

T. C. in Acidic Media

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SUMMARY

Tritium labelling of aromatic compounds in a few acidic media and the intramolecular distributions of radioactivities in the tritiated compounds were investigated. Various kinds of aromatic compounds were treated with powerful acid complex, $TH_2PO_4 \cdot BF_3$, or some other tritiated acids at room temperature. Aromatic compounds having electron-donating substituents were easily labelled with tritium and the radioactivity in each compound was predominantly found at the ortho- and para-positions of aromatic ring. Meanwhile, substituents which withdraw electrons from the aromatic ring inhibit the incorporation of tritium. Benzene and naphthalene were also labelled with tritium only when they were treated with $TH_2PO_4 \cdot BF_3$. This method was, however, not available for the nitrogen containing compounds. The reaction mechanism was also discussed.

INTRODUCTION.

The general procedures for the preparation of labelled organic compounds with tritium may be separated into four categories ⁽¹⁾, Recoil labelling, Isotope exchange reactions (Gas exposure and Catalytic exchange in solution), Direct chemical synthesis and Biochemical methods.

The reaction of recoil tritium atom with organic compounds has been investigated in the field of hot atom chemistry ⁽¹⁾, but this technique is seldom used for preparative labelling with tritium because of the extensive radiation damage during the irradiation and of the formation of by-products at high specific activity.

The tritium gas-exposure method called "Wilzbach labelling" ⁽²⁾ consists of allowing a compound to contact with tritium gas for a number of days or weeks. Although much less radiation damage is induced to the compound in this method than in recoil labelling, vigorous purification is generally required to obtain a radiochemically pure parent compound.

Synthesis by direct chemical methods is the only practical approach for obtaining the compounds with specific labelling and is particularly valuable when high specific activities are required.

Biochemical methods have not yet proved very useful with a few specific exception.

Meanwhile, it is well known that certain aromatic compound dissolved in a tritiated solvent, for example tritiated water, in the presence of an acid catalyst are subjected to the isotopic exchange between tritium atoms of the solvent and hydrogen atoms in the compound. The exchange occurs almost instantaneously on the labile hydrogen atoms attached to nitrogen, oxygen or sulfur atom in the compound and deliberately on the other non-labile hydrogen atoms at the ambient temperature. The exchange ratio of hydrogen in these non-labile positions is known to be much affected by both substituents on the aromatic ring of the compounds and the acidity of the solvents.

Recently, the new tritium labelling method in which powerful acid complex $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ is employed as a catalyst has been proposed by Yavorsky and Gorin⁽³⁾ and extended by Hilton and O'Brien⁽⁴⁾ and Evans⁽⁵⁾. The method is very simple and does not produce substantially unwanted by-products at high specific activity as in the recoil reaction or the Wilzbach technique. Therefore this conventional acid-catalyzed exchange reaction can be regarded as the most useful and simple labelling technique.

In the present studies, the acid-catalyzed hydrogen exchange reactions of a number of organic compounds have been investigated in detail. The tritiated phosphoric acid-boron trifluoride complex and a few other tritiated acids were employed as the tritiating reagents. Furthermore, a suitable degradation scheme was utilized to determine the intramolecular distributions of tritium in a few compounds labelled. The exchange mechanism in this reaction is discussed.

EXPERIMENTAL.

(a) *Materials.*

Tritiated water was purchased from the Radiochemical Center, Amersham, and boron trifluoride was supplied by the Matheson Co.

(b) *Tritiating reagents.*

Tritiated phosphoric acid-boron trifluoride complex;

Phosphoric anhydride (0.3 mol) was weighed quickly into a 200 ml flask, and a rubber top was snapped into place. The flask was cooled in an ice-water, and tritiated water (0.9 mol) was carefully added to it with a syringe through the rubber top. The mixture was stirred with magnetic stirrer until the water had taken up all the P_2O_5 . The specific activity of the phosphoric acid was 1.32×10^7 dpm/g.

Boron trifluoride was introduced slowly into tritiated phosphoric acid while stirring was continued, until the heavy liquid was thoroughly saturated. The specific activities of the complex used in the following experiments were (a) 7.8×10^6 dpm/g (1.3×10^9 dpm/mol) or (b) 4.45×10^6 dpm/g (7.38×10^8 dpm/mol).

Tritiated perchloric acid and tritiated hydrochloric acid;

These acids were prepared by addition of a drop of tritiated water of high specific activity onto hundreds ml. of 70 % HClO_4 and concd. HCl , respectively. The specific activities of these acids were $\text{HClO}_4\text{-THO}$, 3.5×10^9 dpm/g, and HCl-THO , 2.62×10^9 dpm/g, respectively.

(c) *Method of tritiation.*

Compounds that are solid at room temperature (0.075 mol.) were dissolved in 50 g of cyclohexane, and phenol, diphenyl ether and bromobenzene (0.15 mol) were dissolved in 10 g of cyclohexane. Another liquid compounds (0.15 mol) were subjected to tritiation without adding solvent.

In each reaction, 58.8 g of TH_2PO_4 , 20 g of concd. HCl-THO , 20 g of 70 % $\text{HClO}_4\text{-THO}$ or 99.6 g of $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ was used as a tritiating reagent.

The reaction mixture containing one of the above substrates and one of the tritiating reagents was stirred using a teflon-coated magnetic stirrer in a 200 ml flask at room temperature for certain hours. These reactions were carried out heterogeneously because all these substrates were insoluble in the reagents.

After the reaction was stopped, the content in the flask was transferred to a separatory funnel, and the reagent layer was removed from the reaction mixture. In the case of $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ complex, a small amount of pyridine was added onto the substrate layer to remove the excess of BF_3 containing in the layer. The layer was washed with water and then dried over anhydrous sodium sulfate. After solvent was removed under reduced pressure, the product was purified by redistillation or recrystallization from methanol until constant specific radioactivity was indicated.

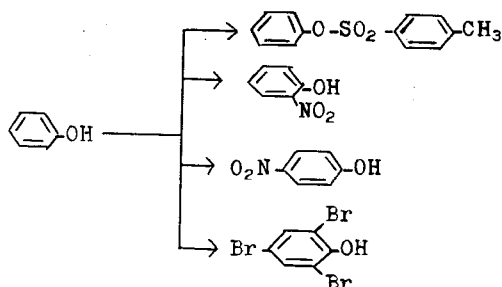
(d) *Degradations of tritiated compounds.*

The tritium-labelled phenol, anisole and naphthalene were converted to tosyl, nitro- and bromo-derivatives respectively, by the usual method, and the toluene was degraded by Bonner's method ^(6,7). The reaction schemes are shown in Schemes 1-4.

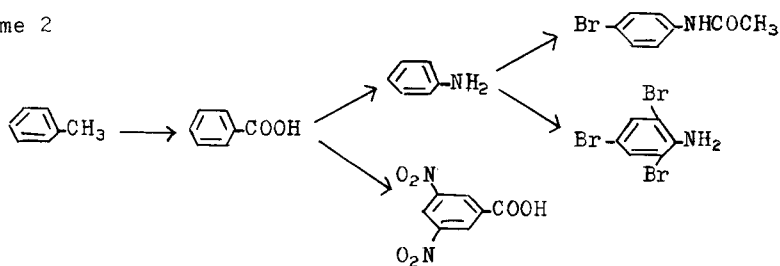
(e) *Tritium assay.*

Scintillator solution for tritium assay of labelled compounds was prepared by dissolving 4 g of PPO. and 0.1 g of POPOP. in 1/liter of toluene. About 10 mg of tritium-labelled organic compound was exactly weighed into the glass vial, and 20 ml of the toluene scintillator described above was added onto it. In the case of the assay of tritiating reagents, 2 ml of hyamine was

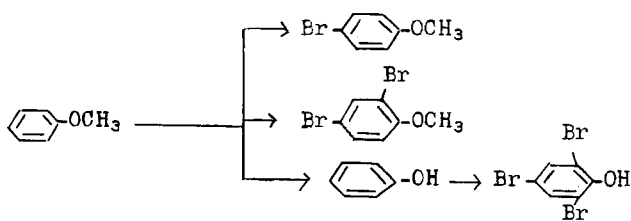
Scheme 1.



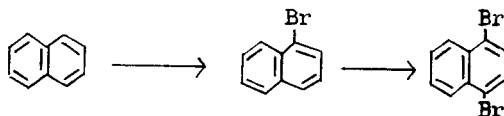
Scheme 2



Scheme 3



Scheme 4



first added to the vial in which about 1 mg each of them had been weighed exactly, and then 18 ml of the toluene scintillator was added onto it.

Radioactivity of each compound was measured with Tri-Carb 314 AX model liquid scintillation counter (Packard instrument Co.) using the internal standard method.

RESULTS AND DISCUSSION.

In order to compare the acidity effect of tritiating reagents on tritium incorporation, several tritiating reagents were mixed with cyclohexane, benzene, or naphthalene dissolved in cyclohexane and each mixture was stirred for 2 or 20 hrs at room temperature. The compounds separated from the reaction mixture were purified by redistillation or recrystallization until constant specific activities were obtained.

The activities of all these compounds were measured with a liquid scintillation counter, and the results are shown in Table I.

TABLE I. Compounds Labelled by Exchange Reaction with Some Tritiated Acids (Reaction for 20 hrs).

Acid-Reagent	Specific Activity dpm/g	Product, dpm/mol.		
		Cyclohexane	Benzene	Naphthalene
TH ₂ PO ₄ · BF ₃	<i>a</i> 7.8 × 10 ⁶	none	3.97 × 10 ⁸	6.6 × 10 ⁸
	<i>b</i> 4.45 × 10 ⁶	none	2.27 × 10 ⁸ *none	
70 % of HClO ₄ -THO	3.5 × 10 ⁹	none	7.69 × 10 ⁴	3.11 × 10 ⁷
Concd. HCl-THO	2.62 × 10 ⁹	none	none	2.28 × 10 ⁶
TH ₂ PO ₄	1.32 × 10 ⁷	none	none	trace

* Reaction for 2 hrs.

It was found that an alicyclic compound such as cyclohexane was not labelled with tritium in any acidic media. Meanwhile, a small amount of tritium incorporation was observed in cases of benzene and naphthalene even in treatment with tritiated hydrogen chloride. Cyclohexane was, therefore used as a favorable solvent in the following experiments.

Among these four acid-catalyzed exchange reactions, the highest tritium incorporation was observed in the reaction of the compounds with $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ catalyst for 20 hrs. Consequently, except for aliphatic or alicyclic compounds, high acidity of tritiating reagent and prolonged reaction time seemed to lead to successful exchange reactions.

TABLE II. Compounds Labelled by Exchange Reaction with $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ (Reaction for 20 hrs).

Compound	$\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$		Product			
			Theo. dpm/mol	Obs.		Yield %
				dpm/mol	$\mu\text{Ci/mol}$	
Benzene	6	<i>a</i>	3.47×10^9	3.97×10^8	179	11.4
		<i>b</i>	1.97×10^9	2.27×10^8	102	11.5
Phenol	6	<i>a</i>	3.47×10^9	1.16×10^9	523	33.4
		*	3.25×10^9	9.46×10^8	426	29.1
	6	<i>a</i>		1.06×10^9	477	30.5
		*		8.77×10^8	395	26.9
Anisole	5	<i>a</i>	3.25×10^9	1.09×10^9	491	33.5
		<i>b</i>	1.84×10^9	4.91×10^8	221	26.7
Toluene	5	<i>a</i>	3.25×10^9	1.10×10^9	495	33.8
		^o <i>b</i>	1.84×10^9	3.39×10^8	153	18.4
Diphenyl ether	10	<i>a</i>	4.00×10^9	1.62×10^9	730	40.5
Thiophenol	6	<i>a</i>	3.47×10^9	5.12×10^8	231	14.8
		<i>b</i>	1.97×10^9	3.56×10^8	160	18.1
Thioanisole	5	<i>b</i>	1.84×10^9	4.22×10^8	190	22.9
Diphenyl sulfide	10	<i>b</i>	2.27×10^9	8.85×10^8	399	39.0
Naphthalene	8	<i>b</i>	2.15×10^9	6.6×10^8	296	30.7
β -Naphthole	8	<i>a</i>	3.78×10^9	1.61×10^9	725	42.6
β -Methoxy naphthalene	7	<i>a</i>	3.64×10^9	1.32×10^9	595	36.3
Cyclohexane	12	<i>a</i>		—		
Nitrobenzene	5	<i>a</i>		—		
Bromobenzene	5	<i>a</i>		—		
Benzoic acid	5	<i>a</i>		—		
Benzaldehyde	5	<i>a</i>		—		
Phenyl methyl ester	5	<i>a</i>		—		
Aniline	5	<i>a</i>		—		

a = 1.3×10^9 dpm/mol (5.86×10^2 $\mu\text{Ci/mol}$).

b = 7.38×10^8 dpm/mol (3.32×10^2 $\mu\text{Ci/mol}$).

* = The value excluding labile tritium.

^o = Reaction for 6 hrs.

The effective labelling by the hydrogen exchange reaction could be conveniently achieved when a number of aromatic compounds were mixed with tritiated phosphoric acid-boron trifluoride complex, and then stirred for 20 hrs at room temperature. The procedures for the purification of the recovered products were similar to those described above. The results are shown in Table II.

In the table, the theoretical specific activity S_x is calculated from the following equation ⁽³⁾ :

$$S_x \times \frac{W_x}{M} = \frac{N}{N+3} \times S_r \times \frac{W_r}{166}$$

where S_x — Specific activity of the compound

S_r — Specific activity of the reagent

M = Molecular weight of compound

N = Number of exchangeable hydrogen positions in the compound

W_x = Weight of compound

W_r = Weight of reagent

From these results, it was found that the compounds having electron donating substituents such as, -OH, -OCH₃, -CH₃ and -O-C₆H₅ at the para position of benzene ring were labelled very easily with tritium, although benzene was rather difficult to be labelled. The substitution by mercapto groups such as, -SH, -SCH₃ and -S-C₆H₅ at the para-position of benzene ring, was also found to accelerate the incorporation of tritium considerably. On the other hand, the existence of electron withdrawing substituents such as, -COOH, -NO₂, -COOEt and -Br at the para-position of benzene ring, inhibited the exchange reaction. This procedure was, however, not available for the compounds having -NH₂ groups, since such compounds immediately react with BF₃ to produce amino compound-BF₃ complexes.

It is well known that rates of electrophilic substitution in aromatic compounds are increased by electron donating substituents and inhibited by electron withdrawing ones attached to the benzene ring, and that the reactivity of substituents decreases in the order of -OH > -OCH₃ > -CH₃ > -O-C₆H₅ > -H.

The results listed in Tables I and II indicate that the order of the tritiation of the compounds is just same as that toward electrophilic substitution reaction. Consequently, the reaction is considered to be exactly in the category of electrophilic substitution reactions of aromatic compounds.

Furthermore, it has long been recognized that a substituent containing divalent sulfur capable of releasing electrons has an effect on the reactivity of attached aromatic groups through its mesomeric ability, although this mesomeric effect of the sulfide function was suggested to be smaller than that

of the oxygen analogues^(8,9). Therefore, aromatic sulfur compounds are expected to be labelled not as easily as corresponding oxygen compounds by means of electrophilic substitution reaction. A series of experiments of the T-H exchange reactions were carried out in the aromatic compounds containing sulfur or oxygen in the substituent in the hope of obtaining further information about this reaction through comparing the substituent effects. The compounds were treated with tritiated phosphoric acid-boron trifluoride complex for 2 hrs at room temperature. The results are shown in Table III.

TABLE III. Compounds Labelled by Exchange Reaction with $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ (Reaction for 2 hrs).

Compound N	$\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$	Product			Yield %	
		Theo. dpm/mol	Obs.			
			dpm/mol	$\mu\text{Ci/mol}$		
Benzene	6	<i>a</i>	—			
Phenol	6	<i>a</i>	3.47×10^9	1.18×10^9	532	34.0
Anisole	5	<i>a</i>	3.25×10^9	9.82×10^8	442	30.2
Toluene	5	<i>a</i>	3.25×10^9	6.78×10^8	305	20.9
Diphenyl ether	10	<i>a</i>	4.00×10^9	3.42×10^7	15.4	0.86
Thiophenol	6	<i>a</i>	3.47×10^9	1.79×10^8	80.6	5.16
Thioanisole	5	<i>a</i>	3.25×10^9	3.19×10^7	14.4	0.98
Diphenyl sulfide	10	<i>a</i>	4.00×10^9	3.39×10^6	1.53	0.08

$a = 1.3 \times 10^9$ dpm/mol (5.86×10^2 $\mu\text{Ci/mol}$).

Tritium was not incorporated in the recovered benzene, contrary to the remarkable incorporation of tritium in phenol and anisole. In both compounds almost the same specific activities as those in the case for the reaction for 20 hrs were found. These observations indicate that the electron donating substituents such as -OH and -OCH₃, mightly accelerate the incorporation of tritium. The exchange ability of various electron donating substituents attached to aromatic ring decreases in order of -OH > -OCH₃ > -CH₃ > -SH > -SCH₃ > -O-C₆H₅ > -H. In other words, the relative reactivities between the substituents containing oxygen and sulfur are concluded to be as follows.



These observations which indicate smaller facility of sulfur containing compounds than that of oxygen analogues for the labelling reaction are identical with the theory which has been pointed out by Ingold *et al.* ^(8,9).

The intramolecular distribution of radioactivity was investigated in four representative compounds in order to obtain detailed evidence of the reaction mechanism. The tritium labelled phenol, toluene, anisole and naphthalene were converted to the appropriate derivatives as shown in Scheme 1-4, respectively. From the specific activities of the individual derivatives, the intramolecular distributions of tritium in the parent compounds were obtained. The results are shown in Tables IV and V.

TABLE IV. Radioactivity of Phenol, Toluene, Anisole, Naphthalene and their Degradation Products.

Compound	Specific Activity (dpm/mol)
Phenol	1.16×10^9
Phenyl tosylate	8.87×10^8
<i>o</i> -Nitrophenol	2.67×10^8
<i>p</i> -Nitrophenol	2.72×10^8
2,4,6-Tribromophenol	6.87×10^7
Toluene	3.39×10^8
Benzoic acid	3.33×10^8
3,5-Dinitro benzoic acid	2.89×10^8
2,4,6-Tribromoaniline	5.30×10^7
<i>p</i> -Bromoacetanilide	2.32×10^8
Anisole	1.09×10^9
<i>p</i> -Bromoanisole	7.77×10^8
2,4-Dibromoanisole	4.59×10^8
2,4,6-Tribromophenol	5.82×10^7
Naphthalene	6.60×10^8
α -Bromonaphthalene	4.96×10^8
1,4-Dibromonaphthalene	3.33×10^8

Electrophilic attack on the compounds having electron donating group is expected predominantly to give substitution at the ortho- and para-positions to the resident groups of aromatic ring.

As is indicated in the Tables IV and V, the ratios of tritium distributions were much more at the ortho- and para-positions than meta-positions in phenol, toluene and anisole, and almost all of the tritium was found at the α -positions in naphthalene. Almost no tritium was found on the aliphatic side chain of toluene and anisole and at β -positions of the naphthalene ring.

TABLE V. Intramolecular Distribution of Tritium Activity in Phenol, Toluene, Anisole and Naphthalene.

Position	Relative Activity			
	Phenol	Toluene	Anisole	Naphthalene
Ortho-	46.0/2	53.8/2	64.0/2	...
Meta-	6.0/2	15.6/2	5.7/2	...
Para-	23.4	31.6	28.9	...
Side-chain	24.7	1.8/3	1.8/3	...
α -	100/4
β -	trace

These observations indicate that sequences of the acid catalyzed hydrogen exchange of aromatic compounds should be described as typical electrophilic substitution reaction.

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REFERENCES

1. ROWLAND, F. S. and WOLFGANG, R. — *Nucleonics*, **14** : (8) 58 (1956).
2. WILZBACH, K. E. — *J. Am. Chem. Soc.*, **79** : 1013 (1957).
3. YAVORSKY, P. M. and GORIN, E. — *J. Am. Chem. Soc.*, **84**, 1071 (1962).
4. HILTON, B. D. and O'BRIEN, R. D. — *J. Agr. and Food Chem.*, **12** : 236 (1964).
5. EVANS, E. A. — "Tritium and its Compounds" Butterworths London (1966).
6. BONNER, W. A. — *J. Am. Chem. Soc.*, **79** : 2469 (1957).
7. WHITE, R. M. and ROWLAND, F. S. — *J. Am. Chem. Soc.*, **82** : 4713 (1960).
8. INGOLD, C. K. and INGOLD, E. H. — *J. Chem. Soc.*, **1926** : 1310 (1926).
9. HOLMES, E. L. and INGOLD, C. K. — *Ibid*, **1926** : 1328 (1926).