

## Tritiation of Organic Compounds by Electrolytic Reduction I. Uracil-5-t from 5-Bromouracil

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### SUMMARY

*Uracil was tritiated to uracil-5-t by electrolytic reduction of 5-bromouracil in tritiated water. No secondary chemical or radiochemical reactions took place.*

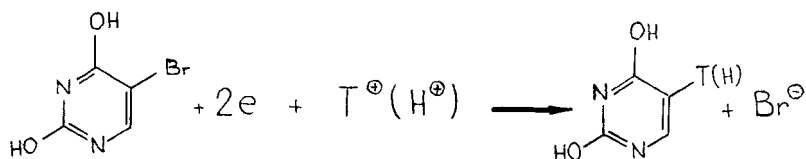
In the search for a rapid and specific procedure of tritiating uracil the electrolytic reduction of 5-bromouracil in tritiated water was investigated. The application of the electrolytic reactions to obtain labelled organic compounds was described only for iodine isotopes <sup>(1)</sup>. Isotopic hydrogen, that is deuterium, was used however in establishing the mechanism of such a reaction <sup>(2)</sup>.

The electrolytic reduction of organic halogen compounds is widely used in organic chemistry <sup>(3)</sup>. As final products of this process both the halogen ion and the hydrogenated species are obtained.

As far as we are aware nobody studied the electrolytic reduction of 5-bromouracil, the mechanism of the reaction being established by polarographic data.

The reduction of 5-bromouracil was achieved using the polarographic data obtained on a dropping mercury electrode in 0.1 N LiClO<sub>4</sub>. In these conditions a polarographic halfwave potential of -1.86 V vs. SCE resulted, the limiting current being proportional to the concentration of 5-bromouracil and to the square root of the mercury pressure. The number of electrons participating in the reduction reaction of 5-bromouracil was established by comparing the experimental limiting current with that obtained for other halogen compounds <sup>(4)</sup>.

As in most cases the electrolytic reduction of mono-halogen compounds appears to involve fission of the carbon-halogen bond with the participation of two electrons simultaneously with the release of the proton from the solvent, one of the possible reaction paths could be the following.



In practice this reaction was performed at a controlled cathode potential, yielding a mixture of uracil-5-t and 5-bromo-uracil. The reaction products were separated and purified by paper chromatography.

The experimental data obtained in the electrolytic reduction of 5-bromo-uracil are given in Table 1.

TABLE 1. Electrolytic reduction of 5-bromouracil in 1 ml tritiated water.

HTO Specific activity mCi/mole	Total activity in products $\mu\text{Ci}$				% Yield	
	Uracil Calculated	Uracil measured	5-Bromo- uracil measured	Specific activity of uracil $\mu\text{Ci}/\text{mmole}$	Chemical	Radio- chemical
1	2	3	4	5	6	7
2.5	6.6	5.3	0	16	75	80
1.9	4.7	3.9	0	13	64	84
1.9	4.6	3.7	0	14	61	80

As it can be seen from Table 1, the lack of activity in 5-bromouracil denotes the absence of secondary tritiation reactions such as isotopic exchange. The radiochemical yield (column 7) is given by the ratio of the columns 3/2. The total calculated activity of uracil-5-t has resulted from the stoichiometric quantity of water required for the reduction of 50 mg of 5-bromouracil expressed in radioactivity units. Almost 90 % of the remaining tritiated water was recovered.

The electrolytic reduction of 5-bromouracil to uracil-5-t is a simple and fast procedure of labelling uracil giving good chemical and radiochemical yields without secondary chemical and radiochemical reactions.

## EXPERIMENTAL PART.

*Procedure.* — A Heyrovsky LP 60 Polarograph was used to collect the data about the reduction of 5-bromouracil. The electrolytic reduction of 5-bromouracil in tritiated water was performed at a controlled potential mercury cathode in the cell represented in Figure 1.

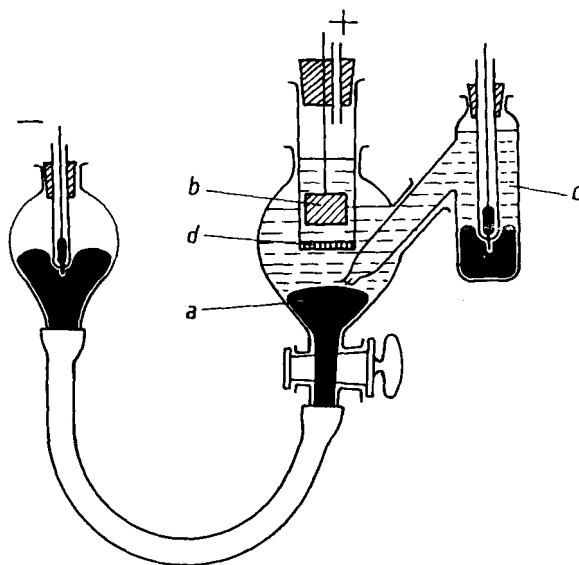


FIG. 1. Cell for the electrolysis of 5-bromouracil at controlled potential : (a) mercury cathode; (b) platinum anode; (c) saturated calomel electrode; (d) sintered glass disc G-4.

In order to accomplish the electrolytic reduction at a controlled potential an additional SCE electrode was employed. Solutions of 50 mg of 5-bromouracil in one ml 0.1 N solution of  $\text{LiClO}_4$  in tritiated water and in normal water were introduced into the cathodic respectively anodic space (Fig. 1). The density of the current was of  $0.1 \text{ A/cm}^2$ . The end of the reaction was indicated by the solution becoming alkaline towards indicator paper.

The reaction mixture was then transferred into a 2 ml glass vessel, attached to a vacuum line, the tritiated water was distilled off and any labile tritium was removed by repeated equilibrations in water followed by distillation.

*Separation and purification of the products.* — The reaction mixture was dissolved in water and separated by paper chromatography in the following mixture of solvents : n-butanol : diethylene-glycol : water (4 : 1 : 1). The  $R_f$ 's were determined in order to establish the reproducibility and the effectiveness of the separation and purification of the products.

*Characterization of the products.* — The two compounds obtained in the electrolytic reduction of 5-bromouracil were analyzed and characterized by UV spectroscopy in water solution with a CF 4 Optica Milano spectrophotometer.

*Determination of the radiochemical purity and of the specific activity.* — Paper chromatographic separations were repeated until constant radioactivity was obtained in each product. The lack of radioactivity in 5-bromouracil showed the absence of a secondary reaction and a good separation from uracil-5-t.

The radioactivity of the products was determined by direct suspension of the paper strips in the scintillant liquid of a Packard Tricarb liquid scintillation spectrometer Model 3375, using internal standards and the external ratio method.

#### ACKNOWLEDGMENTS.

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