Microbial Desaturation of Bis(1-chloro-2-propyl) Ether into a Dichloro Vinyl Ether

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Bis(chloropropyl)ethers are undesirable by-products of the industrial production of propylenoxide and epichlorohydrine by the two-step chlorohydrine process. Such by-products have been released into the environment.[1] They are considered an important class of environmental pollutants due to their persistence and toxicity. Compounds containing an ether bond are poorly biodegradable because the ether bridge is very stable.[2] In addition, metabolites resulting from ether transformation may be toxic for organisms or the organisms may lack the necessary enzymes for further degradation.[3] The aerobic biodegradation of bis(1-chloro-2-propyl)ether (**1**, DDE) by the *Rhodococcus* species strain DTB occurs by scission of the ether bridge resulting in the formation of chloroacetone (**4**), 1-chloropropan-2-ol (**5**) and the transient formation of a so far unknown metabolite.[4] The possible involvement of a flavin-containing monooxygenase and the formation of chloroacetone and 1-chloropropan-2-ol may indicate that scission of the ether bond is initiated by hydroxylation of DDE at C-2, which results in a hemiketal structure **3** that is unstable in aqueous solution and predisposed to spontaneous scission.[4]

It has been suggested by several groups that scission of ether compounds under aerobic conditions occurs via the formation of hemiacetal intermediates, the formation of which was proposed exclusively on the identification of the cleavage products, for example, the resulting alcohols and aldehydes. It was suggested that the conversion of the gasoline compound methyl *tert*-butyl ether into *tert*-butyl alcohol and formaldehyde occurs by hydroxylation, which leads to the formation of hydroxymethyl *tert*-butyl ether.[5] It was proposed that the scission of diethyl ether by the *Graphium* sp. strain ATCC 58400 occurs by a cytochrome P450-mediated hydroxylation of the carbon atom adjacent to the ether bridge, which leads to the formation of a hemiacetal.[6] However, ether-cleaving methane monooxygenase (MMO) is known to catalyze hydroxylation but also desaturation reactions,[7] which after hydration will also result in a hemiacetal structure with ether as substrate.

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In contrast to hydroxylation of the carbon atom adjacent to the ether bridge, hemiacetal structures can also be achieved by hydroxyl shifts or by the addition of water to vinyl ethers.[2,8] For example, such a vinyl ether mechanism was proposed for isochorismate pyruvate hydrolase (EC 3.3.2.1), which catalyzes the conversion of isochorismic acid to 2,3-dihydro-2,3-dihydroxybenzoic acid and pyruvate,[9] while a hydroxyl shift route is operative with glycol monoethyl ether.[10,11]

In order to get further hints for the mechanism of ether scission with DDE, the unknown metabolite was isolated and its structure was determined by MS and NMR spectroscopy. Here we report the transient formation and characterization of an as yet unknown dichloro vinyl ether (DVE) **2** by the *Rhodococcus* sp. strain DTB during growth on DDE **1** (Figure 1).

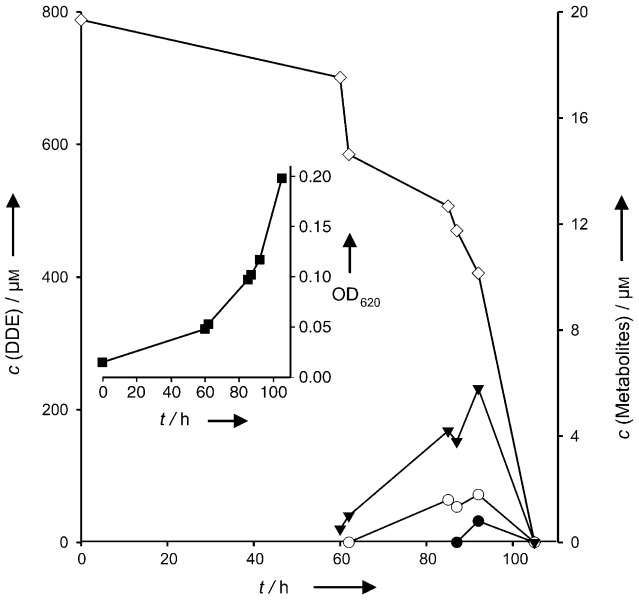

Figure 1. *Batch growth of the* Rhodococcus *sp. strain DTB in minimal medium containing 800 µM DDE* ***1*** *as sole source of energy and carbon. (■) Growth measured by* $OD_{620\,nm}$*; (●) transformation of DDE* ***1****; transient formation of (◇) 1-chloropropan-2-ol (****5****), (○) chloroacetone (****4****) and (▼) metabolite DVE* ***2****. The Figure shows representative values for progress curves observed in repetitive experiments.*

The dichloro vinyl ether was unequivocally identified as 1′-chloro-2′-propyl-3-chloro-2-prop-1-enyl-ether (**2**, DVE) by mass spectrometry and ^1H and ^{13}C NMR spectroscopy; the identification was confirmed by a facile chemical synthesis. DVE **2** is a desaturated product of DDE **1** that contains both chlorines and a reactive vinyl structure. The mass spectrum of DVE **2** showed the molecular ion of DVE **2** at $m/z = 168$ (Figure 2); this agreed with the empirical molecular formula of $C_6H_{10}OCl_2$. Electron impact ionization conditions resulted in major fragment ions at $m/z = 119$ [$M^+ - CH_2Cl$], 92 (base peak, C_3H_5OCl), and 77 (C_3H_6Cl). The prominent ion at $m/z = 92$ showed the natural isotopic pattern of one chlorine ($m/z = 92{:}94$, intensity 3:1) which would correspond to the loss of CH_2CHCH_2Cl [$M^+ - 76$]. Therefore a proton migration according to a McLafferty rearrangement of $M^{\cdot+}$ is postulated under EI-MS conditions

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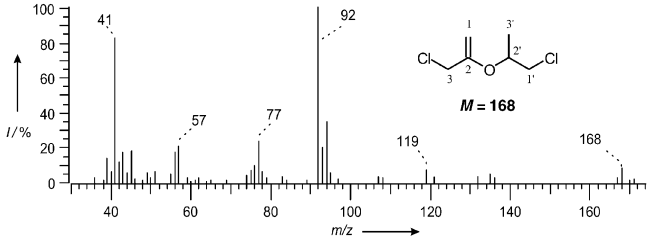


Figure 2. *Mass spectrum (EI) of the isolated metabolite 1′-chloro-2′-propyl-3-chloro-2-prop-1-enyl-ether (**2**, DVE).*

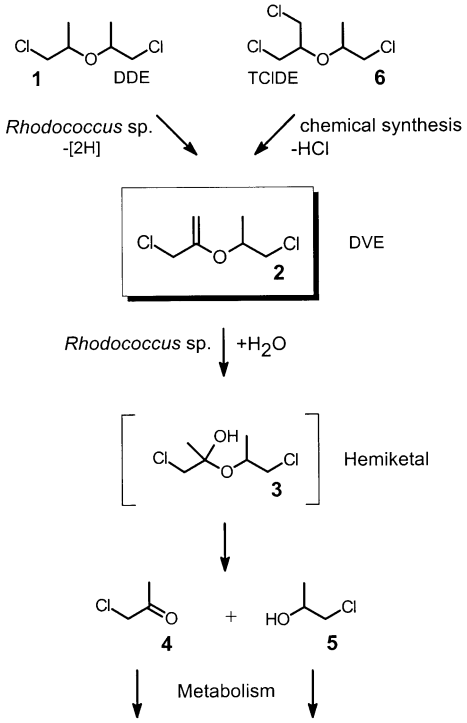
whereby a double bond in the molecule **2** is necessary. The ^{1}H NMR of **2** shows the protons H1a/b (double bond) at 4.23 and 3.96 ppm, respectively, as doublets with $J = 2$ Hz. The ^{13}C NMR data show C-1 at 86.97 ppm and C-2 at 163.58 ppm. These ^{1}H and ^{13}C NMR spectra are in accordance with NMR data predictions.

In order to confirm the structure of DVE **2**, the compound was chemically synthesized by elimination of HCl from 1,3-dichloro-2-propyl-1′-chloro-2′-propyl ether (**6**, TClDE), because selective chemical dehydrogenation of DDE **1** to obtain DVE **2** cannot be achieved easily. Dehydrohalogenation of TClDE **6** with potassium *tert*-butoxide in tetrahydrofuran afforded DVE **2** with a yield of approximately 40% based on epichlorohydrine. Synthetic TClDE **6** and DVE **2** contained approximately 10% of *n*-propyl ether isomers. The impurities could not be separated from the desired TClDE **6** and DVE **2**, and interfered marginally with their NMR spectra. The mass spectrum and the NMR data of the synthetic product **2** confirmed the structure of the isolated metabolite DVE.

The chemical synthesis and the NMR and MS data of the small molecules TClDE **6** and DVE **2** are reported here for the first time.

DVE **2** showed its highest accumulation of up to 5 μM during exponential growth of the *Rhodococcus* sp. strain DTB on DDE **1**. Formation of DVE **2** from DDE **1** and degradation of synthetic DVE **2** with dense cell suspensions pregrown on DDE **1** resulted in the formation of chloroacetone (**4**) and 1-chloropropan-2-ol (**5**) (data not shown). In order to exclude an abiotic chemical cleavage of DVE **1**, control experiments with heat-killed cells were performed. In contrast to the experiments with living cells, DVE **2** did not disappear. Dense suspensions of cells grown on 1-chloropropan-2-ol (**5**) were used as a further control. They converted neither DDE **1** nor the synthetic DVE **2**. The results suggest that the DVE **2**-converting enzymatic activity is induced together with the DDE **1**-degrading activity.

Our data show that DDE **1** can be converted to DVE **2** and subsequently to **4** and **5** by the *Rhodococcus* sp. strain DTB (Scheme 1). The introduction of a C=C double bond at the carbon atom adjacent to the ether oxygen is a suitable strategy for its activation. In comparison to saturated alkyl ethers, vinyl ethers are very reactive compounds that can be easily hydrated. The NMR data of DVE show a high electron density at C-1 and a low electron density at C-2 in comparison to a unsubstituted double bond. The partial positive charge at C-2



Scheme 1. *Conversion of DDE **1** via DVE **2** and hemiketal **3** by the* Rhodococcus *sp. strain DTB and chemical synthesis of DVE **2**.*

allows the attack of a water oxygen at the carbon adjacent to the ether bond; this results in the formation of hemiketal **3**.

The scission of 4-(methoxymethyl)phenol ether via formation of a vinyl ether *p*-quinone methide intermediate by the vanillyl-alcohol oxidase of *Penicillium simplicissimus* and subsequent hydration of the vinyl group has already been proposed.[8] The intermediate quinoid structure of *p*-quinone methide is energetically stabilized due to electronic resonance structures accompanied by a lower energy of this transition state compared to desaturation of DDE **1** into DVE **2**.

Reactive vinyl ethers are known from plasmalogens that occur in mammalian tissue and the high vinyl ether content seems to be a ubiquitous characteristic of electrically active tissues such as myocardial cells.[12] Vinyl ethers are also found in the lipids of bacteria.[13] These lipids are considered to be structurally very similar to platelet activating factor.[14]

In conclusion, DVE **2** has not been described in the literature until now, and here we report the transient bioformation and the chemical synthesis of DVE **2** by different routes (Scheme 1). The aerobic growth of the *Rhodococcus* sp. strain DTB on the environmental pollutant bis(1-chloro-2-propyl)ether (**1**) is accompanied by the transient formation of the dichloro vinyl ether **2**. This as yet unknown vinyl ether was isolated, and its structure was determined by NMR spectroscopy and GC-MS and confirmed as 1′-chloro-2′-propyl-3-chloro-2-prop-1-enyl-ether by a facile chemical synthesis. The formation of vinyl ethers may represent an initial key step for microbial ether scission. The *Rhodococcus* sp. strain DTB might serve as a biocatalyst for a desaturation reaction of ethers into vinyl ethers, which are chemically prepared only by a multistep synthesis.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra of the purified metabolite **2** were recorded on a Bruker AMX 500 (Bruker, Karlsruhe, Germany) and those of the synthetic products **2** and **6** on a 500 MHz Jeol ECP (Japan) with tetramethylsilane as internal standard. Concentrations of the substrate and the metabolites were analyzed by autoinjection GC-FID. GC-FID and GC-MS were performed as previously described.[4] Quantification was performed by correlating peak areas of the sample components with those of authenticated standards (external calibration).

The *Rhodococcus* sp. strain DTB was grown as previously described in mineral salt medium containing an initial concentration of bis(1-chloro-2-propyl)ether (**1**, 1 mM).[4] The transient formation of the metabolite **2** was monitored by analytical GC-FID, until **2** reached its maximum concentration of about 5 μM. A total of 20 L of the bacterial culture was extracted with pentane (100 mL per litre, 3×). After evaporation of the organic solvent, the residue was subjected to liquid chromatography (5 g aluminium oxide/silica gel 2:1 column) and eluted with pentane. Fractions of 1 mL were collected and monitored by GC-FID. Product-containing fractions were pooled and concentrated. In total 3 mg of metabolite **2** were isolated. Transformation experiments with dense cell suspensions were performed as previously described.[4]

Chemical syntheses and physical data: 1,3-Dichloro-2-propyl-1′-chloro-2′-propyl-ether (**6**, TClDE) was prepared as follows.[15] Propylene gas (3.3 L, 0.15 mol) was dissolved in CCl_4 (60 mL) at −20 °C and added to a stirred solution of epichlorohydrine (15 g, 0.16 mol) in CCl_4 (30 mL) also at −20 °C. Chlorine was bubbled into the stirred solution until it turned green, and stirring was continued for another 30 min at −20 °C. The solvent was removed, and the residue was purified by distillation (140 °C, 5 mm Hg) to yield 7.5 g (36 mmol) of TClDE **6** (GC: 85% purity) that contained about 10% of the *n*-propyl isomer.^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 1.26 (t, ^3J(H,H) = 7 Hz, 3H; CH_3), 3.42 (m, 1H; CH(CHHCl)CH_3), 3.48 (m, 1H; CH(CHHCl)CH_3), 3.55 (m, 1H; (ClCHH)$_a$CH), 3.64, (m, 1H; (ClCHH)$_a$CH), 3.60 (m, 1H; (ClCHH)$_b$CH), 3.64, (m, 1H; (ClCHH)$_b$CH), 3.77 (m, 1H; CH(CH_2Cl)CH_3), 3.79 (m, 1H; ($ClCH_2$)$_2$CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta =$ 18.63 (CH_3), 43.45 ($ClCH_2$)$_b$CH), 43.84 ($ClCH_2$)$_a$CH), 47.60 ((CH_2Cl)$CHCH_3$), 76.07 (CH(CH_2Cl)CH_3), 78.16 ($ClCH_2$)$_2$CH); EI-MS (70 eV): (M = 204); *m/z* (%): 193 (2), 191 (7), 189 (5), 158 (8), 157 (6), 156 (75), 155 (85) [$M^+ - CH_2Cl$], 113 (10), 111 (20), 81 (40), 79 (100), 77 (65), 75 (80), 51 (20), 49 (55), 45 (95), 41 (94), 39 (60).

*Synthesis of 1′-chloro-2′-propyl-3-chloro-2-prop-1-enyl-ether (**2**, DVE)*: TClDE (**6**; 1 g, 4.9 mmol) was dissolved in dry THF under N_2, and a solution of potassium *tert*-butoxide in THF (5 mL, 1 M) was added at −18 °C over 20 min. The reaction mixture was warmed to RT over 3 h and stirred overnight (15 h). After the addition of water (35 mL), the mixture was extracted with diethyl ether (100 mL). The organic phase was washed with brine (35 mL), dried (Na_2SO_4), and concentrated in vacuum (300 mm Hg). Distillation (74 °C, 5 mm Hg) yielded 350 mg (2 mmol) of DVE **2** (GC: 80% purity). Further purification was performed by using liquid chromatography (20 g aluminium oxide/silica gel 2:1 column, eluted with pentane) whereby a >90% (GC) pure product DVE **2** was obtained containing about 10% of the *n*-propyl isomer. ^{1}H NMR (500 MHz, [D_{14}]*n*-hexane, 25 °C, TMS): $\delta =$ 1.28 (t, ^3J(H,H) = 7 Hz, 3H; CH_3), 3.18 (m, 1H; CHHCHO), 3.49 (m, 1H; CHHCHO), 3.68 (s, 2H; CH_2CO), 3.96 (d, ^2J(H,H) = 2 Hz, 1H; OCCHH), 4.23 (d, ^2J(H,H) = 2 Hz, 1H; OCCHH), 4.12 (sext, ^3J(H,H) = 7 Hz, 1H; CHO); ^{13}C NMR (125 MHz, [D_{14}]*n*-hexane, 25 °C): $\delta =$ 18.50 (CH_3), 45.40 (CH_2CHO), 46.65 (CH_2CO), 75.00 (CHO), 86.97 (CH_2C), 163.58 (CH_2C).

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Keywords: biotransformations · enzymes · mass spectrometry · *Rhodococcus* species · vinyl ether

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