CHROM. 4404

ANALYSIS OF α-HYDROXY KETONES BY GAS CHROMATOGRAPHY

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(Received September 25th, 1969)

SUMMARY

α-Hydroxy ketones, acetoin and 3-hydroxy-2-pentanone have been isolated from an alcohol–water solution as their 2,4-dinitrophenylhydrazones, and the latter converted in acid solution to the corresponding vicinal diketones by water–steam distillation. Analysis of the diketones formed has been effected by head-space gas chromatography of the distillate by means of an electron-capture detector.

INTRODUCTION

The closely related compounds, diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), 2,3-butanediol and 2-aceto-2-hydroxylactic acid, appear in fermentation solutions, in addition to smaller amounts of their next higher homologues. Gas chromatographic methods have been developed for the analysis of diketones[1–3], glycols[4] and 2-aceto-2-hydroxycarboxylic acids[5] but gas chromatography has only occasionally been used for the analysis of α-hydroxy ketones[6].

The analysis of acetoin by gravimetric[7–9] and colorimetric[10] methods has been based upon the formation of coloured metallic salts of dimethylglyoxime after the oxidation of acetoin to diacetyl, and other colorimetric methods rely upon the colour reaction between diacetyl and creatine[11,12], or between diacetyl and chromotropic acid[13]. The estimated quantity of acetoin is the sum of the α-hydroxy ketones present.

A method is presented here for the analysis of acetoin and 3-hydroxy-2-pentanone, separately, by gas chromatography, after the isolation of these compounds from an alcohol–water solution as 2,4-dinitrophenylhydrazones.

MATERIAL AND METHODS

Preparation of 2,4-dinitrophenylhydrazones of α-hydroxy ketones

The 2,4-dinitrophenylhydrazone of acetoin was prepared both from the dimeric acetoin (research chemical, Aldrich Chemical Co., Inc., Milwaukee 10, Wisc., U.S.A.) and 2-acetolactic acid (synthesised by the KRAMPITZ's method[14]). In the former case, 100 mg of crystals of dimeric acetoin were first washed with diethyl ether and after drying, were dissolved in a small amount of water. For precipitation, 100 ml of 2,4-dinitrophenylhydrazine (analar, BDH Ltd., Poole, Great Britain) reagent solution (2.5 g of reagent/1000 ml 2 *N* HCl) were added. The solution was saturated with

$Na_2SO_4 \cdot 10\ H_2O$ (guaranteed reagent, E. Merck AG., Darmstadt, Germany) to minimise the solubility of the hydrazone; after the solution had been allowed to stand at 4° overnight, the precipitate formed was filtered, washed with water, and recrystallised twice from an alcohol–water (1:1) mixture (m.p. 107°).

For the preparation of the acetoin hydrazone from 2-acetolactic acid, the ester derivative of the latter, 2-aceto-2-acetoxypropionic acid ethyl ester, was synthesised by the KRAMPITZ's method[14]. A sample of 100 mg of the ester was dissolved in 14 ml of 0.1 *N* NaOH solution, and hydrolysed at 40° for 1 h. The liberated acetolactic acid was decarboxylated by means of 10 ml of 9 *M* H_2SO_4 solution, and the solution was kept at 40° for 2 h and then neutralised with solid $NaHCO_3$ (guaranteed reagent, E. Merck AG., Darmstadt, Germany). The precipitation and recrystallisation of 2,4-dinitrophenylhydrazone of acetoin were effected as described above (m.p. 106°).

The 2,4-dinitrophenylhydrazone of 3-hydroxy-2-pentanone was prepared from the ethyl ester of 2-aceto-2-acetoxybutyric acid synthesised by KRAMPITZ's method (*cf.* ref. 5); the same procedures as above then being employed, *viz.* hydrolysation, decarboxylation, precipitation and recrystallisation (m.p. 98°).

Isolation of 2,4-dinitrophenylhydrazones of α-hydroxy ketones from diluted alcohol–water solution by adsorption

In cases where the concentrations of α-hydroxy ketones are no more than some tens of milligrammes per litre or less, the isolation of their hydrazones by precipitation is difficult, because of the solubility of the α-hydroxy ketone hydrazones. The adsorption method[15] developed earlier, with some slight modification, can be used. A model solution of acetoin and 3-hydroxy-2-pentanone was prepared from the synthesised 2-aceto-2-hydroxycarboxylic acid derivatives mentioned above. For this purpose, 10^{-1} mmole (20.2 mg) of 2-aceto-2-acetoxypropionic acid ethyl ester, and $2.5 \cdot 10^{-2}$ mmole (5.4 mg) of 2-aceto-2-acetoxybutyric acid ethyl ester were dissolved in 4 ml of 0.1 *N* NaOH solution, and hydrolysed at 40° for 1 h. After acidification with 2.5 ml of 9 *M* H_2SO_4, the solution was kept at 40° for 2 h during which time, the acetohydroxy acids were decarboxylated. The volume of the solution was adjusted to 10 ml with water, and used as the base solution of α-hydroxy ketones. A sample of 1 ml of the base solution (containing 10^{-2} mmole, or 0.88 mg of acetoin and $2.5 \cdot 10^{-3}$ mmole, or 0.25 mg of 3-hydroxy-2-pentanone) was dissolved in 100 ml of 5 % ethanol–water mixture; in another parallel experiment 50 μl of 2,3-butanediol (puriss., Fluka AG., Buchs, Switzerland) was added to clarify its possible influence upon the analysis of α-hydroxy ketones. The solutions of 2,4-dinitrophenylhydrazine reagent and concentrated hydrochloric acid (guaranteed reagent, E. Merck AG., Darmstadt, Germany) were added in the proportions of 100 ml and 30 ml respectively. The solutions were mixed with a magnetic stirrer overnight at 4°. For precipitation of the excess of reagent present, the solutions were neutralised with solid $NaHCO_3$, and the precipitate formed was filtered off. For the isolation of α-hydroxy ketone hydrazones from the solutions, 3 g of activated carbon (Dargo Grade G-60, Atlas Chemical Industries, Inc., Wilmington, Del., U.S.A.) was added to the solutions and mixed with a magnetic stirrer for 1 h. Carbon was filtered in a glass filter crucible on a layer of 2–4 mm of Hyflo-Super-Cel (a Celite product, Johns-Manville, Lompoc, Calif., U.S.A.) and washed with 200 ml of 94.5 wt. % ethanol (pure, Rajamäki Factories of the Finnish State Alcohol Monopoly, Rajamäki, Finland), and 200 ml of water.

Conversion of 2,4-dinitrophenylhydrazones of α-hydroxy ketones to diketones

For conversion of the α-hydroxy ketone hydrazones adsorbed on the carbon to the corresponding diketones, the carbon was transferred into a micro steam-distillation apparatus and 4 ml of a mixture (1:1) of water and concentrated H_2SO_4 (guaranteed reagent, E. Merck AG., Darmstadt, Germany), and 100 mg of 2-oxoglutaric acid (for biochemistry, E. Merck AG., Darmstadt, Germany) were added. The mixture was distilled with water–steam until the amount of distillate was 100 ml. During the distillation, the α-hydroxy ketones were liberated and oxidised to the corresponding vicinal diketones; these were collected in the distillate.

Experiments to convert α-hydroxy ketone hydrazones to diketones were also made with pure (precipitated and recrystallised) 2,4-dinitrophenylhydrazones of acetoin and 3-hydroxy-2-pentanone. A solution of these hydrazones was made by dissolving 10^{-1} mmole (26.8 mg) of acetoin hydrazone and $2.5 \cdot 10^{-2}$ mmole (7.0 mg) of 3-hydroxy-2-pentanone hydrazone in 40 ml of a mixture (1:1) of concentrated sulphuric acid and water. A part of this solution (4 ml) was transferred into the micro steam-distillation apparatus mentioned above, and distilled with water–steam in the presence of 2-oxoglutaric acid (100 mg) until the amount of distillate was 100 ml.

Analysis of the steam distillates

During the distillation of a mixture of activated carbon and sulphuric acid solution, traces of SO_2 can be formed, therefore 200 μl of acetaldehyde (for synthesis, E. Merck AG., Darmstadt, Germany) was added to each distillate (100 ml) and mixed in thoroughly for the liberation of diketones (*cf.* ref. 3) in the distillate possibly converted to non-volatile sulphites. 24 ml vessels (Fig. 1) were filled to the top with the steam distillates. The flasks were provided with magnetic stirrers, and sealed with membranes of silicone rubber. Each flask was connected to an empty flask by an injection needle and a silicone rubber capillary tube, and each pair, filled and empty, was placed in a water bath at 40°. After the flask had stood for 15 min in the bath, 10 ml of air was injected into the filled flask (Fig. 1). The solution in the flasks was

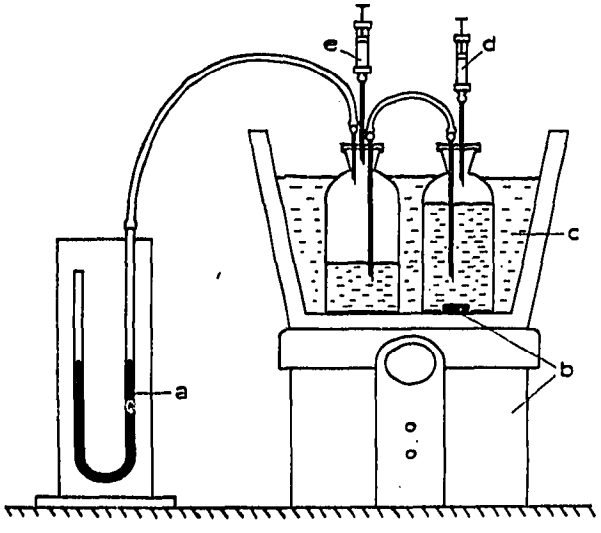

Fig. 1. Sampling system. a = Water manometer; b = magnetic stirrer; c = constant temperature bath; d = syringe for head-space sampling; e = syringe for pressure regulation.

stirred magnetically for another 15 min, and 2-ml aliquots of the gas phase in the head space of the sampling flask (Fig. 1) were taken. The samples were analysed gas chromatographically by a Perkin-Elmer F 11 apparatus. To preclude condensation, the sampling syringe was warmed up 40° before sampling.

Conditions of analysis. Electron capture detector, sensitivity for air peak 10 and for diketone peaks 5; column length 2 m (glass), internal diameter 3 mm, filling ma-

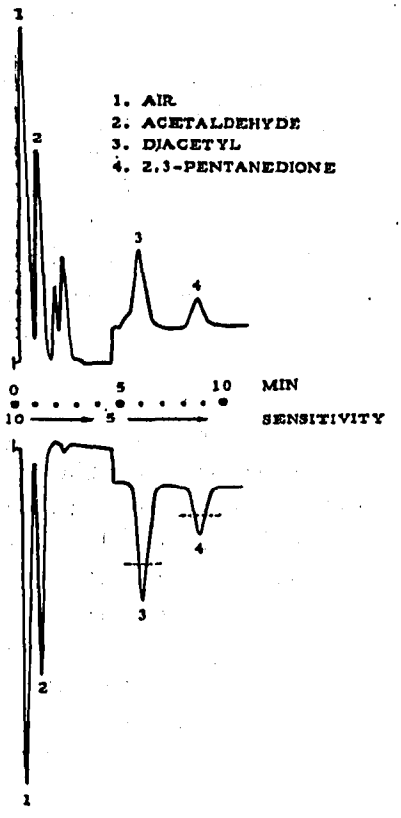

Fig. 2. Head-space gas chromatograms of the steam distillates of acetoin and 3-hydroxy-2-pentanone hydrazones adsorbed on carbon from the solution (upper) and of the equivalent reference mixture of the corresponding pure hydrazones (lower).

terial (two liquid phases) approx. 30 cm packing of Celite 545 + diglycerol (20 wt. %) and approx. 165 cm packing of Celite 545 + 1,2,3-tri(2-cyanoethoxy)propane (TCP) (20 wt. %); carrier gas, nitrogen (99.999 %), inlet pressure 0.7 kp/cm^2, flow rate 52 ml/min.

RESULTS AND DISCUSSION

Fig. 2 reproduces two gas chromatograms. The upper one illustrates vicinal diketones produced during the steam distillation of α-hydroxy ketone hydrazones isolated from the solution by adsorption on carbon, and the lower the vicinal diketones produced during the steam distillation of pure recrystallised hydrazones of α-hydroxy ketones. If the adsorption of α-hydroxy ketone hydrazones from the solution on the carbon, their subsequent separation from the carbon, and the conversion to diketones

during the distillation had been complete, the corresponding diketone peaks in both chromatograms should have been equal in size. To facilitate comparison, the peak heights in the upper chromatogram are indicated by horizontal dotted lines crossing the corresponding peaks for the same components in the reference, lower chromatogram. It can be seen that the peak heights of the diketones in the upper chromatogram are about three quarters of those in the lower chromatogram, and consequently the isolation and conversion procedures are not quite quantitative; nevertheless the method gives reproducible results, and the peak heights change in relation to the change in concentration of α-hydroxy ketones. The two extra peaks, between the peaks of acetaldehyde and diacetyl in the upper chromatogram, are produced by influence of activated carbon upon the sulphuric acid during the distillation.

2,3-Butanediol in the solution studied did not interfere with determination of the α-hydroxy ketones; the chromatogram was identical with that illustrated in the upper chromatogram in Fig. 2.

Preliminary experiments have indicated that the method is suitable for the study of fermentation solutions and beverages.

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