Phytochemistry, Vol. 23, No 9, pp 2059–2060, 1984
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
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SHORT REPORTS

FREE AND BOUND HYDROXYL AND CARBOXYL GROUPS IN THE CUTIN OF *QUERCUS SUBER* LEAVES

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(*Revised received* 6 *February* 1984)

Key Word Index—*Quercus suber*; Fagaceae; leaves; cutin; mesyl-cutin; free hydroxyl groups; hydrogenolysis.

Abstract—The number of free and bound hydroxyl and carboxyl groups of the cutin of *Quercus suber* leaves was investigated by the lithium borohydride hydrogenolysis of mesyl-cutin compared with the lithium borohydride hydrogenolysis of untreated cutin. Fifty per cent of the *vic*-diol groups of the trihydroxy C_{18} acid component and twenty five per cent of the secondary hydroxyl groups of the dihydroxy C_{16} acid component are free. The rest of the secondary and all of the primary hydroxyl groups are esterified; all carboxyl groups are esterified.

INTRODUCTION

Cutin is a lipid biopolyester which is depolymerized by transesterification with methanol containing a catalyst such as sodium methoxide [1] and releases the constituent monomer acids as their methyl esters. The precise structure of the polyester, its MW, the sequence of hydroxy-acids and the numbers of bound and free hydroxyl and carboxyl groups are not known. However, recently a little progress has been made in the understanding of the structure of this polymer by Kolattukudy [2] and Deas and Holloway [3]. Two methods tested for this purpose have given some useful information. One method involves oxidation of any free hydroxyl groups with CrO_3–pyridine complex followed by depolymerization with sodium methoxide in anhydrous methanol. The other method involves treatment of the polyester with methane sulphonyl chloride followed by depolymerization with either $LiAlD_4$ [2] or sodium methoxide–methanol. The latter method applied to the suberin of *Quercus suber* [4, 5] has shown that all the primary hydroxyl groups are esterified, whilst the secondary hydroxyl groups are free.

We now wish to report on our research to determine which monomers of the cutin of the leaves of *Q. suber* bear free and bound hydroxyl groups and free and bound carboxyl groups. For this determination we treated the cutin with methane sulphonyl chloride in pyridine, which converted all of the free hydroxyl groups into their mesylates; this mesylation was complete only after 48 hr in pyridine. The so obtained mesyl-cutin was then depolymerized with $LiBH_4$, which reduced the mesylates to hydrocarbons, according to the following scheme:

$$\text{Cutin-CH}_2\text{OH} \xrightarrow{\text{MeSO}_2\text{Cl}} \text{Cutin-CH}_2\text{OSO}_2\text{Me} \xrightarrow{\text{LiBH}_4} \text{Monomer-CH}_3$$

Table 1. Acetates of alcohols separated by gas chromatography from the depolymerization products of cutin and mesyl-cutin of *Quercus suber*

Peak	R_t (min)	Compound	Class of compound	% cutin	% mesyl-cutin
1	6.50	$CH_3(CH_2)_{11}OAc$	Monoacetate-C_{12}	0.2	0.8
2	10.07	$CH_3(CH_2)_{15}OAc$	Monoacetate-C_{16}	3.2	5.9
3	13.37	$CH_3(CH_2)_{17}OAc$	Monoacetate-C_{18}	0.5	1.0
4	16.90	$CH_3(CH_2)_{19}OAc$	Monoacetate-C_{20}	1.0	1 0
5	17.15	$AcO(CH_2)_{16}OAc$	Diacetate-C_{16}	6.1	17.7
6	20.23	$AcO(CH_2)_8CH=CH(CH_2)_8OAc$	Diacetate-INS-C_{18}	1.4	1.0
7	20.76	$AcO(CH_2)_{18}OAc$	Diacetate-C_{18}	1.8	7.1
8	21.65	$AcO(CH_2)_6CH(OAc)(CH_2)_9OAc$	Triacetate-C_{16}	59.2	43.5
9	24.20	$AcO(CH_2)_{20}OAc$	Diacetate-C_{20}	0.3	1.9
10	24.83	$AcO(CH_2)_8CH(OAc)\,(CH_2)_9OAc$	Triacetate-C_{18}	12.3	17.8
11	27.49	$AcO(CH_2)_{22}OAc$	Diacetate-C_{22}	2.3	1.9
12	27.65	$AcO(CH_2)_8CH(OAc)CH(OAc)(CH_2)_8OAc$	Tetraacetate-C_{18}	11.3	0
13	30.65	$AcO(CH_2)_{24}OAc$	Diacetate-C_{24}	0.6	0.3

The monomers thus obtained were cleanly separated by column and gas chromatography. Comparison of the acetates produced in this way with those obtained from untreated cutin provide information on the nature and numbers of free and bound hydroxyl groups in the polymer. Moreover $LiBH_4$ distinguish carboxylates and carboxylic acids; carboxylates are reduced to the alcohols in both mesyl-cutin and cutin; free carboxylic acids are recovered unchanged following the treatment.

RESULTS AND DISCUSSION

Leaves of *Quercus suber* were thoroughly extracted with organic solvents to remove the nonpolymeric material which embeds cutin. The resulting material containing cutin, pectin and cellulose was treated at 130° in diglyme with $LiBH_4$. The alcohols produced in this way were treated with acetic anhydride and pyridine and the resulting acetates were separated by GC into 13 peaks, corresponding to 13 acetates, as is shown in Table 1. Another sample of the extracted leaves was shaken in pyridine for 48 hr with methane sulphonyl chloride at room temperature under anhydrous conditions. The mesylated residue (mesyl-cutin) so obtained gave IR absorption bands at 1350 and 1170 cm^{-1} (SO_2) without absorption at 3400 cm^{-1} (OH). This material was depolymerized in the same way as the cutin and the monomers fractioned with aqueous alkali into neutral (alcohols) and acidic compounds (phenols). The neutral compounds were acetylated and the resulting acetates were analysed by GC as before into 12 peaks corresponding to 12 acetates as shown in Table 1.

The results obtained show that the cutin monomer 9,10,18-trihydroxyoctadecanoic acid, which bears a *vic*-diol group, has *ca* 50% of this group free in the polymeric form whereas the remainder occurs with one hydroxyl group free and the other esterified. Hydrogenolysis of the cutin afforded 11.3% of the C_{18} tetraacetate which was not detected in mesyl-cutin; therefore both hydroxyl groups could not have been esterified. However, one hydroxyl group of this diol groups was partially esterified because the amount of C_{18} triacetate increased from 12.3% in the cutin to 17.8% in the mesyl-cutin. The monomer 10,16-dihydroxyhexadecanoic acid, from hydrogenolysis of the cutin, afforded 59.2% of C_{16} triacetate and this amount decreased to 43.5% in the mesyl-cutin. This result indicates that the 10-hydroxyl group of the acid was esterified *ca* 75% and that the remainder was free, since the C_{16} diacetate increased from 6.1% in the cutin to 17.7% in the mesyl-cutin. No free carboxylic acid groups were detected in the cutin.

The secondary hydroxyl group of the dihydroxy C_{16} acid and the *vic*-diol group of the trihydroxy C_{18} acid constitute the bulk of the free hydroxyl groups present in the cutin of *Q. suber*, strongly suggesting that the primary hydroxyl groups are preferentially esterified. It is clear that a significant proportion of the secondary hydroxyl groups are also esterified. These results confirm those obtained by Kolattukudy [2] and Deas and Holloway [3] for the cutins of other plant species.

EXPERIMENTAL

Preparation of polyester (cutin) Air dried and powdered leaves of *Q. suber* were exhaustively and successively extracted in a Soxhlet extractor with C_6H_6, H_2O and MeOH. The wt of leaves decreased with this treatment by *ca* 24%.

Preparation of mesylated polyester (mesyl-cutin). Extracted leaves (cutin) (5 g) were shaken in pyridine (50 ml) with $MeSO_2Cl$ (2.5 ml) for 48 hr under anhydrous conditions. The residue was filtered, washed with MeOH and dried under vacuum at 40°.

Depolymerization. $NaBH_4$ (1.85 g; 0.05 mol) was shaken with freshly destilled diglyme (50 ml) and LiCl (2.1 g; 0.05 mol) for 1 hr at room temp. This soln of $LiBH_4$ heated at 130° was used to treat the mesyl-cutin (5 g) under an Ar atmosphere, keeping the mixture at this temp with vigorous shaking for 4 days The reaction mixture was cooled and filtered, the filtrate acidified and extracted with EtOAc. The organic extract was fractioned with aq. NaOH (5%) into two fractions. Removal of solvent from the two fractions gave a mixture of alcohols in the neutral fraction and two phenols in the acidic fraction. In a similar way the extracted leaves were submitted to the same conditions of hydrogenolysis in order to compare cutin with mesyl-cutin.

Monomer identification. Depolymerization of the cutin and mesyl-cutin on a prep. scale led to the isolation by CC of the acetate monomers and later identification by physical and spectroscopic techniques. Reference acetate monomers were obtained from the $LiBH_4$ reduction of suitable Me esters obtained from methanolysis of cutin [6]. The alcohols obtained from cutin and mesyl-cutin were acetylated and the acetates analysed by FID GC on an SE-30 column with temp programming (175–300° at 4°/min).

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