Mechanism of Anaerobic Ether Cleavage

CONVERSION OF 2-PHENOXYETHANOL TO PHENOL AND ACETALDEHYDE BY ACETOBACTERIUM SP.*

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2-Phenoxyethanol is converted into phenol and acetate by a strictly anaerobic Gram-positive bacterium, Acetobacterium strain LuPhet1. Acetate results from oxidation of acetaldehyde that is the early product of the biodegradation process (Frings, J., and Schink, B. (1994) Arch. Microbiol. 162, 199–204). Feeding experiments with resting cell suspensions and 2-phenoxyethanol bearing two deuterium atoms at either carbon of the glycolic moiety as substrate demonstrated that the carbonyl group of the acetate derives from the alcoholic function and the methyl group derives from the adjacent carbon. A concomitant migration of a deuterium atom from C-1 to C-2 was observed. These findings were confirmed by NMR analysis of the acetate obtained by fermentation of 2-phenoxy-[2-13C,1-2H2]ethanol, 2-phenoxy-[1,2-13C2,1-2H2]ethanol, and 2-phenoxy-[1-14C,1,2-13C2,1-2H2]ethanol. During the course of the biotransformation process, the molecular integrity of the glycolic unit was completely retained, no loss of the migrating deuterium occurred by exchange with the medium, and the 1,2-deuterium shift was intramolecular. A diol dehydratase-like mechanism could explain the enzymatic cleavage of the ether bond of 2-phenoxyethanol, provided that an intramolecular H/OC5H5 exchange is assumed, giving rise to the hemiacetal precursor of acetaldehyde. However, an alternative mechanism is proposed that is supported by the well recognized propensity of α-hydroxyradical and of its conjugate base (ketyl anion) to eliminate a β-positioned leaving group.

Ether linkages are comparably stable, and their cleavage requires rather rigorous conditions. Such cleavage reactions represent challenges also to microbes and their enzymes, and this difficulty causes the relative stability of many ether compounds in nature (1).

An important group of xenobiotic ether compounds, the linear polyether PEG* and its derivatives, is released into the environment at high quantities, as lubricants, solubility mediators, hydrophilic moiety of nonionic surfactants and household detergents, or as a constituent of cosmetics and pharmaceutical preparations (2). PEGs were found to be degraded by various bacteria, both in the presence and the absence of molecular oxygen (aerobically, Refs. 2–6; anaerobically, Refs. 7–12). Different reaction mechanisms are involved in PEG degradation, and it is generally accepted that they all involve the formation of a labile intermediary hemiacetal structure (1). In the presence of oxygen, such a hemiacetal can be formed through a monoxygenase-catalyzed hydroxylation of one of the methylene carbon atoms. In the absence of molecular oxygen, generation of such a hemiacetal can be achieved only with substrates containing a free hydroxyl group adjacent to the ether carbon through a hydroxyl shift reaction. Such hydroxyl shift reactions are catalyzed by diol dehydratase (EC 4.2.1.28) and glycerol dehydratase (EC 4.2.1.30) enzymes, with the substrates EG, 1,2-propanediol, or glycerol. The reaction mechanisms of these enzymes have been studied in great detail (13–15). They typically depend on adenosylcobalamin as cofactor, which provides a reversible radical source. Based on these well-studied model systems, it was assumed that anaerobic PEG degradation to acetaldehyde as the first identifiable intermediate may be adenosylcobalamin-dependent as well and may proceed in a way analogous to diol dehydratase, provided that at least one terminal hydroxyl group is free for the required shift reaction (7, 10, 11, 16, 17).

The anaerobic homoacetogenic bacterium Acetobacterium strain LuPhet1 can grow with low molecular weight PEGs as the sole source of carbon and energy but can also use EG or 2-phenoxyethanol as the sole substrate; the latter is fermented to phenol plus acetate (12) as schematized in Fig. 1. In cell-free extracts of this strain, two separate enzyme activities were detected, the one reacting with EG and the other one reacting with phenoxyethanol. Both reactions yield acetaldehyde as the first product. The authors found that the EG-degrading activity was stimulated 3.5-fold by added adenosylcobalamin and was strongly inhibited by cyano- or hydroxocobalamin or by light; the latter effect could be alleviated by adenosylcobalamin addition (12). With this, the EG-degrading enzyme behaved identically to the known diol dehydratases (18). Cleavage of 2-phenoxyethanol, on the other hand, was influenced neither by various corrinoids, including adenosylcobalamin, nor by light (12), indicating that the two enzymes are definitively different proteins and perhaps operate by different reaction mechanisms.

Since 2-phenoxyethanol is a monosubstituted ethylene glycol, it allows us to study the assumed shift reaction in greater detail because theoretically, either the free hydroxyl group or the phenoxy residue can be shifted to form a hemiacetal as an
intermediate. We therefore tried to distinguish between those two possible pathways by application of specifically deuterated and/or \(^{13}C\)-labeled 2-phenoxynethyl preparations to resting cell suspensions of *Acetobacterium* strain LuPhet 1 and subsequent analysis of the produced acetate.

**EXPERIMENTAL PROCEDURES**

**General Methods**—TLC was performed on Silica Gel F\(_{254}\)-precoated aluminum sheets (0.2-mm layer, Merck, Darmstadt, Germany); components were detected by spraying with a ceric sulfate-ammonium molybdate solution followed by heating to \(150^\circ\)C. Silica gel (Merck, 40–63 \(\mu\)m) was used for GC. GC analyses were carried out on a DANI 3800 gas chromatograph (DANI, Monza, Italy) using a homemade glass column (2 m \(\times\) 2 mm inner diameter) packed with 20% Carbowax 20M on Chromosorb W (60–80 mesh). GC parameters were as follows: injector, 220 \(\circ\)C; detector (flame ionization detection), 220 \(\circ\)C; carrier, \(N_2\); (30 ml/min); oven, from 60 to 200 \(\circ\)C at 10 \(\circ\)/min. \(^1H\) and \(^{13}C\) NMR spectra were acquired at 400.132 and 100.613 MHz on a Bruker AVANCE 400 Spectrometer using an Xwin-nmr software package and at 200.133 and 50.330 MHz on a Bruker AC 200 (Bruker, Karlsruhe, Germany) Spectrometer using an Xwin-nmr software package and at 200.133 and 50.330 MHz on a Bruker AC 200 (Bruker, Karlsruhe, Germany) equipped with an ASPECT 2000 data system. Chemical shifts (\(\delta\)) are given in parts per million and were referenced to the signals of CDCl\(_3\) (\(\delta\) \(1.25\) ppm) or to 3-(trimethylsilyl)propionic-2,2,3,3-\(\text{d}_4\) acid sodium salt (\(\delta\) \(0\) ppm) in the case of D\(_2\)O/NaOD (pH \(23.97\)) and the carbonyl group, respectively. \(^{13}C\) NMR signal multiplicities were based on attached proton test spectra. \(^{13}C\) NMR spectra for quantitative analyses were obtained by the inverse gated decoupling pulse sequence and a relaxation delay of 300 m (19). EIMS spectra were run on a VG 7070 EQ mass spectrometer (VG Instruments, Manchester, UK) operating at 70 eV. All reagents were of commercial quality or purified prior to use by standard methods. Ethyl bromo-\(^{12}\)Cacetate, bromo-\(^{13}\)Cacetate, and bromo-\(^{12,13}\)Cacetate were from Aldrich.

**Medium and Growth Conditions**—*Acetobacterium* strain LuPhet 1 (DSM 9077) was grown at 28 \(\circ\)C in the dark in bicarbonate-buffered (30 ml, \(pH\) 7.2) sulfide-reduced (1 mm) freshwater medium (20 ml) with 10 mM 2-phenoxynethyl alcohol as sole organic carbon substrate under a \(N_2/CO_2\) atmosphere (80:20 v/v) as described previously (12). 2-Phenoxyethyl alcohol trihydrate (340 mg, 2 mmol) in ethanol (5 ml) was added to the reaction mixture and then washed with diethyl ether. The organic phase was washed with saturated NaHCO\(_3\) solution, washed with water and diethyl ether (4 ml) and the reaction mixture was diluted with water (10 ml), adjusted to pH 3 by the addition of sodium hydroxide (2M), and freeze-dried. Sodium acetate showed chemical shifts in the range \(\delta_1\) \(8.88–2.03\) (literature 1.90), \(\delta_2\) \(23.8–26.3\) (literature 23.97), and \(\delta_3\) \(182.0–184.4\) (literature 182.02) (21) for the methyl and the carbonyl group, respectively.

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![FIG. 1. Pathway for anaerobic degradation of phenoxyethanol by strain LuPhet 1. The acetaldehyde formed in phenoxyethanol cleavage is oxidized to acetate by an acetaldehyde:acceptor oxidoreductase that forms acetyl coenzyme A. The reducing equivalents are used to reduce carbon dioxide to acetate through the carbon monoxide dehydrogenase pathway (see Ref. 12).](image-url)
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RESULTS

2-Phenoxyethanol dideuterated at carbon-1 (3, D₂-molecules > 98%) was prepared by LiAlD₄ reduction of ethyl 2-phenoxyacetate (2) (22) (Fig. 2A). After the complete fermentation of 3 by Acetobacterium under a N₂/CO₂ atmosphere, sodium acetate was isolated from the culture supernatant and examined by ¹H and ¹³C NMR spectroscopy. It is understood that throughout this study, spectra of sodium acetate (proton-decoupled in the case of ¹³C) were recorded using NaOD/D₂O at pH > 10. The methyl regions of these spectra exhibited peaks assignable to a mixture of mono- and non-deuterated acetate molecules only (Fig. 3, A and B). Monodeuterated molecules are revealed by the typical patterns of ¹H and ¹³C NMR signals due to the CH₃D and ¹³CH₃D groups. In both cases, this pattern consists of a 1:1:1 triplet (27) (JCD = 2.09 Hz, JCD = 19.5 Hz) (28, 29), which is upfield with respect to the non-deuterated methyl group (2ΔH(D) = 13.5 ppb, ΔCD(D) = 0.254 ppm) (29, 30). The presence of non-deuterated molecules besides the monodeuterated ones in the fermentation acetate (~35% as calculated from the integrated peak areas in the ¹H NMR spectrum, taking into account the number of protons of the two species) can be explained by considering additional acetate synthesis from CO₂ by this acetogenic bacterium (12) (Fig. 1). In addition, a partial loss of both deuterium atoms during the conversion of 2-phenoxethanol into phenol and acetate could not be excluded.

When Acetobacterium cells were fed with 2-phenoxo-[2-¹³C]ethanol (7) prepared as shown in Fig. 2B, the resulting acetate was found to be a mixture of dideuterated and non-deuterated molecules in the ratio ~2:5:1. In fact, in the ¹H and ¹³C NMR spectra of this acetate, an upfield quintet (1:2:3:2:1) (27) was present beside the singlets due to the non-deuterated methyl group (2ΔH(D₂) = 27.0 ppb, 2ΔCD₂(D₂) = 0.467 ppm) (29, 30), thus indicating the occurrence of CHD₃ and ¹³CHD₂ groups (Fig. 3, C and D). The complete absence of CHD₂CO₂ and CHD₃CO₂ species in the product from the former and the latter experiment, respectively, clearly resulted from a comparison of the corresponding NMR spectra. The results of the experiments carried out with 2-phenoxethanol bearing the dideuterated methylene group at either position of the glycol unit were in agreement with each other and consistent with the conversion of carbon-1 into the carboxylic group of the acetate and of carbon-2 into the methyl group. The most striking feature of this biotransformation appeared to be the shift of a deuterium (hydrogen) atom from carbon-1 to carbon-2 (Reaction 1).

To gain further insight into the process schematized in Reaction 1, samples of 2-phenoxethanol enriched with ¹³C at 1- and/or 2-position and dideuterated at the alcoholic function, i.e. 3, 9, and 10 (Fig. 4), were prepared from the proper ethyl [¹³C]bromacetate. The quantitative determination of differently labeled species (isotopomers) in the acetate recovered from feeding experiments performed with these samples was based on peak area measurements in ¹H and ¹³C NMR spectra, provided that the latter were obtained by the inverse gated decoupling method (19). The identification of signals due to isotopomer molecules was made possible by exploiting deuterium effects on the shielding of ¹H and ¹³C nuclei as well as spin-spin coupling constants.

After fermentation of sample 8, the ¹H NMR spectrum of the resulting acetate showed signals of CH₃D (triplet) and CH₃H at a ratio from which ~45% dilution of the biotransformation product with de novo synthesized acetate could be calculated (neglecting satellite peaks due to ¹³CH₂D and ¹³CH₃ groups). A
complete retention of the migrating deuterium atom (within
the limits of the experimental error) was indicated by the
$^{13}$CH$_3$/CH$_3$ peak area ratio approximating the value of $^{13}$C
natural abundance (1.1%). In accordance with this assump-
tion, the peak intensities measured in the $^{13}$C NMR spectrum
appeared in the expected proportions, i.e., $1:16:3$ for the $^{13}$CH$_3$
group (singlet at $\delta$ 26.27, acetate coming from the acetogenic
activity of the microorganism), for the $^{13}$CH$_2$D group (triplet
downfield shifted, acetate coming from the 2-phenoxycetanoin sup-
plied), and for the $^{13}$CO$_2$/H$_2$O (singlet at $\delta$ 184.39, corresponding to
the $^{13}$C natural abundance level of the whole acetate recovered
from the fermentation experiment).

Only the peak due to the [13C]carboxylate group was detect-
able in the $^{13}$C NMR spectrum of the acetate arising from the
bioconversion of 9. The $^1$H NMR spectrum displayed singlet at
$\delta$ 2.031 and a 1:1:1:1:1:1 system centered at $\delta$ 2.017, really a
doublet ($^2J_{HC}$ = 5.9 Hz) of triplet ($^2J_{HD}$) in agreement with
the presence of two species only, i.e., CH$_3$-CO$_2$/H$_2$O and CH$_2$D-
$^{13}$CO$_2$/H$_2$O, in the ratio of $1:2$. The absence of the isotopomer
CH$_2$D-2CO$_2$/H$_2$O allows the exclusion of an exchange with the me-
dium of the carbonyl group (at the level of acetyl-CoA) (Fig. 1).

Assuming the participation of the enzyme/coenzyme system
as a hydrogen carrier in the hydrogen 1,2-shift during the
biodegradation of 2-phenoxycetanol, two possibilities could be
envisaged: (i) the hydrogen (deuterium) atom is abstracted
from a substrate molecule, temporarily retained by the enzyme,
and then transferred to another molecule (intermolecular transfer) or (ii) the migrating hydrogen (deuterium) is returned to the same glycolic unit from which it had been abstracted (enzyme-mediated intramolecular transfer). To estimate the relative extent of the two events, compound 10 was administered to a cell suspension of *Acetobacterium* after dilution (18 to 100) with unlabeled 2-phenoxyethanol. When the acetate isolated at the end of this fermentation was examined by $^1$H NMR (Fig. 5A), no signals assignable to the CH$_2$D group were observed, i.e. no signals of a triplet 13.5 ppb upfield shifted from the singlet due to the CH$_3$ group (Fig. 3A). This result was consistent with a complete intramolecularity of the C-1 hydrogen migration. In addition, well resolved systems of satellite peaks were present in the proton NMR spectrum (Fig. 5A) due to isotopomers containing $^{13}$CH$_3$ and $^{13}$CH$_2$D groups. The multiplicity of the system corresponding to the [13C,D]methyl group, i.e. a doublet of 1:1:1:1:1:1 sextets (doublet centered upfield with respect to the CH$_3$ singlet in agreement with the

Fig. 5. $^1$H (400 MHz) (A) and $^{13}$C (50 MHz) NMR spectra (B and C) of acetate isolated from fermentation of a 15.5 mixture of 2-phenoxy-$[1,2-^{13}$C$_2, 1-^2$H$_2]$ethanol (intramolecular isotopic substitution >98%), and 2-phenoxyethanol having natural nuclidic composition. Signal assignments are as follows: a, $^{13}$CH$_2$D-$^{13}$CO$_2$/H$_3$O$_2$; b, CH$_3$-$^{13}$CO$_2$/H$_3$O$_2$; c, $^{13}$CH$_3$CO$_2$/H$_3$O$_2$; d, $^{13}$CH$_2$D-CO$_2$/H$_3$O$_2$; e, $^{13}$CH$_3$-$^{13}$CO$_2$/H$_3$O$_2$. 

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expected deuterium isotope shift), was indicative of a strong prevalence of $^{13}$CH$_2$D-$^{13}$CO$_2$ species among the $^{13}$C-methyl isotopomeric mixture ($J_{MC} = 126.7$ Hz) (31). The presence of a very minor concentration of $^{13}$CH$_3$CO$_2$ isomer was recognizable by the slightly higher intensity of the downfield peak of each sextet and could be explained in terms of natural $^{13}$C abundance in the acetate molecule accompanying the doubly $^{13}$C-labeled ones.

These findings were corroborated by the following considerations: (i) by inspection of methyl and carboxyl regions of the $^{13}$C NMR spectrum (Fig. 5, B and C) the composition of the $^{13}$C isotopomeric mixture was estimated to be: $^{13}$CH$_2$D-$^{13}$CO$_2$ ($a$) = 86% (dt at $\delta 26.05$ and d at $\delta 184.40$; $J_{CD} = 52$ Hz, $J_{CC} = 19.5$ Hz) (28, 31); CH$_2$-$^{13}$CO$_2$ ($b$) = 6% (s at $\delta 184.42$); $^{13}$CH$_3$CO$_2$ ($c$) = 6% (s at $\delta 26.29$); $^{13}$CH$_2$D-$^{13}$CO$_2$ ($d$) = 1% (t at $\delta 26.05$, $J_{CD}$); $^{13}$CH$_3$-$^{13}$CO$_2$ ($e$) = 1% (d at $\delta 26.29$ and d at $\delta 184.40$, $J_{CC}$); (ii) the ratio of the acetate resulting from acetogenic activity to that produced by transformation of 2-phenoxyethanol was found to be 1:2.6. This value was calculated from the ratio between CH$_2$-CO$_2$ molecules (measured as the area of the singlet at $\delta_{1.90}$) and CH$_2$D-CH$_3$CO$_2$ molecules (measured as the total area of the satellite signals, decreased by 1.1% of the CH$_3$ area and then corrected for the number of hydrogen atoms in the monodeuterated methyl group), taking into account the concentration (18%) of the labeled substrate in the sample fermented; (iii) the percentage of isomers $b$ and $c$ in the $^{13}$C isotopomeric mixture was found to be very close to the expected one (±5%) for $^{13}$C-labeled species present at the natural abundance level in the portion of acetate (87% of the total) arising in part (28%) from $^{13}$CH$_2$D-CH$_3$CO$_2$ molecules (measured as the total area of the satellite signals, decreased by 1.1% of the CH$_3$ area and then corrected for the number of hydrogen atoms in the monodeuterated methyl group), taking into account the concentration (18%) of the labeled substrate in the sample fermented; (iii) the percentage of isomers $d$ and $e$, which are present in trace amount in the acetate examined, their formation might depend on hydrogen and CO exchange reactions (32) occurring to a very small extent. Thus, the conversion of 2-phenoxyethanol into acetate appears to be an essentially straightforward process, as shown in Reaction 1, involving an intramolecular hydrogen migration in the first step.

**DISCUSSION**

In the light of a previous report (12) and in light of the results obtained by feeding experiments performed using $^2$H- and $^{13}$C-labeled substrates and resting cell suspensions of *Acetobacterium* strain LuPhet1, the conversion of 2-phenoxyethanol into acetate and phenol can be summarized as follows. Acetate originates from the glycolic moiety of 2-phenoxyethanol through elimination of phenol with formation of acetaldehyde, which is then oxidized in subsequent steps with retention of its molecular integrity (Fig. 1). In the first reaction, the alcoholic function of the substrate becomes a formyl group, whereas the adjacent methylene group is transformed into a methyl group, originating from the glycolic moiety of 2-phenoxyethanol used to dilute the doubly labeled substrate.

![Mechanism of Anaerobic Ether Cleavage](image)

**Fig. 6. Hypothetical reaction mechanisms for anaerobic glycol ether cleavage.** A, commonly accepted reaction mechanism of diol dehydratases ($R = H$); $X$ denotes 5'-deoxyadenosyl radical or a protein-based radical. B, putative mechanism of the enzyme-catalyzed C-O cleavage of phenoxyethanol by *Acetobacterium* sp.; $X$ denotes a protein-based radical. C, alternative pathway for the conversion of $\alpha$-oxo radical 18 into acetaldehyde; $\text{NuH}$, nucleophile (e.g. H$_2$O).

In the light of the previous report (12) and in light of the results obtained by feeding experiments performed using $^2$H- and $^{13}$C-labeled substrates and resting cell suspensions of *Acetobacterium* strain LuPhet1, the conversion of 2-phenoxyethanol into acetate and phenol can be summarized as follows. Acetate originates from the glycolic moiety of 2-phenoxyethanol through elimination of phenol with formation of acetaldehyde, which is then oxidized in subsequent steps with retention of its molecular integrity (Fig. 1). In the first reaction, the alcoholic function of the substrate becomes a formyl group, whereas the adjacent methylene group is transformed into a methyl group with concomitant 1,2-hydrogen shift (Reaction 1). These features are strongly reminiscent of the diol dehydratase-catalyzed reactions for which a generally accepted mechanism is schematized in Fig. 6A for 1,2-ethanediol (11, $R = H$) (18, 33). The whole process encompasses a double H/OH interchange giving rise to the gem-diol (14, $R = H$) (15, 34, 35) that rapidly collapses to the aldehyde 15. Its radical nature has largely been proven (33, 36, 37) and appears to be consistent with the transfer of a hydrogen atom from the C-1 of the substrate to a transient radical ($X'$) and then back to C-2 of the product-related radical (13, $R = H$), generated in turn by a hydroxyl 1,2-shift.

If an analogous rearrangement occurs in the anaerobic degradation of 2-phenoxyethanol (11, $R = C_6H_5$) with formation of the labile hemiacetal (14, $R = C_6H_5$), the migration of the phenoxy group should be assumed given the metabolic correlation between each carbon atom of the glycolic unit of 2-phenoxyethanol and those of the acetate molecule (Reaction 1). Thus, the opposite pathway, i.e. the 1,2-hydroxyl shift suggested previously (11, 12), has to be ruled out.

Considering that no evidence has been given so far for the formation of the hemiacetal (14, $R = C_6H_5$), an alternative mechanism can be envisaged with regard to the subsequent transformation of the radical intermediate (12, $R = C_6H_5$) (Fig. 6A). This mechanism (Fig. 6B), based on the intermediary of the resonance stabilized ($\alpha$-carbonyl $\leftrightarrow$ enoxy) radical 16 (33), is supported by the propensity of ketyls (radical anions) (e.g. 17) to eliminate adjacent leaving groups as a result of their electron-rich character (38, 39). The cleavage of the $\beta$-C,O-bond can also be facilitated by stereoelectronic effects in the appropriate conformation of the radical anion 17 (40). It is well known that $\alpha$-hydroxy radicals are up to 10$^5$ times more acidic than the corresponding alcohols (CH$_3$OH$\rightarrow$CHOH has $pK_a$ values of $\sim$10–12) (41). In addition, a base-promoted hydrogen abstraction as schematized in formula 16 is coherent with the marked lowering of gas-phase C-H bond dissociation energy observed when going from 1-alkanols (e.g. 94 ± 2 kcal mol$^{-1}$ for H-CH$_2$OH) (42) to alcoholate ions (e.g. 85 kcal mol$^{-1}$ for H-CH$_2$O$^-$) (43). $\alpha$-Oxo radicals have been proposed as intermediates in a number of enzymatic reactions (36, 38, 39, 43, 44).

We have found that in the biotransformation of 2-phenoxyethanol, the exchange of the migrating hydrogen atom with the medium occurs only to a negligible extent, if at all, and that its 1,2-shift is intramolecular (even if enzyme-mediated).
The fact that the hydrogen atom abstracted from the C-1 position of the substrate is returned quantitatively to the adjacent position of the same molecule requires that the hydrogen carrier be monoprotic \((XH = Enz-SH \text{ in Fig. 6B})\). It can be noted that such a facet of the phenoxyethanol acetaldehyde lyase recalls the reaction mechanism of adenosylcobalamin-dependent ribonucleotide reductase of \(Lactobacillus leichmannii\), which involves a protein-based cysteinyl radical as a catalytically competent \((XH = Enz-SH \text{ in Fig. 6B})\), thus facilitating the formation of the same molecule requires that the hydrogen carrier be monoprotic \((XH = Enz-SH \text{ in Fig. 6B})\). It can be noted that such a facet of the phenoxyethanol acetaldehyde lyase recalls the reaction mechanism of adenosylcobalamin-dependent ribonucleotide reductase of \(Lactobacillus leichmannii\), which involves a protein-based cysteinyl radical as a catalytically competent intermediate \((43)\). Although the α-oxo radicals appear to be thermodynamically capable of hydrogen abstraction from a thiol group \((XH = Enz-SH \text{ in Fig. 6B})\), given that gas-phase bond dissociation energies of H-SR compounds are in the range \(88–92 \text{ kcal mol}^{-1} \text{ (43)}\) and bond dissociation energy of \(\text{H}-\text{CH}_2\text{COCH}_3\) was estimated at \(\approx 91 \text{ kcal mol}^{-1} \text{ (45)}\), a temporary addition of a nucleophile to the carbonyl group of the radical \(18\) might occur \((\text{Fig. 6C})\). This further step would remove the resonance stabilization in \(18\) \((-8 \text{ kcal mol}^{-1})\) (see Table IV, footnote \(k\) in Ref. 45), thus facilitating the formation of the C-H bond by the intermediate \(19\) to give the labile diol \(20\) (bond dissociation energy for \(\text{H}-\text{CH}_2\text{H}_2\text{R} \approx 98 \text{ kcal mol}^{-1}\) \(\text{ (42)}\). A similar addition (with \(\text{NuH} = \text{H}_2\text{O}\)) has been suggested in the case of the diol dehydratase reaction mechanism \((18, 36, 37, 39)\). It remains to be elucidated whether this reaction mechanism also underlies anaerobic cleavage of PEG and its derivatives.

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