Isolation, Structural Assignment and Insecticidal activity of (1S,2R,3R,4S) 1-Epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate, a Natural Product from *Minthostachys tomentosa*

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Abstract

With the aim to identify the active compounds of *Minthnostachys tomentosa* (1S,2R,3R,4S)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate, has been isolated as the responsible of the insecticidal activity against *Oncopeltus fasciatus*. This product was initially identified on the basis of spectral and synthesis. In order to confirm the stereochemistry, stereoselective synthesis of its enantiomer, (1R,2S,3S,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate, was carried out through a Sharpless reaction. Measurements of insecticidal activities of the different steroisomers revealed that only the natural product is active.

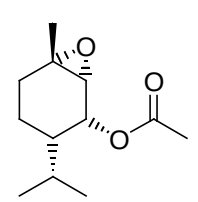
Key words: Minthnostachys, insecticidal, Sharpless, stereoselective, epoxide.

Introduction

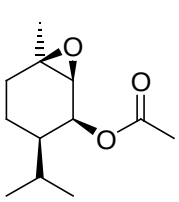
Natural products, with their overwhelming structural diversity, provide an important source of new and effective methods for controlling plagues. Hence, the isolation and identification of new plant metabolites have constituted essential steps towards obtaining new bioactive products.

In this context, certain spices and herbs are known to possess insecticidal activity[1-3], which is frequently present in the essential oil fraction. Among them, herbs belonging to the genus *Minthostachys* Griseb are used as natural insecticides by the local farmers of los Andes from Venezuela to Argentina[4-8].

We wish now to report the isolation from of (1S,2R,3R,4S) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate (**1**) *Minthostachys tomentosa*[9]. A natural product originally named 1,2-epoxymenthyl acetate and tentatively assigned to posses the same structural formula as **1** (without indication of the stereochemistry) has been previously isolated from *Mentha rotundifolia*[10]. The structural and stereochemical assignment of the product isolated in the present work from *Minthostachys tomentosa* is unambiguous and has been based on the independent enantioselective synthesis of its enantiomer (1R,2S,3S,4R) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate.



(1S,2R,3R,4S)-**1**



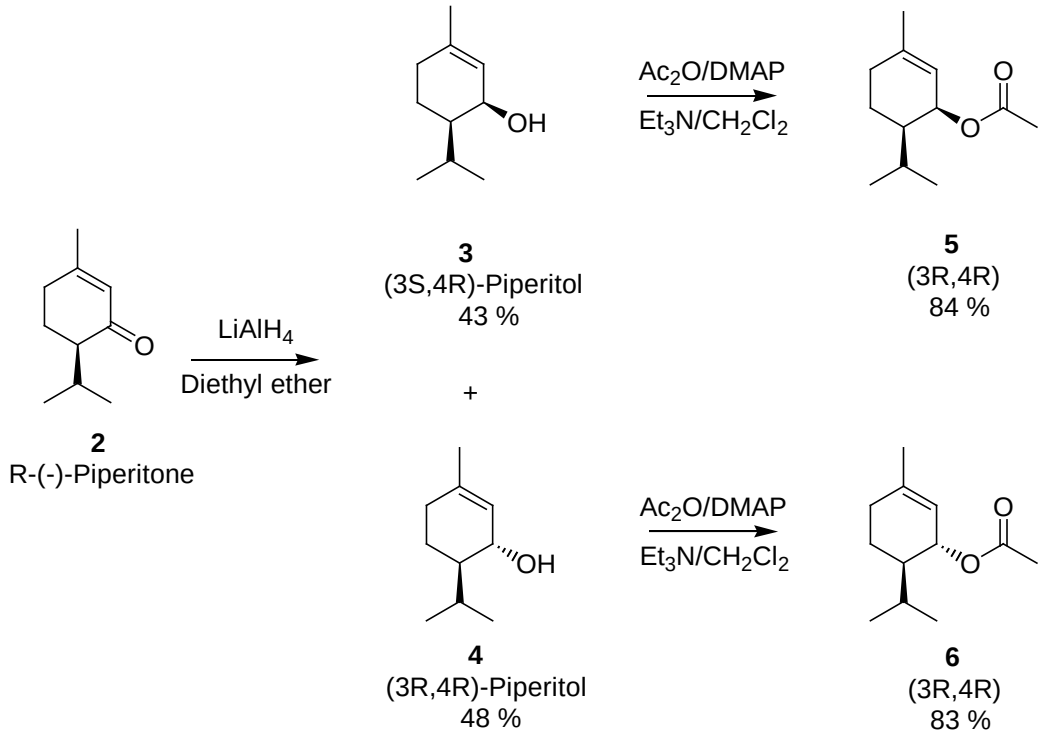
(1R,2S,3S,4R)-**1**

Results and Discussion

The volatile constituents of *Minthostachys tomentosa* were investigated for insecticide activity against *Oncopeltus fasciatus*. The search for the active product led to a fraction containing a major compound which was purified by semipreparative HPLC. The information obtained from the spectral data, together with the natural origin of the product, suggested a diterpene skeleton with two oxygenated groups.

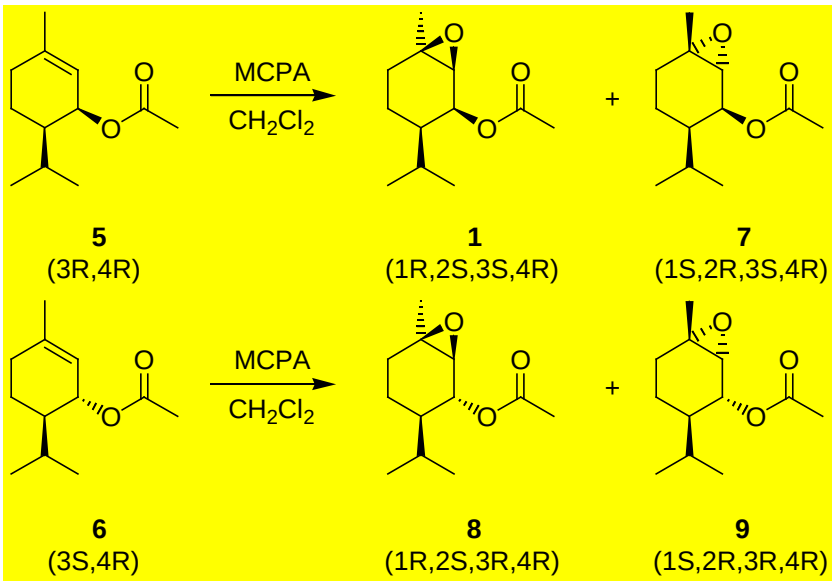
The structure **1** was assigned to the metabolite on the following basis. Its molecular formula, $C_{12}H_{20}O_3$ was established by HRMS (M^+, m/z 212.1418). The mass spectrum showed a fragment corresponding to elimination of acetic acid (*m/z* 152.1199 [$C_{10}H_{16}O$]). The presence of an ester carbonyl group was confirmed by the IR and ^{13}C NMR spectra, which showed bands at ν= 1757 cm^{-1} and δ= 171.0, ppm respectively. The ^{13}C NMR spectrum exhibited also distinct signals at δ= 68.6, 58.7 and 58.6 ppm corresponding to carbons bound to oxygen; two of them were tertiary and the other one quaternary according to the DEPT spectrum. In the ^1H NMR spectrum, the two deshielded methine protons appeared at δ= 5.1 (dd) and 3.3 (d) ppm. The above facts, together with the need to accomodate three insaturations, suggested the presence of an epoxide moiety. The two typical doublets of the isopropyl group at δ= 0.9 and 0.8 ppm were also observed.

As structure **1** presents four different asymetric carbons, a synthetic scheme was planned to draw unambiguous conclusions concerning the skeleton and stereochemistry of the isolated compound.



Initially, a simple racemic synthesis was designed starting from commercially available R-(-)-piperitone (**2**). Thus, reduction of the carbonyl group[11] using $LiAlH_4$ provided an aproximately 1:1 mixture of *Z*- and *E*-piperitol (**3** and **4**). The mixture was easily resolved by column cromatography and the spectroscopic data were fully coincident with those previously reported in the literature[12-13]. Subsequent treatment of both separated alcohols with acetic anhydride in CH_2Cl_2 provided the corresponding acetates (**5** and **6**). Final epoxidation of the acetates using *m*-chloroperbenzoic acid (MCPA) in CH_2Cl_2 gave two pairs of diastereomers [(1R,2S,3S,4R)-**1**/**7** and **8**/**9**], which were also resolved by column cromatography.

All the diastereomers were chromatographically resolved. Moreover, one of the two products obtained by epoxidation of **5** showed the same GC retention time and spectral data as the natural compound isolated from *Minthostachys tomentosa*.



5 (3R,4R) **1** (1R,2S,3S,4R) **7** (1S,2R,3S,4R)

6 (3S,4R) **8** (1R,2S,3R,4R) **9** (1S,2R,3R,4R)

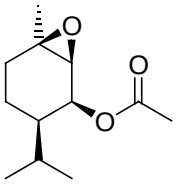
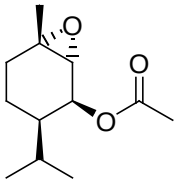
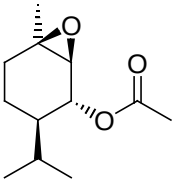
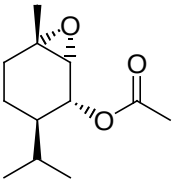
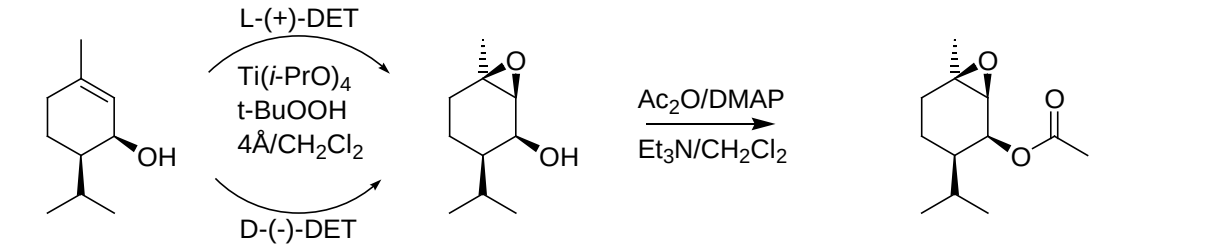
	 **1** (1R,2S,3S,4R)	 **7** (1S,2R,3S,4R)	 **8** (1R,2S,3R,4R)	 **9** (1S,2R,3R,4R)
H-2	3.3, d, *J*= 5 Hz	3.0, d, *J*= 3 Hz	2.8, s	3.2, d, *J*= 2 Hz
H-3	5.1, dd, *J*= 5, 1 Hz	5.4, s	4.9, d, *J*=10 Hz	5.1, dd, *J*= 10, 2 Hz

Table 1. ^{1}H NMR data of characteristic protons in the diastereoisomers.

Thus, once stablished that the proposed structural formula was correct, a stereoselective synthesis was undertaken to elucidate the correct stereochemistry of the natural compound. As for stereochemistry assignment the synthesis of (1S,2R,3R,4S)-**1** or its enantiomer (1R,2S,3S,4R)-**1** would be equally useful, we decided to start again from the more accesible R-(-)-piperitone. In a first attempt reduction of R-(-)-piperitone

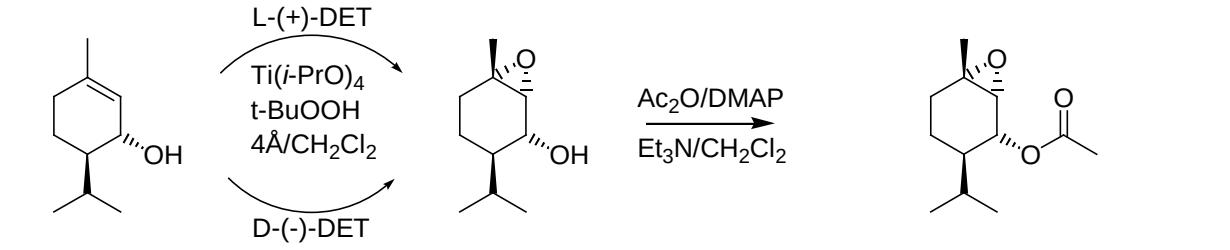
(**2**) was performed with bulky reductive agents such as L-Selectride or LiAl(*t*-BuO)$_4$[14] but unfortunately the results were similar to those obtained with $LiAlH_4$ (see above).



3
(3S,4R)-Piperitol

10
(1R,2S,3S,4R)

1
(1R,2S,3R,4S)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate



4
(3R,4R)-Piperitol

11
(1S,2R,3R,4R)

9
(1S,2R,3R,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate

Trying to improve the stereoselectivity, both piperitols (**3** and **4**) were separately submitted to a Sharpless epoxidation[15-16], using either L-(+)-diethyl tartrate [L-(+)-DET] or D-(-)-diethyl tartrate [D-(-)-DET]. In this case, the reaction was indeed stereoselective (90 % ee), although only one of the two possible epoxides was obtained from each substrate, irrespective of the chiral auxiliary employed. Thus piperitol **3** afforded epoxyalcohol **10** which was initially supposed to posses *trans* arrangement of the deshielded methine protons, according to their coupling constant (J_{23}= 8 Hz). However, when diedric angles were observed it could be noticed that the configuration of cyclohexane ring is enough modified by epoxide ring affording different results as usual[17]. Thus, finally **10** was definetely assigned to the *cis* isomer. Likewise, piperitol **4** gave epoxyalcohol **11**, where the corresponding coupling constant was much lower

(J_{23}= 2 Hz); this indicates a *trans* arrangement of the involved protons revealing an unambiguously *E* geometry. Hence, the absolute configurations of the Sharpless oxidation products obtained from **3** an **4** were unambiguously assigned to be **10** and **11**, respectively. These results, as could be expected, indicate that the stereochemistry of epoxidation is controlled by the presence of the alcohol group.

The next step was acetylation of the epoxyalcohols **10** and **11**. As this reaction does not modify the configuration of any of the four asymetric carbons, stereochemical assignment of the resulting acetates (1R,2S,3S,4R)-**1** and **8** was straighforward. At this point, it was realized that the NMR spectra (^{1}H and ^{13}C) of compound (1R,2S,3S,4R)-**1** were exactly coincident with those of the natural compound. On the other hand, upon measurement of the optical activity it became clear that (1R,2S,3S,4R)-**1** was the optical antipode of the isolated natural product, hence the stereochemistry of the latter is that shown in structure (1S,2SR,3R,4S)-**1**. As the synthetic product was established to be (1R,2S,3S,4R) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate, the natural product is (1S,2R,3R,4S) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate. Moreover, chiral HPLC analysis with optical rotation detector clearly showed that the natural product **1**-(-) and the synthetic product **1**-(+) gave rise to two picks of opposite sign (**Figure 5**).

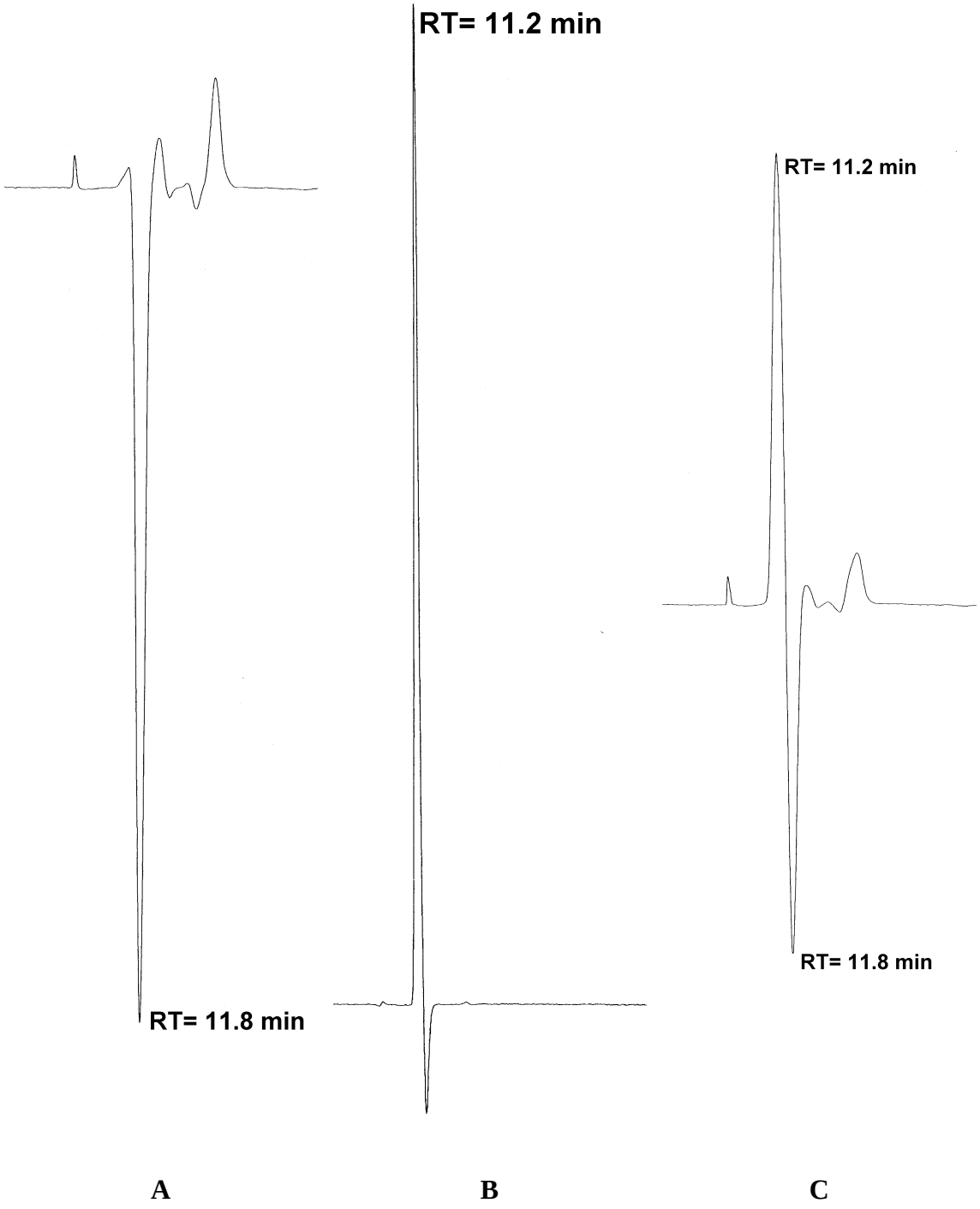


Figure 1. Chiral HPLC chromatogram of: **A** isolated natural product **1**-(+). **B** synthetic product **1**-(-). **C** Mixture of **1** and **2**.

Conclusion

(1S,2R,3R,4S)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate, a natural product, has been isolated from the volatile constituents of *Minthostachys tomentosa*. Its structure has been tentatively assigned based on spectral data and unambiguously confirmed by alternative synthesis of its enantiomer (1R,2S,3S,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate.

Experimental Section

Material and Methods

R-(-)-Piperitone was provided by Acedesa; Fluka comercial *t*-butyl hydroperoxide (80 %) was dried over activated molecular sieves (3 Å) obtaining purity of 88 % with less than 2000 ppm of H_2O; all other chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtanined as liquid ; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ solvent; chemical shifts are reported in δ (ppm) values, using TMS as internal standard. The assignment of ^{13}C signals is supported by DEPT experiments. Mass spectra were obtained under electron impact or chemical ionization; the ratios *m/z* and the relative intensities are reported. Isolation and purification were done by flash column chromatography on silica gel 60 (230-400 mesh). HPLC was performed on a Waters 600 connected to a chiral detector JASCO OR-990. For the determination of enanttiomeric excesses, a Chiracel OD column (250 mm x 4.6 mm) was used. Analytical TLC was carried out on precoated

plates (silica gel 60 F_{254}), and spots were visualized with UV light and/or in a I_2 chamber or with anisaldehyde/ acetic acid/ sulfuric acid/ methanol mixture. Molecular sieves were activated by heating at 220º C in vacuo for 8 hours.

Isolation and Characterization of Natural Compound. 320 g of the dried picked leaves from the mint plant *M. tomentosa* collected in Huaraz were hydrodistilled in a Likens-Nickerson apparatus for 4 h using CH_2Cl_2 as collecting solvent. The resulting extract was dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. The essential oil was submitted to open-column chromatography on silica gel (1:60 w/w) using mixtures of hexane, CH_2Cl_2, AcOEt as eluent. This led to the separation of 6 fractions. The biological activity was localized exclusively in fraction number 3 which killed 100 % of the exposed insects in 72 h at a dose of 50 μg/cm^2. This fraction had the active compound as the major and almost exclusive product. Purification of the active fraction by semipreparative HPLC was achieved using the following conditions: column Spherisorb S5 W, 5 μm (25 x 1.0 cm); mobile phase CH_2Cl_2:EtAcO (98:02, v/v); detection UV (254 nm). This led to the isolation of 5 mg of the pure active compound, whose structure was tentatively assigned to be 1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol , acetate (**1**) on the basis of spectral data. $[\alpha]^{20}_D$= + 93º (c 1.27, $CHCl_3$); HRMS: *m/z* 212.1418 ($C_{12}H_{20}O_3$ requires 212.1412); IR: ν_{max} 2966, 2882, 1757, 1457, 1375, 1236, 1083, 1024 and 985; ^{1}H NMR: δ_H 5.1 (dd, *J* = 5 and 1 Hz, 1H, H-3), 3.3 (d, *J* = 5 Hz, 1H, H-2), 2.1 (s, 3H, C*H*$_3$CO), 2.0 (m, 1H, H-4), 1.7-1.5 (m, 3H, H-6, H-1''), 1.4 (m, 2H, H-5), 1.3 (s, 3H, H-1'), 0.9 and 0.8 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 171.0 (CO), 68.6 (C_3), 58.7 (C_1), 58.6 (C_2), 44.8 (C_4), 31.0 (C_6), 27.6

($C_{1''}$), 22.5 (CH_3CO), 20.8, 20.7 ($3xCH_3$) and 18.3 (C_5); MS: *m/z* 212 (M^+, 2), 169 (9), 154 (100), 153 (52), 134 (6) and 112 (30).

Chemical Syntheses

Reduction of 1-Mehyl-4-(1-methylethyl)cyclohex-1-en-3-one (piperitone).

A solution of R-(-)-piperitone (**2**) (4.0 g; 26.1 mmol) in anhydrous diethyl ether (40 mL) was added dropwise under N_2 to at 0º C a stirred suspension of $LiAlH_4$ (2.1 g; 55.5 mmol) in anhydrous diethyl ether (70 mL). When the addition was finished the mixture was refluxed until all the starting material had been consumed according to TLC analysis. Then, the reaction was quenched by addition of H_2O (2.3 mL), 15% acqueous solution of NaOH (2.3 mL) and distilled H_2O (6.8 mL). After 30 min. of stirring at room temperature the solution was filtered and the liquid obtained was washed with brine, dried and concentrated to dryness. Chromatography of the residue on silica gel using mixtures hexane:diethyl ether (9:1) provided both epimeric alcohols (**3** and **4**) as yellow oils.

(3S,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**3**). 43 % yield; ^1H NMR: δ_H 5.6 (d, *J* = 6 Hz, 1H, H-2), 4.1 (br s, 1H, H-3), 1.9 (m, 2H, H-6), 1.7 (s, 3H, H-1'), 1.8-1.6 (m, 2H, H-4+H-1''), 1.3 (m, 2H, H-5), 1.0 and 0.9 (d+d, *J* = 7 Hz, 6H, 2xCH_3); ^{13}C NMR: δ_C 139.5 (C_1), 123.6 (C_2), 64.9 (C_3), 46.1 (C_4), 31.4 (C_6), 28.3 ($C_{1''}$), 23.3 ($C_{1'}$), 20.9, 20.7 ($2xCH_3$) and 20.4 (C_5); MS: *m/z* 154 (M^+, 7.0), 139 (69), 112 (27), 111 (21), 95 (14), 93 (28), 91 (20), 84 (100), 83 (74), 81 (28), 79 (18), 77 (17), 69 (18), 55 (33) and 41 (64).

(3R,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**4**). 48 % yield; ^1H NMR: δ_H 5.4 (br s, 1H, H-2), 4.0 (br s, 1H, H-3), 2.1-1.9 (m, 4H, H-4+H-6+H-1'), 1.6

(s, 3H, H-1'), 1.2 (m, 2H, H-5), 0.9 and 0.8 (d+d, J = 7 Hz, 6H, 2xCH_3) ; ^{13}C NMR: δ_C 137.2 (C_1), 125.4 (C_2), 68.8 (C_3), 47.6 (C_4), 30.0 (C_6), 26.3 ($C_{1''}$), 23.0 ($C_{1'}$), 21.1, 21.0 (2xCH_3) and 20.7 (C_5); MS: *m/z* 154 (M^+, 7), 139 (61), 121 (7), 112 (24), 111 (20), 93 (30), 91 (22), 84 (100), 83 (73), 81 (18), 79 (17), 77 (18), 69 (16), 55 (34) and 41 (65).

General Procedure for the Synthesis of the Acetates.

A solution of the corresponding prepared alcohol, (2.7 mmol) and a catalitic amount of 4-dimethylamino-pyridine (DMAP) in dry CH_2Cl_2 (15 mL) was cooled at 4º C; then triethylamine (5.4 mmol) and acetic anhidride (5.4 mmol) were added. The mixture was stirred for 4 h 30 min. and after this period was quenched with H_2O, stirred for 30 min. and extracted with CH_2Cl_2. The combined organic extracts were washed with acqueous HCl (5 %) and brine, dried over Na_2SO_4 and concentrated in vacuo. Chromatography of the residue on silica gel provided the corresponding acetate.

(3S,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate (**5**). 84 % yield; $[\alpha]^{20}_D$= -284º (c 1.11, $CHCl_3$); ^{1}H NMR: δ_H 5.6 (d, J = 7 Hz, 1H, H-2), 5.2 (m, 1H, H-3), 2.0-1.8 (m, 2H, H-4), 1.9 (s, 3H, CH_3CO), 1.8-1.7 (m, 2H, H-4+H-1''), 1.6 (s, 3H, H-1'), 1.6-1.3 (m, 2H, H-5), 0.9 and 0.8 (d+d, J = 7 Hz, 6H, 2xCH_3); ^{13}C NMR: δ_C 170.8 (CO), 141.4 (C_1), 119.8 (C_2), 68.5 (C_3), 44.3 (C_4), 31.2 (C_6), 28.3 ($C_{1''}$), 23.3 ($C_{1'}$), 21.3 (C_5), 20.7 and 20.6 (2xCH_3); MS: *m/z* 154 (11), 137 (76), 136 (94), 122 (10), 121 (100), 93 (59), 81 (42) and 41 (39).

(3R,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate (**6**). 83 % yield; $[\alpha]^{20}_D$= +119º (c 1.16, $CHCl_3$); ^{1}H NMR: δ_H 5.2 (br s, 1H, H-2), 5.1 (m, 1H, H-3), 1.9 (s, 3H, CH_3CO), 1.8 (m, 2H, H-6), 1.7-1.5 (m, 2H, H-4+H-1''), 1.5 (s, 3H, H-1'), 1.4-1.2 (m, 2H, H-5), 0.8 and 0.7 (d+d, J = 7 Hz, 6H, 2xCH_3) ; ^{13}C NMR: δ_C 170.5

(CO), 139.1 (C_1), 120.6 (C_2), 71.5 (C_3), 43.5 (C_4), 29.1 (C_6), 26.4 ($C_{1''}$), 22.7 ($C_{1'}$), 20.75 (C_5), 20.9 and 20.4 (2xCH_3); MS: *m/z* 154 (11), 139 (12), 137 (17), 136 (55), 121 (100), 93 (65), 91 (35), 84 (28), 81 (22), 79 (21), 77 (21), 43 (82), and 41 (39).

(1R,2S,3S,4R)-1-Epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (**1**). 84 % yield; This product showed spectral data identical to those reported above for the natural product except the optical activity sign $[\alpha]^{20}_D$= -146º (c 1.15, $CHCl_3$).

(1S,2R,3R,4R)-1-Epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (**9**). 90 % yield; $[\alpha]^{20}_D$= -138º (c 1.81, $CHCl_3$); HRMS (EI): *m/z* 212.1416 ($C_{12}H_{20}O_3$ requires 212.1412); IR: ν_{max} 2950, 2870, 1740, 1370, 1240 and 1100; ^{1}H NMR: δ_H 5.1 (dd, *J* = 10 and 2 Hz, 1H, H-3), 3.2 (d, *J* = 2 Hz, 1H, H-2), 2.1 (s, 3H, C*H*$_3$CO), 1.8-1.6 (m, 2H, H-6), 1.6-1.4 (m, 4H, H-4+H-5+H-1''), 1.3 (s, 3H, H-1'), 0.9 and 0.7 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 171.2 (CO), 72.6 (C_3), 61.3 (C_1), 61.1 (C_2), 38.6 (C_4), 28.1 (C_6), 25.7 ($C_{1''}$), 23.9 (*C*H_3CO), 21.1 (C_5), 20.8, 20.5 and 16.3 (3xCH_3); MS: *m/z* 212 (M^+, 9), 169 (6), 154 (100), 152 (54), 134 (14), 112 (32) and 81 (4).

Syntheses of the diastereoisomeric epoxides.

To a 0º C cooled solution of *m*-chloroperoxybenzoic acid, MCPA, (0.52 mmol) in dry CH_2Cl_2 (13 mL) was added another solution of the corresponding acetate, **6** or **7** (0.42 mmol) in dry CH_2Cl_2 (5 mL) dropwise. After 6 h of stirring the mixture was poured into a acqueous solution of $NaHCO_3$ (10%) and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Chromatography of the residue on silica gel provided both isomeric epoxides.

Epoxides from (3S,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate.

The first eluted epoxide obtained from (3S,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate was obtained with a 60 % yield. $[\alpha]^{20}_D$= -138º (c 1.81, $CHCl_3$); HRMS (EI): *m/z* 212.1410 ($C_{12}H_{20}O_3$ requires 212.1412); IR: ν_{max} 2960, 2930, 2870, 1740, 1370 and 1245; ^{1}H NMR: δ_H 5.4 (s, 1H, H-3), 3.0 (d, *J* = 3 Hz, 1H, H-2), 2.1 (s, 3H, C*H*$_3$CO), 2.0 (m, 1H, H-4), 1.8 (m, 2H, H-6), 1.5-1.3 (m, 3H, H-5+ H-1''), 1.3 (s, 3H, H-1'), 0.9 and 0.8 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 170.7 (CO), 69.7 (C_3), 59.3 (C_2), 58.3 (C_1), 39.0 (C_4), 28.1 (C_6), 24.1 (*C*H$_3$CO), 21.1 ($C_{1''}$), 20.9, 20.4, 20.3 (3xCH_3) and 19.6 (C_5); MS: *m/z* 212 [M^+, 9], 194 (4), 170 (12), 154 (100), 152 (85), 134 (23) and 112 (40). After diastereoselective sequence this product was assigned to the (1S,2R,3S,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (**7**).

The second eluted epoxide was obtained with 32 % yield as a yellow oil which showed spectral data identical to those reported above for the natural product except the optical activity sign.

Epoxides from (3R,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate.

The first eluted epoxide obtained from (3R,4R) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol, acetate was obtained with a 60 % yield; $[\alpha]^{20}_D$= -138º (c 1.81, $CHCl_3$); HRMS (EI): *m/z* 213.1495 ($M+H^+$, $C_{12}H_{21}O_3$ requires 213.1490); IR: ν_{max} 2955, 2875, 1735, 1375, 1240 and 990; ^{1}H NMR: δ_H 4.9 (d, *J* = 10, 1H, H-3), 2.8 (s, 1H, H-2), 2.1 (s, 3H, C*H*$_3$CO), 2.1-1.9 (m, 2H, H-6), 1.6 (m, 4 H, H-4+H-5+ H-1''), 1.3 (s, 3H, H-1'), 0.8, 0.7 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 170.4 (CO), 70.3 (C_3), 61.7 (C_2), 58.3 (C_1), 43.4 (C_4), 30.1 (C_6), 27.6 ($C_{1''}$), 22.3 (*C*H$_3$CO), 21.1 (C_5), 20.4, 16.8 and 16.1 (3xCH_3); MS: *m/z* 213 [$(M+1)^+$, 4], 212 (M^+, 1), 169 (5), 154 (86), 152 (26), 137 (11), 127 (65), 112 (100), 109 (30), 84 (20), 81 (51), 71 (14) and 55 (16). After

diastereoselective sequence this product was assigned to the (1R,2S,3R,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (**8**).

The second eluted epoxide was obtained with 32 % yield. It showed spectral data identical to those reported above for the (1S,2R,3R,4R)-1-Epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (**9**).

Selective synthesis of the epoxides by Sharpless reaction.

A mixture of activated 4 Å molecular sieves and anhydrous CH_2Cl_2 (7 mL) was cooled to –10º C under N_2. Then L-(+)-diethyl tartrate or D-(-)-diethyl tartrate (0.91 mmol) and titanium isopropoxide (1.35 mmol) were added; followed by slowly addition of a solution of dry *t*-butyl hydroperoxide (7.6 mmol) in anhydrous CH_2Cl_2. When the addition was finished (ca. 20 min.) the mixture was cooled at –20º C and a new solution of the corresponding allylic alcohol, **3** or **4**, (4.5 mmol) in anhydrous CH_2Cl_2 (0.7 mL) was added dropwise. The mixture was stirred overnight at –20º C and then H_2O was added (about 20 times the weight of titanium isopropoxide). After 30 min. of stirring at 0º C and 15 min. remaining at room temperature a NaOH (30%) acqueous solution saturated in NaCl (1.0 mL) was added. The mixture was stirred vigorously for 10 min. and after extracted with CH_2Cl_2. The combined organic extracts were filtered trough a celite column and concentrated in vacuo. Chromatography of the residue on silica gel provided the corresponding epoxide.

Both enantiomeric tartrates provided (1R,2S,3S,4R) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**10**) when (3R,4S) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**3**) was used as starting alcohol. Rendiments were about 52 % yield and recovering ca 15 % of starting material. $[\alpha]^{20}_D$= -85º (c 2.93, $CHCl_3$); HRMS (EI): *m/z* 212.1416 ($C_{12}H_{20}O_3$ requires 212.1407); IR: ν_{max} 2950, 2870, 1740, 1370, 1240 and

1100; ^{1}H NMR: δ_H 4.1 (m, 1H, H-3), 3.2 (d, *J* = 8 Hz, 1H, H-2), 2.2-2.0 (m, 2H, H-4+OH), 1.6 (m, 2H, H-6), 1.3 (s, 3H, H-1'), 1.3-1.1 (m, 3H, H-5+H-1"), 1.0 and 0.9 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 64.5 (C_3), 62.1 (C_1), 61.0 (C_2), 46.8 (C_4), 31.0 (C_6), 27.7 ($C_{1"}$), 23.0 (C_5), 20.8, 20.7 and 17.3 (3xCH_3); MS: *m/z* 212 (M^+, 6), 154 (10), 153 (100), 152 (18), 134 (6), 112 (10), 111 (8), 110 (5), 109 (17), 95 (10), 81 (15), 43 (35) and 41 (10).

Both enantiomeric tartrates provided (1S,2R,3R,4R) 1-epoxy-1-ethyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**11**) when (3S,4S) 1-Methyl-4-(1-methylethyl)-1-cyclohexen-3-ol (**4**) was used as starting alcohol. Rendiments were about 52 % yield and recovering ca 15 % of starting material. $[\alpha]^{20}_D$= + 28º (c 1.41, $CHCl_3$); HRMS (EI): *m/z* 212.1416 ($C_{12}H_{20}O_3$ requires 212.1407); IR: ν_{max} 2950, 2870, 1740, 1370, 1240 and 1100; ^{1}H NMR: δ_H 3.7 (t, *J* = 10 Hz, 1H, H-3), 3.2 (d, *J*= 2Hz, 1H, H-2), 2.5 (d, J = 8 Hz, 1H, OH), 2.1 (m, 1H, H-4), 1.8 (m, 2H, H-6), 1.5-1.3 (m, 2H, H-5), 1.3 (s, 3H, H-1'), 1.1-0.9 (m, 3H, H-1"), 0.9 and 0.8 (d+d, *J* = 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 69.7 (C_3), 63.9 (C_1), 62.3 (C_2), 42.6 (C_4), 28.1 (C_6), 25.5 ($C_{1"}$), 24.0 (C_5), 21.2, 20.1 and 16.5 (3xCH_3); MS: *m/z* 212 (M^+, 6), 154 (10), 153 (100), 152 (18), 134 (6), 112 (10), 111 (8), 110 (5), 109 (17), 95 (10), 81 (15), 43 (35) and 41 (10).

Biological assays.

Insects. *Oncopeltus fasciatus* Dallas were mantained at 28 ± 1 °C, 50-60 % relative humidity, 16h/8h (day/night) photoperiod and a diet based on sunflowers seeds.

Entomotoxicity activity. The test was carried out basically according to the contact method of Bowers et al.[18]. Briefly, 15 third-instar *O. fasciatus* nymphs were

confined to a 5 cm Petri dish coated with 50 μg/cm^2 in the case of fractions. Toxicity effects were considered according to the number of insects dead of exposure to the chemicals. All assays were made three times. Controls were run in parallel and received the same amount of acetone as treated insects.

Acknowledgements

The authors acknowledge the financial support of Fundación José y Ana Royo (fellowship to AC), Conselleria de Educació i Ciencia (fellowship to CL). We thank Prof. Dr. Ramón Mestres for providing the *Minthostachys tomentosa* plant.

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Captions

Figure 1. Structure of the isolated natural compound (1S,2R,3R,4S) and its enantiomer (1R,2S,3S,4R).

Figure 2. Synthesis of both diastereoisomeric acetates **5** and **6**.

Figure 3. Synthesis of the natural product and its three diastereoisomers.

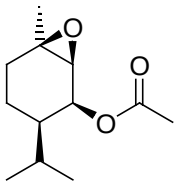
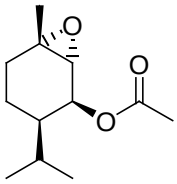
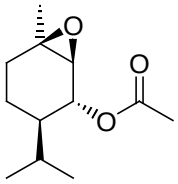
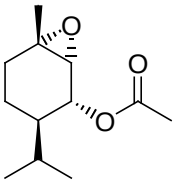
Table 1. ^{1}H NMR data of the characteristic protons in all diastereomers.

Figure 4. Stereoselective synthesis of **1** and **9**.

Figure 5. Chiral HPLC chromatogram of: **A** isolated natural product **1**-(+). **B** synthetic product **1**-(-). **C** Mixture of **1**-(+) and **1**-(-).

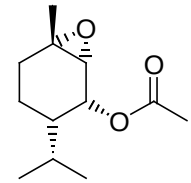
Tables

Table 1

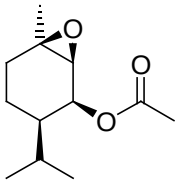
	 **1** (1R,2S,3S,4R)	 **7** (1S,2R,3S,4R)	 **8** (1R,2S,3R,4R)	 **9** (1S,2R,3R,4R)
H-2	3.3, d, *J*= 5 Hz	3.0, d, *J*= 3 Hz	2.8, s	3.2, d, *J*= 2 Hz
H-3	5.1, dd, *J*= 5, 1 Hz	5.4, s	4.9, d, *J*=10 Hz	5.1, dd, *J*= 10, 2 Hz

Figures

Figure 1



(1S,2R,3R,4S)-**1**



(1R,2S,3S,4R)-**1**

Figure 2.

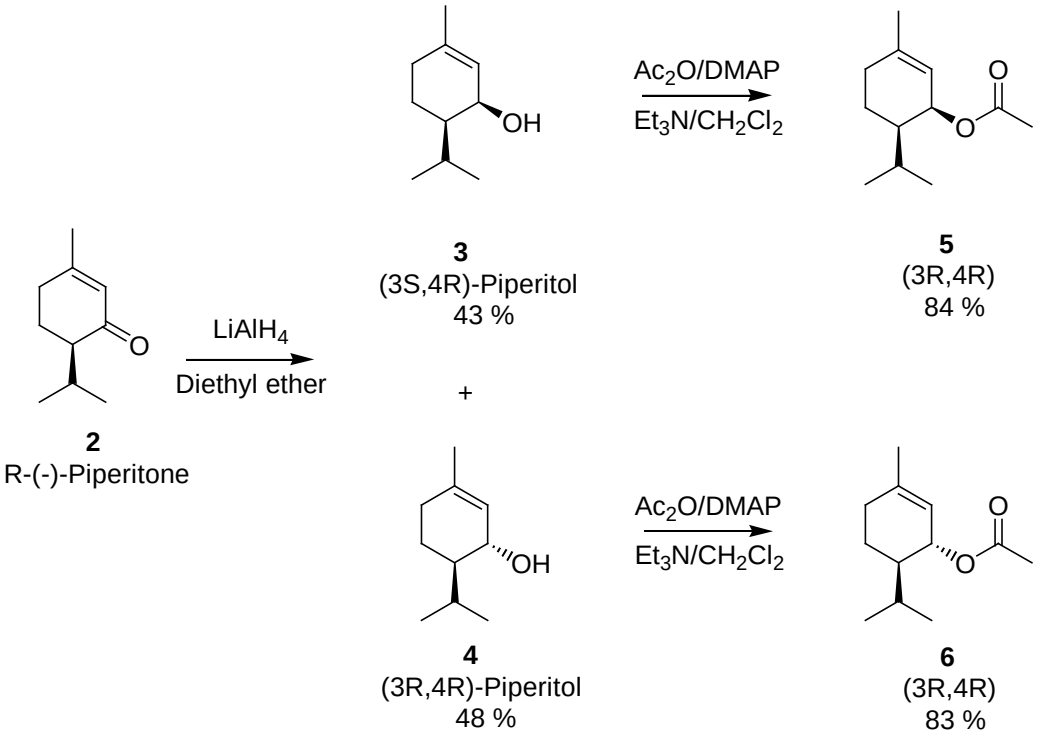

OH
Ac2O/DMAP
Et3N/CH2Cl2
O
O
3
(3S,4R)-Piperitol
43 %
5
(3R,4R)
84 %
LiAlH4
Diethyl ether
O
+
2
R-(-)-Piperitone
OH
Ac2O/DMAP
Et3N/CH2Cl2
O
O
4
(3R,4R)-Piperitol
48 %
6
(3R,4R)
83 %

Figure 3.

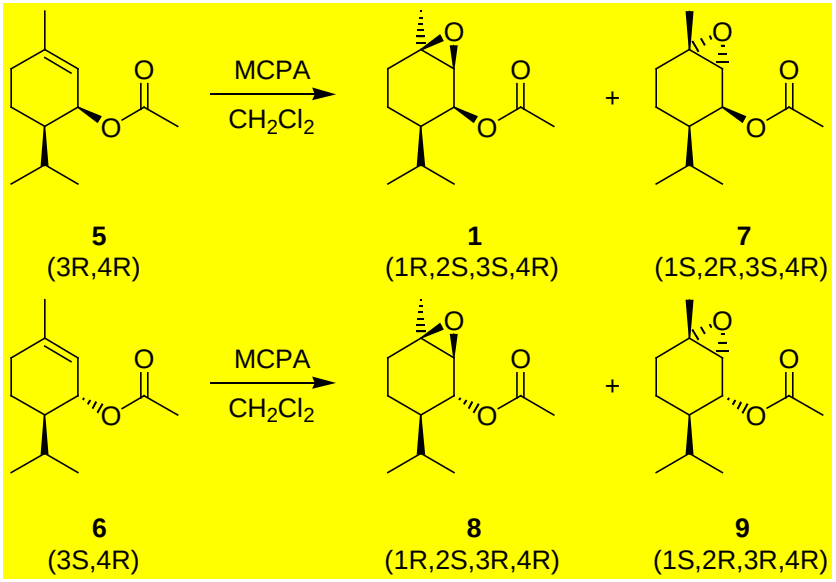

MCPA
CH2Cl2
5
(3R,4R)
1
(1R,2S,3S,4R)
7
(1S,2R,3S,4R)
MCPA
CH2Cl2
6
(3S,4R)
8
(1R,2S,3R,4R)
9
(1S,2R,3R,4R)

Figure 4.

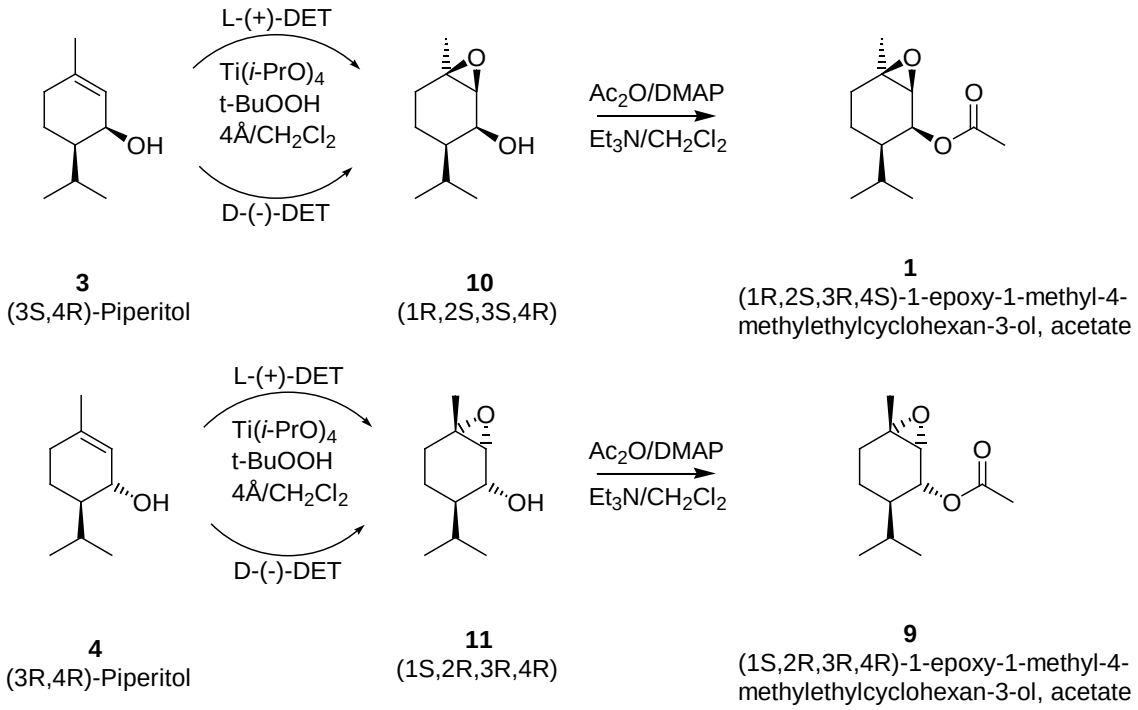

L-(+)-DET
Ti(i-PrO)4
t-BuOOH
4Å/CH2Cl2
D-(-)-DET
OH
O
Ac2O/DMAP
Et3N/CH2Cl2
3
(3S,4R)-Piperitol
10
(1R,2S,3S,4R)
1
(1R,2S,3R,4S)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate
4
(3R,4R)-Piperitol
11
(1S,2R,3R,4R)
9
(1S,2R,3R,4R)-1-epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate

Figure 5.