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Volatiles from Chives (*Allium schoenoprasum*)

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Gas chromatographic and mass spectrometric studies of volatiles of green leaves of chive (*Allium schoenoprasum*) have led to the identification of 2-methyl-2-butenal (tiglic aldehyde), 2-methyl-2-pentenal (earlier found in this laboratory in volatiles of onion), methyl-propyldisulphide. Evidence is presented for the presence of propenyl-propyldisulphide (*cis*- and *trans*-forms). Both dipropyldisulphide and the last mentioned disulphide have a typical onion-like odour and are therefore important flavour compounds in chive. Many peaks have not yet been identified, but methylpropenyl disulphide is possibly one of the sulphur compounds present. Allyl derivatives are generally not found in onion and chive and allyl disulphide is definitely absent.

In connection with studies on sulphur compounds in *Allium* species conducted at this institute a few years ago propenylsulphenic acid, the lachrymatory factor, was identified [1,2] besides the volatiles earlier isolated [3] from onion (other compounds identified were methanol, ethanol, acetaldehyde, propionaldehyde, and 2-methyl-2-pentenal). Volatile sulphur compounds reported by other authors to be found in garlic, leek, and onion are listed in Table 1. In this report the identification of 2-methyl-2-butenal, 2-methyl-2-pentenal, methyl-propyldisulphide, and di-propyldisulphide from green leaves of chives is described, and evidence is presented for the presence of propenyl-propyldisulphide. The presence of methylpropenyldisulphide is also probable.

MATERIAL AND METHODS

The green parts of gardengrown chives were stored at −17°C. About 700 g of the frozen leaves were disintegrated and placed in a tube with glass wool at the bottom and a thermometer at the top end. Three gas-washing bottles containing a total of 30 ml of ethyl ether (purified with $FeSO_4$, alkaline $KMnO_4$ and distilled water, slowly distilled on a 50 cm fractionating column, and stored for not more than 5 days) and a gas flow meter connected in series with the tube. Pure inert gas was allowed to flow through the train at a rate of 100 – 200 ml per min for 6 h. The tube was externally heated so that the thermometer reading was approx. 50°C. The wash-bottles were kept at 0°C. The ether extract was collected and replaced with fresh ether every two hours. The combined ether extracts were dried by cooling to −17°C and decanting, and concentrated to about 2 ml

Table 1. Volatile compounds reported by other authors in *Allium* spp.[4-11]

H_2S	Propyl-SH	Allyl-SH	
Methyl-SH	Propyl$_2$S$_2$	Allyl$_2$S	
Methyl$_2$S	Propyl$_2$S$_3$	Allyl$_2$S$_2$	
Methyl$_2$S$_2$		Allyl$_2$S$_3$	
Methyl$_2$S$_3$			
Methyl-propylS$_2$	Methyl-methanethiosulphinate		$C_{11}S_2$ compd.
Methyl-propylS$_3$	Propyl- » »		
Methyl-allylS	Allyl- » »		
Methyl-allylS$_2$	Propyl-propenethiosulphinate		
Methyl-allylS$_3$	Allyl- » »		
Propyl-allylS$_2$	Propyl-propanethiosulphonate		
Methyl-OH	Formaldehyde	Methyl$_2$CO	
Ethyl-OH	Acet »	Methyl-ethylCO	
Propyl-OH	Propyl »		
Isopropyl-OH	4-Hexen-1-al?	Acetic acid	
Allyl-OH			

by slow distillation on a 30 cm fractionating column. The volume of the residue was further reduced to about 20 μl by distillation in a narrow longnecked tube heated with a small, 10 watt heater 0.5 cm from the bottom end; the solution was added stepwise with a narrow pipette. 2 μl of the concentrate was injected into the gas chormatographic columns. Column A: glass, 1.8 m, 4 mm i. d., 20 % Rheoplex 400 on silanized Chromosorb P, carrier flow rate 50 ml/min, 137°C. Column B: 1 % (w) Na-caproate + 19 % (w) Apiezon L, 120°, other conditions as for A. Instrument: "home-made", argon-tetrode detector,[12] anode voltage ~ 300 V. Compounds eluted from column A were collected in glass traps cooled with liquid oxygen, connections being made with PTFE tubing. The traps were sealed with glass stoppers and stored in solid carbon dioxide until their contents were fed onto column B, from which the purified components were similarly trapped and transferred to a mass spectrometer. The mass spectrometry was performed by T. Moisio.

Reference compounds: methyl$_2$S$_2$, allyl$_2$S, propyl-SH, allyl-SH were obtained commercially. 2-Metyl-2-pentenal was synthesized by E. Honkanen (from propylaldehyde and vinylethylether), allyl-propylS$_2$ and allyl-methylS$_2$ by B. Granroth (by oxidation of a mixture of allyl- and propyl-SH with iodine, and by exchange between methyl$_2$S$_2$ and allyl-SH. Propyl$_2$S$_2$ and ethyl$_2$S$_2$ were made by oxidation of the mercaptans, propylmethylS$_2$ by exchange between methyl$_2$S$_2$ and propyl-SH in alkaline solution, methyl$_2$S$_3$ by alkylation of Na_2S_3,[13] and 2-methyl-2-butenal by aldol condensation from propyl- and acetaldehyde. [14] The following attempts were made to synthesize propenyl-propylS$_2$: 1) *via* the Bunte salt.[15] The 1-propenyl bromide did not react with the thiosulphate even in a sealed ampoule at 110°. This is understandable because the reaction is a nucleophilic substitution and the electron density at the C_1-carbon is high. 2) By base-catalyzed rearrangement of allyl-propylS$_2$ in analogy with the rearrangement of allyl$_2$S.[16] The allyl-propylS$_2$ disappeared, but the corresponding propenyl compound could not be found. 3) Propenyl$_2$S was prepared [16] and treated first with sodium in alcohol to break the sulphide bond and then with propylSH and iodine. 4) Allyl-propylS$_2$ was heated with catalytic amounts of iodine in a sealed tube at 200°C for 1 h. It is known that 2-olefins are isomerized to 1-olefins in this way.[17] In tests 3 and 4, trace amounts of the desired product could be isolated by chromatography on columns A and B from the propyl$_2$S$_2$ present in large amounts in the reaction mixtures. The isolated product was shown by mass spectrometry to have a molecular weight of 148 and to contain two sulphur atoms. The mass spectrum was reminiscent of but not identical with that of allyl-propylS$_2$. A source of uncertainty is that the sample was still contaminated with propyl$_2$S$_2$. The relative retention times of the compound were 1.36 on column A and 1.07 on column B ($R_{\text{Propyl}_2\text{S}_2} = 1$) as compared with 1.36 (col. A) and 0.86 (col. B) for allyl-propylS$_2$. It is suggested that the compound is propenyl-propylS$_2$ though definite proof is lacking.

All reference compounds were purified on columns A and B before mass spectrometry.

RESULTS

Chromatograms of the concentrates are shown in Fig. 1. Components 0 and 1 had a fruity smell, while the others had an onion-like odour. Each band isolated from column A gave rise to several small peaks and one main component when chromatographed on column B, except for band 3, which was resolved into four peaks of about equal size on B.

Fig. 2 shows the retention times of the main components and reference compounds. The solvent fraction which is shown in the figure as a shaded area, contained at least three compounds. One of these had a mercaptan-like odour, another a smooth butter-like odour.

Component 0: Mass 84, mass spectrum identical with that of 2-methyl-2-butenal, *i.e.* tiglic aldehyde.[18] Has not been described previously as a green plant component though it is present in the dry distillation products of guajac resin. Retention times identical.

Component 1: Mass spectrum identical with that of 2-methyl-2-pentenal. Occurs in *Allium cepa* also.[3] Retention times identical.

Component 2: Mass spectrum and retention times identical with those of methyl-propyl S_2. Occurs in *Allium cepa* also.[5]

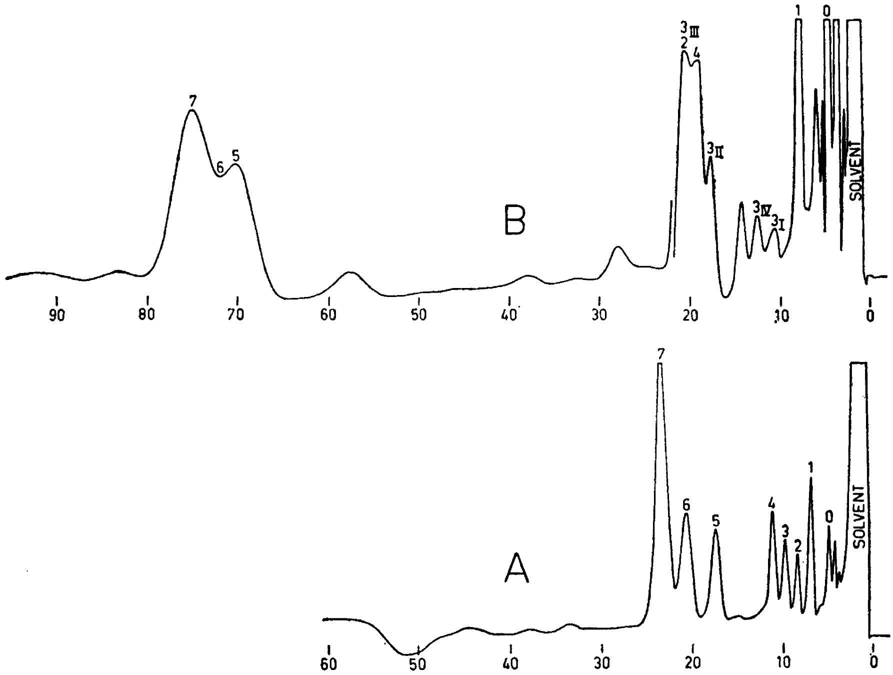

Fig. 1. Gas chromatograms of volatiles from chive. Column A: Rheoplex 400, 20 %, 137°C; Column B: 19 % Apiezon L + 1 % Na-caproate, 120°C.

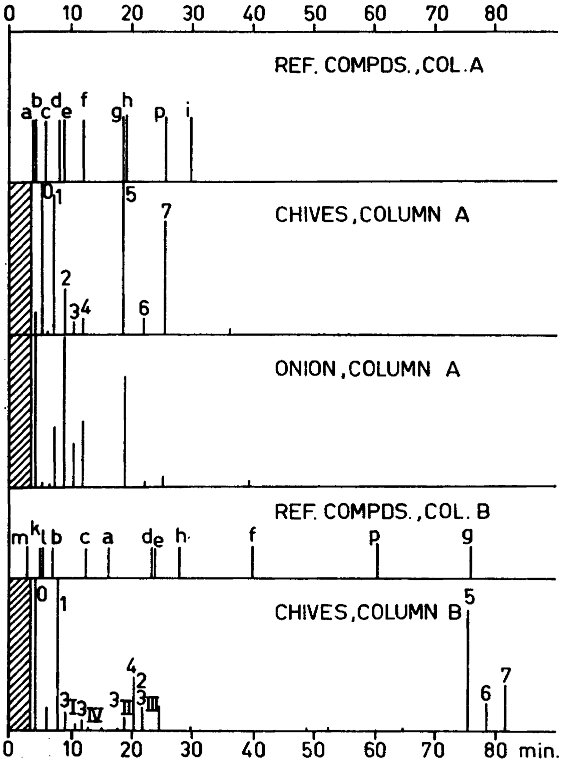

Fig. 2. Gas chromatographic analysis of the volatiles of onion and chive. Columns as in Fig. 1.

Retention times. a = $propyl_2S$, b = $methyl_2S_2$, c = $allyl_2S$, d = $ethyl_2S_2$, e = $methyl\text{-}propylS_2$, f = $ethyl\text{-}propylS_2$, g = $propyl_2S_2$, h = $methyl_2S_3$, i = $allyl_2S_2$, k = propyl-SH, l = butyl-SH, m = allyl-SH, p = $allyl\text{-}propylS_2$.

Component 3_{I}: Mass 98. Mass spectrum suggestive of some of the 2-hexenal isomers.

Component 3_{II}: Mass 112. Mass spectrum suggestive of an aldehyde.

Component 3_{III}: Mass 120. Contains two sulphur atoms.

Component 3_{IV}: Mass spectrum not determined.

Component 4: Mass 120. Contains two sulphur atoms. Mass spectrum similar to that of $methyl\text{-}allylS_2$, but not quite identical. Possibly $methyl\text{-}propenylS_2$.

Component 5: Mass spectrum and retention times identical with those of $di\text{-}propylS_2$.

Component 6: Mass 148. Contains two sulphur atoms. Mass spectrum suggestive of a straight chain disulphide containing one double bond. The mass spectrum was similar to but not identical with that of $allyl\text{-}propylS_2$.

The retention times of compound 6 differ from those of allyl-propylS_2 (21 and 25 min, respectively on col. A, and 72 and 60 min, on col. B).

Component 7: Mass 148. Mass spectrum similar to that of component 6. There is no evidence against the conclusion that the mass spectrum of this compound and that of the synthetic compound believed to be propenyl-propylS_2 are identical, though the result cannot be regarded as conclusive because the compounds were not quite pure. On column B, the retention times of component 7 (75 min) and allyl-propylS_2 (60 min) were different though they coincided on column A (23 min). The retention times of compound 7 and the synthetic product assumed to be the propenyl compound were identical on both columns.

DISCUSSION

Among compounds earlier found in *Allium* species are methyl$_2$ S_2 and methyl$_2$ S_3.[4-11] At the retention time of methyl$_2S_2$ a peak occurs in the chromatograms of chives, but the trisulphide could not be detected (Fig. 2). Ethyl sulphides were not found, nor have they been found in other *Allium* plants.

It is interesting to note that allyl S_2 ($t_R = 30$ min on column A), found in several *Allium* species, [4-11] was not present in chives, nor in chromatograms of onion volatiles (Fig. 2). Even if it were present as a trace component, its amount must be less than 0.2 % of the compounds visible on the chromatogram. On the B column a small peak is visible at the retention time of allyl-propylS_2 (60 min).

Because of their retention times, compounds 6 and 7 are certainly not allyl-propylS_2. The mass spectrometric data, together with the route taken in the synthesis of the compound with the same molecular weight, make it probable that one of them, or both, would be the corresponding propenyl compound(s). Because the *cis*-form of stereoisomers usually elutes before the *trans*-form, the retention time difference being smaller on a non-polar than on a polar column,[19] it is feasible to suggest that peak No. 6 is *cis* and No. 7 *trans*-propenyl-propylS_2. Compound 4 could be methyl-propenylS_2.

The formation of other volatile propenyl sulphur compounds in addition to that isolated by Virtanen and Spåre[1] might be expected from the S-propenyl-cysteine compounds present in *Allium* plants,[20-22] by splitting of a C—S bond, reduction, and coupling with some mercaptan present when the plants are crushed.

A comparison of chromatograms from leaves of chives and onion bulbs indicates that many components are probably identical, though there are quantitative differences (Fig. 2). However, quantitative differences between different samples of chives were observed also.

It is interesting to observe that until the present time allyl compounds have not definitely been found in onion or chive by any method of isolation, gas chromatographic methods included. A small peak at the retention time of allyl-propyldisulphide (Fig. 2), observed in this study on the B column, is not yet sufficient evidence of the presence of this compound. Propenyl-cysteine-sulphoxide in onion and chive, and allyl-cysteinsulphoxide in garlic, have a decisive influence on the physiological properties of these *Allium* species, because the course of enzymatic decomposition depends on the position of the double bonds.[22] Propenylsulphenic acid, the lachrymatory factor of onion, is formed from S-propenylcysteine sulphoxide; allylthiosulphinate, the relatively strong antimicrobial substance of garlic, is formed from S-allyl-cysteine sulphoxide. Also the flavour substances of various *Allium* species depend on the S-alkyl and alkenyl groups of cystein derivatives. The pungent and stimulating flavour of onion and chive is mainly due to di-propyldisulphide and components 6 and 7, which are probably *cis-* and *trans*-propenyl-propyl-disulphides. They have a strong onion-like flavour, whilst the flavour of methyl-propyldisulphide is somewhat cabbagelike. The flavour of garlic is connected with sulphur compounds in which the allyl-group is found. Many other partly unidentified volatiles formed in *Allium* species contribute to the nuances of the flavour.

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REFERENCES

1. Virtanen, A. I. and Spåre, C.-G. *Suomen Kemistilehti* **B 35** (1962) 28.
2. Spåre, C.-G. and Virtanen, A. I. *Acta Chem. Scand.* **17** (1963) 641.
3. Spåre, C.-G. and Virtanen, A. I. *Acta Chem. Scand.* **15** (1961) 1280.
4. Bernhard, R. A., Saghir, A. R., Jacobsen, J. V. and Mann, L. K. *Arch. Biochem. Biophys.* **107** (1964) 137.
5. Challenger, F. and Greenwood, O. *Biochem. J.* **44** (1949) 87.
6. Oaks, D. M., Hartmann, H. and Dimick, K. P. *Anal. Chem.* **36** (1964) 1560.
7. Foley, R. F. *Dissertation Abstr.* **16** (1955) 1323.
8. Carson, J. F. and Wong, F. F. *J. Agr. Food Chem.* **9** (1961) 140.
9. Kohman, E. F. *Food Technol.* **6** (1952) 288.
10. Yoshimura, M. *Chem. Abstr.* **55** (1961) 12568 f.
11. Niegisch, W. D. and Stahl, W. H. *Food Res.* **21** (1956) 657.
12. Wahlroos, Ö. *Acta Chem. Scand.* **16** (1962) 1093.
13. Strecker, W. *Ber.* **41** (1908) 1105.
14. Evans, L. K. and Gilliam, A. E. *J. Chem. Soc.* **1943** 565.
15. Footner, H. B. and Smiles, S. *J. Chem. Soc.* **1925** 2887.
16. Price, C. C. and Snyder, W. H. *J. Org. Chem.* **27** (1962) 4639.
17. Benson, S. W. and Bose, A. N. *J. Am. Chem. Soc.* **85** (1963) 1385.
18. Karrer, W. *Konstitution und Vorkommen der organischen Pflanzenstoffe*, Birkhäuser Verlag, Basel 1958, No. 359.
19. Hively, R. A. *Anal. Chem.* **35** (1963) 1921.
20. Virtanen, A. I. and Matikkala, E. J. *Suomen Kemistilehti* **B 34** (1961) 114.
21. Däbritz, E. and Virtanen, A. I. *Acta Chem. Scand.* **18** (1964) 837.
22. Däbritz, E. and Virtanen, A. I. *Chem. Ber.* **98** (1965) 781.

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