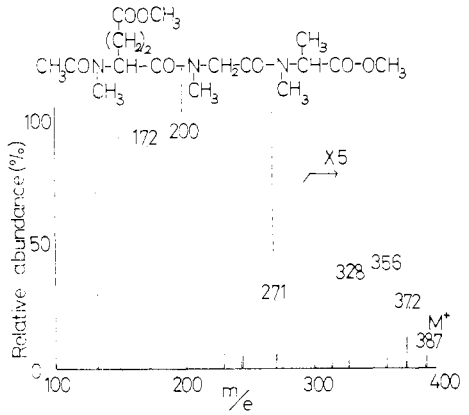


**Figure 3.** A representative chart of the mass spectrum for an acetyl permethyl derivative from L-glutamylglycyl-L-alanine (fraction O-2-2-1).

modified glutamyl residue and another peak at $m/e$ 172 may show a decarbonylated fragment from this residue. Accordingly, the N terminal must be glutamic acid. The difference between 271 and 200 is in agreement with the mass number of a permethylated glycine residue. We can thus interpret the structure of this tripeptide. A similar way of interpretation applied also to other peptides listed in Table IV.

Flavor notes of these isolated peptides were evaluated by a panel of three members. It seemed to be probable that at least four dipeptides, Glu-Asp, Glu-Glu, Glu-Ser, and Thr-Glu, and five tripeptides, Asp-Glu-Ser, Glu-Asp-Glu, Glu-Gln-Glu, Glu-Gly-Ser, and Ser-Glu-Glu (threshold indicated in Table IV), had a flavor quantitatively resembling that of MSG. However, their flavor intensities were weaker than that of MSG. The threshold level of Glu-Gly-Ser, for example, was estimated to be approximately 0.2% in water at pH 5, whereas that of MSG was almost one-tenth of this level under the same conditions. Other flavor peptides were generally comparable to Glu-Gly-Ser in respect to the threshold level.

A mixture of the acidic oligopeptides, though completely free from glutamic acid, is expected to serve as a flavor potentiator which may give a more natural flavor sensation than artificial seasonings. Information obtained from the present study may give a clue to the elucidation of the flavoring activities of peptides occurring in practical fermentation foods such as soy sauces, wines, cheeses, etc.

A study is being undertaken to synthesize the isolated flavor peptides and to elucidate their individual flavor notes in more detail.

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# Analysis of the Lactone Fraction of Lavender Oil (*Lavandula vera* D.C.)

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The lactones extracted from lavender oil after hydrolysis were analyzed by gas chromatography and mass, infrared, and nuclear magnetic resonance spectrometry. Ten lactones were identified. Eight of these have not previously been reported as constituents of lavender oil.

In the literature concerning lavender oil about 100 components were described, among which there are only two lactones. Coumarin was already mentioned in the beginning of this century by workers of Schimmel (1900, 1903) and afterward by other investigators (Ripert, 1937; Bénezet, 1943; Seidel *et al.*, 1944). More recently Klein and Rojahn (1967) reported the presence of 4-methyl-4-vinyl-4-butanolide. This paper describes the identification of ten lactones in lavender oil.

## EXPERIMENTAL SECTION

**Isolation of Lactones.** Lavender oil (1 kg) was added to a 5-l. flask containing a solution of 200 g of KOH (1.5 times the calculated amount based on the saponification value of the oil) in water (200 ml) and ethyl alcohol (1200 ml). The clear, homogeneous mixture was then heated for 1 hr under reflux in order to saponify the oil completely. Afterward water (1 l.) was added and the mixture was shaken vigorously. The aqueous layer, containing the potassium salts of phenols and acids and including the hydroxy acids resulting from ring opening of the corresponding lactones, was drawn off, saturated with sodium chloride, and extracted successively with ether (7 × 100 ml) and dichloromethane (3 × 100 ml) to remove the unsaponifiables. The aqueous phase was brought to pH 9.5 with 12 $N$ $H_2SO_4$. The liberated phenolic compounds were removed by successive extraction with ether (1 × 100 ml,

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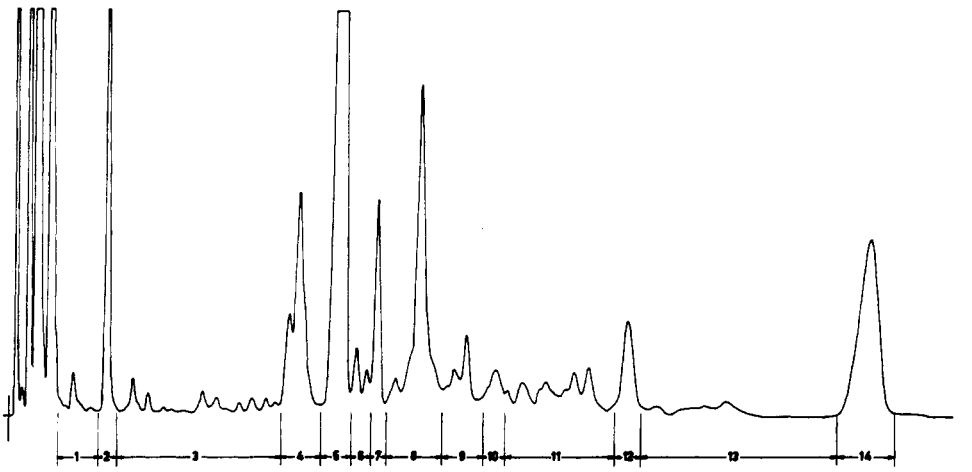

**Figure 1.** Gas chromatogram of the lactone fraction of lavender oil: column, 2 m × ⅛ in. o.d., packed with 20% Carbowax 20M on Embacel (60–80 mesh); temperature programmed from 80 to 200° at 1°/min.

6 × 50 ml) and dichloromethane (3 × 50 ml). Subsequently the alcohol was distilled off in order to minimize the formation of ethyl esters in the next step. The residue was acidified with 12 *N* $H_2SO_4$ to pH 1 and extracted with dichloromethane (7 × 50 ml). The organic phase was dried over anhydrous $Na_2SO_4$ and concentrated by distillation under atmospheric pressure (using a Vigreux column) until a residue of about 1 ml remained.

The hydroxy acids were reconverted to the lactones by addition of 50 mg of *p*-toluenesulfonic acid as a catalyst and by azeotropic removal of water after addition of dry benzene (50 ml). The residue was diluted with dichloromethane to a volume of 200 ml. The remaining acids were removed by washing with 25-ml portions of a saturated solution of $NaHCO_3$, until the pH of the aqueous phase was about 8. In model experiments with 4-butanolide, 5-pentanolide, 4-hexyl-4-butanolide, and 5-pentyl-5-pentanolide no appreciable ring opening was detectable at this pH within the time of the experiment.

The aqueous layers were brought to pH 9 and extracted three times with 15 ml of dichloromethane. After drying the combined organic phases over anhydrous $Na_2SO_4$, the solvent was removed by careful distillation using a Vigreux column.

**Preparative Gas Chromatography, Infrared, and Nmr Spectrometry.** The lactone extract was separated into 14 fractions on an F&M Model 810 gas chromatograph with flame ionization detection and equipped with a 6:1 effluent splitter to permit collection. The instrument was fitted with a 2 m × ⅛ in. o.d. stainless steel column packed with Carbowax 20M (20% by weight) on 60–80 mesh Embacel support. The operating conditions were as follows: injection port temperature, 220°; column temperature, programmed from 80 to 200° at 1°/min; carrier gas flow (He), 25 ml/min; hydrogen flow, 40 ml/min; air flow, 240 ml/min.

Each of the fractions was rechromatographed on a 2 m × ⅛ in. o.d. stainless steel column packed with Silicone OV-17 (10% by weight on Embacel support). The operating parameters were the same as mentioned before. In some cases sufficient amounts of pure material could be trapped from this column for infrared and nmr analysis. The infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord fitted with a 4× beam condensor and a beam attenuator. Nmr spectra were determined on a Varian A-60-A instrument in 10% v/v carbon tetrachloride solutions using tetramethylsilane as an internal standard.

**Combined Gas Chromatography–Mass Spectrometry.** The fractions collected from the Carbowax 20M column were analyzed with a coupled gas chromatograph–mass spectrometer. The gas chromatograph, a Varian Aerograph Model 1220, was equipped with a 30 m × 0.03 in. i.d. glass SCOT (support coated open tubular) column. The column was coated with a suspension of 3 g of Ucon LB 550X, 6 g of ANM rice (Neckar Chemie), and 0.03 g of Aerosil type R972 (DEGUSSA) in 24 g of dichloromethane. The dynamic coating method (a flow of 2 cm/sec was maintained) was used.

The column was operated at a temperature of 120° and a helium flow of 3 ml/min. The outlet of the column was admitted directly to the inlet of a Varian MAT CH5 mass spectrometer. The separations were performed in an all-glass system from the injection port to the center of the ion source. The coupling was achieved by means of an interface specially developed to connect SCOT columns to a mass spectrometer. This interface consisted of an all-glass capillary restriction allowing a helium flow rate of 0.5–1.0 ml/min. The excess effluent was split off at atmospheric pressure. This is realized by inserting the capillary restriction partly in to the SCOT column. The material admitted to the mass spectrometer was conducted to the heart of the ion source by a glass capillary. Concurrent recordings of mass spectra (70-eV ionization chamber) and chromatographic peaks (20-eV ionization chamber) were obtained. The ion source temperature was 210°. The mass spectra and gas chromatographic elution characteristics of the lactones were compared with those of authentic substances examined under identical conditions. Some reference samples which could not be obtained from commercial sources were prepared as follows.

**Synthesis.** *4,4-Dimethyl-2-buten-4-olide.* 4-Methyl-3-pentenoic acid was prepared from malonic acid and isobutyraldehyde in triethanolamine according to Boxer and Linstead (1931). This acid was converted into the unsaturated lactone by bromination and dehydrobromination following the procedure of Franck-Neumann and Berger (1968).

*2-Methyl-4-butanolide.* This lactone was prepared by the method of Kaneko *et al.* (1963). Diethyl methylmalonate was alkylated with 2-chloroethanol and the resulting 2-carbethoxy-2-methyl-4-butanolide was hydrolyzed and decarboxylated with hydrochloric acid.

*4-Methyl-4-vinyl-4-butanolide.* Following the procedure of Papa *et al.* (1954) levulinic acid was ethinylated with sodium acetylide to give 4-methyl-4-ethinyl-4-butanolide. Hydrogenation with Lindlar catalyst afforded 4-methyl-4-vinylbutanolide.

*4-Isopropyl-4-butanolide.* 5-Methyl-2-hexenoic acid was obtained by a Knoevenagel condensation between 3-methylbutanal and diethyl malonate followed by hydrolysis and decarboxylation (Cope *et al.*, 1941). Treatment of this acid with sulfuric acid resulted in a mixture of 4-iso-

**Table I. Lactones Identified in Lavender Oil**

| Fraction no. | Compound | Method of identification[a] | | | | Previously reported |
|---|---|---|---|---|---|---|
| | | Glc | Ms | Ir | Nmr | |
| 4 | 4-Butanolide | + | +[b] | | | |
| | 4,4-Dimethyl-2-buten-4-olide | + | + | +[e] | + | |
| | 2-Methyl-4-butanolide | + | + | | | |
| 5 | 4-Methyl-4-vinyl-4-butanolide | + | +[c] | +[c,f] | | *c* |
| 6 | 4-Methyl-4-vinyl-2-buten-4-olide | | | Tent. | | |
| 7 | 4-Isopropyl-4-butanolide | + | + | + | + | |
| 12 | 4-Hexyl-4-butanolide | + | +[b] | +[g] | | |
| | 5-Pentyl-5-pentanolide | + | +[b] | +[g] | | |
| 13 | Dihydrocoumarin | + | + | + | | |
| 14 | Coumarin | + | +[d] | +[h] | | *i–m* |

[a] Plus sign indicates presence confirmed. [b] McFadden *et al.* (1965). [c] Felix *et al.* (1963). [d] Budzikiewics *et al.* (1964). [e] Angell *et al.* (1960). [f] Klein and Rojahn (1967). [g] Tang (1967). [h] Mecke *et al.* (1965). [i] Schimmel (1900). [j] Schimmel (1903). [k] Ripert (1937). [l] Benezet (1943). [m] Seidel *et al.* (1944).

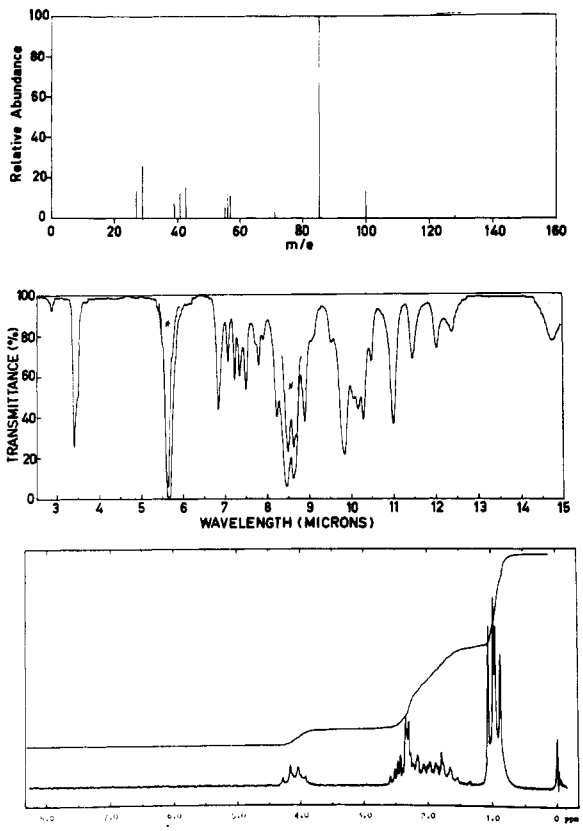


**Figure 2.** Mass, ir, and nmr spectra of 4-isopropyl-4-butanolide: mass spectra *m/e* (relative intensities) 128 (M) (1), 100 (13), 85 (100), 71 (3), 57 (11), 56 (12), 55 (5), 43 (15), 41 (12), 39 (7), 29 (26), 27 (13); ir λ (neat, thickness 0.007 mm) 2.83, 3.37, 3.46, 6.80, 7.03, 7.19, 7.31, 7.46, 7.69, 7.76, 7.86, 8.19, 8.43, 8.59, 8.86, 9.49, 9.79, 10.01, 10.12, 10.24, 10.42, 10.95, 11.40, 11.97, 12.33, 14.71 μ; (asterisk indicates $CCl_4$ solution) 5.59, 8.45, 8.58, 8.66 μ; nmr ($CCl_4$, 60 MHz) δ 0.94 (3 H, d, *J* = 6.5 Hz), 1.01 (3 H, d, *J* = 6.5 Hz), 1.3–2.7 (5 H, m), 4.09 (1 H, m).

propyl-4-butanolide and 5-methyl-5-pentanolide, from which the first lactone was isolated by means of preparative gas chromatography.

## RESULTS AND DISCUSSION

Figure 1 shows a gas chromatogram of the lactone fraction on the Carbowax 20M column. The numbers on the chromatogram indicate the collected fractions. On reinjecting these fractions on the packed Silicone OV-17 column or particularly on the SCOT column, the fractions 2, 5, 7, and 14 turned out to be rather pure.

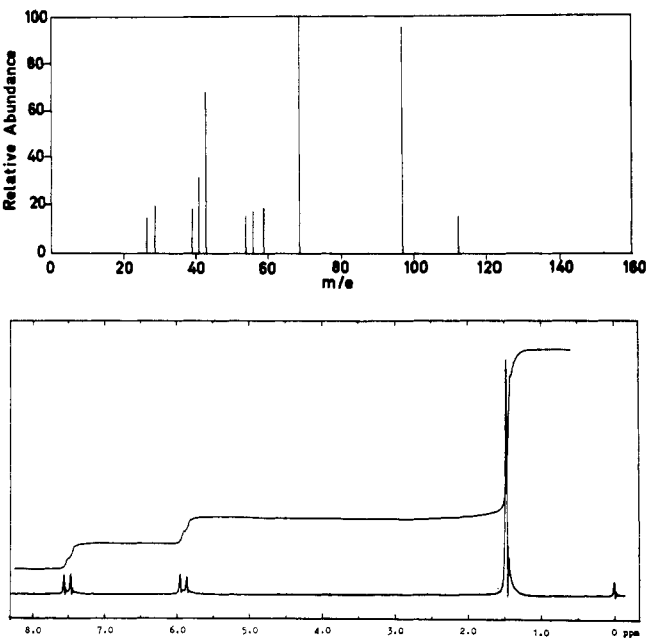


**Figure 3.** Mass and nmr spectra of 4,4-dimethyl-2-buten-4-olide: mass *m/e* (relative intensities) 112 (M) (16), 97 (96), 69 (100), 59 (19), 56 (18), 54 (16), 43 (68), 41 (32), 39 (19), 29 (20), 27 (15); nmr ($CCl_4$, 60 MHz) δ 1.47 (6 H, s), 5.89 (1 H, d, *J* = 5.5 Hz), 7.37 (1 H, d, *J* = 5.5 Hz).

Table I summarizes the lactones found and the techniques used for their identification. Literature references for the spectra of the lactones are also mentioned in the table. The spectra of 4-isopropyl-4-butanolide, 4,4-dimethyl-2-buten-4-olide, 2-methyl-4-butanolide, and dihydrocoumarin are shown in Figures 2, 3, 4, and 5, respectively.

By infrared and mass spectral analysis fraction 2 was shown to consist of ethyl hexanoate. Its presence in the lactone fraction must be explained by reaction of residual ethyl alcohol with hexanoic acid during the isolation procedure. Also fractions 1 and 3 contained ethyl esters of other fatty acids as was shown by infrared and mass spectrometry. Apart from solvent peaks, the same holds for the most volatile part.

From fraction 6 a compound could be isolated that is supposed to be 4-methyl-4-vinyl-2-buten-4-olide on the basis of the infrared spectrum. The spectrum shows a very strong carbonyl absorption doublet at 5.57 and 5.64 μ ($CCl_4$ solution). This doublet, possibly caused by Fermi resonance, occurs at lower wavelengths as compared with published data for α,β-unsaturated γ-lactones (Infrared

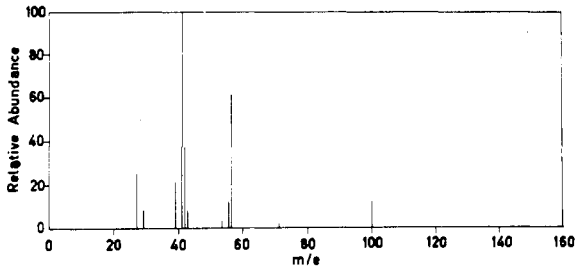


**Figure 4.** Mass spectrum of 2-methyl-4-butanolide: *m/e* (relative intensities) 100 (M) (12), 71 (2), 56 (61), 55 (12), 53 (3), 43 (8), 42 (37), 41 (100), 39 (21), 29 (8), 27 (25).

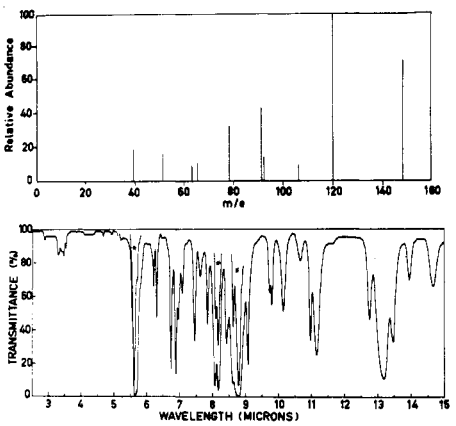


**Figure 5.** Mass and ir spectra of dihydrocoumarin: mass *m/e* (relative intensities) 148 (M) (71), 120 (100), 106 (9), 92 (14), 91 (43), 78 (33), 65 (11), 63 (9), 51 (16), 39 (19); ir λ (neat, thickness 0.007 mm) 2.84, 3.28, 3.38, 3.43, 3.50, 6.18, 6.29, 6.72, 6.87, 6.94, 7.06, 7.44, 7.59, 7.82, 8.05, 8.16, 8.39, 8.60, 9.05, 9.70, 9.77, 10.12, 10.63, 10.93, 11.13, 12.72, 13.14, 13.42, 13.91, 14.62 μ; (asterisk indicates $CCl_4$ solution) 5.60, 5.68, 8.07, 8.14, 8.60, 8.76 μ.

Structural Correlation Tables, 1965; Jones *et al.*, 1959). This shift may be caused by the tertiary vinyl group in the γ position. The same shift was observed by comparison of the carbonyl band position (in $CCl_4$ solution) in the spectra of the saturated lactones 4,4-dimethyl-4-butanolide (5.61 μ) and 4-methyl-4-ethyl-4-butanolide (5.60 μ) with the carbonyl band position in the spectra of 4-methyl-4-vinyl-4-butanolide (5.58 μ) and 4-methyl-4-ethinyl-4-butanolide (5.55 μ).

The lactone 4,4-dimethyl-2-buten-4-olide shows, as expected, a carbonyl doublet (in $CCl_4$ solution) at higher wavelengths (5.62 and 5.67 μ) than in the compound isolated from fraction 6 of lavender oil (5.57 and 5.64 μ). The presence of an unsubstituted double bond in the ring follows from a strong absorption band at 12.28 μ. The presence of a tertiary vinyl group is indicated by characteristic absorptions ($CS_2$ solution) at 6.08 (w), 10.18 (m), and 10.76 μ (s), the methyl group by adsorption at 7.32 μ (m). Further absorptions (in μ) of the suggested compound are 3.24 (w), 8.13 (s), 9.04 (s), 10.45 (s, shoulder), 10.50 (s), 10.59 (s), 11.16 (s) in $CS_2$ solution and 7.10 (w) in $CCl_4$ solution. Great resemblance with the spectra of 4,4-dimethyl-2-buten-4-olide (Angell *et al.*, 1960), 4-methyl-2-buten-4-olide (Angell *et al.*, 1960), and 4-methyl-4-vinyl-4-butanolide (Klein and Rojahn, 1967; Felix *et al.*, 1963) supports the proposed structure.

Fraction 8 contained mainly hexanoic acid that was apparently not removed from the lactone fraction by the saturated solution of $NaHCO_3$. The amounts of the other components in this fraction were too small to allow identification. In fractions 9, 10, and 11 several lactones were located by mass spectrometry but for the same reason their structures could not be established. It is noteworthy that only two compounds with an unsaturated lactone ring were detected. This may be caused by partial formation of oxocarboxylic acids from unsaturated lactones by the action of aqueous alkali (Houben-Weyl, 1963).

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