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Metabolism of tartaric and malic acids in *Vitis:* A review — Part A [1])

by

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Introduction

Quantitative analyses of the acid fraction of *Vitis vinifera* invariably show that in all parts of the vine with the exception of the root tissue (36, 69) tartaric and malic acids represent the predominant acid constituents (lit. cit. in 63, 98), accounting for 70—90 % of this fraction. Other organic acids found in variable, but always low concentrations are: citric, succinic, fumaric, formic, acetic, glycolic, lactic, aconitic, quinic, shikimic and mandelic acids (8, 9, 12, 36, 68, 70). For the commercially important part of the plant, the fruit, the acid content is an important quality factor (3, 98). High acid levels, and in some instances also extremely low concentrations, not only affect the palatability of table grapes, but also influence the suitability of wine-grapes for vinification. Excessive tartness of the fruit is normally correlated with low sugar concentrations, resulting in poor wine quality, whereas low acid levels at harvest can be accompanied either by low or high sugar contents, depending on the preceding climatic conditions; in both cases unbalanced and flat wines are produced. It has been unequivocally demonstrated that the acid content of ripening grapes is mediated by exogenous factors, notably the temperature (20, 23, 34, 37, 38, 64, 93, 94).

Under continuously warm conditions, acid accumulation in the green berry is decreased, and the consumption of malic acid during the subsequent ripening period is enhanced, both phenomena resulting in lower acid content at maturity (7, 72).

In spite of their close chemical resemblance (Fig. 1), tartaric and malic acids display distinctly different accumulation patterns throughout leaf and berry ontogeny.

Fig. 1

[1]) Part B will be published in issue 4 of this volume.

Due to the intensive tartrate formation at the beginning of development in these tissues (24, 36, 74), a correlation between cell divisions and tartrate synthesis has been proposed (21). As a matter of fact, tartrate levels appear to remain remarkably constant after an initial synthetic period, which indicates that the acid, or possibly its salts, are metabolically very inert after their formation. It has been suggested that tartaric acid is remetabolized (35, 74, 87), but the changes in tartrate levels reported were rather small when calculated on a per berry basis, which takes into account the considerable increase in berry volume occurring during ripening (26, 33). In the grape leaf tartrate formation is also restricted to the initial growth phases (36, 54, 96), while in the mature tissue its concentration remains constant. The drastic fall in tartrate concentration observed by KLIEWER (36) in senescing leaves, was attributed by the author to a translocation of the acid into the woody parts of the vine, where indeed higher acid concentrations were determined in late autumn.

Malic acid, on the other hand, accumulates steadily in the fruit after anthesis and fruit set, and reaches a maximum shortly before the beginning of ripening. With the onset of berry softening, colour change and sugar accumulation (véraison, 10) a period of rapid malate consumption is initiated (11, 19, 63, 98). The decrease in malic acid can generally not be explained by a mere increase in berry volume, but actually reflects a remetabolization of stored malate (65, 71, 84). In very young leaves, malic acid levels are constantly low, with perhaps even a tendency to decline immediately after budburst (36, 54, 62). Only when the lamina has attained about one fourth of the final size, malate starts to accumulate rapidly up to a concentration of 1.5—3 mg/g FW.

With progressing maturity of the leaves, the malate content decreases, which must also be due to translocation into lignifying shoots (36). Mobilization of assimilates for storage within the perennial parts of the plant is not uncommon in nature (1), it implies, however, that the acids are translocated within the symplast; the relatively bulky molecules, carrying presumably one or even two negative charges, would have the problem of permeating cellular membranes, unless they are first broken down to smaller units, translocated and finally reassembled at their destination.

It was originally thought that malic and tartaric acids were synthesized solely in grape leaves and transported from there into the berries. However, HALE (21), was able to demonstrate that the fruit is also an important site of acid synthesis. Nevertheless, the grape is essentially dependent on the influx of assimilatory substances, it has an inadequate photosynthetic potential (19, 40) and is unable to ripen after being detached from the plant (63). The main translocated substance in shoots of grapevines was found to be sucrose (39, 85) and malic acid synthesis from exogenous sucrose has been demonstrated (24). It therefore seems likely that the two dicarboxylic acids are not, under normal physiological conditions, translocated from the leaves to the berries, but are locally synthesized from carbohydrate precursors. Accordingly, no transfer of ^{14}C label to the stem or the berries was detectable after injection of radioactive tartrate into one of the major veins of a leaf (70).

The quantitative ratio between malate and tartrate is reported to vary considerably depending on the grape variety. Cultivars such as Carignane, Malbec and Pinot noir have a relatively high malate content at maturity, and the majority of varieties, among them Chasselas, Sémillon and Thompson Seedless, predominantly store tartrate (2, 98). Apart from the differences between malic and tartaric acids with respect to their accumulation patterns in grape berries and leaves, the two acids obviously have basically different biosynthetic and metabolic pathways. This fact has prompted a separation of the present paper into two sections, the first one dealing with the physiology and biosynthesis of tartaric acid, the second concerning the formation and physiological role

of malic acid, which appears to be a central intermediate in the primary metabolism of the grapevine. The article is generally geared towards an overall view of tartrate and malate biosynthesis and regulation in *Vitis vinifera*, and attempts to integrate biochemical and physiological data of the last decade with the long-known facts of grape berry ripening.

Tartaric acid

Occurrence of tartaric acid in higher plants is relatively unusual (60, 79, 80) and the grape appears to be the only fruit of any widespread commercial interest to accumulate this compound in appreciable amounts (69, 88). As in some other angiosperms, notably in *Pelargonium*, tartaric acid in Vitaceae is present as the optically active L-(+)-stereoisomer (92). It has been mentioned in the previous section that throughout the growing season this substance usually represents the quantitatively most important component within the acid pool of leaves and berries of *Vitis vinifera*. In both tissues, tartaric acid biosynthesis, or at least its accumulation, was shown to be intrinsically related to the phenomenon of growth (21, 74). Only leaves up to a certain size and stage of development were found to synthesize the acid at an appreciable rate (77).

1. Characterization and localization of tartrates

It is believed that with progressing maturity of the fruit an increasing part of tartaric acid is stored in the inert form of a sparingly soluble salt. This conclusion bases on the different solubility of free tartaric acid and its potassium (hydrogen) salts in ethanol and dilute mineral acids, respectively (74, 76). Due to the abundance of potassium in grapes, the obvious assumption was made that potassium was the natural counter-cation, and that the cristalline precipitates occurring in grape tissue had to be potassium hydrogen tartrate (2, 95, 98). However, reexamination of the hypothesis using scanning electron microscopy in combination with energy dispersive X-ray analysis of grape berry, leaf and shoot samples revealed that although potassium is indeed the main cation present in these tissues, the insoluble tartrates occurring as highly ordered cristalline bundles (Fig. 2 c) are exclusively calcium salts (this lab, unpubl. data). Circumstantial evidence for this tentative result is offered by the obvious lack of correlation between potassium and tartrate contents in grapes at any stage of development (22). Because the solubility characteristics of dipotassium tartrate, potassium hydrogen tartrate and calcium tartrate are essentially the same as far as the partitioning between ethanol and hydrochloric acid is concerned, this finding does not detract from the hypothesis that tartaric acid is stored predominantly in the salt form. Still, it has to be borne in mind that the ever so slightly soluble tartrates and the free acid are in equilibrium within the storage compartment, and that remetabolization of one species would lead to replenishment by the other. Consequently, other mechanisms to render the presumable metabolic form, the free acid, inert, must also be in play, particularly because unphysiologically low pH-values would have to be maintained within the cytoplasm to guarantee the existence of the fully protonized acid.

In grape material, calcium tartrate is sequestered in huge, specialized cells, the idioblasts (cf. also 99). These cells are regularly arrayed in groups of two or three in the intercostal sections of fully differentiated leaves. A considerably higher incidence of tartrate cells is observed in the serrated tips of the leaf (Fig. 2 a), which is emphasized by a mean titratable acidity of 29.2 mg acid per g fresh material calculated as tartaric acid in the leaf periphery, as opposed to 20.2 mg insoluble acid per g fresh weight in the

rest of the lamina, with the malic acid concentration being equal in both extracts (MALIPIERO, pers. comm.).

The tartrate containing idioblasts are distinguished by their size, which is several times bigger than that of the surrounding cells and naturally by the fact that they contain bundles of crystals arranged in parallel and termed raphides, which more or less fill the entire lumen of the idioblast. These unusual cells are localized between the palisade cells and the spongy tissue of the leaf, adjacent to palisade cells which are somewhat shorter than normal (Fig. 2 b, HASLER, Ph. D. Thesis, in preparation). Attempts to isolate idioblastic protoplasts from grape leaves usually failed, although in some instances tartrate containing cells could be observed in the light microscope before they collapsed in the heat of the light-beam (Fig. 2 c). Interestingly, after disintegration of the protoplast, the individual tartrate needles grew visibly longer, thus indicating that the liquid surrounding the raphides was a solution of highly concentrated tartaric acid, which upon contact with the cation-containing isolation mixture (27) resumed cristallization! It is therefore thought that cations, judging by the otherwise unchanged appearance of the elongated needles most likely calcium ions, represent the limiting factor in tartrate precipitation within the idioblasts. The concept of a physiological role of tartaric acid in scavenging excessive imported calcium, comparable to the function of oxalate in *Canavalia* (16) is supported by the fact that idioblasts are concentrated in the leaf tip area where, due to the presence of numerous hydatodes (Fig. 2 a), local calcium concentrations may by higher than normal.

The assumption that in ripening grape berries practically all tartaric acid is stored as an insoluble salt (76, 98) is somewhat doubtful in the light of the recent findings of MOSKOWITZ and HRAZDINA (1981), who were able to isolate subepidermal vacuoles, containing the free acid, from grapes. It is highly improbable that raphide-containing idioblasts would have survived the isolation procedure, and even if some released tartrate needles were not removed by the subsequent purification, a tartaric to malic acid ratio of approximately 3 : 1 could certainly not be accounted for by these impurities. Also, analyses of the organic acid fraction of grape leaf protoplasts as compared with those of intact leaf tissue did not reveal a lower content of tartaric acid in the protoplast preparation, although no idioblasts were present in the analyzed sample (27). This finding is further supported by earlier results from Geraniaceae (80), where a strict correlation between the acidity of leaf extracts and the potential of the respective species to store tartaric acid was observed. Additionally, a potassium content in the range of 50 pmoles per vacuole, as found in grape material (59), would suffice to neutralize all of the tartaric and malic acid present to the dipotassium salt. Still, vacuolar pH-values between 2.5 and 3 were determined, thus indicating that in certain cases free tartaric acid is stored effectively sequestered within the vacuoles.

2. Biosynthesis of tartaric acid

The differences in ^{14}C-labelling between malate and tartrate, observed after administration of $^{14}CO_2$ under variable assimilatory conditions, presents strong evidence that the two acids are not related metabolically: malic acid is the predominant radioactive substance after dark $^{14}CO_2$ fixation in grape material (58, 66, 81) and in the light metabolic times of 10 s and less already allow detection of radiocarbon in this acid (5, 67). Tartaric acid, on the other hand, fails to incorporate any ^{14}C from radioactive carbon dioxide in the dark (14, 81) and even under photosynthetic conditions, radioactivity in tartrate is detectable only after an extended period of time (14, 74, 81), emphasizing that the compound is a secondary product. With these facts in mind, the possibility of a TCA-cycle intermediate being related to tartaric acid was ruled out (25, 41) and

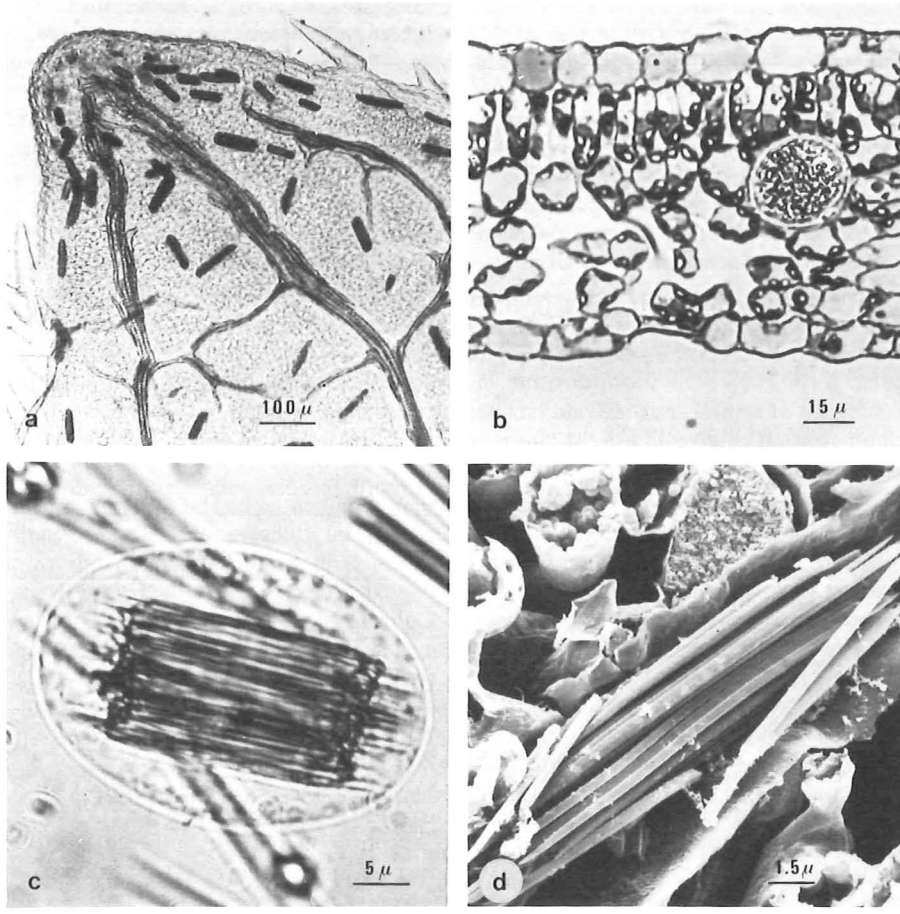

Fig. 2: a) Massing of tartrate idioblasts in the serrated tips of grape leaves; b) transversal section through a grape leaf, showing the localization of a tartrate containing cell; c) tartrate idioblast in an unpurified protoplast preparation from leaves of *Vitis vinifera;* d) scanning electron microcraph of cristalline Ca-tartrate in grape leaves.

the suggestion that a sugar was the more likely precursor was put forward relatively early (81, 89). Although this hypothesis based mainly on stereochemical considerations concerning carbons 1 to 4 of glucose and their configurational similarities to tartaric acid, subsequent experimental data (68) supported this assumption by demonstrating that glucose is indeed an effective metabolic precursor of tartrate in grape material. Approximately 15 % of the total radioactivity was found to be incorporated in tartaric acid within 2 h of administering glucose-U-^{14}C to immature grape berries. In young leaves of *Pelargonium zonale* a close relationship between glucose and tartrate formation was also observed (57). In this case glucose-6-^{14}C, and under photosynthetic conditions also gluconate-6-^{14}C, were more effective in transferring radioactive label to tartrate than glucose-1-^{14}C, thus indicating that the C_3-C_6-moiety is transformed to tartaric acid in *Pelargonium.* It was further established by the same author that

^{14}C-glycolate is as effective a tartrate precursor as glucose in both *Pelargonium* and *Vitis* (55). Nevertheless, because of the labelling patterns of glucose, glycerate and serine, isolated from green grapes and *Pelargonium* leaves after feeding radioactive glycolate, the hypothesis of a direct metabolic link between glycolate, glucose and tartaric acid had to be dismissed. Instead, oxaloglycolic acid was proposed as a common intermediate in the biosynthetic pathways of glucose and tartrate (55). A certain portion of tartaric acid, formed after administration of glycolate-1-^{14}C, was subsequently identified as D-(−)- and/or meso-tartaric acid, while sorbose-U-^{14}C and glucose-U-^{14}C gave rise to L-(+)-tartaric acid exclusively (56). Unfortunately, this experiment did not include stereochemical identification of glycolate-induced tartrate from grape material.

The metabolic scheme of tartrate biosynthesis as proposed by MAROC (55, 56, 57) quite conclusively envisaged two pathways of tartrate formation: one starting from glucose and producing L-(+)-tartaric acid; the second condensing two molecules of glycolate to form D-(−)- or meso-tartrate via reduction of oxaloglycolate. However, the occurrence of some L-(+)-tartrate labelled predominantly in the carboxyl atoms after administration of glycolate-1-^{14}C (56) to *Pelargonium* leaves, is not accounted for by either possibility.

This somewhat confusing situation is further complicated by the findings of RIBÉREAU-GAYON (67), who reported that in grape material glucose-1-^{14}C is more readily transformed to tartaric acid than glucose-6-^{14}C, the former being approximately twice as effective in transmitting ^{14}C-label to the C_4-acid under photosynthetic conditions, whereas no incorporation of radiocarbon from glucose-6-^{14}C into tartrate was detectable in the dark. These results were taken as conclusive evidence for a transformation of the intact C_1-C_4-moiety of glucose to tartrate via a biosynthetic pathway branching off at the pentose phosphate cycle. A reaction sequence via gluconic and 5-ketogluconic acids has been proposed (63, 67), but no explanation for the comparatively high occurrence of label in tartrate after feeding glucose-6-^{14}C in the light was offered.

In 1969, SAITO and KASAI (75) found that ascorbic acid-1-^{14}C is an extremely effective precursor of tartaric acid in grape berries, with transformation rates of more than 70 % within 24 h, while, according to earlier reports, administration of ascorbic acid-6-^{14}C to grape leaves fails to produce any ^{14}C-tartrate (52). On the basis of these results it was concluded that ascorbic acid is an immediate metabolic neighbour of tartaric acid, the reduction of the carbon chain length occurring by cleavage of the C_4-C_5 bond. However, certain experimental peculiarities aroused doubts concerning the nature of this precursor-product relationship:

1. The observed in vivo transformation rate of more than 70 % is suspiciously high, considering that the precursor was fed through the peduncle and a major portion of it may never have reached the tartrate synthesizing cells (cf. 44, 83).
2. With increasing metabolic time from 24 to 48 h, the amount of labelled tartrate decreased by one third, which is certainly unusual in view of the biological half-life of tartaric acid, previously determined to be in the range of 100—150 h (13).
3. Virtually no unmetabolized ^{14}C-ascorbic acid was observed in any experiments with grape material (75), even at developmental stages where tartrate synthesis had obviously ceased (77).

These anomalies seemed to warrant a control experiment in which ascorbic acid-1-^{14}C was added to a grape leaf homogenate immediately before the kill. After extraction with aqueous ethanol, followed by separation on ion exchange columns, 25 % of total ^{14}C-label was found within the anionic fraction. Gas-liquid-chromatographic separation of this group of substances revealed that tartaric acid and an unidentified component

containing presumably two carbon atoms were radioactive (73), thus indicating that a non-enzymic step was involved. Although some circumstantial evidence has since been brought forward to dismiss this fact (92), convincing proof, such as a time course study of tartrate formation from ascorbate, comparable to that conducted on *Pelargonium* (53), is still lacking. Strangely enough, administration of ^{14}C-ascorbate to grape leaves, apices or berries during the tartrate synthesizing stage of development, invariably resulted in a complete disappearance of the tracer (77, 91, 96, 97). It is admittedly not clear why the insinuated non-enzymic cleavage of ascorbate should be transitory in grape material and even non-existent in *Pelargonium* (90) although drastic changes in the concentrations of various cell constituents are bound to take place during fruit set. A repetition of the described experiments with glucose-1-^{14}C and glucose-6-^{14}C as precursors of tartaric acid (67) in grape leaves and berries, showed that the results were very reproducible as far as the relative incorporation of the differentially labelled sugar is concerned (73). Again, transformation of glucose-6-^{14}C amounted to 50—60 % of the value observed with glucose-1-^{14}C, thus emphasizing the fact that there must exist, beyond mere chance, a metabolic possibility for C_6 of glucose to enter the tartrate molecule. In contrast to the incorporation rates, the labelling patterns of tartrate, as determined in these two studies, did not correspond, although in both cases relatively little exchange of ^{14}C-label between the external and internal carbon atoms had occurred. On this evidence it was suggested that in *Vitis* two different pathways of tartaric acid synthesis are effective (73), one retaining the original carbon sequence of glucose with C_1 of the sugar entering C_1 of tartaric acid, while the other includes a formal inversion of the hexose skeleton, analogous to the path of ascorbate synthesis in animals (6). As the common intermediate of these two biosynthetic mechanisms, which were believed to be tissue-specific, pretaric acid was proposed. This compound, a 1,2-dihydroxymethyl hydrogen L-(+)-tartrate, was isolated by KOTERA *et al.* (42) from cultures of *Gluconobacter suboxydans* and shown to be an intermediate in bacterial tartrate synthesis.

The very high incorporation of glycolate-1-^{14}C and -2-^{14}C in combination with the resulting labelling pattern of tartrate (73, 82) were taken as indicative of a third mechanism of tartrate formation, featuring a tail-to-tail condensation of two C 2-molecules, presumably glycolaldehyde, similar to that found in *Pelargonium* (55, 56). However, the stereochemical configuration of tartrate was not determined in these experiments, and the occurrence of traces of radioactive meso-tartaric acid after feeding glycolate-2-^{14}C to grape leaves (70), may indicate that the tartrate isolated by crystallization consisted partly of the meso- and/or the D-(−)-isomer both of which have not been determined quantitatively in grape material. Attempts to influence tartaric acid biosynthesis in grape leaves by inhibiting glycolate production (77), or by physiologically enhancing it (54), proved unsuccessful.

The question whether ascorbic acid or pretaric acid is the more likely direct tartrate precursor must remain unanswered at this stage. However, administration of specifically labelled ascorbic acid to *Vitaceae* invariably resulted in a negligible random distribution of label in the product, with usually more than 95 % of radioactivity entering the carboxyl group(s) of tartrate (30, 75, 91, 96). The cleavage of the carbon chain of exogenous ascorbic acid between C_4 and C_5 consequently appears to be absolutely specific. If one accepts the tenaciously maintained hypothesis that no synthetic pathway for ascorbate including an inversion of the carbon skeleton is realized in higher plants (29, 47, 48, 49, 50, 51), this specificity makes an interpretation of the available data somewhat difficult. After administration of glucose-1-^{14}C to leaves of *Parthenocissus quinquefolia*, 96 % of the label incorporated into tartrate was in the carboxyl atoms

while in the same extract 80 % of the radioactivity in ascorbate was found in C_1. This difference in the degree of randomization between precursor and product is not consistent with the simultaneously observed 99 % specificity of ascorbate cleavage (30). In addition, the approximately 15 % relative inversion determined in ascorbic acid after application of glucose-1-^{3}H must mean that also in *Parthenocissus* there is a mechanism of ascorbate and, by inference, tartrate formation, in which the carbon sequence of glucose is inverted. It seems equally evident from the variable labelling pattern of tartrate after supplying specifically labelled glucose (67, 73) that the relative activities of the respective pathways are subject to changes. While the original hypothesis considered the actual turnover in one or the other reaction sequence to be organ-specific, careful reexamination of all available data reveals that the developmental stage and the accessible carbon source have a stronger influence.

Although ^{14}-C-incorporation from $^{14}CO_2$ into tartrate has been demonstrated to be absolutely light-dependent (14, 81), the number of tartrate idioblasts is not decreased in etiolated leaves from sprouting grape cuttings (this lab, unpubl. data), an observation which has already been made on oxalate accumulating plants (99). It thus appears that tartrate formation is not light-dependent provided that precursors are available, either in the form of remobilized hexose, or possibly as by-product components of the uronic acid group. These alduronic compounds are thought to be subsequently removed by a salvage-type process. Glucuronate, and particularly glucurono-γ-lactone were clearly demonstrated to be relatively good precursors of tartaric acid in grape berries (74, 75), with incorporation rates of the former amounting to 50 % of those observed after feeding ^{14}C-gluconate, and the latter even exceeding gluconate in effectiveness. In contrast to the transformation pattern of sucrose and gluconate, there was no standstill observed in tartrate biogenesis from "uronic compounds" until general berry metabolism seemed to slacken approximately 80 d after flowering. The same is true for ascorbate catabolism (76), while sucrose- and gluconate-induced tartrate synthesis, and, as has been demonstrated in other plants, growth-dependent ascorbate formation, is confined to the time immediately after anthesis (17). At this stage of development photosynthetic products are mainly utilized for the synthesis of structural carbohydrates, and accordingly glucose-6-phosphate dehydrogenase activity in grape berries was also found to be high (28, 72).

The fact that glucose-6-^{14}C does not relay radioactive carbon to tartrate in the dark (67) may indicate that galacturonic rather than glucuronic acid is the actual alduronic precursor of tartrate, as was proposed as an alternative to the gluconate pathway for ascorbic acid synthesis in plants (31, 32). Provided that transformation of glucose to galacturonic acid includes a light-dependent reaction, this would explain the failure of grape tissue to form tartrate from C_6 labelled glucose in the dark, while the same conversion obviously takes place in the light.

The coincidence in physiological behaviour of ascorbate and tartrate, viz. the marked correlation between plant growth and the biogenesis of these compounds (18, 21), as well as the similarities concerning their derivation from a hexose pool via aldonic and/or alduronic acids certainly does support the hypothesis of a precursor-product relationship between ascorbic and tartaric acids. In the physiological context it appears irrelevant, whether the cleavage of ascorbate is enzyme-catalyzed or not.

Precursor experiments with developing plants (4, 46) and wounded potato tubers (45) support the concept of a physiological classification of ascorbate according to the presence or absence of growth dependency (17). A similar scheme for tartrate synthesis, based on data from grape material, would include:

1. Tartrate biogenesis via gluconate-glucono-γ-lactone-ascorbic (or pretaric) acid (lit. rev. in 48), correlated with a high acitivity of the hexose-monophosphate shunt.

2. Tartaric acid synthesis by transformation of alduronic compounds, presumably galacturonic acid, (lit. rev. in 31) possibly in a salvage-type reaction. A function of this mechanism in maintaining a physiological balance of calcium ions cannot be excluded.

The two pathways are not mutually exclusive and appear to be essentially light-independent, provided the respective precursors are available. The importance of the latter statement is emphasized by the complete failure of *in vitro* cultivated grape berries to produce tartaric acid (78).

3. Remetabolization of tartaric acid

The accumulation pattern observed in grape leaves and berries, which is characterized by a sharp initial increase in acid concentration in young tissues and a subsequent levelling off at an essentially constant concentration (22, 36, 54, 74), raises the question, whether the standstill is due to a drastic reduction in tartrate synthesis, or alternatively reflects the steady-state nature of acid formation and utilization. In any case, tartaric acid synthesis appears not to be completely abolished after the period of cell division, since as the leaf area and berry volume increase (10, 11), the amount of tartrate per gram fresh weight remains unchanged.

If translocation of tartaric acid into the woody parts of the plant, as has been described for senescing leaves (36), is excluded as a not strictly metabolic process, there exists no unequivocal indication of remetabolization of endogenous tartrate. The high R. Q. values in the range of 1.6—1.7 at elevated temperatures (37 °C), previously taken as evidence for tartrate respiration (lit. cit. in 61), must rather be attributed to some degree of fermentation within the berries under these conditions (43).

Not unexpectedly, attempts to demonstrate tartrate dissimilation have met with little success. Although ^{14}C-tartrate administered through the severed stem or peduncle was reported to be respired at an appreciable rate in grape tissues (13, 14, 15, 25, 74), in all the experiments where photosynthetic reassimilation can be excluded, no intermediary products of tartrate dissimilation were detectable (84). Interestingly, a circadian rhythm of $^{14}CO_2$ evolution was observed after wick-feeding radioactive tartaric acid to grapes still attached to the vine (86). Still, an explanation of this phenomenon, going beyond trivial speculation, seems difficult. The same group of authors finally succeeded in identifying marginal amounts of malate and monoethyl tartrate after administration of tartaric acid to grape material (87). However, transformations of less than 1 % were registered in the presumptive intermediary compounds, which appears hardly significant, considering that biogenic tartrate was used as tracer, and the isolated malate displayed exactly the labelling pattern of this compound, determined after photosynthetic formation in grape material (81). Also the occurrence of monoethyl tartrate seems problematical, if one takes into account that the tracer was extracted with ethanol/HCl from green grape berries, conditions which are more than likely to induce some degree of esterification.

Since any conclusive evidence concerning the biochemical nature of the dissimilatory pathway is lacking, tartrate respiration as well as its physiological significance must remain enigmatic.

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References

1. ALQUIER-BOUFFARD, A., 1969: Evolution des acides organiques et des glucides du rameau de vigne. Physiol. Vég. **7**, 201—225.
2. AMERINE, M. A., 1956: The maturation of wine grapes. Wines and Vines **10**, 27—38.
3. — — and SINGLETON, V. L., 1972: Wine, 37—50. University of California Press, Berkeley, Los Angeles, London.
4. ARNDT, K., 1970: Beitrag zum Problem der Biosynthese von Vitamin C (Ascorbinsäure) in höheren Pflanzen, untersucht an *Tradescantia albiflora* (KUNTH) und *Triticum aestivum* (L.), Sommerweizen, Sorte Arin. Diss. Universität Bonn.
5. BREM, S., RUFFNER, H. P. and RAST, D. M., 1981: Aspects of malate formation in grape leaves. In: AKOYUNOGLOU, G. (Ed.): Photosynthesis VI. Photosynthesis and productivity, Photosynthesis and Environment, 181—188. Balaban Internatl. Sci. Services, Philadelphia, Pa.
6. BURNS, J. J. zit. in HOLLMANN, S., 1961: Nicht-glykolytische Stoffwechselwege der Glucose, 100—106. Thieme, Stuttgart.
7. BUTTROSE, M. S., HALE, C. R. and KLIEWER, W. M., 1971: Effect of temperature on the composition of "Cabernet Sauvignon" berries. Amer. J. Enol. Viticult. **22**, 71—75.
8. CARLES, J. et ALQUIER-BOUFFARD, A., 1962: Les acides organiques de la Vigne et leurs gradients. C. R. Hebd. Séances Acad. Sci. (Paris) **254**, 925—927.
9. COLAGRANDE, O., 1959: Formazione ed evoluzione degli acidi organici durante la maturazione dell'uva. Ann. Mikrobiol. Enzimol. **9**, 62—67.
10. COOMBE, B. G., 1960: Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. **35**, 241—250.
11. — —, 1976: The development of fleshy fruits. Ann. Rev. Plant Physiol. **27**, 507—528.
12. DELMAS, J., POITOU, N. et LEVADOU, B., 1963: Séparation par chromatographie des principeaux acides organiques dans la feuille de la vigne. Chimie Anal. **45**, 63—65.
13. DRAWERT, F. und STEFFAN, H., 1965: II. Verteilung und Veratmung von zugeführten ^{14}C-Verbindungen. Vitis **5**, 27—34.
14. — — und — —, 1966: III. Stoffwechsel von zugeführten ^{14}C-Verbindungen und die Bedeutung des Säure-Zucker-Metabolismus für die Reifung von Traubenbeeren. Vitis **5**, 377—384.
15. — —, — —, ALLMANN, K. und BACHMANN, O., 1962: Bildung von ^{14}C-Glucose und ^{14}C-Fructose aus ^{14}C-Weinsäure in reifenden Beeren der Rebe. Naturwiss. **49**, 159—160.
16. FRANK, E., 1972: The formation of crystal idioblasts in *Canavalia ensiformis* DC. at different levels of calcium supply. Z. Pflanzenphysiol. **67**, 350—358.
17. FRANKE, W., 1964: Ein Beitrag zur Biosynthese von Ascorbinsäure in Pflanzen. Ber. Dtsch. Bot. Ges. **77**, (143)—(145), Sitzung vom 11. 9.
18. — —, 1968: Der jahreszeitlich unterschiedliche Vitamin-C-Gehalt in Coniferennadeln als Folge von Ascorbinsäuresynthese und -abbau. Z. Pflanzenphysiol. **60**, 30—37.
19. GEISLER, G. und RADLER, F., 1963: Entwicklungs- und Reifevorgänge an Trauben von *Vitis*. Ber. Dtsch. Bot. Ges. **76**, 112—119.
20. GENEVOIS, L., 1958: En quoi la baie de vigne diffère-t-elle de la feuille? Bull. Soc. Franc. Physiol. Vég. **4**, 37—38.
21. HALE, C. R., 1962: Synthesis of organic acids in the fruit of the grape. Nature **195**, 917—918.
22. — —, 1977: Relation between potassium and the malate and tartrate contents of grape berries. Vitis **16**, 9—19.
23. — — and BUTTROSE, M. S., 1974: Effect of temperature on ontogeny of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. J. Amer. Soc. Hort. Sci. **99**, 390—394.
24. HARDY, P. J., 1967: Sucrose breakdown and synthesis in the ripening grape berry. Austral. J. Biol. Sci. **20**, 465—470.
25. — —, 1968: Metabolism of sugars and organic acids in immature grape berries. Plant Physiol. **43**, 224—228.
26. HARRIS, J. M., KRIEDEMANN, P. E. and POSSINGHAM, J. V., 1968: Anatomical aspects of grape berry development. Vitis **7**, 106—119.
27. HASLER, M., RUFFNER, H. P. and RAST, D. M., 1982: High-yield isolation of grape leaf protoplasts as an instrument in physiological research. Experientia **38**, 564—565.
28. HAWKER, J. S., 1969: Changes in the activities of enzymes concerned with sugar metabolism during the development of grape berries. Phytochem. **8**, 9—17.
29. HELSPER, J. P. F. G., KAGAN, L., HILBY, C. L., MAYNARD, T. M. and LOEWUS, F. A., 1982: L-ascorbic acid biosynthesis in *Ochromonas danica*. Plant Physiol. **69**, 465—468.

30. — —, SAITO, K. and LOEWUS, F. A., 1981: Biosynthesis and metabolism of L-ascorbic acid in virginia creeper (*Parthenocissus quinquefolia* L.). Planta **152**, 171—176.
31. ISHERWOOD, F. A. and MAPSON, L. W., 1962: Ascorbic acid metabolism in plants: Part II. Biosynthesis. Ann. Rev. Plant Physiol. **13**, 329—350.
32. JACKSON, G. A. D., WOOD, R. B. and PROSSER, M. V., 1961: Conversion of L-galactono-γ-lactone into L-ascorbic acid by plants. Nature **191**, 282—283.
33. JOHNSON, L. A. and CARROLL, D. E., 1973: Organic acid contents of Scuppernong grapes during ripening. J. Food Sci. **38**, 21—24.
34. KLENERT, M., RAPP, A. und ALLEWELDT, G., 1978: Einfluß der Traubentemperatur auf Beerenwachstum und Beerenreife der Rebsorte Silvaner. Vitis **17**, 350—360.
35. KLIEWER, W. M., 1965: Changes in the concentration of malates, tartrates, and total free acids in flowers and berries of *Vitis vinifera*. Amer. J. Enol. Viticult. **16**, 92—100.
36. — —, 1966: Sugars and organic acids of *Vitis vinifera*. Plant Physiol. **41**, 923—931.
37. — —, 1968: Effect of temperature on the composition of grapes grown under field and controlled conditions. J. Amer. Soc. Hort. Sci. **93**, 797—806.
38. — —, 1973: Berry composition of *Vitis vinifera* cultivars as influenced by photo- and nyctotemperatures during maturation. J. Amer. Soc. Hort. Sci. **98**, 153—159.
39. KOBLET, W., 1969: Wanderung von Assimilaten in Rebtrieben und Einfluß der Blattfläche auf Ertrag und Qualität der Trauben. Wein-Wiss. **24**, 277—319.
40. KOCH, R. und ALLEWELDT, G., 1978: Der Gaswechsel reifender Weinbeeren. Vitis **17**, 30—44.
41. KOLAR, G. F., 1970: Accumulation of fumarate in immature berries of *Vitis vinifera* L. Z. Pflanzenphysiol. **62**, 124—128.
42. KOTERA, U., KODAMA, T., MINODA, Y. and YAMADA, K., 1972: Isolation and chemical structure of new oxidation product of 5-ketogluconic acid, and a hypothetical pathway from glucose to tartaric acid through this new compound. Agricult. Biol. Chem. (Tokyo) **36**, 1315—1325.
43. KRIEDEMANN, P. E., 1968: Observations on gas exchange in the developing Sultana berry. Austral. J. Biol. Sci. **21**, 907—916.
44. — —, 1969: Sugar uptake by the grape berry: A note on the absorption pathway. Planta **85**, 111—117.
45. LANGHANS, D., 1980: Zur Frage der Biosynthesewege der Ascorbinsäure (Vitamin C) in Gewebeschnitten von Speicherorganen höherer Pflanzen am Beispiel von *Solanum tuberosum* L., Sorte Holl. Erstling (Kartoffel) und *Helianthus tuberosus* L., Sorte Bianca (Topinambur). Diss. Universität Bonn.
46. LEHNHARD, A., 1974: Zur Frage der Biosynthesewege der Ascorbinsäure (Vitamin C) in Keimlingen von höheren Pflanzen, am Beispiel von *Sinapis alba* L. und *Borago officinalis*. Diss. Universität Bonn.
47. LOEWUS, F. A., 1963: Tracer studies on ascorbic acid formation in plants. Phytochem. **2**, 109—128.
48. — —, 1980: Biochemistry of L-ascorbic acid. In: STUMPF, P. K. and CONN, E. E. (Eds.): The Biochemistry of Plants, 77—99. Academic Press New York, London, Toronto, Sydney, San Francisco.
49. — —, FINKLE, B. J. and JANG, R., 1958: L-ascorbic acid: a possible intermediate in carbohydrate metabolism in plants. Biochim. Biophys. Acta **30**, 629—635.
50. — — and JANG, R., 1957: Further studies on the formation of ascorbic acid in plants. Biochim. Biophys. Acta **50**, 205—206.
51. — —, — — and SEEGMILLER, C. G., 1956: The conversion of C^{14}-labeled sugars to L-ascorbic acid in ripening strawberries. J. Biol. Chem. **222**, 649—664.
52. — — and STAFFORD, H. A., 1958: Observations on the incorporation of C^{14} into tartaric acid and the labeling pattern of D-glucose from an excised grape leaf administered L-ascorbic acid-6-C^{14}. Plant Physiol. **33**, 155—156.
53. — —, WAGNER, G. and YANG, J. C., 1975: Biosynthesis and metabolism of ascorbic acid in plants. Ann. N. Y. Acad. Sci. **258**, 7—23.
54. MALIPIERO, U., RUFFNER, H. P. and RAST, D. M., 1981: Photorespiration and malate formation in grape leaves. Z. Pflanzenphysiol. **104**, 243—251.
55. MAROC, J., 1967: La conversion du glycolate en glucose et ses relations avec la biogenèse de l'acide tartrique. Physiol. Vég. **5**, 37—46.
56. — —, 1967: La biogenèse d'isomères de l'acide tartrique à partir de L-sorbose-U-^{14}C, de glycolate-1-^{14}C et de glucose-U-^{14}C. Physiol. Vég. **5**, 47—55.
57. MAROC-GYR, J., 1965: Le métabolisme du glucose et du gluconate dans les feuilles de *Pelargonium zonale* L., en relation avec la formation d'acides organiques et, en particulier, de l'acide tartrique. Physiol. Vég. **3**, 167—180.

58. MEYNHARDT, J. T., 1963: Assimilation of ^{14}C-labelled carbon dioxide by Barlinka grape berries. Proc. Nat. Conf. Nuclear Energy, South Africa 456—463.

59. MOSKOWITZ, A. H. and HRAZDINA, G., 1981: Vacuolar contents of fruit subepidermal cells from *Vitis* species. Plant Physiol. **68**, 686—692.

60. NIERHAUS, D. und KINZEL, H., 1971: Vergleichende Untersuchungen über die organischen Säuren in Blättern höherer Pflanzen. Z. Pflanzenphysiol. **64**, 107—123.

61. PEYNAUD, E., 1958: Evolution des acides organiques et des aminoacides du raisin. Bull. Soc. Franc. Physiol. Vég. **4**, 34—37.

62. — — and MAURIE, A., 1958: Synthesis of tartaric and malic acids by grape vines. Amer. J. Enol. Viticult. **9**, 32—36.

63. — — and RIBÉREAU-GAYON, P., 1971: The grape. In: HULME, A. C. (Ed.): The Biochemistry of Fruits and their Products. Vol. II, 171—205. Academic Press London and New York.

64. RADLER, F., 1965: The effect of temperature on the ripening of Sultana grapes. Amer. J. Enol. Viticult. **16**, 38—41.

65. RAPP, A., STEFFAN, H., KUPFER, G. und ULLEMEYER, H., 1971: Über den Säurestoffwechsel in Weinbeeren. Angew. Chemie **22**, 925.

66. RIBÉREAU-GAYON, G., 1964: Méthodes et techniques de l'étude du métabolisme des limbes et des baies de *Vitis vinifera* L. utilisant les molécules marquées par le carbon 14. Thèse Université de Bordeaux.

67. — —, 1968: Étude des méchanismes de synthèse et de transformation de l'acide malique, de l'acide tartrique et de l'acide citrique chez *Vitis vinifera* L. Phytochem. **7**, 1471—1482.

68. — — et RIBÉREAU-GAYON, P., 1963: Utilisation de $^{14}CO_2$ et de glucose-U-$^{14}CO_2$ pour l'étude du metabolisme des acides organiques de *Vitis vinifera* L. C. R. Hebd. Séances Acad. Sci. (Paris) **257**, 778—780.

69. RIBÉREAU-GAYON, P. et RIBÉREAU-GAYON, G., 1969: Études biochimiques sur la composition du raisin et ses variations. Rev. Franç. d'Oenol. **35**, 3me trimestre, 5—16.

70. RUFFNER, H. P., 1973: Biogenese und Translokation von Tartrat in der Weinrebe. Diss. Universität Zürich.

71. — — and HAWKER, J. S., 1977: Control of glycolysis in ripening berries of *Vitis vinifera*. Phytochem. **16**, 1171—1175.

72. — —, — — and HALE, C. R., 1976: Temperature and enzymic control of malate metabolism in berries of *Vitis vinifera*. Phytochem. **15**, 1877—1880.

73. — — und RAST, D. M., 1974: Die Biogenese von Tartrat in der Weinrebe. Z. Pflanzenphysiol. **73**, 45—55.

74. SAITO, K. and KASAI, Z., 1968: Accumulation of tartaric acid in the ripening process of grapes. Plant and Cell Physiol. **9**, 529—537.

75. — — and — —, 1969: Tartaric acid synthesis from L-ascorbic acid-1-^{14}C in grape berries. Phytochem. **8**, 2177—2182.

76. — — and — —, 1978: Conversion of labeled substrates to sugars, cell wall polysaccharides, and tartaric acid in grape berries. Plant Physiol. **62**, 215—219.

77. — — and LOEWUS, F. A., 1979: The metabolism of L-(6-^{14}C)-ascorbic acid in detached grape leaves. Plant and Cell Physiol. **20**, 1481—1488.

78. SKENE, K. G. M. and HALE, C. R., 1970: Organic acid synthesis by grape berries cultured *in vitro*. Phytochem. **10**, 1779—1781.

79. STAFFORD, H. A., 1959: Distribution of tartaric acid in the leaves of certain Angiosperms. Amer. J. Bot. **46**, 347—352.

80. — —, 1961: Distribution of tartaric acid in the Geraniaceae. Amer. J. Bot. **48**, 699—701.

81. — — and LOEWUS, F. A., 1958: The fixation of $^{14}CO_2$ into tartaric and malic acids of excised grape leaves. Plant Physiol. **33**, 194—199.

82. STEFFAN, H., 1968: Untersuchungen über Veränderungen von Inhaltsstoffen in reifenden Beeren der Rebe mit ^{14}C-Verbindungen. Diss. Universität Karlsruhe.

83. — — und RAPP, A., 1979: Ein Beitrag zum Nachweis unterschiedlicher Malatpools in Beeren der Rebe. Vitis **18**, 100—105.

84. — —, — —, ULLEMEYER, H. und KUPFER, G., 1975: Über den reifeabhängigen Säure-Zucker-Stoffwechsel bei Beeren von *Vitis-vinifera*-Sorten, untersucht mit ^{14}C-Verbindungen. Vitis **14**, 181—189.

85. SWANSON, C. A. and EL-SHISHINY, E. D. H., 1958: Translocation of sugars in the Concord grape. Plant Physiol. **33**, 33—37.

86. TAKIMOTO, K., SAITO, K. and KASAI, Z., 1976: Diurnal change of tartrate dissimilation during the ripening of grapes. Phytochem. **15**, 927—930.

87. — —, — — and — —, 1977: Conversion of tartrate to malate and monoethyl tartrate in grape leaves. Phytochem. **16**, 1641—1645.
88. ULRICH, R., 1970: Organic acids. In: HULME, A. C. (Ed.): The Biochemistry of Fruits and their Products. Vol. I, 89—118.
89. VICKERY, H. B. and PALMER, J. K., 1954: The metabolism of the organic acids of tobacco leaves. VII. Effect of culture of excised leaves in solutions of (+)-tartrate. J. Biol. Chem. **207**, 275—285.
90. WAGNER, G. and LOEWUS, F., 1973: The biosynthesis of (+)-tartaric acid in *Pelargonium crispum*. Plant Physiol. **52**, 651—654.
91. — — and — —, 1974: L-ascorbic acid metabolism in Vitaceae. Plant Physiol. **54**, 784—787.
92. — —, YANG, J. C. and LOEWUS, F. A., 1975: Stereoisomeric characterization of tartaric acid produced during L-ascorbic acid metabolism in plants. Plant Physiol. **55**, 1071—1073.
93. WEJNAR, R., 1965: Der Einfluß der Temperatur auf die Bildung der Zucker, Äpfelsäure und Weinsäure in Weintrauben. Ber. Dtsch. Bot. Ges. **78**, 314—321.
94. — —, 1967: Weitere Untersuchungen zum Einfluß der Temperatur auf die Bildung der Äpfelsäure in Weinbeeren. Ber. Dtsch. Bot. Ges. **80**, 447—450.
95. — —, 1969: Untersuchungen zum Säurestoffwechsel reifender Beeren von *Vitis vinifera* L. Flora, Abt. A **160**, 211—216.
96. WILLIAMS, M. and LOEWUS, F. A., 1978: Biosynthesis of (+)-tartaric acid from L-(^{14}C)-ascorbic acid in grape and geranium. Plant Physiol. **61**, 672—674.
97. — —, SAITO, K. and LOEWUS, F. A., 1979: Ascorbic acid metabolism in geranium and grape. Phytochem. **18**, 953—956.
98. WINKLER, A. J., COOK, J. A., KLIEWER, W. M. and LIDER, L. A., 1974: General Viticulture, 138—196. University of California Press, Berkeley, Los Angeles, London.
99. ZINDLER-FRANK, E., 1974: Die Differenzierung von Kristallidioblasten im Dunkeln und bei Hemmung der Glykolsäureoxidase. Z. Pflanzenphysiol. **73**, 313—325.