

UV Degradation of Sesquiterpene Lactones in Chicory Extract: Kinetics and Identification of Reaction Products by HPLC-MS

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Abstract: Inulin, a valuable food ingredient, can be extracted from chicory roots (*Cichorium intybus* L.). However, the direct use of this material is limited, because of the presence of extremely bitter, co-extracted sesquiterpene lactones. Lactucin, a major component, has been degraded by UV irradiation, a process which showed neither temperature (293–313 K) nor initial concentration dependence. An overall half life time of ca. 45 min was determined. The degradation product was identified by HPLC-MS and ¹H NMR and was the result of the addition of a water molecule on the lactucin C(1)–C(10) double bond, with the OH group located at the C(10) position.

Keywords: Degradation product · HPLC-MS · Kinetics · Sesquiterpene lactones · UV degradation

Introduction

Today's consumers prefer foods which taste fresh and natural, contain little or no additives, are nutritious and safe, and bring added health benefits. Inulin and oligofructose are widely used in functional foods throughout the world for their health-promoting and technological properties. They are already key ingredients in a large number of commercial products, the range of which keeps growing due to bustling research and development activity in the field [1]. Some non-food applications have also been proposed for oligosaccharides which including drug delivery, cosmetics and mouth washes [2].

Inulin, which is widely known for its bifidogenic, cholesterol-lowering, texturing or fat replacing properties, is found in chicory roots [1]. A major issue in the re-

covery of food-grade inulin on an industrial scale are the extremely bitter, co-extracted sesquiterpene lactones 1–6 shown in Fig. 1. Yet these compounds are photolabile [3], and the complete disappearance of the bitterness after a UV treatment has been demonstrated [4]. The structure of the degradation product, which should be elucidated for obvious food safety reasons, was however not known in detail. The degradation of lactucin was characterised in the present study.

Extraction and Purification of Lactucin

Freeze-dried, powdered chicory roots (*Cichorium intybus* L.) were extracted with methanol. The methanol was removed and the extract dissolved in water, then treated with cellulase to release bound sesquiterpene lactones. The solution was successively extracted with dichloromethane and ethyl acetate to obtain a mixture of sesquiterpene lactones. This mixture was

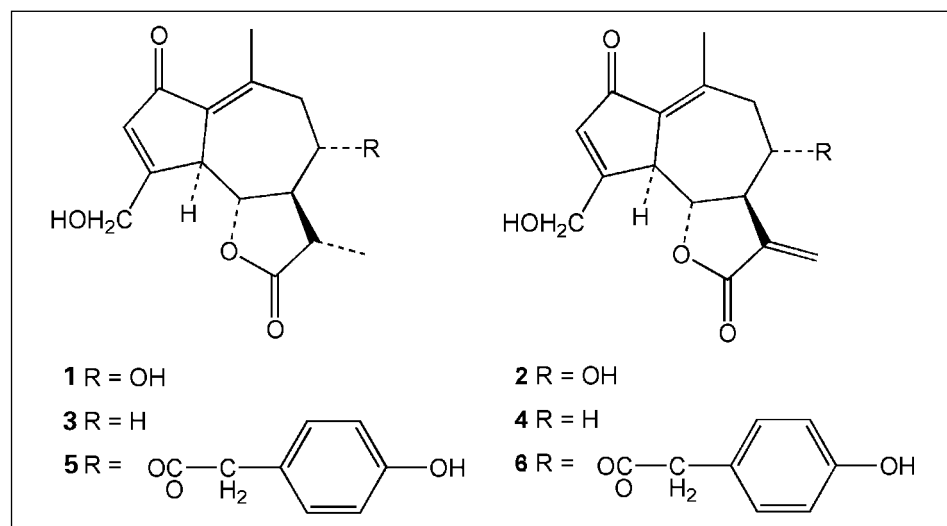


Fig. 1. Sesquiterpene lactones found in chicory root; 1: 11(S),13-dihydrolactucin; 2: lactucin; 3: 11(S),13-dihydro-8-deoxylactucin; 4: 8-deoxylactucin; 5: 11(S),13-dihydrolactucopicrin; 6: lactucopicrin

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analysed by HPLC (UV detection at 262 nm) coupled with mass spectrometry, *i.e.* MS analysis after atmospheric pressure chemical ionisation in the positive mode (APCI, pos). The mixture was further fractionated and for the present study, a solution of pure lactucin was obtained by semi-preparative HPLC.

Kinetics of UV Degradation

UV degradation experiments were carried out to determine the rate constant of the degradation and its dependence on temperature and lactucin concentration. Lactucin solutions were placed in quartz cuvettes and thermostated in a water bath. The absorbance spectra of lactucin (**2**) were then measured as a function of UV (366 nm) irradiation time. A decrease of the maximum at 260 nm, an increase at 220 nm and an isosbestic point at 245 nm were observed (Fig 2). The presence of an isosbestic point indicates that only one (major) degradation product is obtained. The photodegradation of lactucin (**2**) follows a pseudo-first-order kinetics.

No temperature dependence of the rate constant between 293 and 313 K was observed. Also no rate dependence with respect to lactucin concentration ($0.4\text{--}1.7 \times 10^{-5}\text{g/ml}$) was found. An overall rate constant of $2.6 \pm 0.4 \times 10^{-4}\text{s}^{-1}$ was determined, which corresponds to a half life of *ca.* 45 min.

Identification of Degradation Product

HPLC analysis of a lactucin solution after the UV treatment showed that the sesquiterpene lactone degraded to one major compound, **7**. The MS (APCI, pos) spectrum of this compound shows a molecular ion at m/z 295 ($M+H^+$) as well as the fragments already observed for lactucin (Fig 3). This corresponds to the addition of one water molecule to a double bond of lactucin, in accordance with the observed shift from 260 to 220 nm of the absorption wavelength maximum (Fig. 2). Analysing the ^1H NMR spectrum of the degradation product and comparing it to the spectrum of lactucin helped identify the double bond where the water addition takes place. The main difference between these two spectra is observed for the C(15) methyl group signal which is a singlet at 2.4 ppm for lactucin (**2**). In the spectrum of **7**, this signal is absent and two new singlets at 1.42 ppm and 1.23 ppm in a ratio of about 3:1 are observed. This change can be explained by the

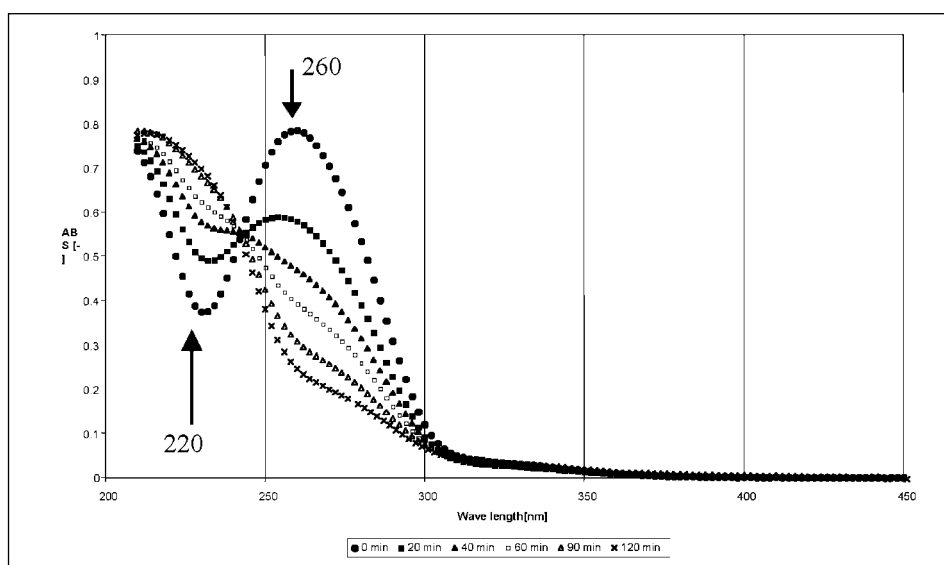


Fig. 2. UV-VIS spectra of lactucin (**2**) ($1.7 \times 10^{-5}\text{g/ml}$) as a function of UV irradiation time at 303K.

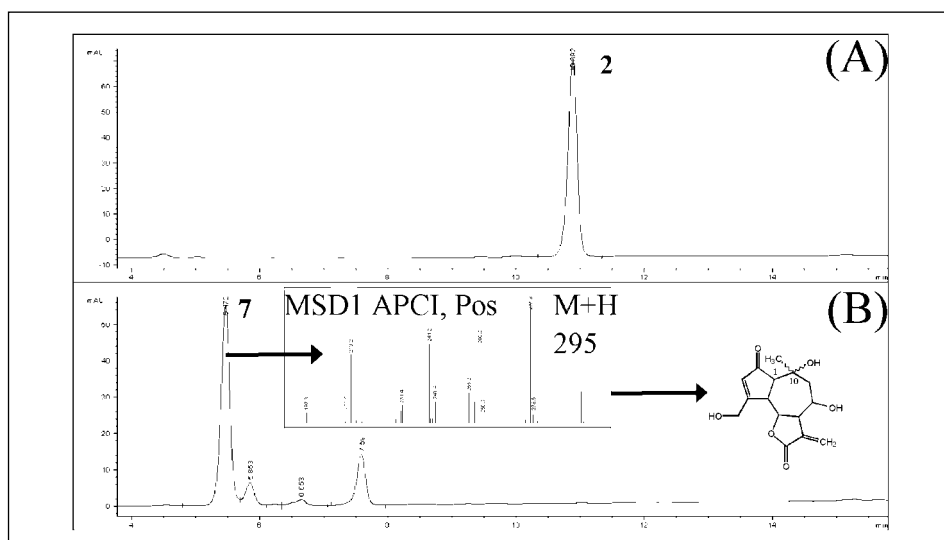


Fig. 3. Chromatograms of lactucin solution ($1.7 \times 10^{-5}\text{g/ml}$): (A) before and (B) after UV degradation. The insert shows the MS spectrum of the major degradation product and the proposed structure.

addition of one water molecule to the C(1)–C(10) double bond, with the OH group located on C(10). The presence of two singlets can be explained by the two possible diastereomers, which result from the addition of one water molecule.

Conclusion

The bitter sesquiterpene lactones co-extracted with inulin from chicory roots can be completely degraded by UV treatment. The kinetics of the degradation process was measured in the case of lactucin (**2**). The unique degradation product has been identified using an HPLC-MS technique, com-

pleted by analysis of ^1H -NMR spectra. The degradation of lactucopicrin (**6**) and 8-deoxylactucin (**4**) very likely follows similar pathways. The food safety of these water adducts should be ascertained in view of future food applications.

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