THERMAL AND RADIOLYTIC DECOMPOSITION OF ^{131}I-19-IODOCHOLESTEROL

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Using accelerated decomposition studies, the thermal and radiolytic decomposition of ^{131}I-19-iodocholesterol was determined. Thermal decomposition was the major cause of decomposition of ^{131}I-19-iodocholesterol in its present formulation. Radiation decomposition does not significantly affect radiochemical purity over the expiration time of the product. A G value of 2.56 and an energy of activation of 34.6 kcal/mole was obtained with ^{131}I-19-iodocholesterol. Radiochemical purity of 70% is obtained in 18 days if stored at 5°C and 3 days if stored at room temperature.

Counsell, et al (*1*) and Beierwaltes, et al (*2*) have reported the synthesis and selective localization in the adrenal gland of ^{131}I-19-iodocholesterol. All previously labeled compounds used to scan the adrenals have been clinically inadequate because of in vivo or in vitro instability (*3,4*).

Iodine-131-19-iodocholesterol is resistant to in vivo dehalogenation without altering the basic cholesterol structure, thus enabling the product to mimic the cholesterol metabolic pathways (*5*).

Using ^{125}I-19-iodocholesterol, Blair, et al (*6*) produced clear visualization of dog adrenal glands with a rectilinear scintillation scanner. Since that time a number of reports from the University of Michigan have dealt with the clinical use of ^{131}I-19-iodocholesterol in the diagnosis of unilateral adrenocortical adenoma (*7*), primary aldosterone adenoma (*8*), Cushing's syndrome (*9*), and adrenal remnants (*10*).

Conversely, conclusive studies on the thermal and radiolytic stability of ^{131}I-19-iodocholesterol have not been carried out. Jones, et al (*11*) followed ^{131}I-19-iodocholesterol stability over five batches used clinically and found up to 40% degradation over a period of 30 days. The objective of this study was to evaluate the effect of heat and specific radioactive concentration on ^{131}I-19-iodocholesterol as it exists in its final dosage form.

EXPERIMENTAL

Radiation decomposition of ^{131}I-19-iodocholesterol. Iodine-131-19-iodocholesterol, 50 mg, was synthesized, solubilized in 2 ml of ethanol and formulated by adding 0.5 ml of polysorbate-80 and 27.5 ml of sodium chloride injection, USP. The final product concentration was 2.58×10^{-6} *M* (1.32 mg/ml) with a specific activity of 50 μCi/mg.

Six 1-ml samples were filled with the ^{131}I-19-iodocholesterol solution and sealed. The ampules were irradiated for 2 hr by a 3,900-Ci ^{60}Co source. Each ampule was positioned according to a predetermined isodose curve. Table 1 shows the radiation dose versus distance associated with each sample.

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TABLE 1. ABSORBED RADIATION DOSES

Sample position	Distance from source (cm)	Dose (rads)
1	not irradiated	control
2	53	7.56×10^3
3	9	6.58×10^4
4	4	1.24×10^5
5	1	1.79×10^5
6	center 26 cm height	2.49×10^5
7	center 13 cm height	3.53×10^5

Radiation dose supplied by 3,900-Ci ^{60}Co source located at Phoenix Memorial Laboratories, University of Michigan, Ann Arbor, Mich.

The total radiation dose which each sample received was calculated using the following equation:

$$\frac{(V)(t)(R)(100\ \text{ergs/gm-rad})}{1.6 \times 10^{-12}\ \text{erg/ev}} = \text{radiation dose (ev/2 hr)}$$

where V is volume of the sample, t is time (hr), and R is radiation level (rads).

After sample irradiation, 5 μl of each sample was spotted on Eastman silica gel precoated thin-layer chromatograms. The chromatograms were developed in ethanol (95%):chloroform (1:1) or ethyl acetate:benzene (1:1) The developed chromatograms were analyzed using a Packard Model 7201 strip scanner (Packard Instrument Co., Downers Grove, Ill.) and chromatogram quantitation accomplished with a planimeter.

Thermal decomposition of ^{131}I-19-iodocholesterol. Four 1-ml samples of the test solution were placed in 5-ml multidose vials. Each vial was immersed in a constant temperature bath at a specific temperature. The temperatures used were 45°, 55°, 75°, and 90°C. At time intervals up to 22 hr, 5-μl samples were withdrawn and spotted on Eastman silica gel chromatograms for analysis as previously described.

RESULTS AND DISCUSSION

The decomposition of a radiopharmaceutical is based on the relative contributions of temperature, light, ionizing radiation, and other physical conditions. By assuming that the effects of the respective conditions are additive and by estimating the effect of each contribution, it is possible to estimate the rate of decomposition of the product. Accelerated decomposition studies can be accomplished with respect to temperature by obtaining a product's decomposition rate at elevated temperatures, preparing an Arrhenius plot, and extrapolating the rate of decomposition at the usual storage temperature (*12*). Expected radiation decomposition can be estimated by diluting a radioactive sample to negate its own radiation effects and then irradiating aliquots of the sample with known quantities of external radiation (*13*). Radiation decomposition has been found to be dependent upon the total-absorbed energy of the sample (radiation dose) and the radiation yield of decomposition (G value) of the product (*13*). Therefore, by determining the G value for the product and by knowing the radiation dose, the effect of radiation on the product can be calculated.

Using thin-layer chromatography, the ^{131}I-19-iodocholesterol had an R_f value of 0.93 ± 0.07 in chloroform:ethanol and 0.45 ± 0.03 in the ethyl acetate:benzene solvent system. An impurity was observed at R_f 0.41 ± 0.06 in chloroform:ethanol and remained at the origin in the ethyl acetate:benzene system. This impurity increased with time and is presently unidentified. Iodide ion had an R_f value of 0.19 ± 0.03 in chloroform:ethanol and remained at the origin in the ethyl acetate:benzene system.

TABLE 2. THERMAL DECOMPOSITION OF ^{131}I-19-IODOCHOLESTEROL

Temperature (°C)	Time (hr)	Percent purity
90	0.0	96
90	1.0	56
90	2.0	39
90	2.5	36
90	3.0	34
Slope = −0.45	Y-intercept = 93.2	Correlation coef. = −.994
75	0.0	95
75	2.0	66
75	4.0	43
75	5.0	37
75	6.0	35
75	20.0	14
Slope = −0.192	Y-intercept = 95.42	Correlation coef. = −.999
55	0.0	82
55	2.0	72
55	4.5	61
55	13.0	36
55	22.0	22
Slope = −0.063	Y-intercept = 81.65	Correlation coef. = −.999
45	0.0	82
45	2.0	80
45	4.5	74
45	13.0	66
45	22.0	53
Slope = −0.019	Y-intercept = 82.39	Correlation coef. = −.993

The results of the thermal decomposition study are given in Table 2 and illustrated in Fig. 1. As the temperature increased, a faster degradation rate was noted. Each sample exhibited a two-component degradation curve, suggesting that decomposition involves multiple competing chemical reactions. An Arrhenius plot (Fig. 2) was made using the initial slope for each curve to allow extrapolation from other temperatures. The initial slope was used to provide the most conservative shelf-life data. Thus it was possible to obtain a degradation rate for ^{131}I-19-iodocholesterol at room temperature (22°C) and when refrigerated (5°C).

At room temperature, ^{131}I-19-iodocholesterol has a thermal decomposition rate of 0.005 hr^{-1}. Knowing that this rate yields a conservative shelf-life value, that the impurity is not iodide ion, and that clinically acceptable scans can be obtained with a

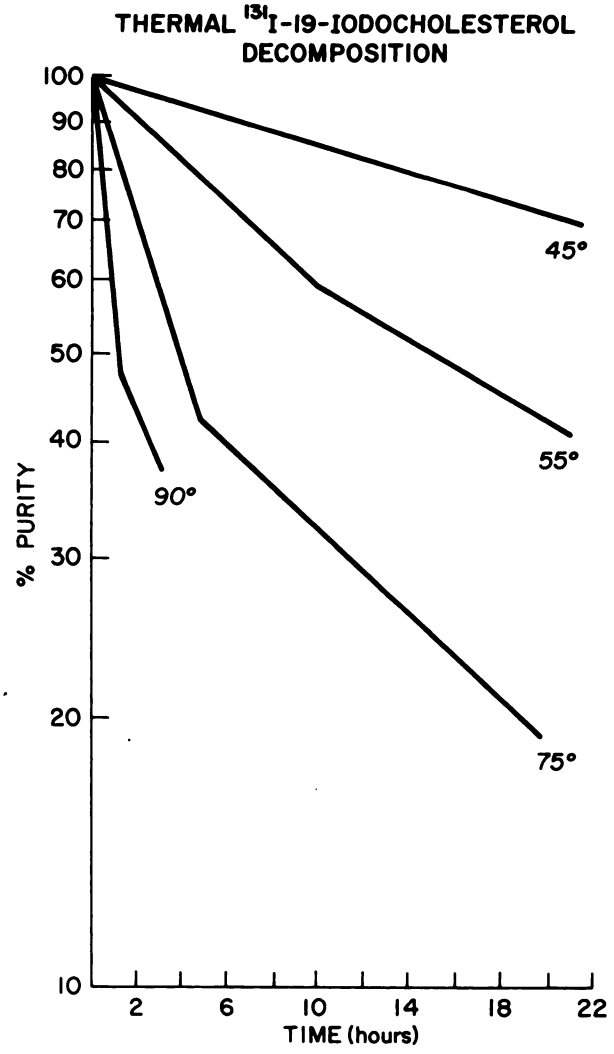

FIG. 1. Logarithmic plot of thermal ^{131}I-19-iodocholesterol decomposition.

70% purity, the product expiration date due to thermal decomposition would be

$$P_{70} = P_{100}\, e^{-0.005\,(t)}$$

or, t = 72 hr or 3 days. When stored in a refrigerator at 5°C, the product has a decomposition rate of 0.00084 hr^{-1}, and thus an expiration date of 17.7 days. A purity of greater than 95% can be attained by passing the ^{131}I-19-iodocholesterol product through a 100-200 mesh Biorad AG 3-x4a anion exchange resin (Biorad Labs, Richmond, Calif.).

Using the slope of the Arrhenius plot, the energy of activation (E_a) for ^{131}I-19-iodocholesterol was found to be 34.6 kcal/mole.

Table 3 and Fig. 3 show the percent of ^{131}I-19-iodocholesterol remaining after each sample was exposed to the ^{60}Co source for different absorbed radiation doses. Using these results, a G value of 2.56 was calculated using the following equation:

$$\text{G value} = \frac{(\text{spec. conc. mg/ml})\,(\text{Avogadro's No.})\,(\Delta\%)}{\text{mg/mole}\;(\Delta\text{ev/ml})}$$

$$= \%\text{-atoms/ev}$$

where $\Delta\%$ is change in percent purity and Δev/ml is change in absorbed energy. Since the maximum specific activity of ^{131}I-19-iodocholesterol currently used is 1.5 mCi/mg with a specific concentration of 4 mCi/ml, the total absorbed radiation energy per milliliter over the physical decay lifetime of the product is

$$\frac{(4\text{ mCi/ml})\,(0.22\text{ MeV})\,(2.2 \times 10^{9}\text{ dpm/mCi})}{0.693/1.15 \times 10^{4}\text{ min}}$$

$$= 3.23 \times 10^{17}\text{ ev/ml}$$

where 0.22 represents an average energy of deposition per decaying atom (beta = 0.20 MeV and gamma = 0.02 MeV). This level of self irradiation as evident from Fig. 3 as well as the calculated low G value shows insignificant contribution to the decomposition of ^{131}I-19-iodocholesterol. Therefore, the primary decomposition of the product is thermal and not radiolytic.

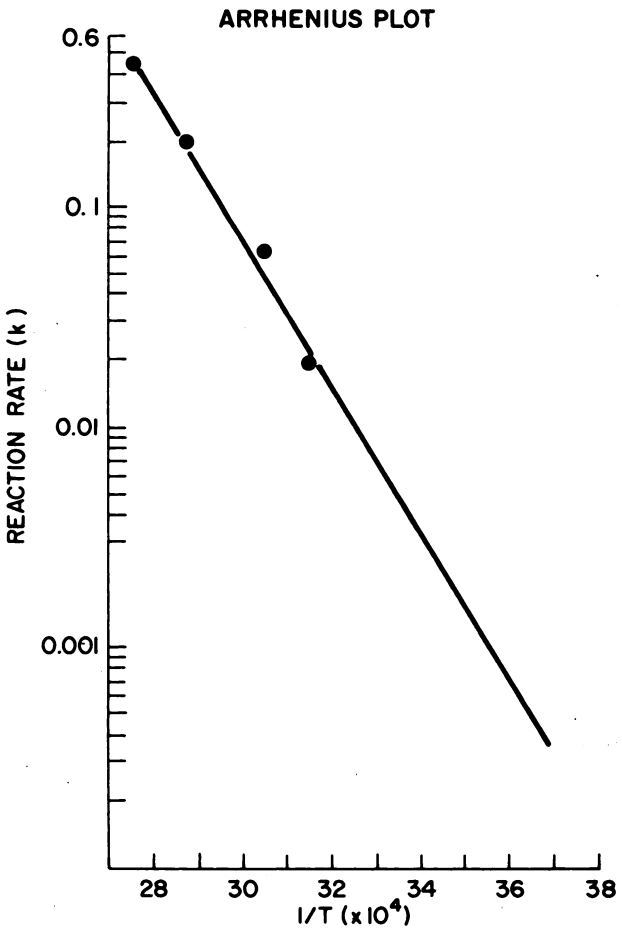

FIG. 2. Arrhenius plot of first portion of curves in Fig. 1.

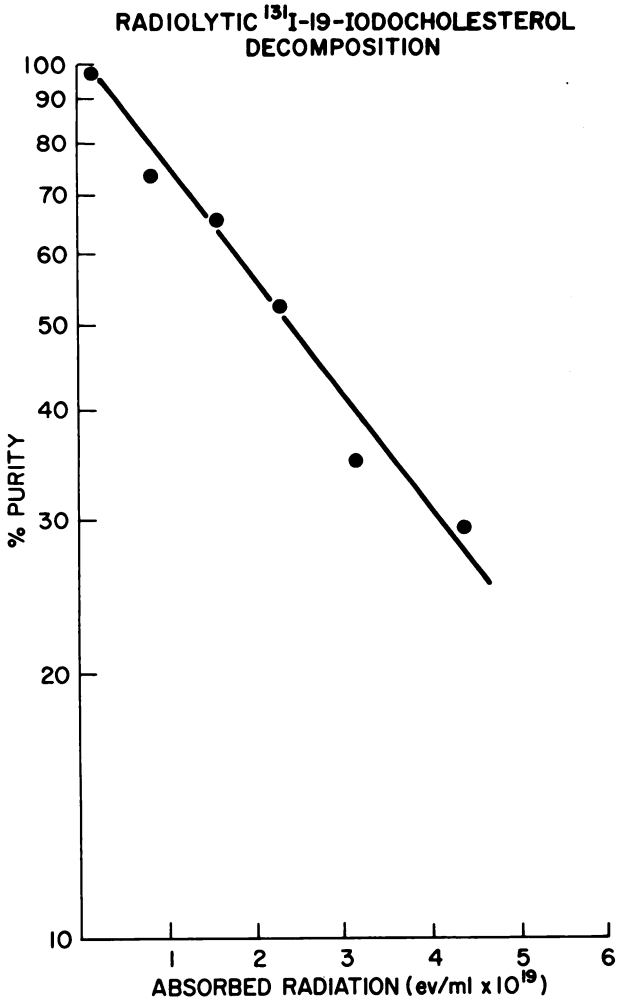

FIG. 3. Logarithmic plot of radiolytic ^{131}I-19-iodocholesterol decomposition.

TABLE 3. RADIATION DECOMPOSITION OF ^{131}I-19-IODOCHOLESTEROL

Sample number	Radiation level (× 10^{19} ev/2 hr)	Percent remaining
1	0.000	96.1
2	0.094	80.1
3	0.820	71.7
4	1.550	63.9
5	2.240	51.7
6	3.120	34.2
7	4.400	29.0

CONCLUSIONS

A G value of 2.56 and an energy of activation of 34.6 kcal/mole was obtained for ^{131}I-19-iodocholesteral. Thermal decomposition is the governing factor of the decomposition rate of ^{131}I-19-iodocholesterol. Radiation decomposition does not significantly affect radiochemical purity over the expiration time of the product. Radiochemical purity of 70% is obtained 17.7 days after synthesis if stored at 5°C and 3 days after synthesis at room temperature.

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