# Identification of Ethyl Acid Tartrate and One Isomer of Ethyl Acid Malate in California Flor Sherry

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Ethyl acid tartrate and the ethyl acid malate isomer having the free carboxylic acid group adjacent to the carbinol group have been isolated from a California Palomino flor sherry wine by gradient elution from an ion exchange column followed by gas chromatographic purification of the methylated (diazomethane) acid esters. Identification was accomplished by comparison of the infrared and mass spectra of the methylated wine esters with synthetic compounds of known structure. The presence of a single isomer of ethyl acid malate in the wine suggests that it is produced enzymatically during the primary fermentation. Hydrogen ion–catalyzed reaction of ethanol with malic acid yields a mixture of two parts of the isomer having the free carboxylic acid group adjacent to the carbinol group and one part of the other isomer.

Ribéreau-Gayon and Peynaud in 1961 (*11*), reviewing earlier research, showed that a number of white Bordeaux wines contained from 1.8 to 6.0 meq. per liter of acid esters, while in comparable reds the range was from 1.3 to 4.7 meq. per liter. These values were obtained by taking the difference between the total and the neutral ester determinations, total esters being measured by a saponification procedure and neutral esters (assumed to be volatile and soluble in petroleum ether) by a distillation or extraction procedure. Ribéreau-Gayon and Peynaud state that the neutral esters are formed principally by biological action, whereas the acid esters are formed predominantly by chemical esterification during the aging of the wine subsequent to the fermentation. Indicated as apt to be present among the acid esters in wines were ethyl acid tartrate, ethyl acid malate, and ethyl acid succinate.

Acid esters have recently been isolated and identified in foods. Yokotsuka and Goto (*19*) found two isomers of monoethyl citrate in Japanese plum liquor and Jansen, Palmer, and Lundin (*5*) isolated asymmetric monoethyl citrate from avocados. Webb, Kepner, and Galetto (*17*) isolated and identified ethyl acid succinate as one of the principal acids in three different types of sherry wines of California. Later, Webb, Ribéreau-Gayon, and Boidron (*18*) found this same acid ester as a constituent of a sample of pure Cabernet Sauvignon wine of the 1959 vintage in Bordeaux. The Bordeaux red wine was 3 years old at the time of analysis, while the sherries varied considerably in age and in method of production. The isolation and identification of ethyl acid tartrate and the ethyl acid malate isomer having the carboxylic acid group adjacent to the carbinol group in California Palomino flor sherry produced by the solera process are the subject of this research report.

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*Experimental*

**Wine.** The wine used was obtained from the last or solera stage of a six-stage fractional blending system. The system was started in 1952 and has used wines produced from Palomino grapes from the University of California Solano County Experimental Vineyards. As operated, no flor is present on the wine in the last two stages of the system. The wine, therefore, more nearly resembles an Amontillado than a fino type.

**Isolation of Acid Esters.** A 5-gallon sample of the sherry was concentrated 10-fold in a low temperature rotary evaporator. The concentrate was placed in a refrigerator overnight to induce crystallization of potassium acid tartrate, which was removed by filtration. The concentrated filtrate was then placed on an ion exchange column (Dowex 1-X 8, 100 to 200 mesh, formate form) and developed by use of gradient elution with continuously increasing concentrations of formic acid (*9*). The fractions from the column were evaporated to dryness and the solid acid residues were analyzed using paper chromatography (*9*).

**Synthesis of Ethyl Acid Tartrate and Its Methyl Ester.** Absolute ethyl alcohol plus an equimolar quantity of tartaric acid together with a few drops of concentrated sulfuric acid was refluxed for 3 hours. The acid ester was separated from the neutral ester and the unreacted acid on an ion exchange column packed with Dowex 1-X 8, 100 to 200 mesh, formate form. Gradient elution, evaporation of the solvent, and analyses of the solid acids by paper chromatography were carried out as described above.

The ethyl acid tartrate was finally purified by converting the acid ester to the methyl ethyl tartrate using diazomethane (*17*) and gas chromatography on a poly(neopentyl glycol adipate) column. To obtain complete separation from dimethyl tartrate, three successive passages through the gas chromatograph were required. The sample was finally purified for infrared analysis by gas chromatography using an SE 30 column.

**Synthesis of Mixed Isomers of Ethyl Acid Malate and Their Methyl Esters.** A mixture containing the two

isomers of ethyl acid malate was synthesized from absolute ethyl alcohol and malic acid by the technique described above. Gas chromatography of the mixture of methyl ethyl malate isomers yielded a single peak when either the poly(neopentyl glycol adipate) or SE 30 column was used.

**Synthesis of Methyl Ethyl Malate with the Carbethoxy Group Adjacent to the Carbinol Group. $C_2H_5OOCCHOHCH_2COOR$; I, R = $CH_3$; I-A, R = H.** A Reformatsky-type reaction following the procedure of Stoll (*16*) and with workup of the reaction products as described by Hauser and Breslow (*4*) was used to synthesize I. The ethyl glyoxylate required for reaction with methyl bromoacetate in this synthesis was prepared by $NaBiO_3$ oxidation of diethyl tartrate (*12*). Ethyl glyoxylate was isolated from the oxidation mixture and purified by vacuum distillation before being used in the Reformatsky reaction.

Small samples of the above synthesized methyl ethyl malate isomer were purified gas chromatographically using the sequence of operations described for the isolation and purification of the material from the wine.

**Synthesis of Methyl Ethyl Malate with the Carbethoxy Group Beta to the Carbinol Group. $C_2H_5OOCCH_2CHOHCOOR$; II, R = $CH_3$; II-A, R = H.** A Reformatsky reaction using methyl glyoxylate and ethyl bromoacetate in a manner analogous to that employed in the previous synthesis was used to prepare II.

Methyl glyoxylate was obtained by $NaBiO_3$ oxidation of dimethyl tartrate which had been prepared by refluxing an excess of anhydrous methanol with tartaric acid in the presence of a few drops of concentrated $H_2SO_4$.

This methyl ethyl malate isomer was also repurified gas chromatographically as in the isolation and purification of the ester obtained from the wine.

**Infrared Spectra.** A Beckman IR-8 infrared spectrophotometer with ultramicro demountable NaCl cells was used in obtaining the infrared spectra of the various samples. In every case samples were run neat after repeated gas chromatographic purification, the last purification being run on the SE 30 column.

**Mass Spectra.** A Bendix Time-of-Flight mass spectrometer, Model 12, was used in determining the mass spectra of the three synthesized and the one wine isolate of methyl ethyl malate.

*Results and Discussion*

On paper chromatographic analysis of the acids separated on the ion exchange column the wine contains an acid which moves with the same $R_f$ as that of synthetic ethyl acid tartrate. Methylation of this acid from the wine and purification on gas chromatographic columns show the methyl ethyl ester to have retention distances identical to synthetic methyl ethyl tartrate on two gas chromatographic columns (Table I). Figure 1 presents a gas chromatogram showing the separation of the various malate and tartrate esters. The finding of dimethyl malate and dimethyl tartrate in the same fraction with the ethyl methyl malate and ethyl methyl tartrate upon gas chromatography indicates that the separation of the free acids from the acid esters on the ion exchange column was not too clean. Gas chromatographic separation of the dimethyl tartrate from methyl ethyl tartrate was relatively easy, but separation of dimethyl malate from methyl ethyl malate was especially difficult.

**Table I. Retention Distances of Methylated Acid Esters from Sherry and of Knowns**

(Centimeters)

| Gas Chromatographic Column | Temp., ° C. | Wine Ester 1 | Wine Ester 2 | MeEt Tartrate | MeEt Malate |
|---|---|---|---|---|---|
| Carbowax 20 M | 170 | | 6.60 | | 6.60 |
| NPGA | 170 | | 6.10 | | 6.15 |
| SE-30 | 115 | | 5.40 | | 5.35 |
| NPGA | 170 | 14.30 | | 14.30 | |
| SE-30 | 121 | 5.25 | | 5.25 | |

Figure 2 shows the infrared spectra of the methyl ethyl tartrate from the wine and a synthetic sample. The agreement of the two spectra is excellent, with the exception of bands between 750 and 800 $cm.^{-1}$ in the wine ester which are due to traces of $CCl_4$ used in transferring the sample from the capillary collection tube to the infrared cell. Thus, evidently California Palomino flor sherry contains small quantities of ethyl acid tartrate and the earlier postulation of Ribéreau-Gayon and Peynaud that ethyl acid tartrate was one of the acid esters in wines is confirmed.

The flor sherry contained an acid which upon paper chromatography of the fractions separated on the ion exchange column had an $R_f$ equal to that of the mixture of isomers of synthetic ethyl acid malate. Upon methylation with diazomethane both the acid from the wine and the mixture of isomers of ethyl acid malate gave bands with equal $R_f$ values on several different gas chromatographic columns (Table I). The authors thus established that California Palomino flor sherry contains at least one of the isomers of ethyl acid malate.

Figure 3 depicts the infrared spectra of the two synthetic isomers of methyl ethyl malate (I and II) and the methyl ethyl malate obtained by diazomethane treatment of the ethyl acid malate isolated from sherry. Examination of these spectra shows, as would be expected from the similarity of the molecules, that there are only very small differences between the two synthesized isomers. Evidently, the spectrum of the wine-derived ester more nearly resembles that of isomer II, which suggests that the wine contains II-A.

The infrared spectra of the two pure synthetic isomers of methyl ethyl malate show that the only significant differences occur in the region of the CH deformation vibrations (1475 to 1340 $cm.^{-1}$). Consideration of the structures of the two methyl ethyl malate isomers suggests that these small differences in the infrared spectra must arise from secondary effects of interactions between the hydroxyl group and either the methyl or the ethyl group of the ester ends of the molecule. By means of Fisher-Taylor-Hirschfelder molecule models very close approaches and hence weak interactions are possible between the hydroxyl group and the alkyl group of the

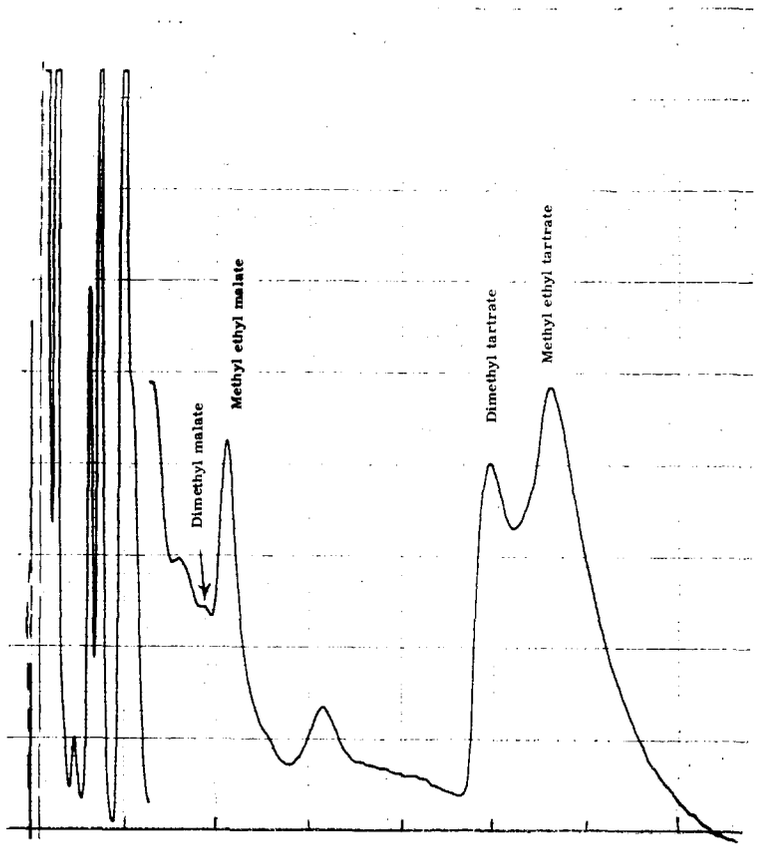


Figure 1. Gas chromatogram showing separation of methylated ethyl acid malate and ethyl acid tartrate

Methylated acids isolated from flor sherry. 10% NPGA on Gas-pack, 1/8 inch × 6 feet, 170° C., 20 ml./minute $N_2$

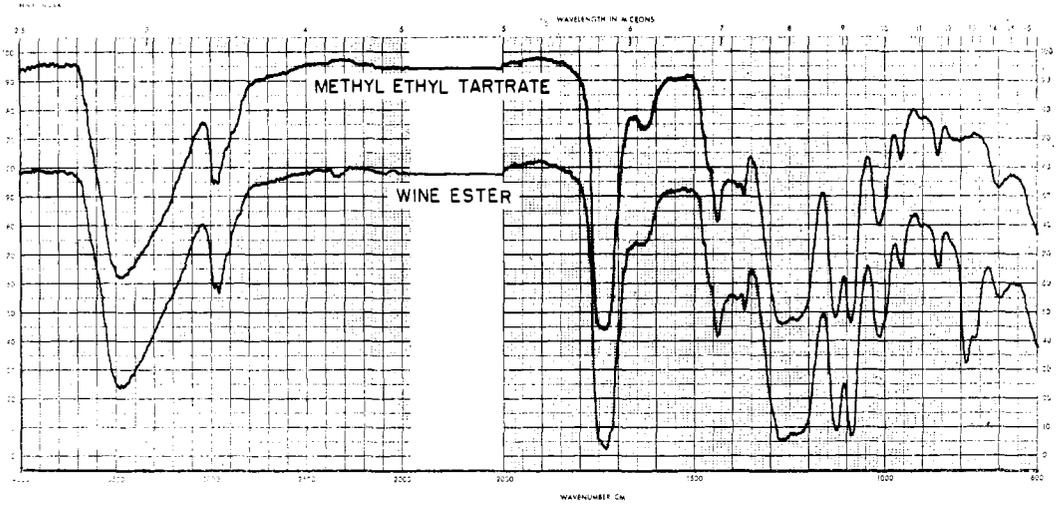


Figure 2. Infrared spectra of methyl ethyl tartrate and methylated ethyl acid tartrate from wine

Beckman IR-8, NaCl ultramicro demountable cell, neat. Wine sample transferred to cell with $CCl_4$

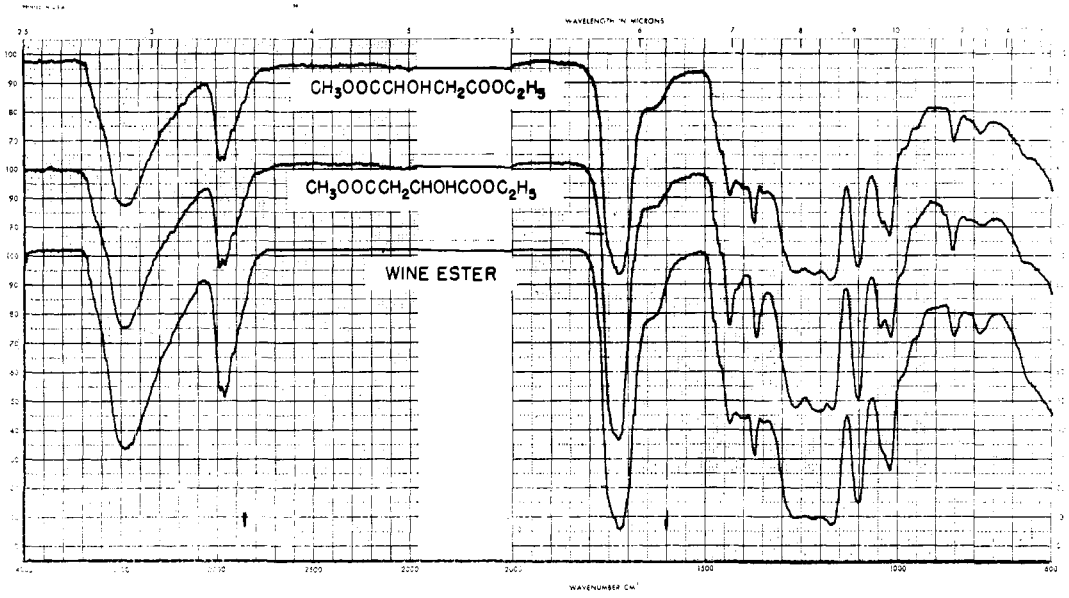


Figure 3. Infrared spectra of two synthetic isomers of methyl ethyl malate and methylated ethyl acid malate from wine

Beckman IR-8, NaCl ultramicro demountable cell, neat
*Upper.* Compound II
*Lower.* Compound I

ester which is farthest removed from the hydroxyl group. According to a number of standard infrared reference works (*2, 7, 10, 15*) and the paper by Francis (*3*) the various bands of interest in these infrared spectra of the methyl ethyl malate isomers are probably due to the following vibrations: 1460 cm.$^{-1}$, $CCH_3$ asymmetrical bending; 1435 cm.$^{-1}$, $OCH_3$ symmetrical bending; 1420 and 1410 cm.$^{-1}$, $CH_2$ adjacent to carbonyl; 1375 cm.$^{-1}$, $CCH_3$ symmetrical bending; 1370 and 1365 cm.$^{-1}$, uncertain—possibly $OCH_2$—plus contribution from some other absorber. The most striking difference between the spectra is the greater intensity of the bands at 1435 cm.$^{-1}$ in the spectrum of isomer I as contrasted with II. According to Francis (*3*), intensification of the absorption owing to the symmetrical bending of the carbon-hydrogen bonds of the methoxy group occurs on close approach of an electron-withdrawing group such as the hydroxyl group. The particular isomer in which band 1435-cm.$^{-1}$ intensification is observed is the one in which close proximity of the ester methoxyl and the hydroxy group is possible and, thus, this interaction may well be responsible for the change in the spectra observed.

Reasoning by analogy, the infrared spectrum for methyl ethyl malate isomer II might be expected to show enhancement of some band owing to interaction between the hydroxy group and the ethoxy group of the ester end of the molecule. The authors have been unable to assign with certainty the bands arising from vibrations of the $OCH_2$ group. The band at approximately 1365 cm.$^{-1}$, which may be partly due to the $OCH_2$ group, is observed to have very small if any difference in intensity between the two ethyl methyl malate isomers. This band, however, is possibly shifted a few wave numbers toward the higher frequency in isomer II. According to Francis (*3*), one normally would expect a decrease in frequency and an augmentation of intensity upon interaction between an electron-withdrawing group such as the hydroxy group and the ethoxy group of the ester.

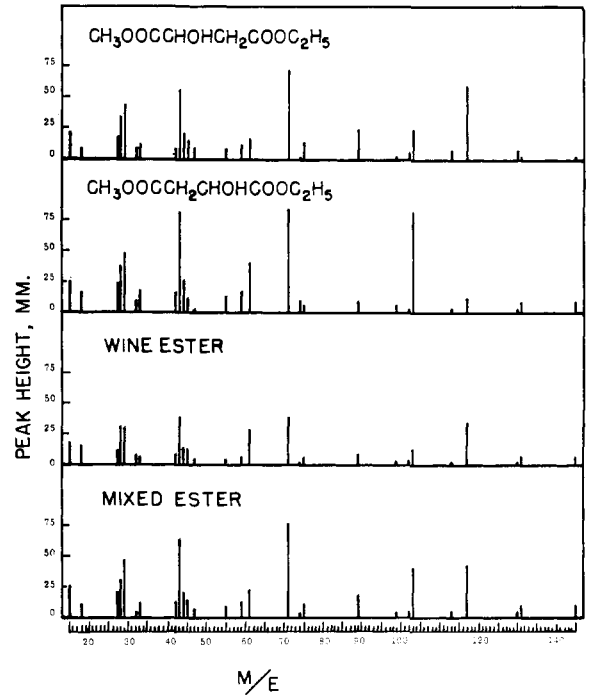


Figure 4. Mass spectra of pure methyl ethyl malate isomers, methylated ethyl acid malate from wine, and methylated ethyl acid malates produced by acid-catalyzed reaction of ethanol and malic acid

Bendix Time-of-Flight mass spectrometer

The anomalous behavior of the 1365-cm.$^{-1}$ band suggests that either, for some reason, there is not close enough approach of the ethoxy group to the hydroxy group to permit significant interaction or that the $OCH_2$- group vibrations do not contribute to the 1365-cm.$^{-1}$ band. The former supposition is probably more likely, as no other band assignable to the ethoxy group is observed to differ in intensity or frequency between the two isomers.

Mass spectra of aliphatic esters have been studied by Beynon, Saunders, and Williams (*1*) and of longer chain dicarboxylic acid esters by Kourey, Tuffly, and Yarborough (*6*). Figure 4 depicts the essential features of the mass spectra for the two synthetic isomers of methyl ethyl malate. The parent peaks for the methyl ethyl malates at *m/e* 176 are so small that they are hardly distinguishable from the background peaks. The base peak at *m/e* 71, in the case of isomer I, probably results from the carbethoxy group minus two hydrogen atoms, while in the case of the other isomer, the peak results from the fragment consisting of the carbmethoxy group attached to a methylene group, the whole unit minus two hydrogen atoms. The very large peak at *m/e* 103, in the case of isomer I, very probably represents the fragment $—CHOHCH_2COOCH_3$. The peak *m/e* 117, in the case of isomer II, probably results from the fragment $—CHOHCH_2COOCH_2CH_3$. The loss of water, observed by Ryhage and Stenhagen (*14*) in their study of long-chain hydroxy esters, apparently does not occur as a simple process in these cases, as peaks at *m/e* 158 are not observed for either isomer. Rather, fragmentation between the carbinol carbon and the central methylene carbon would be preferred.

In calculating the isomeric compositions of the methyl ethyl malate derived from the ethyl acid malate of the wine and of the methyl ethyl malate mixture obtained by simple hydrogen ion–catalyzed esterification, six peaks from the mass spectrometric data which showed relatively large differences between the two isomers and were known to be essentially free of common impurities were chosen. Table II lists the heights of these peaks and the relative abundances calculated for each peak on the basis of peak *m/e* 71 being 100. Compositions of the ester from the wine and the ester from simple esterification were then calculated by linear interpolation between the relative abundance values for the two known synthetic isomers. Examination of the data in Table II shows that within the limits of accuracy of the Bendix Time-of-Flight mass spectrometer the ester derived from the wine acid is 100% the single isomer II. The preliminary indication from the infrared spectra that the ester derived from the wine was predominantly this isomer is thus confirmed and made quantitative.

The ethyl acid malate mixture produced by simple hydrogen ion–catalyzed reaction between malic acid and absolute ethyl alcohol turns out to be 66% isomer II-A. As the hydroxyl group is the only source of asymmetry in the molecule, its presence must be responsible for the fact that the two isomers are not formed in equal quantities. Two effects, steric and inductive, might reasonably be considered the modes by which the hydroxyl group influences the relative rates of esterification on the two ends of the malic acid molecule. The inductive effect of the hydroxyl group (an electron-withdrawing group) would operate to give some dipole character with the positive end nearest the dissociating proton to the oxygen-hydrogen bond of the carboxyl group; the effect is considerably greater in the carboxyl group adjacent to the carbinol group as compared with the carboxyl group on the other end of the molecule. That an inductive effect is active is supported by the observed fact that malic is a stronger acid than succinic. Protonation of the acid, the first step in acid-catalyzed esterification, might be expected to occur less readily when the bonds of the carboxyl group are partially polarized through the inductive effect. On the other hand, the bond polarization owing to the inductive effect of the hydroxyl group may stabilize the carbonium ion intermediate and thus enhance approach of the nucleophylic alcohol. The influence of the dipoles induced by the hydroxyl group on the succeeding steps, proton transfer, loss of water, and deprotonation, are difficult to assess. Similarly, it is difficult to predict what inductive effects would be of significance in the hydrolysis reactions in which ester is reconverted to acid and alcohol. In sum, one cannot predict with certainty

**Table II. Mass Spectrometric Peak Heights and Relative Abundances Used in Calculating Isomeric Compositions of Methyl Ethyl Malate from Wine and from Hydrogen Ion–Catalyzed Esterification**

| | Synthetic Isomers[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | Wine Ester | | [H$^+$] Ester | | Wine Ester Composition, | [H$^+$] Ester Composition, |
| *m/e* | Mm. | RA % | Mm. | RA % | Mm. | RA % | Mm. | RA % | % II | % II |
| 71 | 84 | 100 | 73 | 100 | 40 | 100 | 76.5 | 100 | ... | ... |
| 47 | 3.6 | 4.3 | 8.7 | 11.9 | 5.0 | 12.5 | 6.8 | 8.90 | 107.9 | 60.5 |
| 61 | 41 | 48.8 | 15.2 | 20.8 | 8.5 | 21.2 | 22.3 | 28.2 | 101.4 | 73.5 |
| 89 | 7.1 | 8.45 | 23.8 | 32.6 | 12.8 | 32.0 | 17.7 | 23.2 | 97.6 | 61.0 |
| 103 | 83 | 98.9 | 24.3 | 33.3 | 12.7 | 31.8 | 41 | 53.6 | 97.8 | 69.0 |
| 117 | 11.5 | 13.7 | 59 | 80.8 | 33 | 82.5 | 43 | 56.2 | 102.3 | 63.3 |
| 131 | 2.9 | 3.5 | 12 | 16.4 | 6.8 | 17.0 | 9.4 | 12.3 | 104.6 | 68.2 |
| Mean and av. dev. | | | | | | | | | 101.9 ± 3.0 | 65.9 ± 4.7 |

[a] I. Isomer with carbethoxy group adjacent to carbinol group.
II. Isomer with carbmethoxy group adjacent to carbinol group.

whether or in what manner the inductive effect influences the rates and equilibrium constants for esterification on the two separate ends of the malic acid molecule. The fact remains that the mass spectrometric analysis shows that two thirds of the mixture obtained on reaction of ethanol with malic acid is isomer II and the inductive effect probably plays a part in the unequal distribution.

Examination of molecular models suggests that the steric effect between the hydroxyl group and the carboxyl group would be relatively small. However, as the exact dimensions and configuration of the transition complex involved in the esterification are not known, the hydroxyl group may have some effect, spatially, favoring the esterification of the more remote carboxylic acid group.

The acid ester present in the wine is 100% (within the limits of precision of the experiment) a single isomer, whereas in the case of the acid-catalyzed esterification a mixture of two parts of one isomer and one part of the other was obtained. The most reasonable explanation of this finding is that the production of ethyl acid malate in the wine was an enzymatically mediated reaction. Such an enzymatic reaction could occur either during the primary fermentation when presumably carried out by enzymes of the yeast or as the result of action by "malolactic" organisms—bacteria which usually decarboxylate malic acid to produce lactic acid in a "secondary fermentation." The acid-catalyzed hydrolysis and re-esterification reactions are slow in the wine medium, as none of the ethyl acid malate isomer (I) was found, even though the average age of the wines composing the sherry blend approximated 6 years. Nordström (*8*) favors the idea that ethyl acid succinate is formed during fermentation by reaction of 2-keto-glutarate with NAD and CoASH to give succinyl coenzyme-A which then reacts with the alcohol of the beer or wine. Presumably a parallel series of reactions starting with 2-keto-4-hydroxyglutarate could yield the ethyl acid malate (II-A) actually obtained. 2-Keto-4-hydroxyglutarate is a hydroxyproline metabolite in animal tissues, according to Rosso and Adams (*13*).

If, as these experimental results suggest, all of the ethyl acid malate of wines is enzymatically produced, the increases in total acid esters observed by Ribéreau-Gayon and Peynaud to occur upon long aging of wines must result principally from the production of ethyl acid tartrate and ethyl acid succinate. Unfortunately, because of the symmetry of the tartaric acid and succinic acid molecules, some technique other than simple isolation and identification of isomers would be required to follow the mode of production of acid esters in these cases.

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