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MEASUREMENT OF THE CHANGES IN THE COMPOSITION OF DEVELOPING UNDETACHED GRAPE BERRIES BY USING ^{13}C NMR TECHNIQUES*

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Key Word Index—*Vitis vinifera*; Vitidaceae; grape; fruit development; ^{13}C NMR; sugars; acids.

Abstract—A non-destructive method for detecting changes in composition during the development of unpicked grape berries has been developed. The levels of malate, tartrate, glucose and fructose have been followed by measuring the ^{13}C NMR resonances of these constituents during berry development. The changes correlate well with known changes in berry deformability.

INTRODUCTION

Attempts to measure the developmental changes in plant organs are complicated by variability between samples; non-destructive methods of analysis present one way out of this difficulty but are generally not applicable. In the case of the grape (*Vitis vinifera* L.) berry some progress was made by relating berry deformability with the composition of 2 μl juice samples withdrawn hypodermically every 2 days [1]. Such an approach is open to the criticism that the frequent injury creates abnormalities in softening and/or composition. We have, therefore, attempted to apply the approaches used on single-celled organisms and animal organs where changes in water-soluble compounds have been monitored by NMR studies [2–5]. ^{13}C NMR measurements on dried 'Sultana' berries rehydrated with D_2O indicated the feasibility of the method [A. Marker, unpublished observations].

RESULTS AND DISCUSSION

Figure 1 illustrates the natural abundance ^{13}C NMR spectra of a single attached grape berry both before (a) and after (b) softening. The peaks in these spectra can be ascribed to four compounds; D(+)-glucose, D(−)-fructose, L(+)-tartrate and L(−)-malate. Other peaks, which are unassigned, are thought to account for less than 2% of the total observable ^{13}C signal. Under constant data acquisition conditions the intensities of these peaks can be related to the amount of solute present in the berry. The limit of sensitivity was judged to be *ca* 5 μmol of an individual solute in berries of *ca* 300 mg fr. wt.

The results in Fig. 2, from berries of two cultivars, are in accord with the types of changes in berry composition during these stages of development which have been found previously by other techniques [6]. The relative constancy of tartrate per berry has been shown by Hale [7]. The results show that the rates of increase of glucose and fructose and of decrease in malate, were faster after berry softening than before. This is in agreement with the findings from sequential sampling of juice. However,

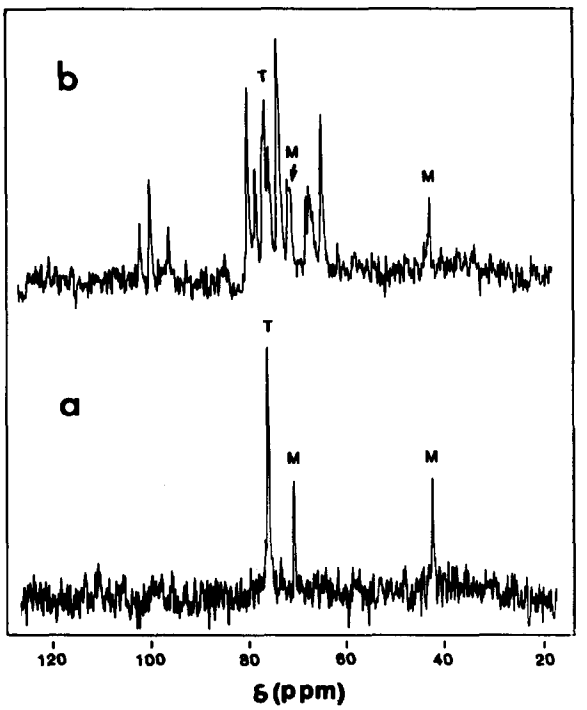

Fig. 1. Natural abundance ^{13}C NMR spectra of a single grape berry attached to its vine (a) before softening and (b) after softening. Chemical shifts [δ values (ppm)] were referenced against an external capillary containing sodium trimethylsilyl propionate. Peaks marked M and T are assigned L(−)-malate and L(+)-tartrate, respectively. All other prominent peaks in spectrum (b) can be ascribed to D(+)-glucose and D(−)-fructose.

*Part 4 in the series "Development of the Grape Berry". For Parts 3 and 2 see refs. [1] and [8].

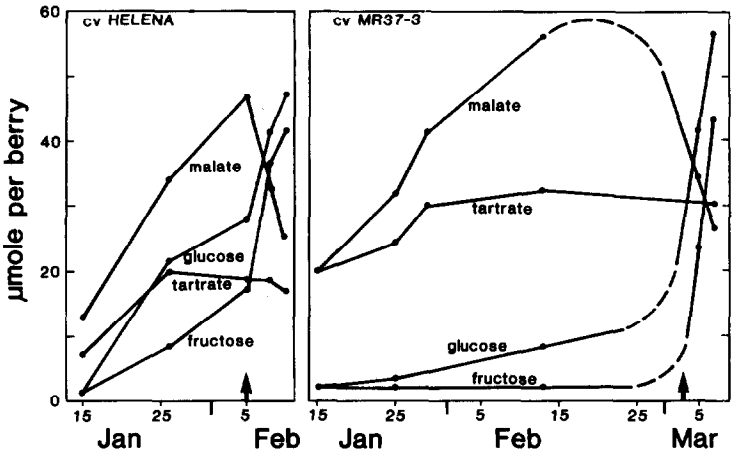

Fig. 2. Changes in accumulated solute composition of individual grape berries during their development monitored using natural abundance ^{13}C NMR techniques. Results from berries of cultivars, Helena and MR 37-3 are illustrated. The dates on which the berries commenced the ripening phase of development are indicated by vertical arrows.

the claim that these faster rates began coincidentally with the time of increased berry deformability [1] could not be verified because of lack of measurements just before and after this event. Nevertheless, the data do not preclude this possibility. The compositional changes during ripening lead eventually to very high levels (*ca* 0.7 M each) of glucose and fructose. The size limitations of the NMR probe however, did not enable us to make these observations on the enlarged fully developed berries. However, this work does show that, in principle, NMR permits the monitoring of molecular processes in living plant tissue in a manner similar to that demonstrated for animal tissue [5].

EXPERIMENTAL

Grapevines and berries. Insertion of a berry into the NMR spectrometer, while still attached to the vine, necessitated that the vine be grown in a metal- and dust-free container and that the berry be less than 9 mm diameter and borne on a clear, straight rachis 130 mm or more long. Seedlessness was thought desirable to increase the effective vol. of the berry and to prevent interference from compounds in the seeds, but this proved unnecessary. Potted vines of many cultivars were selected after setting and their bunches trimmed to single, terminal, small berries. Vines of the cultivars Sultana, Verdeilho, Helena and MR 37-3 (an unnamed hybrid bred by A. Antcliff) were measured; the latter two are reported. Diameter, length and deformability [8] were measured daily during the test period which covered the lag phase and the beginning of the ripening phase of berry growth. The inception of the ripening phase was determined as that day on which the berry deformability reading exceeded a value of 0.4 mm (see method in ref. [1]).

NMR spectroscopy. ^{13}C NMR spectra of the attached grape berry were obtained on a JEOL FX90Q NMR spectrometer operating in pulsed Fourier transform mode at 22.49 MHz with a probe temp. of 24°. Proton noise decoupling was used. The sample was not spun. 6000 accumulations of 5000 Hz spectral width were collected into 8192 double precision words of computer memory using a pulse width of 11 μsec (60°) with an interpulse delay of 2.9 sec. An external ^{2}D lock was employed.

In order to relate accurately peak intensities of successive spectra to each other during the course of the expt, without the aid of an internal reference, special attention was paid to the re-establishment of optimum magnet homogeneity before each data collection. All other spectrometer parameters were not changed. For this purpose a standard sample containing 100 mM D(+)-glucose, D(−)-fructose, L(+)-tartaric acid and L(−)-malic acid dissolved in D_2O was used. Magnetic field homogeneity was optimized using the ^{2}D internal lock signal of the non-spinning sample. The lock signal was then switched over to an external ^{2}D sample and the lock signal suitably phased. The standard sample was removed and replaced with the tube containing the attached berry ready for data accumulation. Special attention was also made to ensure that the berry was repeatedly situated in the centre of the probe irradiation coils by the use of guide marks and a rigid tube holder.

Calculation of solute quantities. Assignment of peaks in the ^{13}C NMR spectra of intact grape berries was achieved by comparison with the ^{13}C NMR spectra of the individual compounds. The chemical shifts (δ) of these peaks in the spectra were referenced against an external capillary containing sodium trimethylsilyl propionate. Because of peak overlap, only those peaks which were clearly resolved were used for estimation purposes, namely δ 42.2 and 70.5 (malate); 75.6 (tartrate); 64.4, 95.8 and 99.6 [D(+)-glucose]; 67.1, 67.6, 71.3 and 101.7 [D(−)-fructose]. For those compounds where more than one peak was available for estimation purposes, internal consistency of the data was sought by comparing the ratios of peak intensities with those of the standard sample; where a ratio differed by more than 35% the value was rejected.

Changes in peak intensities from one NMR data collection to another were used as a measure of the changes in amounts of solutes present in the grape berry. At the end of the NMR expt the berry was immediately removed from its rachis and its solute composition measured by alternative techniques [B. G. Coombe, unpublished observations]. The relative values obtained from NMR analyses were then put on an absolute scale (in μmol) using these values.

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