Selective Enzymatic Oxidation of Aromatic Methyl Groups to Aldehydes

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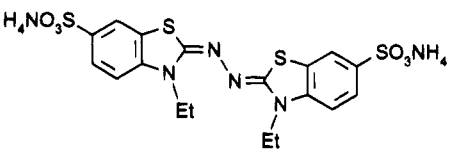
Oxidation of methyl groups on aromatic rings is a frequently used procedure in organic synthesis. However, attempts to achieve a selective oxidation are met with difficulties.[3] Most commonly used are transition metal oxidants. In these cases, the initial oxidation products are often more susceptible to oxidation than the starting material. Once a methyl group is attacked, it is likely to be oxidized to the carboxylic acid. While such reactions readily give benzoic acids in high yields, they are rather difficult to stop at the aldehyde stage. Several special procedures have been developed to suppress further oxidation of the aldehyde to the corresponding acid. Basically, there are two approaches: first, the use of special oxidants that oxidize the aromatic methyl group to the aldehyde, but not further,[4] and second, the application of specific reagents that trap the aldehyde formed.[5] However, all of these procedures exhibit some disadvantages, such as the need for drastic reaction conditions and rather poor yields.

The following method for the selective oxidation of methyl groups on aromatic rings to the corresponding aldehydes is to employ oxygen with laccase/ABTS-$(NH_4)_2$[6] as a catalyst. The finding that oxygen in the presence of this catalyst system is capable of converting aromatic methyl groups into formyl groups is of great importance with respect to the increasing interest in reactions carried out under mild conditions. Since no comparable method with such broad applicability exists, it will certainly find wide acceptance in organic synthesis.

The enzyme laccase (benzenediol:oxygen oxidoreductase; EC 1.10.3.10) is a blue metalloglycoprotein containing four copper atoms in the active sites. It has been intensively investigated because of its ability to degrade biopolymeric structures[7] and because of its usefulness in the synthesis of organic compounds.[8] Laccase is acting as catalyst accomplishing the four-electron transfer from the substrate to molecular oxygen which is reduced to water.[9] Since laccase alone shows no reactivity toward nonphenolic substrates, it is commonly applied together with a cosubstrate or "mediator", mostly the diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS-$(NH_4)_2$[10] (**1**). The mediator acts as a single-electron donor and activator of the enzyme, but does not function as oxidant of the substrate.[11]

Table 1. Representative Examples of Benzyl Alcohols Oxidized to the Corresponding Benzaldehydes by Molecular Oxygen with Laccase/ABTS as Catalyst

starting material	product obtained	yield (%)
toluene	benzaldehyde	92
p-nitrotoluene	*p*-nitrobenzaldehyde	98
m-chlorotoluene	*m*-chlorobenzaldehyde	89
3,4-dimethoxytoluene	3,4-dimethoxybenzaldehyde	90



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The advantages of this enzymatically catalyzed oxygenation are substantial in comparison to existing methods for the oxidation of methyl groups on aromatic rings. The reaction proceeds under physiological conditions[12] at atmospheric pressure and at room temperature. No sidereactions occur because of the high specificity of the catalyst.[13] However, the oxygen-sensitive aldehydes formed may be subject to autoxidation to minor extent by small excessive amounts of oxygen.[14] The average overall yield of aldehydes is about 90%. Since only small amounts of chemical auxiliaries are applied, which remain in the aqueous phase after extraction of the

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(3) For a review see: *Organic Synthesis by Oxidation with Metal Compounds*; Mijs, W. I., de Jonge, C. R. H. I., Eds.; Plenum Press: New York, 1986.

(4) To achieve selective oxidation to the aldehydes, benzeneselenic acid is sometimes referred to as the oxidant of choice. However, yields are poor to fair, and prolonged reaction times have to be applied. For representative examples see: Barton, D. H. R.; Hus, R. A. H. F.; Lester, D. J.; Ley, S. V. *Tetrahedron Lett.* **1979**, 3331.

(5) Most commonly, the benzaldehyde formed is trapped with glacial acetic acid or acetic anhydride as benzylidene diacetate. Among the oxidants used in this method, chromic acid and chromyl acetate give superior results: Nishimura, T. *Org. Synth.* **1955**, *4*, 713.

(6) Laccase from *Coriolus versicolor* was purchased from Mercian Corp., Tokyo, Japan. ABTS-$(NH_4)_2$ [diammonium salt of (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was purchased from Aldrich, Milwaukee, WI.

(7) Moroshi, N.; Wariishi, H.; Muraiso, C.; Nagai, T.; Haraguchi, T. *Mokuzai Gakkaishi* **1987**, *33*, 218–225.

(8) Potthast, A.; Rosenau, T.; Chen, C.-L.; Gratzl, J. S. *Tetrahedron Lett.*, submitted for publication.

(9) The structure of the active site and the processes during oxygen reduction were elucidated in the remarkable works of Solomon et al. For reviews see: Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem. Rev.* **1992**, *92*, 521–542. Solomon, E. I.; Lowery, M. D. *Science* **1993**, *259*, 1575–1581.

(10) The use of other compounds as the mediator for laccase has been described: Call, H. P. International Patent No. WO 92/20857 (PCT/EP92/01086). Bourbonnais, R.; Paice, M. G. *FEBS Lett.* **1990**, *267*, 99–102.

(11) The previous suggestion of ABTS acting as the oxidant must be reevaluated in light of the results obtained (compare to: Jurasek, L.; Archibald, F. S.; Bourbonnais, R.; Paice, M. G.; Reid, I. D. *Appl. Env. Microbiol.* **1993**, *59*(1), 260–266. Call, H. P.; Mücke, I. *Proc. TAPPI Pulping Conf.* Nov 6–10, 1994, San Diego, CA). The investigations carried out in our group demonstrate that, by transferring one electron to the enzyme, the mediator initiates the ability of the enzyme to accomplish the electron transfer from the substrate to dioxygen in two-electron transfer processes. Manuscript in preparation.

(12) The reactions were carried out with numerous methyl aromatics. Usually, 10–20 mmol of the substrate, 0.1 mmol of ABTS-$(NH_4)_2$, and 0.3 mL of laccase solution (laccase activity, 1.1 × 10^4/mL, determined by the *p*-hydroxymandelic acid assay method, see: Agematu, H.; Shibamoto, N.; Nishida, H.; Okamoto, R.; Shin, T.; Murao, S. *Biosci. Biotech. Biochem.* **1993**, *57*, 1877) were kept in a 100 mL flask, with 30–50 mL of acetate buffer (pH 4.5) under vigorous stirring. In the case of starting material with limited solubility in water, the substrate was dissolved in 20 mL of freshly destilled THF or dioxane and added to the buffer solution. A sample was taken when the initial blue color of the solution disappeared. If, at this point, not all starting material was converted, an additional 0.3 mL of laccase and 0.01 mmol of ABTS-$(NH_4)_2$ were added. The course of the reaction was monitored by means of GC or GCMS.

(13) In the early stages of the reaction and in the case of an insufficient amount of catalyst present, the corresponding benzyl alcohol was detected, besides the desired benzaldehyde.

(14) To avoid autoxidation, incubation of the reaction mixture with a sufficient amount of dioxygen was preferred, rather than flushing of the reaction mixture with air or oxygen.

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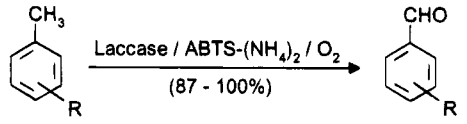
aldehyde with an organic solvent, very pure compounds are obtained requiring no further purification in most cases (Table 1).



R = alkyl (besides Me), alkoxy, nitro, halogen etc.

The major benefit of the reaction lies in its broad applicability. It can be applied to methyl-substituted aromatics, for instance, toluene, and also to methyl-substituted aromatic compounds with + M or − M substituents, such as alkoxytoluenes or nitrotoluenes, respectively, resulting in high yields of the corresponding aldehydes. Phenolic and benzylic hydroxyl groups, as well as aromatic and benzylic amino groups, require protection.[15] The oxidation does not affect other functional groups and structures, for instance, aliphatic OH and NH_2 groups, multiple bonds, and alkyl groups except aromatic methyl groups. The very mild reaction conditions allow the method to be applied to compounds with limited stability. In all cases investigated so far, the oxidation reaction ceased to proceed when the aldehyde was formed, and further oxidation to the acid was not observed.

Presently, investigations are being carried out to extend this selective oxidation to heterocyclic aromatic systems. On the basis of the results obtained so far, laccase/ABTS-promoted oxidations are expected to become a useful tool in organic synthesis.[16]

Supporting Information Available: Procedure and chromatograms of products (6 pages).

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(15) Laccase reacts with phenols and anilines under H-atom (i.e., one proton and one electron) abstraction, leaving behind radicals, which may undergo coupling or other reactions. Benzyl alcohols are oxidized to the aldehydes; see ref 8.

(16) Wong, C. H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 412−432.