

176. 1-*p*-Menthene-8-thiol¹): A Powerful Flavor Impact Constituent of Grapefruit Juice (*Citrus paradisi* MACFAYDEN)

by Edouard Demole, Paul Enggist and Günther Ohloff

Firmenich SA, Research Laboratories, CH-1211 Geneva 8

(9.VI.82)

Summary

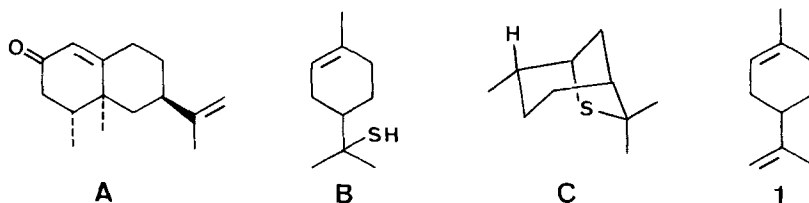
1-*p*-Menthene-8-thiol (**B**) is shown to be a potent character-donating constituent of grapefruit juice, in which it occurs at the ppb-level or below. A convenient synthesis is described for this terpene-thiol, apparently the most powerful flavor compound ever found in nature.

The peel and juice oils from *Citrus spp.* are important flavoring ingredients for the food and beverage industries [1]. Considerable research work has already been done in order to identify many individual constituents of these natural flavors. Since about 1950, both cold-pressed oil and juice of grapefruit (*Citrus paradisi* MACFAYDEN) have been investigated and a total of some 126 volatile constituents were characterized [2] (not including less volatile components such as coumarins [3], sugars, hydroxy-acids, and other water-soluble substances which do not contribute substantially to the specific grapefruit flavor). Among the volatiles identified, nootkatone (**A**) [4] is considered as a primary flavor-impact compound in grapefruit, although it also occurs in most other *Citrus spp.* Recently, however, authors have questioned the true flavoring importance of **A**, even whether or not this ketone is necessary for good grapefruit flavor [5] [6]. In any case, there is no doubt that still unidentified compounds co-occur with nootkatone (**A**) in grapefruit flavor and contribute importantly to the flavor and aroma of grapefruit oil and juice [5-7].

We now report that a prominent and unexpected representative of these hitherto unknown flavor components is 1-*p*-menthene-8-thiol (**B**) [8]. When adequately diluted, this novel, powerful flavor-impact compound displays a genuine, unmistakable aroma of *fresh grapefruit juice* (in which it naturally occurs at or below the ppb-level). The identification of **B** practically settles the long-standing problem of synthetically reproducing the full-bodied flavor of fresh grapefruit juice. Other previously identified S-compounds in grapefruit were hydrogen sulfide [9], carbonyl sulfide [10], methyl sulfide [10], methanediol [10], and sulfur dioxide [10]. Also, a series of bicyclic sulfides related to *p*-menthane were reported to occur in grapefruit

¹) Patent applications relative to the use of 1-*p*-menthene-8-thiol (**B**) have been filed in several countries by Firmenich SA, Geneva (Priority: 23.12.1980).

peel oil [11]. Unfortunately, no rigorous structural proof was provided for the latter substances which bear a close resemblance to 2,8-*epithio-cis-p-menthane* (C) [12a] and to the well-known bicyclic terpene sulfides formed by reaction of elemental sulfur with limonene (1) at elevated temperatures [12].



1. Preparation²⁾ and fractionation of the volatile flavor of grapefruit juice. – About 100 l of commercial canned grapefruit juice³⁾ were subjected to concurrent steam-distillation/heptane⁴⁾-extraction under 100 Torr (b.p. (H₂O) ≈ 52°; b.p. (C₇H₁₆) ≈ 42°) in a *Likens-Nickerson* apparatus [13]. The main flask of this apparatus was large enough to permit treatment of 16–17 l batches of juice for 24 h each time; cooling of the condenser was efficiently ensured by satd. NaCl-solution at –5°. The resulting crude heptane extract (1 l), carefully dried over MgSO₄ and evaporated at 50°/30 Torr, left 7.7 g (0.0077% by weight of starting grapefruit juice) of a concentrate having quite satisfactory organoleptic characteristics⁵⁾. Chromatography of this concentrate on silica gel afforded five fractions, one of which (Fr. 2: 0.165 g, see *Exper. Part*) exhibited a peculiar *sulfurous* odor typical of grapefruit juice. This fraction was examined by capillary GC. (*Carbowax 20 M*, 80–160°, +3.5°/min, 53 m × 0.3 mm column), using a multi-detector instrument⁶⁾ with which it was possible to simultaneously ‘sniff’ the column effluent and selectively detect the N-, P- and S-containing compounds. It was thus observed that the ‘sulfurous odor’ emerged sharply in a practically empty part of the chromatogram, giving rise to a concomitant but very weak response of the S-detector. Essentially the same result (*Fig. 1*) could be attained by semipreparative GC. on packed silicone oil columns previously ‘deactivated’ by treatment with dimethylaminoethanol and ‘Silyl-8’⁷⁾, using temperatures not exceeding 200° at any point of the (gas + vapor)-path (omission of these precautions led in general to complete disappearance of the ‘sulfurous odor’). Sub-fraction 2 H from this separation (*Fig. 1*) was further separated into four sub-groups of constituents (2 Ha–2 Hd) under more efficient GC. conditions. Sub-group 2 Hd, finally examined by GC/MS. coupling, proved to be a mixture of 8-ethoxy-1-*p*-menthene, geranyl

2) This preparation was carried out at our Pilot laboratory under the direction of Dr. A. F. Boschung, to whom we are grateful.

3) ‘Libby’s’, produce of Swaziland.

4) *Puriss.* (*Fluka AG*, Buchs, Switzerland). Very pure solvents were used throughout this work.

5) We thank Dr. P. Dietrich and Messrs. A. Y. Smith and F. Benzi (*Firmenich SA*, Geneva) for having carried out this and many other sensory evaluations.

6) *Fractovap 2900*, *Carlo Erba*, conveniently modified by Drs. I. Flament and E. Paluy (*Firmenich SA*, Geneva) according to *Hřivnáč et al.* [14].

7) *Pierce Chemical Company*, Rockford, Ill., U.S.A.

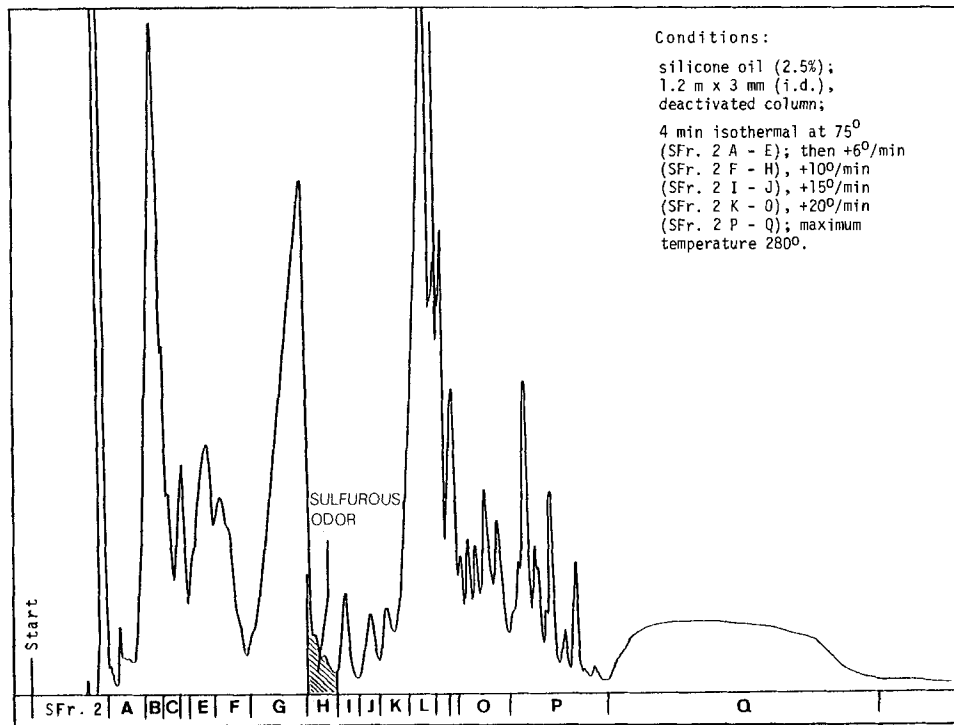


Fig. 1. Semi-preparative GC. separation of Fr. 2 into sub-fractions 2A-2Q

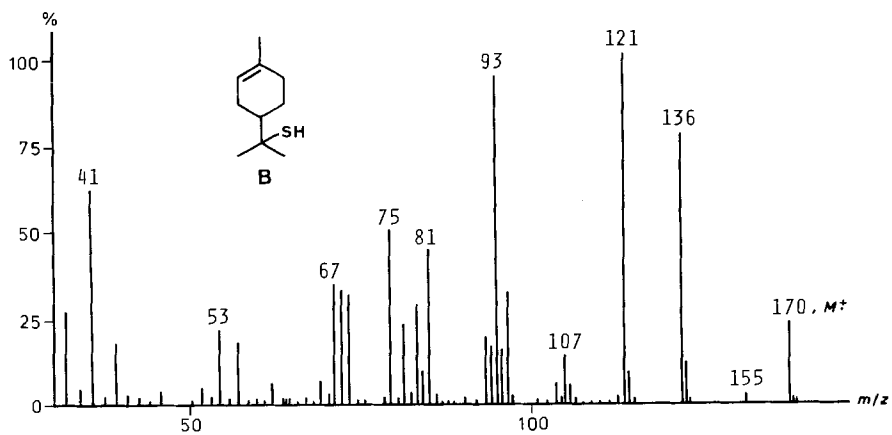


Fig. 2. Mass spectrum of 1-p-menthene-8-thiol (B)

ethyl ether, vitispiranes [15]⁸) (2 stereoisomers), *2endo*- or *2exo*-bornyl acetate, and propyl octanoate. However, beside these major components, there were minute amounts of two unknown compounds, in fact **B** and **C**, which clearly contained one S-atom as evidenced by the ($M^+ + 2$) ions at m/z 172 in their mass spectra (Fig. 2 and 3).

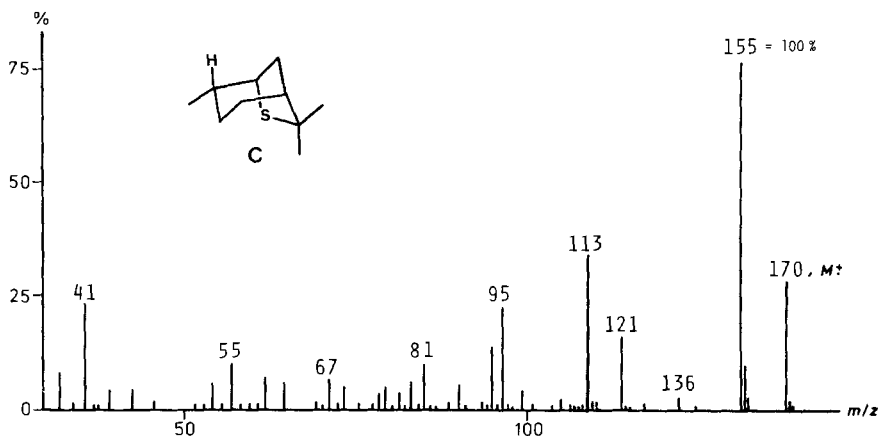
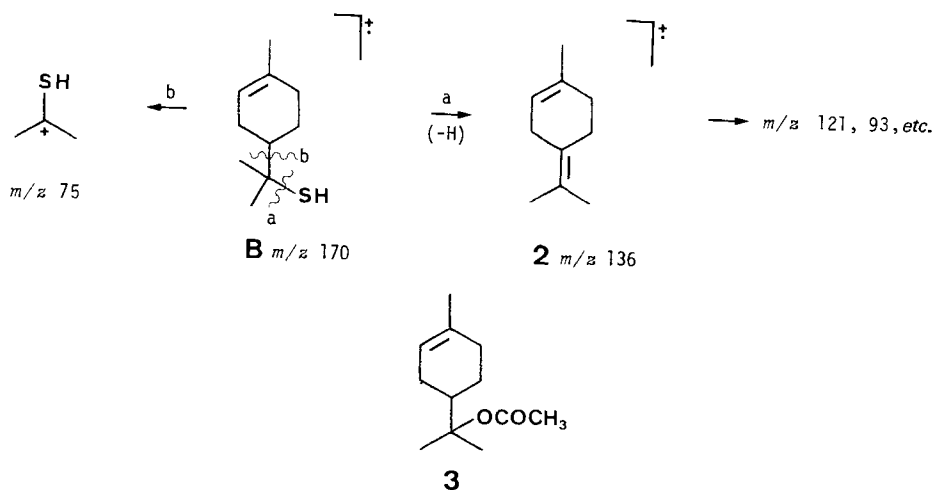


Fig. 3. Mass spectrum of 2,8-epithio-cis-p-menthane (**C**)

2. Structure elucidation and synthesis of 1-p-menthene-8-thiol (B). – A clue to the structure of compound **B** was given by its mass spectrum which exhibits three noticeable features (Fig. 2). First, there is an easy loss of 34 mass units (H_2S or equivalent) leading to the large m/z 136 ion. Second, this and the two other prominent ions at m/z 121 and 93 have practically the same relative abundances (0.77:1.00:0.97) as in the mass spectrum of terpinolene (**2**) [16]. Third, the peak at m/z 75, quite unexpected for a monoterpene [16], is best accounted for by the fragment $(CH_3)_2\dot{C}SH$. As shown in Scheme 1, structure **B** would represent the simplest possibility to reconcile these data. This interpretation was substantiated by the fact that the large peaks at m/z 136, 121 and 93 in the mass spectrum of α -terpinyl acetate (**3**) are virtually identical to those of **B**. To our knowledge, 1-p-menthene-8-thiol (**B**) has been described only once, in a Russian work dealing with the synthesis of thiols by addition of hydrogen sulfide to olefins catalyzed by alkylaluminum dichlorides [8]. However, judging from the scarce data given, this process leads to a low yield of impure **B** when it is applied to limonene (**1**). We devised a more efficient synthetic procedure shown in Scheme 2. Reaction of racemic 8,9-epoxy-1-p-menthene (**4**) [17] with thiourea [18] followed by alkaline treatment of the resulting thiuronium salt **5** afforded 8,9-epithio-1-p-menthene (**6**), the reduction of which with $LiAlH_4$ in refluxing THF proceeded quite regioselectively to give 98% pure (\pm)-1-p-menthene-8-thiol (**B**) (overall yield 41%). The

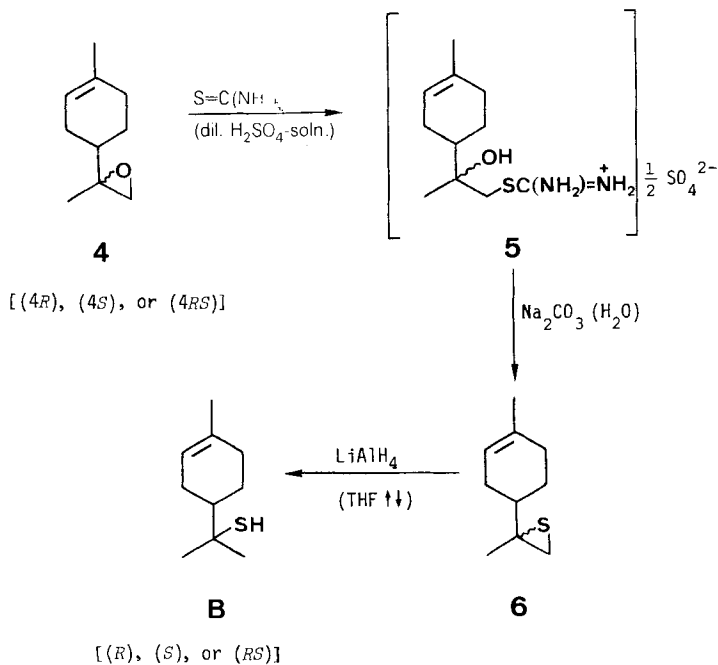
⁸) Formerly identified in grapefruit by Dr. A. F. Thomas (Firmenich SA, Geneva, personal communication).

Scheme 1. Major fragmentation modes of **B** under electron impact



(+)-(R)- and (-)-(S)-**B**, with estimated optical purities of 97 and 77%, respectively, were synthesized in the same way from optically active **4**; this was itself derived from both enantiomeric limonenes (**1**) via known procedures [17] [19] [20]. Synthetic and

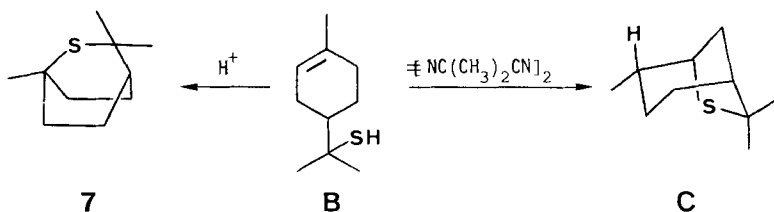
Scheme 2. Synthesis of 1-p-menthene-8-thiol (**B**) and its enantiomers



natural **B** had identical mass spectra (*Fig. 2*) and t_R (capillary GC.) but, at present, no specific rotation data is available for the latter⁹⁾. Pure, liquid **B** smells extremely powerful and nauseous.

3. Preparation of 2,8-epithio-*cis-p*-menthane (C**) from 1-*p*-menthene-8-thiol (**B**).** – The structure of natural **C**, isolated as described above, could not be deduced directly from the meagre spectral data available (mass spectrum only, *Fig. 3*). Fortunately, this frustrating situation was resolved when we found that 1-*p*-menthene-8-thiol (**B**) cyclizes easily either under radical or protic conditions, and that the known 2,8-epithio-*cis-p*-menthane [12a] formed in the former process¹⁰⁾ was identical to **C** (MS., t_R on capillary GC.) (*Scheme 3*). The relative configuration of **C** ([*endo* CH₃-C(1)]) (*p*-menthane numbering), firmly established [12a], implies that the radical cyclization of **B** proceeds stereoselectively *via* antiperiplanar, anti-*Markownikoff* addition of the thiol moiety to the double bond. Analogous, intermolecular addition reactions of thiols are known to exhibit the same type of selectivity [21]. The particular ease of the radical cyclization **B** → **C** explains why these two compounds co-occur in grapefruit juice (we found a **B**/**C**-ratio close to 4:1). This reaction takes place spontaneously at room temperature in the presence of light, usually after a variable induction period. It can be efficiently inhibited, however, by small concentrations (0.1%) of usual antioxidants like 'BHA' (2-*t*-butyl-4-methoxyphenol). Under protic conditions, the cyclization of **B** obeys the *Markownikoff* rule and affords 1,8-epithio-*p*-menthane (**7**) [12] (*Scheme 3*).

Scheme 3. Cyclization reactions of 1-*p*-menthene-8-thiol (**B**)



4. Organoleptic properties of 1-*p*-menthene-8-thiol (B**) and its bicyclic isomer **C**.** – The conspicuous organoleptic properties of **B** had been noticed early in our separation work, well before this compound could be actually isolated from grapefruit flavor and identified. Subsequently, these observations were fully confirmed by

⁹⁾ A reasonable assumption is that **B** could be biosynthetically related to (+)-(*R*)-limonene (**1**) which constitutes, in a state of high optical purity, > 90% of grapefruit oil. Accordingly, **B** most probably occurs as its (+)-(*R*)-enantiomer in nature.

¹⁰⁾ Alternative disulfide formation does not seriously compete with the radical cyclization of **B**, although this oxidative dimerization takes place under particular conditions. Clearly, both steric hindrance around the tertiary thiol function and intramolecularity favor the cyclization of **B**.

sensory evaluations¹¹⁾ which showed the taste detection threshold¹²⁾ of synthetic, racemic **B** to be slightly lower than 1.10^{-4} ppb in water (this corresponding to a concentration of 1.10^{-4} mg of **B** in one metric ton of water). For (+)-(*R*)- and (–)-(*S*)-**B**, the respective values found were 2.10^{-5} and 8.10^{-5} ppb. To the best of our knowledge, these detection thresholds are the lowest ever recorded for a naturally occurring flavor compound [24]. Qualitatively, there is no significant difference between the organoleptic properties of racemic and optically active **B**: while the (–)-(*S*)-enantiomer appears to have a somewhat more fruity and natural aroma than its antipode, (\pm)-**B** behaves just like a mixture of both forms. Regarding the taste detection threshold of 2,8-epithio-*cis-p*-menthane (**C**), it was found to be as high as 9 ppb in water. This value, about 10^5 times greater than that of **B**, indicates that the contribution of **C** to the overall profile of grapefruit flavor should be negligible.

Finally, we also synthesized a number of thiols structurally related to **B** (isomers, saturated analogs, etc.). None of these exhibited organoleptic properties matching those of 1-*p*-menthene-8-thiol (**B**), which appears to be a unique flavor compound.

Experimental Part

General. – Specific rotations $[\alpha]_D^{20}$ were measured in a 0.1 dm tube on a *Perkin-Elmer Model 141* polarimeter. The infrared (IR.) spectra (neat liquids) were taken on a *Perkin-Elmer 720* instrument; characteristic absorption bands are given in cm^{-1} . $^1\text{H-NMR}$. spectra were recorded on a *WH 360 Bruker* instrument, using CDCl_3 as solvent. Chemical shifts are expressed in ppm (δ -scale) downfield from TMS as internal standard; abbreviations: *s*=singlet, *d*=doublet, *m*=multiplet, *J*=spin-spin coupling constant (Hz). Mass spectra (MS.) were measured at 70 eV on an *Atlas CH 4* spectrometer, inlet temperature 130–150°; the molecular ions (M^+) and fragment ions are given as *m/z* with relative peak intensities in % of the base peak. Gas chromatography/MS. data (GC./MS.) were obtained using a gas chromatograph *Carlo Erba, Model 2101 AC (UCON HB 50 5100, 60-180°, 50 m × 0.3 mm glass column)*, coupled to a *Varian MAT 112* or a *Finnigan 4023-c* mass spectrometer. Unless otherwise stated, GC. was performed on *Varian Aerograph (Models 1820-3 and 2720-3)* and *Carlo Erba (Model 2301 AC)* gas chromatographs; carrier gas: He. The liquid-solid chromatographic separation was carried out on 0.05–0.2 mm silica gel for column chromatography (*Merck AG, Art. No. 7734*).

1. Fractionation of volatile grapefruit flavor. – The crude extract (7.7 g) resulting from the concurrent steam-distillation/heptane-extraction [13] of about 100 l of grapefruit juice³⁾ (see general part) was chromatographed as follows on silica gel (250 g):

Fr. 1	hexane/ether 100:0 to 95:5	4.910 g	
Fr. 2	hexane/ether 95:5	0.165 g	(sulfurous odor)
Fr. 3	hexane/ether 94:6 to 92:8	0.210 g	
Fr. 4	hexane/ether 90:10 to 20:80	2.230 g	
Fr. 5	ether/methanol 100:0 to 85:15	0.100 g	
		<u>7.615 g</u>	

¹¹⁾ We are very grateful to Dr. *W. Pickenhagen* and Mr. *A. Furrer (Firmenich SA, Geneva)* for having carefully evaluated our compounds with the cooperation of a tasting panel of 14–20 assessors. The detection thresholds were determined by the ‘triangular tests’ [22] and ‘multiple pairs test’ [23] procedures.

¹²⁾ Taste detection threshold: the lowest concentration at which the taste of a substance is detected, but not necessarily recognized.

Fr. 2 was first separated into 17 sub-fractions (2A to 2Q) by semipreparative GC. as indicated in Fig. 1. More efficient GC. conditions (silicone oil¹³) 5%, 140°, 2.5 m column previously deactivated by treatment with dimethylaminoethanol and *Silyl-8*⁷) then allowed further partition of sub-fraction 2H into four sub-groups of constituents, 2Ha to 2Hd. The sub-group 2Hd, selected for final study by GC./MS. coupling, was found to contain minute amounts ($\leq 0.5\%$) of both S-compounds B and C, characterized by their mass spectra (Fig. 2 and 3). The other, major constituents of the mixture were more common substances (see general part).

2. Synthesis of (\pm)-1-p-menthene-8-thiol (B) (Scheme 2). – 2.1. [18]. Preparation of 8,9-epithio-1-p-menthene (6). (\pm)-8,9-Epoxy-1-p-menthene (4) [17] (17.65 g, 0.116 mol) was added dropwise over 1 h at 5° to a stirred mixture of thiourea (8.82 g, 0.116 mol), water (40 ml), and conc. sulfuric acid (3.48 ml, 0.06 mol). After 10 min further stirring at 5° and 14 h at 20°, the mixture was diluted with 20 ml of water and cooled to 0°. The precipitated crystals, collected, washed with water and dried at 40°/0.01 Torr, represented 30.66 g (95%) of thiuronium salt 5, m.p. 159–160° (uncorrected). A solution of Na₂CO₃ (11.66 g, 0.11 mol) in water (60 ml) was added dropwise over 1 h at 25° to a stirred mixture of 5 (30.66 g, 0.11 mol) with water (430 ml). After 20 min further stirring at 20–25° and 15 min at 50°, the mixture was extracted with pentane (3 \times) and the combined organic layers washed with water (3 \times). Distillation of the crude product (15.16 g) obtained after usual workup gave 10.67 g (57%) of 8,9-epithio-1-p-menthene (6), b.p. 43°/0.001 Torr; $d_4^{20} = 0.991$; $n_D^{20} = 1.522$. – IR.: 1435, 1060, 1380, 920, 795, 805. – ¹H-NMR.: 1.50 and 1.52 (2 s, total 3 H¹⁴); 1.64 (s, 3 H); 1.30–1.60 (m, 2 H); 1.85 (m, 1 H); 2.00 (m, 4 H); 2.34, 2.37, 2.40 and 2.43 (4 s, total 2 H¹⁴); 5.37 (m, 1 H). – MS.: 168 (~ 0 , M⁺, C₁₀H₁₆S), 136 (20), 121 (17), 94 (19), 93 (54), 79 (21), 68 (100), 67 (45), 53 (17).

C₁₀H₁₆S (168.29) Calc. C 71.39 H 9.59% Found C 71.43 H 9.61%

2.2. Preparation of (\pm)-1-p-menthene-8-thiol (B). A solution of 6 (1.00 g, 5.95 mmol) in anhydrous THF (20 ml) was added dropwise, under N₂, to a stirred slurry of LiAlH₄ (0.114 g, 3.00 mmol) in refluxing THF (5 ml). After 1 h further stirring/refluxing, water (6 ml) was cautiously added at 0°. The mixture was stirred for 10 min, 'stabilized' by addition of 5 mg of BHA (2-*t*-butyl-4-methoxyphenol), extracted with ether (3 \times), and the combined organic layers washed twice with satd. NaCl-solution. Usual workup followed by distillation of the crude product (to which a further 5 mg portion of BHA had been added) afforded 0.767 g (76%) of (\pm)-1-p-menthene-8-thiol (B), b.p. 40°/0.001 Torr (98% pure according to capillary GC.); $d_4^{20} = 0.948$; $n_D^{20} = 1.503$. – IR.: 1450, 1365, 1385, 1130, 1155, 800. – ¹H-NMR.: 1.35 (s, 3 H); 1.42 (s, 3 H); 1.57 (s, 1 H); 1.2–1.6 (m, 2 H); 1.66 (s, 3 H); 1.8–2.2 (m, 5 H); 5.39 (m, 1 H). – MS.: see Figure 2.

C₁₀H₁₈S (170.30) Calc. C 70.54 H 10.66% Found C 70.22 H 10.49%

Table. Specific rotations of limonene (1) enantiomers¹⁵) and derived compounds 4, 6 and B

	$[\alpha]_D^{20}$ (R)-	(S)-	Values corrected to 100% optical purity ^{a)}
Limonene (1)	+ 120.5° (neat)	– 96.4° (neat)	$\pm 123.8^{\circ b}$
8,9-Epoxy-1-p-menthene (4) ^{c)}	+ 91.6° (<i>c</i> = 1.54, CHCl ₃)	– 72.9° (<i>c</i> = 1.66, CHCl ₃)	$\pm 93.8^\circ$
8,9-Epithio-1-p-menthene (6) ^{c)}	+ 56.4° (<i>c</i> = 1.72, CHCl ₃)	– 46.6° (<i>c</i> = 1.48, CHCl ₃)	$\pm 58.8^\circ$
1-p-Menthene-8-thiol (B)	+ 79.8° (<i>c</i> = 1.44, CHCl ₃)	– 63.9° (<i>c</i> = 1.91, CHCl ₃)	$\pm 82.0^\circ$

^{a)} Assuming that no epimerization occurred throughout the synthesis. ^{b)} Mean of the values reported for (+)-(R)- and (–)-(S)-limonenes (1) [26]. ^{c)} As mixtures of diastereoisomers (4R,8RS) and (4S,8RS), respectively.

¹³) 'Embaphase', May & Baker, Ltd., Dagenham, England.

¹⁴) Splitting corresponding to a mixture ($\approx 45:55$) of both diastereoisomers of 6 (capillary GC., UCON HB 505100, 100°, 50 m \times 0.3 mm column).

¹⁵) Fluka AG, Buchs, Switzerland, Art. No. 62120 and 62130.

3. Synthesis of (+)-(R)- and (-)-(S)-1-p-menthene-8-thiols ((+)- and (-)-B). – Both enantiomers of **B** were prepared by applying the preceding synthetic procedure (*Scheme 2*) to the optically active 8,9-epoxy-1-p-menthenes (**4**). The latter, resulting from the direct epoxidation [19] of (+)-(R)- and (-)-(S)-limonenes (**1**)¹⁵ under *Payne's* conditions [25], were thus respectively converted into (+)-(R)- and (-)-(S)-**B** via their epithio-derivatives (+)- and (-)-**6**. The specific rotations measured throughout this process in both enantiomeric series are given in the *Table*, together with corresponding values corrected to 100% optical purity.

4. Cyclization of 1-p-menthene-8-thiol (B) under radical conditions [27] (\rightarrow 2,8-epithio-*cis*-p-menthane (**C**)). – A mixture of **B** (0.170 g, 1 mmol), *a,a'*-azo-isobutyronitrile¹⁶ (0.082 g, 0.5 mmol) and 1 ml hexane was stirred for 2 h at 70° under N₂. The cooled mixture, quenched with water, was extracted with hexane (3 \times) and the combined organic layers washed to neutrality. Distillation of the crude product resulting from usual workup afforded 110 mg (65%) of 2,8-epithio-*cis*-p-menthane (**C**) [12a], b.p. \sim 40°/0.001 Torr (91% pure according to capillary GC.), which was finally purified by semi-prep. GC. (*Carbowax 20 M* 15%, 160°, 2.5 m column). – IR.: 1450, 1365, 1155, 1295, 1100. – ¹H-NMR.: 0.77 (*d*, *J* = 6.5, 3 H); 1.40 (*s*, 3 H); 1.43 (*s*, 3 H); 1.20–1.50 (*m*, 2 H); 1.50–1.65 (*m*, 1 H); 1.70–1.80 (*m*, 2 H); 1.83 (*m*, 2 H); 2.40 (*m*, 1 H); 3.27 (pseudo *d*, *J* \sim 5, 1 H). – MS.: see *Figure 3*.

5. Cyclization of 1-p-menthene-8-thiol (B) under protic conditions (\rightarrow 1,8-epithio-p-menthane (7)). – A solution of **B** (0.100 g, 0.59 mmol) and *p*-toluenesulfonic acid (0.050 g) in anhydrous toluene (10 ml) was stirred for 1 h at 70° under N₂. The mixture taken up in ether was washed with 5% KOH-solution (3 \times) and water (2 \times). Usual workup gave 0.094 g of crude **7** [12] (97% pure according to capillary GC.), which was purified by semi-prep. GC. (*Carbowax 20 M* 15%, 150°, 2.5 m column). – IR.: 1450, 1070, 1220, 1350, 1140, 1190. – ¹H-NMR.: 1.10 (*s*, 3 H); 1.37 (*s*, 6 H); 1.40–1.65 (*m*, 5 H); 1.77 (*m*, 2 H); 2.04 (*m*, 2 H). – MS.: 170 (87, M⁺, C₁₀H₁₈S), 155 (100), 136 (32), 121 (53), 97 (42), 95 (41), 87 (26), 81 (22), 41 (39).

REFERENCES

- [1] *R. J. Braddock & J. W. Kesterson*, *J. Food Sci.* **41**, 1007 (1976).
- [2] 'Volatile Compounds in Food', Suppl. 6 (1981), pp. 5.9 and 5.10, edited by *S. van Straten et al.*, Division for Nutrition and Food Research TNO, Zeist, The Netherlands.
- [3] *J. F. Fisher & H. E. Nordby*, *J. Food Sci.* **30**, 869 (1965) and *Tetrahedron* **22**, 1489 (1966); *J. F. Fisher, H. E. Nordby, A. C. Waiss, jr. & W. L. Stanley*, *Tetrahedron* **23**, 2523 (1967); *R. W. Wolford, J. W. Kesterson & J. A. Attaway*, *J. Agric. Food Chem.* **19**, 1097 (1971); *W. L. Stanley & L. Jurd*, *J. Agric. Food Chem.* **19**, 1106 (1971); *J. H. Tatum & R. E. Berry*, *Phytochemistry* **18**, 500 (1979).
- [4] *W. D. MacLeod & N. M. Buigues*, *J. Food Sci.* **29**, 565 (1964); *W. D. MacLeod*, *Tetrahedron Lett.* **1965**, 4779.
- [5] *P. E. Shaw & C. W. Wilson, III*, in 'Citrus Nutrition and Quality' (ACS Symposium Series 143), p. 181, edited by *S. Nagy & J. A. Attaway*, American Chemical Society, Washington D.C. 1980.
- [6] *P. E. Shaw & C. W. Wilson, III*, *J. Agric. Food Chem.* **29**, 677 (1981).
- [7] *K. L. Stevens, D. G. Guadagni & D. J. Stern*, *J. Sci. Food Agric.* **21**, 590 (1970).
- [8] *G. A. Tolstikov, F. Ya. Kanzafarov, Yu. A. Sangalov & U. M. Dzhemilev*, *Neftekhimiya* **19** (3), 425 (1979); *Chem. Abstr.* **91**, 107252q (1979).
- [9] *J. G. Kirchner, R. G. Rice, J. M. Miller & G. J. Keller*, *Arch. Biochem. Biophys.* **25**, 231 (1950).
- [10] *P. E. Shaw, J. M. Ammons & R. S. Braman*, *J. Agric. Food Chem.* **28**, 778 (1980); *P. E. Shaw & S. Nagy*, in 'The Quality of Foods and Beverages. Chemistry and Technology', Vol. 1, p. 361, edited by *G. Charalambous & G. Inglett*, Academic Press, New York 1981.
- [11] *S. R. Srinivas*, 13th ACS Middle Atlantic Regional Meeting, Agric. and Food Chem. Div., March 1979, paper No 7.
- [12] a) *A. W. Weitkamp*, *J. Am. Chem. Soc.* **81**, 3430 (1959); b) *ibid.*, pp. 3434, 3437; c) *C. G. Moore & M. Porter*, *Tetrahedron* **6**, 10 (1959).

¹⁶) *Fluka AG*, Buchs, Switzerland, Art. No. 11630.

- [13] *G. B. Nickerson & S. T. Likens*, *J. Chromatogr.* **21**, 1 (1966).
- [14] *M. Hřivnač, W. Frischknecht & L. Čechová*, *Analytical Chem.* **48**, 937 (1976) and references cited therein.
- [15] *R. F. Simpson, C. R. Strauss & P. J. Williams*, *Chem. and Ind.* **1977**, 663; *K. H. Schulte-Elte, F. Gautschi, W. Renold, A. Hauser, P. Fankhauser, J. Limacher & G. Ohloff*, *Helv. Chim. Acta* **61**, 1125 (1978).
- [16] *A. F. Thomas & B. Willhalm*, *Helv. Chim. Acta* **47**, 475 (1964).
- [17] *F. L. Malanco & L. A. Maldonado*, *Synthetic Commun.* **6**, 515 (1976).
- [18] *F. G. Bordwell & H. M. Andersen*, *J. Am. Chem. Soc.* **75**, 4959 (1953).
- [19] *G. Farges & A. Kergomard*, *Bull. Soc. Chim. Fr.* **1969**, 4476.
- [20] *W. Knöll & C. Tamm*, *Helv. Chim. Acta* **58**, 1162 (1975).
- [21] *H. H. Szmant & J. Baeza H.*, *J. Org. Chem.* **45**, 4902 (1980); *C. Walling & E. S. Huyser*, in 'Organic Reactions', Vol. 13, pp. 173–179, edited by R. Adams *et al.*, John Wiley & Sons, Inc., New York, London 1963.
- [22] *P. Salo*, *J. Food Sci.* **35**, 95 (1970) and references cited therein.
- [23] *D. G. Guadagni, R. G. Buttery & S. Okano*, *J. Sci. Food Agric.* **14**, 761 (1963); *D. G. Guadagni, V. P. Maier & J. G. Turnbaugh*, *J. Sci. Food Agric.* **24**, 1277 (1973).
- [24] *G. Ohloff*, in 'Progress in the Chemistry of Organic Natural Products', Vol. 35, p. 431, edited by W. Hertz, H. Grisebach & G. W. Kirby, Springer-Verlag, Wien, New York 1978.
- [25] *G. B. Payne, P. H. Deming & P. H. Williams*, *J. Org. Chem.* **26**, 659 (1961).
- [26] 'Handbook of Chemistry and Physics', 62nd edition, The Chemical Rubber Co, CRC Press Inc., Florida 1981/82.
- [27] *P. Bakuzis, O. O. S. Campos & M. L. F. Bakuzis*, *J. Org. Chem.* **41**, 3261 (1976).