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Berkeley, California

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FATE OF THE RADICAL INITIATOR IN THE RADIOLYSIS OF CRYSTALLINE CHOLINE CHLORIDE[1]

By Margaret A. Smith and Richard M. Lemmon

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Abstract

A search was made for the termination mechanism in the radical-induced decomposition of irradiated crystalline choline chloride. It appears that the chain propagating radical, $CH_3\dot{C}HOH$ or $\cdot CH_2CH_2OH$, terminates by reaction with another radical [possibly $(CH_3)_3\overset{+}{N}\cdot$] that propagates into its vicinity. Other possible radical termination mechanisms, such as dimerization to a butanediol, or disproportionation to ethanol and acetaldehyde, appear to play only negligible roles.

Crystalline choline chloride, $[(CH_3)_3NCH_2CH_2OH]^+Cl^-$, is of considerable interest to radiation chemists because of its extraordinarily high decomposition G value. Under certain conditions of irradiation, it decomposes by a chain reaction that gives $G_{(-M)}$ values as high as 55,000.[2] The anomolous radiation sensitivity is shown only by the crystalline form; in solution, the compound exhibits normal radiation stability.[3] The main radiolysis products are trimethylamine and acetaldehyde.[4]

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(2) R. O. Lindblom, R. M. Lemmon, and M. Calvin, J. Am. Chem. Soc. 83, 2484 (1961).

(3) R. M. Lemmon, et al., ibid. 80, 2730 (1958).

(4) B. M. Tolbert, et al., ibid. 75, 1867 (1953).

Electron spin resonance studies of the irradiated crystals have shown the appearance of a free radical with a postulated structure approximating $\cdot CH_2CH_2OH \leftrightarrow CH_2CH_2\dot{O}H$[2]. This structure appears to be stabilized by hydrogen bonding and by crystal cage effects. Kinetic studies indicate that the e.s.r.-observed radical acts to initiate, rather than to propagate, the chain reaction.[2]

In the present work a study was made of the fate of the observed free radical among the possible dimerization products, 1,4-butanediol and 2,3-butanediol, and the disproportionation product, ethanol. The results of this work give greater weight to the previously-postulated radical termination mechanism[2] (reaction with a different radical that propagates into the vicinity).

Experimental

Compounds Used.--Two different ^{14}C-labeled choline chlorides were used in this work. The first, $[(CH_3)_3NCH_2{}^{14}CH_2OH]^+Cl^-$, was prepared in this laboratory from commercially available ethyl bromoacetate-1-^{14}C (the procedure was described previously[5]). The second,

(5) R. M. Lemmon and M. A. Smith, J. Am. Chem. Soc. 85, 1395 (1963).

$[(CH_3)_3N{}^{14}CH_2{}^{14}CH_2OH]^+Cl^-$, was prepared from the labeled bromide that was obtained from Nuclear Research Chemicals, Inc., Orlando, Florida. In the cases of both labeled choline chlorides we had evidence [(1) preparation of the chloride from either the bromide or iodide shortly before use, (2) paper chromatography] that the compounds were radiopure.

The 2,3-butanediol, used as a carrier in the search for the labeled compound, was obtained from L. Light and Co., Colnbrook, England. The 1,4-butanediol was obtained from Eastman Organic Chemicals. The trimethylsilylether derivatives of both diols were prepared by the procedure of Sweeley, et al.[6]

(6) C. C. Sweeley, et al., J. Am. Chem. Soc. 85, 2497 (1963).

In the thin-layer chromatographic purification of the ethanol carrier, the ethanol was converted to phenylurethane by reaction with phenylisocyanate according to standard procedures.[7]

(7) S. M. McElvain, "The Characterization of Organic Compounds," MacMillan Co., New York, 1953, pp. 199-200.

Irradiations.--The ^{14}C-labeled crystalline choline chloride was irradiated either by Co^{60} γ rays or by an electron beam from a linear accelerator. In all cases the sample temperature was kept between 25-35°. At a given dose rate and total dose, there is no difference between the effects of γ rays and electron beams on the choline chloride's decomposition. The choice of the radiation used was merely one of convenience.

The choline chloride samples were all given a total radiation dose of approximately 50 megarads. This dose results in approximately 12% decomposition of the choline and higher total doses are known to have little effect in producing further decomposition.[2] All irradiated samples were allowed to stand at room temperature for sufficient time (about 20 hours) to allow the maximum (12%) decomposition to take place.

Ethanol Determinations.--Both gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) of the phenylurethane derivative were used to determine the radioactivity in the ethanol. The GLC was carried out on commercial instruments (Wilkens Instrument Co. models 90 and 350A). These instruments were equipped with flow-through gas proportional counters that permitted monitoring any radioactivity in a GLC peak. More accurate measurements of the total activity in a trapped GLC peak were made by liquid scintillation counting.

A typical experiment using GLC was as follows: A sample of freshly-prepared choline chloride (4.97 μc) was irradiated with an electron beam (total dose 5×10^7 rads, delivered over about ~~16 hrs~~ 1 hr). The sample was dissolved in 500 λ of ethanol carrier, and the ethanol was then removed by vacuum transfer. A 200 λ aliquot of the ethanol was chromatographed on a Castorwax column (5', 1/4", 20% on 60/80 firebrick, 35°, 15 cc He/min)

in four 50-λ injections. The chromatographed ethanol samples were combined, an aliquot withdrawn for radioactivity assay, and the remainder rechromatographed on β-cyanoethyl ether (5', 1/4", 20% on 60/80 firebrick, 70°, 15 cc He/min). Again, an aliquot was withdrawn for radioactivity assay and, on the remainder, a third GLC purification was carried out on a Castorwax column (5', 1/4", 5% on Fluoropak, 80°, 20 cc He/min)

Further determinations of the radiopurity of the ethanol (after it was added as a carrier to irradiated choline chloride) were made by means of thin-layer chromatography of the phenylurethane derivative.[7] The TLC plates were coated with Al_2O_3 ("Aluminum Oxide G" from Research Specialties Co., Richmond, Calif.) that contained 0.5% (by wt.) each of zinc silicate and zinc sulfide. When this mixture is used the entire TLC plate fluoresces under UV light except in the areas where UV-absorbing compounds are located.[8] Preliminary experiments showed that negligible

(8) J. W. Sease, J. Am. Chem. Soc. 70, 3630 (1948).

amounts of UV-absorbing material were removed from this Al_2O_3-phosphor mixture when it was slurried in acetone or ethanol; consequently, the amount of urethane eluted from the TLC chromatograms could be determined spectrophotometrically ($\varepsilon = 1.67 \times 10^4$ at 237 mμ). Two percent acetone in hexane, and 7.5% chloroform in hexane were found to be good developing solvents. The best results were obtained when the chromatograms were run at approximately 4°.

A typical experiment using TLC was as follows: An irradiated (5×10^7 rads) choline chloride-1,2-^{14}C sample (25 mg containing 2.85 μc) was dissolved in 500 λ of methanol and 50 λ of ethanol. The methanol

was removed by GLC (in 50 λ amounts) on a 5 1/4" column of 20% castorwax on 60/80 firebrick, at room temperature, with a helium flow rate of 37 cc/min. The combined ethanol fractions were then transferred on a vacuum line into a flask containing 300 λ of phenylisocyanate (Aldrich Chemical Co., Milwaukee, Wis.); usually the reaction mixture was allowed to stand overnight. The excess phenylisocyanate was removed by evaporation, the resultant crystalline solid was dissolved in 10 cc of acetone, and 200 λ of this solution was streaked on each of five Al_2O_3-TLC plates (20 x 20 cm). After the one-dimensional development of the plates with 2% acetone in hexane, the phenylurethane streak from each plate was eluted with absolute ethanol and the eluates were combined. The phenylurethane was then rechromatographed using 7.5% chloroform in hexane as the solvent; a third chromatography was accomplished by a return to the origianl 2% acetone in hexane system.

Diol Purifications.--Initial attempts to purify the 2,3-butanediol by GLC were unsuccessful. The diol quickly attacks hot tungsten filaments (of the GLC's thermal conductivity cell) and is thereby lost. In addition, because of the low vapor pressure of the diol, it is necessary to operate the GLC at 300°. At this temperature, even when the detector filaments were turned off, about 90% of the diol was destroyed during its passage through the GLC column (poly m-phenyl ether). We next tried to purify the diol by preparation of its phenylisocyanate derivative[7] and paper chromatographic or TLC purification of the derivative. The paper chromatography was unsuccessful because the presence of ultraviolet eluates from the paper itself prevented spectrophotometric estimation of the amount of the derivative eluted from the paper. The

TLC purification was unsuccessful because of our failure to find a suitable adsorbent-solvent combination that would give reasonably compact and reproducible zones. We finally resorted to VPC purification of the trimethylsilyl ethers of the 2,3- and 1,4-diols. This purification worked well.

After preparation of the trimethylsilyl ethers by the method of Sweeley,[6] the ethers were initially purified by GLC on a 10', 1/4" column of 20% Apiezon L on 60/80 firebrick, at 130°, with a helium flow rate of 30 cc/min. In the case of the ether from the 2,3-diol we obtained a split peak; the ether from the 1,4-diol gave a single, unsplit peak. Mass spectra (taken on a CEC Model 130 mass spectrometer) of the two split peaks from the 2,3-diol were essentially identical, and we concluded that we had separated the DL- from the meso compound. Practically identical mass spectra have been reported for a number of aliphatic diasteromers.[9] In later work the Apiezon columns were dis-

(9) K. Biemann, "Mass Spectrometry - Organic Chemical Applications," McGraw-Hill, New York, 1962, p. 144.

carded in favor of poly-m-phenyl ether and SF-96 (see below).

A typical experiment in the search for labeled 2,3-diol was as follows: Forty mg of irradiated choline chloride-1,2-^{14}C, containing 8.63 μc was dissolved in 500 λ of 2,3-butanediol. The diol was removed from the choline by distillation in a small molecular still. It was dissolved in 600 λ of pyridine and to this solution were added 800 λ of hexamethyldisilizane (K and K Laboratories, Plainview, N. Y.) and 400 λ of trimethylchlorosilane (California Biochemical Corp., Los Angeles). The solution was extracted three times with water, dried over $MgSO_4$, and

the product chromatographed (in 50 λ quantities) on the two GLC columns: (1) 10', 1/4" column of 15% SF-96 on 60/80 firebrick, at 140°, He flow 15 cc/min; (2) 10', 1/4" column of 15% poly-m-phenyl ether on Fluoropak, at 150°, He flow 15 cc/min.

The procedures used to prepare and purify the trimethylsilyl ether from the 1,4-diol were the same as for the 2,3-diol with but one exception: SF-96 was used on a Fluoropak support instead of on firebrick.

Results and Discussion

In determining how much radioactivity we would expect to find in the diols or in the ethanol we assume that the previously-postulated decomposition model[2] (below) is correct, at least through reaction (3):

$$\text{Cho} \rightsquigarrow \text{Cho}^* \xrightarrow{\text{fast}} \cdot\text{Cho}\cdot \qquad \text{biradical formation} \qquad (0)$$

$$\cdot\text{Cho}\cdot + \text{Cho} \xrightarrow{k_1} (CH_3)_3\overset{+}{N}H + AcH + (CH_3)_3\overset{+}{N}\cdot + AcH_2\cdot \qquad \text{initiation} \qquad (1)$$

$$AcH_2\cdot + \text{Cho} \xrightarrow{k_2} (CH_3)_3\overset{+}{N}H + AcH + AcH_2\cdot \qquad \text{propagation} \qquad (2)$$

$$AcH_2\cdot + \cdot\text{Cho}\cdot \xrightarrow{k_3} (CH_3)_3\overset{+}{N}H + AcH + AcH_2\cdot \qquad \text{pre-termination} \qquad (3)$$

$$AcH_2\cdot + (CH_3)_3\overset{+}{N}\cdot \xrightarrow{k_4} \text{Cho} \qquad \text{termination} \qquad (4)$$

where

$$\text{Cho} = (CH_3)_3\overset{+}{N}CH_2CH_2OH$$

$$\cdot\text{Cho}\cdot = (CH_3)_3N\cdot\cdot CH_2CH_2OH \text{ --- } Cl^- \qquad \text{(the e.s.r. observed radical)}$$

$$CH_2CH_2\dot{O}H \text{ --- } Cl^-$$

$$AcH_2\cdot = CH_3\dot{C}HOH \text{ ; } AcH = CH_3CHO$$

$$\text{(or } \cdot CH_2CH_2OH\text{)}$$

The chain propagating radical, $AcH_2\cdot$, is written in two ways because previous work[5] has shown that the five hydrogen atoms of the ethanol

moiety have considerable mobility during the radiolysis.

However, the chain termination mechanism might be, not reaction (4), but one of the following:

$$2\ AcH_2^{\bullet} \longrightarrow \underset{\text{2,3-butanediol}}{CH_3CHOHCHOHCH_3} \qquad (a)$$

$$2\ AcH_2^{\bullet} \longrightarrow \underset{\text{1,4-butanediol}}{HO(CH_2)_4OH} \qquad (b)$$

$$2\ AcH_2^{\bullet} \longrightarrow CH_3CH_2OH + CH_3CHO \qquad (c)$$

Under the irradiation conditions used in the present work we would get about 12% decomposition of the choline chloride; this is about the maximum decomposition that occurs when the chain mechanism is operating.[2] Consequently, we have maximized the chances for reactions (a), (b), or (c) to occur.

The production of two $AcH_2^{\bullet}$ radicals requires the decomposition of four cholines [through reaction (1)], or six cholines if reactions (2) and (3) also take place. If either (a) or (b) represent the fate of the free radical, the resultant diol should have at least one-third the activity of the radiation-decomposed choline. With a choline decomposition of 12%, we assume that a resultant diol would have at least 4% of the radioactivity of the starting choline.

If reaction (c) represented the fate of the free radical, the resultant ethanol would have one-sixth the activity of the decomposed choline (or 2% of the starting choline) if reactions (2) and (3) took place. If they did not take place, the ethanol could contain one-fourth of the activity of the decomposed choline. In the tables that follow, the one-sixth figure (equivalent to 2% of the starting choline) is used

as the calculated activity that would appear in ethanol if this compound (and acetaldehyde) were the radical termination products [reaction (c)].

Table I

Activity Appearing in 2,3- and 1,4-Butanediol from Irradiation Choline Chloride-1,2-^{14}C[a]

Expt. No.[b]	Choline chloride irradiated, μc	Carrier diol added, mmoles	Sp. act. expected, dpm/mmole[c]	Sp. act. found, dpm/mmole	% of expected activity
1	8.63	5.81	99,000	49	< 0.1
2	7.29	5.81	83,600	0	0
3	10.93	5.66	128,600	13	< 0.001
4	1.33	5.66	15,700	12	< 0.01

a. Both diols were isolated as the trimethylsilyl ethers; the derivatives were purified on GLC columns of (1) SF-96 and (2) poly m-phenyl ether.

b. Experiments 1 and 2 were done with carrier 2,3-butanediol; experiments 3 and 4 were done with the 1,4-diol.

c. Assuming one diol is produced for every four cholines destroyed.

Table II

Activity Appearing in Ethanol from Irradiated Choline Chloride-1,2-^{14}C

Expt. No.	Choline chloride irradiated, μc	Carrier EtOH added, mmoles	Purification method	Sp. act. expected, μc/mmole[a]	Sp. act. found, μc/mmole	% of expected activity
1	2.85	0.85	TLC[b]	0.067	0.0028	4.2
2	2.85	0.85	TLC[c]	0.067	0.0025	3.7
3	2.85	0.85	TLC[d]	0.067	0.0030	4.5
4	4.79	8.52	GLC[e]	0.011	5.6×10^{-4}	5.1
5	4.79	8.52	GLC[b]	0.011	5.5×10^{-4}	5.5
6	4.79	8.52	GLC[g]	0.011	5.6×10^{-4}	5.0

a. Assuming one ethanol is produced for every six cholines destroyed.

b. One solvent system used: 2% acetone in hexane.

c. Two successive solvents used: (1) 2% acetone in hexane, (2) 7.5% $ChCl_3$ in hexane.

d. Three successive solvents used: (1) 2% acetone acetone in hexane, (2) 7.5% $CHCl_3$ in hexane, (3) 2% acetone in hexane.

e. Castorwax column.

f. Two successive GLC purifications: (1) Castorwax, (2) β-cyanoethyl ether.

g. Three successive GLC purifications: (1) Castorwax, (2) β-cyanoethyl ether, (3) Castorwax.

The results of this work indicate that the radical termination mechanism [reaction (4)] that was previously postulated is essentially correct. Although a few percent of the radicals disproportionate to ethanol and acetaldehyde, most of them (about 95%) terminate by reaction with another radical, presumably $(CH_3)_3\overset{+}{N}\cdot$, that propagates into the vicinity.

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