HPLC and LC–MS Studies of the Transesterification Reaction of Methylparaben with Twelve 3- to 6-Carbon Sugar Alcohols and Propylene Glycol and the Isomerization of the Reaction Products by Acyl Migration

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Abstract

Sugar alcohols and parabens are commonly used ingredients in oral suspension formulations. However, their possible incompatibility because of transesterification reaction is a concern during formulation development. In order to gain more knowledge about the reaction, a high-performance liquid chromatographic (HPLC) method is developed to separate the transesterification reaction products of methylparaben preservative with twelve 3- to 6-carbon sugar alcohols and propylene glycol. It is found that the number of peaks separated or partially separated correlate well with the number of distinct hydroxyl groups present in the sugar alcohol molecules. This means that all the hydroxyl groups in a sugar alcohol molecule can react with methylparaben to form transesterification reaction products. These products are positional isomers that have identical UV spectra with a maximum at 255 nm and the same *m/z* ratio for molecular ions by liquid chromatography–mass spectrometry. When isolated individually, they can isomerize (interconvert) under suitable conditions to form other positional isomers by intramolecular acyl migration. The acyl migration pathway for each of the isolated positional isomers from the transesterification reaction of methylparaben with sorbitol, ribitol, and xylitol is followed by HPLC. Based on the information, a tentative assignment of the six isomer peaks generated from the transesterification reaction between methylparaben and sorbitol is proposed.

Introduction

Sugar alcohols (also known as polyols) such as sorbitol and mannitol are among the most widely used excipients in the pharmaceutical industry. Parabens (a group of alkyl esters of *p*-hydroxybenzoic acid) are commonly used antimicrobial agents in pharmaceuticals, food, and cosmetics. During the sta-

bility study of an experimental batch of an oral suspension formulation that contained sorbitol and paraben preservatives (methylparaben and propylparaben), extra peaks at a level of ~1% of the area of the main drug peak were found near the solvent front of the high-performance liquid-chromatographic (HPLC) chromatograms for samples stored at 30°C for one year. In a subsequent investigation, it was demonstrated that similar peaks could be generated in a mixture of sorbitol and the parabens and that the formation of the extra peaks was affected most significantly by pH and temperature. In a commercially available antacid oral suspension that contains sorbitol and the parabens at pH ~8, similar peaks were found at a much higher level.

It has been proposed by others (1–5) that the extra peaks are the transesterification reaction products between paraben and sugar alcohols. Because of the concern about the adverse effect of the reaction on the effectiveness of the paraben preservatives in pharmaceutical formulation, the kinetics of the transesterification reaction has been studied for parabens with sorbitol, xylitol, erythritol, and glycerol (2). The identities of the reaction products have been investigated for methylparaben and xylitol by liquid chromatography (LC)–mass spectrometry (MS), gas chromatography (GC)–MS, and nuclear magnetic resonance (NMR) (3). However, the attempt to identify the reaction products of methylparaben with sorbitol by MS and NMR after isolation by preparative LC was much less a success because of the fact that only three peaks (one major and two minor) were separated and the isolated products were unstable (2). In theory, six reaction products are possible for sorbitol, but these reaction products are positional isomers and difficult to separate. Others also reported only three (3–5) or four (1) peaks. In our investigation, up to six peaks were separated depending on the chromatographic conditions. The difficulty in separating all possible reaction products may be contributed to the incorrect conclusion made by Hensel et al. (3) that three isomers (or three pairs of diastereomers) were expected

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from the reaction between methylparaben and sorbitol. In order to better understand the reaction mechanism between parabens and sugar alcohols, the separation of all possible reaction products is necessary.

In this study, LC and LC–MS methods were developed to separate (or partially separate) and identify (as a group of isomers) all possible reaction products of methylparaben with twelve 3- to 6-carbon sugar alcohols and propylene glycol. During the study, it was noticed that the positional isomers, when isolated individually by fraction collection, could isomerize (convert) reversibly from one to another. A similar isomerization reaction (which involves intramolecular acyl migration on a glucuronic acid ring) has been studied recently by Nicholson and colleagues using LC–NMR (6–11) and also by others (12–14) for a number of glucuronides. However, an acyl migration reaction on linear sugar alcohol molecules have not been reported. In this study, we will show that useful information can be obtained by an HPLC study on the acyl migration reactions of the transesterification reaction products of methylparaben with sugar alcohols.

Experimental

Reagents

All chemicals used in this study were of reagent grade and used without further purification. D-Sorbitol (99%), D-mannitol (+99%), L-iditol (99%), D-threitol (99%), glycerol (+99.5%), and p-hydroxyl benzoic acid (99%) were obtained from Aldrich (Milwaukee, WI); D-xylitol (99%), D-ribitol (99%), D-arabitol (99%), dulcitol (99%), erythritol (99%), 1,2-propanediol (propylene glycol) (99.9%), and methylparaben (99%) were obtained from Sigma (St. Louis, MO); and D-Talitol (+98%) was obtained from TCI America (Portland, OR). Allitol was specially ordered from Omicron Biochemicals (South Bend, IN). Sodium methyl paraben was provided by Merck Research Laboratories (West Point, PA). Tetrahydrofuran (THF) (99.9%) and acetonitrile (HPLC grade) were supplied by EM Science (Gibbstown, NJ). Trifluoroacetic acid (TFA) was purchased from Pierce (Rockford, IL). The deionized water used for the preparation of all the solutions was purified with a Milli-Q water purification system (Millipore, Bedford, PA).

Generation of transesterification reaction products

The transesterification reaction products of methylparaben with the twelve 3- to 6-carbon sugar alcohols were generated as follows. Approximately 50 mg of sodium methylparaben was added to 5 mL of a 60-mg/mL sugar alcohol aqueous solution. After the pH was adjusted to ~11 with a few drops of 1M sodium hydroxide, the solution was heated in a 90°C oven for 2 h. The stress conditions were chosen so that the reaction could reach equilibrium in a short period of time. Similar reaction conditions have been employed by others (3). The sample solution was then cooled to room temperature and filtered through a 0.45-μm polytetrafluoroethylene syringe filter into an HPLC vial.

The transesterification reaction of methylparaben with propylene glycol was generated in the same way except that a much higher concentration of propylene glycol (5.7M) was used and

the solution was kept in the 90°C oven much longer (48 h).

LC

The HPLC system used in this study was an HP 1090 LC (Hewlett-Packard, Palo Alto, CA) equipped with an autosampler and a diode-array detector (DAD). HP Chemstation software was used to acquire and analyze the chromatographic data. In order to achieve the separation of the transesterification reaction products (positional isomers), two Hewlett Packard Zorbax SB-CN (3.5 μm) columns were coupled in series (4.6 × 75 mm and 4.6 × 150 mm). Column temperature was kept at 40°C. A mixture of 85:15 (v/v) THF–acetonitrile was used as mobile phase B and 0.1% (v/v) TFA in water as mobile phase A. The injection volume was 20 μL. Chromatograms were acquired at 255 nm with a bandwidth of 4 nm. Three different gradient programs (Table I) were employed in this study. Gradient I was used for the separation of the transesterification reaction products of methylparaben with the twelve 3- to 6-carbon sugar alcohols and for the study on the isomerization of the six reaction products of sorbitol. Gradient II was used for the separation of the reaction products between methylparaben and propylene glycol and also for all the LC–MS analyses. Gradient III was used for the study on the isomerization of the reaction products of xylitol and ribitol. The same flow rate (0.60 mL/min) was used for all three gradients.

Study of the isomerization of the transesterification products

The isomerization of the transesterification reaction products of methylparaben with sorbitol, xylitol, and ribitol were studied. The positional isomers that were generated according to the previously mentioned procedure were separated by HPLC using gradient I for the reaction products with sorbitol and gradient III for the reaction products with xylitol and ribitol. The fraction of each individual positional isomer was collected manually into an ice-chilled 2-mL HPLC vial from the outlet of the DAD. Among the six products generated from the reaction of methylparaben with sorbitol, peaks 1, 2, 3, and 4 were well-separated, but peaks 5 and 6 overlapped significantly. Therefore, only the ascending part of peak 5 and the descending part of peak 6 were collected to minimize cross contamination. Before the

Table I. Gradients Used to Separate the Transesterification Reaction Products and for the Study on the Isomerization of the Reaction Products

Gradient I		Gradient II		Gradient III	
Time (min)	%B	Time (min)	%B	Time (min)	%B
0	0	0	4	0	4
5	0	14	4	17	4
5.5	5	16	20	22	70
6.5	11	36	20	27	70
12	11	38	30	30	4
16	70	40	30	39	4
18	70	42	4		
25	0	51	4		
34	0				

fraction collection of peaks 1, 2, and 5, the tip of the capillary leading out of the detector was rinsed with water to avoid carryover. Because the same analytical columns were used for fraction collection, the amount of sample that could be loaded was limited. Approximately 10 collections were required for each peak in order to obtain a sufficient amount of sample for the study. In order to slow down the isomerization of the isolated positional isomers at the collection stage, the vials were kept on a bed of ice between collections. When the fraction collection was done, the mobile phase in the collected samples was evaporated overnight to dryness with a SpeedVac Plus Concentrator (Model SC210A) (Holbrook, NY). The dry samples were then kept temporarily in a –20°C freezer to minimize isomerization.

In order to study the isomerization (acyl migration) pathway for each isolated isomer (peak), the dry product was redissolved in an appropriate amount of water (150 to 450 µL) to obtain a suitable concentration for the study. Approximately 150 µL of the sample was then transferred to a 200-µL HPLC vial insert, and 20 µL of the sample solution was immediately injected onto the HPLC column to obtain an initial chromatogram before any significant isomerization. A small amount (0.5–2 µL) of 0.01–0.05M NaOH was then pipetted into the sample solution remaining in the HPLC vial insert as it was gently vortexed. The sample was again immediately injected onto the HPLC column. After the run was finished (approximately 45 min after the NaOH addition), a second HPLC injection was made to determine the rate of isomerization and the time intervals for future HPLC injections. The rate of isomerization was visually estimated by the appearance of other isomer peaks and their relative peak sizes as compared with those at equilibrium. If the rate of isomerization was too slow to follow the reaction in a realistic time frame, more NaOH was added to the remaining sample solution. This process was repeated until a suitable rate of isomerization was obtained.

LC–MS analyses

The mass spectra of the transesterification reaction products of methylparaben with the twelve 3- to 6-carbon sugar alcohols and propylene glycol were acquired using a Finnigan (San Jose, CA) LCQ Deca ion-trap MS with an ESI source operated in negative ion mode. The HPLC conditions were mainly the same as those described in the LC section with some modifications. Because TFA was not suitable for LC–MS as a result of the significant suppression of ionization, 0.1% formic acid in water was used as mobile phase A. The mobile phase flow (0.60 mL/min) was split using a tee connected after the columns, and approximately 50 µL/min of the flow was introduced into the ESI source. The separation was carried out with gradient II for all transesterification reaction products. The operating parameters of the ESI source were as follows: the sheath gas flow rate was 60, the auxiliary gas flow rate was 5, the spray voltage 4.5 kV, the capillary temperature 350°C, the capillary voltage –41 V, and the tube lens offset –25 V. Mass spectra were acquired in full-scan MS mode with a mass scan

range of 50 amu. The actual mass ranges were chosen such that the molecular ions of the transesterification reaction products would fall approximately in the center of the 50-amu mass scan range.

Results and Discussion

Separation of transesterification reaction products

The transesterification reaction of methylparaben with sugar alcohols results in the replacement of the methoxyl group of methylparaben by one of the alcohol groups of sugar alcohol, as shown in Figure 1 for sorbitol. In order to understand the transesterification reaction of sorbitol and parabens, other related sugar alcohols were also included in this study. The molecular structures of the twelve 3- to 6-carbon sugar alcohols and propylene glycol with their plane of symmetry are shown in Figure 2. The number of transesterification reaction products that can be formed is in theory dependent on the number of unique hydroxyl groups present in the sugar alcohol
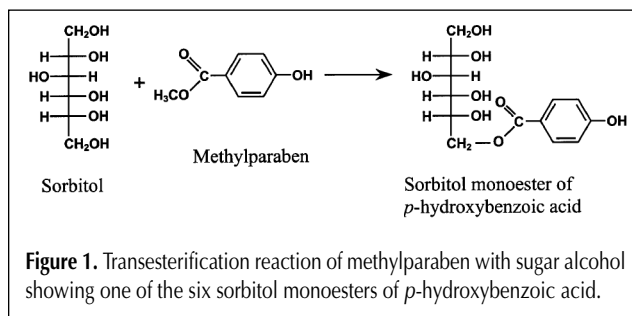

Figure 1. Transesterification reaction of methylparaben with sugar alcohol showing one of the six sorbitol monoesters of p-hydroxybenzoic acid.
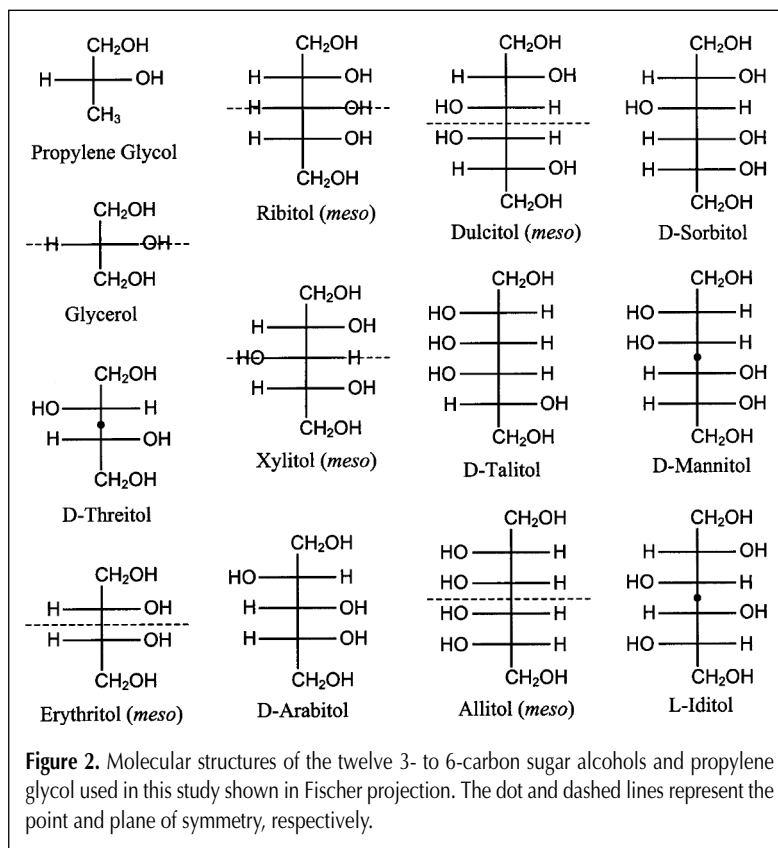

Figure 2. Molecular structures of the twelve 3- to 6-carbon sugar alcohols and propylene glycol used in this study shown in Fischer projection. The dot and dashed lines represent the point and plane of symmetry, respectively.

molecule and the symmetry of the molecule. Allitol, for example, possesses a plane of symmetry between the two middle carbon atoms, and therefore the expected number of transesterification reaction products is three. Sorbitol, talitol, and arabitol, however, have no symmetry element. The number of possible transesterification reaction products should be in theory equal to the number of hydroxyl groups present in the molecule (i.e., six for sorbitol and talitol and five for arabitol). In the previous studies on the transesterification reaction products of methylparaben with sorbitol and other selected sugar alcohols (1–5), C18 columns were used to separate the reaction products. However, because the transesterification reaction products are hydrophilic compounds, their separation using a C18 column is not ideal. In the best case (1), only four out of six possible peaks of the reaction products of methylparaben with sorbitol were separated. In order to increase the retention and improve the separation of the reaction products, cyano columns were used in this study.

During the method development, a Zorbax SB-CN (5 μm, 4.6 × 250 mm) column was first employed and 0.1% TFA in water was used as mobile phase A and pure acetonitrile as mobile phase B. With this system, five peaks (including two majors and three minors) could be separated or partially separated. The two major peaks corresponded well with the two primary reaction products of methylparaben with the two primary hydroxyl groups present in sorbitol because they were expected to be much more reactive than the secondary hydroxyl groups (15). In order to find out if all six hydroxyl groups in sorbitol reacted with methylparaben, a further increase in resolution was required. This was done by coupling in series two Zorbax SB-CN columns (4.6 × 75 mm and 4.6 × 150 mm) with a 3.5-μm particle size. With this setup, one of the three minor peaks broadened significantly, which indicated the possibility of two overlapping peaks. By adjusting the gradient program, that minor peak was resolved into two peaks while the resolution of the two major peaks was completely lost. This experiment showed that all six hydroxyl groups of sorbitol did react with methylparaben to form six transesterification products.

In order to improve the selectivity, mobile phase B (pure acetonitrile) was modified by a few different solvents. The best resolution was obtained with THF as the modifier. Figures 3 and 4 show the separation or partial separation of all possible transesterification reaction products of methylparaben with the twelve 3- to 6-carbon sugar alcohols using 85:15 THF–acetonitrile as mobile phase B and gradient I. It can be seen from these figures that the number of peaks recognizable from the chromatograms correlated well with the expected number of transesterification reaction products. Because the HPLC conditions were optimized for the separation of the reaction products with sorbitol (Figure 3A), they were not ideal for the separation of arabitol and talitol reaction products. In the case of arabitol (Figure 4C), five peaks were indeed found in the chromatogram, although two minor peaks were severely overlapped. For talitol (Figure 3E), two broad minor peaks instead of four were seen in the chromatogram, indicating a possible coelution of the two pairs of minor peaks. It is interesting to note that all the primary reaction products eluted later than the secondary products. This could be related
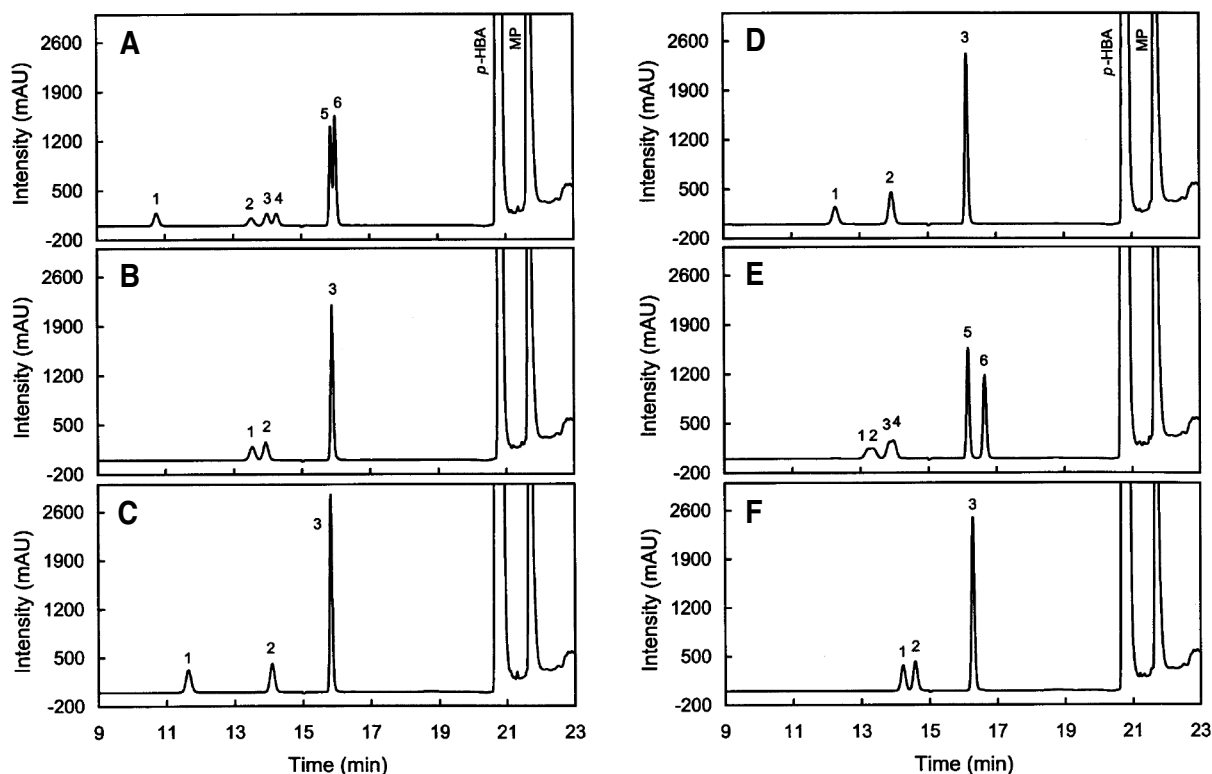

Figure 3. Chromatograms of the reaction products of methylparaben with 6-carbon sugar alcohols: (A) sorbitol, (B) mannitol, (C) iditol, (D) dulcitol, (E) talitol, and (F) allitol.

to the molecular shape rather than the hydrophobicity of the reaction products. When methylparaben reacts with a primary hydroxyl group, the reaction product has a more linear structure, which may allow for a stronger interaction with the stationary phase. It was also observed from the chromatograms that the smaller the sugar alcohol molecule then the longer the retention time of the reaction product because the products become less hydrophilic.

It has been reported that there is no significant reaction between methylparaben and propylene glycol at 90°C and pH 7.3 (2). We found that propylene glycol was indeed much less reactive than sugar alcohols under the same reaction conditions as the twelve 3- to 6-carbon sugar alcohols (i.e., pH ~11 and 90°C for 2 h). In order to clearly show the reaction between propylene glycol and methylparaben, the concentration of propylene glycol was increased to 25 times higher than that of glycerol and the reaction time at 90°C was extended to 48 h. The chromatogram of the two expected reaction products is shown in Figure 5. Because propylene glycol is less hydrophilic than glycerol, its reaction products were found to elute after *p*-hydroxybenzoic acid. In order to obtain a good separation of the reaction products from *p*-hydroxybenzoic acid and methylparaben, gradient II was used.

Theoretically, sugar alcohol monoesters of *p*-hydroxybenzoic acid may react further with methylparaben to form sugar alcohol diesters of *p*-hydroxybenzoic acid because sugar alcohols have more than one hydroxyl group. The mole ratios of methylparaben to the sugar alcohols used in this study ranged from 3:1 to 1:99. If diesters were formed, they would have longer retention times than monoesters because of the presence of two acyl groups. Under the current experimental conditions, however, no such peaks were found in the chromatograms of the transesterification reaction products.

LC–MS study on the transesterification reaction products

In order to ascertain that the peaks separated or partially separated in the chromatograms shown in Figures 3–5 are indeed the transesterification reaction products, the UV and mass spectra of these peaks were acquired by LC–DAD and LC–MS using mobile phase gradient II. It was found that the UV spectra of all the peaks were almost identical to those of methylparaben and *p*-hydroxybenzoic acid with a maximum absorption at 255 nm. This indicated the structural similarity of these reaction products to methylparaben and *p*-hydroxybenzoic acid. The more definitive information was obtained from the LC–MS study.

The initial investigation using LC–MS with electrospray was done in the positive ion mode. However, the mass detector sensitivity could be significantly increased by using electrospray in the negative ion mode even though the mobile phase (0.1% TFA) was acidic. It indicated that the *p*-hydroxyl group of the paraben moiety was easily deprotonated at the electrospray ion source under the current experimental conditions to form the negative molecular ions, (M–H)⁻. Therefore, electrospray in the negative ion mode was employed in this study.

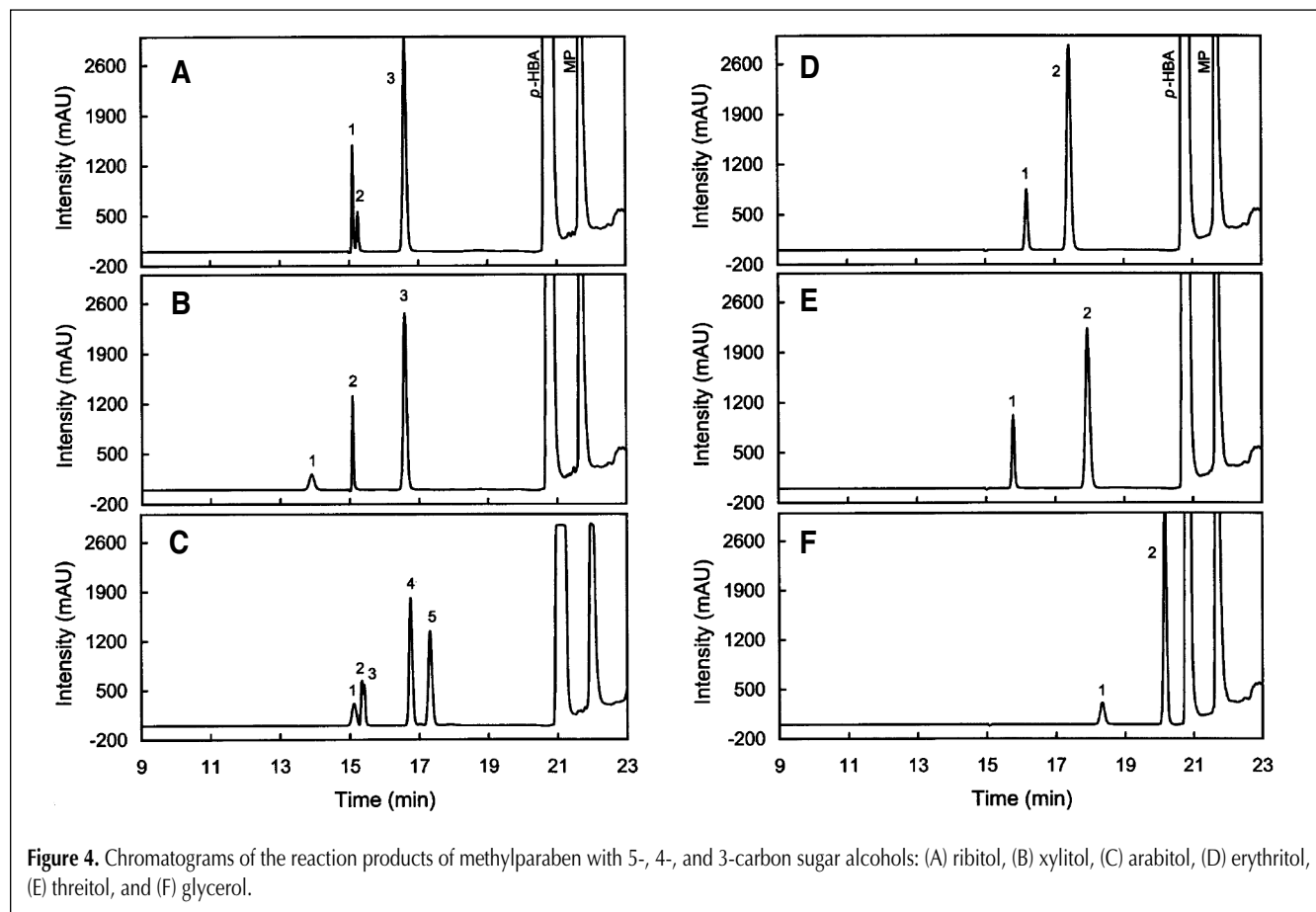Figure 6 shows some typical total ion chromatograms (Fig-

Figure 4. Chromatograms of the reaction products of methylparaben with 5-, 4-, and 3-carbon sugar alcohols: (A) ribitol, (B) xylitol, (C) arabitol, (D) erythritol, (E) threitol, and (F) glycerol.

ures 6A–6C) and mass spectra (Figures 6D–6F) of the transesterification reaction products. Because the mass scan range was limited to 50 amu around the expected molecular ions of the reaction products, *p*-hydroxybenzoic acid and methylparaben were not detected and therefore excluded from the mass chromatograms. All of the peaks shown on the total ion chromatograms were expected to be the reaction products. Because they were acquired using gradient II, the total ion chromatograms were slightly different from those shown in Figures
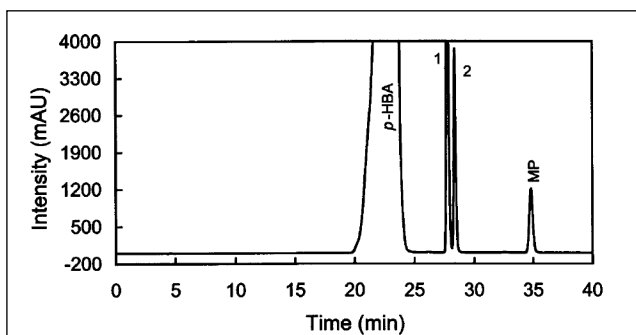

Figure 5. Chromatogram of the reaction products of methylparaben with propylene glycol.

3–5 in terms of peak retention and resolution. For example, the minor peaks 3 and 4 shown in Figure 3A for the reaction products of sorbitol were overlapped on the mass chromatogram in Figure 6C. It was found that all the peaks on the same mass chromatogram had identical mass spectra with the same *m/z* ratio for their molecular ions. The *m/z* ratio matched exactly what was expected based on the molecular weight of sugar alcohol monoesters of *p*-hydroxybenzoic acid. For example, the mass spectra of the five peaks shown in Figure 6C gave the same *m/z* ratio of 301, (M–H)⁻, as shown in Figure 6F, and the molecular weight of the sorbitol monoesters of *p*-hydroxybenzoic acid was 302. The LC–MS data confirmed that all the "unknown" peaks that were separated or partially separated in Figures 3–5 were indeed the transesterification reaction products of methylparaben with sugar alcohols and propylene glycol.

Isomerization of the transesterification reaction products

During this study, effort was made to isolate individually the six positional isomers generated from the reaction of methylparaben with sorbitol by fraction collection for structural identification using other spectroscopic techniques such as NMR. However, it was found that the isolated positional isomers could
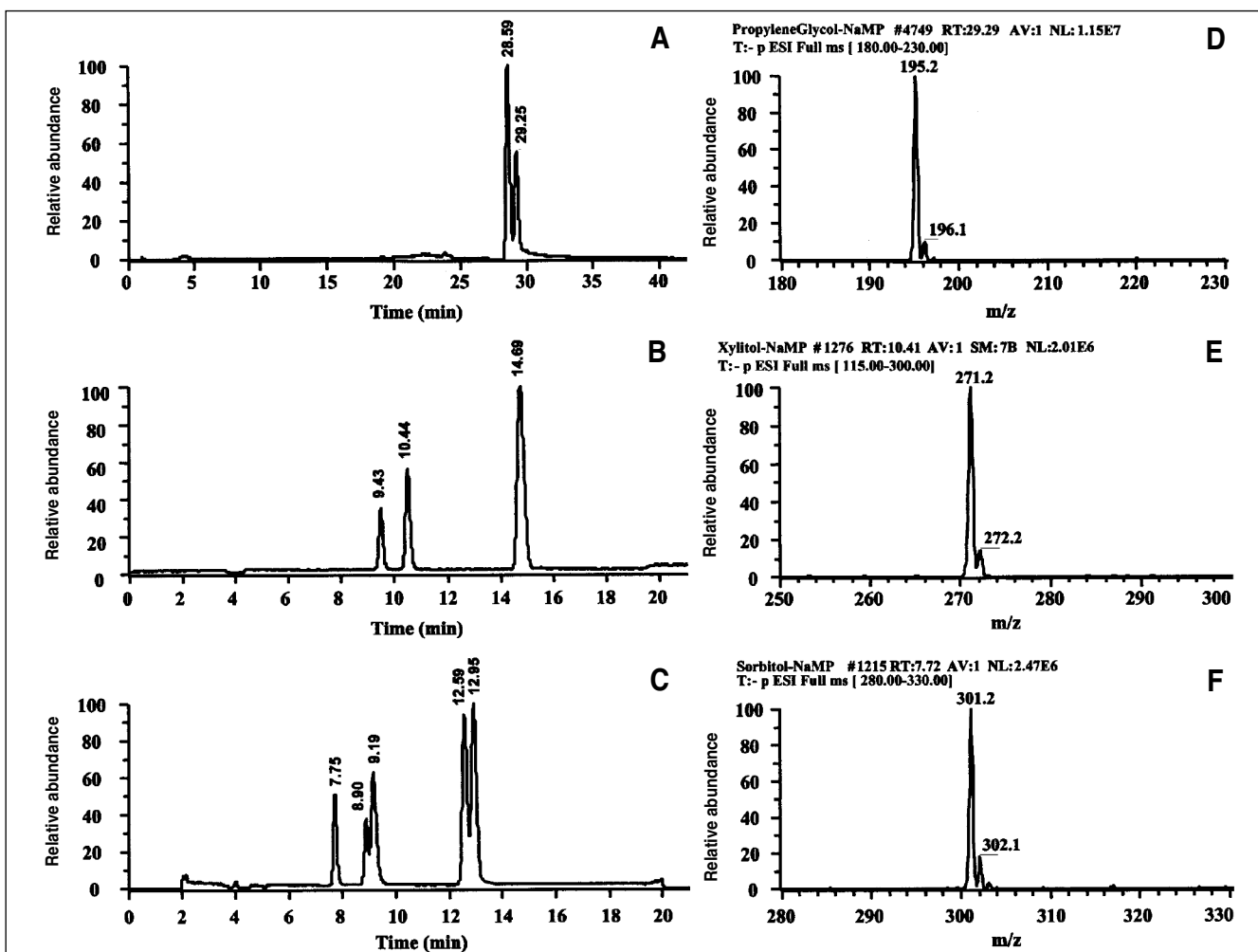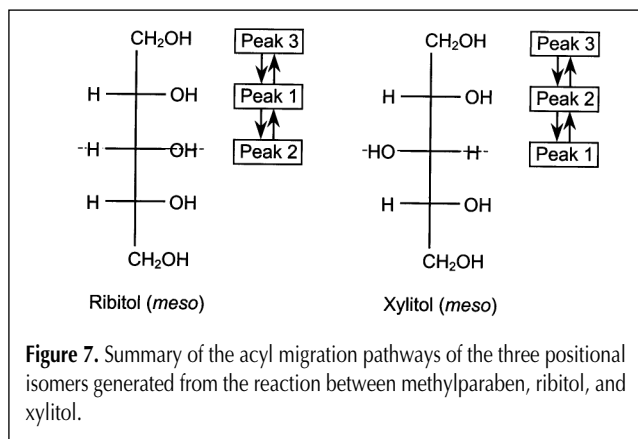

Figure 6. Total ion chromatograms of the transesterification reaction products of methylparaben with (A) propylene glycol, (B) xylitol, and (C) sorbitol. Their typical mass spectra of the isomer peaks are shown in D, E, and F, respectively.

isomerize (convert) reversibly from one to another via intramolecular acyl migration, which under suitable conditions could be followed using the developed HPLC–UV method. For simplicity, a peak and positional isomer were used interchangeably in the following discussion and figures.

After the eluent in the collected fractions was evaporated to dryness using a vacuum concentrator, the dry isomer samples were redissolved in a small amount of water and reinjected immediately onto the HPLC column. In water, the rate of isomerization of the reaction products was slow, but it could be
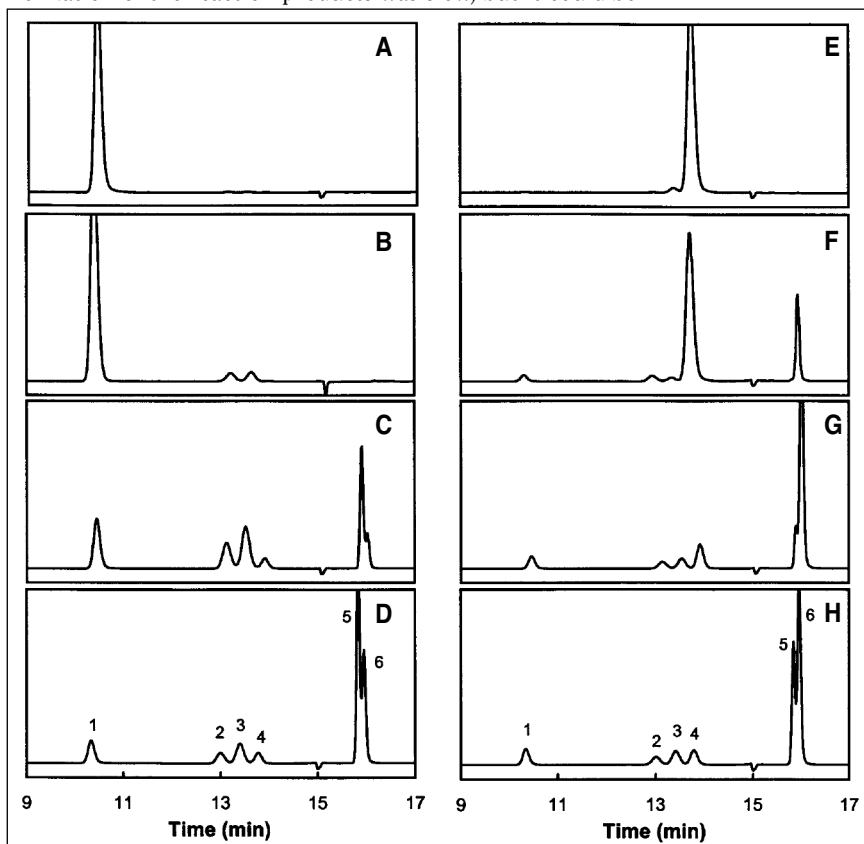

Figure 7. Summary of the acyl migration pathways of the three positional isomers generated from the reaction between methylparaben, ribitol, and xylitol.

drastically increased by the increase in solution pH because the reaction was catalyzed by base. When the pH of the solution containing one isolated isomer was adjusted to approximately 12 using diluted NaOH, the rate of isomerization was so fast that the chromatogram obtained at 15 min after the NaOH addition showed the presence of all six isomer peaks with the distinct relative peak sizes (shown in Figure 3A). No further change in relative peak sizes was found at a later time point. This means that isomerization from one isomer to the other five isomers reached equilibrium.

In order to understand the acyl migration pathway, the isomerization (interconversion) process of the isolated positional isomers from the reaction of methylparaben with ribitol and xylitol was first followed by HPLC because the isomer peaks could be readily assigned based on their relative peak size. For example, there were three isomer peaks as shown in Figures 4A and 4B that correspond with the three distinct hydroxyl groups present in ribitol and xylitol, respectively. Based on the understanding of the relative reactivity of the hydroxyl groups, the largest peak of the chromatogram could be easily assigned to the isomer with the acyl group linked to the primary hydroxyl group, the second largest peak could be assigned to the isomer with the acyl group linked to one of the two equivalent secondary hydroxyl groups, and the smallest peak to the reaction product of the central hydroxyl group. The two secondary isomer peaks should have a peak-area ratio of approximately 2:1 because all of the secondary hydroxyl groups were expected to have similar reactivity. Indeed, the peak-area ratios were found to be 2.3:1 and 1.9:1 for ribitol and xylitol, respectively. The isomerization results show that at each step the acyl group migrated to an adjacent hydroxyl group via a less sterically hindered five-member intermediate as shown in Figure 7, which is similar to acyl migration on a glucuronic acid ring (6–14).

Figure 8 shows typical chromatograms illustrating the isomerization (acyl migration) process of isolated peak 1 and peak 4. In Figures 8A–8D, isolated peak 1 first converted to peaks 2 and 3, which in turn converted to peaks 4, 5, or 6. In Figures 8E–8H, isolated peak 4 initially converted primarily to peak 6 and then to other peaks. This suggests that isomer 1 is on the adjacent hydroxyl group of isomers 2 and 3 and that isomer 4 is on the adjacent hydroxyl group of isomer 6. By following the isomerization process of the other isolated peaks, the complete acyl migration pathway can be obtained. Figure 9 shows two tentative assignments of the peaks based on these observations. Two assignments were given because the two primary monoester isomers cannot be specifically assigned based on this study.

Figure 8. Typical chromatograms of the isomerization process of the isolated transesterification reaction products of methylparaben with sorbitol: (A) isolated peak 1 initially, (B) 1.2 h after the first addition of NaOH (approximately 1.5×10^{-4}M in sample), (C) 17 h after the first addition and 3 min after the second addition of NaOH (totaling approximately 4×10^{-4}M in sample), (D) 1.2 h after the second addition of NaOH, (E) isolated peak 4 initially, (F) 2 h after the addition of NaOH (approximately 4×10^{-4}M in sample), (G) 13 h after NaOH addition, and (H) 66 h after NaOH addition.

Conclusion

This study has demonstrated that all the hydroxyl groups in a sugar alcohol molecule can react with methylparaben to form transesterification reaction products. The number of possible reaction products is dependent on the number of hydroxyl groups present in the sugar alcohol molecule and the symmetry characteristics of the molecule. These reaction products are positional isomers and if isolated individually they can isomerize (convert) reversibly to other isomers. Based on the information obtained from acyl migration reaction, two possible assignments could be made for the six isomer peaks from the transesterification reaction of methylparaben with sorbitol. A definitive assignment of the six isomer peaks may be achieved by an LC–NMR study in the future. Derivatization of an isolated individual positional isomer may also be useful for this purpose.
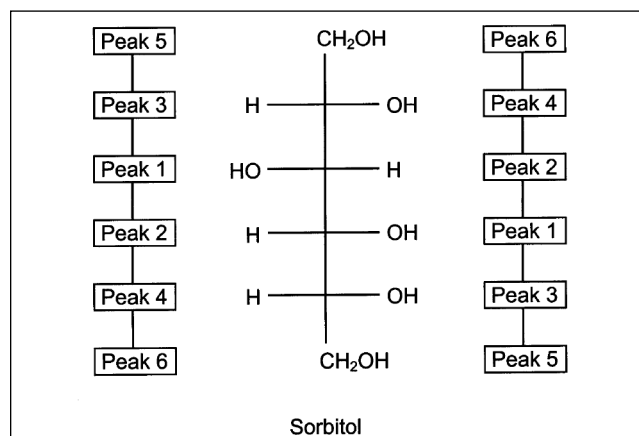

Figure 9. The tentative assignments of the six sorbitol monoesters of *p*-hydroxybenzoic acid to the six isomer peaks.

Acknowledgments

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