

## Tritiations by HTO-promoted aluminium chloride. III<sup>1</sup>. Uracil, adenine, glycine, di- and triglycine.

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### SUMMARY

*Tritiations by means of tritiated water in the presence of anhydrous  $AlCl_3$  in dichloroethane were extended to products which are insoluble in the reaction medium. In the tritiation of uracil and adenine, the molar ratios substrate : HTO :  $AlCl_3$  were varied systematically. It appears that the highest specific activities are obtained with small amounts of  $AlCl_3$  (less than one mole per mole of substrate).*

### INTRODUCTION

In a previous report<sup>(2)</sup> we described the tritiation of pyrimidines by means of tritiated water HTO in the presence of anhydrous aluminium chloride, a reagent which affords one of the strongest acids  $T[AlCl_3OH]$ <sup>(3, 4)</sup>. It was shown<sup>(2)</sup> that there is an optimum time for the tritiation of pyrimidines in dichloroethane, employing for 1 mmole substrate 0.5 mmole HTO and 1 mmole  $AlCl_3$ , whereas in carbon disulphide the activity increases continuously. In the latter solvent, the activities were a magnitude order lower than in dichloroethane solution. These observations and the tritiation of  $C_2H_4Cl_2$ , were interpreted as implying trantritiation from the solvent. These results were valid for compounds which were readily soluble in the reaction medium (pyrimidine, 4,6-dichloropyrimidine) as well as for less soluble compounds (uracil, cytosine) which were not totally dissolved during the reaction.

The aim of the present study was to see whether tritiation by means of  $HTO + AlCl_3$  could be extended to other compounds which were less soluble in the reaction medium (adenine, glycine, di- and triglycine), and to investigate the effect of varying the ratios substrate:catalyst:tritiated water, using as substrates, uracil and adenine. The specific activity of the tritiated water was 10.1 mCi/mmole. The exchanges were effected at a definite temperature for 10 minutes after which the mixture was quenched in ice-water, the precipitated

<sup>1</sup> Previous parts in the series are to be considered refs. (1, 2).

compound was purified and labile tritium was removed. After a preparative thin-layer chromatographic separation, the activities were determined by combustion.

## RESULTS

The variation of the specific activity of uracil and adenine obtained in the presence of various molar ratios of catalyst is presented in table 1. It can be seen that the highest specific activity is obtained with a surprisingly low amount of  $\text{AlCl}_3$ , specifically, 0.25 moles  $\text{AlCl}_3$  for 1 mole uracil and 0.5 moles HTO. The specific activity is smaller for 0.5 moles  $\text{AlCl}_3$ , then increases again for 1 mole  $\text{AlCl}_3$ . Then, there is a smooth decrease of the specific activity with increasing amounts of catalyst, maintaining a fixed amount of substrate and water.

TABLE 1. Specific activity of uracil adenine (1 mmole) tritiated in 8 ml dechloroethane at  $80^\circ$  for 10 minutes with HTO and various amounts of sublimed  $\text{AlCl}_3$ .

HTO (mmole)	$\text{AlCl}_3$ (mmole)	Specific activities $\mu\text{Ci}/\text{mmole}$	
		Uracil	Adenine
0.5	0	0.01	—
	0.1	6.6	—
	0.25	10.1	—
	0.5	1.2	2.37
	1	8.9	—
	2	0.71	1.06
	3	0.62	0.89
	4	0.49	0.73
	5	—	0.51
	6	0.19	—
	7	—	0.35
1	9	—	0.16
	11	—	0.13
	1	1.6	—
	2	10.8	—
	4	0.41	—
	6	0.37	—

This variation of the specific activity with increasing amounts of  $\text{AlCl}_3$  is curious, but the data are reproducible and therefore reliable. A tentative explanation is the following: since  $\text{T}[\text{Al}_2\text{Cl}_6\text{OH}]$  is a stronger acid than  $\text{T}[\text{AlCl}_3\text{OH}]$ , similar to  $\text{H}[\text{Al}_2\text{Cl}_7]$  and  $\text{H}[\text{AlCl}_4]$  <sup>(5)</sup>, the electrophilic tritiation should proceed farther for a molar ratio HTO: $\text{AlCl}_3$  of 1:2 than for 1:1. That

the tritiation also proceeded extensively for a ratio of 2:1 is less readily accountable.

Specific activities of uracil tritiated with varying molar ratios of HTO are presented in table 2. It can be observed that the specific activities obtained with 4 mmoles  $\text{AlCl}_3$ /mmole uracil decrease, whereas with 6 mmoles  $\text{AlCl}_3$ /mmole uracil they increase, with increasing amounts of tritiated water. The specific

TABLE 2. Specific activities of uracil (1 mmole) tritiated for 10 minutes in 8 ml  $\text{C}_2\text{H}_4\text{Cl}_2$  at  $80^\circ$  with HTO and  $\text{AlCl}_3$ .

HTO (mmoles)	$\text{AlCl}_3$ (mmoles)	Specific activity ( $\mu\text{Ci}/\text{mmole}$ )
0.25	1	0.24
0.5		8.9
1		1.6
2		1.2
3		0.80
4	4	0.45
0.5		0.49
1		0.41
2		0.37
0.5		0.19
1	6	0.37
2		0.51
3		0.57

activities at low and constant molar ratio  $\text{AlCl}_3$ /HTO are comparable ( $0.21 \mu\text{Ci}/\text{mmole}$  for 6 mmoles  $\text{AlCl}_3$ /3 mmoles HTO,  $0.19 \mu\text{Ci}/\text{mmole}$  for 4 mmoles  $\text{AlCl}_3$ /2 mmoles HTO), but at high molar ratio  $\text{AlCl}_3$ /HTO, the activities are markedly different, being lower for higher amounts of catalyst, in agreement with the results presented in table 1.

The effect of the simultaneous variation of the molar ratios of  $\text{AlCl}_3$ /uracil and HTO/uracil, at constant molar ratio  $\text{AlCl}_3$ :HTO equal to 2, is presented in

TABLE 3. Specific activity of uracil (1 mmole) tritiated in 8 ml dichloroethane at  $80^\circ$  for 10 minutes with various amounts of HTO and  $\text{AlCl}_3$ .

HTO (mmole)	$\text{AlCl}_3$ (mmole)	Specific activity ( $\mu\text{Ci}/\text{mmole}$ )
0.25	0.5	2.1
0.5	1	8.9
1	2	10.8
2	4	0.37
3	6	0.57

table 3. It can be seen that the highest specific activity of uracil was obtained with 0.5-1 mole  $\text{AlCl}_3$  and 0.25-0.5 mole HTO for 1 mole of uracil.

The course of these variations of the ratios HTO:substrate and  $\text{AlCl}_3$ :substrate is presented in figure 1. Dots indicate ratios for which activity measurements were performed, and circles indicate ratios leading to the highest specific activity. The numbers on the straight lines connecting the different dots correspond to the tables where the respective data are given.

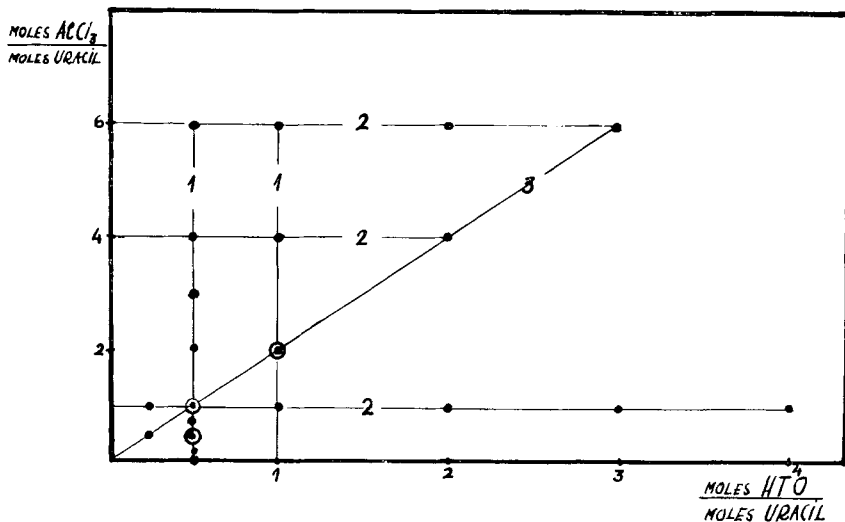


FIG. 1. — Variation of molar ratios  $\text{AlCl}_3$ :uracil and HTO:uracil in tables 1-3 (indicated by numbers on the lines). The location of highest specific activities is shown by circles.

The tritiation of glycine, di- and triglycine was selected because there is no possibility for racemization of the amino-acid residues. The strong acid causes partial hydrolysis of the di- and tripeptide, as shown by thin-layer chromatograms, but the recovery of the labelled product is over 75%. The reproducibility of the results is less satisfactory than in the previous cases, probably due to the exclusively heterogeneous exchange process, and to the high number of labile hydrogen atoms. Therefore, the effect of having increased the molar ratio of the  $\text{AlCl}_3$  catalyst with 1 mmole substrate and 0.5 mmole HTO was obscure and no definite trend was observable. For di- and triglycine the highest specific activities (0.7 and 0.85  $\mu\text{Ci}/\text{mmoles}$  respectively) were obtained with 4 mmoles  $\text{AlCl}_3$ . However, the average specific activities of the three compounds, shown in table 4, indicate that the number of tritium atoms per amino-acid residue is approximately constant in these conditions. The specific activities of glycyl-glycyl-glycine for 0.5, 1, or 2 mmoles of catalyst were lower than for glycine or glycyl-glycine, therefore these values were not taken into consideration in the calculation of average specific activities.

TABLE 4. Average specific activities of glycine, di- and triglycine (1 mmole) tritiated with 0.5 mmole HTO in 8 ml  $C_2H_4Cl_2$  at  $80^\circ$  for 10 minutes in the presence of various amounts of  $AlCl_3$ .

Compound:	Glycine	Glycyl-glycine	Glycyl-glycyl-glycine
mmoles $AlCl_3$ :	0.5-6	0.5-12	3-18
Average specific activities ( $\mu Ci/mmole$ ):	0.18	0.31	0.40

## DISCUSSION

The tritiation of uracil and adenine in these conditions very probably takes place by electrophilic substitution. Hence, decreased electron densities in the aromatic rings will result in lower specific activities. This explains the decreasing specific activities with higher amounts of catalyst (table 1).

The highest specific activities were obtained with molar ratios uracil: $AlCl_3$ :HTO of 1:0.25:0.5, 1:1:0.5 and 1:2:1, proving that the catalyst is not deactivated by coordination with the substrate.

The effect of varying the amount of tritiated water consist in: (i) up to 1 mole per mole  $AlCl_3$ , formation of  $T(AlCl_3OH)$ ; (ii) especially at high molar ratios HTO: $AlCl_3$ , hydrolysis of  $AlCl_3$ , with evolution of gaseous  $TCl$ , and hence loss of activities. With up to 1 mole HTO per mole  $AlCl_3$ , such hydrolysis is unfavourable because it would lower the coordination number of aluminium from 4 as in  $Al_2Cl_6$  to 3 as in  $Cl_2AlOH$ . Therefore most experiments were carried with a molar ratio  $AlCl_3$ :HTO of 2:1.

Optimum conditions for tritiation by this technique appear therefore to be those in which for one mole uracil, the amounts of  $AlCl_3$  and HTO are 0.25-1 mole and 0.5-2 moles respectively. These ratios lead to specific activities of ca.  $10 \mu Ci/mmole$ , i.e. to an uptake of tritium from HTO of ca. 0.2%. Most of the activity goes into the solvent ( $C_2H_4Cl_2$ ) which, as shown previously <sup>(2)</sup>, is involved in the tritiation process.

Notwithstanding the low tritium uptake, tritiation by means of HTO- $AlCl_3$  is a convenient and rapid procedure of labelling, at low specific activities, small amounts of aliphatic aromatic or heterocyclic compounds which are not decomposed by these reagents. It is not convenient for labelling aminoacids or polypeptides.

## EXPERIMENTAL

*Procedure.* Isotopic exchanges were run in flasks under conditions similar to those described previously <sup>(2)</sup>.

*Purification of the reaction products.* Uracil and adenine were filtered off after hydrolysis of the reaction mixture with ice-water. For the removal of inorganic salts, the products were extracted with hot water, then purified by TLC on silicagel G with 30% aqueous ethanol, using an UV light source for locating the spots. The compounds were eluted from the spots with hot ethanol, and labile tritium was removed by repeated (3-4 times) solvations in ethanol followed by evaporation and radioactivity measurement until a constant activity was attained. Glycine, glycyl-glycine (REANAL products) and glycyl-glycyl-glycine (NATIONAL BIOCHEMICALS CORPORATION) were tested by TLC for purity. After the exchange, they were hydrolyzed with ice-water, then passed on a column with DOWEX-50 for removing  $Al^{3+}$ . After concentration the crystallized products were purified by TLC on silicagel G with a mixture of *n*/butanol/water/acetic acid (3:1:1). The spots were located with ninhydrin, eluted with slightly acidified water, evaporated, and brought to constant activity by repeated exchange with ethanol.

*Radioactivity measurements* were made by combustion of the samples and reduction of the condensed water to hydrogen gas, which was measured in two gas counters compensated in length, and using as filling a gas mixture of cyclohexane and argon 1:10<sup>(6)</sup>.

The reproducibility of specific activities obtained on repeating the exchange of uracil or adenine is satisfactory (only the second decimal figure in tables 1 and 2 is affected). It is unsatisfactory with compounds from table 4 which do not reveal a regular pattern of specific activities *versus* the amount of  $AlCl_3$ .

#### REFERENCES

1. MĂNTEȘCU, C. and BALABAN, A. T. — *Canad. J. Chem.*, **41** : 2120 (1963).
2. MĂNTEȘCU, C., GENUȘCHE, A. and BALABAN, A. T. — *J. Labelled Compounds*, **1** : 178 (1965).
3. NENITZESCU, C. D., AVRAM, M. and SLIAM, E. — *Bull. Soc. Chim. Fr.*, 1266 (1955).
4. MILIOTIS, J. A. — *Compt. Rend.*, **245** : 1314 (1959).
5. BROWN, H. C. and BRADY, J. D. — *J. Am. Chem. Soc.*, **74** : 3570 (1952).
6. MĂNTEȘCU, C. and GENUȘCHE, A. — *Studii și Cercetări Chim., Acad. R. S. R.*, **13** : 1101 (1965).