Studies of Tritium-labeled Compounds. VII.⁽¹⁾

The Platinum-catalyzed Exposure Reaction

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In the gas exposure technique developed by Wilzbach⁽²⁾ only a small part of the tritium gas used is actually incorporated into organic compounds, and relatively large quantities of tritium gas and prolonged exposure are required to supply sufficient energy to the system. In a previous work,⁽¹⁾ it was found that the platinum catalyst was the most effective in the promotion of labeling, and it was suggested, on the basis of the large increase in the ratio of nonlabile tritium to labile tritium in salicylic acid in the platinum-catalyzed labeling, that this catalytic action probably is due to the catalytic exchange of hydrogen atoms rather than to the increased absorption of the decay energy in the system. It is evident that tritium gas is adsorbed on the surface of the platinum catalyst, but the question remains of whether or not a similar adsorption of solid organic compounds occurs on the catalyst surface. The fact⁽¹⁾ that the aromatic compounds are usually more efficiently labeled than the aliphatic compounds may be accounted for by assuming that the former is more readily chemisorbed on the catalyst surface through such an interaction as the π-complex formation. In order to elucidate the nature of the chemisorption and to obtain some information on possible reaction paths, it seems useful to investigate the effects of the substituents upon the hydrogen-tritium exchange of the aromatic ring and the tritium distributions within aromatic compounds labeled by different methods. Salicylic acid and benzoic acid were chosen as suitable materials for the determination of tritium distribution. The relative amounts of ring tritiation at the *o*-, *m*- and *p*-positions of salicylic acid were determined by the bromination of the recovered salicylic acid, while those of benzoic acid were determined by first converting it into aniline, which was then brominated to give bromination products, whose tritium activities were determined.

Benzoylvaline was used to determine the relative exchange rates of labile, semilabile and nonlabile hydrogen atoms.

Experimental

Samples. — All the chemicals used were of analytical reagent grade and were not subjected to any further purification. Platinum black was prepared by the hydrogenation of Adams platinum oxide. The tritium gas was purchased from the Radiochemical Centre, Amersham.

Exposures. — Exposures were carried out by the same procedure as was described in the previous paper.⁽¹⁾ Since it was found that the highest specific activity was obtainable when the exposure time was less than one hour, all the reactions were performed for one hour at room temperature.

Purifications. — Radiochemical purifications of benzoic acid and salicylic acid have been described in the previous paper. *p*-Aminobenzoic acid and *p*-aminosalicylic acid were purified according to

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1) Part VI: T. Meshi and T. Takahashi. This Bulletin, 35, 1510 (1962).

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

the solvent extraction procedure of Rydberg and Hanngren.³⁾ Phthalic acid, naphthalene and benzoylvaline were diluted with their respective untritiated compounds and recrystallized from a mixture of ethanol and water until constant specific activities were obtained. Benzene was converted into *m*-dinitrobenzene, which was then purified by repeated recrystallization from ethanol to a constant specific activity. Dimethylaniline was converted into its picrate.

Degradations. — The successive reaction steps used for the determination of tritium distributions of salicylic acid and benzoic acid are as follows:

Salicylic acid $\xrightarrow{Br_2}$ 5-Bromosalicylic acid $\xrightarrow{Br_2}$
3,5-Dibromosalicylic acid

Benzoic acid $\xrightarrow{SOCl_2}$ Benzoyl chloride $\xrightarrow{NH_4OH}$
Benzamide $\xrightarrow{NaOBr}$
Aniline $\xrightarrow{(CH_3CO)_2O}$ Acetanilide $\xrightarrow{Br_2}$
$\xrightarrow{Br_2}$ Tribromoaniline
p-Bromoacetanilide

The scheme used for benzoic acid was used earlier by Bonner⁴⁾ in the determination of the distribution of deuterium.

Benzoylvaline, the labile tritium of which had been removed by treating it with a mixture of water and ethanol, was treated with an alkaline solution to remove the semilabile tritium incorporated at the amide group; then it was hydrolyzed into benzoic acid and L-valine by refluxing it with 6 N-hydrochloric acid.

Tritium Assay. — The tritium activity of each compound was assayed by liquid scintillation counting. An aliquot of a suitable concentration of a compound in ethanol was added to toluene containing 2,5-diphenyloxazole (4 g./l.) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (100 mg./l.), and the solution was counted with a Pachard Tri-Carb liquid scintillation spectrometer cooled to $-10°C$. The efficiency of counting was usually about 13% if the sample was nonquenching. An internal standard consisting of 0.1 ml. of benzene-^3H (2.3×10^5 d. p. m.) was used to correct for the quenching loss and to estimate the efficiency of counting.

Results and Discussion

The data in Table I show the effect of the substituents on the tritium labeling of the aromatic compounds. It may be seen that the amount of nonlabile tritium attached to carbon atoms varies considerably with the nature of the substituents and that the amount of tritium incorporated into the aromatic nucleus is usually small in the substituted compounds, except for salicylic acid. When one compares the amount of tritium incorporation in phthalic acid and phthalic anhydride, one finds that not only the substituents but also the presence of labile hydrogen atoms attached to oxygen or nitrogen substantially lower the amount of tritium incorporation into the aromatic nucleus. If one assumes that the catalytic activity of the platinum catalyst is a consequence of its unfilled d-orbitals and that the chemisorption of the aromatic compounds on a platinum catalyst occurs through the transfer of the π-electrons of the aromatic compounds into the empty d-orbitals of the catalyst, one would expect that the substituents which have higher electron densities than the aromatic rings would monopolize the active centers of the catalyst and that, consequently, the chemisorption of the aromatic ring on the platinum catalyst would be decreased, thus lowering the amount of tritium incorporation into the rings. However, it can not be explained why only the hydroxyl group promotes the isotope exchange of the aromatic ring, as is shown in an experiment carried out with salicylic acid. Garnett et al.⁵⁾ studied the catalytic exchange of hydrogen atom using deuterium oxide and concluded that the stronger the chemisorption, the greater the tendency for a compound to poison the catalyst and, consequently, for the exchange to be less efficient.

The competitive incorporation of tritium was performed with a mixture of benzene with another aromatic compounds, using the amount of the tritium incorporation in benzene as the standard. When one compares the specific activities in Table I and Table II, one finds that the tritium incorporation of benzene decreases greatly in the presence of dinitrobenzene, phthalic anhydride, salicylic acid and dimethylaniline, and that of these compounds, except for dimethylaniline, also does in the presence of benzene. These results are in direct contrast to those obtained with the mixture of benzene and cyclohexane, which has only a very small retarding effect on the tritium incorporation of benzene. (Incidentally, the platinum-catalyzed tritium incorporation of cyclohexane is relatively low.)

It is evident from these results that the bond strength of the chemisorption and the π-complex formation are important factors in the determination of the extent of isotope exchange. It may be considered that the amino group and the nitro group are so strongly chemisorbed on the catalyst surface that the specificity of the catalyst is lost, while the hydroxyl group is relatively weakly chemisorbed so as to cause a more facile chemisorption of the aromatic

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

4) W. A. Bonner, *J. Am. Chem. Soc.*, **79**, 2469 (1957).

5) 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).

TABLE I. EFFECT OF SUBSTITUENTS ON TRITIUM LABELING OF AROMATIC COMPOUNDS*

Compound	Time hr.	Catalyst mg.	Labile tritium A μc./μM	Nonlabile tritium B μc./μM	Increase of a) specific activity	Ratio of B to A %
Benzene	1	50		15.8	298.0	
Naphthalene	1	50		4.9	76.2	
Dimethylaniline	1	50		1.34		
Dinitrobenzene	1	50		0.59	8.1	
Phthalic anhydride	1	50		9.7	212.0	
Phthalic acid	1	50	50.2	1.27		2.6
Benzoic acid	1	50	44.3	2.31	40.3	5.2
p-Aminobenzoic acid	2	100	71.0	0.21		0.30
Salicylic acid	1	50	95.0	14.1	596.0	14.8
p-Aminosalicylic acid	2	100	63.5	0.07		0.10
5-Bromosalicylic acid	2	100	77.6	0.04		0.06

* One hundred milligrams of each sample was exposed to about 100 mc. of tritium gas.

a) An increase in specific activity compared with gas exposure experiment.

TABLE II. RETARDATION EFFECT OF CATALYST POISONS UPON THE H-T EXCHANGE OF BENZENE*

Reaction mixture	Catalyst mg.	Tritium gas mc.	Quantity mmol.	Specific activity μc./μM	Comparison a) of specific activities %
Benzene	50	100	1.30	1.36	8.6
Dinitrobenzene			0.65	0.08	14.0
Benzene	50	100	1.30	3.52	22.2
Dimethylaniline			0.65	1.33	99.2
Benzene	50	100	1.30	6.10	38.4
Phthalic anhydride			0.65	0.22	2.28
Benzene	50	100	1.30	1.50	9.5
Salicylic acid			0.65	0.02 b)	0.14
Benzene	100	200	2.60	14.50	91.5
Cyclohexane			2.60	0.26 c)	—

* All reactions were performed at room temperature for one hour.

a) The values in this column represent the ratio of the specific activities in Table II to those in Table I.

b) This value is the amount of the nonlabile tritium attached to carbon atoms but that of the labile tritium is 70.8 μc./μM.

c) This value was obtained when cyclohexane alone was exposed to the tritium gas in the presence of the catalyst.

nucleus by increasing the electronic density of the aromatic ring. The unexpectedly large decrease in the incorporation of tritium into salicylic acid in the presence of benzene can be explained on the basis of the increased probability of the hydroxyl group of the salicylic acid dissolved in benzene being chemisorbed, which would result in the decreased chemisorption of the nucleus of salicylic acid and the predominant exchange of the labile hydrogen attached to oxygen by the tritium present in the adsorption layer, for the amount of the labile tritium incorporated into salicylic acid is as large as 70.8 μc./μM.

The tritium distribution in benzoic acid in salicylic acid treated with tritium gas was determined by measuring the specific activities of the products derived from their acids; they are shown in Table III and Table IV. It may be seen that, in the gas-exposure experiments, i. e., in the absence of platinum black, the ortho hydrogens exchange at a faster rate than do those of the meta and para positions since salicylic acid has two hydrogens at the meta position but only one at the ortho position.

This is in agreement with the result obtained with benzoic acid,6) toluene,7) chlorobenzene8) and nitrobenzene.8) It may be of interest that, in the gas-exposure technique, the ortho position is always favored. On the other hand, the tritium distribution in benzoic acid and in salicylic acid labeled by the catalyzed exposure method indicates that the predominant exchange occurs at the meta positions of benzoic acid and at the para position to the

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6) K. E. Wilzbach, "Proceedings of the Symposium on Tritium in Tracer Applications" (1957), p. 3.

7) H. J. Ache. W. Herr and A. Thiemann, "Symposium on the Detection and Use of Tritium in the Physical and Biological Sciences," Vienna (1961).

8) F. Cacace, *Int. J. Appl. Rad. Isotopes*, **8**, 82 (1960).

TABLE III. THE DISTRIBUTION OF TRITIUM IN BENZOIC ACID

Method	Labile tritium μc./mg.	Nonlabile tritium μc./mg.	Ortho %	Meta %	Para %
Gas exposure	62.5	0.47	58.3	21.3	20.4
Catalyzed exposure	382.3	18.90	5.0	72.5	22.5

One hundred milligrams of benzoic acid was exposed to 100 mc. of the tritium gas for one hour in the presence of 50 mg. of platinum black.

TABLE IV. THE DISTRIBUTION OF TRITIUM IN SALICYLIC ACID

Method	Labile tritium μc./mg.	Nonlabile tritium μc./mg.	Ortho to OH %	Meta to OH %	Para to OH %
Gas exposure	115.6	0.17	39.0	48.4	13.6
Catalyzed exposure	792.0	102.0	16.9	31.7	51.4

One hundred milligrams of salicylic acid was exposed to 100 mc. of the tritium gas for one hour in the presence of 50 mg. of platinum black.

TABLE V. THE AMOUNT OF TRITIUM IN VARIOUS POSITIONS IN BENZOYLVALINE LABELED BY THE DIFFERENT METHODS*

Method	Time hr.	Labile tritium μc./mg.	Semilabile tritium μc./mg.	Nonlabile tritium μc./mg.	Tritium in ring %	Tritium in side-chain %
Gas exposure	1	77.835	0.015	0.15	92.5	7.5
Catalyzed exposure	1	286.26	8.47	0.27	60.2	39.8

* One hundred milligrams of benzoylvaline was exposed to 100 mc. of the tritium gas for one hour in the presence or in the absence of 100 mg. of platinum black.

hydroxyl group of salicylic acid. From the observed tendency in the tritium distributions, it may well be assumed that, in the catalyzed exposure, the hydrogen-tritium exchange in the aromatic ring may follow the substitution rule for electrophilic aromatic exchange. In the gas exposure method, the reactive tritium species are the energetic tritium ions produced by the radioactive decay of the tritium and by the beta radiation,[9] while in the catalyzed exposure it seems to be a tritium ion or atom formed by the dissociation of the tritium molecule on the catalyst surface. Therefore, it would be natural to expect that the tritium arrangements in benzoic acid and salicylic acid would be different between the gas exposure and the catalyzed exposure, and that, in the catalyzed exposure, just as in the chemical substitution reaction, the reactive tritium ion would be directed in this order: meta>para>ortho in benzoic acid and para>ortho>meta in salicylic acid.

The distribution of tritium in benzoylvaline labeled by both the methods is shown in Table V. It is noticeable that the amount of the semilabile tritium incorporated into the amide group varies remarkably with the different methods. In the catalyzed exposure experiment, the amount of the semilabile tritium becomes much larger than that of the nonlabile tritium, although in the gas exposure experiment the amount of the semilable tritium is less than that of the nonlabile tritium. This also seems to suggest that the chemisorption of the benzoylvaline molecule on the platinum catalyst predominates at the amide group.

As Table V shows, the amount of tritium exchange is the largest for labile hydrogen, intermediate for semilabile hydrogen, and the smallest for nonlabile hydrogen. In other words, the more readily a hydrogen dissociates, the more rapidly it undergoes the exchange with tritium.

Summary

In order to elucidate the nature of the chemisorption in the platinum-catalyzed exposure reaction with tritium, the effects of the substituents upon the hydrogen-tritium exchange of the aromatic ring and upon the tritium distribution in benzoic acid, salicylic acid and benzoylvaline have been investigated. All the substituents except the hydroxyl group decrease the hydrogen-tritium exchange of the aromatic ring, and the compounds carrying those substituents poison the catalyst for the tritium incorporation of benzene. This retardation effect of the substituents upon the

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

hydrogen-tritium exchange of the aromatic nucleus may perhaps be attributed to the preferential adsorption of the substituents on the catalyst surface. The tritium distribution in benzoic acid and salicylic acid suggests that the hydrogen-tritium exchange in the aromatic ring may follow the substitution rule for electrophilic aromatic exchange. The relative hydrogen-tritium exchange rates of benzoylvaline show that the more readily a hydrogen dissociates, the more rapidly it undergoes the exchange.

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