Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 677–683

TETRAHEDRON:
ASYMMETRY

Isolation, structural assignment and insecticidal activity of (−)-(1*S*,2*R*,3*R*,4*S*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate, a natural product from *Minthostachys tomentosa*

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Received 7 February 2001; accepted 23 February 2001

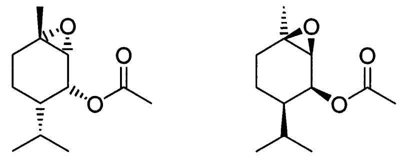
Abstract—(−)-(1*S*,2*R*,3*R*,4*S*)-1,2-Epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate has previously been identified as the active compound of *Minthostachys tomentosa* responsible for the insecticidal activity against *Oncopeltus fasciatus*. Its structure was initially assigned on the basis of spectral data. In order to confirm the structure and to define the stereochemistry, stereoselective synthesis of its enantiomer, (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate, starting from (*R*)-(−)-piperitone, was carried out using a Sharpless reaction as the key step. The natural product is *dextro*-rotatory while the synthetic product is *levo*-rotatory. Measurements of insecticidal activities of the different steroisomers revealed that only the natural product is active. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Natural products, with their overwhelming structural diversity, provide an important source of new and effective methods for controlling all types of bioactivity. 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.[4–9]

We now wish to report the isolation of (−)-(1*S*,2*R*,3*R*,4*S*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate (−)-**1** from *Minthostachys tomentosa*. A natural product originally named 1,2-epoxymenthyl acetate, tentatively assigned the structural formula **1** (without stereochemical assignment), was previously isolated from *Mentha rotundifolia*.[10] The structural and stereochemical assignment of the product isolated herein from *M. tomentosa* is unambiguous and was based on independent enantioselective synthesis of its enantiomer (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate. The natural product (−)-(1*S*,2*R*,3*R*,4*S*)-**1** exhibits insecticidal activity against *Oncopeltus fasciatus*. The activity was found to be enantiospecific, as shown by the fact that the synthetic enantiomer (+)-(1*R*,2*S*,3*S*,4*R*)-**1** was non-toxic to the target insect species (Fig. 1).



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

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

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PII: S0957-4166(01)00094-5

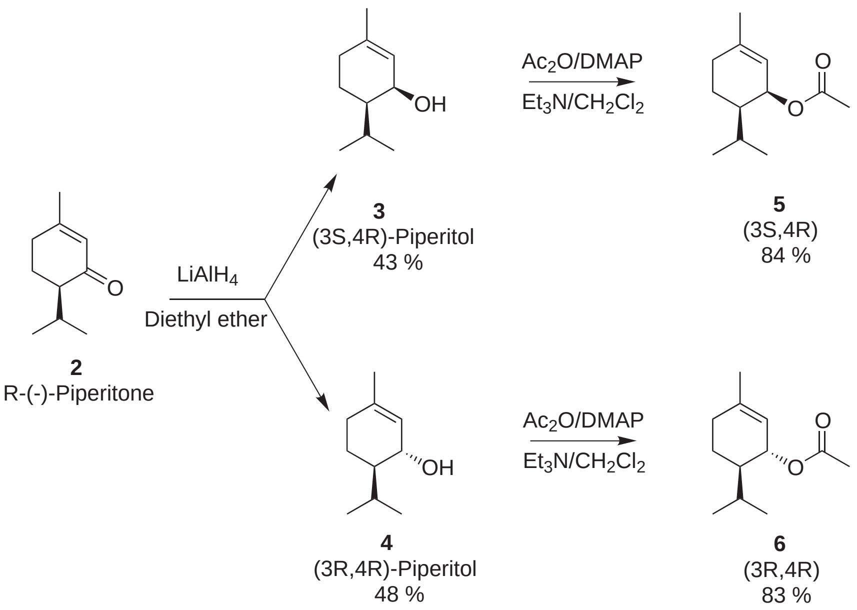


Figure 2. Synthesis of both diastereoisomeric acetates **5** and **6**.

2. Results and discussion

The volatile constituents of *M. tomentosa* were investigated for insecticidal activity against *O. fasciatus*. A search for the active natural product led to a substance which was purified by semi-preparative HPLC, which afforded a pure metabolite that exhibited insecticidal activity against *O. fasciatus* according to the contact method[11] with 100% mortality after 72 h using 50 μg/cm^2 of the compound.

The spectral data and the natural origin of the product suggested a diterpene skeleton with two oxygenated groups. The structure **1** was assigned on the following basis. The 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 $\nu = 1757$ cm^{-1} and $\delta = 171.0$ ppm, respectively. The ^{13}C NMR spectrum also exhibited distinct signals at $\delta = 68.6$, 58.7 and 58.6 ppm corresponding to carbons bound to oxygen, two of them tertiary and the other quaternary according to the DEPT spectrum. In the ^{1}H NMR spectrum, the two deshielded methine protons appeared at $\delta = 5.1$ (dd) and 3.3 (d) ppm. These data, and the need to accommodate three double bond equivalents, suggested the presence of an epoxide moiety. Two doublets typical of the *iso*-propyl group at $\delta = 0.9$ and 0.8 ppm were also observed.

As structure **1** presents four different asymmetric 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[12] using $LiAlH_4$ provided an aproximately 1:1 mixture of (*Z*)- and (*E*)-piperitol **3** and **4** (Fig. 2). The mixture was easily resolved by column chromatography and the spectroscopic data were fully concordant with those previously reported.[13,14] 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, **1**/**7** and **8**/**9**, which were also resolved by column chromatography (Fig. 3).

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

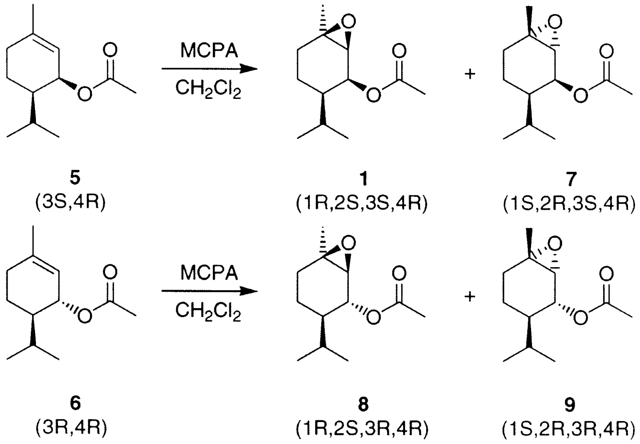
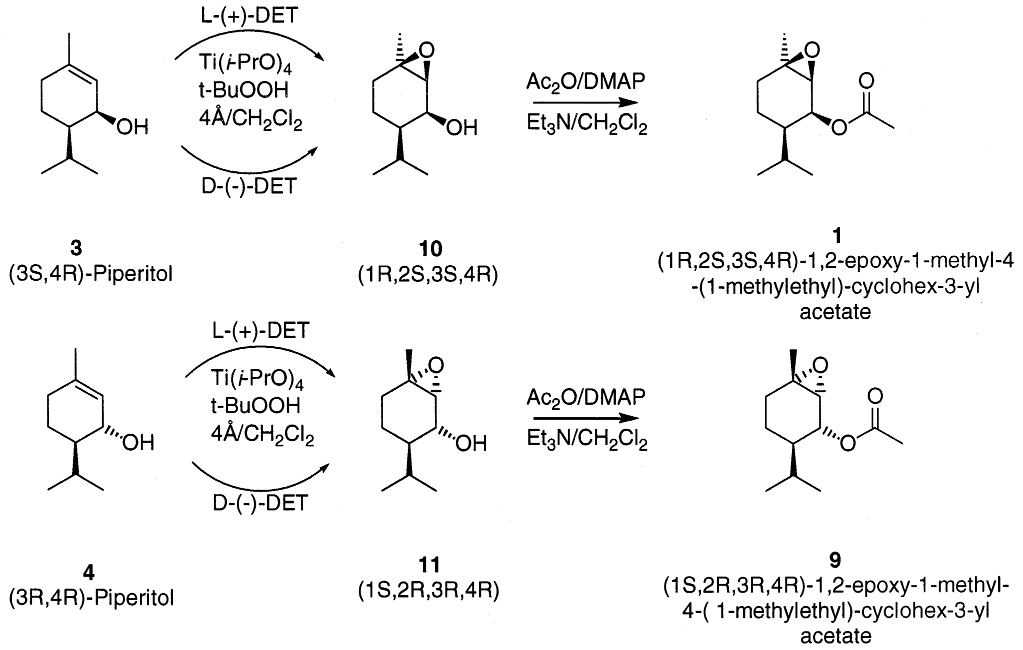


Figure 3. Synthesis of the enantiomeric natural product and its three diastereoisomers.



3
(3S,4R)-Piperitol

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

1
(1R,2S,3S,4R)-1,2-epoxy-1-methyl-4
-(1-methylethyl)-cyclohex-3-yl
acetate

4
(3R,4R)-Piperitol

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

9
(1S,2R,3R,4R)-1,2-epoxy-1-methyl-
4-(1-methylethyl)-cyclohex-3-yl
acetate

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

Thus, once we had established that the proposed structural formula was correct, a stereoselective synthesis was undertaken to elucidate the correct stereochemistry of the natural compound. For stereochemical assignment the synthesis of (−)-(1*S*,2*R*,3*R*,4*S*)-**1** or its enantiomer (+)-(1*R*,2*S*,3*S*,4*R*)-**1** would be equally useful, so we decided to start from the more accessible (*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)$_4$,[15] but unfortunately the results were similar to those obtained with LiAlH$_4$ (see above).

To control the stereoselectivity, both piperitols **3** and **4** were separately submitted to a Sharpless epoxidation,[16,17] using either L-(+)-diethyl tartrate [L-(+)-DET] or D-(−)-diethyl tartrate [D-(−)-DET]. In this case, the reaction was indeed stereoselective (90% e.e.), 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**, while piperitol **4** gave epoxyalcohol **11** (Fig. 4). The structural assignments were based on the vicinal coupling constants between H-1 and H-3, taking into account the dihedral angles in 3D models.[18] These results 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 asymmetric carbons, stereochemical assignment of the resulting acetates (1*R*,2S,3*S*,4*R*)-**1** and (1*S*,2*R*,3*R*,4*R*)-**9** was straightforward. At this point, it was realised that the NMR spectra (^{1}H and ^{13}C) of compound (1*R*,2*S*,3*S*,4*R*)-**1** were exactly concordant with those of the natural compound. Upon measurement of the optical activity it became clear that (1*R*,2*S*,3*S*,4*R*)-**1** was the optical antipode of the isolated natural product; hence the stereochemistry of the latter is that shown in structure (1*S*,2*R*,3*R*,4*S*)-**1**. As the synthetic product was established to be (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate, the natural product must be (−)-(1*S*,2*R*,3*R*,4*S*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate. Moreover, chiral HPLC analysis with optical rotation detector clearly showed that the natural product (−)-**1** and the synthetic product (+)-**1** gave two peaks of opposite sign, as shown in Fig. 5.

The synthetic product (+)-**1** was submitted to the contact method to check its activity against *O. fasciatus*. Remarkably, this compound did not exhibit any significant toxicity to the target insect species; hence it appears that the insecticidal activity of the natural product (−)-**1** is enantiospecific.

3. Conclusion

(−)-(1*S*,2*R*,3*R*,4*S*)-1,2-Epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate was isolated from the volatile constituents of *M. tomentosa*. Its structure was assigned based on spectral data and unambiguously confirmed by an alternative synthesis of its enantiomer (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate. The natural product exhibits significant insecticidal activity, whereas the synthetic product is inactive.

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

4. Experimental

4.1. General methods

(*R*)-(–)-Piperitone was provided by Acedesa (Murcia, Spain) and used as provided; Fluka commercial *t*-butyl hydroperoxide (80%) was dried over activated molecular sieves (3 Å) obtaining a 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 obtained as liquid films; ν_{max} is given for the main absorption bands. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ as 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 ionisation; the *m*/*z* ratios and the relative intensities are reported. Isolation and purification were accomplished by flash column chromatography on silica gel 60 (230–400 mesh). HPLC was performed on a Waters 600 connected to a chiral Jasco OR-990 detector. For the determination of enantiomeric excesses, a Chiracel OD column (250×4.6 mm) was used. Analytical TLC was carried out on precoated plates (silica gel 60 F_{254}), and spots were visualised with UV light and/or in an I_2 chamber or with anisaldehyde–acetic acid–sulfuric acid–methanol mixture. Molecular sieves were activated by heating at 220°C in vacuo for 8 h.

The target insects (*O. fasciatus* Dallas) were maintained at 28±1°C, 50–60% relative humidity, 16 h/8 h (day/night) photoperiod and a diet based on sunflower seeds. The test for the insecticidal activity was carried out basically according to the contact method of Bowers.[11] Briefly, 15 third-instar *O. fasciatus* nymphs were confined to a 5 cm Petri dish coated with 50 μg/cm^2 of chemicals. Toxicity effects were considered according to the number of insects dead after exposure to the chemicals. All assays were performed three times. Controls were run in parallel without the added chemicals.

4.2. Isolation and characterisation of the natural compound

Dried picked leaves (320 g) from the mint plant *M. tomentosa* collected in Huaraz (Peru) 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 subjected to open-column chromatography on silica gel (1:60 w/w) using mixtures of hexane, CH_2Cl_2 and AcOEt as eluent. This led to the separation of six fractions. The biological activity was localised exclusively in fraction number 3 which killed 100% of the exposed insects after 72 h at a dose of 50 μg/cm². This fraction contained the active compound as the almost exclusive product. Purification by semi-preparative HPLC was achieved using the following conditions: column Spherisorb S5 W, 5 μm (25×1.0 cm); mobile phase CH_2Cl_2:AcOEt (98:2, v/v); detection UV (254 nm). This led to the isolation of 5 mg of the pure active compound, whose structure was tentatively assigned as 1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **1** on the basis of spectral data. $[\alpha]_D^{20}=+93$ (*c* 1.27, $CHCl_3$); HRMS: *m*/*z* 212.1418 ($C_{12}H_{20}O_3$ requires 212.1412); IR: ν_{max} 2957, 2926, 2873, 1737, 1373, 1242 and 1105; ^{1}H NMR: δ_H 5.1 (t, $J=5$ 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+ $(CH_3)_2CH$), 1.4 (m, 2H, H-5), 1.3 (s, 3H, C*H*$_3$), 0.9 and 0.8 (d+d, $J=7$ Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 22.5 (CH_3CO), 20.8, 20.7 (3×CH_3) and 18.3 (C_5); MS: *m*/*z* 212 (M^+, 2), 170 (8), 169 (9), 154 (100), 152 (52), 127 (10) and 112 (30).

4.3. Reduction of 1-methyl-4-(1-methylethyl)-cyclohex-1-en-3-one, piperitone 2

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 a stirred suspension of $LiAlH_4$ (2.1 g, 55.5 mmol) in anhydrous diethyl ether (70 mL) at 0°C. When the addition was complete, the mixture was stirred under reflux until all starting material was consumed according to TLC analysis. The reaction was quenched by addition of H_2O (2.3 mL), 15% aqueous solution of NaOH (2.3 mL) and further H_2O (6.8 mL). After stirring at room temperature for 30 min 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 a hexane:diethyl ether (9:1) mixture provided the epimeric alcohols **3** and **4** as yellow oils.

4.4. (3*S*,4*R*)-1-Methyl-4-(1-methylethyl)-cyclohex-1-en-3-ol 3[13,14]

43% yield; ^{1}H NMR: δ_H 5.6 (d, $J=6$ Hz, 1H, H-2), 4.1 (m, 1H, H-3), 1.9 (m, 2H, H-6), 1.7 (s, 3H, C*H*$_3$), 1.8–1.6 (m, 2H, H-4+$(CH_3)_2CH$), 1.3 (m, 2H, H-5), 1.0 and 0.9 (d+d, $J=7$ Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 23.3, 20.9, 20.7 (3×CH_3) and 20.4 (C_5); MS: *m*/*z* 154 (M^+, 7.0), 139 (69), 121 (10), 112 (27), 111 (21), 95 (14), 93 (28), 91 (20), 84 (100), 83 (74), 79 (18), 77 (17), 69 (18), 55 (33) and 41 (64).

4.5. (3*R*,4*R*)-1-Methyl-4-(1-methylethyl)-cyclohex-1-en-3-ol 4[13,14]

48% yield; ^{1}H NMR: δ_H 5.4 (br s, 1H, H-2), 4.0 (m, 1H, H-3), 2.1–1.9 (m, 4H, H-4+H-6+$(CH_3)_2CH$), 1.6 (s, 3H, CH_3), 1.2 (m, 2H, H-5), 0.9 and 0.8 (d+d, $J=7$ Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 23.0, 21.1, 17.1 (3×CH_3) and 20.7 (C_5); MS: *m*/*z* 154 (M^+, 7), 139 (61), 121 (7), 112 (24), 111 (24), 93 (30), 91 (22), 84 (100), 83 (73), 81 (18), 79 (17), 77 (18), 69 (16), 55 (34) and 41 (65).

4.6. General acetylation procedure

A solution of the corresponding alcohol (2.7 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in dry CH_2Cl_2 (15 mL) was cooled at 4°C, then triethylamine (5.4 mmol) and acetic anhydride (5.4 mmol) were added. The mixture was stirred for 4.5 h then quenched with H_2O, stirred for 30 min and extracted with CH_2Cl_2. The combined organic extracts were washed with aqueous HCl (5%) and brine, dried over Na_2SO_4 and concentrated in vacuo. Chromatography of the residue on silica gel provided the corresponding acetate. The racemic forms of acetates **5** and **6** have already been described in the literature.[19,20]

4.7. (3*S*,4*R*)-1-Methyl-4-(1-methylethyl)-cyclohex-1-en-3-yl acetate 5

84% yield; $[\alpha]_D^{20}=-284$ (*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-6+$(CH_3)_2CH$), 1.6 (s, 3H, CH_3), 1.6–1.3 (m, 2H, H-5), 0.9 and 0.8 (d+d, $J=7$ Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 23.3 (CH_3), 21.3 (C_5), 20.7 and 20.6 $(CH_3)_2CH$); MS: *m*/*z* 154 (11), 137 (76), 136 (94), 121 (100), 93 (59), 81 (42), 43 (80) and 41 (39).

4.8. (3*R*,4*R*)-1-Methyl-4-(1-methylethyl)-cyclohex-1-en-3-yl acetate 6

83% yield; $[\alpha]_D^{20}=+119$ (*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+ $(CH_3)_2CH$), 1.5 (s, 3H, CH_3), 1.4–1.2 (m, 2H, H-5), 0.8 and 0.7 (d+d, $J=7$ Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 22.7 (CH_3), 20.8 (C_5), 20.9, 20.4 and 17.5 (3×CH_3); MS: *m*/*z* 154 (11), 139 (10), 137 (4), 136 (25), 121 (85), 93 (100), 91 (40), 84 (85), 81 (35), 79 (38), 77 (37), 43 (85), and 41 (40).

4.9. Epoxidation with *m*-chloroperbenzoic acid

To a solution of *m*-chloroperbenzoic acid, MCPA (0.52 mmol), in dry CH_2Cl_2 (13 mL) cooled to 0°C, a solution of the corresponding acetate **5** or **6** (0.42

mmol) in dry CH_2Cl_2 (5 mL) was added dropwise. After stirring the mixture for 6 h it was poured onto 10% aqueous $NaHCO_3$ solution 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 epoxyacetates.

4.10. Epoxides from (3*S*,4*R*)-1-methyl-4-(1-methylethyl)-cyclohex-1-en-3-yl acetate 5

A mixture of two epoxides was obtained from (3*S*,4*R*)-1-methyl-4-(1-methylethyl)-1-cyclohexen-3-yl acetate; the major compound (60% yield) had the following spectral data: $[\alpha]_D^{20}=-80$ (*c* 1.16, $CHCl_3$); 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; ^1H NMR: δ_H 5.4 (br 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+$(CH_3)_2CH$), 1.3 (s, 3H, C*H*$_3$), 0.9 and 0.8 (d+d, *J*=7 Hz, 6H, $(CH_3)_2CH$); ^{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 (CH_3CO), 21.1 $(CH_3)_2CH$), 20.9, 20.4, 20.3 ($3\times CH_3$) and 19.6 (C_5); MS: *m*/*z* 212 (M^+, 9), 194 (4), 170 (12), 169 (8), 154 (100), 152 (85), 134 (23), 112 (40) and 109 (30). After diastereoselective synthesis (see below) this product was assigned to be (1*S*,2*R*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **7**.

The second eluted epoxide was obtained in 32% yield as a yellow oil. This had identical spectral data to those reported above for the natural product except for the specific rotation, $[\alpha]_D^{20}=-146$ (*c* 1.15, $CHCl_3$). As the available amount was higher, repeated chromatography allowed a more pure product to be obtained. In addition, more accurate specific rotation measurements could be performed. Moreover, after diastereoselective synthesis (see below) this epoxyacetate was assigned to be (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **1**.

4.11. Epoxides from (3*R*,4*R*)-1-methyl-4-(1-methylethyl)-cyclohex-1-en-3-yl acetate 6

A mixture of two epoxides was obtained. The first eluting product was isolated in a 60% yield; $[\alpha]_D^{20}=+29$ (*c* 1.00, $CHCl_3$); 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; ^1H NMR: δ_H 4.9 (d, *J*=10 Hz, 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+$(CH_3)_2CH$), 1.3 (s, 3H, C*H*$_3$), 0.8, 0.7 (d+d, *J*=7 Hz, 6H, $(CH_3)_2CH$); ^{13}C NMR: δ_C 170.4 (CO), 70.3 (C_3), 61.7 (C_2), 58.3 (C_1), 43.8 (C_4), 30.1 (C_6), 27.6 $(CH_3)_2CH$), 22.3 (CH_3CO), 21.1 (C_5), 20.4, 16.8 and 16.1 ($3\times CH_3$); 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 synthesis (see below) this product was assigned to be (1*R*,2*S*,3*R*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **8**.

The second eluting epoxide was obtained in 32% yield. $[\alpha]_D^{20}=-138$ (*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; ^1H 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+$(CH_3)_2CH$), 1.3 (s, 3H, C*H*$_3$), 0.9 and 0.7 (d+d, *J*=7 Hz, 6H, $(CH_3)_2CH$); ^{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 $(CH_3)_2CH$), 23.9 (CH_3CO), 21.1 (C_5), 20.8, 20.5 and 16.3 ($3\times CH_3$); MS: *m*/*z* 212 (M^+, 9), 170 (8), 169 (9), 154 (100), 152 (54), 134 (14), 129 (13), 112 (32) and 81 (4). After diastereoselective synthesis (see below) this product was assigned to be (1*S*,2*R*,3*R*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **9**.

4.12. 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 −10°C under N_2. Then L-(+)-diethyl tartrate or D-(−)-diethyl tartrate (0.91 mmol) and titanium *iso*-propoxide (1.35 mmol) were added; this was followed by slow addition of a solution of dry *tert*-butyl hydroperoxide (7.6 mmol) in anhydrous CH_2Cl_2. When the addition was finished (ca. 20 min) the mixture was cooled at −20°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 −20°C, and then H_2O was added (about 20 times the weight of titanium *iso*-propoxide). After stirring at 0°C for 30 min and then allowing the mixture to warm to room temperature and stirring for a further 15 min, aqueous NaOH (30%) solution saturated in NaCl (1.0 mL) was added. The mixture was vigorously stirred for 10 min and then extracted with CH_2Cl_2. The combined organic extracts were filtered through a Celite column and concentrated in vacuo. Chromatography of the residue on silica gel provided the corresponding epoxide.

Both enantiomeric tartrates provided (1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohexan-3-ol **10** when (3*S*,4*R*)-1-methyl-4-(1-methylethyl)-cyclohexan-3-ol 3 was used as the starting alcohol. Yields were about 52% although ca. 15% of starting material was recovered. $[\alpha]_D^{20}=-85$ (*c* 2.93, $CHCl_3$); HRMS (EI): *m*/*z* 170.1316 ($C_{10}H_{18}O_2$ requires 170.1306); IR: ν_{max} 3600–3200, 2957, 2926, 2868, 1384, 1073 and 842; ^1H 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, C*H*$_3$), 1.3–0.6 (m, 3H, H-5+$(CH_3)_2CH$), 1.0 and 0.9 (d+d, *J*=7 Hz, 6H, $(CH_3)_2CH$); ^{13}C NMR: δ_C 64.5 (C_3), 62.1 (C_2), 61.0 (C_1), 46.8 (C_4), 31.0 (C_6), 27.7 $(CH_3)_2CH$), 22.8 (C_5), 20.8, 20.7 and 17.3 ($3\times CH_3$ MS: *m*/*z* 170 (M^+, 1), 137 (4), 127 (11), 123 (5), 112 (66), 109 (16), 99 (25), 97 (18), 86 (50), 84 (83), 81 (100), 72 (46), 71 (76), 57 (20) and 55 (35). Acetylation of this product under the conditions described above provided (+)-(1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate, (+)-**1**, in 84% yield.

Likewise, both enantiomeric tartrates provided (1*S*,2*R*,3*R*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-1-cyclohexan-3-ol **11** when (3*R*,4*R*)-1-methyl-4-(1-methylethyl)-1-cyclohexen-3-ol **4** was used as a starting alcohol. Yields were about 52% and ca. 15% of starting material was recovered. $[\alpha]^{20}_{D} = +28$ (*c* 1.41, $CHCl_3$); HRMS (EI): *m*/*z* 170.1306 ($C_{10}H_{18}O_2$ requires 170.1306); IR: ν_{max} 3600–3000, 2952, 2931, 2868, 1463, 1373, 1026 and 847; ^1H NMR: δ_H 3.7 (t, $J = 9$ Hz, 1H, H-3), 3.2 (d, $J = 2$ Hz, 1H, H-2), 2.2 (br s, 1H, OH), 2.1 (m, 1H, H-4), 1.8 (m, 2H, H-6), 1.5–0.9 (m, 3H, H-5+$(CH_3)_2C\mathit{H}$), 0.9 and 0.8 (d+d, $J = 7$ Hz, 6H, $(C\mathit{H}_3)_2CH$); ^{13}C NMR: δ_C 69.7 (C_3), 63.9 (C_2), 62.3 (C_1), 42.6 (C_4), 28.1 (C_6), 25.5 $(CH_3)_2\mathit{C}H$), 24.0 (C_5), 21.2, 20.1 and 16.5 ($3\times \mathit{C}H_3$); MS: *m*/*z* 170 (M^+, 1), 137 (4), 127 (20), 123 (5), 112 (98), 109 (20), 99 (37), 97 (18), 84 (45), 81 (100), 72 (46), 71 (76), 57 (25) and 55 (30). Acetylation of this product under the above described conditions provided (1*S*,2*R*,3*R*,4*R*)-1,2-epoxy-1-methyl-4-(1-methylethyl)-cyclohex-3-yl acetate **9** in 90% yield.

Acknowledgements

The authors acknowledge the financial support of Fundación José y Ana Royo (fellowship to AC) and the Generalitat Valenciana (fellowship to CL). We thank Professor Dr. Ramón Mestres for providing the *M. tomentosa* plant.

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