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Sulphur Compounds of the Genus *Allium*

DETECTION OF *n*-PROPYLTHIOL IN THE ONION. THE FISSION AND METHYLATION OF DIALLYL DISULPHIDE IN CULTURES OF *SCOPULARIOPSIS BREVICAILIS*

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The chief constituent of the essential oils of onion and garlic is usually stated to be allyl sulphide (Whitmore, 1937; Bernthsen & Sudborough, 1931; Vass, 1939). This is an erroneous view arising from a statement by Wertheim (1844) and was first contradicted by Semmler (1892). He found no allyl sulphide, but by distilling the oil of onions, *Allium cepa*, under reduced pressure and collecting various fractions he concluded from the boiling point and other physical properties that the main constituent is a disulphide, $C_6H_{12}S_2$, probably propyl allyl disulphide. The oil amounted to only 0.005% of the whole weight of the onions. Semmler (1892) states that oil of garlic, *A. sativum*, contains 6% propyl allyl disulphide, 60% diallyl disulphide, 20% diallyl trisulphide, and some diallyl tetrasulphide.

Kooper (1910) found thiocyanic acid and also allyl thiocarbimide in freshly expressed, weakly acid onion juice. No formaldehyde, acetaldehyde or acrolein was found. Platenius (1935), Platenius & Knott (1941), Sherratt (1943), Currier (1945) and Dyer, Taylor & Hamence (1941) estimated the total volatile sulphur in onions by conversion to sulphate. Walker, Lindegren & Bachmann (1925) found the fungicidal principles in onion juice to be of two types, one of which is non-volatile and stable to heat. The

other is volatile, and passes off from the extracted juice at room temperature within a few hours. No attempt was made by these later authors to identify the compounds in question.

The work of recent investigators. Kohmann (1947) reports the presence of propionaldehyde in onions from evidence based on the melting points and analyses of the 4-nitro- and 2:4-dinitro-phenylhydrazones. He also states that the lachrymatory principle is probably a thioaldehyde, and that sulphur analyses indicate that this may be thiopropionaldehyde. It seems possible that, during the distillation under reduced pressure in presence of water at 50° which Kohmann employed, propionaldehyde might have arisen from propylthiol by the following series of reactions:

This type of reaction was observed by Schöberl (1933, 1936) with many disulphides under mild alkaline conditions and by Challenger & Rawlings (1937) with diethyl disulphide in a closed copper vessel at 210°.

Kohmann (1947) observed the smell of hydrogen sulphide during the treatment with 2:4-dinitrophenylhydrazine. This might also have arisen from the reaction of the monomolecular thioaldehyde with the phenylhydrazine derivative. However, the percentage of sulphur found by Kohmann is also very close to the figure required by propylthiol or dipropyl disulphide. There is no conclusive evidence that the distillate contained a thioaldehyde.

Stoll & Seebeck (1948) have isolated a new amino-acid from garlic, *A. sativum*, and from the crow onion or ramsons, *A. ursinum*, which they name alliin. They have shown this to be L-S-allylcysteine sulphoxide,

and regard it as the parent substance of the allicin obtained by Cavallito & Bailey (1944) from *A. sativum*. Cavallito, Buck & Suter (1944) and Cavallito, Bailey & Buck (1945) considered that allicin was either

or

The results of Stoll & Seebeck favour the first structure. It was pointed out by Challenger (1946) that, owing to the well-known basic properties of the sulfoxides, allicin may be regarded as of sulphonium type. The importance of sulphonium compounds in nature is emphasized by other recent work. Maw & du Vigneaud (1948) find that the dimethyl-β-carboxyethylsulphonium chloride,

isolated from the marine alga *Polysiphonia fastigiata* by Challenger & Simpson (1947, 1948) and also dimethylcarboxymethylsulphonium chloride possess a labile methyl group and support the growth of rats on a diet deficient in methionine but containing homocystine. A similar observation has been made with the last-named substance by Dubnoff & Borsook (1948). On acetylation of alliin with thioacetic acid Stoll & Seebeck (1948) found that the sulfoxide group is simultaneously reduced to a sulphide link giving the N-acetyl derivative of S-allylcysteine. This and the corresponding S-propyl derivative yield allylthiol and n-propylthiol, respectively, on hydrolysis with sodium hydroxide.

The exact bearing of these observations on our detection of n-propylthiol (see p. 89) and on Kohmann's (1947) results cannot at present be assessed. Alliin has, moreover, not yet been reported in the onion.

Aspiration experiments with onions. We find that when a slow stream of sterile air is passed over

freshly chopped, peeled onions n-propylthiol volatilizes and can be characterized as mercury di-n-propylthiol by absorption in mercuric cyanide. This was observed with three separate batches of onions, and the thiol was also converted to the silver and lead derivatives.

n-Propylthiol has not till now been detected in nature, although Challenger & Rawlings (1937) observed its formation from di-n-propyl disulphide in cultures of *Scopulariopsis brevicaulis*. Its high volatility probably prevented its detection by Semmler (1892) or other workers, or it might, in their experiments, have been converted to dipropyl disulphide by the oxygen of the air. It may be identical with the very volatile compound mentioned by Walker *et al.* (1925). These authors state, 'it is quite probable that the volatile substances released upon crushing the tissue do not occur thus exactly as they existed in the host cell'. We cannot say whether free n-propylthiol occurs in the undamaged bulb or whether it is liberated by enzyme action after slicing. We considered the possibility that n-propylthiol may not actually be present in the vapours evolved by the onions, but might arise by the interaction of some more complex substance, e.g. a n-propylthioacetal such as

with the mercuric cyanide used as absorbent. Thioacetals $\text{RCH}(\text{SR}')_2$ react with hot aqueous mercuric chloride giving $\text{R}'\text{SHgCl}$ as shown by Holmberg (1932) and by Taylor (1937); see also Hellström & Holmberg (1935). The two propyl derivatives were not available, but we find that diethylthioformal, $\text{CH}_2(\text{SC}_2\text{H}_5)_2$, and diethylthioacetal,

are inert to cold aqueous mercuric cyanide. There seems, therefore, no reason for doubting the presence of free n-propylthiol in the vapours from the sliced onions.

The literature contains no mention of the occurrence of alkylthiols in oil of onions, but Semmler (1887) states that traces of a thiol accompany the divinyl sulphide which he isolated, after treatment with metallic potassium, from the oil of *Allium ursinum*.

Diallyl disulphide and cultures of Scopulariopsis brevicaulis. Challenger & Charlton (1947) have discussed the possible origin of the diallyl disulphide which occurs in oil of garlic (Semmler, 1892). It was, therefore, considered desirable to examine the metabolism of this compound under biological conditions where chemical change could readily be detected. This seemed convenient in view of the investigation of the behaviour of various sulphur compounds in mould cultures, and especially of dimethyl-β-carboxyethylsulphonium chloride (recently isolated from the alga *Polysiphonia fastigiata* by Challenger

& Simpson, 1947) which is being made by Mr Y. C. Liu in these laboratories. Consequently a study of the fate of diallyl disulphide in cultures of the mould *Scopulariopsis brevicaulis* was undertaken.

We find that this undergoes fission with formation of allylthiol which we have characterized as the mercury, lead and silver derivatives. Methylation also occurs and methyl allyl sulphide is produced; this has been characterized as the addition products with mercuric and platinic chlorides. These findings are in agreement with the work of Challenger & Rawlings (1937) and Blackburn & Challenger (1938) who showed that saturated disulphides R_2S_2 , where R is methyl to n -amyl, in cultures of *S. brevicaulis*, yield the corresponding alkylthiol RSH and the alkyl methyl sulphide $R \cdot S \cdot CH_3$. Challenger & Ellis (1935) found that allylarsonic acid in cultures of the mould is converted to dimethylallylarsine thus showing that the allyl group, when attached to arsenic, is not reduced by the mould. Our results with diallyl disulphide show that this is also true when the allyl group is linked to sulphur.

EXPERIMENTAL

Detection of n-propylthiol in the onion

Treatment of onions. Three separate batches of onions, 1070, 1350 and 1710 g., were peeled, sliced and chopped immediately before use. Air was passed through conc. H_2SO_4 , a 1% $HgCl_2$ solution and two tubes of cotton wool (to remove any traces of S compounds and also micro-organisms), then through the chopped onions in 0.5 l. jars, and finally through various absorbents. Two tubes of aniline removed any thiocarbimide as a thiourea derivative, a tube of 2N-HCl eliminated aniline vapour, two tubes of 4% $Hg(CN)_2$ solution removed thiols, and finally two tubes of 3% $HgCl_2$ solution absorbed the sulphides and disulphides which are known (Challenger & Rawlings, 1937; Blackburn & Challenger, 1938) to pass unchanged through $Hg(CN)_2$. The suitability of this procedure was checked by a preliminary experiment in which the constituents of a mixture of allylthiocarbimide and diallyl disulphide were satisfactorily separated.

Identification of n-propylthiol. Formation of a precipitate in the $Hg(CN)_2$ occurred after 6 hr. and ceased in 2–3 days. The m.p. was 63–65° and, after one recrystallization from alcohol, 66–67°. The mixed m.p. with authentic mercury di- n -propylthiol of m.p. 67° was 67–68°. Mixed m.p. with mercury diallylthiol of m.p. 74° was 60–63°. (Found: C, 20.8; H, 4.06. Calc. for $C_6H_{14}S_2Hg$: C, 20.5; H, 4.00. Mercury diallylthiol, $C_6H_{10}S_2Hg$, requires C, 20.75; H, 2.90%.) The first batch of onions yielded 0.025 g. (equivalent to 10 mg. of propylthiol/kg.).

The second and third batches yielded 0.013 and 0.042 g. of mercury di- n -propylthiol representing 4 and 11 mg. of propylthiol/kg. The m.p. and mixed m.p. determinations confirmed the conclusions of the first experiment.

A portion of the mercury compound was decomposed by 6N-HCl, the thiol passed through water, and into a 20% solution of lead acetate. The lead salt of the thiol had m.p. 92°. The mixed m.p. with lead di- n -propylthiol (m.p.

95°) was 94°. Lead diallylthiol melts at 108°. The remaining lead thiol was decomposed with HCl and converted to the silver derivative. This, when washed with ethanol and ether had m.p. and mixed m.p. 186° (decomp.) with silver n -propylthiol m.p. 186° (decomp.). The corresponding allyl derivative melts at 114°.

Examination of the aniline and of the precipitates in the mercuric chloride. Solution of the aniline in 50% (v/v) aqueous acetic acid left a trace of solid indicating the absence of more than traces of a thiocarbimide in the volatile products.

The precipitate in the first $HgCl_2$ tube from three batches of onions was only 0.008 g. It was insoluble in all common solvents, did not melt below 270° and was probably the mercurichloride of an unsaturated sulphide such as

or the mercurated fission product of a disulphide, e.g. $RCH \cdot HgCl \cdot CHOH \cdot CH_2S \cdot HgCl$ (Smith, 1939). Addition of HCl gave the odour of a thiol suggesting that a product of the second type was present.

Preparation of metallic derivatives of authentic allyl- and n-propyl-thiols. Lead n -propylthiol (Borgstrom, Ellis & Reid, 1929) melts at 94–96°, sintering at 88–89°. The yellow precipitate obtained by us melted at 90–92°. It was recrystallized from benzene, much lead sulphide formed by decomposition by the hot solvent being removed. The m.p. was sharp and constant at 95°. The decomposition of lead dialkylthiols by heat, giving lead sulphide and a dialkyl sulphide, is well known (Klason, 1887; Challenger & Rawlings, 1937). Mercury diallylthiol, $(C_3H_5S)_2Hg$, was prepared by shaking the thiol (2 g.) with aqueous 4% $Hg(CN)_2$ solution (700 ml.; 4.5 mol.) for 2 hr. Reaction was rapid. The sticky, greenish precipitate was separated, washed with water and with ethanol and recrystallized 3 times from ethanol. It formed colourless pearly plates, m.p. 74°. These gradually became pale green in colour. (Found: C, 20.8; H, 2.8; S, 18.95. $C_6H_{10}S_2Hg$ requires C, 20.75; H, 2.90; S, 18.46%.) A specimen which had been kept in a closed tube for 10 years was found to be almost completely black and had an odour of a sulphide.

The lead derivative of allylthiol had m.p. 105–108° and 107–108°, constant, after recrystallizing from benzene. It was again found necessary to remove lead sulphide.

The silver derivatives are insoluble in all common solvents and could not be recrystallized. The allyl derivative melted at 113–114°, darkening above 100°, whereas the n -propyl compound melted between 182 and 190° (decomp.) the m.p. varying with the rate of heating, but always lying within these limits. The m.p. were unchanged after washing the compounds with various solvents. The silver derivatives were fairly stable, but the lead derivatives, especially lead diallylthiol, decomposed on keeping for 2 or 3 days.

Preparation and reactions of reference compounds

Preparation of methyl allyl sulphide. Methylthiol, prepared from S -methylisothiurea sulphate and NaOH (Arndt, 1921), was passed through dilute H_2SO_4 , dried by $CaCl_2$ and absorbed in ice-cold sodium ethoxide. The solution of sodium methylthiol was then added in the cold to the theoretical weight of allyl bromide in ethanol under reflux. After refluxing for 30 min. the mixture was cooled, poured into water and the oil separated and dried over $CaCl_2$. After four distillations, the b.p. was 91–93°, the

figure given by Obermeyer (1887). (Found: C, 54.6; H, 9.45. Calc. for C_6H_8S : C, 54.6; H, 9.10%.) The product was free from thiol, no precipitate being formed on shaking with 4% $Hg(CN)_2$ solution.

Derivatives of methyl allyl sulphide. The compound formed with excess $HgCl_2$ could not be recrystallized and was infusible. A soluble mercurichloride, m.p. 115° , was prepared by shaking together equimolecular quantities of methyl allyl sulphide and $HgCl_2$ solution, and filtering immediately. The *platinichloride* was prepared from platinic chloride (1.0 g.) in water (2–3 ml.) and methyl allyl sulphide (0.8 g.). The solid was extracted with ether; removal of the solvent left a yellow powder which was recrystallized 5 times from dilute ethanol. The m.p. (150°) was unchanged by a sixth recrystallization from chloroform-light petroleum. (Found: Pt, 38.2. $(C_3H_5.S.CH_3)_2PtCl_4$ requires Pt, 38.0; $C_3H_5.S.CH_3.PtCl_4$ requires Pt, 45.8; $C_3H_5S.CH_3.PtCl_4.2H_2O$ requires Pt, 42.2%.)

Action of methyl iodide on methyl allyl sulphide. *Elimination of the allyl group.* Equimolecular quantities of methyl allyl sulphide (1.2 g.) and methyl iodide (1.73 g.) were mixed and left at room temperature for 48 hr. The resulting solid, rubbed with ether and recrystallized from absolute ethanol by addition of ether, had m.p. 206° . The mixed m.p. with authentic trimethylsulphonium iodide of the same m.p. was 206.5° .

Preparation of diallyl disulphide. This was prepared by the thiosulphate method of Stutz & Shriner (1933), bearing in mind the ready decomposition of diallyl disulphide in presence of alkali giving, presumably, allylthiol and allylsulphenic acid $C_3H_5S.OH$ (cf. Schöberl, 1933, 1936). By limiting the time of hydrolysis of the sodium allylthiosulphate to 10 min., thus preventing hydrolysis of the diallyl disulphide, and by thorough washing and drying of the disulphide, an almost pure fraction of b.p. $79^\circ/16$ mm. pressure was obtained after three distillations. Von Braun (1903), who prepared it from sodium disulphide and allyl bromide, gives b.p. $77\text{--}82^\circ/15$ mm. (Found: C, 49.8; H, 7.2; S, 43.1. Calc. for $C_6H_{10}S_2$: C, 49.3; H, 6.9; S, 43.8%.) The product may have contained a small amount of the monosulphide. The absence of thiol was shown by shaking with 4% $Hg(CN)_2$ solution, when no precipitate formed.

By alkaline hydrolysis of sodium allyl thiosulphate in the cold for 30 min., separating the oil and boiling the aqueous liquid to complete the reaction, an improved yield of about 30% was obtained.

Action of diallyl disulphide on $PtCl_4$ and $HgCl_2$. When 2 drops of the disulphide and 0.1 g. of $PtCl_4$ in a small amount of water were mixed an orange solid formed during 24 hr. Most of this was insoluble in ether, ethanol and chloroform and did not melt below 270° . Ether extracted traces of a solid with similar properties. The compound formed from diallyl disulphide and excess of 3% $HgCl_2$ solution was also insoluble and did not melt below 270° .

Diallyl disulphide and Scopulariopsis brevicaulis in bread cultures

Formation of methyl allyl sulphide and allylthiol. Four 1 l. conical flasks, each containing 150 g. of breadcrumbs and 25 ml. of distilled water, were sterilized at 20 lb. pressure (120°) for 25–30 min., inoculated with spores from a potato-

agar subculture of *S. brevicaulis* and incubated for 6 days at 32° and for 3 days at room temperature. Diallyl disulphide (0.25 g.), emulsified with 25 ml. of sterile water, was then added by means of a sterile pipette to each flask. The flasks were connected in series by means of sterile rubber bungs and glass tubing, and sterile air passed through into two tubes of 4% $Hg(CN)_2$, two of $PtCl_4$ and finally two of 3% $HgCl_2$, all in aqueous solutions.

Characterization of allylthiol. After 30 min. a white solid formed in the first $HgCl_2$ tube, probably due to reaction with unchanged diallyl disulphide, and after 2 hr., a precipitate was observed in the first $Hg(CN)_2$ tube. Next day solid had formed in all absorption vessels except the second $Hg(CN)_2$ tube. A total of 0.20 g. (m.p. 73°) was removed from the first $Hg(CN)_2$ tube (0.12 g. after 1 day and 0.08 g. after 7 days). After one recrystallization from ethanol, the m.p. was 74° , unaltered by further recrystallization. The mixed m.p. with authentic mercury diallylthiol of m.p. 74° was 74° and with mercury di-*n*-propylthiol of m.p. 67° was $56\text{--}57^\circ$.

The product was converted to the lead and silver derivatives. The lead diallylthiol melted at $105\text{--}106^\circ$ and after one recrystallization from hot benzene, which removed much lead sulphide (see p. 89) had m.p. $108\text{--}109^\circ$ unaltered by further recrystallization. The mixed m.p. with freshly prepared lead diallylthiol of m.p. 108° was $108\text{--}109^\circ$. The silver derivative after washing with ethanol and ether melted at $111\text{--}113^\circ$. The mixed m.p. with authentic silver allylthiol of m.p. $114\text{--}115^\circ$ was $113\text{--}114^\circ$. In each case darkening occurred above $85\text{--}90^\circ$. These m.p. are less sharp than those of the lead and mercury derivatives, but clearly distinguish between *n*-propyl- and allyl-thiols, as the m.p. of silver *n*-propylthiol, $C_3H_7S.Ag$, is 186° .

Characterization of methyl allyl sulphide. The platinum chloride precipitates were removed after 2 days (0.36 g., m.p. 118°), after 3 days (0.15 g., m.p. $123\text{--}126^\circ$), after 7 days (0.23 g., m.p. $126\text{--}135^\circ$) and after 15 days (0.14 g., m.p. $124\text{--}136^\circ$). After three crystallizations from aqueous ethanol and two from chloroform-light petroleum ($60\text{--}80^\circ$), the constant m.p. was 150° alone and in admixture with synthetic methyl allyl sulphide platinichloride of the same m.p. (Found: Pt, 38.04. $(C_3H_5S.CH_3)_2PtCl_4$ requires Pt, 38.03%.) The unsharp m.p. of the unrecrystallized products were probably due to the presence of some of the compound formed from diallyl disulphide and platinum chloride. On the nineteenth day 0.025 g. of precipitate was removed from the $HgCl_2$ tubes. The amount hardly increased after the second day. It was unmelted below 270° , darkened and sintered above 200° , and was insoluble in all common solvents. Warm dilute HCl gave an odour of a thiol. With NaOH, HgO was precipitated, and only a slight odour of sulphide or disulphide was observed. The results of these tests suggest that the precipitate contained a product of the type $RSHgCl$ or $xRSHgCl.yHgCl_2$ arising from the fission of unchanged diallyl disulphide by $HgCl_2$ (cf. Challenger & Rawlings, 1937) along with a small amount of a mercuric chloride compound of methyl allyl sulphide.

Potassium chloroplatinite, K_2PtCl_6 , was unsuitable as an absorbent for the methyl allyl sulphide, the insoluble product being unmelted below 270° . A second experiment with *S. brevicaulis* and diallyl disulphide gave mercury diallylthiol, m.p. and mixed m.p. 74° , and methyl allyl sulphide platinichloride, m.p. 149° and mixed m.p. 150° . The deposit in the $HgCl_2$ solution on extraction with ethanol yielded a

small amount of methyl allyl sulphide mercurichloride, m.p. 112–114° and 113–114° mixed with an authentic specimen, m.p. 114° (see p. 90). This afforded a second mode of characterization of the mixed sulphide. In a third experiment mercury diallylthiol was again obtained.

SUMMARY

1. *n*-Propylthiol has been detected in the onion, *Allium cepa*, and characterized as the mercuric, lead and silver salts. The thiol was removed from the freshly chopped bulbs in a stream of sterile air and absorbed in mercuric cyanide.

2. The behaviour of diallyl disulphide, the main constituent of oil of garlic, *A. sativum*, has been investigated in cultures of the mould *Scopulariopsis brevicaulis* which convert it to methyl allyl sulphide and allylthiol. These compounds were identified as the platinichloride and the mercuric, lead and silver salts respectively.

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