THE CUTIN ACIDS OF SMOOTH AND RUSSETED "GOLDEN DELICIOUS" APPLES

H. A. M. A. DE VRIES

Botanisch Laboratorium, Universiteit, Nijmegen

SUMMARY

By analyzing the monobasic cutin acids of smooth and russeted "Golden Delicious" apples with gas liquid chromatography, we found that these acids occur in about the same proportions in both kinds of apples. This is also the case for all the cutin acids, after reduction to the parent monobasic acids. From these results, we concluded that there are no great differences between smooth and russeted "Golden Delicious" apples with respect to the polymerized fatty acid material. There is evidence that these parent acids do not occur in the same proportion as the monobasic cutin acids with respect to the chain length.

1. INTRODUCTION

Recent work of SCHWERTFEGER c.s. (1968) disclosed much about russeting on the microscopical level and our results (DE VRIES 1968b) have thrown some light upon this process on the EM level. But, in the literature there are few chemical data of the smooth and russeted apple skins. The apple wax has been analyzed by MAZLIAK (1963), the lipids of the skin by NEUBELLER (1963), and the constituents of the cutin acids of the apple "Cox Orange Pippin" are known from the work of EGLINTON & HUNNEMAN (1968).

The cutin acids are the object of our studies because cutin acids form an important part of the epidermal and cuticle layers where russeting starts. The problem discussed in this paper has two aspects: first, are there differences between the cutin acids of the cuticular (smooth) and suberized (russeted) layers of the apple "Golden Delicious" and second, which are these possible differences? To solve these questions we have compared the cutin acids of smooth apples with two groups of russeted apples. Firstly, a group in which russeting was caused by natural conditions as observed in the orchard and secondly, a group in which it was induced by spraying with copper oxychloride (DE VRIES 1968b). Although we are well aware of the fact that the russeted apples have a cork tissue on the outside, we use in this paper only the designation "cutin acids" which comprises all the fatty acids that can not be removed by boiling in MeOH and $CHCl_3$.

2. MATERIAL AND METHODS

The peelings of smooth and russeted "Golden Delicious" apples in the full-grown stage were refluxed with 0.4% oxalic acid –1.6% ammonium oxalate solution (for 48 hrs, changed every 24 hrs). The waxes were extracted from the

membranes by refluxing with methanol (for 72 hrs, changed every 24 hrs), and the membranes were ground in a Sorvall homogenizer and refluxed for 30 min in $CHCl_3$. After drying the homogenate was hydrolyzed for 20 hrs with 3% KOH in MeOH and the insoluble material was removed by centrifugation. The methanolic solution was evaporated to dryness, acidified, and extracted with ether.

The ether soluble acids were subjected to several procedures:

(a) The acids were methylated with BF_3-methanol reagent (VAN WIJNGAARDEN 1967). The esters were separated by thin layer chromatography (TLC) on Silicagel G in a solvent system of light petroleum (b.p. 40–60)/ether/AcOH (90:10:1). The monobasic acid methyl esters, visualized by exposure to iodine vapors, were scraped off the plate, eluted with ether, and identified by gas liquid chromatography (GLC I). All the other bands were scraped off the plate, eluted with ether, evaporated to dryness and reduced to the saturated parent esters following the method of MATIC (1956). After TLC as described above the resulting monobasic acid methyl esters were identified by GLC (II).

(b) The acids were reduced to the parent acids (MATIC 1956), methylated, and chromatographed as described above. The monobasic methyl esters were again identified by GLC (III).

(c) The fatty acids were methylated and made into the TMSi ethers (EGLINTON & HUNNEMAN 1968) and chromatographed by GLC (IV).

The procedure is shown schematically in *fig. 1*.

GLC was done on a Carlo Erba Fractovap Model C, series 200 AID/2f, using a 1.50 m × 4 mm stainless steel column packed with 10% Apiezon L on chromosorb W 100/120 mesh (column 1). Because many of the peaks were not completely resolved, we also separated the samples on a 0.80 m × 3 mm (column 2) and a 2.00 m × 3 mm (column 3) glass column, both packed with 12% HIEFF 2AP on Chromosorb W (AW) 100/120 mesh on a Becker type 1452/SH chromatograph. Both gas chromatographs were operating with a flame ionization detector. The columns tested for 1300 (column 1 at 240°), 1445 (column 2 at 170°), and 2320 (column 3 at 175°) theoretical plates with methyl palmitate. The nitrogen flow rate at the end of the column was 27 (column 1), 110 (column 2), and 70 (column 3) ml/min. Peak measurement was done by area estimation (peak height × width at half height, PRIMAVESI *c.s.* 1967), and there was good agreement between column 2 and 3. The maximum deviation in the calculated figures is about 10%. But to show traces of material, it is necessary to inject the same sample in a high concentration (cf. *fig. 2* and *3*). The percentages of these traces are too low (<1%) to involve them in the calculation. Therefore, we indicated them with +.

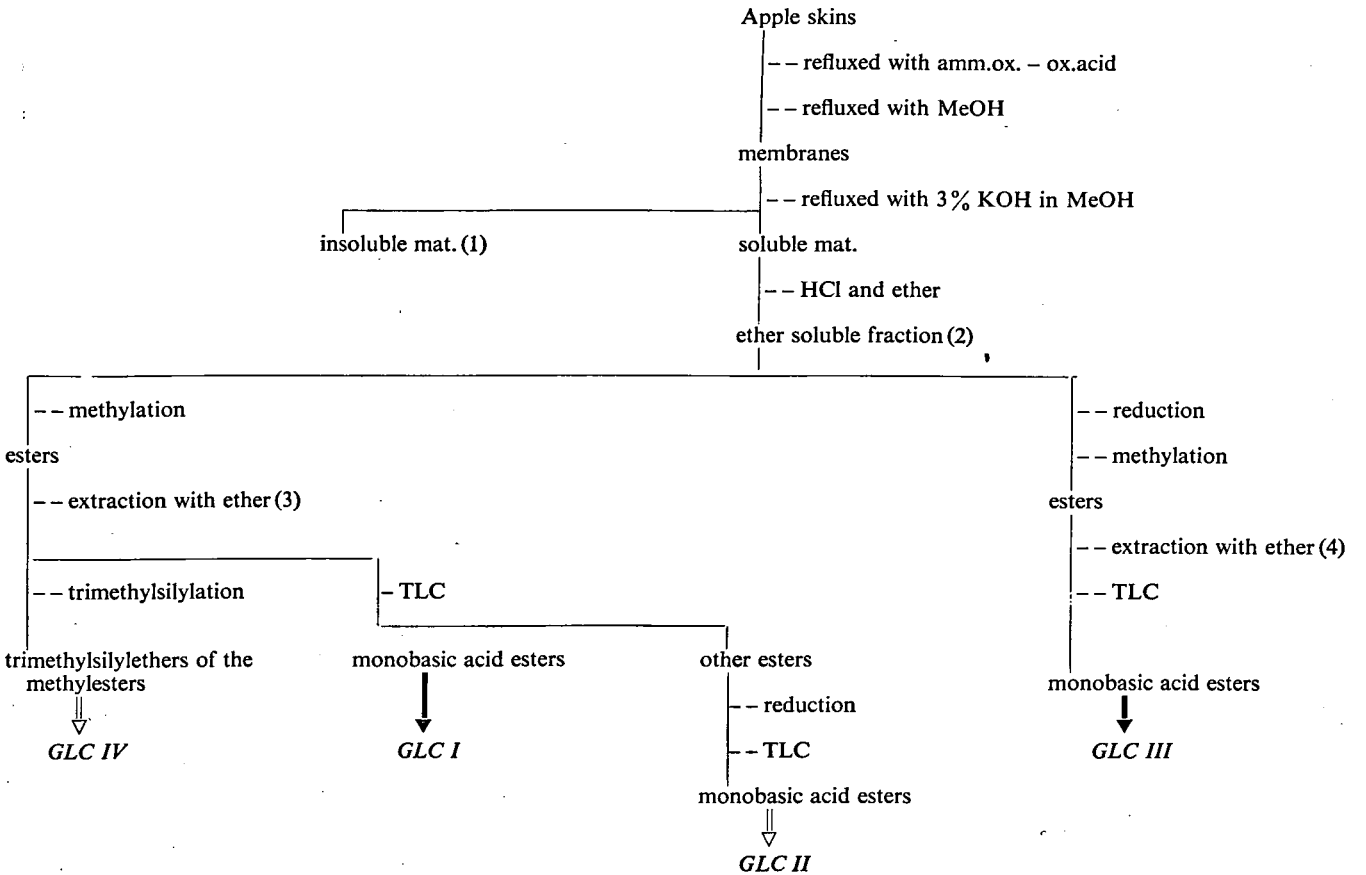

Fig. 1. Procedure to fractionate the monobasic acids for separation by GLC.
(1) the membranes before saponification (100%) contain 20% insoluble material in smooth and 30% in russeted apples.
(2) 60% of the smooth and 55% of the russeted skin is ether soluble.
(3) extraction with heptane or hexane as described by VAN WIJNGAARDEN (1968) renders only one quarter of the material compared with the extraction with ether. The reason for this low figure is the polarity of the esters of the hydroxy acids.
(4) extraction with heptane or hexane gives nine tenths of the material compared with the extraction with ether for which the majority of the hydroxy acids are reduced to saturated fatty acids.

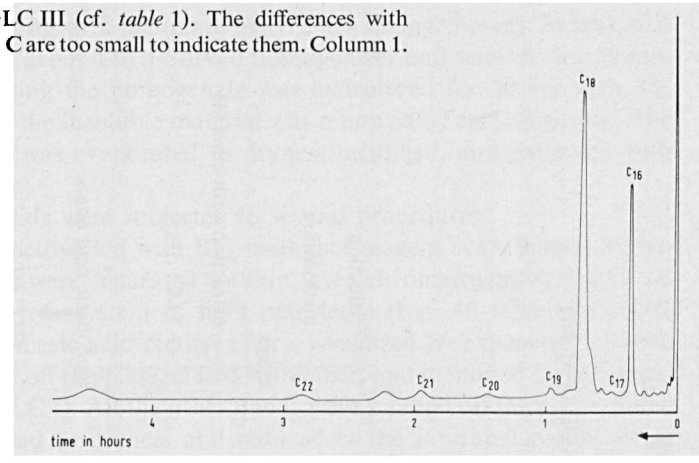

Fig. 2. Group B – GLC III (cf. *table* 1). The differences with Group A and C are too small to indicate them. Column 1.

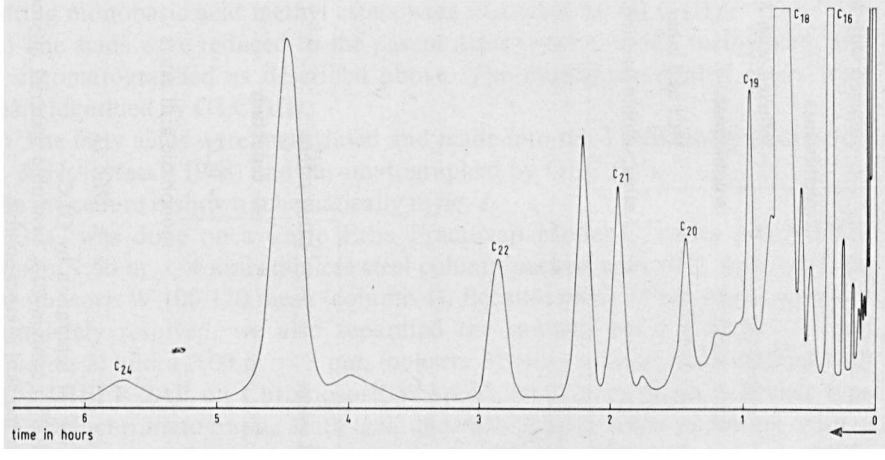

Fig. 3. As *fig.* 2; the same sample injected in a high concentration.

3. RESULTS

We have compared the cutin acids of full-grown smooth apples (Group A) with two groups of russeted apples of the same age. In the first group, russeting came about naturally (Group B) and in the second group (Group C), it was induced by spraying after anthesis with 3% copper oxychloride (DE VRIES 1968b). The degree of russeting for Group B was 65 and for Group C was 80, according to the derivation of the formula of Townsend & Heuberger (KREMER 1967).

3.1. TLC of the cutin acid methyl esters

By using the solvent system light petroleum (b.p. 40–60)/diethyl ether/AcOH (90:10:1) a better separation between the monobasic esters and the rest is achieved than with the solvent system used by EGLINTON & HUNNEMAN (1968)

though the latter gives a separation of all the fractions. No differences in separation are found for the three Groups of cutin esters using these solvents.

3.2. GLC of the monobasic cutin methyl esters on Apiezon L

The constituent acids obtained as methyl esters following the procedure shown in *fig. 1* are shown in *table 1.*

Table 1. Cutin acid distribution in percentages of smooth (Group A) and two groups of russeted apples (Group B & C), identified by three GLC procedures (See *fig.* 1). Separation on 10% Apiezon L.

	C number	GLC I			GLC II			GLC III		
		A	B	C	A	B	C	A	B	C
Methyl decanoate up to Methyl palmitate	10–16	+	+	+	+	+	+	+	+	+
Methyl palmitate	16	14	15	17	18	14	17	24	19	19
Methyl heptadecanoate	17	+	+	+	1	1	1	+	+	+
Unidentified		–	–	–	3	3	3	1	+	1
Methyl oleate + linoleate	18: 1–2	37	39	43	–	–	–	–	–	–
Methyl stearate	18	10	8	10	32	31	38	53	53	53
Unidentified		+	+	+	6	7	5	+	+	+
Unidentified		3	1	1	–	–	–	–	–	–
Methyl nonadecanoate	19	4	3	2	8	7	8	3	2	2
Unidentified		9	6	4	2	2	3	+	+	+
Unidentified		+	1	1	3	2	2	–	–	–
Methyl eicosanoate	20	7	6	6	+	1	1	+	1	1
Unidentified		7	+	+	+	+	+	+	+	+
Unidentified		–	–	–	1	2	2	+	+	+
Methyl heneicosanoate	21	–	–	–	8	10	9	3	3	4
Unidentified		–	–	–	6	4	3	4	5	5
Unidentified		3	2	+	–	–	–	–	–	–
Methyl docosanoate	22	2	15	12	+	+	1	+	3	2
Methyl tricosanoate	23	–	–	–	2	3	+	+	+	+
Unidentified		–	–	–	8	11	6	8	10	11
Methyl tetracosanoate	24	–	–	–	–	–	–	–	+	+
		96	96	96	98	98	99	96	96	98

From GLC I it is clear that there is little difference between the fatty acids in smooth and russeted apples with the exception of the C22 content and the occurrence of an unidentified peak after C 20. The resemblance is even better in GLC II and III (GLC III is shown in *figs. 2* and *3*). One can see that there are no qualitative and only slight quantitative differences between the three groups of apples. The C 16/C 18 ratio's of the Groups A, B and C are 0.32, 0.32, 0.30 (GLC I), and 0.40, 0.36, 0.37 (GLC III) respectively.

3.3. GLC of the monobasic cutin acid methyl esters on HIEFF-2AP

In GLC I (*table 2*, and *fig. 4*) differences can be seen in the C 18 acids between the three Groups A, B, and C; but the C 16/C 18 ratio is about the same: 0.32, 0.32 and 0.30 respectively. In GLC III, however, there is again great resemblance; the C 16/C 18 ratio is in this case 0.40, 0.36 and 0.37 respectively.

Table 2. Cutin acid distribution in percentages of smooth (Group A) and two groups of russeted apples (Group B & C), identified by three GLC procedures (see *fig.* 1). Separation on 12% HIEFF 2AP.

	C number	GLC I			GLC II			GLC III		
		A	B	C	A	B	C	A	B	C
Methyl decanoate up to Methyl palmitate	10–16	+	+	+	+	+	+	+	+	+
Methyl palmitate	16	22	16	17	23	22	20	24	22	22
Unidentified		+	+	+	1	1	2	1	+	+
Methyl heptadecanoate	17	+	+	+	+	+	+	+	+	+
Unidentified		1	1	+	2	1	1	+	1	+
Methyl stearate	18	15	8	7	37	39	45	60	61	59
Unidentified		–	–	–	4	6	6	4	3	4
Methyl oleate	18:1	29	22	28	–	–	–	–	–	–
Unidentified		–	–	–	6	7	6	1	2	1
Methyl linoleate + Methyl nonadecanoate	18:2+ 19	23	18	20	1	+	+	+	+	–
Unidentified		+	2	1	–	–	–	–	–	–
Methyl linolenate	18:3	2	2	2	–	–	–	–	–	–
Methyl eicosanoate	20	3	7	6	3	2	2	+	1	1
Methyl eicosenoate	20:1	1	3	2	–	–	–	–	–	–
Unidentified		2	2	+	6	6	6	2	2	2
Methyl docosanoate + Unidentified	22	1	17	16	+	+	+	2	2	2
Unidentified		–	–	–	+	5	–	–	–	–
Methyl tricosanoate	23	–	–	–	10	4	7	2	2	3
Unidentified		–	–	–	6	5	2	3	3	5
		99	98	99	99	98	97	99	99	99

3.4. GLC of the TMSi-ethers

The crude cutin acid methyl ester fraction is trimethylsilylated (EGLINTON & HUNNEMAN 1968) and the resulting ethers are introduced into the gas chromatograph without previous separation by preparative TLC (GLC IV). Here, also, a good resemblance is found between the smooth and russeted groups on Apiezon L.

4. DISCUSSION

The basic components of cutin from smooth apple skins of "Golden Delicious" are chemically closely related to the suberin of the russeted tissue. Although, the EM structure of the cuticles is different from that of the suberized wall (DE

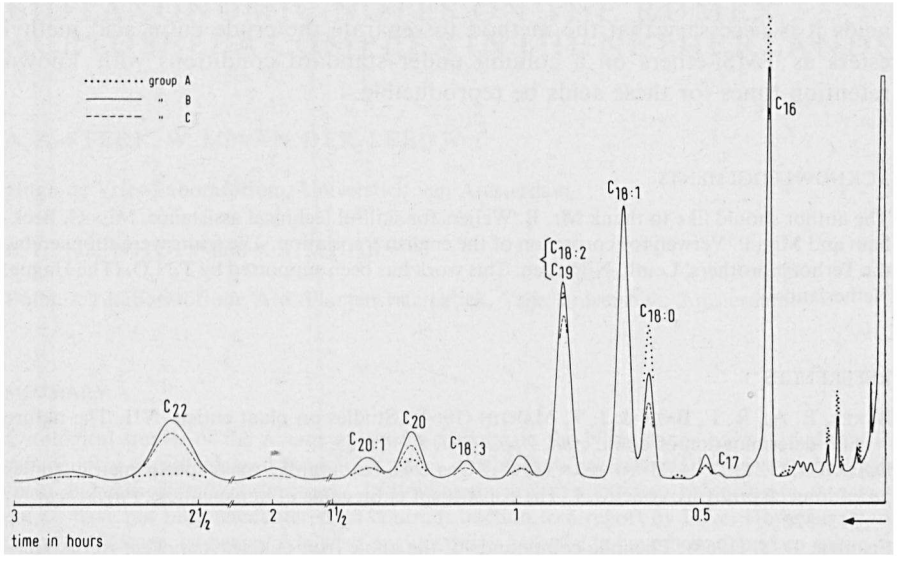

Fig. 4. Group B – GLC I. The differences with Group A (. . .) and with Group C (- - -) are indicated. Column 3.

VRIES 1968) the fatty acid components are very similar. This leads to the conclusion that these components are not responsible for the structure but possibly are accompanying molecules. In this context attention may be given to a suggestion by HUELIN (1959) that carbohydrates and other compounds form part of cutin. Also, BAKER *c.s.* (1964) have observed that membranes containing cutin were attacked much more rapidly by 3% ethanolic potassium hydroxide after previous extraction of pigmented organic material by dilute aqueous alkali. This organic material contains phenolic components according to FISCHER (1956).

EGLINTON & HUNNEMAN have found that in cutin the ω-hydroxybasic acids occur in approximately the same proportion as the individual unsubstituted monobasic acids of the same chain length and saturation. From our results it appears that the fatty acids reduced to their parent acids (GLC III) do not occur in the same proportion as the original monobasic acids (GLC I). However, in both cases, the majority of free fatty acids have a chain length of 16 and 18 C atoms. The fact that there are differences in the proportions in which the various chain lengths of the esters are found may be explained by the different procedures used to obtain GLC II and GLC III.

The differences in percentages obtained by the Apiezon and HIEFF columns can not be explained by us at this moment. But since we used identical samples on both columns we assume that these differences are due to differences inherent to the columns.

For a rapid chemical examination of different types of cuticles, the procedure described seems rather complicated. But, due to the lack of authentic hydroxy-

acids it is necessary that the method to separate the crude cutin acid methyl esters as TMSi-ethers on a column under standard conditions with known retention times for these acids be reproducible.

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