Ozonolysis of Pittsburgh Green homoallyl ether

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Ozonolysis of Pittsburgh Green homoallyl ether

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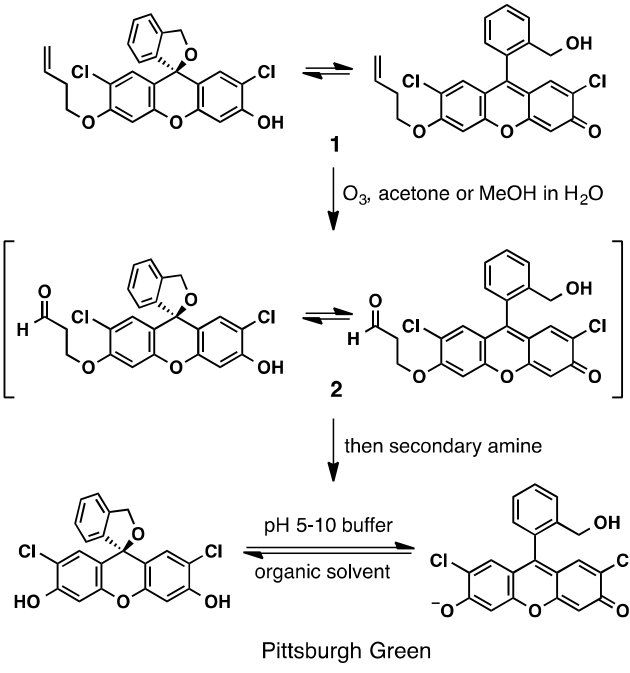
We previously developed Pittsburgh Green homoallyl ether to quantify trace ozone. Independently, problems were reported when the method was used for excess ozone. Here, we discuss the origin of the reported problems and demonstrate that when this method is used according to our previous report, no problems occur.

Fluorogenic or chromogenic chemosensors designed on the basis of a stoichiometric reaction must be used in excess relative to a target analyte to ensure the quantitative nature of the assay method. In other words, only when all the analyte molecules convert a chemosensor to its product, is the amount of the reaction product (= reporter) linearly proportional to the analyte. For example, a reaction-based chemosensor for Cu(II) by the Czarnik group showed a linear response up to 2 μM Cu(II) when the chemosensor concentration was 10 μM.[1] This stoichiometry principle has been widely followed in literature.[2-5]

When ozone is measured using homoallyl ether **1** (Scheme 1), this ether must be used in excess relative to ozone so that the reaction rate is first order and zero order for ozone and ether **1**, respectively.[6] The β-elimination of aldehyde **2** to form Pittsburgh Green was rapid in tissue culture media[6] presumably due to albumin-catalyzed elimination.[7] Although this transformation was slow under non-biological conditions, it could be substantially accelerated by secondary amines.[8] This method was successfully used and improved by the Arakawa group.[9] Since the first report on ether **1**, the ozonolysis of an olefin as part of ozone detection has inspired other research groups to develop new chemosensors for ozone.[5, 10]

In the meantime, others reported potential problems associated with the use of homoallyl ether **1**.[11] First, it was shown that continuous addition of ozone to ether **1** generated 2’,7’-dichlorofluorescein (DCF) in addition to Pittsburgh Green. It should be noted that while we treated ether **1** with less than one equivalent of ozone to form only Pittsburgh Green,[6] they added excess ozone to the same ether.[11] Second, they reported an equilibrium between the spirocyclic form and opened forms of Pittsburgh Green, raising a question about the quantitative nature of the method that we reported. Pittsburgh Green forms its spirocycle in $CDCl_3$, the solvent that was used in their study. However, in a pH 7 buffer, Pittsburgh Green exists as the open form (Scheme 1, bottom), and its spirocycle form is undetectable,[12] which was corroborated by the Ahn group.[13] It was stated that we previously reported that Al_2O_3 and triethylamine catalyzed the β-elimination of aldehyde **2** to form Pittsburgh Green.[11] However, our previous report showed that primary and secondary amines could catalyze such elimination and triethylamine was far inferior, and the use of Al_2O_3 was not discussed.[8]



Scheme 1 Reaction pathway for the conversion of homoallyl ether **1** to Pittsburgh Green through aldehyde **2**.

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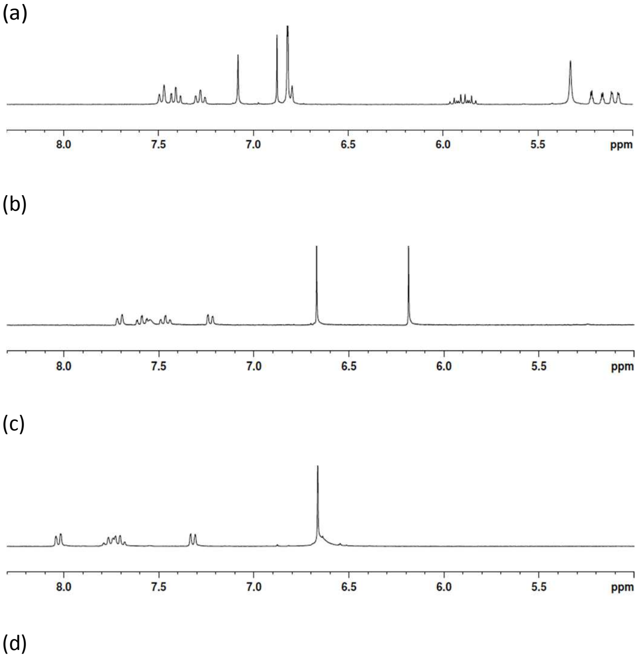
Nonetheless, to experimentally clarify these confounding differences, we decided to further investigate the ozone detection method in question. We first prepared a solution of ether **1** (20 mg, 45 μmol) in MeOH (10 mL) and a saturated solution of ozone in MeOH or acetone. The ozone solutions were prepared by bubbling ozone into a flask containing MeOH or acetone at -78 °C until a faint blue color was observed. We added the saturated ozone solution (3 mL) to the solution of ether **1** at 23 °C and monitored the progress of the reaction by TLC analysis. Upon completion of the reaction with ~10% consumption of ether **1**, an aliquot of the reaction mixture was treated with pyrrolidine (20 μL, 0.24 mmol)[3] and concentrated *in vacuo*. The ^{1}H NMR analysis of this mixture (Fig. 1d) confirmed the absence of DCF (Fig. 1c) and the presence of ether **1** (Fig. 1a) and Pittsburgh Green (Fig. 1b). The addition of Pittsburgh Green or DCF to the product further confirmed the presence of Pittsburgh Green (Fig. 1e) and the absence of DCF (Fig. 1f), respectively. The same reaction was performed in acetone/water (95:5), and the same results were observed.

Table 1 Summary of the oxidation of benzyl alcohol

$$PhCH_2OH \xrightarrow[\textbf{Z}\text{/pH 7 buffer (5:1)}\ \ 0\ ^\circ C]{\textbf{X}\text{; then }\textbf{Y}} PhCHO + PhCO_2H$$

Exp.	**X**	**Y**	**Z**	PhCHO+PhCO$_2$H
A	I_2	none	acetone	not formed
B	O_3 (3 min)	none	acetone	not formed
C	O_3 (3 min)	KI	acetone	formed
D	O_3 (15 min)	none	acetone	formed
E	O_3 (15 min)	none	CH_2Cl_2	formed
F	O_3 (15 min)	none	MeOH	not formed



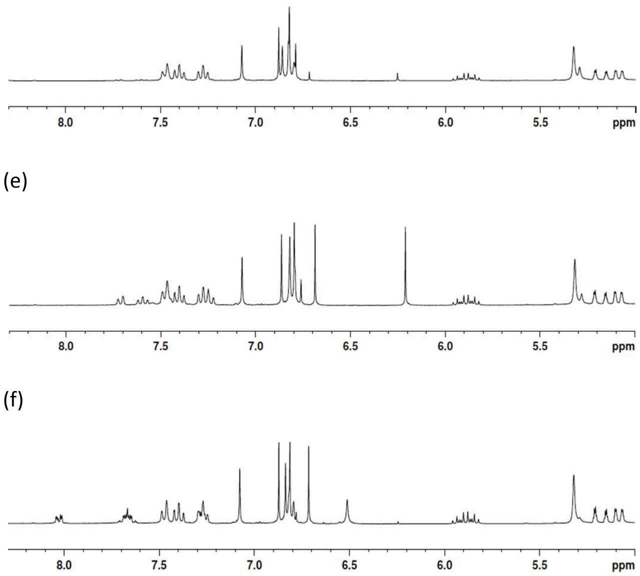


Fig. 1 ^{1}H NMR (300 MHz) spectra of samples in DMSO-d_6 at 293 K. (a) Ether **1** and pyrrolidine; (b) Pittsburgh Green and pyrrolidine; (c) 2',7'-dichlorofluorescein (DCF) and pyrrolidine; (d) Reaction mixture; (e) Reaction mixture and Pittsburgh Green; (f) Reaction mixture and DCF.

To better understand the role played by KI and ozone in the reported oxidation of Pittsburgh Green, benzyl alcohol was chosen as a closely related and better model substrate. We carried out six experiments with benzyl alcohol (41 mg, 0.38 mmol) under the following conditions: in experiment A, I_2 (96 mg, 0.38 mmol), the product of a reaction between KI and ozone,[14-16] was added to a solution of benzyl alcohol in acetone/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL) at 0 °C, and the resulting solution was stirred at the same temperature for 15 min. In experiment B, ozone was bubbled into a solution of benzyl alcohol in acetone/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL) at 0 °C for 3 min and the resulting mixture was stirred for 15 min all at 0 °C. In experiment C, ozone was bubbled into a solution of benzyl alcohol in acetone/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL) at 0 °C for 3 min followed by the addition of KI (~15 mg) and the resulting mixture was stirred for 15 min at the same temperature. In experiments D, E and F, ozone was bubbled into a solution of benzyl alcohol in acetone/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL), CH_2Cl_2/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL) and MeOH/0.5 M pH 7 buffer (5:1 v/v, 1.5 mL) respectively, at 0 °C for 15 min. The progress of these reactions was monitored by TLC analysis. An aliquot of each reaction mixture was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Table 1 summarizes the outcome. The ^{1}H NMR spectroscopic analyses of these crude mixtures indicated that while experiment B did not show the oxidation of benzyl alcohol, in experiment C, ozone in combination with KI led to the formation of benzaldehyde and benzoic acid (Fig. 2d and 2e). This did not occur when benzyl alcohol was treated with I_2

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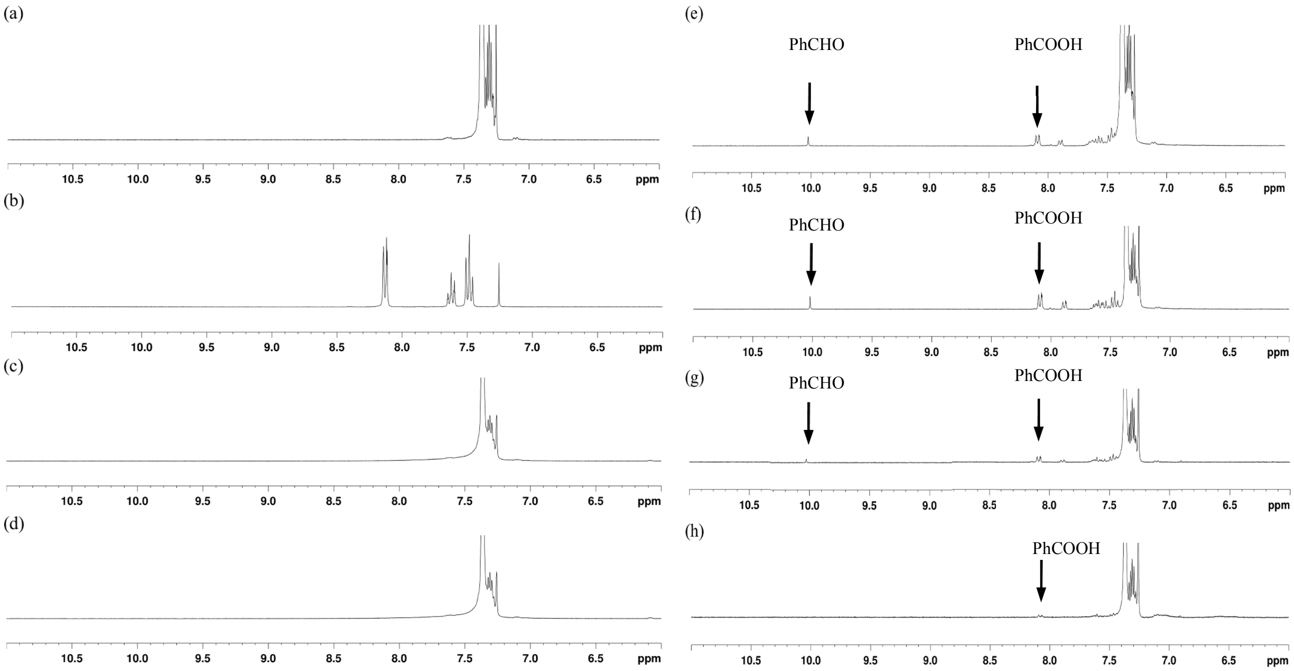

Fig. 2 ^{1}H NMR (300 MHz) spectra of crude reaction mixtures or authentic samples in $CDCl_3$ at 293 K. (a) Benzyl alcohol; (b) benzoic acid; (c) benzyl alcohol and I_2 in acetone/pH 7 buffer (5:1) at 0 °C (experiment A); (d) benzyl alcohol and O_3 in acetone/pH 7 buffer (5:1) at 0 °C (experiment B); (e) benzyl alcohol and O_3 in acetone/pH 7 buffer (5:1) at 0 °C followed by addition of KI (experiment C); (f) benzyl alcohol and excess ozone in acetone/pH 7 buffer (5:1) at 0 °C (experiment D); (g) benzyl alcohol and excess ozone in CH_2Cl_2/pH 7 buffer (5:1) at 0 °C (experiment E); (h) benzyl alcohol and excess ozone in MeOH/pH 7 buffer (5:1) at 0 °C (experiment F).

in the absence of ozone (Fig. 2c). When excess ozone was added, notable amounts of benzaldehyde and benzoic acid were observed (Fig. 2f and 2g) in acetone/water or CH_2Cl_2/water. In Experiment F, it appeared that MeOH was responsible for suppressed oxidation of the substrate (Fig. 2h).

In summary, we investigated the consequence of the ozonolysis of Pittsburgh Green homoallyl ether in the presence of KI or excess ozone. These studies revealed that the combination of ozone and KI or excess ozone by itself could oxidize benzylic alcohols to the corresponding aldehydes and carboxylic acids in acetone or CH_2Cl_2. To our knowledge, this is the first report in which the combination of KI and ozone mediates such oxidation. For simple benzylic alcohols, the combination of ozone and KI or excess ozone alone may be a synthetically viable approach for conversion to benzoic acids. As shown here, such oxidation does not occur under the KI-free reaction conditions that we[6] and others[9] employed in a mixture of a pH 7 buffer and MeOH for the quantification of ozone using ether **1**, further validating these previous publications.

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