Identification of 4-Ethoxy-4-hydroxybutyric Acid γ Lactone [5-Ethoxydihydro-2(3*H*)-furanone] as an Aroma Component of Wine from *Vitis vinifera* var. Ruby Cabernet

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An aroma compound isolated from red table wine made from *Vitis vinifera* var. Ruby Cabernet grapes has been identified as 4-ethoxy-4-hydroxybutyric acid γ lactone [5-ethoxydihydro-2(3*H*)-furanone] through comparison of gas chromatographic retention times and mass and infrared spectra of the wine isolates with synthetic reference material. 4-Ethoxy-4-hydroxybutyric acid γ lactone was synthesized by literature methods. Although the compound has been so far identified only in Ruby Cabernet wines, the biosynthetic pathway proposed for its production through succinsemialdehyde and its ethyl hemiacetal suggests that it should be found in other wines as well.

In an earlier comparison of the volatile materials isolated from Ruby Cabernet and Cabernet-Sauvignon wines, one component was found to be unique to the Ruby Cabernet but was not isolated in quantities large enough to permit its characterization (Webb *et al.*, 1969). An infrared spectrum with definite characteristics of that of a γ lactone was obtained. The spectrum could not be matched with those of the known γ lactones available, however.

γ Lactones with five-carbon or larger groups on the ring are frequently involved in the aroma complexes of foods, as has been summarized by Weurman and van Straten (1969) and other lactones—some with larger rings—are frequently components of compounded flavor mixtures.

It was thus of interest to extract a larger amount of Ruby Cabernet wine and to attempt the complete identification of the previously partially characterized lactone. Its identification as 4-ethoxy-4-hydroxybutyric acid γ lactone is the subject of this report.

EXPERIMENTAL

Isolation of Peak 39 Material from Wines. Peak 39 lactone was isolated from two different wines. The first was a 5-gal sample of Ruby Cabernet (cellar no. 11838) vinified in 1967 from grapes grown in the Solano County vineyard of the University of California, and the second was also a Ruby Cabernet (cellar no. 12131), but a 50-gal sample vinified from grapes from the Solano County experimental vineyard in 1968. Aroma materials were extracted from both of the wines with CH_2Cl_2. For the smaller sample, the solvent was simply gently agitated in a 5-gal glass bottle by means of a magnetically driven Teflon-coated stirring bar, as described by Webb *et al.* (1967, 1969). The solvent layer was replaced with fresh solvent every day until no more aroma materials were being extracted. The larger sample was extracted in a 50-gal stainless steel drum arranged so that it could be rotated slowly on a set of four power driven wheels, as described previously (Muller *et al.*, 1971). An internal drain line and external valve were fitted so that, when the drum head was tilted slightly lower than the bottom, the solvent could all be removed and replaced with fresh solvent. Such changes were made on a daily basis, and the extractions were continued as long as significant amounts of aroma materials were being extracted.

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The extracting solvent was stripped, the free acids were separated, and the neutral essences were fractionated gas chromatographically, as described previously (Webb *et al.*, 1967, 1969; Muller *et al.*, 1971) on a Loenco dual column, dual thermal conductivity detector instrument with nitrogen carrier gas flow rates of 50 ml/min and filament currents of 100 mA. The desired unknown lactone emerged as peak 39 after the peaks for butyrolactone and diethyl succinate and before that of 2-phenethyl alcohol on FFAP columns.

Synthesis of 5-Ethoxydihydro-2(3*H*)-furanone. Although Ducher (1960) has described the isomerization of ethyl 4-oxobutyrate to yield the ethoxy lactone, we synthesized 5-ethoxydihydro-2(3*H*)-furanone (4-ethoxy-4-hydroxybutyric acid γ lactone) from ethyl glycidyl ether (1-ethoxy-2,3-epoxypropane or Epiethylin) following the procedure of Kuwamura and Takahashi (1969). The ethyl glycidyl ether was in turn prepared from epichlorohydrin (1-chloro-2,3-epoxypropane) after the procedure of Fairbourne *et al.* (1932). Final purification of the lactone for mass and infrared analyses was accomplished on an FFAP column programmed from 75 to 250°C (emergence temp. 163°C) followed by passage through an SE-30 column operated isothermally at 135°C. Details of the gas chromatographic purifications have been described previously by Muller *et al.* (1971).

Spectral Analyses of the Samples. Infrared spectra of the wine and synthetic samples were taken with a Beckman IR-8 spectrophotometer using thin films of the samples transferred to NaCl ultramicro cell windows directly from the collection capillaries or with the aid of a small amount of CH_2Cl_2.

Mass spectra of the synthetic and second wine samples were run on a C.E.C. Model 21-104 mass spectrometer, and a small amount of an earlier isolation of peak 39 material from the second wine was submitted to high-resolution analysis of the parent peak on a Varian Associates Model M66 mass spectrometer. Fragmentation in each case was by electron bombardment at a potential of 70 eV, and samples were introduced in small lengths of the collection capillaries by means of the solid probe.

RESULTS AND DISCUSSION

Figure 1 depicts the infrared spectra of the various samples of 4-ethoxy-4-hydroxybutyric acid γ lactone. It is obvious that the wine samples are identical with the synthesized material. The strong absorptions at 1770 cm^{-1} and at 1150

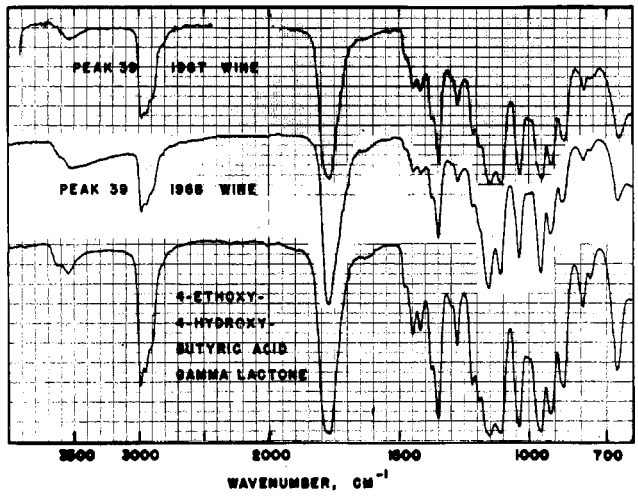

Figure 1. Infrared spectra of peak 39 lactone from two different wines and of synthetic 4-ethoxy-4-hydroxybutyric acid γ lactone

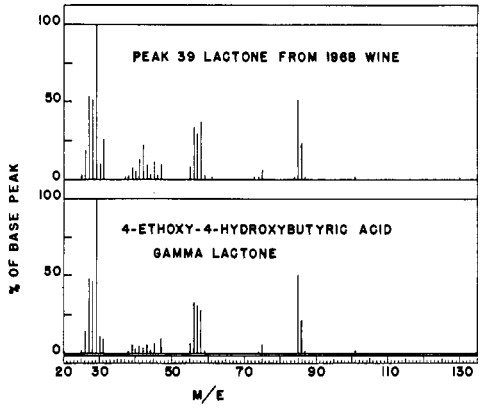

Figure 2. Mass spectra of peak 39 lactone from 1968 wine and 4-ethoxy-4-hydroxybutyric acid γ lactone

cm^{-1} are characteristic of the carbonyl stretching and the –C–O–C– stretching in γ lactones. The absorption at 1420 and at 1350 cm^{-1} can be assigned to asymmetrical and symmetrical bending, respectively, in the methyl group. The 1450 cm^{-1} absorption arises from symmetrical bending (scissoring) of the methylene group adjacent to the carbonyl in the lactone ring and from scissoring of the methylene group in the ethoxy group substituted on carbon-4 of the lactone. The strong absorption at 1110 cm^{-1} can very probably be associated with vibrations of the –C–O–C– system of the ethoxy substituent, since this band is in the proper region (Silverstein and Bassler, 1967) and is not found in the spectra of 4-acetyl-4-hydroxybutyric acid γ lactone or 4,5-dihydroxyhexanoic acid γ lactone. The absorption at 1040 cm^{-1} of 4-ethoxy-4-hydroxybutyric acid γ lactone probably corresponds to the 1065 cm^{-1} absorption of 4-acetyl-4-hydroxybutyric acid γ lactone, and reflects a complex vibration of the whole molecular system.

In Figure 2 are plotted the mass spectra of the synthetic and wine-isolated lactones. It is readily apparent that the two spectra are the same in all critical features. The molecular ion peak at *m/e* 130 is so small that it does not show on both of the plots. For the synthetic, the instrument printout gave a *m/e* 130 peak-height reading of 3.6 and for the wine sample 0.72, with comparable base peak *m/e* 29 readings of 2184 and 298. Loss of the substituting group from the number four carbon atom of the lactone, which is, according to Silverstein and Bassler (1967), McFadden *et al.* (1965), and Honkanen (1965), the most likely mode of rupture of substituted γ lactones, yields the large peaks at *m/e* 85 and 86 of the unsubstituted γ lactone and this ring plus a proton from the ethoxy fragment. Loss of CO_2 from the lactone also leaves a fragment, probably contributing to the *m/e* 86 peak. The instability of the ethoxy fragment is demonstrated by the lack of a significant peak at *m/e* 45. The ethoxy lactone of this research and the acetyl lactone of Augustyn *et al.* (1971) both have as base peak *m/e* 29, while the hydroxyethyl lactone of Muller *et al.* (1969) has the base peak of *m/e* 85 more typical of γ lactones. High-resolution mass determination of the M − 1 peak of the ethoxy lactone with the Varian M66 instrument gave a value of 129.0552, in excellent agreement with the calculated value of 129.0549 for $C_6H_9O_3$.

To date, wines have been shown to contain the following γ lactones, the biosynthetic interrelationships of which are suggested in Figure 3: (1) γ-butyrolactone (VII), Webb and Kepner (1962); (2) 4-carboethoxy-4-hydroxybutyric acid γ lactone (V), Webb *et al.* (1967); (3) (−) 4R:5R or 4S:5S 4,5-dihydroxyhexanoic acid γ lactone (XII), Muller *et al.* (1969); (4) (+) 4R:5S or 4S:5R 4,5-dihydroxyhexanoic acid γ lactone (XII), Muller *et al.* (1969); (5) 4-hydroxy-5-ketohexanoic acid γ lactone (XI), Augustyn *et al.* (1971). One lactam, ethyl pyroglutamate (IV), has been identified (Webb *et al.*, 1967). The very important role played by ethyl 4-oxobutyrate (I) as a key intermediate in the syntheses of most of the lactones is apparent from examination of the figure. Ethyl 4-oxobutyrate (I) has not been rigorously identified as a wine constituent to date, but some preliminary indications of its presence (ms data) have been obtained, and we are continuing our efforts to isolate and identify it. While we have indicated in Figure 3 that the 4-oxobutyrate (I) comes from glutamate (III) *via* 2-oxoglutarate (II), and this route is supported by the finding of relatively large amounts of ethyl pyroglutamate (IV) and 4-carboethoxy-4-hydroxybutyric acid γ lactone (V) in some wines, it is quite possible that some of the 4-oxobutyrate (I) results from transamination of the relatively large amounts of 4-aminobutyrate that are present in fermenting grape juices and possibly also from direct reduction of succinic acid by the yeast at the height of the alcoholic fermentation. Succinic acid, ethyl acid succinate, and diethyl succinate are present in generous amounts in wines toward the end of alcoholic fermentation. Muller *et al.* (1971) have proposed that the failure to observe augmentation of the ethyl 4-hydroxybutyrate (VI) and γ butyrolactone (VII) upon addition of large amounts of glutamic acid (III) to the fermenting must reflects intact assimilation of the amino acid, rather than invalidity of the direct pathway from glutamate (III) to butyrolactone (VII) through ketoglutarate (II) and 4-oxobutyrate (I). It is apparent from the proposed pathways of Figure 3 that the 4-oxobutyrate could have been converted to a number of compounds other than the butyrolactone (VII) that was looked for. Small amounts of ethyl 4-hydroxybutyrate (VI) and γ butyrolactone (VII) are found in nearly all wines, however.

The condensation of pyruvate with ethyl 4-oxobutyrate (I) to yield 4-hydroxy-5-oxohexanoic acid γ lactone (XI) and its reduction products, the isomers of 4,5-dihydroxyhexanoic acid γ lactone (XII), almost certainly involves the thiamin pyrophosphate–pyruvate or pyridoxal-5′-phosphate–pyruvate complex in a reaction such as discussed by Juni (1952), and more recently Schmauder and Gröger (1968). *Via* this reaction, yeasts have been shown to produce 2-oxo-3-hydroxy

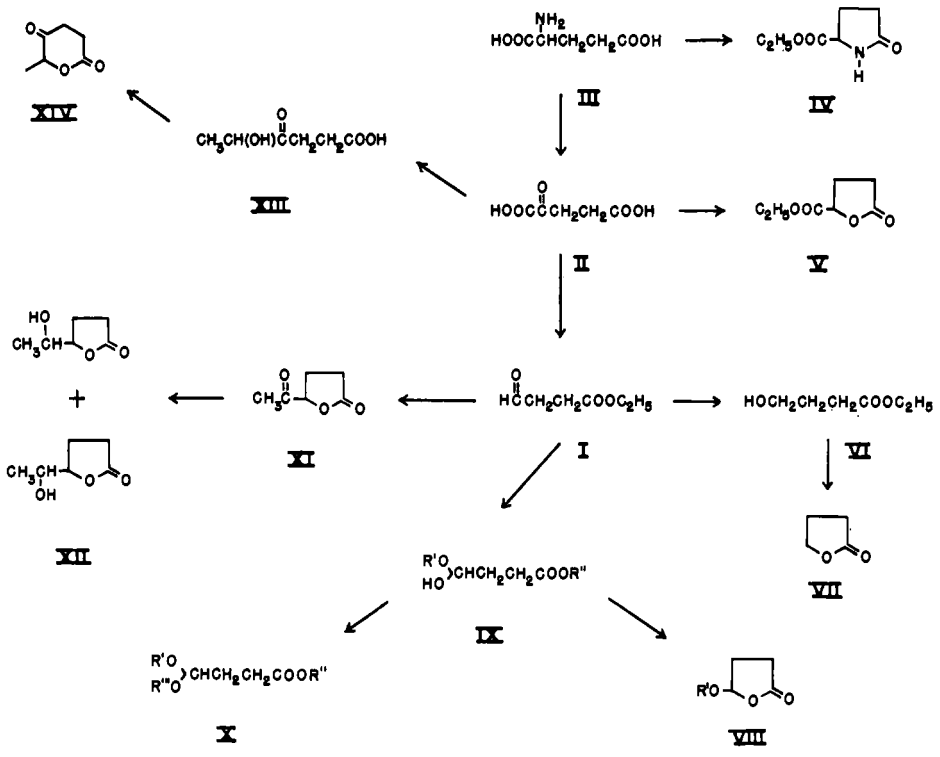

Figure 3. Postulated routes of biosynthesis of a number of wine lactones and related aroma compounds. R′, R″, and R‴ are ethyl or other alkyl groups present as alcohols in the wine

analogs of a number of different types of aldehydes. Hirabayashi and Harada (1969) have demonstrated that yeast in shake culture with acetaldehyde and 2-oxoglutarate (II) can produce 5-hydroxy-4-oxohexanoic acid (XIII). If this reaction also proceeds *via* the thiamin pyrophosphate or pyridoxal-5′-phosphate mediated complex, then the complex must involve the 2-oxoglutarate rather than the acetaldehyde, in order to get the observed positions of the hydroxy and oxo groups. To our knowledge 5-hydroxy-4-oxohexanoic acid (XIII) or its ethyl ester has not been found in wines as the products of normal alcoholic fermentation, but we do have some preliminary indications (ms spectra) of the presence of the Δ lactone (XIV) which would be derived from the acid in acid solutions.

As indicated in Figure 3, we believe that the 4-ethoxy-4-hydroxybutyric acid γ lactone (VIII) reported in this research is derived from ethyl 4-oxobutyrate (I). Whether the lactone is formed directly from the ester–aldehyde (I) with migration of the ethoxy group from carbon 1 to carbon 4 as the ring closes or whether the biosynthesis goes through the hemiacetal ester (IX) is not known. The hemiacetal ester (IX) is likely a highly unstable compound, which would make its identification as an intermediate difficult.

Ethyl 4-oxobutyrate (I) can form a wide range of compounds by reaction with the various alcohols of the wine. One would naturally expect to find a predominance of ethyl groups both as acetals and as esters, but there should be smaller amounts of propyl, isobutyl, 2-methylbutyl, 3-methylbutyl, and 2-phenylethyl substituents giving rise to a large number of different aroma compounds through hydrogen ion catalyzed mass action reactions. Enzymatically mediated reactions, which would occur during or shortly after the fermentation, of course, could produce acetals or esters of 4-oxobutyrate (I) involving alcohols normally present in the wine only in trace concentrations.

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