

NOTES

Labelling of Thioxanthine, Thioguanine and 2-Thio-Uracil with ^{35}S by Isotopic Exchange

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The labelling of TX and TG with ^{35}S has been performed by Moravek and Nejedly (¹), by isotopic exchange with elemental radiosulphur in boiling pyridine solution. The authors do not give any indications regarding the kinetics and the exchange yields. 2-Thio-uracil has been labelled only by synthesis in 1958 by Ziegler (²).

We have done this study, aiming to find some simpler labelling methods than those described in the literature.

1. — MATERIALS

TX and TG have been prepared by the reaction between xanthine and guanine, respectively, and P_2S_5 (^{3, 4}). Their purity was checked both by their melting points (325°C for TX, and 360°C for TG) and by ascendent paper chromatography (⁵).

The TU was a Mann Research Laboratory Inc. New York product.

The elemental ^{35}S was obtained from neutron irradiated KCl, according to our method (⁶). The naphtalene was qualitatively pure. The pyridine was dried over NaOH and freshly distilled. The other solvents have been also freshly distilled.

2. — METHODS

Labelling in solution.

In a first set of experiments we have studied the labelling of TX and TG with elemental ^{35}S in boiling pyridine as a function of time, by using the above mentioned method (¹).

The labelling of TU was tried, by isotopic exchange with ^{35}S in solution of different solvents (ethanol, dioxan) but the yields were too small (less than 5 %) so we abandoned this labelling method.

Labelling with molten ^{35}S .

We have tried the labelling of the three substances, by heating them together with elemental radiosulphur under vacuum, alone or in naphtalene mixture, a method which gave good results in other cases (⁷).

After heating for different periods of time, the labelled TX and TG have been separated from the mixture, purified, and their specific radioactivities measured. The ratio of the specific radioactivities, experimentally

found and calculated for equilibrium, represents the radioactive labelling yield.

The labelling of TU was studied with molten ^{35}S , alone and in presence of naphthalene, as melting medium.

RESULTS

Samples of 100 or 50 mg thioxanthine or thioguanine, with ^{35}S added in trichlorethylene solution, which was subsequently evaporated, were sealed under vacuum in glass vials and heated at 200°C for different periods of time, as indicated in Table I. The labelled substances have been separated from the elemental sulphur by dissolution in hot water, filtration and evaporation to dryness. After recrystallisation, the labelled substances were analysed by paper radiochromatography on Whatman 2 paper with *n*-butanol-water-concentrated ammonia (172 : 18 : 10) for TX and TG, and on Whatman 1 paper with *t*-butanol water (1 : 5) for TU, during 18 hrs. All compounds were found to be radiochemically pure. Radioactivities were measured by using a thin mica window (1.5 mg/cm^2) G. M. counter.

For the labelling of 2-thio-uracil we proceeded in the same manner as above, but adding 100 mg naphthalene to the mixture of 100 mg TU and 0,08 mg ^{35}S . The melting of TU with elemental ^{35}S without adding naphthalene, has given unsatisfactory results. We mention that the labelling in molten naphthalene is not a new technique, being also applied by other authors ^(8, 9).

TABLE I. Labelling of thioxanthine and thioguanine in pyridine solution at 116°C

Heating time h	Substance mg	³⁵ S added		Specific activity μCi/mg S		Exchange yield %
		mg	μCi	calculated for equi- brium	obtained	
<i>Thioxanthine</i>						
1	50	2.38	550	43	14.3	30
3	100	0.8	295	13.5	8.2	60
5	50	4.2	306	20.8	13.8	66
7	200	4.8	510	11	7.7	70
8½	50	4.2	306	20.8	1.4	7
<i>Thioguanine</i>						
1	50	3.96	705	52	8	15
3	148	1.4	534	12	5.1	42
5	50	4.2	306	22.1	12.2	56
6	160	1.7	413	14.5	9	62
7	100	2.6	590	27	6.5	24

(a) *Labelling in pyridine solution.*

The labelling results for TX and TG are shown in Table 1. One can see in this table that for TX, the maximum labelling yield was 70 % after a refluxing time of 7 hours, and 62 % for TG after 6 hours of refluxing.

For TU, the labelling yields were too small, less than 5 %.

TABLE 2. Labelling of thioxanthine and acetylthioguanine with molten ^{35}S at 200° C

Heating time h	Substance mg	³⁵ S added mg μCi		Specific activity μCi/mg S		Exchange yield %
				calculated for equi- brium	obtained	
<i>Thioxanthine</i>						
1	100	0.8	264	12.1	3.2	26
3	100	0.8	272	12.5	5.5	44
6	100	0.8	272	12.5	7	56
7	50	1.2	382	33	17.1	52
8	50	1.2	382	33	16.7	50
9	100	0.8	272	12.5	6.3	50
<i>Acetyl thioguanine</i>						
1	50	1.2	380	41.3	1.5	3.6
3	50	1.2	380	41.3	11.5	28
6	50	1.2	380	41.3	25	60
7	50	1.2	380	41.3	9	22

(b) *With ^{35}S in molten state.*

The results for TX and TG labelling are shown in Table 2. On labelling TX by this way, no anomalous behaviour was observed, but for TG the blackening of the mixture and the forming of a viscous product was noticed. From this product we have obtained very small quantities of labelled TG of low specific radioactivities (see Table 2). Supposing that the black colour and the viscous product were due to the reactivity of amino-group for sulphur, in another set of experiments we first protected this group by acetylation ⁽¹⁰⁾ and then the acetylguanine (ATG) was labelled with ^{35}S in molten naphtalene. After labelling, the ATG was saponified, and a ^{35}S -labelled thioguanine was obtained, having a specific activity lower by 30 % than that of ATG (see Table 3).

On trying to label the 2-thio-uracil with elemental ^{35}S in molten state without naphtalene, low labelling yields were obtained (less than 14 %), while

TABLE 3. ^{35}S thioguanine obtained from acetylthioguanine by saponification ^a

Specific activity $\mu\text{Ci}/\text{mg S}$		Yield %	
in ATG	in TG	from ATG	final
11.5	8.1	70.5	19.8
25.0	18	72	43.2
9	62	69	15.2

^a The final yield is calculated taking in account the initial ^{35}S activity, used for ATG labelling.

TABLE 4. Labelling of 2-thio -uracil with ^{35}S in molten naphtalene ^a

Heating time h	Specific activity $\mu\text{Ci}/\text{mg S}$		Exchange yield %
	calculated for equilibrium	obtained	
1	10.2	3	30
3	10.2	5	49
6	10.2	7.2	70
9	10.2	8.8	86
10	10.2	4.3	42
11	10.2	3.5	34

^a Heating temperature : 200° C.

Sample composition : 2-thio-uracil, 100 mg; ^{35}S : 0.8 mg of total activity 264 μCi ; naphtalene : 100 mg.

on heating in the presence of naphtalene, a much higher yield (86 %) resulted (see Table 4).

We mention the results from the four tables represent the mean of at least two experiments not differing with more than 2 %.

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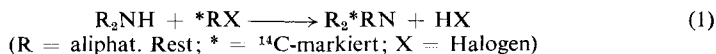
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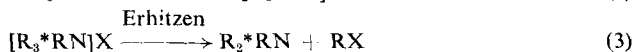
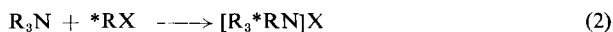
Zur ^{14}C -Markierung von tertiären aliphatischen Aminen durch Austausch von Alkylgruppen

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^{14}C -markierte tertiäre aliphatische Amine sind bereits mehrfach nach der folgenden allgemein anwendbaren Methode dargestellt worden (^{1, 2}) :



Neben dieser Alkylierung eines sekundären Amins mit einem markierten Alkylhalogenid ist auch die Reaktionsfolge



anwendbar (³). Während nach Reaktion (1) auch tertiäre Amine mit drei verschiedenen aliphatischen Gruppen gewonnen werden können, ist Reaktion (3) nur für tertiäre Amine mit drei gleichen Liganden brauchbar.

In der vorliegenden Untersuchung wurde die Frage geprüft, unter welchen Bedingungen die thermische Zersetzung eines quartären Ammoniumhalogenids vom Typ $[\text{R}_4\text{N}]\text{X}$ als Gleichgewichtsreaktion zur ^{14}C -Markierung von tertiären aliphatischen Aminen geeignet ist :



Das Salz $[\text{R}_3* \text{RN}]\text{X}$ kann dabei leicht nach Gl. (2) gewonnen werden.

Harmann, Stewart und Ruben (⁴) fanden, daß bei Raumtemperatur in den Lösungsmitteln Äthylalkohol und Benzol kein Austausch von Methylgruppen zwischen T-markiertem Tetramethylammoniumchlorid (bzw. -jodid) und überschüssigem Trimethylamin stattfindet. Das Gleichgewicht