[CONTRIBUTION FROM THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Effects of Ionizing Radiation on Choline Chloride and its Analogs[1]

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Choline chloride and six analogs have been exposed in the dry, crystalline state to high energy electron and γ-radiation. This investigation has confirmed the abnormal radiation sensitivity of choline chloride. Its G values (molecules decomposed/100 e.v.) were found to be: e^- radiation, 20; γ-radiation, 175. These high values indicate a chain mechanism for the solid state reaction. The G values for the choline analogs were found to range from 1 to 18 for the electron irradiations and from 1 to 32 for the γ-irradiations. Betaine hydrochloride approaches choline chloride in instability toward high-energy electrons but is far more stable in the presence of γ-rays.

The abnormal sensitivity of anhydrous, crystalline choline-methyl-C_1^{14} chloride toward its own radiation[2,3] and the simplicity of its decomposition products (only trimethylamine and acetaldehyde were observed) has led to the present study of the effects of high energy radiation on this compound and on six of its analogs. The objects of this work were to confirm the abnormal sensitivity of the choline chloride molecule and to determine the effects of changes in the molecule on this radiation sensitivity. Electron (2–4 mv.) and gamma (1.23 Mev.) irradiations have been carried out on the dry, crystalline salts maintained under vacuum during the bombardments. The compounds irradiated and the average sensitivities measured are listed in Table I.

TABLE I

RADIATION DECOMPOSITION OF CHOLINE ANALOGS

Compound	$G = \frac{\text{molecules decomposed}^a}{100\text{ e.v.}}$ C^{14}-β	2–4 mv. electrons	1.23 Mev. γ
$[CH_3)_3NCH_2CH_2OH]^+Cl^-$	1250	20[b]	175
$[(CH_3)_3NCH_2CH_2OH]^+I^-$	..	2	5
$[(CH_3)_3NCH_2CH_2OCOCH_3]^+Cl^-$	5	2	4
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	..	2	4
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	..	2	2
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	..	3	12
$[(CH_3)_3NCH_2COOH]^+Cl^-$	..	14	18

[a] These G values should not be considered as more than rough indications of relative stabilities; they may be in error by as much as a factor of two. [b] NOTE ADDED IN PROOF.—More recent work has shown that this G value is dependent on the intensity of the electron beam. A lowered intensity gives a higher G value.

A further objective of this work was to learn more about the effects of radiation on pure organic compounds in the solid state. At the present time, aside from observations on the self-decomposition of C^{14}-labeled compounds[2,3] and the radiation initiation of polymerization,[4] the only reported measurements of the radiation decomposition of solid state pure organic compounds are those on glycine,[5] bromoform,[6] a few hydrocarbons,[7] and some of the carboxylic acids.[8,9]

Experimental

Preparation of Choline Analogs.—All of the compounds used in this work were prepared with very small amounts of carbon-14 in the methyl groups. Choline-methyl-C_1^{14} iodide (sp. act. 0.1 μc./mg.) was prepared by treating $C^{14}H_3I$ with dimethylaminoethanol in methanol solution and recrystallizing the product from absolute ethanol–ether. (All of the preparations, with the exception of trimethyl-3-hydroxypropylammonium chloride, as noted below, were recrystallized from this solvent.) The chloride was prepared from the iodide through conversion to the quaternary base with Ag_2O, followed by titration with HCl. Acetylcholine chloride was prepared by refluxing the labeled choline chloride with acetic anhydride for 2–3 hours. The trimethyl-2-chloroethylammonium chloride was prepared by treating the choline chloride-C^{14} with thionyl chloride at room temperature.

Trimethyl-C_1^{14}-3-hydroxypropylammonium chloride (sp. act. 1.0 μc/mg.) was prepared from labeled trimethylamine and 3-chloropropanol; the product was recrystallized from absolute ethanol–dioxane. Trimethyl-C_1^{14}-ethylammonium chloride (sp. act. 0.1 μc./mg.) was prepared by treating labeled trimethylamine with ethyl iodide, followed by conversion of the quaternary iodide to the chloride.

Betaine-methyl-C_1^{14} hydrochloride was prepared by treating labeled trimethylamine with excess chloroacetic acid in methanol solution at room temperature. The product was separated from chloroacetic acid on a Dowex-50 cation-exchange column. Acceptable carbon, hydrogen, nitrogen and halogen analyses (all within 0.3% of the calculated values) were obtained on all compounds used in these radiation studies.

Irradiation Procedure.—For the self-decomposition experiments, two samples of analytically pure crystalline choline chloride (sp. act. 0.6 and 10.7 μc./mg.) were prepared separately. These samples were stored at room temperature in the dark in sealed ampoules under high vacuum. The first sample was stored for 46 months, the second for 46 days. Another portion of the second sample was stored at −196° (liq. N_2), otherwise all conditions were the same.

For the high energy electron irradiations, 50–200 mg. of the analytically pure sample was placed in a small watch-shaped container. Because of the hygroscopic nature of these compounds, transfers from one container to another were carried out in a dry-box in an atmosphere of dry nitrogen. The flat sides of the container were 1 cm. in diameter, the glass was about 1 mm. thick, and the distance between the glass walls was about 2 mm. After the sample was placed in the container it was re-dried at 100° for several hours under a high vacuum and, finally, the tube was sealed off under vacuum. The irradiations were carried out using the pulsed beam of a 2–4 mv. linear electron accelerator; there were 7.5 pulses (each lasting 2 microseconds) per second. The number of electrons passing through the sample was measured by a brass collector (also 1 cm. in diameter) placed immediately behind the sample holder; this collector was connected to an ammeter and grounded through a 10^6-ohm resistor. Due to such factors as the stoppage of some electrons by the sample itself and by the glass walls, scattering of electrons by sample and container, secondary electron emission, and air ionization, the absolute amount of energy

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission and was presented before the Division of Organic Chemistry at the September, 1954, Meeting of the American Chemical Society.

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(3) R. M. Lemmon, *Nucleonics*, **11**, No. 10, 44 (1953).

(4) A. Charlesby, *ibid.*, **12**, No. 6, 18 (1954).

(5) W. M. Dale, J. V. Davies and C. Gilbert, *Biochem. J.*, **45**, 93 (1949).

(6) W. Minder, *Brit. J. Rad.*, **24**, 435 (1951).

(7) C. S. Schoepfle and C. H. Fellows, *Ind. Eng. Chem.*, **23**, 1396 (1931).

(8) W. L. Whitehead, C. Goodman and I. A. Breger, *J. chim. phys.*, **48**, 184 (1951).

(9) V. L. Burton, THIS JOURNAL, **71**, 4117 (1949).

received by the sample is probably not known within a factor of two. However, the comparative values for the various choline analogs are probably correct to within ±25%. Calculations of the energies received by the samples were made as

$$\text{No. of electrons passing through sample} = N = \frac{(\text{amps})(\text{sec.})}{\text{coulombs/electron}}$$

$$\text{Energy loss in Mev./electron} = \Delta E = \frac{\Delta R}{0.54}\ (\text{Feather's rule for energies} > 0.8\ \text{Mev.})^{10}$$

$$\text{Mev./g./Rep} = (83\ \text{ergs/g./Rep})\left(\frac{6 \times 10^{23}\ \text{e.v.}}{96{,}500 \times 10^{7}\ \text{ergs}}\right)(10^{-6}\ \text{Mev./e.v.}) = 5.2 \times 10^{7}$$

$$\therefore\ \text{Reps received by sample} = \frac{(N)(\Delta E)}{(\text{g.})(5.2 \times 10^{7})}$$

During the irradiations the samples, which received energy at a rate of approximately 10^7 reps/minute, were kept between room temperature and a maximum of 50° by means of an air blast.

The γ-irradiations were carried out with a 100-curie Co^{60} γ-ray source. Through the use of a ceric ion dosimeter[11] this source was found to deliver $1.7 \pm 0.3 \times 10^5$ reps/hour, the uncertainty being due to the variation in results obtained with this dosimeter. As was the case with the electron irradiations, all γ-irradiations were carried out on analytically pure crystalline samples contained in sealed tubes under high vacuum.

The G values,[12] or molecules decomposed per 100 e.v. of absorbed energy, were calculated as follows for both types of irradiations

$$1\ \text{Rep} = 5.2 \times 10^{13}\ \text{e.v./gram}$$

$$\frac{\text{molecules dec.}}{100\ \text{e.v.}} = \frac{\left(\frac{6.02 \times 10^{23}}{\text{mol. wt.}}\right)\left(\frac{\%\ \text{dec.}}{100}\right)}{(\text{Reps})\,\frac{5.2 \times 10^{13}}{100}} = \frac{(\%\ \text{decomposed})\,(1.16 \times 10^{10})}{(\text{Reps})\quad(\text{mol. wt.})}$$

Analytical Procedures.—Two procedures have been used to determine the extent of decomposition of the irradiated materials: reineckate analysis and paper chromatography.

Reineckate analyses have been used to determine the extent of decomposition of all the choline analogs with the exception of the trimethyl-3-hydroxypropylammonium chloride. In applying this method we have in general followed the procedure of Glick[13] in which the quaternary ammonium cation is precipitated out of aqueous solution by the anion of ammonium reineckate, $NH_4^+[Cr(SCN)_4(NH_3)_2]^-$. Before the addition of this reagent, the irradiated compounds were boiled for 15 minutes in 0.1 N KOH solution in order to eliminate any trimethylamine formed by the irradiation, and then neutralized. The precipitated quaternary ammonium reineckate was freed of ammonium reineckate by washing with 1-propanol, then dissolved in acetone, and its concentration determined by means of its optical density at 526 mμ. The molar extinction coefficient for choline reineckate was determined by Glick to be 111; our value is 109. The molar extinction coefficients for the reineckates of choline iodide, trimethyl-2-chloroethylammonium chloride, and trimethylethylammonium chloride were found to be 111, 111 and 109, respectively; these values are all very nearly the same because the optical density at 526 mμ is due only to the reineckate moiety. Acetylcholine was determined as choline by a prior hydrolysis accomplished by boiling for 15 minutes in 0.1 N KOH solution. For all of the analogs for which it was used, the error of the reineckate procedure in determining the amount remaining after an irradiation is about ±5%.

The reineckate procedure was found to be unsuited for the analyses of trimethyl-3-hydroxypropylammonium chloride as no procedure could be found which would separate the reineckate of the quaternary ammonium salt from the ammonium reineckate reagent. Propanol washing was found to dissolve large fractions of the desired reineckate along with the unused reagent.

In the analysis of betaine hydrochloride the titration procedure of Walker and Erlandsen[14] was used on the precipitated reineckate instead of the photometric procedure of Glick. In the former procedure, the acid function of the reineckate-precipitated betaine hydrochloride is determined titrimetrically. The presence of trimethylamine was found not to interfere with the Walker and Erlandsen procedure; however, deviations in results obtained under standard conditions indicate that the error involved in this procedure may be as high as ±10%.

Paper chromatography was also used to measure the extent of decomposition of the methyl-labeled choline analogs. This procedure was possible because the only labeled decomposition product found on either two-dimensional (phenol–water and butanol–propionic acid–water) or one-dimensional (butanol–HCl–water) chromatograms was trimethylamine. This product is formed as the hydrochloride (or hydroiodide) during the bombardments; volatile substances formed were never more than 1% (usually less than 0.5%) of the total weight of the irradiated compound. This lack of volatility presumably is due to radiation polymerization of the product derived from the 2- or 3-carbon chain of the molecule. The trimethylamine was separated from unchanged choline analog by one-dimensional paper chromatography on Whatman No. 1 paper using the n-butanol–concd. HCl–water solvent (4:1:1 by vol.). In the case of the trimethylethylammonium chloride, no separation from trimethylamine was achieved with the butanol solvent. A successful separation was effected, however, using a 95% ethanol–concd. HCl (10:1 by vol.) solvent. The position of the radioactive spots was established by radioautography and the amount of trimethylamine hydrochloride determined by counting directly on the paper. The estimated error in this procedure is ±10%.

Results

The data obtained from the irradiation experiments are summarized in Table II. The G values given in these tables are subject to large errors and therefore can be taken as only rough approximations. The absolute values of total energy absorption for the high energy electron irradiations are not known to better than a factor of two; for the self-irradiation of labeled choline chloride and the γ-irradiation experiments the energies received are probably correct to ±20%. In addition, the G values are subject to the errors introduced by the analytical techniques. These are particularly large when the decompositions are less than 5%.

Discussion

Choline chloride is by far the least resistant to ionizing radiation of the seven members of this series studied and is, by any criterion other than polymerization, the most radiation-sensitive solid state organic compound known at the present time. Its very high G value and lack of diversity of products clearly indicate the presence of a chain mechanism of decomposition. Since about 25–30 e.v. are required to ionize an organic molecule, a G value in the range of about 3 to 4 indicates that, assuming ionization rather than excitation is playing the dominant role, approximately every molecule which is ionized is subsequently irreversibly converted to some other chemical species. In the case of the self-decomposed C^{14}-labeled choline chloride, with a G value of something like 1000, approximately 300 choline ions are decomposed by

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TABLE II

RADIATION DECOMPOSITION OF CHOLINE ANALOGS

Compound	Type of radiation	Reps $\times 10^{-6}$	G values $-M^a$	G values TMA[b]
Choline chloride	C^{14}-β	10.7	...	490[c]
Choline chloride	C^{14}-β	2.5	...	1780
Choline chloride	C^{14}-β	1.5	...	1490[d]
Choline chloride	2–4 mv. e^-	160	...	26
Choline chloride	2–4 mv. e^-	57	23	15
Choline chloride	2–4 mv. e^-	127	14	....
Choline chloride	2–4 mv. e^-	66	20	....
Choline chlorine	Co^{60}-γ (1.23 Mev.)	37	160	....
Choline chloride	Co^{60}-γ (1.23 Mev.)	28	200	190
Choline chloride	Co^{60}-γ (1.23 Mev.)	12	173	152
Acetylcholine chloride	C^{14}-β	10.9	...	4
Acetylcholine chloride	2–4 mv. e^-	220	2	....
Acetylcholine chloride	2–4 mv. e^-	500	...	~0.1
Acetylcholine chloride	2–4 mv. e^-	57	1	....
Acetylcholine chloride	2–4 mv. e^-	240	4	3
Acetylcholine chloride	2–4 mv. e^-	140	1	1
Acetylcholine chloride	Co^{60}-γ (1.23 Mev.)	10	<1	<1
Acetylcholine chloride	Co^{60}-γ (1.23 Mev.)	56	...	5
Acetylcholine chloride	Co^{60}-γ (1.23 Mev.)	90	4	3
Choline iodide	2–4 mv. e^-	70	5	....
Choline iodide	2–4 mv. e^-	240	~0.5	1
Choline iodide	2–4 mv. e^-	160	2	1
Choline iodide	2–4 mv. e^-	230	1	....
Choline iodide	Co^{60}-γ (1.23 Mev.)	15	3	....
Choline iodide	Co^{60}-γ (1.23 Mev.)	28	3	....
Choline iodide	Co^{60}-γ (1.23 Mev.)	16	17[f]	<1
Choline iodide	Co^{60}-γ (1.23 Mev.)	28	5	2
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	2–4 mv. e^-	82	...	3
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	2–4 mv. e^-	390	...	2
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	13	...	6
$[(CH_3)_3NCH_2CH_2CH_2OH]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	27	...	3
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	2–4 mv. e^-	99	4	....
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	2–4 mv. e^-	101	2	....
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	2–4 mv. e^-	150	1	5[e]
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	2–4 mv. e^-	280	1	7[e]
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	16	32	....
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	13	17	....
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	12	<1	12[e]
$[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	33	<1	....
$[(CH_3)_3NCH_2COOH]^+Cl^-$	2–4 mv. e^-	60	18	....
$[(CH_3)_3NCH_2COOH]^+Cl^-$	2–4 mv. e^-	89	11	....
$[(CH_3)_3NCH_2COOH]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	60	19	....
$[(CH_3)_3NCH_2COOH]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	16	17	....
$[(CH_3)_3NCH_2COOH]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	81	4	2
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	2–4 mv. e^-	123	2	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	2–4 mv. e^-	172	13[f]	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	2–4 mv. e^-	208	1	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	2–4 mv. e^-	166	1	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	30	34[f]	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	28	3	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	29	<1	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	52	<1	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	29	<1	....
$[(CH_3)_3NCH_2CH_3]^+Cl^-$	Co^{60}-γ (1.23 Mev.)	50	6	4

[a] The symbol "$-M$"[15] represents the starting material which is permanently altered by the irradiation. These G values were determined by Reinecke salt precipitation. [b] The "TMA" G values were obtained by the paper chromatographic determination of the amount of trimethylamine produced during the irradiation. [c] This result was determined in an earlier paper. See reference 3. [d] Another portion of this sample was stored at $-196°$. It showed no measurable decomposition and, at this temperature, probably has a G value of 3 to 4. [e] The reason for the discrepancy between the two methods of analysis is not known. The reineckate results may be due to interference from some decomposition product other than trimethylamine. [f] This value is almost certainly high and is probably due to an error in carrying out the reineckate analysis.

the energy which is required to ionize a single molecule. Therefore, the decomposition of the labeled choline chloride may be proceeding through a free-

(15) B. M. Tolbert and R. M. Lemmon, *Rad. Research*, in press.

radical chain mechanism which starts with a single +2 choline ion and continues through about 300 ions before the chain reaction is terminated. What part, if any, excited molecules play in the decomposition mechanism, is unknown. The only facts known at present about the postulated chain reaction are: (a) the energy of activation is high enough so that the chain does not proceed at liquid nitrogen temperatures and (b) the presence of iodide (or iodine), in the case of choline iodide, also breaks the chain. One way in which the chain reaction and the chain-breaking step may be taking place is

$$[(CH_3)_3NCH_2CH_2OH]^+ \xrightarrow{-e^-} [(CH_3)_3NCH_2CH_2OH]^{++} \quad (1)$$

$$[(CH_3)_3NCH_2CH_2OH]^{++} \longrightarrow (CH_3)_3N^{\cdot+} + {}^+CH_2CH_2OH$$

$${}^+CH_2CH_2OH \longrightarrow H^+ + CH_2{=}CHOH \quad (2)$$

$$CH_2{=}CHOH \longrightarrow CH_3CHO$$

$$(CH_3)_3N^{\cdot+} + [(CH_3)_3NCH_2CH_2OH]^+ \longrightarrow (CH_3)_3N: + (CH_3)_3N^{\cdot+} + {}^+CH_2CH_2OH \quad (3)$$

Chain-breaking step (in the case of choline iodide) (4)

$$(CH_3)_3N^{\cdot+} + I^- \longrightarrow (CH_3)_3N: + I\cdot$$

Reactions (2) and (3) might then be repeated until the trimethylamine radical ion participated in some reaction other than (3), thus breaking the chain. However, since the crystal structure of choline chloride has not been determined, it is not known whether the spatial arrangement of atoms in a choline chloride crystal would be such as to favor the operation of this mechanism. It is probable that the crystal structures of the choline analogs, when they are accurately known, will do much to explain the great difference in radiation sensitivity between choline chloride and the other choline analogs.

The explanation of the great difference in the *G* values of the C^{14}-beta- and the 2–4 mv. electron-decomposed choline chloride may lie in the far higher flux of electrons in the case of the high energy particles; the higher energy irradiations took place during a few milliseconds (pulsed beam), the low-energy ones during weeks or months. There is, therefore, apparently an intensity effect in which the lengths of the chains are inversely related to the rate of chain starting.

By analogy with the work of Ingold's group[16] on the thermal decomposition of quaternary ammonium bases (Hofmann elimination reaction), it might be expected that trimethyl-2-chloroethylammonium chloride, with an even stronger electron-attracting group on the β-carbon atom, might be even more unstable than choline chloride. However, toward radiation decomposition the former compound is much more stable than choline chloride. Since a chain mechanism apparently is present in the case of choline chloride, but not in any of the analogs (with the possible exception of betaine hydrochloride), the radiation–decomposition reaction is quite different from the thermal decompositions and no analogies can be drawn.

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[CONTRIBUTION FROM THE PACIFIC FISHERIES EXPERIMENTAL STATION]

Marine Sterols. I. Isolation of 24-Methylenecholesterol from Molluscs

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A new sterol, 24-methylenecholesterol, has been isolated by a chromatographic separation of the azoyl esters prepared from the sterols of the oyster (*Ostrea gigas*) and the clam (*Saxidomus giganteus*). Oyster and clam sterols contain 36 and 53% respectively, of this sterol.

It has long been recognized that oysters contain sterols other than cholesterol.[1,2] Ostreasterol has been isolated from several molluscs including the oyster, *Ostrea virginica*.[2] There have been several structures proposed for this sterol but the most recent evidence indicates that it is identical with chalinasterol($\Delta^{5,22(23)}$-campestadiene-3β-ol).[2–4] However, the evidence appears to be based solely on a resemblance of physical properties, a frequently unreliable criterion when applied to sterols. Ostreasterol was first isolated as the most insoluble steryl acetate from *Ostrea virginica* and later it was purified further *via* the tetrabromide.[2,3]

In the present investigation 24-methylenecholesterol was isolated by chromatography of the steryl azoates prepared from the sterols of the oyster (*Ostrea gigas*) and the butter-clam (*Saxidomus giganteus*), on silicic acid. This sterol does not have the same properties as ostreasterol, for when the mixed acetates from *Ostrea gigas* were recrystallized repeatedly, the most insoluble acetate was still a mixture composed of 29% 24-methylenecholesterol and 71% of chromatographic zone 3. Thus judging from the original composition, zone 3 and not zone 2 (24-methylenecholesterol) forms the more insoluble acetate. Zone 3 has been shown to

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