

Electron Transfer as a Potential Cause of Diacetyl Toxicity in Popcorn Lung Disease

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1 Introduction

Bronchiolitis obliterans, a serious lung disease, arises in microwave popcorn plant workers who are exposed to butter-flavored volatiles, particularly diacetyl. Findings indicate that chronic inhalation of butter-flavoring volatiles compromises lung epithelial barrier function (Fedan et al. 2006).

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A considerable amount of information is available on the toxicity of artificial butter-flavoring agents and their associated constituents (Kreiss 2007; ILS 2007). One inhalation toxicity study indicated necrosis of tracheal and nasal epithelium. Necropsy results revealed general pulmonary tissue congestion, focal hyperemia, atelectasis, bloody edema, bronchial edema, and altered thorax in rats that expired after 4-h diacetyl exposures. Histopathological examination indicated the presence of emphysema and hyperemia, as well as necrosis in kidney proximal tubules and swelling of hepatocytes. Subacute exposure to diacetyl caused death, necrotizing rhinitis, necrotizing laryngitis, and bronchitis (Morgan et al. 2008). The foregoing pattern of injury replicates features of human injury observed among workers engaged in processing and packaging of popcorn. Inhalation of diacetyl induced epithelial necrosis and inflammation in the nose, larynx, trachea, and bronchi (Hubbs et al. 2008), also produced were edema, hemorrhage, and ultrastructural changes in the tracheal epithelium. Although the threat of lung disease is most serious among plant workers, one investigation suggests that diacetyl, used as a butter substitute in cooking, puts professional cooks at risk as well (Schneider 2007). Thus far, however, few kitchen-related injuries from diacetyl exposure have been identified. One article describes occupational injury from exposure to diacetyl as an avoidable tragedy caused by corporate and regulatory negligence (Egilman et al. 2007). Because considerable evidence is accumulating concerning diacetyl toxicity, major popcorn makers have finally and recently stopped using the compound (Hanson 2009).

The preponderance of bioactive substances or their metabolites incorporates ET (electron transfer) functionalities which we believe play an important role in many physiological responses. Some chemical functionalities that are engaged in ET include quinones (or phenolic precursors), metal complexes (or complexors), aromatic nitro compounds (or reduced hydroxylamine and nitroso derivatives), and conjugated imines (or iminium species). In vivo redox cycling with oxygen of substances that retain ET functionalities may occur and, thereby, may produce oxidative stress (OS) through generation of reactive oxygen species (ROS), such as hydrogen peroxide, hydroperoxides, alkyl peroxides, and diverse radicals (hydroxyl, alkoxyl, hydroperoxyl, and superoxide). The mechanism of the reaction that occurs between electrons and oxygen produces superoxide, which acts as a precursor for hydrogen peroxide and other ROS. In some cases, ET results in interference with normal electrical effects, e.g., in respiration or neurochemistry. In addition, ET, ROS, and OS have been increasingly implicated in the mode of action of drugs and toxins. The α -dicarbonyl group of diacetyl is a functional moiety that has ET effects, and Niufar et al. (2002) are among the few authors who have addressed this aspect. On preparing this review, we found that there have been increasing numbers of reports that address the bioactivity of diacetyl and its connection to ET. Therefore, it is our goal in this review to propose and explain a novel mechanism for how diacetyl may express its toxic action, along with the electrochemical, metabolic, and cell signaling evidence that support our thesis.

2 Diacetyl Toxicity

2.1 *Electron Transfer*

The literature is incomplete on the question of how diacetyl manifests its toxic action. Notwithstanding, diacetyl and other α -dicarbonyls have been implicated to act through mechanisms that involve ET and possibly ROS and OS, and, in this section, we will summarize the cogent literature on that topic.

Evidence has been presented in various reviews that support involvement of ET-ROS-OS in the toxic action of several categories of chemical substances: therapeutics (Kovacic and Becvar 2000), anticancer agents (Kovacic and Osuna 2000), carcinogens (Kovacic and Jacintho 2001), and toxins (Kovacic et al. 2005; Kovacic and Somanathan 2006a; Kovacic et al. 2002; Poli et al. 1989; Kovacic and Thurn 2005; Kovacic and Somanathan 2005; Kovacic and Cooksy 2005a; Kovacic and Somanathan 2008; Halliwell and Gutteridge 1999). Sometimes, however, no evidence is found for toxic action that derives from participation of ROS or OS. For example, there are no substantial data to support the involvement of ROS in the metabolism of zolpidem (Ambien), although ET may participate (Kovacic and Somanathan 2009a). The toxicity of zolpidem is quite low (Berson et al. 2001), when compared with several other drugs that produce effects through the involvement of ROS (Kovacic et al. 2005; Kovacic and Somanathan 2006a; Kovacic et al. 2002; Poli et al. 1989; Kovacic and Thurn 2005; Kovacic and Somanathan 2005; Kovacic and Cooksy 2005a; Kovacic and Somanathan 2008; Halliwell and Gutteridge 1999). Another feature of zolpidem is that it does not deplete GSH (glutathione), which suggests that the molecule only induces low levels of OS (Berson et al. 2001).

Zolpidem may not be alone. There are other drugs that engage in ET reactions, but do so without appreciable formation of ROS. For example, amsacrine, a 9-anilinoacridine derivative and anticancer agent, appears to exert its cytotoxic action by poisoning of cellular topoisomerase enzymes (Baguley et al. 2003). Evidence indicates, however, that the drug may act as an electron donor in ET reactions, perhaps in a way that involves DNA. Other substances, 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and flavone-8-acetic acid (FAA), also appear to be capable of ET as pyrylium-type species (Kovacic 2005), but do not produce effects through ROS and OS. DMXAA is an anticancer agent and exhibits tumor antivascular activity. Administration of DMXAA or FAA in cultured marine splenocytes results in the synthesis of tumor necrosis factor (TNF), in addition to various cytokines, chemokines, and transcription factors.

Because little is known about the effect of diacetyl on ROS production, it may be that this α -dicarbonyl produces its effects mainly by ET. On the other hand, a recent review provides extensive evidence for the participation of ET-ROS for the vast majority of pulmonary toxins (Kovacic and Somanathan 2009b), which diacetyl certainly is. More work is needed to elucidate the degree to which ROS are involved in diacetyl toxicity.

2.2 Metabolism

Although metabolites often play important roles in therapeutic action and toxicity, evidence suggests that, in the case of diacetyl, the parent toxin is the active agent. In rats, labeled diacetyl was rapidly metabolized and excreted, mainly as carbon dioxide (ILS Inc. 2007). Acetoin may also play a role in lung toxicity as a precursor of diacetyl. Metabolism was mainly by oxidation to diketone at low concentrations, but by reduction to diol at higher levels. Alternatively, since carbonyls condense readily with protein primary amino acid groups, the imine derivative may be physiologically active, as discussed below.

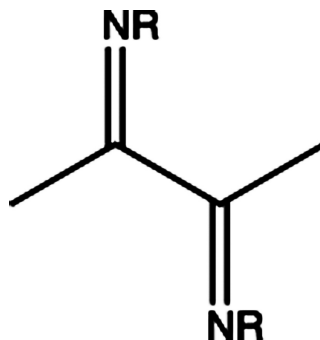
3 A Unifying Mechanism for Pulmonary Toxicity

The lung is a major target for toxicity. The atmosphere is replete with natural and manmade agents that may be toxic by the inhalation route. Some prominent toxic chemicals, or associated categories that manifest their action on the lung, include industrial materials (asbestos, sand, silicates, etc.), particulates from mining and combustion, agricultural chemicals, cigarette smoke, ozone, nitrogen oxides, and miscellaneous types that include diacetyl. Although pulmonary toxins may manifest their actions through many modes of action, we have recently postulated that oxidative processes (ET, ROS, and OS) are often strong contributors to pulmonary damage (Kovacic and Somanathan 2009b). We regard these aforementioned oxidative processes to form the basis of a unifying mechanism that may well describe how pulmonary toxins often produce their effects (Kovacic and Somanathan 2009b). The underlying concept behind this unifying mechanism is that the vast majority of toxins or their metabolites possess ET functionalities, which may undergo redox cycling and, thereby, generate ROS that are capable of injuring various cellular constituents of the lung or other organs by oxidative attack. Such attack is commonly associated with lipid peroxidation and oxidation of genetic materials that may produce DNA strand cleavage and production of 8-hydroxy-D-guanosine (8-OH-DG). Antioxidants (AOs), sourced both naturally in fruits and vegetables, as well as synthetic ones may provide protection from these adverse effects. However, oxidative reactions in tissues are often accompanied by depletion of natural AOs, which further exacerbates toxicity or susceptibility thereto. The mechanistic framework described above is also applicable to some more prominent pulmonary illnesses, such as asthma, COPD (chronic obstructive pulmonary disease), and cancer.

We propose that because diacetyl possesses an ET moiety and is a pulmonary toxin, it is plausible that it acts by oxidative processes known for other pulmonary toxins.

4 Diacetyl: Evidence to Support an ET Mechanism of Action

Electrochemistry can provide valuable insight on mechanisms by which chemicals may produce their effects, because it deals with the energetics of electron uptake, which is relevant to how ET functionalities react in vivo. If, in a biosystem reaction,

Fig. 1 Diacetyl diimine

the reduction potential is measured and found to be more positive than -0.5 V, the possibility exists for ET. This value for diacetyl (at pH 5) is -0.37 V; for diimine derivatives (Fig. 1) this value is -0.45 to -0.49 V (Niufar et al. 2002). Thus, these key substances are within the electrochemical range that may sustain ET reactions *in vivo*, possibly with the formation of ROS. The imines represent models of counter-parts generated by the reaction of diacetyl with the primary amino groups of proteins. Because ET processes are catalytic, only small amounts of the agents would be needed to induce significant physiological effects. This thesis is supported by the observation that α -dicarbonyl has an affinity for electrons (Jacobs 1986); in fact, the detailed nature of the resultant radical anion has been elucidated (Russell 1968).

Valuable additional insight may be gained from evaluating electroreduction data from α -dicarbonyl compounds other than diacetyl (Kovacic 2006). For example, methylglyoxal (CH_3COCHO) possesses a reduction potential in cyclic voltammetry of -0.18 V in the presence of strong acids (Montoya et al. 1993). From polarography, the range of values for the reduction potential ($E_{1/2}$) of this substance was -0.34 to -0.46 V for pH 2.5–5 (Mellado and Montoya 1994a). This range clearly supports the view that these α -dicarbonyl compounds may result in ET reactions in biological systems. It is interesting that methylglyoxal is a prominent member of a host of products that arise from oxidation of sugars, lipids, and amino acids and induces various toxic responses. Wu (2005) and Du et al. (2001) report that some ROS species are generated in such toxic responses, and AOs, if present, offer benefits. The electrochemical characteristics of methylglyoxal, and the degree to which it forms imines from protein interaction, may be important to its biochemical behavior. Unfortunately, research on this aspect of its mode of action has largely been neglected (Choudhary et al. 1997).

The electron uptake efficiency of any ET agent is strongly correlated to its electron affinity (EA). Electron affinity is expressed as the enthalpy change (ΔH) for the process $\text{A} + \text{e}^- \rightarrow \text{A}^-$. The electron uptake is determined by the free energy (ΔG) for this electron attachment, which is related to the EA at any given temperature (T) by $\Delta G = \Delta H - T\Delta S = \text{EA} - T\Delta S$ (ΔS = entropy change). However, because ΔS for this process is roughly independent of the electron acceptor (A), the free energy for the electron uptake is determined almost entirely by the electron affinity. Electron affinities for many organic electron acceptors were obtained by

directly measuring electron absorption coefficients in electron-rich environments, which yield a relative electron uptake constant (K_A). The process requires a direct measurement of the anion/neutral concentration ratio of anthracene to standardize the results through determining an absolute equilibrium constant (K_A) (Briegleb 1964) (See Eq. (1) below).

$$EA(A) = RT \ln K_A = RT[\ln K'_A(A) + \ln K_A(\text{anthracene}) - \ln K'_A(\text{anthracene})]. \quad (1)$$

These measurements of EA are more direct and precise than methods based on the Huckel orbital energies or on relative electron transfer efficiencies that use a common donor.

The EA determined by this method for diacetyl is 0.60 eV. It is illuminating to compare this value for diacetyl with those of prominent ET functionalities, such as quinone and aromatic nitro compounds, and to consider the effect of structural differences. The EA value for diacetyl is within the range of those for quinones (0.54–0.64 eV) and dinitrophenol (0.59 eV) (Briegleb 1964). The strongest acceptors in the quinone category are 2,3-dichloro-5,6-dicyano- *p*-benzoquinone, 2,6-dinitrobenzoquinone, tetracyano- *p*-benzoquinone, 2,3-dicyanobenzoquinone, and bis(cyanomethylene)quinone. EAs of unsubstituted quinones are as follows: 9,10-anthraquinone < 1,4-naphthoquinone \approx 9,10-phenanthrenequinone \approx 1,2-naphthoquinone \approx *p*-benzoquinone < *o*-benzoquinone < 1,8-diphenoquinone. Nitroaromatics are relatively good electron acceptors: 2,4,7-trinitrofluorenone > 1,3,5-trinitrobenzene \approx 1,4-dinitrobenzene > nitrobenzene. It is of particular interest that molecules with two adjacent electrophilic carbonyl groups (e.g., diacetyl, ethyl pyruvate, and dimethyloxaloacetate) are relatively good electron acceptors, and, in this regard, are comparable to trinitrobenzene. These findings represent favorable delocalization of the derived radical anion over the conjugated α -dicarbonyl system and anion stabilization by electronegative oxygen. By comparison, the absence of conjugation in acetylacetone results in a low EA value (0.34 eV).

Diacetyl has long been used to stabilize electrons in biological systems. Diacetyl has been used to function in a fashion similar to oxygen in enhancing the response of bacterial spores to x-rays (Tallentire et al. 1968). Diacetyl, bound to an active site in vivo, may well be present in a hydrogen-bonded complex involving acidic agents in proteins that could be in the form of alcohols, phenols, carboxylic acids, or RNH_3^+ . The favorable influence of H-bonding on electron uptake and ET is indicated by the following data for reduction potentials of diacetyl vs. pH: -0.2 V, pH 1.5; -0.3 V, pH 5; -0.5 V, pH 7 (Niufar et al. 2002).

Electrostatics constitutes those effects determined by the distribution of fixed charges in a system, such as the interactions between two polar groups of a protein. The application of electrostatics to biology and medicinal chemistry has been neglected, which is unfortunate because the literature discloses extensive involvement of electrostatics in living systems. Electrostatics is involved in living systems at a fundamental level, because electromagnetic forces are primarily responsible for the structure of matter (from atoms to more complex substances). Electrical

forces also appear to be a vital factor in the early evolution of life (Gagliardi 2006). Electrostatic force, a component of electromagnetic interaction, has played a dominant role in the dynamics of cell division and other biochemical processes in primitive cells, with the involvement persisting in modern, highly evolved eukaryotic cells. An electrostatic mechanism is believed to play a role in receptor–ligand activity, and therefore in receptor docking (Kovacic et al. 2007a).

A fundamental characteristic of receptor–ligand activity entails a molecular electrostatic potential (MEP) that is associated with ions and dipoles, and diacetyl carbonyl groups have an appreciable dipole character. The exact role of MEP is speculative, but the fields produced may function as conduits for electrons and radicals. Such fields may also affect the energetics associated with the ET process. Electrical fields are also known to influence electron spin (Nowack et al. 2007). Phosphorylation and sulfation play essential roles in cell signaling. The MEP associated with the phosphate and sulfate anions is a key element in linking communication among cells and may also favorably affect the energetics involved (Kovacic et al. 2007b). With this background, it is reasonable to rationalize that electrostatic interactions are involved in the mechanism by which diacetyl produces its effects. We provide some lines of evidence below that support the involvement of electrostatics in ET and in the action of α -dicarbonyls, with emphasis on energetics (Kovacic 2008).

Electrostatic effects have been studied in the plant kingdom. Ishikita and Knapp (2006) reported strong support for electrostatic action that energetically facilitates electron migration in the photosynthetic process. In one bacterial photosynthetic reaction, pheophytin is transferred to a primary quinone (QA), followed by passage to a secondary quinone (QB). A non-heme iron complex is situated midway between the two Qs. Removal of the iron complex causes a 15-fold decrease in ET rate from pheophytin to QA. Computational data support the conclusion of a predominant role for Fe electrostatics in relation to a favorable energetic effect on forward ET. Hence, the electrostatic field of Fe can be regarded as a force that facilitates efficient electron migration.

MEP is a significant force in DNA function, which has not attracted appreciable attention. The MEP results from the presence of repeating phosphate links in the DNA chain, along with associated anions. One review addresses the influence of electrostatics on the chemical behavior of DNA (Kovacic and Wakelin 2001). This article addresses the impact of MEP on ligand binding to DNA, guanine oxidation, axial charge transport, hopping termination, and reactions with charged and uncharged ROS.

In the Hofmeister series, a system designed to classify ions according to their ability to affect water structure, ammonium-type cations and sulfate or phosphate anions are the most active, which is generally in line with their involvement in cell signaling and certain other biological processes (Kovacic 2008).

The evidence that diacetyl and its imine derivatives act through mechanisms that involve ET is based on the following: ease of electron uptake, including having an appropriate reduction potential; stability of the resultant radical anion that

permits time for biological interaction; ability to interact with proteins to form conjugated imines (which may serve to anchor the agent at the receptor and illicit a physiological response); similarity to other bioactive α -dicarbonyls in both electrochemical character and in vivo action; and, finally, the propensity of diacetyl to cause pulmonary damage similar to that caused by selected other pulmonary toxins.

5 Other Relevant α -Dicarbonyls

The literature contains articles that address appreciable numbers of other α -dicarbonyls which are structurally related to diacetyl. The chemical and physiological properties of these other α -dicarbonyls, discussed in the following sections, provide additional support for the mechanistic theme presented for diacetyl.

5.1 *Ethanol Metabolites*

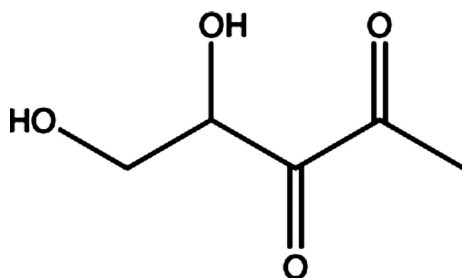
Diacetyl is involved in the metabolism of ethanol, which involvement has been fairly well delineated (Kovacic and Cooksy 2005b; Kovacic and Somanathan 2006b). As ethanol is metabolized, there is an increase in ROS and an initial generation of hydroxyethyl radicals. A key intermediate in the metabolism of ethanol is acetaldehyde, which is converted to acetic acid. Many investigators consider the acetaldehyde to be the main toxin from ethanol consumption. Other reactions that damage tissues as ethanol is metabolized entail condensation with protein and formation of ROS during the oxidation of acetaldehyde to acetic acid.

Oddly, despite their toxicity and the problems emanating from alcohol addiction, scant attention has been paid to the products of acetaldehyde's side reactions, which mostly contain four carbons. Most of these side reactions result in the production of diacetyl, acetoin, and 2,3-butanediol. Acetaldehyde reacts with pyruvate to form acetoin, after which it is oxidized to α -dicarbonyl. One hypothesis that has been advanced is that the side products may be responsible for "hangovers" (Otsuka et al. 1996).

5.2 *Bacterial Cell Signaling Agents*

The α -dicarbonyl functionality is also present in 4,5-dihydroxy-2,3-pentanedione (DPD)(Fig. 2), an important bacterial cell signaling molecule whose activity may be related to that of diacetyl. Cell signaling has attracted attention in higher organisms for some time and has recently been reported to occur in bacteria. If cell signaling is disrupted, it may have substantive impact on living systems. Cell signaling may be important for several reasons, including its role in quorum sensing. It is known that chemical exposure may affect cell signaling. DPD acts as an autoinducer, one of the trigger molecules in quorum sensing. In vivo DPD is in equilibrium with a furanone and a furanosyl-borate diester (AI-2). In one hypothesis, ET and ROS are involved in cell signaling in higher organisms and are also involved in biological conduits, relays, and electrical effects (Kovacic and Pozos 2006).

Fig. 2 4,5-Dihydroxy-2,3-pentanedione (DPD)



As ET agents, diacetyl or its imine derivatives can serve as a model for DPD, since diacetyl possesses a reduction potential amenable to ET in the biological domain. Hence, it is conceivable that DPD and its imine derivatives also may be involved in ET-ROS processes (Kovacic 2007). The presence of hydroxyl groups should facilitate ET by DPD vs. diacetyl, as shown in Fig. 3; these hydroxyl groups stabilize the anionic character of the oxygen molecules via bonding with the partially acidic hydrogens of the alcohol groups. A 2007 article focuses on 3-hydroxytridecane-4-one (CAI-1), an α -hydroxyketone, as a bacterial signaling agent (Higgins et al. 2007). In this chapter, it is pointed out that the α -hydroxyketone acetoin is oxidized *in vivo* to diacetyl. Similar metabolism may occur with CAI-1 to produce an ET α -dicarbonyl that is capable of playing a bacterial cell communication role.

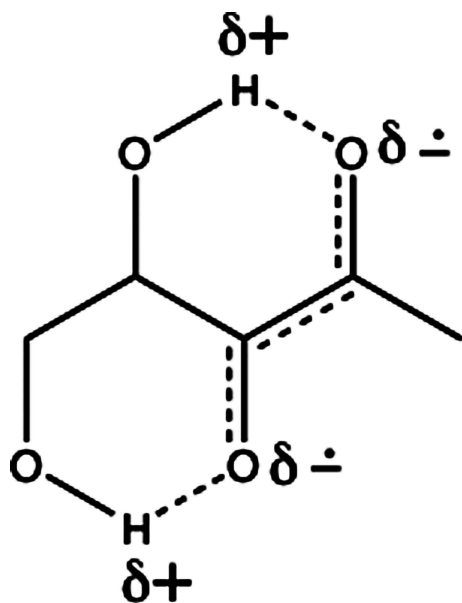


Fig. 3 Hydrogen-bonded radical anion of DPD

5.3 Other Toxins

Exposure to reactive α -dicarbonyl species, of which diacetyl is a member, is known to induce ROS and OS. In addition, one recent report casts light on cellular damage induced by reactive carbonyl species (RCS) (Wondrak et al. 2002). Such toxic entities, mainly in the α -dicarbonyl class, are generated by oxidation of sugars, lipids, and amino acids. Among the more prominent examples are glyoxal, methylglyoxal, and α -dicarbonyls such as 3-deoxyosones from monosaccharides. This category can exert its adverse effects by increasing OS, thereby generating a vicious cycle of ROS and RCS formation. Lately, α -dicarbonyls have attracted appreciable attention as key reactive intermediates in toxicity and involvement in neurodegenerative diseases, for example, Alzheimer's and amyotrophic lateral sclerosis, involving the Maillard reaction, which yields AGEs. As discussed in another section, imines from condensation with protein primary amino groups may be other important participants in the induction of such disease.

5.4 Protein Derivatives

Protein cross-linking via the Maillard reaction has been the subject of much scrutiny. A study was made on the impact of this process on enzyme action (Miller and Gerrard 2005). Protein glycation can generate α -dicarbonyls (see below). Protein function following glycation was examined after treatment of ribonuclease A with various α -dicarbonyl compounds, including diacetyl, which induced cross-linking and impaired activity. The effects of two Maillard reaction inhibitors, namely aminoguanidine and 3,5-dimethylpyrazole-1-carboxamide, were assessed, along with a parallel measurement of the effect on enzyme activity. Evidence indicates that prevention of protein cross-linking does not necessarily preserve enzyme activity. Thus, the data point to the involvement of another mode of action. We suggest that electrochemical properties, including ET, may be important factors in this alternative mode of action.

In a related investigation, odorous products were formed from reactions of diacetyl with amino acids involving the Maillard and Strecker reaction (Pripas-Nicolau et al. 2000). In the presence of cysteine, many of the derivatives formed were heterocyclic in nature.

5.5 Advanced Glycation End Products (AGEs)

Among AGEs are α -dicarbonyls that result from protein-carbohydrate interactions. Accumulated evidence supports the hypothesis that there is mutual enhancement between glycation and oxidation and entails closely linked processes (Traverso et al. 1997). Glucose auto-oxidation plays an essential role in non-enzymatic glycation

of proteins. Glucose and glycated collagen show a catalytic role in lipid peroxidation. Early glycation products can be transformed into AGEs by oxidative processes involving ROS.

Glyoxal and methylglyoxal products result from oxidative degradation of glucose. Condensation of the carbonyl functions, present in these compounds, with primary amino groups of protein occurs and has been described in the literature (Walker and Feather 1983). Non-degraded α -carbonyls such as deoxyosone, gluco-some, and 3-deoxyglucose are also formed and may display potential for ET and formation of ET imines as well.

5.6 Other Bioactive α -Dicarbonyls

Cyclohexane-1,2-dione is an antiviral agent (Tiffany et al. 1957). Diacetyl monoxime, an imine analog, has attracted attention because of its physiological activity, including its effects on cardiac muscle tension, neuromuscular transmission, action potential, and ion currents (Schlichter et al. 1992). This molecule also displays some activity as an antidote for nerve gas (Kovacic 2003). Since the protonated form exhibits a reduction potential of -0.43 V (Niufar et al. 2002), some of its bioresponses may reflect ET-ROS activity. *o*-Quinones represent a special class of α -dicarbonyl compounds that possess reduction potentials amenable to ET with the capacity to produce ROS. Various members of this group are physiologically active as (for example) anti-infective agents (Kovacic and Becvar 2000) or anticancer drugs (Kovacic and Osuna 2000) and, thereby, emulate the profile of substances that fit the ET-ROS-OS mechanistic framework.

A new structural class of antibiotics, represented by marinopyrroles (Fig. 4), was recently reported (Hughes et al. 2008). These antibiotics were obtained from actinomycetes that inhabit ocean sediments. The agents possess potent antibiotic activities

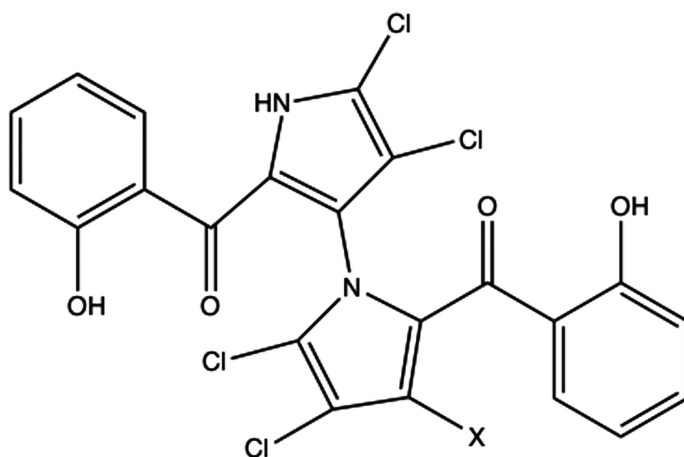


Fig. 4 Marinopyrrole with α -dicarbonyl conjugation

against methicillin-resistant *Staphylococcus aureus*. Extensive evidence has been presented to support ET–ROS–OS involvement in the action of anti-infective agents (Kovacic and Becvar 2000). This evidence has been buttressed by research on a common cell death mechanism for antibiotics that involves ROS (Kohanski et al. 2007). Inspection of the marinopyrrole structure (Fig. 4) reveals the presence of a highly conjugated α -dicarbonyl analog, obtained from conjugation by aromatic nuclei, namely pyrrole and benzenoid. Hence, the electron affinic nature of the moiety should be appreciably greater than for diacetyl. The reduction potential of carbonyls is enhanced by hydrogen bonding, which increases electron attraction and stabilizes the resultant radical anion. It is interesting that the phenolic hydroxyl groups in Fig. 4 are in position to coordinate with the carbonyls, with resultant enhancement of the potential for electron uptake.

A relevant feature of carbonyls is the ease by which they condense with protein primary amino groups to yield imines. As previously mentioned, conjugated imines and iminiums are ET agents and may play important physiological roles in organisms. Two aromatic oximes, zinviroxime (Fig. 5a) and enviroxime (Fig. 5b), have been shown to be potent inhibitors of rhinovirus multiplication in vivo (Kovacic et al 1990). The protonated, iminium-like forms displayed reduction potentials in the range amenable to ET in the biobdomain. A similar situation may pertain to the structure in Fig. 4.

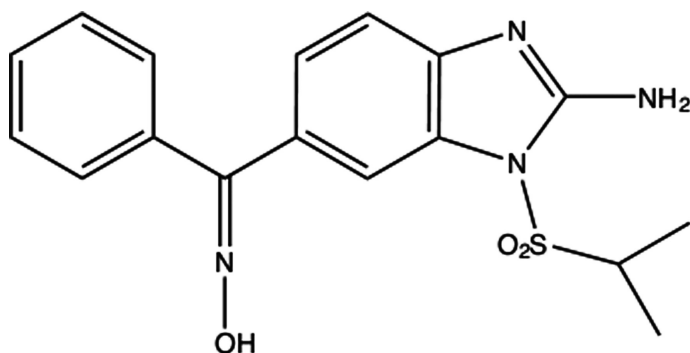


Fig. 5 Zinviroxime (syn) and enviroxime (anti)

These reports show that various α -dicarbonyl derivatives are electron affinic and possess reduction potentials favorable for in vivoET. In addition, the compounds display diverse physiological activities that are compatible with the ET–ROS–OS theme.

6 Other Physiological Effects

When tested for genotoxicity, diacetyl generally exhibited mutagenic activity in *Salmonella typhimurium* (ILS Inc. 2007). ROS are believed to be involved in

the induction of genotoxicity (Kovacic and Jacintho 2001). Sulfite, a reducing or inactivating agent toward ROS, countered the mutagenicity effect of diacetyl (ILS Inc. 2007). When diacetyl was tested in a sister chromatid exchange (SCE) assay, positive results were recorded in Chinese hamster ovary cells. The reducing agent bisulfite significantly diminished the frequency of SCEs and proportion of endoreduplicated cells. Diacetyl completely inhibited cell growth in sarcoma cells at high doses, but only moderately at lower levels. Mutagenesis is believed to reflect the formation of adducts between α -dicarbonyls and guanine residues of DNA, giving rise to conjugated imines that possess ET characteristics and are capable of modifying DNA (Mellado and Montoya 1994b). ET and ROS action may at times apply to both mutagenesis and carcinogenesis, since these processes are closely linked (Kovacic and Jacintho 2001).

Diacetyl has activating and deactivating influences on a number of enzymes and metabolic processes. Diacetyl is known to have increased ornithine decarboxylase activity and DNA synthesis. Moreover, diacetyl inactivated estradiol 17- α -dehydrogenase in the human placenta under ultraviolet light. Imines and oximes of α -dicarbonyl compounds are good complexing agents for heavier metals. Since metal complexes can undergo ET and generate ROS (Kovacic et al. 2005; Kovacic and Somanathan 2006a; Kovacic et al. 2002; Poli et al. 1989; Kovacic and Thurn 2005; Kovacic and Somanathan 2005; Kovacic and Cooksy 2005a; Kovacic and Somanathan 2008; Halliwell and Gutteridge 1999), it is plausible that these complexes possess physiological activity.

7 Summary

Diacetyl, a butter-flavoring component, has recently attracted scientific and media attention because it has been implicated as an agent that induces popcorn lung disease in exposed plant workers. This disease, officially referred to as bronchiolitis obliterans, entails exposure-induced compromise to the lung's epithelial barrier function. In this review, we present a novel molecular mechanism (electron transfer, ET) designed to explain how diacetyl and its imine derivatives might interact to produce lung damage. We relate the fact that diacetyl and related compounds possess reduction potentials amenable to electron transfer (ET) *in vivo*. The electrochemical nature of these toxicants can potentially disrupt normal ET processes, generate reactive oxygen species (ROS), and participate in cell signaling events. Condensation of diacetyl with protein may also play a role in the toxicity caused by this compound. ET is a common feature of toxic substances, usually involving their metabolites which can operate *per se* or through reactions that generate ROS and oxidative stress (OS). Examples of agents capable of ET are quinone and metal compounds, aromatic nitro compounds, and iminium salts. Among compounds that generate ET, the α -dicarbonyl ET class, of which diacetyl is a member, is much less studied. This review emphasizes diacetyl as an agent that acts through oxidative processes to cause its effects. However, we also treat related substances that appear to act by a similar mechanism. This mechanism forms a theoretical framework capable of

describing the mechanism by which diacetyl may induce its effects and is in accord with various physiological activities displayed by other α -dicarbonyl substances. Examples of substances that may act by mechanisms similar to that displayed by diacetyl include cyclohexane-1,2-dione, marinopyrroles, reactive carbonyl species, the bacterial signaling agent DPD, and advanced glycation end products.

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References

- Baguley BC, Wakelin LPG, Jacintho JD, Kovacic P (2003) Mechanisms of action of DNA intercalating acridine-based drugs: How important are contributions from electron transfer and oxidative stress. *Curr Med Chem* 24:2643–2649.
- Berson A, Descatoire V, Sutton A, Fau D, Maulny B, Vadrot N, Feldmann G, Berthon B, Torkjmann T, Pessayre D (2001) Toxicity of alpidem, a peripheral benzodiazepine receptor ligand, but not zolpidem, in rat hepatocytes: Role of mitochondrial permeability transition and metabolic activation. *J Pharmacol Exp Ther* 299:793–800.
- Briegleb G (1964) Electron affinity of organic molecules. *Angew Chem Int Edit* 3:617–632.
- Choudhary D, Chandra D, Kale RK (1997) Influence of methylglyoxal on antioxidant enzymes and oxidative damage. *Toxicol Lett* 93:141–152.
- Du J, Suzuki H, Nagase F, Akhand A.A, Ma XY, Yokoyama T, Miyata T, Nakashima I, (2001) Superoxide-mediated early oxidation and activation of ASK1 are important for initiating methylglyoxal-induced apoptosis process. *Free Rad Biol Med* 31:469–478.
- Egilman D, Mailloux C, Valentin C (2007) Popcorn-worker lung caused by corporate and regulatory negligence: An avoidable tragedy. *Int J Occup Environ Health* 13:85–98.
- Fedan JS, Dowdy J.A, Fedan KB, Hubbs AF (2006) Popcorn worker's lung: In vitro exposure to diacetyl, an ingredient in microwave popcorn butter flavoring, increases reactivity to methacholine. *Toxicol Appl Pharmacol* 215:17–22.
- Gagliardi LG (2006) Electrostatic considerations in nuclear envelope breakdown and reassembly. *J Electrostat* 64:843–849.
- Halliwell B, Gutteridge JMC (1999) *Free Radicals in Biology and Medicine*, Oxford University Press, New York pp 1–897.
- Hanson DG (2009) OSHA withdraws diacetyl proposal. *Chem Eng News* 87:23.
- Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL (2007) The major *Vibrio cholerae* autoinducer and its role in virulence factor production against oxidative stress. *Nature* 450:883–886.
- Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V (2008) Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. *Toxicol Pathol* 36:330–344.
- Hughes CC, Prieto-Davo A, Jensen PR, Fenical W (2008) The marinopyrroles, antibiotics of an unprecedented structure class from a marine *Streptomyces* sp. *Org Lett* 10:629–631.
- ILS (2007) Integrated Laboratory Systems Inc: Chemical Information Review Document for Artificial Butter Flavoring and Constituents Diacetyl [CAS No. 431-03-08] and Acetoin [CAS No. 513-86-0], http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Artificial_butter_flavoring.pdf 2007.
- Ishikita H, Knapp EW (2006) Electrostatic role of the non-heme iron complex in bacterial photosynthetic reaction center. *FEBS Lett* 580:4567–4570.
- Jacobs GP (1986) Non-nitro radiation sensitizers. *Int J Radiat Biol Relat Stud Phys Chem Med* 49:887–890.

- Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins J (2007) A common mechanism of cellular death induced by bactericidal antibiotics. *J Cell* 130:797–810.
- Kovacic P (2003) Mechanism of organophosphates (nerve gases and pesticides) and antidotes: Electron transfer and oxidative stress. *Curr Med Chem* 10:2705–2710.
- Kovacic P (2005) Fundamental, electron transfer mechanism by pyrylium-type ions for the anticancer drugs 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and flavone-8-acetic acid (FAA). *Curr Med Chem-Anti-Cancer Agents* 5:501–506.
- Kovacic P (2006) Novel electrochemical approach to enhanced toxicity of 4 oxo-2-nonenal vs. 4-hydroxy-2-nonenal (role of imine): Oxidative stress and therapeutic modalities. *Med Hypotheses* 67:151–156.
- Kovacic P (2007) Unifying mechanism for bacterial cell signaling (4,5-dihydroxy-2,3-petanedione, lactones and oligopeptides) electron transfer and reactive oxygen species. Practical medical features. *Med Hypotheses* 69:1105–1110.
- Kovacic P (2008) Bioelectrostatics: Review of widespread importance in biochemistry. *J Electrostat* 66:124–129.
- Kovacic P, Becvar LE (2000) Mode of action of anti-infective agents: Focus on oxidative stress and electron transfer. *Curr Pharmaceut Des* 6:143–167.
- Kovacic P, Cooksy AL (2005a) Unifying mechanism for toxicity and addiction by abused drugs: Electron transfer and reactive oxygen species. *Med Hypotheses* 64:357–366.
- Kovacic P, Cooksy AL (2005b) Role of diacetyl metabolite in alcohol toxicity and addiction via electron transfer and oxidative stress. *Arch Toxicol* 79:123–128.
- Kovacic P, Draskovich CD, Pozos RS (2007b) Unifying electrostatic mechanism for phosphates and sulfates in cell signaling. *J Recept Signal Transduct* 27:433–443.
- Kovacic P, Jacintho JD (2001) Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer. *Curr Med Chem* 8:773–796.
- Kovacic P, Kassel MA, Popp WJ, Feinberg BA (1990) Mechanisms of antiviral action: Focus on electron transfer and oxidative stress. *J Biopharmaceut Sci* 1:315–330.
- Kovacic P, Osuna JA (2000) Mechanisms of anti-cancer agents: Emphasis on oxidative stress and electron transfer. *Curr Pharmaceut Des* 6:277–309.
- Kovacic P, Pozos RS, Somanathan R, Shangari R, O'Brien P (2005) Mechanism of mitochondrial uncouplers, inhibitors, and toxins: Focus on electron transfer, free radicals, and structure-activity relationships. *J Curr Med Chem* 5:2601–2623.
- Kovacic P, Pozos RS (2006) Cell signaling (mechanism and reproductive toxicity): Redox chains, radicals, electrons, relays, conduit, electrochemistry, and other medical implications. *Birth Defects Res Part C* 78:333–344.
- Kovacic P, Pozos RS, Draskovich CD (2007a) Unifying electrostatic mechanism for receptor-ligand activity. *J Recept Signal Transduct* 27:411–432.
- Kovacic P, Sacman A, Wu-Weis M (2002) ephrotoxins: Widespread role of oxidative stress and electron transfer. *Curr Med Chem* 9:823–847.
- Kovacic P, Somanathan R (2005) Neurotoxicity: The broad framework of electron transfer, oxidative stress and protection by antioxidants. *Curr Med Chem-CNS Agents* 5:249–258.
- Kovacic P, Somanathan R (2006a) Mechanism of teratogenesis: Electron transfer, reactive oxygen species, and antioxidants. *Birth Defects Res Part C* 78:308–344.
- Kovacic P, Somanathan R (2006b) In: *New Research on Alcohol Abuse and Alcoholism*. Brozner EY (ed), Nova Science, New York, pp 51–58.
- Kovacic P, Somanathan R (2008) Ototoxicity and noise trauma, electron transfer, reactive oxygen, species, cell signaling, electrical effects, and protection by antioxidants: Practical medical aspects. *Med Hypotheses* 70:914–923.
- Kovacic P, Somanathan R (2009a) Zolipidem, a clinical hypnotic that affects electronic transfer, alters synaptic activity through potential GABA receptors in the nervous system without significant free radical generation. *Oxid Med Cell Longev* 2: 116–121.
- Kovacic P, Somanathan R (2009b) Pulmonary toxicity and environmental contamination: Radicals, electron transfer and protection by antioxidants. *Rev Environ Contam Toxicol* 201:41–69.

- Kovacic P, Thurn LA (2005) Cardiovascular toxicity from the perspective of oxidative stress, electron transfer, and prevention by antioxidants. *Curr Vasc Pharmacol* 3:107–118.
- Kovacic P, Wakelin LPG (2001) DNA molecular electrostatic potential, novel perspectives for the mechanisms of action of anticancer drugs involving electron transfer and oxidative stress. *Anti-Canc Drug Des* 16:1–10.
- Kreiss K (2007) Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol* 7: 162–167.
- Mellado JMR, Montoya MR (1994a) CEC mechanisms in the electroreduction of α -dicarbonyl compounds on mercury electrodes. *J Electroanal Chem* 365:71–78.
- Mellado JMR, Montoya MR (1994b) Correlations between chemical reactivity and mutagenic activity against *S. typhimurium* mechanism TA100 for dicarbonyl compounds as proof of mutagenic mechanism. *Mutat Res* 304:261–264.
- Miller AG, Gerrard JA (2005) Assessment of protein function following crosslinking by dicarbonyl. *Ann N Y Acad Sci* 1043:195–200.
- Montoya MR, Zon MA, Mellado JMR (1993) Investigation of the reduction of 1,2-cyclohexanedione and methylglyoxal on mercury electrodes under pure kinetic conditions by linear sweep voltammetry. *J Electroanal Chem* 353:217–224.
- Morgan DL, Flake GP, Kirby PJ, Palmer SM (2008) Respiratory toxicity of diacetyl in C57BL/6 mice. *Toxicol Sci* 103:169–180.
- Niufar NN, Haycock FL, Wesemann JL, MacStay JA, Heasley VL, Kovacic P (2002) Reduction potentials of conjugated aliphatic ketones, oximes and imines: Correlation with structure and bioactivity. *Rev Chem Soc Mex* 46:307–312.
- Nowack KC, Koppens FHL, Nazarov YV, Vandersypen LMK (2007) Coherent control of a single electron spin with electric fields. *Science* 318:1430–1433.
- Otsuka M, Mine T, Ohuchi K, Ohmori S (1996) A detoxification route for acetaldehyde, metabolism of diacetyl, acetoin and 2,3-butanediol in liver homogenate perfused liver of rats. *J Biochem (Tokyo)* 119:246–251.
- Poli G, Cheeseman KH, Dianzani MU, Slater TF (1989) Free Radicals in the Pathogenesis of Liver Injury, Pergamon, New York, pp 1–330.
- Pripis-Nicolau L, de Revel G, Bertrand A, Maujean A (2000) Formation of flavor components by the reaction of amino acids and carbonyl compounds in mild conditions. *J Agric Food Chem* 48:3761–3766.
- Russell GA (1968) In: *Radical Ions*. Interscience. Kaiser ET, Kevan N (eds), New York, pp 91–106.
- Schlichter LC, Pahapill PA, Chung I (1992) Dual action of 2,3-butanedione monoxime (BDM) on K^+ currents in human T lymphocytes. *J Pharmacol Exp Ther* 261:438–446.
- Schneider A (2007) *Seattle Post-Intelligencer*, Dec: 21: p. A.1.
- Tallentire A, Schiller NL, Powers EL (1968) 2,3-Butanedione, an electron-stabilizing compound, as a modifier of sensitivity of *Bacillus megaterium* spores to X-rays. *Int J Radiat Biol* 14: 397–402.
- Tiffany BD, Wright JB, Moffett RB, Heinzelmann RV, Strube RE, Aspergren BD, Lincoln EH, White JL (1957) Antiviral compounds, aliphatic glyoxals, hydroxyaldehydes and related compounds. *J Am Chem Soc* 79:1682–1687.
- Traverso N, Manini S, Cottalasso D, Odetti P, Marinari UM, Pronzato MA (1997) Mutual interaction between glycation and oxidation during non-enzymatic protein modification. *Biochim Biophys Acta* 1336:409–418.
- Walker GR, Feather MS (1983) *The Maillard Reaction in Food and Nutrition*, ACS Symposium Series. 215:ACS, Washington, DC, p 36.
- Wondrak GT, Cervantes-Laurean D, Roberts MG, Qrasem JG, Kim M, Jacobson EL, Jacobson MK (2002) Identification of dicarbonyl scavengers for cellular protection against oxidative stress. *Biochem Pharmacol* 63:361–373.
- Wu L (2005) The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells. *Can J Physiol Pharmacol* 83:63–68.

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