Natural Complex Mixtures of Hydroxy Fatty Acids by ^{1}H Nuclear Magnetic Resonance Spectroscopy

ANDREIA F. SOUSA, ALESSANDRO GANDINI, ARMANDO J. D. SILVESTRE,* and CARLOS PASCOAL NETO

CICECO and Department of Chemistry, University of Aveiro, 3810–193 Aveiro, Portugal

The use of trichloroacetyl isocyanate (TAI) to mark both hydroxyl and carboxyl groups borne by the hydrolysis or methanolysis of suberin fragments (a complex mixture of hydroxy fatty acids), allowed the quantitative assessment of the ratio between carboxyl and hydroxy groups, as well as the ratio between primary and secondary hydroxy groups, to be carried out reliably by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. All the samples thus analyzed displayed an excess of CO_2H (or CO_2CH_3) functions with respect to the OH counterparts, albeit to a variable extent, depending on the procedure adopted to isolate the suberin fragments. The precise knowledge of the molar ratio of these two reactive moieties is fundamental for the correct utilization of suberin monomers in polymerization reactions leading to aliphatic polyesters.

Index Headings: **Hydroxy fatty acids; Functional group analysis; ^{1}H NMR; Suberin; Natural monomers; Oleochemicals.**

INTRODUCTION

Suberin is a widespread natural cross-linked polyester regularly found in the cell walls of plants, where it plays a major role against physical, chemical, and biological aggressions.[1–5] Suberin is indeed referred to as being ubiquitous in plants,[1,2,5] present not only in normal but also in wounded tissues.[2] One of the major sources of this material is the outer bark of cork oak, *Quercus suber* L., representing typically 40 to 60% of cork dry weight.[1,2,5–8] The unique chemical composition of suberin is one of the major factors generally associated with the remarkable properties of cork,[2] together with its physical morphology. Another hardwood species in which suberin is present in abundance is the outer bark of *Betula pendula*, varying between 30 to 60% of the extractive free bark.[2]

This naturally occurring cross-linked polyester is composed of both aliphatic and aromatic domains, as clearly established by Bernards for potato suberin.[1] The aromatic domain is mainly composed of hydroxycinnamic type units, whereas the major components of the aliphatic counterpart are ω-hydroxyalkanoic acids, α,ω-alkanedioic acids, and minor amounts of long chain alkanols and fatty acids.[1–6]

Suberin aliphatic monomeric units can be isolated by alkaline hydrolysis or alcoholysis (most frequently by methanolysis).[2,5] When the former technique is used the depolymerization mixtures are obtained in the form of free carboxylic acids, while in the case of alcoholysis, the corresponding alkyl esters are instead obtained. Gas chromatography–mass spectroscopy (GC-MS) analyses of these mixtures showed that the ω-hydroxyalkanoic and α,ω-alkanedioic acids are characterized by the presence of even-numbered aliphatic chains (C_{16} to C_{26}), with a predominance of the C_{18} and C_{22} homologues. C_{18} homologues containing a 9,10-epoxy group, or the corresponding 9,10-dihydroxy derivative, are often significant components of such fractions.[1–6] The ^{1}H nuclear magnetic resonance (NMR) analyses of depolymerized cork suberin are consistent with the GC-MS evidence just described, showing essentially a predominance of the aliphatic methylenic protons and lower percentages of methoxy protons and of protons attached to carbon atoms bearing hydroxy groups.[6]

This unique chemical composition of the aliphatic suberin fragments makes them promising precursors for the synthesis of polymeric materials from renewable origins, e.g., polyesters.[2,5,9–11] Hence, detailed knowledge of the total amount of reactive functional groups is obviously a critical issue for the efficient synthesis of these macromolecular materials from suberin mixtures, but also for other complex natural mixtures of similar composition. Although this aspect can be estimated by the routinely used GC and/or GC-MS analyses based on the quantification of the individual monomeric components,[6–8] an analytical tool that would provide the ratio of specific functionalities, regardless of the length of the aliphatic chains, in an accurate and rapid manner, would be of major interest, once more not only for suberin components but also for other naturally occurring mixtures of hydroxy fatty acids, particularly when considering the growing interest in the exploitation of oleochemicals[12] as one of the alternatives to petrochemical sources.

A significant number of publications deal with analytical approaches aiming at quantifying the hydroxy and/or carboxylic acid groups of polyesters or other smaller molecules,[13–21] including, for example, the classical titration techniques, which, however, are associated with the use of considerable amounts of solvents and samples and also with difficulties in the end-point detection.[14,20,21] End-group analyses have also been routinely investigated by NMR spectroscopy of the derivatized functional groups,[13,15,17,18] with the advantage of requiring only small amounts of both sample and solvent. One interesting approach in this context is the esterification of the hydroxy and carboxylic acid groups with fluorinated compounds followed by ^{19}F NMR analyses, which usually requires the use of an internal standard.[17,19] Other authors[13,15,18] have reported the use of a trichloroacetyl isocyanate (TAI) at room temperature to generate rapidly and quantitatively derivatives that can be directly analyzed by ^{1}H NMR.

Although, the ^{1}H NMR spectrum of the non-derivatized depolymerized suberin is well known,[6] to the best of our knowledge a derivatization procedure prior to NMR analyses, aimed at gaining relevant quantitative information about its functional groups, has not been reported. In the present paper

Received 25 March 2009; accepted 28 May 2009.
* Author to whom correspondence should be sent. E-mail: armsil@ua.pt.

0003-7028/09/6308-0873$2.00/0

the ^{1}H NMR analyses of the relative contents of both carboxylic and hydroxy (both primary and secondary) groups in suberin depolymerization mixtures after derivatization with TAI is presented. This technique has been demonstrated to be a very useful tool for the characterization of such mixtures in view of their valorization, e.g., in polymer synthesis.

MATERIALS AND METHODS

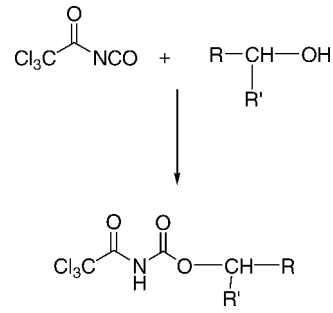
Materials. Trichloroacetyl isocyanate (TAI, ≥97%) and sodium methoxide ($NaOCH_3$, 97%) were purchased from Sigma-Aldrich Chemicals. Potassium hydroxide (KOH, 85%) was purchased from Merck. Deuterochloroform ($CDCl_3$) was purchased from Acros. *Quercus suber* L. cork planks, "Amadia" grade, were sampled in the south of Portugal (Herdade da Moinhola, Amorim Florestal mill, Portugal, March 2005), ground, and the fraction of 40–60 mesh used for the depolymerization experiments.

Suberin Isolation. The suberin depolymerization products were isolated from cork powder samples following two different depolymerization procedures, viz. alkaline methanolysis or alkaline hydrolysis. Alkaline methanolysis[8] was conducted by refluxing the cork powder (≈16 g) in a dry methanol $NaOCH_3$ solution (0.6 M, 2.0 L) for 4 h, followed by filtration of solid residue. The residue was then refluxed for 1 h with dry methanol (0.5 L) and filtered again. The two liquid fractions were mixed, acidified to pH 3–3.5 with aqueous hydrochloric acid (2.0 M), and then extracted three times with ≈300 mL of dichloromethane (DCM). The solvent was removed in a rotary evaporator and the residue vacuum-dried and weighed. Hereafter, this fragment mixture will be referred to as DCM-MDS (dichloromethane methanolysis-depolymerized suberin). A sample of the DCM-MDS extract (≈8 g) was further fractionated by refluxing it with *n*-hexane (250 mL) for four hours, cooling to room temperature, and isolating the *n*-hexane soluble fraction in order to assess the effect of a second purification step over the CO_2CH_3/OH and primary/secondary OH ratios. The *n*-hexane solvent was removed in a rotary evaporator and thereafter the residue was vacuum-dried and weighed. This sample will be referred to hereafter as HEX-MDS.

The alkaline hydrolysis[8] of cork powder (≈20 g) was carried out with a KOH solution (0.5 M, 2 L) in ethanol/water (9:1 v/v) at 70 °C for 1.5 h. The ensuing mixture of hydrolyzed suberin fragments was cooled to room temperature, acidified with aqueous hydrochloric acid (2 M) to pH 3–3.5, and extracted three times with diethyl ether and once with neutral water in order to remove any water-soluble compounds present. The resulting diethyl ether extract was then freed from this solvent in a rotary evaporator, vacuum-dried, and weighed. Hereafter, this fragment mixture will be referred to as HDS (hydrolysis-depolymerized suberin).

Derivatization of Depolymerized Suberin Samples with Trichloroacetyl Isocyanate. Approximately 15 mg of suberin residue was dissolved with $CDCl_3$ (500 μL) in a NMR tube, and an excess of TAI (varying between 45–200 μL) was added at room temperature, in a controlled argon atmosphere to avoid side reactions with moisture. The mixtures were stirred until complete dissolution and the ^{1}H NMR spectrum promptly recorded.

^{1}H Nuclear Magnetic Resonance Analyses. The ^{1}H NMR spectra ($CDCl_3$) were acquired at 300.13 MHz with at least 64 scans, using a Brüker AMX 300 spectrometer. All chemical shifts were expressed as parts per million downfield from tetramethylsilane (TMS) used as the internal standard.



SCHEME 1. Reaction between TAI and a primary hydroxy group (R′=H) or a secondary hydroxy group (R′=alkyl chain).

RESULTS AND DISCUSSION

Analyses of the Functional Groups of MDS. Since the methanolysis depolymerization mixtures were essentially in the form of methyl esters, only the hydroxy groups reacted with TAI (Scheme 1). This condensation was almost instantaneous and occurred for both primary or secondary hydroxy groups, resulting in the formation of a urethane derivative. In order to ensure complete derivatization of the hydroxy groups, three different volumes of TAI were tested, 45, 100, and 200 μL for ≈15 mg of MDS. It was observed that there was no significant difference between the ensuing TAI-MDS ^{1}H NMR spectra, which indicated that typically ≈45 μL of TAI was enough to derivatize all the free OH groups. Given that the TAI molecule is aprotic, when an excess of this reagent is used, no additional resonance appeared in the spectrum.

^{1}H Nuclear Magnetic Resonance Analyses of MDS Samples. A typical ^{1}H NMR spectrum of MDS suberin is shown in Fig. 1a and the characteristic chemical shifts and integrations of both underivatized HEX- and DCM-MDS are shown in Table I. These spectra showed, as the most relevant signals, weak resonances at δ ≈ 0.72–1.05 ppm, assigned to the $C\underline{H}_3$ protons; an intense multiplet around δ 1.25–1.31 ppm, attributed to $C\underline{H}_2$ protons of the alkylic chains; a multiplet at δ 1.61–1.71 ppm, also typical of $C\underline{H}_2$ protons, but in the β position to the hydroxy and ester groups ($C\underline{H}_2CH_2O$ and $C\underline{H}_2CH_2CO_2CH_3$); a weak multiplet at 2.01 ppm, assigned to the allylic $C\underline{H}_2$ protons adjacent to the CH=CH groups; a triplet at δ 2.30 ppm, assigned to the protons of $C\underline{H}_2CO_2CH_3$ groups; a weak triplet at δ 2.34 ppm, assigned to the $C\underline{H}_2$ protons adjacent to free CO_2H groups, resulting from residual hydrolysis reactions (only present in DCM-MDS); and a multiplet at δ ~ 3.64 ppm, assigned to terminal $C\underline{H}_2OH$ protons overlapped with the resonances of mid-chain $C\underline{H}OH$ proton resonance and with an intense and sharp singlet at δ 3.66 ppm, assigned to the protons of the $CO_2C\underline{H}_3$ groups. Finally, a low intensity triplet at δ 5.34 ppm, corresponds to the protons of the $C\underline{H}$=$C\underline{H}$ groups.

In general, the ^{1}H NMR spectra of MDS were consistent with previously published data[6] and obviously with the aliphatic nature of suberin, dominated by the signals arising from the aliphatic methylene protons, in the region between 1.25 and 2.01 ppm, typically representing approximately 70% of all protons. The resonances directly related to the OH and $COOCH_3$ functional groups (δ 3.64 and 3.66 ppm, respectively) represented a smaller percentage of all protons, viz. ≈10%.

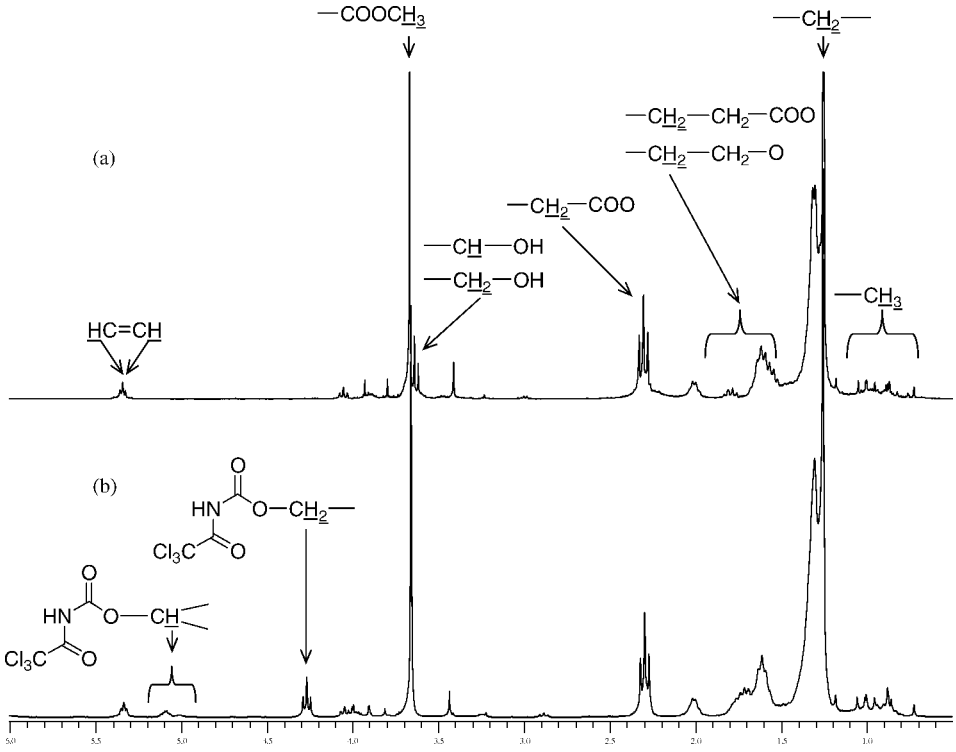


FIG. 1. ^{1}H NMR spectra of HEX-MDS (**a**) before and (**b**) after derivatization with TAI.

Hence, this spectroscopic evidence clearly reflected the formation of depolymerization products, as already suggested by the GC-MS results.

The use of the MDS ^{1}H NMR spectra for the quantitative determination of the reactive functional groups is hampered by the overlapping between $C\underline{H}_2OH$ and $C\underline{H}OH$ (δ 3.64 ppm) and also by the partial overlapping between those two resonances and $CO_2C\underline{H}_3$ resonances, 3.64 and 3.66 ppm, respectively (Fig. 1a). This limitation could be easily overcome in the ^{1}H NMR of the TAI derivatized samples.

^{1}H Nuclear Magnetic Resonance Analyses of the TAI-MDS Samples. The ^{1}H NMR spectra of TAI-MDS showed essentially the same resonances as their MDS counterparts (illustrated for TAI-HEX-MDS in Fig. 1b), except for the resonance assigned to the $C\underline{H}_2OH$ and $C\underline{H}OH$ protons, which shifted from δ 3.64 ppm to 4.27 ppm and 5.00–5.09 ppm, respectively. A new resonance at δ 8.44–10.44 ppm, assigned to NH protons, was also observed (not shown). These shifts and the new NH resonance are due to the formation of the corresponding TAI urethane derivatives[13] (Scheme 1). Hence, the derivatization procedure allows the correct integration of $CO_2C\underline{H}_3$, $C\underline{H}_2O$, and $C\underline{H}O$ resonances to be assessed, since they are no longer overlapping. In this way, the ratio between carboxylic and hydroxy groups can be determined with accuracy, as well as estimating the relative amount of primary and secondary hydroxy groups. Once more, both TAI-HEX- and TAI-DCM-MDS show similar ^{1}H NMR profiles, differing only in the resonance integrations (Table I).

TABLE I. Important ^{1}H NMR resonances of MDS samples before (HEX-MDS and DCM-MDS) and after TAI derivatization (TAI-HEX-MDS and TAI-DCM-MDS).

			Integration[a]			
δ/ppm	Assignment	Multiplicity	HEX-MDS	TAI-HEX-MDS	DCM-MDS	TAI-DCM-MDS
0.72–1.05	$C\underline{H}_3$	m	5.6	6.7	21.0	16.6
1.25, 1.31	$C\underline{H}_2$	m	55.7	59.2	87.6	75.0
1.61–1.71	$C\underline{H}_2CH_2OH$ $C\underline{H}_2CH_2CO_2CH_3$	m	14.0	15.1	26.6	23.1
2.01	$C\underline{H}_2CH{=}CH$	s	2.6	2.5	3.3	3.0
2.30	$C\underline{H}_2CO_2CH_3$	t	7.8	6.7	8.3	6.9
3.64	$C\underline{H}_2OH$ $C\underline{H}OH$	m	2.2	-	4.1	-
3.66	$CO_2C\underline{H}_3$	s	8.6	8.7	9.3	7.6
4.27	$C\underline{H}_2O$-TAI	t	-	1.9	-	1.5
5.00–5.09	$C\underline{H}O$-TAI	m	-	0.8	-	1.1
5.34	$C\underline{H}{=}C\underline{H}$	t	1.0	1.0	1.0	1.0
8.44–10.44	$N\underline{H}$	s	-	tr	-	tr

[a] All values of areas of integration are the average of the spectra of three MDS aliquots.

TABLE II. Important ^{1}H NMR peaks of HDS before and after TAI derivatization (HDS and TAI-HDS, respectively).

δ/ppm	Assignment	Multiplicity	Integration[a] HDS	Integration[a] TAI-HDS
0.70–1.03	CH_3	m	7.6	11.3
1.23, 1.30	CH_2	m	48.8	64.2
1.53–1.82	CH_2CH_2OH $CH_2CH_2CO_2H$	m	12.6	19.4
1.98	$CH_2CH{=}CH$	s	2.4	3.1
2.26	CH_2CO	t	1.3	1.4
2.35	CH_2CO_2H	t	5.0	-
2.42	CH_2CO_2-TAI	t	-	0.4
2.56	CH_2CO_2-TAI	t	-	3.7
2.86	CH_2CO_2-TAI	t	-	0.7
3.65	CH_2OH $CHOH$	m	2.3	-
4.24	CH_2O-TAI	t	-	1.8
5.07	CHO-TAI	m	-	1.0
5.31	$CH{=}CH$	t	1.0	1.0
8.38–10.43	NH	s	-	tr

[a] All values of areas of integration are the average of the spectra of three aliquots.

Analyses of the Functional Groups of the HDS Samples. ***^{1}H Nuclear Magnetic Resonance Analyses of the HDS Samples.*** The ^{1}H NMR spectra of the HDS samples (Table II) showed the following characteristic signals: weak resonances at δ 0.70–1.03 ppm, assigned to the CH_3 protons; a strong multiplet at δ 1.23–1.30 ppm, ascribed to CH_2 protons of the alkylic chains; a multiplet at δ 1.53–1.82 ppm, also typical of CH_2 protons, but in the β position to the hydroxy and free carboxylic groups (CH_2CH_2O and $CH_2CH_2CO_2H$); a weak multiplet at δ 1.98 ppm, assigned to the CH_2 protons adjacent to the CH=CH groups; a weak triplet at δ 2.26 ppm, assigned to the protons of the CH_2CO groups; a triplet at δ 2.35 ppm, assigned to the CH_2CO_2H protons; a multiplet at δ 3.65 ppm, assigned to the CH_2 protons of CH_2OH, overlapped with the resonances of mid-chain CHOH proton; and a low intensity triplet at δ 5.31 ppm, attributed to the protons of the CH=CH groups.

The ^{1}H NMR spectra of HDS samples, just as those of the MDS counterparts, were dominated by the signals arising from the aliphatic methylene protons (≈80% of all protons), whereas the resonances directly related to the OH and CO_2H functional groups played a modest role (7% of all protons). These results agree with previous GC-MS findings,[7,8] which indicated that depolymerization mixtures are composed of structures in which aliphatic chains dominate, but which bear polar groups, mainly constituted of hydroxy and carboxylic moieties.

The partial overlapping of CH_2OH and CHOH resonances at 2.35 and CH_2CO_2H and CH_2CO resonances at 2.26–2.35 ppm in HDS samples again hampers the direct quantitative determination of the ratios CO_2H/OH and between primary and secondary OH's. This limitation can be once more overcome in the corresponding TAI-HDS spectra.

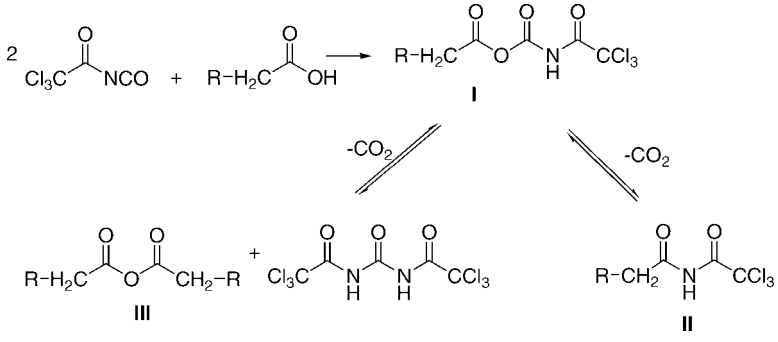
^{1}H Nuclear Magnetic Resonance Analyses of the TAI-HDS Samples. With the HDS samples, TAI reacted with both the hydroxy and the free carboxylic groups. As shown in Scheme 2, the reaction between CO_2H and TAI proceeds forming derivative **I** as the main product. However, the partial decarboxylation of **I** results in the formation of amide **II**, and an anhydride **III**, with the elimination of a biuret.[13]

The ^{1}H NMR spectra of TAI-HDS were similar to their HDS counterparts (Table II), differing only in the resonances associated with the functional groups. These new resonances were a weak triplet at δ 2.42 ppm, assigned to the CH_2 protons adjacent to the amide group (**II**); a triplet at δ 2.56 ppm, attributed to the protons of the CH_2 groups adjacent to the urethane (**I**) moiety; a weaker triplet at δ 2.86 ppm, attributed to the CH_2 protons adjacent to the anhydride group (**III**); a triplet at δ 4.24 ppm, assigned to the protons of the CH_2O-TAI groups; a multiplet at δ 5.07 ppm, attributed to the proton of the mid-chain CHO-TAI groups; and weak resonances at δ 8.38–10.43 ppm, assigned to the NH protons.

The resonances at δ 2.42, 2.56, and 2.86 ppm, related to protons adjacent to the derivatized CO_2H groups and the resonances at δ 4.24 and 5.07 ppm, from protons adjacent the derivatized OH groups, were used for their quantitative determinations.

Determination of the Carboxylic and Hydroxy Functional Group Ratios. The ratio between the number of carboxylic and hydroxy groups (r) present in the different suberin fragments was determined using the ^{1}H NMR results of the TAI-derivatized samples. In the TAI-MDS samples, r was simply calculated from the ratio between the integration area of the resonances of the OCH_3 protons (A_{CO2CH3}) at δ 3.66 ppm and those of the CH_2 and CH protons adjacent to the derivatized hydroxy groups ($A_{CH2O\text{-}TAI}$, $A_{CHO\text{-}TAI}$, respectively) at δ 4.27 ppm and δ 5.00–5.09 ppm, respectively, viz. $r = [A_{CO2CH3}/3]/[A_{CH2O\text{-}TAI}/2 + A_{CHO\text{-}TAI}]$.

In the TAI-HDS samples, the equation $r = [A_{CH2CO2\text{-}TAI}/2]/[A_{CH2O\text{-}TAI}/2 + A_{CHO\text{-}TAI}]$ was used instead, where $A_{CH2CO2\text{-}TAI}$ is the sum of integration areas of the resonances of the CH_2 protons adjacent to the derivatized carboxylic groups at δ 2.42, 2.56, and 2.86 ppm, $A_{CH2O\text{-}TAI}$ is the integration area of the resonances of CH_2 protons adjacent to the derivatized primary hydroxy groups, at δ 4.24 ppm, and $A_{CHO\text{-}TAI}$ is the integration



SCHEME 2. Reaction between TAI and a carboxylic group.

TABLE III. Results of ^{1}H NMR analyses of TAI-derivatized suberin: system; ratio between the number of carboxylic and hydroxy groups (r); and standard deviation (σ).

System	r[a]	σ
DCM-MDS	1.4	0.17
HEX-MDS	1.7	0.04
HDS	1.3	0.12

[a] Each r value was calculated as an average of the ^{1}H NMR spectra of three aliquots of each suberin sample.

TABLE IV. Results of ^{1}H NMR analyses of TAI-derivatized suberin: system; ratio between the number of primary and secondary hydroxy groups [$(A_{\underline{CH}2O\text{-}TAI}/2)/A_{\underline{CH}O\text{-}TAI}$]; and standard deviation (σ).

System	$[A_{\underline{CH}2O\text{-}TAI}/2]/A_{\underline{CH}O\text{-}TAI}$[a]	σ
DCM-MDS	0.7	0.04
HEX-MDS	1.2	0.12
HDS	0.9	0.05

[a] Each ratio was calculated as an average of the ^{1}H NMR spectra of three aliquots of each suberin sample.

area of the resonances of C<u>H</u> protons adjacent to the derivatized secondary hydroxy groups at δ 5.07 ppm.

The average values of r for each suberin sample studied are given in Table III. All samples showed more carboxylic than hydroxy groups. Whereas the higher values were obtained for MDS, viz. between 1.4 and 1.7, depending on the solvent used in the extraction step, for HDS counterparts r was close to 1.3. For MDS, the extraction step with *n*-hexane led to an increase in the CO_2H/OH ratio and therefore different needs in terms of correction in view of their application for polyester synthesis, as will be discussed below.

These data disagree considerably with those from GC-MS analyses of suberin fragments, which gave systematically $r < 1$.[6,8] This discrepancy can be rationalized by the fact that the GC-MS results bore an intrinsic limitation associated with the fact that only about 40% of fragments were in fact identified, as opposed to the present spectroscopic analyses of the TAI-derivatized samples, in which the whole mixture is inspected. We can therefore conclude that all the suberin extracts studied in this work bore a higher content of CO_2H (or CO_2CH_3) groups compared with the OH counterparts.

The ^{1}H NMR analyses of the TAI-derivatized suberin samples were also used to determine the relative abundance of primary and secondary hydroxy groups. These proportions were calculated by the ratio $[A_{\underline{CH}2O\text{-}TAI}/2]/[A_{CHO\text{-}TAI}]$. Results summarized in Table IV showed that HEX-MDS presents the highest relative amount of primary OH groups, followed by HDS, and DCM-MDS (1.2, 0.9, and 0.7, respectively). The use of the fractionation step with *n*-hexane leads to an increase in the $[A_{\underline{CH}2O\text{-}TAI}/2]/[A_{CHO\text{-}TAI}]$ ratio.

The knowledge of the *precise* quantity of functional groups present in suberin fragments is essential, not just for the detailed characterization of these extracts, but also, and especially, in the context of such applications as their use as monomers in the preparation of polyesters, where the relative content of primary and secondary alcohols could have an important influence in properties of the ensuing materials. In fact, in a study of these type of polycondensations,[11] we have shown that polyesters with significantly different physical properties are obtained depending on the use of ω-hydroxy fatty acids or mid-chain hydroxy fatty acids (primary or secondary OH groups, respectively) as monomers.

Additionally, the value of r should be as close as possible to unity in order to ensure the highest molecular weights of the resulting polymers; in the same study mentioned above,[11] we have shown that the lack of stoichiometric balance reported here resulted in rather modest DPs, but a simple readjustment was readily achieved by the addition of an appropriate OH-bearing comonomer, as some preliminary results demonstrated recently.

CONCLUSION

This investigation showed that it is possible to determine the relative amount of carboxylic and hydroxy functional groups of a highly complex mixture of suberin fragments, using a routine laboratory technique, ^{1}H NMR spectroscopy, and a simple derivatization procedure.

For all the suberin fragments analyzed, we found an excess of carboxylic over hydroxy groups, with notable quantitative differences among samples extracted by different procedures. The exact knowledge of r is an essential requisite in certain applications, particularly if the fragments are to be employed as precursors of new macromolecular materials.

The ^{1}H NMR analyses of these suberin mixtures of hydroxyacids also showed that these samples present different amounts of primary and secondary hydroxy groups, varying between 1.2 and 0.7, and depending on the extraction procedure adopted.

Finally, the results obtained clearly show the potentiality of this method to access the functionality ratios in depolymerization mixtures of suberins from different species or other natural sources of hydroxyacids (e.g., lesquerella oil or castor oil) as sources of monomers for macromolecular synthesis.

ACKNOWLEDGMENT

FCT is gratefully acknowledged for a doctorate grant to A. F. Sousa (SFRH/BD/38890/2007).

1. M. A. Bernards, Can. J. Bot. **80,** 227 (2002).
2. A. Gandini, C. Pascoal Neto, and A. J. D. Silvestre, Prog. Polym. Sci. **31,** 878 (2006).
3. J. Graça and S. Santos, Macromol. Biosci. **7,** 128 (2007).
4. P. E. Kalattukudy, "Polyesters in higher plants", in *Advances in Biochemical Engineering/Biotechnology,* W. Babel and A. Steinbuchel, Eds. (Springer-Verlag, Berlin, 2001), p. 1.
5. A. J. D. Silvestre, C. Pascoal Neto, and A. Gandini, "Cork and suberins: major sources, properties, applications", in *Monomers, Polymers and Composites from Renewable Resources*, M. N. Belgacem and A. Gandini, Eds. (Elsevier, Amsterdam, 2008), p. 305.
6. N. Cordeiro, M. N. Belgacem, A. J. D. Silvestre, C. P. Neto, and A. Gandini, Int. J. Biol. Macromol. **22,** 71 (1998).
7. M. H. Lopes, A. M. Gil, A. J. D. Silvestre, and C. P. Neto, J. Agric. Food Chem. **48,** 383 (2000).
8. P. C. R. O. Pinto, A. F. Sousa, A. J. D. Silvestre, C. Pascoal Neto, A. Gandini, C. Eckerman, and B. Holmbom, Ind. Crop Prod. **29,** 126 (2009).
9. N. Cordeiro, M. N. Belgacem, A. Gandini, and C. P. Neto, Ind. Crop Prod. **6,** 163 (1997).
10. A. Olsson, M. Lindstrom, and T. Iversen, Biomacromolecules **8,** 757 (2007).
11. A. F. Sousa, A. J. D. Silvestre, A. Gandini, and C. Pascoal Neto, Chem. Sus. Chem. **1,** 1020 (2008).
12. M. N. Belgacem and A. Gandini, "Materials from vegetable oils: major sources, properties, applications", in *Monomers, Polymers and Composites from Renewable Resources*, M. N. Belgacem and A. Gandini, Eds. (Elsevier, Amsterdam, 2008), p. 39.
13. A. R. Donovan and G. Moad, Polymer **46,** 5005 (2005).

14. I. Fallais, J. Devaux, and R. Jerome, J. Polym. Sci. Pol. Chem. **38,** 1618 (2000).
15. V. W. Goodlett, Anal. Chem. **37,** 431 (1965).
16. D. Lu, J. C. Yuan, L. G. You, and Z. Q. Lei, J. Macromol. Sci. A **45,** 705 (2008).
17. Y. Ma, U. S. Agarwal, J. A. J. M. Vekemans, and D. J. Sikkema, Polymer **44,** 4429 (2003).
18. A. Postma, T. P. Davis, A. R. Donovan, G. X. Li, G. Moad, R. Mulder, and M. S. O'Shea, Polymer **47,** 1899 (2006).
19. M. Rajan, I. Coduga, Y. Q. Ma, F. Mcchioni, and U. S. Agarwal, E-Polymers (2003).
20. G. F. Zakis, *Funcional Analysis of Lignins and Their Derivatives* (Tappi Press, Atlanta, 1992).
21. P. Narayanan, A. Iraqi, and D. J. Colehamilton, J. Mater. Chem. **2,** 1149 (1992).