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Note

Determination of lactucin in roots of chicory (*Cichorium intybus* L.) by high-performance liquid chromatography

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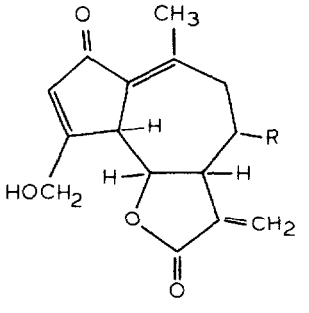
The milk sap of lettuce (*Lactuca* sp.) and the roots and heads of chicory (*Cichorium intybus* L.) contain lactucin and lactucopicrin (Fig. 1), both of which are sesquiterpene lactones responsible for the bitterness of the plants[1–3].



I: R = –OH
II: R = *p*-$HOC_6H_4CH_2COO$–
III: R = –H

Fig. 1. Structure of lactucin (I), lactucopicrin (II) and 8-deoxylactucin (III)[5].

As part of a wider research project, the Sprenger Institute is interested in the total bitterness of chicory heads and consequently in the isolation of lactucin from chicory roots. There are several methods of detecting lactucin and lactucopicrin in extracts of chicory and lettuce: paper chromatography[4], fluorimetry after reaction with potassium cyanide[2] and measurement of the absorbance of potassium cyanide derivatives[3]. Pyrek[5] used high-performance liquid chromatography (HPLC) to separate the two lactones, but did not determine them and gave no information about the conditions used for the separation. Therefore, we have developed an HPLC method for the determination of lactucin from chicory roots.

EXPERIMENTAL

A Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph, equipped with a Model 6000A pump and U6K injector, was used. The detector was a Kratos Spectroflow 773 variable-wavelength UV detector with a 12-μl flow-through cuvette (Kipp & Zonen, Delft, The Netherlands) used at a wavelength of 258 nm (sensitivity in

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terms of absorbance, 0.050 and 0.100) and the integrator was an Infotronics CRS-204 (Techmation, Schiphol-Oost, The Netherlands). The column was a 10 cm × 8 mm I.D. Radial-PAK C_{18} cartridge (Waters Assoc., Cat. No. 84720) with a C_{18} Guard-PAK column (Cat. No. 85824), which were pressurized in a radial compression Z-Module before use (Cat. No. 86500). The solvent was water–methanol (1:1) and the flow-rate was 2.0 ml/min (overpressure 5.2 MPa, 750 p.s.i.). Deionized, doubly distilled water was used and pure methanol was obtained from Merck (Darmstadt, G.F.R., Cat. No. 6007). The solvents were degassed by filtration through a filter of pore size 0.45 μm for organic solvents.

Extraction of chicory roots

Chicory roots kept at 0°C for 1 week were cut into small pieces (about 4 mm^3) under liquid nitrogen and stored at −60°C for 6 weeks. A portion (200 g) of these pieces was suspended in 600 ml of water and sodium chloride (20 g) at room temperature. After 30 min, the suspension was extracted continuously for 30 h with diethyl ether (Merck, Cat. No. 971) in a liquid–liquid extractor[6]. After extraction, the diethyl ether was removed by evaporation and the residue dissolved in 50 ml of 95% ethanol (Merck) to give the chicory root extract.

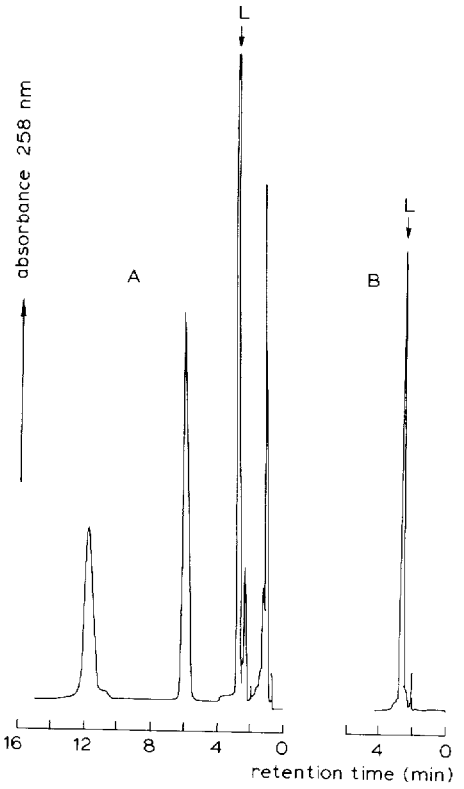

Fig. 2. Chromatograms of chicory root extract (A) and lactucin (L) reference solution (B).

Calibration graph

Several portions of a stock solution of pure lactucin (a gift from Professor D. Barton, Gif-sur-Yvette, France) (116 mg/l of 95% ethanol) were diluted with 95% ethanol in order to obtain reference solutions containing 12, 23, 46, 58, 70, 81, 116, 232, 248, 580 and 1044 ng of lactucin in 5 μl of 95% ethanol. A 5-μl volume of each solution was injected and the corresponding area was integrated against mass of lactucin injected.

RESULTS AND DISCUSSION

Solvents with different proportions of water and methanol were tried for the HPLC of lactucin, and a 1:1 mixture was found to give the best separation as determined with the extract. With this solvent, two rectilinear responses were obtained by injection of the reference solutions, the first for the range 0–100 ng (correlation coefficient $r = 0.921$) and the second for the range 100–1200 ng ($r = 0.995$). The coefficient of variation of this method was 3.4% (eleven replicates in one run) and the recovery was 95%, as established by the spiking technique. Thus the precision and accuracy were satisfactory. The minimum amount of lactucin that could be detected (absorbance 0.001) was 0.6 ng (2.2 pmol).

The chromatograms of lactucin in the reference solution and in the chicory root extract, monitored at 258 nm where lactucin has the greatest absorbance in 95% ethanol[7,8] and in water–methanol (1:1), are shown in Fig. 2. The retention time of lactucin in the chromatograms for the chicory root extract was corroborated by spiking. Only one peak increased in area, at a retention time of 2.6 min (lactucin). In addition, the fraction corresponding to this peak was collected and identified by mass spectrometry (MS) and nuclear magnetic resonance (NMR). These spectra were identical with those obtained by Pyrek[5].

The fraction corresponding to the peak in the chromatogram of the chicory root extract with a retention time of 6.0 min was also collected and identified by MS and NMR. This peak was due to two compounds, distinguished by MS. One of them was 8-deoxylactucin (Fig. 1), in agreement with the spectra obtained by Pyrek[5]. The other compound had the molecular formula $C_{15}H_{20}O_3$ and mass spectrum *m/e* (70 eV) 248 (M^+), 233, 230, 204, 191, 175, 163 and 141, but could not be identified.

The peak with a retention time of 12 min in the chromatogram of the chicory root extract is probably lactucopicrin (Fig. 1), which is a less polar compound and therefore would elute after 8-deoxylactucin.

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