Journal of Labelled Compounds — July-September — Vol. I, No. 3.

Tritiation of pyrimidines by HTO in the presence of aluminium chloride *

C. MANTESCU, A. GENUNCHE, A. T. BALABAN.
Institute of Atomic Physics, P. O. Box 35, Bucharest, Roumania.

Pyrimidine derivatives (pyrimidine, 4,6-dichloropyrimidine, uracil, cytosine and 4-chloro-6-hydroxypyrimidine) were tritiated at the ring by tritiated water in the presence of aluminium chloride in carbon disulphide or in ethylene dichloride (which is also tritiated in these conditions).

In continuation of previous investigations concerning the isotopic hydrogen exchange of pyridine and benzene derivatives with tritiated water [1] we studied the reaction of pyrimidines with HTO in the presence of aluminium chloride.

For one millimole pyrimidine derivative we employed one millimole anhydrous aluminium chloride and half a millimole tritiated water of specific activity $3.9.10^8$ disintegration/second · millimole. The exchanges were performed for various times at definite temperatures with 1,2-dichloroethane and carbon disulphide as solvents. After the prescribed times, the mixture was treated with an excess of ice + water; the organic layer was shaken several times with water in order to remove labile tritium; the solvent was then evaporated and the pyrimidine derivative was purified by recrystallization from dilute alcohol to constant activity (uracil, cytosine, 4-chloro-6-hydroxypyrimidine) or by distillation followed by recrystallization from benzene — ligroin (4,6-dichloropyrimidine). The unsubstituted pyrimidine was separated as the mercury chloride complex several times until a constant activity was reached. The purity of the tritiated compounds was checked by infrared spectroscopy and by inverse isotopic dilution.

The exchanges in dichloroethane at 80° C presented a puzzling maximum activity (Tab. 1), at 10-20 hrs.

Up to the activity maximum, the purity of the product is 100 % (by inverse isotopic dilution), but later, the products have only 70 % purity. A similar degradation of pyrimidine derivatives was observed with tritiated sulphuric

* Received on June 1st 1965.

acid at 20° C for 150 hrs [2]. The influence of substituents on the reactivity of the substrate may be seen by comparing the percentage of exchange after 5 hrs in dichloroethane at 80° C. The reactivity decreases in the following order :

Compounds	Specific activity (μC/mMole)
Uracil	56
Cytosine	56
4-Chloro-6-hydroxypyrimidine	20
Pyrimidine	16
4,6-Dichloropyrimidine	7.5

For longer reaction times, the activity of dichloropyrimidine increases more than that of pyrimidine. At 30° C, no maxima can be observed even for longer reaction times; no degradations of the compounds leading to lower purity are found.

TABLE I. Tritiation of pyrimidines (1 millimole) with 0.5 millimole tritiated water ($3.9.10^8$ disintegration/second.mMole) and 1 millimole $AlCl_3$, in 50 millimoles dichloroethane.

Compound	Temperature °C	Reaction time hrs	Specific activity dps/mMole
Uracil	30	24-144	$4.5.10^4$
	80	5	$2.1.10^6$
	80	10	$6.5.10^6$
	80	15	$5.3.10^6$
	80	20	$2.8.10^6$
	80	36	$1.6.10^6$
	80	48	$1.6.10^6$
4,6-Dichloropyrimidine	30	24-144	$5.7.10^4$
	80	5	$2.8.10^5$
	80	10	$9.8.10^5$
	80	15	$1.1.10^6$
	80	20	$2.6.10^6$
	80	36	$1.8.10^6$
	80	48	$1.8.10^6$
Pyrimidine	80	5	$6.0.10^5$
	80	10	$6.9.10^5$
	80	20	$8.9.10^5$
	80	30	$8.5.10^5$
	80	65	$8.5.10^5$

The pattern of the exchange from Table 1 made it plausible that a second process competes with the tritiation. It is plausible that this process is the tritiation of dichloroethane. Indeed, as shown in Table 2, dichloroethane is tritiated by HTO in the presence of aluminium chloride. Isotopic exchange between uracil and tritiated dichloroethane was also evidenced : starting from 1 millimole inactive uracil and 50 millimoles tritiated dichloroethane ($5.8.10^5$ dps/mMole) in the presence of 1 millimole aluminium chloride, about 72 % of the activity is transferred to the uracil after 5-12 hrs at 80° C. In order to account for the course of tritiations in Table 1, the rate of direct tritiation of dichloroethane by HTO + $AlCl_3$ must be lower than that of transtritiation of dichloroethane by tritiated pyrimidine. The degradation observed at longer times may also be related to the decreases of the specific activity.

In carbon disulphide, uracil is tritiated without any activity maximum (Tab. 3).

TABLE 2. Tritiation of 1 millimole dichloroethane with 0.5 millimoles tritiated water ($3.9.10^8$ disintegration/second.mMole) and 1 millimole $AlCl_3$.

Temperature °C	Reaction time hrs	Specific activity (dps/mMole)
30	24	$2.5.10^3$
80	7	$4.9.10^4$
80	14	$5.6.10^4$

TABLE 3. Tritiation of uracil (1 millimole) with 0.5 millimoles tritiated water and 1 millimole $AlCl_3$ in 50 millimoles carbon disulphide at reflux (46° C).

Reaction time hrs	Specific activity (dps/mMole)
7	$4.2.10^4$
10	$9.8.10^4$
17	$3.0.10^5$
25	$3.0.10^5$

A comparison of the present method for tritiating pyrimidine derivatives by isotopic exchange with previously described procedures using tritiated sulphuric [2] or acetic [3-5] acid (the latter in the presence of palladium) shows the feasibility of the method with simple reagents and short reaction times.

The variation of the amounts of reagents for increasing the yields is at present being investigated.

EXPERIMENTAL PART.

Exchange reactions were performed with magnetic stirring in double-walled Erlenmeyer flasks fitted through ground-glass joints with reflux condensers protected against atmospheric moisture. The constancy of the temperature was ensured by circulating thermostated water between the double walls of the flasks. The amounts of reagents specified in the tables were introduced into the flasks in the following order : pyrimidine, solvent, aluminium chloride and tritiated water. The following reagents were employed : sublimed anhydrous aluminium chloride; 1 2-dichloroethane and carbon disulphide distilled over $AlCl_3$, washed, dried and redistilled; uracil prepared from malic acid and urea in the presence of oleum [6], recrystallized from hot water, m.p. 320° C; 4,6-dichloropyrimidine (m.p. 64° C after distillation), prepared [7] from phosphorus oxychloride, dimethylaniline and 4,6-dihydroxypyrimidine; (Calculated for $C_4H_2Cl_2N_2$: C, 32.24; H, 1.35; N, 18.80; Cl, 47.60; Found : C, 32.11; H, 1.25; N, 19.18; Cl, 46.69) 4-chloro-6-hydroxy-pyrimidine, obtained [8] from the previous dichloropyrimidine by heating on the water bath with 3N hydrochloric acid, treating with ammonia, and reacidifying, m.p. 192-193° C (Calculated for $C_4H_3ClN_2O$: C, 36.8; H, 2.3; N, 21.5; Found : C, 36.9; H, 2.4; N, 21.5); pyrimidine, obtained [7] from the previous dichloropyrimidine by reduction on palladized charcoal (3 %), separated as the mercury chloride complex, and regenerated by distillation over sodium sulphide, b.p. 123.5-124° C, m.p. 21°-22.5° C.

After the prescribed reaction time, the mixture was poured into ice-water, and the organic layer was separated and shaken several times with water.

The purified compounds were converted into water by combustion. The tritiated water was exchanged with acetone at 0° C for one hour, and then the activity of the acetone was measured with a reproducibility of 2 % [9].

ACKNOWLEDGEMENTS.

The autors thank Dr. J. FILIP for gifts of pyrimidine derivatives and for discussions, and Mr. D. FARCASIU for helpful suggestions.

REFERENCES

1. MANTESCU, C. and BALABAN, A. T. — *Canad. J. Chem.*, **41** : 2120 (1963) .
2. FILIP, J., KOUTECKY, Z. and ZIGMUND, I. — Private communication.
3. CROWTER, D. G., EVANS, E. A. and LAMBERT, R. W. — *Chem. and Ind.*, **1960**, 899.
4. EVANS, E. A. and HANFORD, F. G. — *Nature*, **189** : 762 (1963).
5. EIDINOFF, M. L. and KNOLL, J. E. — *J. Am. Chem. Soc.*, **75** : 1992 (1953).
6. DAVIDSON, D. and BAUDISCH, O. — *J. Am. Chem. Soc.*, **48** : 2382 (1926).
7. HULL, R. — *J. Chem. Soc.*, **1951**, 2214.
8. BROWN, D. J. and HARPER, J. S. — *J. Chem. Soc.*, **1961**, 1298.
9. MANTESCU, C. and GENUNCHE, A. — *Rev. Roumaine Chim.* — In the press.