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## REACTION GAS CHROMATOGRAPHY IN SEALED GLASS CAPILLARIES DEHYDRATION, REDUCTION AND OXIDATION REACTIONS, AND THE SYNTHESIS OF REFERENCE COMPOUNDS

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### SUMMARY

The sealed glass capillary technique of reaction gas chromatography, previously described for hydrogenation, hydrogenolysis and ozonolysis, has been extended to include the dehydration of alcohols to olefins, the reduction of carbonyl groups to primary and secondary alcohols, and the oxidation of alcohols to aldehydes and ketones on the microgram and submicrogram scale. The three new techniques have been coupled with those previously described to form the basis of a very convenient and rapid means of synthesising microgram quantities of compounds required for the measurement of retention and mass spectral data.

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### INTRODUCTION

In an earlier publication<sup>1</sup> a technique was described for hydrogenation, hydrogenolysis or ozonolysis of microgram quantities of pure (single peak) compounds collected from the outlet of a gas chromatograph in glass capillaries. These capillaries, containing a small quantity of catalyst when necessary, were filled with either hydrogen or ozone, sealed, and heated to promote the reaction, after which all the reaction products were re-injected into the gas chromatograph by breaking the capillary within the injection port. Successful application of this technique to the analysis of food flavour volatiles<sup>2-6</sup> indicated the possibility of extending the technique to a wide variety of reactions.

In this paper the application of the glass capillary reaction gas chromatographic (GC) technique to the dehydration of alcohols to olefins, the reduction of carbonyls to alcohols, and the oxidation of alcohols to carbonyls is described. The yield of reaction products obtained from a few micrograms of material was usually sufficient to permit their collection from the GC outlet for further reaction. This process could be continued as long as sufficient material remained to produce a significant result. This greatly increased the versatility of the technique, making possible the rapid synthesis of many different compound types in sufficient quantities to obtain GC and mass spectral data. The synthesis of five reference compounds from

2-cyclopentylethanol by this means is described and chromatograms and reaction conditions for the completion of the last two steps in the synthesis of edulan III<sup>7</sup> are given.

## EXPERIMENTAL

A detailed description of the apparatus was given in a previous publication<sup>1</sup>. It consisted of three basic parts, firstly, a collection system which allowed the GC effluent to be condensed in the centre of a glass capillary (100- $\mu$ l microcap) containing a small quantity of catalyst. Secondly, a low pressure, low flow-rate source of a "reactive" or a blanketing gas (hydrogen, ozone or nitrogen) and a micro-flame for sealing the capillaries, and thirdly a device for crushing the capillaries within the GC injector at the completion of the reaction. Reaction conditions were controlled by heating the sealed capillary for a short time in a laboratory oven (typically 160° to 300° for 1 to 10 min).

The laboratory-constructed dual-column GC system was equipped with two 6 m  $\times$  2 mm glass columns, one packed with 3% SF-96 on Chromosorb G and the other with 3% Carbowax 20 M on Chromosorb G, or with a 150 m  $\times$  0.75 mm stainless-steel capillary column coated with SF-96, when increased resolution was required. Samples for mass spectrometry were collected from the heated GC outlet (stream split ratio 5:1) in short stainless-steel traps packed with 10% SF-96 on Chromosorb A<sup>8</sup> and transferred to a similar gas chromatograph equipped with a 150 m  $\times$  0.75 mm OV-101 capillary column connected to an Atlas CH4 mass spectrometer by an open split interface<sup>9</sup>.

Dehydrating agents used were basic and acidic alumina (Woelm, Eschwege, G.F.R.), potassium hydrogen sulphate and hexamethylphosphoric triamide (Aldrich, Milwaukee, Wisc., U.S.A.). Oxidants used were potassium dichromate and potassium permanganate as ground crystals, or coated 10% (w/w) on Gas-Chrom P (Applied Science, State College, Pa., U.S.A.). The reducing agent used was sodium borohydride (Koch-Light, Colnbrook, Great Britain). Hydrogenation catalysts were platinum oxide and platinum on carbon (Fluka. Buchs, Switzerland).

Nitrogen was used as an inert gas to blanket the dehydration, oxidation and reduction reactions.

## RESULTS AND DISCUSSION

### *Dehydration*

Dehydration of alcohols to olefins was first applied to reaction gas chromatography by Drawert *et al.*<sup>10,11</sup>. It is complementary to hydrogenolysis, or "hydrocarbon skeleton chromatography" of Beroza and Sarmiento<sup>12</sup>. Hydrogenolysis tends to remove the terminal carbon atom together with the hydroxyl, to yield predominantly the "parent-minus-one" hydrocarbon. In contrast, dehydration does not remove the terminal carbon atom, and under favourable (mild) conditions the new double bond is formed and remains at the original position of attachment of the hydroxyl group.

To test the application of the glass capillary technique to dehydration, each of the *n*-alcohols from C<sub>5</sub> to C<sub>10</sub> was collected in turn from the GC outlet, on a small bed of acidic alumina, in the same manner as previously described for hydrogenation.

The capillary was then filled with nitrogen, sealed, and heated to 300° for 3 min to produce a reaction, and the reaction products were re-injected into the GC system using the glass capillary crushing device.

From each alcohol, a mixture of olefins was obtained. When the reaction temperature was increased above *ca.* 350°, the alumina turned brown, and extensive decomposition of the alcohol to low-molecular-weight products took place. As the temperature was reduced below 300°, both the yield of olefins and the extent of double bond scrambling were reduced, and the terminal alkene became predominant in the mixture of products. Unreacted alcohol was not seen in the chromatogram of reaction products even at reaction temperatures as low as 150°.

Basic and acidic alumina, and potassium hydrogen sulphate were compared for the dehydration of *n*-heptanol. It was found that basic alumina yielded mainly hept-1-ene (Fig. 1A) whereas acidic alumina yielded all five possible heptenes (Fig. 1B). The position of the double bond in the heptenes was determined by ozonolysis as already described. Potassium hydrogen sulphate gave a result similar to acidic alumina except for the appearance of unreacted heptanol in the chromatogram of the reaction products.

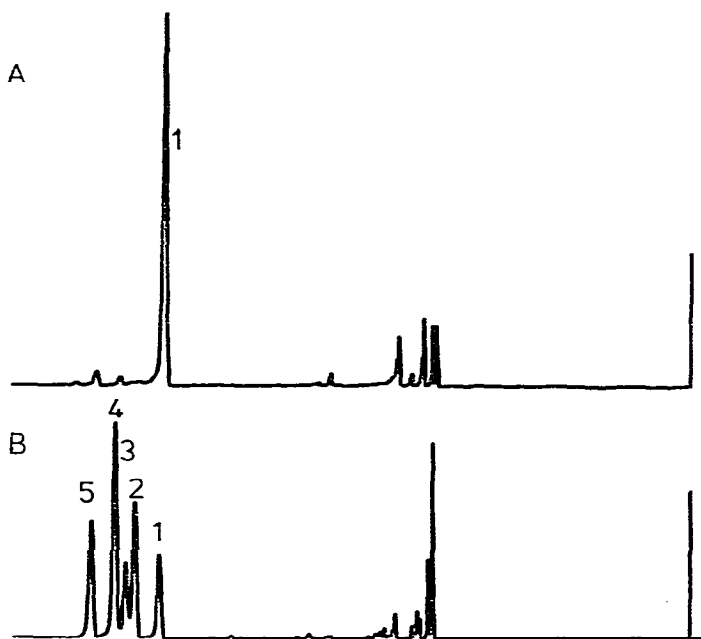


Fig. 1. Dehydration of *n*-heptanol (A) with basic alumina; 300°, 3 min; (B) with acidic alumina; 300°, 3 min. Peaks: 1 = hept-1-ene; 2 and 3 = *cis*- and *trans*-hept-3-ene; 4 and 5 = *cis*- and *trans*-hept-2-ene. Column, 150 m × 0.75 mm stainless-steel capillary packed with SF-96; temperature, 40°; carrier gas (nitrogen) flow-rate, 4 ml/min.

Secondary alcohols were generally easier to dehydrate than primary alcohols. Hexan-2-ol and hexan-3-ol were easily dehydrated with basic alumina at 250° for 3 min whereas *n*-hexanol required a minimum temperature of 300° for 3 min.

### *Reduction of aldehydes and ketones to alcohols*

The reduction of carbonyl groups without the loss of unsaturation is a desirable complement to hydrogenation. The applicability of sodium borohydride reduction to the present technique was examined using *n*-heptanal, *cis*-hept-4-enal, octa-2,4-dienal, octan-4-one and 3-hydroxybutan-2-one as test compounds. A small quantity of sodium borohydride (*ca.* 1 mg) reduced the three aldehydes to primary alcohols at 160° in 3 min. The octan-4-one was reduced more slowly to yield only 30% octan-4-ol after 3 min at 160° and 55% after 10 min in agreement with the slower reduction rate in solution<sup>13</sup>. The usual addition of water as a second reaction step to liberate the alcohol from the sodium complex was not found to be necessary with these simply carbonyls. With 3-hydroxybutan-2-one, it was essential to open the capillary after the reaction with the sodium borohydride, add 1–2  $\mu$ l of water, reseal the capillary and heat it before re-injection of the products into the gas chromatograph. In this way a small yield (10–20%) of butan-2,3-diol was obtained. Klimes *et al.*<sup>14</sup>, using a very similar reduction technique employing aluminium hydride in a carefully dried system, added water as a second reaction step to liberate the alcohol from the aluminium complex.

### *Oxidation of alcohols to aldehydes and ketones*

Application of the present technique to the oxidation of alcohols was examined using *n*-butanol, *n*-hexanol, *n*-heptanol, hexan-2-ol, hexan-3-ol, heptan-2-ol, 2-methylbutanol, 3-methylbutanol, 2-ethylbutanol, and  $\beta$ -ionol as test compounds, and potassium permanganate and potassium dichromate as oxidants. The two oxidants were initially diluted with Gas-Chrom P (10% coating of oxidant applied from an aqueous solution and oven dried), and used at a reaction temperature of 300° to 350° for 3 min for the three *n*-alcohols. In later experiments, finely ground potassium dichromate crystals (0.25–0.5 mg) were used satisfactorily without the diluting effect of the Gas-Chrom P at a slightly lower reaction temperature (typically 250°–280° for 3 min).

The optimum reaction conditions varied from compound to compound, primary alcohols requiring more careful control of the reaction conditions than secondary alcohols. The yield of carbonyl compound was usually in the range 30–40% and was accompanied by up to 30% unreacted alcohol. Attempts to increase the yield by increasing the temperature resulted in extensive decomposition to low-molecular-weight compounds. The colour change produced by the oxidation of as little as 3  $\mu$ g of alcohol could be seen easily, providing visual confirmation that a reaction had taken place.

Fig. 2 is a typical chromatogram for the oxidation of 80 ng of octan-3-ol showing a yield of 33% of octan-3-one, the usual low-molecular-weight decomposition products and 3% of unreacted alcohol.

The oxidation of  $\beta$ -ionol was difficult to control, yielding mainly dehydration products, even at the low reaction temperature of 200°. The maximum yield of  $\beta$ -ionone obtained was only 5%.

### SYNTHESIS OF REFERENCE COMPOUNDS

The glass capillary technique has been successfully used to synthesise micro-

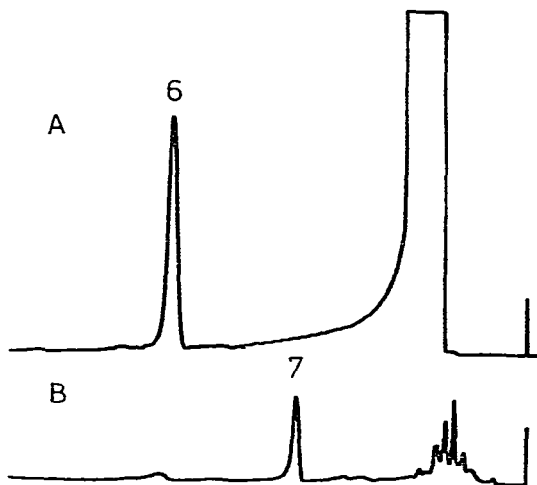


Fig. 2. Oxidation of octan-3-ol. (A) 80 ng of octan-3-ol (6) in ether. (B) Oxidation with  $K_2Cr_2O_7$  at  $280^\circ$  for 3 min to octan-3-one (7). Column, 6 m  $\times$  2 mm glass packed with 3% SF-96 on Chromosorb G; temperature,  $140^\circ$ ; carrier gas (nitrogen) flow-rate, 4 ml/min.

gram quantities of compounds required for their retention and mass spectral data. Fig. 3 illustrates the preparation of five different derivatives of 2-cyclopentylethanol, and lists the reaction conditions used. In the two-step syntheses of ethylcyclopentane and formylcyclopentane, the intermediate compound, vinylcyclopentane, was chromatographed in the normal manner and only the pure compound was collected for the second reaction. Omission of this chromatographic step, by opening the capillary after the first reaction, adding ozone or hydrogen, resealing the capillary and

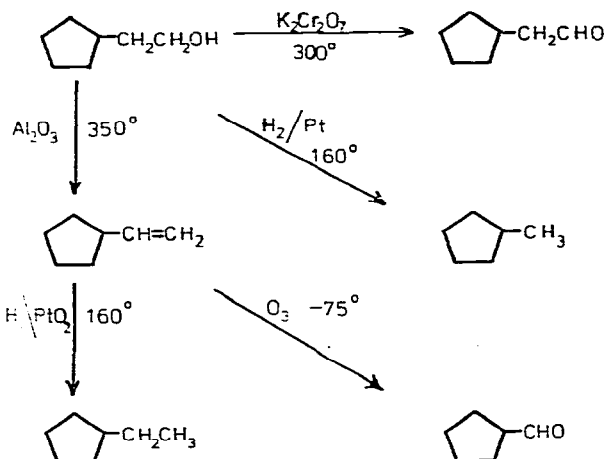


Fig. 3. Reference compounds prepared from 2-cyclopentylethanol. (A) Oxidation to 2-cyclopentylacetaldehyde at  $300^\circ$  for 3 min; oxidant, 10%  $K_2Cr_2O_7$ , on Gas-Chrom P. (B) Dehydration to vinylcyclopentane with basic alumina at  $350^\circ$  during 3 min. (C) Hydrogenolysis to methylcyclopentane at  $160^\circ$  for 10 min; catalyst, Pt/carbon. (D) Ozonolysis of vinylcyclopentane to formylcyclopentane at  $-75^\circ$  during 12 min followed by pyrolysis at  $160^\circ$  for 1 min. (E) Hydrogenation of vinylcyclopentane to ethylcyclopentane at  $160^\circ$  for 10 min; catalyst, Adams  $PtO_2$ .

proceeding to the next reaction is quite feasible. However, this loses the advantage of the chromatographic clean-up of the intermediate and the chance of its verification by retention time.

Sugowdz and Whitfield<sup>7</sup> have reported a synthesis of the edulans by classical means: the last two steps of this synthesis have been duplicated by reaction GC as shown in Fig. 4. Reduction of 3,5,6,7,8,8a-hexahydro-2,5,5,8a-tetramethyl-7-oxo-2*H*-1-benzopyran (peak 8) with sodium borohydride at 160° for 3 min proceeded smoothly, to yield two isomeric 3,5,6,7,8,8a-hexahydro-7-hydroxy-2,5,5,8a-tetramethyl-2*H*-1-benzopyrans, the major of which (peak 9) was dehydrated with potassium hydrogen sulphate at 200° for 3 min to give a product (peak 11) identical in retention time and mass spectrum to an authentic sample of edulan III (3,5,5,8a-tetrahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyran). The dehydration step was difficult to control and the yield of edulan III was low (20%). Most of the peaks in the chromatogram had mass spectra indicative of the loss of both oxygen atoms from the molecule. Alumina (basic and acidic) and hexamethylphosphoric triamide were tried as dehydrating agents with no better results.

In the above examples, 3  $\mu\text{g}$  of starting material was used for the single-step reactions, and 5  $\mu\text{g}$  for the two-step reactions, yielding sufficient material in each case to obtain good quality mass spectral data.

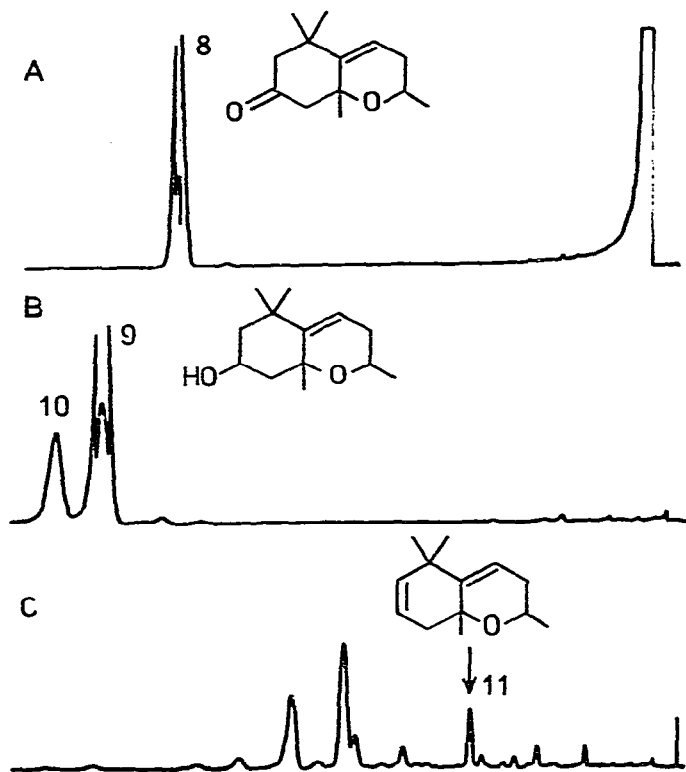


Fig. 4. Synthesis of edulan III. (A) Collection of 5  $\mu\text{g}$  of the ketone (see text) from solution. (B) Two isomeric alcohols produced by reduction of the ketone (8) with  $\text{NaBH}_4$  at 160° for 3 min. (C) Dehydration of the alcohol (9) with  $\text{KHSO}_4$  at 200° for 3 min to yield edulan III (11). Column, 6 m  $\times$  2 mm glass packed with 3% SF-96 on Chromosorb G at 150°; carrier gas (nitrogen) flow-rate, 4 ml/min.

Further applications of the glass capillary reaction GC technique will undoubtedly be developed as the need arises. To obtain the best possible result for any given reaction, both the reaction conditions and the reactant should be chosen to suit the particular compound under study. When very low reaction temperatures are required, due allowance must be made for the heat input which the reaction mixture receives while the capillary and its contents are being heated in the GC injector prior to crushing the capillary. This heat input alone is often sufficient for easy reactions. The maximum reaction temperature is limited only by softening of the glass capillary.

The choice of reactants is not limited to those described above, nor to solid compounds. Liquid reactants may be used provided that they do not interfere with the operation of the GC by forming liquid plugs within capillary columns, giving rise to interfering peaks in the chromatogram, or by accumulating at the front of the column. Incorporation of the liquid reactant in a porous layer open tubular trap, similar to that described by Cronin and Gilbert<sup>15</sup> for the incorporation of platinum catalyst, may be one means of immobilizing a liquid reactant and preventing it from reaching the column in the liquid state.

## CONCLUSION

The flexibility of the glass capillary reaction GC technique has been extended by its successful application to dehydration, reduction and oxidation reactions on the microgram and submicrogram scale. When used in conjunction with high-performance capillary columns, and the established hydrogenation, hydrogenolysis and ozonolysis reactions, it provides a very powerful tool for the identification of compounds which cannot be recognised from their GC and MS data alone.

The synthesis of reference compounds by the present technique has been shown to provide a quick and direct means of obtaining high quality GC and mass spectral data from a few micrograms of starting material, which could if necessary be fractionated from a complex (naturally occurring) mixture using high resolution capillary columns.

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