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Ring substitution influences oxidative cyclisation and reactive metabolite formation of nordihydroguaiaretic acid analogues

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ABSTRACT

Nordihydroguaiaretic acid (NDGA) is a natural polyphenol with a broad spectrum of pharmacological properties. However, its usefulness is hindered by the lack of understanding of its pharmacological and toxicological pathways. Previously we showed that oxidative cyclisation of NDGA at physiological pH forms a dibenzocyclooctadiene that may have therapeutic benefits whilst oxidation to an *ortho*-quinone likely mediates toxicological properties. NDGA analogues with higher propensity to cyclise under physiologically relevant conditions might have pharmacological implications, which motivated this study. We synthesized a series of NDGA analogues which were designed to investigate the structural features which influence the intramolecular cyclisation process and help to understand the mechanism of NDGA's autoxidative conversion to a dibenzocyclooctadiene lignan. We determined the ability of the NDGA analogues investigated to form dibenzocyclooctadienes and evaluated the oxidative stability at pH 7.4 of the analogues and the stability of any dibenzocyclooctadienes formed from the NDGA analogues. We found among our group of analogues the catechols were less stable than phenols, a single catechol-substituted ring is insufficient to form a dibenzocyclooctadiene lignan, and only compounds possessing a di-catechol could form dibenzocyclooctadienes. This suggests that quinone formation may not be necessary for cyclisation to occur and the intramolecular cyclisation likely involves a radical-mediated rather than an electrophilic substitution process. We also determined that the catechol dibenzocyclooctadienes autoxidised at comparable rates to the parent catechol. This suggests that assigning in vitro biological activity to the NDGA dibenzocyclooctadiene is premature and requires additional study.

1. Introduction

The leaves of the plant creosote bush were used commonly in traditional medicines among the Native Americans for diverse beneficial effects.[1–3] The aqueous extract of this shrub, commonly referred to as Chaparral tea, was listed in the American pharmacopeia as an ethnobotanical used to treat tuberculosis, arthritis and cancer.[1] Documented traditional applications of the plant extract include treatment for infertility, rheumatism, arthritis, diabetes, gallbladder and kidney stones, pain and inflammation among many others.[2,3] Creosote bush is rich in lignans, particularly nordihydroguaiaretic acid (NDGA) (up to 15% d.w.).[2] NDGA is generally accepted as responsible for both beneficial and adverse effects associated with this shrub.[2–6] NDGA shows promise in the treatment of multiple diseases, including cardiovascular diseases,[7,8] neurological disorders[9–13] and cancers.[3,14–20] It also potently inhibits viruses such as human immunodeficiency virus (HIV-1), herpes simplex virus (HSV), human papilloma virus (HPV) and influenza virus.[2,21] The radical scavenging[22,23] and antioxidant effects[24] as well anti-inflammatory[25,26] and anti-proliferative properties may be of relevance in different diseases. Despite its broad pharmacological activities, NDGA use is associated with toxicity especially when ingested at higher doses. Hepato- and nephrotoxicity has been associated with NDGA use[24,27–30] and is likely linked to NDGA bioactivation to a reactive *ortho*-quinone.[31–33]

The origin of the dichotomous biological activity observed for NDGA remains unknown. Traditionally creosote bush-based products are prepared by boiling the leaves in water.[3] We previously demonstrated that incubation of NDGA at pH 7.4 gave a

Abbreviations: NDGA, nordihydroguaiaretic acid; cNDGA, NDGA cyclolignan; DMPA, 3,4-dimethoxyphenylacetone; ESI-MS, electrospray ionization-mass spectrometry; GSH, glutathione; GSSG, oxidized glutathione; HPLC, high performance liquid chromatography; HIV-1, human immunodeficiency virus; HSV, herpes simplex virus; HPV, human papilloma virus; NADP(H), reduced nicotine adenine dinucleotide phosphate; TFA, trifluoroacetic acid.

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schisandrin-like dibenzocyclooctadiene lignan,[34] however in the presence of glutathione (GSH) a ring adduct is observed, indicating that NDGA can form an *ortho*-quinone.[31] Biological evaluations of NDGA are commonly conducted under aerobic conditions at pH 7.4 or higher, which may confound the results as biological activity could be the result of NDGA, its *ortho*-quinone, dibenzocyclooctadiene or a combination.[5,35] Traditional conditions of preparation favour oxidation and we suggested that the NDGA dibenzocyclooctadiene may make a contribution to the beneficial effects of NDGA, and that NDGA analogues with a higher propensity to cyclise under physiologically relevant conditions might have pharmacological implications. There are numerous reports of biological activity for naturally-occurring and synthetic dibenzocyclooctadiene lignans,[36–39] including a report that for a series of dibenzylbutanediols the dibenzocyclooctadiene structure enhanced anti-tumor activity and inhibition of MDA-MB-435 breast cancer cells.[40] Pharmacological activity from the uncyclised lignan cannot be ruled out however, as anti-viral properties have been reported for tetramethyl and tetraacetyl NDGA which would not be anticipated to cyclise under these conditions.[21]

The precise mechanism of the intramolecular cyclisation is unknown although it possibly follows one of two pathways (Scheme 1).[34,41–43] We[34] and others[22] have hypothesized that cyclisation occurs through a di-radical mechanism, whereas there are numerous examples where intermolecular coupling of catechols is proposed to occur via electrophilic substitution through an *ortho*-quinone intermediate.[42,44] We therefore propose to identify the structural features which control oxidative metabolism of NDGA-like lignans, including cyclisation. In order to accomplish these goals we plan to: investigate the oxidative stability of NDGA lignan and dibenzocyclooctadiene analogues at pH 7.4 and determine what structural features are necessary for formation of dibenzocyclooctadienes from the NDGA lignan analogues. We synthesized seven NDGA analogues (Fig. 1) and evaluated their stability including lignan half-life at pH 7.4, and product studies to determine their ability to form dibenzocyclooctadienes using our previously established conditions.[34] We were also interested in establishing the oxidative stability of the prepared analogues as stability of a compound in a test medium is an important parameter when investigating biological activity.[45,46]

The analogues were designed to help us understand the structural features which influence the intramolecular cyclisation process. This will be useful in understanding the mechanisms of NDGA autoxidative conversion to dibenzocyclooctadiene lignans and will allow us to control intramolecular cyclisation in the preparation of additional lignan analogues. We anticipate that these studies will provide information on the contribution that dibenzocyclooctadienes play in the in vitro pharmacological activity of NDGA.

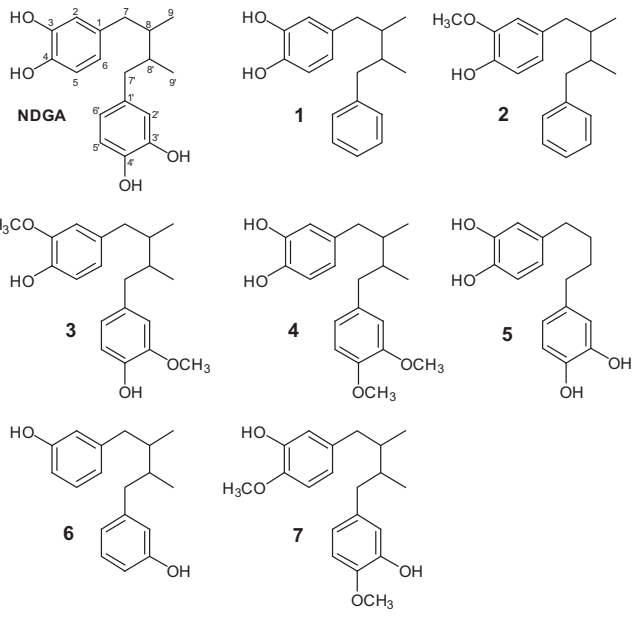
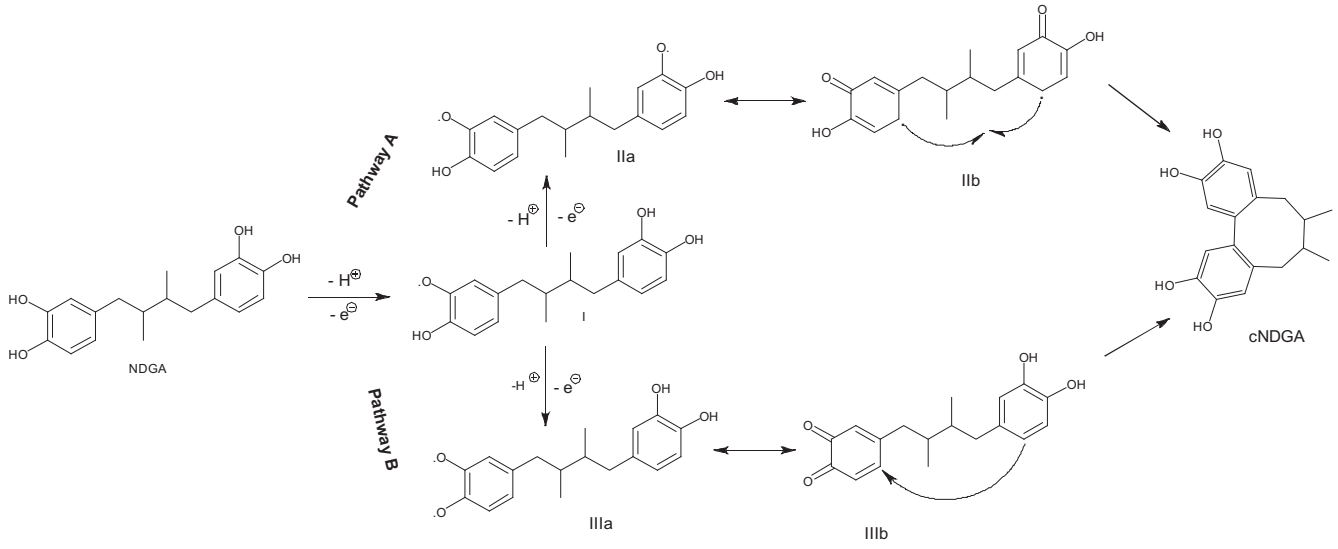


Figure 1. Structures for NDGA and its structural analogues synthesised for this study.

2. Materials and methods

2.1. Materials

Caution: *The following chemicals are hazardous and should be handled carefully:* ***meso***-Nordihydroguaiaretic acid (NDGA, 97%) from *Larrea tridentata*, reduced glutathione (GSH), 3,4-dimethoxyphenylacetone (DMPA), Tyrosinase from mushroom (EC1.14.18.1),



Scheme 1. Intramolecular conversion of NDGA to dibenzocyclooctadiene lignan (cNDGA) via radical-mediated process (pathway A) or electrophilic substitution mechanism (pathway B). Both pathways involve a 2 proton, 2 electron loss followed by isomerisation of a di-radical intermediate IIa–IIb or an *ortho*-quinone IIIa–IIIb and subsequent radical coupling or electrophilic substitution, respectively.