CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS*

IV. GAS CHROMATOGRAPHY OF ALDEHYDE 2,4-DINITROPHENYLHYDRAZONES

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For the purpose of gas chromatographic analysis, the dinitrophenylhydrazones must be converted into volatile compounds. If aldehyde dinitrophenylhydrazones are heated with diketones[4,5], keto aldehydes[4,5] or keto acids[6], the bound aldehydes can be liberated. RALLS[7,8] has developed a method in which the precipitated dinitrophenylhydrazones of aldehydes are heated together with 2-oxoglutaric acid in a glass capillary tube before the sample is subjected to gas chromatography. Some applications and modifications of this method have been reported[9,10]. In the method of KUNITAKE[11], the aldehydes are released from their dinitrophenylhydrazones by heating them in a methanol–sulphuric acid mixture, and then analysed by gas chromatography.

Dinitrophenylhydrazones can be analysed by gas chromatography if they are oxidized with ozone as has already been described in preliminary communications[12–14]. The ozonisation leads to cleavage of the double bond between carbon and nitrogen in the hydrazone[15,16]. This study provides a detailed description of the methods of ozonisation and subsequent gas chromatography. Two successive chromatograms were run in the gas chromatographic analyses. In one run, the mixture of pure aldehyde hydrazones, and in the other the mixture of aldehyde hydrazones isolated by adsorption and elution technique from ethanol–water solution (Part I), were analysed after their ozonisation.

EXPERIMENTAL

Preparation of mixtures of aldehyde hydrazones and their ozonisation

A mixture containing 0.2 mmole of each of the 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, 2-methylbutyraldehyde and furfural, was prepared by weighing and dissolved in 100 ml of methyl formate (purum, Fluka AG). A 10-ml volume of this stock solution was evaporated to dryness and the residue R was dissolved in 1 ml of methyl formate. A dinitrophenylhydrazone mixture M, isolated by adsorption on carbon from the 4 l of 8 wt. % aqueous ethanol from which the bishydrazones had been removed, was eluted from the carbon (Part I) and dissolved in 1 ml of methyl formate. The solutions of the mixtures R and M were cooled to the temperature of a

* For Parts I–III, see refs. 1–3.

dry ice–ethanol mixture. Oxygen containing 2 vol. % of ozone was passed into them at a rate of 10–20 ml/min for a period of about half an hour and then pure oxygen was passed through the solutions for half an hour to remove any excess ozone. The solutions were then allowed to warm to room temperature and subjected to gas chromatography.

The mixtures R and M were also treated with ozone in distilled formic acid. The temperatures of the solutions were then held a few degrees above zero to avoid solidification of the solvent. The resulting solutions were also 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 was NEGS (neopentyl glycol succinate) (Applied Science Laboratories, Inc.) containing phosphoric acid[17]. The proportions by weight of Chromosorb W, phosphoric acid (85 wt. %) and NEGS were 77:3:20. The gas chromatograph was a Perkin-Elmer Fractometer F 6/4 HF. The operating conditions were: carrier gas, helium; flow rate, 74 ml/min; inlet pressure, 2.5 kp/cm^2; temperature 140°; detection by flame ionisation, sensitivity 8; recorder range from 0 to 10 mV; paper speed in the recorder 1/3 in./min; injected volume 5 μl.

The carrier gas was led through a vessel containing formic acid before it passed into the gas chromatograph. The gas picked up some formic acid which prevented the trace compounds, higher homologues of formic acid, from being adsorbed on the column packing and caused these compounds to emerge as peaks with reproducible areas.

Several other columns, including a Perkin-Elmer capillary column, were also tested for their ability to separate carboxylic acids. The best results were, however, obtained in the resolution of trace amounts of carboxylic acids derived from aldehyde hydrazones on the NEGS column.

RESULTS AND DISCUSSION

Gas chromatograms of the carboxylic acids produced by ozonisation of the aldehyde hydrazones

Gas chromatograms of the carboxylic acids produced by ozonisation of the mixtures M and R of aliphatic C_2–C_5 aldehyde- and furfural 2,4-dinitrophenylhydrazones are shown in Fig. 1. The mixture M was isolated from 8 wt. % ethanol by adsorption and subsequent elution; the amounts of the components in this mixture should have been equal to the amounts of the corresponding components in the reference mixture R. The possibility of cleaving the carbon-nitrogen double bond in hydrazones by ozonisation is not generally known, although this reaction has been studied in the case of dinitrophenylhydrazones of steroids[15] and its mechanism has been studied with the dinitrophenylhydrazones of acetone and several aromatic ketones[16]. In preliminary experiments the ozonisation reaction proved to be of value in gas chromatographic studies of dinitrophenylhydrazones[13,14]. Carboxylic acids are formed when the double bonds are broken by ozone[14]. The aromatic moieties of the dinitrophenylhydrazones are mainly converted into *m*-dinitrobenzene and 2,4-dini-

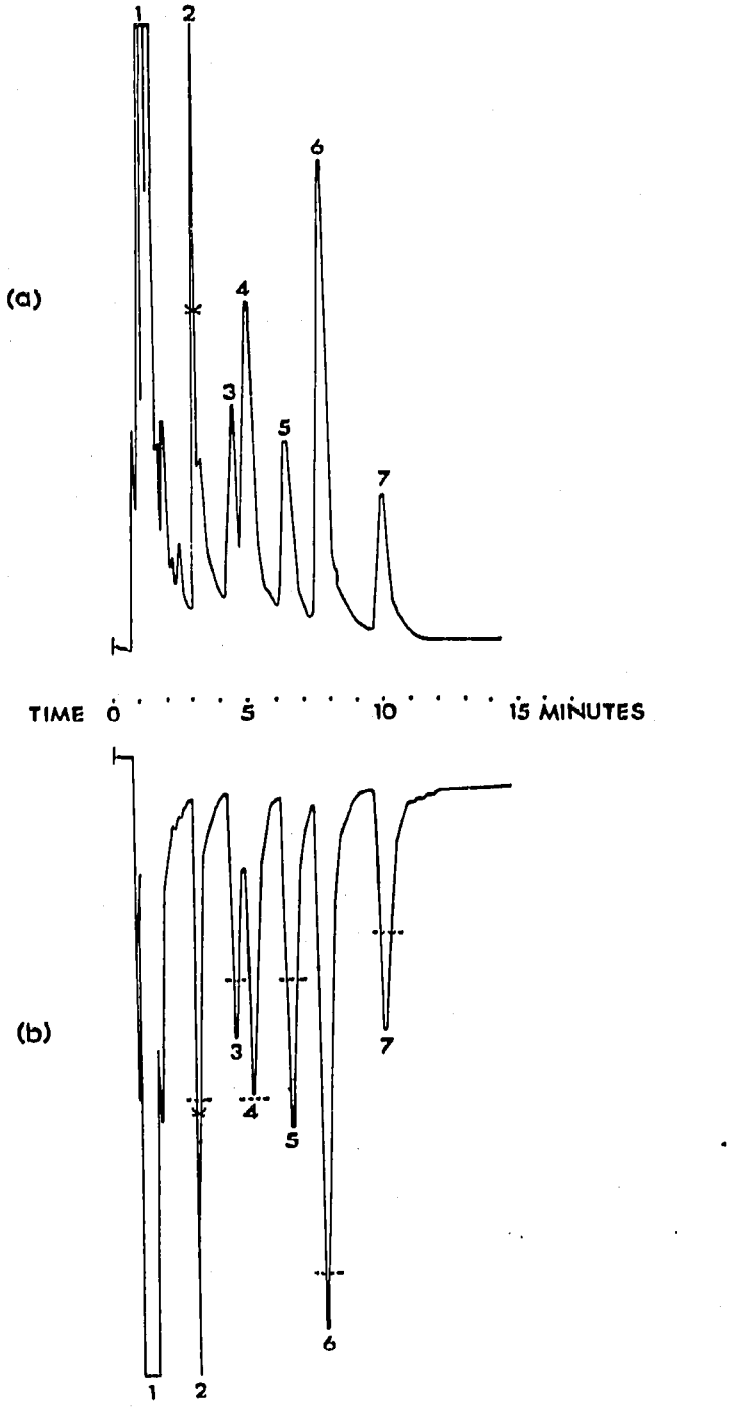

Fig. 1. Gas chromatogram of the carboxylic acids produced by ozone oxidation of the mixture M of 2,4-dinitrophenylhydrazones of aldehydes isolated by adsorption on carbon from aqueous ethanol and elution from the carbon (a), and that of the carboxylic acids produced by ozone oxidation of the reference mixture R of pure aldehyde hydrazones (b). Acetaldehyde is analysed as acetic acid, propionaldehyde as propionic acid, and so on. 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 NEGS containing phosphoric acid, solid support acid-washed Chromosorb W; carrier gas helium, flow rate 74 ml/min, inlet pressure 2.5 kp/cm^2; temperature 140°; detection by flame ionisation, sensitivity 8; injected volume 5 μl. 1 = Solvent; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = butyric acid; 6 = isovaleric acid and 2-methylvaleric acid; 7 = valeric acid.

trophenol[16], but these compounds do not give rise to interfering peaks when the oxidized mixture is subjected to gas chromatography as described above. The gas chromatographic method has several advantages over the paper chromatographic method (Part III) described earlier. Thus, for example, any excess dinitrophenylhydrazine in the sample studied does not interfere with the identification of aldehydes as the corresponding carboxylic acids and hence this method can be used to analyse oxidized mixtures of dinitrophenylhydrazones isolated by adsorption on carbon from aqueous ethanol and elution from the carbon. Also several isomeric aldehydes can be identified by this method. No peak due to a carboxylic acid derived from furfural hydrazone is found in the gas chromatogram, and therefore the paper chromatographic method described earlier must be used to identify this aldehyde.

The ozonisation of the aldehyde hydrazones can be carried out in methyl formate at the temperature of the dry ice–ethanol mixture[14] or in formic acid at temperatures a few degrees above 0°. It is advantageous to use carrier gas containing formic acid in the gas chromatography of the higher homologues of formic acid or to add formic acid to the solution to be analysed before gas chromatography[14] to prevent retention in the column of acids present only in trace quantities. The chromatogram in Fig. 1 refers to the analysis of the ozonisation products of the mixtures M and R in formic acid when the carrier gas contained formic acid. Formic acid itself does not give any peak when a flame ionisation detector is used, but when several microlitres of formic acid are injected simultaneously into the gas chromatograph, several peaks are formed in the chromatogram which evidently originate in the liquid phase in the column. However, these peaks emerge before those of the carboxylic acids derived from the aldehyde hydrazones and are indicated by the word solvent in Fig. 1.

The isothermal gas chromatographic procedure employed is suitable for the identification of lower aliphatic monocarboxylic acids. However, a temperature of 140° seems to be too high for acetic acid and results in a very sharp peak extending beyond the paper. The acetic acid peaks in the gas chromatograms of the two mixtures M and R in Fig. 1 are marked by x's to indicate the peak levels if the sensitivity of the detection had been decreased from the employed 8 to 32.

If the isolation of the dinitrophenylhydrazones from the 8 wt. % aqueous ethanol had been complete, the corresponding peaks in both chromatograms of Fig. 1 should have been equal in size. To facilitate comparison, the peak heights for the components derived from mixture M are indicated by horizontal dotted lines crossing the corresponding peaks for the same components in the reference mixture R.

The peak heights are lower for all the components, except isobutyric acid, derived from mixture M than those for the components derived from reference mixture R. All the components do not seem to have been equally effectively eluted from carbon. The solvent used to elute the compounds from the carbon is naturally of importance. Better results were obtained when the carbon was treated successively with methyl formate and dichloromethane than when either solvent was used alone. An experiment was also carried out in which the pyridine–water azeotrope was used to elute aldehyde hydrazones from carbon, but the chromatogram of the carboxylic acids produced by ozonisation of the eluted aldehyde hydrazones contained a number of additional interfering peaks. The pyridine–water azeotropic mixture is, however, suitable for the elution of keto acid hydrazones after the aldehyde hydrazones have

already been eluted with methyl formate and dichloromethane, or the pyridine–water azeotropic mixture can be used to elute all the hydrazones from the carbon for the analysis of keto acid hydrazones. In the chromatogram of the carboxylic acids derived from mixture M in Fig. 1, there is a small unidentified peak adjoining the acetic acid peak, which does not, however, interfere with the identification of the major components.

When the NEGS column is employed, isovaleric acid and 2-methylbutyric acid emerge at the same time. The gas chromatographic separation of these two acids has been very little studied. Despite repeated attempts, it was not found possible to separate these two acids on a silicone oil–stearic acid column, which has been stated to effect their separation[18]. In experiments with the pure carboxylic acids, it was found that the two acids are separated on a Perkin-Elmer capillary column (50 m long, 0.25 mm internal diameter) coated inside with a (25:1, w/w) mixture of trimer acid (a C_{54} tribasic acid with about 10 % C_{36} dibasic acid content) and dinonylnaphthalene-disulphonic acid[19], especially when a programmed temperature rise is employed. A capillary column requires, however, that the solution of the components under study is at least one hundred times as concentrated as the solutions of the mixtures M and R (1–2 mg/ml) employed in this investigation; this is because only a low proportion of the mixture injected into the gas chromatograph enters the capillary column. When samples of the mixtures M and R were submitted to gas chromatography in the capillary column, only the solvent peaks were visible in the chromatograms. The isomeric aldehydes, isovaleraldehyde and 2-methylbutyraldehyde, can therefore only be identified as a pair by the proposed method.

In contrast to paper chromatography, gas chromatography using a NEGS column can, however, be employed to separate carboxylic acids derived from isomeric aldehydes such as butyraldehyde and isobutyraldehyde and also valeraldehyde and isovaleraldehyde or 2-methylbutyraldehyde. The peak heights of the corresponding carboxylic acids derived from mixtures M isolated by the adsorption and elution technique from identical solutions showed good agreement. Although the hydrazones are not quantitatively recovered by the adsorption and elution technique (Fig. 1), the method can be employed, in view of its reproducibility, to determine unknown quantities of aldehydes in alcoholic solutions by comparing their gas chromatograms with those obtained for standard solutions of aldehydes by the same procedure. The aldehyde hydrazone that is recovered in highest yield, by adsorption and elution, is isobutyraldehyde. Because this aldehyde is less completely precipitated as its dinitrophenylhydrazone than other aldehydes from alcoholic solutions, and as it gives a much weaker colour with fuchsin than other aldehydes[20], the method described here offers the possibility of detecting this aldehyde in fermenting liquors and their distillates more reliably than previously.

SUMMARY

A study has been made of the ozonisation of 2,4-dinitrophenylhydrazones of aliphatic C_2–C_5 aldehydes to the corresponding carboxylic acids, which were analysed by isothermal gas chromatography on a NEGS column. The completeness of the isolation was examined by comparison of the peak heights of the carboxylic acids derived from a mixture of pure aldehyde hydrazones (R) with those of carboxylic

acids derived from a mixture of aldehyde hydrazones (M) isolated by adsorption on carbon from aqueous ethanol and elution from the carbon.

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