Structure Elucidation of a Pungent Compound in Black Cardamom: Amomum tsao-ko Crevost et Lemarié (Zingiberaceae)

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JANUARY 2008

Impact Factor: 2.91 · DOI: 10.1021/jf072707b · Source: PubMed

5 AUTHORS, INCLUDING:

Laurent Wunsche
Firmenich
13 PUBLICATIONS **190** CITATIONS
SEE PROFILE

Christian Vial
Firmenich
17 PUBLICATIONS **329** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structure Elucidation of a Pungent Compound in Black Cardamom: *Amomum tsao-ko* Crevost et Lemarié (Zingiberaceae)

CHRISTIAN STARKENMANN,* FABIENNE MAYENZET, ROBERT BRAUCHLI, LAURENT WUNSCHE, AND CHRISTIAN VIAL

Firmenich SA, Corporate R&D Division, Post Office Box 239, CH-1211 Geneva 8, Switzerland

Natural plant extracts containing taste modifier compounds will gain more commercial interest in the future. Black cardamom, *Amomum tsao-ko* Crevost et Lemarié, used as a spice in Asia, produces a nice refreshing effect in the mouth. Therefore, an ethyl acetate extract was prepared, and constituents were separated by liquid chromatography. Guided by the tasting of each fraction (LC tasting), a new pungent compound was discovered, (±)-*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde. To confirm this new structure, a synthesis was performed starting from cyclopentene-1-carbaldehyde. The Wittig conditions were determined to control the stereochemistry of the ring fusion to prepare (±)-*trans*-(2,3,3a,7a-tetrahydro-1*H*-inden-4-yl) methanol and (±)-*cis*-(2,3,3a,7a-tetrahydro-1*H*-inden-4-yl) methanol. After oxidation, (±)-*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde and (±)-*cis*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde were tasted in water and only the *trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde, present in black cardamom, produced a trigeminal effect in the mouth.

KEYWORDS: (±)-*trans*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carbaldehyde; (±)-*trans*-(2,3,3a,7a-tetrahydro-1*H*-inden-4-yl); trigeminal effect; *Amomum tsao-ko* Crevost et Lemarié

INTRODUCTION

Chemosensation is mediated by taste and smell receptor organs and transmitted to the brain along their cognate nerves. However, the trigeminal nerve also carries sensory information (*1*). Free nerve endings in the facial mucosa are responsible for the detection of sharp, pungent, cold, or hot stimuli, such as capsaicin (*2*). Recent research has shown that the behavior of transient receptor potential (TRP) ion channels involved in the detection of hot or cold temperatures can be modulated by chemical stimuli (*3–5*). Thus, menthol elicits a cold sensation by raising the opening of the TRPM8 channel (*6, 7*). The compounds present in foodstuffs, which interact with TRP receptors, are often derivatives of vanilloid-like capsaicin, gingerol, and eugenol (*8*) or originate from closely related structures, such as piperine and galangal acetate. Polyunsaturated amides, widely distributed in plants, such as Szechuan pepper, *Spilantes acmella*, and Echinacea, also generate trigeminal effects (*9, 10*). In the family of terpene derivatives, menthol, cubebol, and eucalyptol generate a cooling, refreshing sensation and polygodial is described as pungent (*11*). A few other examples, such as phorbol esters, isothiocyanate derivatives, and allicin, are also known (*3, 8*). Very simple molecules, such as ethanol and carbon dioxide can also generate a trigeminal sensation (*12*).

Spices are a good source for natural compounds with oral sensory qualities distinct from olfaction and taste. The ginger family (Zingiberaceae) contains several important plants used as spices in food and compounds producing trigeminal effects, such as gingerols in *Aframomum melegueta* (grains of paradise) or *Zingiber officinale* (ginger) (*13, 14*) or galangal acetate in *Alpinia galanga* (greater galanga) (*15*), which were extensively studied. The sensory properties of black cardamom (*Amomum tsao-ko* Crevost et Lemarié), native in the Eastern Himalayas, are smoky, phenolic, with a fresh citrus, camphor odor reminiscent of green cardamom (*Elettaria cardamomum*) and *Amomum subulatum* Roxb. The smoky flavor comes from a traditional drying procedure over an open fire. Although there are many distinct varieties of black cardamom from China and Vietnam, ranging in pod size from 2 to more than 5 cm, their tastes do not differ much. In central and southern China, the seeds are optional ingredients of the five spice powders. *A. tsao-ko* produces a refreshing and pungent effect, which cannot be solely explained by the presence of 1,8-cineol and other known constituents (*16–21*). This observation led us to a careful analysis of black cardamom, to understand why this spice produces a trigeminal effect. This paper describes the identification of a new compound guided by tasting, and the structure elucidated was confirmed by its synthesis.

* To whom correspondence should be addressed. Telephone: +41-22-780-34-77. Fax: +41-22-780-33-34. E-mail christian.starkenmann@firmenich.com.

10.1021/jf072707b CCC: $37.00
Published on Web 11/20/2007

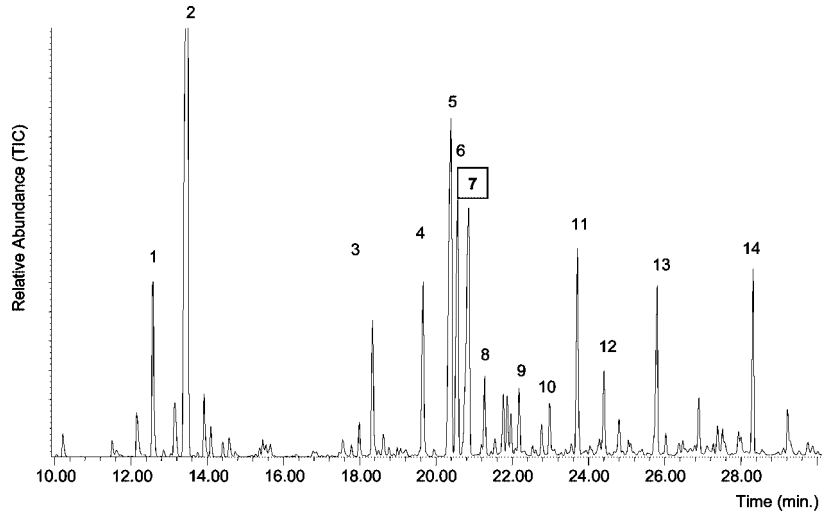


Figure 1. GC/MS (total ion current) on a SPB-1 column of *A. tsao-ko* (Rui Li) ethyl acetate extract.

MATERIALS AND METHODS

General Procedures. Commercially available reagents and solvents of adequate quality were used without further purification. Reactions were performed in standard glassware. Yields were not optimized.

Gas Chromatography/Electron Impact−Mass Spectrometry (GC/EI−MS). An Agilent-GC-6890 system connected to an Agilent-MSD-5973 quadrupole mass spectrometer was operated at an electron energy of ca. 70 eV. Helium was the carrier gas set at a constant flow rate of 0.7 mL/min. Separations were performed on 30 m × 0.25 mm i.d., 0.25 μm fused-silica capillary columns, either coated with SPB-1 or coated with Supelcowax 10 (Supelco, Buchs, Switzerland). The standard oven program was the following: 50 °C for 5 min, then 50−240 °C at 5°/min, and held at 240 °C. Retention indices (I) were calculated by linear extrapolation from the retention times (t_R in minutes) of the analytes and the two closest alkanes. The chiral column was a 25 m × 0.25 mm i.d., 0.25 μm fused-silica capillary column CP-Chirasil-Dex CB (Varian, Zug, Switzerland). The oven temperature was programmed from 100 to 145 °C at 2°/min. The mass spectra are listed as follows: fragment ions m/z (relative intensity).

Time-of-flight GC/MS analyses were performed on a GCT Premier (Waters, Milford, MA). Separations were performed on 30 m × 0.25 mm i.d., 1.0 μm film thickness fused silica capillary columns, coated with a SPB-1 (Supelco). The oven program started at 100 °C with a temperature gradient of 10°/min to 240 °C, at a constant helium flow of 1.0 mL/min. The injection volume was 1.0 μL, and the injector temperature was 250 °C with a split 1:50. The acquisition time was set to 0.5 s with an interscan delay of 0.01 s over a mass range of 1–300 Da. Spectra were recorded using an electron energy of 70 eV, emission current of 600 μA, trap current of 200 μA, and source temperature of 150 °C. Calibration was performed using heptacosa (perfluorotributylamine, mass spectrometry grade, Apollo Scientific LTD, Bradbury, U.K.). Calibration data were collected for 3 min in continuum mode. A total of 180 spectra were summed to generate a 16 point calibration curve from m/z 69 to 614 Da. The curve was fitted to a second-order polynomial such that the standard deviation of the residuals was 0.001 amu or lower. Heptacosa was continuously introduced into the ion source, and the ion m/z 218.9856 was used as a lock mass. Mass spectra and molecular formula were obtained using MassLynx software (Waters). The difference δ, between the exact mass calculated from the molecular formula and that measured, is calculated by the sofware and expressed in ppm ($\delta = (M_{measured} - M_{calculated})/M_{calculated} \times 106$).

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectra. The NMR spectra were recorded on a Bruker-Avance-500 spectrometer at 500.13 and 125.76 MHz, respectively. The solvent was $CDCl_3$. δ Values are in ppm downfield from $(CH_3)_4Si$ (=0 ppm). The assignments by correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC), and nuclear Overhauser effect spectrometry (NOESY) experiments were performed with standard Bruker software (Topspin 1.3) (Bruker Biospin, Fallanden, Switzerland).

Analysis of *A. tsao-ko* Crevost et Lemarié The seeds (1031 g), purchased from ZhaoYang Perfume Trading Shop, number 10-13, JunQi dry vegetable market, XiaoBanQiao town, Kunming City, 650000, Kunming of Yunnan Province, were manually separated from the pods, ground into a fine powder, and macerated at room temperature in ethyl acetate (2000 mL) for 90 min under mechanical stirring. The suspension was decanted, and the solvent was separated, dried (Na_2SO_4), filtered, and concentrated in a Vigreux apparatus at 45 °C under reduced pressure (6 mbar). The resulting oleoresin (41 g) was tasted in plain water at 50 ppm.

The oleoresin was fractionated by two consecutive chromatographic columns using SiO_2 [silica gel 60, 35–70 μm (Toulouse, France) and Lobar LiChroprep size B SiO_2 60, 40–63 μm (Merck, VWR, Nyon, Switzerland)] with 9:1 pentane/ethyl acetate, as a mobile phase. Each fraction was tasted by dipping a smelling strip into it. The solvent was removed by shaking the smelling strip in the air, and then it was placed onto the tongue. We isolated a pungent fraction of 211 mg, which contained mainly peak 7 (68%) and peak 8 (18%), plus minor impurities (**Figure 1**).

GC retention indices: peak 7, $I_{SPB\text{-}1}$ 1254 and I_{SPWAX} 1848; peak 8, $I_{SPB\text{-}1}$ 1270 and I_{SPWAX} 1876. GC/MS$_{CP\text{-}ChirasilDex\ CB}$: the major peak (peak 7) gave two peaks of the same intensity at R_T = 12.7 and 12.9 min (ratio of 1:1); the minor peak (peak 8) also gave two peaks at R_T = 14.5 and 14.9 min. ^{1}H, ^{13}C NMR showed a mixture having signals corresponding to synthetic **1** and **2**. The MS of peak 7 was the same as **1**, and the MS of peak 8 was the same as **2**. Elemental analysis of **1** gave 148.0881 Da ($C_{10}H_{12}O$, 4.8 ppm).

Quatitative Analysis of (±)-*trans*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carbaldehyde, 1. The powder prepared as described above (3 g) was extracted with 15 mL of ethyl acetate containing methyl octanoate (3.3 mg) as an internal standard, during 15 min in an ultrasonic bath. After filtration on a paper filter, the sample was injected in triplicate on GC$_{SPB\text{-}1}$/MS and **1** (peak 7) was quantified by a comparison to the internal standard without applying any response factor. Quantifications were performed on two different lots of *A. tsao-ko* from Vietnam and China, qualities *A. rui li* (three different lots) and *A. he ko* (two different lots).

Reduction of the Fraction Having a Pungent Effect. An aliquot of the fraction obtained above (20 mg) was diluted in ethanol (1 mL) and treated with $NaBH_4$ (~20 mg). The crude mixture was diluted in 0.1% H_2SO_4 (1 mL) and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, concentrated (45 °C, 12 mbar), and purified on a LiChroprep column with a mixture of 1:1 hexane/diethylether, leading to 4.2 mg of **10**.

GC retention indices of the main peak (reduced peak 7): $I_{SPB\text{-}1}$ 1299 and I_{SPWAX} 2115.

Synthesis of 1-Cyclopentene-1-carbaldehyde, 6. Methyl-1-cyclopentene-1-carboxylate (Fluka, Buchs, Switzerland) (20 g, 0.16 mol) was diluted in toluene (200 mL). Vitride (sodium bis(2-methoxyethoxy)aluminum hydride, 3.5 M in toluene (Acros, Chemie Brunschwig A.G., Basel, Switzerland) (100 mL, 0.35 mol) was added at 0 °C dropwise. The reaction was stirred for 90 min, and then the organic phase was washed with brine. The crude product was filtered through a column of 15 × 7 cm SiO_2 with 100% pentane and then eluted with diethyl ether, to give the corresponding alcohol, 14.9 g (yield 95%) after concentration.

This crude product (14 g, 0.143 mol) was diluted in pentane and treated with MnO_2 (118 g, 1.36 mol) overnight. The reaction mixture was filtered through Celite; the solvent was removed by distillation; and the crude product **6** (yield 84%) was used without further purification for the following Wittig reaction.

Synthesis of (±)-Methyl *cis*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carboxylate, 8 (*22*). To methyl 4-(triphenylphosphonio)crotonate bromide **7** (Alfa Aesar, Karlsruhe, Germany) (50 g, 0.113 mol) in methanol (50 mL) was added a freshly prepared sodium methylate solution (2.26 M in methanol). After 15 min, the aldehyde **6** (10 g, 0.104 mol) in tetrahydrofuran (THF, 280 mL) was added and the reaction was stirred overnight at 22 °C. The crude product was extracted with diethylether and washed with 0.5 M H_2SO_4, followed with brine, and then dried over Na_2SO_4, filtered, and concentrated. The crude extract was filtered through a column of 15 × 7 cm SiO_2 with a mixture of 9:1 pentane/diethylether. Compound **8**, 2.1 g (yield 11.7%), was obtained with a GC purity of 80%. The major compounds formed were methyl (2*E*,4*E*)-5-(1-cyclopentene-1-yl)-2,4-pentadienoate and methyl (2*E*,4*Z*)-5-(1-cyclopentene-1-yl)-2,4-pentadienoate.

GC retention indices: I_{SPB-1} 1390 and I_{SPWAX} 1933. ^{1}H NMR: 1.36 (1H, m, H-3), 1.44 (1H, m, H-2), 1.49 (1H, m, H-2′), 1.63 (1H, m, H-1), 2.11 (1H, m, H-1′), 2.16 (1H, m, H-3′), 2.85 (1H, ddd, J = 8, 10, 12 Hz, H-3a), 2.96 (1H, m, H-7a), 3.75 (3H, s, MeO−), 5.79 (1H, dd, J = 3, 9 Hz, H-7), 5.89 (1H, ddd, J = 3, 6, 9 Hz, H-6), 6.88 (1H, d, J = 6 Hz, H-5). ^{13}C NMR: 168.4 (C-10), 137.8 (C-7), 131.0 (C-4), 130.3 (C-5), 120.4 (C-6), 51.5 (MeO−), 38.4 (C-8), 36.4 (C-9), 34.6 (C-3), 34.4 (C-1), 22.7 (C-2). NOESY observed between H-7a and H-3a was in agreement with a *cis* configuration. MS: 178 (50, $M^{+\cdot}$), 149 (35), 147 (30), 119 (90), 105 (45), 91 (100), 77 (28).

Synthesis of (±)-Methyl *trans*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carboxylate, 9. To methyl 4-(triphenylphosphonio)crotonate bromide **7** (22 g, 0.05 mol) in 1,4-dioxane (100 mL) was added potassium carbonate (8.62 g, 0.062 mol) and the aldehyde **6** (4 g, 0.042 mol). The reaction mixture was heated at 70 °C during 5 h. The same workup was described as above. Compound **9**, 1.86 g (yield 24.9%), was obtained with a GC purity of 70%.

GC retention indices: I_{SPB-1} 1368 and I_{SPWAX} 1910. ^{1}H NMR: 1.39 (1H, m, H-1), 1.46 (1H, m, H-3), 1.79 (2H, m, H-2), 1.84 (1H, m, H-1′), 2.21 (2H, m, H-7a and H-3a), 2.26 (1H, m, H-3′), 3.75 (3H, s, MeO−), 6.08 (1H, m, H-6), 6.40 (1H, m, H-7), 6.97 (1H, m, H-5). ^{13}C NMR: 168.0 (C-10), 139.2 (C-7), 134.9 (C-5), 133.6 (C-4), 125.2 (C-6), 51.3 (MeO−), 44.5 (C-8), 42.8 (C-9), 27.2 (C-3), 26.4 (C-1), 22.7 (C-2). MS: 178 (45, $M^{+\cdot}$), 149 (28), 147 (28), 119 (90), 117 (35), 105 (20), 91 (100), 77 (15).

Synthesis of (±)-*cis*-(2,3,3a,7a-Tetrahydro-1*H*-inden-4-yl) Methanol, 11. Compound **8** (130 mg, 0.73 mmol) was reduced with Vitride with the same procedure described above. Compound **11**, 102 mg (yield 93.1%), was obtained with a GC purity of 88%.

GC retention indices: I_{SPB-1} 1311 and I_{SPWAX} 2134. ^{1}H NMR: 1.42 (2H, m, H-2 and H-3), 1.56 (2H, m, H-1 and H-2′), 2.05 (1H, m, H-3′), 2.09 (1H, m, H-1′), 2.51 (1H, m, H-3a), 2.88 (1H, m, H-7a), 4.12 (1H, d, J = 14 Hz, H-10), 4.15 (1H, d, J = 14 Hz, H-10′), 5.48 (1H, m, H-7), 5.74 (1H, m, H-6), 5.75 (1H, m, H-5). ^{13}C NMR: 140.9 (C-4), 130.3 (C-7), 120.5 (C-6), 116.5 (C-5), 66.0 (C-10), 38.4 (C-9), 38.1 (C-8), 34.8 (C-1), 34.0 (C-3), 23.2 (C-2). MS: 150 (40, $M^{+\cdot}$), 132 (39), 119 (58), 117 (60), 91 (100), 79 (28), 77 (22).

Synthesis of (±)-*trans*-(2,3,3a,7a-Tetrahydro-1*H*-inden-4-yl) Methanol, 10. Compound **9** (150 mg, 0.84 mmol) was reduced with Vitride using the same procedure described above. Compound **10**, 112 mg (yield 89%), was obtained with a GC purity of 90%.

GC retention indices: I_{SPB-1} 1299 and I_{SPWAX} 2115. ^{1}H NMR: 1.39, (2H, m, H-1 and H-3), 1.80 (2H, m, H-2), 1.81 (1H, m, H-1′), 1.87 (1H, m, H-3′), 2.11 (2H, m, H-7a and H-3a), 4.22 (1H, d, J = 13.5 Hz, H-10), 4.26 (1H, d, J = 13.5 Hz, H-10′), 5.91 (1H, m, H-5), 5.97 (1H, m, H-6), 6.06 (1H, m, H-7). ^{13}C NMR: 143.7 (C-4), 131.7 (C-7), 125.4 (C-6), 120.3 (C-5), 64.2 (C-10), 44.6 (C-9), 44.4 (C-8), 26.5 (C-1), 25.0 (C-3), 22.8 (C-2). MS: 150 (20, $M^{+\cdot}$), 132 (14), 119 (42), 117 (38), 91 (100), 79 (18), 77 (20).

Synthesis of (±)-*cis*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carbaldehyde, 2. The pure alcohol **11** (32 mg, 0.21 mmol) was oxidized with MnO_2 (185 mg, 2.1 mmol) in CH_2Cl_2 (0.5 mL). After 2 h of stirring at 22 °C, the reaction mixture was filtered through Celite and then through SiO_2 using dichloromethane. The filtration was performed using a glass pipet. Compound **2** was obtained with a GC purity of ∼80%; the major impurity was the aromatic compound **4** (GC < 20%).

Compound **2**. GC retention indices: I_{SPB-1} 1270 and I_{SPWAX} 1876. $GC/MS_{CP\text{-}ChirasilDex\ CB}$ R_T = 14.5 and 14.9 min. ^{1}H NMR: 1.26 (1H, m, H-3), 1.42 (1H, m, H-2), 1.50 (1H, m, H-2′), 1.59 (1H, m, H-1), 2.16 (1H, m, H-1′), 2.22 (1H, m, H-3′), 2.88 (1H, m, H-3a), 2.95 (1H, m, H-7a), 6.03 (1H, m, H-7), 6.04 (1H, m, H-6), 6.63 (1H, m, H-5), 9.50 (1H, s, H-10). ^{13}C NMR: 193.7 (C-10), 141.3 (C-4 and C-7), 140.1 (C-5), 120.4 (C-6), 38.4 (C-8), 34.6 (C-1 and C-3), 33.8 (C-9), 22.9 (C-2). MS: 148 (58, $M^{+\cdot}$), 119 (50), 105 (38), 92 (25), 91 (100), 77 (27).

Compound **4**. GC retention indices: I_{SPB-1} 1307 and I_{SPWAX} 1991. Significant signals from the mixture: ^{1}H NMR, 2.15 (2H, m, H-2), 2.94 (2H, m, H-1), 3.29 (2H, m, H-3), 7.32 (1H, m, H-6), 7.47 (1H, m, H-7), 7.62 (1H, m, H-5), 10.15 (1H, s, H-10); ^{13}C NMR, 192.8 (C-10), 146.3 (C-8 and C-9), 132.3 (C-4), 129.9 (C-7), 129.2 (C-5), 126.7 (C-6), 32.1 (C-1), 31.8 (C-3), 25.2 (C-2); MS, 146 (85, $M^{+\cdot}$), 145 (28), 117 (100), 116 (29), 115 (60), 91 (18).

Synthesis of (±)-*trans*-2,3,3a,7a-Tetrahydro-1*H*-indene-4-carbaldehyde, 1. The pure alcohol **10** (275 mg, 1.8 mmol) was oxidized with MnO_2 (770 mg, 8.6 mmol) in CH_2Cl_2 (30 mL). After 2 h of stirring at 22 °C, the reaction mixture was filtered through Celite and then on SiO_2 using dichloromethane. The filtration was performed using a glass pipet. Compound **1** (220 mg, yield 83%) was obtained with a GC purity of ∼98%.

GC retention indices: I_{SPB-1} 1254 and I_{SPWAX} 1848. $GC/MS_{CP\text{-}ChirasilDex\ CB}$: enantiomers **1a** R_T = 12.7 min and **1b** R_T = 12.9 min, at a ratio of 1:1. ^{1}H NMR: 1.37 (1H, m, H-1), 1.51 (1H, m, H-3), 1.83 (3H, m, H-1′ and H-2), 2.17 (2H, H-7a, H-3a), 2.36 (1H, m, H-3′), 6.24 (1H, ddd, J = 2.2, 4.7, 9.0 Hz, H-6), 6.56 (1H, d, J = 9.0 Hz, H-7), 6.77 (1H, J = 4.7 Hz, H-5), 9.56 (1H, s, H-10). ^{13}C NMR: 193.0 (C-10), 144.2 (C-5), 142.8 (C-4), 141.9 (C-7), 125.3 (C-6), 44.6 (C-8), 41.6 (C-9), 25.8 (C-3), 25.7 (C-1), 23.0 (C-2). MS: 148 (40, $M^{+\cdot}$), 119 (38), 117 (23), 115 (20), 105 (18), 92 (21), 91 (100), 77 (15).

Energy Calculations for 1 and 2. Each molecule was first minimized using the standard Monte-Carlo procedure as implemented in MacroModel using the OPLS_2005 Molecular Force Field (*23*). For each molecule, the lower energy conformer was then minimized at the density functional theory (DFT) level (B3LYP/6.31G**) using the Jaguar software (*24*). The DFT energies were then used to calculate the energy difference. Compound **1**, 463.491484HT; compound **2**, 463.499707HT (corresponding to an energy difference of 5.16 kcal or 21.60 kJ).

Synthesis of (3a*RS*,4*SR*,7a*RS*)-(Octahydro-1*H*-inden-4-yl) Methanol, 15. The alcohol **11** (200 mg, 1.3 mmol) was treated with hydrogen in the presence of methanol (6 mL) and PtO_2 (30 mg). In 1 h at 22 °C, 60 mL of hydrogen was absorbed. The reaction mixture was filtered through Celite, and the solvent was evaporated to give 200 mg of **15** (yield 97%).

GC retention indices: I_{SPB-1} 1316 and I_{SPWAX} 2006. ^{1}H NMR: 1.00 (2H, m, H-5, H-7), 1.21 (1H, m, H-6), 1.34 (1H, m, H-1), 1.39 (1H, m, H-7′), 1.42 (2H, m, H-2), 1.52 (1H, m, H-5′), 1.59 (1H, m, H-3), 1.64 (1H, m, H-1′), 1.70 (1H, m, H-6′), 1.71 (1H, m, H-3′), 1.84 (1H, m, H-7a), 1.88 (1H, m, H-4), 2.01 (1H, dddd, J = 10.0, 10.0, 5.1, 5.1 Hz, H-3a), 3.45 (1H, dd, J = 7.1, 10.5 Hz, H-10), 3.49 (1H, dd, J = 7.1, 10.5 Hz, H-10′). ^{13}C NMR: see **Figure 6**. MS: 136 (33, $M^{+\cdot} - H_2O$), 123 (60), 121 (41), 94 (28), 81 (100), 79 (28), 67 (52), 41 (22).

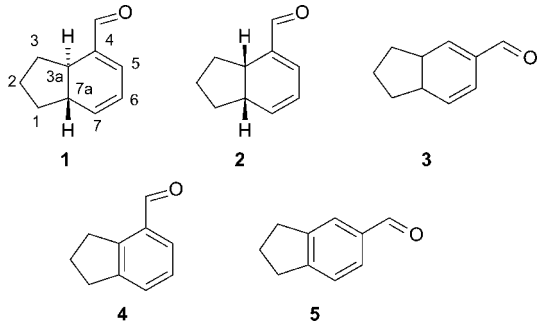


Figure 2. Structures of (±)-*cis*/*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde **1** and **2** and related structures **3**–**5**.

Synthesis of (3a*RS*,4*RS*,7a*SR*)-(Octahydro-1*H*-inden-4-yl) Methanol, 13, and (3a*RS*,4*SR*,7a*SR*)-(Octahydro-1*H*-inden-4-yl) Methanol, 14. The alcohol **10** (200 mg, 1.3 mmol) was treated with hydrogen in the presence of methanol (6 mL) and PtO_2 (30 mg). In 3 h at 22 °C, 50 mL of hydrogen was absorbed. The reaction mixture was filtered through Celite, and the solvent was evaporated. After filtration on SiO_2 (55:45 pentane/diethylether), we obtained 189 mg (yield of 90%) of a mixture of **13** and **14**, in a 77:23 ratio by ^{1}H NMR.

Compound **13**. GC retention indices: I_{SPB-1} 1275 and I_{SPWAX} 1920. ^{1}H NMR: 0.83 (1H, dddd, J = 11.3, 10.8, 10.8, 6.6 Hz, H-3a), 0.95 (1H, m, H-7), 0.98 (1H, m, H-5), 1.09 (1H, m, H-1), 1.10 (1H, m, H-7a), 1.15 (1H, m, H-3), 1.28 (1H, m, H-6), 1.30 (1H, m, H-4), 1.60 (2H, m, H-2), 1.74 (1H, m, H-1′), 1.82 (1H, m, H-6′), 1.85 (2H, m, H-3′ and H-5′), 1.88 (1H, m, H-7′), 3.44 (1H, dd, J = 7.0, 10.6 Hz, H-10), 3.67 (1H, dd, J = 4.3, 10.6 Hz, H-10′). ^{13}C NMR: see **Figure 6**. MS: 154 (3, $M^{+\cdot}$), 136 (23), 123 (95), 121 (28), 81 (100), 79 (25), 67 (45).

Compound **14**. GC retention indices: I_{SPB-1} 1310 and I_{SPWAX} 1989. ^{1}H NMR: 0.94 (1H, m, H-7), 1.04 (1H, m, H-1), 1.23 (1H, m, H-7a), 1.33 (4H, m, H-3, H-5, H-6 and H-3a), 1.55 (3H, m, H-2, H-2′ and H-6′), 1.60 (1H, m, H-3′), 1.73 (1H, m, H-1′), 1.90 (1H, m, H-7′), 1.92 (1H, m, H-5′), 2.07 (1H, m, H-4), 3.63 (1H, dd, J = 8.8, 10.6 Hz, H-10), 3.78 (1H, dd, J = 5.2, 10.6 Hz, H-10′). ^{13}C NMR: see **Figure 6**. MS: 136 (38, $M^{+\cdot} - H_2O$), 123 (25), 121 (98), 94 (41), 93 (45), 81 (100), 79 (48), 67 (62).

RESULTS AND DISCUSSION

Isolation of the Fraction Producing a Trigeminal Effect. The oleoresin prepared from the seeds was analyzed by gas chromatography and mass spectrometry. The major volatiles detected were 1-phellandrene, 1,8-cineol, α-terpineol, neral, (*E*)-2-decenal (co-eluting with geraniol), and geranial. They correspond respectively to peaks 1−6 (**Figure 1**). Peaks 7 (**1**) and 8 (**2**) (**Figure 2**) were unknown, and none of the published data helped to attribute structures to these compounds. Peak 9 had the same mass spectrum as peak 10, and it was attributed to 4-indanecarbaldehyde (**4**); peak 10 was 5-indanecarbaldehyde (**5**); peaks 11−13 were geranyl acetate, (*Z*)-2-decenyl acetate, and (*E*)-2-dodecenal, respectively; peak 14 was unknown; and peak 15 corresponded to nerolidol. These attributions were based on a comparison to the mass spectra of the authentic sample and to the retention indices on both polar and apolar columns. The oleoresin was tasted at 50 ppm in water, and an irritating, pungent, biting effect was perceived.

This extract was fractionated by flash chromatography, and each fraction was tasted using smelling strips. The fractions presenting some pungency were pooled. The major compound was the unknown peak 7, which has a molecular ion of *m*/*z* 148, and the elemental analysis suggested a raw molecular formula of $C_{10}H_{12}O$. The fragmentation pattern (*m*/*z* 105, 91, and 77) is typical of compounds having a benzene ring; the ion at *m*/*z* 119 corresponds to a loss of −CHO. This fraction was

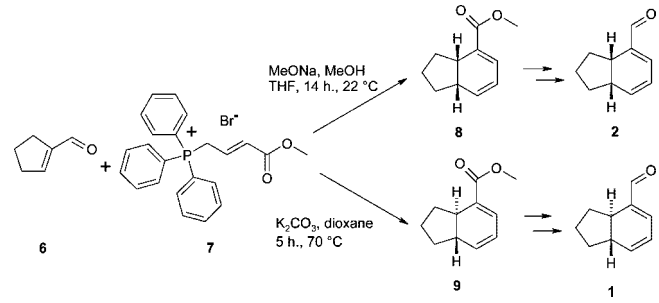


Figure 3. Preparation of compounds **1** and **2**.

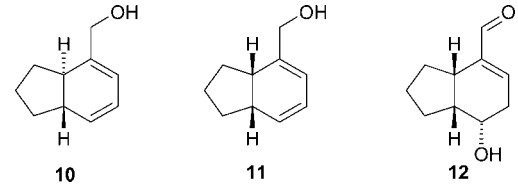


Figure 4. Chemical structures of **10** and **11** obtained either by the reduction of esters **9** and **8** or by the reduction of natural aldehydes **1** and **2**. Compound **12** corresponds to the chemical structure of tsaokoin (*11*).

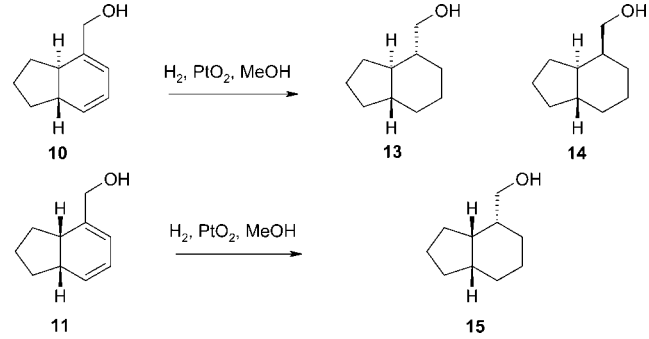
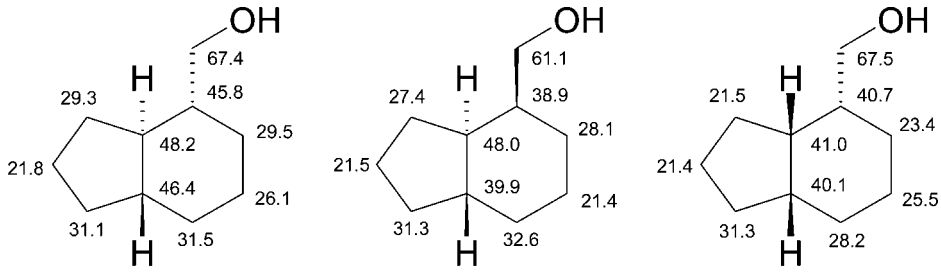


Figure 5. Catalytic hydrogenation of **10** and **11**. Relative stereochemistry of three of the eight possible diastereoisomers.

then fractionated by medium-pressure chromatography. This allowed for a partial structure elucidation of peak 7 by ^{1}H and ^{13}C NMR. The HMBC and COSY NMR 2D correlations showed the backbone of the 8,9-dihydroindane, but the correlations between H-1′, H-2, H-7a, and H-3a were not clear because of overlapping signals. The presence of both 4-indanecarbaldehyde (**4**) and 5-indanecarbaldehyde (**5**) (peaks 9 and 10) (**Figure 1**) in the oleoresin, suggested a possible position for the carbaldehyde on C-4 (**1** or **2**) as well as on C-5 (**3**). The presence of both compounds **4** and **5** has already been reported in *A. tsao-ko* (*25*). It was difficult to obtain peak 7 in high purity, because it was not stable on preparative GC or on reverse-phase preparative high-performance liquid chromatography (HPLC). Therefore, an enriched fraction containing peaks 7 and 8 was reduced with sodium borohydride to transform the aldehyde functionality into a hydroxyl group. The reaction mixture was purified by flash chromatography on SiO_2. The NMR spectrum of the major stereoisomer (reduced peak 7) based on HMBC correlations between C-10 and C-5 and C-9 confirms the presence of the functional group on C-4. It was still not possible to determine the *cis* or *trans* configuration of reduced peak 7 because H-7a and H-3a both have the same chemical shift at 2.11 ppm. The only possible way to determine whether reduced peak 7 corresponds to structure **10** or **11** was by synthesis.

Synthesis of the Authentic Samples (Figure 3). The Wittig reaction with cyclopentene-1-carbaldehyde **6** and methyl 4-(triphenylphosphonio)crotonate bromide **7**, deprotonated with



13 14 15

Figure 6. Display of key elements of ^{13}C NMR chemical shifts of indan-4-yl methanol **13–15**.

sodium methylate in methanol, gave mainly (±)-methyl *cis*-2,3,3a,7a-tetrahydo-1*H*-indene-4-carboxylate **8** (*22*). The NOESY experiment suggests an interaction between H-7a and H-3a, indicating a *cis* stereochemistry, but this evidence alone was insufficient to prove the *cis* stereochemistry. Compound **8** was thus reduced to give **11**. When the Wittig reaction conditions were modified, using K_2CO_3 as a base, it was possible to prepare **9**. The chemical pathway to the 8,9-dihydroindane implies the formation of two double bonds by the Wittig reaction and then cyclization via an intramolecular electrocyclic reaction. Changing the conditions of the Wittig reaction influences the stereochemistry of the pericyclic reaction that leads to the formation of the 8,9-dihydroindane. Using potassium carbonate and dioxane instead of sodium methylate, the reaction gave two products corresponding to noncyclic structures and compound **9** as a side product, which was latter purified, fully analyzed by ^1H and ^{13}C NMR, and then reduced to give **10**. The retention indices of reduced peak 7 ($I_{SPB\text{-}1}$ 1299 and I_{SPWAX} 2115) as well as the ^1H and ^{13}C NMR spectra were compared to those of synthetic **10** and **11**. All data clearly indicate that reduced peak 7 corresponds to the *trans* compound **10** (**Figure 4**).

Synthetic compounds **10** and **11** were oxidized in ethanol with MnO_2 to give **1** and **2**. Again, the retention indices of the natural peak 7 corresponded perfectly to those of the synthetic compound **1** (*trans*) and not compound **2** (*cis*). Compound **2** is more stable than **1** ($\Delta E = 5.16$ kcal) (*24, 25*), which explains the interconversion of **1** into **2**, as observed in extracts of *A. tsao ko* stored 6 months at 4 °C. The oxidation of **11** produced **4** as a side product.

A total of 10 trained panelists tasted both **1** and **2** in plain water at 10 and 50 ppm. At 10 ppm, a strong, short lasting pungency was perceived for compound **1** but not for compound **2**. The tasting was repeated at 50 ppm, and compound **2** was surprisingly still not pungent, except for one panelist, who perceived a faint throat-burning effect. The odor was described as fatty, fishy, fresh almond for compound **1**. Compound **2** is also almond but with a more phenolic, coumarin type of odor. The hydroxyl derivatives **10** and **11** were tasted under the same conditions and were neither hot nor pungent. Compound **10** had a green, fishy, oyster odor, and compound **11** was terpenic, earthy, and citrus. Compounds **10** and **11** were not detected in the natural extract. Compound **1**and **2** obtained by synthesis, as well as natural **1** and **2**, were injected onto a chiral capillary column. This analysis showed that both enantiomers of **1** and **2** are present in *A. tsoa ko* as a racemic mixture.

We received different qualities of Amomums: the *A. tsoa ko* from China and Vietnam and *A. rui li* and *A. he ko* from China. Compound **1** was present at a slightly higher concentration (approximately 0.12–0.09%) in the *A. tsao-ko* qualities compared to the *A. he ko* and *A. rui li* qualities (approximately 0.08–0.05%).

Compound **12** (tsaokoin), which is compound **2** hydroxylated on the C-7 position (**Figure 4**), has a closely related structure and was previously isolated by bioassay-guided purification of the *A. tsoa ko* extract (*26*). Peak 14 (**Figure 1**) had a mass spectrum with *m*/*z* 166 corresponding to the molecular weight of **12** and *m*/*z* 148, 119, 105, and 91, which are exactly the same fragments as those of **1** and **2**. Therefore, we isolated peak 14 from our samples of *A. tsoa ko*. The ^1H and ^{13}C NMR spectra confirm the structure **12**, in full agreement with Moon et al. (*26*). Tsaokoin **12** was tasted at 50 ppm in plain water, and no trigeminal effect was detected. Tsaokoin **12** has a *cis* configuration. Isotsaokoin, the other *cis* diastereoisomer (3a*RS*,7*SR*,7a*SR*)-7-hydroxy-2,3,3a,6,7,7a-hexahydro-1*H*-indene-4-carbaldehyde was also detected in black cardamom.

A very recent paper (*27*) reported for the first time the preparation of **2**, but their ^1H and ^{13}C NMR data corresponds to **1**, according to our interpretation. We decided to confirm our results concerning the *trans* stereochemistry of **1** by catalytic hydrogenation of **10** and **11** (**Figure 5**). Platinum oxide was preferred to palladium to avoid any double-bond migration. From **10**, two isomers were obtained in a 4:1 ratio and were fully characterized as compounds **13** and **14** (**Figure 6**). This result supports the *trans* configuration of **10**, because of the lack of a good facial differentiation for the hydrogenation reaction. Conversely, we expected to obtain only one diastereoisomer from the reduction of **11** because of the good facial discrimination presented by the *cis* configuration. This was indeed the case, and **15** was isolated and fully characterized. The ^{13}C NMR of **15** shows C-8 and C-9 at 40.1 and 41.0 ppm, respectively, whereas there is a clear downfield shift for C-8 and C-9 at 46.4 and 48.2 ppm for **13** (**Figure 6**), in agreement with a *trans*-indanyl stereochemistry (*28*). The ^1H NMR of **13** shows a signal at 0.83 ppm for H-3a, with four coupling constants (ddd, $J = 11.3$, 10.8, 10.8, and 6.6 Hz), which also confirm a *trans* configuration. All of these results clearly differentiate the stereochemistries of **13**, **14**, and **15** and prove that Hong et al. (*27*) synthesized **1** instead of **2**.

To the best of our knowledge, the pungent active compound **1** found in *A. tsao-ko* has never been described before in *Amomum* sp. or in any other natural source.

ACKNOWLEDGMENT

We thank Dr. Horst Sommer for careful discussions, Dr. Jean-Yves de Saint Laumer for energy calculations, Dr. Estelle Delort for elemental analysis, and Mrs. Sandy Frank, apprentice, and Dr. Didier Roguet, curator of the botanical garden of Geneva, Switzerland, for botanical discussions.

LITERATURE CITED

(1) Purves, D.; Augustine, G. J.; Fitzpatrick, D.; Katz Lawrence, C.; LaMantia, A. S.; McNamara, J. O.; Williams, M. S. The chemical senses. *Neuroscience*, 2nd ed.; Sinauer Associates, Inc.: Sunderland, MA, 2001; Chapter 15, pp 317–344.

(2) Rentmeister-Bryant, H.; Green Barry, G. Percieved irritation during ingestion of capsaicin or piperine: Comparison of trigeminal and nontrigeminal areas. *Chem. Senses* **1997**, *22*, 257–266.

(3) Dhaka, A.; Viswanath, V.; Patapoutian, A. TRP ion channels and temperature sensation. *Annu. Rev. Neurosci.* **2006**, *29*, 135–161.

(4) Peterlin, Z.; Chesler, A.; Firestein, S. A. Painful TRP can be a bonding experience. *Neuron* **2007**, *53*, 635–638.

(5) Clapham, D. E. TRP channels as cellular sensors. *Nature* **2003**, *426*, 517–524.

(6) McKermy, D. D.; Neuhausser, W. M. Julius David identification of a cold receptor reveals a general role for TRP chanels in thermo sensation. *Nature* **2002**, *416*, 52–58.

(7) Zucker Charles, S. A cool ion channel. *Nature* **2002**, *416*, 27–28.

(8) Szallasi, A.; Blumberg, P. M. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol. Rev.* **1999**, *51*, 199–211.

(9) Bryant, B.; Mezine, I.; Epple, G. M. Trigeminal sensory stimuli and animal repellents from plants. Patent WO 99/21425, 1999.

(10) Galopin, C. C.; Furrer, S. M.; Goeke, A. Pungent and tingling compounds in Asian cuisine. ACS Symposium Series 867, American Chemical Society, Washington, D.C., 2003; pp 139–152.

(11) Barnes, C. S.; Loder, J. W. The structure of polygodial: A new sesquiterpene dialdehyde from *Persicaria hydropiper* L. *Aust. J. Chem.* **1962**, *15*, 322–327.

(12) Trevisani, M.; Smart, D.; Gunthorpe, M. J.; Tognetto, M.; Barbieri, M.; Campi, B.; Amadesi, S.; Gray, J.; Jerman, J. C.; Brough, S. J.; Owen, D.; Smith, G. D.; Randall, A. D.; Harrison, S.; Bianchi, A.; Davis, J. B.; Geppetti, P. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. *Nat. Neurosci.* **2002**, *5*, 546–551.

(13) Connell, D. W.; Sutherland, M. D. A re-examination of gingerol, shogaol and zingerone, the pungent principle of ginger (*Zingiber officinale* Roscoe). *Aust. J. Chem.* **1969**, *22*, 1033–1043.

(14) Connell, D. W. Natural pungent compounds III. The paradols and associated compounds. *Aust. J. Chem.* **1970**, *23*, 369–376.

(15) Yang, X.; Eilerman, R. G. Pungent principal of *Alpinia galangal* (L.) Swartz and its applications. *J. Agric. Food Chem.* **1999**, *47*, 1657–1662.

(16) Lawrence, B. M. Terpenes in two *Amomum* species. *Phytochemistry* **1970**, *9*, 665.

(17) Volatile Compounds in Food (VCF): database; Nijssen, L. M., van Ingen-Visscher, C. A., Donders, J. J. H., Eds.; version 9.2., TNO Quality of Life, Zeist, The Netherlands, 2007; p 1963.

(18) Dung, N. X.; Bien, L. K.; Leclercq, P. A. The essential oil of *Amomum tsao-ko* Crevost et Lemarie from Vietnam. *J. Essent. Oil Res.* **1992**, *4*, 91–92.

(19) Yi, Z.; Qin, Q.; Guoying, Z.; Zhonghua, X.; Tingli, L. Study on chemical constituents of volatile oils from *Amomum tsao-ko* Crevost et Lemaire. *Zhongcaoyao* **2004**, *35*, 1225–1227.

(20) Zhuqiang, L.; Lei, L.; Wanghua, D.; Rong, H.; Yuanqing, Z. Chemical constituents of essential oil on *Amomum tsao-ko* from Yunnan Province. *Yunnan Zhiwu Yanjiu* **1998**, *20*, 119–122.

(21) Essential oil from seeds of *Amomum tsao-ko* Crevost et Lemarie (Zingiberaceae). II. Oxygen-containing compounds. Sofiya 1991, *31*, 203–211.

(22) Bohlmann, F.; Zdero, C. Ein neue Synthese von Cyclohexadienderivaten. *Chem. Ber.* **1973**, *106*, 3779–3787.

(23) MacroModel, 9.1 ed.; 2007, Schrödinger, LLC: New York, Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel. An integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(24) Jaguar, 6.5 ed.; Schrödinger, LLC: New York, 2005.

(25) Takido, M.; Yoshikawa, Y.; Yamanouchi, S.; Kimura, Y. 1*H*-Indene-2,3-dihydro-4-carboxaldehyde and 1*H*-indene-2,3-dihydro-5-carboxyaldehyde from the seeds of *Amomum* medium. *Phytochemistry* **1978**, *17*, 327–328.

(26) Moon, S. S.; Lee, J. Y.; Cho, S. C. Isotsaokin, an antifungal agent from *A. tsao-ko*. *J. Nat. Prod.* **2004**, *67*, 889–891.

(27) Hong, B.-C.; Tseng, H.-C.; Chen, S.-H. Synthesis of aromatic aldehydes by organocatalytic [4 + 2] and [3 + 3] cycloaddition of α,β-unsaturated aldehydes. *Tetrahedron* **2007**, *63*, 2840–2850.

(28) Metzger, P.; Cabestaing, C.; Casadevall, E.; Casadevall, A. Ring distortion effect on δ carbon-13 values of bicycle[4.n.0]alkanes. *Org. Magn. Reson.* **1982**, *19*, 144–147.

Received for review September 12, 2007. Revised manuscript received November 1, 2007. Accepted November 1, 2007.

JF072707B