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Some Observations on the Periodate Oxidation of Amino Compounds

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Various aliphatic and aromatic amines are oxidized by sodium metaperiodate and these reactions have been studied quantitatively in acidic, unbuffered and basic media. Significant differences have been observed between the behaviour of aliphatic and aromatic amines. Certain compounds also behaved differently under acidic and basic conditions. These reactions are related to the periodate oxidation of amino acids and, from observations on a number of glycine derivatives, a reaction mechanism is proposed for this process.

Periodate oxidation is a powerful method for the structural investigation of small amounts of carbohydrate material, when used under strictly controlled conditions and in the absence of certain components such as aromatic amines, phenols, amino acids etc., which also react with periodate. The usefulness of this technique has resulted from the many systematic studies that have been carried out previously on aliphatic compounds with vicinal hydroxyl groups. To widen the application of this technique, additional studies have been made of the periodate oxidation of phenolic compounds such as lignin (Adler & Hernestam, 1955; Adler, Hernestam & Walldén, 1958) and tyrosine (Clamp & Hough, 1965). Although periodate will oxidize groups other than hydroxyl groups, for example thio and amino groups, the lack of systematic investigations has hampered the application of this technique to the study of carbohydrate in combination with other naturally occurring compounds as in glycoproteins, *O*- and *N*-glycosides etc. Interest in the periodate oxidation of amino acids has led us to survey the various types of aliphatic and aromatic amines that are oxidized and to what extent, but in view of the complexity of the products (Tanabe, 1956, 1957*a*,*b*, 1958) little attempt has been made at this stage to characterize these compounds. Related aspects of the oxidation of glycine derivatives have been investigated because of their relevance to the complex mechanisms involved in the oxidation of amino acids.

METHODS

Periodate oxidation. The conditions of periodate oxidation, determinations of periodate consumption, carbon dioxide liberation and the pH of the final reaction mixtures were as described by Clamp & Hough (1965).

Identification of the oxidation product of p-*aminophenol.* Sodium metaperiodate (3·21g.) was added to an aqueous solution of *p*-aminophenol (0·273g.) at pH2·0 and the volume adjusted to 2l. The solution became mauve and finally orange and at this stage (24hr.) was extracted with methylene chloride (5×200ml.). The combined extracts were concentrated, yielding pungent orange crystals, m.p. 111–113°. After sublimation under reduced pressure, the crystals had m.p. 114–116°, unchanged after repeated sublimation. The mixed m.p. with an authentic sample of *p*-benzoquinone was 114–116°. The infrared-absorption spectra of the two compounds were identical (Found: C, 66·6; H, 3·8; N, Nil. Calc. for $C_6H_4O_2$: C, 66·6; H, 3·7%).

Identification of the oxidation product of hydrazobenzene. Sodium metaperiodate (3·21g.) was added to an aqueous solution (2l.) of hydrazobenzene (0·53g.). After 48hr. the insoluble material was filtered off and recrystallized from ethanol–water. The product was identified as azobenzene by m.p. and mixed m.p. with an authentic specimen, 64–66°, and by identity of the infrared-absorption spectra of the two compounds.

Synthesis of 2-*phenyl*-N-*phenylglycine.* 2-Phenyl-*N*-phenylglycine was prepared by a modified Strekker synthesis (I. D. Spenser, personal communication). Potassium cyanide (21·7g.) and aniline hydrochloride (43·2g.) were suspended in ether (200ml.) and then benzaldehyde (35·4g.) was added. The mixture was left for 12hr. with occasional shaking, and was then filtered. The insoluble material was discarded, and the ethereal solution was concentrated to a yellow solid, which was crystallized from ethanol–water to give crystals (28·3g.) of 2-anilino-2-phenylethylnitrile, m.p. 82–84°. The product (28g.) was mixed with ice-cold concentrated sulphuric acid (85ml.) containing water (15ml.) and the temperature of the solution was allowed to rise slowly to 37° and maintained there for 48hr. The solution was then poured into an excess of ice-cold water and neutralized with 5N-sodium hydroxide solution, the temperature being kept at 0°. After isolation, the precipitate was recrystallized from ethanol–water to yield colourless crystals of phenyl-*N*-phenylglycinamide (19·5g.), m.p. 118–119°. The crystals (5g.) were suspended in 6N-hydrochloric acid (200ml.) and heated under reflux

for 4hr. The solution was cooled, filtered, and adjusted to pH 5·0 with 5N-sodium hydroxide solution. The precipitate was recrystallized from ethanol–water to yield colourless cubes (2·2g.) of 2-phenyl-*N*-phenylglycine, m.p. 185–187° (Found: C, 74·8; H, 6·5; N, 6·2. Calc. for $C_{14}H_{13}NO_2$: C, 74·1; H, 5·74; N, 6·2%).

RESULTS AND DISCUSSION

The results for the uptake of periodate and liberation of carbon dioxide are given as mol./mol. of compound in Tables 1–7. In those oxidations carried out under unbuffered conditions the pH of the final solution is shown. All the reactions were performed under the same conditions of temperature and concentration of reactants.

Although simple aliphatic primary amines, for example *tert.*-butylamine, were not attacked by periodate, dimethylamine and trimethylamine were appreciably oxidized in alkaline solution, but not in an acid environment (Table 1). On the other hand hydroxylamine was rapidly oxidized under both acidic and alkaline conditions, as was methylhydroxylamine, which is reported to yield a *cis*-nitrosomethane dimer (Emery & Neilands, 1960). The oxidation of amino groups is facilitated by either vicinal participating groups, such as hydroxyl, amino (Table 2), carboxyl and thio groups (Nicolet & Shinn, 1939; Fleury, Courtois & Grandchamp, 1949; Clamp & Hough, 1965), or direct attachment to an aromatic ring. The presence of an intervening methylene group, as in 3-aminopropan-1-ol or benzylamine, eliminates participation by these neighbouring groups and these molecules are not oxidized. For amino alcohols to be oxidized in acidic or neutral solution, the vicinal hydroxyl group must be unsubstituted and the amino group either unsubstituted or monosubstituted. Amino acids show a similar requirement for a hydrogen substituent on the nitrogen atom for oxidation to occur (Clamp & Hough, 1965), presumably in order that the hydrogen may be eliminated as a proton during the oxidative process.

The pH-dependence of oxidation of aliphatic amines, particularly tertiary amines, is good evidence for different processes of oxidation in acidic and alkaline solution. This pH-dependence was reversed in aromatic amines, such as aniline, which were more readily oxidized in acidic than in alkaline solution (Table 3). Methylation of the amino group of aniline did not reduce the final consumption of periodate, and in some cases enhanced it. *NN*-Dimethylaniline was rapidly oxidized under acidic and basic conditions, constituting additional evidence that the mechanism of periodate oxidation of aromatic amines differed from that of aliphatic amines. Comparison of the oxidation of aniline with those of the toluidines showed that ring methylation had a significant effect since *p*-toluidine at pH 2·0, and *o*-, *m*- and *p*-toluidine in unbuffered solution consumed 1 mol. of periodate more than aniline under the same conditions. These results were similar to the effect of ring methylation on the oxidation of phenol (Clamp & Hough, 1965). On the other hand, 2,4,6-trimethylaniline (mesidine) consumed less periodate

Table 1. *Periodate oxidation of aliphatic amines*

Experimental details are given in the text. Butylamine was not oxidized under these conditions.

	Consumption of periodate (mol./mol. of compound)			
Time (hr.)	Methylamine	Dimethylamine	Trimethylamine	Hydroxylamine
(*A*) pH 2·0				
7·5	0·0	0·0	0·0	1·22
32·0	0·02	0·03	0·0	1·30
51·0	0·03	0·05	0·0	1·28
75·5	0·04	0·09	0·0	1·35
(*B*) Unbuffered				
7·5	0·0	0·0	0·0	1·40
32·0	0·01	0·05	0·0	1·44
51·0	0·02	0·10	0·0	1·45
75·5	0·01	0·13	0·0	1·50
Final pH	(4·5)	(4·4)	(5·20)	
(*C*) pH 9·0				
7·5	0·05	0·14	0·21	0·88
32·0	0·08	0·23	0·71	0·86
51·0	0·15	0·30	0·98	0·85
75·5	0·12	0·41	1·37	0·85
100·0	—	—	1·73	—
174·0	—	—	2·19	—

Table 2. *Periodate oxidation of diaminoethane, ethanolamine and* NN*-dimethylethanolamine*

Experimental details are given in the text. 3-Aminopropan-1-ol was not oxidized under these conditions.

	Consumption of periodate (mol./mol. of compound)		
Time (hr.)	Diaminoethane	Ethanolamine	*NN*-Dimethyl-ethanolamine
(*A*) pH2·0			
1	0·0	—	0·0
6	0·0	0·32	0·0
28	0·0	0·51	0·0
50	0·32	0·62	0·0
117	0·34	0·77	0·0
165	0·34	—	0·0
(*B*) Unbuffered			
1	0·51	—	0·03
6	0·96	0·98	0·07
28	0·97	1·01	0·19
50	1·00	1·01	0·34
117	1·06	1·05	0·49
165	1·08	—	0·52
Final pH	(4·10)	(8·25)	(7·05)
(*C*) pH9·0			
1	0·95	—	0·03
6	1·27	0·98	0·11
28	1·16	0·99	0·23
50	1·16	0·99	0·40
117	1·27	1·00	0·63
165	1·22	—	0·73

Table 3. *Periodate oxidation of aromatic amines*

Experimental details are given in the text. Benzylamine, azobenzene, benzaldehyde and acetanilide were not oxidized under these conditions.

	Consumption of periodate (mol./mol. of compound)							
Time (hr.)	Aniline	*N*-Methyl-aniline	*NN*-Dimethyl-aniline	*o*-Toluidine	*m*-Toluidine	*p*-Toluidine	Mesidine	Hydrazo-benzene
(*A*) pH2·0								
3	1·37	0·61	1·33	1·37	1·42	2·89	0·71	0·19
24	2·30	1·36	2·68	2·16	2·21	3·29	—	0·60
48	2·45	2·26	3·03	2·31	2·37	3·42	1·15	0·71
70	2·47	2·58	3·27	2·41	2·48	3·54	—	0·75
94	2·62	3·16	3·38	2·44	2·54	3·61	1·07	0·75
166	2·78	3·97	3·73	2·57	2·70	3·79	0·98	0·79
(*B*) Unbuffered								
2	1·37	1·70	1·45	2·27	2·26	0·09	2·65	0·07
19	2·08	3·92	1·95	2·89	2·71	0·56	3·42	—
49	2·22	4·11	2·27	3·12	2·94	1·58	3·63	1·00
72	2·24	4·38	2·37	3·17	3·03	2·00	—	1·00
96	2·28	4·45	2·45	3·22	3·12	2·48	3·81	1·10
163	2·37	4·53	2·65	3·30	3·16	3·20	3·92	1·00
Final pH	(4·45)	(3·50)	(4·40)	(4.60)	(4·05)	(3·70)	(3·95)	(5·35)
(*C*) pH9·0								
5	0·52	0·55	1·08	1·44	1·21	1·36	2·88	0·36
23	1·28	1·63	1·70	—	—	—	—	—
28	1·44	1·81	1·81	2·47	2·09	2·77	3·28	0·46
46	1·80	2·23	2·24	3·01	2·27	2·96	3·50	0·60
75	—	2·60	2·40	3·24	2·49	3·14	—	—
93	—	2·76	2·58	3·35	2·62	3·24	—	—
120	—	2·95	2·75	3·49	2·71	3·42	3·99	—
167	—	—	2·91	3·61	2·78	3·52	4·16	—

Table 4. *Periodate oxidation of hydroxyanilines and* p-*benzoquinone*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound)			
	o-Hydroxyaniline	*m*-Hydroxyaniline	*p*-Hydroxyaniline	*p*-Benzoquinone
(*A*) pH 2·0				
1	1·96	2·93	0·71	0·0
14	2·46	2·98	1·24	0·0
38	2·84	3·03	1·26	0·0
62·5	2·93	3·14	1·28	0·0
140	3·07	3·30	1·28	0·0
(*B*) Unbuffered				
1	1·27	2·95	1·34	0·15
14	1·80	3·35	2·62	0·39
38	2·10	3·64	3·32	0·51
62·5	2·25	3·85	3·64	0·63
140	2·53	4·07	4·06	0·78
Final pH	(4·75)	(3·80)	(4·40)	(3·75)
(*C*) pH 9·0				
1	1·35	2·70	1·26	2·61
14	2·10	2·78	3·42	3·92
38	2·49	3·10	3·83	4·10
62·5	2·72	3·39	4·11	4·33
140	3·14	3·60	4·36	4·43

Table 5. *Periodate oxidation of nitroanilines*

Experimental details are given in the text. 2,4-Dinitroaniline was not oxidized under these conditions.

Time (hr.)	Consumption of periodate (mol./mol. of compound)		
	o-Nitroaniline	*m*-Nitroaniline	*p*-Nitroaniline
(*A*) pH 2·0			
18	0·0	0·22	0·0
48	0·07	0·59	0·19
64·5	0·07	0·75	0·19
144	0·07	1·27	0·45
(*B*) Unbuffered			
18	0·0	0·08	0·0
48	—	—	—
64·5	0·0	0·16	0·0
144	0·04	0·43	0·09
Final pH	(4·45)	(5·25)	(5·50)
(*C*) pH 9·0			
2	0·0	0·0	0·0
71	0·0	0·0	0·0
143	0·04	0·01	0·04

than aniline, only 1 mol. of oxidant reacting at pH 2·0, and unlike the toluidines did not produce a coloured solution. These results suggest that at pH 2·0 the *ortho* and *para* positions of the aromatic ring were involved in the oxidation of aniline. Hydrazobenzene was quantitatively oxidized to azobenzene and, at pH 2·0, *p*-hydroxyaniline (*p*-aminophenol) was converted into *p*-benzoquinone, which was not oxidized further at this pH (Table 4). Unlike most aniline derivatives, these two compounds consumed only 1 mol. of periodate, because the initial product of oxidation was resistant to further attack under these conditions. The oxidation of hydrazobenzene consisted simply of

Table 6. *Periodate oxidation of aromatic derivatives of glycine and related compounds*

Experimental details are given in the text. *N*-(2,4-Dinitrophenyl)-glycine was not oxidized by periodate under these conditions.

	Consumption of periodate (mol./mol. of compound)						Consumption of periodate (mol./mol. of compound)		
Time (hr.)	Aniline	*N*-Phenyl-glycine	2-Phenyl-glycine	*N*-Benzylidene-aniline	2-Phenyl-*N*-phenyl-glycine	Time (hr.)	*N*-Phenyl-glycine ethyl ester	Anthranilic acid	*N*-(2′-Carboxy-phenyl)-glycine
(*A*) pH2·0									
1	0·47	1·35	0·14	0·15	0·22	0·75	0·22	—	1·20
3	1·28	1·83	—	0·45	0·59	5·00	—	2·14	—
5	—	1·86	—	0·62	0·73	18·5	2·00	2·40	2·57
20	1·95	2·77	0·18	1·35	1·61	42·5	2·75	3·00	3·23
26	2·10	2·82	0·19	1·51	1·71	117·25	3·50	3·40	3·50
48	2·32	3·15	0·25	1·63	2·10	162·0	3·93	3·80	—
77	2·42	3·30	0·26	1·71	2·42	211·0	—	3·93	4·30
138	2·54	3·50	0·29	1·78	2·83				
192	2·61	3·61	0·33	1·86	—				
(*B*) Unbuffered									
2	2·03	2·90	0·45	0·17	0·87	0·75	0·27	—	1·75
19	2·50	3·36	—	0·45	1·45	5·00	—	2·49	—
42	2·57	3·51	—	0·95	1·83	18·50	1·27	3·26	3·58
67	2·70	3·56	0·53	1·47	2·18	42·50	1·70	3·64	4·32
91	2·71	3·61	0·62	1·98	2·66	117·25	2·68	4·46	5·20
170	2·84	3·74	0·72	3·42	4·15	162·00	2·81	4·75	5·50
						211·00	3·00	4·97	5·70
Final pH	(4·30)	(4·05)	(6·20)	(3·80)	(3·55)		(4·05)	(3·95)	(3·85)
(*C*) pH9·0									
2	0·55	1·86	0·20	—	1·65	0·75	0·10	—	1·46
19	1·39	2·93	0·47	0·14	3·16	5·00	—	0·34	—
42	1·84	3·30	—	0·21	3·64	18·50	0·42	0·37	2·03
67	2·10	3·50	0·55	0·34	3·96	42·50	1·03	0·53	2·56
91	2·29	3·62	0·61	0·45	4·18	117·25	2·45	0·81	2·57
						162·00	3·08	1·09	2·78
						211·00	3·57	1·17	—

Table 7. *Evolution of carbon dioxide during periodate oxidation of aromatic derivatives of glycine and related compounds*

Experimental details are given in the text. All oxidations were carried out under unbuffered conditions except where otherwise indicated.

	Production of CO_2 (mol./mol. of compound)						
				Anthranilic acid		*N*-(2′-Carboxyphenyl)-glycine	
Time (hr.)	*N*-Phenylglycine	2-Phenyl-*N*-phenylglycine	2-Phenyl-*N*-phenylglycine ethyl ester	Unbuffered	pH 2·0	Unbuffered	pH 2·0
0·1	0·12	—	—	—		0·02	
0·25	0·35	0·48	—	0·03		0·21	
0·4	0·62	0·52	—	0·08		0·23	
0·5	0·69	0·56	—	0·10	0·10	0·25	0·33
1·0	0·72	0·66	—	0·12		0·31	
1·5	0·73	0·72	—	0·13		0·37	
2·0	0·74	0·75	—	0·14	0·22	0·42	0·67
4·0	0·77	0·80	0·02	0·16	0·24	0·62	0·83
6·0	0·82	0·83	0·04	0·18	0·27	0·71	0·93
18·0	0·92	0·87	0·08	0·24		1·01	
23·0	—	0·91	0·09	0·26	0·44	1·13	1·31
25·0	0·96	—	—	0·27	0·45	1·15	1·33
44·0	1·03	0·92	0·12	0·33	0·55	1·29	1·51
52·0	—	0·93	—	0·35		1·33	
74·0	1·04	0·97	0·15	0·40	0·65	1·39	1·65
120·0	1·05	—	0·19	0·45		1·42	

the elimination of two hydrogen atoms, to give azobenzene,

$$Ph{\cdot}NH{\cdot}NH{\cdot}Ph \rightarrow Ph{\cdot}N{:}N{\cdot}Ph$$

whereas that of *p*-hydroxyaniline probably proceeded through an intermediary iminoquinone which subsequently hydrolysed to yield

$$HO{\cdot}Ph{\cdot}NH_2 \rightarrow O{:}Ph{:}NH \rightarrow O{:}Ph{:}O + NH_3$$

p-benzoquinone and ammonia. If electron-withdrawing substituents were present on the amino or benzene functions, such as acetyl or nitro groups respectively, periodate oxidation was considerably decreased (Table 5). An *o*-carboxylic acid group, as in anthranilic acid, which might be expected to show a similar deactivating effect, in fact resulted in an increased consumption of periodate (Table 6). The carboxylic acid group, unlike the nitro group, can participate in the reaction and is lost as carbon dioxide in the process (Table 7). Periodate oxidation of aromatic amines may lead to the intermediate production of free radicals, since aniline and many of its derivatives produced transient, highly coloured solutions. Aniline solutions rapidly become purple, and this colour could be extracted with methylene chloride, but eventually a brown precipitate was formed, which was insoluble in water and only slightly soluble in most organic solvents. *NN*-Dimethylaniline, at pH 9·0, did not produce a precipitate, but underwent a series of colour changes, being at first red and finally green.

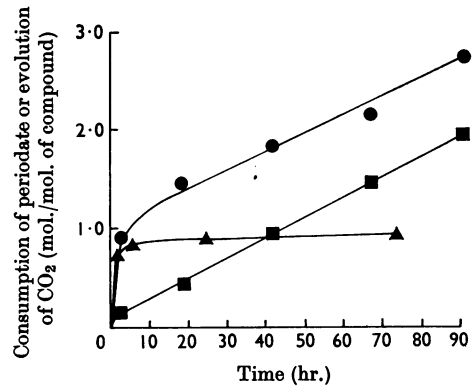
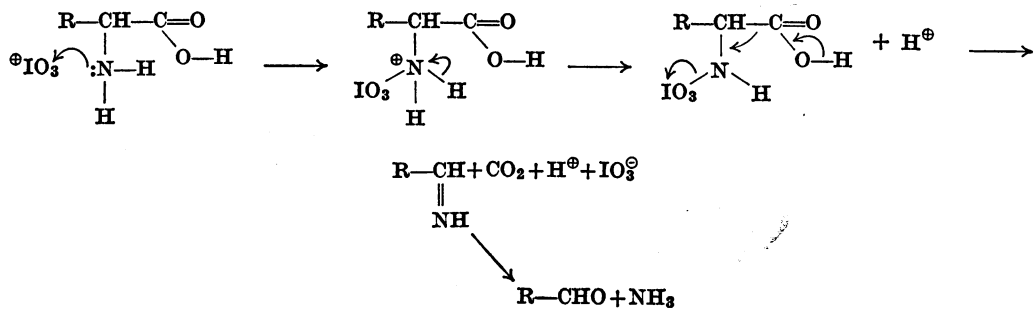


Fig. 1. Consumption of periodate by diphenylglycine (●) and *N*-benzylideneaniline (■). Evolution of carbon dioxide by diphenylglycine (▲) during periodate oxidation.

In view of the fact that periodate oxidation studies have been made on nucleosides (Fox, Yung, Davoll & Brown, 1956) and nucleic acids (Uchida, 1951), the following purine and pyrimidine bases were investigated: adenine, 2,6-diaminopurine, guanine, hypoxanthine, xanthine, uracil, thymine and cytosine. Of these only 2,6-diaminopurine was oxidized, consuming 0·70 mol. of periodate in 90 hr. under unbuffered conditions.

Mechanistic studies (Spenser, Crawhall & Smyth, 1956; Kay & Rowland, 1959) on the reaction of



Scheme 1.

amino acids with various oxidants (but not periodate) have utilized tritium labelling and diphenyl derivatives of glycine. Therefore the above periodate studies were extended to *N*-substituted derivatives of glycine, since it was expected that the free amine would result from the hydrolysis of an intermediary imino compound formed during the periodate oxidation of these derivatives. The release of aromatic amines by this process would result in a greatly increased consumption of periodate, which would be of value in following the course of oxidation. The presence of a phenyl group on the α-carbon atom of glycine (2-phenylglycine) did not markedly alter the rate of oxidation. *N*-Phenylglycine, however, showed an extensive uptake of periodate which was 1 mol. greater than that of aniline. 2 - Phenyl - *N* - phenylglycine (diphenylglycine) also consumed an amount of periodate which was 1 mol. greater than *N*-benzylideneaniline. In unbuffered solution the periodate consumption curves of these pairs of compounds are parallel, but 1 mol. greater for *N*-phenylglycine and diphenylglycine, suggesting that aniline and *N*-benzylideneaniline are intermediates in the periodate oxidation of *N*-phenylglycine and diphenylglycine respectively. Carbon dioxide is lost during the consumption of the first mol. of periodate (Fig. 1) by both *N*-phenylglycine and diphenylglycine, giving rise to *N*-formylideneaniline (Ph·N:CH_2) and *N*-benzylideneaniline (Ph·N:CH·Ph) respectively. Many oxidants other than periodate cause decarboxylation of amino acids by the following general reaction:

$$R{\cdot}CH(NH_2){\cdot}CO_2H + O \rightarrow R{\cdot}CHO + NH_3 + CO_2$$

This reaction cannot proceed through an intermediary imino acid since the tritium label on C-2 is retained after oxidation (Spenser *et al.* 1956; Kay & Rowland, 1959). The initial site of attack by a positively charged oxidant would be on atoms with lone pairs of electrons (Levitt, 1955), that is either the nitrogen or oxygen atoms, and the results in Table 6 favour the former, and are consistent with the mechanism shown in Scheme 1. Such a Scheme is consistent with: (1) the resistance to oxidation of an amino group when the nitrogen atom is substituted with an electron-withdrawing group (for example acetyl); (2) the necessity for a hydrogen substituent on the nitrogen atom, and for a vicinal participating group for oxidation to occur; (3) the fact that esterification has little effect upon the oxidation of an amino acid (Clamp & Hough, 1965); (4) the release of 1 mol. of carbon dioxide during the uptake of the first mol. of periodate by *N*-phenylglycine and diphenylglycine; (5) the subsequent oxidation of these glycine derivatives through aniline and *N*-benzylideneaniline respectively.

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