Transesterification Reactions of Parabens (Alkyl 4-Hydroxybenzoates) with Polyols in Aqueous Solution

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Abstract □ Accelerated stability tests of aqueous solutions containing parabens and polyols were performed using concentrations similar to pharmaceutical and cosmetic formulations. Reaction products were detected in these solutions by HPLC and identified by chromatographic and spectroscopic means. Using xylitol and methylparaben as model reactants, three unknown peaks having the relation 1:2:4 were obtained together with the hydrolysis product 4-hydroxybenzoic acid. Diode array detection gave identical UV spectra for each peak with a maximum at 255 nm. The structures of the isomeric 1-, 2-, and 3-xylityl 4-hydroxybenzoic acid esters were proved by means of LC–MS, GC–MS, and NMR and correlated to the peaks in the HPLC chromatograms. The rate of the transesterification was shown to be highest in strongly alkaline medium (ph 10–11), whereas equilibration of the reaction was optimally balanced at pH 8–9. An increase of polyol concentration enhanced the formation of the esters. The reactivity of different substituted parabens was higher in the case of parabens with a short alkyl ester function. Similar reaction profiles were observed with C3-C6 polyols, but no transesterification took place when aldoses were used.

Introduction

Parabens, especially the methyl, ethyl, and propyl 4-hydroxybenzoates, are the most common preservatives used for pharmaceuticals, cosmetics, and food products. Beside a high antimicrobical activity, advantages of parabens are found in their low tendency toward adsorption in commonly used plastics of primary packaging material and in their good stability in liquid and semiliquid formulations.

Inactivation of parabens, causing diminished preservation of the formulations by interaction with macromolecules, e.g. polyvinylpyrrolidone, polyoxyethylenesorbitol esters,[1] polyoxyalkalene fatty ethers[2] and certain polysaccharides,[3] has been described.

Additionally, degradation of parabens can occur mainly by pH and ion strength dependent hydrolysis to 4-hydroxybenzoic acid.[4] A further possible decarboxylation at pH 7 results in the formation of phenol.[5] Degradation of parabens by transesterification with ethanolamine[6] and alcohols was described,[7] indicating a sharp decrease in the half-life of parabens. Thus far, no data are available on the exact structural features of transesterification products of parabens with polyols, a class of exipients widely used for isotonization of liquid formulations. The aim of the presented study was to evaluate the conditions leading to these interactions with polyols and to clarify the molecular structures of the resulting products.

Experimental Section

Chemicals and Reagents—All chemicals and solvents for chromatography were purchased from Merck (Darmstadt, Germany) in p.a. or Lichrosolv quality. Polyols and carbohydrates were purchased from Aldrich (Steinheim, Germany).

Stress Tests—Parabens and polyols, weighted exactly, were dissolved in potassium phosphate buffer (0.05 mol/L). The pH of the solution was adjusted between 1 and 13 (±0.05) using phosphoric acid or sodium hydroxide solution. The solutions were heated in Reacti Therm hydrolysis tubes (Pierce) at 90 °C. Samples taken from these test solutions were diluted with water 1:20 and used for HPLC. Evaluation of the results was expressed by using the area percent of the relevant peaks in the chromatograms.

HPLC—*Analytical*—HPLC was performed on a Hewlett-Packard HP 1090 system with a diode array detector. Nucleosil C18 (5 μM) was used as the stationary phase in prepacked columns with 125 × 4 mm dimensions (Machery and Nagel). The injection volume was 25 μL; detection was performed by using an UV-detector at 255 nm wavelength. Water (A) and acetonitrile (B) were used as the mobile phase with a flow rate of 1 mL/min. A time program was developed which started with 100% A isocractically for 5 min followed by a linear gradient to 100% B within 15 min.

Calculation of the amounts of side peaks in the chromatograms was performed either by using area percent or by external standard method, using a 1% standard solution of methylparaben reference substance.

HPLC—*Semipreparative*—Isolation of the products was performed on a Waters Delta Semiprep HPLC system. LiChroprep RP18 material (40–63 μm) was used as stationary phase in prepacked columns with 310 × 25 mm dimensions (Merck). Water (A) and acetonitrile (B) were used as eluent at a flow rate of 20 mL/min. A time program was developed, which started with 100% A isocratically for 7 min followed by a linear gradient to 100% B within 14 min. The fractions from the semipreparative eluate were partially evaporated at ≤40 °C and finally lyophilized.

LC–MS—For LC-MS analysis a Finnigan MAT 4600 mass spectrometer equipped with a thermospray ion source, a Waters M600 MS pump, and a GAT 500 UV detector with pressure resistant detection cell were used. A flow rate of 1 mL/min ammonium acetate (0.1 mol/L) was delivered by a Gynkotek 300 B pump for postcolumn addition. Chromatographic conditions were the same as described for the standard analytical HPLC system.

GC–MS—For GC-MS analysis a Finnigan MAT 8230 mass spectrometer coupled to a Varian 3300 GC was used. A 30 m × 0.25 mm DB5 column (Fisons Analytical) with a film thickness of 1 μM served as stationary phase. The temperature program was started at 70 °C and raised to 320 °C at a rate of 15 °C/min (helium, 20 psi). The dried samples were silylated by addition of MSTFA.

NMR—Spectra were acquired on a Bruker AMX 500 instrument using dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal standard.

Results and Discussion

An aqueous buffer solution (pH 6.5 ± 0.05) containing 15% xylitol and 0.13% methylparaben was heated to 90 °C for 1 h. Reversed phase HPLC analysis of the test solution indicated the presence of three unknown reaction products with the relative peak areas of 1:2:4 together with 4-hydroxybenzoic acid (Figure 1). The concentration of the three unknown peaks was about 7% overall related to the starting concentration of methylparaben. These unknown products were not detected when the reaction was carried out with 4-hydroxybenzoic acid instead of parabens, indicating this compound to be inert against xylitol under the chosen conditions.

A mixture containing xylitol, methylparaben, and propylparaben in water at pH 6.5 was stored for 12 month at room

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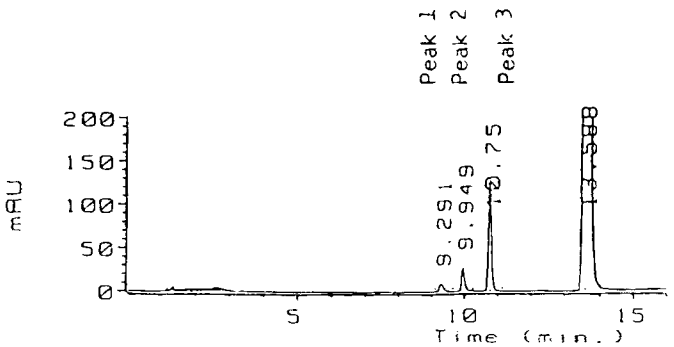

Figure 1—HPLC chromatogram of a solution containing xylitol and methylparaben after 1 h at 90 °C. Detection was performed by determination of UV absorption of eluate from RP-HPLC as described in the Experimental Section.

temperature, in order to determine if such a reaction can occur under real time conditions comparable to that used for storage tests for pharmaceuticals and cosmetics. This long term experiment revealed the same impurity peak profile and identical peak relation of 1:2:4 as obtained by the stress test. The total concentration of the three peaks was about 3.5% referred to the starting material.

The UV spectra of the three unknown peaks in the chromatogram and of 4-hydroxybenzoic acid were recorded by diode array detection and found to be identical to each other with a local absorption maximum at 255 nm.

Separation of the unknown product complex from the other ingredients of the solution (parabens, xylitol, 4-hydroxybenzoic acid) was achieved by means of a semipreparative RP-HPLC method. The fraction obtained in this way contained the questionable components in the unchanged ratio of 1:2:4.

In order to elucidate the molecular structures, we performed simultaneous HPLC–UV and –MS detection. The process of thermospray ionization for mass spectrometric detection was enabled by postcolumn addition of an aqueous ammonium acetate buffer downstream of the UV detector. The three peaks exhibited comparable intensity ratios in the total ion current chromatogram and in the UV trace (as shown in Figure 1). The corresponding mass spectra were identical, showing signals *m/z* 273 as base peak and *m/z* 290 with a relative intensity of 30%. The difference of 17 mass units can be related to $M + H^+$ and $M + NH_4^+$ ions of a molecule with a nominal mass of 272 Da, which is reasonably attributed to xylitol monoesters of 4-hydroxybenzoic acid. The proton NMR spectrum of the semipreparative HPLC fraction corroborates the formation of transesterification products. Especially three multiplets at approximately 4.2, 5.0, and 5.3 ppm indicate the presence of three isomeric esters, attributed to the 1-, 2-, and 3-substituted xylityl 4-hydroxybenzoates, respectively.

Further information concerning the molecular structures were obtained from GC–MS studies after silylation. The GC total ion trace again showed a group of three peaks with increasing intensity in the same elution order as in the HPLC–UV trace. The corresponding EI mass spectra are given in Figure 2. In combination with the CI spectra a molecular weight of 632 Da could be concluded, stating a molecular formula of $C_{27}H_{56}Si_5O_7$, which is in agreement with the penta-TMS derivatives of the monoesters proposed above.

In addition to the known unspecific ions *m/z* 73, 103, 147, 205, and 217, all isomers exhibit an intense ion *m/z* 193 characterizing the (trimethylsiloxy)benzoyl cation. The EI spectra of silylated alditols were described in detail by Peterson.[8] In analogy to this paper we expected to observe cleavage of the xylitol carbon chain between adjacent TMS groups characterizing the different substitution sides. The priminent cleavage ions for the primary ester of peak 3 are *m/z* 325 and 427, indicating a scission between C2 and C3

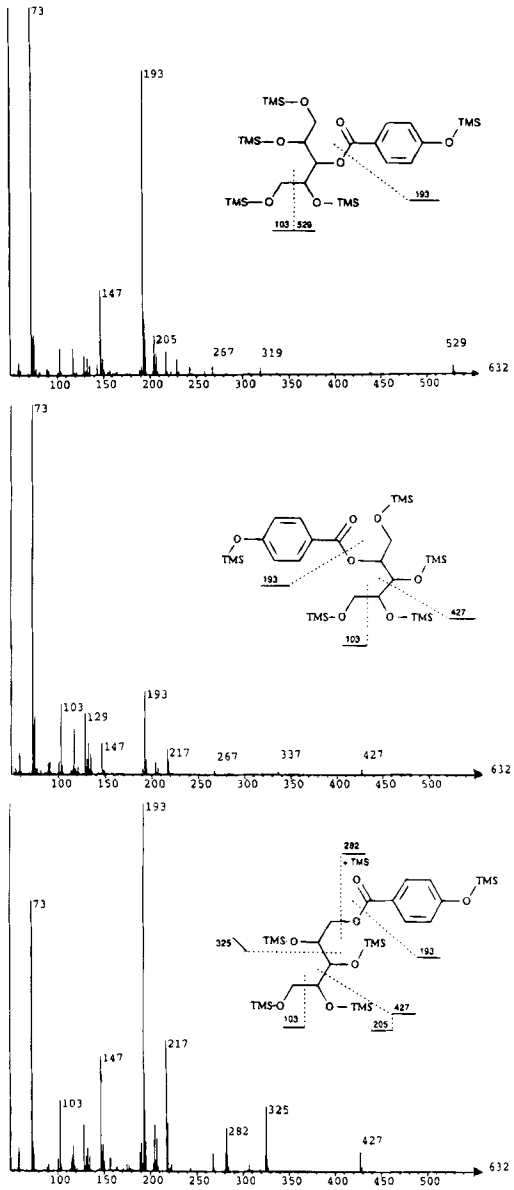

Figure 2—EI mass spectra of the three isomeric silylated xylityl 4-hydroxybenzoic acid esters.

and between C3 and C4 of the polyol backbone with charge retention on the aromatic ester group.

Additionally, a McLafferty type rearrangement of a TMS group is observed,[9] forming an ion *m/z* 282, which can be attributed to silylated 4-hydroxybenzoic acid. This ion is missing for the two secondary esters peak 1 and 2, probably due to steric hindrance in the required transition state for the transfer of the TMS group. Peaks 2 and 1 show only a small or even no signal at *m/z* 427, and therefore this isomers should be substituted at C2 and C3, respectively. The assignment of the substitution pattern is also possible from the CI spectra, where the intensity of the M – 103 ion at *m/z* 529 indicating the loss of a terminal CH_2OTMS group is preferred for peak 1 over peaks 2 and 3.

A time course of this transesterification reaction was studied over 30 h with aqueous solutions containing methylparaben and xylitol between pH 7 and 10 at a stress temprature of 90 °C. The amounts of xylityl esters arising over the test time were determined by HPLC (Figure 3).

The highest reaction rate at this temperature was initially observed at pH 10 followed by a strong hydrolysis of the

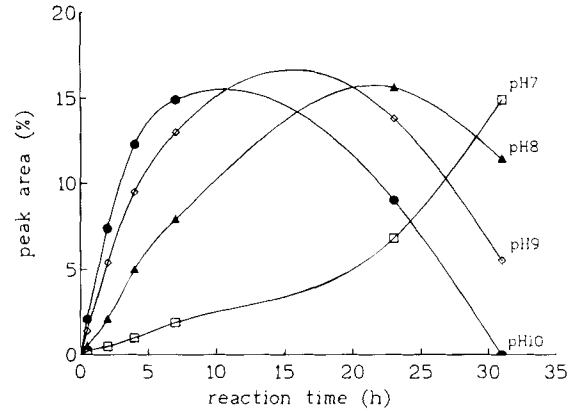

Figure 3—Time course of xylityl ester formation from methylparaben and xylitol at different pH values in aqueous solution at 90 °C.

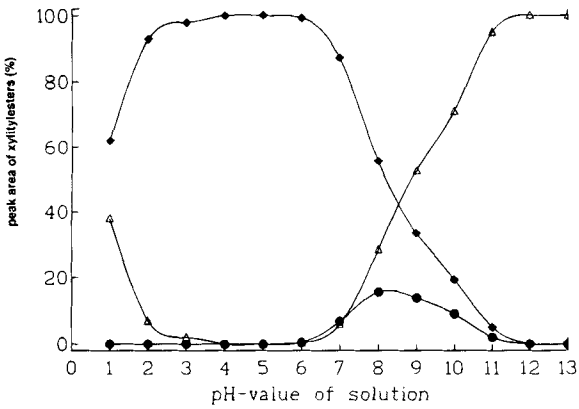

Figure 4—Formation of xylityl esters (○) and 4-hydroxybenzoic acid (△) from methylparaben- (◆) and xylitol-containing reaction mixtures at different pH values (90 °C, 1 h). Peak area was determined by UV observation of eluate from HPLC as described in the Experimental Section.

product after about 8 h. Similar results were obtained at pH 8 and 9, but with a decreased reaction rate and less hydrolysis. At pH 7 a steady increase of the xylityl ester concentration was observed over the time range of 30 h up to about 15%. This was the final concentration which remained constant when the reaction mixture was kept for up to 72 h at the reaction temperature of 90 °C.

In order to define the optimum pH value of the formation of xylityl esters and concerning side reactions, buffered solutions in the pH range of 1–13 were stressed for 23 h at 90 °C. The solutions contained 15% xylitol and 0.13% methylparaben and were checked by HPLC on the amounts of paraben, 4-hydroxybenzoic acid, and xylityl esters. The highest stability of paraben is given between the range of pH 3 and 6, as shown in Figure 4. More acidic or alkaline medium provoked hydrolysis. Formation of xylityl esters occurred only at pH values above 6 with an optimum at pH 8–9. The peak distribution was at all pH values tested was 1:2:4.

Solutions with varying amounts of xylitol and methylparaben were stressed at 90 °C for 3 h, in order to investigate the dependence of the reaction on different educt concentrations. The formation of xylityl esters increased with increasing xylitol concentrations when using reaction mixtures with 500–600 mg/L xlitol and a constant content of 13 mg/L methylparaben (Figure 5a).

In the inverse experiment with a constant xylitol level (159 mg/L) and different amounts of methylparaben (13–100 mg/L), the maximal xylityl ester formation was found at low paraben concentration (Figure 5b). Increasing paraben concentrations obviously diminish the formation of xylitol esters.

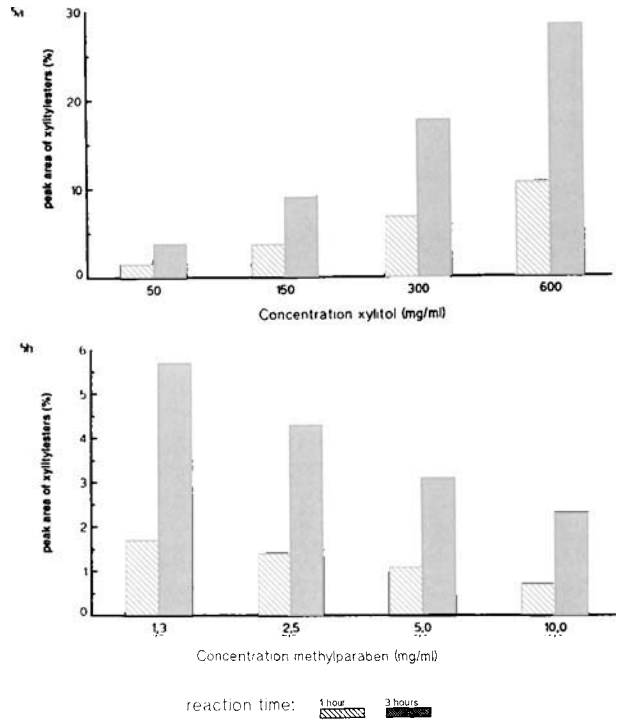

Figure 5—(a) Dependence of xylityl ester formation on the amounts of xylitol at constant methylparaben concentration (150 mg/mL) after 1 and 3 h reaction time at pH 11 and 90 °C reaction temperature. (b) Dependence of xylityl ester formation on the amounts of methylparaben at constant xylitol concentration (1.3 mg/mL) after 1 and 3 h reaction time at pH 11 and 90 °C reaction temperature. Peak area was determined by UV observation of eluate from HPLC as described in the Experimental Section.

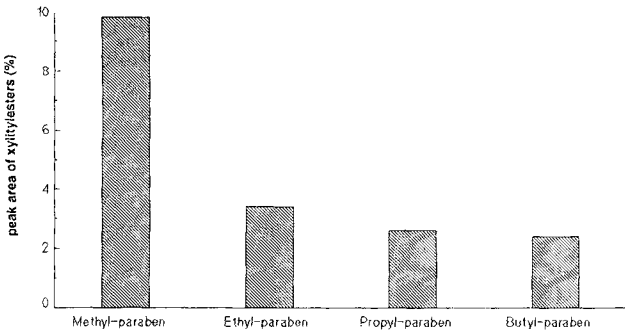

Figure 6—Formation of xylityl esters from the reaction of xylitol with methyl-, ethyl-, propyl-, and butylparaben in aqueous solution (90 °C, 3 h).

Further experiments were performed to elucidate the stability of methylparaben against different alcoholic reactants and of different parabens (methyl-, ethyl-, propyl-, and butylparaben) against xylitol at pH 11 at 90 °C for 3 h.

Figure 6 shows that xylityl esters emerged to a higher degree when short chain esters were used. Butylparaben was 4 times less effective in this system than methylparaben.

Stress tests of methylparaben with various polyols were conducted to quantify by HPLC the occurrence of transesterification products. In all cases the expected number of isomers were detected: C3 polyols (glycerol) gave two isomers, C5 polyols (adonitol, arabitol) three isomers, and sorbitol as C6 polyol also three isomers. No reaction products were observed when using aldoses, e.g. ribose, xylose, or cellobiose.

Conclusions

Sufficient preservation of liquid and semiliquid formulations in pharmaceuticals and cosmetics is essential for the efficacy

and safety of these products. The compatibility tests performed in this study clearly verify interaction between parabens and polyols in aqueous solution, leading to transesterification products with the general structure polyol 4-hydroxybenzoic acid esters (Figure 2). The molecular structures of these products were elucidated by separation of the different peaks with HPLC or GC and mass spectrometric detection. These methods may serve as a powerful tool in analysis of such compounds.

Because of the different hydroxyl groups of the polyols, isomers are formed. Xylitol for example reacts to 1-, 2-, and 3-xylityl monoesters in a constant ratio of 4:2:1, glycerol forms the expected two isomers, and in the case of sorbitol (C-6 polyol) three pairs of diastereomers can be detected.

The formation rate of polyol esters was shown to be maximal at high pH values (9–11) when tested at 90 °C. At pH 6 ester formation was found to take place only to a minor extend and no transesterification reaction (but hydrolysis) is expected at pH values below 5.

One has to keep in mind that pharmaceutical formulations, especially solutions for injections, eyedrops, or liquids for application on mucous membranes, are preferred to be isohydric, which means pH values between 5 and 7. In cases of formulation at the upper limit, a high degree of ester formation has to be expected, espeically at high polyol concentrations.

Transesterification can be avoided in these formulations by lowering the pH (if possible); by using the higher parabens (propyl- or butylparaben), or by lowering the polyol concentration. The application of the higher parabens is limited by their poor solubility in water, and one has to consider the changed physical properties caused by low polyol concentrations (e.g. isotony).

Actually, it seems likely to us that because of the wide distribution of polyols and parabens, many commercial pharmaceutical products, cosmetics, and foods may contain such polyol esters. Especially preserved fruit drinks are worthy of investigation for the presence of such esters. It is speculative, but with a high degree of probability, that they may be considered harmless.

References and Notes

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Abbreviations

CI	Chemical ionization
EI	Electron impact ionization
GC	Gas chromatagraphy
GC–MS	Gas chromatagraphy with mass spectrometric detection
HPLC	High-pressure liquid chromatography
LC–MS	Liquid chromatagraphy with mass spectrometric detection
MSTFA	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide
NMR	Nuclear magnetic resonance
Paraben	Alkyl 4-hydroxybenzoate
RP	Reversed phase
TMS	Tetramethylsilane or trimethylsilyl
UV	Ultraviolet

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