

Identification of *m*-Mentha-3(8),6-diene (Isosylvesterpipolene) in Black Pepper Oil

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m-Mentha-3(8),6-diene (= isosylvesterpipolene; **5**), which has not been found in nature before, was shown by GC/MS analysis and co-injection with a synthetic sample to be present to the extent of 0.12–0.17% in black and green pepper oil. The synthetic reference **5** was prepared by deconjugation of 3-methylcyclohex-2-en-1-one (**1**), subsequent alkylation with lithium 2-lithio-2-methylpropanoate, and decarboxylative dehydration of the resulting hydroxycarboxylic acid **3** employing dimethylformamide dimethyl acetal. On polar GC columns, **5** was eluted between *p*-cymene and terpinolene, and was characterized by an MS similar to that of terpinolene, but with the fragment m/z 121 (59, $[M - \text{Me}]^+$) being less intense than that of the molecular ion at m/z 136 (67, M^+). Since *m*-mentha-3(8),6-diene was also found to the extent of 0.24% in the headspace of ground black peppercorns, it can be considered to be of genuine natural origin, and thus is the first established example of a naturally occurring *m*-menthadiene.

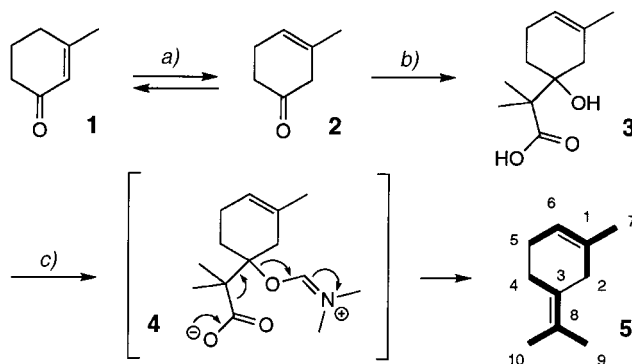
Introduction. – Black pepper oil (*Piper nigrum* L., Piperaceae) has an intense, spicy, and peppery odor, reminiscent of cubebs [1] but with a milder taste which lacks the pungency of the spice. This oil is not only one of the most important flavoring raw materials, it also becomes increasingly fashionable in fine fragrances. In ‘Extreme Polo Sport’ (Lauren, 1998), the extreme amount of around 6% was harmoniously incorporated. ‘Miss Dior’ (Dior, 1947), ‘Comme des garçons’ (Comme des garçons, 1994), and ‘Contradiction’ (C. Klein, 1997) demonstrate that its use is not limited to masculine notes. Of importance with respect to the application in perfumery are the dry-spicy, terpenic top-note aspects of black pepper oil that are mainly due to the monoterpene fraction. In 1992, Kollmannsberger *et al.* [2] reported on two unidentified menthadienes in the monoterpene fraction that could contribute to the characteristic top note of black pepper oil. They assumed these menthadienes to be *meta*-substituted, though the natural occurrence of *m*-menthanes [3] is controversial. Sylvestrene (= *m*-mentha-6,8-diene) and sylveterpinolene (= *m*-mentha-1,3(8)-diene), which have been isolated from different turpentine oils (*Pinus sylvestris* L.), could be artifacts resulting from the acid-catalyzed ring opening of carenes [4].

If *m*-menthadienes are genuine secondary metabolites, they could arise either from an enzymatic degradation of carenes or from the cyclization of a *head-to-head* joined diisoprene pyrophosphate, *e.g.*, 2,7-dimethylocta-1,6-dien-3-yl pyrophosphate. Aromatic products and *p*-menthadienes predominate in the pyrolysis of car-3-ene [5], while dehydrohalogenation in AcOH/NaOAc of sylvestrene dihydrochloride, an addition product of HCl to car-3-ene, was reported to yield mainly a 42:31:27 mixture of *m*-mentha-6,8-diene (= sylvestrene), *m*-mentha-1,8-diene (= isosylvestrene), and *m*-mentha-1(7),8-diene [6]. On GC/MS analysis of black pepper oil, we found an unknown GC peak with a molecular ion M^+ at m/z 136 between the peaks of *p*-cymene

and terpinolene; however, the MS of this unknown compound was significantly different from those of the three sylvestrenes mentioned above [7]. Besides the fragment $[M - \text{Me}]^+$ at m/z 121 which was less significant than M^+ at m/z 136, the MS of the unknown compound was rather quite similar to that of terpinolene. Therefore, and since *m*-mentha-3(8),6-diene (**5**) would be the expected biogenetic cyclization product of a head-to-head joined diisoprene pyrophosphate, we decided to synthesize this *m*-menthadiene to establish its presence in black pepper oil.

Results and discussion. – Our three-step synthesis of *m*-mentha-3(8),6-diene (**5**) started with the deconjugation of 3-methylcyclohex-2-en-1-one (**1**) in the presence of adipic acid as a mild acidic catalyst [8] (*Scheme*). Though the equilibrium ratio **1/2** of 98.8 : 1.2 was quite unfavorable, we were able to enrich 3-methylcyclohex-3-en-1-one (**2**) up to 70% by using a 80-cm vacuum-jacketed *Sulzer* column for distillation at 176–180°. The distillate was further purified by flash chromatography (silica gel) to provide isomerically pure 3-methylcyclohex-3-en-1-one (**2**) on a 70-g scale. According to a general procedure of *Krapcho et al.* [9], enone **2** was then alkylated with the lithium α -lithiocarboxylate of isobutyric acid, prepared by treatment of isobutyric acid with 2 equiv. of lithium diisopropylamide. The crude α,α -dimethyl- β -hydroxycarboxylic acid **3** was isolated by extraction in 79% yield in a sufficiently pure state for direct further transformation.

Scheme. Synthesis of m-Mentha-3(8),6-diene (5) from 3-Methylcyclohex-2-en-1-one (1).



a) $\text{HO}_2\text{C}(\text{CH}_2)_4\text{CO}_2\text{H}$, Δ , distillation. *b)* $\text{Me}_2\text{CLiCO}_2\text{Li}$, THF. *c)* $\text{Me}_2\text{NCH}(\text{OMe})_2$, CH_2Cl_2 .

Following the smooth method of *Eschenmoser* and coworkers [10] for the decarboxylative dehydration of hydroxycarboxylic acids, the crude β -hydroxy acid **3** was next treated with dimethylformamide dimethyl acetal in CH_2Cl_2 [11]. *Via* intermediate **4** (*Scheme*), the isopropylidene group was built up, and we isolated our target molecule **5** in 71% yield, free from double-bond isomers. *Fig. 1* details the spectroscopic assignments of *m*-mentha-3(8),6-diene (**5**) as determined by COSY-DQF, HMQC, and NOESY experiments. The methyl groups Me(9) and Me(10) were discriminated by a NOEDIFF spectrum (irradiation of H–C(2) at 2.65 ppm).

GC/MS Analyses and co-injection of the synthetic reference compound **5** with two commercial black pepper oils unambiguously established the presence of *m*-mentha-

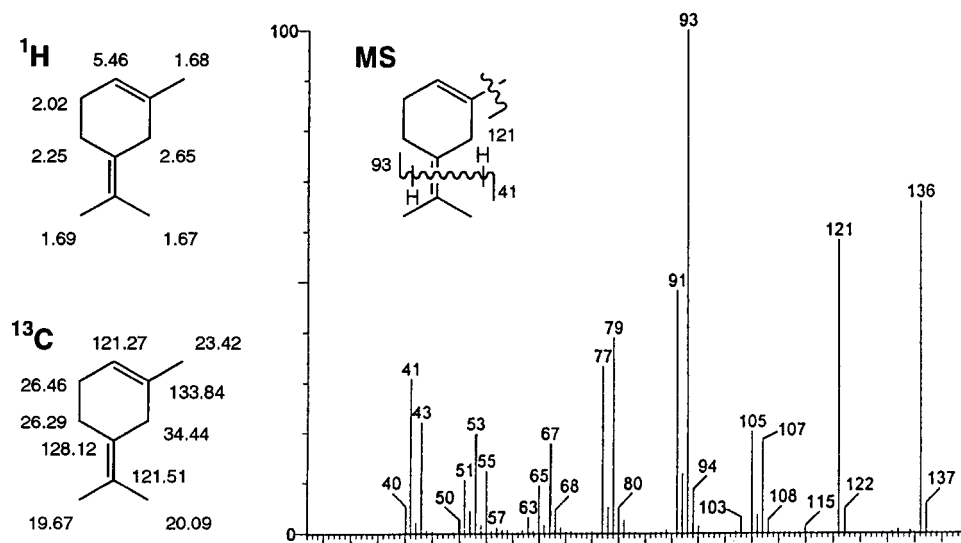


Fig. 1. Spectroscopic Data of *m*-Mentha-3(8),6-diene (**5**). Assignments by COSY-DQF, HMQC, NOESY, and NOEDIFF.

3(8),6-diene (= isosylvestripinolene; **5**) to the extent of 0.12 and 0.16%, respectively, matching the previously unidentified peak between *p*-cymene and terpinolene (Fig. 2). We also found **5** in amounts of 0.24% in the headspace of black peppercorns, of 0.17% in green pepper oil, and of 0.27% in the supercritical fluid extract (SFE) of Muntok pepper [2]. The percentage of *m*-mentha-3(8),6-diene (**5**) in the different pepper oils relative to the hydrocarbons of the monoterpene fraction is almost constant (0.22–0.23%; see Table). This almost constant quantity of **5** in different pepper oils as well as the high content of **5** in the headspace of ground black peppercorns clearly indicate that *m*-mentha-3(8),6-diene (**5**) is of genuine natural origin. Thus, **5** is the first established example of a naturally occurring *m*-menthadiene.

m-Mentha-3(8),6-diene (**5**) was first synthesized in 1972 by Muraleedharan and Verghese [12] by dehalogenation of 1,3,6,8-tetrabromo-*m*-menthane with Zn dust. This tetrabromide was obtained from a 6-% fraction of the dehydrohalogenate of sylvestrene dihydrochloride with aniline. In another synthetic approach [13], dehydration of *m*-menth-6-en-8-ol provided **5** as the 5-% component of a mixture with sylvestrene and the original alcohol. However, no well-directed synthetic approach towards **5** has been reported. The above described simple, straightforward synthesis of **5** from commercially available 3-methylcyclohex-2-en-1-one (**1**), should allow the identification of this unusual *meta*-monoterpene in other natural products.

Olfactory Evaluation. – With the exception of highly unsaturated systems such as the undeca-1,3,5-trienes [14] or the megastigma-4,6,8-trienes [15], hydrocarbons generally show only relatively weak, uncharacteristic odors. A recent structure-odor-relationship study of hydrocarbons [16] [17] indicated that at least two sterically accessible C=C bonds – favorably in conjugation – should be present in a cycloaliphatic

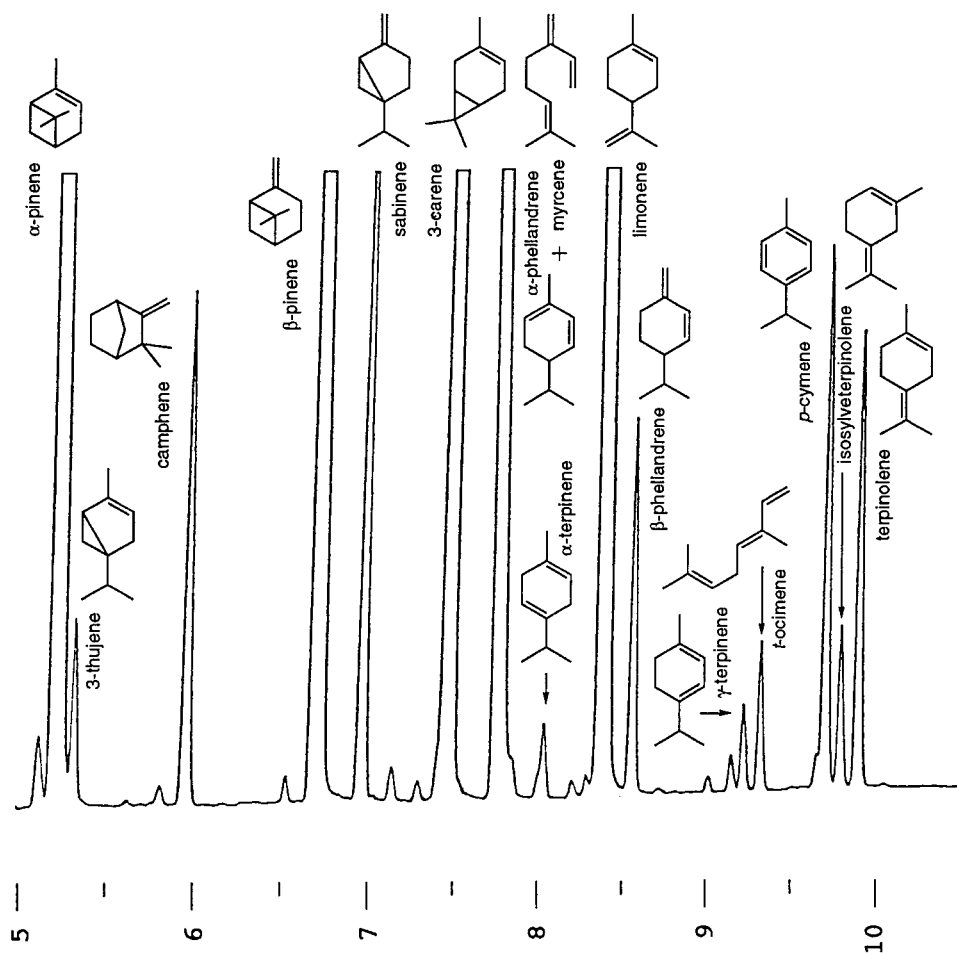


Fig. 2. GC Trace of the monoterpene fraction of black pepper oil (Rocchia Distillerie s.a.s.).
Supelcowax™ 10 column.

Table. Content of *m*-Mentha-3(8)-6-diene (5) in Different Natural Products. Area-% values in the GC (FID).

	Black pepper oil (Rocchia)	Black pepper oil (Kalsec)	Ground black peppercorns (headspace)	Green pepper oil (Synthite)	Muntok pepper SCF extract [2]
Car-3-ene	12.64	7.49	9.25	11.79	22.94
Limonene	18.76	14.75	13.44	22.88	15.78
<i>p</i> -Cymene	0.53	0.43	0.58	1.82	1.37
<i>m</i> -Mentha-3(8),6-diene (5) (in % of monoterpene fraction)	0.16 (0.22)	0.12 (0.23)	0.24 (0.70)	0.17 (0.22)	0.27 (0.42)
Terpinolene	0.46	0.44	0.65	1.25	0.64

hydrocarbon odorant. Limonene with a fresh lemon-like odor and a threshold of 70 ng/l can be considered as a benchmark [16] [17]. *m*-Mentha-3(8),6-diene (**5**) with a pleasant green-herbal, terpenic odor reminiscent of parsley and carrot leaves and a slightly metallic nuance, is much weaker than limonene, with a threshold of only 500 ng/l. Because of this high threshold, **5** does not contribute considerably to the characteristic top note of black pepper oil.

Experimental Part

General. Black pepper oil was obtained from *Rocchia Distillerie s.a.s.*, I-12014 Demonte, Italy, and *Kalsec*, Kalamazoo, Michigan 49005-0511, USA, and green pepper oil from *Synthite Industrial Chemicals Ltd.*, Cochin 682 016, India. Reagents and solvents were purchased from *Fluka* and used without further purification. 3-Methylcyclohex-2-en-1-one is also commercially available: *Fluka 66453*, *Aldrich M3,910-5*, *Lancaster 5090*. Flash chromatography (FC): *Merck silica gel 60*, particle size 0.040-0.063. IR: *Nicolet-510 FT-IR* spectrometer; $\tilde{\nu}$ in cm^{-1} . ^1H - and ^{13}C -NMR: *Bruker-AVANCE-DPX-400* spectrometer, δ in ppm rel. to SiMe_4 , J in Hz. GC/MS: *Fisons-MD-800* instrument, rel. int. in % of the base peak.

3-Methylcyclohex-3-en-1-one (2). A distillation apparatus with an 80-cm vacuum-jacketed *Sulzer* column and a *Perkin* triangle was flushed with N_2 and charged with 3-methylcyclohex-2-en-1-one (**1**; 484 g, 4.40 mol) and adipic acid (64.3 g, 440 mmol). The mixture was heated to 213° (inner temp.; heating mantle) and the column equilibrated at ambient pressure for 4 h. At a rate of ca. 1 drop per 10 s and a stillhead temp. of $176\text{--}180^\circ$, the product was collected for 4 h. This distillate was taken up in pentane (1 l), separated from the aq. layer, and dried (Na_2SO_4) to provide a 7:3 fraction of **2/1** (213 g, 70% based on recovered starting material). The ratio **1/2** in the distillation bulb was 98.8:1.2. FC (silica gel, pentane/*t*-BuOMe, 9:1, R_f 0.52) of the distillate provided isomerically pure **2** (69.2 g, 32%). IR (neat): 1718s (C=O). ^1H -NMR (CDCl_3): 1.73 (*d*, $J = 1.4$, Me–C(3)); 2.39–2.47 (*m*, 2 H–C(5), 2 H–C(6)); 2.78 (*s*, 2 H–C(2)); 5.60 (*t*, $J = 1.4$, H–C(4)). ^{13}C -NMR (CDCl_3): 22.54 (*q*, Me–C(3)), 24.85 (*t*, C(5)), 38.14 (*t*, C(6)); 44.26 (*t*, C(2)); 120.78 (*d*, C(4)); 131.95 (*s*, C(3)); 210.44 (*s*, C(1)). MS (70 eV): 110 (67, M^+), 95 (3, $[M - \text{Me}]^+$), 82 (10, $[M - \text{CO}]^+$), 67 (100, $[M - \text{CO} - \text{Me}]^+$), 53 (31, $[M - \text{CO} - \text{C}_2\text{H}_5]^+$), 39 (30, $[M - \text{CO} - \text{C}_3\text{H}_7]^+$).

2-(1'-Hydroxy-3'-methylcyclohex-3'-en-1'-yl)-2,2-dimethylacetic Acid (3). Lithium diisopropylamide (272 mmol) was prepared by the addition of 1.6M BuLi in hexanes (170 ml, 272 mmol) at -40° under N_2 to a stirred soln. of (*i*-Pr) $_2\text{NH}$ (39 ml, 276 mmol) in THF (650 ml). Stirring was continued at 0° for 20 min, before isobutyric acid (12.0 g, 136 mmol) was added dropwise at -40° . The mixture was heated to 50° for 2 h and then recooled to -40° before **2** (15.0 g, 136 mmol) was added dropwise. Stirring at -40° was continued for an additional 2 h. Then the mixture was poured on ice-water (1 l), and extracted with *t*-BuOMe (4×250 ml). The aq. layer was acidified with 2N aq. HCl and extracted with *t*-BuOMe (4×250 ml). The combined org. extracts were dried (MgSO_4) and evaporated: **3** (21.4 g, 79%) Crude material sufficiently pure for further transformation. IR (neat): 1702s (C=O), 1165m (C–O); 3150, 3400 (br. sh, O–H). ^1H -NMR (CDCl_3): 1.28, 1.30 (2 *s*, 2 Me–C(2)); 1.67 (*s*, Me–C(3')), 1.48–2.33 (*m*, OH, 2 H–C(2'), 2 H–C(5'), 2 H–C(6')); 5.49 (*m*, H–C(4')). ^{13}C -NMR (CDCl_3): 20.77, 20.80 (2 *q*, 2 Me–C(2)); 21.76 (*t*, C(5')); 23.68 (*q*, Me–C(3')); 27.21 (*t*, C(6')); 37.28 (*t*, C(2')); 49.51 (*s*, C(2)); 74.13 (*s*, C(1')); 120.46 (*d*, C(4')); 130.65 (*s*, C(3')); 183.39 (*s*, C(1)). MS (70 eV): 198 (2, M^+), 180 (4, $[M - \text{H}_2\text{O}]^+$), 110 (50, $[M - \text{C}_4\text{H}_8\text{O}_2]^+$), 93 (53, $[M - \text{H}_2\text{O} - \text{C}_4\text{H}_7\text{O}_2]^+$), 88 (100, $\text{C}_4\text{H}_8\text{O}_2^+$), 67 (66, C_5H_7^+), 43 (90, C_3H_7^+).

m-Mentha-3(8),6-diene (= *Isosylveterpinolene* = *1-Methyl-5-(1-methylethylidene)cyclohex-1-ene*; **5**). To a suspension of crude **3** (21.4 g, 108 mmol) in CH_2Cl_2 (270 ml), dimethylformamide dimethyl acetal was added and the clear soln. stirred under reflux for 2 h and at r.t. for 14 h. The mixture was evaporated, the residue taken up in pentane, and the pentane soln. washed with H_2O . After evaporation, the crude product (19.7 g) was purified by FC (silica gel, pentane, R_f 0.87): **5** (9.71 g, 71%). Colorless, odoriferous liquid. IR (neat): 2913s, 2966s, 2842s, 2804s (C–H); 1441m 1375m (CH_2 , CH_3); 1678w (C=C). ^1H -NMR (CDCl_3): 1.67 (*s*, 3 H–C(9)); 1.68 (*s*, 3 H–C(7)); 1.69 (*s*, 3 H–C(10)); 2.02 (*m*, 2 H–C(5)); 2.25 (*t*, $J = 6.2$, 2 H–C(4)); 2.65 (*s*, 2 H–C(2)); 5.46 (*m*, H–C(6)). ^{13}C -NMR (CDCl_3): 19.67 (*q*, C(10)); 20.09 (*q*, C(9)); 23.41 (*q*, C(7)); 26.29 (*t*, C(4)); 26.46 (*t*, C(5)); 34.44 (*t*, C(2)), 121.27 (*d*, C(6)), 121.51 (*s*, C(8)); 128.12 (*s*, C(3)); 133.84 (*s*, C(1)). MS (70 eV): 136 (66, M^+), 121 (58, $[M - \text{Me}]^+$), 107 (18, $[M - \text{C}_2\text{H}_5]^+$), 105 (20, $[M - \text{C}_2\text{H}_5 - \text{H}_2]^+$), 93 (100, C_7H_9^+), 91 (48, C_7H_7^+), 79 (39, C_6H_7^+), 77 (33, C_6H_5^+), 67 (18, C_5H_7^+), 65 (9, C_5H_5^+), 55 (12, C_4H_7^+), 53 (19, C_4H_5^+), 51 (10, C_4H_3^+), 43 (22, C_3H_7^+), 41 (31, C_3H_5^+). Odor: green, herbal, terpenic, parsley, carrot leaves, metallic.

Headspace Sampling. Black peppercorns (30 g; origin: Madagascar), purchased in a local drugstore, were ground and placed in an Erlenmeyer flask. Following the method described by *Neuner-Jehle* and *Etzweiler* [18], the headspace was trapped under quasi-static conditions on a *Porapak Q* (50–80 mesh) microfilter during 6 h by sucking air with a small pump (model 222-4, *SKC Inc.*, PA 15330, USA). Subsequently, the adsorbed volatiles were eluted with hexane/acetone 4:1 (50 μ l), and the extract was analyzed by GC/MS.

Identification of m-Mentha-3(8),6-diene (5). By comparison of the GC/MS spectra and co-injection with synthetic **5** on a *SupelcowaxTM 10* column (*Supelco*; 30 m \times 0.25 mm, film thickness 0.25 μ m). Temperature program: 3 min at 40°, then 20°/min \rightarrow 70°, then 8°/min \rightarrow 250°. Flow: 1 ml/min He. Black pepper oil (*Rocchia*) 0.16 area-% (FID), *i.e.* 0.22% of the monoterpene fraction. Black pepper oil (*Kalsec*): 0.12 area-% (FID), *i.e.* 0.23% of the monoterpene fraction. Headspace of ground black peppercorns: 0.24 area-% (FID), *i.e.* 0.70% of the monoterpene fraction. Green pepper oil (*Synthite*) 0.17 area-% (FID), *i.e.* 0.22% of the monoterpene fraction. SCF Extract of Muntok pepper [2]: 0.27 area-% (FID), *i.e.* 0.42% of the monoterpene fraction. Retention times: Car-3-ene 7.39, limonene 8.29, *p*-cymene 9.58, *m*-mentha-3(8),6-diene (= isosyl-veterpinolene; **5**) 9.67 and terpinolene 9.78 min.

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