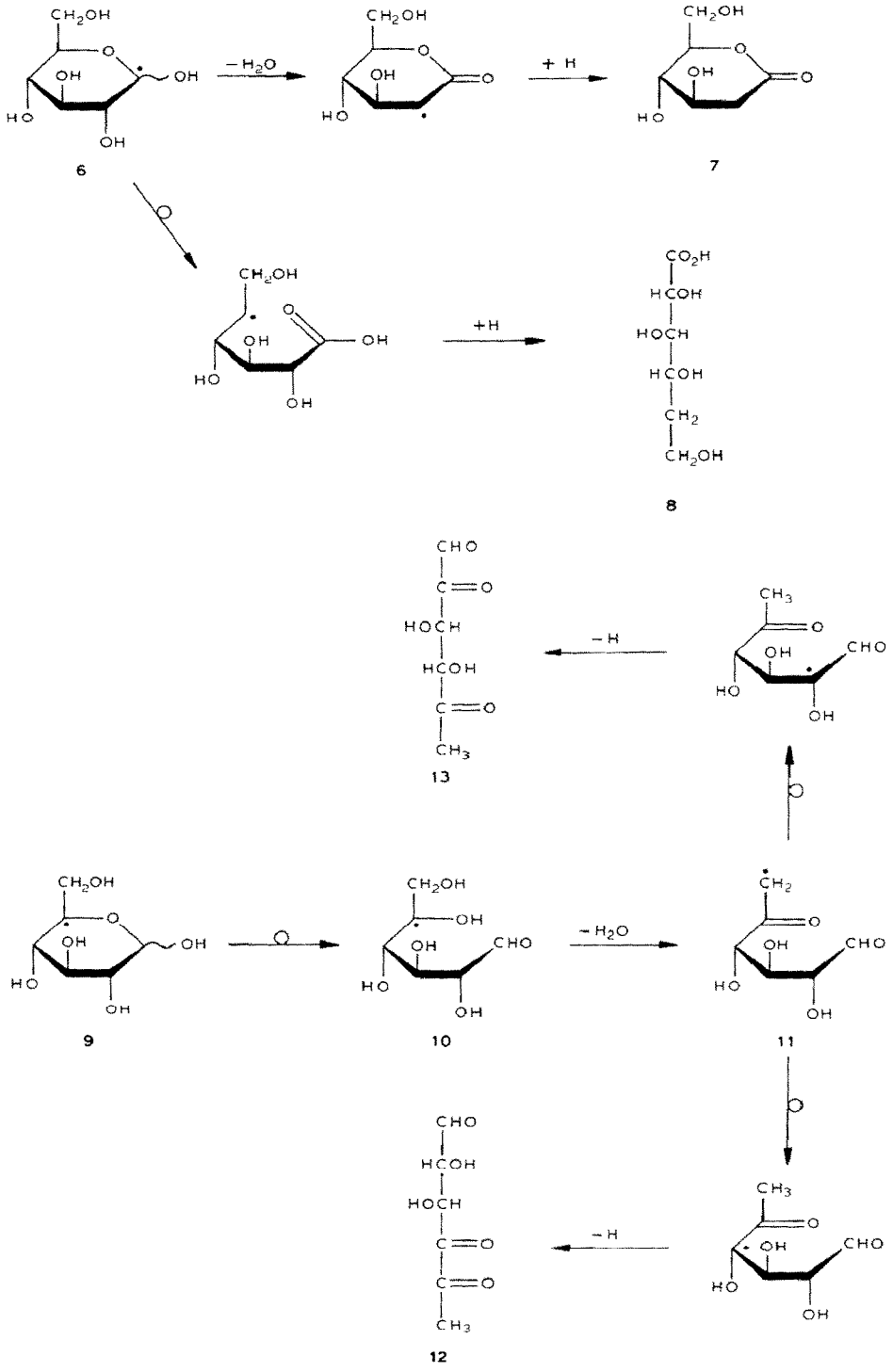
(1291) were sufficiently separated from the main peak for the derivative of glucitol (1325) to be evaluated quantitatively. Taking into account the ratios given in the literature[13] for the two isomers formed by reduction, the yield of D-*xylo*-hexos-4-ulose and the sum of D-*arabino*-hexos-2-ulose and D-*ribo*-hexos-3-ulose could be determined. The sum of the hexosuloses and the hexodialose was evaluated from the ratio of the peak intensities of the methoximated and silylated samples corresponding to the former hexosuloses to the total peak intensities corresponding to the hexosuloses and the hexodialdose.

Peak 1113 in the gas chromatograms of the spectra of the protium forms originating from 2-deoxy-D-*arabino*-hexonic acid and 5-deoxy-D-*xylo*-hexonic acid was evaluated by comparison with 2-deoxy-D-*arabino*-hexitol. This evaluation can give only approximate values because it is based on the assumption that all hexosuloses and the hexodialdose, as well as 2-deoxy-D-*arabino*-hexitol and 5-deoxy-D-*xylo*-hexitol, have the same ionization probability. The results are given in Table I together with the *G*-values reported in the literature for γ-radiolysis of aqueous solutions of D-glucose.

DISCUSSION

The marked similarity in the products formed by the action of ultrasound and ionising radiation on D-glucose indicates that the same mechanisms are involved. With D-glucose as the solute, OH and H radicals abstract carbon-bound hydrogens, leading to glycosyl radicals in positions 1–6, which disproportionate ($2 \cdot COH \rightarrow C{=}O + CHOH$), leading to D-gluconic acid, D-*arabino*-hexos-2-ulose, D-*ribo*-hexos-3-ulose, D-*xylo*-hexos-4-ulose, D-*xylo*-hexos-5-ulose, and D-*gluco*-hexodialdose.

The glucosyl-radical (**6**) is an alkoxyradical adjacent to the lactol bridge and has a high probability of formation. Besides disproportonation leading to D-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gluconic acid, it can lose water or rearrange, leading to 2-deoxy-D-*arabino*-hexonic acid (**7**) or 5-deoxy-D-*xylo*-hexonic acid (**8**).

Hexosdiuloses could originate from hexosuloses as secondary products. However, only 6-deoxyhexosdiuloses are produced, suggesting that a special mechanism is operative which may be associated with cleavage of the lactol bridge. For the 5-radical (**9** → **10**), loss of water could be followed by intramolecular hydrogen abstraction.

The reactions of D-glucose induced by ultrasound in deoxygenated solutions differ in two respects from those of aerated solutions. Ultrasound does not cleave the carbon skeleton and, in addition to oxidation products, deoxy compounds are formed. The non-degraded products observed for ultrasound and γ-radiation are identical, but the yield of deoxy compounds is smaller with the former. Possibly this is connected to the fact that, in a closed system with ultrasound, in contrast to γ-rays, a small amount of oxygen is produced[14], so that, even in deoxygenated solutions, peroxyradicals may participate in the formation of the products.

The results outlined above cast doubt on the widely held opinion that application of ultrasound, in contrast to X-rays, is of no risk.

ACKNOWLEDGMENTS

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