

Chemistry of Hop Constituents. Part 42.¹ The Formation and Characterisation of Sesquiterpene Episulphides in the Essential Oil of Hops

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The novel episulphides, humulene-1,2- and -4,5-episulphide and caryophyllene-4,5-episulphide, identified as components of hop oil, particularly that from hops treated with sulphur during the growing season, have been prepared under very mild conditions by the u.v.-promoted addition of sulphur to the sesquiterpenes, and have been characterised largely by i.r. and ¹H n.m.r. spectroscopy.

GAS chromatography coupled with flame photometric sulphur detection has revealed some twenty sulphur compounds in the essential oil of hops. Several have been identified as dialkyl polysulphides² and as methyl thioesters.^{3,4} Three more compounds⁵ have now been identified as sesquiterpene episulphides.

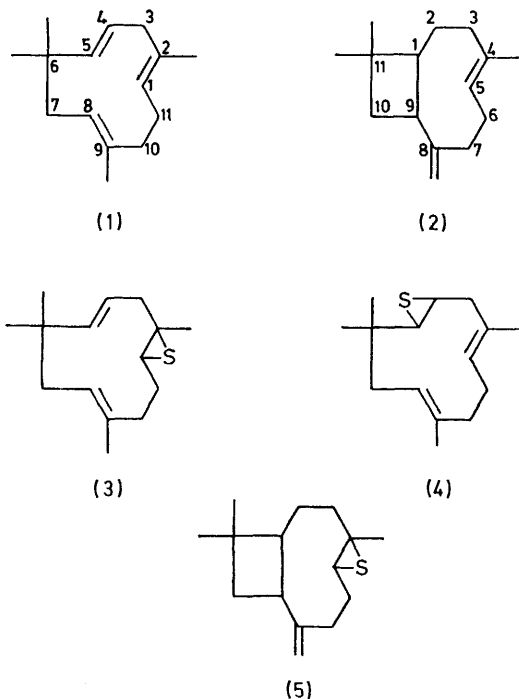
These three sulphur compounds featured prominently in the gas chromatograms of most hop oils examined and were found as major components of the oil from hops that had been heavily dressed with flowers of sulphur during the growing period to control mildew. This last observation led to experiments in which humulene (1) and caryophyllene (2), major components

phyllene reaction mixture, has been identified as 4,5-epithiocaryophyllene (5).

Improved yields of the episulphides (3), (4), and (5), amounting to 20, 5, and 11% severally, were obtained by long irradiation at 254 nm of the sesquiterpenes and sulphur dissolved in cyclohexane. Presumably singlet sulphur is generated and thence triplet sulphur S(³P), by collisional deactivation, which may then add to sesquiterpenoid double bonds. Indeed, in agreement, the three products were also conveniently prepared by u.v. irradiation of benzene solutions of the sesquiterpenes containing an excess of phenyl or ethyl isothiocyanate, conditions well recognised as providing triplet sulphur atoms.⁷

Structural Evidence.—Each of the new sulphur compounds had the molecular composition C₁₅H₂₄S. Hence each was formed by addition of one sulphur atom to the sesquiterpene in question. The major product (3) from humulene gave back humulene (1) when desulphurised with triphenylphosphine, as expected⁸ of an episulphide, and indicating that no skeletal rearrangement had been involved. The product (5) from caryophyllene similarly gave back caryophyllene. That none of the new compounds was a thiol (as might have arisen through reaction with singlet sulphur) was shown by the absence of a precipitate when they were shaken with 20% lead(II) acetate solution and by the absence of i.r. absorption in the 2 600–2 550 cm⁻¹ region. On the other hand, all three compounds gave heavy white precipitates with saturated aqueous mercury(II) chloride, as expected of episulphides (or sulphides).

Confirmation of the episulphide structures came from the i.r. and ¹H n.m.r. spectra. The strong i.r. absorption at 972 cm⁻¹ by the major product (3) from humulene showed that it retained the 4,5-*trans*-disubstituted double bond. The ¹H n.m.r. spectrum (see Table for data) showed a doublet signal (*J* 15.8 Hz) at δ 5.08 from the isolated 5-proton, coupled *trans* across the 4,5-double bond to the 4-proton. The signal from the 4-proton at δ 5.39 was split as a doublet (*J* 15.8 Hz) of double-doublets, the further double-doublet splitting (*J* 9.5, 4.2 Hz) arising from coupling to the two non-equivalent protons of the 3-methylene group. Specific decoupling experiments located the signals from the geminally coupled 3a- and 3b-protons as double doublets at δ 2.91 (*J* 12.6, 4.2 Hz) and 1.78 (*J* 12.6, 9.4 Hz), this non-equivalence indicating the presence of the adjacent



of hop oil, were kept with sulphur at ambient temperature in daylight.⁵ Two of the new sulphur compounds were detected in the humulene reaction mixture by g.c. and these have now been identified as the humulene 1,2- and 4,5-episulphides, (3) and (4). The suggestion made in a preliminary report⁵ that these might be the 1,2- and 8,9-episulphides by analogy with the structures of humulene epoxides II and I⁶ is hereby revised. The third new sulphur compound, detected in the caryo-

¹H N.m.r. data (at 90 MHz) for humulene (1) and for the sesquiterpene episulphides (3), (4), and (5) in CDCl₃ containing SiMe₄

Compound	δ	Intensity	Multiplicity (J/Hz)	Assignment	
(1)	1.06	6H	s	6-Me ₂	
	1.43	3H	ca. d (2)	2-Me	
	1.64	3H	d (2)	9-Me	
	1.91	2H	d (7.6)	7-CH ₂	
	2.07—2.11	4H	c	10-, 11-CH ₂	
	2.51	2H	d (7.3)	3-CH ₂	
	4.88	2H	ca. t (7.6)	1-H	
	4.96		ca. t (7.6)	8-H	
	5.14	1H	d (16)	5-H	
	5.61	1H	dt (16, 7.3)	4-H	
	(3)	1.08	3H	s	6-Me ₂
		1.11	3H	s	
		ca. 1.53	1H	m	11b-H
		1.61	6H	d (2)	9-Me
		1.63		s	2-Me
1.78		1H	dd (12.6, 9.4)	3b-H	
1.93—2.56		5H	c	7-, 10-CH ₂ , 11a-H	
2.63		1H	dd (3.8, 8.5)	1-H	
2.91		1H	dd (4.2, 12.6)	3a-H	
4.98		2H	m	8-H	
5.08			d (15.8)	5-H	
5.39		1H	ddd (15.8, 9.4, 4.2)	4-H	
(4)		0.71	3H	s	6-Me ₂
		1.10	3H	s	
		ca. 1.54	1H	m	3b-H
	1.56	3H	s	2-Me	
	1.63	3H	d (1.2)	9-Me	
	2.13—2.29	6H	c	7-, 10-, 11-CH ₂	
	2.51	1H	d (5.5)	5-H	
	ca. 2.87	2H	m	3a-H, 4-H	
	ca. 4.93	2H	ca. t	1-, 8-H	
	(5)	0.98	6H	s	11-Me ₂
1.01		s			
1.06—1.46		2H	c	3b-, 6b-H	
1.53		7H	s	4-Me	
1.56—1.72			c	2-, 10-CH ₂	
1.99—2.63		6H	c	1-, 3a-, 6a-, 9-H, 7-CH ₂	
2.93		1H	dd (11.4, 3.5)	5-H	
ca. 4.90		1H	ca. s	8-CH ₂ =C	
5.00		1H	ca. s		

1,2-episulphide ring. In contrast, in the parent humulene (1), the 3-methylene protons are equivalent and give a signal at δ 2.51 which is split only as a doublet (*J* 7.3 Hz) by the adjacent 4-proton, showing that the adjoining portions of the molecule are effectively coplanar. The remaining olefinic 8-proton in the episulphide (3) gives a complex multiplet at δ 4.98.

The minor sulphur compound (4) from humulene lacked the 4,5-*trans*-disubstituted double bond, as shown by the absence of the characteristic i.r. absorption at 972 cm⁻¹. The ¹H n.m.r. spectrum likewise showed the absence of the characteristic signals from the olefinic 4- and 5-protons as seen in the corresponding spectra of humulene and 1,2-epithiohumulene. Further confirmation of the 4,5-episulphide structure (4) came from the marked non-equivalence of the 6-*gem*-methyl groups which gave singlets at δ 0.71 and 1.10. The expected signals were given by the olefinic 1- and 8-protons and by the 2- and 9-methyl groups.

The third new sulphur compound (5) derived from caryophyllene showed no i.r. absorption in the 800—840 cm⁻¹ region, indicating that the 4,5-double bond of the

ring had undergone addition. In agreement, the only olefinic signals in the ¹H n.m.r. spectrum were those near δ 5.0 from the protons of the exocyclic vinyl methylene group. There was no evidence from the spectrum of any change other than addition of sulphur to the endocyclic double bond in caryophyllene.

Reactions between elemental sulphur and unsaturated hydrocarbons at 120—200 °C are well documented.⁹ Mostly, the reactions have been conducted at the high end of this temperature range and have been studied because of their connection with the vulcanisation of rubber and the production of sulphurised oils for cutting and lubrication. The u.v.-promoted interaction of elemental sulphur with alkenes at ambient temperature, as in the preparation of the episulphides (3), (4), and (5), appears to be a new discovery. Aspects of the stereochemistry of these products remain to be investigated.

EXPERIMENTAL

U.v. and i.r. spectra were recorded, respectively, with a Pye-Unicam SP 800 and a Perkin-Elmer 157G spectrophotometer. ¹H N.m.r. spectra, obtained with a Bruker WH 90 Fourier-transform instrument, are for solutions in CDCl₃ containing SiMe₄. Mass spectra were recorded with either an AEI MS12 or a Varian CH 5D double-focusing spectrometer. Analytical g.c. (for monitoring all operations) was carried out with a Pye GCV gas chromatograph (3% OV1) equipped with synchronous flame ionisation and flame photometric detection, and preparative g.c. with a Pye 105 instrument employing flame ionisation. Column chromatography used Mallinckrodt silicic acid 2 847 (100 mesh) with 1% dry diethyl ether in light petroleum (b.p. 60—80 °C) as eluant.

Epithiohumulenes (3) and (4).—(a) *From sulphur*. Humulene¹⁰ (7.6 g) and powdered sulphur (6 g) in cyclohexane (170 ml) were irradiated at 254 nm (2.5-W low-pressure mercury lamp) for 323 h. The mixture was filtered, the solvent removed in a current of nitrogen, and precipitated sulphur filtered off. A portion (0.5 ml) of the brown oil was chromatographed (column 47 × 1.5 cm) and fractions rich in the individual epithio-compounds were purified by preparative g.c. (10% Carbowax 20M) to afford compounds (3) and (4) (ca. 10 mg each) as colourless oils.

(b) *From phenyl isothiocyanate*. A solution of humulene (4.1 g) and phenyl isothiocyanate (10.6 g) in benzene (95 ml) was irradiated at 254 nm (6-W lamp) under nitrogen (oxygen-free) for 90 h. After filtration, the solution was concentrated under reduced pressure (ca. 10 mmHg), and then at 55 °C and 0.05 mmHg, to leave a dark brown oil. A portion (3.4 ml) was chromatographed (column 37.5 × 2 cm) and the two concentrates obtained were separately rechromatographed (columns 47 × 1.5 cm) to yield compounds (3) (0.3 g) and (4) (10 mg) as colourless oils.

1,2-*Epithiohumulene* (3) (Found: C, 76.3; H, 10.3; S, 13.8%, *m/e* 236.159 75. C₁₅H₂₄S requires C, 76.2; H, 10.2; S, 13.6%, *M* 236.159 75) has *d*₄²⁵ 0.943 2, λ_{max} (EtOH) 266 nm (ε 220); ν_{max} (film) 1 670w (C=C), 1 450s, 1 388s and 1 367s (*gem*-Me₂), 1 294w, 1 215w, 1 180w, 1 100w, 1 046, 993w, 972s (*trans* CH=CH), 909w, 867w, 834, and 829 cm⁻¹ (C=CH), *m/e* 238 [(*M* + 2)⁺, 3], 236 (*M*⁺, 30), 168 (27), 154 (30), 153 (30), 127 (31), 121 (28), 109 (23), 107 (22), and 93 (100%).

4,5-Epithiohumulene (4) (Found: C, 76.1; H, 10.0%; m/e 236.159 75) has ν_{\max} (film) 1 670w and 1 640w (C=C), 1 450s, 1 389s, and 1 365s (*gem*-Me₂), 1 292w, 1 214w, 1 188, 1 105w, 1 084w, 1 019w, 994w, 930w, 904, 840w, and 820w cm⁻¹ (C=CH), m/e 238 [(M + 2)⁺, 5], 236 (M⁺, 78), 154 (30), 153 (28), 121 (36), 107 (46), 101 (70), 100 (62), 99 (49), and 93 (100%).

Epithiocaryophyllene (5).—(a) *From sulphur*. A solution of caryophyllene (7.2 g) and sulphur (2 g) in cyclohexane (170 ml) was irradiated at 254 nm (2.5-W lamp) for 68 h, filtered, and evaporated. Preparative g.c. (10% Carbowax 20M) of a portion of the residue afforded compound (5) (10 mg) as an oil.

(b) *From ethyl isothiocyanate*. Caryophyllene (15.4 g) and ethyl isothiocyanate (6.5 g) in benzene (70 ml) under nitrogen were irradiated at 254 nm (6-W lamp) for 89 h. The solution was filtered and evaporated under reduced pressure (*ca.* 10 mmHg) and the residue heated at 75 °C and 0.05 mmHg to leave a dark brown oil (7.8 ml). Chromatography ($\times 3$; columns 36 \times 2, 33 \times 2, 48.5 \times 1.5 cm) afforded compound (5) as an oil (0.5 g).

4,5-Epithiocaryophyllene (Found: m/e 236.159 7. C₁₅H₂₄S requires *M*, 236.159 75) has ν_{\max} (CHCl₃) 1 710 (CH₂=C overtone), 1 675, and 1 629 (C=C), 1 459, 1 450, 1 375, and 1 365 (*gem*-Me₂), 1 278, 1 255, 1 245, and 858 cm⁻¹ (CH₂=C), m/e 238 [(M + 2)⁺, 2], 237 (6), 236 (M⁺, 31), 221 (6), 203 (9), 161 (28), 147 (30), 133 (25), 119 (28), 109 (37), 105 (52), 93 (49), 91 (56), 79 (47), 69 (44), 55 (39), and 41 (100%).

Desulphurisation of Episulphides.—The episulphide (3) (56 mg) in dry dibutyl ether (2 ml) was heated with triphenylphosphine (65 mg) at 130 °C for 40 min. G.c. examination of the product showed *ca.* 90% conversion into humulene (1).

The episulphide (5) (60 mg) in dibutyl ether (2 ml) was similarly treated with triphenylphosphine (150 mg) at 134 °C for 1 h. G.c. examination showed *ca.* 99% conversion into caryophyllene (2).

Treatment of the Episulphides with Lead(II) Acetate.—None of the episulphides (10 mg portions) gave any precipitate when shaken in ether (0.5 ml) with 20% lead(II) acetate in water (0.5 ml).

Treatment of the Episulphides with Mercury(II) Chloride.—Shaking the episulphide (10 mg) in ether (0.5 ml) with saturated aqueous mercury(II) chloride (0.5 ml) gave a dense white precipitate. That from the humulene episulphides was collected, washed with water and then cyclohexane, and dried under reduced pressure (P₂O₅) to give the bis[dichloromercury(II)] complex of each [Found: C,

22.9; H, 3.4. C₁₅H₂₄S·(HgCl₂)₂ requires C, 23.1; H, 3.1%].

Identification of Episulphides in Hop Oil.—A solution of steam-distilled hop oil¹¹ (25 μ l) in cyclohexane (0.5 ml) was subjected to g.c. analysis (5 μ l portions) with a Pye GCV gas chromatograph fitted with synchronous flame ionisation and flame photometric detectors. With a temperature programme of 50–200 °C at 3 °C min⁻¹, the retention times of 3 unknown sulphur compounds present were (a) 55.8, (b) 48.2, (c) 60.0 min on a column (2.8 m \times 4 mm) packed with 10% Carbowax 20 M on Chromosorb W AW DMCS 80–100 mesh at a flow rate of 52.6 ml min⁻¹, and correspondingly (a) 36.1, (b) 36.8, and (c) 37.7 min on a column (1.5 m \times 4 mm) packed with 3% OVI on Diatomite CQ 100–120 mesh at a flow rate of 51.7 ml min⁻¹. The 3 components were identified as (a) 4,5-epithiocaryophyllene, (b) 1,2-epithiohumulene, and (c) 4,5-epithiohumulene by g.c. peak enhancement, achieved by injecting in turn solutions prepared by mixing the hop oil solution (5 μ l) with a solution (1 μ l) of episulphide, the concentration of the latter in cyclohexane being adjusted such that, separately, the episulphide solution (1 μ l) gave a peak similar in height to that in the chromatogram of the original hop oil.

Different steam-distilled hop oils contained from 10 to 350 p.p.m. of each episulphide: atypically as much as 0.9% was found.

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