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\textsuperscript{1-3}, which is frequently present in the essential oil fraction. Among them, herbs belonging to the genus \textit{Minthostachys} Griseb are used as natural insecticides by the local farmers of los Andes from Venezuela to Argentina\textsuperscript{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) \textit{Minthostachys tomentosa}\textsuperscript{9}. A natural product originally named 1,2-epoxymenthyl acetate and tentatively assigned to possess the same structural formula as 1 (without indication of the stereochemistry) has been previously isolated from \textit{Mentha rotundifolia}\textsuperscript{10}. The structural and stereochemical assignment of the product isolated in the present work from \textit{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.

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\begin{align*}
(1S,2R,3R,4S)-1
\quad & \quad (1R,2S,3S,4R)-1
\end{align*}
\]
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 \(\nu= 1757 \text{ cm}^{-1}\) and \(\delta= 171.0, \text{ ppm}\) respectively. The \(^{13}\)C NMR spectrum exhibited also distinct signals at \(\delta= 68.6, 58.7\) 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 \(^1\)H NMR spectrum, the two deshielded methine protons appeared at \(\delta= 5.1\) (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 \(\delta= 0.9\) 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\textsuperscript{11} using LiAlH\textsubscript{4} provided an approximately 1:1 mixture of Z- and E-piperitol (3 and 4). The mixture was easily resolved by column chromatography and the spectroscopic data were fully coincident with those previously reported in the literature\textsuperscript{12-13}. Subsequent treatment of both separated alcohols with acetic anhydride in CH\textsubscript{2}Cl\textsubscript{2} provided the corresponding acetates (5 and 6). Final epoxidation of the acetates using m-chloroperbenzoic acid (MCPA) in CH\textsubscript{2}Cl\textsubscript{2} gave two pairs of diastereomers [(1R,2S,3S,4R)-1/7 and 8/9], which were also resolved by column chromatography.

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 \textit{Minhostachys tomentosa}. 
Thus, once established 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 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 but unfortunately the results were similar to those obtained with LiAlH₄ (see above).

![Chemical structures and reactions]

(3S,4R)-Piperitol (3) and (3R,4R)-Piperitol (4) were separately submitted to a Sharpless epoxidation, 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 possess 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. Thus, finally 10 was definitely 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 asymmetric carbons, stereochemical assignment of the resulting acetates (1R,2S,3S,4R)-1 and 8 was straightforward. At this point, it was realized that the NMR spectra (^1H and ^13C) 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,2R,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 commercial t-butyl hydroperoxide (80 %) was dried over activated molecular sieves (3 Å) obtaining purity of 88 % with less than 2000 ppm of H2O; all other chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtained as liquid; νmax is given for the main absorption bands. 1H and 13C NMR spectra were recorded at 300 and 75 MHz, respectively, in CDCl3 solvent; chemical shifts are reported in δ (ppm) values, using TMS as internal standard. The assignment of 13C 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 F254), 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$_2$Cl$_2$ as collecting solvent. The
resulting extract was dried over anhydrous Na$_2$SO$_4$, 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$_2$Cl$_2$, 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$_2$Cl$_2$: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-
methyleneethylcyclohexan-3-ol , acetate (I) on the basis of spectral data. [α]$^{20}_D = + 93º$ (c
1.27, CHCl$_3$); HRMS: $m/z$ 212.1418 (C$_{12}$H$_{20}$O$_3$ requires 212.1412); IR: $\nu_{\text{max}}$ 2966, 2882,
1757, 1457, 1375, 1236, 1083, 1024 and 985; $^1$H NMR: $\delta$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, CH$_3$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, 2xCH$_3$);
$^{13}$C NMR: $\delta$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
(C1), 22.5 (CH3CO), 20.8, 20.7 (3xCH3) and 18.3 (C3); MS: m/z 212 (M+, 2), 169 (9), 154 (100), 153 (52), 134 (6) and 112 (30).

Chemical Syntheses

Reduction of 1-Methyl-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 N2 to a stirred suspension of LiAlH4 (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 H2O (2.3 mL), 15% aqueous solution of NaOH (2.3 mL) and distilled H2O (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: δH 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, 2xCH3); 13C NMR: δC 139.5 (C1), 123.6 (C2), 64.9 (C3), 46.1 (C4), 31.4 (C6), 28.3 (C1”), 23.3 (C1’), 20.9, 20.7 (2xCH3) and 20.4 (C3); 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: δH 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, 2xCH3); $^{13}$C NMR: $\delta$C 137.2 (C1), 125.4 (C2), 68.8 (C3), 47.6 (C4), 30.0 (C6), 26.3 (C1''), 23.0 (C1'), 21.1, 21.0 (2xCH3) and 20.7 (C5); 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 catalytic amount of 4-dimethylamino-pyridine (DMAP) in dry CH$_2$Cl$_2$ (15 mL) was cooled at 4°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$_2$O, stirred for 30 min. and extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with acqueous HCl (5 %) and brine, dried over Na$_2$SO$_4$ 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: $\delta$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$_3$CO), 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, 2xCH3); $^{13}$C NMR: $\delta$C 170.8 (CO), 141.4 (C1), 119.8 (C2), 68.5 (C3), 44.3 (C4), 31.2 (C6), 28.3 (C1''), 23.3 (C1'), 21.3 (C5), 20.7 and 20.6 (2xCH3); 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: $\delta$H 5.2 (br s, 1H, H-2), 5.1 (m, 1H, H-3), 1.9 (s, 3H, CH$_3$CO), 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, 2xCH3); $^{13}$C NMR: $\delta$C 170.5
(CO), 139.1 (C1), 120.6 (C2), 71.5 (C3), 43.5 (C4), 29.1 (C6), 26.4 (C1''), 22.7 (C1'), 20.75 (C5), 20.9 and 20.4 (2xCH3); 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 [α]$_{20}^{D}$ = -146º (c 1.15, CHCl$_3$).

(1S,2R,3R,4R)-1-Epoxy-1-methyl-4-methylethylcyclohexan-3-ol, acetate (9).
90 % yield; [α]$_{20}^{D}$ = -138º (c 1.81, CHCl$_3$); HRMS (EI): m/z 212.1416 (C$_{12}$H$_{20}$O$_3$ requires 212.1412); IR: ν$_{\text{max}}$ 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, CH$_3$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, 2xCH$_3$); $^{13}$C NMR: δ$_{C}$ 171.2 (CO), 72.6 (C3), 61.3 (C1), 61.1 (C2), 38.6 (C4), 28.1 (C6), 25.7 (C1''), 23.9 (CH$_3$CO), 21.1 (C5), 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$_2$Cl$_2$ (13 mL) was added another solution of the corresponding acetate, 6 or 7 (0.42 mmol) in dry CH$_2$Cl$_2$ (5 mL) dropwise. After 6 h of stirring the mixture was poured into a aqueous solution of NaHCO$_3$ (10%) and extracted with CH$_2$Cl$_2$. The combined organic extracts were dried over Na$_2$SO$_4$ 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}= -138^\circ \text{ (c 1.81, CHCl}_3)](\text{HRMS (El): } m/z 212.1410 (C_{12}H_{20}O_3 \text{ requires } 212.1412); \text{IR: } \nu_{\text{max}} 2960, 2930, 2870, 1740, 1370 \text{ and } 1245; {^1}\text{H NMR: } \delta_{\text{H}} 5.4 \text{ (s, } 1\text{H, H-3), 3.0 \text{ (d, } J = 3 \text{ Hz, } 1\text{H, H-2), 2.1 \text{ (s, } 3\text{H, CH}_3\text{CO), 2.0 \text{ (m, } 1\text{H, H-4), 1.8 \text{ (m, } 2\text{H, H-6), 1.5-1.3 \text{ (m, } 3\text{H, H-5+ H-1\''), 1.3 \text{ (s, } 3\text{H, H-1\'}), 0.9 \text{ and } 0.8 \text{ (d+d, } J = 7 \text{ Hz, } 6\text{H, } 2\times\text{CH}_3\text{); } {^{13}}\text{C NMR: } \delta_{\text{C}} 170.7 \text{ (CO), 69.7 \text{ (C}_3\text{), 59.3 \text{ (C}_2\text{), 58.3 \text{ (C}_1\text{), 39.0 \text{ (C}_4\text{), 28.1 \text{ (C}_6\text{), 24.1 \text{ (CH}_3\text{CO), 21.1 \text{ (C}_1\text{)}, 20.9, 20.4, 20.3 \text{ (3xCH}_3\text{) and 19.6 \text{ (C}_5\text{); MS: } m/z 212 \text{ [M}^+\text{, 9], 194 \text{ (4), 170 \text{ (12), 154 \text{ (100), 152 \text{ (85), 134 \text{ (23) and 112 \text{ (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}= -138^\circ \text{ (c 1.81, CHCl}_3)](\text{HRMS (El): } m/z 213.1495 \text{ (M+H}^+, \text{ C}_{12}\text{H}_{21}\text{O}_3 \text{ requires } 213.1490); \text{IR: } \nu_{\text{max}} 2955, 2875, 1735, 1375, 1240 \text{ and } 990; {^1}\text{H NMR: } \delta_{\text{H}} 4.9 \text{ (d, } J = 10, 1\text{H, H-3), 2.8 \text{ (s, } 1\text{H, H-2), 2.1 \text{ (s, } 3\text{H, CH}_3\text{CO), 2.1-1.9 \text{ (m, } 2\text{H, H-6), 1.6 \text{ (m, } 4\text{ H, H-4+H-5+ H-1\''), 1.3 \text{ (s, } 3\text{H, H-1\'}), 0.8, 0.7 \text{ (d+d, } J = 7 \text{ Hz, } 6\text{H, } 2\times\text{CH}_3\text{); } {^{13}}\text{C NMR: } \delta_{\text{C}} 170.4 \text{ (CO), 70.3 \text{ (C}_3\text{), 61.7 \text{ (C}_2\text{), 58.3 \text{ (C}_1\text{), 43.4 \text{ (C}_4\text{), 30.1 \text{ (C}_6\text{), 27.6 \text{ (C}_1\text{)}, 22.3 \text{ (CH}_3\text{CO), 21.1 \text{ (C}_3\text{), 20.4, 16.8 and 16.1 \text{ (3xCH}_3\text{); MS: } m/z 213 \text{ [(M+1}^+, \text{ 4], 212 \text{ (M}^+, 1), 169 \text{ (5), 154 \text{ (86), 152 \text{ (26), 137 \text{ (11), 127 \text{ (65), 112 \text{ (100), 109 \text{ (30), 84 \text{ (20), 81 \text{ (51), 71 \text{ (14) and 55 \text{ (16). After}})}})}}\}

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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$_2$Cl$_2$ (7 mL) was cooled to $-10^\circ$ 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$_2$Cl$_2$. When the addition was finished (ca. 20 min.) the mixture was cooled at $-20^\circ$ C and a new solution of the corresponding allylic alcohol, 3 or 4, (4.5 mmol) in anhydrous CH$_2$Cl$_2$ (0.7 mL) was added dropwise. The mixture was stirred overnight at $-20^\circ$ C and then H$_2$O was added (about 20 times the weight of titanium isopropoxide). After 30 min. of stirring at $0^\circ$ C and 15 min. remaining at room temperature a NaOH (30%) aqueous solution saturated in NaCl (1.0 mL) was added. The mixture was stirred vigorously for 10 min. and after extracted with CH$_2$Cl$_2$. 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. $[\alpha]_{20}^D = -85^\circ$ (c 2.93, CHCl$_3$); HRMS (EI): $m/z$ 212.1416 (C$_{12}$H$_{20}$O$_3$ requires 212.1407); IR: $\nu_{\text{max}}$ 2950, 2870, 1740, 1370, 1240 and
\[ \text{H NMR: } \delta \text{H 4.1 (m, 1H, H-3), 3.2 (d, } J = 8 \text{ Hz, 1H, H-2), 2.2-2.0 (m, 2H, H-4+OH), 1.6 (m, 2H, H-6), 1.3 (s, 3H, H-1'), 1.3-1.1 (m, 3H, H-5+H-1''), 1.0 and 0.9 (d+d, } J = 7 \text{ Hz, 6H, 2xCH}_3; \text{ C NMR: } \delta \text{C 64.5 (C}_3\text{), 62.1 (C}_1\text{), 61.0 (C}_2\text{), 46.8 (C}_4\text{), 31.0 (C}_6\text{), 27.7 (C}_1'\text{), 23.0 (C}_5\text{), 20.8, 20.7 and 17.3 (3xCH}_3; \text{ MS: } m/z 212 (M^+, 6), 154 (10), 153 (100), 152 (18), 134 (6), 112 (10), 111 (8), 110 (5), 109 (17), 95 (10), 81 (15), 43 (35) and 41 (10). \]

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. \([\alpha]_{D}^{20} = +28^\circ\) (c 1.41, CHCl_3; HRMS (EI); \[ m/z 212.1416 (C_{12}H_{20}O_3 \text{ requires 212.1407); IR: } \nu_{\text{max}} 2950, 2870, 1740, 1370, 1240 \text{ and } 1100; \text{ H NMR: } \delta \text{H 3.7 (t, } J = 10 \text{ Hz, 1H, H-3), 3.2 (d, } J = 2Hz, 1H, H-2), 2.5 (d, } J = 8 \text{ Hz, 1H, OH), 2.1 (m, 1H, H-4), 1.8 (m, 2H, H-6), 1.5-1.3 (m, 2H, H-5), 1.3 (s, 3H, H-1'), 1.1-0.9 (m, 3H, H-1''), 0.9 and 0.8 (d+d, } J = 7 \text{ Hz, 6H, 2xCH}_3; \text{ C NMR: } \delta \text{C 69.7 (C}_3\text{), 63.9 (C}_1\text{), 62.3 (C}_2\text{), 42.6 (C}_4\text{), 28.1 (C}_6\text{), 25.5 (C}_1'\text{), 24.0 (C}_5\text{), 21.2, 20.1 and 16.5 (3xCH}_3; \text{ MS: } m/z 212 (M^+, 6), 154 (10), 153 (100), 152 (18), 134 (6), 112 (10), 111 (8), 110 (5), 109 (17), 95 (10), 81 (15), 43 (35) and 41 (10). \]

**Biological assays.**

**Insects.** *Oncopeltus fasciatus* Dallas were maintaned 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.\textsuperscript{18} Briefly, 15 third-instar *O. fasciatus* nymphs were
confined to a 5 cm Petri dish coated with 50 µg/cm² 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.

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References


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

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<td>4.9, d, $J=10$ Hz</td>
<td>5.1, dd, $J=10$, 2 Hz</td>
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</table>
Figures

Figure 1

\[(1S,2R,3R,4S)-1\]  \[(1R,2S,3S,4R)-1\]
Figure 2.

R-(−)-Piperitone

2

LiAlH₄

Diethyl ether

\[ \text{OH} \]

3
(3S,4R)-Piperitol
43%

\[ \text{O} \]

5
(3R,4R)
84%

\[ \text{Ac}_2\text{O}/\text{DMAP} \]

\[ \text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2 \]

\[ \text{OH} \]

4
(3R,4R)-Piperitol
48%

\[ \text{O} \]

6
(3R,4R)
83%
Figure 3.

![Chemical structures and reactions](image-url)
Figure 4.

3 (3S,4R)-Piperitol

L-(+)-DET

Ti(i-PrO)₄
t-BuOOH
4Å/CH₂Cl₂

D-(-)-DET

4Å/CH₂Cl₂

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

OH

Ac₂O/DMAP
Et₃N/CH₂Cl₂

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

9 (1S,2R,3R,4R)

Ac₂O/DMAP
Et₃N/CH₂Cl₂

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

4 (3R,4R)-Piperitol

L-(+)-DET

Ti(i-PrO)₄
t-BuOOH
4Å/CH₂Cl₂

D-(-)-DET

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

OH

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