

Stability of Pulegone and Thujone in Ethanolic Solution

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The stability of the monoterpene ketones (*R*)-(+)-pulegone and α - and β -thujone in aqueous-ethanolic solutions was measured by using a model experimental system. Qualitative and quantitative analysis of degradation products was performed by GC, GC-MS, and GC-FTIR. Under variable light and temperature conditions, pH values, and ethanol concentrations, the degradation products formed from the above chemicals were (*E*)- and (*Z*)-isopulegone, the stereoisomer 8-hydroxy-*p*-menthones, 8-hydroxy- $\Delta^{4(5)}$ -*p*-menthen-3-one, the stereoisomer 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ols, and (*E*)- and (*Z*)-5-methylene-6-methylhept-2-ene.

(*R*)-(+)-Pulegone is the major monoterpene constituent of Pennyroyal oil. This oil, obtained from the leaves and flowering tops of *Mentha pulegium* and *Hedeoma pulegoides*, has been widely used as a fragrance component (Opdyke, 1978), a flavoring agent (Hall and Oser, 1965), and an herbal medicine to terminate pregnancy (Gleason et al., 1969). Pulegone is also present in peppermint and mint oil, obtained from *Mentha piperita* and *Mentha arvensis* (Grundschober, 1979). α -Thujone, one of the major constituents of Dalmatian or garden sage oil, is obtained from partially dried leaves of *Salvia officinalis* by steam distillation. The concentration of β -thujone in this oil is approximately 5%. This oil is used in the food industry and for pharmaceutical preparations (Bauer and Garbe, 1985).

In the Federal Republic of Germany the use of oils of *Herba pulegii*, of synthetic pulegone, or of synthetic thujone to flavor foods was banned (Aromenverordnung, 1981). Concentrations of pulegone and thujone in foods flavored with peppermint and mint oil or thujone-containing essential oils was limited by the European Community (1988). Even though many toxicity and mutagenicity studies were done on mint oil, pulegone, and thujone (Moorthy et al., 1989; Andersen and Jensen, 1984; Rice and Wilson, 1976), the chemicals, including photoproducts, that are formed after their application to foods or cosmetic products are difficult to predict; some may react to produce even more toxic compounds. The aim of this study was to obtain more information about the stability of pulegone and thujone in alcoholic solutions to assess the safety of essential oils.

EXPERIMENTAL PROCEDURES

Chemicals. (*R*)-(+)-Pulegone (purity 92%, as measured by capillary gas chromatography), thujone [purity 83%; 71% (1*S*,4*R*,5*R*)-(-)-thujone (α -thujone) and 12% (1*S*,4*S*,5*R*)-(-)-thujone (β -thujone)], and 2-heptanol (purity 97%) were obtained from Aldrich Chemical Co., Milwaukee, WI. (*E*)- and (*Z*)-5-methylene-6-methylhept-2-ene were synthesized according to the method of Cooke and Lyon (1981), (*E*)- and (*Z*)-isopulegone according to the method of Ohloff et al. (1962), and the 8-hydroxy-*p*-menthone isomers according to the method of Fujita and Morishita (1987). 8-Hydroxy- $\Delta^{4(5)}$ -*p*-menthen-3-one was synthesized according to the method of Schulte-Elte et al. (1971), and the 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ol isomers were synthesized according to the method of Kawai

and Naya (1972). No preparative gas chromatography was applied to separate the structural isomers. Methanol (Baker-analyzed), dichloromethane (Baker-Resi-Analyzed), and pentane (Baker-Resi-Analyzed) were obtained from Baker Chemical Co., Phillipsburg, NJ. Ethanol (100%) was purchased from Gold Shield Chemical Co., Hayward, CA. C₁₈ solid-phase extraction columns (500 mg) from Analytichem International (Harbor City, CA) were used. Chemicals and solvents were used without further purification.

Instrumentation. A Rayonet photochemical reactor Model RPR 100, Southern New England Ultraviolet Co. (Hamden, CT), was used for photochemical irradiation of samples. The reactor was equipped with four 310-nm reactor lamps. UVB intensity was measured near the center of the reactor with a Spectroline UVB meter (Spectronic Corp., Westbury, NY) at 0.68 ± 0.2 mW/cm² (mean \pm SD).

Gas chromatography (GC) was carried out on a Hewlett-Packard (Avondale, PA) Model 5890 gas chromatograph, modified for split injection and equipped with a flame ionization detector. A 30 m \times 0.25 mm i.d. J&W (Folsom, CA) fused silica capillary column coated with DB-Wax (film thickness 0.25 μ m) was temperature-programmed from 50 °C (isothermal for 3 min) to 220 °C at 4 °C/min with helium as carrier gas (25.4 cm/s at 50 °C). Quantitative determinations were carried out by standard controlled calculations using a Spectra Physics (San Jose, CA) SP 4290 computing integrator without consideration of calibration factors for the degradation products.

Mass spectra were recorded on a VG (Danvers, MA) Trio 2 GC-MS system, using the same GC apparatus, column, and conditions mentioned above. The column was directly coupled to the ion source. The temperatures of the GC line and the ion source were 200 and 150 °C, respectively. An electron energy of 70 eV was applied for electron impact spectra as well as for chemical ionization using isobutane as reactant gas.

GC-FTIR spectra were run on a Hewlett-Packard Model 5890A gas chromatograph equipped with a Model 5965A infrared detector. Data processing was done on a 59970 HP IRD Chem Station. The GC column and conditions were the same as mentioned above, except splitless injection (60 s) was applied to match the lower sensitivity of the instrument compared to flame ionization detection (FID) and MS. Vapor-phase spectra were scanned from 4000 to 750 cm⁻¹ at a rate of 4 scans/s and with a spectral resolution of 16 cm⁻¹. Light pipe and GC lines were kept at temperatures 10 °C above the elution temperature of the highest boiling compound of interest.

Results of qualitative analysis were verified by comparison of GC retention data, mass spectral data, and, in part, FTIR vapor-phase data with data from authentic reference substances.

Sample Preparation. Pulegone (486 mg) and 2-heptanol (511 mg) as the internal standard compound were dissolved in 10 mL of methanol. A 1-mL aliquot of each was diluted to 100 mL with 100% ethanol, with 30% ethanol at pH 6.5, with 30% ethanol adjusted to pH 2.5 by 1 M HCl, and with 30% ethanol adjusted to pH 11.5 by 1 M NaOH (see Figure 1). Thujone (503 mg) and 2-heptanol (502 mg) were treated in the same way. Approximately 10 mL of each dilution was transferred to vials with

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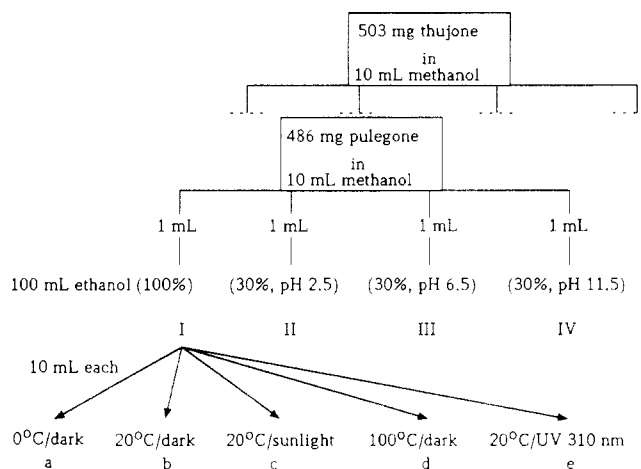


Figure 1. Schematic display of the model system.

Teflon-lined screw caps and stored under the following conditions: 0 °C/in the dark, 20 °C/in the dark, and 20 °C/in the dark. The concentrations of pulegone and thujone were analyzed every 2 weeks by GC. Another 10 mL of each sample was refluxed in the dark at 100 °C for 2 days. Samples for GC were taken every 2 h. Still another 10 mL of each sample was transferred to 15-mL Pyrex vials with Teflon-lined screw caps and irradiated with UV light (310 nm) at room temperature.

Quantitative GC analysis was carried out without further sample preparation. For MS and FTIR investigations, 0.5 mL of sample was diluted with purified water to an ethanol concentration of 5%. The solution was passed through a C_{18} solid-phase extraction column. The column was dried by centrifugation at 3000 rpm for 1 min in a swinging-bucket rotor centrifuge and then eluted with 1 mL of pentane-dichloromethane (2 + 1, v + v). The extract was concentrated under a nitrogen stream.

Four series of control samples that contained only 2-heptanol, pulegone, thujone, or ethanol were prepared and treated in the same way as described above. The UV irradiation experiments of thujone were repeated in a hexane solution because the irradiation product was coeluting with ethanol on the stationary phase used.

RESULTS AND DISCUSSION

Photochemical irradiation of samples was performed in Pyrex tubes at 20 °C with 310-nm UV light to simulate food storage in glass bottles in sunlight. Neither any decrease in the amount of pulegone nor the formation of decomposition products was observed during the first 7 months in any experiment at pH 6.5 (III in Figure 1) and at pH 11.5, except for those involving UV irradiation (IVe). In 100% ethanol, no changes were noticed in any experiment carried out in the dark (Ia, Ib, and Id). At pH 2.5 (II), pulegone readily started to give two isomeric compounds, the amount of which increased slowly at 20 °C in sunlight and in the dark (IIb and IIc) and increased only very slowly at 0 °C in the dark (IIa). These compounds were identified as the isomeric 8-hydroxy-*p*-menthones shown in Figure 2. The menthone derivatives occur naturally in the essential oil of *Schizonepeta tenuifolia* (Fujita and Morishita, 1987) and are minor constituents of Buchu leaf oil (Kaiser et al., 1975). No further decomposition was observed during experiments IIA-d.

On UV irradiation in 100% ethanol (Ie), the concentration of pulegone decreased slowly (Figure 3), leading to only one product. It was identified as 8-hydroxy- $\Delta^{4(5)}$ -*p*-menthen-3-one (Figure 2), a photooxygenation product described earlier by Schulte-Elte et al. (1971) and by Enssley and Carr (1977). The formation of the same hydroxy derivative was noticed after 3 months at 20 °C in sunlight (Ic). During irradiation of pulegone in 30% eth-

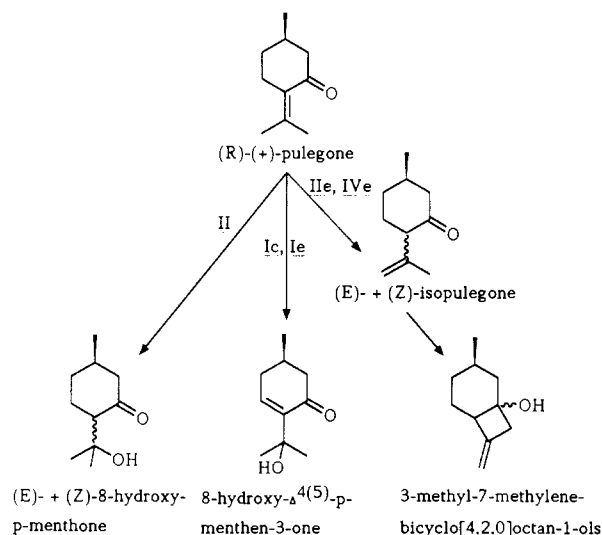


Figure 2. Reactions of (*R*)-(+)-pulegone in ethanolic solutions (I, 100% ethanol; II, 30% ethanol, pH 2.5; IV, 30% ethanol, pH 11.5; c, 20 °C/sunlight; e, 20 °C/UV 310 nm).

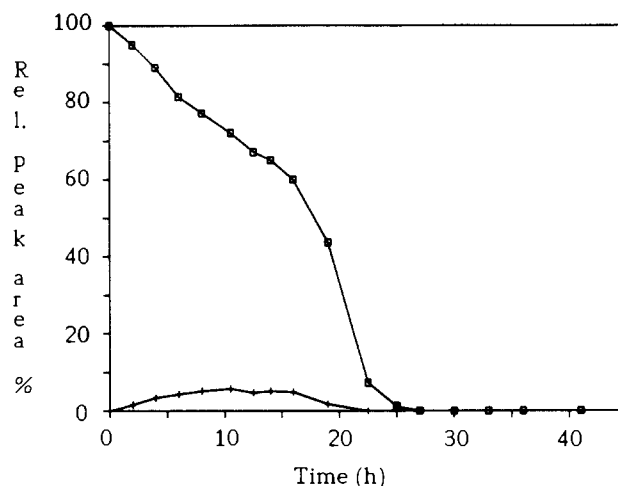


Figure 3. Degradation of (*R*)-(+)-pulegone in 100% ethanol under UV irradiation. \square , (*R*)-(+)-pulegone; $+$, 8-hydroxy- $\Delta^{4(5)}$ -*p*-menthen-3-one.

anol at pH 2.5 (IIe), the concentration of pulegone decreased slowly (Figure 4) and formed four main products (Figure 4). At first, pulegone was isomerized to its vinyl stereoisomer (*E*- and *Z*-isopulegone, probably with preference of the *E* configuration, reaching a maximum concentration after 15 h. The concentration of the second irradiation product, consisting of two isomers, increased steadily during the first 35 h. The chromatographic and spectroscopic data of these compounds revealed the structure of the stereoisomer 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ols (Figure 2), described earlier by Matsui et al. (1967) for the photoirradiation of isopulegone and by Kawai and Naya (1972) for the photoirradiation of pulegone. The methylene cyclobutanol derivatives appeared at a constant ratio of 77:23 and are assumed to be given by a Norrish type II reaction (Shibamoto and Mihara, 1983). At pH 11.5 a rapid decrease of pulegone and increase of the same four compounds were noticed (Figure 5).

During photoirradiation of thujone in 30% ethanol (IIe, IIIe, and IVe), in 100% ethanol (Ie), and in hexane, its concentration decreased rapidly, leading to only two products (Figure 6). They were identified as the isomeric 5-methylene-6-methylhept-2-enes, photochemical products described earlier by Eastman et al. (1963) for the irradiation of thujone as neat liquid or dissolved in either cyclohex-

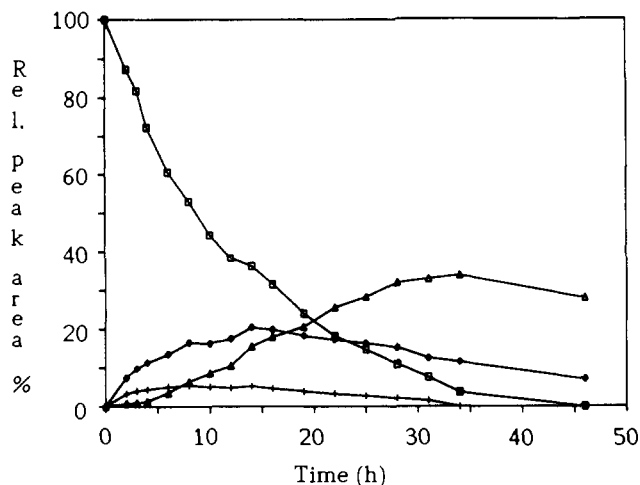


Figure 4. Degradation of (*R*)-(+)-pulegone in 30% ethanol (pH 2.5) under UV irradiation. □, (*R*)-(+)-pulegone; ◇, (+), (*E*)- + (*Z*)-isopulegone; △, the sum of the stereoisomer 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ols.

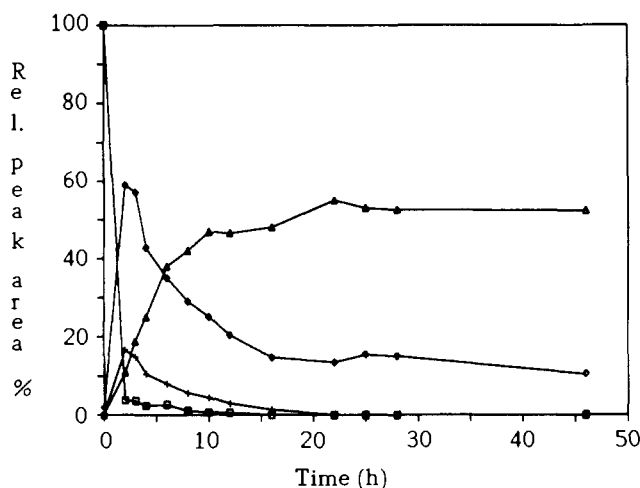


Figure 5. Degradation of (*R*)-(+)-pulegone in 30% ethanol (pH 11.5) under UV irradiation. □, (*R*)-(+)-pulegone; ◇, +, (*E*)- + (*Z*)-isopulegone; △, the sum of the stereoisomer 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ols.

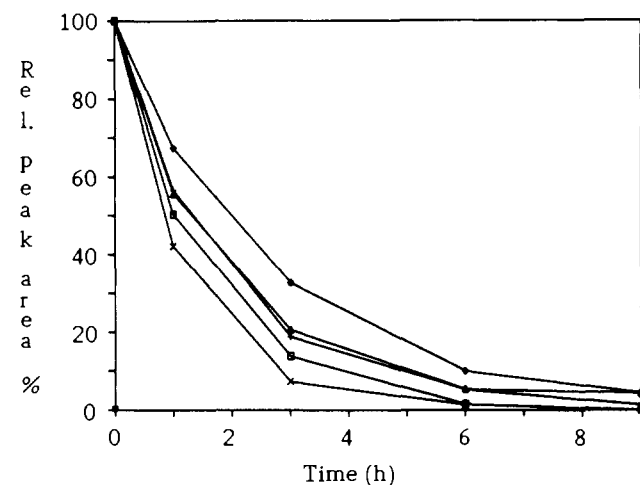


Figure 6. Degradation of thujone under UV irradiation (solvent: □, 30% ethanol, pH 2.5; +, pH 6.5; ◇, pH 11.5; △, 100% ethanol; ×, hexane). The sum of α - and β -thujone is displayed.

ane or 1-propanol. The photodecarbonylation reaction was examined in more detail by Cooke and Lyon (1971, 1981). The rate of decomposition by losing carbon monoxide was 15–30 times faster than that of cyclopentanone or cyclo-

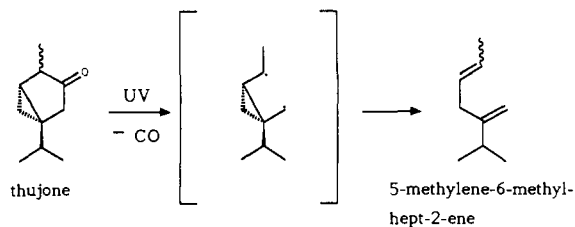


Figure 7. Photochemical decarbonylation of thujone.

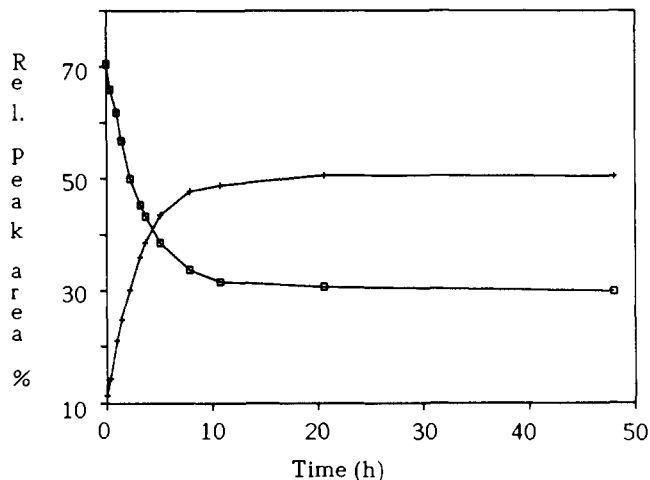


Figure 8. Epimerization of thujone in 30% ethanol, pH 11.5. □, β -thujone; ◇, α -thujone.

hexanone. This is due to the presence of a cyclopropane ring in the homoallylic position (Figure 7).

No change in the thujone concentrations was noticed during the storage experiments in 100% ethanol and 30% ethanol at either pH 2.5 or pH 6.5 (Ia–IIIId). At pH 11.5 a very rapid epimerization of α -thujone to the 5 times less toxic β -thujone (Rice and Wilson, 1976) took place as reported by Hach et al. (1971) and reached an equilibrium of about 1:2 α -thujone to β -thujone after about 20 h (Figure 8). A comparison of the reaction rates at 20 °C in sunlight and in the dark showed that the reaction was independent of the influence of light. At the different temperatures (100, 20, and 0 °C), nearly no difference in reaction rate was observed.

Nothing is known about the toxicity or mutagenicity of the degradation products formed in the present study, except for isopulegone. It was found to have hepatotoxicity and pulmonary toxicity in the mouse (Gordon et al., 1982).

ACKNOWLEDGMENT

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Registry No. Pulegone, 89-82-7; thujone, 546-80-5; ethanol, 64-17-5; (*E*)-isopulegone, 29606-79-9; (*Z*)-isopulegone, 52152-10-0; (*E*)-8-hydroxy-*p*-menthone, 129099-30-5; (*Z*)-8-hydroxy-*p*-menthone, 129099-31-6; 8-hydroxy-4(5)-*p*-menthen-3-one, 83212-19-5; 3-methyl-7-methylenebicyclo[4.2.0]octan-1-ol, 17844-32-5; (*E*)-5-methylene-6-methylhept-2-ene, 33746-69-9; (*Z*)-5-methylene-6-methylhept-2-ene, 33746-70-2.