Quantitative analysis of lactone–oxo acid mixtures by gas chromatography

Optically active lactones of 5-hydroxy fatty acids contribute considerably to the character of butter flavour[1,2]. These lactones can be prepared on a technical scale by microbiological reduction of 5-oxo acids[3,4]. On the other hand, racemic lactones can be prepared by hydrogenation of those 5-oxo acids[5], *e.g.* by catalytic hydrogenation.

Determination of the lactone is possible either by converting the lactone into an anilide[1,2,6], which is then determined spectrophotometrically in the same way as fatty acid anilides[7], or by converting it into a hydroxamic acid[1,2] followed by spectrophotometric determination of the ferric complex[8]. Any non-converted oxo acid can be determined spectrophotometrically as 2,4-dinitrophenylhydrazone[8].

For the simultaneous determination of the two components, a gas chromatographic method has been developed, which is both rapid and selective, and which requires only little material. In this technique the lactone–oxo acid mixture is treated with diazomethane. In this way the oxo acid is converted into its methyl ester, while the lactone remains unchanged.

In the investigation pure methyl 5-oxo decanoate, methyl 5-oxo dodecanoate, 5-decanolide and 5-dodecanolide were used as model substances. The experiments were carried out with a Pye Argon Chromatograph with β-ray ionization detector (^{90}Sr as radioactive source) using Apiezon L as immobile phase. Polyethylene glycol adipate is unsuitable, as in that case 5-decanolide and methyl 5-oxo dodecanoate have nearly equal retention times. The working conditions were: column 120 × 0.4 cm; packing 10 % Apiezon L on Celite (150–178 μ); temperature 175°; pressure 58 cm Hg; flow rate 30 ml/min; detector voltage 750 or 1000 V.

In the analysis of a mixture of 5-dodecanolide and 5-oxo dodecanoic acid the corresponding C_{10}-compounds served as internal standards; for a mixture of C_{10}-components, the corresponding C_{12}-compounds were used as such.

The retention times of the C_{10}- and C_{12}-compounds, relative to 5-dodecanolide, are: 5-dodecanolide 1.00; methyl 5-oxo dodecanoate 0.74; 5-decanolide 0.40; methyl 5-oxo decanoate 0.30. The absolute retention time of 5-dodecanolide is 23 min.

For quantitative analysis the following procedure is applied:

The weighed sample (non-deacidified crude lactone) is dissolved in benzene and the solution made up to a certain volume in a measuring flask. An amount, containing *ca.* 50 mg sample, is pipetted into a round-bottomed flask. A Vigreux column (40 cm) is attached to the flask and the benzene is distilled off under reduced pressure at *ca.* 50°. The Vigreux column is washed with ether, which is added to the distillation residue. The greater part of the ether is evaporated by means of a weak flow of nitrogen and heating to about 25°. A few ml of a solution of diazomethane in ether are then added and after about 10 min the excess of diazomethane and the ether are evaporated under nitrogen. A part of the residue (50–100 μg) is brought on to the gas chromatography column and a chromatogram is recorded. From this chromatogram the amounts of internal standard to be added are determined. They are chosen in such a way that the ratios between standard and component to be determined do not become excessive. In addition it is established from this first chromatogram whether the sample contains components having the same retention time as the standards, in which case a correction factor has to be introduced.

Subsequently the same amount of the sample solution is pipetted into a second round-bottomed flask and to this solution known amounts of the standards (as solutions in benzene) are added. The same procedure is further followed as described above.

From the second chromatogram the amounts of oxo acid and lactone in the sample are calculated by comparing the peak areas of these components with those of the standards. As the oxo acids are chromatographed in the form of methyl esters a conversion factor has to be used to calculate the amount of oxo acid.

Although the dynamic response curves, determined with 0–10 μg methyl 5-oxo decanoate and 0–30 μg 5-dodecanolide, showed a linear behaviour, they displayed a significant difference in slope. This implies that the use of a single internal standard is impossible for the determination of both lactone and oxo acid. Consequently, we used a lactone standard for the determination of lactones and an oxo acid standard for the determination of oxo acids.

The accuracy and reproducibility of the method as tested by analysing mixtures of known composition, were satisfactory. The differences between recovered and added percentages of 5-dodecanolide varied from 0.2 to 1 % for model mixtures containing 10–40 % of this component. A model mixture containing 24.5 mg 5-oxo dodecanoic acid yielded a mean result of 24.3 (95 % confidence interval $\pm$0.5).

From the duplicate determinations of a number of technical samples it appeared that the mean relative standard deviation per determination was *ca.* 3 % for oxo acid and *ca.* 5–6 % for lactone.

In the present investigation only compounds having 10 and 12 carbon atoms have been used. However, the procedure is also suitable for the analysis of mixtures of lactones and oxo acids having other chain lengths. It may also be applied to mixtures of 4-lactones and 4-oxo acids, which are obtained in the analogous preparation of 4-lactones from the corresponding 4-oxo acids[3,4,5].

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