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IDENTIFICATION BY GC—MS OF CYMENE ISOMERS AND 3,7,7-TRIMETHYLCYCLOHEPTA-1,3,5-TRIENE IN ESSENTIAL OILS

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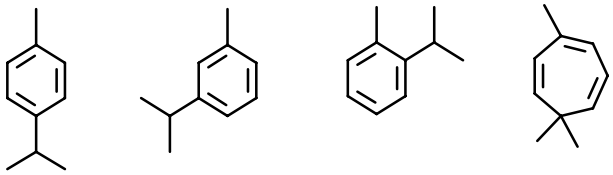
Retention indices of cymene isomers published in popular GC—MS atlases were found to be erroneous by analyzing synthetic samples. The following retention indices (RI) were found using a nonpolar phase (diphenyl:dimethylpolysiloxane, 5:95) for four essential-oil components with indistinguishable mass spectra: 3,7,7-trimethylcyclohepta-1,3,5-triene (RI = 970), m-*cymene (RI = 1022),* p-*cymene (RI = 1024), and* o-*cymene (RI = 1039). The relative distributions of these compounds were evaluated based on the analysis of about 1000 essential oils. Simple methods were given for preparing standard mixtures of isomeric compounds for identification by GC—MS.*

Key words: essential oils, 3,7,7-trimethylcyclohepta-1,3,5-triene, *m*-cymene, *p*-cymene, *o*-cymene, retention indices, GC—MS.

Monoterpenes of the *p*-menthane series are widely distributed in the plant world and are the main components of many essential oils and plant extracts. One of the most common compounds of this type is *p*-cymene (**1**), which is the starting material for synthesizing products for various purposes, mainly oxidized derivatives [1], exhibits biological activity [2], is a component of common spices.

Identification of **1** in plant extracts is often difficult because the isomeric *m*- (**2**) and *o*-isomers (**3**) have mass spectra that are practically indistinguishable from that of *p*-cymene. The situation is further complicated by the fact that GC—MS data in commonly used atlases published in 1989 [3], 1995 [4], and 2001 [5] give erroneous retention indices (RI) and elution orders for *o*- and *p*-cymene. As a result, many published scientific studies of essential oil compositions incorrectly identify cymene isomers. An example of this is a technique for precise analysis of lavander oil [6] in which peaks for cymene isomers are incorrectly identified based on the citation of an atlas [4].

We synthesized samples of cymene isomers **1**-**3**, confirmed their structures by PMR and ^{13}C NMR spectra, and determined their GC—MS properties. As it turned out, there is another component, 3,7,7-trimethylcyclohepta-1,3,5-triene (**4**), with similar properties (see Experimental).



1 **2** **3** **4**

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We used GC—MS to analyze about 1000 samples of commercially available essential oils and oils obtained under laboratory conditions from plants of the families Apiaceae, Asteraceae, Lamiaceae, Pinaceae, Cupressaceae, Salicaceae, Valerianaceae, Ericaceae, Araceae, Betulaceae, Grossulariaceae, Rosaceae, and Rutaceae. Many essential oils and plant extracts contained **1**, most often as a minor component in a mixture generally dominated by *p*-menthane-type derivatives. Small amounts of **2**, **3**, and **4**, mainly in products from conifers, were observed. *m*-Cymene (**2**) was found in some turpentines from pine (*Pinus* spp.), larch (*Larix* spp.), and juniper (*Juniperus* spp.), always as an insignificant (content <0.05%) impurity and always with *p*-cymene (*p*/*m* ratio 15:1 at a minimum). *o*-Cymene (**3**) was found in trace (up to 0.05%) amounts in monoterpene fractions of resins and in extracts and essential oils from various parts of coniferous trees (*P. pityusa* Stev., *P. mugo* Turra., and *P. sibirica* R. Mayr.). The content of **3** was maximum (0.8% of the monoterpene fraction) in turpentine from Japanese larch resin [*L. leptolepis* (Sieb. et Zucc.) Gord.]. An insignificant amount of **4** was found in practically all samples including industrial products from reprocessing of coniferous tree resins and in pine and fir oils, in the monoterpene fraction of pine (*Pinus* spp.), larch (*Larix* spp.), fir (*Abies* spp.), spruce (*Picea* spp.), and microbiota (*Microbiota decussata* Kom.).

The origin of **1**-**4** is not yet clear. Apparently **1** in many instances is an artifact and is formed by oxidation of various *p*-menthane-type derivatives. Compound **4** may be a dehydration product of 3-carene oxidation products. Heating **4** above 300°C leads in turn to its destruction and formation of a mixture of products including *p*- and *m*-cymene [7].

Because the difference in the RI of *p*- and *m*-cymene is only two units, these isomers can be reliably identified only by using standards since noticeable deviations from the table values of the RI can be observed on going from some nonpolar phases to others (and even on going to a column with the same type of phase but from different manufacturers). Several methods can be recommended for preparing standard mixtures containing these isomers: 1) dehydrogenation of limonene produces *p*-cymene; 2) dehydrogenation of 3-carene produces a mixture of *p*- and *m*-cymene; 3) Friedel-Krafts alkylation of toluene by isopropylbromide gives a mixture of *o*-, *m*-, and *p*-cymene; 4) thermal degradation of 3,4-caranediol diacetate produces 3,7,7-trimethylcyclohepta-1,3,5-triene.

EXPERIMENTAL

General Comments. GC—MS was carried out in a Hewlett—Packard 5890/II gas chromatograph with an HP 5972A mass-selective detector, 30-m HP-5 MS quartz capillary column [30 m × 0.25 mm, 0.25 μm stationary phase (diphenyl:dimethylsiloxane copolymer, 5:95), He carrier gas (1 mL/min), vaporizer temperature 280°C, column 50°C (2 min)-10°C/min-280°C, ion source 173°C, interface between GC and MS detector 280°C, ionizing-electron energy 70 eV, data collection 1.2 scans/s for mass range 30-650 amu].

Preparative GC was performed by refurbishing for preparative work a Chrom-5 chromatograph with a flame-ionization detector and N_2 carrier gas. Products were separated using a steel column [4500 × 6 mm, 15% Apiezon L on Chromaton N-AW (0.250-0.315 mm), column temperature 120°C, vaporizer and detector 250°C].

PMR and ^{13}C NMR spectra of $CDCl_3$ solutions were recorded on a Bruker DRX-500 spectrometer (500.13 MHz for ^1H; 125.75 MHz, ^{13}C) at 25°C using solvent signals (δ_C 76.90 ppm, δ_H 7.24) as internal standards.

Limonene Dehydrogenation. Limonene (170 mg, 1.2 mmol) was mixed with conc. H_2SO_4 (0.1 mL) and stirred at room temperature for 15 min. The isomerization products were extracted with hexane (5 × 1 mL). The combined extracts were washed with water (1 mL) and dried over anhydrous $MgSO_4$. Solvent was removed to afford *p*-cymene (**1**, 40 mg) without impurities of other isomers.

3-Carene Dehydrogenation. 3-Carene (330 mg, 2.4 mmol) was treated with conc. H_2SO_4 (0.3 mL) and stirred at room temperature for 30 min. The reaction products were extracted with hexane (5 × 1 mL). The combined extracts were washed with water (1 mL) and dried over anhydrous $MgSO_4$. Solvent was removed to afford a mixture of hydrocarbons (83 mg) containing ~60% of *p*- (**1**) and *m*-cymene (**2**) in a 4:1 ratio.

Toluene Alkylation. A mixture of toluene (4.0 mL, 37.8 mmol) and $AlCl_3$ (0.18 g, 1.3 mmol) was cooled to 0°C, stirred vigorously, treated dropwise over 60 min with *i*-PrBr (1.0 mL, 10.6 mmol), held at 0°C for another 30 min after the addition was finished, and poured onto ice. The organic layer was separated, washed with $NaHCO_3$ solution (1 M, 2 × 1 mL) and water (1 mL), and dried over $MgSO_4$. The excess of toluene was removed to afford a mixture (0.98 g) of **1**, **2**, and **3** in a 2:5:1 ratio.

3,7,7-Trimethyl-1,3,5-cycloheptatriene (4). 3,4-Caranediol diacetate (750 mg, 2.9 mmol) that was prepared as before [8] was heated in a sealed ampul at 230°C for 2 h, cooled to room temperature, diluted with hexane (10 mL), washed with saturated aqueous $NaHCO_3$ (2 × 1 mL) and water (1 mL), and dried over $MgSO_4$. Solvent was removed. The solid was chromatographed over SiO_2 (hexane eluent) to isolate a hydrocarbon fraction (120 mg) containing according to GC **4** (65%) that was then purified by preparative GC.

PMR spectrum (δ, ppm, J/Hz): 0.98 (6H, s, Me_2-7), 2.01 (3H, s, Me-3), 5.10 (1H, d, J = 10.2, H-6), 5.16 (1H, d, J = 10.3, H-1), 5.90 (1H, d, J = 10.3, H-2), 5.98 (1H, dd, J = 1.02, 6.6, H-5), 6.25 (1H, d, J = 6.6, H-4). ^{13}C NMR spectrum ($CDCl_3$): 24.33 (q, $\underline{C}H_3$–C-3), 26.07 [2q, $(\underline{C}H_3)_2$–C-7], 34.78 (s, C-7), 123.94 (d, C-5), 127.03 (d, C-4), 127.28 (d, C-2), 132.28 (d, C-6), 133.59 (d, C-1), 138.46 (s, C-3).

Retention Indices (HP 5 MS Column, Diphenyl:dimethylsiloxane copolymer, 5:95) and Electron-Impact Mass Spectra of 1-4.

***p*-Cymene (1).** RI = 1024. Mass spectrum (*m*/*z*): 134 (27), 120 (10), 119 (100), 117 (12), 115 (5), 105 (2), 104 (2), 103 (3), 93 (2), 91 (17), 77 (5), 65 (4), 41 (2), 39 (2).

***m*-Cymene (2).** RI = 1022. Mass spectrum (*m*/*z*): 134 (32), 120 (10), 119 (100), 117 (13), 115 (7), 105 (3), 104 (3), 103 (4), 93 (2), 92 (2), 91 (20), 79 (2), 78 (2), 77 (6), 65 (4), 63 (2), 51 (2), 41 (2), 39 (2).

***o*-Cymene (3).** RI = 1039. Mass spectrum (*m*/*z*): 134 (31), 120 (10), 119 (100), 117 (13), 115 (7), 105 (2), 104 (3), 103 (4), 93 (2), 92 (2), 91 (20), 79 (2), 78 (2), 77 (5), 65 (4), 63 (2), 58 (2), 51 (2), 41 (2), 39 (2).

3,7,7-Trimethylcyclohepta-1,3,5-triene (4). RI = 970. Mass spectrum (*m*/*z*): 134 (11), 120 (9), 119 (100), 117 (10), 115 (5), 105 (4), 104 (3), 103 (5), 93 (3), 92 (4), 91 (36), 79 (5), 78 (3), 77 (10), 65 (5), 63 (2), 51 (4), 41 (7), 39 (5).

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