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Note

Qualitative and quantitative estimation by thin-layer and gas chromatography of a series of C_{17} oxygenated aliphatic compounds in the avocado (*Persea americana*)

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It is well known that immature avocado flesh has an unplesant bitter-type flavour, which, while not being particularly intense, nevertheless leaves a distinctive, prolonged after-taste on the palate. In a recent study[1,2] Zutano, a pear shaped Mexican type of strong, unpleasant flesh flavour was used, and it was found that at least three acetylenic members of a series of ten C_{17} olefinic and acetylenic oxygenated aliphatic compounds (Fig. 1) recently isolated from the avocado[3–6] contribute to an unpleasant flavour in immature avocado flesh. The acetylenic compounds Ib, IIIb and IVb were found to be the principal unpleasant flavour compounds. Recently, one of the ten compounds (1,2,4-trihydroxy-*n*-heptadeca-16-ene) was found to be an active antibacterial agent[7], and another (1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene) was identified as a plant growth inhibitor[8]. This group of ten compounds constitutes an important factor in the consumer acceptability of certain avocado varieties.

Extraction procedures for isolation of eight colourless crystalline components and two oily components have been described previously[2,5]. The ten components occurred in pairs of which the pairs II and V were major components, pairs I and IV, which had an unpleasant flavour, were present in moderate amounts, and the pair III was a minor constituent. One member of each pair had a vinyl linkage, the other a terminal acetylenic bond. The compounds are shown in order of polarity on thin-layer chromatography (TLC) (silica gel H plates, hexane–acetone (2:1)) from the olefinic compound Ia (R_F *ca.* 0.80) to the acetylenic compound Vb (R_F *ca.* 0.15).

Similar heat-induced off-flavour components are reportedly produced in the processing of ripened avocado flesh[9]. In order to use these compounds in studying the maturation and ripening of the avocado, and because of their suspected significance in the processing of ripened flesh, it was necessary to develop a reliable, accurate and rapid method for their qualitative and quantitative estimation.

The close chemical similarity of the ten compounds (Fig. 1) made it difficult to achieve a relatively simple chemical means of estimating the three acetylenic flavour compounds separately from the other seven compounds. Gas chromatographic (GC) and densitometric methods appeared to be the best means of obtaining a rapid accurate estimation of each of the ten compounds.

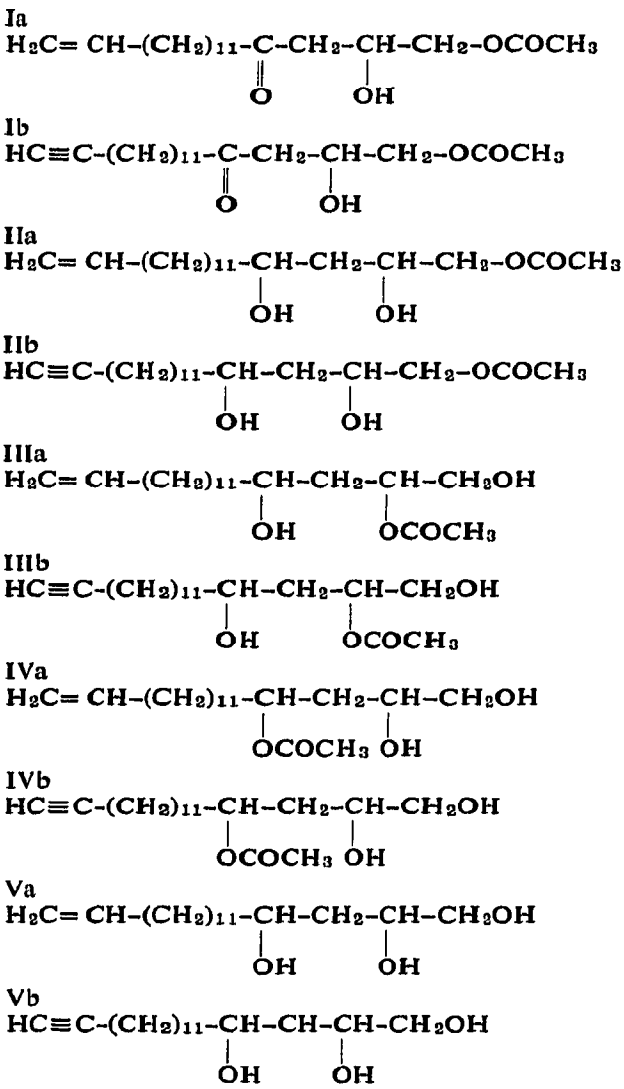

Fig. 1. Oxygenated aliphatic compounds from avocado.

MATERIALS AND METHODS

Gas chromatography

An Aerograph Model A-600-B instrument with a flame ionization detector, and fitted with a 1.8-m stainless-steel column (3.18 mm diameter) was used. The column (3% OV-17 on Chromosorb W, AW, HP, 80–100 mesh) was operated isothermally at 220°. Nitrogen was used as a carrier gas. A standard injector block fitted with a glass injector port was maintained at 240°. All samples were silylated by reacting for 60 min at 20° with N-trimethylsilyldimethylamine[10].

Qualitative estimations of the compounds were determined by retention times and by reference to pure compounds. Standard graphs (peak height × retention time *vs.* sample weight (mg)) were determined for quantitative estimations.

Thin-layer chromatography

For qualitative estimations, glass plates (20 cm × 5 cm) were coated with silica gel H (approximate thickness 0.7 mm) and dried at 100° for 1 h. Samples (*ca.* 10 mg) in acetone were spotted onto the cooled plates which were then developed for 15 min in hexane–acetone (2:1) in closed lined jars at 20°. The developed plates were dried at ambient temperature for 10 min and visualised by concentrated sulphuric acid spray and heating at 150° for 20 min.

Densitometric estimation of compounds

Qualitative and quantitative estimations using densitometric graphs, were obtained from TLC plates (20 cm × 5 cm) which were prepared as above, using silica gel H (approximate thickness 0.7 mm) except that the sample was applied either as a band of medium intensity or quantitatively as a spot, so that optical density readings were in the range 0.10–0.25 O.D. An Elphon single white light source (18W) densitometer was used, with a 1-cm slit. Plates were developed in hexane–acetone solvents (2:1, 1.5:1, 1:1) and visualised.

RESULTS AND DISCUSSION

Gas chromatographic estimation

All samples were silylated before injection. The silylated derivatives were considerably less polar than the parent compounds, and could be readily separated from minor reaction products by elution through Florisil with 3% acetone–hexane (3:97).

A column packed with 3% OV-17 stationary phase was used and a good separation of a mixture of 6 pure compounds was achieved (Fig. 2). Each peak was characterised by comparison with a single pure compound. The problem of the overlap of the peaks for the compounds Ia and IIb could be overcome by an initial separation on 5% $AgNO_3$–silica gel[2,4] of the olefinic and acetylenic components in a crude sample. The acetylenic flavour compound Ib, of longest retention time, could be readily estimated by this method. In the case of the flavour compounds IIIb and IVb, by using pure IVb and the purified oily IIIb (which, because of transesterification[2,5], contained some IIb and IVb as well), characteristic peaks of short retention time could be obtained, and these were compared with Vb.

By preparative TLC, crude extracts from Zutano seed and flesh were treated on 5% $AgNO_3$–silica gel H plates[2] to obtain an olefinic and acetylenic mixture of the five compounds under investigation from each extract. After silylation and GC, each of the olefinic and acetylenic compounds in the pairs I to V could be readily estimated in a crude extract by this method.

To test further the usefulness of the method, standard mixtures by weight of compounds IIb and IIa, and compounds Vb and Va were prepared and silylated. The ratios by weight compared well with mean ratios (±2.5% about a mean of 3 values) determined from peak areas, and satisfactory standard graphs of concentration *vs.* peak height × retention time were obtained for the compounds Ib, IIb and Vb (Fig. 3).

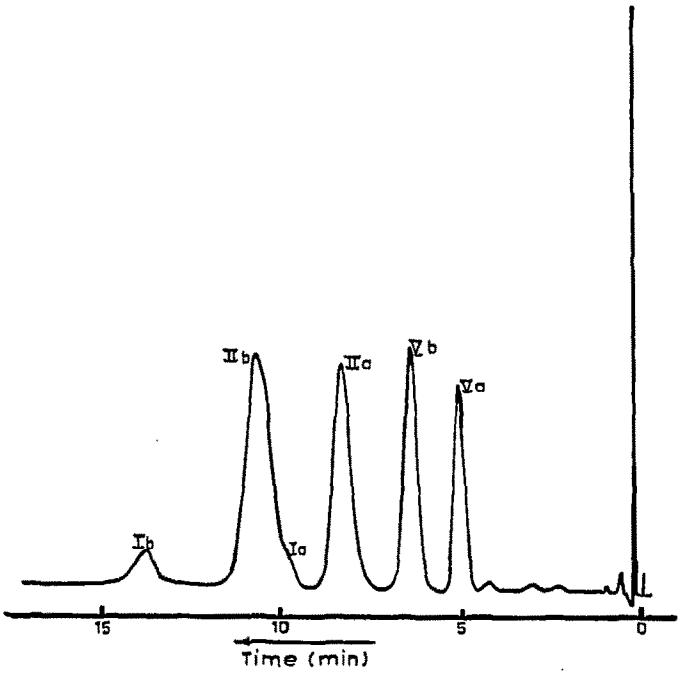

Fig. 2. GC separation of a mixture of 6 silylated compounds. Column: 3% OV-17 on Chromosorb W, AW-DMCS, HP.

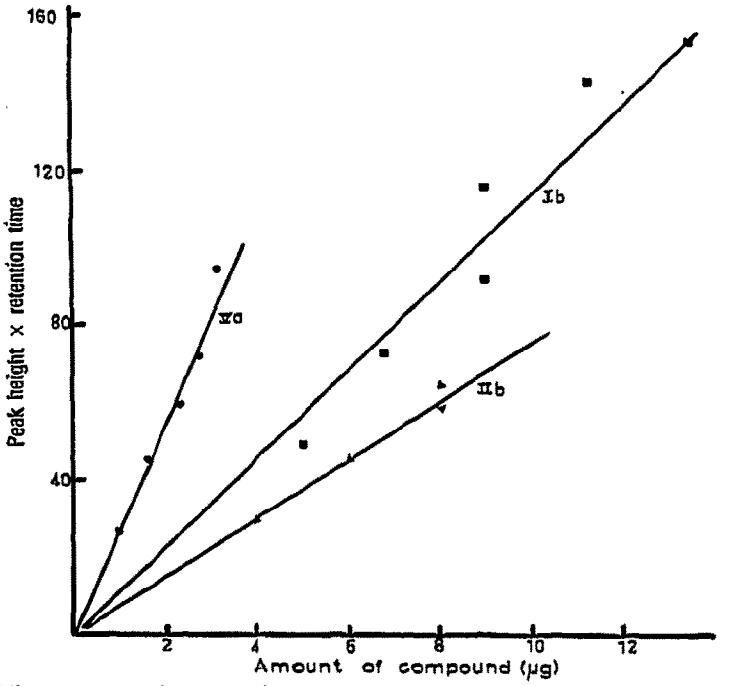

Fig. 3. Quantitation of compounds by GC.

Therefore a reliable qualitative and quantitative estimation of compounds in the pairs I to V could be made by this technique.

Densitometric estimation of compounds

Qualitatively, the ten compounds had the following R_F values on TLC: Ia, 0.80; Ib, 0.75; IIa, 0.55; IIb, 0.50; IIIa, 0.48; IIIb, 0.43; IVa, 0.42; IVb, 0.35; Ia, 0.20; and Vb, 0.15. In order to improve this separation, various other techniques were investigated[11,12], however, it was subsequently determined that silica gel H plates gave the best overall separation and intensity of compact spots, and these plates were used in the densitometric estimations.

Using the Elphon densitometer, it was found that satisfactory results (±5% about a mean of 6 plates of medium light transmittance 0.2–0.7 O.D.) were obtained directly on the dry silica gel H plates, provided the plates were carefully prepared. Standard mixtures by weight compared well with mean area ratios from 6 plates for compounds IIb and IIa, and good separation of densitometric peaks was obtained from a mixture containing Ia: IIb: Vb (1.00:1.03:1.09) (Fig. 4).

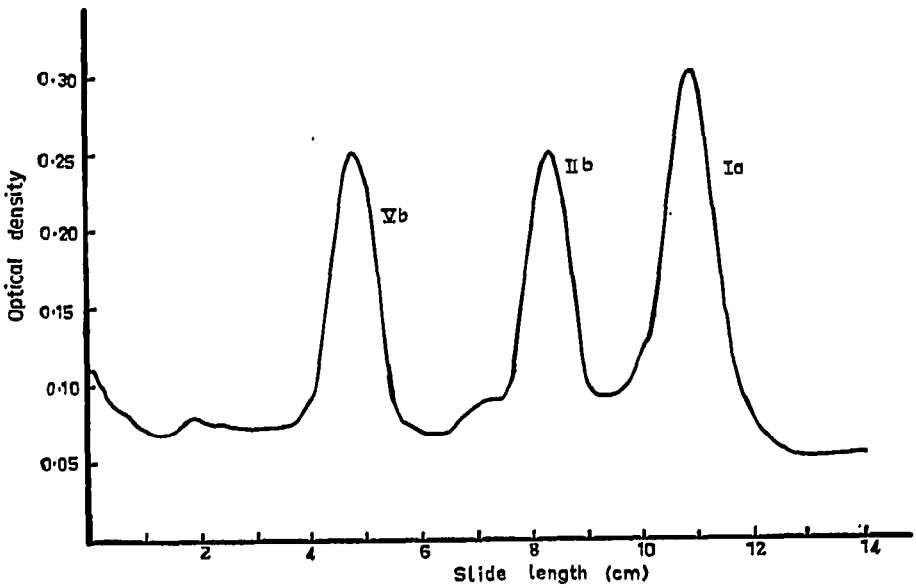

Fig. 4. Densitometric determination of a standard mixture of three compounds.

A linear relationship was found (Figs. 3 and 5) in the low range of concentration (*i.e.* up to ca 12 μg) and it is thought that this might be a characteristic of the sulphuric acid charring reaction. It has been reported[13] that many factors such as optimum wavelength for optical density measurement, and application and concentration of the spot, might all contribute to inconsistencies in the densitometric technique. By using single spots of up to medium intensity, a curvilinear relationship between concentration of a compound and optical density, was determined for concentrations from 12 to 45 μg for Ib, IIb and Vb (Figure 5), and it was evident that only the low range of concentration should be used quantitatively.

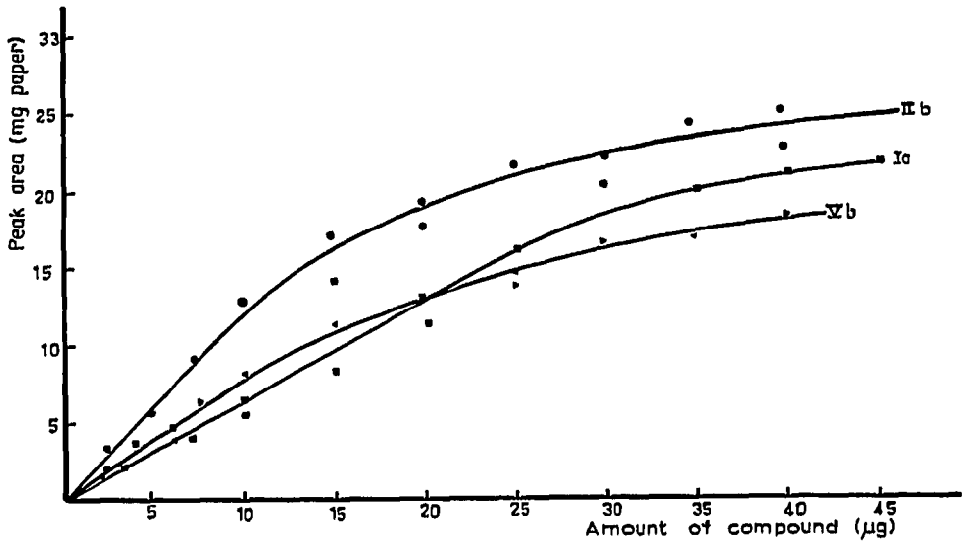

Fig. 5. Quantitation of compounds by densitometry.

Purified olefinic and acetylenic mixtures, obtained by preparative TLC from crude seed and flesh extracts of Zutano, Fuerte, Ryan, Rincon and Edranol, were prepared for densitometric examination on TLC plates[2], and good separation of the five densitometric peaks of interest was obtained.

Therefore taking densitometric measurements in the range 0.10–0.25 O.D., qualitative and quantitative results could be obtained on the pairs I to V by this technique.

CONCLUSIONS

Rapid, accurate, qualitative and quantitative estimation of the ten C_{17} oxygenated aliphatic compounds studied, was possible by either the GC or the densitometric method described above. The GC method is preferred, although quantitatively it would be desirable to duplicate injections to obtain a peak from a particular sample. The densitometric method was accurate enough for routine analysis, but would require up to 6 TLC replications of a particular sample in order to obtain a high degree of accuracy. A more elaborate densitometer than the Elphon used in this study would also assist in reducing errors in densitometric measurements.

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