Analysis of aliphatic biopolymers using thermochemolysis with tetramethylammonium hydroxide (TMAH) and gas chromatography–mass spectrometry

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Abstract—Selected aliphatic biopolyesters (cutins, cuticles and a suberin) isolated from different plants have been analyzed using thermochemolysis with tetramethylammonium hydroxide (TMAH). This method consists of a high-temperature saponification/transesterification, and yields methyl esters of fatty acids and the methyl ethers of alcohols, which are subsequently analyzed by gas chromatography and gas chromatography–mass spectrometry. The main compounds produced from the analyzed samples correspond to the methyl derivatives of long-chain fatty acids, hydroxy fatty acids and α,ω-alkanedioic acids. The composition of the released compounds are similar to those reported in the literature using different depolymerization methods. The main advantage of the procedure is that it is easily performed in glass tubes with very low amounts of sample and without additional derivatization steps prior to gas chromatographic analysis because the products are methylated *in situ*. The method also avoids the laborious and time consuming sample preparation of extractive methods and the use of large amounts of solvents.

Key words—cutin, cuticle, suberin, fatty acids, TMAH/thermochemolysis

INTRODUCTION

Because cutin and suberin play a key mediating role between higher plants and the environment, their chemical composition and structure have been the subject of numerous investigations (Kolattukudy, 1984; Holloway, 1982; Walton, 1990). Cutin, a biopolyester composed of interesterified hydroxy fatty acids, is found as an external protective barrier on aereal plant surfaces (Kolattukudy, 1980, 1984). Cutin is composed primarily of hydroxylated fatty acids with a high proportion of mid-chain oxygenated acids (Espelié *et al.*, 1979, 1980). The monomer composition of cutin varies among different plant species. In general, angiosperm cutins can be broadly classified into three categories according to the chemical lengths of the predominant fatty acid monomers, that is, a C_{16} type, a C_{18} type and a mixed C_{16} and C_{18} type. The cutins of the gymnosperms and other lower plants generally lack the C_{18} monomer acids (Holloway, 1982). Suberin, a similar biopolymer, serves as a protective barrier for subterranean surfaces of plants and its structure is thought to consist of aliphatic polyester domains, composed of aliphatic monomers of long-chain (C_{16}–C_{24}) ω-hydroxy fatty acids and α,ω-dicarboxylic acids, covalently attached to aromatic domains which are in turn attached to the cell wall (Kolattukudy, 1978).

However, despite all the investigations, the intramolecular structure of cutin and suberin is only poorly understood and a detailed characterization of their chemical composition is important for a physiological or chemotaxonomical point of view. Indeed, cutins and suberins are unique to vascular plants and have already been used as a convenient mean for determining the flow of terrestrially derived organic matter in marine environments, and also to discriminate between different vascular plant sources in natural environments (Goñi and Hedges, 1990; Opsahl and Benner, 1995).

Partial depolymerization of the aliphatic biopolyesters has been accomplished using various chemical and enzymatic methods, including hydrogenolysis with $LiAlH_4$, hydrolysis with alcoholic KOH or HCl, and enzymatic degradation with cutinases (Holloway, 1982; Kolattukudy, 1984; Walton, 1990). The identifications of products of the reactions often requires further derivatization to convert the alcohol groups into their trimethylsilyl

or acetyl derivatives and the carboxyl groups into their respective methyl esters, prior to analysis by capillary gas chromatography.

In a number of papers (Holzer *et al.*, 1988; Dworzanski *et al.*, 1990, 1991; Challinor, 1996) natural esters were converted to their respective methyl esters by tetramethyl- or other tetra-alkylammonium hydroxides under pyrolytic conditions. Dworzanski *et al.* (1990, 1991) used the "*pyrolytic methylation*" with tetramethylammonium hydroxide (TMAH) for the generation of chemotaxonomically characteristic profiles of fatty acid methyl esters from different bacterial cells. It was demonstrated that the methyl esters were formed by transesterification reactions (via intermediate tetramethylammonium hydroxide salts) and were not formed by methylation of pyrolytically generated fatty acids. Holzer *et al.* (1989) used a related quaternary amine, trimethylanilinium hydroxide to obtain fatty acid profiles of bacterial cells, while Butte (1983) used trimethylsulphonium hydroxide (TMSH) as a transesterification reagent. Most of the products corresponded to those formed by hydrolysis and quantitative methylation of the fatty acid moieties with partial methylation of the aliphatic hydroxyl groups.

Another technique for analysis of these biopolymers, pyrolysis in the presence of TMAH, has been particularly effective in transforming macromolecular material, composed of esters and phenolic compounds, to monomers by hydrolysis and subsequent alkylation. The volatile monomers are then analyzed quantitatively by gas chromatography/mass spectrometry. Challinor (1989, 1991a,b) first introduced this technique for the simultaneous pyrolysis and methylation of phenolic polymers. Since then, it has been applied to the structural characterization of bio- and geomacromolecules such as lignins (Martín *et al.*, 1995b; Clifford *et al.*, 1995), humic materials (Saiz-Jiménez *et al.*, 1993, 1994; Saiz-Jiménez, 1994a,b; Hatcher and Clifford, 1994; del Río *et al.*, 1994; Chiavari *et al.*, 1994; Martín *et al.*, 1994, 1995a; Fabbri *et al.*, 1996), whole soils, (Schulten and Sorge, 1995; Schulten *et al.*, 1996), asphaltenes and kerogens (Kralert *et al.*, 1995; del Río *et al.*, 1996b) and natural and fossil resins and resinites (Anderson and Winans, 1991; Clifford and Hatcher, 1995). In particular, several biopolyesters such as the cutin from tomato fruit and the suberin from potato tubers have already been successfully analyzed by pyrolysis/TMAH (de Leeuw and Baas, 1993; González-Vila *et al.*, 1996). The main products formed were the methyl derivatives of fatty acids and hydroxylated fatty acids. Challinor (1996) also used the pyrolysis/TMAH for the rapid profiling of fatty acids in a variety of vegetable oils and animal fats.

Several authors (de Leeuw and Baas, 1993; Hatcher and Clifford, 1994; Martín *et al.*, 1994; Challinor, 1995; del Río *et al.*, 1996a) have pointed out that the reaction involved in the TMAH/pyrolysis scheme is one of chemolysis rather than pyrolysis. Sub-pyrolysis temperatures have therefore been found to effectively produce a suite of products similar to that observed at higher pyrolysis temperatures (Hatcher and Clifford, 1994; Clifford *et al.*, 1995). In a recent paper (McKinney *et al.*, 1995), a procedure was outlined for the characterization of lignin at subpyrolysis temperatures in the presence of TMAH using sealed glass tubes. Since then, this procedure has been extended to the characterization of lignin in fresh and degraded woods (Hatcher *et al.*, 1995), coalified woods (McKinney and Hatcher, 1996) and the highly aliphatic and resistant biopolymer cutan present in the cuticle of various plants (McKinney *et al.*, 1996).

In this paper, we investigate the use of the sealed tube reaction with TMAH on a set of aliphatic biopolyesters (cuticle, cutin and suberin) isolated from different plants. The main advantage of this procedure is that it is conducted in a glass microreactor, a glass ampoule, as a one-step thermolysis and methylation, with the products being immediately available for analysis by gas chromatography. The procedure avoids the laborious and time-consuming sample preparation of extractive methods and does not require additional derivatization steps.

MATERIALS AND METHODS

The samples selected for this study were kindly provided by Dr. Karl Espelié and consisted of the cutins isolated from tomato fruit, papaya fruit and lime fruit and the cuticles from *Agave Americana* and apple fruit. The chalazal region of the inner seed coat of grapefruit (*Citrus paradise* INSC hilum) was also selected for this study as an example of a suberin polymer. The procedures for the isolation of the different samples have already been reported (Espelié *et al.*, 1980, 1982, 1983).

The samples (0.5–1.0 mg) were weighed precisely and placed in a glass tube with a measured amount (100 ml) of TMAH (25% w/v in methanol). Triplicate samples of tomato cutin were prepared to verify the reproducibility of the method. The methanol was evaporated under vacuum and the tube sealed under vacuum. The sealed tubes were then placed in an oven at 250°C for 30 min. After cooling to room temperature, the tubes were cracked open and all inside surfaces washed out with methylene chloride (3 × 1 ml). The extracts were combined and reduced to dryness under a stream of nitrogen. The samples were then diluted with a known volume of methylene chloride (100 ml), which contained an internal standard (28.4 ng/ml of *n*-eicosane) and subsequently analyzed by capillary gas chromatography (GC) on a Hewlett Packard 5890 Series II gas chromatograph

and by gas chromatography/mass spectrometry (GC/MS) on a Kratos MS-80 RFA high-resolution gas chromatograph/mass spectrometer system. The columns used for the GC separation were 30 m × 0.25 mm, i.d., fused silica capillary columns (DB-5, J and W). The column was heated at 30°C/min from an injection temperature of 60°C to a temperature of 100°C, at which point the rate was slowed to 6°C/min to a final temperature of 300°C, and held for 10 min. Injector and detector were set at 300°C.

Mass spectra (electron impact mode) were obtained in the mass range from m/z 40 to 600 at a scan rate of 0.6 s/decade of mass with a 0.2 s magnet settling time added. Compounds were identified by their mass spectra and relative retention times. Identification of the monomers liberated by TMAH/thermochemolysis has been made through direct comparison of commercially available mass spectral database using computerized library searching and by analysis of mass spectral fragmentation patterns.

RESULTS AND DISCUSSION

The chromatograms of the products released after TMAH/thermochemolysis of the different aliphatic biopolyesters selected for this study are shown in Fig. 1. The main compounds identified corresponded to the methyl derivatives of long-chain fatty acids, hydroxylated fatty acids (the hydroxylated sites being converted by the TMAH to methyl ethers) and dicarboxylic acids. All these products can be rationalized as derived from alkaline hydrolysis of the polyesters into fatty acid monomers, followed by quantitative methylation of the fatty acid monomers. Since hydroxy groups become methylated after TMAH/thermochemolysis, it would not be then possible to distinguish them from naturally occurring methoxy groups; however, this is not the case since methoxy-fatty acids have not been found as forming part of the structure of cutin and suberin.

Tables 1 and 2 show the list of the identified compounds, as well as their relative distribution in the different samples. The distribution of the compounds identified is similar to that previously reported for the monomeric constituents of these samples using different depolymerization methods (Ray *et al.*, 1995; Espelié *et al.*, 1980, 1983; Holloway, 1973, 1982; Baker and Holloway, 1970; Baker *et al.*, 1982). The cutin of tomato fruit was selected to study the reproducibility of the method. Three replicates were performed and the composition determined. Table 2 shows the mean values and the standard deviation of the abundances of the compounds identified in tomato fruit cutin. The average standard deviation is 8%, which indicates that this procedure is very reproducible. In the ensuing discussion, reference is made to hydroxylated fatty acids with the understanding that it is the methylated ester/ethers that are inferred to be the ones detected in our analysis.

As expected, great differences in composition were found between the suberin sample (the chalazal region of the inner seed coat of grapefruit) and the cutins and cuticles. While the suberin sample yielded mainly C_{16}, C_{18}, C_{22} and C_{24} monomers, the major monomers produced from the cutin and cuticles analyzed in this study have either 16 or 18 carbon atoms or both (Fig. 1), in agreement with literature. The most abundant members of the C_{16} family identified in these samples were the methyl derivatives of hexadecanoic acid (**12**), ω-hydroxyhexadecanoic acid (**16**) and different positional isomers of dihydroxyhexadecanoic acid (**19**,**20**,**21**). Other monomers that can be derived by further oxidation of the common C_{16} monomers were also detected in some cutins. This is the case of the methyl derivative of the 16-hydroxy-10-oxohexadecanoic acid (**17**) released from lime cutin. This agrees with prior studies of citrus fruit cutins, in which large proportions of ω-hydroxy-oxo-hexadecanoic acids were found among the hydrogenolysis and alkaline hydrolysis products of grape fruit, lime, lemon, orange, clementine and mandarine (Deas *et al.*, 1974; Espelié *et al.*, 1980, 1983; Baker and Procopiou, 1975). The methyl derivative of the 10-hydroxyhexadecanoic acid (**14**), also reported in lime cutin and other citrus especies (Ray *et al.*, 1995), was also identified in lime fruit cutin. On the other hand, the major members identified of the C_{18} family of cutin monomers were the methyl derivatives of octadecanoic acid (**15**), octadecenoic acid (**13**), 18-hydroxyoctadec-9-enoic acid (**23**) and 9,10,18-trihydroxyoctadecanoic acid (**30**).

The C_{16} monomers, particularly the methyl derivatives of different positional isomers of dihydroxyhexadecanoic acid, were dominant in the case of tomato fruit, papaya fruit and lime fruit cutins. Tomato fruit cutin also released trace amounts of a C_{18} monomer, the methyl derivative of the 9,10,18-trihydroxyoctadecanoic acid (**30**). Its presence has been shown to be confined to the early stages of fruit development, and is not usually detected in mature tomato fruit cutins (Baker *et al.*, 1982). In the apple fruit and *Agave americana* cuticles, the C_{16} and C_{18} monomers are both dominant, but with a predominance of the C_{16} monomers in the apple fruit cuticle (mainly the methyl derivatives of the 16-hydroxyhexadecanoic acid, **16** and 10,16-dihydroxy-hexadecanoic acid, **21**) and a predominance of the C_{18} monomers in the *Agave americana* cuticle (mainly the methyl derivative of the 9,10,18-trihydroxyoctadecanoic acid, **30**), in agreement with previously published data (Holloway, 1973; Espelié *et al.*, 1982).

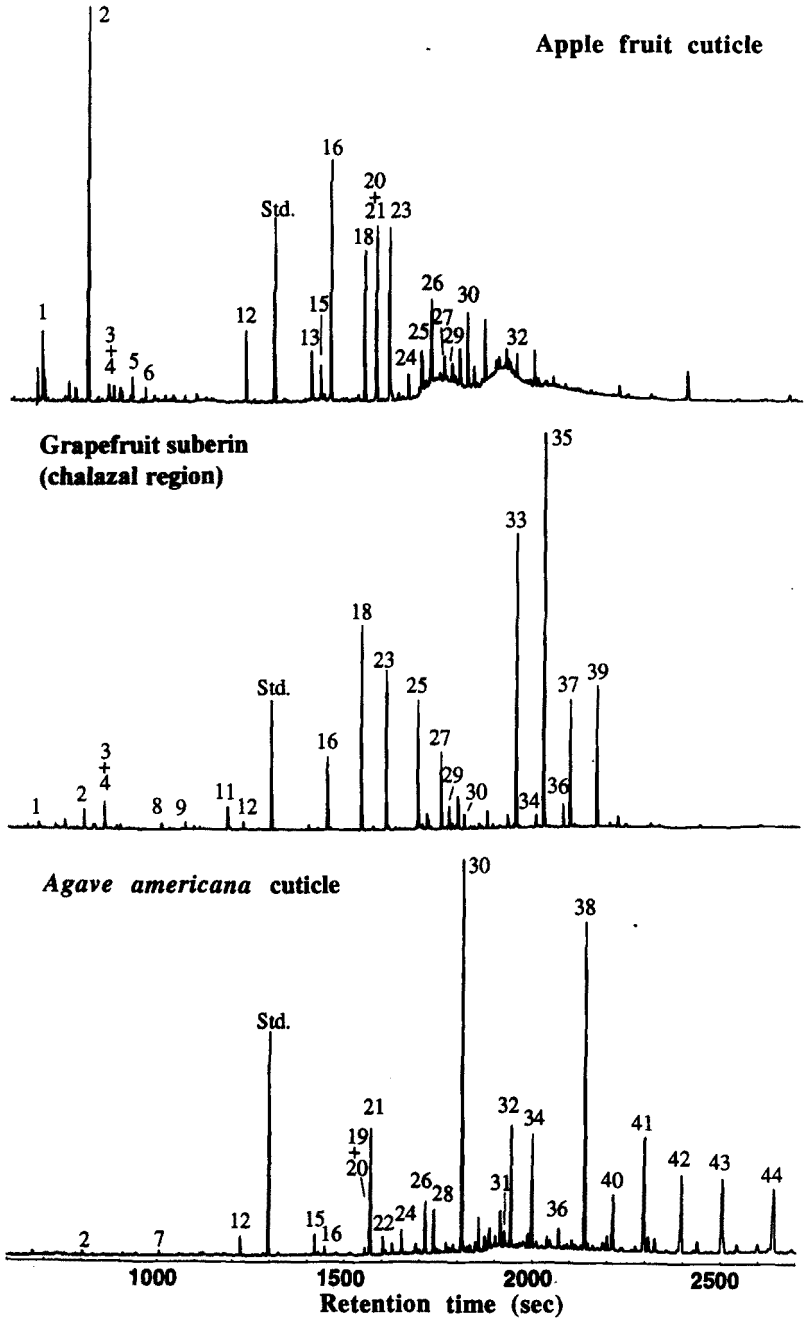

Fig. 1a.

Dihydroxyhexadecanoic acids are the most characteristic and dominant cutin monomers in plants and are usually found as a mixture of positional isomers, with the mid-chain hydroxyl found at either C-7, C-8, C-9 or C-10, with the latter two isomers being the most prevalent ones (Holloway and Deas, 1971). After TMAH/thermochemolysis, the different positional isomers produce the respective methyl derivatives, which under the chromatographic conditions used, coelute in the same chromatographic peak. However, the mass fragmentation pattern of each positional isomer is different and therefore they can be easily detected and quantified by integration of the characteristic fragment ions in the GC–MS. The isomers that were detected in the samples studied were the methyl derivatives of the 8,16-, 9,16- and 10,16-dihydroxy-hexadecanoic acids. Figure 2 shows the structures and mass spectra of the different positional isomers. The characteristic fragment ions in their mass spectra represent cleavage α to the secondary methoxyl group giving a dimethoxy fragment (I) and a meth-

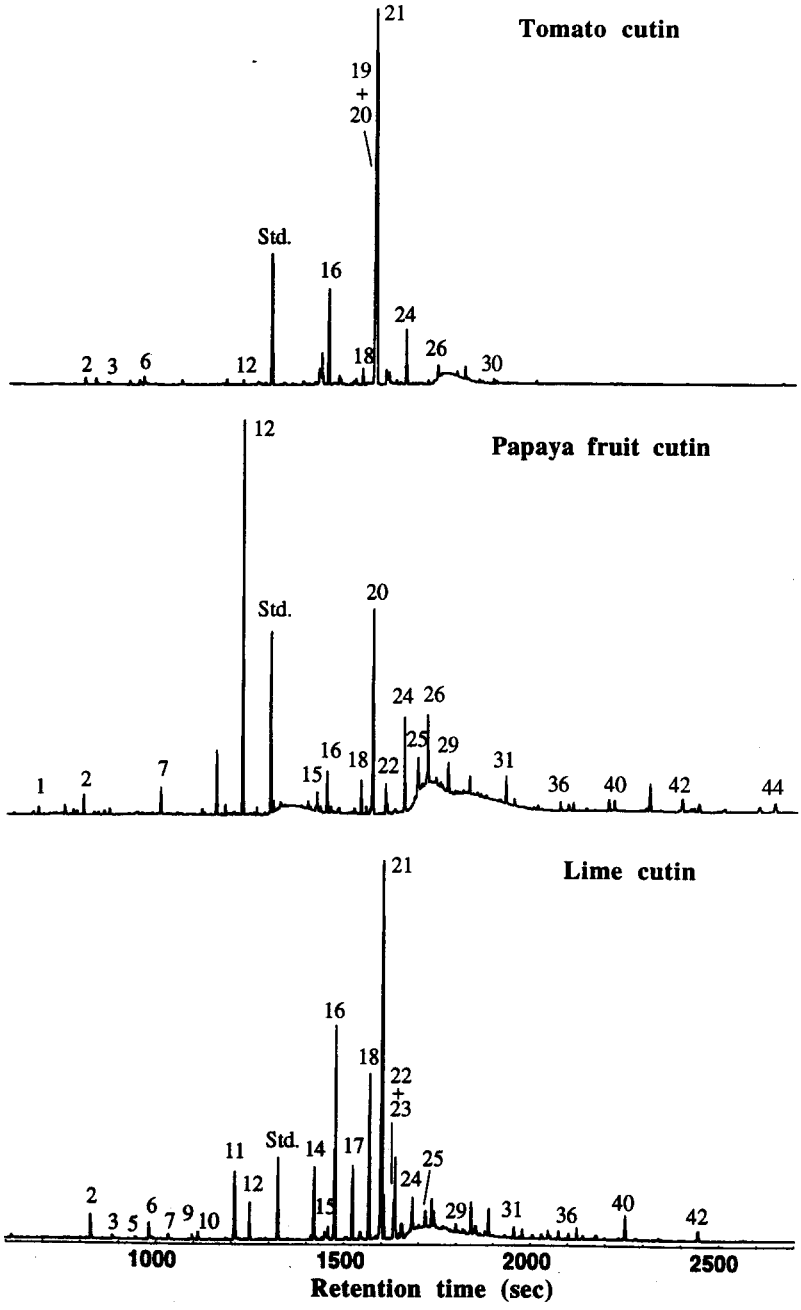

Fig. 1. Gas chromatograms of the TMAH/thermochemolysis products released from the selected samples. The peak numbers correspond to the compounds identified in Tables 1 and 2. Std: internal standard (*n*-eicosane).

oxy methyl ester fragment (II). The relative distribution of the different dihydroxyhexadecanoic acid isomers was calculated using the most intense fragment ions of the individual isomers in the GC–MS, assuming that the sensitivity was the same for each isomer. Table 3 shows the composition of the positional isomers of the methyl derivatives of the dihydroxyhexadecanoic acids in the different samples analyzed, which generally agrees with the relative compositional data published in the literature (Holloway and Deas, 1971; Holloway, 1973; Baker *et al.*, 1982; Espelié *et al.*, 1982; Gérard *et al.*, 1992). Among the different positional isomers of the dihydroxyhexadecanoic acids, the methyl derivative of the 10,16-dihydroxyhexadecanoic acid (**21**) was the most abundant in all the cutins and cuticles analyzed, except in the papaya fruit cutin, in which the methyl derivative of the 9,16-dihydroxyhexadecanoic acid methyl ester (**20**) was present almost exclusively. While in the tomato cutin, the methyl

Table 1. Composition (%) of the TMAH/thermochemolysis products released from the selected samples

Label	Compound	Papaya fruit cutin	Lime fruit cutin	Apple fruit cuticle	Paradise INSC hilum	*Agave americana* cuticle
1	octane-1,8-dioic acid dimethyl ester	0.7	0	3.0	0.6	0
2	nonane-1,9-dioic acid dimethyl ester	1.4	1.3	22.4	1.0	0
3	*cis* 3-(4-methoxyphenyl)-3-propenoic acid methyl ester	0	0.3	0.9	1.4	0
4	3,4-dimethoxybenzenecarboxylic acid methyl ester	0	0	0	0	0
5	decane-1,10-dioic acid dimethyl ester	0	0.3	1.3	0	0
6	*trans* 3-(4-methoxyphenyl)-3-propenoic acid methyl ester	0	1.2	0.8	0	0
7	tetradecanoic acid methyl ester	2.0	0.3	0	0	0.3
8	3,4,5-trimethoxybenzenecarboxylic acid methyl ester	0	0	0	0	0
9	*cis* 3-(3,4-dimethoxyphenyl)-3-propenoic acid methyl ester	0	0.2	0	0	0
10	pentadecanoic acid methyl ester	0	0.4	0	0	0
11	*trans* 3-(3,4-dimethoxyphenyl)-3-propenoic acid methyl ester	0	4.1	0	1.4	0
12	hexadecanoic acid methyl ester	32.6	1.8	3.2	0.4	0.7
13	octadec-9-enoic acid methyl ester	0	0	2.4	0	0
14	10-methoxy-hexadecanoic acid methyl ester	0	3.8	0	0	0
15	octadecanoic acid methyl ester	1.8	0.6	1.6	0	0.8
16	16-methoxy-hexadecanoic acid methyl ester	3.2	12.8	12.1	3.6	0.4
17	16-methoxy-10-oxo-hexadecanoic acid methyl ester	0	3.9	0	0	0
18	hexadecane-1,16-dioic acid dimethyl ester	2.6	9.5	7.3	10.4	0
19/20/21	8,16-, 9,16- and 10,16-dimethoxy-hexadecanoic acid methyl esters	18.6	33.3	9.5	0	6.0
22	eicosanoic acid methyl ester	3.1	2.1	0	0	0.7
23	18-methoxy-octadec-9-enoic acid methyl ester	0	0	9.7	8.5	0
24	mixt. of hydroxy-methoxy hexadecanoic acid methyl ester	7.3	3.7	1.3	0	1.1
25	octadec-9-ene-1,18-dioic acid dimethyl ester	4.0	1.0	3.7	6.9	0
26	9,10,16-trimethoxyhexadecanoic acid methyl ester	5.8	0	4.4	0	2.6
27	dimethoxyoctadecenoic acid methyl ester (tentatively)	0	0	1.5	3.8	0
28	10,18-dimethoxyoctadecanoic acid methyl ester	0	0	0	0	2.1
29	docosanoic acid methyl ester	2.7	0.5	1.3	1.4	0
30	9,10,18-trimethoxy-octadecanoic acid methyl ester	0	0	3.7	0.8	18.8
31	tetracosanoic acid methyl ester	2.2	0.5	0	0	0.9
32	9,10,12,18-tetramethoxyoctadecanoic acid methyl ester	0	0	1.1	0	5.0
33	22-methoxy-docosanoic acid methyl ester	0	0	0	15.7	0
34	hexacosanol methyl ether	0	0	0	0.8	4.6
35	docosane-1,22-dioic acid dimethyl ester	0	0	0	22.2	0
36	hexacosanoic acid methyl ester	0.8	0.5	0	1.2	1.2
37	24-methoxy-tetracosanoic acid methyl ester	0	0	0	6.8	0
38	octacosanol methyl ether	0	0	0	0	15.2
39	tetracosane-1,24-dioic acid dimethyl ester	0	0	0	7.8	0
40	octacosanoic acid methyl ester	1.1	1.6	0	0	3.4
41	triacontanol methyl ether	0	0	0	0	7.1
42	triacontanoic acid methyl ester	2.0	0	0	0	5.9
43	dotriacontanol methyl ether	0	0	0	0	6.6
44	dotriacontanoic acid methyl ester	1.4	0.8	0	0	6.2
	unknowns	6.7	15.5	8.8	5.3	10.4

Table 2. Composition (%) of the products released after TMAH/thermochemolysis of tomato cutin replicates

Label	Compound	Abundance (%)
2	nonane-1,9-dioic acid dimethyl ester	0.4 ± 0.07
3	*cis* 3-(4-methoxyphenyl)-3-propenoic acid methyl ester	0.8 ± 0.06
6	*trans* 3-(4-methoxyphenyl)-3-propenoic acid methyl ester	2.8 ± 0.10
12	hexadecanoic acid methyl ester	0.5 ± 0.06
16	16-methoxy-hexadecanoic acid methyl ester	9.3 ± 1.40
18	hexadecane-1,16-dioic acid dimethyl ester	2.1 ± 0.05
19/20/21	8,16-, 9,16-, 10,16-dimethoxy-hexadecanoic acid methyl ester	72.5 ± 2.45
24	mixture of hydroxy-methoxy hexadecanoic acid methyl ester	5.7 ± 0.30
26	9,10,16-trimethoxyhexadecanoic acid methyl ester	1.0 ± 0.12
30	9,10,18-trimethoxy-octadecanoic acid methyl ester	0.4 ± 0.02
	unknowns	4.5 ± 0.05

derivatives of the dihydroxyhexadecanoic acids account for nearby 70% of the total monomers, in lime cutin they comprise around 33% and papaya fruit cutin around 18%. Minor amounts were found in the cuticles of apple fruit and *Agave americana* (10% and 6% respectively). Some relatively low amounts of hydroxymethoxyhexadecanoic acid methyl ester, corresponding to partially methylated hydroxyl groups, were also identified as products, although in far lower amounts than in pyrolysis/TMAH experiments (de Leeuw and Baas, 1993).

In contrast to dihydroxyhexadecanoic acids, dihydroxyoctadecanoic acids are not common components of cutins. However, the methyl derivative of the 10,18-dihydroxyoctadecanoic acid (**28**) could be identified after TMAH/thermochemolysis of the *Agave americana* cuticle, as also reported by other authors (Matic, 1956). A wide range of positional isomers (9,18-, 11,18- and 12,18-) were found to coelute in the same chromatographic peak, although in minor amounts, as demonstrated by their fragmentation pattern in the mass spectrometer. The main characteristic fragments are *m/z* 187 and 215 (10,18-), *m/z* 201 (9,18-), *m/z* 173 and 229 (11,18-) and *m/z* 159 and 243 (12,18-dimethoxyoctadecanoic acid methyl ester).

Among the trihydroxy fatty acids, the most common are the trihydroxyoctadecanoic acids, in particular the 9,10,18-trihydroxyoctadecanoic acid. Its methyl derivative, the 9,10,18-trimethoxyoctadecanoic acid methyl ester (**30**) was found as an important component of the TMAH/thermochemolysis products of apple fruit and *Agave americana* cuticles. Trihydroxyhexadecanoic acids, in particular the methyl derivative of the 9,10,16-trihydroxyhexadecanoic acid (**26**) was also present in minor amounts in the cutins of tomato fruit and in higher amounts in papaya fruit cutin, apple fruit cuticle and *Agave americana* cuticle. The methyl derivatives of more hydroxylated fatty acids, such as 9,10,12,18-tetramethoxyoctadecanoic acid methyl ester (**32**), were also detected in the cuticles of apple fruit and *Agave americana*.

A series of fatty alcohol methyl ethers and fatty acid methyl esters up to C_{32}, with strong even carbon number predominances and maxima at C_{28}, were also produced from TMAH thermochemolysis of the cuticle of *Agave americana*. These compounds arise from the transesterification of the cuticular waxes.

The suberin sample selected for this study corresponds to the chalazal region of the inner seed coat of grapefruit. This sample yielded primarily the methyl derivatives of the ω-hydroxy fatty acids C_{16}, $C_{18:1}$, C_{22} and C_{24}, and their corresponding dicarboxylic acids. Minor amounts of the methyl derivatives of phenolic compounds (*p*-coumaric acid, **3** and ferulic acid, **9**, **10**) were also detected, as well as minor amounts of 9,10,18-trimethoxyoctadecanoic acid methyl ester (**30**). The distribution of the monomeric constituents is in agreement with previously published work using depolymerization with $LiAlH_4$ and $LiAlD_4$ (Espelié *et al.*, 1980).

It is known that epoxy fatty acids usually occur as major monomers in some cutins (Holloway and Deas, 1973). The use of $LiAlH_4$ reduction or methanolysis shows that large amounts of 9,10-epoxy-18-hydroxy octadecanoic acid occurs among the products of depolymerization of apple fruit and *Agave americana* cuticles (Kolattukudy *et al.*, 1971; Walton and Kolattukudy, 1972; Holloway and Deas, 1973; Espelié *et al.*, 1982). However, no epoxy fatty acids could be identified after TMAH/thermochemolysis. While the method does not affect most monomeric units, the epoxy fatty acids seem to be converted to other compounds. To confirm whether the epoxy group is destroyed during the procedure, an epoxy fatty acid standard, the 9,10-epoxyhexadecanoic acid methyl ester, was subjected to the TMAH/thermochemolysis procedure. It was found that the 9,10-epoxy group is mainly converted to a 9,10-dimethoxy group, with minor amounts of partially methylated hydroxy-fatty acids. Therefore, we would expect a compound such as 18-hydroxy-9,10-epoxyoctadecanoic acid to be mainly converted to the 9,10,18-trimethoxyoctadecanoic acid methyl ester upon treatment with TMAH, and so, the concentration of this compound is likely to reflect the contents of both epoxides and ether-linked components in the aliphatic

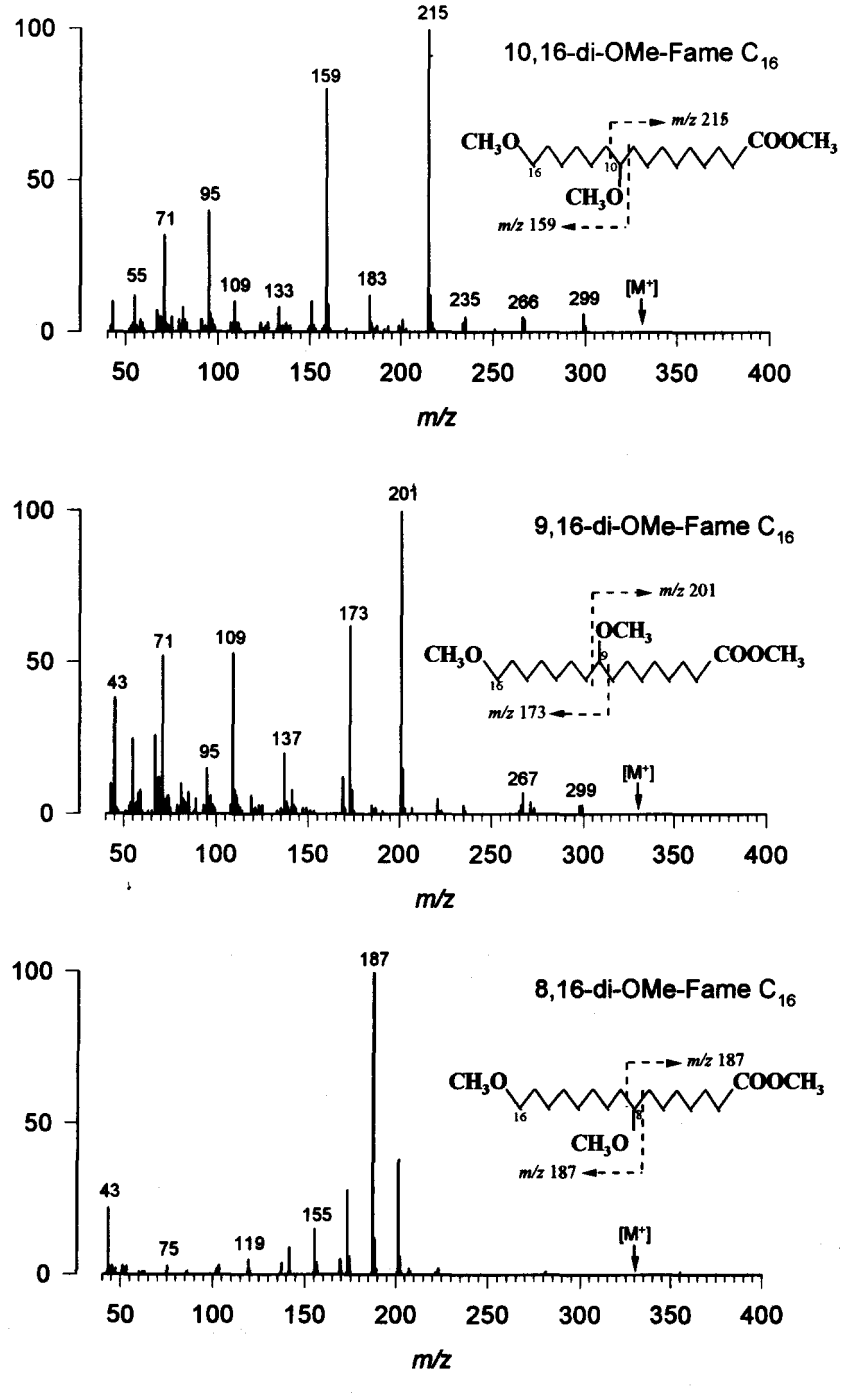

Fig. 2. Structures and mass spectra of the different dimethoxyhexadecanoic acid methyl ester isomers.

Table 3. Composition (%) of the different positional isomers of dimethoxyhexadecanoic acid methyl ester occurring in the different samples

		Papaya fruit cutin	Lime fruit cutin	Apple fruit cuticle	*Agave americana* cuticle	Tomato cutin
19	8,16-dimethoxy-hexadecanoic acid methyl ester	1	3	5	18	6
20	9,16-dimethoxy-hexadecanoic acid methyl ester	98	13	16	10	8
21	10,16-dimethoxy-hexadecanoic acid methyl ester	1	84	79	72	86

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