

## Some factors involved in the decomposition of labeled steroids during storage

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### SUMMARY

*Estrone,6-7-<sup>3</sup>H in benzene has been found to exhibit greater decomposition when stored at -5 °C than when stored at +5 °C. The reason is attributed to lack of uniform dispersal of the solute due to solvent crystallization. The non-distillable residues from commercial solvents have been found to cause rapid destruction of a stored radiochemical if not removed before storage. Data is presented which indicates that the best method for reducing the total amount of decomposition of labeled steroids which are stored for extended periods of time is repurification at regular time intervals.*

In an earlier report <sup>(1)</sup> it was shown that non-radioactive tars normally present after the synthesis of a radioactive steroid could cause serious decomposition if not removed from the steroid before storage. This present report is concerned with a further investigation into the origin of these tars and some other factors influencing the decomposition of labeled steroids. Among the factors investigated were temperature and solvents.

### TEMPERATURE STUDY.

There have been several reports <sup>(2)</sup> demonstrating that the lowering of temperature generally decreases the rate of decomposition of a stored radiochemical. In an excellent review of the modes of decomposition (2d), the lowering of temperature has been one of the suggested means for decreasing decomposition resulting from both secondary and chemical decomposition. However, if the lower temperatures result in the crystallization of the solvent a decrease in the stability of the solute can occur.

A sample of estrone-6,7-<sup>3</sup>H with a specific activity of 42 curies/mole and at a concentration of 1 mc/ml in benzene was divided into three equal

portions. One sample was stored at room temperature, another in the refrigerator at  $+5^{\circ}\text{C}$  and the third at  $-5^{\circ}\text{C}$  in a freezer. The rates of decomposition were obtained by chromatographic scans employing 1% standards to quantitate numbers in the 1 to 5% range. It can be seen in Table I that after 2 months, 18 months and 20 months of storage, the sample stored in the freezer exhibited a greater amount of decomposition than the sample stored at room temperature and in the refrigerator.

TABLE I. Temperature Study with Estrone-6,7- $^3\text{H}$  42 c/mM, 1 mc/ml, Benzene Solvent

	Percent Decomposition		
	2 months	18 months	20 months
Room Temp.	2 %	50 %	75 %
$0^{\circ}$ to $+5^{\circ}\text{C}$	1 %	8 %	15 %
$0^{\circ}$ to $-5^{\circ}\text{C}$	5 %	90 %	100 %

The sample stored in the freezer began to solidify within 48 hours, and during this process aliquots were removed from the non-frozen solvent and counted. A steady increase in the concentration of activity (mc/ml) of the diminishing non-frozen solvent was observed. After complete solidification, the solute was completely unprotected by the solvent matrix, and as such underwent rapid decomposition in spite of the lower storage temperature. The other two samples remained in solution, with the one stored in the refrigerator exhibiting less decomposition than the one stored at room temperature.

This experiment demonstrates that in lowering the temperature, zone localization of the solute may occur with a resulting decrease in stability. It may be beneficial if a crystal structure can be produced which allows the solute to be dispersed evenly throughout the solvent matrix. An amorphous glass giving a uniform distribution of solute in solvent may be desirable and could be produced by quick freezing<sup>(3)</sup>.

#### SOLVENT STUDY.

A labeled steroid that has been highly purified should exhibit no unusual decomposition throughout its early storage life provided its storage is maintained under optimum conditions. Some of the factors that are involved with these conditions are temperature, concentration and solvent.

Several studies were conducted to learn more about the relationship between solvents and the stability of labeled steroids. One such study was the

determination of the solvents which would provide the best environment for the purified steroids. The results of this study on estrone-6,7-<sup>3</sup>H at 42 c/mmole are given in Table II.

TABLE II. Solvent Study on Estrone-6,7-<sup>3</sup>H 42 c/mM, 0° to + 5°C, 1 mc/ml of solvent

	2 months	18 months	20 months
Ethanol : benzene (1 : 9)	0 %	3.5 %	15 %
Ethanol : benzene (1 : 4)	1-2 %	35 %	60 %
Ethanol : benzene (1 : 1)	1-2 %	60 %	80 %
Abs. Ethanol	5 %	90 %	—
Methanol : benzene (1 : 4)	1-2 %	45 %	—
5 % aqueous methanol	5 %	100 %	—
Benzene	1 %	8 %	16.5 %

It can be seen that estrone-6,7-<sup>3</sup>H is reasonably stable in benzene and in benzene : ethanol (9 : 1). The later solvent combination has the advantage that it can dissolve the more polar steroids and that even lower temperatures can be employed without solvent freezing.

Considering the relative values obtained in Table II, it can be seen that larger amounts of ethanol in benzene caused greater decomposition in the estrone-6,7-<sup>3</sup>H samples. This points out the necessity of determining the most favorable composition of a binary mixture of solvents. Ekstrom and Garnett <sup>(4)</sup> have recently pointed out that the rate of radiolysis and the species

TABLE III. Study of Purified vs. Non-Purified Solvents with Estrone-6,7-<sup>3</sup>H, 42 c/mmole 1 mc/ml solv. 0° to 5°C, 2 months. Percent Decomposition.

Solvent	Conc. tars 500 ml Solvent	Charcoal Column	Distillation	Distillation and Charcoal Column
Benzene	0.5 %	0.3 %	0 %	0.4 %
Methanol	60 %	9.2 %	0.9 %	7.9 %
Dioxane	15 %	1.5 %	0.7 %	0.4 %
Methylene chloride	55 %	50 %	4 %	35 %
Formamide	0.7 %	0.4 %	0.6 %	0.4 %
Hexane	3.5 %	1.6 %	1.1 %	1.0 %
Ethanol (gold shield)	6.3 %	1.3 %	0.3 %	2.1 %
Ethanol (3A)	3.6 %	0.9 %	0.7 %	0.6 %

of radiolysis products of a binary mixture of deuterated benzene and non-deuterated ethanol varies with the relative composition of ethanol in benzene.

Frankel and Nalbandov <sup>(5)</sup> have demonstrated that various solvents can have a deleterious effect on the stability of steroids. They also examined a variety of methods for the removal of the harmful agents causing the deleterious effects. Another study has appeared by Crosby and Aharonson <sup>(6)</sup> which demonstrated that commercial solvents contain fluorescent residues that could be removed by passage over a charcoal column and by distillation. Our own study was an effort to see if these fluorescent tars were causing decomposition, thereby linking the work of Frankel and Nalbandov with that of Crosby and Aharonson.

In Table III a list is given of some common solvents which are employed in the synthesis and purification of steroids. In each case 500 ml of commercial grade solvent \* were evaporated to dryness *in vacuo* at 50 °C\*\* and the residue redissolved in a milliliter of distilled solvent. This was added to estrone-6,7-<sup>3</sup>H 42 c/mmole and stored as indicated. The solvent tars which caused the most damage originated from commercial methanol. Passing the solvents through a charcoal column decreased the amounts of decomposition except in the case of methylene chloride. Distillation was even more favorable. The fact that distillation and then charcoaling together were little better than charcoaling alone demonstrated that an impurity was picked up after passing the distilled solvent through the charcoal column. The results of Table III show that a simple distillation of commercial solvents is sufficient to remove the non-distillable residues and that a greatly increased shelf life can be realized.

One experiment was made with these tars to see if heavy metal ions such as iron and copper were, in part, responsible for the decomposition. The idea that these ions may be implicated was advanced by Harold Varon \* in his work on the removal of heavy metal ions from the adsorbents on TLC plates. Accordingly, the residue from the concentration of commercial methanol was assayed for iron and copper content \*\*\*, dissolved in methylene chloride and washed with a versene solution. See Table IV. The results of storing these tars with estrone-6,7-<sup>3</sup>H before and after the heavy metal ions had been removed are given in Table V.

\* Commercial grade solvents were obtained from the following sources :

- I Fisher — Dioxane and methylene chloride
- II Baker and Adamson — Benzene, methanol and hexane
- III Eastman — Formamide
- IV Commercial Solvent Corp. — 95 % Ethanol (3A) and 100 % ethanol (gold shield).

\*\* This quantity was selected in an effort to simulate the amount of solvent tars which would result from the combining and distilling of a large number of chromatographic fractions in a normal purification.

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TABLE IV. Analysis of non-distillable tars from 100 ml of commercial Methanol

Sample	Fe	Cu
Untreated tars	130 $\mu\text{g} \%$ (or 1.3 $\mu\text{g}/\text{sample}$ )	70 $\mu\text{g} \%$ (or 0.7 $\mu\text{g}/\text{sample}$ )
Versene washed tars	0 % (sensitivity 30 $\mu\text{g} \%$ )	0 % (sensitivity 30 $\mu\text{g} \%$ )

TABLE V. Estrone-6,7- $^3\text{H}$ , 42 c/mmole, 1 mc/ml Benzene : Ethanol (9 : 1) 5 °C, 5 weeks stored in the presence of methanol tars before and after washing with 0.2 % versene solution

Sample	Treatment	% Decomposition
Estrone-6,7- $^3\text{H}$	No tars	0 %
Estrone-6,7- $^3\text{H}$	Untreated tars	6.5 %
Estrone-6,7- $^3\text{H}$	Washed tars	1.5 %

It can be seen that the solvent tars which were washed with a 0.2 % versene solution caused little decomposition. It is possible that the versene extraction removed, in addition to heavy metal ions, other substances which could have been the cause of the decomposition.

Figure 1 are curves obtained from the data from Tables I and II. Regrettably there are only four points including the initial time zero, when the estrone-6,7- $^3\text{H}$  was radiochemically pure. The notable thing is the similar shapes of the curves. None are linear, in spite of the fact that decomposition was hastened by the alteration of temperature or unfavorable solvents. The initial decomposition is almost linear, but then, suggestive of an autocatalytic process, the curves rise sharply.

The practical implication of these curves is that radiochemicals should be stored under optimum conditions and purity checks performed at regular intervals. When a few percent of impurities accumulate the radiochemicals should be purified. A delay of several months could result in considerable decomposition particularly if that delay occurs when the slopes of the decomposition curves are increasing rapidly.

In summary, the best methods for increasing the shelf life of any steroid is to remove all of the radiochemical and chemical impurities present at the

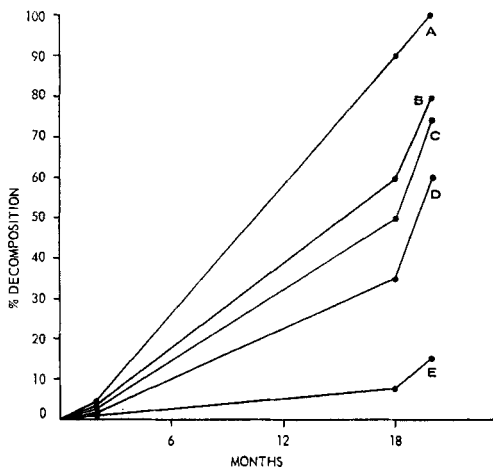


FIG. 1 Decomposition of estrone-6,7-<sup>3</sup>H, 42 c/mmole during storage. A. −5 °C in benzene; B. +5 °C in benzene : ethanol (1 : 1); C. room temperature in benzene; D. +5 °C in benzene : ethanol (4 : 1); E. +5° C in benzene.

time of synthesis. The solvents employed for both synthesis and storage should be distilled and benzene or benzene-ethanol (9 : 1) should be utilized as the solvent pair. When radiodecomposition begins to surpass a few percent the radiochemical should be purified for further storage.

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