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Aryl Hydroxylation of 3-Methylacetanilide[4-$^{2(3)}$H] by Several Model Systems[1]

Yukio Mori, Kazumi Toyoshi,[2a] and Shigeo Baba[2b]

Gifu College of Pharmacy[2a] and Tokyo College of Pharmacy[2b]

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The reactions of tritiated or deuterated 3-methylacetanilide with photolysis of pyridine-N-oxide, *m*-chloroperbenzoic acid, photolysis of hydrogen peroxide and the Fenton or Udenfriend system has been investigated.

The retention of tritium (21%) obtained on the 4-hydroxylation of 3-methylacetanilide-4-^{3}H with pyridine-N-oxide photolysis is corresponding to the tritium retention in the enzymic hydroxylation by rat *in vivo* and *in vitro* metabolisms,[7] and the retention value (12%) observed in the reaction with *m*-chloroperbenzoic acid to the results obtained in the metabolism by rat pretreated with 3,4-benzpyrene or 3-methylcholanthrene.[5] Isotopic hydrogen in the 3-methyl-4-hydroxyacetanilide produced from 3-methylacetanilide-4-$^{2(3)}$H by Fenton or Udenfriend system was also retained. The reactions with these systems under a few conditions are presented.

The NIH shift, the intramolecular migration of aromatic ring substituents such as deuterium, tritium, halogens, and alkyl groups during the metabolism of aromatic substrates to phenols, may be considered as a fundamental phenomenon associated with the action of monooxygenases.[3] Such migrations are so characteristic of the enzyme reactions that oxidants which do not produce them may no longer be regarded as meaningful models for monooxygenases. Detailed studies on model hydroxylating systems which exhibit the NIH shift should permit further elucidation of the mechanism of the more complex enzymic oxidations.[4]

In a previous report,[5] we investigated the NIH shift during the conversion of 3-methylacetanilide[4-$^{2(3)}$H] to 4-hydroxy-3-methylacetanilide by animals *in vivo* or *in vitro* metabolism, and reported that the retention values of isotopic hydrogen in the phenolic product vary from 11 to 47% under various conditions. It is of interest whether the NIH shift is similarly observed in the 4-hydroxylation of 3-methylacetanilide[4-$^{2(3)}$H] by several model systems as the enzyme reactions. Therefore, this paper deals with aryl hydroxylations of the labeled 3-methylacetanilide by such nonenzymic reactions as photolysis of pyridine-N-oxide, *m*-chloroperbenzoic acid, photolysis of hydrogen peroxide, and the Fenton or Udenfriend system.

Table I shows the tritium retention during these chemical hydroxylations of 3-methylacetanilide[4-^{3}H]. 4-Hydroxy-3-methylacetanilide was produced in 0.03±0.01% yields by photolysis of pyridine-N-oxide from the substrate. Pyridine-N-oxide photolysis is of great value as a mechanistic model for enzymic oxidation, since this system is capable of many oxidation reactions typical of mixed function oxidases and exhibits the NIH shift in which the retention values are similar to those observed during hydroxylation of several substrates with microsomes.[6] Tritium retention during hydroxylation of 3-methylacetanilide[4-^{3}H]with this system was also the same level as those obtained with rat *in vivo* and *in vitro*.[7]

1) This was presented at the 2nd Meeting of Association of Bioorganic Chemistry, Kyoto, March 1974.
2) Location: *a*) *Mitahora higashi 5-6-1, Gifu*; *b*) *Horinouchi 1432-1, Hachioji, Tokyo.*
3) J. Daly, D. Jerina, and B. Witkop, *Experientia*, **28**, 1129 (1972).
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6) D.M. Jerina, D.R. Boyd, and J.W. Daly, *Tetrahedron Letters*, **1970**, 457.
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Table I. Tritium Retention in 3-Methyl-4-hydroxyacetanilide Derived from 3-Methylacetanilide-4-^{3}H by Several Oxidants

Oxidizing system	% retention of tritium[a]
Pyridine-N-oxide, $h\nu$	21.1±1.8
m-Chloroperbenzoic acid	12.0±0.3
H_2O_2, $h\nu$	9.9±1.3
Fe(II), EDTA, ascorbic acid, H_2O_2[b]	9.2±1.0

a) Deviations from the mean are presented for experiments which were repeated at least four times.
b) The reaction was carried out in air.

In the case of *m*-chloroperbenzoic acid, the product was obtained in 3.0±0.5% yields. The hydroxylating species in this system may be same as trifluoroperacetic acid which also exhibits the NIH shift with many substrates.[8] The retention value in the product corresponds to the results obtained with *in vivo* and *in vitro* metabolisms by rats pretreated with 3,4-benzpyrene or 3-methylcholanthrene.[5]

Tritium was also retained approximately 10% in 4-hydroxy-3-methylacetanilide produced by photolysis of hydrogen peroxide or the Udenfriend system, which is known to generate a hydroxyl radical.[9,10] It has, however, been reported that model hydroxylating systems involving hydroxyl radicals do not cause the NIH shift.[8] So, the reactions of 3-methylacetanilide with the Fenton or Udenfriend system, using also the deuterated substrate, were further investigated under various conditions.

Table II. Yield (%) in the 4-Hydroxylation of 3-Methylacetanilide by Fenton or Udenfriend System

System[a]	Atmosphere		
	air	O_2	N_2
H_2O_2	1.3±0.6	—	1.4±0.5
Ascorbate	1.3±0.6	1.5±0.4	n.d.[b]
H_2O_2, ascorbate	3.9±0.6	4.5±0.3	4.5±0.7

a) In the mixture of Fe(II), EDTA and phosphate buffer(pH 6.3).
b) The product was not detected on thin-layer chromatography.

No primary isotope effect was observed in the aryl hydroxylation of 3-methylacetanilide, since the rates of hydroxylation with a system consisting of ferrous ion, ethylenediaminetetraacetate (EDTA), H_2O_2 or/and ascorbic acid in air, oxygen, and nitrogen atmosphere are the same in the nonlabeled, deuterated and tritiated rings. The chemical yields are shown in Table II. If H_2O_2 and ascorbic acid were omitted from the reaction mixture, any products was not formed. If O_2 was omitted in the Udenfriend reaction (Fe^{2+}/EDTA/ascorbate), the 4-hydroxylated product was not also formed (Table II). With other conditions there were no significant differences in yields between nitrogen and oxygen or air atmosphere. Yield with a system consisting of H_2O_2 and ascorbic acid was about 3 times that of H_2O_2 or ascorbic acid in three atmospheres.

Table III presents the retentions of isotopic hydrogen in 4-hydroxy-3-methylacetanilide produced from $^{2(3)}$H-3-methylacetanilide with the Fenton or Udenfriend system. Heavy hydrogens were retained 8—10% in the product obtained from the 4-labeled substrate by the

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Table III. Retention of Isotopic Hydrogen in 3-Methyl-4-hydroxyacetanilide Derived from Labeled 3-Methylacetanilide by Fenton or Udenfriend System

Label position	Oxidizing system[a]	% retention of deuterium	% retention of tritium
4-$^{2(3)}$H	H_2O_2	10.5±1.7	8.5
	ascorbate	8.2±1.5	8.8
	H_2O_2, ascorbate	8.4±1.1	9.2±1.0
5-$^{2(3)}$H	H_2O_2, ascorbate	96.5±1.1	98.8±1.8

a) The other components of reaction mixture were described in Table II.

both systems. Moreover, the retentions of tritium and deuterium in these hydroxylations and chemical yields were independent on the pH of the reaction mixture (pH 6.3—9.0).

No significant difference in the retention of isotopic hydrogen between the Fenton and Udenfriend systems was observed, even though there is strong evidence that a hydroxyl radical is the actual hydroxylating species when H_2O_2 is the oxidizing agent in the latter system but is not when O_2 is presented.[11] Infrared (IR) and nuclear magnetic resonance (NMR) analyses of the product obtained from 3-methylacetanilide[4-^{2}H] suggested that deuterium retained was present at 5 position. Isotopic hydrogens at adjacent to the position of hydroxylation were not apparently lost during the conversion of the 5-labeled substrate to the 4-hydroxylated product.

There is a report that the treatment of 4-chloro-8-methoxyquinoline with H_2O_2 in glacial acetic acid leads to the production of 3-chloro-8-methoxy-4-quinolinol, indicating a migration of chlorine atom.[12] The present work, however, may be first observation that isotopic hydrogen was retained in the product of hydroxylation by the Fenton or Udenfriend system.

Experimental

The position of labeling in the deuterated or tritiated 3-methylacetanilide was established by IR and NMR analyses of the corresponding deuterated compound.[7] The product prepared in each chemical hydroxylation was identified by mp, elemental analysis of the product obtained from the unlabeled substrate under the same conditions, and comparison of its spectroscopic data with the authentic sample. In all reactions, extreme care was taken to ensure complete separation of isomeric phenols since cross-contamination of the 4-hydroxylated product by either 5- or 6-hydroxylated isomer would adversely affect the measurement of deuterium or tritium content. The isolation of the product and the determination of deuterium or tritium in the product have been described previously.[7] Percentage retention of tritium in the product was determined by dividing its specific radioactivity by that of the substrate.

Photolysis of Pyridine-N-oxide——Solution of 0.67 mmoles of 3-methylacetanilide [4-^{3}H] and 10.5 mmoles of pyridine-N-oxide were irradiated (low pressure mercury lamp, 2536 Å) in 50 ml CH_2Cl_2 for 5 hr with nitrogen bubbled through the solution. After addition of 10 ml of 1.6N HCl, the 4-hydroxylated product was isolated from an ether extract by thin-layer chromatography and quantitated by colorimetric assay. Carrier was then added and the material recrystallized to constant specific radioactivity.

***m*-Chloroperbenzoic Acid**——0.67 mmoles of 3-methylacetanilide[4-^{3}H] was hydroxylated to 4-hydroxy-3-methylacetanilide by stirring at room temperature with 2 mmoles of *m*-chloroperbenzoic acid in 25 ml CH_2Cl_2 for 5 hr. Product was isolated by pouring the reaction mixture into an excess of $NaHCO_3$ solution which was then extracted several times with ether. The combined extracts were dried (Na_2SO_4) and concentrated.

Photolysis of Hydrogen Peroxide——1 mmoles of 3-methylacetanilide[4-^{3}H] and 30% H_2O_2 solution (10 mmoles) were dissolved in 25 ml CH_3CN. The solution was photolysed for 3 hr at room temperature under bubbling N_2 using low-pressure Hg lamp (2536 Å). After irradiation, the remaining peroxide was decomposed by the addition of $NaHCO_3$ solution (10 mmoles). The solution was then separated into organic and aqueous layers. The organic layer was separated from the aqueous layer which was extracted with ethyl acetate.

Fenton, Udenfriend and Modified Fenton System——Each flask contained 1.2 mmoles of $^{2(3)}$H-3-methylacetanilide, 0.3 mmoles of ferrous sulfate, 1.6 mmoles of disodium EDTA, 17.6 mmoles of H_2O_2 or/and 2.3

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12) T. Fujita and J.M. Price, *J. Org. Chem.*, **33**, 3004 (1968).

mmoles of L-ascorbic acid and 6 mmoles of phosphate buffer, pH 6.3—9.0. After stirring for 2 hr at room temperature in air, oxygen or nitrogen atmosphere, the product was extracted into ethyl acetate several times. The combined extracts were dried over Na_2SO_4 and concentrated before separation by chromatography. Deuterium content in the phenolic product was determined by mass spectrometry using a direct probe inlet system on a Hitachi RMU7L mass spectrometer.

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Studies on Heterocyclic Compounds. XVII.[1] Reaction of 6-Mercapto-1,3-Dimethyluracil with Electrophilic Reagents[2]

Tsuneo Itoh, Mieko Honma, and Haruo Ogura

School of Pharmaceutical Sciences, Kitasato University[3]

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Attempts to prepare 5-oxothiopyrano[2,3-*d*]pyrimidine derivatives by the reaction of 6-mercapto-1,3-dimethyluracil (I) with various acetylenecarboxylates and ethyl ethoxymethylenecyanomalonate have led instead to the synthesis of substituted thiopyrimidine-2,4-dione. The condensation of (I) with diethyl ethoxymethylenemalonate yielded readily the corresponding 5-oxothiopyrano[2,3-*d*]pyrimidine-2,4-dione. Elimination of ethoxycarbonyl group in V obtained 1,3-dimethyl-5-oxothiopyrano[2,3-*d*]pyrimidine-2,4-dione.

Some pyrido[2,3-*d*]pyrimidines exhibit interesting antibacterial activities[4] and antitumor activities,[5] and compounds with this ring system have been extensively investigated.[6]

Little is known, however, of the chemistry of the 8-sulfur isostere, the thiopyrano[2,3-*d*]-pyrimidine system,[7] which may be of potential biological importance. This paper deals with a successful synthesis of 1,3-dimethyl-5-oxothiopyrano[2,3-*d*]pyrimidines by reaction of 6-mercapto-1,3-dimethyluracil (I) with electrophilic reagents.

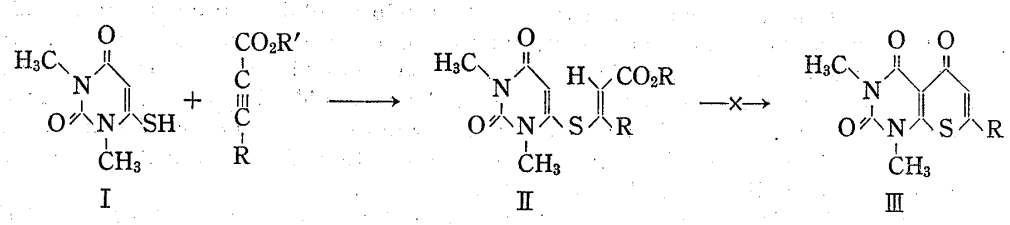

Chart 1

When compound I was treated with methyl propiolate in chloroform, a single product was obtained in good yield. Proton magnetic resonance (PMR), mass spectral and elementary

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