LABELLING WITH TRITIUM OF CERTAIN PYRIMIDINE DERIVATIVES BY DEIODINATION*

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A simple method for labelling of pyrimidine derivatives by tritium is described. The method essentially consists of heating the 5-iodopyrimidine in dimethyl sulfoxide containing 5% ^3H_2O. The specific activity obtained by this method is comparable with the specific activity of the product prepared by catalytic reaction.

During our studies on exchange reactions of 5-substituted pyrimidines we observed that the pyrimidine can be obtained in a medium of DMS* containing an aqueous solution of a halogenide. We have made use of this observation to obtain pyrimidines tritium-labelled at position 5. Unlike with the conventional methods of labelling pyrimidines at position 5 by exchange reactions using tritium gas[1-3], the chief advantage of the procedure described here is that it requires a considerably simpler equipment.

EXPERIMENTAL

5-IOA and 5-IUrd were prepared according to Prusoff[4], 5-IC according to Johnson and Johns[5], and 5-ICtd according to Chang and Welch[6]. 5-IUp was a generous gift of Dr A. Holý of this Institute. Tritiated water (3·15 Ci/ml) and DMS (b.p. 85°C at 25 Torr) were purchased from Sojuzchimexport, USSR. The deiodination of individual iodo derivatives was carried out in sealed glass ampules 1 ml in volume immersed in a Wood metal bath. For each experiment, always the equivalent quantity was taken of the corresponding iodo derivative (13·5 μmol, equal to 5 mg of 5-IUrd), dissolved in 0·5 ml of DMS containing a certain amount of water. The reaction mixtures were fractionated by descending chromatography on Whatmann No 3 in the following systems: *A* n-butanol saturated with water, *B* n-butanol–acetic acid–water (4 : 1 : 5), *C* 2-propanol–ammonia–water (7 : 1 : 2), *D* ethyl acetate saturated with 0·05M phosphate buffer at pH 6·0. The configuration of sugar component was established by electrophoresis using the apparatus

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** Abbreviations used: Urd, uridine; U, uracil; 5-IUrd, 5-iodouridine; 5-IU, 5-iodouracil; 5-IUp, 5-iodouridine 3′(2′)-monophosphate; 5-BrUrd, 5-bromouridine; Ctd, cytidine; 5-ICtd, 5-iodocytidine; C, cytosine; 5-IC, 5-iodocytosine; OA, orotic acid; 5-IOA, 5-iodoorotic acid; DMS, dimethyl sulfoxide.

of Markham[7] and 0·1M triethylammonium borate buffer at pH 7·5 and 27 V/cm, 1 h. The spectra were measured in Unicam SP 700 spectrophotometer. The radioactivity of the samples was determined in Tricarb 314 X scintillation spectrometer (Packard, USA).

RESULTS AND DISCUSSION

The investigation of the temperature dependence of the deiodination of 5-IUrd in DMS containing 5% of water showed that during 3 h 0·24 μmol of Urd (1·78%) was formed at 120°C, 0·64 μmol (4·7%) at 140°C, and 3·74 μmol (27·7%) at 160°C. The reaction was carried out also at 175°C, its yield, however, could not be evaluated because of the decomposition of the reaction components. For the investigation of the time dependence of the reaction, the temperature 160°C was chosen and the remaining conditions were left unaltered. The time profile of the deiodination (Fig. 1) points to 4 h as the most convenient period. The yield of Urd was 4·6 μmol, *i.e.* 34%. These experiments were carried out with nonradioactive water. The optimum quantity of water contained in DMS (Table I) appears to be 5 and 10%, respectively. Since the content of U-^{3}H in reaction mixture increases simultaneously with the increasing quantity of water in DMS, it is better to use the 5% concentration. The reaction was carried out also in different solvents conventionally employed for exchange reactions (acetic acid, dioxane, pyridine, and dimethylformamide). The effect of the presence of potassium fluoride, potassium chloride, and potassium hydroxide was also examined. Under these conditions, however, the results of the experiment were relatively worse. Deiodination reactions were investigated also with another compounds: 5-IU, 5-IOA, and 5-ICtd (Table II). The deiodination of 5-IUp, even under relatively mild conditions (100°C, 1 h), does not give rise to Up-5-^{3}H but results in dephosphorylation and 5-IUrd is formed.

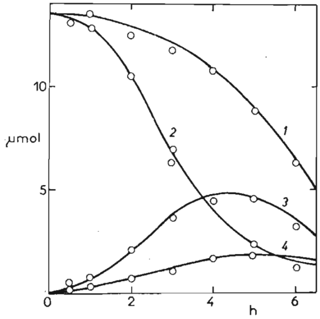


FIG. 1

Time Profile of Deiodination

1 IUrd + Urd + U, *2* IUrd, *3* Urd, *4* U. Temperature: 160°C; solvent: DMS containing 5% of water; chromatographic system: ethyl acetate saturated with phosphate buffer (0·05M, pH 6·0).

TABLE I

Effect of Quantity of Water in DMS on Deiodination of 5-IUrd

Conditions: 160°C; 4 h; 13·5 μmol of 5-IUrd.

% of water-^{3}H in DMS	water-^{3}H/5-IUrd μmol/μmol	Found in reaction mixture		
		Urd-^{3}H μmol	sp.a. μCi/μmol	sp.a. rel.[a]
1·25	25	4·05	8·3	0·15
5·00	100	4·60	16·5	0·29
10·00	200	4·75	15·9	0·28
20·00	400	4·72	15·9	0·28
30·00	600	4·65	16·4	0·29

[a] sp.a. rel. denotes the ratio of the specific activity of the product to the specific activity of the used water-^{3}H.

TABLE II

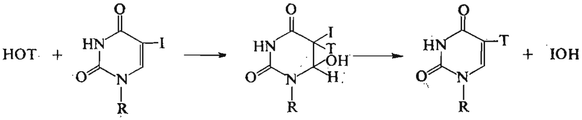
Results of Deiodination Reactions Carried out in Presence of Water-^{3}H under Optimum Conditions

Quantity of starting iodo derivative 13·5 μmol; solvent DMS containing 5% of water-^{3}H of 56·7 μCi/μmol.

Starting material	Temperature °C	Time h	Product	μmol	%	μCi/μmol	sp.a. rel.[a]
5-IUrd	160	4	Urd-5-^{3}H	4·60	34·0	16·5	0·29
			U-5-^{3}H	1·78	13·2	15·9	0·27
			5-IUrd	4·51	33·4	—	—
5-IU	160	4	U-5-^{3}H	5·96	44·0	23·9	0·42
			5-IU	5·02	37·1	—	—
5-IOA	110	2	OA-5-^{3}H	2·01	14·8	10·5	0·19
			U-5·6-^{3}H	0·55	7·4	33·9	0·60
			5-IU-6-^{3}H	7·88	58·1	17·8	0·31
			5-IOA	2·85	21·1	—	—
5-IC	140	4	C-5-^{3}H	7·07	52·1	13·5	0·24
			U-5-^{3}H	0·58	4·4	15·8	0·27
			5-IC	0·25	1·8	—	—
5-ICtd	140	2	Ctd-5-^{3}H	4·72	34·9	16·4	0·29
			C-5-^{3}H	3·52	26·0	16·9	0·30
			5-ICtd	0·67	4·9	—	—

[a] sp.a. rel. denotes the ratio of the specific radioactivity of the product to the specific radioactivity of the used water-^{3}H.

In case that merely one position has been labelled, the maximum value of the ratio of specific radioactivity of the obtained compound to the specific radioactivity of tritiated water is 0·5. The values of this ratio obtained by us (Table I and II) vary around 0·3, *i.e.* 60% of the theoretical value and are comparable to those obtained in certain catalytic exchange reactions of elemental hydrogen for halogen. The proof of the location of tritium at position of the pyrimidine ring was effected by bromination of Urd-5-^{3}H according to Wang[8] and following the known addition-elimination mechanism. We assume that the probably reaction scheme is as follows:



We have investigated to prove the intermediate stage by means of deuterated DMS on NMR spectra. The recorded value was, however, too small relatively to the record of deuterated water.

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