2,4,5-Trithiahexane from Photolysis of Dimethyl Disulfide

Irradiation of dimethyl disulfide with ultraviolet (UV) (2537 Å) light at room temperature for 16 h gave a 15% yield of 2,4,5-trithiahexane, a known component of cabbage.

Dimethyl disulfide occurs in a number of foods (cf. Schutte, 1974). The authors (Buttery et al., 1976) recently studied the volatile flavor components of cabbage, broccoli, and cauliflower confirming the previously found dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide but also characterizing for the first time the related unusual compound 2,4,5-trithiahexane ($CH_3SCH_2SSCH_3$). It seemed reasonable that the 2,4,5-trithiahexane could be formed from a free-radical reaction involving $CH_3S\cdot$ and CH_3SSCH_3 in the cabbage. The action of ultraviolet (UV) light on dimethyl disulfide is known to give the free radical $CH_3S\cdot$. However, in the several studies reported on the photolysis of dimethyl disulfide (e.g., Parker and Kharasch, 1959; Sayamol and Knight, 1968; Carlson and Knight, 1973; Inaba and Ogoro, 1969) the formation of 2,4,5-trithiahexane has not been reported. The authors undertook to reinvestigate the photolysis of dimethyl disulfide to specifically look for 2,4,5-trithiahexane.

EXPERIMENTAL SECTION

Dimethyl disulfide (1 ml, Eastman Organic Chemicals No. 1825) was placed in a Vicor tube under a nitrogen atmosphere and irradiated with quartz mercury arc UV lamps (2537 Å, total of 35 W) for 16 h. The product was analyzed directly by gas–liquid chromatography (GLC) using a 1 m × 0.63 cm o.d. aluminum column packed with 80–100 mesh Chromosorb P coated with 10% Tween 20, collecting the samples in 150 × 3 mm Pyrex glass tubes. The GLC conditions were column temperature 150 °C and flow rate 40 ml/min helium.

Mass Spectra. Samples collected from the GLC column were analyzed using batch introduction with a modified Consolidated 21-620 mass spectrometer. Ionization voltage was 70 V.

Authentic 2,4,5-Trithiahexane. This was synthesized by the reaction of CH_3SNa with $ClCH_2SCl$ using the method of Brintzinger and Schmahl (1954) as described previously by the authors (Buttery et al., 1976).

RESULTS AND DISCUSSION

Analysis of the products of the irradiation of dimethyl disulfide for 16 h by GLC separated mostly unchanged dimethyl disulfide together with a 15% yield of 2,4,5-trithiahexane whose mass spectrum (molecular ion 140, other important ions at 61, 45, 125, 108, and 94; cf. Buttery et al., 1976) and GLC retention time were consistent with that of the authentic sample.

There is not much dimethyl disulfide in raw cabbage and it is doubtful whether 2,4,5-trithiahexane could be formed by the action of sunlight on cabbage during the growing period. Dimethyl disulfide is chiefly produced when cabbage is cooked. The formation of 2,4,5-trithiahexane probably also occurs during the cooking. Schutte (1974) has outlined a likely mechanism for the formation of dimethyl disulfide in foods from the breakdown of the amino acid methionine to CH_3SH which is oxidized by atmospheric oxygen to CH_3SSCH_3. The formation of $CH_3S\cdot$ is probably part of this process and could react with dimethyl disulfide already formed to give the 2,4,5-trithiahexane present in cabbage.

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Patulin Content of Juice and Wine Produced from Moldy Grapes

Patulin is a mycotoxin produced by a number of fungal spoilage organisms, some of which may be present on moldy grapes. Juices and wines produced from Rauschling and Gamay grapes (moldy at harvest) and from Concord and V.53263 grapes (moldy after extended storage) were analyzed for patulin. Patulin was present in the juices but was not detected in a partially fermented juice (Gamay) and the wines. *Penicillium* spp. were the predominant microflora of samples of the Rauschling grapes, from which were isolated strains of *P. expansum* that produced patulin and citrinin.

The mycotoxin patulin (4-hydroxy-4*H*-furo[3,2-*c*]-pyran-2(6*H*)-one) is a metabolite of several *Aspergillus* and *Penicillium* spp. (Scott, 1974) and of *Byssochlamys fulva* and *nivea* (Escoula, 1975). Patulin occurs widely in commercial apple juice in North America (Stoloff, 1975) and this problem deserves attention as there is some in-

Table I. Processing and Storage Conditions of Grapes and Patulin Content of Their Products

Pressing method	Cultivar	Condition of fruit[a]	Wt of lot, kg	Storage conditions			Patulin concn, ppm			
							Juice			
				t, days	T, °C	R.H., %	Free flow	Press	Total[b]	Wine
Cold press	V.53263	M	55	12 + 15	1 + 8	97 + 85	0.38	0.33[c,d]	0.36	ND[e]
	(French hybrid)	M	55	12 + 15	1 + 8	97 + 85	0.60	0.38	0.50	
	Rauschling (*Vitis vinifera*)	M	43	15	1	97			0.72	ND
Hot press	Concord	M	48	21	8	85	3.8	4.5[d]	4.2	
	(*V. labrusca*)	M	51	21	8	85	1.0	0.88	0.94	ND
		M	48	21	8	85	1.5	1.4	1.5	
Hot enzyme press		M	51	21	8	85	3.2[c]	1.9[f]	2.7	ND
		M	53	21	8	85	1.0	1.1	1.1	
Crush	Gamay Beaujolais	M	23	2	1	97	0.03			
Skin fermentation	(*V. vinifera*)	M					ND	ND		ND
Crush		H	16	2	1	97	ND			
Skin fermentation		H					ND	ND		ND

[a] M = moldy; H = healthy. [b] Patulin concentration of total juice yielded was calculated for all but the Rauschling sample from the volume and patulin content of the free flow and press juice fractions. [c] Mean of duplicate analyses. [d] Identity of patulin confirmed by GC-MS. [e] ND = not detected. [f] Mean of triplicate analyses.

dication that patulin may be carcinogenic. Subcutaneous injection of 0.2 mg of patulin twice weekly into male rats for 61–64 weeks produced sarcomas at the site of injection (Dickens and Jones, 1961). The presence of patulin and patulin-producing strains of *Penicillium expansum* in naturally rotten apples has been amply demonstrated (Harwig et al., 1973a). *P. expansum* can also form patulin in inoculated peaches, pears, apricots, plums, and cherries (Buchanan et al., 1974; Lovett et al., 1974). Although *Byssochlamys* and patulin-producing strains of *P. expansum* have been isolated from grapes (Anslow et al., 1943; King et al., 1969; Sommer et al., 1974), no studies have been published on the potential of this fruit to support patulin production and hence give rise to patulin in processed grape juice. We have been able to demonstrate this potential by detecting appreciable amounts of patulin in juices prepared using different processing methods and made from four cultivars of spoiled, naturally infected grapes.

EXPERIMENTAL SECTION

Materials. The grapes were obtained from the experimental vineyard of the Horticultural Research Institute of Ontario. Extended storage was used to induce heavy mold growth on V.53263 and Concord grapes. Two *vinifera* cultivars, Rauschling and Gamay Beaujolais, harvested in 1975 were badly spoiled on the vine. Healthy Gamay was obtained by selecting the unspoiled clusters from the lot when they were received from the vineyard. Further information on the grapes and on the storage conditions is presented in Table I.

Juice from V.53263 and Rauschling grapes was prepared by cold pressing. Hot pressing was used to prepare Concord juice. The destemmed and crushed grapes were heated to 63 °C and held at that temperature for 30 min prior to pressing. The hot pressing with enzyme treatment was the same as hot pressing, except 1 g/50 kg of commercially available pectic enzyme preparation (Irgazyme-100, Ciba-Geigy) was added to the crushed grapes when the temperature reached 63 °C. Gamay juice was prepared from the destemmed and crushed grapes after 3 days of fermenting on the skin at 21 °C; juice samples were also taken right after crushing of the Gamay grapes.

A medium capacity hydraulic press was used for pressing. Two percent rice hull was mixed with the crushed grapes. The grape mass was packed into nylon press cloth. The packed "cheeses" were weighed down with wooden blocks (14.5 kg) and allowed to stand for 15 min. To press the material, the pressure was gradually raised to 70 kg/cm^2 in about 6 min. The juice released prior to and during pressing was labeled "free flow" and "press", respectively.

Grape, pomace, and juice samples were frozen at −27 °C until analysis.

Wine making followed generally accepted micro-vinification methods, except that the use of sulfur dioxide or its salts was avoided. A mixed wine yeast culture was used to initiate the fermentation which was carried out at 21 °C. The fermenting material was sufficiently chaptalized with cane sugar to reach 12% by volume alcohol content. The wine was racked twice and detartared by chilling. The wines were bottled and stored at 6 °C.

Analysis of Samples. Thawed juices and the wines were analyzed for patulin by the method of Scott and Kennedy (1973); emulsions were separated by centrifugation at 1500 rpm. In most cases, two-dimensional thin-layer chromatography (TLC), on silica gel or alumina layers using ethyl ether or chloroform–acetone (9:1, v/v, CA) followed by toluene–ethyl acetate–90% formic acid (5:4:1, v/v/v, TEF), was also employed for final analysis of wine extracts. Pomace samples from V.53263 grapes were drained and blotted dry and a 100-g subsample was extracted by blending 3 times with ethyl acetate; an aliquot equivalent to 50 g of pomace was added to the chromatographic column used in the method of Scott and Kennedy (1973). The concentration of patulin standard was 25 or 30 μg/ml. The presence of patulin in all positive juice samples was confirmed by TLC in the additional solvent systems ether and CA; addition of formic acid (5%) to the 3-methyl-2-benzothiazolinone hydrazone spray reagent improved sensitivity with these neutral solvents. Patulin isolated by preparative TLC from two juice extracts was further confirmed by gas chromatography–mass spectrometry of its trimethylsilyl ether, using a Perkin-Elmer 990 gas chromatograph (3 ft × 0.125 in. i.d. glass column packed with 3% OV-101 on 100–120 mesh Chromosorb WHP, operated at 150 °C following 6 °C/min program from 100 °C) coupled to a Hitachi Perkin-Elmer RMS-4 mass spectrometer (operated at 80 eV).

Samples of patulin-containing juices (50 ml) from 6 different lots of grapes were extracted with 75 ml of chloroform–ethyl acetate (2:1, v/v) for citrinin analysis.

Extracts were filtered through a pad of anhydrous sodium sulfate, evaporated to dryness under nitrogen, and examined by TLC on silica gel D-B (Camag). Chromatograms developed in solvent system TEF were examined under long-wave ultraviolet light.

Mycological Examination. Thawed grapes of the Rauschling cultivar were quartered and the quarters plated on each of the following agar media: potato dextrose, potato dextrose acidified to pH 3.5, tomato juice, and malt extract. This was also done for pieces of thawed, crushed, moldy, and healthy Gamay grapes. Plates were examined periodically over 2–3 weeks and the most predominant molds identified by genus. Some of the green *Penicillium* spp. that grew from grape tissue resembled *Penicillium expansum* when examined visually, and after single spore isolation were cultured on a semi-micro scale (Scott et al., 1970) in order to test for patulin and citrinin production. Patulin was detected by TLC with each of the solvent systems TEF and CA using phenylhydrazine hydrochloride as spray reagent (Scott and Somers, 1968). For detection of citrinin, chloroform extracts were extracted with 1.25% sodium bicarbonate solution, which was then acidified and reextracted with chloroform; TLC plates were developed in ethyl acetate–acetone–water (5:5:2, v/v/v) (Hald and Krogh, 1973) and TEF.

RESULTS AND DISCUSSION

Patulin was detected in juices produced from moldy grapes of four cultivars in concentrations ranging from 0.03 to 4.5 ppm (Table I). No significant difference was found in patulin content of free flow or press juices obtained from a given lot of moldy grapes (V.53263 or Concord), although with the Concord juices there was some variation between lots. Of particular interest is the observation that high concentrations of patulin can survive the hot pressing treatment. Furthermore, the addition of pectolytic enzyme during this hot pressing of the Concord grapes did not result in destruction of patulin. Traces of patulin appeared to be present at the absolute limit of detection (about 0.06 ppm, solvent system TEF) in pomaces remaining after cold pressing of the 2 lots of V.53263 grapes.

Patulin was not detected in the wines, for which detection limits were of the order of 0.03 ppm, nor in the partially fermented Gamay juice (<0.02 ppm) (Table I). This suggests that patulin is destroyed or converted into other compound(s) during fermentation. Similar observations were reported by Harwig et al. (1973b). They found that added patulin rapidly disappeared from apple cider during alcoholic fermentation. Pohland and Allen (1970) demonstrated that patulin is unstable in the presence of SO_2. The addition of SO_2 or its compounds is standard practice in commercial wine making (Amerine and Joslyn, 1970). To prevent the destruction of patulin, SO_2 was not added in the present study at any stage of the wine making process. However, SO_2 is formed during fermentation from sulfate naturally present in grape juice (Wurdig and Schlotter, 1967; Eschenbruch, 1974). Wurdig and Schlotter (1967) found that 13 to 114 ppm (average 47 ppm) of SO_2 was produced in 20 fermentations. Sulfite formation during fermentation and the instability of patulin in the presence of SO_2 could explain the absence of patulin in wine, cider, and partially fermented grape juice. The fate of patulin in wine is not known. The possibility exists that the modified compound is still toxic; therefore, moldy fruits should not be used for wine (or cider) making.

The mycological examination showed that in the Rauschling grapes, which had been stored at 1 °C for 15 days, green *Penicillium* spp. were the predominant component of the microflora. Twenty to sixty-five percent of the pieces of grape plated on the four media showed growth of these *Penicillium* spp. only. *Byssochlamys* spp. were not detected. Five of six single spore isolates taken from colonies visually resembling *P. expansum* produced patulin and citrinin. *P. expansum* strains from apple have previously been reported to produce both these mycotoxins (Harwig et al., 1973a). Two of these six isolates were further examined for more definitive identification. They were strongly pathogenic to apple and morphologically resembled *P. expansum* as described by Raper and Thom (1968). It is concluded that *P. expansum* is likely to have been the mold responsible for the presence of patulin in grape juice made from the Rauschling grapes. Since the grapes were not examined immediately after harvest, it is not possible to say whether the patulin was formed on the vine or during storage at 1 °C. Blue mold rot due to *Penicillium* spp. is known to sometimes affect grapes before harvest (Harvey and Pentzer, 1960). On the crushed healthy and moldy Gamay grapes, *Penicillium* spp. occurred as commonly as in the Rauschling grapes but they always grew in the presence of other molds such as *Rhizopus*, *Cladosporium*, *Botrytis*, *Aspergillus niger*, and yeasts.

Citrinin did not accompany patulin in the two V.53263, three Concord, and one Rauschling grape juice samples analyzed for this mycotoxin. The detection limit was approximately 0.02 ppm. Recoveries of citrinin added at 1 ppm to partially fermented juice from healthy Gamay grapes, which was then analyzed immediately, were estimated to be 60 and 100% in 2 experiments. It is possible that citrinin, if formed, is unstable in grape juice, as in apple juice (Harwig et al., 1973a), but stability studies were not carried out.

In conclusion, we have demonstrated that grapes are capable of supporting patulin production by natural fungal populations, resulting in the presence of patulin in the processed juice. If such moldy grapes are included in the manufacture of grape products, the presence of patulin, particularly in view of its appreciable stability in grape juice (Scott and Somers, 1968), would constitute a potential health hazard.

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