

## CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS

## IX. THE CARBONYL COMPOUNDS IN CRUDE SPIRITS\*

PENTTI RONKAINEN, SAARA BRUMMER AND HEIKKI SUOMALAINEN

*Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki (Finland)*

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The total aldehyde content of crude Finnish grain spirits, as determined by the fuchsin method of HÄHNEL<sup>1-3</sup>, and expressed as acetaldehyde, varies from 30 to 100 mg/l, and that of Finnish crude sulphite spirits from 300 to 500 mg/l. The main component of the carbonyl compounds in these two grades of spirit is acetaldehyde<sup>4,5</sup>, and some of its higher homologues present in trace quantities have also been identified in spirits after they had first been concentrated by distillation<sup>4,5</sup>. In this study the carbonyl compounds in spirits were identified, without previous concentration of the components by distillation, by applying the methods developed for isolation (Part I) and chromatography (Parts II and IV). A method for quantitative estimation of the aldehydes in a spirit has also been examined.

## EXPERIMENTAL

*Materials and isolation of the components*

The crude grain spirit studied here was produced by the Koskenkorva factory of the Finnish State Alcohol Monopoly and contained, as determined by HÄHNEL's method, 58 mg of aldehydes per l. The crude sulphite spirit examined was from a sulphite spirit factory in the centre of Finland, and contained 374 mg of aldehydes per l. Several 400-ml volumes of each grade of spirit were diluted with water to an alcohol content of about 8 wt.%. A solution of dinitrophenylhydrazine (2.5 g/l in 2*N* hydrochloric acid) was added to the diluted spirits, 60 ml to the diluted grain spirit, and 320 ml to the diluted sulphite spirit. The solutions were left to stand overnight at room temperature. An abundant precipitate (S) was separated from the diluted sulphite spirit samples, but only a small precipitate (G) from the diluted grain spirit samples. These precipitates were preserved for subsequent thin-layer chromatography. A second pair of samples of the diluted spirits was treated similarly with dinitrophenylhydrazine, and left to stand overnight. The precipitated dinitrophenylhydrazone mixtures (S and G) were collected by filtration. The filtrates were treated with carbon and filtered, and the aldehyde hydrazone mixtures (S<sub>A</sub> and G<sub>A</sub>) eluted from the carbon successively with methyl formate and dichloromethane (*cf.* Part I). The precipitates isolated from these samples were kept for subsequent gas chromatography.

\* For Parts I-VIII, see *J. Chromatog.*, 27 (1967) 374, 380, 384; 28 (1967) 253, 259, 263, 440, 443.

*Identification*

The first pair of precipitated hydrazone mixtures S and G were dissolved in 160 ml of pyridine, and a mixture R of pure bishydrazones of glyoxal, methylglyoxal, diacetyl and 2,3-pentanedione, containing 0.02 mmole of each, was dissolved in 250 ml of pyridine. Volumes of 4  $\mu$ l of these solutions were applied side by side on an activated thin layer of Silica Gel HF<sub>254</sub>, and the mixtures were resolved with a solvent system containing 34 volumes of benzene, 5 volumes of petroleum ether, and 1 volume of ethyl acetate (*cf.* Part II).

The second pair of dinitrophenylhydrazone mixtures S and G isolated by precipitation from the diluted crude sulphite and crude grain spirits, and precipitates S<sub>A</sub> and G<sub>A</sub> isolated from the same solutions after filtration by the adsorption and elution technique (*cf.* Part I), were dissolved in formic acid (S in 5 ml, G, S<sub>A</sub> and G<sub>A</sub> in 0.5 ml), ozonated (*cf.* Part IV) and analysed by isothermal gas chromatography on a NEGS column.

*Quantitative estimation of aldehydes in a crude grain spirit*

For the estimation of the quantities of the major aldehydes in crude grain spirit, three standard solutions (I, II, III, each 400 ml) containing freshly distilled acetaldehyde, propionaldehyde, isobutyraldehyde and isovaleraldehyde in aldehyde-free 94 wt. % ethanol were prepared. The amounts of the aldehydes in the solutions are given in Table I.

TABLE I

	<i>Acetaldehyde</i> (mmole)	<i>Propionaldehyde</i> (mmole)	<i>Isobutyraldehyde</i> (mmole)	<i>Isovaleraldehyde</i> (mmole)
Solution I	0.430	0.004	0.004	0.004
Solution II	0.500	0.007	0.007	0.007
Solution III	0.570	0.010	0.010	0.010

Every solution was diluted with water until the ethanol content was about 8 wt. %. Three 400-ml volumes of crude 94 wt. % grain spirit (from the same container) were similarly diluted with water. To each diluted solution was added the same volume (60 ml) of acid dinitrophenylhydrazine solution and the hydrazones formed were isolated by adsorption on carbon from the solutions and elution from the carbon and analysed, after ozonation, as carboxylic acids by gas chromatography.

## RESULTS AND DISCUSSION

Fig. 1 reproduces a thin-layer chromatogram of hydrazone mixtures S and G isolated by precipitation from diluted crude sulphite and crude grain spirits. The main purpose of this run was that of identifying the dicarbonyl compounds in the two grades of spirit. Diacetyl and 2,3-pentanedione were identified in both the grain and sulphite spirits. It has been reported that diacetyl is present in sulphite spirits<sup>6-8</sup>, but apparently 2,3-pentanedione has not been identified in spirits; however, it has been found quite recently that it occurs in beer<sup>9,10</sup>, and in whisky and brandy<sup>11</sup>.

A second pair of dinitrophenylhydrazone mixtures S and G isolated by precipi-

tation from the diluted samples of crude spirits, and precipitates  $S_A$  and  $G_A$  isolated by the adsorption and elution technique from the same solutions after removal of the precipitates S and G, were analysed by gas chromatography; the results are indicated in Figs. 2 and 3. Mixture G, precipitated from the crude grain spirit, was found to contain only traces of acetaldehyde dinitrophenylhydrazone (Fig. 2, lower chromatogram of carboxylic acids produced by ozonation). The greater part of the hydrazones of acetaldehyde and other aldehydes possibly present in low concentrations thus remained in solution. These compounds ( $G_A$ ) can, however, be recovered by adsorption on carbon from the solution and elution from the carbon and analysed after ozonation as carboxylic acids (Fig. 2, upper chromatogram). The dominating aldehydes are seen to have been acetaldehyde, isobutyraldehyde, isovaleraldehyde and/or 2-methylbutyraldehyde and propionaldehyde, whereas butyraldehyde, valeraldehyde and caproaldehyde were present in trace quantities. Neither butyraldehyde nor valeraldehyde has apparently been previously identified in grain spirits.

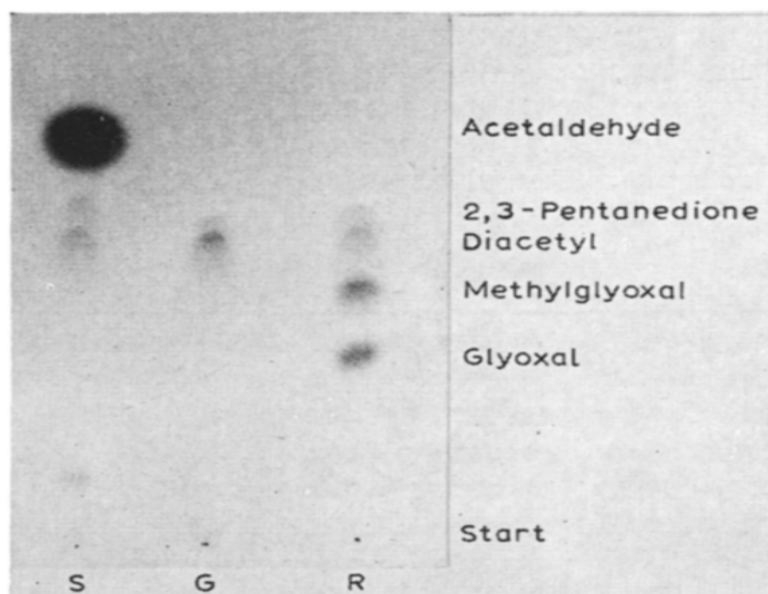


Fig. 1. Thin-layer chromatogram of mixtures S and G of 2,4-dinitrophenylhydrazones isolated by precipitation from crude sulphite (S) and crude grain (G) spirits, and of a reference mixture R of pure bishydrazones. The chromatographic solvent was benzene-petroleum ether-ethyl acetate (34:5:1) and adsorbent Silica Gel HF<sub>254</sub>. The amount of each of the reference compounds applied to the thin layer was  $3 \times 10^{-7}$  mmole.

The mixture (S) of dinitrophenylhydrazones isolated by precipitation from the diluted sample of crude sulphite spirit and the hydrazone mixture ( $S_A$ ) isolated by the adsorption and elution technique from the same solution after removal of the precipitate S contained almost the same number of components (Fig. 3). The amount of precipitated hydrazones was so large that the volume of formic acid which was required to dissolve them for analysis was ten times the volume required to dissolve the dinitrophenylhydrazones isolated by adsorption on carbon from the filtrate and elution from the carbon. The major aldehyde components in the mixtures S and  $S_A$  were found, when analysed as the corresponding carboxylic acids by gas chromatography, to be acetaldehyde, isovaleraldehyde and/or 2-methylbutyraldehyde, isobutyraldehyde and caproaldehyde. The precipitate  $S_A$  was also found to contain butyraldehyde.

Peaks due to enanthic acid (heptanoic acid) and caprylic acid (octanoic acid) are seen in the chromatogram of Fig. 3. The corresponding aldehydes were not, however, identified by paper chromatography, whereas the dinitrophenylhydrazone of caproaldehyde was identified on the paper chromatogram. The enanthic and caprylic acids

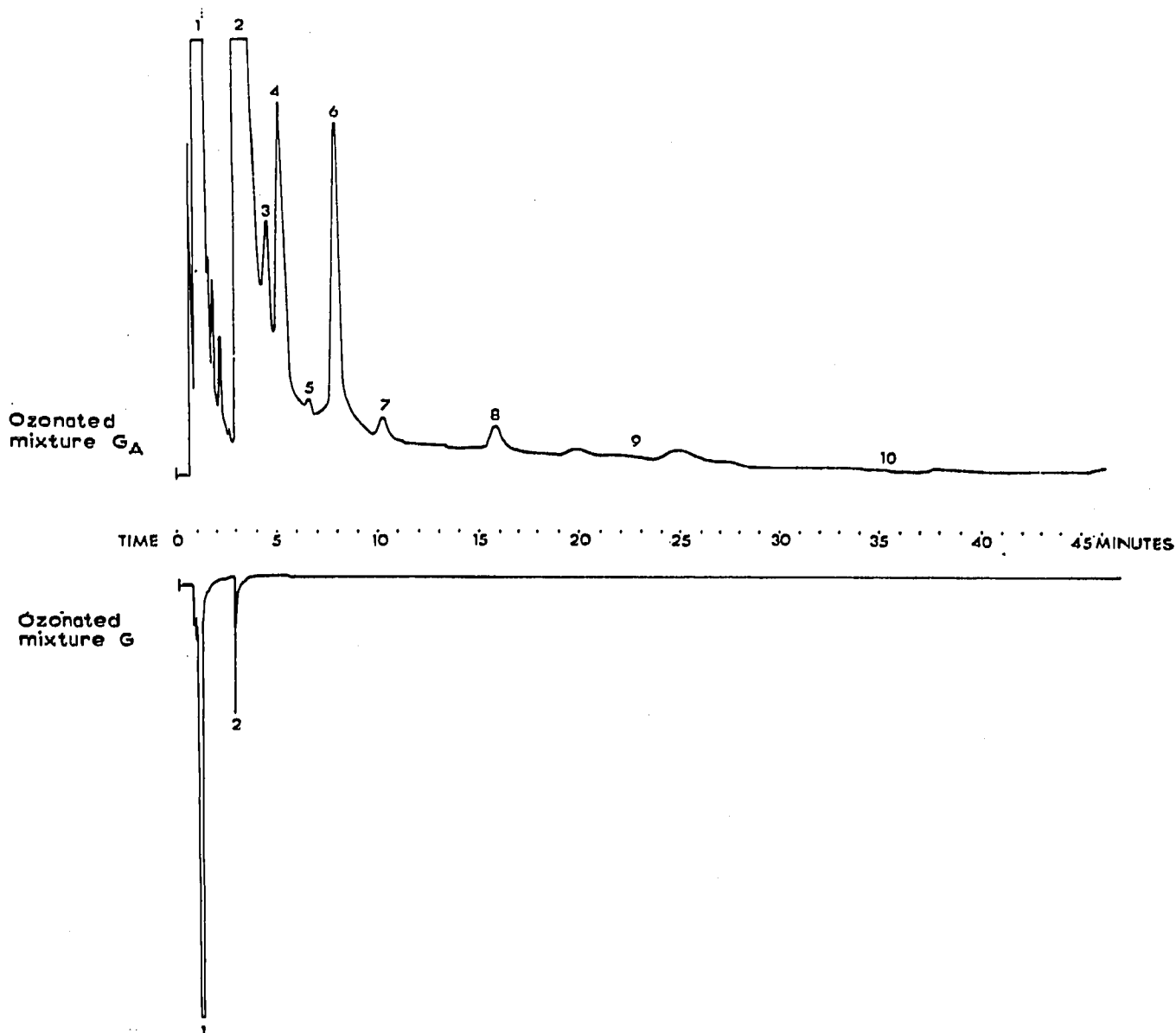


Fig. 2. Gas chromatogram of carboxylic acids produced by ozone oxidation of the 2,4-dinitrophenylhydrazones G isolated by precipitation from 400 ml of crude grain spirit after dilution with water, and that of the carboxylic acids produced by ozone oxidation of the hydrazones G<sub>A</sub> isolated by adsorption on carbon from the same solution after filtration and eluted from the carbon. Acetaldehyde is analysed as acetic acid, propionaldehyde as propionic acid, and so on. The hydrazone mixtures G and G<sub>A</sub> were each dissolved in 0.5 ml of formic acid for the ozonation and subsequent gas chromatography. 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, 73 ml/min; inlet pressure, 2.5 kp/cm<sup>2</sup>; temperature, 140°; detection by flame ionisation: sensitivity, 4; 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; 7 = valeric acid; 8 = caproic acid (hexanoic acid); 9 = enanthic acid (heptanoic acid); 10 = caprylic acid (octanoic acid).

were evidently formed in the ozonation of the dinitrophenylhydrazones from a high-molecular weight, possibly unsaturated, carbonyl compound present in these mixtures. Butyraldehyde has not been identified positively in sulphite spirits previously. Propionaldehyde was not identified as propionic acid; the very large peak of acetic acid probably obscured its peak (Fig. 3).

In the quantitative estimation by gas chromatography of the amounts of

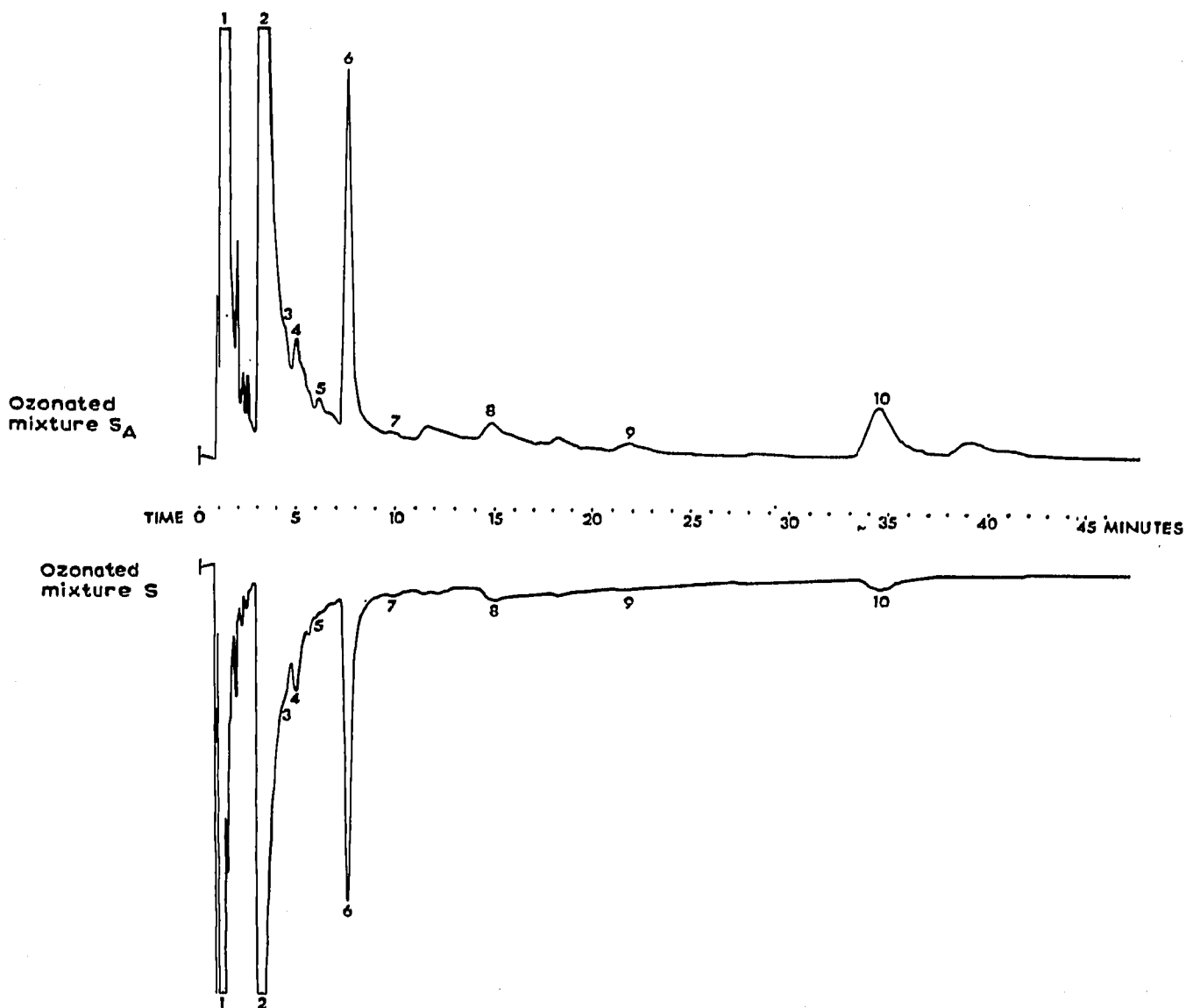


Fig. 3. Gas chromatogram of carboxylic acids produced by ozone oxidation of the 2,4-dinitrophenylhydrazones S isolated by precipitation from 400 ml of crude sulphite spirit after dilution with water, and that of the carboxylic acids produced by ozone oxidation of the hydrazones  $S_A$  isolated by adsorption on carbon from the same solution after filtration and eluted from the carbon. Acetaldehyde is analysed as acetic acid, propionaldehyde as propionic acid, and so on. The hydrazone mixture S was dissolved in 5 ml and the mixture  $S_A$  in 0.5 ml of formic acid for the ozonation and subsequent gas chromatography. 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, 76 ml/min; inlet pressure, 2.5 kp/cm<sup>2</sup>; temperature, 140°; detection by flame ionisation: sensitivity, 4; injected volume, 5  $\mu$ l. For designations, see the legend of Fig. 2.

aldehydes in a crude grain spirit, the areas of the peaks in the chromatograms were determined by multiplying the peak heights by the peak half width<sup>12</sup>. To increase the areas of the peaks and the accuracy of their measurement, the chart speed of the recorder was increased to 2 in. per min from the usual 1/3 in. per min, and for the same reason the column temperature was lowered when the acetic acid produced from acetaldehyde hydrazone was analysed. The data for the standard solutions are plotted and joined by straight lines in Fig. 4. The abscissa gives the aldehyde concentration in millimoles in 400 ml of the 95 wt. % ethanol and the ordinate the area of the peak of the carboxylic acid derived from the aldehyde in square centimetres.

The points marked by symbols  $\times$ ,  $\circ$  and  $\Delta$  in Fig. 4 represent the amounts of the components in the crude grain spirit deduced from the peak areas. Each symbol refers to one of three spirit samples. Because all the samples were taken from the same container, the distances between the symbols on each line are due to differences in the adsorption and elution. To eliminate the differences in peak areas due to differences in

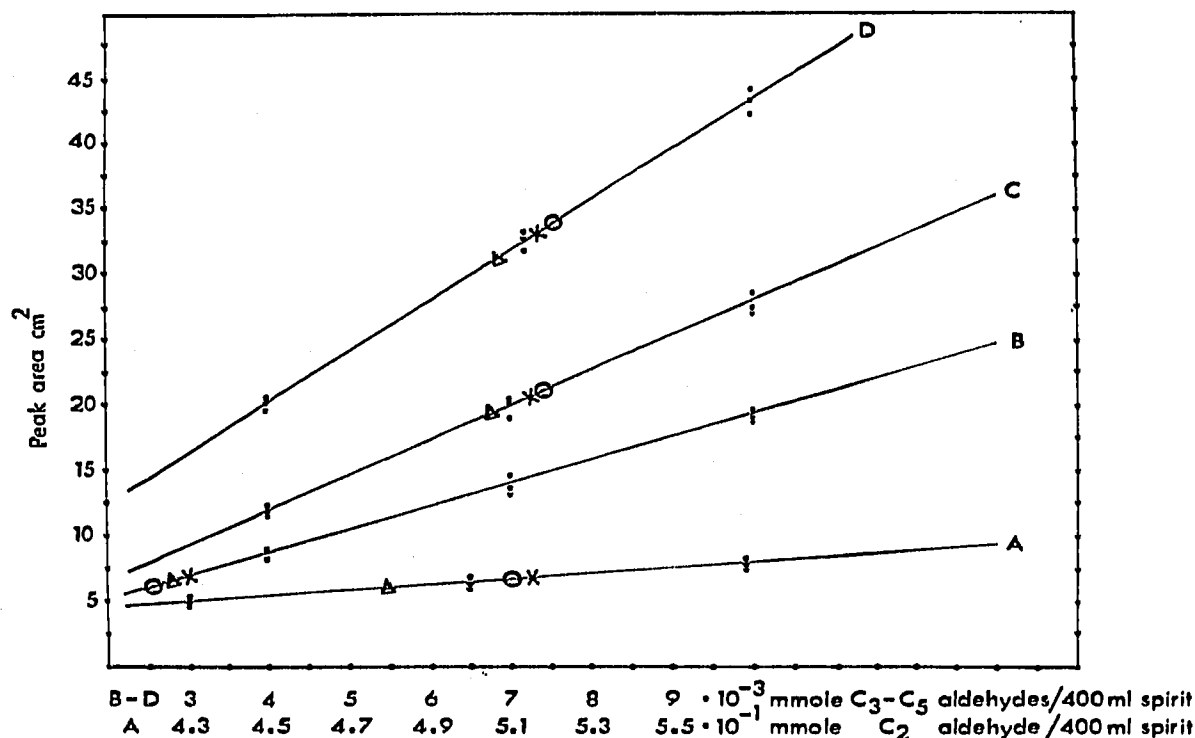


Fig. 4. Calibration lines plotting peak areas of carboxylic acids in gas chromatograms against the numbers of millimoles of aldehydes dissolved in 400-ml volumes of 94 wt. % ethanol. The lines were drawn through observed values marked by points. The aldehydes were isolated as 2,4-dinitrophenylhydrazones by adsorption on carbon from the ethanol, after its dilution with water, and eluted from the carbon and the hydrazones were then converted for gas chromatography into the corresponding carboxylic acids by treatment with ozone. Line A refers to acetic acid derived from acetaldehyde; the gas chromatograms were run using a column temperature of 110° and detector sensitivity of 688. The lines B, C and D refer to propionic acid, isobutyric acid and isovaleric acid, respectively, derived from the corresponding aldehydes; the gas chromatograms were run using a column temperature of 140° and a detector sensitivity of 8. The other conditions in the gas chromatographic runs were: 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<sup>2</sup>; detection by flame ionisation; recorder range from 0 to 5 mV; paper speed, 2 in./min; injected volume, 5  $\mu$ l. Peak areas for carboxylic acids derived similarly from the aldehydes present in three samples of a crude grain spirit are marked by the symbols  $\times$ ,  $\circ$  and  $\Delta$ .

injected volumes every symbol in Fig. 4 gives the mean of three values from three gas chromatograms.

The crude grain spirit thus contained, as means of the three mean values (the three symbols in Fig. 4), 1.25 mmole/l (55 mg/l) of acetaldehyde, 0.018 mmole/l (1.5 mg/l) of isovaleraldehyde and/or 2-methylbutyraldehyde, 0.018 mmole/l (1.3 mg/l) of isobutyraldehyde, and 0.007 mmole/l (0.4 mg/l) of propionaldehyde.\*

The areas of the peaks in the chromatograms and the corresponding concentrations of aldehydes in the solutions were subjected to regression analysis on an Elliott 803 electronic computer at the Computer Centre of the Suomen Kaapelitehdas Osakeyhtiö (Finnish Cable Works Co.), Helsinki, to determine the mean concentrations of the aldehydes in the crude grain spirit and the 99 % confidence limits of these mean concentrations. The following mean aldehyde concentrations and their 99 % confidence limits were evaluated as: acetaldehyde  $55 \pm 8$  mg/l; isovaleraldehyde and/or 2-methylbutyraldehyde  $1.5 \pm 0.1$  mg/l; isobutyraldehyde  $1.3 \pm 0.1$  mg/l and propionaldehyde  $0.4 \pm 0.1$  mg/l. The mean values are the same as the mean values obtained by the graphical method (Fig. 4).

#### SUMMARY

Carbonyl compounds in crude Finnish sulphite and grain spirits have been isolated by precipitation as their 2,4-dinitrophenylhydrazones, and the unprecipitated hydrazones by adsorption on carbon and elution from the latter. The dicarbonyl compounds were identified as their bishydrazones in thin-layer chromatography. Diacetyl and 2,3-pentanedione were found present in these two grades of spirit. Aldehydes were analysed by isothermal gas chromatography on a NEGS column, after ozone oxidation of the hydrazones to the corresponding carboxylic acids. Acetaldehyde proved to be the dominating component, whereas C<sub>3</sub>-C<sub>6</sub> aldehydes were present in smaller quantities. The amounts of acetaldehyde, propionaldehyde, isobutyraldehyde and isovaleraldehyde and/or 2-methylbutyraldehyde in a crude grain spirit were estimated quantitatively by the gas chromatographic method mentioned, using standard solutions of these compounds for reference. The confidence limits of the values were estimated on an electronic computer.

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\* The total aldehyde content expressed as acetaldehyde as measured by the fuchsin method was  $58.5 \pm 0.3$  mg/l as the mean of ten determinations.