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Hemisynthesis of Dihydroumbellulols from Umbellulone: New Cooling Compounds

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Although menthol is a common ingredient used in food products, other molecules also evoke coolness through stimulation of the somatosensory system. To discover new molecules having cooling properties, we virtually screened the chemical structures of terpenes and sesquiterpenes to find structures that are similar to (−)-menthol. We realized that dihydroumbellulols could be good candidates. Although their occurrence was reported in *Hyptis pectinata* Poit, we were unable to obtain these molecules from the plant or to prove their natural occurrence. Therefore, we extracted (−)-(*R*)-umbellulone from *Umbellularia californica* Nutt. The (−)-(R)-umbellulone was reduced to prepare (1R,2*R*/*S*)-1-isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-ol, (1*R*,4*R*/*S*)-1-isopropyl-4-methylbicyclo-[3.1.0]hexan-2-one, and (1*R*,2*RS*,4*RS*)-1-isopropyl-4-methylbicyclo[3.1.0]hexan-2-ols, named dihydroumbellulols. Sensory analysis suggested that (1*R*,2*R*,4*S*)-dihydroumbellulol has a pleasant, trigeminal cooling effect, about 2−3 times less cooling than (−)-menthol, with a weak odor slightly reminiscent of eucalyptol. In addition, a previously unreported compound was discovered, (−)-(1*R*)-1-isopropyl-4-methylenebicyclo[3.1.0]hexan-2-one.

KEYWORDS: Trigeminal; cooling; umbellulone; dihydroumbellulols; *Umbellularia californica* Nutt

INTRODUCTION

A cooling effect can be produced by several molecules, mainly derived from terpenes and sesquiterpenes (*1*, *2*). The coolest natural molecule is apparently (−)-menthol (**1**). Other structural analogues bearing an oxygen atom in position 3 of the *p*-menthane framework have cooling activity. (−)-Isopulegol (**2**) has one-fifth as much cooling power as (−)-menthol and is used commercially in combination with (+)-*cis*- and (−)-*trans*-*p*-menthane-3,8-diol (**3**). Another natural cooling molecule is (−)-menthone (**4**), which is weakly cooling and comparable to (±)-piperitone (**5**) (*2*). Several other terpenes, such as verbenol (**6**) and eucalyptol (**7**), are slightly cooling (**Figure 1**), and only one sesquiterpene is described as cooling, cubebol (**8**) (*3*). Many artificial cooling compounds are menthol derivatives (e.g., esters, ethers, ketals) (*4*−*10*) or have very different chemical structures (*11*−*14*). Such compounds are not discussed in this paper.

The flavor industry is intent on finding new molecules that produce trigeminal effects. Two approaches are followed to discover new natural molecules: high-throughput screening of plant extracts, with the help of cell-based assays, or a traditional method that consists of focusing only on botanical species having specific taste properties. The question is how to find these plants. Undertaking a botanical trek is an option, as in having discussions with ethnobotanists or chefs. The approach presented in this paper is quite different, however, because the idea came from investigations of chemical structures. We noticed a structure similarity between dihydroumbellulols and menthol. Unfortunately, the occurrence of these molecules was described only in *Hyptis pectinata* Poit by Malan et al. (*15*). We obtained this plant to isolate the dihydroumbellols, but it did not contain these molecules. However, the similarity between menthol and dihydroumbellol can also be seen in (−)-menthone (**4**) and umbellulone **10**; therefore, we searched for a natural occurrence of umbellulone **10**.

The presence of (−)-(*R*)-umbellulone (**10**) in *Umbellularia* essential oil was reported by Power and Lees in 1904 (*16*). The natural occurrence of (−)-(*R*)-umbellulone (**10**) is frequently mentioned from many different plants, such as cold-pressed mandarin peel oil from Cuba (*17*), *Lippia sidoides* Cham. (*18*, *19*), *Cupressus lusitanica* Mill. (*20*), *Tanacetum cadmeum* (Boiss.) Heywood (*21*−*23*), *Daucus gingidium* L. subsp. *gingidium* (*24*), and *Cymbopogon jwarancusa* (Jones) Schultz (*25*). (−)-(*R*)-Umbellulone (**10**) is also present in the bark of *Umbellaria californica* (Hook. & Arn.) Nutt (*26*). Umbellulols **13** and **14** are also reported to be present in nature in *C. lusitanica* Mill. (*27*), *Salvia officinalis* L. (*28*), *Zanthoxylum simulans* Hance fruits (*29*), and *Eucalyptus camaldulensis* var. *brevirostris* (Miq.) Blakely (*30*), but most investigators have not discussed which stereoisomer was present or have indicated that the assignment was only tentative.

The first preparation of dihydroumbellulols from umbellulone was reported in a paper by Wienhaus and Todenhofer in 1929, in which they described an odor resembling isomenthol, but no taste characteristics were discussed (*31*). The present paper describes the preparation of dihydroumbellulols, clarifies data about their

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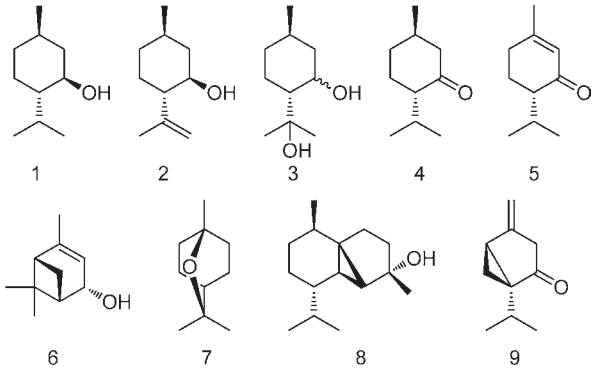

Figure 1. Cooling compounds with a terpenyl skeleton.

absolute configurations, and describes the taste evaluation of their cooling properties.

MATERIALS AND METHODS

General. Commercially available reagents and solvents of adequate quality were used without further purification. Mineral water for tasting was from Henniez (Henniez, Switzerland). Optical rotations were recorded with a Perkin-Elmer 241 polarimeter, with the cell thermostated at 20 °C (l = 0.1 dm).

^{1}H and ^{13}C NMR Spectra. The NMR spectra were recorded on a Bruker Avance-500 spectrometer (Fällanden, Switzerland) at 500.13 and 125.76 MHz or on a Bruker DPX-400 at 400.13 and 100.61 MHz. The solvent was $CDCl_3$. δ values are in parts per million downfield from $(CH_3)_4Si$ (= 0 ppm). The assignments by correlated spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond coherence (HMBC) experiments were performed with standard Bruker software (XWINNMR 3.1).

Gas Chromatography (GC)−Electron Impact−Mass Spectrometry (MS). An Agilent-GC-6890 system connected to an Agilent-MSD-5973 quadrupole mass spectrometer (Palo Alto, CA) was operated at ca. 70 eV. Helium was the carrier gas set at a constant flow rate of 0.7 mL/min. Separations were performed on fused-silica capillary columns, coated with either SPB-1 or Supelcowax (Supelco, Buchs, Switzerland; 30 m × 0.25 mm i.d., 0.25 μm). The standard oven program was as follows: 50 °C for 5 min, increased to 240 °C at 5 °C/min, and then held at 240 °C. Linear retention indices (LRIs) were calculated by linear interpolation from the retention times of the analytes and the two closest alkanes. The GC-chiral column was a fused-silica capillary column (Beta Dex, Supelco, 30 m × 0.25 mm i.d., 0.25 μm). The oven temperature was programmed at 50 °C for 5 min and then increased to 220 °C at 5 °C/min. Mass spectra are listed as follows: fragment ions m/z (relative intensity).

Preparation of the Essential Oil. The leaves were collected in August 2008 from the Botanical Garden of Geneva, Switzerland (CJBG coll. no. 19861536). Two weeks after collection, the leaves (381 g) were ground in the presence of water (2.3 L) in a KitchenAid food processor. The water was then distilled off under vacuum on a rotary evaporator at 50 °C. More water was added to the remaining dry green residue and redistilled off. The distillate was then saturated with NaCl and extracted with pentane twice. The organic phase was dried on $MgSO_4$ and filtered. The solvent was removed by distillation on a Vigreux column and a crude oil obtained (13.64 g).

Identification of (−)-(1*R*)-1-Isopropyl-4-methylenebicyclo[3.1.0]-hexan-2-one (9). The essential oil (6.4 g) was flash chromatographed on SiO_2 (512 g) with 95% toluene and 5% tetrahydrofuran (THF). Fraction 1 (0.87 g) contained the unknown peak at 20% purity by GC−total ion current (TIC) peak area. (−)-(*R*)-Umbellulone (**10**) was in the next fraction (2.97 g, 71% purity by GC). Both fractions were further purified.

Fraction 1 was then rechromatographed on SiO_2 (79 g) (silica 32-63, 60 A, Brunschwig, Basel, Switzerland) with the same solvent system to give the unknown (158 mg, 90% purity by GC-TIC). Finally, this fraction was injected 10 times on prep-GC (Varian Star 3600, column 2.5 m, i.d., 0.3 cm, phase SP2100 10% on a Chromosorb 80−100 mesh). Injectors and detectors were set at 250 °C. The program started at 120 °C for 20 min and then increased to 240 °C at 20 °C/min. The structure of **9** was elucidated by ^{1}H NMR: δ 0.94 (3H, q, J = 6.8 Hz, H-9), 0.96 (3H, q, J = 6.8 Hz, H-8), 1.11 (1H, dd, 4.8, 4.0 Hz, H-6), 1.31 (1H, ddd, J = 7.8, 4.8, 2.0, H-6′), 2.09 (1H, sept, J = 6.8 Hz, H-7), 2.46 (1H, dd, J = 7.8, 4.0, H-5), 2.68 (1H, ddd, J = 21.2, 1.4 1.4 Hz, H-3), 2.92 (1H, dddd, J = 21.2, 2.4, 2.4, 2.0 Hz, H-3′), 4.76 (1H, br s, H-10), 5.01 (1H, br s, H-10′); ^{13}C NMR δ 18.4 (t, C6), 19.6 (q, C8), 19.6 (q, C9), 26.2 (d, C7), 33.5 (d, C5), 40.7 (t, C3), 46.7 (s, C1), 105.8 (t, C10), 142.9 (s, C4), 211.4 (s, C2); GC retention indices $LRI_{SPB\text{-}1}$ 1113 and LRI_{SPWAX} 1567; MS, 150 ($M^{\bullet+}$), 135 (15), 108 (100), 107 (80), 91 (65), 83 (45), 79 (70), 77 (55).

Isolation of (−)-(1*R*,5*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-one, (*R*)-Umbellulone (10). Fraction 2 (2.97 g) was also chromatographed on SiO_2 (270 g) by using the same solvent system. We obtained (−)-(1*R*,5*S*)-1-isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-one (**10**) (1.675 g, 98% purity by GC-TIC). Analyses were in agreement with published data (*37*). GC retention indices: $LRI_{SPB\text{-}1}$ 1143 and LRI_{SPWAX} 1637, $[\alpha]_D$ = −29° (c 1) EtOH.

Preparation of (1*R*,2*R*)-1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-ol (13) and (1*R*,2*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-ol (14). (−)-(*R*)-Umbellulone (**10**) (545 mg) was reduced with $LiAlH_4$ (39 mg) in Et_2O at 22 °C for 90 min. The reaction was poured on 1 M HCl at 0 °C. The compound was extracted twice with Et_2O, and the organic phases were dried on anhydrous Na_2SO_4, filtered, and concentrated. We obtained 0.412 mg of crude oil (yield = 75%), a mixture of isomers **13** (24%) and **14** (76%). Both isomers were separated by chromatography on SiO_2 (54 g) by using cyclohexane, EtOAc, and THF in a ratio of 93:6:1. (1*R*,2*R*)-Umbellulol (**13**) (114 mg, yield = 21%) and (1*R*,2*S*)-umbellulol (**14**) (152 mg, yield = 28%) were obtained. Analyses were in agreement with published data (*37*) (**Table 1**). GC retention indices: (1*R*,2*R*)-umbellulol (**13**), $LRI_{SPB\text{-}1}$ 1094 and LRI_{SPWAX} 1596; (1*R*,2*S*)-umbellulol (**14**), GC $LRI_{SPB\text{-}1}$ 1103 and LRI_{SPWAX} 1607.

Preparation of (1*R*,4*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-2-one (11) and (1*R*,4*R*)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-2-one (12; Trivial Name, Thujanone). (−)-(*R*)-Umbellulone (**10**) (1369 mg) was reduced in EtOH in the presence of Pd/C 5% (100 mg) with H_2 (148 mL, volume absorbed) at atmospheric pressure. The catalyst was filtered off on Celite and the solvent distilled. We obtained 0.662 g (yield 65%) of (1*R*,4*S*)-dihydroumbellulone (1*R*,4*S*)-2-thujanone (**11**); isomer **12** was also formed, but it represented < 10% by GC. Both isomers were separated on an SiO_2 medium-pressure Lobar B column by using pentane and Et_2O in 98:2 mixtures. Analyses were in agreement with published data (*37*) (**Table 1**). GC retention indices: (1*R*,4*S*)-2-thujanone (**11**), $LRI_{SPB\text{-}1}$ 1114 and LRI_{SPWAX} 1502, $[\alpha]_D$ = −76.5° (c 1) EtOH; (1*R*,4*R*)-2-thujanone (**12**), $LRI_{SPB\text{-}1}$ 1112 and LRI_{SPWAX} 1508.

Preparation of (1*R*,2*R*,4*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]-hexan-2-ol (15), (1*R*,2*S*,4*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-2-ol (16), (1*R*,2*R*,4*R*)-1-Isopropyl-4-methylbicyclo [3.1.0]hexan-2-ol (17), and (1*R*,2*S*,4*R*)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-2-ol (18). (−)-(*R*)-Umbellulone (**10**) (2.987 g) was submitted to catalytic hydrogenation as described earlier. After we removed Pd/C 5% by filtration, the crude mixture was directly treated with $NaBH_4$ (0.630 g) in EtOH at 20 °C for 15 h. The reaction mixture was then added to 1 M HCl at 0 °C and extracted with Et_2O. We obtained 1.874 g (yield = 73%) of a mixture of the four possible isomers: **15**, **16**, **17**, and **18** (74, 16, 4, and 6% by GC-TIC, respectively). These isomers (516 mg) were separated on Lobar B normal phase SiO_2 by using 2 L of a 95% pentane and 5% Et_2O mixture and then 0.5 L of a 9:1 mixture of solvent. We obtained all four pure isomers for analysis in an elution sequence of 7.7 mg of (1*R*,2*S*,4*R*)-**18**, 13 mg of (1*R*,2*S*,4*S*)-**16**, 189 mg of (1*R*,2*R*,4*S*) -**15**, and ∼1 mg of (1*R*,2*R*,4*R*)-**17**. GC retention indices: (1*R*,2*R*,4*S*)-dihydroumbellulol (**15**), $LRI_{SPB\text{-}1}$ 1103 and LRI_{SPWAX} 1576, $[\alpha]_D$ = −72.5° (c 1) EtOH; (1*R*,2*S*,4*S*)-dihydroumbellulol (**16**), $LRI_{SPB\text{-}1}$ 1119 and LRI_{SPWAX} 1613, $[\alpha]_D$ = −77.8° (c 1) EtOH; (1*R*,2*R*,4*R*)-dihydroumbellulol (**17**), $LRI_{SPB\text{-}1}$ 1108 and LRI_{SPWAX} 1589, $[\alpha]_D$ = +42°* (c 1) EtOH (*measured, −47.3° for a sample containing 78% of **15**); (1*R*,2*S*,4*R*)-dihydroumbellulol (**18**), $LRI_{SPB\text{-}1}$ 1109 and LRI_{SPWAX} 1581, $[\alpha]_D$ = +21.3° (c 1) EtOH (NMR in **Table 1**).

Preparation of (1*R*,2*S*,4*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]-hexan-2-ol 16 for Tasting. Ketone **11** (0.75 g, 4.9 mmol) was heated in the presence of aluminum isopropoxide (3.3 g, 16 mmol) in isopropanol (42 mL) at 90 °C for 48 h. After workup, compound **16** was purified by medium-pressure chromatography on a Lobar 440-37 LiChroprep Si 60 column (40−63 μm) (Merck, Darmstadt, Germany) and eluted with

Table 1. ^{1}H and ^{13}C NMR Assignments for Compounds **11**−**18**, Measured in $CDCl_3$[a]

	11			**12**			**13**		
position[b]	δ (multiplicity)		coupling const	δ (multiplicity)		coupling const	δ (multiplicity)		coupling const
C/H	δ ^{1}H	δ ^{13}C	*J*(HH), Hz	δ ^{1}H	δ ^{13}C	*J*(HH), Hz	δ ^{1}H	δ ^{13}C	*J*(HH), Hz
1		43.7 (s)			41.6 (s)			36.0 (s)	
2		215.0 (s)			215.2 (s)		4.96 (br s)	78.9 (d)	
3	1.73 (ddd)	40.8 (t)	18.0, 9.7, 1.5	1.63 (d)	41.8 (t)	18.0	4.93 (mc)	125.4 (d)	
3′	2.12 (dd)		18.0, 8.8	2.35 (ddd)		18.0, 7.6, 1.6			
4	2.49 (mc)	28.4 (d)		2.28 (mc)	28.8 (d)			148.8 (s)	
5	1.91 (mc)	31.8 (d)		1.7 (dd)	33.6 (d)	7.7, 4.5	1.49 (ddd)	31.4 (d)	7.4, 3.2, 1.4
6	0.91 (mc)	13.4 (t)		0.9 (mc)	17.6 (t)		0.46 (mc)	21.6 (t)	
6′	0.98 (mc)			1.09 (mc)			0.66 (dd)		7.4, 3.8
7	2.03 (sept)	25.8 (d)	6.7	1.99 (sept)	25.9 (d)	6.7	1.55 (sept)	31.5 (d)	6.7
8	0.92 (d)	19.8 (q)	6.7	0.9 (d)	19.4 (q)	6.7	0.97 (d)	19.5 (q)	6.7
9	0.89 (d)	19.5 (q)	6.7	0.95 (d)	19.8 (q)	6.7	1.00 (d)	20.0 (q)	6.7
10	1.08 (d)	18.2 (q)	6.7	1.06 (d)	22.6 (q)	6.7	1.75 (t)	16.3 (q)	1.4

	14			**15**			**16**		
position[b]	δ (multiplicity)		coupling const	δ (multiplicity)		coupling const	δ (multiplicity)		coupling const
C/H	δ ^{1}H	δ ^{13}C	*J*(HH), Hz	δ ^{1}H	δ ^{13}C	*J*(HH), Hz	δ ^{1}H	δ ^{13}C	*J*(HH), Hz
1		41.1 (s)			38.1 (s)			38.8 (s)	
2	4.42 (br s)	78.4 (d)		4.40 (t)	74.9 (d)	7.6	4.17 (d)	76.1 (d)	5.1
3	5.12 (br s)	123.6 (d)		0.74 (ddd)	39.1 (t)	13.1, 10.6, 7.6	1.07 (ddd)	39.8 (t)	14.3, 10.9, 5.1
3′				1.99 (ddd)		13.1, 7.6, 7.6	1.59 (dd)		14.3, 7.5
4		151.2 (s)		2.15 (mc)	31.2 (d)		2.5 (mc)	31.6 (d)	
5	1.61 (mc)	29.9 (d)		1.07 (mc)	28.6 (d)		1.29 (mc)	27.1 (d)	
6	−0.07 (mc)	22.6 (t)		0.22 (dd)	6.2 (t)	7.7, 5.1	0.09 (mc)	4.9 (t)	
6′	0.96 (dd)		7.1, 3.5	0.66 (dd)		5.1, 3.8	0.25 (dd)		8.0, 5.3
7	2.46 (sept)	24.0 (d)	6.7	1.56 (sept)	31.0 (d)	6.7	2.27 (sept)	24.3 (d)	6.7
8	0.72 (d)	18.5 (q)	6.7	0.97 (d)	20.0 (q)	6.7	0.99 (d)	21.0 (q)	6.7
9	1.11 (d)	22.3 (q)	6.7	0.98 (d)	20.6 (q)	6.7	0.67 (d)	18.4 (q)	6.7
10	1.81 (t)	16.4 (q)	1.4	0.93 (d)	17.9 (q)	6.7	0.97 (d)	17.6 (q)	6.7

	17			**18**		
position[b]	δ (multiplicity)		coupling const	δ (multiplicity)		coupling const
C/H	δ ^{1}H	δ ^{13}C	*J*(HH), Hz	δ ^{1}H	δ ^{13}C	*J*(HH), Hz
1		37.8 (s)			38.8 (s)	
2	4.55 (br s)	73.4 (d)		4.18 (d)	76.0 (d)	5.7
3	1.28 (mc)	39.2 (t)		1.31 (d)	39.8 (t)	14.9
3′	1.66 (dd)		13.3, 7.8	1.76 (ddd)		14.9, 7.8, 5.7
4	2.03 (mc)	32.6 (d)		2.06 (mc)	34.1 (d)	
5	0.91 (mc)	30.3 (d)		1.20 (dd)	29.2 (d)	8.1, 3.8
6	0.36 (dd)	10.7 (t)	7.9, 5.0	−0.07 (dd)	8.9 (t)	5.1, 3.8
6′	0.64 (dd)		5.0, 3.8	0.35 (dd)		8.1, 5.1
7	1.52 (sept)	31.0 (d)	6.7	2.25 (sept)	25.1 (d)	6.7
8	0.98 (d)	19.9(q)	6.7	1.02 (d)	21.2 (q)	6.7
9	1.03 (d)	20.7 (q)	6.7	0.73 (d)	18.7 (q)	6.7
10	0.94 (d)	22.6 (q)	7.0	1.13 (d)	23.5 (q)	6.7

[a] 2D experiments COSY, HSQC, and HMBC were performed for all compounds. [b] Numbering according to **Figure 2**.

hexane/Et_2O to give **16** (0.24 g, yield = 32%, 100% purity by GC). Fractions containing a mixture were not considered.

Preparation of (±)-Ethyl-1-isopropyl-2-(*N*-methoxy(*N*-methyl)-carbamoyl)cyclopropanecarboxylate (22). 2-Bromopropane (98.4 g, 0.8 mol) was added during 2 h at room temperature to 400 mL of THF and Mg turnings (19.2 g, 0.8 mol). After an additional 2 h, the clear solution was added to (MeO)MeNH·HCl (38.8 g, 0.4 mol) and **21** (63.7 g, 0.28 mol) in 520 mL of THF at −20 °C. The reaction was held at −10 °C for 1 h. After the usual workup, 63.8 g of **22** was obtained (64% purity by GC, crude yield = 95%) and used for the next step. ^{1}H NMR δ 4.13 (mc, 2H), 3.78 (s, 3H), 3.19 (s, 3H), 2.28 (br s, 1H), 1.78 (dd, *J* = 5.9, 4.7 Hz, 1H), 1.59 (sept, *J* = 6.8 Hz, 1H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.99 (mc, 1H); ^{13}C NMR δ 171.1 (s), 170.5 (s), 61.5 (q), 60.6 (t), 39.7 (s), 34.3 (d), 32.6 (q), 23.4 (d), 20.1 (q), 19.2 (q), 16.8 (t), 14.2 (q); MS: 198 ($M^{\bullet+}$), 183 (100), 155 (75), 55 (35), 83 (30), 109 (15).

Sensory Evaluation. *Subjects*. Thirty trained panelists from Firmenich S.A., Geneva, Switzerland, were asked to evaluate the perceived cooling intensity.

The tastings were performed in compliance with appropriate protocol, and informed consent was obtained for all the panelists.

Stimuli. Solutions of 100 and 50 mg/L of dihydroumbellulol **15** were prepared by prediluting the product using 2 and 1% ethanol, respectively, and were then diluted in mineral water (Henniez). Thus, the quantity of the alcoholic solution in the tasted solutions was 5 g/L (0.5%). Next, the ethanol concentration of the other tasted solutions, (−)-menthol (50 mg/L) and blanks, were adjusted to contain 0.5% ethanol. All dilutions were made on a w/w basis.

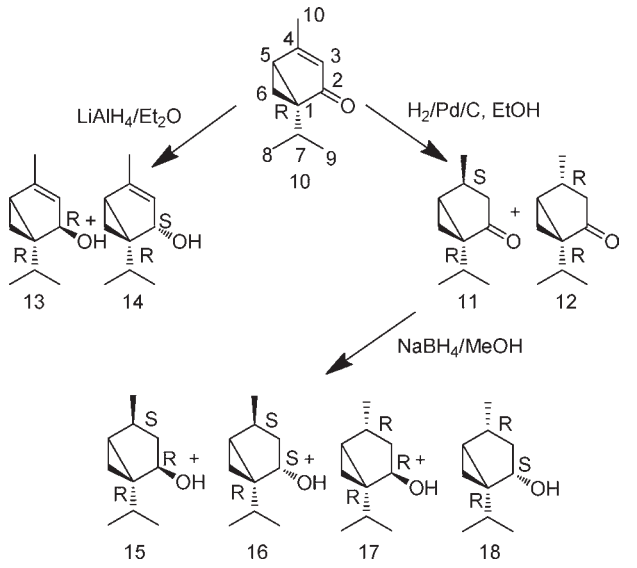

Figure 2. Preparation of dihydroumbellulol stereoisomers from optically active natural (*R*)-(+) umbellulone.

Testing Protocol. To avoid any fatigue or sensation overlap, subjects tasted only one sample per session. Each subject received a sample (30 mL) in a cup and was asked to put on a nose clip and then to sip the entire sample into their mouth and to start a timer at the same time. Subjects spit the sample out after 5 s. Starting when they had the sample in their mouth, they had to concentrate on the cooling perception. They evaluated the time at which they started to perceive the cooling sensation (T_{begin}), the maximum perceived cooling intensity and its corresponding time (I_{max} and T_{max}), the time when the cooling sensation started to decrease, and, finally, the perceived cooling intensity 3 min after they had sipped the sample (I_{end}) or the time when the cooling sensation disappeared, if it was < 3 min (T_{end}). Each evaluation was rated on a linear scale from *not at all* to *very intense*. Intensity answers were coded from 0 to 10. The solution of dihydroumbellulol **16** at 50 ppm was evaluated twice and that of the blank three times to demonstrate reproducibility of sensory measurements.

Statistical Analysis. Analysis of variance (ANOVA) tests (one-factor completely randomized design, followed by Duncan's multiple-comparison test) were performed on each parameter (T_{begin}, I_{max}, T_{max}, T_{dec}, I_{end}, and T_{end}) to compare data obtained from the cooling compounds and the blanks. The probability obtained for each of these tests indicates, for the parameter under consideration, whether the compounds have been perceived as significantly different or not. Significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Umbellularia californica leaves furnished 3.6% essential oil, which contains 30% eucalyptol, 30% (*R*)-umbellulone, and 8% of an unknown. The remaining compounds are α- and *β*-pinene, sabinene, myrcene, thymol, and methyleugenol, which is consistent with previous documented analysis (*31*−*34*). The unknown compounds had a GC-MS fragmentation pattern that was not documented in the National Institute of Standards and Technology (NIST) or the Wiley mass spectral library or in our Firmenich database. The fragmentation pattern was the same as for umbellulone but with 135 as the major fragment instead of 107. The molecular weight was also 150, which probably indicates the same raw molecular formula, $C_{10}H_{14}O$. Therefore, this compound was isolated by flash chromatography and further purified by preparative GC. The ^{1}H NMR clearly indicates the presence of two vinylic protons at 5.01 and 4.76 ppm, corresponding to an exocyclic double bond. This was confirmed by ^{13}C NMR displaying a singlet at 142.9 ppm and a triplet at 105.8 ppm. The presence of a triplet at 13.4 ppm in ^{13}C NMR and two protons at 0.91 and 0.98 in ^{1}H NMR unambiguously designated the structure as **9**.

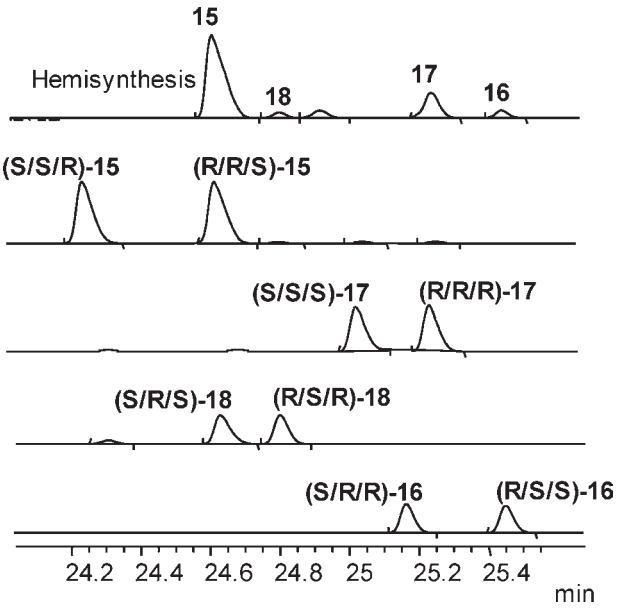

Figure 3. GC-FID trace (top) shows injection on a chiral column (Beta Dex 225, 30 m) of the crude mixture obtained after reduction of naturals **12** and **13**. The lower traces correspond to synthetic racemic dihydroumbellulols.

Catalytic reduction with H_2 Pd/C 5% gave the same **11** and **12** compounds obtained when umbellulone was used as the starting material, which confirms our assignation. Compound **9**, a regioisomer of **10**, is apparently a new structure, as it cannot be found in the Beilstein database or by using SciFinder. The odor profile of **9** is close to that of **10**, and when tasted at 50 mg/L in water, the same mild cooling trigeminal effect was perceived as for **10**. The smell of this essential oil is aggressive, which is representative of the odor of crushed fresh leaves.

The structural similarity of (−)-(*R*)-umbellulone (**10**) to (−)-menthone (**4**) prompted us to check whether the reduced forms produce a trigeminal effect. The reduction of the C=C double bond of (*R*)-umbellulone by catalytic hydrogenation leads to the formation of two diastereoisomers with a clear stereofacial preference for **11** (90% GC) and **12** (10% GC). This catalytic reduction of **10** was studied (*35*−*37*), and the stereochemistry of the major compound formed was assigned to **11**. We were able to purify **11** and **12** by medium-pressure chromatography to compare their ^{1}H and ^{13}C NMR data. The chemical shift of the methyl in position 4 is at 18.2 ppm for compound **11** and at 22.6 ppm for compound **12**. The methyl of compound **11** is more shielded and therefore shifted upfield as a result of the gamma effect. This finding is in agreement with published data for compound **11** (*38*) (**Figure 2**).

To have access to whole isomers of dihydroumbellulols, we used a second approach: we first reduced the carbonyl of umbellulone with $LiAlH_4$, which gave 25 and 75% of **13** and **14**, respectively. These two compounds were separated by flash chromatography and fully characterized by ^{1}H and ^{13}C NMR. The stereochemistry assignment was also in agreement with published data (*37*, *39*).

The mixture of compounds **12** and **13** was reduced with $NaBH_4$ to give **15**, **16**, **17**, and **18** (74, 16, 4, and 6%, respectively) (**Figures 2** and **3**). All isomers were isolated and fully characterized by ^{1}H and ^{13}C NMR (**Table 1**). The major difference is in the shielding of the C and H at position 7. The reduction of pure compound **11** gave only two compounds with fixed *S* stereochemistry at C4: **15** and **16**. Reduction by $NaBH_4$ or $LiAlH_4$ occurs by a transfer of hydride to the carbon; although the detailed nature of

the chemical complex is still not clear, this mechanism is now accepted. To prepare more of **16**, we reduced ketone **11** by the Meerwein−Ponndorf−Verley reaction, involving a cyclic transition state that can change the stereofacial attack of the hydride. This was in fact the case; the reduction of **11** was in favor of **16** (70%) over **15** (30%). Only isomers **15** and **16** were in sufficient quantities to be tasted by a panel of 25 subjects. The absolute configuration can be assigned to **15** and **16** on the basis of the chemical shift of the proton on C2, which is shifted upfield for the exo configuration **16** (*38*). To obtain isomers **17** and **18**, we reduced the compounds having a double bond between C3−C4 **13** and **14**, respectively, by catalytic hydrogenation in MeOH. We obtained **15** and **17** with a low yield from **13** as a result of the hydrogenation of the cyclopronane ring as a major pathway, and compound **14** gave, exclusively, a substituted cyclopentanol, as well as a substituted cyclopentanone.

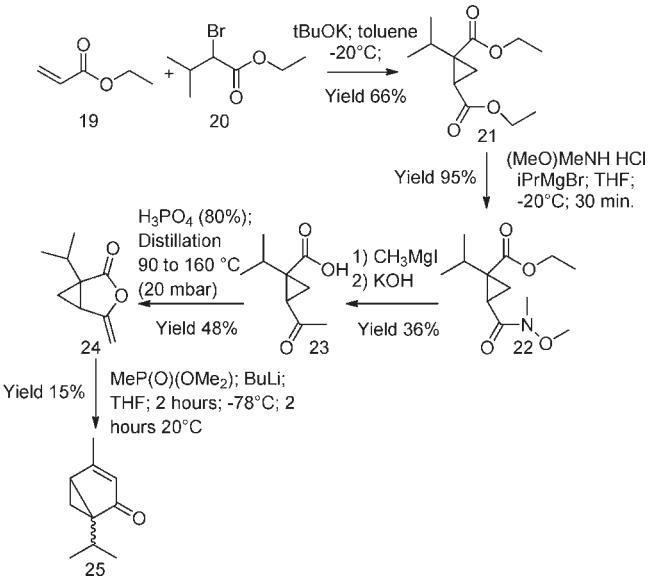

Figure 4. Synthesis of racemic umbellulone. Step 1, cyclopropanation, was performed according to the protocol of Bonavent et al. (*44*). Steps 2 and 3 are an adaptation of the protocol of Benayache et al. (*43*), but the remaining steps were done according to the protocol of ref *43*. THF, tetrahydrofuran.

To confirm that we obtained only optically active dihydroumbellulols, we decided to synthesize a racemic mixture. A staightforward synthetic route would have been to isomerize 4-methyl-2-(propan-2-ylidene)cyclopentanone in acidic conditions into 2-isopropyl-4-methylcyclopent-2-enone (*39*) followed by cyclopropanation. Unfortunately, we were not able to repeat the procedure described (*39*). Only four syntheses of racemic umbellulone have been reported. The most recent synthesis was published by Baeckstroem et al. in 1985 (*40*). This procedure described a photochemical transformation, over 40 h, of thymol in triflic acid. Umbellulone was obtained in a complex mixture. No isolated yield was reported. We repeated this experiment, and the percentage GC of umbellone was around 1%. Klein and Rojahn in 1965 reported the preparation of **10** from photo-oxidation of (+)-α-thujene; they obtained 19.5% of a mixture of regioisomers of hydroxylated (+)-α-thujene (*41*). The diazomethyl isopropyl ketone can be reacted with methyl methacrylate to give an intermediate, with 35% yield, which can then be converted to umbellulone, but the final yield was not reported (*42*). We tried to avoid working with dangerous diazo compound derivatives. A multistep synthesis was published by Benayache et al. in 1977 (*43*). This synthesis was repeated, starting from ethyl acrylate **19** and the bromo ester **20**, to give compound **21** (*44*). To avoid double addition, we prepared the Weinreb amide **22** and added organomagnesium to **22**, followed by saponification to give **23** in a moderate yield (36%). This was the only modification made to the published procedure (*44*). Lactone **24** was formed from **23** by

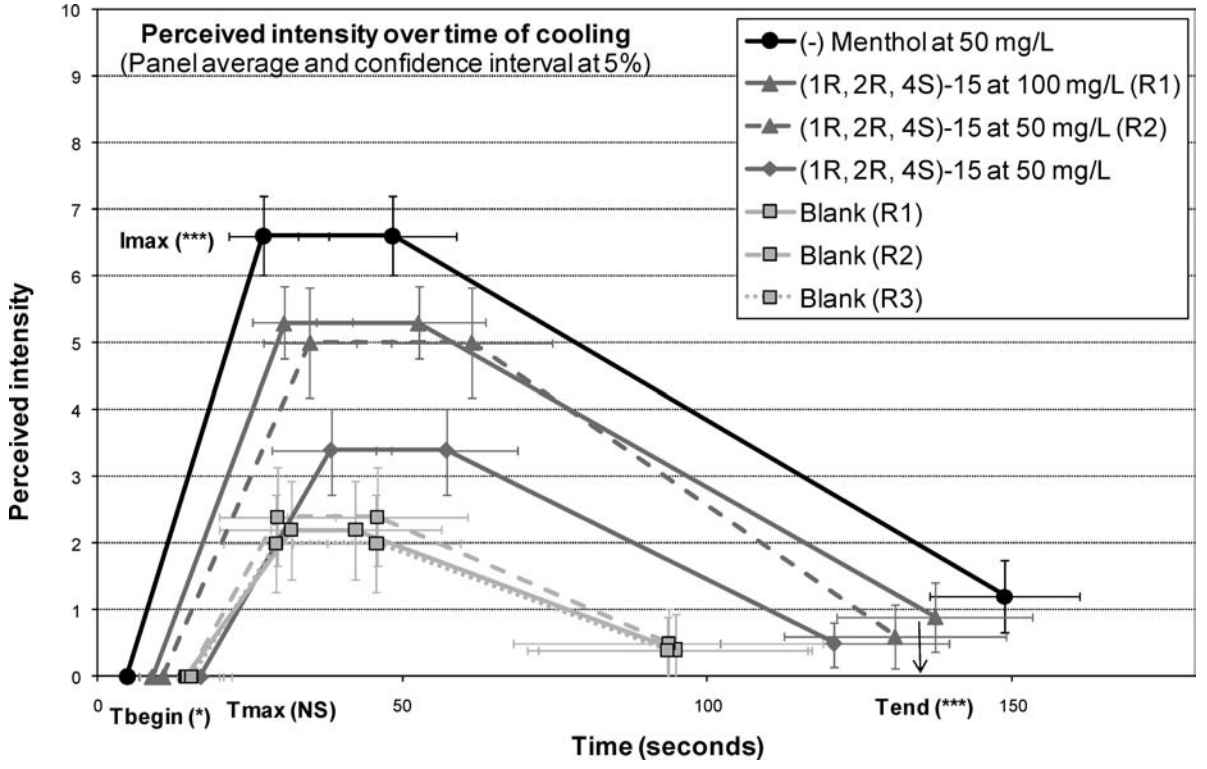

Figure 5. Perceived intensity: (−)-menthol at 50 mg/L, (1*R*,2*R*,4*S*)-**15** at 100 mg/L in two repetitions; (1*R*,2*R*,4*S*)-**15** at 50 mg/L; and three blanks. R, repetition.

dehydration in acidic conditions. Lactone **24** was then transformed into racemic umbellulone **10** by a Wittig-type reaction with poor yield (15%). The overall yield of the synthesis, repeated several times, was between 1 and 2%, which was enough to have all isomers on hand (**Figure 4**). The racemic dihydroumbellulols were prepared from the reduction of racemic umbellulone **25** in the conditions described for the optically natural (*R*)-umbellulone. The racemic mixture was tasted at 50 mg/L in water by five subjects. The cooling effect was comparable to the cooling effect of **15**.

All derivatives were tasted at 50 mg/L in water containing 2.5 g/L EtOH by a group of eight experienced panelists. After they assessed the trigeminal effect with the nose clip on, we asked them to remove it and to comment on the odor profile. (*R*)-Umbellulone (**10**) was described as slightly cooling and numbing with a thymus, minty, phenolic-like odor. The mixture of (*R*,*R*/*S*) **11**−**12** in a 9:1 ratio was described as having a cooling, fresh odor: minty, clove, medicinal. Compounds **13** and **14** were not cooling and refreshing, but described several times as having a sweet odor. This odor was also the weakest compared with those of the other compounds and described as terpenic, green, and medicinal. Finally, dihydroumbellulols **15**−**18** were the most cooling and refreshing, and the odor was described as weak, tarragon, green, earthy, woody, and terpenic. These results prompted us to better define the cooling effect of **15**, the major isomer, and to compare it with (−)-menthol.

Dihydroumbellulol **15** was tasted in water at two concentrations of 50 and 100 mg/L. The perceived intensity over time was determined by 30 subjects. The reference was (−)-menthol at 50 mg/kg. The solutions contained the ethanol (0.25%) used for dilution, and we noted that the blanks were also rated as cooling by some subjects. Three repetitions of the blank tasting (R1; R2; R3) showed this background noise (**Figure 5**).

This sensory evaluation confirmed the cooling effect of **15**, having the same type of cooling profile over time compared with (−)-menthol (no significant differences for the time parameters at a confidence level of 95% with ANOVA). The I_{max} values showed that **15** is weaker than the (−)-menthol by about 2−3 times (significant difference for I_{max}). Note that it is difficult to assess small differences for persistent trigeminal effects in the mouth by means of a panel of judges. Nevertheless, the cooling measurements for **15** at 100 ppm (two replicates) and for the blanks (three replicates) are reproducible (no significant differences regardless of the parameter). Furthermore, the two concentrations of **15** at 50 and 100 mg/L are well discriminated (I_{max} is significantly higher and T_{begin} smaller for the samples at 100 mg/L) (**Figure 5**). A synthetic racemic mixture of **15**−**18**, in the same ratio as for the optically actives, was also tasted by five judges, and no significant differences were noted.

In conclusion, we demonstrated for the first time that dihydroumbellulol **15** is cooling. Although their natural occurrence was reported by Malan et al. (*15*), we were not able to confirm it. Assessment of trigeminal effects, such as pungency, tingling, prickling, and cooling, is difficult because of the temporal chemesthetic sensation, which takes time to develop and decay (*45*, *46*). The most popular protocol to evaluate trigeminal effects is the "sip and spit" procedure, which was used in this study. Alternatively, half-tongue tasting with paper strips could be used to directly compare trigeminal intensities. We found that when subjects sipped water at room temperature, the rating of cooling intensity was not zero and was even higher with water containing 0.5% ethanol.

ABBREVIATIONS USED

COSY, correlated spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC heteronuclear multiple bond coherence; GC, gas chromatography; MS, mass spectrometry; LRI, linear retention index; THF, tetrahydrofuran; TIC, total ion current; ANOVA, analysis of variance.

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