RAPID EXAMINATION OF CUTIN ACIDS BY GAS LIQUID CHROMATOGRAPHY

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SUMMARY

Application of a simple and rapid method for separation of cutin acids, by gas liquid chromatography, if carried out on the methyl esters of their trimethylsilyl derivatives has been found possible without previous, more complicated, separation by thin layer chromatography. For comparative research one chromatogram renders sufficient information. The resemblance between the cutin acids from smooth and from russeted apple skin of "Golden Delicious" has been established again. However, some interesting differences occur between the cutin acid pattern of smooth tissue and tissue scarred by mechanical injury.

1. INTRODUCTION

For our research on the phytopathology of the apple skin a rapid and reproducible method was required for gaschromatographical analysis of the cutin acids. Since the cutin acids consist largely of hydroxy acids, it is necessary to prepare derivatives with relatively short retention times. Therefore the cutin acids were methylated with diazomethane and subsequently trimethylsilylated. The latter chemical reaction has been described by VANDENHEUVEL (1967) and it has been used both for analysis of the hydroxy fatty acids from animal tissue (CAPELLA *c.s.* 1968) and from plant tissue, particularly from the cuticle of the apple (EGLINTON & HUNNEMAN 1968).

2. MATERIAL AND METHODS

The ether soluble cutin acids of "Golden Delicious" apples were obtained as previously described (DE VRIES 1969). The cutin acids were subjected to several procedures:

a. The cutin acids were methylated with diazomethane and separated by thin layer chromatography (TLC). All the bands were scraped off the plate with the exception of the one on the starting point, eluted with ether, trimethylsilylated according to EGLINTON & HUNNEMAN (1968) and identified by gas liquid chromatography (GLC) (*cf. fig. 1*; GLC 1).

b. The acids were separated by TLC. All the bands were scraped off the plate again with the exception of the one on the starting point, eluted with ether, methylated and trimethylsilylated as described under (a) (*cf. fig. 1*; GLC 2).

c. The acids were methylated, trimethylsilylated and separated by GLC without previous TLC (*cf. fig. 1*; GLC 3).

GLC analyses were carried out using a Becker gaschromatograph type 1452/

SH equipped with a temperature programming unit of our own make, using a 1.90 m × 3.5 mm coiled stainless steel column, packed with 3% SE-30 on Chromosorb W (NAW) 100/120 mesh. This column tested for 6200 theoretical plates with methyl palmitate at 210°, if the temperature is programmed at 4.5°/min. from 125–210°. The nitrogen flow rate at the end of the column was 25 ml/min at 210°. Amounts of material present were determined by area measurement (cutting out and weighing the peaks).

For comparison the commercially available higher monobasic acids and samples of the following authentic acids, sent to us by Professor Brieskorn, have been used: hexadecane-1, 16-dioic; 9, 10-dihydroxyoctadecane-1, 18-dioic; 9, 10-dihydroxyoctadecanoic; and a mixture of 9, 10, 18-trihydroxy octadecanoic and 10, 16-dihydroxyhexadecanoic acid. We also compared our chromatograms with those of EGLINTON & HUNNEMAN (1968).

3. RESULTS

3.1. Separation of the cutin acid derivatives by GLC

The different procedures to fractionate cutin acids of full-grown smooth "Golden Delicious" have been compared by GLC as described in *fig. 1*. The chromatograms are shown in *fig. 2*. The different acids have been identified by use of the authentic standards, and by means of the results obtained in an experiment carried out according to EGLINTON & HUNNEMAN (1968). In this experiment we injected the six bands resulting from methylation and TLC separatedly. When the six groups of cutin acids were compared with the same examined by EGLINTON & HUNNEMAN (1968) only slightly different hRF values were found e.g. 85, 77, 64, 50, 40 and 26 in case the lenght of run was 10 cm and 78, 67, 55, 45, 35 and 20 in case the lenght of run was 15 cm.

Comparison of the three GLC procedures reveals that there are no differences

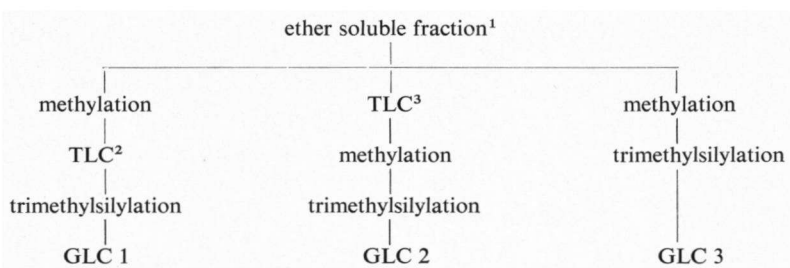

Fig. 1. Procedure to fractionate the ether soluble fraction for separation by GLC.
(1) see DE VRIES 1969, p. 591.
(2) separation by TLC, Silicagel G, diethyl ether/n-hexane/methanol (40/10/1, v/v) *cf.* EGLINTON & HUNNEMAN (1968).
(3) as (2), but with solvent system: $CHCl_3$/methanol/acetic acid (43/3/1, v/v).

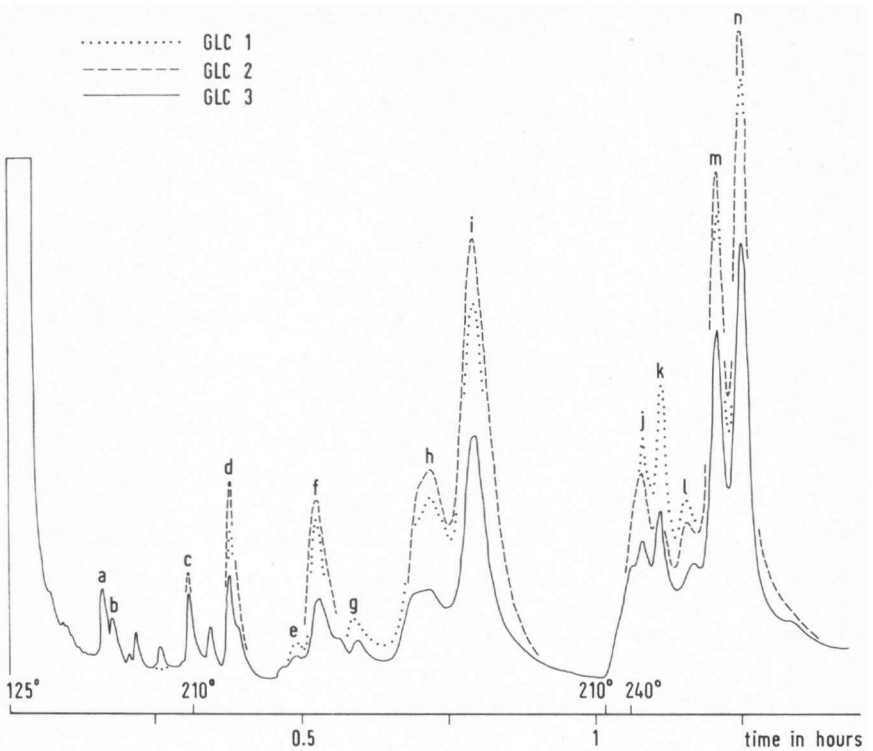

Fig. 2. GLC 1, 2 and 3 of the cutin acids as methylesters and TMSi ethers; temperature successively programmed at 4.5°/min from 125–210°, isothermal at 210° during 42 min, and programmed at 12°/min from 210–240°. The six groups of cutin acids are represented by the following peaks: monobasic acids, c (C 16) and d (C 18); dibasic acids, b, (d) and e; ω-monohydroxymonobasic acids, f (C 16), g and h (C 18); vic-dihydroxydibasic acids, a, j, k and l; dihydroxymonobasic acids, i (10,16-dihydroxyhexadecanoic); trihydroxymonobasic acids, m and n (both are 9, 10, 18 trihydroxyoctadecanoic acids). The differences with GLC 1(...) and GLC 2 (---) are indicated.

between GLC 1 and GLC 3, not even after accurate area measurements. The differences of both GLC 1 and GLC 3 with GLC 2 are mainly confined to the dihydroxydibasic acids. GLC 3 is most suitable for comparative research, because this procedure is much faster, since previous separation by TLC of the cutin esters before analyzing the esters as their trimethylsilyl (TMSi) ethers is not necessary, and since the material which stays behind on the starting point, when using GLC 1, also stays behind on the column when using GLC 3.

3.2. Comparison of the cutin acids of smooth and russeted skins of "Golden Delicious" by means of GLC 3

Fig. 3 shows the chromatograms of the cutin acids of smooth skin (Group A), of chemically russeted skin induced by copper oxychloride (Group C), and of russeted material induced by mechanical wounding (Group D). Group B, con-

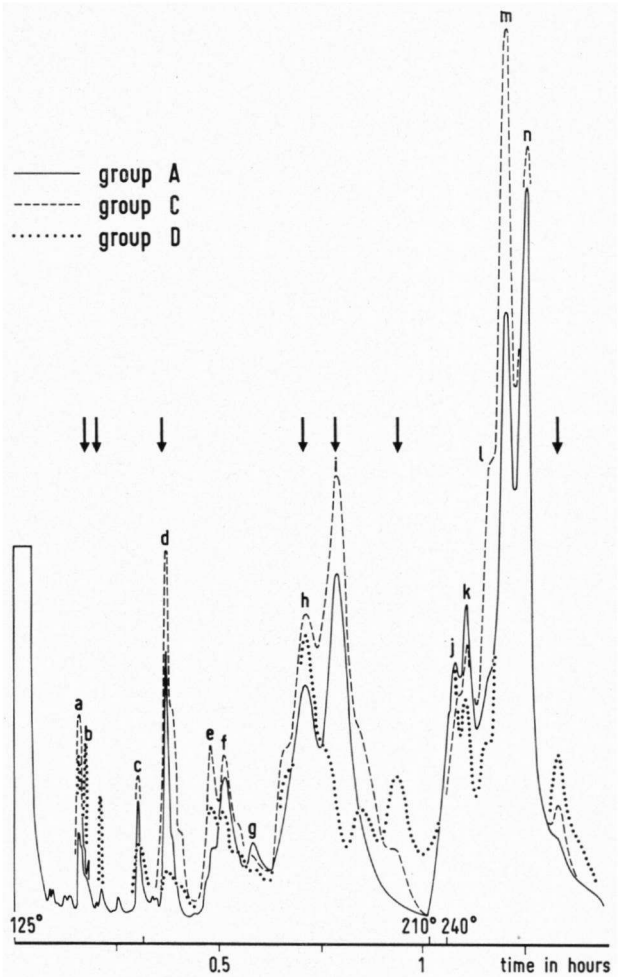

Fig. 3. The cutin acids of smooth skin (Group A), chemically russeted tissue (Group C), scar tissue (Group D). The differences between Group A and C, and Group D are indicated with arrows. Peak a–d, e–i, and j–n in Group A respectively; 9%, 35%, 56%; in Group C: 10%, 39%, 52%; and in Group D: 9% 31%, 60%.

sisting of naturally russeted material, was not analyzed because of the resemblance with Group C (DE VRIES 1969).

The differences between Group A and C are small. However, there are quantitative differences between Group A and C on the one and Group D on the other hand. Remarkable is the very low concentration of dihydroxymonobasic acids (peak i). Other differences have been indicated by arrows in the figure.

4. DISCUSSION

Since our previous paper (DE VRIES 1969) we have improved the separation of the crude cutin acid methyl esters by separating them as their TMSi ethers in GLC. This method proved to be rapid and reproducible in identifying the acids on the chromatograms. There is good agreement between our results and the work of EGLINTON & HUNNEMAN (1968) except for the higher concentration of trihydroxymonobasic acids. This may be due to the fact that EGLINTON & HUNNEMAN used a different apple-variety.

The differences between the cutin acids of smooth and russeted skins of "Golden Delicious" apples are not qualitative but quantitative only. Skin on which russeting was induced by mechanical wounding (Group D) contains a higher percentage of monohydroxymonobasic as well as dihydroxydibasic and trihydroxymonobasic acids than normal skin (group A). Chemically this means two respectively four functional groups in the molecules. Consequently the cutin acids of Group D have about the same chance to form polycondensates.

ACKNOWLEDGEMENTS

The author wishes to thank Professor Dr. C. H. Brieskorn, Universität Würzburg, for the samples of the authentic hydroxy acids, Mr. B. Weijers for skillful technical assistance and Miss P. Verweij for translating the manuscript. This work has been supported by T.N.O. (The Hague, Netherlands).

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