Studies on Tritium-labeled Compounds. VI⁽¹⁾. *The Platinum-catalyzed Exposure Technique*

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The gas exposure technique developed by Wilzbach[2] has greatly simplified the labeling of many organic compounds, including such biologically active materials as insulin[3]. Its use is somewhat restricted, however, by the fact that multicuric quantities of tritium are required to obtain levels of activity, satisfactorily high for tracer experiments.

Up to the present, workers have tried to increase the labeling by adding to the system such external activation energy as is obtained from electric discharge[4-7], microwave discharge[7], ultraviolet irradiation[7], and γ-radiation[5]. These methods increase the specific activities remarkably, but they also produce a large amount of decomposition products. The aim of our experiments is also to obtain higher labeled products by the Wilzbach method but not by adding external activation energy. We have also tried to increase the efficiency of labeling by a larger exploitation of the β-ray energy of tritium.

Since the β-particle from tritium has a range of only 0.7 mg./cm^2, complete absorption of energy occurs within the layers of 7 μ if the organic material has a specific weight of 1 g./cm^3. Therefore, it is quite clear that labeling will occur at or near the surface of the organic compound.

The possibility of increasing the labeling efficiency by reducing the size of the particle of the organic compound is very limited, as Rosenblum et al.[8] have already shown.

Recently Wenzel et al[9] reported that higher specific activities in the range of 2∼130 fold were obtained by adsorbing the compound on charcoal. When hydrogenation catalysts were applied to a salicylic acid-T_2 system, a large increase in specific activity was observed. This paper presents the results of this new modification for labeling organic compounds by the Wilzbach procedure.

Experimental

Chemicals.—All chemicals used were of analytical reagent grade and were not subjected to any further purification.

Platinum black was prepared by hydrogenation of Adams platinum oxide[10] made from chloroplatinic acid.

Palladium black was prepared by hydrogenation of palladium oxide[11] made from palladium chloride. Raney nickel was developed from a Ni-Al alloy consisting of 50% nickel by the W-2 method. The tritium gas was purchased from the Radiochemical Center, Amersham, and was 98% T_2, the rest being H_2.

Exposure. — Glass ampoules with a volume of about 5 ml. and with a breakoff seal were used for exposure.

All the samples were thoroughly mixed with 100 mg. of the catalysts and then brought into a reaction vessel. The reaction vessel was connected with the apparatus described in the previous paper[1] and was throughly degassed by pumping.

After some hours of evacuation, about 100 mc. of tritium was transferred to the reaction vessel by means of the Toepler pump, and then the capillary was sealed off. After the exposure, the reaction vessel was remounted on the apparatus, the breakoff

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[1] Part V: Y. Sato, T. Takahashi and T. Meshi, *Radioisotopes*, **10**, 488 (1961).

[2] K. E. Wilzbach, *J. Am. Chem. Soc.*, **79**, 1013 (1957).

[3] C. Holt and L. Holt, *Naturwiss.*, **12**, 289 (1958).

[4] K. E. Wilzbach and L. M. Dorfman, *J. Phys. Chem.*, **63**, 799 (1959).

[5] F. L. Jackson, G. W. Kittinger and F. P. Krause, *Nucleonics*, **18**, 102 (1960).

[6] Y. Sato, T. Meshi, T. Takahashi and N. Sugimoto, *Japanese J. Pharm. and Chem.*, **32**, 317 (1960).

[7] T. Westermark, H. Lindroth and B. Enander, *Intern. J. Appl. Rad. Isotopes*, **7**, 331 (1960).

[8] C. Rosenblum and H. T. Meriwether, Proceedings of the Symposium on Advances in the Tracer Applications of Tritium (1958), p. 3.

[9] M. Wenzel and H. Wollenberg, Symposium on the Detection and Use of Tritium in the Physical and Biological Sciences, Vienna (1961).

[10] R. Adams, V. Voohees and R. L. Shriner, "Organic Syntheses", Col. vol. I, (1948), p. 463.

[11] R. L. Shriner and R. Adams, *J. Am. Chem. Soc.*, **46**, 1683 (1924).

seal was opened, and the tritium was transferred to a storage vessel.

Purifications. — The exposed products were dissolved in a hydroxylic solvent, and the catalyst was filtered off.

The solvent was removed from the filtrate by distillation in vacuo. This operation was repeated three times to remove the exchangeable tritium completely. The residue was recrystallized from a suitable solvent and radiochemically purified through paper-chromatography, using Toyo Roshi No. 526. L-Valine and thiamine were purified with *n*-butanol-acetic acid-water (4 : 1 : 1), inositol with *n*-butanol-ethanol-water (4 : 1.1 : 1.9), and predonisolone with toluene-methanol (3 : 1). Sublimation was chosen for purification of salicylic acid, and solvent extraction for that of benzoic acid. The radiochemical purity was established by cutting the chromatograms of the labeled compounds in 10 mm. segments, extracting each segment with distilled water or alcohol, and counting the tritium activity of each extract in a windowless gas-flow counter.

Measurement of Radioactivity. — The specific activities of the tritiated products were determined by liquid scintillation counting. An aliquot of a suitable concentration of a compound in a mixture of alcohol and water was added to toluene dissolving phenylbiphenyloxadiazole (4 g./l.) and 1,4-di(2,5-phenyloxazole)-benzene (100 mg./l.) and was counted with a Pachard Tri-Carb liquid scintillation spectrometer cooled to −10°C. This system had an efficiency of approximately 13% if the sample was nonquenching.

An internal standard was used to correct for quenching and to estimate the efficiency of counting.

Results

Listed in Table I are the results of the new method of labeling with tritium catalyzed by three different typical hydrogenation catalysts.

As is shown in Table I, the platinum catalyst is the most effective in promoting tritium labeling. With platinum oxide, there is also an increase in the specific activity, but the increase is much lower because most of the tritium is used to form the tritium oxide. The addition of a small amount of platinum oxide plus platinum, however, gave a greater

TABLE I. INCREASE OF THE SPECIFIC ACTIVITY BY THE CATALYZED EXPOSURE TECHNIQUE*

Compound	Catalyst mg.	Tritium gas mc.	Exposure time days	Specific activity μc./mg.	Increase of specific activity	Recovery %
Salicylic acid	Control	150	2	0.05	1	78
Salicylic acid	Charcoal (100)	150	5	0.06	1.2	53
Salicylic acid	Raney nickel (100)	150	2	0.06	1.2	59
Salicylic acid	Nickel oxide (100)	150	2	0.007		62
Salicylic acid	Pd black (100)	150	3	13.8	276	48
Salicylic acid	Pd–PdO (50 : 50)	150	3	20.0	400	49
Salicylic acid	Pt black (100)	150	5	108	2018	33
Salicylic acid	Pt–$PtO_2 \cdot H_2O$ (65 : 35)	150	5	146	2920	30
Salicylic acid	$PtO_2 \cdot H_2O$ (100)	150	5	1.0	20	54
Salicylic acid	Control	100	3	0.068	1	76
Salicylic acid	Charcoal (100)	100	3	0.090	1.3	49
Salicylic acid	Charcoal (200)	100	3	0.060		45
Salicylic acid	Pd black (100)	100	3	11.8	173	48
Salicylic acid	Pt black (100)	100	3	88	1290	37
Salicylic acid	Pt black (200)	100	3	128	1880	31
L-Valine	Control	100	20	0.30	1	80
L-Valine	Charcoal (100)	100	20	0.30	1	66
L-Valine	Charcoal (200)	100	20	0.26		63
L-Valine	Pd black (100)	100	7	0.57	1.9	72
L-Valine	Pt black (100)	100	20	8.6	29	62
L-Valine	Pt black (200)	100	20	6.2	21	59
Predonisolone	Control	100	22	0.40	1	67
Predonisolone	Charcoal (100)	100	22	0.28		63
Predonisolone	Pt black (100)	100	22	2.32	5.8	60
Benzoic acid	Control	100	5	0.10	1	74
Benzoic acid	Pt black (100)	100	5	21.0	210	53
Inositol	Control	100	4	0.24	1	68
Inositol	Pt black (100)	100	4	4.55	19	41
Thiamine	Control	100	7	0.95	1	68
Thiamine	Pt black (100)	100	7	3.05	3.2	56

* One hundred mg. of each sample was exposed to tritium gas.

TABLE II. SPECIFIC ACTIVITY OF TRITIUM IN LABILE AND NONLABILE POSITIONS IN SALICYLIC ACID AS A FUNCTION OF TIME OF EXPOSURE TO TRITIUM GAS*

Condition	Exposure time hr.	Total tritium incorporated *A*, μc./mg.	Increase of *A*	Nonlabile tritium *B*, μc./mg.	Increase of *B*	Survival *B*/*A*
Salicylic acid	1	5.4	1	0.041	1	0.76
+Pt black	1	660	122	100	2440	15.2
Salicylic acid	3	5.7	1	0.036	1	0.63
+Pt black	3	555	97	91	2530	16.4
Salicylic acid	10	5.9	1	0.024	1	0.42
+Pt black	10	426	72	84	3500	19.7
Salicylic acid	30	6.0	1	0.020	1	0.33
+Pt black	30	372	63	78	3900	21.0

* One hundred mg. of salicylic acid was exposed to about 100 mc. of tritium either in the presence or in the absence of the platinum catalyst.

TABLE III. EFFECT OF TEMPERATURE ON LABELING OF L-VALINE*

Platinum catalyst mg.	Exposure time days	Temperature °C	Specific activity μc./mg.	Increase of specific activity	Recovery %
	7	Room temp.	0.45	1	80
100	7	Room temp.	106	235	62
	4	80	0.58	1.3	68
100	4	80	2.25	5	58

* One hundred mg. of L-valine was exposed to about 100 mc. of tritium.

increase in specific activity than that of platinum alone. This is probably due to the fact that tritium gas is more readily adsorbed on platinum oxide than on platinum. The conditions employed and the results obtained in the labeling of six compounds by the catalyzed exposure technique are listed in Table I. The increase in labeling varies considerably with different compounds, but it is present in all of the compounds studied.

The aromatic compounds are more readily labeled than the aliphatic compounds. Table II shows the rates of the labeling of salicylic acid in the presence of or in the absence of the platinum catalyst.

In the absence of the catalyst, the total amount of tritium incorporated increases with the exposure time, while the amount of nonlabile tritium attached to the carbon atoms decreases with the time. In the presence of the catalyst, both the total amount of tritium (*A*) and the amount of nonlabile tritium (*B*) decrease with the exposure time beyond one hour.

These results indicate that the exposure for a longer time greatly increases the radiation damage in salicylic acid and so that the highest specific activity is obtainable when exposure is less than one hour. This is in contradiction to the report of Rydberg et al.[12] that the specific activity generally increased with the exposure time when *p*-aminosalicylic acid was exposed to tritium gas.

The data in Table III show the effect of temperature on labeling.

The amount of labeling increases with the exposure temperature without the catalyst, but in the presence of platinum the increase in the specific activity at 80°C is much lower than that at room temperature. Probably the amount of the adsorption of tritium gas on the platinum catalyst is substantially dependent on the temperature.

Discussion

The favorable effect of the platinum catalyst in the system is obviously related to the properties of platinum, as is shown in Table I. One probable effect of the platinum addition is to increase the absorption of β-ray energy from tritium in the system by making the contact closer between tritium and organic molecules on the catalyst surface by chemisorption.

The exchange reaction between tritium and hydrogen atoms of organic molecules is undoubtedly induced by the radioactive decay of the tritium and by the beta radiation associated therewith, as is shown below[13]:

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[12] J. Rydberg and A. Hanngren, *Acta Chem. Scand.*, **12**, 332 (1958).

[13] T. H. Pratt and R. Wolfgang, *J. Am. Chem. Soc.*, **83**, 10 (1961).

$$T_2 \xrightarrow{-\beta^-} HeT^+ \qquad (1)$$

$$RH \rightsquigarrow \text{Ions and excited species} \qquad (2)$$

$$T_2 \dashrightarrow \text{Ions and excited species} \qquad (3)$$

where RH is the organic compound being irradiated.

If the tritium is to be used efficiently without adding external activation energy to the system, it is necessary to promote such ionization and excitation reactions as 2 and 3 by completely absorbing the decay energy in the system and also to increase the probability of reactions between HeT^+ and the organic compound.

When tritium and an organic compound are sealed in an ampoule without a catalyst, the beta energy is dissipated by the formation of ions and of excited molecules of both constituents and by the loss due to the absorption to the wall.

When the catalyst is present in the system, the wall loss should be small; the decay energy would be spent efficiently in the formation of the ions and of the excited molecules by the chemisorption of tritium on the catalyst.

The chemisorption of molecular tritium and organic molecules on the surface of the catalyst also increases the probability of reactions between HeT^+ and organic molecules.

Another effect of platinum addition may be seen in the catalytic exchange of hydrogen atoms, an exchange which has been employed for the preparation of tritium-labeled compounds using tritium oxide[14]. Farkas et al.[15] have already demonstrated the catalytic exchange of hydrogen atoms between molecular tritium and the nonpolar solvent, cyclohexane.

In the absence of a catalyst, the ratio of the total tritium to the nonlabile tritium attached to carbon atoms was less than one per cent, while in the presence of the catalyst, the B/A ratio increased to more than 15%, as is shown in Table II.

If the labeling is promoted only by increased absorption of the decay energy in the system, the B/A ratio must be independent of the presence of the catalyst.

Therefore, the great increase in the B/A ratio by platinum addition should be attributed to the catalytic exchange of hydrogen atoms, which presumably occurs through CH bond rupture. The relative amount of exchange for aliphatics is lower than for aromatics, as is shown in Table I. The observed tendency in the relative amount of exchange may be attributed to different strengths of chemisorption and to the fact that chemisorption of unsaturated hydrocarbons on a platinum catalyst occurs through π-complex formation.

It may be considered that chemisorption involving aliphatic compounds is relatively weak, or, alternatively, that strong chemisorption may occur only at very active, and hence less abundant, catalyst sites. In the case of salicylic acid, doubling the amount of platinum in the system increases the specific activity, but in that of valine, an increase in the amount of catalyst causes no increase in tritiation.

Furthermore, there is apparently an optimum concentration of platinum beyond which an increase in the amount of catalyst reduces the specific activity of the compound. The decrease in specific activity with the increase in platinum concentration beyond an optimum can be explained on the basis of an increasing probability of the ion neutralization and of the disappearance of the excited molecules before an effective collision (one leading to labeled-product formation) can occur.

On the other hand, charcoal also adsorbs the organic compounds, and so an increase in labeling is expected. However, a drop in the specific activity was observed when the amount of charcoal was doubled.

It could be that a part of the β-ray energy is used in vain to ionize and excite the charcoal, because the tritium gas is not adsorbed on charcoal at room temperature[9], and that consequently the efficiency of the labeling decreases.

The data in Table III indicate that the chemisorption of tritium gas on the catalyst is a very important factor in promoting the labeling. The addition of the platinum catalyst appears to promote the radiolysis of organics, but the amount of radiolysis by-products is not very large, as is shown in the recovery column of Table I.

Salicylic acid became somewhat brownish in color after 3 days of irradiation, and only 31 mg. of a radiochemically pure product could be obtained. This recovery of 31% is presumably due to the increased radiation decomposition caused by the more intense irradiation.

The low recovery in the presence of charcoal can be ascribed to the incomplete elution of the charcoal-adsorbed compound, because only about 70% of the compound used was recovered when the test blank of elution was carried out with 20 ml. of alcohol. The platinum-addition method has some advantages over the original exposure technique in that the amount of tritium required is reduced and in that the time required for labeling becomes substantially

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[14] J. L. Garnett, L. J. Henderson and W. A. Sollich, Symposium on the Detection and Use of Tritium in the Physical and Biological Sciences, Vienna (1961).

[15] A. Farkas and L. Farkas, *Trans. Faraday Soc.*, 35, 917 (1937).

shorter—a few day by this new technique as compared to a few weeks by the Wilzbach procedure for obtaining the same amount of activity.

Summary

To increase the efficiency of labeling according to the Wilzbach procedure by absorbing the β-ray energy of tritium in the system, three typical hydrogenation catalysts were applied to the salicylic acid-T_2 system.

The platinum catalyst was found to be most effective in promoting the labeling. The results obtained in labeling six compounds by the platinum-catalyzed-exposure technique showed that by this procedure higher specific activities in the range of 3～1880 fold were obtained.

The increase in labeling changed considerably with different compounds, and the relative amount of labeling for aromatics was higher than for aliphatics.

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