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Hydrogen Exchange Reaction of Aminobenzoic Acid. I. Comparison between Wilzbach Tritiation and Acid Catalyzed Hydrogen Exchange Reaction

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Wilzbach tritiation in *ortho*-, *meta*- and *para*-aminobenzoic acids was compared with acid catalysed hydrogen exchange reaction and bromination. The acid catalysed hydrogen exchange reaction of the acids in 4 N DCl occurred at *ortho* and *para* to amino group of the acids and it was confirmed that the reaction was a typical electrophilic substitution. Tritium incorporation in the acids by Wilzbach labelling was high at *ortho* and *para* to amino group of the acids although the order of reactivity on Wilzbach labelling did not completely agreed with that in acid hydrogen exchange reaction. This seems to suggest that HeT^+ ion would participate in the labelling predominantly.

Although the detailed mechanism of Wilzbach labelling is not fully worked out, it has been confirmed that the labelling occurred *via* two different processes, initiated by HeT^+ ions and the decay electrons which were produced from tritium molecule.[2] Recently Cacace and Caronna reported that on Wilzbach tritiation of toluene, the reaction responsible for HeT^+ ions appeared to be similar to the electrophilic aromatic substitutions occurring in solution.[3] According to these considerations, it is plausible that the similarity would be observed more distinctly on Wilzbach tritiation of polar aromatic molecule. In the present paper, Wilzbach tritiation in *ortho*-, *meta*-and *para*-aminobenzoic acids (ABAs) was compared with acid catalysed hydrogen exchange reaction and bromination.

The reactivities among *o*-, *m*- and *p*-ABAs on Wilzbach tritiation were examined. Each same amount of the ABAs was sealed in an ampule with tritium gas. The specific activities of the labelled ABA were determined (Table I). Table I shows that the difference of the reactivities among the ABA was very little. The tritium distribution in the labelled ABAs was determined by measuring the specific activities of the halogenated derivatives of the ABAs. The halogenations of the ABAs were indicated in Chart I. and the tritium distribution in the labelled ABAs were shown in Table II. Garnett and his co-workers reported

Table I. The Specific Activities of Tritiated *o*–, *m*– and *p*–Aminobenzoic Acids Labelled by Wilzbach Method

	Aminobenzoic acid		
	ortho	*meta*	*para*
Specific activity (μCi/mmole)	10.7	9.1	15.4

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[1] Location: *9–1, 4–Chome, Anagawa, Chiba–shi, Chiba*; *a*) On leave from *Morishita Pharmaceutical Company, Ltd.*

[2] K.E. Wilzbach, *J. Am. Chem. Soc.*, **79**, 1013 (1957); T.H. Pratt and R. Wolfgang, *ibid.*, **83**, 10 (1961); S. Wexler, *ibid.*, **85**, 272 (1963); P. Riesze and K.E. Wilzbach, *J. Phys. Chem.*, **62**, 6 (1958); K. Yang and P.L. Gant, *ibid.*, **66**, 1619 (1962); P.L. Gant and K. Yang, *J. Chem. Phys.*, **30**, 1108 (1959); *ibid.*, **31**, 1589 (1959); *ibid.*, **32**, 1757 (1960).

[3] F. Cacace and S. Caronna, *J. Am. Chem. Soc.*, **89**, 6848 (1967).

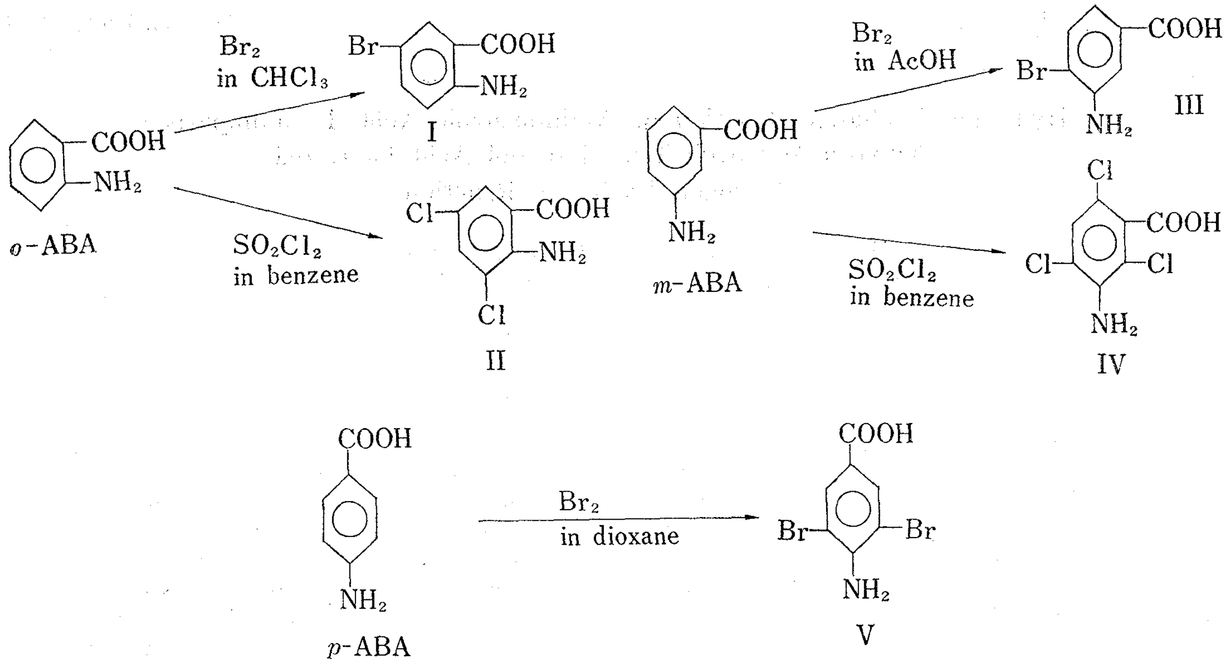
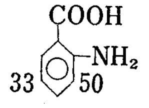
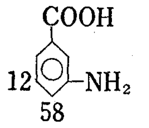
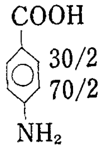


Chart 1

Table II. The Tritium Distribution in the Labelled Aminobenzoic Acids

Compound	Specific activity dpm/mmol	%	Tritium distribution at position indicated %
o–Aminobenzoic acid	4.34×10^5	100	
2–Amino–5–bromobenzoic acid	2.92×10^5	67	
2–Amino–3,5–dichlorobenzoic acid	7.37×10^4	17	(4)+(6)=17
m–Aminobenzoic acid	4.27×10^5	100	
3–Amino–4–bromobenzoic acid	1.78×10^5	42	
3–Amino–2,4,6–trichlorobenzoic acid	5.11×10^4	12	
			(2)+(6)=30
p–Aminobenzoic acid	6.69×10^5	100	
4–Amino–3,5–dibromobenzoic acid	2.01×10^5	30	

that the most reactive site on Wilzbach tritiation of *o*-ABA was *ortho* position to the amino group.[4] Our data of *o*-ABA agreed closely with their results.

Acid catalysed hydrogen exchange reaction was carried out in 4 N deutero-hydrochloric acid solution. After each of the ABAs, dissolved in 4 N DCl, was heated at 120° for an appropriate times in a sealed tube, they were followed by NMR spectroscopy in further report.[5] The comparison of deuteration efficiencies among the ABAs was shown in Table II and the kinetical data of the exchange reaction in *m*-ABA was given in Fig. 1. Moreover, when acetic acid-D was used as acid catalyst in the exchange reaction of *o*-, *m*- and *p*-ABAs, only protons to *ortho* and *para* of *o*-ABA was exchangeable and the deuteration ratio was 65% in 5-position

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[4] J.L. Garnett, S.W. Law, and A.R. Till, *Aust. J. Chem.*, **18**, 297 (1965).
[5] M. Matsuo, T. Matsuo, Y. Kasida, and T. Kondo, *Chem. Pharm. Bull.* (Tokyo), submitted.

and 46% in 3-position by heating at 120° for 20 hours. These results show that the order of the reactivities among the ABAs was *ortho* > *para* > *meta,* and *ortho* and *para* positions to amino group of the ABAs were deuterated under these conditions.

The bromination of the ABAs was carried out in the appropriate solvents by using bromine and the most reactive site in each ABA molecule was examined. Since the main products in the bromination were 2-amino-5-bromobenzoic acid, 3-amino-4-bromobenzoic acid and 4-amino-3,5-dibromobenzoic acid, it was confirmed that 5-position of *o*-ABA, 4 of *m*-ABA and 3 and 5 of *p*-ABA were more reactive in each ABA molecule.

Although it has been considered that acid catalysed hydrogen exchange reaction and bromination of aromatic compound were typical electrophilic substitutions and these reaction species attacked to the more electronegative site in aromatic compound, there is the marked difference between these reactions in *m*-ABA; that is, 2-position was the most active position in the hydrogen exchange reaction, however 4-position in bromination. The difference may be explained by bulkiness of bromine molecule. On the other hand, it is interested that the tritium incorporation into 4-position was more efficient on Wilzbach labelling of *m*-ABA. It appears that *ortho* position of carboxyl group in ABA system was deactivated on Wilzbach labelling. In *o*-ABA, the most reactive position on Wilzbach tritiation above mentioned, differing from that on electrophilic substitution, was *ortho* to the amino group. Garnett and his co-workers described that this might be explained with participation of radical reaction mechanism.³⁾ Although, in detail, tritium distribution pattern of ABAs on Wilzbach labelling has not completely agreed with that in the acid hydrogen exchange reaction, the tritium incorporation by Wilzbach labelling tends to be high at the more electronegative position in the molecules and this seems to suggest that HeT^+ ion would participate in the labelling predominantly.

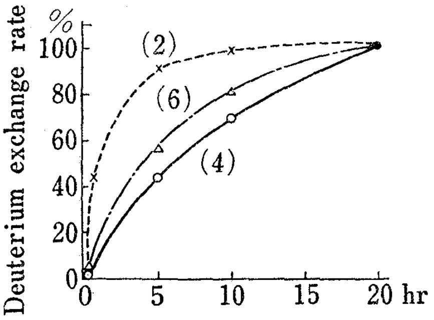

Fig. 1. The Hydrogen Exchange Reaction of *m*-Aminobenzoic Acid in 4 N DCl[a]

[a] The reaction temperature: 120°, and the figure in the parenthesis indicates the position of aromatic proton in *m*-ABA.

Experimental

Materials—— *o*-, *m*- and *p*-ABAs were commercially available. Tritium gas (>98%) was obtained from Radiochemical Centre (Amersham). Deuterium oxide (99.75%) and 20% deutero-hydrochloric acid (deuterium purity 99%) were purchased from E. Merk (Darmstadt). Deutero-acetic acid was prepared from acetic anhydride and deuterium oxide.

Isotope Analyses——All deuterium analyses were carried out with NMR spectrometry as described in the next paper.⁵⁾ The radioactivities of tritium were determined by using a Tri-Carb liquid scintillation spectrometer.

Wilzbach Tritiation——Each of *o*-, *m*- and *p*-ABAs(250 mg respectively) was sealed in a special breakable ampule (Fig. 2) with tritium gas (1 Ci) for a week at room temperature.

The labelled materials, diluted with inactive carrier, were purified by recrystallization from water containing active charcoal until a constant specific activity was obtained.

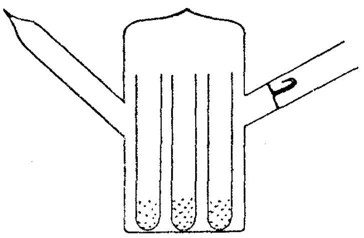

Fig. 2.

Halogenation——2-Amino-5-bromobenzoic Acid (I): *o*-ABA (200 mg) was dissolved in $CHCl_3$ (15 ml). A mixture of bromine and $CHCl_3$ (1:20 v/v) was added to the solution for 5 min with stirring until bromine color appeared in the reaction mixture. The precipitate was filtered and recrystallized from a mixture of EtOH and benzene. The yield was 70%. mp 218° (lit. 220°). *Anal.* Calcd. for $C_7H_6O_2NBr$: C, 38.92; H, 2.80; N, 6.48. Found: C, 39.14; H, 2.93; N, 6.61.

2-Amino-3,5-dichlorobenzoic Acid (II): *o*-ABA (200 mg) and sulfuryl chloride (2 ml), dissolved in benzene (30 ml), were refluxed on a boiling water bath for 1 hr and the reaction mixture was evaporated to dryness *in vacuo*. The residue was recrystallized from benzene with active charcoal. The yield was

Table III. The Comparison of Deuteration Efficiencies among *o*-, *m*- and *p*-Aminobenzoic Acids with 4 N DCl Catalyst[a]

Compound	Position in the molecule	Deuteration (%)
o-Aminobenzoic acid	3	83
	5	100
m-Aminobenzoic acid	2	44
	4	2
	6	8
p-Aminobenzoic acid	3 and 5	98

[a] the reaction time: 1 hr the reaction temperature: 120°

15%. mp 226° (lit. 223°). *Anal.* Calcd. for $C_7H_5O_2NCl_2$: C, 40.81; H, 2.45; N, 6.80. Found: C, 40.96; H, 2.53; N, 6.72.

3-Amino-4-bromobenzoic Acid (III): *m*-ABA (274 mg), dissolved in acetic acid (10 ml), was treated with acetic acid solution (2 ml) of bromine (0.11 ml) for 5 min with stirring. The precipitate was filtered, washed with acetic acid and recrystallized from a mixture of H_2O and EtOH. The yield was 24%. mp 223° (lit. 225°). *Anal.* Calcd. for $C_7H_6O_2NBr$: C, 38.92; H, 2.80; N, 6.48. Found: C, 39.09; H, 2.84; N, 6.49.

3-Amino-2,4,6-trichlorobenzoic Acid (IV): *m*-ABA (274 mg) and sulfuryl chloride (5 ml) with benzene (30 ml) were refluxed on a boiling water bath for 2 hr and were evaporated *in vacuo*. The residue was recrystallized from a mixture of H_2O and EtOH with active charcoal. The yield was 68%. mp 161°. *Anal.* Calcd. for $C_7H_4O_2NCl_3$: C, 34.96; H, 1.68; N, 5.82. Found: C, 34.63; H, 1.73; N, 5.94.

4-Amino-3,5-dibromobenzoic Acid (V): *p*-ABA (274 mg) was dissolved in dioxane (10 ml). Bromine (0.2 ml) in dioxane (6 ml) was added to the solution for 10 min with stirring. The precipitate was filtered off and the filtrate was evaporated *in vacuo*. The residue was recrystallized from EtOH. The yield was 22%. mp >330° (lit. 330°). *Anal.* Calcd. for $C_7H_5O_2NBr_2$: C, 28.51; H, 1.71; N, 4.75. Found: C, 28.69; H, 1.85; N, 4.84.

Acid Catalysed Hydrogen Exchange Reaction——After labil hydrogens in ABAs were replaced with deuterium, each ABA (50 mg) and 4N DCl (1 ml) were heated at 120° for the appropriate times. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOH and was alkalized with ammonia. The solution was evaporated *in vacuo*. The residue was purified with recrystallization from water. When *o*-ABA was deuterated by using deutero-acetic acid as acid catalyst, *o*-ABA (100 mg) with deutero-acetic acid (2 ml) was heated at 120° for 20 hr.

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