

## The Volatile Carbonyls and Alcohols in the Flavour Substances of Onion (*Allium cepa*)

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When the volatile substances in the onion were studied, two unknown carbonyl compounds were found in addition to acetaldehyde and propionaldehyde. One of the unknown substances was chemically characterized as 2-methyl-2-pentenal. The other carbonyl compound, which was present in very small amounts, has not yet been identified. On the basis of its UV-spectrum and behaviour on the paper chromatogram, it may be a saturated aldehyde. 2-Methyl-2-pentenal is formed enzymatically in crushed onion. The only alcohols found were methanol and ethanol.

In our studies on the flavour substances of crushed onion (*Allium cepa*), our attention was also drawn to the carbonyl compounds. When air was sucked through a homogenized onion mass with the aid of a vacuum pump and the airborne vapours were led into a dilute solution of 2,4-dinitrophenylhydrazine, a number of 2,4-dinitrophenylhydrazones (DNPHones) were precipitated. Acetaldehyde DNPHone and propionaldehyde DNPHone could be identified by paper chromatography. In addition, a spot containing an unknown aldehyde DNPHone was found.

In order to identify the unknown compound, the experiment was repeated on a larger scale and the precipitate of DNPHones was divided into fractions on a cellulose powder column. In addition to the unknown aldehyde DNPHone, another unknown DNPHone was detected in small amounts in the first fractions collected. DNPHones of the following carbonyl compounds were thus separated:

- a) the minor unknown, designated as Y
- b) the major unknown, designated as X
- c) propionaldehyde
- d) acetaldehyde

Acetaldehyde (d) DNPHone was identified by its m.p., by its mixed m.p with an authentic specimen, by paper chromatography, and by its UV-spectrum. The UV-spectra of the DNPHones are not very specific, but they can be

used to divide the DNPHones into classes, *e.g.* into saturated aldehydes, 2-en-1-als, 2,4-dien-1-als, and methylketones<sup>1</sup>.

Propionaldehyde (c) DNPHone was identified by the same criteria as acetaldehyde DNPHone.

The unknown X, which was crystallized twice from methanol and then formed red, rhombohedral crystals, was investigated more closely in order to determine its structure. Elementary analysis of the X—DNPHone (Found C 51.91; H 5.04; N 20.00; O 23.21) gave the empirical formula  $C_6H_{10}O$  for the carbonyl compound (Calc. for its DNPHone: C 51.79; H 5.07; N 20.14; O 23.00). The compound took up two atoms of bromide and accordingly contained one double bond. The unknown carbonyl compound might thus have been 2-hexenal, which is also called leaf aldehyde. This compound was synthesized<sup>2</sup>. The m.p. of its DNPHone was 143°C, in agreement with the m.p. reported in the literature; the m.p. of the X—DNPHone was 155°C, and the mixed m.p. 120°C. The nonidentity of the two compounds was thus established.

On the basis of the IR-spectra of different hexenals and the unknown DNPHone, it appeared that X is not 2-, 5-, or 3-hexenal. Regarding the identity of X and 4-hexenal, the IR-spectra gave no conclusive proof.

In order to establish the identity or nonidentity of the X—DNPHone and 4-hexenal DNPHone definitely, the latter was synthesized. The m.p. of the synthetic compound was 120°C. The bromine addition products of 4-hexenal DNPHone and X—DNPHone had different melting points, the former melting at 90—92°C and the latter at 105—110°C. They also separated on paper chromatograms. Geometric isomerism was not hence the cause of the difference in the melting points. The isolated compound X was not therefore 4-hexenal.

On the basis of these results it was obvious that the unknown X was not a hexenal. It seemed probable that the compound X had a branched carbon chain.

The unknown carbonyl compound X was liberated from its DNPHone by transderivation with levulinic acid by a modification of the method of Keeney<sup>3</sup> which seemed to increase the yield. The resulting solution of the free carbonyl in methylene chloride was ozonized, and the ozonide split with an alkaline silver oxide suspension. A test for keto acid was positive. The keto acid formed was identified as pyruvic acid by paper chromatography. In addition, acetic acid and a small amount of propionic acid were formed. For comparison, 2-hexenal liberated from its DNPHone was ozonized in a similar way. The test for keto acid was negative. Butyric acid and small amounts of propionic and acetic acids were formed. The ozonization of 2-hexenal thus seems to proceed as expected (with the formation of butyric acid), although small amounts of other products, including acetic and propionic acids are produced.

The formation of pyruvic acid as an ozonization product of X suggested that the structure of the carbonyl compound might be  $CH_3-CH_2-CH=C(CH_3)-CHO$ . A study of the literature revealed that the m.p. of 2-methyl-2-pentenal DNPHone is close to that of the isolated X—DNPHone. 2-Methyl-2-pentenal was synthesized by the method of Häusermann<sup>4</sup>. After being crystallized several times from methanol, the compound formed beautiful, red, rhombohedral crystals similar to those of the isolated X—DNPHone.

The m.p. and the mixed m.p. with the isolated compound were both 155°C (uncorr.). The UV-spectra were identical and the compounds were not separated on a paper chromatogram. On the basis of all known facts, the hitherto unknown aldehyde in onion vapour may safely be considered as 2-methyl-2-pentenal.

Niegisch and Stahl<sup>5</sup> concluded from mass-spectrometric evidence that the higher aldehyde present in onion vapour is 4-hexenal and not a condensation product of propionaldehyde which they erroneously assumed to be  $\alpha$ -methyl- $\beta$ -methyl-acrolein.

The minor unknown carbonyl Y was isolated in an amount so small that we have not had enough of its DNPHone for a more detailed study. The UV-spectrum, however, corresponds to that of a saturated aldehyde DNPHone. On the paper chromatogram it is inseparable from authentic hexenal DNPHone.

In order to elucidate the origin of 2-methyl-2-pentenal, the enzymes of onion were destroyed with trichloroacetic acid (TCA), and the volatile carbonyls distilled with steam into an ice-chilled DNPH solution. The DNPHones formed were separated on a cellulose powder column. The other aldehydes were present as usual, but at most only traces of 2-methyl-2-pentenal. In another experiment the onion was homogenized with water and the resulting pulpy mass was left to stand for one hour before TCA was added. After steam distillation, the DNPHones were fractionated as above. 2-Methyl-2-pentenal DNPHone was present in normal amounts. The results suggest that this carbonyl may be formed enzymatically in crushed onion. Because the compound was also formed in an oxygen-free solution, oxygen may not be needed for the reaction. As the amount of propionaldehyde in onion is comparatively large, it is possible that 2-methyl-2-pentenal is formed from propionaldehyde by a condensation reaction.

The volatile alcohols among the flavour substances of onion were converted into their 3,5-dinitrobenzoates as described below and chromatographed on paper. By comparison with known derivatives, they were found to be methanol and ethanol. No other volatile alcohols were isolated.

## EXPERIMENTAL

Air was sucked through a homogenate of 9.5 kg of onions and 12 l of water with the aid of a vacuum pump. The vapours were trapped in a dilute 2,4-dinitrophenylhydrazine solution. After four days 595 mg of crude DNPHone mixture was collected.

*Paper chromatography of DNPHones.* Sheets (24 × 24 cm) of chromatographic filter paper were immersed in a 25 % solution of dimethylformamide in ethanol for 5 min and blotted between two filter papers. The unknowns were applied in chloroform solution (10–20  $\mu$ l contained 5–10  $\mu$ g) to the still moist paper. The paper was immediately rolled into a cylinder and development effected with *cyclohexane* saturated with dimethylformamide employing upward flow.

*Cellulose powder column chromatography of DNPHones.* A cellulose powder column (7.5 × 60 cm) was prepared in the "wet" way by suspending the powder in ethanol. The column was then saturated with dimethylformamide by percolating a 15 % ethanolic solution of the latter through it and washed with *cyclohexane* saturated with dimethylformamide until the effluent contained only one phase. The crude DNPHone mixture was dissolved, added to the column, and eluted with the same solvent.

*Determination of the number of double bonds.* The determinations were made according to the method of Rossmann<sup>6</sup> on the unknown and on synthetic 2-hexenal DNPHone. Four determinations on 2-hexenal DNPHone gave a mean double bond number of 1.27,

and four determinations on the unknown a mean double bond number of 1.03. The unknown DNPHone contained accordingly one double bond in the carbonyl part of the molecule.

*Preparation of 4-hexenal DNPHone.* 4-Hexenol was synthesized by the method of Brandon *et al.*<sup>7</sup> The corresponding aldehyde, 4-hexenal, was prepared by rapid low-temperature dichromate oxidation of this alcohol according to the method of Delaby and Guillot-Allègre<sup>8</sup>. After the resulting ether solution had been shaken with a dilute solution of DNPH and then washed with 2 N HCl and water, it was evaporated to dryness in vacuum and the residue crystallized from methanol. The crystals were sublimed in vacuum, crystallized from methanol and dried. The product consisted of beautiful, yellow-brown needles melting at 120°C (uncorr.). (Found: C 50.69; H 4.93; N 20.64; O 23.97. Calc. for C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>: C 51.79; H 5.07; N 20.14; O 23.00).

*Preparation of 4,5-dibromo-hexenal DNPHone.* Bromine was rapidly added to 5 ml of 4-hexenal until a permanent colour remained. The excess bromine was destroyed by shaking the mixture with a solution of sodium hydrogen sulphite and the product washed. The oxidation and preparation of the DNPHone were carried out as above. The product consisted of oily, yellow crystals melting at 90–92°C.

*Preparation of dibromo-X-DNPHone.* 12.8 mg of the X-DNPHone was dissolved in chloroform. A solution of bromine (1 ml in 20 ml of chloroform) was added until a permanent bromine colour remained. The excess bromine was destroyed as above and the solution evaporated to dryness in vacuum. When crystallized, the residue yielded yellow crystals melting at 105–110°C.

*Transderivation of DNPHone.* 25 mg of the DNPHone and 25 ml of levulinic acid reagent (levulinic acid: N HCl = 9:1 (v/v)) were placed in a test tube provided with a vertical condenser tube. The test tube was warmed on a steam bath for 25 min and the contents washed into a separatory funnel with 60 ml of water and extracted with three 15 ml portions of methylene chloride. The methylene chloride solution was extracted with a half-saturated sodium bicarbonate solution until no more colour could be extracted and then washed with water. The resulting methylene chloride solution, the colour of which indicated the transderivation to be incomplete, was transferred to a small distillation flask together with an equal volume of distilled water. The methylene chloride was distilled off first, and subsequently water, both into the same flask. Water was distilled until the small stream in the condenser was observed to consist of only one phase. The distillates were shaken and the aqueous layer discarded. The methylene chloride solution of the parent carbonyl was dried.

*Ozonization of the free carbonyl.* Ozone in approximately 5-fold excess was bubbled through the ice-cold carbonyl solution. The solution was evaporated to dryness in vacuum within one hour, and the residue treated in the usual way with alkaline silver oxide suspension and filtered (80 ml).

*Preparation of keto acid DNPHone.* 20 ml of a dilute solution of DNPH in hydrochloric acid was added to 10 ml of the above filtrate and warmed for 20 min on a water bath. The mixture was extracted with methylene chloride until the coloured matter had been transferred into the methylene chloride. The methylene chloride solution was then extracted with a saturated bicarbonate solution, acidified with sulphuric acid, and again extracted with methylene chloride. The resulting methylene chloride solution was subjected to paper chromatography.

*Paper chromatography of the keto acid DNPHones.* The keto acid DNPHones were chromatographed on Whatman No. 1 paper which was developed through downward displacement with butanol-ethanol-water (5:1:4). The chromatogram of the keto acid DNPHones derived from X showed spots which did not separate from authentic pyruvic acid DNPHone spots over a distance of 50 cm and an unidentified minor spot near the solvent front.

*Enzymatic experiments.* An onion (80 g fresh wt.) was chilled in dry ice, crushed, and homogenized in 150 ml of 10% TCA. The homogenate was distilled with steam into 100 ml of a dilute DNPH solution until about 200 ml had been collected. After overnight storage, the distillate was extracted with methylene chloride. The methylene chloride solution was extracted with hydrochloric acid in order to remove excess DNPH, washed, and evaporated to dryness. The residue, 25 mg in all, consisted of oily, red crystals with a pungent odour. Fifteen milligrams of crystals were fractionated on a cellulose powder column (1.5 × 75 cm). Only one fraction contained traces of 2-methyl-2-pentenal DNPHone.

Another onion (44 g fresh wt.) was homogenized with 150 ml of water and the homogenate left to stand for one hour. Fifteen grams of TCA was then added, the homogenate steam-distilled, and the distillate worked up and fractionated as above. 2-Methyl-2-pentenal DNP-Hone appeared in nine fractions in an amount far exceeding that recovered in the preceding experiment.

In a third experiment an onion (131 g fresh wt.) was homogenized in 150 ml of oxygen-free water in an oxygen-free atmosphere (purified nitrogen) in a special apparatus and the homogenate left to stand in this atmosphere under a low pressure for one hour. 18 g of TCA dissolved in 30 ml of boiled, warm water was added through a stopcock and mixed with the homogenate. The homogenate was removed with a low pressure to the steam distillation apparatus and worked up as above. Eleven fractions contained 2-methyl-2-pentenal DNP-Hone in an amount approximately equal to that recovered in the preceding experiment.

*The volatile alcohols.* 194 g of onions were homogenized in a Waring blender with 450 ml of distilled water and immediately transferred to a flask and steam distilled. The distillation was discontinued when 600 ml of distillate had been collected. The distillate was then exhaustively percolated with peroxide-free ether and the ether solution (250 ml) dried ( $\text{CuSO}_4$ ) and filtered. One gram of 3,5-dinitrobenzyl chloride was then added and the solution was shaken until all had dissolved. After standing for 24 h, the ether was distilled off in vacuum. The residue was heated on a steam bath for a few minutes and dissolved in peroxide-free ether. The ether solution was extracted with water and distilled to dryness in vacuum. The residue (41 mg) was dissolved in 2 ml of chloroform and subjected to paper chromatography.

*Paper chromatography of alkyl 3,5-dinitrobenzoates.* Sheets (24 × 24 cm) of chromatographic filter paper (Whatman No. 4) were immersed in a 25 % ethanolic solution of dimethylformamide for 5 min and blotted between two filter papers. The compounds were applied in chloroform solution (3–15  $\mu\text{l}$ ) to the still moist paper and the paper immediately rolled into a cylinder and developed with decaline saturated with dimethylformamide by the ascending technique. The spots on the dried paper were visible in ultraviolet light.

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