Title
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Publication Date
1958-04-01

UCRL 3982

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DISCLAIMER

THE RADIATION SENSITIVITY OF CRYSTALLINE CHOLINE CHLORIDE

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Crystalline choline chloride, $[(CH_3)_3NCH_2CH_2OH]^+Cl^-$, has been shown[1, 2] to have an extremely high radiation sensitivity. Its G value (molecules decomposed per 100 ev of input energy) ranges from 100 to 1000 depending on both the method of irradiation (fast electrons, gamma rays, or C^{14} beta particles) and the rate of energy input. In contrast, practically all organic compounds, regardless of structure or physical state, are decomposed by ionizing radiation with G values in the range of 1 to 10. In order to shed light on the mechanism of this unique radiation sensitivity, the following investigations have been carried out: (1) Nineteen crystalline analogs of choline chloride were prepared and the radiation sensitivities of these compounds determined. (2) The radiation sensitivity of crystalline choline chloride was compared with that of the compound in solution. (3) The rate of decomposition of the crystalline compound at low temperatures was determined. (4) The nature of the free radicals involved in the radiation decomposition was investigated by the method of electron-spin resonance spectroscopy.

EXPERIMENTAL

Preparation of Choline Analogs

The other choline salts (bromide, iodide, nitrate, sulfate, acetate, and cyanide) were prepared from commercial choline chloride by conversion to the quaternary base with Ag_2O, followed either by titration with the appropriate acid or by passage through an ion-exchange column containing the desired replaceable anion. The preparation of the carnitine hydrochloride, $[(CH_3)_3NCH_2CH(OH)CH_2COOH]^+Cl^-$, has been described previously.[3] The 2-hydroxypropyl trimethyl ammonium chloride was prepared by reacting trimethylamine with 1-chloro-2-propanol in aqueous-alcoholic solution. The ethyl analogs of choline chloride (with one, two, or three methyl groups replaced by a like number of ethyl groups) were synthesized by reacting either methyl iodide or ethyl iodide with the appropriate aminoethanol, followed by conversion of the iodide to chloride. The phenyl analog was prepared from dimethylaniline and ethylene chlorohydrin. Dimethyl bis (2-hydroxyethyl) ammonium chloride was obtained from the reaction of two moles of methyl iodide with one mole of diethanolamine, followed by conversion of the iodide to chloride. The 2-hydroxyethyl trimethyl phosphonium chloride was synthesized from trimethylphosphine[4] and ethylene chlorohydrin.

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All the choline analogs were recrystallized from ethanol-water or dimethylformamide solutions. Acceptable carbon, hydrogen, nitrogen, and halogen analyses (all within 0.3% of the calculated values) were obtained for all the analogs used in this work.

Irradiation Procedures

Two sources of radiation were used in this work: A 3-5 Mev linear electron accelerator and a 100-curie Co^{60} γ-ray source. A description of these sources, their use in the irradiation experiments, and the estimation of radiation doses were given previously.[1,5] For the low-temperature irradiations, nitrogen gas (from boiled liquid nitrogen) was forced through the target area at such a rate that the sample was maintained at -170±20°.

Analytical Procedures

Three analytical techniques were used to determine the extent of radiation decomposition of the choline analogs. Two of these (reineckate analysis and paper chromatography) were described earlier.[4] The third procedure was to precipitate the undecomposed choline analog with sodium tetraphenylboron.[6]

Electron-Spin Resonance Spectra

The electron-spin resonance (ESR) spectra were taken at a frequency of 9.6×10^9 cycles per second on a recording differentiating spectrometer similar to the one developed by Beringer and Castle.[7] Before a sample was placed in the cavity, the upper half of the tube in which it was contained was annealed in a burner flame in order to remove the F centers. The upper half was then cooled and the tube inverted so that the sample was situated in glass which had no paramagnetism.

In order to investigate the ESR spectrum of irradiated choline chloride it was necessary to prepare the compound selectively deuterated (hydrogen atoms completely replaced by deuterium atoms) in the methyl groups, on the α-carbon of the ethanol group, or on the β-carbon of the ethanol group. The methyl-deuterated compound was synthesized by the methylation of ethanolamine with CD_3Br; this latter compound was made from completely deuterated acetic acid by the Hunsdiecker (silver salt) reaction. The α-deuterated choline chloride was prepared from the reduction of ethyl N,N-dimethylglycine with $LiAlD_4$, followed by quaternization with methyl iodide. The β-deuterated compound was prepared by the following route: $CD_3CO_2D \longrightarrow BrCD_2CO_2C_2H_5 \longrightarrow (CH_3)_2NCD_2CO_2C_2H_5 \longrightarrow (CH_3)_2NCD_2CH_2OH \longrightarrow [(CH_3)_3NCD_2CH_2OH]^+Cl^-$.

RESULTS

Decomposition of Crystalline Choline Analogs

The data obtained from the irradiation of crystalline choline chloride and its analogs are summarized in Table I. Since G values vary somewhat depending on the percentage of decomposition, all these values were obtained at exactly 10% decomposition.

TABLE I

Radiation Decomposition of Crystalline Choline Analogs

Compound	G Value at 10% Decomposition	
	Fast electrons	γ rays
$[(CH_3)_3NCH_2CH_2OH]^+ Cl^-$	98	354
$[(CH_3)_3NCH_2CH_2OH]^+ Br^-$	30	92
$[(CH_3)_3NCH_2CH_2OH]^+ I^-$	1.0	2.5
$[(CH_3)_3NCH_2CH_2OH]^+ NO_3^-$	1.4	4.9
$[(CH_3)_3NCH_2CH_2OH]_2^+ SO_4^=$	14	29
$[(CH_3)_3NCH_2CH_2OH]^+ CH_3CO_2^-$	8.2	11
$[(CH_3)_3NCH_2CH_2OH]^+ CN^-$	19	16
$[(CH_3)_3NCH_2CH_2OCOCH_3]^+ Cl^-$	2.7	3.5
$[(CH_3)_3NCH_2CH_2CH_2OH]^+ Cl^-$	1.9	4.8
$[(CH_3)_3NCH_2CH(OH)CH_3]^+ Cl^-$	6.7	6.9
$[(CH_3)_3NCH_2CO_2H]^+ Cl^-$	16	15
$[(CH_3)_3NCH_2CH(OH)CH_2CO_2H]^+ Cl^-$	14	14
$\left[(CH_3)_2^{C_2H_5}NCH_2CH_2OH\right]^+ Cl^-$	6.1	26
$\left[^{(C_2H_5)_2}_{CH_3}NCH_2CH_2OH\right]^+ Cl^-$	6.0	7.9
$[(C_2H_5)_3NCH_2CH_2OH]^+ Cl^-$	5.5	14
$\left[^{C_6H_5}_{(CH_3)_2}NCH_2CH_2OH\right]^+ Cl^-$	4.8	1.9
$[(CH_3)_2N(CH_2CH_2OH)_2]^+ Cl^-$	5.6	14
$[(CH_3)_3NCH_2CH_2Cl]^+ Cl^-$	4.3	2.6
$[(CH_3)_3NCH_2CH_3]^+ Cl^-$	1.6	1.7
$[(CH_3)_3PCH_2CH_2OH]^+ Cl^-$	4.9	4.9

A comparison of the radiation sensitivities of normal and deuterated choline chloride is presented in Table II.

TABLE II

Radiation Sensitivity of Normal and Deuterated Choline Chlorides

Compound		G value[a]
Choline chloride,	$[(CH_3)_3NCH_2CH_2OH]^+Cl^-$	75
α-d analog,	$[(CH_3)_3NCH_2CD_2OH]^+Cl^-$	30
β-d analog,	$[(CH_3)_3NCD_2CH_2OH]^+Cl^-$	67
Me-d analog,	$[(CD_3)_3NCH_2CH_2OH]^+Cl^-$	80

(a) Each sample received 2.5×10^6 rads of Co^{60}-radiation during a period of 13 minutes.

Decomposition of Choline Chloride in Solution

The G values found for choline chloride in water or ethanol solutions are recorded in Table III.

TABLE III

Radiation Sensitivity of Choline Chloride in Solution

Solvent	Conc. mg./ml.	Megarads (γ rays)	% Decomp.	$G = \frac{\text{molecules decomp.}}{100\ \text{e.v. to soln.}}$
Water	9.4	4.3	15	2.2
"	33	16	20	2.9
"	52	16	14	3.2
"	130	18	15	4.4
"	205	58	18	4.4
"	405	177	16	2.5
Abs. EtOH	24	125	5	2.8
"	99	108	3	1.9

Irradiation of Crystalline Choline Chloride at -170°

Eighteen samples of crystalline choline chloride were irradiated in the electron beam at -170 ± 20°. The samples all received 4×10^6 rads, an energy dose which would cause 10% decomposition at room temperature. Five of the samples were kept at -196° until analyses. The samples were then dissolved in water as they were warming up to room temperature, and the analyses then performed. Other samples were kept at room temperature for varying times before solution and analysis. The amounts of decomposition in these samples are recorded in Table IV.

TABLE IV

Radiation[a] Decomposition of Crystalline Choline Chloride--
Irradiations at -170°, Storage at Room Temperature

No. of Samples	Time of Storage, hours	Average Percent Decomposition
5	0	0
2	2	1
2	4	5
2	7	7
2	10	7
5	15	9

(a) 4×10^6 rads of 3 Mev electrons

Electron Spin Resonance Spectra of Irradiated Crystalline Choline Chlorides

The ESR derivative spectra of electron-irradiated crystalline choline chloride and some of its analogs are shown in Figures 1a, 1b, and 1c. The spectra of the deuterated choline chlorides are compared in Figure 2.

CONCLUSIONS

The following conclusions may be advanced concerning the mechanism of the radiation decomposition of choline chloride:

1. The compound decomposes via a free-radical chain mechanism.
2. With the exception of choline bromide, no crystalline analog of choline chloride shows abnormal radiation instability.
3. Choline chloride in solution is stable towards ionizing radiation. The compound's great radiation sensitivity is therefore a function only of its crystal structure.
4. The data of Table II show that α-deuterated choline chloride is significantly less radiation-sensitive than the other analogs. This indicates an involvement of the α position in the transition state of either the propagating or terminating reaction of the free-radical chain mechanism.
5. The free radical in irradiated, crystalline choline chloride has a half-life of several hours at room temperature.
6. The ESR spectrum of irradiated, methyl-deuterated choline chloride is the same as that of the irradiated normal compound; this spectrum may be interpreted on the basis of an unshared electron interacting with four hydrogen atoms. The irradiated α- and β-deuterated compounds both exhibit an ESR spectrum which may be interpreted as an unpaired electron interacting with only two hydrogen atoms. In none of the ESR spectra is there any suggestion of nitrogen "splitting" (three equal peaks). Since the unpaired electron appears to be shared equally between the two carbons, it is probable that the free radical has an ethylene oxide structure. That is, the electrophilic character of the oxygen is felt equally by both carbon atoms. One resonance form of a radical that would satisfy the above observation is

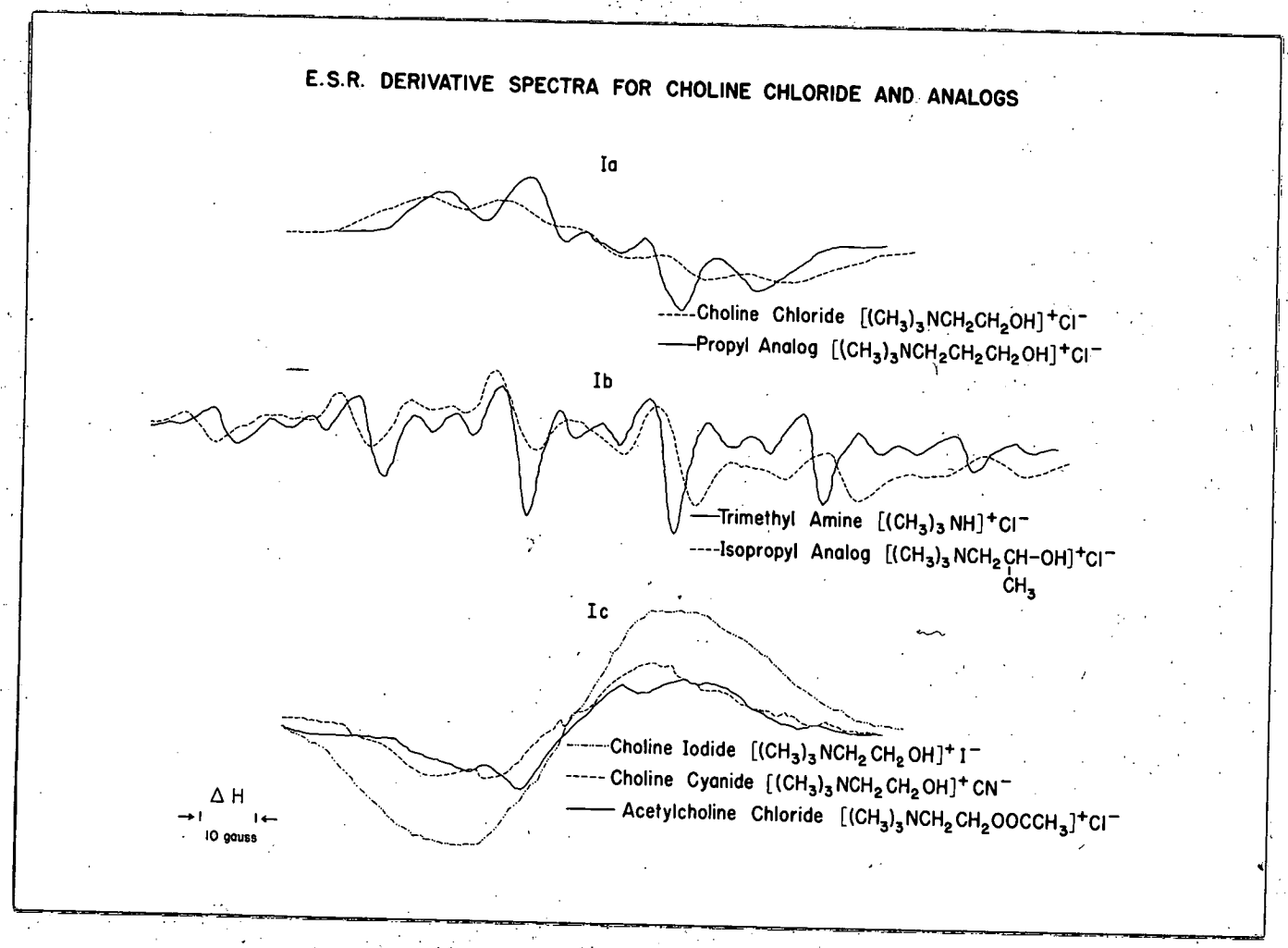

Figure 1

ESR Derivative Spectra for Choline Chloride and Analogs

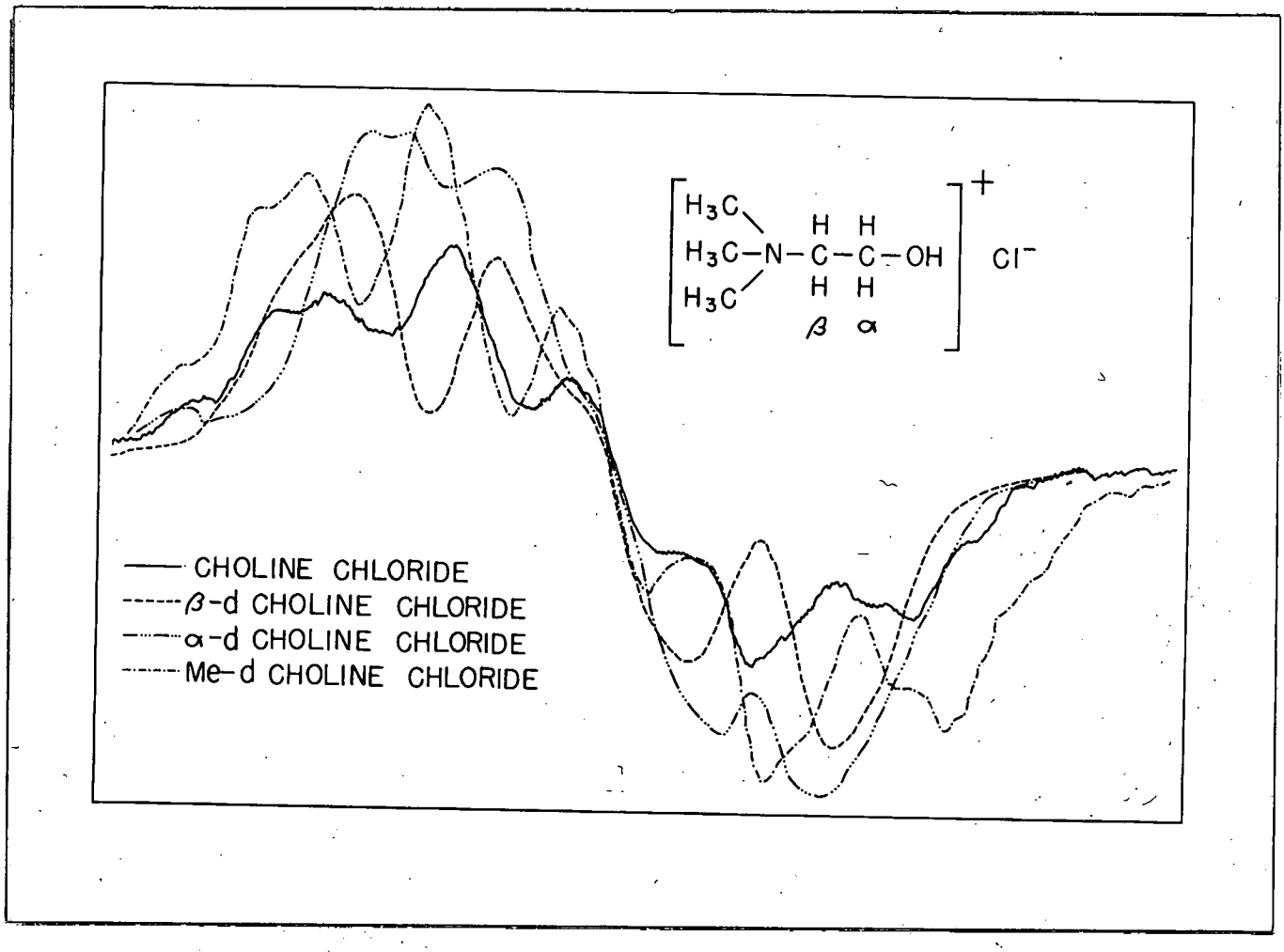

Figure 2
ESR Derivative Spectra of Deuterated Choline Chlorides

7. The ESR spectra for irradiated choline chloride and its propyl analog are similar. The same type of structure proposed above for the radical ion of choline is compatible with the propyl analog signal. The ESR spectrum for the irradiated isopropyl analog is very similar to that of irradiated trimethylamine hydrochloride. It is not obvious what radical will give this structure, but is probably contains a nitrogen atom. The lack of fine structure in the spectra obtained from choline iodide, choline cyanide, and acetylcholine chloride (Figure 1c) makes difficult any conclusions about the radical obtained on the irradiation of these compounds.

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