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Extractives of cork (*Quercus suber* L.): chemical composition of dichloromethane and supercritical CO_2 extracts

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Abstract

The polymeric matrix of the cork is mainly composed of suberin, lignin and polysaccharides and contains also extractives. Typical components of the extracted mixtures are triterpenes belonging to friedelane, lupane and steroid families and long chain alkanes and alkanols. Two extractive methods have been used, the conventional extraction with a solvent (SE) using dichloromethane (DCM) and the innovative method which uses as solvent supercritical CO_2 (SFE) at moderate pressures (200–250 bar). Identification and quantitation of the individual triterpenoids and steroids present in the extracts have been carried out by means of the computer aided ^{13}C NMR analysis without previous separation. Friedelin was found as the main component of the extracts. The main difference between the two extracts concerned the contents of sitost-4-en-3-one which represented 15% of the CO_2 extract against 3% in the DCM extract. Similarly, β-sitosterol was found in CO_2 extract (3%) but not in DCM extract.

Keywords: Triterpenes; Cork; Supercritical extraction; ^{13}C NMR

1. Introduction

Cork is produced by the cork cambium in the outer bark of *Quercus suber* L. Because of a remarkable set of properties, this raw material is of considerable importance in several industries. If Portugal and Spain are the most important producers of cork, *Q. suber* is also widespread in Corsica and Sardinia.

Cork consists essentially of suberin (Kolattukudy, 1978), lignin and cellulose and contains also a small amount of extractives, fatty acids, terpenes, long chain aliphatic compounds and saccharides (Pereira, 1988). Several triterpenes such as friedelin, betulin, betulinic acid and β-amyrin and a few steroids, β-sitosterol, sitost-4-en-3-one and campesterol, were identified in cork extracts (Ribas-Marques and Fernandez-Salgado, 1974; Talpatra et al., 1978; Caldas et al., 1985; Patra and Chaudhuri, 1988; Moiteiro et al., 1991). All of the aforementioned compounds were first isolated by chromatographic techniques and then characterized by spectroscopic methods.

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Various solvents were used in order to obtain extractives from cork (dichloromethane (DCM), ethanol, water), among them dichloromethane gave the best yields (Pereira and Baptista, 1993). Conversely, supercritical CO_2 extraction has never been applied to cork whereas this technique procures numerous advantages such as (i) low temperature processing meaning thermosensitive products do not deteriorate, (ii) absence of any harmful solvent residue in the final product, (iii) possibility to modulate the selectivity of the supercritical solvent to obtain the fractionation of complex mixtures (Cherchi et al., 2001; De Gioannis et al., 2001) and (iv) water excepted, carbon dioxide is one of the cheapest industrial solvent in use today.

The aim of the present work is to check the ability of supercritical CO_2 to extract triterpenes from cork and, if so, to compare the influence of the extraction process (supercritical CO_2 versus dichloromethane) on the chemical composition of the triterpene fraction of cork extracts.

2. Materials and methods

Plant material: Cork samples were taken from *Quercus suber* L. trees growing in the Sardinian region of Sorgono. The samples were classified as medium quality classes from an operator working at a cork stopper industry located in Sorgono. After removal of the outer surface each cork chunk was ground to 40–60 mesh.

Dichloromethane extraction (*SE*): Cork powder was extracted on a Soxhlet apparatus with dichloromethane (25 ml/g) for 8 h. The extract yield, calculated from fresh material was ca. 6%.

Supercritical extraction (*SFE*): Supercritical CO_2 extraction was performed in a laboratory apparatus equipped with a 400 ml extraction vessel previously described (Cherchi et al., 2001). The extractor was charged with about 50 g of cork previously grounded to a mean particle size of about 500 μm. The mean diameter of the particles was determined by mechanical sieving. A CO_2 flow rate of 0.8 kg/h was used in all experiments. CO_2 (purity 99%) was supplied by SIO (Società Italiana Ossigeno, Cagliari, Italy). To improve the yield of extractives, several runs were carried out at different pressures (200–250 bar). The extract yield, expressed as the percentage of extract recovered with respect to the weight of the material charged in the extractor, was ca.7% at the optimum extraction conditions: 50 °C and 220 bar. At 6 h the extraction rate is bigger and after this time no significant increments on the recovery of the total extract were observed.

^{13}C *NMR spectra*: they were recorded on a Varian VXR-300 Fourier Transform spectrometer operating at 75.43 MHz for ^{13}C, using 10 mm diameter tubes, in deuterochloroform, with all shifts (δ) referred to internal tetramethylsilane (TMS). Parameters—pulse width (PW): 10 μs (flip angle 45°); acquisition time: 1.3 s and relaxation delay D_1: 2 s (total recycling time 3.3 s) for 64 K data table with a spectral width (SW) of 19.194 Hz (254 ppm); composite phase decoupling (CPD) of the proton band; digital resolution of 0.58 Hz/pt. The spectra were recorded with 150 and 144 mg of the cork extracts, respectively, for CO_2 and dichloromethane extracts, diluted in 3 ml of $CDCl_3$, 10 000 acquisitions were accumulated for each sample.

Identification and quantitation of triterpenes: ^{13}C NMR was carried out on the whole sample without previous separation of the components. Identification of the components was led according to an experimental procedure and a computerized method developed in our laboratory and optimized for triterpenes (Castola et al., 1999). The components were identified by comparison of the values of the carbon chemical shifts in the mixture spectrum with those of reference spectra compiled in a computerized data bank. Each compound is identified by taking into account three parameters, directly available from the computer program: (i) the number of observed signals with respect to that expected, (ii) the difference between the chemical shift of each signal in the mixture and in the reference ($\Delta\delta$), (iii) the number of overlapped signals of carbons belonging to two components which possess fortuitously the same chemical shift.

The mass percentage (mass%) of each triterpene was calculated taking into account the mean intensity of signals of five selected carbons following a procedure recently developed by our group which provides reasonable accuracy (Castola et al., 1999). The calculated amount m_C (mg) of each triterpene, was determined using the formula (1) where m_D = amount (mg) of diglyme, M_D = molecular weight of diglyme and M_T = molecular weight of the considered triterpene (the factor 2 is due to the symmetry of diglyme).

$$m_C = \frac{2I_T M_T m_D}{I_D M_D} \quad (1)$$

The subsequent mass percentage of each triterpene was calculated using formula (2) where m_E is the amount of crude extract diluted in $CDCl_3$.

$$\%m_C = \frac{m_C 100}{m_E} \quad (2)$$

3. Results

The same sample of powdered cork was divided into two parts which were extracted using dichloromethane and supercritical CO_2. The yield of extract obtained from both techniques was quite similar 6 and 7% (w/w) versus fresh material, respectively.

The results obtained for the two extracts are reported in Table 1. The spectra of both samples exhibited several signals belonging to long chain linear compounds, particularly a huge peak at 29.71 ppm corresponding to most methylenes of these components. Other signals belonged to triterpenes and steroids (Castola et al., 1999). Comparison of the chemical shifts of the mixture spectra with those of our reference spectra compiled in a laboratory made library led to the identification of friedelin, betulin, betulinic acid, β-sitosterol and sitost-4-en-3-one (Figs. 1 and 2). For all identified components, at least 83% of the expected signals were observed. The number of overlapped signals ranged from 2 to 13, this observation means that for any component, at least 13 peaks belonged solely to one molecule (Table 1).

Friedelin was the major component of the dichloromethane extract (21.0%) while betulinic acid, betulin and sitost-4-en-3-one represented, respectively, 7.3, 2.7 and 2.9%. β-Sitosterol and 3-α-hydroxyfriedelan-2-one, which are present in some extracts of cork (Castola et al., 1999), were not identified in this sample which means that they are not present or present under the detection threshold of the NMR spectrometer, i.e. 0.5–1%. Similarly, friedelin (20.4%) was the main component of the supercritical CO_2 extract. The contents of betulinic acid and betulin (2.1 and 1.4%, respectively) were lower than in the dichloromethane extract. Conversely, the content of β-sitosterol was around 3% and overall, the content of sitost-4-en-3-one reached 15% of the CO_2 extract. Sitost-4-en-3-one represented more than 35% of the identified triterpenes. A so high percentage was not previously observed in dichloromethane extract of cork from Corsica and Sardinia (Castola et al., 2001).

From these results we concluded that supercritical CO_2 is a good solvent for the extraction of the triterpenes and steroids of cork with a yield similar to that obtained using dichloromethane (7% versus 6%) which is one of the best solvent. Moreover, supercritical CO_2 extracted more steroids than dichloromethane and particularly sitost-4-en-3-one (about 1% of the weight of the cork sample).

Table 1
Identification and quantitative determination of triterpenes in the supercritical CO_2 and dichloromethane extracts of cork[a]

Components	Supercritical CO_2					Dichloromethane				
	NMR	OS	I_T	m_C	$\%m_C$	NMR	OS	I_T	m_C	$\%m_C$
Friedelin	30/30	3	46.70	30.6	20.4	30/30	3	38.00	30.2	21.0
Betulinic acid	28/29	12	4.93	3.2	2.1	29/29	13	13.16	10.5	7.3
Betulin	24/29	11	3.13	2.1	1.4	29/29	12	4.90	3.9	2.7
β-Sitosterol	28/28	9	6.59	4.3	2.9	–	–	–	–	–
Sitost-4-en-3-one	27/27	10	34.44	22.5	15.0	24/27	2	5.17	4.1	2.9

[a] Amount of diglyme m_D = 11.4 and 13.1 mg, molecular weight M_D = 134 g/mol, mean peak intensity of its methylenes I_D = 109.71 and 104.75 for CO_2 and dichloromethane extracts, respectively. Molecular weight of friedelin, betulinic acid, betulin, β-sitosterol and sitost-4-en-3-one M_T = 426, 456, 442, 413 and 412 g/mol, respectively. Weighted amount of extracts m_E = 150.0 and 144.0 mg for CO_2 and dichloromethane extracts, respectively. NMR: number of observed carbons with respect to the number of expected signals; OS: number of overlapped signals of carbons which possess the same chemical shift; I_T: mean peak intensity of the selected carbons of each triterpene (see text); m_C: mass (mg) of each triterpene measured by ^{13}C NMR; $\%m_C$: mass percentage measured by ^{13}C NMR.

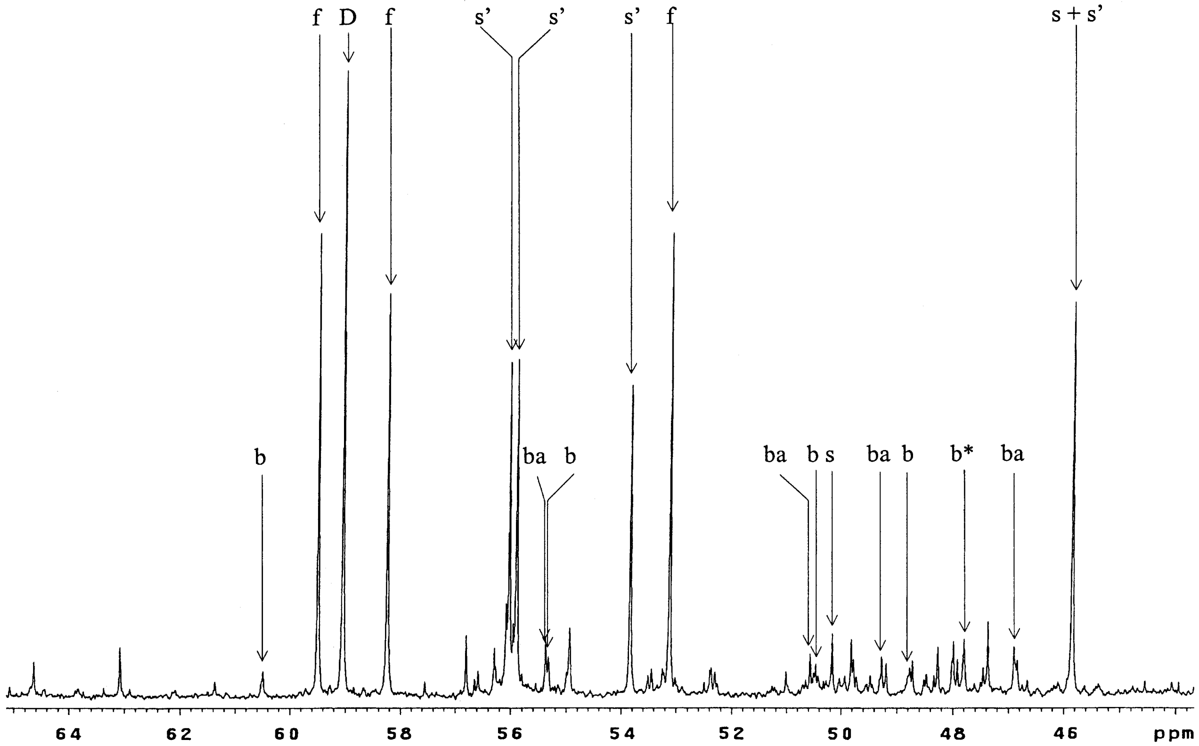

Fig. 1. Part of the spectrum of the supercritical CO_2 extract of cork: D, methyls of diglyme; f, friedelin; s′, sitostost-4-en-3-one; s, β-sitosterol; ba, betulinic acid; b, betulin; b*, overlapped peaks of betulin.

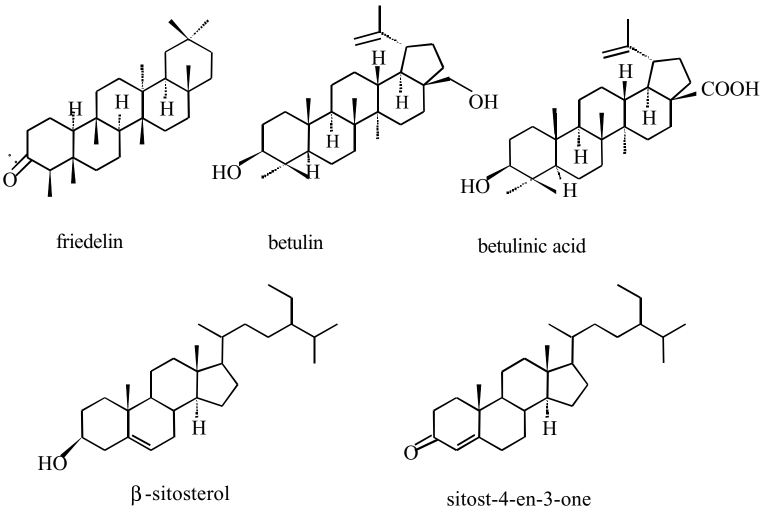

Fig. 2. Structure of the triterpenes identified in the extracts of cork.

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