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ANALYSIS OF QUINIC ACID ESTERS OF HYDROXYCINNAMIC ACIDS IN PLANT MATERIAL BY CAPILLARY GAS CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Capillary gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods for separation and determination of *cis* and *trans* isomers of nine caffeoyl-, *p*-coumaroyl- and feruloylquinic acids in plant extracts are presented and compared. GC analysis requires highly deactivated SE-30 capillaries because of the sensitivity of the silylated compounds and the high temperatures involved. The production of these capillaries is described. HPLC analysis is performed with an RP-18 column and a gradient elution with 2% acetic acid–methanol. Both methods allow the separation of the *trans* isomers and most of the *cis* isomers. For the various samples, quantitative results of GC and HPLC separation agree well.

INTRODUCTION

Quinic acid esters of hydroxycinnamic acids[1] are of interest in plant physiology and food chemistry because of their ubiquitous occurrence in plants. The most common compounds of the class are esters of caffeic acid, especially chlorogenic acid and neochlorogenic acid. They are often found together with *p*-coumaric acid and ferulic acid esters. In contrast, sinapic acid esters are rare, except in a few plant families like Brassicaceae. Table I lists the investigated substances. The old and not the IUAPAC nomenclature has been chosen to allow a simple comparison to former investigations.

Because of the chemical similarity of the different cinnamoylquinic acids and their possible isomers, high separation efficiency is needed to resolve all compounds. For each acid there are three positional isomers each having two *cis–trans* isomers. Capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been shown to be the appropriate methods. So far, GC[2–5] and HPLC[6,7] have only been used to determine some of the hydroxycinnamoylquinic acids. Moreover, none of these studies considered *cis* isomers, which are formed by brief exposure to UV light during sample preparation and storage.

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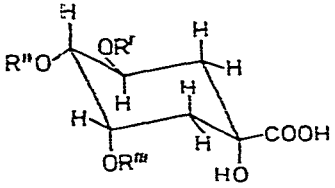
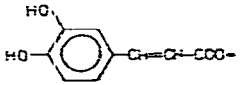
TABLE I

INVESTIGATED CINNAMOYL QUINIC ACIDS

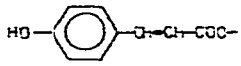
Quinic acid



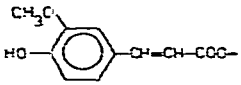
R_1 = Caffeic acid



R_2 = *p*-Coumaric acid



R_3 = Ferulic acid



Compound	*R'*	*R''*	*R'''*
3-Caffeoylquinic acid (chlorogenic acid)	R_1	H	H
4-Caffeoylquinic acid (cryptochlorogenic acid)	H	R_1	H
5-Caffeoylquinic acid (neochlorogenic acid)	H	H	R_1
3-*p*-Coumaroylquinic acid	R_2	H	H
4-*p*-Coumaroylquinic acid	H	R_2	H
5-*p*-Coumaroylquinic acid	H	H	R_2
3-Feruloylquinic acid	R_3	H	H
4-Feruloylquinic acid	H	R_3	H
5-Feruloylquinic acid	H	H	R_3

EXPERIMENTAL

Reference samples

Only chlorogenic acid is commercially available (Roth, Karlsruhe, G.F.R.). By heating chlorogenic acid with a buffer of pH 7 its isomerization yielded the other caffeoylquinic acids[8]. The *p*-coumaroylquinic acids were isolated from unripe apples by paper chromatography, as were the feruloylquinic acids from green Robusta coffee. They were isomerized in the same way as chlorogenic acid. All compounds were affirmed by mass and NMR spectroscopy.

Sample extraction and prepurification

A 100-g amount of fruit material was twice extracted with 1 l of 80% methanol for 30 min in an atmosphere of nitrogen at room temperature. The combined extracts were concentrated to about 350 ml in a rotary vacuum evaporator at less than 40°C and then purified in a preliminary way by polyamide column chromatography.

About 50 g of polycaprolactam powder (MN-Polyamid-SC-6, 0.05–0.16 mm; Macherey, Nagel & Co, Düren, G.F.R.) were suspended in methanol–water (1:1), packed into a 250 × 35 mm I.D. tube, first washed with 1 l of methanol–formic acid (995:5) and then with 1 l of water. The aqueous extract was added to the polyamide column, which then was rinsed with 800 ml of water followed by 800 ml of methanol. The hydroxycinnamoylquinic acids were eluted with 800 ml of methanol–formic acid

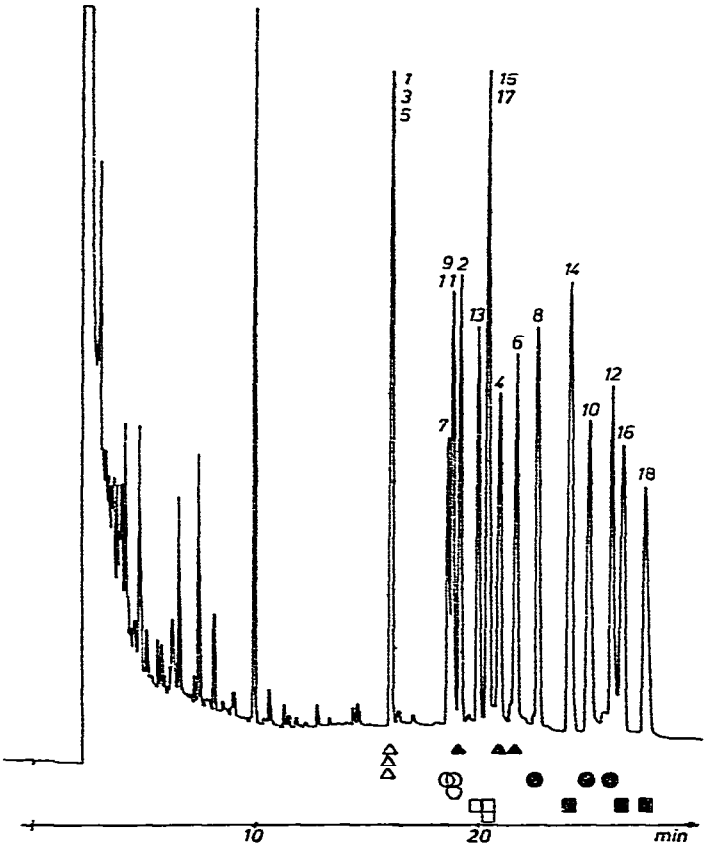


Fig. 1. Gas chromatogram of hydroxycinnamoylquinic acids (trimethylsilyl derivatives). Column: SE-30 (wall-coated open tubular column, 37 m × 0.27 mm I.D.), temperature 220–270°C at 4°C/min. Injector and detector temperatures: 300°C. Carrier gas: nitrogen, 0.9 ml/min. Attenuation: 1 × 32. Splitting ratio: 1:20. Chart speed: 40 cm/h. Quinic acids: 1 = *cis*-3-*p*-coumaroyl; 2 = *trans*-3-*p*-coumaroyl; 3 = *cis*-4-*p*-coumaroyl; 4 = *trans*-4-*p*-coumaroyl; 5 = *cis*-5-*p*-coumaroyl; 6 = *trans*-5-*p*-coumaroyl; 7 = *cis*-3-feruloyl; 8 = *trans*-3-feruloyl; 9 = *cis*-4-feruloyl; 10 = *trans*-4-feruloyl; 11 = *cis*-5-feruloyl; 12 = *trans*-5-feruloyl; 13 = *cis*-3-caffeoyl; 14 = *trans*-3-caffeoyl; 15 = *cis*-4-caffeoyl; 16 = *trans*-4-caffeoyl; 17 = *cis*-5-caffeoyl; 18 = *trans*-5-caffeoyl; △, *cis*-, ▲, *trans*-*p*-coumaroyl; ○, *cis*-, ●, *trans*-feruloyl; □, *cis*-, ■, *trans*-caffeoyl.

(995:5). The eluate was concentrated to 50 ml at 40°C under vacuum. This served as a stock solution for analysis by GC and HPLC.

Capillary GC

GC analysis was performed with a Carlo Erba 2150 gas chromatograph equipped with a Grob splitter, a glass capillary and a flame ionization detector. Borosilicate glass capillaries (0.27 mm I.D., 0.8 mm O.D.) were leached, flushed and dehydrated according to Grob *et al.*[9]. A simple and excellent deactivation is achieved by silylation with N,O-bis(trimethylsilyl)acetamide (BSA). Nine tenths of the capillary were filled with BSA and the ends were sealed in a flame. The capillary was kept at 110°C for 20 h, then flushed with toluene and pentane (2 ml of each for a 50-m capillary) and dried at 100°C in a nitrogen flow for about 30 min. The capillary was coated by the static method[10] using a 0.2% solution of the coating material in pentane. 3 m at both ends were discarded.

Derivatization of the samples. A 0.5-mg amount of (+)-catechin (Roth) was added as an internal standard to 2–10 ml of the stock solution depending on its concentration, and the sample was evaporated to dryness. Derivatization was performed with 1 ml of BSA–trimethylchlorosilane (TMCS) (20:1) at 70°C for 1 h.

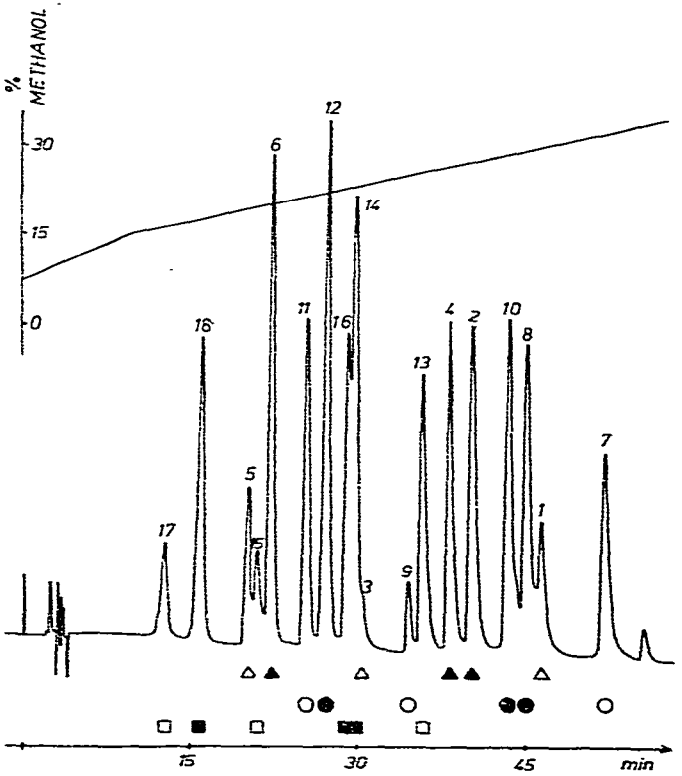


Fig. 2. High-performance liquid chromatogram of the hydroxycinnamoylquinic acids. Column: LiChrosorb RP-18. Detection: 320 nm. Flow-rate: 0.8 ml/min. Gradient elution: solvent A = 2% acetic acid, B = methanol; from 7% B to 15% B in 10 min, then from 15% B to 35% B in 50 min. Peaks as in Fig. 1.

Quantitative analysis of the chromatograms was done with a Hewlett-Packard 3390 A integrator. Each compound was calibrated with chlorogenic acid. With an injection volume of 1 μl and splitting ratio of 1:20, the detection limit for each substance is 10 ng, that means 1 ppm for the original concentration in the fruit.

HPLC

HPLC analysis was performed with a Pye Unicam HPLC chromatograph (LC XPD pump, LC XP gradient programmer, LC UV detector) equipped with a Rheodyne 7125 injection valve (10 μl) and a reversed-phase C_{18} column (LiChrosorb RP-18, 5 μm, 250 × 4 mm I.D.; E. Merck, Darmstadt, G.F.R.). The stock solutions can be used without modification although in some cases dilution was useful.

For quantitative analysis the HP 3390 A integrator was used. Calibration was done with the free acids (caffeic, *p*-coumaric and ferulic acid) and their mass concentrations were calculated with respect to their molecular weights.

RESULTS AND DISCUSSION

Prepurification

Polyamide columns are generally used to isolate phenolic compounds from plant extracts. Because of their carboxyl group in addition to the phenolic structure the hydroxycinnamoylquinic acids are strongly adsorbed by polyamide. On one hand this fact permits their separation from other phenolics like hydroxycinnamoylglucose and most of the flavonoids. On the other it poses the problem of desorption without modification or decomposition.

The usual method of desorption by an alkaline solution cannot be applied because of the formation of positional isomers and rapid oxidation, especially of caffeic acid esters. We have tried an elution with methanol–0.05 *N* HCl (9:1), but when concentrating the eluate we observed that the increase in concentration of the acid caused a methylation.

Both problems are avoided by eluting with methanol–formic acid (995:5). Using this method no modification of any compound was found. This was shown in the case of chlorogenic acid with a recovery of 98%.

For GC analysis this preliminary purification is absolutely necessary because otherwise perturbating substances would be silylated in a similar way. It is also important for HPLC because it simplifies the chromatograms, which means an increase in reliability.

Derivatization

When performing the derivatization for GC by use of pure BSA, a permutation of positional isomers takes place in some cases. Probably this is due to the alkaline nature of BSA and is avoided by the addition of the strongly acidic TMCS.

Capillary GC

As silyl derivatives of hydroxycinnamoylquinic acids are very sensitive to contact with incompletely deactivated surfaces, a high degree of inertness even at high temperatures was required. Most commercially available capillaries did not provide either the required inertness or the separation efficiency, especially after longer periods

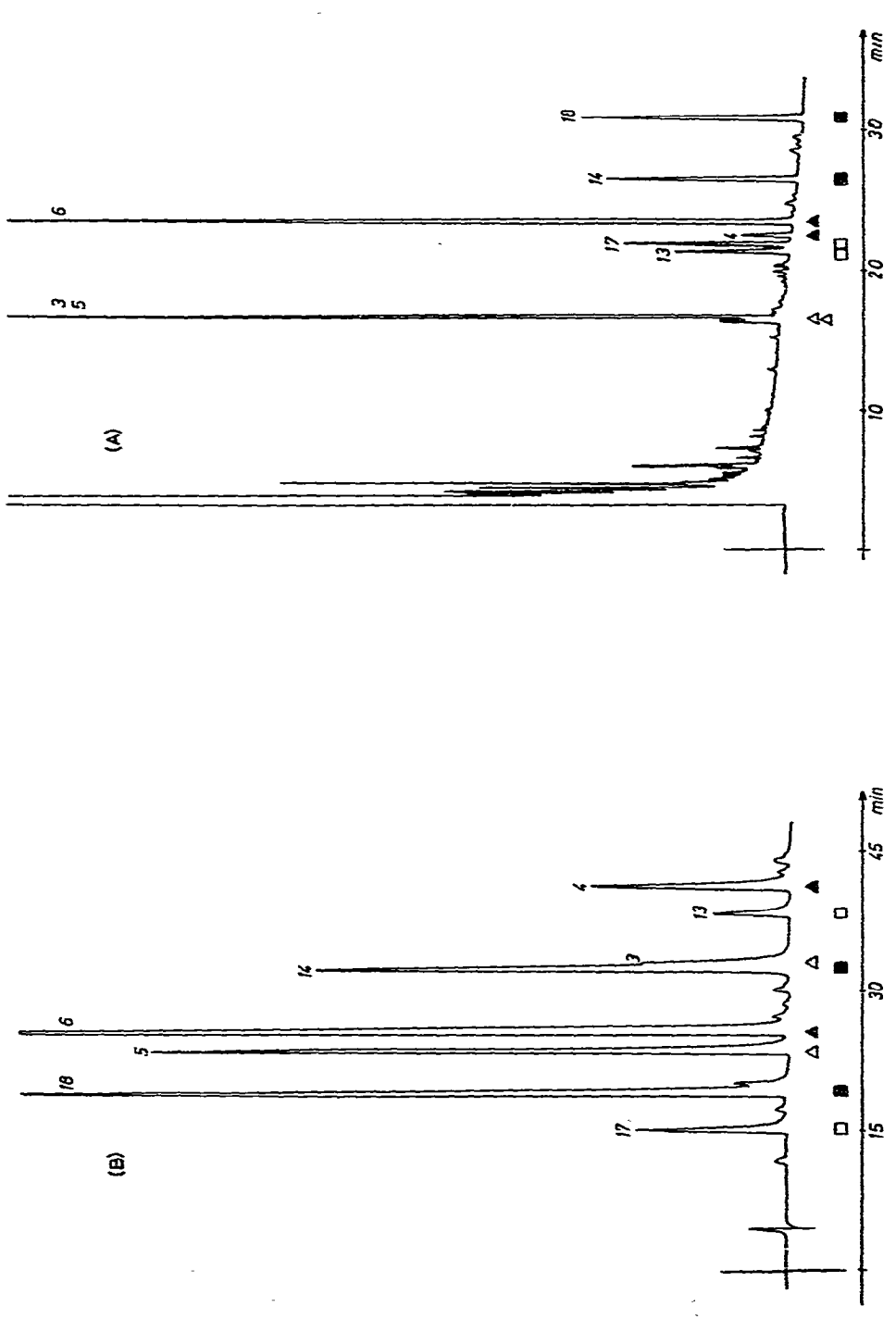


Fig. 3. GC (A) and HPLC (B) separation of an extract of the morello cherry "Schattenmorelle Bockelmann". Peaks and conditions as in Figs. 1 and 2.

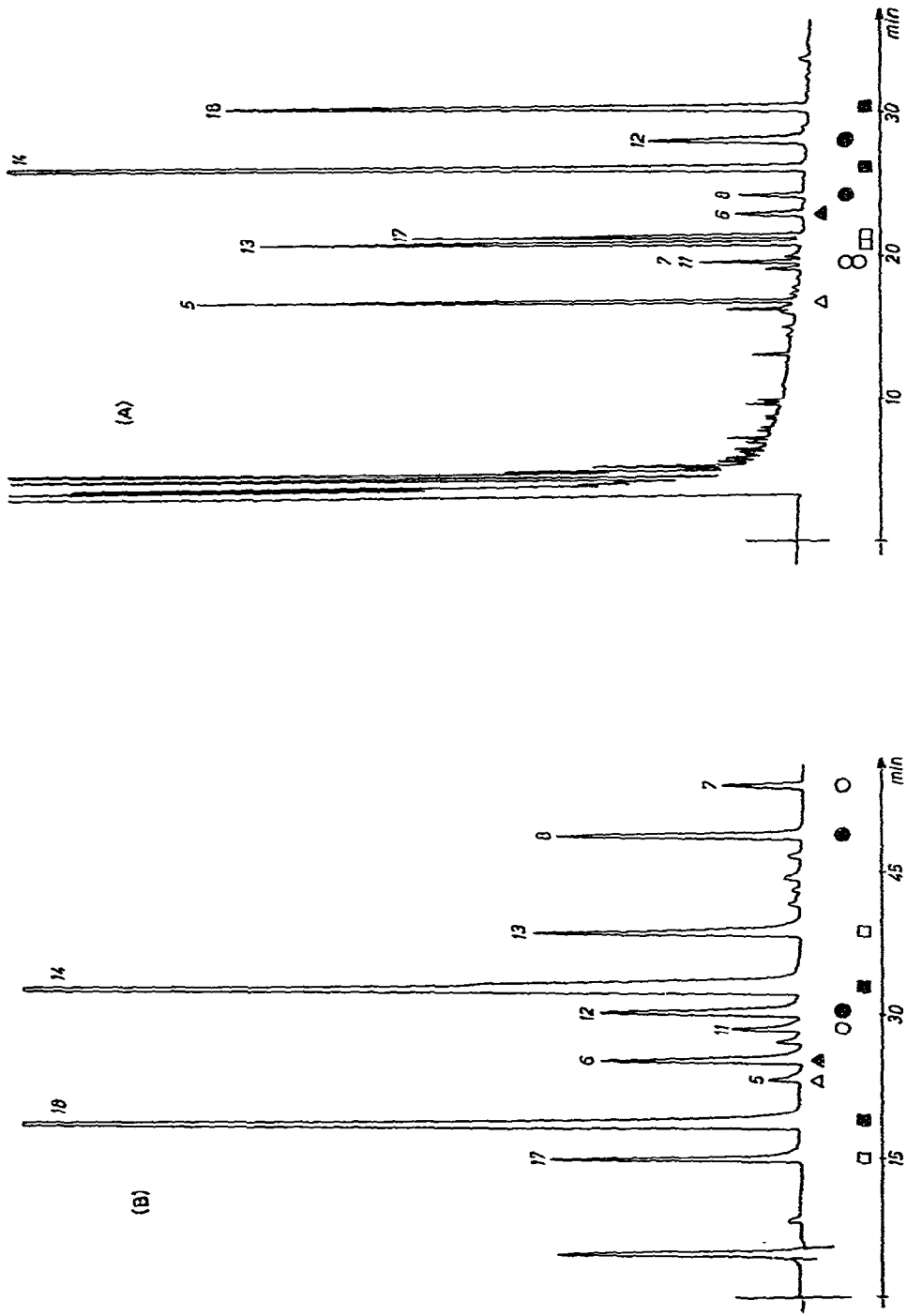


Fig. 4. GC (A) and HPLC (B) separation of an extract of the apricot "Mombacher Frühe". Peaks and conditions as in Figs. 1 and 2.

of use. Therefore a procedure has been developed which guaranties suitable capillaries of constant quality. These capillaries ($\approx$40 m) had a separation number according to Kaiser[11] (determined with undecane and dodecane at 90°C) between 35 and 45 and their quality could be maintained for more than half a year.

A comparison of the different stationary phases SE-30, SE-52, Dexsil 300 and 400 revealed that the less polar phase SE-30 showed the best separation. For all hydroxycinnamoylquinic acids and for all stationary phases the three *cis* isomers were eluted before the *trans* isomers and the positional isomers in the sequence 3-, 4-, 5-.

HPLC

In contrast to GC, HPLC does not require derivatization nor specially prepared columns.

On RP-18 the positional isomers were eluted contrary to GC in the sequence 5-, 4-, 3- when using a gradient elution with 2% acetic acid and methanol. The *cis* and *trans* isomers are grouped together. Surprisingly the sequence of the *cis* and the *trans* isomers differs for each positional isomer. For all hydroxycinnamoylquinic acids the *cis* isomers are eluted first for the 4- and 5-isomers, while the *trans* isomers are eluted first for the 3-isomers.

Quantitation

For both GC and HPLC, calibration could not be performed by the reference samples since sufficient amounts of pure substances were not available. Therefore in GC all compounds are calibrated with chlorogenic acid. It is assumed that the response of the compounds varies only slightly, because the structures and the molecular weights are very similar. In HPLC the free hydroxycinnamic acids in *trans* form have been used for calibration because the UV-absorption is believed to be the same for the free acids and the esters. Between chlorogenic acid and caffeic acid a difference of 4% was found. The results were corrected neither for this difference nor for the possible difference of the extinction of the *cis* and *trans* isomers.

Both chromatographic techniques have been applied to 20 different fruit samples and the results from the two methods agreed within 10%. This value is the maximum deviation that has been observed and there seems to be no systematic difference. The agreement is evidence that the assumptions made for calibration are reasonable and that no important effects have been negelected. The concentrations of the hydroxycinnamoylquinic acids found in pome and stone fruit, will be reported elsewhere.

HPLC may be preferred because there is no danger of decomposition and because it is easier to apply. On the other hand GC allows coupling with a mass spectrometer which provides a quick and reliable identification.

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REFERENCES

1 K. Herrmann, *Prog. Chem. Organ. Nat. Prod.*, 35 (1978) 73.
2 J. L. Wilson, W. J. Dunlap and S. H. Wender, *J. Chromatogr.*, 35 (1968) 329.
3 J. T. Kung, W. S. Ryder and J. R. Feldman, *Assoc. Sci. Int. Cafe*, (1968) 223.
4 A. Fleuriet and J. J. Macheix, *J. Chromatogr.*, 74 (1972) 339.
5 E. Bombardelli, B. Gabetta and E. M. Martinelli, *Fitoterapia*, 48 (1977) 143.
6 J. Krause and D. Strack, *J. Chromatogr.*, 176 (1979) 465.
7 L. Nagels, W. van Dongen, J. de Brucker and H. de Pooter, *J. Chromatogr.*, 187 (1980) 181.
8 M. L. Scarpati and P. Esposito, *Ann. Chim. (Rome)*, 54 (1964) 56.
9 K. Grob, G. Grob and K. Grob, Jr., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 31.
10 J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.
11 R. Kaiser, *Chromatographie in der Gasphase, II Kapillar-Chromatographie, Dünnfilm- and Dünnschichtkapillar-GC*, Bibliographisches Institut, Mannheim, Wien, Zürich, 1975.