Identification of Two Isomers of 4,5-Dihydroxyhexanoic Acid Gamma Lactone in Californian and Spanish Flor Sherries

Carlos J. Muller,[1] Linda Maggiora, Richard E. Kepner, and A. Dinsmoor Webb

Two major components of the aroma extracts of one Californian and two Spanish Flor Sherries are identified as (−) 4R:5R or 4S:5S 4,5-dihydroxyhexanoic acid gamma lactone and (+) 4R:5S or 4S:5R 4,5-dihydroxyhexanoic acid gamma lactone; diastereomeric forms of 5-(1-hydroxyethyl)dihydro-2-(3H) furanone. Identifications were accomplished from the IR, MS, and NMR spectra of the sherry lactones and those of the known lactones synthesized from *trans*-4-hexenoic acid. Optical activity in the sherry lactones strongly indicates their production by enzyme mediated routes. The possibility that these lactones may be produced during the period of film growth of *Saccharomyces beticus* on the surface of the sherry is discussed.

In an investigation of two samples of Spanish fino sherry and one sample of a California palomino flor sherry reported earlier, Webb *et al.* (1967) were unable to identify completely two of the major high boiling components (peaks 60 and 61). Preliminary findings from infrared, mass, and NMR spectra indicated that the two components were hydroxy lactones. The small amounts of the materials obtained, and some difficulty in separating the gas chromatographic peak 60 material from diethyl tartrate, precluded complete characterization.

Although the odors of these two components, when smelled upon emergence from the gas chromatographic column, were generally wine-like, they could not be said to be characteristic of flor sherry. The material from peak 61 was more intense in odor than that from peak 60. The relatively large size of peaks 60 and 61 made positive identification of these components very desirable. Their unequivocal characterization is the subject of this report.

EXPERIMENTAL

Isolation and Purification of Wine Lactones. Using the techniques described by Webb *et al.* (1967), larger quantities of the wine lactones than previously available were obtained from the California Palomino flor sherry. Repeated gas chromatographic separations on FFAP and SE-30 columns eventually provided adequate amounts of pure materials from peaks 60 and 61.

Synthesis of Trans-4-Hexenoic Acid. Following the procedure of Julia *et al.* (1961) cyclopropyl methyl ketone (Aldrich Chemical Co. C 12000-6) was reduced with $NaBH_4$ to cyclopropyl methyl carbinol. Hydrobromination of the carbinol, followed by preparation of the Grignard reagent in the usual manner and addition of dry ice, afforded, after hydrolysis, a 70 to 30 mixture of *trans*- and *cis*-4-hexenoic acid. The acid mixture was separated gas chromatographically on a 10-foot × $^1/_4$-inch FFAP column in a Loenco Model 70 instrument using He at 60 ml. per minute flow rate and thermal conductivity detectors. The desired *trans*-4-hexenoic acid was collected and showed the characteristic IR absorption at 695 cm^{-1} (Figure 1) of trans substituted double bonds.

Synthesis of Racemic 4R:5R and 4S:5S 4,5-Dihydroxyhexanoic Acid Gamma Lactone, I. Cis hydroxylation of the olefin was carried out by the following modification of the procedure by Robinson and Robinson (1925). A 0.23-gram (2 mmole) sample of the purified *trans*-4-hexenoic acid was added to a cold (0° C.) stirred solution of an excess (3 mmole) of KOH in 10 ml. of H_2O. To this solution, 2.66 ml. of 0.5*M* $KMnO_4$ solution was added slowly with stirring. After settling and decanting, the supernatant liquid was acidified with 15% H_2SO_4 to the methyl orange end point and the lactone was extracted with ten 2-ml. portions of diethyl ether. After drying over anhydrous $MgSO_4$, the solvent was distilled off through a small Vigreux column and the residue was chromatographed on the FFAP column at 200° C. (Yield: 40%; retention time on FFAP, 9 minutes; essentially odorless.)

Synthesis of Racemic 4R:5S and 4S:5R 4,5-Dihydroxyhexanoic Acid Gamma Lactone, II. Trans hydroxylation of the olefin was carried out by the following modification of the procedure of Swern *et al.* (1945). A 0.25-gram sample of 30% H_2O_2 was mixed with 0.50 gram of glacial acetic acid and heated for 30 minutes at 75° C. The mixture was cooled and added dropwise to 0.23 gram of *trans*-4-hexenoic acid with stirring. The solution was then heated at 40° C. for 30 minutes, cooled, and extracted with ten 2-ml. portions of diethyl ether. After evaporation of the solvent as above, the residue was chromatographed on the FFAP column at 200° C. (Yield: 73%; retention time on FFAP, 12 minutes; weak wine-like odor.)

Measurement of Physical Characteristics of Samples. Infrared spectra were determined with a Beckman IR-8 Spectrophotometer using thin films of the pure compounds on NaCl ultramicro cells. Low resolution mass spectra were determined with a Varian Associates Model M66 mass spectrometer. NMR spectra were measured in deutero-chloroform (TMS as internal standard) with a Varian Associates Model A-60A instrument. Optical rotations of the natural samples from the wine were measured to a precision of ±0.01° with a Zeiss Precision Polarimeter at 578 nm in a 1-cm. microtube at room temperature (23° C.). For rotation measurements, 17.17 mg. of wine lactone from peak 60 was dissolved in H_2O to give 0.200 ml. of solution and, in an analogous manner, 16.12 mg. of wine lactone from peak 61 was made to 0.200 ml. in H_2O.

RESULTS AND DISCUSSION

The two racemic mixtures of the diastereoisomeric forms of 4,5-dihydroxyhexanoic acid gamma lactone, which were essential for the identification of the natural sherry lactones,

Department of Chemistry and Department of Viticulture and Enology, University of California, Davis, Calif. 95616

[1] Present address, Department of Chemistry, Louisiana Polytechnic Institute, Ruston, La. 71270

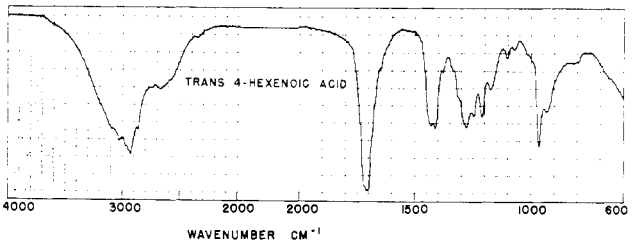


Figure 1. Infrared spectrum of *trans* 4-hexenoic acid
Beckman IR-8, NaCl ultramicro demountable cell, neat

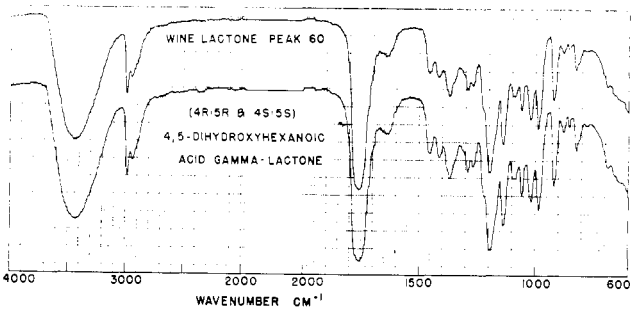


Figure 2. Infrared spectra of sherry lactone from peak 60 and synthetic 4R:5R and 4S:5S 4,5-dihydroxyhexanoic acid gamma lactone

Beckman IR-8, NaCl ultramicro demountable cell, neat

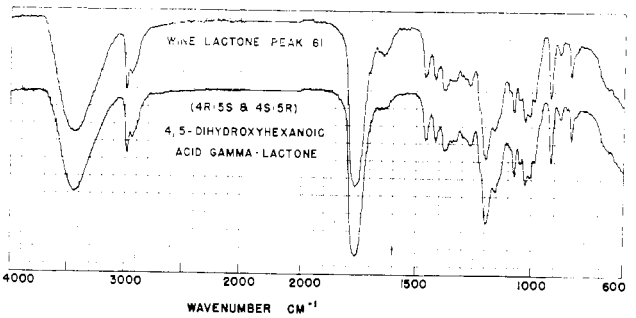


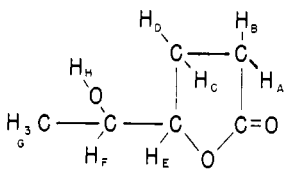
Figure 3. Infrared spectra of sherry lactone from peak 61 and synthetic 4R:5S and 4S:5R 4,5-dihydroxyhexanoic acid gamma lactone

Beckman IR-8, NaCl ultramicro demountable cell, neat

were synthesized by cis and trans hydroxylation of *trans*-4-hexenoic acid. The infrared spectrum of the *trans*-4-hexenoic acid, given in Figure 1, shows the characteristic adsorption at 965 cm.$^{-1}$ of trans disubstituted double bonds. Figure 2 depicts the infrared spectra of the gas chromatographically purified lactone of peak 61 from the sherry wine and that of the synthetic racemix mixture of 4R:5R and 4S:5S 4,5-dihydroxyhexanoic acid gamma lactone, I. Figure 3 presents the IR spectra for the gas-chromatographically purified lactone of peak 61 from the sherries and that of the synthesized racemic mixtures of 4R:5S and 4S:5R 4,5-dihydroxyhexanoic acid gamma lactone, II. Examination of Figures 2 and 3 shows that, in each case, the IR spectrum of the lactone from the wine is identical with that of the known racemate of synthetic lactones. The broad strong band at 3436 cm.$^{-1}$ is due to the —OH stretching under conditions permitting intermolecular hydrogen bonding. The very large band at 1762 cm.$^{-1}$ is characteristic of the —C=O stretching and that at 1192 cm.$^{-1}$ of the —CO— stretching in gamma lactones. The small, rather broad band at 1415 cm.$^{-1}$ possibly is due to the —OH bending in the secondary alcohol. The many differences in the intensities and positions of the bands in the finger-print region (900–1300 cm.$^{-1}$) between the spectra of Figure 2 and those of Figure 3 result from the fact that the compounds are diastereomers. Examination of molecular models shows that in the case of the 4S:5S, 4R:5R racemic mixture, it is possible for the hydrogen of the secondary alcohol to approach very close to the ether oxygen of the lactone ring with the possibility of formation of a five-member hydrogen-bonded ring lying in nearly the same plane as the lactone ring. In the case of the other diastereomer, the plane of the ring containing the hydrogen bond (if it is formed) is nearly perpendicular to the plane of the lactone ring. It seems reasonable that these differences would have profound secondary effects on the many vibrational modes of the molecules, and thus be reflected as differences in the finger-print region of the spectra.

Figures 4 and 5 present the nuclear magnetic resonance spectra of the wine lactones of peaks 60 and 61. The spectra of the synthesized lactones are not presented as they were identical in all essentials with those of the unknowns—that of the compound from cis hydroxylation of *trans*-4-hexenoic acid being essentially identical to that of wine lactone peak 60 and that of the product of trans hydroxylation of the *trans* acid being similar to that of wine lactone peak 61.

The chemical shift assignments of the protons for lactone I (wine peak 60), as designated below, are as follows: δ 2.60, multiplet, H_A and H_B; 2.10, multiplet, H_C and H_D; 4.36, multiplet, H_E; 3.79, multiplet, H_F; 1.25, doublet, H_G; 3.28 ppm, singlet H_H.



With the exception of the methyl group protons, all protons on the molecule are non-equivalent. The assignments of the protons as indicated are consistent with the above structure for the lactone. The integrated areas also agree quite well with the numbers of protons indicated in the above assignments. In the case of the diastereomeric lactone II (wine peak 61), the multiplet for the H_C and H_D protons has shifted downfield resulting in a complex multiplet which integrates reasonable well for the four protons (H_A, H_B, H_C, H_D) on the ring. The multiplet for H_f has also shifted downfield and overlapped somewhat with the multiplet for H_E resulting in less symmetrical peak patterns. The integrated areas are again consistent with an assignment of protons for lactone II comparable to that indicated above for lactone I. The chemical shift of the alcohol group proton, H_H, varies in the different spectra with the differences in concentration of the lactones, as expected.

Low resolution mass spectrometry of the four samples gave nearly identical mass spectra. The spectrum obtained from wine lactone from peak 60 is presented in Figure 6 as being typical. Honkanen *et al.* (1965) state that gamma lactones give extremely weak parent peaks, and Silverstein and Bassler (1967) mention that having an alkyl substituent present on the fourth carbon makes the parent peak even weaker. In the present case, with substitution of a hydroxyethyl group on carbon number four, the parent peak is scarcely distinguishable from the back-ground noise of the spectrum. The base peak is *m/e* 85, corresponding to rupture of the ring side-chain C—C bond. McFadden *et al.* (1965), Friedman and Long (1953), and Honkanen (1965) all report *m/e* 85 as the base peak for C_6 to C_{18} gamma lactones. The intense peak

at *m/e* 86 (nearly as intense as the base peak) observed in the current research is not typical of gamma lactones with saturated straightchain alkyl substituents on C_4 and no doubt reflects the presence of the secondary hydroxy group in the side chain. Abstraction of the alcoholic proton from the side chain seems the most rational procedure for obtaining the peak *m/e* 86. High-resolution mass spectrometry confirms the assignments for the *m/e* 85 and *m/e* 86 peaks, as it shows that these ions have the empirical formulas $C_4H_5O_2$ and $C_4H_6O_2$. High-resolution mass spectrometry shows the ion at *m/e* 115 to be $C_5H_7O_3$, which corresponds to the loss of a CH_3 group from the parent molecule. Also shown is a very small peak at *m/e* 112 due to $C_6H_8O_2$ arising from loss of one molecule of water from the parent molecule, and a somewhat larger peak at *m/e* 102 corresponding to the ion $C_5H_{10}O_2$ resulting from the loss of a CO fragment. Low resolution mass spectrometry shows significant peaks at *m/e* 45 and 44 which very probably correspond to the hydroxyethyl fragment produced by breaking the C—C bond between the ring and side chain, and to this fragment minus one proton. According to Silverstein and Bassler (1967), gamma butyrolactone gives strong peaks at *m/e* 27, 28, 29, 41, and 42. These peaks are observed in this research indicating the presence of gamma butyrolactone as a unit of the larger molecular structure. In addition, the 4,5-dihydroxyhexanoic acid gamma lactone isomers give significant peaks at *m/e* 43 which may be due to the CH_3CO fragment, and peaks at *m/e* 58, 57, 56, and 55, which could result from a double break in the ring portion of the parent molecule to split out the CH_3-CHOHCH ion, or from cleavage of the alkyl group from the ring followed by scission of the ring to give CO and the residual CH_2CH_2CHO, and subsequent loss of 1, 2, or 3 protons.

The fact that the mass spectra for the two natural isomers and the two racemic mixtures are identical in all essential details, the fact that high resolution mass spectrometry shows the large fragments observed are those expected from these molecules, and the fact that the smaller fragments correspond with those listed in the literature for gamma butyrolactone proves beyond reasonable doubt that these wine components are truly isomers of 4,5-dihydroxyhexanoic acid gamma lactone.

Repeated measurement of the rotation of wine lactone from peak 60 gave an average value of $-0.53° \pm 0.01°$, which calculates to a specific rotation, $[\alpha]_{578}^{23} = -31°$. For wine lactone from peak 61 the measured rotation was $+0.04 \pm 0.01°$ giving a calculated specific rotation, $[\alpha]_{578}^{23} = +5°$. Because of the small observed rotation for sample 61, the relative error is large, but for lactone 60 with the larger observed rotation, the relative error is of less significance. Finding optical activity in these compounds is of significance in that it implies enzymatic mediation in their synthesis. Tang and Jennings (1968) have proposed biosynthetic pathways for gamma and delta lactones found in apricots, peaches, and pineapples. Their postulated mechanism, involving a C_4 unsaturated fatty acid as intermediate, is attractive. They postulate hydration of the double bond to produce both gamma and delta hydroxy acids which then would lactonize spontaneously at the low pH of fruit tissues. What is required in the present case, of course, is an enzymatically mediated process that would put hydroxyl groups on both the fourth and fifth carbon atoms and do it stereospecifically.

It is interesting that of the several types of sherries (flor, baked, submerged culture), investigated in detail in this laboratory (Webb, *et al.*, 1964), 4,5-dihydroxyhexanoic acid gamma lactones have so far been observed only in flor sherries.

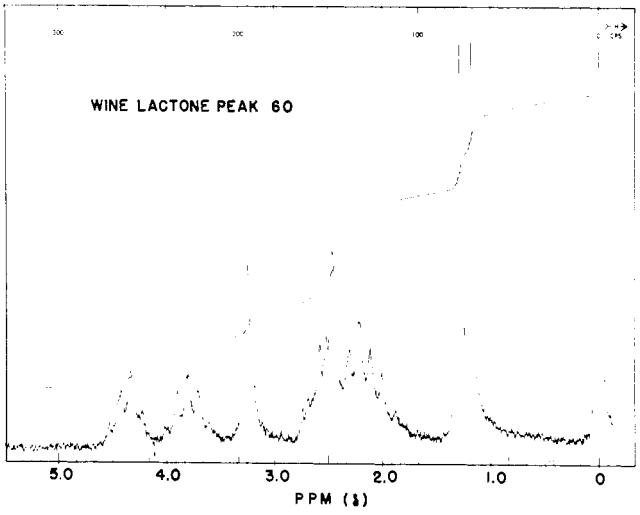


Figure 4. NMR spectrum of sherry lactone from peak 60
Varian Associates Model A-60A; in deuterochloroform

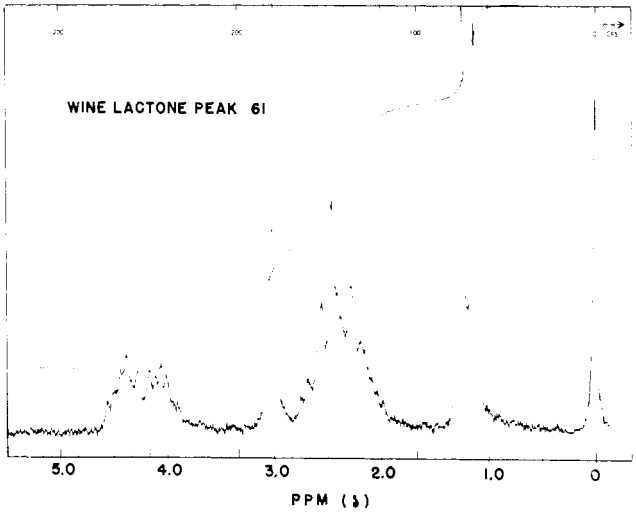


Figure 5. NMR spectrum of sherry lactone from peak 61
Varian Associates Model A-60A, in deuterochloroform

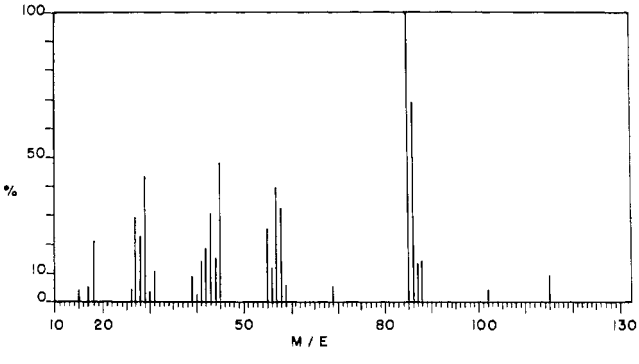


Figure 6. Mass spectrum of sherry lactone from peak 60
Varian Associates Model M66 mass spectrometer

Further, high boiling constituents in the aroma extracts of a number of different wine types have been investigated gas-chromatographically without the presence of these lactones being noticed (Webb, 1967). In four cases, White Riesling (Van Wyk *et al.*, 1967), two Cabernet-Sauvignons and one Ruby Cabernet (Webb *et al.*, 1969) investigated in this labora-

tory, a careful and detailed search for these high boiling lactones showed that, within the limits of detection, they were not present. Aroma materials can be present in the finished wine as a result of their presence in the grape, as the result of their production by yeast action on the sugars or other constituents of the grape during alcoholic fermentation, as the result of normal cellar operations and bacterial changes, and, in the case of film sherries, as the result of the particular metabolism of the yeast growing on the surface of the wine under microaerophyllic conditions. That the lactones could not be found in the White Riesling and Cabernet wines suggests that they are not formed during alcoholic fermentation of sugar by yeasts. Similarly, their absence from the baked sherry (produced from Palomino grapes) suggests that they are not natural constituents of this grape variety. Bacterially induced changes and changes produced by cellar operations are generally common for all wine types. One infers, therefore, that the two lactones are most likely the result of the film-stage growth of *Saccharomyces beticus*. This problem is being investigated further.

ACKNOWLEDGMENT

We thank E. P. Painter for help in measuring the rotations, Jack Maroski for help with the measurement of the NMR spectra, and Allan Duffield, Robert Ross, and Kei Miyano for help in running and interpreting the mass spectra.

LITERATURE CITED

Friedman, L., Long, F. A., *J. Amer. Chem. Soc.* **75,** 2832 (1953).
Honkanen, E., Moisio, T., Karvonen, P., *Acta Chem. Scand.* **19,** 370 (1965).
Julia, M., Julia, S., Tchen, S. Y., *Bull Soc. Chim., France* **1961,** 1849.
McFadden, W. H., Day, E. A., Diamond, M. J., *Anal. Chem.* **37,** 89 (1965).
Robinson, G. M., Robinson, R., *J. Chem. Soc.* **127,** 175 (1925).
Silverstein, R. M., Bassler, G. C., "Spectrometric Identification of Organic Compounds," 2nd Ed., John Wiley and Sons, New York, 1967.
Swern, D., Billen, G. N., Findley, T. W., Scanlan, J. T., *J. Amer. Chem. Soc.* **67,** 1786 (1945).
Tang, C. S., Jennings, W. G., J. AGR. FOOD CHEM. **16,** 252 (1968).
Van Wyk, C. J., Kepner, R. E., Webb, A. D., *J. Food Sci.* **32,** 669 (1967).
Webb, A. D., Chapter 9 in "Chemistry and Physiology of Flavors," H. W. Schultz, E. A. Day, L. M. Libbey, editors, Avi Pub. Co., Westport, Conn. (1967).
Webb, A. D., Kepner, R. E., Galetto, W. G., *Amer. J. Enol. Viticult.* **15,** 1 (1964).
Webb, A. D., Kepner, R. E., Maggiora, L., *Amer. J. Enol. Viticult.* **18,** 190 (1967).
Webb, A. D., Kepner, R. E., Maggiora, L., *Amer. J. Enol. Viticult.* **20,** 16 (1969).

Received for review March 27, 1969. Accepted June 19, 1969. Research supported in part by a grant from the California Wine Advisory Board.