

Tomoya Yokoyama · Akihiko Nakagawa  
Fumiko Konishi · Yuji Matsumoto

## Investigation on hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions III: effects of the origin of active oxygen species

Received: February 15, 2011 / Accepted: May 2, 2011 / Published online: August 19, 2011

**Abstract** Carbohydrate model compounds methyl  $\beta$ -D-glucopyranoside (MGP $\beta$ ), methyl  $\alpha$ -D-glucopyranoside (MGP $\alpha$ ), and methyl  $\beta$ -D-mannopyranoside (MMP $\beta$ ) and the deuterium compounds of MGP $\beta$  labeled at the anomeric or C-2 positions (MGP $\beta$ -1D, MGP $\beta$ -2D) were reacted with active oxygen species (AOS) generated in situ by reactions between O<sub>2</sub> and a co-treated phenolic lignin model compound, 4-hydroxy-3-methoxybenzyl alcohol (VAlc), under conditions simulating oxygen delignification (0.5 mol/l NaOH, 0.36 mmol/l Fe<sup>3+</sup>, 1.1 MPa O<sub>2</sub>, 95°C). MGP $\beta$  was degraded more than MGP $\alpha$  but less than MMP $\beta$  when the pairs MGP $\beta$ /MGP $\alpha$  and MGP $\beta$ /MMP $\beta$ , respectively, were treated, which indicates that the configurational differences at the anomeric and C-2 positions influence the reactivity of AOS toward these compounds. When the pairs MGP $\beta$ /MGP $\beta$ -1D and MGP $\beta$ /MGP $\beta$ -2D were treated, no clear kinetic isotope effects were observed in either case. These results contrasted with those obtained when another phenolic compound, 2,4,6-trimethylphenol (TMPh), was used as the AOS generator instead of VAlc under exactly the same conditions. Clear kinetic isotope effects were observed when using TMPh. Because it is not easily accepted that the anomeric and C-2 hydrogen abstractions are minor reaction modes only for AOS generated in the VAlc system, it is suspected that the AOS do not show any clear kinetic isotope effect even though the AOS abstract an objective hydrogen.

**Key words** Active oxygen species · Bleaching · Carbohydrate · Kinetic isotope effect · Lignin · Hydroxyl radical

T. Yokoyama (✉) · A. Nakagawa · F. Konishi · Y. Matsumoto  
Laboratory of Wood Chemistry, Department of Biomaterial Sciences,  
Graduate School of Agricultural and Life Sciences, The University of  
Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan  
Tel. +81-3-5841-5264; Fax +81-3-5841-2678  
e-mail: yokoyama@woodchem.f.p.a.u-tokyo.ac.jp

Part of this article was presented at the 54th Lignin Symposium, Shizuoka, Japan, October 2009, and the 77th Pulp and Paper Research Conference, Tokyo, Japan, June 2010

### Introduction

It is still a problem that oxygen delignification is accompanied by severe damage to carbohydrates. To overcome this problem, it is necessary to further understand the chemistry involved in oxygen delignification. The degradation of carbohydrates is not caused by the direct attack of O<sub>2</sub> but by active oxygen species (AOS), which are mainly generated by reactions between phenolic units in lignin and O<sub>2</sub>.<sup>1–3</sup> Among AOS, hydroxyl and oxyl anion radicals (HO· and O<sup>-</sup>) are believed to be mostly responsible for the degradation of carbohydrates. Several research groups have focused on the reactivity of HO· and have contributed to progress in the understanding of the chemistry of oxygen delignification.<sup>4–15</sup>

It is generally accepted that HO· abstracts a hydrogen connected to a C-2 or C-3 carbon in carbohydrates, which introduces a carbonyl group and results in depolymerization via the  $\beta$ -elimination mechanism.<sup>10</sup> In contrast, Guay et al. proposed that HO· mainly attacks the anomeric position of carbohydrate model compound methyl  $\beta$ -D-glucopyranoside (MGP $\beta$ ), although the mechanism that they proposed should be further examined.<sup>12</sup> Against this background, we initiated a study to examine which position of MGP $\beta$  is predominantly attacked by AOS generated in situ by reactions between O<sub>2</sub> and phenolic compound 2,4,6-trimethylphenol (TMPh) under conditions faithfully simulating practical oxygen delignification.<sup>16,17</sup>

When a pair of carbohydrate model compounds, i.e., MGP $\beta$  and methyl  $\alpha$ -D-glucopyranoside (MGP $\alpha$ ), or MGP $\beta$  and methyl  $\beta$ -D-mannopyranoside (MMP $\beta$ ), was reacted together with AOS generated in situ by reactions between O<sub>2</sub> and TMPh, the degradation of MGP $\beta$  was greater than that of MGP $\alpha$ , whereas MMP $\beta$  was degraded more than MGP $\beta$ , suggesting that configurational differences between each pair of compounds influence the reactivity of AOS toward these compounds.<sup>16,17</sup> Another pair of carbohydrate model compounds, MGP $\beta$  and the corresponding deuterated compound labeled at the anomeric position, methyl  $\beta$ -D-(1-<sup>2</sup>H)glucopyranoside (MGP $\beta$ -1D), or MGP $\beta$  and the

compound labeled at the C-2 position, methyl  $\beta$ -D-(2- $^2$ H) glucopyranoside (MGP $\beta$ -2D), was also treated under conditions identical to those for the treatments of the pairs MGP $\beta$ /MGP $\alpha$  and MGP $\beta$ /MMP $\beta$ . Clear kinetic isotope effects were observed in both cases, which clearly indicate that the anomeric and C-2 hydrogens are abstracted by the AOS.<sup>16,17</sup>

In this article, phenolic lignin model compound 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol, VALc) was applied as the AOS generator instead of TMPH. Pairs of carbohydrate model compounds, MGP $\beta$ /MGP $\alpha$ , MGP $\beta$ /MMP $\beta$ , MGP $\beta$ /MGP $\beta$ -1D, and MGP $\beta$ /MGP $\beta$ -2D, were reacted with AOS generated in situ by reactions between O<sub>2</sub> and VALc under conditions identical with those employed in our previous articles.<sup>16,17</sup> These experiments examined whether the degradation of the carbohydrate model compounds is dependent on the kind of AOS generator, and whether the kind or the amount of AOS generated, or both, in the VALc system is different from those in the TMPH system. Chemical structures of the model compounds used and referred to in this article are shown in Fig. 1.

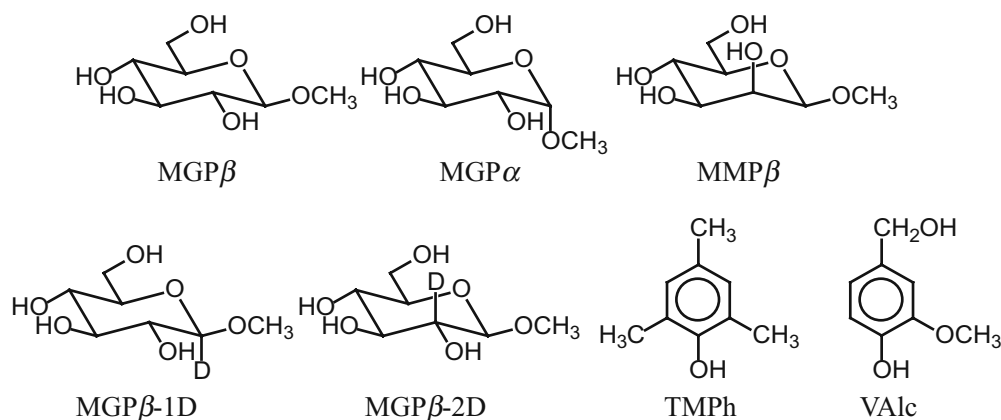
## Materials and methods

### Materials

MGP $\beta$ , MGP $\alpha$ , and TMPH (Tokyo Chemical, Tokyo, Japan) were recrystallized from a mixture of hexane/ethanol, ethanol, and hexane, respectively. MMP $\beta$  hemi-isopropylate was purchased from Toronto Research Chemicals (North York, ON, Canada) and purified as previously described.<sup>17</sup> VALc (Wako, Osaka, Japan) was recrystallized from a mixture of ethanol/hexane. MGP $\beta$ -1D was synthesized from D-glucono-1,5-lactone (Wako).<sup>16</sup> MGP $\beta$ -2D was synthesized from D-(2- $^2$ H)glucose (Cambridge Isotope, Andover, MA, USA).<sup>17</sup>

Semiconductor grades (99.99+%) of NaOH and FeCl<sub>3</sub> (Sigma-Aldrich Japan, Tokyo, Japan) were used without further purification. All the other chemicals (Wako) were used without further purification. Ultra-high-purity water produced by reverse osmosis filtration (Puric-Z, Organo, Tokyo, Japan) was used in all the experiments.

**Fig. 1.** Chemical structure of model compounds used and referred to in this study. MGP $\beta$ , methyl  $\beta$ -D-glucopyranoside; MGP $\alpha$ , methyl  $\alpha$ -D-glucopyranoside; MMP $\beta$ , methyl  $\beta$ -D-mannopyranoside; MGP $\beta$ -1D, MGP $\beta$ -2D, the deuterium compounds of MGP $\beta$  labeled at the anomeric or C-2 positions; TMPH, 2,4,6-trimethylphenol; VALc, 4-hydroxy-3-methoxybenzyl alcohol



### Oxygen-alkali treatment

The reaction solution (300 ml) contained NaOH (0.5 mol/l), FeCl<sub>3</sub> (0.36 mmol/l), VALc (9.0 mmol/l), and a pair of carbohydrate model compounds, MGP $\beta$ /MGP $\alpha$ , MGP $\beta$ /MMP $\beta$ , MGP $\beta$ /MGP $\beta$ -1D, or MGP $\beta$ /MGP $\beta$ -2D (4.0 mmol/l each). The solution was transferred into a Teflon-coated stainless steel vessel (Taiatsu Techno, Tokyo, Japan) and O<sub>2</sub> was pressurized to 1.1 MPa. The vessel was heated to 95°C in 10 min and then maintained at that temperature for 360 min with stirring. The reaction time was defined as 0 when the temperature reached 95°C. At prescribed times, a portion of the solution was withdrawn for the quantification of the model compounds.

### Quantification of residual model compounds

For the quantification of the carbohydrate model compounds, all the work-up and analytical methods were exactly the same with those described in our previous articles.<sup>16,17</sup> VALc was quantified together with the carbohydrate model compounds.

## Results and discussion

The novel features of the reaction system employed in this study have been introduced previously.<sup>16</sup> The AOS generator (VALc) and each pair of the carbohydrate model compounds were treated together, and AOS generated in situ by reactions between O<sub>2</sub> and VALc degraded the carbohydrate model compounds. The degradation of the carbohydrate model compounds observed was compared with those obtained in our previous articles, in which the same pairs of carbohydrate model compounds were reacted with AOS generated in situ by reactions between O<sub>2</sub> and TMPH under the exact same conditions as those employed in this study.<sup>16,17</sup>

MGP $\beta$  and MGP $\alpha$  were found to be stable in oxygen-alkali treatment under conditions identical to those employed in this study when the AOS generator was absent from the system.<sup>16</sup> MMP $\beta$  was slightly labile under the same conditions, even in the absence of the AOS generator when

compared with the above two compounds.<sup>17</sup> However, the lability is small enough to consider that it does not largely affect the degradation of  $\text{MGP}\beta$  and co-treated  $\text{MGP}\beta$  in the reaction with AOS.<sup>17</sup> This information indicates that the degradations of the carbohydrate model compounds observed and discussed in the following sections are mainly caused by AOS generated in situ by reactions between  $\text{O}_2$  and VALc.

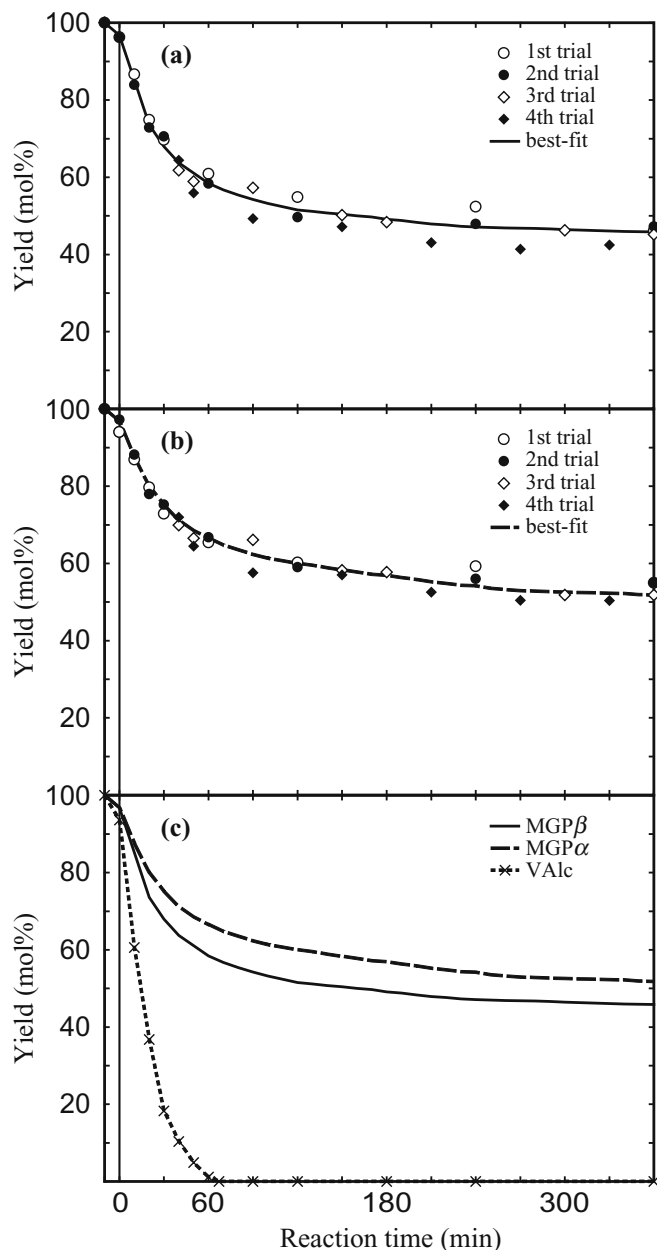
VALc was degraded slower than TMPH was.<sup>16,17</sup> VALc disappeared from the solution at a reaction time of about 60 min (Fig. 2c), while about 45 min was the corresponding time for TMPH.<sup>16,17</sup> This result is in accordance with those

obtained previously.<sup>18</sup> It is considered that AOS readily generated by reactions between  $\text{O}_2$  and VALc are mainly responsible for the degradation of the carbohydrate model compounds until VALc disappears from the solution. Chain-type reactions gradually increase their contribution to the degradation of the carbohydrate model compounds and become dominant after this time.<sup>3,16,19</sup>

Differences in the degradation rates between  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  in the VALc system

$\text{MGP}\beta$  and  $\text{MGP}\alpha$  were reacted together with AOS generated by reactions between  $\text{O}_2$  and VALc to examine the effect of the configurational difference at their anomeric positions on the reaction of these compounds with AOS. The recovery levels of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  are individually shown in Figs. 2a and 2b, respectively, although both compounds were treated together. The lines shown in Figs. 2a and 2b are the best-fit curves that are presented together in Fig. 2c. The degradation of VALc is also shown in Fig. 2c.

The degradation of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  was extensive until VALc disappeared, and slower degradations of these compounds were observed after the disappearance of VALc. A difference in the degradation rates of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  was evident before the disappearance of VALc, and this difference gradually and slightly decreased with progress of the reaction after the disappearance of VALc. It was confirmed that  $\text{MGP}\beta$  is degraded more than  $\text{MGP}\alpha$  because of the configurational difference at their anomeric positions. It was suggested that the configurational difference affects the reactivity of these compounds in the reaction with AOS readily generated by reactions between  $\text{O}_2$  and VALc before the disappearance of VALc but does not largely influence the of chain-type reactions that occur after the disappearance of VALc. These results seem to be similar to those obtained when the AOS generator was TMPH.<sup>16</sup>

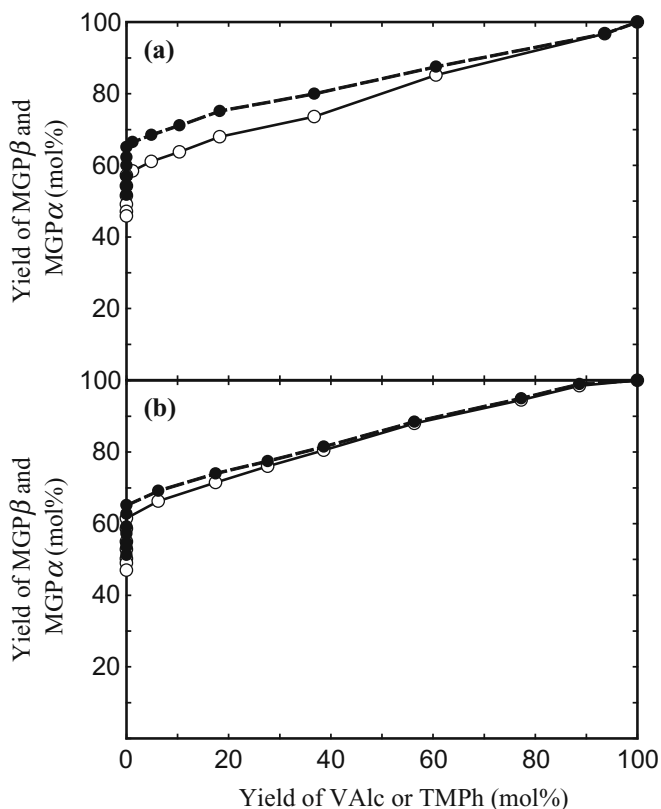


**Fig. 2a-c.** Change in the mol% yield of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  when subjected together to oxygen-alkali treatment in the presence of VALc. **a**  $\text{MGP}\beta$ , **b**  $\text{MGP}\alpha$ , **c** joint indication of two best-fit lines and VALc

Effect of the kind of AOS generator on the degradation of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$

The detailed degradation behaviors of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  in the VALc system are slightly different from those in the TMPH system. Figures 3a and 3b show how the degradation of  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  accompanies the degradation of VALc and TMPH, respectively. Figure 3b was created from the data observed by Konishi et al.<sup>16</sup> The data points on the y-axes show the yields observed after the disappearance of the AOS generators until the end of the reactions.

The difference in the degradation rates between  $\text{MGP}\beta$  and  $\text{MGP}\alpha$  is larger in the VALc system than that in the TMPH system. This larger difference in the VALc system seems to mainly result from the greater degradation of  $\text{MGP}\beta$  in the VALc system than in the TMPH system before the AOS generators disappear. When the AOS generators disappear, the degradation of  $\text{MGP}\beta$  is greater by about 5% in the VALc system than in the TMPH system, whereas  $\text{MGP}\alpha$  seems to be degraded only slightly less in the VALc

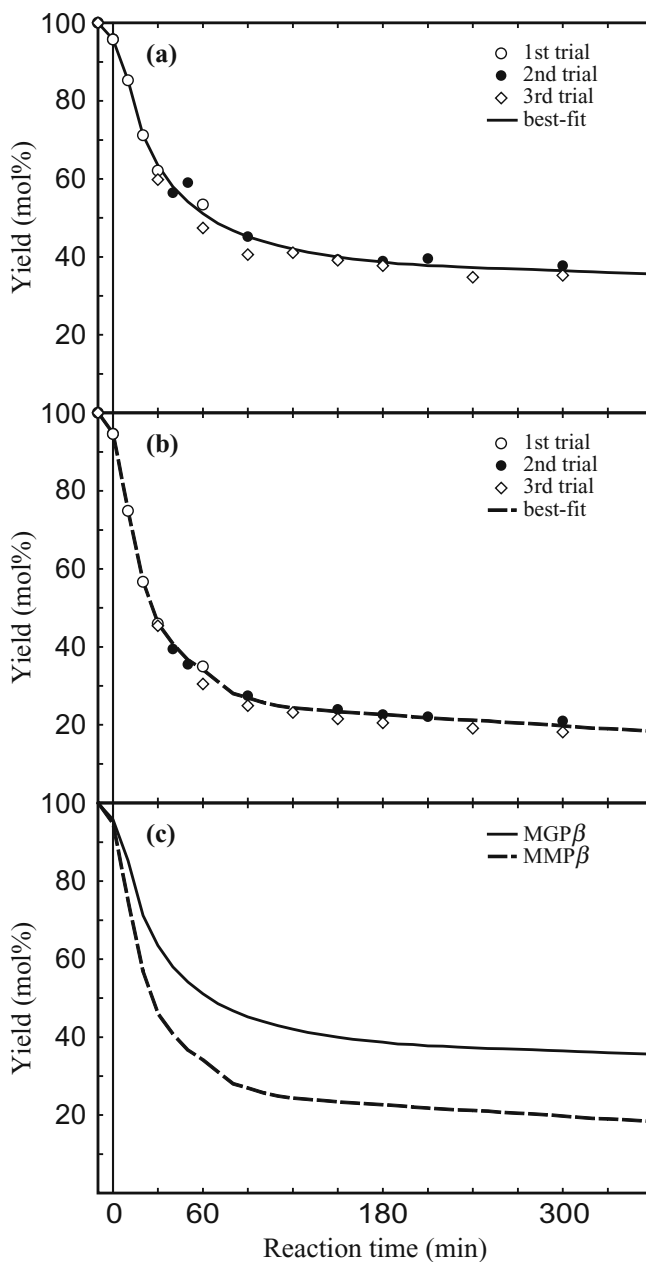


**Fig. 3a,b.** Correlation of the degradations of the carbohydrate model compounds MGP $\beta$  and MGP $\alpha$  and the AOS generator (VALc or TMPH). **a** VALc system, **b** TMPH system, *open circles*, MGP $\beta$ ; *closed circles*, MGP $\alpha$

system than in the TMPH system. However, the yields of MGP $\beta$  and MGP $\alpha$  were not different in both systems at the end of the reactions owing to the smaller degradation of MGP $\beta$  in the VALc system than in the TMPH system after the disappearance of the AOS generators. The evolution of the difference in the degradation rates between MGP $\beta$  and MGP $\alpha$  seems to be complete at a time when about 40% of VALc still remains (Fig. 3a). On the other hand, the difference gradually grows in the degradation rates of MGP $\beta$  and MGP $\alpha$  until TMPH disappears (Fig. 3b). All these observations suggest that the amount or the type of AOS generated, or both, are different for the two reaction systems and this difference results in the different degradations of MGP $\beta$  and MGP $\alpha$  between these systems. The difference in the amount or the type of AOS generated, or both, between these systems influences the degradation of MGP $\beta$  more than that of MGP $\alpha$ .

Difference in the degradation rates between MGP $\beta$  and MMP $\beta$  in the VALc system

MGP $\beta$  and MMP $\beta$  were reacted together with AOS generated by reactions between O<sub>2</sub> and VALc to examine the effect of the configurational difference at their C-2 positions on the reaction of these compounds with AOS. The recovery



**Fig. 4a–c.** Change in the mol% yield of MGP $\beta$  and MMP $\beta$  when subjected together to oxygen-alkali treatment in the presence of VALc. **a** MGP $\beta$ , **b** MMP $\beta$ , **c** joint indication of two best-fit lines

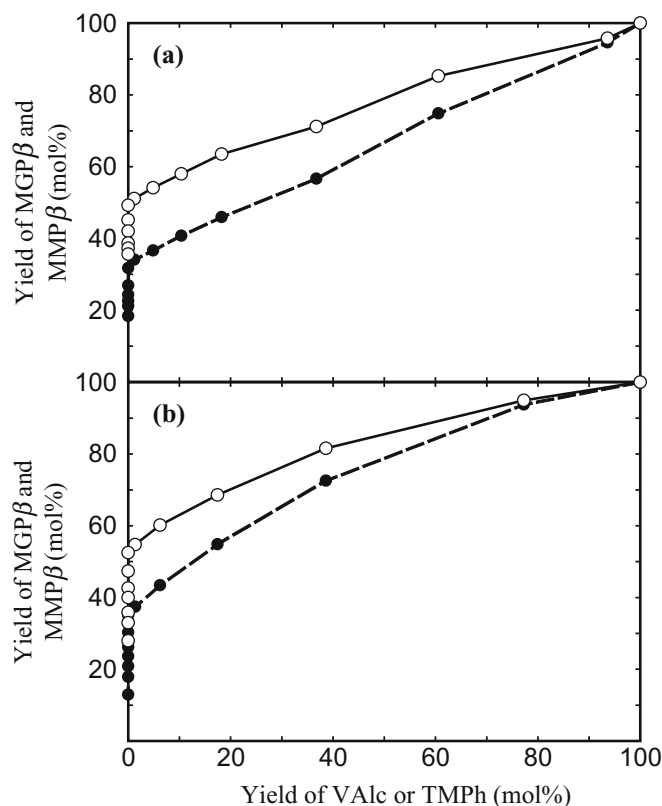
levels of MGP $\beta$  and MMP $\beta$  are individually shown in Figs. 4a and 4b, respectively, although both compounds were treated together. The lines shown in Figs. 4a and 4b are the best-fit curves and are presented together in Fig. 4c. The degradation rate of VALc was almost the same as that shown in Fig. 2c.

The degradation of MGP $\beta$  and MMP $\beta$  was extensive until VALc disappeared, after which slower degradation of these compounds was observed. The difference in the degradation rates between MGP $\beta$  and MMP $\beta$  was evident before the disappearance of VALc, and remained constant after its disappearance. It was confirmed that MGP $\beta$  is

degraded less than  $MMP\beta$  because of the configurational difference at their C-2 positions. It was suggested that the configurational difference affects the reactivity of these compounds in the reaction with AOS readily generated by reactions between  $O_2$  and VALc before the disappearance of VALc, but this difference does not largely influence the operation of chain-type reactions after the disappearance of VALc. The degradation of  $MGP\beta$  and  $MMP\beta$  was much greater than that of  $MGP\beta$  and  $MGP\alpha$  (Fig. 2). It should be noted that the degradation of  $MGP\beta$  was greater when treated together with  $MMP\beta$  than in the co-treatment with  $MGP\alpha$ , although both pairs,  $MGP\beta/MMP\beta$  and  $MGP\beta/MGP\alpha$ , were treated under exactly the same conditions. This fact indicates that the degradation of  $MGP\beta$  is influenced by the other co-existing carbohydrate model compound. It was suggested that the amount or the type of AOS generated, or both, is dependent not only on the AOS generator but also on the substrate of carbohydrate model compound. All these observations seem to be similar to those obtained when TMPH was the AOS generator.<sup>17</sup>

#### Effect of the kind of AOS generator on the degradation of $MGP\beta$ and $MMP\beta$

The detailed degradation behaviors of  $MGP\beta$  and  $MMP\beta$  in the VALc system are slightly different from those in the



**Fig. 5a,b.** Correlation of the degradations of the carbohydrate model compounds  $MGP\beta$  and  $MMP\beta$  and the AOS generator (VALc or TMPH). **a** VALc system, **b** TMPH system, *open circles*,  $MGP\beta$ ; *closed circles*,  $MMP\beta$

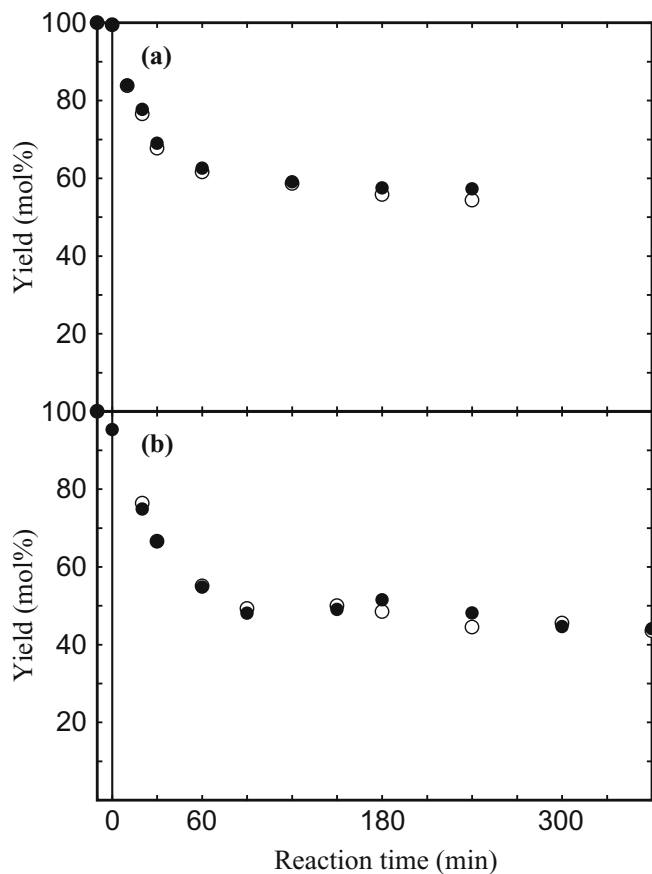
TMPh system. Figures 5a and 5b show how the degradation of  $MGP\beta$  and  $MMP\beta$  accompanies the degradation of VALc and TMPH, respectively. Figure 5b was created from data recorded in a previous study.<sup>17</sup> The data points on the y-axes show the yields observed from the disappearance of the AOS generators until the end of the reactions.

$MGP\beta$  and  $MMP\beta$  are degraded more in the VALc system than in the TMPH system before the AOS generators disappear. The recoveries of  $MGP\beta$  and  $MMP\beta$  are lower by about 3% and 6%, respectively, in the VALc system than in the TMPH system, when each AOS generator has disappeared. On the other hand, the degradation of  $MGP\beta$  and  $MMP\beta$  is smaller in the VALc system than in the other system after the disappearance of the AOS generators, and consequently, more of both compounds remained in the VALc system than in the TMPH system at the end of the reactions. The evolution of the difference in the degradations of  $MGP\beta$  and  $MMP\beta$  seems to be complete by the time about 40% of VALc still remains (Fig. 5a). In contrast, the difference evolves gradually in the degradations of  $MGP\beta$  and  $MMP\beta$  until TMPH disappears (Fig. 5b). All these observations suggest that the amount or the type of AOS generated, or both, is different for the two reaction systems.

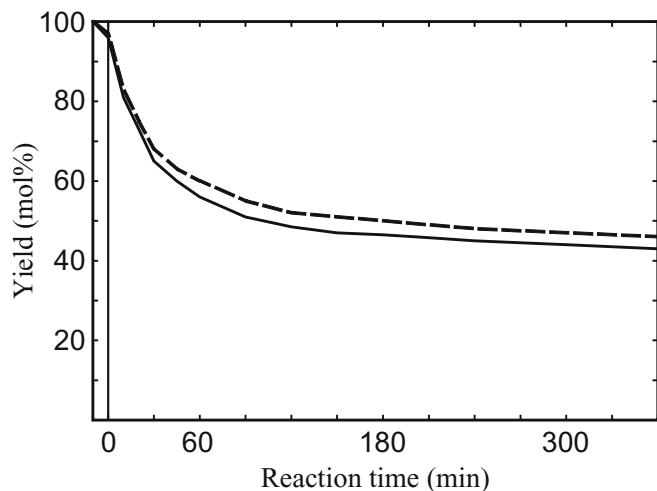
#### Effect of the kind of AOS generator on the hydrogen abstraction reaction by AOS

$MGP\beta$  and  $MGP\beta$ -1D were reacted together with AOS generated by reactions between  $O_2$  and VALc to examine whether the anomeric hydrogen is abstracted by the AOS. Figures 6a and 6b show the recoveries of both  $MGP\beta$  and  $MGP\beta$ -1D in the first and second trials, respectively. The recoveries of  $MGP\beta$  and  $MGP\beta$ -1D in the first trial were not exactly the same as those in the second trial period. However, what is important here is that the degradation of  $MGP\beta$  was not different from that of  $MGP\beta$ -1D in both trials, which confirmed that no clear kinetic isotope effect was observed. This result is in great contrast to that obtained when TMPH was used as the AOS generator.<sup>16</sup> A clear kinetic isotope effect was observed by the greater degradation of  $MGP\beta$  than that of  $MGP\beta$ -1D when TMPH was the AOS generator. Figure 7 shows the recoveries of both  $MGP\beta$  and  $MGP\beta$ -1D when these were reacted together with TMPH, which is a copy of Fig. 6c from a previous article.<sup>16</sup> A difference evolved in the degradation of  $MGP\beta$  and  $MGP\beta$ -1D until TMPH disappeared, and then the degradation remained constant after the disappearance of TMPH. It is safe to conclude that some AOS readily generated by reactions between  $O_2$  and TMPH certainly abstract the anomeric hydrogen, but the same AOS are clearly not generated in the VALc system.

$MGP\beta$  and  $MGP\beta$ -2D were reacted together with AOS generated by reactions between  $O_2$  and VALc to examine whether the C-2 hydrogen is abstracted by the AOS. Figures 8a–c show the recoveries of  $MGP\beta$  and  $MGP\beta$ -2D in the first, second, and third trials, respectively. In all three trials, the degradation of  $MGP\beta$ -2D was not greatly different from

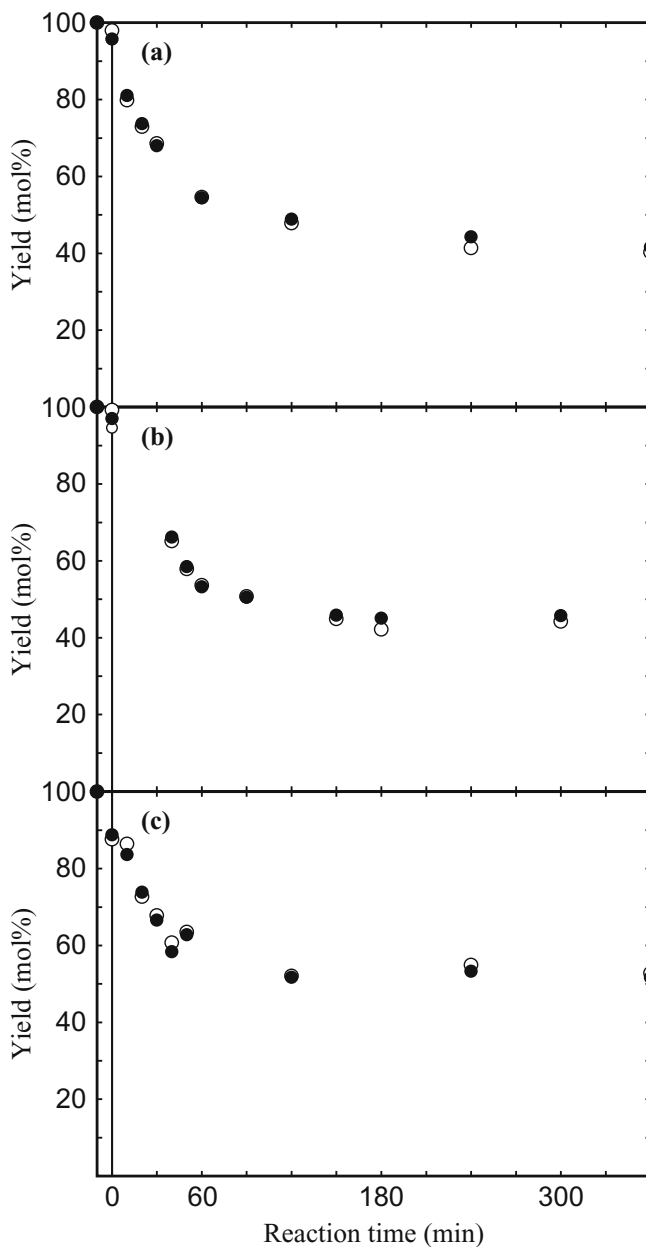


**Fig. 6a,b.** Change in the mol% yield of MGP $\beta$  and MGP $\beta$ -1D when subjected together to oxygen-alkali treatment in the presence of VALc. Open circles, MGP $\beta$ ; closed circles, MGP $\beta$ -1D, **a** first trial, **b** second trial



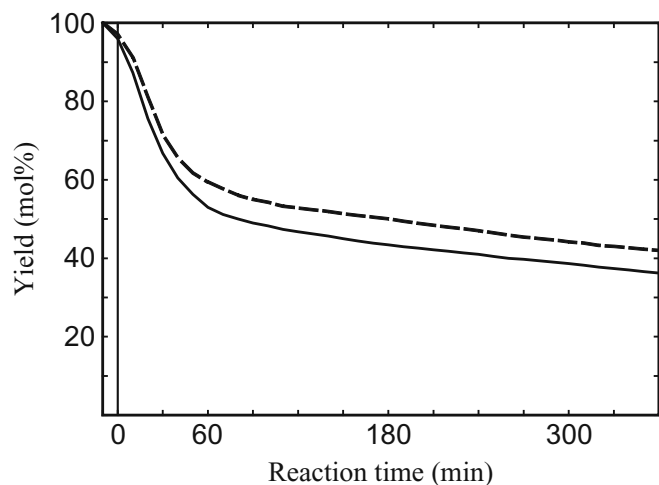
**Fig. 7.** Change in the mol% yield of MGP $\beta$  and MGP $\beta$ -1D when subjected together to oxygen-alkali treatment in the presence of TMPH. This figure is the same as Fig. 6c in a previous article.<sup>16</sup> Solid line, MGP $\beta$ , dashed line, MGP $\beta$ -1D

that of MGP $\beta$ ; this small uncertain difference was not enough to positively judge that a kinetic isotope effect was observed. This result is in great contrast to that obtained when the AOS generator was TMPH.<sup>17</sup> A clear kinetic



**Fig. 8a-c.** Change in the mol% yield of MGP $\beta$  and MGP $\beta$ -2D when subjected together to oxygen-alkali treatment in the presence of VALc. Open circles, MGP $\beta$ ; closed circles, MGP $\beta$ -2D, **a** first trial, **b** second trial, **c** third trial

isotope effect was observed by the greater degradation of MGP $\beta$  than that of MGP $\beta$ -2D when TMPH was the AOS generator. Figure 9 shows the recoveries of both MGP $\beta$  and MGP $\beta$ -2D when these were reacted together with TMPH; this figure is a copy of Fig. 5c from a previous article.<sup>17</sup> The difference in degradation between MGP $\beta$  and MGP $\beta$ -2D evolved until TMPH disappeared, and remained constant after its disappearance. It is safe to conclude that some AOS readily generated by reactions between O<sub>2</sub> and TMPH certainly abstracted the C-2 hydrogen, but the same AOS are not generated in the VALc system.



**Fig. 9.** Change in the mol% yield of MGP $\beta$  and MGP $\beta$ -2D when subjected together to oxygen-alkali treatment in the presence of TMPh. This figure is the same as Fig. 6c in a previous article.<sup>17</sup> *Solid line, MGP $\beta$ , dashed line, MGP $\beta$ -2D*

Consideration of the absence of a clear kinetic isotope effect in the VALc system

In our previous studies, MGP $\beta$ /MGP $\beta$ -1D and MGP $\beta$ /MGP $\beta$ -2D were subjected to alkaline H<sub>2</sub>O<sub>2</sub> treatments under conditions similar to those employed in this study (0.5 mol/l NaOH, 0.36 mmol/l Fe<sup>3+</sup>, 58.8 mmol/l H<sub>2</sub>O<sub>2</sub>, 95°C) except that no AOS generator was added and the O<sub>2</sub> was not pressurized.<sup>16,17</sup> Several radicals were generated in this system via the decomposition of H<sub>2</sub>O<sub>2</sub>. It has reasonably been assumed that O<sup>-</sup> is the main, and most likely the only, AOS that can attack the carbohydrate model compounds in alkaline H<sub>2</sub>O<sub>2</sub> treatments.<sup>17</sup> No clear kinetic isotope effects were observed in alkaline H<sub>2</sub>O<sub>2</sub> treatments of each pair of carbohydrate model compounds.<sup>16,17</sup>

There are three possible phenomena that could result in the observation of no kinetic isotope effects in the degradations between MGP $\beta$  and MGP $\beta$ -1D and between MGP $\beta$  and MGP $\beta$ -2D both in the VALc system and in the alkaline H<sub>2</sub>O<sub>2</sub> treatment (reaction with O<sup>-</sup>): (1) the abstractions of the anomeric and C-2 hydrogens are actually minor reaction modes for AOS generated in the VALc system and O<sup>-</sup>, (2) the AOS and O<sup>-</sup> do not show any clear kinetic isotope effect even though these species certainly abstract an objective hydrogen, (3) the AOS and O<sup>-</sup> do not directly abstract a hydrogen connected to a carbon of MGP $\beta$  but primarily the hydrogen from a hydroxyl group. Because (1) is not easily accepted, (2) and (3) seem to be more relevant. We are now trying to examine the possibilities of (2) and (3).

It can be safely concluded that at least some AOS other than O<sup>-</sup> that are generated in the TMPh system, but not in the VALc system, certainly abstract the anomeric and C-2 hydrogens of MGP $\beta$ . It is suspected, however, that AOS

generated in the VALc system also abstract these hydrogens without showing clear kinetic isotope effects.

**Acknowledgments** The authors gratefully acknowledge financial support from the Japan Society for the Promotion of Science [Grant-in-Aid for Young Scientists (A), No. 20688007].

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