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DIMETHYL TRISULPHIDE, ITS MECHANISM OF FORMATION IN HOP OIL AND EFFECT ON BEER FLAVOUR

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The precursor of dimethyl trisulphide (DMTS) in hops is destroyed by sulphur dioxide during kilning, but then re-forms slowly during storage. A redox mechanism is suggested and *S*-methylcysteine sulphoxide is postulated as the DMTS-precursor.

The effect of DMTS on beer flavour is described. The flavour threshold value for DMTS in a commercial beer has been found to be 0·10μg/litre.

Key words: *flavour, hop oil, hops, sulphur compound, sulphuring.*

Introduction

In a previous communication,[11] we reported the existence of a sulphur compound which was generally much more prominent in the steam distilled oil obtained from hops which had not been treated with sulphur dioxide during the normal hop kilning process. This compound is 2,3,4-trithiapentane, or dimethyl trisulphide (DMTS), and seemingly it is formed during steam distillation from a labile precursor which is destroyed by sulphur dioxide.[9] The presence or absence of DMTS in steam distilled hop oil apparently provided a convenient indicator of whether or not the hop sample had been treated with sulphur dioxide during kilning. However, recent investigations have revealed that, on storage, even at 4°C, the DMTS-precursor is gradually re-formed in sulphur dioxide-treated hops. This paper postulates a mechanism for the destruction, initiated by sulphur dioxide, of the DMTS-precursor and its subsequent reformation, which occurs during storage of the hops.

DMTS has been found to have an extremely low flavour threshold in beer (*ca* 0·10 μg/litre). It could therefore contribute markedly to beer flavour when hops containing the DMTS-precursor are used in the copper. Unfortunately, using gas chromatography in combination with a headspace sampling technique, DMTS could not be detected in beer below a level of *ca* 100 μg/litre, *i.e.* one thousand times the taste threshold level.

Results and Discussion

Samples of both Fuggles and Wye Northdown hops (1975 crop), which had been kilned with sulphur dioxide, originally yielded, on stream distillation, oils containing low levels of DMTS. The steam distilled oils from the same hops, after an 18 month period of storage at 4°C, were analysed by GC, using a flame photometric sulphur detector, and shown to be much enriched in DMTS (see Table I). In fact, DMTS was the major sulphur component of both oils, and in neither case was it possible to distinguish reliably between these oils and those obtained from the corresponding non-sulphur dioxide-treated hops. Similarly, a sample of sulphur dioxide-treated Northdown hops (1977 crop), also found to have a very low level of DMTS-precursor originally, underwent a small increase in the level of DMTS in the steam distilled oil during the first 5½ months of storage.

The destruction of DMTS-precursor by sulphur dioxide, and its reappearance during subsequent storage, suggests a redox mechanism. Hence a portion of the 1975 Northdown hops which had been kilned with sulphur dioxide was stood overnight with 5% aqueous sulphur dioxide prior to steam distillation. This yielded an oil containing virtually no DMTS. Portions of the 1977 sulphur dioxide-treated Northdown hops, on storage in open vessels at ambient temperature for up to 3½ months, underwent significant increases in the level of DMTS in the steam distilled oil. Another portion of the same hops on treatment overnight with 2% aqueous hydrogen peroxide also yielded, on steam distillation, an oil much enriched in DMTS. From the above observations it would appear that the situation can be represented by the simple redox mechanism shown in Fig. 1. Furthermore, since hops which are not kilned with sulphur dioxide also appear to undergo an increase in the level of DMTS-precursor during storage, it is likely that green hops initially contain some

TABLE I. Effects of Various Treatments on Levels of DMTS in Hop Oil.

Hop	SO_2 Treatment during kilning	Other treatment	DMTS Flame photometric response (× 1/64)
1975 Northdown	Yes	—	2
1975 Northdown	Yes	19 Months storage at 4°C	520
1975 Northdown	Yes	26 Months storage at 4°C followed by treatment with 5% aq. sulphur dioxide.	1
1975 Northdown	No	—	300
1975 Northdown	No	27 Months storage at 4°C	1440
1975 Fuggles	Yes	—	8
1975 Fuggles	Yes	26 Months storage at 4°C	370
1977 Northdown	Yes	—	2
1977 Northdown	Yes	2 Months storage at 4°C	4
1977 Northdown	Yes	5½ Months storage at 4°C	19
1977 Northdown	Yes	1 Month storage at 4°C followed by 1 month at 20°C	44
1977 Northdown	Yes	2 Months storage at 4°C followed by 3½ months at 20°C	136
1977 Northdown	Yes	3 Months storage at 4°C followed by treatment with 2% aq. hydrogen peroxide	540
1977 Northdown	Yes	5½ Months storage at 4°C followed by treatment with 1000 ppm *S*-methylcysteine sulphoxide	60
1977 Northdown	No	—	55
1977 Northdown	No	2 Months storage at 4°C	90

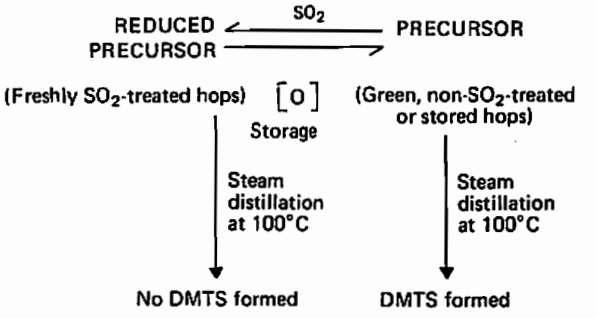


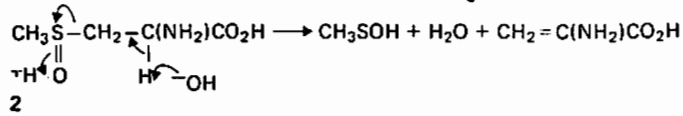
Fig. 1. Mechanism of DMTS-precursor destruction and reformation.

'reduced' precursor, *i.e.* they contain a mixture of precursor and reduced precursor.

S-Methylcysteine sulphoxide, which is known to occur naturally in many plants, *e.g.* cabbage,[19] turnip,[8] and broccoli,[8] appears to fit the criteria required of the DMTS-precursor.

Maruyama postulated a mechanism for the formation of DMTS from *S*-methylcysteine sulphoxide during the cooking of several brassicaceous vegetables.[7] This involved reaction between hydrogen sulphide (evolved during cooking) and methansulphenic acid, formed as an unstable intermediate by β-elimination from the sulphoxide (Fig. 2).

1



2

$$2CH_3SOH + H_2S \longrightarrow \underset{\text{DMTS}}{CH_3SSSCH_3} + 2H_2O$$

3

$$CH_2=C(NH_2)CO_2H + H_2O \longrightarrow CH_3COCO_2H + NH_3$$

Fig. 2. Postulated reaction sequence leading to formation of DMTS from *S*-methylcysteine sulphoxide.

Anderson and Howard showed that hops contain significant quantities of hydrogen sulphide.[1] Although dry hops contain *ca* 15% wt/wt protein, they contain only low levels (*ca* 0·1%) of amino acids and, furthermore, *S*-methylcysteine sulphoxide appears not to be one of those previously identified.[18] However, we have now tentatively identified *S*-methylcysteine sulphoxide as being present in a batch of hops known to be rich in the DMTS-precursor. The amino acids were removed from an aqueous extract of the hops by treatment with cation exchange resin, and then extracted from the resin with ammonia. After removal of the ammonia, the amino acid extract was examined by thin layer chromatography using cellulose plates with two different eluants, and silica gel plates with one eluant. The plates were treated with a reagent which is specific for sulphoxides,[20] although it was observed that the sensitivity of this spot test was very much reduced when used with silica gel plates. The R_F values obtained are given in Table II. Discrepancies in the values obtained for *S*-methylcysteine sulphoxide run separately and in admixture with the hop amino acid extract are likely to be due to the effects of the other components in the mixture (revealed, by the ninhydrin test, to number between 15 and 20).

Johnson *et al*[6] showed that various sulphoxides were reduced to the corresponding sulphides by treatment with aqueous sulphur dioxide. Similarly, Snow *et al*,[17] showed that methionine sulphoxide was reduced to methionine by aqueous bisulphite at acid pH. Kilning hops in the presence of sulphur dioxide would therefore be expected to cause reduction of *S*-methylcysteine sulphoxide to the sulphide. Furthermore, methionine, in various natural materials, has been shown by Slump *et al*[16] to become oxidized slowly to the sulphoxide. *S*-Methylcysteine would therefore be expected to behave similarly, causing an increase on storage in the level of *S*-methylcysteine sulphoxide in hops which had been kilned with sulphur dioxide.

S-Methylcysteine sulphoxide was refluxed for 3 hr in aqueous solution buffered at pH 4·5 (the conditions under which conventional steam distillation of hops is carried out). The experiment was then repeated with hydrogen sulphide bubbling slowly through the reaction mixture. Yields of DMTS were 0·9 and 15·9% respectively, DMTS being the major volatile product in the presence of hydrogen sulphide.

A portion of the Northdown hops (1977 crop) which had been kilned with sulphur dioxide, on treatment with *ca* 1000 ppm *S*-methylcysteine sulphoxide followed by steam distillation at 100°C, yielded an oil containing an enhanced level of DMTS. Similar experiments, using respectively *S*-methylcysteine and methionine sulphoxide, produced oils in which the level of DMTS was unaffected. Although only a relatively small increase in DMTS level was obtained using *S*-methylcysteine sulphoxide, this may have been as a result of inadequate penetration of the sulphoxide into the hop material, from whence the hydrogen sulphide originates. The same result was obtained even when the hops were milled prior to steam distillation. That no increase in DMTS level was obtained using methionine sulphoxide is presumably due to the inability of this compound to undergo the required β-elimination, (see Fig. 2).

An alternative possible mechanism for DMTS formation in hop oil involves the redox couple methanthiol/dimethyl disulphide (DMDS), where DMDS represents the precursor and methanthiol the reduced precursor. DMTS can be formed from DMDS as a result of an insertion reaction with elemental sulphur.[14] The sulphur could either be present on the hops[15] or be derived from hydrogen sulphide during steam distillation. This possibility was ruled out, however, when it was shown that the level of DMDS in hop oil was not reduced by overnight treatment of the hops with 5% aqueous sulphur dioxide prior to steam distillation.

S-Methylcysteine sulphoxide thus fits all the criteria required of the DMTS-precursor, but final confirmation must await isolation.

The Effect of DMTS on Beer Flavour

The flavour threshold of a sample of pure DMTS in a commercial light ale was determined, by a method based on a combination of the multiple comparison test of Clapperton[3] and the multiple pairs test of Guadagni *et al*,[4] to be 0·10 μg/litre, with upper and lower confidence limits of respectively 0·16 and 0·06 μg/litre. This threshold value is considerably lower than those reported (33 μg/litre and 7·5 μg/litre respectively) for the corresponding mono- and disulphides.[1] A beer, to which had been added 0·15 μg/litre of DMTS, was analysed using the flavour profile technique

TABLE II. Results of T.L.C. Analysis of Hop Amino Acid Extract.

T.L.C. System		R_F values		
Adsorbant	Eluant	*S*-Methylcysteine sulphoxide	Hop amino acid extract	Hop amino acid extract + *S*-methylcysteine sulphoxide
Cellulose	nBuOH/AcOH/H_2O 74:19:20	0·14	0·17	0·14
Cellulose	lutidine/EtOH/H_2O/$HNEt_2$ 55:25:20:2	0·21	0·19	0·20
Silica Gel	nBuOH/AcOH/H_2O 74:19:20	0·09	0·06	0·06

of Clapperton.[2] The beer received high scores for terms such as *cooked vegetable*, *onion-like* and *sulphury*, and was considered by the tasters to have been adversely affected.

Dry hopping, a process by which the aroma and flavour of the essential oil of hops may be imparted to beer, consists of standing beer in contacts with dry hops in the cask for a period of up to 3 weeks.[13] However, DMTS is not expected to be important in the process of dry hopping. Any precursor present in hops used for dry hopping, even if transferred to the beer, would not be subjected to sufficient heat treatment for DMTS formation. However DMTS could well affect beer flavour adversely when non-sulphur dioxide-treated hops, or sulphur-dioxide-treated hops which have been stored for a sufficiently long time, are used in the copper, either for bittering or as a late addition to impart hop character.

Similarly, addition of hop oil preparations produced by conventional steam distillation of hops at 100°C might well produce in beer a level of DMTS in excess of its threshold concentration. However, aqueous emulsions of hop oil produced by low temperature steam distillation of hops[12] do not contain a detectable level of DMTS, and so would not be expected to cause problems in this way.

We are examining possible methods for measuring beer DMTS levels at concentrations as low as 0·10 μg/litre. Using the modification of a beer headspace sampling technique[21] currently employed for the determination of DMS, DMTS was not detected in beer at levels below *ca* 100 μg/litre. Far more sensitive procedures are therefore necessary. It is likely that the method for concentrating and estimating beer volatiles devised by Pickett *et al*[10] will prove sensitive enough for determining DMTS at or around its threshold concentration.

Experimental

Hop oils were isolated by steam distillation at atmospheric pressure according to the procedure recommended by the Institute of Brewing.[5] Samples of hop oil were stored at 4°C in glass ampoules sealed under vacuum.

Treatment of hops with sulphur dioxide was carried out by thoroughly shaking hops (100 g) in a 5 litre round bottomed flask with 5% aqueous sulphur dioxide (300 ml) and storing the flask overnight at ambient temperature prior to steam distillation in the normal manner. Treatment of hops with hydrogen peroxide was carried out in the same way, using 2% aqueous hydrogen peroxide (300 ml).

Treatment of hops with *S*-methylcysteine, *S*-methylcysteine sulphoxide and methionine sulphoxide, respectively, was carried out by spraying the hops (100 g) with solutions of the amino acid (0·1 g) in deionized water (10 ml), prior to overnight storage and steam distillation as usual.

Gas-chromatography was carried out using the Pye GCV gas chromatograph fitted with synchronous flame ionization and flame photometric detection. Samples (5 μl) of solutions of hop oil (25 μl) in cyclohexane (0·5 ml) were chromatographed using a glass column (9 ft × ¼ in) packed with 10% carbowax 20M on chromosorb W AW DMCS 80–100 (Perkin Elmer Ltd.), a nitrogen carrier gas flow rate of 45 ml/min and a temperature programme of 50°C to 200°C at 3°C/min.

S-Methylcysteine sulphoxide was prepared by treating *S*-methylcysteine (10 mmol.) with 3·8% aqueous hydrogen peroxide (10 mmol) at 60°C for 2 h. The product was shown by T.L.C. on both silica gel (Polygram Sil G/UV_{254} 300, Machery-Nagel & Co.), using propan-2-ol/water, 2:1 as eluant, and cellulose (Polygram MN 300, Machery Nagel & Co.), using butan-1-ol/acetic acid/water, 74:19:20 as eluant, to be one component almost completely free of starting material. The product gave positive results with ninhydrin, and also with a sulphoxide-specific spot test reagent,[20] and exhibited the following spectral features: νmax (nujol) 1012s & 1025s $(S{=}O)^{1}_{CH}$ δ(D_2O) 2·83 (3H, s, CH_3 S=O), 3·10–3·62 (2H, m, O=SCH_2), 4·24 (1H, m, $CH(NH_2)$ CO_2 H).

Reaction between S-methylcysteine sulphoxide and hydrogen sulphide.—*S*-Methylcysteine sulphoxide (0·67 mmol) was boiled for 2 hr under reflux in aqueous soln. (20 ml) buffered at pH 4·5, through which hydrogen sulphide was bubbling slowly, and to which had been added *n*-tetradecane (1·5 ml). The mixture was then steam distilled for 1 h when n-tetradecane and the volatile hydrolysis products were collected. The concentration of DMTS in the n-tetradecane was determined by a GC method based on comparison of peak height (FID) with a calibration plot of peak height versus DMTS concentration. The result was compared with that obtained from a control experiment carried out in the absence of hydrogen sulphide.

Dimethyl trisulphide was prepared pure as described previously.[9]

Preparation of Hop Amino Acid Extracts.—Non-sulphur dioxide-treated hops (1975 Wye Northdown) (50 g) were freed of resins and oil by extraction with diethyl ether for 20 h in a Soxhlet apparatus. The hop cones were thorougly dried, before being mixed with deionized water (*ca* 1 litre) and heated to 50°C in a water bath. The mixture was stirred frequently during 6 hr, and the hops were then filtered off at the pump and washed well with further portions of warm water to yield a total aqueous extract of *ca* 2 litres. This extract was reduced in volume to *ca* 500 ml by rotary film evaporation, and then shaken for 2 h with Amberlite cation exchange resin IR120H (30 g). The resin was filtered off at the pump, washed with a large excess of deionized water, and finally extracted with 1N ammonium hydroxide (5 × 100 ml). This solution was rotary film evaporated to dryness, the residue being redissolved in water and evaporated several times to ensure thorough removal of ammonia. The residue was finally dissolved in deionized water (5 ml) and chromatographed as described below.

Thin Layer Chromatography of Hop Amino Acid Extracts.—Portions of the hop amino acid extract (1 μl) were chromatographed on cellulose plates with both butan-1-ol/acetic acid/water, 74:19:20 and 2,6-lutidine/ethanol/water/diethylamine, 55:25:20:2 as eluants, and on silica gel plates with the first mixture mentioned above as eluant. Visualization of the plates was made by treatments with (i) a ninhydrin reagent (0·4% ninhydrin in methanol/chloroform, 1:5, containing 2% acetic acid when used with the lutidine eluant and (ii) a sulphoxide-specific spot test reagent.[20]

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References

1. Anderson, R. J., & Howard, G. A., *Journal of the Institute of Brewing*, 1974, **80**, 357.
2. Clapperton, J. F., *Journal of the Institute of Brewing*, 1973, **79**, 495.
3. Clapperton, J. F., *Journal of the Institute of Brewing*, 1974, **80**, 164.
4. Guadagni, D. G., Maier, V. P. & Turnbaugh, J. G., *Journal of Science, Food and Agriculture*, 1973, **24**, 1277.
5. Howard, G. A., *Journal of the Institute of Brewing*, 1970, **76**, 381.
6. Johnson, C. R., Bacon, C. C. & Rigau, J. J., *Journal of Organic Chemistry*, 1972, **37**(6), 919.
7. Maruyama, F. T., *Journal of Food Science*, 1970, **35**, 540.
8. Morris, C. J. & Thompson, J. F., *Journal of the American Chemical Society*, 1956, **78**, 1605.
9. Peppard, T. L., & Sharpe, F. R., *Phytochemistry*, 1977, **16**(12), 2020.
10. Pickett, J. A., Coates, J., & Sharpe, F. R., *Journal of the Institute of Brewing*, 1976, **82**, 228.
11. Pickett, J. A., Peppard, T. L., & Sharpe, F. R., *Journal of the Institute of Brewing*, 1976, **82**, 288.
12. Pickett, J. A., Peppard, T. L., & Sharpe, F. R., *Journal of the Institute of Brewing*, 1977, **83**, 302.
13. Rabak, F., *Wallerstein Communications*, 1944, **20–22**, 173.
14. Reid, E. E., "*Organic Chemistry of Bivalent Sulphur*", Vol. 3, New York: Chemical Publishing Co., 1965, p365.

15. Sharpe, F. R., & Peppard, T. L., *Chemistry and Industry*, 1977, 664.
16. Slump, P., & Schreuder, H. A. W., *Journal of Science, Food and Agriculture*, 1973, **24**, 657.
17. Snow, J. T., Finley, J. W., & Kohler, G. O., *Journal of Science, Food and Agriculture*, 1976, **27**, 649.
18. Stevens, R., *Chemical Reviews*, 1967, **67**, 19.
19. Synge, R. L. M., & Wood, J. C., *Biochemical Journal*, 1956, **64**, 252.
20. Thompson, J. F., Arnold, W. N., & Morris, C. J., *Nature*, 1963, **197**, 380.
21. White, F. H., & Parsons, R., *Proceedings of the European Brewery Convention, Nice*, 1975, 721.