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# DIURNAL CHANGE OF TARTRATE DISSIMILATION DURING THE RIPENING OF GRAPES

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**Key Word Index**—*Vitis labruscana*; Vitaceae; grape; berries; dissimilation; diurnal variation; tartrate; sucrose.

**Abstract**—L(+)-tartrate-[U-$^{14}$C] or sucrose-[U-$^{14}$C] was fed into grape berries and $^{14}CO_2$ evolution was determined. $^{14}CO_2$ evolution from L(+)-tartrate-[U-$^{14}$C] was slightly higher in mature than immature berries, and that from sucrose-[U-$^{14}$C] was higher in immature than mature ones. $^{14}CO_2$ evolution from L(+)-tartrate-[U-$^{14}$C] was irregular throughout the day until 2 or 3 weeks after flowering. This stage shifted to regular $^{14}CO_2$ evolution until 6 or 7 weeks after flowering, and the mode of $^{14}CO_2$ evolution showed diurnal variation; higher in the day than at night. Then the stage without variation of $^{14}CO_2$ evolution followed 10 weeks after flowering. These observations indicate that tartrate is not biochemically inert in grape berries, while the amount of $^{14}CO_2$ evolution from sucrose-[U-$^{14}$C] was higher at night than in the day through the whole ripening process, except in the early stage.

## INTRODUCTION

Tartrate accumulates in several higher plants [1–4], but its physiological roles have remained obscure. Grape is the best known plant accumulating tartrate, and has been the material for intensive work on tartrate. The tartrate content per berry gradually increases with ripening and reaches a plateau after the coloring stage [5,6]. It has been shown that synthesis of tartrate is more active in young berries than mature ones [6–8], and we have proposed a biosynthetic pathway via ascorbate [9]. On the other hand, only a few reports dealing with tartrate dissimilation in higher plants have appeared [10,11], and all of these results were negative. Hardy, however, observed $^{14}CO_2$ evolution from grape berries administered with D,L-tartrate-[1,4-$^{14}$C] [12]. Saito and Kasai similarly demonstrated $^{14}CO_2$ evolution from grape berries of which the peduncle was dipped into L(+)-tartrate-[1,4-$^{14}$C] [6], and they confirmed the dissimilation of tartrate in grape berries. In both experiments, "detached clusters" were used and, furthermore, D,L-tartrate was used as the feeding substrate in the former one, though tartrate occurs in the L-(+)-form in grapes [13].

This paper is concerned with the dissimilation of L-(+)-tartrate in grape berries still attached to the tree in comparison with that of sucrose under natural conditions. Diurnal variation of $^{14}CO_2$ evolution from grape berries was observed for both substrates. In the following description, tartrate refers to L(+)-form, unless otherwise specified.

## RESULTS

Tartrate-[U-$^{14}$C] or sucrose-[U-$^{14}$C] was fed to grape berries at different stages of ripening and the $^{14}CO_2$ evolution was measured. Typical results of $^{14}CO_2$ evolution for several days after feeding are shown in Fig. 1. In this case, berries 47 days after flowering were employed and corresponded to the middle stage of ripening. Just after feeding, rapid evolution of $^{14}CO_2$ was observed for both substrates. Then $^{14}CO_2$ evolution decreased gradually until 100 hr prior to almost steady $^{14}CO_2$ evolution. A similar pattern of the decline of $^{14}CO_2$ evolution was also observed at early and later stages of ripening. At

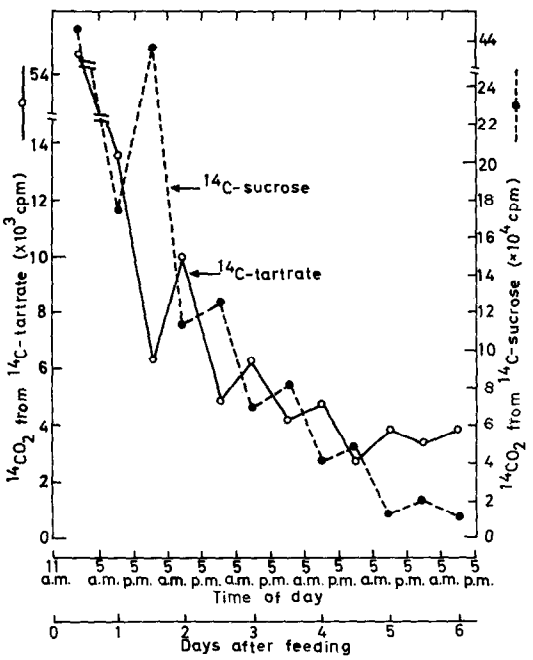


Fig. 1. The decline of $^{14}CO_2$ evolution with time of day. Berries 47 days after flowering were used. 4 $\mu$Ci of tartrate-[U-$^{14}$C] or sucrose-[U-$^{14}$C] were fed to the berries.

Table 1. Incorporation of substrates in grapes and $^{14}CO_2$ evolution

| Substrate | Date fed (days after flowering) | $^{14}CO_2$ evolved* (A) ($\mu$Ci) | Remaining radioactivity† (B) ($\mu$Ci) | Radioactivity incorporated (A + B) ($\mu$Ci) | Ratio (A/A + B) (%) |
|---|---|---|---|---|---|
| Tartrate -[U-$^{14}$C] | 8 | 0.144 | 0.400 | 0.54 | 26.5 |
| | 60 | 0.544 | 1.21 | 1.75 | 31.1 |
| Sucrose -[U-$^{14}$C] | 8 | 0.607 | 1.22 | 1.83 | 33.2 |
| | 60 | 0.538 | 1.54 | 2.08 | 25.8 |

Tartrate-[U-$^{14}$C] or sucrose-[U-$^{14}$C] (4 $\mu$Ci) was fed to the grape berries. *$^{14}CO_2$ evolved for 14 days after feeding. †Total radioactivity remaining in berries 14 days after feeding. Sampled berries were extracted with 80% EtOH and then 50 mM HCl (soluble fraction). The insoluble fraction was dried and then applied with sample oxidizer (Packard: Tri-Carb, 305). The remaining radioactivity is the summation of $^{14}C$ in soluble and insoluble fractions.

the later stage, however, the steady $^{14}CO_2$ evolution appeared 80 hr after feeding both substrates. In order to estimate the dissimilation of tartrate in grape berries, the ratio of the total amount of $^{14}CO_2$ evolution to the radioactivity incorporated into berries was calculated. As shown in Table 1, 27% of $^{14}C$ was found as $^{14}CO_2$ for 14 days after feeding tartrate-[U-$^{14}$C] at early stages, and at later stages, 31% was found as $^{14}CO_2$. On the other hand, when sucrose-[U-$^{14}$C] was fed to the berries, 33 and 26% was found as $^{14}CO_2$ for 14 days at early and later stages, respectively. Thus, $^{14}CO_2$ evolution from tartrate-[U-$^{14}$C] was higher at the later stage than at an early stage, while that from sucrose-[U-$^{14}$C] was higher at the early stage than at the later stage. In Fig. 1 we noticed that there is high evolution of $^{14}CO_2$ from tartrate-[U-$^{14}$C] and low evolution from sucrose-[U-$^{14}$C] in the day time. But at the early stage, the above regularity was not observed, and at later stages the regularity was preserved only in the case of sucrose-[U-$^{14}$C] and the variation of $^{14}CO_2$ evolution was not observed in the case of tartrate-[U-$^{14}$C].

The above results indicate a diurnal change in the dissimilation of tartrate and sucrose, and we have therefore investigated particularly the diurnal change in $^{14}CO_2$ evolution from labeled tartrate and sucrose, administered

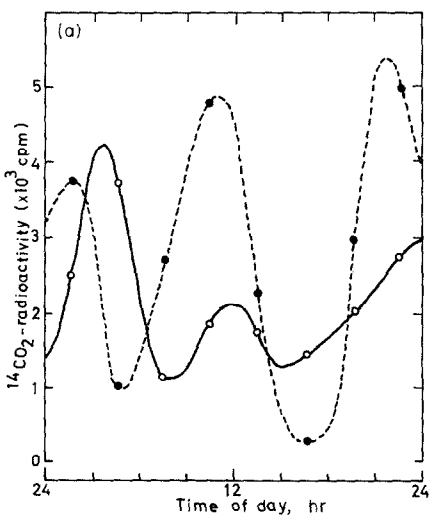


Fig. 2(a).

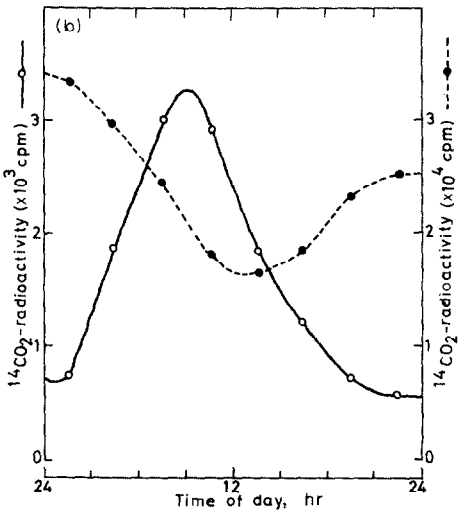


Fig. 2(b).

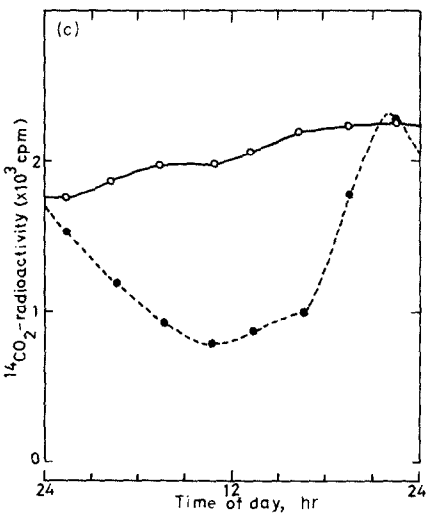


Fig. 2(c).

Fig. 2. The change of $^{14}CO_2$ evolution from tartrate-[U-$^{14}$C] (○--○) or sucrose-[U-$^{14}$C] (●—●) in the berries. 4 $\mu$Ci of each substrate were fed to the berries.

| Berries used for feeding (days after flowering) | Days after feeding | $^{14}CO_2$ evolution after feeding ($\times 10^3$ cpm) |
|---|---|---|
| (a) | 6 | 7 | 182 (tartrate-[U-$^{14}$C]) 828 (sucrose-[U-$^{14}$C]) |
| (b) | 28 | 4 | 105 (tartrate-[U-$^{14}$C]) 1100 (sucrose-[U-$^{14}$C]) |
| (c) | 74 | 14 | 837 (tartrate-[U-$^{14}$C]) 828 (sucrose-[U-$^{14}$C]) |

at three stages of ripening. Almost all remaining radioactivity in the berries 14 days after feeding tartrate-[U-$^{14}$C] was found in the tartrate fraction, and it is therefore thought that the $^{14}CO_2$ evolved is derived from tartrate-[U-$^{14}$C] in this experiment. Irregular $^{14}CO_2$ evolution was observed at early stages of berry development, from 8 days to 2 or 3 weeks after flowering (Fig. 2a). When the berries developed further, a regular corre-

spondence of $^{14}CO_2$ evolution with the time of day appeared, and the amount of $^{14}CO_2$ evolution was higher in the day than at night, as shown in Fig. 2b. The times corresponding the maximal and minimal rate of $^{14}CO_2$ evolution were 8–11 a.m. and 11 p.m.–2 a.m., respectively. This regularity continued until 6 or 7 weeks after flowering, and subsequently disappeared (Fig. 2c). Figures 2a–2c also shows the results when sucrose-[U-$^{14}$C] was used as the feeding substrate. Regularity of $^{14}CO_2$ evolution appeared 2 weeks after flowering, and was preserved through the whole subsequent ripening process. In this case, the amount of $^{14}CO_2$ evolution was higher at night than in the day (Figs. 2b, 2c).

## DISCUSSION

$^{14}CO_2$ evolution from tartrate-[U-$^{14}$C] in grape berries was observed and previous results [6] were confirmed. 27% (at the early stage) or 31% (at the later stage) of tartrate-[U-$^{14}$C] was converted to $^{14}CO_2$, and $^{14}CO_2$ evolution was slightly higher at the later stage than the early stage, which is consistent with the results of Saito and Kasai [6]. This dissimilation rate of tartrate suggests that tartrate is not the final product of metabolism in grape berries. It has been observed that the synthesis of tartrate in grape berries occurs in young fruits and is not detectable 62 days after flowering. The content of tartrate gradually declines from 75 days after flowering [6]. The higher $^{14}CO_2$ evolution and the decrease of tartrate content in berries at the later stage may be due to the activation of a tartrate dissimilation system or an increase in the free form of tartaric acid. Only the free form of tartrate, which is a minor part of the total tartrate in berries [6], may be dissimilated. But $^{14}CO_2$ evolution from sucrose-[U-$^{14}$C], on the other hand, was higher at the early stage than the later stage. The decrease in dissimilation of sucrose is consistent with the accumulation of sugars at the ripe stage [16].

The rapid decrease of $^{14}CO_2$ evolution after feeding both substrates (Fig. 1) is thought to be due to the rapid dilution of tartrate-[U-$^{14}$C] or sucrose-[U-$^{14}$C] with endogenous tartrate or sucrose and the conversion of labeled substrates to metabolically inactive forms or sites; salt forms (tartrate) or accumulating sites (sucrose).

The amount of $^{14}CO_2$ evolution from tartrate-[U-$^{14}$C] in grape berries showed variation with time of day, and the mode of variation changed with the development of the berries. Irregular $^{14}CO_2$ evolution was observed until 2 or 3 weeks after flowering. This stage shifted to a stage of diurnal variation until 6 or 7 weeks after flowering. Then a stage without variation of $^{14}CO_2$ evolution followed 10 weeks after flowering. The diurnal change of enzymatic activity concerning tartrate is probably induced by light or temperature changes or alternatively there may be a change in the amount of substrates with time of day. The organic acid (principally malate and tartrate) content in immature grape berries is about 10% higher in the day than at night [4,20] and it is well known that the organic acid content in CAM plants shows diurnal variation [17–19]. As mentioned above, the major part of the tartrate in grape berries occurs in the salt forms and a smaller part of it in the free form. If only free tartrate is metabolized in grape berries, the variation of $^{14}CO_2$ evolution shown in the present experiment may depend on the variation in the amount of free form tartrate with time of day.

It is known that the growth of grape berries is biphasic [21]. In the first growth phase, organic acid content (malate and tartrate) increases, and in the second growth phase, sugar content increases and organic acid content (mainly malate) decreases. The stage of irregular and regular $^{14}CO_2$ evolution in the present experiment almost corresponds to the first growth phase, and the stage without variation of $^{14}CO_2$ evolution to the second growth phase. The stage of 8 and 60 days after flowering shown in Table 1 correspond to the first and second growth phase, respectively.

## EXPERIMENTAL

Berries of *Vitis labruscana* B. "Delaware" from a vineyard at Kyoto University were used.

*Preparation of labelled compounds.* Tartrate-[U-$^{14}$C] was prepared by the procedure of ref. [6] as follows; the cluster was exposed to 10 mCi of $^{14}CO_2$ for 72 hr, and then detached. Berries were extracted with 80% EtOH and the residue was re-extracted with 50 mM HCl. These extracts were passed through columns of Amberlite IR-120B($H^+$) and IR-45($OH^-$). Organic acids adsorbed on Amberlite IR-45 were eluted with 2 M ammonium carbonate, and concentrated *in vacuo*. Concentrated organic acids were separated by Si gel column chromatography, according to a modified version of the method of refs [14,15]. Fractions containing tartrate were collected and then extracted with $H_2O$. The $H_2O$ phase was brought to about pH 8 with $Ba(OH)_2$. The barium tartrate was converted to the free form by adding Amberlite IR-120B($H^+$). About 100 $\mu$Ci of tartrate-[U-$^{14}$C] was obtained. Sucrose-[U-$^{14}$C] was purchased from the Radiochemical Centre, Amersham, England.

*Administration of labelled compounds.* Tartrate-[U-$^{14}$C] (4 $\mu$Ci, 26.2 $\mu$Ci/mmol) or sucrose-[U-$^{14}$C] (4 $\mu$Ci, 10 mCi/mmol) was fed into grape clusters growing on the tree and feeding was carried out with a cotton thread one end of which pierced through the peduncle and the other was dipped in the vessel containing the labelled compounds.

*Trapping of $^{14}CO_2$ evolved from grape berries.* After feeding the labelled compounds, the cluster was placed in a glass vessel under a cover. The $^{14}CO_2$ evolved was led by suction pump to a test tube containing 10 ml of ethanolamine in ethylcellosolve. In order to collect the $^{14}CO_2$ evolved in a given period of time, an apparatus with timer and electromagnetic valves permitted successive introduction of gas to the $CO_2$-trapping tubes. $^{14}$C-radioactivity was measured with a liquid scintillation counter and quenching was corrected by the external standard ratio method.

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