# CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS*

## V. GAS CHROMATOGRAPHY OF KETO ACID METHYL ESTERS

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Various methods involving pyrolysis, heating with acids, and ozonisation[5,6] (*cf.* Part IV), have been applied as preliminary treatment in attempts to convert the keto acid methyl ester 2,4-dinitrophenylhydrazones into volatile compounds for gas chromatography. As the ozonisation method gave reproducible results, its applicability was studied in greater detail. The ozonisation severs the double bond between carbon and nitrogen in the keto acid ester hydrazones, and leads to formation of the corresponding keto acid methyl esters, which do not further decompose in the presence of ozone, and can be analysed by gas chromatography as has been described in a preliminary communication[7]. In this study, a detailed description is given of the methods of ozonisation of keto acid ester hydrazones and subsequent gas chromatography of the liberated esters. Two successive chromatograms were run in the gas chromatographic analyses. In one run, the mixture of pure keto acid methyl ester hydrazones was analysed after ozonisation. In the other, the mixture of keto acid hydrazones isolated by adsorption and elution technique from ethanol–water solution (Part I) was esterified and analysed after ozonisation.

### EXPERIMENTAL

*Esterification of 2,4-dinitrophenylhydrazones of keto acids*

For esterification, a solution containing 0.5 g of the 2,4-dinitrophenylhydrazone of a keto acid in diethyl ether (pure, Rikkihappo Oy) was cooled in an ice–water bath and treated with an excess of a solution of diazomethane in diethyl ether that had been precooled in an ice–water bath. Immediately after the solution had been thoroughly mixed by shaking, the ether was evaporated and the precipitated ester was recrystallized from a (1:4, v/v) mixture of methyl formate (guaranteed reagent, E. Merck AG) and methanol (guaranteed reagent, E. Merck AG).

*Preparation and ozonisation of mixtures of keto acid ester hydrazones*

A mixture containing 0.2 mmole of each of the pure 2,4-dinitrophenylhydrazones of the methyl esters of pyruvic, 2-oxobutyric, 2-oxoisovaleric, 2-oxoisocaproic, 2-oxo-3-methylvaleric, levulinic, 2-oxoglutaric and oxalacetic acids was prepared by weighing and dissolved in 100 ml of methyl formate (purum, Fluka AG). A 10-ml aliquot of the resulting solution was evaporated to dryness in the Rotavapor and

* For Parts I–IV, see refs. 1–4.

the residue R, containing 0.02 mmole of each component, was dissolved in 1 ml of a (1:4, v/v) mixture of dichloromethane (guaranteed reagent, E. Merck AG) and methanol (guaranteed reagent, E. Merck AG). A mixture of the same keto acid hydrazones isolated from 4 l of the 8 wt. % aqueous ethanol solution by adsorption on carbon and by selective elution (first the aldehyde hydrazones with methyl formate and dichloromethane and then the keto acid hydrazones with azeotropic pyridine–water mixture) from the carbon (Part I) was treated with methanol containing hydrogen chloride to liberate the acid hydrazones from their pyridinium salts. After evaporation of the solvent, the residue was esterified with diazomethane in diethyl ether at 0°. The resulting ester mixture was isolated and dissolved in 1 ml of (1:4, v/v) dichloromethane–methanol mixture. This mixture is designated M. The solutions of the mixtures M and R were cooled to the temperature of a dry ice–ethanol mixture, and oxygen containing about 2 vol. % of ozone was passed into both solutions at a rate of 10–20 ml/min for 2.5 h, after which pure oxygen was passed through the solution for half an hour to remove any excess ozone. After the solutions had warmed to room temperature, they were analysed by gas chromatography.

*Gas chromatography*

The column was a stainless steel tube 4 m long and 3 mm in inner diameter. The solid support was Chromosorb W (acid-washed, 60–80 mesh, Johns-Manville Products Corp.) and the liquid phase DEGA (diethylene glycol adipate) (Applied Science Laboratories, Inc.) containing phosphoric acid[8]. The proportions by weight of Chromosorb W, phosphoric acid (85 wt. %) and DEGA were 77:3:20. The gas chromatograph was a Perkin-Elmer Fractometer F 6/4 H F. The operating conditions were: carrier gas helium, flow rate 65 ml/min, inlet pressure 1.8 kp/cm$^2$; temperature programme 100–180°/2.5°/min; detection by flame ionisation, sensitivity 8; range of the recorder from 0 to 10 mV; paper speed in the recorder 1/3 in./min; injected volume 5 $\mu$l.

## RESULTS AND DISCUSSION

*Gas chromatograms of the keto acid methyl esters*

Gas chromatograms of the keto-acid methyl esters derived from keto acid methyl ester 2,4-dinitrophenylhydrazones are shown in Fig. 1. The structural isomers 2-oxoisocaproic acid and 2-oxo-3-methylvaleric acid were well separated by gas chromatography on a DEGA column. As already shown previously, the dinitrophenylhydrazones of these two acids and their esters are resolved less effectively on a thin layer[9,10]. The heights of the peaks in the gas chromatograms of mixture M are marked by horizontal dotted lines on the corresponding peaks of the gas chromatogram of the reference mixture R to facilitate comparison. The peaks of corresponding components in the two chromatograms should have been equal in height. The peaks of methyl levulinate differ the most in height. This is due to the incomplete elution of its hydrazone and possibly to its partial decomposition during the vaporisation of the eluant. The gas chromatographic method is, however, so sensitive that levulinic acid can nevertheless be identified by this method. The peaks of the methyl pyruvate in the gas chromatograms of the mixtures M and R are much closer in height than the peaks of the other methyl esters. This is due to the decarboxylation of the oxalacetic

acid present in the solution from which the M was isolated to pyruvic acid.

The methods developed for the gas chromatographic separation of keto acids are suitable only for keto monocarboxylic acids. The dinitrophenylhydrazones of 2-oxoglutaric acid and oxalacetic acid yielded on treatment with diazomethane ester derivatives from which ozone liberated esters that did not give peaks on gas chromatography. The esters in question are monoesters and they boil at temperatures too high for analysis by the gas chromatographic technique used. When the diesters formed by heating the free keto dicarboxylic acids in methanol containing hydrogen chloride and thionyl chloride[11] were analysed by the gas chromatographic method used, they gave peaks. In addition, the dinitrophenylhydrazones prepared from the dimethyl esters of the above-mentioned two keto dicarboxylic acids were found to

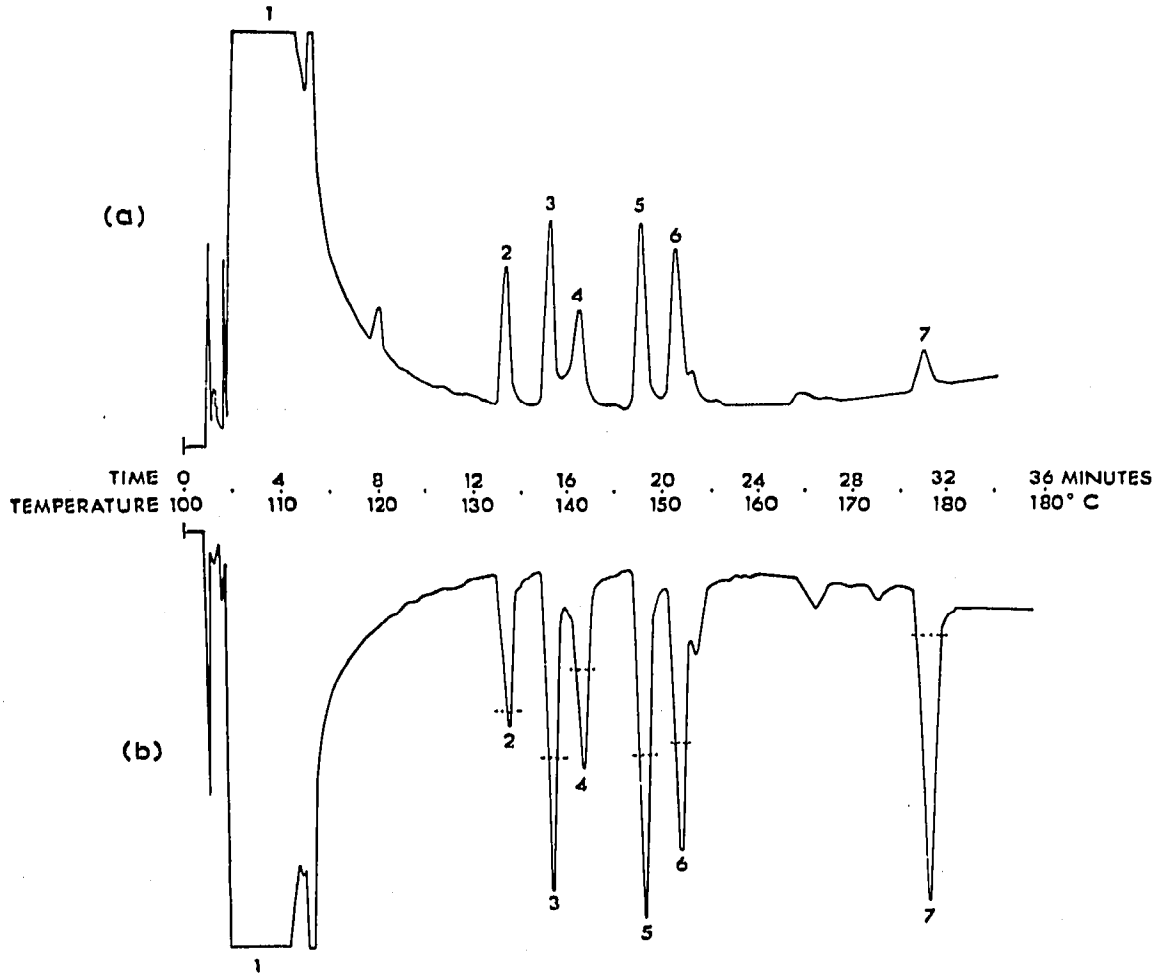


Fig. 1. Gas chromatogram of the keto acid methyl esters liberated by ozone oxidation of the mixture M of 2,4-dinitrophenylhydrazones of keto acid methyl esters (a), and that of the keto acid methyl esters liberated by ozone oxidation of the reference mixture R of pure keto acid methyl ester hydrazones (b). The dotted lines on the latter chromatogram give the heights of the corresponding peaks in the upper chromatogram. 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 65 ml/min, inlet pressure 1.8 kp/cm$^2$; temperature programme 100–180°, 2.5°/min; detection by flame ionisation, sensitivity 8; injected volume 5 $\mu$l. 1 = Solvent; 2 = methyl pyruvate; 3 = methyl 2-oxoisovalerate; 4 = methyl 2-oxobutyrate; 5 = methyl 2-oxo-3-methylvalerate; 6 = methyl 2-oxoisocaproate; 7 = methyl levulinate.

migrate on a thin layer at rates that differed from the rates of the monoester hydrazones of the keto dicarboxylic acids.

The peak heights of the keto acid methyl esters in the gas chromatograms of mixtures M which were isolated as acid hydrazones by the same adsorption and elution technique from identical solutions and then esterified and ozonisated showed good agreement. A number of additional peaks are seen in the chromatograms of the mixtures M and R in Fig. 1. In separate experiments with individual keto acid methyl ester hydrazones it was found that the small peak adjoining the peak of methyl 2-oxoisocaproate is due to a by-product formed when the dinitrophenylhydrazone of methyl pyruvate is treated with ozone and the small peaks between peaks 6 and 7 are due to by-products formed in the ozonisation of the dinitrophenylhydrazones of methyl 2-oxoisocaproate and methyl 2-oxo-3-methylvalerate. These additional peaks may sometimes be relatively large, especially when one of the decomposing compounds, for example pyruvic acid, is a strongly dominating component in the mixture of keto acids that is to be analysed.

SUMMARY

A study has been made of the ozonisation of 2,4-dinitrophenylhydrazones of eight keto acid methyl esters to corresponding methyl esters, and analysis of the latter by programmed gas chromatography on a DEGA column. The completeness of the isolation of the components from aqueous ethanol was examined by comparing the peak heights of the keto acid methyl esters liberated by ozonisation of a mixture of pure keto-acid methyl ester hydrazones (R) with those of methyl esters liberated by ozonisation of a mixture of keto acid methyl ester hydrazones (M) isolated as keto acid hydrazones by adsorption on carbon from ethanol–water solution and elution from the carbon, and then esterified.

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