Chromatographic identification of carbonyl compounds VIII. The carbonyl compounds in a fermented glucose solution*

The previously described thin-layer chromatographic method[1] (*cf.* Part VI) for the analysis of keto acid 2,4-dinitrophenylhydrazones has been applied for identification of the keto acids present in sugar solutions fermented by yeast[2,3]. The keto acids were converted into their 2,4-dinitrophenylhydrazones, which were isolated by extraction[2–4]. In this study, the isolation of carbonyl compounds from a fermented solution was effected by the adsorption and elution technique developed (Part I) and in this case, in contrast to methods applied earlier, the aliphatic aldehydes were also

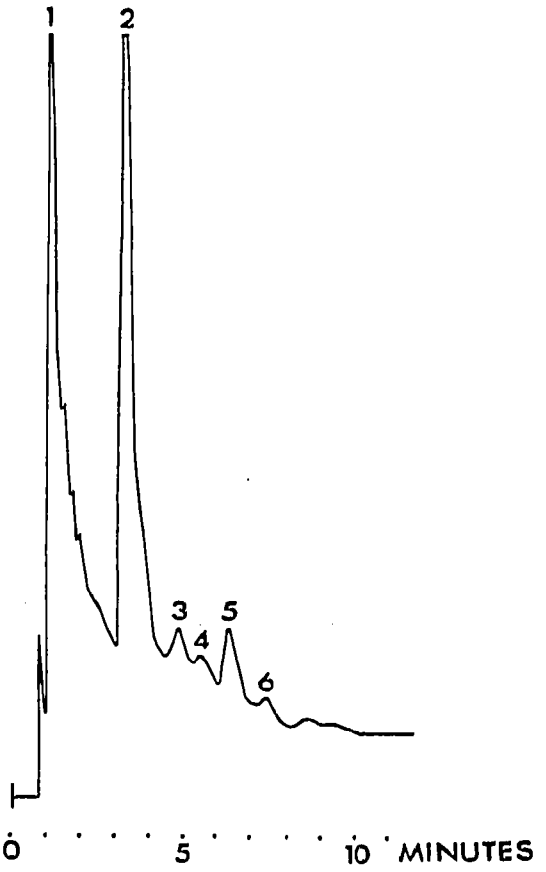

Fig. 1. Gas chromatogram of carboxylic acids produced by ozone oxidation of a mixture of 2,4-dinitrophenylhydrazones of aldehydes isolated by adsorption on carbon from a fermented glucose solution and elution from the carbon. Acetaldehyde is analysed as acetic acid, propionaldehyde as propionic acid, and so on. Conditions: column length, 4 m; internal diameter, 3 mm; liquid phase, NEGS containing phosphoric acid; solid support, acid-washed Chromosorb W; carrier gas, helium; flow rate, 75 ml/min; inlet pressure, 2.5 kp/cm^2; temperature 140°; detection by flame ionisation; sensitivity, 1; injected volume, 5 μl. 1 = Solvent; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = butyric acid; 6 = isovaleric acid and/or 2-methylbutyric acid.

isolated as their hydrazones. In the analyses of aldehydes and keto acids, the gas chromatographic methods developed previously (see Parts IV and V) were applied.

* For Parts I–VII of this series, see *J. Chromatog.*, 27 (1967) 374, 380, 384; 28 (1967) 253, 259, 263, 440.

Experimental

The fermentation was effected by the addition of 20 g of commercial baker's yeast (Rajamäki Factories of the Finnish State Alcohol Monopoly) to 2 l of 12 % glucose solution. In about 12 h at 30°, the glucose had been fermented to approximately 0.1 %, and the fermentation was interrupted by cooling. The yeast was separated on a Büchner funnel and washed. A 60-ml volume of 2,4-dinitrophenylhydrazine solution (2.5 g of the reagent in 1 l of 2 *N* hydrochloric acid) was added to the filtrate. No

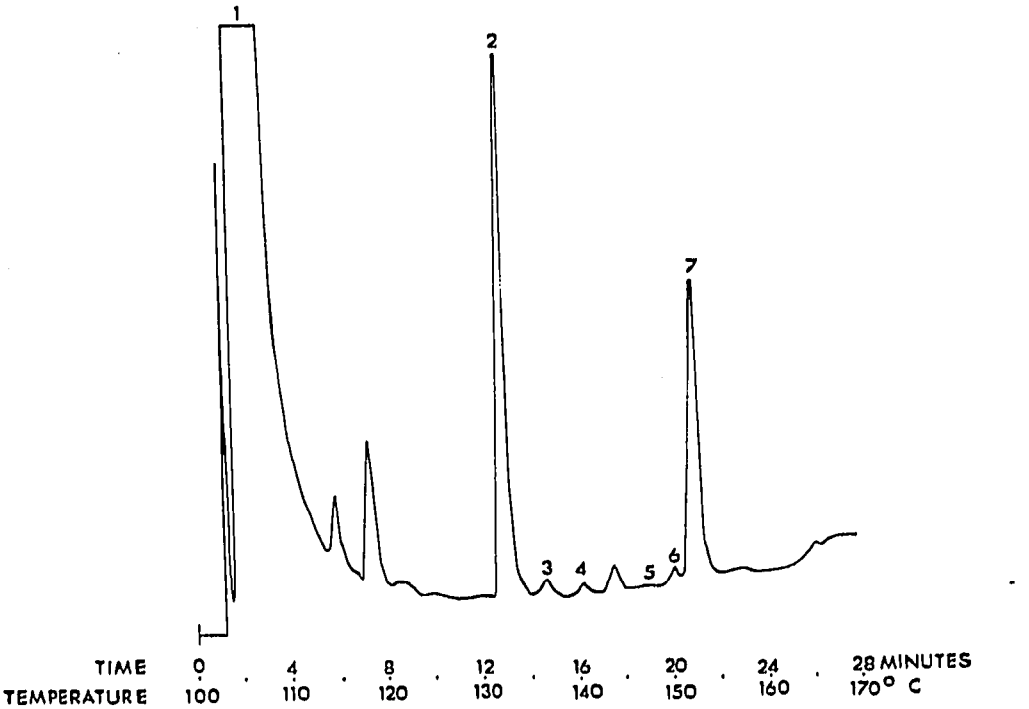

Fig. 2. Gas chromatogram of keto acid methyl esters liberated by ozone oxidation from a mixture of 2,4-dinitrophenylhydrazones of keto acid methyl esters produced by esterification with diazomethane of the keto acid hydrazones isolated by adsorption on carbon from a fermented glucose solution and elution from the carbon. Conditions: column length, 4 m; internal diameter, 3 mm; liquid phase, DEGA containing phosphoric acid; solid support, acid-washed Chromosorb W; carrier gas, helium; flow rate, 63 ml/min; inlet pressure, 1.8 kp/cm^2; temperature programme, 100–170°/2.5°/min; detection by flame ionisation; sensitivity 8; injected volume, 5 μl. 1 = Solvent; 2 = methyl pyruvate; 3 = methyl 2-oxoisovalerate; 4 = methyl 2-oxobutyrate; 5 = methyl 2-oxo-3-methyl valerate; 6 = methyl 2-oxoisocaproate; 7 = unknown derivative of pyruvic acid.

precipitate was formed after standing overnight at room temperature. The hydrazones were isolated from the solution after its neutralisation with dilute ammonia by adsorption on activated carbon (*cf.* Part I). Aldehyde hydrazones were eluted from the carbon with methyl formate and dichloromethane, successively. The extract, dissolved in 0.5 ml of formic acid, was ozonizated and analysed by isothermal gas chromatography on a NEGS column. The keto acid hydrazones were then eluted from the carbon with an azeotropic pyridine–water mixture at reduced pressure. The keto acid hydrazones extracted were first liberated from their pyridinium salts by treatment with methanol containing hydrogen choride and then esterified with diazomethane in ether at 0°. The mixture of the methyl ester hydrazones was dissolved for ozonation and subsequent gas chromatography in 0.5 ml of a dichloromethane–methanol (1:4,

v/v) mixture. A temperature programme and a DEGA column were used in the chromatographic run.

Results

Gas chromatograms showing the resolution of derivatives of carbonyl compounds formed in glucose fermentation by baker's yeast under anaerobic conditions are shown in Figs. 1 and 2. The aldehydes were identified as carboxylic acids (Fig. 1) and the keto acids as their methyl esters (Fig. 2). On the basis of the chromatogram in Fig. 1, the aldehyde present in greatest amount in the fermented solution was acetaldehyde and the other aldehyde components were propionaldehyde, isobutyraldehyde, butyraldehyde and isovaleraldehyde and/or 2-methylbutyraldehyde. Pyruvic acid is the dominating keto acid (Fig. 2); it gives rise to two peaks (*cf.* Part V, Fig. 1) of which that designated "2" is due to methyl pyruvate, and that designated "7" is due to an unknown derivative of pyruvic acid formed in the ozonization of methyl pyruvate dinitrophenylhydrazone. The trace components are the same as have previously been found present in fermented liquors by thin-layer chromatography of their dinitrophenylhydrazones, *viz.* 2-oxobutyric acid, 2-oxoisovaleric acid and 2-oxoisocaproic acid[2]. There is only a very small peak due to 2-oxo-3-methylvaleric acid in the chromatogram of Fig. 2; this acid was, however, identified when a greater detector sensitivity was employed. There is one unidentified peak between the peaks 4 and 5 in Fig. 2. The keto acids in the fermented glucose solution were also analysed by thin-layer chromatography of their hydrazones. 2-Oxoglutaric acid, which cannot be identified in the gas chromatographic method used, was then found to be present in an amount of the same order of magnitude as pyruvic acid (*cf.* ref. 2).

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