Designing green plasticizers: influence of alkyl chain length on biodegradation and plasticization properties of succinate based plasticizers

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Abstract

Phthalate diesters such as di (2-ethylhexyl) phthalate (DEHP) are considered ubiquitous contaminants and are poorly biodegraded in the environment. Moreover, both the parent compound and stable metabolites such as mono (2-ethylhexyl) phthalate (MEHP) are linked to several negative impacts on the environment and human health. Earlier work established that saturated diester compounds, such as succinates, showed better biodegradation characteristics and comparable plasticizer properties compared to DEHP. In this work we examine the effect of alkyl chain length of succinate molecules on plasticizer and biodegradation properties. This included both the side chains (n-ethyl to n-octyl) as well as substituents on the middle part of the succinate molecule. We showed that the common soil bacterium Rhodococcus rhodocrous could rapidly break down all unsubstituted succinates, without the appearance of stable metabolites. Furthermore, the organisms used the plasticizer metabolites as carbon source. The introduction of a large cyclohexyl substituent on the succinate resulted in a poorer degradation rate. Glass Transition Temperature (Tg) measurements were performed to evaluate plasticizer properties and showed that longer side chains reduced the Tg more efficiently, while large cyclohexyl substituents on the succinate decreased this effect. However, all compounds performed better or equal to DEHP at reducing the Tg.

Keywords

Green Plasticizer, Phthalates, Biodegradation, Succinate

Introduction

Among the many additives to polymers that serve to improve certain properties, plasticizers account for a large fraction, as they can make up to 40% of the overall material (Mersiowsky et al., 2001). These are usually relatively small compounds, which serve to render polymers flexible and workable (Sears and Darby, 1982). Most plasticizers are not covalently bound to the polymer matrix which means that they can leach out of the material (Jaeger and Rubin, 1970). Approximately 80% of all plasticizers produced worldwide are used in poly(vinyl chloride) (PVC) formulations (Stevens, 1999; Murphy, 2001) and about 90% of the plasticizers used for PVC are diesters of phthalic acid (Murphy, 2001).

Di (2-ethylhexyl) phthalate (DEHP) is particularly well studied owing to its wide range of toxic effects (Lloyd and Foster, 1988; Akingbemi et al., 2001; Foster et al., 2001; Gazouli et al., 2002; Akingbemi et al., 2004; Horn et al., 2004). Studies have found DEHP in samples from many different types of environments including the interior of buildings (Thuren and Larsson, 1990; Bauer and Herrmann, 1997; Cartwright et al., 2000; Staples et al., 2000; Butte et al., 2001; Becker et al., 2004; Horn et al., 2004). Due to this, DEHP and its metabolites are generally considered as ubiquitous environmental contaminants (Wams, 1987). DEHP and its stable metabolites mono (2-ethylhexyl) phthalate (MEHP), 2-ethyl hexanol and 2-ethyl hexanoic acid have been shown to be toxic to microorganisms (Nalli et al., 2002; Horn et al., 2004) and linked to antiandrogenic activities in humans (Richburg and Boekelheide, 1996; Swan et al., 2005; Pant et al., 2008; Fan et al., 2010; Piche et al., 2012).

Several bans on the use of phthalates in various jurisdictions (EU/2005/84/EC, 2005; CPSIA, 2008; HPA, 2010) have spurred interest in developing “greener plasticisers”. Research has been done for example on modified dibenzoate (Firlotte et al., 2009; Kermanshahi et al., 2009b), oligo-esters made from ε-caprolactone (Shi et al., 2011) and small diesters based on four carbon diacids (Erythropel et al., 2012). Stuart et al. worked with diesters of succinic acid, a natural molecule involved in the citric acid cycle of mammals, and showed that it was compatible with PVC (Stuart et al., 2010). In this work, we present data demonstrating the influence of structural characteristics on plasticizing as well as biodegradation

properties of various succinate-based plasticizers. Alkyl chain length was varied either by altering the alcohol used or the central diacid leading to several series of homologous compounds.

Materials and Methods

Plasticizers

Two of the plasticizers tested, diethyl succinate (DES; 99%) and di (2-ethylhexyl) phthalate (DEHP; 99%) were purchased from Sigma Aldrich. All others (Figure 1) were synthesized using a Dean-Stark esterification as described earlier (Erythropel et al., 2012).

Dihexyl 2-methyl succinate (DHMS) 2-methyl succinic acid (4.45 g, 33.7 mmol, 99%, Sigma Aldrich) and 1-hexanol (6.46 g, 63.2 mmol, 98%, Sigma Aldrich) were dissolved in 100 ml of toluene (99%, Sigma Aldrich) with catalytic amounts of concentrated sulphuric acid (Fisher Scientific). This mixture was refluxed at 120 °C over night in a 250 ml flask. A Dean-Stark trap was used between the flask and the reflux condenser to remove water. After cooling, the clear solution was washed with three aliquots of 50 ml of a saturated aqueous $NaHCO_3$ solution. The combined aqueous phases were set aside to recover any monoester produced and the combined organic phases were washed with deionized water, dried with sodium sulphate (Anachemia) and then the solvents were removed on a rotatory evaporator at 95° C at a pressure of 1.2 kPa. (Büchi RE III with Heidolph Rotavac Valve Control). A colorless, oily liquid was obtained. Yield: 89%.

All other syntheses were carried out in a similar manner, keeping the molar ratio of diacid to alcohol at 1 to 2. Whenever possible, the anhydride was used preferentially over the free diacid. The purity of each compund was verified by NMR spectroscopy, the spectra are shown in Appendix A. The yield of each reaction was obtained as follows: DBS: 90%; DHS: 99%; DOS: 82%, DEHS: 81% (Erythropel et al., 2012); DHMS: 89%; DHCHS: 88%; DHMCH: 85%.

Succinic anhydride (1.0 g, 10.0 mmol, 99%, Fisher Scientific) and 1-butanol (0.78 g, 10.5 mmol, 99%, Fisher Scientific) were dissolved in 10 ml of chloroform (99%, Fisher Scientific) and heated to 80 °C in a hermitically sealed vial. Once a clear solution was obtained, the vial was allowed to cool to

room temperature and the chloroform was removed on a rotatory evaporator (Büchi RE III with Heidolph Rotavac Valve Control). The products were recrystallized from hexanes (99%, Fisher Scientific).

All other succinate monoesters were prepared in a similar manner, keeping an equimolar ratio of succinic anhydride (or acid in some cases) to alcohol. Again, the structures of the products were verified using NMR, the spectra are shown in Appendix A. The yield of each reaction was obtained as follows: MBS: 85%; MHS: 45%; MOS: 70%.

The synthesis of the mixture of monohexyl 2-methylsuccinate (MH2MS) and monohexyl 3-methylsuccinate (MH3MS) differed significantly as it was recovered as a by-product from the synthesis of the corresponding diester, DHMS (as described above). The combined aqueous phases from the washing step were brought to pH 2 with hydrochloric acid (36%, Sigma Aldrich), and extracted with three aliquots of 30 ml of chloroform. The combined organic phases then washed with a saturated aqueous solution of $NaHCO_3$. Then this aqueous phase underwent a chloroform extraction followed by one more cycle of extractions. The solvent in the final organic phase was removed using a rotatory evaporator (Büchi RE III with Heidolph Rotavac Valve Control).

Extrusion

A conical intermeshing twin-screw extruder (Haake Minilab, Thermo Electron Corporation, screw diameter 5/14 mm conical, screw length 109.5 mm) was used to incorporate the plasticizers into unplasticized PVC (UPVC, Solvay Benvic, France). The batch feed size was 3 g, rotation speed of the screws was set to 60 min^{-1} and the operating temperature was between 110 °C and 130 °C. Due to the difference in viscosity between UPVC and the liquid plasticizers, the addition of plasticizer had to be carried out in a series of steps. The first step was to prepare a blend containing 20 parts per hundred rubber (phr, 16.6 wt.%), 4 phr of epoxidized soy bean oil (Chemtura Corporation) as heat stabilizer and 5 phr of stearic acid (Fisher Scientific) as lubricant. This was collected and recycled through the extruder a second time to ensure homogeneity. This blend of 20 phr served as master batch to prepare blends of higher

plasticizer in steps that added an additional 20 phr of the plasticizer each time. No additional amounts of heat stabilizer or lubricant were added.

Differential Scanning Calorimetry

Temperature modulated differential scanning calorimetry (TA Instruments Q100) was carried out to determine glass transition temperatures. Samples were cut into slices of 1-2 mg each, which were placed into a standard DSC pan (TA Instruments, model #070221). A top was crimped on and the total weight and weight of the sample was recorded. Pans were loaded into the autosampler of the instrument, along with an empty pan for calibration. After quenching the sample at -90 °C for 5 min, a heating rate of 2 °C min^{-1} was applied, which was superimposed by a sinusoidal modulation of 1.27 °C with a period of 60 s. Once 100 °C was reached, the sample was held at this temperature for 5 min. A preliminary cycle was done to erase the thermal history of the sample. The data from the second cycle was analysed using the software TA Universal Analysis, plotting reversible heat flow against temperature to determine the glass transition temperature according to ASTM D-3418 (ASTM D-3418, 2003).

Biodegradation study

The microorganism *Rhodococcus rhodocrous*, American Type Culture Collection (ATCC) 13808 was used to evaluate the biodegradation properties of the pure plasticizers. For each measurement, it was necessary to extract the entire contents of a shake flask. Taking several samples from one flask led to errors because of the difficulty in obtaining a homogeneous sample. For each experiment, sets of 500 ml Erlenmeyer flasks fitted with foam caps were prepared, containing 100 ml Minimum Mineral Salt Medium (MMSM: 6 g L^{-1} Na_2HPO_4, 4 g L^{-1} NH_4NO_3, 4 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.014 g L^{-1} Na_2EDTA, 0.01 g L^{-1} $CaCl_2 \cdot 2H_2O$ and 0.01 g L^{-1} $FeSO4 \cdot 7H_2O$, Fisher Scientific), 10 mM pure plasticizer and 0.1 g L^{-1} yeast extract. Other sets of flasks were prepared the same manner, but these included 2 g L^{-1} hexadecane as an easily used carbon source. The flasks were autoclaved at 121 °C and 100 kPa for 15 minutes (Steris Amsco Lab 250), cooled and then inoculated with 1 ml of cell broth from a previously

grown culture, using sterile techniques in a laminar fumehood (Baker Company, Model VBM600). The flasks were cultured in an incubator-shaker at 30 °C and 140 RPM (Multitron II, Infors AG).

The complete contents of one flask of each set were extracted on day 0. Subsequent flasks were extracted at intervals of 1 to 5 days depending on the degradation rate. All of these extractions were done in the same manner. First, the contents were adjusted to pH 2 with concentrated sulphuric acid and then extracted with 20 ml of chloroform containing 2 g L^{-1} pentadecane, as an internal standard. The isolated organic phase was stored at 4 °C until analysis.

In order to compare the results of the several biodegradation experiments, rate constants for the first hydrolysis step of the diesters were approximated using a first order fit. With these, values for half lives were calculated (Table 1 and Table 2).

Gas Chromatography

Determination of plasticizer concentrations in the extracts of the flasks, as well as detection and quantification of metabolites, was done using a gas chromatograph (Trace GC Ultra with AI3000 Autosampler, Thermo Scientific). After appropriate dilution of the samples, 1 µL was injected into the GC, which was fitted with a Restek RTX®-5 column (length 30 m, 0.32 mm internal diameter, 0.25 µm film). A flame ionization (FID) was used. In order to calculate concentrations from the ratios of peak areas of the compounds to those of the internal standard pentadecane, calibration curves were prepared for each compound of interest.

NMR Spectroscopy

^{1}H - Nuclear Magnetic Resonance spectroscopy was carried out using one of two spectrometers: Varian Mercury-300 (^{1}H = 300 MHz) and Varian Unity-500 (^{1}H = 500 MHz). The solvent used for all measurements was deuterated chloroform ($CDCl_3$), with tetramethylsilane (TMS) as internal standard. The chemical shifts δ are indicated in ppm.

Statistics

Statistical Analysis was done using the software GraphPad Prism 5; one-sample t-tests and one-way ANOVA tests with Bonferroni post test were carried out as indicated. A p value less than 0.05 was taken as significant.

Results

Syntheses

The synthesis of the monoester of methyl succinic acid resulted in two isomers, depending on which carboxylic function was esterified, as shown in Figure 1. Figure 2 contains the relevant parts of the gas chromatogram (a) as well as the NMR spectrum (b). By integrating the GC data it can be seen that one of the monoesters was preferentially formed during synthesis. The identity of the major and minor isomers was resolved with NMR. This is shown in an enlargement of the region for the single proton on the optical centre of the methylated carbon atom between the two carboxylate groups of the diacid core of either isomer (see Figure 1 for structures). The higher shifted signal belongs to the proton in MH3MS, as this proton is in the β-position to the COOH group, resulting in a slightly higher chemical shift, while the corresponding proton in MH2MS is in the γ-position to the COOH group, but in the β-position to the ester function. These results suggest that the peak at 8.8 min belongs to MH2MS (smaller peak areas), and the peak at 8.9 min to MH3MS (larger peak areas). Since the pure compounds were not obtained, the calibration curve for the unsubstituted monohexyl succinate was used, assuming the effect of the added methyl group would have a negligible effect in gas chromatography.

Plasticizing Studies

Glass Transition Temperature (Tg) data for blends of 40 phr (28.6 wt.%) in PVC are shown in Figure 3. Comparing the data for the branched di (2-ethylhexyl) succinate and the unbranched dihexyl succinate there was no significant difference in Tg reduction (one sample t-test, $p = 0.2254$). A significant statistical difference was found between DHS and DEHP, however (one sample t-test, $p = 0.0002$).

Figure 4 shows the glass transition temperatures for several succinates with varying substituents on the central part of the molecule, while the alcohol used to esterify all compounds was hexanol. Amongst all data, a significant statistical difference was found between the means (one-way ANOVA, $p < 0.0001$). DHS was not statistically different than DHMS (addition of a methyl group), however it was statistically different than DHMCH and DHCHS (Bonferroni post test, both $p < 0.0001$), which both contain larger substituents. DHMS was also found statistically different than DHMCH and DHCHS (Bonferroni post test, both $p < 0.0001$). The results for both compounds with large substituents, DHCHS and DHMCH, were found to not be significantly different.

Biodegradation Studies

Figure 5 shows examples of typical biodegradation experiments. Figure 5A shows the biodegradation pattern for dihexyl succinate. The parent compound is quickly hydrolysed and a corresponding increase in monohexyl succinate and hexanol are observed, however by day 3 and day 7, respectively, the presence of these compounds have decreased to trace amounts. No significant build-up of any other metabolite, including the corresponding hexanoic acid, was detected. Figure 5A also shows the concentration of hexadecane in the solution and its concentration only starts decreasing once all of the other compounds have disappeared.

Figure 5B shows the biodegradation pattern for the dihexyl ester of the methyl-substituted succinate. An equally quick hydrolysis of the parent compound is observed, and there is only a temporary build-up of the metabolite hexanoic acid. As expected, two metabolites with very close retention times in GC could be detected. These corresponded to the two structurally different monoesters MH2MS and MH3MS. After day 3, the concentrations of the monoesters remained at a constant ratio of approximately 5:1 of MH3MS to MH2MS.

Table 1 shows data for the half lives and time of maximum concentration during the biodegradation studies conducted with succinate esters of varying side chain length as well as results for several substituted succinates with constant side chain length. No data is presented for the small diethyl

succinate and its metabolites because of their very quick removal from the broth, as well as for the bulky dihexyl 2-cyclohexylsuccinate, which showed almost no hydrolysis of one of the ester bonds, resulting in no significant amount of metabolites detected.

For the remaining compounds, a quick hydrolysis of the diester was observed, except for the compound in which R'' was altered from a hydrogen to a cyclohexyl group (Figure 1). Table 1 summarizes the observed metabolites and their stability. Complete removal of the corresponding monoester within the timeframe of the degradation experiments was only observed if R' was six carbons or less and no hydrogen was replaced in the central structure of the succinate molecule (R''). Both corresponding alcohol and acid were quickly removed for all compounds with R' equal to or smaller than six.

Table 2 shows a comparison of half lives of selected succinate compounds being biodegraded with and without hexadecane present. For all compounds, hexadecane did not affect the half life and the onset of hexadecane use was correlated to the disappearance of the plasticizer compounds.

Discussion

DEHP has been the object of intense scrutiny in North America and Europe due to its potential toxic effects. A safe replacement of DEHP requires the design of compounds that adequately plasticizes PVC, biodegrades upon disposal, and avoids the buildup of stable and toxic metabolites. In this work we present a series of succinate plasticizers with good biodegradation kinetics and similar plasticizing properties compared to DEHP.

Earlier work has shown that there is a strong correlation between the glass transition temperature of PVC mixtures and their tensile properties such as elongation at break and secant modulus (Erythropel et al., 2012). From the glass transition temperature measurements in Figure 3, it is clear that the length of the alcohol portion of the plasticizers is an important aspect of their plasticizing properties. It should be

noted that increasing the length of the alcohol used to esterify by one carbon atom must increase the length of the longest chain of atoms in these plasticizers by two carbon atoms. The longer the alcohol used to make the diester plasticizer, the more effective the diester is at lowering the Tg. A decrease in Tg indicated that the PVC was becoming more flexible. So, these data can be interpreted as showing that as the plasticizer molecule becomes longer the interaction with the polymer chains becomes more effective at reducing the crystallinity of the PVC.

The Tg data for the branched 2-ethylhexyl succinate is almost identical to the Tg of the succinate made with hexanol. While the 2-ethyl containing compound has a higher molecular weight, the lengths of the longest chains of atoms in the two plasticizers are identical (Figure 1). By comparison, the Tg of the succinate made with octanol (longest chain of C and O atoms of 22 atoms) is significantly lower than that for the compound made with 2-ethyl hexanol, despite that these two compounds have an identical molecular weight. This supports the argument that the relative length of the plasticizer is the important factor in lowering Tg (Figure 3).

The data for the modified succinate plasticizer, in which one of the central hydrogen atoms has been replaced with a methyl group, shows little or no effect on Tg reduction, compared to the non-substituted succinate with the same alcohol (Figure 4). Again, this is suggesting the key factor is the length of the longest chain of atoms. Adding a larger group to the central diacid also significantly affects the overall plasticizing properties. When succinic acid is substituted with a cyclohexyl group (DHCHS) or when the central part of the diacid is part of a cyclohexane ring (DHMCH), Tg reduction is not as effective. These results can still be explained by considering the properties of these compounds relative to the plasticizer made with simple succinic acid. The cyclohexyl substituent would hinder rotation about the central bond of the plasticizer and no rotation is possible in case of the DHMCH. Rotational freedom is considered to play an important role in plasticizing abilities of an additive (Erythropel et al., 2012) and thus it is not surprising that these two compounds are poorer plasticizers than the compound made from the simpler succinic acid.

All of the above compounds are thermally stable. However, it has been shown that plasticizers inevitably leach out of the formulations and enter the environment (Jaeger and Rubin, 1970; Thuren and Larsson, 1990; Staples et al., 1997; Cartwright et al., 2000; Becker et al., 2004; Horn et al., 2004). In some cases, these released compounds can be found to have long half lives and therefore accumulate in the environment. It would be desirable to develop plasticizers that can be biodegraded quickly and not produce stable toxic metabolites.

Rhodococcus rhodocrous, a common soil bacterium, was chosen as the organism due to its known ability to degrade a broad range of compounds, including aliphatic hydrocarbons (Jones and Goodfellow, 2012). Previous studies have shown that *R.rhodocrous.* has the capability to hydrolyse diester plasticizers rapidly in the timeframe of these experiments, thus allowing to compare relative hydrolysis rates between different compounds (Nalli et al., 2002; Nalli et al., 2006c, Sauvageau et al., 2009).

Taken together, the biodegradation data for all of the plasticizers tested here are consistent with the mechanism of biodegradation worked out for other diesters in previous experiments (Nalli et al., 2006b; Nalli et al., 2006c; Kermanshahi et al., 2009a). In the presence of bacteria, the diesters are first hydrolysed to the corresponding monoester and an equivalent of the alcohol used to synthesize the diester. The monoester is then hydrolysed to ultimately yield the diacid and two equivalents of the corresponding alcohol. The alcohols are then oxidized to yield the corresponding carboxylic acids and will readily undergo β-oxidation (Nalli et al., 2006a) . The intermediate aldehydes are inferred but were not observed, which can be explained by the volatility of these compounds as explained in earlier work (Nalli et al., 2006c).

As with the plasticizing properties in PVC mixtures, the type of alcohol used to make the succinate diesters influences the rate of biodegradation Table 1. The longer the aliphatic chain of the alcohol – and thus of the overall length of the plasticiser – the slower is the overall degradation rate. This can be attributed to the increasing hydrophobicity of the compounds as the length of the hydrophobic portions of the plasticizers increase. This would lead to a decrease in the water-solubility of the compounds, which would make a plasticizer less available to the microbes in their aqueous environment.

The di (2-ethylhexyl) succinate is degraded more slowly than the equally long dihexyl succinate, yet only slightly more quickly than the di-n-octyl succinate (Table 1). This could be attributed to its 2-ethyl branches adding to the hydrophobicity of the molecule but it is just as likely that the ethyl branches, located near the ester bond, are sterically hindering its interaction with the appropriate enzymes.

It is important to note that all of the succinate diesters were degraded in a few weeks, which is significantly faster than the rate for the commercial plasticizer DEHP. In earlier work with di (2-ethylhexyl) esters, we were able to show that one of the key parameters to hydrolysis of the ester bonds is the structure of the central part of the diester molecule (Erythropel et al., 2012). With unsaturated diesters that had their two ester groups in a cis orientation, similar to DEHP, the first hydrolysis step to the monoester was very slow. Yet when saturated compounds, such as a succinate diester were used, the first hydrolysis step was much faster. With the compounds in this study, the change in the alcohol used makes biodegradation even more facile.

While it is important that the first hydrolysis step is much quicker than that observed for DEHP, it is also essential to consider the possibility of the accumulation of stable metabolites. As expected, the use of straight-chained alcohols was advantageous with regard to the metabolites as reported earlier (Nalli et al., 2006a). None of these alcohols resulted in accumulation of stable, toxic metabolites (Table 1) as was observed with any di (2-ethylhexyl) compounds, namely 2-ethyl hexanoic acid (Nalli et al., 2002, 2006a, 2006b; Erythropel et al., 2012).

The monoester of DEHP, MEHP, has been studied extensively due to its suspected effect on the endocrine system (Richburg and Boekelheide, 1996; Swan et al., 2005; Pant et al., 2008; Fan et al., 2010; Piche et al., 2012). MEHP has long been suspected as the active metabolite of DEHP, although more recent work has shown 2-ethylhexanal and 2-ethyl hexanol to also be biologically active (Piche et al., 2012). We showed that the stability of the monoesters is influenced by the choice of the central diacid (Erythropel et al., 2012), and in this study we show the influence of the side chain on the monoester stability. The monoesters were observed in the degradation experiments with all of the compounds (Table 1). The shortest diester, diethyl succinate, degraded quickly as did its monoester as it was not observed.

The next two compounds in the series, dibutyl and dihexyl succinate (Figure 5A), did generate small amounts of their monoesters, however these were hydrolysed within a week. The exact time is obscured by the fact that monoesters continued to be released as the parent diesters were hydrolyzed. It was also found that the removal of this compound was even faster in the presence of a second carbon source; hexadecane. Future studies will evaluate the toxicity and potential antiandrogenic nature of these compounds.

The longest monoester in this series was monooctyl succinate and this was the slowest to biodegrade. This means that, as observed with the parent diesters, as the series of compounds becomes longer, the rate of biodegradation of the monoesters becomes slower. Again, this is probably related to the solubility of these compounds in water. However, none of these monoesters are as intractable as those observed with some of the compounds, such as MEHP, studied in earlier work (Nalli et al., 2002; Horn et al., 2004; Sauvageau et al., 2009). Another important consideration is that the earlier work was done in the presence of hexadecane. This work shows that while hydrolysis of the monoester is faster when hexadecane is present, it is not essential (Table 2).

The study's results with the compounds made with the branched alcohol, 2-ethylhexanol, show these to be stable as has been reported previously (Nalli et al., 2006c; Erythropel et al., 2012). It should be noted that without the presence of hexadecane as an easily used carbon source, almost none of the 2-ethylhexyl diester was degraded during the course of the experiment (data not shown). However, it was not essential when the unbranched alcohols were used. Table 2 shows that there was no significant difference between any of the pairs of experiments with or without hexadecane in the media. In fact, in every case, there was a pattern of diauxic growth and there was no significant decrease in hexadecane concentration until the last of the plasticizer remnants had been removed (Table 2). Figure 5A shows this behaviour in an experiment with dihexyl succinate. This shows that the bacteria use the released alcohols and/or succinic acid as carbon sources for growth, which is not surprising as succinic acid is part of the TCA cycle. These results are important because rapid metabolism of the fragments released after the initial steps of biodegradation reduces concerns about their potential environmental impact. On the other

hand, the ethyl branch in the branched compounds blocks β-oxidation, making this a poor carbon source to support growth of the organism and resulting in an accumulation of 2-ethylhexanoic acid.

The other metabolites are alcohols and acids and these were observed for every type of compound studied (Table 1). As stated above, the straight chain alcohols or the resulting organic acids can be metabolized and accumulation does not seem to be significant. The longest compounds, octanol and octanoic acid, are significantly slower to degrade than the others, but this is again attributed to relative solubility in water and these compounds are not expected to reach significant concentrations in the environment as many microbes would be expected to use all of the straight chain alcohols and acids as carbon sources.

The effects of modifying the central diacid on biodegradation were more complex. The addition of a methyl group to succinic acid did not prevent hydrolysis of the first ester bond (Figure 5B). However, the addition of a cyclohexyl group completely inhibited degradation of the diester (Table1). This compound would be significantly less polar and hence less soluble in water. However, the dramatic change in biodegradation rate would seem to indicate that the bulky cyclohexyl substituent also interferes with the interaction with the enzyme to affect the first hydrolysis step. This can also be seen in the pattern of the first hydrolysis step with dihexyl methylsuccinate. There are two possible monoesters depending on which of the ester bonds was hydrolyzed, MH2MS and MH3MS (see Figure 1). These two monoesters had significantly different rates of production meaning that one ester bond was more susceptible to hydrolysis than the other. This cannot be attributed to steric hindrance of the methyl substituent on hydrolysis of the diester because this would cause a reduction in the rate of hydrolysis of the ester bond closer to the methyl substituent. In fact, the opposite is true so there may be a slight preferential stabilization of an intermediate in the preferred hydrolysis pathway.

However, there appears to be a steric inhibition of the biodegradation of the monoesters that are produced by this first step. Neither monoester is degraded quickly. This was not observed for the degradation of the monoester from the unsubstituted dihexyl succinate (Figure 5A).

Conclusion

Based on an analysis of both the plasticizing properties and the biodegradation properties of the new diesters, the majority of them are potential green plasticizers. All of the compounds showed plasticization properties comparable to DEHP but there were trends that showed the importance of structure. Within the range tested, an increasing overall molecule length positively influenced the plasticizing properties of unsubstituted succinate compounds, while a negative influence on plasticizer properties was found when alkyl substituents were added to the middle part of the succinate compounds. This negative influence became larger with increasing size of these substituents, which relates to the ability of these compounds to rotate around the axis connecting the ester functions.

Structure was important to biodegradation of these compounds: larger, less water-soluble compounds were slower to disappear. There is some evidence that steric hindrance near the ester bonds inhibited the rate of hydrolysis. However, the overall observation was that most of these compounds could be biodegraded and most of the metabolites were also removed quickly. Earlier work on biodegradation of some commercial plasticizers had shown that the presence of an easily degraded carbon source was important. The biodegradation of most of the diesters in this study was faster if hexadecane was included in the medium but it was not essential. The metabolites such as small diacids and unbranched alcohols were readily used as carbon sources by the bacterium.

Thus, a compromise has to be made between longer molecules favourable for plasticization and shorter molecules favourable for biodegradation. A good compromise found here would be dihexyl succinate. It was a comparable plasticizer to DEHP based on the Tg measurements and it was completely biodegraded in about one week, including all observed metabolites.

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