

## CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS

## I. ISOLATION OF CARBONYL COMPOUNDS FROM SOLUTIONS AS THEIR 2,4-DINITROPHENYLHYDRAZONES

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(Received September 29th, 1966)

The synthesis of dinitrophenylhydrazones on the preparative scale is generally very simple<sup>1</sup> and relatively high yields are obtained under favourable conditions<sup>1-3</sup>. If the solution under study contains several carbonyl compounds of different types, which may, in addition, vary greatly in amount, the components present in trace amounts may not be precipitated, especially if the solution also contains ethanol. In such cases the precipitation is less quantitative the higher the alcohol content of the solution studied<sup>4,5</sup>. The precipitation of the hydrazones is also complicated by the mutual interaction of the components, the extent of precipitation being different depending on whether individual compounds or their mixtures are present<sup>4</sup>.

It is possible to concentrate volatile carbonyl compounds present in trace quantities by distillation before precipitation. Equilibrium distillation experiments have shown that it is advantageous to dilute concentrated alcohol solutions with water to alcohol concentrations of 20% or less before removal of the aldehydes by distillation<sup>6</sup>. On the basis of equilibrium distillations it is not, however, possible to determine how long batch distillation should be continued in order that the greater part of the aldehydes be transferred to the distillate. Neither is it possible to estimate the yield. Batch distillation studies show that the aldehydes are mainly collected in the first distillate fractions when dilute alcoholic solutions are distilled<sup>5</sup>. Considerable losses of aldehydes occur, however, in the distillation; the proportion lost varies for different aldehydes, but is of the order of 20-50%<sup>5</sup>. Evidently the losses are partly due to the tendency of the aldehydes to undergo polymerization during the distillation. Thus, batch distillation does not seem to be a suitable method for the concentration of aldehydes when their levels are low (of the order of a few milligrams per liter). In such cases the high alcohol content of the distillate requires that it must be diluted again with water before the precipitation, and even without the losses the amounts of the compounds recovered would be so small that their precipitation would not succeed.

The unprecipitated dinitrophenylhydrazones can in some cases be recovered from the solutions by solvent extraction. Extraction methods for the isolation of neutral carbonyl compounds have been studied only to a limited extent<sup>7</sup>, but a great many methods have been developed for the recovery of dinitrophenylhydrazones of keto acids from their solutions by solvent extraction<sup>8-15</sup>. Although these methods differ in respect of the solvents employed, they do not differ in principle.

An excess of dinitrophenylhydrazine solution is added to the solution under study and the hydrazones formed and excess reagent are extracted with an organic solvent. The keto acid hydrazones are removed from the extract with an alkali metal carbonate or ammonia solution and from the latter, after acidification, with an organic solvent. The methods are fairly complicated and it has been found that the extracted precipitate contains besides keto acid hydrazones also aldehyde hydrazones, if the latter are present in abundance in the solution, and dinitrophenylhydrazine, which may interfere with the subsequent chromatographic separation<sup>15</sup>. It has also been established that some keto acids are incompletely extracted, for instance, by a sodium carbonate solution from ethyl acetate<sup>14</sup>, and the many variations in pH during the extraction may lead to isomerization<sup>11,16</sup> and partial decomposition<sup>17</sup> of some of the keto acid hydrazones.

The present experimental investigation has had as its purpose the development, with the aid of model carbonyl compounds, of a method for the isolation of dinitrophenylhydrazones of carbonyl compounds from ethanol-water solutions for the subsequent analysis of these derivatives by appropriate chromatographic methods, which can also be applied in the study of fermented liquors and alcohol distillates. The model compounds comprised mainly carbonyl compounds such as aldehydes, keto acids and dicarbonyl compounds that had previously been identified or which were expected to be present in fermented liquors and/or alcohol distillates. The number of model compounds was nineteen. Also the presence of excess reagent and its interference was taken into account in the experimental investigation.

## EXPERIMENTAL

### *Sources and purification of the carbonyl compounds*

Most of the carbonyl compounds used as reference standards were commercial products and only two, 2-methylbutyraldehyde and 2-oxo-3-methylvaleric acid, were expressly synthesized for the investigation. The commercial carbonyl compounds were purified by distillation or recrystallization before use. The grades of purity, sources, and boiling points or melting points of the compounds are given in Table I.

2-Methylbutyraldehyde was prepared from 2-methyl-1-butanol (practical, Fluka AG) by oxidation with dichromate-sulphuric acid<sup>18</sup> and purified by distillation. 2-Oxo-3-methylvaleric acid was prepared by condensing *sec.*-butylmagnesium chloride and diethyl oxalate (purum, Fluka AG) and saponifying the resulting ester<sup>19,20</sup>. The acid was purified by recrystallization from dichloromethane (guaranteed reagent, E. Merck AG). The aqueous solutions of glyoxal and methylglyoxal were evaporated to dryness *in vacuo* and the residues were distilled from phosphorus pentoxide<sup>21,22</sup>. The last two keto dicarboxylic acids in Table I were recrystallized from a 1:10 (v/v) mixture of acetone (guaranteed reagent, E. Merck AG) and dichloromethane (guaranteed reagent, E. Merck AG) at the temperature of a dry ice-ethanol mixture.

### *Preparation of solutions of reference carbonyl compounds*

The alcohol employed in the experiments was absolute ethanol (99.9 wt. %, Danisco AS) that had been distilled from aluminium and potassium hydroxide to remove aldehydes<sup>23</sup>. Its boiling point was 77.9°/746 mm Hg. The distilled ethanol did not give any colour in the fuchsin test for aldehydes. Immediately after the

TABLE I

CARBONYL COMPOUNDS USED IN THE INVESTIGATION, THEIR QUALITIES, SOURCE AND OBSERVED BOILING OR MELTING POINTS

<i>Carbonyl compound, quality and source</i>	<i>Boiling point (°C/mm Hg)</i>	<i>Melting point (°C)</i>
Acetaldehyde, guaranteed reagent, E. Merck AG	20/746	—
Propionaldehyde, purum, Fluka AG	48/760	—
Butyraldehyde, puriss., Fluka AG	74/760	—
Isobutyraldehyde, purum, Fluka AG	64/760	—
Valeraldehyde, puriss., Fluka AG	102/751	—
Isovaleraldehyde, purum, Fluka AG	92/751	—
2-Methylbutyraldehyde, synthesized	90-92/746	—
Furfural, guaranteed reagent, E. Merck AG	160/756	—
Glyoxal, aqueous solution (40%), Fluka AG	50-51/755	—
Methylglyoxal, aqueous solution (39%), Fluka AG	68-70/758	—
Diacetyl, puriss., Fluka AG	89-90/770	—
Pyruvic acid, puriss., Fluka AG	55-56/ 9	—
2-Oxobutyric acid, puriss., Fluka AG	64/ 9	—
2-Oxoisovaleric acid, puriss., Fluka AG	64/ 9	—
2-Oxoisocaproic acid, puriss., Fluka AG	76/ 9	—
2-Oxo-3-methylvaleric acid, synthesized	— —	37
Levulinic acid, pure, E. Merck AG	134/ 9	—
2-Oxoglutaric acid, puriss., Fluka AG	— —	113
Oxalacetic acid, puriss., Fluka AG	— —	163-166

distillation, the ethanol was diluted with distilled water to obtain two solutions, one containing 8% and the other containing 96% ethanol by weight. A stock solution of every one of the nineteen carbonyl compounds studied was prepared by diluting 2 mmoles of compound to 1 litre with 8 wt. % ethanol. These nineteen solutions were stored in the dark in a refrigerated room at 4°. As required, small volumes of these stock solutions were allowed to warm to 20° and the volumes required in the experiments were measured with pipettes. Oxalacetic acid proved to be the least stable in solution of the carbonyl compounds studied and for this reason a new stock solution of this compound in 8 wt. % aqueous ethanol was prepared before each experiment.

#### *Preliminary experiments on the isolation of carbonyl compounds from solutions*

Preliminary experiments were carried out to isolate carbonyl compounds present in low concentrations in aqueous ethanol solutions as their dinitrophenylhydrazones. A 2.5-ml volume of each of the stock solutions or 0.005 mmole of every one of the nineteen carbonyl compounds was added to two vessels and the mixture in the first vessel was diluted to one litre with 94 wt. % ethanol and that in the other to 1 l with 8 wt. % ethanol. An excess of the acid dinitrophenylhydrazine solution was added to the two resulting solutions which were then mixed with magnetic stirrers. A precipitate formed only in the dilute aqueous ethanol solution, and this precipitate was formed only by the dicarbonyl compounds; there was no precipitate when these were absent.

Attempts were made to remove the unprecipitated hydrazones from the concentrated and dilute aqueous ethanol with a number of solid adsorbents. It was found that many grades of activated carbon rapidly decolourized the dilute aqueous ethanol solution, but were unable to decolourize the concentrated aqueous ethanol

solution. Various solvents were used to remove the adsorbed compounds from the carbons. Dichloromethane, methyl formate, pyridine and diethylamine proved to be the most effective eluants. Dichloromethane and methyl formate eluted only the aldehyde hydrazones and a part of the excess dinitrophenylhydrazine, while pyridine and diethylamine eluted the hydrazones of the aldehydes and keto acids as well as the excess reagent.

#### *Isolation methods*

Ten millilitres of the stock solution of each of the nineteen carbonyl compounds (0.02 mmole of each component) were added to a flask and the mixture was diluted to 4 l with aldehyde-free 8 wt. % aqueous ethanol. The concentration of acetaldehyde, the compound of lowest molecular weight, was hence 0.9 mg/4 l, and the concentration of 2-oxoglutaric acid, the compound of highest molecular weight, 2.9 mg/4 l. A little more than one and a half times the calculated volume of acid dinitrophenylhydrazine solution (60 ml of solution containing 2.5 g of 2,4-dinitrophenylhydrazine in 1000 ml of 2 N hydrochloric acid) was added to the solution, which was then mixed with a magnetic stirrer overnight. The precipitate, containing only bishydrazones according to the preliminary experiments, was collected on a Jena G 4

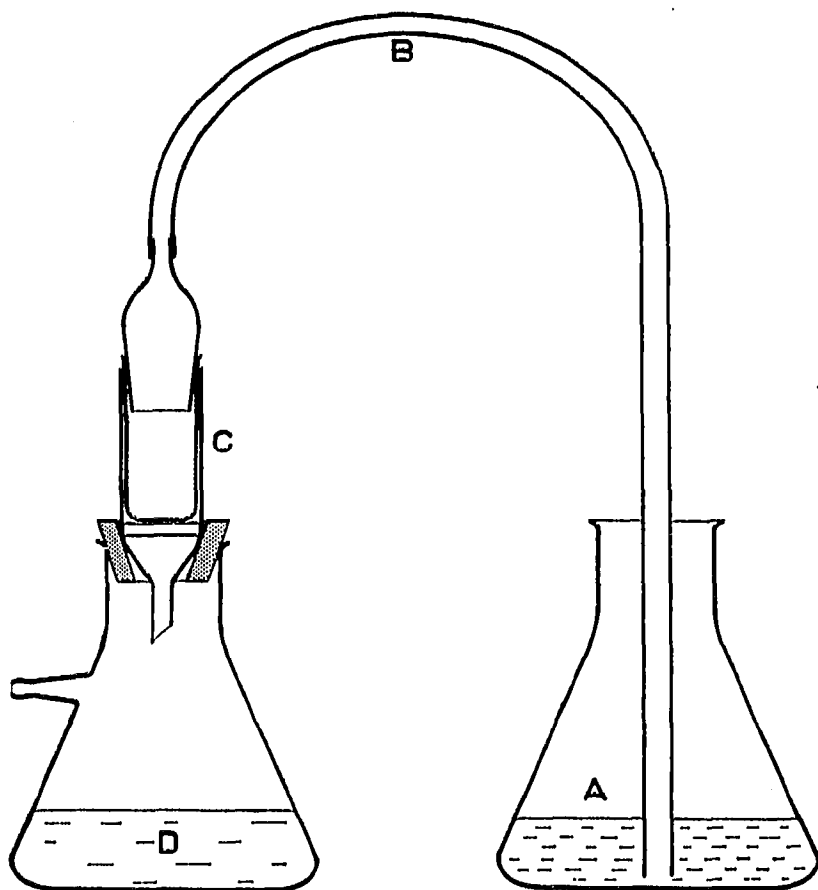


Fig. 1. Apparatus for removing carbon from solutions. A = vessel containing solution from which carbon is to be removed; B = Teflon tubing; C = glass filter crucible supporting a cellulose thimble which is pressed down against the filter plate by an inverted funnel attached to B; D = suction flask to collect the filtrate.

glass filter crucible, washed with 2 *N* hydrochloric acid and distilled water, and dried over phosphorus pentoxide in a vacuum desiccator. The filtrate was neutralised with 2 *N* ammonia to pH 6.9 as measured with a Beckman Model H 2 pH-meter and then 3 g of activated carbon (Dargo Grade 60, Atlas Powder Co.) was added to it. The carbon-containing solution was mixed by shaking from time to time during the course of 2 h to promote the adsorption of the coloured matter. The solution was then filtered through a cellulose thimble (Extraktions-Hülsen No. 603, Carl Schleicher & Schüll, Dassel/Kr. Einbeck Pachtbetrieb der Büttenpapierfabrik Hahnemühle GmbH) in the apparatus shown in Fig. 1, which permits the rapid filtration of relatively large liquid volumes. The filtrate was colourless. The carbon remaining in the container was rinsed into the thimble with portions of the filtrate. When all the carbon was in the thimble, it was washed with 8 wt. % aqueous ethanol and dried in the vacuum desiccator. The dinitrophenylhydrazones of the aldehydes were extracted from the carbon in a Soxhlet apparatus, first with methyl formate (purum, Fluka AG) and then with dichloromethane (guaranteed reagent, E. Merck AG), 24 h with each solvent. The extracts were combined and the solvents evaporated in a Rotavapor unit. The dichloromethane retained by the carbon and the thimble was evaporated *in vacuo* and the retained dinitrophenylhydrazones of the keto acids were extracted in a nitrogen atmosphere from the carbon with an azeotropic mixture of pyridine ("Baker Analyzed" Reagent, J. T. Baker Chemical Co.) and water in the Soxhlet apparatus at a pressure of 17 mm Hg, where the solvent mixture boiled at 18°. The extraction was continued for about 48 h and the condenser was cooled with an ethanol-water mixture at -10 to -15°. The pyridine-water extract was finally evaporated to dryness in the Rotavapor. An attempt was also made to extract all the dinitrophenylhydrazones adsorbed on the carbon with the pyridine-water azeotropic mixture. The extracted mixture is, however, difficult to analyse and this single elution step is to be preferred when only keto acids, and particularly the unstable oxalacetic acid are to be determined.

## RESULTS

It was found that of nineteen carbonyl compounds, each present in a concentration of 0.005 mmole/l in 8 wt. % aqueous ethanol, only the dicarbonyl compounds glyoxal, methylglyoxal and diacetyl precipitate from the solution as bis[(2,4-dinitrophenyl)hydrazones]. The unprecipitated monohydrazones of the remaining carbonyl compounds can be isolated from the solution by adsorption on activated carbon and elution from the carbon in a Soxhlet apparatus with organic solvents. The mixture of 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, 2-methylbutyraldehyde and furfural was eluted from the carbon successively with methyl formate and dichloromethane. The mixture of 2,4-dinitrophenylhydrazones of pyruvic acid, 2-oxobutyric acid, 2-oxoisovaleric acid, 2-oxoisocaproic acid, 2-oxo-3-methylvaleric acid, levulinic acid, 2-oxoglutaric acid and oxalacetic acid was then eluted from the carbon with an azeotropic pyridine-water mixture at low pressure. The last-mentioned hydrazone decarboxylated mainly to pyruvic acid hydrazone during

\* The azeotropic mixture of pyridine and water boils at 94° at normal pressure and contains 57 % water by weight<sup>24</sup>.

these elutions. If all the monocarbonyl compounds were eluted from the carbon in one group with the azeotropic pyridine-water mixture the 2,4-dinitrophenylhydrazone of oxalacetic acid did not decarboxylate completely during this single elution step.

#### SUMMARY

The isolation of traces of aliphatic aldehydes, furfural, dicarbonyl compounds and keto acids as their 2,4-dinitrophenylhydrazones from dilute aqueous ethanol for their subsequent identification by chromatography has been investigated. Bishydrazones of dicarbonyl compounds were isolated by precipitation and monohydrazones of aldehydes and keto acids by adsorption on activated carbon and selective elution from the latter—first aldehyde hydrazones and then keto acid hydrazones.

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