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Iron (III)-mediated degradation of α-asarone and characterization of its major degradation products by UPLC-MS/MS and NMR

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α-Asarone, the main bioactive phytochemicals of *Acorus* species, is widely used in the treatment of respiratory disorders. The solution stability study of α-asarone was investigated in the presence of various metal ions. α-Asarone was found to be unstable in the presence of the metal ions Fe^{3+}, Cu^{2+} and Al^{3+}, in which the induction of Fe^{3+} was highly prone to the degradation of α-asarone. Thus, an iron (III)-mediated forced degradation study of α-asarone was carried out. One oxidative and four dimeric products were formed after the degradation of α-asarone. The complete mass fragmentation patterns for α-asarone and its degradation products (DPs) were established by UPLC-MS/MS in the positive ionization mode, and their structural confirmation was accomplished with ^{1}H and ^{13}C NMR. Then, the mechanistic pathways for the formation of all DPs were postulated. Finally, the oxidation degradation behavior and mechanism of α-asarone in the presence of oxidative stressors viz., hydrogen peroxide and azobisisobutyronitrile were explored.

1. Introduction

α-Asarone, chemically known as (E)-2,4,5-trimethoxy-1-propylbenzene, is one of the main active components of *Acorus* species in India and China (Sandeep et al. 2014; Chamorro et al. 1993). In 1979, Junshou Zhang et al. isolated α-asarone from the volatile oil of the *Acorus* plant, and prepared an α-asarone injection, which showed effects on reducing phlegm, relieving cough as well as asthma, and was useful to cure large lobular pneumonia and bronchial inflammation. At present, α-asarone is clinically available in China in several dosage forms, including tablet, capsule and injection, for the treatment of respiratory disorders. Modern pharmacological show that α-asarone reveals positive effects in the treatment of depression, anxiety, Alzheimer's, and Parkinson's disease (Chellian 2017). The mechanism of α-asarone in the treatment of neurodegenerative processes has been described (Chen et al. 2020; Lam et al. 2019; Chellian et al. 2016; Huang et al. 2013) and suggests that it could be a promising drug. Although many studies were devoted to the pharmacological and toxic effects of α-asarone, there are few reports about the stability of α-asarone.

Forced degradation studies are of great significance in investigating the stability of drugs and the selectivity of analytical methods in the development of drug products. According to ICH and PhRMA guidelines (ICH Q1A 2003; Reynolds et al. 2002), forced degradation studies should include thermolysis, oxidation, hydrolysis and photolysis, if the drug or the impurities of the drug are sensitive to trace metal, forced degradation studies should investigate the stability of drug exposure to the various metal ions, and strategies should be developed to avoid risk. Transition metals, such as Fe^{3+} and Cu^{2+}, which widely found in process pipes, excipients, reagents, glassware etc., are always difficult to avoid and completely remove from the entire drug development and manufacturing process. Some specific cases of drug degradation mediated by metal ions indicate that it is of great significance to evaluate the drug sensitivity to metal ions (Hong et al. 2004; Harmon et al. 2006; Wang et al. 2015; Nanda et al. 2017; Dotterer et al. 2011). However, metal-induced forced degradation studies are rare in pharmaceutical companies or research institutions (Steven et al. 2001).

It is already known that the α-asarone is photo-unstable and easy to transform into its cis-isomer (β-asarone) under the light. A report has described the light-induced degradation behavior of α-asarone, and several degradation products were detected by HSGC-MS, but exact structural elucidation was insufficient due to the small amount of products (Lander and Schreier 1991). In this study, the influence of metal ions on the stability of α-asarone was investigated by adding metal ions to drug solution. Depending on the actual condition, the metal ions to be investigated were selected as Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+} and Ni^{2+}. This study attempts to confirm the chemical structures of the major degradation products of α-asarone in the presence of metal ions, and explore the iron (III)-mediated degradation mechanism. Besides, the mechanisms of oxidation reactions of α-asarone were explored. The information will be valuable for understanding the chemical stability of α-asarone and screening for appropriate process and storage conditions.

2. Investigations, results and discussion

2.1. HPLC analysis of the stability and stressed samples

2.1.1. HPLC analysis of the stability samples

This study was carried out to explore the stability of α-asarone solution in the presence of metal ions. Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+} and Ni^{2+} were selected to simulate the possible conditions in the actual manufacturing process. According to the ICH Q3D: impurity elements guideline (ICH Q3D 2003), the element Ni belongs to Class 2A and the element Cu belongs to Class 3. It is indicated that Ni may be present in the drug product with relatively high probability, and Cu is at risk in inhalation and injection products. The permitted daily exposure (PDEs) of the elements Ca, Mg, Fe, Al, Mn and Zn have not been established and classified. In the

injection route, the PDE of Ni is 22 µg/ day and that of Cu is 340 µg/ day. According to the commonly used concentration of metal ions described in the references (Liu et al. 2012; Chen et al. 2007; Hisao et al. 1987), and to ensure that the concentration of metal ions was higher than the concentration of potential metal ions, the concentrations of Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Mn^{2+} and Zn^{2+} in the α-asarone solution were set at 100 µg/mL, and the concentration of Ni^{2+} was set at 10 µg/mL. The HPLC analysis results are shown in Fig.1, α-asarone solution remained stable in the presence of metal ions Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Ni^{2+}, while it was highly prone to degradation in the presence of Fe^{3+}, Cu^{2+} and Al^{3+}, especially under the mediation of Fe^{3+}, α-asarone degraded by more than 90% degradation in 10 days.

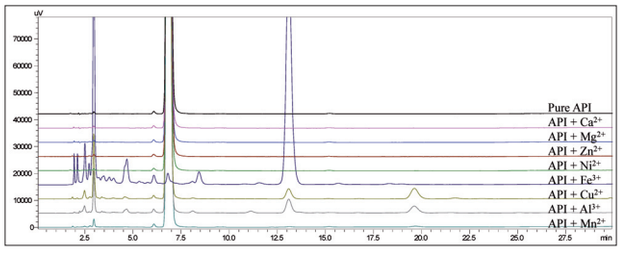

Fig. 1: Chromatograms of stability of α-asarone solution in the presence of different metal ions.

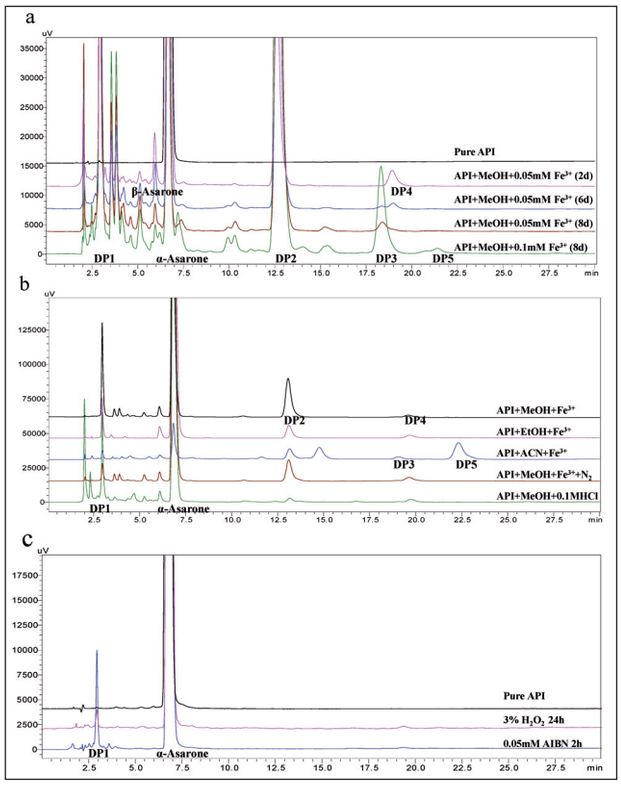

Fig. 2: HPLC chromatograms showing the DPs in iron (III)-stressed samples at different time intervals (a), the behavior on the iron (III)-induced degradation of α-asarone under different conditions (b), and oxidative degradation undergone by α-asarone in the presence of 3% H_2O_2 and 0.05 mM AIBN (c).

2.1.2. HPLC analysis of the degradation behavior of stress samples

The iron (III)-mediated degradation behavior of α-asarone solution was investigated with HPLC-DAD after subjecting it to various stress conditions in order to understand its degradation pathway and mechanism. As shown in Fig. 2a, three DPs, DP1, DP2 and DP4, were present in iron (III)-mediated stressed sample dissolved in methanol, and as the degradation time was extended or the addition of iron (III) increased, DP3 and DP5 were observed, but DP4 faded away. The effects of different solvents on the iron (III)-induced degradation of α-asarone are shown in Fig. 2b, the degradation behavior in methanol and ethanol added with iron (III) was found to be similar, but the degradation rate of the ethanol system was slower. The impurity profile of the acetonitrile system was different from that of the two protic solvents, DP1, DP2, DP3and DP5 were found but the DP5 as the main impurities, moreover, degradation rate was much faster. Nitrogen was beneficial to the stability of α-asarone solution, which could slow down the degradation rate, and help for a significant decrease in the formation of DP1, so it could be speculated that the formation mechanism of DP1 is related to the oxygen. In 0.1M HCl, the degradation of α-asarone took place with the major formation of the DP1, suggested that the impurity may also be produced with acid acting as catalysis.

Iron ions are often used for oxidative degradation, and the most common mechanism of metal-induced drug degradation is metal-catalyzed oxidation reaction. In order to compare the different degradation behavior and mechanism of α-asarone under different oxidation conditions, hydrogen peroxide and the autooxidation initiator AIBN were used for oxidative degradation. As shown in Fig. 2c, only DP1 was present in H_2O_2 and AIBN assisted-drug degradation in the suitable degradation degree.

2.2. Structural characterization of degradation products

The DP1 is relatively polar in nature, as it eluted much earlier than the drug and other DPs. The molecular formula of DP1 was established as $C_{10}H_{12}O_4$ by ESI-MS data in which the observed *m/z* 196.0 was calculated for $[M+H]^+$ 197.0 and $[M+Na]^+$ 218.9 (Fig. 3b). A comparison of the molecular formula of DP1 with the α-asarone standard revealed two less carbons, two less hydrogens and one more oxygen incorporated into DP1, implying that DP1 is an oxidation product of α-asarone. Based on the second order mass spectrums of $[M+H]^+$ peaks of α-asarone and DP1 (Fig. 3a, 3b), the structure was proposed and the main fragmentation pathway were shown as Fig. 5, and the complete mass fragmentation pattern of α-asarone was shown in Fig. 4. The structure was further confirmed based on the NMR results (Fig. 6). An inspection of NMR ($CDCl_3$) spectra indicated that products contained the same trimethoxyphenyl structure as α-asarone. A comparison of the ^1H and ^{13}C NMR data of DP1 with α-asarone showed the significant downfield shift of H-8 (δ_H 189.53) and C-7 (δ_C 10.29) and the lack of one methyl carbon (-CH_3), olefin hydrogen and methyl protons (=CH-CH_3), indicating that the propenyl was substituted by an aldehyde group and the degradation product to be 2,4,5-trimethoxybenzaldehyde (asaryldehyde).

DP2 exhibited the molecular formula of $C_{24}H_{30}O_6$ by ESI-MS, in which the observed *m/z* 414.1 was calculated for $[M+H]^+$ 415.1, $[M+NH_4]^+$ 432.1 , $[M+Na]^+$ 437.1 and $[M+K]^+$ 453.1 (Fig. 3c). By comparing the formula of DP2 with that of α-asarone ($C_{12}H_{16}O_3$), it appeared that DP2 would be a dimeric degradant of α-asarone in of some sort, and the dimer structure was further confirmed based on six methoxy peaks at δ_H 3.37, 3.55, 3.84, 3.85, 3.86 and 3.91 ppm (each 3H, s, OCH_3×6) in ^1H NMR spectrum (Fig. 6). DP2 has two less hydrogen atoms than a simple dimer, and belongs to the type of M + M -2H= 2(M-H) dimer. In ^1H NMR spectra of DP2, two methyl peak at δ 1.05 ppm (s, -CH_3) and δ 1.73 ppm (s, =C-$\underline{CH_3}$), two methine protons at δ 2.21 ppm (q, -$\underline{CH}$-CH_3), δ 4.73 ppm (d, Ar-$\underline{CH}$-CH), and an isolated olefinic protons at δ 6.51 ppm were found. Combined with the generation of the characteristic fragment ion *m/z* 247.0 $[C_{15}H_{19}O_3^+]$, *m/z* 231.9 $[C_{14}H_{16}O_3^+]$ and *m/z* 215.9 $[C_{14}H_{16}O_2^+]$, we speculated that the lost two hydrogens may due to a ring formed by the double bond of one molecule of α-asarone replaced by another molecule, and the structure of DP2 was identified as magnoshinin. The compound has been reported as a new neolignan isolated from *Magnolia saliciforia* maxim (Kikuchi et al. 1983). The fragmentation pattern of the DP2 at 20 eV collision energy in MS^2 spectrum was proposed as shown in Fig. 7.

The MS spectra of DP3, DP4 and DP5 were similar. All of them displayed two ions with their *m/z* values at 417.1 and 439.1, corre-

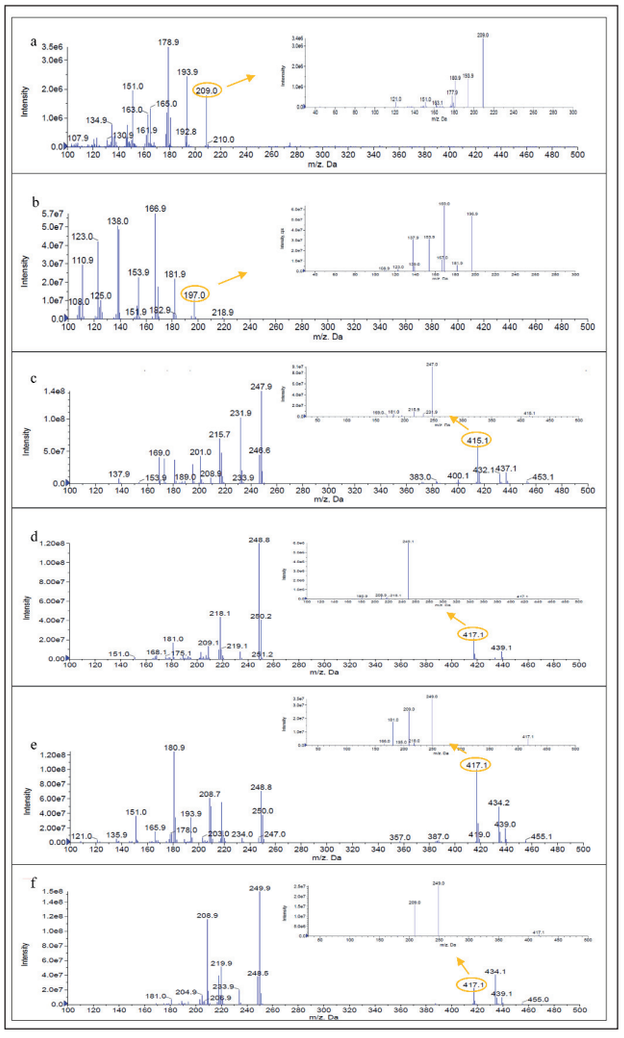

Fig. 3: UPLC–MS^2 spectra of α-asarone (a) and DP1 (b), DP2 (c), DP3 (d), DP4 (e) and DP5 (f).

sponding to the protonated and sodium adduct ions of DPs, respectively, which matched a formula of $C_{24}H_{32}O_6$ (exact mass 416.2), and the twice molecular weight of α-asarone (Fig.3d, 3e, 3f). The MS^2 spectrum of DP3 also showed resemblance to that of DP4 and DP5, the presence of distinctive ions at *m/z* 249.0 and *m/z* 209.0 were found, and the *m/z* 209 corresponding to the protonated adduct ions of α-asarone. It could be speculated that DP3, DP4 and DP5 would be isomers, all of which are dimeric degradants of α-asarone in the type of 2M. Meanwhile, the characteristic fragment ion of *m/z* 249.0 [$C_{15}H_{21}O_3^+$] and *m/z* 209.0 [$C_{13}H_{19}O_2^+$] in MS^2 spectrum, suggested that the DP3, DP4 and DP5 may have arisen from the reaction of α-asarone with trimethoxybenzylcarbonium, which was generated through the interaction of α-asarone with the iron (III). The NMR data of the three impurities showed differences, as shown in Fig. 8. There is a symmetric characterization in the structure of DP4, with the methyl peak at δ 1.18 ppm (d, 6H), two pairs methine protons at δ 1.72-1.81 ppm (m, 2H) and δ 3.26 ppm (d, 2H), respectively, and two pairs aromatic protons. The structure can be deduced, but there are multiple isomers in this structure (Fig. 9), which are very confusing. After a careful comparison (Ryu et al. 2002; Mahindru et al.1993; Yamamura et al. 1978), DP4 was identified as 1β,2α,3β,4α-1,2-dimethyl-3,4-bis-(2,4,5-trimethoxyphenyl)-cyclobutane (magnosalin). The spectral data of DP3 and DP5 were significantly different, although they both contained a triplet peak of a methyl proton (-CH_2-$\underline{CH_3}$). DP3 showed four aromatic protons and one olefinic proton, while DP5 showed three aromatic protons, suggesting that they were formed by two molecules of α-asarone linked in different ways, one was linked by the carbon on the side chain and the other was linked by the carbon on the benzene ring. In addition, the presence of a double peak at δ 1.17 ppm and a triple peak at δ 3.74 ppm in the proton NMR showed the presence of a methyl proton (-CH-$\underline{CH_3}$) and a methine proton (-CH_2-$\underline{CH}$) in DP5, while a singlet peak at δ 1.65 ppm and a double peak at δ 4.29 ppm showed the presence of a methyl proton (-C-$\underline{CH_3}$) and a methine proton (-CH-$\underline{CH}$) in DP3. Finally, the two impurities, DP3 and DP5 were respectively identified as 3-ethyl-2-methyl-3-(2",4",5"-trimethoxy)phenyl-1-(2',4',5'-trimethoxy)phenyl-1-propene, and 1-ethyl-4,5,7-trimethoxy-2-methyl-3-(2,4,5-trimethoxyphenyl)-2,3-dihydro-1H-indene. The fragmentation patterns of the protonated DPs (*m/z* 417.1) were proposed as shown in Fig. 10, 11, 12, respectively.

2.3. *Plausible formation mechanism of degradation products*

The proposed mechanism pathway of iron (III)-mediated degradation of α-asarone as shown in Fig. 13. α-Asarone degraded to

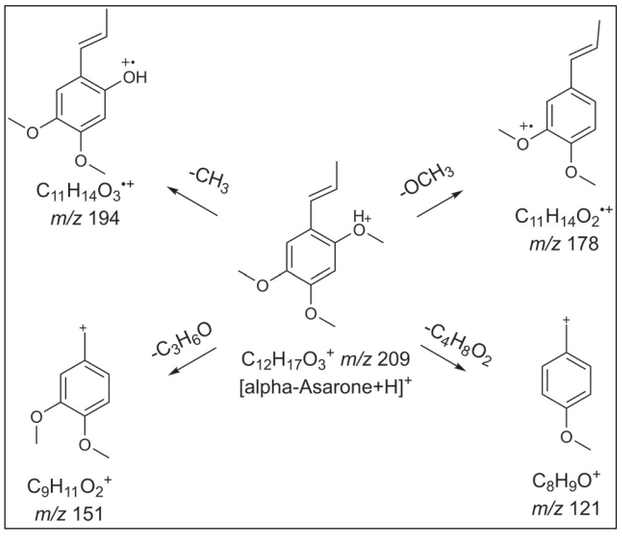

Fig. 4: Proposed mass fragmentation pathway of protonated α-asarone.

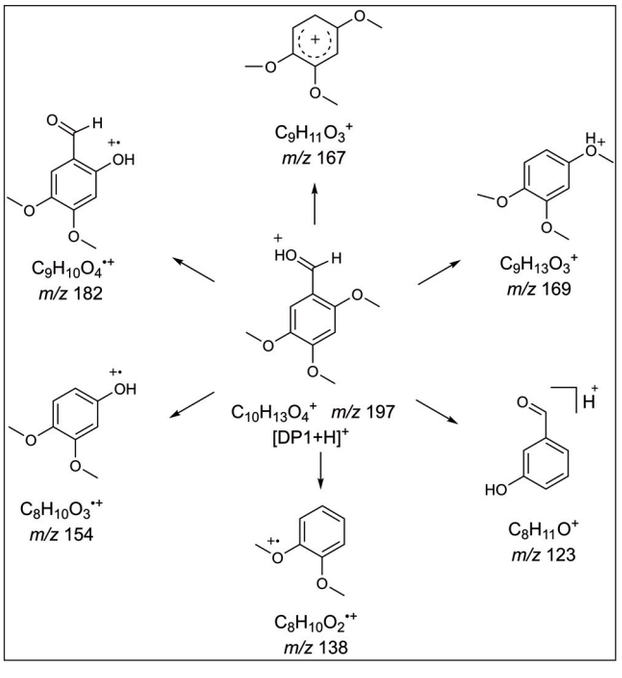

Fig. 5: Proposed mass fragmentation pathway of protonated DP1.

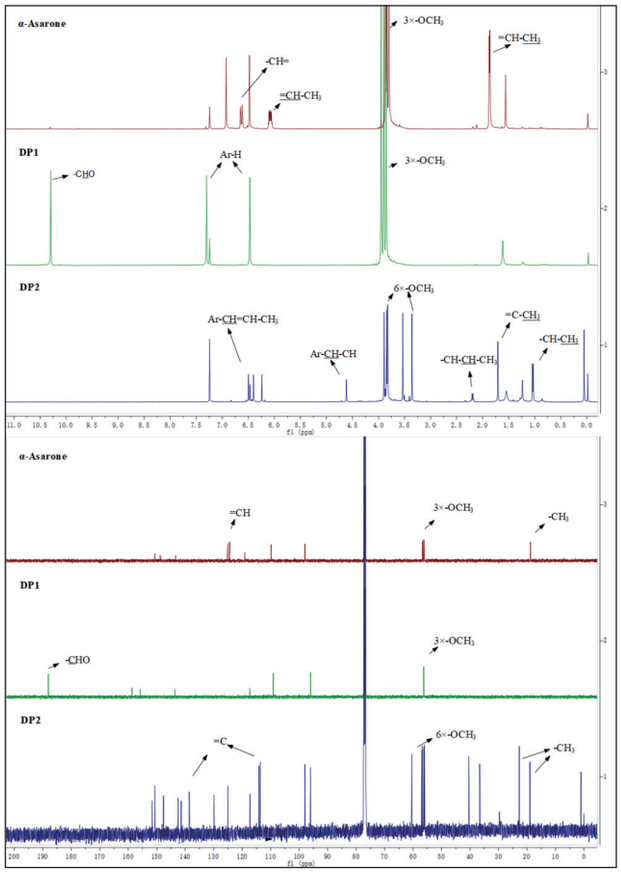

Fig. 6: Overlaid ^{1}H NMR and ^{13}C NMR spectra of α-asarone., DP1 and DP2.

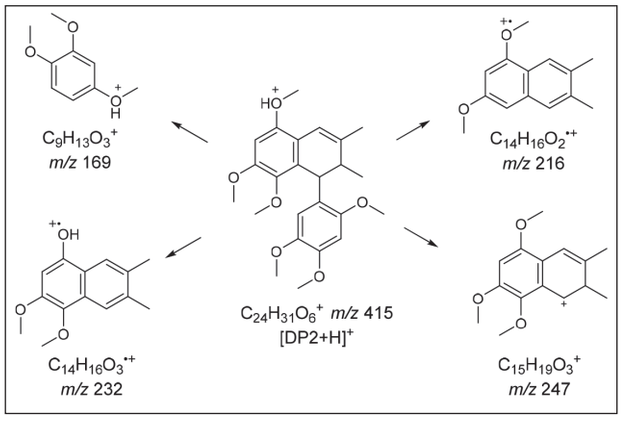

Fig. 7: Proposed mass fragmentation pathway of protonated DP2.

generate an oxidation product with the catalysis of iron (III), its mechanism may be for the catalytic oxidation reaction of metal ions. There are two possible ways: one is the transition metals can activate molecular oxygen, make the triplet state oxygen into a singlet state oxygen, then singlet oxygen acts an oxidative mediator and results in a series of one-electron reduction steps, including the generation of superoxide anion radical and the initiation of free radical oxidation reaction; The other is the strong oxidation of iron (III) could reduce the activation energy of direct reaction between drugs and oxygen, so that single electron transfer occurs between drugs and oxygen, and finally an oxidative impurity, DP1 was produced.

Four dimer degradation products were formed in the presence of a trace of iron (III), and the possible mechanism is that iron (III) could act as a catalyst to activate the electron-starved center of

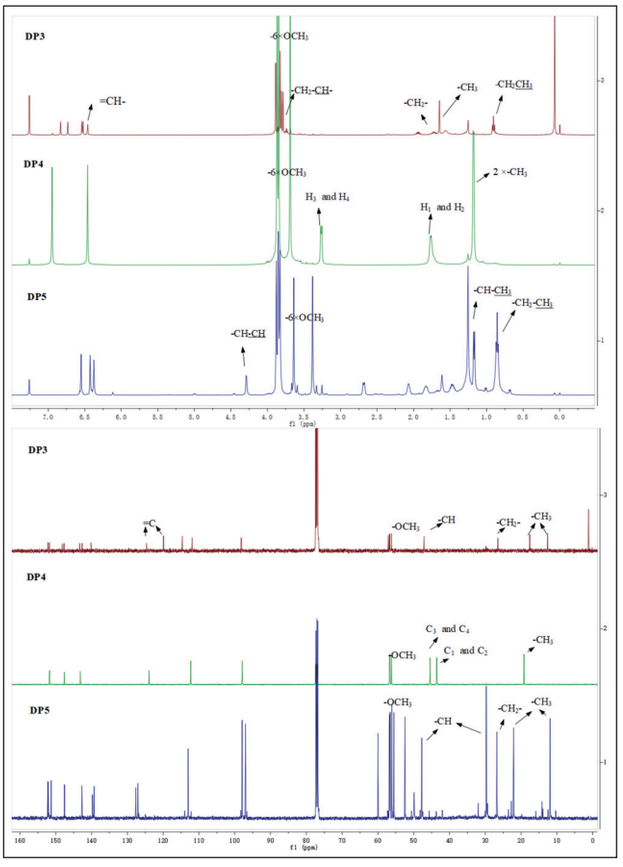

Fig. 8: Overlaid ^{1}H NMR and ^{13}C NMR spectra of DP3, DP4 and DP5.

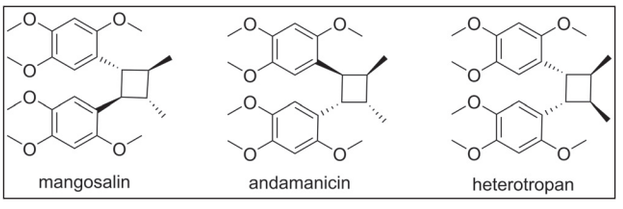

Fig. 9: Proposed structure of DP4 and its stereos.

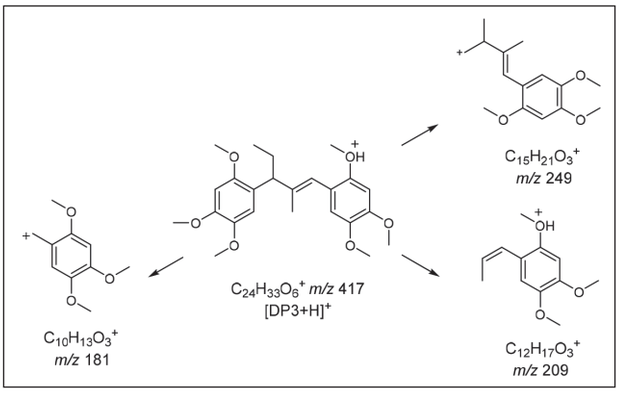

Fig. 10: Proposed mass fragmentation pathway of protonated DP3.

the drug to form a carbocation. The double bond of in the α-asarone can provide electrons to attack the carbocation, resulting in the occurrence of dimerization reaction and the generation of self-coupling products. Transition metals can also catalyze the isomerization of the C=C bond in olefin, involving the electrophilic cracking of the C-H bond of allyl (Ayusman and Ta Wang 1984). α-Asarone can produce different carbon cations intermediates, and

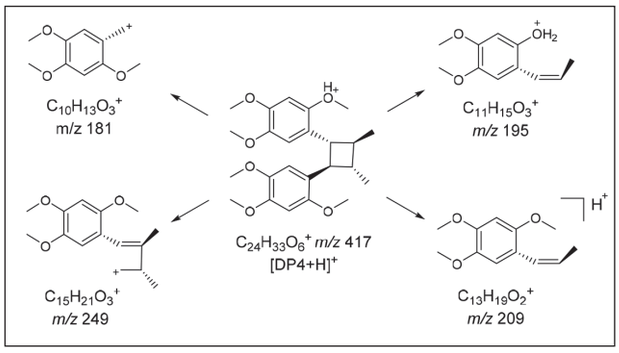

Fig. 11: Proposed mass fragmentation pathway of protonated DP4.

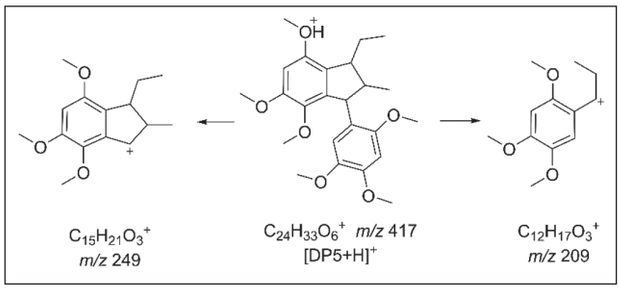

Fig. 12: Proposed mass fragmentation pathway of protonated DP5.

thus form the different dimer impurities. There exists a relationship of mutual transformation between dimer DPs, for example, DP4 gradually disappeared but DP3 began to produce, with reaction time extended, and DP2 had been on the rise, so it was speculated that the secondary degradation of DP4 may have occurred, and it may transform into DP2.

The proposed mechanistic pathway for the formation of DP1 upon treatment of α-asarone with hydrogen peroxide and AIBN as depicted in Fig. 14. The electron-deficient carbon-carbon double bond of α-asarone was attacked by the hydrogen peroxide, and underwent epoxidation reactions. Epoxides were further degraded to produce DP1. The thermal decomposition of the free radical initiator AIBN ejected nitrogen, leaving two cyanoalkyl radicals which can react rapidly with oxygen to form peroxy radicals. The peroxy radicals reacted with the allyl position of α-asarone to form the drug radicals, which subsequently reacted with oxygen to form the DP1.

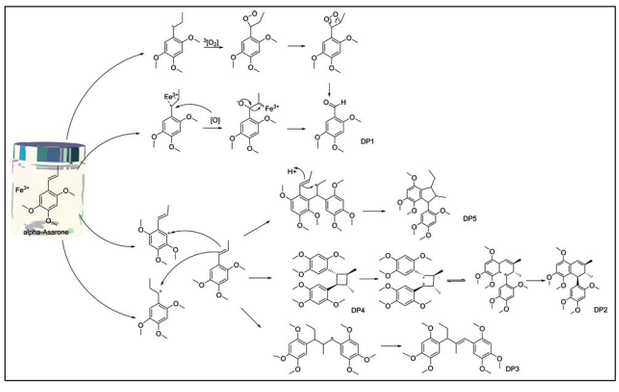

Fig. 13: Proposed mechanism of iron (III)-mediated degradation of α-asarone.

3. Experimental

3.1. Materials and reagents

α-Asarone was obtained from Guangxi Yikang Pharmaceutical Co., Ltd (China). All metal salts were purchased from Sinopharm Chemical Reagent Co., Ltd (China). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were supplied by Merck

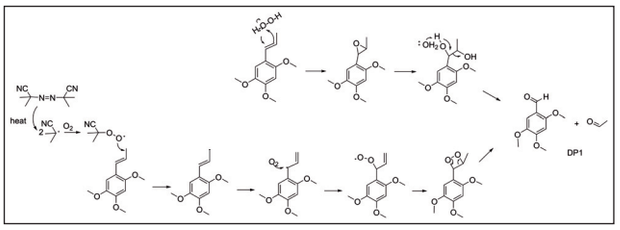

Fig. 14: Proposed mechanism of oxidative degradation of α-asarone.

(Germany). Ammonium formate and tetrahydrofuran (HPLC grade) were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd (Shanghai, China). All other commercially available reagents and chemicals used were of analytical grade. HPLC grade water was obtained from Youpu Series Ultra Pure Water system (Chengdu super pure technology co., Ltd, China).

3.2. HPLC-DAD conditions

Samples were analyzed on an HPLC system (Agilent Technologies, USA) equipped with a diode array detector (DAD). The chromatography was carried out on a Zorbax RX-C18 column (250 ×4.6 mm, 5 µm, Agilent, USA), using a mixture of methanol-water (70:30, v/v) as the mobile phase with a flow rate of 1.0 mL/min. The column temperature was 30 °C and injection volume was 20 µL. The UV detection was at 258 nm with spectra collected from 210 to 400 nm.

3.3. Solution stability and stress studies

3.3.1. Solution stability in the presence of different metal ions

Seven kinds of metal salts were respectively dissolved in water to prepare 2.1 mg/mL of metal ion stock solutions, and 0.21 mg/mL of nickel ion stock solution was prepared. α-Asarone stock solutions (4 mg/mL) were divided into penicillin vials (2 mL per vials), then 100 µL of different metal ion stock solutions were added, respectively. The mixtures were capped and shaken well, then kept at 60 °C for 10 days. Samples were appropriately diluted with the mobile phase before injecting into HPLC system.

3.3.2. Solution stress studies

Ferric chloride hexahydrate was dissolved in water to prepare 40 mg/mL of iron (III) stock solutions, and α-asarone stock solution of 4 mg/mL was prepared in methanol. 5 mL of α-asarone stock solution and 70 µL of the iron (III) stock solution were mixed to give the stress testing solution, then incubated in a water bath at 40 °C. The final concentration of the iron (III) was 0.05 mM. An appropriate amount of $NH_3{\cdot}H_2O$ was added to the solution to precipitate with metal ions and stop the degradation reaction. The mixture was centrifuged (1.3×10^4 rpm, 1 min), then supernatant was diluted to analyze with HPLC.

The effects of solvents, oxygen and pH on the iron (III)-mediated degradation behavior of α-asarone were investigated. The α-asarone stock solutions of 4 mg/mL were prepared in ethanol and acetonitrile, respectively, and the same stress testing was conducted as above. The effect of oxygen on the formation of the degradation products was illustrated by filling nitrogen into the reaction solution. The pH of iron (III) stock solution is acidic, an experiment was performed to assess whether acid condition has effect on generating the impurities, hydrochloric acid (0.1M) was used to conduct a control test. All the samples were observed for their degradation at 48h.

Another study was carried out to explore the oxidative degradation mechanisms of α-asarone exposed to 3% H_2O_2 and 0.05 mM AIBN, respectively. α-Asarone stock solutions (8 mg/mL) was prepared in methanol, and AIBN solution (0.1 mM) was prepared in acetonitrile. 30% H_2O_2 was diluted with water to prepare 6% H_2O_2 Equal volume of oxidative stressors and α-asarone stock solution were mixed to give the stress testing samples, respectively. Sample mixed with H_2O_2 was kept at room temperature for 24 h, and sample mixed with AIBN was kept at 40 °C for 2h.

The blank samples were prepared and exposed to same conditions as that of the stress samples. All of samples were diluted with mobile phase before HPLC analysis.

3.4. Isolation of degradation products

For isolation the major degradation products, an HPLC system (Shimadzu, Japan) equipped with a SPD-20A detector was used. In order to achieve good separation and obtain high purity DPs, different DPs were isolated by the following liquid phase conditions.

For DP1, preparative HPLC conditions was performed on Shim-pack PRC ODS column (250×20 mm, 5 µm; Shimadzu, Japan). Isocratic elution was applied using methanol-water (60:40, v/v) as the mobile phase. The ultraviolet detection wavelength was set at 258 nm and 278 nm, respectively. The flow rate was set at 10 mL/min, while the injection volume was 2.0 mL.

For DP2, preparative condition was carried out on a semi-preparative column: Xtimate C18 (250×10 mm, 5 µm, Welch, China). The mobile phase system consisted of 50% acetonitrile tetrahydrofuran solution-water (50:50, v/v) and gradient elution programmed as follows: 50% water linear to 45% water at 6min and then linear to 40% water at 17 min. The ultraviolet detection wavelength was set at 258 nm and 278 nm, respectively. The flow rate was set at 3.0 mL/min, while the injection volume was 1.0 mL.

For DP3 and DP5, preparative HPLC conditions was performed on Shim-pack PRC ODS column (250×20 mm, 5 μm, Shimadzu, Japan). Isocratic elution was applied using methanol-water (80:20, v/v) as the mobile phase. The ultraviolet detection wavelength was set at 258 nm and 290 nm, respectively. The flow rate was set at 8.0 mL/min, while the injection volume was 3.0 mL.

For DP4, preparative condition was carried out on a semi-preparative column: Xtimate C18 (250 ×10 mm, 5 μm, Welch, China). The mobile phase system consisted of 50% acetonitrile tetrahydrofuran solution-water (50:50, v/v) and gradient elution programmed as follows: 50% water isocratic for 1 min and then a linear gradient to 45% water over 16 min. The ultraviolet detection wavelength was set at 258 nm and 293nm, respectively. The flow rate was set at 3.0 mL/min, while the injection volume was 1.0 mL.

Fractions containing >95% purity of the required DPs were respectively collected and pooled together; concentrated to remove solvent.

3.5. Identification of major degradation product

3.5.1. UPLC-MS/MS conditions

An SCIEX Exion LC coupled to a mass spectrometer Q Trap AB SCIEX 4500 was used for identification the major degradation products. The Zorbax Elicapse C18 column (100×2.1 mm, 1.8μ m, Agilent, USA) was used, and the column temperature maintained at 30 °C. The mobile phase consisted of a mixture of 10 mM ammonium formate (A) and acetonitrile (B). Gradient elution was carried out at a constant flow of 0.2 mL/min, with a linear gradient from A-B (30:70, v/v) to A-B (20:80, v/v) over 8 min. The injection volume was 1.0 μL.

Electrospray ionization (ESI) in the positive ionization mode was employed for the analysis. The conditions were as follows: ion spray (IS), 4500 V; temperature (TEM), 450 °C; curtain gas (CUR), 35 psi and ion source gas (GS1 and GS2) at 40 psi. Nitrogen was used as the nebulizer gas, curtain gas and collision gas. The declustering potential (DP) and collision energy (CE) were 80 V and 20 eV, respectively. The mass range was set at *m/z* 100~500. The data acquisition and processing in the UPLC-Q Trap system was carried out using the Analyst software, version 1.7.1 (AB SCIEX, Foster City, CA).

3.5.2. Nuclear magnetic resonance spectroscopy (NMR)

The ^{1}H and ^{13}C NMR data for the DPs of α-asarone dissolved in chloroform-d were acquired on a Bruker BioSpin GmbH spectrometer on 500 MHz and 126 MHz, respectively. The chemical shift values were reported in ppm relative to chloroform-d (δ_H = 7.26 ppm, δ_C = 77.16 ppm) as internal standard.

Conflicts of interest: None declared.

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