# THE SURFACE WAXES OF THE SULTANA VINE (*VITIS VINIFERA* cv. THOMPSON SEEDLESS)

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*Summary*

The surface wax from leaves, fruits, and stems of the sultana vine obtained by extraction with cold chloroform has been analysed by chromatographic methods. The composition of the hydrocarbon, alcohol, and acid fractions was determined by gas–liquid chromatography. No considerable changes in the amount of wax during fruit or leaf development has been observed. Leaves were found to have only 0·008–0·011 mg wax per square centimetre, whereas 0·09–0·11 mg wax per square centimetre was found on grapes. The surface wax of grapes at all developmental stages contained oleanolic acid as the major constituent, but this compound was present only in small amounts on leaves. That part of all waxes soluble in light petroleum had a similar composition.

Normal aldehydes were found to be present in grape wax and in the wax from mature leaves. Wax from young leaves of a size of less than 30 $cm^2$ contained no aldehydes but instead a series of esters of normal acids with one alcohol. This was not positively identified but showed a retention time approximating to that of nonanol or decanol.

## I. Introduction

The chemical composition of the grape has attracted many investigators, but the lipids and particularly the surface waxes from grapes and leaves have received comparatively little attention. The earliest investigators isolated oenocarpol alcohol (Etard 1892) and vitin (Seifert 1893) from grapes. These compounds were later shown to be probably identical with oleanolic acid by Markley, Sando, and Hendricks (1938) who examined the lipids from milled pomace of Concord grapes (*Vitis labrusca*). However, these lipids would have included, besides the surface waxes, those from the fruit flesh and from the grape-seed oil. Using chromatographic methods Radler and Horn (1965) have found that the surface waxes of ripe sultana grapes consist largely of oleanolic acid with lesser amounts of n-alcohols, n-aldehydes, esters, free acids, and n-hydrocarbons. An extension of this work has been the determination of the amount and composition of the surface waxes of different parts of the sultana vine at several developmental stages.

## II. Material and Methods

Grapes, leaves, and stems of the sultana cultivar Thompson Seedless and of a mutant of sultana—"Bruce's Sport" (Antcliff and Webster 1962)—were collected from the experimental vineyards of the Horticultural Research Section located at Merbein, Vic., during the seasons 1963–64 and 1964–65.

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The surface waxes of fresh plant material were extracted by immersion in cold chloroform ($3 \times 10$ sec), following the suggestion of Martin (1960). Larger amounts

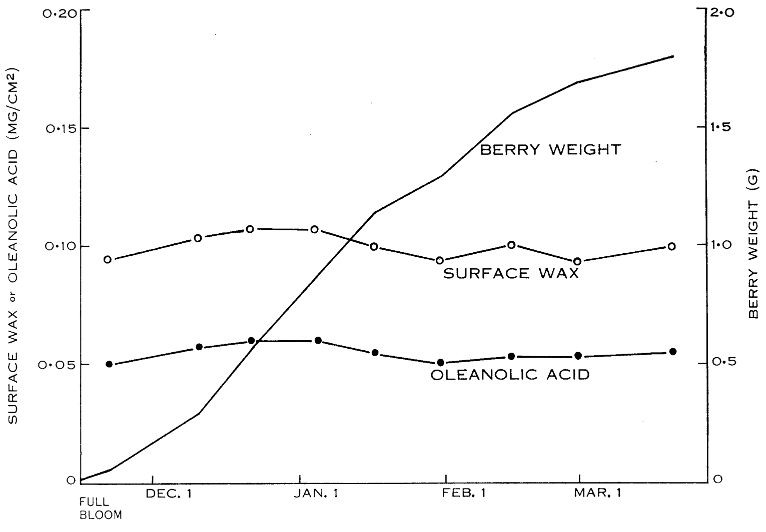


Fig. 1.—Average amount of surface wax and oleanolic acid on the cuticle of developing sultana grape berries.

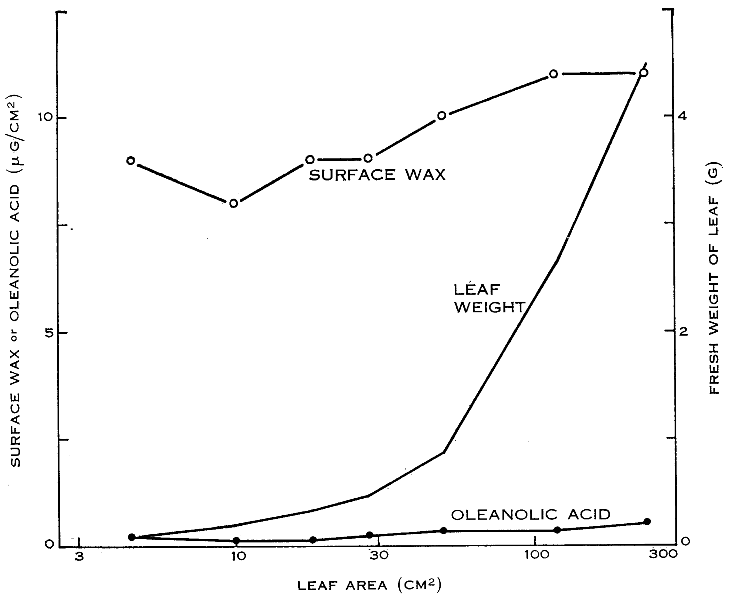


Fig. 2.—Amount of surface wax and oleanolic acid on the cuticle of leaves from sultana vines at different stages of development. The leaf size is plotted logarithmically. Leaves collected December 4, 1964.

of surface wax were extracted by pouring three lots of fresh cold chloroform over the plant material. Dried grapes were extracted for 3 hr with chloroform (Soxhlet). The surface area of leaves was estimated according to the method of Williams (1954), and the surface area of grapes was calculated from the berry volume, with the assumption that the berries are round.

The methods of examination of the waxes were essentially as described by Radler and Horn (1965). The chloroform extracts were re-extracted with light petroleum (b.p. 40–60°C). The soluble fraction (soft wax) was chromatographed on aluminium

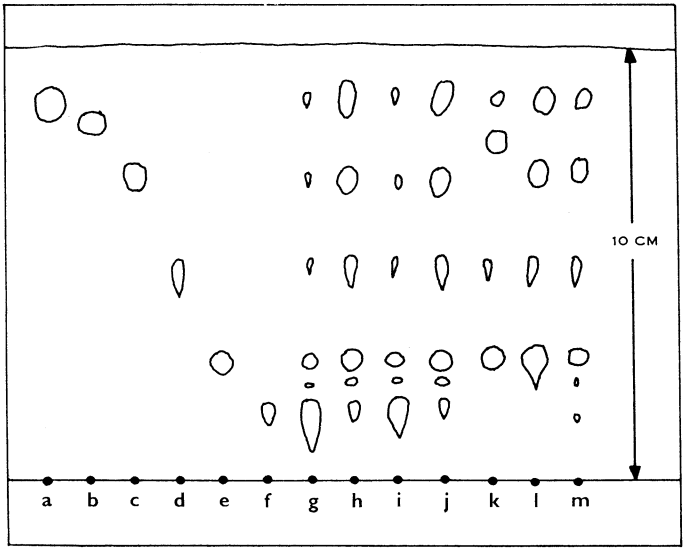


Fig. 3.—Thin-layer chromatogram of wax from sultana grapes and leaves using silica gel activated at 90°C for 30 min. Solvents: light petroleum–ether–acetic acid (70 : 30 : 1·5 v/v). Spray reagent: 5% $K_2Cr_2O_7$ in 40% $H_2SO_4$.

*a*, 10 μg of the hydrocarbon fraction from sultana grapes;
*b*, 10 μg octyl docosanoate;
*c*, 10 μg of the aldehyde fraction from mature sultana leaves;
*d*, 20 μg docosanoic acid;
*e*, 20 μg of a mixture of the n-alcohols 24 : 0 and 28 : 0;
*f*, 20 μg oleanolic acid;
*g*, 50 μg of whole wax from young sultana grapes;
*h*, 50 μg of the light petroleum-soluble part of the wax from young sultana grapes;
*i*, 50 μg of whole wax from mature sultana grapes;
*j*, 50 μg of the light petroleum-soluble part of the wax from mature sultana grapes;
*k*, 50 μg of whole wax from young sultana leaves;
*l*, 50 μg of whole wax from mature sultana leaves;
*m*, 50 μg of whole wax from sultana stems.

oxide, eluting in turn with light petroleum (b.p. 40–60°C), ether (saturated with water), ether plus 1% ethanol, chloroform plus 1% ethanol, and acetic acid. The fractions so obtained were then examined by gas–liquid chromatography. The waxes and all fractions were also analysed by thin-layer chromatography, which was employed as a check of their uniformity. Since grape waxes have been shown to contain aldehydes, which are destroyed by chromatography on alumina (Radler and Horn 1965), this fraction of mature leaves and the corresponding ester fraction from young leaves were obtained by chromatography on silicic acid.

The esters of long-chain fatty acids with the normal $C_6$, $C_8$, and $C_{10}$ alcohols were prepared for reference purposes. 1 g of docosanoic acid (Eastman Kodak), which contained considerable amounts of normal $C_{16}$, $C_{18}$, $C_{20}$, and $C_{24}$ acids, was refluxed for 3 hr with 10 g of the respective alcohol with an addition of 0·5 ml conc. sulphuric acid as catalyst. After addition of light petroleum the sulphuric acid was washed out with water and the surplus alcohol was removed under vacuum at 100°C. Thin-layer chromatography showed that the esterification was complete.

The oleanolic acid content of the wax was determined by the Liebermann–Burchard reaction (Brieskorn and Hofmann 1962).

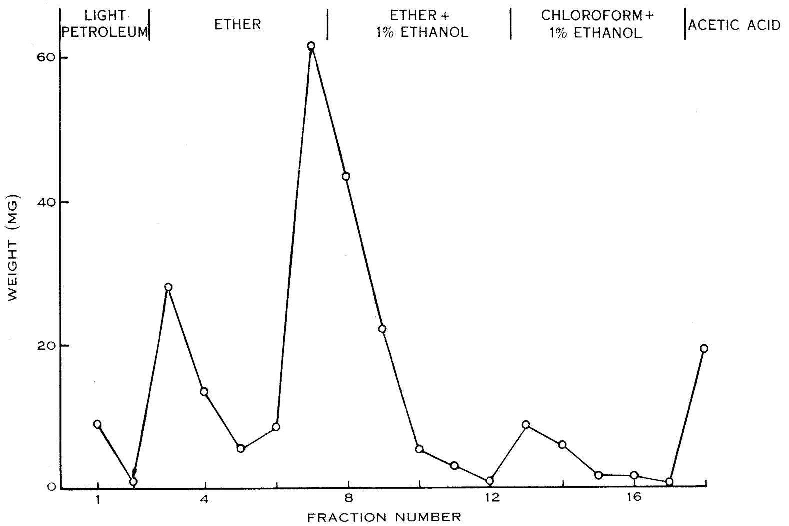


Fig. 4.—Fractionation of the light petroleum-soluble part of wax from young sultana grapes on a column of aluminium oxide (20 g, activity grade II). Fraction No. 1 was 80 ml and fraction No. 18 100 ml, all other fractions 20 ml.

## III. Results

Results have shown that the cuticular wax of the grape (the "bloom") is already present on very young fruit and the amount increases in step with the growth of the fruit. This may be seen from Figure 1, where the ratio wax weight per surface area hardly changes during the development of the fruit. The same result applies to the main constituent oleanolic acid.

Like the grape wax, the proportion of leaf wax (expressed as weight per area) also changes little during growth, only a slight increase being indicated (Fig. 2). The amount of wax on leaves is much lower (*c.* 10 $\mu g/cm^2$) compared with the value for the wax of grapes (*c.* 0·1 $mg/cm^2$). Oleanolic acid, the major constituent of grape wax, is present on leaves in very small amounts only.

Most of the leaf and stem wax was soluble in light petroleum, whereas the larger part of the fruit wax, consisting mainly of oleanolic acid, was insoluble in that solvent (hard wax). Thin-layer chromatography showed that the light petroleum-soluble fractions of waxes from grapes, stems, and mature leaves are similar in their overall composition (Fig. 3). The wax from young leaves contains a compound that is not present in the wax of grapes or mature leaves. The waxes soluble in light petroleum were fractionated, by chromatography on aluminium oxide, into hydrocarbons, an "ester–aldehyde" fraction, free alcohols, and free acids. Figure 4 shows a typical fractionation of a wax and similar figures were obtained with all waxes. The quantitative results are compiled in Table 1. The composition of the light petroleum-soluble fractions of all the waxes appears to be similar. If the content of oleanolic acid is not included, the hydrocarbon fraction, the ester–aldehyde fraction, the free alcohol fraction, and the free acid fraction of the waxes vary only between 2–4, 13–20, 40–59, and 4–27%, respectively.

Table 1

MAIN FRACTIONS OF THE SURFACE WAX OF DIFFERENT PARTS OF THE SULTANA VINE

Values expressed as a percentage of the whole wax (extracted with chloroform). Percentage of whole wax of light petroleum-soluble fraction is given in parenthesis

| Fraction | Leaf | | Stem | Grape | | | Mature Grape of Mutant |
|---|---|---|---|---|---|---|---|
| | Young | Mature | | Young | Mature | Dried | |
| Light petroleum-soluble | 77 | 85 | 98 | 31 | 12 | 44 | 23 |
| Oleanolic acid | 1 | 1 | 5 | 45 | 65 | 50 | 68 |
| Hydrocarbon | 2 (2) | 2 (2) | 3 (3) | 1 (4) | 1 (5) | 1 (2) | 1 (2) |
| "Ester–aldehyde" | 10 (14) | 11 (13) | 13 (13) | 6 (20) | 2 (15) | 7 (15) | 4 (16) |
| Alcohol | 48 (62) | 61 (71) | 40 (40) | 17 (56) | 5 (42) | 22 (49) | 14 (59) |
| Acid | 8 (10) | 8 (10) | 27 (27) | 2 (7) | 1 (4) | 6 (13) | 1 (6) |
| Not eluted | 9 (12) | 3 (4) | 16 (16) | 5 (13) | 3 (34) | 8 (21) | 3 (17) |

The hydrocarbon, alcohol, and acid fractions were further separated according to chain length by gas–liquid chromatography. A typical gas–liquid chromatogram of these fractions is presented in Figure 5.

Table 2 gives the composition of the hydrocarbon fraction of the waxes. The hydrocarbons are mainly normal paraffins of odd and even carbon number ranging from n-$C_{18}$ to n-$C_{35}$, with traces of lower homologues. Unidentified hydrocarbons comprise 1–6% of the total. In the leaf wax the chain length shows a maximum at n-$C_{29}$, which is not shown by the other waxes. The wax of young grapes contains a higher amount of hydrocarbons with shorter chain lengths (maximum at $C_{21}$) than the waxes from the other parts of the sultana vine. The hydrocarbons of the mutant Bruce's Sport are similar to the original variety.

The alcohol fractions of all parts of the sultana vine have a similar composition (Table 3). The predominant alcohol of the grape wax is n-$C_{26}$, leaf wax contains more of n-$C_{28}$, and in the stem wax both alcohols are found in almost equal quantities. The alcohol fractions of all waxes contain, in varying amounts, one unidentified

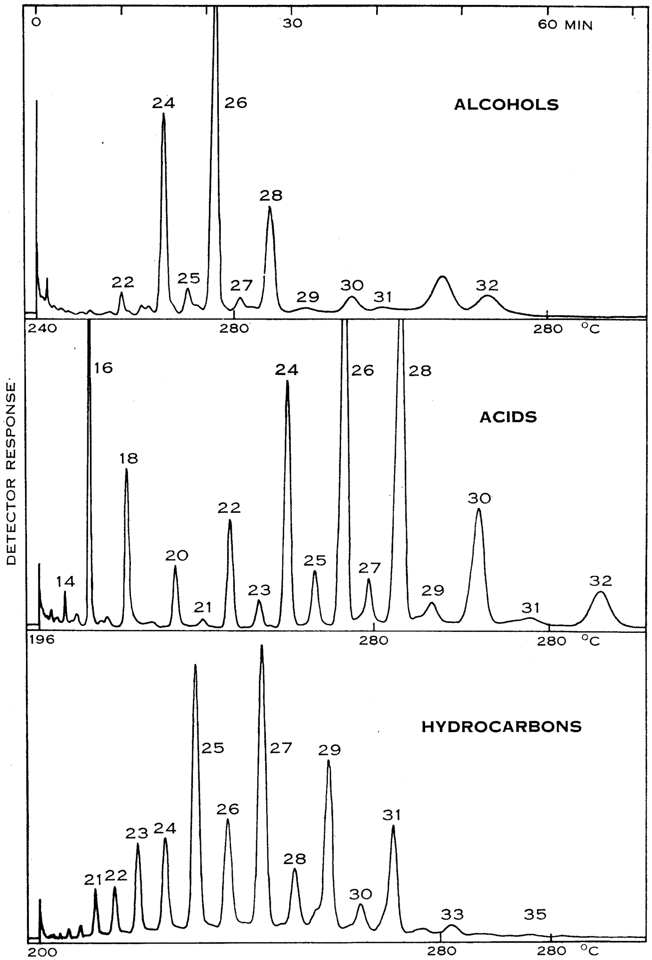


Fig. 5.—Separation of the alcohol, acid, and hydrocarbon fractions of wax from sultana grapes by gas–liquid chromatography. The numbers at the peaks indicate the chain length of the corresponding compound. The alcohols and acids are separated after conversion to acetates and methyl esters respectively. Column: 15% silicone grease on Chromosorb W (80–100 mesh). Column temperature: linear-programmed as indicated; nitrogen flow rate 50 ml/min.

compound (or series of compounds) with an $R_F$ value (on thin-layer chromatography) lower than the normal alcohols (Fig. 3). Since this spot gave a weak Liebermann–Burchard reaction and a red colour with phosphoric acid spray reagent [50% (v/v) in water] it may be a triterpene alcohol or sterol.

The general pattern of the distribution of the acids (Table 4) which range from n-$C_{14}$ to n-$C_{32}$ is more uniform. There is no distinct maximum as with the alcohols and the distribution of the acids resembles that of the hydrocarbons. Only the acids of the young leaf wax show a pronounced maximum at n-$C_{24}$. The waxes of the mature fruit of the sultana and of the mutant contain the acids with the chain lengths n-$C_{24}$ and n-$C_{26}$ in similar amounts. Dried grapes contain more of the n-$C_{28}$ acid, but it is difficult to assess the significance of this finding because of the different extraction method used to remove the wax of dried grapes.

TABLE 2

COMPOSITION OF THE HYDROCARBON FRACTION OF THE SURFACE WAX FROM DIFFERENT PARTS OF THE SULTANA VINE

Values as percentage of the total fraction; tr., $<0\cdot3\%$; +, $<1\%$

| Carbon No. | Leaf | | Stem | Grape | | | Mature Grape of Mutant |
|---|---|---|---|---|---|---|---|
| | Young | Mature | | Young | Mature | Dried | |
| <18 | + | + | + | 2 | + | 1 | 1 |
| 18 | + | tr. | tr. | + | tr. | tr. | + |
| 19 | + | tr. | tr. | 7 | + | + | + |
| 20 | + | tr. | tr. | 1 | + | + | + |
| 21 | + | + | + | 21 | 2 | 2 | 4 |
| 22 | 2 | 1 | 1 | 2 | 3 | 2 | 3 |
| 23 | 4 | 4 | 3 | 16 | 7 | 5 | 8 |
| 24 | 3 | 3 | 3 | 4 | 7 | 6 | 4 |
| 25 | 8 | 10 | 16 | 11 | 19 | 19 | 20 |
| 26 | 3 | 6 | 5 | 2 | 5 | 9 | 3 |
| 27 | 17 | 17 | 21 | 10 | 18 | 21 | 16 |
| 28 | 4 | 8 | 4 | 2 | 4 | 5 | 2 |
| 29 | 37 | 30 | 16 | 10 | 15 | 14 | 14 |
| 30 | 3 | 3 | 3 | 2 | 3 | 2 | 2 |
| 31 | 14 | 12 | 17 | 4 | 11 | 9 | 15 |
| 32 | | + | 2 | | 1 | + | + |
| 33 | + | tr. | 3 | 2 | + | 1 | + |
| 34 | | | + | | | + | |
| 35 | | | 2 | 3 | + | 1 | |
| Sum of minor unknown peaks | 1 | 6 | 3 | | 2 | | 6 |

The ester–aldehyde fraction has only been analysed from young and mature leaves by separation on silicic acid. The presence of aldehydes in the wax of mature leaves was confirmed by analysis of this fraction by gas–liquid chromatography. The chain-length distribution of the main components of the aldehydes of mature sultana leaves was: < n-$C_{26}$ 3%, n-$C_{26}$ 10%, n-$C_{27}$ 3%, n-$C_{28}$ 28%, n-$C_{29}$ 3%, n-$C_{30}$ 40%, n-$C_{31}$ 2%, n-$C_{32}$ 11%.

No aldehydes could be isolated from young leaves of a size of less than 30 $cm^2$. From the young leaves a fraction was obtained by chromatography on silicic acid which showed strong ester bands at 1737 and 1175 $cm^{-1}$ and no appreciable hydroxyl absorption between 3200 and 3500 $cm^{-1}$. Thin-layer chromatography of this fraction showed as a minor impurity a spot corresponding to the normal esters present in wax and with an $R_F$ value similar to hydrocarbons. Hydrolysis of the ester fraction (8 mg) yielded acids (6 mg) and only traces of unsaponifiable material. The unknown esters from young leaves could be separated according to chain length by gas–liquid chromatography (Fig. 6). The composition of the unknown ester fraction was identical to the methyl esters, derived therefrom by methylation with methanol–sulphuric acid (Table 5) and the logarithm of the retention times of both series

Table 3

COMPOSITION OF THE FRACTION CONTAINING FREE ALCOHOLS FROM THE SURFACE WAX OF DIFFERENT PARTS OF THE SULTANA VINE

Values expressed as percentage of total acetyl ester; tr., $<0\cdot3\%$; +, $<1\%$

| Carbon No. | Leaf | | Stem | Grape | | | Mature Grape of Mutant |
|---|---|---|---|---|---|---|---|
| | Young | Mature | | Young | Mature | Dried | |
| <20 | tr. | tr. | tr. | tr. | tr. | 4 | tr. |
| 20 | tr. | tr. | tr. | + | + | + | tr. |
| 21 | tr. | tr. | tr. | tr. | + | tr. | tr. |
| 22 | 2 | 1 | 2 | 3 | 1 | 1 | 2 |
| 23 | tr. | + | + | + | + | + | + |
| 24 | 10 | 7 | 7 | 16 | 19 | 17 | 20 |
| 25 | + | + | 2 | 2 | 2 | 3 | 2 |
| 26 | 25 | 21 | 31 | 41 | 39 | 31 | 40 |
| 27 | 1 | + | 2 | 2 | + | 2 | 2 |
| 28 | 32 | 42 | 28 | 20 | 17 | 13 | 20 |
| 29 | 1 | 1 | 1 | + | + | 2 | + |
| 30 | 18 | 16 | 8 | 5 | 7 | 4 | 6 |
| 31 | | | | | | 2 | |
| 32 | 9 | 4 | 7 | 3 | 5 | 7 | 3 |
| 34 | + | tr. | tr. | 2 | tr. | tr. | + |
| Sum of minor unknown peaks | + | 5 | 11 | 3 | 8 | 14 | 3 |

plotted against the carbon numbers formed a straight line (Fig. 7). This indicates that the acids were of an homologous series and were esterified with one alcohol only. The comparison of this ester series from young leaves with the esters of acids with n-hexanol, n-octanol, and n-decanol led to the conclusion that this alcohol has a chain length around $C_9$ or $C_{10}$ (Table 6). An alcohol of such characteristics could be recovered by hydrolysis of the "unknown ester" from young leaves, if the unsaponifiable material was not evaporated to dryness under vacuum (water-pump), as alcohols of this chain length are volatile under these conditions. The peak of the alcohol from the leaf esters appeared between those of n-octanol and n-decanol at 135°C on a silicone grease column. The unknown alcohol could be nonanol or a higher homologue with a branched chain. This alcohol is obviously not unsaturated as bromination of the esters from young sultana leaves did not result in a change of their chromatographic characteristics.

## IV. Discussion

Sultana grapes contain about 0·09–0·11 mg wax per square centimetre, in agreement with values reported by Dudman and Grncarevic (1962), whereas leaves contain only one-tenth of this amount. During growth the waxes of grapes and grape leaves show only little or no increase in the amount per surface area. Silva Fernandes, Batt, and Martin (1964) have observed an increase of about 50–100% in the amount of surface wax per square centimetre in the growth of apples, but little change in the amount of surface wax of growing pears.

Table 4

COMPOSITION OF THE FRACTIONS CONTAINING FREE FATTY ACIDS FROM THE SURFACE WAX FROM DIFFERENT PARTS OF THE SULTANA VINE

Values expressed as percentage of the methylated fraction; tr., <0·3%; +, <1%

| Carbon No. | Leaf | | Stem | Grape | | | Mature Grape of Mutant |
|---|---|---|---|---|---|---|---|
| | Young | Mature | | Young | Mature | Dried | |
| <14 | tr. | tr. | tr. | tr. | tr. | 1 | tr. |
| 14 | + | tr. | + | tr. | + | + | + |
| 15 | tr. | tr. | tr. | + | tr. | tr. | tr. |
| 16 | 4 | 3 | 3 | 9 | 6 | 10 | 4 |
| 17 | tr. | + | tr. | 1 | + | tr. | tr. |
| 18 | 4 | 4 | 3 | 12 | 7 | 6 | 6 |
| 19 | 1 | + | tr. | 1 | | + | + |
| 20 | 3 | 9 | 6 | 5 | 3 | 4 | 7 |
| 21 | tr. | 1 | + | + | + | + | + |
| 22 | 10 | 17 | 14 | 7 | 3 | 5 | 4 |
| 23 | + | 3 | 2 | + | 1 | 2 | + |
| 24 | 36 | 23 | 22 | 15 | 14 | 11 | 19 |
| 25 | 1 | 2 | 2 | 2 | 4 | 4 | 3 |
| 26 | 19 | 12 | 20 | 16 | 14 | 18 | 17 |
| 27 | + | 1 | 2 | 1 | 2 | 3 | 4 |
| 28 | 7 | 9 | 14 | 9 | 12 | 21 | 14 |
| 29 | + | + | + | + | 3 | 3 | 3 |
| 30 | 3 | 5 | 4 | 3 | 8 | 7 | 8 |
| 31 | + | | tr. | + | 3 | + | + |
| 32 | + | + | 1 | + | 4 | 3 | 3 |
| Unknown | 8 | 10 | 4 | 15 | 15 | 1 | 5 |

The analysis of the composition of the cuticular waxes of different parts of the sultana vine showed that these waxes are not uniform. Grapes contain in the cuticle a high content of oleanolic acid, whereas this compound is present only in low quantities on the cuticle of leaves and stems. It is not a general feature that triterpene acids are present on fruits only. Apples and apple leaves contain 40% and more ursolic acids in the cuticular wax (Silva Fernandes, Batt, and Martin 1964) and olive

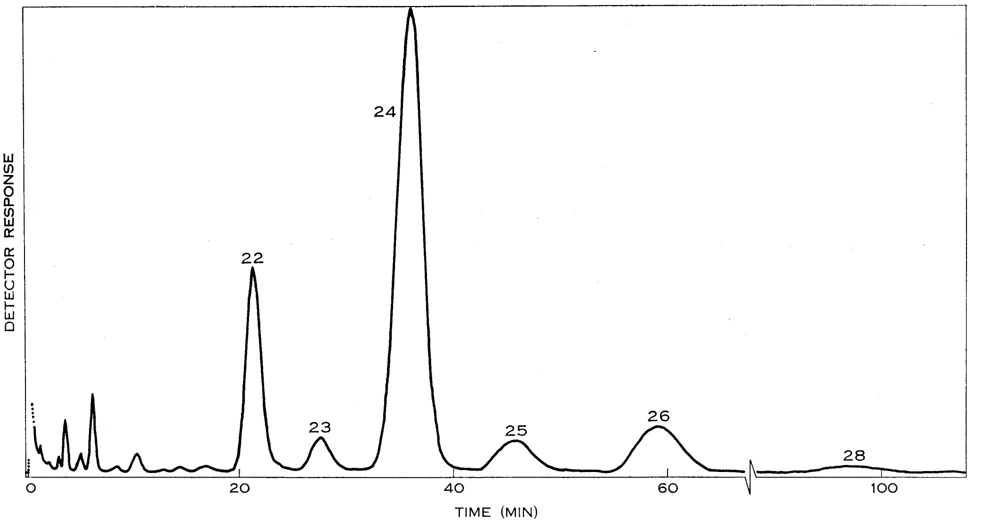


Fig. 6.—Separation of the esters from the wax of young sultana leaves by gas–liquid chromatography. The numbers at the peaks indicate the chain length of the acid of the corresponding ester. Column: 15% silicone grease on Chromosorb W (80–100 mesh). Column temperature: 280°C; nitrogen flow rate 48 ml/min.

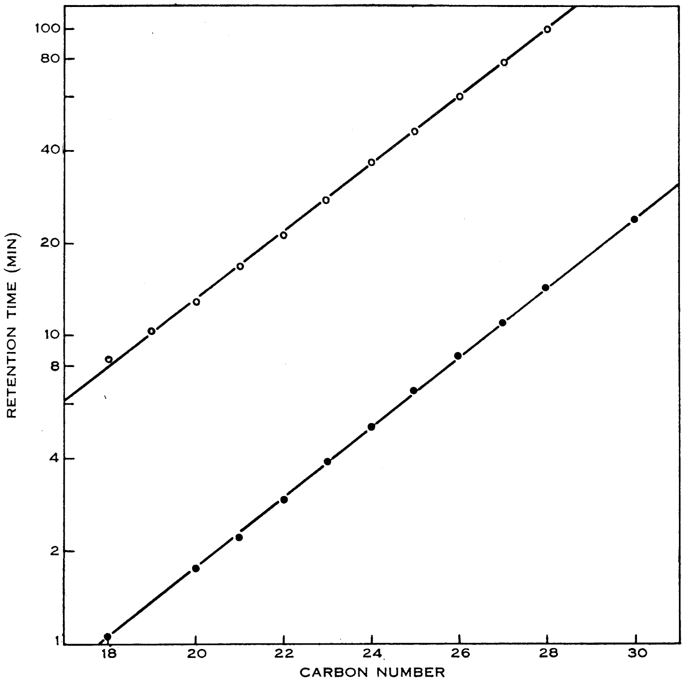


Fig. 7.—Composition of the esters of wax from young sultana leaves. Retention times of the esters from leaves and the derived methyl esters are plotted on a logarithmic scale against the carbon numbers of the acids. Column: 15% silicone grease on Chromosorb W (80–100 mesh). Column temperature: 280°C; nitrogen flow rate 48 ml/min.

leaves are a rich source of oleanolic acid. Markley, Sando, and Hendricks (1938) have isolated oleanolic acid from grape pomace and they discussed the significance of the occurrence of different triterpene acids in leaves and fruit on one species. They

TABLE 5

COMPOSITION OF THE "ESTER FRACTION" OF THE YOUNG SULTANA LEAF AND OF THE METHYL ESTERS OBTAINED FROM THIS FRACTION

| Carbon No. of Acid | Esters from Young Leaf (%) | Methyl Esters (%) |
|---|---|---|
| <22 | 4 | 2 |
| 22 | 16 | 18 |
| 23 | 3 | 1 |
| 24 | 60 | 62 |
| 25 | 5 | 1 |
| 26 | 9 | 11 |
| 27 | — | 1 |
| 28 | 2 | 4 |
| 30 | — | — |

were referring, however, to a paper of Kuwada and Matsukawa (1933) describing the isolation of ursolic acid not from the vine grape but from the leaves of the bear grape (*Arctostaphylus uva ursi*).

TABLE 6

RELATIVE RETENTION VALUES OF ESTERS OF NORMAL ACIDS WITH NORMAL ALCOHOLS AND THE ESTERS FROM SULTANA LEAVES (METHYLPALMITATE = 1·0)

Column: 15% silicone grease on Chromosorb W (80–100 mesh). Column temperature: 280°C; nitrogen flow rate: 48 ml/min. The retention time of methylpalmitate is 38 sec

| Carbon No. of Acid | Methyl Ester | Hexyl Ester | Octyl Ester | Decyl Ester | Ester from Leaf Wax |
|---|---|---|---|---|---|
| 16 | 1·00 | 3·00 | 5·00 | 8·25 | 7·75 |
| 18 | 1·62 | 5·12 | 8·50 | 13·8 | 12·5 |
| 20 | 2·75 | 8·62 | 14·3 | 23·4 | 19·7 |
| 22 | 4·62 | 14·7 | 24·2 | 39·5 | 32·9 |
| 24 | 7·87 | 24·3 | 40·0 | 65·3 | 56·5 |
| 26 | 10·2 | | | | 92·2 |
| 28 | 22·4 | | | | 150 |

The composition of that fraction of the wax soluble in light petroleum is quite similar in all parts of the grape. The exception is the young leaf, which contains a series of esters with the same alcohol component instead of aldehydes. It is unlikely that those components are converted to the aldehydes at later stages of the leaf development. Probably these esters are not formed beyond a certain stage of development of the leaf. It is tempting to speculate that this shift in the formation of esters to aldehydes might be correlated to the periods of cell division and cell elongation in leaf growth of grapes. It is noteworthy that the n-$C_{24}$ acid is the major component of the free acid fraction and the esters in the wax of young sultana leaves.

It has been demonstrated that the soft wax fraction of the grape determines the water permeability of the cuticle (Radler 1965); the hard wax fraction, consisting mainly of oleanolic acid, together with the rest of the cuticle (i.e. cutin, cuticular layers, pectin, cellulose) does not prevent the water permeability to any great extent. The cuticular wax of the leaves contains only low quantities of oleanolic acid and thus consists mainly of the fractions that are active in the prevention of water permeability.

## V. Acknowledgments

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