UDC 547.551 : 542.943.6 : 546.157-33

60. **Hiroya Tanabe**: Studies on the Periodic Acid Oxidation of N-Glycosides. XIV.[1] Periodic Acid Oxidation of Anilines. (7).

(*National Hygienic Laboratory**)

In the preceding paper of this series,[1] reaction mechanism of aniline oxidation products (six kinds of anilino-*p*-benzoquinone derivatives) with periodic acid was described mainly on the basis of mutual reaction between anil and free hydroxyl radicals. Accordingly, a new modified mechanism by which some of these products were formed was proposed. The bases upon which the aniline oxidation with periodic acid was assumed to proceed in free radical reactions were derived from the following facts: (1) The reaction occurred easily in many kinds of hydrous organic solvents of various dielectric constants; (2) the kind of oxidation products formed had almost no relation to the pH value of the reaction solution; and (3) the products are very similar to those obtained by light irradiation of aniline. It may be reasonable to assume from these facts that the oxidation is a free radical reaction, but there is not sufficient evidence in the above facts to show the presence of free radicals in the reaction solution. Subsequently, experiments were carried out in order to obtain direct proof that oxidation of aniline with periodic acid proceeds in free radical reactions and some satisfactory results were obtained.

Among many kinds of reactions reported for evidence of the presence of free radicals of very labile intermediates in the reaction solution, the following tests were thought to be the most reliable and universally applicable.

(1) A certain stable free radical is added to a reaction solution to obtain a stable addition compound which consists of the stable free radical added and the free radical produced as a labile intermediate during the reaction.[2]

(2) A readily polymerisable olefin compound is added to the reaction solution under such conditions that the monomer does not easily polymerise without a starter, in order to obtain a polymer formed with the intermediate free radical, as a starter, produced during the reaction.[3]

(3) An active compound capable of being replaced by the free radical is added to the reaction solution in order to obtain the substituted derivative with the intermediate free radical produced during the reaction.[3b,4]

Goldschmidt, *et al.*[2] was the first to employ the foregoing first method (1) to prove the presence of anil free radical in the oxidation of aniline with lead(II) dioxide in dehyd. ether and established the mechanisms of main process of aniline oxidation with oxidizing agents of the second class.[1] However, in the presence of water, the stable free radical used in this method was attacked easily by the oxidizing agent, and periodic acid does not react with aniline in anhydrous organic solvents. Thus, the method (1) is not applicable for the present purpose.

The methods (2) and (3) can both be employed in aqueous solution, but (2) seems to be more general than (1) as a test for the presence of free radicals. Therefore, this method was applied in the present case using methyl methacrylate as the monomer.

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* Tamagawa, Yoga-machi, Setagaya-ku, Tokyo (田辺弘也).

1) Part XIII: This Bulletin, **7**, 177(1959).

2) cf. S. Goldschmidt, *et al.*: Ber., **55**, 3216(1922).

3) cf. a) G.S. Park, *et al.*: Trans. Faraday Soc., **42**, 155(1946); b) M.C.R. Symons: J. Chem. Soc., **1955**, 2794.

4) cf. a) J. Weiss, *et al.*: J. Chem. Soc., **1949**, 2074; b) Fr. Richter, H. Stenzl: Helv. Chim. Acta, **22**, 970(1939).

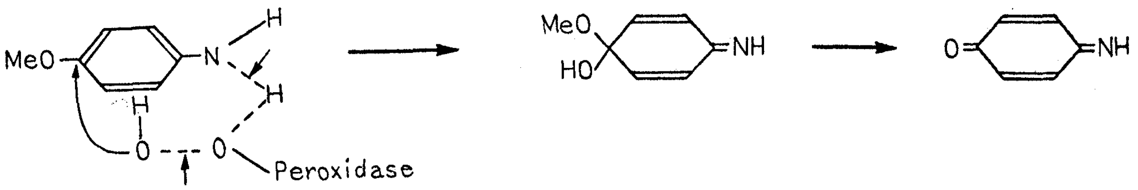
Experiments are carried out at room temperature, excluding light and oxygen. The concentrations of aniline and periodic acid are about $10^{-3}M$, and about $10^{-1}M$ concentration of methyl methacrylate is used at the beginning of the reaction. Otherwise, deposition of a large amount of aniline oxidation product will make it difficult to detect the formation of a polymer, and too high a concentration of free radical will result in the failure of this test process.[5] In the present experiments, methyl methacrylate polymerised only in the presence of both aniline and periodic acid, and a large amount of flocculent polymer deposited after 10 hours. This stage was preceded by a uniform turbidity throughout the solution. When iodate was used instead of periodate in this experiment no polymerisation resulted and solution remained colorless and clear for a long time (Table I).

TABLE I. Reaction with Methyl Methacrylate

Reagent added to methyl methacrylate		Formation of polymer after	
		10 hr.	48 hr.
Aniline		—	—
$NaIO_4$		—	—
Aniline+$NaIO_4$ Aniline+$K_4I_2O_9$+$KHCO_3$	in Vacuum	++	+++
	in N_2	++	+++
	in air	—	++
Aniline+KIO_3		—	—

Thus, it became obvious that the periodic acid oxidation of aniline is a free radical reaction.

In the preceding paper of this series,[1] it was also assumed that the free hydroxyl radical plays an important part in the production of some kinds of anilino-*p*-benzoquinones. Saunders, *et al.*[6] also assumed that a similar mechanism occurred in the oxidation of anisidines with H_2O_2-peroxidase, but they seemed to assume that the oxidation



proceeds only in bimolecular reactions, since they stated in their report that "in this scheme it is not necessary to picture the hydroxyl radical as being free⋯." However, judging from the structure of the product in the oxidation of aniline with periodic acid in the present series of work, it is necessary to presume at least a part of the hydroxyl radicals produced to be free in the solution, even if for a short time, and it is desirable to detect the presence of free hydroxyl radical in the reaction solution. It is reasonable to think that some polymer molecules obtained above would contain one or two hydroxyls as end groups but in the polymer obtained under the conditions mentioned above, the degree of polymerisation is more than 1000,[7] and it would be very difficult to detect the presence of hydroxyl groups in it.

Consequently, the method (3) was finally adopted. Compounds capable of being replaced by free radicals, such as pyridine[4b] and nitro derivatives of benzene[4a] have been used as detection agents, but since it was found[8] that the reaction of free hydr-

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[5] a) M.C.R. Symons: J. Chem. Soc., **1955**, 275; b) F.S. Dainton: *Ibid.*, **1952**, 1533.

[6] B.C. Saunders, *et al.*: *Ibid.*, **1951**, 2115.

[7] Degree of polymerization of poly(methyl methacrylate), obtained by the method of emulsion-polymerisation at 40°, $\bar{P}=4\sim40\times10^3$. (cf. "Kobunshi Jikken Koza," **6**, 61).

[8] N. Uri, *et al.*: Nature, **166**, 869(1950); J. Am. Chem. Soc., **75**, 2754(1953).

oxyl radical and benzoic acid produced *o*-, *m*-, and *p*-hydroxybenzoic acids in the ratio of 2:2:1, benzoic acid has been mainly used for detection of the presence of free hydroxyl radical in solution.

Aniline, periodic acid, and benzoic acid were mixed in water in a respective concentration of about $10^{-2}M$ and the presence of salicylic acid was detected by means of ferric chloride after 10 hours (Table II).

TABLE II. Reaction with Benzoic Acid

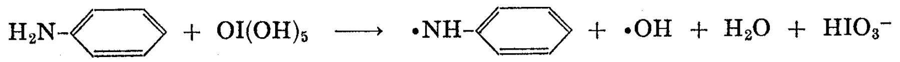
Reagent added to benzoic acid	Violet coloration with $FeCl_3$
$NaIO_4$	—
$NaIO_4$+aniline	++*
KIO_3+aniline	—
$NaIO_4$+aniline	—

* Violet color obtained had a visible absorption (λ_{max} 520~530 mμ just after coloration) identical with that obtained from an authentic salicylic acid.

Thus it became obvious that in oxidation of aniline with periodic acid, free hydroxyl radical is produced and, as in general, the following reaction scarcely occurs except when R is OH.[6b)]

$$H_2O + \cdot R \longrightarrow HO\cdot + HR$$

It seems certain, therefore, that the free hydroxyl radical is doubtless produced by the reaction between aniline and periodic acid according to the following formula



The foregoing two experimental results have proved beyond doubt the mechanism of oxidation of aniline with periodic acid reported previously. It is not known why a diphenyl derivative or phenylhydroxylamine is not produced in spite of the presence of both anil and free hydroxyl radical at the same time in the reaction solution.

The writer expresses his gratitude to Prof. S. Akiya of Tokyo Medico-Dental University for his kind guidance and encouragement, to Prof. T. Okamoto of the University of Tokyo for his useful advices, to Dr. T. Kariyone, Director of the Laboratory, for giving him facilities for the present study, and to Dr. I. Kawashiro, Head of the Food Division of the Laboratory, for his kind encouragement.

Experimental

Polymerisation Test of Methyl Methacrylate (MMA)—MMA was purified as follows: Commercial MMA was washed alternately with the same volume of satd. $NaHSO_3$ solution to remove the peroxide and one-half its volume of 5% NaOH solution saturated with NaCl to remove the hydroquinone, and the same volume of water. After drying over Na_2SO_4, it was distilled under a reduced pressure, in oxygen-free nitrogen stream, with a small quantity of S as inhibitor. $b.p_{140}$ 53°. The purified MMA was kept below 5° in a sealed bottle, in the dark.

Water was distilled twice from a strong alkaline permanganate, in all-glass apparatus which was cleaned with the same reagent.

The following solutions were prepared as reagents.

(a) 1.5 cc. pure MMA dissolved in 100 cc. of water.

(b) 1.0 g. $NaIO_4$ (recrystallised 5 times from water) dissolved in 100 cc. of water.

(c) 0.10 cc. aniline (distilled just before use) dissolved in 100 cc. of water.

(d) 1.0 g. KIO_4 (recrystallised twice from water), 0.3 g. of KOH (guaranteed reagent), and 0.5 g. of $KHCO_3$ (ultimate substance for volumetry) dissolved in 100 cc. of water.

(e) 1.0 g. KIO_3 (ultimate standard for volumetry) dissolved in 100 cc. of water.

In a Thumberg tube, 0.4 cc. of (b) diluted with 10 cc. of (a) was placed in the main tube, and 0.6 cc. of (c) diluted with 10 cc. of (a) was placed in the side tube. Then the tubes were connected and evacuated under cooling until vigorous boiling set in and the space was filled with oxygen-free N_2. This procedure was repeated three times and the tube was left in the dark at room temperature. After 5~6 hr., the solution became uniformly turbid and in 10 hr. flocculent polymer deposited.

Tests on mixtures of MMA and aniline, and of MMA and HIO_4, were carried out just the same as above. Tests on mixtures of MMA, alkaline HIO_4, and aniline, and of MMA, HIO_3, and aniline were also carried out as above, using solutions (d) and (e). The results obtained are given in Table I.

Hydroxylation Test of Benzoic Acid—Following solution was prepared as a reagent besides (b), (c), and (d).

(f) 0.12 g. benzoic acid (guaranteed reagent) dissolved in 100 cc. of water.

In a Thumberg tube, 4 cc. of (b) diluted with 10 cc. of (f) was placed in the main tube, 6 cc. of (c) diluted with 10 cc. of (f) in a side tube, and the test was carried out as for the polymerisation test of MMA After 10 hr., the reaction mixture was filtered, the filtrate was made alkaline with Na_2CO_3, and extracted twice with 30 cc. of $CHCl_3$ to remove pigments. After making the aqueous solution acid with HCl, it was extracted with 20 cc. of Et_2O. The Et_2O layer was placed in a small test tube with 1 cc. of water, the solvent was evaporated, and $FeCl_3$ solution was added to the residual solution. The reaction mixture may be colored directly with $FeCl_3$ after adjusting its acidity to pH 2 with $HClO_4$, but adjustment of pH is rather complicated. Below pH 2, because of too high acidity, and above pH 2, because of a formation of ferric periodate, the reaction mixture does not color with $FeCl_3$ solution even if salicylic acid is present. Moreover, the oxidation products of aniline with HIO_4 contained a violet-colored substance and the coloration was not clear. Thus, the procedure with extraction mentioned above seemed to be more suitable.

From the mixtures of benzoic acid and HIO_4, of benzoic acid and HIO_3, and of aniline and HIO_4, no appearance of violet color was observed by the treatment mentioned above. These results are summarized in Table II.

Summary

Methyl methacrylate polymerised in a concentration of about $10^{-3}M$ in the reaction system of aniline oxidized with periodic acid, and benzoic acid was oxidized in a concentration of $10^{-2}M$ to salicylic acid in the same system. From these facts it was proved that the oxidation of aniline is a radical reaction and a free hydroxyl radical is produced, thereby proving the correctness of presumptions reported in the previous paper of this series.

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61. Itiro Yosioka, Shintaro Takahashi, Hiroshi Hikino, and Yasuko Sasaki: Studies on the Constituents of *Atractylodes*.[*1] III.[1] Separation of Atractylol into Eudesmol and Hinesol.

(*Faculty of Pharmacy, University of Osaka*[*2])

It had been reported that a crystalline sesquiterpenoid alcohol, atractylol, had been isolated from the essential oil derived from the rhizomes of a certain *Atractylis* species (Compositae). Recently, the authors[1] determined that the original plant which gave the so-called atractylol was *Atractylodes lancea* De Candolle and not *Atractylis ovata* Thunberg as had been believed, and that atractylol, which the earlier workers[2,3] had handled, might have been contaminated with other sesquiterpenoids and thus not completely pure.

Regarding previous works carried out on the chemical structure of this substance,

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[*1] Paper presented at the Annual Meeting of the Pharmacognostical Society of Japan, Sendai, September 6, 1958.

[*2] Toneyama, Toyonaka, Osaka-fu (吉岡一郎, 高橋真太郎, ヒキノヒロシ, 佐々木靖子).

1) Part II. S. Takahashi, H. Hikino, Y. Sasaki: Yakugaku Zasshi, **79**, 544(1959).

2) J. Gadamer, T. Amenomiya: Arch. Pharm., **241**, 22(1903).

3) S. Takagi: Yakugaku Zasshi, **473**, 565(1921).