

On the Occurrence of a New
Lactone Compound,
trans-4-Methyl-5-hydroxy-
hexanoic Acid Lactone, in Milk

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During a gas-chromatographic comparison of flavour compounds in milk produced with various feeds, we have observed only very small differences. Also the so-called 0-milk produced on an odourless, purified diet^{1,2} (cellulose, starch, sucrose, urea and ammonium salts as the nitrogen source) contains the same main flavour compounds as normally produced milk. Accordingly, the flavour compounds in milk are to a considerable degree formed in the organism of the cow (in the rumen and/or metabolic processes in the liver or mammary gland) from carbohydrates and are derived only partly from special flavour substances or their precursors in the plant material fed.

The typical main flavour compounds in milk are the even carbon number δ -C₆-C₁₄-lactones. Odd carbon number δ -lactones and some γ -lactones have also been found in milk. In 0-milk the total lactone content, especially the content of odd carbon number δ -lactones, seems to be somewhat higher than in normal milk.

An interesting new lactone compound (C₇H₁₂O₂), moving between the δ -C₆- and δ -C₇-lactones in a gas chromatography column, has been isolated, in the first place from 0-milk. Later, traces of this compound were detected in normal milk also. About 10 μ g of this new lactone compound was isolated from 1 kg 0-milk fat. In milk produced with pasture feeding the content of the lactone is only 1/20 of that found in 0-milk. The odour of the compound resembles that of the normal δ -C₆-lactone, but is somewhat weaker. Since its concentration is so low, it has probably a very small effect on the milk flavour.

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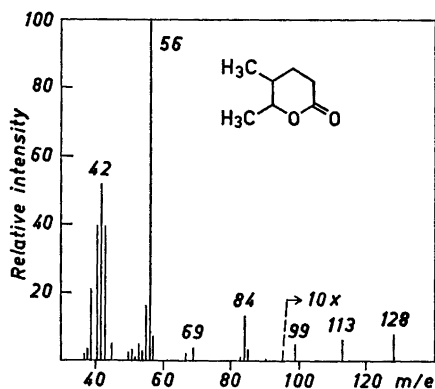


Fig. 1. Mass spectrum of *trans*-4-methyl-5-hydroxyhexanoic acid lactone.

The mass spectrum of the new compound (Fig. 2) differs distinctly from the mass spectra of normal δ -lactones³ and indicates possibly a branched-chain δ -lactone structure for this compound. To solve this problem four isomeric branched-chain δ -lactones, namely 2-, 3-, 4-, and 5-methyl-5-hydroxyhexanoic acid lactones, were synthesized and their mass spectra determined.⁴ It appeared then that the mass spectra of the unknown compound and 4-methyl-5-hydroxyhexanoic acid lactone (Fig. 1) were similar. The synthetic 4-methyl-5-hydroxyhexanoic acid lactone was resolved on a gas chromatography column into two peaks (geometric isomers), of which the faster-moving isomer (1) had

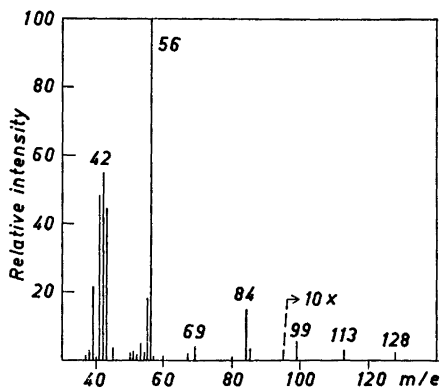


Fig. 2. Mass spectrum of the new lactone compound in 0-milk.

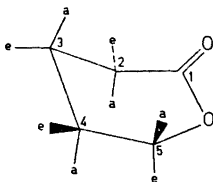


Fig. 3. Schematic diagram of the half-chair conformation of 4-methyl-5-hydroxyhexanoic acid lactone.

the same retention time as that of the compound found in milk. In order to determine its geometric configuration the synthetic 4-methyl-5-hydroxyhexanoic acid lactone mixture was separated by a preparative scale gas chromatography column (3/8" \times 3 m NPGS-column at 150°C) into the pure isomers (1 and 2) which were subjected to NMR-analysis.

Collective chemical and spectroscopic evidence that δ -lactones in solution have the half-chair conformation has been reported.⁶ Thus the methyl groups and H-atoms in compounds 1 and 2 lie in nearly axial (a) or equatorial (e) positions (see Fig. 3). Under NMR test the protons of axial methyl groups or hydrogens are shielded by the β -located C—C or C—O bonds; the proton signals are expected to be at a higher field than the signals of the corresponding equatorial groups or H-atoms.⁶ A large J_{45} short-range coupling appears generally only between axial protons, the other possible J_{45} 's being much smaller ($J_{aa} \approx 7$ cps, $J_{ae} \approx J_{ee} \approx J_{ea} \approx 3$ cps on average for acetylated sugars⁶).

Proton resonance spectra of 1 and 2 as solutions of low concentration (2–3 %) in tetrachloromethane were run with TMS as internal reference, using a Perkin-Elmer spectrometer model R10 at the University of Turku and recorded on calibrated paper. The lowest field signal of the H-atom at carbon 5 appeared, with isomer 1, at $\tau_{H5} = 6.05$ ppm as a quintet ($J = 7$ cps = J_{45} and J_{CH_3H}) and with isomer 2 at $\tau_{H5} = 5.55$ ppm as a quartet of doublets ($J_{45} = 3$ cps and $J_{CH_3H} = 7$ cps), including some broadening from long-range couplings. The methyl group signals were two doublets, of which the lower field signal belongs most probably to the CH_3 at carbon 5, due to the neighbouring electro-negative oxygen atom. Thus 1 has $\tau_{CH_34} =$

9.0 ppm and $\tau_{CH_35} = 8.66$ ppm, and 2 has $\tau_{CH_34} = 9.0$ ppm and $\tau_{CH_35} = 8.71$ ppm.

4-Methyl-5-hydroxyhexanoic acid lactone may have four possible conformations (I–IV) from two configurations (*cis* and *trans*):

Isomer	Conformation				Configura- tion
	4a	4e	5a	5e	
I	H	CH ₃	H	CH ₃	<i>trans</i>
II	H	CH ₃	CH ₃	H	<i>cis</i>
III	CH ₃	H	CH ₃	H	<i>trans</i>
IV	CH ₃	H	H	CH ₃	<i>cis</i>

NMR data obtained for 1 support strongly conformation I: J_{45} as large coupling can be only J_{aa} . τ_{H5} is 0.5 ppm higher than τ_{H5} of isomer 2, representing the normal difference in chemical shifts between axial and equatorial protons of pyranose rings.⁶ τ_{CH_35} of isomer 1 is slightly lower than the corresponding chemical shift of 2, supporting equatorial conformation for CH_3 in 1. 2 has a small J_{45} coupling and τ_{H5} is lower than that of isomer 1, indicating an equatorial position for the hydrogen atom at C5. This is confirmed by the τ_{CH_35} , which being slightly higher than the corresponding value of isomer 1 points to an axial position for the methyl group at C5. The methyl group at C4 is likely to be in the same spatial arrangement in both isomers, since their signals have the same chemical shifts. Thus conformation II can be deduced for isomer 2. Accordingly, the new lactone compound found in milk must have the *trans* configuration, since it has the same retention time as isomer 1.

The occurrence of a branched-chain lactone compound in milk is evidently connected with the biosynthesis of branched-chain fatty acids by rumen bacteria. It is known that the concentration of branched-chain fatty acids in milk fat is increased when the protein constituents in feed are substituted by urea.^{2,7}

The flavour compounds were isolated from the milk fat by vacuum carbon dioxide distillation.⁸ The first separation of the flavour concentrate was performed with a preparative-scale gas chromatography column (3/8" \times 3 m NPGS-column) using temperature programming from 150° to 240°C. The fractions collected were purified further on EGS or Carbowax 1500 columns and then fed into a gas chro-

matograph-mass spectrometer unit. The effluent gas (He) from the gas chromatograph was pumped out in a molecular separator made from Teflon tubing⁹ (ID 0.3 mm, 10 m long, at 280°C). The mass spectra were determined using an exponential voltage scan³ and recorded in 20 sec with a Visicorder 1706 oscillograph.

4-Methyl-5-hydroxyhexanoic acid lactone was synthesized according to the method of Dew and Rai,¹⁰ starting from ethyl acrylate and recorded in 2-methyl-3-ketobutanoate.

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Synthesis of Mesyloxyacetic Acid and some Derivatives

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In connection with studies concerning the relations of the structures and physical properties of alkylating agents, especially alkyl alkanesulfonates, to their mutagenic and toxic activities,^{1,2} attention was given to mesyloxyacetic acid and some of its derivatives. Ethyl mesyloxyacetate³ has been obtained from the bromoacetate and silver mesylate in acetonitrile but mesyloxyacetic acid,⁴ prepared *via* glycolonitrile, has only recently been briefly mentioned. The acid, its amide and its methyl ester have now been prepared from suitable halogen derivatives. Mesyloxyacethydroxamic acid was obtained, in low yield, from mesyloxyacetyl chloride.

Experimental. Melting points were determined on a Kofler Micro hot stage. IR-spectra were measured on a Perkin-Elmer IR 221 spectrophotometer. The solvents used were of analytical grade.

Mesyloxyacetic acid. A solution of bromoacetic acid (35 g) and an excess of silver mesylate (56 g) in acetonitrile (175 ml) was refluxed for 5 h. The solution was filtered, the precipitate was washed with acetonitrile and the combined solutions were evaporated to dryness *in vacuo*. The residue was extracted with acetone, the extract was evaporated to dryness and the crude acid (32 g) was crystallised from ethyl acetate to give mesyloxyacetic acid as needles, m.p. 114–115° (lit.⁴ m.p. 113–114°) (Found: C 23.4; H 3.92; S 20.8. C₃H₆O₃S requires C 23.3; H 3.78; S 22.6) ν_{\max}^{KBr} 1740, 1360, 1170 cm⁻¹.

*Mesyloxyacetyl chloride.*⁴ Thionyl chloride (18 g) was added to mesyloxyacetic acid (9 g). The mixture was refluxed for 3 h and excess thionyl chloride was distilled off in an atmosphere of dry argon. The acid chloride (7.5 g) was distilled, b.p. 99–100°/2 mm, n_D^{25} 1.4608. (Found: C 21.0; H 2.90; S 18.6; Cl 20.4. C₃H₆O₂SCl requires C 20.9; H 2.92; S 18.6; Cl 20.6.) ν_{\max}^{pure} 1820, 1360, 1180 cm⁻¹.

Mesyloxyacetamide. A solution of silver mesylate (12 g) in acetonitrile (50 ml) was added to a solution of iodoacetamide (9 g) in